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# Mosquitoes and Their Control

Second Edition

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ISBN 978-3-540-92873-7 e-ISBN 978-3-540-92874-4  
DOI 10.1007/978-3-540-92874-4  
Springer Heidelberg Dordrecht London New York

Library of Congress Control Number: 2009931710

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*Cover illustration:* Courtesy of Roger Eritja, Spain, Centro Agricoltura Ambiente, Italy, Roland Kuhn and Norbert Becker, Germany.

*Cover design:* WMXDesign GmbH, Heidelberg, Germany.

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

## Foreword to the Second Edition

The magnitude of the role played by mosquitoes in the scheme of human life is undisputed. It is some 100 years since mosquitoes came to be recognised and incriminated as playing a highly significant part in the affairs of humans. Mankind has been plagued by mosquito nuisance and mosquito-borne diseases since the dawn of human history, resulting in immeasurable suffering and economic losses. Mosquitoes transmit some of the deadliest diseases known to man, such as malaria, yellow fever, dengue, encephalitis, filariasis, and a hundred or so other infections, claiming many millions of lives. Despite much research, and decades of mosquito control work around the world, mosquitoes remain a major global public health problem. In many developing countries, the eradication of classic mosquito-borne diseases still seems a long way off in the future. Some developed countries have experienced the introduction and establishment of exotic mosquito species, and of mosquito-borne diseases formerly unknown in these regions. Increased global travel has contributed to the current trends in re-emerging mosquito-borne infectious diseases, exemplified by increasing numbers of imported malaria cases, and recent outbreaks of West Nile and Chikungunya fever have given rise to growing public concern. Seasonal outbreaks of nuisance mosquito populations, which plague ecologically sensitive tourist and urban areas, cause significant economic damage and constitute major problems globally.

The second edition of “Mosquitoes and Their Control” has been thoroughly updated since the first edition was published in 2003. This has been achieved through a review of the very extensive and rapidly-growing global literature on mosquitoes. The objective of this revised and expanded text is to provide a broad and balanced description of the biology of mosquitoes, their significance as disease vectors, and the techniques available for their management. The authors present this in a clear, concise, readable fashion throughout with a variety of specially prepared line illustrations and photographs. As a result, this book is appropriate for students, scientists, mosquito professionals and the lay reader alike. The text provides a summary of this important group of insects, of which over 3500 species have been recorded worldwide, across five continents. Significant sections have been added on mosquito-borne diseases, such as malaria, arboviruses, and filariases, and their vectors worldwide. Among others, the most important Sub-Saharan malaria vectors are discussed in detail, including seven species of the *Anopheles Gambiae* Complex.

The content is conveniently divided into separate sections and chapters on specific topics. The book opens with sections on systematics, morphology, and the biology of mosquitoes, followed by an extensive review of their medical significance. The chapter on mosquito research reviews a wide range of practical techniques that are used by those involved in mosquito research and surveillance, in both the laboratory and

the field. Additional sections contain keys to the identification of larval and adult mosquito genera and species present in Europe, and genera and species of important vector/nuisance female mosquitoes in Africa, Asia, Australia, and the Americas. This is followed by a detailed and instructive account of the morphology, ecology, and distribution of all the discussed species belonging to 13 genera including: *Anopheles*, *Aedes*, *Armigeres*, *Haemagogus*, *Ochlerotatus*, *Psorophora*, *Verrallina*, *Culex*, *Culiseta*, *Coquillettidia*, *Mansonia*, *Orthopodomyia*, and *Uranotaenia*. Accurate species identification is central to any successful mosquito control or mosquito-borne disease surveillance programme.

The final section is dedicated to the control of mosquitoes, and covers biological, chemical, and physical techniques, as well as genetic control, environmental management and personal protection. The section on biological control contains important information on mosquito predators, parasites, and pathogens and is of special significance in the light of the increasing interest in finding alternatives to conventional pesticides. The section on mosquito control concludes with a review of integrated mosquito management, showing how these various approaches may be used collectively to improve effectiveness, while ensuring the safety of humans and the environment.

The authors' very extensive knowledge and experience in their respective topics, together with the information so generously contributed by others working in this field, have been combined to produce this comprehensive and unique treatise.

This text book will serve as a valuable resource for vector ecologists, medical entomologists, and professionals involved in the biology, ecology, systematics and the control of mosquitoes world-wide. We hope that the primary beneficiaries will be the students, scientists, and professionals dealing on a day-to-day basis with mosquitoes and their control. However, society as a whole stands to gain enormously from more effective and environmentally responsible mosquito management programmes designed on the basis of a more comprehensive understanding of mosquitoes and the issues around their control, as presented in this enlightening book.

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# Foreword to the First Edition

Mankind has been plagued by mosquitoes as nuisances and as vectors of mosquito-borne diseases for centuries, resulting in inestimable economic losses and indeterminate human suffering. Mosquitoes transmit some of the deadliest diseases known to man malaria and yellow fever—as well as dengue, encephalitis, filariasis and a hundred or so other maladies. In spite of decades of mosquito control efforts throughout affected regions worldwide, this scourge has not left us and our present-day overpopulated, jet-linked world remains on the edge of resurgence and outbreaks of old and new mosquito-borne disease epidemics.

Ninety-two mosquito species of more than 3200 recorded worldwide, traverse the European continent. In Europe malaria was eradicated ca. 50 years ago. Current trends in re-emerging mosquito-borne infectious diseases, exemplified by increasing numbers of imported malaria cases and recent outbreaks of West Nile fever (WNV) virus, however, have given rise to growing public concern. Seasonal outbreaks of nuisance mosquito populations, which plague ecologically sensitive tourist and urban areas cause significant economic damage and constitute the major problem in Europe.

This book is the product of a monumental task of collecting, processing and organising vital information on the mosquito populations of Europe. It presents a multitude of information on the bionomics, systematics, ecology and control of both pestiferous (nuisance) and disease vectors in an easily readable style providing practical guidance and important information to both professional and layman alike. It is conveniently divided into four parts containing sixteen chapters. Part one deals with general information on systematics, morphology and biology of mosquitoes, their medical significance and a very useful subchapter on mosquito research that includes important techniques and technologies utilised in mosquito surveys for sampling eggs, larvae and adult mosquito populations. Part two contains keys to identification of larval and adult mosquito genera. Part three gives a very detailed and instructive account of the morphology, ecology and distribution of all 92 European species included in eight genera: *Anopheles*, *Aedes*, *Ochlerotatus*, *Culex*, *Culiseta*, *Coquilletidia*, *Orthopodomyia* and *Uranotaenia*. This part is extremely important for species identification in any successful mosquito control or mosquito-borne disease surveillance programme.

Part four dealing with control of mosquitoes is well ordered in seven sections: biological, chemical and physical control as well as personal protection, integrated pest management, implementation of survey methodologies and management. This information is particularly necessary for a comprehensive approach to both studying and controlling mosquitoes. This part distinctly demonstrates the tremendous experience that the authors possess over decades in mosquito biology and control.



The section on biological control contains important information on predators, parasites and pathogens is of special significance in light of the increasing interest in replacing detrimental chemical pesticides with environmentally friendly integrated biological control technologies.

The authors of this book collectively possess a vast amount of knowledge and experience in the relevant topics and they have meticulously gathered and incorporated the experience and knowledge of others to compose the first and only comprehensive treatise on the subject of European mosquitoes.

This book is a valuable tool for vector ecologists, entomologists, and all those involved with mosquito control, biology, ecology and systematics in Europe. It's primary beneficiaries will be the students, scientists and professionals dealing on a day-to-day basis with mosquitoes and their control. Society as a whole stands to gain from improved, environmentally responsible mosquito management programmes designed on the basis of the broader understanding of mosquitoes and their control provided in this enlightening book.

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## Preface to the Second Edition

The first edition of this book (Becker et al. 2003) included comprehensive and globally relevant chapters on Mosquito Systematics, Biology, Morphology, Medical Importance, Research Techniques, and Control. However, the identification keys and descriptions were limited to European mosquito species. Nonetheless, the reviews (Olejníček and Gelbič 2004) and the rapid sale of the first edition, indicated that the book was widely appreciated and accepted by both scientists and professionals.

Having had time to reflect on the first edition, the authors realised the need for a second edition and agreed that the scope required widening to include an overview of selected important vector and nuisance mosquito species world-wide. This 2nd edition is intended to be useful not only to readers in Europe, but to readers around the world.

The additional list of mosquito species we chose to include, was compiled using our own knowledge and experience, and that of our colleagues in various regions, and of course utilising the very extensive literature.

Despite the natural division of the mosquito fauna of the world into zoo-geographical regions, in our work we have adopted a continental approach. This allows us to present a simpler overview of the current situation, and to provide an understanding of the global distribution of important nuisance and disease vector mosquitoes, irrespective of the science of zoo-geography.

Given that the tribe Aedini is the largest and most polyphyletic group of mosquitoes, we therefore agree with the work of Reinert (2000c) and Reinert et al. (2004, 2006, 2008), in revising it and establishing monophyletic genera. We believe that progress should be made towards establishing genera as “monophyletic” or natural groups of species; that is to say groups including an ancestor and all of its descendants.

However, several groups of mosquito taxonomists and editors of major professional journals have not accepted the latest nomenclature proposed by Reinert et al. (2004, 2006, 2008), and have even returned *Ochlerotatus* (Reinert 2000c) to the sub-generic rank after several years of extensive usage.

As a compromise, the placement of genera within the tribe Aedini in this edition, is based on “traditional” generic and sub-generic affiliations recognised prior to and including the separation of *Aedes* and *Ochlerotatus* by Reinert (2000c). In this text, the “latest” placement of genera proposed by Reinert et al. (2004, 2006, 2008) is indicated in square brackets following the “traditional” generic names. Those who would prefer to follow the nomenclature before Reinert (2000c) can treat the name

*Ochlerotatus* as equivalent to *Aedes*. Finally, for those species whose status did not change after Reinert (2000c), we have not provided additional information.

We have restructured and broadened chapters such as “Mosquito Research Techniques” and “Medical Importance”, to make this text more useful to our colleagues working outside Europe.

In general, we sincerely hope that we have produced a text that will be useful and interesting to entomologists in general, and particularly to professionals in public health battling the scourges of mosquito nuisance and mosquito-borne diseases.

The authors

## Preface to First Edition

Throughout the world, mosquitoes interact with man in many different ways. However, despite the very extensive research on these interactions, there remains much that has not yet been fully understood. The attempt by the authors of this volume is to highlight the importance of a basic knowledge of mosquito biology as a basis for successful control operations.

Compared to Curculionidae, the largest family in the animal kingdom, with 35,000 known species, Culicidae, numbering more than 3,200 species, could be ranked as a family of only a small-to-moderate size. Even though yield losses caused by weevils could be estimated in billions of dollars, mosquitoes are able to carry many lethal diseases in their bodies. By the time you have read this Preface (5 min), ten human lives would have been lost because of plasmodian infections. Apart from being the well-known vectors of life-threatening diseases, in some parts of the world, mosquitoes may also occur in enormous numbers thus causing a significant reduction in human life quality and serious economic damage, for instance, in livestock.

Ramsdale and Snow (1999) published a list of currently recognised European mosquito taxa with synonyms. They included 98 species in 7 genera and 17 subgenera. The authors of this volume, in deciding upon the species that should be covered, have come to the conclusion that the pertinent species are those that were recorded on more than one occasion at least, where type specimens have been deposited, identification material has been available and where the position regarding the validity of the species is satisfactory. In addition to these requirements, information about the geographical distribution with substantiating references was considered. According to this, 92 species and subspecies belonging to 8 genera and 18 subgenera are described and included in the keys.

The following species or subspecies previously reported in Europe are not included in the list: *Oc. gilcolladoi* (Villa, Rodriguez and Llera 1985) [*Dahlia gilcolladoi*] was named after a form from central Spain differing in certain features of larval chaetotaxy from both *Oc. echinus* and *Oc. geniculatus*. The position regarding the validity of this species is unsatisfactory. Type specimens were not deposited, and material is not available for examination (Ramsdale and Snow, 1999). *Oc. thibaulti* (Dyar and Knab, 1909) is a Nearctic species in Europe, recorded from the river Dnieper, Ukraine (Gutsevitch and Goritskaya, 1970), but no longer found there according to Gutsevitch and Dubitsky (1987). *Oc. atropalpus* (Coquillett, 1902) is not considered here because it was reported only once from Italy and it is not considered as a permanent species in the European area.

For the species *Oc. krymmontanus* Alekseev 1989, *Oc. coluzzii* Rioux, Guilvard and Pasteur 1998, *Oc. duplex* Martini 1926 and *Cx. deserticola*, short notes are given in the description of the species.

The principal objective of the taxonomy and morphology chapters is to provide an identification key to both adult females and males as well as to fourth instar larvae, which incorporate all the 92 species. At the beginning of Chaps. 6–8, keys for the identification of genera are given. Recently, Reinert (2000c) divided the composite genus *Aedes* Meigen into two genera, *Ochlerotatus* Lynch Arribalzaga and *Aedes*. Adults of these genera are distinguished primarily on the basis of genitalic characters that require dissection. For this reason, the species belonging to these genera are treated in a single key. The illustrated keys are followed by a detailed description of the morphology, biology and distribution of each species. The morphological terms engaged in this volume are somewhat changed from those used by Harbach and Knight (1980, 1981), taking into consideration homology, phylogeny and their general use among the dipterous insects.

When a specimen has been keyed out, it should be compared with the description of that species to use the additional information as a cross-check. The description of the species is given according to the examination of the Peus collection, as well as a considerable number of species sampled in different European regions and various literature sources.

Characters of fourth instar larvae are often clearer than those of the adults, and many taxonomists prefer to identify mosquitoes in this stage. Since there is a certain degree of variation among the larvae of a species, it is best to identify, if possible, from a series of specimens. Rare exceptions to key characters can almost always be found. More important variations and their relative frequency are indicated in the systematic section of the species.

In the keys, the larval chaetotaxy of the thorax and abdomen is not used to such an extent as by other authors. This seems to be a little bit “old fashioned”, but on the other hand, quite often setae may be broken off, lying in a misleading or barely visible position in slide preparations or in the worst case, are totally missing. If the latter occurs, an experienced eye is needed to see the alveola or tubercle of the missing seta.

Although eggs, early instar larvae and pupae of most European species are known, they are more difficult to identify than adults and fourth instar larvae. Rearing eggs to fourth instar larvae and pupae to adults is easier and less time consuming than to identify them in the early stages.

The authors suggest that the user should study the sections on general morphology before starting to identify specimens. The user should also be familiar with the proper sampling and mounting techniques of adults and larvae, because the presence of a full set of scales, setae and other features is essential for identification. Updated mosquito distribution throughout Europe, together with the bioecological conditions to be met for each species, should also help in the species identification. The territory of Europe, despite not being a distinct zoogeographical region, is chosen in an attempt to provide for the first time a unique key for the whole European region.

The authors' intention is to encourage and give support to every person who intends to start, or already has some experience in, mosquito control. The concept of the book is also based on several fields of knowledge that are important for everyone who deals with mosquitoes. Overviews on mosquito taxonomy, morphology, biology, biological and chemical control measures are given, to complete the information needed for a comprehensive approach to both studying and controlling mosquitoes.

Mosquito control measures are dependent on many complex and interacting factors ranging from biological (species-dependent), abiotic and physical factors influencing the phenology and abundance of mosquitoes (terrain features, climate, types

of breeding sites etc.) to administrative, organisational and most certainly economical conditions. A decision to use one or another mosquito control measure is highly dependent on a basic knowledge of all aspects of the target species, and the impact of a chosen control method on the target and non-target organisms, as well as on the environment. A professional control programme should combine cost effectiveness, acceptable level of mosquito population suppression, and an environmentally sensitive approach. In some situations, it is possible to rely on inexpensive and simple methods such as applying fragments of copper wire into flower pots for the control of *Ae. albopictus* larvae (Bellini, pers. comm.), while in others only pure biological, highly selective control measures are allowed, such as in the Upper Rhine Valley, where almost the entire mosquito control programme in the river floodplains has relied solely on *B. thuringiensis israelensis* larviciding for several decades. Sections on different approaches in mosquito control (chemical, biological, integrated or personal protection) provide basic information about different methods of using products with different formulation and toxicological features, effectiveness on target species and their impact on non-target organisms. Information from this part of the volume attempts to serve as a basis for an appropriate mosquito control operation, allowing the user to live in relative security from some vector-borne diseases, to alleviate the effects of abundant nuisance populations, to re-establish wetlands and to share and enjoy nature by conserving the biodiversity, by using environmentally friendly control tools.

Up to now, a comprehensive book in which the taxonomy, biology and dispersal of all currently known European mosquito species are described, as well as the options for their control, has been missing. This volume should fill the gap and be a valuable help to scientists and indeed all those interested in, or working in any of the fields related to, Culicidae. It should provide guidance to field workers concerned with mosquito control and those who wish, for example, to learn more about the behaviour of the species in their region, about mosquito breeding sites, or about the mosquito control techniques and options that may be suitable for each specific environment. Since there is still much information that for some, despite greatly increased access to the Internet, may be difficult to acquire, we have tried to include and summarise all the available information, so that entomologists can apply it to their own situations.

The authors



# Acknowledgments

We are most indebted to Paul Schädler, President of the German Mosquito Control Association (KABS, Waldsee, Germany) and the members of the KABS-Scientific board, Volker Storch (University of Heidelberg, Germany), Herbert W. Ludwig<sup>†</sup> (University of Heidelberg), Wolfgang Schnetter<sup>†</sup> (University of Heidelberg), Hartwig Rihm (Mayor of Au), Gisbert Dieter<sup>†</sup> (Mayor A.D. of Lampertheim, Germany), Paul Glaser (GFS, Germany), Herrmann Magin<sup>†</sup> (GFS, Germany), and Hans Wirnshofer (Mayor A.D. Ketsch, Germany, Administrative Director of KABS), for their support in all aspects of mosquito control in Germany and in preparing this text.

We are grateful for the substantial help in the preparation of the manuscript to Thin Thin Oo (KABS, Germany), Richard Russell (University of Sydney, Australia), Major Dhillon (Northwest MVCD, Corona, USA), Mir Mulla (University of California, Riverside, USA), Ute Timmermann (KABS, Germany), Andreas Arnold (KABS, Germany), Klaus Hoffmann (KABS, Germany), Thomas Weitzel (KABS, Germany), Christian Weisser (KABS, Germany), Antje Lohmann (KABS, Germany), Ernst-Jan Scholte (National Institute for Public Health and the Environment, Centre for Infectious Disease Control, Bilthoven, The Netherlands), Thania Guaycurus (Oswaldo Cruz Institute, Rio de Janeiro, Brazil), and Wanderli Tadei (INPA, Manaus, Brazil).

During the preparation of the manuscript, authors received valuable information provided by our colleagues and friends worldwide. We thank the following contributors, who supplied a variety of important and useful information: Carlos Aranda (Consell Comarcal del Baix Llobregat, Spain), Romeo Bellini (Centro Agricoltura Ambiente, Italy), Roger Eritja (SCM, Consell Comarcal del Baix Llobregat, Spain), Raul Escosa (CODE, Ebro Delta, Spain), Remi Foussadier (E.I.D., Rhone-Alpes, France), Jose Carlos Galvez (SCM, Huelva County Council, Spain), Aleksandra Gliniewicz (National Institute of Hygiene, Poland), Raymond Gruffaz (E.I.D., Rhone-Alpes, France), Karch Said (SIAP Paris, France), Bart Knols (K&S Consulting, Netherlands), Beata Kubica-Biernat (University of Gdynia, Poland), Christophe Lagneau (E.I.D. Mediterranee, France), Peter Lüthy (University of Zürich, Switzerland), Jan Lundström (University of Uppsala, Sweden), Eduard Marques i Mora (SCM, Roses Bay and Lower Ter, Spain), Jean-Pierre Mas (E.I.D. Atlantique, France), Elzbieta Lonc (University of Wroclaw, Poland), Enrih Merdic (University of Osijek, Croatia), Spiros Mourelatos (Environment, Public Health and Ecodevelopment, Greece), Odile Moussiegt (E.I.D. Mediterranee, France), Francoise Pfirsch (Mosquito Control Organization, Bas-Rhin, France), Dirk Reichle (KABS, Germany), Frantisek Rettich (National Institute of Public Health, Czech Republic), Katarzyna Rydzanicz (University of Wroclaw, Poland), Martina Schäfer (University of Uppsala, Sweden),



Francis Schaffner (ETH, Zürich, Switzerland), and Asghar Talbalaghi (Alessandria Abatement District, Italy).

The authors express appreciation for the skillful and diligent preparation of the illustrations to Miguel Neri (University of San Carlos, Cebu, Philippines), Michael Gottwald (University of Heidelberg, Germany), Tara Petrić (Novi Sad, Serbia), Vlada Vojnić-Hajduk (Novi Sad, Serbia), and Kyaw Kyaw (Ludwigshafen, Germany).

We are grateful to numerous individuals and organizations who assisted in various ways toward the final form of this book, especially to Matthias Beck (KABS, Germany), Geoffrey L. Brown (Heidelberg, Germany), Djamal Chakhmaliev (Heidelberg, Germany), Jeff Charles (Institute Pasteur, Paris, France), Culinex GmbH (Ludwigshafen, Germany), Ernest Dankwa and Peter DeChant (Valent BioSciences, USA), Jessica Fussell (Pioneer, USA), Elisabeth and Albert Gasparich (Eppelheim, Germany), Annette Hinsky-Billhard, Stefanie Keller, Ingrid Lützel (Gesellschaft zur Förderung der Stechmückenbekämpfung-GFS, Waldsee, Germany), Thomas Heeger (Böhl-Iggelheim, Germany), Mikael Henriksson (Borga, Finland), Icybac GmbH (Speyer, Germany), Thomas Imhof (Heidelberg, Germany), Michael Kinzig (Heidelberg, Germany), Olaf Becker (Ludwigshafen, Germany), Daniela Aedes Lanzalaco (Heidelberg, Germany), Steven Krause (Valent BioSciences, USA), Roland Kuhn (Mainz, Germany), Charles Myers (Glendora, USA), Beate Ruch-Heeger (Böhl-Iggelheim, Germany), Willem Takken (University of Wageningen, The Netherlands) and Srećko and Esther Zgomba (Toronto, Canada).

# Contents

## Part I General Aspects

<b>1 Systematics</b> .....	3
<b>2 Biology of Mosquitoes</b> .....	9
2.1 Oviposition.....	10
2.2 Embryonic Development .....	13
2.3 Hatching.....	13
2.4 Larvae.....	16
2.5 Pupae.....	19
2.6 Adults.....	19
2.6.1 Emergence.....	19
2.6.2 Mating.....	20
2.6.3 Dispersal and Host-Seeking Behaviour .....	21
2.6.4 Feeding.....	22
2.7 Survival During Dry Seasons and Hibernation.....	24
2.7.1 Egg Stage .....	24
2.7.2 Larval Stage .....	24
2.7.3 Adult Stage.....	24
<b>3 Medical Importance of Mosquitoes</b> .....	25
3.1 Malaria .....	25
3.2 Arboviruses .....	30
3.2.1 <i>Togaviridae (Alphavirus)</i> .....	31
3.2.2 <i>Flaviviridae (Flavivirus)</i> .....	34
3.2.3 <i>Bunyaviridae (Bunyavirus)</i> .....	37
3.3 Filariasis .....	39
3.4 Future Perspectives and Conclusions.....	40
<b>4 Mosquito Research Techniques</b> .....	43
4.1 Sampling Mosquito Eggs.....	43
4.1.1 <i>Anopheles</i> Eggs.....	43
4.1.2 Egg Rafts.....	43
4.1.3 <i>Aedes/Ochlerotatus</i> Eggs .....	44
4.1.4 Eggs in Artificial Oviposition Sites .....	45
4.2 Sampling Mosquito Larvae and Pupae .....	46

4.3	Sampling Adult Mosquitoes in the Field .....	47
4.3.1	Sampling Flying Mosquitoes .....	47
4.3.2	Adult Mosquito–Outdoor Resting Catches .....	47
4.3.3	Adult Mosquito–Indoor Catches .....	47
4.3.4	Bait Catches .....	48
4.3.5	Adult Mosquito Traps .....	49
4.3.6	Mark-Release-Recapture Techniques .....	51
4.4	Laboratory Based Research Techniques .....	51
4.4.1	Rearing Mosquitoes .....	51
4.4.2	Preserving Mosquitoes .....	53
4.4.3	Mosquito Blood Meal Identification .....	55
4.4.4	Methods for Measuring the Physiological Stage .....	55
4.4.5	Morphological and Taxonomic Techniques .....	56
4.5	Assessing the Activity of Insecticides and Repellents on Mosquitoes .....	58
4.5.1	Insecticide Susceptibility Testing .....	58
4.5.2	Assays of Insecticide Deposits on Surfaces (e.g. Walls or Nets) .....	60
4.5.3	Assays of Efficacy of ULV Insecticide Treatments in the Field .....	60
4.5.4	Assays of Efficacy of Mosquito Repellents .....	60
4.6	Conclusion .....	61
<b>5</b>	<b>Morphology of Mosquitoes</b> .....	<b>63</b>
5.1	Adults .....	63
5.1.1	Head .....	63
5.1.2	Thorax .....	67
5.1.3	Abdomen .....	72
5.2	Larvae .....	76
5.2.1	Head .....	78
5.2.2	Thorax .....	80
5.2.3	Abdomen .....	80
5.3	Pupae .....	84
 <b>Part II Identification Keys; Morphology; Ecology and Distribution of European Species</b>		
<b>6</b>	<b>Key to Female Mosquitoes</b> .....	<b>91</b>
	Genera .....	91
6.1	Genus <i>Anopheles</i> .....	92
6.2	Genera <i>Aedes</i> and <i>Ochlerotatus</i> .....	95
6.3	Genus <i>Culex</i> .....	106
6.4	Genus <i>Culiseta</i> .....	108
6.5	Genus <i>Coquillettidia</i> .....	111
<b>7</b>	<b>Key to Male Mosquitoes</b> .....	<b>113</b>
	Genera .....	113
7.1	Genus <i>Anopheles</i> .....	115
7.2	Genera <i>Aedes</i> and <i>Ochlerotatus</i> .....	118

7.3	Genus <i>Culex</i> .....	128
7.4	Genus <i>Culiseta</i> .....	132
7.5	Genus <i>Coquillettia</i> .....	134
<b>8</b>	<b>Key to Mosquito Fourth Instar Larvae</b> .....	135
	Genera .....	135
8.1	Genus <i>Anopheles</i> .....	137
8.2	Genera <i>Aedes</i> and <i>Ochlerotatus</i> .....	141
8.3	Genus <i>Culex</i> .....	154
8.4	Genus <i>Culiseta</i> .....	159
8.5	Genus <i>Coquillettia</i> .....	162
<b>9</b>	<b>Subfamily Anophelinae</b> .....	163
9.1	Genus <i>Anopheles</i> (Meigen) .....	164
9.1.1	Subgenus <i>Anopheles</i> (Meigen) .....	165
	<i>Anopheles algeriensis</i> (Theobald 1903) .....	165
	<i>Anopheles Claviger</i> Complex .....	166
	<i>Anopheles claviger s.s.</i> (Meigen 1804) .....	166
	<i>Anopheles petragnani</i> (Del Vecchio 1939) .....	168
	<i>Anopheles hyrcanus</i> (Pallas 1771) .....	169
	<i>Anopheles Maculipennis</i> Complex .....	170
	<i>Anopheles atroparvus</i> (Van Thiel 1927) .....	173
	<i>Anopheles beklemishevi</i> (Stegnii and Kabanova 1976) .....	173
	<i>Anopheles labranchiae</i> (Falleroni 1926) .....	174
	<i>Anopheles maculipennis s.s.</i> (Meigen 1818) .....	174
	<i>Anopheles melanoon</i> (Hackett 1934) .....	175
	<i>Anopheles messeae</i> (Falleroni 1926) .....	175
	<i>Anopheles sacharovi</i> (Favre 1903) .....	175
	<i>Anopheles subalpinus</i> (Hackett and Lewis 1935) .....	176
	<i>Anopheles marteri</i> (Senevet and Prunelle 1927) .....	177
	<i>Anopheles plumbeus</i> (Stephens 1828) .....	178
9.1.2	Subgenus <i>Cellia</i> (Theobald 1902) .....	180
	<i>Anopheles cinereus</i> (Theobald 1901) .....	180
	<i>Anopheles cinereus hispaniola</i> (Theobald 1903) .....	181
	<i>Anopheles multicolor</i> (Cambouliu 1902) .....	182
	<i>Anopheles sergentii</i> (Theobald 1907) .....	183
	<i>Anopheles superpictus</i> (Grassi 1899) .....	185
<b>10</b>	<b>Subfamily Culicinae</b> .....	187
10.1	Genus <i>Aedes</i> (Meigen) .....	187
10.1.1	Subgenus <i>Aedes</i> (Meigen) .....	188
	<i>Aedes cinereus</i> (Meigen 1818) .....	189
	<i>Aedes geminus</i> (Peus 1970) .....	191
	<i>Aedes rossicus</i> (Dolbeskin, Gorickaja and Mitrofanova 1930) .....	192
10.1.2	Subgenus <i>Aedimorphus</i> (Theobald) .....	193
	<i>Aedes vexans</i> (Meigen 1830) .....	194
10.1.3	Subgenus <i>Fredwardsius</i> (Reinert) .....	196
	<i>Aedes vittatus</i> (Bigot 1861) .....	196

10.1.4	Subgenus <i>Stegomyia</i> (Theobald) .....	198
	<i>Aedes aegypti</i> (Linnaeus 1762) .....	198
	<i>Aedes albopictus</i> (Skuse 1895) .....	201
	<i>Aedes cretinus</i> (Edwards 1921).....	203
10.2	Genus <i>Ochlerotatus</i> (Lynch Arribalzaga) .....	204
10.2.1	Subgenus <i>Finlaya</i> (Theobald).....	205
	<i>Ochlerotatus echinus</i> (Edwards 1920) .....	205
	<i>Ochlerotatus geniculatus</i> (Olivier 1791).....	206
10.2.2	Subgenus <i>Ochlerotatus</i> Lynch Arribalzaga .....	208
	<i>Ochlerotatus annulipes</i> (Meigen 1830).....	209
	<i>Ochlerotatus behningi</i> (Martini 1926) .....	211
	<i>Ochlerotatus berlandi</i> (Seguy 1921).....	212
	<i>Ochlerotatus cantans</i> (Meigen 1818).....	214
	<i>Ochlerotatus Caspius</i> Complex .....	216
	<i>Ochlerotatus caspius</i> (Pallas 1771).....	216
	<i>Ochlerotatus cataphylla</i> (Dyar 1916).....	218
	<i>Ochlerotatus Communis</i> Complex .....	219
	<i>Ochlerotatus communis</i> (De Geer 1776).....	220
	<i>Ochlerotatus cyprius</i> (Ludlow 1920) .....	221
	<i>Ochlerotatus Detritus</i> Complex .....	222
	<i>Ochlerotatus detritus</i> (Haliday 1833).....	223
	<i>Ochlerotatus diantaeus</i> (Howard, Dyar and Knab 1913)....	224
	<i>Ochlerotatus dorsalis</i> (Meigen 1830) .....	226
	<i>Ochlerotatus Excrucians</i> Complex .....	228
	<i>Ochlerotatus euedes</i> (Howard, Dyar and Knab 1913) ....	228
	<i>Ochlerotatus excrucians</i> (Walker 1856).....	229
	<i>Ochlerotatus surcoufi</i> (Theobald 1912).....	231
	<i>Ochlerotatus flavescens</i> (Müller 1764) .....	231
	<i>Ochlerotatus hexodontus</i> (Dyar 1916) .....	233
	<i>Ochlerotatus hungaricus</i> (Mihalyi 1955).....	234
	<i>Ochlerotatus impiger</i> (Walker 1848).....	236
	<i>Ochlerotatus intrudens</i> (Dyar 1919) .....	237
	<i>Ochlerotatus leucomelas</i> (Meigen 1804) .....	238
	<i>Ochlerotatus Mariae</i> Complex .....	240
	<i>Ochlerotatus mariae</i> (Sergent and Sergent 1903).....	240
	<i>Ochlerotatus zammitii</i> (Theobald 1903).....	242
	<i>Ochlerotatus mercurator</i> (Dyar 1920) .....	242
	<i>Ochlerotatus nigrinus</i> (Eckstein 1918) .....	243
	<i>Ochlerotatus nigripes</i> (Zetterstedt 1838) .....	245
	<i>Ochlerotatus pionips</i> (Dyar 1919).....	246
	<i>Ochlerotatus pulcritarsis</i> (Rondani 1872) .....	248
	<i>Ochlerotatus pullatus</i> (Coquillett 1904).....	249
	<i>Ochlerotatus punctodes</i> (Dyar 1922) .....	250
	<i>Ochlerotatus punctor</i> (Kirby 1837).....	251
	<i>Ochlerotatus riparius</i> (Dyar and Knab 1907) .....	253
	<i>Ochlerotatus sticticus</i> (Meigen 1838) .....	255
10.2.3	Subgenus <i>Rusticoidus</i> (Shevchenko and Prudkina).....	256
	<i>Ochlerotatus lepidonotus</i> (Edwards 1920).....	257
	<i>Ochlerotatus quasirusticus</i> (Torres Canamares 1951) .....	258

	<i>Ochlerotatus refiki</i> (Medschid 1928).....	259
	<i>Ochlerotatus rusticus</i> (Rossi 1790).....	261
	<i>Ochlerotatus subdiversus</i> (Martini 1926).....	263
10.3	Genus <i>Culex</i> Linnaeus.....	264
10.3.1	Subgenus <i>Barraudius</i> (Edwards).....	265
	<i>Culex modestus</i> (Ficalbi 1889).....	265
	<i>Culex pusillus</i> (Macquart 1850).....	267
10.3.2	Subgenus <i>Culex</i> (Linnaeus).....	268
	<i>Culex brumpti</i> (Galliard 1931).....	269
	<i>Culex laticinctus</i> (Edwards 1913).....	270
	<i>Culex mimeticus</i> (Noe 1899).....	271
	<i>Culex perexiguus</i> (Theobald 1903).....	273
	<i>Culex pipiens</i> Complex.....	275
	<i>Culex pipiens pipiens</i> (Linnaeus 1758).....	275
	<i>Culex pipiens pipiens</i> biotype <i>molestus</i> (Forskal 1775)..	277
	<i>Culex pipiens quinquefasciatus</i> (Say 1832).....	278
	<i>Culex torrentium</i> (Martini 1925).....	279
	<i>Culex theileri</i> (Theobald 1903).....	280
10.3.3	Subgenus <i>Maillotia</i> (Theobald).....	282
	<i>Culex hortensis</i> (Ficalbi 1889).....	282
10.3.4	Subgenus <i>Neoculex</i> (Dyar).....	283
	<i>Culex impudicus</i> (Ficalbi 1890).....	284
	<i>Culex martinii</i> (Medschid 1930).....	285
	<i>Culex territans</i> (Walker 1856).....	286
10.4	Genus <i>Culiseta</i> Felt.....	288
10.4.1	Subgenus <i>Allotheobaldia</i> (Broelemann).....	289
	<i>Culiseta longiareolata</i> (Macquart 1838).....	289
10.4.2	Subgenus <i>Culicella</i> (Felt).....	290
	<i>Culiseta fumipennis</i> (Stephens 1825).....	291
	<i>Culiseta litorea</i> (Shute 1928).....	292
	<i>Culiseta morsitans</i> (Theobald 1901).....	294
	<i>Culiseta ochroptera</i> (Peus 1935).....	296
10.4.3	Subgenus <i>Culiseta</i> (Felt).....	297
	<i>Culiseta alaskaensis</i> (Ludlow 1906).....	298
	<i>Culiseta annulata</i> (Schrank 1776).....	299
	<i>Culiseta bergrothi</i> (Edwards 1921).....	301
	<i>Culiseta glaphyoptera</i> (Schiner 1864).....	303
	<i>Culiseta subochrea</i> (Edwards 1921).....	305
10.5	Genus <i>Coquillettidia</i> (Dyar).....	306
10.5.1	Subgenus <i>Coquillettidia</i> (Dyar).....	307
	<i>Coquillettidia buxtoni</i> (Edwards 1923).....	307
	<i>Coquillettidia richiardii</i> (Ficalbi 1889).....	308
10.6	Genus <i>Orthopodomyia</i> (Theobald).....	310
	<i>Orthopodomyia pulcripalpis</i> (Rondani 1872).....	310
10.7	Genus <i>Uranotaenia</i> (Lynch Arribalzaga).....	312
10.7.1	Subgenus <i>Pseudoficalbia</i> (Theobald).....	312
	<i>Uranotaenia unguiculata</i> (Edwards 1913).....	312

### Part III Identification Keys, Morphology, Ecology, and Distribution of Important Vector and Nuisance Species – World-Wide

<b>11 Africa</b> .....	317
11.1 Key to African Female Mosquitoes .....	318
11.2 Species Description.....	322
<i>Anopheles (Cellia) funestus</i> (Giles 1900) .....	322
<i>Anopheles Gambiae Complex</i> .....	323
<i>Anopheles (Cellia) gambiae s.s.</i> (Giles 1902) .....	324
<i>Anopheles (Cellia) arabiensis</i> (Patton 1905) .....	325
<i>Anopheles (Cellia) quadriannulatus s.l.</i> (Theobald 1911).....	325
<i>Anopheles (Cellia) bwambae</i> (White 1985).....	326
<i>Anopheles (Cellia) melas</i> (Theobald 1903) .....	326
<i>Anopheles (Cellia) merus</i> (Dönitz 1902).....	326
<i>Anopheles (Cellia) pharoensis</i> (Theobald 1901) .....	326
<i>Culex (Culex) antennatus</i> (Becker 1903).....	328
<i>Culex (Culex) univittatus</i> (Theobald 1901).....	329
<i>Mansonia (Mansonioides) africana</i> (Theobald 1901) .....	330
<i>Mansonia (Mansonioides) uniformis</i> (Theobald 1901) .....	330
<b>12 Asia</b> .....	333
12.1 Key to Asian Female Mosquitoes .....	334
12.2 Species Description.....	339
<i>Anopheles (Anopheles) lesteri</i> (Baisas and Hu 1936).....	339
<i>Anopheles (Anopheles) sinensis</i> (Wiedemann 1828) .....	340
<i>Anopheles (Cellia) culicifacies</i> (Giles 1901) .....	341
<i>Anopheles (Cellia) dirus</i> (Peyton and Harrison 1979).....	342
<i>Anopheles (Cellia) flavirostris</i> (Ludlow 1914) .....	343
<i>Anopheles (Cellia) fluviatilis</i> (James 1902) .....	344
<i>Anopheles (Cellia) maculatus</i> (Theobald 1901) .....	345
<i>Anopheles (Cellia) minimus</i> (Theobald 1901) .....	346
<i>Anopheles (Cellia) stephensi</i> (Liston 1901).....	347
<i>Anopheles (Cellia) sundaicus</i> (Rodenwaldt 1925) .....	348
<i>Culex (Culex) tritaeniorhynchus</i> (Giles 1901).....	349
<b>13 Australia</b> .....	351
13.1 Key to Australian Female Mosquitoes.....	351
13.2 Species Description.....	356
<i>Anopheles (Cellia) farauti s.l.</i> (Laveran 1902) .....	356
<i>Ochlerotatus (Mucidus) alternans</i> (Westwood 1835).....	357
<i>Ochlerotatus (Finlaya) notoscriptus</i> (Skuse 1889).....	358
<i>Ochlerotatus (Ochlerotatus) camptorhynchus</i> (Thompson 1868) ....	360
<i>Ochlerotatus (Ochlerotatus) normanensis</i> (Taylor 1915) .....	360
<i>Ochlerotatus (Ochlerotatus) sagax</i> (Skuse 1889) .....	362
<i>Ochlerotatus (Ochlerotatus) theobaldi</i> (Taylor 1914) .....	363
<i>Ochlerotatus (Ochlerotatus) vigilax</i> (Skuse 1889) .....	364
<i>Culex (Culex) annulirostris</i> (Skuse 1889).....	365
<i>Culex (Culex) sitiens</i> (Wiedemann 1828) .....	366
<i>Coquillettidia (Coquillettidia) xanthogaster</i> (Edwards 1924) .....	367

<b>14 Central and South America</b> .....	369
14.1 Key to Central and South American Female Mosquitoes .....	369
14.2 Species Description.....	374
<i>Anopheles (Anopheles) calderoni</i> (Wilkerson 1991) .....	374
<i>Anopheles (Anopheles) pseudopunctipennis</i> (Theobald 1901) .....	374
<i>Anopheles (Anopheles) punctimacula</i> (Dyar and Knab 1906).....	375
<i>Anopheles (Nyssorhynchus) albimanus</i> (Wiedemann 1820).....	376
<i>Anopheles (Nyssorhynchus) aquasalis</i> (Curry 1932).....	377
<i>Anopheles (Nyssorhynchus) darlingi</i> (Root 1926).....	378
<i>Anopheles (Nyssorhynchus) nuneztovari</i> (Gabaldon 1940).....	380
<i>Haemagogus (Haemagogus) janthinomys</i> (Dyar 1921).....	381
<i>Ochlerotatus (Ochlerotatus) albifasciatus</i> (Macquart 1838) .....	382
<i>Ochlerotatus (Ochlerotatus) scapularis</i> (Rondani 1848).....	383
<i>Culex (Culex) nigripalpus</i> (Theobald 1901) .....	384
<i>Mansonia (Mansonia) titillans</i> (Walker 1848).....	385
<b>15 North America</b> .....	387
15.1 Key to North American Female Mosquitoes .....	387
15.2 Species Description.....	391
<i>Ochlerotatus (Ochlerotatus) sollicitans</i> (Walker 1856) .....	391
<i>Ochlerotatus (Ochlerotatus) taeniorhynchus</i> (Wiedemann 1821) ....	393
<i>Psorophora (Grabhamia) columbiae</i> (Dyar and Knab 1906).....	394
<i>Psorophora (Janthinosoma) ferox</i> (von Humboldt 1819).....	395
<i>Culex (Culex) restuans</i> (Theobald 1901) .....	396
<i>Culex (Culex) salinarius</i> (Coquillett 1904).....	397
<i>Culex (Culex) tarsalis</i> (Coquillett 1896).....	398
<i>Coquillettidia (Coquillettidia) perturbans</i> (Walker 1856).....	400
<b>Part IV Control of Mosquitoes</b>	
<b>16 Biological Control</b> .....	405
16.1 Introduction.....	405
16.2 Predators .....	406
16.2.1 Vertebrate Predators .....	406
16.2.2 Invertebrate Predators .....	410
16.3 Parasites .....	415
16.3.1 Nematodes .....	415
16.4 Pathogens .....	416
16.4.1 Fungi .....	416
16.4.2 Protozoa .....	418
16.4.3 Bacteria .....	419
16.4.4 Viruses .....	431
<b>17 Environmental Management of Mosquitoes</b> .....	433
17.1 Introduction.....	433
17.2 Environmental Management of Mosquitoes in Urban Areas.....	434
17.2.1 Denying Mosquito Access to Urban Areas, and into Homes .....	434
17.2.2 Construction Sites .....	434



17.2.3	Water Storage Containers .....	434
17.2.4	Drainage Systems .....	435
17.2.5	Sewage and Waste-Water Processing.....	435
17.2.6	Cemeteries .....	436
17.2.7	Urban Sanitation .....	436
17.3	Environmental Management of Mosquitoes in Rural Areas.....	436
17.3.1	Agriculture .....	436
17.3.2	Natural Wetlands.....	437
17.4	Environmental Management of Mosquitoes and Human Issues .....	438
17.4.1	Community Participation.....	438
17.4.2	Regulations for Environmental Management of Mosquitoes .....	439
<b>18</b>	<b>Chemical Control</b> .....	441
18.1	History.....	441
18.2	Insecticide .....	442
18.2.1	Classification of Insecticides .....	443
18.2.2	Insecticide Formulations.....	446
18.2.3	Insecticide Application Techniques .....	448
18.3	Chemical Groups of Insecticides .....	449
18.3.1	Chlorinated Hydrocarbons.....	449
18.3.2	Organophosphates.....	452
18.3.3	Carbamates.....	458
18.3.4	Pyrethroids .....	459
18.3.5	Insect Growth Regulators (IGRs) .....	466
18.3.6	Novel Insecticide Classes .....	470
18.4	Management and Monitoring of Insecticide Resistance.....	471
18.4.1	Resistance Mechanisms .....	472
18.4.2	Resistance Surveillance .....	472
18.4.3	Resistance Management .....	474
<b>19</b>	<b>Physical Control</b> .....	477
19.1	Introduction.....	477
19.2	Physical Control of Immature Mosquitoes .....	477
19.2.1	Oil .....	477
19.2.2	Surface Films and Polystyrene Beads.....	478
19.3	Physical Control of Adult Mosquitoes.....	481
19.3.1	Other Technology: A Novel Technology or New Tool .....	481
19.4	Conclusions.....	481
<b>20</b>	<b>Genetic Control of Mosquitoes</b> .....	483
20.1	Introduction.....	483
20.2	Population Elimination Via the Sterile Insect Technique (SIT).....	484
20.2.1	Introduction.....	484
20.2.2	Rearing and Sexing.....	484
20.2.3	Male Sterilization.....	485
20.2.4	SIT in Practice .....	485

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20.3	Population Replacement .....	486
20.3.1	The principle of Population Replacement.....	486
20.3.2	Refractoriness to Pathogens .....	486
20.3.3	Genetic Drivers .....	487
20.4	Ethical, Legal and Social Implications of the Use of Genetic Techniques for Mosquito Control .....	488
20.4.1	Absence of Community Participation.....	489
20.4.2	International Committee for Genetic Control Work .....	489
<b>21</b>	<b>Personal Protection .....</b>	<b>491</b>
21.1	Introduction.....	491
21.2	Impregnated Bednets.....	491
21.3	Repellents Against Mosquitoes.....	493
21.3.1	Repellents on Skin or Clothing .....	493
21.3.2	Mosquito Coils.....	494
21.3.3	Vapourizing Mats .....	496
21.3.4	Liquid Vapourizers.....	496
<b>22</b>	<b>Implementation and Integration of Mosquito Control Measures .....</b>	<b>499</b>
22.1	Introduction.....	499
22.2	Prerequisites for the Successful Implementation of the IVM programme .....	501
22.2.1	Entomological Research (Monitoring) .....	501
22.2.2	Mapping of the Breeding Sites .....	503
22.2.3	Selection of Appropriate Tools .....	504
22.2.4	Effective Dosage Assessment .....	505
22.2.5	Design of the Control Strategy.....	505
22.2.6	Training of Field Staff.....	506
22.2.7	Governmental Application Requirements.....	506
22.2.8	Community Participation .....	506
22.2.9	Registration of Insecticides .....	507
22.2.10	Routine Treatments .....	508
22.2.11	Public Information Systems .....	509
	<b>References .....</b>	<b>511</b>
	<b>Subject Index.....</b>	<b>553</b>
	<b>Taxonomic Index .....</b>	<b>565</b>



# Introduction

The mosquitoes (family Culicidae) are at the centre of worldwide entomological research because of their importance as vectors of a wide range of debilitating viral and parasitic diseases affecting both humans and animals.

More than half of the world's population lives under the risk of becoming infected by mosquitoes that carry the causative agents of diseases such as malaria, dengue, Chikungunya, West Nile fever, Japanese encephalitis or lymphatic filariasis. Estimates made by the World Health Organisation (WHO) show that 247 million people became ill in 2006 and about one million people died (WHO 2008) from mosquito-borne diseases. Although approximately three quarters of all mosquito species occur in the humid tropics and subtropics, mosquitoes are a problem not only in these regions. They also cause a considerable nuisance or can occasionally transmit pathogens to humans in temperate latitudes, for example, the West Nile virus epidemic in the USA, or the outbreak of Chikungunya fever, in Italy in 2007 (Depoortere et al. 2008).

The floodwater mosquitoes, such as *Aedes vexans*, are common nuisance species along rivers world-wide. The snow-melt mosquitoes, e.g. *Ochlerotatus communis*, *Oc. punctor* and *Oc. hexodontus*, occur in swampy woodlands and tundra areas. The halophilous species, *Oc. caspius* and *Oc. detritus*, breed particularly in the shallow lagoons along the coasts of southern Europe, Asia Minor and North Africa, and the rock-pool mosquito *Oc. mariaae* along parts of the Mediterranean rocky coasts. Mass outbreaks of all those species can cause a major nuisance. *Culex pipiens pipiens* biotype *molestus*, which is commonly known as the “house mosquito” because of its presence within close proximity to human settlements, can be a nuisance in temperate zones, as can the closely related *Cx. p. quinquefasciatus* in the tropics. Mosquitoes are extremely successful organisms due to their ability to adapt to a wide range of habitats. They are found throughout the world, except in permanently frozen areas. Mosquito larvae colonise a wide range of water bodies, temporary and permanent, highly polluted as well as clean, large or small, stagnant or flowing, and even the smallest accumulations such as water-filled buckets, flower vases, old tyres, bromeliads, hoof prints or leaf axils. Adult mosquitoes vary greatly in their bionomics, e.g. in terms of the host-seeking, biting and dispersal behaviour, and strategy for reproduction. Their significant medical importance and their troublesome behaviour have historically attracted the interest of scientists. Their importance as vectors of malaria and yellow fever was suspected by Joseph Nott in 1848. In 1878 Sir Patrick Manson showed that the roundworm *Wuchereria bancrofti* is transmitted by *Cx. p. quinquefasciatus*. Only 3 years later, Carlos Finley postulated that yellow fever was trans-

mitted by mosquitoes, which was later proved by Walter Reed and his co-workers in 1901. Sir Ronald Ross made a further pioneering discovery in Hyderabad, India in 1897, when he recognised the importance of the anophelines as vectors of malaria. The discovery of the transmission cycles of most vector-borne diseases led to the recognition that mosquitoes represented a major scourge to humans, which in turn triggered the development of mosquito control. The foundations for mosquito control were established at the beginning of the 20th Century. William C. Gorgas, a member of the Army Medical Corps, USA, dedicated most of his professional life to the control of yellow fever, with special focus on control of the vector mosquito *Ae. aegypti* [*Stegomyia aegypti*]. By the turn of the 20th Century, he succeeded in suppressing yellow fever in Panama, and set the basis for the construction of the Panama Canal. Gorgas was the first to recognise that only by the implementation of an integrated control programme, could the severe burden of vector-borne diseases be reduced. His approach comprised draining the breeding sites, vegetation cutting to reduce preferred resting sites of adult mosquitoes, treatment of water bodies with oil derivatives to suppress immature mosquito stages, screening and quarantine of infected people to interrupt the transmission, as well as killing the adult mosquitoes to reduce the vector-density and vector-human contact (Le Prince 1910; Le Prince and Orenstein 1916).

The development and use of DDT as a residual insecticide, initially achieved phenomenal success in the control of mosquitoes. In the 1950s, it was believed that malaria would be eradicated by the use of DDT and chloroquine, but disillusionment quickly followed, because mosquitoes became resistant to the insecticide in many areas. In addition, the control efforts against the vectors were not entirely beneficial, as the widespread use of non-specific and highly persistent insecticides caused a number of toxicological and eco-toxicological problems. Despite considerable efforts by national and international organisations such as the WHO, it could be argued that the main outcome to date is simply the prevention of a more dramatic increase in vector-borne diseases. The Roll Back Malaria partnership (RBM) – an international alliance of over 90 organisations – such as WHO, UNICEF, World Bank, USAID, the Global Fund for AIDS, Tuberculosis and Malaria (GFATM) and many non-governmental organisations (NGOs) was set up in 1998 to highlight the existence of low-cost and effective interventions for malaria control. RBM is the main instrument through which African leaders were hoping to achieve the goals of the Abuja Declaration of 2000, where 53 African heads of state pledged to reduce malaria mortality by 50% by the year 2010. The RBM Programme is mainly based on insecticide-treated bed nets (long-lasting nets), indoor residual spraying and effective diagnosis and treatment of malaria cases.

But since its inception, it seems that the 2010 target to halve malaria-related deaths will not be achieved. This attempt demonstrates that other choices for vector control strategy have to be implemented. It is imperative to recognize that integrated vector management (IVM), as a basic sound approach, should be incorporated into mosquito-borne disease control programmes. Larviciding, breeding site reduction, improvements in housing facilities and effective public education efforts should be, together with health care measures, a key part of any further internationally adopted strategies in vector-borne disease control.

The risk of becoming infected with a vector-borne disease has increased again not only in the tropics but also in Europe and USA, as shown by the outbreak of

Chikungunya fever in Italy in 2007 (Angelini et al. 2007) and West Nile virus in the USA. Italy's outbreak of Chikungunya is the first known disease transmission by the invasive vector, the Asian tiger mosquito (*Ae. albopictus* [*St. albopicta*]) in Europe, and triggered action by both experts and public in the EU community. Overall, the greater mobility of people to and from endemic areas, the intensified international trade, as well as the changing climate, will further encourage the spread and establishment of exotic diseases and invasive species in formerly safe areas. The Asian tiger mosquito originates from Southeast Asia, where its developmental stages occur in water-filled tree-holes, coconut shells, bamboo stumps and similar water collections. Over time, this species has adapted to breeding in artificial containers such as water barrels, car tyres or other places where small pools of water may collect. This mosquito has undergone an astonishing expansion of its range within the last few decades. Since 1979, *Ae. albopictus* has been found in Africa, the Americas and Europe, and more recently also in the Pacific region. It is expected to spread to tropical and subtropical regions, and occasionally to regions with moderate climates. Like *Ae. albopictus*, the "Asian bush" or "Asian rock pool" mosquito *Oc. japonicus japonicus* [*Hulecoeteomyia japonica*] is also an invasive species that has been established outside of its native range (Japan, Korea, China and Russia) for more than a decade. It was first recorded in North America in 1998 and in Europe (France) in 2000 (Schaffner et al. 2003, 2009; Williges et al. 2008). Since *Oc. j. japonicus* is generally found in more northern climates within its native range (whereas *Ae. albopictus* was originally a more tropical species), this species is able to establish itself more successfully in moderate climate zones like Central Europe (e.g. Switzerland, Belgium, Germany). Both species are able to colonise a wide range of natural and artificial breeding sites (discarded tyres, flower vases, catch basins, bird baths, tree-holes, rock pools, etc.). Due to the resistance of their eggs to desiccation, cold (diapausing larvae in eggs), and the relative lack of preference concerning their host type (e.g. humans, mammals and birds), these two invasive species have rapidly built up populations in newly colonised geographic regions (Pluskota et al. 2008). The international trade in used tyres and ornamental flowers has facilitated their spread over large distances and between continents.

The essential foundation for successful action against the mosquitoes requires not only an integrated mosquito management (IMM) concept, in which all appropriate methods for control are used, but also knowledge of the biology and ecology of the target organisms. The importance of a vector or nuisance species is determined above all by its physiological characteristics, such as reproduction, migration, host-seeking and biting behaviour. Accurate identification is a basic pre-requisite to a study of the autecology of a species as well as its biocoenotic relationship in the ecosystem. All these efforts should result in an improvement of the quality of life for humans by reducing the mosquito abundance by enhancing control measures based on IVM principles. All approaches should favour effective methods with low toxicological profile and minor environmental impact to contribute to the preservation of wetland biodiversity. Exchange of information and knowledge in the broadest sense should support sound mosquito control programmes worldwide.

Exciting achievements in molecular biology such as the sequencing of the genomes of the malaria parasite *Plasmodium falciparum* and the mosquito vector *Anopheles gambiae* give hope for the development of new drugs, vaccines, genetic engineering of malaria-resistant mosquitoes, and the release of insects carrying dominant lethal genes (RIDL). However, these new tools should not lead to the neglect of already developed and efficient strategies for IVM.

It is only by implementing IMM/IVM with inputs from remote sensing and GIS technology, climatology, geology, biology, ecology, medicine, social sciences and animal and human behaviour, to genetics and molecular biology, that we will be able to provide innovative tools for solving the most pressing problems in the control of nuisance/vector mosquitoes and vector-borne diseases.

# Part I

## General Aspects



# Chapter 1

## Systematics

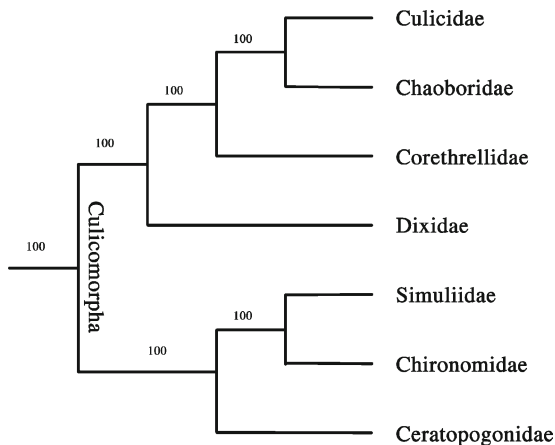
Hennig's (1966) concept of hierarchies of monophyletic taxons based on common ancestors (with plesiomorphies or retained primitive, ancestral characters) and shared synapomorphies (homologous shared characters inferred to have been present in the nearest common ancestor but not in earlier ancestors nor in the taxa outside this group), has provided the theoretical basis for taxa formation. During the first decades following its proposal, it resulted in the establishment of cladistic trees where recency of common ancestry is the sole criterion for grouping of the taxa. The problem of ranking taxa and tree formation in a Darwinian evolutionary context was the next scientific step (Eldridge and Cracraft 1980). Opinions about what different nodes represent, how to deal with branch lengths, and how to rank monophyletic entities, have become part of a scientific field of its own (Britton et al. 2007). Applying both morphologic and genetic taxonomic methods, and working with different groups of characters to reveal evolutionary relationships between insect orders or families as monophyletic groups, is now becoming standard. In the new millennium this work has resulted in several new hypotheses of phylogenetic trees of Insecta. Wheeler et al. (2001) established the relationship between Diptera and Strepsiptera. Grimaldi and Engel (2005) in their comprehensive work on extinct and extant Insecta, summarized different hypotheses for Diptera. They accepted five suborders of Lower Diptera (Nematocera): Tipulomorpha, Psychodomorpha, Culicomorpha, Blephariceromorpha and Bibionomorpha. They placed Anisopodidae as a sistergroup to Brachycera (all higher Diptera) and discarded Nematocera as a paraphyletic group (including a most recent common ancestor and some, but not all, of its descendants). This view has been strongly advocated by Amorim et al. (2006). They recognised seven suborders/infraorders instead of Nematocera and

added Brachycera as the eighth suborder/infraorder for the rest of the Diptera. The monophyly of most of these suborders is now accepted. However in Fly Tree (Yeats et al. 2006, <http://www.inhs.uiuc.edu>), the term Nematocera is still used for infra-orders Ptychoptermorpha, Culicomorpha, Blephariceromorpha, Bibionomorpha, Psychodomorpha, but superfamily Tipuloidea is now a sistergroup to Brachycera.

Cameron et al. (2007) in a molecular pilot study on Insecta, mainly Diptera, using several genes including mitochondrial ones, and several methods for tree building, reached consensus results for some already accepted suborders/infraorders in Diptera. However, the discussion of the relationships of families within monophyletic infraorders in Lower Diptera is still going on.

The infraorder Culicomorpha including the Culicidae, Chaoboridae, Dixidae and the Chironomidae was already regarded as a monophyletic group by Hennig (1973). This has been accepted in the first catalogue of Culicidae by Knight and Stone (1977). In a more recent analysis of Culicomorpha, the Corethrellidae, Simuliidae and Ceratopogonidae were also included, and the Thaumaleidae and the Nymphomyiidae were regarded as a sistergroup (Becker et al. 2003, Fig. 1.1). A consensus about the monophyly of Culicomorpha is given by Grimaldi and Engel (2005), with the Culicidae, Chaoboridae, Corethrellidae, Dixidae, Ceratopogonidae, Chironomidae, and Simuliidae included. The position of Thaumaleidae and Nymphomyiidae as a sistergroup, is still under discussion (Fig. 1.1).

At the family level, the monophyly (sharing a single ancestral form) of the family Culicidae with three subfamilies Anophelinae, Culicinae and Toxorhynchitinae, was established by Edwards (1932), and subsequently accepted by Belkin (1962a) and Knight and Stone



**Fig. 1.1** The phylogeny of the monophyletic infraorder Culicomorpha after Saether (2000) from a strict consensus and majority rule cladogram

(1977). The first attempt to elucidate higher phylogeny within subfamilies by creating tribes, was made by Belkin (1962a). He discussed the general assumption that the Anophelinae were closer to the stem of the superfamily, but did not press it further because of lack of evidence. A cladistic phylogenetic study of the family by Harbach and Kitching (1998) has now confirmed the basal position of the Anophelinae as a subfamily within the family Culicidae. They also partly affirmed the currently used higher ranks within the family and regarded Anophelinae and Culicinae as valid monophyletic subfamilies, but lowered the Toxorhynchitinae to tribal rank within the Culicinae (Fig. 1.2). Their phylogeny of ten tribes broadly corresponds to the classic divisions. They discussed the relationship between lower categories as genera and brought the tribes Aedini and Mansoniini into one sister group, and the tribes Culicini and Sabethini into another. Relationships within the Aedini were not fully resolved. Reinert (2000c) raised the subgenus *Ochlerotatus* Lynch Arribalzaga of the genus *Aedes* Meigen to generic rank, placed other former subgenera of *Aedes* in the new genus, and divided it into two sections of species based on larval and pupal characters (Fig. 1.3a,b). We now have the results of four further analyses by Reinert et al. (2004, 2006, 2008, 2009). These are presented in Fig. 1.3a,b. Until an independent analysis with other phylogenetic methods and an evolutionary molecular analysis of the additional material are available, the scientific discussion of the phylogeny of Aedini will go on. Reinert et al. (2008)

regarded the “phenetic clustering” method (based on general or overall similarity) of Kumar et al. (2007) as irrelevant to the conclusions drawn from the cladistic analyses of Reinert et al. (2004, 2006).

At the level of genera, the “new” genus *Ochlerotatus* sensu Reinert, Harbach and Kitching (RHK) now contains some 264 species most of which are unassigned to the 14 genera currently recognised by authors. For those involved in practical research, and in writing and publishing, these changes have caused confusion and have resulted in a rebuttal of the “new” nomenclature by many scientists, editors of international journals, and also by mosquito control managers. Russel (2006) made a useful analysis of “pros” and “cons” for the proposed changes. For this edition, the same nomenclature as in the first edition, with minor changes, is used. On a practical level this will keep the identification of species consistent. Until a more precise analysis of evolutionary pathways of characters within and between the different RHK genera, and a new analysis using accepted scientific phylogenetic methods, are carried out on the material, the scientific use of all proposed generic changes by RHK will be contentious on scientific grounds.

Up until Knight and Stone (1977), 2,960 valid species in 34 genera and 120 subgenera were recognised worldwide. Since then more species, genera, and subgenera have been described, synonymized, or resurrected. With the results of Reinert et al. (2004, 2006, 2008, 2009) added, the numbers have risen to 111 genera and 137 subgenera (Reinert 2009) containing 3,517 species (<http://mosquito-taxonomic-inventory.info>). More revisions of genera or subgenera, especially on tropical material, will become available. These revisions will contribute to the understanding of the characters and evolutionary processes which have made the Culicidae such a successful group which, as shown by the fossil record, has existed in its current shape since the Mesozoic (Grimaldi and Engel 2005).

All very closely related taxonomic entities such as subspecies, species complexes, and genetically, behaviourally, or physiologically different strains or populations are important in the analyses of vector capacity and therefore in any approach to vector control. The example of the Anopheles Gambiae Complex shows this. The introduction of new biochemical and recently developed molecular methods has opened up a new field of research. However, such studies should be

**Subfamily Anophelinae**Genus *Anopheles* (*An.*)Subgenera *Anopheles*, *Baimaia*, *Cellia*, *Kerteszia*, *Lophopodomya*, *Nyssorhynchus*, *Stethomyia*Genus *Bironella* (*Bi.*)Subgenera *Bironella*, *Brugella*, *Neobironella*Genus *Chagasia* (*Ch.*)**Subfamily Culicinae**

Tribus Aedeomyiini

Genus *Aedeomyia* (*Ad.*)Subgenera: *Aedeomyia*, *Lepiothauma*

Tribus Aedini (given separately in Fig. 1.3a,b)

Tribus Culicini

Genus *Culex* (*Cx.*)Subgenera *Acalleoemyia*, *Acallyntrum*, *Aedinus*, *Afroculex*, *Allimanta*, *Anoedioporpa*, *Barraudius*, *Belkinomyia*, *Carrollia*, *Culex*, *Culiciomyia*, *Eumelanomyia*, *Kitzmilleria*, *Lasiosiphon*, *Lophoceraomyia*, *Maillotia*, *Melanoconion*, *Micraedes*, *Microculex*, *Neoculex*, *Nicaromyia*, *Oculeomyia*, *Phenacomyia*, *Phytotelmatomyia*, *Sirivanakarnius*, *Tinolestes*Genus *Deinocerites* (*De.*)Genus *Galindomyia* (*Ga.*)Genus *Lutzia* (*Lt.*)Subgenera *Insulalutzia*, *Lutzia*, *Metalutzia*

Tribus Culisetini

Genus *Culiseta* (*Cs.*)Subgenera *Allotheobaldia*, *Austrotheobaldia*, *Climacura*, *Culicella*, *Culiseta*, *Neotheobaldia*, *Theomyia*

Tribus Ficalbiini

Genus *Ficalbia* (*Fi.*)Genus *Mimomyia* (*Mi.*)Subgenera *Etorleptiomyia*, *Ingramia*, *Mimomyia*

Tribus Hodgesiini

Genus *Hodgesia* (*Ho.*)

Tribus Mansoniini

Genus *Coquillettidia* (*Cq.*)Subgenera *Austromansonia*, *Coquillettidia*, *Rhynchotaenia*Genus *Mansonia* (*Ma.*)Subgenera *Mansonia*, *Mansonioides*

Tribus Orthopodomyiini

Genus *Orthopodomyia* (*Or.*)

Tribus Sabethini

Genus *Isostomyia* (*Is.*)Genus *Johnbelkinia* (*Jb.*)Genus *Kimia* (*Km.*)Genus *Limatus* (*Li.*)Genus *Malaya* (*Ml.*)Genus *Maorigoeldia* (*Mg.*)Genus *Onirion* (*On.*)Genus *Runchomyia* (*Ru.*)Subgenera *Ctenogoeldia*, *Runchomyia*Genus *Sabethes* (*Sa.*)Subgenera *Davismyia*, *Peytonulus*, *Sabethes*, *Sabethinus*, *Sabethoides*Genus *Shannoniana* (*Sh.*)Genus *Topomyia* (*To.*)Subgenera *Suaymyia*, *Topomyia*Genus *Trichoprosopon* (*Tr.*)Genus *Tripteroides* (*Tp.*)Subgenera *Polylepidomyia*, *Rachionotomyia*, *Rachisoura*, *Tricholeptomyia*, *Tripteroides*Genus *Wyeomyia* (*Wy.*)Subgenera *Antunesmyia*, *Caenomyiella*, *Cruzmyia*, *Decamyia*, *Dendromyia*, *Dodecamyia*, *Exallomyia*, *Hystatamyia*, *Menolepis*, *Nunezia*, *Phioniomyia*, *Prosopolepis*, *Spilonympha*, *Wyeomyia*, *Zinzala*

Tribus Toxorhynchitini

Genus *Toxorhynchites* (*Tx.*)Subgenera *Afrorhynchus*, *Ankylorhynchus*, *Lynchiella*, *Toxorhynchites*

Tribus Uranotaeniini

Genus *Uranotaenia* (*Ur.*)Subgenera *Pseudoficalbia*, *Uranotaenia***Fig. 1.2** Classification of Culicidae (subfamily/ subgenus) after Becker et al. (2003) and WRBU 2009 (<http://www.wrbu.org>) and abbreviations after Reinert (2009)

Becker et al. 2003		Reinert et al. 2004, 2006, 2008, 2009	
Genus	Subgenus	Genus (abbreviation)	Subgenus
		<i>Abraedes</i> ( <i>Ab.</i> )	
		<i>Acartomyia</i> ( <i>Ac.</i> )	
<i>Aedes</i>	<i>Aedes</i> , <i>Aedimorphus</i> , <i>Alanstonea</i> , <i>Albuginosus</i> , <i>Belkinius</i> , <i>Bothaella</i> , <i>Cancaeraedes</i> , <i>Christophersiomyia</i> , <i>Diceromyia</i> , <i>Edwardsaedes</i> , <i>Fredwardsius</i> , <i>Huaedes</i> , <i>Indusius</i> , <i>Isoaedes</i> , <i>Leptosomatomyia</i> , <i>Lorrainea</i> , <i>Neomelaniconion</i> , <i>Paraedes</i> , <i>Pseudarmigeres</i> , <i>Scutomyia</i> , <i>Skusea</i> , <i>Stegomyia</i>	<i>Aedes</i> ( <i>Ae.</i> ) ' <i>Aedes</i> ' s.a. s.a. = sensu auctorum = in the sense of previous authors	
		<i>Aedimorphus</i> ( <i>Am.</i> )	
		<i>Alanstonea</i> ( <i>As.</i> )	
		<i>Albuginosus</i> ( <i>Al.</i> )	
<i>Armigeres</i>	<i>Armigeres</i> , <i>Leicesteria</i>	<i>Armigeres</i> ( <i>Ar.</i> )	<i>Armigeres</i> , <i>Leicesteria</i>
<i>Ayurakitia</i>		<i>Ayurakitia</i> ( <i>Ay.</i> )	
		<i>Aztecaedes</i> ( <i>Az.</i> )	
		<i>Belkinius</i> ( <i>Bc.</i> )	
		<i>Bifidistylus</i> ( <i>Bf.</i> )	
		<i>Borichinda</i> <sup>1</sup> ( <i>Bc.</i> )	
		<i>Bothaella</i> ( <i>Bo.</i> )	
		<i>Bruceharrisonius</i> ( <i>Br.</i> )	
		<i>Cancaeraedes</i> ( <i>Ca.</i> )	
		<i>Catageomyia</i> ( <i>Cg.</i> )	
		<i>Catatassomyia</i> ( <i>Ct.</i> )	
		<i>Christophersiomyia</i> ( <i>Cr.</i> )	
		<i>Collessius</i> ( <i>Co.</i> )	<i>Alloeomyia</i> , <i>Collessius</i>
		<i>Cornetius</i> <sup>2</sup> ( <i>Cn.</i> )	
		<i>Dahlana</i> ( <i>Da.</i> )	
		<i>Danielsia</i> ( <i>Dn.</i> )	
		<i>Dendroskusea</i> ( <i>Ds.</i> )	
		<i>Diceromyia</i> ( <i>Di.</i> )	
		<i>Dobrotworskyius</i> ( <i>Db.</i> )	
		<i>Downsiomyia</i> ( <i>Do.</i> )	
		<i>Edwardsaedes</i> ( <i>Ed.</i> )	
		<i>Elpeytonius</i> ( <i>El.</i> )	
<i>Eretmapodites</i>		<i>Eretmapodites</i> ( <i>Er.</i> )	
		<i>Finlaya</i> ( <i>Fl.</i> )	
		<i>Fredwardsius</i> ( <i>Fr.</i> )	
		<i>Georgecraigius</i> ( <i>Ge.</i> )	<i>Georgecraigius</i> , <i>Horsfallius</i>
		<i>Geoskusea</i> ( <i>Ge.</i> )	
		<i>Gilesius</i> ( <i>Gi.</i> )	
		<i>Gymnometopa</i> ( <i>Gy.</i> )	
<i>Haemagogus</i>	<i>Conopostegus</i> , <i>Haemagogus</i>	<i>Haemagogus</i> ( <i>Hg.</i> )	<i>Conopostegus</i> , <i>Haemagogus</i>
		<i>Halaedes</i> ( <i>Ha.</i> )	
<i>Heizmannia</i>	<i>Heizmannia</i> , <i>Mattinglyia</i>	<i>Heizmannia</i> ( <i>Hz.</i> )	<i>Heizmannia</i> , <i>Mattinglyia</i>
		<i>Himalaius</i> ( <i>Hi.</i> )	
		<i>Hopkinsius</i> ( <i>Hk.</i> )	<i>Hopkinsius</i> , <i>Yamada</i>
		<i>Howardina</i> ( <i>Hw.</i> )	
		<i>Huaedes</i> ( <i>Hu.</i> )	
		<i>Hulecoeteomyia</i> ( <i>Hl.</i> )	
		<i>Indusius</i> ( <i>In.</i> )	
		<i>Isoaedes</i> ( <i>Ia.</i> )	
		<i>Jarnellius</i> ( <i>Ja.</i> )	
		<i>Jihlieni</i> ( <i>Ji.</i> )	
		<i>Kenknightia</i> ( <i>Ke.</i> )	
		<i>Kompia</i> ( <i>Ko.</i> )	
		<i>Leptosomatomyia</i> ( <i>Lp.</i> )	
		<i>Levua</i> ( <i>Le.</i> )	
		<i>Lewnielsenius</i> ( <i>Ln.</i> )	
		<i>Lorrainea</i> ( <i>Lo.</i> )	
		<i>Luius</i> ( <i>Lu.</i> )	
		<i>Macleaya</i> ( <i>Mc.</i> )	<i>Chaetocruimyia</i> , <i>Macleaya</i>
		<i>Molpemyia</i> ( <i>Mo.</i> )	
		<i>Mucidus</i> ( <i>Mu.</i> )	<i>Mucidus</i> , <i>Pardomyia</i> <sup>3</sup>
		<i>Neomelaniconion</i> ( <i>Ne.</i> )	
<i>Ochlerotatus</i>	Section I <i>Chaetocruimyia</i> , <i>Finlaya</i> , <i>Geoskusea</i> , <i>Halaedes</i> , <i>Kenknightia</i> , <i>Levua</i> , <i>Macleaya</i> , <i>Molpemyia</i> , <i>Mucidus</i> , <i>Nothoskusea</i> , <i>Ochlerotatus</i> , <i>Protomacleaya</i> , <i>Pseudoskusea</i> , <i>Rhinoskusea</i> , <i>Rusticooidus</i> , <i>Zavortinkius</i> Section II <i>Abraedes</i> , <i>Aztecaedes</i> , <i>Gymnometopa</i> , <i>Howardina</i> , <i>Kompia</i>	<i>Ochlerotatus</i> ( <i>Oc.</i> )  ' <i>Ochlerotatus</i> ' s.a.	<i>Buvirilia</i> , <i>Chrysoconops</i> , <i>Culicada</i> , <i>Culicelsa</i> , <i>Empihals</i> , <i>Gilesia</i> , <i>Juppius</i> , <i>Lepidokeneon</i> , <i>Ochlerotatus</i> , <i>Pholeomyia</i> , <i>Protoculex</i> , <i>Pseudoskusea</i> , <i>Rusticooidus</i> , <i>Woodius</i>  ' <i>Finlaya</i> ' s.a., ' <i>Protomacleaya</i> ' s.a.
<i>Opifex</i>		<i>Opifex</i> ( <i>Op.</i> )	<i>Nothoskusea</i> , <i>Opifex</i>
		<i>Paraedes</i> ( <i>Pr.</i> )	
		<i>Patmarksia</i> ( <i>Pm.</i> )	
		<i>Petermattinglyius</i> ( <i>Pe.</i> )	<i>Aglaonotus</i> , <i>Petermattinglyius</i>

<sup>1</sup>Harbach et al. 2007<sup>2</sup>Huang 2005; <sup>3</sup>Reinert 2006a**Fig. 1.3a** Classification of Aedini (genus/subgenus) and abbreviations after Reinert (2009)

Becker et al. 2003		Reinert et al. 2004, 2006, 2008, 2009	
Genus	Subgenus	Genus (abbreviation)	Subgenus
		<i>Phagomyia</i> (Ph.)	
		<i>Polyleptomyia</i> (Po.)	
		<i>Pseudarmigeres</i> (Pa.)	
<i>Psorophora</i>	<i>Grahamia, Janthinosoma, Psorophora</i>	<i>Psorophora</i> (Ps.)	<i>Grahamia, Janthinosoma, Psorophora</i>
		<i>Rampanyia</i> (Ra.)	
		<i>Rhinoskusea</i> (Rh.)	
		<i>Sallumia</i> (Sl.)	
		<i>Scutomia</i> (Sc.)	
		<i>Skusea</i> (Sk.)	
		<i>Stegomyia</i> (St.)	<i>Actinotrix, Bohartius, Heteraspidion, Huangmyia, Mukwaya, Stegomyia, Xyele, Zoromorplus</i>
		<i>Tanakaius</i> (Ta.)	
		<i>Tewarius</i> <sup>4</sup> (Te.)	
<i>Udaya</i>		<i>Udaya</i> (Ud.)	
		<i>Vansomerenis</i> (Va.)	
<i>Verrallina</i>	<i>Harbachius, Neomacleaya, Verrallina</i>	<i>Verrallina</i> (Ve.)	<i>Harbachius, Neomacleaya, Verrallina</i>
		<i>Zavortinkius</i> (Za.)	
<i>Zeugomyia</i>		<i>Zeugomyia</i> (Ze.)	

<sup>4</sup>Reinert 2006b

**Fig. 1.3b** Classification of Aedini (genus/subgenus) and abbreviations after Reinert (2009)

complemented by renewed high resolution studies of the external morphology of all life-stages to find species differences. Use of a combination of different methods will improve the chances of identifying vector species within a sibling species group. Therefore more studies of inter- and intraspecific variability of the different morphological life-stages are also much needed. Cross mating studies also improve understanding of relationships. The difficulties in separating species on morphological characters alone are shown by the numerous synonyms available for worldwide species such as *Culex pipiens* s.s. This indicates the amount of variability in external morphological phenotypic characters such as body size, colour, amount and colour of scaling and number and positions of setae in different life-stages, within one species in different geographic areas. Field studies of behaviour and physiology of adults and larvae will also add much to our knowledge and recognition of species. Such studies are important not only for taxonomic clarity but also for practical vector recognition and vector control.

Molecular research advances have created a separate branch of molecular research on Insecta, which also has bearings on systematics (Gilbert et al. 2005). Now it is possible to retrieve DNA from very small sources, and also from museum material. This step will be of the utmost importance in highlighting phylogenetic problems in species groups, as well as enabling the analysis of difficult species complexes. Munsterman's (1995) summary of advances in molecular culicid sys-

tematics has been followed by many new studies, which have established genetic relationships between species and within species complexes. Results from several authors up to the end of 2006 were discussed by Reinert et al. (2008) in relation to positive genus congruence with their ranking of *Aedes*, *Ochlerotatus* and *Stegomyia*. The analysis by Shepard et al. (2006) is a good example of molecular phylogeny based on subunits of ribosomal DNA sequences from 37 northern Nearctic and Holarctic species. In Simuliidae, Moulton (2001) obtained results that corresponded with classic phylogeny, by combining ribosomal and mitochondrial subunit sequences with phylogenetic analysis.

Molecular analysis is of particular interest for the study of difficult culicid vector species complexes, such as the Neotropical Anopheles Albitarsis Complex containing four species and possibly a fifth molecular species (Wilkerson et al. 2005), and the Australian Anopheles Annulipes Complex (Foley et al. 2007). The latter has been found to have 17 members of two geographically distinct clades. Harrison (2007) showed how ongoing molecular research on the South USA Anopheles Crucians Complex, can raise practical vector problems as well as create serious taxonomical difficulties. He found that the Crucians Complex, which was formerly considered to include three species, in fact contained two more sibling species with inseparable females. His discussion of which species in reality may be present at a site, if no reared material

from larvae are available to help identify a species of a complex, is a useful illumination of the problems facing practical control work. Therefore for both scientific taxonomy and practical vector control reasons, sibling species or species complexes are important targets for

analysis, using a combination of all available methods. The basis for such studies are comprehensive field sampling, the careful curating of collections of hatched material, and the ongoing support of taxonomic knowledge throughout long-term investigations.

## Chapter 2

# Biology of Mosquitoes

Regarding their special adaptational mechanisms, mosquitoes are capable of thriving in a variety of environments. There is hardly any aquatic habitat anywhere in the world that does not lend itself as a breeding site for mosquitoes. They colonise the temporary and permanent, highly polluted as well as clean, large and small water bodies; even the smallest accumulations such as water-filled buckets, flower vases, tyres, hoof prints and leaf axes are potential sources.

In temporarily flooded areas, along rivers or lakes with water fluctuations, floodwater mosquitoes such as *Aedes vexans* or *Ochlerotatus sticticus* develop in large numbers and with a flight range of several miles, become a tremendous nuisance even in places located far away from their breeding sites (Mohrig 1969; Becker and Ludwig 1981; Schäfer et al. 1997).

In swampy woodlands, snow-melt mosquitoes such as *Oc. cantans*, *Oc. communis*, *Oc. punctor*, *Oc. hexodontus*, and *Oc. cataphylla*, encounter ideal conditions for development in pools that are formed after the snow melts or after heavy rainfall.

In floodplains along coastal areas, the halophilous species (preferring brackish or salt water habitats) such as *Oc. taeniorhynchus*, *Oc. sollicitans*, *Oc. vigilax*, *Oc. caspius*, *Oc. detritus*, develop in huge numbers. Larvae of the *Anopheles* can be found in association with other mosquito species in fresh- or salt-water marshes, mangrove swamps, rice fields, grassy ditches, edges of streams as well as in small temporary water collections. Many species prefer habitats either with or without vegetation.

Tree-holes are the habitat of arboreal species such as *Oc. geniculatus*, *Ae. cretinus*, *Anopheles plumbeus* and *Orthopodomyia pulcripalpis*.

Species like *Cx. p. pipiens*, *Ae. aegypti* [*St. aegypti*] *Ae. albopictus* [*St. albopicta*] or *Oc. j. japonicus* can even breed in a variety of small water containers such

as rain-water drums, tyres, cemetery vases, small clay pots, etc.

Furthermore, their capacity to adapt to various climatic factors or changing environmental conditions is fascinating. For instance, *Ae. albopictus*, the Asian tiger mosquito is originally a tropical species. In the course of a climate-related evolutionary adaptation it developed a photoperiodic sensitivity. When days are shorter, the photoperiodically sensitive female inhabiting a temperate climate, lays eggs that are different from the eggs that she lays when days are longer. Eggs laid during shorter days, are dormant and do not hatch until the following season, ensuring the species' survival through the winter.

This ability to adapt to moderate climatic conditions and the fact that the eggs are resistant to desiccation and survive for more than a year, including the capability of adaptation to artificial breeding sites such as tyres and flower pots, make *Ae. albopictus* or *Oc. j. japonicus* successful species. This has contributed to its spread globally via international trade in plants like *Dracaena* spp. ("lucky bamboo") and tyres. Within hours or days they can be transported from one country or continent to another by cars, aircrafts or trans-oceanic containers (Madon et al. 2002).

These are but a few examples, which illustrate the tremendous ecological flexibility of mosquitoes successfully adapted to.

Like all Diptera, mosquitoes exhibit complete metamorphosis. All mosquitoes need aquatic habitats for their development, although *Aedes/Ochlerotatus* spp. can lay their eggs in moist soil. After hatching they pass through four larval instars and a pupal stage when the transformation into an adult takes place. Most species are unautogenous: following copulation, the females have to take a blood-meal to complete the egg development.

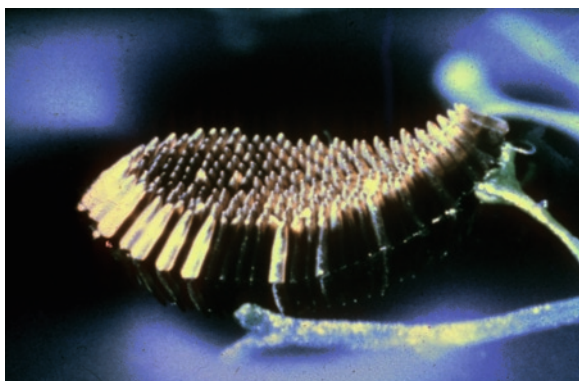
Only a few species have populations that are autogenous. They first develop egg batches without a blood-meal (e.g. *Cx. p. pipiens* biotype *molestus*).

## 2.1 Oviposition

Female mosquitoes lay between 50 and 500 eggs, 2–4 days (or longer in cool temperate climates) after the blood-meal. In general, the mosquitoes can be divided into two groups depending on their egg-laying behaviour (Barr 1958) and whether or not the embryos enter into a period of dormancy (externally triggered resting period) or diapause (genetically determined resting period).

In the first group, females deposit their eggs onto the water surface either singly (*Anopheles*) or in batches (e.g. *Culex*, *Uranotaenia*, *Coquillettidia*, *Orthopodomyia* and subgenus *Culiseta*, Fig. 2.1).

The *Culex* females lay their eggs in rafts comprising several hundred eggs locked together in a boat-shaped structure. During oviposition, the females stand on the water surface with the hind-legs in a V-shaped position. The eggs are released through the genital opening and grouped together between the hind-legs, forming a raft where the eggs stand vertically on their anterior poles attached together by chorionic protrusions (Clements 1992). The anterior pole of each egg has a cup-shaped corolla with a hydrophilic inner surface, which lies on the water surface, the outer surface is hydrophobic. The resulting surface tension helps to



**Fig. 2.1** Egg raft of *Culiseta annulata* (size approx. 5 mm)

keep the egg-raft in position and when rafts drift to their aquatic boundaries they tend to remain there. Immediately following oviposition, the eggs are soft and white, but they sclerotize and darken within 1–2 h.

Anophelines lay single eggs while standing on the water surface or hovering above it. The eggs of this subfamily are adapted for floating and can easily be damaged by desiccation.

The embryos of the first group do not enter dormancy or diapause and hatch when the embryonic development is completed. Species producing nondormant eggs usually have several generations each year. Their developing stages are found for the most part in more permanent waters where one generation succeeds another during the breeding season. The number of generations depends on the length of the breeding season, as well as the abiotic and biotic conditions, and most importantly, it is the temperature which influences the speed of development.

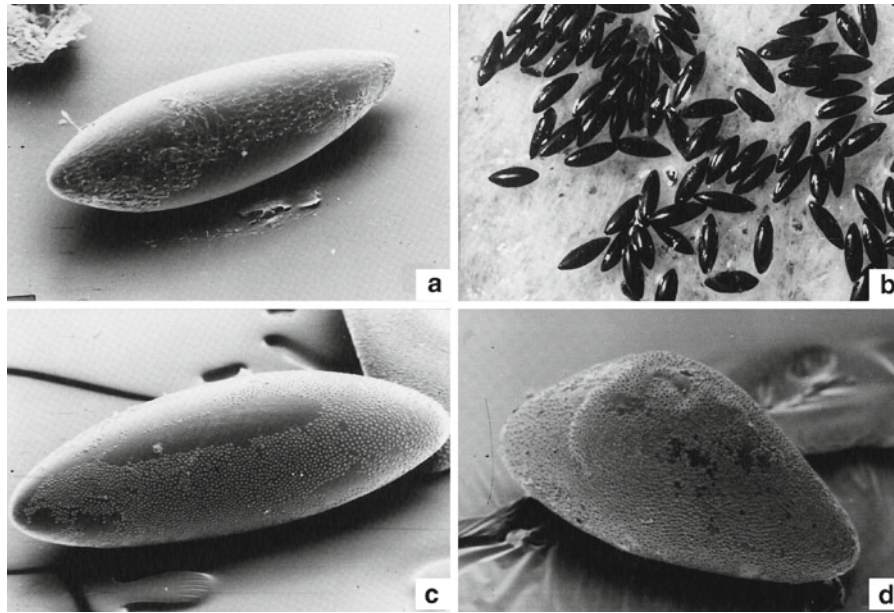
The parameters that determine the choice of a breeding site by the females laying their eggs onto the water surface are still unknown for many species. Factors such as water quality, incidence of light, existing eggs, available food, and local vegetation are decisive factors in selecting a favourable breeding site.

For *Cx. p. pipiens*, it is known that the content of organic material in the water plays an important role in attracting the females about to lay eggs. Apparently, gaseous substances such as ammonia, methane, or carbon dioxide, which are released when organic material decomposes, create an effect of attracting the females of *Cx. p. pipiens* (Becker 1989b). They recognise that such a site has adequate food and that favourable conditions prevail for the development of their brood.

A few other examples illustrate that the egg-laying behaviour reflects the ecological conditions in the breeding site. The submerged larvae and pupae of *Coquillettidia* obtain the oxygen they need by inserting their siphon into the aerenchyma (air filled tissue) of certain plants under water. Therefore, in order to ensure the development of the larvae, the females must recognise the appropriate aquatic plants at the time they lay their eggs in order to ensure the development of the larvae.

The second group lays eggs which do not hatch immediately after oviposition (Fig. 2.2). The egg-laying behaviour of the floodwater mosquitoes (e.g. *Ae. vexans*), and the subgenus *Culicella* of genus *Culiseta*, which lay





**Fig. 2.2** Eggs of *Ae. vexans* (a: SEM-photo, 50X; b: light microscopical photo 8X, *Oc. cantans* (c, SEM, 50X), *Oc. rusticus* (d, SEM, 50X)

their eggs singly, not on the water surface but into the moist soil, which is subsequently flooded when water levels rise, is most interesting. The eggs are laid into small depressions or between particles of moss with a high degree of soil moisture which protect the sensitive eggs from drying-out during the embryogenesis (Barr and Azawi 1958; Horsfall et al. 1973). For *Ae. vexans* and *Oc. caspius*, which breed in flooded areas where the levels fluctuate frequently, the appropriate egg-laying behaviour is crucial to ensure successful development of the immature stages. A suitable egg-laying site for floodwater species must meet the following prerequisites:

- (a) The substrate must be wet enough at the time the eggs are laid in order to ensure that the freshly laid eggs which are very sensitive to any water loss, do not desiccate before their impermeable endochorion has been tanned and the wax layer of the serosal cuticle is formed (Horsfall et al. 1973; Clements 1992);
- (b) There must be a subsequent and sufficient flooding of the soil where the eggs have been laid, so that the complete process can take place from hatching all the way to the imago emergence;
- (c) The water body for subsequent breeding should have as few mosquito predators as possible, to ensure that the larvae are not preyed upon by natural enemies when they hatch.

The ability of a floodwater mosquito female to find appropriate places for egg-laying which guarantees maximum breeding success is not yet fully understood. However, respect is due to these tiny insects which have adapted their behaviour to overcome the hostile conditions in their breeding sites. If the females chose to lay their eggs in low-lying areas with almost permanent water-flow, they would encounter crucial disadvantages: low-lying areas are flooded for long periods of time and have, therefore, a very unfavourable alternating sequence of dry and flooding spells, therefore, allowing only very small populations of floodwater mosquitoes to develop. Areas with almost permanent water-flow generally have a high concentration of natural enemies, such as fish, so that the risk of being preyed upon would be very high for the mosquito larvae.

However, flooded areas with a very short period of water-flow are also unfavourable egg-laying sites, because they entail the risk of premature drying-out. This kind of terrain becomes flooded for a short period of time and only in the years when water is abundant, thus the wet and dry sequences are not favourable for the development of several consecutive generations. These areas dry very rapidly following a flood so that the *Aedes* or *Ochlerotatus* eggs run the risks of desiccation since the developing embryos are very sensitive to water loss.

Figure 2.3, shows the preferred egg-laying sites of the floodwater mosquitoes (e.g. *Ae. vexans*). Usually, these areas consist of dense vegetation and silty soil, with the reed (e.g. *Phragmites australis*) being highly attractive to ovipositing female mosquitoes.

Usually, the reed zone precisely demarcates the mid-water level in riparian systems, since reeds need a good amount of water.

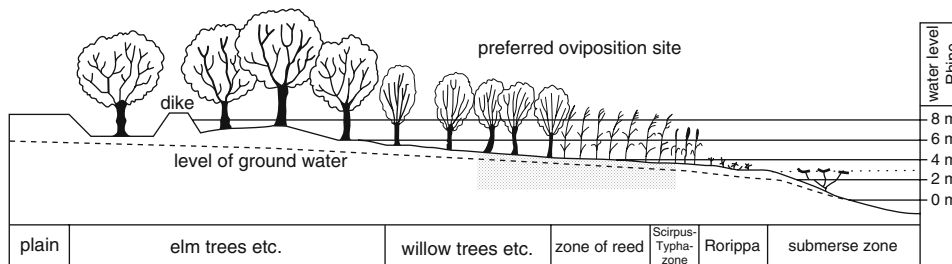
The fluctuating curve of the Rhine river, for example, makes it quite clear how important this egg-laying behaviour is for the development of the floodwater mosquitoes (Fig. 2.4). In a zone of between 4 and 5 m, there is the ideal timing of dry and wet periods, with optimum water-flow to guarantee the development of a large number of individuals in a population. Furthermore, predators, especially fish are usually low in numbers or absent.

But how do these female floodwater species find the optimum oviposition site? Gravid females obviously recognise the wet, silty, riparian clay soil as an

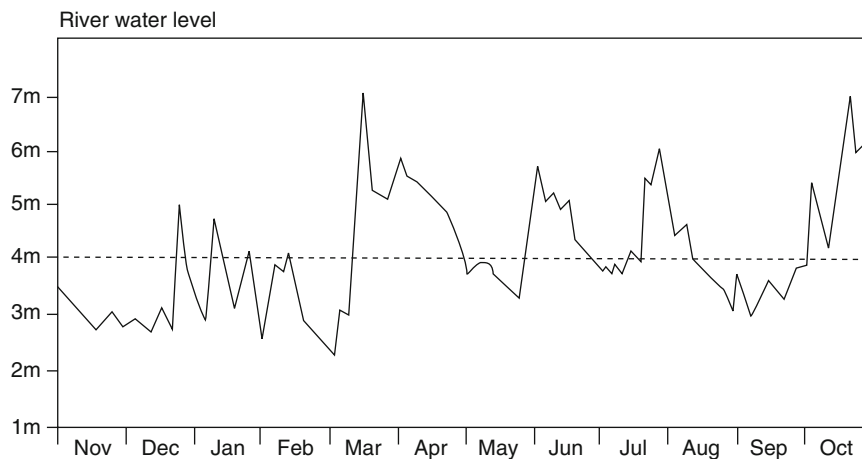
appropriate egg-laying substrate and are attracted to it. However, these criteria alone are not enough, given that during rainy periods there would be many other places which would apparently appear adequate due to their high moisture content.

It is likely that the floodwater mosquitoes are able to differentiate between various soil types. The soil in most floodplains consists of a high percentage of clay and low percentage of humus or organic materials, in contrast to many other soils (Ikeshoji and Mulla 1970; Strickman 1980a,b; Becker 1989b).

It is also possible that flooded areas along the river-banks produce pheromone-like odours, which may be recognised by the female mosquitoes and which induce them to lay their eggs. These odours could come from eggs, which have already been laid in the soil, or from particular associations of plants which are indicators of a specific moisture level in the soil, and the occurrence of regular floods. As a whole, the ovipositional behaviour of the floodwater mosquitoes shows an astonishing



**Fig. 2.3** Transsection through the Upper Rhine Valley with zones of vegetation and preferred oviposition sites of floodwater mosquitoes (e.g. *Ae. vexans*)



**Fig. 2.4** River water levels in Central Europe favor the development of the eggs of floodwater mosquitoes

degree of adaptation to their habitat, an adaptation which has developed through the course of evolution.

## 2.2 Embryonic Development

Mosquito embryogenesis has been described in detail by Clements (1992). The embryonic development starts almost immediately after the eggs have been laid. Depending on the temperature, it takes about 2–7 days or more until the embryos are fully developed.

The course of embryonic development also reflects a special adaptation to various abiotic conditions in the larval habitat (Becker 1989b). The nondormant eggs of the *Culex*, *Coquillettidia*, *Uranotaenia*, *Orthopodomyia* and the subgenus *Culiseta*, usually hatch after a short time when the embryonic development is completed. The length of time required is dependent almost entirely on temperature. At a temperature of 30°C, the *Cx. p. pipiens* larvae hatch 1 day after the eggs have been laid, at 20°C and 10°C it takes 3 and 10 days respectively, and at 4°C, the embryonic development of the *Cx. p. pipiens* cannot be completed (Fig. 2.5).

The embryonic development of the *Aedes/Ochlerotatus* species usually takes significantly longer, for

instance, larvae of *Ae. vexans* are ready to hatch in 4–8 days after oviposition, when the eggs are kept at 25 and 20°C, respectively (Horsfall et al. 1973; Becker 1989b). Hatching experiments with freshly laid *Ae. vexans* eggs kept at 20°C have shown that 8 days after the eggs had been laid, almost 50% were ready to hatch. This means that the embryonic development of *Cx. p. pipiens* usually takes only half as long as that of *Ae. vexans*, which assures a quick generation renewal of the former. The relatively slow embryonic development of floodwater *Aedes/Ochlerotatus* species can be explained by the fact that these mosquitoes lay their eggs in flooded areas where there are few ecological factors requiring rapid embryonic development. It usually takes more than one week until the next flooding occurs. Therefore, there is little ecological advantage in the fast sequence of generations that would result from rapid development of the embryos.

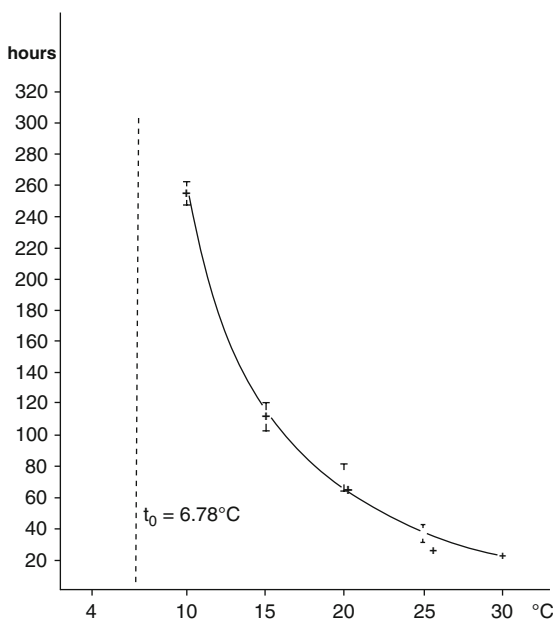
## 2.3 Hatching

*Aedes* and *Ochlerotatus* mosquitoes have developed a highly sophisticated mechanism which regulates the hatching process, as a direct adaptation to the greatly fluctuating abiotic conditions existing in the temporary waters where these mosquitoes breed (Gillett 1955; Telford 1963; Horsfall et al. 1973; Beach 1978; Becker 1989b). The timing of larval hatching to coincide with the presence of ideal developmental conditions, is a prerequisite for successful development in temporary water bodies.

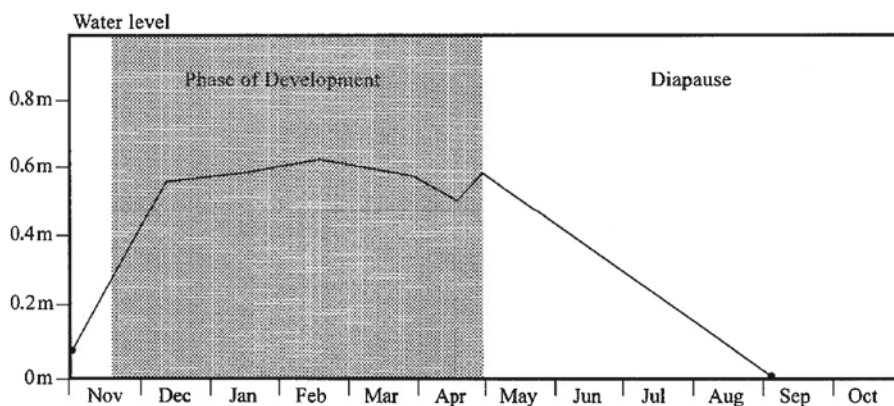
The difference in hatching behaviour between the snow-melt mosquitoes (e.g. *Oc. cantans*, *Oc. communis*, and *Oc. rusticus*) and the floodwater mosquitoes (e.g. *Ae. vexans*) clearly illustrates the extent to which the hatching behaviour of each *Aedes/Ochlerotatus* species is adapted to the abiotic conditions in their respective breeding sites.

The breeding waters of snow-melt mosquitoes, for example depressions and ditches in marshy regions covered frequently with alder trees in central Europe, are usually flooded for long periods of time with relatively cold water.

In Fig. 2.6, the phases of development and diapause of the univoltine (monocyclic, one generation/year) snow-melt mosquitoes, are shown as a function of the water level variation of a pool in the swampy woodlands in central Europe.



**Fig. 2.5** Duration of the embryonic development of *Cx. p. pipiens* at various temperatures ( $t_0$  = no development possible according to Tischler 1984)



**Fig. 2.6** Phases of development and diapause of univoltine snow-melt mosquitoes in a pond in Central Europe

The breeding sites of the snow-melt mosquitoes are usually flooded in the late fall and after the snow has melted. The water level usually reaches its peak in early spring. Under normal conditions it eventually recedes slowly but steadily throughout the summer until the pools are dry again.

Snow-melt mosquitoes have adapted perfectly to these conditions in their breeding sites, by their diapause patterns and appropriate reaction to the hatching stimuli. After the eggs have been laid usually in early summer, the embryos of the majority of the snow-melt mosquitoes automatically enter diapause. They are unable to hatch during the summer months, thus avoiding the risk of premature emergence during the dry spells of summer.

In central Europe, some species, *e.g.* the snow-melt mosquito *Oc. rusticus* and *Cs. morsitans*, are ready to hatch at the beginning of winter after sensing the continuous temperature decrease during autumn. The diapause of most snow-melt mosquitoes (*e.g.* *Oc. cantans* and *Oc. communis*) is interrupted when the temperature has dropped in autumn and the cold winter period has set-in. Consequently, these mosquitoes are ready to hatch during the snow-melt in the next spring and shortly afterwards. This factor, along with their ability to hatch in very cold and oxygen-rich waters, enables these mosquitoes to be ready to hatch at a time when favourable water level conditions are present. After hatching, the semipermanent water in forest pools provides ideal conditions for slow development. In central Europe, this usually takes place between the end of April and the beginning of May.

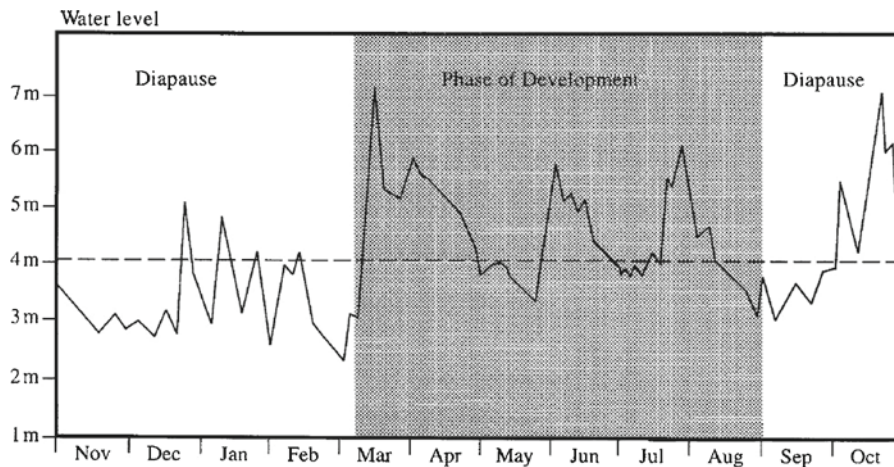
Even more sophisticated, is the hatching behaviour of the floodwater mosquitoes. In Fig. 2.7, the phases of

development and diapause of *Ae. vexans* are shown as a function of the water level variation (for *e.g.* the Rhine river).

Unlike the semipermanent status of the water in the breeding sites of snow-melt mosquitoes, the breeding sites of the floodwater mosquitoes are characterized by temporary water-flow caused by rapid, substantial fluctuations in the water level of the rivers following heavy rains in early and mid-summer. By contrast, late summer and winter are usually marked by extensive periods of low water levels. As a consequence of this, the best developmental conditions for larvae of floodwater mosquitoes occur between April and September, therefore, these mosquitoes diapause during autumn, winter, and early spring (Telford 1963). Due to the extremely variable nature of the water-flow, floodwater mosquitoes have to hatch during summer when high water temperatures enable rapid development to take place. Moreover, their being multivoltine (polycyclic, several generations/year), makes sense from an ecological viewpoint, since they can go through several generations coinciding with the fluctuations in the water level. This factor is mainly responsible for the huge reproduction rate of these species, often creating a tremendous nuisance.

If the hatching behaviour of the floodwater mosquito is analyzed in detail, a well-adjusted control mechanism can be seen, which is mainly influenced by the following factors:

- (a) Dissolved oxygen is present during periods of high water levels, most of the flooded areas along rivers are covered with flowing and oxygen-rich water. Hatching at that time would create the risk of the



**Fig. 2.7** Phases of development and diapause of the floodwater mosquito *Ae. vexans* in Central Europe

larvae being swept away. Moreover, fish usually invade deep flooded areas in search for food. In order to avoid these dangers, the floodwater mosquitoes have developed a specific hatching behaviour. The decline in dissolved oxygen when water is stagnant, usually triggers hatching of the larvae of the floodwater mosquitoes (Hearle 1926; Gjullin et al. 1941; Borg and Horsfall 1953; Travis 1953; Judson 1960; Horsfall et al. 1958, 1973; Burgess 1959; Becker 1989b). They hatch only when the flooded pools become stagnant and the oxygen content starts to decrease very rapidly (due to bacterial degradation processes). The decreasing level of oxygen in the water signals the unhatched larvae that the water will remain stagnant at the time of their hatching, thus ensuring that the risk of being washed away, has diminished. Moreover, the bacterial action causing decomposition of organic material ensures that the larvae will have an adequate food supply. In addition, the shallow, stagnant waters are not a suitable environment for the larvae's main predator, the fish.

- (b) Water temperature plays a fundamental role in the hatching process of the floodwater mosquito. Premature hatching during cold weather would greatly delay the development of the larvae, since the process is very temperature dependent.

Since floodwater mosquitoes usually diapause from autumn until springtime, the temperature-dependent hatching behaviour of *Ae. vexans* also ensures that the larvae will not hatch before the temperature of the

water is warm enough to permit rapid development. This ensures that the brood will not dry out due to rapid changes in the water levels. For instance, in the Upper Rhine Valley, the *Ae. vexans* larvae hatch in springtime when the water temperature reaches 10°C or more. An interesting phenomenon is the seasonal-related hatching process. Following the cold winter phase and after the subsequent increase in temperature, a small percentage of the *Ae. vexans* larvae are ready to hatch even at 4°C. The adaptation of the temperature-dependent hatching behaviour to the climatic conditions and water-flow in an area in central Europe can be demonstrated with the example of the hatching response to water of 15°C. In springtime, the hatching rate reaches its peak at 15°C. In the months of March and April, apart from the gradually increasing water temperatures during springtime, the water level usually rises due to rainfall. This creates favourable conditions for the development of the floodwater mosquitoes. On the other hand, water of the same temperature induces a reduced hatching response during late summer and autumn. Larvae would not be able to complete their development if they were to hatch at a temperature of 15°C in October or November, since at this time of year the falling temperatures prolong the larval development, while the water level is receding.

It is remarkable that there are also differences in the hatching behaviour of the *Ae. vexans* populations in different river systems. It appears that the *Aedes/Ochlerotatus* species are adapted to the hydrological characteristics of each river system. In river systems

with a lower discharge of water, the periods of inundation are usually shorter, which means that the development of the mosquito has to be rapid. Therefore, the *Aedes* and *Ochlerotatus* mosquitoes breeding in these areas have an extended diapause until summer, to allow a faster development at higher temperatures.

Let us have a closer look at the diapause of the ready-to-hatch larvae inside the eggs of the floodwater mosquito *Ae. vexans*, especially concerning the change from a state of hatching inhibition to one of hatching readiness. This process is called “conditioning”, whereas, the onset of hatching inhibition is called “deconditioning” (Horsfall 1956b; Horsfall and Fowler 1961; Clements 1963; Horsfall et al. 1973).

The factors that are most likely to have an influence on diapause, or on hatching inhibition and readiness, are temperature fluctuations, varying degrees of moisture in the air and soil as well as changes in the day-length (Brust and Costello 1969). Larvae of *Ae. vexans* are able to hatch to some extent during a flood in the same year as that in which the embryogenesis was completed, providing that the temperature remains above 20°C. Decreasing temperatures below 15°C lead to a hatching inhibition in autumn. After a cold (winter) phase, temperatures of 10°C and above have a conditioning effect, and interrupt the diapause in the next spring. However, it is worth mentioning that after a cold spell, the readiness to hatch is positively correlated with the rise in temperature. The higher the temperature during egg laying and the lower the temperature in winter, the higher is the hatching response in the following summer. The complex diapause behaviour allows the larvae of *Ae. vexans* to distinguish between favourable developmental conditions in springtime, and unfavourable conditions in late summer. It is remarkable that in winter even extreme temperatures well below the freezing point, will not kill the diapausing larvae.

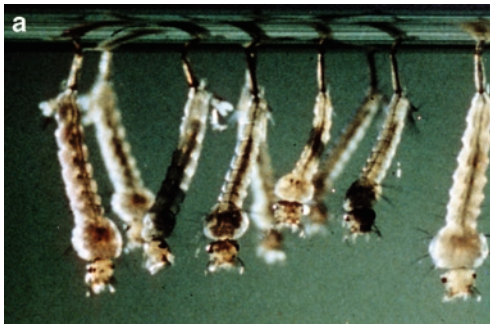
Another behaviour that represents a sophisticated adaptation to the very variable water-flow in the breeding sites is what is referred to as “hatching in installments”. Even within a batch of eggs laid by one female subjected to the same microclimatical conditions, not all of the larvae hatch uniformly. Without a cold phase, only a few individuals from a freshly laid egg batch of one female are ready to hatch, whereas, after a cold phase, the readiness to hatch is far greater. Apart from their inherited variability, the conditions that each egg had experienced (for instance, the location of the egg in the ovariole during maturation, the timing of the oviposition, as well as differing microclimatic factors

at the egg-laying site) determine whether a larva will hatch under certain conditions or not. Thus, the larvae hatch “in installments” (Wilson and Horsfall 1970; Becker 1989b). For instance, soil samples containing the eggs of *Ae. vexans* and kept at 25°C, were flooded several times, with dry phases of 4 weeks between each flooding step. After the first flooding, 57% of the total number of larvae hatched, 10% after the second, 25% after the third, and 8% hatched the fourth time (Becker 1989). This behaviour pattern assures long-term survival for mosquito species that develop in temporary bodies of water. If, for example, all of the larvae were to hatch at the same time under ideal hatching conditions and if, as a consequence of a sudden dry spell, all of the breeding sites were to dry-out before the brood could complete its developmental cycle. One single natural event could virtually wipe out the entire mosquito population. By “hatching in installments”, the floodwater mosquito population can survive such potentially catastrophic events. There still remains a large contingent of unhatched larvae in the breeding area. At the time of the next flooding, these larvae will still have an opportunity to successfully produce a new generation without new eggs having to be laid. Incidentally, this also happens after a larvicidal treatment. It is remarkable that the unhatched larvae are able to persist for at least 4 years without losing their ability to hatch (Horsfall et al. 1973).

After the content of oxygen decreases in the breeding site, the larva initiates the shell rupture by pressing the so-called “egg tooth”, an egg burster located posterior-dorsally on the head capsule of the larva, onto the egg shell. As a result, the shell splits along a particular line at the anterior end of the egg. A cap (anterior part of the egg shell) breaks away and the larva escapes by swallowing water into the gut which forces the body from the shell (Clements 1992). The whole process of hatching takes only a few minutes.

## 2.4 Larvae

The legless (apodous) larval body is divided into three distinct parts: (a) the head with mouth-parts, eyes and antennae; (b) the broader thorax and (c) the abdomen which is composed of seven almost identical segments and three modified posterior segments. These posterior segments bear four anal papillae to regulate the electrolyte levels. At the abdominal segment VIII, a siphon in culicines, or only spiracular lobes in anophelines, are



**Fig. 2.8** (a) Larvae of *Aedes vexans*



**Fig. 2.8** (b) Larva of *An. plumbeus*



**Fig. 2.8** (c) Larva of *Coquillettidia richiardii* attached to plant tissue (photo, Hollatz)

developed where the tracheal trunks open at spiracles for the intake of oxygen. Usually the culicine larvae hang head downwards from the water surface (Fig. 2.8a). Anopheline larvae lie horizontally at the water surface. Their body is held horizontally by specialized setae (palmate setae), the notched organ located at the anterior margin of the prothorax and the spiracular lobes which are flush with the dorsal surface of the larval body and have direct contact with the air (Fig. 2.8b).

When the larvae leave the water surface, the lobes are retracted, the spiracles are closed and reach the water surface, the flaplike four or five spiracular lobes are pulled into their extended position by the surface

tension forces. A gland adjacent to the larval spiracles, secretes hydrophobic substances to avoid the influx of water into the respiratory system.

The larvae of the *Coquillettidia* and *Mansonia* live submerged. They therefore possess a siphon which is modified for piercing submerged parts of aquatic plants to obtain oxygen from the aerenchyma. The spiracular apparatus at the distal end of the siphon contains hooks and a saw-like blade with teeth to pierce the plant tissues. These larvae have a more sessile habit, hanging head downwards whilst attached to the plant tissues and filtering the water column for food (Fig. 2.8c). They are therefore not easily recognised by predators such as fish.

The larval food consists of microorganisms, algae, protozoa, invertebrates and detritus. On the basis of their feeding behaviour they may be classified into filter or suspension feeders, browsers or predators. The filter feeders collect food particles suspended in the water column (especially larvae of the *Culex*, *Coquillettidia*, the subgenus *Culiseta* or to some extent the *Aedes/Ochlerotatus* larvae). The larvae of the filter feeders, typically hang on the water surface filtering the water column beneath the surface by beating their head brushes (lateral palatal brushes) towards the preoral cavity. This generates water currents which carry food particles towards the mouth (Dahl et al. 1988). Mosquito larvae are usually not discriminatory in what they ingest. However, the size of the particles are usually less than 50  $\mu\text{m}$ . Larvae can also move slowly in the water column while filter feeding. The browsers (e.g. most *Aedes* and *Ochlerotatus* species) collect food by re-suspension, scraping or shredding particles, microorganisms, algae, and protozoa from the surface of submerged substrates or the microbial film at the air–water interface (the *Anopheles* larvae). Even small portions of dead invertebrates and plants can be bitten-off with the mouth parts.

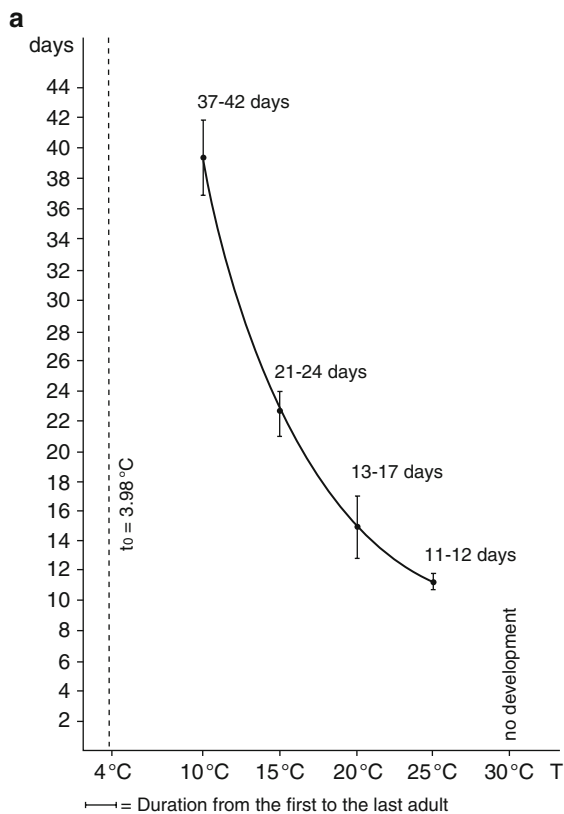
The anopheline larva hangs horizontally under the water surface with its dorsal side uppermost and the mouthparts directed downwards. When feeding, the larva rotates its head 180° and creates a water-current by beating its head brushes to collect the food organisms on the surface film.

Predacious larvae of the genera *Toxorhynchites*, *Aedes*, *Psorophora* and *Culex* can feed upon insects (often other mosquito larvae).

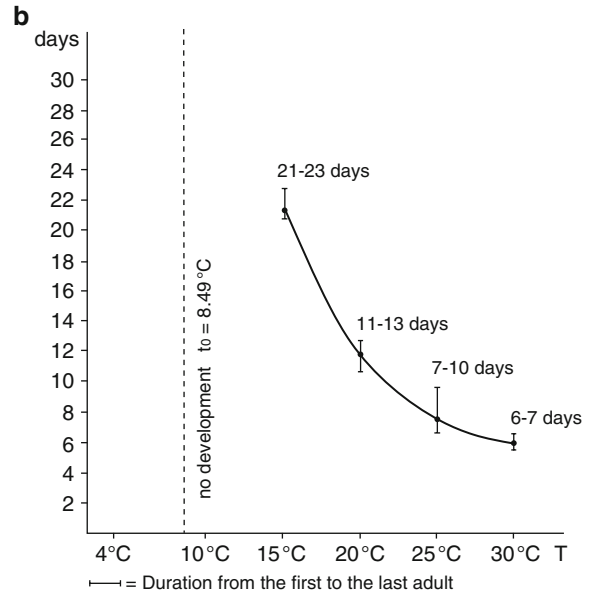
Disturbances of the water surface cause the larvae to dive for short periods of time. They dive by flexing the abdomen and moving backwards. When the larvae return to the water surface, they swim backwards until their abdomens come into contact with the surface.

Larvae moult four times at intervals, before reaching the pupal stage. At each moult, the head capsule is increased to the full size characteristic of the next instar, whereas the body grows continuously. Thus the size of the head capsule is a fairly good morphometric indicator for the larval instar. Each moult is coordinated by the relative concentrations and interactions of juvenile hormone and ecdysone, a molting hormone.

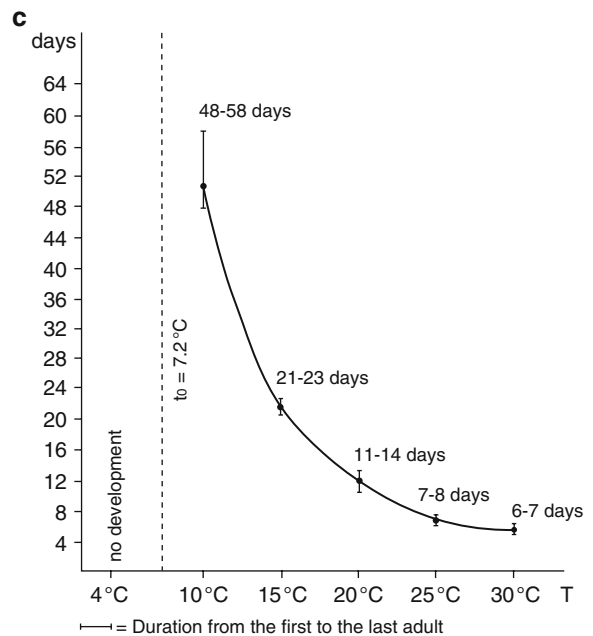
The development of larvae is temperature dependent. There are great differences in the optimum temperature for the development of different mosquito species (Figs. 2.9a-c). For instance, the snow-melt mosquitoes can complete their development at temperatures as low



**Fig. 2.9 (a)** Larval and pupal development of *Oc. cantans* in relation to water temperature



**Fig. 2.9 (b)** Larval and pupal development of *Ae. vexans* in relation to water temperature



**Fig. 2.9 (c)** Larval and pupal development of *Cx. pipiens* in relation to water temperature



as 10°C, whereas they are incapable of developing successfully at temperatures above 25°C (Fig. 2.9a). Usually they hatch in southern and central Europe during February, or later in the northern parts, and the adults emerge 2–3 months later. Larvae of those species which overwinter in the larval stage such as the *Oc. rusticus* or *Cs. morsitans*, will survive in water close to freezing, or even in waters which become regularly coated with ice.

In contrast, the floodwater mosquitoes (*e.g. Ae. vexans*), develop successfully at higher temperatures within a short period of time, usually 6–7 days from hatching to emerging at 30°C (Fig. 2.9b). Larvae of *Cx. p. pipiens* can successfully develop in a wide range (10–30°C) of temperatures (Fig. 2.9c). The development of the aquatic stages also represents an adaptation to the ecological environment in breeding waters.

Mosquito larvae (*e.g. Ae. vexans*) sometimes aggregate in particular places at the breeding sites. This crowding effect may be a mechanism to reduce the chance of predation of any single larva.

## 2.5 Pupae

The pupae are also aquatic, the pupal stages usually lasting about 2 days, however, this period may be reduced or extended, at higher or lower temperatures respectively. During the pupal stage, the process of metamorphosis takes place. Some larval organs are histolysed, whilst the body of the adult is formed through the development of imaginal disks (cells or groups of cells that remained quiescent in the larval body until the pupal stage). In particular, the fat body of the larva is transferred to the adult stage and used as a source of vitellogenines for autogenous egg formation or as a reserve for hibernation.

Characteristically, the head and thorax of the pupae are fused into a prominent cephalothorax which carries anterolaterally two respiratory trumpets (see Chap. 5, Fig. 5.18), which are connected to the mesothoracic spiracles of the developing adults to provide oxygen. The abdomen terminates with two paddles and is kept flexed under the cephalothorax. When at rest, the pupae float motionless at the water surface, with the tip of the trumpets and the palmate setae of the first abdominal segment in contact with the water surface. The hydrophobic rims of the trumpets protrude through the water surface for respiration. An air bubble between the appendages of

the cephalothorax makes the pupa positively buoyant. Mosquito pupae are quite mobile (unlike the pupae of most other insects). When the pupa is disturbed, it dives by straightening the abdomen and spreading the paddles rapidly flexing the abdomen which has retained the larval musculature. In contrast to larvae which have to swim actively to the water surface, the pupa floats passively back to the surface after diving. Pupae of most mosquitoes are relatively tolerant of desiccation and adults can emerge successfully even if pupae have been stranded, or when the breeding sites have almost dried out. Unlike larvae, pupae do not feed.

In *Mansonia* and *Coquillettidia*, the trumpets are modified for penetrating plant tissues. The pupae, like the larvae, take the oxygen from the aerenchyma of the submerged parts of the plants.

## 2.6 Adults

### 2.6.1 Emergence

The final stage of metamorphosis is completed when gas is forced between the pupal and the pharate adult cuticle, and into its midgut. The pupa straightens the abdomen into a horizontal position, and by swallowing air it further increases the internal pressure. The cephalothoracic cuticle of the pupa then splits along the ecdysial line and the adult slowly emerges from the pupal skin (Fig. 2.10). The emerging adult moves cautiously to avoid falling onto the water surface, whilst its appendages still remain partly in the exuvia. In this phase, the emerging individual is highly susceptible to



**Fig. 2.10** Emerging adult mosquito

strong winds and predators such as water striders and spiders.

The pupae of the genus *Coquillettidia* are fixed to the plant tissues in the water body. At the end of the pupal development they have to float to the water surface. Therefore, the tips of the pupal trumpets break to release the pupa from the plant before emergence (Mohrig 1969).

After emergence, the adult increases the haemolymph pressure which causes the legs and wings to stretch. It then immediately ejects droplets of fluid to empty the gut, while air is dispelled from the gut some hours later. Within a few minutes it is able to fly when the soft cuticle has sclerotized. However, 1–1.5 days more are required for males and females respectively to adjust their metabolism (Gillett 1983).

There is also a difference between male and female sexual maturity at the time of emergence. Male mosquitoes are not sexually mature at emergence as they have to rotate their hypopygium through 180° before they are ready to mate, which takes about 1 day. Therefore, the males in the population usually emerge 1–2 days before the females in order to achieve sexual maturity at the same time as the emerging females. Since the pupal stage of the two sexes appears to be about the same length, the shortening in development of males takes place primarily in the larval stage. Consequently, the male pupae and adults of a population are smaller in size than the corresponding females.

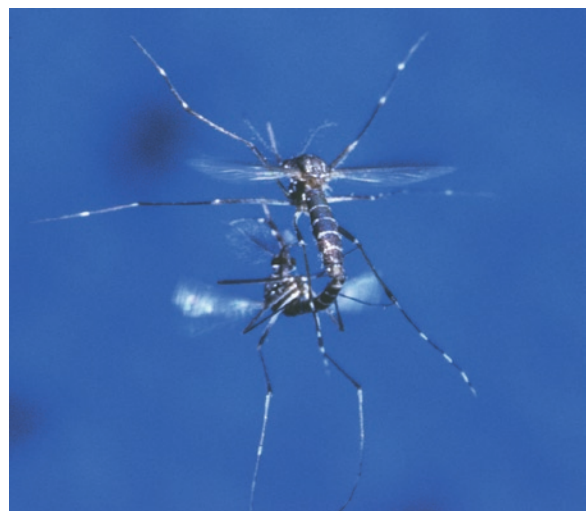
Following emergence, the adults are ready to begin their life cycle again of mating, feeding and oviposition.

### 2.6.2 Mating

Mating of most mosquitoes in the palaeartic region takes place when females enter swarms of flying males. Such a swarm can consist of only a few or up to several thousands of male mosquitoes. Usually, males form swarms over a marker at low light intensities especially in the evening and morning. Markers are projecting objects which contrast with the surroundings such as bushes. When swarming, males face into the wind and fly forward and backwards, up and down over the marker. This oscillating flight pattern is often called “dancing flight”. The sound produced by the male wing beat has a frequency of  $\sim 600\text{ cs}^{-1}$ . The frequency of the wing beat of the females is lower than that of the males at  $500\text{--}550\text{ cs}^{-1}$  and even lesser when engorged.

The plumose antennae of the males are especially receptive to the sound generated by the female. The flagellum of the antenna starts to vibrate and stimulate the Johnston’s organ which is located in the swollen second segment (pedicel) of the antenna (Clements 1963; McIver 1982). Contact pheromones may also be involved in the mating behaviour.

When a female enters a swarm it will be seized immediately by a male. Usually the male and female copulate face to face when flying outside the swarm (Fig. 2.11). The copulation requires a complex merging of the male and female reproductive structures. Usually it takes less than half a minute for the male to deposit the spermatozoa in the bursa copulatrix of the female (Clements 1963), the sperm then moves to the spermathecae. The male accessory gland secretions contain a substance known as matronae, which after copulation makes the female unreceptive for the rest of her life. The females store sufficient sperm in their spermathecae to fertilize several egg batches without further copulation. In contrast to females, male mosquitoes may mate many times. The time and preferred location of swarming is species-specific. Swarming (eurygamy) is not necessary for all species, and some species may mate without it (stenogamy). Usually, mating takes place soon after emergence, since biting females are almost invariably inseminated. After insemination the search for a host to obtain a blood-meal is the next important phase in the reproductive life of the female.



**Fig. 2.11** Mating mosquitoes (Photo courtesy of Roland Kuhn, University of Mainz, Germany)

### 2.6.3 Dispersal and Host-Seeking Behaviour

In most mosquito species, oogenesis can only be completed when the females take a blood-meal. Therefore, they have developed a complex host-seeking behaviour to locate a potential host. Primarily, the location of the host is based on olfactory, visual and thermal stimuli. Females possess numerous antennal receptors which respond to host odours. The main olfactory stimuli are carbon dioxide, lactic acid, octenol, acetone, butanone and phenolic compounds. The host-seeking process may differ within species depending on the season and the availability of certain hosts. However, it can usually be divided into three phases (Sutcliffe 1987):

- (a) Non-oriented dispersal behaviour which enhances the likelihood of the female coming into contact with stimuli derived from a potential host.
- (b) Oriented host location behaviour resulting from contact with host stimuli. The strengths of the stimuli are increased as the mosquito and host come closer together;
- (c) Attraction to a suitable candidate host, once the female has identified it in her immediate vicinity.

The extent of the non-oriented dispersal behaviour differs from species to species. In general, it can be separated into (a) species which usually breed and rest close to the habitat of their hosts and therefore do not fly long distances (most container breeders, e.g. *Cx. p. pipiens*); (b) species which disperse moderate distances from their breeding or resting places to the host's habitats (some species of snow-melt mosquitoes, e.g. *Oc. rusticus*); (c) species, which migrate considerably long distances to invade new habitats for biting, and/or egg-laying when suitable habitat is available (some floodwater mosquitoes, e.g. *Ae. vexans*).

The flight behaviour is influenced by temperature, humidity, illumination levels, wind velocity and the physiological stage of a female. For instance, most *Aedes/Ochlerotatus* species migrate during the twilight when the temperature is dropping and the humidity is increasing. They are usually more active on moonlit nights (Bidlingmayer 1964).

Species with a tendency for extensive flight activities usually show two different non-oriented dispersal behaviours (Provost 1953), a drift with the

wind (passive migration) and an active dispersal (appetitive flight).

During passive migration, the mosquitoes ascend in swarms and use the wind to drift long distances and may occur suddenly in large numbers far away from their breeding sites. This non-oriented flight activity is especially influenced by the speed and direction of the wind and guiding landmarks. The passive migration in swarms occurs only a short time after emergence (Bidlingmayer 1985).

During the appetitive flight, female mosquitoes usually disperse actively at least 24 h after emergence. They fly upwind when the wind velocities are below mosquito flight speed (1 m/s) (Bidlingmayer and Evans 1987). The flight against the wind increases the likelihood of encountering stimuli deriving from a host. However, strong winds prevent active dispersal. This behaviour is species-specific and depends on various features of the terrain and meteorological factors. The microclimate influenced by the vegetation type, which causes increased humidity and reduced wind, strongly affects the dispersal behaviour. Therefore, females usually fly close to the ground or slightly above the top of the vegetation. According to the preferred microclimate requirements, some species occur in greatest numbers in open areas (mostly strong flyers), others in woodlands (woodland species are moderate flyers), a third group prefers edges of fields and forests and finally the fourth group comprises the urban domestic species, which are usually weak flyers (Gillies 1972; Bidlingmayer 1975). Experiments show that *Ae. vexans* migrate approximately 1 km/night during warm and humid weather periods at moderate wind speed. Increasing numbers of *Ae. vexans* females have been caught in CDC-traps at a distance of about 5 km from their breeding place 8 days after emergence, and within 2 weeks at a distance of 10 km or more. Clarke (1943a,b) recorded migration distances of marked *Ae. vexans* females of 22 km and Gjullin and Stage (1950) and Mohrig (1969) up to 48 km.

In contrast, snow-melt mosquito species stay near their breeding sites and do not regularly migrate long distances (Schäfer et al. 1997). In mark-release-recapture experiments Joslyn and Fish (1986) collected *Oc. communis* females at distances up to 1,600 m from their breeding sites. Nielsen (1957) reported a maximum flight range of about 1,600 m for *Oc. communis* and *Ae. cinereus* with an average dispersal range of less than half this distance.

In Germany, *Oc. rusticus* females have been found resting in the forest during daytime and flying to the forest edge and the adjoining fields with approaching dusk. Females preferred to disperse along rows of trees in open areas. It can be assumed that in the absence of other attractants or adverse meteorological conditions, the flight of these mosquitoes was guided by the reduced level of illumination beneath the forest canopy, the contrast in illumination with the adjoining forest edge as well as the visual image of the rows of the trees. The mosquitoes obviously follow their hosts, mostly red deer, when these animals browse in the meadows near the forest. The flight distance observed was only a few hundred meters (Schäfer et al. 1997).

Dispersal serves mostly to bring these blood sucking insects into contact with a suitable signal from a potential host animal. It is likely therefore, that species which breed in areas where few hosts are available develop a stronger tendency for migration than those which breed in the vicinity of their hosts. For instance, *Cx. p. pipiens* which breeds in human settlements migrates usually less than 500 m. It is likely that host-seeking females will find stimuli from a suitable host within a few hundred meters.

In field studies, it was shown that both a horizontal and a vertical dispersal behaviour assists the host-seeking process. Females of *Aedes* spp. (*Ae. vexans*, *Ae. rossicus*, and *Ae. cinereus*) and *Ochlerotatus* spp. (*Oc. sticticus*), were most frequently captured in traps at ground level up to a height of 4 m whereas, at a height of 10 m, *Cx. p. pipiens* was by far the most abundant (99.2%) species. There is an interaction between the availability of suitable hosts and the distribution of mosquito species. For blood-seeking females of ornithophilic species (*Cx. p. pipiens* and *Cs. morsitans*), it is an advantage to search for birds in the canopy. In contrast, the *Aedes* and *Ochlerotatus* species prefer mammals as hosts, which explain the dominance of these species at ground level.

After encountering the host stimuli, the female mosquito changes its behaviour from the non-oriented flight pattern to an oriented host location. Initially, the mosquito is responsive to the host odour and then it uses this odour to track the host from a distance of >20 m. It is the release of carbon dioxide by the host, and the change in concentration of carbon dioxide in combination with other stimuli, which elicits behavioural responses. Mosquitoes are sensitive to very small changes in carbon dioxide levels. The receptors

on mosquito palpi show responses to changes as small as 0.01% (Kellogg 1970). There are many other components of host breath and odour which stimulate the antennal receptors of female mosquitoes when mixed with carbon dioxide. For instance, lactic acid is an activating and orientating stimulus for mosquitoes, but only if carbon dioxide is also present in the air stream (Smith et al. 1970; Price et al. 1979). Interaction or synergism of the components of the host odour in attracting a given species is a very complex process which developed in the course of evolution between the insect and the target organisms. The components of the host odour only stimulate the mosquito female when they occur in a distinctive mixture typical of the host. This enables it to distinguish between different hosts and to trace the plume as a series of packets, lamellae and filaments of odour mixed and dispersed by wind (Murlis 1986). The female mosquito flies upwind in a zigzag pattern which holds the mosquito within the plume and brings it closer to the odour source. In the final stages of orientation, mosquitoes especially those which bite during daytime or at twilight, use visual contact to locate the host. The compound eyes serve to discriminate between form, movement, light intensity, contrast and colour. Mosquitoes respond particularly to blue, black and red colours, whereas least attraction is caused by white and yellow (Lehane 1991). It is unlikely that the utilization of colour information is well developed in mosquitoes active at night, but they may be particularly sensitive to intensity contrast between the background and the target. When the mosquito is in close proximity of the host, it may also distinguish between three-dimensional targets and infrared radiation may also be involved in host location.

In the immediate vicinity of the host, odour is important once again as well as body heat. Mosquitoes can easily detect temperature differences of 0.2°C. Water vapour in short-range orientation-attraction may also play a role (Lehane 1991).

#### 2.6.4 Feeding

Mosquitoes have well-developed piercing/sucking mouthparts. Males feed on plant juices as a source of carbohydrates, the female mouthparts are developed to pierce the skin of the host to obtain blood for egg maturation (Magnarelli 1979; Clements 1992).

The mouthparts are extended into a proboscis which consists of the stylet bundle (fascicle) enclosed by the labium when the mosquito is not feeding. The stylet bundle is formed of six long, thin stylets: the labroepipharynx, hypopharynx, and two of both maxillae and mandibles. These stylets are held tightly together by the labium. The mandibles are sharply pointed and used to rupture the skin for passage of the other stylets. The maxillae have a pointed tip and recurved teeth at their distal ends. They are the main penetrative elements of the mouthparts, which are thrust alternately by using the teeth to anchor themselves in the tissues. Thus the labroepipharynx (food channel) and hypopharynx (salivary channel) are penetrated together into the tissue (Robinson 1939). The penetration of the stylets into the tissue is caused by an alternating rotary movement of the head. Four muscles are associated with each maxilla, to protract and retract the maxillary stylets (Clements 1992).

After the females land on the host, they may probe the skin a few times with their labellae while they are searching for a capillary for the intake of blood. Thickness and temperature of the skin are probably important probing stimuli for mosquitoes, since the surface temperature of the skin is related to the number of blood vessels in the skin (Davis and Sokolove 1975). Sensilla on the ventral side of the pair of labellae and on the distal part of the labium contain receptors for stimuli that may indicate a suitable site for piercing the skin.

Having successfully punctured the skin, the female starts ingesting blood. When feeding, only the stylet bundle penetrates the skin, while the labium becomes progressively bent backwards as the mosquito probes deeper into the tissue. Two pumps, the cibarial pump below the clypeus and the pharyngeal pump, are the sucking organs which pump the blood or plant juices into the gut.

It is important for the female mosquito that the blood remains in a liquid form to enable the mosquito to complete the blood-meal. To prevent coagulation, the mosquito injects saliva into the wound which usually contains anticoagulants similar to hirudin produced by blood-sucking leeches (Parker and Mant 1979). The introduction of saliva into the host tissue usually stimulates an immune response which can cause an inflammatory reaction of the host at the site of the wound. These wounds often cause irritation and the scratching by the host can cause bacterial infection. When the stylets reach a blood vessel, blood-associated components (*e.g.* ADP



**Fig. 2.12** *Anopheles* female ingesting blood (Photo courtesy of Roger Eritja, Barcelona, Spain)

and ATP) function as phagostimulants, and the mosquito starts to take-in blood through the food channel.

The female mosquito can ingest more than three times its mean body weight (Nayar and Sauerman 1975). Larger species such as *Oc. cantans* can ingest more than 6  $\mu$ l and smaller species such as *Ae. cinereus* only 3.7  $\mu$ l. The blood and especially its protein ingredients are essential for egg-production by the anautogenous females. Only a few autogenous species such as *Cx. p. pipiens* biotype *molestus* are able to produce their first egg-batch without a bloodmeal (Weitzel et al. 2009). When they originate from the eutrophic breeding sites (*e.g.* septic tanks) where the larvae can develop a prominent fat body due to the high level of nutrition, the fecundity is quite high, but still lower than after a blood-meal by the female. The fat body is obviously enough to complete ovarian development without a further blood-meal. The blood is used more for egg production and less as a source of energy. Both sexes of mosquitoes require plant juices as an energy source, mostly for flight. Plant sugars such as floral nectar, damaged fruits and honeydew are the main energy source during the adult life of both sexes (Briegel and Kaiser 1973).

Mosquitoes differ in their feeding and resting behaviour. Species which preferably feed indoors are called endophagic (endophagy), and those which feed mainly outdoors, are called exophagic (exophagy). The females which rest outdoors after feeding or during the day outdoors are called exophilic (exophily), and those which rest indoors are called endophilic (endophily). Ornithophily is expressed when females prefer to feed

on birds (ornithophilic species), zoophily when they feed on other animals (zoophilic species) and the term anthropophily is used when they prefer to feed on humans (anthropophilic species).

## 2.7 Survival During Dry Seasons and Hibernation

In tropical areas, the dry season survival mechanisms of mosquitoes, such as *Anopheles gambiae*, is one of the most vexing deficiencies in the understanding of the biology of major malaria vectors. Two survival strategies could be observed: continuous reproduction throughout the year and embryo dormancy in moist soil for at least several days (Minakawa et al. 2001).

Mosquitoes in temperate zones have developed efficient overwintering mechanisms in the egg, larval or adult stages. Some species such as *Oc. rusticus* and *Cs. morsitans* can overwinter in more than one stage, e.g. in the larval as well as the egg stage. Several factors, especially the latitude (cold) and hydrological conditions (droughts) determine the duration of hibernation and can differ within one species according to the latitude.

### 2.7.1 Egg Stage

Hibernation in the egg-stage is practiced by most of the *Aedes/Ochlerotatus* species in the temperate zone. Their diapause is induced in such a way that they do not hatch when unsuitable climatic and hydrological conditions prohibit successful development to the adult stage (Sect. 2.3). The occurrence of larvae of these species overwintering in the egg stage can vary greatly within the species of *Aedes* and *Ochlerotatus*. Within some species, the hatching time is closely related to snow-melt, others hatch in late spring or summer.

### 2.7.2 Larval Stage

Some mosquitoes are known to overwinter in the larval stage and can even survive for days in breeding

sites with a frozen surface. During the cold season, their metabolism is reduced and the larval development is delayed. For instance, larvae of *Oc. rusticus* and *Cs. morsitans*, which hatched in autumn, hibernate in the second and third larval instar. The high content of dissolved oxygen in cold water or bubbles of oxygen under the ice enable the larvae to cover their demand of oxygen for survival. However, during a severe winter, the mortality rate can be very high. Some anopheline species such as *Anopheles claviger* and *An. plumbeus* hibernate as larvae in pools or tree-holes, respectively. Usually, hibernation takes place in the third or fourth larval stage in water bodies that do not entirely freeze or only for a short time. This is also true for the hibernating larvae of *Or. pulcripalpis*. In contrast to the above-mentioned species, the larvae of *Coquillettidia richiardii*, which usually hibernate in the third or fourth-instar are not sensitive to long frost periods, because they live submerged in permanent water bodies.

Larvae of *Cx. p. pipiens* can frequently be found during winter. Whereas the females of anautogenous, ornithophilic, eurygamous *Cx. p. pipiens* are overwintering in diapause, its biotype *molestus* reproduces during the winter. Therefore, all developing stages of this biotype can be found in the breeding habitat (usually underground breeding sites) within the temperate zones during winter.

### 2.7.3 Adult Stage

Most mosquito species (*Culex*, *Culiseta*, *Uranotaenia* and *Anopheles*) overwinter as adult females. They seek hibernating shelters (locations free of frost such as caves, stables, cellars, canals and earth burrows) during autumn and leave these sites in spring when the temperatures increase. Usually, females of these species use the remaining larval fat body and feed intensively on plant juices during autumn to synthesize huge lipid reserves for diapausing. Females of some species within the *Anopheles Maculipennis* Complex can take occasional blood-meals during winter to withstand the long periods of starvation (Clements 1992).

## Chapter 3

# Medical Importance of Mosquitoes

Mosquitoes are responsible for the transmission of many medically important pathogens and parasites such as viruses, bacteria, protozoans, and nematodes, which cause serious diseases such as malaria, dengue, yellow and Chikungunya fever, encephalitis or filariasis (Kettle 1995; Beaty and Marquardt 1996; Lehane 1991; Eldridge and Edman 2000). Transmission can be mechanical (*e.g.* Myxoma virus causing myxomatosis in rabbits) or biological. The latter is more complex because it involves an obligatory period of replication and/or development of the pathogen or parasite in the vector insect. Due to their blood-sucking behaviour, mosquitoes are able to acquire the pathogens or parasites from one vertebrate host and pass them to another, if the mosquito's ecology and physiology is appropriate for transmission. Highly efficient vectors have to be closely associated with the hosts and their longevity has to be sufficient enough to enable the pathogens/parasites to proliferate and/or to develop to the infective stages in the vector. For successful transmission, multiple blood-meals are necessary.

In terms of morbidity and mortality caused by vector-borne diseases, mosquitoes are the most dangerous animals confronting mankind. They threaten more than three billion people, in the tropical and subtropical regions and have also substantially influenced the development of mankind, not only socio-economically but also politically. Undoubtedly, insect-transmitted pathogens leading to epidemics and pandemics have been instrumental in the development, decline, and fall of empires, for, *e.g.* Greece and Rome. Malaria was the dominant health problem in the latter days of the Roman Empire (Bruce-Chwatt and de Zulueta 1980). The Roman marshes were notorious for "malaria" (bad air).

### 3.1 Malaria

Human malaria caused by protozoans (*Plasmodium* spp.) continues to be the most important vector-borne disease. It affects more than 100 tropical countries, placing 3.3 billion people (more than 40% of the world population) at risk as of 2006. The disease is responsible for no less than 300 million infections and more than one million deaths annually, mainly among children below the age of five, with more than 90% of them living in tropical Africa (WHO 1997b,c; 2008). The enormous loss of lives and days of labour, the costs for treatment of patients, and the negative impact of the disease on development, makes malaria a major socio-economic burden. The annual costs of malaria in Africa alone were estimated to be almost two billion US\$ (WHO 1993, 2008).

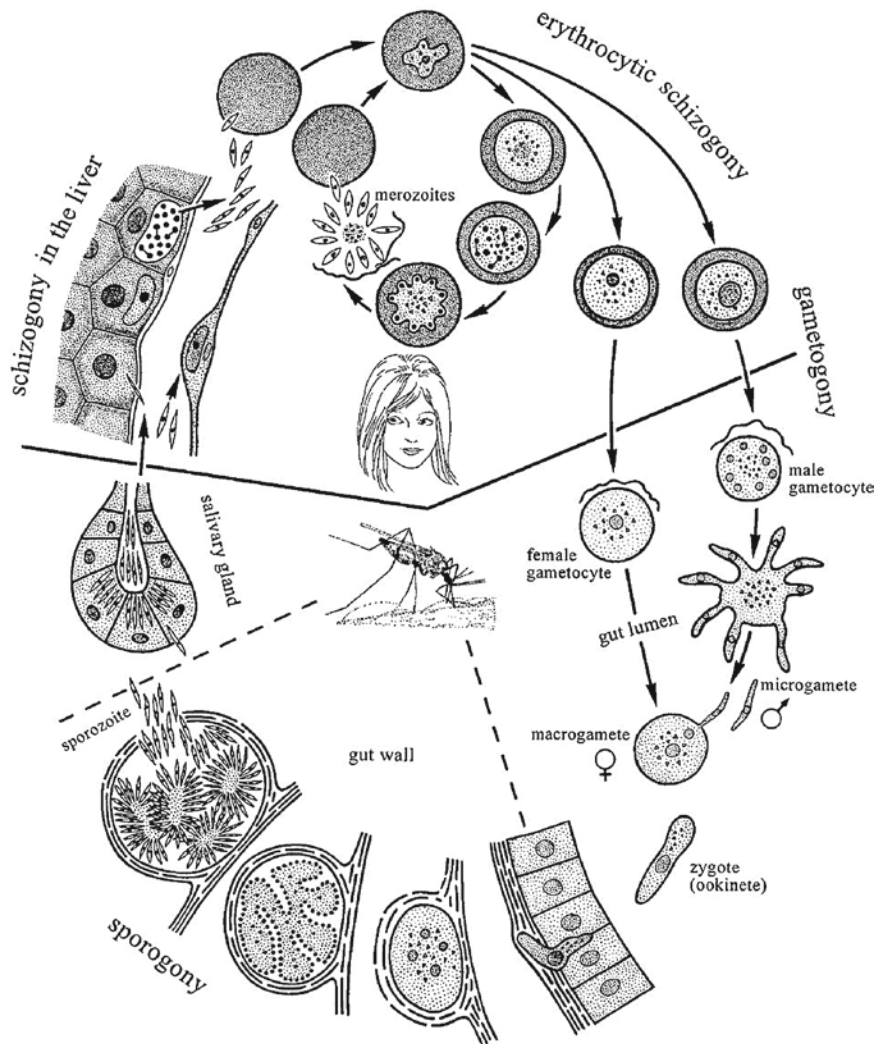
At least four species of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*) cause human malaria and are transmitted solely by the anopheline mosquitoes. There are more than 400 species of *Anopheles* world-wide and of these about 40 species are important vectors of human malaria. The most important vectors in Sub-Saharan Africa and the most efficient malaria vectors world-wide belong to the *Anopheles Gambiae* Complex. Of the seven species of this complex, *An. gambiae s.s.* and *An. arabiensis* (species A and B) are the most serious vectors of *P. falciparum* (the most serious malarial pathogen) in Africa. Due to their anthropophilic behaviour and physiological feasibilities, they possess a higher vector capacity than other closely related sibling species with no or low vector capacity. Apparently, pathogen/parasite and vector (mosquito) either adapted to each other during the course of evolution, resulting in coexistence, or the pathogen/parasite was repelled. This phenotypic

and genotypic plasticity exacerbates the difficulties in the identification of vector populations and the implementation of effective surveillance and control/management strategies. However, new tools such as the PCR-technique, enables scientists to use specific genetic markers to distinguish between sibling species and in the investigations of defined populations.

About 20 *Plasmodium* species occur in other primates, a similar number in other mammals, and about 40 each in birds and reptiles (Garnham 1980, 1988).

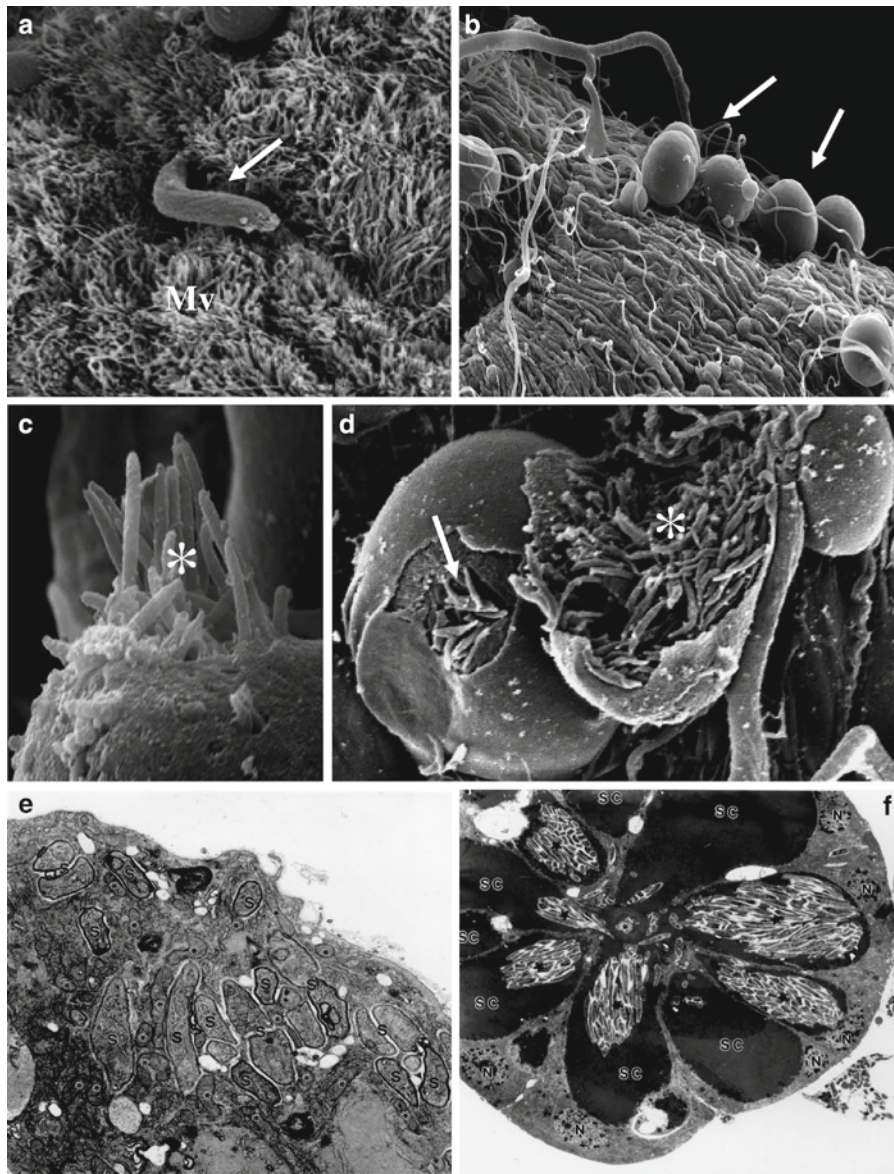
The *Plasmodium* species have a complex replication and transmission cycle with the sexual replication in mosquitoes and the asexual replication in vertebrates (Fig. 3.1). Shortly after the ingestion of blood from

infected vertebrates containing sexual forms of the parasite, the gametes fuse in the mosquito gut to form a zygote which elongates and develops into a motile ookinete (Fig. 3.2a). It penetrates to the outside of the midgut epithelium, settles there and forms an oocyst (Fig. 3.2b). Meiotic and subsequent mitotic divisions (sporogony) within the oocyst result in the formation of many haploid, spindle-shaped sporozoites which burst the wall of the oocyst and migrate through the haemocele and accumulate in the salivary glands (Fig. 3.2c–f). The infected mosquito is now able to inject the sporozoites with saliva into the next host. Once inside a vertebrate, the sporozoites infect the parenchyma of the liver where they immediately



**Fig. 3.1** Life cycle of malaria parasites in *Anopheles* and the human host





**Fig. 3.2** (a)–(d) (from a scanning electron microscope): (a) *Plasmodium* ookinete (arrow) attached to the mosquito midgut microvillar epithelium (Mv). The anterior part of the parasite is already invading between the microvilli. (b) View of an infected midgut (6 days after the infective bloodmeal). Several immature oocysts (arrows) are attached to the outer midgut wall. (c) Mature oocyst releasing the sporozoites to the haemocoel (10 days after infection). (d) Two broken oocysts in different phases (12 days of infection). One oocyst at the beginning of sporozoite release

(arrow) and the other opened showing tens of sporozoites ready to escape. (e, f) (from a transmission electron microscope): (e) Initial phase of salivary gland invasion by sporozoites (10 days after infection). The whole cytoplasm of the secretory cell is full of sporozoites(s). (f) Late phase of salivary gland invasion (15 days after infection). All sporozoites are inside the secretory cavities (sc) forming longitudinal arrangements (\*). Some parasites are in the salivary duct (N=nucleus). (Micrographs courtesy of Paulo Pimenta, Fundacao Oswaldo Cruz, Brazil)

undergo a cycle of exoerythrocytic schizogony, or develop to latent hypnozoites which sometimes later undergo schizogony to cause relapse. In each large schizont, several thousand merozoites are formed and

released into the circulating blood (end of the prepatent period), where they invade the erythrocytes to commence the erythrocytic schizogony. In the infected erythrocyte, the merozoite becomes a feeding trophozoite

and then, when a fully grown schizont, produces a small number of new merozoites (Garnham 1966). The merozoites burst the erythrocytes, are released and then invade other erythrocytes to repeat the schizogonic cycle. Each release of the merozoites from the erythrocytes causes an attack of malaria with fever and other clinical signs. The length of the schizogonic cycle determines the interval between the fever attacks.

*P. falciparum* causes malignant tertian, and *P. vivax* and *P. ovale* benign tertian malaria recurring at 48 h intervals (fever attack at the third day); and *P. malariae* quartan malaria recurring at 72 h intervals (Kettle 1995). After several cycles of schizogony, some trophozoites do not produce merozoites but become gametocytes which have to be ingested by anopheline mosquitoes to conclude the cycle of development.

In humans, malignant malaria caused by *P. falciparum*, is the most severe form resulting in life-threatening complications such as anemia and cerebral malaria. This is a frequent cause of mortality in children and can kill up to 25% of non-immune adults within 2 weeks. This form of malaria is called Malaria tropica and occurs mostly in tropical and subtropical areas, being limited by a summer isotherm of 20°C which is necessary to complete the sporogony of the parasite in the mosquito. In contrast, *P. vivax* can complete the sporogony and gametogony in mosquitoes in areas with a summer isotherm of 16°C (Wernsdorfer 1980).

In the long battle against malaria, resource shortages, absence of proper infrastructure, and lack of knowledge and training as well as resistance to chloroquine, other antimalarial drugs, environmental constraints and use of pesticides, have collectively impeded progress in malaria prevention and control especially in Africa for many years (WHO 2000, 2008; Etang et al. 2004; Mukabana et al. 2006). The global campaigns against malaria such as the Roll Back Malaria (RBM) programme led by major international organizations, aim to mitigate these long-standing shortcomings. Extending facilities for rapid case detection and treatment, prophylactics, personal protection based on the use of long-lasting insecticide-treated bednets (LLINs) against adult vector-mosquitoes, indoor residual spraying (IRS) and epidemic preparedness are cornerstones of these current strategies in malaria vector control in Africa and worldwide (Makundi et al. 2007; Mboera et al. 2007; Protopopoff et al. 2007a,b; RBM 2005). Control of vector-mosquito populations in their aquatic larval habitats offers an additional opportunity to sig-

nificantly enhance the protection afforded by existing vector management strategies and consequent malaria reduction (Killeen et al. 2000a,b; Fillinger and Lindsay 2006; Dongus et al. 2007; Walker and Lynch 2007; Worrall 2007).

In Europe, malaria threatened human life until the first half of the twentieth century. Although the impact of the disease was more severe in Southern Europe, it is well documented that even in Northern Europe, malaria was a well-known hazard of life (Marchant et al. 1998). It is known that for instance, Napoleon lost large numbers of soldiers due to malaria when he invaded the Upper Rhine Valley in Germany. The two main *Plasmodium* species found in Europe were *P. vivax* and *P. falciparum*. While the former occurred throughout the continent, the latter was restricted to Southern Europe (Jetten and Takken 1994). In Northern Europe, the parasite must have been *P. vivax* because of its adaptation to the moderate climate. Furthermore, it is likely that the parasite could survive as hypnozoites in the human liver during phases too cold for mosquito transmission (Marchant et al. 1998). Nowadays, *P. vivax* seldom causes a lethal disease, which suggests that *P. vivax* has evolved into a reduced virulence over the last century (Kettle 1995).

Before World War II, endemic malaria was spread throughout Europe (Bruce-Chwatt and de Zulueta 1980). The most endemic malaria areas were found in the south, where a continuous transmission occurred from spring to autumn. Greece was considered to be the country with the highest incidence of malaria. In the early 1930s, the number of people in Greece infected with malaria annually, ranged from one to two million. Other severe epidemics were reported from the Dalmatian coast in Croatia, in coastal areas in Southern Spain, in the south of Italy and the island of Sardinia. In Central Europe, the malaria incidence was much lower than in the south (Jetten and Takken 1994). In Northern and Western Europe, the transmission of the disease was discontinuous with annual maxima. Malaria epidemics were mainly restricted to coastal areas in Southern Sweden, Southern Finland, Denmark, the Netherlands, Belgium, Germany and northern France. In the eastern parts of Europe, malaria was mainly recorded from Southern Ukraine and along the lower Volga River. After World War II, malaria slowly disappeared from the continent. This was mainly due to the reduction in natural breeding habitats through improved agricultural techniques, improved socio-economic conditions and

better sanitation practices. An important role was also played by the malaria eradication campaigns with the application of residual insecticides and the availability of new drugs. The last reported focus of indigenous malaria in continental Europe disappeared in Greek Macedonia in 1975 (Bruce-Chwatt et al. 1975).

There are indications that malaria was mainly transmitted in Europe by the species of the *Anopheles Maculipennis* Complex, which are widely distributed in the Palearctic region. However, the distribution of *Anopheles maculipennis s.l.*, was not directly related to the distribution of malaria (Jetten and Takken 1994). What was the reason for the “Anophelism without malaria”? Following intensive research, it was established that the former described species, *An. maculipennis*, is not a single species but a species complex consisting of more than a dozen separate species, of which eight occur in Europe (White 1978). The knowledge of species complexes containing species that are morphologically very similar but differ greatly in their vector competence, has generated interest in the control of malaria by genetic manipulation (Crampton et al. 1990; Crampton 1992; Crampton and Eggleston 1992; Kidwell and Ribeiro 1992; Carlson 1995; Rai 1995).

In addition to the species of the *Anopheles Maculipennis* Complex, some other European anophelines are known to be potential malaria vectors, such as *An. claviger*; *An. sergentii*, *An. cinereus hispaniola*, *An. algeriensis*, *An. superpictus* and *An. plumbeus*. The latter is increasingly becoming interesting to malariologists because in recent decades, it has proliferated in huge numbers as a result of its adaptation from natural (tree-holes) to artificial breeding sites (underground water catch basins and septic tanks contaminated with organic waste). In recent studies it has been demonstrated that in contrast to *An. atroparvus* which is more or less refractory to *P. falciparum*, *An. plumbeus*, is able to develop oocysts of *P. falciparum* when fed with blood containing gametocytes (Marchant et al. 1998). Although *An. plumbeus* lives in close proximity to man, the risk of malaria epidemics is very low due to good malaria notification and the absence of cases of indigenous transmission. However, the effect of global warming which favours the completion of the sporogonic cycle of *Plasmodium* in anophelines, and the hundreds of malaria cases mostly caused by *P. falciparum* acquired in the tropics and imported to Europe, could increase the risk of some indigenous malaria transmission, even though malaria epidemics can be excluded in most

parts of Europe. An increase in temperature accelerates not only the development of mosquitoes in their breeding sites, but also other phases of their life cycle, such as the frequency of blood meals, the duration of the gonotrophic cycle (time from a blood meal to the final development of eggs) and their longevity. A reduction in the duration of the gonotrophic cycle increases the frequency of blood meals and, therefore, also the probability of transmitting disease agents such as *Plasmodium* spp. (Dhiman et al. 2008; Snow 1999; Becker 2008).

Increase in international travel has added to the complexity of this problem, not only infected people import the parasite, infected anophelines can also be transported by aircraft from one continent to another. These insects can become a threat for people working in or living close to international airports. Since 1963, more than 60 cases of “airport malaria” have been reported in Europe. In 1994, in the vicinity of the Charles de Gaulle airport in Paris alone, seven people were infected by *P. falciparum* although they had never visited the tropics. Similar cases have been reported from Gatwick Airport in London and Frankfurt International Airport in Germany.

In North America malaria was widespread, except in central areas up until the early twentieth century, with an estimated 600,000 cases occurring in 1914. The principal vectors were *Anopheles freeborni* (mainly in the Western USA) and *An. quadrimaculatus* (mainly in eastern, central and southern areas). Both species are still widespread. *P. vivax* was the main pathogen. Organised malaria control programmes were established in 1914 by the US Public Health Service, and then continued to be supported and organised by a succession of other regional or national organizations including the Tennessee Valley Authority, the National Malaria Eradication Programme and Centers for Disease Control and Prevention (CDC). Following intensive house-spraying with DDT in the post World War II years, indigenous malaria was eventually considered eradicated in the USA in 1951.

Since the 1970s, there has been an overall upward trend in the number of malaria cases in the USA, with ~1,000–1,500 cases per year being reported in recent years. The majority are imported, *i.e.* the victim acquired the disease whilst overseas. However, in addition, there have been 63 cases of locally transmitted malaria reported between 1957 and 2003, probably arising from infection of indigenous mosquitoes by imported malaria cases.

In Central and South America, malaria has been a major problem despite recent advances, and still remains a significant threat. Across the region, a number of anopheline species are involved in transmission. *An. albimanus* is very widespread, occurring from the southern states of the USA in the north, to Northern Peru in the south, including the Caribbean region. It is a tropical lowland species, occurring most commonly on coastal plains and along waterways. *An. darlingi* is also an important vector, found most commonly in forested areas, and occurring from Mexico to Argentina, and from the Atlantic to the Pacific coasts. Other species of importance include *An. aquasalis*, *An. albitarsis*, and *An. pseudopunctipennis*.

*P. vivax* is the most common parasite in the Americas, constituting around 75% of cases. Most of the rest is caused by *P. falciparum*, with *P. malariae* also occurring in limited areas. Malaria control has faced a variety of challenges including insecticide resistance, budgetary issues, the emergence of urban malaria, and human migration. Nonetheless, there has been a downward trend in malaria cases from 1.1 million in 2,000, to ~770,000 in 2007 (PAHO).

In Australia, malaria formerly occurred sporadically in the northern areas. A sudden outbreak of malaria caused by *P. falciparum* at Fitzroy Crossing (Western Australia) in 1934 resulted in 165 deaths, but by 1981 the disease was declared eradicated. The vectors originally responsible for transmission are not known with certainty, but *An. farauti* was likely to have been an important vector, with other species such as *An. amictus*, *An. bancroftii* and *An. hilli* also playing a role. The exact detail of the species responsible is complicated by several of these species existing as complexes. Currently, about 700–800 malaria cases are imported annually, and a very few cases of locally transmitted malaria also occur.

Sub-Saharan Africa remains the most complex and challenging malarious region. Political instability, military activity, uprooted human populations, poverty and urbanization, collectively complicate the situation. In some former malaria-free upland areas in East Africa, cases are now being reported, which has been suggested as indicating a climate-change effect.

Across the African continent, 93% of the population lives in areas with endemic malaria or in areas at risk of epidemics. There are about 270 million clinical cases annually, with 95% of the global malaria deaths in this

continent. The main vectors are *An. gambiae s.s.*, *An. arabiensis* and *Anopheles funestus*, with *Anopheles pharoensis* also important in some areas. The Anopheles Gambiae Complex contains some of the most efficient malaria vectors (anthropophilic and endophilic) and occur in both rural and urban areas.

*P. falciparum* remains the most important parasite, with *P. malariae* and *P. ovale* also occurring. *P. vivax* is found in East and Southern Africa, but appears to be absent from West Africa.

Asia spans a very wide range of regions, with great diversity of climate, habitat, and socio-economic status. Vector importance and parasite prevalence is similarly varied. Important vectors in Western and Central Asia include *An. sacharovi*, *An. stephensi*, *An. culicifacies*, *An. fluviatilis*, and *An. superpictus*. In the tropical Far East, a wide range of important species occur, including *An. dirus*, *An. sundaicus*, *An. minimus*, *An. farauti*, and *An. sinensis*. This range occupies a broad range of habitats, from streams, rice fields, polluted urban water bodies, to rain forests.

*P. falciparum*, *P. malariae* and *P. vivax*, all occur in this continent, with the proportion of *P. falciparum* increasing in South East Asia (WHO 2000). The total number of malaria cases in the South-East Asian region is ~2.5 million annually, with the majority occurring in India, with ~4% of the world malaria mortality occurring in this region. The trend since 1996 has been a gradual decrease, although this may not include fluctuations on a local scale. As in other parts of the world, military conflicts and human migration exacerbate malaria problems.

### 3.2 Arboviruses

Arboviruses (arthropod-borne-viruses) are defined as viruses that replicate in arthropods and are transmitted by arthropods to vertebrates. The arthropod becomes infected by feeding on blood from an infected vertebrate during viraemia (virus circulation in the peripheral blood vessels), and after proliferation in the vector, the virus can be transmitted to another vertebrate-host (horizontal transmission). Arboviruses can also be passed from one arthropod generation to another by transovarial transmission (vertical transmission). Thus, some of these viruses are known to be capable of

overwintering in the egg stage of the vector (e.g. a few *Aedini* species).

More than 300 arboviruses are listed by Francki et al. (1991) and more than 500 by Karabatsos (1985). Approximately 100 viruses infect humans and 40 infect livestock (Monath 1988). The most important viruses transmitted by mosquitoes to humans or other vertebrates are found in three families: the *Togaviridae* with the genus *Alphavirus* (e.g. Chikungunya, Sindbis, Equine Encephalitis and Ross River viruses), *Flaviviridae* with the genus *Flavivirus* (e.g. Yellow fever virus, Dengue 1–4 viruses, West Nile virus, Japanese and St. Louis encephalitis viruses) and the *Bunyaviridae* with the genera *Bunyavirus* (e.g. the California group), and *Phlebovirus* (Rift Valley virus) (Murphy et al. 1995; Eldridge and Edman 2000). Human arboviral diseases are classified by the major clinical symptoms they cause such as encephalitis, febrile illness accompanied by rash and arthritis as well as haemorrhagic fever. Infections can cause a wide range of mild or severe symptoms with significant morbidity and mortality, especially in tropical countries.

### 3.2.1 *Togaviridae* (*Alphavirus*)

Within this family, only the members of the genus *Alphavirus* are arthropod-borne. They replicate in the cytoplasm of host cells. The genome is a single strand of positive sense RNA with 11–12 kb. After replication, the nucleocapsids are released from the host cell forming an envelope when they push through the host cell membrane (Eldridge and Edman 2000). The viruses of a size of 70 nm are most enzootic and infect mostly small mammals and birds. Humans are usually dead-end hosts (exceptions Chikungunya and Ross River viruses) because they do not circulate enough viruses to infect vector mosquitoes. Transovarial transmission from one mosquito generation to the next can be possible.

#### 3.2.1.1 Chikungunya Virus

Chikungunya viral infections in humans result after an incubation time of 2–4 days with a sudden onset of illness, fever, chills, headache, photophobia, arthralgia and arthritis affecting multiple joints (“Chikungunya”

comes from Swaheli, meaning “that which bends up”, referring to the severe joint pain). The fever usually subsides after 2–3 days. Other symptoms like joint pain can last for about a week. However, some severe cases can require months of recovery, especially when older people are infected. Although unusual, people above the age of 60 can die due to Chikungunya viral infection induced complications.

Chikungunya virus is distributed in Africa and Asia and in 2007, a few cases occurred in Europe (Italy) for the first time. During epidemics, the African tiger mosquito *Aedes aegypti* [*Stegomyia aegypti*] and/or the Asian tiger mosquito *Aedes albopictus* [*Stegomyia albopicta*] are the main vectors transmitting the disease from human to human. In Africa, nonhuman primates (monkeys) are thought to serve as reservoir hosts from which the virus can spread to humans. Mosquito species other than *Aedes* (*Stegomyia*) spp. can be involved in the enzootic transmission.

Chikungunya virus epidemics have recently drawn a great amount of attention after there were sudden outbreaks in 2005 in Réunion (a French island), when more than 300,000 people were infected, and again in 2006/2007 in India, when more than two million people were infected with the Chikungunya virus. In July–October 2007, the first epidemic of this tropical disease broke out in Italy. One person travelling from Kerala, India, to Italy developed symptoms of Chikungunya fever at the end of June. Subsequently, there were approximately 280 cases of Chikungunya fever in the Ravenna Province. The disease symptoms include high fever, limb/muscle and joint pain. Most of the time, the disease is relatively harmless, and haemorrhaging seldom occurs. However, older people are more prone to dying from this viral infection. In the Ravenna region, an 83-year old man died of this disease. As a result, it is now required to report cases when haemorrhagy occurs. In addition, the laboratories have to report these cases according to the European “Prevention of Infection Regulations” (ECDC 2008).

Chikungunya virus was detected in *Ae. albopictus* females, indicating that the newly introduced exotic pathogens which led to this epidemic, were transmitted by the Asian tiger mosquito which was introduced in Italy in 1990. The degree to which indigenous mosquitoes (other than the newcomer, Asian tiger mosquito) can act as vectors for the Chikungunya virus, and whether the viruses can be passed on by

transovarial transmission during egg development, is now being examined.

### 3.2.1.2 Ross River Virus

Ross River virus is endemic in Australia, Papua New Guinea and neighbouring islands. Although not fatal, infections are debilitating, causing fever, rash and polyarthritis. Joint pain can last for weeks or even years thus reducing productivity of people and harming the economy (Mckenzie and Smith 1996). The virus was first isolated from *Ae. vigilax*, but a wide range of mosquito species including *Ae. aegypti* [*St. aegypti*], *Ae. polynesiensis* [*St. polynesiensis*], and *Ma. uniformis* show a high degree of vector competence (Kay and Aaskov 1988). Thus a further spread of the virus is not unlikely.

The virus was first isolated from passerine birds, but mammals like kangaroos and wallabies are considered as major reservoir hosts for enzootic transmission. *Stegomyia* species may transmit the virus from human to human (Eldridge and Edman 2000).

### 3.2.1.3 Eastern Equine Encephalomyelitis (EEE) Virus

EEE virus is present in North, Central and South America and the Caribbean. EEE was first recognised in Massachusetts, USA in 1831, when 75 horses died of encephalitic illness. Epizootics in horses have continued to occur regularly in the United States. The virus was first isolated from infected horse brains in 1933 (Giltner and Shahan 1933). In 1938, the first confirmed human cases were identified when 30 children died of encephalitis in northeastern USA. These cases coincided with outbreaks in horses in the same regions. The fatality rate in humans was 35% and there is currently no cure for human infections (Scott and Weaver 1989).

EEE virus is capable of infecting a wide range of vertebrates including mammals, birds, reptiles and amphibians. The virus is maintained in nature through a bird-mosquito cycle. The ornithophilic mosquitoes, *Culiseta melanura* and *Cs. morsitans* are primarily involved as vectors. Several species, *Cq. perturbans*, *Ae. vexans*, *Oc. sollicitans* and *Oc. canadensis* are bridge vectors which transmit the viruses from avian

to mammalian populations including humans (Morris 1988).

In horses, symptoms occur 1–3 weeks after infection and begin with high fever which usually lasts for 1–2 days. During the fever period, nervous signs such as sensitivity to sound and restlessness and periods of excitement and restlessness appear. Brain lesions appear causing drowsiness, drooping ears, circling and abnormal gait. The horse usually suffers complete paralysis and death 2–4 days after symptoms appear. Mortality rates among horses with the eastern strain range from 70 to 90% and 50–90% among infected humans.

### 3.2.1.4 Western Equine Encephalomyelitis (WEE) Virus

WEE occurs in North, Central and South America, but most cases have been reported from the plains of the western and central United States. In the complex life cycle, mainly birds, small mammals are also involved. Important vectors are *Cx. tarsalis* (western half of North America), but *Oc. dorsalis* or *Oc. albifasciatus* (Argentina) may also be involved. In 1930 and 1958, the largest outbreaks occurred in California when 6,000 horses and almost 1,000 human cases were involved (Reeves 1990). Clinical cases of horses are often fatal, but compared to EEE virus infections the mortality rate is low (3–15%). The WEE virus is comprised of six serotypes which vary in their virulence. In humans, infections can cause a range of illness from no symptoms to headache and fever, to aseptic meningitis to encephalitis. People with more severe disease can have sudden high fever, headache, drowsiness, irritability, nausea, and vomiting, followed by confusion, weakness, and coma. Infants often suffer seizures. Symptoms usually appear in 5–10 days after the bite of an infected mosquito.

### 3.2.1.5 Venezuelan Equine Encephalomyelitis (VEE) Virus

VEE affects equine species such as horses, donkeys and zebras, which are also the amplifying vertebrate host for epizootic virus transmission. However, small rodents and in some locations birds may be involved in the transmission cycles. After infection, equines may suddenly die or show progressive central nervous

system disorders. Healthy human adults who become infected by the virus may experience influenza-like symptoms. However, people with weakened immune systems, children or older people in rare cases, become severely ill or die. The incubation period from the inoculation of the virus until the febrile response generally is 0.5–5 days.

In 1936, VEE was first recognised as a disease of concern in Venezuela following a major outbreak of equine encephalomyelitis. From 1936 to 1968, equines in several South American countries, suffered devastating outbreaks. In the following years, the disease moved north throughout Central America reaching Mexico and Texas in 1971. Outbreaks are usually related to an increase in mosquito populations. Several mosquito species are involved in the transmission cycle including the species of *Aedes*, *Ochlerotatus* (e.g. *Oc. taeniorhynchus*), *Anopheles*, *Culex*, *Deinocerites*, *Mansonia*, and *Psorophora*.

### 3.2.1.6 O’Nyong-Nyong (ONN) Virus

ONN virus occurs only in Africa and is closely related to the Chikungunya virus. The name is derived from a Ugandan dialect and refers to joints being weak. The symptoms are similar to those of Chikungunya infections. *An. funestus* and *An. gambiae s.s.* are the most important vectors. ONN virus infection in vertebrates other than humans has not been reported (Eldridge and Edman 2000).

### 3.2.1.7 The Sindbis Virus Complex

In 1952, the Sindbis virus was first isolated from *Culex* mosquitoes in the Egyptian village called Sindbis, near Cairo. It is known from Europe, Africa, Asia and Australia. The first human case occurred in Uganda 1961.

Four viruses of the Sindbis complex (Sindbis/subtypes Ockelbo, Whataroa, Babanki, and Kyzylgach viruses) are widely spread in the Old World (Strauss and Strauss 1994). In terms of the nucleotide sequences, European strains are more or less identical with the strains in Africa and it can be assumed that they were transported by birds migrating between the two continents (Shirako et al. 1991; Norder et al. 1996).

Antibodies of Sindbis virus have been detected in birds of several orders, e.g. Passeriformes, Galliformes, Anseriformes and Ciconiiformes and also in domestic and wild mammals, e.g. in bovids, in several European countries (Lundström 1999). Additionally, Sindbis viruses were isolated from hamsters and frogs as well as from sentinel chickens and rabbits (Gresikova et al. 1973; Kozuch et al. 1978; Aspöck 1996).

In humans, Sindbis virus infections have resulted in hundreds of clinical cases in Northern Europe, especially in Sweden, Finland, Russia and Norway (Lundström et al. 1991). The disease results in a rash, as well as pain in muscles and joints with joint swelling and fever (Espmark and Niklasson 1984).

In Europe, Sindbis virus was isolated for the first time in Sweden in 1984 from *Culiseta* spp. (Niklasson et al. 1984) and later from *Cx. p. pipiens* and/or *Cx. torrentium*, *Cs. morsitans* and *Ae. cinereus* (Francy et al. 1989) as well as from mosquitoes in Russia, Norway and Germany (Lvov et al. 1984; Norder et al. 1996; Jöst et al. 2010). The virus circulates in bird populations during the summer and is transmitted by the ornithophilic mosquitoes *Cx. p. pipiens* and/or *Cx. torrentium* (Lundström 1994). In contrast to these *Culex* species, *Aedes/Ochlerotatus* species such as *Ae. cinereus*, are less specific concerning host choice. They bite birds as well as humans and are thus able to transmit the virus infection into the human population (Aspöck 1996; Lundström 1999). In Europe, outbreaks of Sindbis diseases occur usually in July and August and disappear at the end of autumn. The initiation of Sindbis virus transmission in summer is not fully understood, the virus may either overwinter in the mosquito vectors or in the bird hosts. Reinfections can be caused each year by infected birds migrating from Africa.

### 3.2.1.8 Viruses of the Semliki Forest Complex

Semliki Forest virus was first isolated from Uganda in 1944. Nine viruses of this complex occur in the Old World (Strauss and Strauss 1994). In Western Europe, the only record of human disease possibly caused by a virus of the Semliki Forest complex is from Albania (Eltari et al. 1987; Lundström 1999). The viruses could possibly occur in humans and other mammals (e.g. bovids and ovids) at low frequencies. Clinical cases have not been reported from Western Europe. Although antibodies have been detected in a number of birds

(e.g. *Ardea cinerea*, *Acrocephalus* spp., *Remiz pendulinus*) and viruses have been isolated from *Ochlerotatus euedes* in central Russia (Mitchell et al. 1993), these viruses may be considered as minor epidemiological importance in Europe.

### 3.2.2 Flaviviridae (Flavivirus)

The *Flaviviridae* gets its name from the Yellow fever virus (*flavus* in Latin means “yellow”). The *Flaviviridae* contain some of the most dangerous arboviruses in the world such as Yellow fever virus, Dengue virus, West Nile virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus and Rocio virus. All belong to the genus *Flavivirus* and are transmitted by mosquitoes.

The enveloped spherical *Flaviviridae* with a diameter of 40–60 nm contain a single-stranded positive sense RNA.

#### 3.2.2.1 Yellow Fever Virus

Yellow fever is historically the most important and dangerous mosquito-borne disease and still an important cause of haemorrhagic illness in many African and South American countries despite the existence of an effective vaccine. The attribute “yellow” refers to the jaundice symptoms that affect some patients. Devastating epidemics of yellow fever broke out in the 1700s in England, France and Spain. In the nineteenth century, more than 300,000 people are believed to have died in Spain. Until the early part of the twentieth century, in regions of North and Central America, Caribbean and Europe, the spread of *Ae. aegypti* [*St. aegypti*] caused severe yellow fever epidemics. In 1900, the research of Carlos Finlay, Walter Reed and colleagues proved the role of *Ae. aegypti* in the transmission cycle of Yellow fever virus which initiated preventive efforts against the vector and spread of the disease. In 1927, the Yellow fever virus was isolated by Adrian Stokes in West-Africa (Sosa 1989; Staples and Monath 2008). However, despite all efforts, the disease still remains as a threat to humans in Africa and Central/South America. As of 2001, the World Health Organization (WHO) estimates that yellow fever causes 200,000 illnesses and 30,000 deaths every year in unvaccinated populations. Yellow fever is absent in

Asia, most probably due to the immunologic cross-protection by the exposure of humans to other related viruses or reduced vector efficiency (Eldridge and Edman 2000).

After the bite of an infected mosquito, the virus first replicates locally and then spreads to the rest of the body via the lymphatic system and establishes itself throughout organ systems, including the heart, kidneys, adrenal glands and the parenchyma of the liver; high viral loads are also present in the blood. The incubation period in humans ranges from 3 to 5 days until the victims develop malaise, nausea, fever, chills, vomiting, constipation, rapid heartbeat, back pain, and prostration. Due to liver malfunction, jaundice usually appears on day 2 or 3 after the first signs of the disease. Patients develop haemorrhagic symptoms, liver and renal failure and lapse into delirium and coma, frequently followed by death. The fatality rate is higher than 50% in patients developing jaundice (Eldridge and Edman 2000).

Numerous vertebrates, especially primates are susceptible to the virus which enables the virus to persist in enzootic cycles. Two distinct transmission cycles can be distinguished:

- (a) The jungle or sylvan cycle in tropical America and Africa. Mostly monkeys are the principle vertebrate hosts infected mainly by *Haemagogus* mosquitoes in the forest canopy in tropical America or by *Ae. africanus* in Africa. In gallery forests or savannahs in Africa, vectors of the yellow fever can be *Ae. (Stegomyia) bromeliae* (East and Central Africa) or *Ae. vittatus* in West Africa. Humans entering the forests for searching for food or harvesting wood are infected by mosquito bites and carry the virus to human settlements.
- (b) The urban cycle which begins when infected humans returning to their villages or cities where *Ae. aegypti* carries the virus from human to human and eventually result in epidemics.

Mass vaccination and vector control programmes in human settlements can reduce the burden of yellow fever (Norrby 2007).

#### 3.2.2.2 Dengue Virus

Dengue is a mosquito-borne disease caused by four serotypes of the Dengue virus (DEN-1, DEN-2, DEN-3



and DEN-4). The illness usually begins 5–7 days after the infective bite (intrinsic incubation period). The clinical features of dengue include fever with rash, severe headache, pain behind the eyes, muscle and joint pains (myalgias and arthralgias – severe pain gives it the name “*break-bone fever*”). The dengue rash usually appears first on the lower limbs and the chest; in some patients, it spreads over most of the body. There may also be gastritis with a combination of associated abdominal pain, nausea, vomiting, or diarrhoea.

Recovery from infection by one serotype provides lifelong immunity against that virus but confers only partial and transient protection against subsequent infection by the other three serotypes. There is good evidence that sequential infection with different serotypes increases the risk of developing dengue haemorrhagic fever (DHF). The infection with one serotype initiates the production of different antibodies neutralizing the viruses of this serotype and antibodies which are not able to neutralize viruses of another serotype acquired by a second infective bite. The virus of that serotype can proliferate in the epithelial cells of blood vessels and make them permeable for blood serum which enters the body tissue and cause haemorrhagic manifestations. The blood pressure of the patient sinks dramatically.

DHF is a potentially deadly complication that is characterized by high fever and haemorrhagic manifestations, often with enlargement of the liver and in severe cases, circulatory failure. The illness begins with a sudden rise in temperature accompanied by facial flush, other flu-like symptoms and sometimes convulsions. The high fever usually continues for 2–7 days. After a few days of high fever the patient’s condition can suddenly deteriorate, the temperature drops, followed by the circulatory failure and the patient can rapidly go into a critical state of shock and die within 12–24 h, or may quickly recover following appropriate medical treatment.

The first reported epidemics of dengue fever occurred in 1779–1780 in Asia, Africa and North America. It indicates that these viruses and their mosquito vectors have had a worldwide distribution in the tropics for more than 200 years. During most of this time, dengue fever was a mild, nonfatal disease until the 1950s when DHF cases first occurred during dengue epidemics in the Philippines and Thailand. Today DHF has become a leading cause of hospitalization and death among children in regions with dengue epidemics.

Dengue and DHF have become a major international public health concern and is found in tropical

and subtropical regions around the world, predominantly in urban and semiurban areas. The global incidence of dengue and DHF has grown dramatically in recent decades especially with the dramatic increase in international travel. Some 2.5 billion people (40%) of the world’s population – in more than 100 countries in Africa, Asia, the Western Pacific region, the Caribbean, as well as Central and South America and the Eastern Mediterranean, live under the risk of Dengue virus infection (Halstead 1980, 1982, 1992; Becker et al. 1991; Gratz 1999; WHO 2008). In 2007 alone, there were more than 890,000 reported cases of dengue in the Americas, of which 26,000 cases were DHF. WHO currently estimates that annually there may be 50 million dengue infections worldwide. An estimated 500,000 people with DHF require hospitalization each year, a high proportion being children. The case fatality rate is about 2.5%.

In Europe, one of the most recent outbreaks of dengue occurred in Athens during 1927 and 1928, when the dengue viruses caused a devastating epidemic of fever and polyarthritis. Approximately one million people (80% of the inhabitants at that time) were infected with more than 1,500 fatalities (Papaevangelou and Halstead 1977). Dengue cases also occurred in Spain, Italy, Austria (Vienna had a large epidemic in 1941) and the former Yugoslavia. In Europe, especially in countries bordering the Mediterranean Sea, people may still suffer from this disease.

*Ae. aegypti* (primary vector in urban areas) and *Ae. albopictus* (secondary vector in suburban/rural areas) are the vectors of dengue. The mosquitoes acquire the dengue virus while feeding on an infected person. After virus incubation for 8–10 days (extrinsic incubation period), the infected mosquito is capable of transmitting the virus during a blood meal for the rest of its life. Infected female mosquitoes may also transmit the virus to their offspring by transovarial transmission, but the role of this in sustaining transmission of the virus to humans has not yet been defined.

Infected humans are the main amplifiers of the virus, serving as a source of the virus for uninfected mosquitoes. The virus circulates in the blood of the infected human during the fever period for 2–7 days. *Aedes* (*Stegomyia*) females acquire the virus when they feed on an individual during this period.

The spread of dengue is not only attributed to the expanding geographic distribution of the four Dengue viruses, but also to their mosquito vectors. *Ae. aegypti* populations increase especially in areas that are

favourable for mosquito breeding, *e.g.* where household water storage in containers is common and where solid waste disposal services are inadequate. Increased global travel by airplane provides the ideal mechanisms for infected humans transporting Dengue viruses between regions, countries or even continents, resulting in a frequent exchange of Dengue viruses and other pathogens. The exposure to various serotypes of the Dengue virus is usually followed by DHF outbreaks.

There is no specific treatment for dengue (no vaccine is available currently), but appropriate medical care frequently saves the lives of patients with the more serious dengue haemorrhagic fever. The best preventive measures include intensive public education emphasizing the elimination/reduction of the breeding sites of *Ae. aegypti* improved water supply systems (*e.g.* potable water supply), overhead tanks/cisterns should be mosquito-proofed, containers should be covered with tight-fitting lids or screens to avoid egg laying by mosquitoes. The use of larvicides or predators such as copepods can also reduce the vector population.

### 3.2.2.3 West Nile Virus

West Nile virus was first isolated from a human in the West Nile district of Uganda in 1937 and later from many other vertebrate hosts including horses, dogs, rodents and bats (Eldridge and Edman 2000). West Nile virus is widely distributed and the virus has been described in Africa, Europe, the Middle East, West and Central Asia, Oceania, and most recently, North America. The ecology was characterized in Egypt in the 1950s. Work et al. (1953), isolated WNV from Hooded Crows and Rock Pigeons in the Nile Delta. Later, in 1955, Work et al. postulated that the indigenous wild birds are potential reservoirs of WNV in the Nile Delta. Outbreaks of WNV encephalitis in humans have occurred in Algeria in 1994, Romania in 1996–1997, the Czech Republic in 1997, the Democratic Republic of the Congo in 1998, Russia in 1999, the United States from 1999 and Israel in 2000. Epizootics of disease in horses occurred in Morocco in 1996, Italy in 1998, in the United States from 1999 until the present, and France in 2000 and in birds in Israel in 1997–2001 (Zgomba and Petric 2008).

The virus became recognised as a cause of severe human meningitis or encephalitis (inflammation of the spinal cord and brain) in elderly patients during

an outbreak in Israel in 1957. Equine disease was first noted in Egypt and France in the early 1960s (Kunz 1969; Aspöck 1979; Filipe 1990; Vesenjak et al. 1991).

West Nile virus circulates usually in birds with *Cx. p. pipiens* being the most likely enzootic vector (Mouchet et al. 1970). In Europe, virus isolates are known from *Cx. p. pipiens* in the city of Bucharest, *Culex modestus* in southern France (Hannoun et al. 1964), from *An. maculipennis s.l.* in Portugal (Filipe 1972) and from *Oc. cantans* in former Czechoslovakia (Labuda et al. 1974). Obviously virus amplification can occur in a wide range of birds (*e.g.* Passeriformes, chickens, ducks, geese and pigeons). There is strong evidence that the West Nile virus may have been sporadically introduced from Africa to Europe by migrating birds. The virus is transmitted in a bird–mosquito cycle in endemic areas in Europe.

Humans and other mammals are infected when suitable mosquito bridge vectors (mosquitoes biting birds and humans like *Cx. p. pipiens* biotype *molestus* and *Culex p. quinquefasciatus*) are abundant. However, the possibility of transovarial transmission, with the virus surviving the winter in hibernating *Culex* females in Europe cannot be excluded, as transovarial transmission has been demonstrated in the laboratory by Baqar et al. (1993).

Following infection, it takes 3–6 days until the symptoms of the disease occur, *e.g.* malaise with fever, headache and muscle pain, sore throat, rash and swollen lymph nodes (Lundström 1999). The disease usually lasts for about 1 week. In most endemic areas it is a childhood disease, as adults have acquired immunity (Manson-Bahr and Bell 1987; Tesh 1990). However, severe cases of meningoencephalitis, myocarditis, acute pancreatitis and hepatitis are reported, which can be fatal. In 1935, 1942 and the early 1960s outbreaks occurred in the Camargue (Southern France) with benign to fatal encephalitis symptoms (Panthier et al. 1968). More recently, West Nile fever epidemics were reported from southeastern Romania (the Danube plains and the city of Bucharest) with 393 acute human cases and a case fatality rate of 4.3% in 1996 (17 deaths). In 1997, 14 cases with two deaths occurred between July and the end of September (Ceianu, pers. comm.). In Europe virus isolates are known from France, Portugal, former Czechoslovakia and Romania. Serological surveys have revealed antibodies in human sera from Albania, Austria, Germany, Greece, Italy,

Poland, Spain and former Yugoslavia (Hubalek and Halouzka 1999; Lundström 1999; Hrnjakovic-Cvjetkovic et al. 2006). In addition to humans, West Nile virus has also been isolated from horses with encephalomyelitis in France, Italy and Portugal (Jourbert et al. 1970; Filipe 1972; Cantile et al. 2000). Antibodies have been detected in other domestic mammals, *e.g.* bovids as well as in wild mammals, *e.g.* mice, bats and even in the water snake, *Natrix natrix* (Aspöck 1996).

The outbreak of West Nile fever in North America is remarkable. West Nile fever first appeared on this continent in New York in the summer of 1999 when 62 cases and seven fatalities were recorded. During the next 5 years, it spread across the country. In 2003, 9,862 cases and 264 fatalities were recorded in 46 States. Since that time West Nile fever occurred regularly in more than 40 States with thousands of infected people and fatality rates of 2.7–4.1% (CDC 2008).

In the USA West Nile virus has dramatically reduced populations of several common bird species, including robins (*Turdus migratorius*), chickadees (*Parus atricapillus*) and especially crows (*Corvus brachyrhynchos*) which have declined in some regions by 50%. Sixty two mosquito species belonging to a wide range of genera have been found positive for West Nile virus in the United States since 1999 (Reisen et al. 2004). Besides mosquitoes, West Nile virus has also been isolated from ticks. The role of ticks in the transmission cycle of the virus is not understood so far (Eldridge and Edman 2000).

### 3.2.3 *Bunyaviridae* (*Bunyavirus*)

*Bunyaviridae* are vector-borne viruses with the exception of Hantaviruses, which are transmitted through aerosol contact with mice feces. There are five genera currently recognised in the family. In addition to topsoviruses, which mainly infect plants, *Bunyavirus*, *Hantavirus*, *Nairovirus* and *Phlebovirus*, infect animals. Only the genus *Bunyavirus* includes the viruses which are transmitted by mosquitoes to humans (exception Rift Valley fever, a *Phlebovirus*, which can also be transmitted by mosquitoes). The genome consists of a negative-stranded RNA with three segments.

Three groups of bunyaviruses have been recorded, namely the California group including the California

encephalitis, Inkoo, La Crosse encephalitis, Tahyna, Snowshoe hare, Trivittatus and Jamestown Canyon viruses, the Bunyamwera complex including the Bunyamwera, Batai and Cache Valley viruses, and the Turlock group including the Lednice virus only in Europe (Lundström 1999; Eldridge and Edman 2000).

#### 3.2.3.1 The California Serogroup

Viruses of the California group need mammals as amplification hosts (Lundström 1994, 1999; Aspöck 1996). Antibodies have been detected in wild and domestic mammals such as hares, rabbits, bovids, cervids (reindeer), and in carnivores as well as in hedgehogs (*Erinaceus europaeus*). Humans are also susceptible, infection of this serogroup in humans include mild infections to severe CNS diseases such as encephalitis or involvement of the respiratory system. From the public health point of view, the La Crosse (in North America) and the Tahyna virus (in Europe) are the most important viruses (Calisher and Karabatsos 1988; Lundström 1999, Eldridge and Edman 2000).

#### California Encephalitis Virus

This virus was first detected from *Ae. melanimon* mosquitoes from the Central Valley in California and later from *Oc. dorsalis* from Utah (Reeves 1990; Smart et al. 1972). Antibodies of the virus have been isolated from many mammals such as raccoons, skunks, opossums and woodrats. Infected humans may develop encephalitis.

#### Inkoo Virus

The Inkoo virus is circulating widely in Northern Europe. Although discovered 40 years ago little is known about the disease associations and immune response in humans (Putkuri et al. 2007).

#### La Crosse Encephalitis Virus

La Crosse virus is another important arbovirus in North America (following West Nile and St. Louis virus). It causes febrile illnesses accompanied by fever,

headache, vomiting, nausea, lethargy and coma. The virus is perpetuated in an enzootic cycle involving mosquitoes such as the tree-hole mosquito *Oc. triseriatus* and small mammals such as squirrels. The virus can be transmitted transovarially by mosquitoes (Watts et al. 1973). Approximately 70 cases are reported each year.

### Tahyna Virus

The Tahyna virus is widespread in Europe and Asia and also detected in Africa (Traavik et al. 1978; Aspöck 1979; Pilaski and Mackenstein 1985; Danielova 1992; Lundström 1994; Eldridge and Edman 2000). Infected people develop influenza-like symptoms which can lead to morbidity. It has frequently been isolated from *Ae. vexans*, but also from other species such as *Ae. cinereus*, *Oc. sticticus*, *Oc. cantans*, *Oc. flavescens*, *Oc. caspius*, *Cs. annulata*, *Cx. modestus*, *Cq. richiardii* (Lundström 1994; Aspöck 1996) and even in biting midges *Culicoides* spp. (Ceratopogonidae) (Halouzka et al. 1991). Danielova and Ryba (1979) proved in experiments that the virus can be transmitted transovarially in *Ae. vexans*. Therefore, it can be assumed that the virus overwinters within the eggs and with the mass development of *Ae. vexans* after spring floods, the virus circulation starts again. Virus overwintering in females of *Culiseta* spp. and *Culex* spp. seems to be of minor importance (Chippaux et al. 1970). Antibodies have been detected in many mammals, such as rabbits, wild boars, deer, cattle, swine and foxes. They serve as reservoirs for the virus and do not develop signs of illness.

### Snowshoe Hare Virus

Snowshoe hare (SSH) virus has been reported from the northwestern USA, Alaska and Canada. As the name suggests, the Snowshoe Hare (*Lepus americanus*) is thought to be an important host, but evidence of infection has been found in many wild species such as rodents, carnivores, ungulates as well as in domestic species – chickens, dogs, horses and cattle. Many different species of mosquitoes can become infected and transmit the virus. These include many species of the genera *Ochlerotatus* and *Aedes* as well as the species of *Culiseta* and of *Culex*. Most isolations of this virus

have been from snowpool mosquitoes. The virus can be transmitted transovarially and may also persist in cold weather. Disease in humans takes the form of infection and inflammation of the brain such as meningitis and encephalitis (Fauvel et al. 1980). Some cases of clinical illness due to SSH virus probably go unrecognised. However, it appears that infection is much more common than the disease itself.

### Trivittatus Virus

This virus occurs in North America and can cause disease with CNS symptoms. Antibodies have been found in rabbits, squirrels, opossums and raccoons. The vectors are *Oc. trivittatus* and *Oc. infirmatus*, other species belonging to the genera *Culex* and *Anopheles* may be involved (Pinger et al. 1975).

### Jamestown Canyon Virus

This virus has been involved in a few cases of human disease including encephalitis. Besides humans, large mammals such as deer and other cervids and domestic livestock can be infected. Snowpool mosquitoes such as *Oc. tahoensis* and *Oc. punctator* are the primary vectors (Eldridge and Edman 2000).

## 3.2.3.2 The Bunyamwera Complex

### Bunyamwera Virus

This virus occurs in Africa where it was first isolated in mosquitoes from Uganda. Infected humans develop a febrile illness, including headache and anthralgia. Besides humans, the virus has been found in a wide variety of mammals, including goats, sheep and rats (Eldridge and Edman 2000).

### Batai Virus

The Batai virus (strain: Calovo virus) is mainly transmitted by *An. maculipennis s.l.* (Bardos and Cupkova 1962). However, some isolates have also been obtained from *An. claviger*, *Cq. richiardii* and *Oc. communis*

(Francy et al. 1989; Traavik et al. 1985). It can be assumed that the virus overwinters in hibernating anopheline females. Following infection, humans can develop febrile disease with bronchopneumonia, catarrh and gastritis (Sluka 1969). However in Europe, Batai virus antibodies are usually found in less than 1% of the human population (Lundström 1999). Bovids are often infected with Batai virus. Antibodies have also been detected in pigs and deer; birds are not important for the virus circulation. This virus has been reported from several European countries, e.g. Finland, Sweden, Germany, Austria, former Czechoslovakia and Yugoslavia (Lundström 1999).

#### Cache Valley Virus

Cache Valley virus may be responsible for defects in human and animal foetuses as well as for a human case of haemorrhagic disease in the USA. The virus has been isolated in many mosquito species belonging to the genera *Culiseta*, *Aedes*, *Ochlerotatus*, *Psorophora* and *Anopheles*.

#### 3.2.3.3 The Turlock Group

##### Lednice Virus

The Lednice virus has been isolated from *Cx. modestus*. It is likely that the virus is transmitted vertically in the *Culex* population. Only rarely are vertebrates, mainly birds, infected (Aspöck 1996; Lundström 1999). Antibodies have never been found in humans or wild mammals, except in two hares (Wojta and Aspöck 1982), so the Lednice virus seems to be of no epidemiological importance.

### 3.3 Filariasis

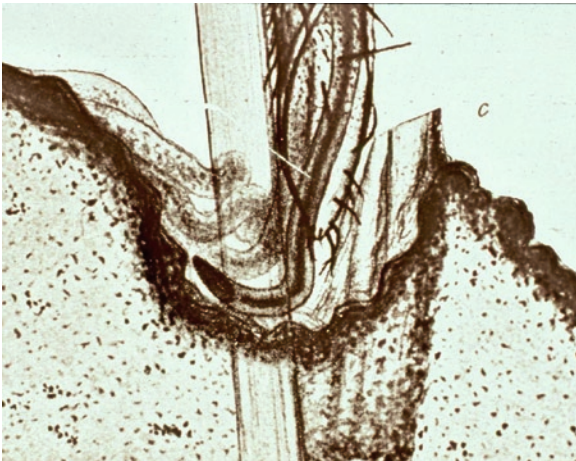
In the tropics, lymphatic filarial diseases affect an estimated 120 million people in 80 countries throughout the tropics and subtropics of Asia, Africa, the Western Pacific, and parts of the Caribbean and South America. Most of the infections, about 90%, are caused by *Wuchereria bancrofti*. In Asia, the disease can also be caused by *Brugia malayi* and *B. timori*. An estimated

905 million people are directly exposed to the infection transmitted by various genera of mosquitoes, the most important being *Cx. p. quinquefasciatus* and *Mansonia* spp. (Eldridge and Edman 2000). One-third of the people infected with the disease live in India, one third are in Africa and most of the remainder are in South Asia, the Pacific and the Americas. In tropical and subtropical areas where lymphatic filariasis is well-established, the prevalence of infection is continuing to increase. A primary cause of this increase is the rapid and unplanned growth of cities which result in break-down in environmental sanitation which usually creates numerous breeding sites for the mosquitoes that transmit the disease.

Lymphatic filariasis is thought to have affected humans for approximately 4,000 years. Artifacts from ancient Egypt (2,000 B.C.) and the Nok civilization in West Africa (500 B.C.) show possible “elephantiasis” symptoms. The first clear reference to the disease appears in ancient Greek literature, where scholars differentiated the often similar symptoms of lymphatic filariasis from those of leprosy.

Human filarial nematode worms have a complicated life cycle, which primarily consists of five stages. Following mating, the female worm produces millions of microfilariae measuring 244–296 µm by about 10 µm. They are sheathed and have usually nocturnal periodicity which is an adaptation to the biting habit of the vector. The microfilariae migrate into lymph and enter the blood stream reaching the peripheral blood. A mosquito ingests the microfilariae during a blood meal. After ingestion, the microfilariae lose their sheaths and penetrate through the wall of the proventriculus and cardiac portion of the midgut to reach the thoracic muscles of the mosquito. There, the microfilariae develop into second-stage larvae and subsequently into third-stage larvae. This usually takes 7–21 days. The third-stage larvae migrate through the haemocoel to the proboscis of the mosquito, ready to enter the punctured skin following the mosquito bite (Fig. 3.3). During the next months, up to a year, the larvae moult through two more stages, maturing into the adult worm that commonly reside in the lymphatic system of humans. They live for 4–6 years, producing millions of immature microfilariae that circulate in the blood.

Most of the symptoms of filariasis are caused as a consequence of the adult worms living in the lymph system in the network of nodes and vessels that maintain the delicate fluid balance between the tissues and blood and are an essential component for the body's



**Fig. 3.3** Worm larvae penetrating into the host skin

immune defence system. Tissue damage caused by the worms restricts the normal flow of lymph fluid. The disease usually is not life threatening, but it can permanently damage the lymph system and kidneys. Because the lymph system does not function correctly, fluid collects and causes swelling in arms, breasts, legs, and in the genital area of males. The name for this swelling is lymphedema. The entire leg, arm, or genital area may swell several times its normal size (hence the name “elephantiasis”). Also, the swelling and the decreased function of the lymph system make it difficult for the body of the victim to fight germs and infections. This causes hardening and thickening of the skin referred to as “elephantiasis”. Some of the symptoms are caused by the body’s immune response to the parasite, but most are the result of bacterial infection of the skin where normal defences have been partially lost due to underlying lymphatic damage. Careful cleaning can be extremely helpful in healing the infected surface areas in both slowing and even more remarkably, reversing much of the overt damage that has occurred already. The symptoms usually appear 5–18 months after the infectious mosquito bite.

The psychological and social stigma associated with the aspects of this disease, are immense. Because of its prevalence often in remote rural areas, on the one hand, and in disfavoured periurban and urban areas, on the other, lymphatic filariasis is primarily a disease of the poor.

In Europe, filariasis is of no medical importance.

The dog heartworm, *Dirofilaria immitis* (Onchocercidae) causes canine cardiovascular dirofilariasis in dogs and other canids, but rarely cats (Boreham and Atwell 1988). Infection in humans is asymptomatic because the worm does not mature in humans. The adult worms are 12–31 cm in length. Usually they are found in the heart (hence heartworm) and the pulmonary artery of the host. Infected animals can suffer from cardiac insufficiency and heart failure. Mature female worms release microfilariae into the blood. They exhibit a nocturnal periodicity and circulate in the peripheral blood vessels, an adaptation to the biting habit of the potential mosquito vectors. Lok (1988) listed 26 species of mosquitoes, incriminated on the basis of field or laboratory studies, as vectors of *D. immitis*. Most vectors are found in the genera *Aedes/Ochlerotatus*, *Anopheles* and *Culex* (Eldridge and Edman 2000). When ingested by a mosquito, the microfilariae penetrate the gut epithelium and enter the Malpighian tubes where a certain percentage grow and moult to the infective third larval stage within about 2 weeks, under favourable temperate climatic conditions. The infective larvae migrate to the mosquito mouthparts and enter the labium from which they penetrate into the host skin when the mosquito is biting (Fig. 3.3). The development to the adult worms in the heart and pulmonary artery of the vertebrate host takes several months, and after more than 6 months microfilariae are produced again. In addition, other dog-infecting filarioids are *Dipetalonema* spp. or *Mansonella* spp. which have a similar biology as *D. immitis* (Aranda et al. 1998).

*D. repens*, the causative agent of the canine cutaneous dirofilariasis is endemic in large parts of Southern and Eastern Europe, however, increasing numbers of autochthonous cases in dogs have been reported in recent years from northern countries including Germany (Pantchev et al. 2009).

### 3.4 Future Perspectives and Conclusions

In conclusion, the family Culicidae has had, and continues to have, a more negative impact on human well-being, health and socio-economic development, than any other group of animals.

There are many examples from history, where endemic or epidemic insect-borne disease have affected the pattern of human settlement, changed the outcome of military campaigns, or simply acted as a drain on the rate of development. Even in the early years of the twenty-first century, as shown in this chapter, despite at least a century of determined scientific and technological advance, the impact of mosquito-borne disease continues. Approximately 30% of the world's human population still lives with the threat of insect-borne disease.

In some areas, the numbers of people at risk of disease has actually increased, as a result of the vector and/or pathogen expanding or consolidating its range. Such changes may take place as a result of trade or movement of people, or in changes to the environment itself.

In some cases, the movement of pathogens from one geographical region to another has resulted in the spread of diseases. The introduction of West Nile virus into the USA for the first time, in 1999, possibly on an infected pet bird, allowed indigenous mosquitoes and birds to become infected. This enabled the disease to spread rapidly (within 5 years from the East Coast to the West Coast) across the continent, placing another 0.5 billion people at risk (Reisen et al. 2004). The very recent introduction of Chikungunya virus into Northern Italy, probably on a single infected person, brought the virus and previously introduced vector, *Ae. albopictus*, into contact, resulting in the first known European outbreak of this disease.

Alternatively, increases in the incidence of insect-borne disease may take place as a result of a decline of the effectiveness of control programmes, perhaps through insecticide resistance or through political changes. Military conflict, through its displacement of human populations, and its damage to infrastructure and abject poverty often results in outbreaks of mosquito-borne diseases. Those living in refugee camps are particularly at risk. Changes in political structures, such as the dismantling of the Soviet Union in the 1990s, can also create opportunities for mosquito populations to develop in the absence of monitoring or control programmes, resulting in a resurgence of malaria in some territories.

For rural communities, the city has often appeared as place of opportunity. The human drift into towns and cities has been taking place for millennia, and it is now estimated that for the first time, the proportion of the human population living in cities exceeded 50%. In

many individual countries, the urban population now exceeds 80%. Where the rate of urbanization exceeds the rate at which services and infrastructure can be established, there is the risk of allowing pest species, particularly mosquitoes, to become established. Improper water storage and disposal, whether in residential areas or on construction sites, and the operation of small farms within urban areas tend to create opportunities for mosquitoes. Mosquito control in urban areas brings very different challenges from those encountered in rural communities.

Overall, the outlook for mosquito-borne disease is not positive. In recent decades, there appears to have been more bad news than good. The need for good medical entomologists is as strong as it ever was. Changing environmental conditions creates the danger of novel host–virus interactions that may lead to potentially disastrous results in the future. To conclude, it can be stated that the risk for the reintroduction and transmission of mosquito-borne diseases has and will increase rapidly, due to the increased populations, lack of proper housing, poverty and mobility of humans, the international trade and the changing climate.

The trade with used tyres and possibly “lucky bamboo” (*Dracaena* spp.) is responsible for the introduction of *Ae. albopictus* into most parts of Europe. The introduction of *Ae. albopictus* and *Oc. j. japonicus*, into the United States under similar circumstances, is another well established fact. *Ae. albopictus* was found for the first time in the continental United States in used tyres and retrograde cargo returning from Asian ports (Pratt et al. 1946; Eads 1972). The first record of establishment of this species in the continental US was reported by Sprenger and Wuithiranyagool (1986), when a large population was discovered breeding in used tyres (shipped from Japan) in Houston, Texas. Within a short period (1985–1999), widespread infestations of *Ae. albopictus* were reported from 28 states east of the Mississippi River (Moore 1999). More recently, this species was introduced for the first time on the west coast of the United States, into California in June 2001. Developmental stages and adult specimens were discovered in shipments of *Dracaena* sp. plants packaged in standing water, arriving in refrigerated maritime containers at the Los Angeles and Long Beach Harbours. *Ae. albopictus* as a container breeder

has adapted very well to breeding in such manmade containers (Madon et al. 2002).

*Oc. j. japonicus* is another exotic species which was recently introduced into the United States and in Europe (Schaffner and Chouin 2003; Eritja et al. 2005; Peyton et al. 1999). The most likely mode of introduction of *Oc. j. japonicus* may have been via used tyres exported to the United States. This subspecies prefers to breed in a variety of natural as well as artificial containers. It is a day-time biter and is known to readily bite humans.

These are just a few examples of mosquito vectors and their capability of readily adapting to different environmental and climatic conditions. Increase in international travel and global commodities, increased urbanization, change of climate with increasing temperatures and unusual heavy rainfalls may lead to an extension of the range of mosquito vectors and mosquito-borne diseases and contribute to the complexity of occurrences in world-wide vector-borne disease.



## Chapter 4

# Mosquito Research Techniques

Basic knowledge about the distribution, abundance, seasonality, and ecology of different mosquito species is essential for a successful control campaign against these insects. For example, knowledge of the population dynamics and migration behaviour of the target organisms are crucial to the design of a control strategy. In parasitological and epidemiological studies, the interaction between the parasite or pathogen, and the vector and host, must be evaluated in order to suppress mosquito-borne diseases successfully. In the initial phases of all mosquito control campaigns, detailed entomological studies are likely to be carried out. In this chapter, the most important methods of mosquito research are presented. A complete review of mosquito sampling techniques and the analysis of collected data is given by Silver (2008).

### 4.1 Sampling Mosquito Eggs

Mosquitoes lay their eggs singly or in egg rafts in many different habitats, such as swamps, marshes or pools, as well as in a great variety of small, natural, and artificial water collections, such as tree-holes, rock pools or man-made containers. Some females lay their eggs on the water surface, whereas others lay them onto the moist soil at the breeding site, on the edge of the water or on the wall of natural or artificial containers. The determination of egg densities in natural habitats provides not only a better understanding of the egg-laying behaviour of the various mosquito species, but can also aid in predicting future larval populations and possible control areas.

The range of egg-laying behaviour and of the physical characteristics of the eggs require various collect-

ing techniques to be employed, depending on the species and habitat.

#### 4.1.1 *Anopheles* Eggs

*Anopheles* females lay their eggs on the water surface where they float due to air-filled chambers formed from the outer layer of the egg, the exochorion. Often, the eggs group themselves into net-like structures until the larvae hatch. Less than 2 mm in size, the eggs are hardly recognizable with the naked eye. Eggs can be sampled from the water surface using a light-coloured dipper. However, a dipper whose bottom surface has been cut out and replaced with a fine wire mesh is preferred. This modification allows the dipper to be pulled through the water, skimming the surface and thus collecting the eggs from the surface. The mesh is then washed with water into a light-coloured plastic dish, where the eggs can be collected with a pipette. In the absence of a dipper, a metal ring (10 cm diameter) with a nylon mesh stretched across the surface can be also used. A wooden handle may be attached for easier use (WHO 1975).

#### 4.1.2 Egg Rafts

Species of the genera *Culex*, *Uranotaenia*, *Coquillettidia*, and subgenus *Culiseta* of the genus *Culiseta* lay their eggs in rafts on the water surface. With a size of several millimeters, the egg rafts can be easily seen and sampled by fine forceps, pipettes, or small nets.

### 4.1.3 *Aedes/Ochlerotatus* Eggs

*Aedes* and *Ochlerotatus* females lay their tiny eggs into the moist substrate of the breeding sites. It is extremely difficult to recognise the eggs in situ even with the help of a magnifying lens, therefore, soil samples from the breeding sites have to be taken. For the determination of the egg density the so-called “flooding method” or the “saltwater method” can be employed. For the estimation of the number of eggs per surface unit, it is important to standardize the soil samples (Becker 1989b; Silver 2008). For this purpose, a metal frame of angle iron (20×20×2.5 cm) is suitable. The frame can be driven into the ground with a hammer until the angled side is flush with the ground surface. Using a trowel, the soil along the bottom of the frame can be cut horizontally to a depth of 2.5 cm. The soil sample can then be carefully transferred to a plastic bag, and labeled with the location and date of sampling. In this manner, the *Aedes/Ochlerotatus* eggs should remain in their natural state.

Females prefer particular sites for egg deposition. It is known that the moisture and quality of the soil, or the plant associations, which indicate a special flooding sequence or degree of soil moisture, are important factors in determining where the female mosquito lays her eggs. This usually results in a heterogeneous distribution of eggs. In order to secure the necessary data from the different zones, it is therefore recommended to take the samples in transects, to determine the variation in egg densities in a potential breeding site. Along the bank of a breeding site, samples should be taken at equal distances beginning at the deepest point, and continuing to the upper margin of the breeding site. This ensures that the areas with the highest densities of *Aedes/Ochlerotatus* eggs will be recorded. Often the highest numbers of eggs are laid in bands around pools subject to fluctuations in water level. The bands indicate the zones above the water edge with a high content of moisture during the time of mass oviposition by the gravid females. Until processing, the soil samples should be stored out of the sun to avoid drying of the soil and damage to the eggs. If the samples are kept for several days or weeks, the soil should be regularly moistened.

When the “flooding method” is applied to calculate the relative egg density of a certain breeding site, the standardized soil samples should be flooded with water

at a temperature which aids the hatching of the larvae (~20°C). It is recommended to use water with an initially high oxygen content. The decrease in oxygen caused by the metabolism of the microbes in the soil stimulates the hatching of the larvae. Ascorbic acid, or yeast and sugar, can be added for further reduction of oxygen to increase the hatching stimulus. The hatched larvae can be collected a day or two after flooding, counted, and identified after being reared to the fourth larval instar or adult stage. Because of sequential hatching (batches of larvae hatch during consecutive flooding), samples with high egg densities should be repeatedly flooded. The standing water must be decanted so that the soil can dry out until the next flooding procedure takes place. The alternate flooding and drying raises the hatching stimulus of the eggs that remain in the soil. Even after several floodings, larvae will still continue to hatch.

As the *Aedes/Ochlerotatus* larvae may be in diapause and therefore unable to hatch even when suitable hatching conditions are provided, it is important to interrupt the diapause and so condition the larvae for hatching, before the samples are treated. For instance, when eggs of *Aedes vexans* are sampled during winter, the samples must be stored for at least 2 weeks at temperatures above 20°C, so that larvae are able to hatch. The higher the difference between the low and high temperature, the higher is the response to the hatching stimulus.

In contrast to the flooding method, the “saltwater method”, described by Horsfall (1956b), is less time consuming and ensures close to 90% recovery rate of the eggs. The principle behind this method is that the density of an aqueous solution is increased by adding salt, thereby causing the eggs with a density lower than the saline solution, to float to the surface.

If the soil samples are taken from the field during summer, the samples should be stored at 5°C for at least 2 weeks to prevent the larvae (*e.g. Ae. vexans*) from hatching. To wash the eggs out of the soil, the sample should be placed in a tub and flooded with cold water (below 10°C). The cold water further reduces the hatching stimulus. The flooded sample should be carefully and uniformly mixed, and lighter particles (leaves, wood) can be removed from the surface of the water (Butterworth 1979). The water and the finer, suspended particles in the water have to be carefully decanted. This procedure should be repeated several times. Then salt (sodium chloride) is added to the

water, until a 100% saturated salt solution has been achieved and the salt crystals no longer dissolve. This solution has a higher density than the eggs. By thoroughly mixing the sample, the eggs rise to the surface, where they can be collected by means of a pipette or with a filter paper and counted. The eggs can be determined either by rearing the larvae, or in some species the specific pattern of the egg shell (chorion) can also be used for species determination.

Another method for collecting the eggs is the use of a sequence of meshes with a decreasing mesh-size to separate the eggs from the soil.

Due to the similar egg-laying behaviour and physical properties of the eggs of the subgenus *Culicella* within the genus *Culiseta*, the same methods can be employed.

#### 4.1.4 Eggs in Artificial Oviposition Sites

Mosquito species have preferred breeding habitats, which can differ greatly in abiotic and biotic factors, such as water quality, light intensity, available food or vegetation. The knowledge of the critical factors in the choice of a breeding site by a certain mosquito species allows the construction of artificial oviposition sites or traps. Oviposition traps (ovitrap) can be useful tools in surveillance programmes for certain mosquito species, such as mosquitoes breeding in artificial containers, tree-holes or rock-pools.

In survey programmes *Aedes albopictus* [*St. albopicta*] and *Aedes aegypti* [*St. aegypti*] are monitored mainly by means of ovitraps (Fay and Eliason 1966; Pratt and Jakob 1967; Jakob and Brevier 1969a,b; Evans and Brevier 1969; Thaggard and Eliason 1969; Chadee and Corbet 1987, 1990; Freier and Francy 1991; Service 1993; Bellini et al. 1996; Reiter and Nathan 2001).

Population levels of both species can be determined by using a sufficient number of ovitraps in a certain area (Mogi et al. 1990; Bellini et al. 1996). Commonly used ovitraps consist of a dark plastic or glass jar which is painted black on the outside, about 8 cm in diameter at the top and 5 cm at the bottom, with a height of 12.5 cm. When the ovitraps are placed in the field, a strip of hard-board or masonite (2 × 12 cm) with a smooth and a rough surface is attached vertically with a paper clip to the inside of the jar so that the rough side is facing towards the center of the jar (Service 1993). Then, approximately 200 ml of dechlorinated tap water is poured in. A small

hole in the plastic jar above the water line prevents water overflow during heavy rainfall. Usually the females of *Ae. albopictus* and *Ae. aegypti* lay their eggs just above the water line on the rough side of the strip. At regular intervals (once a week) the strips are changed and the water replaced. The number of eggs can be counted on the strips using a dissecting microscope.

Plastic buckets, wash tubes or other receptacles containing several liters of infusion of hay, brewer's yeast, dog biscuit, alfalfa or sewage water can be used as oviposition traps for *Culex p. pipiens* and *Cx. p. quinquefasciatus* (Yasuno et al. 1973; Sharma et al. 1976; Leiser and Beier 1982; Reiter 1983, 1986; O'Meara et al. 1989; Becker 1989b). The *Culex* mosquitoes are apparently attracted by gaseous compounds such as ammonia, methane, or carbon dioxide, which are released when organic material decomposes in the water. Fatty acids and n-capric acid are also oviposition attractants for females of *Cx. p. pipiens*. In order to study the egg-laying behaviour, or the population density of the *Culex* mosquitoes, egg rafts can be sampled at regular intervals. It can be assumed that in human settlements with limited numbers of natural breeding sites, the *Culex* population may be effectively reduced by destroying the egg rafts sampled in oviposition traps at regular intervals. In addition to attractants, an insect growth regulator (IGR) may be added to the water in the oviposition traps to prevent the development of adult mosquitoes, or to sterilize them.

Gravid female mosquitoes in search of oviposition sites may be sampled using a gravid trap first developed by Reiter (1983). The attractive component of a gravid trap consists of an open-topped container of a water-based infusion (Fig. 4.1). Situated just above



Fig. 4.1 Gravid trap

the surface of the water is an updraft collection port, leading into a collection container, connected to an extract fan. Gravid females active on the infusion surface are sucked up the port and held in the collection container. The infusion used is typically prepared by soaking hay in water for several days, but the attractiveness of other types of infusions has been determined (Burkett-Cadena and Mullen 2008; Jackson et al. 2005). Gravid traps are typically used to monitor *Culex* mosquitoes (Tsai et al. 1989), but may also be successfully used to monitor *Oc. j. japonicus* (Scott et al. 2001). In terms of their trapping efficiency compared to light traps, gravid traps appear particularly useful against *Culex quinquefasciatus* (DiMenna et al. 2006).

## 4.2 Sampling Mosquito Larvae and Pupae

Many sampling techniques are used to assess the population of the aquatic stages of mosquitoes. They are especially employed to study the population dynamics of mosquitoes, or to estimate population densities pre- and post-larvicidal application. Although mosquito larvae occur in a wide range of habitats, some simple techniques can be employed for the assessment of the larval population. The most commonly used tool is the dipper, which may vary in size and shape. Soup ladles, white plastic or enamel bowls or photographic trays with a capacity of a few hundred milliliters to 1 liter are inexpensive and easily used tools for collecting larvae. For comparative purposes it is recommended to use standardized dippers. In most programmes the “standard pint dipper” is used, consisting of a white plastic container measuring 11 cm in diameter and with a capacity of 350 ml (Dixon and Brust 1972; Lemenager et al. 1986). The white background aids accurate counting of larvae. A wooden handle is attached to the dipper to reach water bodies from a distance, and to avoid disturbing the water, which causes larvae to dive during sampling (Fig. 4.2).

According to the size of the surface of the larval habitat, a sufficient number of dips have to be taken to estimate the number of developmental stages. In larger ponds (>20 m<sup>2</sup>) 10–20 samples along the edges and in the center should be taken. The num-



**Fig. 4.2** Assessment of larval densities by using a standard pint dipper

ber of various larval instars and pupae per dip should be separately recorded. By calculating the volume of water inhabited by larvae, a rough estimation of the aquatic mosquito population can be made (Papierok et al. 1975; Croset et al. 1976; Mogi 1978). Silver (2008) suggests more precise methods for population estimates based on data evaluation by regression statistics. In large habitats, a plankton net attached to a handle can be used to catch a relatively large number of larvae within a short time. The net should be drawn through the water in a figure-eight pattern to sample the larvae. In small collections of water such as tree-holes, sampling can be difficult. Larvae can be pipetted directly from the surface or can be collected by siphoning out the water.

A sufficient number of larvae/pupae have to be transported to the laboratory alive for species determination, or alternatively fourth-instar larvae can be preserved in 70% ethanol in the field. In the larval stage only fourth-instar larvae are suitable for species determination. Earlier instars have to be reared to the fourth-instar or adult stage. For transportation to the laboratory, a glass or plastic container with a close-fitting cap should be 3/4 filled with water from the site. The larvae can be pipetted into the container, or the contents of a dipper or net catches can be released into the container. The containers must be marked carefully recording the date and location of sampling.

In the laboratory, larvae can be killed by hot water (60°C) and transferred into preserving media for further handling. Alternatively the larvae can be identified at the fourth-instar, or reared to the adult stage in a

mosquito breeder for confirmation of the larval identification (see rearing of mosquitoes).

In *Ae. aegypti* control programmes, whether intended for the control of yellow fever, dengue or DHF, several standardized larval assessment indices have been established. The House Index is the percentage of houses in which larvae or pupae are found, while the Container Index is the percentage of water-holding containers, that contain active immature stages (Connor and Monroe 1923). Subsequently the Breteau Index was established and used, defined as the number of positive containers per 100 houses (Breteau 1954). However, none of these indices takes account of the numbers of insects in each container. More recently, Focks and Chadee (1997) proposed that counts of pupae, and calculations of pupae/person were more meaningful figures in terms of assessing the risk of disease transmission.

### 4.3 Sampling Adult Mosquitoes in the Field

A very wide variety of sampling techniques for adult mosquitoes are available, such as direct human bait catches (HBC), netting, collecting with aspirators, suction or attractant traps, *e.g.* containing carbon dioxide (CO<sub>2</sub> traps). Several factors such as the weather conditions, activity pattern, host-seeking and resting behaviour, as well as the physiological stages of the mosquito, will determine the composition of the catch in terms of the species and the sexes. Here, only the more commonly used techniques will be discussed. Silver (2008) provides more detailed information.

#### 4.3.1 Sampling Flying Mosquitoes

Traps that sample mosquitoes, without relying on the mosquito to actively orientate towards a potential host or other attractant, are relatively few.

##### 4.3.1.1 Truck Traps

Truck traps are used to sample mosquitoes in flight. They consist of a funnel-shaped net fixed to the roof of

a vehicle, with the large open end pointing forwards. The funnel leads into a small collecting container. The vehicle is driven through the area to be sampled, ideally on a fixed route and at a steady speed, and at intervals the vehicle is stopped and the insects removed from the collecting container (Tsai et al. 1989). This trap is also suited to establishing mosquito flight times.

##### 4.3.1.2 Electrocutation Traps

Gilles et al. (1978) developed an electrified wire grid that killed and collected mosquitoes that blundered into it from various directions. The grid was placed between breeding sites and the villages in which the mosquitoes obtained blood meals, and the catches of mosquitoes moving to and from these locations, were related to meteorological conditions. More recently electrified nets have also been used to catch mosquitoes attracted to various host odours (Torr et al. 2008).

#### 4.3.2 Adult Mosquito–Outdoor Resting Catches

Many mosquito species rest outdoors, either in vegetation or in natural openings or crevices. Those resting in holes may be sampled by the construction of pit shelters. These typically consist of a ca. 1.5 m square hole dug into the ground (water-table permitting), and a smaller horizontal hole then dug into each vertical face of the pit. At intervals the collector enters the pit (beware of wildlife, *e.g.* snakes) and with the aid of a torch and aspirator, collects the mosquitoes resting in the horizontal holes (Bhatt et al. 1989).

Adult mosquitoes resting in vegetation may be sampled with a sweep net (Holck and Meek 1991), or directly with an aspirator.

#### 4.3.3 Adult Mosquito–Indoor Catches

The natural host seeking, movement, and resting behaviour of mosquitoes in and around buildings, may be assessed by a range of techniques. In addition, these techniques may also be used to assess the changes resulting from insecticide treatment.

Mosquitoes resting inside buildings may be collected by use of a torch and aspirator. Typically such assessments are carried out in the morning, as there may be a progressive departure of mosquitoes over the day, or predation by ants or geckos. Alternatively, the indoor resting mosquitoes may be collected by spreading a white sheet on the floor of the selected room, and then treating the room with a non-residual pyrethrin spray. The dead and knocked-down mosquitoes may then be collected from the sheet a few minutes later (Magbity et al. 1997). However, frequent use of pyrethrins in a particular room may result in changes in mosquito resting behaviour in that room.

Entry and exit of mosquitoes from buildings may be assessed using traps fitted over existing window openings. Depending on the orientation of the trap, these collect mosquitoes either entering or leaving the building. Typically, these consist of a net-covered funnel fitted over the window, leading into a holding cage. Depending on the study, the mosquitoes may be removed in the morning and/or evening. Siting traps over windows with overhanging roof eaves, may provide protection from direct sunlight, or rain (Magbity et al. 1997).

Instead of using existing buildings, to which the behaviour of mosquitoes will differ, due to differences in design, location and use of the buildings, standardized experimental huts may be built and used instead (WHO 2006). These typically consist of a small one-room building, which may be occupied by people or animals, and which may be fitted with window and verandah traps. The hut may be built on a raised plinth, incorporating an ant barrier, to reduce predation of mosquitoes. Indoor resting collection, and pyrethrin spray collections may also be carried out, depending on the programme.

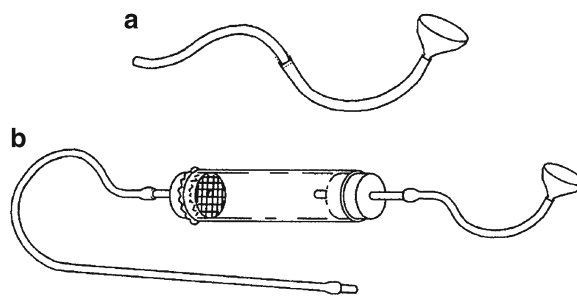
#### 4.3.4 Bait Catches

The bait or host for a blood-meal (*e.g.* humans or animals) emit olfactory stimuli or kairomones, which are attractants for the host-seeking mosquitoes. Female mosquitoes respond to compounds such as carbon dioxide from the exhaled breath, host odour (*e.g.* lactic acid) emanating from sweat, water vapour, body heat and vision aided by the movement of the host (Bar-Zeev

et al. 1977; McIver 1982; Takken and Kline 1989; Takken 1991; Lehane 1991; Becker et al. 1995b, Petric et al. 1995). A traditional method of assessing the adult population is to count females landing on a human, and to express the number of females per unit of time. The catch can be performed in hourly or bi-hourly intervals over a 24-h period to assess the activity pattern of the female mosquitoes in relation to abiotic factors such as temperature, relative humidity, light intensity or wind speed. For instance, the biting activity of many mosquitoes is often highest during sunset, when the temperature is still high and the humidity is increasing.

Depending on the number of mosquitoes, the collector might expose only a part of the body (leg or arm) or the whole body, to collect the mosquitoes with an aspirator. Widely used aspirators consist of a clear plastic tube, which can be fitted with a two-hole stopper to hold the intake and exhaust tubes, made from flexible clear plastic (Fig. 4.3b). The top of the intake tube should hold a small funnel to aid the sampling of landing mosquitoes, and the exhaust tube should be protected with a fine-mesh nylon net to limit inhalation of undesirable material. The plastic tubes can be removed and capped by snap-on caps. Mosquitoes can be killed by placing a small piece of cotton wool absorbed with a very small quantity of a killing agent (*e.g.* ethyl acetate), into the tube (ensure that the tube material is unaffected by the killing agent). If plugs of cotton wool or paper strips are added to the tube, movement of the dead, fragile mosquitoes and loss of scales can be avoided.

A simple aspirator can be easily constructed by joining two flexible plastic tubes with slight differences in diameters, so that they fit snugly within each other. A nylon net fixed between the joined tubes



**Fig. 4.3** Simple (a) and chamber aspirator (b) for collecting adult mosquitoes

prevents the inhalation of mosquitoes by the collector (Fig. 4.3a). The catch can then be blown into a separate killing chamber.

The use of a drop-net is advisable when the number of mosquitoes is excessive (Fig. 4.4). The trap consists of a bell-shaped net with three metal hoops which can be fixed on a branch of a tree just above the collector. The two lower hoops have a diameter of 1 m with cords fixed on the hoops which the collector can pull the lower hoop up for exposure to mosquitoes. After an exposure time, ranging from 2 to 10 min or longer, the net is dropped. The collector then samples all mosquitoes inside the drop-net, with an aspirator.

#### 4.3.4.1 Animal Bait Catches

Many important vector species for human disease transmission may also feed on animals. Catches on a variety of animal baits, such as cattle, goats, pigs, rodents and various birds may therefore be carried out to determine the role and extent of zoophily (Amerasinghe et al. 1999; Russell 1987). Catches may involve removing mosquitoes directly from the tethered animal host with an aspirator or, less directly, by

collecting resting mosquitoes from inside an animal shelter (Kanojia et al. 2003).

#### 4.3.5 Adult Mosquito Traps

It is known that mosquito species respond differently to various kairomones and to individual attractiveness of the collectors, resulting in variations in catch sizes and species composition. To allow a more standardized monitoring of adult mosquito populations in large areas without the need for additional collectors, many types of mosquito traps have been developed to attract different target species. In particular, a wide range of mosquito light traps have been developed and tested. Some rely solely on a conventional incandescent filament light bulb as the main source of attraction, others use an ultra-violet light source, while others supplement the light source with carbon dioxide or another chemical attractant (Petric et al. 1999). Traps may also incorporate a photosensitive switch which turns the light and motor off during daylight hours, and closes a valve to prevent the mosquitoes from escaping. A variety of models are now commercially available, and for a full review of light traps, see Silver (2008). The EVS model



Fig. 4.4 Collecting mosquitoes with a drop-net

has been successfully used for many years and is described in detail below.

#### 4.3.5.1 Carbon Dioxide-Baited Light Trap

The CO<sub>2</sub>-baited CDC mosquito trap (also referred to as Encephalitis virus surveillance trap) was first developed in the mid 1950s (Nelson and Chamberlain 1955). Further improvements were published by Sudia and Chamberlain (1962). Since then, many additional modifications have been made (Newhouse et al. 1966; Smith and Downs 1970; Johnson et al. 1973; Rohe 1974; Smith et al. 1979; Williams et al. 2009). The top part consists of a 3.5 l plastic dry ice container. The walls and snap-on polyethylene lid are insulated with polyethylene foam to prevent rapid sublimation of the dry ice that is placed in the container during use. In the lower part of the container, two to four holes of 0.5 cm in diameter allow the sublimated CO<sub>2</sub>, which is the primary attractant, to escape and flow downwards over the lower part of the trap. The middle part of the trap consists of a plastic tube with holders for three 1.5 V dry cell batteries, which provide power for a fan and a subminiature 1.5 V, 70 mA lamp, which can be operated by an off/on switch. Female mosquitoes, attracted mainly by the carbon dioxide, enter through an opening and are sucked downward by airflow created by the plastic fan attached to a small motor into a 30-cm long nylon netting catch bag (Fig. 4.5). In routine monitoring programmes the trap is baited with approximately 1 kg dry ice, which is enough to catch mosquitoes during one night.

A carrying handle is provided, along with a metal chain to facilitate hanging from a tree branch or other object. The traps are set up in the late afternoon and removed the next morning. The catch, including the net, can be transferred into a container with dry ice or other killing agents to kill the mosquitoes. In the laboratory the species composition is determined. Catches at regular intervals (*e.g.* each fortnight) yield valuable information about the seasonality and population size of the adult mosquitoes. Furthermore, the comparison of the catches in controlled and uncontrolled areas allows an estimation of the reduction of the mosquito population by control operations. Under favourable conditions in areas with mass breeding sites, more than 15,000 female *Ae. vexans* can be caught in one EVS-trap per night.



**Fig. 4.5** Carbon dioxide baited trap

Male mosquitoes are not attracted by CO<sub>2</sub> traps, but can respond to light traps. They are best collected in the field when they are swarming close to their breeding sites. The males of most species usually swarm above prominent landmarks where they can be caught by a long-handled insect net.

#### 4.3.5.2 Recently Introduced Mosquito Traps

In addition to the range of conventional mosquito light traps used by mosquito control organizations for many years, a number of novel mosquito trapping or monitoring devices have been introduced over the last decade. Some incorporate novel lures, other have alternative air-flow systems, while other use novel technology to power the fan.

The Mosquito Magnet™, was one of the first of the new generation of traps (Fig. 4.6). This is a free-standing machine, that was originally intended more for its use as a local mosquito control device, than for moni-





**Fig. 4.6** The Mosquito Magnet™

toring and survey work. It uses a container of propane gas as an internal energy source. The propane is converted into carbon dioxide by a platinum catalyst, and the heat produced by the reaction is used to generate enough electricity to drive a small fan generating an updraft. The device incorporates a replaceable lure that releases 1-octen-3-ol into the carbon dioxide plume. The machine will run continuously for up to 21 days, after which time the propane gas cylinder and the octenol lure need replacement. Mosquitoes and other biting insects such as phlebotomine sandflies, are attracted by the plume of carbon dioxide and 1-octen-3-ol emitted by the machine, and are then sucked into a net cage by the fan. Kline (2002) evaluated several propane powered traps and found them highly effective for collecting mosquitoes. Dennett et al. (2004) conducted field trials at an old tyre dump and found that the Mosquito Magnet caught more individuals and more species than other traps tested.

Other updraft traps, and also counter flow traps, have now been introduced. The Biogents Sentinel trap (BGS) incorporates a proprietary odour lure, which improves the number and diversity of mosquitoes caught compared to the EVS trap (Irish et al. 2008).

Semi-field tests with the BGS trap in Tanzania showed that it caught more *Anopheles gambiae* than the Mosquito magnet (Schmeid et al. 2008).

### 4.3.6 Mark-Release-Recapture Techniques

Mark-release-recapture techniques may be used in the field to follow the behaviour and dispersion of marked mosquitoes, to determine survivorship of mosquitoes, or as a tool to estimate population size.

These approaches require a marking technique that lasts for a useful period of time, but which has little effect on the insects' behaviour. Fluorescent pigments applied to adults are most commonly used, and these may be applied directly to batches of laboratory-reared or field caught insects, or self-marking devices have been used in which the mosquitoes become marked with pigment when leaving the natural breeding site (Niebylski and Meek 1989). However, other marking techniques have also been used, including staining of the larvae, which then produce stained adults (Paing and Naing 1988). In general, recapture rates are low, hence relatively large numbers of mosquitoes need to be marked and released. Tietze et al. (2003) released 43,000 mosquitoes over 3 days, trapping the mosquitoes using carbon dioxide baited traps positioned up to 2.8 km from the release point, and found a recapture rate of about 0.5%. In this study, the population size was estimated, using the Lincoln Index, as 3.8–9.4 million adult mosquitoes.

## 4.4 Laboratory Based Research Techniques

### 4.4.1 Rearing Mosquitoes

It is often necessary to breed mosquitoes to the adult stage in order to identify them. Frequently, the fourth larval instar, its exuviae, or the exuviae of the pupae or the adult are needed before identification confirmation can be made. The breeding of mosquitoes is also essential for assessing insecticide efficacy, and the study of the bionomy of the species. A wide range of different

techniques have been developed for rearing various mosquito species in the laboratory (Gerberg 1970).

Larvae should be kept in water obtained from the breeding sites, to assist successful development to the adult stage. If the immature stages of the mosquitoes have to be transferred into clean water, either distilled or rainwater should be used. If water from the original breeding site is used it is not usually necessary to add food. However, if distilled water is used, or when large numbers of larvae are reared, then food in the form of powdered fish food, dried yeast or liver powder should be added. Only small quantities of food should be added to avoid fouling of the rearing medium. A regular change of water or supply of oxygen is necessary if excess food is accumulating, to avoid anaerobic conditions and scum formation. Before emergence of the adults, fourth-instar larvae or pupae are transferred into a "mosquito breeder". The mosquito breeder consists of two clear polystyrene containers. The water sample with larvae is placed into the bottom portion. A screw-on lid between the two sections contains a funnel through which the emerging adults can fly into the upper part. Refrigerating the apparatus enables removal of the adults. Alternatively, they can be reared to adults in an improvised container, such as a jam jar. As pupae develop, the jar should be covered with fine netting held by a rubber band, or alternatively a larger glass container may be put upside-down over the jar, so that the emerging mosquitoes cannot escape.

When larger numbers of larvae are reared they should be placed inside a mesh cage. These can be constructed by making a cuboidal frame with a side of about 30 cm, covered with nylon netting. On one side, a circular opening of about 10 cm should be left to allow access into the cage, *e.g.* to remove containers or mosquitoes using an aspirator. A sleeve should be sewn in the opening to prevent the escape of mosquitoes. It can be knotted when access is not required. A density of not more than 1,000 larvae per liter of water should be kept in shallow containers to avoid overcrowding. On a daily basis, pupae can be removed with pipettes and transferred into separate containers with clean water, also kept in the mesh cage.

The rearing temperature should be adapted to the species requirements. For snow-melt mosquitoes it should be about 20°C, and for summer species, about 25°C. Adult mosquitoes can be fed with a sugar solution soaked into cotton wool or with wet raisins.

Only a few species can be successfully reared in the laboratory for several generations, due to the fact that only a few species mate readily in artificial small cages. However, artificial techniques of mating are sometimes used. Males are glued on the edges of glass Petri-dishes and decapitated with small scissors. Then the tip of the abdomen of the anesthetized female is moved towards the tip of the abdomen of the male at an angle of 45° for grasping and copulation. Afterwards, the female is transferred to a cage and fed with blood (Cosgrove et al. 1994). Some researchers feed the mosquitoes on themselves, or on small mammals such as mice or chickens. A license may be required for such use of laboratory animals. A few species are able to mate in small cages and to lay eggs without a blood-meal, such as the *Cx. p. pipiens* biotype *molestus*. This mosquito is therefore very often kept in cultures where containers with water for laying the egg rafts, and a sugar solution or raisins as food, are provided.

Females can also be collected in the field from a host when they take their blood-meal. A glass tube which is covered with cotton wool at the bottom is carefully placed onto the female when it starts to suck blood. When the blood-meal is completed (about 2 min) the glass container with the blood-fed mosquito is covered with a nylon mesh and kept for several days at about 20°C. Raisins can be placed on the nylon mesh as additional food. The cotton wool is kept wet to keep the humidity high, and when *Aedes/Ochlerotatus* species are captured the wet cotton could also serve for egg laying. Females of *Culex* and *Anopheles* need a small water body for egg laying. The time from the blood-meal until the deposition of the eggs can be measured. The number of eggs can be counted, the morphology of the eggs can be described, or hatching experiments can be conducted with defined egg batches.

The structure and colouration of the chorion of *Anopheles* eggs is of crucial importance in distinguishing certain species, *e.g.* within the *Anopheles Maculipennis* Complex. To enable the females to lay eggs, they need to be handled gently both during sampling and in the laboratory. The engorged females of the complex can be easily obtained within animal shelters in any part of a year, depending on the overwintering behaviour of the species. Test tubes (diameter 15 mm, length 75 or 150 mm) lined with wet 10 mm wide strips of filter paper can be used for direct sampling of single females within shelters. After the

specimen has been captured, the tube is closed with a cotton wool ball and transferred to the laboratory. For successful egg laying, the filter paper needs to be kept damp until the end of the gonotrophic cycle.



**Fig. 4.7** Arrangement of culicine larvae on a slide

## 4.4.2 Preserving Mosquitoes

### 4.4.2.1 Larvae

The best method for rapidly killing larvae is to drop them into hot water (60°C), which leaves the specimens in a nicely distended condition. After a few minutes they are transferred to a preserving fluid, which can be MacGregor's solution (10 ml 5% borax, 80 ml distilled water, 10 ml formaldehyde and 0.25 ml glycerin) or 70% alcohol, with 1% glycerin added if specimens are to be prevented from drying out when in long-term storage. The specimens can be examined by means of a dissecting microscope in the preserving fluid, or stored in small glass containers with rubber-lined screw caps to avoid fast evaporation. For quick reference, the glass containers must be clearly labelled. Labels in pencil on a strip of paper can be put into the bottle; those in waterproof ink can be stuck to the outside of the bottle. The label should contain at least the location of collection, type of breeding site, date of sampling and name of the collector.

For permanent preparations, larvae are mounted in Caedax, Canada balsam, Eukit, Euparal or Histomount. For this purpose, the larvae should first be transferred with some fluid into a solid, small glass vessel. When very late fourth-instar larvae are mounted it may be necessary to macerate the larvae before dehydration. It is recommended to leave the larvae in a 5% solution of potassium or sodium hydroxide overnight, or as an alternative, to heat them below the boiling point in the same fluid for about 10 min. The caustic solution should be removed and 5% acetic acid added for several minutes to neutralize the liquid. Then the larvae should be dehydrated in graded ethyl alcohols (50, 70, 80, and 96%), iso-propanol and xylene, with each step lasting at least 30 min. The larvae should be kept for 10 min in a 1:1 mixture of xylene and mounting media when Caedax or Canada balsam is used. Xylene is not needed when mounted in Euparal, which is the most

widely used material. The fluids must be carefully pipetted off and then replaced with the next fluid by means of a Pasteur pipette to avoid damaging the delicate setae, which are important taxonomic characteristics. Finally, a large drop of viscous mountant is to be put on a slide before the larva is transferred by featherweight forceps.

When culicine larvae are mounted, it is necessary to cut off the last abdominal segments and to arrange this part alongside the rest of the body. Using two micro-needles the specimen should be worked to the slide surface, dorsal side upwards, with the head and the end of abdomen (lateral side up) toward the long-side and across the slide (Fig. 4.7). Then, a coverslip is gently placed onto the specimen, starting from the abdominal end of the larva rather than the head, which will help keep the larva in position. If needed, more fluid mountant should be added from the side to apply the coverslip.

A label with data for the specimen, including information on the location of sampling and breeding site as well as date of sampling and name of the collector, should be placed to the right of the specimen. The label with the name of the specimen and instar stage should be placed on the left, with the specimen between the two labels. The same procedure can be used to preserve larval skins.

### 4.4.2.2 Pupae

Slide mounts of pupae and pupal skins can be made in the same way as described above. When adults are reared from larvae and pupae it is useful to keep the adults, the fourth-instar larval and the pupal skins together in the collection.

To mount the pupal skin, the cephalothorax should be cut from the abdomen and cut open ventrally to lay flat on the slide alongside with the abdomen (dorsal side up).

#### 4.4.2.3 Adults

Adult mosquitoes are preferably killed with ether or ethyl acetate vapour. This is done by placing a small piece of cotton, which has absorbed several drops of the killing agent, into a glass vessel containing the mosquitoes.

Some field workers also use cigarette smoke in the absence of other killing agents, or a killing bottle with a layer of Plaster of Paris containing cyanide on the bottom. The dead mosquitoes are pinned and kept dry. The mosquitoes should be pinned soon after killing because the soft bodies can be more easily handled than fragile, dry insects, which tend to disintegrate. For this reason, dry specimens should be relaxed in a humidity chamber prior to pinning. The dried mosquitoes should be separated from one another on a filter paper in a Petri-dish and kept for 12–24 h in a desiccator containing water to soften the specimens. Care should be taken to avoid drops of condensation falling on the specimens.

The mosquito should then be removed by fine-pointed forceps and pinned using the double-mounted method. In this method, a large stainless steel pin about 38 mm long, no. 2 in thickness, holds the stage where the mosquito is mounted. The mosquito is attached with a small (about 12 mm) stainless steel point (minuten pin), and usually two labels with all necessary data similar to those for permanent preparations of larvae (Fig. 4.8).

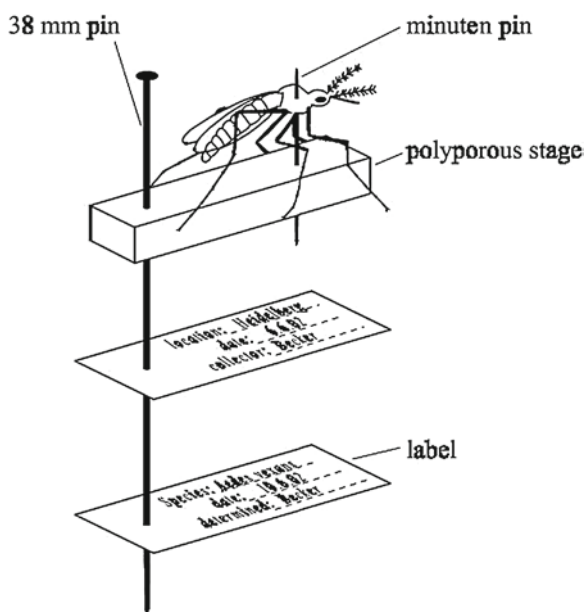


Fig. 4.8 Mounted adult mosquito

Stage materials can be strips of cardboard, cork, polystyrene board, polyporous or expanded polyethylene foam. The narrow strips should be equal in size, usually 13 mm long and a few millimeters in width. First, the minuten pin for the mosquito is put through the stage about 2 mm from one end. Then, the mosquito is placed upside down on a sheet of polystyrene; the prepared stage holding the minuten pin is picked up with entomological pinning forceps; and the end of the minuten pin is inserted from the ventral side into the thorax of the specimen between the legs, pushing through until the point of the pin reaches the scutum. The head of the mosquito should face away from the end of the stage, and the abdomen and legs along the stage. It is recommended to pull the minuten pin through the stage so that the abdomen of the mosquito remains horizontal to the stage, but avoid arranging the legs, as they could be damaged. A gentle puff of air from behind the mounted specimen will spread wings into a position so that they do not cover the abdomen. The stage should be carefully held between the fingertips of one hand by the end in which the large pin will be inserted. About 1 mm from the end of the stage, the large pin should be pushed through until the upper third of the pin remains above the stage. This allows room for holding the mounted specimen and for putting the two labels below the stage. Labels should be of good quality card and written in India ink, using a fine pen of uniform size and always attached to the pin in the same position. On the label pushed next to the stage, the locality of sampling, date and collector's initials are recorded. The label with the identity of the specimen should be placed below this. A pinning block is useful for a uniform and neatly presented collection of specimens (Snow 1990).

A simpler and widely used method, described below, also provides good protection to the mosquito and provides easy handling of the mounted mosquito (Bohart and Washino 1978). The freshly killed, frozen or newly relaxed mosquito is attached in a horizontal position by the left pleuron to a drop of clear fingernail varnish on the tip of a triangular cardboard or cork strip, of at least 7 mm in length, which is located at the top of a 38 mm pin. The scutum should face away, and the legs towards the pin. Then, the cardboard or cork strip is pulled down to about two-thirds of the pin's length, to leave enough room for the two labels.

After drying for about 1–2 days (if possible in a refrigerator) the insects should be transferred into a

wooden cork-lined storage box with a fumigant (*e.g.* crystals of naphthalene) to avoid pest damage.

The external genitalia of males are sometimes essential for identification. After the mosquito is killed the tip of the abdomen is cut between segment VII and VIII with optical scissors or fine forceps. The end of the abdomen is placed in a solution of warm sodium or potassium hydroxide for about 15 min for maceration. Then, the genitalia are transferred to glacial acetic acid in order to neutralize the maceration fluid and to dissolve any remaining fat droplets. They are dehydrated and mounted as described for larvae (see Sect. 4.4.2.1). To obtain the best view of the genitalia, they should be placed with the ventral surface facing upwards before the cover slip is placed in position. The balsam should be viscous enough and in sufficient quantity to ensure that the specimen is not flattened. The remainder of the male is pinned, and both the slide preparation and the pinned male are labelled to indicate their relationship.

#### 4.4.3 Mosquito Blood Meal Identification

Identification of host preferences of mosquitoes is central to understanding their potential to act as vectors of human diseases. Although animal and human landing rate catches, or use of baited traps, will give useful information on this behaviour, analysis of the source of the blood meal in captured mosquitoes is widely used to understand the natural feeding preferences of mosquitoes.

Precipitin testing was introduced by Bull and King (1923) and became the standard technique used over many years. The technique typically involved the addition of the unknown blood meal to a narrow tube (a capillary is used by some researchers) in which a layer of antiserum has already been placed at the bottom of the tube. The formation of a ring at the interface of the two reagents indicates a positive reaction, *i.e.* the blood meal is from the same species as the antiserum. Other serological techniques that have been reported, but less widely used, include the fluorescent antibody technique (Gentry et al. 1967), and the latex agglutination test (Boorman et al. 1977).

Since the 1980s however, techniques based on enzyme linked immuno-sorbent assay (ELISA) have

become more widely used. Burkot et al. (1981) initially described an indirect micro titration technique in which blood meals taken by *Ae. triseriatus* could be identified to species within rodents and canines. The period between feeding and analysis was critical, with 100% of the blood samples being immuno-positive within 8 h of feeding, but by 16 h after feeding, only 40% of samples were immuno-positive. Gomes et al. (2001) compared precipitin and ELISA techniques and found that the precipitin showed greater specificity, while the ELISA test showed greater sensitivity.

Most recently, a number of DNA techniques have been used for blood-meal identification, including DNA profiling. Using DNA profiling of four polymorphic human loci, Benedictis et al. (2003) established profiles for the residents in a community, and then allocated blood meals taken by wild *Ae. aegypti* mosquitoes to individual residents. Cytochrome b has also proved useful in establishing the identity of mosquito blood meals. Lee et al. (2002) used a polymerase chain reaction heteroduplex assay to separate blood meals taken by wild caught *Cx. tarsalis* from a large numbers of bird species. Results showed that it was possible to determine avian hosts for up to 7 days postfeeding, while blood from mammalian hosts with non-nucleated erythrocytes had to be assayed sooner.

#### 4.4.4 Methods for Measuring the Physiological Stage

The vector capacity of a mosquito species depends to a great extent on the ability of the females to have several blood-meals, which in turn enables the vector mosquito to transmit pathogens or parasites from a previous infection. In this respect, the knowledge of the physiological stage of a mosquito is of epidemiological importance. It can be determined by the assessment of the number of gonotrophic cycles through which a mosquito female has passed. The gonotrophic cycle for species that require a single blood-meal for each batch of eggs, is defined as the time period needed from the blood-meal until the oviposition of the eggs. After a blood-meal, the basal oocytes within the ovarian tubes (ovarioles) in the ovaries develop into mature eggs. As a result, the

epithelium surrounding a single ovariole is stretched by the expanding oocyte. When the egg is deposited, the epithelial sheet that surrounded the egg during its development shrinks, leaving a residual lump or a knot-like dilatation. Following the next blood-meal the next sequence of oocytes located more distally in the direction of the germarium of the ovariole, continue their development. After the deposition of the next batch of mature eggs a second series of dilatations remains in the ovarioles. Therefore, the number of dilatations corresponds to the number of previous ovipositions. This allows an estimation of the longevity of the individual mosquito, and its importance as a vector. The more the number of blood-meals, the greater is the possibility for the mosquito to acquire and transmit pathogens.

Furthermore, the age or the physiological stage of a mosquito can also be determined by examining the changes in the ovarian structure. During the first gonotrophic cycle the coiled ends of the tracheoles are unwound by the swelling of the ovaries. These uncoiled tracheoles indicate parity (eggs developed) whereas nulliparity (no eggs developed) is indicated by still coiled tracheoles.

For the examination of the ovaries, the mosquito must be dissected. After the female mosquito is killed, it is placed in a drop of water on a microscope slide. The mosquito is held with a fine mounted needle on the thorax, and with a second needle the intersegmental membrane, between abdominal segments VII and VIII, is cut. The gut and the ovaries can then be pulled out with forceps. The gut is cut and the ovaries are inspected. For the examination of the dilatations of the ovarioles, the ovarian sheet has to be removed and the ovarioles have to be separated with a needle. For examination using a microscope, the ovaries are covered with a coverslip.

The appearance of the abdomen can also be used to determine whether a mosquito has engorged blood, or to assess the stage of egg development. When the abdomen of the mosquito is thin it is unfed, or has passed through a gonotrophic cycle. In freshly fed females the abdomen (midgut) is filled with red blood, which gradually becomes dark red. As the eggs develop, the ovaries increase in size, and the red colour disappears. In the gravid females, in most cases a trace of dark blood is left and ovaries occupy almost the whole abdomen.

## **4.4.5 Morphological and Taxonomic Techniques**

### **4.4.5.1 Morphological Studies**

Although the morphological characteristics of most of the mosquito species are described, there is still need for further studies on character variation within individuals and populations of a species. Not only the number and size of scales and setae can vary but also the colouration, size or other features. In particular, the comparison of species from different areas can reveal new results on variation. However, in some cases the morphological features are not sufficient for species identification, and therefore other techniques have to be employed.

### **4.4.5.2 Cytodiagnostic Methods for the Identification of Sibling Species**

The morphological similarity of the members of mosquito species complexes (so-called sibling species) in the larval and/or adult stages, required the development of tools for species identification which were not based on external morphological characters. Genetic mapping is of great importance to distinguish between species, subspecies or ecotypes, and for investigations of population genetics. Various types of maps can be constructed (Hillis 1996):

- (a) Preparations of polytene chromosomes made from larval salivary glands or ovarian nurse cells, provide the basis for physical maps, where the specific banding patterns of polytene chromosomes are illustrated in photomicrographs or drawings. For several decades polytene chromosomes have been particularly useful in chromosomal studies, due to their specific properties.
- (b) Genetic or linkage maps are maps where the relative position of genes and the distances between them are plotted.
- (c) Restriction maps and
- (d) Whole genome or molecular maps.

The typical karyotype of culicines consists of three pairs of homomorphic chromosomes. Typical for the karyotype of anophelines is one pair of heteromorphic sex chromosomes, as well as two pairs of autosomes.

In certain mosquito tissues the chromosomes replicate repeatedly without mitosis-like events, so that hundreds of sister chromatids remain synapsed. This multiple DNA duplication is accompanied by alignment and condensation of the DNA strands, thus forming specific patterns of bands and interbands. These banded polytene chromosomes occur in larval and adult tissues, such as the salivary glands and midgut epithelium, the malpighian tube cells, as well as in the ovarian nurse cells of adult females. Banding sequences can change by rearrangements within or between chromosome arms, *e.g.* by inversions or translocations. Chromosome aberrations are typical of certain mosquito populations. Banding patterns can therefore be used as markers for distinguishing among species and ecotypes. Differences in banding, *e.g.* due to inversions, have proved to be a useful tool in distinguishing members of sibling species complexes, and for resolving interrelationships between species.

The polytene or so-called giant chromosomes are easily seen using a microscope at high magnification (view at  $\times 1,000$  with oil immersion lens and phase contrast). In the culicine mosquito *Cx. p. pipiens* and the anopheline mosquitoes, high quality squashes of polytene chromosomes can be obtained from the salivary glands of the fourth-instar larvae. The specimen is placed in 5% Carnoy's fixative (mixture of one part of glacial acetic acid and three parts of 95% ethanol) on a glass slide. The abdomen is cut off and the thorax is slit open dorsally along the mid-line. By pulling on the head, the glands attached to the head are pulled out of the thorax. Then the salivary glands are cut free and transferred to a slide in a droplet of Carnoy's fixative, stained for about 5 min by adding two droplets of a mixture of acetic and lactic acids with orcein (2% by weight of orcein powder is dissolved in 1 part 85% lactic acid and 1 part glacial acetic acid, and this concentrated stain is then again diluted 1:3 with 45% acetic acid). The tissue is carefully squashed by placing a coverslip on top of the preparation and pressing it with a finger. Thus the cells are broken and the chromosomes are spread. Species, sex and karyotype composition of larvae from various habitats can be determined by the examination of the chromosome preparations with a microscope. This technique can be successfully employed for population studies on the Anopheles Maculipennis Complex. Methods for making polytene chromosome preparations have been described by French et al.

(1962), Green (1972), Hunt (1973), Green and Hunt (1980) and Graziosi et al. (1990).

#### 4.4.5.3 Biochemical and Molecular Methods in Studies on Systematics

Taxonomy utilizes homologous morphological characters for the identification of species and evaluation of relationships among the taxa. The phenotype depends both on the genotype and environmental conditions, so that even characters depending on a monomorphic gene locus, may vary between individuals or populations of a distinct species. Consequently, separation of closely related species may be difficult using variable, quantitative characters.

Molecular methods for analyzing variation in DNA, and biochemical investigation of gene products, are widely used in support of taxonomic studies. Generally, investigations that incorporate both morphological and molecular approaches will provide the most substantiated descriptions and interpretations of diversity within and between the taxa.

In taxonomy and evolutionary research, protein-electrophoresis is a widely used and highly efficient technique. For this, homogenates of animals or organ samples are applied to a gel (polyacrylamide, agarose or starch) and exposed to an electrical field under ionic buffer conditions. The migration of dissolved proteins is determined by their charge, size and shape. Depending on these factors, the proteins segregate to bands in the gel and then are visualized by unspecific or, in the case of enzymes, by the substrate transformation coupled staining. Differential migration of the same protein is the result of a change of its amino acid sequence. Proteins are primary gene products, so changes in their amino acid sequence are caused by changes in the underlying DNA sequence, *i.e.* there are different alleles on their gene locus. When various species possess different alleles exclusively, these fixed alleles may be used as genetic markers and enhance the identification of species by morphological traits. By investigation of several proteins, the resulting data are suitable for the quantification of genetic variation within populations (*e.g.* degree of polymorphism and heterozygosity), detection of species boundaries, phylogenetic relationships and evolutionary processes (Harris and Hopkinson 1976).

Investigation of DNA has been driven particularly by the development of the polymerase chain reaction (PCR) that allows the amplification of small amounts of DNA million-fold, and makes DNA accessible for many purposes. There are several methods of DNA analysis: DNA–DNA-hybridization estimates the amount of sequence divergence between genomes, but provides no information on the structural background of differences. Fragment and restriction analysis such as variation in fragment size (*e.g.* VNTR), restriction site variation (RFLPs, RAPDs, DNA fingerprinting) and others, provide some information about genome organization, and are suitable for detection of individual and species relationships. By DNA sequencing, exact information about the genotype is obtained that allows solid conclusions to be drawn about molecular evolution and phylogeny (Murphy et al. 1996).

All these techniques provide important and useful information about biodiversity and evolution, but the techniques used in any particular study should always be selected according to the overall aim and resources of the investigation.

## **4.5 Assessing the Activity of Insecticides and Repellents on Mosquitoes**

### **4.5.1 Insecticide Susceptibility Testing**

The WHO has developed test kits for the assessment of insecticide susceptibility of adult and larval mosquitoes. The kits typically consist of containers and tubes for holding and manipulating the insects, together with various insecticide treated substrates which are commercially available. Insecticides from the main insecticide classes, including organochlorines, organophosphates, carbamates, and pyrethroids are available. Discriminating doses, *i.e.* those concentrations that kill susceptible individuals, but allow survival of resistant individuals, have been established for the most important species, and are available. WHO typically sets the discriminating dose as double the  $LC_{99}$  obtained by extrapolation of the log-probit line. Standardized data recording forms are available, and these may be returned to

WHO to assist with centralized monitoring of insecticide resistance status (WHO 2001a).

#### **4.5.1.1 Assessing the Susceptibility of Adult Mosquitoes**

The adult mosquito susceptibility kit consists of two interconnected plastic tubes, separated by a gate mechanism. One tube is lined with clean paper, and the other lined with paper treated with a discriminating dose of the chosen insecticide. Typically, adult mosquitoes will be collected from the area under study, or reared from immature stages. A batch of mosquitoes is introduced into the clean tube, and then moved through the gate onto the insecticide treated paper in the other tube. After a fixed exposure period (ranging from 0.5 to 4 h, depending on mosquito species and insecticide) on the insecticide, the mosquitoes are then moved back through the gate onto the clean paper. Mortality is then assessed after a fixed period, typically 24 h after exposure. Control mosquitoes are exposed to untreated paper, but otherwise handled identically. Where mortality is in the range 98–100%, the mosquitoes are considered susceptible. Mortality in the range 80–97% suggests the possibility of resistance that needs confirmation, while mortality of <80% suggests resistance (WHO 1998b).

The technique may be used to determine the susceptibility status of mosquitoes prior to the start of treatment programmes, and should be used at intervals to detect any changes in the susceptibility status of the insects over the course of the programme, and as part of the investigation of any local control failures.

#### **4.5.1.2 Assessing Susceptibility of Larval Mosquitoes**

Laboratory efficacy tests to establish the activity of different larvicides against a particular species, or to assess the efficacy of a given insecticide against different strains, may be relatively easily carried out in the laboratory. The larvicide is prepared as a stock solution (typically 10 g insecticide/l concentration), and then diluted to give a range of relatively widely spaced serial dilutions, spanning the likely active concentration. Replicate disposable



cups or other containers are filled with about 100 ml of each larvicide concentration. For conventional and bacterial larvicides, batches of 25 third or fourth-instar larvae are introduced to each container, and mortality assessed 1 or 2 days later. For insect growth regulators (IGRs), the test will run longer to allow for pupation and adult emergence, and the larvae will need feeding. Efficacy of IGRs is typically expressed not as mortality, but as % inhibition of adult emergence (WHO 2005b).

#### 4.5.1.2.1 Assessing the Potency of Microbial Larvicides

Standardized methods have been developed to determine the  $LC_{50}$  values for microbial larvicides using standard formulations (e.g. IPS 82 for *Bacillus thuringiensis israelensis* tests and SPH 88 for *B. sphaericus* tests) as reference products. The activity of IPS 82 has been assigned the value of 15,000 ITU/mg and of SPH 88 the value of 1,700 ITU/mg).

##### Bioassay Procedure

The following procedure for bioassays is recommended in order to allow an accurate and standardized measurement of the potency of the *B. thuringiensis israelensis* and *B. sphaericus* products:

Fifty mg of the standard microbial agent powder is weighed and poured into a 20 ml penicillin flask, to which 10 ml of deionized water and 15 glass balls (6 mm diameter) are added. This suspension is vigorously homogenized on a flask shaker for 10 min at 700 strokes/min. Only one stock dilution is made in a test tube of 22 mm diameter: 0.2 ml of the initial suspension are added to 19.8 ml of deionized water. The test tube is agitated for a few seconds on an agitator at maximum speed.

From this dilution (50 mg of product/l), subsequent dilutions are immediately made in plastic cups which have been already filled with 148 ml of deionized water; using precision pipettes or micropipettes. Aliquots of 120, 90, 60, 30 and 15  $\mu$ l are added to the cups in order to obtain final concentrations of 0.04; 0.03; 0.02; 0.01 mg and 0.005 mg/l respectively of IPS 82 (*B. thuringiensis israelensis*) or SPH 88 (*B. sphaericus*).

Four replicate cups are used for each concentration and for the control.

Twenty-five early fourth-instar larvae of *Ae. aegypti* (when *B. thuringiensis israelensis* is to be tested) or 25 early fourth-instar larvae of *Cx. pipiens* (when *B. sphaericus* is to be tested). Each batch of larvae in 2 ml water is put into each cup using a Pasteur pipette. The use of early fourth-instars is very important, and should be strictly adhered to. A small amount of food (ground mouse diet) is added to each cup in order to avoid excessive mortality caused by starvation when the bioassay is run longer than 24 h.

A comparable initial suspension and series of dilutions are prepared in the same way with the test preparations, but with a range of dilutions exceeding that of the standard, to ensure that a reliable regression line can be obtained.

The results of an initial range-finding bioassay using only two widely spaced concentrations of the test material, can be used to select the concentrations used in the full assay more accurately, and as a partial replicate of the full bioassay.

Each series of bioassays will involve at least 500 larvae exposed to the standard treatment; 100 larvae as controls, and 500–1,000 larvae exposed to the test preparations. All tests should be conducted at 25°C ( $\pm$  1°C).

The mortality data are recorded after 24 and 48 h by counting both dead and living larvae. The second reading is useful in routine work to confirm the previous data, and to check for the possible intervention of factors other than microbial toxins. If some pupae emerge, they should be taken out and not included in the mortality count.

When control mortalities exceed 5%, the percentages observed in the treated containers should be corrected using Abbott's formula (Abbott 1925). Test series with control mortalities greater than 10% should be discarded. Mortality concentration regression lines should be drawn on logarithmic paper. Then the  $LC_{50}$  of the series treated with the standard and with the test preparations are read and the potency (titre) of the test material determined by the following formula:

$$\frac{\text{activity of the standard (ITU)} \times LC_{50} \text{ of standard}}{LC_{50} \text{ of test preparation}}$$

The potency or titre of the product is expressed in ITUs (International Toxic Units). For improved precision such bioassays should be repeated on at least three different days, and the standard deviation calculated.

A very wide range of literature exists on the use of larvicides and assessments, and WHO (2005b) provides a useful overview.

### **4.5.2 Assays of Insecticide Deposits on Surfaces (e.g. Walls or Nets)**

The WHO has also established standardized kits and procedures for the measurement of the efficacy of deposits of insecticides applied to walls and bed nets (WHO 2006). The test kit consists of a transparent plastic cone, with a hole at the apex. The cone is fixed to a surface, such as an insecticide-treated wall, using adhesive, nails, rubber bands, etc. A strip of plastic foam may be fixed to the base of the cone to ensure a tight fit against an uneven wall surface. Adult mosquitoes are then introduced into the cone through the hole at the apex, which is then plugged with cotton wool. After a fixed exposure period, typically 1 h, the mosquitoes are removed with an aspirator, transferred to a clean holding container such as a net-covered paper cup, and mortality assessed after 24 h. Control mosquitoes are exposed to an untreated wall, but are otherwise handled in the same manner as the treated mosquitoes. Procedures for assaying treated bed nets are essentially similar (WHO 2005a).

Such tests enable the quality of treatments to be assessed, and allow the longevity of the insecticide deposit to be determined (WHO 2006).

### **4.5.3 Assays of Efficacy of ULV Insecticide Treatments in the Field**

The efficacy of ULV adulticide treatments is typically assessed using caged mosquitoes positioned in the treated area. Cages may be cuboidal, cylindrical or be comprised of modified WHO adult mosquito susceptibility test kits, but all will have the maximum possible area of open mesh, to allow penetration of air-borne droplets. Cages may be left in the open, or positioned under vegetation or within buildings (Perich et al. 1990). There are concerns that the mesh of the cage may prevent insecticide droplets reaching the mosquitoes inside, while there are also concerns that insecticide droplets deposited on the cage mesh may then be available to the insects by tarsal contact. To avoid the risk of artificial contamination, some researchers transfer the mosquitoes from the exposure cage to a holding cage shortly after treatment (Bunner et al. 1989). After exposure in the field, treated mosquitoes would nor-

mally be transferred to the laboratory, supplied with sugar solution on a cotton pad, and mortality recorded at 24 h after exposure. Control mosquitoes may be located outdoors in an untreated area for a similar time to the treated mosquitoes, before being returned to the laboratory for holding and assessment.

### **4.5.4 Assays of Efficacy of Mosquito Repellents**

Personal protection is an important component of any programme intended to reduce nuisance biting or the transmission of mosquito disease. A very wide range of compounds have been proposed and evaluated as mosquito repellents, with botanical extracts in particular having been widely evaluated.

A number of standardized evaluation techniques have been established, but in addition, a number of alternative approaches have been developed by individual researchers.

In broad outline, the standardized techniques typically involve application of a known dosage of the test repellent to a human volunteer's arm (research involving human volunteers will in many countries be covered by legislation and will require ethics approval), and then exposing the treated skin to unfed mosquitoes (ASTM 2006a; US EPA 1999; WHO 1996b). The time of first bite, or the number of bites over a fixed time, are recorded. Depending on the objectives of the test, a range of doses are evaluated in order to determine the minimum effective dose, or a given dose is evaluated repeatedly over time in order to determine the duration of effectiveness. Initial tests will be carried out in a laboratory, but field tests will be required to show the efficacy under realistic conditions (ASTM 2006b). Tests should be very carefully designed to eliminate or compensate for factors which may affect results, including variation in the human subject due to, e.g. washing, activity, perspiration or underlying human to human variation, and variation in the mosquitoes due to age, parity, previous sugar feeding or larval diet, and other factors including cage size and light intensity (Barnard 2005).

Alternative techniques for the evaluation of repellents include the use of wind tunnels with human breath as an attractant (Sharpington et al. 2000), or the

use of a membrane feeding technique as opposed to a human arm (Cockcroft et al. 1998).

## 4.6 Conclusion

The above-mentioned techniques represent those that are used more commonly in mosquito research. Mosquitoes are a group of insects that have attracted considerable research attention not only because of their intrinsically

interesting and specialized biology, but also because of their major impact on human health. In practice, research and operational programmes will use a selection of these techniques to build up a better understanding of the insect, the way in which it relates to its environment, and the impact of control techniques. Despite the extensive literature on research methodology, new techniques are regularly developed, as new questions are asked, or new technology becomes available. Researchers are encouraged to review the literature, both historical and current, before finalizing their planned research programme.



## Chapter 5

# Morphology of Mosquitoes

### 5.1 Adults (Fig. 5.1)

Mosquitoes differ from all other members of the Nematocera by having a long scaled proboscis (labium and stylets), always longer than the thorax, which projects forward together with the maxillary palps (Fig. 5.1). The latter are as long as or longer than the proboscis in males of most species and females of the genus *Anopheles*. The head, thorax, and abdomen are covered with scales and setae; the extent of coverage is genus specific. The legs, wing margins, and wing veins are typically clothed with scales. The closest resemblance of the body shape is found within the families of slender, long-legged crane flies (Tipulidae) and non-biting midges (Chironomidae), the latter often being mistaken for mosquitoes, especially around artificial lights at night. However, none of these families have mouthparts for piercing and sucking. The short mandibulate mouthparts of the Tipulidae are of the biting and chewing type, and articulated on the tip of a prolonged, beak-like gnathocephalon. The Chironomidae usually have a reduced gnathocephalon and biting mouthparts. In addition, the Chironomidae possess a conspicuously humped thorax, and often particularly long, forward-facing fore legs.

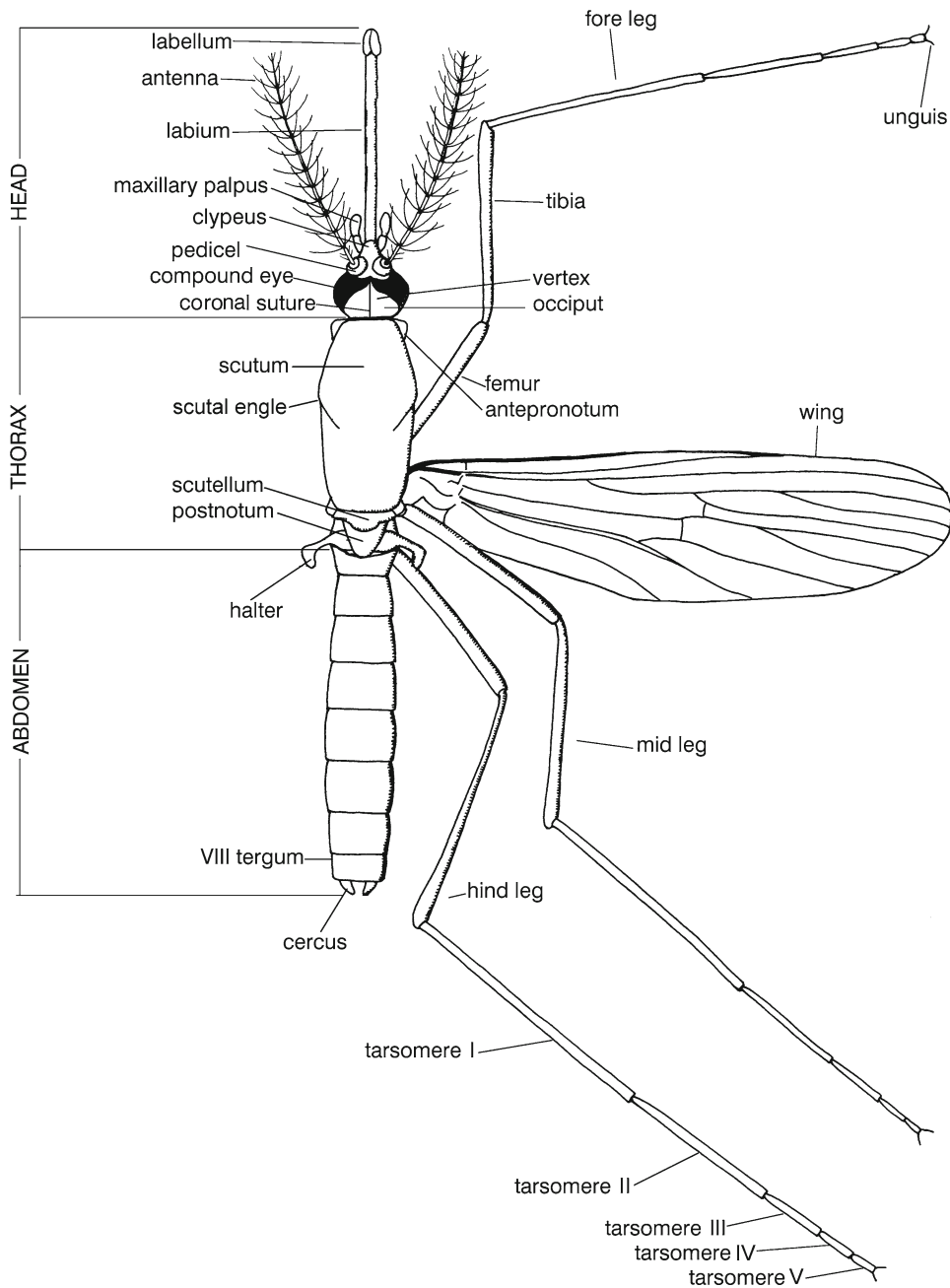
The males of most mosquito species clearly differ from the females by having plumose antennae and long, hairy maxillary palps (Fig. 5.2a,b). The first 12 flagellomeres of a male antenna bear dense, long setae, which are at least as long as the head capsule. The setae on the last flagellomere are shorter than the head capsule, and of similar length to those on the female antenna.

Unusually for Diptera, the integument of mosquitoes is quite extensively covered with scales. Essentially, scales are flattened setae containing pigment and often have a striate surface, which produces optical effects,

giving some mosquitoes a physicochemical or physical colouration. The shiny, metallic blue, green and purple appearance is mostly found within tropical species (e.g. *Haemagogus* spp., *Sabethes* spp.). In most other species the colour of the scales may vary from white to almost black but is usually referred to as pale or dark in the description of the species. Pale and dark scales can be intermixed wherever they occur. They may also be grouped together, forming the specific contrasting patterns, which will be referred to as rings on all legs, stripes on the scutum, and bands on the abdominal terga. Scale colouration can change in different light sources and after long-term storage. The overall body colour can be influenced by the colour of the integument which may shine through the scales. The abdominal terga and sterna are densely covered by scales in the subfamily Culicinae, while the sterna, and usually also the terga, are wholly or largely devoid of scales in the subfamily Anophelinae. Like all other Diptera, the mosquito head, thorax, and abdomen are covered with setae of different length, shape, and colouration which can have significant genus and species-specific taxonomic importance.

#### 5.1.1 Head (Figs. 5.2 and 5.3)

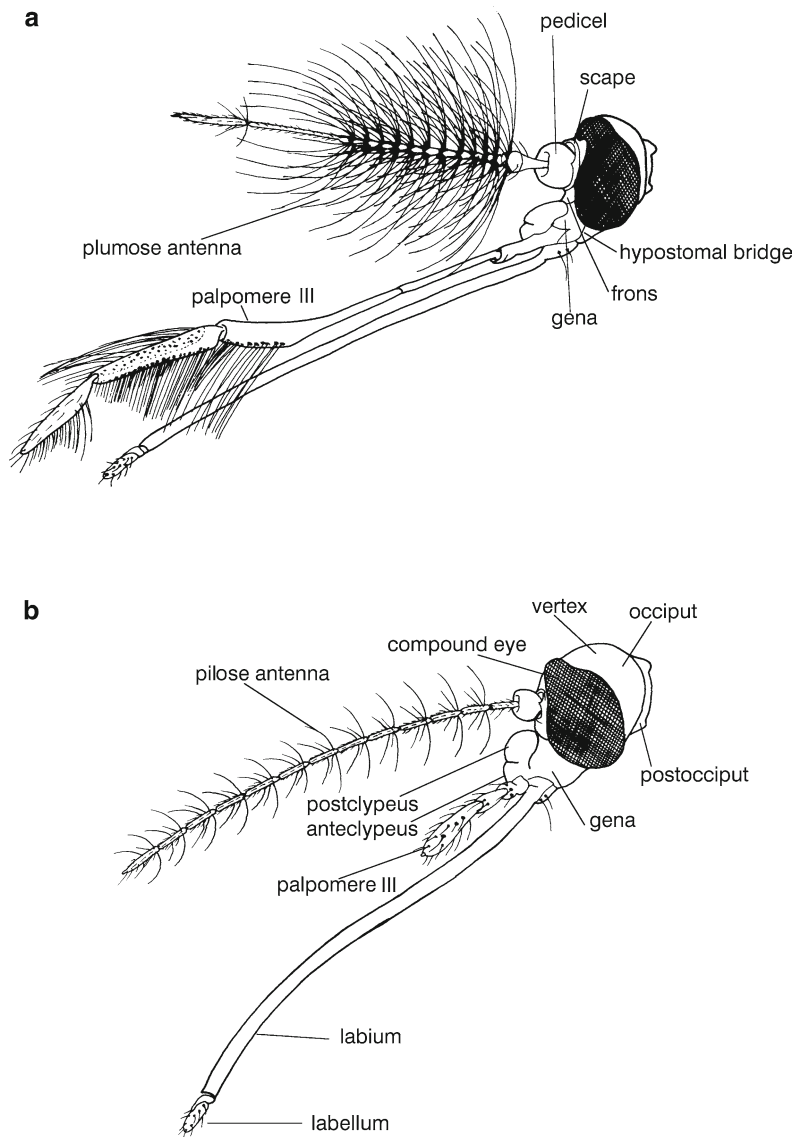
Mosquitoes, like all Pterygota (winged insects) and Thysanura, have annulated antennae. Their basal segment (scape) is collar shaped and hidden behind an enlarged, spherical second segment, the pedicel (Fig. 5.2a). In the Chironomidae and Culicidae the pedicel is specially enlarged to accommodate a highly developed mechano- and sound-receptor, the Johnston's organ. The remaining 13 divisions of the antenna together constitute the flagellum and are entirely without intrinsic muscles.



**Fig. 5.1** General outline of a female culicine mosquito (after Marshall 1938)

Consequently, those divisions are named annuli or flagellomeres instead of segments, the latter term being used only for the partitions of the insect body having intrinsic musculature. The antennae are usually subequal to the proboscis but could be distinctly longer in the genera *Deinocerites* and *Galindomyia*. The exterior of the head is composed of several sclerites

amalgamated to form the head capsule (Figs. 5.2 and 5.3). Compound eyes occupy a substantial portion of the head, and if they meet in the middorsal line, this condition is then called holoptic. The epicranial suture is quite indistinct. Its median part is called the coronal suture and the two branches above the scape are called the frontal sutures. The region of

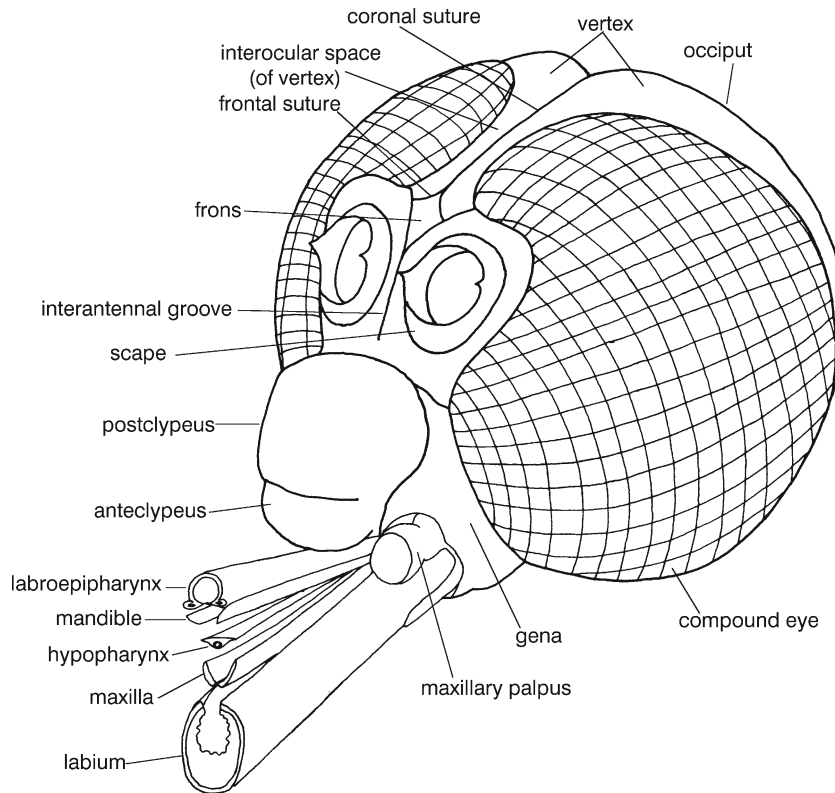


**Fig. 5.2** Head of (a) male; (b) female culicine mosquito (after Wood et al. 1979)

the head below the frontal sutures is the frons, above them lies the vertex, though the precise limits of the former areas are not easily defined. A narrow strip of the vertex between the compound eyes is called the interocular space. At the back of the head, between the vertex and the cervix, or neck, the occiput and the narrow ring-like postocciput are located. The vertex and occiput may be covered with truncated or forked erect scales and narrow, curved, or broad decumbent scales. The nearest structure, found immediately anterior to the frons, is the bulge of the gnathocephalon, called the rostrum in some other orders of insects.

The gnathocephalon is composed of the anteclypeus and postclypeus dorsally, of the genae laterally, and of the hypostomal bridge ventrally.

The proboscis, which is articulated with the gnathocephalon, is almost straight and cylindrical in many genera, but is laterally compressed with the apical third evenly curved downwards (*Armigeres* spp.) or strongly recurved and tapering towards the apex (*Toxorhynchites* spp.). In *Malaya* spp. the tip of the proboscis is inflated, turned upwards and setose. It is made of six slender stylets (Fig. 5.3), the labroepipharynx or labro-palatum (a more or less fused structure made of an outer wall,



**Fig. 5.3** Head regions and mouthparts (after Clements 1992)

the labrum, and an inner wall, the epipharynx), the paired mandibles, the hypopharynx and the paired maxillae. These are lodged in the groove of the elongated labium (prementum) which ends with a distally articulated pair of labella, thought to represent the modified labial palps (Figs. 5.1 and 5.2). Between the labella, the ligula, a short pointed lobe is situated, and in its shallow groove lies the tip of the stylet fascicle. The mandibles and the maxillary laciniae are modified into long piercing stylets. The latter, being stronger and serrated apically, are the main piercing organs used to perforate the skin of the host. The labroepipharynx, which bears three pairs of sensillae on its tip, and the other stylets form a fascicle which probes until it enters a blood capillary of a host. The saliva which acts as an anticoagulant, is injected through the hypopharynx. The blood is taken via a channel made mostly of the labroepipharynx. The mandibular and maxillary stylets are reduced or absent in females of *Toxorhynchites* spp. and *Malaya* spp. which feed on nectar. They have a functional labroepipharynx and hypopharynx. The labium, being only a protective sheath

for the stylets, is looped backwards during feeding and does not penetrate the host tissue. The labium of *Toxorhynchites* spp. females is rigid.

In males, both mandibles and maxillary laciniae are reduced or altogether lacking and cannot be used for piercing. Nectar and other sugary juices are simply imbibed through the tubular labroepipharynx.

The well developed maxillary palps are articulated below the clypeus, dorsolaterally to the proboscis (Figs. 5.1 and 5.2). Palps are divided into five divisions which are clearly visible in both sexes of *Anopheles* and in the males of almost all the other genera. The females of other genera frequently have an atrophied basal segment and apical annuli, therefore, their palps appear to have fewer segments. The three basal divisions are true segments, having intrinsic muscles (Clements 1992). Although the two apical divisions are not true segments they will be referred to as segments or all five as palpomeres, for the convenience in species description. The palps of most female mosquitoes are less than half the length of the proboscis (except in some *Armigeres* spp. and *Mucidus* spp.).



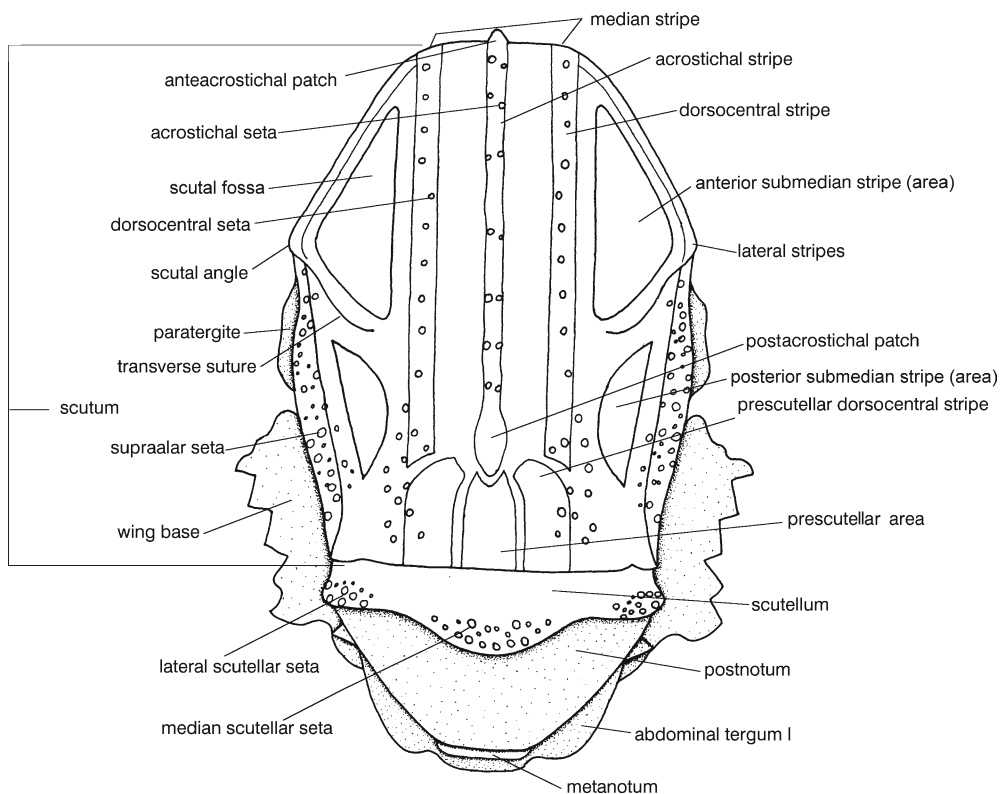
Only in anopheline females (except *Bironella* spp.) are the palps of similar length to the proboscis. In almost all male mosquitoes the maxillary palps are as long as, or longer than the proboscis. Exceptions can be found in males of genus *Uratonotaenia* and subgenus *Aedes*, where the palps are of similar length as in females.

### 5.1.2 Thorax (Figs. 5.4–5.8)

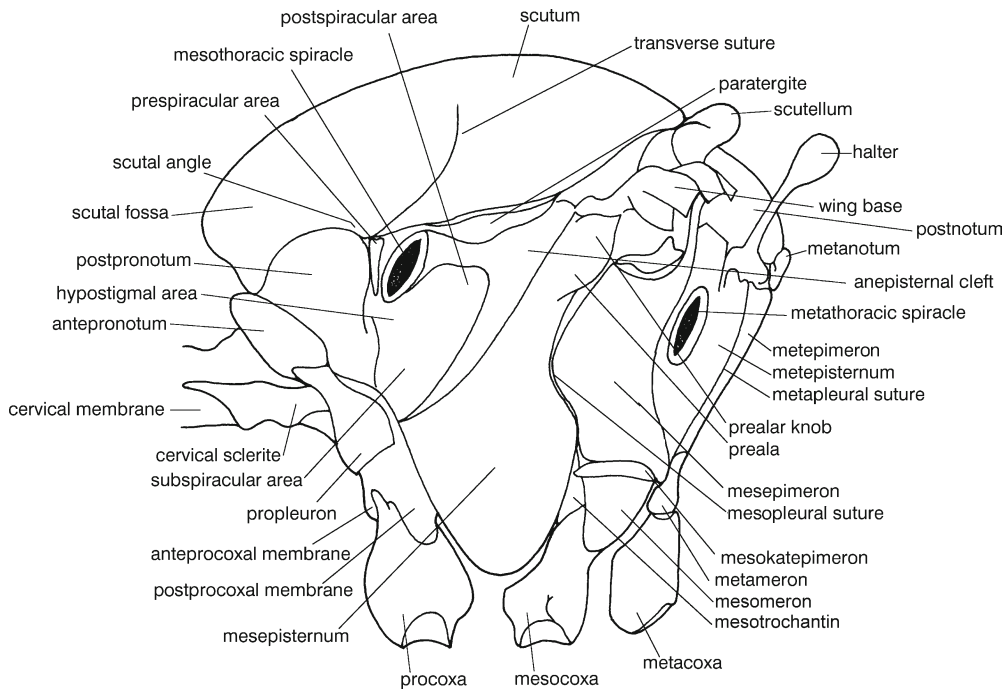
The three thoracic segments are known as the prothorax, mesothorax and metathorax. In mosquitoes and all other Diptera, where only the fore wings are used for flight, the mesothorax is the best developed segment. The prothorax and metathorax are reduced to little more than leg bearing collars fore and aft. The hind (metathoracic) wings are modified into gyroscopic organs, called the halteres. Each of the thoracic segments is divisible into four main regions, the dorsal tergum or notum, the ventral sternum and the two

lateral pleurae. These regions are differentiated into separate sclerites which are termed as tergites, sternites and pleurites respectively. The sclerites of the thorax, according to the segments to which they belong, are denoted by the prefixes pro, mes(o) and met(a).

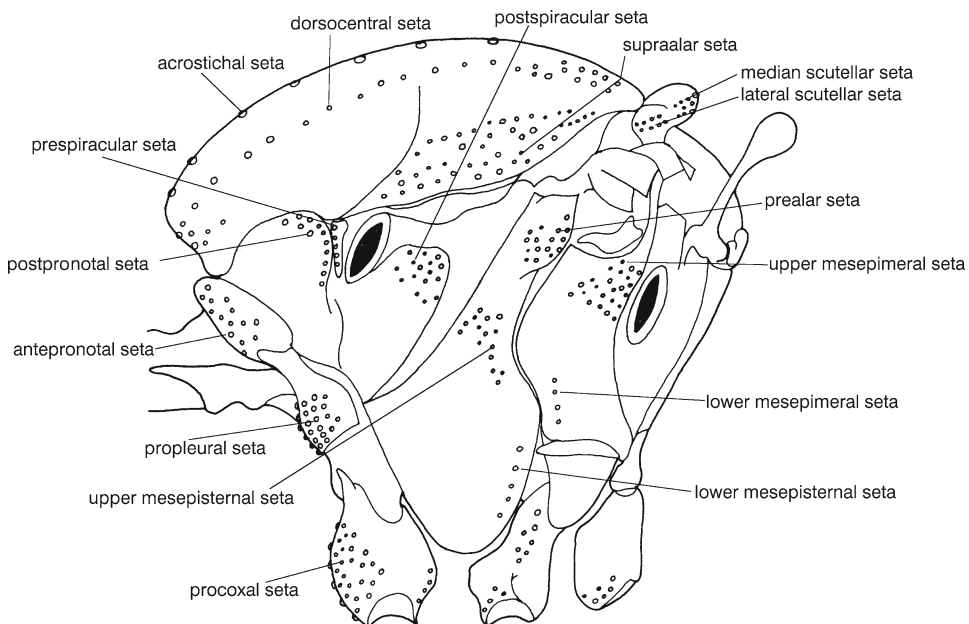
The pronotum is seemingly absent medially but well developed laterally. It is separated into an anterior lobe like division, the antepronotum, and a flattened posterior division, the postpronotum which appears to be part of the mesopleuron (Fig. 5.5). The lobes of the antepronotum are very large, protruding and closely approaching at the midline of the scutum in the genera *Haemagogus*, *Sabethes* and *Wyeomyia*. The mesonotum of Diptera is clearly subdivided into prescutum, scutum and scutellum, the fourth tergite, the postnotum, being generally hidden beneath the scutellum. The prefix meso is usually omitted for dipterous insects because those subdivisions do not occur in the weakly developed metanotum, and the pronotum is usually simple or bipartite. In mosquitoes (Figs. 5.4 and 5.5), the transverse suture separating the prescutum and scutum is not fully developed, hence the term scutum



**Fig. 5.4** Dorsal view of thorax



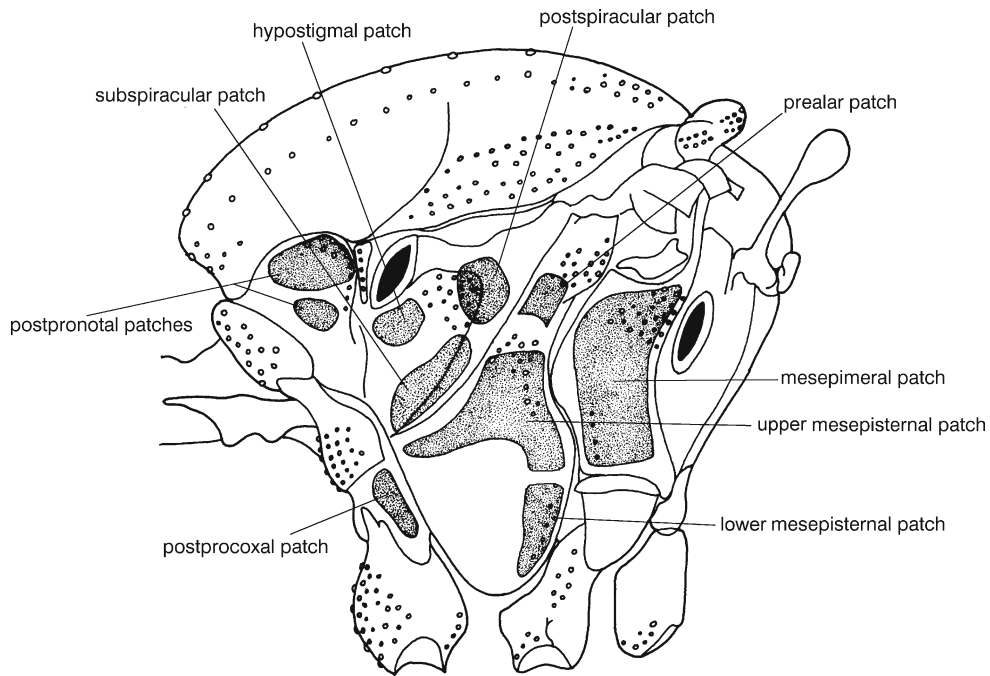
**Fig. 5.5** Lateral view of thorax – pleurites



**Fig. 5.6** Lateral view of thorax – setation

is used for both mesotergites. The scutum is the principal dorsal area of the thorax and is sometimes incorrectly referred to as the mesonotum in the mosquito literature.

The lateral scutal margin above the mesothoracic spiracle bears a tubercle named the scutal angle from which the transverse suture extends. The scutal fossa is



**Fig. 5.7** Lateral view of thorax – scale patches

a slightly depressed area anterior to the scutal angle, which lies between the scutal margin and the dorsocentral row of setae. A small elongated sclerite of mesonotal origin, the paratergite, is situated anterior to the wing root and in some species it is not apparent from above. The scutum is followed by the scutellum (Figs. 5.4 and 5.5). Its posterior margin may be evenly convex (genera *Anopheles* and *Toxorhynchites*) or three lobed (most genera of Culicinae and genus *Chagasia*). An additional, well developed sclerite of intersegmental origin, the postnotum, bears the phragma to which the dorsal longitudinal flight muscles are attached. The metanotum is reduced to a narrow, weakly sclerotized transverse band (Figs. 5.4 and 5.5). The metapostnotum is usually not visible.

The postpronotum usually bears a vertical row of setae close to its posterior margin, the postpronotal setae (Fig. 5.6). Sometimes they may cover the mesothoracic spiracle and can easily be mistaken for the prespiracular setae (an important diagnostic character for the genera *Culiseta* and *Psorophora*). It is therefore advisable to carefully focus on the point where the setae are inserted. Setae may be concentrated in several areas of the scutum or scattered over most of its surface. As far as the identification of the mosquitoes is concerned, the main setae are inserted in three

pairs of, more or less dispersed, longitudinal rows (Fig. 5.4). The setae of the acrostichal rows are articulated close to each other and to the median line. The second pair of dorsocentral rows of setae is more dispersed towards the end of the scutum. Finally two rows of scattered supraalar setae occupy the posterior parts of the lateral margins of the scutum. The scutellum in species of the genus *Anopheles* bears a uniform row of backwardly directed setae along its evenly rounded posterior margin. In the other genera that have a trilobed scutellum, the setae are arranged in three sets, a median and two groups of lateral scutellar setae. The postnotum is usually bare.

Narrow or broad scales may cover the whole scutum or only some of its areas, the amount of scaling varies between different genera. The scales may be entirely of the same colour on the whole scutal surface, or differently coloured producing usually linear, longitudinal and patch-like scale patterns which are usually species specific and consequently important for identification. Therefore it is important to catch, handle and mount specimens with special care to prevent scales of the central scutal area from being rubbed off, or to collect larvae/pupae and let adults emerge in the laboratory. It seems that, although a variety of colour patterns occur, most of them are based on a common template.

The nomenclature devised here for the principal elements of these patterns, is derived from Berlin (1969) who regarded the general distribution of the setae mentioned above (Fig. 5.4). The acrostichal stripe is always a very narrow row of scales confined to the median line of the scutum. It may be absent, or often it is reduced to an anterior or antecrostichal patch and a usually larger posterior or postacrostichal patch of scales. If present, the acrostichal stripe divides the much broader median stripe which is located between the dorsocentral rows of setae. The paired dorsocentral stripes lie in the dorsocentral areas. They can be subdivided into inner and outer dorsocentral stripes. For convenience, if only one of them is present it will be simply named the dorsocentral stripe. The lateral stripes border the scutum to varying degrees from the anterior border to the wing base, and may be broken into several prescutal or supraalar fragments. Between the dorsocentral and lateral stripes are the moderately broad, lanceolate or crescent shaped submedian stripes. The stripes are usually subdivided into anterior and posterior portions. Lateral to the prescutellar area lie the prescutellar dorsocentral stripes, which can be fused with the dorsocentral stripes and/or the postacrostichal patch of scales above (Fig. 5.4). The prescutellar area is often bare.

The pleuron in advanced insects has become an enlarged, flattened and sclerotized part of the thoracic wall, which it rigidly supports (Fig. 5.5). It is in general transversely divided into two main pleurites, the anterior episternum and the posterior epimeron. These are separated by the pleural suture, and may be further divided into upper and lower pleurites which are denoted by the prefixes an- and kat-, thus anepisternum refers to the upper pleurite of episternum and katepimeron to the lower pleurite of epimeron. A small pleurite, the trochantin, is often present near the lower margin of the episternum. Another small pleurite, the meron (meso- and meta-) was originally the posterior part of the basicoxite which has become completely separated from the coxa and forms a part of the thoracic wall. In a wing-bearing segment the pleuron develops a dorsal (alar) articular process for the wing.

In mosquitoes the elongated, lobe shaped propleuron is located between the antepronotum and the procoxa (Fig. 5.5). The membrane which articulates the procoxa with the prothorax is arbitrarily separated into an anterior part lying between the prosternum and procoxa, the anteprocoxal membrane, and the posterior

part between the propleuron, mesepisternum and procoxa, named the postprocoxal membrane.

The mesopleural suture divides the mesopleuron transversely into a large anterior mesepisternum and a smaller posterior mesepimeron. The mesepisternum is further subdivided into two pleurites, an upper mesanepisternum and a lower mesokatepisternum. The former pleurite bears the mesothoracic spiracle. It is small, displaced towards the postpronotum and often divided into four areas (Fig. 5.5). The prespiracular area is very narrow, confined between the postpronotum and the anterior margin of the spiracle. A small area immediately below the spiracle is called the hypostigmal area. The smooth subspiracular area lies below it and is usually connected to the sclerotized postspiracular area. Because the names of the four mentioned areas are so widely accepted and used in mosquito morphology, there is no practical reason to use the designation mesanepisternum for the description of the whole pleurite. Consequently, the mesokatepisternum is referred to as the mesepisternum. The membranous anepisternal cleft lies above the mesepisternum, which is particularly well developed towards the ventral side. There it frequently unites with the opposite mesepisternum separating the fore and mid coxae from one another, thus concealing the mesosternum. The very narrow upper portion of the mesepisternum or preala, bears the lobe-like prealar knob. The mesepimeron, which lies behind the mesopleural suture, is also divided into a large upper mesanepimeron and a very narrow, bare, inconspicuous mesokatepimeron. The latter is not mentioned in the description of the species and consequently, the mesanepimeron will be referred to as the mesepimeron (Fig. 5.5). Below the mesopleural suture lies the small triangular mesotrochantin. Slightly above and immediately behind the mesocoxa and just below the mesokatepimeron, is the small triangular mesomeron located.

The metapleuron, like all other sclerites of the metathorax, is greatly reduced. It is divided by the metapleural suture in an anterior triangular or trapezoid metepisternum, which bears the metathoracic spiracle and the haltere, and the posterior stripe-like metepimeron (Fig. 5.5). The small metameron lies above the metacoxa.

Propleural setae are grouped above the lower border of the propleuron (Fig. 5.6). The prespiracular area bears the taxonomically important prespiracular

setae, present in the genus *Culiseta* among European mosquitoes and some other genera worldwide (e.g. *Psorophora*), except *Aedes*, *Ochlerotatus*, *Coquillettia*, and *Culex*.

The subspiracular area rarely supports setae, whereas the postspiracular area may bear the taxonomically significant postspiracular setae. Three groups of setae are typical for the mesepisternum. Displaced towards the wing base, inserted in the prealar knob, is the group of prealar setae. The upper mesepisternal setae are grouped at the level where the mesepisternum narrows into the preala, and the lower mesepisternal setae are located along the posterior margin, anterior to the mesotrochantin. The mesepimeron bears two groups of setae, the lower mesepimeral setae located at the anterior lower corner, behind the upper mesepisternal setae, and the upper mesepimeral setae at the posterior upper corner, anterior to the metathoracic spiracle (Fig. 5.6).

The postpronotum is usually covered with scales of various size. Sometimes the patch is divided into an upper and lower portion, called the upper postpronotal patch and lower postpronotal patch (Fig. 5.7). Both anteprocoxal and postprocoxal membranes may be covered with scales in some species, the patches are named anteprocoxal and postprocoxal patch respectively. A group of scales on the hypostigmal area is, if present, consequently named the hypostigmal patch. The subspiracular area may bear scales, the subspiracular patch. The postspiracular patch of scales can extend posteriorly onto the anepisternal cleft. The scales of the mesepisternum are usually grouped into two patches, the prealar one below the prealar knob, and the mesepisternal one blended to varying degrees with the upper and lower mesepisternal setae. The mesepisternal patch may be subdivided into an upper and lower portion (Fig. 5.7). The upper half of the mesepimeron is usually covered with scales, the mesepimeral patch. It can extend downwards towards the lower margin of the mesepimeron to a varying degree.

The sternum in advanced insects is often greatly reduced in width and partly enfolded between the legs, while the two sternal apophyses are often united on a common base to form a Y-shaped furca. The prosternum in Culicidae is the region lying anteriorly between the procoxae, connected dorsolaterally with the propleuron. It bears a distinct median suture and sometimes setae or scales. The scaling of the prosternum is of taxonomic significance in some *Aedes* and *Ochlerotatus* species. The mesosternum is obscured by

the mesepisterna, which are fused ventrally, and is restricted to the region between the mesocoxae, having a strong median suture. The metasternum is reduced to a narrow crescent shaped sclerite.

Adult mosquitoes have three pairs of legs, one pair on each of the thoracic segments (Fig. 5.1). They are referred to as fore legs, mid legs and hind legs. Each leg consists typically of six segments, the coxa, trochanter, femur, tibia, tarsus and pretarsus, the first five being almost entirely covered with scales. The tarsus is subdivided into five tarsomeres. These are, similarly to the flagellomeres, differentiated from true segments by the absence of intrinsic muscles. The basal tarsomere I articulates with the distal end of the tibia. Between the tarsomeres there is no articulation; they are connected by a flexible membrane so that they are freely movable (Chapman 1982). The distal tarsomere V bears the pretarsus which consists of a pair of claws, or unguis, a ventral unguis tractor plate where the tendon of the flexor muscle of the claws is inserted, the paired pad-like or setose pulvilli, if present, one under each claw, and a median spine-like empodium. The hind tarsus bears only one claw in females of the genus *Limatus* (Smith 1973). The tarsal claw may be simple or have a subbasal tooth. In females of most of the genera, except some of *Aedes*, *Ochlerotatus* and *Psorophora*, all tarsal claws are simple and of quite similar shape on all three pairs of legs. The shape of the tarsal claws of the fore legs can be used as a diagnostic character, especially in the genus *Ochlerotatus*. In males, the outer claws of the fore and mid legs are much bigger than the inner claws, modified for grasping the females. The two claws of the hind legs are similar and may be simple or denticulated.

The scaling of the coxa and trochanter is not usually used as a character for species identification. However, pale scales can form specific patterns on a usually dark background of the femur, tibia and tarsus, generally in the shape of longitudinal stripes and/or rings. The surfaces of the segments are described as if the leg is held stretched straight out, forming a right angle with the longitudinal body axis. Thus, it can be referred to as an anterior and posterior, dorsal and ventral, as well as an anterodorsal surface, etc. (Wood et al. 1979). The femur, when characteristically coloured, has basally more pale scales than apically.

In all Diptera the mesothoracic wings are used for flight; the metathoracic are modified to form small vibrating organs known as halteres, which control the equilibrium during flight. The wings consist of upper

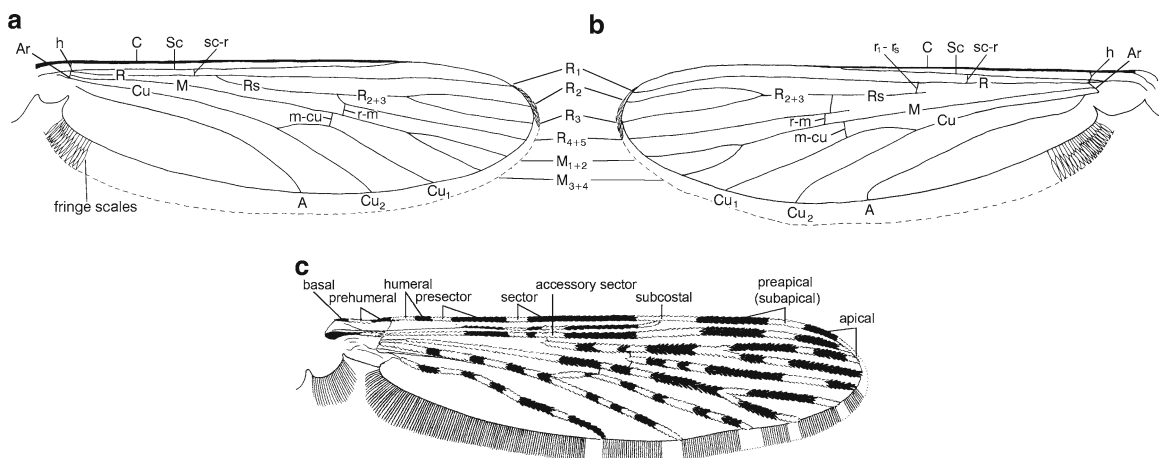
and lower epidermal layers, not fused along certain strengthening tubes, the wing veins. The complete system of wing veins is called the venation. Beginning from the anterior margin of the mosquito wing (Fig. 5.8), the first unbranched vein is the costa (C), which passes around the apex of the wing and forms its anterior margin. The subcosta (Sc) is located closely behind the costa and is also undivided. The radius (R) forks into an anterior branch R<sub>1</sub> and a posterior branch, or radial sector Rs, which branches again into R<sub>2+3</sub> and R<sub>4+5</sub>. Vein R<sub>2+3</sub> divides once more into R<sub>2</sub> and R<sub>3</sub>, while R<sub>4+5</sub> remains unbranched. The fourth vein, or media (M), bifurcates apically into M<sub>1+2</sub> and M<sub>3+4</sub>. Likewise, the fifth vein, or cubitus (Cu), divides into Cu<sub>1</sub> and Cu<sub>2</sub>. Finally, there is one anal vein (A) present. All veins are relatively straight but in *Bironella* spp. Cu<sub>1</sub> and M are wavy at their distal parts. The longitudinal veins may be connected by six different cross veins. Two of them are situated close to the wing base, the humeral vein (h) stretches from C to Sc and the arculus (Ar) from R to M and Cu. The other four cross veins are displaced towards the wing apex. They are the subcostal-radial vein (sc-r) extending from Sc to R, the sectorial vein r<sub>1</sub>-r<sub>3</sub> from R<sub>1</sub> to Rs (apparent in *Anophelinae*, Fig. 5.8b), the radio-medial vein (r-m) from R<sub>4+5</sub> to M and the medio-cubital vein (m-cu) from M to Cu. The veins divide the wing area into cells, the name for each cell is derived from that of the vein forming its anterior margin. Where two veins have fused or make one part of the anterior border, the cell is named after the posterior component. Thus, when veins R<sub>4</sub> and R<sub>5</sub> coalesce, as in mosquitoes, the area behind it is called cell R<sub>5</sub> (Davies 1992).

Almost all wing veins are both dorsally and ventrally covered with scales. The scales may be narrow or broad

and are conspicuously broad and asymmetrical in *Mansonia* spp. The cross veins are usually without scales, except, e.g. in the genera *Anopheles* and *Culiseta*. The wing margin, from the apex of the costa until the level of the base of the anal vein, is fringed with a row of scales. The veins and the wing margin are usually covered with dark scales, which can aggregate to form distinct spots (e.g. some species of *Anopheles*, *Culiseta* and *Ochlerotatus*). Pale scales may be scattered or grouped into alternating patches along the veins in a pattern of considerable taxonomic value (Fig. 5.8c). In males, the scales are less dense and more easily rubbed off, even though of similar colouration and distribution as in the females. The same is true for the scaling pattern of other body parts. As a consequence the scaling pattern of the males will not be discussed in detail in the species description nor used in the keys.

### 5.1.3 Abdomen (Fig. 5.1, 5.9–5.12)

Basically the insect abdomen consists of eleven segments (only present in some primitive orders) and the nonsegmental telson (absent in most insects). An abdominal segment consists of a sclerotized tergum and sternum joined by membranous pleural regions. If a tergum or sternum is further subdivided each sclerite is named tergite or sternite, respectively. Numerous reductions and amalgamations of segments can occur in the abdomen, especially in the last abdominal segments with the functions of mating, egg laying and disposal of feces. In both sexes, segments X and XI are generally represented by a dorsal lobe, or epiproct, and two



**Fig. 5.8** Wing of (a) Culicinae; (b) Anophelinae (c) nomenclature of wing spots in certain *Anopheles* spp.

lateroventral lobes, or paraprocts. In mosquitoes the first segment of the abdomen is somewhat reduced. The median part of tergum I is often not visible from above. In such cases only lateroterga are apparent (Fig. 5.4). The rest of the pregenital segments, II–VIII in males and II–VII in females, are well developed. The posterior part of each segment overlaps the anterior part of the next segment; the segments are joined by an intersegmental membrane. The pleural membranes of segments I–VII bear a pair of spiracles anterolaterally (Knight and Laffoon 1971). In males, the segment IX supports the reproductive opening and is highly modified into a well developed copulatory apparatus, the hypopygium. The postgenital segments X and XI are reduced, fused and partly telescoped into segment IX, forming the proctiger. In males, both the proctiger and, in some species of *Culiseta*, the abdominal segment VIII may be of taxonomic value. In females, the genital segment VIII is well developed but shorter than segment VII. The opening of the internal reproductive organs lies posterior to the border of segment VIII. Segment IX is reduced to less than a third of the size of the preceding segment and bears the rest of the postgenital segments, the proctiger (epiproct and paraprocts), and the cerci, which support oviposition. Most of these structures are partly telescoped into segment VIII. The terga and sterna of the eight easily distinguishable segments (except sternum I) may be entirely clothed with decumbent scales or covered with setae only.

Insect genitalia in both sexes are said to be derived from modified abdominal appendages, present in other arthropods and used for mating and egg laying. Basically, each genital appendage, the gonopod, has been regarded as consisting of a limb base, the gonocoxite, which shoulders an apical appendage, the gonostylus, and a median process, the gonapophysis, arising from its basal region. Some or all of these parts may be reduced, obliterated or fused, therefore the homologies of the various structures are not known with certainty for all orders of advanced insects (Davies 1992).

The male genitalia are said to be derived from appendages of the genital segment (IX). In some insects such as the Ephemeroptera these consist of a pair of lateral claspers, the gonocoxite with the gonostylus. They grip the female in copulation, together with a pair of parameres, which guide the median intromittent organ, the aedeagus. Parameres of many orders are most probably not homologous throughout the class of insects. But together with paired rudiments, the mesomeres, from which the aedeagal structures develop, are

suggested to represent the divided gonapophyses of abdominal segment IX (Davies 1992). For mosquitoes, Horsfall and Ronquillo (1970) showed the development of the aedeagal complex from the genital primordia of segment IX. Some authors claim that claspers of mosquitoes are parameres and not homologous with the claspers of Ephemeroptera (Snodgrass 1935; Seguy 1967). Thus parameres and claspers should not be confused, as the latter most probably are derivatives of segment IX as in other nematocera (Dahl 1980). In the present work the widely accepted nomenclature of gonocoxite and gonostylus is applied for practical reasons and without implications of the true origin.

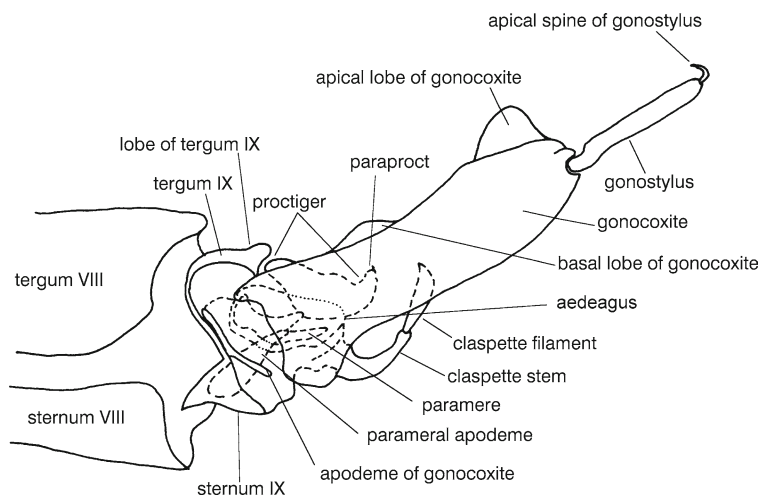
The uncertainty of origin of the various structures and consequently the variation in terminology are further complicated by the fact that a few hours after emergence of the males, the abdominal segments VIII and IX rotate through 180°. This inverts the terga, sterna and genitalia into an upside-down position. Thus, the aedeagus lies above the anus instead of being under it and the hindgut is twisted over the reproductive duct. However, it is unanimously accepted for all the structures to be referred to as though they were in their correct, prerotational, morphological position. All drawings of the genitalia are consequently of dorsal, prerotational view (Figs. 5.10–5.12). The synonyms most frequently used for the genital structures are given in brackets.

The tergum, sternum and pleurae of segment IX are usually fused into a complete ring (Fig. 5.9). The tergum IX is often bilobed, each lobe bearing a various number of strong setae. These are not always shown in the drawings of the hypopygia, except in cases of taxonomic importance. The largest unit of the male genitalia is the gonocoxite (basimere, basistyle, coxite, sidepiece or paramere according to some authors) which articulates with sternum IX (Fig. 5.9). The gonocoxite may be a sclerotized, truncate cone or may have the dorsal and ventral surfaces mesally separated by a median membrane. It may lack lobes, as in most *Anopheles* and some *Aedes* and *Ochlerotatus* species, or be variously lobed. These lobes are named with the appropriate combination of position adjectives (*e.g.* basal lobe, apical lobe, subapical lobe) without intent to imply homologies (Knight and Laffoon 1971). The gonocoxite and its lobes are covered with sparse or dense setae of different length, width and shape, sometimes with scattered scales. The movable, elongated appendage, the gonostylus (clasper, distimere or dististyle) is articulated with the gonocoxite at or close to its apex. The gonostylus usually bears one or more setae,

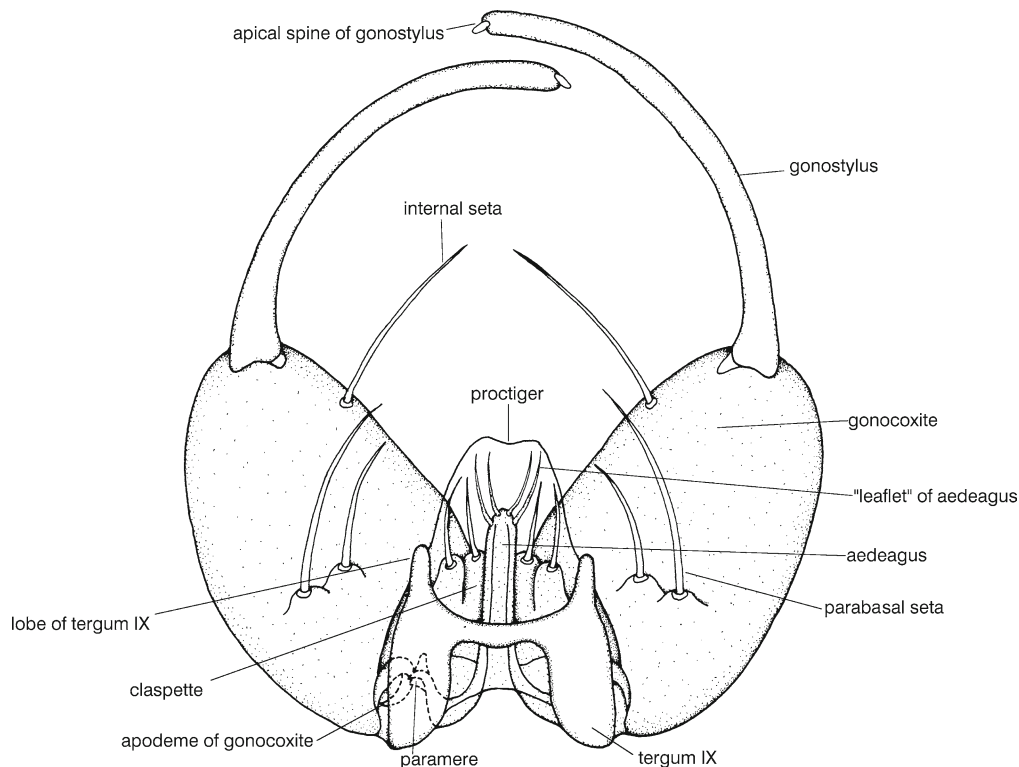
the apical spines of gonostylus (Wood et al. 1979), which are often short, spine or peg-like and situated apically or near the apex of the gonostylus. Dorsomesally to the gonocoxite and laterally to the aedeagus, a paired sclerite, the paramere, is articulated with the gonocoxal and parameral apodemes, thus supporting the aedeagus (Fig. 5.9). Some authors apply the term phallosome to the complex of structures made up of the aedeagus,

parameres and parameral apodemes, while in this publication these structures will be referred to separately as in Harbach and Knight (1980).

The aedeagus (mesosome, phallosome), is the median structure of the genitalia. It may be quite variable in shape but is often bulb or flask shaped (Figs. 5.10 and 5.11). It lies medially, above the ventral surfaces of the gonocoxites and below the proctiger (the posi-

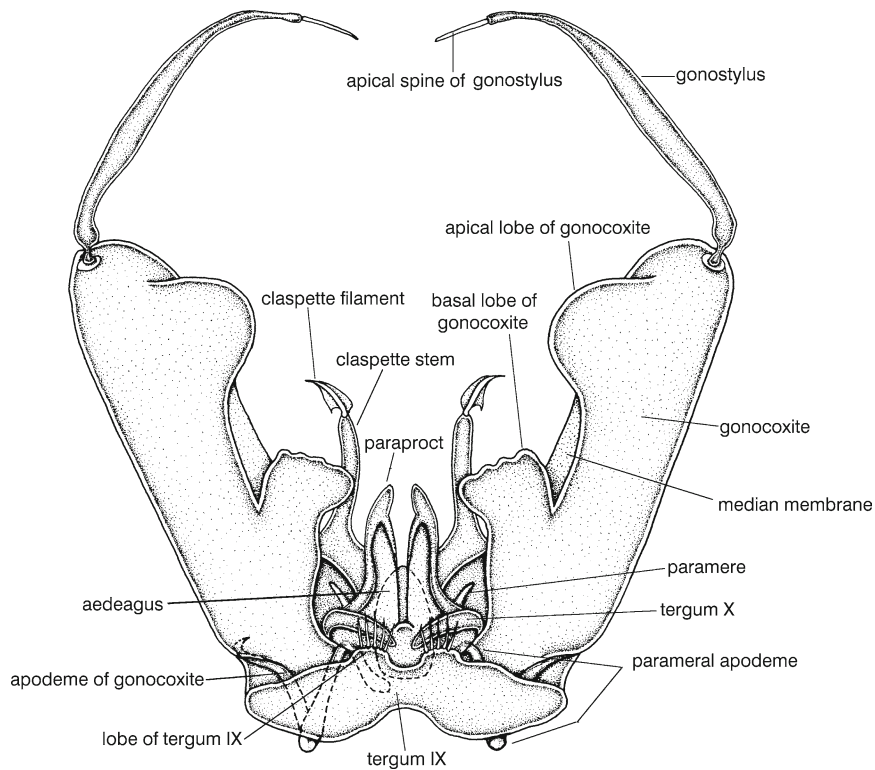


**Fig. 5.9** End of abdomen of *Aedes/Ochlerotatus* sp. – male, lateral view



**Fig. 5.10** Hypopygium of *Anopheles* sp.





**Fig. 5.11** Hypopygium of *Aedes/Ochlerotatus* sp.

tion is switched after rotation). The lateral borders of the aedeagus are usually sclerotized and form two darkly coloured plates, the lateral aedeagal plates.

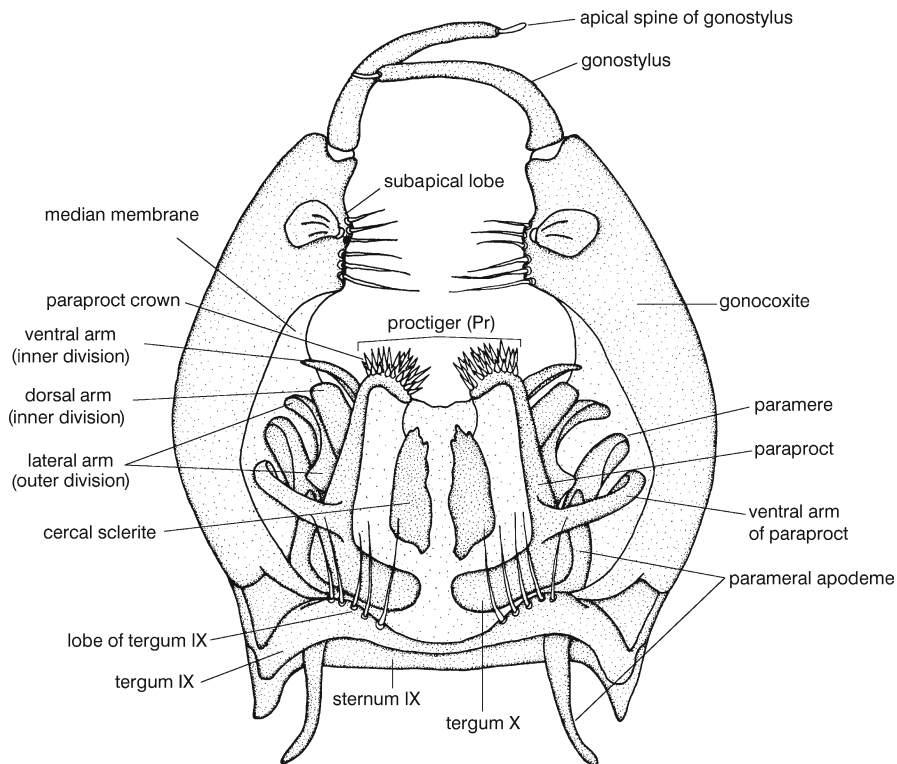
Dorsal to the aedeagus lies the proctiger (anal segment, anal cone, tenth segment), a complex formed by segment X, the cercal sclerite and the paraprocts (Figs. 5.9–5.11). The proctiger bears the anus and is largely membranous. Usually the sclerotized parts of the proctiger are tergum X, a portion of the cercal sclerite and the paraprocts (tenth sternite) (Figs. 5.11 and 5.12). Tergum X is often developed as a pair of dorsally directed arm-like tergites, arising laterobasally on the proctiger and sometimes confluent with the basal part of the paraproct. The paraproct is paired, usually a well sclerotized sclerite, situated laterally on the proctiger. Its strongly sclerotized apex can be denticulated or covered with numerous short spine-like setae (in *Culex*).

Largely membranous projections of the gonocoxites support variably shaped ventromesal lobes, the claspettes (harpes, harpagones), which are well developed in *Anopheles*, *Aedes*, *Ochlerotatus* and *Psorophora* but absent in *Culex* and other genera (Figs. 5.9–5.12). The male genitalia of different gen-

era and related species often differ considerably in detail and are therefore of great taxonomic value.

In most Anophelinae, one or more strong, spine-like setae (parabasal setae), are sunk into a cuticular socket or elevated on a tubercle, both located dorsobasally on the gonocoxite (Fig. 5.10). Additionally, a strong spine-like seta, the internal seta (mesal seta), is mesally attached to the gonocoxite of many species. One or more pairs of spine-like or leaf-like setae, the leaflets of aedeagus (aedeagal setae), are often attached to the apex of the aedeagus. The proctiger is usually greatly reduced or membranous (cone shaped). It is hardly visible, especially after decolouration with KOH during slide preparation, so it is not shown in any drawing of anopheline hypopygia. Only some anophelines (subgenus *Nyssorhynchus*) have well sclerotized paraprocts. The claspettes of *Anopheles* may be divided longitudinally into two or three lobes, the inner, median and outer lobe. All of these can bear spine-like or flattened spatula-like setae.

In the genera *Aedes* and *Ochlerotatus*, the typical claspette is divided into a proximal, more or less cylindrical portion, the claspette stem, and a single leaf shaped division, the claspette filament (Fig. 5.11).



**Fig. 5.12** Hypopygium of *Culex* sp.

In the subgenus *Aedes*, the apex of the claspette is bifurcate (claspetteoid) with the two branches bearing a few setae, while in *Aedimorphus* and *Stegomyia* it is of variable shape, covered with numerous setae or bare.

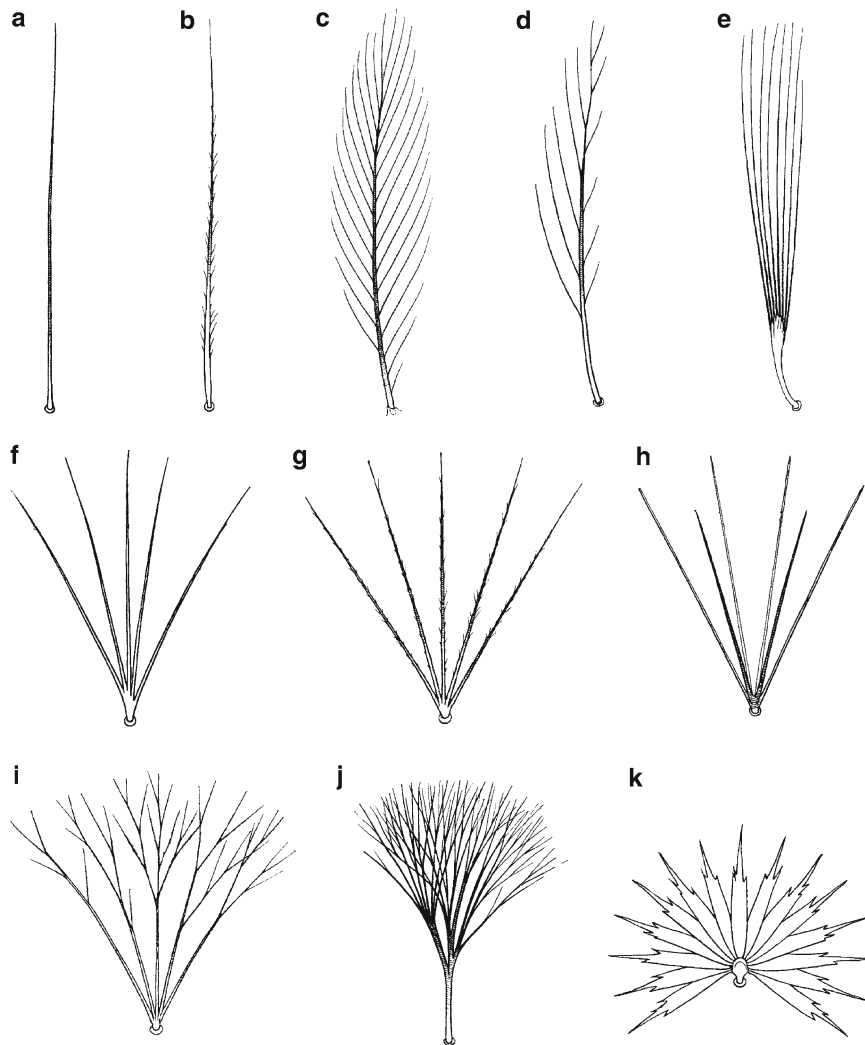
In *Culex* (Fig. 5.12), the apex of the paraproct is called the paraproct crown. It may bear a group of dense, short setae, be denticulate or with a combination of both. The lateral aedeagal plates are particularly well developed and subdivided into a lateral portion, the outer division, and a mesal portion, the inner division. The outer division is often further differentiated into lateral arms or teeth, the inner division of some species is further divided into a dorsal and a ventral arm. Laterobasally from the paraproct originates a ventrally pointed process, the ventral arm of the paraproct (basal arm of tenth sternite, basal arm of anal segment).

Mosquito females do not possess a true appendicular ovipositor. The posterior segments of the abdomen form a short apparatus modified for egg laying which acts as a functional ovipositor. The structures of the postgenital segments are greatly reduced. The best developed are the postgenital plate and cerci. The cerci (Fig. 5.1) are long and readily visible in

most species of the genera *Aedes*, *Ochlerotatus* and *Psorophora* while in other genera the cerci are short and inconspicuous.

## 5.2 Larvae

The body of the mosquito larva is divided into three principal parts, the completely sclerotized head capsule, the flattened thorax composed of three fused segments which is distinctly broader than the two other parts in fully grown instars, and the abdomen which consists of ten segments. Mosquito larvae are distinguished from all other dipterous larvae by the combination of the following characters: the presence of distinct labral brushes (lateral palatal brushes), exceptions are found in carnivorous larvae, e.g. *Toxorhynchites* spp., the expanded thorax and the tubular or cylindrical breathing tube, the siphon. The siphon is located on the dorsal surface of abdominal segment VIII in all genera, except *Anopheles*, *Bironella* and *Chagasia*. In the larvae of Dixidae, which possess labral brushes but lack a siphon, the thoracic segments are not fused or broad-



**Fig. 5.13** Types of larval setae: (a) simple; (b) aciculate; (c) pinnate or plumose; (d) lateral branched; (e) fan like; (f, g) branched; (h) stellate; (i, j) dendriform; (k) palmate (after Marshall 1938)

ened. In the larvae of the Chaoboridae, especially of *Mochlonyx* spp. which have a siphon, the mouthparts are converted for a predacious life and not used for filter feeding. They have long, ventrally curved prehensile antennae, adapted to capture aquatic prey. In addition, the chaoborid larvae possess conspicuous hydrostatic organs, one pair in the thorax, the other in abdominal segment VII, which allow the larvae to float in a horizontal position in the water.

Mosquito larvae pass through four larval instars. During their development various diagnostic characters change, e.g. the size of the head capsule, the number of pecten teeth or the number of branching of certain setae may increase. For this reasons most larval identification is based on the fourth-instar. This may require

rearing of earlier instars until the fully grown fourth-instar is reached. The following description of the larval morphology is mainly confined to those characters which are of taxonomic importance and used in the keys. Although the larvae of the anopheline and culicine subfamilies differ from one another in many respects, they are structurally similar. Therefore, they are described together, directing attention to those characters which are exhibited by one or the other subfamily.

The larval body is ornamented with 222 pairs of setae (Forattini 1996). Their arrangement (called chaetotaxy) and structure, are important taxonomic features. The setae may be simple or variously branched (Fig. 5.13). A simple seta is undivided and usually cylindrical and apically attenuated. An aciculate seta

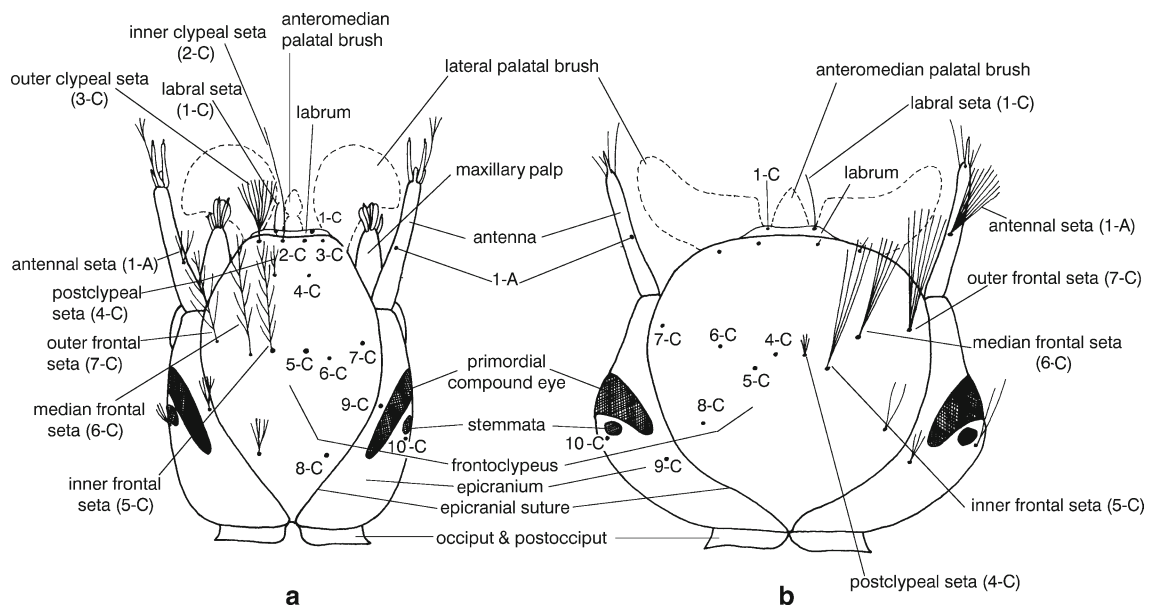
consists of a main stem with lateral secondary branches which are short and delicate. The branching may be sparse or dense. A branched seta consists of a long or short stem split into two or a few more divisions usually approximately equal in length, none of them can be recognised as the continuation of the main stem. A branched seta, which has no apparent stem and is divided practically at the base into several more or less equal divisions, is called a tuft. A stellate seta has a very short stem, is basally branched, the stiff branches radiating from the stem at various angles. A pinnate or plumose seta consists of a main stem with regularly arranged, long lateral branches, which usually shorten towards the apex. A lateral branched seta has a main stem with lateral branches, irregularly arranged and often irregular in length. A fan-like seta, which can be found in the ventral brush of the larva, consists of a very stout stem, usually apically widened, and many branches. A dendriform seta has various stems with branches that are divided and subdivided so that they resemble the branches of a tree. Specialized setae, the so called palmate setae, characteristic of the genera *Anopheles*, *Bironella* and *Chagasia*, can be found on several abdominal segments. A palmate seta consists of a short stem from which leaf shaped, flattened branches, called the leaflets, extend. They may have smooth or serrated margins. The number of the leaflets, their shape and marginal serration can vary in

different species. Sometimes the leaflets are narrowed or shouldered more or less abruptly at or beyond the middle, and thus divided into a wider proximal part, or blade, and a narrower distal part, called the filament. Palmate setae support the larval body at the water surface film while feeding. When the anopheline larvae lie just beneath the water surface, the setae are held in an open position and the leaflets are spread to at least  $180^\circ$ . When the larvae dive, the leaflets become folded and hold an air bubble inside which is used to break the surface film at the next rise to the surface.

### 5.2.1 Head (Fig. 5.14)

Although the heads of culicine and anopheline larvae resemble each other in structural and other features, they are noticeably different in shape. In most species of culicine larvae, the head is significantly broader than it is long, whereas the head of anopheline larvae is normally longer than broad, but the ratio of the length to the width may vary in different species.

The head capsule is formed by four sclerotized plates, the frontoclypeus (dorsal apodeme), a large sclerite forming the dorsal aspect of the head, and two epicranial plates (lateralia), covering the lateral surfaces as well as the ventral surface and the fused



**Fig. 5.14** Head of larva – dorsal view (a) Anophelinae; (b) Culicinae

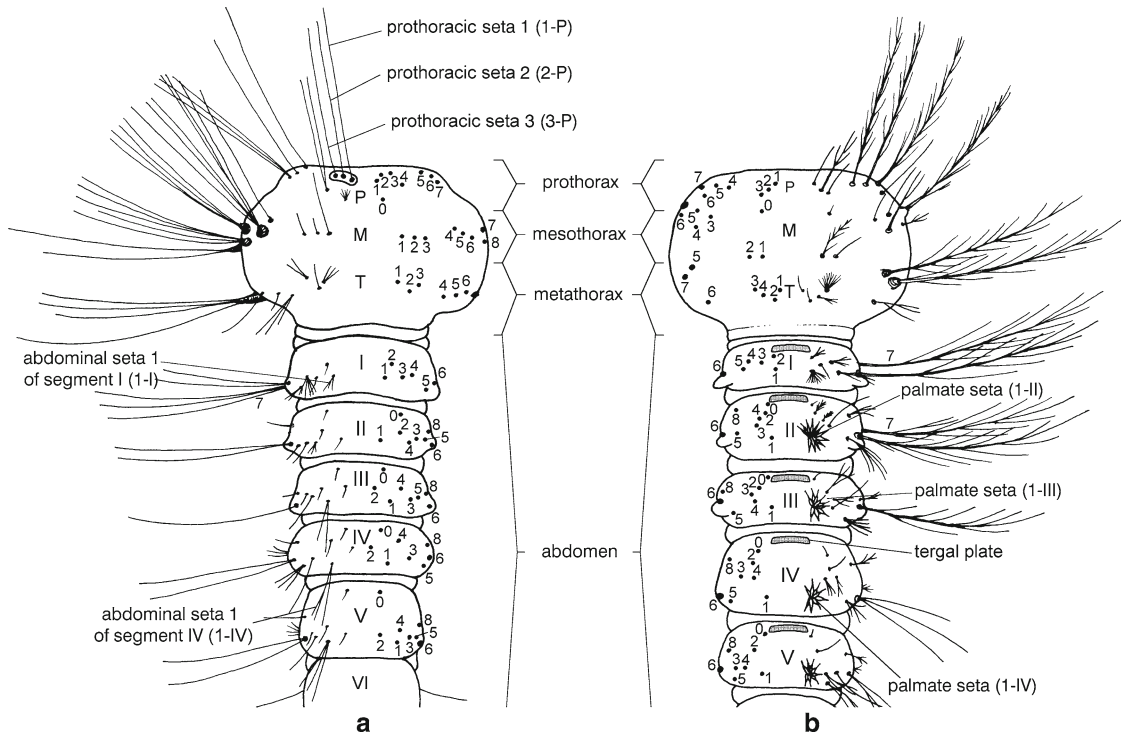
collar-shaped occiput and postocciput. The first three plates meet dorsally in the epicranial suture. In some species, the frontoclypeus has areas of darker colouration which gives rise to a specific pattern, sometimes of diagnostic value (e.g. in *An. algeriensis*). To evaluate this character correctly, the larval head must be examined under low magnification. The crescent shaped labrum is located anterior to the frontoclypeus. Its well developed ventral surface or palatum carries the conspicuous lateral palatal brushes. Each lateral palatal brush is composed of numerous long, sometimes sigmoid setae. The brushes can be moved rapidly and synchronously to produce currents in the surrounding water and thereby attract floating food particles. The presence or absence of serrations on the setae of the lateral palatal brushes may distinguish those larvae that brush food particles from those that sweep the water as filter feeders. In predatory larvae, the brushes have only a few, stout, prehensile setae. The median part of the palatum bears a brush, the anteromedian palatal brush, which is much less developed than the lateral palatal brushes. The larval mouthparts are rarely used for taxonomic purposes and they will not be discussed in detail here. Besides the labrum, they consist of two pairs of flattened sclerites, the mandibles and maxillae and the mentum (labium). The maxillary palps arise close to the base of each maxilla. They are much more developed in anophelines than in culicines. For particular descriptions of the mouthparts see Clements (1992) and Pucat (1965) and of the lateral palatal brushes Dahl (2000), Dahl et al. (1988), and Harbach and Knight (1980).

The antennae, which arise at the anterolateral corners of the head, are slender and slightly tapering sensory appendages but could be enlarged, broad and flattened in the genus *Aedeomyia* or divided with a freely articulated distal portion in the genus *Mimomyia*. They may be shorter than the head and either straight or slightly curved (e.g. genera *Aedes* and *Ochlerotatus*, except *Oc. diantaeus*, and subgenus *Culiseta*) or as long as or longer than the head and evenly curved (e.g. genera *Culex* and *Mansonia* and subgenus *Culicella*). Each antenna bears six setae, which are numbered 1-A to 6-A. The antennal seta 1-A projects from the shaft of the antenna; it can be simple and inconspicuous (e.g. *Oc. geniculatus*) or mostly has multiple branches of large size (e.g. *Cs. morsitans*). Its position on the antennal shaft is often of diagnostic importance. Setae 2-A to 6-A are minute and located at the antennal apex.

The antennal shaft is usually covered with small anteriorly projecting spines or spicules, only in the subgenus *Finlaya* and in *Oc. pulcritarsis* is the surface of the shaft smooth.

Two pairs of eyes are situated medio-laterally at the epicranial plates. The dark crescent shaped anterior patches are the primordial compound eyes of the future adult showing through the larval skin. The smaller simple larval eyes (stemmata) are located just behind them. Posterior to the frontoclypeus and the epicranial plates lies the occipital foramen, the opening of the cranium to which the cervix (neck) is attached.

The head bears up to 18 symmetrically paired setae, which are numbered 0-C to 17-C. The letter "C" is used to indicate that the setae are located on the head or caput. Two more setae, affiliated with the letter "C", C-18 and C-19, are articulated to the cervical sclerite. The setae which are of diagnostic importance and used for identification in the keys, arise from the frontoclypeus and are numbered 2-C to 7-C (Fig. 5.14). They provide their diagnostic indication not only by their relative position but also by their length, degree of branching and other characteristics. One additional seta, 8-C also arises from the frontoclypeus. Seta 1-C is prominent, forwardly directed, arising on the labrum. The inner and outer clypeal setae 2-C and 3-C are located close to the anterior margin of the frontoclypeus. In culicines they are either greatly reduced in size or invisible, or absent. In anophelines they are among the most conspicuous setae, and their position in relation to one another is used to separate several subgenera within *Anopheles*. The postclypeal seta 4-C is located at some distance behind the clypeal seta in anophelines and it is more weakly developed. In most culicine species the postclypeal setae 4-C are very short, usually multiple branched and situated closer to the midline than the frontal setae. The frontal setae consist of three pairs, the inner (5-C), median (6-C) and outer (7-C) frontals. In *Anopheles* larvae the frontal setae arise more or less side by side, forming a transverse row. Usually they are plumose, except e.g. in *An. plumbeus*, which has very short and simple setae. In the other genera, except a few *Aedes* species, setae 6-C are displaced forward, sometimes even in front of 4-C, forming a triangle with its two companions 5-C and 7-C. The frontal setae are mostly well developed and branched. The outer frontal setae 7-C arise near the edge of the frontoclypeus close to the antennal bases. They are often more densely branched



**Fig. 5.15** Thorax and abdomen – dorsal setation (a) Culicinae; (b) Anophelinae

than the inner or median frontal setae. In larvae of *Ur. unguiculata* setae 5-C and 6-C are unique, they are long and stout. Setae 9-C and 10-C are located at the epicranium, below the primordial compound eyes, the latter being displaced more laterally. All other setae arise on the ventral side of the head.

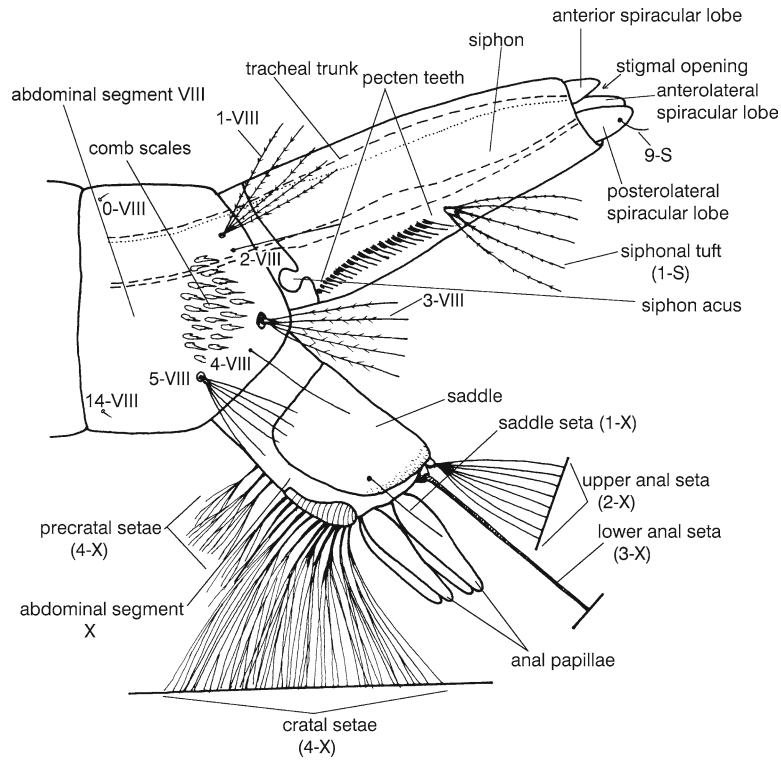
### 5.2.2 Thorax (Fig. 5.15)

The thorax is the most conspicuous part of the larvae. Its cuticle is mainly or entirely membranous, and during the growth of the instars it becomes increasingly larger, relative to the head. Just before pupation of the fourth-instar larvae, it is much broader than the head. As in the adult, the thorax consists of three segments, the pro-, meso-, and metathorax. The segments are completely fused, their borders can only be determined by the arrangement of the setae in three distinct sets. The symmetrically paired setae are numbered 0-P to 14-P on the prothorax, 1-M to 14-M on the mesothorax and 1-T to 13-T on the metathorax. The numbering starts with the pair of setae closest to the middorsal line and

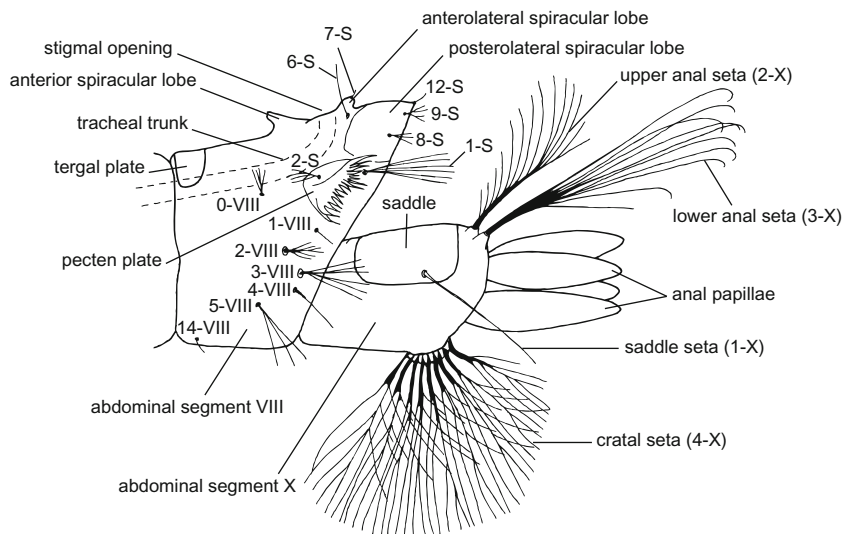
ends with the one nearest the midventral line, the only exception being seta 0-P which is articulated lateral to 1-P and displaced towards the mesothorax. Many of the 42 pairs of thoracic setae may be useful for identification, but only setae 1-P to 3-P are used in the larval keys, because there exist other convenient characters for identification, especially on the head and the last abdominal segments. Setae 1-P to 3-P usually arise very close to each other in a line and may, at a first glance, be mistaken for branches of a single seta. They are often situated on a sclerotized tubercle.

### 5.2.3 Abdomen (Figs. 5.15– 5.17)

The larval abdomen consists of ten segments, the first seven segments closely resemble each other. Abdominal segment I bears 13 pairs of setae and each of segments II–VII has 15 pairs. When referring to a seta, its number is followed by the segment number, for example 3-VI refers to seta 3 on abdominal segment VI. The numbering of the setae follows the same principle as described for the thorax. Of all pairs of abdominal setae available on segments I–VII, only a few are used



**Fig. 5.16** Lateral view of larval abdomen – Culicinae



**Fig. 5.17** Lateral view of larval abdomen – Anophelinae

for identification in the keys. In anophelines, seta 1 is of palmate type in some or all abdominal segments (Fig. 5.15b). The number of segments with fully developed palmate setae, and the shape of the branches, varies between species. To distinguish between the two species so far known in the Anopheles Claviger Complex, the branching of the antepalmate setae 2-IV and 2-V is a useful characteristic. These setae are located anterior to the palmate setae and closer to the dorsomedian line, than the setae 3-5 of the segments. The most laterally placed seta 6 is the longest and most conspicuous on segments I-VI. In European culicines, no palmate setae are present (Fig. 5.15a). In the genus *Culex*, setae 1 on segments III-V show a diagnostic number of branches useful in separating species of *Cx. p. pipiens* and *Cx. torrentium*. In anophelines, each of the abdominal segments I-VII bears a sclerotized tergal plate anteriorly, and may also have one or more smaller plates posterior to it near the centre of each segment. Usually, these plates do not occur in culicine larvae, except *Or. pulcripalpis* which in the fourth-instar larva has sclerotized plates of different sizes dorsally on segments VI-VIII.

Segment VIII is entirely different from the preceding segments. It bears the only functional external openings of the metapneustic respiratory system, the spiracles, which are located posteriorly on the dorsal surface of the segment. In culicines, the spiracles are situated at the tip of a long, tubular and cylindrical organ called the siphon (Fig. 5.16). In anophelines, the siphon is almost completely reduced to the spiracular plate and often is said to be absent (Fig. 5.17).

On each side of segment VIII a number of decumbent scales can be found. The whole structure is called the comb (Fig. 5.16). Each comb scale is directed posteriorly and fringed with small spines. All spines may be of the same length or the median, or terminal spine can be exceptionally distinct and longer than the others. The shape of the spines can only be studied adequately under sufficient magnification, therefore this character is used in the keys only to separate a few species within the genera *Aedes*, *Ochlerotatus* and *Culex*. The number of comb scales varies greatly with the species, from 5 to 7 to more than 100 and they may be arranged in a single row, a double row or in an irregular pattern. The total number of comb scales, with its range of variability, is of diagnostic value. In *Ur. unguiculata*, the comb scales arise from the posterior margin of a sclerotized lateral plate. In *Or. pulcripal-*

*pis*, the comb scales are much more elongated than in the other genera and form two regular rows. In fourth-instar larvae of anophelines there is no structure which corresponds to the culicine comb (Fig. 5.17).

In culicines, five out of the seven pairs of setae on the lateral sides of segment VIII are located posterior to the comb scales. They cannot be homologized with the setae on the first seven segments, except the ventral most one which is consequently named 14-VIII instead of 6-VIII. All setae are numbered, successively from dorsal to ventral, 0-VIII to 14-VIII. Only 1-VIII to 5-VIII are distinct (Fig. 5.16) and in the fourth instar larvae the setae 1-VIII, 3-VIII and 5-VIII are usually strongly branched, whereas the intermediate ones, 2-VIII and 4-VIII, are shorter and nearly always simple, except in *Coquillettidia*. Both 0-VIII and 14-VIII are minute and of no taxonomic importance.

At the base of the siphon, two small lateral projections arise close to its ventral margin, each called acus. They are the points of muscle attachment which enable the siphon to be bent posteriorly. In all species of the subgenus *Stegomyia*, the acus is absent and in *Ae. vittatus* it is very indistinct. The siphon of the culicines is one of the most useful structures for identification. Its shape and proportions vary considerably. Very often the siphonal index is used for identification, its value is expressed as the ratio of the length of the siphon to its basal width. Harbach and Knight (1980) and Belkin (1962) suggested measuring the width of the siphon at its middle. In many cases such measurement is difficult and quite subjective. For this reason it is suggested to measure the width of the siphon at its base (Gutsevich et al. 1974; Forattini 1996). The index may vary from less than 2.0 in some species, such as *Oc. mariae*, to over 7.0 in others, like *Cx. hortensis*. In some *Culiseta* species the ratio of the siphonal length to the width of its apex is diagnostic. The siphon also carries a pair of ventrolateral rows of stout, sclerotized spines, the pecten teeth. Each row is known as the pecten. A single pecten tooth usually has 1-4 lateral denticles on its ventral margin. In culicines the pecten teeth commonly increase uniformly in size toward the apex. All teeth may be evenly spaced, but in some species the distal pecten teeth may be more widely and irregularly spaced than the others. In the keys they are referred to as being detached apically. The proportion of the siphon to which the pecten extends from the base, is of taxonomic importance. It may be short, with a few teeth which do not exceed the middle of the siphon, or



it may extend almost to the apex of the siphon, as in *Oc. cataphylla*. In *Cs. longiareolata* the pecten consists of a row of teeth which are widely spaced along the entire siphon. In *Cs. fumipennis* the stout teeth continue on in an irregularly row of large spines. In the subgenus *Culiseta* typical pecten teeth are found only at the base of the siphon. They are then followed by a row of thin, hair-like setae. Among the European culicine species, the pecten is absent in the species of the genus *Coquillettidia* and in *Or. pulcripalpis*, and elsewhere in the genera *Armigeres*, *Mansonia*, *Orthopodomyia* and *Toxorhynchites*.

In anophelines which lack an elongated siphon, the pecten is situated immediately below the spiracles and consists of a row of alternate long and short teeth arising from the posterior side of a triangular sclerite, the pecten plate (Fig. 5.17). The two plates on each side of segment VIII are connected by a U-shaped band. The setae 0-VIII to 14-VIII are located ventrally to the pecten and are less conspicuous than in culicines.

The siphon and spiracular plate bear 13 pairs of setae designated 1-S to 13-S. In culicine larvae, the siphon is adorned with one or more pairs of tufts or simple setae on its ventral and/or lateral surface, the siphonal setae 1-S (Fig. 5.16). If there is more than one pair of setae 1-S present, the basalmost is named 1a-S, the next one towards the apex of the siphon 1b-S and so on, proceeding distally (Darsie and Ward 1981). Larvae of the genus *Culex* have several pairs of tufts, they may be arranged symmetrically and sometimes the penultimate tuft is found to be situated dorsally out of line with the others, or they may arise in a more or less zig-zag row. The total number of siphonal tufts, their length compared to the width of the siphon at the point of articulation of each 1-S, and their position on the siphon, is often characteristic. A single paired siphonal tuft is usually present, either at the base, as in *Culiseta*, which is the principal diagnostic feature of this genus, or between the mid length and the apex of the siphon, as in other culicines. The position of 1-S with respect to the pecten is used to separate some species of *Ochlerotatus*. Usually 1-S is attached distally to the pecten, but sometimes it is attached basally to the distal pecten teeth, and is then described as being attached within the pecten. The number and length of the branches of 1-S may vary and are used for species identification. Occasionally additional setae can be found on the dorsal surface of the siphon (subgenus *Rusticoidus*). In anopheline larvae, seta 1-S is articu-

lated behind the pecten plate (Fig. 5.17). Seta 2-S is located dorsoapically, closely to the spiracular apparatus in culicines and on the pecten plate in anophelines. The spiracles are surrounded by the spiracular plate. It is a five-lobed valve which closes the openings during submersion of the larva. These lobes are the anterior spiracular lobe and two pairs of anterolateral and posterolateral spiracular lobes. They bear several setae, 3-S to 13-S, of which only 9-S, the second one below the apices of the posterolateral, largest valves, are used in the keys. It is elongated, thickened and hook-shaped in some *Ochlerotatus* species. In the genus *Coquillettidia* the spiracular plate is highly modified, bearing inner and outer spiracular teeth at the apex and a row of teeth on the anterior surface known as the saw, for piercing and penetrating submerged parts of plants in order to obtain oxygen. Similar modifications are not found in any other European genus, but may be characteristic of larvae of all *Mansonia*, some *Culex* (subgenus *Lutzia*), *Mimomyia* and *Hodgesia* species occurring elsewhere.

Segment IX is reduced, its remnant is visible as a small ring at the base of the anus bearing segment X in many species, but in some others, like in *Ur. unguiculata*, this rudiment disappears entirely. It does not exist as a separate morphological unit and is of no taxonomic importance.

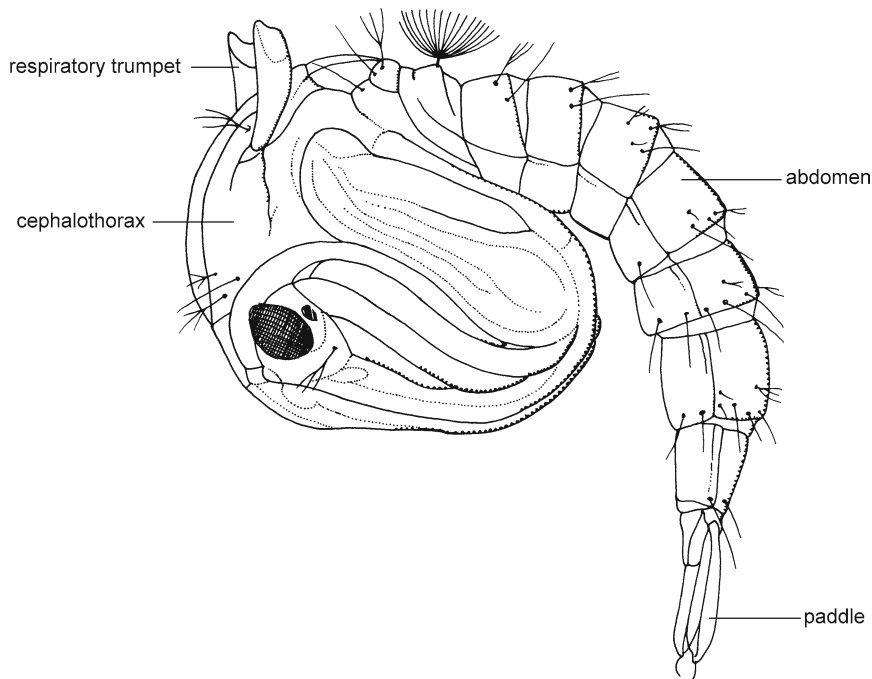
The most posterior, or anal segment X is narrower than the others, forms an angle to the ventral side of segment VIII and bears four pairs of setae, 1-X to 4-X. It possesses a curved, sclerotized plate called the saddle. Although in many species the plate is saddle-shaped and extends down the lateral sides of the segment to various degrees, e.g. almost reaching to the midventral line in *Oc. punctodes*, it completely encircles the anal segment in others. The shape of the saddle is of diagnostic value in many species. It bears a lateral saddle seta, 1-X. In the fourth-instar larva the saddle seta arises well within the saddle, closer to its posterior margin than to its ventral margin. Its length compared to the length of the saddle is frequently used for identification. The posterior margin of the saddle may bear denticles or spines of varying shape. Dorsally at the distal end, the anal segment bears two long, paired setae, the upper anal seta 2-X and the lower anal seta 3-X. In anophelines both pairs are composed of multiple branched setae, which are often hooked and support the larvae while in horizontal position (Fig. 5.17). In most culicines, seta 2-X is multiple branched and

3-X is a long stout seta which is usually either simple or only slightly branched (Fig. 5.16). Along the mid-ventral line of the anal segment, close to its apex, arise a number of long setae 4-X, which make up the ventral brush or fin. The number of 4-X can vary from two, a few (e.g. *Malaya*, *Sabethes*, *Wyeomyia*) to many more (e.g. *Ochlerotatus*). All setae of the ventral brush are fan-like and they act as a rudder during swimming. Some or all of these setae are attached to a heavily sclerotized network of bars which resembles a ladder or grid for greater basal support. In anophelines, all setae arise from the grid, while in culicines, the setae may be situated partly on and partly proximal to the grid. Those setae attached to the grid are called the cratal setae and those attached to the segment anterior to the grid are called the precratal setae (Fig. 5.16). The number of precratal setae is an important feature to separate various *Ochlerotatus* species. Sometimes it is not easy to differentiate between the cratals and precratals, especially when they are close together. In this case, attention should be focused on the proximal end of the grid and the first tuft which is attached to it. Then the number of tufts anterior to it should be counted. The anal segment terminates with two pairs of flexible, papilliform structures, the anal papillae which surround the anus and are involved in osmo-

regulation. The length of the anal papillae varies remarkably in different species. In salt marsh species and others which are associated with brackish or alkaline water, the anal papillae are extremely short. In some species the length of the papillae depends upon the physico-chemical conditions of the water in which the larvae develop, as it is well known for *Oc. caspius*. Usually the two pairs are of the same length, but occasionally one pair may be longer than the other. The length and shape of the anal papillae are regularly used for identification but often they are broken off or hardly visible, especially in mounted specimens.

### 5.3 Pupae (Fig. 5.18–5.21)

The mosquito pupae provide less distinct characters for identification than the larvae or adults. Although there are differences in the external morphology and chaetotaxy of the pupae in different genera and even at the species level, no attempt is made here to include the pupae in the keys. It is easier to rear pupae, which are collected in the field to the adult stage and then identify them. However, occasionally there may be difficulties in separating certain closely related species in the adult



**Fig. 5.18** Pupa – lateral aspect of the body

or larval stage, e.g. *Cs. annulata* and *Cs. subochrea*, or *An. claviger s.s.* and *An. petragrani*. In these cases some pupal characters are needed for proper identification. Thus, a brief overview of the external morphology and chaetotaxy of mosquito pupae is provided.

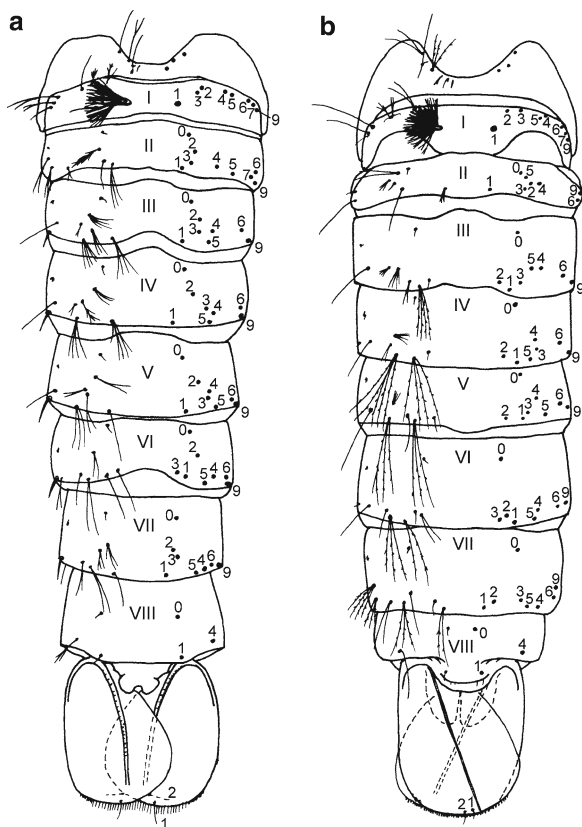
The body of the pupa consists of a large globular anterior portion, the cephalothorax, and a narrower articulated abdomen, which is kept flexed under the cephalothorax and used to propel the individual while swimming (Fig. 5.18). Mosquito pupae, unlike the pupae of most other insects, are quite mobile and can rapidly dive from the water surface when disturbed. Usually they remain at the water surface most of the time, with the paired respiratory trumpets in contact with the air.

The general appearance of the mosquito pupa with its division into only two obvious parts is mainly due to the morphology of the pupal case. The head with the sheaths of the mouthparts together with the thorax with the sheaths of the wings and legs appear to form one structure, the cephalothorax. The flattened head with the mouthparts is located at the front of the cephalothorax. The mouthparts are bent beneath it along the ventral surface to its posterior part, like a keel. The pupal cuticle is transparent and the compound eyes of the adult are visible at the sides of the head and behind these, the stemmata of the former larva. The antennae arise in front of the compound eyes in the upper part, and are directed backwards in a curved line over the sides of the thorax. The broad convex scutum of the mesothorax extends on the anterior dorsal surface of the cephalothorax. Along its median dorsal line a crest-like ridge or median keel is visible. This ridge forms the line of weakness, the ecdysial line, along which the cuticle splits before the emergence of the adult. The respiratory trumpets project from the sides of the scutum. In pupae of culicine mosquitoes the respiratory trumpets are long and cylindrical. An exception, as with the siphon of the larvae, can be found in the genera *Coquillettidia* and *Mansonia*. In this case, the trumpets taper apically and have a strong sclerotized hook, adapted for piercing submerged parts of aquatic plants. In pupae of anopheline mosquitoes, the respiratory trumpets are shorter and broad, with a flap-like appearance. The base of the trumpet is very flexible allowing it to be easily moved into position when brought to the water surface. A tracheal trunk, which leads from the base of the trumpet to the developing mesothoracic spiracle of the imago, is visible through the cuticle. The wing bases from which the pupal wing sheaths pass downwards on the sides of the

cephalothorax, are located posterior to the respiratory trumpets. Between the sheaths of the wings and mouthparts, the sheaths of the legs are visible, the tarsi being curled up under the wing apex.

The abdomen of the pupa is flattened dorsoventrally and consists of nine segments, the last of which is very small and carries the terminal paddles (Figs. 5.18 and 5.19). The segments are sclerotized, connected with intersegmental membranes and freely movable on each other in flexion and tension, but with little or no lateral movement. The first abdominal segment bears a pair of conspicuous palmate setae (1-I), which resemble those of the *Anopheles* larvae and have the same function in supporting the pupal body at the water surface in the upright position. Again the genera *Coquillettidia* and *Mansonia* are exceptions. Due to the permanently submerged life of the pupae, the seta 1-I is not of the palmate type, but short and simple. Both ventrally and laterally, the first abdominal segment is largely membranous. Abdominal segments II to VII are more or less the same size. They consist of completely sclerotized rings without pleural membranes between the terga and sterna. Segment VIII is smaller than the preceding segments and bears on its dorsal side a median caudal lobe of tergum IX. The lateral lobes of the tergum IX are developed into the paddles, and the sternum IX is an indistinct transverse band. The paddles are irregularly oval in shape with a narrow base, and overlap by about half of their width. They are the main organs of movement of the pupae. They are provided with a median longitudinal strengthening or midrib. Close to the end of the midrib arises an apical seta (1-P). In pupae of *Anopheles* a small accessory seta (2-P) is present above the apical seta on the ventral side (Fig. 5.19a), while in *Culex* the accessory seta arises dorsally side by side with 1-P (Fig. 5.19b). In the genera *Coquillettidia* and *Mansonia*, both the apical and accessory setae are absent. In many species the margin of the paddle is fringed with spicules or small spines which are of taxonomic significance.

Caudal and ventral to the median caudal lobe lies medially a small rudiment of segments X and XI, the anal lobe or proctiger. In female mosquitoes it is widened and carries a more or less distinct pair of cercal lobes. Between the bases of the paddles, caudal and ventral to the anal lobe, arises a sheath, enclosing the developing genitalia of the adult, the genital lobe. In



**Fig. 5.19** Abdominal setation of pupa – dorsal view, (a) Anophelinae; (b) Culicinae

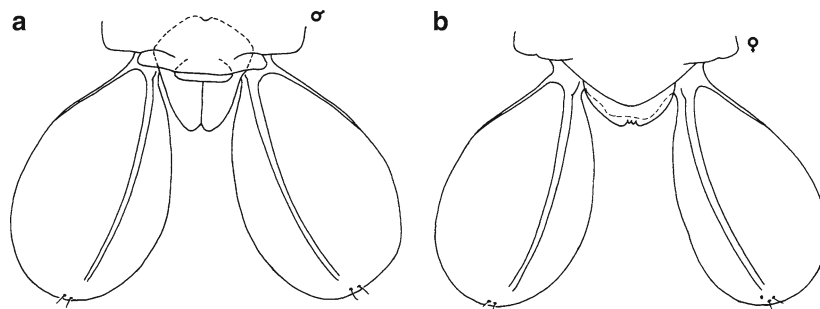
males the lobe is relatively large, conical and clearly divided, almost completely separated by a deep median fissure which extends closely to its base (Fig. 5.20a). In females the lobe is smaller and of similar size to the cercal lobes, with a more or less blunt end, and the median fissure is short and does not divide the lobe completely (Fig. 5.20b). The sex of the pupae can be readily determined by the shape and the size of the

genital lobe in relation to the size of the paddles, and the extent of the median fissure.

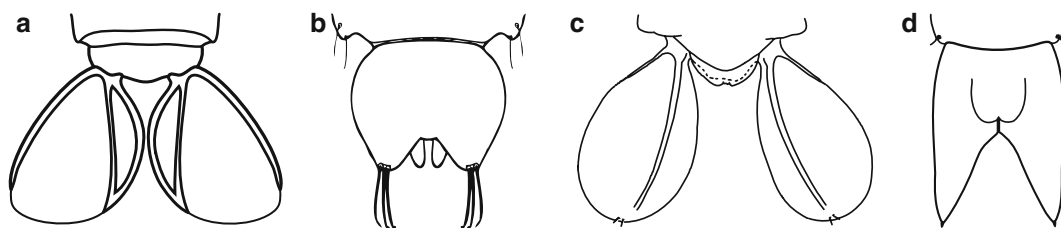
Another separation technique may also be used in the laboratory. If larvae are reared in aerated water and overfed, the female pupae are distinctly larger than those of the males and the abdomen is almost of the same width as the cephalothorax, while in male pupae the abdomen is distinctly narrower than the cephalothorax. Bearing in mind that the development of male larvae into pupae is usually faster than that of females, sexing according to the size and general appearance of the pupae can be used with quite high accuracy. Both separation techniques can be applied to living individuals and are useful in a number of laboratory experiments, e.g. for single pair rearing without the necessity of handling the adults.

The abdominal segments bear paired dorsal and ventral setae. Except for segments I and VIII, they resemble those of the larvae. Together with the form of the respiratory trumpets and the characteristics of the paddles, the chaetotaxy of the pupal abdomen is mainly used for description and identification of species. The nomenclature used in this work follows that of Harbach and Knight (1980) and is given in Fig. 5.19. Characteristic of the pupae of the subfamily Anophelinae are setae 9 of the abdominal segments III to VII. They are located at the lateral posterior corner of the segments and have the form of stout spines (Fig. 5.19a). In pupae of the Culicinae, the setae do not arise quite at the corner but a little anterior to it and they are usually small and simple, rarely branched (Fig. 5.19b). Within the Culicinae, the genera *Coquillettidia* and *Mansonia* again show an exception in the chaetotaxy by having scarcely developed abdominal setae.

The shape and structure of the terminal appendages or paddles may be used to separate mosquito pupae



**Fig. 5.20** Abdomen of pupa, (a) male; (b) female



**Fig. 5.21** Abdomen of pupa, (a) Chaoboridae; (b) Chironomidae; (c) Culicidae; (d) Dixidae (after Cranston et al. 1987)

from those of the closely related families of Chaoboridae, Chironomidae and Dixidae. As mentioned above, the paddles of culicid pupae are provided with a median longitudinal strengthening or midrib. Chaoborid pupae have paddles quite similar to those of mosqui-

toes, but they are stiffened by three veins or ribs. Despite great variations within the family, the paddles of chironomid pupae lack a midrib and they are never completely separated. In Dixidae the pupal abdomen ends with sharply tapered caudal lobes (Fig. 5.21).

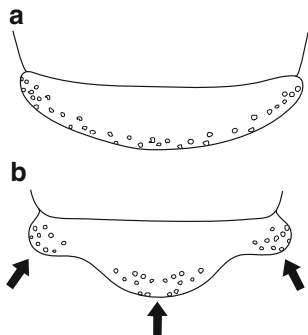
**Part II**  
**Identification Keys; Morphology;**  
**Ecology and Distribution of European Species**

# Chapter 6

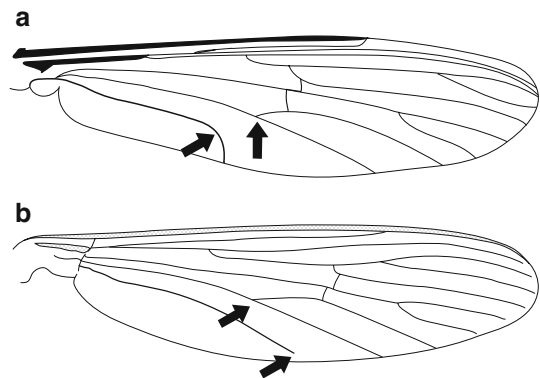
## Key to Female Mosquitoes

### Genera

- 1 Palps as long as proboscis. Scutellum evenly rounded and uniformly setose (Fig. 6.1a). *Anopheles* (p 164)
- Palps distinctly shorter than proboscis. Scutellum trilobed, setae arranged in three sets (Fig. 6.1b).  
Abdominal terga and sterna densely covered with scales. .... 2
- 2 (1) Anal vein (A) sharply bent apically, ending slightly before or at the same level as furcation of cubitus (Cu) (Fig. 6.2a).....*Uranotaenia unguiculata* (p 312)
- Anal vein (A) evenly curved, ending distinctly beyond fork of cubitus (Cu) (Fig. 6.2b). .... 3

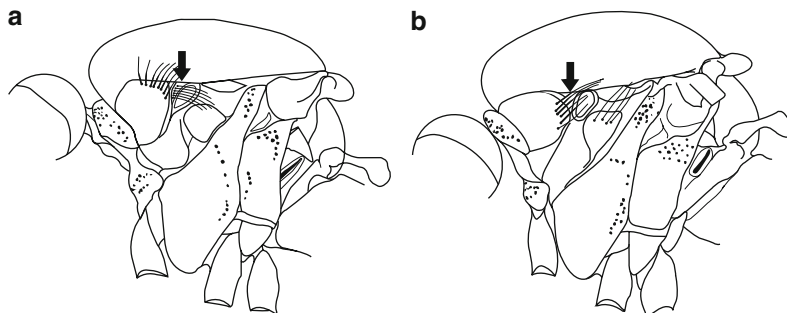


**Fig. 6.1** Scutellum of: (a) *Anopheles* sp.; (b) *Aedes* sp.



**Fig. 6.2** Wing of: (a) *Ur. unguiculata*; (b) *Ae. vexans*

- 3 (2) Prespiracular setae present (Fig. 6.3a)..... *Culiseta* (p 288)
- Prespiracular setae absent (Fig. 6.3b). .... 4



**Fig. 6.3** Lateral view of thorax of: (a) *Cs. annulata*; (b) *Oc. geniculatus*

- 4 (3) Tarsomere I of fore legs longer than tarsomeres II to V together. Tarsomere IV of fore legs reduced, not longer than broad (Fig. 6.4a). ..... *Orthopodomyia pulcripalpis* (p 310)  
 Tarsomere I of fore legs usually shorter than tarsomeres II to V together. Tarsomere IV of fore legs not reduced, distinctly longer than broad (Fig. 6.4b). ..... 5

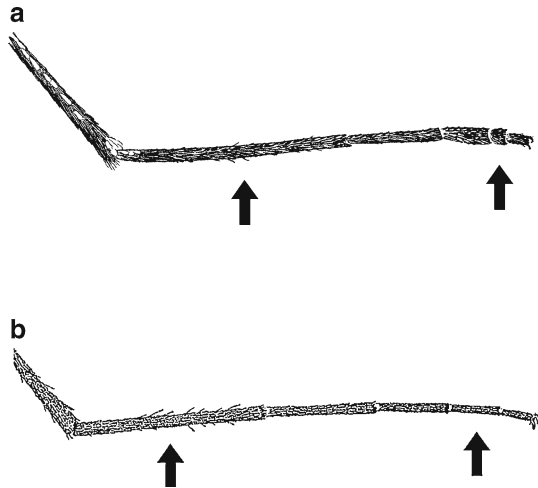


Fig. 6.4 Fore tarsus of: (a) *Or. pulcripalpis*; (b) *Aedes* sp.

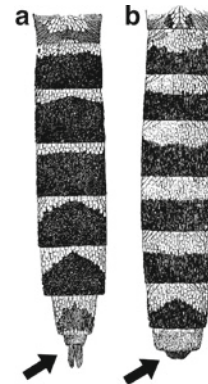


Fig. 6.5 Dorsal view of abdomen of: (a) *Aedes* sp.; (b) *Culex* sp.

- 5 (4) Postspiracular setae present. Claws usually with subbasal tooth. Abdomen tapering apically, cerci long, easy visible (Fig. 6.5a). ..... *Aedes* and *Ochlerotatus* (p 187, 204)  
 Postspiracular setae absent. Claws simple, without subbasal tooth. Abdomen rounded apically, cerci short, hardly visible (Fig. 6.5b). ..... 6  
 6 (5) Pulvilli present. Wing scales narrow (Fig. 6.6a). ..... *Culex* (p 264)  
 Pulvilli absent. Wing scales usually broad and conspicuous (Fig. 6.6b). ..... *Coquillettidia* (p 306)

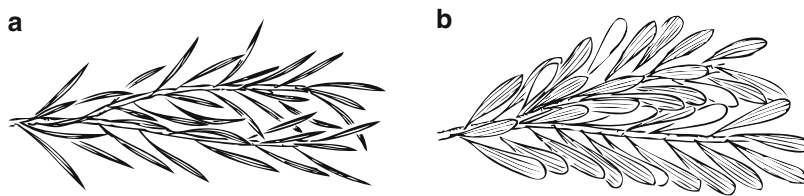
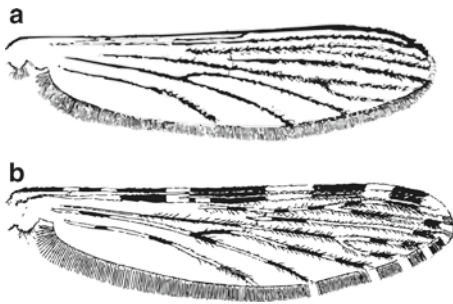


Fig. 6.6 Wing scales of: (a) *Culex* sp.; (b) *Coquillettidia* sp.

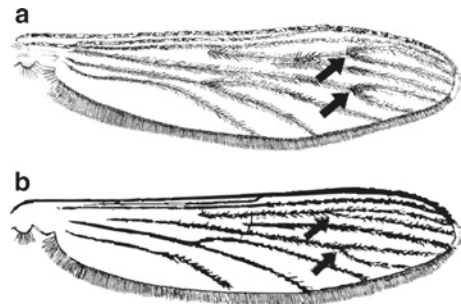
### 6.1 Genus *Anopheles*

- 1 Wing veins entirely dark scaled (Fig. 6.7a). ..... 2  
 Wing veins covered with dark and pale scales, forming contrasting spots at least on costa (C), radius (R) and R<sub>1</sub> (Fig. 6.7b). ..... 7  
 2 (1) Dark scales on wing veins more dense in some areas forming spots, mainly on cross veins and furcations. Furcation of R<sub>2+3</sub> and M situated at the same distance from base of wing (Fig. 6.8a). ..... 3  
 Dark scales evenly distributed, not aggregated into spots. Furcation of R<sub>2+3</sub> usually situated slightly closer to base of wing than furcation of M (Fig. 6.8b). ..... 4



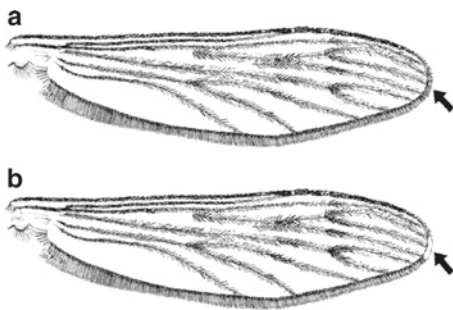


**Fig. 6.7** Wing of: (a) *An. marteri*; (b) *An. hispaniola*

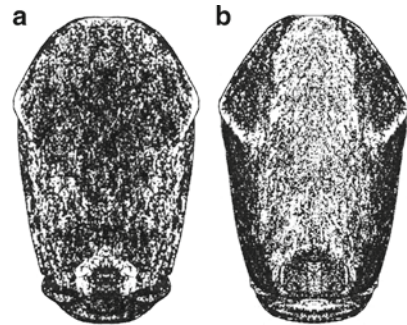


**Fig. 6.8** Wing of: (a) *An. maculipennis s.l.*; (b) *An. marteri*

- 3 (2) Scutum more or less pale brown, without a pale longitudinal stripe, dark spots on wing usually inconspicuous, scales of wing fringe unicolourous dark, without a pale patch at the apex (Fig. 6.9a). ..... *An. sacharovi* (p 175)  
 Scutum dark brown with a broad pale longitudinal stripe, dark spots on wing conspicuous, scales of wing fringe with a pale patch at the apex (Fig. 6.9b). ..... all other members of **Anopheles Maculipennis Complex** (p 170)
- 4 (2) All erect scales on vertex dark brown. Scutum unicolourous brown, without a median stripe of pale scales (Fig. 6.10a). ..... *An. algeriensis* (p 165)  
 Erect scales on median part of vertex white or cream coloured, lateral parts dark. Scutum with median stripe of pale scales (Fig. 6.10b). ..... 5

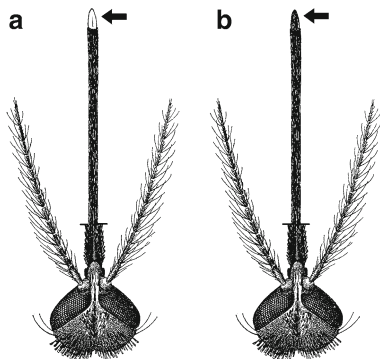


**Fig. 6.9** Wing of: (a) *An. sacharovi*; (b) *An. maculipennis s.l.*

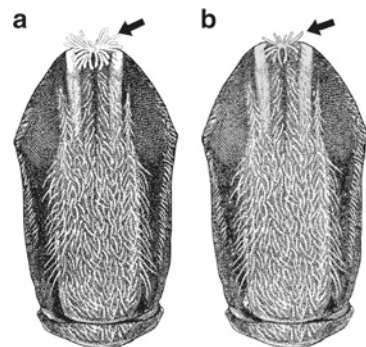


**Fig. 6.10** Scutum of: (a) *An. algeriensis*; (b) *An. plumbeus*

- 5 (4) Apex of proboscis pale scaled (Fig. 6.11a). ..... *An. marteri* (p 177)  
 Apex of proboscis dark scaled (Fig. 6.11b). ..... 6

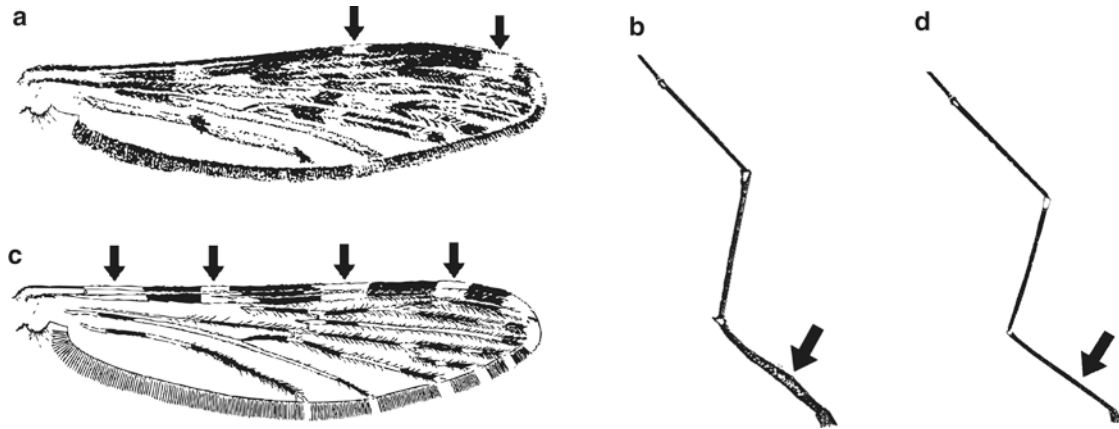


**Fig. 6.11** Head of: (a) *An. marteri*; (b) *An. plumbeus*



**Fig. 6.12** Scutum of: (a) *An. plumbeus*; (b) *An. claviger s.l.*

- 6 (5) Body blackish grey with a leaden tinge. Antecroscichal patch well developed, distinct, snow white (Fig. 6.12a). ..... *An. plumbeus* (p 178)  
 Body yellowish brown or brown. Antecroscichal patch weakly developed, yellowish (Fig. 6.12b). .....  
 ..... *An. claviger s.s.* and *An. petragrani* (p 166, 168)
- 7 (1) Costal margin of wing with 2 pale spots situated in the apical half. Base of fore femur distinctly swollen (Fig. 6.13a, b). ..... 8  
 Costal margin of wing with more than 3 pale spots along its entire length. Base of fore femur not swollen (Fig. 6.13c, d). ..... 9



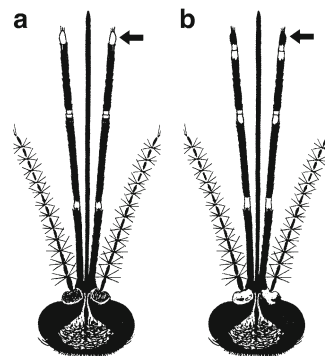
**Fig. 6.13** Wing and fore leg of: (a, b) *An. hyrcanus*; (c, d) *An. superpictus*

- 8 (7) Tarsomere IV of hind leg mostly dark, pale only at apex (Fig. 6.14a). ..... *An. hyrcanus* (p 169)  
 Tarsomere IV of hind leg entirely pale scaled (Fig. 6.14b). ..... *An. hyrcanus* var. *pseudopictus* (p 170)
- 9 (7) Palps pale at apex (Fig. 6.15a). ..... 10  
 Palps dark at apex, rarely with a small white spot (Fig. 6.15b). ..... 11
- 10 (9) Dark scales predominate on wing veins  $R_s$  to Cu. Vein  $R_{4+5}$  almost entirely dark scaled. Pale spot at the apex of wing fringe small (Fig. 6.16a). ..... *An. sergentii* (p 183)  
 Pale scales predominate on wing veins  $R_s$  to Cu. Vein  $R_{4+5}$  almost entirely pale scaled. Pale spot at the apex of wing fringe large (Fig. 6.16b). ..... *An. superpictus* (p 185)

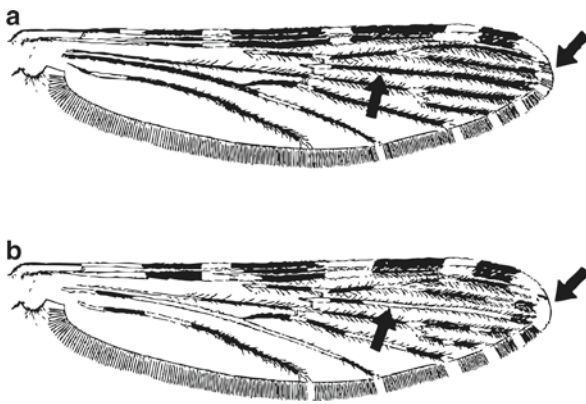


**Fig. 6.14** Hind tarsus of:

(a) *An. hyrcanus*; (b) *An. hyrcanus* var. *pseudopictus*



**Fig. 6.15** Head of: (a) *An. sergentii*; (b) *An. multicolor*



**Fig. 6.16** Wing of: (a) *An. sergentii*; (b) *An. superpictus*

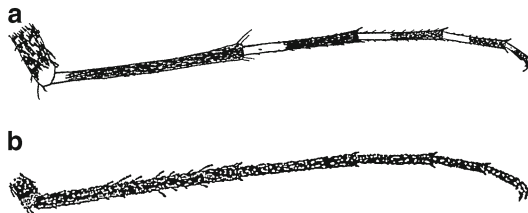


**Fig. 6.17** Wing of: (a) *An. multicolor*; (b) *An. cinereus hispaniola*

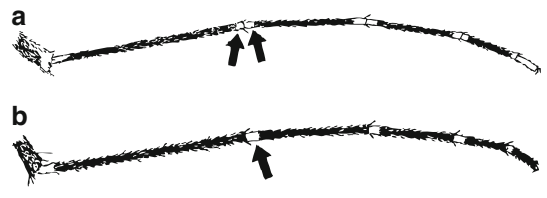
- 11 (9) Pleurites with a few pale scales. Submedian and lateral areas of scutum with scattered pale scales. Base of costa pale scaled (Fig. 6.17a). ..... *An. multicolor* (p 182)
- Pleurites without scales. Submedian and lateral areas of scutum without scales, sometimes a few hair-like pale scales present, but confined to the extreme anterior margin of scutum. Base of costa dark scaled (Fig. 6.17b). ..... *An. cinereus hispaniola* (p 181)

**6.2 Genera *Aedes* and *Ochlerotatus***

- 1 Tarsomeres with pale rings, usually more distinct on hind legs, rings sometimes very narrow (pale rings better visible against a dark background and with a blue light filter) (Fig. 6.18a). ..... 2
- Tarsomeres without pale rings (Fig. 6.18b). ..... 20

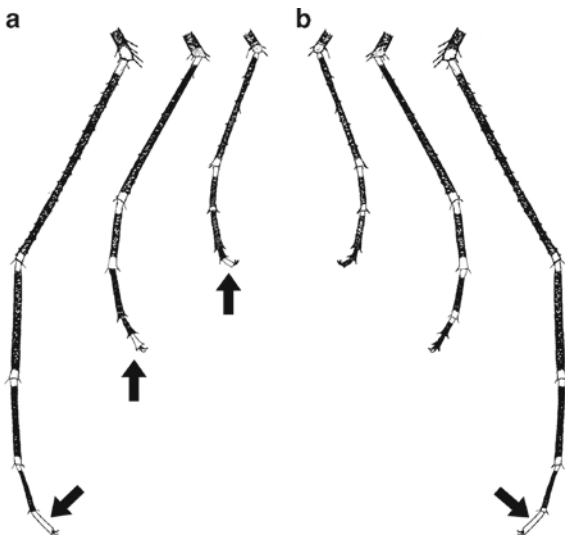


**Fig. 6.18** Hind tarsus of: (a) *Oc. cantans*; (b) *Ae. rossicus*

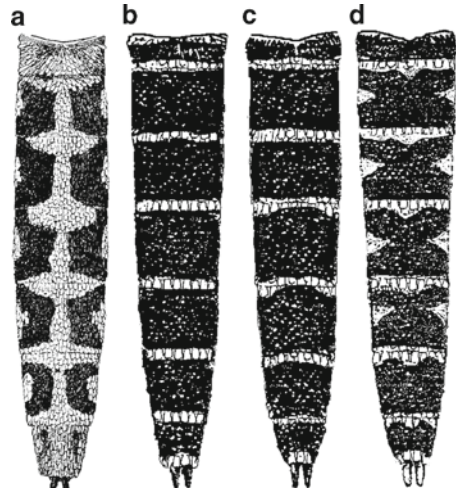


**Fig. 6.19** Hind tarsus of: (a) *Oc. caspius*; (b) *Ae. vexans*

- 2 (1) Each pale ring embraces two tarsomeres, the apex of one and the base of the following tarsomere (Fig. 6.19a). ..... 3
- Pale rings present only at base of tarsomeres (Fig. 6.19b). ..... 6
- 3 (2) Wing veins uniformly dark scaled (in *Oc. berlandi* isolated pale scales sometimes present on wing veins and legs). Tarsomere V of all legs entirely pale scaled (Fig. 6.20a). ..... *Oc. berlandi* and *Oc. pulcritarsis* (p 212, 248)
- Wing veins with pale and dark scales. Only tarsomere V of hind legs entirely pale scaled (Fig. 6.20b). ..... 4

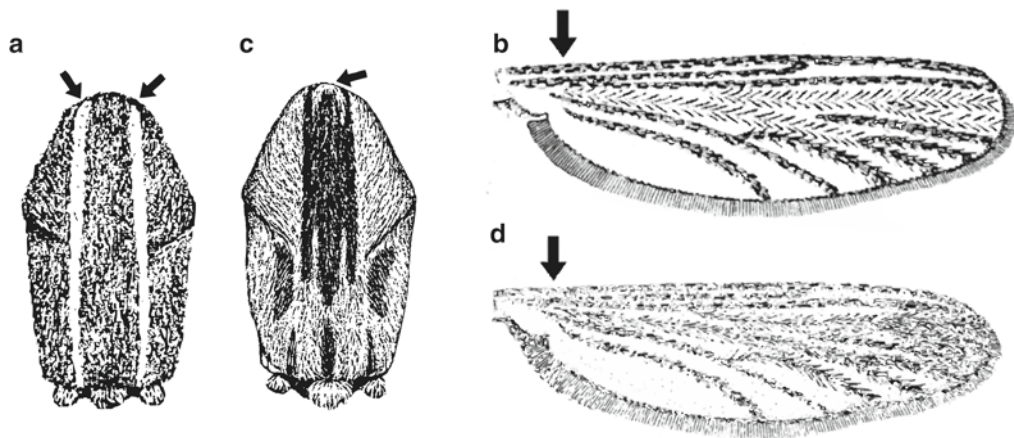


**Fig. 6.20** Fore, mid and hind tarsi of:  
(a) *Oc. berlandi*; (b) *Oc. dorsalis*



**Fig. 6.21** Dorsal view of abdomen of:  
(a) *Oc. caspius*; (b–d) *Oc. mariae* s.l.

- 4 (3) Terga with a median longitudinal pale band (in *Oc. caspius* rarely restricted to tergum II). The last terga sometimes almost completely covered with pale scales (Fig. 6.21a). ..... 5
- Terga without a median longitudinal pale band. Pale scales form narrow basal transverse bands of varying pattern (Fig. 6.21b–d). ..... *Oc. mariae* and *Oc. zamitii* (p 240, 242)
- 5 (4) Scutum fawn coloured, with two narrow dorsocentral white stripes, reaching to the posterior margin of scutum. Wing veins with dark and pale scales more or less evenly mixed (Fig. 6.22a,b). .....  
     ..... *Oc. caspius* (p 216)
- Scutum with a dark brown median stripe, reaching to the prescutellar dorsocentral area. Median stripe posteriorly ornamented with a pair of narrow white lines. Posterior submedian area usually dark brown scaled. Lateral parts of scutum greyish-white. Base of C, Sc, and R predominantly pale scaled (Fig. 6.22c, d). ..... *Oc. dorsalis* (p 226)

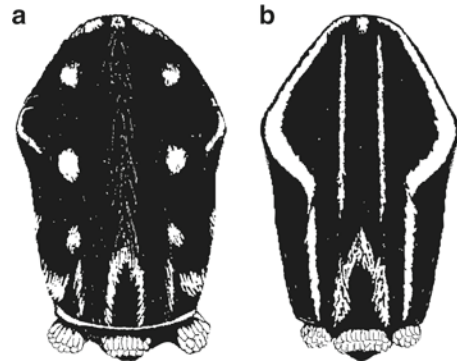


**Fig. 6.22** Scutum and wing of: (a, b) *Oc. caspius*; (c, d) *Oc. dorsalis*

- 6 (2) Proboscis as long as fore femur or slightly shorter. Scutellum with broad, white scales (Fig. 6.23a). ..... 7  
 Proboscis distinctly longer than fore femur. Scutellum with narrow, yellowish or pale and curved scales (Fig. 6.23b)..... 10

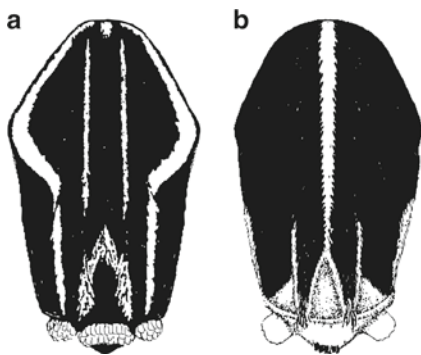


**Fig. 6.23** Scutellum of: (a) *Ae. aegypti*; (b) *Ae. vexans*

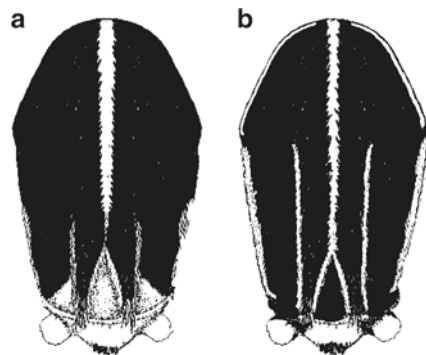


**Fig. 6.24** Scutum of: (a) *Ae. vittatus*; (b) *Ae. aegypti*

- 7 (6) Scutum with 2 to 3 pairs of small white spots, distributed along the dorsocentral area (Fig. 6.24a). Tibiae of hind legs with white rings in the middle. .... *Ae. vittatus* (p 196)  
 Scutum with one or more longitudinal white stripes (Fig. 6.24b). Tibiae of hind legs entirely dark scaled ..... 8
- 8 (7) Scutum without acrostichal stripe on anterior part, but with two narrow white dorsocentral stripes separated from anterior margin. Lateral white stripes broad, continuing over transverse suture to the end of scutum, lyre shaped (Fig. 6.25a). .... *Ae. aegypti* (p 198)  
 Scutum with a white acrostichal stripe extending from the anterior margin to the beginning of the prescutellar area, where it forks to end at the anterior margin of scutellum. If lateral stripes are present, they are narrow and do not continue over transverse suture, never lyre shaped (Fig. 6.25b). ..... 9



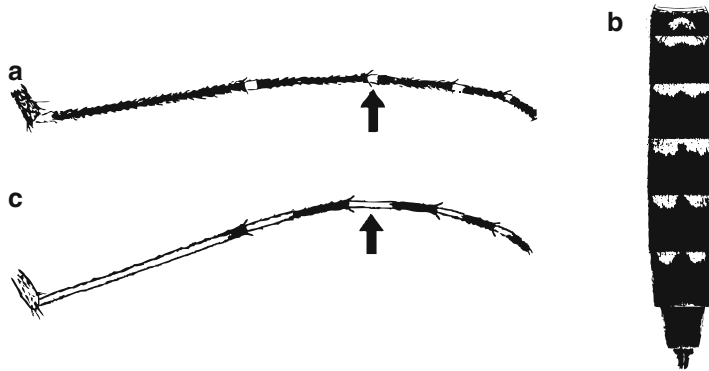
**Fig. 6.25** Scutum of: (a) *Ae. aegypti*; (b) *Ae. albopictus*



**Fig. 6.26** Scutum of: (a) *Ae. albopictus*; (b) *Ae. cretinus*

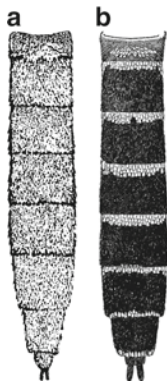
- 9 (8) Acrostichal stripe broad. Posterior dorsocentral white stripes narrow, short, not reaching to middle of scutum (Fig. 6.26a). .... *Ae. albopictus* (p 201)  
 Acrostichal stripe narrow. Posterior dorsocentral white stripes narrow, long, reaching to middle of scutum slightly posterior to the level of scutal angle (Fig. 6.26b). .... *Ae. cretinus* (p 203)

- 10 (6) Pale rings on tarsi very narrow, usually not exceeding more than 1/4 the length of the tarsomeres. Terga with white transverse basal bands constricted in the middle giving them a bilobed pattern (Fig. 6.27a, b). ..... *Ae. vexans* (p 194)  
 Pale rings on tarsi broad. Tarsomere III of hind legs with the pale ring embracing at least 1/3 of its length (Fig. 6.27c). ..... 11

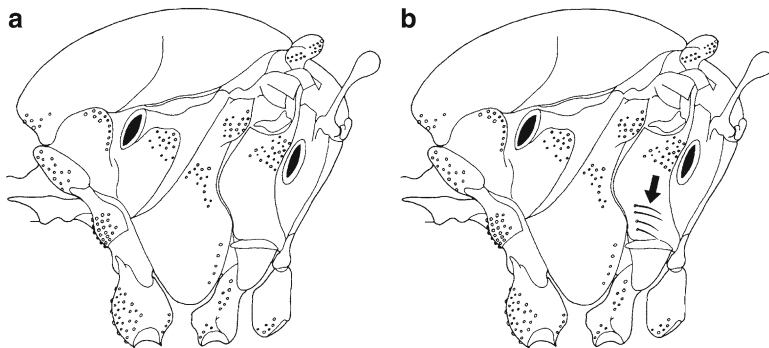


**Fig. 6.27** Tarsus (a) and abdomen (b) of *Ae. vexans* and tarsus (c) of *Oc. flavescens*

- 11 (10) Dorsal surface of terga with pale scales, sometimes mixed with isolated dark scales (Fig. 6.28a). .... 12  
 Dorsal surface of terga with scattered dark scales (not isolated). Sometimes dark scales predominate (Fig. 6.28b). ..... 13



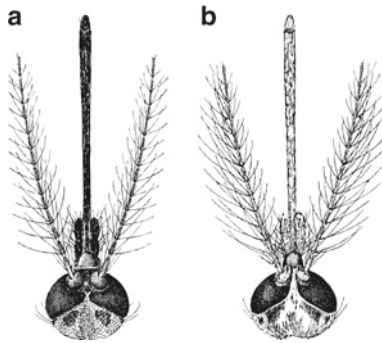
**Fig. 6.28** Dorsal view of abdomen of:  
 (a) *Oc. flavescens*; (b) *Oc. cantans*



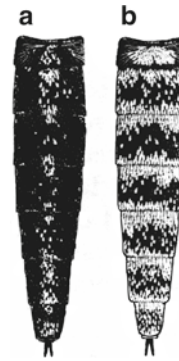
**Fig. 6.29** Lateral view of thorax of:  
 (a) *Oc. flavescens*; (b) *Oc. cyprius*

- 12 (11) Scutum covered with copper or golden brown scales. Scales on terga straw coloured, distinctly paler than scutal scales. Pleural scales also paler compared to scutal scales. Lower mesepimeral setae absent (Fig. 6.29a). ..... *Oc. flavescens* (p 231)  
 Scutum covered with golden yellowish scales. Scales on terga usually ochre yellowish, not distinctly differing in colour from the scales of the scutum. Pleurites with cream coloured scales, colouration similar to scutum. Lower mesepimeral setae present (Fig. 6.29b). ..... *Oc. cyprius* (p 221)

- 13 (11) Palps, proboscis and wing veins uniformly dark scaled, occasionally isolated pale scales may be present at tip of palps or at base of costa (C) (Fig. 6.30a). ..... *Oc. mercurator* (p 242)  
 Palps, proboscis and wing veins, or at least one of them, with scattered or grouped pale scales (Fig. 6.30b). ..... 14

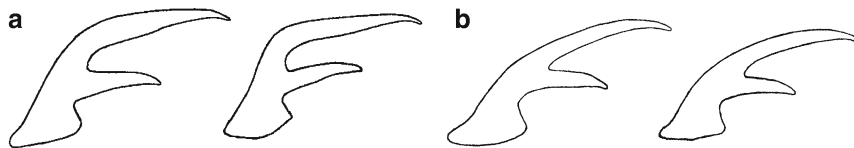


**Fig. 6.30** Head of: (a) *Oc. mercurator*; (b) *Oc. excrucians*



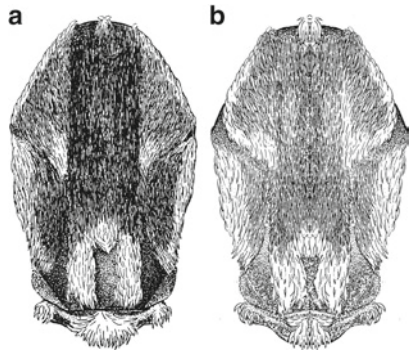
**Fig. 6.31** Dorsal view of abdomen of: (a) *Oc. behningi*; (b) *Oc. excrucians*

- 14 (13) Terga almost completely covered with dark scales dorsally, without pale transverse bands. Pale scales usually forming diffuse patches in the midline of the terga (Fig. 6.31a). Scutum usually uniformly coloured with small, bronze or rust coloured scales. .... *Oc. behningi* (p 211)  
 Terga with scattered pale scales not forming patches or with more or less distinct pale transverse bands dorsally (Fig. 6.31b). Scutum with a dark median stripe or with diffuse pale patches..... 15
- 15 (14) Claw strongly and abruptly bent beyond the base of subbasal tooth. Subbasal tooth nearly parallel enclosing an angle of less than 25°. Claw more or less sinuous beyond bend (Fig. 6.32a).  
 ..... *Oc. excrucians* (p 229)  
 Claw evenly curved. Subbasal tooth clearly diverging, enclosing an angle of at least 30°. Apex of claw not sinuous (Fig. 6.32b). ..... 16

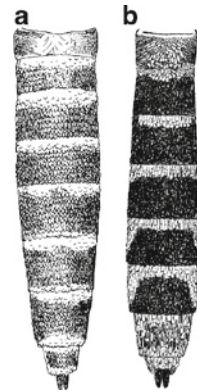


**Fig. 6.32** Claws of: (a) *Oc. excrucians*; (b) *Oc. riparius*

- 16 (15) Scutum with a more or less well defined median stripe formed by brown scales (Fig. 6.33a). ..... 17  
 Scutum without a well defined median dark brown stripe (Fig. 6.33b). ..... 18



**Fig. 6.33** Scutum of: (a) *Oc. riparius*; (b) *Oc. euedes*



**Fig. 6.34** Dorsal view of abdomen of: (a) *Oc. riparius*; (b) *Oc. annulipes*

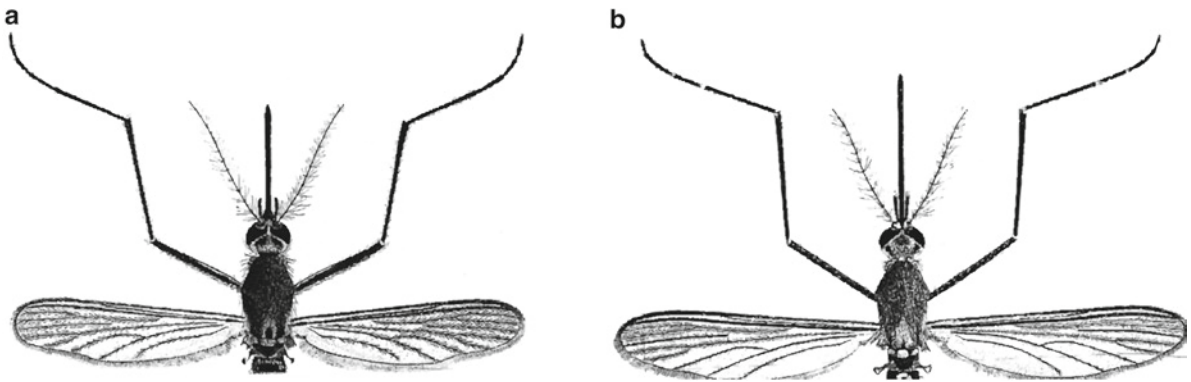
- 17 (16) Median stripe of scutum dark brown or bronze. Integument reddish brown. Terga with distinct white or pale basal transverse bands, sometimes interrupted in the middle on terga II–V forming indistinct pale triangular patches laterally. Apical bands of pale scales present at least on segments VI–VIII (Fig. 6.34a). Lower postpronotal patch with sickle-shaped pale scales. ....*Oc. riparius* (p 253)
- Median stripe of scutum chocolate brown, golden brown or fawn coloured (sometimes not so distinct). Integument brownish, mesepimeral sclerite honey coloured. Terga with distinct yellowish basal transverse bands, scattered light scales might be present at the apex (Fig. 6.34b). Lower postpronotal patch with broad white scales. ....*Oc. annulipes* (p 209)
- Note:** To separate *annulipes* from *cantans*, the hind supraalar setae may be considered. They are straw coloured in the former and dark coloured in the latter. General body colouration should also be considered, see species description.
- 18 (16) Scutum covered with dark brown or bronze brown scales. Usually a pair of white submedian patches present just beyond scutal angle (Fig. 6.35a). White basal transverse bands on terga of variable width, sometimes indistinct. More or less numerous white scales scattered among the dark distal part of the terga. ....*Oc. cantans* (p 214)
- Scutum covered with reddish brown, golden or bronze scales. Submedian pale patches absent (Fig. 6.35b). At least anterior terga with narrow basal transverse bands. Numerous scattered white scales apically, sometimes forming narrow transverse apical bands. .... 19



**Fig. 6.35** Scutum of: (a) *Oc. cantans*; (b) *Oc. euedes*

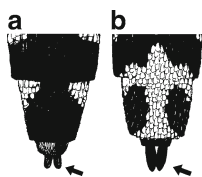


- 19 (18) Dorsocentral stripes of pale scales start at some distance from anterior margin of scutum, extending to level of transverse suture, white lateral stripes continuing over transverse suture and reaching the end of dorsocentral stripes. .... *Oc. euedes* (p 228)  
 Dorsocentral stripes of pale scales usually start at about the level of transverse suture reaching close to the posterior margin of scutum. White lateral stripes sometimes indistinct, transverse suture with well visible stripes of white scales. .... *Oc. surcoufi* (p 231)
- 20 (1) Proboscis not longer than fore femur (Fig. 6.36a). .... 21  
 Proboscis distinctly longer than fore femur (Fig. 6.36b). .... 22

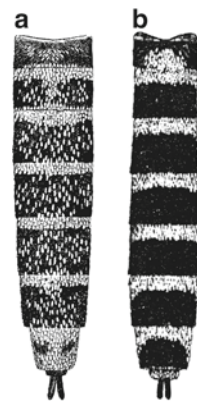


**Fig. 6.36** Head and thorax of: (a) *Ae. cinereus*; (b) *Ae. vexans*

- 21 (20) Scales of scutum fawn brown, scales of terga dark brown, colouration distinctly different. Scales of pleurites yellowish white, scales of sterna yellowish. .... *Ae. cinereus* and *Ae. geminus* (p 189, 191)  
 Scales of scutum and terga unicolourous, dark brown. Scales of pleurites and sterna greyish white. .... *Ae. rossicus* (p 192)
- 22 (20) Cerci short, blunt (Fig. 6.37a). Pale patches on terga silvery, with metallic sheen. .... 23  
 Cerci long, tapering (Fig. 6.37b). Pale scales on terga, if present, without silvery sheen. .... 24

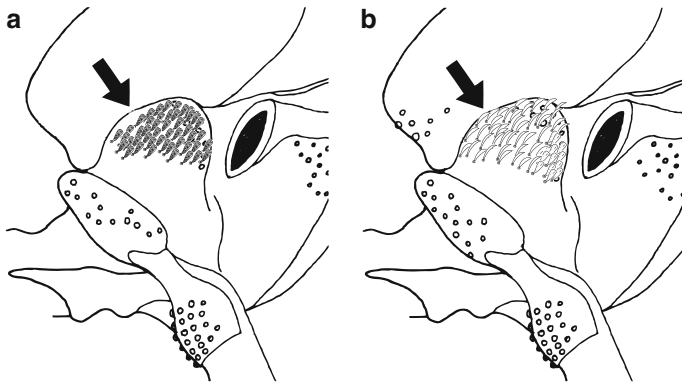


**Fig. 6.37** Abdominal end of:  
 (a) *Oc. geniculatus*; (b) *Oc. rusticus*

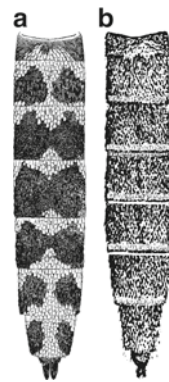


**Fig. 6.38** Dorsal view of abdomen of:  
 (a) *Oc. detritus*; (b) *Oc. cataphylla*

- 23 (22) Scutellum with narrow yellowish scales. .... *Oc. geniculatus* (p 206)  
 Scutellum with broad whitish scales. .... *Oc. echinus* (p 205)
- 24 (22) Pale scales present or predominating in apical half of terga (Fig. 6.38a). .... 25  
 Apical half of terga with dark scales, pale scales forming basal bands or lateral spots (Fig. 6.38b).  
 ..... 30
- 25 (24) Upper scales of postpronotum broad, straight and black (Fig. 6.39a). Scutum with a dark median stripe,  
 which can be divided into two stripes by a narrow acrostichal stripe of paler scales. .... 26  
 Upper scales of postpronotum often narrow, curved (Fig. 6.39b). If broad and straight, the colour is  
 yellowish or light brown, not black. Scutum usually without dark median stripe, if present, the colour  
 of the stripe is bronze or yellowish. .... 28
- 26 (25) Basal transverse bands on terga distinct, usually widened middorsally. Often at least the apical 2-3 terga  
 with a continuous median longitudinal band (Fig. 6.40a). .... 27  
 Basal transverse bands on terga present, sometimes not well defined and not widened in the middle. Pale  
 scales scattered on apical part of terga (sometimes predominating) and occasionally forming narrow  
 apical bands (Fig. 6.40b). .... *Oc. refiki* (p 259)

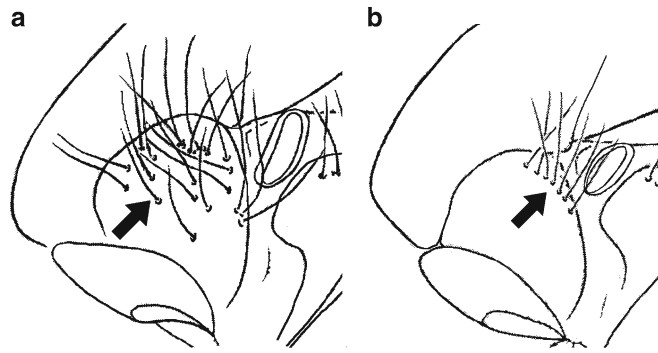


**Fig. 6.39** Lateral view of thorax of:  
 (a) *Oc. refiki*; (b) *Oc. detritus*



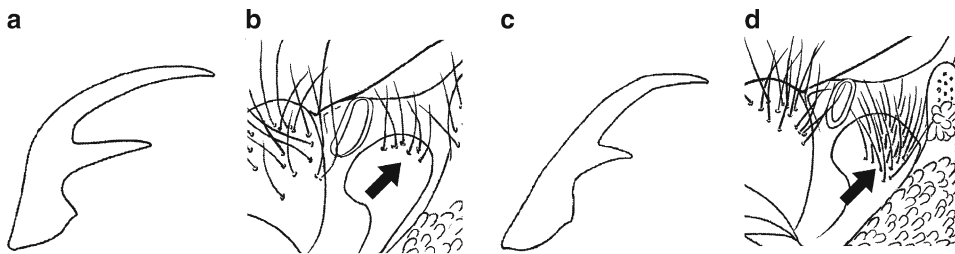
**Fig. 6.40** Dorsal view of abdomen of:  
 (a) *Oc. rusticus*; (b) *Oc. refiki*

- 27 (26) Subcosta with scattered pale scales, most numerous at its apex. .... *Oc. rusticus* (p 261)  
 Subcosta without pale scales. .... *Oc. quasirusticus* (p 258)
- 28 (25) Terga with transverse bands of pale scales. Scattered pale scales usually present in apical part of terga  
 (Fig. 6.38a). .... *Oc. detritus* (p 223)  
 Pale transverse bands absent. Terga completely covered with pale scales or with scattered dark scales  
 sometimes forming diffuse spots. .... 29
- 29 (28) Postnotum with a group of scales. .... *Oc. lepidonotus* (p 257)  
 Postnotum without a group of scales. .... *Oc. subdiversus* (p 263)
- 30 (24) Scutum with dense, long and black setae. Postpronotal setae scattered on entire postpronotum  
 (Fig. 6.41a). .... 31  
 Setae of scutum not that long and fewer, often brown or golden. Postpronotal setae only  
 present along posterior margin, sometimes with a few setae confined to dorsal margin of postpronotum  
 (Fig. 6.41b). .... 32



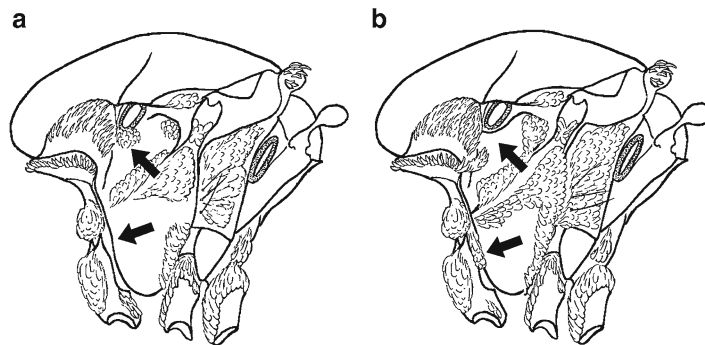
**Fig. 6.41** Lateral view of thorax of: (a) *Oc. impiger*; (b) *Oc. cataphylla*

- 31 (30) Tarsal claws sharply bent in the middle. Apical part of claw nearly parallel to long subbasal tooth (most distinct on fore legs). Postspiracular area with 10 or less setae (Fig. 6.42a, b). ..... *Oc. impiger* (p 236)  
 Tarsal claw evenly curved. Apical part of claw not parallel to short subbasal tooth (enclosing an angle of more than 45°). Postspiracular area with 14 or more setae (Fig. 6.42c, d). ..... *Oc. nigripes* (p 245)



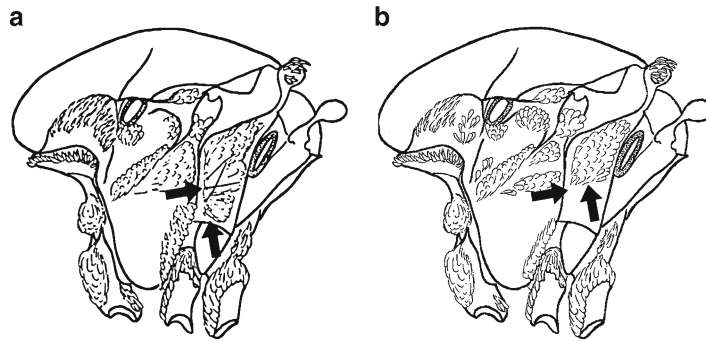
**Fig. 6.42** Claw and lateral view of thorax of: (a, b) *Oc. impiger*; (c, d) *Oc. nigripes*

- 32 (30) Wing veins with pale and dark scales intermixed, especially on C and R<sub>1</sub>. ..... 33  
 Wing veins without pale scales, if present, pale scales restricted to basal part of veins. .... 34  
 33 (32) Proboscis uniformly dark scaled. .... *Oc. cataphylla* (p 218)  
 Proboscis speckled with more or less numerous pale scales, especially in the middle. ....  
 ..... *Oc. leucomelas* (p 238)  
 34 (32) Hypostigmal patch of scales present, postprocoxal patch of scales absent (Fig. 6.43a). ..... 35  
 Hypostigmal patch of scales absent, postprocoxal patch of scales present or absent (Fig. 6.43b). .... 36



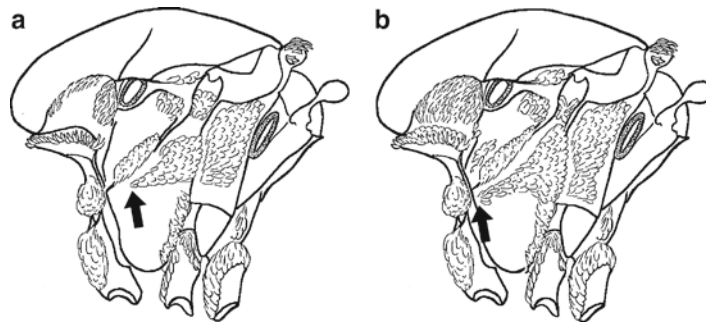
**Fig. 6.43** Lateral view of thorax of: (a) *Oc. pullatus*; (b) *Oc. punctor*

- 35 (34) Mesepimeral patch of white scales reaching lower margin of mesepimeron. Lower mesepimeral setae present (Fig. 6.44a). ..... *Oc. pullatus* (p 249)  
 Mesepimeral patch of white scales not reaching lower margin of mesepimeron. Lower mesepimeral setae usually absent (Fig. 6.44b). ..... *Oc. intrudens* (p 237)



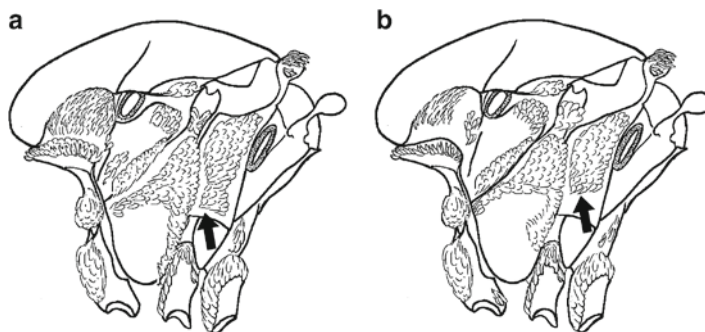
**Fig. 6.44** Lateral view of thorax of: (a) *Oc. pullatus*; (b) *Oc. intrudens*

- 36 (34) Upper mesepisternal patch of scales not reaching anterior angle of mesepisternum, or the lower edge ending above the level of anterior angle (Fig. 6.45a). ..... 37  
 Upper mesepisternal patch of scales reaching anterior angle of mesepisternum or at least a few scales situated close to it or below the level of anterior angle (Fig. 6.45b). ..... 38



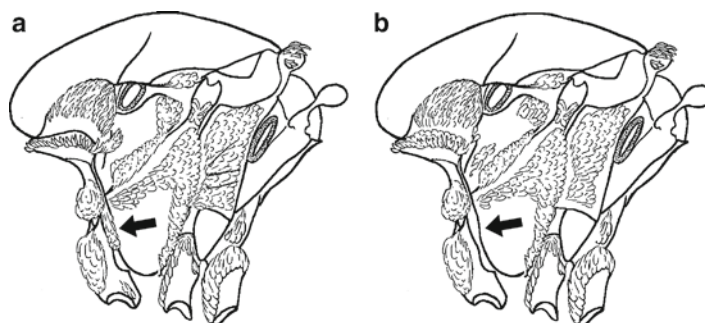
**Fig. 6.45** Lateral view of thorax of: (a) *Oc. diantaeus*; (b) *Oc. communis*

- 37 (36) Upper mesepisternal patch of scales large, not divided into two portions (Fig. 6.45a). Abdominal terga with triangular patches of pale scales, patches usually connected by a basal transverse band on the last segments. .... *Oc. diantaeus* (p 224)  
 Upper mesepisternal patch of scales small, divided into two or more portions (Fig. 6.44b). All abdominal terga with pale transverse basal bands. .... *Oc. intrudens* (p 237)  
 38 (36) Mesepimeral patch of scales reaches lower margin of mesepimeron (Fig. 6.46a). ..... 39  
 Mesepimeral patch of scales ends distinctly above lower margin of mesepimeron (Fig. 6.46b). ..... 42



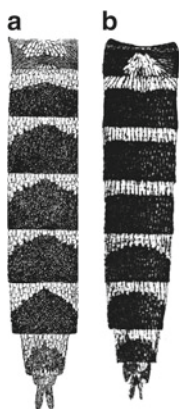
**Fig. 6.46** Lateral view of thorax of: (a) *Oc. communis*; (b) *Oc. sticticus*

- 39 (38) Postprocoxal scales present (Fig. 6.47a). ..... 40
- Postprocoxal scales absent (Fig. 6.47b). ..... *Oc. communis* (p 220)

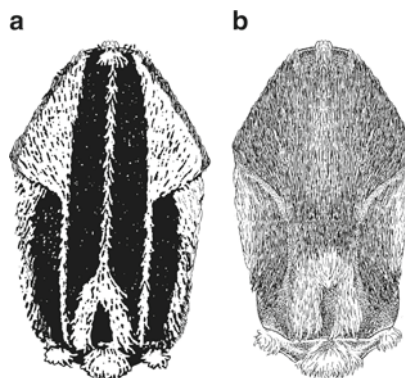


**Fig. 6.47** Lateral view of thorax of: (a) *Oc. punctor*; (b) *Oc. communis*

- 40 (39) Pale bands of terga II–V distinctly confined in the middle (Fig. 6.48a). .....  
 ..... *Oc. punctor* and *Oc. punctodes* (p 251, 250)
- Pale bands of terga II–V of uniform width or only slightly confined in the middle (Fig. 6.48b). ..... 41

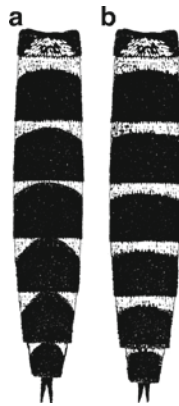


**Fig. 6.48** Dorsal view of abdomen of: (a) *Oc. punctor*; (b) *Oc. hexodontus*



**Fig. 6.49** Scutum of: (a) *Oc. pionips*; (b) *Oc. hexodontus*

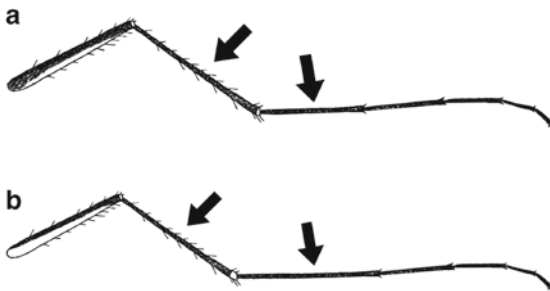
- 41 (40) Scutum with dark brown median stripe, occasionally divided into two dorsocentral stripes by an acrostichal row of yellowish scales (Fig. 6.49a). Base of costa covered with dark scales, rarely a few pale scales present. .... *Oc. pionips* (p 246)
- Scutum more or less uniformly covered with yellowish brown scales (Fig. 6.49b). Base of costa with numerous pale scales often forming large patch. .... *Oc. hexodontus* (p 233)
- 42 (38) Hind tibia generally covered with light scales on anterior surface. .... 43
- Hind tibia with dark scales on anterior surface. .... *Oc. hungaricus* (p 234)
- 43 (42) Wing veins usually entirely dark scaled. Terga II–IV with pale basal bands distinctly constricted in the middle, on following terga basal bands interrupted in the middle forming triangular pale patches at lateral sides (Fig. 6.50a). First flagellomere yellowish at the base, second and third not distinctly shortened. .... *Oc. sticticus* (p 255)
- Wing veins with scattered pale scales at base of C, entire Sc and M proximal to the cross veins. Terga with broad pale basal bands, slightly, if at all, constricted in the middle (Fig. 6.50b). First flagellomere entirely black, second and third distinctly shortened. .... *Oc. nigrinus* (p 243)



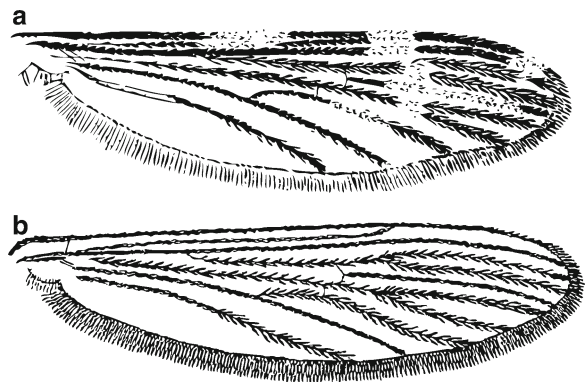
**Fig. 6.50** Dorsal view of abdomen of:  
(a) *Oc. sticticus*; (b) *Oc. nigrinus*

**6.3 Genus Culex**

- 1 Tarsomere I of hind legs distinctly shorter than hind tibia (Fig. 6.51a). .... 2
- Tarsomere I of hind legs as long as or slightly longer than hind tibia (Fig. 6.51b). .... 3

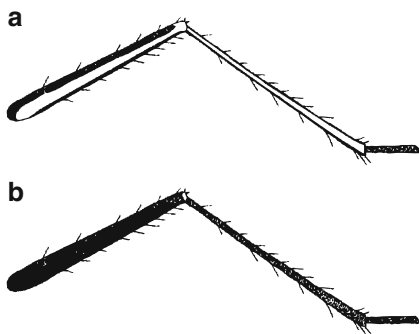


**Fig. 6.51** Hind leg of: (a) *Cx. modestus*; (b) *Cx. p. pipiens*

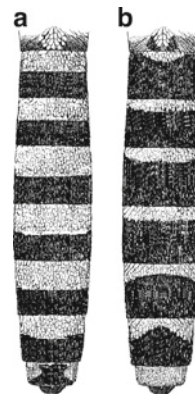


**Fig. 6.52** Wing of: (a) *Cx. mimeticus*; (b) *Cx. p. pipiens*

- 2 (1) Terga with continuous lateral longitudinal bands of pale scales. Pale scales sometimes form more or less developed, triangular patches, which are usually connected. .... *Cx. modestus* (p 265)  
 Terga without continuous lateral longitudinal bands. More or less developed patches of pale scales at the lateral basal margins of terga present. .... *Cx. pusillus* (p 267)
- 3 (1) Tarsi with pale rings, which are sometimes narrow. Wings with large, prominent spots of pale scales, especially on C (Fig. 6.52a). .... *Cx. mimeticus* (p 271)  
 Tarsi without pale rings. Wing veins mainly covered with dark scales (in *theileri* a few pale scales may be present on C) (Fig. 6.52b). .... 4
- 4 (3) Terga uniformly covered with reddish brown scales, without pale transverse bands. *Cx. martinii* (p 285)  
 Terga with more or less developed transverse bands formed by white or yellowish scales. .... 5
- 5 (4) Terga with pale basal bands, sometimes reduced to lateral triangular patches. .... 6  
 Terga with pale apical bands, sometimes reduced to lateral spots or completely absent. .... 10
- 6 (5) Femora and tibiae of fore and mid legs with a distinct anterior pale longitudinal stripe, rarely only the fore femur with a pale stripe (Fig. 6.53a). Pale basal bands on abdominal terga usually triangularly extended posteriorly. .... *Cx. theileri* (p 280)  
 Femora and tibiae of fore and mid legs without anterior pale longitudinal stripe (Fig. 6.53b). .... 7



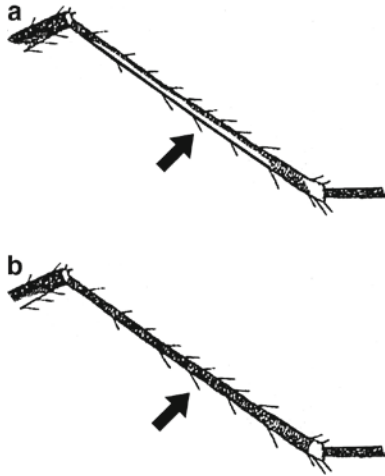
**Fig. 6.53** Fore femur and tibia of:  
 (a) *Cx. theileri*; (b) *Cx. p. pipiens*



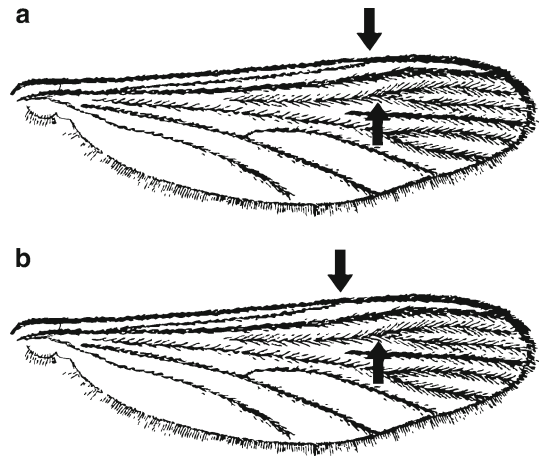
**Fig. 6.54** Dorsal view of abdomen of:  
 (a) *Cx. laticinctus*; (b) *Cx. p. pipiens*

- 7 (6) Proboscis with an indistinct pale median ring. .... *Cx. brumpti* (p 269)  
 Proboscis dark scaled. .... 8
- 8 (7) Basal bands on abdominal terga broad and white, covering 1/2 to 2/3 of each tergum (Fig. 6.54a). Tibia of hind leg with a prominent apical pale spot. .... *Cx. laticinctus* (p 270)  
 Basal bands on abdominal terga narrow, usually covering less than 1/2 of each tergum (Fig. 6.54b). Tibia of hind leg without apical pale spot. .... 9
- 9 (8) Basal bands on terga formed by white scales. Hind tibia with more or less distinct longitudinal anterior pale stripe (Fig. 6.55a). .... *Cx. perexiguus* (p 273)  
 Basal bands on terga formed by yellowish scales. The bands are sometimes reduced or pale scales form patches at the sides of the terga. Hind tibia without longitudinal pale stripe (Fig. 6.55b). ....  
 .... *Cx. p. pipiens*, *Cx. p. quinquefasciatus* and *Cx. torrentium* (p 275, 278, 279)
- 10 (5) Apical bands narrowed or interrupted in the middle on some terga. .... *Cx. impudicus* (p 284)  
 Apical bands well developed, not interrupted in the middle. .... 11

- 11 (10) Palps with dark and pale scales. End of Sc nearly aligned with furcations of  $R_{2+3}$  and M (Fig. 6.56a). Pale apical bands on terga relatively broad, distinctly widened in the middle of some segments. Apex of hind tibia with a pale spot. .... *Cx. hortensis* (p 282)
- Palps with dark scales. End of Sc distinctly displaced towards the wing base compared to furcations of  $R_{2+3}$  and M (Fig. 6.56b). Pale apical bands on terga relatively narrow, without widening in the middle. Apex of hind tibia without a pale spot. Abdomen usually greenish ventrally. .... *Cx. territans* (p 286)



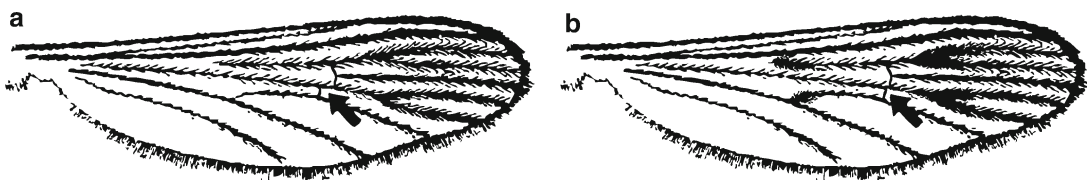
**Fig. 6.55** Hind tibia of: (a) *Cx. perexiguus*; (b) *Cx. p. pipiens* and *Cx. torrentium*



**Fig. 6.56** Wing of: (a) *Cx. hortensis*; (b) *Cx. territans*

**6.4 Genus Culiseta**

- 1 Scutum with distinct longitudinal pale stripes (resemble a lyre in shape). Femora and tibiae with well defined pale spots and stripes. Costa mainly covered with pale scales. .... *Cs. longiareolata* (p 289)
- Scutum without distinct longitudinal stripes. Femora and tibiae without pale stripes, uniformly dark scaled or with scattered light scales. Costa completely or mainly dark scaled. .... 2
- 2 (1) Cross veins r-m and m-cu well separated. The distance between them usually at least the length of m-cu (Fig. 6.57a). Wings usually without dark spots, otherwise with an indistinct dark spot at the base of  $R_{4+5}$ . .... 3
- Cross veins r-m and m-cu aligned or slightly separated. If separated, the distance between them is usually not longer than m-cu (Fig. 6.57b). Wings weakly or distinctly spotted (spots sometimes absent in *Cs. glaphyroptera*). .... 6



**Fig. 6.57** Wing of: (a) *Culicella*; (b) *Culiseta*



- 3 (2) Proboscis with scattered pale scales, mainly in its middle part. Sterna usually with dark scales arranged in a pattern of an inverted “V” (Fig. 6.58a, b). ..... 4  
 Proboscis uniformly dark scaled, rarely with a few pale scales in the middle (in *Cs. morsitans*), or pale scales predominating (in *Cs. ochroptera*). Sterna without a pattern of an inverted “V”, pale and dark scales diffused (Fig. 6.58c, d). ..... 5

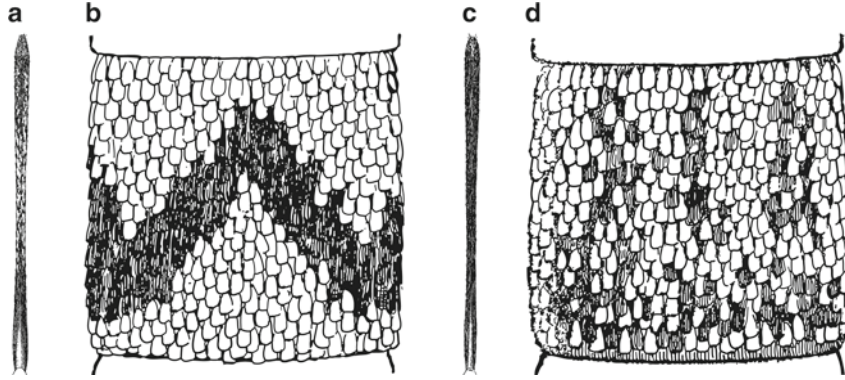


Fig. 6.58 Proboscis and sternum of: (a, b) *Cs. fumipennis*; (c, d) *Cs. morsitans*

- 4 (3) On fore legs pale rings include apical and basal parts between tarsomeres III–IV and IV–V (Fig. 6.59a). ..... *Cs. fumipennis* (p 291)  
 On fore legs apical parts of tarsomeres III and IV entirely dark scaled (Fig. 6.59b). ..... *Cs. litorea* (p 292)

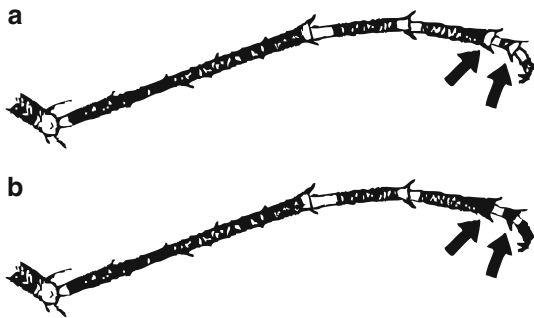


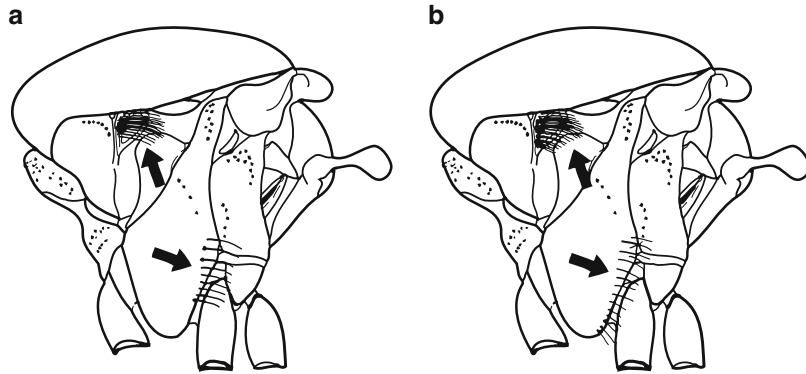
Fig. 6.59 Fore tarsus of: (a) *Cs. fumipennis*; (b) *Cs. litorea*



Fig. 6.60 Hind tarsus of: (a) *Cs. glaphyroptera*; (b) *Cs. annulata*

- 5 (3) Terga with narrow basal pale bands. Scales on wing veins evenly distributed, not forming spots. Tibia of fore leg mainly dark brown scaled. .... *Cs. morsitans* (p 294)  
 Terga with narrow indistinct basal and apical pale bands, sometimes absent. Tergum VIII completely covered with pale scales. Indistinct dark spot at the base of  $R_{4+5}$  may be present. Tibia of fore leg mainly yellowish scaled. .... *Cs. ochroptera* (p 296)
- 6 (2) Tarsi dark (Fig. 6.60a). ..... 7  
 Tarsi with white rings (Fig. 6.60b). ..... 8
- 7 (6) Palps dark with scattered pale scales, eyes distinctly bordered with light scales. Spots on wing veins always present, sometimes indistinct. Usually not more than 15 prespiracular setae and not more than 10 lower mesepisternal setae present (Fig. 6.61a). .... *Cs. bergrothi* (p 301)

Palps entirely dark scaled, eyes not bordered with light scales. Spots on wing veins absent or indistinct. Number of prespiracular setae 16-22 and of lower mesepisternal setae 12-18 (Fig. 6.61b). .....  
 ..... *Cs. glaphyoptera* (p 303)

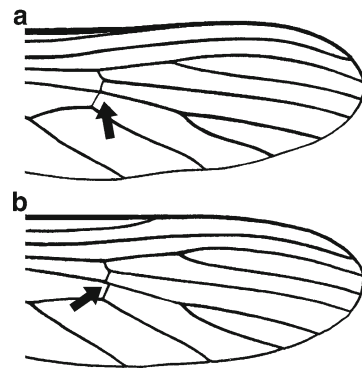


**Fig. 6.61** Lateral view of thorax of: (a) *Cs. bergrothi*; (b) *Cs. glaphyoptera*

8 (6) Femora with subapical pale rings. Tarsomere I of hind legs with a median white ring (Fig. 6.62a). ..... 9  
 Femora without subapical pale rings. Tarsomere I of hind legs without a median white ring (Fig. 6.62b). .....  
 ..... *Cs. alaskaensis* (p 298)



**Fig. 6.62** Hind tarsus of: (a) *Cs. annulata*; (b) *Cs. alaskaensis*



**Fig. 6.63** Wing of: (a) *Cs. annulata*; (b) *Cs. subochrea*

9 (8) Costa (C) usually completely dark scaled or isolated pale scales could be present on C, Sc and R. Cu entirely dark scaled. Dark spots on wings distinct. Terga with distinct white basal bands, pale scales absent in apical half of terga. Cross veins r-m and m-cu aligned (Fig. 6.63a). ..... *Cs. annulata* (p 299)  
 Costa, Sc and R with scattered pale scales. Cu with more or less numerous pale scales. Dark spots on wings indistinct. Terga with indistinct pale basal bands formed by yellowish scales (not white), pale scales are also present among the dark scales in the apical half of the terga. Cross veins r-m and m-cu slightly separated (Fig. 6.63b). ..... *Cs. subochrea* (p 305)

## 6.5 Genus *Coquillettidia*

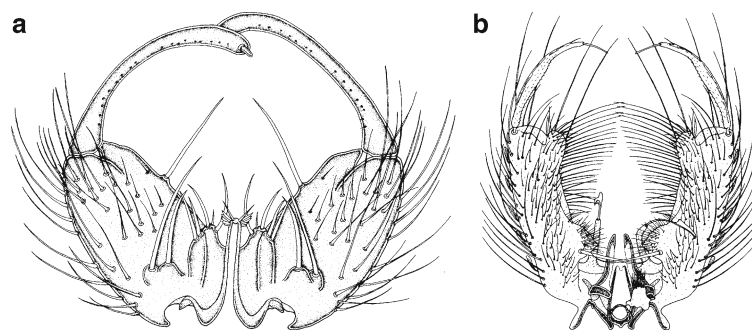
- 1 Tarsomeres with pale rings. Proboscis and palps with numerous pale scales. Wing veins with broad dark and pale scales intermixed. .... *Cq. richiardi* (p 308)
- Tarsomeres without pale rings. Proboscis and palps uniformly blackish brown. Wing veins uniformly dark scaled. .... *Cq. buxtoni* (p 307)

# Chapter 7

## Key to Male Mosquitoes

### Genera

- 1 Gonocoxite without lobes. Gonostylus about as long as gonocoxite. Sclerotized paraproct absent. Proctiger, if present, membranous, cone-shaped and hardly visible (Fig. 7.1a). ..... *Anopheles* (p 164)  
 Gonocoxite with 1 or 2 lobes. Lobes sometimes rudimentary or absent (subgenus *Finlaya*). Gonostylus shorter than gonocoxite. Proctiger different, sclerotized paraproct present (Fig. 7.1b). ..... 2



**Fig. 7.1** Hypopygium of: (a) *An maculipennis s.l.*; (b) *Oc. mercurator*

- 2 (1) Gonocoxite with only one subapically located lobe covered with spines and setae. Apex of paraproct with abundant spines or rows of numerous denticles (paraproct crown) (Fig. 7.2a). ..... *Culex* (p 264)  
 Gonocoxite with 2 lobes (basal and apical), if only one lobe is present, it is situated at the base of the gonocoxite, lobes absent in subgenus *Finlaya*. Apex of paraproct without spines or rows of numerous denticles (Fig. 7.2b). ..... 3  
 3 (2) Apical spine of gonostylus longer than the maximum width of gonostylus (Fig. 7.3a). If the spine is shorter than the maximum width of the gonostylus, it is articulated subapically (Fig. 7.3b). If the spine is absent, the gonostylus is divided at its base (Fig. 7.3c). ..... *Aedes* and *Ochlerotatus* (p 187, 204)  
 Apical spine of gonostylus shorter than or equal to the maximum width of the gonostylus (Fig. 7.3d). ..... 4

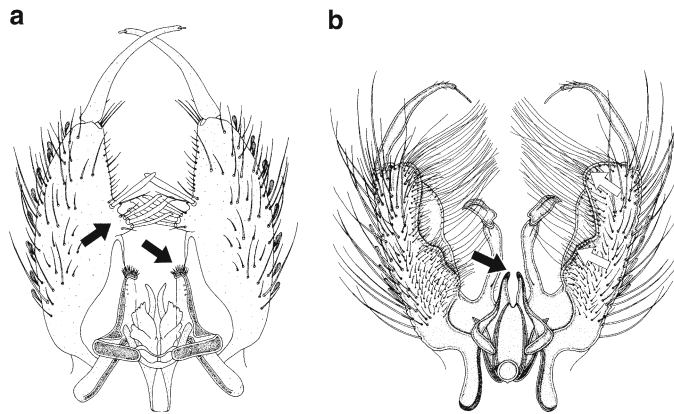


Fig. 7.2 Hypopygium of: (a) *Cx. modestus*; (b) *Oc. annulipes*

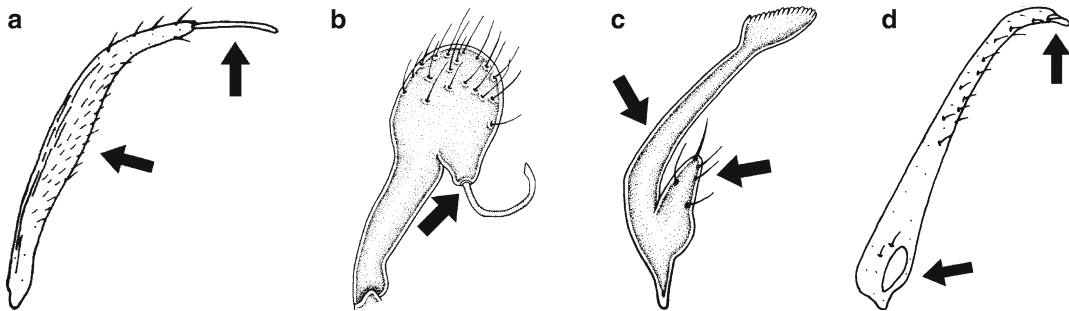


Fig. 7.3 Gonostylus of: (a) *Oc. riparius*; (b) *Ae. vittatus*; (c) *Ae. rossicus*; (d) *Cs. morsitans*

- 4 (3) Basal lobe of gonocoxite with one strongly sclerotized rod like spine (Fig. 7.4a)....*Coquillettidia* (p 306)
- Basal lobe of gonocoxite with at least 2 spines or strong setae (Fig. 7.4b)..... 5

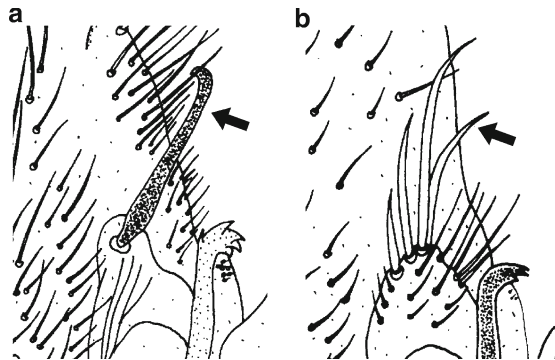
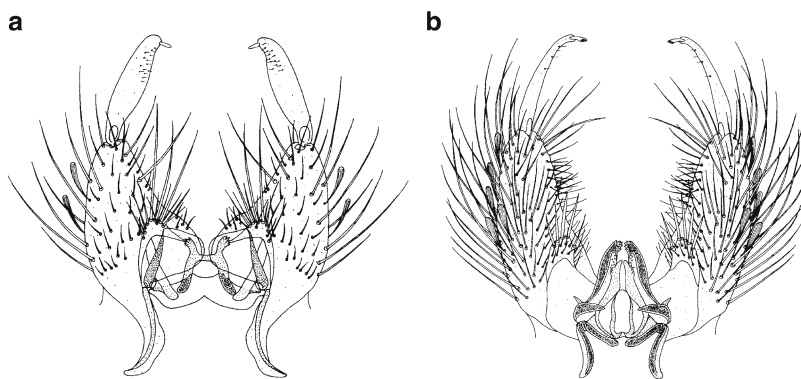


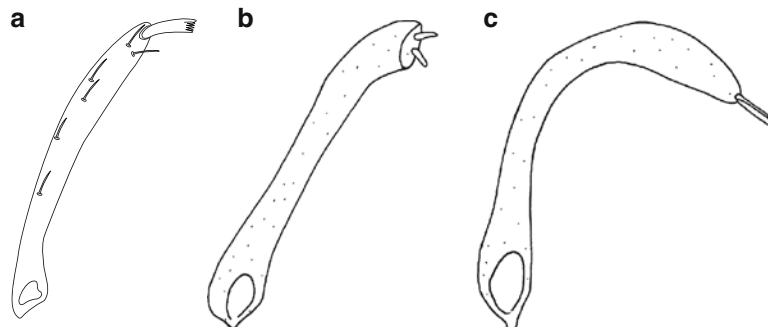
Fig. 7.4 Basal lobe of gonocoxite of: (a) *Cq. richiardi*; (b) *Cs. fumipennis*

- 5 (4) Gonocoxite broad and short, plumpy in appearance with a small, flattened basal lobe. Gonostylus broad, flattened dorsoventrally. Proctiger lobe shaped, membranous (Fig. 7.5a).....*Uranotaenia unguiculata* (p 312)
- Gonocoxite more elongated, with a distinct basal lobe. Gonostylus narrow, not flattened dorsoventrally (Fig. 7.5b)..... 6



**Fig. 7.5** Hypopygium of: (a) *Ur. unguiculata*; (b) *Or. pulcripalpis*

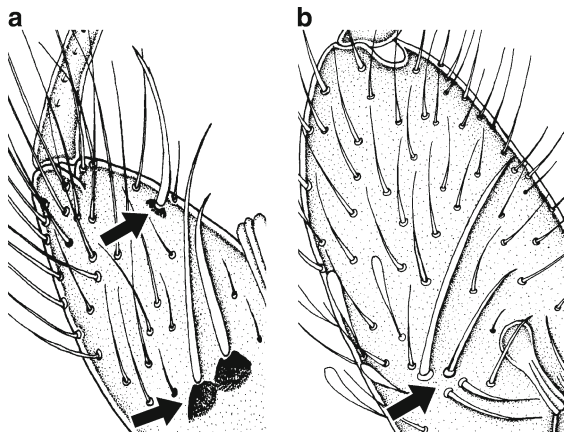
- 6 (5) Apical spine of gonostylus fingered at the apex. Spine as long as the maximum width of the gonostylus (Fig. 7.6a)..... ***Orthopodomyia pulcripalpis*** (p 310)  
 Apical spine of gonostylus not fingered (there may be 2 spines at the apex of the gonostylus in *Cs. longiareolata*, Fig. 7.6b). Spine distinctly shorter than the maximum width of the gonostylus (except in *Cs. glaphyroptera*, Fig. 7.6c). ..... ***Culiseta*** (p 288)



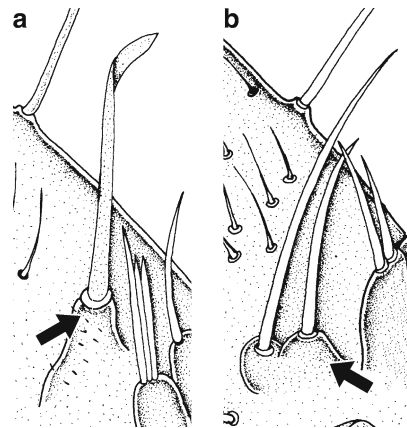
**Fig. 7.6** Gonostylus of: (a) *Or. pulcripalpis*; (b) *Cs. longiareolata*; (c) *Cs. glaphyroptera*

### 7.1 Genus *Anopheles*

- 1 Base of gonocoxite with 1-3, usually 2, parbasal setae. At least one of them originated from a tubercle (tubercles weakly developed in *An. plumbeus*). Internal seta present (subgenus *Anopheles*, Fig. 7.7a) .... 2  
 Base of gonocoxite with 4-7, usually 6, parbasal setae. None of them elevated on a tubercle. Internal seta absent (subgenus *Cellia*, Fig. 7.7b). ..... 7  
 2 (1) Base of gonocoxite with 1 parbasal seta (Fig. 7.8a). ..... ***An. algeriensis*** (p 165)  
 Base of gonocoxite with 2-3 parbasal setae (Fig. 7.8b). ..... 3

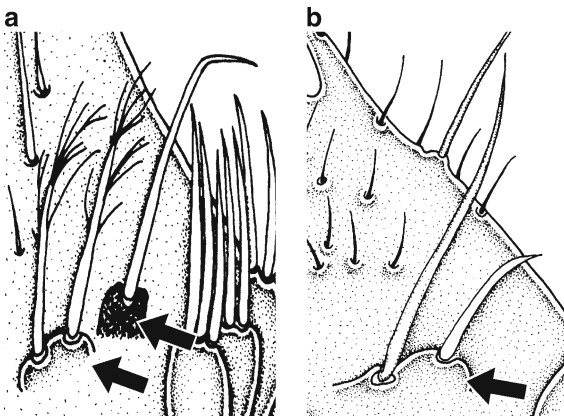


**Fig. 7.7** Gonocoxite of: (a) *An. marteri*; (b) *An. superpictus*

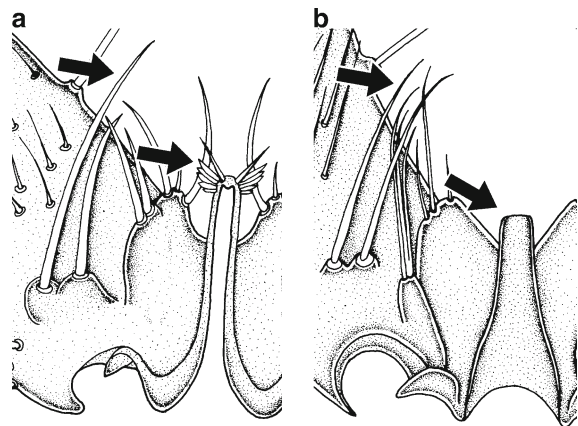


**Fig. 7.8** Base of gonocoxite of: (a) *An. algeriensis*; (b) *An. maculipennis s.l.*

- 3 (2) Base of gonocoxite with 3 parbasal setae, inner 1 simple, the 2 outer setae apically branched (Fig. 7.9a).  
 .....*An. claviger s.s.* and *An. petragrani* (p 166, 168)
- Base of gonocoxite with 2 simple parbasal setae (Fig. 7.9b). ..... 4

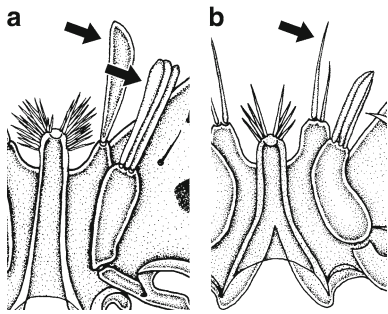


**Fig. 7.9** Base of gonocoxite of: (a) *An. claviger s.l.*; (b) *An. hyrcanus*

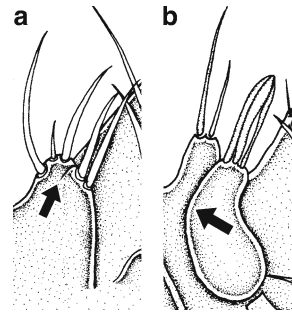


**Fig. 7.10** Aedeagus and parbasal setae of: (a) *An. maculipennis s.l.*; (b) *An. plumbeus*

- 4 (3) Aedeagus with leaflets. Parbasal setae of different length, outer seta longer than inner seta (Fig. 7.10a)..... 5
- Aedeagus without leaflets. Parbasal setae of approximately the same length, tubercles weakly sclerotized (Fig. 7.10b).....*An. plumbeus* (p 178)
- 5 (4) Parbasal setae arising from a strongly sclerotized base (Fig. 7.7a). Outer and inner claspette lobes bear flattened spatula-like or longer sabre-like setae (Fig. 7.11a). ..... *An. marteri* (p 177)
- Parbasal setae arising from a weakly or not sclerotized base (Fig. 7.8b). At least the inner claspette lobe bears spine like setae (Fig. 7.11b)..... 6
- 6 (5) Claspette lobes not well defined, bearing only spine like setae of variable length and shape (Fig. 7.12a). ..... **Anopheles Maculipennis Complex** (p 170)
- Claspette lobes well defined. Setae of outer lobe fused into broad, spatula like processes, inner lobe with 2 spine-like setae (Fig. 7.12b)..... *An. hyrcanus* (p 169)

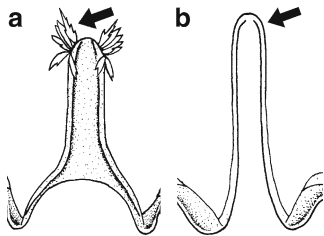


**Fig. 7.11** Claspette of: (a) *An. marteri*; (b) *An. hyrcanus*

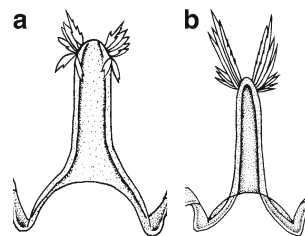


**Fig. 7.12** Claspette of: (a) *An. maculipennis s.l.*; (b) *An. hyrcanus*

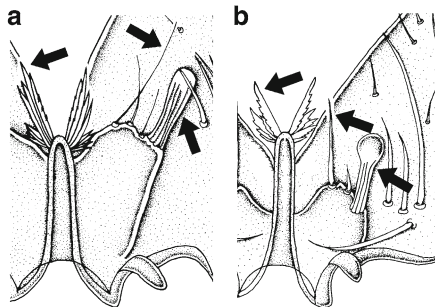
- 7 (1) Leaflets of aedeagus present (Fig. 7.13a)..... 8
- Leaflets of aedeagus absent (Fig. 7.13b)..... *An. multicolor* (p 182)
- 8 (7) Leaflets of aedeagus short, distinctly shorter than half the length of the aedeagus (Fig. 7.14a).....
- ..... *An. superpictus* (p 185)
- Leaflets of aedeagus well developed, longest leaflet almost half as long as the aedeagus (Fig. 7.14b)..... 9
- 9 (8) Longest spine-like seta of claspette distinctly longer than spatula-like seta. The longest leaflet of aedeagus is less than 1/3 longer than the second longest. Leaflets weakly serrated (Fig. 7.15a).....
- ..... *An. cinereus hispaniola* (p 181)
- Longest spine-like seta of claspette of similar length as spatula-like seta. The longest leaflet of aedeagus is distinctly longer than the second longest. Leaflets strongly serrated (Fig. 7.15b)..... *An. sergentii* (p 183)



**Fig. 7.13** Aedeagus of: (a) *An. superpictus*; (b) *An. multicolor*



**Fig. 7.14** Aedeagus of: (a) *An. superpictus*; (b) *An. cinereus hispaniola*

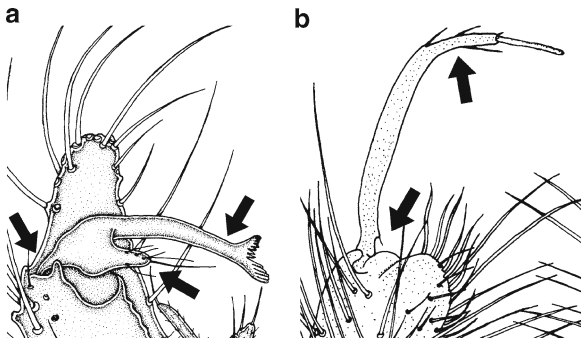


**Fig. 7.15** Claspette and aedeagus of: (a) *An. cinereus hispaniola*; (b) *An. sergentii*

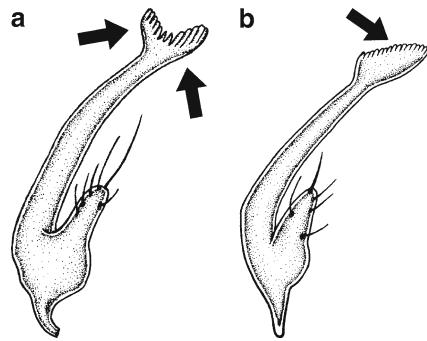


**7.2 Genera *Aedes* and *Ochlerotatus***

- 1 Apex of gonocoxite exceeding articulation point of gonostylus, which is divided into two branches (Fig. 7.16a). Palps several times shorter than the proboscis, as in females ..... 2
- Gonostylus arising at the apex of gonocoxite, simple, not divided into 2 branches (Fig. 7.16b). Palps about as long as the proboscis..... 4
- 2 (1) Longer branch of gonostylus forked into 2 prongs at the apex (Fig. 7.17a)..... 3
- Longer branch of gonostylus not forked at the apex, denticulated at outer apical margin (Fig. 7.17b)..... *Ae. rossicus* (p 192)

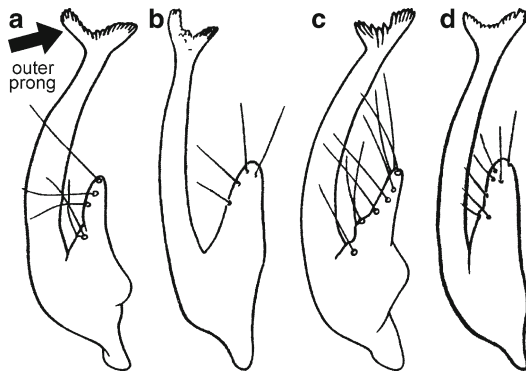


**Fig. 7.16** Apex of gonocoxite and gonostylus of: (a) *Ae. cinereus*; (b) *Oc. communis*

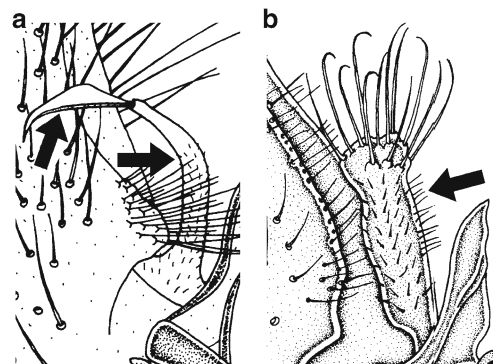


**Fig. 7.17** Gonostylus of: (a) *Ae. cinereus*; (b) *Ae. rossicus*

- 3 (2) Outer prong of the fork usually longer than or equal to the inner one (Fig. 7.18a,b).. *Ae. geminus* (p 191)
- Inner prong of the fork usually longer than the outer one (Fig. 7.18c,d)..... *Ae. cinereus* (p 189)
- Note:** Transitional forms present, specific status of *geminus* uncertain.
- 4 (1) Typical claspette (divided into stem and filament) present (Fig. 7.19a)..... 9
- Typical claspette absent (Fig. 7.19b)..... 5

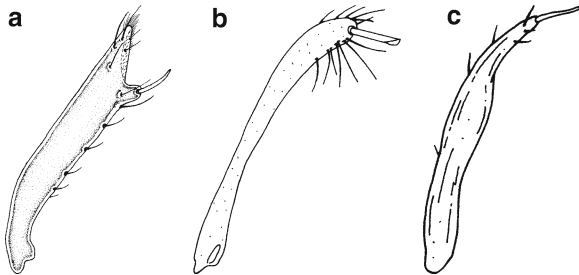


**Fig. 7.18** Gonostylus of: (a, b) *Ae. geminus*; (c, d) *Ae. cinereus*

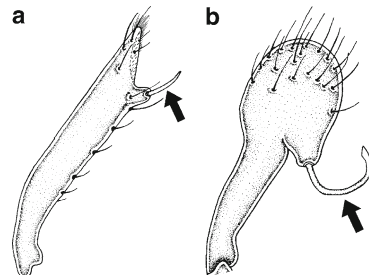


**Fig. 7.19** Claspette of: (a) *Oc. nigripes*; (b) *Ae. vexans*

- 5 (4) Gonostylus distinctly expanded apically (Fig. 7.20a). Claspettes of different shape, elongated, well separated from basal part of gonocoxite. .... 6  
 Gonostylus slightly expanded apically or evenly tapering (Fig. 7.20b,c). Claspette lobe like, seem to be inner basal part of gonocoxite and covered with dense setae, some of them may be enlarged, spine like..... 7

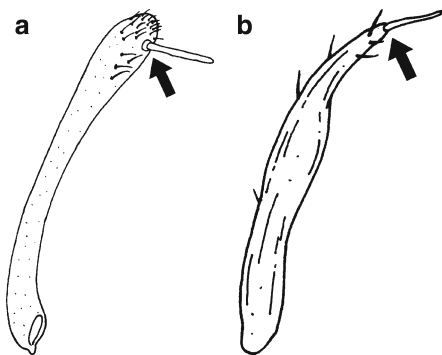


**Fig. 7.20** Gonostylus of: (a) *Ae. vexans*; (b) *Ae. albopictus*; (c) *Ae. aegypti*

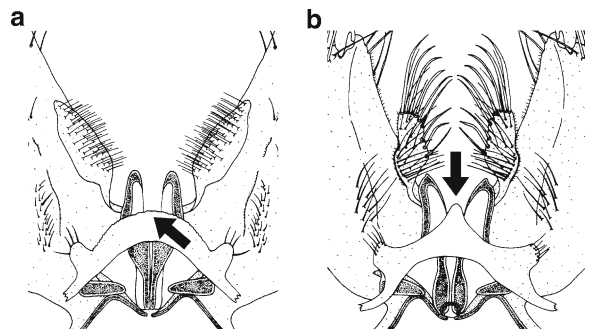


**Fig. 7.21** Gonostylus of: (a) *Ae. vexans*; (b) *Ae. vittatus*

- 6 (5) Gonostylus gradually expanded to apex. Spine of gonostylus articulated subapically, straight (Fig. 7.21a).....*Ae. vexans* (p 194)  
 Gonostylus abruptly expanded apically, flask shaped. Spine of gonostylus articulated subapically, strongly curved (Fig. 7.21b).....*Ae. vittatus* (p 196)  
 7 (5) Apical part of gonostylus slightly broader than median part. Spine of gonostylus articulated subapically (Fig. 7.22a)..... 8  
 Apical part of gonostylus distinctly narrower than median part, tapering. Spine of gonostylus articulated at the apex (Fig. 7.22b). .... *Ae. aegypti* (p 198)



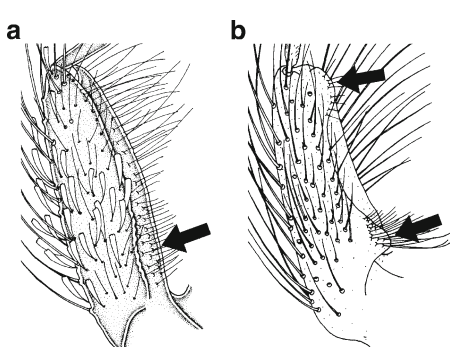
**Fig. 7.22** Gonostylus of: (a) *Ae. cretinus*; (b) *Ae. aegypti*



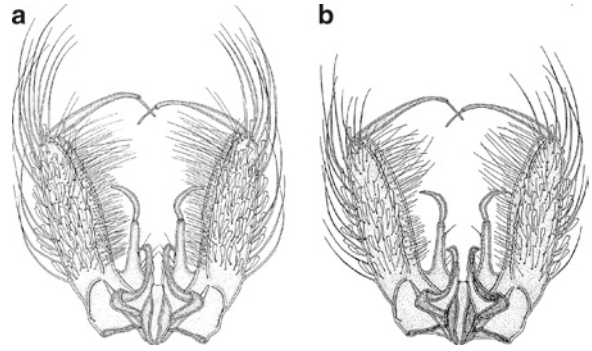
**Fig. 7.23** Claspette lobes and tergum IX of: (a) *Ae. cretinus*; (b) *Ae. albopictus*

- 8 (7) Setae below subapical spine of gonostylus usually scattered (Fig. 7.22a). Claspette lobes covered with short setae. Median part of tergum IX evenly rounded (Fig. 7.23a).....*Ae. cretinus* (p 203)  
 Setae below subapical spine of gonostylus usually forming a row (Fig. 7.20b). Claspette lobes covered with long setae, several of them stronger, spine like, curved at the apex. Median part of tergum IX pointed (Fig. 7.23b).....*Ae. albopictus* (p 201)

- 9 (4) Gonocoxite without lobes. Several tubercles bearing thin setae may be present at the inner base of gonocoxite (Fig. 7.24a)..... 10
- Gonocoxite with more or less distinct basal and apical lobes, or at least basal lobe present (Fig. 7.24b)..... 11



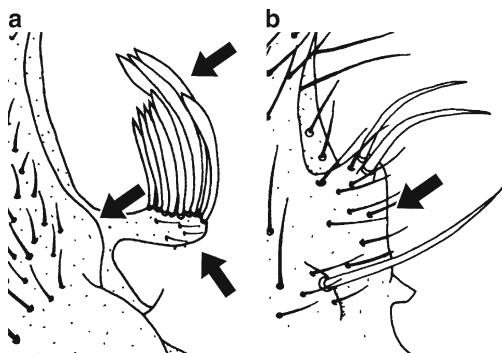
**Fig. 7.24** Gonocoxite of: (a) *Oc. geniculatus*; (b) *Oc. nigripes*



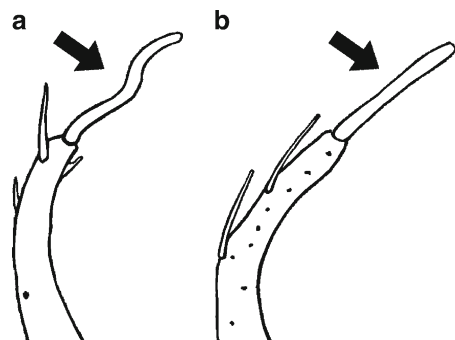
**Fig. 7.25** Hypopygium of: (a) *Oc. echinus*; (b) *Oc. geniculatus*

- 10 (9) Setae of gonocoxite very long and dense (Fig. 7.25a).....*Oc. echinus* (p 205)
  - Setae of gonocoxite short and less dense (Fig. 7.25b).....*Oc. geniculatus* (p 206)
- Note:** Males of the two species are difficult to separate.

- 11 (9) Basal lobe of gonocoxite usually divided, one lobe with several long, lanceolate, flattened setae which may be slightly or strongly curved (Fig. 7.26a)..... 12
- Basal lobe of gonocoxite usually undivided, without a row of several lanceolate, flattened setae, but 1 or 2 could be present (Fig. 7.26b)..... 16



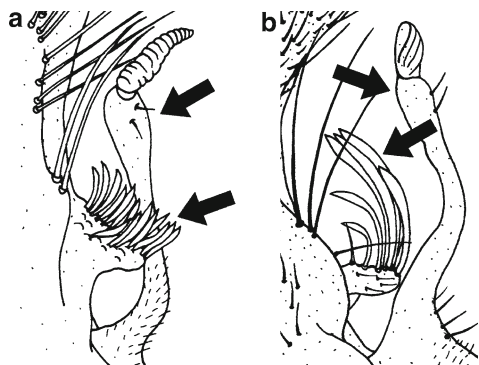
**Fig. 7.26** Basal lobe of gonocoxite of: (a) *Oc. rusticus*; (b) *Oc. intrudens*



**Fig. 7.27** Apex of gonostylus of: (a) *Oc. rusticus*; (b) *Oc. refiki*

- 12 (11) Apical spine of gonostylus distinctly S-shaped (Fig. 7.27a).....*Oc. rusticus* (p 261)
- Apical spine of gonostylus straight or slightly curved (Fig. 7.27b)..... 13

- 13 (12) Division of basal lobe which bears lanceolate setae elongated, at least in basal part. Claspette filament distinctly transversely striated (Fig. 7.28a)..... 14  
 Division of basal lobe which bears lanceolate setae arising gradually from gonocoxite, more or less conical. Claspette filament not distinctly striated (Fig. 7.29a). .... 15
- 14 (13) Claspette stem broader at the apex than at the base. Number of slightly curved lanceolate setae on basal lobe 15–16, arranged in several rows (Fig. 7.28a)..... *Oc. refiki* (p 259)  
 Apex and base of claspette stem have more or less equal width. Number of strongly curved lanceolate setae on basal lobe 6-8, located at the distal margin (Fig. 7.28b).....*Oc. quasirusticus* (p 258)
- 15 (13) Base of gonocoxite with one lobe, bearing a group of lanceolate, flattened setae (Fig. 7.29a).....  
 .....*Oc. lepidonotus* (p 257)  
 Base of gonocoxite with two or more lobes. Only one lobe bears lanceolate, flattened setae, the others with hair like setae (Fig. 7.29b). ....*Oc. subdiversus* (p 263)

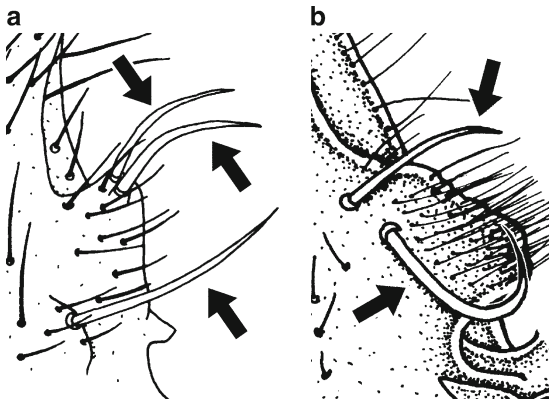


**Fig. 7.28** Claspette and basal lobe of gonocoxite of:  
 (a) *Oc. refiki*; (b) *Oc. quasirusticus*

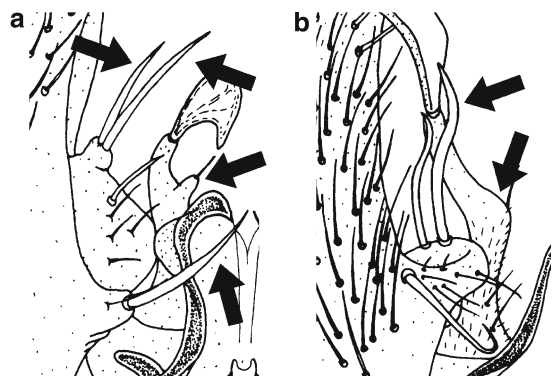


**Fig. 7.29** Base of gonocoxite of:  
 (a) *Oc. lepidonotus*; (b) *Oc. subdiversus*

- 16 (11) Basal lobe of gonocoxite with 3 setae distinctly larger than the others (Fig. 7.30a)..... 17  
 Basal lobe of gonocoxite with 0-2 setae which are larger than the others (Fig. 7.30b)..... 19



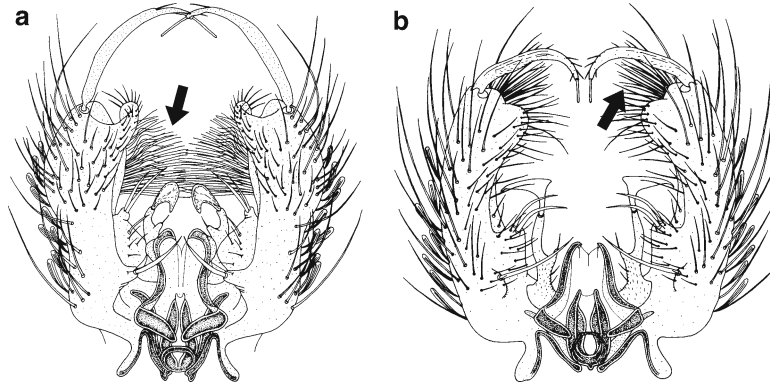
**Fig. 7.30** Basal lobe of gonocoxite of:  
 (a) *Oc. intrudens*; (b) *Oc. caspius*



**Fig. 7.31** Basal lobe of gonocoxite and claspette of:  
 (a) *Oc. diantaeus*; (b) *Oc. pullatus*

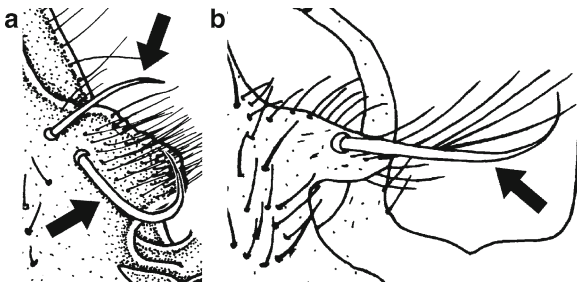
- 17 (16) Basal lobe of gonocoxite with all 3 large setae spine like. Claspette stem with a thorn shaped process beyond the middle (Fig. 7.31a)..... 18  
 Basal lobe of gonocoxite with 1 or 2 of the larger setae flattened and lanceolate. Claspette stem without a thorn shaped process, bent and swollen in the middle (Fig. 7.31b)..... *Oc. pullatus* (p 249)

- 18 (17) Apical half of gonocoxite with very dense setae directed more inwardly (Fig. 7.32a). ..... *Oc. diantaeus* (p 224)  
 Only small subapical zone with dense setae directed more distally (Fig. 7.32b) ..... *Oc. intrudens* (p 237)

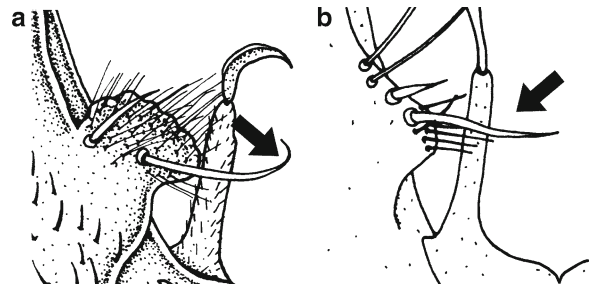


**Fig. 7.32** Hypopygium of: (a) *Oc. diantaeus*; (b) *Oc. intrudens*

- 19 (16) Basal lobe of gonocoxite with 2 spine like setae (Fig. 7.33a). ..... 20  
 Basal lobe of gonocoxite with 0-1 spine like setae (Fig. 7.33b). ..... 22

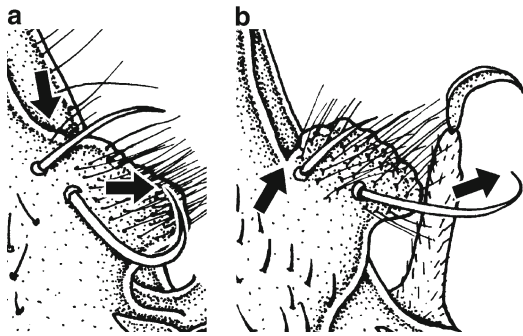


**Fig. 7.33** Basal lobe of gonocoxite of:  
 (a) *Oc. caspius*; (b) *Oc. impiger*

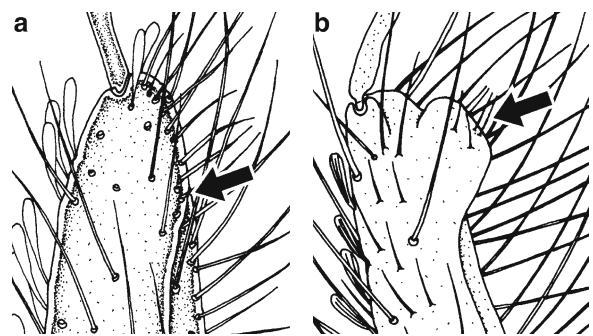


**Fig. 7.34** Basal lobe of gonocoxite of:  
 (a) *Oc. dorsalis*; (b) *Oc. hungaricus*

- 20 (19) Larger spine of basal lobe hook shaped (Fig. 7.34a). ..... 21  
 Larger spine of basal lobe not hook shaped, slightly curved in the middle (Fig. 7.34b) ..... *Oc. hungaricus* (p 234)
- 21 (20) Basal lobe gradually arising from gonocoxite, spines situated close together, larger spine strongly curved apically (usually tip extending backwards to almost the middle of the spine) (Fig. 7.35a) ..... *Oc. caspius* (p 216)  
 Basal lobe of gonocoxite slightly constricted at base, spines widely separated, larger spine slightly curved apically (usually tip extending backwards to not more than one third of the spine) (Fig. 7.35b) ..... *Oc. dorsalis* (p 226)  
**Note:** The structure of the hypopygium of the two species is very similar and difficult to distinguish, intermediate forms are common.
- 22 (19) Apical lobe of gonocoxite small, weakly developed or absent (Fig. 7.36a). ..... 23  
 Apical lobe of gonocoxite well developed (Fig. 7.36b). ..... 26

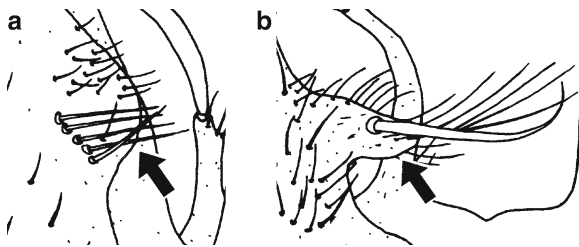


**Fig. 7.35** Basal lobe of gonocoxite of:  
(a) *Oc. caspius*; (b) *Oc. dorsalis*

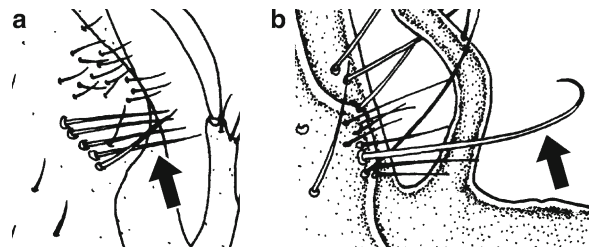


**Fig. 7.36** Apical lobe of gonocoxite of:  
(a) *Oc. pulcritarsis*; (b) *Oc. cataphylla*

- 23 (22) Basal lobe of gonocoxite indistinct, weakly developed (Fig. 7.37a)..... 24
- Basal lobe well developed (Fig. 7.37b)..... 25

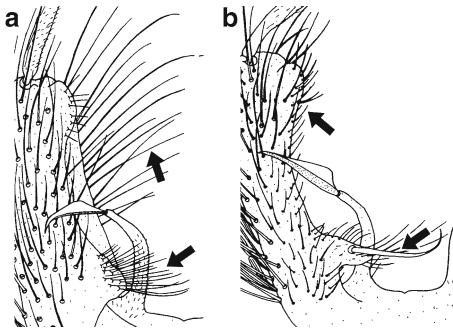


**Fig. 7.37** Basal lobe of gonocoxite of:  
(a) *Oc. mariae s.l.*; (b) *Oc. impiger*

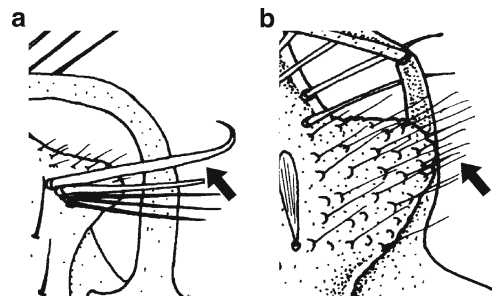


**Fig. 7.38** Basal lobe of gonocoxite of:  
(a) *Oc. mariae s.l.*; (b) *Oc. berlandi*

- 24 (23) Longer setae on basal lobe thin, of equal length and thickness. Strong spine like setae absent (Fig. 7.38a)..... *Oc. mariae* and *Oc. zammitii* (p 240, 242)
- Longer setae on basal lobe distinctly differ in length and thickness, 1 strong spine like seta present (Fig. 7.38b)..... *Oc. pulcritarsis* and *Oc. berlandi* (p 248, 212)
- 25 (23) Gonocoxite with long setae predominating on inner side. Basal lobe with setae of more or less equal thickness (Fig. 7.39a)..... *Oc. nigripes* (p 245)
- Gonocoxite with short setae predominating on inner side. Basal lobe with one seta distinctly stronger than the others (Fig. 7.39b)..... *Oc. impiger* (p 236)
- 26 (22) Basal lobe of gonocoxite with one spine or with at least one enlarged seta among thinner setae (Fig. 7.40a)..... 27
- Basal lobe without a spine or enlarged seta. All setae on basal lobe with more or less the same length and width (Fig. 7.40b)..... 40

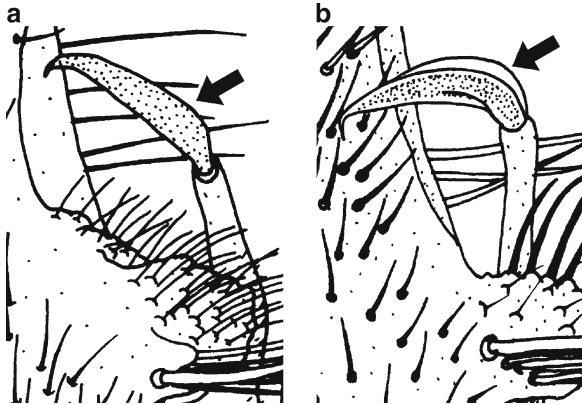


**Fig. 7.39** Gonocoxite of: (a) *Oc. nigripes*; (b) *Oc. impiger*

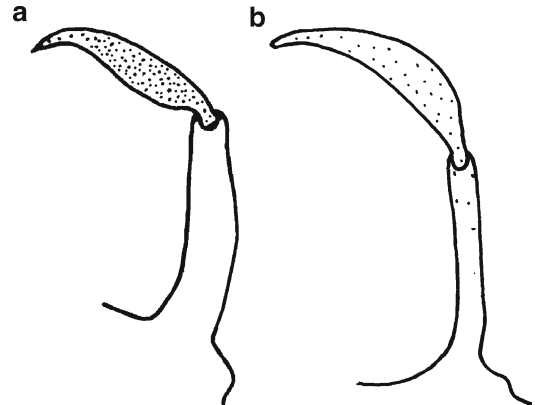


**Fig. 7.40** Basal lobe of gonocoxite of: (a) *Oc. cataphylla*; (b) *Oc. behningi*

- 27 (26) Claspette filament evenly sclerotized, without transparent wings (Fig. 7.41a)..... 28
- Claspette filament differentiated into a well sclerotized ridge and 1 or 2 weakly sclerotized, transparent wings (Fig. 7.41b)..... 29

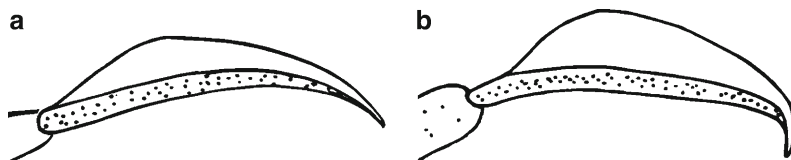


**Fig. 7.41** Claspette filament of: (a) *Oc. punctor*; (b) *Oc. communis*



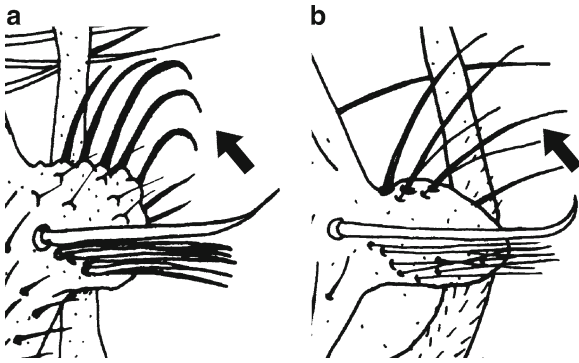
**Fig. 7.42** Claspette of: (a) *Oc. hexodontus*; (b) *Oc. punctodes*

- 28 (27) Claspette filament relatively short, shorter than the stem, strongly sclerotized (Fig. 7.42a)..... *Oc. hexodontus* and *Oc. punctor* (p 233, 251)
- Claspette filament relatively long, of almost the same length as the stem, weakly sclerotized (Fig. 7.42b)..... *Oc. punctodes* (p 250)
- 29 (27) Wing narrow, of more or less similar width along the whole claspette filament (Fig. 7.43a)..... 30
- Wing broad, distinctly widening the claspette filament at any section between its base and apex (Fig. 7.43b)..... 32

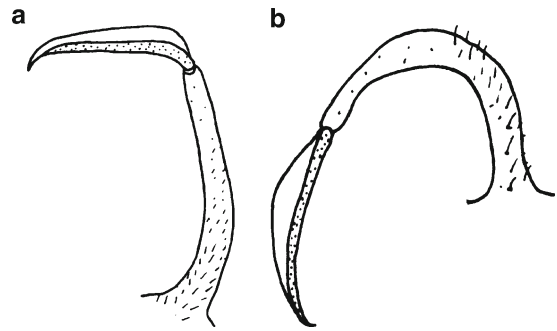


**Fig. 7.43** Claspette filament of: (a) *Oc. leucomelas*; (b) *Oc. cataphylla*

- 30 (29) Upper part of basal lobe with a row of apically strongly curved, sometimes, hooked setae (Fig. 7.44a).....*Oc. communis* (p 220)  
 Upper part of basal lobe with straight or slightly curved setae, never hooked (Fig. 7.44b). ..... 31

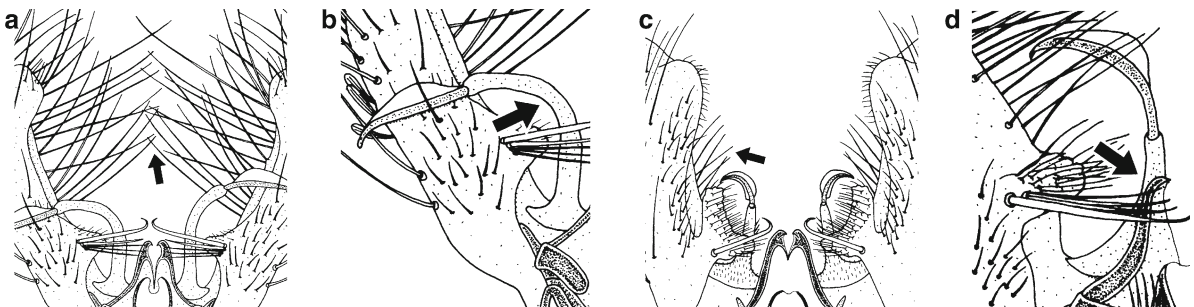


**Fig. 7.44** Basal lobe of gonocoxite of: (a) *Oc. communis*; (b) *Oc. pionips*



**Fig. 7.45** Claspette of: (a) *Oc. pionips*; (b) *Oc. leucomelas*

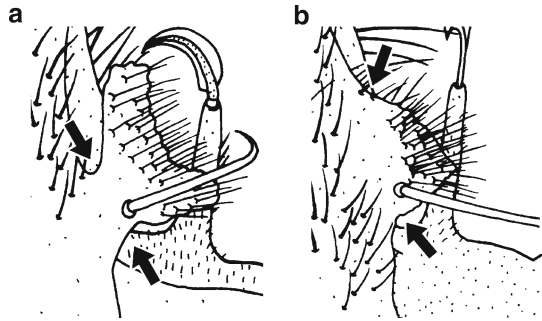
- 31 (30) Claspette stem straight or slightly curved (Fig. 7.45a).....*Oc. pionips* (p 246)  
 Claspette stem strongly curved (Fig. 7.45b). ..... *Oc. leucomelas* (p 238)  
 32 (29) Setae above basal lobe of gonocoxite long, usually overlapping in the middle. Claspette stem strongly curved in the middle (Fig. 7.46a,b).....*Oc. cataphylla* (p 218)  
 At least several setae immediately above basal lobe of gonocoxite short, not overlapping in the middle, or if all setae are long, claspette stem more or less straight or slightly curved in the apical part (*Oc. detritus*) (Fig. 7.46c,d). ..... 33



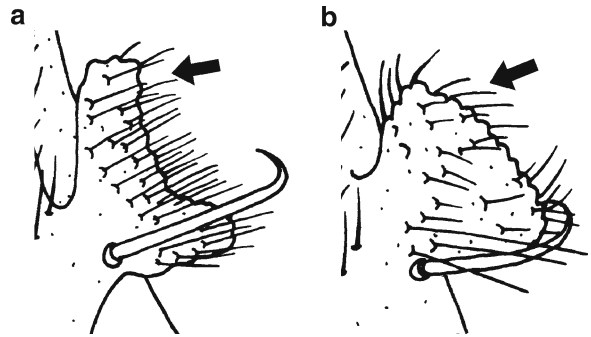
**Fig. 7.46** Gonocoxite and claspette of: (a, b) *Oc. cataphylla*; (c) *Oc. sticticus*; (d) *Oc. detritus*



- 33 (32) Basal lobe of gonocoxite constricted at its base (Fig. 7.47a)..... 34
- Basal lobe gradually arising from gonocoxite (Fig. 7.47b)..... 35

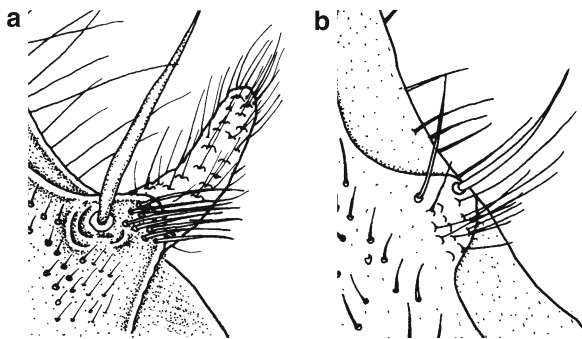


**Fig. 7.47** Basal lobe of gonocoxite of:  
(a) *Oc. sticticus*; (b) *Oc. mercurator*

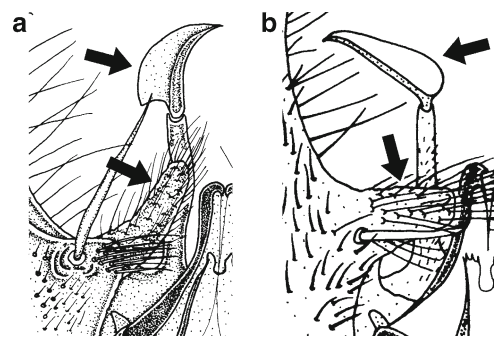


**Fig. 7.48** Basal lobe of gonocoxite of:  
(a) *Oc. sticticus*; (b) *Oc. nigrinus*

- 34 (33) Basal lobe of gonocoxite more or less crescent shaped, its upper part slender (Fig. 7.48a) ..... *Oc. sticticus* (p 255)
- Upper part of basal lobe of gonocoxite broad and rounded (Fig. 7.48b)..... *Oc. nigrinus* (p 243)
- 35 (33) Basal lobe of gonocoxite longer than broad at its base (Fig. 7.49a)..... 36
- Basal lobe distinct but not longer than broad at its base (Fig. 7.49b)..... 37



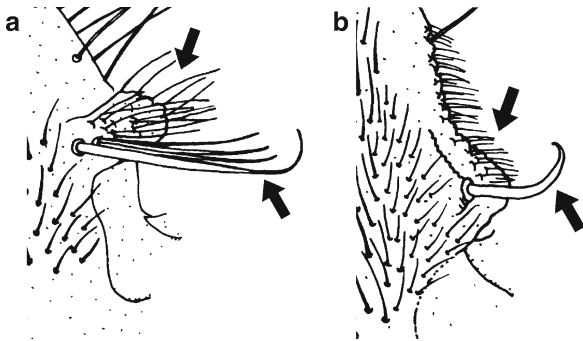
**Fig. 7.49** Basal lobe of gonocoxite of:  
(a) *Oc. cantans*; (b) *Oc. cyprius*



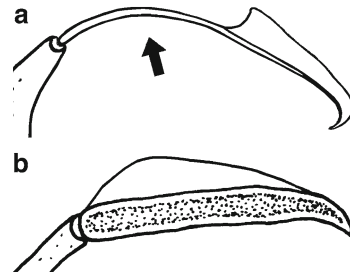
**Fig. 7.50** Basal lobe of gonocoxite and claspette of:  
(a) *Oc. cantans*; (b) *Oc. riparius*

- 36 (35) Basal lobe of gonocoxite narrow and markedly elongated. Claspette filament extremely broad, at least half as wide as long (Fig. 7.50a)..... *Oc. cantans* (p 214)
- Basal lobe of gonocoxite slightly longer than broad at its base. Claspette filament moderately broad, less than 1/4 of its length (Fig. 7.50b). ..... *Oc. riparius* (p 253)

- 37 (35) Basal lobe of gonocoxite more or less conical, with 1 spine and long setae (Fig. 7.51a). ..... 38  
 Basal lobe of gonocoxite more flattened, with 1 spine and short, dense setae (Fig. 7.51b). .....  
 ..... *Oc. flavescens* (p 231)

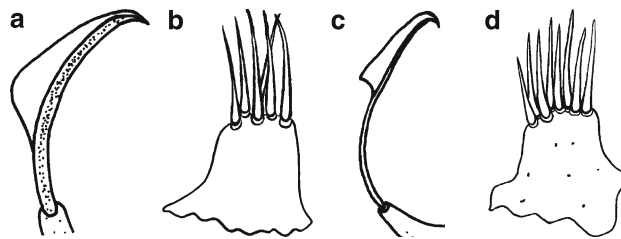


**Fig. 7.51** Basal lobe of gonocoxite of: (a) *Oc. detritus*; (b) *Oc. flavescens*



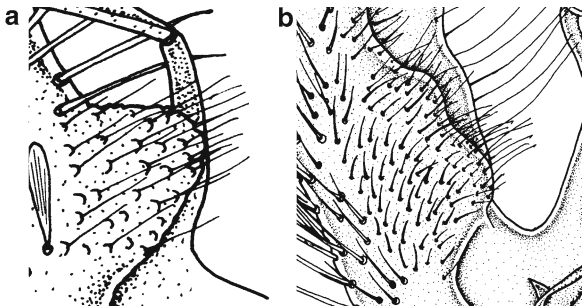
**Fig. 7.52** Claspette filament of: (a) *Oc. mercurator*; (b) *Oc. cyprius*

- 38 (37) Claspette filament with long stalk, widened from about or beyond the middle (Fig. 7.52a)..... 39  
 Claspette filament without stalk, widened from its base (Fig. 7.52b)..... *Oc. cyprius* (p 221)  
 39 (38) Claspette filament evenly widened into a wing from about its middle. Lobe of tergum IX with 3-8 spine-like setae (Fig. 7.53a,b)..... *Oc. detritus* (p 223)  
 Claspette filament abruptly widened into a wing beyond the middle. Lobe of tergum IX with 6-12 spine-like setae (Fig. 7.53c,d)..... *Oc. mercurator* (p 242)

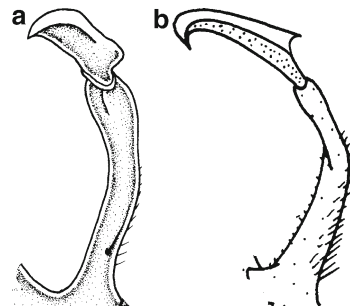


**Fig. 7.53** Claspette filament and lobe of tergum IX of: (a, b) *Oc. detritus*; (c, d) *Oc. mercurator*

- 40 (26) Basal lobe of gonocoxite conical, more or less as long as broad at the base (Fig. 7.54a). ..... *Oc. behningi* (p 211)  
 Basal lobe of gonocoxite flattened, indistinct or absent (Fig. 7.54b). ..... 41  
 41 (40) Claspette stem stout, slightly swollen at the apex. Claspette filament slightly swollen beyond the middle (Fig. 7.55a)..... *Oc. annulipes* (p 209)  
 Claspette stem slender, tapering apically. Claspette filament tapering gradually towards the apex (Fig. 7.55b)..... 42

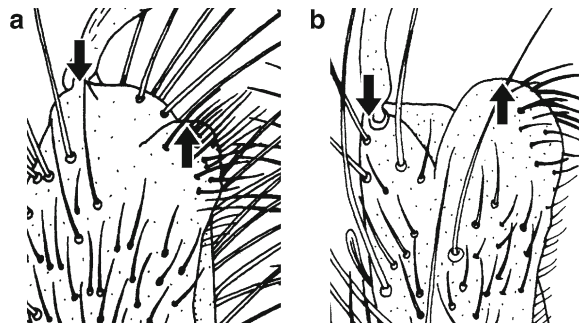


**Fig. 7.54** Basal lobe of gonocoxite of: (a) *Oc. behningi*; (b) *Oc. annulipes*



**Fig. 7.55** Claspette of: (a) *Oc. annulipes*; (b) *Oc. excrucians*

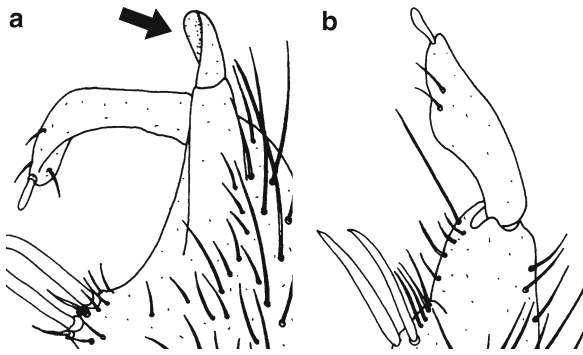
- 42 (41) Apical lobe small, not reaching the level of gonostylus articulation (Fig. 7.56a). .....  
 .....*Oc. excrucians* and *Oc. surcoufi* (p 229, 231)
- Apical lobe prominent, protruding above the level of gonostylus articulation (Fig. 7.56b).....  
 .....*Oc. euedes* (p 228)



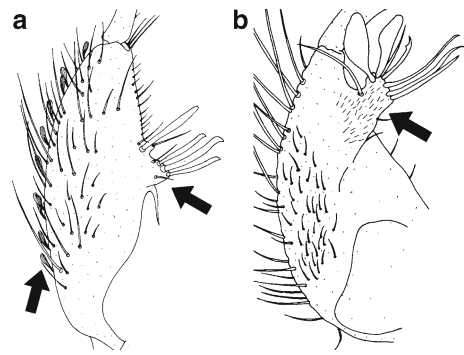
**Fig. 7.56** Apical lobe of gonocoxite of: (a) *Oc. excrucians s.l.*; (b) *Oc. euedes*

**7.3 Genus Culex**

- 1 Gonocoxite with a broad, flattened, sclerotized process at the apex, extending distinctly beyond the base of the gonostylus (Fig. 7.57a). .....*Cx. hortensis* (p 282)
- Gonocoxite without a process at the apex (Fig. 7.57b)..... 2
- 2 (1) Gonocoxite with small scales on the outer surface. Lobe of gonocoxite located slightly beyond the middle (Fig. 7.58a)..... 3
- Gonocoxite without scales on the outer surface. Lobe of gonocoxite located well beyond the middle (Fig. 7.58b)..... 4

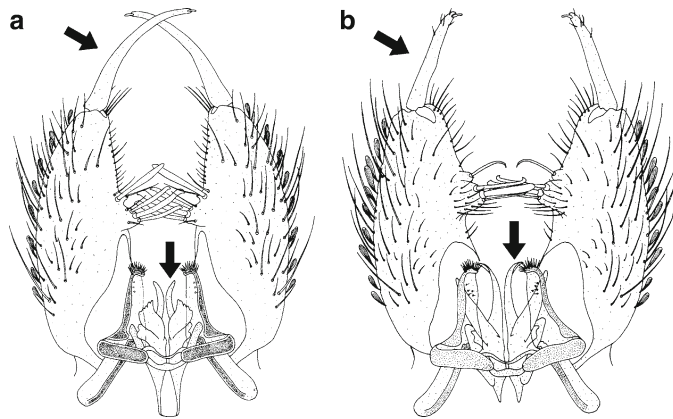


**Fig. 7.57** Apex of gonocoxite of: (a) *Cx. hortensis*; (b) *Cx. martinii*



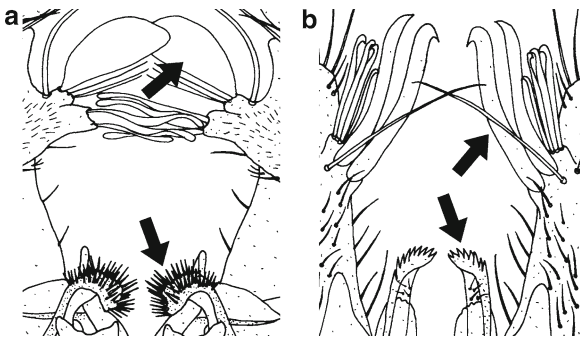
**Fig. 7.58** Gonocoxite of: (a) *Cx. modestus*; (b) *Cx. brumpti*

- 3 (2) Gonostylus relatively long, more or less half as long as gonocoxite. Ventral arm of aedeagus short, its apex not extending beyond the apex of paraproct (Fig. 7.59a)..... *Cx. modestus* (p 265)  
 Gonostylus short, distinctly shorter than half the length of gonocoxite. Ventral arm of aedeagus long, its apex extending beyond apex of paraproct (Fig. 7.59b)..... *Cx. pusillus* (p 267)

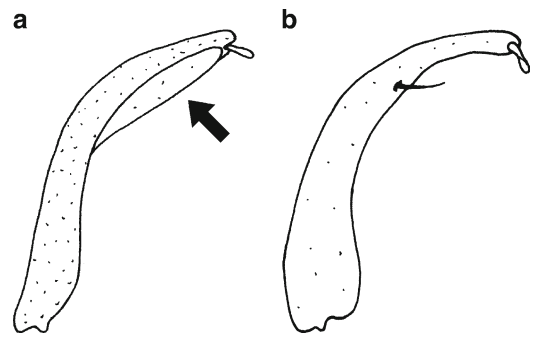


**Fig. 7.59** Hypopygium of: (a) *Cx. modestus*; (b) *Cx. pusillus*

- 4 (2) Lobe of gonocoxite with one or several transparent, broad, oval or lanceolate scale like setae. Apex of paraproct with several rows of spines (Fig. 7.60a)..... 5  
 Lobe of gonocoxite with distinctly sclerotized broad and narrow setae. Transparent scale like setae absent. Apex of paraproct with a row of large denticles, sometimes in addition to the denticles several rows of short spines could be present (Fig. 7.60b). ..... 12  
 5 (4) Gonostylus expanded beyond the middle (Fig. 7.61a)..... 6  
 Gonostylus not expanded beyond the middle, tapering apically (Fig. 7.61b). ..... 7

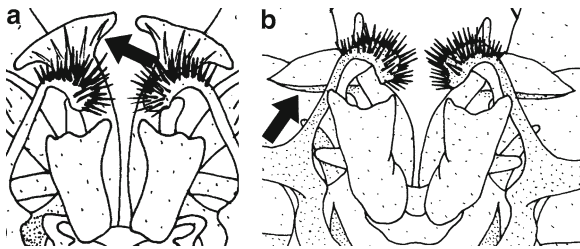


**Fig. 7.60** Gonocoxite and paraproct of: (a) *Cx. perexiguus*; (b) *Cx. territans*

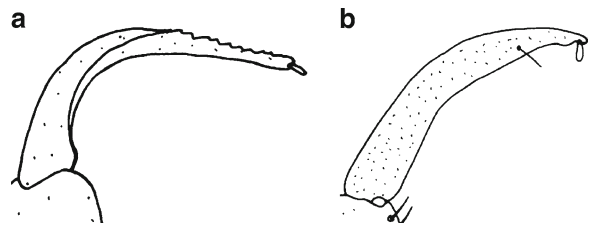


**Fig. 7.61** Gonostylus of: (a) *Cx. perexiguus*; (b) *Cx. mimeticus*

- 6 (5) Ventral arm of aedeagus slender, extended apically into a fan shaped process which bears 5 spines. The spines do not exceed the upper border of the fan (Fig. 7.62a).....*Cx. brumpti* (p 269)  
 Ventral arm of aedeagus stout, with a concave apex not bearing any spines (Fig. 7.62b). *Cx. perexiguus* (p 273)

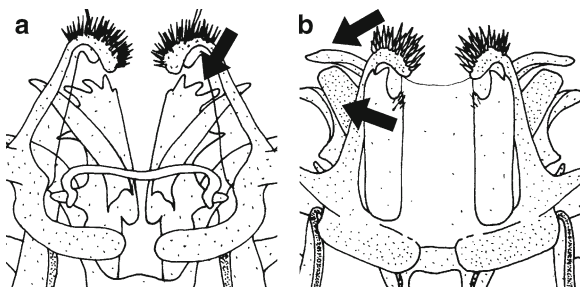


**Fig. 7.62** Aedeagus of: (a) *Cx. brumpti*; (b) *Cx. perexiguus*

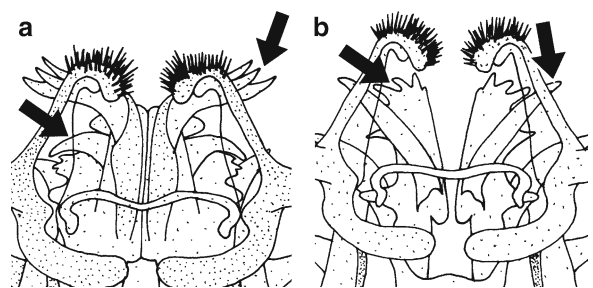


**Fig. 7.63** Gonostylus of: (a) *Cx. laticinctus*; (b) *Cx. theileri*

- 7 (5) Gonostylus sharply bent (Fig. 7.63a).....*Cx. laticinctus* (p 270)  
 Gonostylus gradually curved (Fig. 7.63b). ..... 8  
 8 (7) Ventral arm or both, dorsal and ventral arms of aedeagus with denticles or teeth (Fig. 7.64a)..... 9  
 Dorsal and ventral arms of aedeagus without denticles or teeth (Fig. 7.64b)..... 10



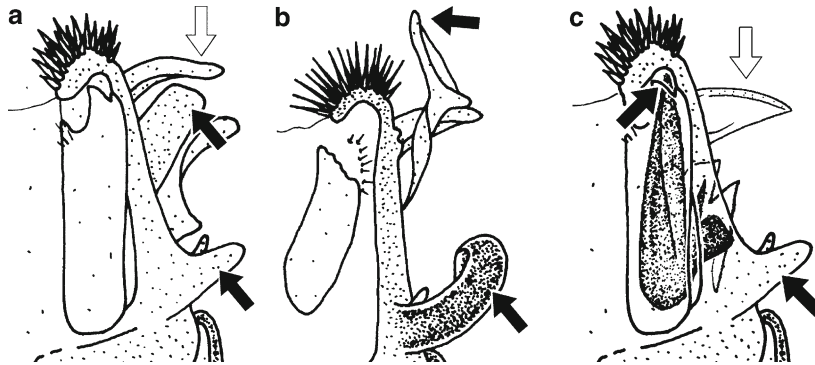
**Fig. 7.64** Aedeagus of: (a) *Cx. theileri*; (b) *Cx. p. pipiens*



**Fig. 7.65** Aedeagus of: (a) *Cx. mimeticus*; (b) *Cx. theileri*

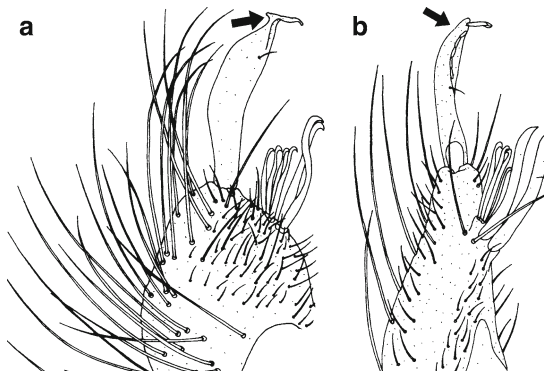
- 9 (8) Ventral arm of aedeagus with 1-2 denticles, dorsal arm usually with 3 finger-like processes (Fig. 7.65a).....*Cx. mimeticus* (p 271)  
 Ventral arm of aedeagus with 2-4 strong lateral teeth, dorsal arm simple, slender, pointed (Fig. 7.65b).....*Cx. theileri* (p 280)

- 10 (8) Dorsal arm of aedeagus tube like, truncate apically or straight and pointed, never twisted. Ventral arm of paraproct usually weakly developed, transparent (slightly sclerotized) (Fig. 7.66a,c)..... 11  
 Dorsal arm of aedeagus twisted and pointed apically. Ventral arm of paraproct well developed, strongly sclerotized (Fig. 7.66b)..... *Cx. torrentium* (p 279)
- 11 (10) Ventral arm of aedeagus slender, sickle shaped (Fig. 7.66a)..... *Cx. p. pipiens* (p 275)  
 Ventral arm of aedeagus broad, leaf like (Fig. 7.66c)..... *Cx. p. quinquefasciatus* (p 278)

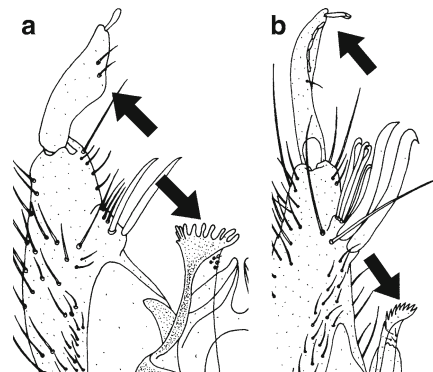


**Fig. 7.66** Aedeagus and paraproct of: (a) *Cx. p. pipiens*; (b) *Cx. torrentium*; (c) *Cx. p. quinquefasciatus*

- 12 (4) Gonocoxite with many long and conspicuous setae on its outer surface. Gonostylus constricted subapically, then expanding into a “T”-shaped apical part (Fig. 7.67a)..... *Cx. impudicus* (p 284)  
 Gonocoxite with less conspicuous setae on its outer surface. Apex of gonostylus not “T”-shaped, gradually tapering from the middle (Fig. 7.67b)..... 13



**Fig. 7.67** Gonocoxite of: (a) *Cx. impudicus*; (b) *Cx. territans*

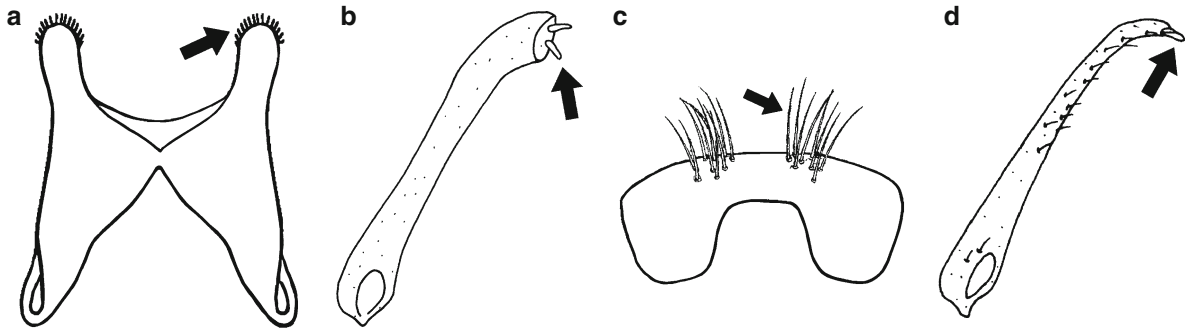


**Fig. 7.68** Gonocoxite and paraproct of: (a) *Cx. martinii*; (b) *Cx. territans*

- 13 (12) Gonostylus short, markedly widened beyond the middle. Apex of paraproct widened with a convex row of denticles (Fig. 7.68a)..... *Cx. martinii* (p 285)  
 Gonostylus elongated, usually evenly tapering from base to apex. Apex of paraproct not widened, with an inwardly curved row of denticles (Fig. 7.68b)..... *Cx. territans* (p 286)

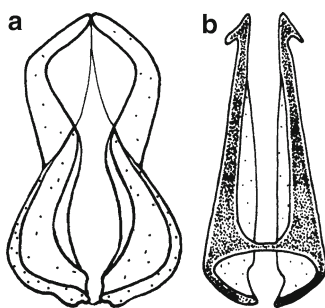
**7.4 Genus *Culiseta***

- 1 Tergum IX laterally expanded into two long and slender, sclerotized lobes bearing tiny spine like setae at the apex. Gonostylus broadened apically, blunt, with 2 short, pointed apical spines (Fig. 7.69a,b)..... *Cs. longiareolata* (p 289)
- Lobe of tergum IX small or indistinct, bearing long setae. Gonostylus tapering apically, apical spine single (Fig. 7.69c,d)..... 2

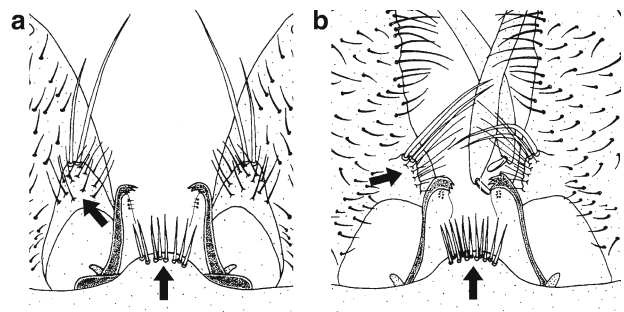


**Fig. 7.69** Tergum IX and gonostylus of: (a, b) *Cs. longiareolata*; (c, d) *Cs. morsitans*

- 2 (1) Aedeagus usually oval, slightly sclerotized (Fig. 7.70a) (in *Cs. bergrothi* the oval shaped aedeagus is slightly sclerotized at least in the lateral parts). ..... 3
- Aedeagus usually elongated, conical. Lateral plates strongly sclerotized, pointed and well separated at the apex (Fig. 7.70b). ..... 7



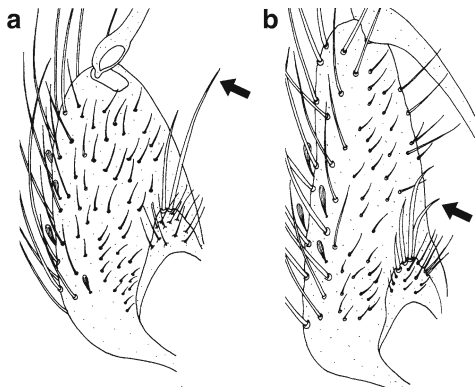
**Fig. 7.70** Aedeagus of: (a) *Cs. litorea*; (b) *Cs. alaskaensis*



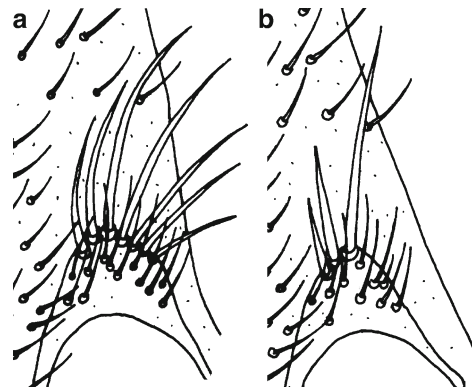
**Fig. 7.71** Basal lobes and tergum VIII of: (a) *Cs. litorea*; (b) *Cs. bergrothi*

- 3 (2) Basal lobe of gonocoxite well developed, elongated. Median lobe of tergum VIII with a row of less than 10 strong spine like setae (Fig. 7.71a)..... 4
- Basal lobe of gonocoxite weakly developed. Median lobe of tergum VIII with a row of usually more than 10 (4–18) strong spine like setae (Fig. 7.71b)..... *Cs. bergrothi* (p 301)

- 4 (3) Gonocoxite plump in appearance, more or less twice as long as its basal width. Basal lobe of gonocoxite with 2 stout setae, longer seta reaching apex of gonocoxite (Fig. 7.72a).....*Cs. litorea* (p 292)  
 Gonocoxite elongated, at least 2.5 times as long as its basal width. Basal lobe of gonocoxite with 2 or more (usually 3-8) stout setae, no seta reaching apex of gonocoxite (Fig. 7.72b)..... 5

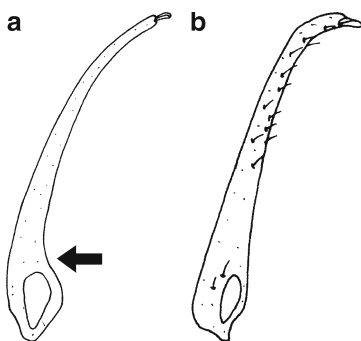


**Fig. 7.72** Gonocoxite of: (a) *Cs. litorea*; (b) *Cs. fumipennis*

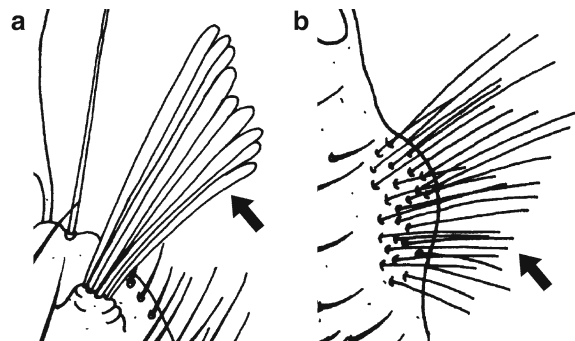


**Fig. 7.73** Basal lobe of gonocoxite of: (a) *Cs. ochroptera*; (b) *Cs. morsitans*

- 5 (4) Basal lobe of gonocoxite with 5-8 strong setae (Fig. 7.73a)..... *Cs. ochroptera* (p 296)  
 Basal lobe of gonocoxite with 2-4 strong setae (Fig. 7.73b)..... 6  
 6 (5) Gonostylus long and slender, abruptly constricted shortly beyond the base (Fig. 7.74a).....  
 .....*Cs. fumipennis* (p 291)  
 Gonostylus more stout, without abrupt constriction (Fig. 7.74b)..... *Cs. morsitans* (p 294)



**Fig. 7.74** Gonostylus of: (a) *Cs. fumipennis*; (b) *Cs. morsitans*

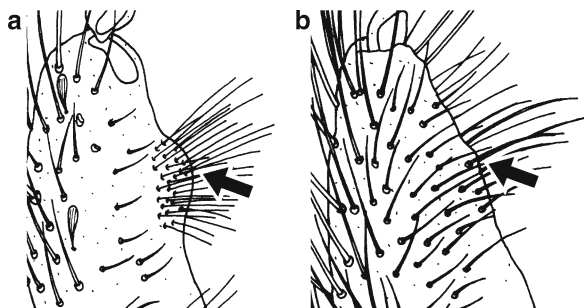


**Fig. 7.75** Apical lobe of gonocoxite of: (a) *Cs. glaphyroptera*; (b) *Cs. alaskaensis*

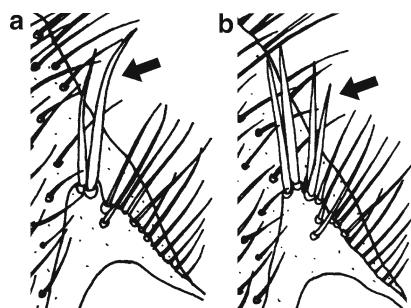
- 7 (2) Apical lobe of gonocoxite with several long lanceolate setae (Fig. 7.75a)..... *Cs. glaphyroptera* (p 303)  
 Apical lobe of gonocoxite with thin short setae or absent (Fig. 7.75b)..... 8



- 8 (7) Apical lobe of gonocoxite well developed and slightly convex, densely covered with short setae (Fig. 7.76a)..... *Cs. alaskaensis* (p 298)  
 Apical lobe of gonocoxite indistinct or absent (Fig. 7.76b)..... 9



**Fig. 7.76** Apical lobe of gonocoxite of:  
 (a) *Cs. alaskaensis*; (b) *Cs. annulata*

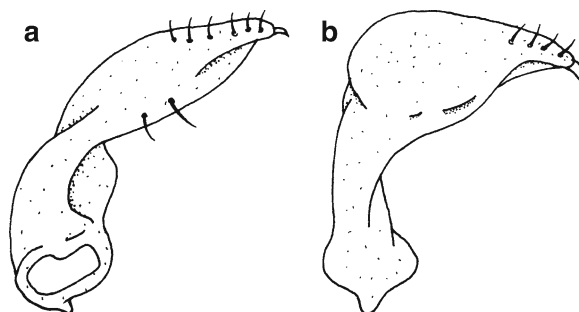


**Fig. 7.77** Basal lobe of gonocoxite of:  
 (a) *Cs. annulata*; (b) *Cs. subochrea*

- 9 (8) Basal lobe of gonocoxite with 2 (rarely 3) setae distinctly stouter than the others (Fig. 7.77a). Median lobe of tergum VIII usually without stout setae, rarely a few present (1–4) ..... *Cs. annulata* (p 299)  
 Basal lobe of gonocoxite with 3–5 setae distinctly stouter than the others (Fig. 7.77b). Median lobe of tergum VIII with several stout setae (usually 4)..... *Cs. subochrea* (p 305)

## 7.5 Genus *Coquillettidia*

- 1 Base of gonostylus slightly constricted below expanded portion, and then sharply narrowed in the middle part (Fig. 7.78a)..... *Cq. richiardii* (p 308)  
 Base of gonostylus broad, continuation stem like, expanded apically into a bulbous structure (Fig. 7.78b)..... *Cq. buxtoni* (p 307)



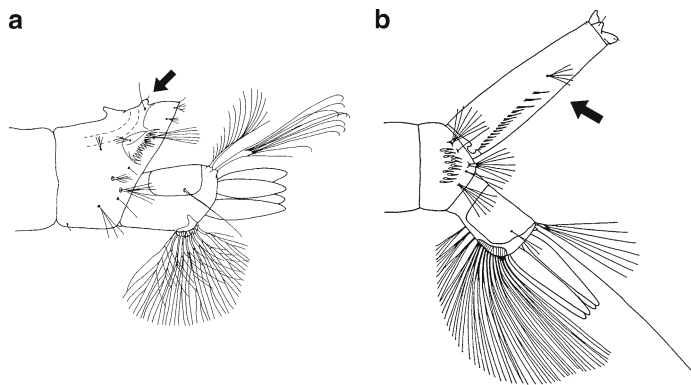
**Fig. 7.78** Gonostylus of: (a) *Cq. richiardii*; (b) *Cq. buxtoni*

# Chapter 8

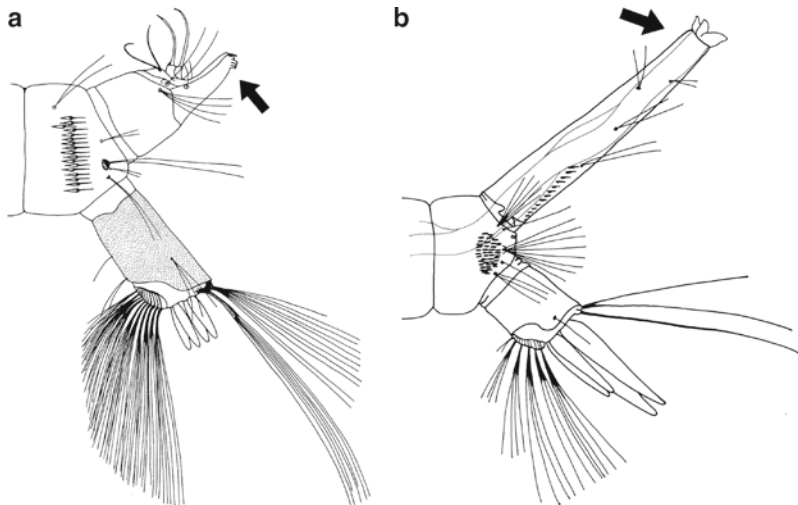
## Key to Mosquito Fourth Instar Larvae

### Genera

- 1 Abdominal segment VIII without elongate siphon (Fig. 8.1a) ..... *Anopheles* (p 164)
- Abdominal segment VIII with elongate siphon (Fig. 8.1b) ..... 2
- 2 (1) Siphon short, apex strongly sclerotized and pointed, with saw-like apparatus for cutting and piercing plant tissues (Fig. 8.2a) ..... *Coquillettia* (p 306)
- Siphon longer, apex not pointed, without such an adaptation (Fig. 8.2b) ..... 3

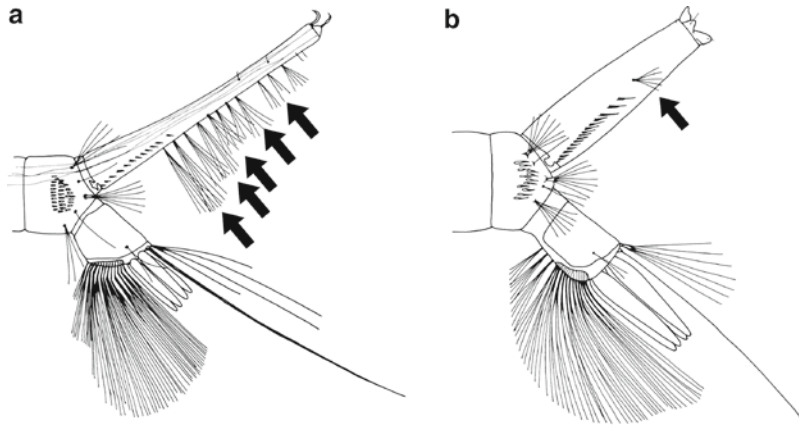


**Fig. 8.1** Abdominal segment VIII of: (a) *Anopheles* sp.; (b) *Aedes* sp.

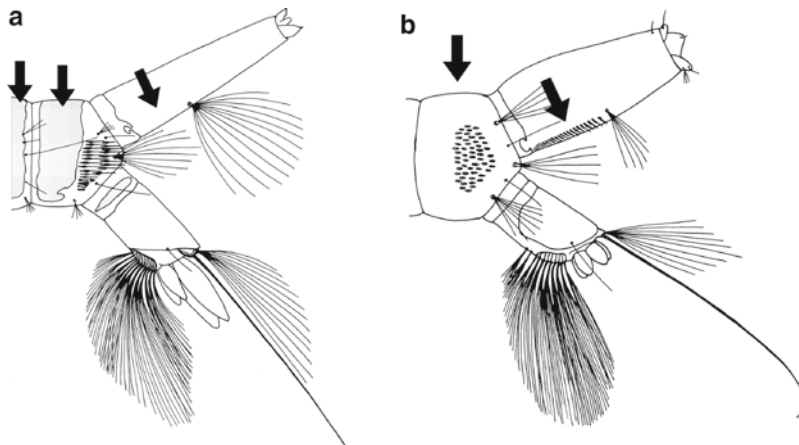


**Fig. 8.2** Siphon of: (a) *Cq. richiardii*; (b) *Cx. p. pipiens*

- 3 (2) Siphon with several pairs of siphonal tufts (1-S) (Fig. 8.3a). ..... *Culex* (p 264)  
 Siphon with one pair of siphonal tufts (Fig. 8.3b). ..... 4
- 4 (3) Pecten absent. Abdominal segments VI–VIII with more or less developed sclerotized plates dorsally (Fig. 8.4a). ..... *Orthopodomyia pulcripalpis* (p 310)  
 Pecten present. Abdominal segments VI–VIII without sclerotized plates dorsally (Fig. 8.4b). ..... 5

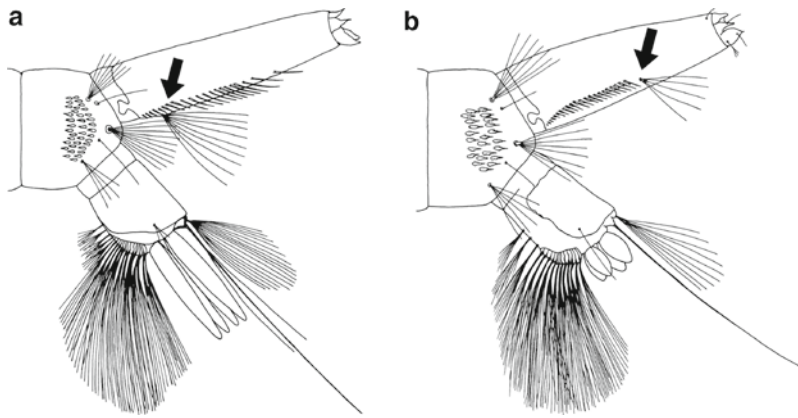


**Fig. 8.3** Siphon of: (a) *Cx. hortensis*; (b) *Ae. vexans*

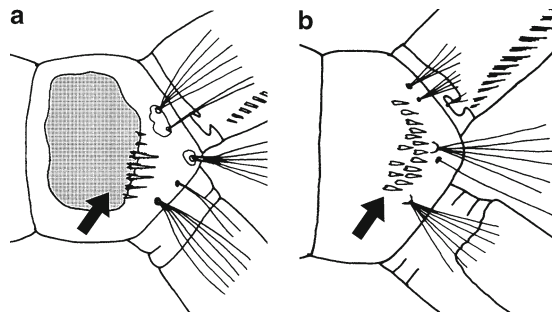


**Fig. 8.4** End of abdomen of: (a) *Or. pulcripalpis*; (b) *Oc. detritus*

- 5 (4) Siphonal tuft (1-S) inserted at the base of the siphon (Fig. 8.5a). ..... *Culiseta* (p 288)  
 Siphonal tuft inserted close to the middle or near the apex of the siphon (Fig. 8.5b). ..... 6
- 6 (5) Abdominal segment VIII with sclerotized plates laterally, comb scales arising from the posterior margin of the plates (Fig. 8.6a). ..... *Uranotaenia unguiculata* (p 312)  
 Abdominal segment VIII without sclerotized plates laterally. Only comb scales are present and they never arise from sclerotized plates (Fig. 8.6b). ..... *Aedes* and *Ochlerotatus* (p 187, 204)



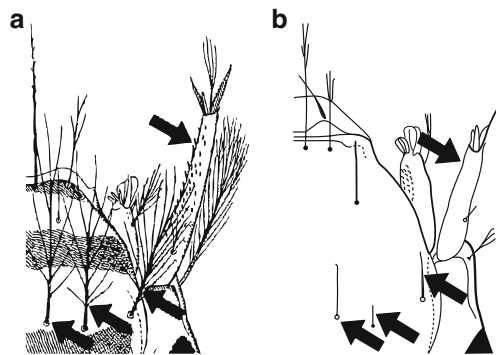
**Fig. 8.5** Siphon of: (a) *Cs. annulata*; (b) *Oc. caspius*



**Fig. 8.6** Abdominal segment VIII of: (a) *Ur. unguiculata*; (b) *Ae. cinereus*

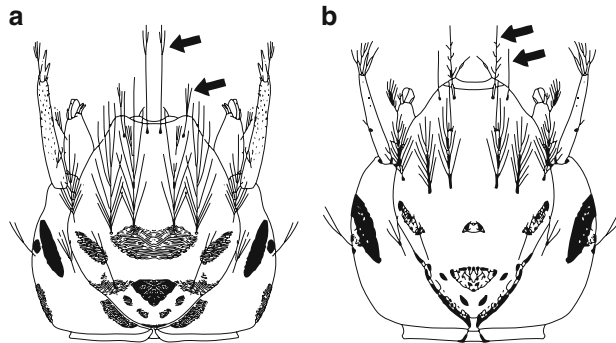
**8.1 Genus *Anopheles***

- 1 Frontal setae (5-C to 7-C) long, pinnate. Antenna covered with spicules, at least on its inner surface (Fig. 8.7a)..... 2
- Frontal setae short, single. Antenna not covered with spicules (Fig. 8.7b). .....*An. plumbeus* (p 178)



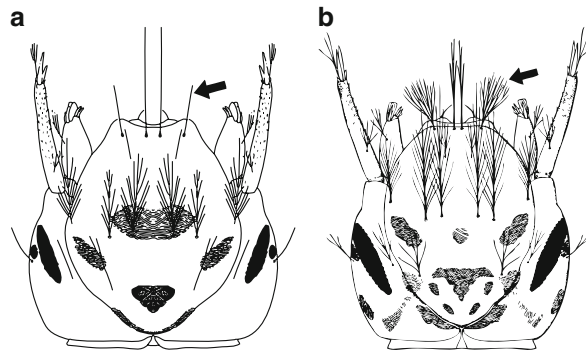
**Fig. 8.7** Head of: (a) *An. algeriensis*; (b) *An. plumbeus*

- 2 (1) Inner clypeal setae (2-C) situated close together, closer to each other than to outer clypeal setae (3-C) (Fig. 8.8a). ..... 3  
 Inner clypeal setae widely separated, closer to outer clypeal setae than to each other (Fig. 8.8b). ..... 8



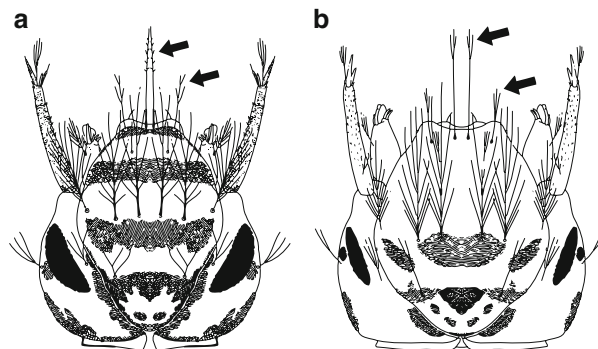
**Fig. 8.8** Head of: (a) *An. claviger s.s.*; (b) *An. superpictus*

- 3 (2) Outer clypeal seta (3-C) simple or aciculate (Fig. 8.9a) (2-3 apical branches in *An. claviger s.l.*) ..... 4  
 Outer clypeal seta dendriform (Fig. 8.9b). ..... 7



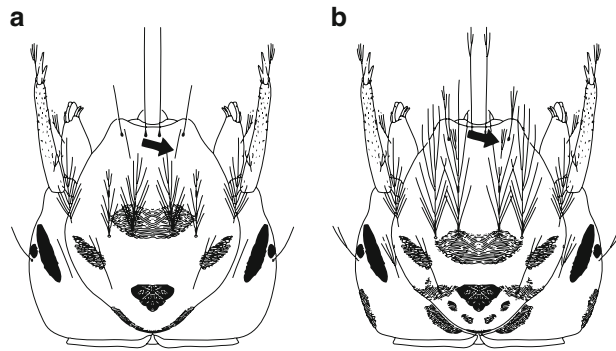
**Fig. 8.9** Head of: (a) *An. marteri*; (b) *An. maculipennis s.l.*

- 4 (3) Frontoclypeus with 3 dark transverse bands. Clypeal setae (2-C and 3-C) aciculate (Fig. 8.10a). .....  
 ..... *An. algeriensis* (p 165)  
 Frontoclypeus spotted but not banded. Clypeal setae simple or with 2-3 apical branches (Fig. 8.10b). ... 5



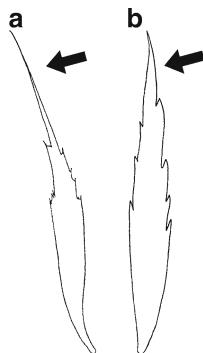
**Fig. 8.10** Head of: (a) *An. algeriensis*; (b) *An. claviger s.s.*

- 5 (4) Postclypeal seta (4-C) single, sometimes with 2 branches (Fig. 8.11a). Antepalpmate setae on abdominal segments IV and V (2-IV and 2-V) with 1-3 branches..... 6  
 Postclypeal seta with 2-5 branches (Fig. 8.11b). Antepalpmate setae on abdominal segments IV and V with 3-5 branches. Palmate setae on abdominal segment II (1-II) with 10–15 leaflets.....  
 ..... *An. claviger* s.s. (p 166)

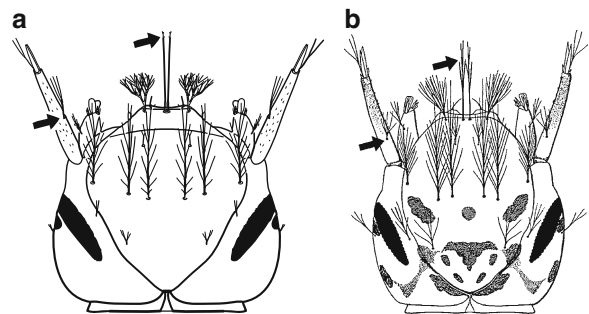


**Fig. 8.11** Head of: (a) *An. marteri*; (b) *An. claviger* s.s.

- 6 (5) Leaflets of palmate setae terminating in a long filament which is 1/3 as long as the blade (Fig. 8.12a). Antepalpmate setae on abdominal segments IV and V (2-IV and 2-V) with 1 or rarely 2 branches .....*An. marteri* (p 177)  
 Leaflets of palmate setae with slightly elongated apex but without terminal filaments. (Fig. 8.12b). Antepalpmate setae on abdominal segments IV and V with 2-3 branches. Palmate setae on abdominal segment II (1-II) with more than 15 leaflets .....*An. petragnani* (p 168)
- 7 (3) Inner clypeal seta (2-C) with short apical branches. Antennal seta (1-A) inserted in the middle or slightly below the middle of antenna (Fig. 8.13a). ..... *An. hyrcanus* (p 169)  
 Inner clypeal seta with long apical branches. Antennal seta inserted in basal 1/4 to 1/3 of antenna (Fig. 8.13b). .....**Anopheles Maculipennis Complex** (p 170)

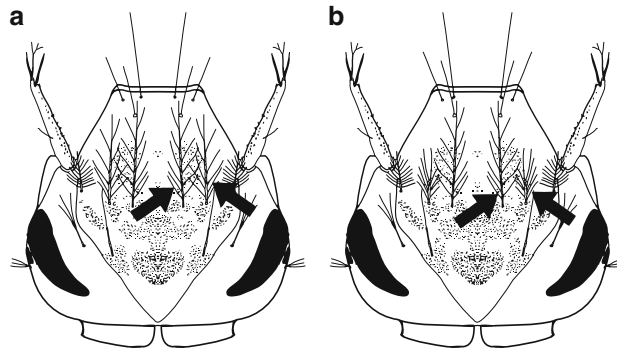


**Fig. 8.12** Leaflet of palmate seta of: (a) *An. marteri*; (b) *An. petragnani*



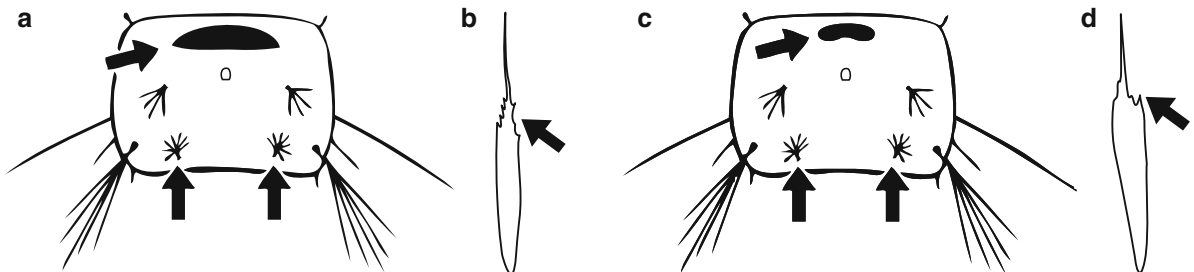
**Fig. 8.13** Head of: (a) *An. hyrcanus*; (b) *An. maculipennis* s.l.

- 8 (2) Inner frontal setae (5-C) slightly longer than median frontal setae (6-C) (Fig. 8.14a) ..... 9  
 Inner frontal setae distinctly longer than median frontal setae (Fig. 8.14b)..... 10



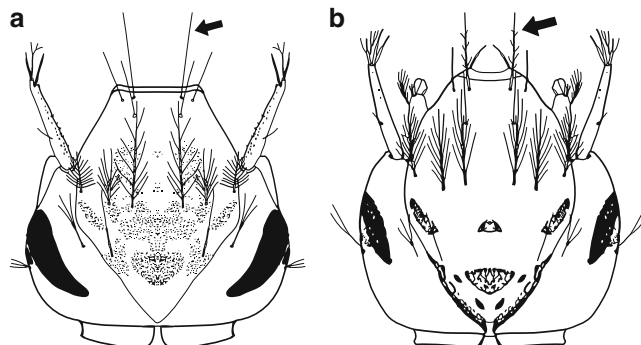
**Fig. 8.14** Head of: (a) *An. sergentii*; (b) *An. multicolor*

- 9 (8) Sclerotized tergal plate on abdominal segment VII broad, broader than distance between palmate setae (1-VII). Filament of abdominal palmate setae long, more than half as long as the blade (Fig. 8.15a, b).....*An. sergentii* (p 183)  
 Sclerotized tergal plate on abdominal segment VII relatively small, smaller than distance between palmate setae. Filament of abdominal palmate setae short, about half as long as the blade (Fig. 8.15c, d) .....*An. cinereus hispaniola* (p 181)



**Fig. 8.15** Abdominal segment VII and leaflet of palmate seta of: (a, b) *An. sergentii*; (c, d) *An. cinereus hispaniola*

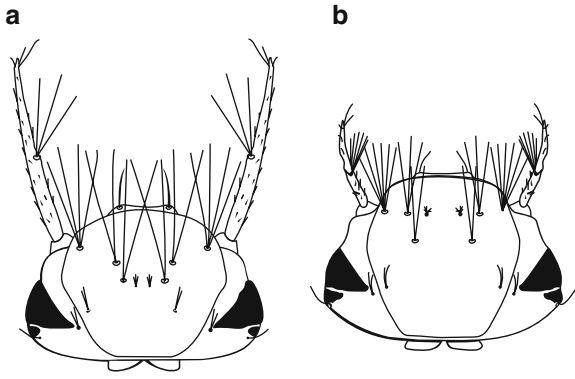
- 10 (8) Inner clypeal setae (2-C) simple (Fig. 8.16a). Typical palmate setae on metathorax (1-T) absent.....  
 .....*An. multicolor* (p 182)  
 Inner clypeal setae with short branches (aciculate) (Fig. 8.16b). Typical palmate setae on metathorax present.....*An. superpictus* (p 185)



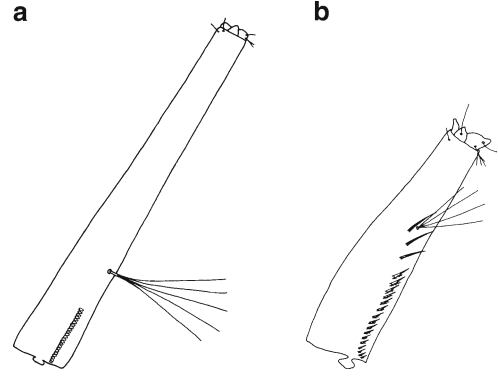
**Fig. 8.16** Head of: (a) *An. multicolor*; (b) *An. superpictus*

**8.2 Genera *Aedes* and *Ochlerotatus***

- 1 (2) Antenna distinctly longer than the head (Fig. 8.17a) ..... *Oc. diantaeus* (p 224)
- Antenna as long as or shorter than the head (Fig. 8.17b) ..... 2

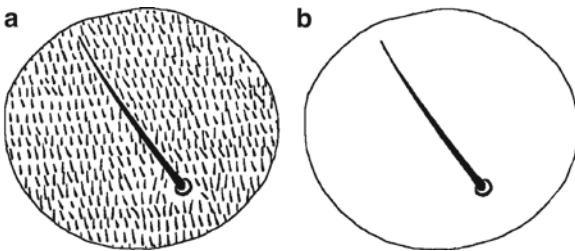


**Fig. 8.17** Head of: (a) *Oc. diantaeus*; (b) *Oc. riparius*

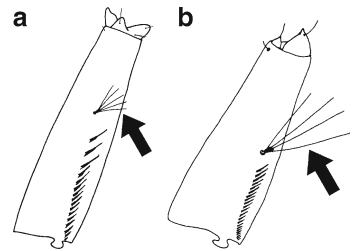


**Fig. 8.18** Siphon of: (a) *Oc. berlandi*; (b) *Oc. euedes*

- 2 (1) Siphon extremely long and slender, siphonal index at least 5.5 (Fig. 8.18a) ..... *Oc. berlandi* (p 212)
- Siphon otherwise, siphonal index never exceeds 5.5 (Fig. 8.18b) ..... 3
- 3 (2) Entire body surface covered with dense rows of spicules (Fig. 8.19a) ..... *Oc. cyprius* (p 221)
- Body surface without spicules. Some indistinct spicules may be present on the last abdominal segments (Fig. 8.19b) ..... 4



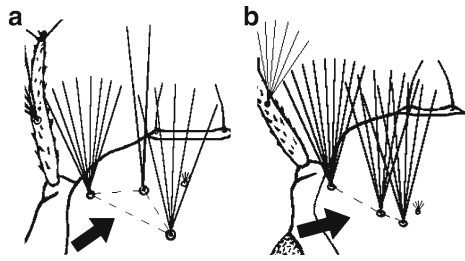
**Fig. 8.19** Fragment of integument of:  
(a) *Oc. cyprius*; (b) *Ae. vexans*



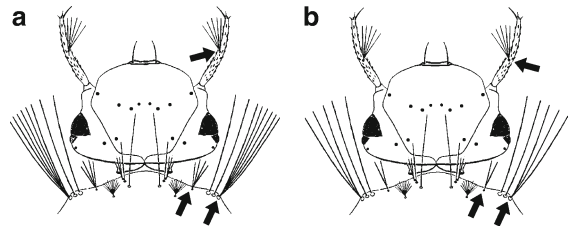
**Fig. 8.20** Siphon of: (a) *Ae. vexans*; (b) *Oc. hexodontus*

- 4 (3) Siphonal tuft (1-S) short, about half as long as the width of the siphon at the point of its origin, or shorter. Tuft inserted well beyond the middle of siphon (Fig. 8.20a) ..... 5
- Siphonal tuft long, at least 2/3 as long as the width of the siphon at the point of its origin, or longer. Tuft may be inserted before or beyond the middle of siphon (Fig. 8.20b) ..... 7
- 5 (4) Frontal setae (5-C to 7-C) arranged in an triangular pattern (Fig. 8.21a). Median setae of labral brush serrated apically ..... *Ae. vexans* (p 194)
- Frontal setae arranged in an arc-like row (Fig. 8.21b). All setae of labral brush simple ..... 6



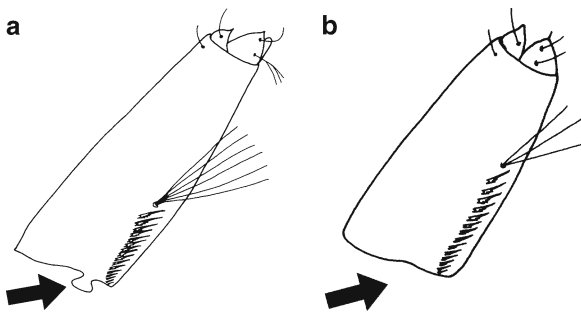


**Fig. 8.21** Head of: (a) *Ae. vexans*; (b) *Ae. cinereus*

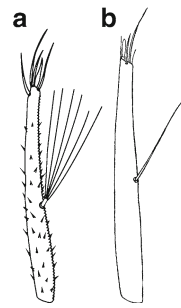


**Fig. 8.22** Head and prothorax of: (a) *Ae. rossicus*; (b) *Ae. cinereus*

- 6 (5) Antennal seta (1-A) inserted in the middle of antenna. Prothoracic seta 4-P with 4 branches, 7-P with 5-6 branches (Fig. 8.22a). ..... *Ae. rossicus* (p 192)
- Antennal seta inserted slightly before the middle, at 2/5 of the length of antenna. Prothoracic setae 4-P with 2 branches, 7-P with 3 branches (Fig. 8.22b) *Ae. cinereus* and *Ae. geminus* (p 189, 191)
- 7 (4) Base of siphon with acus (Fig. 8.23a). ..... 8
- Base of siphon without acus. (indistinct in *Ae. vittatus*) (Fig. 8.23b). ..... 45

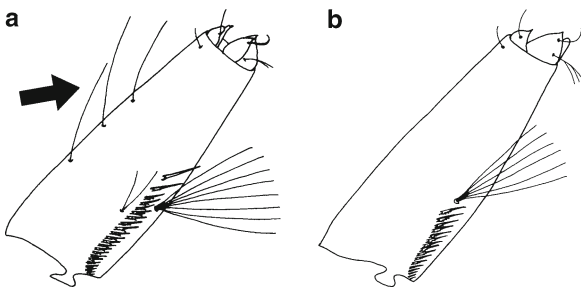


**Fig. 8.23** Siphon of: (a) *Oc. punctor*; (b) *Ae. aegypti*

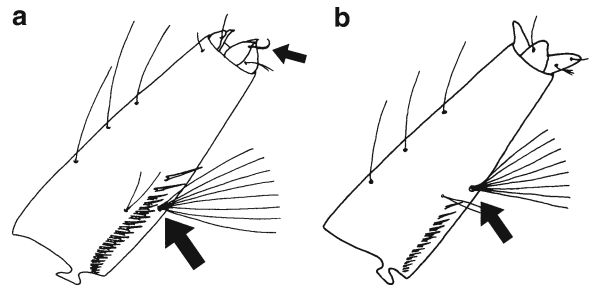


**Fig. 8.24** Antenna of: (a) *Oc. rusticus*; (b) *Oc. geniculatus*

- 8 (7) Antennae covered with more or less numerous spicules (Fig. 8.24a). ..... 9
- Antennae without spicules (Fig. 8.24b). ..... 43
- 9 (8) Dorsal surface of siphon with several pairs of additional setae (Fig. 8.25a). ..... 10
- Dorsal surface of siphon without additional setae (Fig. 8.25b). ..... 14
- 10 (9) Siphonal tuft (1-S) attached within the distal pecten teeth (Fig. 8.26a). ..... 11
- Siphonal tuft attached beyond the distalmost pecten tooth (Fig. 8.26b). ..... 13

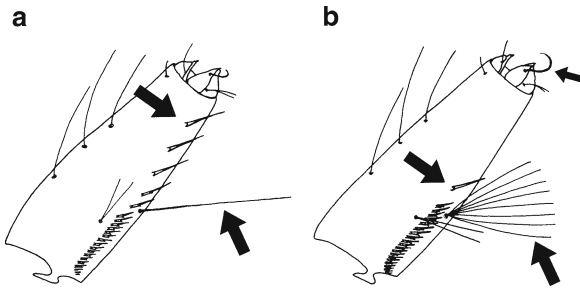


**Fig. 8.25** Siphon of: (a) *Oc. rusticus*; (b) *Oc. punctor*

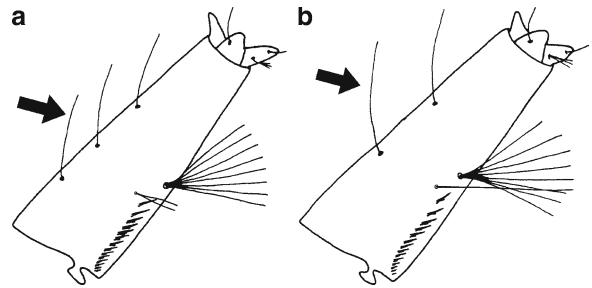


**Fig. 8.26** Siphon of: (a) *Oc. rusticus*; (b) *Oc. refiki*

- 11 (10) Siphonal seta (1-S) simple. 3-4 distal pecten teeth atypical, spine-like, widely spaced, almost reaching apex (Fig. 8.27a). ..... *Oc. subdiversus* (p 263)
- Siphonal seta with 5-8 branches. 1-3 distal pecten teeth widely spaced, but not reaching to apical 1/3 of siphon (Fig. 8.27b). ..... 12
- 12 (11) Prothoracic seta 1-P simple. Seta 9-S small (Fig. 8.26a). ..... *Oc. rusticus* (p 261)
- Prothoracic seta 1-P with 3 branches. Seta 9-S large, hook shaped (Fig. 8.27b). *Oc. quasirusticus* (p 258)

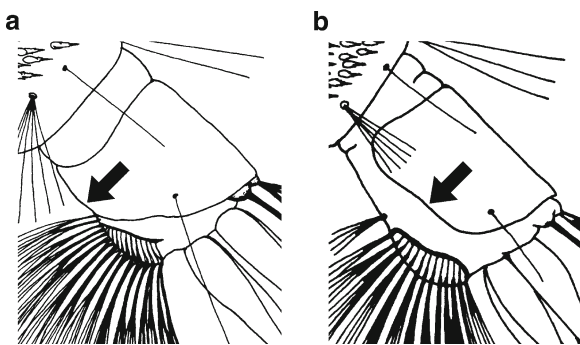


**Fig. 8.27** Siphon of: (a) *Oc. subdiversus*; (b) *Oc. quasirusticus*

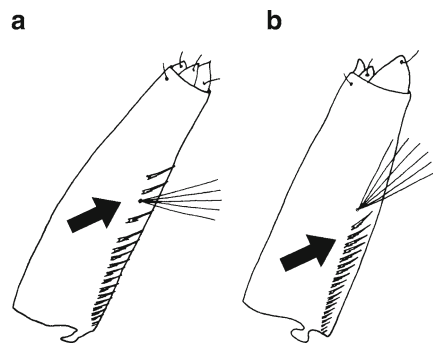


**Fig. 8.28** Siphon of: (a) *Oc. refiki*; (b) *Oc. lepidonotus*

- 13 (10) Dorsal surface of siphon with 3 pairs of additional setae (Fig. 8.28a). ..... *Oc. refiki* (p 259)
- Dorsal surface of siphon with 2 pairs of additional setae (Fig. 8.28b). ..... *Oc. lepidonotus* (p 257)
- 14 (9) Saddle completely surrounding anal segment or ventral margins of the saddle are separated by a very narrow gap (Fig. 8.29a). ..... 15
- Saddle extending to the lateral parts of the anal segment to various degrees, but leaving the ventral part of segment uncovered (Fig. 8.29b). ..... 18
- 15 (14) Distal pecten teeth (one to three) detached (Fig. 8.30a). ..... *Oc. nigripes* (p 245)
- Pecten teeth evenly spaced, close together (Fig. 8.30b). ..... 16

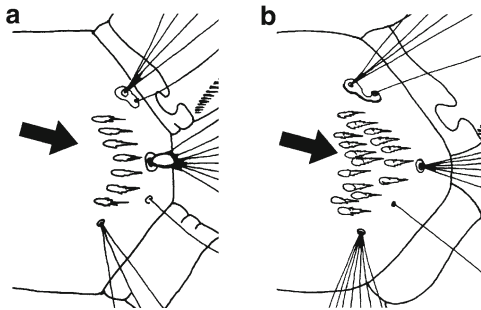


**Fig. 8.29** Anal segment of: (a) *Oc. punctor*; (b) *Oc. cataphylla*

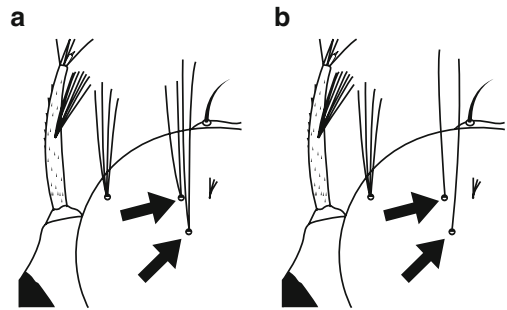


**Fig. 8.30** Siphon of: (a) *Oc. nigripes*; (b) *Oc. punctodes*

- 16 (15) Number of comb scales 6-9. (Fig. 8.31a)..... *Oc. hexodontus* (p 233)  
 Number of comb scales 10-30. (Fig. 8.31b)..... 17

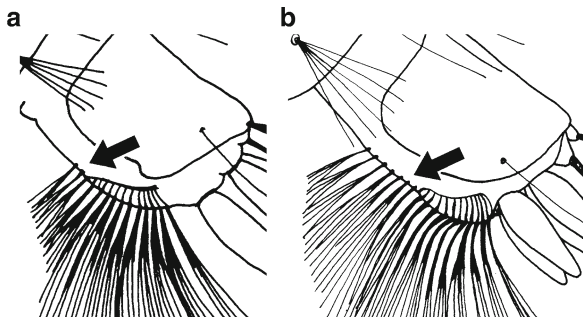


**Fig. 8.31** Abdominal segment VIII of: (a) *Oc. hexodontus*; (b) *Oc. punctor*

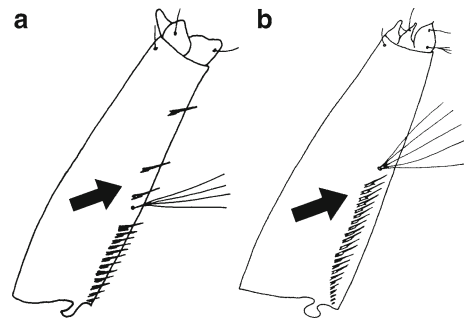


**Fig. 8.32** Head of: (a) *Oc. punctor*; (b) *Oc. punctodes*

- 17 (16) Saddle completely surrounding anal segment. Inner (5-C) and median (6-C) frontal setae with 1-3 branches (Fig. 8.32a)..... *Oc. punctor* (p 251)  
 Lower margins of the saddle are separated by a very narrow gap. Inner and median frontal setae single (Fig. 8.32b). ..... *Oc. punctodes* (p 250)
- 18 (14) Anal segment with 1-3 precratal setae (4-X) (Fig. 8.33a). ..... 19  
 Anal segment with 4-6 precratal setae, sometimes up to 10 (Fig. 8.33b)..... 33

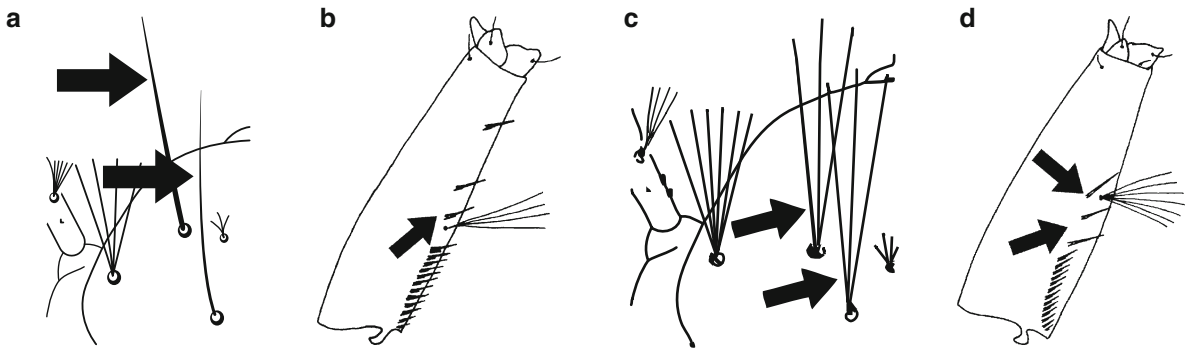


**Fig. 8.33** Anal segment of: (a) *Oc. intrudens*; (b) *Oc. annulipes*



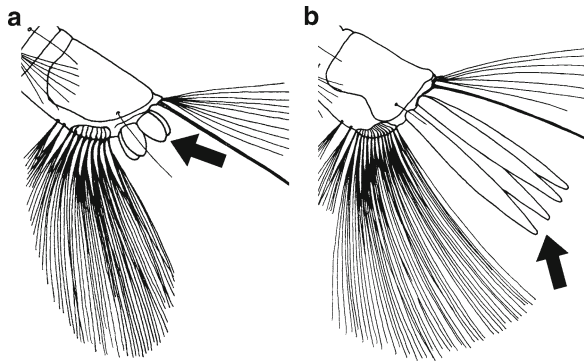
**Fig. 8.34** Siphon of: (a) *Oc. cataphylla*; (b) *Oc. caspius*

- 19 (18) Distal pecten teeth (1-4) detached (Fig. 8.34a). ..... 20  
 Pecten teeth evenly spaced, close together (Fig. 8.34b). ..... 21
- 20 (19) Inner (5-C) and median (6-C) frontal setae single. All detached pecten teeth (2-4) located beyond the insertion point of the siphonal tuft (1-S) (Fig. 8.35a,b)..... *Oc. cataphylla* (p 218)  
 Inner and median frontal setae with 3-5 branches. Detached pecten teeth usually located below insertion point of the siphonal tuft, the distalmost tooth may be located slightly beyond it (Fig. 8.35c,d).....  
 ..... *Oc. intrudens* (p 237)

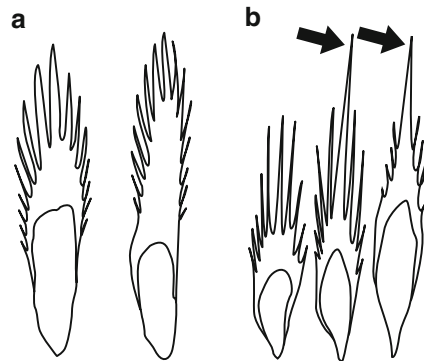


**Fig. 8.35** Head and siphon of: (a, b) *Oc. cataphylla*; (c, d) *Oc. intrudens*

- 21 (19) Anal papillae usually much shorter than the saddle, rarely as long as or up to 1.3 times longer than the saddle (Fig. 8.36a)..... 22
- Anal papillae distinctly longer than the saddle, at least 1.3 times longer than the saddle (Fig. 8.36b)..... 25

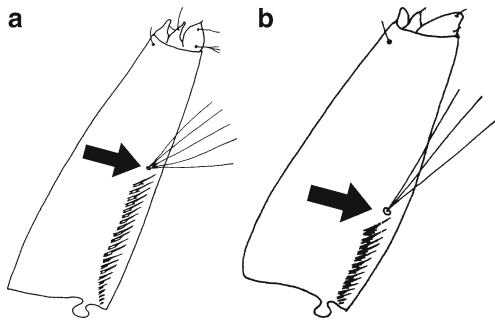


**Fig. 8.36** Anal segment of: (a) *Oc. detritus*; (b) *Oc. communis*

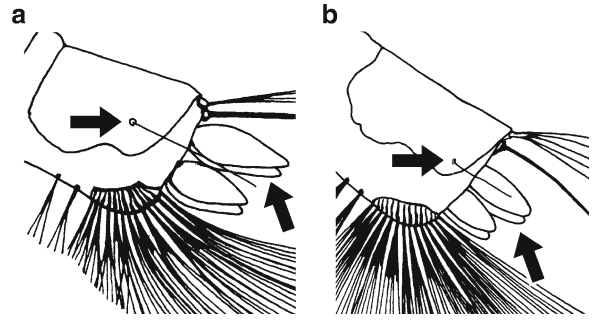


**Fig. 8.37** Comb scales of: (a) *Oc. detritus*; (b) *Oc. caspius*

- 22 (21) Comb with more than 40 scales. Comb scales blunt ended (median spine is not distinctly longer than the others) (Fig. 8.37a). Inner frontal seta (5-C) with 2-3 branches ..... *Oc. detritus* (p 223)
- Comb with less than 35 scales. Comb scales pointed (median spine always distinctly longer than the others, at least in some scales) (Fig. 8.37b). Inner frontal seta usually single, sometimes with 2 branches ..... 23
- 23 (22) Siphonal tuft (1-S) situated beyond the middle of siphon (Fig. 8.38a) ..... *Oc. caspius* (p 216)
- Siphonal tuft usually situated below, rarely slightly beyond the middle of siphon (Fig. 8.38b) ..... 24

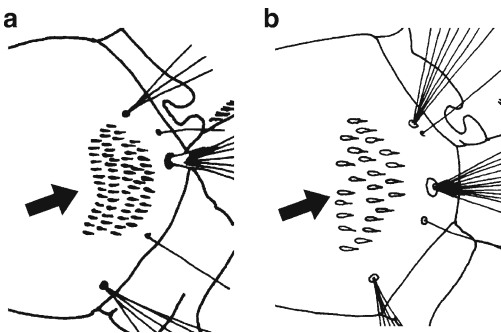


**Fig. 8.38** Siphon of: (a) *Oc. caspius*; (b) *Oc. leucomelas*

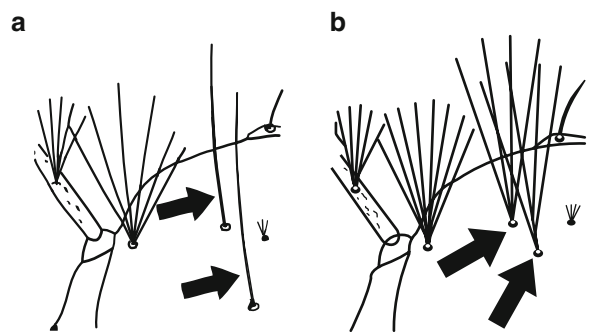


**Fig. 8.39** Anal segment of: (a) *Oc. leucomelas*; (b) *Oc. dorsalis*

- 24 (23) Anal papillae tapering. Saddle seta (1-X) long, nearly as long as the saddle (Fig. 8.39a) ..... *Oc. leucomelas* (p 238)
- Anal papillae rounded. Saddle seta half as long as the saddle (Fig. 8.39b).....*Oc. dorsalis* (p 226)
- 25 (21) Comb with more than 40 scales (Fig. 8.40a) ..... 26
- Comb with less than 40 scales (Fig. 8.40b). ..... 28

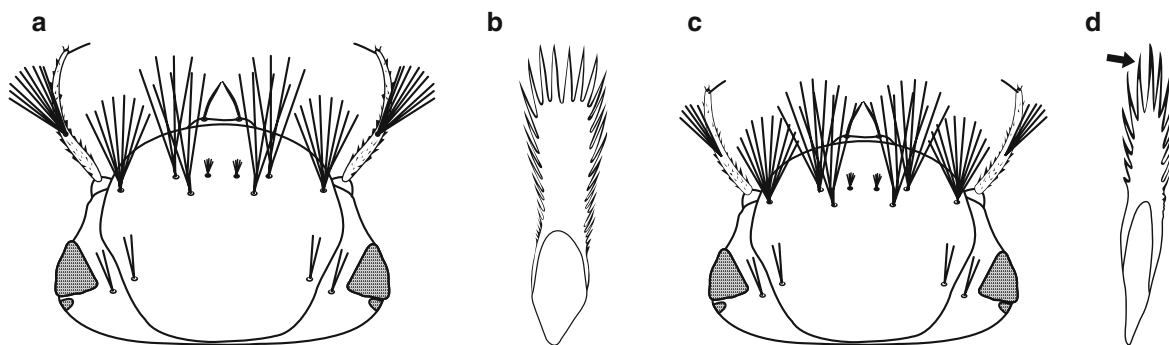


**Fig. 8.40** Abdominal segment VIII of:  
(a) *Oc. communis*; (b) *Oc. sticticus*



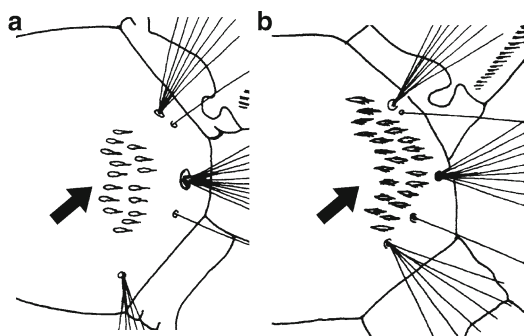
**Fig. 8.41** Head of: (a) *Oc. communis*; (b) *Oc. pionips*

- 26 (25) Inner (5-C) and median (6-C) frontal setae single, rarely one seta with 2 branches (Fig. 8.41a). ..... *Oc. communis* (p 220)
- Inner and median frontal setae multiple branched, with 3 or more branches (Fig. 8.41b)..... 27
- 27 (26) Antennae long, about 2/3 as long as the head or slightly longer. General appearance of comb scales blunt. Elongated median spine absent, all spines of similar length (Fig. 8.42a,b). *Oc. pionips* (p 246)
- Antennae shorter, about half as long as the head. General appearance of comb scales pointed. At least some lateral scales with the median spine distinctly longer than the others (Fig. 8.42c,d). .....*Oc. pullatus* (p 249)

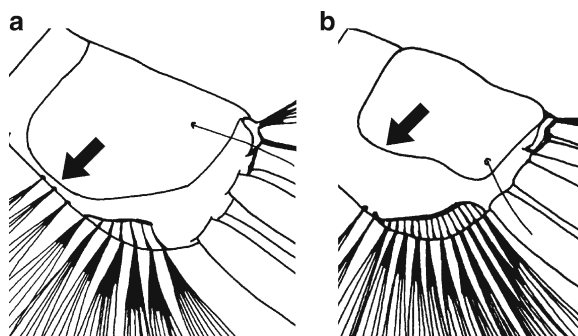


**Fig. 8.42** Head and comb scale of: (a, b) *Oc. pionips*; (c, d) *Oc. pullatus*

28 (25)	Number of comb scales 6–16 (Fig. 8.43a).....	29
	Number of comb scales more than 16 (Fig. 8.43b).....	30



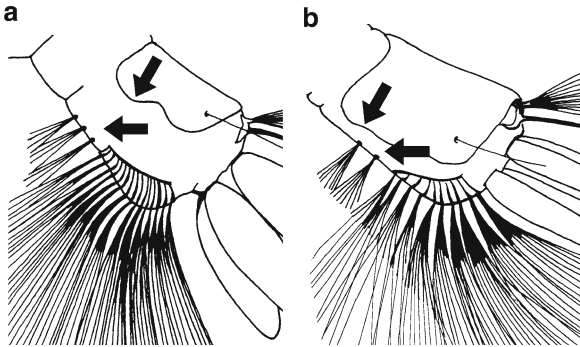
**Fig. 8.43** Abdominal segment VIII of:  
(a) *Oc. nigrinus*; (b) *Oc. hungaricus*



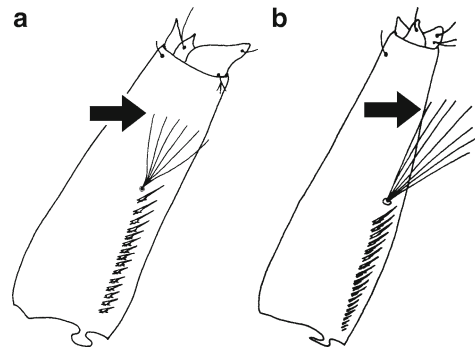
**Fig. 8.44** Anal segment of:  
(a) *Oc. nigrinus*; (b) *Oc. impiger*

29 (28)	Saddle almost completely covering lateral parts of anal segment (Fig. 8.44a).....	<i>Oc. nigrinus</i> (p 243)
	Saddle more plate shaped, extending slightly beyond lateral half of anal segment (Fig. 8.44b).....	<i>Oc. impiger</i> (p 236)

- 30 (28) Inner frontal seta (5-C) single. Anal segment with 3 precratal setae (4-X), saddle extending at most to half of lateral sides (Fig. 8.45a) ..... *Oc. hungaricus* (p 234)  
 Inner frontal seta with 2 or more branches. If one of the pairs of setae is single, anal segment with 1-2 precratal setae and saddle extending to at least 2/3 of lateral sides (atypical *Oc. sticticus*) (Fig. 8.45b)..... 31



**Fig. 8.45** Anal segment of:  
 (a) *Oc. hungaricus*; (b) *Oc. mercurator*

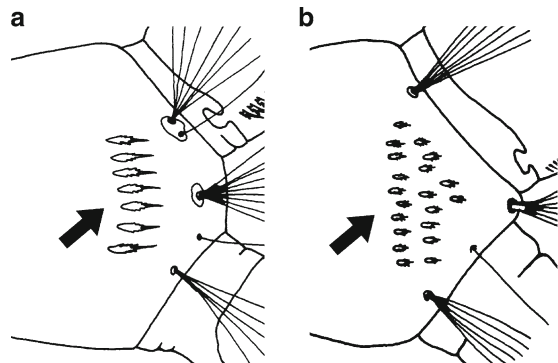


**Fig. 8.46** Siphon of: (a) *Oc. sticticus*; (b) *Oc. pullatus*

- 31 (30) Siphonal tuft (1-S) short, never longer than the width of the siphon at its insertion point (Fig. 8.46a)..... *Oc. sticticus* (p 255)  
 Siphonal tuft long, distinctly longer than the width of the siphon at its insertion point (Fig. 8.46b)..... 32
- 32 (31) Prothoracic setae 2-P and 3-P distinctly shorter and thinner than 1-P (Fig. 8.47a).....  
 ..... *Oc. mercurator* (p 242)  
 Prothoracic setae 2-P and 3-P nearly as long and strong as 1-P (Fig. 8.47b) ..... *Oc. pullatus* (p 249)

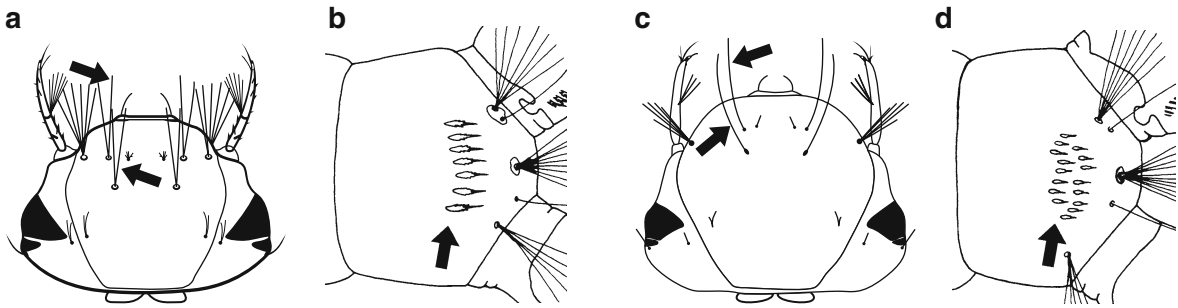


**Fig. 8.47** Prothoracic setae 1-P to 3-P of:  
 (a) *Oc. mercurator*; (b) *Oc. pullatus*



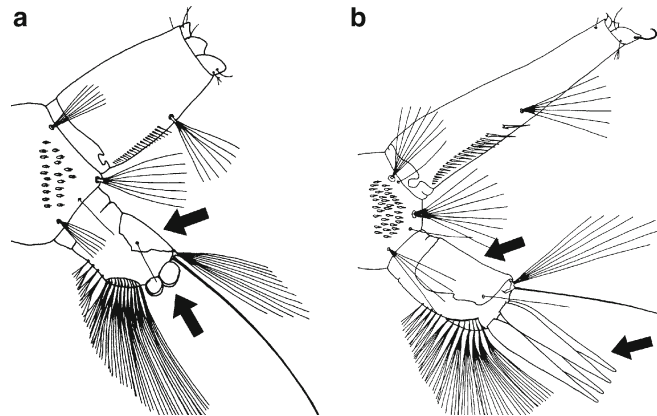
**Fig. 8.48** Abdominal segment VIII of:  
 (a) *Oc. riparius*; (b) *Oc. mariae* s.l.

- 33 (18) Number of comb scales 6–12, rarely 15–17 in *Oc. nigrinus* (Fig. 8.48a) ..... 34  
 Number of comb scales 15–45 (Fig. 8.48b)..... 35
- 34 (33) Inner (5-C) and median (6-C) frontal setae with 2-3 branches. Comb scales arranged in one row (Fig. 8.49a,b). Siphonal index 3.5–4.0..... *Oc. riparius* (p 253)  
 Inner and median frontal setae single (rarely one seta with 2 branches). Comb scales arranged in 2 (rarely 3) rows (Fig. 8.49c,d). Siphonal index never exceeds 3.0, usually 2.0–2.5... *Oc. nigrinus* (p 243)



**Fig. 8.49** Head and abdominal segment VIII of: (a, b) *Oc. riparius*; (c, d) *Oc. nigrinus*

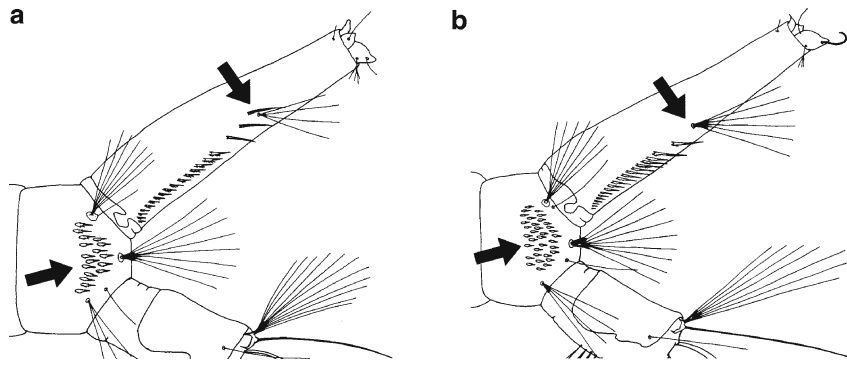
- 35 (33) Siphonal index less than 2.0. Anal segment with weakly developed saddle, lateral part triangular. Anal papillae very short and spherical (Fig. 8.50a) ..... *Oc. mariae* and *Oc. zammitii* (p 240, 242)  
 Siphonal index more than 2.0. Anal segment with well developed saddle, lateral part more or less rectangular. Anal papillae shorter or longer than the saddle, tapering (Fig. 8.50b)..... 36



**Fig. 8.50** End of abdomen of: (a) *Oc. mariae s.l.*; (b) *Oc. excrucians*

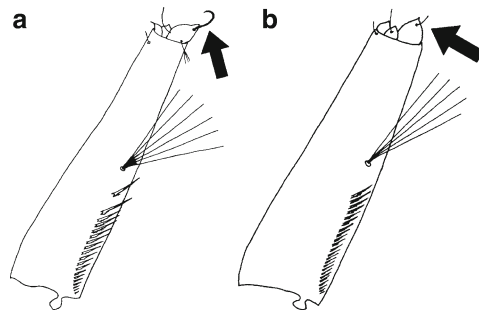
- 36 (35) Number of comb scales less than 20. Siphonal tuft (1-S) short, inserted distinctly beyond the middle of siphon (Fig. 8.51a) ..... *Oc. euedes* (p 228)  
 Number of comb scales more than 20. Siphonal tuft usually long, inserted at about the middle of siphon (Fig. 8.51b)..... 37





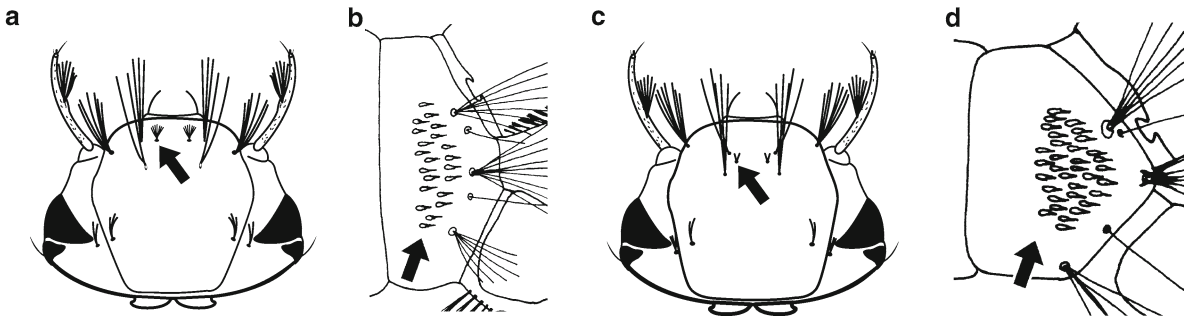
**Fig. 8.51** End of abdomen of: (a) *Oc. euedes*; (b) *Oc. excrucians*

- 37 (36) Seta 9-S on posterolateral flap of stigmal plate strong and hooked or curved (Fig. 8.52a)..... 38
- Seta 9-S on posterolateral flap of stigmal plate relatively weak, slightly curved (Fig. 8.52b) ..... 40



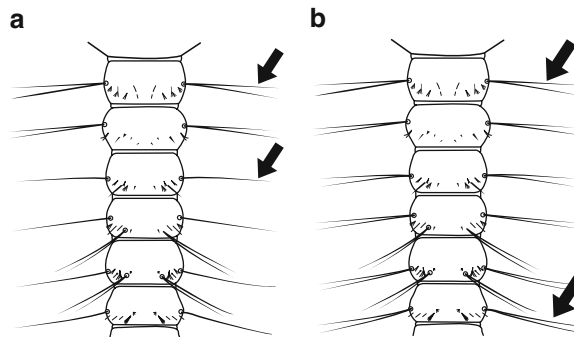
**Fig. 8.52** Siphon of: (a) *Oc. excrucians*; (b) *Oc. mercurator*

- 38 (37) Postclypeal seta (4-C) with 6-8 branches. Comb with less than 30 scales (Fig. 8.53a,b).....
- ..... *Oc. behningi* (p 211)
- Postclypeal seta with 2-3 short, thin branches. Comb with more than 30 scales (Fig. 8.53c,d)..... 39



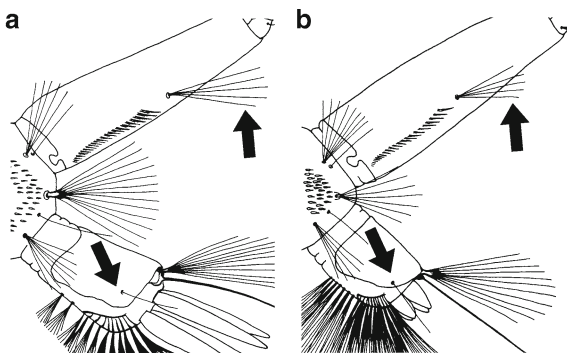
**Fig. 8.53** Head and abdominal segment VIII of: (a, b) *Oc. behningi*; (c, d) *Oc. surcoufi*

- 39 (38) Abdominal seta 6 with 2 branches on segments I and II, single on segments III–VI (Fig. 8.54a)..... *Oc. excrucians* (p 229)  
 Abdominal seta 6 with 2 branches on segments I–VI (Fig. 8.54b)..... *Oc. surcoufi* (p 231)

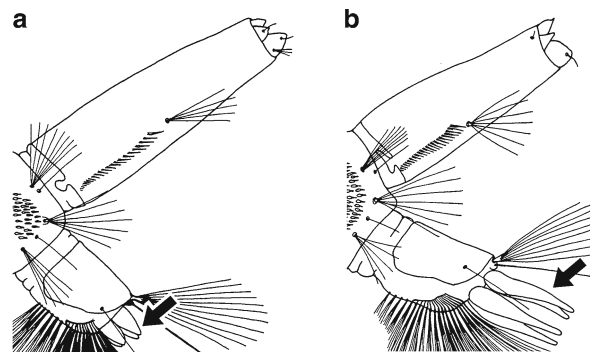


**Fig. 8.54** Abdominal segments I–VI of: (a) *Oc. excrucians*; (b) *Oc. surcoufi*

- 40 (37) Saddle seta (1-X) short, distinctly shorter than the saddle. Siphonal tuft (1-S) distinctly longer than the width of the siphon at the point of its insertion (Fig. 8.55a)..... *Oc. mercurator* (p 242)  
 Saddle seta long, about as long as the saddle. Siphonal tuft (1-S) about as long as the width of the siphon at the point of its insertion (Fig. 8.55b)..... 41

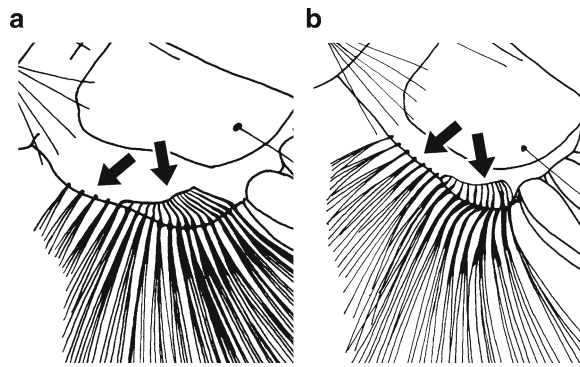


**Fig. 8.55** End of abdomen of:  
 (a) *Oc. mercurator*; (b) *Oc. flavescens*



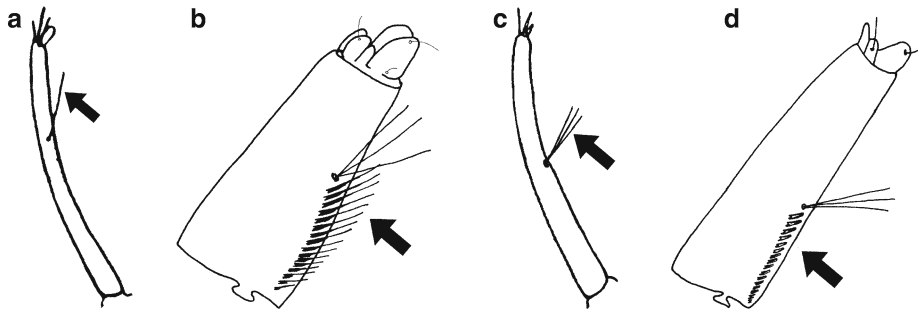
**Fig. 8.56** End of abdomen of:  
 (a) *Oc. flavescens*; (b) *Oc. cantans*

- 41 (40) Anal papillae short, about half as long as the saddle. Siphonal index more than 3.0 (Fig. 8.56a).  
 ..... *Oc. flavescens* (p 231)  
 Anal papillae long, about as long as the saddle or longer. Siphonal index usually less than 3.0 (Fig. 8.56b)..... 42
- 42 (41) Ventral brush with 4–6 precratal setae (4-X) and 15–20 cratal setae (4-X) (Fig. 8.57a).....  
 ..... *Oc. cantans* (p 214)  
 Ventral brush with 6–10 precratal setae and 10–15 cratal setae (Fig. 8.57b)..... *Oc. annulipes* (p 209)



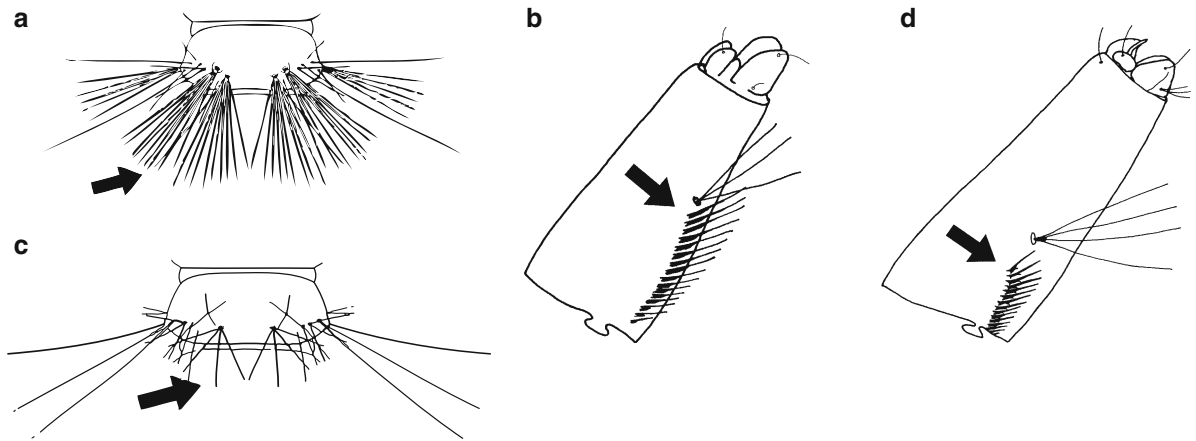
**Fig. 8.57** Anal segment of: (a) *Oc. cantans*; (b) *Oc. annulipes*

- 43 (8) Numerous stellate setae on thorax and abdomen. Antennal seta (1-A) single, pecten teeth long and spine like (Fig. 8.58a,b)..... 44  
 Stellate setae absent. Antennal seta with 3-4 short branches, pecten teeth short, not spine-like, with a broad base (Fig. 8.58c,d).....*Oc. pulcritarsis* (p 248)



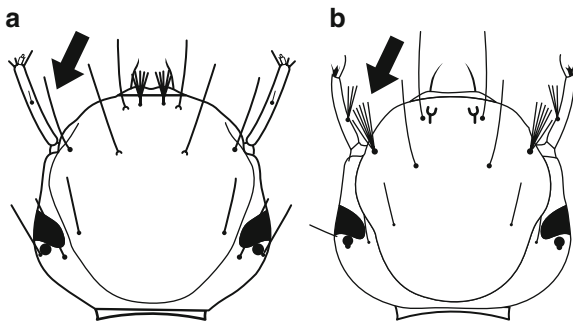
**Fig. 8.58** Antenna and siphon of: (a, b) *Oc. echinus*; (c, d) *Oc. pulcritarsis*

- 44 (43) Branches of stellate setae on abdominal segment I longer than length of the segment. Pecten at least half as long as the siphon (Fig. 8.59a,b) .....*Oc. echinus* (p 205)  
 Branches of stellate setae on abdominal segment I about the same length as the segment. Pecten 1/4-2/5 as long as the siphon (Fig. 8.59c,d) ..... *Oc. geniculatus* (p 206)

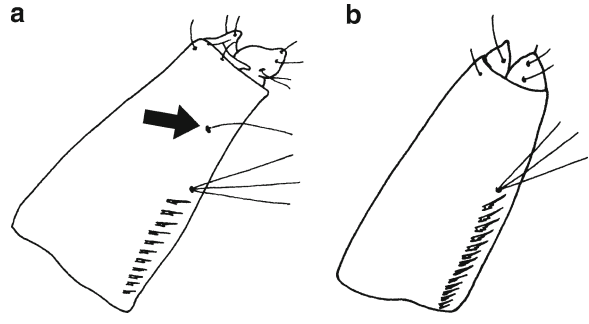


**Fig. 8.59** Abdominal segment I and siphon of: (a, b) *Oc. echinus*; (c, d) *Oc. geniculatus*

- 45 (7) Outer frontal seta (7-C) single (Fig. 8.60a)..... 46
- Outer frontal seta usually with 2 or more branches, rarely single (Fig. 8.60b) ..... 47

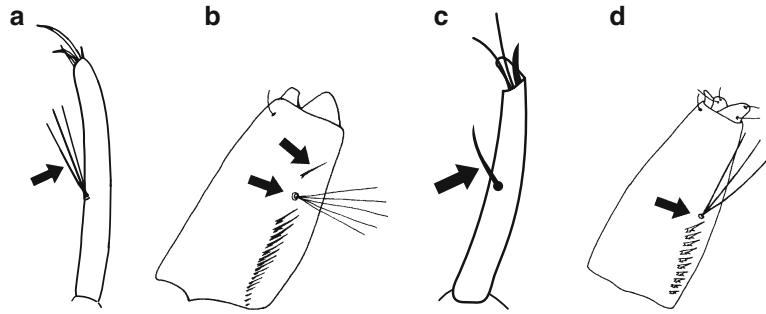


**Fig. 8.60** Head of: (a) *Ae. aegypti*; (b) *Ae. vittatus*



**Fig. 8.61** Siphon of: (a) *Ae. cretinus*; (b) *Ae. aegypti*

- 46 (45) In addition to the siphonal tuft (1-S), a single seta is inserted laterally within the apical 1/3 of the siphon (Fig. 8.61a)..... *Ae. cretinus* (p 203)
- Additional lateral seta absent (Fig. 8.61b) ..... *Ae. aegypti* (p 198)
- 47 (45) Antennal seta (1-A) with 2-3 branches. Siphonal tuft (1-S) inserted at about 2/3 the length of the siphon and within the pecten. Distalmost pecten tooth spine like and apically detached (Fig. 8.62a,b) ..... *Ae. vittatus* (p 196)
- Antennal seta single. Siphonal tuft inserted slightly beyond the middle of the siphon, distal to the pecten (Fig. 8.62c,d)..... *Ae. albopictus* (p 201)

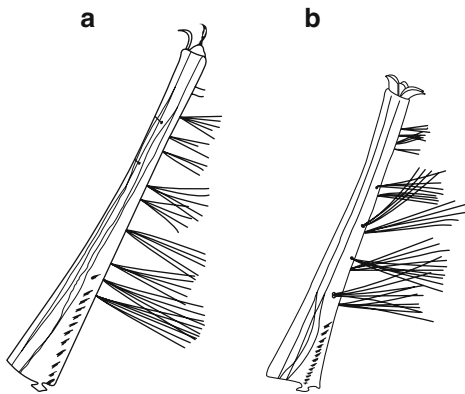


**Fig. 8.62** Antenna and siphon of: (a, b) *Ae. vittatus*; (c, d) *Ae. albopictus*

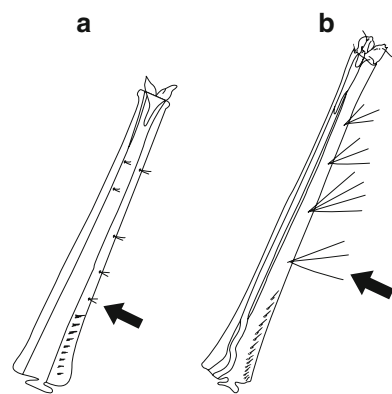
### 8.3 Genus *Culex*

- 1 Siphon long and slender, siphonal index 6.5 or more (Fig. 8.63a) ..... 2
- Siphon shorter, siphonal index usually less than 6.0 (Fig. 8.63b) ..... 7

**Note:** The siphonal index in specimens of *Cx. perexiguus*, *Cx. brumpti* and *Cx. territans* may vary between the above defined groups, consequently they are treated under both branches of the key

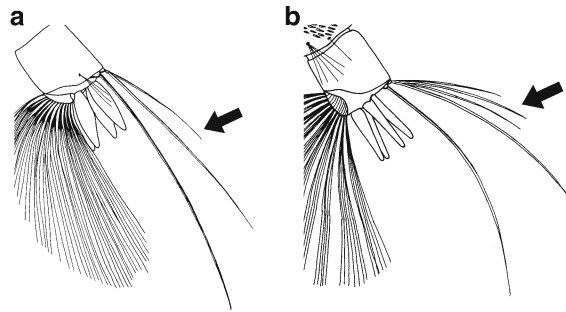


**Fig. 8.63** Siphon of: (a) *Cx. hortensis*; (b) *Cx. mimeticus*



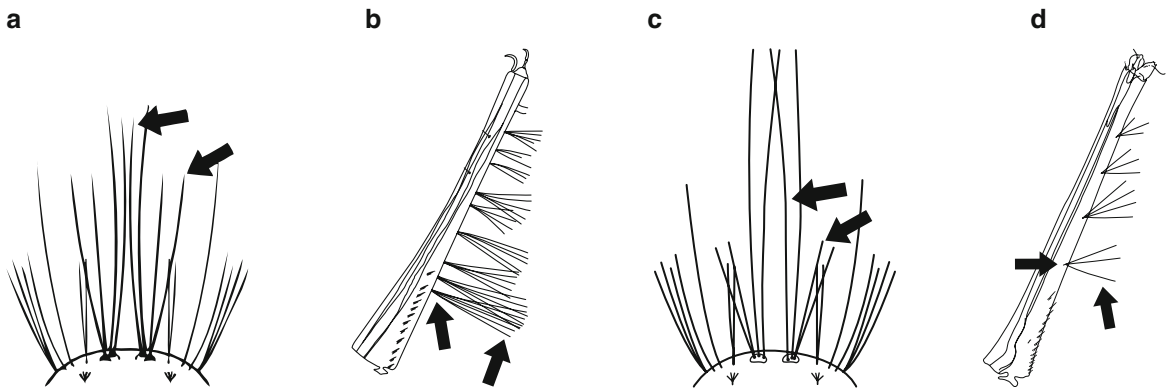
**Fig. 8.64** Siphon of: (a) *Cx. brumpti*; (b) *Cx. territans*

- 2 (1) Basal siphonal tuft (1a-S) shorter than the width of siphon at the point of its origin (Fig. 8.64a) ..... 3
- Basal siphonal tuft equal or longer than the width of siphon at the point of its origin (Fig. 8.64b) ..... 4
- 3 (2) Upper anal seta (2-X) with 2 branches (Fig. 8.65a) ..... *Cx. perexiguus* (p 273)
- Upper anal seta with 3-4 branches (Fig. 8.65b) ..... *Cx. brumpti* (p 269)



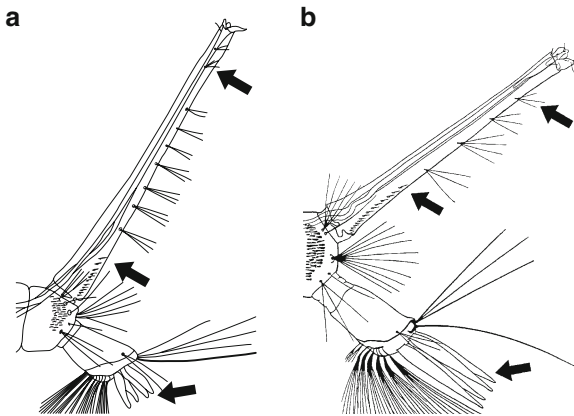
**Fig. 8.65** Anal segment of: (a) *Cx. perexiguus*; (b) *Cx. brumpti*

- 4 (2) Prothoracic seta 3-P nearly as long as 1-P, always longer than the half of 1-P. Basal siphonal tuft (1a-S) more or less 3 times longer than the width of siphon at the point of its origin. At least one siphonal tuft clearly inserted within the pecten (Fig. 8.66a,b). ..... *Cx. hortensis* (p 282)
- Prothoracic seta 3-P never exceeding half the length of 1-P. Basal siphonal tuft of variable length, usually not more than twice as long as the width of siphon at the point of its origin. Usually no siphonal tuft inserted within the pecten, rarely one tuft may be inserted close to the last pecten tooth (Fig. 8.66c,d). ..... 5

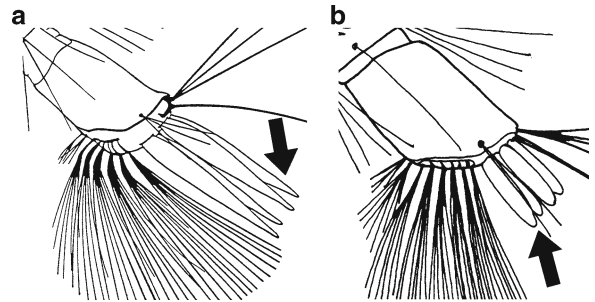


**Fig. 8.66** Prothorax and siphon of: (a, b) *Cx. hortensis*; (c, d) *Cx. territans*

- 5 (4) Siphon usually evenly tapering towards the apex. More than 1, usually 2, apical siphonal tufts inserted laterally. Pecten occupying 1/5 of the siphon length. Anal papillae half as long as the saddle (Fig. 8.67a). ..... *Cx. martinii* (p 285)
- Siphon distinctly widened at the apex. Only one apical siphonal tuft inserted laterally. Pecten occupying more than 1/4 of siphon length. Length of anal papillae variable (Fig. 8.67b)..... 6

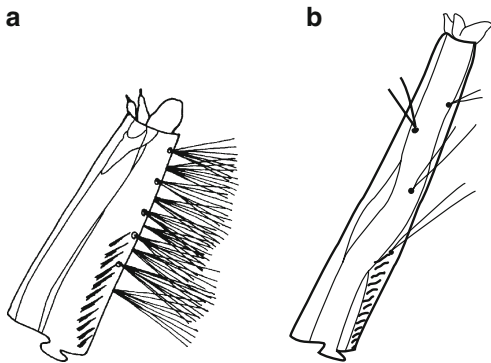


**Fig. 8.67** End of abdomen of: (a) *Cx. martinii*; (b) *Cx. territans*

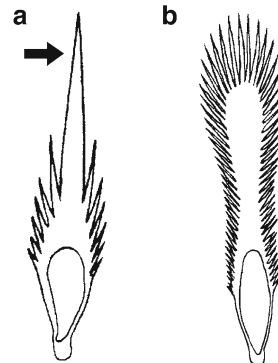


**Fig. 8.68** Anal segment of: (a) *Cx. territans*; (b) *Cx. impudicus*

- 6 (5) Anal papillae nearly as long as or longer than saddle, pointed (Fig. 8.68a). ..... *Cx. territans* (p 286)
- Anal papillae about half as long as saddle, blunt ended (Fig. 8.68b). ..... *Cx. impudicus* (p 284)
- 7 (1) Siphon short, siphonal index at most 3.0. Siphonal tufts (1-S) arranged in a zigzag row on ventral side (Fig. 8.69a). ..... *Cx. pusillus* (p 267)
- Siphon longer, siphonal index at least 4.0 (Fig. 8.69b). ..... 8

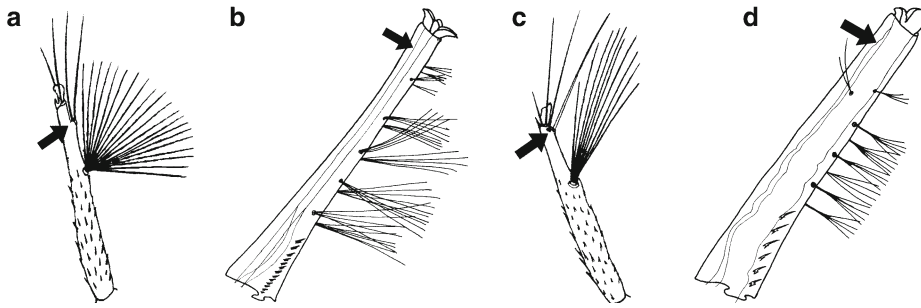


**Fig. 8.69** Siphon of: (a) *Cx. pusillus*; (b) *Cx. p. pipiens*



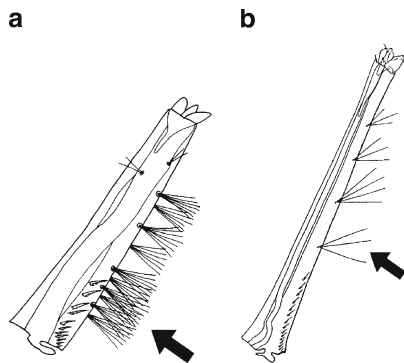
**Fig. 8.70** Comb scale of: (a) *Cx. mimeticus*; (b) *Cx. territans*

- 8 (7) Comb scales with a distinct median spine longer than the others (Fig. 8.70a). ..... 9
- Comb scales without a distinct median spine. All spines at the apex of more or less the same length (Fig. 8.70b). ..... 10
- 9 (8) Subapical setae (2-A, 3-A) inserted at about 2/3 the distance between antennal seta (1-A) and tip of the antenna. Main tracheal trunks narrow, less than half as wide as the siphon at its apical 1/3 (Fig. 8.71a,b) ..... *Cx. mimeticus* (p 271)
- Subapical setae inserted close to the tip of antenna. Main tracheal trunks broad, at least half as wide as the siphon at its apical 1/3 (Fig. 8.71c,d). ..... *Cx. theileri* (p 280)

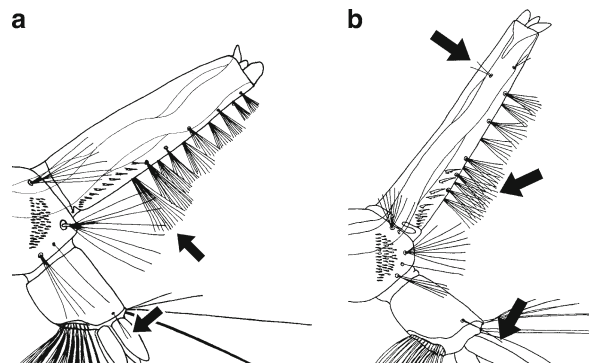


**Fig. 8.71** Antenna and siphon of: (a, b) *Cx. mimeticus*; (c, d) *Cx. theileri*

- 10 (8) Several basal siphonal tufts not paired, inserted at the ventral surface forming a zigzag row. If paired, inserted very close together, near the ventral midline (Fig. 8.72a). ..... 11  
 All siphonal tufts paired. The pairs more widely separated, inserted ventrolaterally or laterally (Fig. 8.72b). ..... 12



**Fig. 8.72** Siphon of:  
 (a) *Cx. laticinctus*; (b) *Cx. territans*

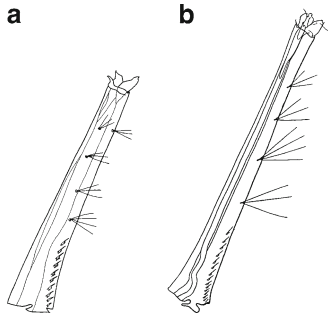


**Fig. 8.73** End of abdomen of:  
 (a) *Cx. modestus*; (b) *Cx. laticinctus*

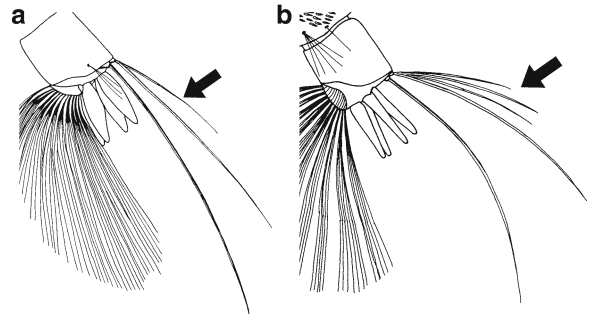
- 11 (10) All siphonal tufts (1-S) arranged in a ventral zigzag row. The length of the tufts suddenly drops towards the apex of the siphon. Usually one tuft inserted within the pecten. Saddle seta (1-X) with 2-3 branches (Fig. 8.73a). ..... *Cx. modestus* (p 265)  
 Not all siphonal tufts arranged in a ventral zigzag row, penultimate tuft arising from the lateral surface of siphon. The length of the ventral tufts gradually decreases towards the apex of the siphon. Usually 3 tufts inserted within the pecten. Saddle seta single, sometimes with 2 branches (Fig. 8.73b). ..... *Cx. laticinctus* (p 270)



- 12 (10) All siphonal tufts (1-S) shorter than or equal to the width of the siphon at the point of their insertion (Fig. 8.74a). ..... 13  
 At least some siphonal tufts distinctly longer than the width of the siphon at the point of their insertion (Fig. 8.74b). ..... 14

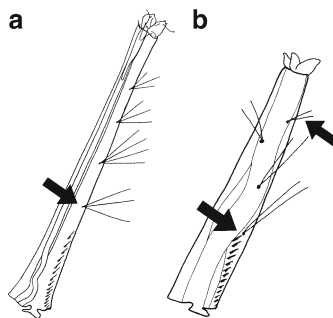


**Fig. 8.74** Siphon of: (a) *Cx. perexiguus*; (b) *Cx. territans*



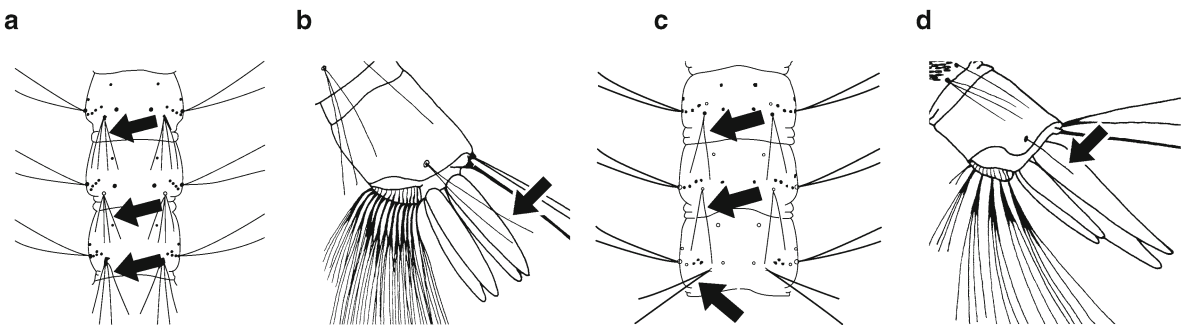
**Fig. 8.75** Anal segment of: (a) *Cx. perexiguus*; (b) *Cx. brumpti*

- 13 (12) Upper anal seta (2-X) with 2 branches (Fig. 8.75a). ..... *Cx. perexiguus* (p 273)  
 Upper anal seta with 3-4 branches (Fig. 8.75b). ..... *Cx. brumpti* (p 269)  
 14 (12) All siphonal tufts longer than the width of the siphon at the point of their insertion. Basalmost tuft usually inserted apart from the last pecten tooth (Fig. 8.76a). ..... *Cx. territans* (p 286)  
 Apicalmost siphonal tuft as long as or shorter than the width of the siphon at its point of insertion. Basalmost tuft usually inserted close to the last pecten tooth (Fig. 8.76b). ..... 15



**Fig. 8.76** Siphon of: (a) *Cx. territans*; (b) *Cx. p. pipiens*

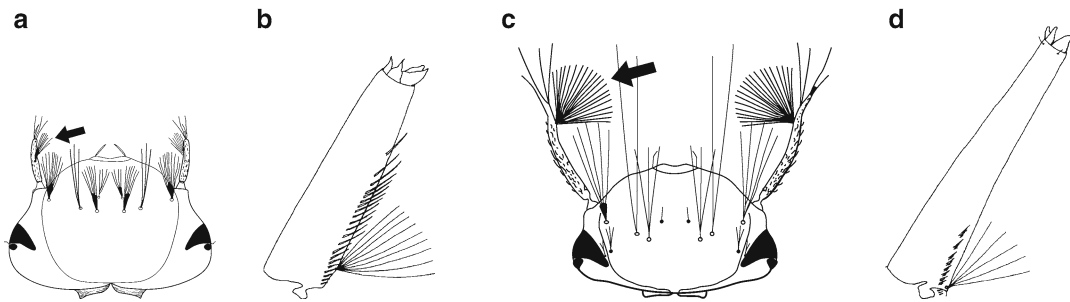
- 15 (14) Metathoracic seta 1-T longer than half of the length of 2-T. Seta 1 on abdominal segments III-V (1-III to 1-V) usually with 4-5 branches (sum of the branches on one side usually more than 10). Saddle seta (1-X) usually with 2 branches. (Fig. 8.77a,b)..... *Cx. torrentium* (p 279)  
 Seta 1-T shorter than half of the length of 2-T. Seta 1 on abdominal segments III-V usually with 1-2 branches (sum of the branches on one side usually 6 or less). Saddle seta usually single. (Fig. 8.77c,d)..... *Cx. p. pipiens* and *Cx. p. quinquefasciatus* (p 275, 278)



**Fig. 8.77** Abdominal segments III–V and anal segment of: (a, b) *Cx. torrentium*; (c, d) *Cx. p. pipiens*

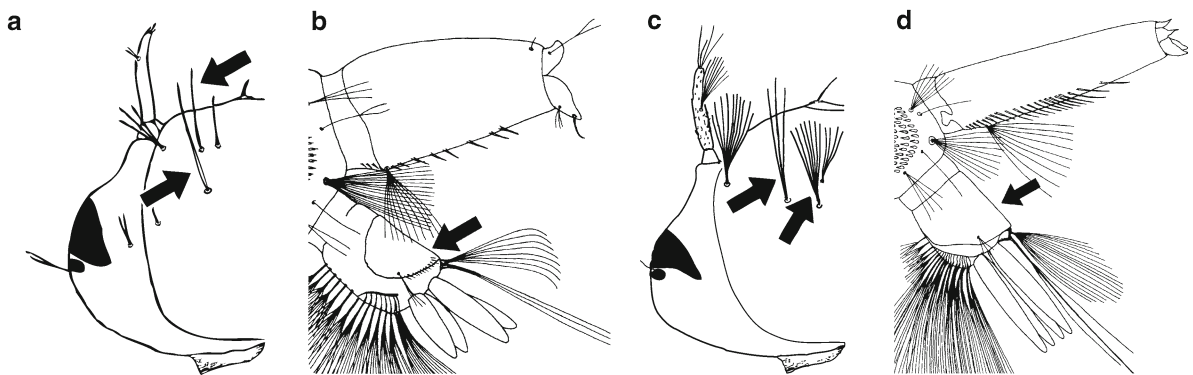
### 8.4 Genus *Culiseta*

- 1 Antenna shorter than the head, seta 1-A weakly developed. Siphon short, siphonal index less than 4.0 (Fig. 8.78a,b). ..... 2
- 2 Antenna longer than the head, seta 1-A well developed. Siphon long and slender, siphonal index more than 5.0 (Fig. 8.78c,d). ..... 7



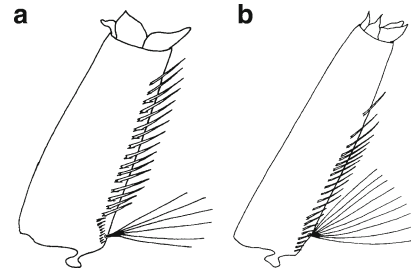
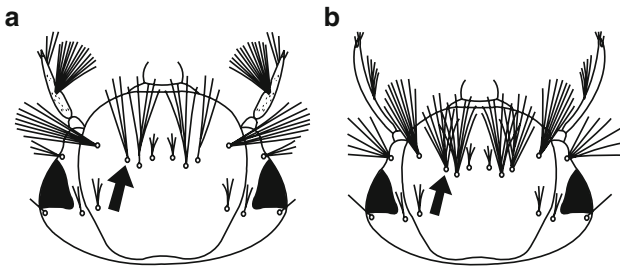
**Fig. 8.78** Head and siphon of: (a, b) *Cs. annulata*; (c, d) *Cs. morsitans*

- 2 (1) Inner (5-C) and median (6-C) frontal setae single. Siphonal index 2.0 or less. Saddle plate shaped, not surrounding anal segment (Fig. 8.79a,b). ..... *Cs. longiareolata* (p 289)
- Inner and median frontal setae multiple branched. Siphonal index more than 2.0. Saddle completely surrounding anal segment (Fig. 8.79c,d). ..... 3



**Fig. 8.79** Head and end abdomen of: (a, b) *Cs. longiareolata*; (c, d) *Cs. annulata*

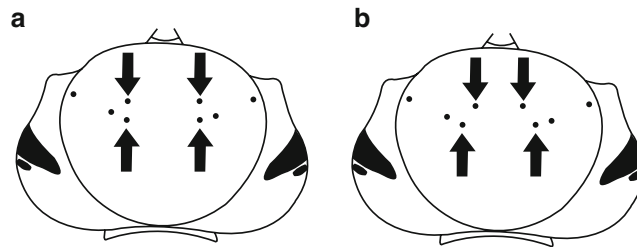
- 3 (2) Antenna less than half as long as the head. Median frontal seta (6-C) with 1-3 branches. (Fig. 8.80a). Number of comb scales usually less than 50 ..... 4  
 Antenna at least half as long as the head. Median frontal seta with 4-8 branches. (Fig. 8.80b). Number of comb scales usually more than 60 ..... 6



**Fig. 8.80** Head of: (a) *Cs. alaskaensis*; (b) *Cs. glaphyoptera*

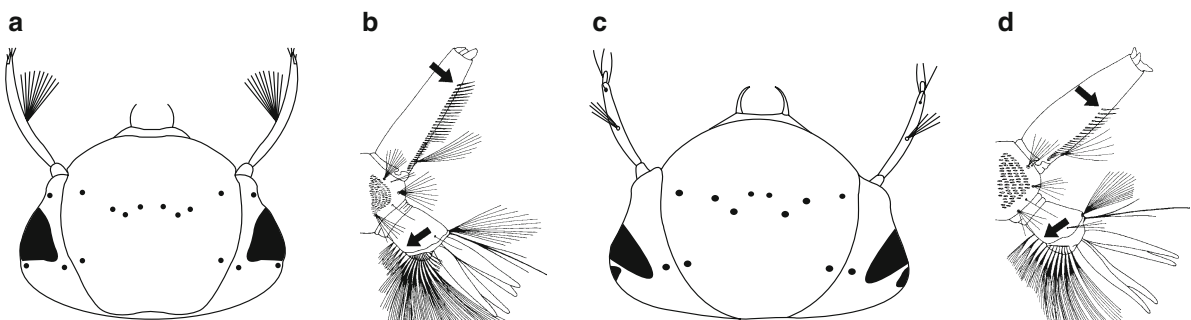
**Fig. 8.81** Siphon of: (a) *Cs. alaskaensis*; (b) *Cs. annulata*

- 4 (3) Siphonal index less than 3.0, siphon slightly tapering apically (Fig. 8.81a). ..... *Cs. alaskaensis* (p 298)  
 Siphonal index 3.0–4.0, siphon distinctly tapering apically (Fig. 8.81b). ..... 5  
 5 (4) Distance between postclypeal setae (4-C) equal to or longer than distance between inner frontal setae (5-C) (Fig. 8.82a). ..... *Cs. annulata* (p 299)  
 Distance between postclypeal setae distinctly shorter than distance between inner frontal setae (Fig. 8.82b). ..... *Cs. subochrea* (p 305)



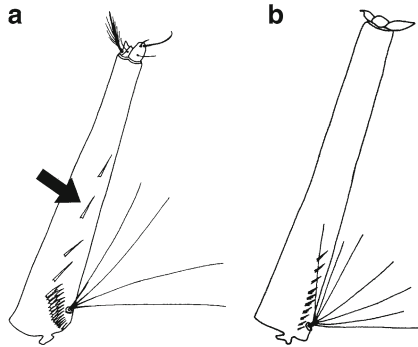
**Fig. 8.82** Head of: (a) *Cs. annulata*; (b) *Cs. subochrea*

- 6 (3) Antenna 2/3 as long as the head. Pecten spread over 3/4 the length of siphon. Ventral brush with 5 precratal setae (4-X) (Fig. 8.83a,b). ..... *Cs. glaphyoptera* (p 303)  
 Antenna half as long as the head. Pecten spread over 2/3 the length of siphon. Ventral brush with 3-4 precratal setae (Fig. 8.83c,d). ..... *Cs. bergrothi* (p 301)



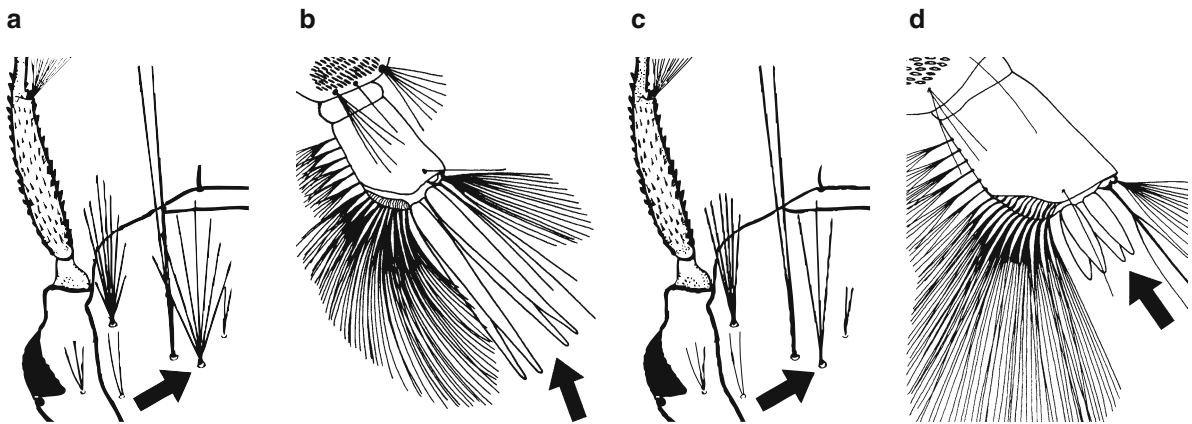
**Fig. 8.83** Head and end of abdomen of: (a, b) *Cs. glaphyoptera*; (c, d) *Cs. bergrothi*

- 7 (1) In addition to the typical pecten teeth, the siphon bears spine-like setae irregularly scattered on its ventro-lateral surface (Fig. 8.84a). ..... *Cs. fumipennis* (p 291)  
 Siphon with typical pecten teeth only (distal 1-2 teeth may be detached and atypical, spine like setae in *Cs. ochroptera*) (Fig. 8.84b). ..... 8



**Fig. 8.84** Siphon of: (a) *Cs. fumipennis*; (b) *Cs. ochroptera*

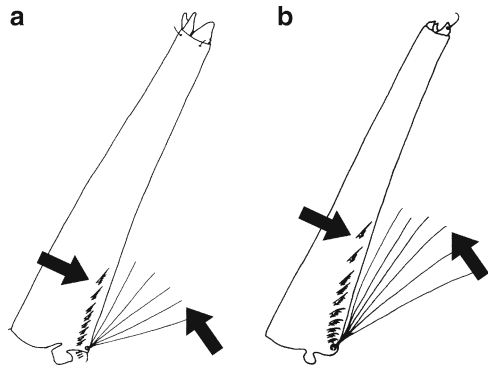
- 8 (7) Inner frontal seta (5-C) with 5-9 branches. Anal papillae 1.5–2.0 times longer than the saddle (Fig. 8.85a,b). ..... *Cs. ochroptera* (p 296)  
 Inner frontal seta with 2-4 branches. Anal papillae shorter than the saddle (Fig. 8.85c,d). ..... 9



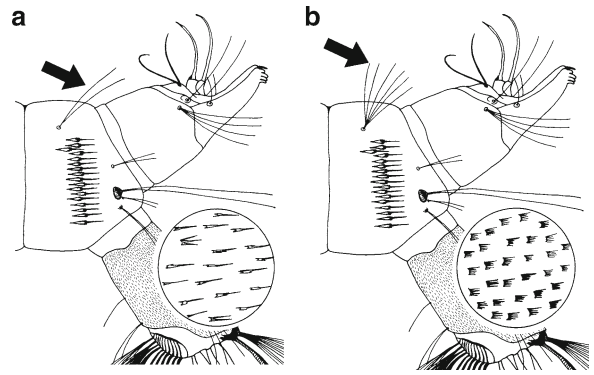
**Fig. 8.85** Head and anal segment of: (a, b) *Cs. ochroptera*; (c, d) *Cs. morsitans*

- 9 (8) Pecten confined to basal 1/4 of siphon. Length of siphonal tuft (1-S) usually 1/3 or less than length of siphon (Fig. 8.86a). ..... *Cs. morsitans* (p 294)  
 Pecten confined to basal 1/3 of siphon. Length of siphonal tuft usually more than 1/3 length of siphon (Fig. 8.86b). ..... *Cs. litorea* (p 292)

**Note:** Both species show variation and overlapping in these characters and are difficult to distinguish.



**Fig. 8.86** Siphon of: (a) *Cs. morsitans*; (b) *Cs. litorea*



**Fig. 8.87** End of abdomen of: (a) *Cq. richiardi*; (b) *Cq. buxtoni*

### 8.5 Genus *Coquillettidia*

- 1 Seta 1-VIII with 2-4 branches. Saddle completely covered with short and stout, usually single spicules, rarely 2-3 grouped on a common base (Fig. 8.87a).....*Cq. richiardi* (p 308)
- Seta 1-VIII with 5-7, usually 6 branches. Spicules on saddle grouped in rows of 2-8 (usually 5-6) on a common base (Fig. 8.87b). .....*Cq. buxtoni* (p 307)

## Chapter 9

### Subfamily Anophelinae

In adults of this subfamily, at least the first abdominal segment (tergum I) is devoid of any scales. In general the development of scaling has not reached the same level as in the subfamily Culicinae; often the abdomen is covered with fine setae only. The palps of both sexes are approximately the same length as the proboscis. The larvae have no discernible respiratory siphon and seta 1 of most abdominal segments is usually of the palmate type.

The subfamily comprises only three genera: *Anopheles*, *Bironella* and *Chagasia*. The genus *Bironella* Theobald includes eight species divided between three subgenera (Fig. 1.2) that are confined to the Australasian Region. They are easily distinguished from *Anopheles* by wing veins  $Cu_1$  and M being wavy at their distal parts and thoracic setae 1-M and 3-T in larvae that are both palmate. The adults are seldom seen in nature and rarely bite man. *Chagasia* Cruz is a small and rare genus; its four species are exclusively distributed in the Neotropical region. Adults of this genus are mainly characterized by the somewhat trilobed scutellum with a set of setae on each lobe, wings without distinct spots as in most *Anopheles* and the large claws on the fore and mid legs of the males. The larvae of *Chagasia* have the anterior flap of the spiracular apparatus produced into a long spine-like process and the palmate setae on the abdominal segments are characteristically shaped. Most of the species of the subfamily, including all European species, belonging to the genus *Anopheles* Meigen have a worldwide distribution with more than 450 described species, species complexes, subspecies and varieties. The genus *Bironella* of the subfamily, including its three subgenera *Bironella* Theobald, *Brugella* Edwards and *Neobironella* Tenorio was synonymized with the genus *Anopheles* Meigen and redefined as an informal group within the subgenus *Anopheles* (Sallum et al. 2000). However, this synonymy was not supported by later

studies of Sallum et al. (2002) and Harbach and Kitching (2005), which suggest that, at the present, *Bironella* should be regarded as a subgenus of *Anopheles*.

Adult anophelines (except *Chagasia*) are usually recognised at once by their attitude when at rest on walls or other objects. The proboscis is held straight out in line with the body axis, not at an angle as in culicine mosquitoes, and the body is strongly tilted downwards to the head end. This causes the abdomen to point away from the surface and the whole body to form an angle with the surface. The majority of species form angles of 30–45° with the surface on which they rest, but in some species, such as *Anopheles hyrcanus*, this angle may even approach 90°. The tip of the labium and the palps are usually brought close to the surface, almost being in contact with it. In addition, adult anophelines generally have longer legs than culicines. In females the palps are elongated, about the same length as the proboscis. They are held closely adjacent to the proboscis (except during feeding), so that the palps and the proboscis appear to be a single organ. When the insect is dead and the tissue somewhat dried, the elongated palps separate and are easily recognised. In males, as in nearly all other mosquitoes, the palps are also elongated. The two apical segments of the palps are swollen and laterally flattened, giving them a club like appearance. The larvae of anophelines are distinguishable from all other mosquitoes by their feeding behaviour. They usually rest under the water surface in a horizontal position and feed on particles from the surface film. The head can be rotated through 180° toward both sides, so that the ventral side is upward and the mouthparts are in contact with the surface. At the sides of the anterior margin of the thorax are two lobed, reversible organs (notched organs) which can be retracted and together with the palmate setae on the abdominal segments, support the body when attaching into a horizontal position at the surface film.

## 9.1 Genus *Anopheles* Meigen

Members of the genus *Anopheles* are usually medium sized to small mosquitoes with long and slender legs and relatively narrow wings. Their general colouration may be variable from grey, brown or almost black to whitish or pale, but without a metallic shine. The proboscis is straight, long and slender. The palps are usually as long as the proboscis in both sexes, rarely, slightly shorter in some tropical females. The palps of females consist of five slender palpomeres, except in *An. plumbeus* that has a slightly swollen palpomere V. The clypeus is usually longer than it is broad, and triangular in shape. The occiput is covered with erect, forked scales. The scutum is slightly convex and not strongly arched, and clothed with setae of various colours. The scutellum is evenly rounded with a more or less regular row of long setae. Prespiracular setae are usually present. Pulvilli are absent, and tarsal claws of females are without a subbasal tooth. The wings are relatively narrow, often with dark or pale scales forming characteristic spots or evenly dark scaled and without spots; cross veins are usually well separated. The integument of the terga is usually dark, covered with various coloured setae and usually without scales. The abdomen is parallel sided, with a blunt end. The cerci of the females are short, rounded and inconspicuous, and only one spermatheca is present.

The male hypopygium with a conical gonocoxite, is about twice as long as it is broad, usually without an apical and basal lobe, but with prominent parbasal setae of variable number and shape in place of a basal lobe. The gonostylus is approximately as long as the gonocoxite, curved, with the apical spine of the gonostylus short and blunt ended. The claspettes are divided into two or more lobes, each lobe bearing long, various shaped setae at its apex. The aedeagus is tubular, bare or with apical aedeagal leaflets of various shape.

The head of the larvae is as long as or longer than it is broad, and antenna shorter than the head, and rod-shaped. Antennal seta (1-A) is small and single or more prominent and multiple branched. Two pairs of clypeal setae, the inner (2-C) and outer (3-C), are situated at the anterior margin of the head. The frontal setae (5-C to 7-C) are of pinnate or plumose type, except in *An. plumbeus* that has short and single frontal setae. Most setae of the thorax are usually plumose, varying in length, and arranged in three sets, corresponding to the thoracic segments. Seta 1 of abdomi-

nal segments I–VII is usually of the palmate type, it is often rudimentary on segments I and II, and well developed on segments III–VII. The lateral setae of the first three segments (6-I to 6-III) are long and plumose. Each segment has 1 or 2 dorsal sclerotized plates positioned medially. The spiracular apparatus is situated at the posterior margin of the dorsal side of abdominal segment VIII flush with its surface. On the sides of segment VIII pecten plates are located, each with a posterior row of conspicuous teeth. Both plates are connected by a narrow sclerotized band, bordering the spiracular apparatus posteriorly. The saddle is plate-shaped, and does not encircle the anal segment. The ventral brush consists of a group of fan-like setae, but without precratal setae. The anal papillae are usually blunt ended, and about the length of the anal segment.

The genus *Anopheles* is divided into seven subgenera (Fig. 1.2). Members of the subgenera *Kerteszia* Theobald, *Lophopodomys* Antunes, *Nyssorhynchus* Blanchard and *Stethomyia* Theobald have a mainly neotropical distribution. Only species of the subgenera *Anopheles* Meigen and *Cellia* Theobald are represented in the Palaearctic region, which includes Europe. A new subgenus *Baimaia* Abraham is introduced for the unusual crabhole species, *An. kyondawensis* Abraham, in Southeast Asia (Harbach et al. 2005). The phylogenetic relationships of *Lophopodomys* and *Stethomyia* with subgenus *Anopheles* and their subgeneric status are uncertain (Sallum et al. 2000, 2002; Harbach and Kitching 2005).

The eggs of *Anopheles* are commonly laid singly and directly on the water surface, where they lie horizontally, several of them often adhering to one another and forming characteristic configurations of triangles, stars or ribbons due to the surface tension and the shape of the eggs. The egg of the anopheline is usually boat shaped with pointed ends and has a flattened or slightly concave upper surface or deck and a more convex lower surface. The fringe or fringed surrounds the whole, or parts, of the upper surface. At the sides of the egg are the floats or air cells, which may interrupt the fringe in the middle. The number of air cells is characteristic for different species. In species of the *Anopheles Maculipennis* Complex the ornamentation of the upper surface of the egg and the form of the intercostal membrane (surface of the air cells) are important diagnostic tools for identification.

Larvae of *Anopheles* are usually found in natural water bodies and rarely in artificial collections of water. The preferred breeding places include permanent and

semi permanent ponds, pools, puddles or ditches with a growth of emergent aquatic vegetation. They can also be found in rice fields, swamps or margins of rivers and lakes, where the water flow is reduced through shelters. Only a few species show exceptions, like *An. plumbeus*, which is a typical tree-hole breeder, and *An. multicolor*, which exhibits a preference for brackish water.

Although adult females seek their blood meal from early evening on and then continue to be active throughout the night, they are considered predominantly biters during the crepuscular period after sunset and during dawn (Bates 1949).

### 9.1.1 Subgenus *Anopheles* Meigen

The wing is often entirely without pale spots; if pale spots are present (*An. hyrcanus*), the costal margin is covered with one or two of them in its apical half. The cross veins and bifurcations of  $R_{2+3}$  and M are dark scaled. In the male genitalia, 1-3, usually 2, parbasal setae are situated at the base of the gonocoxite with at least one of them arising from a more or less distinct tubercle. Internal setae are present. The larvae of the subgenus *Anopheles*, except those of *An. plumbeus*, are characterized by the branched antennal seta (1-A) which arises from the inner surface of the antennal shaft, and the inner clypeal setae (2-C) arising close together, their bases often nearly touching. In *An. plumbeus*, seta 1-A is single and the pair of setae 2-C is more widely separated, the distance between them is nearly the same as the distance between 2-C and the outer clypeal setae (3-C). In all species of the subgenus, the leaflets of the palmate setae are lanceolate, not abruptly narrowed or shouldered, and have a more or less developed terminal filament. The subgenus *Anopheles* is widely distributed and occurs with approximately 150 other species in all zoogeographical regions. In Europe, 15 species of the subgenus (including *An. daciae*) are recorded with a distribution range throughout the whole region.

#### *Anopheles (Anopheles) algeriensis* Theobald 1903

**Female:** *An. algeriensis* differs from all other European species of the subgenus *Anopheles* which have unspotted wings by the absence of white or cream coloured scales on the vertex, and by the scutum which is uni-

coloured and may vary from reddish-brown to brown or dark brown, showing no trace of darkening at the sides (Fig. 6.10a). The characteristic median stripe of pale scales on the scutum, that stretches at least along the anterior half in *An. marteri*, is also absent. The proboscis and palps are brown to dark brown, the latter nearly as long as the former. The erect head scales are broad, and dark brown throughout. The scutum and scutellum are brown with blackish setae, and the lobes of the antepnotum are dark brown. The colour of the pleurites is uniformly dark to greyish brown with 2 prespiracular setae, 3 prealar setae, 2 upper mesepisternal setae, and 10 upper mesepimeral setae. The lower mesepisternal setae and lower mesepimeral setae are absent. The legs are uniformly dark, very rarely the hind tarsi show indistinct pale ringing. The wing veins are covered with dark scales that are uniformly distributed. The abdominal terga are mainly dark brown without transverse bands.

**Male** (Figs. 7.8a and 9.1): The genitalia are characterized by the gonocoxite with only one apically curved parbasal spine-like seta situated on a distinct lobe. Above it, another prominent pointed seta, the internal seta, arises from the inner side of the gonocoxite near the apex. The claspettes are 3-lobed, the outer lobe with 2-3 spine-like pointed setae located close together, the middle lobe with 2 flattened setae inwardly curved at the apex and the inner lobe with 3 long, thin and pointed setae. The aedeagus has 2-3 pairs of long narrow leaflets.

**Larva:** The antenna is slightly curved, dark and spiculate throughout its inner surface. The antennal seta (1-A) is short with 5-6 branches arising close to the base of the antenna. The inner clypeal seta (2-C) is long, with a few minute branchlets along the apical half of the shaft. The outer clypeal seta (3-C) is about half as long as the inner clypeals (2-C), with 2-3



**Fig. 9.1** Hypopygium of *An. algeriensis*



branches near the apex. The postclypeal seta (4-C) is single, rarely with 2 branches at the apex. One of the most suitable features to separate larvae of *An. algeriensis* from those of the closely resembling *An. claviger s.l.* is the pattern of dark markings on the head. In *An. algeriensis*, at least three transverse dark bands run across the frontoclypeus (Fig. 8.10a). The most anterior band is located behind the postclypeal setae, the median band behind the frontal setae, and the posterior band is situated close to the base of the frontoclypeus. In *An. claviger s.l.* the frontoclypeus is dark spotted, but never banded. The palmate setae on abdominal segments III–VII (1-III to 1-VII) each consist of 16–18 leaflets with a weakly developed terminal filament and a conspicuous sawed margin. The sclerotized tergal plate on segment VIII is as broad as, or broader than the distance between the palmate setae on segment VII (1-VII). The saddle seta (1-X) arises well within the margin of the saddle.

**Biology:** The larvae are found in natural or artificial, well shaded, and more or less fresh, water bodies, such as ditches, canals or flooded pools with a rich overgrowth of vegetation (*Phragmites* sp.). They often occur in shaded permanent pools among thick sedge or in swamps and marshes. Owing to their tolerance of slight salinity, they are occasionally found in high numbers in brackish water. They are associated with larvae of *An. maculipennis s.l.* and *Ur. unguiculata*, but rarely with *An. claviger s.l.*, *Cx. hortensis* or *Cx. theileri*. In central Europe, adults of *An. algeriensis* first occur in early summer and the species usually hibernates in the larval stage, but in its southern range in northern Africa, both adults and larvae can be found during the winter months. In this area the larvae can be found in clear, cool, mountain streams or in wells and cisterns (Senevet and Andarelli 1956). *An. algeriensis* is considered to be an exophilic mosquito; the adults rest outside in dense grassy vegetation and readily attack their hosts, humans and animal, in the open preferably at dusk and dawn. They rarely enter houses or stables.

**Distribution:** *An. algeriensis* is widely distributed throughout the Mediterranean region and northern Africa. In central Europe, it is recorded in England, Germany, northern France, Hungary and Bulgaria. In the eastern part of Europe there is one record from the west coast of Estonia (Gutsevich et al. 1974). The eastward extension of *An. algeriensis* includes the coastal plain area of Turkey to the northern slopes of the Caucasus and through Middle Asia.

**Medical importance:** Although the vectorial capacity of *An. algeriensis* is high (e.g. it can easily be infected with *P. falciparum* in the laboratory), it is considered as a secondary vector due to its exophily. *An. algeriensis* is rarely encountered in villages and an abundance of its population, even in the open field, is rarely high.

### Anopheles Claviger Complex

It has been shown by Coluzzi (1960, 1962) and Coluzzi et al. (1965) that *An. claviger* is a species complex with at least two distinct members, *An. claviger s.s.* (Meigen) and *An. petragani* Del Vecchio. These two sibling species are distributed in the western Mediterranean subregion and differ distinctly in their larval and pupal morphology and larval and adult behaviour. The question remains unsolved if other described forms, e.g. *missiroli* Del Vecchio from Italy and *pollutus* Torres Canamares from Spain, so far treated as synonyms of *An. claviger*, are also distinct species or varieties of the nominative form.

### *Anopheles (Anopheles) claviger s.s. (Meigen 1804)*

**Female:** *An. claviger s.s.* is distinguished from *An. marteri* by its entirely dark palps without pale scales at the tip and from the similar *An. plumbeus* by its decidedly larger size and its general brownish colouration. The pleurites and lateral parts of the scutum are fawn brown or light brown, whereas *An. plumbeus* is a small mosquito with dark brown or bluish pleurites with the lateral parts of the scutum forming a distinct contrast to the pale scales of the median scutal area. In *An. claviger s.s.* the median pale stripe is always visible, but it is not so distinctly contrasted. In old and dried-up specimens with an indistinct colouration, the different length of the palpomeres may be of more help in the separation of the two species. In *An. claviger s.s.* the last segment of the palps is less than half as long as the penultimate segment, whereas in *An. plumbeus* it exceeds half the length of the penultimate segment. *An. claviger s.s.* has a uniformly dark brown proboscis and palps. The antennae are brown, the vertex has a tuft of whitish and cream coloured scales and the setae are anteriorly directed. The occiput has narrow pale scales. The integument of the scutum is brown, with narrow to moderately broad pale scales forming the

median stripe, which is broad and covers more than half of the scutum. The antecrostichal patch of pale scales is faintly developed (Fig. 6.12b). The scutellum is brown, darker in the middle with dark setae on its posterior margin. The pleurites of the thorax are brown, and the legs are brown or dark brown with a few pale scales at the articulations. The scales on the wing veins are dark, evenly distributed and do not form dark spots. The abdomen is brown, with indistinct pale apical bands and long, pale brown setae.

**Male** (Fig. 9.2): The gonocoxite has 3 parabasal setae: the inner one, a single seta tapering and arising on a distinct lobe, the two outer setae located close together and apically branched. The internal seta is inserted on the inner side of the gonocoxite near the apex, and the gonostylus is relatively thick with a short apical spine. The claspettes are made up of three distinct lobes, the outer lobe carries 2-3 lanceolate blades, the middle one 2, and the inner one, 2-3 pointed spines. The aedeagus has 2-3 pairs of leaflets at its apex.

**Larva:** The antenna is about half as long as the head with small spicules on the inner surface, decreasing in size towards the tip, and rarely absent from the distal third. The antennal seta (1-A) is short, with 4-7 branches, and located near the base of the antenna. The inner clypeal seta (2-C) is long, nearly as long as the antenna; the setae are situated close together and are single or sometimes with 2-3 branches at the apex. The outer clypeal seta (3-C) is rarely single, more often with 2-3 apical branches, and the postclypeal seta (4-C) is short and thin, with 2-4, rarely 5 branches

(Fig. 8.11b). The frontal setae (5-C to 7-C) are strongly branched. Dark markings on the frontoclypeus are restricted to spots on the posterior part of it, and not banded as in *An. Algeriensis*. The palmate setae on the abdominal segment II (1-II) have 10-15 leaflets. The palmate setae on abdominal segments III-VII (1-III to 1-VII) have lanceolate leaflets with an elongated apex but without a long filament. The antepalmate setae on segments IV and V (2-IV and 2-V) have 4-5 branches; on the rare occasions when they have 3 branches, all branches are of the same length. The sclerotized, tergal plate on abdominal segment VIII is not as broad as that of the palmate setae on segment VII (1-VII). The saddle seta (1-X) arises just outside the margin of the saddle.

**Biology:** The larvae are found in a wide variety of habitats, but show a preference for clean water and for bodies of water with a more or less permanent character. They are found in cool water in the shade, e.g. weedy pools sheltered by trees or among growth of reeds at the edges of ponds or lakes, where they are often associated with the larvae of *An. atroparvus*, *Cs. annulata*, *Cx. impudicus* and *Cx. theileri*. The larvae are also common in ditches overgrown with weed, in the Mediterranean region they are frequently found in wells and water containers. In mountainous areas the larvae breed among the aquatic vegetation on the margins of small well shaded mountain streams. In the northern range of its distribution, the larvae are often found together with *Cs. morsitans* and *Oc. punctor*. The larvae hibernate, and the adults appear in early spring. They can be found during the summer months until late autumn. In southern England, maximum biting activity of adult females was observed in May and September showing that *An. claviger* is bivoltine in this area (Service 1973b). The females deposit their eggs not directly on the water surface, like other *Anopheles* species, but above the water level into the wet soil. The females die off before the end of the year. Hibernation takes place in the 3rd or 4th larval stage in water bodies that do not entirely freeze. In the southern range of its distribution, the larval development is not interrupted in winter, but is merely very slow at that time. The larvae are very sensitive to disturbance and promptly descend to the bottom of the breeding sites after sensing the slightest movement of the water. *An. claviger s.s.* is a more exophilic species; the adult females do not readily enter houses or stables, but bite in the open, outside villages (exophagy). *An. claviger*

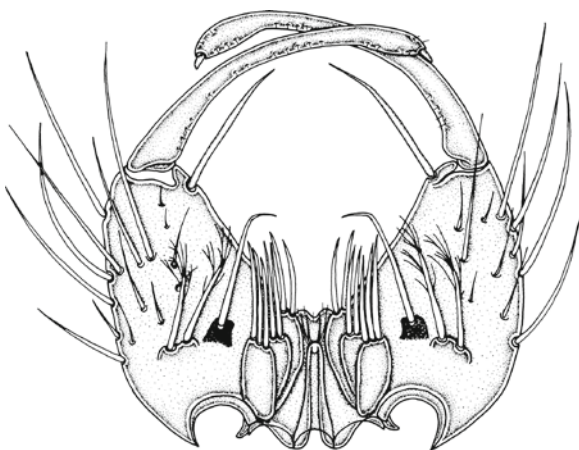


Fig. 9.2 Hypopygium of *An. claviger*

*s.s.* is a zoophilic species, and its preferred hosts are large domestic animals. Sampling carried out in human dwellings and stables revealed that more females of *An. claviger s.s.* fed on humans (3.3%) than was the case for *An. maculipennis s.l.* (2.3%) (Tovornik 1974). Autogenous populations are reported from Italy (Rioux et al. 1975).

**Distribution:** The species is widely distributed in the Palaearctic region. Its northern range runs through central Scandinavia, Estonia and the St. Petersburg area. It is found throughout nearly all of Europe from central Sweden and Norway to northern Africa and in the Caucasus and Crimea area. In the mountains of Central Asia, it is found at an altitude of up to 2,000 m and its range stretches to Iraq, Iran and Pakistan. In the western Mediterranean area it is sympatric with *An. petragrani*, but not as common (Ramos et al. 1978).

**Medical importance:** *An. claviger s.s.* is a potential vector of malaria. Although its epidemiological importance is not significant due to its small populations, it is well known as a principal malaria vector in the eastern Mediterranean region (Postiglione et al. 1973)

**Note on systematics:** *An. claviger* was first described by Meigen in 1804 as *Culex bifurcatus*. After the generic change, the name *An. bifurcatus* was used for a long time, but this usage was inadmissible because *bifurcatus* was a name given earlier by Linnaeus for the males of *Cx. pipiens*.

### ***Anopheles (Anopheles) petragrani* Del Vecchio 1939**

**Female:** *An. petragrani* as a member of the Anopheles Claviger Complex can only be separated from the nominative form by larval and pupal characters. Coluzzi (1960) reported that the adult females have a darker colouration than *An. claviger s.s.*, but this character is of less value for taxonomic separation and the females of the two species are particularly difficult to distinguish.

**Male:** In the male hypopygium, as in the adult females, no distinct differences can be found to distinguish *An. petragrani* from *An. claviger s.s.*

**Larva:** In *An. petragrani*, the postclypeal setae (4-C) are usually single, rarely bifid. The palmate setae of abdominal segment II (1-II) usually have more than 15 leaflets with slightly elongated apices (Fig. 8.12b). The antepalmate setae on abdominal segments IV and

V (2-IV and 2-V) have 2 or 3 branches, and if 3-branched, the median one is shorter than the outer branches. In *An. claviger s.s.*, the postclypeal setae (4-C) are short and thin, with 2-4, rarely 5 branches. The palmate setae on abdominal segment II (1-II) have 10-15 leaflets. The antepalmate setae on segments IV and V (2-IV and 2-V) have 4-5 branches, rarely 3-branched and if so, they are all of the same length.

**Pupa:** A reliable morphological character, which facilitates the separation of the two species, is found in the length of seta 9 on abdominal segments IV and V (9-IV and 9-V). In all anophelines, seta 9 on abdominal segments III-VII is developed into a short, stout, dark spine arising from the extreme posterior corner of the segment. According to Coluzzi (1960), in *An. claviger s.s.* the spine of abdominal segment IV (9-IV) is less than half as long as the spine of abdominal segment V (9-V), whereas 9-IV is more than half as long as 9-V in *An. petragrani*.

**Biology:** The larvae of *An. petragrani* are able to tolerate slightly higher water temperatures than *An. claviger s.s.* In Sardinia they are found from February through July and from October to December in fresh-water rockholes, ditches, drainage canals, and the edges of streams and rivers preferably in shady situations (Marchi and Munstermann 1987). Pires et al. (1982) reported larvae breeding in southern Portugal along shaded mountain streams with submerged green algae at an altitude of 600 m. The water surface was covered with *Lemna* sp. and the larvae were associated with those of *An. atroparvus* and *Cx. impudicus*. Ribeiro et al. (1989) found *An. petragrani* in Portugal from about sea level up to an altitude of 1,750 m, and in central Portugal, 87% of its recorded findings are above 500 m. They also reported a marked host preference of the adult females for livestock such as pigs and cows.

**Distribution:** *An. petragrani* seems to be restricted to the western Mediterranean subregion, where it is largely sympatric with *An. claviger s.s.* It is the prevalent species of the Anopheles Claviger Complex in southern Italy, Sardinia and Corsica, but has not been recorded from east of the Italian peninsula. Its whole range of distribution has not yet been fully clarified.

**Medical importance:** *An. petragrani* seems to be a strongly zoophilic species, which often appears in small numbers only. It apparently plays no role in malaria transmission.

***Anopheles (Anopheles) hyrcanus* (Pallas 1771)**

**Female:** The proboscis is dark brown. The palps are nearly as long as the proboscis, and dark brown with a pale apex and 3 narrow pale rings at the joints of palpomeres II–III, III–IV and IV–V. The clypeus is dark brown with a large tuft of dark scales positioned laterally on each side. The antennae are dark brown, and the basal 5–7 flagellomeres have a few white scales. The vertex has a tuft of anteriorly directed white narrow scales between the eyes. There are white erect scales on the dorsal surface of the occiput and blackish brown upright scales at its sides. The scutum is brown with a stripe of greyish narrow scales in the middle. Often the median stripe is divided by dark longitudinal stripes into 2 or 4 narrow greyish stripes. In addition, a few pale scales are present on the anterior margin of the scutum. The postnotum is light brown, and the pleurites are brown. The legs are brown, but lighter on the ventral surfaces of the femora and the inner surfaces of the tibiae, and the bases of the front femora are distinctly enlarged (Fig. 6.13b). The tarsi are dark brown, and the fore and mid tarsi have white rings at the apices of tarsomeres I–III. Tarsomere IV of the hind tarsi is also pale at the apex (Fig. 6.14a), and entirely pale in var. *pseudopictus* (Fig. 6.14b). Sometimes the white ring extends to parts of the hind tarsomere V. The wing veins are covered with dark and pale scales, forming contrasting dark and pale spots. The dark costal margin of the wing is interrupted by 2 pale areas in the apical half; the more proximal spot includes C and  $R_1$ , and the spot near the apex extends from C to  $R_2$  (Fig. 6.13a). White scales particularly dominate on Cu and A, and sometimes these veins are almost entirely white scaled. The fringe scales of the wing are white at the apex, but otherwise brownish. It has to be mentioned that *An. hyrcanus* exhibits a considerable amount of intra-specific variation in the wing markings. The dark to white area ratio may vary as well as the discreteness and vividness of the spotting. The abdomen is dark with long, dense, brown or golden setae, and sternum VII has a tuft of dark greyish scales at its apical half.

**Male:** Tergum IX has long lateral lobes which are enlarged at their apices. The gonocoxite has 2 parbasal setae, unequal in length, the outer seta being longer than the inner, and neither is inserted in a sclerotized base (Fig. 9.3). The internal seta is inserted near the middle of the gonocoxite, and its length slightly



**Fig. 9.3** Hypopygium of *An. hyrcanus*

exceeds that of the tip of the gonocoxite. The outer lobe of the claspettes has setae fused into a flattened spatula like process, and the inner lobe has 2 single pointed setae. The aedeagus has several pairs of leaflets at the apex. These leaflets are delicate, and about 1/3 the length of the aedeagus.

**Larva:** The larvae of *An. hyrcanus* resemble those of the *Anopheles Maculipennis* Complex, but can easily be distinguished by the characteristics given in the keys. The head is longer than the width. The antennae are straight with small spicules located on the inner aspect and a conspicuous antennal seta (1-A), which is multiple-branched (7–8 branches) and at least half as long as the antenna. It arises about midway on the antennal shaft or slightly below it. The inner clypeal setae (2-C) are situated close together, with short apical branches (Fig. 8.13a). The outer clypeal seta (3-C) is multiple-branched, and dendriform. The frontal setae (5-C to 7-C) are long and plumose. The palmate setae on abdominal segments I and II are rudimentary, but well developed on segments III–VII, with 17–24 leaflets ending in indistinct terminal filaments. The sclerotized tergal plates on the abdominal segments are comparatively broader than the length.

**Biology:** The larvae of *An. hyrcanus* can be usually found in reasonably clean, stagnant, sun-exposed water bodies, rich in aquatic vegetation. They are found especially in rice fields and associated irrigation systems, in swamps and similar locations in the open, e.g. pools, margins of lakes or ditches with grassy edges. Elsewhere, the larvae breed at the edges of slowly moving waters such as grassy streams and canals or road side ditches. They exhibit a tolerance to a slight degree of salinity and can also be found in coastal and inland marshes.

In rice fields in northern Greece, the larvae are associated with *An. sacharovi* and *Cx. modestus*. From the coastal plains of Turkey, Postiglione et al. (1973) reported the presence of larvae in association with those of *An. maculipennis s.l.*, *An. superpictus* and *An. algeriensis*. At a temperature of about 20°C the larval development of *An. hyrcanus* lasts 14–16 days (Senevet and Andarelli 1956). The adults occur in small numbers in late April and May but the size of the population increases towards autumn. *An. hyrcanus* produces 2–4 generations/year. The biting behaviour of the adult females is predominantly exophilic (outdoors), but the degree of exophily is known to differ between places. Usually they rest outdoors in bushes and other dense vegetation during the day time. They rarely enter houses, but are common in cattle sheds or shelters, from where they return into the open after blood feeding. Livestock, or when these are not available as hosts, humans are readily attacked in the open field at dusk or in the night. Occasionally, feeding is observed during the day time in shaded situations. Autogenous populations have been reported from Kirgisia (Rioux et al. 1975).

**Distribution:** In Europe *An. hyrcanus* is widely distributed throughout the northern Mediterranean countries, from Spain, southern France, Italy, and Greece to Turkey. In central Europe it is reported from Hungary (Toth 2003), Slovakia (Halgos and Benkova 2004) and the Czech Republic (Votypka et al. 2008; Sebesta et al. 2009). It is distributed in the Ukraine, southern Russia, southern Kazakhstan, the Caucasus and middle Asia. Together with closely related species it is common in southeast Asia and its eastern distribution range includes China, Japan and Korea.

**Medical importance:** Because of its exophilic behaviour, *An. hyrcanus* has never been regarded as a significant vector of malaria in the Mediterranean region. With regard to the probability of changes in human behaviour (e.g. increased mobility of humans or increase in the number of seasonal workers in the rice and cotton fields), its role as a potential malaria vector should not be ignored.

**Note on systematics:** *An. hyrcanus* is one of the most widespread and common species of the genus *Anopheles*, and certainly one of the most variable. It has an enormous distribution range throughout the Palaearctic from the Atlantic in the west to the Pacific in the East and the Oriental region in the south. Due to its variability, a number of different forms have been

described from different localities as variations or subspecies of the nominative form. Some of these forms from south-east Asia are now considered as distinct species within the *An. hyrcanus* sibling species group, e.g. *An. sinensis* Wiedemann, *An. nigerrimus* Giles, *An. paeditaeniatus* (Leicester) and others (Reid 1953; Harrison 1972). The Palaearctic variations include the form *mesopotamiae* from western Asia with a lighter, diffused colour pattern of the wing caused by more pale scales intermixed on the wing veins and a western Palaearctic form *pseudopictus* with tarsomere IV of the hind legs entirely white, found mainly in southern and southeastern Europe. Glick (1992) treated *An. pseudopictus* as a distinct species being clearly separated from *An. hyrcanus* by its sympatric distribution throughout Turkey, Iran and Afghanistan with apparently no evidence of hybridization. However, Gutsevich (1976) reported a wide variation of *An. hyrcanus* in the extent of the pale ring on the hind legs and found intermediate forms. Because Glick's judgment was solely based on one character of female morphology and no investigations on the morphology, of the male genitalia, the developing stages or cross-mating experiments have been carried out since, the opinion not to treat the European form *pseudopictus* as a distinct species nor a subspecies of *An. hyrcanus* is favored here.

### **Anopheles Maculipennis Complex**

The mosquitoes of the *Anopheles Maculipennis* Complex are the classical example of a "species complex", comprising various sibling species. Before 1925, it was reported that malaria was transmitted by the malaria mosquito "*An. maculipennis*". Further research on the distribution and ecology of this mosquito uncovered considerable irregularities. It was found that the distribution of malaria and the distribution of *An. maculipennis* were not closely correlated. In some areas where individuals of *An. maculipennis* were abundant, the incidence of malaria was low or absent. This situation was characterized as anophelism without malaria (Bates 1940). Furthermore, differences in the biology and behaviour of various populations were discovered. In some regions the larvae were restricted to fresh water, in others, to brackish water; the adult females preferred to feed on humans in some areas and

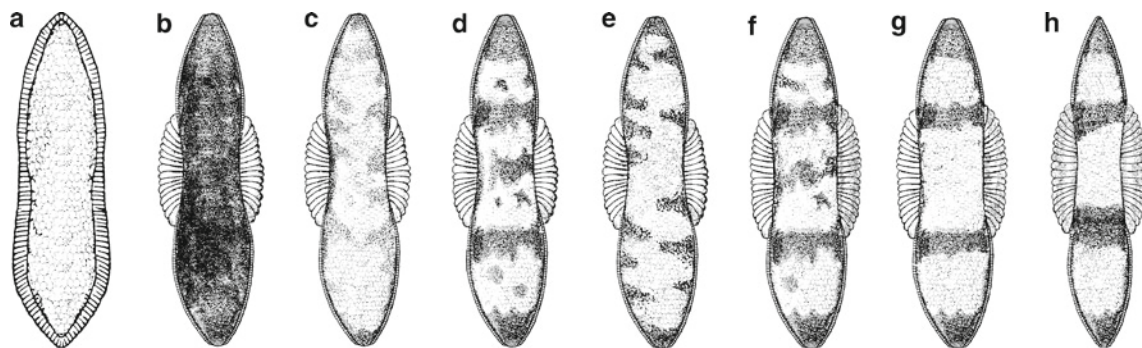
elsewhere they mainly fed on livestock. Exceptional differences in the swarming and mating behaviour (stenogamy, eurygamy) between certain populations were also observed. The first evidence for the existence of a complex of sibling species was brought up by Falleroni (1926), who described distinct morphological differences of the chorion pattern of the eggs in populations with a different biology. Unfortunately, Falleroni's observations lay dormant for five years and were not rediscovered until 1931. Research of Martini et al. (1931), Van Thiel (1933) and Hackett and Missiroli (1935) gave more evidence of the presence of a complex and by the end of the 1930s, most of the present species of the complex were identified (Bates 1940). Studies using cross-breeding experiments, cytotoxic methods or enzyme electrophoresis confirmed the existence of the different species within the complex (Stegnii and Kabanova 1976; Bullini and Coluzzi 1978; Suzzoni-Blatger et al. 1990). The complex comprises of at least a dozen reproductively isolated but morphologically similar species in the northern hemisphere (White 1978) and it can be expected that more members will be found with the use of the above men-

tioned advanced techniques of identification, e.g. *An. daciae* (Nicolescu et al. 2004).

Once the existence of a complex of sibling species was established, some morphological differences between adults and larvae were also found. The females may be separated by the form and shape of scales on certain wing veins, the males differ in the form and length of the spines on the outer claspette lobe and larvae may be distinguished by the overall number of branches of the four antepalpal setae on abdominal segments IV and V (2-IV and 2-V, four setae together). Unfortunately, there exists an intra-specific variation of the characters and overlapping between the species is not uncommon. Therefore, it is necessary to investigate a series of specimens with statistical analysis of the results, which is not applicable for individual identification. Thus the morphological identification is still based largely on characters of the egg (markings of the dorsal exochorion, presence of floats and their size, position and texture). Engorged females can be directly sampled into vials, e.g. from animal shelters, and allowed to lay their eggs for individual identification.

Identification key for European species of the *Anopheles Maculipennis* Complex based on characters of the eggs (after White 1978):

- 1 Egg without floats (but rudimentary floats may develop at low temperatures), egg surface uniformly pale (frosty-white) from pole to pole (Fig. 9.4a) ..... *sacharovi*  
Egg with fully developed floats, egg surface dark, barred or mottled..... 2
- 2 (1) Intercostal membranes of floats (surface of the air cells) smooth..... 3  
Intercostal membranes of floats rough (finely corrugated) ..... 5
- 3 (2) Surface of egg entirely dark (Fig. 9.4b) ..... *melanoon*  
Surface of egg otherwise, barred or mottled ..... 4
- 4 (3) Surface of egg softly patterned with wedge-shaped black marks on a pale background, pale almost at the tip (Fig. 9.4c) ..... *atroparvus*  
Surface of egg with pattern of 2 transverse dark bars near the ends of floats, poles dark and remainder of the surface irregularly mottled (Fig. 9.4d) ..... *subalpinus*
- 5 (2) Surface of egg richly patterned with wedge-shaped dark marks on frosted pale background, tip of poles dark and narrow (Fig. 9.4e) ..... *labranchiae*  
Surface of egg marked with 2 transverse dark bars near the end of the floats, with or without other pattern (mottled background)..... 6
- 6 (5) Transverse dark bars on surface forming part of diffuse mottled pattern (Fig. 9.4f) ..... *messeae*  
Transverse dark bars on upper surface sharply contrasted with unmottled pale background colour..... 7
- 7 (6) Tips of eggs less acutely pointed, chorion of upper egg surface relatively rough, width of egg between floats about 17% of egg length (Fig. 9.4g)..... *maculipennis s.s.*  
Tips of eggs more acutely pointed, chorion of upper egg surface relatively smooth, width of egg between floats about 12% of egg length (Fig. 9.4h)..... *beklemishevi*



**Fig. 9.4** Eggs of *Anopheles Maculipennis* Complex: (a) *sacharovi*, (b) *melanoon*, (c) *atroparvus*, (d) *subalpinus*, (e) *labranchiae*, (f) *messeae*, (g) *maculipennis s.s.*, (h) *beklemishevi*

Morphological characters of adults and larvae shared between the members of the complex:

**Female:** Dark or medium brown in colour, although there is a wide variation in colouration and size. Individuals of southern origin are usually lighter and smaller. The characteristic features, which differ from all other European *Anopheles* species, are the wings with an aggregation of dark scales forming several distinct spots (Fig. 6.8a). The proboscis is dark brown, and the palps are nearly as long as the proboscis, and of the same colour. The antennae are brown coloured. The vertex has a tuft of long, whitish, anteriorly directed narrow scales and setae. The occiput has erect dark brown scales. The scutum has a broad greyish median stripe tapering anteriorly and usually 2-3 indistinct brownish stripes on its anterior half. The lateral parts of the scutum are brown anteriorly and blackish brown posteriorly (for different colouration patterns of *An. sacharovi* see under species description). The antechrostichal patch is made of pale, long and thin scales. The scutellum is brown with golden narrow scales, the postnotum is pale brown, and the pleurites are dark brown. The femora are dark brown on the dorsal side and pale brown on the ventral side, the tibiae are brown but slightly paler at their apices, and the tarsi are dark brown. The wings have dark scales unevenly distributed forming several dark spots close to the cross veins, base of  $R_s$  and furcations of  $R_{2+3}$  and M. The furcations of  $R_{2+3}$  and M are situated at about the same distance from the wing base. The fringe of the wing has a patch of pale scales at the apex of the wing (Fig. 6.9b). *An. sacharovi* has dark spots on the wing veins which are more indistinct and the fringe of the wing is uniformly dark (Fig. 6.9a). The abdomen is brown or

blackish brown with long, golden brown, narrow scales.

**Male** (Fig. 9.5): The gonocoxite has 2 single parabasal setae positioned on small, but distinct tubercles, and the setae are unequal in length, with the outer one being longer. The internal seta is inserted near the middle of the gonocoxite. The gonostylus is well developed, and is longer than the gonocoxite, with a short apical spine. The claspette lobes are not well defined, and have spine-like setae of variable length and shape situated close together but not fused. The aedeagus is long and narrow with leaflets at the apex.

**Larva:** Very variable in pigmentation and size according to their different habitats. Larvae from the northern parts of Europe are usually larger with a darker head capsule. The head is longer than the width, and the antenna is nearly straight, sparsely spiculate, and about 2/3 as long as the head. The antennal seta (1-A) is small, with 4-6 branches arising at the basal



**Fig. 9.5** Hypopygium of *An. maculipennis s.l.*

1/3 to 1/4 of the antennal shaft (Fig. 8.13b). The inner clypeal setae (2-C) are situated close together, with long apical branches. The outer clypeal seta (3-C) is dendriform (Fig. 8.9b). The frontal setae (5-C to 7-C) are long and plumose. The palmate setae on abdominal segments I and II are rudimentary, but well developed on segments III–VII, with 16–24 leaflets which are slightly wider in the middle. The terminal filament is nearly 1/3 as long as the leaflet.

### ***Anopheles (Anopheles) atroparvus* Van Thiel 1927**

**Biology:** The larvae can be found in a variety of stagnant, semi-permanent or permanent, clean breeding sites, both in saline and fresh water, but they show a slight preference for brackish water. They may occur in canals, ditches, marshes in coastal areas, river margins, pools in river beds, rice fields, and are sometimes even found in septic tanks. The water bodies are usually exposed to the sun and carry a considerable amount of filamentous green algae and other floating and submerged vegetation. In their southern distribution range, the larvae are often found together with those of *Cx. theileri*, *Cx. impudicus*, and *Cx. p. pipiens* (Ramos et al. 1978). *An. atroparvus* hibernates as the adult female and usually shows incomplete diapause. After seeking shelter in stables or dwellings in autumn, the females remain active during winter time and may irregularly take blood-meals without subsequent oviposition. This habit mainly contributed to the indoor transmission of winter malaria in Great Britain, Netherlands, and other parts of Europe at the beginning of the twentieth century. The problem disappeared in the late 1940s, due to improved socio-economic conditions. The duration of the diapause depends on the length of day and also on temperature and thus varies with the latitude of the distribution of *An. atroparvus*. It may last from September–April in northern Europe or from November–February in southern Europe. The females are mainly zoophilic and prefer different domestic animals according to their availability in different areas, but also readily feed on humans. They usually rest indoors, predominantly in stables and man-made shelters. Although swarming of the males before mating has been observed on several occasions, it is considered to be a vestigial characteristic, playing only a minor part in the actual mating behaviour of the sexes (Cambournac and Hill 1940). Usually the adults of *An. atroparvus* do not swarm before mating (stenogamy)

and mate almost entirely indoors. Flight ranges of *An. atroparvus* females of at least 3 km have been reported (Cambournac and Hill 1938).

**Distribution:** It is a largely a littoral species occurring from southeastern Sweden to Portugal along the coasts of the Atlantic, Baltic, and Mediterranean Sea. In southern and southeastern Europe it has a patchy distribution, e.g. from northern Italy and inland areas of central Italy through southwestern parts of Russia and the coastal area of the Black Sea. In Serbia and Macedonia it is widespread in the lowlands, but conspicuously dominant only in areas with saline/alkaline soils in the Pannonean Plain (Adamovic 1980). In Portugal it is the most common, most abundant, and most widely distributed *Anopheles* species throughout the country (Ribeiro et al. 1988). *An. atroparvus* was more common in the past, but has a diminished distribution today. In the Netherlands, industrial pollution is considered to be one reason for the decrease of the species abundance (Jetten and Takken 1994).

### ***Anopheles (Anopheles) beklemishevi* Stegnii and Kabanova 1976**

**Biology:** The larvae may occur in breeding sites quite typical for *An. messeae*. Stegnii and Kabanova (1976) reported a finding of larvae in a pond that was heavily polluted with organic material and aquatic vegetation was absent. The larvae could be found only along the edges of the pond. They seem to be more tolerant of the cold than larvae of *An. messeae*, and thus show an adaptation to the continental climate. So far little is known about the biology and the biting behaviour of the females. Many characteristics of this species seem to be similar to those of *An. maculipennis s.s.*, e.g. its zoophily (preference for feeding on animals). The adults usually rest indoors. In northern Sweden, Jaenson et al. (1986a) collected females from livestock shelters. *An. beklemishevi* was found resting in cattle sheds, horse stables, and pigsties without showing a preference for a particular species of domestic animal. It is an eurygamous species, e.g. swarming occurs before mating and the species undergoes complete winter diapause of a duration that is inversely correlated with the photoperiod (White 1978).

**Distribution:** *An. beklemishevi* is endemic to the cooler highlands and northern latitudes of Russia and the Baltics (White 1978). It can be found in Siberia,



northern Sweden and Finland (Korvenkontio et al. 1979; Jaenson et al. 1986a) and is also recorded in several localities in southern Finland (Utrio 1979).

**Note on systematics:** *An. beklemishevi* was the first mosquito species ever to be named and described primarily from cytogenetic evidence, rather than from a morphological description (White 1978).

### ***Anopheles (Anopheles) labranchiae* Falleroni 1926**

**Biology:** Larvae can be found in sunlit, stagnant fresh and brackish water containing horizontal vegetation in coastal areas in Europe and in some inland regions (Jetten and Takken 1994). They may occur in coastal marshes, sheltered edges of flowing streams, rice fields, grassy pools and a number of similar breeding sites. In Europe, larvae of *An. labranchiae* are usually associated with brackish water, where the salinity of the breeding sites can reach up to 10 g/l (Weyer 1939). In Sardinia, where other members of the complex are virtually absent, the larvae are almost entirely limited to fresh water breeding sites. On the island they can be found in ponds, ground-flooded pools, streams, ditches, drainages or canals (Aitken 1954a; Marchi and Munstermann 1987). *An. labranchiae* larvae are better adapted to warmer waters than those of *An. atroparvus*. Hibernation takes place in the adult female stage in dark sheltered situations, e.g. in stables and/or other natural cavities. Diapause may be complete or incomplete, e.g. occasional blood-feeding is possible during the winter months. The females principally feed on humans and they persistently try to enter bedrooms at night (Hackett and Missiroli 1935), but domestic animals may also serve as hosts. *An. labranchiae* is predominantly an endophilic species, indoor resting sites include human dwellings, stables, animal shelters, etc. Occasionally they may be found resting outdoors in various natural shelters, such as tree cavities. Swarming before mating was reported by Horsfall (1955), but in the laboratory adults were found to mate in small cages of 50×50×100 cm (Hackett and Bates 1938). The flight range of the species is considered to be 2–5 km (Senevet and Andarelli 1956).

**Distribution:** *An. labranchiae* has a restricted distribution in southern and southeastern Europe. It has been reported in the south east of Spain, Corsica, coastal areas of Italy, Sardinia, Sicily, and the Dalmatian

coast. In northern Africa, the species occurs in Morocco, Algeria and Tunisia.

### ***Anopheles (Anopheles) maculipennis s.s.* Meigen 1818**

**Biology:** The larvae mainly occur in cold clear waters of upland areas, but they can also be found in plains and coastal zones (Hackett and Missiroli 1935). Typical breeding sites are sheltered areas in running streams, river edges, rice fields, or artificial pools. In mountainous regions in central Europe, the species can be found at altitudes of >1,000 m, and occur there as the only member of the complex. Altitudes of 2,190 m–2,300 m are reported from Bulgaria and Turkey (Bozkov 1966; Postiglione et al. 1973). *An. maculipennis s.s.* shows a better adaptation to breeding in small water bodies without vegetation and artificial collections of water with a wide fluctuation of the daily temperature than *An. messeae* (Mohrig 1969). Therefore, this species seems to be better suited for survival in cultivated land that was widely created in Europe in the past decades through water management campaigns and the use of extensive agriculture techniques. The larvae can frequently be found in waters with a high content of organic matter together with those of *Cx. p. pipiens*. Hibernation of adult females is complete, but it can be of relatively short duration in warmer climates (Senevet and Andarelli 1956). The winter months are usually passed in abandoned buildings without a potential host. The adults normally have little contact with humans, the females are considered to be strongly zoophilic, feeding mainly on cattle (Weyer 1939), but also on pigs and chicken (Tovornik 1980). Nonetheless, in case of a shortage of livestock, *An. maculipennis s.s.* may feed on humans as well, both outdoors and indoors (Barber and Rice 1935). It is usually an endophilic species; the daytime resting sites are stables and dwellings. The adults are eurygamous, usually swarming before mating.

**Distribution:** *An. maculipennis s.s.* is widely distributed throughout Europe. With the exception of the southern parts of the Iberian Peninsula it is recorded in nearly every European country. The range extends eastwards to southwest Asia and the Persian Gulf. It is considered to be more of a continental species with demands for humidity considerably lower than those of *An. messeae* and *An. atroparvus*.

***Anopheles (Anopheles) melanoon* Hackett 1934**

**Biology:** Larvae of *An. melanoon* are typically found in sunlit stagnant water bodies with a large surface area and some vegetation (Jetten and Takken 1994). They may occur in marshes, edges of rivers and lakes, ponds, and ground pools. In Sardinia, larvae can be found in fresh-water ground pools, streams and water collections with a sunny exposure in the springtime and shadier situations during the summer months (Aitken 1954a). Elsewhere, the species is reported to breed in rice fields (Hackett and Missiroli 1935). Hibernation takes place in the adult female stage with complete diapause. The winter quarters are not well known, but may be the same as in other members of the complex, e.g. *An. maculipennis* s.s., *An. messeae* and *An. subalpinus*. *An. melanoon* females reveal a zoophilic preference, they mainly feed on cattle and have only occasionally been recorded as feeding on humans, both indoors and outdoors. Cattle stables are reported as daytime resting shelters (Ribeiro et al. 1980), but others described the species as semi-exophilic (Artemiev 1980). The adults are eurygamous. *An. melanoon* is a rare species with a limited distribution range. So far, it has never been found in the same numbers as other members of the complex.

**Distribution:** *An. melanoon* seems to be confined to southwestern and southern Europe. It has been found in Portugal and Spain (one record each), Corsica, Sardinia, and Italy. More eastern records probably refer to *An. subalpinus*.

***Anopheles (Anopheles) messeae* Falleroni 1926**

**Biology:** The larvae are typically found in cool, fresh, stagnant water bodies with an abundant growth of submerged vegetation. They occur at the edges of rivers and lakes, in swamps, flood plains, ponds and ditches. In central Europe, the larvae are restricted to inland areas and fresh water habitats and avoid breeding sites with a high content of organic matter. *An. messeae* prefers larger water bodies which are available mainly in lowlands with poorly regulated ground water level (Mohrig 1969). It is the predominant species of the *Anopheles Maculipennis* Complex in inundation areas and flood plains of larger rivers, e.g. the Danube, Sava, Rhone or Rhine rivers. It is

rare or almost completely absent along the coastlines and in mountainous regions. Hibernation takes place in the adult female stage, the diapause is complete. The winter months are usually passed in abandoned buildings. The females are essentially zoophilic species feeding almost exclusively on domestic animals; thus contact with humans is largely suppressed in an agricultural area with livestock (Jetten and Takken 1994). Blood meals are taken from humans only when the density of *An. messeae* is very high and there is a shortage of livestock, but they may also attack humans in houses (Barber and Rice 1935; Dahl 1977). Artemiev (1980) described the species as endophilic as it was found resting during the daytime in stables, barns, and cellars as well as in human buildings. The adults are eurygamous.

**Distribution:** *An. messeae* is the most widespread member of the complex. Its range stretches in the northern Palaearctic region from the Atlantic coast in the west, through Scandinavia, across northern and central Europe and Asia as far as into China. The species is virtually absent in southern Europe, it cannot be found on the Iberian peninsula, southern Italy, and in the eastern Mediterranean region. *An. messeae* is regarded as being comparatively more susceptible to high temperature and low humidity than *An. atroparvus* and this behaviour might limit the southern distribution of the species.

***Anopheles (Anopheles) sacharovi* Favre 1903**

Adults of this species are most readily distinguished from the other members of the complex by the lighter colouration of the mesonotum and some wing characters. The pale median stripe on the scutum, characteristic of the other members of the complex is absent. The lateral parts of the scutum are yellowish brown, more or less the same as in the middle part. The scales of the wing fringe are uniformly dark, without a pale patch at the wing apex (Fig. 6.9a). The dark spots on the wings, particularly in males, are less conspicuous than in the other members of the complex and barely distinguishable in old worn out specimens. The eggs of *An. sacharovi* are unique in the lack of the air floats (Fig. 9.4a), but in the south of its distribution range, where development continues throughout the year, eggs deposited in winter may have rudimentary floats. Usually the larvae of *An. sacharovi* are smaller than

the larvae of the other members of the complex, and the outer clypeal setae are relatively longer.

**Biology:** Larvae can be generally found in open, sun exposed shallow water bodies with abundant surface vegetation, both in fresh and saline waters. They are more tolerant to salinity than other members of the complex (e.g. *An. maculipennis s.s.*), and usually occur in coastal swamps and marshes, lagoons and nearby streams, irrigation drains, and roadside ditches, rice fields, grassy ponds, pools, or seepages. In rice fields, the larvae are often found together with those of *Cx. modestus*. The larvae are not very mobile and rarely leave the water surface, but if they do, they return after a short time (Gutsevich and Dubitzky 1987). *An. sacharovi* is the most thermophilic species of the *Anopheles Maculipennis* Complex, and water temperatures of the breeding sites up to 39°C during daytime are not uncommon. Hibernation takes place in the adult female stage, and the individuals preferably seek stables and other animal shelters for overwintering. When the temperatures are suitable, they may take blood meals occasionally during winter. Hibernation starts earlier and lasts longer than in other members of the complex (Martini 1931). In spring, adults are not very abundant, the maximum population is usually reached between July and August. The females predominantly feed on humans but also on cattle when available. Even during the daytime they may be persistent biters in sheltered situations (Postiglione et al. 1973). In some places they become numerous and attack humans in large numbers (Gutsevich and Dubitzky 1987). *An. sacharovi* is usually an endophilic species; adults are frequently found in stables and human dwellings during the day, and leave their daytime shelters during the hours of darkness. Occasionally they can also be found resting outdoors: recorded outdoor shelters include hollows and cavities under bridges and earth banks, hollow trees or rock cavities (Postiglione et al. 1973). Saliternik (1957) caught marked mosquitoes at a distance of 3.5 km from the release point. The flight range of *An. sacharovi* may exceed this distance, if the blood source is far from the breeding places.

**Distribution:** In southern Europe, *An. sacharovi* is mainly a coastal species and distributed in the eastern Mediterranean sub-region. It can be found in Corsica, Sardinia, Sicily, in coastal areas of Italy, Greece, former Yugoslavia and Albania, south of the Balkan peninsula and in the western and southern coastal plains of Turkey. The range extends eastwards from the Near

East through middle Asia, Iran, and Afghanistan to China. In its continental distribution range, *An. sacharovi* is a characteristic species for areas with a dry and hot climate.

### ***Anopheles (Anopheles) subalpinus* Hackett and Lewis 1935**

**Biology:** Larvae can be found in various types of sunlit, stagnant water bodies where aquatic vegetation is present. They occur in swamps, edges of lakes, flooded rice fields, or ponds and avoid shady places and small water collections. Regular breeding sites can be found up to an altitude of about 1200 m (Postiglione et al. 1973). Hibernation of adult females is complete, and caves and abandoned buildings are chosen as winter quarters (Senevet and Andarelli 1956). *An. subalpinus* is regarded as being a strongly zoophilic species, which rarely feeds on humans. Females are endophilic and can be found during the day in large numbers in stables and barns, and very rarely in human dwellings or buildings; the adults are eurygamous.

**Distribution:** *An. subalpinus* is a southern European mosquito with a range from the Iberian Peninsula, through the northern Mediterranean countries to the lowlands around the Caspian Sea.

**Note on systematics:** According to genetic studies and widespread surveys, *An. subalpinus* was formerly regarded as a subspecies of *An. melanoon*. It should merely represent an alternative egg phenotype of *An. melanoon*. The two forms of eggs apparently are intergrading conspecific varieties that occur in pure populations in limited geographical areas (Bates 1940; White 1978). Based on material from Portugal, *An. melanoon* and *An. subalpinus* were treated as two distinct species on account of the differences of their eggs and the apparent area of sympatry of both forms in southwestern Europe (Ribeiro et al. 1980; Ramos et al. 1982). Furthermore, evidence was provided of reproductive isolation between sympatric populations of the two species (Cianchi et al. 1987). Subsequently, regarding *An. subalpinus* as a distinct species of the *Anopheles Maculipennis* Complex was accepted by others (Ward 1992).

**Medical importance of the species of the *Anopheles Maculipennis* Complex:** The anthropophilic species of the complex, such as *An. atroparvus* in coastal Europe, *An. labranchiae* around the west-

ern Mediterranean region and *An. sacharovi* around the eastern Mediterranean region are the three main malaria vectors in the complex. All three species tend to be most prevalent in relation to saltwater habitats. *An. labranchiae* and *An. sacharovi* are important vectors throughout their distribution range. The medical importance of *An. atroparvus* depends on local conditions, e.g. presence of the species in large numbers, or transmission of winter malaria (availability of human hosts in or close to overwintering shelters). The more zoophilic species, e.g. *An. maculipennis s.s.*, *An. beklemishevi*, *An. messeae*, *An. melanoon* and *An. subalpinus* which are usually associated with freshwater habitats, play a minor role in malaria transmission. Nevertheless, at least *An. maculipennis s.s.* and *An. messeae* are responsible for malaria transmission in limited regions of Europe where high incidences of the species and a shortage of domestic animals occur.

**Notes on systematics:** After the detection of a new species within the *Anopheles Maculipennis* Complex with cytotaxonomic techniques by Stegnii and Kabanova (1976) and evidence of the presence of other hitherto unknown members, White (1978) resurrected two members of the complex, previously regarded as synonyms, to specific rank, namely: *An. martinius* Shingarev 1926, with an eastern distribution in central Asia, from synonymy with *An. sacharovi*, and *An. sicaulti* Roubaud 1935, found in northern Africa, from synonymy with *An. labranchiae*. Several North American mosquitoes clearly belong to the *Anopheles Maculipennis* Complex, e.g. *An. aztecus* Hoffmann 1935, *An. earlei* Vargas 1943, *An. freeborni* Aitken 1939, *An. occidentalis* Dyar and Knab 1906.

### ***Anopheles (Anopheles) marteri* Senevet and Prunelle 1927**

**Female:** Resembles *An. claviger* in general appearance, most easily distinguished from it by the whitish apex of the proboscis and a usually present pale apical spot on the fringe of the wing. The proboscis is without pale rings, and its apex is distinctly paler than the brown basal part (Fig. 6.11a). The palps are subequal to the proboscis, with scattered pale scales at the base. The terminal segment of the palps is at least half as long as the penultimate one. The occiput bears a tuft of whitish scales. The scutum has pale scales which form

a median stripe, the sublateral and lateral areas are dark and bare. An antecroscichal patch of pale scales is present. The lateral scutellar setae are longer than the median ones. The prespiracular setae are brown and often absent. The femora, tibiae, and tarsi are blackish brown with a blue metallic shine. The femora and tibiae have a pale scaled apex and the tarsi are without rings. Tarsomere I of the fore leg is distinctly longer than tarsomeres II–V combined. The scales on the wing veins are dense and all dark, and the wings are without spots (Fig. 6.7a). The wing fringe is made of lanceolate scales of unequal length, with a white apical spot.

**Male:** The palps are as long as or slightly shorter than the proboscis. The gonocoxite has two strong parbasal setae, curved at the end, arising from a strongly sclerotized base (Fig. 9.6). The outer seta is longer and thinner than the inner seta. A crescent shaped internal seta is inserted at the apical 1/3 of the gonocoxite, and numerous long thin setae cover the same portion of its dorsal surface. The claspettes have two lobes. The outer lobe bears three flattened, spatula-like setae and the inner lobe has a single, longer, sabre-like seta. The aedeagus has 8-10 or more long leaflets on each side.

**Larva:** The antennae have a dark, spiculate apex. The antennal seta (1-A) has 3-4 moderately long branches inserted in the basal half of the antenna, close to the middle. The frontoclypeus is spotted but not banded. The clypeal setae are all single. The inner clypeal setae (2-C) are closer to each other than to the



**Fig. 9.6** Hypopygium of *An. marteri*

outer (3-C), and more than twice as long as the outer setae. The postclypeal setae (4-C) are of variable length, and as long as the outer clypeal setae (3-C) which extend beyond the anterior margin of the frontoclypeus (frequent character of larvae from Portugal, Greece, Turkey, Iran and Tadjikistan populations), or are short and do not surpass the margin (larvae from Spain, Algeria, Tunisia, Israel and Jordan). The frontal setae (5-C to 7-C) are plumose, with 6-7 pairs of lateral branches. The end of the inner frontal seta (5-C) hardly reaches the base of the postclypeal seta (4-C). The prothoracal seta 1-P is well developed, with a strong shaft, pinnately branched, similar to 2-P and 4-P. Seta 3-P is single, rarely 2-branched. Seta 1-T on the metathorax of palmate type, with 10-11 leaflets. The abdominal seta 1-I is short and spine-like. The palmate setae 1-II to 1-VII are well developed, with 15-19 leaflets, sometimes more. The leaflets are narrowed in 2-3 irregular steps beyond the middle, forming a filament that is at least 1/3 as long as the blade (Fig. 8.12a). The antepalmate setae on abdominal segments IV and V (2-IV and 2-V) are single, rarely with 2 branches. The abdominal setae 6-I to 6-III are pinnately branched.

**Biology:** *An. marteri* is a polycyclic species often found in mountainous regions. In Sardinia and Corsica, the species was collected from sea level up to 1,000 m (Aitken 1954a), and in Tadjikistan from 900 to 1,600 m (Keshishian 1938). Females hibernate in natural shelters. Spring is the preferred season for larval development but a second, autumnal peak has been registered as well (Logan 1953; Senevet and Andarelli 1956). Larvae have been found from March-September in shaded, clear and cool water in rock pools and mountain streams and springs, with rocky bottom and scarce vegetation. They usually breed in acidic water (pH 5.5-6.0) within a temperature range of 15-22°C (Senevet and Andarelli 1956; Ribeiro et al. 1987). The larvae can be found in association with those of *An. atroparvus*, *An. claviger* s.s., *An. petragrani*, *Cx. p. pipiens* and *Cx. territans* (Gutsevich et al. 1974; Ribeiro et al. 1987). *An. marteri* females are sylvatic, exophagic and markedly zoophilic.

**Distribution:** It is a southern Palaearctic species, which is distributed from Portugal and North Morocco to Tadjikistan. In Europe it is recorded in Albania, Bulgaria, Corsica, Greece, Portugal, Sardinia, Sicily, and Spain.

**Medical importance:** According to its strong preference for animal hosts, *An. marteri* is most probably of minor importance as a vector of human diseases (Ribeiro et al. 1988).

**Notes on systematics:** Since 1927 when Senevet and Prunelle described *An. marteri* as a new species, the closely related *An. sogdianus* was described in Tadjikistan (Keshishian 1938) and *An. marteri* var. *conquensis* in Spain (Torres Canamares 1946). Later, *An. sogdianus* was given subspecific status under *An. marteri* after Beklemishev (in Boyd 1949). Ribeiro et al. (1987) stated that *An. marteri* is a polymorphic, monotypic species, and that the names *sogdianus* and *conquensis* were only the morphs that have to be treated as junior synonyms of *marteri*. The authors hypothesize that the clinical distributions of the morphs is temperature dependent, with a January isotherm of +10°C separating *marteri/conquensis* in the south from *sogdianus* in the north. The proposed status was accepted by Ward (1992).

#### ***Anopheles (Anopheles) plumbeus* Stephens 1828**

**Female:** *An. plumbeus* can be distinguished from the similar *An. claviger* by its smaller size and its general darker, leaden colouration. The pleurites of the thorax and the lateral parts of the scutum are blackish brown, forming a distinct contrast to the pale or ashy-grey median part of the scutum. Furthermore, the wings are more densely scaled and darker in appearance than those of *An. claviger*. The proboscis and palps are black, with the palps being approximately the same length as the proboscis with the apical segment more than half as long as the penultimate one. The pedicel is brown, and the flagellum is blackish brown with black setae. The vertex has a tuft of narrow, pure white scales, which is directed anteriorly, and yellowish longer setae. The occiput is covered in its median part with whitish lanceolate and erect forked scales, laterally with black erect forked scales. Lateral parts of the scutum are blackish brown, with a median longitudinal grey stripe which covers at least 1/3 of the width of the scutum. The anterior margin of the scutum has a well developed anteachrostichal tuft of pure white, narrow scales (Fig. 6.12a). The scutellum is brown with dark setae, its posterior margin is slightly concave at the sides, and the postnotum is dark brown. The pleurites

are blackish brown with 5-6 prespiracular setae. The legs are black or blackish brown, the coxae and ventral surfaces of the tibiae are slightly paler. The wings are densely covered with dark brown, lanceolate scales and not spotted; the cross veins are well separated. The abdomen is black, and covered with pale brown or dark setae with a golden tinge.

**Male** (Fig. 9.7): The gonocoxite bears 2 two strong parabasal setae of approximately the same length. They arise directly from the surface of the gonocoxite, not from a tubercle. The internal setae are inserted near the middle of the gonocoxite. The apical spine of the gonostylus is short. The claspettes are divided into two lobes, the outer lobe bears three setae, which might be slightly flattened and situated close together but are not fused. The inner lobe bears one short hair-like seta and two to three spine-like setae of variable length, at least one of which is slightly bent at the apex. The aedeagus is short and broad, without spines or leaflets.

**Larva:** Larvae of *An. plumbeus* are at once distinguished from all other European species of the genus *Anopheles* by the frontal setae (5-C to 7-C) which are reduced in size and are single (Fig. 8.7b). The head is more or less oval, uniformly dark brown, and the primordial compound eyes are weakly developed. The antennae are approximately 1/3 of the length of the head, straight and smooth. The antennal seta (1-A) is very short and single, situated close to the base. The clypeal setae (2-C and 3-C) are thin and sparsely

branched. The distance between the pair of inner clypeal setae (2-C) is smaller or almost the same as the distance between the inner (2-C) and outer clypeal setae (3-C). The postclypeal setae (4-C) are short and single, and situated wide apart. The distance between them is larger than the distance between the outer clypeal setae (3-C). Seta 1 of abdominal segment I (1-I) is short and single. The palmate setae on abdominal segments II–VI (1-II to 1-VI) are conspicuous, but inconspicuous or rudimentary on segment VII. Each palmate seta consists of 14–15 lanceolate leaflets with a pointed apex, but without a terminal filament, their margin may be slightly serrated in the apical half. The lateral setae on segments I–VI (6-I to 6-VI) are large and pinnately branched. Each abdominal segment ventrally carries two pairs of stellate setae. The pecten plate is usually composed of teeth of more or less the same length. The saddle is covered with numerous spicules. The ventral brush has 17–19 fan-like setae arising from a common base. The anal papillae are shorter than the saddle.

**Biology:** Larvae of *An. plumbeus* develop almost exclusively in tree-holes. The breeding water is usually dark brown owing to the dissolved tannins and pigments derived from the wood and has a high concentration of salts in combination with a deficiency of oxygen (Mohrig 1969). The larvae are ordinarily found in tree-holes in beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*), elm (*Ulmus* sp.), sycamore (*Acer pseudoplatanus*), lime (*Tilia* sp.), oak (*Quercus* sp.), birch (*Betula* sp.), horsechestnut (*Aesculus hippocastanum*) and others, often together with larvae of *Oc. geniculatus*. Further associates may be larvae of *Or. pulcripalpis*, *Oc. berlandi*, *Oc. Echinus*, and *Oc. pulcritarsis*. The eggs of *An. plumbeus* are not laid on the water surface but on the side of the tree-hole and hatching occurs when the hole is flooded. Thus, the number of generations per year depends on the hydrological situation. Hibernation takes place in the egg or larval stage. The larvae which hatch in autumn usually grow into the second and third-instar by the end of the year, but do not pupate until the next spring. They spend most of the time at the bottom of the hole and can survive long periods when the water surface is frozen, but high mortality can be observed when the breeding water and the mud at the bottom are entirely frozen during a long period (Mohrig 1969). In spring, a major proportion of the larvae hatch from hibernated eggs. Occasionally, especially in periods of drought, larvae of *An. plumbeus* may also occur in artificial containers, rock holes, or in ground depressions in shaded situations

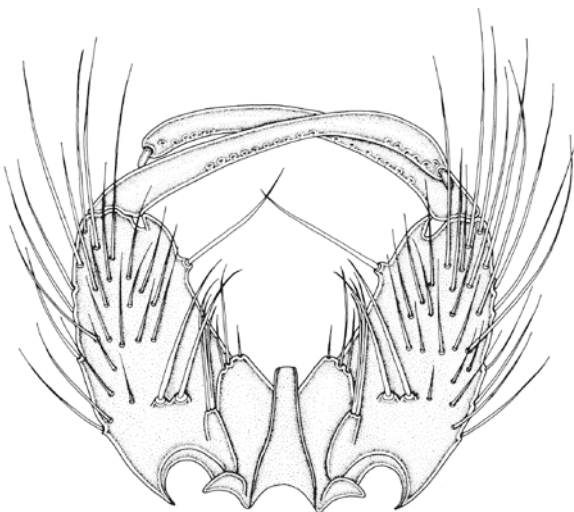


Fig. 9.7 Hypopygium of *An. plumbeus*

containing a rich infusion of fallen leaves (Aitken 1954a). In central Europe the adults usually occur from late spring on and are present until the end of September. They can be found from sea level up to an altitude of 1,200 m (Senevet and Andarelli 1956), and in the southern part of its range the species occurs in forests and in mountainous areas up to altitudes of 1,600–2,000 m (Gutsevich et al. 1974). Females are persistent biters and they are most active during the crepuscular period, feeding principally on mammalian blood, including that of humans (Service 1971a). Occasionally they have been observed to attack humans during the day in shaded situations along forest edges. Some populations have shown a strong anthropophilic preference (Petric 1989). Because of its preferred larval habitats, *An. plumbeus* is mainly found in forests and rural areas, but considerable populations may also be found in urban situations, where the larvae develop in tree-holes in gardens or parks. However, it has adapted its breeding habit to widely available artificial breeding sites below the ground, such as catch basins and septic tanks with water contaminated with organic waste. Therefore, in central Europe *An. plumbeus* has increased in numbers during the last decades and can be a major nuisance species in human dwellings especially when unused septic tanks support mass breeding.

**Distribution:** *An. plumbeus* is widely distributed throughout Europe wherever there are deciduous trees in which rot holes can be found. It is also distributed in the northern Caucasus, in the Middle East south to Iran and Iraq and in North Africa. A similar species which is found in India and Pakistan is considered by most experts to be the species *An. barianensis* James, but Gutsevich et al. (1974) pointed out that there are no distinct differences between *An. plumbeus* and *An. barianensis*. In North America, *An. plumbeus* is replaced by an allied species, *An. barberi* Coquillett, which closely resembles it (Marshall 1938).

**Medical importance:** Although laboratory studies have shown that *An. plumbeus* can successfully be infected with *P. vivax* and *P. falciparum* (Weyer 1939; Marchant et al. 1998) and that the species is an efficient carrier of malaria, it is considered to be of minor epidemiological importance at the present time because of its ecology. In the past, *An. plumbeus* played a major role as a vector in forests in Caucasian resorts (Gutsevich et al. 1974) and probably has been responsible for two recorded cases of locally transmitted malaria in London, UK (Shute 1954).

### 9.1.2 Subgenus *Cellia* Theobald

Members of this subgenus are characterized as follows: In the adults, the costa (C) has four or more pale spots and the cross vein areas and furcations of  $R_{2+3}$  and M are pale scaled. In males the base of the gonocoxite bears 4–7, usually 6, parbasal setae, situated close together and not arising from distinct tubercles. The internal seta is absent. The larvae of the subgenus *Cellia* have a single and small antennal seta (1-A) which is situated on the outer side of the antennal shaft. The inner clypeal setae (2-C) are wide apart, situated closer to the outer clypeal setae (3-C) than to each other. The leaflets of the palmate setae are not lanceolate, but always abruptly narrowed or shouldered, thus divided into a blade and a terminal filament. The subgenus *Cellia* is mainly distributed in the Oriental and Ethiopian regions and is not found in the Nearctic. In Europe, the distribution range of the subgenus is confined to the Mediterranean region, where three species and one subspecies of *An. cinereus* can be found. Therefore, *An. cinereus* is described here, despite the complicated situation of its real distribution.

#### *Anopheles (Cellia) cinereus* Theobald 1901

**Female:** The proboscis and palps are extraordinarily long and slender. The palps commonly have four pale rings. The basal three rings are broad, subequal, and extend to both segments at the articulations of palpomeres II–III, III–IV and IV–V. The distalmost ring is often very narrow, sometimes indistinct or absent. When present and well developed, it occupies the apical third of palpomere V. Palpomeres IV and V are long and when combined, distinctly longer than palpomere III. Glick (1992) described the palps of *An. cinereus* as having three rings and a dark apex which is usually true for *ssp. hispaniola*. The first flagellomere is speckled with white scales. The occiput has a well developed tuft of white scales. The scutum has a median stripe of long, usually narrow pale scales, and the submedian and lateral areas are devoid of scales. The femora and tibia are dark with distinct pale spots on the femorotibial and tibiotarsal articulations. The fore and mid tarsi are entirely dark or have very narrow pale apical rings, but usually only on tarsomere I. The hind tarsi have very

narrow white apical rings on tarsomeres I–IV. Tarsomere V is entirely dark scaled. The costa has five large dark spots, and its very base is dark scaled.  $R_{4+5}$  are mainly pale scaled, dark scaling of cubitus (Cu) is quite variable, and the anal vein (A) usually has three dark spots with no pale fringe spot at its end.

**Male:** Palpomeres IV and V are very long; their combined lengths exceed that of palpomere III. The palps have 3–4 pale rings, the basalmost ring is sometimes indistinct, and the apicalmost ring is distinct. The gonocoxite has 3–6 subequal parabasal setae. The claspette lobe is undivided, and the inner seta is nearly twice as long as the lobe. Two additional outer setae may be present. The aedeagus has 7–10 pairs of long leaflets, some of them distinctly broadened and serrated. The longest leaflet is almost half as long as the aedeagus.

**Larva:** The frontoclypeus is marked with dark spots of variable size and shape. The antenna is sparsely spiculate. The antennal seta (1-A) is single, and inserted at about 1/3 the length of the shaft, nearly as long as the width of the antenna at the point of its insertion. The clypeal setae are all single. The inner clypeal setae (2-C) are closer to the outer (3-C) than to each other, and 1.5–2.0 times as long as the outer setae. The length of the postclypeal setae (4-C) is variable, often subequal to the outer clypeal setae (3-C). The frontal setae (5-C to 7-C) are strong and pinnately branched (3–5 pairs of branches are evenly distributed over the entire main stem). The prothoracal seta 1-P is well developed, pinnately branched, and similar to 2-P and 4-P but distinctly shorter. Seta 3-P is short and single. The abdominal seta 1-I is short, variable in shape, and bifurcated or plumose. The palmate setae on abdominal segment II (1-II) are small, with 6–9 leaflets. The palmate setae on abdominal segments III–VII (1-III to 1-VII) have 13–15 leaflets abruptly narrowed in one or several irregular steps forming a filament which is about half as long as the blade (Fig. 8.15d). The abdominal setae 6-IV to 6-VI are pinnately branched.

**Biology:** *An. cinereus* is a polycyclic species. The most preferred breeding sites are edges of swamps, streams, irrigation ditches, rock pools and marshy pools. The larvae prefer moderately shaded water bodies with a slightly alkaline pH ranging from 7.7 to 8.3 (Evans 1938). The females are zoophilic and frequently enter shelters of domestic animals. Occasionally they can be encountered inside human dwellings.

**Distribution:** Eastern Afrotropical region from Sudan and Ethiopia to South Africa, Arabian Peninsula

***Anopheles (Cellia) cinereus hispaniola*  
(Theobald 1903)**

**Female:** Similar to the nominative form in all stages, but easily distinguished from the other European members of the subgenus *Cellia*. It differs from *An. multicolor* by having a dark base of costa (C) (Fig. 6.17b) and a bare submedian scutal area, from *An. sergentii* by having a mainly pale scaled  $R_{4+5}$  and from *An. superpictus* by having the apex of the palps dark and three relatively short dark spots on the anal vein (A). The palps usually have three pale rings, on both sides of articulations between palpomeres II–III, III–IV and IV–V, with a dark apex. Rarely, the very tip may be white but the middle of palpomere V remains dark scaled. The hind tarsomeres have indistinct pale apical rings, sometimes reduced to a few scattered white scales. Other characters are similar to *An. cinereus*.

**Male (Fig. 9.8):** The gonocoxite has 4–7 (usually 6) short parabasal setae of similar size and length. The inner seta on the claspette lobe is about as long as the lobe, and one or two shorter median setae may be present. Several outer setae are fused to form a conspicuous broad spatula-like process, which is distinctly shorter than the inner seta. The aedeagus has 7–8 pairs of long, broad weakly serrated leaflets.



**Fig. 9.8** Hypopygium of *An. cinereus hispaniola*



**Larva:** The frontal setae (5-C to 7-C) are poorly developed, with a few branches arising near the base of the main stem. The inner frontal seta (5-C) is slightly longer than the median frontal seta (6-C). The lateral abdominal setae 6-I to 6-V are pinnately branched, weakly pinnated on segments IV and V.

**Biology:** A polycyclic, orophilic (preference for higher altitudes) species, which can be found from sea level to 2,334 m (Logan 1953). The larvae breed on hilly grounds, most numerous from mid summer on in the mountains of Sardinia (Aitken 1954a). The preferred breeding sites are sandy and gravelly edges of streams in shallow, clear water, but larvae can also be found in irrigation ditches and swamps (Senevet and Andarelli 1956). According to the same authors, the larvae were found in waters with salinity ranging from 0 to 2.9% and a pH value ranging from 5.0 to 7.0. When disturbed, the larvae may remain below the surface of the water for one hour or more (Aitken 1954a). Often they breed in association with larvae of *An. labranichiae*. The females express zoophily and exophily, but readily bite humans in the open (Ribeiro et al. 1988).

**Distribution:** Mediterranean region, North Africa, French Equatorial Africa, Sinai Peninsula and Transjordan (Knight and Stone 1977; Ribeiro et al. 1980). In Europe, the species is registered in Portugal, Spain, Italy and Greece.

**Medical importance:** The species is a potential vector of malaria in southern Europe (MacDonald 1957). However, owing to its exophilic and exophagic behaviour it is regarded as a vector of minor importance (Jetten and Takken 1994).

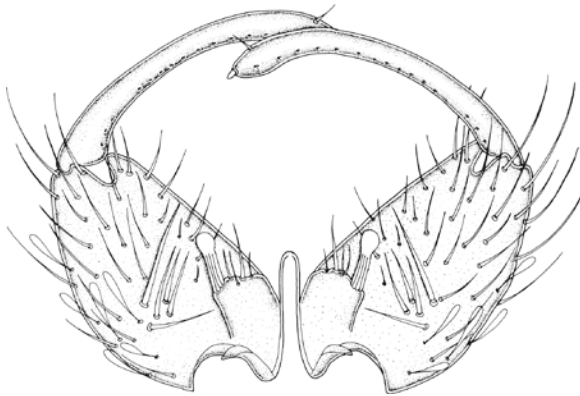
**Notes on systematics:** The status of the name *hispaniola* is still rather undefined. Knight (1978) and Knight and Stone (1977) treated both *An. cinereus* and *An. hispaniola* as valid species of the subgenus *Cellia* Theobald. The status of *An. hispaniola* was changed to synonymy of *An. cinereus* after Dahl and White (1978) with no further explanation, which was acknowledged by others (Ward 1984). Finally, the status of *hispaniola* was changed from species (former change to synonymy ignored) to subspecies of *An. cinereus* (Ward 1992) following Ribeiro et al. (1980). The authors stated that owing to a very low degree of morphological differences and absence of information concerning hybridisation between the forms, it is most advisable to treat *hispaniola* as a subspecies. Nevertheless, Ramsdale (1998) stated that the name *hispaniola* should be regarded as a junior primary synonym of *cinereus*, until further evidence is given.

### ***Anopheles (Cellia) multicolor* Cambouliu 1902**

**Female:** The proboscis is dark brown, and the palps are nearly as long as the proboscis with three pale rings at the joints of palpomeres II–III, III–IV and IV–V. The proximal ring is very narrow with pale scales mainly at the apex of palpomere II and sometimes with a few scales at the base of palpomere III. The median and distal rings are broader than the proximal ring. Half of palpomere V is dark. The clypeus is light brown, the flagellum brown, and the pedicel and the first 3–4 flagellomeres have a few pale scales. The vertex has long pale scales and setae forming a frontal tuft, the occiput in the upper half has pale scales, and dark scaled at the sides. The integument of the scutum is brown, the lateral areas somewhat darker than the median. The submedian and lateral areas are covered with narrow creamy scales, and the setae on the scutum are black. The scutellum is light brown, the pleurites brown with some pale scales, and about 3–5 prespiracular setae. The tibiae are prominently pale apically, the tarsi minutely pale apically, with pale scales not forming definite pale rings, and the hind tarsi may be completely dark. The wing veins are covered with narrow to moderately broad scales, and the cross veins are well separated. The wing is extensively pale scaled, and not well ornamented due to the paleness of the dark scales. The costa (C) is largely pale, with dark areas reduced in some specimens, and pale at the extreme base (Fig. 6.17a). The anal vein (A) is mainly pale with three small dark spots. There are pale fringe spots at the ends of all the veins except the anal vein (A). The abdomen is brown, entirely devoid of scales, but with dark setae, and the first abdominal segment is more densely covered with setae.

**Male** (Fig. 9.9): The gonocoxite is slightly longer than broad. The base of the gonocoxite has 5 rather slender parabasal setae. The outermost seta is long and straight, arising from slightly above the other setae. The claspette is not clearly divided into distinct lobes, has a long and slender spine-like seta, several smaller setae and a broad, spatula-like seta situated at the outer border. The aedeagus is distinctly shorter than half the length of the gonocoxite, and leaflets and spines of the aedeagus are absent.

**Larva:** The larva of *An. multicolor* closely resembles that of *An. superpictus*. The antenna has numerous spicules along the inner border, and the antennal seta (1-A) is usually situated close to the base of the



**Fig. 9.9** Hypopygium of *An. multicolor*

shaft and is single. The apex of seta 1-A is sometimes divided into 2 or 3 extremely fine branches. The inner clypeal setae (2-C) have widely separated bases. Both inner and outer clypeal setae (2-C and 3-C) are single (Fig. 8.16a), the outer seta is about  $2/3$  the length of the inner seta. The postclypeal setae (4-C) are single, as long as or longer than the outer clypeals, with their distal ends reaching beyond the bases of the clypeal setae. The frontal setae are pinnately branched, and the inner frontals (5-C) are usually distinctly longer than the median frontals (6-C) (Fig. 8.14b). The palmate setae are undeveloped or rudimentary on abdominal segments I and II (1-I and 1-II), and variably developed on segments III–VII, or sometimes undifferentiated. If fully developed, the leaflets are more or less uniformly pigmented and narrow. The shoulder is well marked, with a long filament about  $2/3$  the length of the blade, rather broad at the base and sharply pointed distally. The pecten plate has 7–8 long teeth usually alternating with 1–2 short teeth, finely serrated on the basal half. The arrangement is sometimes regular, but often irregular. The sclerotized tergal plates are small, and on abdominal segment V the width of the plate is about  $1/3$  of the distance between the pair of palmate setae (1-V). A small, single accessory plate is present near the centre of segment V. The saddle seta (1-X) is long and single, and the anal papillae are very short.

**Biology:** The larvae are generally to be found in inland or coastal breeding places in semi-arid regions in brackish and fresh water, but they show a preference for saline desert waters. They occur in salt pans, oases, small pools with or without aquatic vegetation, and sometimes in unused shallow wells. They are never found in rice fields (Christophers 1933). The larvae are

able to tolerate a high content of salinity, which may occasionally reach the point of saturation. In North Africa, they were found in association with larvae of *Oc. caspius*, *Cs. longiareolata* and *An. cinereus hispaniola* (Senevet and Andarelli 1956). The authors reported larval findings throughout the year with two peaks, the first between April and July and a second peak from November to December. Although *An. multicolor* has a wide distribution range, very little is known about the adult behaviour. The females are considered to feed on humans. In Egypt they readily enter houses and bite at night (Kirkpatrick 1925). The same author reported considerable flight ranges; adults have been found up to 13 km from the nearest possible breeding places.

**Distribution:** *An. multicolor* is mainly a desert species distributed in North Africa and much of Southwest Asia. Its range extends from Cyprus, Egypt, Palestine and Anatolia eastwards to West Pakistan. It has been reported in Spain in the province of Murcia and the Canary Islands (Romeo Viamonte 1950).

**Medical importance:** The role of *An. multicolor* as a vector of malaria is still uncertain. Several authors have considered it to be an important vector on epidemiological grounds, but no naturally infected females have been found. Other authors regard its role as doubtful even in areas of mass breeding (Gillies and de Meillon 1968).

### ***Anopheles (Cellia) sergentii* (Theobald 1907)**

**Female:** Resembling *An. superpictus*, but differs from the latter by its smaller size and the wing ornamentation. The proboscis is dark brown, long and slender. The palps are nearly as long as the proboscis, of uniform thickness from the apex to the base, and the last palpomere is very short and entirely pale scaled (Fig. 6.15a). In addition to the pale tip of the palps, two more narrow, but well marked pale rings are present at the joints of palpomeres II–III and III–IV. The clypeus is dark brown. The pedicel is dark brown, and without scales. The flagellum is brown with black setae and some narrow pale scales on the first and often on the succeeding flagellomeres. The vertex has pale lanceolate scales, which are directing forwards, and the frontal tuft is not well developed but is distinct. The occiput in the upper part has pale forked scales and at the sides has dark forked scales and setae. The eyes are bordered with broad scales. The scutum is brown, the lateral

areas and transverse suture are somewhat darker than the median area, but not distinct and varies between the specimens. The scutum is covered with lanceolate hair-like scales and dark setae. The scutellum is brown with dark setae. The pleurites are entirely devoid of scales, with 2 prespiracular setae, and 8 upper mesepimeral setae. The coxae are without scales, the femora are uniformly dark except for pale markings at the tips of the femora and tibiae of all the legs. The tarsi are entirely dark, without pale rings. The wing has predominating dark scales on veins  $R_s$  to Cu, and vein  $R_{4+5}$  is usually almost entirely dark scaled, but may show an indistinct pale area about the middle. The extreme base of the radius (R) is entirely pale. Pale spots are also present at the furcations of  $R_{2+3}$  and M, at the cross veins, at the bases of Cu and A and at the middle of  $Cu_2$ . The wing fringe is well marked with pale spots at the ends of all veins except the anal vein (A) (Fig. 6.16a). The abdomen has no scales, is blackish brown, and covered with light setae.

**Male** (Fig. 9.10): The gonocoxite is slightly longer than it is broad. The base of the gonocoxite has 4-5 parbasal setae of varying length irregularly scattered over the surface and sometimes slightly curved at their apices. The claspette is not divided into lobes, and presents a massive unit, with a spatula-like seta on the outer border and a stout spine-like seta of similar length on the inner border. Between them is a second spine-like seta of half the length of the others. The aedeagus is long and slender, with 3-5 pairs of more or less broad leaflets. The longest leaflet is distinctly lon-

ger than the second longest, and all the broad leaflets are serrated through most of their length.

**Larva:** The antenna is covered with spicules along its inner border, and the antennal seta (1-A) is inserted at the basal 1/3 of the antennal shaft, and is short and single. The clypeal setae are usually single, rarely do the outer clypeals (3-C) have 2 branches. 3-C is slightly longer than half the length of the inner clypeals (2-C). The bases of the inner clypeal setae are not that much separated as in the other members of the subgenus *Cellia*, but the distance between them is nearly twice the distance between the inner and outer clypeals. The postclypeal setae (4-C) are usually single, about the same length as the outer clypeals, with their distal ends reaching well beyond the bases of 2-C and 3-C. Occasionally 4-C may be divided into 2-4 branches. The frontal setae (5-C to 7-C) are pinnately branched, and the inner frontals (5-C) are slightly longer than the median frontals (6-C) (Fig. 8.14a). The typical palmate setae are well developed on abdominal segments III-VII, with 17-18 large leaflets, which are more or less uniformly pigmented. The filament is long, slender and pointed, and more than half the length of the blade (Fig. 8.15b). The sclerotized tergal plates are broad, nearly as broad as the distance between the palmate setae on the first five abdominal segments and broader than the distance between the palmate setae on segments VI-VIII (Fig. 8.15a). The last tergal plate occupies more than 1/3 of segment VIII. The pecten plate has 6-8 long and 4-8 short spine-like teeth. The saddle seta is long and single.

**Biology:** The breeding places of *An. sergentii* are quite variable. Larvae can be found in rice fields, irrigation channels, slow moving streams, small pools in river beds or ponds with a rich aquatic vegetation, and grassy swamps from overrunning irrigation ditches or streams. In the Canary Islands, it was found in pools in ravines, especially in small rock pockets (Christophers 1929). *An. sergentii* often occurs together with larvae of *An. sacharovi* and *An. superpictus*, and other associates are *An. multicolor*, *Cx. p. pipiens* and *Cs. longiareolata* (Senevet and Andarelli 1956). Adults are rarely found from spring to early summer and are most prevalent in late summer and autumn (Martini 1931). Hibernation may take place in both the larval and adult stage. Adult females commonly enter any kind of habitation, including houses, and readily bite humans soon after dark, with the main activity period being during the night. They appear to be moderate flyers, and have been found in large numbers 2.5 km from the nearest possible breeding place (Christophers 1933).



Fig. 9.10 Hypopygium of *An. sergentii*

**Distribution:** *An. sergentii* has a wide distribution in the southern Mediterranean region. Its range stretches from North Africa, through the Middle East to Pakistan. It is recorded in the Canary Islands (Romeo Viamonte 1950) and in Europe in Sicily (Coluzzi and Sabatini 1995). Senevet and Andarelli (1956) reported a doubtful record in Bulgaria.

**Medical importance:** Although natural infections were rarely found, the species is considered as a potential vector of malaria on epidemiological grounds. In Palestine it was regarded as the principal malarial vector (Saliternik 1955).

### *Anopheles (Cellia) superpictus* Grassi 1899

**Female:** A variable species, especially in the scaling of the palps, with whitish scales on the wings and legs. The four white scale patches on the costa (C) and subcosta (Sc) may be of variable length. The proboscis is dark, palpomeres III and IV have basal and apical white rings, and palpomere V is nearly completely covered with white scales. The vertex is dark, and the occiput has light, erect scales and an anterior patch of flat white scales. The scutum has a median stripe of pale greyish scales, but is otherwise dark, and the scutellum has some white scales. The pleurites are dark with some greyish tinge, pale setae, and no distinct patches of scales. The costa (C) and subcosta (Sc) have four white areas, somewhat variable in length. Predominant pale scales are present on veins  $R_s$  to Cu, and vein  $R_{4+5}$  is usually almost entirely pale scaled. The pale spot at the apex of the wing fringe is large (Fig. 6.16b). Because of the numerous pale scales the whole wing has a light impression. The legs are dark, the coxae are without scales, the femora have a lighter ventral surface, the knee spot is indistinct or absent and the tarsomeres have some white basal scales, sometimes forming indistinct rings. The abdomen has long yellowish setae.

**Male** (Fig. 9.11): The gonocoxite is rounded and short with very few scales and five strong parabasal setae, the two innermost setae are the shortest and the outermost one the longest. The gonostylus is long, bent at the apex, with a small apical spine. The claspette is not divided into lobes, but is broad, bearing several setae. The outer seta is spatula-like, the inner seta is long and spine-like. The aedeagus is short, less than



**Fig. 9.11** Hypopygium of *An. superpictus*

half as long as the gonocoxite, and bearing several very short leaflets (Fig. 7.13a).

**Larva:** The inner clypeal seta (2-C) has short branches (Fig. 8.16b), and the outer clypeal seta (3-C) is single and about half as long as 2-C. The inner frontal seta (5-C) is distinctly longer than the median frontal seta (6-C). Typical palmate setae on the metathorax are present (1-T). The palmate setae on abdominal segments II–VII (1-II to 1-VII) have 13–19 leaflets, with well developed, serrated and pointed filaments. The pecten plate has 4 long strong and 5 smaller teeth.

**Biology:** The larvae are found in slow running, warm and not polluted waters, e.g. in pools at the edges of rivers or in irrigated rice fields. They also occur in shallow pools in dry river beds (Gutsevich et al. 1974). They are able to tolerate some amount of salinity but avoid eutrophic and muddy waters. They are found in smaller numbers early in the summer (first generation) and can occur in great numbers in autumn (further generations). The larval development may be rapid in high temperatures but usually takes nearly a month. The females may survive a cold climate, and can remain active throughout the cold season. They bite humans and animals, and biting activity reaches its peak at sunset. They are both endo- and exophilic.

**Distribution:** In Europe the species is distributed in the Mediterranean region and occurs also in adjacent areas in Asia Minor and northern Africa. It has a southern

Palearctic distribution throughout Transcaucasia and middle Asia and is recorded as far as Pakistan and north-western India.

**Medical importance:** The species is reported as being an important vector of malaria in middle Asia (Gutsevich et al. 1974).

**Note on systematics:** Knight and Stone (1977) listed seven subspecies, described from Greece, Tashkent, and Baluchistan. There are two more subspecies mentioned in the European literature (Martini 1931; Peus 1967). Thus the variability of the species needs to be further investigated.

## Chapter 10

### Subfamily Culicinae

Most of the mosquito species of the world belong to this subfamily, which is subdivided into 11 tribes. The adults exhibit a high morphological variability ranging from species with little scaling to those with explicit patterns of scales of different colours, from white to black and even a metallic appearance, as well as from small to large species. Many species have prominent patterns of scales and setae on the scutum. The scutellum is trilobed with setae grouped on the lobes, except in Toxorhynchitini, which have an evenly rounded scutellum. The short female palps and the trilobed scutellum clearly distinguish the Culicinae from the Anophelinae. The legs are often scaled in a characteristic pattern and the claws in some tribes have a subbasal tooth which can be species specific. The wings are often broader than those of Anophelinae and have the cross veins r-m and m-cu well expressed. They have usually three spermathecae (receptaculum seminalis). The structure of the male hypopygium, especially the aedeagal apparatus is often more complex and in some tribes the gonocoxite may be enriched with well developed lobes. The head capsule of the larvae is more or less squared or rounded, and the antennae are of variable length. The larval thorax and abdomen are ornamented with long but less spiculate setae and lack, in the European species, palmate setae. The elongated siphon with the plate-like, or in Mansoniini piercing, spiracular apparatus distinguishes the larvae of the Culicinae from those of the Anophelinae. The pupae have long trumpets; their openings being less wide than they are in Anophelinae. The chorionic pattern of the eggs varies with the mode of egg laying and can be used for genus, and in some cases, for species identification.

The culicine females belong to the fiercest biters especially in the cold temperate regions. They stand

more parallel to their resting surface in contrast to most of the females of Anophelinae. Some species have developed autogeny, the ability to lay eggs without a previous blood meal, usually in extremely cold regions and also when they inhabit confined spaces. The eggs can be laid either on the water surface for direct development in rafts (*Culex*, subgenus *Culiseta*, *Coquillettidia*) or into the substrate as single eggs. These may be drought resistant and able to overwinter for up to several years (most *Aedes* and *Ochlerotatus* species).

In tropical and subtropical regions, the main portion of the mosquito fauna belongs to the tribes of Culicini, Sabethini, Mansoniini and others not occurring in Europe. Most of the Aedini species belonging to the genera *Ochlerotatus* and *Aedes* are found in the temperate zones, and they dominate in the most extreme northern cold areas of the Holarctic region.

#### 10.1 Genus *Aedes* Meigen

The scaling and setation pattern is extensive and variable. The colour of the integument varies from brownish to blackish. The proboscis is long, straight, and always entirely scaled. The palps of the female are usually very short but in some species can extend to half the length of the proboscis. The vertex is scaled, and the occiput has erect scales. The scutum has a more or less species specific pattern of broad and/or narrow scales. The lobes of the scutellum are scarcely scaled, but have groups of setae. The prespiracular area is without setae, but postspiracular setae are present. The postpronotum and sometimes also the postprocoxal membrane have pale scale patches. The mesepisternum, mesepimeron, and mesomeron have

groups of setae and patches of more or less broad scales. The coxae are scaled, and the femora and tibiae have distinct light and dark scaling patterns. Tarsomere IV is always distinctly longer than tarsomere V. The pulvilli are usually barely developed, and setose. The claws usually have a subbasal tooth; the European species of the subgenus *Aedes* have simple claws on all legs. The wing veins are covered with numerous dark scales, and sometimes scattered pale scales are present, most frequently on the costa (C), subcosta (Sc) and radius (R). The abdomen is covered with flat and more or less broad scales. The end of the abdomen differs from other Culicinae by its tapered last segments, and usually distinct cerci, which are elongated and rarely rounded. The scaling patterns of males is similar to those of females but often with less scaling on the pleurites. The palps of males are as long as or longer than the proboscis, and only rarely shorter (subgenus *Aedes*). The antenna has numerous whorls of setae. Tergum IX usually has two lateral lobes bearing a variable number of setae. The gonocoxite has more or less developed basal and apical lobes, but the lobes are sometimes absent. The gonostylus is of variable length, and can be simple or divided. The paraproct is sclerotized, and usually not fused, with a more or less acute tip. The claspettes are present but of variable shape and structure. The aedeagus is pear shaped, rounded or elongated. The head of the larva is oval, usually wider than long, and with a rounded frontal margin. The antenna is about half as long as the head, occasionally longer than the head, and usually covered with more or less numerous spicules. The antennal seta 1-A is usually multiple-branched, inserted at about the middle of the antennal shaft, and the inner frontal seta (5-C) is usually inserted in front of the median frontal seta (6-C). Branching of the prothoracic setae 1-P to 7-P is often species specific. Abdominal segment VIII has between a few to numerous comb scales arranged in one or more irregular rows. The siphon is usually moderately long, and only one siphonal tuft (1-S) is present. It is never located at the base of the siphon, but often inserted distal to the last pecten tooth, at about or beyond the middle of the siphon. The saddle does not usually encircle the anal segment, and seta 1-X is always located on the saddle, and is often single. The ventral brush has a variable number of precratal and cratal tufts (4-X). The anal papillae are of variable lengths and shapes.

The genus *Aedes* comprises more than 40 subgenera. Some of them are strictly tropical, confined to the Neotropical, Afrotropical, Oriental, and/or Australian regions. In the European region, species of the subgenera *Aedes*, *Aedimorphus*, *Fredwardsius*, and *Stegomyia* can be found. Some of the most feared vectors belong to the genus *Aedes*. They can transmit diseases such as Yellow Fever and Dengue as well as other arboviruses from several different families causing encephalitis in humans and equines and transmit dog heart worm, filariae and bacteria.

### 10.1.1 Subgenus *Aedes* Meigen

Members of the subgenus *Aedes* are characterized by the palps which are short in both sexes and the proboscis which is usually about as long as the fore femur. The vertex is covered with narrow curved scales, the occiput with flat broad scales. The patches of scales on the pleurites are weakly developed and the lower mesepimeral setae are absent. The wings of the adults are dark scaled. The most characteristic features are found in the male genitalia. The gonostylus is subapically inserted, unequally bifurcated and does not support an apical spine. The shorter branch of the gonostylus bears several setae on its distal half. Typical claspettes, divided in a more or less slender stem and a flattened appendage or filament, are absent. Instead of this, a process which arises from the dorsal part of the basal lobe is developed. It can be simple or unequally bifurcated. If present, both branches bear distinct setae on their apices. The larvae closely resemble those of the subgenus *Aedimorphus* and the genus *Ochlerotatus*.

Knight and Stone (1977) included more than 20 species in the subgenus, but earlier Reinert (1974) transferred nearly half of them, mostly from the Oriental region, into the subgenus *Verallina*. This transfer was acknowledged by Knight (1978). At present, 12 species are listed under the subgenus *Aedes*; three from the Oriental, one from the Australian and eight species from the Holarctic region, but none are found in the Ethiopian or Neotropical regions.

**General remarks on the systematics:** The taxonomic status of the species of the subgenus *Aedes* found in the Palaearctic region is still uncertain. Gutsevich et al. (1974) treated *Ae. cinereus*, *Ae. rossicus*,

and *Ae. esoensis* as subspecies of the nominative form *Ae. cinereus*. This opinion is not shared, because there is a large overlap between *Ae. cinereus* and *Ae. rossicus* in Europe; very often the larvae occur in the same breeding sites and transitional forms are not known. There is no doubt that the Palaearctic species can be divided into two groups – first, the *cinereus*-group with *Ae. cinereus*, *Ae. geminus*, and *Ae. sasai* described from Japan, second, the *esoensis*-group with the two eastern species *Ae. esoensis* and *Ae. yamadai* and *Ae. rossicus* from Europe. The members of each group closely resemble each other. Whether the two other species of the subgenus, *Ae. dahuricus* (Danilov 1987) and *Ae. mubiensis* (Luh and Shih 1958), belong to one of these groups remains unresolved so far.

Peus (1972) treated *Ae. rossicus* as a subspecies of the eastern *Ae. esoensis* and this opinion was followed by others (Ward 1984). Although the record of *Ae. rossicus* from Japan by Hara (1958) was based on a misidentification (Tanaka et al. 1975) and there is apparently no overlap between the two forms, the arguments given by Peus are not convincing. There are clear and distinct differences between the two species in the shape of the shorter branch of the gonostylus and the structure of the claspettes. In *Ae. rossicus*, the shorter branch of the gonostylus is nearly as half as long as the main branch and the claspettes are divided into a longer and a shorter branch. In *Ae. esoensis* the shorter branch of the gonostylus is only one third as long as the main branch and the claspettes are simple. Furthermore the white basal bands on the abdominal terga are always absent in *Ae. rossicus* and generally present in *Ae. esoensis*, at least as patches (Tanaka et al. 1979). Thus until the distribution and status of all the forms involved are clarified, *Ae. rossicus* should be considered as a valid species.

### ***Aedes (Aedes) cinereus* Meigen 1818**

**Female:** Medium sized to rather small mosquitoes. The proboscis is dark brown with lighter scales on its ventral surface, and is about as long as the fore femur (Fig. 6.36a). The palps are entirely dark brown. The head is mainly covered with flat dark scales, the vertex with golden narrow curved scales and lateral parts of the occiput with broad yellowish scales. The integument of the scutum is reddish brown, covered with golden brown narrow scales which are paler on the lat-

eral margins and above the wing roots, giving the scutum a fawn brown colouration. The scutellum has dark setae and pale narrow scales on each lobe. The integument of the pleurites is light brown with patches of broad yellowish white or creamy scales on the propleuron, postspiracular area, mesepisternum, and mesepimeron. The scales on the postpronotum are narrow and hair-like; often the lower portion is paler than the upper one. The prealar area is not scaled, but covered with setae. The mesepimeron is bare on the lower half, and lower mesepimeral setae are absent. The anterior part of the fore coxa has a patch of brown scales. The femora and tibiae are dark scaled, with paler scales on their posterior surfaces. There are small, indistinct patches of pale scales on the apices of the femora, the tarsi are entirely dark brown scaled, and pale rings are absent. The wing veins are covered with dark scales. The terga have dark brown scales on the dorsal surface, but without pale transverse bands. The lateral patches of pale scales on each tergum are usually joined, forming longitudinal stripes at the sides of the abdomen, which are not readily visible in dorsal view. The sterna have yellowish white scales. The apex of the abdomen is pointed, with cerci of short or moderate length.

Owing to the general colouration, adult females of *Ae. cinereus* may, at first glance, be confused with *Cx. modestus*, but the pointed end of the abdomen and cerci, the claw with subbasal tooth, and the lack of pulvilli easily identify it as a member of the genus *Aedes*.

**Male:** The lobes of tergum IX are as long as broad, widely separated, and each lobe bears several slender spine-like setae. The gonocoxite is about twice as long as broad, conical, with scales, and long setae on its outer surface (Fig. 10.1). The basal lobe is well developed. The basal lobe as well as the part distal to it is covered with dense long setae, and the apical lobe is absent. The gonostylus is inserted well before the apex of the gonocoxite and is unequally divided. The inner and broader branch reaches between 1/3 and 1/2 of the length of the main outer branch, tapers towards the apex, and is rounded at its tip. It bears several setae on the lateral margin. The longer main branch is slightly curved and bifurcated at the apex giving it a fishtail-like appearance. The outer branch of the fork is usually shorter than the inner branch. Their lengths may vary and often the prongs are nearly the same length, but the outer branch is never longer than the inner branch (Fig. 7.18c,d). The prongs appear to be flattened

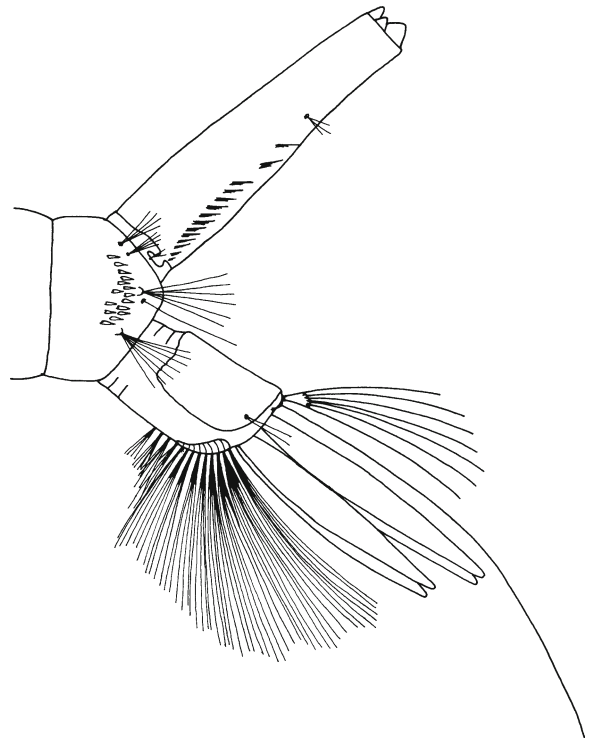




**Fig. 10.1** Hypopygium of *Ae. cinereus*

dorsoventrally and are usually stouter than in *Ae. geminus*. The claspettes are unequally bifurcated with a long and slender branch usually bearing 1–6 long setae and a shorter branch usually bearing one apical and 2–3 subapical setae. The paraproct is heavily sclerotized, slender and rod shaped with a narrow apex. The lateral plates of the aedeagus are heavily sclerotized, closed at the base and apex, with the apical half expanded.

**Larva:** The head is distinctly broader than long, and the antennae, slender and nearly as long as the head. The antennal tuft (1-A) is inserted slightly below the middle at about 2/5 of the length of the shaft (Fig. 8.22b). The setae of the labral brush are simple, not denticulated. The postclypeal seta (4-C) is inserted anterior to the frontal setae, and is small and multiple-branched. The frontal setae (5-C to 7-C) are arranged in a posteriorly curved row (Fig. 8.21b). The inner (5-C) and median (6-C) frontal setae have 5 or more branches, rarely 3–4, the outer frontal seta (7-C) is long and multiple-branched. The comb of abdominal segment VIII usually has 10–16 scales arranged in a double row partly. The individual scale is long with a strong apical spine and small lateral spines. The siphon is slender; the siphonal index 3.0–4.0 (Fig. 10.2). The pecten consists of about 13–21 weakly sclerotized teeth reaching beyond the middle of the siphon, and the distal pecten teeth are unevenly and more widely spaced than the basal teeth. The siphonal tuft (1-S) is



**Fig. 10.2** Larva of *Ae. cinereus*

inserted distal to the pecten and usually consists of 3–6 short branches. The saddle is longer than it is wide and extends to the middle of the lateral sides of abdominal segment X or beyond it. The saddle seta (1-X) is double-branched and shorter than the saddle. The ventral brush consists of 8–10 tufts of cratal setae (4-X) on the common base, and 2–4 shorter tufts of precratal setae. The anal papillae are long, at least twice as long as the saddle.

**Biology:** The larvae can be found in various habitats, but most often they occur at the edges of semi-permanent, partly shaded pools of flood plains, in sedge marshes or *Sphagnum* sp. bogs and at the edges of lakes covered by emergent vegetation. The larvae also occur in woodland pools, but they require a higher temperature for larval development than the typical snow-melt mosquitoes. *Ae. cinereus* larvae can be found in these pools when they are subsequently reflooded after rainfall. The larvae usually hatch at a temperature of 12–13°C and the development starts at 14–15°C, the optimum temperature being 24–25°C (Mohrig 1969). Under these conditions the larvae develop very rapidly and finish their immature stages within 8–10 days. The adults occur

later than the typical snow-melt mosquitoes *Oc. rusticus*, *Oc. communis*, *Oc. cantans*, or *Oc. punctor*. In central Europe the larvae can be found from April on, in the northern parts of Europe a couple of weeks later, and the adults first occur usually in May and through the summer months until late September. The findings of larvae in *Sphagnum* sp. bogs and other acido-oligotrophic habitats indicate that *Ae. cinereus* seems to be slightly acidophilic. The females feed principally on mammals and bite human hosts readily when available. They attack in numbers at dusk and dawn, but do not bite in an exposed sunlit situation. During the day they rest in the low vegetation, but feed readily after arrival of the prospective host biting those parts of the host within the vegetative cover (Wesenberg-Lund 1921). The migration range of *Ae. cinereus* seems to be low, the species is practically never seen in the open unshaded field. It has at least two generations per year, in its more northern range only one generation per year may occur. The oviposition takes place in the summer months in suitable dried up depressions prone to subsequent flooding, and hibernation takes place in the egg stage. In many localities *Ae. cinereus* occurs in masses and causes great annoyance to walkers or people seeking recreation in forested areas.

**Distribution:** *Ae. cinereus* is distributed in the northern Holarctic region and widely spread over Europe. It can be found from Finland to Italy and from Spain to the eastern shores of the Baltic Sea and the North Caucasus. It is distributed in Middle Asia, Kazakhstan and Siberia, the Far East and North America.

### ***Aedes (Aedes) geminus* Peus 1970**

**Female:** *Ae. geminus* closely resembles *Ae. cinereus* and can be identified with certainty solely through hypopygial characteristics. Peus (1972) considered both as sibling species and described the adults of *Ae. geminus* in the typical form usually being smaller than the adults of *Ae. cinereus* and the females of the latter usually with a lighter colouration in the typical form than the females of the former. However, the size of an adult is closely related to the nutritional situation during the immature stages and females of *Ae. cinereus* show a variation in their colouration, e.g. darker forms may exist, thus these characteristics are of no value for identification of the two species.

**Male (Fig. 10.3):** In *Ae. geminus* the basal lobe is less developed than in *Ae. cinereus* and the covering with long setae seems not to be as dense as it is in the latter species. Whereas in this case it is recommended to compare individuals of the two species to get a clear picture, the immediately visible difference between the two species is the shape of the apical fork of the gonostylus. It is bifurcated in both species, but in *Ae. geminus* the outer branch is longer than the inner one (Fig. 7.18a, b). In *Ae. cinereus* the length of the outer branch never exceeds that of the inner branch. In the typical form the prongs appear to be thinner and much slender in *Ae. geminus* and they are more or less rounded, whereas in *Ae. cinereus* they are often flattened. The claspette is divided into two branches, the shorter branch bearing 2–3 setae, and in *Ae. cinereus* this branch bears usually 3–4 setae.

**Larva:** No specific differences, neither in the chaetotaxy nor in other characteristics, can be found in the larvae of *Ae. geminus* and *Ae. cinereus*. Both show individual variability, e.g. in the number of the small accessory siphonal setae on the dorsal part of the siphon.

**Biology:** So far, the information available about the biology of *Ae. geminus* is scanty. There is a large overlap in the preferred breeding sites with *Ae. cinereus*, and very often both species can be found together in the same water bodies. Peus (1972) reported *Ae. geminus* to have a lower tolerance against acidic habitats,



**Fig. 10.3** Hypopygium of *Ae. geminus*

though he could not find the species in mesotrophic and higher acido-oligotrophic swamps, where *Ae. cinereus* was numerous. As *Ae. cinereus*, *Ae. geminus* has at least two generations per year and the males form mating swarms of only 10 individuals or less. The females are anthropophilic and can cause great annoyance, when present in large numbers.

**Distribution:** Because of the close similarity to *Ae. cinereus* it is difficult to assess the whole range of distribution for *Ae. geminus*. Records of *Ae. cinereus* earlier than 1970 or those which are solely based on females or larvae are at best questionable. Lvov (1956) worked on the specific status and distribution of *Ae. esoensis* and *Ae. cinereus* in the eastern Palaearctic region with findings of transitional forms, but never mentioned the characteristic form of the apical fork of the gonostylus which is typical for *Ae. geminus*. It is most likely that *Ae. geminus* does not occur in this region and is distributed mainly in middle and western Europe (Peus 1972). The species is recorded with certainty from England, northwestern France, Germany, Poland, Czech Republic and southern Sweden. It has also been identified from the southern and eastern shores of the Baltic Sea (Peus 1972).

### ***Aedes (Aedes) rossicus***

#### **Dolbeskin, Gorickaja and Mitrofanova 1930**

**Female:** *Ae. rossicus* is easy to distinguish from the morphologically similar *Ae. cinereus* by comparing the colour of the thorax and the abdomen. In *Ae. cinereus* the colour of the scutum is fawn brown with light brown pleurites, the colour of the abdomen is dark brown, distinctly different from the colour of the thorax. In *Ae. rossicus*, the scutum, the integument of the pleurites and the abdomen, are more or less of the same dark brown colour, and the differences in colouration of the thorax and the abdomen are indistinct. In addition, the scales on the pleurites and the sterna are pale yellowish or yellowish white in *Ae. cinereus* and greyish white in *Ae. rossicus*, producing a distinct contrast between the colour of the terga and sterna in the latter species. The proboscis of *Ae. rossicus* is dark scaled on its dorsal surface, the ventral surface has mixed pale and dark scales mainly on the basal half, and the palps are intermixed with pale and dark scales. The head has narrow pale and broad dark brown scales on the vertex. The lateral parts of the

occiput are covered with broad light coloured whitish grey scales, producing a distinct contrast to the scales on the vertex. The eyes are bordered with a narrow row of whitish scales. The scutum has narrow brown scales, often with an inconspicuous pale stripe on the lateral margins, which is more distinct on the posterior part. The upper part of the postpronotum has narrow dark scales, and on the lower part the scales are broad, flat, and of a lighter colour. The prealar area has whitish scales and setae, the scales on the mesepisternum do not reach the anterior angle, and the scales on the mesepimeron do not reach the lower margin. The abdomen has dark brown scales on the dorsal surface (the same colour as on the scutum), without pale transverse bands. The lateral pale scales on each tergum are fused, forming longitudinal stripes at the sides of the abdomen, which are hardly visible from above.

**Male:** The general morphology of the hypopygium is very similar to that of *Ae. cinereus*. A readily visible difference is the shape of the longer branch of the gonostylus, which is never apically bifurcated. It is somewhat flattened distally and rounded at the apex, and the outer margin has fine spatula shaped serrations (Fig. 10.4). The apical part of the gonocoxite, distal to the point of insertion of gonostylus, tapers more abruptly and seems to be more slender than in *Ae. cinereus*. The claspette is divided into two branches,



**Fig. 10.4** Hypopygium of *Ae. rossicus*

the shorter branch is broad with 3–4 setae, two of them strong and spine-like, and usually stronger than the setae in *Ae. cinereus*. The longer branch of the claspette is slender with 1–2 setae.

**Larva** (Fig. 10.5): The larva of *Ae. rossicus* is very similar to that of *Ae. cinereus*. Both species show subtle differences in certain characteristics, e.g. the point of origin of the antennal seta (1-A) and the setation of the prothorax, as indicated in the keys (Fig. 8.22a). Often it is hardly visible and difficult to decide if the antennal tuft is located in the middle of the antenna or slightly below it, sometimes the prothoracic setae may be broken or lost. In these cases one needs to rear the larvae until the adults are hatched and then differentiate between the two species in the adult stage.

**Biology:** The larvae occur from mid March to early September in small temporary pools, where they can be found together with those of *Ae. cinereus* and *Ae. geminus*, but apparently the first generation of *Ae. rossicus* occurs earlier in the year than in the two latter species. Larvae of *Ae. rossicus* can also be found in large numbers in the inundation areas of rivers, often associated with *Ae. vexans* and *Oc. sticticus*. They are rarely found in swampy woodlands in acid breeding

waters (Becker and Ludwig 1981). In the inundated forests of the Upper Rhine valley, the females are severe biters and readily attack humans even during the day. They rarely leave the protected and shaded areas for host-seeking, their migration range is limited, thus they are only occasionally found in the open field. Adults are found during the summer months usually from early May to October. Occasionally single individuals can be found in November when other species of the genera *Aedes* and *Ochlerotatus* have already disappeared; apparently they have a higher tolerance against the cold. It is assumed that *Ae. rossicus* produces several generations per year in the temperate zones, in its more northern distribution range it could be less (1 or 2 generations per year). Hibernation takes place in the egg stage.

**Distribution:** The western European range of its distribution is not so well known as yet. It is supposed that *Ae. rossicus* is distributed in the West as far as the Atlantic Ocean. The species is recorded in Sweden, Norway, France, Germany, Hungary, the Czech Republic, and former Yugoslavia and can be found in the Ukraine and north of the Caucasus to the western slopes of the Ural. In the eastern Palaearctic it can be found in the northern regions of the Far East. The record of *Ae. rossicus* from Japan (Hara 1958) was based on a misidentification (Tanaka et al. 1975).

### 10.1.2 Subgenus *Aedimorphus* Theobald

The females are characterized by the numerous erect forked scales on the vertex and occiput. The achrosetical and dorsocentral setae are well developed and numerous. The ante- and postpronotum are usually covered with narrow curved scales, and postspiracular setae are present. The mesepisternum has several upper mesepisternal setae, and the mesepimeron has no setae or scales in its lower part. Sternum VIII has a deep median V-shaped notch apically, and the cerci are long and narrow. In males the gonostylus is usually complex with an expanded apical portion, and typical claspettes divided into a stem; and winged filaments are absent. The antennae of the larvae are moderately pigmented and slightly curved distally. The postclypeal seta (4-C) is very small and usually multiple-branched. The pecten has 10–23 teeth, distal 2–4 teeth spine-like

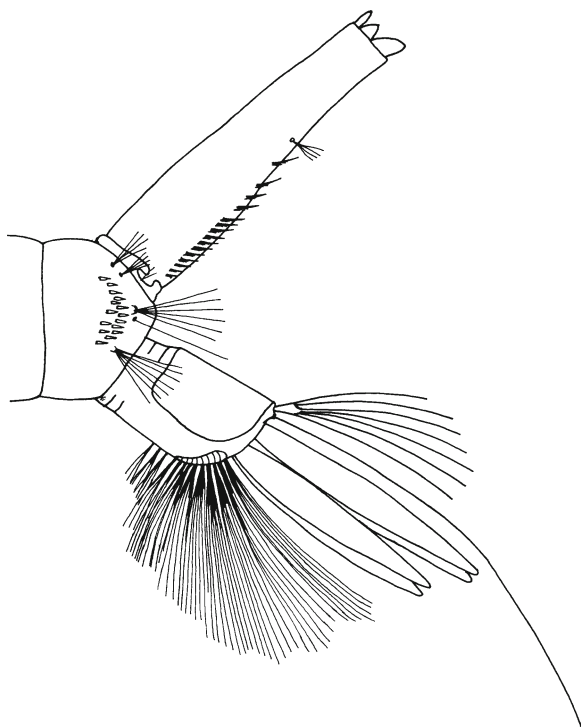


Fig. 10.5 Larva of *Ae. rossicus*

and apically detached. The siphonal tuft (1-S) is located well beyond the middle of the siphon.

The structure of the female genitalia and some male characteristics resemble the species of the subgenus *Stegomyia*; therefore a close relationship between the two subgenera is assumed. The subgenus *Aedimorphus* embraces approximately 110 species and subspecies. The majority of them can be found in the Ethiopian and Oriental regions. Only one species of *Aedimorphus*, namely *Ae. vexans*, occurs in Europe.

***Aedes (Aedimorphus) vexans* (Meigen 1830)**  
**[*Aedimorphus vexans*]**

**Female:** Tarsomeres II and III of the fore legs, tarsomeres I–IV of the mid legs, and all the tarsomeres of the hind legs have narrow basal pale rings which usually do not exceed more than 1/4 of the length of the tarsomeres (Fig. 6.27a). Compared to other European *Ochlerotatus* species with pale rings on the legs, e.g. *Oc. annulipes*, *Oc. cantans*, *Oc. flavescens*, or *Oc. excrucians*, the rings are much narrower. The proboscis and palps are dark scaled, and the palps have some white scales apically. The head is covered with narrow curved decumbent pale and dark scales and numerous dark brown erect forked scales which extend anteriorly to the interocular space. The scutal integument is dark brown, and the scutum is covered with narrow curved dark scales and narrow pale scales forming indistinct patches on the anterior submedian, and prescutellar dorsocentral areas, as well as on the transverse suture. The acrostichal and dorsocentral setae are well developed. The postspiracular area has a large patch of narrow curved or moderately broad pale scales. The upper and lower mesepisternal scale patches are present. The mesepimeron has a patch of broad pale scales in the upper part. The tibiae are dark scaled dorsally and light scaled ventrally. The wing veins are covered with moderately broad dark scales and isolated pale scales at the bases of the costa (C) and subcosta (Sc). The abdominal terga have white basal bands with the distal parts dark scaled. The basal bands on terga III–VI are distinctly narrowed in the middle, forming a bilobed pattern (Fig. 6.27b). Old and worn females, which have lost most of their scales, can still unequivocally be identified by the distinct V-shaped notch at the apical margin of sternum VIII.

**Male:** Tergum IX is strongly bilobed with 6–11 setae on each lobe. The gonocoxite is long and mod-

erately broad, with scattered scales on the lateral and ventral surfaces. The basal and apical lobes are absent. The gonostylus gradually expands towards the apex. The spine of the gonostylus is articulated subapically arising from a small tubercle, and is straight (Fig. 10.6). The claspette has a moderately broad basal part, and the apex is slightly expanded and rounded, with a crown of numerous spine-like setae, some of them curved apically. The claspette filament is absent. The paraproct has a pointed apex, and the aedeagus is strongly sclerotized, with lateral plates connected at the base.

**Larva:** The antenna is less than half as long as the head, with numerous spicules scattered over the shaft. The antennal seta (1-A) with 5–10 branches, is inserted below the middle of the antenna. The median setae of the labral brush are serrated apically, a valuable characteristic to distinguish *Ae. vexans* from the similar larvae of *Ae. rossicus* and *Ae. cinereus*, both of which have simple setae. The frontal setae (5-C to 7-C) are arranged in a triangular pattern (Fig. 8.21a), the median frontal seta (6-C) is situated in front of the inner frontal seta (5-C); 5-C has 1–5 branches, 6-C has 1–2 (rarely 3) branches, and 7-C has 6–12 (usually 7–9) branches. The comb with 7–13 scales is arranged in 1–2 irregular



**Fig. 10.6** Hypopygium of *Ae. vexans*

rows (Fig. 10.7). Each comb scale has a long, stout pointed median spine and small spines at the base. The siphonal index is usually 2.3–3.0. The pecten has 13–18 teeth, the apical 2–3 teeth are larger than the others and detached. The basal teeth are with 1–3 lateral denticles. The siphonal tuft (1-S) is situated well beyond the middle of the siphon, with 3–8 short branches, and about half as long as the width of the siphon at the point of origin. The saddle reaches far down the sides of the anal segment, and the saddle seta (1-X) has 1–2 branches. The ventral brush has pre-cratral setae (4-X). The anal papillae are distinctly longer than the saddle.

**Biology:** *Ae. vexans* is a polycyclic species predominantly breeding in inundated areas such as floodplains of rivers or lakes with fluctuating water levels. The preferred breeding sites are temporary water bodies with neutral to alkaline water, which are present only a few days to weeks after a flood, such as flooded meadows, poplar cultures, willow and reed areas. Usually the larvae hatch in large numbers if the water temperature exceeds 9°C. In central Europe they occur in springtime when the typical snow-melt mosquitoes, such as *Oc. cantans* are already hatched. After flooding, the larvae hatch

within a few minutes to hours when the flooded water becomes stagnant and the content of oxygen decreases. The hatching behaviour of the larvae is adjusted to the temporary water conditions. After the completion of the embryogenesis, which may last 4–8 days (about 1 week at 20°C), not all the larvae hatch after flooding, but only a proportion of them (“hatching in installments”). If one population of larvae fail to complete the development due to drying out, a second population can develop during a following flood even if no additional eggs are laid. The hatching rate is particularly high at high water temperatures and after the completion of a diapause, which lasts from September to early March of the following year in temperate zones. If suitable hatching conditions fail to exist (*e.g.* lack of floods during summer), the eggs are capable of surviving for a long time (at least 5 years).

*Ae. vexans* as a “summer species” has an optimum temperature of 30°C for its development. At a water temperature of 30°C, the development from hatching of the first-instars to emergence of the adults lasts 1 week; at a temperature of 15°C it is 3 weeks. *Ae. vexans* frequently becomes the dominant species during the summer months rich in floods and is often the most important nuisance mosquito in temperate climate zones. Often, hundreds of larvae per liter of water can be encountered, that is frequently >100 million larvae per hectare. Owing to the pressure of a large population, after mass emergence, the adults frequently migrate long distances from their breeding sites to find a host for the blood meal and thus may become a serious nuisance, not only close to the breeding sites, but also far away from their breeding waters. A migration of up to 15 km (according to the circumstances, the flight capacity is about 1 km/night), occasionally even a multiple of that, could be proven. The immigration of females into human settlements, *e.g.* gardens and parks, can cause a considerable nuisance. After the blood meal, the females lay the eggs 5–8 days at the earliest into damp depressions. A female can lay >100 eggs after a single blood meal; occasionally after repeated blood meals, multiple egg batches are laid. The preferred hosts are mammals. Both females and males imbibe plant juices in order to cover their energy requirements. However, no eggs are developed without a blood meal. Under optimum conditions, *Ae. vexans* needs less than 3 weeks from hatching of one generation to the hatching of the larvae of the next

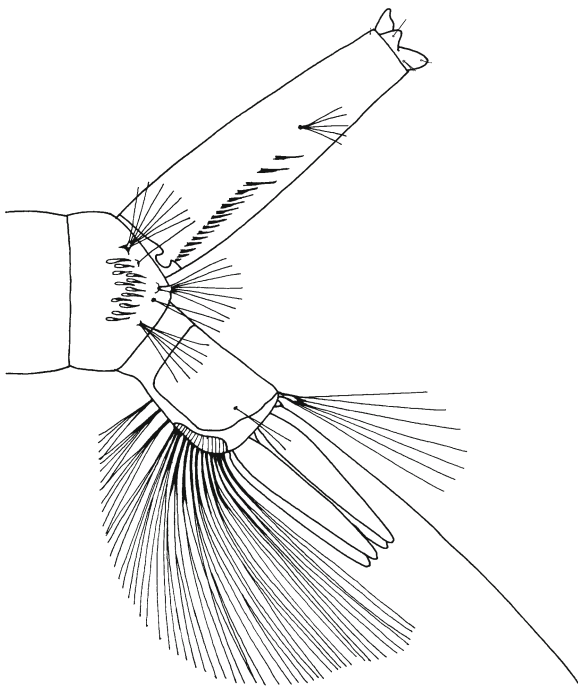


Fig. 10.7 Larva of *Ae. vexans*

generation (development in water: approx. 6 days; copulation: approx. 2 days; blood meal: approx. 2 days; egg development: approx. 5 days and embryogenesis about 4 days). It is suspected that only a part of the emigrated population returns to the original breeding sites after the blood meal, while a considerable part of the population do not return and lay eggs far away from their original breeding sites. Therefore, the migration leads to a natural regulation of the population densities.

**Distribution:** *Ae. vexans* is distributed almost worldwide and can be found in nearly every country in Europe.

**Medical importance:** *Ae. vexans* has many attributes of an ideal vector species. It is widely distributed, can become very abundant, often at the same time when virus activity is at its peak, it feeds readily on humans and domestic animals, and it has been found naturally infected with various arboviruses (Reinert 1973). Natural infections with western equine encephalomyelitis (WEE) virus, eastern equine encephalomyelitis (EEE) virus and California encephalitis (CE) group viruses have been reported from North America (Wallis et al. 1960; McLintock et al. 1970; Hayes et al. 1971; Sudia et al. 1971). In Europe, *Ae. vexans* is involved in the transmission of Tahyna virus (Aspöck 1965; Mattingly 1969; Gligic and Adamovic 1976; Lundström 1994).

**Note on systematics:** Three subspecies of *Ae. vexans*, ssp. *arabiensis* (Patton 1905), ssp. *nipponii* (Theobald 1907) and ssp. *vexans* (Meigen 1830), have been described (Reinert et al. 2009).

### 10.1.3 Subgenus *Fredwardsius* Reinert

The establishment of the monotypic subgenus *Fredwardsius* is based on the type species, *Ae. vittatus* (Reinert 2000a). The species has long been recognised to not fully conform to any recognised subgenus of *Aedes*. Edwards (1932) included *Ae. vittatus* in the subgenus *Stegomyia* Theobald and placed it in a monotypic group (Group D), apart from other species of the subgenus. Later on, Huang (1977) transferred *Ae. vittatus* from subgenus *Stegomyia* Theobald to subgenus *Aedimorphus* Theobald, mainly based on the structure of the male hypopygium. After a comparison of *Ae. vittatus* with all currently recognised subgenera and genera in the tribe Aedini, Reinert (2000a) found the species shared some characteristics with the subgenera *Stegomyia* and

*Aedimorphus*, but possessed unique and unusual characteristics, that are of subgeneric rank. The combination of these characteristics distinguishes *Fredwardsius* from all other subgenera and genera within the tribe Aedini, e.g. 2–6 well developed lower mesepimeral setae in the female, the greatly expanded distal portion of the gonostylus in the male hypopygium, and the position and development of head setae 4-C to 7-C in fourth-instar larvae. For a more detailed morphological description of the subgenus, see Reinert (2000a).

#### ***Aedes (Fredwardsius) vittatus* (Bigot 1861) [*Fredwardsius vittatus*]**

**Female:** The proboscis is as long as the fore femur. Its median part has a band of whitish scales of varying width, which is better developed ventrally than on the dorsal surface. The palps have a few white scales in the middle and the apex is broadly pale scaled. The clypeus has lateral patches of white scales. The antennae are shorter than the proboscis, and the pedicel and first flagellomere are white scaled on the median and lateral parts. The vertex has decumbent curved blackish brown scales and black, erect forked scales. There are two dorsolateral patches of white decumbent scales behind the eyes. The scutum is mainly covered with narrow, curved, blackish brown scales. Three pairs of prominent silvery whitish spots are present, located close to the dorsocentral areas (Fig. 6.24a). The anterior two pairs are usually larger than the posterior pair, which is situated close to the wing roots. All three lobes of the scutellum have broad white scales, and a few black scales may be present at the apex of the middle lobe. Broad white scale patches are present on the ante- and postpronotum, propleuron, postprocoxal membrane, and sub- and postspiracular areas. The mesepisternum has upper and lower scale patches. The mesepimeral patch is located in the upper part of the mesepimeron. The femora of all the legs have a white ring close to their apices and white knee spots, and the hind femur is more extensively white scaled at the base. The hind tibia has a distinct median white ring. The tarsi of the fore and mid legs have narrow white basal rings on tarsomeres I–III, and tarsomeres IV and V are entirely dark. The tarsi of the hind legs have broad white basal rings on tarsomeres I–IV, but tarsomere V is entirely white scaled. The wing veins are mainly dark scaled, with a few broad white

scales at the base of the costa (C) and a few scattered white scales on the costa (C) and radius (R). The abdominal terga are predominantly covered with black scales. Tergum I has a median longitudinal white band extending to near the apical margin, terga II–VII have narrow white basal bands, and lateral curved white markings separated from the basal bands. Sterna I–VI have lateral and central areas of scattered white scales, and sternum VII is extensively white scaled. Sternum VIII has a deep notch at the middle, and the cerci have dark scales.

**Male:** Tergum IX is narrowed in the middle, with a distinct setose lobe on each side. The gonocoxite is elongate, and about three times as long as it is wide at its base, without distinct basal and apical lobes (Fig. 10.8). The gonostylus has a very characteristic shape, being greatly expanded apically. The apical spine is situated at the base of the expanded apical part, and is long and strongly curved. The claspette is large, with a narrow base, is distinctly swollen from about the middle, and is covered with numerous small setae. The aedeagus is small with several well developed recurved teeth on the lateral and apical parts.

**Larva:** The antenna is slightly more than half as long as the head, and is covered with very small spicules, although sometimes the antenna is smooth. The antennal seta (1-A) is situated slightly below the middle

of the antennal shaft, usually with 2–3 branches (Fig. 8.62a). The postclypeal seta (4-C) has 2–3 branches, the inner and median frontal setae (5-C and 6-C) are single, and the outer frontal seta (7-C) has 5–7 branches. The comb consists of 6–9 (usually 8) large comb scales, each scale with a long, pointed median spine and a few thin, short spines close to its base. The siphon is sclerotized along its entire length, except for a very small area at the extreme base, and the acus is indistinct. The siphon slightly tapers towards its apex (Fig. 10.9). The siphonal index is 2.0 or slightly more. The pecten usually has 15–25 well sclerotized teeth, occupying about 2/3 of the length of the siphon. The distalmost pecten tooth is spine-like, and apically detached (Fig. 8.62b). The siphonal tuft (1-S) is situated below the distalmost pecten tooth, with 3–6 branches, which are about as long as the width of the siphon at the point of origin. The saddle seta (1-X) is short and single. The upper anal seta (2-X) has 4–6 branches, and the lower anal seta (3-X) is single and long. The ventral brush has 5–7 tufts of cratal setae (4-X) and 3–4 tufts of precratal setae. The anal papillae are more than twice as long as the saddle, and pointed.

**Biology:** Females prefer to lay their eggs above the water level of rock pools, occasional utensils, boats, wells, or tree-holes (Mattingly 1965). Boorman (1961) found that deep rock holes usually contained



Fig. 10.8 Hypopygium of *Ae. vittatus*

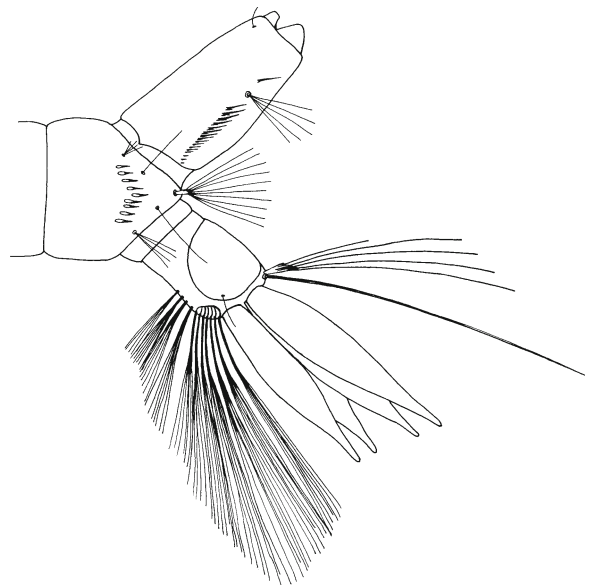


Fig. 10.9 Larva of *Ae. vittatus*



larvae of *Ae. vittatus*, particularly those where the water was clear and the bottom covered with a layer of mud and a few dead leaves. *Ae. vittatus* is particularly well suited to such an environment, as its eggs can tolerate desiccation for many years, and the very rapid larval development minimizes the likelihood of the immature stages being killed by the pools drying out. In Nigeria, larval and pupal development were completed within 6 days (Service 1970a). Larvae of *Ae. vittatus* were found in rock pools in association with those of *Cx. hortensis*, *Cx. mimeticus*, *An. claviger*, and *Cs. longiareolata*. In France, *Ae. vittatus* is mainly a crepuscular species, but its biting activity continues during most of the night (Rioux 1958). In the Mediterranean region, the species is found from late spring to early autumn. Its seasonal incidence depends both on local rainfall and on the water level of rivers. There are records of equal biting intensity both indoors and outdoors. *Ae. vittatus* females sometimes attack humans in large numbers (Gutsevich et al. 1974).

**Distribution:** Mediterranean subregion, Afro-tropical and Oriental regions. Its northernmost distribution range is probably limited by the 10°C isotherm. Even if the species can survive cold winters in the north, summer temperatures must be sufficiently high to enable the completion of at least one gonotrophic cycle.

#### 10.1.4 Subgenus *Stegomyia* Theobald

Members of this subgenus are rather small, rarely medium sized mosquitoes. According to Gutsevich et al. (1974) and Huang (1979) the following combination of characteristics can be found within species of *Stegomyia*. The male palps are more than half the length of the proboscis, often slightly longer than the proboscis, and with 5 palpomeres. The female palps are up to 1/4 the length of the proboscis, 4- or sometimes 5-segmented and when present, palpomere V is minute. The vertex is largely covered with broad and flat decumbent scales, erect forked scales are not numerous and restricted to the occiput. The scutum has a characteristic pattern of light scales for each species. The acrostichal and spiracular setae are absent, and postspiracular setae present. The scutellum has broad scales on all the lobes, and the postnotum is bare. The hind tarsus has a basal white ring at least on tarsomere I, and the wings have

narrow scales on all the veins. The abdominal terga have white basal bands and often white lateral spots, and the cerci are relatively short. In the male genitalia, the basal and apical lobes of the gonocoxite are absent. The gonostylus is usually simple and elongated, or sometimes it is expanded apically or at the base. Typical claspettes, which are divided into a stem and a filament are modified into a structure which is usually lobed, and located at the base of the gonocoxite and covered with numerous setae. The aedeagus is divided into two plates and is strongly toothed apically. In the larvae, the antennae are without spicules, the antennal seta (1-A) is usually small and single. The postclypeal seta 4-C is well developed and branched, and the median frontal setae (6-C) are situated in front of the inner frontal setae (5-C). The comb scales are arranged in a single row, and the base of the siphon has no acus. The ventral brush has 8–10 cratal setae (4-X) on a common base, without any pre-cratal setae.

The subgenus is confined to the Old World and mainly occurs in the tropical and subtropical zones throughout this region, except *Ae. aegypti* and *Ae. albopictus* which have been introduced through commerce into the New World. It seems that in *Stegomyia* there are a few widely distributed species and a number of specialized endemic species (Huang 1979). In Europe the subgenus is represented (or was partly represented in the past) by three species which could be found in the southern countries of the continent. In the family Culicidae the subgenus *Stegomyia* is one of the most important subgenera in view of the transmission of pathogens and parasites, it includes several vectors of human filariasis and a number of severe viral diseases.

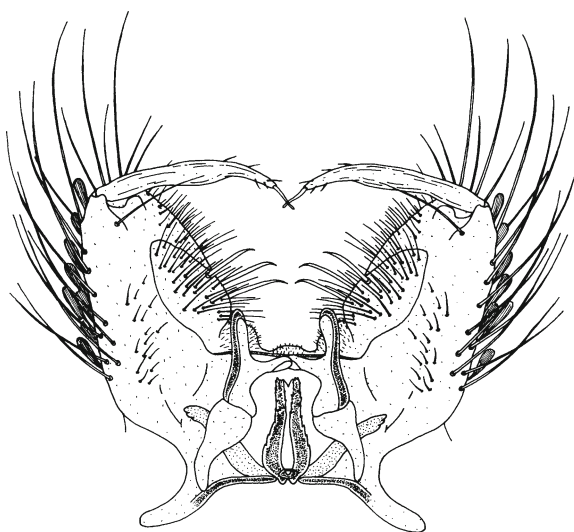
#### ***Aedes (Stegomyia) aegypti* (Linnaeus 1762) [*Stegomyia aegypti*]**

**Female:** A medium sized dark species with contrasting silvery white ornamentation on the head, scutum, legs, and abdomen. *Ae. aegypti* is easily recognised and distinguished from the other members of the subgenus by the white scutal markings which form the typical “lyre-shaped” pattern of the species (Fig. 6.25a). The proboscis is dark scaled, the palps 1/5 the length of the proboscis with white scales on the apical half, the clypeus with lateral white scales, and the pedicel with large patches of white scales at the sides. The

vertex has a median line of broad white scales from the interocular space to the back of the occiput, and white scales also on the sides, separated by patches of dark scales. Erect scales are restricted to the occiput, and are all pale. The scutum is predominantly covered with narrow dark brown scales, with a distinctive pattern of light scales as follows: a small antecroscistichal patch of white scales, a pair of narrow dorsocentral stripes of narrow pale yellowish scales close to the midline extending to the anterior 2/3 of the scutum, a short postacroscistichal stripe of white scales just in front of the prescutellar area where it forks forming prescutellar dorsocentral stripes which end at the margin of the scutellum, broad lateral presutural stripes which continue over the transverse suture covered with crescent-shaped white scales followed by submedian stripes of narrow white scales reaching the posterior margin of the scutum, and a patch of broad white scales on the lateral margin just in front of the wing root. The scutellum has broad white scales on all the lobes and a few broad dark scales at the apex of the mid lobe (Fig. 6.23a). The postpronotum has a patch of broad white scales and some dark and pale narrow scales in the upper part, and the paratergite has broad white scales. The postspiracular area is without scales, but there are patches of broad white scales on the propleuron, and subspiracular and hypostigmal areas. Mesepisternal and mesepimeral patches are present, divided into an upper and lower portion, but not connected. The upper mesepisternal scale patch does not reach the anterior corner of the mesepisternum. The mesepimeron has no lower mesepimeral setae. The coxae have patches of white scales, and all femora have white knee spots, the fore and mid femora with a narrow white longitudinal stripe on the anterior surface. All the tibiae are dark anteriorly, the fore and mid tarsi have a white basal band on tarsomeres I and II, the hind tarsus has a broad basal white band on tarsomeres I–IV, and tarsomere V is all white. The claws of the fore and mid tarsi have a subbasal tooth, and the claws of the hind tarsi are simple. The wing veins are all dark scaled except for a small spot of white scales at the base of the costa (C). Tergum I has white scales laterally and a median pale patch, terga II–VI have basal white bands and basolateral white spots separate from the bands, and tergum VII has lateral white spots only. Sterna II–IV are largely pale scaled, V and VI have predominantly dark scales, and VII is dark except for a small lateral pale patch.

**Male:** The palps are as long as the proboscis with white basal rings on palpomeres II–V. The last two segments are slender and upturned with only a few short setae. The posterior margin of tergum IX is deeply concave in the middle, and the lateral lobes are prominent with 3 apical setae. The gonocoxite is slightly more than twice as long as wide, with scales restricted to the lateral and ventral surfaces (Fig. 10.10). The gonostylus markedly narrows apically and is curved, with a pointed apical spine (Fig. 7.20c). The claspettes are large, lobe-like, appressed to and occupy most of the mesal surface of the gonocoxite, with numerous setae and several stronger setae, 3 or 4 of which are bent at the tip. The paraproct has an inwardly directed mesal arm, and the aedeagus is strongly serrated.

**Larva:** The antennae are about half as long as the head and without spicules. The antennal seta (1-A) is small and single, and inserted slightly beyond the middle of the antennal shaft. The postclypeal (4-C) and median frontal (6-C) setae are displaced far forward toward the anterior margin of the head. 4-C is situated slightly anterior to 6-C, and is well developed with 4–7 short branches. The frontal setae (5-C to 7-C) are long and single (Fig. 8.60a), and the outer frontal seta (7-C) very rarely have 2 branches. At the base of the meso- and metathoracic setae (9-M to 12-M and 9-T to 12-T) there is a long stout spine which is pointed and hooked at the tip. The comb consists of a single irregular row of 6–12 scales, each



**Fig. 10.10** Hypopygium of *Ae. aegypti*

comb scale has a long median spine and strong smaller spines at the base forming a “triffid” appearance. The siphon is moderately pigmented, the siphonal index is about 1.8–2.5, and the acus is not developed (Fig. 10.11). The pecten has 8–22 teeth (usually 10–16), evenly spaced or sometimes the distalmost tooth is detached apically, and each tooth has 1–4 lateral denticles. The siphonal tuft (1-S) has 3 or 4 branches, usually inserted close to the distal pecten tooth and just beyond the middle of the siphon. The saddle reaches far down the lateral sides of the anal segment, the saddle seta (1-X) usually has 2 short branches. The ventral brush has 8–10 tufts of cratal setae (4-X), and the precratal setae are absent. The anal papillae are about 2.5–3 times the length of the saddle, sausage-like, and rounded apically.

**Biology:** In subtropical climates the species is found almost always in the close vicinity of human settlements. The larvae occur in a wide variety of small artificial containers and water recipients of all kinds, both inside and outside of human habitations in gardens and within a radius of 500 m around dwellings, *e.g.* in earthenware pots and water tanks for storing water, uncovered cisterns, rainfilled empty cans or flower pots, broken bottles or discarded motor vehicle tyres. If vegetation surrounds the settlements, the larvae may breed in tree-holes, leaf axils, bamboo stumps, or coconut shells after heavy rainfall. They can also be found in any artificial and natural water

collection in harbors and on ships. The breeding water is mostly clean or has a moderate content of organic matter. The larvae spend a long time under water feeding on the bottom of their breeding sites. The eggs are resistant to desiccation and are deposited close to the waterline in the mentioned recipients. At a temperature of 27–30°C the larvae will hatch 2 days after egg deposition, pupation occurs after 8 days and the adults emerge from the pupae 9–10 days after the eggs have been laid. The females feed predominantly during the day in shaded places and only occasionally during the night in lit rooms. Human blood seems to be preferred to that of domestic animals (Carpenter and La Casse 1955); the blood feeding interval is only about 2–4 days. The adults are frequently found resting indoors, *e.g.* in cupboards, closets or behind doors. They do not migrate over long distances, and rarely fly more than several hundred meters from their breeding sites. The species is supposed to have had its origin in Africa and subsequently spread over vast areas, mainly the Tropics.

*Ae. aegypti* is one of the most suitable mosquitoes for laboratory colonies and has been extensively used as a test organism for laboratory research in many fields. At a constant rearing temperature of 22–28°C the species has some outstanding advantages in respect of colonization: the adults and larvae are easy to handle, rearing is possible in nearly every type of cage and breeding container, and mating takes place even in the smallest space. The females readily feed on a variety of small mammals offered as a blood source and the eggs can be stored for months, if necessary, without losing viability. The available literature about *Ae. aegypti*, its biology and medical significance is numerous; a well recognised monograph was published by Christophers in 1960.

**Distribution:** This cosmopolitan species is distributed in the tropical, subtropical, and warm temperate regions of both hemispheres. Its range is mainly limited by the 10°C cold-month isotherms where breeding can continue all year round (Christophers 1960). Certain populations may extend their summer range considerably north of this line, *e.g.* in North America specimens may be found at the 40° northern latitude in southern Illinois and Indiana, but they are not able to survive during cold winter months; this prevents the establishment of permanent populations. In Europe, prior to 1945, all Mediterranean countries and most major port cities had reported at least occasional

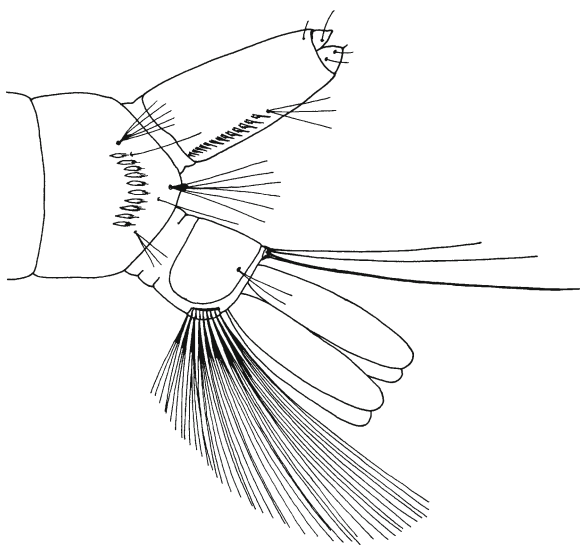


Fig. 10.11 Larva of *Ae. aegypti*

introductions of *Ae. aegypti* (Mitchell 1995). It could be found in Portugal, Spain, France, Italy, former Yugoslavia, Greece and Albania, but has now been eradicated or has become rare in many countries where it was previously common.

**Medical importance:** As the major vector of yellow fever virus, *Ae. aegypti* has long been notorious as the “yellow fever mosquito”, but it is also an important vector of dengue and several other viral infections.

**Synonymy:** The species in question has appeared under various other names in the past. The number of names, which are now accepted as synonyms is large and listed in Knight and Stone (1977). Synonyms that are often found in the early literature are *Stegomyia fasciata* of Theobald 1901 (described as *Culex fasciatus* by Fabricius 1805), *Aedes calopus* Meigen 1818, and *Aedes argenteus* of Edwards 1921 (described as *Culex argenteus* by Poiret 1787). From about 1935 on, the earliest name, *aegypti* given by Linnaeus in 1762, was accepted and is in general use until today.

**Note on systematics:** A paler variation of the type form, var. *queenslandensis* (Theobald) exists and a subspecies *formosus* (Walker) is characterized through its markedly darker appearance. The latter form is confined to Africa south of the Sahara and has been recorded from the forest or bush away from human settlements, breeding in natural places.

***Aedes (Stegomyia) albopictus* (Skuse 1895)  
[*Stegomyia albopicta*]**

**Female:** The proboscis is dark scaled, about the same length as the fore femur, and the palps are 1/5 the length of the proboscis with white scales on the apical half. The clypeus is bare and entirely dark. The vertex has broad white scales, and the occiput in the middle is white scaled with dark scales at the sides; erect scales are usually absent. The scutum is mainly covered with narrow dark scales, with a prominent acrostichal stripe of narrow white scales which narrows posteriorly, and extends from the anterior margin of the scutum to the beginning of the prescutellar area where it forks to the end at the margin of the scutellum (Fig. 6.26a). On each side a slender posterior dorsocentral white stripe does not reach the middle of the scutum, but extends about midway to the level of the scutal angle. The

supraalar white stripe is incomplete, there is a patch of broad white scales on the lateral margin just before the level of the wing root and a few narrow white scales over the wing root. The scutellum has broad white scales over all the lobes with an apical area of dark scales on the mid lobe. The postpronotum has a large patch of broad white scales and some narrow dark ones in the upper part, and the paratergite has broad white scales. The postspiracular area is without scales, and the subspiracular area has white scales. The mesepisternal patch is divided into large upper and lower patches of white scales. The mesepimeron has connected upper and lower scale patches which form a V-shaped white scale patch, with the open V directed backwards. The coxae have patches of white scales, the fore and mid femora are dark anteriorly and paler posteriorly with apical pale spots. The hind femur has a broad white longitudinal anterior stripe widening at its base and slightly separated from the apical white scale patch. The tibiae are all dark. The fore and mid tarsi have narrow basal white bands on tarsomeres I and II, the hind tarsus has broad basal white bands on tarsomeres I–IV, and tarsomere V is all white. The claws are simple without a subbasal tooth. The scales on the wing veins are all dark except for a small spot of white scales at the base of the costa (C). Tergum I has white scales laterally, terga II–VII have basolateral white spots. In addition, terga III–VI have narrow basal white bands, which widen laterally and do not connect with the spots.

**Male:** The palps are longer than the proboscis with white basal rings on palpomeres II–V. The last two segments are slender and upturned with only a few short setae. The posterior margin of tergum IX has a conspicuous horn-like median projection and a small setose lobe on each side. The gonocoxite is approximately twice as long as wide with a patch of setae on the basomesal area of the dorsal surface (Fig. 10.12). The gonostylus is simple, elongated, distinctly swollen apically and has a few thin setae. The spine of the gonostylus is inserted subapically and is blunt ended. The claspettes are large, lobe-like, and occupy most of the mesal surface of the gonocoxite, with numerous long setae and several stronger setae, a few of which are curved apically.

**Larva:** The head is approximately as long as it is wide. The antennae are about half as long as the head, and without spicules. The antennal seta (1-A) is single and small and situated close to the middle of the antennal

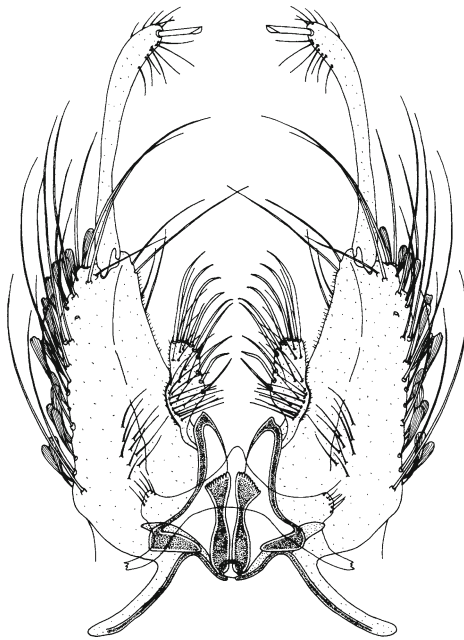


Fig. 10.12 Hypopygium of *Ae. albopictus*

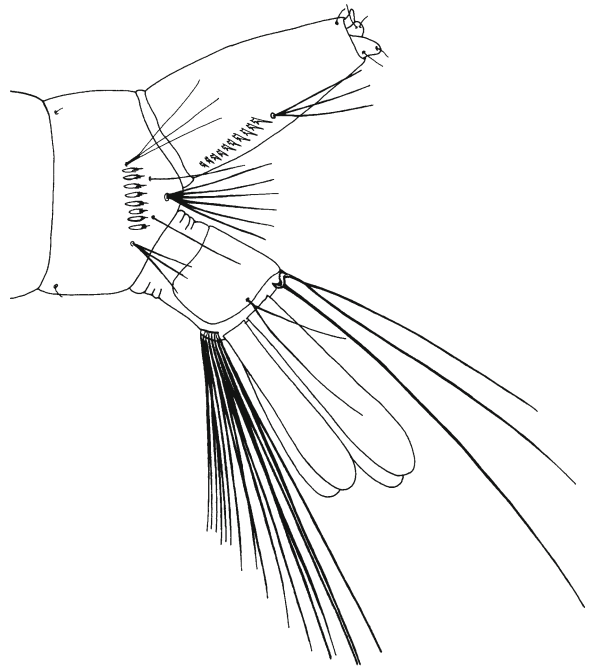


Fig. 10.13 Larva of *Ae. albopictus*

shaft (Fig. 8.62c). The postclypeal seta (4-C) is located close to the anterior margin of the head. It is well developed with 6–15 branches and a short stem. The median frontal seta (6-C) is displaced anteriorly, and has 1–2 branches, the inner frontal seta (5-C) is situated posterior to 6-C, and is longer and single, and the outer frontal seta (7-C) usually has 2–3 branches. The comb consists of 6–13 (usually 8–10) long slender scales in a single row, and each comb scale has a large pointed median spine and fine denticles or fringes at its base. The siphon is short and tapers distinctly from the middle, the siphonal index is 1.7–2.5 (Fig. 10.13). The number of pecten teeth is between 8 and 14, they are evenly spaced, and each tooth usually has 2 lateral denticles. The siphonal tuft (1-S) has 2–4 branches, and is inserted beyond the distalmost pecten tooth, slightly beyond the middle of the siphon (Fig. 8.62d). The saddle extends to the ventral margin of the anal segment, and the saddle seta (1-X) usually has 2 branches, at least one of them distinctly longer than the saddle. The upper anal seta (2-X) has usually 2 branches, which are rarely single, but the lower anal seta (3-X) is single. The ventral brush has 8 tufts of cratal setae (4-X), but the precratal setae are absent. The anal papillae are sausage-like, and about 8 times the length of the saddle.

**Biology:** The immature stages occur in a variety of small natural and artificial containers, *e.g.* in tree-holes, bamboo stumps, coconut shells, rock holes, plant axils or palm fronds, and in flower pots, tin cans, water jars, metal and wooden buckets or drums, broken glass bottles, or discarded motor vehicle tyres (Huang 1972). The eggs are resistant to desiccation, which facilitates their transport in used tyre casings, even over long distances. Continuous breeding throughout the year takes place in tropical and subtropical areas, but in more temperate climatic zones, such as Europe, populations of *Ae. albopictus* are found which show embryonic diapause and overwinter in the egg stage. Several generations per year may occur. Adult females predominantly feed on humans, but may also bite other mammals including rabbits, dogs, cows and squirrels or occasionally avian hosts, *e.g.* Passeriformes or Columbiformes. This feeding behaviour indicates that *Ae. albopictus* is well suited for transmission of a variety of arboviruses that use mammals and birds as their main hosts (Mitchell 1995). To feed on humans the females readily enter dwellings during dusk and night, but may also be found biting during daytime outside houses in shaded areas. *Ae. albopictus* is an abundant species in East Asia causing great nuisance wherever it occurs and, although it was not present before 1990, it

has become a major pest species in some areas of northern Italy.

**Distribution:** In the past *Ae. albopictus* was mainly distributed in the Oriental Region and Oceania and thus it got its popular name, the “Asian Tiger Mosquito”. In the Palaearctic it occurred in Japan and China. In 1985 it was discovered for the first time in the New World (Houston, Texas) and this was the beginning of a rapid spread and discovery of recently introduced populations of *Ae. albopictus* in many parts of the world (Mitchell 1995). It is now present in over 25 states of the USA and in several countries of South America and Africa. Specimens have been found in Australia and New Zealand, but breeding populations have not so far become established there. In Europe, *Ae. albopictus* has probably been present in Albania since at least 1979 (Adhami and Reiter 1998; Adhami and Murati 1987). In the early 1990s it was passively introduced in Italy, owing to the international trade of used tyres which provide a suitable habitat for the eggs. The species was first detected in Genoa in September 1990 (Dalla Pozza and Majori 1992) followed by a rapid spread into other areas of northern and central Italy (Romi 1995). Since 1999 *Ae. albopictus* has been found in various southern and central European countries, including France (Schaffner et al. 2001), Montenegro (Petric et al. 2001), Belgium (Schaffner et al. 2004), Switzerland (Flacio et al. 2004), Greece (Samanidou-Voyadjoglou et al. 2005), Croatia (Klobucar et al. 2006), Spain (Aranda et al. 2006), the Netherlands (Scholte et al. 2007), and Germany (Pluskota et al. 2008). Another way in which the eggs and larvae of *Ae. albopictus* spread was through the trade of the ornamental plant *Dracaena* sp. (“lucky bamboo”). These plants are packaged in standing water during shipment and permit an “ideal insectary in transit” which lead to the introduction of *Ae. albopictus* from Asia to California (Madon et al. 2004).

**Medical importance:** *Ae. albopictus* is a vector of dengue viruses and a competent transmitter for numerous other arboviruses as well as *Dirofilaria immitis* (dog heartworm).

### ***Aedes (Stegomyia) cretinus* Edwards 1921** **[*Stegomyia cretina*]**

**Female:** The proboscis is completely dark scaled, the palps are about 1/4 the length of the proboscis with

whitish scales dorsally on the apical half and dark scales ventrally. The clypeus is bare, and the pedicel has white scales anteriorly. The vertex has a broad median stripe of broad white scales, and the occiput has a lateral stripe of broad white scales and extensive broad white scaling below, all dark scales are broad and flat. There are narrow white scales at the eye margin, and the erect forked scales are dark. The scutum has narrow dark scales, and an acrostichal stripe of narrow white scales extends from the anterior margin to the beginning of the prescutellar area, where it forks and ends just before the margin of the scutellum (Fig. 6.26b). Dorsocentral white stripes are present on the posterior part of the scutum extending from just posterior to the level of the scutal angle to near the lateral lobes of the scutellum; these stripes are narrow and composed of narrow white scales. The scutum is bordered with a lateral prescutal stripe of narrow white scales which reaches the scutal angle, where after a minute break it continues on with broad white scales and terminates with a few narrow white scales just before the margin of the lateral lobes of the scutellum. The scutellum has broad white scales on all the lobes and a small apical area of dark scales on the mid lobe. The ante- and postpronotum are largely covered with broad white scales. The pleurites have several patches of broad white scales, some of them very densely scaled. The wing veins are dark scaled except for a conspicuous basal spot of pale scales on the costa (C). The fore femur anteriorly has sparse white scales on the basal half and a small white knee spot, posteriorly it is white and the fore tibia is dark. The fore tarsomeres I and II have basal white rings, and tarsomeres III–V are dark. The mid femur is dark anteriorly except for a few white scales at the base and a conspicuous white knee spot, the mid tibia is dark, the mid tarsomeres I and II with basal white rings, tarsomeres III–V dark. The hind femur anteriorly is white almost to its apex and has a conspicuous white knee spot, the hind tibia is dark, and hind tarsomeres I–III have a basal white ring, and hind tarsomere IV has an extreme tip which is dark, but tarsomere V is white. Abdominal terga II–IV have narrow white basal bands slightly constricted in the middle and not connected to the broad lateral white patches. Sterna II–IV are largely covered with white scales, and sterna V–VII with basal white bands.

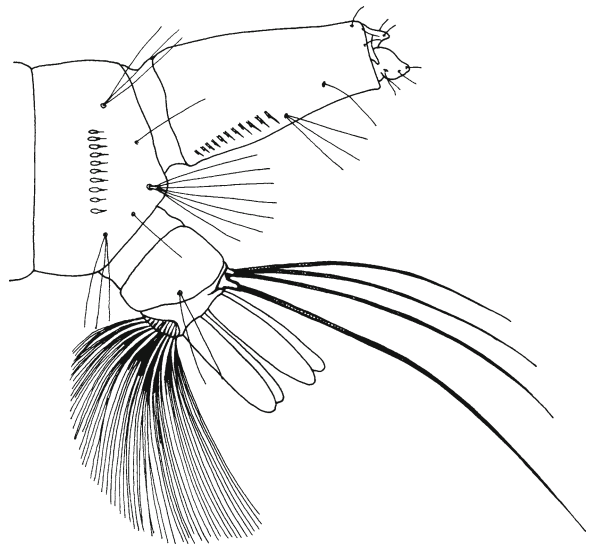
**Male:** The median part of tergum IX is evenly rounded, and the small lateral lobes are strongly



**Fig. 10.14** Hypopygium of *Ae. cretinus*

sclerotized with 2–4 fine setae. The gonocoxite is oblong, the gonostylus is long and slender, and dilated at the apex where it bears a number of fine setae and a long, slender subapical spine (Fig. 10.14). This spine is markedly longer and more slender than that of *Ae. albopictus* (Mattingly 1954). The claspettes narrow apically, and are covered with setae of varying length, but no specialized setae are present.

**Larva:** The antenna is smooth, and the antennal seta (1-A) is minute and single, located at just beyond midway from the base. The outer frontal seta (7-C) is single. The comb has 9–13 scales arranged in one row with exceedingly minute basal denticles. The siphonal index is about 2.0 (Fig. 10.15). The pecten has 10–13 teeth, some with the main denticle longer and more finely drawn out. The siphonal tuft (1-S) has 3 branches, located near the middle of the siphon with a single seta situated laterally in the apical third (Fig. 8.61a). The saddle reaches the ventral margin of the anal segment and has a smooth distal edge, the saddle seta (1-X) is single or 2-branched, the upper anal seta (2-X) has 2–3 branches, and the lower anal seta (3-X) is single. The anal papillae are longer than the saddle, but at most 2/3 of the length of the siphon.



**Fig. 10.15** Larva of *Ae. cretinus*

**Biology:** Little appears to be known about the biology of *Ae. cretinus* (Lane 1982). Gutsevich et al. (1974) reported that larvae were found in tree-holes together with those of *An. plumbeus*, *Oc. geniculatus*, and *Or. pulchripalpis* and that adult females were biting humans in shaded areas.

**Distribution:** Cyprus, Greece, Crete, Georgia and Turkey.

## 10.2 Genus *Ochlerotatus* Lynch Arribalzaga

This genus was established by the division of the composite genus *Aedes* into 2 genera, *Aedes* and *Ochlerotatus*, and restoration of the former subgenus *Ochlerotatus* of genus *Aedes* to generic rank. A further subdivision of *Ochlerotatus* into two sections is proposed (Reinert 2000c). This classification is primarily based on female and male genitalic characteristics, e.g. the aedeagus in the male hypopygium consisting of two lateral plates that usually bear teeth laterally and/or apically in *Aedes*, and the aedeagus being simple and scoop-like, trough-like, or tube-like in *Ochlerotatus*. Supplemental features supporting the partition of the genera include characteristics of the fourth-stage larvae and pupae. For a detailed morphological description

and delimitation of *Aedes* and *Ochlerotatus*, see Reinert (2000c). Of the European mosquito species, members of the subgenera *Finlaya*, *Ochlerotatus* and *Rusticoides* are included in *Ochlerotatus*, all of which belong to Sect. 10.1.

### 10.2.1 Subgenus *Finlaya* Theobald

The subgenus comprises medium to large species. The length of the palps in the females ranges from very short to 2/3 the length of the proboscis. The head has a mixture of narrow and broad scales, with erect forked scales on the vertex and occiput. The scutum has prominent white and dark scale patterns and variable setation, the dark scales are black or have a metallic tinge. The pleurites have patterns of white scales, and are more extensively scaled in females than in males. The mesepimeral setae are sometimes absent. White knee spots are present, and all tarsomeres are dark. The wing veins are covered with dark scales. The abdominal terga have more or less distinct lateral white patches, sometimes with white basal bands, and the cerci are short and blunt. The length of the male palps varies from half the length of the proboscis to much longer than it. Tergum IX has well developed lobes and more or less spine-like setae. The hypopygium has an elongated gonocoxite, without distinct basal or apical lobes but defined fields of setation. The gonostylus is long and slender, and the claspette is divided into a stem and a filament of different shapes. The paraproct is usually heavily sclerotized, and the aedeagus is pear shaped. The head of the larva is rounded, and the antenna is usually shorter than the head, with a single antennal seta (1-A). The frontal setae (5-C to 7-C) are single or may have 2 branches. The abdominal segments are usually covered with stellate setae. The comb scales are large and arranged in a single row. The saddle does not encircle the anal segment, and precratal tufts are present. The ventral and dorsal anal papillae are of different sizes.

The subgenus with its nearly 200 species is one of the largest in the genus *Ochlerotatus*. It is mainly distributed in Asia, Australia, and Africa. It is a polymorphic subgenus of which a species of North and South America has been revised and placed in other subgenera (Zavortink 1972). It is doubtful whether the two

European species, *Oc. echinus* and *Oc. geniculatus*, really belong to *Finlaya*.

#### ***Ochlerotatus (Finlaya) echinus* Edwards 1920 [*Dahlia echinus*]**

**Female:** Closely related to *Oc. geniculatus* with a similar morphology in all stages. The female of *Oc. echinus* differs from that of *Oc. geniculatus* by the patterns of scaling on the head, thorax, abdomen and legs. The proboscis and palps are dark scaled, the head has black setae, the scales on the vertex are dark and with lateral white patches, and there is no white scale border around the eyes. The scutum has two dorsocentral stripes of narrow dark bronze scales divided by a creamy white acrostichal stripe. The supraalar dark scale patch is nearly fused with the dorsocentral stripes. The scutellum has broad whitish scales. The postpronotal scales are whitish, and the mesepisternum and mesepimeron have patches of creamy scales, distinct against the dark integument. The femora have a white ventral stripe and white knee spot. The tibiae and tarsomeres are entirely black scaled. The wings have rather narrow blackish scales. The abdominal terga are dark scaled with narrow white basal bands, sometimes interrupted in the middle from tergum V on, and on all segments extended laterally into triangular whitish patches. Abdominal segment VIII is broad, and the cerci are short and rounded.

**Male (Fig. 10.16):** The general shape of the hypopygium is similar to that of *Oc. geniculatus* except for the denser setation of the gonocoxite toward the tip. The claspette stem has a stout seta near the middle and some thin setae at the base, and the claspette filament is hook-like.

**Larva:** The antenna is more than half as long as the head (longer than in *Oc. geniculatus*), smooth and is not covered with spicules. The antennal seta (1-A) is single (Fig. 8.58a). The inner frontal seta (5-C) is single, the median frontal seta (6-C) is single or double and the outer frontal seta (7-C) usually has 2–4 branches. Branches of the stellate setae on abdominal segment I are longer than the length of the segment (Fig. 8.59a). The number of comb scales is 11–18, arranged in one row. Each scale is elongated with lateral spines, and a prominent median spine is absent. The siphonal index is 2.5–3.6, and the acus is well



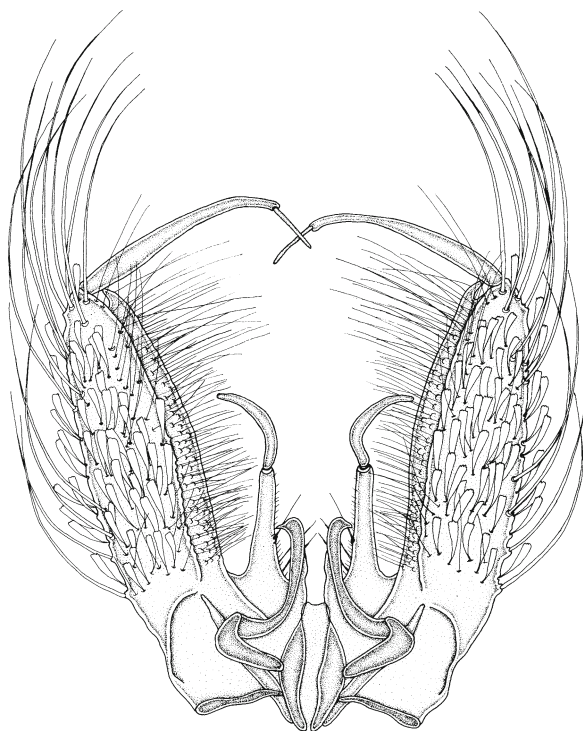


Fig. 10.16 Hypopygium of *Oc. echinus*

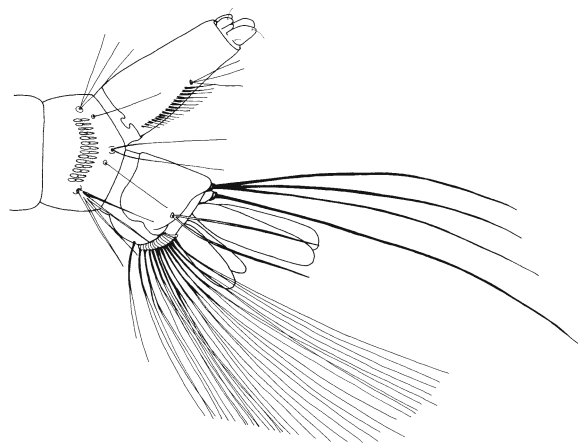


Fig. 10.17 Larva of *Oc. echinus*

developed (Fig. 10.17). The pecten has 15–27 teeth and occupies at least the basal half of the siphon, each tooth is very long and spine-like. The siphonal tuft (1-S) is inserted beyond the middle of the siphon, with 2–4 branches. The anal segment is not entirely encircled by the saddle, and 1–2 precratal tufts (4-X) are present. The anal papillae are broad and long, and the dorsal pair is twice as long as the ventral pair.

**Biology:** Larvae have been found in the same habitats as *Oc. geniculatus*. In Anatolia and Bulgaria they may also occur in root holes of olive trees. Not much is known of the biology of the larvae, they are supposed to feed on microorganisms in the tree-holes in the same way as the larvae of *Oc. geniculatus* do. In Portugal, larvae and adults were found in August and September (Ribeiro et al. 1988).

**Distribution:** In Europe this species is confined to the Mediterranean region and has been reported in Portugal, Italy, Greece, and Bulgaria where it has been found in abundance along the Black Sea coast.

**Note on systematics:** Edwards (1920) originally placed this species in the subgenus *Ochlerotatus* and transferred it to *Finlaya* later (Edwards 1932). The same considerations regarding the subgenus affiliation

of *Oc. geniculatus* also apply to *Oc. echinus*. As the types of most of the synonymous species of *Oc. geniculatus* are lost, apparent synonymy to *Oc. echinus* is not resolved. From Madeira and the Canary Islands another species within the subgenus *Finlaya*, *Oc. eatoni* Edwards, has been reported (Knight and Stone 1977).

#### ***Ochlerotatus (Finlaya) geniculatus* (Olivier 1791) [*Dahlia* *geniculata*]**

**Female:** Dark scales with a violet tinge especially on the abdomen, the white and blackish pattern of the scutum, the conspicuous white knee spots and the blunt cerci immediately distinguish the females from all other females of the genus *Ochlerotatus* except the closely related *Oc. echinus*. The proboscis and palps are black scaled, and the vertex is dark with a median light stripe and a narrow band of whitish scales around the eyes. The scutum has two dorsocentral black stripes which sometimes fuse into one anteriorly, or are otherwise completely separated by a pale acrostichal stripe. The submedian and lateral areas of the scutum have creamy or silvery grey scales. Dark anterior and posterior submedian stripes are present, and the scutellum has narrow yellowish scales. The pleurites have patches of broad, whitish scales. The legs are dark, the femora have a white knee spot, and the tibiae and tarsomeres are entirely black scaled. The fore and mid claws have a subbasal tooth. The wing veins are covered with dark brownish scales. The abdominal terga are black scaled with conspicuous white triangular lateral patches on

segments II–VII. Sternum VIII is unusually broad, and the cerci are broad and rounded (Fig. 6.37a).

**Male:** Tergum IX has somewhat elongated lobes and 4–5 spine-like setae on each lobe. The hypopygium superficially resembles that of the species of the *Ochlerotatus* Excrucians Complex by lacking a basal lobe, but is distinguished from it by the absence of an apical lobe (Fig. 10.18). The long and evenly tapered gonocoxite has two areas of dense setation, a basal one with shorter setae, and an apical one with long setae. The claspette stem is short with several setae, and the filament is narrow and somewhat shorter than the stem. The paraproct is heavily sclerotized and bent at the tip, and the aedeagus is pear shaped.

**Larva:** The larvae are distinguished from those of all other subgenera of *Aedes* and *Ochlerotatus* (except *Oc. echinus*) by the numerous stellate setae on the thorax and abdomen, the broad and unequally long anal papillae and the single row of large comb scales. The antenna is half as long as the head, smooth, and not covered with spicules (Fig. 8.24b). The antennal seta (1-A) is usually single. The inner frontal seta (5-C) is usually single, the median frontal seta (6-C) has 1–2 branches and the outer frontal seta (7-C) has 2–4 branches. All setae on the prothorax are 2-branched, except for 2-P. Most of the setae on the rest of the thorax and abdomen are of the stellate

type. The branches of the stellate setae on abdominal segment I are about the same length as the segment (Fig. 8.59c). The number of comb scales is 8–15 arranged in a single row. Each individual scale is elongated with a swollen base, a strong median spine, and small lateral spines at the base. The siphonal index is 2.3–3.2 (Fig. 10.19). The number of pecten teeth is 15–19, and each tooth is long, spine-like, with a few indistinct denticles at the base. The pecten usually occupies less than the basal half of the siphon. The siphonal tuft (1-S) is situated at about the middle of the siphon or slightly below it, with 4–5 branches. The anal segment X is not completely encircled by the saddle, and the latter has a row of long microtrichia at the distal part. The ventral brush is made up of 7–10 cratal tufts (4-X) and 1–2 precratal tufts. The anal papillae are broad and longer than the saddle, with the ventral pair being shorter.

**Biology:** The larvae live in tree-holes at various heights and in open tree stumps of different deciduous trees as *Quercus* sp., *Fagus* sp., *Alnus* sp., *Betula* sp., and *Juglans* sp. They also occur in mixed forests in old trees and can occasionally be found in ground pools together with *Or. pulcripalpis*, *An. plumbeus*, and *Oc. pulcritarsis*, but very rarely in coniferous forests. The species hibernates in the egg stage in northern areas, and in the larval stage in more southern regions. The

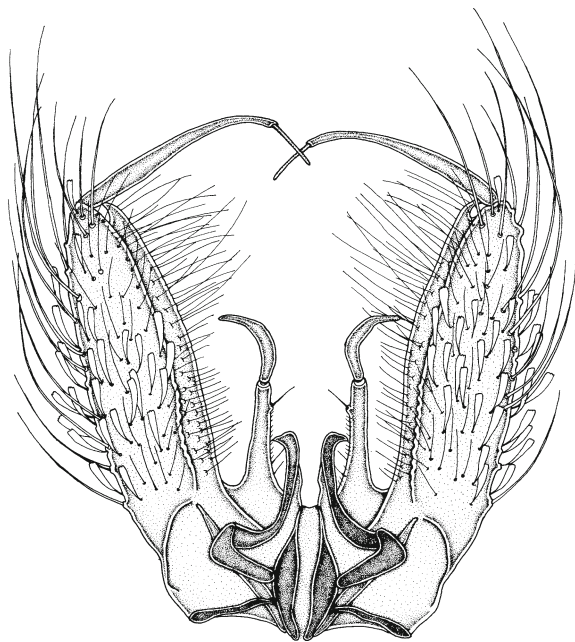


Fig. 10.18 Hypopygium of *Oc. geniculatus*

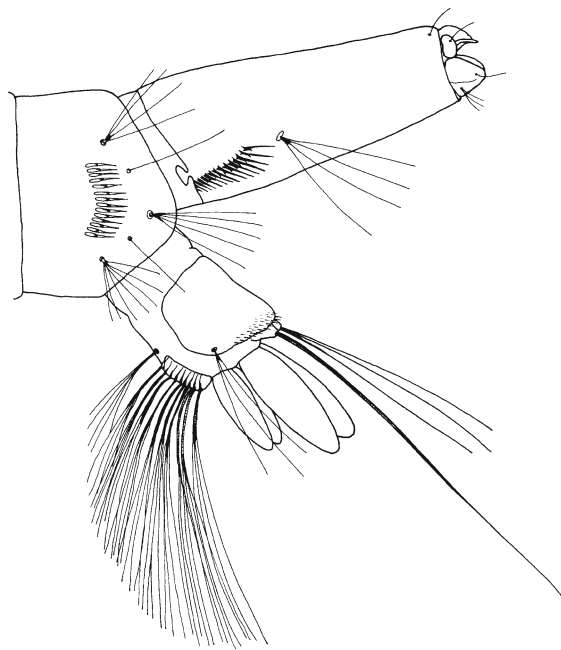


Fig. 10.19 Larva of *Oc. geniculatus*

adults appear during the summer as the development depends on spring and summer rains collected in the tree-holes. Females are day and crepuscular biters and readily feed on humans. In southeastern Europe they occur in masses, viciously attack humans in the open, but rarely enter urban areas.

**Distribution:** Found in the Palaearctic region, known in most European countries, and its northernmost limit follows that of deciduous or mixed forests. In the Mediterranean region it is reported in northern Portugal, Sardinia, Italy mainland, Greece, and extends east to the Caucasus. Also reported from North Africa to Asia Minor.

### 10.2.2 Subgenus *Ochlerotatus* Lynch Arribalzaga

The flagellomeres of the female antennae are prolonged distally, the palps are only 1/3 to 1/4 the length of the proboscis, the latter being longer than the fore femur. The scale pattern on the vertex and occiput is variable, often with numerous erect forked scales on the occiput and mixed narrow and broad scales on the vertex and along the eye margin. The thorax in most species has a dark grey to dark brown or blackish integument. The scutum is covered with scales and with rows of acrostichal, dorsocentral and supraalar setae. The scutellum has three lobes and groups of setae and a few narrow scales. The pleurites are extensively scaled with patches of mostly pale to whitish scales. Prespiracular setae are absent. A postprocoxal patch of whitish scales is present in some species. The legs are mostly covered with dark scales, but pale scales may be scattered or grouped to form a knee spot or basal or apical rings, mainly on the tarsomeres. All the tarsal claws have an additional subbasal tooth, and the pulvilli are setous or inconspicuous. The wings are predominantly dark scaled, both the costa (C) and subcosta (Sc) may have patches of paler scales, and in some species the wing veins are covered with mixed dark and pale scales. The abdomen has elongated cerci and the usually narrowed last segments give the impression of being pointed. The scaling of the abdomen is extensive on both the terga and sterna. It can be rather uniform or display various patterns of banding or mixed colours. The scale patterns on the thorax, legs, wings, and abdomen are often used for species identification.

The proboscis of the males is often not longer than the fore femur, and the palps are usually longer than the proboscis, but sometimes as long as the proboscis or shorter. The tarsal claws of the front and mid legs have prolonged main and subbasal teeth. Tergum IX always has two more or less expressed lateral lobes which usually bears a group of strong or spine-like setae. The gonocoxite in most species has basal and apical lobes, sometimes one or both less expressed, indistinct, or absent. The gonostylus is simple with an apical spine. The paraproct has pointed tips, sometimes inwardly curved. Typical claspettes are present, divided into a stem and a filament. The aedeagus is pear shaped, elongated, or rounded.

The antennae of the larvae have a multiple-branched antennal seta (1-A), usually inserted at about the middle of the antennal shaft. The lateral palatal brushes are well developed for suspension feeding or brushing. The postclypeal seta (4-C) is inconspicuous, and multiple-branched. The inner frontal seta (5-C) is often situated in front of the median frontal seta (6-C), both pairs being single to multiple-branched. Prothoracic setae 1-P to 7-P are single to 3-branched. The number of comb scales is variable from a few to many, arranged in a single or irregular rows. The siphon is well developed, with siphonal seta (1-S) usually inserted at about the middle of the siphon. The pecten has more or less spaced teeth of significant shape. The saddle partly or fully encircles the anal segment, and the saddle seta (1-X) is usually single. The cratal and precratal tufts (4-X) are well developed. The anal papillae are of variable shape and size.

Of the nearly 200 species of the subgenus described worldwide, more than half are distributed in the Holarctic region and nearly a quarter in each of the Australian and the Neotropical regions. Only a few species are found in the Oriental and African regions.

In the western Palaearctic and throughout Europe, Alphavirus, Flavivirus, and three different groups of Bunyavirus were found in a few isolates from the *Ochlerotatus* species, such as *Oc. cantans*, *Oc. caspius*, *Oc. communis*, *Oc. flavescens*, *Oc. hexodontus*, *Oc. punctator*, and *Oc. sticticus* (Traavik et al. 1985; Lundström 1994; Aspöck 1996). Some other parasites have been reported from the *Ochlerotatus* species in Europe, such as the bacterium *Francisella tularensis*. In North America virus vector capacity is documented for several species of the subgenus (Reeves 1990; Beaty and Marquardt 1996).

Morphological heterogeneity in the subgenus is great; several species groups show distinct characteristics. As long as no worldwide analysis of the subgenus exists, discrepancies of grouping the species within different regions will prevail. Edwards (1932) first revised and designed species groups and subgroups of *Ochlerotatus* on a worldwide base including Holarctic, South American, Oriental, and Australian species. A complete grouping of all European members of the subgenus *Ochlerotatus* does not exist so far. Based on the suggestions for the species grouping given for the Palaearctic region (Martini 1931), the Fennoscandian region (Natvig 1948), Germany (Mohrig 1969), and the former USSR (Gutsevich et al. 1974), the following classification is given regarding the European species which are included in the keys.

### Annulipes Group

Large to medium sized mosquitoes. The tarsi have pale basal rings, which are broad at least on some tarsomeres. Identification of the females is sometimes very difficult and arbitrary. The basal lobe of the gonocoxite is never constricted at the base. The apical lobe is well developed. The claspette filament is winged. The larvae usually have 4–6 precratal setae (4-X). The species included in this group are: *annulipes*, *behningi*, *cantans*, *cyprius*, *euedes*, *excrucians*, *flavescens*, *mercurator*, *riparius*, *surcoufi*.

### Caspus Group

The tarsi have pale rings embracing two tarsomeres, the apex of one and the base of the following tarsomere. The apical lobe of the gonocoxite is weakly developed or absent. The species included in this group are: *berlandi*, *caspus*, *dorsalis*, *mariae*, *pulcritarsis*, *zamitii*.

### Communis Group

Usually medium sized mosquitoes. The tarsi are entirely dark scaled. The claspette filament is differentiated into a well sclerotized ridge and weakly sclerotized transparent wings. The species included in this group are: *cataphylla*, *coluzzii*, *communis*, *detritus*, *hungaricus*, *impiger*, *nigrinus*, *leucomelas*, *pionips*, *sticticus*.

### Intrudens Group

The tarsi are entirely dark scaled, but particular differences can be found in the male hypopygium,

which are unique for this group. The basal lobe of the gonocoxite has 3 setae distinctly larger than the rest. One basal seta is well separated from the two distal setae. Dense long setae cover the inner surface of the apical half of the gonocoxite to a large extent. The claspette stem has a thorn shaped process or is distinctly swollen and bent at about the middle. The species included in this group are: *diantaeus*, *intrudens*, *pullatus*.

### Punctor Group

The tarsi are entirely dark scaled, but members of this group differ from the others by the structure of the abdominal segment X of the larvae. The saddle completely or almost completely surrounds the anal segment leaves a very narrow gap ventrally. In the male hypopygium the claspette filament is evenly sclerotized without a narrow transparent wing. The species included in this group are: *hexodontus*, *nigripes*, *punctor*, *punctodes*.

### *Ochlerotatus (Ochlerotatus) annulipes* (Meigen 1830)

**Female:** The general colouration of the integument is more brownish and the scaling more yellowish than in *Oc. cantans*, but less golden than *Oc. riparius*. The females of these three species resemble each other superficially, however the scale pattern of the scutum is different. The proboscis of *Oc. annulipes* is predominantly creamy white with mixed dark scales. The palps have mixed dark and pale scales, and a sometimes distinct basal light ring. The head has bronze scales and a lateral patch or stripe of creamy white scales. The scutum has a defined median stripe of brown or fawn coloured scales, and the lateral parts are covered with cream coloured or greyish scales. The antepnotum and propleuron have whitish scales. The postpronotum in the upper half has narrow bronze scales, and broad yellowish ones in the lower half. A few postprocoxal scales are usually present. A hypostigmal patch is absent, but two distinct patches are present on the sub- and postspiracular areas. The mesepisternum has three distinct patches and a few scattered scales at its upper edge. The mesepimeron is covered with creamy

white scales in the upper half, and a few scales are present on the lower half and on the mesomeron. The coxae have scattered light scales, the femora are mostly yellowish scaled, the front leg occasionally has a dark spot above the knee, the mid leg is somewhat darker on the dorsal side, and all legs have white knee spots. The tibiae are light scaled, especially the fore tibia, or otherwise speckled with dark scales. There are basal rings of whitish scales on tarsomeres I–V, except tarsomere V of the fore legs, which is usually entirely dark scaled. The tarsal rings are variable in width, but usually wider than in *Oc. cantans*. The wing veins are covered with intermixed dark and pale scales, the pale scales are usually more yellowish than in *Oc. cantans*. The abdomen has basal white bands on terga I–VII, the last segments rarely have very narrow apical bands. All the terga have some light scales mixed among the darker ones (Fig. 6.34b). The sterna are usually yellowish scaled, with some speckled dark scales.

**Male:** Tergum IX has two well developed lateral lobes, each with 4–6 strong setae. The basal lobe of the gonocoxite is indistinct or absent, without strong spine-like setae, but numerous thin setae (Fig. 10.20). The apical lobe is well developed. The gonocoxite bears a large amount of long and dense setae at the distal part of its inner ventral surface. This is a unique character within the species of the Annulipes Group. The gonostylus is curved, and tapers towards

the apex. The claspette stem is stout and slightly swollen at the apex, the filament is broad and less than half as long as the stem, and slightly swollen beyond the middle.

**Larva:** Very similar to that of *Oc. cantans* but with a more tapered distal part of the siphon. The antennae are shorter than the head, and the antennal seta (1-A) has 3–4 branches, inserted at about the middle of the antennal shaft. The inner and median frontal setae (5-C and 6-C) have 2–3 branches. The prothoracic formula 1-P to 7-P is as follows: 1 (short, single to 2-branched); 2 (moderately long, single); 3 (long, single); 4 (shorter than 2-P, single); 5 and 6 (single, long); 7 (long, 3–4 branches). The number of comb scales ranges between 30 and 40, and each scale has a long median spine (Fig. 10.21). The siphonal index is usually less than 3.0, and the pecten teeth are similar to that of *Oc. cantans*. The siphonal tuft (1-S) has 5–7 branches, situated at about the middle of the siphon. Seta 9-S is prominent, but not stout. The saddle does not encircle the anal segment but covers 2/3 of its lateral sides. There are usually six or more precratal tufts (4-X) present, which separates the larvae from those of *Oc. cantans*, that usually have less precratals. The anal papillae are about as long as the saddle or longer.

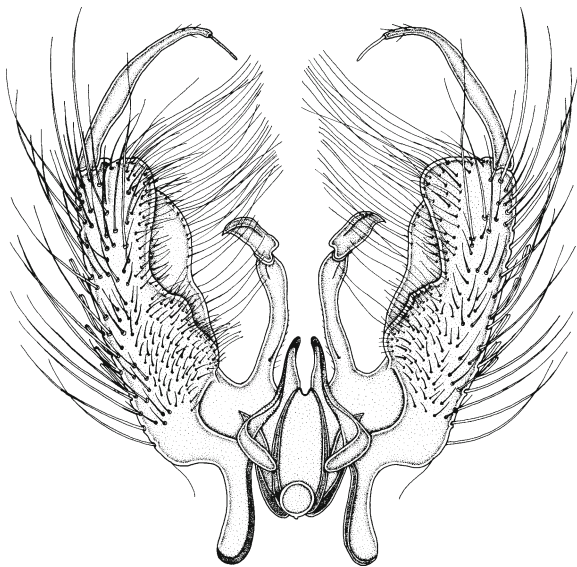


Fig. 10.20 Hypopygium of *Oc. annulipes*

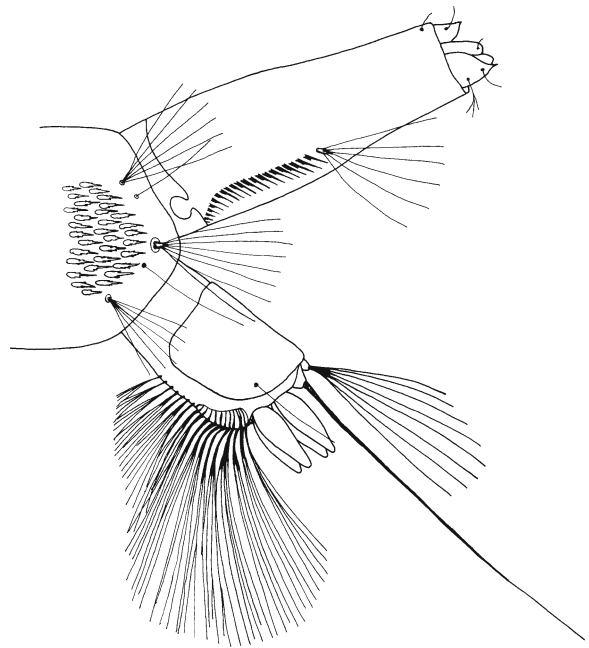


Fig. 10.21 Larva of *Oc. annulipes*

**Biology:** *Oc. annulipes* is a monocyclic species occurring in spring. It is widespread throughout Europe, but most abundant in the central parts of the continent, where it can be very dominant locally. It overwinters in the egg stage and the larvae occur at the same time as *Oc. cantans* or slightly later. They breed in open meadow pools, at forest edges and inside deciduous forests, preferably in semipermanent pools with leaf detritus, where they are often found together with larvae of *Oc. cantans*. In more open habitats they occur together with larvae of *Oc. flavescens*, *Oc. riparius* and *Oc. excrucians*. The males are found around the breeding sites for several days after emergence. The females are day biters with a crepuscular activity in the areas of high abundance. They are present over several weeks to months during late spring and summer depending on local climate.

**Distribution:** *Oc. annulipes* is a western Palaearctic species reported from southern Scandinavia to the Mediterranean region. The separation of larvae and females of *Oc. cantans* and *Oc. annulipes* from different areas may be difficult because of the variation of characteristics such as colouration and setation.

**Medical importance:** Tahyna virus has been isolated from *Oc. cantans/annulipes* in Austria (Lundström 1999).

### ***Ochlerotatus (Ochlerotatus) behningi* (Martini 1926)**

**Female:** A medium to large species. *Oc. behningi* has a more pronounced blackish integument and setation than other members of the Annulipes Group. The setae on the pleurites rarely have a golden shine. The proboscis is dark, with intermixed creamy scales along its whole length, and mostly pale scales in the middle. The palps are dark scaled, mixed with a few white scales. The antennae are covered with very dark setae, the pedicel with some white scales. The vertex is covered with creamy scales intermixed with some black ones, forming an indistinct patch, and the occiput has a mixture of narrow golden and dark erect scales and a broad midstripe of yellowish flat scales. The scutum is usually entirely covered with small, narrow golden bronze or rust coloured scales, sometimes indistinct creamy white posterior submedian stripes are present, and the scutellum is covered with dark scales. The antepronotum has a few bronze

scales and the propleuron has some whitish scales. The postpronotum has narrow scales, more than two third of them are bronze, the rest are creamy coloured. A postprocoxal scale patch is present. The sub- and postspiracular areas have a continuous whitish scaling. The mesepisternum has three distinct patches, an upper patch with creamy scales, a prominent median patch reaching the anterior margin and a small lower patch along the posterior border of the pleurite. One patch covers the upper half of the mesepimeron. The coxae have prominent light scale patches. The femora have mixed whitish and dark scales, with white knee spots. The tibiae as well as tarsomeres I have a somewhat darker pattern. Tarsomeres II–IV have broad whitish basal bands covering up to half of their length, and tarsomere V is dark with a few light scales on the fore and mid legs. The wing veins have dark scales intermixed with a few or many creamy white scales. Some amount of colour variation in scaling might be expected in the females. The abdominal terga are predominantly covered with brownish scales, with scattered creamy white or yellowish scales. Pale scales usually form diffuse median patches, and sometimes short longitudinal stripes (Fig. 6.31a). Transverse pale basal or apical bands are absent. The sterna have dominantly black scales and a few white basal, narrow bands or lateral patches. The cerci have a few white scales among the dark ones.

**Male:** Tergum IX has a deep notch and the protruding lateral lobes have numerous thin setae. The gonocoxite is short and stout, with a conical basal lobe which bears many setae of uniform length and width (Fig. 10.22). The apical lobe is well developed. The gonostylus is curved apically, and is narrow with a slender apical spine. A relatively shorter claspette stem differentiates the species from *Oc. cantans*, *Oc. riparius*, *Oc. flavescens* and *Oc. excrucians* males. The claspette filament is narrow, and approximately as long as the stem.

**Larva:** Similar to that of *Oc. excrucians* and often it is difficult to distinguish between the species. The antennae are short with many distinct spicules, and the antennal seta (1-A) is located below the middle of the antennal shaft. The postclypeal seta (4-C) has 6–8 branches (Fig. 8.53a). The inner frontal seta (5-C) has 2–4 branches, and the median frontal seta (6-C) has 2–3 branches. A report on material from the Don Basin referred to both setae as being double. The prothoracic formula 1-P to 7-P is as follows: 1 (double, short, thin);

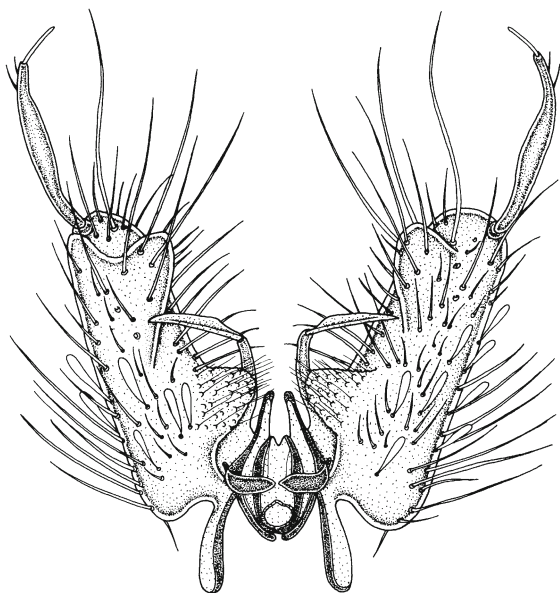


Fig. 10.22 Hypopygium of *Oc. behningi*

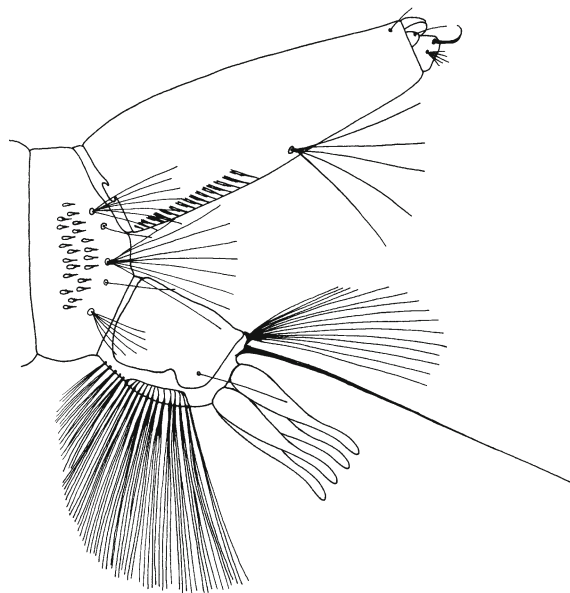


Fig. 10.23 Larva of *Oc. behningi*

2–4 (single); 5 (double); 6 (single); 7 (triple). The number of comb scales is 18–28 (usually 20–24) arranged in irregular rows (Fig. 10.23). Individual scales have a very protruding median spine and insignificant lateral spines. The siphon is more or less tapered at the apex, and the siphonal index is 3.0–4.0. The pecten teeth are usually evenly spaced, sometimes the two distalmost teeth may be detached apically. Each pecten tooth has a few lateral denticles at its base. The siphonal tuft (1-S) is inserted beyond the middle of the siphon, with 5 branches. The saddle does not encircle the anal segment but covers most of it. Five to 6 precratal tufts (4-X) are present, and the anal papillae are at least as long as the saddle.

**Biology:** The species belongs to the early summer species and seems to be monocyclic. Little is known about its general biology. Martini (1931) reported mass occurrence of females as fierce day biters in open spaces from the plains along the Volga river in the Saratov area. The species occurred later than *Ae. vexans*. Females were also found in higher mountainous areas, this could indicate that the females may fly long distances to search for a blood meal.

**Distribution:** The distribution is not very well known. It has been recorded in Russia, Ukraine, Slovakia and Poland which seem to confine the species to a eastern European distribution. It has not been reported from the East Palaearctic.

**Note on systematics:** As no male belongs to the type series (Martini 1931), the missing characteristics and the amount of variation need further studies. The species certainly needs a closer analysis of female, male and larval characteristics and comparisons with species of similar colouration.

#### ***Ochlerotatus (Ochlerotatus) berlandi* (Seguy 1921)**

**Female:** The palps are predominantly dark scaled, with scattered pale scales in the middle, and the tip of the palps is white scaled. The vertex is pale to whitish with some diffuse darker scales which are more numerous at the occiput forming a dark triangular patch. The eye margin is covered with long setae. The scutum is mainly covered with pale golden scales, with distinct patches and stripes of dark brown scales. Pale golden scales form a broad median stripe and two lateral stripes. Dark scales form distinct patches on the anterior and posterior submedian areas. Dark posterior dorsocentral stripes extend from the transverse suture to the end of the scutum. Post- and subspiracular scale patches are present. The mesepisternum has a prealar scale patch, and the mesepisternal patch is divided into a larger upper portion and a smaller lower portion. The mesepimeron has two whitish scale patches on its

upper half. The femora and tibiae are dark scaled, but scattered pale scales may be present. The tarsi have white apical and basal rings usually present on tarsomeres I and II of the front and mid legs, and on tarsomeres I–III of the hind legs. Tarsomere V of all legs is entirely pale scaled (Fig. 6.20a). The wing veins are dark scaled; rarely a few isolated pale scales may be present. The abdominal terga have creamy white basal bands which are usually slightly widened laterally, and sometimes expanded into triangular patches. The sterna are black scaled, with more or less developed pale lateral patches, sometimes almost connected in the middle.

**Male:** The hypopygium is very similar to that of *Oc. pulcritarsis*, no constant differences can be found (Fig. 10.24). *Oc. berlandi* has the lobes of tergum IX well developed and widely separated, and each lobe bears 5–8 spine-like setae. The gonoxocite is about three times as long as it is broad. The basal lobe is weakly developed with one strong, apically recurved, spine-like seta (Fig. 7.38b). The other setae on the basal lobe are thin and of variable length. The apical lobe is indistinct. The gonostylus is somewhat widened in the middle, with a slender apical spine. The claspette filament is slender and longer than the stem.

**Larva:** The head is broader than long, and the antennae are almost as long as the head. The antennal

tuft (1-A) is inserted beyond the middle of the antennal shaft. The postclypeal seta (4-C) is well developed and multiple branched. The inner frontal seta (5-C) is the most prominent of all the frontal setae, and usually has 9 branches. The median frontal seta (6-C) is situated almost at the same level as 4-C, is less developed than 5-C, and usually has 8 branches. The outer frontal seta (7-C) is well developed, long and usually has 10 branches. The comb usually has 16–20 comb scales arranged in several irregular rows, each scale with a well developed median spine. The siphon is very long and slender, the siphonal index is 5.5–7.8 (Fig. 10.25). The pecten consists of 19–29 small and blunt teeth. The siphonal tuft (1-S) is long, more than twice the width of the siphon at the point of its origin, and is inserted distinctly below the middle of the siphon, with 3–5 branches. The saddle reaches far beyond the half of the lateral sides of the anal segment. The saddle seta (1-X) is much longer than the saddle, and is single. The upper anal seta (2-X) has 4–6 branches, the lower anal seta (3-X) is single, and

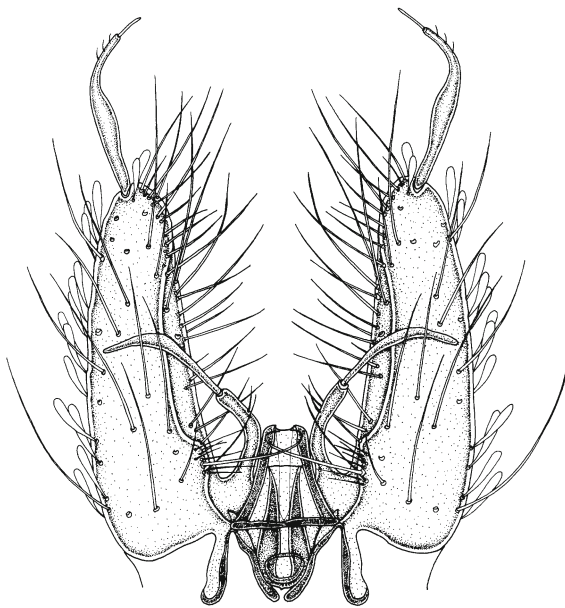


Fig. 10.24 Hypopygium of *Oc. berlandi*

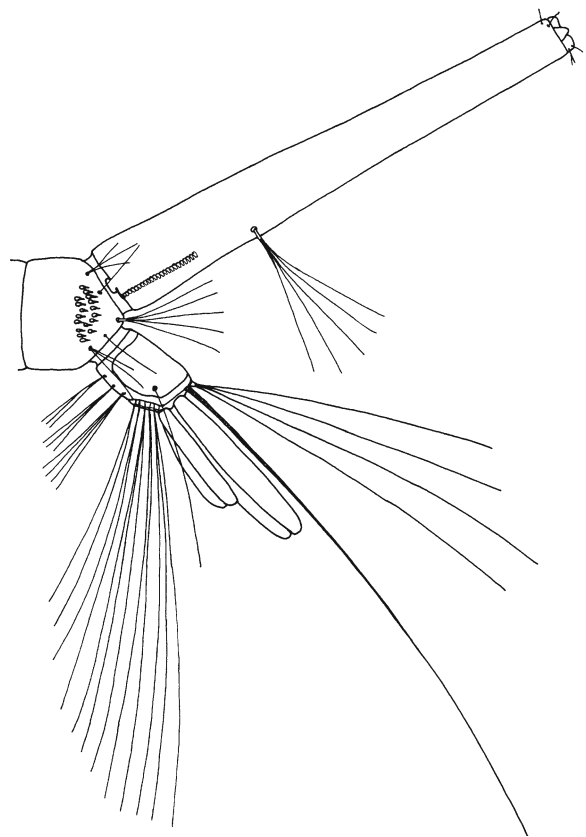


Fig. 10.25 Larva of *Oc. berlandi*



about as long as the siphon. The ventral brush has 3 precratal setae (4-X). The anal papillae are elongated, sausage shaped, and much longer than the saddle, with the dorsal pair being longer than the ventral pair.

**Biology:** The species hibernates in the larval or pupal stage and usually has two generations per year. Larvae can be exclusively found in tree-holes, very often in those of *Platanus orientalis*, *Quercus ilex*, *Q. suber* and *Sophora japonica*, preferably in alkaline waters rich with organic materials. They can usually be found in association with larvae of *Oc. echinus*, *An. plumbeus* and *Or. pulcripalpis* (Ramos 1983). Under laboratory conditions, at a temperature of 24°C, larval development lasts for 24 days and the pupal stage for about 7 days. Under natural conditions, the larval development lasts for approximately 4 months (Ramos 1983). Adult females are mainly zoophilic, but readily bite humans either outside or inside human habitations (Ribeiro et al. 1988).

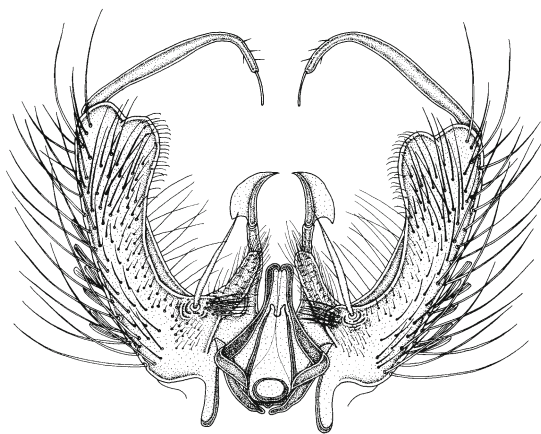
**Distribution:** *Oc. berlandi* is endemic to the Mediterranean region and has been recorded in Portugal, Spain, France, Italy, Greece, and in Morocco, Algeria, and Tunisia. It was the prevalent tree-hole species (together with *Oc. geniculatus*) of Sardinia during a five year survey (Marchi and Munstermann 1987).

### ***Ochlerotatus (Ochlerotatus) cantans* (Meigen 1818)**

**Female:** Its greyish integument with dominant dark, blackish brown scaling and fewer scattered white or yellowish white scales on the body and wings distinguishes *Oc. cantans* from *Oc. annulipes*, which has a more yellowish scaling. The white rings on the legs are not as broad as in *Oc. annulipes*, *Oc. behningi*, and *Oc. riparius*. The proboscis has no or few white scales. The palps are dark with a few white scales at the tip. The clypeus has a dark brown integument. The antennae have dark segments with brownish setae, and the pedicel has a few white scales. The vertex is white scaled with a lateral spot of brown scales. The occiput has brownish scales and two median stripes of white scales. The colouration of the scutum is very variable. Typically it is covered with dark brown or bronze brown scales, the lateral parts with greyish white or creamy scales, but sometimes these scales are light brown. A pair of distinct whitish submedian patches

are usually present just beyond the scutal angle and sometimes narrow whitish submedian stripes run from the patches to the posterior margin of the scutum (Fig. 6.35a). The scutellum has white and brown scales and light setae. The postpronotum has yellow and narrow upper scales, white and somewhat broader lower scales; and the postprocoxal membrane is bare. The white patches of the post- and subspiracular areas are fused. The mesepisternum has one upper and two distinct lower patches of white scales, and the mesepimeron has a patch of white scales. The coxae have white scales, and the femora and tibiae have mixed dark and pale scales. Tarsomere I of all the legs has more or less mixed scales, tarsomeres II–V have moderately broad white basal rings (Fig. 6.18a), except tarsomere V of the fore legs which is entirely dark scaled. The wings are predominantly dark scaled. Usually a few white scales are scattered on the costa (C) and on some other veins. Abdominal terga I–VIII have white basal bands, sometimes narrow and indistinct. The apical parts of the terga have more or less numerous scattered pale scales (Fig. 6.28b). Sterna I–VIII are whitish with darker lateral patches, and the cerci are predominantly dark scaled and elongated.

**Male:** The lobes of tergum IX have numerous strong setae. The hypopygium is of a different shape to that of the other members in the Annulipes Group (Fig. 10.26). The basal lobe of the gonocoxite is slender, and distinctly elongated with a large spine-like seta at the base. The apical lobe is present, and covered with short setae. The gonostylus is long with a curved tip. The paraproct is long and narrow. The claspette filament is shorter than the stem, prominently



**Fig. 10.26** Hypopygium of *Oc. cantans*

winged, and at least as long as it is wide. The shape of the spine-like seta of the basal lobe and the claspette filament seem to be variable characteristics. The aedeagus is elongated and tapered.

**Larva:** The antennae are shorter than the head, and the antennal tuft (1-A) is inserted slightly beyond the middle of the antennal shaft. The inner frontal seta (5-C) has 3–5 branches, the median frontal seta (6-C) has 2–3 branches and the outer frontal seta (7-C) has 7–8 branches. The pthoracic formula of setae 1-P to 7-P is as follows: 1 (short, double to triple); 2 (medium long, single); 3 (very long, single); 4 (short, single); 5 and 6 (long, single); 7 (long, triple). The number of comb scales is 28–40 (usually about 35) arranged in an irregular patch. Each scale has a moderately long median spine. The siphonal index is approximately 3.0 or less (Fig. 10.27). The pecten teeth are evenly spaced, and each tooth has 3–4 lateral denticles at the base. The siphonal tuft (1-S) is inserted distal to the last pecten tooth about the middle of the siphon, with 5–12 branches. The saddle does not encircle the anal segment, and covers 3/4 of its lateral sides. The saddle seta (1-X) is single, and about as long as the saddle. Usually 4–6 precratal tufts (4-X) are present, quite frequently less than in *Oc. annulipes*. The anal papillae are usually as long as or longer than the saddle.

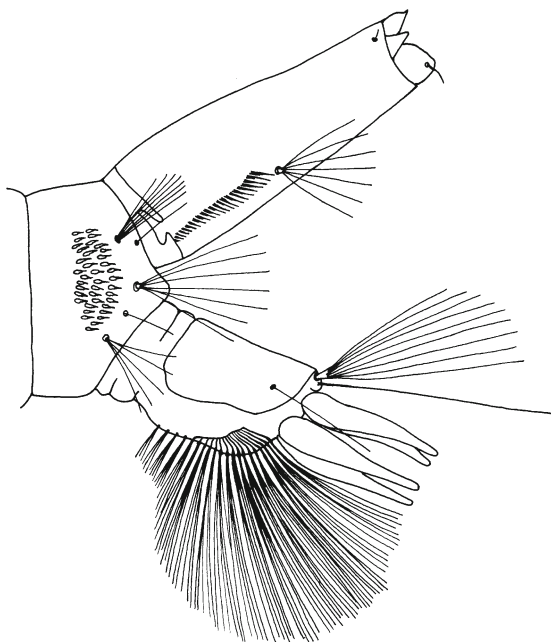


Fig. 10.27 Larva of *Oc. cantans*

**Biology:** The larvae develop rather early in spring in Southern and Central Europe, in northern areas they occur somewhat later but also belong to the early species. Larvae are present until late spring or early summer in varying numbers, depending on the amount of inundation and insolation of the habitat. The species is predominantly monocyclic, but capable of a bicyclic occurrence; the further north, the more obligate monocyclic it is. Under special circumstances another batch of eggs may hatch in late summer. It will mostly originate from unhatched eggs from the spring generation or even the previous year. Eggs are hibernating, and larval development lasts from 2 months to less than 4 weeks, entirely depending on regional temperature regimes. The larvae occur in open permanent or semipermanent meadow pools and dominate in deciduous or mixed forest pools with scarce aquatic vegetation and a thick layer of leaves on the bottom of the pools. In these habitats they may occur together with larvae of *Oc. annulipes*, *Oc. communis*, *Oc. punctor* and occasionally with late spring species, such as *Ae. cinereus* or *Ae. geminus*. The adults emerge shortly after the trees turn green. It has been reported that females need a period of rest in vegetation before they search for a blood meal, but local populations differ in this behaviour and some attack soon after emergence. Biting females are encountered most abundantly in lowland regions from late March to June in the Mediterranean region and from late May to early August at the southern borders of the taiga zone. The wide distribution range of *Oc. cantans* over all of Europe comprises plenty of local variations in time of occurrence and biting habits. The life span of the females is between 1 and 2 months. They are frequently found in dense vegetation but also fly over short distances to open spaces such as pastures and river lowlands to feed on cattle and sheep. Their daily biting cycle on humans seems to be bimodal with peaks during dawn and dusk. No autogenous forms have been reported.

**Distribution:** The species has a western Palaearctic distribution and occurs from the taiga zone in the north to the Mediterranean region in the south.

**Medical importance:** Flavivirus and Bunyavirus isolates have been reported from Slovakia and Austria (Lundström 1994, 1999). On material from Slovakia it was shown that the species is susceptible to infection with Tahyna virus.

### Ochlerotatus Caspius Complex

Two apparently morphologically identical forms of *Oc. caspius* designated as “species A” and “species B” were detected by electrophoretic analysis of wild populations from Italy (Cianchi et al. 1980). As no morphological and/or biological differences were attributed to those sibling species, they will be treated together under the description of the nominative form. Another species, described as *Aedes duplex*, was recorded from the European part of Russia by Martini (1926). The two male specimens particularly exhibit differences in their hypopygium with the basal lobe bearing 4 spine-like setae instead of 2 setae. As no further record of any stage of *Ae. duplex* has been made since that time, it is more probable that the two sampled males are only aberrant specimens, thus *Ae. duplex* should not be regarded as a distinct species and member of the *Ochlerotatus Caspius* Complex.

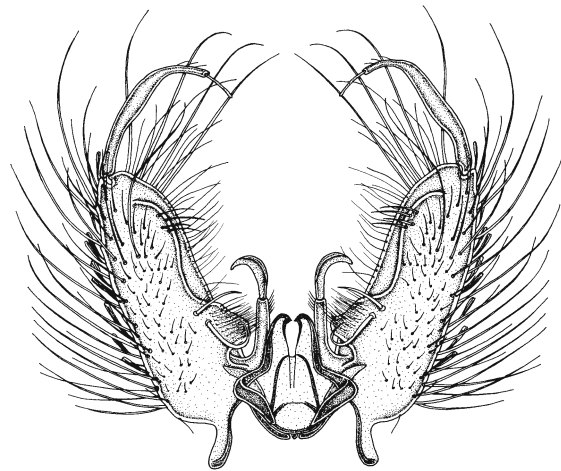
### *Ochlerotatus (Ochlerotatus) caspius* (Pallas 1771)

**Female:** *Oc. caspius* is very similar to *Oc. dorsalis* in general colouration, but is usually distinguished from the latter by two dorsocentral white stripes which run over the bright fawn coloured scutum. However, the colouration of *Oc. caspius* is subject to considerable variation. The proboscis and palps are covered with brown and white scales (Fig. 6.30b). The vertex has white and yellowish brown scales intermixed. The scutum has two narrow, white dorsocentral stripes running continuously from its anterior to posterior margin (Fig. 6.22a). The stripes may also be wide and diffuse, and if more yellowish, indistinct against the light brown background, but when the scales are well preserved, the distinction from the scutal pattern of *Oc. dorsalis* is quite easy. Even in species with the scales rubbed off from the central part of the scutum, the anterior and/or posterior parts of the longitudinal stripes are often well preserved and visible. The scales on the pleurites are broad and white. Tarsomeres I and II of the fore and mid legs and tarsomeres I–IV of the hind legs have white or cream-coloured basal and apical rings (Fig. 6.19a). The light rings are sometimes indistinct, and hind tarsomere V is entirely white scaled. The wing veins are covered with mixed dark and pale scales (Fig. 6.22b). At the basal quarter of the costa (C), the dark and pale scales are of more or less

the same number or the dark scales predominate. The abdominal terga are dark brown scaled, with yellowish scales dorsally and white scales laterally. The terga have basal and apical yellowish bands which are widest in the middle. A longitudinal middorsal yellowish stripe is present, but of varying length (Fig. 6.21a). It is usually present on terga II–IV, otherwise only vaguely expressed by a median widening of the transverse bands. In some specimens the median stripe is present on tergum II only (this pattern resembles that of *Cs. annulata*). The lateral sides of the terga are ornamented with central, triangular, white patches. Tergum VII has mixed dark and pale scales.

**Male (Fig. 10.28):** The basal lobe gradually arises from the gonocoxite, and is not constricted at the base. Two spine-like setae arise from it, one seta is longer and sharply hooked at the apex, the tip of the hook extends backwards to almost the middle of the spine, and the shorter seta is straight or slightly curved (Fig. 7.35a). The apical lobe of the gonocoxite is inconspicuous, almost bare dorsally. The claspette filament is about as long as the stem, with a narrow unilateral wing.

**Larva:** Similar to those of *Oc. dorsalis*, *Oc. detritus*, *Oc. leucomelas* and *Oc. flavescens*. Average values of some quantitative traits can be used to distinguish between larvae of *Oc. caspius* and *Oc. dorsalis*, but only at population levels (Milankov et al. 1998). The antenna is about half as long as the head, with sparse tiny spicules. The antennal seta (1-A) is inserted slightly below the middle of the antennal shaft, usually



**Fig. 10.28** Hypopygium of *Oc. caspius*

with 9 branches which are half as long as the antenna. The postclypeal seta (4-C) has 3–5 short, thin branches. The inner frontal seta (5-C) is inserted well below the median frontal seta (6-C), both are single, or less frequently 2-branched, but rarely does one of the setae have 3 branches. The outer frontal seta (7-C) has 7–10 branches. The mesothoracic seta 1-M is single and moderately long. The comb consists of 18–28 (usually 20–25) variegated scales arranged in 2–3 irregular rows (Fig. 10.29). At least some of the scales have a distinct median spine (Fig. 8.37b). The siphon slightly tapers in the apical half, and the siphonal index is 1.8–2.6. The pecten has 17–26 (usually 20–22) evenly spaced teeth, the basalmost four teeth are rudimentary. The pecten extends slightly beyond the middle of the siphon. The siphonal tuft (1-S) has 5–10 branches inserted beyond the middle of the siphon. The saddle covers more than half of the lateral sides of the anal segment. The saddle seta (1-X) is about half as long as the saddle, and is single. The lower anal seta (3-X) is longer than the siphon, and single. The ventral brush is made up of 12–17 cratal tufts (4-X) and 2–3 precratal tufts. The anal papillae are short, 0.3–0.9 times as long as the saddle, and lanceolate. The ventral pair is shorter than the dorsal pair.

**Biology:** *Oc. caspius* is a polycyclic, halophylic species. Sometimes only one generation per year is

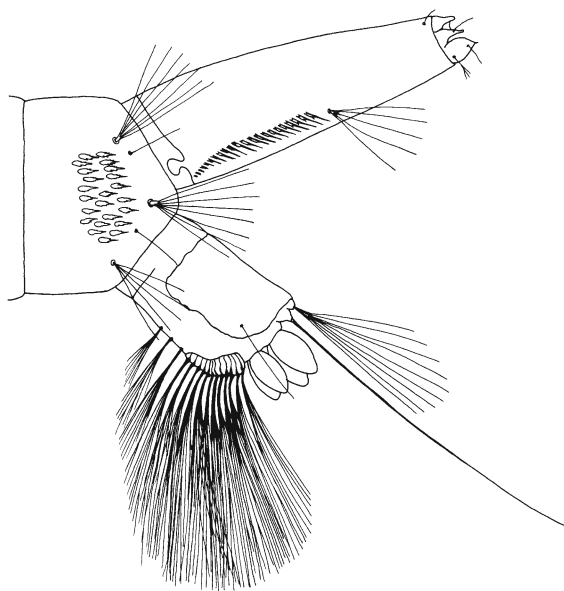


Fig. 10.29 Larva of *Oc. caspius*

produced due to the nature of the breeding site. The species overwinters in the egg stage, the first occurrence of larvae varies with the latitude, but generally takes place at the beginning of the year, this may be February–March in southern parts of Europe. It is regarded as a seaside mosquito that readily breeds in inland salt marshes and freshwaters with 0.5 g NaCl/l (Pires et al. 1982). It is a common species in the Atlantic and Mediterranean coastal marches and rock-holes. The breeding sites in the coastal areas of Portugal are usually restricted to altitudes <50 m (Ribeiro et al. 1989). The larvae develop in open or shaded waters, permanent or temporary water bodies formed by the snow melt, river floods or coastal marshes subjected to intermittent flooding and rice fields, usually with little vegetation and muddy bottom, often with a high concentration of salt, which may reach values of up to 150 g/l (Bozicic-Lothrop 1988). The acidity of the breeding sites recorded in Portugal ranged from pH 6.0–7.0 (Pires et al. 1982). The most characteristic freshwater habitats are river valleys, where larvae can breed in large numbers in the floodplains. They can be associated with larvae of many mosquito species, such as *An. atroparvus*, *An. maculipennis*, *Ae. vexans*, *Ae. vittatus*, *Oc. annulipes*, *Oc. cantans*, *Oc. detritus*, *Oc. intrudens*, *Oc. mariae*, *Oc. sticticus*, *Cx. p. pipiens*, *Cx. theileri*, *Cx. impudicus*, *Cs. annulata* (Bozkov et al. 1969; Ramos et al. 1978; Pires et al. 1982; Knoz and Vanhara 1982; Marchi and Munstermann 1987). Although females are strongly exophagic, they enter inhabited areas, houses, and cattle sheds if they occur in masses. The females readily bite humans and animals both in rural and urban areas (Gutsevich et al. 1974). They often bite during the day and night, but usually most actively search for a blood meal at dusk. Females are repelled by the lights of standard CDC miniature light traps. The species has a high resistance to heat and drought. Females actively search for blood at temperatures ranging from 11.5 to 36°C and relative humidity ranging from 47 to 92% (Petric 1989). They may migrate for long distances, up to 10 km. Autogenous development of *Oc. caspius* was detected in Uzbekistan (Chinaev 1964).

**Distribution:** It is a Palearctic species which is more common in southern and dry regions than *Oc. dorsalis* which is of Holarctic origin. *Oc. caspius* is distributed from Europe to Mongolia, north and west China, north Africa, west and middle Asia. In Europe it can be found from England to the central parts of

Russia, and from the southwest to the Mediterranean basin. The distributions of the sympatric *Oc. caspius* and *Oc. dorsalis* overlap in most of Europe.

**Medical importance:** West Nile virus (WNV), Tahyna virus, and the bacterium *Francisella tularensis*, the causative agent of tularemia, could be detected in natural populations (Detinova and Smelova 1973). *Oc. caspius* may have played a role in the spread of tularaemia and transmission of Tahyna and rabbit myxoma viruses in former Czechoslovakia, France, and Portugal (Bardos and Danielova 1959; Joubert 1975; Pires et al. 1982).

**Note on systematics:** Most authors consider *Oc. caspius* and *Oc. dorsalis* as separate species according to morphologic and genetic differences (Edwards 1921; Natvig 1948; Mohrig 1969; Lambert et al. 1990; Milankov et al. 1998, 2000). Concerning the morphological variation within *Oc. caspius*, Kazantsev (1931) found that the light, "sand-coloured" *Oc. caspius* developed in water with a high salinity, whereas mosquitoes breeding in fresh water had a more contrasting colouration. Specimens with intermediate morphological characteristics are common in Europe (Bozicic-Lothrop 1988) as also in more eastern regions of the former USSR (Gutsevich 1977). Three intermediate types of scutal colouration and two intermediate types of male genitalia are described in Europe, but no correlation between water salinity and scutal pattern or development of the basal lobe of the gonocoxite were found (Bozicic-Lothrop 1988). All the above mentioned differences could suggest the possible presence of subspecies or more than one species in Europe, which was documented by Cianchi et al. (1980).

### ***Ochlerotatus (Ochlerotatus) cataphylla* (Dyar 1916)**

**Female:** The proboscis is entirely dark scaled, which is the main character to distinguish the species from *Oc. leucomelas*, which has the proboscis speckled with numerous pale scales. The palps of *Oc. cataphylla* are dark scaled, with numerous scattered white scales. The scutum is usually covered with reddish brown scales, with lateral areas of paler scales. Sometimes a broad median stripe and two posterior submedian stripes of darker scales are present. The pleurites are extensively covered with scales. The upper part of the postpronotum is mostly brown scaled, and the lower part has

pale scales. Postpronotal setae are present along the posterior margin of the postpronotum (Fig. 6.41b). The postprocoxal membrane has a patch of pale scales. The subspiracular area is more or less entirely covered with scales, and the hypostigmal and postspiracular patches are fused. The upper and posterior part of the mesepisternum are extensively scaled, the scales extending near to the anterior angle. The scales on the mesepimeron end before its lower margin, and mesepimeral setae are present. The femora anteriorly have pale and dark scales. The tibiae and tarsi are mostly dark scaled, but with pale scales especially on the ventral surface, and the tarsi are without white rings. The tarsal claws are small and evenly curved. The wing veins have pale scales on the base of the costa (C) and scattered pale scales along the costa, subcosta (Sc) and  $R_1$ ; the remaining veins are dark scaled. The terga are dark scaled with broad basal bands of white scales (Fig. 6.38b), sometimes the last terga are mainly covered with white scales.

**Male:** The lateral lobes of tergum IX have 4–13 (usually 6–8) short spine-like setae. The gonocoxite has well developed basal and apical lobes. All the setae located on the inner side of the gonocoxite above the basal lobe are inwardly directed, and usually overlapping in the middle (Fig. 10.30). The basal lobe is conical with a group of medially directed long setae, one of which is strong, spine-like, and apically curved. The



**Fig. 10.30** Hypopygium of *Oc. cataphylla*

paraproct is sclerotized with inwardly curved tips. The claspette stem is strongly curved, and the claspette filament is half as long as the stem, with a broad unilateral wing. The aedeagus is more or less conical.

**Larva:** The antenna is less than half as long as the head. The antennal seta (1-A) has 3–5 branches, located at about the middle of the antenna. The post-clypeal seta (4-C) has 2–3 short branches. The inner and median frontal setae (5-C and 6-C) are single, the median frontals are located in front of the inner frontals, and the outer frontal seta (7-C) has 3–6 branches (Fig. 8.35a). The comb is composed of 10–30 (usually 25) scales arranged in 2–3 irregular rows (Fig. 10.31). Each scale has a long-terminal spine and smaller spines near the base. The siphonal index is about 3.0. The pecten is made up of 13–25 teeth, occupying about 3/4 of the length of the siphon. The 2–4 distalmost teeth are larger and apically detached, all are located beyond the siphonal tuft (1-S). 1-S is situated approximately in the middle of the siphon, with 3–5 branches. The saddle extends about 2/3 to 3/4 down the sides of the anal

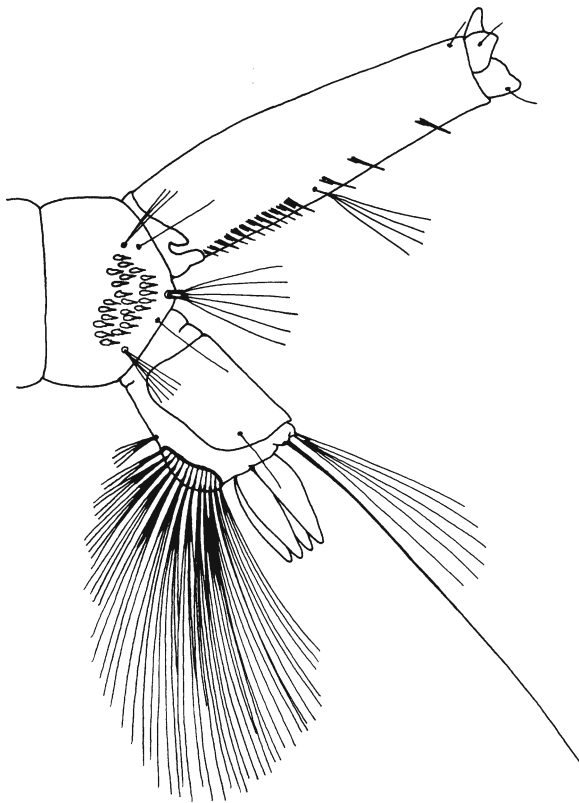


Fig. 10.31 Larva of *Oc. cataphylla*

segment, and the saddle seta (1-X) is single and short. The upper anal seta (2-X) has 5–8 branches, and the lower anal seta (3-X) is single. The ventral brush has 1–2 precratal tufts (4-X). The anal papillae are of variable length and tapered.

**Biology:** *Oc. cataphylla* is a monocyclic species. The most common breeding sites are forest pools in swampy woodlands, e.g. alder forests, with neutral to alkaline water. The pools are usually devoid of submerged vegetation but frequently covered with dead leaves at the bottom. In addition, larval populations are recorded from open areas, e.g. inundated meadows (Wesenberg-Lund 1921; Natvig 1948; Monchadskii 1951). The larvae hatch immediately after the snow thaw when meltwaters or heavy rainfall flood the depressions. The larvae are usually associated with those of *Oc. rusticus*, *Oc. cantans*, and *Ae. cinereus*. Occasionally the larvae of acidophilic species, e.g. *Oc. punctator* and *Oc. communis* are found in the same breeding sites. In central Europe the adults appear usually in the first half of April, before those of *Oc. cantans* and *Oc. rusticus*. The copulation swarms of male mosquitoes can frequently be observed at a height of about 1 m in shaded areas with bushes. Usually the females of *Oc. cataphylla* are a nuisance only in forest areas, where they can bite even during daytime. Repeated blood meals and ovipositions are possible (Carpenter and Nielsen 1965).

**Distribution:** Holarctic, Eurasia, and North America, northern to southern Europe. In northern Europe the species occurs in the tundra, in central Europe in swampy forests, in southern Europe mainly in mountainous areas.

### Ochlerotatus Communis Complex

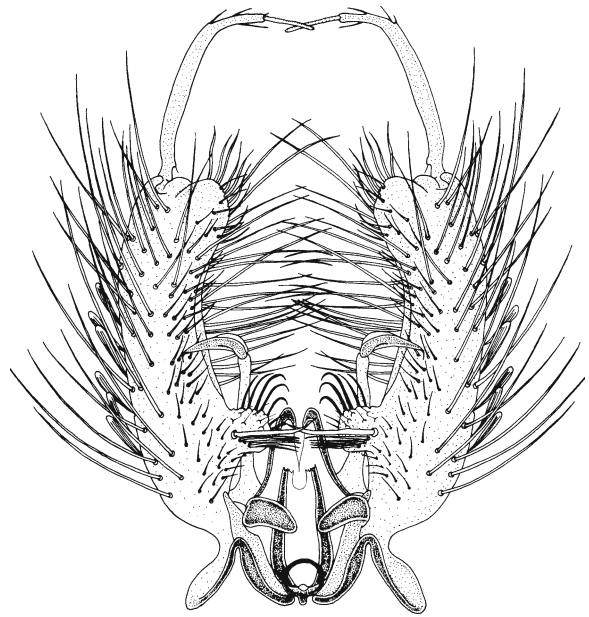
Owing to differences in the reproductive physiology, habitat preferences, swarming behaviour, emergence, and dispersal patterns, the complex is composed of two or more sibling species (Chapman and Barr 1964; Ellis and Brust 1973; Schutz and Eldridge 1993). Morphometric and electrophoretic comparisons of populations of the *Ochlerotatus Communis* Complex in North America revealed 4 sibling species, namely *Oc. communis*, *Oc. nevadensis*, *Oc. tahoensis*, and *Oc. churchillensis*. The females of the latter species are found to be autogenous, whereas the other three species are anautogenous (Brust and

Munstermann 1992). According to variable morphological characteristics in European populations, it is feasible to expect more sibling species of the complex in Europe as well. However, electrophoretical analysis of populations from northern Sweden and Germany has not provided evidence of any differences (Weitzel et al. 1998).

***Ochlerotatus (Ochlerotatus) communis*  
(De Geer 1776)**

**Female:** The proboscis and palps are dark scaled, the palps rarely have a few white scattered scales. The scutal scale pattern is rather variable, but typically the scutum is covered with yellow to golden scales. A broad median stripe and posterior submedian stripes of dark bronze scales are present. They are separated by narrow stripes of pale scales, which are sometimes fused. The scutellar and supraalar setae are dark brown. A postprocoxal scale patch is absent (Fig. 6.47b), the hypostigmal patch is usually absent, or occasionally with only a few pale scales. The upper mesepisternal patch extends to the level of the anterior angle, and the mesepimeral patch extends to the lower margin of the mesepimeron (Figs. 6.45b and 6.46a). Lower mesepimeral setae are present. The femora, tibiae, and tarsomeres I of all legs are mostly dark scaled dorsally and with a few whitish scales ventrally, the remaining tarsomeres are dark scaled. The tarsal claws are curved with a long subbasal tooth. The wing veins are covered with dark scales, and a few pale scales are scattered at the base of the costa (C) and radius (R). The terga are dark scaled with broad basal bands of white scales.

**Male:** The lobes of tergum IX are covered with 8–10 short spine-like setae. The hypopygium is similar to that of *Oc. pionips* (Fig. 10.32). The basal lobe of the gonocoxite is rounded, concave at its lower part, with one long, strong spine-like seta. Mesal to it, is a row of closely spaced, inwardly directed long setae. The upper part of the basal lobe has a row of long, prominent, widely spaced, apically strongly curved, or sometimes hooked setae. The apical lobe of the gonocoxite is well developed and rounded apically. The paraproct is strongly sclerotized apically. The claspette stem is long, and the claspette filament is shorter than the stem, and

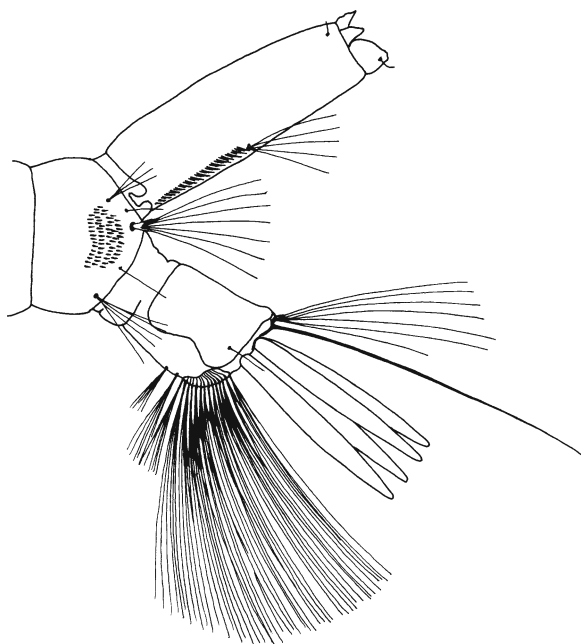


**Fig. 10.32** Hypopygium of *Oc. communis*

heavily sclerotized with a narrow unilateral wing. The aedeagus is conical, rounded, and notched at the apex.

**Larva:** The antenna is nearly half as long as the head, and the antennal seta (1-A) is situated at about the middle of the antennal shaft, with 6–7 branches. The median frontal seta (6-C) is situated in front of the inner frontal seta (5-C), both setae are single and rarely one seta has 2 branches. The outer frontal seta (7-C) has 4–8 branches (Fig. 8.41a). The number of comb scales varies from 40–70, but the average is 60 scales (Fig. 10.33). The scales are arranged in an irregular triangular patch, each individual scale without a prolonged terminal spine, thus appearing to be rounded apically. The siphonal index is 2.3–3.2, usually about 2.8. The pecten has 17–26 evenly and closely spaced teeth which do not extend to the middle of the siphon. The siphonal tuft (1-S) has 5–9 branches which are about as long as the width of the siphon at the point of insertion. The saddle extends about 3/4 down the sides of the anal segment, and the saddle seta (1-X) is distinctly shorter than the saddle, and is single. The ventral brush has 2, rarely 3, precratal setae (4-X). The anal papillae are distinctly longer than the saddle.

**Biology:** *Oc. communis* is usually a monocyclic snow-melt mosquito, which is one of the most frequent mosquitoes of swampy forests. The preferred



**Fig. 10.33** Larva of *Oc. communis*

breeding sites are acidic water bodies which are filled with water during the snow-melt or spring rainfall. Larvae can mainly be found in depressions and ditches without vegetation but with a dense layer of dead leaves at the bottom. Often they are found in strongly acidic waters, e.g. with *Sphagnum* sp., with a pH-value of little more than 3.0. They appear only rarely or are absent in waters with neutral reaction, e.g. in inundated areas of large rivers. Most of the larvae hatch at temperatures of little more than 0°C, when the breeding sites are still partly covered with ice. In central Europe the larvae hatch from February onwards, adults emerge usually in April. In the laboratory, the optimum temperature for larval development is 25°C. At this temperature the development to the adult stage is completed within 18 days, but above 30°C and below 4°C the development is not completed. In central Europe, females are troublesome for warmblooded creatures in forest areas from April onwards, particularly during twilight. Females do not migrate long distances from the breeding sites. Usually the population decreases from July on, but Scherpner (1960) found isolated freshly hatched larvae of *Oc. communis* in August.

**Distribution:** Holarctic, North America, and Eurasia. The species is found from northern Europe to the Mediterranean area.

### *Ochlerotatus (Ochlerotatus) cyprius* (Ludlow 1920)

**Female:** A large species with a light integument and golden to yellowish scaling. Natvig (1948) described *Oc. cyprius* as variable in colour, the golden tinge is sometimes lost and the scales are more or less whitish or yellowish orange in some specimens. It is similar to *Oc. flavescens*, and for separation of the two species, see the description of the latter. The proboscis is yellowish with a few dark scales at the labellum. The palps are 1/4 of the length of the proboscis, with mixed golden and greyish scales. The head is pale scaled, with some dark scales laterally. The antenna is yellowish at the base, with the distal flagellomeres more brownish. The integument of the scutum is light brown with yellowish and golden narrow scales, and sometimes a weak dark median stripe and short dark lateral stripes are present. The postpronotum has narrow golden scales and golden setae. A postprocoxal patch is present. The mesepisternum has two separate cream coloured scale patches, the lower patch reaching the anterior margin. The mesepimeral scale patch nearly reaches the lower margin of the mesepimeron, and the lower mesepimeral setae are present (Fig. 6.29b). The fore and mid femora have yellowish scales mixed with dark ones, the hind femur apically has dark scales, and yellowish knee spots but these are usually indistinct. The tibiae are predominantly yellowish scaled with scattered black scales, which are more numerous towards the apical parts of the mid and hind tibiae. The tarsomeres have broad, yellowish basal rings and dark scaling apically, which is more pronounced at the mid and hind legs. The wing veins are yellow scaled with scattered dark scales. The abdomen dorsally and ventrally has ochre yellowish broad scales mixed with isolated dark scales, and the terga sometimes have more dark scales laterally which never form a continuous transverse band. The cerci are predominantly dark scaled.

**Male:** Tergum IX has 6–11 spine-like setae on each of the lateral lobes. The gonocoxite is long and slender, and the basal lobe is well developed, with one spine-like seta and several long setae of different widths (Fig. 10.34). The apical lobe is prominent with rather short setae. The gonostylus is long and slender, with an elongated apical spine. The paraproct has a sclerotized and recurved tip. The claspette stem is long and slender, and evenly curved. The claspette filament



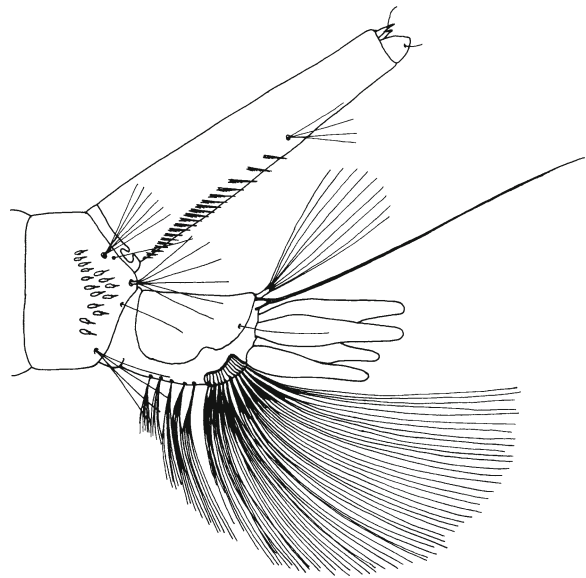


**Fig. 10.34** Hypopygium of *Oc. cyprius*

is more than half as long as the stem, slightly triangular, and unilaterally winged from the base, without a stalk. The aedeagus is elongated and pear shaped with a narrow opening at the apex.

**Larva:** The entire body is densely covered with dark spicules (Fig. 8.19a), which identifies the large larvae from all other European *Aedes* and *Ochlerotatus* species. The antennae are shorter than the head, and the antennal seta (1-A) has 1–3 branches. The inner and median frontal setae (5-C and 6-C) are single or double. The prothoracic setae according to Peus (1937) follow the formula: 1 and 2 (single); 3 (2-branched); 4 (single, short); 5 (2-branched); 6 (single); 7 (3-branched). The number of comb scales is 9–15 arranged in an irregular row (Fig. 10.35). The siphon is long and slender, and moderately tapering. The pecten usually has 19–21 pecten teeth, with several distal teeth detached. Each individual pecten tooth has many lateral denticles. The siphonal tuft (1-S) is situated beyond the distalmost pecten tooth, with three to four branches. The anal segment is not encircled by the saddle, the saddle seta (1-X) is about as long as the saddle, the number of precratal tufts (4-X) is 4–5, and the anal papillae are longer than the saddle.

**Biology:** The species hibernates in the egg stage. Larvae occur in semipermanent pools along inundated river shores and in snow-melt pools. Peus (1937) found the larvae always in the middle of the pools with an average water depth of 50–80 cm and regarded the species as preferring cold water for development. In Sweden one larva was found in late May in a cold well



**Fig. 10.35** Larva of *Oc. cyprius*

and adults were caught resting nearby on a flooded meadow surrounded by a deciduous forest (Dahl 1975). The larvae occur together with *Oc. flavescens*, *Oc. excrucians*, *Oc. cantans*, and *Oc. riparius*. Adults appear until July and from more eastern areas the species is recorded as being a mid spring species (Gutsevich et al. 1974). The females are aggressive biters, they can attack their hosts in sunlit situations (Peus 1937). In Siberia *Oc. cyprius* is sometimes a vicious biter (Gutsevich et al. 1974).

**Distribution:** It is a rare species in western European regions, reported in Sweden, Finland, Germany, Slovakia, and Poland. It can also be found along the eastern shores of the Baltic Sea, the eastern distribution range stretches from the Ukrainian steppe to Central Kazaksthan.

### **Ochlerotatus Detritus Complex**

Laboratory studies carried out using isoenzyme identification techniques gave evidence for the existence of sibling species within the complex (Pasteur et al. 1977). The “species A” and “species B”, as they were then called, occurred sympatrically in the Carmargue and showed reproductive isolation. Further ecological and distributional differences between the apparently morphologically identical siblings, e.g. frequent auto-geny in “species A” which is an exception in “species

B”, have been recorded (Agoulon et al. 1997; Schaffner 1998); “species A” has finally been named *coluzzii* (Rioux et al. 1998).

***Ochlerotatus (Ochlerotatus) detritus*  
(Haliday 1833)**

**Female:** The species is easily distinguished from all other *Ochlerotatus* members with unringed tarsi by its abdominal colouration. The apical parts of the terga are usually covered with dark and pale scales, with dark scales predominating on the first segments and pale scales on the last segments. The proboscis and palps have dark and pale scales intermixed, and the clypeus is black. The vertex has yellowish white narrow curved scales and blackish brown erect forked scales. The integument of the scutum is brown, and more or less uniformly covered with yellowish brown scales, lighter on the posterior part. Stout black setae are more numerous above the wing bases. The scutellum has patches of yellowish white scales on each lobe. The integument of the pleurites is dark brown with patches of broad, flat yellowish white scales. The mesepimeral scale patch does not reach the lower margin of the mesepimeron, and lower mesepimeral setae are present. The anterior part of the fore and mid femora are conspicuously speckled with pale scales, the tibiae and tarsi have dark scales and more or less numerous scattered pale scales, pale tarsal rings are absent. The wing veins are covered with broad pale and dark scales, sometimes the pale scaling is reduced. The abdominal terga have pale transverse basal bands of more or less uniform width, the apical parts of the terga have dark and pale scales intermixed (Fig. 6.38a). The sterna are mostly pale scaled.

**Male:** The lateral lobes of tergum IX have 3–8 straight, spine-like setae. The gonocoxite is about three times as long as it is wide (Fig. 10.36). The basal lobe is more or less conical, and covered with fine setae arranged in an irregular row and 1 conspicuous strong spine-like seta, which is curved apically. The apical lobe is well developed carrying straight setae of moderate length. The gonostylus is curved, tapers apically, and the apical spine of the gonostylus is long. The paraproct is well sclerotized in the apical part. The claspettes have a short stem, the distal third of the stem is sometimes constricted. The claspette filament is as long as or longer than the stem, with a long stalk, and



**Fig. 10.36** Hypopygium of *Oc. detritus*

slightly bent from the end of the stalk on. The convex side of the filament is evenly widened from about its middle. The aedeagus is small and oval.

**Larva:** The head is broader than long. The antenna is short, about half the length of the head, slightly curved and moderately spiculate. The antennal seta (1-A) is inserted at about the middle of the antennal shaft, with 5–8 branches. The postclypeal seta (4-C) has 2–3 thin and short branches. The inner and median frontal setae (5-C and 6-C) are usually 2–3 branched, 5-C may rarely have 3–5 branches, but is always situated behind 6-C. The outer frontal seta (7-C) has 7–12 branches. The comb consists of more than 40 scales, usually 45–60, arranged in a triangular patch, each individual scale is blunt ended and fringed with spines of more or less the same length (Fig. 8.37a). The siphon slightly tapers from the middle, and the siphonal index is 2.2–2.8 (Fig. 10.37). The pecten is composed of about 20 (18–27) evenly spaced teeth, rarely the distalmost tooth may be slightly detached; and each pecten tooth has 2–3 ventral denticles. The siphonal tuft (1-S) is inserted at about the middle of the siphon, beyond the distalmost pecten tooth, with 6–10 (usually 8) branches. The saddle reaches more than half way down the sides of the anal segment. The saddle seta (1-X) is single, and about as long as the saddle. The upper anal seta (2-X) has 8–11 branches, and the lower anal seta (3-X) is single and longer than the siphon. The ventral brush has 1–3 precratal setae (4-X), but most often only 1 precratal

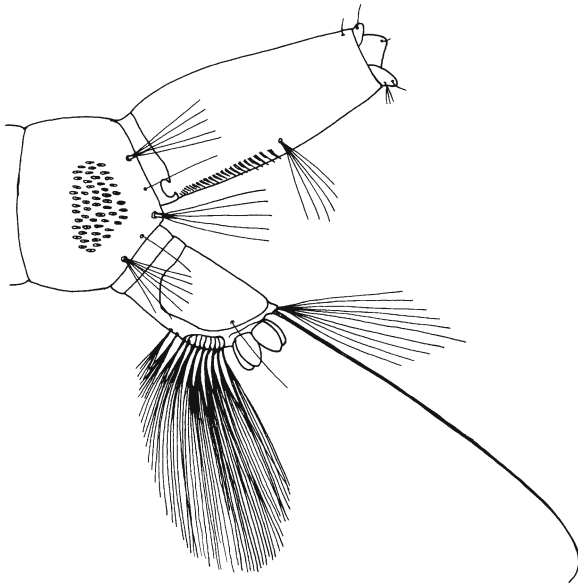


Fig. 10.37 Larva of *Oc. detritus*

seta is present. The anal papillae are very short and rounded.

**Biology:** *Oc. detritus* is a polycyclic species which may have up to three generations per year, according to the latitude of its occurrence. Usually the species hibernates in the egg stage (Gutsevich et al. 1974) and the first larvae occur some weeks later than the majority of the typical snow-melt mosquitoes, when the water temperature of the breeding sites exceeds 10°C (Martini 1931; Mohrig 1969). Hibernating larvae were reported from England, late hatching larvae developed into the fourth-instar during autumn and did not pupate before March of the next year (Service 1968b). *Oc. detritus* is a typical halophilic species and the larvae occur almost exclusively in breeding habitats with an exceptionally high content of salinity, only occasionally they are found in fresh water. Preferred breeding sites are brackish waters in estuaries and coastal marshes. Larvae can be found in semipermanent ponds in open *Salicornia* and *Tamarix* marshes (depending on fluctuation of the water level), and in stagnant drainage channels or lagoons, with little aquatic vegetation. They are sometimes found together with larvae of *Oc. caspius* and *Oc. dorsalis*, but more often, due to the tolerance of extreme salinity, the species occurs alone in its breeding site. Adults can be found from late March until November in England (Cranston et al. 1987), in central Europe they are recorded from early

May until September (Martini 1931; Mohrig 1969). The females are persistent biters and readily attack humans, often in large numbers. They may feed during the day, but are predominantly active at dusk. Rioux (1958) considered *Oc. detritus* as a typical exophilic species which enters buildings only occasionally, but Cranston et al. (1987) reported frequent entering of houses to feed (endophagy), although no resting indoors. Along with *Oc. caspius*, *Oc. detritus* is the most common coastal species in Europe causing considerable annoyance in villages at the seaside and in their vicinity. Migration distances of about 6 km are recorded from Britain (Marshall 1938), in southern France the flight range of *Oc. detritus* is estimated to be approximately 20 km (Rioux 1958). The differences in the biology and behaviour might be attributed to the presence of sibling species of the complex.

**Distribution:** *Oc. detritus* is a Palaearctic species, it occurs along most of the European coast lines, e.g. Northern and Baltic Sea as well as the Atlantic Ocean and around the Mediterranean basin. Furthermore the species has a scattered distribution in saline inland areas in Europe, northern Africa, and southwest Asia. It is possible that the southern range of its distribution is dominated by the sibling species *Oc. coluzzii*.

#### ***Ochlerotatus (Ochlerotatus) diantaeus* (Howard, Dyar and Knab 1913)**

**Female:** The large dark legged female has a dark brown to greyish integument, golden brownish setae and scales and some blackish brown scaling with a slight metallic shine. The proboscis and palps are blackish brown. The head has pale golden setae between the eyes and golden, narrow, curved and erect forked scales on the vertex and occiput. The scutum has narrow pale golden or whitish scales and a broad median or two slightly divided dorsocentral stripes of narrow dark bronze scales and an anterolateral dark stripe. The postpronotum has pale yellow or whitish narrow curved scales. The postprocoxal and hypostigmal scale patches are absent. The upper mesepisternal patch of white broad scales does not reach the anterior margin of the mesepisternum, and the lower edge of the patch ends slightly above the level of the anterior angle (Fig. 6.45a). The mesepimeral patch does not reach the lower margin of the mesepimeron. The pleural setae are pale golden. The coxae have white broad

scales and yellowish setae. The fore and mid femora are dark scaled anteriorly, white scaled posteriorly, sometimes with basal whitish scaling. The hind femur has more dominant basal white scaling, and a whitish knee spot. The tibiae and tarsomeres are entirely dark scaled. The wing veins are covered with dark scales. The abdominal terga have dark scales, sparsely intermixed with white scales and with large white triangular lateral patches. The lateral patches are connected by distinct white basal bands at least on terga IV–VII, and sometimes narrow white basal bands are present also on terga II and III. The sterna have whitish scales, with indistinct dark triangular scale patches apically. Segment VIII and the cerci are entirely black scaled.

**Male:** Tergum IX has 6–8 strong, spine-like setae on each lobe. The hypopygium shows two characteristics which distinguish it from that of the other members of the *Intrudens* Group (Fig. 10.38). The first is the group of dense setae on the inner apical surface of the gonocoxite and the second is the distinct shape of the claspette filament. The basal lobe of the gonocoxite bears three spine-like setae, two of them located close together at about the middle of the gonocoxite, and the third one is widely displaced towards the base of the gonocoxite. The apical lobe is well developed. Dense long inwardly directed setae are located on the apical

half of the gonocoxite. The gonostylus is slender and curved with a long apical spine, and usually one additional seta close to the apex. The paraproct has broad sclerotization. The claspette stem has a thorn-like process beyond the middle. The claspette filament is broad and somewhat crescent shaped. The aedeagus is pear shaped.

**Larva:** The larvae differ from all other European *Aedes* and *Ochlerotatus* species by the antennae, which are distinctly longer than the head (Fig. 8.17a). The head is broad, the antennae have numerous spicules. The antennal seta (1-A) has 2–4 long branches. The inner and median frontal setae (5-C and 6-C) have 2–5 branches. The thorax has the prothoracic formula 1-P to 7-P as follows: 1 and 2 (single); 3 (2-branched); 4–6 (single); 7 (2-branched). The comb has 8–13 scales, each individual scale with a long and strong median spine. The siphon is long and tapering, the siphonal index is 3.2–3.7 (Fig. 10.39). The last two pecten teeth are apically detached, and the siphonal tuft (1-S) is inserted beyond the distalmost pecten tooth, usually with 7–8 branches. The anal segment is not completely encircled by the saddle, but the saddle reaches far down the lateral sides. The saddle seta (1-X) is single, and shorter than the saddle. The lower anal seta (2-X) is single and usually longer than the siphon. The ventral



Fig. 10.38 Hypopygium of *Oc. diantaeus*

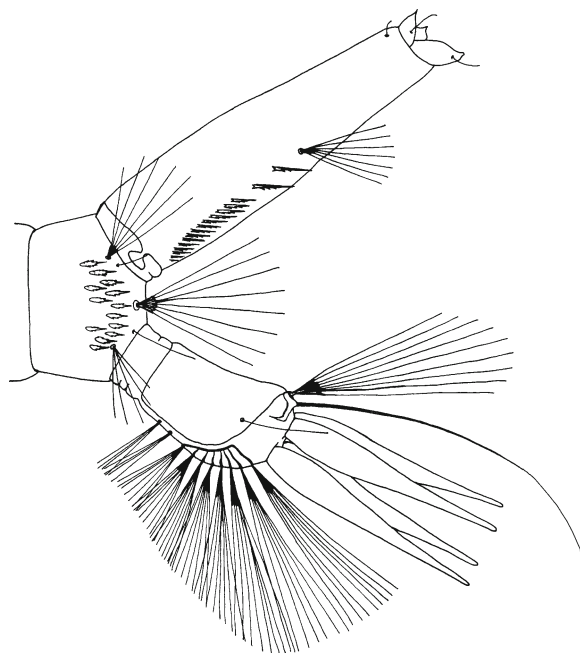


Fig. 10.39 Larva of *Oc. diantaeus*

brush has 2–3 precratal setae (4-X). The anal papillae are very long and slender, and longer than the saddle.

**Biology:** The species hibernates in the egg stage and is monocyclic. Larvae occur early in spring in temporary water bodies formed after snow-melt. They may be found in boggy localities, in shaded ditches and pools in mixed forests, or in open water pools in more northern and taiga areas. Usually the bottom of the breeding sites is covered with abundant leaf debris. Wood et al. (1979) suggest that the exceptionally long antennae might indicate a peculiar feeding behaviour. The larvae tolerate acidic conditions and occur together with several common species, such as *Oc. cantans*, *Oc. cataphylla*, *Oc. communis*, *Oc. pionips*, *Oc. punctor*, and *Cs. morsitans*. The adults emerge later than those of *Oc. communis*, owing to delayed larval development (Gutsevich et al. 1974). Males can be attracted to mammals where females are present and biting (Jaenson 1985). Females have been reported to feed on humans in southeastern Russia.

**Distribution:** A northern Holarctic species, it occurs in central and northern Europe and does extend into the taiga and the subarctic zone. It can be found in the southeastern parts of Russia and is also recorded from Canada and northernmost United States.

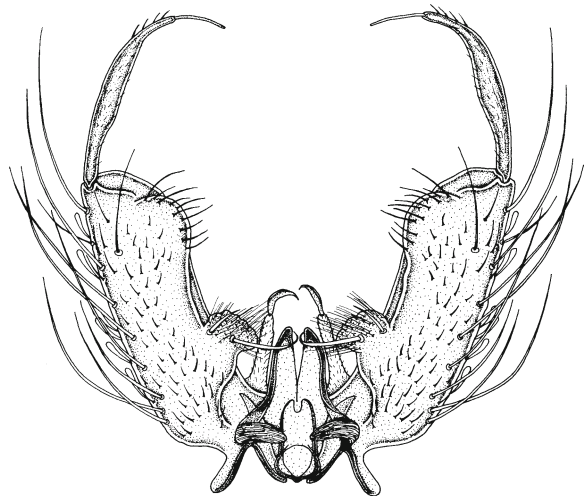
### ***Ochlerotatus (Ochlerotatus) dorsalis* (Meigen 1830)**

**Female:** Most easily distinguished from the similar *Oc. caspius* by its scutal markings and colouration. The scale pattern on the scutum can be variable, but usually is found as described below. The acrostichal and dorsocentral stripes on the scutum of *Oc. dorsalis* are fused to form a dark brown median stripe extending to the pale prescutellar dorsocentral stripes. Posteriorly the stripe is ornamented with a pair of narrow, white lines. There is also a well defined pair of dark posterior submedian stripes. The scales on the other areas of the scutum are ashy white (Fig. 6.22c). Two distinct dorsocentral white stripes, which can be found on the scutum of *Oc. caspius*, are always absent. The proboscis is covered with dark scales, sometimes with scattered pale scales on its middle third. The pleural scales are narrow, and creamy to straw coloured. The tarsomeres have pale rings both basally and apically (Fig. 6.20b). The wing veins are predominantly covered with light scales (Fig. 6.22d). Dark scales are restricted

to the apical portion of the costa (C),  $R_1$ ,  $R_3$  and forked portions of media (M) and cubitus (Cu). The basal quarter of the costa is exclusively white scaled, a character which can be used to distinguish the species from *Oc. caspius*. The abdominal terga have a whitish grey longitudinal stripe which may not be present on all segments. Indistinct, narrow basal and apical light transverse bands of more or less uniform width are present. Blackish brown or black scales are restricted to two rectangular areas on terga I–V, terga VI and VII are mostly light scaled.

**Male:** The hypopygium is very similar to that of *Oc. caspius* (Fig. 10.40). The basal lobe of the gonocoxite is constricted at the base with two widely separated spine-like setae. The apex of the longer seta is sometimes not hook shaped, otherwise the tip does not extend backwards to more than one third of the spine (Fig. 7.35b). The shorter spine-like seta is straight. The apical lobe is inconspicuous, and usually covered with numerous setae. The claspette filament is shorter than the stem, and strongly curved.

**Larva:** The characteristics separating larvae of *Oc. dorsalis* from those of *Oc. leucomelas* and *Oc. caspius* are not very obvious. The antenna is about half as long as the head, with sparse spicules. The antennal seta (1-A) has 4–7 branches articulated in the middle of the antennal shaft or slightly below it, and is not more than half as long as the antenna. The postclypeal seta (4-C) is short, with 2–5 thin branches. The inner frontal seta (5-C) is situated posterior



**Fig. 10.40** Hypopygium of *Oc. dorsalis*

to the median frontals (6-C), and both pairs are single, or more frequently with 2 branches (in Nearctic populations 5-C is usually single, rarely double on one side and 6-C is single). The outer frontal seta (7-C) has 4–8 branches (usually 5–6). Seta 1-M has 2 long branches, which may be used as a characteristic to distinguish the species from *Oc. caspius*. The comb has 13–35 (usually 20–25) scales arranged in 2–3 irregular rows (Fig. 10.41). The scales are variable in shape, as in *Oc. caspius*. The siphonal index is 2.5–3.0, rarely less. The pecten has 14–23 evenly or slightly irregularly spaced teeth not reaching the middle of the siphon. The siphonal tuft (1-S) has 3–8 (usually 4–5) branches, and is inserted in the middle of the siphon or slightly below it, usually closer to the base than in *Oc. caspius*. The saddle is reduced to the dorsal half of the anal segment, is strongly pigmented and with a sharply defined lower margin. The saddle seta (1-X) is half as long as the saddle. The anal papillae are rounded, their length greatly varies with the salinity of the breeding water. They are about 1.3

times as long as the saddle in fresh water, but not more than 0.3–0.4 times the length of the saddle in saline water (Gutsevich et al. 1974).

**Biology:** *Oc. dorsalis* is a polycyclic and halophilic species that hibernates in the egg stage. There are usually 2–4 generations per year in central Europe depending on the number of floodings. The larvae occur mainly in small, open water bodies and swamps, they are found in permanent or temporary water bodies formed by melted snow, floods, rainfall or groundwater such as inland pastures, roadside and drainage ditches. Females preferably oviposit around saline water bodies but the ground near fresh waters can be chosen as well. The larvae can be found in waters with a salt content of up to 12% and a pH value which ranges from 7.0 to 9.3 (Chapman 1960). They are numerous in the Atlantic and Mediterranean coastal marshes but prefer inland saline areas in the Palaearctic region (Bozicic-Lothrop 1988). Adults can be very numerous in some localities such as river valleys, saline ponds, and lakes. The females attack humans readily inflicting painful bites (Richards 1956). They are also characteristic of open habitats in mixed forests and pastures. Blood-searching activity is recorded from 9 to 30°C and 52 to 92% relative humidity in the southern part of central Europe (Petric 1989). The peak of daily biting activity occurs in late afternoon and early evening, but females can be quite active during the night also. They can also search for blood during the day in open areas (Cranston et al. 1987). Females are exophagic but readily enter houses, tents, and wards (Waterston 1918). Autogeny is rare, but was detected in Nevada (Chapman 1962).

**Distribution:** *Oc. dorsalis* is a widespread Holarctic salt marsh species in Europe, central Asia, China, northern Russia, and North America. In Europe the distribution range extends north to Scandinavia (Natvig 1948) and south to Greece (Pandazis 1935).

**Medical importance:** *Oc. dorsalis* transmits Western Equine Encephalitis virus (WEE), St. Louis Encephalitis virus (SLE), California Encephalitis virus (CE) in the United States (Hammon and Reeves 1945; Hammon et al. 1952). Japanese Encephalitis virus (JE), and the bacterium *Francisella tularensis*, the causative agent of tularemia, were isolated from natural populations (Detinova and Smelova 1973).

**Note on systematics:** Three species closely related to *Oc. dorsalis* are the Nearctic *Oc. melanimon* and *Oc. campestris* as well as the Palaearctic *Oc. caspius*.

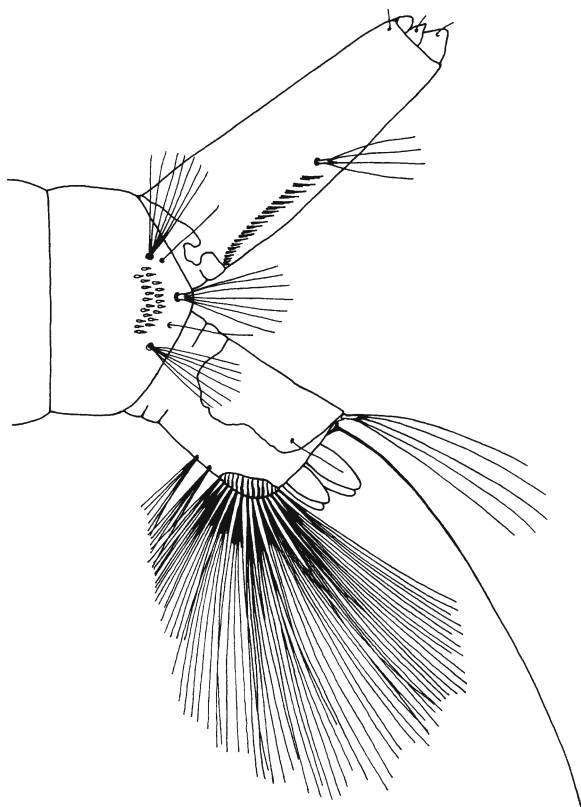


Fig. 10.41 Larva of *Oc. dorsalis*

The current status of *Oc. dorsalis* is discussed under the description of *Oc. caspius*.

### Ochlerotatus Excrucians Complex

The complex is composed of the Holarctic species *Oc. excrucians* and *Oc. euedes*, some other Nearctic species, e.g. *Oc. aloponotum*, and the Palaearctic, European species *Oc. surcoufi*. The current status of the sibling species within the complex is rather unsatisfactory. There exists a great variation between populations from different geographical areas. Apart from differences in the general body colouration of females of *Oc. excrucians*, the shape of the tarsal claw also varies in populations within both North America and Europe (Wood et al. 1979; Dahl 1984). The variation is expressed by the angle formed between the main and the subbasal tooth, as well as the degree of bending of the former. The “wide type” claw described by Wood et al. (1979) as a southern Canadian variant of *Oc. excrucians* is quite similar to some specimens from Italy, France and Germany, designated as *Oc. surcoufi* by Arnaud et al. (1976), another “wide type” variant was also found in Sweden (Dahl 1984). Further confusion arises because the European members of the complex, *Oc. excrucians*, *Oc. surcoufi* and *Oc. euedes*, are not very well studied. However, Arnaud et al. (1976) suggested the substitution of all earlier *Oc. excrucians* records in Europe by its European form *Oc. surcoufi*. As no worldwide comparison of geographic variations and redescriptions of all stages of the species in question has been carried out, this suggestion has not been followed.

### *Ochlerotatus (Ochlerotatus) euedes* (Howard, Dyar and Knab 1913)

**Female:** For separation from *Oc. excrucians* refer to the description of *Oc. surcoufi*. *Oc. euedes* is usually distinguished from the latter species by the scutal scale pattern. In *Oc. euedes* the dorsocentral stripes of pale scales start at some distance from the anterior margin of the scutum and extend to the transverse suture. The pale lateral stripes continue over the transverse suture and reach the end of the dorsocentral stripes (Fig. 6.35b). In *Oc. surcoufi* the dorsocentral stripes of pale scales usually start at about the level of the transverse suture and reach close to the posterior margin of the scutum.

The white lateral stripes are sometimes indistinct, and the transverse suture is covered with clearly visible stripes of white scales. The abdominal terga of *Oc. euedes* have dark scales and narrow basal bands at least on the first segments, otherwise median and lateral patches of white scales and sometimes narrow apical white bands are present. The cerci are dark with scattered light scales.

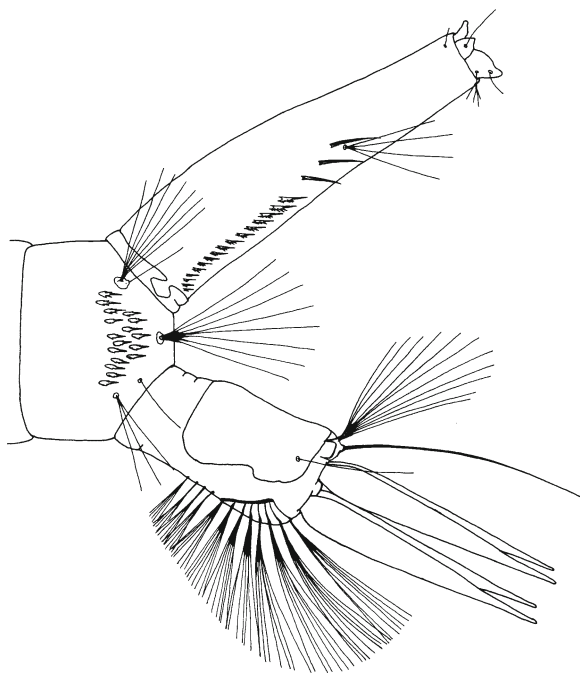
**Male:** The hypopygium is similar to those of the other members of the complex (Fig. 10.42). The only difference may be found in the shape of the apical lobe. In *Oc. euedes* it is well developed, usually protruding above the level of the gonostylus articulation, whereas the apical lobe in *Oc. excrucians* and *Oc. surcoufi* usually does not reach the articulation of the gonostylus.

**Larva:** The large larvae are very similar to those of *Oc. excrucians* (Fig. 10.43). The number of comb scales is 11–19 arranged in 2–3 irregular rows. The siphon is more evenly tapered from the base to the apex, and not as abruptly narrowed as in *Oc. excrucians*. The distalmost pecten tooth is situated well beyond the middle of the siphon, almost reaching 1-S. The siphonal tuft (1-S) is inserted distinctly beyond the middle of the siphon, and the branches of 1-S are shorter than in *Oc. excrucians*.

**Biology:** The species is monocyclic and hibernates in the egg stage. In Europe, it breeds in the same kind of habitats and occurs at the same time as *Oc. excrucians*, *Oc. cantans*, and *Oc. annulipes*.



Fig. 10.42 Hypopygium of *Oc. euedes*



**Fig. 10.43** Larva of *Oc. euedes*

**Distribution:** Reported in the eastern and northern states of the United States and Canada (Wood et al. 1979; Darsie and Ward 1981). In Europe it has been recorded from southern Scandinavia and, sometimes under the synonym of *Oc. beklemeshevi* Denisova 1955, from Finland, Poland, Lithuania, and European Russia (Snow and Ramsdale 1999). Very little European material is available.

**Medical importance:** Alphavirus has been reported from *Oc. euedes* in central Russia (Lundström 1999).

**Note on systematics:** *Oc. euedes* Howard, Dyar and Knab 1913, was resurrected from synonymy of *Oc. excrucians* and regarded as a separate species. At the same time, *Oc. beklemishevi* Denisova 1955, reported from the former USSR and Poland, was put under synonymy of *Oc. euedes* (Wood 1977; Knight 1978).

### ***Ochlerotatus (Ochlerotatus) excrucians* (Walker 1856)**

**Female:** A large species, similar in size and body scale patterns to *Oc. euedes* and *Oc. surcoufi*. The best identification character for females of *Oc. excrucians* is the shape of the fore unguis, which is bent in a sharp angle with a very narrow space between the unguis and the

subbasal tooth. The integument colour varies from dark in northern specimens to brownish in more southern ones. The colour of scales varies from white to creamy white, and the dark scales from nearly black to very dark brown. The body setae are mostly brownish golden. The proboscis is dark scaled, sometimes with a white scale patch in the middle. The palps have white rings at the basal part of palpomeres I and II. The head has some prominent blackish to bronze erect forked scales. The vertex and occiput have mixed scaling, and a more or less expressed white median stripe and a regular dark spot laterally. The scutum is covered with narrow, bronze scales, which are usually lighter on the lateral parts, sometimes with an indistinct broad median stripe of darker scales. The upper postpronotum has bronze, narrow scales and a patch of broad white ones at the lower part. A postprocoxal patch is present, and distinct white subspiracular and postspiracular patches are usually present. In northern specimens the mesepisternum is covered with three distinct white patches. The variability in southern populations is expressed by a larger median patch which may be fused with the lower patch. The mesepimeron has a white patch which occupies half of the sclerite, but never reaches its lower margins. The femora are thoroughly whitish scaled with a few dark scales and dark, irregular bands close to the white knee spots. The tibiae have mixed scaling, which on the fore and mid leg is somewhat lighter, and the amount of white and dark scales may vary. Tarsomere I of all the legs has mixed scales and more or less well defined white basal rings, tarsomeres II–IV have well defined white rings, except for the fore leg, which has an entirely dark scaled tarsomere IV. Tarsomere V of the hind leg has a white basal ring. The fore leg has the main tooth of the unguis sharply bent, almost parallel to the subbasal tooth. However, in northern parts of Europe a “wider” form exists, which nevertheless has the typical bent characteristic of an *Oc. excrucians* unguis (Fig. 6.32a). The wing veins are mainly dark scaled, with pale scales usually present on the costa (C) and subcosta (Sc), and the other veins with a variable amount of pale scales. The terga are dark scaled, with narrow pale basal bands or patches and more or less numerous scattered pale scales in the apical part (Fig. 6.31b). The irregular yellowish basal bands of the terga, which may extend laterally on the last segments, may be a distinct feature of the northernmost populations. The sterna are nearly entirely whitish scaled, with the exception of sterna



II–V which have dark apical edges, and the cerci have dark scales.

**Male:** The hypopygium of *Oc. excrucians* is very similar to those of *Oc. euedes* and *Oc. surcoufi* (Fig. 10.44). From the latter species it has not been separated in much of the European material. The lobes of tergum IX bear 5–7 more or less stout setae. The basal lobe of the gonocoxite is flat, moderately developed or indistinct, almost absent. It is covered with many short setae of similar length and thickness. The apical lobe usually does not reach the articulation of the gonostylus. The gonostylus is slender, curved apically, and somewhat flattened with a moderate long apical spine. The claspette stem is slender, tapers apically, and the claspette filament gradually tapers towards the apex. The aedeagus is elongated.

**Larva:** Large in size, the antenna is shorter than the head with well developed spicules. The antennal seta (1-A) is inserted at about the middle of the antennal shaft. The postclypeal seta (4-C) has 2–3 short, thin branches. The inner frontal seta (5-C) has 2–3 branches, the median frontal seta (6-C) has 2 branches, and the outer frontal seta (7-C) has 6–7 branches. The prothoracic formula 1-P to 7-P is as follows: 1 (double, long); 2 (single, medium long); 3 (4–5 very thin, short branches); 4 (medium long, thin); 5 and 6 (long, single); 7 (3-branched). The

abdominal seta 6 of segments I and II (6-I and 6-II) has 2 branches, but is single on the rest of the segments (Fig. 8.54a). The number of comb scales is 30–38 arranged in a more or less triangular patch, each individual scale with a conspicuous median spine. The siphonal index is 3.4–4.5, and the distal part of the siphon is distinctly tapered (Fig. 10.45). The pecten has 15–24 pecten teeth, with 1–3 teeth apically detached. The siphonal tuft (1-S) is positioned at about the middle of the siphon, beyond the distalmost pecten tooth, with 6 long branches. Seta 9-S is thickened and transformed into a prominent hook. The saddle extends beyond the middle of the lateral part of the anal segment. The ventral brush has 4–6 precratral tufts (4-X). The anal papillae are slender, and longer than the saddle.

**Biology:** The larvae start to hatch from the overwintering eggs in early spring. More often they hatch later in the year and can be found until the middle of summer in shaded, permanent pools. Only one generation has been recorded from all the different habitats. They occur in greater numbers in open semipermanent or permanent pools or pits with vegetation like *Typha* sp. or *Carex* sp. together with larvae of *Oc. cantans* and *Ae. cinereus*. They can also be found in less abundance in a variety of other habitats, especially in mixed forest regions. Larvae have been observed to feed upon *Euglena* sp. and larger protozoans. Larval development



Fig. 10.44 Hypopygium of *Oc. excrucians*

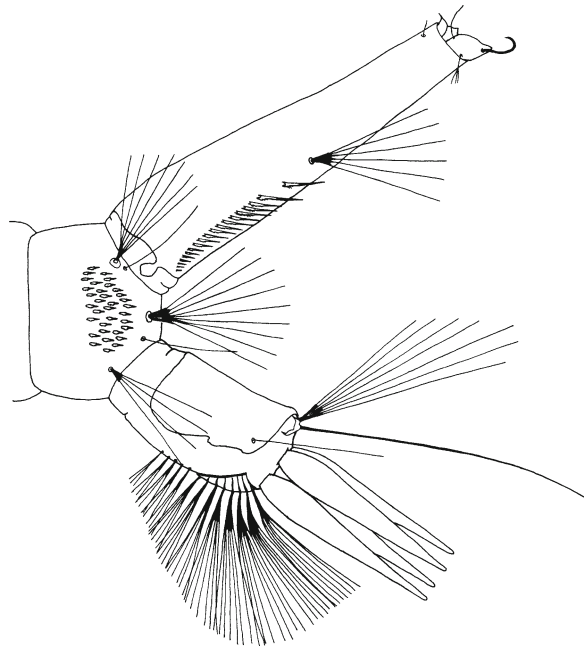


Fig. 10.45 Larva of *Oc. excrucians*

can last from a few to many weeks depending on the water temperature. The females are fierce day biters (for that reason they got the name *excrucians*) and can be found until autumn.

**Distribution:** *Oc. excrucians* is a Holarctic species. Its actual distribution for central and southern Europe is difficult to state, as some earlier records may contain a mixture of sibling species.

***Ochlerotatus (Ochlerotatus) surcoufi*  
(Theobald 1912)**

**Female:** Very similar to *Oc. excrucians*. The most reliable characteristic to distinguish between females of these species is found in the tarsal claws of the fore leg. In *Oc. surcoufi* the main tooth is evenly curved, and the subbasal tooth is diverging, not parallel. The abdominal terga have mixed light and dark scales. Tergum I has a white patch, the following terga have both basal and apical yellowish bands. The apical bands are often reduced to a narrow line, and the basal bands are sometimes laterally irregular. Tergum VII has light scales predominating.

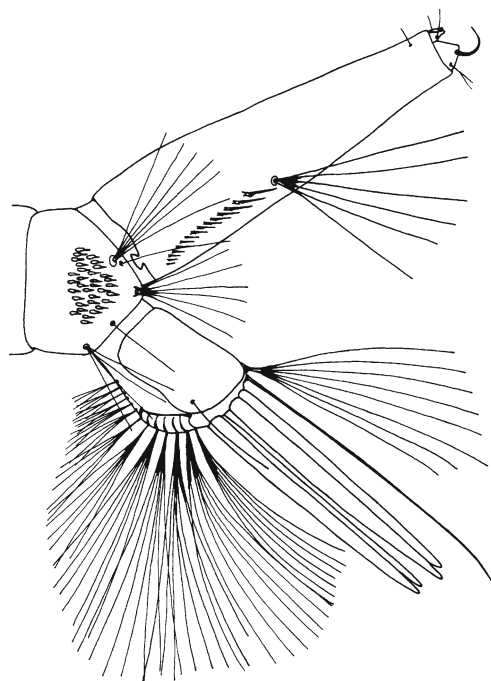
**Male:** The hypopygium is very similar to that of *Oc. excrucians*, apparently morphologically identical.

**Larva:** Similar in most characteristics to *Oc. excrucians*. A striking difference is found in setae 6 on abdominal segments I to VI (6-I to 6-VI) all of which have 2 branches (Fig. 8.54b), whereas in *Oc. excrucians* only setae 6-I and 6-II have 2 branches, and setae 6-III to 6-VI are single. The siphon is long, and tapered towards the apex, the siphonal index is 2.8–3.1 (Fig. 10.46). Seta 9-S is hooked, and not as strong as in *Oc. excrucians*. The ventral brush has 5–6 precatal tufts (4-X).

**Biology:** In the Pyrenees, the species seems to occur in the same habitats as *Oc. excrucians*. The eggs and larvae were found in inundation areas in a Pyrenean valley. Females were caught on humans in the same habitat (Arnaud et al. 1976).

**Distribution:** *Oc. surcoufi* has so far been reported in France (near Paris and the eastern Pyrenees), and on material from Germany and Italy according to Arnaud et al. (1976).

**Note on systematics:** The species has been resurrected from synonymy with *Oc. excrucians* based on European material by Arnaud et al. (1976). In their dis-



**Fig. 10.46** Larva of *Oc. surcoufi*

cussion of the *Ochlerotatus Excrucians* Complex the authors required biometric and genetic analysis of all sibling species to clarify their status.

***Ochlerotatus (Ochlerotatus) flavescens*  
(Müller 1764)**

**Female:** *Oc. flavescens* can be distinguished from other *Aedes* and *Ochlerotatus* species by the abdominal terga which are almost entirely covered with light scales, with only a few scattered dark scales, a characteristic, which is shared only with *Oc. cyprius*. The scutum of *Oc. flavescens* is covered with copper or golden brown scales and the terga with straw coloured scales, which are distinctly paler than the scutal scales. In contrast, *Oc. cyprius* has golden yellowish scales on the scutum and usually ochre yellowish scales on the terga, with the scutum and terga being of more or less the same colour. The proboscis and palps of *Oc. flavescens* have dark and yellowish scales, with the yellowish scales on the proboscis less numerous in the distal half. The vertex and occiput have narrow golden scales

and brown erect forked scales, and broad cream coloured scales laterally. The scutellum has narrow yellowish or light brown scales and brown setae on the lobes. The antepronotum has yellow bronze scales. The postpronotum has upper narrow, bronze scales, and lower broader, cream coloured scales. The postprocoxal membrane usually has a patch of pale scales. The hypostigmal, subspiracular and postspiracular scale patches are well developed. The mesepisternum has pale yellow scales, the upper patch reaching its anterior angle. Pale scales on the mesepimeron end slightly before its lower margin, and lower mesepimeral setae are absent (Fig. 6.29a). The femora are brown with yellowish scales intermixed, pale on the posterior surface. The tibiae are mostly yellow scaled, tarsomeres II–IV of the mid and hind legs with broad basal rings of whitish scales (Fig. 6.27c). The wing veins are covered with narrow yellowish scales and scattered dark scales. The terga are covered with straw-coloured scales, sometimes mixed with isolated dark scales (Fig. 6.28a). The sterna have pale yellowish scales.

**Male:** The lobes of tergum IX are short and narrow, with 5–7 long spine-like setae. The basal lobe of the gonocoxite is slightly flattened with a strong medially directed spine and numerous short setae extending to the middle of the gonocoxite (Fig. 10.47). The apical lobe is prominent, and rounded apically. The gonostylus is slightly expanded in the middle, and the apical spine of the gonostylus is long and slender. The paraproct is strongly sclerotized, and pointed at the apex. The claspette stem is short and straight, slightly tapered distally, with 2–3 long setae on the inner margin near the base. The claspette filament is about as long as the stem, with a cylindrical base and a plate shaped widening on the convex side of the filament, and the apex of the filament is distinctly curved. The aedeagus is long, cylindrical, and notched at the apex.

**Larva:** One of the largest *Ochlerotatus* larva. The antennae are less than half as long as the head, and entirely covered with distinct spicules. The antennal seta (1-A) is located close to the middle of the antennal shaft, with 5–8 branches reaching near the tip. The inner (5-C) and median (6-C) frontal setae have 2–4 branches, and the outer frontal setae (7-C) have 6–9 branches. The number of comb scales is 17–36 (usually 20–27) arranged in three irregular rows (Fig. 10.48). Each individual scale has a strong median spine and several smaller spines at the side, which are about half as long as the median spine. The siphonal index is

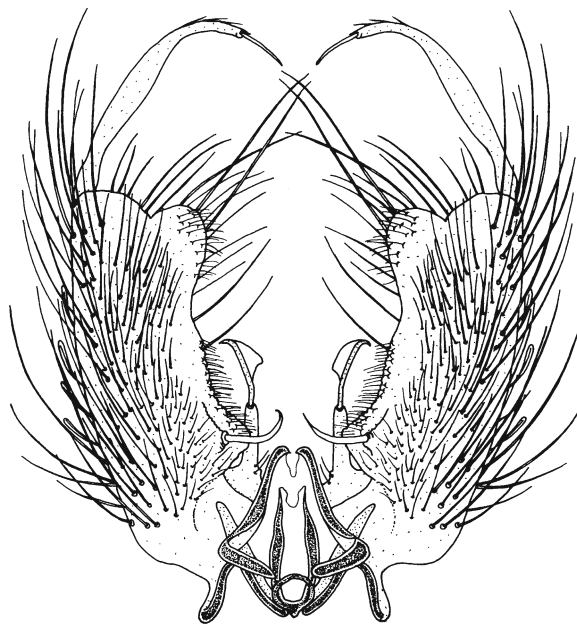


Fig. 10.47 Hypopygium of *Oc. flavescens*

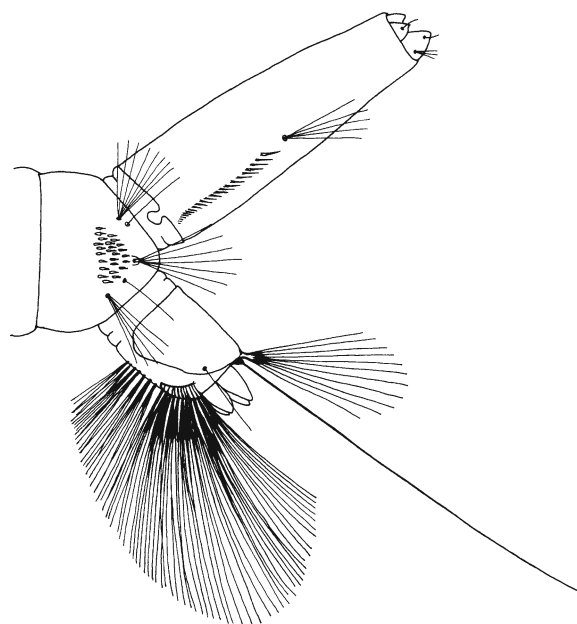


Fig. 10.48 Larva of *Oc. flavescens*

3.4–4.0. The pecten has 17–28 teeth not reaching to the middle of the siphon, and 1 or 2 distal teeth may or may not be apically detached. The siphonal tuft (1-S) has 4–7 branches, situated in the middle of the siphon, about as long as the width of the siphon at the point of its origin. The saddle extends about 3/4 of the way

down the sides of the anal segment. The saddle seta (1-X) is single and approximately as long as the saddle. The upper anal seta (2-X) has >7 branches, and the lower anal seta (3-X) is single and longer than the siphon. The ventral brush has 18–19 tufts of cratal setae (4-X) and 5–7 precratal setae extending nearly to the base of the segment. The anal papillae are usually short, and about half as long as the saddle.

**Biology:** *Oc. flavescens* usually produces only one generation per year (Mihalyi 1959; Trpis 1962). After overwintering in the egg stage the larvae hatch during spring time. They occur predominantly in reeded areas which are not totally shaded. They can frequently be found in brackish marshes along coasts (Gjullin et al. 1961; Mohrig 1969), but also in partly shaded temporary fresh water ponds in floodplains (Hearle 1929; Rempel 1953). The immature stages of this species are tolerant to a wide range of salinity. Usually they occur in neutral or slightly alkaline water, associated with larvae of *Oc. cantans*, *Oc. leucomelas*, *Ae. rossicus*, and *Oc. sticticus*. However, in saline water they can be found together with the larvae of *Oc. detritus*, *Oc. caspius*, or *Oc. dorsalis*. The adults prefer open areas when they are searching for a blood meal. The biting peak of adults occurs during dusk and dawn. Wesenberg-Lund (1921) observed swarming of males near sunset, above nettles. Although *Oc. flavescens* is widely distributed it usually does not occur in great numbers.

**Distribution:** It is a Holarctic species, reported from Eurasia and North America. In Europe the species is widely distributed except for some Mediterranean countries.

### ***Ochlerotatus (Ochlerotatus) hexodontus* (Dyar 1916)**

**Female:** A medium sized species, similar to that of *Oc. punctor*, but can be distinguished from the latter by the pale bands on abdominal sterna III–VI which are of uniform width or only slightly constricted in the middle and the large patch of pale scales at the base of the costa (C). The proboscis and palps are dark scaled, the palps rarely with a few white scales. The head has narrow dark brownish scales and erect forked scales dorsally. The pedicel usually has some whitish scales. The scutum is more or less uniformly covered with rusty brown or yellowish brown scales, usually without a darker median pattern, and occasionally with a pair of indistinct dark

submedian stripes (Fig. 6.49b). The supraalar and scutellar setae are yellowish or yellow brown. The prosternum is scaled with yellow white scales, the upper 3/4 of the postpronotum with rust brown scales. The postprocoxal membrane has pale scales. A hypostigmal scale patch is absent, the subspiracular patch is divided into an upper and lower portion, and the postspiracular scale patch is well developed. The mesepisternum has a pale upper patch extending to the anterior angle, narrowly separated from the prealar patch, and the lower mesepisternal patch is confined to the posterior margin. The mesepimeron has yellowish scales extending to near the lower margin, and 1–3 mesepimeral setae are present. The femora are dark scaled, speckled with pale scales, and pale on the posterior surface. The tibiae and tarsi are dark brown with scattered pale scales especially towards the apices of the tarsomeres, but pale basal rings on the tarsomeres are absent. The wing veins are covered with dark scales, and pale scales are present at the base of the costa (C), and subcosta (Sc) often forming a large patch. The abdominal terga are dark scaled with basal bands of greyish white scales of uniform width, sometimes slightly constricted in the middle (Fig. 6.48b). The sterna are covered with greyish pale scales.

**Male:** The colouration and scaling are much like the females except that the prosternum is not scaled. The hypopygium of *Oc. hexodontus* (Fig. 10.49) is similar to that of *Oc. punctor*; see the description of the latter species.



**Fig. 10.49** Hypopygium of *Oc. hexodontus*

**Larva:** Very similar to that of *Oc. punctor*, but may be distinguished from the latter by the size and number of comb scales. In *Oc. hexodontus* the number of comb scales is usually 6–9, the individual scales are large, and usually longer than the distalmost pecten tooth. In *Oc. punctor* the number of comb scales is >10, and individual scales are shorter than the distal three pecten teeth. In Nearctic populations there also exist differences in the thoracal setation (Wood et al. 1979), which is probably also true for European populations. In *Oc. hexodontus* seta 5-P is branched and setae 1-M, 1-T and 3-T are usually short and multiple branched. In *Oc. punctor* seta 5-P is usually single, and 1-M, 1-T and 3-T are longer and usually single or double. *Oc. hexodontus* has antennae much shorter than the head, and the antennal seta (1-A) is small, and inserted slightly below the middle of the antennal shaft, with multiple branches not reaching to the tip. The inner (5-C) and median (6-C) frontal setae are usually single, rarely with 2 branches, and the outer frontal setae (7-C) have 3–6 branches. The siphon is straight, tapers in the apical half, and the siphonal index is about 3.0 (Fig. 10.50). The pecten has 12–22 evenly spaced teeth situated in the basal half of the siphon. The siphonal tuft (1-S) is located beyond the distalmost pecten tooth, usually with 4–5 branches. The anal segment is entirely encircled by the saddle, and the saddle seta (1-X) is single and about as long as the saddle. The ventral

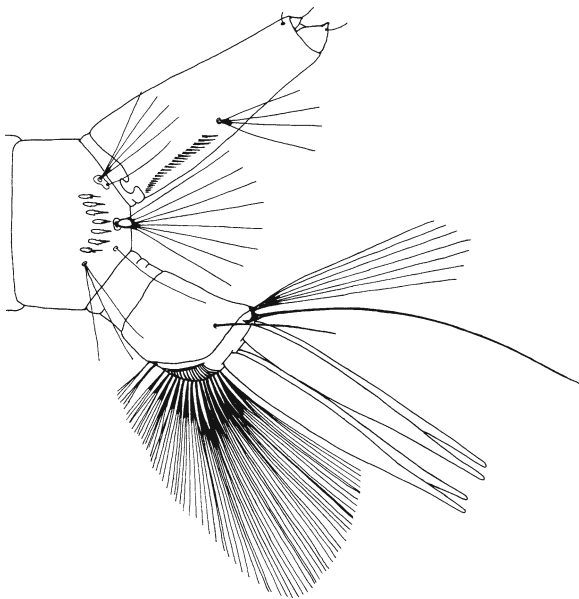
brush has 16–18 cratal setae (4-X) and 2, rarely 1, pre-cratal setae. The anal papillae are lanceolate, and usually twice as long as the saddle or longer.

**Biology:** *Oc. hexodontus* is a monocyclic species which is one of the dominant mosquitoes in areas with long severe winters and short summers. The species occurs in tundra areas close to the tree border where the numerous immature stages develop in oligotrophic snow-melt ponds. In high mountain regions, the larvae can be found in small snow-melt pools with little or no vegetation. The larvae hatch at water temperatures of just above 0°C. The larvae are frequently found associated with those of other snow-melt mosquitoes, such as *Oc. communis*, *Oc. punctor*, *Oc. pionips*, and *Oc. pullatus*. The adults of *Oc. hexodontus* can also occur in mountainous regions close to the tree border and furthermore, in larger numbers if the number of the other species has already decreased. The females are very aggressive biters and may approach their hosts even during strong winds.

**Distribution:** *Oc. hexodontus* is a Holarctic species found in North America and Eurasia, from northern Scandinavia to eastern Siberia, subarctic tundra, and taiga, and above the tree line in mountainous regions.

#### ***Ochlerotatus (Ochlerotatus) hungaricus* (Mihalyi 1955)**

**Female:** A small species, with blackish brown scaled proboscis and palps. The occiput has narrow whitish scales dorsally and broad whitish scales and scattered dark scales laterally, which may form a small patch. The scutum is covered with greyish white scales and a median stripe of dark brown scales, which is divided into two prescutellar dorsocentral stripes not reaching the posterior margin of the scutum. The scutellum has pale narrow scales and light and dark brown setae on the lobes. The postprocoxal membrane is without scales, and a hypostigmal scale patch is absent. The upper mesepisternal scale patch reaches the anterior angle of the mesepisternum, and the mesepimeral scale patch does not reach the lower margin of the mesepimeron. The femora of the fore legs are predominantly pale scaled in the basal half, and the tibiae of the hind legs have dark scales on the anterior surface. The tarsomeres are dark scaled, and pale basal rings are absent. The wing veins are covered with dark scales,



**Fig. 10.50** Larva of *Oc. hexodontus*

and a few isolated pale scales may be present at the base of the costa (C). The abdominal terga have blackish brown scales and pale basal bands which are slightly narrower in the middle and connected with pale lateral triangular patches. The sterna have whitish scales and broad apical dark bands.

**Male:** The gonocoxite is about three times as long as it is wide at the base, and is covered with scales and setae of different length (Fig. 10.51). The basal lobe of the gonocoxite is well developed and conical, with 2 strong spine-like setae of different length and several short setae. The longer spine is slightly curved in the middle, the other spine is short and straight (Fig. 7.34b). The apical lobe is weakly developed and indistinct, with numerous short setae. The gonostylus gradually tapers towards the apex, and the apical spine is moderately long and slender. The paraproct is inwardly curved and pointed. The claspette stem is short and straight, the claspette filament has a long stalk and a broad unilateral wing at the convex side. The aedeagus is tubular shaped.

**Larva:** The head is very dark, blackish brown. The antenna is slightly more than half as long as the head. The antennal seta (1-A) is located distinctly below the middle of the antennal shaft, at about 1/3 of the length from the base, with 5–8 branches. The inner frontal seta (5-C) is single, the median frontal seta (6-C) has 1–2 branches, and the outer frontal seta (7-C) has 6–8 branches. The prothoracic formula (1-P to 7-P) is as follows: 1 (long, single); 2 (medium long, single); 3 (short, 2–4 branched); 4 (short, single); 5 and 6 (long, single); 7 (long, 2-branched). The comb has 16–24 (average 20) scales, arranged in 2 irregular rows (Fig. 10.52). Each individual scale has a long median spine and several shorter spines at the sides. The siphon is cylindrical, only slightly tapering towards the apex, and the siphonal index is 2.0–2.4. The pecten has 19–24 evenly spaced teeth, reaching beyond the middle of the siphon. Each pecten tooth has several lateral denticles at its ventral margin. The siphonal tuft (1-S) has 3–6 branches, situated well beyond the middle of the siphon and well separated from the distalmost pecten tooth. The saddle extends approximately half way down the sides of the anal segment, and the saddle seta (1-X) is single and shorter than the saddle. The upper anal seta (2-X) has 8–11 branches, the lower anal seta (3-X) is single and longer than the siphon. The ventral brush has 13–16 cratal setae (4-X) and 3 precratal setae. The anal papillae are lanceolate,

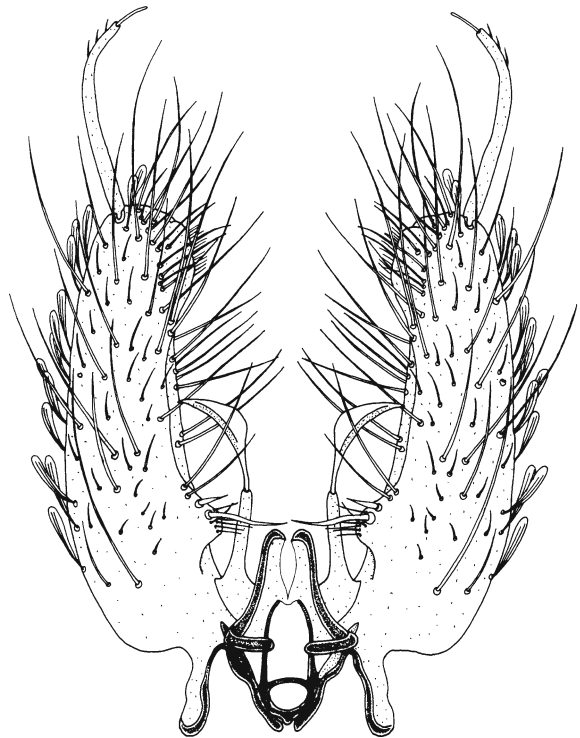


Fig. 10.51 Hypopygium of *Oc. hungaricus*

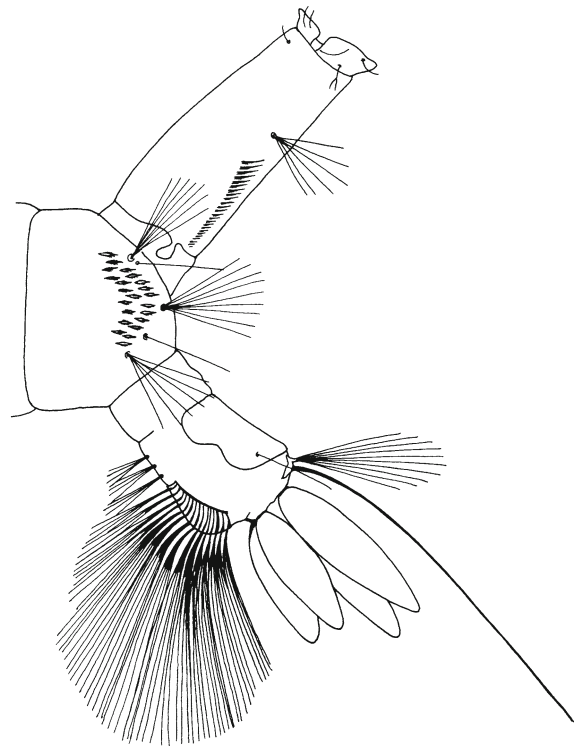


Fig. 10.52 Larva of *Oc. hungaricus*

distinctly longer than the saddle, and the dorsal pair is longer than the ventral pair.

**Biology:** Because the distribution range of the species seems to be limited, little is known about its biology. Larvae are typically found in the floodplains of larger river systems. Mihalyi (1961) collected the larvae in shaded floodwater pools under trees along the Danube river. The species has been found from April to September, thus more than one generation per year is assumed. The females are severe biters and readily feed on humans in shaded situations even during the day.

**Distribution:** *Oc. hungaricus* has so far been reported from Austria, Slovakia, and Hungary (Snow and Ramsdale 1999).

### ***Ochlerotatus (Ochlerotatus) impiger* (Walker 1848)**

**Female:** This small species may sometimes be mistaken for small specimens of *Oc. punctor*. Its integument is brownish grey with conspicuously long and black setae on the scutum. The proboscis and palps are entirely black scaled. The vertex has a spot of dark brown scales. The occiput is covered with erect black setae and white scales. The antenna is black, and the pedicel has some scattered white scales. The scutum has a median stripe of bronze narrow scales, and a lateral stripe of white narrow scales. The scutellum has white narrow scales. The postpronotum has a few bronze scales, otherwise broad white scales, and postpronotal setae are scattered on the entire postpronotum (Fig. 6.41a). A postprocoxal patch is present, the subspiracular and postspiracular scale patches are present, but a hypostigmal patch is absent. The number of postspiracular setae is 10 or fewer (Fig. 6.42b). The mesepisternum has three, more or less, distinct white scale patches, the middle one not reaching the anterior margin. The mesepimeron has a large white patch of scales. All the coxae have white scales, the femora and tibiae of all the legs are dark with scattered white scales, which are less numerous than in *Oc. nigripes*, the tibiae have conspicuous black setae, and all the tarsomeres are dark scaled. The tarsal claws are sharply bent in the middle (Fig. 6.42a). The wing veins are usually entirely dark scaled, a few pale scales are sometimes present at the base of the costa (C) and radius (R). The scaling of the legs and wings seems to vary between Scandinavian and other

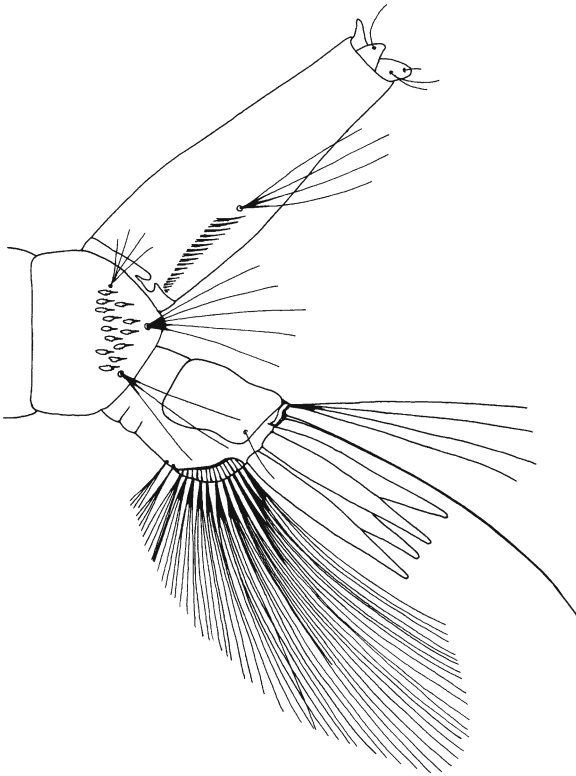
circumpolar populations. The abdominal terga have broad basal white bands.

**Male:** The lobes of tergum IX are rounded, with long setae. The gonocoxite has short setae predominating on the inner side (Fig. 10.53). The basal lobe of the gonocoxite is well developed, and conical, with one spine-like seta. The apical lobe is small and indistinct. The gonostylus is slender, and somewhat broadened in the middle. The paraproct is broadly sclerotized. The claspette filament is about as long as the stem, with a unilateral wing, and the aedeagus is elongated.

**Larva:** The small fourth-instar larva can easily be mistaken for earlier instars of *Oc. hexodontus* or *Oc. punctor* where the saddle is not fully developed and does not encircle the anal segment. The antennae are very short. The inner and median frontal setae (5-C and 6-C) are always single, and the outer frontal seta (7-C) has 3 branches. The prothoracic formula 1-P to 7-P is as follows: 1 (single or 2-branched, short); 2 (single, little longer); 3 (single, long); 4 (single, short); 5 (single or 2-branched, long); 6 (single, long); 7 (3-branched, long). The number of comb scales is 10–14, each scale has a long median spine and several short spines at the base. The siphonal index is 2.8–3.0. The pecten teeth are evenly spaced, each tooth with one long, lateral denticle (Fig. 10.54). The siphonal tuft (1-S) is situated slightly below the middle of the siphon, with 4–6 long branches. The saddle covers approximately half of the anal segment. The ventral brush has 2 tufts of precratal



**Fig. 10.53** Hypopygium of *Oc. impiger*



**Fig. 10.54** Larva of *Oc. impiger*

setae (4-X), and the anal papillae are longer than the saddle (Dahl 1997).

**Biology:** The species is monocyclic. The larvae appear very early in the year and can be found in ponds at the borders of birch-willow shrubs in subarctic areas. They develop fast in snow-melt ponds, preferably with some rotten plant material on the bottom. The small females are most obnoxious daytime biters in the subarctic and arctic areas of Europe. Females bite soon after emergence. However, they quickly leave their hosts and are thus not easy to catch on humans. The biology of the species, together with that of *Oc. nigripes*, has been more thoroughly studied in arctic Canada (Wood et al. 1979).

**Distribution:** *Oc. impiger* is a circumpolar species and in North America it is found, together with *Oc. nigripes*, under extreme arctic conditions. It seems to have a more southern limit than the latter species. In Europe the distribution of *Oc. impiger* is restricted to the tundra of Scandinavia, but its whole distribution range is not well known; it has been recorded in southern Norway (Natvig 1948) and in the tundra of Russia (Gutsevich et al. 1974).

### ***Ochlerotatus (Ochlerotatus) intrudens* (Dyar 1919)**

**Female:** A dark greyish species without metallic lustre in the scales. The presence of a hypostigmal patch of pale scales distinguishes the females from those of *Oc. communis*, *Oc. diantaeus*, and *Oc. nigrinus*. However, the patch is composed of a few scales, which may often be rubbed off in old specimens. The proboscis is black scaled, the palps have scattered white scales, and the head has some pale golden setae between the eyes and yellowish white narrow scales on the vertex. The scutum has two dorsocentral stripes of narrow curved bronze scales, divided by a narrow pale acrostichal stripe, which is sometimes indistinct. The anterior submedian and whole lateral areas are covered with pale scales, and two distinct posterior submedian dark stripes are present. The scutellum has pale yellowish scales and setae. The postpronotum has yellowish-brown narrow curved scales and some whitish scales at the posterior corner. The post-procoxal patch is absent, and a hypostigmal patch is usually present. The sub- and postspiracular areas have patches of white scales. The upper mesepisternal patch of scales is small, and divided into two or more portions, not reaching the anterior margin of the mesepisternum. The prealar patch of scales is displaced apically, and the lower third of the mesepimeron is without scales. The lower mesepimeral setae are usually absent (Fig. 6.44b). The coxae have white scaling, the rest of the legs are almost entirely dark scaled, the fore femur sometimes with some scattered white scales. The wing veins are almost entirely dark scaled, and the base of the costa (C) and radius (R) sometimes have a few white scales. The abdominal terga are dark scaled, with distinct basal white bands.

**Male:** The hypopygium is similar to those of *Oc. diantaeus* and *Oc. pullatus*, but differs in the shape of the claspette stem and the setation on the apical half of the gonocoxite (Fig. 10.55). The lobes of tergum IX are prominent, with a row of 4–7 spine-like setae. The basal lobe of the gonocoxite has 3 large setae which are all spine-like, one of them well separated from the others. The apical lobe of the gonocoxite is well developed, and a small subapical zone of the gonocoxite is covered with long dense setae directed more distally. The gonostylus is curved, and tapers towards the apex. The claspette stem has a thorn shaped process at about its middle. The claspette filament is strongly bent. The





Fig. 10.55 Hypopygium of *Oc. intrudens*

aedeagus is pear shaped, relatively short and with a wide opening.

**Larva:** The head is broader than long, and the antenna is shorter than the head, and covered with spicules. The antennal seta (1-A) is short and inserted slightly below the middle of the antennal shaft. The inner frontal seta (5-C) has 3–5, usually four branches and the median frontal seta (6-C) has 3–5, usually three branches (Fig. 8.35c). The number of comb scales is 12–17, each individual scale is elongated and pointed with lateral spines along the basal part. The siphon is long and slightly tapered (Fig. 10.56). Usually 2–3 distalmost pecten teeth are large, spine-like, without lateral denticles and more widely spaced. The distalmost tooth may be located slightly beyond the point of 1-S insertion. The siphonal tuft (1-S) is inserted slightly beyond the middle of the siphon. The anal segment is not entirely encircled by the saddle, the number of precratal tufts (4-X) is 1–2, and the anal papillae are slender and longer than the saddle.

**Biology:** The species is monocyclic at least in the northern parts of its distribution range. Whether it can have two generations in southern areas is not known. Eggs overwinter and larvae are found from early spring to early summer in many types of temporary pools, which can be located in forests, without submerged vegetation but covered with dead leaves at the bottom, or in more open areas in grassy or snow-melt water pools as well as along floodplains. The larvae

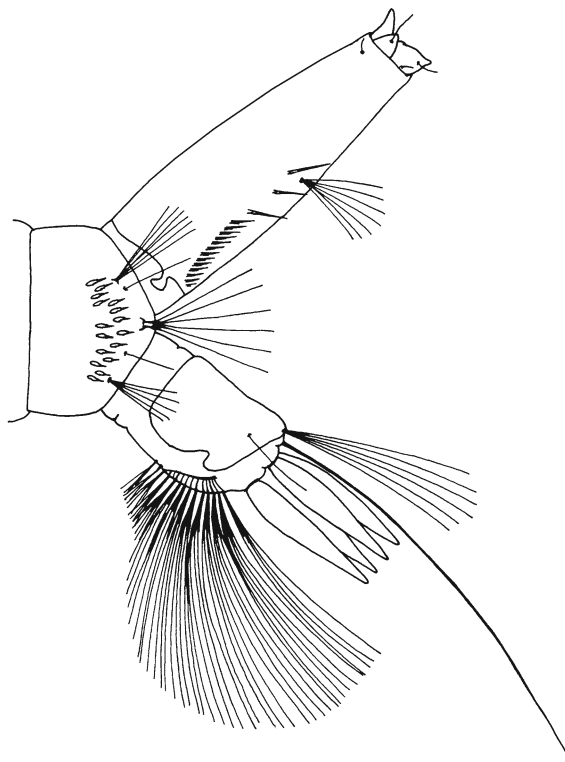


Fig. 10.56 Larva of *Oc. intrudens*

are often found together with those of *Oc. communis*, *Oc. diantaeus*, and *Oc. punctor*, but they emerge before any of these other species (Barr 1958; Wood et al. 1979). The first adults occur from early spring to midsummer depending on the latitude. The females are locally dominant, fierce biters which also enter houses.

**Distribution:** It is a Holarctic species, recorded from central to northern, and eastern to southeastern Europe, it occurs from the steppe in southeastern Europe to the taiga in the north. The distribution range does not seem to extend into western Europe.

***Ochlerotatus (Ochlerotatus) leucomelas*  
(Meigen 1804)**

**Female:** *Oc. leucomelas* is similar to *Oc. cataphylla* in sharing the character of intermixed pale and dark scales on the wing veins, but differs from the latter in the proboscis which is speckled with numerous pale scales, whereas in *Oc. cataphylla* the proboscis is

uniformly dark scaled. The palps are dark brown with scattered pale scales. The vertex and occiput have narrow yellowish white scales and dark erect forked scales, and pale decumbent scales at the sides. The scutum has golden bronze scales, the lateral and prescutellar areas usually have straw coloured scales. The scutellum is dark brown with narrow yellowish white scales. The postpronotum has broad white scales, more yellowish in the upper part, and the scales of the pleurites and legs are as for *Oc. cataphylla*. The postprocoxal membrane has a patch of pale scales. The hypostigmal, subspiracular and postspiracular scale patches are fused. The scales of the mesepisternum do not extend to its anterior angle and the mesepimeral scale patch ends slightly before its lower margin of the sclerite, lower mesepimeral setae are present. The tibiae and tarsi are mostly dark scaled, with pale scales especially on the ventral surface. The tarsi do not have pale rings. The wings are covered with dark scales and numerous scattered pale scales on all veins. The terga have broad basal bands of white scales, the distal parts are mainly dark scaled, and sometimes the last terga are predominantly covered with white scales. The sterna are almost entirely whitish scaled, with some dark scales at the base.

**Male:** The lobes of tergum IX usually have >10 short spine-like setae which are directed slightly outwards, in *Oc. cataphylla* these setae are usually less numerous and directed distally. The gonocoxite is long and slender, with numerous long setae on the inner surface, and the basal setae do not overlap in the middle (Fig. 10.57). The basal lobe is conical with a medially directed, apically strongly recurved long spine and several long setae. The apical lobe of the gonocoxite is well developed and rounded at the apex. The paraproct is strongly sclerotized in the lateral parts, with an inwardly pointed apex. The claspette stem is long and slender, and strongly curved. The claspette filament is very long, with a small plate shaped unilateral widening beginning at the base of the filament and ending distinctly before the apex. The aedeagus is cylindrical and notched at the apex.

**Larva:** The head is broader than long. The antennae are nearly half as long as the head, with weakly developed spicules, usually ventrally located in rows. The antennal seta (1-A) is located in the middle of the antennal shaft with 3–6 branches which are half as long as the antenna. The postclypeal seta (4-C) has 4 short branches, the median frontal seta (6-C) is situ-

ated in front of the inner frontal seta (5-C), and both pairs are single, 5-C rarely with 2 branches, and the outer frontal seta (7-C) has 3–6 branches. The comb consists of 18–30 scales (average 24) arranged in 2–3 irregular rows (Fig. 10.58). Each individual scale varies in shape, the dorsal scales are without a long median spine, the ventral scales have a prominent median spine, and both types have several spines at the sides. The siphon tapers in the apical third, and the siphonal index is 2.5–3.1. The pecten has 13–18 (usually 15)



Fig. 10.57 Hypopygium of *Oc. leucomelas*

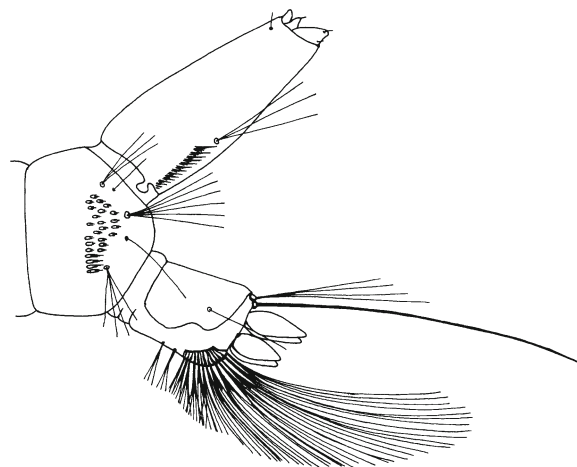


Fig. 10.58 Larva of *Oc. leucomelas*

more or less evenly spaced teeth located in the basal third of the siphon. The siphonal tuft (1-S) is situated below the middle of the siphon with 3–8 branches. The saddle extends down to 2/3 of the sides of the anal segment, and the saddle seta (1-X) is nearly as long as the saddle. The lower anal seta (3-X) is single, the upper anal seta (2-X) has 4–9 branches, and is half as long as 3-X. The ventral brush has 15–18 tufts of cratal setae (4-X) and 1–3 precratal setae. The shape of the cratal setae is characteristic, the branching of the tufts starts far from the base, thus the stem of each tuft is very long. The anal papillae are not longer than the saddle and are tapered apically, with the ventral pair shorter than the dorsal pair.

**Biology:** *Oc. leucomelas* is a monocyclic snow-melt mosquito. The larvae occur early in the year after the snow-melt but seldom in extensive numbers. They are usually found at the edge of forests, in flooded meadows or reeded areas together with larvae of *Oc. cantans*, *Oc. communis*, *Oc. rusticus*, *Ae. rossicus*, and *Oc. sticticus*. In shadowed areas the number of larvae usually decreases. They also occur in slightly saline waters associated with *Oc. detritus*, e.g. in flooded meadows along coasts, thus the larvae are obviously tolerant to variations in the salinity of the breeding sites. The pH can be slightly acidic to alkaline (Natvig 1948). In central Europe the adults usually emerge in May and they decrease in numbers by early July.

**Distribution:** *Oc. leucomelas* is a European species. It is mainly distributed in northern, central, and eastern Europe and has a limited distribution in the southern European countries, where it is solely reported in Spain.

### Ochlerotatus Mariae Complex

Three sibling species, *Oc. mariae*, *Oc. zammitii*, and *Oc. phoeniciae* [*Acartomyia phoeniciae*], have been recognised so far in the complex (Coluzzi and Sabatini 1968; Coluzzi and Bullini 1971; Coluzzi et al. 1976; Bullini and Coluzzi 1982). Apart from slight morphological differences between the species, a varying degree of hybrid sterility was demonstrated. The sterility involves both hybrid sexes between *Oc. phoeniciae* and the other two members of the complex, while only F<sub>1</sub> hybrid males were found to be sterile in the cross between *Oc. mariae* and *Oc. zammitii*. The larvae of

all species have an apparently identical adaptation to breeding in rock pools along the Mediterranean coasts. The distribution range is western Mediterranean for *Oc. mariae* and eastern Mediterranean for *Oc. zammitii* and *Oc. phoeniciae*. The latter has been recorded from the coasts of Cyprus, southeastern Turkey, Lebanon and Israel (Coluzzi et al. 1974). No overlap between these distributional ranges has been recorded so far. According to the updated checklist of European mosquitoes by Ramsdale and Snow (1999), *Oc. mariae* and *Oc. zamitii* correspond to the aforementioned geographical “isolation” within the Mediterranean basin, except for the Greek record of *Oc. marie*, which most probably is *Oc. zammitii*.

### *Ochlerotatus (Ochlerotatus) mariae*

(Sergent and Sergent 1903)

[*Acartomyia mariae*]

**Female:** The species differs from *Oc. caspius* in the ornamentation of the scutum, the black scaling of the legs, and in the absence of a longitudinal light stripe on the abdomen. The proboscis is long and slender, with dark brown scales, sometimes mixed with whitish scales in the middle, giving the impression of being ringed. The palps are 1/6 of the length of the proboscis, and covered predominantly with dark brown scales, with a white tip and some scattered whitish and cream coloured scales. The scutum is largely covered with rust brown to golden scales, usually with some whitish scales, which may sometimes form an indistinct, variable scutal pattern. The scutal setae are predominantly blackish brown. The scutellum has three groups of white sickle shaped scales and golden brown or dark setae. The pleurites have a brown integument and small patches of white scales. A postprocoxal patch of white scales is present. The ventral surfaces of the femora are white scaled, and the anterior surfaces and tibiae are predominantly dark scaled with speckled white scales. The tarsi are conspicuous by their white apical and basal rings on some tarsomeres. Tarsomere V of the fore and mid legs are whitish with some scattered dark scales, and tarsomere V of the hind leg is completely white scaled. The wing veins are covered with dark and pale scales, the pale scales more numerous on the costa (C), subcosta (Sc) and radius (R). The colouration of the abdominal segments is variable (Figs. 6.21b–d). The general appearance is dark, the terga have narrow

basal bands of pale scales which are usually widened laterally into triangular spots. The sterna are covered with whitish and cream coloured scales and dark lateral spots in the apical half, and the cerci are slightly projected.

**Male:** The lobes of tergum IX have 4–6 long setae. The basal lobe of the gonocoxite is moderately convex, densely covered with setae, some of which may be slightly thicker and longer than the others, but strong spine-like setae are absent. The apical lobe of the gonocoxite is indistinct (Fig. 10.59). The claspette has a short and straight stem, and the filament is nearly as long as the stem, narrow and slightly curved, and without a transparent wing. The aedeagus is more or less tubular.

**Larva:** The antenna is shorter than the head, slightly curved, with weakly developed spicules. The antennal seta (1-A) is situated in the middle of the antennal shaft, with 6–9 branches. The postclypeal seta (4-C) is very thin and short, and branched. The inner and median frontal setae (5-C and 6-C) are single, the median frontals are situated in front of the inner frontals, and the outer frontal seta (7-C) usually has 7 branches. The comb is composed of 16–25 scales arranged in 2–3 irregular rows, each individual comb scale with a distinct median spine and a varying number of smaller spines of different size at the base (Fig. 10.60). The siphon is short, slightly tapered, and the siphonal index is 1.4–2.0. The pecten consists of 15 or more thin teeth which are longer distally, reaching to the middle of the siphon. Each pecten tooth usually has 4 or more lateral denticles at the base. The siphonal tuft (1-S) is situated

slightly beyond the middle of the siphon, with 6–7 branches, which are as long as the width of the siphon at the point of its origin. The saddle is weakly developed, extending slightly to the sides of the anal segment. The saddle seta (1-X) is single. The upper anal seta (2-X) has 12–14 branches, the lower anal seta (3-X) is single, and more than twice as long as the siphon. The ventral brush has 11–13 cratal tufts (4-X) and 4–5 precratal tufts. The anal papillae are very short and spherical.

**Biology:** The larvae can be found exclusively in rock pools on the sea shore, often in the surf zone. The usual concentration of salt in such pools is 2–4%, but the larvae are able to tolerate a much higher concentration, up to 20% (Rioux 1958). In the Mediterranean region *Oc. mariae* has several generations per year and its larvae are commonly found from March to October. Full embryonic diapause is observed when eggs are incubated at relatively low temperatures (<16°C) and a short photoperiod. A photoperiod also induces a remarkable change in oviposition behaviour of *Oc. mariae* females. Coluzzi et al. (1975) demonstrated that the adult females readily oviposit when originating

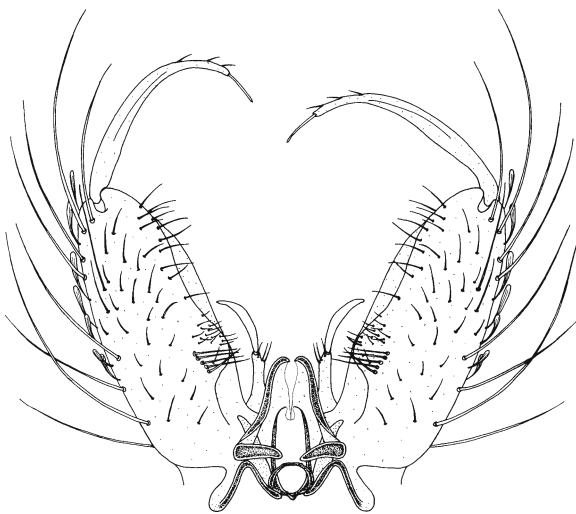


Fig. 10.59 Hypopygium of *Oc. mariae*

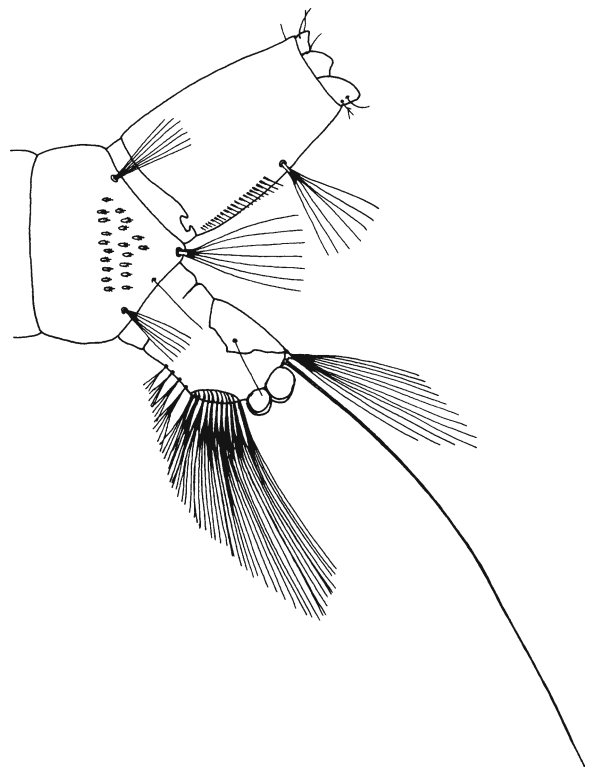


Fig. 10.60 Larva of *Oc. mariae*

from larvae reared at a long day photoperiod, whereas they are very reluctant to oviposit in the same situation when reared at a short day photoperiod. This species can frequently cause nuisance in rocky coastal Mediterranean areas.

**Distribution:** *Oc. mariae* which belongs to the so called “Tyrrhenean” type of the complex inhabits western Mediterranean coasts, ranging from the Algarve in southern Portugal to the Italian western coast, including the western coast of Sicily. It can also be found in Tunisia, Algeria and the Balearic Islands (Coluzzi et al. 1975; Ribeiro et al. 1988).

**Medical importance:** According to Ribeiro et al. (1988) *Oc. mariae* is not known as a vector of any disease, but Gutsevich et al. (1974) reported that the species transmits the parasite of bird malaria, *Plasmodium relictum*.

***Ochlerotatus (Ochlerotatus) zammitii*  
(Theobald 1903)  
[*Acartomyia zammitii*]**

Very similar to *Oc. mariae* in all stages. It seems to be somewhat more robust in appearance with a more distinct colouration pattern. Whereas the scutum of *Oc. mariae* is without special ornamentation, *Oc. zammitii* sometimes has a scutum with two light creamy white longitudinal stripes. The hypopygium of the male is identical to that of *Oc. mariae*, but the larvae differ from those of the latter species by the more numerous spicules on the antenna and the pecten teeth usually having fewer than 4 lateral denticles at the base (Seguy 1924; Darsie and Samanidou-Voyadjoglou 1997).

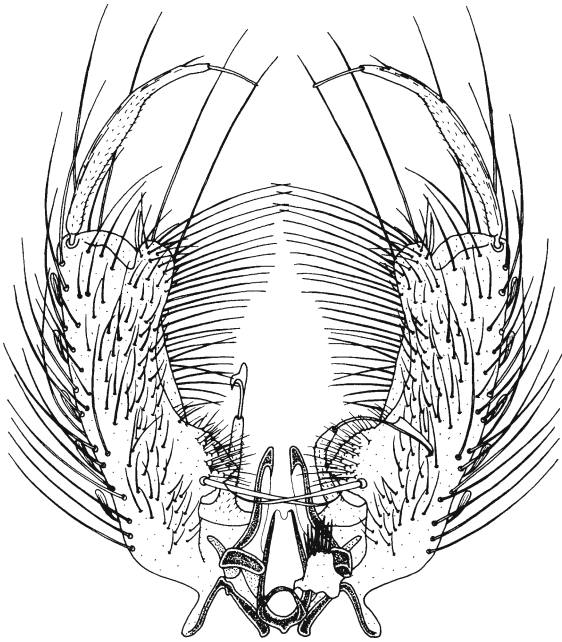
**Distribution:** Adriatic coasts, eastern coast of Sicily, the whole Island of Malta, and Ionean and Aegean coasts (Labuda 1969; Regner 1969; Coluzzi et al. 1974).

***Ochlerotatus (Ochlerotatus) mercurator*  
(Dyar 1920)**

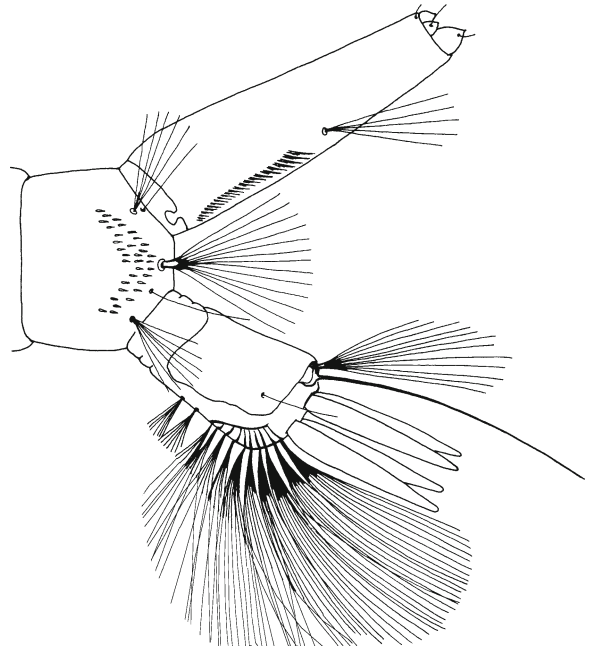
**Female:** The proboscis and palps are dark scaled, and sometimes the palps have a few pale scales at the tip. The pedicel has mixed pale and dark scales. The vertex is yellowish scaled, and the occiput has a pair of dark lateral spots (Fig. 6.30a). The anteppronotum and lower part of the postpronotum are pale scaled, and the upper part of the postpronotum has brown scales. The scutum has a broad median stripe of dark reddish brown scales,

sometimes divided by a narrow acrostichal stripe of pale scales. The posterior submedian areas are brown scaled, and all other areas of the scutum are covered with yellowish scales. A postproxocal scale patch is present, the sub- and postspiracular patches are present, but a hypostigmal patch is absent. The mesepisternum has a prealar patch and upper and lower mesepisternal patches of pale scales, the upper patch not reaching the anterior angle of the mesepisternum. Almost all of the mesepimeron is covered with pale scales. The femora and tibiae of the fore and mid legs have mixed white and dark scales on the anterior surface, and white scales predominate on the posterior surface. The hind femur is light scaled, and the hind tibia has a longitudinal light stripe. The tarsi are predominantly dark scaled with pale basal rings. Tarsomeres I of all the legs have a diffuse basal ring and scattered pale scales almost reaching to the apex. Tarsomeres II and III of all the legs have a more or less distinct basal ring, which is broadest on hind tarsomere III, where it embraces about half of the length of the tarsomere. Tarsomeres IV and V of the fore legs and tarsomere V of the mid legs are usually entirely dark scaled. Tarsomere V of the hind legs sometimes has a few white scales at the base. The tarsal claws are relatively large, and bent near the middle at some distance from the base of the subbasal tooth. The wing veins are entirely dark scaled, sometimes some isolated pale scales are present at the base of the costa (C). The terga are predominantly dark scaled, terga I and II with basal white bands reduced to the median part. The rest of the terga have fully developed transverse basal white bands, which are widened laterally into triangular patches, and are most distinct on terga VI and VII. Narrow apical bands are sometimes present on the last segments. The cerci are long and distinctly projecting.

**Male:** The lobes of tergum IX have 6–12 spine-like setae. The gonocoxite is elongated, with well developed basal and apical lobes (Fig. 10.61). The inner surface of the gonocoxite is covered with long inwardly directed setae, and at least several setae located just above the basal lobe do not overlap in the middle. The basal lobe is conical, and densely covered with thin setae and one medially directed very long spine-like seta which is slightly curved at the apex. The gonostylus is somewhat broadened in the middle, with a slender apical spine. The claspette filament is longer than the stem, with a long stalk, abruptly broadened into a unilateral wing beyond its middle. The paraproct is



**Fig. 10.61** Hypopygium of *Oc. mercurator*



**Fig. 10.62** Larva of *Oc. mercurator*

strongly sclerotized in its apical part, and the aedeagus is elongated.

**Larva:** The head is wider than long. The antenna is about half as long as the head, and is covered with spicules. The antennal seta (1-A) is inserted slightly below the middle of the antennal shaft, with 8–13 (usually 9–10) branches. The inner frontal seta (5-C) has 3–6 branches, usually four or more are present on one side, and the median frontal seta (6-C) has 1–4 branches. The prothoracic formula 1-P to 7-P is as follows: 1 (long, single, rarely 2 branches); 2 (short, single); 3 (short, 2–3 branches) (Fig. 8.47a); 4 (short, single); 5 (long, 2–3 branches); 6 (long, single); 7 (long, 3 branches). The comb is composed of 23–36 (usually about 30) comb scales arranged in several irregular rows (Fig. 10.62). Each scale has indistinct median and lateral spines which become smaller towards the base. The siphon tapers towards the apex, and the siphonal index is 3.3–3.5. The pecten consists of 20–29 (usually 24) closely spaced teeth occupying from about 1/3 to slightly less than half of the siphon length. Each pecten tooth has 2–6 lateral denticles. The siphonal tuft (1-S) is distinctly longer than the width of the siphon at the point of its origin, with 4–7 (usually 5) branches. The saddle covers more than half of the lateral sides of the abdominal segment. The saddle seta (1-X) is distinctly shorter than the saddle, and

is single, or rarely with 2 branches. The upper anal seta (2-X) has 8–13 branches. The ventral brush has 2–6 precratal setae (4-X). The anal papillae are slightly pigmented, tapered, and distinctly longer than the saddle.

**Biology:** The species may have two generations per year. The larvae can be found from May onwards in semipermanent water bodies, such as ditches and small ground pools, or less frequently in permanent waters in open marshes or in old river arms. Adults were encountered from the end of June until the beginning of September. Gutsevich and Dubitsky (1987) stated that nowhere is the species very numerous.

**Distribution:** Northern Holarctic region, in Europe found in the northern part of European Russia as well as in the mountains of Crimea and Caucasus.

#### ***Ochlerotatus (Ochlerotatus) nigrinus* (Eckstein 1918)**

**Female:** Closely related to *Oc. sticticus* and very similar to it in all stages. The proboscis and palps are dark scaled. The occiput has dark erect forked scales at the sides. Flagellomere I of the antennae is entirely dark scaled and slightly swollen, and flagellomeres I–III are distinctly shorter than the others. The pattern of the

scutum is similar to that of *Oc. sticticus*, and the hypostigmal and postprocoxal scale patches are absent. The upper mesepisternal scale patch extends to the anterior angle of the mesepisternum, and the pale scales on the mesepimeron end distinctly above its lower margin. The wing veins are covered with dark scales, with scattered pale scales at the base of the costa (C), the entire length of the subcosta (Sc) and the media (M), proximal to the cross veins. The abdominal terga are dark scaled, with broad basal pale bands of more or less uniform width (Fig. 6.50b). On some terga the bands may be slightly, if at all, constricted.

**Male** (Fig. 10.63): The differences between the hypopygia of *Oc. nigrinus* and *Oc. sticticus* are subtle but usually obvious when directly compared to each other. In *Oc. nigrinus* the basal lobe of the gonocoxite is not as much constricted at the base as it is in *Oc. sticticus* and its upper part is broad and rounded, not slender and crescent shaped (Fig. 7.48b). The apical lobe arises abruptly from the gonocoxite, is widest at its base and rounded in its middle part, and not apically rounded as in *Oc. sticticus*. In all other characteristics the male genitalia of the two species are nearly identical.

**Larva:** Similar to that of *Oc. sticticus*, but can be distinguished by the inner (5-C) and median (6-C)

frontal setae which are usually single, very rarely 2-branched (Fig. 8.49c). The antenna is slightly less than half as long as the head, and the antennal seta (1-A) is situated at about the middle of the antennal shaft, with 3–5 branches not reaching the tip. The number of comb scales is usually 10–12, rarely >20 (Fig. 10.64). Each scale is more elongated and the median spine slightly longer than in *Oc. sticticus*. The siphon is short and straight, and the siphonal index is about 2.5. The pecten teeth are more or less evenly spaced, reaching beyond the middle of the siphon. The siphonal tuft (1-S) is situated slightly beyond the distalmost pecten tooth, with 4–7 branches. The saddle extends far down the sides of the anal segment, and the saddle seta (1-X) is single and shorter than the saddle. The ventral brush usually has 4, rarely 3 precratal setae (4-X). The anal papillae are longer than the saddle, and of varying length.

**Biology:** *Oc. nigrinus* prefers to breed in open terrain, preferably flooded meadows in river depressions, mostly associated with *Ae. vexans*, however, it is not so widely distributed and by far not so numerous as *Ae. vexans* (Eckstein 1918, 1920; Peus 1933).

**Distribution:** Southern Scandinavia, Finland, Denmark, Germany, France, Poland, Estonia, northern Urals to western Siberia.



Fig. 10.63 Hypopygium of *Oc. nigrinus*

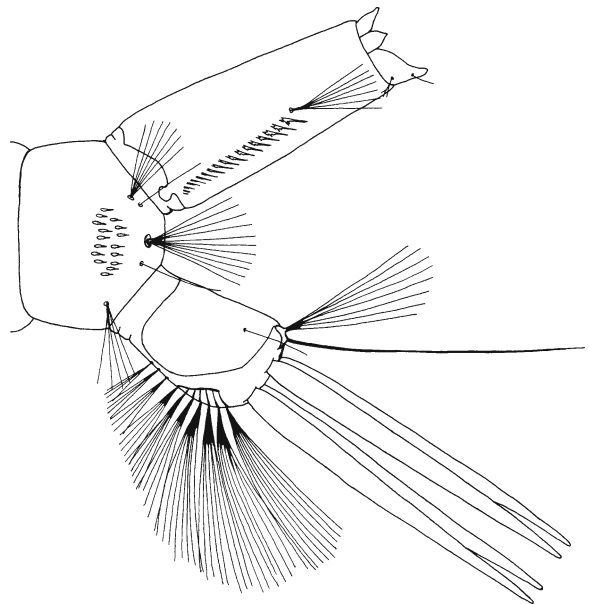
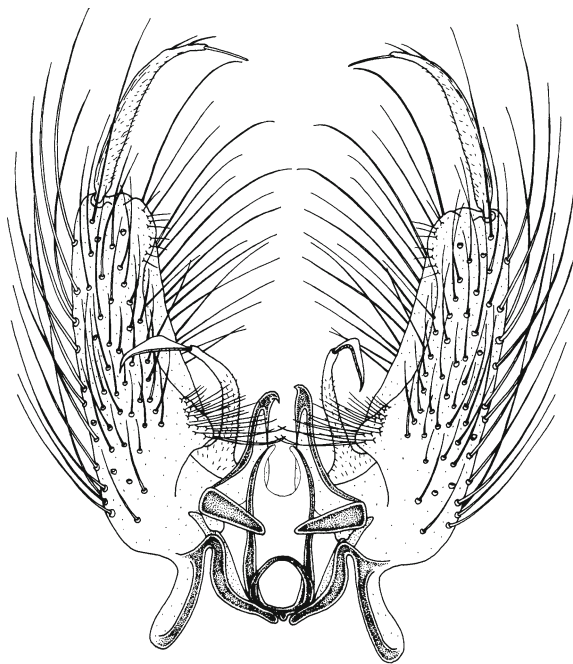


Fig. 10.64 Larva of *Oc. nigrinus*

***Ochlerotatus (Ochlerotatus) nigripes*  
(Zetterstedt 1838)**

**Female:** A medium to large species, easy to identify by its dense blackish body setation, especially on the scutum, its blackish brown integument, and the entirely white scaling of the pleurites. The proboscis is entirely dark scaled, and the palps are dark with some scattered white scales. The head is covered with dark brownish scales, and the occiput with some golden brown erect scales, mixed with grey and dark scales. The antenna is dark greyish, and the pedicel has mixed pale and dark scales. The scutum and scutellum have prominent and dense black or very dark brown setae. The scutum is covered with narrow dark brown scales, occasionally with spots of light scales laterally and on the prescutellar dorsocentral area. All the light scale patches on the pleurites are white. The postpronotum has narrow dark brown scales, and a lower patch either with narrow or broad white scales, and setae scattered on the entire postpronotum. A postprocoxal patch is usually present, but a hypostigmal patch is absent. The subspiracular and postspiracular areas have white scale patches. The number of postspiracular setae is 14 or more (Fig. 6.42d). The mesepisternum has dense upper and lower patches of narrow white scales. The mesepimeron has a broad upper whitish scale patch. The wing veins are covered with dark scales, and pale scales are usually present at the base of the costa (C) and radius (R), sometimes also at the bases of other veins. All the coxae have white scale patches and long dark setae. The femora, tibiae and tarsomeres I of all legs are dark scaled with scattered paler brownish scales. Tarsomeres II–V are dark scaled, with a few pale scales sometimes present on tarsomeres IV and V. The abdominal terga have white basal bands. The sterna are entirely covered with white scales, and the cerci are covered with dark scales and are very setous.

**Male:** The lateral lobes of tergum IX have numerous, heavily sclerotized setae, a characteristic which is shared with *Oc. impiger*. The gonocoxite has long setae predominating on its inner side (Fig. 10.65). The basal lobe of the gonocoxite is well developed, with numerous setae of different length, but of more or less the same thickness. The apical lobe is small and indistinct. The gonostylus is broad, and the apical spine of the gonostylus is slender. The paraproct has inwardly curved tips. The claspette stem tapers towards the apex, and the claspette filament is more than half as

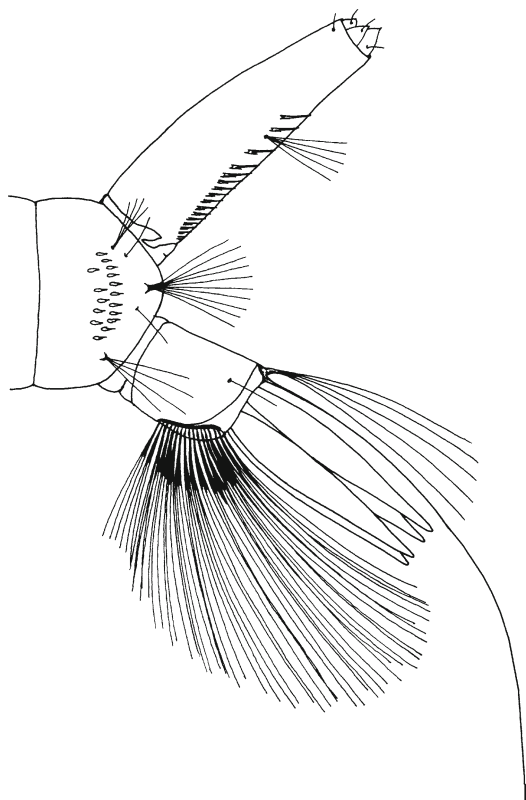


**Fig. 10.65** Hypopygium of *Oc. nigripes*

long as the stem, with a unilateral wing. The aedeagus is oval shaped with a rather wide opening.

**Larva:** The antenna is short and less than half as long as the head. The antennal seta (1-A) is situated beyond the middle of the antennal shaft, and has 1–3 short branches. The inner and median frontal setae (5-C and 6-C) are usually single, sometimes with 2 branches. The prothoracic setae are heavily acidulated, the formula 1-P to 7-P is as follows: 1 (1–2 branches, very short); 2 (single, medium long); 3 (3-branched); 4 (1–3 branches, medium long); 5 and 6 (2–3 branches); 7 (multiple-branched). The number of comb scales is 8–19 and each individual scale has a long median spine and insignificant small lateral spines. The siphonal index is 2.6–3.1 (Fig. 10.66). The pecten has 1–3 distal teeth apically detached, with at least one tooth located beyond the siphonal tuft (1-S). Each pecten tooth is elongated with only one long lateral denticle. The siphonal tuft (1-S) is as long as or longer than the width of the siphon at the point of its insertion, with 2–6 branches. The saddle completely encircles the anal segment, but sometimes a narrow gap may be present at the ventral surface. The saddle seta (1-X) is short, and less than half as long as the saddle. The anal papillae are at least 1.5 times longer than the saddle.





**Fig. 10.66** Larva of *Oc. nigripes*

**Biology:** It is a monocyclic species. In the Canadian tundra (beyond 80°N) females were observed to oviposit only in direct sunlight during the warmest part of the day. Eggs were laid several cm above the water level at the edges of ponds. Such an oviposition behaviour is connected with the search for south facing sites where the snow melts earliest in the next season (Corbet and Danks 1975). In Europe, the larvae have been found in high mountains in Dalarna, Sweden, but mostly occur in the northernmost tundra in willow and birch shrub marshes in the arctic zone in temporary water bodies formed by snow-melt. They are encountered in deep and less oligotrophic pools, as well as in shallow puddles surrounded by *Carex* sp. at higher treeless elevations in the Scandinavian mountains. The larvae are easily disturbed and may spend a long time at the bottom of their breeding sites. The larval development lasts several weeks, adults emerge a little later than those of *Oc. impiger*. Copulation takes places during swarming (Corbet 1965). Females were observed attempting to feed on various birds and mam-

mals (Wood et al. 1979), facultatively autogeny is reported by Corbet (1964). The females are not as aggressive biters as *Oc. impiger* females.

**Distribution:** *Oc. nigripes* is one of the highest arctic, circumpolar species. It mainly occurs north of the tree line in the entire northern hemisphere. Together with *Oc. impiger* its distribution range extends further to the north than any other mosquito species.

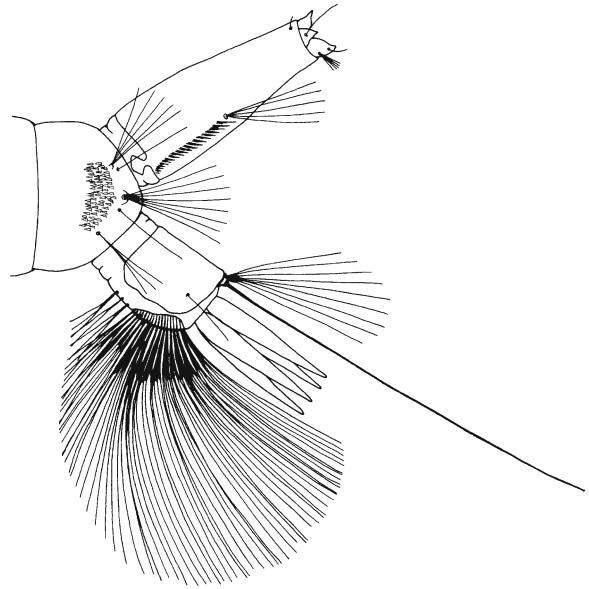
### ***Ochlerotatus (Ochlerotatus) pionips* (Dyar 1919)**

**Female:** Similar to *Oc. communis* but can be distinguished from the latter by the presence of the postprocoxal scale patch. A medium sized species, usually a little larger than *Oc. communis*. The proboscis and palps are entirely dark scaled. The head has narrow yellowish brown scales dorsally and broad appressed yellowish scales laterally and numerous pale erect forked scales. The scutum is covered with golden bronze or yellowish grey scales, with a dark brown median stripe, occasionally divided by a narrow acrostichal stripe of yellowish scales, and a posterior submedian stripe of dark brown scales (Fig. 6.49a). The scutellum has narrow curved yellowish scales and light brown to brown setae on the lobes. The postpronotum has narrow brown scales dorsally, becoming pale ventrally. The postprocoxal membrane has a patch of pale scales. A hypostigmal patch is absent, but the subspiracular and postspiracular scale patches are well developed. The upper and lower mesepisternal scale patches are fused with the prealar patch, reaching the anterior angle of the mesepisternum. The mesepimeron has a pale scale patch reaching the lower margin, and 1–4 lower mesepimeral setae are usually present. The femora are dark brown scaled with scattered pale scales, and the posterior surface is pale scaled. The tibiae and tarsi have dark scales, but pale basal rings on the tarsomeres are absent. The wing veins are covered with narrow dark scales, and occasionally a few pale scales may be present at the base of the costa (C) and radius (R). The abdominal terga are dark scaled, each with a pale basal band of more or less uniform width or slightly widening at the sides. The sterna are covered with whitish scales, and more or less distinct narrow dark apical bands are present.

**Male:** The hypopygium is very similar to that of *Oc. communis* and sometimes they are difficult to



**Fig. 10.67** Hypopygium of *Oc. pionips*



**Fig. 10.68** Larva of *Oc. pionips*

distinguish (Fig. 10.67). The upper part of the basal lobe bears a row of long, prominent setae, which are straight or only slightly curved, but never strongly curved or hooked as in *Oc. communis* (Fig. 7.44a,b). No other distinct differences exist in the hypopygia of the two species. In case the identification by the hypopygia alone is uncertain and the whole specimen is available, the length of the palps and the postprocoxal scale patch should be taken into consideration. In males of *Oc. pionips* the palps are slightly shorter than the proboscis and the postprocoxal scale patch is present. In males of *Oc. communis* the palps are longer than the proboscis and the postprocoxal scale patch is absent.

**Larva:** The head is wider than long. The antenna is about  $2/3$  as long as the head, slender and slightly curved with spicules. The antennal seta (1-A) is situated slightly below the middle of the antennal shaft, with 7–13 (usually 9) branches which do not reach the tip. The postclypeal seta (4-C) is small, with 3–5 short branches. The inner (5-C) and median (6-C) frontal setae have 3–5 (rarely 6) branches (Fig. 8.42a), and in *Oc. communis* both pairs are single, rarely with 2 branches. The outer frontal seta (7-C) has 5–9 branches. The comb consists of more than 60 scales arranged in an irregular patch (Fig. 10.68). Each individual scale is without a prolonged median spine, thus appears to be rounded apically, and the lateral

margin is fringed with short spines, decreasing in size towards the base (Fig. 8.42b). The siphon is straight, tapers towards the apex, and the siphonal index is 2.5–3.0. The pecten has 18–24 evenly spaced teeth, confined to the basal half of the siphon. The siphonal tuft (1-S) is located beyond the distalmost pecten tooth at about the middle of the siphon, with 4–9 branches, which are slightly longer than the width of the siphon at the point of its insertion. The saddle extends far down the sides of the anal segment, and the saddle seta (1-X) is single and a little shorter than the saddle. The upper anal seta (2-X) has 9–13 branches, the lower anal seta (3-X) is single, and is much longer than the siphon. The ventral brush has 17–21 tufts of cratal setae (4-X) and 2–3 precratal setae. The anal papillae are lanceolate, pointed, and longer than the saddle.

**Biology:** *Oc. pionips*, like *Oc. communis*, is a typical monocyclic snow-melt mosquito, whose larvae hatch during the snow-melt in early spring. Its breeding sites are snow-melt ponds in boggy forests up to an altitude of > 1,000 m, where larvae often are associated with those of *Oc. communis*, *Oc. hexodontus*, and *Oc. punctor*. Gjullin et al. (1961) observed that *Oc. pionips* develops slower than the associated *Aedes/Ochlerotatus* species. The adults seem to appear later and are often less numerous than the other snow-melt mosquitoes.

**Distribution:** Holarctic species, occurs in North America and northern Eurasia.

***Ochlerotatus (Ochlerotatus) pulcritarsis*  
(Rondani 1872)**

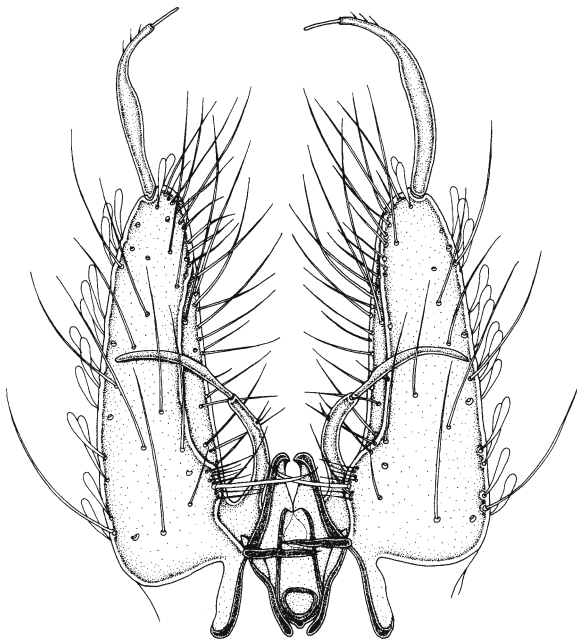
**Female:** Very similar to the females of *Oc. berlandi*. A slight difference exists in the scutal colouration pattern. Whereas the scutum of *Oc. berlandi* has clearly contrasting by dark and pale golden scales, *Oc. pulcritarsis* exhibits a weaker pattern of pale and dark scales on the scutum, and looks rather uniformly golden brownish in colour. However, the median and lateral stripes may be somewhat lighter than the submedian spots.

**Male (Fig. 10.69):** The hypopygium is very similar to that of *Oc. berlandi*. The spine-like seta on the basal lobe of the gonocoxite is usually less curved at the apex, and rarely hooked.

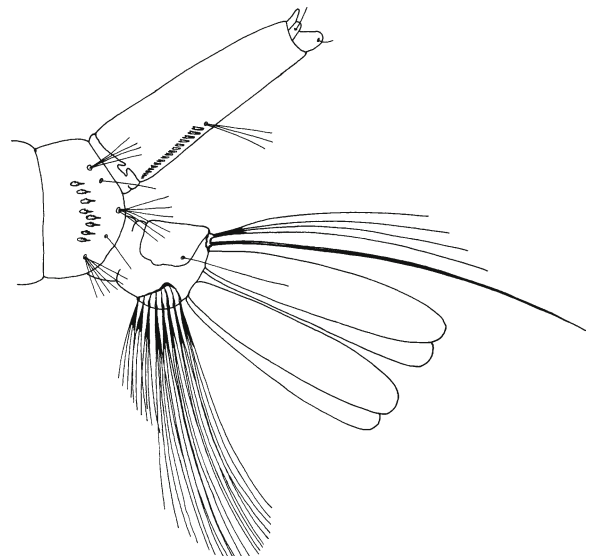
**Larva:** The head is square shaped, and somewhat broader than long. The antenna is almost as long as the head, and the antennal shaft is smooth. The antennal seta (1-A) has 3–4 branches, inserted at about the middle of the antennal shaft (Fig. 8.58c). The postclypeal seta (4-C) is small and multiple-branched. The frontal setae (5-C to 7-C) are well developed and multiple-branched. The comb consist of 6–10 (usually 8) scales

arranged in one row (Fig. 10.70). Each scale is large with a well developed median spine, and small spines on the basal half of the scale. The siphon is dark, almost black, slightly tapering in the apical half, and the siphonal index is 4.0–5.0. The pecten consists of 18–22 evenly spaced teeth. Each individual tooth is blunt ended with 3–6 heavily sclerotized lateral denticles. The siphonal tuft (1-S) has 3–4 branches, is about as long as the width of the siphon at the point of origin, and is situated below the middle of the siphon. The saddle covers about half of the anal segment. The saddle seta (1-X) is longer than the saddle and is single. The upper anal seta (2-X) has 4–5 branches of variable length. The lower anal seta (3-X) is longer than the siphon, and single. The anal papillae are very long, sausage shaped, and several times longer than the saddle.

**Biology:** Hibernation takes place in the egg stage, and the species has usually two generations per year, but sometimes only one generation occurs. The larvae can be found in tree-holes, stumps and among roots of deciduous trees, such as *Quercus* sp., *Platanus* sp., and *Ulmus* sp., the latter being preferred, as well as in olive tree-holes (Shannon and Hadjinicolaou 1937). The water temperature of the breeding sites never exceeds 21°C even in southern European climatic conditions. Similar observations were made along the Black Sea shore in Bulgaria (Bozkov et al. 1969). Larval development may last up to two months. Adult females are



**Fig. 10.69** Hypopygium of *Oc. pulcritarsis*



**Fig. 10.70** Larva of *Oc. pulcritarsis*

anthropophilic; they bite outdoors during daytime (Rioux 1958). Adult mosquitoes of both sexes were found in stables and houses in a village where no trees were present. This may indicate that the species is facultatively zoophilic and could breed in artificial containers or have a considerable migrating capacity (Shannon and Hadjinicolaou 1937).

**Distribution:** *Oc. pulcritarsis* is principally a species of the Mediterranean region. Its northern distribution range reaches as far as the Czech Republic. It is also found in central and southeastern Asia.

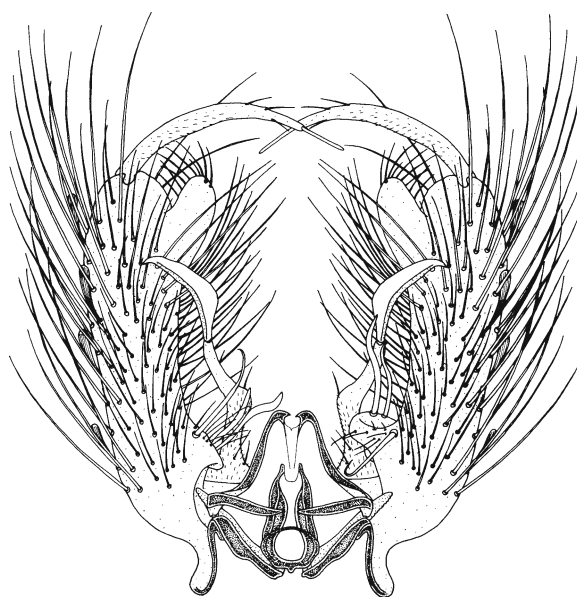
**Note on systematics:** One subspecies, *ssp. asiaticus* Edwards is reported in Uzbekistan, Turkmenia, and Pakistan.

### ***Ochlerotatus (Ochlerotatus) pullatus* (Coquillett 1904)**

**Female:** A medium sized species. The proboscis is dark scaled, and the palps are predominantly dark scaled with a few scattered pale scales at the joints of the palpomeres. The clypeus is blackish brown, and the pedicel is dark brown with a few pale scales. The vertex and occiput are covered with pale yellowish narrow scales and erect forked scales of the same colour on the dorsal part and usually broad appressed yellowish white scales laterally. The integument of the scutum is black, and the scutum is covered with yellowish brown narrow curved scales, disconnected by several bare longitudinal stripes and areas to which they contrast, mainly due to the exposure of the dark integument. The transverse suture, prescutellar area, and lateral ends of the scutum are also devoid of scales. The setae of the scutum are usually golden brown, sometimes blackish brown, and are more numerous on the posterior part. The scutellum has narrow pale scales and yellowish brown setae on the lobes. The pleurites have patches of broad yellowish white scales, a hypostigmal patch of scales is present, and the postprocoxal membrane is bare. The mesepisternal patch of scales is divided into an upper and lower portion, and the upper patch does not reach the anterior margin of the mesepisternum (Fig. 6.43a). The mesepimeral patch of scales reaches near the lower margin of the mesepimeron, and 1–5 lower mesepimeral setae are present (Fig. 6.44a). The femora have dark brown and pale scales intermixed, but are darker apically with a pale knee spot. The tibiae and tarsomere I are dark brown, and speckled with pale scales, espe-

cially on the ventral surface. The remaining tarsomeres are entirely dark scaled. The wing veins are covered with narrow dark scales, and with patches of pale scales at the base of the costa (C), radius (R) and anal vein (A). Abdominal tergum I has a broad patch of white scales, terga II–VII are blackish brown scaled with a basal transverse band of white scales which are sometimes slightly widened laterally, and the abdominal sterna are mostly white scaled. The cerci are exceptionally long and conspicuous.

**Male:** The lobes of tergum IX have 5–7 short stout setae. The gonocoxite is long and slender, about three times as long as it is wide, with its inner surface densely covered with long setae (Fig. 10.71). The basal lobe of the gonocoxite is prominent and has 3 setae which are distinctly larger than the rest, one long stout apically curved spine-like seta inserted dorsally and two sinuous, flattened and slightly lanceolate setae arising from the ventral surface of the basal lobe (Fig. 7.31b). The apical lobe is well developed, thumb-like, with numerous setae at the tip. The gonostylus is about half as long as the gonocoxite, curved, slightly expanded before the middle, bearing a few small setae close to the apex, and the apical spine of the gonostylus is long and slender. The paraproct is heavily sclerotized apically. The claspette stem is long, swollen, and strongly bent in the middle, without a thorn shaped process, the basal half is stout, and the distal half is slender. The



**Fig. 10.71** Hypopygium of *Oc. pullatus*

claspette filament is shorter than the stem, prominently winged on the convex side, and the aedeagus longer than wide.

**Larva:** The head is slightly wider than long. The antenna is about half as long as the head, slightly curved, and covered with spicules. The antennal seta (1-A) is inserted at the basal third of the antennal shaft, with about 5 branches, which do not reach to the tip of the antenna. The postclypeal seta (4-C) is small, with 4–5 branches, the frontal setae are multiple-branched, the inner (5-C) and median (6-C) setae have at least 3 branches (mostly 4–6), and the outer frontal seta (7-C) has 8–13 branches (Fig. 8.42c). The prothoracic setae 2-P and 3-P are nearly as long and strong as 1-P (Fig. 8.47b). The comb consists of 40–60 scales arranged in a large triangular patch, the lateral scales are more or less pointed, with a longer median spine, and fringed with smaller lateral spines. The pecten has 15–25 teeth, which are evenly spaced and situated close together (Fig. 10.72). The siphon uniformly tapers towards the tip from near the middle, and the siphonal index is 3.0–3.5. The siphonal tuft (1-S) is situated more or less at the middle of the siphon, beyond the distalmost pecten tooth, with 5–8 branches. The anal segment has a saddle which extends more

than half way down the side, and the saddle seta (1-X) is single and shorter than the saddle. The upper anal seta (2-X) has 6–10 branches, and the lower anal seta (3-X) is long and single. The ventral brush has about 12–15 tufts of cratal setae (4-X) and 1–3 precratal tufts. The anal papillae are about twice as long as the saddle, and pointed.

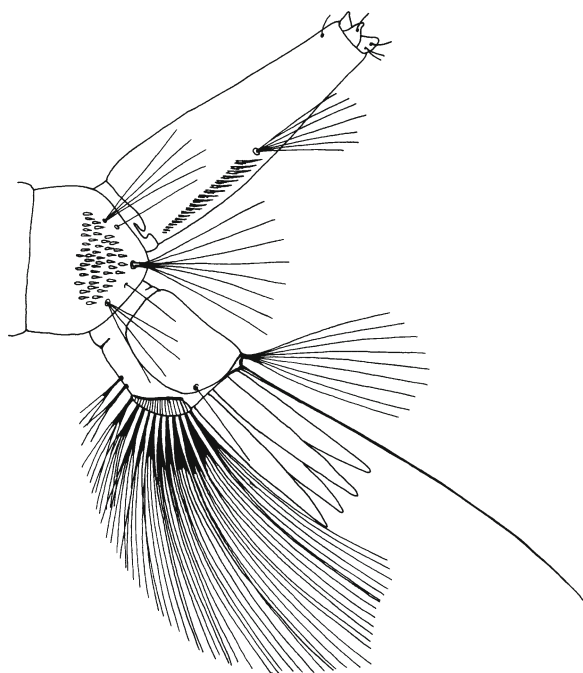
**Biology:** *Oc. pullatus* produces one generation per year. The larvae hatch from overwintering eggs from early spring until late summer, depending on the elevation of their occurrence. They can be found in the tundra mostly in small clear snow-melt pools and in mountainous regions in a variety of breeding sites, e.g. in puddles and pools, without vegetation, created by overflow of mountain streams or after heavy rainfall, small clear lakes with a rocky bottom or boggy holes (Kaiser et al. 2001). The larvae may be associated with those of other mountainous *Aedes/Ochlerotatus* species, e.g. *Oc. communis* and *Oc. punctor*. Their development lasts longer than that of their associates and consequently the adults occur later, mainly in the summer months. The females readily attack their hosts during any time of the day in forested areas. Male swarming takes place after sunset in openings of the forest. The species is usually found in small numbers, but adults can be abundant in some localities, generally far remote from human habitations (Carpenter and La Casse 1955).

**Distribution:** *Oc. pullatus* is a northern Holarctic species with a disjunct distribution range. In central and southern Europe it is restricted to mountainous regions, including the Pyrenees, Alps, Dinari Mountains, Tatra, Carpathians, Balkans, Rhodopi Mountains and can be found up to very high elevations (2,000 m and higher). In the northern parts of its distribution range it occurs in the arctic tundra, lowlands and plains in Eurasia as well as in North America.

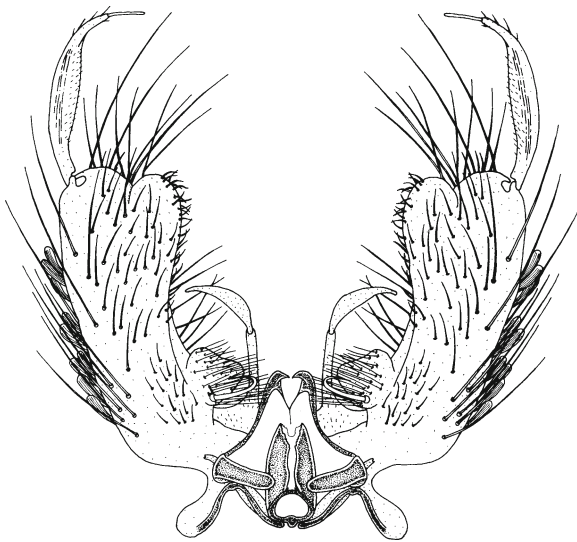
### ***Ochlerotatus (Ochlerotatus) punctodes* (Dyar 1922)**

**Female:** The adult females cannot be distinguished from those of *Oc. punctor*. Differences occur mainly in the larval stage (Knight 1951).

**Male** (Fig. 10.73): Separable from *Oc. punctor* by the shape of the basal lobe of the gonocoxite and the claspette filament. The basal lobe has an irregular triangular shape, and the basal part of the lobe abruptly



**Fig. 10.72** Larva of *Oc. pullatus*

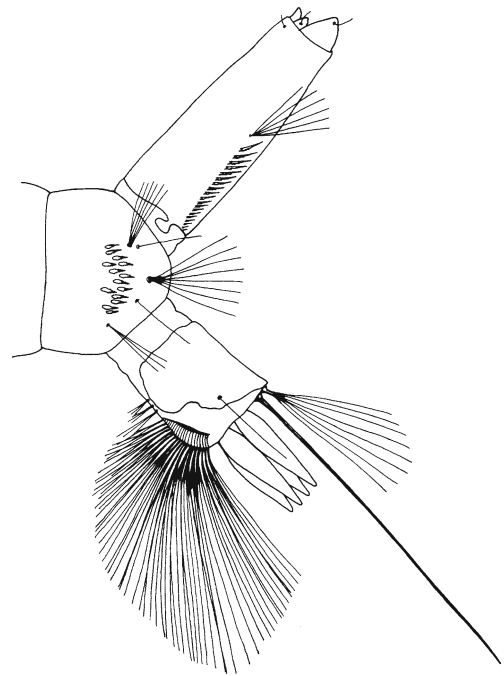


**Fig. 10.73** Hypopygium of *Oc. punctodes*

arises from the gonocoxite, whereas in *Oc. punctor* the basal part of the lobe arises more gradually from the gonocoxite. The claspette filament is relatively long and narrow, and of almost the same length as the stem and weakly sclerotized, but in *Oc. punctor* the filament is shorter than the stem and strongly sclerotized (Fig. 7.42b). Carpenter and La Casse (1955) stated that the spine of the basal lobe is more weakly developed in *Oc. punctodes* and sometimes is difficult to separate from the long setae at the base of the lobe. In *Oc. punctor* the spine of the basal lobe is strongly developed and distinctly different from the long setae at the base of the lobe.

**Larva:** The inner (5-C) and median (6-C) frontal setae are usually single (Fig. 8.32b), whereas in *Oc. punctor* both pairs are usually 2-branched. The saddle does not completely encircle the anal segment, and extends to near the midventral line (Fig. 10.74). The anal papillae are usually much shorter than in larvae of *Oc. punctor*, varying from much shorter to slightly longer than the saddle.

**Biology:** *Oc. punctodes* belongs to the typical salt marsh fauna in subarctic regions (Frohne 1953). The larvae occur predominantly in the more saline waters, whereas the larvae of the closely related *Oc. punctor* prefer fresh water habitats. In these habitats the larvae of *Oc. punctodes* can be found together with those of *Oc. punctor*, *Oc. communis*, *Oc. impiger*, *Oc. excrucians*, and *Oc. flavescens*. Frohne (1953)



**Fig. 10.74** Larva of *Oc. punctodes*

found newly hatched larvae of *Oc. punctodes* in Alaska at the end of April and a few larvae were still present in August.

**Distribution:** *Oc. punctodes* is a subarctic species. It is reported from Alaska and in Europe from Norway, Sweden, Finland, and European Russia.

#### ***Ochlerotatus (Ochlerotatus) punctor* (Kirby 1837)**

**Female:** A medium sized species. The proboscis and palps are dark scaled. The occiput has golden yellowish narrow scales and yellow erect forked scales dorsally, with broad appressed creamy white scales laterally. The scutum is covered with yellowish brown scales, usually with a median stripe of dark brown scales and occasionally divided by an acrostichal stripe of yellowish scales, and the posterior submedian areas with dark brown scales. The scutellum has yellowish brown scales and light brown setae on the lobes. The prosternum has no scales on the anterior surface, or occasionally has scattered pale scales, but is not as extensively covered with scales as in *Oc. hexodontus*. The postpronotum has narrow yellowish brown scales anteriorly and broader paler scales at the

posterior margin. The postprocoxal membrane has pale scales (absent in *Oc. communis*). A hypostigmal patch is absent, the subspiracular patch is divided into an upper and lower portion, and the postspiracular patch is well developed. The upper and lower mesepisternal scale patches are fused, and extend to the anterior angle of the mesepisternum, narrowly separated from the prealar patch (Fig. 6.47a). The scales on the mesepimeron extend to its lower margin, and 1–5 lower mesepimeral setae are present. The femora, tibiae and tarsomeres are mostly dark scaled. The claws of the fore legs are elongated, and gradually curving distal to the subbasal tooth. The wing veins are usually entirely dark scaled, with a few pale scales present occasionally (less than in *Oc. hexodontus*, *Oc. communis*, and *Oc. pionips*) at the base of the costa (C). The abdominal terga are dark scaled with basal bands of white scales, which are distinctly confined in the middle or even interrupted on the more anterior terga (Fig. 6.48a). The sterna are covered with greyish white scales, and the apices of some of the dark sterna are scaled medially.

**Male:** The lobes of tergum IX are sclerotized, each bearing several spine-like setae. The gonocoxite is about three times as long as it is wide, the basal lobe of the gonocoxite is well developed, and more or less triangular shaped (Fig. 10.75). The basal part has a row of long setae, a long apically recurved spine, and

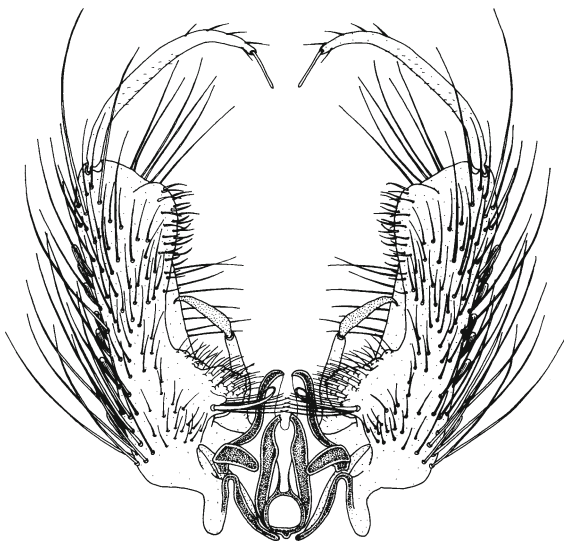
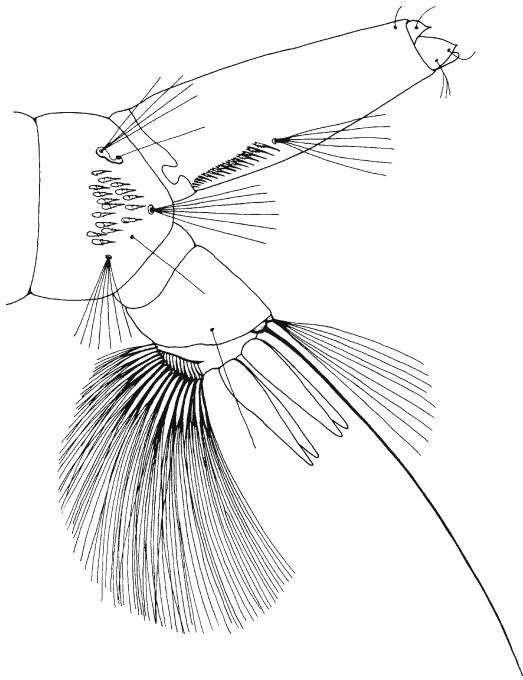


Fig. 10.75 Hypopygium of *Oc. punctor*

the apical part is densely covered with short setae. The apical lobe is broadly rounded with short curved setae extending downwards to near the middle of the gonocoxite. The gonostylus is slightly expanded in the middle with several small setae before the apex, and the apical spine of the gonostylus is slender. The paraproct is strongly sclerotized, with the apex inwardly pointed. The claspette stem is short and slightly curved near the middle. The claspette filament is shorter than the stem, lanceolate, wide in the middle, strongly sclerotized and not transparent, with a curved apex. The aedeagus is cylindrical, and notched at the apex.

**Larva:** Very similar to that of *Oc. hexodontus*. For separation of the two species see the description of the latter. The antennae are less than half as long as the head, and covered with numerous spicules. The antennal seta (1-A) is situated in the middle of the antennal shaft or slightly below it, with 4–7 branches not reaching the tip. The postclypeal seta (4-C) has 2–4 short branches. The inner (5-C) and median (6-C) frontal setae have 1–3 branches, usually 2-branched, and the outer frontal seta (7-C) has 2–8 branches (Fig. 8.32a). The comb has 10–25 small scales, usually arranged in 2–3 irregular rows or a triangular patch, and each scale has a prominent median spine and several smaller spines in the basal part. The siphon tapers in the apical half, and the siphonal index is about 3.0 (Fig. 10.76). The pecten has 14–26 evenly spaced teeth confined to the basal half of the siphon. The siphonal tuft (1-S) is located beyond the distalmost pecten tooth, with 3–9 branches, and is about as long as the width of the siphon at the point of origin. The saddle completely encircles the anal segment, and the saddle seta (1-X) is usually as long as or a little longer than the saddle. The upper anal seta (2-X) has 5–9 branches, and the lower anal seta (3-X) is single and long. The ventral brush has 16–19 tufts of cratal setae (4-X) and 1–2 precratal setae. The anal papillae taper and are of variable length but are always distinctly longer than the saddle.

**Biology:** *Oc. punctor* is a snow-melt mosquito, which has a preference for swampy forests with boggy waters. The larvae hatch during the snow-melt, when the water temperature is only a little above 0°C. While Monchadskii (1951) and Horsfall (1955) found larvae only in springtime, they occur in southern Germany also in the summer after strong rainfall; sometimes together with the larvae of *Ae. cinereus*



**Fig. 10.76** Larva of *Oc. punctor*

and *Cs. alaskaensis* (Peus 1929; Vogel 1933, 1940; Becker and Ludwig 1981). Some larvae may overwinter together with those of *Oc. rusticus* and *Cs. morsitans*. Larvae of this acidophilic species can be found in great numbers in boggy waters with *Sphagnum* sp. growth, where the pH-value can be less than 4.0. The optimum temperature for the development of *Oc. punctor* is 25°C, however the lowest mortality rate is found at temperatures of 15°C. At 25°C larval and pupal development lasts 10–17 days, at 20°C 15–22 days, at 15°C 20–26 days and at 10°C 33–41 days. At 30°C the larvae die in the first and second larval stages. In early spring the larvae of *Oc. punctor* occur together with those of *Oc. communis*, and a little earlier than the larvae of *Oc. diantaeus* and *Ae. cinereus*. In central Europe, the adults occur in the second half of April, mostly later than those of *Oc. communis*, but earlier than *Oc. cantans*. The adults prefer sheltered terrain and seldom migrate out of the forest. Their peak biting activity is during dusk, on sultry days and in strongly shaded situations they can be troublesome even during daytime.

**Distribution:** *Oc. punctor* is a Holarctic species and can commonly be found in North America and

Eurasia. In Europe it is distributed from Scandinavia to the Mediterranean region.

***Ochlerotatus (Ochlerotatus) riparius*  
(Dyar and Knab 1907)**

**Female:** The species has a brownish integument. The proboscis is predominantly white scaled and the palps are dark scaled with scattered white scales or a narrow white band. The vertex and occiput are covered with bronze golden narrow scales and a small lateral, white patch. The scutum has narrow, bronze and golden gleaming scales, and a broad median stripe of darker scales is usually present. Small pale anterior submedian patches close to the transverse suture may be present, and the prescutellar bare space is usually surrounded by pale scales (Fig. 6.33a). The scutellum has bronze scales and a diffuse pale patch on the median lobe. The postpronotum is covered with narrow bronze scales in the upper half and with narrow sickle shaped pale scales in the lower half. Small white scale patches on the antepronotum and propleuron are present, and the postprocoxal membrane has a small white patch. A small hypostigmal patch of scales is sometimes present. The postspiracular area and paratergite have pale scale patches. The mesepisternum has three distinct patches along the posterior margin, and the mesepimeral patch of pale scales covers a little more than half of the mesepimeron. The coxae have white scale patches, the fore femur and all the tibiae have mixed scales dorsally, with white scales ventrally; and the femora of the mid and hind legs are a little darker. Tarsomeres I of all the legs are predominantly white scaled with a diffuse basal ring (sometimes absent), tarsomeres II–IV have a basal white band of a different width, tarsomeres V are entirely dark scaled, and the tarsal claws are evenly curved (Fig. 6.32b). The wing veins are covered with dark greyish scales, which on the costa (C) and subcosta (Sc) are mixed with white scales. The abdominal terga have distinct white or pale basal bands, which are sometimes diffused or interrupted in the middle forming indistinct triangular patches laterally. All terga have scattered white scales apically which usually form apical bands at least on segments VI–VIII (Fig. 6.34a). The sterna are covered with broad white scales.



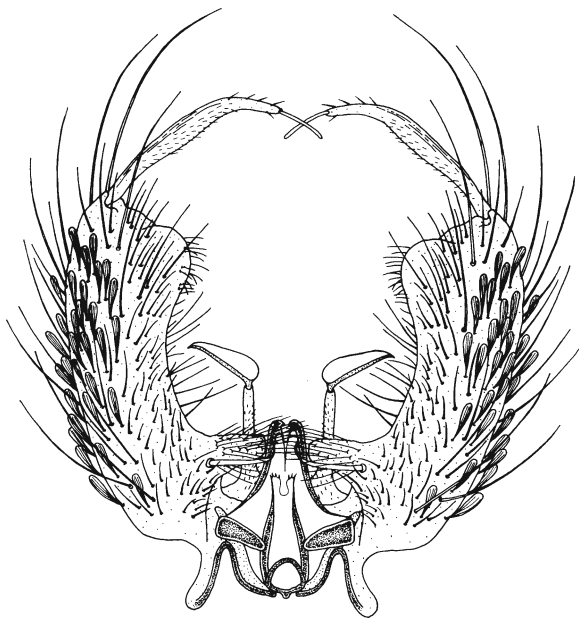


Fig. 10.77 Hypopygium of *Oc. riparius*

**Male:** The lateral lobes of tergum IX have 4–7 setae. The shape of the hypopygium is similar to that of *Oc. cantans*. The basal lobe of the gonocoxite is slightly longer than broad at its base, and not that elongated as in the latter species, with one long apically curved spine-like seta (Fig. 10.77). The apical lobe is well developed, and covered with short, thin setae. The gonostylus is somewhat broadened in its middle part, with a slender apical spine. The claspette stem is slender, and the claspette filament is moderately broad, and unilaterally winged. The aedeagus is elongated and pear shaped.

**Larva:** The antenna is shorter than the head and covered with numerous spicules. The antennal tuft (1-A) is situated at about the middle of the antenna, with moderately long branches not reaching the tip. The inner and median frontal setae (5-C and 6-C) usually have 2 branches (Fig. 8.49a), 5-C is sometimes 3-branched. The prothoracic formula (1-P to 7-P) is as follows: 1 (long, single); 2 and 3 (short, single); 4 (medium, single); 5 and 6 (long, single); 7 (long, 2–3 branches). The number of comb scales is 6–12, arranged in one row (Fig. 10.78). Each individual scale is elongated with a long median spine. The siphonal index ranges between 3.5 and 4.0. The 2–3 last pecten teeth are apically slightly detached. Each pecten tooth has one larger and 2–3 smaller

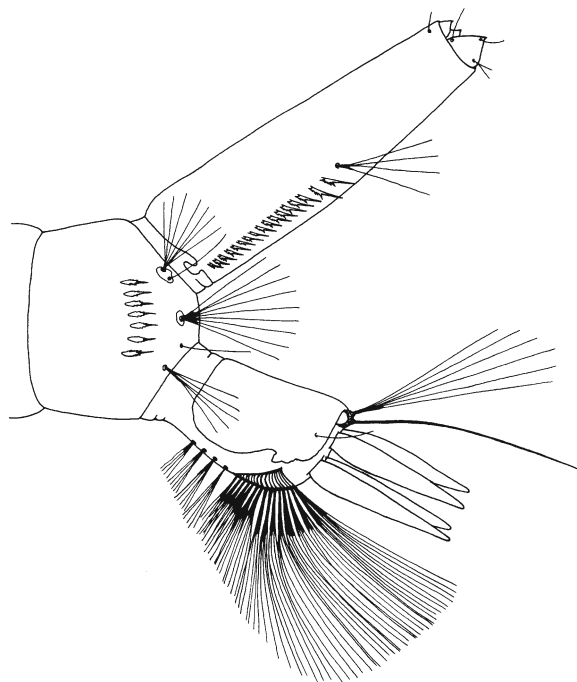


Fig. 10.78 Larva of *Oc. riparius*

lateral denticles. The siphonal tuft (1-S) is situated beyond the middle of the siphon and beyond the distal-most pecten tooth, with 4–5 long branches. Seta 9-S is rather long and curved, but is not transformed into a strong hook. The saddle does not encircle the anal segment, but covers most of its lateral sides. The saddle seta (1-X) is single and shorter than the saddle. The ventral brush has 4–6 precratal tufts (4-X). The anal papillae are about as long as the saddle.

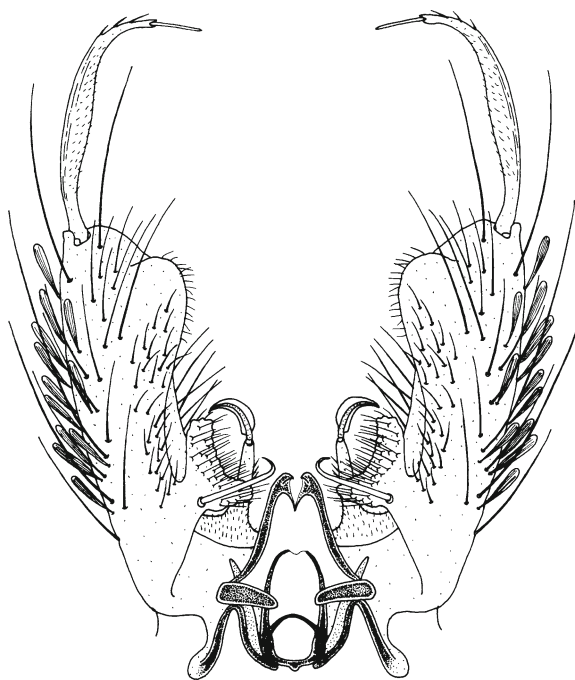
**Biology:** The species is uncommon or rare over most of its range (Wood et al. 1979), thus its biology is not well known. It hibernates in the egg stage and is probably monocyclic, although females can be found throughout the season. The larvae and adults occur at the same time as *Oc. excrucians* and *Ae. cinereus*. Breeding habitats are pools at mixed forest edges, or pools in deciduous forests, usually rich in leaf debris on the bottom. Larvae can also be found in peat bogs in the open (Gutsevich et al. 1974).

**Distribution:** It is a Nearctic and northern Palaearctic species, which has mostly been reported in northern and central Europe. It is usually rare, sometimes locally abundant, but is rather widespread in temperate areas.

***Ochlerotatus (Ochlerotatus) sticticus*  
(Meigen 1838)**

**Female:** A medium sized species, with the proboscis and palps dark scaled. The pedicel has pale scales on its median part, and flagellomere I is yellowish at the base. Flagellomeres I–III are of the same length as the others. The vertex is predominantly covered with pale yellowish scales, and the occiput with pale erect forked scales. The scutum has a dark, median longitudinal stripe sometimes separated by a narrow acrostichal stripe of pale scales. The lateral parts of the scutum are pale yellowish scaled, and the posterior submedian stripe is reddish to dark brown. The upper part of the postpronotum has dark scales, and the lower third is mostly pale scaled. The postprocoxal membrane is without scales, and a hypostigmal patch is absent. The subspiracular and postspiracular scale patches are well developed. The mesepisternum has greyish white scales, and the upper mesepisternal patch extends to near the anterior angle, narrowly separated from the prealar patch. The mesepimeral patch of scales ends distinctly above the lower margin of the mesepimeron, and the lower mesepimeral setae are absent. The tibiae and tarsi are dark scaled dorsally and mostly pale scaled ventrally, and tarsomeres V of all the legs are mostly dark scaled. The tarsal claws are moderately and evenly curved, each with a small subbasal tooth. The wing veins are usually entirely dark scaled, occasionally some isolated pale scales may be present at the base of the costa (C). The abdominal terga are dark scaled, terga II–IV with pale basal bands distinctly constricted in the middle, and on the following terga the basal bands are interrupted forming triangular pale patches at the lateral sides (Fig. 6.50a).

**Male (Fig. 10.79):** The basal lobe of the gonocoxite is constricted at its base, the apical part is slender and not attached to the gonocoxite, and is more or less crescent shaped (Fig. 7.48a). The lobe is densely covered with short setae and a rather large and prominent spine, which is curved apically and located at the constricted base. The apical lobe is well developed, gradually arising from the gonocoxite, apically rounded, and covered with short setae. The gonostylus is slightly expanded in the middle, with several small setae close to the apex. The apical spine of the gonostylus is long and slender. The paraproct is strongly sclerotized, inwardly curved and pointed at its apex. The claspette stem is short and straight, and the filament is short,



**Fig. 10.79** Hypopygium of *Oc. sticticus*

about half as long as the stem, with a small bilateral wing. The wing abruptly expands close to the base of the filament, and more or less gradually tapers towards the apex. The aedeagus is pear shaped.

**Larva:** The antenna is nearly half as long as the head, covered with less numerous, but prominent, coarse spicules. The antennal seta (1-A) is inserted slightly below the middle of the antennal shaft, with 4–5 branches not reaching the tip. The postclypeal seta (4-C) is small, and situated between the median frontal setae (6-C), with 1–4 short branches. The inner frontal seta (5-C) has 2–4 branches, the median frontal seta (6-C) is usually 2-branched, and the outer frontal seta (7-C) usually has 5 branches. The number of comb scales is 18–27 arranged in 2–3 irregular rows (Fig. 10.80). Each individual scale has a median spine 1.5 times the length of the subapical spines. The siphon is straight, gradually tapering towards the apex, and the siphonal index is 2.5–3.0. The pecten teeth are more or less evenly spaced extending beyond the middle of the siphon, and the siphonal tuft (1-S) is inserted beyond the pecten, with 4–6 branches not exceeding the width of the siphon at the point of insertion. The saddle extends far down the sides of the anal segment, and the saddle seta (1-X) is single and shorter than the

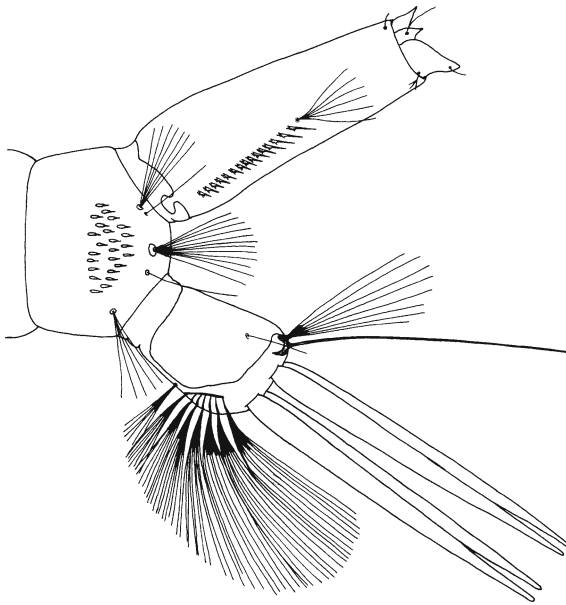


Fig. 10.80 Larva of *Oc. sticticus*

saddle. The ventral brush has 1–2 precratal setae (4–X). The anal papillae are long and pointed, and often 2.0–2.5 times longer than the saddle.

**Biology:** *Oc. sticticus* is a polycyclic species. The larvae occur mainly in temporary water bodies after floods and are regularly associated with those of *Ae. vexans*; it is often the second most frequent mosquito after *Ae. vexans*. The larvae can certainly hatch at lower water temperatures (<8°C) than the larvae of *Ae. vexans*. In central Europe they are associated with the hatching of the larvae of *Ae. rossicus*, *Ae. cinereus* and *Oc. cantans*, after floods during spring time, when *Ae. vexans* is not ready to hatch in masses. However, the peak development occurs during floods in summer. In contrast to *Ae. vexans*, *Oc. sticticus* has an unequivocal preference for shaded breeding waters in the flood plains, which are often covered by trees; the pH-value of the waters usually ranges from neutral to alkaline. The optimum temperature for the development of *Oc. sticticus* is about 25°C. The development from hatching of first-instar larvae to the emergence of the adults lasts 6–8 days at 25°C, 10–14 days at 20°C, 18–19 days at 15°C and 37 days at 10°C. The females can migrate considerable distances when searching for a blood meal, distances of more than 20 km have been observed (Hearle 1926). Adults of *Oc. sticticus* stay predominantly in covered terrains, e.g. in flood plains of river systems covered with trees

where the females frequently become a nuisance. The peak of biting activity of females is at twilight; however, they can also bite during the day in shaded situations. The females may lay up to 150 eggs in shady, damp depressions which will become flooded by rising water levels.

**Distribution:** The Holarctic species is widespread in Europe and occurs from northern Europe to the Mediterranean area and to Siberia in the East. It has also been reported from North America.

### 10.2.3 Subgenus *Rusticoidus* Shevchenko and Prudkina

The adults are large sized mosquitoes with numerous erect forked scales on the occiput and lateral parts of the vertex. The scutum is covered with narrow scales including most of the prescutellar area, and the scutellum with curved narrow scales and numerous long setae on all lobes. The postpronotum has only broad flat scales. Both the anteprocoxal and postprocoxal membrane have a patch of broad pale scales. The pleurites are extensively covered with pale scales and numerous setae. The abdominal terga have dark scales but with extensive pale scaled areas. In the male genitalia tergum IX has narrow lobes on each side with several short setae. The basal lobe of the gonocoxite bears several long lanceolate setae, and the claspette filament is short and more or less onion or triangular shaped. The fourth-instar larvae of European *Rusticoidus* species are easily distinguished from all other *Aedes* and *Ochlerotatus* species by having several pairs of setae inserted dorsally at the siphon and a variable branched small seta located laterally, usually close to the distal pecten teeth in addition to the siphonal tuft (1-S).

The members of the subgenus were previously placed in the former subgenus *Ochlerotatus* of *Aedes* as a separated globus (*Feltianus*) or group (*rusticus*-group) (Martini 1931; Edwards 1932) and this classification was followed by others (Natvig 1948; Mohrig 1969). In 1973, Shevchenko and Prudkina established the new subgenus *Rusticoidus* as a monotypic subgenus, based on the structures of the male genitalia and the larval siphon of *Oc. refiki*, but confusion has existed since then as to which species should be included in the subgenus. According to Reinert (1999a) the following European species are now included in the subgenus *Rusticoidus*:

*Oc. krymmontanus*, *Oc. lepidonotus*, *Oc. quasirusticus*, *Oc. rusticus*, *Oc. refiki*, and *Oc. subdiversus*. Additionally, two North American species, *Oc. bicristatus* and *Oc. provocans*, were transferred from the former subgenus *Ochlerotatus* to *Rusticoidus* (Reinert 2000b).

*Oc. krymmontanus* was described from the southern slopes of the Crimean Mountains (Aleksiev 1989). Owing to the lack of material for examination and the uncertain specific status, the species is not included here.

### ***Ochlerotatus (Rusticoidus) lepidonotus* (Edwards 1920)**

**Female:** Can be distinguished from other *Rusticoidus* species by the presence of whitish scales on the postnotum. The proboscis and palps are black scaled, the palps with scattered pale scales. The vertex and occiput have narrow yellowish scales and dark erect forked scales, and the lateral parts of the head have whitish scales. The pedicel is black with dense white scales, and the clypeus is blackish brown. The integument of the scutum is blackish brown, and the scutum is covered with narrow yellowish scales. The scutellum has pale yellowish scales and setae on the lobes. The postnotum is dark brown, with a group of narrow whitish scales. The pleurites are extensively covered with pale scales, and the postpronotum with light yellowish or brown scales. The femora and tibiae have light yellowish scales, except for black scales at the apices. The tarsi are dark brown scaled with scattered white scales on the entire part of tarsomere I and the basal part of tarsomere II. The wing veins are covered with intermixed yellowish white and dark scales, with dark scales predominantly located on the costa (C), radius 1 ( $R_1$ ), and anal vein (A). The abdominal terga are entirely covered with greyish white scales, with a more or less prominent median part with black scales on terga II–V. The sterna are entirely whitish scaled.

**Male (Fig. 10.81):** The base of the gonocoxite has one lobe arising gradually from the gonocoxite, more or less conical in shape, with a group of lanceolate, flattened setae, but additional smaller lobes are absent. The apical lobe is less developed than in other members of *Rusticoidus*, and is hardly visible. The gonostylus is strongly curved apically, with several small setae near the apex, and the apical spine of the gonostylus is long and straight. The paraproct is inwardly curved at the



**Fig. 10.81** Hypopygium of *Oc. lepidonotus*

apex. The claspette stem is relatively short and thick, and more or less straight. The claspette filament is short, and of an irregular triangular shape. The aedeagus has a broadly rounded apex and small lateral denticles.

**Larva:** The head is wider than long. The antenna is less than half as long as the head, and is entirely covered with spicules. The antennal seta (1-A) is located slightly below the middle of the antennal shaft, with 5–6 branches. The postclypeal seta (4-C) is short with 2–3 thin branches. The inner frontal seta (5-C) has 3–4 branches, the median frontal seta (6-C) has 1–3 branches and the outer frontal seta (7-C) has 8–10 branches. The number of comb scales is 6–11, arranged in an irregular row (Fig. 10.82). Each scale is large with a prominent median spine, and sometimes 1 or 2 lateral spines of almost the same size as the median spine. The siphon tapers from the basal third, the siphonal index is 2.7–4.1, and the dorsal surface of the siphon has 2 pairs of setae. A single additional seta which is longer than the width of the siphon at the point of its insertion, is located on the lateral side of the siphon close to the distalmost pecten tooth. The pecten has 9–21 teeth (average 13–15), sometimes 1–2 distalmost teeth are apically detached, and the pecten does not extend beyond the basal half of the siphon. The siphonal tuft (1-S) is inserted slightly below the middle of the siphon, beyond the distalmost pecten

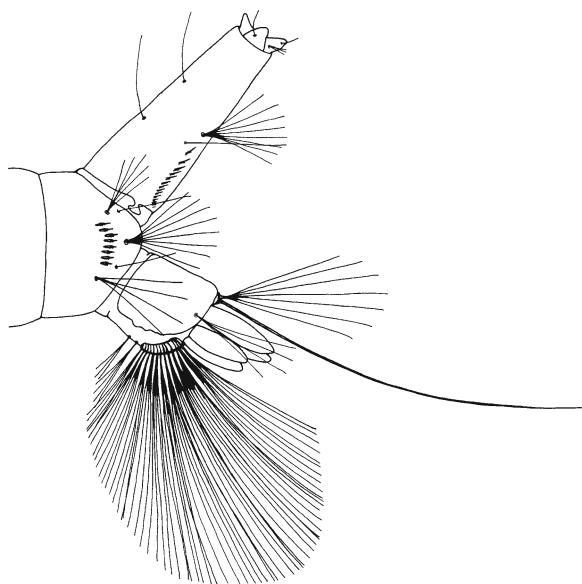


Fig. 10.82 Larva of *Oc. lepidonotus*

tooth, with 8–13 branches. The saddle extends far down the sides of the anal segment, and the saddle seta (1-X) has 3–5 branches, and is longer than the saddle. The upper anal seta (2-X) has 7–9 branches, the lower anal seta (3-X) is single and much longer than the siphon. The ventral brush has 14–16 tufts of cratal setae (4-X) and 2 precratal setae. The anal papillae are lanceolate, usually shorter than the saddle, and the dorsal pair is longer than the ventral pair.

**Biology:** *Oc. lepidonotus* is a rare species and so far recorded from southeastern Europe only; thus little information is available about its biology. It is apparently a monocyclic species, which hibernates in the egg stage (Medschid 1928). Larvae occur by early spring after heavy rainfall or snow-melt. They may be associated with larvae of *Oc. detritus* when the breeding sites are slightly saline (Gutsevich et al. 1974). Adults are predominantly found at the end of April and during May. Martini (1931) reported numerous females biting horses at dawn in Asia Minor.

**Distribution:** Greece and Turkey.

### ***Ochlerotatus (Rusticoides) quasirusticus* (Torres Canamares 1951)**

**Female:** Very similar to *Oc. rusticus* in all stages, and the females particularly, closely resemble each other.

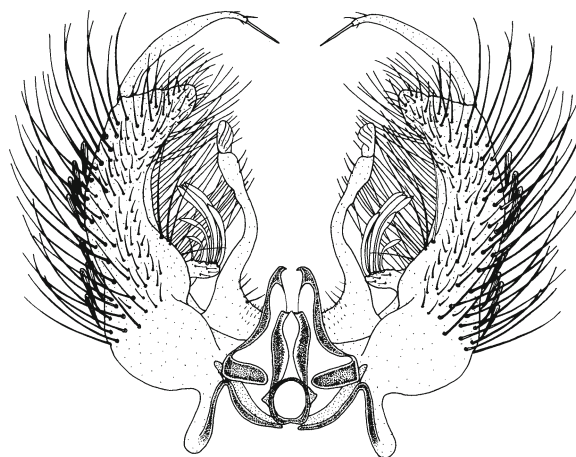


Fig. 10.83 Hypopygium of *Oc. quasirusticus*

The scutal pattern is less distinct than in *Oc. rusticus*, the scutum is covered with narrow yellowish brown scales, disconnected by several bare longitudinal stripes at the acrostichal, anterior dorsocentral, and posterior submedian areas to which they contrast, mainly due to the exposure of the dark integument. The subcosta (Sc) is without pale scales, but there are no other distinct differences.

**Male:** The lobes of tergum IX are situated close together, with 5–8 short spine-like setae. The gonocoxite is more or less straight, not as rounded as in *Oc. rusticus* (Fig. 10.83). The basal lobe of the gonocoxite is well elongated, the distal margin has 6–8 strongly curved lanceolate setae, and the apical portion of the basal lobe has 2–3 long and strong setae (Fig. 7.28b). The gonostylus is distinctly bent in the apical third, and the apical spine of the gonostylus is long, slender, and nearly straight, but never S-shaped as in *Oc. rusticus*.

**Larva:** The antennae have large and strong spicules, more developed than in *Oc. rusticus*. Prothoracic seta 3-P has 3 branches. 1–2 distalmost pecten teeth are apically detached, and situated beyond the middle of the siphon (Fig. 10.84). The siphonal seta (1-S) is attached within the pecten. Dorsal additional pairs of setae are longer than the width of the siphon at its base, as opposed to being shorter than the width of the siphon at its base in *Oc. rusticus*. The lateral additional seta is usually single, about as long as the width of the siphon at the point of origin, and longer than in *Oc. rusticus*. Seta 9-S is strongly developed, large and

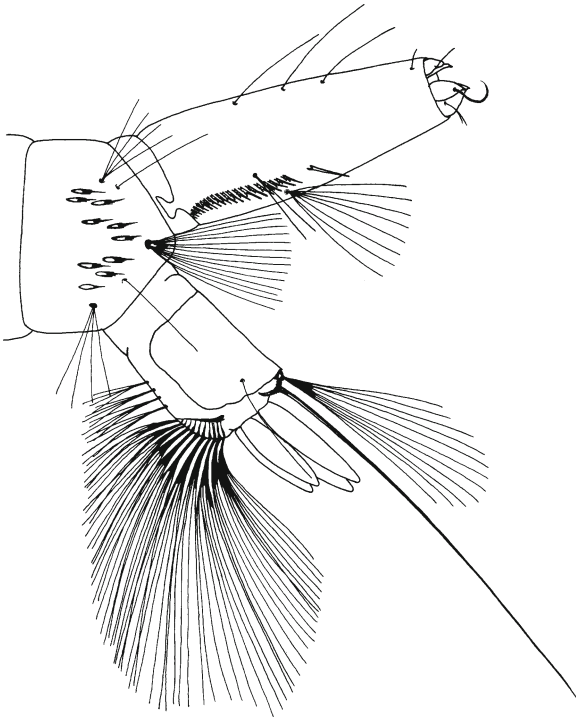


Fig. 10.84 Larva of *Oc. quasirusticus*

hooked. The anal papillae are distinctly longer than the saddle, with the dorsal pair nearly twice as long as the saddle.

**Biology:** Very similar to that of *Oc. rusticus*. Some larvae hatch in late autumn and hibernate in the larval stage, others hatch in early spring of the following year (Encinas Grandes 1982). They are predominantly found in permanent water bodies in forests or in ditches and flooded meadows. Often they are associated with larvae of *Oc. refiki*, *Oc. excrucians*, *Cx. hortensis*, *Cs. fumipennis*, and *An. atroparvus* (Torres Canamares 1951). Adults can be found from May to June and they are often encountered close to their breeding sites, indicating that their migration ability is limited. The females are mainly zoophilic but also feed on humans, predominantly in shaded situations.

**Distribution:** *Oc. quasirusticus* is so far reported only from Spain, where it belongs to one of the most abundant *Ochlerotatus* species in certain regions (Encinas Grandes 1982). Owing to the great similarity to *Oc. rusticus* it was probably overseen or misidentified in the mosquito collections of other countries in the past, thus its whole distribution range is not clari-

fied yet. The species is believed to occur also in North Africa (Encinas Grandes 1982).

### ***Ochlerotatus (Rusticoides) refiki* (Medschid 1928)**

**Female:** *Oc. refiki* closely resembles *Oc. rusticus*, but differs from the latter in the colouration of the terga. The proboscis and palps are predominantly dark scaled, with scattered pale scales of varying number, mainly at the base of the proboscis. The vertex and occiput have yellowish white narrow scales and erect forked scales of the same colour. The pedicel is dark with a row of white scales at the base, and the clypeus is dark brown. The integument of the scutum is blackish brown, and covered with yellowish scales and a median stripe of dark scales, sometimes divided by a narrow yellowish acrostichal stripe. The scutellum has whitish narrow scales on the lobes. The postprocoxal membrane has pale scales, and the postpronotum has broad flattened scales, dark brown or black in the upper part and white in the lower part. The hypostigmal and spiracular scale patches are fused. White scales on the mesepisternum extend more or less to the anterior angle and to the lower margin, and the mesepimeral patch extends to the lower margin of the mesepimeron. The femora are predominantly pale scaled, the tibiae and tarsomeres I mostly have dark scales, and tarsomeres II–V are entirely dark scaled. The wing veins are covered with dark scales, with scattered pale scales at the base of the costa (C), subcosta (Sc), and radius (R). The colour of the abdomen varies greatly. The terga are often covered with dark scales and indistinct pale basal bands not wide in the middle and scattered pale scales at the distal part of the terga, sometimes with a narrow band of pale scales at the apical margin. Sometimes pale scales predominate on all terga, with only a few scattered dark scales (Fig. 6.40b). In contrast to *Oc. rusticus* the pale scales on the terga never show a tendency to form a longitudinal stripe in the middle.

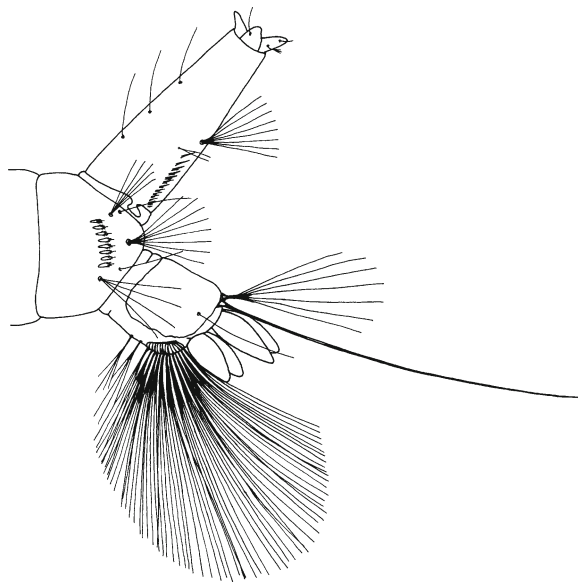
**Male:** The lobe of tergum IX has 5–10 short spine-like setae. The basal lobe is situated close to the middle of the gonocoxite, the ventral division with an elongated basal part is covered with 15–16 slightly curved lanceolate setae arranged in several rows (Fig. 10.85). The dorsal division of the basal lobe is small, and situated apically to the ventral part with 2 long and strong setae directed towards the apex. The gonostylus is bent



**Fig. 10.85** Hypopygium of *Oc. refiki*

apically and the apical spine of the gonostylus is slender and more or less straight. The paraproct is strongly sclerotized, inwardly curved at the apex, and pointed. The claspette stem is long, curved at the basal part, and swollen at the apex. The claspette filament is elongated and pointed at the apex, and transversely striated. The aedeagus is moderately rounded at the apex with small lateral denticles.

**Larva:** The head is wider than long. The antenna is less than half as long as the head, is slightly curved, and covered with spicules. The antennal seta (1-A) is located at about the middle of the antennal shaft, with 5–6 short branches. The postclypeal seta (4-C) is thin, with thin branches. The median frontal seta (6-C) is usually single, occasionally with 2–3 branches, and is situated before the inner frontal seta (5-C), which has 2–5 (usually 3) branches. The outer frontal seta (7-C) usually has 6–9 branches. The number of comb scales is 6–11 arranged in a row, and each individual scale has a strong median spine and several shorter spines at the base (Fig. 10.86). The siphon is straight, gradually tapers towards the apex, and the siphonal index is 3.0–4.0. The dorsal surface of the siphon has 3 pairs of additional setae, and another seta with 2–5 short branches is located on the lateral side of the siphon close to the distalmost pecten tooth. The pecten has



**Fig. 10.86** Larva of *Oc. refiki*

12–21 teeth occupying the basal third of the siphon, each tooth has a prominent main spine and 3–4 lateral denticles. Sometimes 1–2 distalmost pecten teeth are apically detached. The siphonal tuft (1-S) has 6–9 branches, and is located at about the middle of the siphon or slightly below it, but always beyond the distalmost pecten tooth. The saddle extends down to near the lower margin of the anal segment, and the saddle seta (1-X) has 1–3 branches, and is longer than the saddle. The upper anal seta (2-X) has 7–9 branches, and the lower anal seta (3-X) is single and longer than the siphon. The ventral brush has 13–15 tufts of cratal setae (4-X) and 2–3 precratal setae. The anal papillae are lanceolate and about as long as the saddle.

**Biology:** The biology of *Oc. refiki* is similar to that of *Oc. rusticus*. It is a rare species. The early larval stages of this monocyclic snow-melt mosquito can be found after heavy rainfall in late autumn. They can survive even when the breeding sites are covered with ice during wintertime. However, the majority of the population overwinters in the egg stage and hatching takes place during the snow-melt early in the year. Typical breeding sites are semipermanent water bodies in swampy woodlands, e.g. with *Alnus glutinosa*, occasionally larvae can be found in flooded meadows (Vogel 1933). Larvae of *Oc. refiki* are often associated with those of *Oc. rusticus*, *Oc. cantans*, and *Oc. cata-*

*phylla*. The waters of the breeding sites have usually a neutral to alkaline pH-value. In central Europe the larvae pupate in April and the first adults occur at the end of April or early May. They prefer shaded areas where they may bite humans and mammals even during daytime; however, the biting activity is usually highest at dusk. The adults do not migrate much and prefer to stay close to their breeding sites in shaded areas.

**Distribution:** In Scandinavia, *Oc. refiki* has been reported from Sweden (Dahl 1975). It is widely distributed in the other parts of Europe, e.g. France, Spain, Italy, Switzerland, Germany, Czech Republic, former Yugoslavia, Slovakia, Hungary, and Romania. Outside Europe the species can be found in Asia Minor.

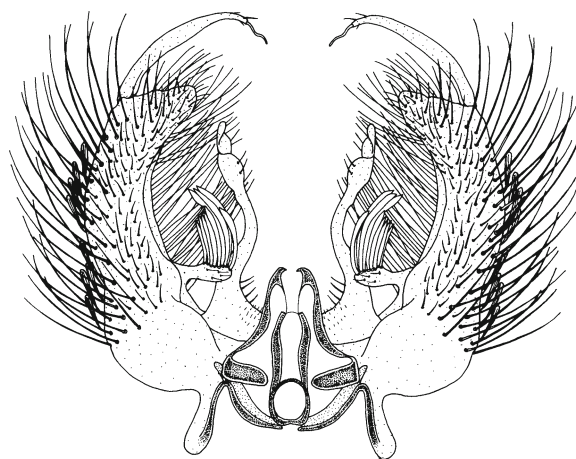
### ***Ochlerotatus (Rusticoides) rusticus* (Rossi 1790)**

**Female:** A large mosquito, with a dark scaled proboscis and palps, and a few scattered pale scales at the base of the proboscis. The vertex and occiput have narrow yellowish white scales, and the eyes are bordered with narrow white scales. The pedicel is dark brown with a circle of whitish scales, and the clypeus is blackish brown. The integument of the scutum is blackish brown, covered with golden bronze scales and a median stripe of dark scales, usually divided by a narrow acrostichal stripe, but sometimes there are two more dark stripes in the posterior submedian areas. The lateral parts of the scutum have cream coloured scales. The scutellum is dark brown with narrow yellowish white scales and pale setae on the lobes. The postprocoxal membrane has pale scales. The pleurites are extensively covered with yellowish or white scales. The postpronotum has broad flattened scales, blackish brown in the upper part and whitish in the lower part. The hypostigmal and spiracular scale patches are fused. The mesepisternum has scales extending to the anterior angle and lower margin, the scales of the mesepimeron extend more or less to the lower margin, and lower mesepimeral setae are present. The femora have pale yellowish scales on the ventral surface and dark scales on the dorsal surface, the tibiae and tarsomeres I have pale and dark scales intermixed, and tarsomeres II–V are almost entirely dark scaled. The wing veins are predominantly covered with dark scales, with scattered pale scales at the base of the costa (C) and on the subcosta

(Sc), and are most numerous at the apex of the subcosta. Abdominal tergum I has 2 patches of yellowish white scales and pale setae, the other terga are dark scaled with pale basal bands which are usually widened middorsally and show a tendency to form a longitudinal stripe in the middle, at least on the apical terga (Fig. 6.40a). Dark parts of the terga often have scattered pale scales. The sterna are predominantly whitish scaled.

**Male:** The apical margin of tergum VIII is densely covered with long, inwardly curved setae. The lobe of tergum IX has 5–7 short spine-like setae. The gonocoxite has dense long setation on the entire inner surface (Fig. 10.87). The basal lobe of the gonocoxite has a constricted stem-like base and a group of lanceolate, flattened setae arranged more or less in a row. The apical lobe is well developed, protruding beyond the level of the insertion point of the gonostylus, with numerous short setae. The gonostylus is slightly curved at the apex with several small subapical setae, and the apical spine of the gonostylus is twisted and distinctly S-shaped. The paraproct is strongly sclerotized, and inwardly curved at the apex. The claspette stem is long, distinctly curved in the middle, and slightly swollen at the apex. The claspette filament is short, more or less onion-like, without a plate shaped widening, and is transversely striated. The aedeagus is moderately rounded at the apex with small lateral denticles.

**Larva:** The head is wider than long. The antennae are approximately half as long as the head, slightly curved, and adorned with numerous spicules



**Fig. 10.87** Hypopygium of *Oc. rusticus*



(Fig. 8.24a). The antennal seta (1-A) is located at about the middle of the antennal shaft or slightly below it, with 5–6 branches. The median frontal seta (6-C) has 2, occasionally 3, branches situated before the inner frontal seta (5-C), which has 3, sometimes 2, branches, and the outer frontal seta (7-C) usually has 8 branches. The number of comb scales is 10–18, arranged in two irregular rows, each individual scale with a strong median spine, 1–2 shorter lateral spines and small spines at the base. The siphon is straight, tapering in the apical half, and the siphonal index is approximately 3.0–3.5 (Fig. 10.88). The dorsal surface of the siphon has 3, occasionally 4, pairs of additional setae; another seta with 1–2 thin branches is located on the lateral side of the siphon beyond the median pecten teeth. The pecten has 15–25 teeth not extending to the apical third of the siphon, the basal and median teeth have 2–3 lateral denticles, 1–3 distal pecten teeth are detached and spine-like. The siphonal tuft (1-S) is located at about the middle of the siphon within the distal pecten teeth, with 6–8 branches. The saddle extends about 3/4 of the way down the sides of the anal segment, and the saddle seta (1-X) is single, and nearly as long as the saddle.

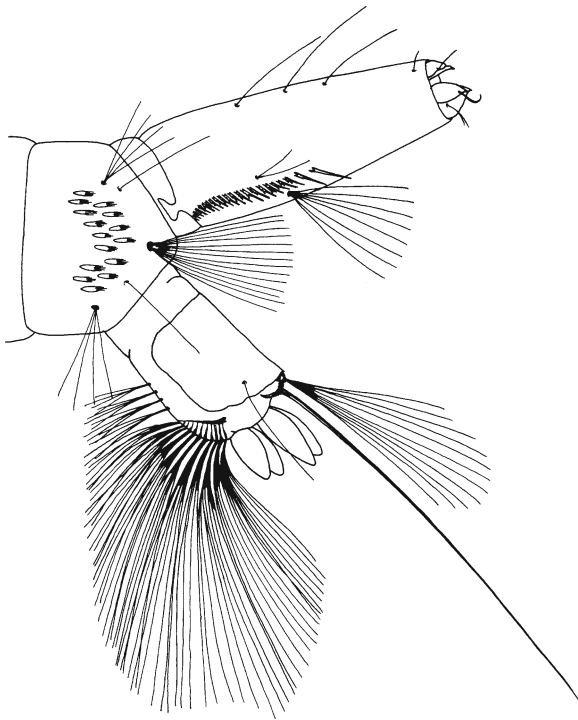


Fig. 10.88 Larva of *Oc. rusticus*

The upper anal seta (2-X) has more than 6 branches, and is half as long as the lower anal seta (3-X) which is single and longer than the siphon. The ventral brush has 11–16 tufts of cratal setae (4-X) and 3–4 precratal setae. The anal papillae are about half as long as the saddle, and the dorsal pair is longer than the ventral pair.

**Biology:** *Oc. rusticus* is a monocyclic snow-melt mosquito which predominantly occurs in swampy woodlands with a high level of ground water; occasionally it can be found in floodplains. Larvae are able to hatch during heavy rainfall in autumn when the water level rises. The diapause of these larvae is terminated by the decreasing temperatures in autumn. Usually the larvae which hatch in autumn hibernate in the second and third larval instar; they can even survive under a closed coverage of ice. The high content of dissolved oxygen in cold water or bubbles of oxygen under the ice which are produced by assimilating plants enable the larvae to cover their demand of oxygen and to survive; they are usually attached to these oxygen bubbles. However, during a severe winter the mortality rate can be very high. For this reason *Oc. rusticus* usually does not occur in areas where the isotherm of January is less than  $-1^{\circ}\text{C}$  (Kirchberg and Petri 1955). Often the larvae of *Oc. rusticus* are associated with those of *Cs. morsitans* or with overwintering larvae of *An. claviger*. A second larval population of *Oc. rusticus* and *Cs. morsitans* hatches from hibernating eggs in early spring shortly after the snow-melt. Thus, first and fourth-instars of these two species can be found together with numerous first-instar larvae of *Oc. communis* and *Oc. punctor* which usually hatch in early spring from hibernating eggs. Typical breeding sites are ditches or deeper depressions with vegetation, e.g. *Carex* sp. or *Phragmites* sp. The larvae of *Oc. rusticus* are seldom found in shallow water bodies because of the high risk that the entire water body may freeze. Larvae are found preferably in breeding sites with a pH-value of 5.0–8.0; they are rare or absent in water bodies with pH values less than 5.0. The optimum temperature for the larval development in the laboratory is  $15\text{--}20^{\circ}\text{C}$ . The time of development is about 66 days at a constant temperature of  $10^{\circ}\text{C}$ , 28–29 days at  $15^{\circ}\text{C}$  and 23–25 days at  $20^{\circ}\text{C}$ . Although the larvae of *Oc. rusticus* belong to the first occurring species of snow-melt mosquitoes, they pupate and emerge after the species which hatch in early spring from hibernating eggs such as *Oc. communis* and *Oc. punctor*. In central Europe adults usually emerge at the end of

April. Females are vicious biters in shaded situations. The adults prefer to stay in forested areas and do not migrate long distances, usually not more than 2 km (Schäfer et al. 1997).

**Distribution:** *Ae rusticus* is widely distributed throughout Europe and can also be found in North Africa and Asia Minor.

***Ochlerotatus (Rusticoides) subdiversus*  
(Martini 1926)**

**Female:** The proboscis and palps are dark scaled, often with some scattered pale scales. The vertex has whitish narrow scales, and the occiput has pale and dark scales. The integument of the scutum is blackish brown, the scutum has a median stripe of yellowish bronze scales, lighter in the median and darker in the lateral part, but sometimes the stripe is indistinct. The scutellum has narrow whitish scales and pale setae on the lobes. The postnotum is blackish brown without a group of scales. The pleurites have dense patches of silvery to yellowish scales, and the upper part of the postpronotum has straight or slightly curved bronze scales. Postprocoxal and hypostigmal patches are present. The femora and tibiae have light and dark scales intermixed, and the tarsi are dark scaled with numerous pale scales on tarsomeres I and the basal part of tarsomeres II. The wing veins are predominantly covered with narrow dark scales, and pale scales are present mainly at the base and the anterior part of the wing. The abdominal terga have greyish white scales and a varying number of dark scales, forming indistinct spots, and pale transverse bands are absent. There is great variation in the colouration of the species. Gutsevich et al. (1974) described a light form with numerous pale scales on the abdominal terga, proboscis, and palps of females. Transitional forms have also been found.

**Male:** The apical margin of tergum VIII is densely covered with long, thin setae. The lobes of tergum IX are situated close together, with 7–10 short, thick setae. The base of the gonocoxite has two or more lobes, but only one lobe bears a group of lanceolate, flattened setae, the other smaller lobes have long hair-like setae (Fig. 10.89). The apical lobe is weakly developed with short, slightly curved setae. The gonostylus is curved, with several small setae near the apex, and the apical spine of the gonostylus is more or less straight. The

paraproct is well sclerotized, and inwardly curved at the apex. The claspette stem is long and slightly curved in the basal part. The claspette filament is short, and more or less triangular shaped with a pointed apex. The aedeagus is not as broadly rounded at the apex as in *Oc. lepidonotus*, and has small lateral denticles.

**Larva:** The head is wider than long. The antenna is about half as long as the head, and densely covered with spicules. The antennal seta (1-A) is located at about the middle of the antennal shaft, with 3 branches. The post-clypeal seta (4-C) is short, with 2 branches. The inner frontal seta (5-C) has 2 branches, the median frontal seta (6-C) has 2–3 branches and the outer frontal seta (7-C) has 5 branches. The number of comb scales is usually 14–15 arranged in two irregular rows, and each scale is large with a prominent median spine and small spines at the base. The siphon is straight, tapering in the apical third, and the siphonal index is 3.0–3.3 (Fig. 10.90). The dorsal surface of the siphon has 3–4 pairs of additional long setae, and another thin seta with 2 branches is located on the lateral side of the siphon close to the median pecten teeth. The pecten has the 3–4 distalmost teeth atypical, spine-like, widely spaced, almost reaching the apex of the siphon. The siphonal tuft (1-S) is single, and nearly twice as long as the width of the siphon at the point of its origin. It is attached at about the middle of the siphon within the pecten. The saddle extends down 3/4 of the sides of the anal segment, and the saddle seta (1-X) is single and as long as the saddle. The upper anal seta (2-X) usually has 11 branches, and the lower anal seta (3-X) is single and about as long as the siphon. The ventral brush has 13–15 tufts of cratal



**Fig. 10.89** Hypopygium of *Oc. subdiversus*

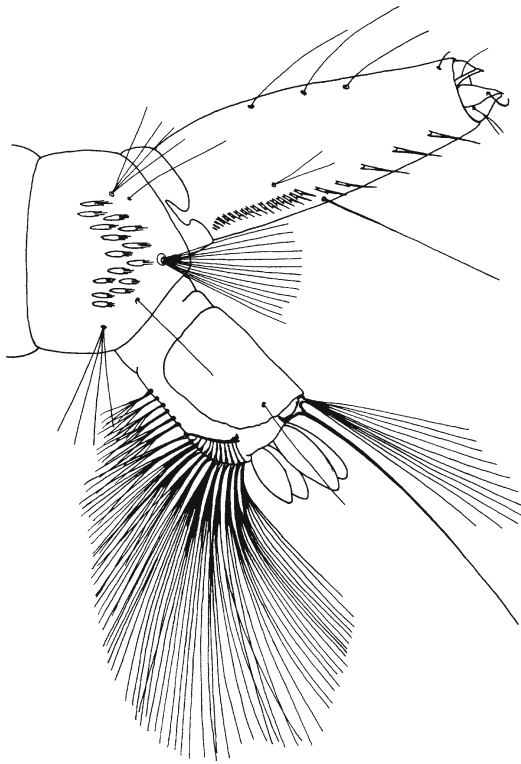


Fig. 10.90 Larva of *Oc. subdiversus*

setae (4-X) and 6–7 precratal setae. The anal papillae are lanceolate, shorter than the saddle, and the dorsal pair is longer than the ventral pair.

**Biology:** *Oc. subdiversus* is a monocyclic species. Larvae occur in early spring after heavy rainfalls or snowmelt. Adults may be very numerous in some localities and massive attacks of biting females on humans and live stock have been observed (Gutsevich et al. 1974).

**Distribution:** European part of Russia to Kazakhstan and southern Siberia.

### 10.3 Genus *Culex* Linnaeus

Members of this genus are usually small to medium sized species with sparse pleural scaling. The scutellum is distinctly trilobed, prespiracular setae are absent, all claws are without a subbasal tooth. The abdomen is blunt ended with short, oval cerci. The last three characteristics separate *Culex* species from nearly all *Aedes* and *Ochlerotatus* species. The antenna is as long as or shorter than the proboscis, and is cov-

ered with numerous setae. The proboscis is usually dark, sometimes with scattered pale scales, and the length of the female palps is about 1/3 that of the proboscis, and most often shorter. The eyes meet in the middorsal line or are slightly separated, the vertex has numerous erect scales, and the occiput has narrow and broad scales. The scutum is covered with narrow scales and a full pattern of setae, but acrostichal setae are sometimes absent. The scaling on the pleurites is reduced and variable. The dorsal margin of the mesomeron is not in line with the hind coxa. Tarsomere I of the hind legs is as long as or longer than the hind tibia (except in subgenus *Barraudius*), pulvilli are present in most species. The wing has narrow scales on all veins and a long radial fork. All abdominal segments are nearly equally broad, usually banded or with lateral triangular patches of pale scales. The males are smaller than the females, but scaling and setation are quite similar. The antenna has numerous flagellate whorls, and the palps are long with five palpomeres exceeding the length of the proboscis. The morphology of the *Culex* hypopygium is very complicated and belongs to one of the most complex structures in the family of Culicidae. Usually the gonocoxite is devoid of scales (except in subgenus *Barraudius*) and bears only one lobe, which is displaced mesally; hence, the name subapical lobe. It may be divided and ornamented with several setae of different length and shape. Typical claspettes are absent, and the aedeagal apparatus is of a complicated structure with several characteristics of unknown homology. These surround the phallosome, which is dorsally covered by the membranous proctiger and the sclerotized and fused or unfused parts of the paraproct, which distally bear a more or less crown-like area of spines or denticles. The head of the larva is broader than long. The antenna is spiculate in most species and longer than the head in many species. The antennal tuft (1-A) usually has multiple branches situated in the distal half of the antennal shaft. The mouthparts are adapted to filter feeding with well developed anteromedian and lateral palatal brushes with an exception in the predacious larvae of the tropical subgenus *Lutzia*, which have the labrum and the brushes modified for capturing prey. The frontal seta 5-C is located distally to 6-C, and all setae are usually multiple-branched. Setae 1-P to 3-P are located on a common sclerotized tubercle. Abdominal seta 4-I is plumose, but never palmate, and setae 6-I to 6-VII have a variable number of long

branches. The number of comb scales is usually between 40 and 60, and each individual scale is relatively small. The siphonal index is variable, and the siphon is usually slender and moderately to extremely long. At least 4–6 siphonal setae (1-S) are inserted along the ventral surface of the siphon, either paired or arranged in a straight or zig-zag row. The pecten teeth in some species are widely spaced distally. The saddle usually completely encircles the anal segment, and the saddle seta (1-X) is often branched. The precratal tufts (4-X) are usually reduced in number or absent, and the anal papillae are variable in shape and size.

The genus *Culex* with more than 750 described species from 24 subgenera world wide comprises only a few non tropical species. In Europe, species of the subgenera *Barraudius*, *Culex*, *Maillotia* and *Neoculex* can be found and most of them have a Mediterranean and/or central European distribution.

Several tropical *Culex* species from Asia and Africa are well known for transmission of lymphatic filariasis and various viral diseases.

### 10.3.1 Subgenus *Barraudius* Edwards

Members of the subgenus are small brownish species. The proboscis of the female is shorter than the fore femur, the margin of the eyes is usually ornamented with narrow scales, and the vertex has erect and broad light scales. The scutum has uniform brownish scales, and the scutellum is usually light scaled. The hind tarsomere I is distinctly shorter than the hind tibia. The abdominal terga are dark scaled without transverse pale bands, but basolateral pale patches of scales may be present, and the sterna have light scales. The palps of the male are longer than the proboscis, without long setae, but are covered with a few short spines. The gonocoxite is covered with small scales on the outer surface. The subapical lobe of the gonocoxite arises slightly beyond the middle, not as far apically situated as in the majority of other *Culex* species, with a number of spine-like or hair-like setae; any transparent, broad scale-like setae are absent. The gonostylus is slender, the paraproct apically has a group of small spines forming a paraproct crown, and the aedeagus has dorsal and ventral arms. The head of the larva is broader than long, the

antenna is as long as the head or slightly longer and more spiculate towards the tip, and the antennal seta (1-A) is multiple-branched. The comb scales are numerous, small and elongated. The pecten occupies about half of the siphon length; each tooth has several lateral denticles. The siphonal tufts (1-S) are arranged in a zig-zag row on the ventral side of the siphon, and the main tracheal trunks are broad. The saddle entirely surrounds the anal segment, precratal setae (4-X) are absent, and the anal papillae are short.

The small subgenus *Barraudius* embraces only four species so far, *Cx. richeti* Brunhes and Venhard is only known in Nigeria and *Cx. inatomii* Kamimura and Wada reported from Japan. The two other members of *Barraudius*, *Cx. modestus* and *Cx. pusillus*, are partly distributed in the European region.

#### ***Culex (Barraudius) modestus* Ficalbi 1889**

**Female:** The proboscis is dark brown, paler on its ventral surface from the base to the middle, and slightly swollen at the apex. The palps, clypeus and flagellum of the antenna are dark brown. The vertex has dark brown setae which are directed anteriorly between the eyes. The head is covered with brown or yellowish narrow scales, with some broader pale scales on each side, and the occiput has dark brown erect and forked scales. The integument of the scutum is brown, and covered with chestnut-brown scales, rather lighter on the scutellum and in front of it. Fine, blackish setae are scattered mainly along the margin of the scutum, along the dorso-central stripes and above the wing roots. The setae are more conspicuous and longer on the scutellum. The pleurites are pale brown, with small patches of pale scales on the mesepisternum and upper mesepimeron, 3–6 postpronotal setae, and one lower mesepimeral seta is present. The legs are mainly dark brown, the fore and mid femora have pale scales on the posterior surface, and the hind femur is pale except for the dorsal surface which is brown scaled, and the pale knee spot is distinct. The tibiae are dark brown dorsally, with pale scales on the ventral surface. All tarsomeres are dark scaled, and the hind tarsomere I is shorter than the hind tibia (Fig. 6.51a). The wings are entirely dark scaled, and the cross veins are well separated. The terga are dark brown scaled, transverse pale bands are

absent, but lateral pale patches usually form a continuous pale border on either side of the abdomen. The sterna are uniformly covered with pale yellowish scales. The abdomen is blunt ended, which separates the species from the similarly coloured females of *Ae. cinereus*.

**Male:** The palps are almost devoid of setae, and are longer than the proboscis. The long palps separate the males from the similarly coloured males of *Ae. cinereus* which have palps which are considerably shorter than the proboscis. The gonocoxite is approximately twice as long as it is wide with more or less dense scales on its outer surface (Fig. 10.91). The lobe of the gonocoxite is situated slightly beyond the middle of the gonocoxite and is divided into two distinct tubercles. The proximal one bears 2–3 spines of different size, 1 or 2 of them may be curved apically, and the more distal tubercle carries 2 strong setae. Broad transparent scale-like setae are absent. The gonostylus is long and slender (longer than in the similar *Cx. pusillus*), usually more than half as long as the gonocoxite, evenly tapering apically and curved in the apical half. The apex of the paraproct has one row of spines forming a paraproct crown. The ventral arm of the aedeagus is short, only slightly curved or nearly straight at the apex and not extending beyond the paraproct crown. The dorsal arm of the aedeagus is conspicuously bent upwards.

**Larva:** The antenna is moderately spiculate, slightly longer than the head, curved, darkly pigmented at the base and distinctly narrowing from the insertion point of the antennal seta (1-A) to the apex. Seta 1-A is inserted beyond the middle of the antennal shaft, and is half as long as the antenna, with 15–25 branches. The inner frontal seta (5-C) has 3–5 branches, the median frontal seta (6-C) has 3–4 branches and the outer frontal seta (7-C) has 7–8 branches. The comb consists of a patch of 50 or more fringed scales which are more or less rounded apically. The siphon is straight, the main tracheal trunks are broad, and the siphonal index is about 4.0–5.0 (Fig. 10.92). The pecten has about 12 relatively widely spaced teeth situated in the basal half of the siphon, and most of the teeth have 4–5 lateral denticles. Setae 1-S has 10–12 tufts arranged in a more or less ventral zig-zag row. The basalmost tuft arises proximal to the distalmost pecten tooth, and the distalmost tuft is located close to the apex of the siphon. Each tuft is slightly shorter, or occasionally slightly

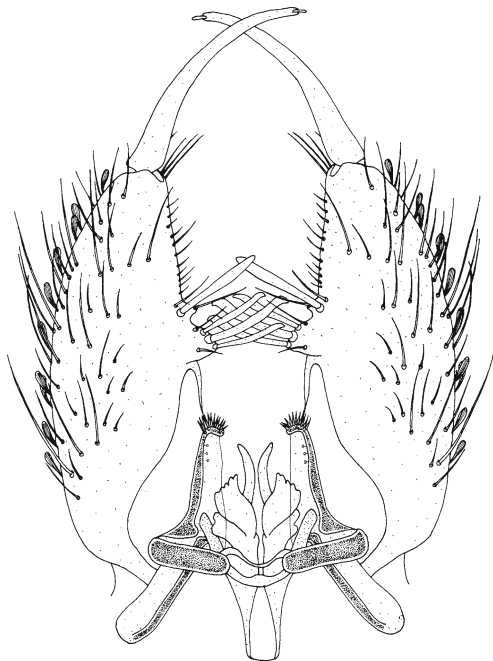


Fig. 10.91 Hypopygium of *Cx. modestus*

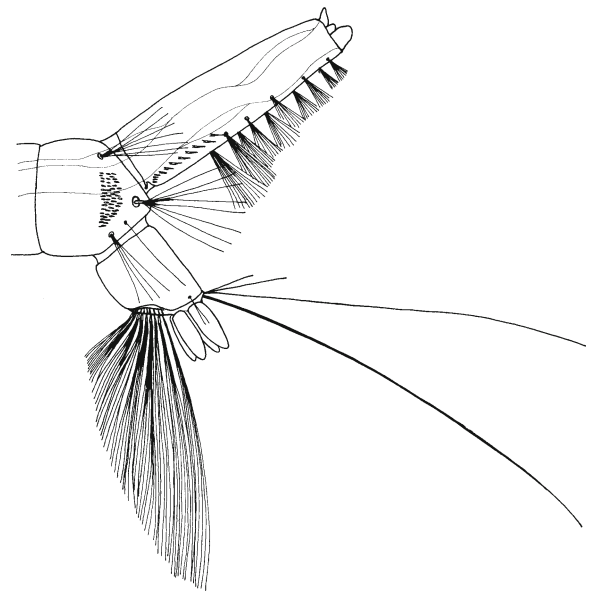


Fig. 10.92 Larva of *Cx. modestus*

longer than the width of the siphon at the point of its insertion. The saddle is as long as it is wide or slightly longer and completely encircles the anal segment. The saddle seta (1-X) is small with 2–3 branches. The upper anal seta (2-X) has 3–4 branches, one branch longer than the others, and the lower anal seta (3-X) is long and single. The ventral brush has 10–13 cratal setae (4-X). The anal papillae are shorter than the saddle, slender, and tapering.

**Biology:** The larvae show a preference for shallow sunlit habitats and are frequently found on meadows, in irrigation channels, inundation areas of rivers, or rice fields. Other common breeding waters are ground pools, ponds, swamps, and marshes with rich vegetation; the water may be fresh or slightly saline. In southern Europe they are mainly found in salt water marshes (Ribeiro et al. 1988) and rice fields. The larvae occur from late spring until late autumn and they are often found together with those of the *Anopheles* species. In central Europe the seasonal maximum of the adult population is recorded from the beginning of July to late September. Usually the females do not enter buildings, but readily bite humans outside, often during the day at sun and wind exposed places. They may cause a considerable nuisance in some regions, especially in late summer when the floodwater *Aedes* and *Ochlerotatus* species have already vanished.

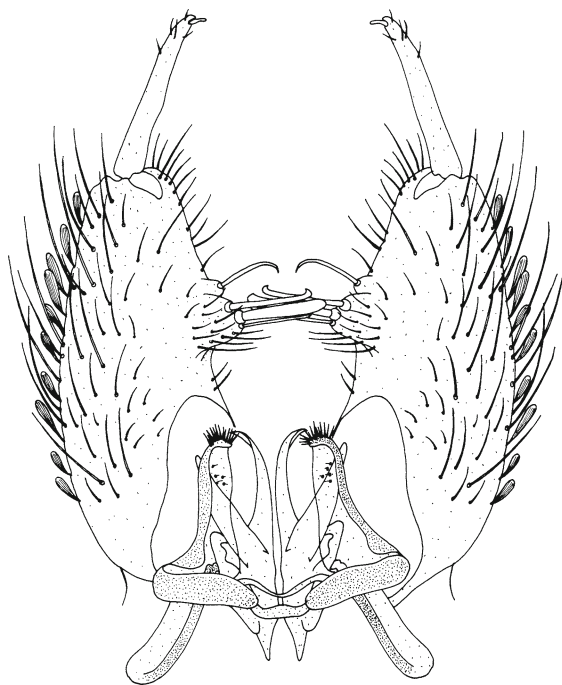
**Distribution:** *Cx. modestus* is widely distributed in the Palaearctic region from England to southern Siberia. It is recorded from middle and southwest Asia, northern India, and northern Africa. In Europe it is a common species in the southern and central countries.

**Medical importance:** The species has repeatedly been reported as an arbovirus vector of two different Bunyaviremia, Tahyna and Lednice (Lundström 1994) and is also regarded as a potential vector of WNV (Ribeiro et al. 1988). In addition, it has been found naturally infected with tularemia (Gutsevich et al. 1974)

### ***Culex (Barraudius) pusillus* Macquart 1850**

**Female:** It can be distinguished from the closely related *Cx. modestus* by its commonly darker colouration and the separated basolateral pure white patches of scales on the abdominal terga, which do not form a continuous longitudinal stripe usually present in *Cx.*

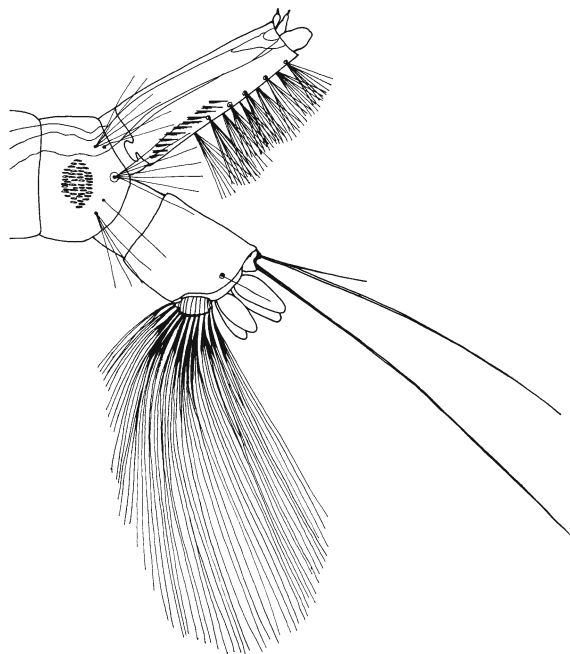
*modestus*. The proboscis, palps and clypeus are dark brown, the proboscis is apically swollen and shorter than the fore femur. The pedicel is dark brown with a tuft of brown scales, and the flagellum is dark brown. The occiput is brown with golden narrow scales and dark forked scales, and the sides are scattered with whitish scales. All decumbent scales of the vertex are narrow and golden, with a few dark setae, directing anteriorly between the eyes. The integument of the scutum is brown, and covered with brown narrow scales and blackish brown setae. The scutellum is brown, occasionally with a pale greenish shine, with brown coarse setae. The pleurites are brown with large patches of pale scales, but prealar scales are absent. The femora have brown scales, usually slightly paler on the ventral surface, with an indistinct knee spot. The tibiae and tarsomeres are dark brown scaled, and the hind tarsomere I is shorter than the hind tibia. The wings are entirely dark scaled, and the cross veins are well separated. The abdominal terga are dark brown with separated basolateral spots of white scales, and the sterna are uniformly covered with whitish grey scales.



**Fig. 10.93** Hypopygium of *Cx. pusillus*

**Male:** The gonocoxite has scales on the outer surface (Fig. 10.93). The lobe of the gonocoxite is situated slightly beyond the middle of the gonocoxite, with numerous setae and 2–3 strong spines of different sizes which are recurved apically, one of them may be more or less flattened. A single strong seta is situated more distally on a small separated elevation. The gonostylus is straight or slightly curved, relatively short, less than half as long as the gonocoxite, with a broad base and tapering apically. 4–5 small setae are located on the outer surface of the gonostylus close to its apex. The apex of the paraproct is densely covered with small spines forming a paraproct crown. The ventral arm of the aedeagus is long and curved at the apex and usually extends beyond the paraproct crown. To distinguish *Cx. pusillus* males from those of the similar *Cx. modestus*, the shape of the gonostylus and the length of the ventral arm of the aedeagus should be taken into consideration as indicated in the key. These two characteristics are usually sufficient for a positive identification.

**Larva:** The body is nearly transparent, pale yellowish green, and the head and siphon are slightly pigmented. The antennae are weakly spiculate, approximately as long as the head, straight, darkly pigmented at the base and distinctly narrowing from the insertion point of the antennal seta (1-A) to the apex. Seta 1-A is inserted slightly below the middle of the antennal shaft, and is half as long as the antenna or slightly more, with 15–27 branches. The frontal setae are long, the inner and median frontals (5-C and 6-C) have 2–3 branches, and the outer frontal seta (7-C) has 7 branches. The comb consists of a patch of 50 or more long fringed pointed scales (Fig. 10.94). The siphon is short, more or less cylindrical, the main tracheal trunks are broad, and the siphonal index is about 3.0. The pecten has 11–13 teeth, the distalmost teeth extending slightly beyond the middle of the siphon, and each individual tooth is long and slender with 1–3 lateral denticles. Setae 1-S have 8–10 tufts arranged in a more or less zig-zag row along the ventral surface of the siphon. 2–3 basalmost tufts are situated between the distal pecten teeth. The tufts with about 6 or more branches, are each at least as long as the width of the siphon at the point of its insertion. The anal segment is slightly shorter than the siphon, the saddle is approximately as long as it is wide, and completely encircles the anal segment. The



**Fig. 10.94** Larva of *Cx. pusillus*

saddle seta (1-X) is short with 1–2 branches. The upper anal seta (2-X) has 2–3 branches, 1 branch is longer than the others, and the lower anal seta (3-X) is long and single. The ventral brush has about 12 cratal setae (4-X). The anal papillae are half as long as the saddle, and the dorsal pair is slightly longer than the ventral pair.

**Biology:** *Cx. pusillus* is apparently a halophilic species. The larvae are mainly found in coastal breeding places, e.g. saline marshes and swamps, lagoons or stagnant pools with or without vegetation. Occasionally they occur in saline inland waters such as salt lakes or oases, but rarely in fresh water. Common associates in the breeding waters are larvae of *Oc. caspius*, *An. multicolor*, and *An. pulcherrimus*. *Cx. pusillus* is not a very common species, therefore little is known about its biology and adult behaviour. They are frequently found between September and November (Martini 1931). Females have never been observed entering dwellings or stables and probably do not feed on humans (Senevet and Andarelli 1959).

**Distribution:** Eastern Mediterranean, Middle East, southwest Asia, and northern Africa. In Europe, *Cx. pusillus* is solely reported from eastern and southern Greece (Samanidou-Voyadjoglou and Darsie 1993). This may represent its northernmost record.

### 10.3.2 Subgenus *Culex* Linnaeus

The body of the females is small to medium sized, and the proboscis in some species has a pale ring in the middle, but otherwise is entirely dark scaled. The vertex has numerous erect scales, and the occiput mainly has narrow scales with a few broad scales. The setal and scale patterns of the scutum and pleurites are as for the genus *Culex*. Lower mesepimeral setae are usually absent, but in some species 2–3 setae may be found. The coxae have a few scales, and wing scaling is as for the genus *Culex*. The abdominal terga may or may not have basal pale bands. The sterna are usually scaled and some species have laterosternal patches of pale scales. The proboscis of the males is usually lacking a pale ring. The gonocoxite is without scales, and the gonostylus is flattened and bent, and an apical spine is present. The structure of the aedeagus is very complex with differently shaped plates of outer and inner divisions. The paraproct bears distally a crown-like area composed of heavy spines. The antenna of the larva is spiculate, and is usually not longer than the head. Other morphological characteristics and setation are as for the genus *Culex*.

The subgenus *Culex* comprises the most species of the genus, but the main portion of it is of tropical distribution. In Europe two species occur in northern parts, the rest in central and southern Europe. *Cx. pipiens* occurs throughout the continent, whereas the southern and northern limits of *Cx. torrentium* are not fully understood. Other species have a more southern distribution or are limited to the Mediterranean and adjacent areas.

#### *Culex (Culex) brumpti* Galliard 1931

**Female:** According to Galliard (1931) *Cx. brumpti* is a relatively large mosquito. The proboscis is dark with an indistinct pale median ring, the scutum is covered with brownish scales, sometimes slightly paler on its posterior part, and the pleurites have 5 patches of pale scales. The legs have pale spots at the femoro-tibial and tibio-tarsal joints, and the tarsi are all dark, without pale rings. The wings are entirely dark scaled. The abdominal terga have basolateral triangular pale spots.



Fig. 10.95 Hypopygium of *Cx. brumpti*

**Male (Fig. 10.95):** The subapical lobe of the gonocoxite has a group of 3 long spine-like setae, two broad, transparent scale-like setae and 2 shorter narrow setae, one of them curved at the apex. At the base of the lobe there is an additional long, narrow hair-like seta. The gonostylus is expanded beyond the middle and then tapers apically. The apex of the paraproct has several rows of stout spines. The dorsal arm of the aedeagus is robust basally and directed outward at an angle in the form of a sharp tooth, and the ventral arm of the aedeagus is slender and curved, and extended apically in a fan shaped process (Fig. 7.62a).

**Larva:** Relatively large in size, with a curved antenna, abruptly narrowed distal from the insertion point of the antennal seta (1-A). 1-A is situated at 2/3 length of the antennal shaft, with multiple branches. The inner (5-C) and median (6-C) frontal setae have 2–3 branches, the outer frontal seta (7-C) has 6–8 branches. The comb is arranged in a patch of 22–24 fringed scales (Fig. 10.96). The siphon is long and slender, the siphonal index is about 6.0–7.0. The siphonal setae (1-S) consist of 4–6 pairs of tufts, each with 2–3 branches. The tufts are very short, much shorter than the siphon at the point of insertion, and all tufts arise beyond the distalmost pecten tooth. The pecten has about 8–11 teeth occupying the basal quarter to a third of the siphon. The main tracheal trunks are broad. The anal segment is completely encircled by the saddle, the upper anal seta (2-X) has



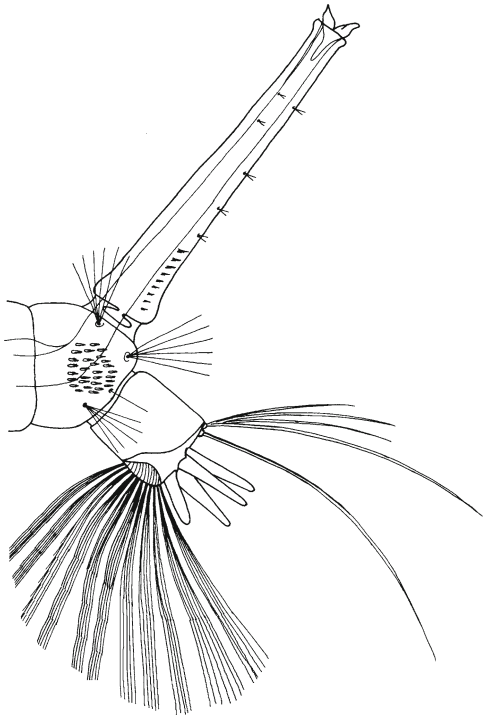


Fig. 10.96 Larva of *Cx. brumpti*

3–4 branches of varying length, and the lower anal seta (3-X) is single and nearly as long as the siphon. The ventral brush has about 11 tufts of cratal setae (4-X). The anal papillae are pointed and nearly as long as the saddle.

**Biology:** Because of its limited distribution range, little information is available on the biology of *Cx. brumpti*. In Corsica, the larvae were found in pools with aquatic vegetation, and in river beds (Galliard 1931; Aitken 1954a). Both authors sampled the larvae in August. Nothing is known about the feeding behaviour of adult females.

**Distribution:** Corsica, Sardinia.

### ***Culex (Culex) laticinctus* Edwards 1913**

**Female:** The proboscis is dark, but slightly paler on the basal half of the ventral surface. The palps are usually entirely dark scaled, but palpomere III may have a few apical pale scales. The clypeus and flagellum are dark brown, and the pedicel and flagellomere

I have a few pale scales. The erect forked scales of the vertex are light yellowish brown, and the curved scales are white to pale yellowish. The integument of the scutum is brown, and the scutal scales are light yellowish-brown, with paler scales located anteriorly, laterally, and on the prescutellar area. The scutal setae are prominent, brown, and darker than the scales. The scutellum has narrow pale curved scales and darker setae on all three lobes. The pleural integument is yellowish-brown, with yellowish to golden-brown setae and patches of narrow pale scales. Postspiracular and prealar scales are absent, the prealar area has 7–14 setae, and usually 2–3 lower mesepimeral setae are present. The fore coxae have pale scales on the anterior surface, and the mid and hind coxae have a longitudinal area of pale scales. The fore and mid femora have dark scales anteriorly and pale scales on the posterior surface, and inconspicuous pale knee spots are present. The hind femur is mainly pale scaled with a dorsal stripe of dark scales widening to encircle the distal end. The tibiae are dark scaled anteriorly, whitish posteriorly, and the apex of the hind tibia has a prominent pale spot. The tarsi are entirely dark scaled. The wings are dark scaled with a short line of pale scales on the costa (C) located near the humeral cross vein (h). Tergum I has a median posterior patch of pale scales, terga II–VII have broad pale basal bands, covering 1/2 to 2/3 of each segment, and tergum VIII has lateral patches of pale scales (Fig. 6.54a). Sterna I–VII have cream coloured scales, and sternum VIII is usually entirely white scaled.



Fig. 10.97 Hypopygium of *Cx. laticinctus*

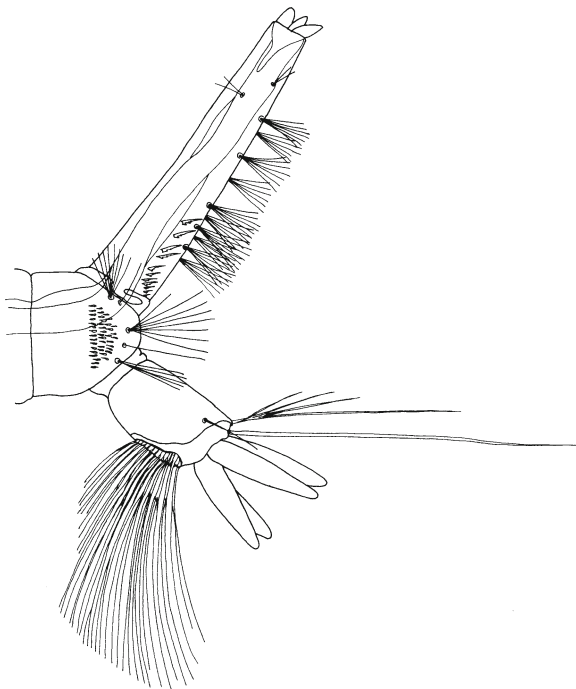
**Male:** The tergum IX has small lobes bearing 4–9 small setae. The gonocoxite is stout, with dense patches of long and short setae around the apex and near the subapical lobe (Fig. 10.97). The lobe is distinctly divided but not prominent, the proximal portion has 3 stout setae, slightly flattened and bent distally, and the distal portion has 2 setae similar in shape, and a crescent shaped, scale-like seta with a pointed apex. The gonostylus is relatively short, sharply bent in the middle and then tapering towards the apex, with a crest of small sharp ridges close to the tip (Fig. 7.63a). The apex of the paraproct has several rows of spines, and the ventral arm of the paraproct is well developed and curved.

**Larva:** The head is wider than long, and the antenna is about 2/3 the length of the head, curved and strongly spiculate, with a darker distal part. The antennal seta (1-A) has about 25 branches (19–30). Seta 1-C is long and rather stout, and the postclypeal seta (4-C) is short and single. The inner frontal seta (5-C) usually has 3–5 branches, the median frontal seta (6-C) usually has 4 branches, both reaching just beyond the anterior margin of the head, and the outer frontal seta (7-C) has about 7 branches, longer than

5-C and 6-C. The comb consists of >30 evenly fringed scales (Fig. 10.98). The siphon is straight, evenly tapering towards the apex, and the siphonal index averages 3.5 (2.8–4.6). The siphonal setae (1-S) consist of about 7 pairs of tufts arranged in a more or less ventral zig-zag row, with the penultimate pair of tufts displaced laterally. Each tuft has 6–9 branches about as long as the width of the siphon at its points of origin, and 2–3 basalmost pairs of tufts arising within the pecten. The lateral and most distal tufts are smaller and have fewer branches than the others, and are shorter than the width of the siphon at the point of insertion. The pecten has 10–16 long curved teeth with 3–4 basal denticles, extending to near the middle of the siphon. The saddle completely encircles the anal segment, and is longer than broad, the saddle seta (1-X) is small, usually single, sometimes with 2 branches, the upper anal seta (2-X) has 4–5 branches of varying length, and the lower anal seta (3-X) is single. The ventral brush has about 7 pairs of multiple-branched cratal setae (4-X), and precratal setae are absent. The anal papillae are pointed, and about as long as the saddle.

**Biology:** *Cx. laticinctus* seems to have been more common in the past than it is today (Harbach 1988). It was frequently collected in cisterns and concrete basins or artificial pools, tanks and barrels in gardens (Aitken 1954a). Nowadays it is more often found in stream pools, rock pools, swamps or ditches. The larvae usually occur in fresh water, but are occasionally found in brackish water. They are often collected with larvae of other *Culex* sp., *Anopheles* sp., *Cs. annulata*, *Cs. longiareolata* and *Ur. unguiculata*. *Cx. laticinctus* is mainly to be found in the summer months, although a few specimens may occur during the rest of the year. Adult females have never been observed entering houses (Senevet and Andarelli 1959), and it is not known whether they bite humans (Harbach 1988). Ribeiro et al. (1988) noted that it is apparently a zoophilic species with little or no medical importance.

**Distribution:** The range of *Cx. laticinctus* extends from the Canary Islands eastwards through the countries around the Mediterranean Sea, Somalia, Ethiopia, Sudan, the Arabian Peninsula, to the Middle East, and southwestern Asian countries. In Europe it is reported in Portugal, Spain, Romania, Italy, Greece (Crete), and former Yugoslavia.

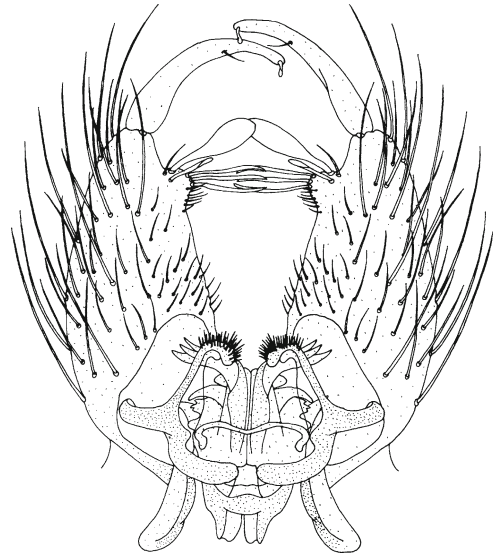


**Fig. 10.98** Larva of *Cx. laticinctus*

### *Culex (Culex) mimeticus* Noe 1899

**Female:** It is the only European *Culex* species with spots of pale scales on the wing veins and pale rings on the tarsomeres. The head has narrow pale yellowish scales dorsally, brownish laterally. The lateral patches of broad white scales are quite prominent. The palpomeres I–III are covered with blackish brown scales, and the apex of palpomere V is usually white scaled. The proboscis is predominantly dark scaled, with a broad median white ring. The scutum is covered with yellowish to golden scales in the middle, and greyish or whitish scales laterally. The pleurites have patches of white scales. The femora and tibiae of the fore and mid legs are usually entirely blackish brown scaled anteriorly, sometimes speckled with some pale scales, and the posterior surface is white scaled. The anterior surface of the hind tibia usually has a distinct longitudinal stripe of pale scales. Tarsomeres I–IV of all the legs have pale rings extending to both sides of articulation, and the basal rings are broader. The wing veins are predominantly covered with blackish brown scales, but numerous pale scales form a characteristic spotted pattern (Fig. 6.52a). The anterior margin of the wing has three white spots, the first spot at the middle of the costa (C), envelops (C) and subcosta (Sc), the second at half distance toward the apex, envelops C, Sc and  $R_1$ , and the third spot near the apex of the wing, usually envelops C,  $R_1$  and  $R_2$ . Pale scales are also present at the furcations of  $R_{2+3}$  and M, the broad midportion of  $R_4$ , the midportion of  $Cu_1$  and the basal portion of the anal vein (A).  $Cu_2$  is almost entirely covered with blackish brown scales, only a very short apical part may be pale scaled. The abdominal terga are dark brown to black with transverse pale basal bands (usually 1/3 as wide as the tergum) and large lateral pale spots. Terga VI and VII have narrow apical bands. The sterna are white scaled, except for a narrow apical portion.

**Male:** Similar to the female, but the contrast between the dark and light scales on the wing veins seems to be weaker. The palps are longer than the proboscis, with blackish brown scales and usually 3 dorsal patches of pale scales, one broad median patch on palpomere III, and two narrow basal patches on palpomeres IV and V, and the apex of palpomere V is covered with pale scales. The proboscis has a median pale ring. The antennae are brown scaled, with pale rings. The



**Fig. 10.99** Hypopygium of *Cx. mimeticus*

gonocoxite has relatively short setae predominant on the inner surface (Fig. 10.99). The subapical lobe has a broad, leaf shaped appendage and usually 4–6 large flattened setae. The gonostylus tapers apically. The ventral arm of the aedeagus has 1–2 denticles, and the dorsal arm usually has three finger-like processes (Fig. 7.65a). The apical crown of the paraproct is large, and made up of numerous spines. The ventral arm of the paraproct is thin, and the apex is slightly curved ventrally.

**Larva:** Similar to the larvae of the subgenus *Neoculex*. The main tracheal trunks are narrow, less than half as wide as the siphon. The head is relatively broad, often dark. The antenna is as long as the head, usually light with a darker apex, with the basal 2/3 covered with tiny spicules. The antennal seta (1-A) is multiple-branched, and inserted just above the spiculate region of the antennal shaft. The position of the subapical setae (2-A and 3-A) as well as the width of the tracheal trunks are valuable discriminative characteristics between *Cx. mimeticus* and *Cx. theileri*. In the former species the subapical setae are inserted between 1/3 and 1/2 of the distance between the apical setae (4-A to 6-A) and the antennal seta (1-A) (Fig. 8.71a); in the latter, they are located adjacent to the apical setae. The labral seta (1-C) of *Cx. mimeticus* is relatively thickened in the middle, and often denticulated in the apical part. The postclypeal seta (4-C) has 2–4 branches, the

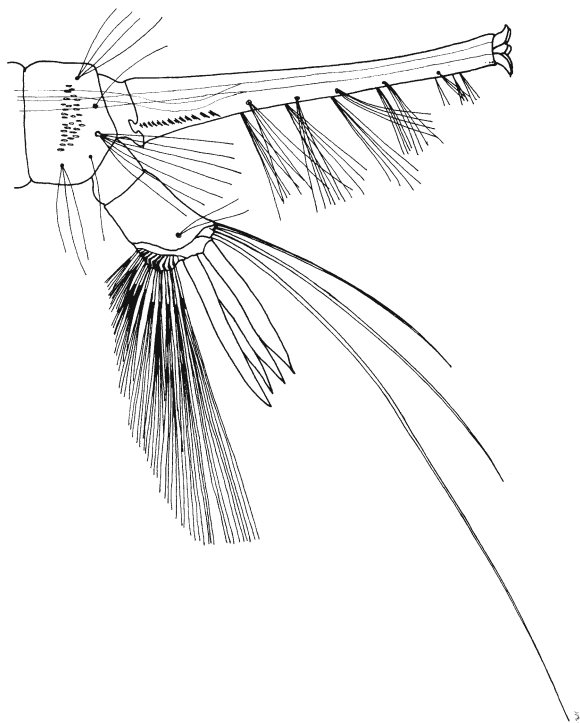


Fig. 10.100 Larva of *Cx. mimeticus*

inner frontal seta (5-C) has 3–5 branches, the median frontal seta (6-C) has 2–3 branches, and the outer frontal seta (7-C) is 5–7 branched. The comb has 20–35 scales (usually 25–30), each scale with a distinct, strong median spine and lateral rows of small spines (Fig. 8.70a). The siphon is straight, gradually tapering apically, and the siphonal index is 4.5–7.0, usually about 6.0 (Fig. 10.100). The number of pecten teeth is 12–18 (usually 13–15), most often evenly spread over the basal third of the siphon. Five to six pairs of siphonal setae (1-S) are inserted beyond the pecten. The basalmost 3–4 pairs of tufts have 4–5 branches, they are at least twice as long as the width of the siphon at the point of their insertion. The two distal pairs of tufts have 2–3 thin branches, their length equal to the siphon width at the point of their insertion, and a penultimate tuft is articulated laterally. The saddle entirely encircles the anal segment, and the saddle seta (1-X) has 2 branches. The upper anal seta (2-X) has 2 branches, with the dorsal branch more than half as long as the ventral branch. The lower anal seta (3-X) is single and about as long as the siphon. The anal papillae are 1.5–2 times longer than the saddle.

**Biology:** *Cx. mimeticus* is a polycyclic, orophilic (associated with mountainous regions) species. It is quite common in some parts of the Mediterranean region. The larvae occur in small, shallow pools in dried-up streams and torrent beds containing growth of *Spirogyra* (Aitken 1954a), rock pools and in shallow margins and backwaters of rapidly flowing mountain streams overgrown with aquatic vegetation. The breeding water is usually crystal clear with a pH ranging between 5.2 and 6.0 (Ribeiro et al. 1977). The larvae may be associated with those of *An. atroparvus*, *An. claviger*, *An. marteri*, *An. petraghani*, *An. superpictus*, *An. cinereus hispaniola*, *Cx. p. pipiens*, *Cx. theileri*, *Cx. perexiguus*, *Cx. hortensis*, *Cx. impudicus*, *Cx. territans*, and *Cs. longiareolata* (Gutsevich et al. 1974; Ribeiro et al. 1977). They are reported from an altitude of 3,055 m in Tibet (Feng 1938). The species is moderately orophilic in Portugal, occurring from 150 to 1,100 m (Ribeiro et al. 1989). The females are zoophytic, but occasionally enter houses and bite humans (Sicart 1951).

**Distribution:** Oriental region, southern parts of the Palearctic (Mediterranean, Middle East, Iran, Nepal, Tibet, India, Vietnam, China, Japan). In Europe it is recorded in the southern parts of the continent in France, Italy, former Yugoslavia, Macedonia, Bulgaria, Greece, Cyprus, and Russia.

### ***Culex (Culex) perexiguus* Theobald 1903**

The species is closely related to *Cx. univittatus* and exhibits a very similar external morphology in all life stages. Thus the following description of *Cx. perexiguus*, based on Harbach (1988), will highlight the differences between the two species as there is some chance that *Cx. univittatus* might occur in some parts of the Mediterranean region. However, Eritja et al. (2000) recognised *Cx. univittatus* as a species occurring in Spain according to findings of Encinas Grandes (1982).

**Female:** A small sized mosquito. The proboscis is dark, with whitish scales ventrally except at the base. The pedicel and flagellomeres have few pale scales. The head has golden brown scales, and the vertex is pale scaled with forked brown scales laterally. The integument is brownish, the scutal scales are narrow and golden brown and pale scales form a pair of faint submedian spots. The postpronotum has golden brown

scales which become paler posteriorly. The upper and lower posterior border of the mesepisternum and anterior part of the mesepimeron have patches of white scales. The legs are dark scaled on the anterior surface, and the hind tibia has a more or less distinct longitudinal anterior pale stripe (Fig. 6.55a). The tarsomeres are dark scaled dorsally, with pale scales ventrally. The wings are dark scaled, with a short line of pale scales on the costa (C). The abdominal terga have slightly convex white basal bands connected with the basolateral patches. The sterna are whitish scaled, sometimes with dark scales, scattered or in patches.

**Male:** Tergum IX has two slightly elevated lobes with long, spaced setae. In *Cx. univittatus* the lobes seem to be almost flat and the coverage of setae is denser. The gonocoxite has numerous long and short setae (Fig. 10.101). The subapical lobe is slightly divided; the proximal portion has 3 long and strong setae, and the distal portion has three shorter hair-like setae and one broad, spatulate seta. In *Cx. univittatus* the latter seta is narrower. The gonostylus is expanded beyond the middle, and has two setae and a short apical spine. The aedeagus has a stout ventral arm and concave apex, without any spines, which is shorter than the dorsal arm (Fig. 7.62b). In *Cx. univittatus* the ventral arm is as long as the dorsal arm, and they seem to be in line in a lateral view of the aedeagus (prepara-

tion necessary). The ventral arm of the paraproct is long and recurved at the apex.

**Larva:** The head is broader than long, and the antennal seta (1-A) has 19–27 branches. The frontal setae 5-C and 6-C usually have 2–3 branches. The number of comb scales is 35–55, each individual scale is elongated and rounded apically. The siphon is usually long and slender, and the siphonal index may vary (Fig. 10.102). The number of pecten teeth is 8–15; the larger basal teeth are widely spaced, each with 3–4 lateral denticles. The siphonal seta (1-S) consists of five pairs of lateral tufts usually positioned beyond the pecten. In *Cx. univittatus* the number of pairs is 5–6 and they are inserted more ventrolaterally, with the two basalmost tufts (1a-S and 1b-S) attached within or close to the pecten. All tufts in both species have short branches not exceeding the width of the siphon at the point of insertion. The saddle entirely encircles the anal segment, and the anal papillae are short.

**Biology:** The species is common during summer and autumn. The larvae can be found in many kinds of stagnant water collections, e.g. clean to moderately polluted swamps, ponds, streams, pools, wells, usually with emergent vegetation, and occasionally in man-made containers. The adult females probably prefer to feed on birds (Harbach 1988). This has also been reported for *Cx. univittatus* from Turkmenia (Gutsevich



Fig. 10.101 Hypopygium of *Cx. perexiguus*

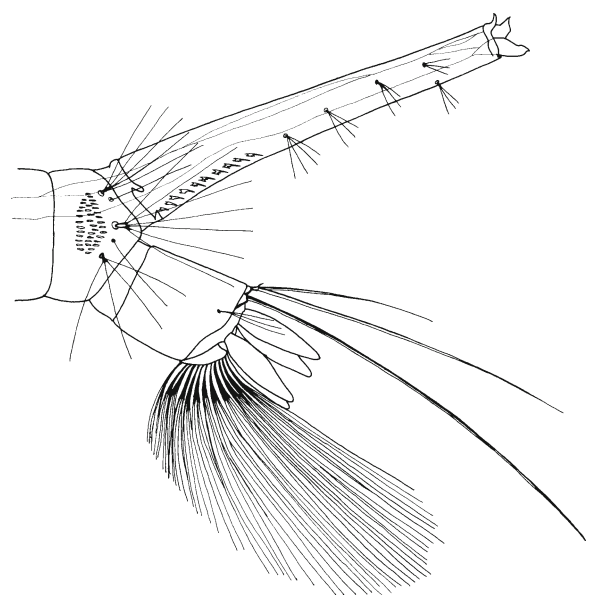


Fig. 10.102 Larva of *Cx. perexiguus*

et al. 1974). Martini (1931) reported the species (as *Cx. univittatus*) as biting humans inside houses at night.

**Distribution:** This species is recorded in Portugal, Spain, Italy including Sicily, Macedonia, Bulgaria, Greece, and Turkey (Snow and Ramsdale 1999). *Cx. perexiguus* is found in Asia Minor, south western Asia towards India, and in northern Africa (Harbach 1988).

**Medical importance:** From Israel and Egypt *Cx. perexiguus* has been reported as a vector of WNV (Harbach 1988).

**Notes on systematics:** The species name which has hitherto been used for European material is *Cx. univittatus*, but Harbach (1999) stated that *Cx. univittatus* is restricted to the temperate highlands in the East African Subregion of the Afrotropical Region and identified a few specimens from Greece, Italy, and Turkey as *Cx. perexiguus* based on characteristics of the male genitalia and larvae. He suggested regarding the species which occurs in southern Europe, that it should be rather *Cx. perexiguus* than *Cx. univittatus*, although Eritja et al. (2000) confirmed the presence of *Cx. univittatus* in Spain.

### Culex Pipiens Complex

The complex consists of several species, subspecies, forms, races, physiological variants, or biotypes according to various authors. At present it includes the names *Cx. pipiens pipiens* Linnaeus, *Cx. p. pipiens* biotype *molestus* Forskal, *Cx. p. quinquefasciatus* Say, *Cx. p. pallens* Coquillett, *Cx. restuans* Theobald, and *Cx. torrentium* Martini in the Holarctic as well as two Australian members, *Cx. australicus* Dobrotworsky and Drummond and *Cx. globocoxitus* Dobrotworsky.

The status of the three first names has been taxonomically stabilized by designation of neotypes (Sirivanakarn and White 1978; Harbach et al. 1984, 1985). It is now generally accepted that the former *Cx. pipiens molestus* (Harbach et al. 1984) is not separated from the subspecies *Cx. pipiens pipiens* and is designated as a biotype, as no genetical differences have been found (Bourguet et al. 1998). However, new data based on protein electrophoresis revealed a significant genetic distance between the two forms (Becker et al. 1999).

The females of the complex are very difficult to separate in field material. In several reared populations it took eight variables and a discriminant analysis to

discern between *pipiens*, *molestus*, and *quinquefasciatus* females and overlapping was considerable (Kruppa 1988). Thus, there is no reliable characteristic yet for discrimination between *pipiens* and *molestus*.

The former *Cx. quinquefasciatus* Say and *Cx. quinquefasciatus pallens* Coquillett are currently regarded as subspecies of *Cx. pipiens* (Miller et al. 1996). They freely hybridize but show a difference in the male hypopygial morphology (Kruppa 1988). *Cx. pipiens pipiens* and *Cx. torrentium* are two separate sibling species (Harbach 1985; Dahl 1988; Harbach 1988; Miller et al. 1996) defined by genetic characteristics and different morphology in some life stages.

### *Culex (Culex) pipiens pipiens* Linnaeus 1758

**Female:** A medium sized mosquito, with a yellowish brown to dark brown integument. The antennae are dark, and the pedicel and flagellomere I have a few tiny white scales. The palps are mainly black scaled, and the proboscis has cream coloured scales ventrally. The head has dark forked scales and some paler scales laterally. The scutum has delicate golden brown scales, which are lighter laterally. The scutellum has narrow, pale yellow scales and dark setae. The postpronotum has golden brown scales. The pleurites have yellowish or white scale patches on the mesepisternum. Postspiracular and prealar scales are absent, or rarely a few scales may be present. The absence of scales in *Cx. p. pipiens* provides an almost reliable characteristic for separation from *Cx. torrentium* females which have a few prealar scales when undamaged. The coxae have a small patch of dark scales, the femora have a yellowish apical border but otherwise are dark scaled, and the hind femur has mostly whitish scales. The tibiae and tarsi are dark scaled, the hind tibia lacks a longitudinal pale stripe (Fig. 6.55b). The scaling of the wings is dark, and the subcosta (Sc) intersects the costa (C) beyond the furcation of  $R_{2+3}$  (Fig. 6.52b). The abdominal terga are predominantly dark scaled; tergum II has a small basomedian whitish spot, and terga III–VII have whitish to yellowish, narrow basal bands which expand laterally (Fig. 6.54b). The sterna are yellowish scaled.

**Male:** The subapical lobe of the gonocoxite has seven large, simple and one broad scale like setae (Fig. 10.103). The gonostylus is broad with several small and two spiniform setae apically. The aede-

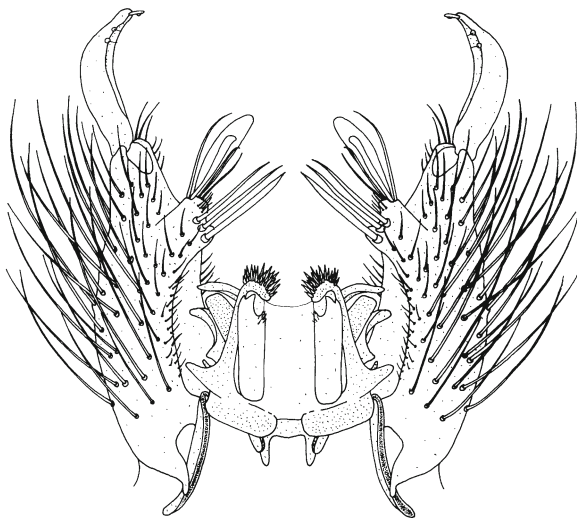


Fig. 10.103 Hypopygium of *Cx. p. pipiens*

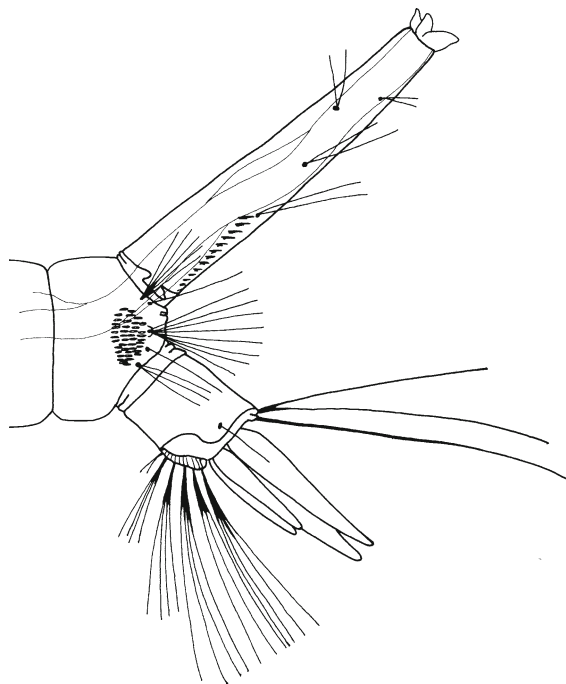


Fig. 10.104 Larva of *Cx. p. pipiens*

gus has several complicated plates and lobes of which the ventral and dorsal arms are diagnostic characteristics and are used for the so called DV/D formula (Sirivanakarn and White 1978; Ishii 1991). The shape of the ventral arm is slender, blade-like with a sharply pointed apex. The dorsal arm is tubular, stout, and distinctly truncated at the apex (Fig. 7.64b). The ventral arm of the paraprot varies in length from being an inconspicuous knob to a conspicuous extension which is never recurved. The paraprot has numerous stout setae forming a brush-like crown. It is possible to distinguish it from *Cx. torrentium* by the blunt apex of the dorsal arm and a different shape of the ventral arm of the paraprot, which is long, sclerotized, and recurved at the apex in *Cx. torrentium*.

**Larva:** The head is wider than long, and the antenna is shorter than the head. The postclypeal seta (4-C) is short and simple, and all frontal setae are long. The inner frontals (5-C) have 5–6 branches, the median frontal seta (6-C) is 4–5 branched, and the outer frontals (7-C) have 6 branches. The prothoracic setae 1-P to 3-P are long and single, 4-P has 2 branches and is somewhat shorter than the other setae, 5-P and 6-P are single and long; 7-P is 2-branched and long. The metathoracic seta 1-T is shorter than half the length of 2-T. The number of comb scales is about 40, each individual scale is short and widened at the apex, and evenly fringed. The siphon is slender, evenly tapered towards the apex, and the siphonal index

ranges between 4.8 and 5.0 (Fig. 10.104). The number of pecten teeth is 13–17, evenly spaced up to seta 1a-S. Each pecten tooth has a long pointed tip and three lateral denticles. The siphonal tuft (1-S) consists of four widely spaced 2-branched pairs of setae which arise distal to the pecten in an irregular row. The anal papillae are elongate, and the dorsal pair is twice as long as the saddle. The larvae of the species complex are very similar. Those of *Cx. p. pipiens* and *Cx. torrentium* can only be distinguished from each other when the specimens are very well preserved; differences in abdominal setation are as indicated in the keys (Fig. 8.77c). The larvae of *Cx. p. quinquefasciatus* have a significantly shorter siphon (the siphonal index is about 4.0).

**Biology:** Females usually do not feed before entering winter shelters; the specimens which have taken a small blood meal before overwintering have a poorer chance of surviving (Mitchell and Briegel 1989). Overwintering females lay their eggs on the water surface in batches as egg rafts of usually 150–240 eggs. The larvae hatch within 1 or 2 days and complete their development to adults in about one to a few weeks, depending on the temperature. They are able to inhabit nearly every kind of water source. The first larvae

often occur together with those of *Anopheles* species and can be found in semi permanent waters, larger pools with vegetation, rice fields, along river edges in still zones, and in inundation areas; occasionally even in tree-holes. The larvae frequently occur in man-made water bodies such as flooded cellars, construction sites, water barrels and tin cans, metal tanks, ornamental ponds, and containers in gardens and in churchyards. They even tolerate a small amount of salinity and can occur in rock pools. The species can develop up to several generations per year depending on climatic conditions. The females are anautogenous, ornithophilic, eurygamous, and diapausing in wintertime. Occasionally they have been observed to feed on wild mammals or on mice in the laboratory. In southern France, anautogenous populations with stenogamous males of *Cx. p. pipiens* have been reported from the field. Its biotype *molestus* occurs more frequently in human environments.

**Distribution:** *Cx. p. pipiens* is widespread in the Holarctic region and found throughout Europe. Its distribution seems to be more northern in the easternmost European parts than in Scandinavia. Its northern borders throughout Europe need renewed analysis, as it has not always been distinguished from *Cx. torrentium*. It has been introduced into Australia and also into South America and eastern and South Africa. In the southeasternmost areas of the northern hemisphere *Cx. p. pipiens* occurs sympatrically with *Cx. p. quinquefasciatus*.

**Medical importance:** *Cx. p. pipiens* seems to play a minor role as an arbovirus vector in Europe. Nevertheless, one report of high incidence of WNV from Romania (Nicolescu 1998) and a record of low incidence of Ockelbo virus from wild caught females (Lundström 1994) demonstrate the capacity of the species for arboviruses.

**Notes on systematics:** The species is highly variable in its pattern of integument and scaling colour, hence it has numerous synonyms worldwide. From European material nine valid synonyms exist (Knight and Stone 1977; Knight 1978; Ward 1984, 1992). Two further invalid subspecies descriptions on European material and now treated as synonyms, are the former *Cx. p. torridus* Iglisch and *Cx. p. erectus* Iglisch (Dahl 1988; Harbach 1988). There are different views as to the specific status of *Cx. pipiens* and *Cx. quinquefasciatus*. They are still regarded as one species based on their mode of hybridisation and molecular studies

which have not confirmed a species specific status (Bourguet et al. 1998). However, the males can be separated by morphological characteristics (Sirivanakarn and White 1978; Kruppa 1988).

### ***Culex pipiens pipiens* biotype *molestus* Forskal 1775**

The biotype cannot be distinguished from *Cx. p. pipiens* on a single morphological characteristic in adults. Kruppa (1988) found a statistical difference in the larval mentum between *pipiens*, *quinquefasciatus*, and *molestus* in reared strains. No reliable genetic marker was found to separate *molestus* from *pipiens* (Bourguet et al. 1998). However, four biological criteria for the biotype *molestus* have been established: autogeny, stenogamy, anthropophily, and facultative diapause.

**Female and male:** Colouration, setae, and scaling of head, thorax, abdomen, wings, and legs as in *Cx. p. pipiens* (Harbach et al. 1984). The males are stenogamous, meaning copulation with females in very limited spaces without swarming is possible. This has led to a search for male morphological characteristics to distinguish between *pipiens* and *molestus* (Oljenicek and Zoulova 1994). The first character was found in the length of palpomere IV, which gave a reliable difference from that of *molestus*, being 1.5 times longer than that of *pipiens*, a result in contrast to earlier observations (Harbach 1988). The second character is the average length of the antennal seta compared to the average length of the antenna, expressed in an antennal index. This index is 3.5 in *pipiens* and ranges between 4.1 and 4.4 in *molestus*. These morphometric parameters were measured on reared material.

**Larva:** The morphological characteristics are so variable that only identification based on eight characters with statistical analysis gave some discrimination between *pallens*, *molestus*, and *pipiens*. More reliable is the discrimination between all three mentioned taxa against *Cx. p. quinquefasciatus* (Kruppa 1988). However, it is claimed by many authors that a difference in the siphonal length can be found with a shorter siphon in *molestus* than in *pipiens* (Oljenicek and Zoulova 1994). Previously, this was correlated with highly polluted larval habitats characterized by a high content of ammonia (Gabinaud et al. 1985).



**Biology:** A well-accepted biological character of the biotype *molestus* is the autogeny of the females, which might even occur in several forms (obligate or facultative) as among other culicid species (O'Meara 1985; Clements 1992). Without taking any blood meal the females lay much fewer eggs and can bite readily after the first batch (Harbach et al. 1984). Females do not have an obligate diapause. They can reproduce throughout the winter in dark, warm urban habitats containing water or overwinter in unheated cellars or similar man-made shelters. The mortality in winter shelters is very high (95–100%) and mainly caused by the lack of fat body reserves obtained in the larval stage, by entomopathogenic fungi and spiders. Males and unmated females do not survive the winter (Petrić 1985; Petrić et al. 1986). The males are stenogamous; they readily mate in confined spaces without swarming. The most common larval habitats are dark and moist cellars of large buildings in towns, underground sewage constructions, and man-made water containers in dark, humid places.

**Distribution:** Females of the complex, registered as *molestus* because of biting humans both indoors and outdoors, have been reported from many of the largest cities throughout Europe and temperate regions of the world. There are records even from very northern cities in the European parts of Russia. No recent summary of the occurrence of *molestus* throughout Europe or other continents is available.

**Medical importance:** One of the major bridge vectors of WNV worldwide. *Cx. p. pipiens* biotype *molestus* and *Cx. restuans* account for more than 80% of the total risk, a surrogate for human WNV infections in the northeastern United States. The threat of these two species is nearly 16 times higher than that for the four other important vectors of WNV, *Oc. japonicus*, *Ae. vexans*, *Oc. trivittatus*, and *Cx. salinarius* (Kilpatrick et al. 2005).

**Notes on systematics:** *Cx. molestus* was described as a species by Forskål 1775 on Egyptian material. Harbach et al. (1984) selected a neotype and at the same time stated that *molestus* was not a valid species. All claims to identify *molestus* on morphological characteristics failed. This might depend on an extensive gene flow between the populations. A certain hybridization between the forms has been postulated for natural conditions and was tested in laboratory experiments (Chevilon et al. 1995).

### ***Culex (Culex) pipiens quinquefasciatus* Say 1823**

Closely resembling *Cx. p. pipiens* in colouration, differing in the yellowish brown not reddish or dark brown scales on the scutum and in the structure of the lateral aedeagal plates of the hypopygium (Gutsevich et al. 1974). In *Cx. p. quinquefasciatus* the inner division is simple, represented by a broad leaf like ventral arm which is strongly divergent laterally and the dorsal arms parallel or subparallel and distally strongly tapered into a point (Sirivanakarn 1976) (Fig. 7.66.c). In *Cx. p. pipiens* the ventral arm is sickle shaped, smaller, shorter, and narrower and the dorsal arms divergent and uniformly thick, with a truncated apex (Fig. 7.66a, Fig. 10.103).

The females of the two subspecies cannot be distinguished with certainty but in some regions such as Australia, the difference in the pale banding on the terga may enable separation. In *Cx. p. quinquefasciatus* the distinct basolateral pale spots are apparently not connected with the evenly broad or slightly medially produced basal pale bands, whereas in *Cx. p. pipiens* biotype *molestus* the pale bands on the terga are not constricted laterally (Russell, pers. commun.).

**Biology:** The “Southern House Mosquito” is one of the most troublesome mosquitoes, biting, like other *Culex*, almost exclusively at night (Edwards 1941). It occurs abundantly in houses and in practically all types of human and animal shelters in urban communities throughout the tropics at diverse altitudes up to 2,300 m above sea level (Mara 1945). The larvae can be found in any type of habitat which contains water ranging from fresh and clear to brackish, turbid and polluted with decayed organic matter from garbage and human waste accumulated in ground pools, ditches, drains, sewages, dumping areas, latrines, septic tanks, and in various kinds of artificial containers. In general, *Cx. p. quinquefasciatus* is uncommon in small containers, but common in large ones (Sirivanakarn 1976). The adults are fairly abundant throughout the year and are commonly found resting during the day in dark places in wardrobes, bathrooms, under tables, and similar places inside houses. The females are vicious biters at night, indoors or outdoors and feed principally on blood of humans from sunset until dawn. They also frequently attack birds such as poultry and, to a lesser extent, other domestic animals, including dogs, cats, and pigs. The range of hosts for the blood meal is broad but different populations vary

in this respect. The females are stenogamous and not autogenous and they can develop several generations per year without diapause (Dobrotworsky 1965; Gutsevich et al. 1974).

**Distribution:** Widespread throughout the tropics and subtropics of the world.

**Medical importance:** *Cx. p. quinquefasciatus* is one of the most serious pests of man in the tropics and has been incriminated as the principal vector of nocturnal periodic bancroftian filariasis in various parts of the Oriental region. It is an important vector of *Brugia malayi* (Malayan filariasis) and *Dirofilaria immitis* (dog heartworm) and some plasmodia of birds. In some parts of Southeast Asia, it has also been reported to be a host of certain arboviruses, notably Chikungunya and Japanese encephalitis but there is apparently no conclusive evidence to indicate that it plays a role in the natural transmission of these diseases. This may in part be due to the fact that it rarely bites reservoir animals such as pigs infected with Japanese encephalitis virus. It is a vector of St. Louis encephalitis and Western Equine encephalitis in the Americas (Gutsevich et al. 1974; Sirivanakarn 1976). *Cx. p. quinquefasciatus* is the predominant vector of WNV in the southeastern and much of the western parts of the United States (Reisen et al. 2004; Kilpatrick et al. 2005).

### ***Culex (Culex) torrentium* Martini 1925**

The species is very similar to *Cx. p. pipiens* in all stages, except for larval seta 1-T (Harbach et al. 1985), the prealar scale patch (Service 1968a), the pointed and twisted apex of the dorsal arm of the aedeagus and the curved ventral arm of the paraproct in the male hypopygium, and the pattern of the egg chorion (Dahl 1988). Wild caught females are larger in average body size than those of *Cx. p. pipiens*. This character is very subtle and needs much experience to recognise it. Martini (1931) highlights some differences in scaling which seem to give some guidance for specimens from central and east Europe.

**Female:** The proboscis is dark with a whitish mid-part and pale scaling ventrally, and the palps are dark brown scaled. The antenna is dark with a whitish tinge. The head has yellowish flat scales on the occiput and vertex and around the eyes, the setae are golden to dark brown. The scaling on the scutum is brownish, apically lighter, and on the pleurites is brownish with

some whitish scales. A patch of prealar scales is present in most newly emerged specimens. The coxae have patches of light scales, the femora are dark, ventrally whitish, with a white knee spot. The tibiae are dark scaled, ventrally whitish, and the hind tibia has no longitudinal pale stripe. All the tarsomeres are dark brown with some light ventral scaling. The wing veins are covered with brownish, elongated scales. The abdomen is dark brown with pale yellowish basal bands on all segments which are not expanded laterally.

**Male:** Although there seem to be some constant features in the scaling of the proboscis and legs of the males, the most reliable characteristics for distinguishing *Cx. p. pipiens* from *Cx. torrentium* are found in the hypopygium (Fig. 10.105). The dorsal arm of the aedeagus is pointed and twisted at the apex and not blunt as in *Cx. p. pipiens*. In *Cx. torrentium* the ventral arm of the paraproct is always long and recurved, without much variability in shape. In *Cx. p. pipiens* it varies from being vestigial to conspicuous, but is never recurved. The shape of the cercal sclerite of the paraproct is broader and shorter in *Cx. torrentium* than in *Cx. p. pipiens*.

**Larva** (Fig. 10.106): The larvae differ from those of *Cx. p. pipiens* and its biotype *molestus* in some very subtle characteristics. Harbach et al. (1985) found some differences in the thoracic and abdominal setation as indicated in the keys (Fig. 8.77a).



**Fig. 10.105** Hypopygium of *Cx. torrentium*

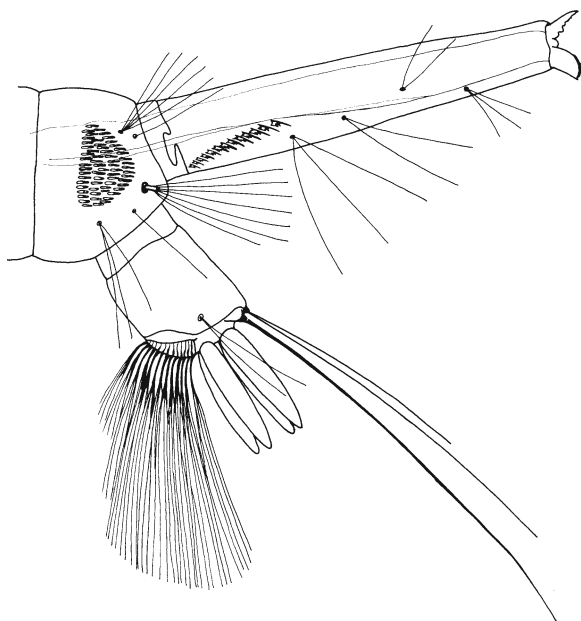


Fig. 10.106 Larva of *Cx. torrentium*

**Biology:** The larvae occur throughout the warmer season, often together with those of *Cx. p pipiens* in both unpolluted and polluted habitats, such as edges of slow running streams, in vegetation at borders of lakes, semipermanent pools, marshy areas, man-made containers, and reservoirs of sewage plants. The development seems to be slower than in *Cx. p pipiens* which might result in only one generation per year in northern areas. Females are ornithophilic and have never been reported to bite humans, not even in laboratory colonies. Both sexes are most active in nectar feeding between 2200 and 0300 hours (Andersson and Jaenson 1987). However, in captivity the females live longer and lay their eggs more scattered through time and in smaller batches. The pattern of the eggs' exochorion is more widely spaced in *Cx. torrentium* than in *Cx. p pipiens*, this characteristic needs verification from other geographic areas (Dahl 1988).

**Distribution:** The species seems to have a wide distribution in the temperate Palaearctic region and neither its southernmost or northernmost borders are well established.

**Medical importance:** In northern Europe, Ockelbo virus (Alpha virus) has been isolated from *Cx. pipiens/Cx. torrentium*. In the laboratory, the vector competence of *Cx. torrentium* was much higher than that of *Cx. pipiens* (Lundström 1994).

### *Culex (Culex) theileri* Theobald 1903

**Female:** A large mosquito, with a brown general coloration. The most striking feature is the presence of a longitudinal pale stripe on the anterior surface of all the femora and tibiae, particularly on the fore and mid legs. The palps are predominantly brown scaled, palpomeres III and IV have pale scales dorsally, sometimes largely pale scaled. The proboscis is brown scaled with more or less numerous pale scales predominating in the middle, but not forming a distinct ring. The head has white, yellowish or golden brown narrow decumbent scales and brown erect scales. The scutum has golden brown scales medially, yellowish white laterally, and is whitish at the prescutellar area. The postspiracular area has a broad patch of pale scales. The upper and lower mesepisternal patches of scales are fused forming a stripe at the posterior margin of the mesepisternum. The femora and tibiae of the fore and mid legs have an anterior longitudinal white stripe along their whole length (Fig. 6.53a), and sometimes the fore femur has a linear row of white spots instead. The tarsomeres of the fore and mid legs are entirely dark. Tarsomere I of the hind leg has a distinct white stripe on its anterior surface, but the remaining tarsomeres are completely brown scaled. The basal half of the costa (C) has pale scales. Sometimes the subcosta (Sc) and radius (R) also carry some pale scales (Aitken 1954a). The other wing veins are entirely dark scaled. There are pale scales on tergum I and sometimes median scale patches on terga II and III. Terga IV–VII have broad basal pale bands which are usually triangularly produced posteriorly, sometimes with median yellowish dots, which may form a pale median longitudinal stripe. Tergum VIII is completely covered with yellowish scales.

**Male:** The palps are longer than the proboscis at least for the length of palpomere V, brown scaled, with more or less numerous pale scales which sometimes form rings. The lobe of tergum IX has 10–12 setae. The subapical lobe of the gonocoxite has 4 flattened blade-like setae, a thin hair-like seta and a leaf shaped seta which is relatively broad and elliptical (Fig. 10.107). The gonostylus is sickle shaped with a slender subapical spine. The ventral arm of the aedeagus has 2–4 strong lateral teeth, and the dorsal arm is simple and pointed apically.

**Larva:** The antennal shaft is strong, 3/4 of the length of the head, with a darker base and apical part. The



Fig. 10.107 Hypopygium of *Cx. theileri*

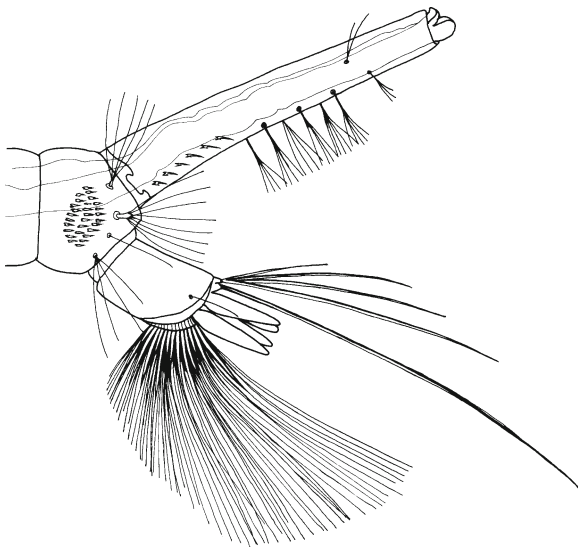


Fig. 10.108 Larva of *Cx. theileri*

antennal seta (1-A) is multiple-branched (up to 30 branches), and the subapical setae 2-A and 3-A are inserted close to the apex (Fig. 8.71c), and not separated from the apical seta as in *Cx. mimeticus*. The labral seta (1-C) is dark, strong, spiniform, and gradually tapering. The postclypeal seta (4-C) is 1–3 branched. The inner

frontal seta (5-C) is 2–5 branched, the median frontal seta (6-C) has 2–4 branches, and the outer frontal seta (7-C) is 6–10 branched. The number of comb scales ranges between 12 and 44, usually with 20–30 scales (Fig. 10.108). Each individual scale has a strong median spine, with or without lateral rows of smaller spines. The siphon slightly tapers apically, and the siphonal index ranges from 4.0 to 8.0, usually 5.0–6.0. The main tracheal trunks are broad, at least half as wide as the siphon, with an oval cross section. The pecten is restricted to the basal third of the siphon, with 5–15 widely spaced teeth. Five, sometimes six pairs of siphonal setae (1-S), are inserted beyond the pecten (an odd number of setae could be present as well). The three basal pairs are subequal in length, 4–6 branched, situated in a zig-zag row on the ventral surface of the siphon, and are about as long as the width of the siphon at the point of insertion. The two distal pairs are weaker and shorter, inserted laterally and subventrally, with 2–5 branches. The saddle seta (1-X) has 1–3 branches, the upper anal seta (2-X) has 3–4 branches, and the lower anal seta (3-X) is single and longer than the siphon. The ventral brush has 14 well developed cratal setae (4-X), without precratal setae. The anal papillae are about as long as the saddle, slender, pointed, and both pairs are of similar length.

**Biology:** A polycyclic species recorded from a broad range of elevations. It is reported to be common at altitudes of 1,000–3,000 m in the Himalayan areas (Sirivanakarn 1976). The larvae occur in spring in flooded meadows, stagnant or slowly moving streams, ditches, rock pools, drains, swamps, and rice fields but also frequently in artificial containers and strongly polluted waters (Aitken 1954a; Sirivanakarn 1976; Ramos et al. 1978). They usually breed in fresh or slightly saline water (2 g NaCl/l), but tolerate a salinity up to 16.6 g NaCl/l and pH 5.5–9.5 (Gutsevich et al. 1974; Ramos et al. 1977; Pires et al. 1982). The larvae can often be found in association with those of *An. algeriensis*, *An. atroparvus*, *An. claviger*, *An. labranchiae*, *An. maculipennis*, *An. superpictus*, *Ae. vexans*, *Ae. vittatus*, *Oc. caspius*, *Oc. detritus*, *Cx. modestus*, *Cx. laticinctus*, *Cx. p. pipiens*, *Cx. perexiguus*, *Cx. hortensis*, *Cx. impudicus*, *Cs. longioreolata*, *Cs. annulata*, *Ur. unguiculata* (Coluzzi 1961; Bozkov et al. 1969; Ramos et al. 1977; Pires et al. 1982). The females are zoophilic, but sometimes feed on humans and bite mainly in the open, occasionally in large numbers, also entering houses and other buildings (Gutsevich et al. 1974; Ramos et al. 1977).

**Distribution:** Scattered through the Ethiopian (south, east and north Africa), Palaearctic (Mediterranean, Ukrainian steppes, Crimea), Middle East, and the east Oriental region (India, Burma, China). In Europe it is reported from Portugal, Spain, France, Italy, former Yugoslavia, Greece, Hungary, Bulgaria, and Ukraine.

**Medical importance:** In South Africa, Sindbis virus and WNV were isolated from wild populations (McIntosh 1975). *Cx. theileri* is known to be a carrier of Rift Valley Fever virus and canine *Dirofilaria* in North Africa and Portugal (Smith 1973; Ribeiro et al. 1983, 1988).

### 10.3.3 Subgenus *Maillotia* Theobald

The palps of the females are much shorter than the proboscis. The head has mixed white and dark to golden scales. The scutum and pleurites have many whitish and dark, somewhat narrow scales, sometimes in more distinct patterns. Prealar scales and usually postspiracular scales are present. A white knee spot is present, and the tarsi are uniformly dark scaled. The abdomen may or may not have pale basal bands or lateral patches. The wing veins are covered with long, narrow dark scales. Mohrig (1969) mentioned an important wing characteristic, which separates species of the subgenus *Maillotia* from those of the subgenera *Culex* and *Neoculex*. In *Maillotia*, the ending of the subcosta (Sc) into the costa (C) corresponds roughly with the branching of veins  $R_{2+3}$ . The palps of the males are longer than the proboscis. The gonocoxite has a mesally displaced subapical lobe bearing broad, heavy spines and a flat apical distention which reaches beyond the joint of the gonocoxite and gonostylus. The latter is bent and broad with several setae and one apical spine. The aedeagus is insignificant, the paraproct is broad and crowned with denticles and stout spines. The head of the larvae is usually broader than long. Seta 3-P is nearly as long as setae 1-P and 2-P. The comb scales are arranged in an irregular patch, each individual scale is usually elongated and fringed with numerous thin spines. The siphon is very long and slender. The pecten has widely spaced teeth, and the siphonal tufts (1-S) are arranged in a more or less regular ventral row covering at least 2/3 of the siphonal length, and some distal tufts are laterally displaced. The cratal setae (4-X) are situated close to the anal papillae.

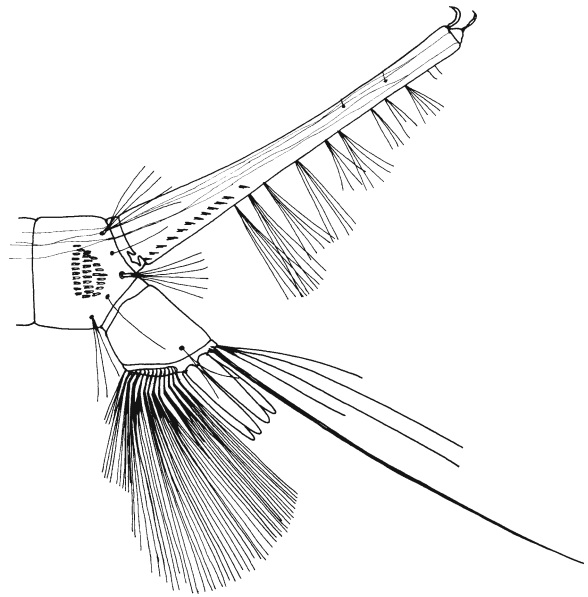
The small subgenus consists of about ten species only. The majority of this is distributed in the Ethiopian region including Madagascar or in westernmost Asia. One species, namely *Cx. hortensis*, is regularly found in the European region with a distribution in the Mediterranean area and central Europe. Another species of *Maillotia*, *Cx. deserticola*, which was transferred from subgenus *Neoculex* (Harbach 1985), has a very limited distribution in Europe. There is one doubtful record from Corsica (Schaffner 1998) and one confirmed record from the Zaragosa Province in Spain (Ramos et al. 1998). The species was found in an area well known as a faunistic refuge regarding other taxa of insects (Eritja et al. 2000). Because of its isolated occurrence in only one location, *Cx. deserticola* is not included in the keys, and no detailed morphological description is given.

#### *Culex (Maillotia) hortensis* Ficalbi 1889

**Female:** The general appearance of the female is similar to *Cx. territans* with greyish unspotted wings but usually paler scaling on the scutum and thorax. The relative broad pale apical bands on the abdominal terga and their distinct median widening at least on some terga differentiate the females from the *Cx. territans* and other *Culex* species in Europe. The proboscis is usually entirely dark scaled, sometimes with scattered pale scales on the ventral surface. The scaling of the palps is variable, often with pale scales at the apex forming a ring on the palpomere V, but sometimes entirely dark scaled. The occiput has light scales, the eyes are bordered with whitish scales, and the vertex is whitish with some dark scales. The scutum has dark setae and is mostly brownish scaled, very often whitish, narrow scales form lateral stripes, and the scutellum always has whitish narrow scales. The mesepisternum and mesepimeron have a few pale scale patches. The coxae have white scales on the ventral surface, and the femora are white scaled, the hind femur has dark scales dorsally. White knee spots are present, and the tibiae and tarsomeres are dark with a few white scales on the ventral surface. The apex of the hind tibia has a white spot which is sometimes difficult to detect. The wing veins are entirely dark scaled, except for the basal 1/5 of the costa which has pale scales. The end of Sc is nearly aligned with the furcations of  $R_{2+3}$  and M (Fig. 6.56a). Terga I–III have broad pale apical bands



**Fig. 10.109** Hypopygium of *Cx. hortensis*



**Fig. 10.110** Larva of *Cx. hortensis*

with a median widening, the rest of the terga have narrow pale apical bands.

**Male:** The palps are almost completely devoid of setae. The lobes of tergum IX are inconspicuous. This species is easily differentiated from all other European members of the genus *Culex* by a broad, flattened, sclerotized process at the apex of the gonocoxite (Fig. 10.109), which extends distinctly beyond the base of the gonostylus. The gonostylus is bent, and the aedeagus has a dorsal and ventral bridge. The paraproct has denticles and several rows of spines.

**Larva:** The head is wider than long, and the antennae are long and slender, apically with extremely long spines. The antennal seta (1-A) has about 10 branches, and the antennal shaft is covered with several short spicules around the insertion point of 1-A. The inner and median frontal setae (5-C and 6-C) have 2 branches, and the outer frontal seta (7-C) has at least 5 branches. The prothoracic seta 3-P is nearly as long as 1-P and 2-P (Fig. 8.66a). The comb scales are arranged in an irregular triangular spot, and each individual scale may be of two shapes, either long, narrow and pointed or shorter and rounded apically. The siphon is long and slender, and the siphonal index is between 6.5 and 8.0, with at least 4–5 pairs of long siphonal tufts (1-S) situated in a more or less ventral row (Fig. 10.110). The number of pecten teeth is 12, and they are widely spaced towards the middle of the

siphon. The ventral brush consists of 12–14 tufts of cratal setae (4-X).

**Biology:** Little is known about the phenology and general biology of *Cx. hortensis* as the species is rather uncommon and seems to occur in large numbers only sporadically. The larvae usually occur in clear water with a certain amount of algae and other vegetation, but also in rice fields, small ponds, unused wells, or garden pots. Hibernation takes place in the female stage; daytime resting sites are dark places, e.g. wooden stables. The females usually do not feed on humans.

**Distribution:** In Europe, *Cx. hortensis* is frequently found in the Mediterranean region. It occurs on the Canary Islands and is distributed through Spain, France, Italy, and Greece up to central Europe, where it is rarer. It can also be found in north Africa, Middle Asia, and India.

### 10.3.4 Subgenus *Neoculex* Dyar

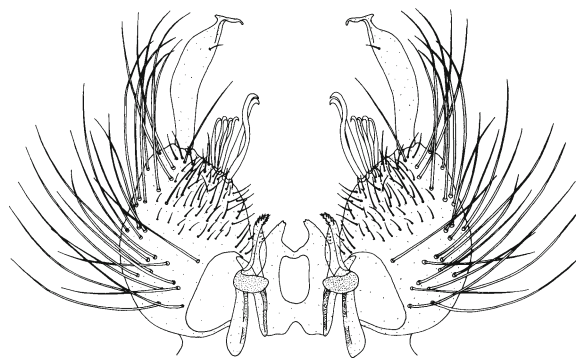
Small to medium sized species, the vertex has erect and narrow scales. The palps are not longer than one quarter of the length of the proboscis, with few scales. The scutum has stout acrostichal and dorsocentral setae, and is covered with narrow uniform scales and bare areas in

between. The pleurites are sparsely scaled, and the coxae are without scales. The tarsi are dark scaled. Pale transverse bands of the terga, if present, are located at the apex of each tergum. The palps of the males are as long as or longer than the proboscis, and the claws of the hind legs have a subbasal tooth. The gonocoxite is covered with more or less dense setae, but typical scales are absent. The subapical lobe has spine-like or hair-like setae, broad transparent scale-like setae are absent, and the gonostylus is simple. The apex of the paraproct is usually adorned with one row of large denticles. The aedeagus is simple, formed by one pair of sclerites connected by 1 or 2 transverse tergal bridges. The thorax and abdomen of the larvae have minute spicules, visible under high magnification (200×) only. The numerous comb scales are slender and elongated, and bordered with thin spines. The siphon is long and slender, sometimes slightly widening at the apex, the siphonal index is usually more than 7.0, and the main tracheal trunks are narrow. The pecten teeth are slender, covering between 1/5 and 1/3 the length of the siphon. The siphonal tufts are situated in an irregular row at the ventral surface of the siphon.

The subgenus *Neoculex* consists of <30 species, mainly distributed in Africa and the Australian region. Three distinct species are reported from the European region. A remarkable fact is that no member of the subgenus *Neoculex* is known to take its blood meal from humans. In nature they were observed to feed exclusively on frogs, small mammals and birds (Mohrig 1969). The egg laying behaviour seems to be typical for the subgenus (Mattingly 1970). The egg rafts are usually not laid directly upon the water surface, but above the water line. The hatching larvae reach the water by wriggling down after emergence.

### ***Culex (Neoculex) impudicus Ficalbi 1890***

**Female:** A small black mosquito, which differs from the similar *Cx. territans* by the apical pale bands on the abdominal terga which are narrowed in the middle, sometimes interrupted and incomplete, forming triangular lateral patches. The pale spot at the apex of the hind tibia, typical for *Cx. hortensis*, is weakly defined or completely absent. The proboscis is dark brown scaled. The occiput has greyish brown setae, a row of white flat scales around the eye margin, and a pair of long brown setae projecting forward between the eyes. The scutum



**Fig. 10.111** Hypopygium of *Cx. impudicus*

is covered with greyish brown or blackish narrow scales. The acrostichal and dorsocentral setae are brown, and long blackish setae are situated at the margins of the scutum and at its posterior part. The integument of the pleurites is brown or ashy grey with patches of pale scales on the antepronotum, propleuron, mesepisternum and mesepimeron, and one lower mesepimeral seta is present. The coxae have patches of pale scales, and the femora are dark scaled on the dorsal surface, with pale scales ventrally, and an indistinct knee spot. The hind tibia is dark at the apex or with only a few pale scales, and all the tarsi have dark scales. The wings are entirely dark scaled. The terga are dark scaled with median narrowed or interrupted apical bands. The sterna are usually pale scaled, occasionally two basal patches of dark scales may be present on each side of some sterna.

**Male:** The palps are well covered with setae. The gonocoxite is prominently exposed (hence the specific name), compact in appearance, slightly longer than broad at its base and covered with numerous long and conspicuous setae on its outer surface (Fig. 10.111). The densely setose gonocoxite is unique for this species and makes identification of males of *Cx. impudicus* an easy matter. The subapical lobe is situated beyond the middle of the gonocoxite with two long pointed setae which are recurved at their apices, one of which has a more or less sigmoid form. Additionally, the lobe bears several smaller, pointed and apically recurved setae and numerous short, thin, straight setae. The gonostylus is constricted subapically, then extends into a “T”-shaped apical part, a feature unique among European *Culex* members. The apex of the paraproct is inwardly curved with a row of several mesally directed denticles and several spines. The aedeagus is made up of two simple plates connected by 2 transverse bridges

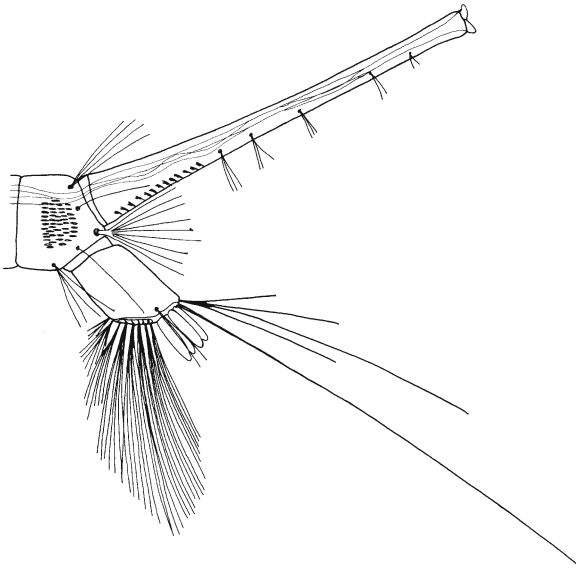


Fig. 10.112 Larva of *Cx. impudicus*

at the base and close to the apex, with 3-4 small denticles at their apices.

**Larva:** The larvae are extremely difficult to distinguish from those of *Cx. territans*. Both species closely resemble each other in chaetotaxy and other characteristics. As indicated in the keys, *Cx. impudicus* usually has anal papillae blunt ended and about half as long as the saddle, subequal in length (Fig. 10.112). On the other hand, in *Cx. territans* larvae the anal papillae are pointed, usually as long as the saddle, and the ventral pair may be slightly shorter than the dorsal. Unfortunately, the length of the papillae may vary between specimens, and other distinct characteristics to distinguish between the two species do not exist.

**Biology:** The larvae can be found in fresh and stagnant water, generally with considerable vegetation and in well-shaded places (Aitken 1954a). They occur in pools, small ponds, rock pools, ditches, marshes or along the edges of streams. Occasionally, they are found in rice fields (Rioux 1958). The larvae are often associated with those of *An. maculipennis s.l.*, *An. claviger*, and *Cs. annulata*. In northern Africa, they frequently occur together with larvae of *An. algeriensis*, *Cx. hortensis*, *Cx. p. pipiens*, or *Cs. longiareolata* (Sicart 1940; Senevet and Andarelli 1959). Hibernation seems to take place in the adult stage. The larvae are most abundant between May and August; the population peak of the adults is usually reached in late summer. Like the other European members of the subgenus

*Neoculex*, the females of *Cx. impudicus* prefer to feed on amphibians and are not known to feed on humans.

**Distribution:** *Cx. impudicus* is mainly distributed in the Mediterranean region. It is a common species in southern and central Portugal (Ribeiro et al. 1988) and reported from Spain, France, Italy, Corsica, Sardinia, Sicily, and Greece. In northern Africa it can be found in Algeria and Tunisia and it is recorded from Iran (Knight and Stone 1977).

### ***Culex (Neoculex) martinii* Medschid 1930**

**Female:** A small mosquito, which differs from the other members of the subgenus *Neoculex* and those of the subgenus *Culex* by the abdominal terga, which are dorsally covered with uniform reddish brown scales, and pale apical or basal transverse bands are absent. The general abdominal colouration of *Cx. martinii* is similar to *Cx. modestus*, but it is distinguished from the latter species by the longer hind tarsomere I and by the wing venation. In *Cx. modestus* the subcosta (Sc) ends distinctly distal to the level of the cross vein (r-m), whereas in *Cx. martinii* Sc ends more or less at the level of r-m. The proboscis is dark scaled, slightly swollen at the apex, the palps are very short with dark scales, and the clypeus is brownish. The vertex and occiput have small brownish scales, with patches of broader pale scales at the sides. The integument of the

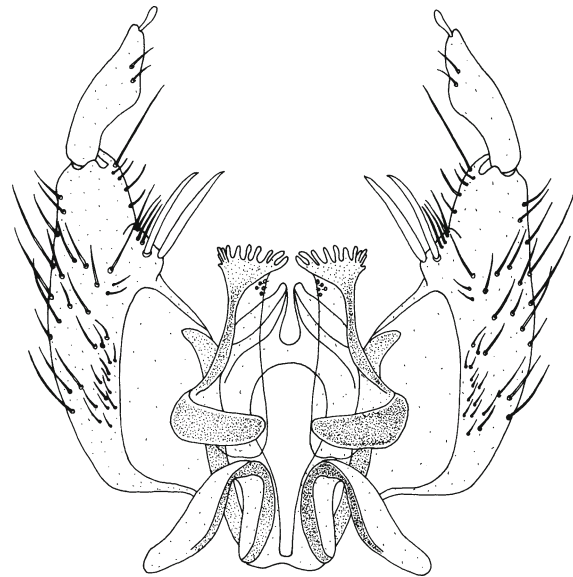


Fig. 10.113 Hypopygium of *Cx. martinii*



scutum is yellowish, and covered with small, golden brown scales and long brownish setae. The integument of the pleurites is brownish-yellowish with dark setae. The femora have brownish scales, slightly lighter on the ventral surface, and a knee spot is absent. The tibiae and tarsi are entirely dark scaled, and the tarsi are without pale rings. The wings have dark scales on all veins. The abdominal terga are reddish brown, without pale transverse bands, and the sterna are entirely yellowish pale scaled.

**Male:** The palps are dark, and longer than the proboscis by about the length of palpomere V, and not swollen apically. The gonocoxite is short and stout (Fig. 10.113), slightly more than twice as long as it is wide, and the subapical lobe is situated well beyond the middle of the gonocoxite, with 2 moderately long, stout spines and a row of several stronger setae located distal to the spines. The gonostylus is short and broad, distinctly widened beyond the middle, with 2 small setae situated close together at about  $2/3$  of its length. The apical spine of the gonostylus is relatively long, and blunt ended. The apex of the paraprocit is widened with a convex row of apical denticles and several small setae inserted subapically. The aedeagus is divided into two simple plates connected by apical and basal transverse bridges.

**Larva:** The head is distinctly broader than long. The antenna is about  $3/4$  the length of the head, slightly curved in the basal part and entirely covered with spicules. The antennal seta (1-A) has 22–26 branches. The inner frontal seta (5-C) is 2-branched, the median frontals (6-C) are exceptionally long, reaching close to the apex of the antenna, with 1–2 branches, and the outer frontal seta (7-C) has about 5 branches. The comb is composed of a patch of 35–40 elongated scales, each scale is apically rounded and fringed with thin spines. The siphon is long and slender, evenly tapered towards the apex, the siphonal index is 7.5–11.0, and the main tracheal trunks are narrow (Fig. 10.114). The pecten has 11–16 teeth occupying the basal  $1/5$  of the siphon, and each pecten tooth has 3–4 short lateral denticles. 4–6 pairs of siphonal tufts (1-S) are situated in an irregular row at the ventral surface of the siphon, 1–2 apical tufts are laterally displaced, and usually the basalmost tuft (1a-S) is inserted beyond the last pecten tooth. Each tuft has 2–5 branches, slightly longer than the width of the siphon at the point of insertion, and the apical tufts are shorter. The anal segment is elongated, and entirely surrounded by the saddle. The saddle seta

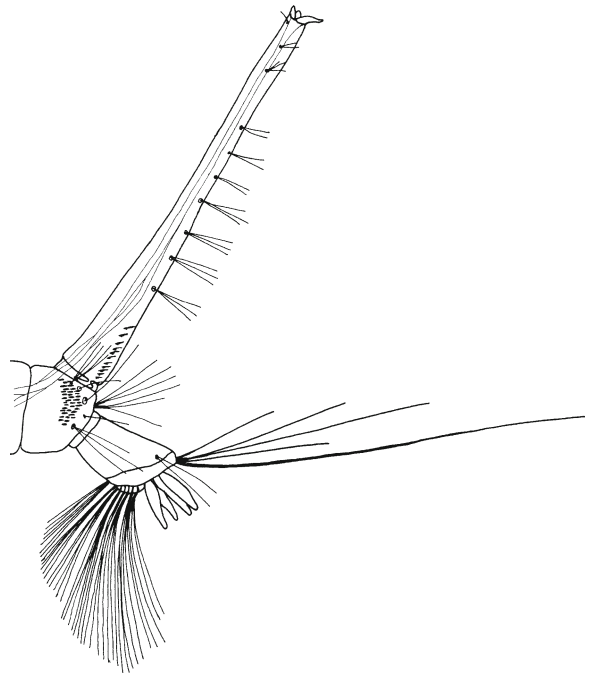


Fig. 10.114 Larva of *Cx. martinii*

(1-X) has 2 branches, the upper anal seta (2-X) has 4 branches, and the lower anal seta (3-X) is single. The ventral brush is composed of 11–12 tufts of cratal seta (4-X), and the anal papillae are about half as long as the saddle.

**Biology:** *Cx. martinii* is not a very common species, thus the information about its biology is very limited. In Germany, a considerable number of larvae were found once in a boggy alder forest in October (Mohrig 1969). It is not known if this is the preferred breeding site of the species or whether it breeds in a wide variety of habitats. Nothing is known about the number of generations per year in Europe, the hibernation principles, the time of appearance of the adults, their period of main flight activity or host-seeking behaviour. It seems likely that the species does not feed on humans but prefers to bite amphibians and/or birds, like the other European members of the subgenus *Neoculex*.

**Distribution:** *Cx. martinii* is mainly distributed in the eastern Mediterranean region, Asia Minor, and middle Asia. It is reported from Italy, former Yugoslavia, Croatia, Hungary, Turkey, Germany, and in northern Africa from Morocco.

***Culex (Neoculex) territans* Walker 1856**

**Female:** The proboscis and palps are dark scaled, and the proboscis is slightly swollen at the apex. The clypeus and pedicel are dark brown, the first flagellomere is slightly swollen, and the flagellum is dark brown with black setae. The occiput has narrow curved pale to golden scales and brown erect forked scales, and broad whitish scales laterally. The integument of the scutum is usually light brown, sometimes darker, and covered with narrow light brown scales, paler scales on the anterior and lateral margins of the scutum and the prescutellar space. Dark setae at the margin of the scutum are relatively long. The scutellum is brown with greyish narrow scales and long black setae. The antepnotum is densely covered with pale scales. The pleurites are dark brown or greyish with patches of broad whitish scales. The femora are dark scaled on the anterior surface, and pale posteriorly, with small pale knee spots. The tibiae are dark anteriorly and pale on the ventral surface, and the apex of the hind tibia is without a pale spot. The tarsi are entirely dark scaled, although a pale stripe may be present on tarsomere I. The wings are entirely covered with narrow dark scales, and the cross veins are well separated. The end of Sc is distinctly displaced towards the wing base compared to the furcations of  $R_{2+3}$  and M (Fig. 6.56b). Tergum I has a median patch of pale scales, and the

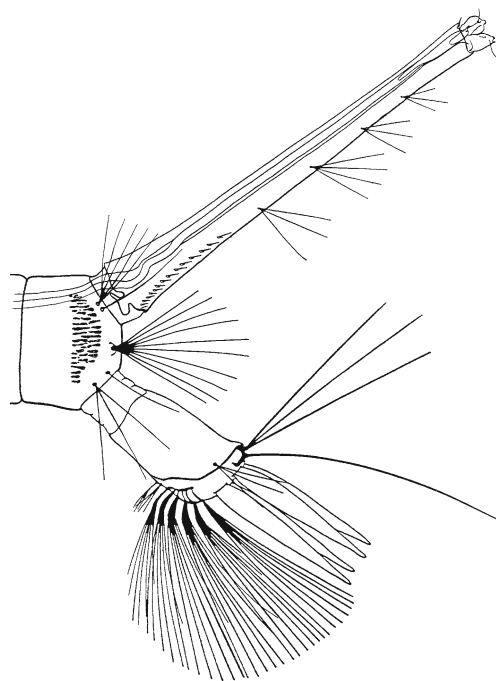
remaining terga are dark brown scaled with evenly narrow apical bands of pale scales usually joining a small pale triangular patch on each side. Tergum VIII is almost entirely dark. The sterna have pale scales often with a greenish tinge.

**Male:** The last two palpomeres have long setae. The subapical lobe of the gonocoxite has 2 basalmost setae which are long and broad, flattened, slightly sinuous and with recurved apices (Fig. 10.115). A few other setae are located distally, similar in appearance, but smaller, and additionally 1 or 2 long and hair-like setae are present. The gonostylus usually tapers evenly from the base towards the apex. The apical spine of the gonostylus is somewhat expanded at the tip. The paraproct is without ventral arms, and the paraproct crown has an inwardly curved row of denticles. The aedeagus consists of two simple plates which are connected by transverse bridges at the base and close to the apex; the plates are ornamented with small denticles at their apices.

**Larva:** The head is distinctly broader than long, the antennae are about as long as the head, inwardly curved, densely covered with spicules from the base to the insertion point of the antennal seta (1-A), and distinctly narrowed from the insertion point to the



**Fig. 10.115** Hypopygium of *Cx. territans*



**Fig. 10.116** Larva of *Cx. territans*

apex. Seta 1-A has 25–32 branches, and is situated at about 2/3 the length of the antennal shaft. The frontal setae are long, the inner (5-C) usually has 2 branches, the median (6-C) is usually single and situated nearly in front of the inner setae, and the outer frontal seta (7-C) has 8–9 branches. The comb has 50 or more, apically rounded scales which are completely fringed (Fig. 8.70b). The siphon is long and slender, with a siphonal index of 6.0–7.0, tapering to near the apex, where it slightly but distinctly expands, and the main tracheal trunks are narrow (Fig. 10.116). The pecten has 12–16 teeth occupying the basal ¼ to 1/3 of the siphon, each pecten tooth with 1 or 2 rather stout lateral denticles on their ventral margin. Seta 1-S consists of 4–6 pairs of tufts, usually commencing at the point where the pecten ends, and rarely the basalmost tuft may arise slightly within the pecten. Each tuft has 2–4 branches of variable length, usually twice as long as the width of the siphon at the point of its origin. The distalmost tuft is shorter and arises slightly out of line laterally. The saddle completely encircles the anal segment and is spiculate on the dorso-apical part, the saddle seta (1-X) is usually 2-branched, the upper anal seta (2-X) has about 4 branches, and the lower anal seta (3-X) is very long and is single. The ventral brush has about 7 pairs of multiple-branched cratal setae (4-X). The anal papillae are pointed, and are usually as long as the saddle, the ventral pair may be slightly shorter than the dorsal pair.

**Biology:** In the northern regions, *Cx. territans* has probably only one generation per year, but in the southern parts of its distribution range, it is polycyclic, as is typical for other *Culex* species. Preferred larval habitats are permanent bodies of water such as ponds, swamps, pools along streams, edges of lakes, or drainage channels with a slow water-flow, often associated with dense vegetation. The larvae prefer cooler water in shaded situations (Mohrig 1969) and are often found together with those of *An. maculipennis s.l.* and *An. claviger*. Gutsevich et al. (1974) reported about larval habitats being in strong sunshine in temperate latitudes and completely shaded in southern regions. The larvae are rarely found in heavily polluted water. In the Nearctic they also occur in artificial containers and other small bodies of water (Wood et al. 1979). Adult females reappear from hibernation in early spring and the first larvae can be found from the end of April or early May, until September, the pop-

ulation maximum is reached in late summer. The females are not known to bite humans, but predominantly feed on amphibians, especially *Rana* sp., reptiles and birds.

**Distribution:** *Cx. territans* is widely distributed throughout Europe. Its range stretches into central Asia and northern Africa. In the Nearctic region it is found in Canada and the United States including Alaska.

**Note on systematics:** *Cx. territans* was formerly synonymized with *Cx. apicalis* Adams, which was originally described from Arizona, United States. After a revision of the subgenus *Neoculex* in the United States, Bohart (1948) gave evidence that they are two distinct species with a distribution of *Cx. apicalis* in the southern parts of the United States and that of *Cx. territans* in the northern states and Alaska. The European species were first identified as *Cx. apicalis*, but it was shown by Mattingly (1953) and others that they were identical with *Cx. territans*. Thus, all European records in the literature of *Cx. apicalis* prior to 1950 actually referred to *Cx. territans*.

## 10.4 Genus *Culiseta* Felt

The genus was formerly known under the name *Theobaldia* Neveu-Lemaire 1902 but later on it was realized that this name was already being used by a genus of molluscs since 1885. According to the rules of nomenclature the name *Theobaldia* was consequently replaced by the name *Culiseta* Felt.

The genus embraces mainly medium-sized to large, dark mosquitoes. The females have a straight proboscis and short palps. Prespiracular setae are present, usually of pale colour, but postspiracular setae are absent. The prealar area has setae, but is usually found without scales. The base of the radius (R) has a few setae which are more numerous on the ventral surface of the wing. The abdomen is blunt ended, the cerci are short and rounded apically, the claws are simple, without a subbasal tooth, and pulvilli are absent. In males the length of the palps may vary in individuals of the different subgenera. The gonocoxite is rather long, a basal lobe is present, but an apical lobe may be present or absent. The gonostylus is simple, the apical spine is not longer than its maximum width (except in *Cs. glaphyroptera*), and

claspettes are absent. The larvae are large to very large with the head being wider than long. The comb scales are numerous and blunt ended, and the siphonal tuft (1-S) is always present, inserted near the base of the siphon, and the pecten is present. The anal segment is completely surrounded by the saddle (except in *Cs. longiareolata*), which is pierced by one or more tufts of precratal setae (4-X).

The larvae of the genus are generally found in semi-permanent and permanent pools, rarely in other locations. As regards the feeding behaviour of the adult females, some species are known to feed exclusively on birds, but others, especially those of the subgenus *Culiseta*, readily attack humans and other mammals and are known to be severe biters.

The distribution of the genus *Culiseta* is almost world wide, but largely confined to the more temperate zones of the Holarctic region. It is a relatively small genus including approximately 40 valid species and subspecies, which are spread over seven subgenera. Throughout the European region, 10 species of three subgenera, *Allotheobaldia*, *Culicella*, and *Culiseta*, are recorded.

In the European members of the genus *Culiseta*, both adult and larval morphological characteristics can be found to distinguish the three subgenera. Such an extent of congruity in larval and adult subgeneric characteristics cannot be found in any other European genus of the Culicidae. Furthermore, the subgenera *Culiseta* and *Culicella* exhibit striking differences regarding their larval and adult behaviour, which will be described in more detail in the subgeneric sections.

#### 10.4.1 Subgenus *Allotheobaldia* Broelemann

The females have a conspicuous colouration pattern on the scutum, and the palps of the males are shorter than the proboscis, and distinctly swollen at the apex. The gonocoxite is without an apical lobe, and tergum IX has two prominent lateral lobes. The head of the larva is small with mouthparts adapted to feed on the substrate. The antennae are short, with a weakly developed antennal seta (1-A). The siphon is short and not sclerotized at the base, and the saddle is weakly developed and plate shaped. The females deposit the eggs in boat-

shaped rafts on the water surface, similar to the species of the genus *Culex*.

The subgenus *Allotheobaldia* is represented by only one species, *Cs. longiareolata*, which is distributed in the southern Palaearctic region.

#### *Culiseta (Allotheobaldia) longiareolata* (Macquart 1838)

**Female:** *Cs. longiareolata* can easily be distinguished from all other European species of the genus *Culiseta* by its distinct longitudinal pale stripes on the scutum, which resemble a lyre in shape and the femora and tibiae with pale scales aggregated into conspicuous spots or stripes. The proboscis is blackish brown, the palps are dark brown with pale scales, the latter predominating on the dorsal part. The tips of the palps are almost entirely pale. The antennae are blackish brown, and the pedicel and first two flagellomeres have white scales. The head has dense white scaling along the margins of the eyes, broad white scales also in the median line of the vertex and on the lateral parts of the occiput. The scutum has light brown, narrow scales. A narrow acrostichal stripe of pale scales extends from the anterior margin to the scutellum. In addition, there are narrow dorsocentral and lateral stripes, which are connected over the transverse suture. The scutellum and pleurae have patches of white scales except on the upper part of the postpronotum, where the scales are of a creamy yellowish colour. The legs are blackish brown with pale spots and longitudinal stripes on the femora, tibiae and tarsomeres I. All the tarsi have pale basal bands on tarsomeres I–III, and tarsomere V is usually entirely dark. The wing veins are covered with dark scales except the costa (C), which is covered with pale scales along its entire anterior surface. Dark scales are aggregated at the base of  $R_5$ , cross veins (r-m and m-cu) and the furcations of M and Cu giving an appearance of spots. The cross veins are well separated. The scales of the terga vary in colour, but usually form broad white basal bands. A mixture of yellowish creamy and brown scales is more frequently found on the last terga. Tergum VIII is usually entirely white scaled.

**Male** (Fig. 10.117): The main characteristic that distinguishes *Cs. longiareolata* from all other European members of *Culiseta* is the conspicuous tergum IX, which is expanded laterally into two long and slender, sclerotized lobes bearing tiny spine-like setae at their



Fig. 10.117 Hypopygium of *Cs. longiareolata*

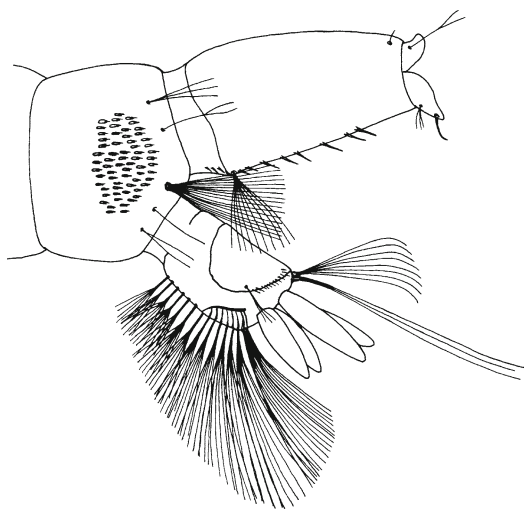


Fig. 10.118 Larva of *Cs. longiareolata*

apices (Fig. 7.69a). The gonostylus is broadened apically, bluntly ending with two short, pointed subapical spines. The aedeagus is thick and strongly sclerotized.

**Larva:** The antennae are short, and the antennal seta (1-A) is articulated in the apical third of the antenna, is short and usually 2-branched, rarely with 1 or 3 branches. The head has single inner and median frontal setae (5-C and 6-C), rarely 2-branched, and

outer frontal setae (7-C) with 3–4 branches (Fig. 8.79a). The number of comb scales shows great variation (40–75), and seta 3-VIII is strongly developed with multiple branches (Fig. 10.118). The siphon is more or less conical in shape, with an index between 1.5 and 2.0. The pecten has 7–13 short teeth arranged in an irregular row and occupying up to 80% of the length of the siphon. The basalmost teeth are smaller and inserted in the unsclerotized basal part of the siphon. The siphonal tuft (1-S) is shorter than seta 3-VIII, with 10–15 branches arising close to the margin of the sclerotized part of the siphon. The anal segment has a plate shaped saddle extending along about half of its lateral sides. The saddle has dense short spines at its posterior margin, the saddle seta (1-X) is short, not more than half the length of the saddle. The length of the anal papillae varies according to the salinity of the breeding water, but is 0.5–1.5 times as long as the anal segment.

**Biology:** The larvae can be found in rock holes and in any kind of artificial container, e.g. wooden and metal barrels, or tanks built of concrete, and wells. Rarely do they occur in natural water bodies like pools, ditches, and drain canals. The larvae are able to tolerate a slight salinity and a high degree of pollution and are often likely to be found together with those of *Cx. p. pipiens* and *Cx. mimeticus*. At temperatures of 20–25°C the larval development lasts about 20–22 days. The larvae spend most of their time at the surface of the breeding site and rarely descend to the bottom. The pupae of *Cs. longiareolata* are able to lie passively on the bottom of their breeding sites for a length of time (Peus 1954). Hibernation takes place in the larval stage. In the temperate climatic zones adults can be found from February to November. The females of *Cs. longiareolata* do not enter dwellings and rarely bite humans outside but are regarded as vectors of blood parasites in birds. In populations from Turkmenia, autogenous egg deposition has been observed (Rioux et al. 1975).

**Distribution:** In Europe, *Cs. longiareolata* is widely distributed in the Mediterranean region from Spain and Portugal in the west to the European part of Turkey in the east, in France as far north as Paris, and in Switzerland and southern England. It has also been recorded from the Canary Islands, Madeira, and the Azores. This species can be found in southern Ukraine and the lower Volga area as far as the northern slopes of Caucasus. Outside Europe the distribution stretches

from middle and southwest Asia, to India and Pakistan and middle Africa.

#### 10.4.2 Subgenus *Culicella* Felt

The wings are usually without spots, except in *Cs. ochroptera* which may have an indistinct dark spot at the base of  $R_{4+5}$ . The cross veins (r-m and m-cu) are well separated, the distance between them being at least the length of m-cu (Fig. 6.57a). The indistinct tarsal pale ring shows the tendency to include both the basal and apical parts of the tarsomeres. The palps of the males are as long as or longer than the proboscis, the gonocoxite is without an apical lobe, and the aedeagus is weakly sclerotized. The antennae of the larvae are longer than the head, with antennal seta (1-A) arising from a point near to the apex, well developed and multiple branched in the form of a broad fan. The mouthparts are adapted for suspension feeding. The siphon is long and slender, with an index of  $>5.0$ . The pecten consists of a few small inconspicuous teeth at the base of the siphon, except for *Cs. fumipennis*, which has in addition to the pecten teeth several stout, spine-like setae on the ventrolateral surface of the siphon. The external morphology of the larvae is adapted to their behaviour as suspension feeders. Usually they are found breathing at the water surface, but they can spend hours submerged. They are sometimes attached to active respiratory parts of plants from which they may take air bubbles. Others lie on the ground of the breeding sites with their backs towards the bottom, producing a flow of water with their mouthparts and filtering microorganisms. The species of the subgenus hibernate in the larval or egg stage. The eggs are deposited singly on the ground above the residual water level, as species of the genera *Aedes* and *Ochlerotatus* usually do. In the European region the subgenus *Culicella* consists of four species.

#### *Culiseta (Culicella) fumipennis* (Stephens 1825)

**Female:** *Cs. fumipennis* is a large mosquito with a dark brown scutum and unspotted wings. The abdominal terga are dark brown scaled with basal, yellowish white bands of uniform width. The legs are largely dark with narrow pale rings at the bases of the tarsomeres.

It closely resembles *Cs. litorea* and *Cs. morsitans* but differs from the latter in having a mainly dark proboscis with pale scales laterally and ventrally in its middle third and the dark scales on most of the abdominal sterna are usually aggregated to form an inverted "V" (Fig. 6.58a,b). In *Cs. morsitans* the proboscis is usually entirely dark scaled and the dark and pale scales on the abdominal sterna are intermixed. However, these two characteristics show some variation, e.g. the proboscis of *Cs. morsitans* with a few scattered pale scales or sometimes the abdominal scale colouration patterns of *Cs. fumipennis* are very indistinct. The most reliable diagnostic characteristics are the narrow pale basal rings on all tarsomeres of the hind legs of *Cs. fumipennis*. In *Cs. morsitans* tarsomeres IV and V of the hind legs are entirely dark. There are no other distinct characteristics to distinguish females of these two species. The differences between *Cs. fumipennis* and *Cs. litorea* may be found on the fore legs. In *Cs. fumipennis* the pale rings include the apical and basal parts between tarsomeres III–IV and IV–V (Fig. 6.59a), and in *Cs. litorea* the apical parts of tarsomeres III and IV are entirely dark scaled (Fig. 6.59b).



Fig. 10.119 Hypopygium of *Cs. fumipennis*

**Male** (Fig. 10.119): The gonocoxite is slender and conical. The basal lobe has 3–4 strong setae. The aedeagus is weakly sclerotized, with lateral sclerites converging apically. The male genitalia of *Cs. fumipennis* and *Cs. morsitans* can be separated with certainty only when directly compared to each other. In *Cs. fumipennis* the gonostylus is much slender and abruptly constricted shortly beyond the base, the ratio of its median width to its entire length is about 1:16. In *Cs. morsitans* the gonostylus is stout, and more gradually tapered towards the apex and the ratio of the median width to the entire length is about 1:12. The median lobe of tergum VIII bears up to 3 stout spine-like setae in *Cs. fumipennis*, and 3–7 strongly sclerotized longer setae in *Cs. morsitans*. These two characteristics combined should enable one to distinguish between the two species with a certain confidence.

**Larva:** In the larval stage *Cs. fumipennis* is easy to distinguish from all other European members of the subgenus *Culicella* by its siphon (Fig. 10.120). It bears, in addition to the pecten teeth, large isolated spine-like setae irregularly scattered on its ventrolateral surface. The most distal one extends well beyond the middle of the siphon. The antennae are longer than the head, the

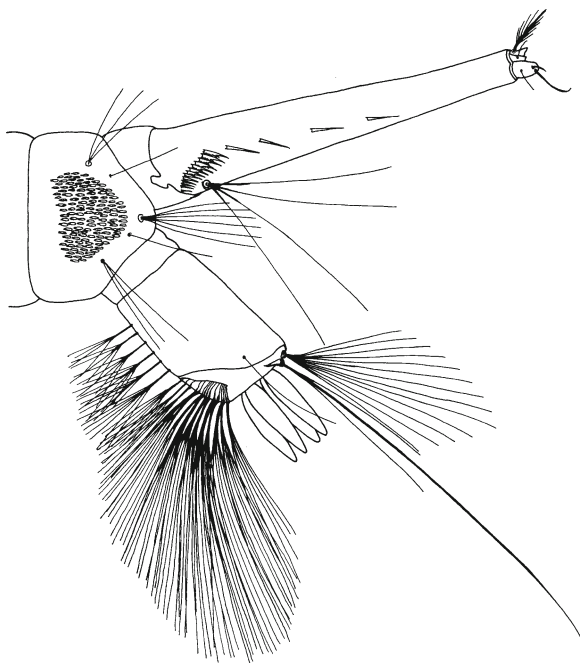


Fig. 10.120 Larva of *Cs. fumipennis*

antennal seta (1-A) is large and multiple-branched. The inner (5-C) frontal seta is 2–4 branched, the median (6-C) is 2-branched and the outer (7-C) has 5–6 branches. The comb consists of 120–160 long and slender scales fringed with small, fine spines. Seta 3-VIII has 4–6 long branches. The siphonal tuft (1-S) consists of 4–5 branches and is distinctly longer than half of the siphon. A pair of conspicuous long, multiple-branched tufts is situated dorsally near the tip of the siphon. Seta 9-S on the posterolateral valves is strongly developed, and hook shaped. The anal segment is long, completely surrounded by the saddle with 6 tufts of precratal setae (4-X) piercing through it, and the saddle seta (1-X) is single. The anal papillae are lanceolate, and about half as long as the saddle.

**Biology:** In Sardinia, larval catches were recorded from January through April (Marchi and Munstermann 1987), but in the central and more northern parts of Europe, the larvae of *Cs. fumipennis* first occur in spring during the months of April and May. The preferred breeding sites are open, unshaded water bodies such as shallow temporary pools with rich vegetation or covered with duckweed (*Lemna* sp.). The larvae also occur among the grassy margins of permanent ponds or swamps. They are often associated with larvae of *Cs. morsitans*, *Cx. territans* and *Cx. hortensis*, occasionally with those of *An. claviger*. They feed on microorganisms and spend most of their time submerged. Very rarely the larvae can be found in water with a high salinity (Martini 1931). Little is known about the feeding habits of the adult females. They were never observed to enter any kind of dwellings or bite humans and domestic animals. It is likely that they do not feed on mammals, but take their blood meals from birds or reptiles, like *Cs. morsitans*. Although adult females were captured in the middle of the year, in July and August, it is not known if *Cs. fumipennis* has more than one generation per year.

**Distribution:** *Cs. fumipennis* is a Holarctic species, widely distributed throughout the whole of Europe, from southern Scandinavia to the east Baltic and southwards to the Ukraine and northern Caucasus. It is recorded from nearly every country in central and southern Europe and occurs around the Mediterranean basin to northern Africa.

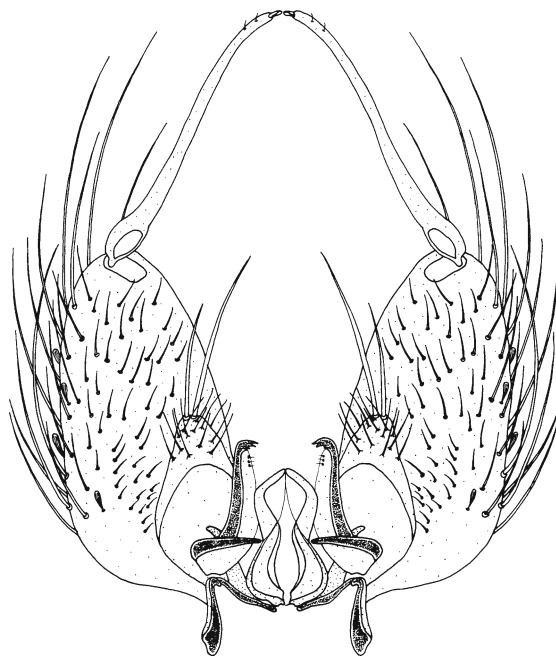
**Notes on systematics:** *Cs. setivalva* was formerly regarded as a valid species (Knight and Stone 1977) but was put under synonymy of *Cs. fumipennis* (Danilov 1984; Ward 1992).

***Culiseta (Culicella) litorea* (Shute 1928)**

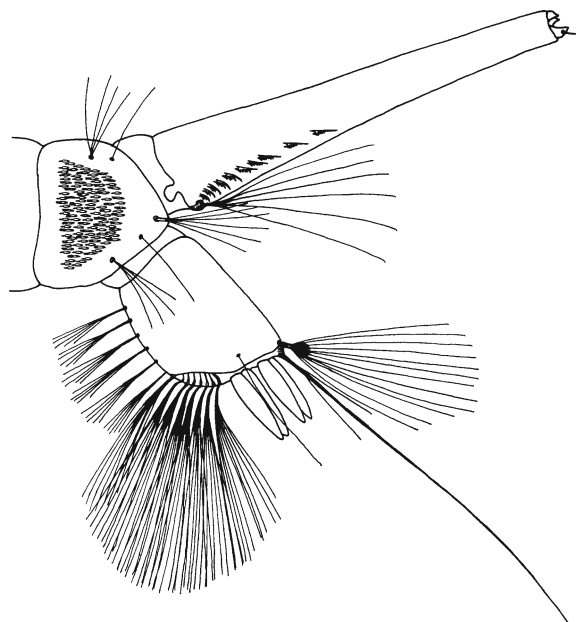
**Female:** *Cs. litorea* was first described as a variety of *Cs. morsitans* (Shute 1928), but subsequently raised to a specific rank, mainly because of striking differences in the male hypopygium (Marshall and Staley 1933). The females, like those of *Cs. fumipennis*, have largely pale scaled sterna with a pattern of dark scales forming an inverted “V”, but the pattern is not always visible, especially in pinned specimens. In this case *Cs. litorea* might be easily confused with *Cs. morsitans*. The separation between *Cs. litorea* and *Cs. fumipennis* is far more difficult. According to Marshall (1938) the pale rings on the last two tarsal joints of the fore and mid legs are less distinct in *Cs. litorea* than in *Cs. fumipennis*, and on the hind legs of *Cs. litorea* the rings are either inconspicuous or absent. Because none of these differences are absolute, it might be impossible to identify *Cs. litorea* females with certainty, especially if the determination is based on a single or a few specimens.

**Male:** The male genitalia are very distinctive and the only reliable means to distinguish *Cs. litorea* from its close relatives. The gonocoxite is more or less twice as long as it is wide, parallel sided and blunt ended (Fig. 10.121). The basal lobe is elongated and bears 2 stout setae, one of which reaches as far as or beyond the apex of the gonocoxite. In *Cs. fumipennis* and *Cs. morsitans* the gonocoxite is more slender and conical, tapering towards the apex and none of the conspicuous stout setae of the basal lobe reaches the apex of the gonocoxite. Furthermore, the male palps of *Cs. litorea* are usually shorter than the palps of the two other species. Marshall and Staley (1933) found the ratio of the length of the palps to the length of the proboscis to be about 1.14–1.22 in *Cs. litorea* and about 1.33–1.40 in *Cs. morsitans*, but Service (1970b) reported an overlap of this character in the two species, which is therefore not always reliable for a positive identification. Marshall (1938) mentioned a further diagnostic character. The base of the gonostylus appears to be distinctly more bulbous in *Cs. litorea* than in the two other species.

**Larva** (Fig. 10.122): The larvae of *Cs. litorea* are readily distinguished from those of *Cs. fumipennis* by having no isolated spine-like setae in addition to the pecten teeth, but the separation from *Cs. morsitans* is less reliable. The only characteristic which might allow identification of fourth-instar larvae of the two species



**Fig. 10.121** Hypopygium of *Cs. litorea*



**Fig. 10.122** Larva of *Cs. litorea*

can be found in the length of the siphonal tuft (1-S) compared to the length of the siphon. In British populations Marshall (1938) found in *Cs. morsitans* the



length of 1-S usually less than, and in *Cs. litorea* more than, 0.4 times the length of the siphon. However, Aitken (1954a) found larvae of *Cs. litorea* from Sardinia with the length of 1-S being less than 0.4 times the length of the siphon, and Rioux (1958) was unable to distinguish larvae of the two species from the Mediterranean area by this characteristic. There is a considerable overlap in the above mentioned ratios between the two species, which invalidates reliable larval identification (Service 1970b).

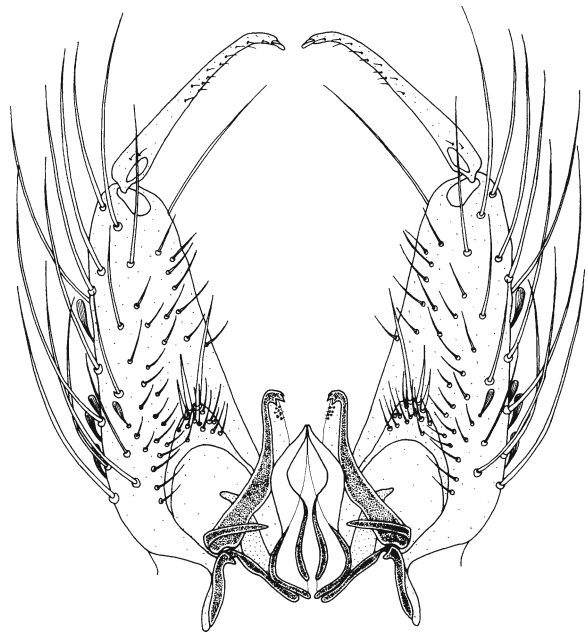
**Biology:** The biology of *Cs. litorea* closely resembles that of *Cs. morsitans* in many aspects. Larval habitats are pools, small ponds or ditches, but unlike *Cs. morsitans*, which can commonly be found in both open (sunlit) and densely shaded situations, the larvae of *Cs. litorea* are always restricted to habitats of the first type. It is mainly a coastal species that can tolerate slightly brackish water, but larvae are not restricted to salt water habitats, where they can often be found together with those of *Oc. detritus*. Frequently they can be found in semipermanent fresh water habitats or channels with a rich growth of vertical vegetation (Rioux 1958). Aitken (1954a) found larvae in a large coastal fresh water marshes, containing abundant growth of cattails (*Typha* sp.). *Cs. litorea* feeds principally on avian blood, occasionally a few females were found to bite reptiles or mammals, including humans (Cranston et al. 1987). Hibernation takes place in the larval stage and they are able, like *Cs. morsitans*, to survive under a cover of ice during the winter months (Rioux 1958).

**Distribution:** *Cs. litorea* is known from Ireland, England, France, Spain, and Italy (Sardinia) in Europe and from Algeria. It has a more restricted distribution than *Cs. morsitans*.

#### ***Culiseta (Culicella) morsitans* (Theobald 1901)**

**Female:** The general appearance of *Cs. morsitans* is in many respects very similar to *Cs. litorea* and *Cs. fumipennis* in all stages. The few characteristics which might facilitate distinction between the three relatives are given in the descriptions of the two latter species. *Cs. morsitans* is a rather large species. The proboscis is uniformly dark scaled, occasionally with a few pale scales in its middle third, and slightly swollen in its apical part (Fig. 6.58c). The clypeus is dark brown, the palps are about one quarter of the

length of the proboscis, and are mainly dark scaled with a few pale scales at the tip. The pedicel is brown, and the flagellum of the antennae is blackish brown with black setae. The vertex has long dark setae, and the occiput has narrow yellowish-white scales and dark erect forked scales dorsally and broad yellowish-white scales laterally. The integument of the scutum is dark brown, with narrow brown and yellowish-golden scales. There is golden scaling mainly on the acrostichal and dorsocentral stripes, on either side of the prescutellar area, on the anterior part of the supraalar area and on the anterior submedian area. The scutellum is dark brown with patches of narrow yellowish-white scales and long dark setae on the lobes. The mesepisternum, mesepimeron and lower part of the postpronotum have small patches of whitish scales. The prespiracular setae are yellowish, numerous, but postspiracular setae are absent. The femora and tibiae are dark brown, with pale scales at the ventral surface. Pale knee spots are well developed, and all tibiae have pale rings at their apices. The tarsi are dark with faint pale rings involving both ends of the joints. Rings at the joints of tarsomeres III–IV and IV–V are inconspicuous or absent on the fore and mid legs and always absent on the hind legs.



**Fig. 10.123** Hypopygium of *Cs. morsitans*

The wing veins are entirely dark scaled, without spots, and the scales are narrow and dark brown. The cross veins r-m and m-cu are widely separated and without scales. The terga are dark brown with narrow yellowish white basal bands. The sterna are mainly covered with pale scales, less numerous dark scales are irregularly scattered, not forming any pattern (Fig. 6.58d).

**Male** (Fig. 10.123): The hypopygium is very similar to that of *Cs. fumipennis*; for differential characteristics see the description of the latter. The posterior margin of tergum VIII has a broadly rounded lobe bearing a group of 3–7 stout, spine-like setae. The lobe of tergum IX is slightly elevated with long hair-like setae (Fig. 7.69c). The gonocoxite is conical, about 2.5 times as long as it is wide at its base, tapering apically, and the basal lobe of the gonocoxite is well developed with 2–4 conspicuously stout setae. The gonostylus is slightly bulbous at the base, gradually tapering towards the apex. The apical spine of the gonostylus is short and stout. The paraprocts are strongly sclerotized, with 2, rarely 3 apical teeth. The aedeagus is weakly sclerotized and pointed at the apex.

**Larva:** Very similar to *Cs. litorea*; to distinguish between the two species in the larval stage see the

description of the latter. The head is exceptionally large in relation to the body, and >1.5 times wider than long. The antennae are as long as or slightly longer than the head, spiculate, and curved with a darkly pigmented, tapering apex. The antennal seta (1-A) is large, forming a fan-like tuft with 18–25 branches, inserted at the upper third or quarter of the antennal shaft and reaching well beyond its tip. The postclypeal seta (4-C) is small and single, and situated anteriorly to the frontal setae. The inner (5-C) and median (6-C) frontal setae have 2–3 branches, 6-C is very long (Fig. 8.85c). The outer frontal seta (7-C) has 6–8 branches. The prothoracic setae (1-P to 7-P) are very long, usually with 1–2 branches. The lateral abdominal setae on segments I and II (6-I and 6-II) have 3–4 branches, those on segments III–VI (6-III to 6-VI) are single. The comb consists of >90 scales closely arranged in a large triangular patch (Fig. 10.124). The individual scales are long and narrow in the middle, slightly widened at the base, with the apical part rounded and fringed with small spines laterally and apically. The siphon is straight, long and slender, and slightly tapered towards the apex. The siphonal index ranges from 5.0 to 7.0, and the pecten consists of 6–11 teeth which are confined to the basal fifth or quarter of the siphon. The smaller basal teeth usually arise from the membrane proximal to the siphon, and the distal teeth are detached. The siphonal tuft (1-S) has 4–5 branches, and is distinctly longer than the basal width of the siphon. The anal segment is long and narrow, and completely ringed by the saddle. The saddle seta (1-X) is single, and slightly shorter than the saddle. The ventral brush consists of 12–15 cratal setae (4-X) and 5–6 precratal setae which pierce the saddle. The anal papillae are variable in length, lanceolate, and the dorsal pair might be slightly shorter than the ventral pair.

**Biology:** *Cs. morsitans* is a monocyclic species. The eggs are deposited during early summer in the moist substrate above the residual water level. Hatching occurs in autumn when heavy rainfall leads to a rise of the water level in the breeding site. Usually the larvae grow to the second or third-instar in the same year. Depending on the weather conditions, fourth-instar larvae can sometimes be found prior to November, but pupation never occurs until the next spring (Marshall 1938). During wintertime the larval development is postponed. The larvae often descend to the bottom of the breeding site,

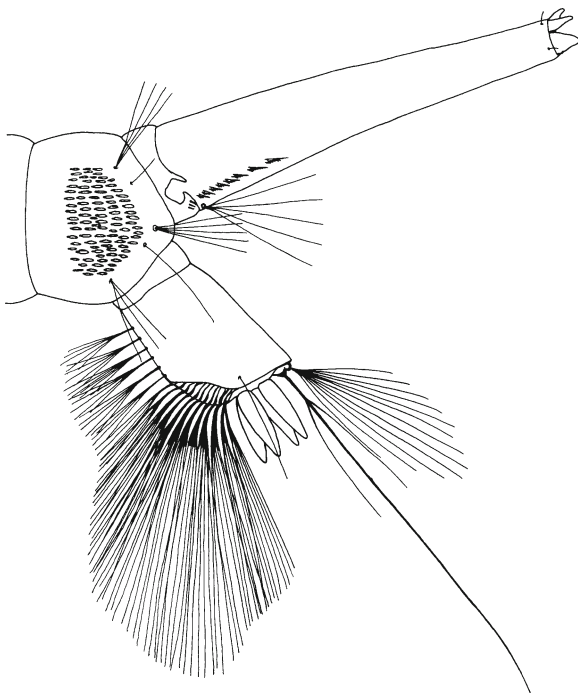


Fig. 10.124 Larva of *Cs. morsitans*

where they lie in an inverted position with their head setae and tip of the siphon in contact with the ground. They are able to survive for considerable periods under a cover of ice, but complete freezing of the breeding site leads to a high mortality. Although hibernation usually takes place in the larval stage, newly hatched first-instar larvae can be observed in early spring. This is in accordance with the situation in the Nearctic region, where *Cs. morsitans* overwinters in the egg stage in most of Canada and first-instars appear from April on (Wood et al. 1979). In Europe, the larvae can be found from autumn to spring/early summer in a variety of breeding sites, including pools, small ponds or ditches and even in slowly flowing waters. They occur predominantly in swampy woodlands and temporary water bodies in forests or at their edges in both, open and shaded situations. They are able to tolerate a considerable amount of salinity and also develop in slightly brackish water. In springtime the larvae can often be found together with those of *Oc. rusticus* and first-instars of *Oc. punctor* and *Oc. communis*, and later in the year they occur together with larvae of *An. claviger*. In Britain they have been found with larvae of *Cs. fumipennis* and *Cs. litorea* (Cranston et al. 1987). The adults emerge from April on and can be observed until October. The females take their blood meal mainly from birds and occasionally from reptiles and small mammals. It is assumed that in central Europe the females attack humans very rarely, if ever, but Horsfall (1955) reported that *Cs. morsitans* was a serious pest in eastern Europe and in the former USSR. The daytime resting sites of the adults are hollows of trees and uninhabited buildings or cellars, but they are rarely found in leafy shrubbery or in grassy vegetation (Service 1971b).

**Distribution:** *Cs. morsitans* is widely distributed throughout the Palaearctic region. It is very common in almost every European country and its range stretches from southern Scandinavia into northern Africa and from the Northern Sea and Atlantic Ocean eastwards to west Siberia and southwest Asia. The form *dyary* (Coquillett) that was formerly regarded as a distinct species, but recently put under synonymy of *morsitans* (Wood et al. 1979; Ward 1984), can be found in the Nearctic region.

**Medical importance:** *Cs. morsitans* was found to be a carrier of Ockelbo virus in Sweden (Francy et al. 1989).

### ***Culiseta (Culicella) ochroptera* (Peus 1935)**

**Female:** Females of *Cs. ochroptera* may be confused with *Cs. morsitans* at first glance, but they are usually smaller in size and more slender than the latter. The most striking differences are the generally more brownish colouration of *Cs. ochroptera* (general colouration of *Cs. morsitans* more greyish) and the tibiae of the fore legs which are predominantly yellowish scaled, whereas the fore tibiae of *Cs. morsitans* are mainly dark scaled. The proboscis of *Cs. ochroptera* is usually densely covered with pale scales, with a dark apex. The palps are dark brown, sometimes with pale scales at the apices of palpomeres III and IV. The antennae are dark brown, and the base of flagellomere I is whitish-yellow. The head has narrow pale scales and broad erect black scales. Along the margin of the eyes there are long, curved, dark setae. The anterior part of the scutum is uniformly golden brown or rusty brown without pale scales. The supraalar and prescutellar areas have narrow, whitish brass coloured scales, which usually form indistinct, narrow, longitudinal stripes from the middle of the scutum. The scutellum is brown with patches of whitish brass coloured scales on the lobes. The posterior margin of the scutellum has dark, slightly curved setae. The postnotum is ochre brown coloured. The postpronotum has uniformly brown scales. The femora are dark on the anterior surface, and pale yellow on the posterior surface with a white patch of scales at their apices. The tibiae of the fore legs are pale yellowish scaled except for a narrow longitudinal dark stripe on the anterior surface. The tibiae of the mid and hind legs are pale on the anterior and posterior surface and dark on the ventral and dorsal surface. The tarsi of the mid and hind legs have narrow pale basal rings on tarsomeres I–III and sometimes a few pale scales at their apices, and tarsomeres IV and V are entirely dark. The pale rings are often indistinct. The costa (C) has a varying number of pale or ochre coloured scales along the entire anterior margin, but other scales on the wing veins are dark. At the base of  $R_{4+5}$  the dark scales may be aggregated to form a small but distinct dark spot. The colouration of the abdominal terga may be variable. Typically the terga are brown scaled with yellowish scales forming indistinct narrow basal and apical bands, and tergum VIII is completely pale scaled. Sometimes the apical bands or both the apical and basal bands are absent, and isolated pale scales are scattered at the bases of the terga. The

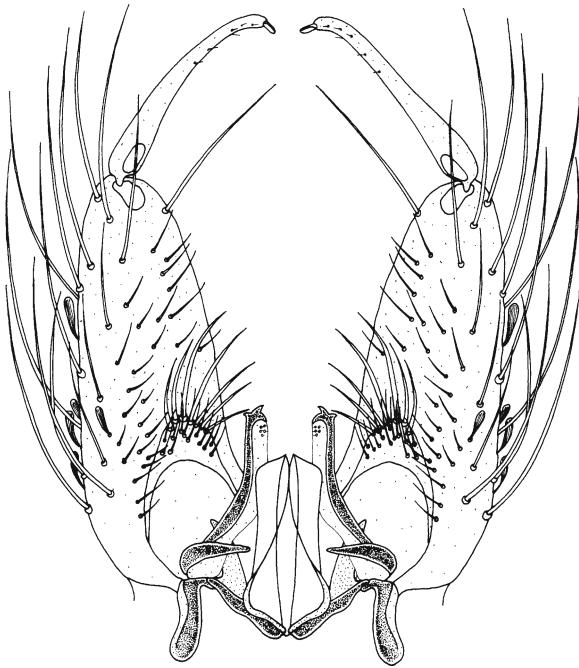


Fig. 10.125 Hypopygium of *Cs. ochroptera*

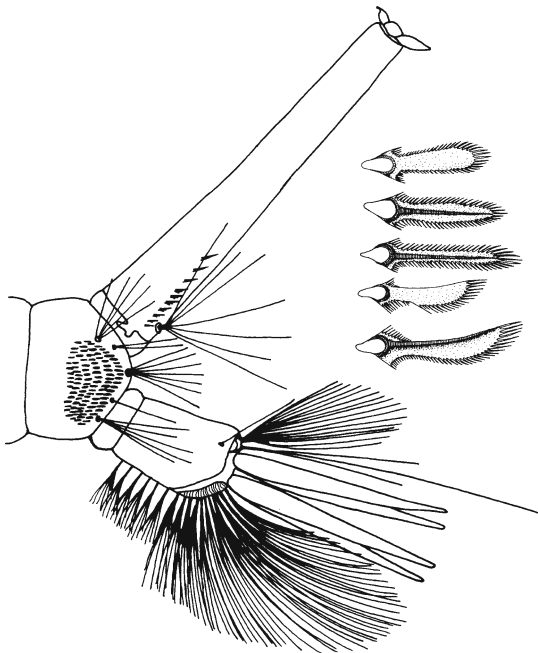


Fig. 10.126 Larva of *Cs. ochroptera* and enlarged comb scales

sterna have a diffuse pattern of intermixed pale and dark scales.

**Male:** The median lobe of tergum VIII has 6–8 strong setae arranged in an irregular row, and the lobes of tergum IX usually have 7–10 slightly curved setae. The gonocoxite is elongated, and is at least 2.5 times as long as it is wide at its base (Fig. 10.125). The basal lobe of the gonocoxite has 5–8 strong setae, which are slightly inwardly curved. The paraproct is strongly sclerotized, with 1–2 teeth at the apex. The aedeagus is more or less oval, and weakly sclerotized.

**Larva:** The larvae of *Cs. ochroptera* resemble superficially those of *Cs. morsitans*, but they are readily distinguishable from the latter by having the inner frontal seta (5-C) with 5–9 branches (Fig. 8.85a) and the comb scales at the posterior margin of the comb with strongly sclerotized longitudinal mid ridges (Fig. 10.126). In *Cs. morsitans* the inner frontal seta is 2–3 branched and the comb scales lack sclerotized mid-ridges. *Cs. ochroptera* has an antenna which is slightly longer than the head, dark pigmented at the base and in the tapering apical part. The postclypeal seta (4-C) has 2–4 small branches, the median frontal seta (6-C) is always 2 branched, and the outer frontal seta (7-C) has 7–13 branches. The comb consists of 60–95 scales of different shape, some with a dark longitudinal mid ridge. The siphonal index is 5.0–7.0, and the siphon slightly tapers apically. The pecten has 7–10 teeth, the 2–3 distal teeth are single spines without lateral denticles on the ventral margin and more widely spaced. The siphonal tuft (1-S) has 5–10 branches. The anal segment is longer than broad, and the saddle completely surrounds the segment. The upper anal seta (2-X) has 12–23 branches, and the lower anal setae (3-X) is 2–3 branched. The ventral brush has 10–22 tufts of cratal setae (4-X) and 5–8, usually 6, precratal setae. Often only 1 precratal seta is not situated on the saddle. The anal papillae are narrow and lanceolate, and 1.5–2.0 times longer than the saddle.

**Biology:** *Cs. ochroptera* is a very rare mosquito and therefore the knowledge about its biology is scanty. In central and eastern Europe the larvae can be found in peat bogs, where they sometimes appear associated with larvae of *Cs. alaskaensis*. In the east of their distribution range they can also occur in large shallow marshes, in forest ponds, and ditches or at the muddy shores of lakes. It seems likely that *Cs. ochroptera* has at least two generations per year and that hibernation apparently takes place in the larval or the egg stage,

but Gutsevich et al. (1974) reported hibernation of adult females in the eastern Ukraine. The females rarely bite humans; they seem to feed mainly on birds and amphibians.

**Distribution:** Forest zones of the Palaearctic region, from central Europe through west Siberia to northeast China. Its southernmost distribution range reaches Romania. It is also distributed from Sweden and Finland southeast to the Caucasus.

### 10.4.3 Subgenus *Culiseta* Felt

The cross veins (r-m and m-cu) are in one line or slightly separated, and the distance between them is not longer than the length of m-cu (Fig. 6.57b). If the tarsi are pale ringed, the rings are confined to the basal parts of each tarsomere. The palps of the males are as long as or longer than the proboscis, and the last two segments are distinctly swollen. The aedeagus is strongly sclerotized, and pointed towards the apex and often curved or hooked. The larvae have antennae shorter than the head, and the antennal seta (1-A) is weakly developed. The siphon is short, and the index is  $<4.0$ . The pecten is short and often prolonged towards the end of the siphon by a row of long, thin setae. The larvae are mainly bottom feeders, they spend most of their time submerged at the bottom of their breeding sites feeding on the substrate. The females of the subgenus hibernate as adults. They deposit the eggs in boat shaped egg rafts on the surface of the water like the species of the genus *Culex*. In the Palaearctic region the subgenus is represented by five species and one subspecies.

#### *Culiseta (Culiseta) alaskaensis* (Ludlow 1906)

**Female:** *Cs. alaskaensis* is a large mosquito resembling both *Cs. annulata* and *Cs. subochrea* in having a median longitudinal pale band on tergum II and conspicuous spots on the wings, resulting from aggregations of dark scales in certain areas. But unlike *Cs. alaskaensis*, the other two species are ornamented with a subapical white ring on the femora and a median white ring on tarsomeres I of all legs. In addition, the pale basal rings on tarsomeres II–IV are much less distinct than in the other two species. The proboscis

and palps are mainly dark scaled with a few scattered pale scales in the basal half of the proboscis and throughout the palps. The antennae are dark brown, and the pedicel and first flagellomere have yellowish-white scales on the inner surface. The occiput has narrow curved whitish scales and dark erect forked scales on its dorsal part and broad whitish scales on its lateral part. The integument of the scutum is dark brown, with dark and whitish scales. Two indistinct longitudinal narrow stripes or patches of pale scales may be visible, and the lateral parts of the scutum are usually lighter than the median part. The scutellum has narrow whitish scales and dark setae on the lobes. The pleurites have sparse patches of narrow curved pale scales, and the prespiracular setae are yellowish. The femora anteriorly are dark brown with pale scales intermixed, and the posterior surface and apices are white scaled, with no subapical pale ring. The tibiae are dark brown with scattered pale scales, and the tarsi are dark with pale basal rings on tarsomeres II–III of the fore and mid legs and on tarsomeres II–IV of the hind legs. Tarsomere I of the hind leg is without a

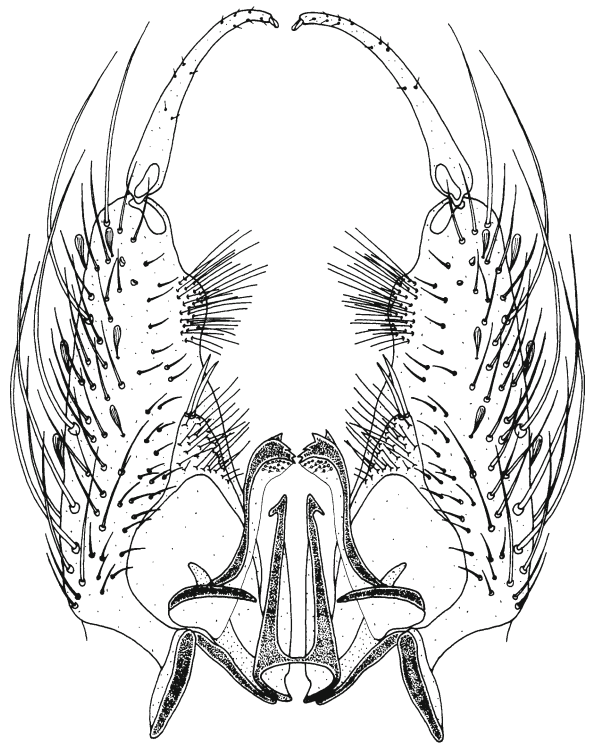


Fig. 10.127 Hypopygium of *Cs. alaskaensis*

median light ring (Fig. 6.62b). The wing veins have narrow dark scales, which are aggregated in some areas forming conspicuous spots. The costa (C), subcosta (Sc) and  $R_1$  have scattered pale scales throughout their length. The base of the subcosta (Sc) bears a dense tuft of yellowish setae on the ventral side of the wing. The terga are blackish brown with rather broad basal white bands, which are widened laterally, especially on the last segments. The sterna are white scaled, with a few dark scales intermixed.

**Male** (Fig. 10.127): The two hypopygial characteristics which distinguish *Cs. alaskaensis* from *Cs. annulata* and *Cs. subochrea* are firstly a group (2–10) of short dark stout setae located in the middle of the posterior margin of tergum VIII, and secondly the slightly convex apical lobe of the gonocoxite which is covered with short thin setae. Both characteristics are absent in *Cs. annulata* and *Cs. subochrea*. The lobe of tergum IX has a row of long curved setae. The gonocoxite is covered with many long setae on the outer surface, and the basal lobe has 2, or rarely 3, strong setae. The gonostylus is curved and tapered apically, and the apical spine is short and bifurcated. The aedeagus is sclerotized, long, conical, tapered, and hooked at the apex.

**Larva:** The antennae are less than half as long as the head, and the antennal seta (1-A) is multiple-

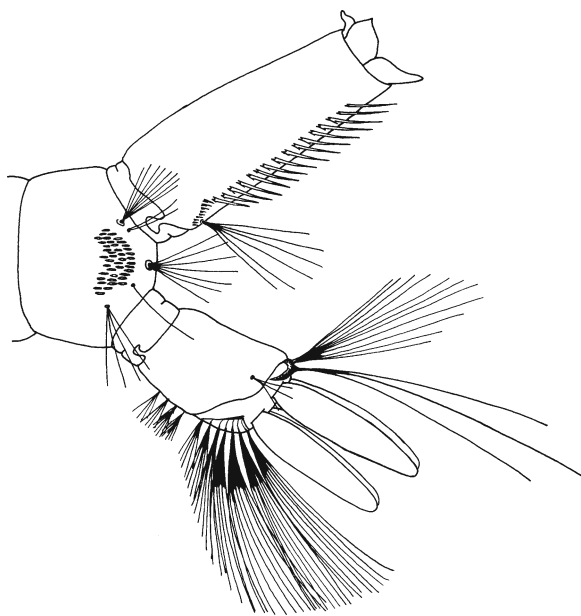


Fig. 10.128 Larva of *Cs. alaskaensis*

branched and inserted near the middle of the antenna. The postclypeal seta (4-C) is short, thin and 3-branched. The inner frontal seta (5-C) has 5–7 branches, the median (6-C) is 2–3 branched and the outer (7-C) has 8–11 branches (Fig. 8.80a). The comb consists of 35–50 comb scales arranged in a triangular patch (Fig. 10.128). The siphon is short and broad, slightly tapered apically, with a siphonal index of 2.5–3.0. The pecten has 6–8 spine-like teeth on the basal 1/5 of the siphon followed by an even row of 16–18 hair-like setae extending to near the apical quarter of the siphon. The anal segment is completely surrounded by the saddle, and the saddle seta (1-X) is inconspicuous, and much shorter than the saddle. The ventral brush is well developed with 3–4 precratal setae (4-X), at least two of them piercing the saddle. The anal papillae are of variable length, at least as long as the saddle, and pointed.

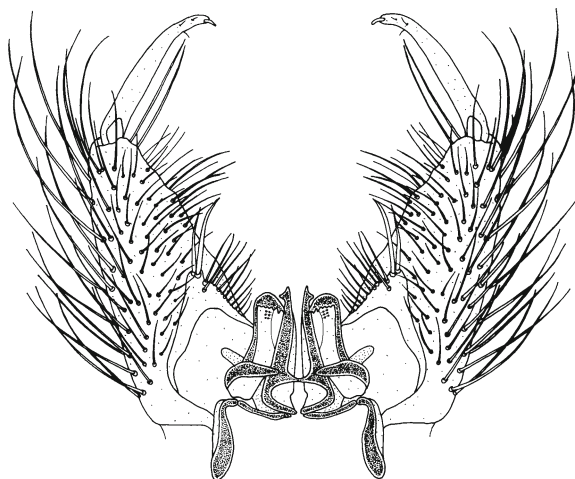
**Biology:** Larvae of *Cs. alaskaensis* can be found from late spring on, in a variety of habitats. They favour small open pools formed by melting snow which do not dry up in summer. These pools usually have a considerable amount of fallen leaves at the bottom and little aquatic vegetation, in the tundra they inhabit swamps. The larvae are often associated with those of *Oc. excrucians*, *Oc. flavescens* and *Oc. cantans*. In the northern parts of its distribution range *Cs. alaskaensis* is apparently a monocyclic species with one generation per year. In the more temperate southern regions several generations per year can be expected (Mohrig 1969). The species apparently hibernates as adult females in tree cavities, caves, and cellars, often together with *Cx. pipiens*. The females leave their winter habitats usually earlier than other mosquito species. *Cs. alaskaensis* frequently feeds on humans. In the tundra zones it is well known as the large snow-melt mosquito that readily attacks humans and reindeer in early spring.

**Distribution:** *Cs. alaskaensis* is a Holarctic species that is typical of the boreal and tundra zones of Fennoscandia, Siberia and Alaska. In the Palaearctic it is distributed from Britain and Norway in the west to the far East. In central Europe, its southern distribution stretches towards the northern slopes of the Alps. In this part of its distribution range, *Cs. alaskaensis* is usually restricted to the higher mountains. Outside Europe it can be found in mountainous regions of Iran, Pakistan, and northern India.

**Notes on systematics:** A subspecies of *Cs. alaskaensis*, ssp. *indica* Edwards 1920, has been described with a distribution range from the Caucasus to Middle Asia and southern India. Its general colouration is much lighter than the nominate form, the scutum is covered with golden yellowish scales and the pale basal bands on the terga are often broader. The male genitalia and larvae do not distinctly differ from the nominate form.

### ***Culiseta (Culiseta) annulata* (Schrank 1776)**

**Female:** *Cs. annulata* is a large, dark brown mosquito with whitish markings on the abdomen and the legs. It can be distinguished from *Cs. alaskaensis* by the presence of subapical white rings on the femora and conspicuous white rings in the middle of tarsomeres I. *Cs. annulata* is closely related to *Cs. subochrea*, the characteristics distinguishing the two species are given in the description of the latter. The proboscis of *Cs. annulata* is speckled with pale and dark scales, which are darker in the apical part, and the labellum is dark brown. The clypeus is dark brown, the palps are dark with scattered pale scales, which are especially abundant at the apices and with a conspicuous pale spot at the joint of palpomeres II–III. The antennae are dark brown, and the pedicel has a few whitish scales on the inner surface. The head has pale narrow scales and dark erect forked scales on the occiput, and the eyes are bordered with yellowish white scales and dark, stout setae. The scutum has narrow dark brown and pale scales. The posterior submedian area has two pale spots, and the prescutellar area has whitish scales. The scutellum is brown with whitish scales and black setae, and the postnotum is brown or dark brown. The pleurites have patches of broad, whitish scales, and the postpronotum is predominantly pale scaled. Hypostigmal, subspiracular, and postspiracular patches are present. The mesepimeral patch of scales almost reaches the lower margin of the mesepimeron. Prespiracular setae and lower mesepimeral setae are present. The legs have dark brown scales and conspicuous white rings. The femora are predominantly dark scaled with scattered pale scales, distinct white subapical rings and pale knee spots, and the tibiae have pale and dark scales intermixed basally and are white scaled apically. Tarsomere I has a noticeable white ring in the middle and white rings

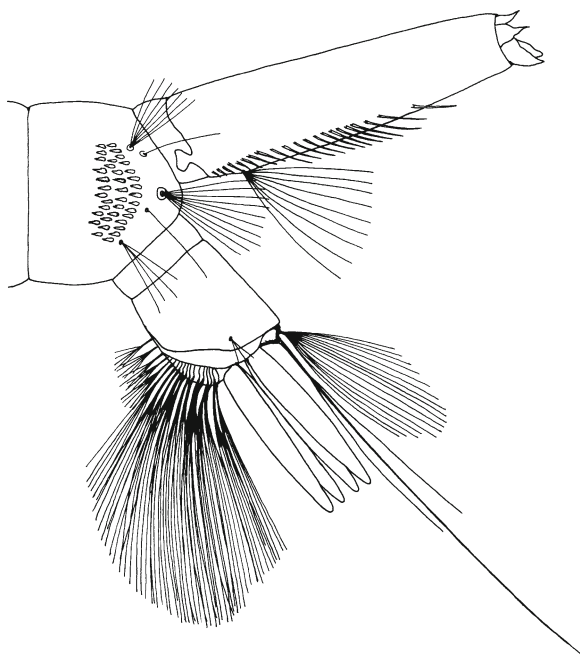


**Fig. 10.129** Hypopygium of *Cs. annulata*

also at the bases of tarsomeres II–IV, and tarsomeres V of all the legs are entirely dark scaled (Fig. 6.62a). The wings are largely covered with dark scales, which are aggregated to form distinct dark spots at the base of  $R_s$ , at the cross veins and the furcations of  $R_{2+3}$  and M. Some scattered pale scales are mainly found in the basal part of the costa (C), subcosta (Sc), and radius (R). The cubitus (Cu) is entirely dark scaled. The cross veins (r-m and m-cu) usually form a straight line (Fig. 6.63a). The abdominal terga have whitish basal bands, and the apical parts are uniformly dark scaled. Tergum II has a narrow basal band and a characteristic longitudinal median white band. Tergum VIII is predominantly pale scaled. The sterna have yellowish white scales.

**Male:** The posterior margin of tergum VIII is usually without stout setae or occasionally a few setae may be present. The lobe of tergum IX has 8–12 hair-like setae. The gonocoxite is conical, gradually tapered towards the tip (Fig. 10.129). The basal lobe of the gonocoxite is well developed, with 2 (rarely 3) strong setae conspicuously stouter than the rest. The apical lobe is usually absent or indistinct. The gonostylus is long and slender, with a short apical spine. The paraproct is strongly sclerotized, recurved, with apical teeth, and the sclerites of the aedeagus are separated and also strongly sclerotized.

**Larva:** The head is broader than long, and the antennae are less than half as long as the head and straight. The antennal seta (1-A) is inserted just beyond the middle of the antennal shaft, with 10–15 branches,



**Fig. 10.130** Larva of *Cs. annulata*

not reaching to the apex of the antenna. The distance between the postclypeal setae (4-C) is about the same as the distance between the inner frontal setae (5-C) (Fig. 8.82a). 5-C has 4–8 branches, the median frontal seta (6-C) has 1–3 branches, and the outer seta (7-C) has 6–14 branches (Fig. 8.78a). The comb usually consists of 35–50 scales, rarely more (Fig. 10.130). The individual scales are slightly narrowed in the middle part, blunt ended and uniformly fringed with small spines. The siphon distinctly tapers apically, with a siphonal index of 3.2–4.0. The siphonal tuft (1-S) is inserted close to the base of the siphon, usually with 9–10 branches, and is about as long as the width of the siphon at the base. The pecten has 11–18 spine-like teeth, followed by a row of 11–21 thin, hair-like setae occupying approximately  $\frac{2}{3}$  the length of the siphon. The saddle completely encircles the anal segment, and its ventral surface is only half as long as its dorsal surface. The saddle seta (1-X) is much shorter than the saddle, usually with 3 branches. The upper anal seta (2-X) has 13–19 branches, and the lower anal seta (3-X) has 3 branches. The ventral brush usually has 16–18 cratal setae (4-X) and 2–3 precratal setae, 1 or 2 of which perforate the saddle. The anal papillae are lanceolate, and usually as long as the saddle.

**Biology:** In central Europe the larvae usually occur from early spring onwards. The population increases in the summer months and reaches its maximum in September. Depending on the latitude of its occurrence, *Cs. annulata* may have 1–3 generations per year. The eggs are laid in rafts, which are composed of approximately 200 eggs. The species breeds in a wide variety of permanent and semipermanent habitats including natural and artificial water recipients, both in open and shaded situations. Larvae can be found in stagnant pools, ponds, ditches, water troughs, and other artificial containers such as barrels collecting rain water. Dense populations could be found in tanks containing manure water, so it seems probable that a high content of nitrogen provides an additional attraction for the females to lay their eggs (Mohrig 1969). The larvae are able to tolerate a high content of salinity and can also be found in brackish water (Marshall 1938). Aitken (1954a) found larvae breeding in a tree-hole in Corsica. In artificial containers they are often found in association with those of *Cx. p. pipiens*. In natural breeding sites the larvae occur together with those of *Cs. subochrea* and *Cs. morsitans* (Natvig 1948). Hatching of *Cs. annulata* larvae takes place 3–5 days after egg deposition, the larval development depends on the temperature. Martini (1931) estimated the overall time from egg deposition to the emergence of the adult being 18 days at a temperature of 20–23°C and 16 days at a temperature of 24–27°C, above 31°C no larvae survived. Usually the species hibernates in the adult stage and the first females appear in early spring when they leave their winter shelters. At this time they readily attack humans and mammals during the day, but in the summer months they show a more nocturnal biting activity and frequently enter houses or stables to feed on humans or domestic animals. Occasionally they may also take their blood meal from birds. In former Yugoslavia, the females could be sampled more efficiently in CO<sub>2</sub> baited traps than on humans (Petric 1989). The females of *Cs. annulata* can often be found inside houses even during the day from early autumn on, when they search for their winter habitats. They hibernate in cellars, attics of dwellings, or in sheds of domestic animals, where they can be extremely annoying during wintertime, when the hibernation is interrupted by rising temperatures or humidity. Winter habitats can also be found far away from human settlements in tree cavities, stacks of



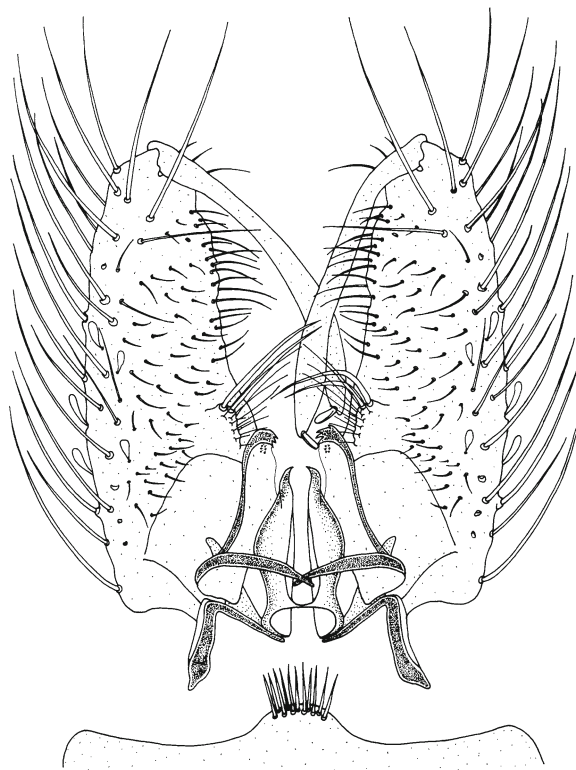
wood, or other natural shelters. When the winter is mild, or in the southern range of its distribution, hibernation can also take place in the larval stage.

**Distribution:** *Cs. annulata* is widely distributed throughout Europe, but it is more common in the north than in the south, where it seems to be largely replaced by *Cs. longiareolata* (Edwards 1921). The distribution range of *Cs. annulata* extends into northern Africa, Asia Minor, and southwest Asia.

**Medical importance:** The species is known to be a potential vector of Tahyna virus (Ribeiro et al. 1988) and transmitter of some plasmodia of birds (Gutsevich et al. 1974).

### ***Culiseta (Culiseta) bergrothi* (Edwards 1921)**

**Female:** The integument is dark brownish with some lighter pleural parts. The colour of the light scales is variable, from white to creamy white. The antennae have some white scales on the pedicel, and the eyes are bordered with white scales. The proboscis is covered with black scales, and the palps are dark with scattered pale scales. The vertex has white flat scales, and the occiput has upright narrow golden scales and brown setae. The scutum is covered with narrow bronze scales and a more or less distinct varying pattern of white scales and bronze brown setae. White broader scales cover the front, sides, and back of the scutum in an irregular pattern and form a narrow, incomplete acrostichal stripe. Dorsocentral stripes of narrow white scales and a postacrostichal patch of white scales are usually present forming a scutal pattern similar to that of *Cs. subochrea*. However, submedian stripes are not present. The scutellum is covered with whitish scales and light setae. The anteprenotal and propleural setae are long and dominating, the postpronotum has narrow bronze and some lower, broader whitish scales. The mesepisternal patch is large with elongated white scales in its upper part, and the mesepimeron is covered with scales in its upper third. Usually not more than 15 prespiracular setae and not more than 10 lower mesepisternal setae are present (Fig. 6.61a). The coxae of the fore and hind legs have long, conspicuous setae. The femora have white scales on the ventral surface and a mixture of white and dark scales on the dorsal surface. The tibiae are dark with either light dorsal stripes or mixed scales, especially on the front legs,



**Fig. 10.131** Hypopygium of *Cs. bergrothi*

and the knee spots are white. Tarsomeres I–V of all the legs are dark, with occasionally some white scales on tarsomere I; the claws are simple. The wing veins are covered with elongated, dark scales, and spots are always present, although often inconspicuous. The spots result from aggregations of dark scales at the furcations of  $R_{2+3}$  and M and the cross veins (r-m and m-cu). The lining up of r-m and m-cu can vary from nearly a straight line to a slight displacement of m-cu towards the wing root, but never distal to r-m. The abdomen is dark with pale basal bands on the terga, which are narrower in the last segments. The sterna are pale with some scattered dark scales.

**Male:** Somewhat smaller than the males of other northern European *Culiseta* species. They share the feature of having an extremely long tarsomere I of the fore leg (exceeding the length of tarsomeres II–V of about one fifth) with males of *Cs. fumipennis*. The median lobe of tergum VIII has 4–18, usually >10, strong spine-like setae (Fig. 7.71b). The gonocoxite has long and numerous setae laterally and apically (Fig. 10.131). The basal lobe is weakly developed,

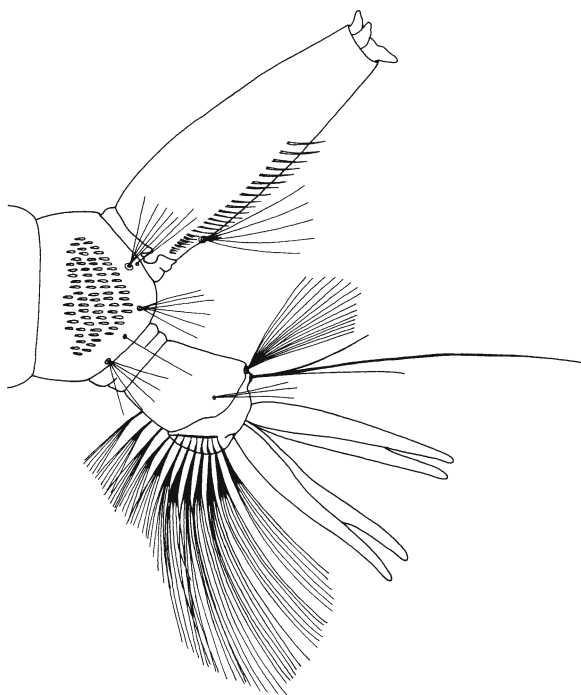


Fig. 10.132 Larva of *Cs. bergrothi*

with two strong spine-like setae of almost equal length. The apical lobe is indistinct, and covered with dense setae. The gonostylus is long, more than half as long as the gonocoxite, and the apical spine is blunt ended. The paraproct is strongly sclerotized, with a curved apex, bearing 3–4 teeth. The aedeagus is oval shaped, slightly sclerotized in the lateral parts with sigmoid, pointed tips.

**Larva:** The head is considerably broader than long, and the antennae are about half as long as the head. The antennal seta (1-A) has 4–6 branches situated slightly proximal to the middle of the antennal shaft (Fig. 8.83c). The frontal setae 5-C and 6-C are nearly in a row, with multiple branches. The prothoracic setae are all very long. Numerous comb scales are arranged in a triangular spot (Fig. 10.132). Each scale has a long, narrow stem, which is rounded apically and fringed with small spines. The siphon is slightly tapered apically, with a siphonal index of 3.0–3.5. The pecten has 12–15 spine-like teeth, and each tooth has 2–3 slender lateral denticles, followed by a row of longer hair-like setae which reach distally about  $2/3$  the length of the siphon. The anal segment is entirely encircled by the saddle, and the

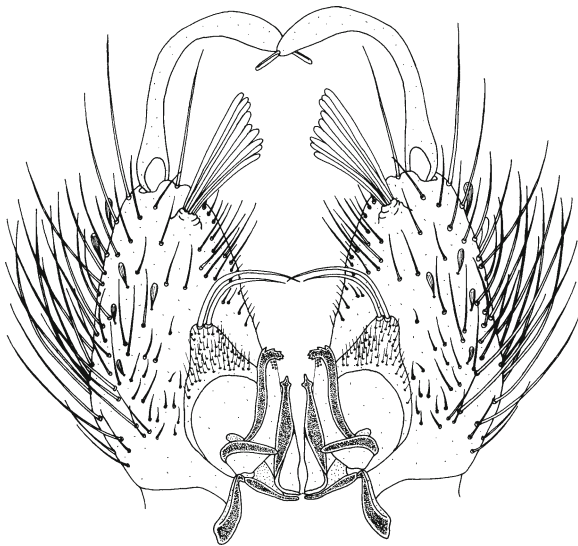
ventral brush has 3–4 precratal setae (4-X), usually two of them piercing the saddle. The anal papillae are very long and slender.

**Biology:** In the northern parts of its distribution range the species breeds in open pools and has only one generation per year. The larvae inhabit swamps and pools in the southern tundra and shaded forest pools in coniferous and mixed forests, they may also tolerate polluted water. In the southern parts of its distribution range several generations per year may occur. Hibernation takes place in the female stage (Gutsevich et al. 1974) and larvae can be found together with other early spring species, such as *Oc. communis*. In Scandinavia the larvae have been recorded until the summer months. The females are most abundant from June to August. They predominantly bite cattle and other mammals and seldom enter houses.

**Distribution:** This northern species is found throughout the tundra and northern taiga zone of the Palearctic, as well as in high mountainous areas both in Scandinavia (Natvig 1948) and in Japan (Tanaka et al. 1979).

#### ***Culiseta (Culiseta) glaphyoptera* (Schiner 1864)**

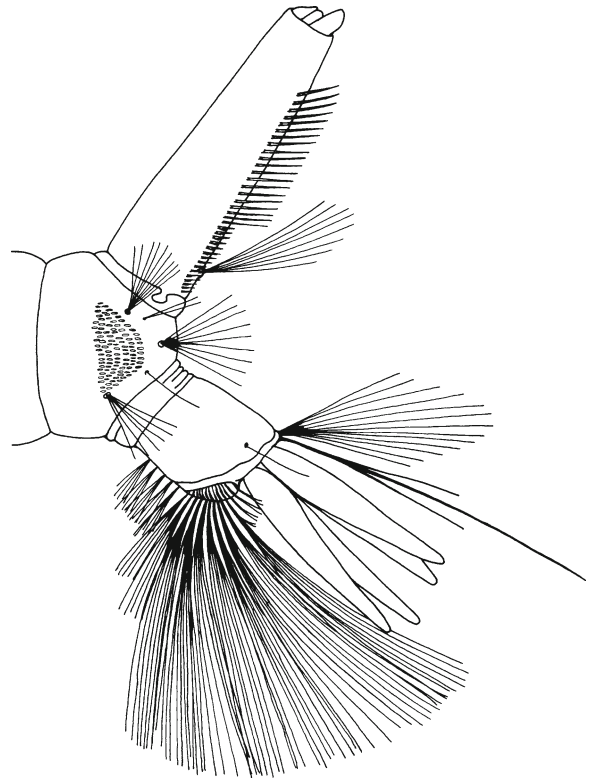
**Female:** *Cs. glaphyoptera* is very similar to *Cs. bergrothi*, the other member of the subgenus *Culiseta* with entirely dark tarsomeres. However, it is easy to distinguish males and females of these two species (Natvig 1948). In *Cs. bergrothi* the eyes are distinctly bordered with light scales and the palps are dark with scattered pale scales. On the other hand, in *Cs. glaphyoptera* the eyes are not bordered with light scales and the palps are entirely dark. The head has whitish scales laterally, the vertex has a tuft of short light scales between the eyes, and the occiput in the posterior part has upright dark brown scales. The proboscis and palps are dark brown. The integument of the scutum is dark brown with golden brown sickle-shaped scales, which form a narrow acrostichal stripe on the anterior part of the scutum and two dorsocentral and lateral, more indistinct, stripes. The scutellum is dark with scales of the same colour as on the scutum. The pleurites of the thorax have patches of whitish flat scales. The lobes of the antepronotum are blackish brown with pale narrow scales. The number of prespiracular setae usually ranges from 16 to 22 and that of the lower mesepistern-



**Fig. 10.133** Hypopygium of *Cs. glaphyoptera*

nal setae from 12 to 18 (Fig. 6.61b). The legs are dark scaled, and the posterior surface of the femora and tibiae has light scales, which sometimes form a longitudinal stripe, the knee spot is distinct and yellowish white. The tarsomeres are entirely covered with dark brown scales, and pale rings are absent (Fig. 6.60a). The wing veins are dark scaled, and the cross veins are slightly separated. Wing spots are absent or only weakly developed. When present, the spots result from aggregations of dark scales at the furcations of  $R_{2+3}$  and M and the cross veins (r-m and m-cu). The terga are dark brown with indistinct bands of light brown to pale scales in the basal third of each tergum. The sterna are more or less uniformly covered with yellowish white scales, or sometimes narrow bands of brownish scales are present at their posterior margins.

**Male:** The lobe of tergum IX has numerous long, thin setae. The gonocoxite is relatively short, and the basal lobe is well developed with two long and prominent setae which are strongly bent in the middle (Fig. 10.133). The apical lobe has a characteristic tuft of long scale-like setae. The gonostylus is long and expanded subapically, and the apical spine of the gonostylus, unlike in other species of the subgenus *Culiseta*, is long. The aedeagus is cylindrical in its proximal part, tapered beyond the middle, and the tips are strongly sclerotized.



**Fig. 10.134** Larva of *Cs. glaphyoptera*

**Larva:** The head is broader than long, and the antennae are  $2/3$  the length of the head, and slightly curved inwardly. The antennal seta (1-A) is inserted close to the middle of the antennae and is about half as long as the antennal shaft, usually with 10 branches (Fig. 8.83a). The postclypeal setae (4-C) are situated before the inner frontals and are close together. The frontal setae are well developed, the inner frontals (5-C) have 7–9 branches reaching to the labrum, the median (6-C) have 5–7 branches and the outer (7-C) are 8–12 branched. The comb consists of approximately 70 scales, which are arranged in a crescent shaped patch (Fig. 10.134). The comb scales are elongated with a broad base and rounded apex. The siphonal index is 3.4–3.5. The pecten has 15–21 spine-like teeth followed by a row of 19–22 longer hair-like setae reaching to the apical quarter of the siphon. The siphonal tuft (1-S) is inserted close to the base of the siphon, with 8 branches which are distinctly longer than the width of the siphon at the point of its origin. The anal segment is completely surrounded by the saddle, which is half as long ventrally as it is dorsally. The saddle

seta (1-X) is short with 1–2 branches. The upper anal seta (2-X) usually has 14 branches, forming a fan, and the lower anal seta (3-X) has 3–4 branches. The ventral brush has 13–16 tufts of cratal setae (4-X) on the common base, with five precratal setae, three of which pierce the saddle. The anal papillae are 1.5–2.5 times longer than the saddle, and pointed.

**Biology:** *Cs. glaphyoptera* can only be found in mountainous regions. The larvae are mostly restricted to partly shaded, cool breeding sites. They usually occur in the beds of small mountain rivers or streams and in depressions in larger rocks, where the water remains after the first flood in spring. They prefer water with fallen leaves and a rich amount of detritus rather than a clean stony bottom with a poor development of algae and other organisms. Occasionally the larvae can be found in ground pools close to small brooks and sources together with *Cx. territans*. Apfelbeck (1928) reported common findings of *Cs. glaphyoptera* larvae in tree-holes of mountain forests in former Yugoslavia, where they were found in association with typical tree-hole breeders such as *An. plumbeus* and *Oc. geniculatus* during the summer months. Later in the year, at the end of November, the larvae disappeared, but adults could be found in sheltered situations, such as in caves. *Cs. glaphyoptera* hibernates in the adult stage. Little is known about the feeding behaviour of the females. It seems likely that they feed on birds or small mammals which inhabit the forest.

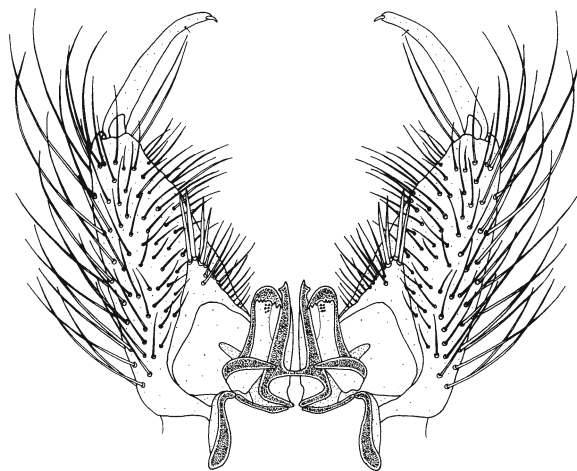
**Distribution:** The species is widely distributed throughout the mountains of central and southeastern Europe. In addition, it has been found in the mountainous regions of western Ukraine and Crimea. It was thought that *Cs. glaphyoptera* must also be present in the northern parts of Europe, but Natvig (1948) examined material from Scandinavia and clearly demonstrated that all specimens formerly referred to as *Cs. glaphyoptera* were in fact *Cs. bergrothi*. As a consequence, *Cs. glaphyoptera* was erased from the list of Fennoscandian mosquitoes (Dahl 1997).

#### ***Culiseta (Culiseta) subochrea* (Edwards 1921)**

**Female:** *Cs. subochrea* is very similar to the closely related *Cs. annulata* in all stages and only the differences between the two species are mentioned here. The scutal integument and most of the scutal scales are yellowish in *Cs. subochrea*, whereas in *Cs. annu-*

*lata* the integument is more brownish and the pale scutal scales are creamy white. The legs of *Cs. subochrea* are more speckled than those of *Cs. annulata* owing to a greater number of pale scales scattered on the femora, tibiae, and tarsomeres I; hence, in the general appearance the contrast of pale and dark colour of the legs is not so conspicuous. The subapical white rings on the femora are less distinct and the yellowish white basal rings on the tarsi are much broader than in *Cs. annulata*. The spots on the wings formed by aggregated dark scales are less obvious in *Cs. subochrea* and in addition to some pale scales on the costa (C), subcosta (Sc), and radius (R), more or less numerous pale scales are scattered along the cubitus (Cu). In *Cs. annulata* vein Cu is entirely dark scaled. In *Cs. subochrea* the cross veins r-m and m-cu are usually slightly separated, the distance between them not being longer than the vein m-cu (Fig. 6.63b). In *Cs. annulata* the cross veins usually form a straight line. The terga of *Cs. subochrea* have indistinct pale bands formed by yellowish scales, the dark areas in the apical half of the terga are scattered with more or less numerous yellowish scales. In *Cs. annulata* the terga have more distinct whitish basal bands and the apical half of the terga lack pale scales, they are uniformly dark scaled.

**Male (Fig. 10.135):** In *Cs. subochrea* the basal lobe of the gonocoxite carries 3–5 strong setae conspicuously stouter than the rest and the median lobe of tergum VIII usually bears several stout setae. In *Cs.*



**Fig. 10.135** Hypopygium of *Cs. subochrea*

*annulata* the number of strong setae on the basal lobe is 2, rarely 3 and the median lobe of tergum VIII is usually devoid of stout setae. Furthermore, Peus (1930) referred to the number of thin setae on the lobe of tergum IX, which ranges from 13 to 24 in *Cs. subochrea* and from 8 to 12 in *Cs. annulata*. Unfortunately, these characteristics are variable and overlap to a certain degree, thus they cannot be precisely used in all cases, especially if the identification is based on a single hypopygium with 3 strong setae on the basal lobe. In this case it is most likely that the specimen belongs to *Cs. subochrea* when the median lobe of tergum VIII bears several stout setae and to *Cs. annulata*, when the stout setae are absent.

**Larva:** In *Cs. subochrea* the distance between the postclypeal setae (4-C) is less than the distance between the inner frontal setae (5-C) (Fig. 8.82b), whereas in *Cs. annulata* both pairs of setae are usually about the same distance apart. Again, the two ranges of variation overlap considerably and it is not always possible to distinguish larvae of the two species according to this characteristic. No other structural differences between the larvae of the two species seem to exist, but Ribeiro et al. (1977) pointed out that in the material from Portugal, the length of the siphonal tuft (1-S) is distinctly shorter than the width of the siphon at the base in *Cs. subochrea*, and the length of 1-S is about the same as the width of the siphon at its base in *Cs. annulata*. So far this observation has not been verified with material from other locations.

**Pupa:** A characteristic which seems to be generally constant and enables one to distinguish between *Cs. subochrea* and *Cs. annulata* with certainty, is found in the pupal stage. The minute denticles which fringe the pupal paddle are long and pointed in the former species and considerably shorter and blunt ended in the latter one.

**Biology:** Owing to the rare findings of *Cs. subochrea* in western and central Europe, the biological data are scanty, but its biology seems to be similar to that of *Cs. annulata*. Hibernation usually takes place in the adult female stage preferably in farm buildings and cellars, but in its southern distribution range the larvae may also hibernate and the dormant phase of the females does not last very long. Several generations per year may occur. As in *Cs. annulata*, the larvae can be found both in fresh water habitats, like ditches, ponds or garden tanks, and habitats with a varying degree of salinity up to  $> 1/3$  that of sea water (Marshall

1938). Larvae were found in rice fields with clean water, associated with those of *An. atroparvus* and *Cx. pipiens* (Ribeiro 1988), but they exhibit a remarkable preference for saline breeding sites (Rioux 1958; Mohrig 1969; Gutsevich et al. 1974). The females bite humans and domestic animals and they are more exophagic than those of *Cs. annulata*. They were reported to feed on humans during the day and far away from dwellings (Rioux 1958). An autogenous egg laying of one female reared in the laboratory was observed (Marshall 1938).

**Distribution:** *Cs. subochrea*, a Palaearctic species, can be found in nearly all European countries, reaching from southern Fennoscandia to the Mediterranean region including western and central Europe, but it is not a very common mosquito. Its range stretches into north Africa and the near East and it is widely distributed in middle Asia.

**Note on systematics:** The taxonomic status of *Cs. subochrea* is still controversial. It was originally regarded as being merely a variety (desert form) of *Cs. annulata*, but then rose to species rank due to its distribution not being confined to desert areas and the conspicuous colouration of the adults (Edwards 1921). Likewise, Peus (1930) referred to several hypopygial, pupal, and larval characteristics differentiating the two species and this was confirmed by Marshall (1938). Later it was brought back to subspecies status of *Cs. annulata* by Maslov (1967), in accordance with the opinion of other Russian authors (Shtakelberg 1937; Monchadskii 1951). This change was based on the comparison of characteristics and their variability of both forms in almost all of the distribution range. The authors stated an overlap in distribution of the subspecies and its nominative form in western and central Europe (Gutsevich et al. 1974). Based on material from Portugal, *Cs. subochrea* was re-ranked as a valid species by Ribeiro et al. (1977) and this was followed by others (Ward 1984). This opinion is supported by the fact that larvae of both forms could be found sympatrically in the same breeding sites in many localities of western and central Europe (Peus 1951; Aitken 1954a; Rioux 1958). The specific characteristics were always retained but intermediate forms are not known, indicating that the gene flow between both forms is inhibited. Further research, based on morphological and genetical studies, demonstrate that *Cs. subochrea* should be regarded as a distinct species (Cranston et al. 1987).

## 10.5 Genus *Coquillettidia* Dyar

The proboscis of the females is moderately long (about 1.5 times longer than the thorax), and uniformly broad. The palps are short, 1/4 the length of the proboscis or shorter. The vertex is covered with numerous erect forked scales. The acrostichal, dorso-central, and lateral setae of the scutum are well developed. The scales on the scutum are usually narrow, and decumbent. Prespiracular setae are absent in all subgenera, and postspiracular setae are absent in the subgenus *Coquillettidia*. The upper mesepisternal setae are well developed, and upper mesepimeral setae are usually present. The mesepisternal and mesepimeral patches are small, with decumbent pale scales. The legs usually have pale rings. Tarsomere I of the hind legs is shorter than the hind tibia. The claw lacks a subbasal tooth, and typical pad-like pulvilli are absent. The wing veins are covered with a mixture of pale and dark, narrow, and broad scales. The abdomen is truncated, and segment VIII is short and broad. The cerci are short and blunt. The palps of the males are about as long as the proboscis, and palpomere V is generally covered with numerous long setae. The fore and mid legs have a pair of claws, unequal in shape and size. Abdominal segment VIII has a distinctly sclerotized tergum, and segment IX is bilobed with sparse long setae. The basal lobe of the gonocoxite is small but distinct, and tapers apically. It bears one or more setae as long as or longer than the lobe. The gonostylus is usually short, enlarged apically with a short apical spine. Claspettes are absent, and the apex of the paraproct is pointed or denticulated. The head of the larva is wider than long, and slightly sclerotized. The antennae are extremely long, at least 1.5 times as long as the head. The part beyond the articulation of the antennal seta (1-A) is slender and whip-like. Abdominal segment I has the lateral tracheal branches extended into a pair of heart shaped air-sacs which lie partly in the metathorax. The siphon is short, conical, and strongly sclerotized. The lobes of the spiracular openings are dark, sclerotized, and folded together. They form a tapered saw-like piercing apparatus for penetrating aquatic plant tissue in order to obtain oxygen. A similar modification of the siphon is characteristic for larvae of some other none-European genera or subgenera, e.g. *Culex* (*Lutzia*), *Mimomyia* (*Mimomyia*), and *Hodgesia* (Gillett 1972).

Abdominal segment X is long and slender, and the saddle completely encircles the segment.

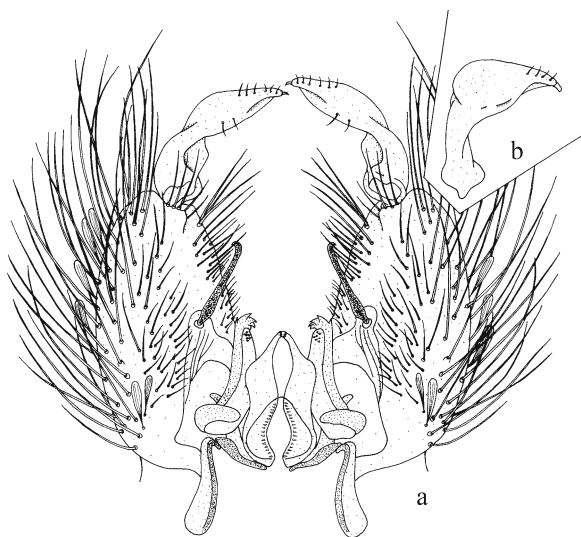
Larvae of all members of the genus obtain the oxygen from the submerged parts of aquatic plants by penetrating the plant tissue with their highly specialized siphon. The pupae are also attached to plant tissues in order to take oxygen from the aerenchyma by their sclerotized, pointed respiratory trumpets. The cuticle of the trumpets is softened subapically at a line of weakness, and breaks off prior to the time of adult emergence. The females lay the eggs on the water surface in rafts of variable shape.

The species are grouped in three subgenera, *Austromansonina* (only one representative from New Zealand), *Rhynchotaenia* (13 species mostly recorded from the Neotropical, some from the southern Nearctic region), and *Coquillettidia*. The last subgenus contains 43 species, more than half of them are distributed in the Ethiopian region, the rest in the Oriental and Australian regions, one in the Nearctic region. Only two species of the subgenus occur in the Palearctic region, these are *Cq. buxtoni* and *Cq. richiardii*.

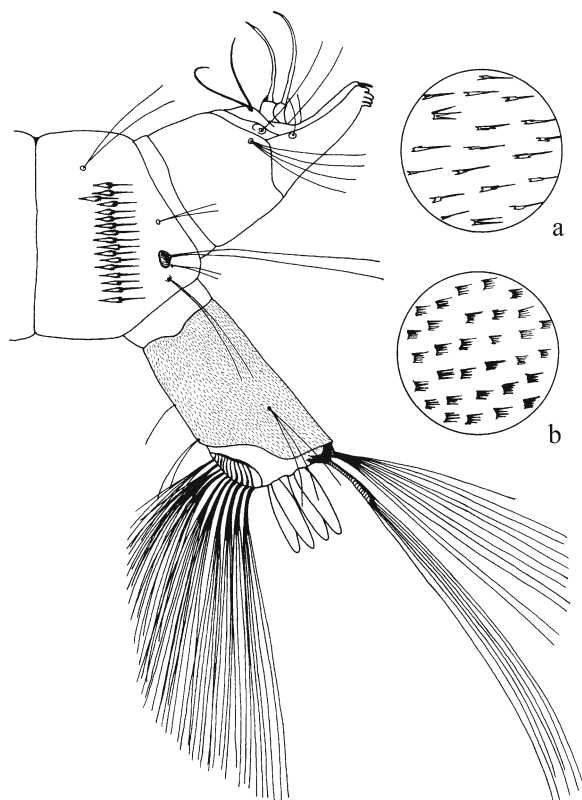
### 10.5.1 Subgenus *Coquillettidia* Dyar

#### *Coquillettidia* (*Coquillettidia*) *buxtoni* (Edwards 1923)

**Female:** Readily distinguished from *Cq. richiardii* by the presence of exclusively or mostly dark coloured scales on the proboscis, palps, wing veins, and tarsomeres. The scales are blackish brown pigmented with structural metallic purple. The head has pale decumbent scales and brown upright scales. The scutum has golden brown scales, which are paler around the wing base and at the posterior margin. The femora and tibiae are dark scaled with scattered ochre coloured scales. The latter form an indistinct longitudinal stripe on the ventral surface and spots at the apex. The dark scales on the wing veins are narrower than in *Cq. richiardii* but still broader than in members of the genus *Culex*. The terga are mainly dark coloured with a violet hue, whitish scales are restricted to triangular basolateral patches, sometimes connected by narrow basal bands, and the sterna have pale basal bands.



**Fig. 10.136** Hypopygium of (a) *Cq. richiardii* and gonostylus of (b) *Cq. buxtoni*



**Fig. 10.137** Larva of *Cq. richiardii* and spicules on the saddle of (a) *Cq. richiardii*; (b) *Cq. buxtoni*

**Male:** The palps are longer than the proboscis. The hypopygium is quite similar to that of *Cq. richiardii*. The lobes of tergum IX possess 4–5 thin setae. The main difference between the two species is found in the shape of the gonostylus. In *Cq. buxtoni* the basal half of the gonostylus is stem-like, not constricted in the middle. The apical half is considerably bulky and then gradually narrows towards the apex. The outer margin of the gonostylus bears four small and tiny setae apically (Fig. 10.136b).

**Larva:** Closely resembles that of *Cq. richiardii*. The head is about 1.5 times wider than long. The post-clypeal seta (4-C) is multiple-branched. The inner frontal seta (5-C) is short and 8-branched. The median frontal seta (6-C) has 5–7 branches and the outer frontal seta (7-C) has 9 branches. The comb consists of 16–22 scales arranged in an irregular row, dorsally in a partly doubled row. The individual scales have a pointed terminal spine. Seta 1-VIII has 5–7, usually 6 branches (Fig. 8.87b), setae 2-VIII and 3-VIII are 2-branched, and 4-VIII and 5-VIII are usually 3–4 branched. Setae 2-VIII, 4-VIII and 5-VIII are at most half as long as seta 3-VIII. The abdominal segment X resembles that of *Cq. richiardii* but the saddle is covered with numbers of rows containing 2–8 spicules on a common base (Fig. 10.137b). The saddle seta (1-X) is 4-branched.

**Biology:** Because of its limited distributional range and rarity, the data on the biology of the species are scanty. The larvae were found attached to the roots of *Acorus* sp. and *Typha* sp. (Coluzzi and Contini 1962). Eggs are laid in boat-shaped rafts (Guille 1975). The females bite humans in open areas (Gutsevich et al. 1974).

**Distribution:** Mediterranean subregion of the Palaearctic. In Europe the species is present in Spain, France, and Italy, and also reported from Romania and Ukraine (Snow and Ramsdale 1999).

### ***Coquillettidia (Coquillettidia) richiardii* (Ficalbi 1889)**

**Female:** The scales on the wing veins are much broader than those of any other European species (Fig. 6.6b). The apex of the proboscis is slightly broader and distinctly darker than the preceding portion, and sometimes the pale scales form a median ring. The base of the proboscis has intermixed

yellowish and brown scales, sometimes with the dark scales predominating. A pale ring is present in the middle of tarsomere I of all the legs, a pattern similar to that of *Cs. annulata* and *Cs. subochrea*. The palps are short, not exceeding 1/4 the length of the proboscis, and are covered with mixed yellowish and brown scales. The vertex has yellowish golden narrow, curved, decumbent scales and dark erect forked scales. The scutum is brown coloured, with narrow, curved brown and golden scales. The mesepisternal and mesepimeral patches have broad, whitish scales. The femora and tibiae are basally sprinkled with yellowish and brown scales, and apically pale scaled. Tarsomere I of all the legs has a pale ring in the middle, which is sometimes indistinct or absent. If so, the legs are mainly covered with pale scales. Broad pale basal rings are usually present at tarsomeres I–III of the fore legs and all tarsomeres of the mid and hind legs. Pale rings are particularly distinct on the hind tarsomeres. The wing veins are covered with broad, intermixed yellowish and brown scales. The terga are brown scaled, with scattered pale scales more numerous at their bases. Basolateral triangular patches of yellowish scales are present, and the scales may form inconspicuous basal bands which are constricted in the middle similar to those of *Oc. punctor*. The sterna are pale scaled.

**Male:** The palps are nearly as long as the proboscis. The lobes of tergum IX have 8–10 setae. The gonocoxite is short and stout. The basal lobe is heavily sclerotized with a strong rod-like spine. The gonostylus is widened basally just above the articulation to the gonocoxite, distinctly constricted and flexed in the middle, enlarged again at the beginning of the distal third and then tapered apically (Fig. 10.136a). The outer side of the gonostylus has 6–7 tiny setae and two more setae on its inner side, just beyond the middle. The apical spine of the gonostylus is short. The apex of the parapect is strongly sclerotized and denticulated.

**Larva:** The head is wider than long. The antennae are very long, 1.5–2.0 times longer than the head. The long-terminal filament is hardly visible on a white background. The antennal seta (1-A) has 15–20 branches. The postclypeal seta (4-C) usually has 5–6 branches, situated anterior to the frontal setae. The inner frontal seta (5-C) is short, matching the postclypeal seta. The median frontal seta (6-C) is long, with 4–5 branches. The outer frontal seta (7-C) has 9 branches. The comb consists of an irregular row of

10–25 scales, each individual scale has a well developed terminal spine (Fig. 10.137). Seta 1-VIII is inserted into the dorsal half of abdominal segment VIII, with 2–4 branches. Setae 2-VIII, 3-VIII, 4-VIII and 5-VIII are articulated medioventrally and have 2–4 branches. The siphon is very short and conical, and forms a piercing apparatus. The siphonal tuft (1-S) is inserted ventrolaterally near the middle of the siphon, and pecten teeth are absent. In addition to 1-S, two pairs of single setae and two pairs of curved spine-like setae with hooked ends are present which support the penetration of the siphon into the plant tissue. Abdominal segment X is elongated, longer than wide, and is completely encircled by the saddle. The saddle is covered with short and stout, usually single spicules, rarely 2 or 3 on a common base (Fig. 10.137a). The saddle seta (1-X) is 2–3 branched, inserted quite apart from the posterior margin. The upper (2-X) and lower (3-X) anal setae are multiple-branched, 2-X is half as long as 3-X. The cratal setae (4-X) have 10–14 tufts and 2 precratal setae (4-X) are widely separated. The anal papillae are lanceolate, subequal in length, and shorter than the saddle.

**Biology:** The species has one generation per year in the north (Service 1969) and 2–3 generations in the south (Gutsevich et al. 1974). The females deposit the eggs in rounded rafts. The larvae hatch in intervals of up to 2 weeks after oviposition (Guille 1975) and usually hibernate in the third or fourth-instar. Larvae and pupae live submerged and obtain oxygen from the aerenchyma of aquatic plants and move very little. Breeding sites may be various permanent water bodies rich in *Acorus* sp., *Typha* sp., *Phragmites* sp., *Glyceria* sp., *Sparganium* sp., *Ranunculus* sp., and *Carex* sp. (Shute 1933; Natvig 1948; Guille 1976). Pupation takes place from end of May to early June. In Serbia, blood searching females have been recorded from June to September, but usually have a seasonal peak during July (Petric 1989). Females can be very numerous and a severe nuisance to humans and domestic animals, in the surroundings of fresh waters or slightly saline marshes, lakes, old river beds, and estuaries. Also frequent indoor feeding on humans has been recorded in England (Shute 1933) and occasionally in Portugal (Ribeiro et al. 1988). Nuisance is usually restricted to the surroundings of the breeding sites but females can use ascendant air currents to invade, in considerable number, areas up to altitudes of 800–900 m (Gilot et al. 1976). Females prefer to



feed on mammals (Service 1968c; Ribeiro et al. 1988; Petric 1989) but may also take their blood meal from birds (Service 1969) and amphibians (Shute 1933). Jaenson et al. (1986b) found the species in horse stables and human bait collections. In England, the peaks of biting activity occurred after sunset and just after sunrise (Shute 1933; Service 1969), while a nocturnal biting activity was typical for a population from Serbia (Petric 1989). Biting activity was recorded at a temperature between 9 and 26°C and a relative humidity between 30 and 92%. Swarming of males could be observed one hour after sunset and at dawn (Marshall 1938). In the laboratory, copulation was observed in small cages of 40 × 40 × 120 cm. The species has been reported as autogenous, but some females may be unable to develop the first egg batch without taking a blood meal (Guille 1975).

**Distribution:** *Cq. richiardii* is a common species throughout Europe and widely distributed in the western Palaearctic region.

**Medical importance:** Females infected with WNV, and Omsk haemorrhagic fever virus (OHF) were detected in wild populations (Detinova and Smelova 1973).

**Note on systematics:** Guille (1975) stated that some authors consider the nearctic species *Cq. perturbans* as a geographic race of *Cq. richiardii*. Similarity in both morphological and biological characteristics of the two species should stimulate further investigation.

## 10.6 Genus *Orthopodomyia* Theobald

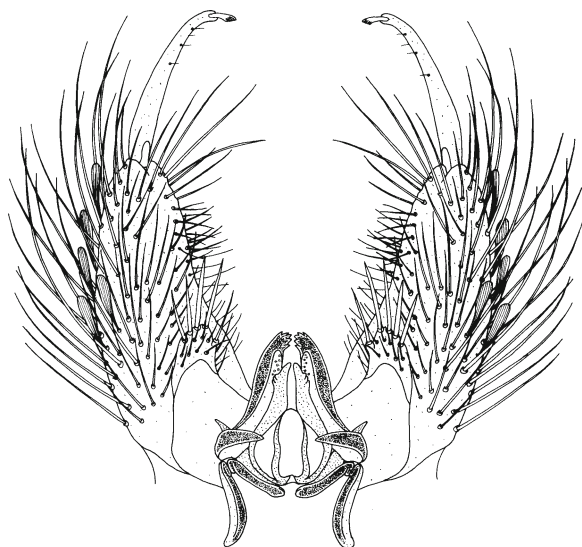
The palps of the females are 1/3 as long as the proboscis. The vertex is covered with erect forked scales. The postpronotum usually has 2 setae, and postspiracular setae are absent. Tarsomeres I on the fore and mid legs are distinctly longer than the other four tarsomeres together. The combined length of tarsomeres IV and V is shorter than tarsomere III of the fore and mid legs. The abdomen is parallel sided with a truncated end, the cerci are blunt and moderately projecting. The palps of the males are slender, and about as long as the proboscis. Palpomere V (sometimes also palpomere IV) is greatly reduced without dense, long setae. The hypopygium is similar to those of the genus *Culiseta* and subfamily Anophelinae. The general colouration of the larvae is red or pink, and violet-blue before pupation.

The antennal seta (1-A) is confined to the basal half of the antennal shaft, with 4 or more branches. The inner and median frontal setae (5-C and 6-C) are long, multiple-branched. The setae of the thorax and abdomen are very long, particularly the lateral setae 6-III to 6-VI. Conspicuous sclerotized plates are present on the dorsal surface of all or at least on one of the segments VI–VIII. The siphon is without a pecten but with a single pair of siphonal tufts (1-S). The siphonal index is at least 2.5, often much more. The saddle completely encircles the anal segment. The ventral brush is made up of 12 or more cratal setae (4-X). The dorsal pair of the anal papillae is longer than the ventral pair. The larvae develop in tree-holes, bamboo stumps, axils of bromeliads (arboreal or dendrolimnocolous species). All species are rare, none of them being known as a nuisance.

The small genus comprises 24 species spread throughout the Neotropical and Oriental regions, a few of them ranging northwards into the Palaearctic and Nearctic regions, some are isolated on Madagascar and Mauritius, but none are found in continental Africa. *Or. pulcripalpis* is the only member of the genus found in the Palaearctic region (Gutsevich et al. 1974; Dahl and White 1978).

### *Orthopodomyia pulcripalpis* (Rondani 1872)

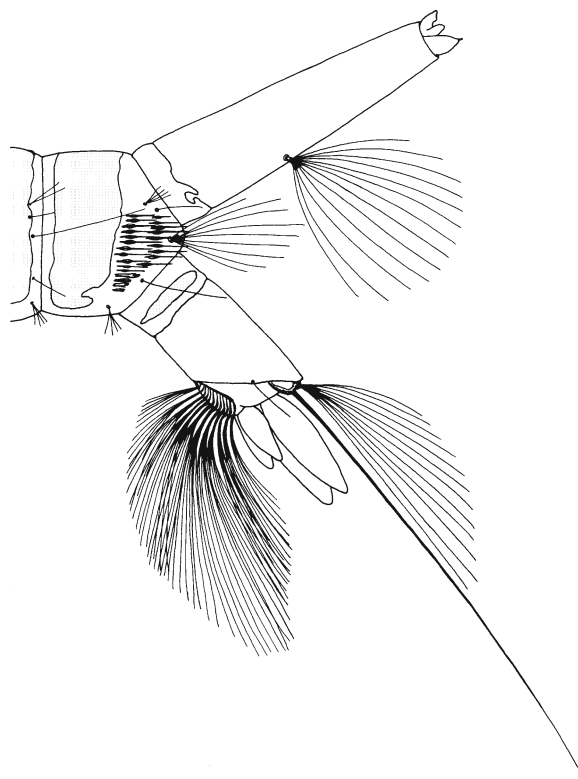
**Female:** It differs from most of the other Palaearctic mosquitoes by its conspicuous pattern of white scales on a black background producing several easily recognised characteristics. The proboscis has black scales and a moderately broad ring of white scales within the apical half. The palps are nearly half as long as the proboscis, black, with a white ring at the base and in the middle, and the apex is white. The antennae are black, the pedicel is covered with white scales, and 3–5 basal flagellomeres may have a median line of white scales. The head is black, and covered with a mixture of black and white scales. The posterior margin of the eyes dorsally is covered with white scales. The scutum is covered with narrow black scales, distinctly ornamented with three pairs of narrow, white longitudinal stripes. A dorsocentral pair of stripes extends from the anterior margin to the posterior third of the scutum, where it is broken. It continues on over the prescutellar dorsocentral area as a wider stripe converging and ending on the scutellum. The second pair



**Fig. 10.138** Hypopygium of *Or. pulcripalpis*

of stripes is confined to the posterior submedian area. The third pair of stripes almost completely borders the scutum. All the legs are covered with black scales with a metallic shine, with white spots on the femoro-tibial and tibio-tarsal articulations. The dorsal surface of the femora is speckled with white scales. Tarsomere IV of the fore legs is not as long as it is broad (Fig. 6.4a). The fore and mid tarsi are entirely black scaled, sometimes with weakly developed, narrow basal white rings, most conspicuous at the base of tarsomere I of the mid legs. Tarsomeres I–IV of the hind legs have white rings at both ends of each tarsomere, and tarsomere V is completely white. The wing veins have dark brown scales, and the bases of the subcosta (Sc) and radius (R) have patches of silvery white scales. The abdomen is blackish brown, covered with scales of the same colour as the scutum, with moderately broad basal bands of white scales.

**Male:** The palps are distinctly longer than the proboscis, black scaled, with white rings at the articulations of the palpomeres, and palpomere V is entirely white. The basal lobe of the gonocoxite is conical, with 4–5 large spine-like setae (Fig. 10.138). The gonostylus is relatively narrow, and slightly expanded in the basal half. The apical spine of the gonostylus is inserted laterally close to the apex, and subdivided into five finger-like serrations. It is longer than the width of the gonostylus at the point of insertion. Claspettes are absent.



**Fig. 10.139** Larva of *Or. pulcripalpis*

**Larva** (Fig. 10.139): In all instars the larvae are easily recognised by the absence of pecten teeth and the pinkish colouration. The head is dark and rounded. The antennae are straight, < 1/4 the length of the head, without spicules. The antennal seta (1-A) is articulated at the end of the basal third of the antennae, with 3–7 short fan-like branches. The postclypeal setae (4-C) are long, with 5–7 branches. The frontal setae are long and multiple-branched. The inner (5-C) is 5–8 branched, the median (6-C) is 9–10 branched extending well beyond the anterior margin of the head, and the outer (7-C) is of similar length and branching as 4-C. The thorax has very long lateral setae. The abdomen distinctly narrows towards the end, with long lateral setae. The section of the main tracheal trunks within the thorax and abdominal segments V–VII is enlarged. Sclerotized plates are usually present on abdominal segment VI, and always present on segments VII and VIII (Fig. 8.4a). The plate is broadest on segment VII and extends down to its lateral sides. On segment VIII it is narrower but nearly encircles the segment. The plate on segment VI is small and narrow,

and restricted to its dorsal part. The comb consists of 23–30 scales arranged in two rows. Most of the scales have a pointed terminal spine. Seta 3-VIII is strongly developed, resembling the siphonal tuft. The siphonal index is 3.5–4.0. The siphonal tuft (1-S) is composed of 8–13 long branches, inserted at the basal 2/5 of the siphon. The pecten is absent, and narrow sclerites are laterally positioned in front of the saddle. The saddle completely encircles the anal segment, and is narrowed ventrally. The saddle seta (1-X) is short and inserted at the posterior margin of the saddle. The upper anal seta (2-X) forms a short, asymmetrical fan with 9–14 branches, the length of the branches increases progressively towards the lower anal seta (3-X). 3-X is single and very long. The ventral brush has 12–14 cratal setae (4-X). The anal papillae are lanceolate, and the dorsal pair is twice as long as the ventral pair.

**Biology:** It is a polycyclic species, the larvae occur throughout the season from May to October in southern Bulgaria (Bozkov et al. 1969) and from June to October in Greece. Hibernation takes place in the fourth larval stage; the larvae can survive the freezing of the water surface (Shannon and Hadjinicolaou 1937). The larvae breed in water with a high pH-value, in tree-holes (dendrolimnocolous) or holes in the roots of elm, oak, beech, horsechestnut, olive, and plane-tree (Shannon and Hadjinicolaou 1937; Munstermann et al. 1985). Large tree-holes that can support a permanent presence of water are preferred breeding sites. Larvae are often found in association with those of *An. plumbeus*, *Oc. pulcritarsis*, *Oc. geniculatus*, *Oc. echinus*, and *Oc. berlandi* (Rioux 1958; Coluzzi 1968; Bozkov et al. 1969). An ornithophilic host preference has been reported (Ribeiro et al. 1988). Any attempt to feed the females on humans in the laboratory failed (Shannon and Hadjinicolaou 1937). According to Gutsevich et al. (1974) the females rarely bite humans and are most active during the day in shaded places.

**Distribution:** *Or. pulcripalpis* is a Palearctic species, mainly distributed in the Mediterranean region, Black Sea coast, and Transcaucasia. In Europe it extends northwards to Belgium and southern England.

**Medical importance:** The vectorial capacity of the genus *Orthopodomyia* is not well studied, although many of its members prefer birds as hosts and probably play a role in amplification of avian arboviruses (Zavortink 1968).

**Note on systematics:** The original spelling *pulcripalpis*, first published by Rondani in 1872, was replaced by the spelling *pulchripalpis* by Verrall in 1901 and adopted by Knight and Stone (1977). The correct original spelling was revived after Snow (1985) and adopted by Ward (1992).

## 10.7 Genus *Uranotaenia* Lynch Arribalzaga

The species of the genus *Uranotaenia* are generally small dark mosquitoes, which are characterized by having short palps in both sexes, the proboscis is usually swollen at the tip and the antennae of the males are very plumose. The scutum has stripes or spots of flat metallic shining scales, the anteprenotal lobes are well separated. The pleural setae are reduced in number, and the scales usually form only 1 or 2 patches or stripes. The wings exhibit a characteristic venation pattern with the anal vein (A) sharply bent apically, ending before or at the same level as the furcation of the cubitus (Cu). The abdomen of the females is blunt ended, and the cerci are short and rounded. The male genitalia have a short, somewhat conical shaped gonocoxite with a moderately developed basal lobe. The gonostylus is short with a small apical spine, and claspettes are absent. The larvae are small with a dark head and short antennae. The inner (5-C) and median (6-C) frontal setae are stout and spine-like in many species. The abdominal segment VIII is covered with characteristic sclerotized plates on its lateral parts. The pecten teeth and siphonal seta (1-S) are present and the saddle completely encircles the anal segment. The larvae rest with their bodies almost parallel to the water surface, unlike most culicines and may be, at the first glance, confused with anophelines. Eggs may be deposited either in boat shaped rafts or laid singly on the water. Very little is known about the adult feeding behaviour.

*Uranotaenia* is a relatively large genus, which occurs mainly in regions with tropical climates and only a few species are found in the more temperate zones of the Holarctic region. About 207 valid species have been recorded in the genus, which is divided into two subgenera, namely *Uranotaenia* Lynch Arribalzaga and *Pseudoficalbia* Theobald (Peyton 1972; Knight and Stone 1977; Ward 1984, 1992). The only species occur-

ing in the Palearctic region, *Ur. unguiculata*, is placed in the subgenus *Pseudoficalbia*, which is characterized by having no suture between the prealar area and the mesepisternum.

### 10.7.1 Subgenus *Pseudoficalbia* Theobald

#### *Uranotaenia (Pseudoficalbia) unguiculata* Edwards 1913

**Female:** A dark mosquito notable for its conspicuous stripes of flat silvery scales along the lateral margin of the scutum. It can be distinguished from all other European species by the shape of its anal vein, which is sharply bent apically and ends slightly before or at the same level as the furcation of the cubitus (Cu) (Fig. 6.2a). The proboscis is blackish brown with patches or an indistinct line of pale scales on the ventral surface, and markedly swollen at the apex. The antenna is brown, and the pedicel has light scales. The head is mainly dark scaled with silvery scales along the eye margins and on the occiput. The scutum is covered with blackish or dark brown scales. Distinct lateral stripes of flat silvery scales stretch from the anterior margin to the wing base. A similar stripe runs across the pleurites stretching from the anteprepronotum to the mesepimeron. The scutellum has blackish brown scales. The legs are largely dark, with patches of white scales at the tips of the femora and tibiae, and pale longitudinal stripes on the anterior surface of the fore and mid femora. The tibiae often have a diffuse pale ring in the middle, with dark brown tarsomeres. The wing veins are dark scaled, and the bases of the subcosta (Sc) and radius (R) have pale scales. The terga are covered with dark brown iridescent scales, often with triangular spots of white scales which can be found mainly on the last segments, and the sterna have light scales.

**Male:** The palps are considerably shorter than the proboscis. The gonocoxite is broad and short, and irregularly conical shaped (Fig. 10.140). The small flattened basal lobe bears several long, stout setae. The gonostylus is broad and flattened dorsoventrally with the inner margin sigmoid shaped, and the apical spine of the gonostylus is pointed.



Fig. 10.140 Hypopygium of *Ur. unguiculata*

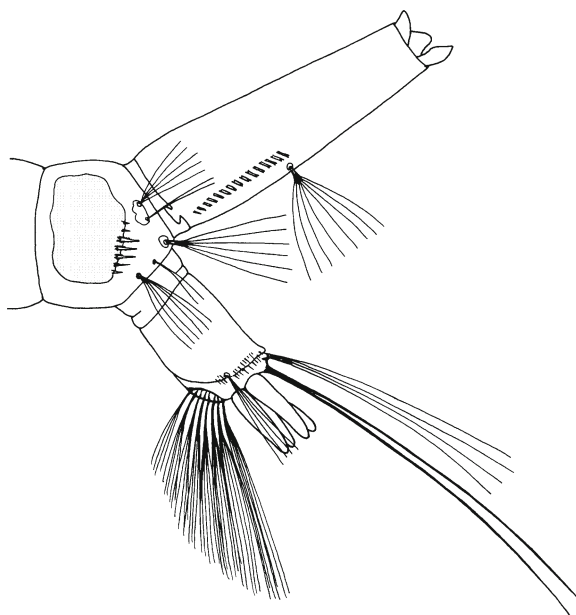


Fig. 10.141 Larva of *Ur. unguiculata*

**Larva** (Fig. 10.141): It is easily recognised by its sclerotized plates on the lateral parts of abdominal segment VIII, from which 5–8, usually 6, dark spine-like comb scales arise in a row along the posterior margin. The antennae are short, almost without spicules and with a tiny single antennal seta (1-A), which arises close to the middle of the antennal shaft. The head is dark and slightly broader than long with the base of the labrum located more anteriorly than in other species. The mouth brushes are distinctly bent

apically. The labral seta (1-C) is strongly developed. This is an adaptation to the feeding behaviour of the larva, which feeds on the surface film from below, while bending its head backwards with the body in a horizontal position. The inner (5-C) and median (6-C) frontal setae are long, stout and single, 5-C rarely 2–3 branched. The inner setae are located close together behind the median setae. The outer frontal setae (7-C) have 4–7 branches. Abdominal segment VIII has the above mentioned sclerotized plates laterally. The siphon is nearly conical and slightly tapered towards the apex, with a siphonal index of 3.2–4.0. The pecten consists of 13–20 weakly pigmented teeth, with the distal tooth reaching to the point of insertion of the siphonal tuft (1-S). The tuft has 7–12 branches and arises near the middle of the siphon. The saddle completely encircles the anal segment, and its posterior margin is covered with distinct tiny spicules. The saddle seta (1-X) is 3–5 branched, and is about the same length as the saddle. The ventral brush has 8–11 multiple-branched tufts of cratal setae (4-X), and pre-cratal setae are absent. The anal papillae are lanceolate, pointed or rounded at the end and generally shorter than the anal segment.

**Biology:** The favoured larval breeding sites of *Ur. unguiculata* are pools, ditches, or canals with stagnant or little flow of water and a rich growth of aquatic vegetation. They are also common in shallow

shores of lakes, which are overgrown with *Lemna* sp., *Scirpus* sp., and *Phragmites* sp. They can also be found often in shaded localities. The larvae prefer fresh water and are only occasionally found in water with a slight salinity. They often occur together with larvae of *An. hyrcanus*, *An. sacharovi*, *Cx. p. pipiens*, *Cx. modestus*, and *Cx. theileri*. Larvae of *Ur. unguiculata* can be found from May to early October with a peak in August. The adults are most abundant in late summer, this may lead to the presumption that the species hibernates in the adult stage. It seems likely that the females of *Ur. unguiculata* do feed on blood, but rarely bite humans or mammals even though they may be capable of doing so. In a population from Turkmenistan autogeny has been observed (Rioux et al. 1975). Other species of the genus are known to feed on amphibians (Remington 1945).

**Distribution:** *Ur. unguiculata* is a frequent species throughout the Mediterranean region. In Europe, its distribution range stretches as far north as Germany (Becker and Kaiser 1995). In eastern Europe the species can be found in the southern Ukraine and the Volga delta with further occurrence in Middle and southwest Asia to Iran and Pakistan.

**Note on systematics:** A subspecies of *Ur. unguiculata*, ssp. *pefflyi* is recorded from the Arabian peninsula (Knight and Stone 1977).

## Part III

# Identification Keys, Morphology, Ecology and Distribution of Important Vector and Nuisance Species – Worldwide

Reaching the final number of species to be included on the list was not an easy task, especially for the nuisance species since the literature about this aspect of their impact was quite scarce and outdated. Additionally, the question: “Are the primary vector mosquitoes also nuisance mosquitoes?” was difficult to answer. Clearly with species such as *Ae. aegypti*, *Cx. p. quinquefasciatus*, *An. funestus* and *An. gambiae* (Gillies and deMeillon 1968, Doannio et al. 2006, Kulkarni et al. 2007), they are both. However in many cases it is difficult to establish the relative importance of mosquito nuisance and mosquito-borne disease. Even for one species, factors such as local meteorology, socio-economic development, or the occurrence or introduction of pathogens, may alter the relative importance of the two types of impact in different parts of its range. In selecting species to include in this book, we have gave higher priority to disease transmission, as this can occur even with low numbers of mosquitoes, while nuisance occurs only when relatively high populations are present.

The importance of *Anopheles* is confined almost exclusively to the tropical and subtropical parts of the world, corresponding primarily to their role in transmission of malaria. In many temperate areas, such as most of Europe and the United States, socio-economic development and public health measures have succeeded in eradicating malaria. For example, no anophelines from the U.S.A. have been included in the key or in the species description, because of their low current status as potential vectors in this region.

Of the ca. 3500 species of Culicinae, the large majority are harmless to man. Most of them are adapted to diverse animal hosts and feed on human blood only occasionally, if at all (*Malaya*, *Toxorhynchites*). Many are either so rare as to be of no importance, or so rural and modest in habits that they cannot be regarded as

nuisance and/or vector species. Perhaps about 5% are to be regarded as representing a serious threat on account of their vicious biting habits and/or vectorial capacities, based upon a preference for human blood as food.

The selected species are divided into two groups. The first includes those species that are recognised as major disease vectors and/or which are responsible for severe biting nuisance, and which are therefore considered to be of primary importance. For these species a description of the fourth-instar larvae and the adult female, the biology, medical importance and distribution range are provided. The second group includes those species with lower vectorial capacity and/or limited distribution, that are considered to be of secondary importance. Although some species responsible for locally important biting nuisance, and/or which are vectors of potential or secondary importance have therefore been excluded from the keys and description, most of these species are nonetheless mentioned in the introductory paragraphs for each continent, and their general distribution according to Walter Reed Biosystematics Unit (WRBU) is given.

Keys are provided for adult females only, bearing in mind that it is the blood-seeking individuals that are most often encountered. In addition, we provide descriptions for the larval stage as well as species biology and distribution. However, descriptions of male genitalia are omitted given that encountering male mosquitoes is less likely, and adult male sampling is usually carried out only in proximity to breeding sites.

All the characters used in the keys have been selected according to the species composition in each continent.

Some cosmopolitan nuisance and/or vector species are included in the keys of most continents (*Ae. aegypti*, *Ae. albopictus*, *Cx. p. pipiens* biotype *molestus* and

*Cx. p. quinquefasciatus*) and have been described in the chapter on the European mosquitoes. Some other widely distributed species are included in the keys of more than one continent, and described under the continent where they are most important.

Having selected only a few out of hundreds of species in each region, it was not possible to create short,

precise yet completely unambiguous keys. In order to cope with this problem and to improve the level of accuracy, some important additional characters were usually included (in smaller font and after a dash at each thesis/antithesis) to guide the reader through the numerous genera and species of the region, that are not mentioned in the text nor included in the key.

## Chapter 11

### Africa

There is no doubt that with statistics of several hundred bites/man/night inflicted by *Cx. p. quinquefasciatus*<sup>1</sup> and *Cx. antennatus* in Kinshasa (Coene 1993; Karch et al. 1993), these mosquitoes could be classified as both voracious biters and important vectors, at least in some countries. Unfortunately, the vectorial capacity, pest status, and distribution range cannot be defined so clearly for other important species. African anophelines of secondary importance that could be involved in malaria transmission are *An. labranchiae*<sup>1</sup> (North Africa), *An. merus* (costal East Africa) and *An. sergentii*<sup>1</sup> (North Africa). *Ae. furcifer* [*Diceromyia furcifer*] (with a patchy distribution in eastern, western and southern Africa) may also be involved in the transmission of Chikungunya, dengue and yellow fever viruses (Germain et al. 1980; Jupp 1996; Diallo et al. 1998).

Apart from being potential vectors of malaria, yellow fever, Sindbis, Chikungunya, Rift Valley fever and West Nile viruses (Huang 1990; Jupp 1996), species included in the next group can cause considerable nuisance where they occur: *An. multicolor*<sup>1</sup> (northern Africa); *An. rufipes* (central and southern Africa); *Ae. albocephalus* (south eastern, southern and south western Africa); *Ae. circumluteolus* [*Neomelaniconion*

*circumluteolum*] (central and southern Africa); *Ae. africanus* [*Stegomyia africana*] (central Africa); *Oc. caspius*<sup>1</sup> (northern Africa); *Oc. detritus*<sup>1</sup> (northern Africa); *Oc. caballus* (eastern and southern Africa); *Cx. duttoni* (central and southern Africa) and *Cx. p. pipiens* biotype *molestus*<sup>1</sup> (northern Africa) (Scirocchi et al. 1990; Jupp 1996; Hassan and Onsi 2004; Muturi et al. 2007). Furthermore, *Ae. africanus*, *Ae. luteocephalus* [*Stegomyia luteocephala*] (Tropical Africa), *Ae. opok* [*Stegomyia opok*] (central Africa), *Ae. taylori* [*Diceromyia taylori*] (central and southern Africa) could be important dengue vectors in the Afro-tropical region (Rueda 2004).

In addition, *Cx. sitiens* is listed as a major pest in Australia and consequently described there, but is also included in the key to African females, although it is of lesser importance there. The same is true for *Cx. tritaeniorhynchus*, described in the Asia section. *Cx. neavei* is given in the key only in order to avoid confusion (misidentification) with the very similar *Cx. univittatus*.

For further reading on the morphology, identification, biology, distribution, and disease vector status of the species mentioned, the reader is referred to Kerr (1933), Evans (1938), Edwards (1941), De Meillon (1947), Lewis (1948), Hopkins (1952), Muspratt (1955, 1956), Gillies and De Meillon (1968), Mattingly (1971), Smith (1973), McIntosh (1986), Anonymous (1993, 2000), Jupp (1996), Huang (2001), Rueda (2004).

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<sup>1</sup>Species described in Chaps. 9 and 10 concerning European mosquitoes



### 11.1 Key to African Female Mosquitoes

- 1 Palps as long as proboscis. Scutellum evenly rounded or slightly trilobed and uniformly setose (Fig. 11.1a). Abdominal terga and sterna completely or largely missing scales. – Proboscis is neither strongly recurved nor tapering towards apex. *Anopheles* ..... 2
- Palps distinctly shorter than proboscis. Scutellum trilobed, setae arranged in three sets (Fig. 11.1b). Abdominal terga and sterna uniformly and densely covered with scales. – Proboscis is neither strongly recurved nor tapering towards apex. Prespiracular setae absent. Anal vein (A) evenly curved, ending distinctly beyond furcation of cubitus (Cu). Alula with narrow fringe scales. .... 4

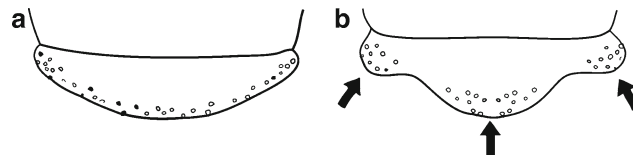


Fig. 11.1. Scutellum of: (a) *Anopheles* sp.; (b) *Aedes* sp.

- 2 (1) Abdominal segments with laterally projecting tufts of dark scales on terga II–VII (Fig. 11.2a). – Hind tarsomeres I–IV at least with apical pale rings, hind tarsomere V and about apical half of IV pale. Wing with abundant pale areas, costa (C) with at least 4 pale spots. .... *An. pharoensis* (p 326)
- Abdominal segments without laterally projecting tufts of scales (Fig. 11.2b). – Hind tarsomeres IV and V not entirely pale, sometimes almost entirely dark. .... 3

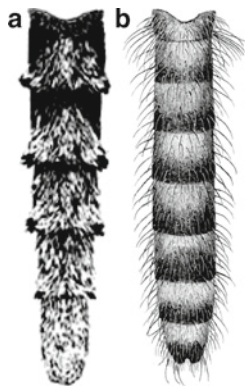


Fig. 11.2 Abdomen of: (a) *An. pharoensis*; (b) *Anopheles* sp.

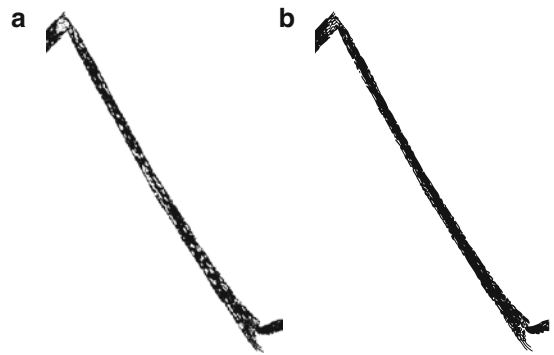


Fig. 11.3 Tibiae of: (a) *An. gambiae* s.l.; (b) *An. funestus*

- 3 (2) Legs speckled with pale scales (sometimes sparsely) (Fig. 11.3a). – Palps with 3–4 pale rings. Tarsomeres I–IV with conspicuous pale rings at least at their apices. Wing with a pale interruption on vein R<sub>1</sub> (sometimes fused with the preceding pale area) within the third main dark area (Fig. 7.3a). Pale fringe spots at terminations of all veins, sometimes absent at tips of Cu<sub>2</sub> and/or A. .... **Anopheles Gambiae Complex** (p 323)

Legs not speckled, mainly dark (Fig. 11.3b). – Palps with 3 pale rings, terminal comprising the apex. Subapical pale ring much narrower than subapical dark ring. Hind tarsomeres dark or with narrow apical pale rings. Wing without pale interruption within third main dark area. Pale spots on the wing not confined to costa (C) and R<sub>1</sub>. Costa with at least one pale spot on its basal half. Basal area of vein R<sub>1</sub> entirely pale, preaccessory dark spot on R<sub>1</sub> absent or if present, narrower or only slightly broader than adjoining pale spots. Vein Cu<sub>1</sub> with one pale spot. Tip of anal vein (A) dark, no fringe spot present. .... *An. funestus* (p 322)

- 4 (1) Postspiracular setae absent (Fig. 11.4a). Pulvilli present, well developed (pad-like). – Antenna with flagellomere I about as long as flagellomere II. Erect forked scales not restricted to occiput, numerous on vertex too. Scutum without silvery spots. Scutellum with all scales narrow. Paratergite without scales. Femora and tibiae either without longitudinal stripes, or if stripes are present on any of them, the hind tibia has a pale apical spot and the anterior stripe does not reach the apex. Tarsomere I of fore legs usually shorter than tarsomeres II–V together. Tarsomere IV of fore legs not reduced, distinctly longer than broad. All tarsal claws simple. Wing scales narrow. Abdomen rounded apically, cerci short, hardly visible. *Culex*..... 5
- Postspiracular setae present (Fig. 11.4b). Pulvilli absent or not well developed (hair-like). – Back of the head, pleurites, and posterolateral corners of abdominal terga not covered with broad silvery scales. .... 10

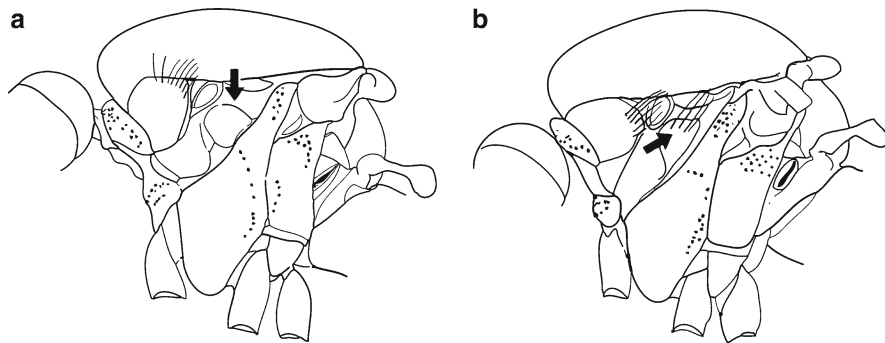
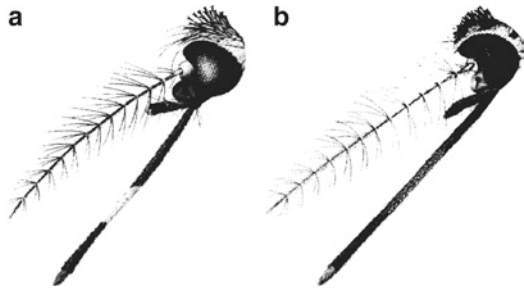


Fig. 11.4 Lateral view of thorax of: (a) *Culex* sp.; (b) *Mansonia* sp.

- 5 (4) Proboscis with distinct broad median pale ring (Fig. 11.5a). Lower mesepimeral setae absent. Tarsomeres of all legs with narrow, indistinct basal and apical pale rings. Rings usually more distinct on tarsomeres I–III, on bases of tarsomeres and on hind legs..... 6
- Proboscis without pale ring in the middle but often with a pale midventral area (Fig. 11.5b). Usually only one lower mesepimeral setae present. Tarsi all dark. .... 7
- 6 (5) Anterior surface of mid femur with numerous scattered pale scales (Fig. 11.6a). Tibiae sometimes with indistinct longitudinal pale stripes. Small apical patches or bands of pale scales sometimes present on tergum VII and/or VIII..... *Cx. sitiens* (p 366)
- Anterior surface of mid femur without scattered pale scales (Fig. 11.6b). Tibiae dark anteriorly. Tergum VII and/or VIII without apical bands or patches. – Anterior and middle part of scutum predominantly dark scaled, numerous pale scales present near scutellum..... *Cx. tritaeniorhynchus* (p 349)

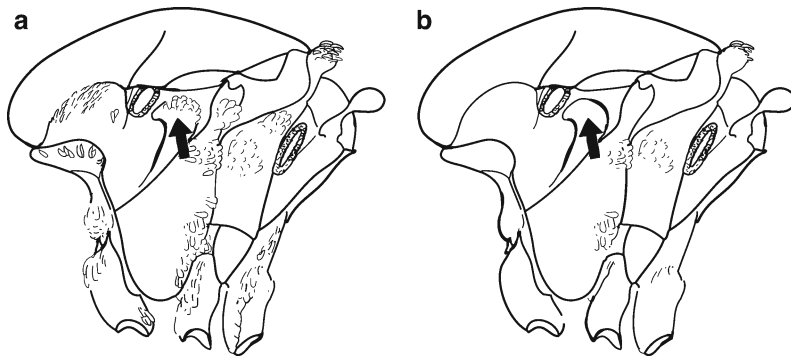


**Fig. 11.5** Proboscis of: (a) *Cx. sitiens*; (b) *Cx. univittatus*



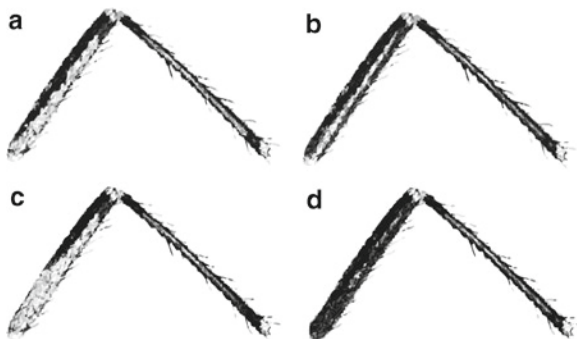
**Fig. 11.6** Mid leg of: (a) *Cx. sitiens*; (b) *Cx. tritaeniorhynchus*

- 7 (5) Postspiracular scales present (Fig. 11.7a). Prealar scales present. Costa (C) with small spot of pale scales on lower edge close to base. .... 8
- Postspiracular scales absent (Fig. 11.7b). Prealar scales absent. Wing scales entirely dark. .... 9

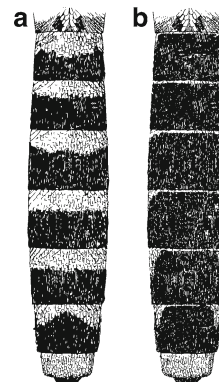


**Fig. 11.7** Lateral view of thorax of: (a) *Cx. univittatus*; (b) *Cx. p. quinquefasciatus*

- 8 (7) Mid femur with longitudinal pale stripe rarely broken (Fig. 11.8b). Hind femur with long dark apical dorsal stripe starting within 1/5 from its base (Fig. 11.8a). Terga with broad white basal transverse bands rounded posteriorly and confluent with basolateral patches. .... *Cx. univittatus* (p 329)
- Mid femur without longitudinal pale stripe or rarely broken stripe is present (Fig. 11.8d). Hind femur with short dark apical dorsal stripe starting within about 1/2 from its base (Fig. 11.8c). Terga with narrow white basal transverse bands sometimes reduced to median spot. Bands or spots absent on terga VI–VII. .... *Cx. neavei*



**Fig. 11.8** Hind and mid femora and tibia of: (a, b) *Cx. univittatus*; (c, d) *Cx. neavei*



**Fig. 11.9** Abdomen of: (a) *Cx. p. pipiens*; (b) *Cx. antennatus*

- 9 (7) Terga with well developed pale basal bands (Fig. 11.9a). – Scutal scales usually fawn (pale brown) coloured (reddish-brown in *Cx. p. pipiens*). Pale spot at tip of hind tibia inconspicuous. Veins  $R_2$  and  $R_3$  just about 2.5 times as long as  $R_{2+3}$ , ..... *Cx. p. pipiens biotype molestus* and *Cx. p. quinquefasciatus* (p 277, 278)
- Terga, at least the first few, without pale basal bands (Fig. 11.9b). – Mesepimeron with a distinct patch of scales in the middle. Tergum V with small basolateral patch of pale scales and terga VI and VII with broad lateral longitudinal stripe of pale scales. .... *Cx. antennatus* (p 328)
- 10 (4) Claws of fore legs with subbasal tooth or wing scales narrow (Fig. 11.10a) or both. – Proboscis without a white ring. Head with erect forked scales not numerous, restricted to occiput. Scales of scutellum all broad. Postprocoxal membrane without scales. Femora with white knee spot. Mid femur without three large white patches on anterior surface. Hind tarsomere V entirely white. *Aedes* subgenus (*Stegomyia*). ..... 11
- Claws all simple. Wing scales usually broad and conspicuous (Fig. 11.10b), many asymmetrical. – Erect forked scales numerous, not restricted to occiput. Subspiracular area without scales. *Mansonia* ..... 12

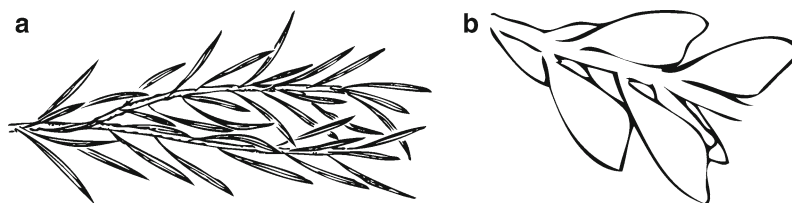


Fig. 11.10 Wing scales of: (a) *Aedes* sp.; (b) *Mansonia* sp.

- 11 (10) Scutum without acrostichal stripe on anterior part, but with two narrow white dorsocentral stripes separated from anterior margin. Lateral white stripes broad, continuing over transverse suture to the end of scutum, lyre shaped (Fig. 11.11a). – Clypeus with white scale patches. Mesepimeron with two well separated white scale patches. Anterior portion of mid femur with a longitudinal white stripe..... *Ae. aegypti* (p 198)
- Scutum with a white acrostichal stripe extending from the anterior margin to the beginning of the prescutellar area, where it forks to end at the anterior margin of the scutellum (Fig. 11.11b). – Clypeus without white scale patches. Mesepimeron with white scale patches forming V-shaped pattern. Anterior portion of mid femur without a longitudinal white stripe. .... *Ae. albopictus* (p 201)

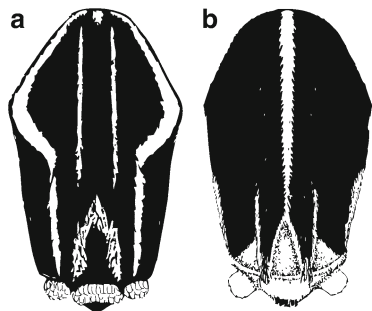


Fig. 11.11 Scutum of: (a) *Ae. aegypti*; (b) *Ae. albopictus*

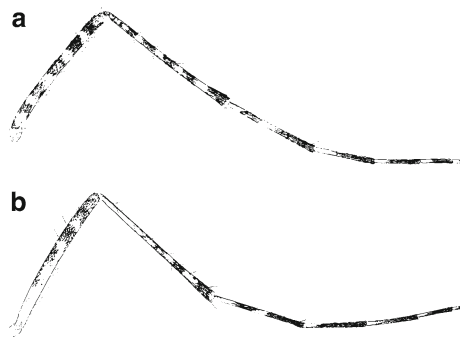


Fig. 11.12 Hind leg of: (a) *Ma. africana*; (b) *Ma. uniformis*

- 12 (10) Submedian area of scutum with three pairs of pale patches or stripes. Median area without distinct dorsocentral stripes of pale scales. Anterior surfaces of mid and hind tibiae with about 6–10 detached pale patches (Fig. 11.12a). Basal half of hind femur with rings of dark scales reaching ventral margin. .... *Ma. africana* (p 330)
- Submedian area of scutum with a pair of continuous pale longitudinal stripes. Central area of scutum with dorsocentral stripes of pale scales. Anterior surfaces of mid and hind tibiae with mostly confluent creamy white patches (Fig. 11.12b). Basal half of hind femur paler, dark scales restricted to upper anterior surface. .... *Ma. uniformis* (p 330)

## 11.2 Species Description

### *Anopheles (Cellia) funestus* Giles 1900

**Female:** A very small dark species. Proboscis dark scaled, palps with 3 pale rings of variable but moderate width, the apical pale ring embracing entire palpomere V or only a part of it, but tip of palps always pale, the median pale ring at joint of palpomeres III and IV, the dark intervening ring usually considerably longer than either pale ring, the basal pale ring at apex of palpomere II and sometimes including base of palpomere III. Vertex with pale erect scales, darker erect scales on occiput, frontal tuft moderately developed, with about 6–10 long, whitish scales. Integument of scutum with a median pale area and contrasting dark brown areas laterally, median area clothed with very narrow pale scales. Integument of pleurites dark brown, with pale transverse bands, prespiracular setae sometimes absent, when present, very small and about 1–3 in number. Fore coxae sometimes with a few scales on anterior part, remainder without scales. Legs predominantly dark scaled, tibiae usually with small apical pale spots (Fig. 11.3b). The hind tarsi are usually entirely dark, but sometimes the scales at the extreme apices of the tarsomeres are obscurely pale, giving the appearance of very narrow indistinct rings. Wing veins predominantly dark scaled, the degree of pale and dark scaling variable. Costa (C) usually with 4 pale spots always markedly shorter than the corresponding dark areas, but varying in width, basal quarter of costa usually entirely dark, sometimes with one pale interruption. Vein

$R_{4+5}$  usually entirely dark scaled, sometimes with a pale area in the middle. Wing fringe with pale spots at the terminations of the veins except anal vein (A), spots sometimes very small and indistinct. Integument of abdomen dark brown, devoid of scales, including the cerci, and clothed with numerous pale setae.

**Larva:** A small dark species with unusually large tergal plates. Head capsule with two dark brown pigmented transverse bands, antenna about half as long as the head, usually heavily pigmented, with numerous and conspicuous spicules. Antennal seta (1-A) minute, inserted at about 1/4 to 1/3 from the base of the antennal shaft. Inner and outer clypeal setae (2-C and 3-C) slender, single, the pair of 2-C well separated, 3-C slightly more than half as long as 2-C. Postclypeal seta (4-C) single, distinctly shorter than 3-C, exceeding the base of 2-C by about 1/2 of its own length, frontal setae (5-C to 7-C) long and plumose. Prothoracic setae 1 and 2 (1-P and 2-P) well developed, arising from large sclerotized tubercles, which are broadly fused, 1-P sometimes with markedly flattened stem, 2-P twice as long as 1-P or longer, with 14–16 branches, 3-P about half as long as 1-P, single. Metathoracic palmate seta (1-T) well developed with slender lanceolate leaflets. The ventral surfaces of the abdominal segments bear conspicuous rows of spicules, especially evident on segments IV–VI. Palmate setae well developed on abdominal segments I–VII (1-I to 1-VII), those of segments I and II smaller than the others. Typical leaflets on segments III–VII light in colour distally with a contrasting dark pigmented area just basal to shoulders, filaments long and slender, serrate at base

and sharply differentiated, about half as long as the blades. Tergal plates very large and sometimes heavily pigmented, on abdominal segments IV–VII they are more than half as long as the segment, and distinctly wider than the distance between the bases of the palmate setae of each segment, the shape of the plates is rather variable, but commonly somewhat rectangular with the posterior margin slightly convex (Fig. 11.13). Setae 0 on segments III–VII (0-III to 0-VII) more prominent than in most other Anophelines, usually with 1–2 branches situated on the tergal plate, occasionally at or just outside its lateral margin. Lateral abdominal setae 6 on segments IV and V (6-IV and 6-V) long, usually with about 3 branches. Pecten commonly of irregular appearance, with 5–6 long and 6–7 short teeth, arrangement variable, the short teeth and some long ones finely serrated in basal half, saddle seta (1-X) long and single.

**Biology:** Larvae of *An. funestus* can mainly be found in bodies of clear water that are either large and more or less permanent, e.g. swamps, weedy banks of streams, rivers, furrows or ditches, protected portions of lake shore, ponds, etc., especially when weedy, or water such as seepages, which are fed from underground permanent sources, and sometimes in flooded rice fields (Evans 1938). The larvae show little tolerance for saline waters and they have never been found in brackish water in nature; they may stay submerged for

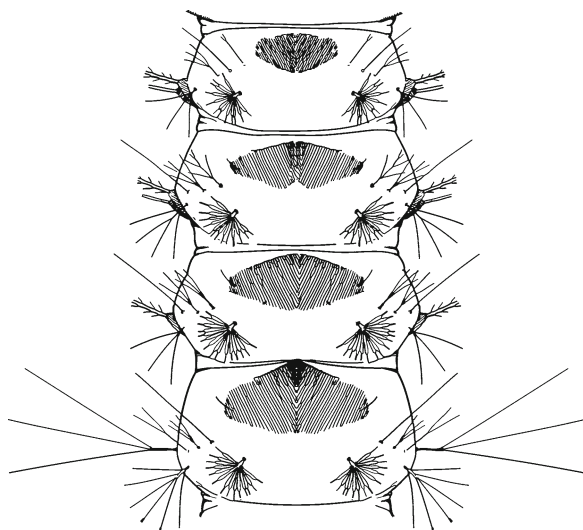
long periods when disturbed. Apart from its important role in malaria transmission, *An. funestus* may act as a major pest and is closely adapted to humans in many aspects of its behaviour. It is one of the most anthropophilic mosquitoes known, in many areas attacking humans even in the presence of abundant alternative hosts such as sheep and cattle. The females usually feed inside houses after 2200 hours up to dawn, entering of the houses starts one hour after sunset with a peak around 2300 hours (Gillies and De Meillon 1968). After the blood meal they stay inside the houses and rest on the upper part of the walls or in the roof. In many areas *An. funestus* spends the greater part of its adult life in houses, which has made it one of the most vulnerable species to control with residual insecticides (Gillies and De Meillon 1968).

**Distribution:** Widely distributed in tropical and southern Africa

**Medical importance:** *An. funestus* is generally regarded as one of the most important vectors in Tropical and South Africa, and is a vector of bancroftian filariasis (Evans 1938; Gillies and De Meillon 1968).

### *Anopheles Gambiae* Complex

This complex comprises, so far, a group of seven sibling species. Some of them are the major vectors of human malaria and other diseases in Sub-Saharan Africa, others are of little importance or vary in importance with local conditions. Formerly regarded as a single species with ecological salt water variants, the complex has been shown to include at least four freshwater species, *An. gambiae* s.s., *An. arabiensis*, *An. quadriannulatus* s.s. (species A), and *An. quadriannulatus* species B, one species which breeds in highly mineralized water, *An. bwambae*, and two salt water species, *An. melas* and *An. merus*. No morphological characters that can universally be applied for differentiation have been found, and although characters for separating the species at the population level have been demonstrated by Coluzzi (1964), the identification of individual specimens is often impossible (Gillies and Cotzee 1987). The separation of the sibling species was formerly based on differences in the banding pattern of polytene chromosomes in the ovarian nurse cells (Hunt 1973; della Torre 1997) and enzyme variation detected via gel electrophoresis



**Fig. 11.13** Larva of *An. funestus*, terga I–IV

(Mahon et al. 1976; Miles 1979), and it is now mainly based on molecular techniques (Scott et al. 1993; Fanello et al. 2002). Thus, a description of the general morphological characters of females and larvae is given, followed by a short summary of the bionomics and distribution of the species of the complex. For further information on the distribution see Coetzee et al. (2000).

**Female:** A medium sized species. Proboscis entirely dark scaled, labellum somewhat lighter. Palps with 3–4 pale rings. When 3 rings are present, the apical one is broad, including the entire palpomere V and the apex of palpomere IV. The subapical and basal rings are much narrower, located at the apices of palpomeres II and III. The broad apical ring may be interrupted by a dark area, resulting in the formation of 4 pale rings. Pedicel with a few pale scales. Vertex with a prominent frontal tuft of long pale setae projecting forwards. Integument of scutum and pleurites of variable colour, usually light brown or greyish. Median area of scutum covered with creamy or yellowish scales of moderate width except near transverse suture, where the scales are broad but somewhat transparent. Upper and lower mesepisternum with groups of 3–5 setae each, 8–13 upper mesepimeral setae usually present. Femora, tibiae, and tarsomeres I of all legs spotted, speckled, or mottled to various extents (Fig. 11.3a). Fore tarsi with broad pale rings involving both ends of tarsomeres I–III, tarsomere IV with a pale ring at apex, tarsomere V entirely dark. Hind tarsi with narrow, pale rings at apices of tarsomeres I–IV, tarsomere V usually entirely dark. Wing field largely pale but with much variation of the pale and dark markings. A pale interruption on vein  $R_1$  (sometimes fused with the preceding pale area) within the third main dark area. Pale fringe spots at terminations of all veins, sometimes absent at tips of  $Cu_2$  and/or A. Additionally a pale fringe spot usually present between  $Cu_2$  and anal vein (A) and basal to vein A. Integument of abdomen light brown, mainly closed with pale setae. Tergum VIII and sometimes tergum VII with a variable number of narrow yellowish scales, sterna usually devoid of scales.

**Larva:** Antenna covered with small and inconspicuous spicules. Antennal seta (1-A) short and single, inserted below the middle of the antennal shaft. Inner clypeal seta (2-C) slender, aciculate or with a varying number of fine lateral branches in the distal half. The pair of 2-C inserted wide apart, much closer to 3-C than to each other. Outer clypeal seta (3-C) usually less than

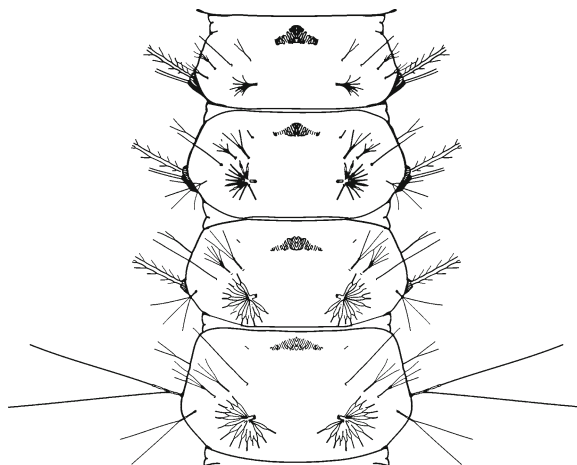


Fig. 11.14 Larva of *An. gambiae* s.s., terga I–IV

half as long as 2-C, single or with few lateral branches, postclypeal seta (4-C) very slender and shorter than 3-C, with 1–3 branches. Frontal setae (5-C to 7-C) plumose, middle frontal seta (6-C) much shorter than inner frontal seta (5-C) and outer frontal seta (7-C) about half as long as 5-C or less. Prothoracic seta 1 (1-P) without conspicuous basal tubercle, poorly developed, with 5–14 branches, 2-P with small basal tubercle, about 1.5 times as long as 1-P, with 10–13 branches, 3-P about half as long as 2-P, single. Palmate seta on abdominal segment I (1-I) poorly developed, with about 7–11 narrow undifferentiated leaflets, palmate setae 1–III to 1–VII fully developed, usually small, with about 14–18 lanceolate and distinctly shouldered leaflets, the filament about 1/2 as long as the blade. Lateral abdominal setae on segments IV–VI (6-IV to 6-VI) split into 2–3 slender branches near base (Fig. 11.14). Tergal plates very small, that on segment V not wider than half the distance between the bases of the pair of palmate setae (1-V). Pecten with about 4–5 long teeth regularly alternating with groups of 3–6 short teeth (arranged more irregularly with no definite pattern in *An. melas*), only the short teeth finely serrated. Saddle seta (1-X) single.

#### ***Anopheles (Cellia) gambiae* s.s. Giles 1902**

This species is regarded to be currently in the state of diverging into two different species, the Mopti (M) and Savannah (S) strains, although the two strains are still considered as a single species (Gentile et al. 2001;

della Torre et al. 2005). The genome of *An. gambiae* s.s. was sequenced in 2002, but there is controversy over the choice of the strain used.

**Biology:** The larvae may occur in a great variety of breeding places, especially in shallow open collections of water completely or partially exposed to direct sunlight, e.g. ground pools, transient rain pools, drains, car tracks, hoof prints around ponds, pools left by receding rivers, water holes and rainwater collections in depressions (Gillies and De Meillon 1968). More permanent habitats such as recently flooded rice fields and edges of seasonal swamps are also occupied by the larvae of this species. In all mentioned breeding sites they are often associated with the larvae of *An. arabiensis*. *An. gambiae* s.s. is capable of growing very rapidly; under optimal conditions, the development from the egg to the adult may be completed within 6–8 days (De Meillon 1947). The females are regarded as being highly anthropophilic and readily bite humans mainly indoors but also outdoors. The main biting activity takes place during the night, particularly from midnight to 0400 hours. Depending on local populations, the resting and feeding behaviour is diverse. Resting indoors after the blood meal is widespread, but it is also known that populations in the West African savannah show a tendency to fly out of houses after feeding (Gillies and Coetzee 1987). The females take their first blood meal usually 24 h after emergence; a high proportion of them feed again the same night after oviposition. The dispersal capacity of the females is considered to be low; usually they are found within 1–3 km from their breeding sites. Apart from this, the long range transport by planes or ships is well documented, e.g. the invasion and establishment of the species in north eastern Brazil in the 1930s, followed by the most devastating epidemics of malaria in tropical America with > 20,000 fatal cases. A joint eradication campaign finally led to the extinction of this alien species in Brazil in 1941 (Soper and Wilson 1943).

**Distribution:** Tropical and southern Africa

**Medical importance:** This species is one of the most important vectors of malaria in Africa; wherever it occurs it is responsible for intense disease transmission. Apart from its association with high endemic malaria it is also responsible for epidemics and is a vector of periodic bancroftian filariasis as well (Gillies and De Meillon 1968).

### ***Anopheles (Cellia) arabiensis* Patton 1905**

**Biology:** As noted above, no major differences in the larval biology of *An. arabiensis* and *An. gambiae* s.s. have been discovered. The females of *An. arabiensis* are also highly anthropophilic, but when alternative mammalian hosts are available, they show a much greater tendency than the females of *An. gambiae* s.s. to feed on these animals. In addition, they show a higher tendency to feed and rest outdoors (exophagic, exophilic). In general *An. arabiensis* tends to predominate in arid savannas, whereas *An. gambiae* s.s. is the dominant species in humid forest zones (Coetze et al. 2000).

**Distribution:** Tropical and southern Africa, Saudi Arabia, Yemen

**Medical importance:** As with *An. gambiae* s.s., *An. arabiensis* is an important vector of malaria wherever it occurs.

### ***Anopheles (Cellia) quadriannulatus* s.l. Theobald 1911**

The sibling species A and B of *An. quadriannulatus* are recognised as allopatric members of the *Anopheles* Gambiae Complex, based on evidence of genetic incompatibility between crosses of southern African and Ethiopian populations. *An. quadriannulatus* s.s. (species A) is widespread in southern Africa, whereas *An. quadriannulatus* species B occurs in Ethiopia (Hunt et al. 1998; Fettene et al. 2002; Fettene and Temu 2003).

**Biology:** The breeding preferences of the larvae of *An. quadriannulatus* s.l. are not essentially different from those of the other freshwater species. The larvae could be found in semi permanent pools in dry river beds (Gillies and Coetzee 1987). The females exhibit a strong zoophilic biting behaviour, e.g. biting of cattle and other domestic animals is very common. The biting activity reaches a peak before midnight and decreases towards dawn. This species shows a tolerance to relatively cool conditions on highland plateaux (White 1974).

**Distribution:** Widespread in southern Africa (*An. quadriannulatus* s.s.), reported from Mozambique, South Africa, Swaziland, Uganda, Zambia, Zimbabwe, and Ethiopia (*An. quadriannulatus* species B).

**Medical importance:** Because of its strong zoophilic biting behaviour, this species is not regarded as an important vector of malaria.



### ***Anopheles (Cellia) bwambae* White 1985**

The main characteristic of *An. bwambae* is the exceptional broad apical band of the female palps, which includes the entire palpomere V and nearly the apical half of palpomere IV (White 1973, 1985). No further morphological characters in the larvae, pupae or eggs exist to enable identification of individual specimens (Gillies and Coetzee 1987).

**Biology:** The larvae mainly breed in sun-exposed ground pools with muddy and polluted water, which is highly mineralized (sulphate, chloride, bicarbonate) and derived from hot springs. In the type locality, breeding is intense and continuous throughout the year (Gillies and Coetzee 1987). The females attack humans outdoors in large numbers, but also enter houses when human settlements are present. The outdoor resting sites of the adults are primarily tree trunks and buttresses in the forest (White 1973).

**Distribution:** Only known from the small area of the Rift Valley, Uganda

**Medical importance:** Compared to *An. gambiae* s.s. this species is of minor importance as a vector of malaria (White 1973).

### ***Anopheles (Cellia) melas* Theobald 1903**

*An. melas* has long been recognised as a variety of *An. gambiae* and is the most distinct member of the complex. The integument of the adults is somewhat darker than in the freshwater species and the speckling of the legs is much reduced. In the larvae, the pecten with the long and short teeth arranged irregularly with no definite pattern separates this species from those of *An. merus* as well as the freshwater species (Gillies and De Meillon 1968).

**Biology:** The larvae occur mainly in mangroves, salt marshes, lagoons and tidal swamps in pools and ponds. They are capable of breeding in waters with very high salinity; the eggs of *An. melas* show a markedly higher tolerance to desiccation than the other members of the complex (Gillies and De Meillon 1968). The adults usually do not disperse far from their saline habitats. They are regarded as being strongly anthropophilic, biting man indoors and outdoors. When biting indoors the females usually leave the habitations soon after the blood meal. The outdoor resting sites include shaded earth banks, tree

trunks and underneath vegetation (Gillies and De Meillon 1968).

**Distribution:** West African coastal salt marsh areas. Recorded from Angola, Benin, Cameroon, Congo, Cote d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Gabon, Gambia, Ghana, Guinea, Guinea Bissau, Liberia, Madagascar, Mauritius, Nigeria, Senegal, Sierra Leone, Togo

**Medical importance:** Due to a much reduced sporozoite rate compared to *An. gambiae* s.s. this species plays a minor role in malaria transmission, except when present in very large numbers (Bryan 1983). *An. melas* is also capable of transmitting Bancroftian filariasis (Blacklock and Wilson 1941).

### ***Anopheles (Cellia) merus* Dönitz 1902**

**Biology:** The larvae often occur in large brackish lagoons, ponds, swamps and pools that are flooded at spring tides and subsequently diluted by rainfall; the water is sometimes dark and shows a high content of organic pollution (Gillies and De Meillon 1968). The females are regarded as being zoophilic with cattle being the preferred host, but in the absence of domestic animals, they readily bite humans indoors and outdoors and can be caught resting indoors by day in considerable numbers. The outdoor resting sites are similar to those of *An. melas*.

**Distribution:** Brackish habitats in eastern Africa, inland as well as coastal. Reported from the Comoros, Kenya, Madagascar, Mauritius, Mozambique, Somalia, South Africa, Swaziland, Tanzania, Zimbabwe

**Medical importance:** As with *An. melas* the importance of *An. merus* as a vector is limited, but in localities where it is abundant, it may play a major role in malaria transmission. In addition *An. merus* can act as a vector of Bancroftian filariasis (Bushrod 1981).

### ***Anopheles (Cellia) pharoensis* Theobald 1901**

**Female:** A pale species of medium size. Proboscis dark scaled, palps shaggy in basal portion, with 4 pale rings at apices of palpomeres II–V, sometimes additional pale scales present on dorsal and median surface, clypeus pale brown to blackish. Pedicel with a few small scales, flagellum dark. Vertex with greyish white erect scales and a prominent frontal tuft of elongated

white scales, occiput covered with dark erect scales. Integument of scutum pale greyish, with a darker median line and dark bare posterior dorsocentral stripes and dark bare oval spots in front, scutum more or less evenly covered with whitish or greyish scales, setae dark. Scutellum clothed with whitish scales and dark setae. Integument of pleurites usually pale yellowish brown, mesepisternum with several spots of whitish flat scales, prespiracular setae usually very fine, upper mesepimeral setae numerous. Coxae usually with numerous white scales, femora and tibiae prominently pale scaled on inner surface, otherwise speckled, with irregular bands, patches or spots of pale scales. Fore and mid tarsi with tarsomeres I and II broadly pale at apices, tarsomeres III usually more narrowly pale apically, tarsomeres IV and V dark or sometimes with white tips. Hind tarsi with broad pale apical rings on tarsomeres I–IV, tarsomere V entirely pale scaled. Wing veins covered with pale and dark scales forming contrasting spots in a variable pattern. Costa (C), usually with three large dark areas and one small dark apical area, basal 1/5 of costa usually mainly pale, or with 1 or 2 small dark spots. Radius (R) similar to costa but often with pale interruptions in dark areas and basally mainly pale, or with dark areas mostly broken up into a variable number of small spots. Other wing veins predominantly pale scaled, number of dark spots variable. Wing fringe with pale spots at the terminations of all the veins, an additional pale spot sometimes present in basal portion. Terga II–VII densely clothed with greyish or yellowish scales, which are more numerous towards their basal margins, and with prominent lateral projecting tufts of dark scales (Fig. 11.2a). Sterna of most segments with numerous broad, yellowish and whitish scales, cerci densely clothed with scales.

**Larva:** Colour usually green, but often pale yellowish brown, sclerotisation of head capsule extremely variable. Antenna densely covered with small spicules, antennal seta (1-A) minute, inserted at about 1/4 from the base of antennal shaft. Inner clypeal seta (2-C) long, pinnately branched on distal half, the pair of 2-C inserted wide apart, the distance between the bases of the pair of 2-C distinctly larger than the distance between 2-C and 3-C. Outer clypeal seta (3-C) dendriform, usually divided into 2–3 unequal branches near the base and these subdivided to form about 20–45 ultimate branches. Postclypeal seta (4-C) usually about half as long as 2-C, with 2–5 branches, frontal setae (5-C to 7-C) long and plumose. Prothoracic setae 1 and

2 (1-P and 2-P) well developed, arising from a well sclerotized common tubercle. 1-P with the stem slightly flattened and sometimes rather elongated, with 20–24 branches and about 3/4 as long as 2-P, 3-P slightly shorter than 1-P, single. Palmate setae developed on abdominal segments I–VII (1-I to 1-VII), 1-I with about 15 leaflets, which usually have well defined shoulders and filaments, but these may be barely indicated. Palmate setae on abdominal segments II–VII (1-II to 1-VII) of variable shape in the width of the leaflets, number of serrations and length of the filaments. Lateral abdominal setae 6 on segments IV–VI (6-IV to 6-VI) with 2 branches. Tergal plates of moderate size, the width of those on abdominal segments IV and V about half the distance between the palmate setae following them (Fig. 11.15). Pecten usually with 3–5 long teeth and groups of short teeth in between, short teeth with conspicuous secondary spicules. Saddle seta (1-X) single.

**Biology:** The larvae are primarily found in large vegetated fresh water swamps, but also breed along lake shores and among floating plants such as *Pistia* and *Potamogeton*. They are also found in reservoirs, rice fields, streams, ditches and overgrown wells (Gillies and De Meillon 1968). The females feed predominantly on domestic animals, but enter houses readily at night and bite humans; the main daytime resting sites are outdoors, particularly in the vegetation. They start biting in large numbers soon after dusk, the attack continuing at a high level until midnight. In arid regions

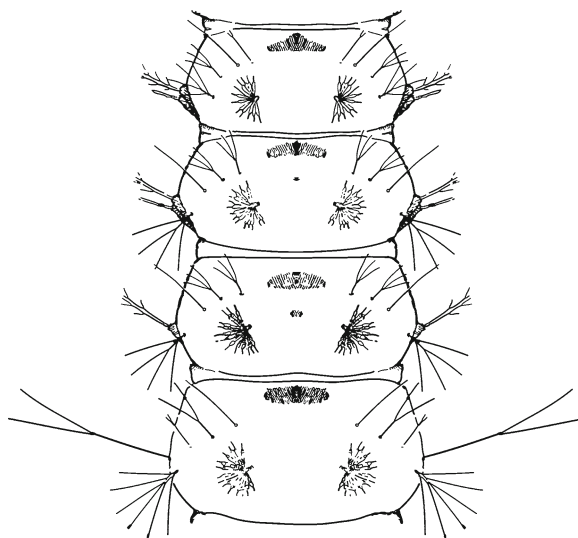


Fig. 11.15 Larva of *An. pharoensis*, terga I–IV

*An. pharoensis* is capable of remarkably long migratory flights of 29–45 km (Gillies and De Meillon 1968).

**Distribution:** Tropical and southern Africa, Egypt, Israel, Syria

**Medical importance:** A well known vector of malaria in Egypt. In tropical Africa, it is of lesser importance compared to *An. gambiae s.l.* and *An. funestus* (Gillies and De Meillon 1968).

### ***Culex (Culex) antennatus* (Becker 1903)**

**Female:** A small brown species with prominent lateral longitudinal pale stripes on abdominal terga VI and VII. Proboscis predominantly dark scaled with paler scales on ventral surface of distal half, palps about 1/5 the length of proboscis, dark scaled. Antenna blackish, pedicel with some small scales or setae on mesal side. Head with numerous erect forked dark scales, curved decumbent scales mostly pale yellowish. Integument of scutum brown, scutum clothed with reddish to golden brown curved scales with some pale yellowish scales on front margin and prescutellar area, scutellum with very fine curved pale yellowish scales on all lobes. Integument of pleurites brownish yellow, patches of pale scales present on upper and lower part of mesepisternum and upper and anterior part of mesepimeron, pleurites with 6–9 prealar setae, 4–5 upper mesepisternal setae, 5–9 lower mesepisternal setae, 5–8 upper mesepimeral setae, and 1 lower mesepimeral seta. Femora and tibiae mainly dark scaled on anterior surface, pale posteriorly, hind femur mainly pale scaled, with dorsal line of dark scales gradually widening to the apex, all tarsi dark scaled, somewhat paler scales ventrally. Wing veins entirely dark scaled, rarely with some pale scales at base of costa (C), haltere pale, more or less same colour as pleurites. Terga II–VII almost entirely brown scaled, terga II–V with small basolateral patches of pale scales, which are usually not visible from above, terga VI and VII with prominent lateral longitudinal pale stripes, tergum VIII largely pale scaled (Fig. 11.9b). Venter entirely pale scaled, sternum VIII with lateral patches only, median area devoid of scales.

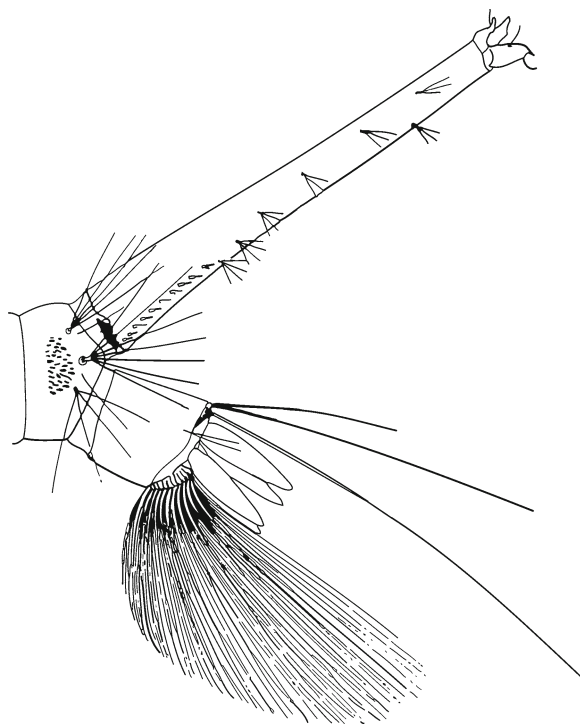
**Larva:** Very closely resembling the larvae of *Cx. perexiguus* and *Cx. univittatus*. In *Cx. antennatus* the labral seta (1-C) is longer, the inner (5-C) and especially median (6-C) frontal setae nearly always have 2 branches, rarely 3-branched. The siphon is generally slightly longer, siphonal index usually more than 6.0

(Fig. 11.16) and the larger distal pecten teeth with larger and fewer (2–3) lateral denticles than in the two other species. In living larvae of *Cx. antennatus* the abdominal segments III and V with dark pigment granules giving the abdomen a two-banded appearance to the naked eye, which is absent in the others (Harbach 1988).

**Biology:** The larvae of *Cx. antennatus* can mainly be found in stagnant fresh water bodies, they rarely breed in polluted water (Hopkins 1952). Common larval habitats include rice fields, stream pools, springs, ponds, swamps, ditches, seepages, and animal footprints. The breeding places usually contain emergent vegetation (Harbach 1988). Females feed primarily on livestock and man. They bite both indoors and outdoors from late afternoon on and in the evening and seek resting sites away from buildings (Zimmerman et al. 1985). In suburban areas of Kinshasa, Zaire *Cx. antennatus* was recorded as the major nuisance species (Karch et al. 1993).

**Distribution:** Egypt, Israel, Ethiopian Region south to Angola and Bechuanaland, Iran

**Medical importance:** *Cx. antennatus* has been found naturally infected with West Nile virus in Egypt (Taylor et al. 1956) and Rift Valley Fever virus in Nigeria (Lee 1979).



**Fig. 11.16** Larva of *Cx. antennatus*

### ***Culex (Culex) univittatus* Theobald 1901**

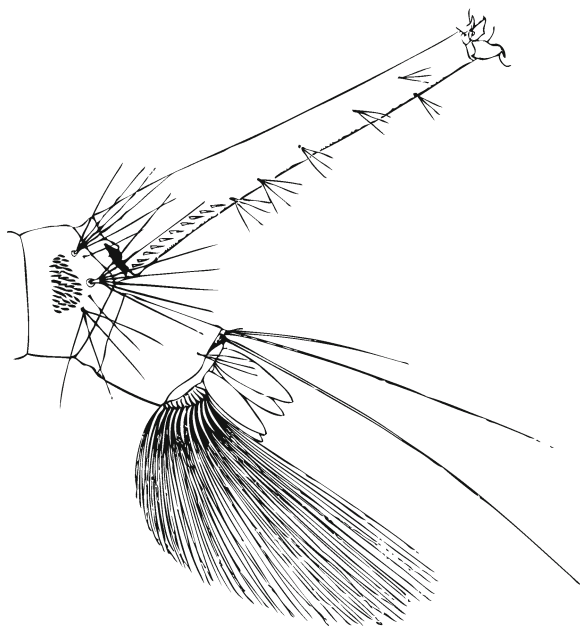
The typical form of this species may be recognised without much difficulty by the presence of an anterior pale stripe on the mid femur stretching over its whole length (Jupp 1971), a similar stripe on the hind tibia and the presence of postspiracular and prealar scale patches. The species is closely related to *Cx. perexiguus* (see description of European species) and exhibits a very similar external morphology in all life stages. In Africa, adults could be confused with *Cx. antennatus*, *Cx. neavei*, *Cx. quasiguiarti* and *Cx. simpsoni* (Edwards 1941).

**Female:** Legs largely dark, front and mid femora and tibiae in many specimens with vague lines of white scales extending their whole length anteriorly (Fig. 11.8b). The hind femur also bears white scales anteriorly on the basal 1/2 continued as a narrow pale stripe to near the apex (Fig. 11.8a). Hind and sometimes the mid tibia with a line of whitish scales extending most of the length on the anterior surface but separate from the conspicuous white spot at the tip (Edwards 1941; Sirivanakarn 1976). The abdominal terga have pale basal bands that are rather broad, slightly convex on terga II–V and narrow and even in width on the rest of the terga, confluent with basolateral patches but may be disconnected and sometimes reduced to small median basal spots or rarely absent. The sterna are creamy-white scaled, usually with dark apicolateral patches and a dark median line (Jupp 1996).

**Larva:** Besides the great similarity to *Cx. perexiguus* the larva resembles those of *Cx. decens*, *Cx. tritaeniorhynchus* and *Cx. antennatus*. From *Cx. decens* it may be separated by its shorter siphon and the possession of two branches of abdominal setae 6 on segments III–VII (6-III to 6-VII), from *Cx. tritaeniorhynchus* by the much shorter siphon and the fact that the antennal seta 1-A is inserted beyond 2/3 (at about 3/4) from the base of the antennal shaft, and from *Cx. antennatus* by the fact that, in the latter species, the inner frontal seta (5-C) is nearly always 2-branched and the pecten teeth have the lateral denticles larger and fewer (2–3 against 3–5) (Hopkins 1952). The siphon is usually long and slender, and the siphonal index may vary between 5.9 and 7.6, a higher index corresponding to larger specimens. The number of pecten teeth is 10–14, each tooth with 3–5 lateral denticles, spreading little beyond the basal 1/4 of the siphon (Fig. 11.17). The most distal tooth may be widely spaced. The siphonal seta (1-S) consists of 5–6 pairs of

ventrolateral tufts with 2–4 branches (Gutsevich et al. 1974). The two basalmost tufts (1a-S and 1b-S) are attached within or close to the pecten. The tufts are inserted more laterally toward the apex. The main tracheal trunks are broad with oval cross section. The anal papillae are variable in length; the dorsal pair is slightly longer than the ventral pair (Hopkins 1952).

**Biology:** The larvae are most common in pools in marshy land and at the edges of swamps but occur also in stagnant or semistagnant streams and ditches, borrow pits, etc. Vegetation is usually present, but there is seldom dense shade. It prefers fresh water but according to Gutsevich et al. (1974) could develop in slightly saline water (with 0.2% of salt and pH of 7.2 to 9.7) or even in strongly saline waters of up to 1% (Senevet 1947). Mara (1945) reported larvae from man-made containers at altitudes above 2,000 m in Eritrea in association with those of *Ae. aegypti*, *Cx. p. quinquefasciatus*, *Cx. laticinctus* and *Cs. longiareolata*. Gutsevich et al. (1974) stated that females feed mainly on birds, while Jupp (1996) reported a great variation in anthropophily between populations. According to Lewis (1947), *Cx. univittatus* and *Cx. neavei* have different biting habits in the Sudan, the latter being far more anthropophilic than the former, but De Meillon (1947) found *Cx. univittatus* to be a persistent biter of humans and monkeys in Bechuanaland.



**Fig. 11.17** Larva of *Cx. univittatus*

**Distribution:** *Cx. univittatus* is common throughout a large part of the Afro-tropical Region with the exception of the forested areas (Edwards 1941; Harbach 1988).

**Medical importance:** Despite the statement that females mainly feed on birds, Gutsevich et al. (1974) reported *Cx. univittatus* to be an important vector of West Nile virus in Asia but this most probably applies to *Cx. perexiguus* (Reuben et al. 1994). In South Africa, *Cx. univittatus* is a primary vector of both Sindbis and West Nile viruses (Jupp 1996).

**Notes on systematics:** Jupp (1971) resurrected *Cx. neavei* Theobald 1906 to species status from *Cx. univittatus* var. *neavei* (Edwards 1922). *Cx. univittatus* and *Cx. neavei* are morphologically and biologically distinct allopatric species in South Africa. At two of three localities of overlapping of the distribution ranges, few intermediates were detected, but it was considered that these were accidental hybrids and that the two species largely coexist at these places without interbreeding. Reproductive isolation was due to differences in mating behaviour (Jupp 1971) (see also comments under *Cx. perexiguus*).

### ***Mansonia (Mansonioides) africana* (Theobald 1901)**

**Female:** It closely resembles *Ma. uniformis* differing from the latter in having the pale scales of the scutum aggregated into spots but not forming stripes. The scutum usually bears three groups of pale greyish scales laterally, on the anterior and posterior submedian areas and the third and largest spot above the wing root, usually another patch of greyish scales is present in front of the prescutellar space. In *Ma. africana* the white markings of the femora are more sharply defined and the tibiae also have distinctly defined white spots (Fig. 11.12a). The short stout spines along the apical margin of tergum VIII differ slightly in arrangement between the two species. In *Ma. africana* the lateral spines are straight and more widely separated from the median ones, sternum VIII with several small lobes, not broadly bilobed as in *Ma. uniformis*.

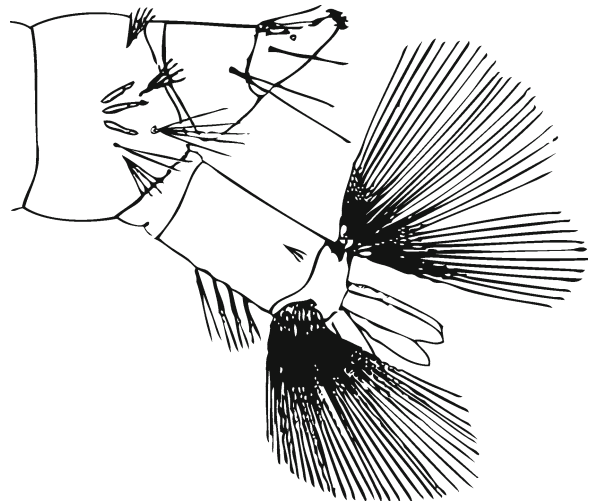
**Larva:** Nearly identical with that of *Ma. uniformis*, only separable from the latter by the form of seta 3 on abdominal segment VIII (3-VIII), which usually is long and has 2 branches in *Ma. uniformis*, while that of *Ma. africana* is much shorter and has 3–5 branches (Fig. 11.18).

**Biology:** There is a great similarity between *Ma. africana* and *Ma. uniformis* in appearance and behaviour of biting, mating and oviposition. Furthermore, the two species often occur together in nature (Laurence 1960). The breeding places are mainly the same, *Ma. africana* has presumably a slightly more marked preference for moderately clean water. Larvae are found in permanent water habitats with floating and emergent vegetation (Hopkins 1952). Eggs are laid in compact masses of about 150 eggs, each attached to the ventral surfaces of leaves of aquatic plants, mainly of water lettuce (*Pistia stratiotes*) (Horsfall 1955). Females are vicious biters, particularly at night, and they readily feed on man and other mammals outdoors and inside dwellings (Muspratt 1955; Laurence 1960). *Ma. africana* is everywhere recognised as a pestiferous mosquito; human blood is its preferred blood meal (Kerr 1933).

**Distribution:** Tropical and southern Africa

### ***Mansonia (Mansonioides) uniformis* (Theobald 1901)**

**Female:** A medium sized mosquito of yellowish brown appearance, legs with many pale markings and ringed tarsi. It closely resembles *Ma. africana* (for differences see under the latter species). Proboscis shorter than fore femur, mottled but mostly pale on basal 3/4 and predominantly dark apically. Clypeus pale brown, palps about 1/3 as long as the proboscis, covered with



**Fig. 11.18** Larva of *Ma. africana*

mixed white, yellow and dark scales, tip with whitish scales. Pedicel yellowish brown, with several dark short setae on ventromesal side. Vertex covered with pale narrow curved scales and brown erect forked scales, whitish broad scales laterally along the eye margin. Integument of scutum brown, scutum covered with narrow golden and pale scales, pale scales often with a greenish tint and forming broad continuous submedian stripes. Prescutellar dorsocentral area with pale scales, prescutellar area bare. A few pale scales scattered in anteromedian area, 3–5 pairs of acrostichal setae on anterior part of scutum. Scutellum brown, each lobe with pale narrow curved scales and 5–8 long brown setae, each lateral lobe with 1–4 additional short setae. Postpronotum with white narrow curved scales and 7–14 postpronotal setae arranged in a single row. Integument of pleurites brown, patches of white broad scales on propleuron, upper and lower posterior mesepisternum and upper mesepimeron. Pleural setae pale yellowish brown, located on propleuron, postspiracular area, along upper and posterior margins of mesepisternum and upper part of mesepimeron. A row of 2–5 lower mesepimeral setae along the anterior margin in the middle of mesepimeron. Fore and mid coxae with both dark and pale scales, hind coxa without scales or with only a few pale scales at apex. Hind legs with femora mostly pale scaled on basal half (Fig. 11.12b), otherwise dark scaled with mottling and patches of pale scales, tibiae likewise, fore and mid tarsomeres I–III and hind tarsomeres I–V with broad pale basal rings, tarsomeres I of all legs with median pale ring as well. Wing veins mottled with very broad and asymmetrical dark and yellow scales, fringe scales slender and all dark. Haltere with dark scaled knob. Tergum I with a median patch of yellow scales, occasionally intermixed with a few dark scales, remaining terga mostly dark scaled with some mottling and pale lateral patches, tergum VIII with many short stout spines along apical margin, lateral spines curved and slightly separated from the median ones. Sterna mostly pale scaled, sternum VIII bilobed, lobes largely rounded.

**Larva:** Head distinctly broader than long. Antenna at least 1.5 times as long as the head, with an indistinct dark ring at base and another at level of origin of antennal seta (1-A), antennal shaft with numerous spicules. Antennal seta (1-A) inserted at basal 1/3 of shaft, with 10–18 branches, A-2 and A-3 inserted at apical 1/3 of antennal shaft, long and reaching distinctly beyond tip

of antenna. Ocular area covered with anteriorly directed denticles. Labral seta (1-C) long and conspicuous. Postclypeal seta (4-C) with 2–4 branches, inner frontal seta (5-C) with 2–5 branches, both very small and difficult to detect. Median frontal seta (6-C) also small, with 4–9 branches, outer frontal seta (7-C) larger, usually with 5–10 branches. Prothoracic seta 1–3 (1-P to 3-P) long, reaching slightly beyond anterior margin of head, 1-P with 2 branches, 2-P and 3-P single. Abdominal seta 6 on segments I–VI (6-I to 6-VI) single. Comb usually with 2 scales, individual scale long and apically rounded, without fringe of small spines. Seta 3 on abdominal segment VIII (3-VIII) always long and with 2 branches (Fig. 11.19). Siphon short and conical, heavily sclerotized beyond the middle, modified for piercing tissues of plants, siphonal seta (1-S) with 2 subequal branches arising before the heavily sclerotized part, pecten absent. Anal segment longer than wide, completely ringed by the saddle, saddle seta (1-X) short, with 3–6 branches, inserted well before the posterior margin of the saddle. Upper and lower anal setae (2-X and 3-X) about the same length, multiple branched. Ventral brush well developed, with 10–11 cratal setae (4-X) and 2–4 small precratal setae (4-X) piercing the saddle. Anal papillae shorter than the saddle, blunt ended.

**Biology:** The larvae of *Ma. uniformis* are primarily found in open swamps not shaded by trees, especially lake shore swamps, and in swampy rivers. They may also be encountered in large natural ponds and

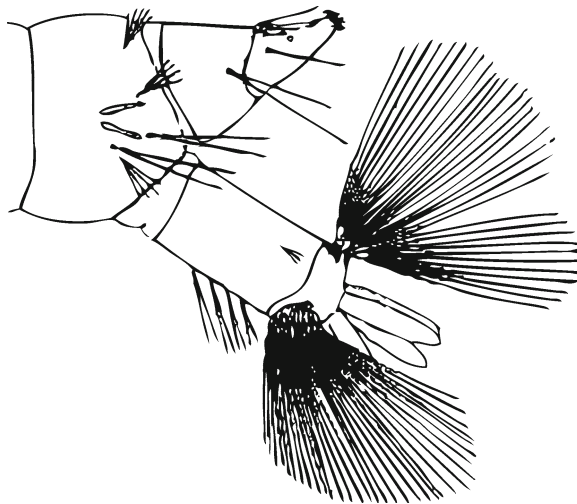


Fig. 11.19 Larva of *Ma. uniformis*

slow moving streams and in old overgrown burrow pits (Hopkins 1952; Tanaka et al. 1979). They have the unique larval structure of a piercing siphon which allows them to attach to a wide range of aquatic grasses, sedges and aquatic plants such as water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*). With the modified siphon they pierce stems and roots of aquatic vegetation in order to obtain oxygen. The eggs are attached to the ventral side of leaves of aquatic plants. Adults are stenogamous and mate readily in small cages (Laurence 1960). The first blood meal is taken approximately 24 h after emergence and immediately after each oviposition. *Ma. uniformis* females are more aggressive during the night but will feed during the day in protected or shaded areas, they enter houses only to feed

(Stojanovic and Scott 1966). The females feed readily on humans as well as on a wide variety of mammals, the flight range of the adults is limited to about 3–6 km making this species more of a nuisance to those living near fresh water areas. *Ma. uniformis* can prove a major pest problem in parts of Africa and northern Australia (Russell 1996).

**Distribution:** Ethiopian, Oriental and Australasian Regions, Japan

**Medical importance:** In the tropical region the species is the primary vector of *Wuchereria bancrofti* (Bancroftian filariasis) and *Brugia malayi* (Malayan filariasis) as well as Chikungunya fever (Horsfall 1955; Stojanovich and Scott 1966). Ross River virus (RR) has been isolated from wild caught *Ma. uniformis* in Northern Australia (Russell 1993).

## Chapter 12

### Asia

In addition to the anopheline species that were included in the key and descriptions for the Asian continent, many others could also be considered as primary malaria vectors but with a relatively restricted distribution (*An. aconitus* from India to Indonesia, *An. arabiensis* on the Arabian Peninsula, *An. sacharovi*<sup>1</sup> from the Middle East to Afghanistan, *An. sergentii*<sup>1</sup> from the Middle East and Arabian Peninsula to Pakistan, *An. superpictus*<sup>1</sup> from the Middle East and Central Asia), or widely distributed secondary vectors such as the day biting *An. pulcherrimus* (from the Middle East through Central to South and East Asia) (Gutsevich et al. 1974; Anonymous 1999, <http://www.wrbu.org>), or even pests of a different territorial scale (*An. superpictus*, *An. pulcherrimus*). *An. multicolor*<sup>1</sup> is considered to be of primary importance for Asia, hence is included in the key but described in Chap. 9.

In South, Southeast and East Asia, females of *Armigeres subalbatus* may bite voraciously throughout the day and may be involved in the transmission of *Wuchereria bancrofti*, the causative agent of Bancroftian filariasis (La Casse and Yamaguti 1950; Thurman 1959; Tanaka et al. 1979). Some members of the genus, such as *Ar. aurolineatus*, *Ar. malayi*, *Ar. annulipalpis* and *Ar. flavus*, are quite widespread, but many others are important pests of limited distribution, especially in coconut growing areas. They are primarily forest mosquitoes but some members of the genus show potential for becoming domestic pests (Macdonald 1960; Lee et al. 1988). Because of their important pest status, their relatively restricted distribution and the strong similarities between many species, they are included in the key within the genus *Armigeres*. Another day biter worth mentioning is *Oc. togoi*

[*Tanakaius togoi*], a possible vector of *W. bancrofti*, *Brugia malayi*, *Dirofilaria immitis* and Japanese encephalitis (JE) virus (Tanaka et al. 1979). Many other *Aedes* and *Ochlerotatus* species are widespread noxious pests in temperate Asia such as *Ae. cinereus*<sup>1</sup>, *Ae. vexans*<sup>1</sup>, *Oc. caspius*<sup>1</sup>, *Oc. communis*<sup>1</sup>, *Oc. dorsalis*<sup>1</sup>, *Oc. excrucians*<sup>1</sup>, *Oc. fitchii*, *Oc. hexodontus*<sup>1</sup>, *Oc. impiger*<sup>1</sup>, *Oc. nigripes*<sup>1</sup>, *Oc. pionips*<sup>1</sup>, *Oc. punctor*<sup>1</sup>, *Oc. sticticus*<sup>1</sup>, *Oc. vigilax* (Gutsevich et al. 1974). Most of them are described in Chap. 10 and should be identified using the key for European mosquitoes (Chaps. 6–8).

The notorious pest and dengue vector species *Ae. aegypti*<sup>1</sup> [*Stegomyia aegypti*] and *Ae. albopictus*<sup>1</sup> [*Stegomyia albopicta*] are widespread from the Middle East to Southeast Asia, China and Japan, and are also included in the key. In addition, mosquitoes of the *Oc. niveus* [*Downsiomyia nivea*] subgroup could be important dengue vectors in the Oriental Region (Rueda 2004).

*Mansonia* females (*Ma. annulata*, *Ma. indiana* and *Ma. uniformis*) readily bite man and can also successfully transmit *B. malayi* (Carter 1950; Wharton 1962). They are widely distributed through South, Southeast and East Asia. *Ma. uniformis* is included in the key as it is the most widespread and significant pest species in the mosquito fauna of three continents (Africa, Asia and Australia – AAA). The closely related *Cq. richiardii*<sup>1</sup> readily attacks man across the Middle East and Central Asia (Gutsevich et al. 1974).

Together with the most important Asian *Culex* mosquito, *Cx. tritaeniorhynchus*, which is described below, the cosmopolitan human pests *Cx. p. pipiens* biotype *molestus*<sup>1</sup> (also a vector of Sindbis and West Nile viruses, and primary vector of periodic Bancroftian filariasis) and *Cx. p. quinquefasciatus*<sup>1</sup> (a primary vector of *W. bancrofti*, vector of avian malaria, and dog

<sup>1</sup> Species described in Chaps. 9 and 10 concerning European mosquitoes.



heartworm) are also included in the key (Sirivanakarn 1976; Harbach 1988). The same is true for the primarily bird and pig feeding bridge vector of AAA distribution, *Cx. sitiens*, responsible for transmission of Japanese B encephalitis and *B. malayi* in Thailand (Harbach 1988).

Two zoophilic *Culex* species distributed in South, Southeast and East Asia will readily bite man in the absence of an animal host. *Cx. gelidus* is a potential vector of Japanese encephalitis (JE) in Malaysia and

Thailand (Bram 1967a) and *Cx. vishnui* is an important vector of JE (Sirivanakarn 1976).

For further reading on the morphology, identification, biology, distribution and disease vector status of the species mentioned, the reader is referred to Christophers (1933), Barraud (1934), Reid (1953), Stojanovich and Scott (1965a,b), Bram (1967a), Cagampang-Ramos and Darsie (1970), Mattingly (1971), Huang (1972, 1979), Smith (1973), Sirivanakarn (1976), Tanaka et al. (1979), Harrison (1980), Rattanarithikul (1982),

Harbach (1988), Darsie and Pradhan (1990, 1991), Reuben et al. (1994), Anonymous (1999), Ree (2003), Rueda (2004).

### 12.1 Key to Asian Female Mosquitoes

- 1 Palps as long as or slightly shorter than proboscis. Scutellum evenly rounded or slightly trilobed and uniformly setose (Fig. 12.1a). Abdominal terga and sterna completely or largely missing scales – Proboscis is neither strongly recurved nor tapering towards apex. *Anopheles*..... 2
- 2 Palps distinctly shorter than proboscis. Scutellum trilobed, setae arranged in three sets (Fig. 12.1b). Abdominal terga and sterna uniformly and densely covered with scales. – Proboscis is neither strongly recurved

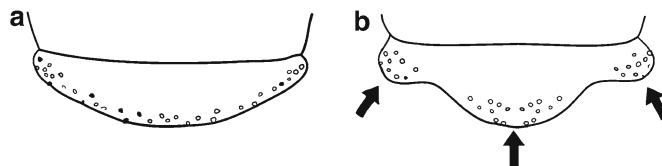


Fig. 12.1 Scutellum of: (a) *Anopheles* sp.; (b) *Aedes* sp.

nor tapering towards apex. Tip of proboscis is neither swollen, upturned nor hairy. Antenna long, flagellomeres slender, cylindrical. Scutum without narrow median longitudinal stripe of broad decumbent silvery white scales. Prespiracular setae absent. Postnotum without patch of setae. Tarsomere I of fore legs usually shorter than tarsomeres II–V together. Tarsomere IV of fore legs not reduced, distinctly longer than broad. Anal vein (A) evenly curved, ending distinctly beyond furcation of cubitus (Cu)..... 12

- 2 (1) Costa (C) with two pale spots excluding humeral and basal spots (these spots usually not present in *An. lesteri* and *An. sinensis*) subgenus *Anopheles* (Fig. 12.2a). – Palps with pale rings. Basalmost pale ring not

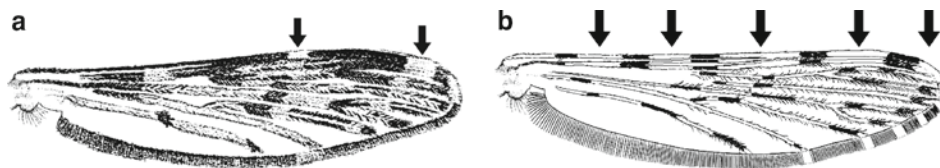


Fig. 12.2 Wing of: (a) *An. hyrcanus*; (b) *An. multicolor*

broader than others. Hind femur without pale ring. Femorotibial joint of hind legs without tuft of black and white scales. Apical pale rings on hind tarsi narrow, tarsomere IV usually without basal pale ring. Basal 1/4 of costa completely dark scaled or speckled with pale scales. Pale apical fringe spot usually present. Anal vein (A) pale with two dark spots. *An. hyrcanus* group ..... 3

Costa with at least four pale spots in addition to humeral and basal spots (Fig. 12.2b). Subgenus *Cellia* ..... 4

- 3 (2) Mid coxa with a distinct upper patch of pale scales. Pale apical fringe spot quite large, stretches at least from  $R_1/R_2$  to  $R_{4+5}$ . Basal dark spot on cubitus (Cu) small, separated by its own length from middle dark spot on anal vein (A). Pale fringe spot at the end of cubitus ( $Cu_2$ ) usually present (Figure 12.3a). – Wing pattern blurred. Some pale scales scattered on  $R_1$  between subcostal and preapical pale spots. Apical dark spot on anal vein (A) longer than that on  $Cu_2$ . ..... *An. sinensis* (p 340)
- Mid coxa without upper patch of pale scales (a few scales occasionally present). Pale apical fringe spot usually very small, stretches between  $R_1/R_2$  and  $R_3$ . Basal dark spot on cubitus (Cu) fairly long, approach-

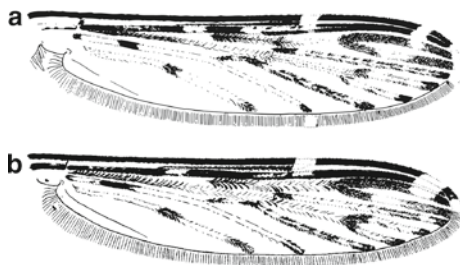


Fig. 12.3 Wings of: (a) *An. sinensis*; (b) *An. lesteri*



Fig. 12.4 Hind leg of: (a) *An. sundaicus*; (b) *An. minimus*

ing to within its own length or less of the middle dark spot on anal vein (A). Pale fringe spot at the end of vein  $Cu_2$  absent (Fig. 12.3b)..... *An. lesteri* (p 339)

- 4 (2) Femora and tibiae speckled with pale and dark scales to a variable extent, sometimes spotted and ringed (Fig. 12.4a)..... 5
- Femora and tibiae not speckled (Fig. 12.4b). – Fore tarsomeres entirely dark-scaled or with very narrow pale rings. .... 8



Fig. 12.5 Hind tarsus of: (a) *An. maculatus*; (b) *An. sundaicus*

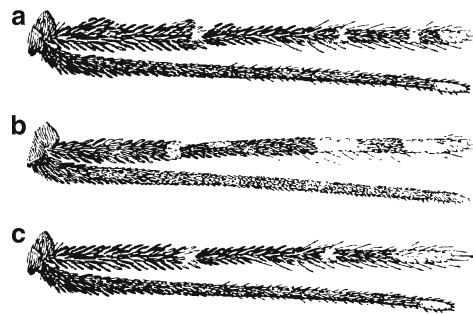


Fig. 12.6 Palps and proboscis of: (a) *An. dirus*; (b) *An. stephensi*; (c) *An. sundaicus*

- 5 (4) Hind tarsomere V and apical part of IV entirely pale-scaled (Fig. 12.5a). – Palps with three distinct pale rings. Vein  $R_2$  short, usually less than twice the length of vein  $R_{2+3}$ . Vein  $R_{4+5}$  with two dark spots. Abdominal terga II and III without scales or with some pale curved and/or narrow spatulate scales on posteromedian area. Tergum IV without scales or with a few posteromedially. Terga V–VII with numerous pale scales but occasionally only on tergum VII. Dark scales usually present only on last two terga. Posterolateral corners of terga VII and/or VIII, rarely also tergum VI, with patches of dark scales. Abdominal sterna without scale tufts..... *An. maculatus* (p 345)
- Hind tarsomeres III–V predominantly dark scaled, usually with narrow pale apical rings (Fig. 12.5b)..... 6
- 6 (5) Palps with four pale rings (Fig. 12.6a). – Apical and subapical pale rings unequal in width. Hind legs with tibiotarsal joint broadly and conspicuously ringed with white scales. .... *An. dirus* (p 342)
- Palps with three pale rings (Fig. 12.6b) ..... 7
- 7 (6) Palps with apical and subapical pale rings equal in width. Apart from rings palps usually speckled with pale scales (Fig. 12.6b)..... *An. stephensi* (p 347)

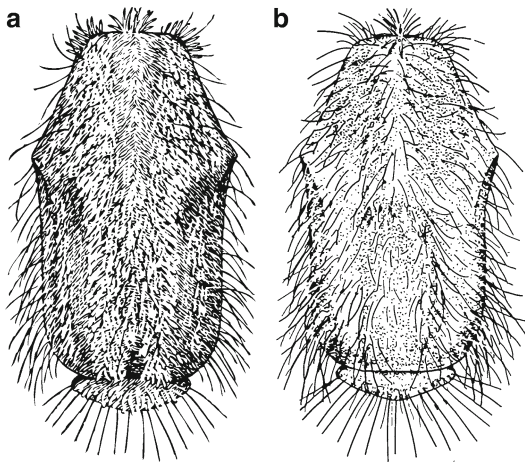


Fig. 12.7 Scutum of: (a) *An. multicolor*; (b) *An. culicifacies*

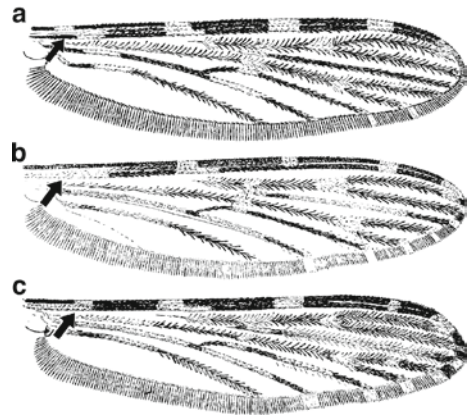
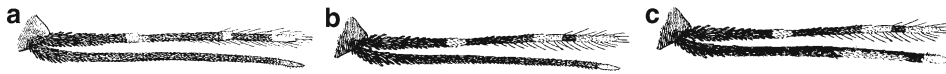


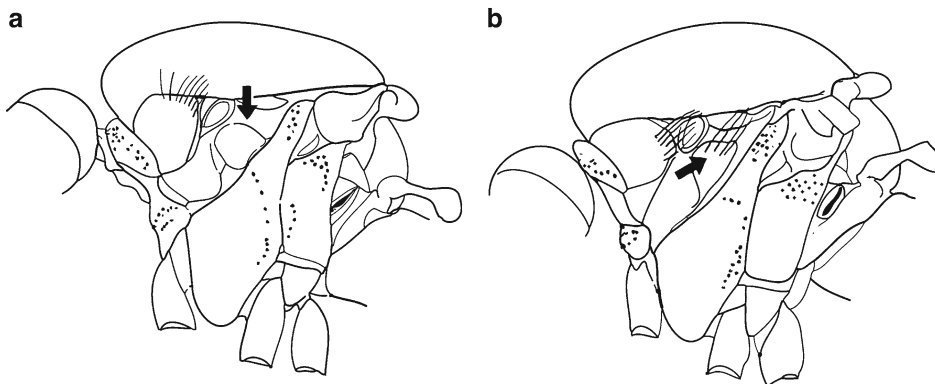
Fig. 12.8 Wing of: (a) *An. culicifacies*; (b) *An. fluviatilis*; (c) *An. minimus*

- Subapical pale ring much narrower than apical and equal to basal ring. Palps usually not speckled (Fig. 12.6c)..... *An. sundaicus* (p 348)
- 8 (4) Median, fossal and supraalar areas of scutum covered with narrow but distinct creamy scales, somewhat broader on fossae (Fig. 12.7a). – Apex of palps dark. Fore tarsomeres with narrow apical pale rings nearly twice the width of tarsomere..... *An. multicolor* (p 182)
- Median area of scutum without scales (setae present), or with slender seta-like pale scales at postacrostichal and prescutelar areas (Fig. 12.7b). – Apex of palps pale. Legs entirely dark scaled, or some tarsomeres with apical pale rings or dorsal patches not wider than tarsomere width..... 9
- 9 (8) Base of radius (R) below humeral cross vein (h) with a patch of grey or black scales (Fig. 12.8a). – Subapical dark ring on palps much wider than apical pale ring. Fore tarsomeres dark scaled. Vein  $R_{4+5}$  usually dark except at base. .... *An. culicifacies* (p 341)
- Base of radius (R) below humeral cross vein (h) with only white or yellowish scales (Fig. 12.8b,c). – Vein  $R_{4+5}$  usually mainly pale scaled..... 10



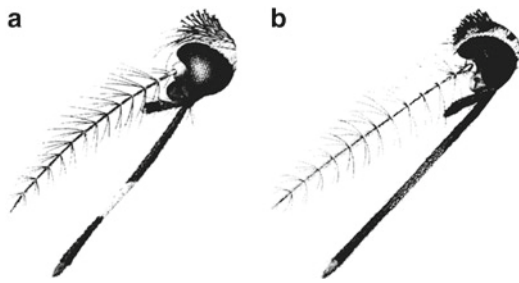
**Fig. 12.9** Palps and proboscis of: (a) *An. fluviatilis*; (b) *An. minimus*; (c) *An. flavirostris*

- 10 (9) Palps with subapical dark ring wider than apical pale ring and 3–5 times wider than narrow subapical pale ring (Fig. 12.9a). ..... *An. fluviatilis* (p 344)  
 Palps with subapical dark ring variable, from slightly wider (mostly twice) than apical and subapical pale rings, to much smaller or even absent (Fig. 12.9b). – Costa usually with presector pale spot or at least some pale scales basal to sector pale spot. Wing fringe without pale spot at termination of anal vein (A) ..... 11



**Fig. 12.10** Lateral view of thorax of: (a) *Culex* sp.; (b) *Mansonia* sp.

- 11 (10) Proboscis usually entirely dark scaled (Fig. 12.9b). Fore tarsomeres I–IV with very small dorsoapical pale patches or pale rings (mainland Southeast Asia and Indian subregions). ..... *An. minimus* (p 346)  
 Proboscis usually with ventral pale scale patch (Fig. 12.9c). Fore tarsomeres entirely dark scaled (confined to Philippines and Indonesia). ..... *An. flavirostris* (p 343)
- 12 (1) Postspiracular setae absent (Fig. 12.10a). All tarsal claws simple. Pulvilli present, well developed (pad-like). – Antenna with flagellomere I about as long as flagellomere II. Erect forked scales not restricted to occiput, numerous on vertex too. Scutellum with all scales narrow. Wing scales narrow. Alula with narrow fringe scales. Abdomen rounded apically, cerci short, hardly visible. *Culex* ..... 13  
 Postspiracular setae usually present (Fig. 12.10b) and/or claws on fore legs with subbasal tooth. Pulvilli absent or not well developed (hair-like) ..... 15

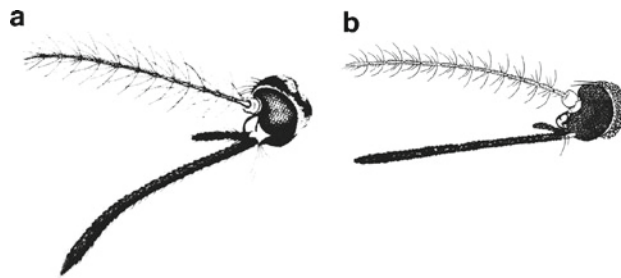


**Fig. 12.11** Proboscis of: (a) *Cx. sitiens*;  
(b) *Cx. p. quinquefasciatus*



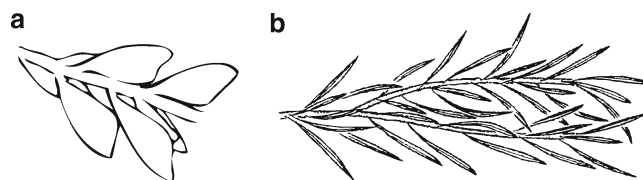
**Fig. 12.12** Mid leg of: (a) *Cx. sitiens*; (b) *Cx. tritaeniorhynchus*

- 13 (12) Proboscis with distinct broad median pale ring (Fig. 12.11a). Lower mesepimeral setae absent. Tarsomeres of all legs with narrow, indistinct basal and apical pale rings. Rings usually more distinct at bases of hind tarsomeres I–III..... 14



**Fig. 12.13** Proboscis of: (a) *Armigeres* sp.; (b) *Aedes* sp.

- Proboscis without pale ring in the middle but often with a pale midventral area (Fig. 12.11b). Usually one lower mesepimeral setae present. Tarsi all dark. – Postspiracular patch of pale scales absent..... *Cx. p. pipiens biotype molestus* and *Cx. p. quinquefasciatus* (p 277, 278)
- 14 (13) Anterior surface of femora (particularly mid femur) with numerous scattered pale scales (Fig. 12.12a). Narrow apical bands and/or small median apical patches of pale scales sometimes present on tergum VII and/or VIII..... *Cx. sitiens* (p 366)
- Anterior surface of mid femur without scattered pale scales (Fig. 12.12b). Tibiae dark anteriorly. Tergum VII and/or VIII without apical bands or patches. – Anterior and median part of scutum dark scaled, pale scales present only near scutellum..... *Cx. tritaeniorhynchus* (p 349)



**Fig. 12.14** Wing scales of: (a) *Mansonia* sp.; (b) *Aedes* sp.

- 15 (12) Proboscis laterally compressed with apical 1/3 slightly curved downwards (Fig. 12.13a). – Postspiracular scales present. Postspiracular setae present and palps less than 1/3 of proboscis or postspiracular setae absent and palps at least half as long as proboscis. .... *Armigeres*  
 Proboscis more or less straight and cylindrical (Fig. 12.13b). – Postspiracular area bare. .... 16
- 16 (15) Claws all simple. Wing scales mostly broad and many conspicuously asymmetrical (Fig. 12.14a). Abdomen rounded apically, cerci short, hardly visible. *Mansonia* – Erect forked scales numerous, not restricted

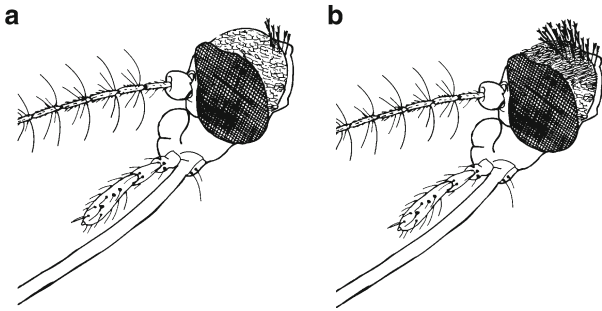


Fig. 12.15 Head of: (a) *Aedes (Stegomyia)* sp.; (b) *Aedes (Ochlerotatus)* sp.

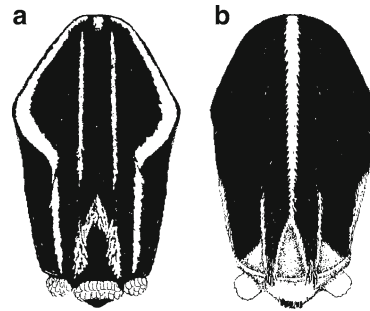


Fig. 12.16 Scutum of: (a) *Ae. aegypti*; (b) *Ae. albopictus*

to occiput, decumbent scales narrow. Submedian area of scutum with a pair of continuous pale longitudinal stripes. Central area of scutum with dorsocentral stripes of pale scales. Anterior surfaces of mid and hind tibiae with mostly confluent creamy white patches. Basal half of hind femur paler, dark scales restricted to upper anterior surface. .... *Ma. uniformis* (p 330)

Claws usually with subbasal tooth. Wing scales mostly narrow, if broad then not conspicuously asymmetrical (Fig. 12.14b). Abdomen tapering apically, gradually from segment V–VII or segment VII distinctly narrower than VI. – Pleural scale patches well developed. .... 17

- 17 (16) Decumbent scales of vertex largely broad. Erect scales not numerous, restricted to occiput (Fig. 12.15a). Cerci relatively short. – Terga with white basolateral patches and at least some with white basal bands. *Aedes* subgenus (*Stegomyia*) ..... 18

Decumbent scales of vertex largely narrow and/or, if scales of vertex are largely broad,

erect scales numerous, not restricted to occiput (Fig. 12.15b). Abdominal segment VII usually distinctly narrower than VI. Cerci usually long, easy visible. *Aedes, Ochlerotatus* ..... (see key for Europe) (p 95)

- 18 (17) Scutum with a white acrostichal stripe extending from the anterior margin to the beginning of the prescutellar area, where it forks to end at the anterior margin of scutellum (Fig. 12.16b). *Ae. albopictus* (p 201)  
 Scutum without acrostichal stripe on anterior part, but with two narrow white dorsocentral stripes separated from anterior margin. Lateral white stripes broad, continuing over transverse suture to the end of scutum, lyre shaped (Fig. 12.16a)..... *Ae. aegypti* (p 198)

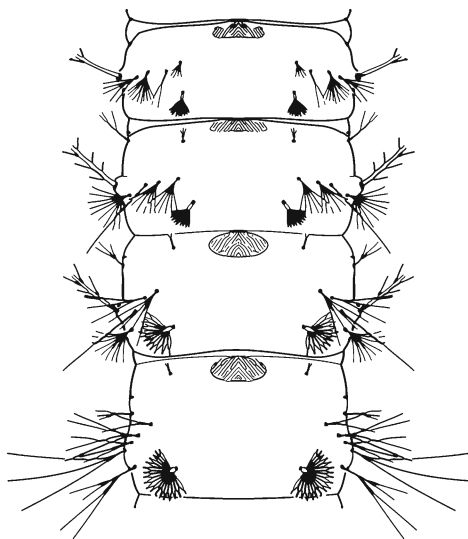


Fig. 12.17 Larva of *An. lesteri*, terga I-IV

## 12.2 Species Description

### *Anopheles (Anopheles) lesteri*

Baisas and Hu 1936

**Female:** Very similar to that of *An. sinensis*, it differs from the latter chiefly by the combination of the following characters: costa (C) usually entirely dark on basal half, without scattered pale scales, humeral cross vein (h) bare; median area of vein  $R_{4+5}$  with fewer dark scales or often entirely pale, pale fringe spot at termination of vein  $Cu_2$  usually absent (Fig. 12.3b). Mid coxa often bare of scales, if few scales are present, not forming a distinct patch as in *An. sinensis*.

**Larva:** Almost indistinguishable from that of *An. sinensis*, lateral abdominal seta 6 on segments IV and V (6-IV and 6-V) usually with 3 branches (Fig. 12.17), 6-III rarely with > 20 branches, 6-I usually with < 21 branches, but characters are variable. Seta 9-II with 5-9 branches (10-18 in *An. sinensis*).

**Biology:** Larvae are found in cool, clean, ground water habitats that include marshes, ground pools, ponds, rice fields and other collections of water. Compared to the larvae of *An. sinensis*, those of *An. lesteri* seem to prefer places which are cooler and more shaded (Tanaka et al. 1979). This can be observed especially in rice fields where both species are often found together. Larvae of *An. sinensis* tolerate shal-

lower, sunnier water than those of *An. lesteri*. As a result, *An. sinensis* appears 2-4 weeks earlier than *An. lesteri*, which is particularly abundant late in the season when the rice plants shade the water (Wilkerson et al. 2003). The females of *An. lesteri* readily enter houses to bite man and seek for resting sites; the species may hibernate in both the egg and adult stages.

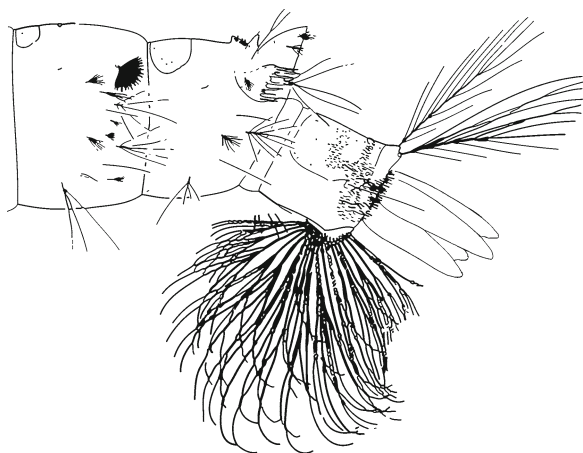
**Distribution:** Borneo, China, Guam, Japan, Korea, Malaysia, Philippines, Thailand, Vietnam.

**Medical importance:** *An. lesteri* is considered as the main vector of malaria in eastern and southern China, Japan and Korea (Harrison and Scanlon 1975; Tanaka et al. 1979; Wilkerson et al. 2003).

### *Anopheles (Anopheles) sinensis*

Wiedemann 1828

**Female:** Proboscis dark scaled, palps slightly shorter than proboscis, predominantly dark scaled, with white scales at tip and 3 narrow, sometimes indistinct pale rings at the joints of palpomeres II-III, III-IV and IV-V. Pedicel dark brown, with few small pale scales, clypeus brown to dark brown, with a tuft of dark brown scales laterally. Vertex with pale erect scales on median part and dark erect scales laterally, frontal tuft with long pale scales projecting forward, numerous dark setae along eye margin. Integument of scutum light brown, usually with a dark brown median stripe extending backwards to prescutellar area and dark dorsocentral stripes on anterior half. Scutum sparsely covered with very fine curved yellowish scales and longer stout golden setae, scutellum clothed with long yellowish setae and small fine curved pale scales. Integument of pleurites light brown to greyish, usually with darker areas forming an upper and lower dark band, sometimes with a few scales on lower mesepisternum, lower mesepimeral setae absent. Legs dark brown on dorsal surface, pale ventrally, fore femur swollen towards the base. Coxae, particularly mid coxae, with a distinct patch of pale scales. Tarsi predominantly dark scaled, fore and mid tarsomeres I-III and hind tarsomeres I-IV with narrow apical pale rings, which are sometimes incomplete, rings on fore tarsi usually broader than those on mid and hind legs. Wing veins covered with pale and dark scales forming contrasting spots. Costa (C) frequently with scattered pale scales on basal half, with subcostal and preapical pale spots, subcostal pale spot includes costa



**Fig. 12.18** Larva of *An. sinensis*

(C), tip of subcosta (Sc) and vein  $R_1$ , preapical pale spot includes costa (C), and veins  $R_1$  and  $R_2$ . Humeral cross vein often with one or a few scales. Wing apex with large pale fringe spot, extending at least to termination of vein  $R_{4+5}$ . Vein  $R_{4+5}$  with basal and apical dark spots and an area in between with mixed pale and dark scales. Media (M) usually dark scaled on basal half or more, cubitus (Cu) with short basal dark spot. Vein  $Cu_1$  with dark basal and apical scales,  $Cu_2$  pale scaled except for dark scales at apex. Anal vein (A) pale scaled except small dark spot in the middle and dark scaled tip, pale fringe spot at termination of vein  $Cu_2$ , usually present (Fig. 12.3a). Haltere stem pale, knob with dark scales. Integument of abdomen dark brown, clothed with long yellowish setae, devoid of scales except a median tuft of erect dark scales close to the apical margin of sternum VII.

**Larva:** Head slightly longer than wide. Antenna about half as long as the head, straight, with spicules largely confined to inner surface, antennal seta (1-A) long, inserted slightly below the middle of antennal shaft, with multiple branches. Inner clypeal seta (2-C) single, the pair of 2-C situated close together, outer clypeal seta (3-C) about half as long as 2-C, strongly dendriform. Postclypeal seta (4-C) usually with 3 branches, frontal setae (5-C to 7-C) long and plumose. Prothoracic seta 1 (1-P) not arising from a sclerotized tubercle, single or with 2–3 branches near apex, 2-P with a prominent sclerotized base, plumose, 3-P longer than 1-P, single. Palmate setae partially developed with pale and slender leaflets on abdominal segments I and II (1-I and 1-II), 1-III to 1-VII well developed, pig-

mented and rather large, leaflets slightly serrated in apical part with sharply pointed tips. Lateral abdominal setae 6 on segments IV and V (6-IV and 6-V) usually with 2 branches. Tergal plate on segment VIII large, about 2/3 as long as wide. Pecten with 7–9 long teeth and about double that number of short teeth. Saddle seta (1-X) as long as or longer than the saddle, upper and lower anal setae (2-X and 3-X) multiple branched, ventral brush with about 9 pairs of cratal setae (4-X). Anal papillae tapering, of variable length (Fig. 12.18).

**Biology:** Larvae are found in shallow habitats, usually with fresh water and emergent vegetation and exposed to direct sunlight, they are characteristic of open agricultural lands (particularly rice fields). They have also been collected in ground pools, pools beside rivers, marshes, stream margins, ditches, seepages, and shallow grassy ponds. In mountainous areas they are confined to the valleys (Reid 1968; Harrison and Scanlon 1975). The females are regarded as being zoophilic, but readily bite humans (Strickman et al. 2000). They feed indoors or outdoors and tend to leave the houses after taking a blood meal.

**Distribution:** Cambodia, China, India, Japan, Korea, Malaysia, Singapore, Taiwan, Thailand, Vietnam.

**Medical importance:** Possible vector of *Brugia malayi* and vector of malaria in China (Harrison and Scanlon 1975).

### ***Anopheles (Cellia) culicifacies* Giles 1901**

**Female:** Small to medium sized species with a yellow brown culicine-like appearance. Proboscis dark scaled, paler at the tip. Palps slightly shorter than proboscis with 3 narrow pale rings, the apical ring involving the entire palpomere V, and two narrow rings at joints of palpomeres II–III and III–IV. Dark area between apical and preapical pale rings much wider than apical ring. Pedicel dark grey or brown, without scales, vertex with pale erect scales, long pale scales forming the frontal tuft weakly developed or absent. Integument of scutum pale greyish with darker acrostichal and dorsocentral stripes, scutum usually devoid of scales except some long erect pale scales at the front margin, sparsely clothed with short pale setae (Fig. 12.7b). Scutellum covered with long and short dark setae. Pleurites without scales, about 4 prelar setae and about 13 upper mesepimeral setae present, lower mesepimeral setae



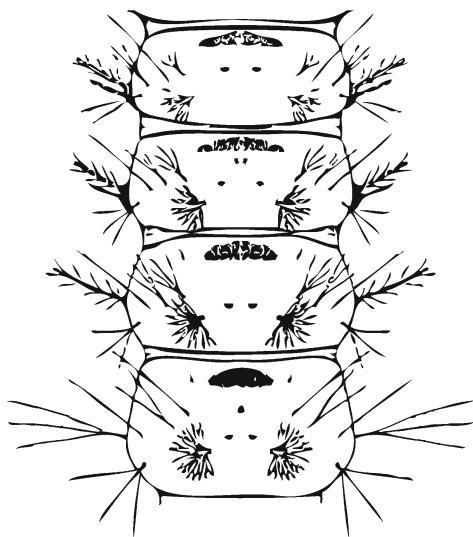


Fig. 12.19 Larva of *An. culicifacies*, terga I-IV

absent. Legs predominantly dark scaled, femora and tibiae with few pale scales at apices, tarsi usually not ringed or some tarsomeres with narrow apical pale rings or patches. Wing with a variable pattern of dark and pale spots. Costa with a humeral pale area at base and with an opposing dark area on  $R_1$ , vein  $R_{4+5}$  mainly or entirely dark scaled. Wing fringe with conspicuous apical pale spot between apices of  $R_1$  and  $R_3$ , with only 2 indistinct pale spots at terminations of  $M_{3+4}$  and  $Cu_1$  (Fig. 12.8a). Integument of abdomen light to dark brown, entirely devoid of scales, clothed with long pale brown setae.

**Larva:** Antenna long and slender, somewhat darker near apex, with short spicules on inner surface, antennal seta (1-A) short and single, inserted at about 1/3 from base at the outer surface of the antennal shaft. Inner and outer clypeal setae (2-C and 3-C) single, the pair of 2-C long and inserted wide apart, 3-C about 2/3 to 3/4 as long as 2-C. Postclypeal seta (4-C) single, distinctly shorter than 3-C, frontal setae (5-C to 7-C) long and plumose, 5-C much longer than 6-C and 7-C. Prothoracic seta 1 and 2 (1-P and 2-P) arising from separate basal tubercles, both with a stout stem and pinnately branched, 1-P distinctly shorter than 2-P, 3-P single, about half as long as 2-P. Palmate setae developed on abdominal segments I-VII (1-I to 1-VII), seta 1-I less developed than the others. Leaflets uniformly pigmented, apices slightly darker, filament long and

pointed, with a broad base, at least half as long as the blade. Lateral abdominal seta 6 on segments IV-VI (6-IV to 6-VI) with 2-4 branches split at some distance from base, tergal plates  $<1/2$  the width of the corresponding segments, a pair of small submedian oval plates present (Fig. 12.19). Pecten with 3-4 long and 10-13 short teeth, all teeth finely serrated on basal half. Saddle seta (1-X) single, about 1.5 times as long as the saddle.

**Biology:** *An. culicifacies* is regarded as a temporary pool mosquito, the larvae are found in fresh water in irrigation ditches, rain pools, pools in riverbeds, along the margins of small streams, partially shaded or fully exposed to sunlight, in shallow tanks, pits and wells. The habitats may contain all types of vegetation, such as submerged, emergent or floating, and often with abundant green algae (Harrison 1980). When disturbed, the larvae move rapidly to the bottom of their breeding sites where they may remain immobile for up to 3-5 min. They are rarely found in brackish water and are uncommon in rice fields. The adults show an unusual behaviour that may be useful in identification, they rest with the body horizontal to the surface like culicine mosquitoes (Harrison 1980). In some localities females are zoophilic, feeding actively on cattle and birds at dusk, but in most areas they prefer humans as a host; they are primarily endophagic, and rest inside human and bovine shelters after the blood meal. Flight ranges of up to 3 km have been observed (Foote and Cook 1959).

**Distribution:** Afghanistan, Bahrain, Cambodia, China, Eritrea, Ethiopia, India, Iran, Iraq, Laos, Myanmar, Nepal, Oman, Pakistan, Sri Lanka, Thailand, Vietnam, Yemen.

**Medical importance:** This species is one of the most important malaria vectors wherever it occurs.

#### ***Anopheles (Cellia) dirus* Peyton and Harrison 1979**

**Female:** Proboscis uniformly dark scaled, labium somewhat lighter. Palps slightly shorter than proboscis, predominantly dark scaled, with a pale apex and 3 narrow apical pale rings on palpomeres II-IV, the ring of palpomere IV is slightly larger than those on palpomeres II and III (Fig. 12.6a). Vertex clothed with erect pale scales and a small tuft of long pale scales projecting forward, occiput with dark erect

scales. Integument of scutum light brown to brown with darker spots above transverse suture and posterior prescutellar area, anterior margin of scutum with a tuft of long thin pale scales, otherwise scaling of scutum sparse, and with long dark setae along posterior lateral margin. Scutellum clothed with a few short, pale scales and long dark setae. Integument of pleurites light brown with darker areas forming a distinct band on upper portion. Mesepisternum with 3–7 upper and 1–4 lower mesepisternal setae; 4–6 prealar and 3–7 upper mesepimeral setae present. Wing veins covered with pale and dark scales forming a contrasting pattern, pale scales on most veins light cream, scales of presector pale spot and sector pale spot shiny white, strongly contrasting with other pale spots. Humeral pale spot usually present and prominent, rarely absent. Haltere with pure white scales on knob. Femora, tibiae, and tarsomeres of all legs with extensive pale speckling, hind leg with a prominent broad pale ring covering the tibio-tarsal joint. Tergum VII with few scales on apical margin, tergum VIII covered with narrow, elongated pale golden scales on apical half, sternum VII with a median patch of dark scales at posterior margin, sternum VIII with basolateral patches of pale scales.

**Larva:** Integument of head entirely light brown to yellowish, sometimes with dark spots. Antenna about half as long as the head, sparsely spiculous. Antennal

seta (1-A) inserted below middle of antennal shaft, usually at 1/3 from the base. Inner clypeal seta (2-C) long, single with minute branches on apical half, outer clypeal seta (3-C) single, less than half as long as 2-C. Postclypeal seta (4-C) with 1–2 branches, usually extending forward to near or beyond base of 2-C. Frontal setae (5-C to 7-C) long and plumose, 5-C conspicuously longer than the antenna and extending beyond the anterior margin of the head. Prothoracic seta 1 (1-P) arising from a prominent sclerotized tubercle, either entirely joined or partially connected by posterior bridge or sometimes entirely separate from common tubercle of setae 2-P and 3-P. Tubercle of seta 1-P with a prominent spine arising from its posterior margin, setae 1-P and 2-P with a stout stem, plumose, 3-P sometimes with 1–2 branches, usually single. Palmate setae on abdominal segments I and II (1-I and 1-II) small, weakly developed, with narrow, nearly transparent lanceolate leaflets, palmate setae 1-III to 1-VI fully developed, with 15–22 moderately broad, lanceolate leaflets, usually with smooth margins, rarely with minute serration close to apices (Fig. 12.20). Lateral abdominal setae 6 on segments IV–VI (6-IV to 6-VI) usually with 2–3 branches. Pecten with 3–5 long teeth alternating with 7–12 short teeth. Saddle spiculous at posterior margin, saddle seta (1-X) long, single, inserted inside or at ventral margin of saddle.

**Biology:** The immature stages are particularly abundant in the rainy season and found in various small, shallow shady temporary ground pools, animal footprints, puddles on foot paths, pools in dry stream beds, springs, streams, wheel ruts, rock pools, bamboo stumps, and depressions in hollow logs, sometimes with organic matter and turbid water (Sallum et al. 2005). The females readily bite man indoors and outdoors from early evening on, with a peak biting activity between 2000 and 2300 hours.

**Distribution:** Cambodia, China, Laos, Thailand, Vietnam.

**Medical importance:** Primary vector of human Plasmodium parasites in forested and hilly forested areas throughout its distribution range (Kobayashi et al. 2004; Trung et al. 2004). However, *An. dirus* seems to be adapted to human changes in the environment and it is suspected to be an efficient vector of human malarial parasites in areas of commercial tree crop plantations and gem mining areas (Sallum et al. 2005).

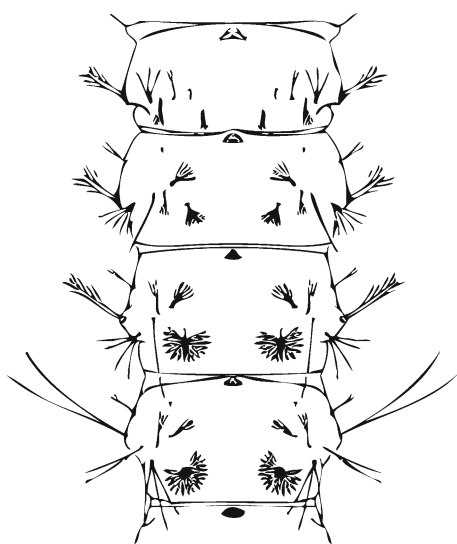


Fig. 12.20 Larva of *An. dirus* s.s., terga I-IV

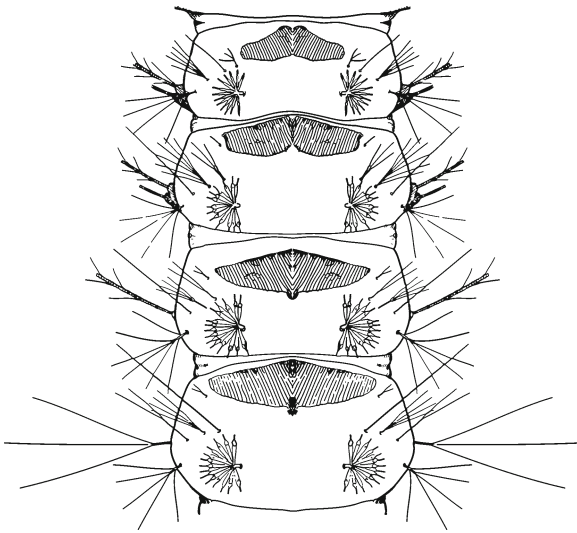


Fig. 12.21 Larva of *An. flavirostris*, terga I-IV

**Note on systematics:** The *Anopheles Dirus* Complex includes seven distinct species, namely *An. dirus s.s.*, *An. cracens*, *An. scanloni*, *An. baimaii*, *An. elegans*, *An. nemophilous*, and *An. takasagoensis*. All members of the complex may be distinguished by slight morphological differences in the females as well as in the fourth larval stage (Sallum et al. 2005). Additionally, the complex can be separated based on distinct banding patterns on the sex chromosomes and the salivary gland polytene chromosomes (Baimai et al. 1988).

#### ***Anopheles (Cellia) flavirostris* (Ludlow 1914)**

**Female:** Closely related to *An. minimus* and regarded as a subspecies of *minimus* for a long time. The main differences as indicated in the keys, the distinct pale scaling on the apical half of the proboscis in *An. flavirostris* may consist of a fairly definite patch of yellowish scales ventrally and laterally beginning near the middle or the apical third and narrowing toward the tip, usually separated from the labella by darker scales (Fig. 12.9c).

**Larva:** A reliable character to separate *An. flavirostris* from *An. minimus* involves the development and branching of seta 0 on abdominal terga IV-VII. In *An. flavirostris* setae 0-IV to 0-VII are small, with 1-2 branches, whereas in *An. minimus* setae 0-IV to 0-VII

are large, particularly on tergum IV, with 2-6 branches, rarely single. Another difference may be found in the shape of the large tergal plate on abdominal tergum II. In *An. flavirostris* the posterior margin of the plate is concave (Fig. 12.21), whereas in *An. minimus* the posterior margin is convex.

**Biology:** The larvae are typically found in slow flowing streams of clear fresh water with grassy margins, in ground pools, irrigation channels, drains and shallow wells. This species is particularly prevalent in streams opened up to sunlight through land settlement or lumbering operations (King 1932). The females feed on both man and cattle and are regarded as being exophilic. Harrison (1980) considered *An. minimus* and *An. flavirostris* geographically isolated, with the latter confined mainly to the Philippines, much of Indonesia, and Sabah, Malaysia. All the previous records of *An. minimus* in these areas should be regarded as *An. flavirostris*.

**Distribution:** Indonesia, Malaysia, Philippines.

**Medical importance:** Primary malaria vector in the Philippines and has been the main target of malaria eradication efforts for a long time (Harrison 1980). It has also been incriminated as a vector of *W. bancrofti*.

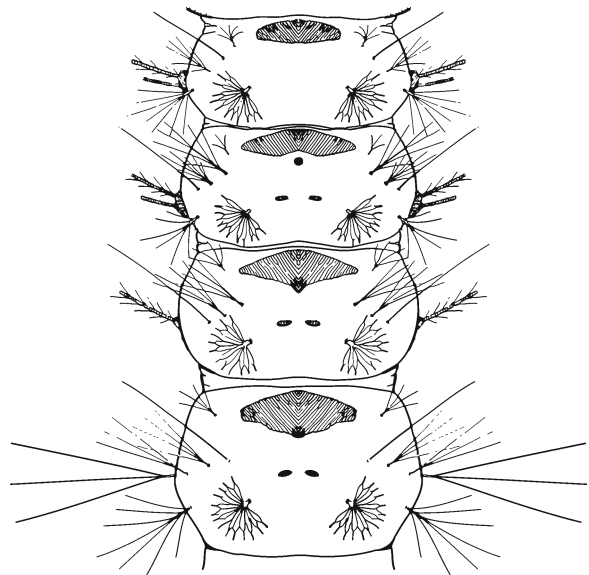


Fig. 12.22 Larva of *An. fluviatilis*, terga I-IV

### ***Anopheles (Cellia) fluviatilis* James 1902**

**Female:** Very similar to *An. minimus*, reliable differences are found only in the palpal ringing patterns. The palps with 3 pale rings, a broad apical ring and 2 narrow subapical and basal rings at joints of palpomeres II–III and III–IV, the subapical pale ring much shorter than the apical, intervening dark ring broad, usually 3 or more times as broad as the subapical pale ring; proboscis all dark (Fig. 12.9a). Another character which may separate the two species can be found on the costa (C), which is entirely dark scaled in the basal third in *An. fluviatilis* (Fig. 12.8b), whereas *An. minimus* has a presector pale spot of variable length (Fig. 12.8c).

**Larva** (Fig. 12.22): Almost not distinguishable from that of *An. minimus*.

**Biology:** The immature stages of *An. fluviatilis* can be found in essentially the same type of habitat as those of *An. minimus*, they breed especially at grassy edges of foothill streams and in stream pools, springs, and irrigation channels, sometimes at the edges of swamps and lakes, in drains, ponds, and tanks (Christophers 1933). The larvae have never been observed in large numbers, dispersed breeding over immense areas is responsible for the importance of this species (Foote and Cook 1959). The females readily bite man and cattle, they will enter houses and feeding generally occurs before midnight, they are regarded as strong fliers (Christophers 1933). Adults are recorded at altitudes up to 2,000 m in Kashmir, in southern India they are usually not found below 300 m.

**Distribution:** Afghanistan, Bahrain, Bangladesh, China, India, Iran, Iraq, Kazakhstan, Nepal, Oman, Pakistan, Saudi Arabia, Sri Lanka, Taiwan, Vietnam, Yemen.

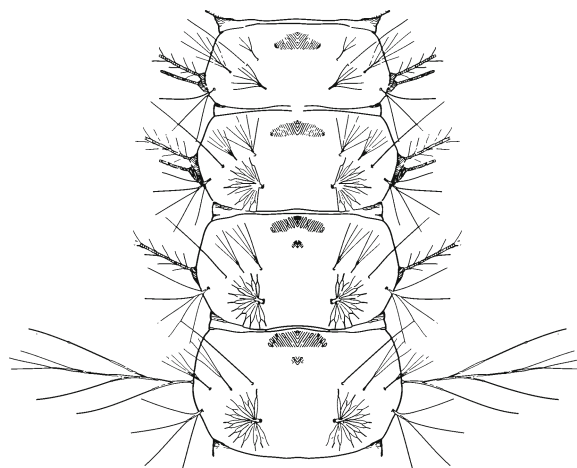
**Medical importance:** An important malaria vector, at least in parts of the Middle East and India (Reid 1968).

### ***Anopheles (Cellia) maculatus* Theobald 1901**

**Female:** Proboscis dark, palps about as long as proboscis, with erect dark scales towards the base, and with 3 distinct white rings, the apical and preapical rings broad and of about equal width, separated by a narrow dark ring, and a narrow basal pale ring at the joint of palpomeres II and III. Pedicel with

small pale scales. Vertex with a well marked frontal tuft of long whitish scales, occiput with numerous erect pale scales, darker scales behind. Integument of scutum light brown to greyish, with indistinct darker lines along acrostichal and dorso-central areas, and distinct dark spots above the transverse suture. Median area covered with creamy white scales, a lateral stripe of rather broad scales present. Scutellum clothed with narrow scales and moderately long pale setae. Integument of pleurites light brown, with paler areas forming a lower band, mesepisternum usually with some scales. Mid coxae with a few pale scales, femora, tibiae, and tarsomeres I of all legs extensively speckled with pale scales. Hind tarsi with a broad apical pale ring which covers the entire tarsomere V and the apical part of tarsomere IV and two broad rings at joints of tarsomeres II–III and III–IV (Fig. 12.5a). Wing with 4 large dark spots involving costa (C), subcosta (Sc) and  $R_1$ , radius (R) usually entirely pale at base and with the middle dark spot interrupted by small pale areas. Small dark spots on wing field variable in size. Anal vein (A) usually with 3 dark spots, more dark spots at base of Cu and apices of  $Cu_1$ ,  $Cu_2$ ,  $M_1$  and  $M_2$ , pale fringe spots at the terminations of all veins. Abdomen with a variable amount of scales on terga, usually increasing in number toward the posterior segments, mixed with long pale setae, small tufts of dark scales at posterior corners of terminal terga may be present.

**Larva:** Antenna with spicules on inner surface, antennal seta (1-A) inserted at about 1/3 from base



**Fig. 12.23** Larva of *An. maculatus*, terga I–IV

of antennal shaft. Inner and outer clypeal setae (2-C and 3-C) single, sometimes finely branched on distal half. The pair of 2-C inserted wide apart, 3-C slightly more than half as long as 2-C. Postclypeal seta (4-C) single, placed nearly in posterior line with 2-C, slightly shorter than 3-C and reaching well beyond the bases of the inner and outer clypeals. Prothoracic seta 1 and 2 (1-P and 2-P) with separate conspicuous basal tubercles, both with a stout stem and pinnately branched, 1-P short, single. Seta 1-I not of palmate type, 1-II weakly developed, leaflets without or with indistinct filaments. Palmate setae well developed on abdominal segments III–VII (1-III to 1-VII), leaflets broad, with a varying amount of pigmentation, filament well differentiated, 1/3 to 2/3 the length of the blade, sharply pointed. Lateral abdominal seta 6 on segments V and VI (6-V and 6-VI) with 3–6 branches, 6-IV with 4–7 branches (Fig. 12.23). Tergal plates small to medium sized, pecten similar to that of *An. stephensi*.

**Biology:** The larvae of *An. maculatus* are primarily found in hilly areas in partially shaded small pools along the margins of fast flowing streams and rivers with grassy edges, but also in drains, springs, borrow pits, rice fields, polluted water, hoof prints and artificial containers (Christophers 1933; Stojanovich and Scott 1966). Often they are especially numerous in recently cleared areas where trees have been cut and the soil is disturbed (Reid 1968). The female feed readily on man and cattle at night, they frequently enter houses but leave immediately after the blood meal; during the day they rest outdoors in low vegetation.

**Distribution:** Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Philippines, Taiwan, Thailand, Vietnam.

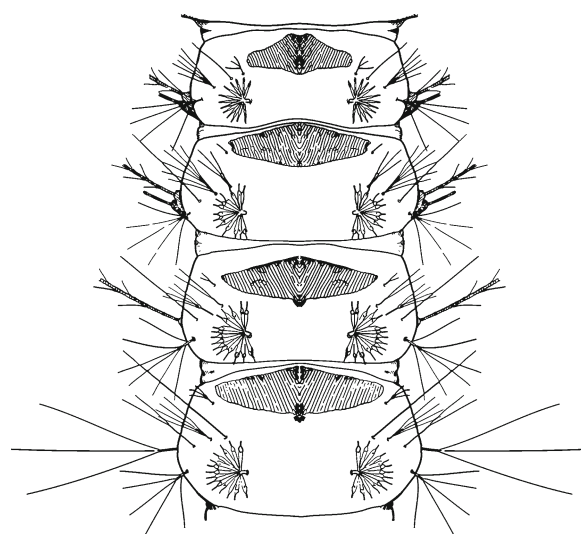
**Medical importance:** Malaria vector and a vector of *W. bancrofti* (Reid 1968).

### ***Anopheles (Cellia) minimus* Theobald 1901**

**Female:** Proboscis dark scaled, palps as long as proboscis, with 3 pale rings, a narrow basal ring at joint of palpomeres II and III, and 2 broad apical rings, the subapical ring as broad as the apical ring, the intervening dark ring usually as long as the preapical ring, rarely longer (Fig. 12.9b). Clypeus dark brown and

without scales, vertex with pale erect scales, long slender whitish scales forming a prominent frontal tuft. Scutum with a median pale area and contrasting darker areas laterally, sometimes with faint dark acrostichal and dorsocentral lines, small median tuft of pale scales on anterior margin of scutum, median area clothed with pale setae and setose scales. Scutellum covered with short pale setae and a row of long dark setae. Integument of pleurites light to dark brown, often with continuous pale transverse band. 2–4 prealar setae, 3 upper and 3–4 lower mesepisternal setae, 3–8 upper mesepimeral setae present, lower mesepimeral setae absent. Coxae pale, devoid of scales, legs uniformly dark scaled, tarsi without pale rings or with very narrow faint pale patches or rings at the joints (Fig. 12.4b). Wings with dark and pale spots forming a variable colour pattern, base of costa (C) usually with a presector pale spot or at least a few pale scales on one wing, costa (C) with 4–5 pale spots of varying width. Wing fringe with pale spots at termination of all veins except anal vein (A) (Fig. 12.8c). Integument of abdomen dark brown, clothed with light and dark setae, entirely devoid of scales.

**Larva:** Antenna straight, dark distally, spiculate on inner surface, antennal seta (1-A) inserted at about 1/3 from base of antennal shaft. Inner and outer clypeal setae (2-C and 3-C) single, stout, 3-C about 1/2 to 2/3 as long as 2-C, postclypeal seta (4-C) single and slen-



**Fig. 12.24** Larva of *An. minimus*, terga I–IV

der, about half as long as and extending beyond the base of 2-C, frontal setae (5-C to 7-C) long and plumose. Prothoracic setae 1 and 2 (1-P and 2-P) arising from basal sclerotized tubercles, which sometimes may be narrowly fused, 1-P less than half as long as 2-P, both with a stout stem and multiple branches, 3-P single. Metathoracic palmate seta (1-T) well developed with slender lanceolate leaflets. Palmate setae well developed on abdominal segments I–VII (1-I to 1-VII), that of segment I smaller than the others. Leaflets light in colour distally with a contrasting dark pigmented area just basal to shoulders, filament slender, serrated at base and well differentiated, about half as long as the blades. Tergal plates very large on segments III–VII, more than half the width of the segment, distinctly wider than the distance between the bases of the palmate setae of each segment, enclosing small median posterior tergal plates. Setae 0 on segments III–VII (0-III to 0-VII) usually large and prominent with 1–4 branches arising outside the tergal plate. Lateral abdominal setae 6 on segments IV–VI (6-IV to 6-VI) long, with 3–4 branches (Fig. 12.24). Pecten with 4–8 long and 6–10 short teeth, length of the long teeth variable, all teeth finely serrated in basal half. Saddle seta (1-X) single, longer than the saddle, anal papillae slightly shorter than anal segment.

**Biology:** *An. minimus* inhabits foothill and hilly areas, the larvae are most commonly found along the shaded grassy edges of clear, sunlit and slowly flowing streams and springs, less frequently margin of swamps, irrigation channels, and rice fields. The larvae normally attach to grass and vegetation with their hooked upper and lower anal setae (2-X and 3-X) to resist currents (Harrison 1980). Other larval habitats include rock pools, sand pools next to streams, and seepage pools. The females readily attack man and cattle and are found in houses and cattle sheds, they are regarded as being anthropophilic and endophagic. Resting collections on vegetation and piled wood around bovine shelters at night also proved to be a good collection technique for this species (Harrison 1980). The extensive use of DDT spraying during the 1940s to 1960s obviously led to a selection pressure toward a zoophilic behaviour and has favoured the survival of that portion of the population feeding outdoors and not coming into contact with DDT.

**Distribution:** Bangladesh, Cambodia, China, India, Japan, Laos, Malaysia, Pakistan, Taiwan, Thailand, Vietnam.

**Medical importance:** Important malaria vector and also vector of *W. bancrofti* (Reid 1968).

**Note on systematics:** There are four sibling species recognised in the Anopheles Minimus Complex, namely species A, B, C, and E (Green et al. 1990; Somboon et al. 2000). Harrison et al. (1990) considered species A as being probably *An. minimus* Theobald.

### *Anopheles (Cellia) stephensi* Liston 1901

**Female:** Medium sized species. Proboscis dark scaled, palps with a broad pale ring at the apex and a broad pale ring at the joint of palpomeres III and IV, the dark area in between is distinctly narrower than either ring, a narrow pale ring at the joint of palpomeres II and III, and patches of pale scales on the dorsal surface of palpomere III, which are characteristic for the species (Fig. 12.6b). Pedicel with small pale scales. Vertex with a well marked frontal tuft of long whitish scales, occiput with numerous erect pale scales. Integument of scutum light brown to greyish, lateral areas distinctly darker and contrasting with the median area, and a darker spot at the presutellar area. Median stripe covered with pale narrow scales and lateral tufts of white scales, supraalar area with a distinct stripe of pale scales. Scutellum clothed with whitish scales and long yellowish setae. Integument of pleurites light brown, bare of scales, with 2–3 prespiracular setae and 5–6 upper mesepimeral setae. Femora and tibiae speckled with pale scales, those of hind legs usually with pale scales on ventral surfaces. Tarsi predomi-

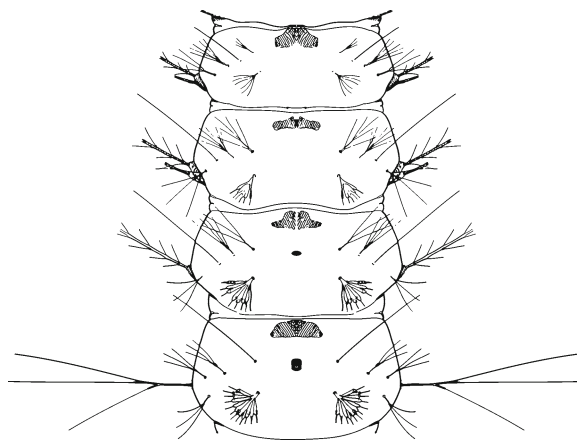


Fig. 12.25 Larva of *An. stephensi*, terga I–IV

nantly dark scaled, fore tarsus with apical and basal pale rings at joints of tarsomeres I–II and II–III, mid and hind tarsi with apical pale rings more narrow, hind tarsomere V entirely dark scaled. Wing with 4 large dark spots at anterior margin involving costa (C), subcosta (Sc) and  $R_1$ . Small dark spots at bases of  $M_1$ , cubitus (Cu),  $Cu_1$ , and anal vein (A), wing fringe usually with spots of pale scales at terminations of all veins. Abdomen clothed with long pale setae and terga VII and VIII heavily covered with narrow scales and pale setae.

**Larva:** Antenna with spicules on inner surface, antennal seta (1-A) inserted at about 1/3 from base of antennal shaft. Inner and outer clypeal setae (2-C and 3-C) single, the pair of 2-C inserted wide apart, 3-C about 2/3 as long as 2-C. Postclypeal seta (4-C) single, about as long as 3-C and reaching well beyond the bases of the inner and outer clypeals. Frontal setae (5-C to 7-C) long and plumose. Prothoracic seta 1 and 2 (1-P and 2-P) arising from separate basal tubercles, both pinnately branched, 1-P slightly shorter than 2-P. Palmate setae well developed on abdominal segments III–VII (1-III to 1-VII), leaflets uniformly pigmented, with a short filament, which is broad at base and about 1/4 as long as the blade. Seta 1-I not of palmate type, 1-II poorly developed (Fig. 12.25). Lateral abdominal seta 6 on segments IV–VI (6-IV to 6-VI) with 3–4 branches split at some distance from base, tergal plates rather small. Pecten with 3–5 long and 8–11 short teeth, all teeth serrated on basal half.

**Biology:** Under natural conditions, the larvae of *An. stephensi* can be found in clean water in stream or river pools, shaded or exposed to the sun, in drains, pools, and irrigation channels. In urban areas they are found in a wide variety of artificial containers, including cisterns, wells, flooded cellars, tubs and fountains, and any kind of artificial reservoirs (Christophers 1933). The larvae are very shy and rapidly sink to the bottom when disturbed, and they may remain down for long periods (Stojanovich and Scott 1966). The females readily feed on man and rest commonly in dwellings and cow sheds.

**Distribution:** Afghanistan, Bahrain, Bangladesh, China, Egypt, India, Iran, Iraq, Oman, Pakistan, Saudi Arabia, Thailand.

**Medical importance:** Important vector of malaria on the Arabian Peninsula and large cities in the Indian subcontinent.

### *Anopheles (Cellia) sundaicus* (Rodenwaldt 1925)

**Female:** Medium sized species with conspicuous speckled legs. Proboscis entirely dark scaled, labelum somewhat lighter. Palps as long as proboscis, predominantly dark scaled with a broad apical pale ring, and 2 narrow preapical pale rings, the apical ring includes the entire palpomere V and the apical portion of palpomere IV and is about as long as the preceding dark area (Fig. 12.6c). Pedicel sometimes with a few minute scales. Occiput covered with erect pale scales, vertex with a tuft of long pale scales projecting forward. Integument of scutum greyish brown, with darker areas laterally. Anterior margin of scutum with a median tuft of narrow pale scales, broader scales laterally. Median part of scutum covered with short pale hair-like curved scales, scutellum clothed with long pale brown setae. Pleurites with a few scales sometimes present on mesepisternum, upper mesepimeral setae numerous. Wing veins covered with pale and dark scales forming characteristic spots. Preapical dark spot longer than pale areas, dark spots other than those on costa (C) and radius (R) mostly small. Pale fringe spots at terminations of all veins and between anal vein (A) and base of wing, an additional pale fringe spot between  $Cu_2$  and anal vein (A) very rarely present. Coxae without scales, femora, tibiae, and tarsomeres I of all legs with conspicuous pale yellowish speckling. Mid and hind femora with pale scaling on

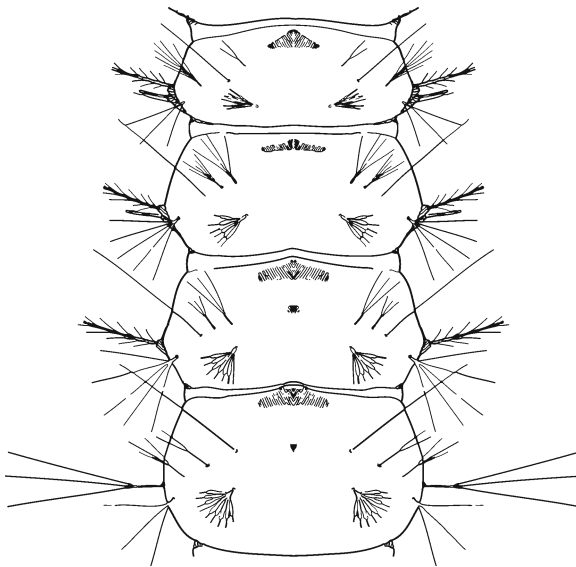


Fig. 12.26 Larva of *An. sundaicus*, terga I–IV

ventral surface, all femora and hind tibiae with pale scales at apices. Fore tarsi with pale apical rings on tarsomeres I–III and narrow basal rings on tarsomeres II–IV, tarsomere V entirely dark scaled. Scaling of mid tarsi similar but pale rings narrower, hind tarsi with apical rings on tarsomeres I–IV, tarsomere V entirely dark scaled (Fig. 12.4a). Integument of abdomen dark to light brown, clothed with golden setae and a few pale scales at posterior margins of terga and sterna VII and VIII and cerci.

**Larva:** Antenna rather slender, antennal seta (1-A) inserted at about the middle of the antennal shaft. Inner clypeal seta (2-C) slender, single, the pair of 2-C inserted wide apart, much closer to 3-C than to each other. Outer clypeal seta (3-C) about half as long as 2-C, single, postclypeal seta (4-C) slightly shorter than 3-C, arising rather far back and extending beyond bases of 2-C and 3-C. Frontal setae (5-C to 7-C) long and plumose. Prothoracic seta 1 (1-P) without conspicuous basal tubercle, slender, with 8–15 branches, 2-P with indistinct basal tubercle, about 1.5 times as long as 1-P, with 10–13 branches, 3-P about half as long as 2-P, single. Palmate seta on abdominal segment I (1-I) weakly developed, with 3–6 moderately broad leaflets but usually without obvious serrations or well defined filaments, 1-II to 1-VII fully developed, leaflets evenly pigmented, with sharply defined filaments with pointed tips and narrow base, nearly as long as the blade. Lateral abdominal setae on segments IV–VI (6-IV to 6-VI) long and split into 2–3 slender branches near base. Tergal plates very small and narrow, posterior plates present on segments III–VII (Fig. 12.26). Pecten with about 4–5 long and 10–11 short teeth, the number and length very variable. Posterior margin of saddle with numerous spicules, lower anal seta (3-X) with 6–8 long branches. Anal papillae about twice as long as anal segment.

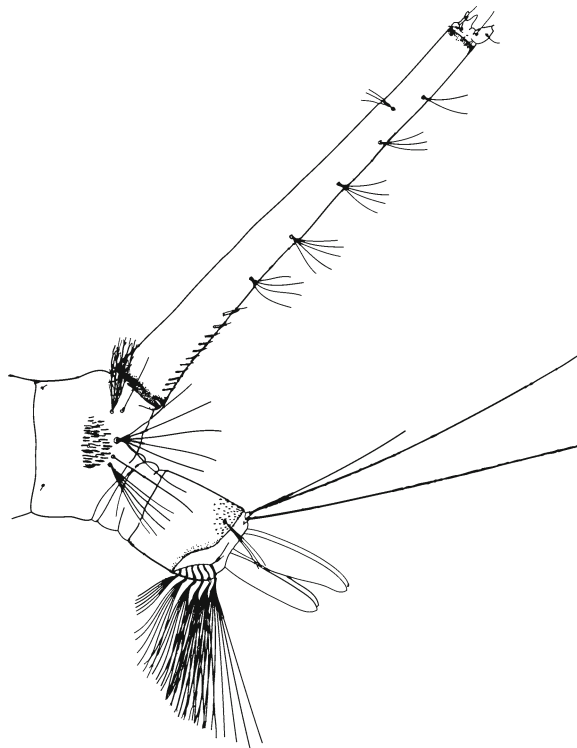
**Biology:** *An. sundaicus* is primarily a coastal species, the larvae are mainly found in sunlit brackish pools or salt swamps with vegetation and algae, but they also breed in small tidal creeks and pools without vegetation and sometimes inland in fresh water in various places (Reid 1968). Females prefer to feed on cattle but readily bite man indoors and outdoors, the main biting activity takes place during the early evening and, to a lesser extent, throughout the night. The adults rest by day both outdoors and indoors, the females are strong fliers, a flight range of up to 5 km is recorded (Christophers 1933).

**Distribution:** Bangladesh, Cambodia, China, India, Indonesia, Malaysia, Singapore, Taiwan, Thailand, Vietnam.

**Medical importance:** This species is an important vector of malaria throughout its distribution range (Reid 1968).

### *Culex (Culex) tritaeniorhynchus* Giles 1901

**Female:** Relatively small, reddish brown species. Proboscis predominantly dark scaled, with a narrow median pale ring, sometimes pale scaling extending to the base ventrally. Palps about 1/5 the length of the proboscis, entirely dark scaled or sometimes with pale scales on apex of palpomere IV. Antenna dark, pedicel yellowish to light brown. Head with decumbent scales predominantly golden or yellowish, erect scales of vertex entirely dark brown, lateral patch of broader scales yellowish white. Integument of scutum reddish brown to brown, scutum predominantly covered with narrow dark reddish brown or golden brown scales, a few paler scales at anterior and



**Fig. 12.27** Larva of *Cx. tritaeniorhynchus*



lateral margin, and prescutellar area. Scutellum covered with pale yellowish scales. Integument of pleurites reddish brown. Mesepisternum with small patches of pale scales in upper portion and lower posterior margin, mesepimeron with a small anterior upper scale patch and sometimes with a few scales among the upper mesepimeral setae. Lower mesepimeral setae absent. Femora and tibiae dark scaled anteriorly, with pale scales posteriorly (Fig. 12.12b). Tarsi predominantly dark scaled with narrow basal and apical pale rings on tarsomeres I–IV, more distinct on fore legs. Wing veins entirely dark scaled, sometimes with a short line of pale scales at the base of the costa (C). Haltere pale scaled, knob with dark scales. Tergum I with median posterior patch of dark scales, terga II–VII with narrow, slightly convex basal pale bands and basolateral pale patches, tergum VIII largely pale scaled with narrow apical dark band. Sterna mostly pale scaled, sometimes with small dark lateral patches.

**Larva:** Head slightly wider than long. Antenna about 0.7 times as long as the head, with numerous spicules, dark at the base and narrowing toward the apex. Antennal seta (1-A) inserted at about 2/3 from the base of the antennal shaft, with multiple branches. Labral seta (1-C) relatively short and sharply pointed, dark. Postclypeal seta (4-C) single, about as long as the distance between the bases of the pair. Frontal setae (5-C to 7-C) long and aciculate, inner frontal seta (5-C) with 3–4 branches, median frontal seta (6-C) with 2–3 branches, outer frontal seta (7-C) about as long as 5-C and 6-C, with 5–10 branches. Prothoracic setae 1–3 (1-P to 3-3P) long and single, 4-P strong with 2 branches. Lateral abdominal seta 6 on segments III and IV (6-III and 6-IV) with 2–3 branches, 6-V and 6-VI usually with 2 branches. The comb consists of about 40–50 small evenly fringed scales arranged in a broad

oval patch. Siphon long and slender, slightly tapering towards apex, siphonal index about 5.7–7.0. The pecten is composed of 10–15 teeth at the basal 1/4 of the siphon, distal pecten teeth with five to six lateral denticles. The siphonal seta (1-S) consists of 5–6 pairs of widely spaced tufts, inserted subventrally, except the subapical tuft (1e-S), which is inserted laterally. All tufts with 3–4 branches as long as or slightly longer than width of siphon at point of insertion (Fig. 12.27). Anal segment completely encircled by the saddle, saddle seta (1-X) usually with 2–3 branches, shorter than the saddle. Upper anal seta (2-X) with 2–3 branches, lower anal seta (3-X) single, longer than the siphon. Ventral brush with 6 pairs of cratal setae (4-X). Anal papillae elongate, about as long as the saddle.

**Biology:** The larvae can be found in various temporary and permanent ground water habitats that are sunlit and contain vegetation, including ground pools, streams, swamps, shallow marshes, irrigation ditches, rice fields, and animal hoof prints (Bram 1967a; Harbach 1988). The females feed primarily on domestic animals such as cattle and pigs, but will bite man in their absence (Bram 1967a). They mainly bite outdoors between sunset and midnight, but may enter cattle sheds and dwellings and bite man during any time of the night (Gutsevich et al. 1974; Sirivanakarn 1976).

**Distribution:** Widely distributed throughout the Oriental region extending west into the Middle East, eastern Mediterranean and large parts of the Ethiopian region, also found in the southeastern and eastern Palaearctic region.

**Medical importance:** Most important vector of Japanese encephalitis virus (JE) in the oriental region, particularly in Southeast Asia (Bram 1967a; Sirivanakarn 1976).

## Chapter 13 Australia

In addition to *An. farauti* described below, two more malaria vectors of secondary importance in Australia are *An. annulipes s.l.* (inland distribution NSW, VIC, SA, TAS, QLD, NT, WA) and *An. bancroftii* (northern Australia). The former species may be involved in the transmission of human filaria, dog heartworm and Ross River virus.

Russell (2006) stated that “whatever happens from here with mosquito nomenclature, we can all be assured that in Australia, as in other continents, *Aedes* by any name will bite as badly”. In addition to those included in the first group of species responsible for serious biting nuisance (Russell personal commun.), the following three can also cause biting nuisance in Australian wetlands, but with no evidence from the field for any role in the transmission of human disease: *Oc. bancroftianus* (inland regions of NSW, NT, QLD, SA, VIC, WA); *Oc. vittiger* (NSW, NT, QLD, SA, VIC) and *Verrallina funerea* (costal northern NSW, NT, QLD). In addition to being a significant nuisance pest in some localities, *Cq. linealis* (coastal and inland NSW, VIC, SA) should also be considered as a vector

of Ross River and Barmah Forest viruses (Russell et al. 1999, <http://medent.usyd.edu.au>).

*Cx. p. quinquefasciatus*,<sup>1</sup> is a significant nuisance in many urban areas, *Ae. aegypti*<sup>1</sup> is an important pest and vector particularly in northern Queensland, and the invasive *Ae. albopictus*,<sup>1</sup> are also included in the key.

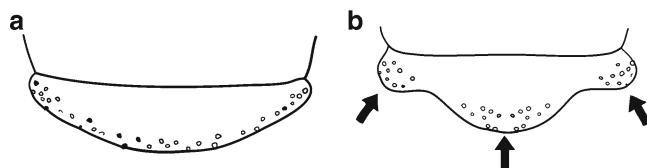
Moreover, *Ae. cooki* [*Stegomyia cooki*], *Ae. hebrideus* [*Stegomyia hebridea*], *Ae. hensilli* [*Stegomyia hensilli*], *Ae. polynesiensis* [*Stegomyia polynesiensis*], *Ae. rotumae* [*Stegomyia rotumae*], *Ae. scutellaris* [*Stegomyia scutellaris*], *Oc. notoscriptus* [*Rampomyia notoscripta*] may also be important dengue vectors for the South Pacific Islands and Australian Region (Rueda 2004).

For further detailed information on morphology, identification, biology, distribution and disease vector status of the species mentioned, the reader is referred to Skuse (1889), Edwards (1924), Mackerras (1927), Lee (1944), Marks (1949), Mattingly (1961, 1971), Belkin (1962a,b), Dobrotworsky (1965), Huang (1968), Tyson (1970), Smith (1973), Lee et al. (1988), Rueda (2004), Reinert and Harbach (2005b).

### 13.1 Key to Australian Female Mosquitoes

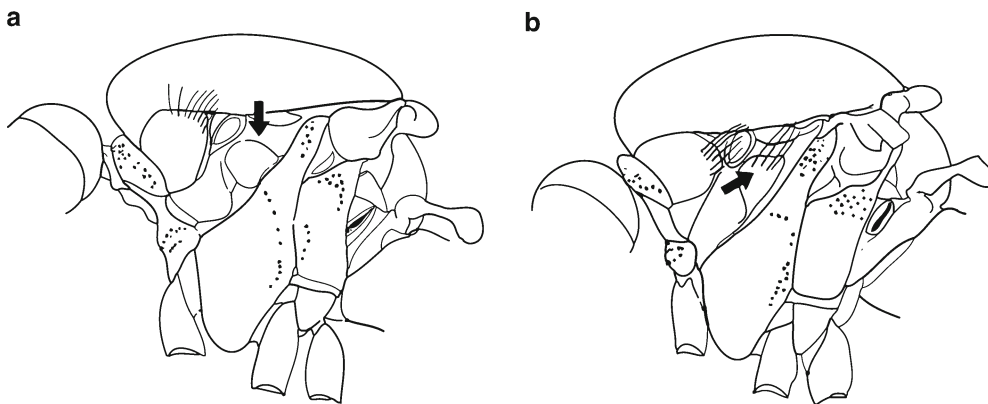
- 1 Palps about as long as proboscis. Scutellum evenly rounded or slightly trilobed and uniformly setose (Fig. 13.1a). Abdominal terga and sterna completely or largely missing scales. – Wing with contrasting pattern of dark and light scales, veins  $R_{3+4}$ , M, and  $Cu_1$  evenly curved beyond cross vein m-cu, *Anopheles*. Proboscis dark-scaled except for narrow inconspicuous pale ring at apex and rarely a few pale scales ventrally on basal half. Scutum speckled with broad decumbent yellowish scales. Knob of haltere dark-scaled. .... *An. farauti* (p 356)
- Palps distinctly shorter than proboscis. Scutellum trilobed, setae arranged in three sets (Fig. 13.1b). Abdominal terga and sterna uniformly and densely covered with scales. – Prespiracular setae absent. Anal vein (A) evenly curved, ending distinctly beyond furcation of cubitus (Cu). Alula with fringe of narrow scales. .... 2

<sup>1</sup>Species described in Chap. 10 concerning European mosquitoes.



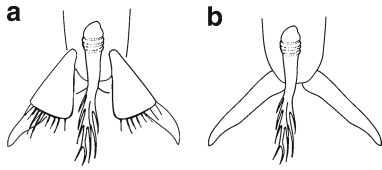
**Fig. 13.1** Scutellum of: (a) *Anopheles* sp.; (b) *Aedes* sp.

- 2 (1) Postspiracular setae absent (Fig. 13.2a). All tarsal claws simple, without subbasal tooth. – Tarsomere I of fore legs usually shorter than tarsomeres II–V together. Tarsomere IV of fore legs not reduced, distinctly longer than broad. Abdomen rounded apically, cerci short, hardly visible. .... 3
- Postspiracular setae present (Fig. 13.2b) and/or at least the claws of fore tarsi with subbasal tooth. – Proboscis not strongly curved. Palps less than 2/3 as long as proboscis. .... 6

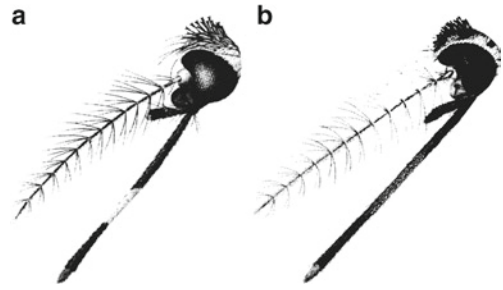


**Fig. 13.2** Lateral view of thorax of: (a) *Culex* sp.; (b) *Mansonia* sp.

- 3 (2) Pulvilli present, well developed (pad-like) (Fig. 13.3a). Hind tarsal claws very small and inconspicuous. *Culex* ..... 4
- Pulvilli absent or not well developed (hair-like) (Fig. 13.3b). Hind tarsal claws quite large and conspicuous. *Coquillettidia*. – Scutum usually uniformly yellowish to orange, scutal scales scanty, very narrow. Abdominal terga with light golden scales and some dark purplish scales. Legs dark scaled, hind femur with pale golden scales on basal 1/3 of anterior surface. .... *Cq. xanthogaster* (p 367)
- 4 (3) Proboscis with distinct median pale ring (Fig. 13.4a). Lower mesepimeral setae absent. At least some tarsomeres with distinct basal or basal and apical pale rings. – No broad erect scales in front of supraalar setae. One or more of abdominal terga II–VI with more or less complete transverse pale bands. Pale bands on terga II–IV largely or entirely basal. .... 5
- Proboscis without median pale ring but often with pale midventral area (Fig. 13.4b). Usually only one lower mesepimeral setae present. Tarsi without distinct pale rings. – Pale spot at tip of hind tibia inconspicuous. Terga with well developed pale basal transverse bands. Sterna pale, without large black central patches. .... *Cx. p. quinquefasciatus* (p 278)

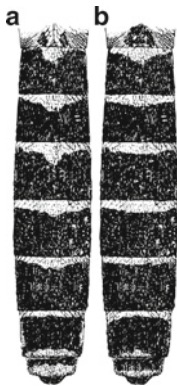


**Fig. 13.3** Pulvilli of: (a) *Culex* sp.; (b) *Coquillettida* sp.

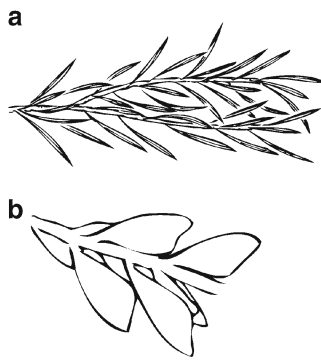


**Fig. 13.4** Proboscis of: (a) *Cx. sitiens*; (b) *Cx. p. quinquefasciatus*

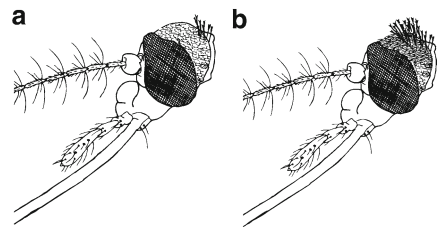
- 5 (4) Fore tibia usually with a line of small pale spots on anterior surface among row of setae. Terga dark scaled with pale basal bands extended medially (Fig. 13.5a). Sterna pale scaled with incomplete apical dark bands. – Hind tarsomeres I–IV with pale basal rings, tarsomere V all dark. .... *Cx. annulirostris* (p 365)
- Fore tibia usually without pale spots on anterior surface among row of setae. Terga with basal bands not extended medially (Fig. 13.5b). Sterna with apical dark bands complete. – Mid and hind tibiae with indistinct longitudinal pale stripes. Mid femur usually with numerous scattered pale scales on anterior surface. *Cx. sitiens* (p 366)
- 6 (2) Wing scales usually narrow, if broad then not strongly asymmetrical (Fig. 13.6a). Claws of fore legs usually with subbasal tooth. .... 7
- Wing scales all broad and conspicuously asymmetrical (especially on Cu) (Fig. 13.6b). Claws all simple, without subbasal tooth. – Erect forked scales numerous, not restricted to occiput. Lower mesepimeral setae present. Scutum with dorsocentral stripes of pale scales and a pair of continuous pale longitudinal stripes on submedian area. .... *Ma. uniformis* (p 330)
- 7 (6) Head with decumbent scales mainly broad. Erect scales not numerous, restricted to occiput (Fig. 13.7a). – Proboscis without a white ring. Scutum with white/silvery scales in lines or patches. Scales of scutellum all broad. Femora with white knee spot. Tarsi with conspicuous pale markings. Hind tarsomere V entirely white. *Aedes* subgenus (*Stegomyia*) ..... 8
- Head with decumbent scales largely narrow and/or (if scales of vertex are largely broad) erect scales numerous, not restricted to occiput (Fig. 13.7b). .... 9



**Fig. 13.5** Abdomen of: (a) *Cx. annulirostris*; (b) *Cx. sitiens*



**Fig. 13.6** Wing scales of: (a) *Aedes* sp.; (b) *Mansonia* sp.



**Fig. 13.7** Head of: (a) *Aedes (Stegomyia)* sp.; (b) *Ochlerotatus* sp.

- 8 (7) Scutum without acrostichal stripe on anterior part, but with two narrow white dorsocentral stripes separated from anterior margin. Lateral white stripes broad, continuing over transverse suture to the end of scutum, lyre shaped (Fig. 13.8a). – Clypeus with white scale patches. Mesepimeron with two well separated white scale patches.....*Ae. aegypti* (p 198)  
 Scutum with a white acrostichal stripe extending from the anterior margin to the beginning of the prescutellar area, where it forks to end at the anterior margin of scutellum (Fig. 13.8b). – Clypeus without white scale patches. Mesepimeron with V-shaped white scale patch.....*Ae. albopictus* (p 201)
- 9 (7) Decumbent scales of vertex largely broad. Cerci short, broad and blunt (Fig. 13.9a). *Ochlerotatus* subgenus (*Finlaya*) – Proboscis with a distinct median pale ring. Scutum with silvery stripes forming a lyre shaped pattern. Postpronotal patch of dark scales with a small but distinct group of silvery scales at lower posterior portion. Mid femur with a median longitudinal silvery stripe. Wing scales all dark. ....*Oc. notoscriptus* (p 358)  
 Decumbent scales of vertex largely narrow. Cerci long and slender, tapering (Fig. 13.9b). .... 10

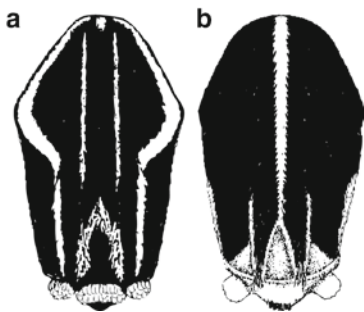


Fig. 13.8 Scutum of: (a) *Ae. aegypti*; (b) *Ae. albopictus*

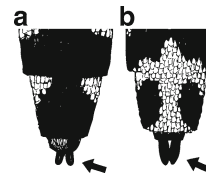


Fig. 13.9 End of abdomen of: (a) *Ochlerotatus (Finlaya)* sp.; (b) *Ochlerotatus (Ochlerotatus)* sp.

- 10 (9) Palps 2/3 length of proboscis (Fig. 13.10a). Wing membrane conspicuously clouded on base and across furcation of  $R_s$  and cross veins r-m, m-cu. *Ochlerotatus* subgenus (*Mucidus*) – Scutum with tufts of twisted erect pale scales. Legs more or less shaggy. Wing very densely scaled with mixed brown, pale yellowish and white scales. .... *Oc. alternans* (p 357)  
 Palps less than 1/3 length of proboscis (Fig. 13.10b). Wing membrane usually not spotted, uniformly clear or uniformly clouded. *Ochlerotatus* subgenus (*Ochlerotatus*)..... 11

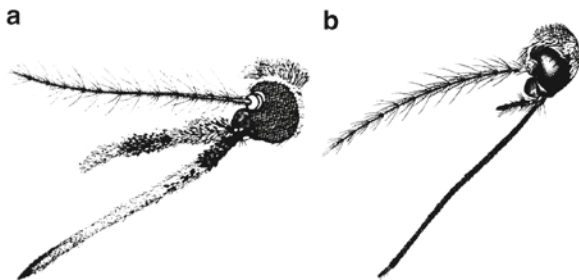
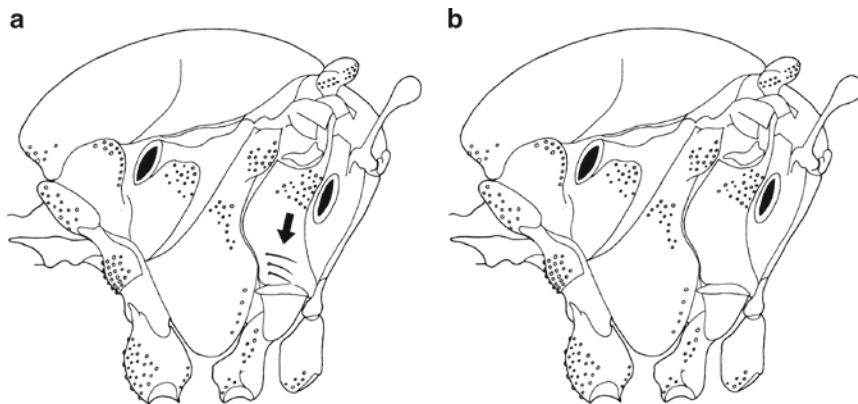


Fig. 13.10 Palps of: (a) *Ochlerotatus (Mucidus)* sp.; (b) *Ochlerotatus (Ochlerotatus)* sp.



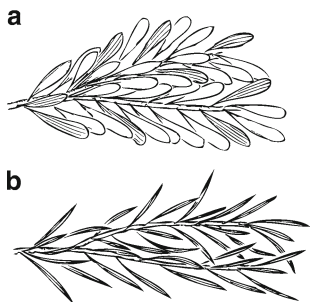
Fig. 13.11 Hind tarsus of: (a) *Oc. vigilax*; (b) *Oc. sagax*

- 11 (10) Tarsi of all legs with distinct white basal rings (Fig. 13.11a). – Head and sides of scutum with narrow scales. .... 12  
 Tarsi without distinct white basal rings (Fig. 13.11b). Scutum with bronzy-brown and creamy white scales. Scutellum with narrow scales only. At least one strong lower mesepimeral seta present. Wing dark scaled, pale scales, if present, relatively few and only along anterior border. Abdomen with distinct pale basal transverse bands. .... *Oc. sagax* (p 362)
- 12 (11) Wings entirely dark scaled. At least one lower mesepimeral seta present (Fig. 13.12a). Femora speckled with white scales anteriorly ..... *Oc. camptorhynchus* (p 360)  
 Wings with at least some white scales. Lower mesepimeral seta absent (Fig. 13.12b)..... 13

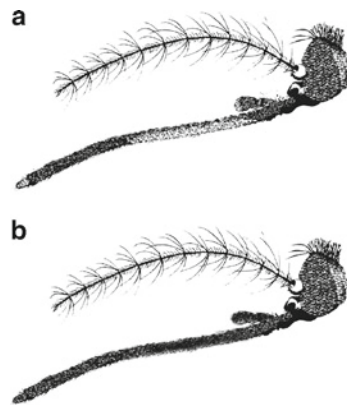


**Fig. 13.12** Lateral view of thorax of: (a) *Oc. camptorhynchus*; (b) *Oc. vigilax*

- 13 (12) Wings with numerous symmetrical broad scales (Fig. 13.13a). – Pale basal bands of abdominal terga narrow, sometimes incomplete, the dark portions of the terga usually conspicuously speckled with pale scales, especially on terminal segments. .... *Oc. theobaldi* (p 363)  
 Wing scales normal in size and shape (Fig. 13.13b)..... 14



**Fig. 13.13** Wing scales of: (a) *Oc. theobaldi*; (b) *Oc. vigilax*



**Fig. 13.14** Proboscis of: (a) *Oc. vigilax*; (b) *Oc. normanensis*

- 14(13) Basal 2/3 of proboscis with ventrolateral pale scaling sometimes forming complete ring (Fig. 13.14a). Hind tarsomere V with a broad white ring.....*Oc. vigilax* (p 364)  
 Proboscis dark scaled, sometimes with a few scattered pale scales on basal half. (Fig. 13.14b). Hind tarsomere V without, or with very narrow, basal white ring..... *Oc. normanensis* (p 360)

## 13.2 Species Description

### *Anopheles (Cellia) farauti* s.l. Laveran 1902

**Female:** Medium sized species, proboscis entirely dark scaled except for a very narrow apical pale ring, labellum light brown. Palps as long as proboscis and dark scaled, usually with 3–4 pale rings, the apical and subapical rings broad, sometimes with variable number of paler scales dorsally near base. Pedicel with a few small white scales. Integument of head dark throughout, occiput with erect scales short and broad, white dorsally and dark laterally, vertex with a frontal tuft of very long slender white scales forwardly directed. Integument of scutum light yellow brown, with dark spots anterior to scutal angle and a dark prescutellar area. Scutum sparsely covered with moderately broad decumbent yellowish scales, scales somewhat longer anteriorly and above wing roots. Scutellum with a few whitish scales smaller than on scutum, scutal and scutellar setae golden brown. Integument of pleurites usually with light and darker areas, anteprepronotum with a dorsal patch of broad erect dark scales and numerous dark setae. Upper mesepisternum with 5–7 setae and a small patch of broad decumbent light scales, lower mesepisternum usually with 4 setae and a small patch of similar scales, 4–10 upper mesepimeral setae present, all pleural setae rather light. Legs with light brown to blackish scales and with yellowish white scale markings. Femora and tibiae extensively speckled with pale scales, tarsomere I of all tarsi with pale apex and a variable number of pale spots. Fore tarsomere I–IV pale at base and apex, tarsomere V pale or dark, all tarsomeres usually much paler ventrally. Mid and hind tarsomeres II–IV with narrow apical pale rings, tarsomeres V usually all dark. Wing with contrasting pattern of dark and pale scales, costa (C) with 4 large dark spots and 3 small dark spots basally, usually a small dark sector spot between basal and median dark spots. Wing fringe dark scaled, with pale spots at apices of all veins. Haltere pale at base,

knob dark scaled. Integument of abdomen dark brown, setae pale golden, more numerous on last segments, scales absent on terga I–V and sterna I–VI, a few scales on terga VI and VII and sternum VII, rather dense scaling on tergum VIII and sternum VIII.

**Larva:** Head strongly and unevenly pigmented, usually with a conspicuous pattern of dark areas. Antenna strongly pigmented, covered with small spicules, antennal seta (1-A) minute and single, arising near base on outer surface of antennal shaft. Inner clypeal setae (2-C) widely separated, inserted closer to outer clypeal setae (3-C) than to each other. 2-C usually strongly aciculate, 3-C more than half the length of 2-C, frequently barbed. Postclypeal seta (4-C) usually with 1–2 branches and extending beyond insertion points of 2-C and 3-C. Frontal setae (5-C to 7-C) plumose, gradually decreasing in size. Prothoracic setae 1–3 (1-P to 3-P) usually arising from a heavily sclerotized common tubercle, stem of 1-P usually strongly swollen, seta 3-T usually with lanceolate leaflets, not strongly palmate. Abdominal seta 1-I usually distinctly palmate, always with distinct flattened leaflets, palmate setae 1-II to 1-VII well developed, leaflets usually narrow and serrated at least on those of middle segments.

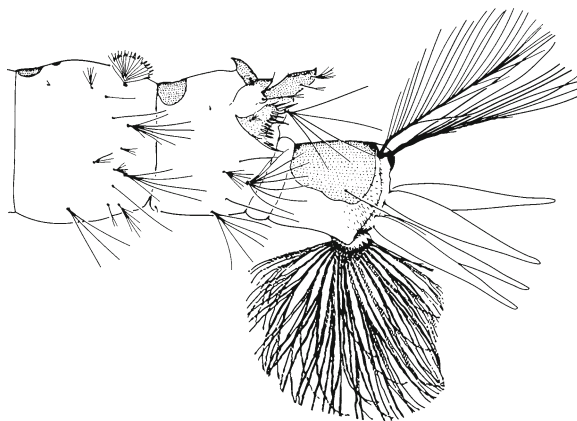


Fig. 13.15 Larva of *An. farauti*

Abdominal setae 6-IV and 6-V usually with 2 branches. Anal segment with conspicuous spicules at posterior margin, saddle strongly pigmented and reaching half way down the ventral sides of the anal segment, saddle seta (1-X) arising at some distance from ventral and posterior margins of saddle (Fig. 13.15). Upper caudal seta (2-X) with lateral branches, slightly shorter than lower caudal seta (3-X). Ventral brush with 7–9 pairs of cratal setae (4-X), anal papillae about twice as long as saddle, pointed.

**Biology:** The natural breeding sites of *An. farauti* consist primarily of river and stream margins with vegetation, springs, seepage areas, ponds, lagoons, and temporary ground pools of all sizes, in open coastal areas or in wide river valleys. *An. farauti* has a definite preference for open sunlit areas but is frequently found in large numbers under partial shade. Very often the breeding habitats are associated with flottage and emergent or surface vegetation (Belkin 1962a). The larvae are remarkably tolerant to organic pollution and may occur in brackish water with a salinity up to 70% of that of sea water. When the population becomes extremely abundant, the larvae will breed in artificial containers to a limited degree. Larvae have been reported from boats, canoes, tanks, oil drums, water collections in canvas, and even in small tin cans (Horsfall 1955). *An. farauti* does not utilize water collections in or on plants or plant parts, such as tree-holes, leaf axils, coconut shells, or cacao pods. Females feed on a wide variety of hosts, including humans. They feed on humans very readily and are most inconspicuous when approaching to feed; their bites are usually not felt at all. Feeding activity usually begins at dusk and probably continues through the night to dawn. Occasional attacks in the daytime have been reported not only in shaded areas but even in bright sunlight (Belkin 1962a). The females feed most commonly in the open but will enter dwellings as well; some remain there after the blood meal. Generations are continuous, with a marked decline in population during parts of the year when precipitation is low. The flight range of the females is as far as 1.6 km, usually less (Horsfall 1955). Only one blood meal is required for the development of the first batch of eggs; oviposition occurs 48–72 h after engorgement (Bryan 1974).

**Distribution:** Australia, New Hebrides, Solomon Islands, New Guinea, Moluccas, Bismarck Archipelago, Indonesia.

**Medical importance:** Primary malaria vector, efficient vector of human filariasis (*Wuchereria bancrofti*) (Belkin 1962a).

**Note on systematics:** *An. farauti* is a member of the Anopheles Punctulatus Complex, which comprises of at least four distinct species: the nominative form *An. punctulatus*, *An. farauti No.1*, *An. farauti No.2*, and *An. koliensis* (Belkin 1962a; Bryan 1974). Adults may be distinguished by the colouration patterns of the proboscis; the larvae differ in the ratios of the clypeal setae 2-C and 3-C and in the structure of the prothoracic setae 1-P to 3-P. The members of the complex are undoubtedly the most important vectors of malaria and filariasis wherever they occur. Because of its wide distribution range, *An. farauti* is the most important, and sometimes the only, vector in the South Pacific (Belkin 1962a).

### ***Ochlerotatus (Mucidus) alternans* (Westwood 1835)**

**Female:** A very large mosquito, speckled with yellow, light brown, and white scales and shaggy palps, legs and abdomen. Palps about 2/3 length of proboscis which is darker at apex and base but paler in the middle. Antenna slightly shorter than proboscis, light brownish yellow, pedicel with a conspicuous patch of small broad white scales. Central portion of vertex largely with narrow scales, brownish yellow laterally, and a median longitudinal stripe of pure white scales; sides of vertex with some broad brownish yellow scales followed by broad white scales, erect scales very numerous and long, largely brownish yellow. Scutum with mottling of brown, white and yellowish broad and narrow scales but in an indefinable pattern, acrostichal and dorsocentral setae short, very numerous. Scutellum with numerous white scales on all lobes, some scales short and decumbent, majority long and erect. Pleurites with numerous broad white scales, pleural setae golden or very pale, lower mesepimeral setae in a patch of about 10 near the middle of sclerite. Coxae with white and scattered yellowish scales, femora and tibiae largely yellow with some white scales at their bases. Tarsi with brown or light brown scales, and with conspicuous whitish rings, basal and median on tarsomere I and basal on tarsomeres II–V. Light rings indefinite or sometimes absent on fore and mid legs, conspicuous on hind leg. Wing veins very densely



covered with brown, pale yellowish and white broad scales, wing fringe with dark and pale spots. Scaling of terga II–VII largely yellow, with variable basal lateral and basal median patches of white scales, sometimes connected by narrow transverse basal white bands, sterna largely whitish scaled, distal ones with yellow or brownish apicolateral patches.

**Larva:** Large and distinct individuals with the mouth brushes modified for predation, the brushes are reduced in number, curved distally and strongly thickened. The head is moderately pigmented, the posterior portion somewhat darker, antenna short and slender,  $<1/2$  as long as the head, distal portion darker. The antennal seta (1-A) is situated well beyond the middle of the antenna and usually has 2 branches. The labral seta (1-C) is long and slender and the clypeal (4-C) and frontal setae (5-C to 7-C) are single. Integument of thorax and abdomen without distinct spicules, prothoracic setae 1-P to 3-P single, 4-P with 3–4 branches. The comb is arranged in an irregular patch of about 5–6 rows of short scales which are broadened and fringed apically. The siphon is rather long, slightly tapering toward its apex, the siphonal index is about 3.7–4.5. The pecten extends to about  $1/3$  from the base of the siphon and consists of about 20 or more slender teeth, which are more or less evenly spaced, the basal teeth are very short, each tooth usually has a strong lateral denticle. The siphonal tuft (1-S) is multiple branched and inserted beyond the pecten well below the middle of the siphon (Fig. 13.16). The saddle is moderately large extending more or less half way down the sides of the anal segment and bears short and strong spines on its posterior dorsal border. The saddle seta (1-X) is single, the upper (2-X) and lower (3-X) anal setae are both single, arising from a prominent process with strong ventral sclerotisation. The ventral brush consists of about 7–8 cratal setae (4-X) and 5–6 precratal setae extending over the greater part of the midventral line of the anal segment. Anal papillae short and pointed, about  $1/4$  the length of the anal segment.

**Biology:** The larvae are mainly found in brackish water swamps on the coast along with those of *Oc. vigilax*, but also in fresh water ground pools inland often associated with larvae of *Oc. vittiger*. They are predacious on other mosquito larvae. The females of *Oc. alternans* can reach reasonably high levels in many areas following extended periods of rain (Russell 1996). They are very aggressive biters and will attack

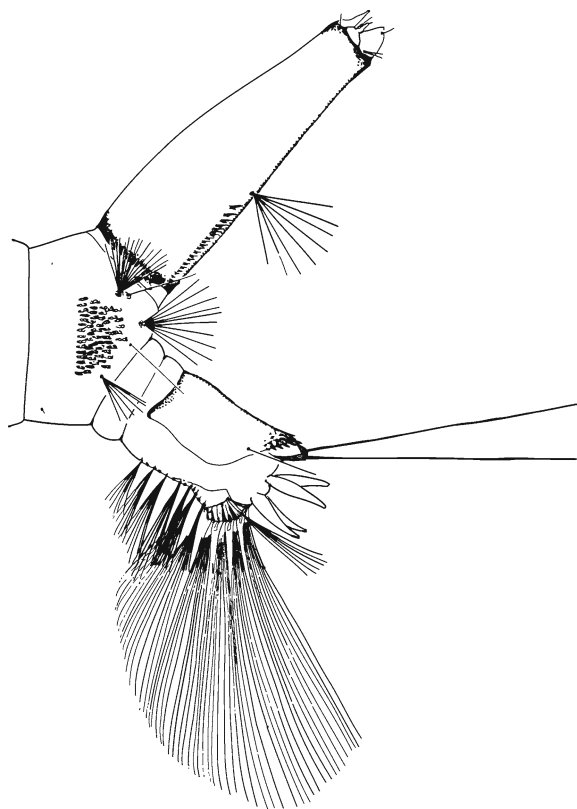


Fig. 13.16 Larva of *Oc. alternans*

humans and other animals throughout the day and night and may disperse several kilometers from their larval habitats in search of a blood meal.

**Distribution:** East and Northern Australia, New Guinea, New Caledonia.

#### ***Ochlerotatus (Finlaya) notoscriptus* (Skuse 1889)**

**Female:** A small to medium sized dark species with conspicuous silvery markings on the scutum and ringed legs. Proboscis predominantly dark scaled with a distinct narrow median pale ring, palps dark with a varying number of silvery scales at the tip, pedicel of antenna with a large mesal patch of silvery scales. Vertex with a rather conspicuous but short frontal tuft of silvery or yellowish scales, a narrow complete line of densely packed silvery white scales around the eye margin, erect scales dark, rather numerous but short. Scutum covered with narrow

dark scales and with silvery, sometimes golden scales arranged in narrow stripes forming a very conspicuous lyre shaped pattern. A narrow median acrostichal stripe extends to prescutellar area where it divides into more or less definite prescutellar stripes, a pair of anterior dorsocentral stripes, yellowish or golden, sometimes reduced or absent, a pair of lateral stripes to scutal angle where each broadens and curves over the transverse suture and then continues as prescutellar dorsocentral stripes, a pair of supraalar stripes usually strongly developed, a pair of conspicuous large patches of broad silvery scales in front of wing roots. Acrostichal and dorsocentral setae strongly developed. All lobes of scutellum with broad flat silvery or slightly yellowish scales, paratergite with conspicuous silvery scales. Pleurites with several patches of broad silvery scales, lower mesepimeral setae absent. Coxae with patches of silvery scales, femora dark with narrow longitudinal silvery stripes from base to near apex on anterior and posterior surfaces. Tibiae with anterior and posterior stripes of silvery or whitish scales, usually nearly reaching apex, short or poorly developed on anterior surface of fore tibia and posterior surface of hind tibia. Hind tarsus with conspicuous large basal white rings and usually very narrow apical incomplete rings on tarsomeres I–IV, tarsomere V variable from completely white to completely dark. Wing scales all dark. Abdominal terga largely dark scaled with basolateral silvery patches or narrow bands separated from lateral patches, sterna mostly pale scaled with more or less distinct basolateral silvery markings, distal segments may be predominantly dark.

**Larva:** Head light brown, antenna about half as long as the head, straight, uniform in width, without spicules, antennal seta (1-A) inserted well beyond the middle of antennal shaft, single. Labral seta (1-C) very stout and conspicuously hooked in distal half, postclypeal seta (4-C) small, with multiple branches, inner and median frontal setae (5-C and 6-C) single, 5-C displaced anteriorly, outer frontal seta (7-C) with 3–4 branches. Prothoracic seta 1 (1-P) 2-branched, 2-P single, 3-P with 3 branches. The comb consists of 19–24 fringed scales arranged in 2 or 3 rows. Siphon short and stout, siphonal index usually about 1.7–2.5, siphonal tuft (1-S) with 3–4 branches, inserted well beyond the middle of the siphon (Fig. 13.17). The pecten consists of 12–13 teeth, individual tooth with a prominent lateral denticle. The saddle covers about

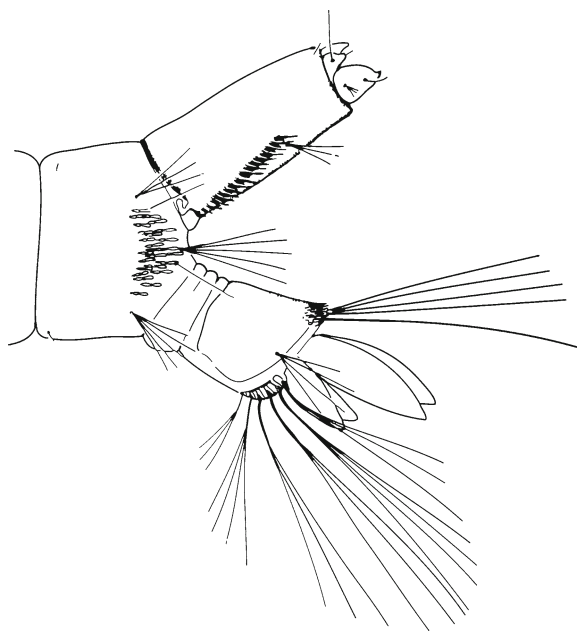


Fig. 13.17 Larva of *Oc. notoscriptus*

3/4 of the anal segment, spicules at its posterior dorsal margin well developed but relatively short. Saddle seta (1-X) 2–4 branched, upper anal seta (2-X) with 3–4 branches, lower anal seta (3-X) single. Ventral brush with 6 pairs of cratal setae (4-X), precratal setae absent. Anal papillae pointed, the dorsal pair about as long as the saddle, the ventral pair shorter.

**Biology:** *Oc. notoscriptus* is a semi domestic species breeding readily in artificial containers of all types, e.g. water tanks, tin cans, flower pots and in coconut husks and shells. The natural breeding places are usually tree-holes but the larvae can also be found in leaf axils of various plants and even rock and ground pools. Females readily attack humans by day in shaded areas but also feed during evening, night and early morning. Overwintering takes place in the larval stage. *Oc. notoscriptus* is one of the major domestic pest species in south eastern Australia.

**Distribution:** Australia, New Guinea, New Zealand, New Caledonia.

**Medical importance:** *Oc. notoscriptus* is believed to be a suitable vector for Barmah Forest virus, Ross River virus and heartworm in dogs (Russell 1996).

***Ochlerotatus (Ochlerotatus) camptorhynchus***  
(Thompson 1868)

**Female:** A medium sized dark mosquito with pale rings on all legs. Proboscis extensively mottled, particularly on ventral side, sometimes almost completely pale scaled, palps about 1/5 the length of proboscis, mottled. Pedicel dark with a few pale scales and black setae. Vertex usually clothed with narrow curved and erect forked pale scales, elongate patches of narrow bronze scales close to posterior eye margin. Integument of thorax dark brown. Scutum uniformly clothed with narrow curved golden scales although there are a few paler median patches particularly close to its posterior margin. Pleurites with several patches of broad white scales, 3–4 lower mesepimeral setae present (Fig. 13.12a). Femora, tibiae and tarsomeres I of all legs mottled with white scales, tarsomeres II–V with pale basal rings. Wings completely dark scaled. Abdominal terga dark scaled, usually with patches of white scales on terga I and II and convex basal bands on terga III–VI, but these bands may be either narrow and almost straight or triangular, sometimes reaching the posterior margin of the terga. Sterna pale scaled with median and sometimes apicolateral patches of dark scales, the patches may be reduced to a few scales.

**Larva:** Antenna short, <1/2 as long as the head, antennal seta (1-A) inserted beyond the middle of the antennal shaft. Inner frontal seta (5-C) with 3–4 branches, median frontal seta (6-C) with 2–3 branches, outer frontal seta (7-C) with 8–10 branches. Prothoracic setae 1-P with 2 branches, 2-P single; 3-P with 2–4 branches. The comb consists of 24–33 fringed scales arranged in an irregular or triangular patch. The siphonal index is about 2.0–2.3, the pecten consists of 22–24 teeth, each tooth with 3–4 lateral denticles. The siphonal tuft (1-S) has 7–8 branches and is inserted well beyond the middle of the siphon. The saddle covers about half of the anal segment, saddle seta (1-X) single, upper anal seta (2-X) with 6–7 branches, and lower anal seta (3-X) single. The ventral brush consists of 18–19 tufts, usually with 2–3 precratal setae (4-X). The anal papillae are very small and globular, but prominent (Fig. 13.18).

**Biology:** *Oc. camptorhynchus* breeds mainly in brackish swamps and marshes in open country; it is typically a coastal species but occurs in inland riverine areas with brackish influence. Adults may be active

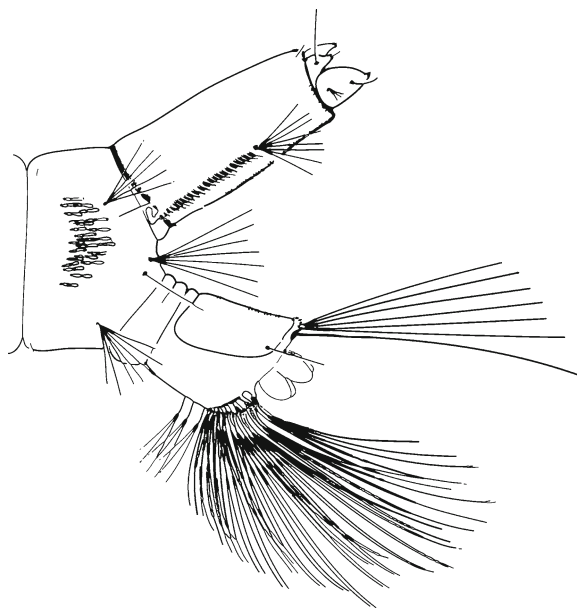


Fig. 13.18 Larva of *Oc. camptorhynchus*

throughout the year in southern parts of Australia, and may disperse widely from their larval habitats. They are extremely vicious biters and readily attack humans and other animals including birds during the day, at dusk and after sunset. The species is a major pest in coastal areas in southern Australia.

**Distribution:** Southern coasts of Australia.

**Medical importance:** Ross River virus has been isolated from this mosquito in Victoria and Tasmania (Russell 1996).

***Ochlerotatus (Ochlerotatus) normanensis***  
(Taylor 1915)

**Female:** A small to medium sized species. Proboscis and palps dark scaled (Fig. 13.14b), sometimes with a few scattered pale scales on basal half of proboscis. Pedicel brown, clothed with small flat dark brown or mixed dark and white scales. Integument of head dark brown, vertex and occiput clothed with narrow curved silvery white scales and narrow curved bronze black scales, and numerous dark brown erect forked scales. Scutum covered with fine narrow curved bronze black scales and scattered golden scales, the dark scales always predominating. Acrostichal and dorsocentral setae strong and dark, numerous dark setae also lateral

and above the wing roots. Scutellum brown, clothed with narrow curved silvery scales, the median lobe sometimes with some dark scales also, 6–10 long dark setae on each lobe. Integument of pleurites brown, antepronotum with narrow curved silvery scales, sometimes with narrow curved or broader dark scales above, postpronotum with mixed narrow curved dark and silvery scales. Patches of narrow curved scales on sub- and postspiracular areas and paratergite, patches of broad silvery scales on upper portion and lower posterior margin of mesepisternum, prealar area and upper part of mesepimeron. Pleural setae dark or pale, 2–6 upper mesepisternal setae, a row of 5–8 long and several shorter lower mesepisternal setae, about 12–15 prealar setae, 10–14 upper mesepimeral setae, lower mesepimeral setae absent. Legs dark scaled with mottled femora and tibiae, tarsomeres with narrow pale basal rings. Wings dark scaled, extensively mottled with pale scales on all veins, although the mottling is much reduced toward the apex of the wing. Haltere pale, with white scaled knob, occasionally with a few dark scales also. Integument of abdomen dark brown. Tergum I with mixed dark and pale scales medially and white lateral patches. Terga II–VII dark scaled with basal pale bands or median patches, usually discontinuous with large basal lateral white patches, the bands may be narrow or fairly wide, straight or somewhat produced medially. Sterna usually dark scaled, with mottling of white scales mainly laterally toward the base, sternum VII often entirely dark scaled, cerci long and dark.

**Larva:** Head about  $2/3$  as long as broad, antenna approximately  $3/4$  as long as the head, slightly curved, tapering toward its apex and clothed with spicules, more dense in the basal half. The antennal seta (1-A) has 3–9 branches arising at about the middle of the antennal shaft. The labral seta (1-C) is straight, slender and pointed. Inner frontal seta (5-C) 2–3 branched, median frontal seta (6-C) single, outer frontal seta (7-C) with 1–4 branches, but usually with 2 branches. The prothoracic setae (1-P to 3-P) are all single, of different lengths and arise from a common sclerotized tubercle, 3-P is the longest and strong, 2-P is less than half as long as 3-P and fine. The comb consists of 13–20 slender scales arranged in 2–3 rows, each scale with an elongated median spine and a narrow base. The siphonal index is approximately 3.0, the siphon is slightly tapering beyond the insertion point of the siphonal tuft (1-S), and the tracheal trunks are very narrow.

The siphonal tuft (1-S) with 5–13 long branches, arises slightly below the middle of the siphon. The tuft is more than twice as long as the width of the siphon at the point of its insertion (Fig. 13.19). The pecten extends over the basal  $1/4$  to  $1/3$  of the siphon, consisting of 5–13 teeth each with 1–4 rather variable lateral denticles, the apical or preapical denticle being prominent. The distalmost tooth is the longest, and they decrease in size basally, the most basal often being very small and scale-like. The saddle covers slightly  $>1/2$  of the anal segment, with a very irregular ventral margin and a row of short pointed spicules at its posterior margin. The saddle seta (1-X) is single and about half as long as the saddle. The upper anal seta (2-X) has 9–17 branches, about twice the length of the saddle, the lower anal seta (3-X) is single, and about twice as long as 2-X. The ventral brush consists of 12–14 cratal setae (4-X), each tuft with 9–16 branches, 1–2 precratal setae (4-X) present. The anal papillae are long, tapering and pointed, 2–3 times longer than the saddle; the lower pair is slightly longer than the upper pair.

**Biology:** Larvae of *Oc. normanensis* can be found in temporary ground pools and swampy areas resulting from heavy rain, fully or partially exposed to the sun. Frequently the larvae may be encountered together

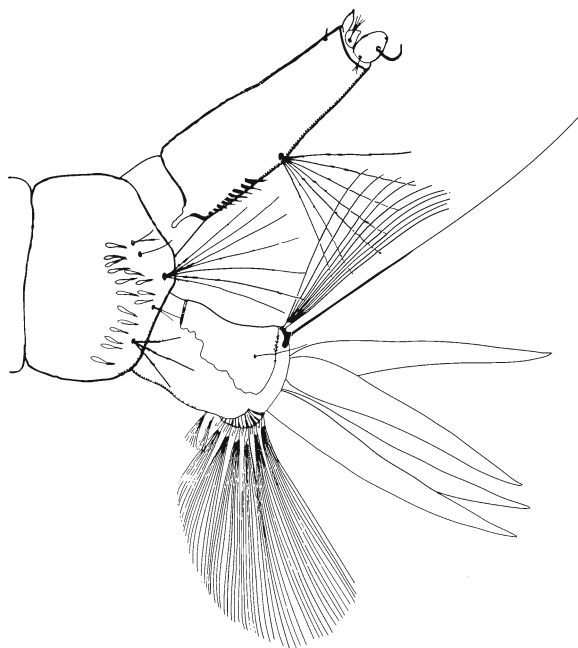


Fig. 13.19 Larva of *Oc. normanensis*

with those of *An. annulipes* s.l., *Oc. alternans*, *Oc. vigilax*, *Oc. vittiger* and *Cx. annulirostris*. The pools may occur in natural depressions, dry water courses, or man-made excavations, including country road gutters and hoof prints. The water may be clear or muddy, and sometimes slightly polluted by animal manures. Females of *Oc. normanensis* readily bite man during the day (Marks 1949). It is a common species in northern Australia where it is a major nuisance mosquito.

**Distribution:** Australia.

**Medical importance:** Vector of Murray Valley Fever and Ross River virus (Russell 1996).

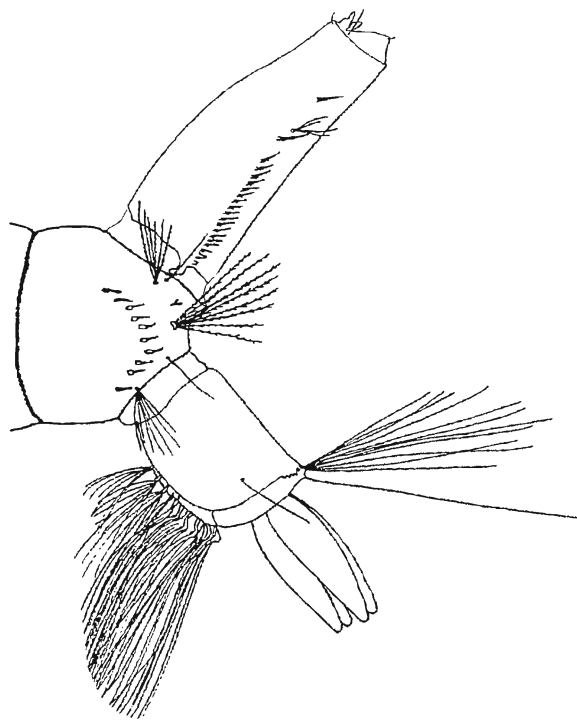
### ***Ochlerotatus (Ochlerotatus) sagax* (Skuse 1889)**

**Female:** A moderately large species of dark appearance with distinct pale scaling on abdomen and pleurae. Proboscis dark scaled, palps sometimes mottled at base, pedicel with narrow pale scales on dorsal surface. Vertex clothed with narrow pale scales, occiput with numerous creamy erect forked scales. Integument of scutum dark, scutum covered with narrow bronze scales, some larger pale scales laterally and around prescutellar area. Scutellum with narrow pale scales and long setae on all lobes. Integument of pleurites dark brown, densely clothed with several patches of broad pale scales. Mesepimeral scale patch not reaching the lower margin of mesepimeron, 3–8 lower mesepimeral setae present. Coxae with decumbent pale scales, fore and mid femora mottled, hind femur mostly pale scaled and mottled over most of its length, with dark apex. All tibiae and basal half of tarsomeres I extensively mottled, rest of tarsi dark scaled (Fig. 13.11b), or at most with traces of white rings on tarsomeres I and II. Wings predominantly dark scaled, some pale scales present at base of costa (C) and sometimes also at bases of subcosta (Sc) and vein  $R_1$ . Haltere with stem and knob pale scaled. Terga dark scaled with moderately broad pale basal bands often extended medially and sometime reaching the apical margin on last segments, sterna predominantly pale scaled, with small apicolateral patches of dark scales.

**Larva:** Head wider than long, antenna  $<1/2$  as long as the head, slightly swollen basally, and spiculate. Antennal seta (1-A) inserted at about the middle of antennal shaft or slightly below, with 3–6 branches. Postclypeal seta (4-C) with 2–3 small branches, inner and median frontal setae (5-C and 6-C) single, 5-C

sometimes with 2 branches, outer frontal seta (7-C) with 4–8 branches. Prothoracic setae 1 and 2 (1-P and 2-P) single, 3-P with 1–2 branches. The comb is composed of 8–14 scales arranged in a single row, individual comb scales with prominent terminal spine and fringed with small spicules basally. Siphon slightly tapering toward apex, siphonal index 2.3–2.8. Siphonal tuft (1-S) inserted at about the middle of siphon, rather small, distinctly shorter than the width of siphon at the point of its origin, with 3–4 branches. The pecten consists of 20–25 teeth with at least one detached tooth inserted beyond siphonal tuft (1-S) (Fig. 13.20). Saddle almost entirely encircling anal segment, saddle seta (1-X) single, upper anal seta (2-X) with 9–12 branches, lower anal seta (3-X) single. The ventral brush consists of 14–16 tufts of cratal setae (4-X), 1–2 precratal setae present. Anal papillae lanceolate, about as long as or longer than the saddle.

**Biology:** The larvae of *Oc. sagax* can be found in temporary ground pools, shallow pools, roadside ditches or irrigation channels with fresh water, exposed to the sun or shaded, with or without vegetation. They breed in the open, but are more common in and near woodland.



**Fig. 13.20** Larva of *Oc. sagax*

(Dobrotworsky 1965; Liehne 1991; Russell 1993). The females readily bite man and domestic animals in the daytime, but also feed at dusk, night and dawn. The adults are generally most abundant in spring; the species can be a major pest in spring in some riverine areas, and particularly after flooding even in summer and autumn (Russell 1993).

**Distribution:** Widely distributed in southeastern Australia.

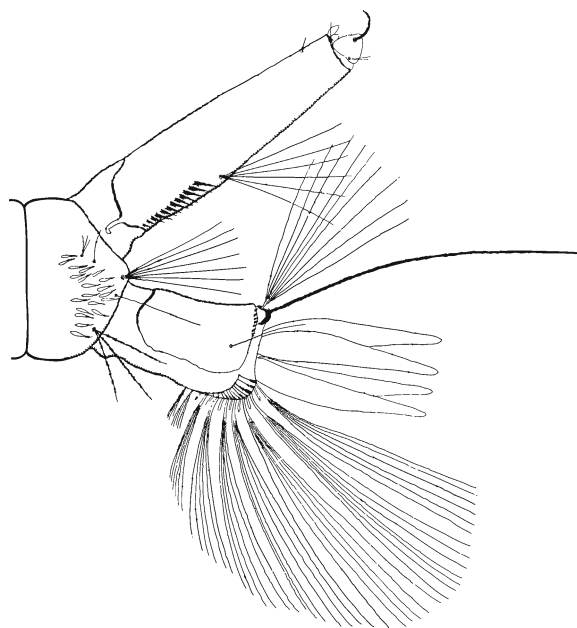
***Ochlerotatus (Ochlerotatus) theobaldi***  
**(Taylor 1914)**

**Female:** A medium sized species of mottled dark appearance. Proboscis mainly dark scaled or mottled but with extensive pale area on ventral surface, palps with whitish scales medially and at apex, and sometimes with some mottling also. Head with broad pale scales laterally and along the eye margin, erect forked scales dark bronzy, pedicel with several pale scales. Scutum covered with narrow curved golden and creamy scales, usually with some admixture of bronze black scales on the transverse suture and along the midline, some larger pale areas toward the rear. Pleurites with several patches of pale scales. Femora and tibiae moderately to extensively mottled with pale scales, hind legs with femur, tibia and tarsomere I mottled, all tarsomeres with basal white rings, although tarsomere V may be all dark. Wing dark scaled with extensive mottling of broad symmetrical pale scales on all veins (Fig. 13.13a). Abdominal terga dark with pale lateral patches and basal bands which may not be complete and there may be some mottling on terminal segments, sterna pale scaled with some scattered dark scales or apical bands or lateral patches.

**Larva:** Head about 2/3 as long as broad, antenna about 2/3 the length of the head, slightly curved and tapering, clothed with spicules. Antennal seta (1-A) about 1/2 as long as antenna, with 4–7 branches inserted slightly below the middle of the antennal shaft. Labral seta (1-C) long, straight and slender, postclypeal seta (4-C) minute with 6–10 branches. Inner frontal seta (5-C) with 2 branches, median frontal seta (6-C) single, outer frontal seta (7-C) with 2–4 branches, arising a short distance behind base of antenna. Prothoracic setae 1–3 (1-P to 3-P) without sclerotized tubercles, 1-P with 1–3 branches, about 1/2 as long as 2-P, which is single, 3-P long and strong.

The comb consists of 16–24 slender pointed scales arranged in 2–3 rows, each with a coarse fringe basally and a prominent median spine. Siphon slightly tapering toward apex, siphonal index 3.3–3.9. The pecten is restricted to the basal 1/4 of the siphon and consists of 9–15 teeth, each tooth with 2–5 lateral denticles, the distalmost tooth is the longest, the teeth decreasing in size toward the base, the most proximal often being very small and scale-like. Siphonal tuft (1-S) with 4–9 long branches arise slightly below the middle of siphon. Seta 9-S on the ventral valve is very stout and forwardly curved (Fig. 13.21). Saddle covers about 2/3 of the anal segment, with irregular ventral margin and a row of short pointed spicules at its posterior margin. Saddle seta (1-X) single, about 2/3 as long as saddle. Upper anal seta (2-X) with 7–13 branches, about twice as long as saddle, lower anal seta (3-X) single. The ventral brush consists of 15–17 tufts, each with 6–10 branches, with 1–2 precratal setae (4-X). Anal papillae long and pointed, about twice as long as the saddle.

**Biology:** Larvae of *Oc. theobaldi* have been found in clear or slightly muddy rain water in grassy edged gutters of country roads, in small rain filled pools and in hoof prints (Marks 1949). Adults may become active in spring and be apparent throughout the year in



**Fig. 13.21** Larva of *Oc. theobaldi*

warmer areas providing natural flooding or irrigation. In inland areas, after heavy rains, they may appear in large numbers, sometimes invading towns. Day biting is usually apparent as the species readily attacks humans and other animals but they will bite also in the evening and at night in forests, scrubs or open country. *Oc. theobaldi* may act as a major pest following extensive rain or flooding in western parts of its distribution range and can disperse for many kilometers when there are major larval populations.

**Distribution:** Australia.

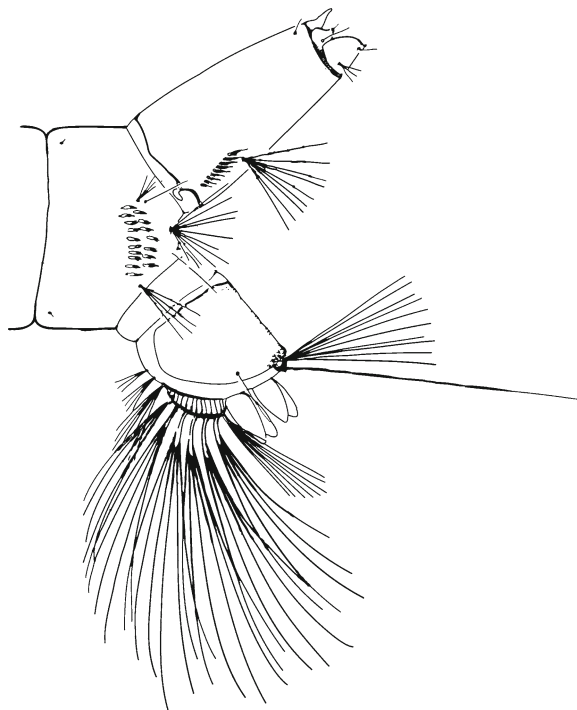
**Medical importance:** The species was successfully infected with Murray Valley encephalitis virus in laboratory studies and Ross River virus has been isolated from natural populations, but its role in transmission of human disease is doubtful (Russell 1996).

***Ochlerotatus (Ochlerotatus) vigilax*  
(Skuse 1889)**

**Female:** A medium sized mosquito of dark appearance with pale ringed legs. Proboscis with yellowish or light golden scales on basal 2/3 on ventral side (Fig. 13.14a), sometimes forming a complete large ring. Palps <1/5 the length of proboscis, largely dark scaled with pale apex. Pedicel of antenna with a few small dark scales. Head with narrow decumbent scales, pale posteriorly and sometimes in the middle, dark anteriorly, erect scales dark, lateral and ventral broad scales largely pale. Integument of scutum dark brown, scutal scales all narrow, mostly dark bronze brown with some pale golden scales intermixed. Scutellum with narrow yellowish scales on all lobes, paratergite with a patch of small whitish scales near the middle. Pleurites with several patches of broad pale scales, namely propleural patch, postspiracular patch, upper and lower mesepisternal patches, mesepimeral patch restricted to upper portion of sclerite, lower mesepimeral setae absent (Fig. 13.12b). Femora and tibiae largely dark scaled, mottled with pale scales, particularly ventrally and on hind leg. Hind tarsus dark scaled with pale basal rings on tarsomeres I–V (Fig. 13.11a). Wing predominantly dark scaled (Fig. 13.13b) with scattered pale scales mainly along the anterior border, some scattered pale scales on the other veins, particularly along cubitus (Cu), haltere knob largely pale scaled. Abdominal terga largely dark scaled, terga II–VI with straight basal pale bands and lateral pale patches, apices of terga V and VI

with some pale scales. Sterna predominantly pale scaled with dark apicolateral patches.

**Larva:** Antenna about half as long as the head, slender and adorned with small, sharply pointed spicules of varying number. Antennal seta (1-A) with 3–4 branches inserted at about the middle of antennal shaft. Labral seta (1-C) long and slightly curved, inner frontal seta (5-C) with 1–2 branches, median frontal seta (6-C) single, outer frontal seta (7-C) with 6–7 branches. Prothoracic seta 1 (1-P) single, 2-P and 3-P with 2 branches. The comb scales are arranged in an irregular triangular patch, individual scale small, strongly fringed and usually with a differentiated median spine. Siphon short and stout, siphonal index about 1.5–2.0. The pecten with 8–12 teeth, evenly and closely spaced, not reaching to the middle of the siphon (Fig. 13.22). Siphonal tuft (1-S) with 7–9 branches inserted within or beyond the pecten. Saddle almost covering anal segment but incomplete, with a few short spicules at its posterior margin, saddle seta (1-X) single. Ventral brush with 12–14 cratal setae (4-X), 1–3 precratal setae present. Anal papillae short, narrow and pointed.



**Fig. 13.22** Larva of *Oc. vigilax*

**Biology:** *Oc. vigilax* primarily breeds in brackish waters in mangrove swamps and salt marshes, occasionally it is found in rock holes and freshwater ground pools. Adults are most abundant in the summer months and are active from mid spring through autumn; the females are very aggressive biters and attack humans and domestic animals readily during the day in sheltered areas, but also at evening and night, with a peak about sunset (Belkin 1962a). This species is essentially coastal and associated with estuaries and mangrove zones, but is reported to be a powerful flier and may be found at considerable distances from the larval habitats (Mackerras 1927). *Oc. vigilax* is the major coastal pest species in large parts of southern and eastern Australia, because it can disperse and be windblown for many kilometers and can create nuisance problems over large and diverse areas.

**Distribution:** Coasts of Australia, New Guinea, New Caledonia, Philippines, Indonesia, Thailand, Indochina, Formosa, Solomon Islands, Fiji Islands, New Zealand.

**Medical importance:** Because of repeated virus isolations from natural populations, *Oc. vigilax* is regarded as the major vector of Ross River and Barmah Forest viruses in coastal areas of New South Wales, the species is also known to carry dog heartworm (Russell 1996).

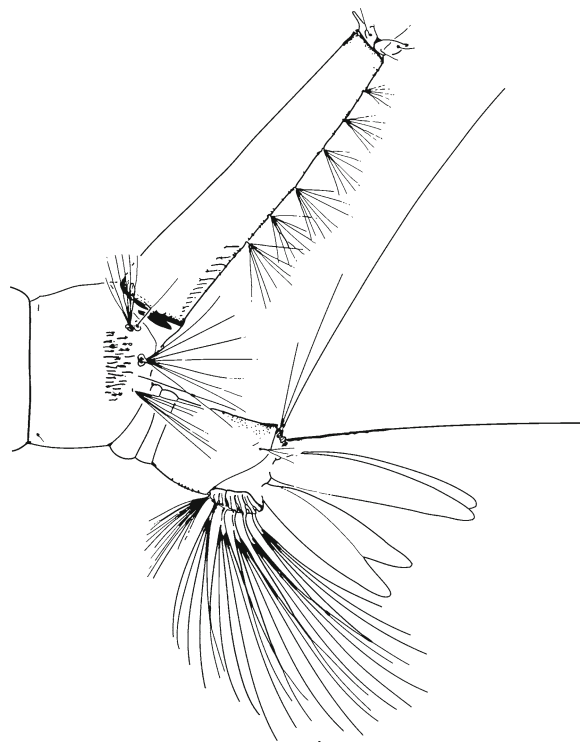
### ***Culex (Culex) annulirostris* Skuse 1889**

**Female:** A medium sized mosquito of brownish to dark appearance, extremely similar to *Cx. sitiens* and very difficult to distinguish between females of the two species. *Cx. annulirostris* differs in the following characters: Median pale ring of proboscis broader, about 1/4 of its total length or slightly more. Acrostichal and dorsocentral setae more strongly developed and longer than in *Cx. sitiens*. Anterior surface of fore femur evenly speckled with pale scales along the whole length, anterior surface of fore tibia with a row of small yellowish spots among anterior setae, anterior surface of hind femur extensively speckled with pale scales. Scales on all wing veins more numerous and longer than in *Cx. sitiens*. Terga II–V with basal pale bands relatively broad, moderately to strongly produced in the middle (Fig. 13.5a), not straight and narrow as in *Cx. sitiens*, basal band on tergum II sometimes incomplete or represented by a median triangular pale patch.

Terga VII–VIII with narrow, basal bands, tergum VIII usually broadly pale at apex, narrow apical dark bands on sterna often incomplete.

**Larva:** As with the females, the larvae of *Cx. annulirostris* closely resemble those of *Cx. sitiens*, differing from the latter in the following characteristics: labral seta (1-C) dark, slender and spiniform, frontal setae (5-C to 7-C) and antennal seta (1-A) stronger and darker than in *Cx. sitiens*. All abdominal setae darker and stronger, 7-I with 2 branches. Comb scales usually arranged in 4 irregular rows. Siphonal index extremely variable, ranging from 5.0 to more than 10.0. Siphonal setae (1-S) with 5 or 6 pairs of tufts, each with 6–10 branches, arranged in subventral rows (Fig. 13.23), none of the distal tufts detached laterally (as in *Cx. sitiens*). Saddle completely encircling anal segment, ventral part only slightly narrowed, saddle seta (1-X) usually with 3 branches. Anal papillae slender, tapering apically, usually about as long as the saddle or slightly longer.

**Biology:** *Cx. annulirostris* utilizes a variety of ground pools as breeding habitats, the larvae can frequently be found in pools, ponds, swamps, ditches,



**Fig. 13.23** Larva of *Cx. annulirostris*



channels, and rice fields containing emergent vegetation. On occasion, they have been collected also from cacao shells and bamboo stumps (Dobrotworsky 1965; Delfinado 1966; Sirivanakarn 1976). In the South Pacific, the larvae have been reported to breed in almost any type of permanent or temporary water, standing or flowing, clean or with a very high organic content, fresh or brackish, in open sunlit as well as in strongly shaded situations. They have been commonly found breeding in canoes and in various types of artificial containers such as shallow wells, cement tanks or rock pools containing algae (Belkin 1962a). In south eastern Australia breeding places include freshwater wetlands and low-lying grassy areas that are commonly inundated following rain as well as irrigation. The adults are generally active from spring to late autumn, they are regarded as the major summer pest of inland riverine areas, and the flight range is about 5–10 km (Russell 1996). The females feed readily on humans but also on other mammals and birds. It is a very vicious mosquito, which may bite during the day but is particularly active after sunset, both indoors and in the open (Dobrotworsky 1965). *Cx. annulirostris* is considered as a nuisance or serious pest of humans in most of its distribution range.

**Distribution:** Southern and western Australasian Region, Fiji Islands, Micronesia, South Pacific, Indonesia, Philippines.

**Medical importance:** *Cx. annulirostris* is an efficient vector of a range of arboviruses in the laboratory and has been incriminated in field studies as a vector of many arboviruses (including Murray Valley encephalites, Kunjin, Barmah Forest, Ross River viruses and Japanese encephalites) in Australia. It is also able to carry dog heartworm and is probably a major vector of myxomatosis (Russell 1996). In New Guinea it is an important vector of *W. bancrofti* (Belkin 1962a).

### ***Culex (Culex) sitiens* Wiedemann 1828**

**Female:** Medium sized species, proboscis dark scaled, with clearly marked median pale ring which occupies about 1/5 of its total length (Fig. 13.4a). Palps about 1/5 of the length of proboscis, with some pale scales at apex, remainder dark, sometimes with pale scales laterally at base. Pedicel yellow, mesal surface dark brown with tiny setae and scales. Narrow decumbent scales of vertex

pale, erect scales usually entirely dark brown, occiput with lateral patch of broad whitish scales. Integument of scutum dark brown; scutum mottled with variable spots of coarse dark and pale scales, dark brown to golden brown scales usually predominant, pale yellow to golden scales on anterior and lateral margins, across the middle and around prescutellar area. Acrostichal and dorsocentral setae short and indistinct, supraalar setae strong and dense. Scutellum with pale yellowish scales and several strong setae. Anteprepronotum with several narrow dark or golden brown scales, postpronotum with dark brown or golden brown scales in the middle and pale scales posteriorly. Patches of pale scales on propleuron, upper and lower mesepisternum and mesepimeron distinct. Numbers of pleural setae are 9–17 prealar, 4–8 upper mesepisternal, 5–10 lower mesepisternal, 7–12 upper mesepimeral, lower mesepimeral setae absent. Scale patch of fore coxa pale on upper surface, largely dark or with a mixture of dark and pale scales on lower surface, scale patch on mid coxa dark above, pale below, scale patch on hind coxa entirely pale. Femora with whitish knee spots, anterior surface of fore femur predominantly dark scaled with a more or less definite whitish stripe on basal half, apical half speckled with pale scales, posterior surface whitish scaled. Anterior surface of mid femur dark scaled except at base, speckled with pale scales, posterior surface whitish scaled. Anterior surface of hind femur largely pale, with narrow band of dark scales at apex. Fore tibia largely dark scaled, without a row of pale spots among anterior setae (as in *Cx. annulirostris*), mid and hind tibiae largely dark, sometimes speckled with pale scales. Tarsi mainly dark scaled, hind tarsomere I pale scaled ventrally, tarsomeres I–III with narrow apical and basal pale rings, tarsomeres IV and V entirely dark scaled. Wing scales dark on all veins, sometimes some pale scales present at base of costa (C). Tergum I with a median patch of dark scales, terga III–VIII with narrow, straight basal pale bands connected with elongated basolateral pale spots, pale basal band on tergum II somewhat widened in the middle (Fig. 13.5b). Terga VII and VIII additionally with narrow apical pale bands, that of tergum VII often strongly produced in the middle. Sterna II–VII with broad basal pale bands and narrow apical dark bands.

**Larva:** Antenna shorter than the head, with numerous spicules mostly restricted to basal part, distal part darker with a few spicules laterally. Antennal seta (1-A) inserted well beyond the middle of antennal shaft, multiple

branched, setae 2-A and 3-A inserted subapically. Labral seta (1-C) strongly flattened, dark, apex usually blunt. Postclypeal seta (4-C) single, inner frontal seta (5-C) with 5–8 branches, median frontal seta (6-C) with 3–6 branches (usually 4), outer frontal seta (7-C) with 8–10 branches. Prothoracic setae 1-P to 3-P strong, unequal in length and single, 4-P shorter than the former, with 2 branches. Abdominal seta 6-I usually 3 branched, 7-I about as long as 6-I, always single, 6-II with 3 branches, 6-III to 6-VI with 2 branches. The comb is composed of about 35–40 small scales arranged in a broad triangular patch, individual comb scale rather short and evenly fringed with small spines. Siphonal index 3.6–4.9, siphon with a dark basal ring and evenly tapering toward the apex. The pecten consists of 6–17 evenly spaced teeth restricted to the basal 1/4 to 1/3 of the siphon, the distalmost teeth with 7–8 lateral denticles. Siphonal tufts (1-S) usually with 7 pairs of multiple branched setae, the basal tufts with 5–8 branches each, about twice as long as diameter of siphon at point of origin. One distal pair (1e-S) distinctly shorter and inserted laterally, the others in an irregular row near ventral midline, the distalmost pair (1g-S) about as long as 1e-S (Fig. 13.24). Saddle completely encircling the anal segment, ventral part distinctly narrowed, dorsal posterior margin with numerous spicules. Saddle seta (1-X) single and rather long, upper anal seta (2-X) with 3–6 branches, lower anal seta (3-X) single. Ventral brush usually with 12 tufts of cratal setae (4-X), anal papillae very short, rounded, about half as long as saddle.

**Biology:** *Cx. sitiens* breeds primarily in coastal brackish water habitats including pools, puddles, ponds, wells, ditches and rock pools, in tidal marshes and mangrove swamps. The larvae are occasionally found in artificial containers such as canoes, boats, cement tanks, jars, cans in the vicinity of sea beaches, harbors or piers in populated areas. The larvae and pupae are very adaptable and show a high tolerance of salinity, occurring in fresh, brackish and even pure sea water (Hopkins 1952; Delfinado 1966; Sirivanakarn 1976; Harbach 1988). Females feed primarily on birds and pigs, but will bite man as well; in some areas of the South Pacific *Cx. sitiens* is reported as a vicious mosquito (Bram 1967a).

**Distribution:** *Cx. sitiens* occurs in coastal areas of the Oriental Region, south western Asia, eastern Africa, Madagascar, Ryukyu Archipelago, Korea, northern Australia and many islands in the South Pacific.

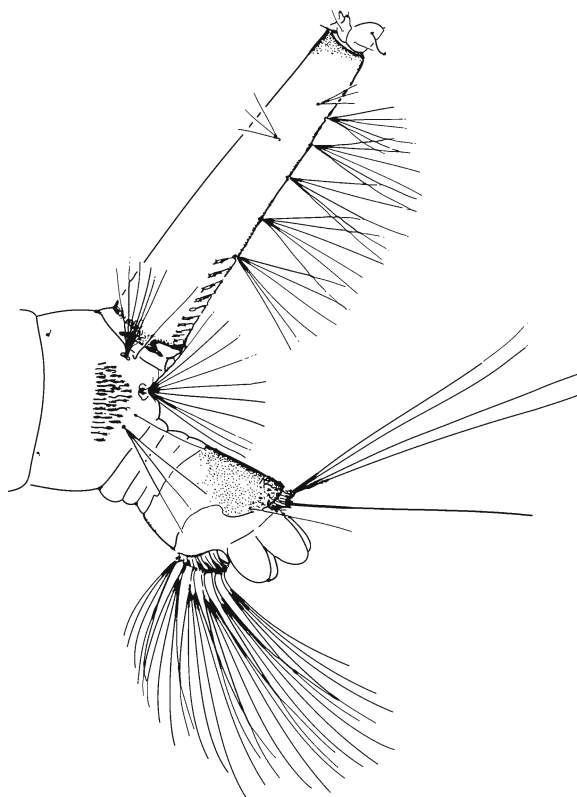


Fig. 13.24 Larva of *Cx. sitiens*

**Medical importance:** Possible vector of Japanese encephalitis (JE). It has been found naturally infected with *Brugia malayi* in Thailand (Harbach 1988).

***Coquillettidia (Coquillettidia) xanthogaster* (Edwards 1924)**

**Female:** A very distinctive mosquito of yellowish to orange appearance. Proboscis and palps dark scaled, palps about 1/5 the length of the proboscis. Pedicel with setae but no scales. Integument of head yellowish or light brown, decumbent scales golden, numerous laterally, erect scales golden to light brown, very long. Integument of scutum usually uniformly yellowish, sometimes darkened on midline, supraalar and prescutellar areas; scutal scales scanty, very narrow. Scutellum usually dull light brown or yellowish, without any distinct scales. Pleurites uniformly yellowish, scales all broad, silvery. Mesepisternum with scales

largely confined to lower portion, mesepimeron with a small patch of scales. Postspiracular setae absent, one lower mesepimeral seta present. Legs dark scaled, hind femur with pale golden scales on basal 1/3 of anterior surface. Wing veins uniformly covered with dark narrow scales, haltere knob with light brown scales. Abdominal terga with light golden scales and some dark purplish scales, sterna largely golden scaled, darker scales in indistinct triangular apical median patches.

**Larva:** Head slightly broader than long, uniformly pigmented. Antenna very long, more than twice as long as the head, distal part of antenna flexible, about as long as or longer than the basal part, which is covered with small but distinct spicules. Antennal seta (1-A) inserted at about the apical 1/3 of basal part, with multiple branches. Labral seta (1-C) very dark and stout, moderately long, postclypeal seta (4-C) usually with 4 branches. Frontal setae (5-C to 7-C) multiple branched, gradually increasing in length. Prothoracic setae 1 and 2 (1-P and 2-P) single, 3-P about half as long as the others, with 4-5 branches. Abdominal segments II-V with seta 3 (3-II to 3-V) rather strongly developed, usually with 1-2 branches. The comb consists of 7-9 scales arranged in a single row, individual scale long and slender, with a prominent median spine and fringed with small lateral spicules. Setae 3-VIII to 5-VIII inserted very close together, seta 3-VIII very long, always single and usually inconspicuously feathered. Siphon short and conical, heavily sclerotized at the base and beyond the middle, modified for piercing plant tissue, siphonal seta (1-S) usually with 4 branches. Anal segment much longer than wide, completely encircled by the saddle, which is moderately pigmented except for a dark basal ring, and with numerous small spicules dorsally. Saddle seta (1-X) short, multiple branched, inserted dorsally near the posterior margin of the saddle, 3 pairs of short, multiple branched accessory setae present on saddle as well, resembling the saddle seta (Fig. 13.25). Upper and lower anal setae

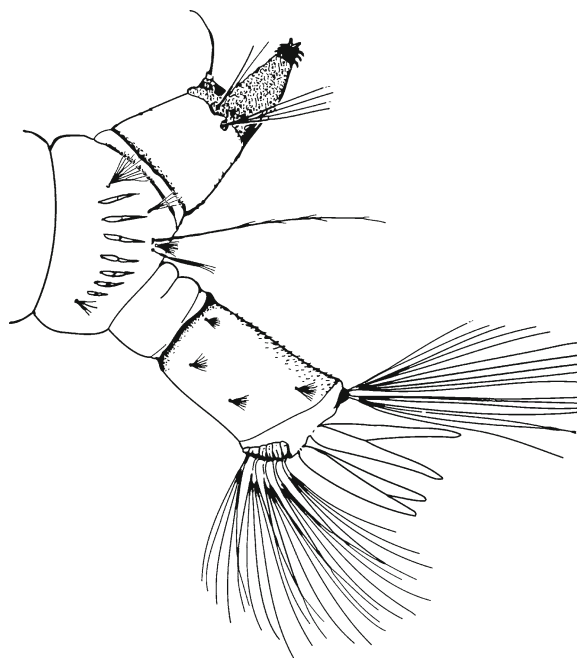


Fig. 13.25 Larva of *Cq. xanthogaster*

(2-X and 3-X) with multiple branches, unequal in length. Ventral brush with 8 tufts of cratal setae (4-X), precratal setae absent. Anal papillae about as long as the saddle, pointed.

**Biology:** The larvae of *Cq. xanthogaster* can be found in grassy freshwater swamps, lagoons and creeks, usually heavily shaded and overgrown with various aquatic plants (Belkin 1962a). Eggs are laid in rafts on the water surface. The adults rest primarily on vegetation in the immediate vicinity of their breeding sites, the females are persistent and vicious biters in early morning and during the day close to the breeding places (Perry 1949). This species is a major pest in many parts of northern Australia (Russell 1996).

**Distribution:** Australia, New Hebrides, New Caledonia.

## Chapter 14

# Central and South America

A survey of mosquito breeding places in a municipal cemetery in Curitiba, Paraná State, Brazil, revealed about 60,000 flower vases, with the potential to support a population of approximately 12 million *Cx. p. quinquefasciatus*<sup>1</sup> and *Oc. fluviatilis* [*Georgecraigius fluviatilis*] larvae per year (Lozovei and Chahad 1997).

The primary Central and South American pest species *Oc. albifasciatus*, a vector of WEE, occurs in very large numbers in Argentina, and at densities above the economic threshold of 2,500 mosquitoes captured per night, will affect beef and dairy production (Gleiser and Gorla 2007). Two more important *Ochlerotatus* pest species; *Oc. taeniorhynchus* and *Oc. sollicitans* are troublesome in Central America and Caribbean. *Oc. mediovittatus* [*Gymnometopa mediovittata*] could be an important dengue vector in Central America and Venezuela (Rueda 2004).

However, *Cx. p. quinquefasciatus*<sup>1</sup> is the main nuisance mosquito throughout Central and South America, especially in urban areas. Over 200 bites/person/night have been recorded in several areas of the Martinique island (Yebakima 1996). It is included in the key together with *Cx. p. pipiens*<sup>1</sup>, *Ae. aegypti*<sup>1</sup> and *Ae. albopictus*<sup>1</sup>, the cosmopolitan and invasive species described in Chap. 10 (culicine mosquitoes of Europe). *Psorophora*

*ferox* is described in the North America section, having the status of a major pest there too. It is also widespread (from Mexico to Argentina) throughout Central and South America and together with other *Psorophora* sp. is a great nuisance in some areas (Anonymous 1992, 1994, 1998a–c, 2002). Accordingly, *Ps. ferox* is included in the key of Central and South America and described in Chap. 15 (North America).

In addition to the seven primary vectors of malaria, four other anophelines of secondary importance *An. albittarsis* (Guatemala to Argentina, most important in Central America), *An. aztecus* (Central America), *An. bellator* (Caribbean Islands and Brazil) and *An. cruzii* (Costa Rica to Argentina) are also worth mentioning (Anonymous 1992, 1994, 1998a–c, 2002).

For further, more detailed information on the morphology, identification, biology, distribution, and disease vector status of the species mentioned, the reader is referred to Lane (1953), Garcia and Ronderos (1962), Cova Garcia et al. (1966), Bram (1967b), Mattingly (1971), Smith (1973), Vargas (1974), Cova Garcia and Sutil Oramas (1977), Faran (1980), Faran and Linthicum (1981), Clark-Gil and Darsie (1983), Darsie (1985), Mitchell and Darsie (1985), Wilkerson and Strickman (1990), Rueda (2004), Reinert et al. (2005).

### 14.1 Key to Central and South American Female Mosquitoes

- 1 Palps about as long as proboscis. Scutellum evenly rounded or slightly trilobed and uniformly setose (Fig. 14.1a). Abdominal terga and sterna completely or largely missing scales. – Proboscis not strongly recurved and not tapering towards apex. *Anopheles*. Integument of scutum without a silvery longitudinal stripe. Wing scales of two or more colours. Vein R<sub>4+5</sub> with several pale spots or largely pale scaled. Hind femur without a tuft of erect scales on distal third. Hind tarsomeres II–V not all with pale rings, sometimes entirely dark scaled..... 2

<sup>1</sup>Species described in Chap. 10 concerning European mosquitoes.

Palps distinctly shorter than proboscis. Scutellum trilobed, setae arranged in three sets (Fig. 14.1b). Abdominal terga and sterna uniformly and densely covered with scales. – Proboscis not strongly recurved and not tapering towards apex. Postnotum without a tuft of setae. Squama with fringe of setae. Anal vein (A) ending well beyond the furcation of cubitus (Cu). Veins  $R_2$  and  $R_3$  at least as long as vein  $R_{2+3}$ , usually much longer. .... 8

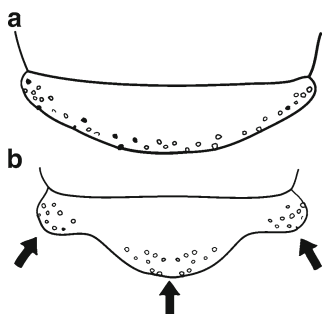


Fig. 14.1 Scutellum of: (a) *Anopheles* sp.; (b) *Aedes* sp.

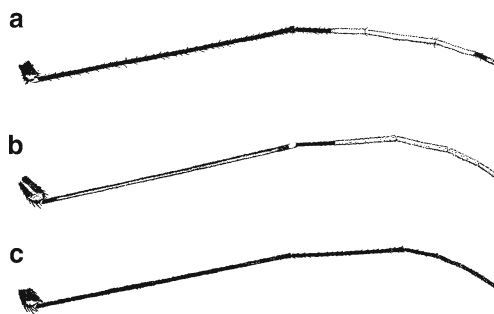


Fig. 14.2 Hind tarsus of: (a) *An. albimanus*; (b) *An. darlingi*; (c) *An. pseudopunctipennis*

- 2 (1) Tarsomeres III and IV of hind legs entirely white (Fig. 14.2a,b). Acrostichal and dorsocentral areas of scutum with numerous scales. *Anopheles* subgenus (*Nyssorhynchus*) – At least half of anal vein (A) pale scaled. At least some of abdominal terga II–VII with scales and posterolateral scale tufts. .... 3
- Tarsomeres III and IV of hind legs not entirely white, could be completely dark (Fig. 14.2c). Acrostichal and dorsocentral areas at most with scattered scales. *Anopheles* subgenus (*Anopheles*) – Hind leg without distinct, large tibiotarsal patch of pale scales ..... 6
- 3 (2) Tarsomere V of hind legs with a basal dark scaled ring (Fig. 14.2a). .... 4
- Tarsomere V of hind legs completely pale scaled (Fig. 14.2b). – Palpomere IV with scattered white scales on mediolateral surface. Scutellum usually with more than 12 large, dark setae on posterior margin. Anteromedian mesepimeral patch of pale scales present. Upper mesepimeral patch absent. Costa (C) with basal dark spot about four times as long as second pale spot. Vein  $R_3$  with three dark spots. Sternum I completely devoid of scales. .... *An. darlingi* (p 378)
- 4 (3) Palpomere IV all dark or with yellow to golden brown (never white or creamy) scales on mediolateral surface (narrow apical pale ring sometimes present). Palpomere V entirely pale. Tarsomere V of fore legs usually entirely dark scaled. Abdominal tergum II without a pair of posterolateral tufts of dark scales (Fig. 14.3a). .... *An. albimanus* (p 376)
- Palpomeres IV and V almost entirely pale scaled, separated by a dark ring. Tarsomere V of fore legs variable. Abdominal tergum II with pair of posterolateral tufts of dark scales (Fig. 14.3b). – Anteromedian part of mesepimeron without a patch of pale scales. Upper mesepimeron usually without pale scales. Tarsomere IV of fore legs predominantly dark scaled, or if pale in more than apical 1/3, then at least the apical half of fore tarsomere V pale scaled. Tarsomere II of hind legs with basal dark ring covering at least 1/4 length of tarsomere. If dark ring covers less than 1/4, then humeral light spot on costa (C) less than 1.5 length of basal dark spot on C. Pale wing spots at least on costa (C) and radius (R) creamy to yellowish, not white. Subcostal pale spot of costa (C) usually less than 1/2 the length of subcostal dark spot. .... 5



Fig. 14.3 Palps of: (a) *An. albimanus*; (b) *An. aquasalis*

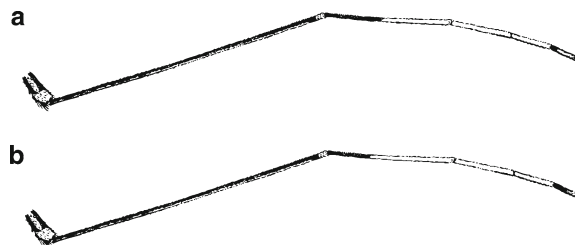
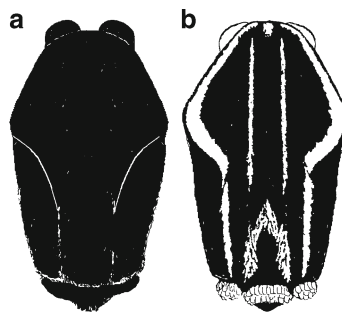


Fig. 14.4 Hind tarsus of: (a) *An. aquasalis*; (b) *An. nuneztovari*

- 5 (4) Tarsomere II of hind legs with basal dark ring covering 1/3 to 1/2 length of tarsomere. Hind tarsomere V with a narrow dark basal ring (Fig. 14.4a). (separation from *An. benarrochi* uncertain) ..... *An. aquasalis* (p 377)
- Tarsomere II of hind legs with basal dark ring covering less than 1/3 the length of the tarsomere. Hind tarsomere V with basal half dark (Fig. 14.4b). – Costa (C) with humeral pale spot less than twice the length of basal dark spot. .... *An. nuneztovari* (p 380)
- 6 (2) Tarsi entirely dark scaled (Fig. 14.2c). – Costa (C) with only two pale spots. Anal vein (A) with less than four dark spots. .... *An. pseudopunctipennis* (p 374)
- Tarsi not entirely dark scaled (Fig. 14.5a,b). – Hind tarsomeres I–IV with several irregular dark and pale scaled spots or rings. Hind tarsomere V mainly pale scaled with median dark ring or spot. Wing not indented at junction of subcosta (Sc) and costa (C). Scales of wing broad, mainly on basal portion of wing. Costa with two pale spots near the junction of the subcosta. Abdomen usually with short dorsolateral scale tufts. .... 7
- 7 (6) Upper mesepimeron with a small patch of pale scales. Hind tarsomere V usually pale scaled, sometimes with a small dark spot (Fig. 14.5a). .... *An. calderoni* (p 374)
- Upper mesepimeron bare. Hind tarsomere V usually with a median dark ring (Fig. 14.5b). .... *An. punctimacula* (p 375)

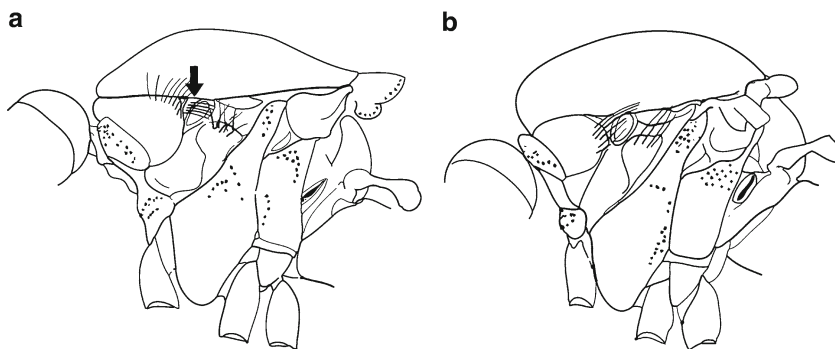


**Fig. 14.5** Hind tarsus of: (a) *An. calderoni*;  
(b) *An. punctimacula*



**Fig. 14.6** Dorsal view of thorax of: (a) *Haemagogus* sp.;  
(b) *Ae. aegypti*

- 8 (1) Prespiracular setae present (Fig. 14.7a). – Postspiracular setae present. Abdomen tapering apically, cerci long, easy visible, *Psorophora*. Scutum with dark brown and golden yellow scales mixed, without forming specific pattern. Femora without subapical ring of pale scales. Apices of femora without erect scales. Tarsomeres IV and V and often apex of tarsomere III of hind tarsus pale scaled. Wing veins uniformly dark scaled or relatively few pale scales present at anterior border. Abdominal terga with apicolateral patches of pale scales. .... *Ps. ferox* (p 395)
- Prespiracular setae absent (Fig. 14.7b). – Antenna subequal to proboscis, not short, thick and tapering. Flagellomere I without a prominent tuft of setae or scales. Mid and hind femora without large tuft of semi erect scales. .... 9



**Fig. 14.7** Lateral view of thorax of: (a) *Psorophora* sp.; (b) *Ochlerotatus* sp.

- 9 (8) Lobes of antepronotum very large, narrowly separated at midline of scutum, commencing before anterior margin of scutum (Fig. 14.6a). Scutum covered with broad, decumbent scales with bright metallic shine (green, blue, copper or bronze with silver scales only in supraalar area), *Haemagogus*. – Pleurites with single vertical line of pale scaled patches. Lower mesepisternal seta well developed (separation from *Hg. capricorni* uncertain). ..... *Hg. janthinomya* (p 381)
- Lobes of antepronotum small and broadly separated, commencing beyond anterior margin of scutum (Fig. 14.6b). Scutum covered with narrow scales without metallic shine. – Flagellomere I of similar length as flagellomere II. Apex of proboscis not distinctly swollen. Tarsomere I of fore legs usually shorter than tarsomeres II–V together. Tarsomere IV of fore legs not reduced, distinctly longer than broad. .... 10
- 10 (9) Postspiracular setae present (Fig. 14.8a) and/or claws of fore legs with subbasal tooth. .... 11
- Postspiracular setae absent (Fig. 14.8b). All tarsal claws simple. – Antennae not longer than proboscis. Flagellomere I and II of similar length. Vertex and occiput with narrow decumbent scales. Scutum with acrostichal setae. Usually no more than 1 or 2 lower mesepimeral setae present. Hind tarsal claws very small and inconspicuous. All tarsi with well developed pulvilli. Hind tarsomere I as long as or longer than hind tibia. Tarsomeres usually without distinct white rings. When pale rings are present, they are narrow and basal. Wing scales usually narrow. Costa (C) without alternating brown and yellow scaled areas. *Culex* subgenus (*Culex*) ..... 15

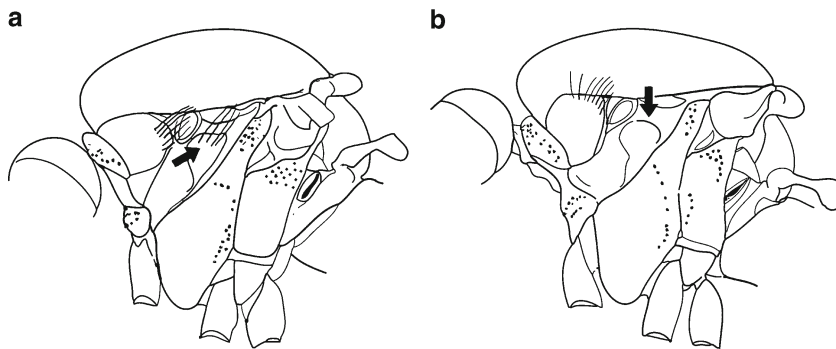


Fig. 14.8 Lateral view of thorax of: (a) *Ochlerotatus* sp.; (b) *Culex* sp.

- 11 (10) Wing scales usually narrow, if broad then not strongly asymmetrical (Fig. 14.9a). Claws of at least fore legs usually with subbasal tooth. .... 12
- Wing scales mostly broad and many conspicuously asymmetrical (Fig. 14.9b). Claws all simple, without subbasal tooth. *Mansonia* subgenus (*Mansonia*) – erect forked scales numerous, not restricted to occiput. Palps about 1/3 as long as proboscis. Palpomere III more than twice as long as palpomere II. Scutum without anterolateral golden scaled spots. Postspiracular setae present. Upper mesepimeral scale patch present. Femora speckled with pale scales, without rings or spots. Hind tibia without erect scales. Hind tarsomere I without a median pale ring. Abdominal terga with dark and pale scales. Abdominal terga VII and VIII with row of fine spines apically (on tergum VIII visible only when dissected). .... *Ma. titillans* (p 385)
- 12 (11) Decumbent scales of vertex largely broad. Erect scales not numerous, restricted to occiput (Fig. 14.10a). – Scutum with white or silvery scales in lines or patches. Scales of scutellum all broad. Femora with white knee spot. *Aedes* subgenus (*Stegomyia*) ..... 13
- Decumbent scales of vertex largely narrow and/or (if scales of vertex are largely broad) erect scales numerous, not restricted to occiput (Fig. 14.10b). *Ochlerotatus* ..... 14

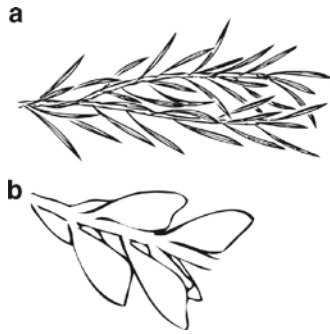


Fig. 14.9 Wing scales of: (a) *Aedes* sp.; (b) *Mansonia* sp.

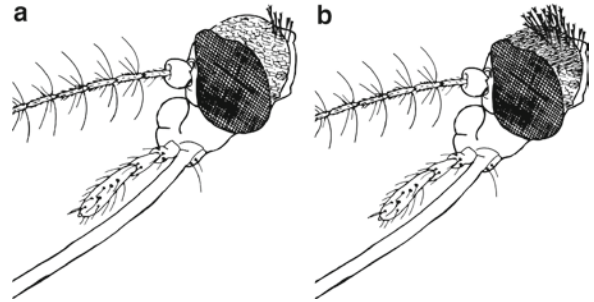


Fig. 14.10 Head of: (a) *Aedes (Stegomyia)* sp.; (b) *Ochlerotatus* sp.

- 13 (12) Scutum with a white acrostichal stripe extending from the anterior margin to the beginning of the prescutellar area, where it forks to end at the anterior margin of scutellum (Fig. 14.11a).  
 ..... *Ae. albopictus* (p 201)  
 Scutum without acrostichal stripe on anterior part, but with two narrow white dorsocentral stripes separated from anterior margin. Lateral white stripes broad, continuing over transverse suture to the end of scutum, lyre shaped (Fig. 14.11b)..... *Ae. aegypti* (p 198)

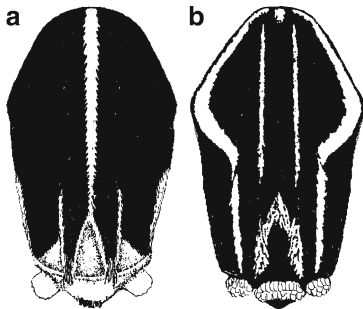


Fig. 14.11 Scutum of: (a) *Ae. albopictus*; (b) *Ae. aegypti*

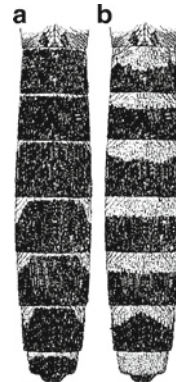


Fig. 14.12 Abdomen of: (a) *Cx. nigripalpus*; (b) *Cx. p. pipiens*

- 14 (12) Wing veins with pale and dark scales. – Scales on wing intermixed, not forming distinct spots. Terga with a median longitudinal pale stripe. .... *Oc. albifasciatus* (p 382)  
 Wing veins entirely dark scaled. – Hind tibia with distinct pale stripe on anterior surface, sometimes encircling tibia. Hind tarsomeres completely dark scaled. Scutum with large patch of silvery white/yellowish scales on anterior 2/3. Posterior 1/3 often with narrow stripes (e.g. prescutellar dorsocentral stripe) in continuation of large pale scaled patch. Terga without pale basal bands, basolateral spots can be present. Posterior terga usually with indistinct longitudinal pale stripe. .... *Oc. scapularis* (p 383)



- 15 (10) Pale basal bands on abdominal terga usually absent, if present they are narrow, or pale scaling restricted to lateral patches (Fig. 14.12a). – Palps short, less than 1/4 length of proboscis. Lower mesepimeral seta present. Mesepisternum with two patches of broad pale scales, each patch usually of less than 6 scales. Tergum VII mostly covered with dark scales. .... *Cx. nigripalpus* (p 384)
- Abdominal terga with a rather broad (about 1/4 of the terga) basal transverse band of pale scales (Fig. 14.12b). Bands rounded on posterior margin and constricted sublaterally, rather narrowly joining (sometimes disconnected) lateral pale patches. – Pale spot at tip of hind tibia inconspicuous. ....  
 ..... *Cx. p. pipiens biotype molestus* and *Cx. p. quinquefasciatus* (p 277, 278)

## 14.2 Species Description

### *Anopheles (Anopheles) calderoni* Wilkerson 1991

**Female:** *An. calderoni* is very similar to *An. punctimacula* in all live stages and the two species are very difficult to differentiate; nevertheless, both are regarded as distinct species. In *An. calderoni* the upper mesepimeron is clothed with a small patch of pale scales, and the hind tarsomere V usually is pale scaled (Fig. 14.5a), sometimes with a dark spot, whereas in *An. punctimacula* the mesepimeron is bare of any scales and the hind tarsomere V usually has a median dark ring. Additional differences exist in the ornamentation and colour pattern of the wing veins, and in the larvae, in the mean number of branches of certain setae (Fig. 14.13); for more information see Wilkerson (1991).

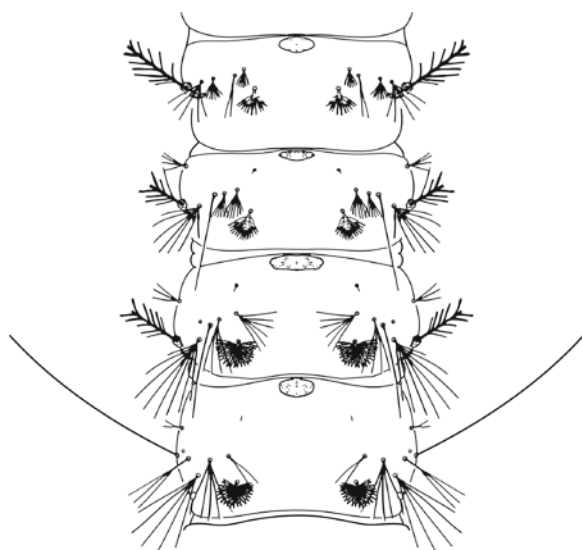


Fig. 14.13 Larva of *An. calderoni*, terga I–IV

**Biology:** *An. calderoni* has been encountered only at elevations < 250 m (north western Peru) and between 200 and 400 m (western Venezuela) (Rubio-Palis and Moreno 2003). The larvae are found in small streams, small irrigation canals and swamps, mostly in dense emergent vegetation, but especially associated with *Typha* sp. (Wilkerson 1991).

**Distribution:** Peru, Venezuela.

**Medical importance:** This species must be regarded as an important malaria vector at least in the lowlands of northwestern Peru.

### *Anopheles (Anopheles) pseudopunctipennis* Theobald 1901

**Female:** Medium sized species. Proboscis dark scaled, palps about as long as the proboscis, yellowish white at tip and with 2 narrow white rings at joints of basal palpomeres, remainder of palps dark scaled. Integument of head brown, occiput covered with erect forked scales, which are whitish in the middle, otherwise dark brown. Scales of vertex pale, a prominent set of long frontal scales directing forward. Integument of scutum dark brown, with a median broad greyish stripe, which is sparsely covered with narrow yellowish scales, acrostichal and dorsocentral setae pale, the dark lateral areas with longer dark setae. Scutellum with long brown and yellowish setae. Integument of pleurites brown with some darker areas. Legs almost entirely dark scaled, apices of femora and tibiae with whitish scales, tarsi dark (Fig. 14.2c). Wing veins covered with dark and pale scales arranged in a well defined pattern of dark and pale spots and lines. Basal portion of costa (C) dark scaled, with two pale spots at junction of subcosta (Sc) and near apex of wing, vein  $R_{4+5}$  narrow white at base, followed by a dark area, a broad white area and a narrow dark tip, anal vein (A) with basal

half white, apical half dark, tip white. Haltere stem pale, knob dark scaled. Integument of abdomen dark brown, clothed with brown setae, scales absent.

**Larva:** Antenna  $<1/2$  as long as the head, smooth on outer surface, spiculate on inner surface. Antennal seta (1-A) short, usually single, inserted below the middle of the antennal shaft. Inner clypeal setae (2-C) situated close together, closer to each other than to outer clypeal setae (3-C), all setae long, subequal in length and single. Postclypeal seta (4-C) long and single, all frontal setae (5-C to 7-C) long and plumose. Prothoracic seta 1 (1-P) short, usually with 2–4 branches beyond the base, 2-P and 3-P arising from a common tubercle, 2-P long and stout, with many lateral branches, 3-P single, about twice as long as 1-P. Palmate setae on segments I and II (1-I and 1-II) rudimentary, well developed on segments III–VII (1-III to 1-VII), leaflets long and slender, with serrations beyond the middle. Lateral abdominal seta 6 on segments I–III (6-1 to 6-III) long and plumose. Posterior spiracular plates each with a long and slender sclerotized projection arising from the posterior margin. These are bent upward at right angles to the plates and project through the water surface (Fig. 14.14). The pecten plate with 7–8 long teeth alternated by 1 or 2 shorter teeth.

**Biology:** The larvae are frequently found in sunny habitats including sidepools of streams, in lagoons and canals, in swamp meadows, or poorly kept drainage systems (Aitken 1954b). They are abundant in shallow, receding streams in mountainous areas during the dry

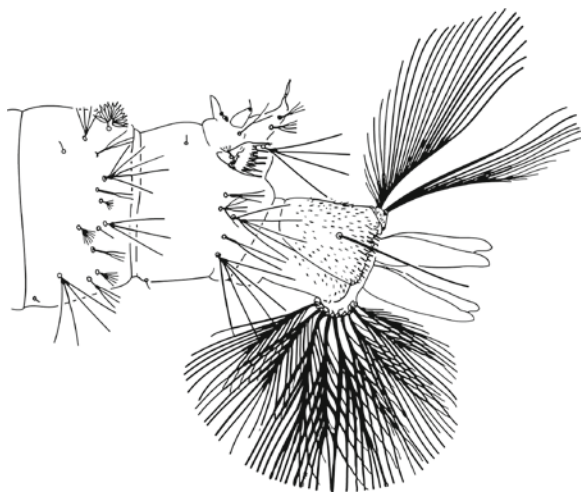


Fig. 14.14 Larva of *An. pseudopunctipennis*

season when the streams are not subject to flushing by heavy rainfall (Carpenter and La Casse 1955). A common character of all breeding sites is the presence of floating vegetation and particularly green algae. The feeding habits and host preferences of the adults appear to differ markedly in the different regions, raising the possibility of different forms or varieties. In its more southern distribution range the species is anthropophilic, the females feed at night and will enter houses to take a blood meal. A flight range of 6–10 km is recorded (Gorham et al. 1973).

**Distribution:** *An. pseudopunctipennis* is probably the most widespread anopheline mosquito in the New World, ranging from the western and southern United States through Central America, Colombia, Venezuela and the Antilles and southward following the Andes to north eastern Argentina. The species can be found from lowland to dry mountains and plateaus up to an altitude of about 2,600 m.

**Medical importance:** *An. pseudopunctipennis* is regarded as an important vector of malaria in some but not all areas of its wide distribution range.

#### *Anopheles (Anopheles) punctimacula* Dyar and Knab 1906

**Female:** A large species with speckled legs. Proboscis long and slender, dark scaled. Palps predominantly dark scaled, palpomere V with basal white ring, palpomere IV with narrow basal white ring, palpomere III often with a few white scales at the base. Pedicel dark brown with a few pale scales. Vertex with a tuft of long pale scales projecting forward, a few whitish scales along the eye margin, occiput covered with erect forked scales, pale anteriorly, dark posteriorly. Integument of scutum grey, with 3 larger conspicuous dark spots, anterior margin of scutum with a prominent median tuft of long white setae and elongated white scales. Scutum clothed with long yellowish setae, scutellum with a median dark spot, covered with long dark setae. Mesepimeron without any scale patches. Femora and tibiae predominantly dark scaled with many white spots on dorsal surfaces. Fore tarsomere I dark with several white spots and white apex, tarsomeres II–IV with white scales at base and apex, tarsomere V entirely white scaled. Mid tarsomere I dark with several white spots and white apex, tarsomeres II–IV with several white rings, tarsomere V with

a dark ring in the middle. Hind tarsomere I dark on dorsal surface, yellowish ventrally, the dorsal part with several white spots, tarsomere II with narrow white rings at base and apex and several white spots, tarsomeres III and IV with white rings at base, apex and middle, tarsomere V usually with a dark ring in the middle (Fig. 14.5b). Wing veins covered with broad pale and dark scales forming a characteristic pattern. Costa (C) with 3 prominent dark spots, involving also subcosta (Sc) and radius (R). Subapical white spot very small, cubitus (Cu) with pale and dark scales intermixed. Wing fringe predominantly dark, interrupted by conspicuous white spots. Halter stem pale, knob dark and pale. Integument of abdomen dark brown, covered with long brown setae. Lateral tufts of dark brown scales on terga II–VII.

**Larva:** Antenna sparsely spiculous, antennal seta (1-A) very large, about 2/3 the length of the antenna, with multiple branches, inserted at basal 1/3 of antennal shaft. Inner clypeal seta (2-C) long, single, the pair of 2-C situated close together, outer clypeal seta (3-C) nearly as long as 2-C, usually dendriform beyond the middle, sometimes with a few branches apically. Postclypeal seta (4-C) small, with 2–3 branches, frontal setae (5-C to 7-C) long and plumose. Prothoracic setae 1 to 3 (1-P to 3-P) not arising from a common tubercle, P-1 about 1/2 as long as 2-P, with long lateral branches, 2-P long and strong, pinnately branched, 3-P short and single. Palmate setae rudimentary on segments I and II (1-I and 1-II), well developed on abdominal segments III–VII (1-III to 1-VII), with 15–20 leaflets. Individual leaflet short and broad, strongly pigmented at the base, margin serrated beyond the middle, apex smooth, pointed. Tergal plate on abdominal segment VIII larger than the others. Lateral abdominal setae 6 of segments IV and V (6-IV and 6-V) long and slender, single, seta 6-VI with 2–4 branches, seta 5-VIII with 3–5 branches. The pecten plate bears 15–16 short and long teeth regularly alternating, about 7 long teeth. Saddle seta (1-X) not inserted on the saddle, but close to its ventral margin (Fig. 14.15).

**Biology:** The larvae of *An. punctimacula* may be found in jungle pools, temporary grassy pools, swamps, and along mountain streams up to an elevation of 1,500 m. The breeding sites are usually well shaded and rich in organic matter (Ross and Roberts 1943; Horsfall 1955). The females are regarded as being zoophilic with a preference for domestic animals, but they

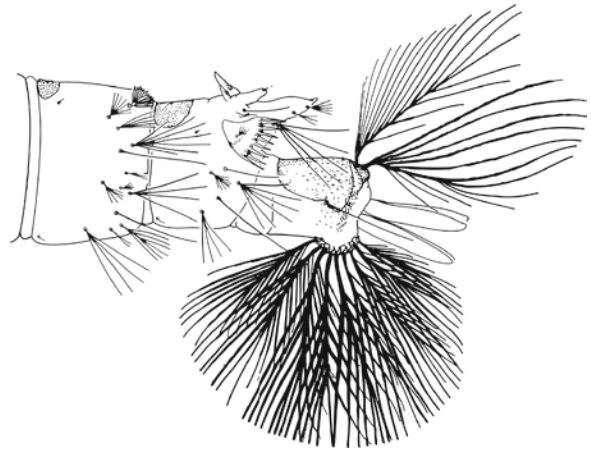


Fig. 14.15 Larva of *An. punctimacula*

also enter dwellings and readily feed on humans; their flight range is about 3 km.

**Distribution:** Central America, Mexico, Venezuela, Trinidad, Colombia, Brazil, Peru, Ecuador, Bolivia, Argentina.

**Medical importance:** *An. punctimacula* is an efficient malaria vector in much of its distribution range.

#### ***Anopheles (Nyssorhynchus) albimanus* Wiedemann 1820**

**Female:** Medium sized greyish species. Proboscis dark scaled, palps about as long as proboscis, predominantly dark scaled, palpomere V entirely white (Fig. 14.3a), apices of palpomeres II and III with narrow ring of white scales. Occiput with white erect forked scales dorsally, dark scaled laterally, vertex with frontal tuft conspicuous, white. Flagellomere I of antenna with a conspicuous tuft of white scales. Integument of scutum grey or greyish brown with 3 conspicuous dark spots. Scutum clothed with grey scales and pale setae, the setae more numerous laterally. Scutellum covered with greyish scales and yellowish brown setae. Anteprenotum with a patch of erect dark scales in upper part, upper and lower part of mesepimeron with a few pale scales. Prespiracular setae present, postspiracular setae absent. Femora and tibiae predominantly dark scaled, sparsely speckled with pale scales, with white scales at apices, mid and hind femora each with a pale subapical spot. Fore tarsus with tarsomere I narrowly white

ringed at apex, tarsomeres II and III white apically, tarsomeres IV and V entirely dark. Mid tarsus with narrow indistinct white rings at apices of tarsomeres I and II, tarsomeres III–V entirely dark. Hind tarsus with tarsomere I entirely dark except for a few white scales at apex, tarsomere II dark on basal half and white on apical half, tarsomeres III and IV entirely white, tarsomere V dark basally and white apically (Fig. 14.2a). Wing scales dark and yellowish white, arranged on the veins in contrasting lines and spots, haltere with the stem pale, knob dark scaled. Integument of abdomen dark brown to black, clothed dorsally with long setae and few pale scales, the scales more numerous in the middle of the last segments. Tufts of dark scales present laterally on terga III–VII, absent on tergum II. Sterna V–VII with a few scattered pale scales laterally, a conspicuous submedian patch on sternum II.

**Larva:** Antenna shorter than the head, with very strong short spicules on inner surface, antennal seta (1-A) short, only slightly longer than width of antennal shaft at point of origin, inserted on basal 1/3 of shaft, with 2–4 branches. Inner and outer clypeal setae (2-C and 3-C) single, finely to strongly aciculate, their bases about equally spaced one from another. Postclypeal seta (4-C) single or apically branched, frontal setae (5-C to 7-C) long, plumose. Prothoracic setae 1 and 2 (1-P and 2-P) arising from a common sclerotized tubercle, 1-P about half as long as 2-P, both setae with shaft thickened and with many lateral branches, 3-P small, single. Metathoracic palmate seta (3-T) with numerous slender transparent leaflets. Palmate setae well developed on abdominal segments I–VII (1-I to 1-VII), smaller on segments I and II, leaflets slender, with smooth margins, not serrated. Lateral abdominal seta 6 on segments I–III (6-I to 6-III) long, plumose, on segments IV–VI (6-IV to 6-VI) long and usually single (Fig. 14.16). The pecten plate has 3–4 long teeth alternated by 2–3 shorter teeth laterally and 6–7 shorter teeth in the middle.

**Biology:** The larvae of *An. albimanus* are found in a wide variety of mostly permanent water habitats, either fresh or brackish, e.g. irrigation ditches, ground pools, ponds, and shallow margins of streams, lakes and swamps; water filled hoof prints in cow pastures are also often inhabited (Carpenter and La Casse 1955). The various breeding sites usually contain abundant floating, emergent vegetation

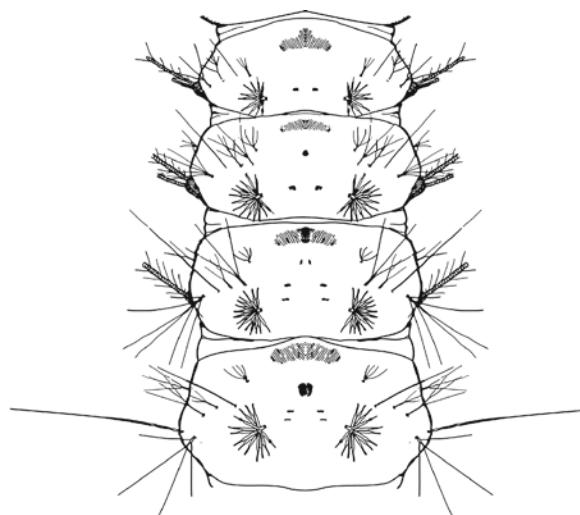


Fig. 14.16 Larva of *An. albimanus*, terga I–IV

and floating algae, and frequently have muddy bottoms and turbid or polluted water. The larvae exhibit a strong preference for breeding sites exposed to full sunlight, they are rarely found in densely shaded pools (Belkin et al. 1970). The adults are strong fliers and feed readily on humans and domestic animals, particularly horses and cattle. They may also enter houses to feed at night, but usually return to the jungle or breeding grounds before daylight. (Carpenter and La Casse 1955). The species may be found throughout the year, predominantly in the lowlands, its elevation limit may be restricted to less than 500 m.

**Distribution:** Antilles, Central America, Mexico, United States (Florida, Texas), Venezuela, Surinam, Brazil, Ecuador, Colombia, Peru, Uruguay.

**Medical importance:** *An. albimanus* is generally regarded as the principal lowland vector of malaria throughout much of Central America, northern South America and the West Indies.

#### ***Anopheles (Nyssorhynchus) aquasalis* Curry 1932**

**Female:** Medium sized species, proboscis slender, dark scaled. Palpomere V entirely white scaled, palpomere IV with narrow dark rings at base and apex, palpomere III with a narrow white ring at apex, rarely with an indistinct line of pale scales dorsally (Fig. 14.3b).

Pedicel dark with a few pale scales, vertex with a tuft of long pale scales projecting forward. Integument of scutum greyish brown, with a darker median line and 3 conspicuous dark spots, covered with curved pale scales and dark setae, supraalar setae long and pale. Scutellum covered with pale scales and long dark setae. Femora and tibiae with dark scales on dorsal surfaces and yellowish scales ventrally. Fore tarsomere I dark dorsally and yellowish ventrally, with a narrow apical pale ring, tarsomeres II and III with more than the basal half dark scaled and pale scales apically, tarsomeres IV and V entirely dark. Mid tarsomeres I–III narrowly ringed with pale scales at the apex, tarsomeres IV and V entirely dark scaled. Hind tarsomere I dark scaled dorsally and yellowish ventrally, tarsomere II with 1/3 to 1/2 of the basal portion with dark scales, remainder white, tarsomeres III and IV all white, tarsomere V with a narrow dark basal ring (Fig. 14.4a). Wing veins covered with pale and dark scales producing pale and dark spots of variable appearance, second pale spot on costa (C) usually longer than the following dark spot. Haltere stem pale, knob dark. Integument of abdomen dark brown, covered with long brown setae, terga II–VII with lateral tufts of dark brown scales.

**Larva:** Antenna with dense spicules on inner surface, antennal seta (1-A) short, inserted well below the middle of the antennal shaft, with 3–4 branches. Inner and outer clypeal setae (2-C and 3-C) long, single, distinctly feathered beyond the middle. The distance between the bases of the pair of 2-C slightly shorter than the distance between 2-C and 3-C. Postclypeal seta (4-C) with 2–3 branches, frontal setae (5-C to 7-C) long and plumose. Prothoracic seta 1 (1-P) of palmate type, with 12–15 lanceolate leaflets, not arising from a common tubercle with 2-P, which is long with a strong shaft and laterally branched, 3-P long, single. Palmate setae developed on abdominal segments I–VII (1-I to 1-VII), 1-I with about 14 leaflets, not pigmented and much smaller than 1-II to 1-VI, which have about 20 narrow and lanceolate leaflets. Tergal plates oval, largest on abdominal segment VIII. Lateral abdominal setae 6 of segments IV to VI (6-IV to 6-VI) long and single, rarely with 2 branches (Fig. 14.17). The pecten plate bears about 14 straight teeth, long and short teeth irregularly arranged.

**Biology:** *An. aquasalis* is a typical brackish water breeder, the larvae can primarily be found in swamps, usually mangroves, pools, and ditches along the sea

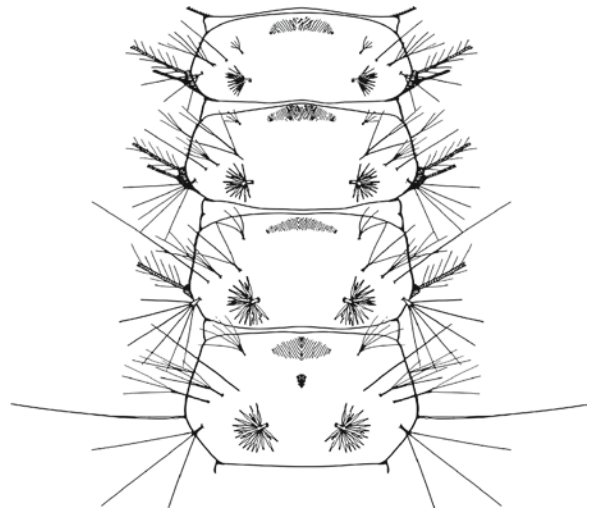


Fig. 14.17 Larva of *An. aquasalis*, terga I–IV

coast, however, it is capable of breeding in fresh water, and sometimes is collected several kilometers from the coast. The habitats may be permanent or temporary, and with or without aquatic vegetation. Females readily feed on humans and livestock; they are usually exophilic and exophagic, and show a crepuscular biting behaviour with a major peak at dusk and a minor peak at dawn (Berti et al. 1993). After the blood meal they rest in the nearby vegetation, such as tall grasses. The flight range of this species does not usually exceed 3–5 km (Horsfall 1955). Adult populations may occur in great numbers, especially during the rainy season.

**Distribution:** Panama Canal Zone, Colombia, Ecuador, Brazil, Guianas, Venezuela, Trinidad, Tobago, Lesser Antilles, Costa Rica, Nicaragua.

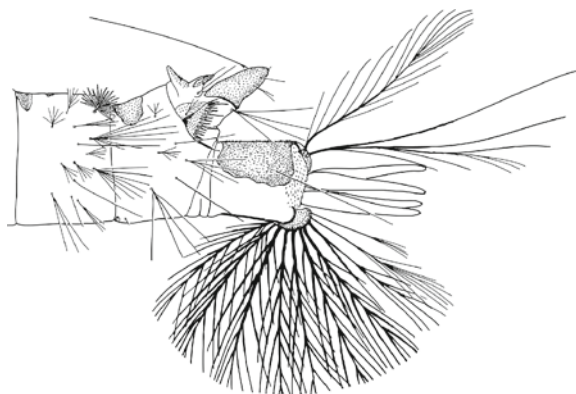
**Medical importance:** *An. aquasalis* is regarded as the main vector of coastal *Plasmodium vivax* malaria from north eastern Venezuela to southern Brazil (Fleming 1986).

#### ***Anopheles (Nyssorhynchus) darlingi* Root 1926**

**Female:** Medium sized greyish species, proboscis slender, dark scaled. Palpomere V entirely white scaled, palpomere IV dark at base and apex with a few white scales in the middle, palpomere III dark with a conspicuous white ring at apex and a few white scales dorsally, palpomere II dark scaled with a few white scales at apex. Pedicel dark with a few pale scales.

Vertex with a tuft of long pale scales projecting forward, occiput covered with erect forked scales, pale anteriorly, dark posteriorly. Integument of scutum dark grey, with a median longitudinal black line and 3 conspicuous dark spots, anterior margin of scutum with a prominent tuft of long white setae and white scales. Scutum clothed with curved yellowish white scales and short dark setae, scutellum covered with pale scales and long dark setae. Femora and tibiae with dark scales on dorsal surface and yellowish scales ventrally. Fore tarsomere I dark scaled, with a narrow apical pale ring, tarsomeres II and III with a broad apical white ring, tarsomeres IV and V entirely dark scaled. Mid tarsomeres I–III narrowly ringed with pale scales at the apex, tarsomeres IV and V entirely dark scaled. Hind tarsomere I dark scaled dorsally, somewhat paler ventrally, with a very narrow apical white ring, tarsomere II with the basal half dark, apical half white, tarsomeres III–V all white scaled (Fig. 14.2b). Wing veins covered with pale and dark scales in a more or less variable pattern, second pale spot on costa (C) usually much shorter than the following dark spot. Subcostal and preapical pale spots both small. Haltere with stem pale, knob dark. Integument of abdomen dark brown, terga with yellowish scales in the middle and lateral tufts of dark brown scales, sternum I devoid of scales, cerci with brown and white scales.

**Larva:** Antenna sparsely spiculate, mainly on inner surface, antennal seta (1-A) short, inserted at the basal 1/3 of the antennal shaft, with 2–4 branches. Inner and outer clypeal setae (2-C and 3-C) long and slender, single, finely aciculate, the pair of 2-C inserted closer to each other than they are to 3-C. Postclypeal seta (4-C) with 2–4 branches, frontal setae (5-C to 7-C) long and plumose. Prothoracic seta 1 (1-P) of stellate type, arising from a distinct tubercle, with about 8 branches radiating from a short stem, 2-P long with a strong shaft and laterally branched, 3-P about 1/4 as long as 2-P, single. Palmate setae developed on abdominal segments I–VII (1-I to 1-VII), 1-I and 1-VII smaller than the others, each seta with 17–20 lanceolate, smooth and sharply pointed leaflets. Tergal plate on abdominal segment VIII much larger than tergal plate on segment VII. Lateral abdominal setae 6 of segments IV–VI (6-IV to 6-VI) long and slender, single. A unique character for the species is found on the spiracular plate. Laterally on the posterior margin arises a pair of prominent tubercles, bearing at their tips very fine, single and long setae (Fig. 14.18). These setae extend dorsally and



**Fig. 14.18** Larva of *An. darlingi*

project up through the water surface into the air when the larvae are resting. The pecten plate bears 3–4 long teeth and 11–13 short ones with a single long tooth at the ventral end. The other long teeth are more or less evenly spaced, the last two or three teeth at the dorsal end of the pecten are short ones.

**Biology:** The larvae of *An. darlingi* occur in fresh water or water of low salt content usually in partial shade, sometimes in full sunlight. Usually they can be found among floating vegetation or debris in ditches, irrigation canals, stream pools, ponds, lakes or lagoons, especially in larger water bodies. The water is always clear, never turbid or polluted (Root 1926; Komp 1942). It is evidently a mosquito which requires high humidity and rainfall and seems to be susceptible to dry seasons. The species is found predominantly along large river valleys and is not present in areas where the dry season is long (Linthicum 1988). The females are endophagic and endophilic, they prefer human hosts to domestic animals and readily enter dwellings and bite humans, predominantly at night. In much of its distribution range it is the most important domestic species (Ross and Roberts 1943). The post-feeding resting sites in houses are usually vertical surfaces mostly within 2 m of the floor (Horsfall 1955).

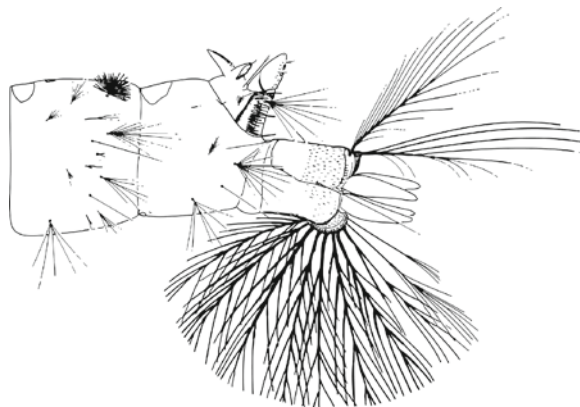
**Distribution:** Brazil, Argentina, Bolivia, Peru, Colombia, Venezuela, Guianas, Honduras, Guatemala, Belize, Mexico.

**Medical importance:** *An. darlingi* is evidently the most efficient and important malaria vector throughout its distribution range because of its domestic habits and its high susceptibility to *Plasmodium* spp. (Linthicum 1988).

***Anopheles (Nyssorhynchus) nuneztovari***  
**Gabaldon 1940**

**Female:** Medium sized to large species, with the integument generally dark to very dark brown and a considerable contrast between light and dark regions. Proboscis slender, entirely dark scaled. Palps predominantly dark scaled, palpomeres II and III with narrow apical white rings, palpomere IV with a broad median white ring, sometimes apical part also white scaled, palpomere V usually entirely white scaled, occasionally with a few dark scales at base. Occiput covered with many erect scales, pale anteriorly becoming progressively darker on the posterior portion and laterally. Vertex with a few decumbent whitish scales and a conspicuous tuft of long pale scales projecting forward. Clypeus bare, pedicel with a dorsolateral row of light scales. Anterior margin of scutum with a median tuft of elongated whitish scales. Scutum clothed with small, lanceolate, yellowish white, decumbent scales on acrostichal area, extending from anterior margin to prescutellar area, and dorsocentral stripe, on scutal fossal and supraalar area, additionally with numerous moderately long, dark setae. Scutellum covered with light scales and very long, dark setae. Upper and lower mesepisternal scale patches present, mesepimeral scale patch usually absent. 1–2 long, upper mesepisternal setae and about 7 long, pale mesepimeral setae present. Coxae with small patches of pale scales, mid femur with a distinct whitish knee spot. Fore tarsomeres I–IV with narrow to moderately broad pale apical rings, tarsomere V pale in apical half. Mid tarsomeres I and II with a narrow apical pale rings, tarsomeres III and IV usually all dark scaled, sometimes tarsomere III with a narrow apical pale ring, tarsomere V with apical part pale. Hind tarsomere II dark in basal 1/3, tarsomere III and IV usually entirely pale scaled, very rarely dark basally, tarsomere V with basal half dark, apical half pale (Fig. 14.4b). Wing veins covered with pale and dark scales in a diagnostic pattern as described in the keys. Light spots usually creamy, not white, at least on anterior veins. Large dark wing spots distinct. Wing fringe dark scaled, with pale spots at terminations of most veins and a pale spot between base of wing and anal vein (A). Integument of abdomen dark brown, covered with long brown setae, terga II–VII with lateral tufts of conspicuous dark brown scales.

**Larva:** Head moderately to very heavily pigmented, slightly longer than broad. Antenna more than half as long as the head, usually lighter than remainder of head, moderately spiculate, mainly on inner surface. Antennal seta (1-A) short, with 5–7 branches, about as long as width of antenna at point of insertion, inserted at about 1/4 from base of antenna. Inner and outer clypeal setae (2-C and 3-C) single, feathered in apical half, the pair of 2-C widely separated, 3-C slightly shorter than 2-C. Postclypeal seta (4-C) with 1–4 branches, moderately long and usually extending to near or beyond base of 2-C, frontal setae (5-C to 7-C) long and plumose. Prothoracic setae 1 and 2 (1-P and 2-P) usually sharing a common sclerotized tubercle, sometimes base of 1-P not sclerotized. 1-P of palmate type with 9–12 pigmented, moderately broad leaflets, 2-P with 12–18 branches, 3-P small and single. Metathoracic seta 3-T of palmate type, with 11–15 semitransparent, narrow leaflets. Palmate setae developed on abdominal segments I–VII (1-I to 1-VII), 1-III to 1-VI with 23–31 long pointed leaflets, 1-I and 1-VII with fewer and slightly shorter leaflets. Lateral abdominal setae 6 of segments IV–VI (6-IV to 6-VI) long and single. Pecten with 15–19 variable long and short teeth, usually one long tooth in the middle and 2–3 long teeth dorsally. Anal segment with short thin spicules posteriorly, saddle with irregular ventral margin (Fig. 14.19). Saddle seta (1-X) single, distinctly longer than the saddle, inserted near its ventral margin. Upper and lower anal seta (2-X and 3-X) single, plumose or laterally branched. Anal papillae about as long as the saddle, and pointed.



**Fig. 14.19** Larva of *An. nuneztovari*

**Biology:** The larvae of *An. nuneztovari* are usually found in fresh water habitats in open marshy areas, ponds and lakes often at the grassy margins, small or large permanent or temporary ground pools, animal or wheel tracks, and along stream margins, fully exposed to sunlight or partially in shade (Faran 1980). Aquatic vegetation may be abundant and algae are often present. They may also be encountered in the interior or in clearings within the forest, and in areas of secondary growth (scrub) such as around villages. The females exhibit a variable feeding and resting behaviour in different parts of their distribution range, however during the peak season they are regarded as being predominantly endophagic and endophilic, readily feeding on humans at night. The principal time the females enter dwellings is 2200–2400 hours. After the blood meal this species usually rests 1 m high or less on the walls inside houses (Faran 1980).

**Distribution:** Venezuela, Guianas, Brazil, Bolivia, Colombia, Panama.

**Medical importance:** *An. nuneztovari* is a major vector of malaria in western Venezuela and northern Colombia as well as in Suriname.

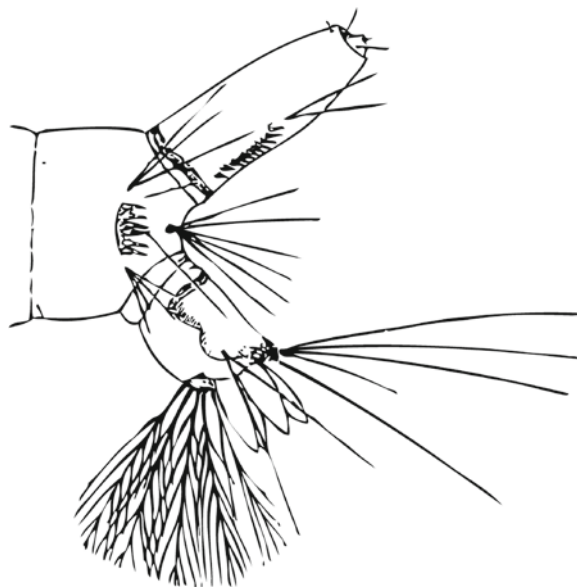
### ***Haemagogus (Haemagogus) janthinomys* Dyar 1921**

**Female:** Distinctive species ornamented with green or blue scales of metallic shine that are visible even under a hand lens. Dark scales of proboscis, palps, wings and legs predominately purple, with some violet reflections. Palps short, about 1/6 the length of the proboscis. Decumbent scales of vertex and occiput bluish with green tinge. Anteprenotal lobes well developed (Fig. 14.7a), much larger than in other genera, clothed with greenish blue scales. Scutum covered with dark green and bluish green scales, sometimes silvery scales on supraalar area, scutellum with dark green to bright blue scales. Fore and mid coxae with patches of purple scales near middle, hind femur with silvery scales on anterior surface in a conspicuous stripe to near apex. Dark scales of terga mixed with purple scales with some violet reflections and dark bluish green scales at distal margins of terga V–VII, more numerous laterally, dark scales of sterna purple with bluish green scales distally.

**Larva:** Head rounded, antenna short, adorned with a few spicules only, antennal seta (1-A) single, inserted at about the middle of the antennal shaft. Postclypeal

seta (4-C) small, multiple branched, inner frontal seta (5-C) single, placed far forward, median frontal seta (6-C) single and long, outer frontal seta (7-C) with 2–4 branches. Integument of thorax densely spiculate. Prothoracic setae 1 to 3 (1-P to 3-P) arising from a common tubercle, P-1 and P-3 with multiple branches, 2-P single. The comb consists of 6–8 scales; individual comb scales are elongated, with a prominent median spine and laterally fringed with minute spines. The teeth arise from a sclerotized plate, one or more scales occasionally detached from the plate. Siphonal index 2.5–2.9, pecten with about 10–14 teeth extending to near the middle of the siphon. Siphonal seta (1-S) with 2–3 branches, inserted beyond the pecten. Anal segment slightly longer than broad, the saddle reaching half way down the segment. Spines on posterior margin of saddle well developed, much elongated and conspicuous dorsally. Saddle seta (1X) long, with 2 or more branches (Fig. 14.20). Upper anal seta (2-X) with 3–5 branches, lower anal seta (3-X) long and single. Ventral brush well developed, precratal setae (4-X) absent, grid strongly sclerotized. Anal papillae 1.0 to 1.5 times the length of the saddle, and pointed.

**Biology:** *Hg. janthinomys* is found almost exclusively in primary tropical rain forest throughout most of its range and is decidedly arboreal in habit (Arnell 1973). It breeds primarily in tree-holes, the eggs are laid above the water line and hatch when flooded. Eggs can



**Fig. 14.20** Larva of *Hg. janthinomys*



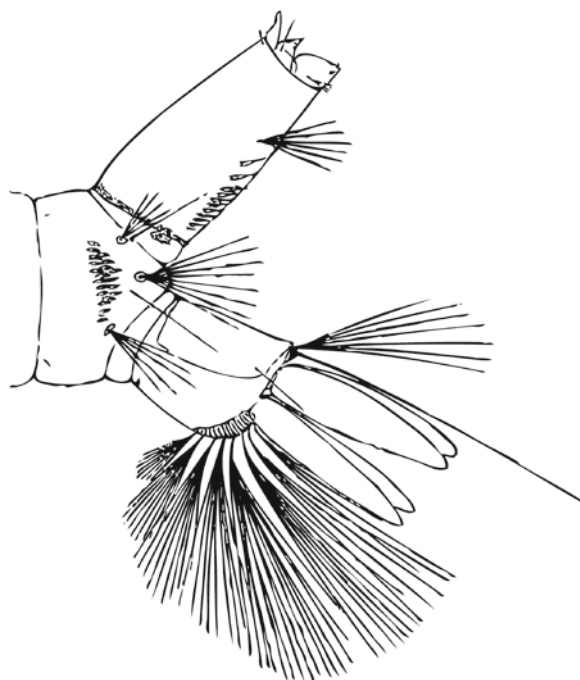
remain viable for many months, sometimes multiple flooding and desiccation cycles are required to induce hatching. Adults of some *Haemagogus* spp. do not fly far from their breeding sites in the tree canopy, where they feed on monkeys. However, *Hg. janthinomys* will readily leave the tree canopy and feed at ground level on forest ruminants and humans, especially in damaged forests and along forest edges. Adults feed during the day between 0600 and 1800 hours, with a single peak of activity between 1000 and 1600 hours. *Hg. janthinomys* usually feeds on the lower limbs and feet of humans. The females can survive 2–3 months in the laboratory, although survival rates in nature are much lower. In the rain forests of Central America, the species reaches its maximum density at elevations between 100 and 1,000 m, the population size is strongly related to the rainy season (Anonymous 2002).

**Distribution:** Argentina, Bolivia, Colombia, Costa Rica, Ecuador, French Guiana, Guyana, Honduras, Nicaragua, Panama, Paraguay, Peru, Suriname, Tobago and Trinidad and Venezuela.

**Medical importance:** This species is the primary vector of sylvatic yellow fever which is endemic in several areas in South America, it may become even more important with the deforestation process continuing.

### ***Ochlerotatus (Ochlerotatus) albifasciatus*** **(Macquart 1838)**

**Female:** Proboscis dark scaled, palps short, about 1/5 the length of the proboscis, concolourous. Pedicel blackish, flagellum of antenna about as long as proboscis. Occiput covered with narrow pale scales and pale forked erect scales, broader dark scale laterally. Integument of scutum brown, scutum with rather broad submedian stripes of coppery brown scales, remainder of scutum yellowish scaled. Scutal setae predominantly dark, lighter above wing root, scutellum with pale scales and setae. Pleurites and coxae with patches of whitish scales. Femora, tibiae and tarsomeres I predominantly dark scaled, speckled with pale scales, tarsomeres I sometimes with an indistinct basal ring, tarsomeres II–V entirely dark scaled. Wing veins clothed with pale and dark scales intermixed, halter with stem and knob blackish. Terga dark scaled, with a median longitudinal pale stripe which broadens toward the apical margins of each tergum, and basolateral spots of pale scales. Sterna almost entirely pale scaled.



**Fig. 14.21** Larva of *Oc. albifasciatus*

**Larva:** Head broader than long, antenna  $<1/2$  as long as the head, sparsely spiculate. Antennal seta (1-A) small, inserted beyond the middle of the antennal shaft. Prothoracic seta 1 (1-P) with 3 branches, 2-P small and single. The comb usually consists of  $>18$  short scales arranged in two irregular rows; individual comb scale with a prominent median spine and laterally fringed with small spines. Siphon short and stout, siphonal index about 2.0. The pecten with 9–13 teeth reaching to near the middle of siphon, the two distal-most teeth slightly detached and without lateral denticles (Fig. 14.21). The saddle covers about half of the anal segment, saddle seta (1-X) single. Upper anal seta (2-X) with multiple branches, lower anal seta (3-X) long and single.

**Biology:** The larvae can be found in temporary shallow ground pools of variable size, with or without vegetation, mainly in the flood plains of river systems or along lake shores. In the Andean valleys, breeding sites were encountered at an elevation of 2,300 m (Ludueña Almeida and Gorla 1995). Females deposit the eggs on muddy soils, the larval development may last less than 8 days, when the temperatures are appropriate. In the subtropical zones of its distribution range, adult activity and larval development may continue

throughout the year, in the more temperate areas *Oc. albifasciatus* is a typical multivoltine flood water mosquito and especially abundant after heavy rainfall. Females are persistent biters and readily attack humans and domestic animals, mainly at dusk. Besides being a serious pest to humans, the species may cause great economic loss in cattle farms.

**Distribution:** Brazil, Bolivia, Paraguay, Chile, Uruguay, Argentina, from tropical to temperate areas.

**Medical importance:** *Oc. albifasciatus* is a competent vector of the Western equine encephalitis virus (WEE) (Mitchell et al. 1987).

### ***Ochlerotatus (Ochlerotatus) scapularis* (Rondani 1848)**

**Female:** Small to medium sized species. Proboscis dark scaled, usually distinctly paler ventrally. Palps short, <1/5 the length of the proboscis, dark scaled. Pedicel brown, darker on inner surface. Occiput with broad median patch of narrow decumbent whitish scales, broad decumbent brown and whitish scales laterally. Erect forked scales numerous, extending over most of vertex and occiput, straw coloured. Integument of scutum dark brown, scutum with a large rounded spot of light scales from anterior promontory to scutal angle and posteriorly to near level of posterior end of paratergite, sometimes with posterior extensions along dorsocentral line to scutellum and surrounding prescutellar bare space, scales silvery white, often yellowish on lateral border. Posterior part of scutum predominantly with dark scales, acrostichal and anterior dorsocentral setae absent. Scutellum with pale scales on the lobes. Pleurites with patches of broad whitish scales. Scales on mesepisternum not reaching anterior angle, separate from prealar scale patch, upper part of mesepimeron with patch of scales. Lower mesepimeral setae absent. Fore and mid legs with conspicuous white stripes on posterior surfaces of femora and basal tarsomeres, tibiae dark, posterior surfaces pale. Hind leg with basal 2/3 of femur pale scaled and a conspicuous white stripe on anterior surface of tibia, occasionally nearly encircling tibia, and continued on tarsomeres I and II. Wing veins covered with narrow, dark scales, halter largely pale. Terga covered with dark scales, terga II–VII with large basolateral white patches and basomedian patches of pale scales,

more obvious posteriorly and often forming an indistinct longitudinal band. Sterna mostly pale scaled, small apicolateral patches of dark scales on distal segments.

**Larva:** Head distinctly wider than long. Antenna uniform in width, about half as long as the head, sparsely spiculate, antennal seta (1-A) small, with about 3 branches, inserted near middle of antennal shaft, not reaching its tip. Postclypeal seta (4-C) small, with 2–3 branches, inner and median frontal setae (5-C and 6-C) inserted far forward, single, outer frontal seta (7-C) with multiple branches, reaching beyond insertion point of antennal seta (1-A). Prothoracic setae 1 and 2 (1-P and 2-P) single, 3-P slightly shorter, with 1–2 branches. Lateral abdominal seta 6 on segments I and II (6-I and 6-II) usually with 2 branches, 6-III to 6-VI single. The comb consists of 20–27 scales arranged in irregular double to triple rows, individual scales evenly fringed apically. Siphonal index 2.0–2.6, pecten with about 11–18 evenly spaced teeth extending slightly beyond the middle of the siphon. Siphonal seta (1-S) multiple branched, much shorter than the width of the siphon at the point of its origin, inserted beyond distalmost pecten tooth. Anal segment completely ringed by the saddle, saddle seta (1-X) single, shorter than the saddle. Lower anal seta (3-X) long, single, upper anal seta (2-X) with multiple branches, less than half as long as 3-X. Ventral brush large, with about 8 pairs of cratal setae (4-X), precratal setae absent. Anal papillae usually more than twice as long as the saddle, pointed (Fig. 14.22).

**Biology:** *Oc. scapularis* breeds in a wide variety of temporary or semipermanent freshwater habitats, primarily temporary rain filled or stream overflow pools, but also in swamps and marshes in either sunlit or in partial shade (Carpenter and La Casse 1955; Belkin et al. 1970). The females attack humans readily, they are especially active during the afternoon and early evening, but will bite at any time when disturbed. They may cause a considerable nuisance problem and are able to disperse as far as 4 km from their breeding sites. In areas of prolonged association with humans, *Oc. scapularis* is adapting to human habitations and is becoming more concentrated around dwellings in rural and semirural areas, even entering buildings to bite (Arnell 1973).

**Distribution:** From the southwestern United States through Central and South America to Argentina, Greater Antilles, Trinidad.

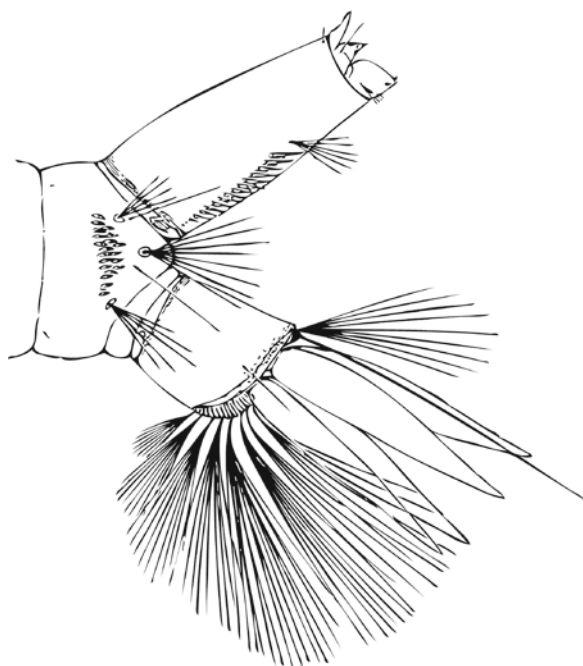


Fig. 14.22 Larva of *Oc. scapularis*

### ***Culex (Culex) nigripalpus* Theobald 1901**

**Female:** Medium sized species. Proboscis dark scaled, usually paler ventrally on basal half, palps short, <1/4 as long as proboscis, dark scaled. Occiput with narrow curved pale golden brown scales and dark erect forked scales dorsally, with a patch of broad whitish scales laterally. Integument of scutum brown, scutum covered with fine dark bronze brown scales. Scutellum with dark bronze brown scales and brown setae on all lobes. Postpronotum with narrow dark bronze scales in upper part, pleurites with few or no scales. Integument of mesepimeron lighter than remainder of pleurites, a few small broad pale scales on upper and lower part of mesepisternum and a few narrow scales on upper mesepimeron. Legs dark scaled, with metallic blue green reflection, posterior surface of femora and tibiae pale scaled. Wing scales narrow, all dark. Terga clothed with dark brown to black scales, often with metallic reflections. Narrow pale basal bands occasionally present on some segments, basolateral pale patches present on terga V–VII (Fig. 14.12a), sterna pale scaled.

**Larva:** Antenna shorter than the head, distinctly constricted beyond insertion point of antennal seta (1-A), the portion below the constriction pale and spicu-

late, the portion beyond the constriction darker and with few spicules. Antennal seta (1-A) large, multiple branched, inserted at apical 1/3 of antennal shaft, reaching well beyond the tip of the antenna, labral seta (1-C) rather stout, attenuate apically. Postclypeal seta (4-C) short, single, inner and median frontal setae (5-C and 6-C) usually with 3 branches, extending beyond anterior margin of head. Outer frontal seta (7-C) long, multiple branched. Thorax densely clothed with fine spicules. Prothoracic setae 1 to 3 (1-P to 3-P) long, single, 4-P long, usually with 2 branches, 5-P and 6-P long, single, 7-P long, with 2–4 branches. Abdominal setae 6-I and 6-II usually with 3 branches, 6-III and 6-VI slightly shorter, usually with 2 branches. The comb is composed of >30 scales arranged in a triangular patch, individual comb scale rounded apically and fringed with small spines. Siphon long and slender, siphonal index 6.0–8.0. The pecten consists of about 12 (9–15) teeth restricted to basal 1/4 of siphon, individual pecten tooth with 2–6 lateral denticles. Siphonal setae (1-S) with 4 pairs of tufts inserted beyond the pecten, basalmost tuft (1a-S) usually with 2 branches, occasionally single, as long as or longer than the basal width of the siphon, second and third tufts (1b-S and 1c-S) usually with 2–3 branches and inserted somewhat laterally, distalmost tuft (1d-S) small, with 1–3 branches (Fig. 14.23). Anal segment completely ringed by the saddle; saddle with numerous strong spicules on posterior margin. Saddle seta (1-X) usually single, sometimes with 2 branches, slightly shorter than the saddle. Upper anal seta (2-X) with 3 branches, 1 long and 2 short, lower anal seta (3-X) long and single. Ventral brush well developed, with about 6–7 pairs of cratal setae (4-X), precratal setae absent. Anal papillae variable in length, 1–3 times as long as the saddle, tapered apically.

**Biology:** The larvae of *Cx. nigripalpus* can be found in semipermanent or permanent habitats in ditches, grassy pools, and marshes. Occasionally they are found in leaf axils of plants or artificial containers (Carpenter and La Casse 1955). The larvae are able to tolerate a wide range of conditions in their breeding sites, such as salinity, sunlight, organic matter, etc. (Belkin et al. 1970). *Cx. nigripalpus* is generally regarded as an outdoor species, but where adults are numerous they readily enter houses. Both the adults and larvae may be found throughout the year in their southern distribution range (Carpenter and La Casse 1955). The females readily bite humans and are regarded as a major pest in the southern United States (Darsie and Day 2004).

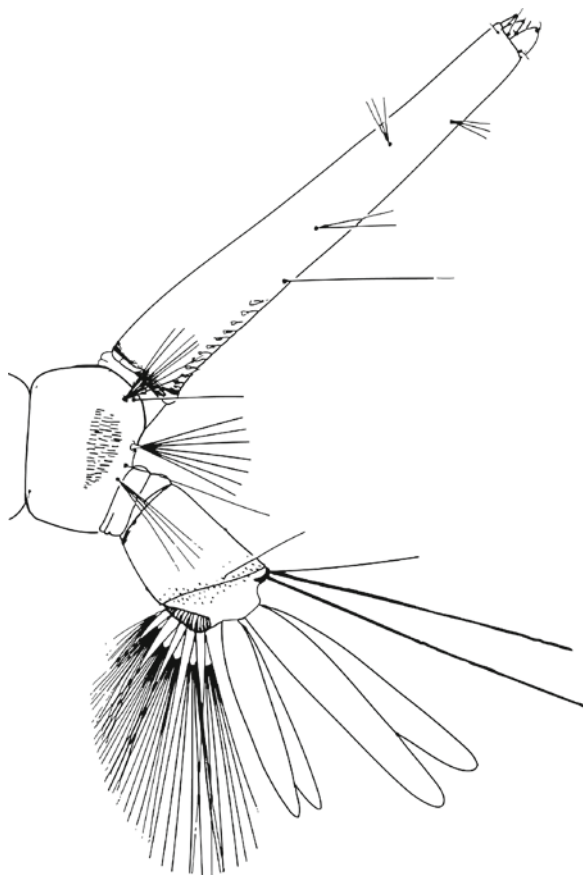


Fig. 14.23 Larva of *Cx. nigripalpus*

**Distribution:** Antilles, southern United States, Mexico, Central America, northern South America.

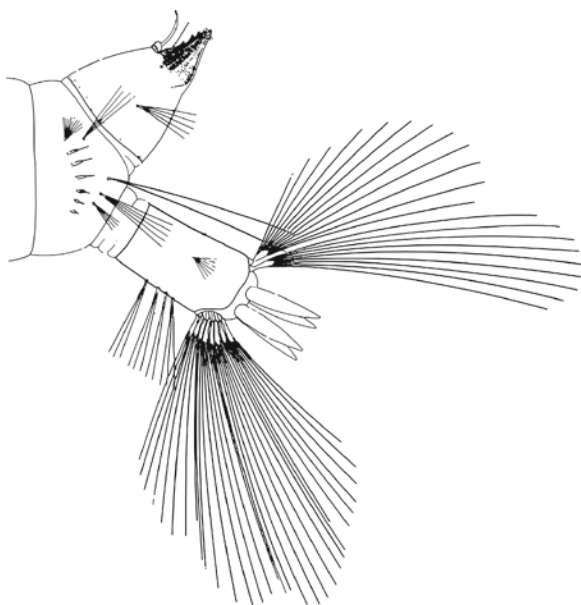
**Medical importance:** Principal vector of St. Louis encephalitis (SLE) and important vector of West Nile virus (WNV) in Florida (Darsie and Day 2004) and northeastern United States (Kilpatrick et al. 2005).

### ***Mansonia (Mansonia) titillans* (Walker 1848)**

**Female:** Medium sized brown to dark brown species moderately speckled with whitish scales on palps, wings, femora, tibiae, tarsomeres I and abdomen. Proboscis dark scaled, speckled with pale scales, and with a narrow white ring beyond the middle. Palps about 1/3 as long as the proboscis, predominantly dark scaled, with scattered white scales. Pedicel brown, darker on inner surface, a few whitish scales on dorsal and inner surfaces. Occiput with pale lanceolate scales

and numerous dark erect forked scales. Integument of scutum dark brown, scutum clothed with narrow curved dark brown scales intermixed with golden brown lanceolate scales, whitish to golden brown scales present, primarily on supraalar and prescutellar dorsocentral areas. Scutal setae very numerous. Scutellum with whitish to golden narrow scales and dark brown setae on all lobes. Scaling of pleurites sparse, small patches of whitish scales on upper part of mesepisternum and along its lower posterior margin, a few scales on upper part of mesepimeron, 3 or more lower mesepimeral setae present, upper mesepimeral setae numerous, postspiracular setae present. Femora and tibiae dark scaled, speckled with pale scales, posterior surface of mid and hind femora predominantly pale scaled, very small pale knee spots more or less distinct on all femora. Tarsomeres I–IV of fore and mid legs each with a narrow but conspicuous basal white ring, all tarsomeres of hind leg with white basal rings. Wing veins covered with very broad, strongly asymmetrical scales, predominantly dark but with scattered whitish scales throughout. Haltere stem pale, knob dark scaled. Tergum I with a small median patch of pale scales. Terga II–VII predominantly dark scaled, with more or less numerous pale scales laterally and with few to many yellow and whitish scales apically. Apex of tergum VII with a row of short stout pointed spines. Tergum VIII with many short stout spines in a curved posterior row and an irregular anterior row. Sterna II–VII with an irregular and variable mixture of dark and pale scales.

**Larva:** Head distinctly broader than long. Antenna about twice as long as the head, sparsely clothed with small but distinct spicules on basal half. Antennal seta (1-A) multiple branched, inserted at a notch on basal third of antennal shaft, a pair of long stout setae (2-A and 3-A) arising near the middle of the shaft and extending slightly beyond its tip. Labrum distinctly produced at base of labral seta (1-C). Postclypeal seta (4-C), inner and median frontal setae (5-C and 6-C), small, with multiple branches, outer frontal seta (7-C) moderately long with multiple branches. Prothoracic seta 1 (1-P) long, usually with 2–3 branches, 2-P short, single, 3-P short, with multiple branches. Abdominal setae 6 on segments I–VI (6-I to 6-VI) single. The comb consists of 6–8 scales arranged in a single row, the dorsal scales are long and slender, with a prominent median spine and fringed with small lateral spinules on basal part, ventrally the scales are much



**Fig. 14.24** Larva of *Ma. titillans*

smaller (Fig. 14.24). Siphon short and conical, heavily sclerotized beyond the middle, modified for piercing plant tissue. Siphonal seta (1-S) usually with 4–5 branches arising before the heavily sclerotized part, pecten absent. Anal segment longer than wide, completely encircled by the saddle; saddle seta (1-X) short, usually with 6 branches, inserted well before the posterior margin of the saddle. Upper and lower anal setae (2-X and 3-X) with multiple branches, 2-X slightly

shorter than 3-X. Ventral brush well developed, with about 4 pairs of cratal setae (4-X) and 4 precratal setae piercing the saddle. Anal papillae shorter than the saddle, and pointed.

**Biology:** Eggs are deposited as masses glued to the undersides of leaves of aquatic plants such as *Pistia sp.* (Horsfall 1955). After hatching, the larvae attach themselves to the submerged roots of aquatic plants from which they obtain oxygen. The pupae also remain attached to the roots of the plants until time for emergence of the adults. Water lettuce (*Pistia stratiotes*) and water hyacinth (*Eichhornia crassipes*) are believed to be the preferred host plants for the larvae and pupae of *Ma. titillans*, but some other host plants are also involved (Carpenter and La Casse 1955; Belkin et al. 1970). The females are fierce biters and readily attack humans and domestic animals, they are most active at dusk and again at dawn but will feed at any time after sundown. The females are known to fly several kilometers from swamps, marshes, ponds, and lakes where their immature stages develop. The species is often extremely abundant and constitute a serious pest problem to humans and livestock.

**Distribution:** Southern United States (Florida, Texas), Mexico, Central and South America, Antilles.

**Medical importance:** The Venezuelan equine encephalitis (VEE) virus has been isolated from wild caught *Ma. titillans*; the species is known to be a vector of filariasis (Carpenter and La Casse 1955).

# Chapter 15

## North America

Anyone who has identified a litre jar packed with mosquitoes collected in a CDC trap from North Dakota to find 10,000 *Ae. vexans*<sup>1</sup> for every one *Oc. melanimon*, knows that all mosquitoes are not created equal. The person collecting a hundred *Ae. albopictus*<sup>1</sup> for every one *Cx. p. quinquefasciatus*<sup>1</sup> in landing rate counts in Texas has an even deeper understanding of that fact (McKnight 2005).

It is not surprising that *Ae. vexans*<sup>1</sup> and *Ae. albopictus*<sup>1</sup> were the top two on a list of the 13 most voracious biters across the USA (McKnight 2005). Consequently, because of their major pest importance, they are included in the key but their description is given under Europe. *Cx. p. pipiens* biotype *molestus*<sup>1</sup> and *Cx. p. quinquefasciatus*<sup>1</sup> are also included in the keys being the top pest species and important vectors of West Nile virus.

Some other species also cause significant nuisance in a number of states of the USA (*Oc. dorsalis*<sup>1</sup>) and Canada: *Oc. hexodontus*<sup>1</sup> (being the main pest), *Ae. vexans*<sup>1</sup>, *Oc. communis*<sup>1</sup>, *Oc. dorsalis*<sup>1</sup>, *Oc. impiger*<sup>1</sup>, *Oc. nigripes*<sup>1</sup>, *Oc. punctor*<sup>1</sup>, *Oc. sollicitans*<sup>1</sup> and *Cq. perturbans*. Except

for the last species, they are described and keyed under the chapters on the mosquitoes of Europe.

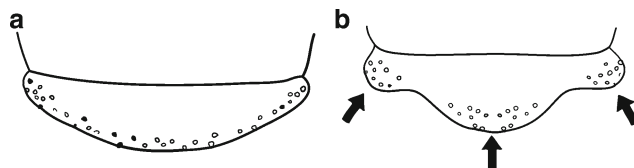
The second tier of mosquitoes causing nuisance with either confirmed or potential vector capability in the USA are, according to their ranking (McKnight 2005): *Oc. trivittatus*, *Oc. nigromaculis*, *Oc. canadensis canadensis*, *Cs. inornata*, *Oc. triseriatus*, *Cs. incidens*, *An. crucians*, *Cx. erythrothorax*, *Oc. sierrensis*, *An. freeborni*, *Cx. nigripalpus*, *An. punctipennis*, *Oc. melanimon*, *Oc. atlanticus*, *An. quadrimaculatus*, *Oc. stimulans*, *Oc. increpitus*, *Oc. sticticus*<sup>1</sup>, *Oc. japonicus*. Many are involved in virus transmission and some, such as *An. quadrimaculatus*, *An. freeborni*, and *An. pseudopunctipennis*, are potential vectors of malaria in North America.

For further reading on the morphology, identification, biology, distribution, and disease vector status of the species mentioned, the reader is referred to Carpenter and La Casse (1955), Mattingly (1971), Smith (1973), Wood et al. (1979), Darsie and Ward (1981, 2005), Rueda (2004).

### 15.1 Key to North American Female Mosquitoes

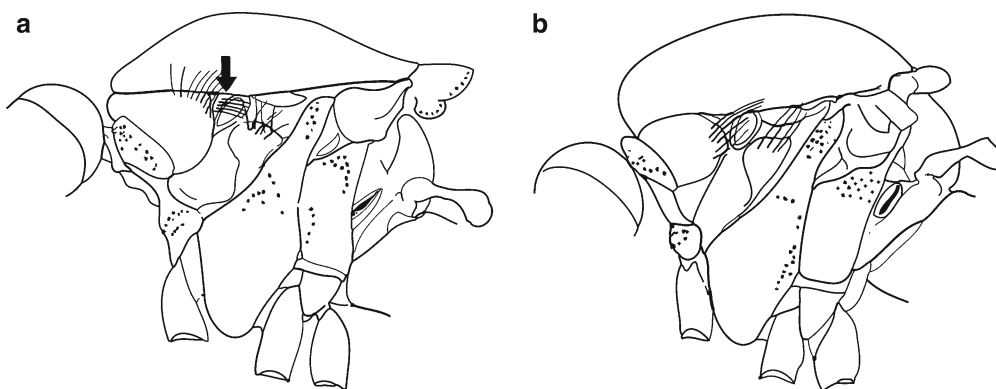
- 1 Palps about as long as proboscis. Scutellum evenly rounded or slightly trilobed and uniformly setose (Fig. 15.1a). Abdominal terga and sterna completely or largely missing scales. – Proboscis not tapering towards apex and not strongly recurved. .... *Anopheles*
- Palps distinctly shorter than proboscis. Scutellum trilobed, setae arranged in three sets (Fig. 15.1b). Abdominal terga and sterna uniformly and densely covered with scales. – Proboscis not tapering towards apex and not strongly recurved. Postnotum without a tuft of setae. Veins R<sub>2</sub> and R<sub>3</sub> at least as long as vein R<sub>2+3</sub>, usually much longer. Anal vein (A) ending well beyond the furcation of cubitus (Cu). Squama with fringe of setae. .... 2

<sup>1</sup> Species described in Chap. 10 concerning European mosquitoes.



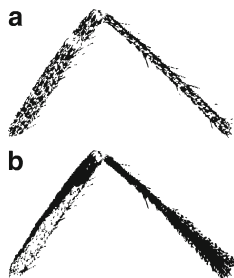
**Fig. 15.1** Scutellum of: (a) *Anopheles* sp.; (b) *Aedes* sp.

- 2 (1) Prespiracular setae present (Fig. 15.2a). – Postspiracular setae present. Abdomen tapering apically, cerci long, easily visible. *Psorophora*..... 3  
 Prespiracular setae absent (Fig. 15.2b). – Lobes of antepronotum are small and broadly separated, commencing beyond anterior margin of scutum. Scutum covered with narrow scales without metallic shine. Tarsomere I of fore legs usually shorter than tarsomeres II–V together. Tarsomere IV of fore legs not reduced, distinctly more long than broad..... 4

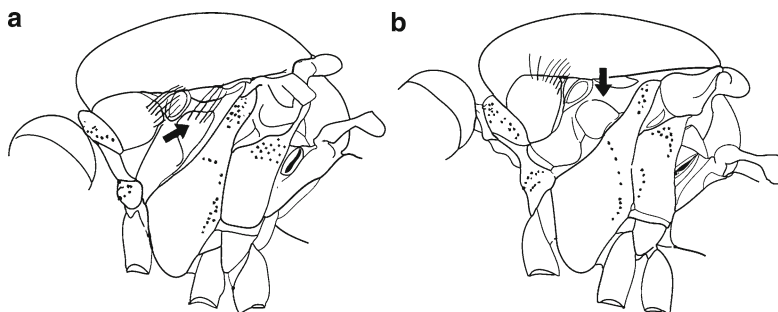


**Fig. 15.2** Lateral view of thorax of: (a) *Psorophora* sp.; (b) *Ochlerotatus* sp.

- 3 (2) Femora usually with a narrow subapical ring of pale scales (Fig. 15.3a). Wing veins with pale and dark scales. – Tarsomere I of hind legs with basal and median pale rings. Pale and dark scales on wing veins randomly mixed (not grouped). ..... *Ps. columbiae* (p 394)  
 Femora without subapical ring of pale scales (Fig. 15.3b). Wing veins uniformly dark scaled or if a few pale scales are present, they are restricted to the anterior border (veins C and Sc). – Scutum with dark brown and golden yellow scales mixed, without forming specific pattern. Apices of femora without erect scales. Abdominal terga with apicolateral patches of pale scales. Tarsomeres IV and V and often apex of tarsomere III of hind tarsus pale scaled. .... *Ps. ferox* (p 395)



**Fig. 15.3** Hind leg of: (a) *Ps. columbiae*;  
 (b) *Ps. ferox*



**Fig. 15.4** Lateral view of thorax of: (a) *Ochlerotatus* sp.; (b) *Culex* sp.

- 4 (2) Postspiracular setae present (Fig. 15.4a) and/or claws of fore legs with subbasal tooth. – Wing scales usually narrow, if broad then not conspicuously asymmetrical. (for Canadian nuisance species see key for Europe)..... 5  
 Postspiracular setae absent (Fig. 15.4b). All tarsal claws simple. .... 9
- 5 (4) Proboscis without a pale ring (Fig. 15.5a). .... 6  
 Proboscis with distinct broad median ring of pale scales (Fig. 15.5b). Decumbent scales of vertex largely narrow and/or (if scales of vertex are largely broad) erect scales numerous, often not restricted to occiput. *Ochlerotatus* ..... 8

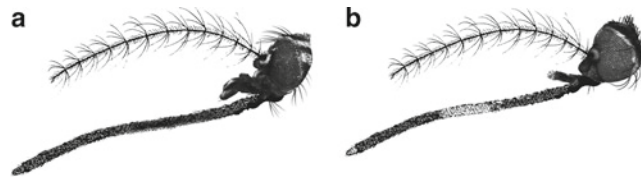


Fig. 15.5 Proboscis of: (a) *Ae. aegypti*; (b) *Oc. sollicitans*

- 6 (5) Proboscis as long as fore femur or slightly shorter (Fig. 15.6a). Erect scales not numerous, restricted to occiput. Scutellum with broad, white and straight scales. – Scutum with white or silvery scales in stripes or patches. Femora with white knee spot. *Aedes* subgenus (*Stegomyia*) ..... 7  
 Proboscis distinctly longer than fore femur (Fig. 15.6b). Erect scales numerous, not restricted to occiput. Scutellum with narrow, yellowish or pale and curved scales. – Basal pale rings on tarsi very narrow, usually not exceeding more than 1/4 of the length of the tarsomeres (pale rings better visible against a dark background and with a blue light filter). Terga with white transverse basal bands constricted in the middle giving them a bilobed pattern. .... *Ae. vexans* (p 194)

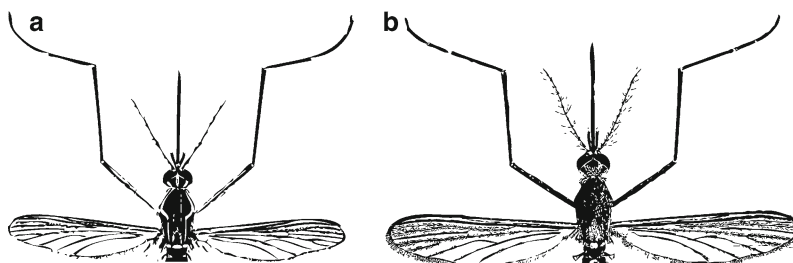


Fig. 15.6 Head and thorax of: (a) *Ae. aegypti*; (b) *Ae. vexans*

- 7 (6) Scutum with a white acrostichal stripe extending from the anterior margin to the beginning of the prescutellar area, where it forks to end at the anterior margin of scutellum (Fig. 15.7a). *Ae. albopictus* (p 201)  
 Scutum without an acrostichal stripe on anterior part but with two narrow white dorsocentral stripes separated from anterior margin. Lateral white stripes broad, continuing over transverse suture to the end of scutum, lyre shaped (Fig. 15.7b)..... *Ae. aegypti* (p 198)



- 8 (5) Wing veins with pale and dark scales. Terga with a median longitudinal pale stripe (sometimes disconnected) (Fig. 15.8a). – Hypostigmal scales present. Tarsomere I of hind tarsi with conspicuous pale basal and median rings. .... *Oc. sollicitans* (p 391)  
 Wing veins dark scaled, few pale scales present at the base of costa (C). Terga without a median longitudinal pale stripe, pale scales form relatively narrow basal transverse bands (Fig. 15.8b).  
 ..... *Oc. taeniorhynchus* (p 393)

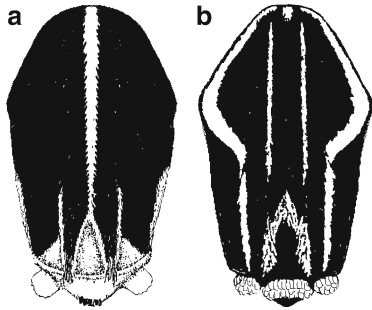


Fig. 15.7 Scutum of: (a) *Ae. albopictus*; (b) *Ae. aegypti*

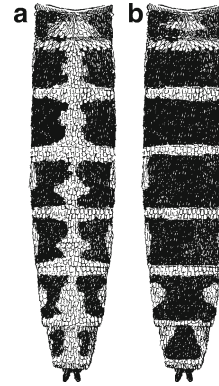


Fig. 15.8 Abdomen of: (a) *Oc. sollicitans*; (b) *Oc. taeniorhynchus*

- 9 (4) Hind tarsal claws very small and inconspicuous. All tarsi with well developed pulvilli. Wing scales usually narrow (Fig. 15.9a). – Antennae not longer than proboscis. Flagellomere I and II of similar length. Vertex and occiput with narrow decumbent scales. Scutum with acrostichal setae. Pale scales on abdominal terga grouped mainly on basal part of terga forming bands and/or lateral patches. *Culex* subgenus (*Culex*)..... 10  
 Hind tarsal claws not very small. Pulvilli absent. Most of wing scales broad and conspicuous (Fig. 15.9b).  
 – Tarsi ringed. Tarsomere I of hind tarsus with a median pale ring. .... *Cq. perturbans* (p 400)

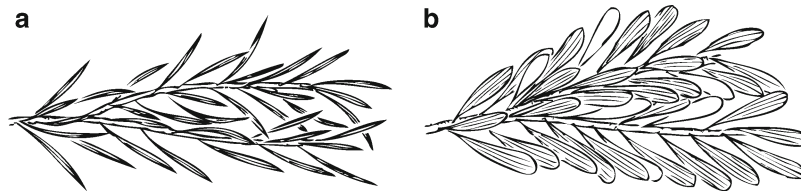
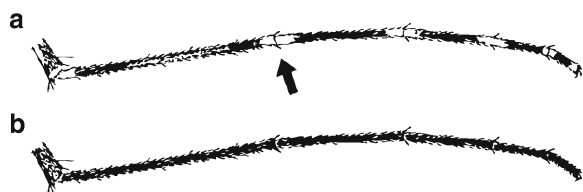
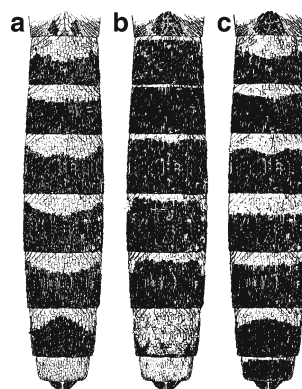


Fig. 15.9 Wing scales of: (a) *Culex* sp.; (b) *Coquillettidia* sp.

- 10 (9) Tarsomeres with distinct basal and apical pale rings, particularly on hind legs (Fig. 15.10a). – Proboscis with a pale ring near the middle. Anterior surface of femora and tibiae with a longitudinal stripe of pale scales or rows of pale patches. Hind tarsi with rather broad basal and apical pale rings. A V-shaped dark marking on all abdominal sterna. .... *Cx. tarsalis* (p 398)  
 Tarsomeres usually without pale rings (Fig. 15.10b). When pale rings are present, they are indistinct, narrow and basal. – Integument of scutum, pleurites, and coxae light brown, brown or dark brown but never reddish brown. .... 11



**Fig. 15.10** Hind tarsus of: (a) *Cx. tarsalis*; (b) *Cx. p. pipiens*



**Fig. 15.11** Abdomen of: (a) *Cx. p. quinquefasciatus*; (b) *Cx. salinarius*; (c) *Cx. restuans*

- 11 (10) Abdominal terga with rather broad (about 1/4 of the terga) basal bands of pale scales (Fig. 15.11a)..... 12  
 Pale basal bands on abdominal terga usually narrow or pale scaling restricted to lateral patches (Fig. 15.11b). – Pleurites with several patches of broad pale scales, each patch usually comprised of 6 or more scales. Terga VII and VIII almost completely covered with yellowish scales.....*Cx. salinarius* (p 397)
- 12 (11) Pale basal transverse bands rounded on posterior margin and constricted sublaterally, rather narrowly joining (sometimes disconnected) lateral pale patches, tergum VIII predominantly pale scaled (Fig. 15.11a). Scutum without spots of pale scales.....  
 ..... *Cx. p. pipiens biotype molestus* and *Cx. p. quinquefasciatus* (p 277, 278)  
 Pale basal transverse bands with nearly straight posterior margin and broadly joining the lateral patches (particularly on terga III–V), tergum VIII predominantly dark scaled (Fig. 15.11c). Scutum usually with submedian spots of pale scales close behind scutal angles.....*Cx. restuans* (p 396)

## 15.2 Species Description

### ***Ochlerotatus (Ochlerotatus) sollicitans* (Walker 1856)**

**Female:** A medium sized dark brown species with ringed proboscis, speckled wings and conspicuously pale ringed tarsi. Proboscis dark scaled, with a white ring near the middle (Fig. 15.5b). Palps dark, more than 1/4 as long as the proboscis with a few pale scales at the tip. Pedicel brown, with pale scales on inner and dorsal surfaces, clypeus prominent, dark brown, without scales. Occiput with a broad median patch of narrow golden yellow scales, submedian patches of narrow, dark, bronze brown scales and

broad, flat, pale yellowish scales, and small lateral patches of dark scales. Erect forked scales on central part of occiput pale, those on lateral region dark, a dense tuft of pale setae projecting between the eyes. Integument of scutum black, scutum densely covered with narrow, golden brown scales dorsally, and dark bronze brown scales laterally, anterior margin and prescutellar area with somewhat paler scales, often a pair of narrow, rather indefinite, submedian stripes of pale yellow to golden yellow scales extending nearly the full length of scutum. Scutal setae dark in anterior part, golden in posterior part. Scutellum with golden scales and darker setae on the lobes. Paratergite with pale scales. Integument of pleurites and coxae brown, pleurites with several patches of broad pale scales. Scale

patch on mesepisternum reaching to anterior angle, not distinctly separated from prealar patch. Mesepimeron bare on lower portion. 0–1 lower mesepimeral setae present. Femora and tibiae extensively speckled with pale scales, posterior surface pale, knee spots white. Hind leg with tarsomere I ringed with pale scales at base and a yellowish ring at the middle, tarsomeres II–IV with broad, pale basal rings, tarsomere V entirely white scaled. Fore and mid tarsi similarly marked but with rings narrower on tarsomeres I–III, absent on tarsomere IV. Tarsomere V of front tarsus varies from entirely dark to nearly all white, tarsomere V of mid tarsus mostly white, with some scattered dark scales. Wing veins covered with broad, dark and pale scales evenly intermixed, dark scales predominating on costal edge. Haltere entirely pale scaled. Tergum I with a median patch of yellowish white scales, terga II–VII with a median longitudinal pale stripe and narrow basal transverse pale bands and small lateral pale patches (Fig. 15.8a). Sterna all predominantly pale scaled, some dark scales on distal segments.

**Larva:** Head broader than long, antenna about half as long as the head, sparsely clothed with spicules, antennal seta (1-A) a multiple tuft, inserted near the middle of the antennal shaft. Postclypeal seta (4-C) small, 2–3 branched, inner and median frontal setae (5-C and 6-C) long, usually single, outer frontal seta (7-C) with 6–10 branches. Prothoracic seta 1 (1-P) long, single, 2-P about half as long as 1-P, single, 3-P short, usually with 1–2 branches. The comb consists of 11 to 21 comb scales, arranged in an irregular patch, individual comb scale with a long median spine and fringed with small lateral spines. Siphon stout, siphonal index 2.0–2.5, tracheal trunks broad. The pecten is composed of about 15–27 evenly spaced teeth reaching slightly beyond the middle of the siphon, occasionally 1–2 apical teeth are slightly detached, individual pecten tooth with 1–3 lateral denticles. Siphonal tuft (1-S) with 4–8 branches, inserted beyond the pecten, about as long as the apical width of the siphon. Anal segment completely ringed by the saddle, the saddle without distinct spicules at its posterior margin (Fig. 15.12). Saddle seta (1-X) single, shorter than the saddle. Upper anal seta (2-X) multiple branched, lower anal seta (3-X) long and single. The ventral brush is large with 7–8 pairs of cratal setae (4-X), precratal setae absent. Anal papillae variable in length, usually short, but may be as long or longer than the saddle.

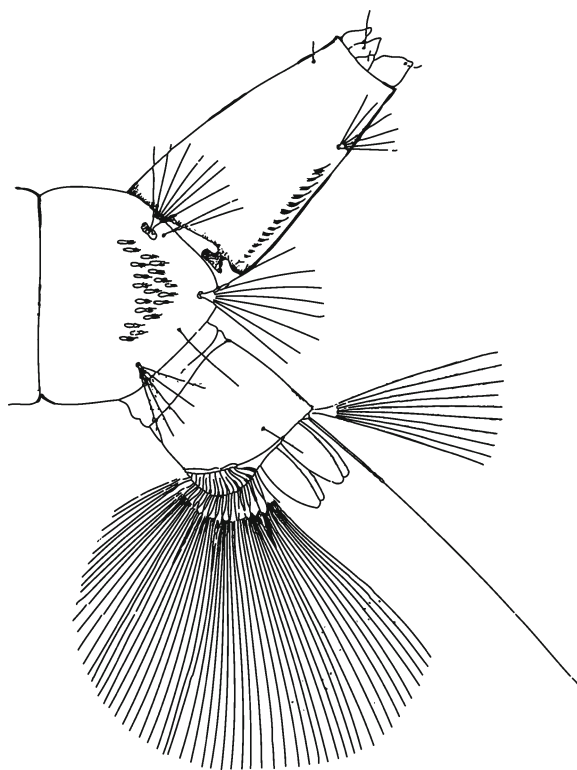


Fig. 15.12 Larva of *Oc. sollicitans*

**Biology:** The larvae of *Oc. sollicitans* occur mostly in salt marshes and mangrove swamps in coastal areas, they may breed even in footprints in salt marshes and coral rockholes (Belkin et al. 1970). The species has been found also in brackish water swamps in many of the inland states in the US (Carpenter and La Casse 1955). *Oc. sollicitans* is multivoltine, overwintering in the egg stage. The eggs are laid on the moist mud of salt marsh pools, they are able to hatch within minutes after being flooded, and the development of the immature stages may be complete within a week after hatching (Wood et al. 1979). The larvae and adults are most abundant from April or May to October in the marshes along the northern Atlantic coast. The adults are strong fliers and often migrate in large numbers to communities many km from the salt water marshes in which they breed. The females are persistent and troublesome biters, attacking humans at any time during the day or night, even in strong wind and may cause a great nuisance. The adults rest in the vegetation during the daytime and will attack anyone invading their resting sites, even in full sunlight.

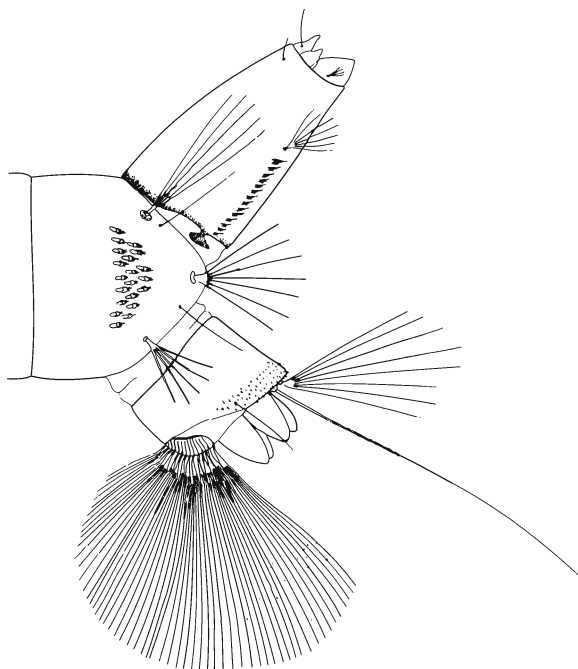
**Distribution:** Nearctic Region (eastern coastal and inland saline areas), Greater Antilles.

***Ochlerotatus (Ochlerotatus) taeniorhynchus*  
(Wiedemann 1821)**

**Female:** A medium sized to rather small, dark species with ringed tarsi and banded abdomen. Proboscis dark scaled, with a conspicuous white ring near the middle, palps short, about 1/5 the length of the proboscis, dark with white scales at the tips. Pedicel brown, with a patch of silvery scales on inner side. Occiput with a median patch of pale to golden brown lanceolate scales, a large patch of broad decumbent white scales laterally, the eye margins narrowly white scaled. Broad decumbent scales predominantly whitish, a few dark scales dorsally and in a lateral patch. Erect scales very numerous, usually all dark, sometimes pale on central part, setae dark brown, dense on vertex and projecting between eyes. Integument of scutum dark brown, scutum clothed with narrow dark golden brown scales becoming pale yellowish on the anterior margin, the prescutellar space and above the wing roots. Paratergite with small broad whitish scales, scutal setae short and dark. Scutellum with pale yellowish scales and brown setae on all lobes. Pleurites dark brown, with patches of broad flat whitish scales, present on propleuron, postspiracular and subspiracular areas. Patch on mesepisternum extending about halfway to anterior angle, separate from prealar patch. Mesepimeron with a patch of scales on upper half, lower half bare. Sometimes one lower mesepimeral seta present. Femora and tibiae dark scaled, pale on posterior surface, knee spots white. Fore and mid tarsi dark, with narrow basal pale rings on tarsomeres I–III, on tarsomeres IV and V the rings are reduced or absent. Hind tarsi dark, tarsomeres I–IV each with a broad basal white ring, tarsomere V usually entirely pale. Claws of fore and mid leg all with subbasal tooth, hind claws simple. Wing scales all narrow and dark except for a few pale scales at base of costa (C), haltere pale scaled. Tergum I with a median patch of dark scales, a few white scales often intermixed. Terga II–VI dark scaled with narrow pale basal bands and conspicuous pale lateral patches, the two patches on terga VI and VII are visible from a dorsal view, apices of terga VI and VII with a narrow row of pale scales, cerci slender, black (Fig. 15.8b).

**Larva:** Head broader than long, antenna less than half as long as the head, slightly tapering towards apex, spicules very small and sparse. Antennal seta 1

(1-A) small, with 2–3 branches inserted slightly before the middle of the antennal shaft not reaching the tip. Postclypeal seta (4-C) small, 2–3 branched, inner and median frontal setae (5-C and 6-C) long, single, outer frontal seta (7-C) short, multiple branched. Prothoracic seta 1 (1-P) long, single, 2-P short, single, 3-P short, usually with 2 branches. The comb consists of about 9 to 20 scales arranged in an irregular patch, individual scales small, apically rounded, prominent median spine absent. Siphon stout, siphonal index 1.5–2.0, tracheal trunks broad. The pecten is composed of 11–17 short evenly spaced teeth, reaching the middle of the siphon or slightly beyond. Siphonal seta (1-S) multiple branched, inserted beyond the pecten, shorter than the width of the siphon at the point of its insertion (Fig. 15.13). Anal segment completely ringed by the saddle, saddle with numerous spicules at its posterior margin. Saddle seta (1-X) single, shorter than the saddle. Upper anal seta (2-X) multiple branched, lower anal seta (3-X) long and single. Ventral brush well developed, usually with 8 pairs of cratal setae (4-X), precratal setae absent. Anal papillae usually much shorter than the saddle.



**Fig. 15.13** Larva of *Oc. taeniorhynchus*

**Biology:** The natural breeding sites of *Oc. taeniorhynchus* are temporary pools in mangroves and grass salt marshes in coastal areas, these breeding sites are influenced by tides and rainfall. Larvae have also been found in inland brackish water swamps, in areas far removed from the coast (Carpenter and La Casse 1955). Occasionally, they breed in fresh water in temporary ground pools and in irrigation and stream overflows in the coastal lowlands (Belkin et al. 1970). The species reaches its greatest abundance along the coastal regions of the southern United States and the Caribbean region, usually following high tides or a combination of high tides and heavy rains during the summer and early fall. The females are severe biters and will attack at any time during the day when they are disturbed. In late afternoon and particularly at dusk and after, they actively search for blood and will attack humans and livestock outdoors and will also invade dwellings (Belkin et al. 1970). As with *Oc. sollicitans*, they are strong fliers and often migrate in large numbers many km away from their breeding grounds. The males begin to swarm at twilight over the top of bushes or small trees, these swarms usually last not longer than 30 min (Apperson 1991). In the warmer parts of continental US, *Oc. taeniorhynchus* is the common coastal species and sometimes appears in enormous numbers when the conditions are favourable (Howard et al. 1917).

**Distribution:** American coasts and inland saline areas, Massachusetts to Brazil and California to Peru, Antilles, Galapagos Islands.

**Medical importance:** *Oc. taeniorhynchus* is a natural vector of dog heartworm (*Dirofilaria immitis*) and Venezuelan equine encephalitis (VEE) (Apperson 1991).

### ***Psorophora (Grabhamia) columbiae* (Dyar and Knab 1906)**

**Female:** Medium sized to rather large species. Proboscis dark scaled at base and apex, with a large, median ring of yellowish white scales. Palps short, about 1/4 as long as proboscis, dark scaled with pale tip. Pedicel dark brown, with a patch of pale scales on inner surface. Occiput clothed dorsally with narrow curved white to pale violet scales and numerous black erect forked scales, a dorsolateral patch of broad flat dark scales followed by broad whitish to light brown scales laterally. Setae along margins of eyes numerous, black. Integument of scutum blackish brown, scutum covered with fine narrow bronze brown to blackish scales, and narrow pale scales along the edge of

scutum, on the prescutellar area, a patch above wing base, and a small submedian patch near middle of scutum. Scutellum with long narrow whitish scales and dark setae on the lobes. Pleurites and coxae blackish brown, with patches of broad whitish scales. Mesepisternum with few scales reaching the anterior angle, prealar patch present. Mesepimeron with scales on upper portion not reaching the lower margin. Femora dark brown scaled, with white scales intermixed, posterior surfaces largely pale scaled, each femur with a narrow subapical pale ring, knee spots white (Fig. 15.3a). Tibiae dark scaled with numerous small white spots on dorsal surfaces. Hind tarsi with basal white rings on all tarsomeres, tarsomere I with a median white ring as well. Fore and mid tarsi similarly marked but with white rings reduced or lacking on tarsomere IV, absent on tarsomere V. Tarsal claws all simple, without subbasal tooth. Wing scales rather broadly triangular, dark brown and pale intermixed, the dark ones predominating, wing fringe entirely dark scaled. Tergum I with a median patch of pale scales, terga II and III with white to pale yellowish triangular scale patch apically, divided into paired submedian patches on terga IV–VII. Sterna with dark and pale scales intermixed, the pale ones predominating, the black ones tending to form median apical spots on distal terga.

**Larva:** Antenna shorter than the head, slightly tapering towards apex, sparsely covered with spicules. Antennal seta (1-A) long, multiple branched, inserted near middle of antennal shaft. Postclypeal seta (4-C) small, multiple branched, all frontal setae (5-C to 7-C) with more than 4 branches, their insertion points more or less in a straight line. The comb consists of 5–6 scales located on the posterior margin of a weakly sclerotized plate, individual comb scale thorn shaped, with the larger basal spines about 1/3 as long as the prominent median spine. Siphon moderately widened in the middle and tapering towards the apex, siphonal index about 3.0, tracheal trunks broad. The pecten is composed of 3–6 widely spaced teeth, not reaching the middle of the siphon, individual pecten tooth with 1 lateral denticle. Siphonal tuft (1-S) about as long as apical width of siphon, multiple branched, inserted at distal third of siphon (Fig. 15.14). Anal segment longer than wide, completely ringed by the saddle, saddle seta (1-X) small, with 2–3 branches. Upper anal seta (2-X) short, multiple branched, lower anal seta (3-X) long, single. Ventral brush well developed, extending along the ventral line almost to the base of the anal segment. Anal papillae long, about 2–3 times as long as the saddle, evenly tapering and pointed.

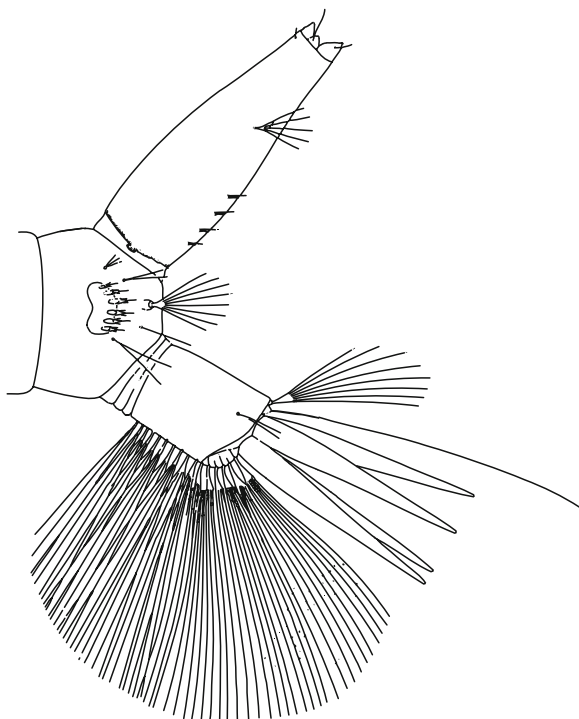


Fig. 15.14 Larva of *Ps. columbiae*

**Biology:** The females lay their eggs on damp soil, in depressions subject to flooding by rainfall or overflow from streams and irrigation canals (Carpenter and La Casse 1955). Areas covered by rice provide good oviposition sites for *Ps. columbiae*, whereas areas bare of vegetation are of lesser importance (Horsfall 1942). Larvae can also be found in all sorts of ground puddles but principally in open ones, often without vegetation and muddy and in ditches along roads. The overwintering eggs hatch in early summer, immediately after they have become flooded. There may be several generations per year (Wood et al. 1979). The larval development is relatively short, usually requiring 4 to 10 days. The females are persistent biters, attacking humans and domestic animals at any time during the day or night. In the southern United States, the species can be an abundant and troublesome pest, especially in rice fields (Horsfall 1942). Because of its abundance in rice fields, *Ps. columbiae* is commonly referred to as the dark rice field mosquito.

**Distribution:** United States, Canada, Grand Cayman Islands, Mexico.

***Psorophora (Janthinosoma) ferox*  
(von Humboldt 1819)**

**Female:** Small to medium sized, dark species, dark scales predominantly metallic with purplish iridescence. Scales on scutum mixed dark and golden, without definite pattern, hind tarsus with tarsomeres IV and V completely white scaled. Proboscis slightly longer than fore femur, palps about 1/4 as long as proboscis, with dark scales of metallic shine. Antenna slightly shorter than proboscis, pedicel light to dark brown, mesal surface with a few short dark setae but no scales. Integument of head dark brown, shining. Occiput covered dorsally with broad curved whitish yellow to golden yellow scales, paler anteriorly, with broad decumbent yellowish scales laterally. Erect forked scales yellowish, numerous on posterior half of occiput. Integument of scutum dark brown to black, shining. Scutum sparsely clothed with rather broad dark brown and golden yellowish scales in no definite pattern, the dark scales more abundant in the median area. Scutal setae brown, paratergite bare. Scutellum with rather broad yellowish white scales and dark brown setae on the lobes. Pleurites densely clothed with greyish white scales. Hypostigmal, subspiracular and postspiracular patches present. Scales on mesepisternum reaching lower margin and anterior angle, mesepimeral patch reaching near lower margin. Coxae with patches of silvery white scales, femora dark with deep violet shine, pale on posterior surface, knee spots poorly developed. Tibiae and tarsi of fore and mid legs dark violet, tarsomeres IV and V of hind tarsus and often the apex of tarsomere III are white scaled. Apical part of hind tibia and tarsomeres I and II of hind tarsus with long and erect scales, appearing rather shaggy and with purple reflection (Fig. 15.3b). All claws with pronounced subbasal tooth, those of hind legs smaller. Wing scales dark violet, narrow. Haltere stem pale, knob dark scaled. Tergum I with a median patch of dark violet scales, terga II–VI predominantly dark scaled with violet reflection, with apicolateral triangular patches of yellowish golden scales. Sterna II–VI predominantly yellowish, with scattered violet scales not forming bands, sternum VII mainly dark violet scaled.

**Larva:** Head distinctly broader than long. Antenna much longer than the head, clothed with conspicuous sharp spicules, antennal seta 1 (1-A) long, multiple branched, inserted near middle of antennal shaft. Labral seta (1-C) long, slightly curved, postclypeal

seta (4-C) small, with 2–3 branches. Inner and median frontal setae (5-C and 6-C) equal in length, usually with 2 branches, 6-C occasionally with 3 branches, outer frontal seta (7-C) multiple branched, insertion points of 5-C to 7-C nearly in a straight line. The comb is composed of 6–8 scales in a curved row on the posterior margin of a weakly sclerotized plate, individual comb scale thorn shaped, the larger basal spines about 1/3 as long as the prominent median spine. Siphon moderately to strongly widened in the middle and tapering towards the apex, siphonal index about 4.0, acus distinct. Pecten with 3–5 widely spaced teeth on basal 1/4 of siphon, individual pecten tooth with 1–3 lateral denticles. Siphonal tuft (1-S) minute, much shorter than apical width of siphon, multiple branched and inserted laterally beyond middle of siphon, sometimes difficult to detect (Fig. 15.15). Anal segment longer than wide, completely ringed by the saddle, saddle seta (1-X) short and inconspicuous, usually multiple branched. Upper anal seta (2-X) short, multiple branched, lower anal seta (3-X) long, single. Ventral brush usually with 8 cratal setae (4-X) on a very poorly developed grid, and about 10–12 precratal setae extending almost the entire length of the anal segment and piercing the saddle. Anal papillae much longer than the saddle, slender and gradually tapering to a sharp point.

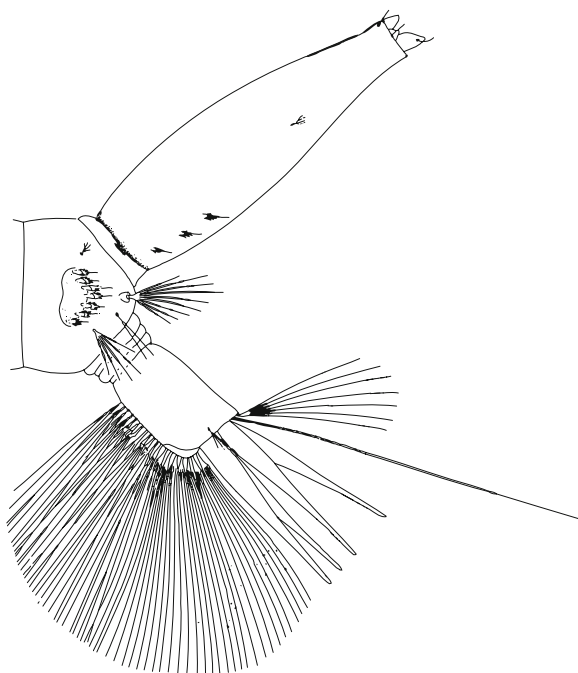


Fig. 15.15 Larva of *Ps. ferox*

**Biology:** The larvae of *Ps. ferox* can be found in temporary rain filled pools, particularly in or near forested areas, in overflow pools along streams, and occasionally in potholes in stream beds after summer rains, usually in a shaded situation. The development of the immature stages is very fast, and they occur from March to November in the southern United States and from early May to September in the north (Carpenter and La Casse 1955). Larvae and pupae are easily alarmed and stay for long periods at the bottom of their breeding sites, where they are difficult to see among the numerous dead leaves and other organic debris (Belkin et al. 1970). The females are persistent and painful biters feeding during the day in the shade of bushes or wooded areas and even attacking in the open on cloudy days. Their main activity is after dark and they prefer to remain within forested areas (Wood et al. 1979).

**Distribution:** Southeastern Canada, eastern United States, South and Central America, Greater and Lesser Antilles

### *Culex (Culex) restuans* Theobald 1901

**Female:** Medium sized species. Proboscis dark scaled with some pale scales on ventral surface, palps short, less than 1/5 as long as proboscis, dark scaled. Occiput clothed with narrow curved yellowish scales and dark brown erect scales dorsally, with broad yellowish scales laterally. Integument of scutum light brown, scutum clothed with fine narrow curved golden brown scales, paler on anterior and lateral margins and on the prescutellar dorso-central area. A pair of distinct pale scaled submedian spots usually present near middle of scutum. Scutellum with narrow pale golden scales and brown setae on the lobes. Pleurites with small patches of broad pale scales on upper parts of mesepisternum and mesepimeron. Legs dark scaled with metallic blue-green reflection. Femora and tibiae with pale scales on posterior surfaces and apices, tarsi entirely dark scaled or with indistinct pale basal rings. Wings entirely clothed with narrow dark scales. Tergum I with a median patch of dark bronze brown scales, terga II–VII dark brown scaled, each with a more or less straight basal band of yellowish scales, usually broadly joined with basolateral patches of pale scales (Fig. 15.11c). Sterna mostly pale scaled.

**Larva:** Antenna shorter than the head, evenly tapering towards the apex, spiculate. Antennal seta (1-A)

multiple branched, inserted near middle of antennal shaft, not reaching to its tip. Postclypeal seta (4-C) short, with 2–3 branches, inner and median frontal setae (5-C and 6-C) with 4–8 branches, outer frontal seta (7-C) multiple branched. Prothoracic setae 1 and 2 (1-P and 2-P) long, single, 3-P long, with 1–2 branches. Lateral abdominal seta 6 on segments I and II (6-I and 6-II) with 2 branches, 6-III to 6-VI long and single. The comb consists of 30–40 scales arranged in an irregular triangular patch, individual comb scale rounded apically and fringed with small spines. Siphon slightly widened near middle and tapering towards the apex, siphonal index 4.0–5.0. The pecten is composed of about 12 to 20 teeth on the basal 1/3 of the siphon, individual pecten tooth with 1–4 lateral denticles. The siphonal setae (1-S) consist of 3 pairs of long single setae, unequal in length and irregularly placed on the siphon and a pair of small sub-apical setae with 2–3 branches, all setae inserted beyond the pecten (Fig. 15.16). Anal segment completely encircled by the saddle, saddle with spicules on its posterior margin, saddle seta (1-X) with 1–2 branches, slightly shorter than the saddle. Upper and lower anal setae (2-X and 3-X) single, 2-X almost as long as 3-X. Ventral brush well developed, confined to the grid, precratal setae (4-X) absent. Anal papillae about 2–3 times as long as the saddle, tapered.

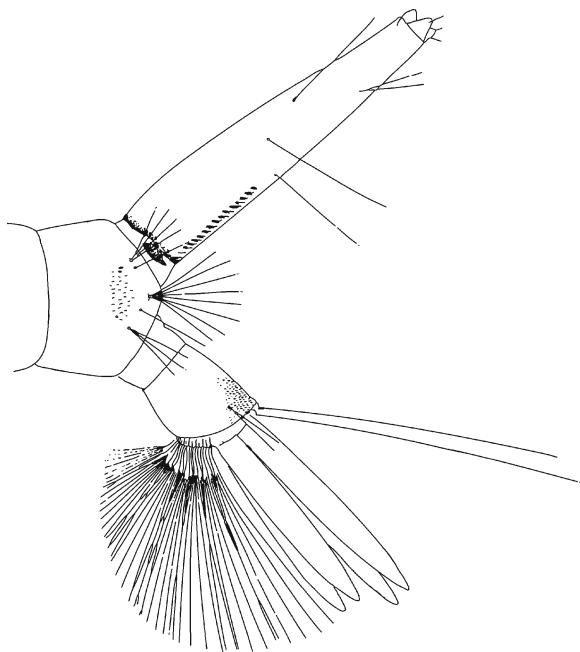


Fig. 15.16 Larva of *Cx. restuans*

**Biology:** The larvae of *Cx. restuans* can be found in a wide variety of aquatic habitats, such as ditches, temporary puddles with decaying vegetation, pools in streams, rock and woodland pools, and in various artificial containers (Carpenter and La Casse 1955). As with *Cx. pipiens*, the females of *Cx. restuans* hibernate in basements, caves, and hollow trees (Wood et al. 1979). The population reaches its peak in the spring and early summer throughout most of its range, and occurs in lesser numbers during late summer and autumn. The females prefer to feed on birds, but mammals are also readily attacked, they are regarded as painful biters. They feed on humans outdoors at dusk and during the day in shaded areas (Breeland et al. 1961).

**Distribution:** United States, southern Canada, Mexico.

**Medical importance:** Viruses of Eastern and Western equine encephalitis (WEE and EEE) were isolated from wild caught *Cx. restuans* females (Carpenter and La Casse 1955). According to Kilpatrick et al. (2005) *Cx. restuans* together with *Cx. p. pipiens* biotype *molestus* may be responsible for up to 80% of the human West Nile Virus (WNV) infections in the northeastern United States.

### ***Culex (Culex) salinarius* Coquillett 1904**

**Female:** Medium sized species. Proboscis dark scaled, usually paler on ventral side, palps short, less than 1/5 the length of the proboscis, dark scaled. Occiput clothed dorsally with narrow curved golden brown scales and dark erect forked scales, yellowish white scales along the margins of the eyes, and with a patch of broad whitish scales laterally. Integument of scutum dark brown, scutum covered with narrow curved golden brown scales, somewhat paler on the anterior and lateral margins and on the prescutellar dorsocentral area. Scutellum with narrow yellowish scales and brown setae on all lobes. Pleurites with several small patches of broad pale scales, each patch usually with more than 6 scales. Legs dark scaled, posterior surfaces of femora and tibiae largely pale scaled, tarsi entirely dark scaled. Wing veins covered with narrow dark scales. Tergum I with a median area of dark brown scales, terga II–VI predominantly dark brown scaled with metallic bluegreen reflection, often with narrow to



moderately broad basal bands and basolateral patches of pale scales. Tergum VII and VIII often entirely covered with yellowish scales (Fig. 15.11b), sterna with yellowish white scales.

**Larva:** Antenna shorter than the head, constricted beyond the insertion point of the antennal seta (1-A), the part below the constriction pale and spiculate, the part beyond the constriction darker and with few spicules. Antennal seta (1-A) large, multiple branched, inserted at apical 1/3 of the antennal shaft, reaching well beyond its tip. Postclypeal seta (4-C) short, single, inner frontal seta (5-C) long, with 3–6 branches, median frontal seta (6-C) long, with 3–4 branches, outer frontal seta (7-C) long, multiple branched. Prothoracic setae 1 and 2 (1-P and 2-P) long, single, 3-P long, with 1–2 branches. Lateral abdominal seta 6 on segments I and II (6-I and 6-II) with 3 branches, 6-III to 6-VI usually with 2–3 branches. The comb consists of about 50 small scales arranged in an irregular triangular patch, individual comb scale broadly rounded apically and fringed with subequal spines. Siphon long and slender, siphonal index about 6.0–7.0. The pecten is composed of about 10–16 teeth extending on the basal 1/4 of the siphon, individual pecten tooth with 2–4 lateral denticles. Usually 4–5 pairs of siphonal setae (1-S) present, with 2–4 branches each and inserted beyond the pecten. The proximal seta (1a-S) as long or longer than the basal width of the siphon, the penultimate seta inserted laterally (Fig. 15.17). Anal segment completely encircled by the saddle, saddle seta (1-X) with 2 branches, sometimes single, slightly shorter than the saddle. Upper anal seta (2-X) with 1 long and 2 shorter subequal branches, lower anal seta (3-X) long, single. Ventral brush well developed, confined to the grid, precratal setae (4-X) absent. Anal papillae varying in length, usually 1.0–1.5 times as long as the saddle, pointed.

**Biology:** The larvae of *Cx. salinarius* are found in various habitats either in fresh or foul water, in swampy edges of lakes, grassy pools, ditches, ponds, marshes of various types, and occasionally in rain barrels, cattle tracks, and sometimes in stump holes (Carpenter and La Casse 1955). Larval development begins early in the season and continues at a rather uniform rate throughout the summer and into early fall in most of its distribution range (Ross 1947). In the extreme south, larvae and adults can be found at any time during the year, but farther north the females hibernate. The adults are frequently found resting dur-

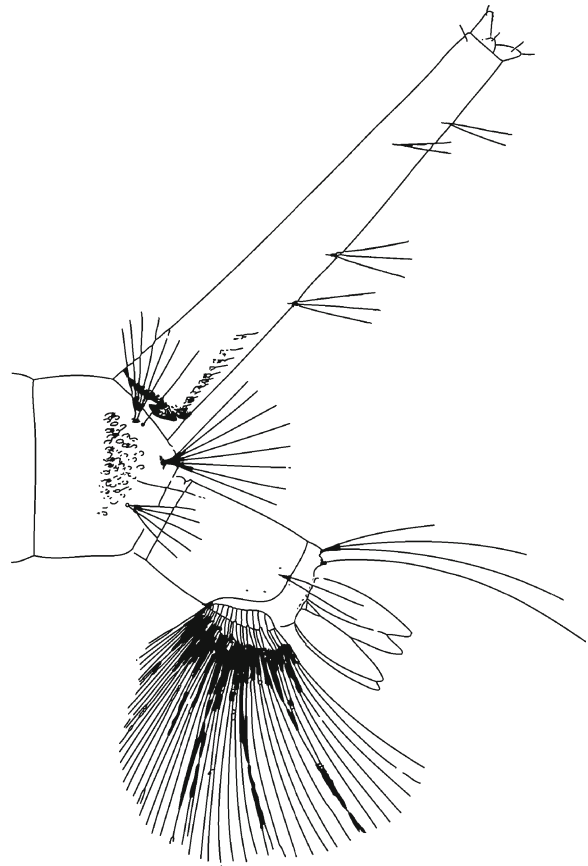


Fig. 15.17 Larva of *Cx. salinarius*

ing the daytime in shelters outside buildings. *Cx. salinarius* reaches its greatest numbers in the Atlantic and Mexican Gulf coastal regions. The females bite readily outdoors and occasionally enter dwellings to feed on humans.

**Distribution:** Eastern United States, south eastern Canada, Mexico, Bermuda.

**Medical importance:** *Cx. salinarius* is an important vector of West Nile virus (WNV) in the northeastern United States (Kilpatrick et al. 2005).

### ***Culex (Culex) tarsalis* Coquillett 1896**

**Female:** Medium sized species. Proboscis mostly dark scaled, with a rather broad white ring just beyond the middle. Palps short, dark scaled except for a few white scales at the tip and at the apex of palpomere III. Occiput with narrow white scales in

a median triangular patch, narrow white scales around the eye margins. Erect forked scales on dorsal surface dark, a few pale ones on the median area, broad white scales on the lateral region of occiput. Integument of scutum dark brown to black, scutum covered with narrow reddish brown scales dorsally, narrow whitish scales along lateral margin anterior to transverse suture, on prescutellar dorsocentral area, supraalar area, and narrow posterior dorsocentral stripes. Two small mid-dorsocentral whitish spots often present. Scutellum with narrow whitish scales and brown setae on all lobes. Pleurites with small patches of broad whitish scales confined to posterior margin of mesepisternum and upper part of mesepimeron. Upper part of postspiracular area with a row of whitish scales. Posterior surfaces of femora and tibiae pale scaled, anterior surfaces dark brown, with a median longitudinal stripe of white scales or a row of pale spots on anterior surfaces of femora. Femora and tibiae with white scales at apices. Hind tarsi with broad basal and apical white rings (Fig. 15.10a). Fore and mid tarsi with tarsal bands narrower on tarsomeres I–III, reduced or absent on tarsomeres IV and V. Wing veins covered with narrow dark scales, a few white scales usually at base of costa (C) and scattered along subcosta (Sc). Tergum I with a median patch of dark brown scales, usually with a few pale scales intermixed, tergum II dark scaled, with a median basal triangular patch of pale scales. Terga III–VII dark scaled, with prominent basal bands of white or yellowish-white scales, tergum VIII often entirely pale scaled. Sterna mostly pale scaled, with a V-shaped marking of dark scales on each sternum.

**Larva:** Antenna more than  $2/3$  as long as the head, distinctly constricted beyond the insertion point of the antennal seta (1-A), the portion below the constriction spiculate, dark near base, remainder pale, portion beyond constriction darker and with fewer spicules. Antennal seta (1-A) large, multiple branched, inserted at distal  $1/3$  of antennal shaft, reaching well beyond its tip. Postclypeal seta (4-C) small, single. Frontal setae (5-C to 7-C) multiple branched, of more or less equal length. Prothoracic setae 1 and 2 (1-P and 2-P) long, single, 3-P long, with 1–2 branches. Lateral abdominal seta 6 on segments I–V (6-I to 6-V) usually with 3 branches, 6-VI with 2–3 branches. The comb consists of about 50 small scales arranged in

an irregular triangular patch, individual comb scale rounded apically and fringed with subequal spines. Siphon slender, evenly tapering towards apex, siphonal index about 4.5–5.5. The pecten is composed of about 10–15 teeth extending on the basal  $1/3$  of siphon, individual pecten tooth with 1–5 lateral denticles. Five pairs of multiple branched siphonal setae (1-S) present, all inserted in a straight ventral line, the proximal pair (1a-S) often inserted near or slightly below apex of pecten (Fig. 15.18). Anal segment completely encircled by the saddle, saddle seta (1-X) usually with 2–3 branches, occasionally single, shorter than the saddle. Upper anal seta (2-X) with 3 branches, 1 branch nearly as long as the lower anal seta (3-X), which is single. Ventral brush well developed, confined to the grid, precratal setae (4-X) absent. Anal papillae varying in length, usually 1.0–1.5 times as long as the saddle, tapering.

**Biology:** The larvae can be found in permanent and semipermanent water bodies in a variety of habitats including ditches, irrigation systems, ground

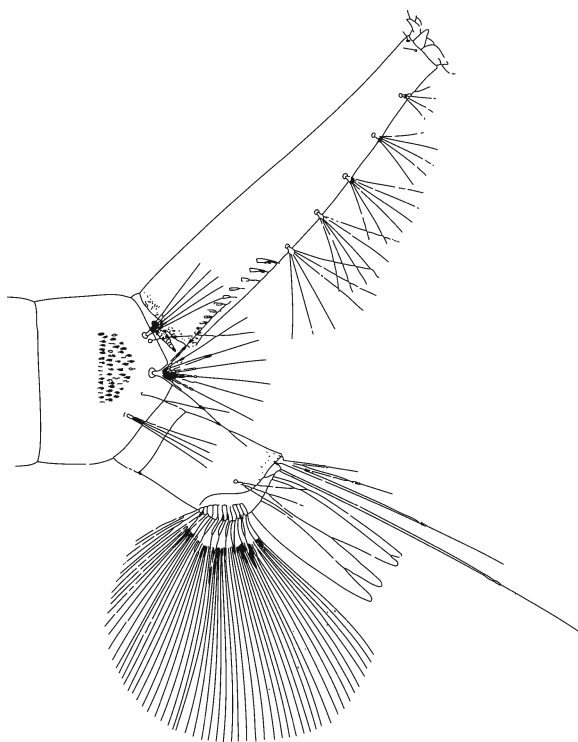


Fig. 15.18 Larva of *Cx. tarsalis*

pools, marshes, pools in stream beds, rain barrels, hoofprints, and ornamental pools (Ross 1947; Carpenter and La Casse 1955). Foul water in corrals and around slaughter yards appear to be favourite larval habitats in many localities. In colder climates, adult females pass the winter in hibernation in basements, cellars, caves, abandoned mines, and rodent burrows (Carpenter and La Casse 1955; Wood et al. 1979). Larval development usually begins during the late spring and continues until early autumn throughout most of its range, with several generations produced per year. Although females of *Cx. tarsalis* feed on domestic and wild birds like other *Culex*, they seem to prefer mammals as hosts (Wood et al. 1979). They are painful and usually persistent biters, attacking at dusk and preferably after dark, and readily entering dwellings for blood meals. During the day they hide in sheltered places. This mosquito is a serious pest to livestock, poultry, and humans (Lungstrom 1954).

**Distribution:** United States, south western Canada, Mexico.

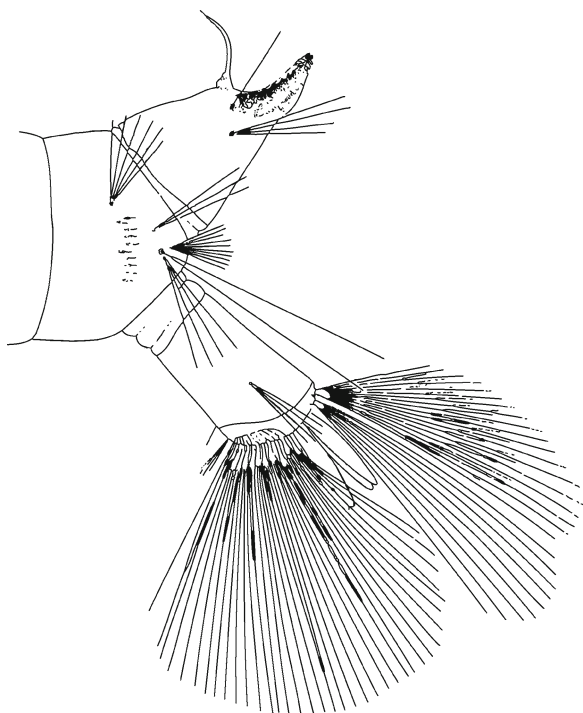
**Medical importance:** The vector competence of this species has been demonstrated not only in the laboratory, but as a major vector in outbreaks of encephalitis viruses in California (Reeves 1990). *Cx. tarsalis* is believed to be the main vector of Western equine encephalitis virus (WEE) under natural conditions. The virus has been isolated from wild caught females on several occasions in areas in which the disease was both epidemic and epizootic. The viruses of both St. Louis and California Encephalitis (SLE and CE) have been isolated from this mosquito (Carpenter and La Casse 1955). *Cx. tarsalis* together with *Cx. p. quinquefasciatus* play an important role in the West Nile virus (WNV) transmission in most of the western United States (Goddard et al. 2002; Reisen et al. 2004, 2008).

***Coquillettidia (Coquillettidia) perturbans*  
(Walker 1856)**

**Female:** Moderately large species. Proboscis dark scaled apically, speckled with yellowish scales basally and with a broad median ring of pale scales. Palps about 1/5 as long as proboscis, mottled with dark and pale scales. Pedicel light brown on outer surface, darker and with a patch of pale scales on inner sur-

face. Occiput with yellowish lanceolate scales and dark erect forked scales, a few pale forked scales on vertex. Integument of scutum dark brown and black, scutum covered with pale yellowish scales and a few smaller brown scales, submedian area of scutum without scales. Scutellum with few pale golden scales and brown setae on all lobes. Pleurites with patches of greyish white scales confined to posterior margin of mesepisternum and upper part of mesepimeron. Prespiracular and postspiracular setae absent. Femora and tibiae speckled with pale and dark scales, the apices of the femora almost entirely dark-scaled, hind tibia with a ring of pale scales near the apex. Tarsomere I of all legs dark scaled, with a narrow white ring basally and a broader white ring a little beyond middle, tarsomeres II–V each with basal half white, apical half dark. Wing scales broad, mixed dark and yellow, the dark scales predominating. Terga dark scaled, with yellowish basolateral patches and occasionally with narrow basal pale bands. Sterna with dark and pale scales intermixed, the pale scales more numerous on the basal part of each sternum, abdomen bluntly ended.

**Larva:** Head much broader than long. Antenna slender and long, more than twice as long as the head, sparsely spiculate basally. Antennal seta (1-A) with multiple branches, arising from a notch on basal third of antennal shaft, a pair of short setae inserted at the middle of the shaft. Postclypeal seta (4-C) small, multiple branched, frontal setae (5-C to 7-C) multiple branched, 5-C much shorter than 6-C and 7-C. Prothoracic setae 1 to 3 (1-P to 3-P) arising from a common sclerotized tubercle, 1-P and 2-P single, 3-P with multiple branches. The comb consists of 8–15 scales arranged in a single irregular row, individual comb scale with a very prominent median spine and fringed on basal half with short spinules. Siphon short, strongly attenuated and heavily sclerotized beyond middle, the attenuated part of siphon bears saw-like projections dorsally and strong hooks apically, forming a stout piercing organ. Siphonal seta (1-S) a multiple tuft, arising before the heavily sclerotized part of the siphon, pecten absent. Anal segment elongated, much longer than wide, completely encircled by the saddle. Saddle seta (1-X) a short multiple tuft, inserted well before the posterior margin of the saddle. Upper and lower anal setae (2-X and 3-X) multiple branched, 2-X shorter than 3-X. Ventral brush well developed, cratal setae (4-X) usually restricted to the grid but often with



**Fig. 15.19** Larva of *Cq. perturbans*

1–2 minute precratal setae piercing the saddle. Anal papillae shorter than the saddle, pointed (Fig. 15.19).

**Biology:** The eggs are laid in floating rafts on the surface of water in areas of heavy emergent vegetation. Larvae are mainly found in permanent bogs and

marshes. After hatching, the small larvae attach themselves with their modified siphon to the roots or submerged stems of plants where they remain throughout development. The pupa also attaches itself to plants by means of the modified respiratory trumpets and remains there until the adult is ready to emerge (Carpenter and La Casse 1955). The larvae are associated mainly with plants of *Typha*, *Sagittaria*, *Nymphaea*, *Juncus*, *Phragmites*, *Carex* and *Calla* (Ross 1947). They are difficult to collect, as they quickly drop off the host plant whenever they are disturbed. The winter is passed in the larval stage, there appears to be no larval diapause, and the adults emerge in the spring and summer (Wood et al. 1979). The females are fierce biters and usually take their blood meal during the night. They occasionally attack humans during daylight hours in shady places. The adults are strong fliers with a flight range of more than 10 km and are important pests in communities near shallow lakes, swamps, and marshes which are partly overgrown with emergent aquatic vegetation.

**Distribution:** United States, southern Canada, Mexico.

**Medical importance:** The virus of Eastern Equine Encephalitis (EEE) has been isolated from wild caught females of *Cq. perturbans* in southern USA (Carpenter and La Casse 1955). It plays a minor role in human West Nile virus (WNV) infections in the northeastern United States (Kilpatrick et al. 2005).

**Part IV**  
**Control of Mosquitoes**

# Chapter 16

## Biological Control

### 16.1 Introduction

Biological control, in the broadest sense, is defined as the reduction of the target population by the use of predators, parasites, pathogens, competitors, or toxins from microorganisms (Woodring and Davidson 1996). Biological control aims to reduce the target population to an “acceptable” level and, at the same time, to avoid adverse effects to the ecosystem. As far as mosquito control is concerned, biological control measures should integrate the protection of humans from mosquitoes with conservation of the biodiversity, while avoiding toxicological and eco-toxicological effects. As a result, the regulatory power of the ecosystem is maintained by protecting the existing community of mosquito predators.

The use of beneficial organisms for the control of mosquitoes was first recognised in the late nineteenth century, when attempts were made by introducing predators such as dragonflies (Lamborn 1890). However, mass breeding and successful introduction of predators, such as hydra, flatworms, predacious insects or crustaceans, often introduces a range of problems. However, such problems did not occur, or occurred only to a limited extent, with the use of fish, such as the mosquito fish, *Gambusia affinis* (western mosquitofish) and *G. holbrooki* (eastern mosquitofish), which were successfully introduced into many countries to control mosquito larvae in the early 1900s (Bellini et al. 1994; Legner 1995; Walton 2007; Chandra et al. 2008).

With the discovery and large-scale use of synthetic insecticides in the 1940s and 1950s, biological control of mosquitoes was unfortunately no longer considered to be an important method.

However, the initial euphoria that greeted the success of synthetic insecticides rapidly dissipated as resistance

subsequently developed within the target populations. Moreover, despite the beneficial effects of traditional insecticides, they also often have unwanted characteristics, such as their non-selectivity which frequently causes ecological damage. As public awareness of environmental issues increased, regulations controlling the application of chemicals were tightened. As a result, a renaissance in the biological control of mosquitoes took precedence in the 1960s and 1970s. By 1964, Jenkins had already listed more than 1,500 parasites, pathogens, and predators as potential candidates for biological control. Today, the literature on mosquito antagonists is immense (Notestine 1971; Lacey and Lacey 1990; Legner 1995; Medrano 1993; Quiroz-Martínez and Rodríguez-Castro 2007; Mogi 2007).

One of the major advantages of biological control measures is that existing predators are conserved, which will in turn assist the control effort by preying upon newly-hatched mosquito larvae after the control operation, thereby, considerably enhancing the efficacy of the current control measures.

By promoting the conservation of existing populations of predators, parasites or pathogens, there are two major strategies for the augmentation of the population of mosquito antagonists.

“*Inoculation*” refers to the release of small numbers of predators, parasites, or pathogens into the habitat of the target organisms. The antagonists become established, they reproduce and multiply under favourable living conditions in the new habitat, resulting in a sustained suppression of the target population which can be achieved by successive generations of mosquito enemies. For instance, the inoculation of fish into newly-flooded rice fields is a very common practice in mosquito control (Bellini et al. 1994; Walton 2007).

“*Inundation*” means the release of an overwhelming number of predators, parasites, pathogens, or their

toxins into the mosquito habitat. Such mass release of organisms or applied pathogens (toxins) can have an immediate effect through a significant reduction of the target population. For instance, inundation control is successfully practiced with microbial pathogens which are produced in artificial cultures, e.g. *Bacillus thuringiensis israelensis* (*B.t.i.*) and *B. sphaericus* (*B.s.*). Only rarely do the antagonists become established in the habitat, for example, *B.s.* is able to recycle under certain conditions (Becker et al. 1995a).

A prerequisite for the successful use of predators, parasites or pathogens is the precise knowledge of the biology of the antagonist in question and its interaction with the ecosystem. For example, the introduction of foreign faunal elements such as predators, risks damaging or displacing existing populations of predators. For instance, introduced fish may reduce numbers of aquatic insects, crustaceans or amphibians which would otherwise be effective predators of mosquito larvae. Rare indigenous species which do not feed on mosquito larvae may also be endangered. A thorough understanding of predator/prey or parasite/host relationship is therefore of fundamental importance for the successful and ecologically sound use of antagonists. Different species of mosquitoes inhabit very different habitats, and have developed various life strategies by adapting to habitats with very different abiotic and biotic conditions. Antagonists can only successfully reduce a target population if their own life strategy is adapted to the target population.

## 16.2 Predators

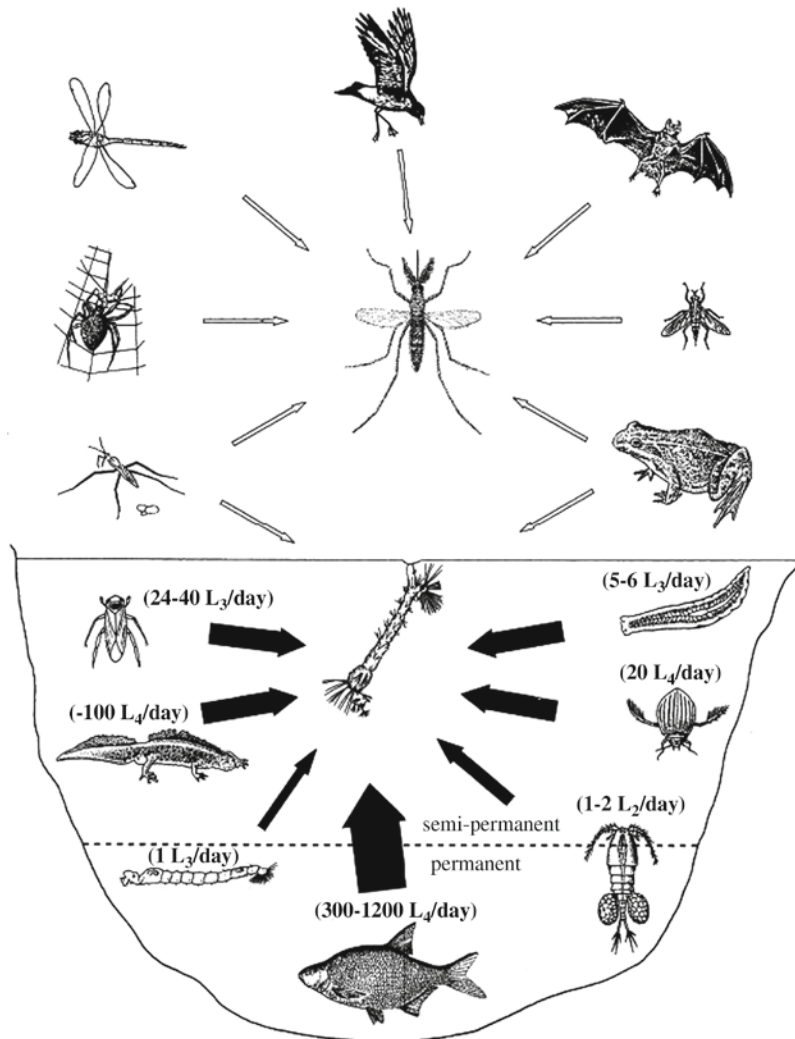
In general, predators of immature mosquito stages are more effective than predators of adults (Fig. 16.1). As a rule, mosquito larvae and pupae are concentrated at their breeding sites and are more easily available to predators than the widely dispersed adults. Moreover, adult mosquitoes evade many predators as they are mostly nocturnal. Mosquitoes have the characteristics of typical *r*-strategists (meaning, a high rate of reproduction and a relatively short life cycle). Predators are particularly effective if they have a similarly high rate of reproduction and/or a high rate of feeding, like fish.

### 16.2.1 Vertebrate Predators

#### 16.2.1.1 Fish (Osteichthyes)

The best known aquatic predator of mosquitoes is the mosquito fish, *G. affinis*, which is native to south-east United States, eastern Mexico, and the Caribbean, and the common guppy, *Poecilia reticulata*, which is native to tropical South America. Both fish are effective predators because their upward-facing mouth enables them to consume mosquito larvae living on or close to the water surface. Other biological attributes of these viviparous fish are their high reproduction rate, small size (3–6 cm), and high tolerance to variations in temperature, organic pollution, and salinity. *G. affinis* can survive water temperatures below 13°C and even overwinters in areas with short periods of frost, but *P. reticulata* is restricted to subtropical and tropical climates. The latter is used particularly in urban areas against *Cx. p. quinquefasciatus*, which usually occurs in masses in highly polluted water bodies, but the efficacy of the fish can be variable (Sjogren 1972; Dua et al. 2007).

*Gambusia affinis* is the most widely disseminated organism for mosquito control (Walton 2007). As early as 1937, Hackett reported on the value of *G. affinis* for the control of malaria in Europe. It is thought that *G. affinis* may have contributed significantly to the reduction of malaria in Turkey and Iran (Tabibzadeh et al. 1970; Inci et al. 1992). In the USA, mosquito fish are commonly bred by local mosquito abatement districts and selectively released for control, in an Integrated Mosquito Management (IMM) programme. In California, they are used successfully in rice fields against the immature stages of *An. freeborni* and *Cx. tarsalis*. Whereas stocking at a rate of more than 500 female mosquito fish/ha in rice fields gave excellent control of *Cx. tarsalis* (Hoy and Reed 1971; Steward et al. 1983), significant reduction rates against *An. freeborni* were only achieved when more than 4,000 fish/ha were used (Kramer et al. 1987a, b, 1988a, b). The inundative release of 4,800 mosquito fish/ha was effective against *Ps. columbiae* in Arkansas rice fields (Davey and Meisch 1977a–c). In California, a reduction rate of >70% was observed in a population of *Cx. quinquefasciatus* by the inundative release of mosquito fish in urban underground storm drains. Generally, mosquito fish are most effective in water bodies where the



**Fig. 16.1** Importance of some mosquito predators in the food web (size of arrows refers to importance)

vegetation is less dense and the mosquito larvae are more accessible.

The South American species *P. reticulata* is a popular aquarium fish which has been introduced into many countries through the ornamental fish trade. This guppy frequently inhabits sewage ditches in subtropical and tropical countries, where it definitively contributes to the reduction of larval populations of *Cx. p. quinquefasciatus*, the main vector of bancroftian filariasis. Sasa and Kurihara (1981) discussed the use of guppies in filariasis control programmes. In Sri Lanka, free-living fish were caught and introduced into mosquito breeding sites (Sabatinelli et al. 1990). In Malaysia, the fish are also used in water containers for the control of

*Ae. aegypti*, the vector of dengue and dengue haemorrhagic fever (DHF). In Southern California, Sjogren (1971) and Mian et al. (1985, 1986) successfully tested guppies against mosquitoes in sewage plants.

Before the discovery of *B.t.i.*, the use of fish was lauded as being one of the most successful biological weapons for the control of mosquitoes. Many critics, however, disapprove of the use of mosquito fish because of their concern for the native fish fauna. *G. affinis* is omnivorous and feeds not only on invertebrates, which can themselves be useful predators, but also on the eggs and offspring of indigenous fish. The introduction of *G. affinis* may have some adverse effects on extensive aquatic predator populations



consisting of water beetles, water bugs, copepods, dragonflies or urodelans and can greatly repress economically important fish species such as carp. Schoenherr (1981) and Lloyd (1987) reported that more than 30 species of native fish have been adversely affected by the introduction of *G. affinis*.

For this reason, it is essential to study the biology of predacious organisms with special reference to prey selectivity, reproductive potential, and their benefit as predators in relation to costs and the environmental damage they might cause, before they are actually released. The ecosystem must also be carefully evaluated to determine the potential effect of the released organisms on the existing biota. This process is even more important if non-indigenous species are to be released (Hurst et al. 2006; Chandra et al. 2008).

The indiscriminate use of *G. affinis* for mosquito control is no longer recommended by the World Health Organization because of its aggressiveness towards numerous aquatic organisms and also because of its doubtful contribution to the control of mosquito-borne diseases (Service 1983).

Fish are also, occasionally, introduced because of their ability to consume aquatic vegetation. As predators, they can reduce the number of mosquitoes, but as consumers of aquatic plants, they can also reduce the niches for the larval development of mosquitoes. In California, the subtropical cichlids *Tilapia zilli*, *Oreochromis mossambica*, and *O. hornorum* were found to be useful (Asimeng and Mutinga 1992).

Because of the risk to an existing community which can result from the introduction of non-indigenous organisms, native fish should be used as a component in an integrated mosquito management (IMM) programme. In China, the grass carp, *Ctenopharygodon idella*, and the common carp, *Cyprinus carpio*, are used in rice fields as young fish. During flooding of the rice fields, the fish not only contribute to the control of mosquito breeding but also feed on rice pests, such as grasshoppers, which fall onto the water surface. In addition, the fish also manure the rice cultures with their excreta. Furthermore, the grass carp, as a plant feeder, prevents the growth of weeds. The beneficial effects of the grass carp led to an increase in the total rice yield by more than 20%. If the rice fields are drained before the harvest, the fish are kept for another year in permanent main irrigation ditches. Because of their relatively fast growth, carp can subsequently be used for personal requirements, either as a source of

proteins or for sale (Xu, personal communication; ICMR Bulletin 2000).

Representatives of the Cyprinodontidae produce hard-shelled eggs that are resistant to drought. In China, *Oryzias latipes* is also used in rice fields to control mosquitoes (Xu, personal communication; Sugiyama et al. 1996). These widely distributed fish grow to about 4 cm in length and feed predominantly on mosquito larvae or insects falling onto the water surface. Despite their small size, one fish eats on average, 51 *Anopheles* or 118 *Culex* fourth-instar larvae/day. In Asia, a species belonging to the labyrinth fish, such as *Macropodus opercularis*, *M. chinensis*, and *Tanichthys albonubes*, are avid feeders of mosquitoes.

In the Americas, the cyprinodontid *Cynolebias bellottii* breeds in temporary waters. The eggs can survive dry periods. Therefore, the fish can be successfully used as predators in rice fields (Coykendall 1980; Walters and Legner 1980; Gerberich and Laird 1985).

Shallow floodlands, which form mass breeding sites for mosquitoes, can be deepened into permanent water bodies or connected by ditches to accommodate fish if it is considered environmentally sound. The fish and their offspring are then able to invade the breeding sites of floodwater mosquitoes such as *Ae. vexans*. In field investigations in Germany, native fish species demonstrated an impressive feeding performance. Fish, of approximately 5 cm in length, were kept in cages in mosquito breeding sites and fed with fourth-instar larvae of *Ae. vexans*. At water temperatures averaging 22°C, the following feeding rates of fourth-instar larvae/day were recorded: *Cyprinus carpio*: 302; *Carassius carassius*: 238; *Tinca tinca*: 185; *Gasterosteus aculeatus*: 178; *Abramis brama*: 148; *Rutilus rutilus*: 147; *Alburnus alburnus*: 113; *Leucaspius delineatus*: 99; *Scardinius erythrophthalmus*: 80 and *Gobio gobio*: 63. Feeding rates of more than 1,000 *Aedes/Ochlerotatus* larvae within 12 h were shown by the larger species *C. carassius* and *S. erythrophthalmus* (Gebhard 1990).

### 16.2.1.2 Amphibians (Amphibia)

Urodela (newts) and their larvae are important predators of immature stages of mosquitoes (Sack 1911; Martini 1920b; Twinn 1931; Blum et al. 1997). In feeding experiments in Europe, *Triturus cristatus* and *T. vulgaris* proved to be voracious consumers of mos-

quitoes (Kögel 1984). Whereas 2-week-old larvae of *T. cristatus* devoured about 15 third-instar larvae of *Cx. p. pipiens*, 5–10-week-old *Triturus* larvae captured ~100 fourth-instar larvae/day. The feeding rate of *T. vulgaris* and *T. cristatus* is approximately the same.

In contrast to urodelans, anurans have little effect as predators on mosquitoes. In a 3-year study in the floodplains of the Rhine River 2,163 anuran specimens of the taxa *Rana arvalis*, *R. temporaria*, *R. dalmatina*, *R. esculenta* s.l., *Hyla arborea*, *Bufo bufo*, and *Pelobates fuscus* were subjected to stomach flushing (Blum et al. 1997). Most of the prey species observed were beetles (Coleoptera), springtails (Collembola), snails (Gastropoda), spiders (Araneae), ants (Formicidae), and woodlice (Isopoda), which all belong to the epigeal fauna. Only 0.1% of the prey were Culicidae, mostly adult *Ae. vexans*. The only anuran known to consume Culicidae at a higher rate is *Bombina orientalis*. Lac (1958) reported 5.7% and 16.7% of *Cx. pipiens* and *An. maculipennis* s.l. larvae respectively.

### 16.2.1.3 Birds (Aves)

In general, birds are not considered to be important predators of mosquitoes, although mosquitoes are a source of food for some bird species (Blotzheim 1985). For example, the duck, *Anas platyrhynchos*, was repeatedly observed filtering out larvae of *Ae. vexans* from mass breeding sites along the Rhine River valley.

There are two main reasons for the relatively insignificant role of birds as predators of adult mosquitoes. Firstly, the activity phases of mosquitoes and birds do not overlap to any great extent. Most mosquitoes are active at dusk, whereas the majority of birds search for food during the day when most of the mosquito species are resting in vegetation. Secondly, floodwater mosquitoes occur irregularly, only after flooding and are, therefore, not available as a stable food-source in seasons when flooding does not occur. As a consequence, insects, such as midges (Chironomidae) which breed mainly in permanent waters and appear in large numbers more or less every year, are a more reliable source of food for birds than floodwater mosquitoes.

An analysis of the diet of birds living in the floodplain areas of the Upper Rhine Valley (Germany) showed that the portion of mosquitoes in the diet of the investigated bird species is low (Timmermann and

Becker 2003). To determine the composition of the birds' diet, the neck-ring method was applied to nestlings of the House Martin (*Delichon urbica*), the Reed Warbler (*Acrocephalus scirpaceus*), the Pied Flycatcher (*Ficedula hypoleuca*), the Great Tit (*Parus major*), and the Blue Tit (*Parus caeruleus*). The aerial insect abundance in these areas was also investigated using a cone trap on a vehicle.

The main activity period of the nuisance mosquitoes (e.g. *Aedes vexans*) in the area was during dusk, whereas the frequency with which the flight-hunting House Martin fed its nestlings began to decrease approximately 60 min before sunset. This may explain, in part, the low numbers of mosquitoes found in the diet of the House Martin nestlings. Out of a total of 6,761 insects which were identified from the nestlings' food, less than 1% belonged to the family Culicidae (Fig. 16.2). These birds preferred feeding their nestlings aphids (Aphidina, during first brood; Staphylinidae and Brachycera, during second brood).

Additionally, 140 food samples were taken from birds which collect insects from or near the vegetation. Within these samplings, only 5 mosquitoes were found (4.4% of the bird's diet); although, car trap samples over many years indicate that adult mosquitoes are present each summer in these areas. The Great Tit and Blue Tit preferred to feed their nestlings with larvae of Lepidoptera, while the Reed Warbler and Pied Flycatcher preferred mainly flightless invertebrates (e.g. caterpillars, spiders, aphids). Among the Dipterans in the bird diet, larger species (e.g. Tipulidae, Syrphidae) were more common. Mosquitoes did not make up a significant proportion of the birds' diets.

### 16.2.1.4 Bats (Mammalia: Chiroptera)

Whereas the majority of insectivorous birds are daylight hunters, bats, in contrast, usually hunt during the night. Therefore, the activity patterns of both bats and most of the mosquito species show a considerable overlap. After dusk, the bats emerge from their roost, and at the same time the flight activity of mosquitoes is increasing.

World-wide, there are about 1,000 known bat species, the majority being exclusively insectivorous and exhibiting a large variety of hunting strategies. Most effective mosquito hunters might be gleaning species, e.g. Bechstein's bats (*Myotis bechsteinii*), that take

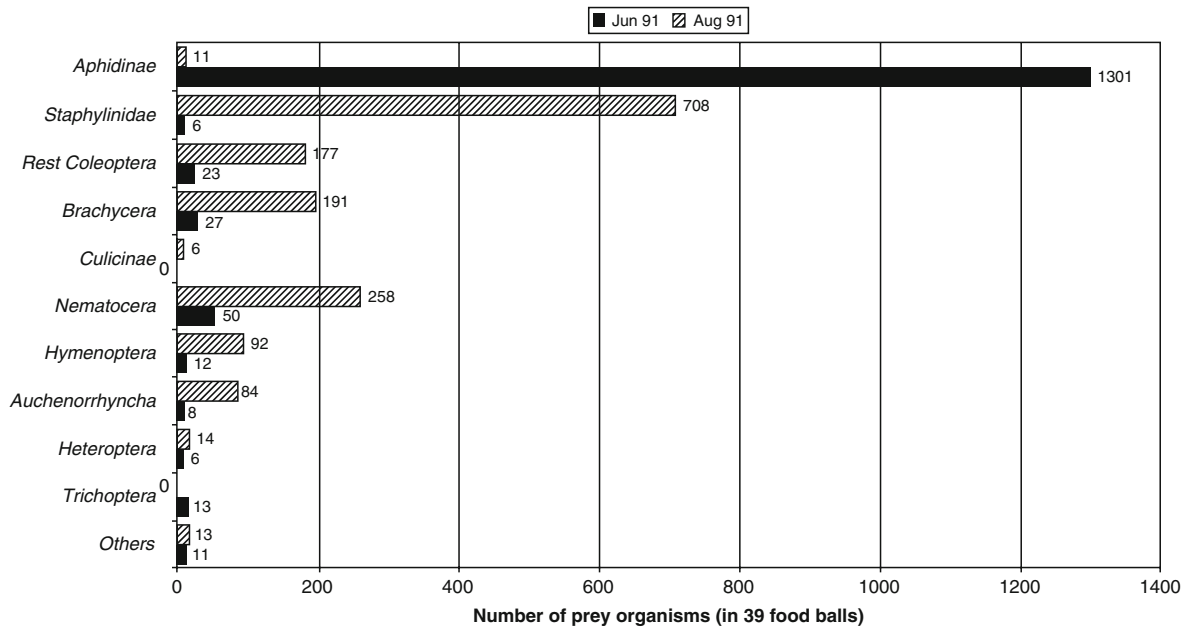


Fig. 16.2 Prey of house martin *Delichon urbica* in the Upper Rhine area

their prey from foliage and hunt closely to vegetation, or species like Daubenton's bats (*M. daubentonii*), that fly and hunt closely over the surface of open water bodies (Arnold et al. 1998).

Mosquito feeding by bats was recorded repeatedly, mostly by analyzing guano samples, but the amount of mosquitoes consumed varied considerably. Studies on the diet of bats, carried out throughout Europe, showed a great variety of nocturnal insects providing food resources for bats, such as moths (Lepidoptera), beetles (Coleoptera), lacewings (Planipennia), caddisflies (Trichoptera), mayflies (Ephemeroptera), and midges (Chironomidae) (Swift et al. 1985; Rydell 1986; McAney et al. 1991; Wolz 1993). A study of the trophic ecology of Daubenton's and Nathusius' bats in southwest Germany (Arnold et al. 2000) showed that mosquitoes are not an important food source for these bat species. Under rare conditions, when they occur in dense populations, mosquitoes might become a major prey for some bat species, like the Northern Bat (*Eptesicus nilssonii*) that relies heavily on mosquitoes and other dipteran insects during the summer in northern Sweden (Rydel 1990).

The majority of bat species are opportunistic hunters that focus on insects occurring in large numbers on their hunting grounds (Gloor 1995), therefore, it is

generally considered today that bats are beneficial in terms of controlling pest insects (Tuttle 2000). However, bats are not considered an effective component in mosquito control programmes, but they are an important group of organisms which should be protected by the use of target-specific control agents.

### 16.2.2 Invertebrate Predators

Countless invertebrates are known as predators of mosquitoes, especially, of the larvae. The biology and importance of the predators have been investigated in numerous studies (Lamborn 1890; Hinman 1934; Köhlhorn 1961; Jenkins 1964; James 1967; Service 1977; Kögel 1984; Collins and Washino 1985; Quiroz-Martinez and Rodriguez-Castro 2007). Although invertebrates have been shown to be effective predators of mosquitoes, they are seldom used in control programmes due to the great difficulties and the high costs involved in mass rearing of these organisms. Nevertheless, their role as mosquito predators is beyond dispute. Mosquitoes can rarely develop in large numbers at breeding sites where predacious invertebrates are abundant. In this section, different groups of

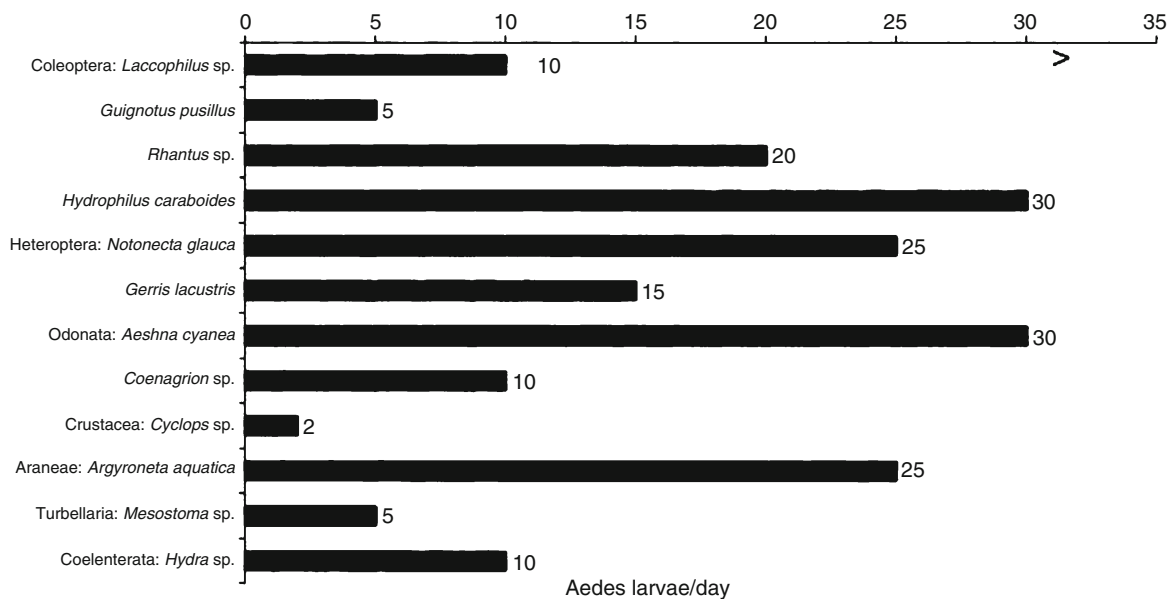


Fig. 16.3 Average feeding rate of some invertebrate predators of mosquito larvae

invertebrates and their importance as predators will be discussed (Fig. 16.3).

### 16.2.2.1 Hydra (Coelenterata)

In feeding experiments, polyps of the genus *Hydra* killed, on average, between 6 and 21 mosquito larvae/day. Polyps frequently contribute considerably to the reduction of mosquitoes in permanent water bodies (Qureshi and Bay 1969). Because of their beneficial effects, *Chlorohydra viridissima* (Pallas) were used in a number of field experiments (Lenhoff 1978; Cress 1980).

### 16.2.2.2 Flatworms (Turbellaria)

Flatworms are one of the most thoroughly investigated groups of predators (Legner 1991, 1995). Turbellarians, like *Mesostoma* sp., secrete slime to catch mosquito larvae. When the larva sticks to the mucous secretion and tries to escape, the flatworm becomes aware of its prey, smothers it and sucks it up with its pharynx.

Some *Mesostoma* species, such as *M. ehrenbergii*, can make a significant contribution to the reduction of mosquitoes. Case and Washino (1979) reported a considerable reduction of *Cx. tarsalis* and *An. freeborni*

populations by *Mesostoma* sp. in California's rice fields, which led to an extensive investigation into the mass rearing of these flatworms.

In central Europe, *Bothromesostoma* sp. sometimes occurs in abundance in ephemeral waters where it can completely suppress *Ae. vexans* populations (Becker 1984). This flatworm is an efficient predator which feeds mainly on mosquito larvae and has consequently adapted its way of life to that of the prey. Like their prey, the turbellarians can survive dry periods as drought-resistant eggs and hatch during summer floods. At high summer temperatures, they develop within a week, to 7 mm long organisms to the reproductive stage and produce ~20 eggs. In laboratory experiments, a turbellarian consumed up to 67 mosquito larvae during its development.

### 16.2.2.3 Spiders and Mites (Arachnids)

Spiders are commonly known to be predators of adult mosquitoes, particularly at day resting places and shelters for hibernation (Service 1973a). Some species are hunters of aquatic organisms. Representatives of Pisauridae and Lycosidae, hunt on the water surface and occasionally capture mosquito larvae, pupae or emerging adults (Bishop and Hart 1931). Their

importance as predators is, however, considered to be slight (Kühlhorn 1961).

One of the most effective arachnid predators of mosquito larvae is the aquatic spider *Argyroneta aquatica*. This fascinating species builds webs under the water to catch its prey. In aquarium experiments, the maximum feeding rate was 29 fourth-instar mosquito larvae/day (Kögel 1984). In permanent pools, these spiders can lead to a significant reduction of mosquitoes.

Water mites (Hydrachnellae) can also contribute to the suppression of larval mosquito populations. For example, in laboratory experiments the small water mite *Piona nodata* consumed up to 18 mosquito larvae/day (Kögel 1984). When the adult mosquitoes emerge, some mites attach themselves to the emerging adult and use it as a carrier to disperse to new habitats.

#### 16.2.2.4 Crustaceans (Crustacea)

The tadpole shrimp, *Triops cancriformis*, is considered to be a predator of mosquito larvae. The shrimp eggs can survive dry periods for several years and the larvae hatch after flooding, often in association with *Aedes/Ochlerotatus* larvae. In California, attempts were made to control *Aedes/Ochlerotatus* and *Psorophora* species with *T. longicaudatus* (Tietze and Mulla 1987, 1991).

Among the crustaceans, copepods are important predators of mosquito larvae (Miura and Takahashi 1985; Marten and Reid 2007). It is more their occurrence in enormous numbers than their rate of feeding which makes them important predators of mosquitoes. In laboratory experiments, *Megacyclops viridus* and *Acanthocyclops vernalis* consumed 1–2 first and second-instar larvae of *Ae. vexans*/day (Kögel 1984). Hintz (1951) reported that *Cyclops* sp. caught about 5 first and second-instar larvae of *Ae. aegypti*/day. Because of their small size, copepods mainly capture early instar mosquitoes, but as predators, they are only effective when present as adults before the mosquito larvae hatch. The use of *Mesocyclops aspericornis*, which has been introduced into artificial containers, wells, and terrestrial crab burrows, has led to a reduction of >90% of *Ae. aegypti* and *Ae. polynesiensis* in Asia (Riviere et al. 1987a, b; Kay et al. 1992). Similar results have been achieved by Vu et al. (1998) in north-

ern Vietnam, where copepods of the genus *Mesocyclops* were used for the effective control of *Ae. aegypti* by inoculation into wells, large cement tanks, ceramic jars, and other domestic containers. The use of *Mesocyclops* sp. was complemented by community participation with respect to recycling, to eliminate unused and discarded containers.

#### 16.2.2.5 Insects (Insecta)

##### Odonata

The importance of dragonflies (Odonata) as mosquito predators has been known for a long time. Both the nymphs and the adults catch mosquitoes (Kögel 1984; Sebastian et al. 1990). Dragonfly nymphs usually have a long development and, therefore, occur mainly in permanent waters. In feeding experiments, anisopteran nymphs proved to be extremely voracious. According to Kögel (1984), nymphs of *Aeshna cyanea* consumed up to 100 mosquito larvae/day (average 30 larvae/day). Zygopteran nymphs, such as *Coenagrion puella*, are usually less efficient as predators (on average, 10 third-instar larvae/day).

In rice fields in Japan, ~208 dragonfly nymphs/m<sup>2</sup> were recorded. Such mass occurrences classified them among the most important predators of mosquitoes in rice field ecosystems (Mogi 1978).

##### Heteroptera

The majority of water bugs (Hydrocorisa and Amphicorisa) are voracious consumers of mosquitoes. However, there is a significant variation in feeding behaviour of different families.

*Corixidae*: In feeding experiments, one of the most abundant corixids in central Europe, *Sigara striata*, captured 2–3 early larval instars of *Ae. vexans*/day. The larger but less common species, *Corixa punctata* (1 cm), consumed up to 45 larvae/day, and even *Cymatia coleoprata* (6 mm) consumed up to 47 early larval instars of *Ae. vexans*/day (Kögel 1984). Although the Corixidae are usually common in mosquito breeding sites, their importance as mosquito predators is rather slight because of their mostly omnivorous feeding habits (Washino 1969).

*Naucoridae*: One of the most common species in Europe, *Ilyocoris cimicoides*, proved to be extremely



**Fig. 16.4** *Ranatra linearis* feeding on a mosquito larva

voracious. The feeding rate of adult bugs was on average, >20 mosquito larvae/day, and their nymphs consumed as many as 35 larvae of *Ae. vexans*/day (Kögel 1984).

**Nepidae:** The widely distributed water scorpion, *Nepa cinerea*, consumed 10–18 fourth-instar larvae of *Ae. vexans* daily (Kögel 1984). This heteropteran inhabits the shallow littoral of stagnant waters, where it is certainly one of the most important predators. *Ranatra linearis* can also frequently be observed feeding on mosquito larvae (Fig. 16.4).

**Notonectidae:** Amongst the Heteroptera, notonectids are known for their voracious feeding on mosquito larvae in permanent and semipermanent waters (Hinman 1934; Hazelrigg 1975, 1976; Murdoch et al. 1984; Legner 1995). For this reason, *Notonecta undulata* and *N. unifasciata* were mass-cultured for use in rice fields in California (Sjogren and Legner 1989). In experiments with *N. glauca*, the daily average feeding rate was 25 third-instar larvae of *Ae. vexans* (Kögel 1984).

**Pleidae:** Although *Plea leachi* (size 2–3 mm) is one of the smallest predators, it is able to catch up to 20 mosquito larvae/day (Kögel 1984).

**Gerridae and Hydrometridae:** The Gerridae and Hydrometridae live on the water surface, usually in large numbers. They feed mainly on insects, which fall onto or emerge from the water surface. *Gerris lacustris* can sometimes be observed seizing mosquito larvae out of the water. Whereas this hunting behaviour is rather an exception in the Gerridae, *Hydrometra stagnorum* is more specialized for pulling mosquito larvae and even pupae out of the water. In laboratory experiments, *Hydrometra* sp. captured up to 15 mosquito larvae/day (Pruthi 1928).

## Coleoptera

Because of their abundance and great voracity, many water beetles and their larvae are effective predaceous aquatic insects (Baldwin et al. 1955; Trpis 1960; Köhllhorn 1961; James 1966, 1967; Bay 1972; Service 1973a; Nelson 1977; Kögel 1984). Their ability to inhabit and reproduce in great numbers in various mosquito-breeding habitats further enhances their significance. The following section briefly outlines the differences between individual representatives of the different water beetle families as mosquito predators.

**Dytiscidae:** Dytiscids are the most important predators among the water beetles (Nelson 1977). Not only the large species of the subfamily Dytiscinae, but also the medium-sized species of the subfamily, Colymbetinae, and the small sized species of the subfamilies, Hydroporinae, Noterinae, and Laccophilinae can show high rates of feeding on mosquito larvae. The large larvae of *Dytiscus marginalis* and *D. circumflexus* feed predominantly on larger creatures such as tadpoles and small fish. However, their young larvae can consume more than 100 *Ae. vexans* fourth-instar larvae/day. Among the medium-sized dytiscids, *Rhantus* species are the most efficient predators. In laboratory experiments, the adults of *R. consputus* and *R. pulverosus* captured up to 40 third and fourth-instar larvae/day (Kögel 1984). While swimming, they are able to rapidly to seize the prey with their front legs. Not only the adults of *Rhantus* spp., but also their larvae are frequently found in temporary *Aedes/Ochlerotatus* breeding sites, where they feed mainly on mosquito larvae. Depending on the larval stage, they consume between 4–6 *Ae. vexans* larvae up to a maximum of even 20 fourth-instar larvae/day. The adults of *Agabus* species do not consume as many larvae of *Aedes/Ochlerotatus* as do *Rhantus* spp. adults. In Europe, *Agabus* spp. are especially abundant in swampy alder forests populated by the immature stages of snow-melt mosquitoes. Among the dytiscids, the representatives of the Hydroporinae and Laccophilinae achieve very high feeding rates. In laboratory experiments, the larvae of *Coelambus impressopunctatus* captured up to 8 third- and fourth-instar larvae of *Ae. vexans*; the larvae of *Hygrotus inaequalis* and *Hyphydrus ovatus*, up to 3 *Aedes/Ochlerotatus* larvae, and *Hydroporus palustris*, up to a maximum of 10 *Aedes/Ochlerotatus* larvae/day

(Kögel 1984). Whereas the adults of these beetles capture fewer mosquito larvae than do their larvae, the adults of *Laccophilus* spp. are highly voracious. Even the very small species *Guignotus pusillus* (about 2 mm long) can contribute to the reduction of newly-hatched first-instar mosquito larvae. Some beetles of the subfamily Colymbetinae, such as *Colymbetes fuscus* are quoted as potential predators of mosquito larvae (Kühlhorn 1961; Jenkins 1964).

**Gyrinidae:** Because of their mode of living on the water surface, gyrids are voracious predators mainly of *Anopheles* larvae (Laird 1947; James 1966).

**Spercheidae:** Although the adults of this family are not particularly carnivorous, the larvae are considered to be voracious predators with a feeding rate of up to 13 first and second-instar larvae of *Ae. vexans*/day (Kögel 1984).

**Hydrophilidae:** Adult hydrophilids are known to be herbivorous. However, the larvae of some species feed on mosquito larvae and are, therefore, relatively important predators of mosquitoes (Nielsen and Nielsen 1953; Hintz 1951). Remarkably, the larvae of *Helochares obscurus* can capture up to 14 larvae/day (Kögel 1984). Of all the beetle larvae tested in the laboratory, those of *Hydrophilus caraboides* proved to be the most voracious. In laboratory experiments, they consumed a maximum of 67 larvae, with an average of 30 fourth-instar *Ae. vexans* larvae/day. The larvae are frequently observed on the water surface seizing prey with their well-developed mandibles.

## Trichoptera

The importance of caddisfly larvae as predators of mosquitoes has been pointed out in many publications (Martini 1920b; Baldwin et al. 1955; James 1961, 1966; Service 1973a). They are the most important predators of snow-melt mosquitoes in semi-permanent water bodies in swampy woodlands. The 2–3 cm long larvae of *Phryganea* sp. and *Limnephilus* sp. have often been observed capturing larvae of snow-melt mosquitoes.

## Diptera

Among the Diptera, carnivorous larvae of Culicidae and Chaoboridae are common predators of mosquito

larvae. In North America, species of the genus *Toxorhynchites* have been closely studied for decades as antagonists of nuisance/vector mosquitoes (Gerberg and Visser 1978; Trpis 1981; Lane 1992). The females of these carnivorous mosquitoes, which occur mainly in warm climates, do not suck blood but feed on nectar. They prefer to lay their eggs in natural and artificial water containers where the voracious and cannibalistic larvae feed upon other mosquito larvae. They are, therefore, suitable for the control of container-breeding mosquitoes. *Toxorhynchites* sp. were reared and released to control *Ae. aegypti* and *Ae. albopictus*, which breed predominantly in artificial containers (Riviere et al. 1987b; Miyagi et al. 1992; Tikasingh 1992). The inundating release or inoculation of *Toxorhynchites* spp. females, combined with the application of adulticides, can lead to a significant reduction of vector mosquitoes (Focks et al. 1986). The advantage of this process is that *Toxorhynchites* spp. females, when searching for breeding sites, can naturally disperse into habitats that are difficult for humans to find and treat. Unfortunately, the level of control is usually not satisfactory because of the insufficient number of breeding sites often used by *Toxorhynchites* spp. for oviposition, the production of a low number of eggs and the lack of synchrony between predator–prey life cycles. Other species of mosquitoes, which feed on larvae of Culicidae, occur mainly in the tropics and belong to the genera *Anopheles*, *Armigeres*, *Culex*, *Eretmapodites* and *Psorophora*.

In Europe, where *Toxorhynchites* species do not occur, other dipterans closely related to Culicidae, such as the Chaoboridae, are important predators of mosquitoes. The larvae of Chaoboridae can remain motionless horizontally in the water body, where they seize their prey with antennae modified for capturing. In the breeding sites of snow-melt mosquitoes, the larvae of *Mochlonyx culiciformis* can contribute substantially to the reduction of mosquito larvae. *Mochlonyx culiciformis* have adapted their development to that of its' prey, therefore, its' larvae can frequently suppress mosquito populations significantly, particularly if the larval development of the predator precedes that of the mosquito. In laboratory experiments, a fourth-instar larva of *M. culiciformis* captured, on average, eight early instars of mosquitoes or one fourth instar larva/day (Becker and Ludwig 1983). Taking into consideration the long period of

development of about 2 months and the frequent abundance of *M. culiciformis* larvae in snow-melt waters, its importance as a of snow-melt mosquitoes is evident (Chodourowski 1968). In semi-permanent and permanent waters, the larvae of *Chaoborus* spp. are efficient predators and appear mostly in late summer with dense populations. With an average feeding rate of 4 early instar mosquito larvae/day, they are not quite so voracious as *M. culiciformis* (Skierska 1969).

Dipterans which can be predacious on adult mosquitoes are the Dolichopodidae, Empididae, Ceratopogonidae, and Muscidae (genus *Lispe*) (Lamborn 1920; Peterson 1960; Laing and Welch 1963; Service 1965; Clark and Fukuda 1967).

## 16.3 Parasites

Parasites, in this context, are understood as multicellular invertebrates which complete at least one phase of their development within a single host. The most important parasites of mosquitoes are mermithid nematodes.

### 16.3.1 Nematodes

Two families of nematodes are of importance as insect parasites, the Steinernematidae and the Mermithidae (Petersen 1985; Weiser 1991).

The Steinernematidae are effective parasites of terrestrial insects, particularly of larvae that develop in the soil. The use of nematodes such as *Steinernema* spp. or *Heterorhabditis* spp. for the control of Diptera, including *Musca domestica*, is still rather controversial. As for mosquito larvae, it was found that they can only be successfully infected in the laboratory. (Gaugler and Kaya 1990).

Aquatic mermithid parasites are more important for the biological-control of mosquitoes. Several species of mermithid nematodes have been tested as biological-control agents, in various parts of the world (Petersen 1985; Rojas et al. 1987; Vladimirova et al. 1990; Platzer 2007).

Females lay their eggs in the substrate of mosquito breeding sites, where they can survive periods of drought. When flooded and when environmental conditions are suitable, the young nematode larvae (10–20 mm) hatch from their eggs and penetrate as pre-parasites through the cuticle of the target larvae, by piercing the integument of the host and entering through the narrow opening. The larvae grow into post-parasites inside the host larva in a little over a week, or up to several weeks in cold climates. The post-parasite (1–3 cm long) leaves the host by boring through the larval cuticle, which leads to the death of the host larva (Fig. 16.5). The post-parasites grow to sexual maturity in the substrate of the water body, forming females and males. After copulation, the females lay eggs in the soil of streams or ponds where they remain until the mermithid larvae hatch under favourable conditions, migrate to the surface and search for a suitable host. In a few cases, infected host larvae are able to pupate and emerge (Blackmore 1994). In this way infected adults spread the parasites from pool to pool or even reinfect upstream habitats



**Fig. 16.5** Post-parasite of *Romanomermis culicivorax* inside an *Aedes* larva and boring through the cuticle of the larva (Picture courtesy of B. Spreier, Germany)



when they die on the edge of aquatic habitats and release the parasite.

Several species of *Romanomermis* (*R. culicivorax*, *R. iyengari*, *R. nielsenii*) are of great interest for the control of mosquitoes because their life cycle is completed within only a few weeks, and they can be mass-reared. Appropriate methods for the mass-rearing of *R. culicivorax* have been developed (Petersen 1980). A moist substrate of sand containing eggs for the inoculation of mosquito breeding sites became available commercially during the 1970s. Unfortunately, these parasites could not be widely used because of difficulties with transportation and with maintenance of the eggs, as well as with sensitivity of the nematodes towards particular environmental conditions, such as low water temperature or high salinity.

## 16.4 Pathogens

Macroorganisms such as fish have been used for decades as biological control tools in many mosquito control programmes. However, fish and other predators as well as mermithid nematodes have specific ecological requirements and can only be used where their preferred living conditions are met. The life cycle of the predator is frequently not adapted to that of the target organism so that it is unable on its own to bring about an effective reduction of the target population. Mass rearing and release of the predators or parasites is often expensive or even impossible. This limits their large-scale use in a number of specific habitats. Special attention has, therefore, been given to the search for microbial-control agents.

Over the past few decades, efforts on an international scale have led to the discovery of a great variety of pathogens, including entomopathogenic fungi, protozoa, bacteria, and viruses (Chapman et al. 1972; Weiser 1991; Davidson and Becker 1996; Becnel 2006).

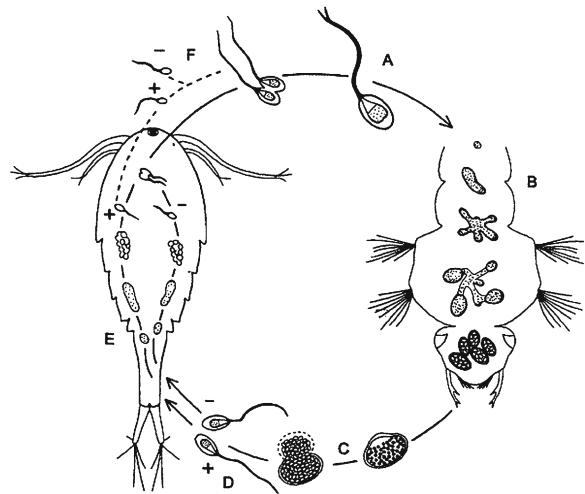
### 16.4.1 Fungi

The fungi that most commonly attack Diptera, belong to the following three groups: (1) Mastigomycotina,

(2) Entomophthorales, and (3) Deuteromycetes. The Mastigomycotina are mainly aquatic organisms. They develop motile zoospores, which are able to propel themselves through the water by means of their flagella. Once they have located a suitable host, they penetrate and develop a mycelium. Fungal pathogens of Diptera are known in the groups Blastocladales, Chytridiales, and Lagenidiales.

About 30 species of *Coelomomyces* belong to the Blastocladales, and infections with *Coelomomyces* spp. are known from more than 50 mosquito species. At first, it proved difficult to infect mosquito larvae in the laboratory. Successful infections were possible on a regular basis, when Whisler et al. (1974) showed that there is an alternation of hosts in which copepods as well as mosquito larvae are involved (Fig. 16.6). Upon infection, a mycelium forms inside the mosquito larva. Yellowish coloured sporangia form at the tips of the hyphae, and the haploid zoospores are developed in these by meiosis (Figs. 16.7–16.9).

Once the zoospores are released, they infect copepods, such as *Acanthocyclops vernalis*, in which a heterothallus is formed which produces isogametes. A biflagellated zygote is formed through fusion of the isogametes. This is the only infectious stage for mos-



**Fig. 16.6** Life cycle of *Coelomomyces psorophorae*. (a) Zygote infective for mosquito larvae; (b) development of hyphal bodies (mycelium) and sporangia at the tip of hyphae; (c) release of zoospores; (d)  $+I-$  zoospores infect copepods; (e) each zoospore develops to a thallus and gametangia which release isogametes;  $+I-$  isogametes fuse to the mosquito infecting zygote (Whisler et al., 1974)



**Fig. 16.7** Larva of *Ae. vexans* infected with *Coelomomyces psorophorae*



**Fig. 16.8** Formation of a sporangium of *Coelomomyces psorophorae* at the tip of a hypha in an anal papilla of an *Ae. vexans* larva

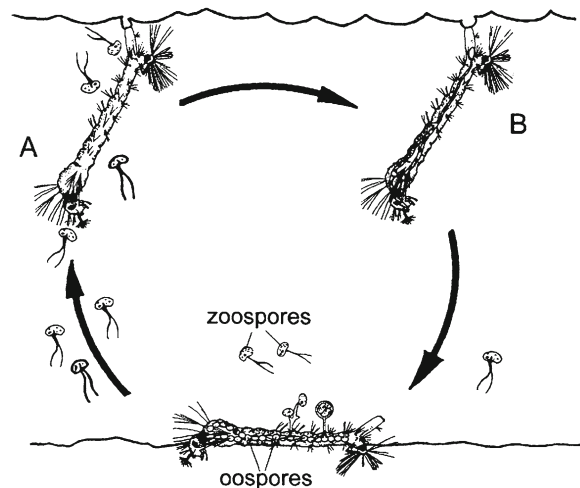


**Fig. 16.9** Sporangium of *Coelomomyces psorophorae* (SEM, magnification 850 $\times$ )

quito larvae. It penetrates through the cuticle into the larval haemocoel. The initial hopes that a satisfactory parasite for mosquito control had been discovered, soon proved unrealistic due to this complex life cycle and associated difficulties in the mass production of the fungi. The same is true for *Coelomyxidium* sp. (Chytridiales).

*Lagenidium giganteum* (Lagenidiales), which goes through an asexual and a sexual developmental stage, has shown the most promising results (Lacey and Undeen 1986; Kerwin and Washino 1988; Kerwin 1992). When introduced into a mosquito breeding site, the motile biflagellated zoospores attach themselves to the larval cuticle. Upon penetration, a fungal mycelium forms inside the larval haemocoel which results in the death of the larva within 2–3 days. In the cadaver, sporangia are developed in which asexually produced zoospores are formed. The zoospores are released through vesicles on the surface of the larval cadaver. Several further asexual cycles take place or the fungus undergoes a sexual cycle which terminates with the formation of oospores which are also infectious for mosquito larvae and are characterized by their great persistence at the breeding site (Fig. 16.10).

After successfully rearing *L. giganteum* in an artificial medium, the fungus was released in large-scale field trials (Kerwin and Washino 1988). High levels of infection were achieved when masses of zoospores were applied. Unfortunately, zoospores are shortlived and disappear from the habitat in the absence of mosquito hosts. Storage of the zoospores is



**Fig. 16.10** Life cycle of *Lagenidium giganteum* (after Woodring and Davidson). (a) biflagellated zoospores (magnified compared to larva) encyst on the cuticle of the mosquito larva; (b) after penetration mycelium develops in the larva and kills it within two to three days. In the cadaver sporangia with exit tubes for the release of asexual zoospores or sexual oospores by fusion of hyphal segments are developed

also difficult. On the other hand, the oospores can persist in the environment, but unfortunately, they only germinate asynchronously, and consequently high-density mosquito populations are only rarely eliminated by a knock-down effect. A combination of different agents which deliver a knock-down and a long-term effect, such as *B.t.i.* preparations, together with oospores of *L. giganteum*, could improve the efficacy of the latter.

The Entomophthorales form various conidia which mainly infect terrestrial insects or their immature stages. *Entomophthora culicis* can infect mosquitoes as they emerge from their pupae (Weiser 1991).

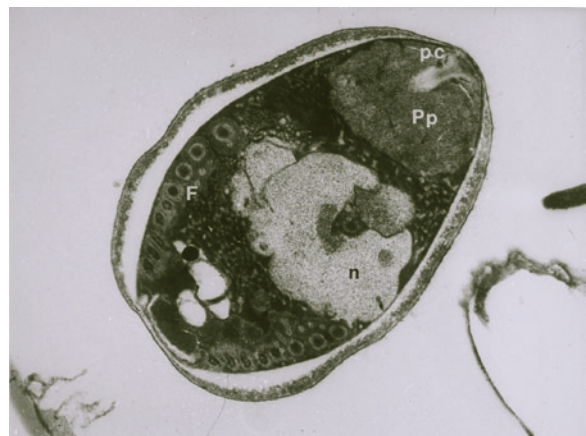
Deuteromycete fungi, such as *Beauveria* sp., *Metarhizium* sp., *Paecilomyces* sp. or *Verticillium* sp., are not host-specific and are not primary pathogens of Diptera. However, their conidia can infect adult mosquitoes, for example, in their shelters during hibernation or resting. In recent years, *Beauveria bassiana* and *Metarhizium anisopliae* have been tested as biological-control agents against adult malaria mosquitoes such as *An. gambiae s.l.* and *An. stephensi* with very encouraging results (Scholte et al. 2003, 2004, 2005). In laboratory studies, the infection of *An. stephensi* with *Beauveria brassiana* reduced malaria transmission by 98% (Blanford et al. 2005). Similar results have been achieved with *An. gambiae s.l.* (Achonduh and Tondje 2008). The infected mosquitoes died before they became infectious to humans, and transmission of *Plasmodium* sp. was also reduced by interrupting mosquito feeding. In a field study, black cotton sheets impregnated with conidia of *M. anisopliae* were hung from the ceilings in five traditional houses in a rural Tanzanian village. Mosquitoes were then collected from these houses for 3 weeks and checked for longevity and fungal infection. About 23% female *An. gambiae* became infected, resulting in a shortening of the life span by 4–6 days compared to mosquitoes collected in five untreated houses. Calculations showed that the use of fungal-impregnated sheets could significantly reduce the number of infective bites in malarial areas from once/night to once every 3 weeks or even less. Apart from its pathogenicity to e.g. *An. gambiae* and *Cx. quinquefasciatus*, *M. anisopliae* exhibits characteristics such as ease and cheap mass-production, considerable shelf-life, safety for vertebrates, and world-wide distribution to make it an attractive agent for bio-control of adult mosquitoes in sub-Saharan Africa.

Other fungi, such as *Tolyopocladium* sp. and *Culicinomyces* sp., are infectious to mosquito larvae and can be produced in artificial media. When the spores of *Culicinomyces clavisporus* are ingested by mosquito larvae, they penetrate the gut wall and proliferate in the larval body. After the death of the larva, the fungus sporulates on the surface of the cadaver. However, effective use of this fungus in mosquito control is unlikely because of its lack of persistence and adequate recycling ability, difficulties in storage, and the high dosages required.

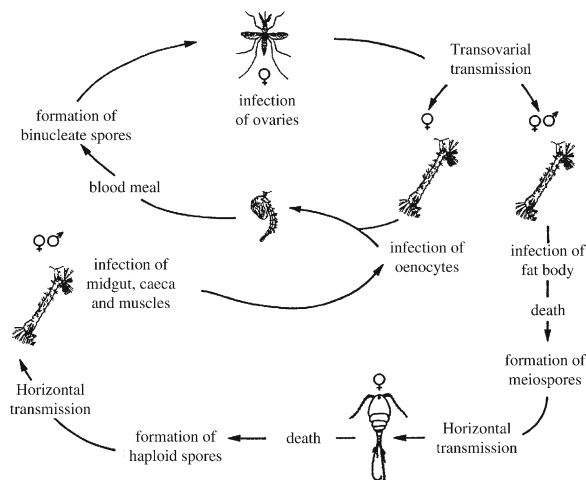
### 16.4.2 Protozoa

The protozoa contain the largest number of mosquito parasites. The best studied are the microsporidian intracellular parasites. Larvae infected with microsporidia can easily be recognised even in the field by their milky-white colour. However, despite the great scientific interest of this group of parasites, no one has yet succeeded in using them as microbial-control agents. This is mainly due to their complex life cycle, which makes mass production difficult, and their frequently low pathogenicity and persistence.

A common feature of all microsporidia is the development of spores which contain a polar filament internally (Fig. 16.11). This is ejected in the host's gut, and



**Fig. 16.11** Ultrastructure of a spore of *Amblyospora* sp. (F = polar filament, Pk = polar body, Pp = polaroplast, n = nucleus)



**Fig. 16.12** Life cycle of *Amblyospora* spp. (after Woodring and Davidson)

in this way, the infectious nucleus is able to penetrate into the host. Two groups of microsporidia are known as mosquito pathogens. The first group, which includes the genera *Nosema* and *Vavraia*, develops asexually and forms only one type of spore. Under laboratory conditions, infection of mosquito larvae can be achieved relatively simply, by feeding them with spores. The second group, which includes the genera *Amblyospora* and *Parathelohania*, has a complex developmental cycle (Fig. 16.12).

An infected mosquito female transmits the spores in a vertical direction, transovarially, to the next generation. In the mosquito larva, the spores can divide meiotically, develop countless haploid spores in the larval fat body and are released when the larva dies (Fig. 16.12).

If spores are ingested by a copepod, they reproduce asexually once again (Andreadis 1985; Sweeney et al. 1985). If, on the death of the copepod, the haploid spores are released and ingested by a mosquito larva, the sexual phase begins with fusion of the cells, which again form diploid spores. Female mosquito larvae which are only lightly infected, can again transmit the diploid spores transovarially to the larvae of the next generation. This complex developmental cycle prevents any mass-rearing of the parasites and makes it unlikely at present for these microsporidia to be used in mosquito control (Sweeney and Becnel 1991; Micieli et al. 2000; Becnel and Johnson 2000).

### 16.4.3 Bacteria

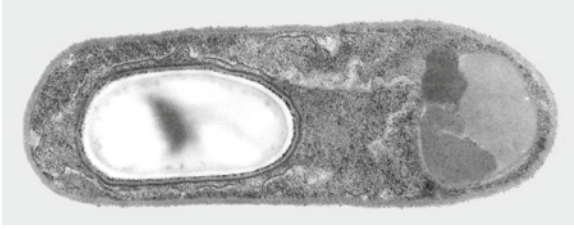
Mosquitocidal bacteria have been known since the early 1960s when the first strains of *Bacillus sphaericus* with larvicidal activity were discovered (Kellen and Meyers 1964). However, these strains were not sufficiently toxic to merit commercial development. The discovery of the gram-positive, endospore-forming soil bacterium *B.t. ssp. israelensis* (*B.t.i.*) in the Negev desert of Israel in 1976 (Goldberg and Margalit 1977) and of the potent strains of *B.s.* in recent years, have inaugurated a new chapter in the control of mosquitoes and blackflies (Singer 1973; Weiser 1984; Becker and Margalit 1993). The newly-discovered subspecies of *B.t.* is target-specific and highly toxic to larvae of most mosquito species and blackfly larvae. New strains of *B.s.*, such as strain 2362, isolated from an adult blackfly in Nigeria (Weiser 1984), and strain 2297, isolated in Sri Lanka (Wickramasinghe and Mendis 1980), are much more potent than the first isolates and are particularly active against larvae of *Culex* species and *An. gambiae* (Ragoonansingh et al. 1992; Fillinger et al. 2003; Majambere et al. 2007). New strains are still being isolated, which are at least as active as the current commercial strain 2362 (Park et al. 2007).

The actinomycete, *Saccharopolyspora spinosa*, was collected from the soil in a rum distillery on a Caribbean Island in 1982. During fermentation, the active compound, spinosad, which contains a tetracyclic ring system to which two different sugars are attached, is produced by the bacteria (Thompson et al. 1997; Sparks et al. 1998). Spinosad-based products have an excellent environmental and mammalian toxicological profile and are effective as bio-control agents against a broad range of agriculturally important insect pests as well as against mosquitoes and tsetse flies (de Deken et al. 2004; Romi et al. 2006).

#### 16.4.3.1 *Bacillus thuringiensis israelensis* (*B.t.i.*)

This *Bacillus* produces protein toxins during sporulation that are concentrated in a parasporal body (PSB), called the protein crystal (Fig. 16.13).

These proteins are highly toxic to mosquito and blackfly larvae. The selectivity of the *Bacillus* toxins derives from a variety of factors.



**Fig. 16.13** *B. thuringiensis israelensis* with spore (left) and parasporal body, so-called protein crystal (right). (Micrographs courtesy of J.-F. Charles, Pasteur Institute, Paris)

- (1) The target-organism must ingest the protein crystal (inactive protoxin), and this depends on its feeding habits.
- (2) The protoxin has to be solubilized in the alkaline midgut milieu of the target insect ( $\text{pH} > 10$ ).
- (3) The target-organism must possess suitable proteases to convert the protoxin into biologically active toxins.
- (4) The target-organism must possess surface receptors (glycoproteins) on the midgut epithelial cells to which the toxins can bind.

This process disturbs the osmo-regulatory mechanisms of the cell membrane, thereby, swelling and bursting the midgut cells (Fig. 16.14). Non-target organisms do not activate the protoxin into the toxin because of their acidic gut milieu, or remain undamaged because of the lack of specific receptors on their intestinal cells

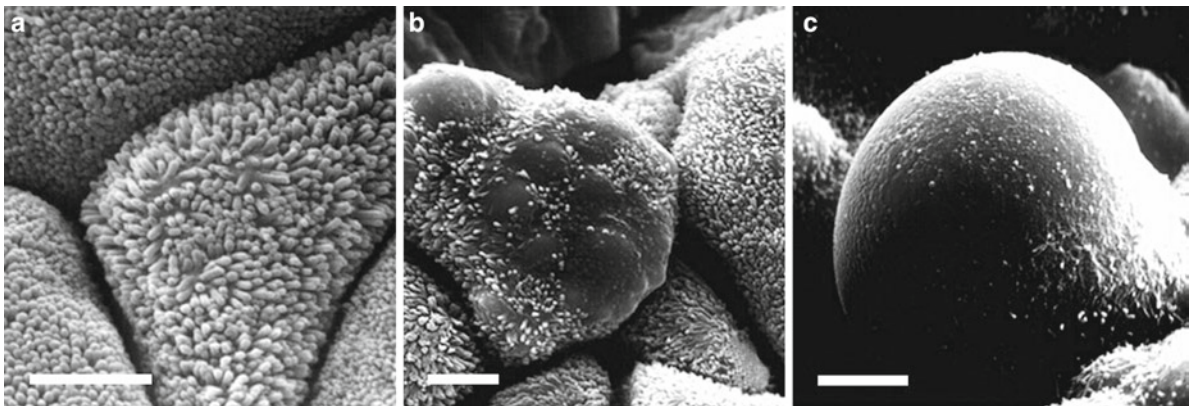
(Charles et al. 2000; Lüthy and Wolfersberger 2000; Boisvert 2005).

The insecticidal effect of *B.t.i.* emanates from the parasporal body, which contains four major toxin proteins of different molecular weight, referred to as the Cry4A (125 kDa), Cry4B (135 kDa), Cry10A (58 kDa) and Cry11A (68 kDa) (Delecluse et al. 1996). These toxins bind to specific glycoprotein receptors on the larval midgut brush border (Charles and Nielsen-LeRoux 1996a,b).

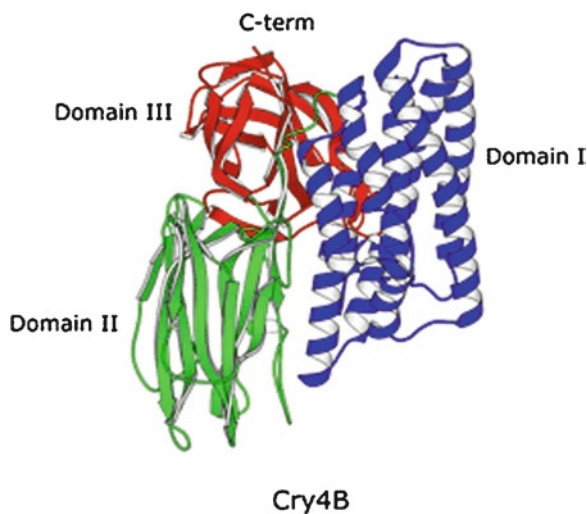
A fifth toxin, called the CytA protein (27 kDa), binds to lipids and does not exhibit the specific binding mechanism which the Cry proteins do (Höfte and Whiteley 1989; Federici et al. 1990; Priest 1992).

The protoxin structure contains three domains (Fig. 16.15). Domain I consists of a bundle of seven alpha-helices, which can insert into the gut cell membrane, creating a pore through which ions can pass freely. Domain II contains three antiparallel beta-sheets, which are thought to bind to receptors in the midgut. Domain III is a beta-sandwich, which obviously prevents further cleavage of the active toxin by gut proteases (Li et al. 1991).

Neither the spore nor the living bacilli appear to be involved in the insecticidal process. The more or less spherical PSB is formed at the end of sporulation and consists of three types of protein inclusions separated by thin layers. The largest inclusion is round, of slight electron density and occupies approximately 50% of the total volume of the PSB. The second type of inclu-



**Fig. 16.14** (a) midgut epithelium of a healthy *Ae. aegypti* larva; (b) 30 min after ingestion of the *B. thuringiensis israelensis* protein crystals, swelling of midgut cells and reduction of microvilli; (c) 1 h after ingestion cell is about to lyse (micrographs courtesy of J.-F. Charles, Pasteur Institute, Paris)



**Fig. 16.15** The *B.t.i.* toxin (*Cry4B*) structure (Graph courtesy of Chanan Angsuthanasombat, Mahidol University, Bangkok, Thailand)

sion (about 20% of the volume of the PSB) is moderately electron-dense and rod-shaped, and the third type of inclusion (about 25% of the PSB) is spherical and highly electron-dense (Ibarra and Federici 1986; Federici et al. 1990).

The mosquitocidal properties of each single solubilized and purified protein have been evaluated in many studies (Beltrao and Silva-Filha 2007; Alam et al. 2008). All tests have shown that each type of protein is mosquitocidal, but none is nearly as toxic as the intact PSB. This high toxicity of the PSB is caused by a synergistic interaction of the 25 kDa protein (split from the 27 kDa protein) with one or more of the higher molecular weight proteins (Ibarra and Federici 1986; Chilcott and Ellar 1988; Chang et al. 1993). It is assumed that CytA may function as a Cry11A receptor in the mosquito midgut (Perez et al. 2005). It is thought that the synergism in the mode of action among the proteins reduces the probability of resistance.

The high toxicity of the PSB to a great variety of mosquito and blackfly species is the most remarkable property of *B.t.i.* Only at significantly higher dosages are some other Nematocera species affected, but no other organisms are harmed.

It has been shown in many studies that plasmids with a molecular weight of 60–94 MDa play an essential role in the crystal toxin production. Cloning and

characterization of the different toxin genes have been accomplished (Bourgouin et al. 1986; Thorne et al. 1986; Ward and Ellar 1988). These results open the possibility of cloning the various toxin genes into host organisms to increase the persistence or mosquitocidal properties.

#### 16.4.3.2 *Bacillus sphaericus* (*B.s.*)

In addition to *B.t.i.*, a second spore-forming bacterium, *B.s.*, has become increasingly important in recent years. The high potential of *B.s.* as a bacterial-control agent lies in its spectrum of efficacy and its ability to recycle or to persist in nature under certain conditions, which means that long-term control can be achieved (Hertlein et al. 1979; Mulligan et al. 1980; Lacey 1990; Ludwig et al. 1994; Silva-Filha et al. 2001). The time-span between retreatments can thus be extended and personnel costs reduced. This opens up the possibility of a successful and cost-effective control of *Culex* species, particularly of *Cx. pipiens* as vector of West Nile virus and *Cx. p. quinquefasciatus*, which is the most important vector of lymphatic filariasis, usually breeding in highly polluted water bodies in urban areas.

*B. sphaericus* can easily be identified by its round spore located terminally in a swollen sporangium (Fig. 16.16). Most recent knowledge indicates that *B.s.* toxins kill only mosquito larvae and, when higher dosages are applied, larvae of Psychodidae also. Certain mosquito species, such as *Cx. p. quinquefasciatus* and *An. gambiae*, are highly susceptible, whereas, *Ae. aegypti* larvae are more than 100-fold less susceptible. Blackfly larvae, other insects, mammals, and other non-target organisms are not susceptible to *B.s.* toxins. A particularly attractive feature



**Fig. 16.16** *B. sphaericus* with round spore and the parasporal protein inclusion (dark structure on the right side of the spore) which is located in a coated “spore crystal complex” (micrograph courtesy of J.-E Charles, Pasteur Institute, Paris)

of *B.s.* is its potential to persist and recycle under certain field conditions. Appropriate formulations have shown a significant residual activity against larvae of *Culex p. pipiens* and *Cx. p. quinquefasciatus* in highly polluted breeding habitats (Hertlein et al. 1979; Nicolas et al. 1987; Davidson and Yousten 1990; des Rochers and Garcia 1984; Lacey 1990; Becker et al. 1995).

The mosquitocidal efficacy of *B.s.* is based on two different types of toxins: (a) parasporal protein inclusions called binary toxin (Bin) and (b) mosquitocidal toxins (Mtx1: 100 kDa; Mtx2: 31 kDa; Mtx3: 36 kDa). The parasporal protein inclusions are located in a coated “spore crystal complex” and consist of proteins of two different molecular weights, a 42-kDa toxin (BinA) and a 51-kDa binding domain (BinB). Both are required for a high level of mosquitocidal activity and are crystallized during sporulation (Broadwell et al. 1990; Baumann et al. 1991; Berry et al. 1991; Priest 1992; Davidson and Becker 1996). The Mtx toxins do not form crystals and degrade quickly upon synthesis during the vegetative stage (Park et al. 2007). The mode of action and receptor-binding is similar to that in *B.t.i.* (Davidson 1988; Davidson and Yousten 1990; Charles et al. 1996b, 2000).

These two microbial-control agents were rapidly developed with the support of industry, universities, and national and international organizations, such as the World Health Organization (WHO). The WHO Pesticide Evaluation Scheme (WHOPES), which promotes and coordinates the testing and evaluation of pesticides for public health, have evaluated microbial-control agents as safe and efficient control agents for public health programmes (WHO/CDS/WHOPES/2004.8). Following extensive safety tests and environmental impact studies, the bacilli were quickly put into use. This rapid exploitation was aided by a series of useful properties of the bacterial-control agents. In addition to the relative ease with which they can be mass-produced, bacterial-control agents are highly efficient, environmentally safe, easy to handle, stable when stored, cost-effective, and suitable for integrated mosquito management (IMM) programmes based on community participation. Furthermore, the costs for development and registration of these agents are many times lower than those of a conventional chemical insecticide. The risk of resistance, especially when *B.t.i.* products are used, is much lower compared to conventional insecticides.

### 16.4.3.3 Environmental Safety

The exceptional environmental safety of bacterial-control agents has been confirmed in numerous laboratory and field tests. The U.S. Environmental Protection Agency (USEPA) categorizes the risk posed by *B.t.* strains to non-target organisms as minimal to nonexistent and approved the use of *B.t.i.* formulations as early as 1981. In safety tests on representative aquatic organisms, it was shown that, in addition to plants and mammals, none of the taxa tested, such as Cnidaria, Turbellaria, Rotatoria, Mollusca, Annelida, Acari, Crustacea, Ephemeroptera, Odonata, Heteroptera, Coleoptera, Trichoptera, Pisces, and Amphibia, appeared to be affected when exposed in water containing large amounts of bacterial preparations (Table 16.1; Becker and Margalit 1993; Boisvert and Boisvert 2000).

Even within the Diptera, the toxicity of *B.t.i.* preparations are restricted to mosquitoes and a few nematoceros families (Colbo and Undeen 1980; Miura et al. 1980; Ali 1981; Garcia et al. 1981; Molloy and Jamnback 1981; Margalit and Dean 1985; Mulla et al. 1982; WHO/IPCS 1999). In addition to mosquito and blackfly larvae, only those of the closely related Dixidae are sensitive to *B.t.i.* Larvae of Psychodidae, Chironomidae, Sciaridae, and Tipulidae are generally far less sensitive than those of mosquitoes or blackflies (Table 16.2).

In contrast to *B.t.i.*, the toxins of *B.s.* are toxic to a much more restricted range of target insects. Blackfly larvae as well as other insects (except for Psychodidae), mammals, and other non-target organisms are not susceptible to *B.s.*

Toxicological tests were carried out using various mammals. *B.t.i.* preparations, when given orally, sub- and percutaneously, intraperitoneally, ocularly, through inhalation and scarification, appeared to be innocuous even at high dosages of  $10^8$  bacteria/animal (WHO 1982a,b, 2000).

Another important aspect is the widespread occurrence of both bacilli in the soil. They are natural components of the soil micro-ecosystem and not an artificial, man-made product, where toxic residues may remain after application against nuisance/vector species.

### 16.4.3.4 Ease of Handling

No special equipment is required for the application of bacterial-control agents. Generally, simple knapsack sprayers are adequate for accessible breeding sites.

**Table 16.1** Organisms not affected by *B. thuringiensis israelensis*

Taxa	Dosage (ppm)	Species
Cnidaria	100	<i>Hydra</i> sp.
Turbellaria	180	<i>Dugesia tigrina</i> , <i>Bothrosomostoma personatum</i>
Rotatoria	100	<i>Brachionus calyciflorus</i>
Mollusca	180	<i>Physa acuta</i> , <i>Aplexa hypnorum</i> , <i>Galba palustris</i> , <i>Anisus leucostomus</i> , <i>Bathymophalus contortus</i> , <i>Hippeutis complanatus</i> , <i>Pisidiuim</i> sp.
Annelida	180	<i>Tubifex</i> sp., <i>Helobdella stagnalis</i>
Acari	180	<i>Hydrachnella</i> sp.
Crustacea	180	<i>Chirocephalus grubei</i> , <i>Daphnia pulex</i> , <i>Daphnia magna</i> , Ostracoda, <i>Cyclops strenuus</i> , <i>Gammarus pulex</i> , <i>Asellus aquaticus</i> , <i>Orconectes limosus</i>
Ephemeroptera	180	<i>Cloëon dipterum</i>
Odonata	180	<i>Ischnura elegans</i> , <i>Sympetrum striolatum</i> , <i>Orthetrum brunneum</i>
Heteroptera	180	<i>Micronecta meridionalis</i> , <i>Sigara striata</i> , <i>Sigara lateralis</i> , <i>Plea leachi</i> , <i>Notonecta glauca</i> , <i>Ilyocoris cimicoides</i> , <i>Anisops varia</i>
Coleoptera	180	<i>Hyphydrus ovarus</i> , <i>Guignotus pusillus</i> , <i>Coelambus impressopunctatus</i> , <i>Hygrotus inaequalis</i> , <i>Hydroporus palustris</i> , <i>Ilybius fuliginosus</i> , <i>Rhantus pulverosus</i> , <i>Rhantus consputus</i> , <i>Hydrobius fuscipes</i> . <i>Anacaena globulus</i> , <i>Hydrophilus caraboides</i> , <i>Berosus signaticollis</i>
Trichoptera	180	<i>Limnophilus</i> sp.
Pisces	180	<i>Esox lucius</i> , <i>Cyprinus carpio</i> , <i>Perca fluviatilis</i>
Amphibia (larvae)	180	<i>Triturus alpestris</i> , <i>Triturus vulgaris</i> , <i>Triturus cristatus</i> , <i>Bombina variegata</i> , <i>Bufo bufo</i> , <i>Bufo viridis</i> , <i>Bufo calamita</i> , <i>Rana esculenta</i> , <i>Rana temporaria</i>

Standard ULV, airblast or mist blower equipment may also be used. In dense vegetation or wide-spread breeding sites, aerial applications are preferable. Rotary seeders or pressurized air sprayers are suitable for the

application of granules. Safety precautions are minimal (compared to the use of toxic agents). Because of the rapid knock-down effect and the high level of efficiency, the success of the treatment can generally be monitored within a day or two after application.

**Table 16.2** Susceptibility of larvae of nematoceran and brachyceran flies to *B. thuringiensis israelensis* (tested Product: VectoBac TP, Potency: 5000 ITU/mg)

Family	Dosage (ppm)	Mortality (%)
Simuliidae		
<i>Simulium damnosum</i> s.l.	0.4	100
Culicidae		
<i>Aedes/Ochlerotatus</i> spp.	0.2	100
Dixidae		
<i>Dixa</i> spp.	2	100
Chaoboridae		
<i>Chaoborus</i> spp.	180	No effect
Psychodidae		
<i>Psychoda alternata</i>	1	100
Sciaridae		
<i>Bradysia</i> sp.	3	90
Chironomidae		
<i>Chironomus</i> sp.	1.8	90
<i>Xenopelopia</i> sp.	4	50
Tipulidae		
<i>Tipula</i> spp.	30	50
Ceratopogonidae	180	No effect
Syrphidae		
<i>Tubifera</i> sp.	180	No effect

#### 16.4.3.5 Cost-Effectiveness

Compared to conventional insecticides, the application of bacterial-control agents is cost-effective when integrated control strategies are designed. For instance, the German Mosquito Control Association (Kommunale Aktionsgemeinschaft zur Bekämpfung der Stechmückenplage-KABS), mosquito abatement project in Germany effectively suppresses mosquitoes emerging from a floodplain area of >600 km<sup>2</sup> involving an average 100 km<sup>2</sup> of actual breeding grounds/year.

The total annual budget of the KABS is €3 million. More than 3 million residents of the area are effectively protected from a serious nuisance (campaign costs/person/year: € 0.92). In a cost-benefit analysis, the benefit for the welfare of the society in this region 3.8 times higher than the costs for the campaign (Hirsch and Becker, 2009). Environmental considerations which cannot be expressed in monetary terms, should also be included in these economic calculations.



### 16.4.3.6 Lack of Potential for Resistance Development

The development of resistance to chemical insecticides represents a serious problem. Bacterial-control agents, however, appear less likely to provoke resistance because their mode of action is more complex (Davidson 1990; Wirth et al. 2005). However, in comparison, resistance in the stored grain pest, *Plodia interpunctella*, to the Lepidoptera specific *B. thuringiensis kurstaki* (*B.t.k.*) has been demonstrated in the laboratory (McGaughey 1985). Studies have shown that the commercial use of *B.t.* preparations in agriculture can lead to resistance within a few years. For example, the diamondback moth, *Plutella xylostella*, which was repeatedly treated with *B.t.k.* preparations on farms in Hawaii, was found to be 41 times more resistant than populations that were only minimally exposed to *B.t.k.* (Tabashnik et al. 1990). Similar resistance phenomena have not yet been observed with *B.t.i.* formulations.

Resistance studies have been conducted by the KABS with populations of *Ae. vexans* which were constantly exposed to *B.t.i.* products over a period of more than 25 years and were, therefore, subjected to constant and intense selection pressure. These mosquitoes were compared with *Ae. vexans* populations taken from a remote location which had never been exposed to *B.t.i.* products and had never been under selection pressure. No reduction in the sensitivity of these mosquitoes to *B.t.i.* products could be detected (Becker and Ludwig 1993; Ludwig and Becker 2005). Similar results were obtained by Kurtak et al. (1989) and by Hougard and Back (1992). They found that after 10 years of intensive applications of *B.t.i.* in West Africa, the susceptibility of the blackfly, *Simulium damnosum*, had not changed.

The complex mode of action of *B.t.i.* toxins partly explains the relative absence of resistance. The lethal changes in the midgut cells are induced only by the synergistic effects of the different toxin proteins present in the parasporal body of *B.t.i.* This combination reduces the likelihood of resistance. On the other hand, when the gene encoding a single toxin protein was cloned into a microorganism and then fed to larval mosquitoes, resistance was induced within a few generations (Georghiou and Wirth 1997).

Resistance is less likely when there is a variable gene pool of target populations. The large size of many mosquito and blackfly populations, thus, inhibits the development of resistance because only a portion of

the population is exposed to the toxin. Floodwater mosquitoes and most blackflies migrate considerably. This behaviour produces a substantial gene flow within their populations which should at least slow the onset of resistance.

However, resistance to *B.s.* toxins has been demonstrated in both the laboratory and the field (Yuan et al. 2000). In southern France, a population of *Cx. pipiens* developed a level of resistance of more than 16,000-fold after 18 applications of *B.s.* treatments (Sinegre et al. 1994). Nielsen-LeRoux et al. (1995) demonstrated in a laboratory population of *Cx. pipiens*, that resistance at a level of 100,000-fold to *B.s.* binary toxin can be caused due to a change in the receptor on midgut brush-border membranes. In other cases, binding to the receptor took place, but there was no toxicity. In all cases, resistance was shown to be recessive (Charles and Nielsen-LeRoux 1996b). It seems that the risk of resistance to bacterial toxins is inversely proportional to the complexity of the mode of action, which is definitely less complex with *B.s.* than with *B.t.i.* toxins.

### 16.4.3.7 Formulations

A basic requirement for the successful use of bacterial-control agents is the development of effective formulations suited to the biology and habitats of the target-organisms. Formulations are available as water dispersible granules (e.g. Vectobac WDG and Vectolex WDG), wettable powder (WP), fluid concentrates (e.g. Vectobac 12AS and Aquabac), corn-cob (Vectobac G and Vectolex G), pellets, tablets (e.g. Vectobac DT/Culicex tablets), briquettes, and ice granules containing the bacilli toxins (Table 16.3).

Biorational mixtures of toxins of both bacilli (*B.t.i.* and *B.s.*), such as VectoMax,<sup>®</sup> inhibit specific advantages. Mixed populations of culicine and/or *Anopheles* populations can be successfully controlled by applying a single product. The likelihood for resistance against *B.s.* can be significantly reduced or even avoided (Zahiri et al. 2002).

Genetic engineering techniques can be used to improve mosquito larvicides based on both bacteria *B.t.i.* and *B.s.* as well as to reduce the risk for resistance against *B.s.* toxins. The best of these recombinants contain all major *B.t.i.* endotoxins, specifically, Cry4A,

**Table 16.3** Some commercially available *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* products used in mosquito and blackfly control programmes

Product	Formulation	Potency (ITU/mg)
<i>B. thuringiensis israelensis</i> (against mosquito and blackfly larvae)		
Aquabac <sup>®1</sup>	Primary powder	7,000
Icepearls <sup>3</sup>	Ice granules	120
Teknar HP-D <sup>®2</sup>	Fluid concentrate	1,200
Teknar TC <sup>®2</sup>	Technical powder	10,000
Teknar G <sup>®2</sup>	Granules	200
VectoBac I2AS <sup>®2</sup>	Fluid concentrate	1,200
VectoBac TP <sup>®2</sup>	Technical powder	5,000
VectoBac WG <sup>®2</sup>	Water dispersible granules	3,000
VectoBac DT <sup>®2,4</sup>	Tablets	2,200
Culindex Tab plus <sup>®2,4</sup>	Tablets	2,200
Lossquito <sup>®5</sup>	Fluid concentrate	1,200
<i>Bacillus sphaericus</i> (against mosquito larvae)		
Spherimos <sup>®2</sup>	Fluid concentrate	120
VectoLex WDG <sup>2</sup>	Water dispersible granules	650 BsITLJ/mg
VectoLex <sup>®2</sup>	Granules	50BsITLJ/mg
Combined <i>B.t.i./B. sphaericus</i> product		
VectoMax G <sup>2</sup>	Granules	50BsITU/mg
VectoMax WSP <sup>2</sup>	Water soluble pouches	50 BsITU/mg

<sup>1</sup>Becker Microbial Products, USA

<sup>2</sup>Valent BioSciences Corp., USA

<sup>3</sup>Icybac GmbH, Germany

<sup>4</sup>Culindex GmbH, Germany

<sup>5</sup>BioDalia, Israel

Cry4B, Cry11A, and Cyt1A, plus the binary (Bin) endotoxin of *B.s.* The presence of Cyt1A in these recombinants, which synergizes Cry toxicity and delays resistance to these proteins and the *B.s.* binary toxin, should enable long-term use of these recombinants with little if any development of resistance (Federici et al. 2007).

A few hundred grams or even less of powder, half to two litres of liquid concentrate or a few kilograms of granules/ha, are usually enough to kill all mosquito larvae. In some situations, a long-term effect can be achieved if larger amounts are used (Becker and Margalit 1993; Becker and Rettich 1994; Russell et al. 2003; Rydzanicz et al. 2009). With the production of tablets, water-soluble pouches or briquette formulations, progress has been made towards achieving long-term effects (Kahindi et al. 2008; Su 2008).

Tablet formulations, based on *B.t.i.* or *B.s.* sterilized by  $\gamma$ -radiation to prevent contamination of drinking

water with spores, can be successfully used for control of container-breeding mosquitoes such as *Cx. p. pipiens* or *Ae. aegypti* or even against *An. gambiae* (Becker et al. 1991; Kroeger et al. 1995; Mahilum et al. 2005).

In addition to commercially available granules based on ground corn-cobs, sand granules can also serve as a carrier for wettable powder formulations: 50 kg fire-dried quartz sand (grain size 1–2 mm) with 0.8–1.4 l of vegetable oil (as a binding material) and 1.8 kg of *B.t.i.* powder (activity >5,000 ITU/mg) should be mixed in a cement mixer. This mixture is sufficient to treat 2–3 ha. Recently, more cost-effective granules were developed in the form of ice pellets (Becker 2003). Ice granules can be easily produced when water suspensions containing the bacterial toxins are frozen into small ice cubes or “pearls” (3–5 mm) and kept in cold storage rooms until used. The advantages of using ice granules are:

- (1) The toxins are bound in the ice pellet, so loss of active material by friction during application is avoided.
- (2) As the specific weight of ice is less than that of water, the ice pellets remain in the upper water layer where they release the toxins into the feeding-zone of mosquito larvae as they melt.
- (3) The ice pellets penetrate dense vegetation and do not stick to leaves even when it is raining and is less sensitive to wind.
- (4) There is increased swath because the friction is reduced due to the physical properties of ice.
- (5) The production is cost-effective and
- (6) the “carrier” is water.

The amount of active material/ha can, thus, be significantly reduced when compared with granules based on sand.

When appropriately stored, most preparations based on bacterial toxins can be kept for long periods without losing activity. Experience has shown that powder or corn-cob formulations lose no or only little of their activity even after many years in storage. On the other hand, the activity of fluid concentrates may be more labile. Preparations should, therefore, be re-tested in bioassays according to WHO guidelines when they have been stored for more than a year.

Standardized methods for bioassays have been developed to determine the LC<sub>50</sub> values using standard formulations (de Barjac 1983; Dulmage et al. 1990; Skovmand and Becker 2000). The procedure for bioassays is described in Chap. 4.

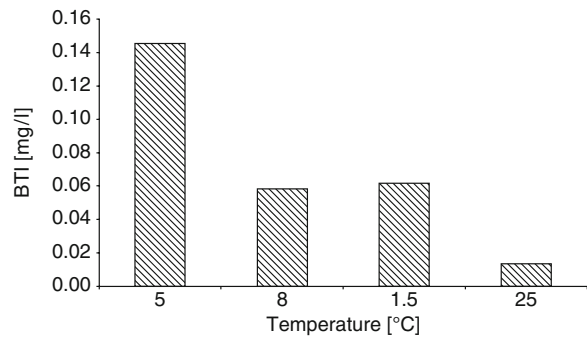
### 16.4.3.8 Factors Influencing the Efficacy of Bacterial-Control Agents

In addition to the different susceptibility of various mosquito species to bacterial toxins, a variety of factors can influence their efficacy (Becker et al. 1993; Ludwig et al. 1994; Puchi 2005, Sorensen et al. 2007). This efficacy depends upon the developmental stage of the target-organisms, their feeding behaviour, organic content of the water, the filtration effect of target-larvae as well as that of other non-target organisms, photosensitivity, and other abiotic factors, such as water temperature and depth, the sedimentation rate, as well as, the shelf-life of *B.t.i.* and *B.s.* formulations (Mulla et al. 1990; Becker et al. 1992). The long-term effect is also strongly influenced by the recycling capacity of the agent (Aly 1985; Becker et al. 1995a).

**Species and Instar Sensitivity:** Larvae lose their sensitivity to bacterial toxins as they develop (Becker et al. 1992). For instance, the second-instar larvae of *Ae. vexans* are about 11 times more sensitive than the fourth-instar larvae, at a water temperature of 25°C (second-instars:  $LC_{90} = 0.014 \pm 0.007$  mg/l; fourth-instars:  $LC_{90} = 0.149 \pm 0.004$  mg/l). The differences in sensitivity are less at temperatures between 8 and 15°C. Nonetheless, the second-instars of *Ae. vexans* are more than twice as sensitive as the fourth-instars at a water temperature of 15°C (second-instars:  $LC_{90} = 0.062 \pm 0.025$  mg/l; fourth-instars:  $LC_{90} = 0.145 \pm 0.004$  mg/l). In field experiments, only half the dosage required to kill the third-instar larvae is needed for the second-instar larvae. If the fourth-instar larvae are dominant, then the dosage must be doubled again. It is, therefore, recommended that control measures commence while the larvae are at an early developmental stage.

Large differences in sensitivity can also be distinguished between various mosquito species due to differences in their feeding habits and their ability to activate the protoxin and the toxin to be bound to midgut cell receptors. For instance, larvae of *Cx. pipiens* were found to be 2–4 times less susceptible to *B.t.i.* toxins than Aedini larvae of the same instar. By contrast, larvae of *Cx. pipiens* are highly sensitive to the toxins of *B.s.*, whereas *Aedes/Ochlerotatus* larvae are much less so.

**Temperature:** The feeding rates of mosquito larvae are influenced by water temperature (Fig. 16.17). For instance, the feeding rate of *Ae. vexans* decreases as temperature decreases, and this is accompanied by a reduction in consumption of bacterial toxins. In



**Fig. 16.17** Impact of the temperature on the *B. thuringiensis israelensis* sensitivity of second-instar larvae of *Aedes vexans* (values are given as  $LC_{90}$ )

bioassays, the second-instar larvae of *Ae. vexans* are more than ten times less sensitive at 5°C than at 25°C (5°C:  $LC_{90} = 0.145 \pm 0.065$  mg/l; 25°C:  $LC_{90} = 0.0142 \pm 0.007$  mg/l) (Becker et al. 1992). The same effect was found when the fourth-instar larvae were tested. Application of bacterial formulations should be conducted at a temperature threshold of 8°C when Aedini species are to be controlled (Fig. 16.17).

**Size of the Water Body:** Because bacterial toxins diffuse throughout the entire body of water, deep water requires higher rates than shallow water of the same surface area. Because larvae of many mosquito species feed near the surface, effectiveness depends on the concentration and durability of the toxins in the upper portion of the water body.

**Larval Density:** Bioassays with *Ae. vexans* have shown that with higher numbers of larvae, the amounts of *B.t.i.* toxins must be increased (Becker et al. 1992). At a density of 10 fourth-instar larvae/150 ml water, the  $LC_{50}$  value was  $0.0162 \pm 0.004$  mg/l; with 75 larvae, the  $LC_{50}$  was ~7 times higher ( $LC_{50} = 0.1107 \pm 0.02$  mg/l). The presence of other filter-feeding organisms, such as Cladocera, cause similar effects. In tests with *B.t.i.* preparations, the  $LC_{50}$  and  $LC_{90}$  values were 5 and 6 times higher at a density of 90 *Daphnia* sp./150 ml than were those without *Daphnia* sp.

**State of Nutrition:** The state of nutrition and the amount of available food influence the sensitivity of mosquito larvae to microbial-control agents. In laboratory studies, two or three times more *B.t.i.* toxins were required for an equal level of mortality in the presence of added food (or polluted water) compared with clean water (Mulla et al. 1990).

*Sunlight:* Although radiation, such as that from a Cobalt<sup>60</sup> source, is well suited for the sterilization of *B.t.i.* products without significantly reducing their toxicity (Krieg 1986; Becker 2002), strong sunshine appears to reduce their larvicidal effect. For example, *B.s* preparations were active for >3 times longer in shaded water than in water exposed to the sun. The LC<sub>90</sub> values obtained with *B.t.i.* powder (6,000 ITUs/mg) in bioassays with third-instar *Cx. p. pipiens* in sunny sites (6,000–12,000 lux for 7 h) and in shaded sites (<150 lux) at the same time and under identical conditions ( $t=25 \pm 1^\circ\text{C}$ ) were very different (Becker et al. 1992). The LC<sub>90</sub> value was  $0.054 \pm 0.008$  ppm under shaded conditions, whereas it was ~4 times higher at sunny sites (LC<sub>90</sub> =  $0.235 \pm 0.036$  ppm).

*Recycling Processes:* A particularly attractive feature of *B.s.* is its high efficacy against *Culex* and *Anopheles* species and its potential to persist and recycle under certain field conditions (Des Rochers and Garcia 1984). In laboratory tests, it was shown that the presence of mosquito larval cadavers in the water contributes to the maintenance of toxic levels of *B.s.* Larval cadavers seem to contain all the nutrients necessary, both for vegetative multiplication of the bacteria and for toxin synthesis associated with the sporulation process. Aly (1985) was also able to demonstrate, experimentally, the germination of *B.t.i.* in the gut of Aedini larvae. When compared with other environmental conditions, it seems that larval cadavers are of crucial importance for the recycling processes (Becker et al. 1995a).

It is important to understand the impact of all these factors on routine treatments, particularly because it allows the correct calculation of the optimal dosage, the selection of the right formulation in various environmental situations, and the optimal timing for application against different mosquito species (Becker and Rettich 1994).

#### 16.4.3.9 Suitability for Integrated Mosquito Control Programmes with Community Participation

Bacterial-control agents are particularly well suited for the use in integrated programmes because their toxic effect is selective, so they do not affect predatory organisms. These agents and predators can therefore be included as additional elements in an integrated management strategy. The effect of predators can continue after the bacterial-control agents have been

applied. This, indirectly, produces a sustained suppressive effect (Mulla 1990; Becker 1992).

Bacterial-control agents can be mixed with other larvicides, such as preparations that are applied to alter the surface film which enhances diffusion over the water surface and destroys mosquito pupae that are not affected by microbial toxins (Roberts 1989).

The WHO “primary health care concept” increasingly seeks to involve local residents in the search for solutions to health care problems. Bacterial-control agents have a considerable safety advantage over synthetic insecticides because neither the operator nor the occupants of treated sites become exposed to potentially dangerous chemicals. For this reason, such preparations are particularly well suited for use by volunteers.

Applications of bacterial toxins do not harm beneficial organisms, such as honey bees, silkworms, or aquatic animals such as fish, shrimps or oysters. These formulations can, therefore, be used in ecologically sensitive areas. Because they are biodegradable, no toxic residues remain after their use. Their environmental safety permits bacterial-control agents to be accepted both by officials and the general public. Indigenous isolates found in the country in question can be used.

#### 16.4.3.10 Use of Microbial-Control Agents in Integrated Mosquito and Vector Management Programmes

Many control programmes aim at integrated biological control (IBC) strategies, which collectively affect the protection of humans against mosquitoes and the conservation of biodiversity with minimal impact on the environment. When the ecosystem is compared with a web and each group of organisms represents one single mesh, the control strategy is to effectively reduce one single mesh (representing mosquitoes) without affecting other meshes in the “food web.”

This goal could only be optimally achieved when microbial-control agents are used as a component in the overall IBC strategy. The conservation and encouragement of predators are important components of IBC. Therefore, microbial agents and biological control methods are integrated with environmental management (e.g. improving of ditch systems for regulating water levels and creating permanent habitats for aquatic predators, such as fish). IBC programmes have been successfully implemented in the US, Canada and Europe.

For the successful implementation and use of microbial-control agents, the following prerequisites are necessary (see chapter 22): entomological investigations, precise mapping and logging data on all major breeding sites, assessment of effective dosages in bioassays and conducting field tests, adaptations of application techniques compatible to the requirements in the field, design of the control strategy as well as training of the field staff and finally, dealing with governmental bureaucracies.

Mosquitoes breed in a great variety of habitats. Almost everywhere, where stagnant waters are present, the probabilities of finding mosquito larvae will be high. Therefore, the formulations and dosages considered, must be compatible with the ecology of breeding sites and factors affecting the efficacy of microbial-control agents.

Two different modes of application of *B.t.i.* preparations should be considered: aerial and ground applications. Helicopters equipped with conventional buckets, booms, and nozzles or rotary mist atomizers and/or fixed-wing aircrafts are commonly used to treat densely vegetated or/and large surface areas. For ground treatments, the knapsack sprayers or motorized backpack blowers are the conventional equipment used with diluted material or granular formulations.

Various commercial products of *B.t.i.* and *B.s.* are used in many countries, on almost all continents (Becker and Margalit 1993; Russell et al. 2003; Puchi 2005; Boisvert 2005; Kahindi et al. 2008). In North America, microbial-control agents have been successfully used for ~25 years in nuisance insect control programmes and since the beginning of the new millennium to control mosquitoes transmitting West Nile virus. In Quebec, each year, more than 50 tons of formulations of *B.t.i.* are used against spring species (*Aedes* and *Ochlerotatus*) and summer species (*Culex*, *Coquillettia*, *Anopheles*) to reduce populations of potential vectors of West Nile virus. Similarly, in other Canadian provinces and/or various states in the US and in Germany, adulticides are seldom used to control mosquito populations.

For the past 3 decades in Germany, *B.t.i.* and *B.s.* preparations have been successfully used against floodwater mosquitoes (*Ae. vexans*) and *Culex* spp. (*Cx. p. pipiens* biotype *molestus*). Over 3,000 km<sup>2</sup> of breeding sites have been treated with *B.t.i.* preparations in the past 3 decades, resulting in a reduction of the mosquito population every year by >90%. The flood plains of the Rhine are usually inundated several times each summer. The extent of the

flooding depends on the snow-melt in the Alps and on rainfall, and it is necessary to constantly monitor the water flow in the Rhine and in the flood plains. During flooding, *Ae. vexans* and other floodwater species hatch within minutes or hours, at temperatures exceeding 8°C. Prior to control measures, the larval density and the larval stages are checked by dip samples at representative breeding sites to justify actions taken and to establish correct dosages and the appropriate formulations applied. One day after the application, spot samplings by dipping are taken to check mosquito density to establish treatment efficacy.

Depending upon the extent of the flooding, 20–30% of the potential breeding sites (~600 km<sup>2</sup>) are treated routinely by the 400 collaborators of the KABS. About 200–400 g of Vectobac WDG (3,000 ITU/mg) or 0.5–1 litre of the liquid concentrate Vectobac 12AS (1,200 ITU/mg) are dissolved in 9–10 litres of filtered pond water for each hectare treated and applied by knapsack sprayers (Fig. 16.18). For polluted breeding sites or



**Fig. 16.18** Ground application of microbial-control agents by means of knapsack sprayers

when late instar larvae are present, the dosage has to be increased.

During the most severe floods, usually a third of the area has to be treated with *B.t.i.* granules which are dispensed with the aid of a helicopter (dosages: 10–20 kg/ha). From 1981 to 2009, 100 tons of *B.t.i.* powder and fluid concentrate and almost 1,000 tons of *B.t.i.* ice and sand granules mixed with quartz sand, vegetable oil and *B.t.i.* powder were used, to treat thousands of hectares of floodwater mosquito breeding sites by ground application or by helicopters (Fig. 16.19).

Control of urban mosquito species is mainly carried out by the residents. To assist with this, KABS provides information on the biology of *Cx. p. pipiens* biotype *molestus* and the appropriate control measures. Vectobac® DT/Culindex® tablets, which contain toxins of one or both bacilli, have been very successful. They kill *Culex* larvae in water containers over a period of several weeks. In drainage systems and large cesspools with eutrophic water bodies, *B.s.*, as a liquid or powder formulation, is applied against *Culex* larvae. Each year,



**Fig. 16.19** Aerial application of *B.t.i.* granules

about 1 million *Culindex-B.t.i./B.s.* tablets are successfully used by residents against *Cx. p. pipiens*, especially in rainwater containers.

#### 16.4.3.11 Monitoring the Programme

Approximately 8% of the KABS budget is invested in monitoring mosquito populations, mosquito resistance and environmental impact. All the studies conducted to date, have demonstrated that the introduction of *B.t.i.* and *B.s.* have reduced the numbers of nuisance mosquitoes to an acceptable level without any adverse effects on the diversity and beauty of the ecosystem.

*Monitoring Mosquito Abundance:* Monitoring populations of adult mosquitoes is achieved by setting adult mosquito traps placed at strategic sites throughout the flood inundation area. The CO<sub>2</sub>-baited CDC/EVS traps are set overnight twice/month, from April to September. Mosquitoes trapped from areas where no control measures have been undertaken serve as points of reference and are compared to the numbers of mosquitoes trapped from treated areas, to determine efficacy of control measures. It has been shown that since the widespread application of *B.t.i.* preparations in 1981, mass occurrences of mosquitoes have been successfully suppressed. Naturally, these control strategies have received an extremely positive reception among the local residents.

#### 16.4.3.12 Monitoring Environmental Impact

It became essential to document the environmental impact of *B.t.i.* and *B.s.* preparations, to provide a scientific basis for rebutting arguments commonly raised to deter mosquito control by lobby groups. Before large-scale application of microbial-control agents was undertaken, the most important members of various aquatic groups (Cnidaria to Amphibia) were screened in the laboratory and in small-scale field trials for their susceptibility to microbial-control agents. This study showed that in addition to mosquitoes (Culicidae) and blackflies (Simuliidae), only a few species of nuisance midges (*e.g.* Chironomidae) were affected by *B.t.i.* toxins. For the most part, these nuisance midges were much less susceptible to *B.t.i.* toxins than the target-organisms.

The development of insects in treated and untreated waters is regularly monitored using emergence traps. The occurrence and abundance of insects in treated areas is routinely assessed by light traps. All investigations have shown that whereas the numbers of *Aedes/Ochlerotatus* mosquitoes are drastically reduced, all other insects continue to develop in the water and, as winged adults, provide a food source for birds, amphibians, and bats.

#### 16.4.3.13 Monitoring Resistance

Mosquito populations should be checked at regular intervals for resistance development. Resistance has not yet been detected after more than 25 years of *B.t.i.* applications (Ludwig and Becker 2005). To prevent development of resistance to *B.s.* in *Culex*, *B.s.* and *B.t.i.* preparations are used alternately in the overall management plan for this species. Combined products like VectoMax which contains toxins of both bacilli can be used against mixed populations of floodwater and *Culex* spp. and so reduce the risk for resistance against *B.s.* toxins.

#### 16.4.3.14 The Role of Microbial-Control Agents in Malaria Control Programmes

The recently initiated global campaign against malaria, particularly in Africa, led by major international organizations, such as Roll Back Malaria (RBM), World Health Organization (WHO), President's Malaria Initiative (PMI), Bill and Melinda Gates Foundation (BMGF), and Global Fund to fight AIDS, tuberculosis and malaria (GFATM), aim to significantly reduce malaria-related mortality after full implementation of the programme. The cornerstones of these strategies include the extension of the facilities for rapid case-detection and disease treatment, prophylactics, personal protection, such as long-lasting insecticide treated nets (LLINs) against adult vector-mosquitoes and indoor residual spraying (IRS) (RBM 2005; Makundi et al. 2007; Protopopoff et al. 2007a,b; Aregawi et al. 2008).

The emergence of resistance to both, pesticides and drugs, is the most dangerous and difficult problem to solve (Etang et al. 2004; N'Guessan et al. 2007). Furthermore, the use of insecticide treated bed-nets

(ITNs) and indoor spraying, focus only on the control of endophagic and endophilic anophelines, exophagic and exophilic species could still transmit malarial pathogens.

Therefore, research on the development of locally viable IVM/IMM strategies, including larval control and development, testing and/or implementation of sustainable, environmentally-sound, and cost-effective alternative strategies to DDT use, was strongly encouraged (WHO 2004b). Mosquito larval control, by use of larval source management (LSM), is the management of mosquito breeding sites using larvicides, such as *B.t.i.* or *B.s.* preparations, natural predators or implementing water management techniques to reduce vector populations. Contrary to adult vectors, the developmental stages of mosquitoes are usually confined in great numbers within relatively small aquatic habitats, therefore, control efficacy is greatly enhanced.

Control of vector-mosquito populations in their aquatic larval habitats offers an opportunity to significantly enhance the protection afforded by existing vector management strategies and consequent malaria reduction (Killeen et al. 2000a,d; Fillinger and Lindsay 2006; Dongus et al. 2007; Walker and Lynch 2007; Worrall 2007). Vector-mosquito population suppression, afforded by larval control, may also reduce selection pressure towards resistance to insecticides utilized for ITN/LLIN and IRS by reducing the rate of insect-chemical contact in localized populations.

Specifically, the use of *B.t.i.* and *B.s.* preparations for mosquito-larval control has been demonstrated to be highly effective for the control of malaria vector-mosquitoes in Africa (Killeen et al. 2002a,b; Yohannes et al. 2005; Fillinger and Lindsay 2006; Majambere et al. 2007; Walker and Lynch 2007; Fillinger et al. 2008).

Cost analysis also indicates that bio-control of larval mosquito populations is within the cost-range of other interventions that have been described as among the most cost effective. Worrall (2007) concluded that the cost/person, protected by larval control under various African scenarios, range from US\$0.94–2.50/person/year, correlating negatively with human population density. Fillinger and Lindsay (2006) suggested an even lower estimate of less than US\$0.90/person/year.

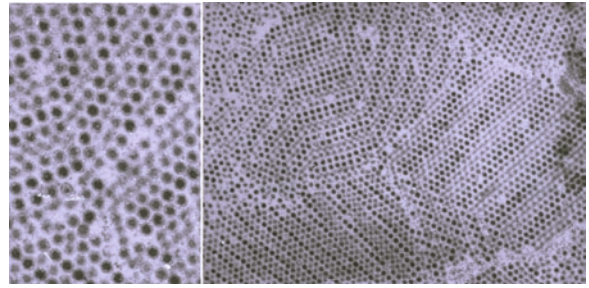
Cost is not the only factor that should be considered, control should also be ecologically sound. The cost analyses above, did not consider the additional benefits of using biological larval control, namely,

reducing the use of chemical larvicides and development of insecticide resistance, lack of impact on non-target organisms, and safety of workers and the human population at large.

#### 16.4.4 Viruses

None of the viruses, at present, are suitable for the control of Diptera, although a number of viruses have been isolated from different members of the order (Hunter-Fujita et al. 1998).

Nuclear polyhedrosis virus and cytoplasmic polyhedrosis virus are both known as common pathogens of Lepidoptera, but these viruses are less frequent in Dipteran populations and usually have low pathogenicity; therefore, they are not practical for controlling mosquitoes.



**Fig. 16.20** Iridescent viruses in the fat body of an *Aedes cantans* larva, 160 000 (left) 7500× (right)

Iridescent virus infections are most common in mosquito larvae (Fig. 16.20). The infected larvae are blue-green or violet iridescent. None of these viruses or any of the parvoviruses, known to be infectious for *Ae. aegypti*, *Cx. p. pipiens* or *Simulium vittatum*, can yet be used as potential microbial-control agents (Weiser 1991).



# Chapter 17

## Environmental Management of Mosquitoes

### 17.1 Introduction

The concept of environmental management for mosquito control spans a broad range of measures, designed to suppress mosquito populations and the risk of disease transmission. In 1980, the WHO Expert Committee on Vector Biology and Control defined three basic categories of environmental management for vector control:

- Environmental modification: long-lasting physical transformation of vector habitats.
- Environmental manipulation: temporary changes to vector habitat as a result of planned activity to produce conditions unfavourable to vector breeding.
- Changes to human habitation or behaviour – efforts to reduce human/vector/pathogen contact.

Before the introduction of synthetic insecticides in the mid twentieth century, environmental management measures were the primary techniques used for mosquito control and disease reduction. In Malaysia, Watson (1911) showed that draining and filling swamp areas resulted in a dramatic fall in the numbers of malaria cases. Ross (1913) described the importance of “proofing” buildings against mosquito entry as an essential part of malaria prevention. In the USA, ditching of salt marshes became very widespread in the 1930s to abate mosquito nuisance (Carlson 2006). The advent of highly effective synthetic insecticides in the mid-twentieth Century initially resulted in a wane in interest in environmental management measures, but in recent decades there has been a resurgence in incorporating these concepts into integrated mosquito management (IMM) programmes. This is a result of several factors, including the onset of insecticidal resistance, concern over the potential for unwanted environmental

or safety impacts of pesticide use, the need for more sustainable approaches to pest and vector management, and the desirability of communities taking more ownership of local pest and vector management.

Environmental management of mosquitoes may include a very wide range of interventions, such as proper water storage containers, proper water management practices (including every possible effort to reduce stagnant water), modifications to existing sewage works, changes in rice husbandry and laser-leveling (if available) of agricultural fields to drastically reduce stagnant water at tail-ends of fields, redesign of bus shelter roofs, ditching of salt marshes, routine collection and proper disposal of certain items of urban refuse, making earnest attempts to screen-out adult mosquitoes from entering homes, training of health department personnel and community activists, soliciting cooperation and support of local politicians, and the preparation and distribution of educational leaflets about mosquitoes to communities. Such interventions often require a multidisciplinary approach, perhaps involving entomologists, civil engineers, agronomists, ecologists, behavioural scientists, economists, local politicians, and community representatives.

Environmental management concepts may sometimes be difficult to elucidate and/or implement. The efficacy of environmental management measures, such as source reduction, may not bear a simple linear correlation with the numbers or proportion of mosquito breeding sites removed. Gu et al. (2006) argued that source reduction exerts its effect not only by reducing the number of adult mosquitoes produced, but also by extending generation times by forcing the remaining gravid females to spend more time searching for oviposition sites. Whatever the mechanisms involved, the benefits of source reduction are substantial, especially

because these concepts result in long-term reduction of mosquito breeding sites. Newton and Reiter (1992) used a deterministic model of dengue transmission to argue that techniques such as source reduction, that provided a long-term reduction of *Aedes aegypti* [*St. aegypti*] populations, were actually more effective and sustainable than ultra low volume (ULV) insecticide treatment, in combating dengue outbreaks.

## 17.2 Environmental Management of Mosquitoes in Urban Areas

Urban areas provide an ideal habitat for many mosquito species, with abundant availability of blood meals, and a wide range of water bodies, ranging from flower vases at cemeteries, bromeliad plants and rain-filled coconuts, to highway drainage structures and faulty sewage systems. Appropriate environmental management interventions will range from the design of urban structures, to the provision of information to cemetery visitors, to public information websites. The following sections will highlight some of the more important and well-documented examples of environmental management concepts in reducing mosquito breeding sites in urban areas, but is by no means exhaustive.

### 17.2.1 Denying Mosquito Access to Urban Areas and into Homes

A number of mosquito species tend to disperse and feed close to ground level. Bellini et al. (1997) working with *Ae. caspius* in the Po delta in Italy, found that this species flies close to ground level, and proposed that a barrier of 3-4 m height and 2.7 km long, would prevent mosquitoes moving from wetlands to residential and tourist areas, without the need for insecticide use. Although such techniques have been previously proposed, there is little evidence of them being used in practice.

Proofing dwellings against the entry of mosquitoes is one of the oldest techniques used to reduce nuisance biting and disease transmission (Ross 1913). However, although such techniques and details have become part of vernacular architecture around the world, they have

received little critical attention and evaluation. Lindsay and Snow (1988) found that the number of *An. gambiae* in houses in Gambia with closed eaves was 43% less than in houses with open eaves. Children who slept without bed nets were found to be at lower risk of malaria in houses without eaves openings, as compared to houses with open eaves.

On the island of Sao Tome off the coast of west Africa, houses built on stilts had about half the number of *An. gambiae* in them than houses built at ground level (Charlwood et al. 2003). This tendency for greater mosquito activity close to ground level is also seen in *Cx. quinquefasciatus*, where Howell and Chadee (2007) found that the numbers of mosquitoes resting in houses on stilts was significantly reduced compared to houses built at ground level.

Lindsay et al. (2003) reviewed a range of modifications to dwellings and concluded that they were often of low cost, were long-lasting, and had the potential to reduce the intensity of malaria transmission in many parts of the world.

### 17.2.2 Construction Sites

Construction sites will often host a variety of water bodies, including water storage containers, water-filled excavations, flooded basements, wheel ruts, and temporary drainage systems. Such habitats will host a range of mosquitoes, including *Aedes*, *Culex* and *Anopheles* species (Baumgartner 1987; BoonTeng 1997). In some cases, mosquito breeding within a site is believed to be responsible for a disease outbreak amongst those working on the site itself (Adak et al. 1994). Prevention of mosquito breeding in such areas is typically achieved by a combination of education and awareness for developers on the need to avoid standing water, and law enforcement where guidance and regulations are not followed (WHO 1986; Seow-Boo 2001).

### 17.2.3 Water Storage Containers

In many areas, water supply is not available directly from a main system to every home, instead it is supplied via a community standpipe, pump, well, or vehicles, and will then typically be stored in or close to the home in a water container(s). Even where running-water is available in the home, then the supply may be intermit-

tent or unreliable, which eventually results in local storage (Barrera et al. 1995). Water storage containers around the home are typical breeding sites for *Ae. aegypti* and *Ae. albopictus* [*St. albopictus*], but may also support a number of other species (Brown et al. 1992; Kusumawathie et al. 2002). In terms of environmental management, preventing mosquito production from such sources is typically achieved by use of tight-fitting lids over the containers (Kittayapong and Struckman 1993). However, the degree to which such lids are used in practice may depend on the intended use of the water and the size and shape of containers available. Phuanukoonnon et al. (2005) working in Thailand found that 96% of drinking water containers were covered by well-fitting lids, while only 36% of containers of water intended for washing, were covered.

### 17.2.4 Drainage Systems

Given the function of building and highway drainage systems, they are particularly prone to the formation of standing water, especially where blockages, leaks or malfunctions occur. Surveys in Queensland, Australia showed that 14% of roof gutters with standing water contained either *Ae. aegypti* or *Oc. notoscriptus* larvae (Montgomery and Ritchie 2002). The authors emphasized the need to include gutters in source reduction exercises. In Singapore, the gutters on city bus shelters were commonly blocked and therefore supported mosquito breeding. A new bus shelter was designed to avoid the need for gutters, which prevented this problem (Seow-Boo 2001). Surface water drainage systems, such as stormwater catch basins and sumps often favour the development of mosquito larvae (Kwan et al. 2008), partly because of the accumulation of organic debris that may impede flow and result in stagnant water. Preventing and resolving mosquito problems in such areas requires extensive multiagency cooperation, and may involve challenging and sometimes unique changes requiring extensive construction/modification (a very costly proposition), including re-shaping of structures and channels, changes to covers of sumps to prevent mosquito access, or removal of emergent aquatic vegetation (Metzger et al. 2002, 2008; Messer 2002; Kluh et al. 2002; Fox and Absher 2002). Cairncross et al. (1988) working in Pondicherry showed that the choice of drain cross-section in urban

areas was particularly important in determining the degree of *Culex* breeding, with L-shaped drains being much less important than U-shaped drains in terms of mosquito production.

### 17.2.5 Sewage and Waste-Water Processing

Sewage processing facilities, through the presence of large volumes of water, rich in nutrients, and often at slightly elevated temperature, can provide highly favourable conditions for mosquito development. Such facilities are typically located close to population centres, hence create the possibility of biting nuisance, or even the risk of disease transmission.

Ensink et al. (2007) working in Faisalabad, Pakistan, found that poorly maintained urban wastewater stabilization ponds supported large populations of several mosquito species, including *Cx. quinquefasciatus*, *An. stephensi*, and *Anopheles culicifacies*. A subset of the ponds was fitted with a screen to remove large debris from the incoming water, floating debris and emergent vegetation were removed from the ponds, and cracks in the concrete lining of the ponds were mended. In the ponds subject to this intervention, the percentage of water samples positive for both *Anopheles* and *Culex* larvae, fell dramatically compared to the control ponds.

Epibane et al. (1993) studied the mosquito fauna in sewage treatment ponds in California, in which water hyacinth (*Eichornia* sp.) was used as part of the water treatment process. Despite the active aeration of the ponds, and the presence of *Gambusia* sp, they supported large populations of various *Culex* species. A grid of garden sprinklers were installed and operated nightly, to provide a pulsating pattern of falling water across the surface of the ponds. The water spray was considered to prevent, deter, and disrupt oviposition on the water surface, resulting in a reduction in larval counts by about 95%. Similar results were obtained by Ayler (1991) using a sprinkler system on stagnant water tanks on sewage treatment plants in Florida.

Whelan (1998) discusses a range of design features of waste-water processing facilities, such as steep sides to water holding ponds, exposed location to enable wind action on the water surface to create unfavourable conditions for oviposition and larval development, the

absence of emergent vegetation, and choice of disposal routes for the fully processed water.

Where processed urban waste-water is disposed of by application to free-water surface constructed wetlands, large areas of favourable mosquito habitat may be created. A variety of techniques have been developed and assessed to mitigate mosquito problems (Walton 2002, 2003). Thullen et al. (2002) found that deepening the water, but at the same time creating numerous small raised hummocks within the water successfully improved water quality and also reduced mosquito numbers. Alternatively, mosquito numbers may be minimized by periodic draining and drying of the wetland cells, providing that it coincides with a period of little or no rainfall (Mayhew et al. 2004). Sanford et al. (2003) found that the duration of drying was critical, with two weeks drying being the period most likely to produce large larval mosquito numbers when the cell was re-flooded.

### 17.2.6 Cemeteries

Cemeteries are potentially significant in terms of mosquito production, because of their presence in urban areas, the use of large numbers of small artificial water containers (*i.e.* vases for flowers), numerous adult mosquito resting sites amongst graves, and availability of carbohydrate from flowers. In Brazil, cemeteries have been recorded with up to 6,100 artificial breeding sites per ha (Vezzani 2007). Mosquito species known to occur commonly in flower vases include *Ae. albopictus*, *Ae. aegypti*, *Cx. pipiens*, *Ochlerotatus* spp. (*e.g.* *Oc. japonicus*), and *Culiseta* spp. Cemeteries are culturally sensitive areas, so simply removing or prohibiting vases is not appropriate, and some cemetery staff may earn an income from replacing fresh flowers in vases. Depending on the local situation, source reduction possibilities may include:

Frequent emptying of water-filled containers, although in practice this may be very difficult to carry out.

Filling flower vases with wet sand or soil.

Replacing real flowers with artificial flowers in vases with a perforated base.

Education of cemetery staff and visitors about the importance of preventing mosquito breeding (Schmidt 2003).

Regulations to prevent visitors introducing water-filled containers into the cemetery, although in practice such regulations may be ignored (Vezzani and Velasquez 2003).

### 17.2.7 Urban Sanitation

The immature stages of container-breeding mosquitoes, such as *Ae. aegypti* and *Ae. albopictus*, will be found not only in municipal features of the environment such as drains and water storage or processing facilities, but also in a vast number of smaller and often ephemeral water bodies. Although DF/DHF vectors are very dependent on such habitats, malaria and filariasis vectors may also be found frequently in such niches (Biswas et al. 1992). These urban water bodies may be semi-natural, *e.g.* cut ends of bamboo, coconut shells, tree-holes, bromeliad plants, or they may be artificial *e.g.* empty drink cans, plant pots, ant traps, used vehicle tyres, etc. In the urban environment, there may be a great diversity and number of such containers. Manrique-Saide et al. (2008) classified *Ae. aegypti* breeding sites in Mexico into 15 different basic categories. Focks and Chadee (1999) working in Trinidad found that the density of containers holding *Ae. aegypti* ranged from 61 to 399/ha.

Destruction, disposal, recycling or regular emptying of such containers and larval sites can have a significant effect on larval indices, and on disease transmission (Soedarmo 1994). However, such improvements are often difficult to achieve by direct action by health or city authorities, because the majority of containers may be on private land to which access is problematic, and because container numbers could increase again if behaviour of residents remain unchanged. As an alternative to direct action, organizations responsible for vector control have often embarked either on programmes of community participation (see Sect. 17.4.1 below), and/or legislation requiring landowners or occupiers to remove potential breeding sites (see Sect. 17.4.2 below).

## 17.3 Environmental Management of Mosquitoes in Rural Areas

### 17.3.1 Agriculture

Rice production is widespread throughout tropical and subtropical regions and often (but not always) involves flooding of rice fields. Flooded rice fields are recognised as major potential sources of both nuisance and disease vector mosquitoes in various regions.

Insecticide use for control of rice pests may provide collateral control of these mosquitoes, and insecticides may also be applied specifically for control of mosquito larvae. Nonetheless, mosquito problems persist in rice fields. A number of approaches have been used to prevent mosquito breeding by introducing changes in agricultural practices. In Sri Lanka, for example, a programme was implemented to educate rice farmers in the benefits of adjusting field levels to avoid uneven flooding and puddles, in preventing seepage from the levees around rice fields, in ensuring that the water flow in channels feeding paddies is fast enough to prevent breeding, and in periodic drainage of paddies before mosquito emergence could occur. Results showed a significant drop in *Anopheles* spp. densities, but had a limited impact on *Culex* and *Aedes* numbers (Yasuoka et al. 2006).

Keiser et al. (2005) used alternate flooding and drying of rice fields as part of an integrated programme to limit production of culicine vectors of Japanese encephalitis.

Alternative approaches include encouraging the water-fern (*Azolla microphylla*) to grow on the water in the rice fields. Rajendran and Reuben (1991) showed that when fully established, the mat of vegetation produced by the water-fern can reduce numbers of *Cx. tritaeniorhynchus* and other species by up to 80%. However, mosquito populations still reached problematic levels in the period before the relatively slow-growing fern became established.

Lacey and Lacey (1990) have provided a comprehensive review of the history and practice of environmental management of mosquitoes in rice production.

### 17.3.2 Natural Wetlands

Mosquito production in salt marsh or freshwater wetlands can be intense, and this can create problems where residential or resort areas are situated close to such habitats. Such problems have been recognised in Australia (*Oc. vigilax*), the USA (*Ae. sollicitans*), Europe (*Ae. caspius*), and elsewhere. Historically, such problems were often addressed by complete drainage of the marsh, or by filling and reclamation (Carson 2006). More recently, with the recognition that natural wetlands are an important wildlife and biodiversity resource, such measures have largely ceased in many

countries, and in some areas there are movements to reinstate former wetland areas (Walton 2002, 2003). Given the wildlife importance of saline and fresh water wetlands, environmentally acceptable alternatives to insecticide use for mosquito control in these areas have been developed.

On salt marshes, a wide variety of environmental management approaches to mosquito management have been used. The most widely used techniques are discussed below:

Grid-ditching was very widely used in the USA during the 1930s, with an estimated 90% of the coastal salt marshes on the Atlantic coast grid-ditched to reduce mosquito populations (Clarke et al. 1984). The process consisted of the excavation of parallel ditches at 50–100 m intervals across the marsh, connecting to tidal channels. Efficacy of the technique was limited, and channels frequently became blocked by silt where they entered tidal water.

Open-marsh water management is a process of excavating channels across the marsh, sometimes using rotary excavators, which link otherwise isolated pools to each other and to the tidal source. It appears to work by flushing mosquito larvae out of the marsh into areas where they are unable to survive, allowing larval predators such as fish to have access to and colonise the pools, and also appears to alter the characteristics of the site, thereby reducing mosquito oviposition. Runnelling is a variation of open-marsh water management that uses shallower channels (Dale et al. 1993).

Impoundment is a process of constructing raised bunds (levees) around the marsh, and has been widely used since the 1950s. The bunds are typically punctuated by culverts that allow the water level within the marsh to be altered by controlling water flow. Typically, water levels would be held high during the summer when the risk of mosquito breeding is highest, and as a result mosquito production could be almost entirely prevented. Rotational impoundment management is a fine-tuning of the process that aims to manage mosquito populations and keep broader environmental impact to a minimum, by limited flooding during the summer followed by extensive flooding in autumn (Carlson 2006).

There is extensive literature on the environmental impact of such changes on salt marsh ecology. The efficacy and impact of the changes is likely to result not only from selection of the intervention, but on the quality with which the work is carried out. Dale and Knight (2006) in Australia, compared the environmental impact of grid-ditching, open-water marsh management, and runnelling, using ten different ecological parameters. They concluded that although none of the techniques completely destroyed the marsh, grid-ditching had the most impact, while runnelling (*i.e.* a version of Open-Marsh Water Management) had the least negative impact, while

still providing a useful reduction in mosquito populations. Carlson (2006) in Florida concluded that a mixture of salt marsh management techniques were beneficial to local ecology, and a combination of rotational impoundment management, and open-marsh water management was the most acceptable approach.

Natural freshwater wetlands may also be very significant in terms of mosquito production. Schäfer et al. (2004) working in Sweden raised concerns about the risk of mosquito nuisance and of transmission of Sindbis virus by mosquitoes from natural wetlands and from wetland areas constructed for biodiversity or amenity purposes. Proposals included limiting the size of constructed wetland areas, and their proximity to human settlements.

In central Europe, the larger rivers, such as the Rhine and Danube, formerly had extensive natural flood plains that were flooded during the spring snow melt, and at intervals over the rest of the year. These natural flood plains contained many permanent and semi-permanent water bodies, which acted as reservoirs for a variety of beneficial wildlife, that provided a check on the mosquito populations, particularly *Ae. vexans*, which tended to develop during periods of flooding. However, a process of canalisation has taken place in many areas, and many natural flood plains were reclaimed. Although flood catchment areas were provided, the water table is lower and the water coverage is temporary, resulting in reduction of their value as reservoirs of beneficial wildlife. However, in recent years there has been a process of environmental management of existing flood plains to re-create permanent and semi-permanent water bodies. This has enabled the establishment of a larger and more diverse population of beneficial vertebrates and invertebrates.

## 17.4 Environmental Management of Mosquitoes and Human Issues

### 17.4.1 Community Participation

Some environmental management measures, such as changes to drainage or waste-water processing, are largely the responsibility of governmental agencies or utility

companies. However, the removal of smaller but no less important larval sites, such as a variety of small containers, are best implemented by the local community.

Techniques for raising awareness and gaining cooperation of the community are not limited to use in environmental management, but are also relevant to other areas such as the use of insecticide treated nets. In practice, community-driven campaigns are often preceded by preliminary survey work (*e.g.* Knowledge, Attitudes, Perceptions – KAPs) to assess the residents' existing knowledge and understanding of the issues relating to mosquito problems and management in their area. This enables the final intervention to be more appropriately targeted.

Interventions intended to gain community support and cooperation may consist of any or all of the following:

- Direct communication with residents, health centre visitors, farmers, community leaders, etc., via public meetings, workshops, school visits, theater events, workplace meetings, and practical demonstrations.
- Distribution of brochures and literature to individual households.
- Mass media (posters, newspaper, radio, television, internet).

The preference of *Ae. aegypti* and *Ae. albopictus* for household and peridomestic containers has traditionally led to a strong emphasis on improving public awareness and cooperation in DF and DHF control campaigns (Becker 1992). However, community mobilization toward environmental management is just as relevant to malaria (Kibe et al. 2006), filariasis (Panicker and Dhanda 1992), and West Nile virus control (Schaffner 2008).

Formerly, such campaigns were planned in the context of education alone, *i.e.* the belief that if the facts concerning mosquitoes and the risk of infestation were supplied to communities, the necessary action would then inevitably take place as a result. More recently, however, campaigns have become more sophisticated. Geounuppakul et al. (2007) reported on a programme aimed at enhancing individual empowerment, ownership, and self-esteem in relation to malaria control. The objective was that as a result of the intervention, individuals and communities would acquire the drive and self-confidence to adopt, implement, and maintain appropriate malaria control measures.

However, detailed assessments of the effectiveness of public awareness and public behavioural-change campaigns are relatively sparse. The general view is that although dissemination of information may be readily achieved, transforming this into public action is more challenging. Certainly, programmes to enhance public participation in vector control must be locally led, as beliefs, superstitions, practices, and ecological conditions greatly differ from area to area. Seow-Boo (2001) suggests that sociologists and behavioural scientists are key to ensuring the effectiveness of community-based programmes.

Schreiber and Cuda (1994) found that in Florida, the impact of distribution of information to residents varied according to the socio-economic status of the community. Significant reductions in numbers of containers were achieved in low-income communities, but not in middle- to high-income communities. Schreiber and Morris (1995) assessed the effectiveness of printed educational materials intended to encourage residents to remove containers and potential mosquito development sites from their properties in Florida. Communities were provided with either no information, black-and-white printed information, or full-colour printed information. Results showed that in terms of the numbers of potential mosquito development containers remaining on residential properties, the black-and-white leaflet resulted in no improvement compared to the provision of no information, while the full-coloured information resulted in a significant reduction.

Heintze et al. (2007) carried out a systematic review of published studies on the effectiveness of community-based interventions for control of dengue vectors. The studies reviewed, included a wide range of measures directed at local communities, including mass media (posters, radio, television, newspaper, etc.), direct distribution of materials to individual households, and meetings with residents and community leaders. The review concluded that although community-based approaches, together with other related interventions, were able to reduce *Aedes*

larval indices, the effectiveness of the individual components of the programme were unclear. Further work would be required to identify which components were actually effective.

#### **17.4.2 Regulations for Environmental Management of Mosquitoes**

Although the environmental management of mosquitoes is ideally achieved with the support, cooperation, and involvement of the general public, there may be occasions where that support is not forthcoming from particular individuals or organizations. Given that mosquitoes may present a threat to the general public, whether as a nuisance or as a disease vector, some countries or states have passed legislation intended to reduce the risk of mosquito breeding (*i.e.* statutory environmental management), which could be used against particular individuals or organizations where their action or inaction might create a risk to public health.

In the USA, *Ae. albopictus* is particularly associated with used tyre disposal sites. The state of Illinois has passed legislation to limit the storage of used tyres and to require proper disposal and recycling (Novak 1995). In Singapore, the Control of Vectors and Pesticide Act empowers city authorities to inspect premises for potential mosquito breeding sites, to require the owner or occupier to abate breeding sites, and to perform the necessary work and recover costs if need be (Seow-Boo 2001). Fines may be imposed (and progressively increased) if subsequent offences occur. However, the author stresses that removal of breeding sites through increased awareness is the preferred approach, rather than relying on the threat of prosecution. Nonetheless, where owners or occupiers persistently fail to discharge their responsibility, legislation can be useful as the last resort. In Italy, regulations also exist that require residents to remove mosquito breeding sites from their property, but local authorities are sometimes reluctant to use legal powers to address mosquito problems.

## Chapter 18

# Chemical Control

### 18.1 History

The development and evolution of the chemical industry has led to many major advances in pest, vector, and pathogen control. However the beneficial effects of these advances has not been felt equally around the world. In many developing countries, and even in some developed countries, pests and vectors are still causing problems for which technical solutions already exist.

In attempting to review the present status of insecticides used for mosquito control, it may be helpful to briefly examine the recent past.

The first recorded use of a synthetic organic insecticide, dinitro-*o*-cresol, occurred in 1892, and by the 1930s a range of such compounds had been discovered and found limited use (Cremlyn 1978). From the 1920s onwards, the increasing potency of insecticides as tools for insect control led to their growing predominance as preferred method for insect control.

A series of dramatic discoveries during the late 1930s provided new synthetic insecticides, which had enormous potential for widespread use, and which further reinforced the emphasis on a chemical approach to insect control.

In 1939, the “wonder” insecticide of the chlorinated hydrocarbon group, dichlorodiphenyltrichloroethane, best known as DDT, was introduced. This was soon followed by the development of organophosphates (OP) in Germany, which had been evolving since 1932, when Lange and von Krueger first synthesized organofluorophosphate esters. In the early 1950s, carbamates were created in Switzerland. Between the 1960s and 1970s, the discovery and development of photostable pyrethroids, mainly in Japan and the UK, led to profound changes in the

practice of insecticide use. These exceptionally potent, biodegradable compounds may be used in the field at rates as low as 20 g/ha, which is 10–100-fold lower than more conventional insecticides and eventually leads to a lower burden of residues in the environment. Unfortunately, although they are an impressive achievement, the synthetic pyrethroids are not the perfect answer to the insecticide problem, since they have a high toxicity on many aquatic species, vertebrates, invertebrates, and several beneficial insects.

The adverse effects of indiscriminate and excessive use of DDT, were dramatically portrayed by the publication of Rachel Carson’s “*Silent Spring*” (1962). As a result of the problems with chlorinated hydrocarbons, alternative methods were explored. These alternatives included insecticides developed as an outcome of rational leads from basic entomological research on metabolic disrupters, moult inhibitors, and behaviour modifiers of insects. Highly potent inhibitors of chitin synthesis in the insect integument were developed in the early 1970s in the Netherlands. Since the target site of action for these chemicals is known and is susceptible to disruption only in certain species at certain times during the life cycle, these materials are thought to have fewer serious deleterious effects on nontarget species. A second example is based on the discoveries that insect juvenile hormones regulate many developmental functions in insects and that they are unique to arthropods. This initiated the synthesis of juvenoids in the early 1980s for selective insect control. As with other alternative strategies for insect control, the full potential of juvenoids in selective and environmentally sound insect control has not been fully realized to date.



Increased research on genetic control of vectors was carried out during the 1960s and 1970s. Numerous areas of genetic techniques such as translocations, cytoplasmic incompatibility, meiotic drive and sterile hybrids were investigated, but few practically applicable. Similarly, much research was conducted on chemosterilisers, but none of the compounds became operational in vector control programmes. It is also important to note the extensive research on the evaluation and development of biological agents such as viruses, bacteria, protozoa, fungi, nematodes, and other parasites, predators, and pathogens. So far, with the exception of bacterial agents, none of these have emerged as widely useful tools in vector control (Mulla 1994).

The use of *B. thuringiensis* var. *israelensis* (*B.t.i.*), which was introduced into integrated mosquito management (IMM) at the beginning of the 1980s, is discussed in detail in Chap. 16.

The effects of pesticides on nontarget organisms and the environment have been a source of worldwide contention for more than 20 years, and form the basis for most legislation intended to control or prohibit the use of specific pesticides. The most readily identified adverse effects of pesticides on nontargets were those of the persistent organochlorine insecticides (e.g. DDT) and their metabolites or conversion products on certain species of fish and birds. Consequences less readily identifiable include the effects of pesticide residues in food and the environment, on humans and domestic animals.

Due to difficulties in recognizing the diversity of the ecological relationships between nontarget and target organisms, mistakes were made in the nonselective use of pesticides. However, these mistakes do not appear to have had permanent or irreversible consequences on nontargets and the environment. Great efforts have been made to safeguard the protection and preservation of nontarget organisms.

These environmental problems were not the only ones to become evident as synthetic organic insecticides came into widespread use. Resistance rapidly increased in a number of agriculturally and medically important insect species. The need for higher dosages and more frequent applications of insecticides to combat these pesticide-induced problems has, on occasion, been disastrous, especially when these control products were used indiscriminately.

As late as in 1955, *Plasmodium* spp. infected approximately 500 million people causing malaria throughout the world. The annual death rate from this debilitating disease was reduced from 6 million in 1939 to 2.5 million in 1965 and to less than 2 million today. Although a clinical manufacturing facility has been opened to produce a vaccine that uses a weakened form of malaria parasite, as a main component of "whole-parasite" vaccine, it is still to be seen if today's technology will allow it to overcome obstacles that have prevented production so far. Through the judicious use of insecticides, similar progress has been made in reducing the incidence of other important tropical diseases. However, in spite of the progress made, it appears that there still remains the ever-lurking danger to humans from mosquito-borne diseases such as malaria, dengue fever, encephalitis, yellow fever, Chikungunya fever, lymphatic filariasis, etc. The number of deaths resulting from all wars appears insignificant compared to the toll inflicted by vector-borne diseases.

The current strategy in successfully implementing pest and vector management programmes is to carefully select from a variety of techniques, the combination of control options that is best suited to achieve the objectives, and at the same time to preserve the ecological balance. This approach, which has to maintain a high flexibility, has gained much support not only among scientists, but also among the general public in the last 20 years, and is referred to as integrated pest management (IPM) and integrated vector management (IVM) in public health programmes.

Since vector population management constitutes an important element in the current global strategy for the control of major vector-borne diseases, chemical control remains an important component in an integrated approach to vector control especially during epidemic/epizootic situations.

## 18.2 Insecticides

When millions of humans are killed or disabled annually from vector-borne diseases, and the global damage by insects, vector-borne diseases, weeds, and rodents is estimated at >\$100 billion annually, it becomes apparent that the control of various harmful organisms is essential for the future development of human health, agriculture, and industry. In the process

of accommodating these vital human requirements, pesticides have consequently become an indispensable part of global integrated vector management programmes.

### 18.2.1 Classification of Insecticides

Insecticides used in mosquito control belong to four major chemical groups, chlorinated hydrocarbons, OPs, carbamates, and pyrethroids, and a special class referred to as insect growth regulators (IGRs).

The first generation of insecticides are the stomach poisons, such as arsenicals. The second generation includes the contact insecticides: chlorinated hydrocarbons, OPs, carbamates, and pyrethroids. After having extensively studied the various physiological effects of the juvenile hormone, Williams in 1976 suggested that their analogs (juvenoids) could be used as insect-specific control agents to which pest species may be unable to develop resistance. He referred to this class of compounds as “third-generation pesticides.” The fourth generation of insecticides is derived from the entomopathogenic abilities of some microbials. Commercial preparations based on bacteria have been available since the beginning of the 1980s and

form an important component in mosquito control management. The microbial agents play a substantial role in mosquito control programmes in Europe and the United States, and are discussed in Chaps. 11 and 16.

On an average, 547 tonnes of active ingredients of organochlorines, 437 of OPs, 24 of carbamates, and 162 tonnes of pyrethroids were reportedly used annually for vector control at the global level during 2003–2005 (Table 18.1). A recent publication by Zaim and Jambulingam (2007) on global insecticide use in vector-borne disease control gives an overview on chemicals by class of insecticides used to control vectors worldwide. The use of organochlorines, exclusively DDT, was reported to World Health Organization Pesticide Evaluation Scheme (WHOPES) only from the African Region. The use of OPs was reported from all regions; about 84% of their total use was in the region of the Americas and 1–6% in countries from other regions. Compared with other classes of insecticides, the use of carbamates is limited, with 80% of their total use globally in the African region, the remaining use was in the region of the Americas (11%), the South-East Asia region (8%), and the East Mediterranean region (1%). Carbamates were not used in the Western Pacific Region and in the European region. The use of pyrethroids was higher in countries from the region of the Americas (57% of total use)

**Table 18.1** Global use of insecticides for vector control reported to WHOPES, in kg of active ingredient, by class of insecticide and WHO region, 2003–2005 (Zaim and Jambulingam 2007)

Year	Class	WHO region V/VHO region						All regions
		African	Americas	Eastern Mediterranean	European	South-East Asia	Western Pacific	
2003	OC	423,868	0	0	0	0	0	423,868
	OP	19,251	151,509	21,619	6,350	22,326	6,483	227,538
	C	3,900	3,835	1,000	0	1,600	0	10,335
	PY	8,407	123,007	9,282	1,288	14,992	14,957	171,933
2004	OC	389,210	0	0	0	0	0	389,210
	OP	327	401,508	19,594	3,600	6,691	3,816	435,536
	C	321	2,473	400	0	1,600	0	4,794
	PY	1,201	81,482	21,092	872	9,300	13,499	127,446
2005	OC	827,648	0	0	0	0	0	827,648
	OP	15,543	550,465	22,230	0	44,644	15,760	648,642
	C	56,701	1,735	465	0	2,560	0	61,461
	PY	31,211	71,355	15,752	2,072	5,645	60,203	186,238
Average	OC	546,909	0	0	0	0	0	546,909
	OP	11,707	367,827	21,148	3,317	24,554	8,686	437,239
	C	20,307	2,681	622	0	1,920	0	24,230
	PY	13,606	91,948	15,375	1,411	9,979	29,553	161,872

C carbamate; OC organochlorine; OP organophosphate; PY pyrethroid

**Table 18.2** Global use of insecticides for vector control reported to WHOPEs, in kg of active ingredient, by type of application and class of insecticide, 2003–2005 (Zaim and Jambulingam 2007)

Type of application	Class of insecticide	Amount of insecticide used (kg active ingredient)			
		2003	2004	2005	Average
Indoor residual spraying	Organochlorines	423,868	389,210	827,648	546,909
	Organophosphates	48,312	32,070	91,232	57,205
	Carbamates	6,242	4,417	61,235	23,965
	Pyrethroids	82,164	71,183	55,128	69,492
Treatment of mosquito nets	Pyrethroids	15,838	4,694	31,819	17,450
Larviciding	Organophosphates	90,725	89,426	83,136	87,762
Space spraying	Organophosphates	84,243	311,948	473,994	290,062
	Pyrethroids	49,675	50,336	98,021	66,011

**Table 18.3** Insecticides used most extensively for vector control reported to WHOPEs, in kg of active ingredient, 2003–2005 (Zaim and Jambulingam 2007)

Class	Compound	2003	2004	2005	Total	Average
Organochlorines	DDT	423,868	389,210	827,648	1,640,726	546,909
Organophosphates	Chlorpyrifos	2,998	2,425	7,041	12,464	4,155
	Chlorpyrifos-methyl	9,048	2,931	6,460	18,439	6,146
	Fenthion	3,924	3,205	3,019	10,148	3,383
	Fenitrothion	19,515	56,565	97,084	173,164	57,721
	Malathion	97,741	243,714	428,955	770,410	256,803
	Pirimiphos-methyl	4,654	38,316	29,372	72,342	24,114
	Temephos	89,338	87,738	76,519	253,595	84,532
Carbamates	Bendiocarb	1,600	2,056	59,759	63,415	21,138
	Propoxur	8,735	2,738	1,702	13,175	4,392
Pyrethroids	Alpha-cypermethrin	19,394	29,436	26,102	74,932	24,977
	Bifenthrin	171	732	803	1,706	569
	Cyfluthrin	3,988	3,221	3,852	11,061	3,687
	Cypermethrin	80,548	26,581	21,293	128,422	42,807
	Deltamethrin	16,874	18,267	48,005	83,146	27,715
	Etofenprox	5,256	4,355	5,248	14,859	4,953
	Lambda-cyhalothrin	6,887	16,725	4,956	28,568	9,523
Permethrin	38,582	25,236	72,708	136,526	45,509	

than in other regions. The European region reported the lowest (1%) use of pyrethroids (Table 18.2).

About 60% of the insecticides reportedly used for vector control (all classes of insecticides) during 2003–2005 were for indoor residual spraying (IRS), followed by space treatments (30.7%), larviciding (7.6%) and insecticide treated mosquito nets (1.5%).

The use of organochlorines and carbamates at the global level was limited to IRS in the endemic areas of developing countries. Of the total annual use of OPs, however, 66% was for space treatments, 20.2% for larviciding and 13.2% for IRS.

While about 43% of the total use of pyrethroids was for indoor residual application, and 45% for space spraying, the remaining 11% was used for treatment of mosquito nets. Though the use of pyrethroids for

space spraying and for treatment of mosquito nets increased over the 3-year reported period, it showed a decline for indoor residual application. The quantity for organochlorines, OPs and carbamates used for residual applications, however, increased considerably (Table 18.3).

All the above mentioned compounds include substances that cause mosquito mortality and may be categorized in various ways. They could also be classified according to their uses on the stage of insect life cycle acted upon: larvicides or adulticides (Table 18.4), or by their mode of action against the insects: stomach poison, contact poison, fumigant, etc.

One of the WHO classifications of insecticides is on the basis of the acute toxicity to laboratory animals.

The hazard of a compound is assessed by determining the lethal effect on test animals, usually rats, when applied to their skin (dermal toxicity) or when it is ingested (oral toxicity). The dose, which is statistically determined to be lethal to 50% of the test animals ( $LD_{50}$ ), is assumed to be a measure of the hazard to man and other mammals. The latest WHO-recommended classification of pesticides by hazard was issued in 2004 (Table 18.5).

Finally, insecticides can be classified as inorganic, natural organic, and synthetic organic. Most of the insecticides used today for mosquito control fall in the last category. A list of insecticides, which may be used in mosquito control according to the WHO Operational Manual on the application of insecticides for control of the mosquito vectors of malaria and other diseases (WHO 2004) is given in Table 18.6.

**Table 18.4** Main uses of synthetic organic insecticides by chemical group

Chemical group	Larvicides	Adulticides	
		Space application	Residual application
Chlorinated hydrocarbons			X
Organophosphorus	X	X	X
Carbamates		X	X
Pyrethroids	X	X	X

**Table 18.5** WHO criteria for classifying pesticides by hazard (WHO 2006)

Class of toxicity by physical state of product or formulation	$LD_{50}$ for rat (mg/kg body weight)			
	Oral		Dermal	
	Solids	Liquids	Solids	Liquids
IA Extremely hazardous	5 or less	20 or less	10 or less	40 or less
IB Highly hazardous	>5–50	>20–200	>10–100	40–400
II Moderately hazardous	>50–500	>200–2,000	>100–1,000	400–4,000
III Slightly hazardous	>500	>2,000	>1,000	>4,000
IIIU a.i. unlikely to present acute hazard in normal use	>2,000	>3,000	-	-

**Table 18.6** List of insecticides that may be used in mosquito control, WHO (2004)

Active ingredient	Use	Chemical type or class	Toxicity class
Allethrin	L, A	PY	III
Alpha-cypermethrin	A	PY	II
Bendiocarb	A	C	II
Bifenthrin	A	PY	II
Bioresmethrin	A	PY	III
Chlorpyrifos	L, A	OP	II
Chlorpyrifos-methyl	L	OP	II
Cypermethrin	A	PY	III U
Cyfluthrin	A	PY	II
DDT	A	OC	II
Deltamethrin	L, A	PY	II
Diazinon	L, A	OP	II
Dichlorvos	L, A <sup>a</sup>	OP	I B
Diflubenzuron	L	IGR	III U
Etofenprox	A	PY	III U
Fenitrothion	L, A	OP	II
Fenthion	L, A	OP	I B
Jodfenphos	L, A	OP	III U

(continued)

**Table 18.6** (continued)

Active ingredient	Use	Chemical type or class	Toxicity class
Lambda-cyhalothrin	A	PY	II
Malathion	L, A	OP	III
Methoprene	L	IGR	III U
Methoxychlor	A	OC	III U
Naled	L, A	OP	II
Permethrin <sup>b</sup>	L, A	PY	II
Pirimiphos-methyl	L, A	OP	III
Propoxur	A	C	II
Pyrethrins	A	n.o.	II
Pyriproxyfen	L	IGR	III
Resmethrin	A	PY	III
surface film	L	SF	-
Temephos	L	OP	III U

<sup>a</sup>Fumigant may be dangerous in oil solution

<sup>b</sup>May be dangerous in oil solution

Use: *L* larvicide; *A* adulticide; *IGR* Insect growth regulator

Chemical type: *OC* Chlorinated hydrocarbons; *OP* Organophosphates; *C* carbamates; *PY* pyrethroids;

*SF* Surface film; *n.o.* natural organic

### 18.2.2 Insecticide Formulations

The active ingredient (a.i.) of an insecticide is rarely suitable for application without further processing. Once the active ingredient is manufactured it is then formulated, that is to say it is processed into a usable form for direct application, or for dilution prior to an application. The formulation is the form in which the pesticide is available at the market.

It is usually necessary to add other nonpesticidal substances to the a.i. so that the resulting formulation allows ease of handling, transportation, safety, application, effectiveness, and storage. To meet these needs, insecticides may be formulated in a number of ways, to enhance their effectiveness, simplify their preparation for application, and meet different requirements of larviciding and adulticiding. Each formulation may be supplied in a variety of concentrations. The concentration of a formulation is designated either on a weight-to-weight basis, when both the a.i. and the formulation are solid, such as DDT 5% dust, or on a weight-to-volume basis, when the a.i. is solid and the formulation liquid, such as chlorpyrifos 400 g/l emulsifiable concentrate.

By improvement of the formulations, the effectiveness, persistence and/or safety of insecticides can also be increased. An example is the recent development of a slow-release larvicidal formulation, designed to last longer when applied into the water. Larviciding,

especially with microbial agents or IGRs, can be made more persistent and affordable by appropriately developed formulations, which release the agent slowly and at the appropriate depth where it will be most easily encountered by the target larvae.

The safety of space treatments has been increased by development of water-based formulations or replacement of emulsifiable concentrates (EC) by suspension concentrates.

*Technical concentrates* (TC/TP) are liquids or powders containing a high concentration of a.i., which can then be manufactured, packaged, shipped, or reformulated into more dilute formulations. Chemicals are rarely used in this form, except for some ultra-low-volume (ULV) adulticides.

*Solution concentrates* are liquids containing a high concentration of an a.i. that has been diluted with oil or petroleum solvents. These SC may also be used undiluted for ULV applications, and are commonly used for thermal aerosol application (fogging) when further diluted with highly refined oils or kerosene.

*Emulsifiable concentrates* (EC) consist of active ingredient dissolved in an organic solvent combined with emulsifying agents. These products can then be diluted with water to an appropriate concentration in the spray tank before use. The emulsifying agent or agents cause the technical product and organic solvent solution to disperse evenly in the water when mixed by stirring. It is sometimes necessary to maintain a level

of agitation in order to keep the chemical evenly dispersed in the tank. ECs are used for larviciding when dispersion throughout water is required and they may also be used undiluted as ULV applications. They are occasionally used for residual applications to impervious surfaces such as houses in urban and semiurban areas.

*ULV formulations*, as a rule, have a low volatility, so reducing the evaporation of airborne droplets. ULV insecticides are applied as mists or aerosols in space applications. By definition, ULV treatment is an application technique in which less than 5 l/ha of liquid insecticide is applied. This method of operation has resulted in substantial savings through speed and reduction in labor requirements, but demands very accurate application, since the droplet size of ULV formulation is of considerable importance.

Droplet size affects:

- (a) The chances of penetrating through vegetation and other obstacles to reach the target
- (b) The total number of droplets/unit volume of the product applied and the toxicity of the average droplet
- (c) The distribution of droplets on treated surfaces and
- (d) The time for which a droplet remains airborne, and therefore its ability to drift downwind.

Both favorable and unfavourable results from using ULV applications have been reported. Disparity in results obtained has been primarily due to weather conditions, application rate and the degree of penetration into the target area. As might be expected, applications at higher label rates together with good penetration of dwellings have proved to be the best control of adult vector populations (Gratz 1991).

*Water dispersible powders or Wettable powders* (WDP/WP) are, as the name suggests, insoluble powders that resemble dust, but which may be dispersed in water. The technical product can be either a solid or liquid. Liquid technical products and concentrates can be absorbed onto an inert solid, and then used as a water dispersible powder. Solid formulations are ground to a fine powder and surface-active agents added to promote dispersion of the particles without excessive foaming when they are added to water. Wettable powders form a suspension rather than a solution when added to water, and need regular agita-

tion. The diluents in some wettable powders are abrasive and can wear down pumps and nozzles, which have to be regularly checked and replaced (Wade 1997). Most applications inside houses are accomplished with WDP. They are usually applied at high volume rates with low concentrations of the a.i.

*Soluble powders* (SP) are similar to WP, except they form true solutions in water. Since they dissolve completely in water, they do not need constant agitation nor are they known to abrade equipment.

*Granules* (GR) are inert carriers impregnated or coated with the solution concentrate of a technical product. They are made by applying a liquid formulation of an active ingredient to coarse particles of an absorptive material such as attapulgite, clay, or corn cobs, or to a nonsorbent material such as sand or other mineral or nonmineral substance. Even frozen water in the form of ice cubes, may be used as a carrier for some materials such as microbial agents, and has been recently used successfully for aerial treatments of flooded habitats (Becker 2003). Granular treatments can be made at any time of the day since they can be applied aerially in winds up to 20 km/h without significant drift.

*Vapourizing strips* are special formulations of insecticides, mainly for household use, which are usually made from polymeric materials containing a volatile compound. They release vapours that are lethal to the insects when exposed to air or heat.

*Aerosols*: The active ingredients must be soluble in the volatile, pressurized petroleum solvent to which a propellant is added. Fluorocarbon propellant was used initially, but has now been largely replaced by the less detrimental propane/butane mixtures, or with carbon dioxide. When the petroleum solvent is atomized, it evaporates rapidly leaving the microdroplets of toxicant suspended in the air. Aerosols commonly produce droplets well below 10  $\mu\text{m}$  in diameter. The push-button variety of aerosol dispenser was first developed during World War II. More recently, total release aerosols have been designed to discharge the entire contents in a single application and are available for home owners as well as for commercial operators. They are effective primarily against flying insects and provide little residual effect.

*Tablets* (usually water dispersible tablets WT) and *Slow release systems* blend an a.i with a material from which it will be released or evaporate at a controlled rate in the course of time. Ingredients that are volatile

or subject to degradation, *e.g.* by sunlight, remain active much longer in these formulations.

*Microencapsulated products* are a form of a slow-release formulation in which the a.i. is enclosed in a material such as polyamide, neoprene, polyvinyl chloride or polyester. The active ingredient can diffuse from the matrix. Microcapsule suspensions (CS/MC) are of two types:

- (a) Porous wall capsules usually containing diazinon, chlorpyrifos, permethrin, etc., through which the a.i. is slowly released over time
- (b) Nonporous capsules (*e.g.* fenitrothion), remain intact until ruptured or burst by tarsal contact with insect vectors

Encapsulated materials generally demonstrate a reduced absolute mortality together with extended residuality (Wade 1997). To this date, the formulations offering the most lasting residual effect are those in which the a.i. is present in microemulsion together with a surface film forming system "Secondary system EWs" (S-EW).

*Pellets and briquettes* are larger in size than granules, varying in size from 1.5 cm in diameter for pellets (tablet-like shape) to several cm for briquettes. The size and weight of briquettes vary depending on the carrier and amount of other additives present. Both pellets and briquettes release the a.i. as they dissolve and disintegrate in mosquito-breeding habitats.

*Extended residual briquettes* (XR) are designed to release effective levels of an a.i. over a longer period into the environment. Release of the a.i. occurs by dissolution of the briquette. XR-briquettes are of a special value in permanent, hard-to-reach breeding sites, or in situations where a pre- or post flood treatment provides a long-term effect.

### 18.2.3 Insecticide Application Techniques

The success of an insecticide application programme depends very much on the quality and performance of the equipment used. Manuals on mosquito control refer to a wide range of application equipment for delivering insecticide to the target-site. In this section, the more commonly used application equipment in mosquito control is briefly described, according to WHO/CTD/WHOPES (1997).

*Hand-operated compression sprayer:* These sprayers are designed to apply insecticides onto surfaces with which the insect will come into contact, or into a breeding site. The formulation and water are either mixed before filling the tank or mixed within it. The tank is then pressurized by a hand-operated plunger. A trigger on the lance of the sprayer controls the release of the material through a nozzle. Filtering the water while filling the sprayer, routine maintenance by qualified personnel and routine nozzle checks and calibration, are essential in maintaining the equipment's effectiveness (Wade 1997). Abrasion from particles in the water can cause deterioration of the nozzle opening, resulting in an excessive increase in the control product applied, which in turn may result in application of an incorrect or uneven dosage rate. A drawback of the hand-operated compression sprayer is that as the tank is emptied, pressure decreases causing a fall in the discharge rate, unless the sprayer is provided with a pressure regulator. Many of the problems with these sprayers at the treatment-sites are related to a lack of proper cleaning at the end of each day.

*Mist blowers (power-operated):* These can either be portable or vehicle-mounted. Portable knapsack mist blowers are powered by a two-stroke engine producing a high velocity air stream in which the insecticide formulation is atomized as a fine mist. The output rate can be adjusted through flow restrictors. At high flow-rates, large droplets are produced. Large droplets coat surfaces, whereas the smaller droplets act as an aerosol impacting insects in flight or at rest. Although the formulation is diluted in water, the overall volumes applied with mist blowers are still relatively small. Knapsack mist blowers can cover a large area in a relatively short time, and provide ease of access to areas where vehicle-mounted equipment cannot reach. The disadvantage of the portable knapsack mist blowers is the risk of burns from the engine, and discomfort caused by heat, vibration, and noise.

*Aerosol generators (power-operated):* These are often referred to as cold aerosol or cold foggers, and are typically used to apply ULV adulticide treatments with either technical insecticide (rarely), or formulations diluted in oil or water. Only formulations recommended for ULV use by the manufacturer should be applied by this equipment. These machines can be hand-held or truck-mounted, depending on their size. The volume of material used per unit area is much lower than with thermal foggers or mist blowers.

**Table 18.7** Insecticides suitable as cold aerosol sprays and for thermal fogs for mosquito control (WHO 2006)

Insecticide	Chemical	Dosage of ai. <sup>b</sup> (g/ha)		Toxicity: oral LD <sub>50</sub> of ai. <sup>a</sup> for rats (mg/kg body weight)
		Cold	Thermal	
Chlorpyrifos	OP	10–40	150–200	135
Cyfluthrin	PY	1–2	-	500
Cypermethrin	PY	1–3	-	7,180
Cyphenothrin	PY	2–5	-	2,250–2,640
Deltamethrin	PY	0.5–1.0	-	>2,940 <sup>c,d</sup>
D-phenothrin	PY	5–10	-	>10,000
Etofenprox	PY	10–20	10–20	>40,000
Fenitrothion	OP	250–300	270–300	503
Fenthion	OP	150	-	330 <sup>d</sup>
Malathion	OP	112–693	500–600	>4,000
Naled	OP	56–280	-	430
Permethrin <sup>d</sup>	PY	5–10	-	>4,000 <sup>b,c</sup>
Pirimiphos-methyl	OP	230–330	180–200	2,018
Propoxur	C	100	-	95
Zeta-cypermethrin	PY	1–3	-	86

PY Synthetic pyrethroid; OP organophosphorus; C Carbamate

<sup>a</sup>ai. active ingredient

<sup>b</sup>Because of their low dermal toxicity and on the basis of experience with their use, these products have been classified in the WHO Hazard Classification in Class III, Table 5 (WHO/PCS/94.2)

<sup>c</sup>Dermal toxicity

<sup>d</sup>Also used in mixtures with knock-down agents or synergists

By using ULV aerosol generators, a larger area can be covered more quickly. Portable ULV aerosol generators may also be used for indoor treatment, when access is difficult with compression sprayers. With ULV aerosol generators, the calibration of the flow rate and the uniformity of the droplet size is particularly important.

*Thermal foggers (power-operated):* These machines, which are either portable or vehicle-mounted, are preferred by some vector control agencies where the dense fog generated is perceived (mistakenly) as indicating high efficacy. In most parts of Europe, however, the dense, visible fog may raise safety concerns among the public. The droplet size is far less controlled than with ULV machines, and a wide range of droplet sizes from 1–200 µm are produced. In such situations, some insecticide will be wasted due to convection currents or early fallout. Moreover, the heat from these thermal foggers can be detrimental to the insecticide used.

*Aerial application equipment:* Large-scale and emergency vector control programmes often employ aircraft to apply insecticides. The aircraft are well suited for the rapid treatment of large areas where there is no access to the target-site, or when vegetation is dense. Aerial treatment can be used for adulticiding and larviciding applications. Accurate placement of the

insecticide formulation can be more difficult with aircraft than with ground application equipment. However, currently available computerized technology now provides very accurate treatment of the target-area.

Insecticides, which have a rapid knock-down effect on mosquitoes and are relatively harmless to other organisms, are best suited for space treatments. Organophosphorous compounds first became widely used for such applications, and various formulations of carbamates and synthetic pyrethroids are now available. According to the WHO operational manual on the application of insecticides for control of mosquito vectors (2006a), there are 15 active ingredients suitable for cold aerosol and thermal fogs applications (Table 18.7).

## 18.3 Chemical Groups of Insecticides

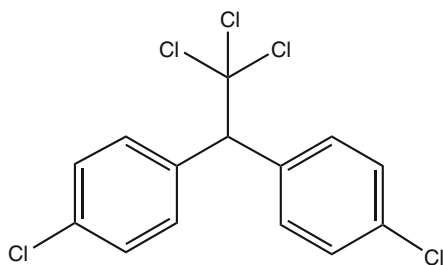
### 18.3.1 Chlorinated Hydrocarbons

The chlorinated hydrocarbons contain carbon, chlorine, and hydrogen. They are also referred to by other names: organochlorines, chlorinated organics, chlorinated insecticides. The chlorinated hydrocarbons can



be divided into subgroups according to structural differences, but they have in common high chemical stability, low solubility in water, moderate solubility in organic solvents, and a low vapour pressure (Hill and Waller 1982). The stability and solubility of the chlorinated hydrocarbons means that they are highly persistent, and this may lead to long-term contamination of the environment and gradual bio-accumulation in animals at the higher end of the food chain. For these reasons they have been banned by most developed countries. The broad spectrum of activity of these compounds, their persistence and their hazard to the environment explain why their use in insect pest management is largely considered inappropriate, although there are still situations where this group is still an important control option, *e.g.* in some malaria vector control programmes in endemic countries.

**DDT:** 1,1,1-trichloro-2,2-di  
(4-chlorophenyl)ethane



DDT is probably the best known and paradoxically, the most infamous chemical of the 20th century. It has been recognised as the most useful insecticide ever developed.

The great advantages of DDT include its persistence and its relative inexpensiveness to produce, so that it may be readily used on a large scale to protect people living in disease endemic areas of the world. Outstanding success was achieved by residual house-spraying against endophilic malaria vectors. Bruce-Chwatt (1971) pointed out that DDT has saved some 15 million lives by malaria control alone.

As is well known, some insecticides have caused problems during the past few decades for two reasons: pest resistance and environmental pollution. The first of these began with DDT-resistant flies in

1947 (Busvine 1978); a problem which grew steadily, but so insidiously that it has attracted little attention except from those concerned with pest/vector control. On the other hand, in the 1960s, the problem of environmental residues became front-page news. As could be expected, there was a widespread official reaction in the form of safety regulations. The result was a virtual banning of organochlorines in many countries.

DDT still, however, remains an important component in malaria control in some rural areas or countries, where it is available and legal to use and where the local vector species are still susceptible (Chavasse and Yap 1997). Even so, the assessment of the efficacy/resistance to DDT in an area is essential for planning an effective malaria control strategy.

DDT still continues to be a substantial part of vector control programmes in a number of countries. With the increase in IRS in many internationally and nationally funded malaria control programmes and affirmation by WHO that DDT is appropriate for use in the absence of longer-lasting insecticide formulations in some endemic malaria settings, DDT has been reintroduced as a major malaria control intervention in Africa (Coleman et al. 2008). A total of 10 countries from the African region reported the use of DDT to control malaria vectors. An estimated annual average of 547 tonnes of DDT was used (2004–2007) to control malaria (Zaim and Jambulingam 2007).

Resistance to DDT was first noted just 11 years after its introduction (WHO 1957). Because DDT-resistant insects were usually fully susceptible to subsequent classes of insecticide, such as the organophosphates, carbamates and synthetic pyrethroids, these classes of insecticides replaced DDT. Later on, as resistance developed, many programmes made an attempt to revert back to DDT as the insecticide of choice. However, because DDT and pyrethroids share a common target site in the sodium channel of insects (Soderlund and Bloomquist 1989), cross-resistance had developed to both classes of insecticides in many locations due to a mutation in the target-site (*kdr*) (Martinez-Torres et al. 1998; Ranson et al. 2000).

The production and use of DDT are strictly restricted by an international agreement (Stockholm

Convention on Persistent Organic Pollutants; [www.pops.int/documents/convtex](http://www.pops.int/documents/convtex) 2007). The Convention's objective is to protect both human health and the environment from persistent organic pollutant substances, DDT being identified as one from the group. However, exemption was given for the production and public health use of DDT for IRS to control vector-borne diseases, mainly because of absence of equally effective and efficient alternatives. Among the 12 insecticides currently recommended for this intervention by WHO, DDT is the one with the longest residual activity when applied on walls and ceilings (6–12 months, depending on dosage and nature of substrate). There are strict conditions to be met when using DDT. Prerequisites for safe and effective implementation of IRS include susceptibility status of vectors, proper monitoring of insecticide resistance and all relevant information reported to WHO (Zaim and Jambulingam 2007). The overview of the recent global use of DDT is given in Table 18.8.

### 18.3.1.1 Mode of Action

In insects, DDT produces tremors throughout the body and hyperexcitability, subsequently followed by loss of movement (ataxia). Beyond this point, an apparent paralysis develops, which may be completed over several hours after application.

Although the compound produces symptoms consistent with a toxic action on the nervous system, there are differences between DDT and other groups

of nerve-acting insecticides, indicating some differences in the mode of action. DDT disturbs the balance of sodium and potassium ions within the sensory neurons, thereby causing spontaneous firing, and preventing normal passage of impulses. This major symptom of DDT poisoning, results from its ability to induce repetitive firing on sense organs, giant axons, presynaptic terminals, and smaller nerve fibers. Whether a given neuron fires repetitively or not depends upon the membrane properties of the nerve cell itself, and on the elevation of the negative after-potential, which contributes to the repetitive discharge. The relative effectiveness of this after-potential in generating repetitive firing depends upon the properties of the sodium gate of the particular fiber. A further aspect of this process is that DDT interferes with the stabilizing action of  $Ca^{2+}$  at the axonal surface, and therefore leads to membrane destabilization. The disruption is transmitted to the rest of the nervous system, causing the muscles to twitch, which is usually followed by convulsions and death. DDT is a relatively slow acting insecticide, and has the unusual quality of being more toxic to insects as the ambient temperature is reduced, a negative correlation that is also characteristic of some pyrethroids. Gammon (1978) showed that the chemical had excitatory effects on both peripheral and central neurons and the effects on central neurons became more pronounced as the temperature was reduced.

A few striking points concerning DDT should be highlighted to understand some of the well documented problems associated with it. One of the most remarkable

**Table 18.8** Global use of DDT for vector control reported to WHOPES, in kg of active ingredient, WHO African Region, 2003–2005, (Zaim and Jambulingam 2007)

WHO region	Country	2003	2004	2005
African	Eritrea	NA	6,552	10,109
	Ethiopia	272,243	255,163	275,195
	Madagascar	45,000	30,000	0
	Mauritius	872	899	625
	Mozambique	NA	NA	307,688
	Namibia	52,143	25,837	39,611
	South Africa	53,610	62,112	65,575
	Swaziland	NA	NA	7,538
	Zambia	NA	8,648	13,308
	Zimbabwe	NA	NA	108,000
	Total	10 countries	423,868	389,210

NA denotes data not available

points of DDT is its chemical stability. This important property results in having a long half-life in the soil, the aquatic environment, and in plant and animal tissues. It is not readily broken down by microorganisms, enzymes, heat or ultra violet light. Secondly, DDT has been reported in the literature to be probably the most water-insoluble compound ever synthesized. However, it is soluble in lipids and, as a consequence of its resistance to metabolism, it is readily stored in the fatty tissue of any animal ingesting it, either alone or dissolved in food, even when it is part of another animal. It accumulates in every predator, as well as in those that eat plants bearing traces of DDT. The principal result of this movement in the food chain is bioaccumulation.

The history of DDT's development and usage shows that there is a need for an informed and cautious approach to pesticide usage. It is essential to conduct basic research before we encourage widespread application of any particular product, to enable us to identify and restrict, if not to avoid those products that may pose dangers to the environment and to health.

### 18.3.2 Organophosphates

The chemically less stable OP insecticides partially replaced the persistent chlorinated hydrocarbons. The term OP is usually used as a generic name to include all insecticides containing phosphorus. The OPs have several other commonly used names: organic phosphorous, phosphorus insecticides, phosphorus esters or phosphoric acid esters. They are all derived from phosphoric acid. The OPs have two distinctive features, a lower chemical stability and they are generally much more toxic to vertebrates than the organochlorine insecticides. Their lower persistence brought them into use as alternatives to persistent organochlorines, particularly to DDT. However, unfortunately, resistance to OPs also developed, and became more widespread as these compounds were more widely used. This situation led to a plea by WHO to encourage commercial companies to continue the search for alternative compounds suitable for pest control (WHO 1976).

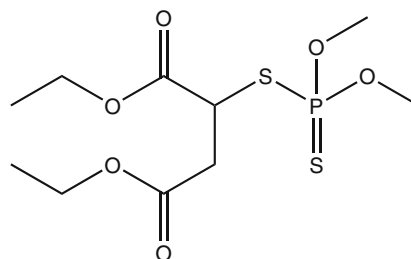
However, in the recent report of WHO, (Zaim and Jambulingan 2007) the OPs continue to be used in vector control in the second half of the 20th and the beginning of 21st century.

#### 18.3.2.1 Mode of Action

OPs exert their toxic action at synapses by inhibiting cholinesterase (ChE), an important enzyme in the nervous system. It is well established that as OPs inhibit acetylcholinesterase (AChE), the acetylcholine (ACh) concentration then rises in treated insects (reviewed by Corbett 1974). OPs interact with AChE in the same way as ACh does, phosphorylating the same serine hydroxyl that ACh acetylates. OPs inhibit the enzyme almost irreversibly, with dephosphorylation occurring over a period measured in weeks or months, contrary to some other chemical groups, such as carbamates, where the action of decarbamylation occurs from minutes to less than 8 h (Taylor 1980). This has led some to refer to OPs as irreversible inhibitors and carbamates as reversible ones. This inhibition, resulting in the accumulation of acetylcholine, interferes with the neuromuscular junction, producing rapid twitching of muscles and final paralysis of the insect. However, the precise sequence of events between enzyme inhibition and death is still not fully understood. The excess acetylcholine has widespread harmful effects, which may include the uncontrolled hormone release known to accompany insect poisoning (Maddrell 1980).

Several insecticides which have been performing an important role in mosquito control worldwide, belong to the group of OPs. Perhaps the most important one is malathion, which is also the oldest and most widely used aliphatic OP.

**Malathion:** 2-(dimethoxyphosphinothioylthio)butanedioic acid diethyl ester



Malathion was introduced in 1950, for agriculture, urban pest control and mosquito control. Because of its fast action and exceptionally low acute toxicity to

humans and other warm blooded animals, it became the insecticide of choice.

As a public health insecticide, malathion has found its most common use as a ULV formulation to control adult mosquitoes. Its low mammalian toxicity allows the application of the compound in urban areas where dense mosquito populations are a nuisance or threaten human health. Many of the ULV applications of malathion are carried out by aircraft or helicopters. Aerial ULV malathion applications are still effective at a rate of 320 g/ha, in regions where resistance to OPs has not yet developed. The application equipment has to be calibrated to dispense droplets small enough to remain suspended in the air, but large enough to contain a sufficient amount of insecticide to kill an adult mosquito. Results of aerial ULV treatments worldwide show that success of ULV applications depend greatly on weather conditions. Above all, the wind speed has to be less than 3 m/s, and the time of application preferably dusk, which coincides with the activity of the species being controlled. The presence of inversion conditions, are also important in maximizing efficacy of the application.

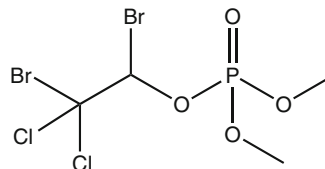
Malathion is also applied by ULV ground equipment. A maximum vehicle speed of 16 km/h for such treatments often limits the area that can be treated during the period of mosquito activity. Any increase in the speed of the vehicle should only take place if the insecticide output is also increased correspondingly. Fortunately, most truck-mounted cold aerosol generators used for ULV applications are also equipped with variable flow-control systems. These systems, when properly calibrated, automatically adjust to dispense the correct amount of insecticide relative to the vehicle speed. The effect of operating pressure on the droplet volume median diameter (VMD) at various flow rates for malathion ULV applications is well documented. Nevertheless, for any particular situation the aerosol droplets have to be collected, counted, and measured using an appropriate procedure: collect droplets on Teflon coated plates, count them using a compound microscope at a magnification 200 $\times$ , take into consideration the insecticide spread factor (for malathion 0.69) and analyze the data. Dukes et al. (1990) determined that an increase in the flow rate required a corresponding increase in the blower pressure, to maintain the labeled malathion (Cythion) droplet median diameter of 17  $\mu$ m. For a flow rate of 254.3 ml/min, at the maximum vehicle speed of 32 km/h, a machine pressure of 7–8 psi

(48.3–55.2 kPa) is required to consistently achieve the droplet size criteria on the Cythion label.

As the efficacy of ULV applications against mosquitoes is based on the premise of an airborne droplet impinging on a flying mosquito, this method is often ineffective against indoor resting species, due to the decreased number of droplets reaching individual target mosquitoes. In such situations, ULV adulticiding is not recommended due to the behaviour pattern of the species.

The broad-spectrum, nontarget activity of malathion makes this compound undesirable in most natural environments.

**Naled:** 1,2-dibromo-2,2-dichloroethyl dimethyl phosphate



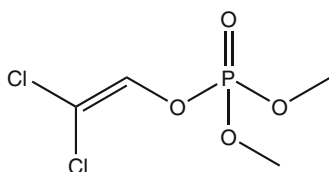
Naled (Dibrom) has been a widely used brominated aliphatic OP with low mammalian toxicity. Naled was one of the choices for adult mosquito control throughout the United States, but has been withdrawn from further use (Mulla 1994). In the Americas, it was also widely applied in large mosquito-breeding areas by ULV or conventional applications. Mount et al. (1968) reported moderate efficacy of Naled with caged *Oc. taeniorhynchus* when ULV treatments were applied at a dosage of approximately 9.0 ml a.i./ha. The results presented by Linley et al. (1988) showed that ULV treatments with Naled did not effectively control *Oc. taeniorhynchus*. In Florida, ULV treatments with Naled with vehicle-mounted equipment were used by many mosquito control districts, but also had some impact on *Culicoides* spp. (Ceratopogonidae).

Howard and Oliver, (1997) based on 11 years' analysis (1984–1994) of data from New York swamp habitats of the primary enzootic vector (*Cs. melanura*) of eastern equine encephalitis (EEE), showed the effect of Naled treatments on the vector population. Naled treatments accomplished only short-term reductions in the mosquito abundance. Despite repeated applications,

populations of *Cs. melanura* increased 15-fold in some parts of the treated area, which discredits the suggestion that Naled applications could reduce the risk of EEE.

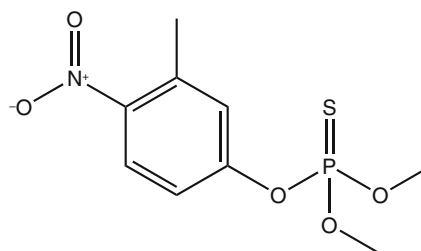
Hurricanes and tropical storms often have a significant impact on mosquito-borne diseases because they result in an increase in mosquito-breeding habitats caused by flooding. In the USA, when mosquito abundance and potential disease emergence are imminent, aerial applications of Naled are implemented, as in the post-hurricane Rita situation when Air Force Aerial Application Flight took action in four Texas counties. A significant (90%) decline in mosquito abundance was observed as determined by post treatment evaluation (Breidenbaugh et al. 2008). In 2004, Florida experienced an extraordinary hurricane season with four major hurricanes traversing the state within 3 months. Because of the potential threat of arboviral diseases, including West Nile virus (WNV) and EEE, ULV aerial applications with Naled were initiated. Although Naled has been associated with adverse human health effects following ULV aerial applications (CDC 2003), the extent to which humans are exposed to Naled during large-scale aerial applications has yet to be accurately quantified. To estimate the potential health risk, CDC and Florida Department of Health assessed human exposure to ULV aerial application of Naled. The data suggest that aerial applications of Naled do not result in increased levels accumulated in humans, provided that the product is used according to label instructions (Duprey et al. 2008). In large-scale mosquito control programmes, Naled is typically applied via aircraft with the inert carrier, naphtha. For effective mosquito control, the maximum rate for ULV surface and aerial applications is typically c. 200 ml/ha. These ULV applications produce very fine droplets that stay aloft and kill mosquitoes on contact. ULV applications are utilized to minimize exposure and risks to people, wildlife, and the environment, (U.S. EPA, 2002).

**Dichlorvos (DDVP):** 2,2-dichlorovinyl dimethyl phosphate



DDVP is also an aliphatic OP with a very high vapour pressure, giving it strong fumigant qualities. Apart from being known as an a.i. used in vapourizing strips, from which it is slowly released, DDVP also serves as an effective mosquito adulticide applied in a mixture with kerosene or diesel (Vapona 7 or Nuvan 7) by thermal fog generators. Thermal fogs using dichlorvos can achieve an immediate and high level of reduction of adult outdoor mosquitoes. The disadvantage of such treatments is generally low persistence, and therefore DDVP treatments must be repeated at fairly short intervals to ensure continued suppression of adult mosquito populations. Since the mammalian toxicity of the product is very high (oral LD<sub>50</sub> for rats is 56 mg/kg), DDVP is in WHO toxicity class 1b, and its use has been discontinued in most European countries. The other disadvantage is its very poor selectivity to the entomofauna. Its only advantage is the speed of action as an adulticide, an advantage in epidemic situations.

**Fenitrothion:** Dimethoxy-(3-methyl-4-nitrophenoxy)-thioxophosphorane

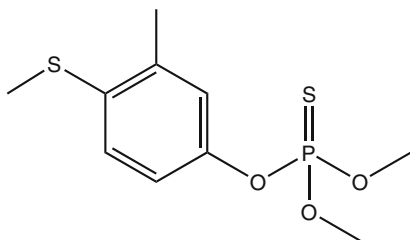


Fenitrothion is a phenyl derivate of OPs (with a benzene ring), and this moiety is critical in improving stability compared with the aliphatic OPs. Fenitrothion under various trade names (Acotion EC 20, Fenitrothion 20 EC, etc.) has been extensively used in Europe, especially as a larvicide in closed water systems (sewage and waste-water containers). However, in France it has produced resistance in *Aedes/Ochlerotatus* and *Culex* species and also in extensively irrigated crops in Spain, where resistant strains of *An. atroparvus* and *Cx. theileri* have developed (Grandes and Sagrado 1988). Studies conducted by Wesson (1990) have demonstrated that the larvae of 26 strains of *Ae. albopictus* [*St. albopicta*] from the United States, Brazil, southeast Asia, and Japan, showed different levels of susceptibility to fenitrothion when it was applied at the baseline susceptibility concentration of

0.02 mg a.i./l. Rodríguez et al. (2007) showed that when *Ae. aegypti* from Cuba, with a high level of temephos resistance, was subjected to temephos selection to evaluate the potential of this OP for mosquito control, after six generations of selection, little or no cross-resistance was observed to the OPs, malathion and fenitrothion.

Insecticidal properties of fenitrothion microcapsules (CS) as a residual formulation were studied on *An. albimanus* females (Kawada et al. 1995). Residual efficacy of the microencapsulated formulation at the rate of 1 g/m<sup>2</sup> was almost equivalent to that of WP at the rate of 2 g/m<sup>2</sup> on a plywood surface and was superior to that on an unglazed pottery surface. Greater than 50% mortality was maintained for >28 weeks after treatment by fenitrothion CS on a mud surface with 10 min contact at the rate of 2 g/m<sup>2</sup>. Microcapsule particles were observed to be mechanically broken (trampled) by mosquito contact and the amount of fenitrothion released from the CS increased as contact time increased.

**Fenthion:** Dimethoxy-[3-methyl-4-(methylthio)phenoxy]-thioxophosphorane



Fenthion is another phenyl derivate with contact and stomach action and broad-spectrum activity against sucking and biting pests. In addition to agriculture, fenthion also has uses in veterinary applications and in public health vector control.

The product was commonly applied peri-focally during the *Ae. aegypti* eradication campaign in the Americas. Fenthion is used as a component in an overall mosquito control programme in some regions of Florida.

The U.S. EPA concluded that fenthion applications, especially when repeated at frequent intervals, may cause mortality in a variety of bird species, including raptors feeding on exposed birds (EPA 2001).

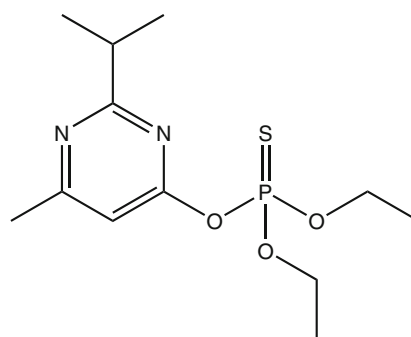
The European Commission (EU 2004) concluded that fenthion cannot be included in Annex I to

Directive 91/414/CE, based on possible risks to birds. In general, uses of fenthion were withdrawn by the EU in 2005. Concerns over the potential adverse effects of fenthion on estuarine biota appear to be unclear, although there were records of fenthion acute LC<sub>50</sub> figures for mosquitoes, which exceeded those for some mysids and pink shrimp. The degree to which the risk might be apparent for nontarget estuarine communities depends on various factors such as mixing and dilution of the product, characteristics of local habitats, weather conditions, and potential for cumulative effects of repeated applications during a season (Clark et al. 1987).

Wesson (1990) showed in a larval bioassay that fenthion can be successfully used in controlling *Ae. albopictus* at a rate of 0.02 mg a.i./l. This rate was effective in suppressing 25 out of 26 tested strains of this species. The LC<sub>95</sub> values for the remaining strain (originating from the US) showed a 4-fold higher tolerance compared to susceptible strains.

Fenthion is sold under various trade names such as Baytex, Lebaycid, and Queletox. The most important formulation types are EC and WP. The products are mostly registered and sold in the Middle and Far East and Africa.

**Diazinon:** Diethoxy-[(2-isopropyl-6-methyl-4-pyrimidinyl)oxy]-thioxophosphorane

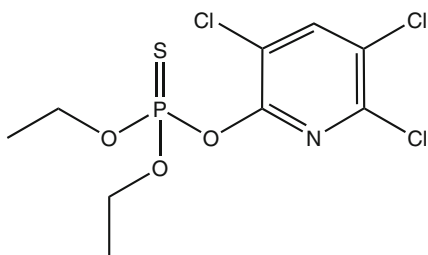


Diazinon was probably the first heterocyclic OP derivate from this group. The heterocyclics have generally longer-lasting residues than many of the aliphatic or phenyl derivatives. Diazinon was first available in 1952 and is sold under a variety of brand names, including DZN and Knox Out 2FM. The product is chemically related to malathion and chlorpyrifos.

Diazinon has agricultural, commercial, and household uses, but household uses predominate. It has been commonly used as a residual fly spray and as an adulticide against indoor mosquitoes. It has a relatively low mammalian toxicity, and has a very good record for urban pest control, largely because it is effective against a wide range of household insect pests.

Inside living organisms, diazinon is transformed into a molecule called diazoxon. Diazinon, and the more potent diazoxon kill insects by interfering with nervous system function. Not all of diazinon's toxicological effects stem from its inhibition of AChE. Diazinon and other OPs inhibit numerous enzymes with molecular structures that are similar to AChE. According to EPA, "Organophosphates are efficiently absorbed by inhalation, ingestion, and skin penetration," Exposure by "multiple routes can lead to serious additive toxicity." EPA (April 2000) estimated exposure via multiple routes following both lawn care application of liquid formulations and crack-and-crevice indoor applications as exceeding "level of concern." For exposures following residential applications, a single application can lead to exposure via all three routes.

**Chlorpyrifos:** *O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate



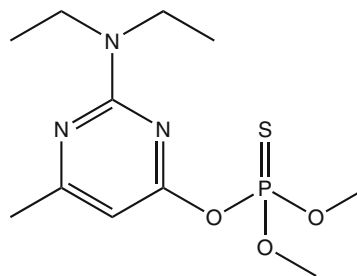
Chlorpyrifos is a heterocyclic OP and as the a.i. in the formulation Dursban E 48, has been used as a larvicide to control *Aedes/Ochlerotatus*, *Anopheles* and *Culex* species in many countries in the tropics, North America and Europe. However, resistance appeared quickly when it was applied frequently in *Culex* habitats in southern France (Sinagre et al. 1984). In California, Schaefer and Dupras (1970) demonstrated that application of chlorpyrifos resulted in a fairly long-term control of mosquito larvae in polluted water sources. Mosquito abatement districts used chlorpyrifos to treat waste-water.

However, in 1974, control failures in these habitats were observed (Georghiou et al. 1975). Susceptibility tests conducted by Wesson (1990), showed that some of the 26 *Ae. albopictus* (*St. albopicta*) strains (from Brazil and southeast Asia) exposed at a concentration of 0.01 mg a.i./l, displayed tolerance to this insecticide. However, the majority of strains tested were still susceptible to the chlorpyrifos concentration used.

Chlorpyrifos in various formulations has provided very good control and long persistence in a variety of container types (Glancoy et al. 1968). Its mammalian toxicity would exclude it from use in potable water containers, but it is a useful product in larval habitats such as flower vases at cemeteries, and in small wastewater containers. Chlorpyrifos has also been used for ULV treatments in the US.

The use of this compound in open-water systems inhabited by fish is not recommended because of toxicity to fish.

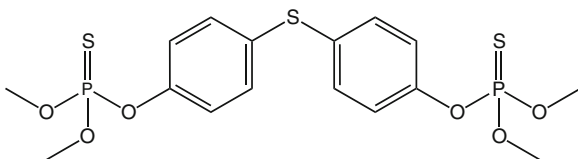
**Pirimiphos methyl:** 0, [2-(diethylamino)-6-methyl-4-pyrimidinyl] 0,0-dimethyl phosphorothioate



Pirimiphos methyl, also a heterocyclic OP, (trade name Actellic), became the insecticide of choice in some endemic malarial regions after resistance was recorded in Anopheline species to some other OPs, carbamates and to some synthetic pyrethroids. Resistance of *An. sacharovi* in Turkey to phoxim, chlorpyrifos, propoxur, and tolerance to malathion, was reported by Ramsdale (1975) and Davidson (1982). However, surface treatment of pirimiphos-methyl 50% EC at 0.9 g/m<sup>2</sup> caused a significant decrease in parous rate and 96.9% reduction in resting density. The persistence on different surface materials for 7 weeks post application resulted in mortality rates of 73–98% (Kasap et al. 1992). For residual mosquito control, pirimiphos methyl may be

used at rates of up to 2 g a.i./m<sup>2</sup>. The optimum volume will vary with the type of surface encountered. Mosquito larvae in shallow water (~10 cm deep) may be controlled with 1 part of pirimiphos-methyl 50% EC mixed with 99 parts of water (0.5%) and applied at a volume of 100 l/ha (10 ml/m<sup>2</sup>). At rates of 0.5–0.1% a.i., depending on the water depth, suppression of mosquito larvae, with retreatments after 5–8 days, can be achieved.

**Temephos:** [4-(4-dimethoxyphosphinothioxyloxyphenyl)sulfanylphenoxy]-dimethoxy-sulfanylidene-phosphorane



Temephos is a heterocyclic OP, available under the trade name Abate. It has been used as a mosquito larvicide for a longer period of time than most other products. Due to its very low mammalian toxicity, with an LD<sub>50</sub> of 2,030 mg/kg (WHO class III N), it is used in various aquatic habitats, including water intended for human consumption. The only insecticide compounds that have been toxicologically approved as safe for use in potable waters are temephos, permethrin, methoprene, and products based on *B. thuringiensis israelensis* (WHO 1991; WHO IPCS 1992). The disadvantage of temephos is a disagreeable smell.

Temephos has a different profile from other larvicides (IGRs and microbials) used in the control of mosquito larvae, in that it is effective against all larval instars of *Aedes/Ochlerotatus*, *Culex* and *Anopheles*. However, it also affected populations of *Podura aquatica* (Collembola) and immature stages of *Odonata*, *Ephemeroptera*, *Coleoptera*, and *Diptera: Chironomidae* (Zgomba et al. 1983; Zgomba 1987).

Sand granule formulations containing 1–5% a.i. have been successfully used as larvicides in a wide range of flood-water situations controlling *Aedes/Ochlerotatus*. The application rate depends on water quality and ranges from 10 to 20 kg/ha for 1% temephos. It can be applied by hand, by ground equipment, from a boat or by helicopter and fixed-wing aircraft.

A reduction of adult density by up to 95.4% was achieved when temephos was applied in 1% sand granule formulation to water-bodies containing larvae of *Ae. aegypti* in Thailand. When temephos EC was applied in India and Burkina Faso, the period of larval control following a single application, lasted up to 8 weeks (Geevarghese et al. 1977; Hervy and Karnbou 1978).

Resistance to temephos has appeared in some areas following intensive use of the compound. It was reported by Sinegre (1984) in parts of France, in agricultural zones of Spain by Grandes and Sagrado (1988), in the Dominican Republic by Mekuria et al. (1991) and recently in India, Baruah (2004).

An increased level of tolerance elsewhere, such as in the Caribbean islands (Georghiou et al. 1987) and French Polynesia, has been reported, but it is assumed that the level of temephos resistance is not high enough to disrupt its operational use. Wesson (1990) examined the OP larval susceptibility of 26 *Ae. albopictus* strains. Three U.S. strains and one Japanese strain had LC<sub>95</sub> values at or above the diagnostic concentration, which for temephos was 0.04 mg a.i./l. The tolerance level of one strain from the Americas increased 3-fold. All Brazilian and Southeast Asian strains had LC<sub>95</sub> values below the diagnostic concentration. Following reports by several authors, Breaud (1993) reviewed mosquito resistance to temephos in Florida. The most abundant *Culex* and *Aedes/Ochlerotatus* species already showed tolerance to temephos in 1979 (6× and 39×), with a tendency to develop resistance to other larvicides and adulticides from the OP group in the region. In laboratory studies using temephos and fenthion, two commonly used larvicides under the Urban Malaria Scheme in India showed that the LC<sub>50</sub> and LC<sub>90</sub> values for temephos against *Ae. aegypti* were 0.0177 and 0.0559, for *An. stephensi* 0.0148 and 0.0472, *Cx. quinquefasciatus* 0.0157 and 0.0480, and for *Cx. vishnui* group of mosquitoes 0.043 and 0.0118 ppm, respectively. The results obtained, revealed that there is a 62.8 and 94.12 times increase in the LC<sub>50</sub> and LC<sub>90</sub> of *Cx. quinquefasciatus*, which indicates that the species has developed resistance to temephos. The 6.32 and 8.34-fold increase in *Ae. aegypti* and 2.27 and 2.34-fold increase in LC<sub>50</sub> and LC<sub>90</sub> values of *An. stephensi* are indicative of development of tolerance against temephos. Reports from South America demonstrate (Seccacini et al. 2008) that acquired resistance by *Ae. aegypti* to the product is widespread.



Thavara et al. (2005) tested the efficacy of temephos 1% GR at 1 and 10 g/200 l water in jars (0.05 and 0.5 mg a.i./l). Their results showed that even at 1 g/200 l water, which is 1/20 of the recommended dosage used in the control programme, the level of control was about 100% for up to 20 weeks or longer.

Temephos has been used for the control of *Ae. aegypti* in Thailand for over 30 years. The current dosage used in the *Ae. aegypti* control programme in Thailand is 10 g of 1% sand granules/100 l water in water-storage containers. Thavara et al. (2005) showed that the magnitude of release-profile and efficacy of temephos 1% GR (sand granules) against *Ae. aegypti* larvae in water-storage containers was adequate in the initial period of 2–3 weeks after treatment. Following this period, the efficacy of the granules increased substantially where 92–100% inhibition of emergence even at the lowest dosage of 1 g/100 l (0.05 mg/l a.i.) was obtained for an additional 5 weeks. Based on this evidence, it is desirable to study the efficacy of lower dosages of temephos than those currently used in *Ae. aegypti* control. It was also suggested (Mulla et al. 2004) that the use of controlled-release formulations or sachets that are retrievable during cleaning and washing could be more practical, desirable and cost-effective. In those many tropical and sub-tropical countries where DF and DHF are considered the most important vector-borne diseases of public health importance, (Gubler et al. 1998), *Ae. aegypti* and *Ae. albopictus* play a crucial role in their transmission. At present, the only effective method is to combat the vector mosquitoes, primarily using insecticides. Controlling or preventing DF and DHF is therefore still heavily dependent on the use of insecticides, although insecticide resistance development represents a threat.

### 18.3.3 Carbamates

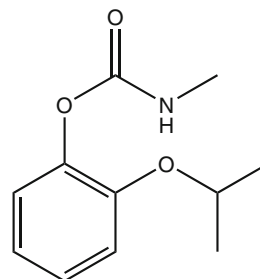
Carbamates are derivatives of carbamic acid, and were first introduced in 1951 by the Geigy Chemical Company in Switzerland. However, early compounds lacked stability, and it was not until the discovery of the highly active *N*-methyl carbamates that this group was developed, and became an important group of insecticides.

#### 18.3.3.1 Mode of action

The mode of action of carbamates is basically the same as that of OPs. They affect the activity of AChE, which catalyzes the hydrolysis of ACh, the chemical neurotransmitter, which acts at synapses in the nervous system of the insect. Following the inhibition of the enzyme caused by carbamylation, ACh accumulates thus prolonging the action of the neurotransmitter at the cholinergic synapses. The resulting hyperexcitation of the nervous system is accompanied by convulsions, paralysis and ultimately leads to death of the insect (Eldefrawi 1985). However, in the case of carbamates, the enzyme inhibition is more easily reversed than with OPs and the insects can recover at lower dosages (Dent 1991).

Carbamates have a broad spectrum of activity and usually act by contact or stomach action. They have been used effectively against vectors that have developed resistance to the organochlorines and OPs.

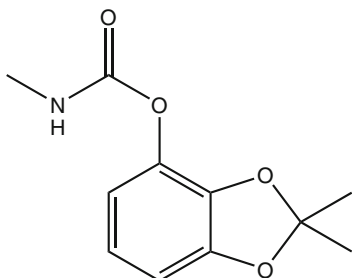
#### Propoxur: 2-isopropoxyphenyl *N*-methylcarbamate



One of the most commonly used products containing propoxur, as a contact and stomach poison, is Baygon. Endophilic mosquitoes can be effectively suppressed by residual treatments of walls and ceilings of human dwellings with propoxur WP. The residual application using a knapsack sprayer will typically provide 3 months of control. For outdoor area treatments to control mosquitoes by ground or aerial ULV application, Baygon ULV 120 may also be used. Ground treatments with propoxur ULV may be carried out with undiluted material at a flow rate of 250 ml/min, and a vehicle speed of 8 km/h, or with a correspondingly increased amount at higher speeds.

To examine the potential for synergism between carbamate and pyrethroid insecticides, Corbel et al. (2003) tested permethrin and propoxur as representatives of these two classes of compounds. Larvicidal activity of both insecticides was assessed separately and together on a susceptible strain of *Cx. quinquefasciatus*. When mixed at a constant ratio (permethrin : propoxur 1:60 based on  $LC_{50}$ ), significant synergy occurred between them. Synergistic effects were attributed to the complementary modes of action by these two insecticide classes acting on different components of nerve impulse transmission. Apart from raising new possibilities for *Culex* control, it seems appropriate to consider using such mixtures or combinations for insecticide-treated mosquito nets in situations with insecticide-resistant *Anopheles malaria* vectors.

**Bendiocarb:** 2,2-Dimethyl-1,3-benzodioxol-4-yl)  
N-methylcarbamate



Bendiocarb has been widely used as a residual household insecticide (Ficam 80 WP) by pest control operators against cockroaches and flies, and in public health adult mosquito control, both as a residual spray and for ground or aerial ULV applications.

Evans (1993) showed that deposits of bendiocarb caused very low irritation of mosquitoes without repellency, which makes it suitable for use where residual insecticides are selectively applied to the mosquito resting-sites within buildings. Selective treatments are sometimes used in house spraying for vector control, in order to reduce insecticide use. Arredondo-Jimenez et al. (1992) reported that selective applications of bendiocarb were equally effective for control of *An. albimanus* in Mexico. Asinas et al. (1994) showed that in the Philippines, *An. flavirostris*

could be controlled by selective applications at a rate of 40 ml/m<sup>2</sup> and a dosage of 400 mg a.i./m<sup>2</sup>.

### 18.3.4 Pyrethroids

Pyrethroids are a new class of highly active synthetic insecticides. They emerged from prolonged efforts to improve the biological activity and chemical stability of the natural pyrethrins, long known for their insecticidal effects. Natural pyrethrins consist of a mixture of insecticidal esters, extracted from the flower heads of *Chrysanthemum* spp.

Commercial production of “Dalmatian insect powder” ground from flowers of *C. cinerariaefolium* started in Dalmatia, and was widely used by 1840 (Casida 1980). Flower production then moved to California, and at the beginning of the twentieth century, cultivation started in Japan, well known for its long existing chrysanthemum horticulture tradition. However, between 1935 and 1940, the extract from pyrethrum flowers grown in Kenya replaced the Japanese product, because it contained a higher concentration of pyrethrins. Since the development of pressurized aerosol containers in 1941 for the delivery of pyrethrum, the product could be applied in droplets smaller than 30 μm, which increased the efficacy and cost-effectiveness of the pyrethrins. Because of its low mammalian toxicity, pyrethrins is commonly used for the control of house flies, mosquitoes, and other indoor pests. Pyrethrum extract is also commonly incorporated into burning coils, the smoke of which repels, knocks-down, or kills mosquitoes within homes (Baillie and Wright 1985).

The effectiveness of pyrethrins was first enhanced by the addition of synergists such as piperonyl butoxide (PBO) in 1949. Synergists are not insecticidal by themselves, but increase the potency of pyrethrum formulations by inhibiting their biodegradation (Yamamoto 1973).

Synthetic pyrethroids, which are more stable than pyrethrins, have partially replaced or supplemented the use of the three other major classes of insecticides in several areas of pest/vector control. An active area of pyrethroid use is in the control of various vectors and nuisance mosquitoes. Besides causing a knock-down effect, pyrethroids may also have a repellent or anti-feeding action.

There is evidence that pyrethroids can also be effectively applied as mosquito larvicides. This potentially allows application of permethrin as a larvicide in an IMM programme, because permethrin has an approval for use in drinking water at a concentration of 15 mg/l (WHO 1991). However, the use of pyrethroids in water bodies inhabited by fish is not recommended due to toxicity to fish.

A large number of pyrethroid compounds have been synthesized during the last few decades, some of which are exceptionally potent. As a result, research aimed at producing new insecticidal compounds has involved not only chemical substitution within molecules of interest, but also resolution and purification of the most active isomers. The widespread distribution of insecticide-impregnated mosquito nets is a major component of the WHO global strategy for malaria control, especially in sub-Saharan Africa, where more than 90% of the world's malaria cases are reported annually (Lines 1996). To date, six pyrethroids – the only group of insecticides currently considered suitable for impregnation of mosquito nets – have been evaluated and recommended (WHO/CDC/WHOPES/2001.2) for this purpose. They are: alpha-cypermethrin, cyfluthrin, deltamethrin and lambda-cyhalothrin (alpha-cyano pyrethroids), and etofenprox and permethrin (non cyano pyrethroids).

Beside malaria prevention, protection against nuisance insects, especially *Cx. quinquefasciatus*, which keep people awake at night, is also an important motivation for the use of mosquito nets. However, pyrethroid resistance in this species is already widespread in the tropical world, including Africa.

#### 18.3.4.1 Mode of action

Considerable progress in the synthesis of these materials has been made, since the mode of action of pyrethroids has become better understood (reviewed by Elliott 1980). Pyrethroids are neurotoxic to insects, and flying insects are in general, rapidly knocked-down. The lethal activity of pyrethroids seems to involve action on both peripheral and central neurons, while the knock-down effect is most probably produced by peripheral intoxication.

The close similarity in action between pyrethroids and DDT is an interesting phenomenon, which is still far from being understood. Pyrethroids share

many characteristics with DDT; one intriguing feature is that they become more toxic to insects as the temperature is lowered (a negative temperature coefficient).

Commercially available natural pyrethrins and their “first generation” synthetic analogs, such as allethrin and resmethrin, are broken down by UV light, as in sunlight. Hence there is an advantage of application at night. The second group of these potent insecticides is a wide range of “second generation” photostable analogs, such as permethrin and cypermethrin.

Pyrethroids are also commonly mixed with the synergist piperonyl butoxide (PBO), which slows down the metabolic degradation of the active ingredients.

Pyrethroids, as broad-spectrum insecticides, are also toxic to beneficial insects, including honeybees and predators of common pests. Fish toxicity is of particular concern in view of the potential use of pyrethroids in aquatic habitats for the control of mosquito larvae, against which pyrethroids have demonstrated excellent activity (Mulla et al. 1978). Selective control of mosquito larvae without affecting fish can nonetheless be achieved by using the correct dosage. Application rates of 2 ppb deltamethrin or 45 ppb permethrin had no effects on reproduction of mosquito fish, while mosquito larvae were effectively eliminated (Mulla et al. 1981).

Pyrethroids now have many uses in addition to conventional surface or space treatments. For example, pyrethroid aerosols are now widely used in aircraft, to prevent the transfer of mosquitoes from areas in which mosquito-borne disease is endemic, to unaffected areas. This usage is carried out under the International Health Regulations. Pyrethroids are also widely used to treat clothing, to prevent mosquitoes and other blood-feeding arthropods from alighting. Bednets are also very widely treated with pyrethroids, to deter biting by nuisance and disease vector mosquitoes. A very wide range of domestic insecticide products, including aerosols, coils, and vapourizing devices, also contain various pyrethroid active ingredients.

#### 18.3.4.2 Resistance

By 1992, pyrethroid resistance had already been detected in at least 40 arthropod species, and this number will have increased considerably since then

(Georghiou, 1992). Cross-resistance or multiple-resistance generated by DDT and pyrethroids is the main mechanism by which this resistance has arisen. Resistance to pyrethroids was exhibited first by those insects that were already resistant to DDT, *e.g.* house flies, (Busvine 1951; Sawicki 1978) and some mosquito species (*Cx. tarsalis*, *Ae. aegypti* and two *Anopheles* spp.) as reported by Prasittisuk and Busvine (1977) and Plapp and Hoyer (1968).

Much information on pyrethroid resistance has been derived from studies on the house fly. The most important of several pyrethroid-resistance mechanisms is knock-down (kd) resistance, a mechanism which results in broad resistance to pyrethroids, DDT, and DDT analogs. Kd resistance induced by DDT confers inherent cross-resistance against the knock-down effect of pyrethroids, and vice versa. Developing resistance against pyrethroids may curtail the otherwise promising prospects for the use of these compounds as pest and vector control agents.

Pyrethroid resistance of malaria vectors has already developed in several malarious countries and is increasingly common in Africa (Hargreaves 2000), and the absence of a suitable alternative insecticide class for impregnation of mosquito nets may undermine the gains in malaria control and personal protection being made through improved coverage with treated nets.

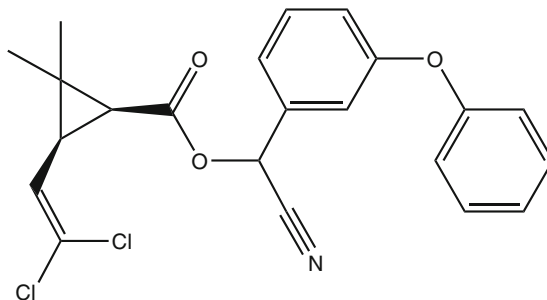
The efficacy of a pyrethroid used for impregnation of mosquito nets is the result of the insecticide's intrinsic activity and the behaviour of the target mosquito in response to it. This is of particular relevance for fast-acting insecticides, such as pyrethroids and DDT with knock-down and irritant properties. The intrinsic activity can be tested with adult mosquitoes using WHO cones. However, natural avoidance behaviour, blood-feeding inhibition and mortality can be obtained only in field conditions.

Recent field studies in pyrethroid-resistant areas of Côte d'Ivoire, in experimental huts N'Guessan (2001) and on a larger scale (Dossou-Yovo 2000), indicated that pyrethroid-impregnated mosquito nets reduce malaria transmission despite a high frequency of the knock-down resistance (*kdr*) gene. WHO recommended that this should be confirmed in other studies, especially where pyrethroid-resistance mechanisms other than the *kdr* gene may be involved.

When selecting pyrethroids for mosquito control and personal protection, specific attention should be given to the various properties of these insecticides,

the behavioural response of the target mosquito species, and the pyrethroid resistance status in the area.

**Alpha-cypermethrin:** (*S*)- $\alpha$ -cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (*R*)- $\alpha$ -cyano-3-phenoxybenzyl-(1*S*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate



Alpha-cypermethrin is a nonsystemic, broad-spectrum pyrethroid with rapid knock-down activity. It is effective upon contact and through ingestion against target pests at relatively low application rates. As other members of the chemical group, it acts by preventing transmission of nerve impulses, by blocking the passage of sodium ions through channels in nerve membranes, thus preventing signals passing down axons. Typically, this intoxication results in a rapid "knock-down" (kd) and mortality.

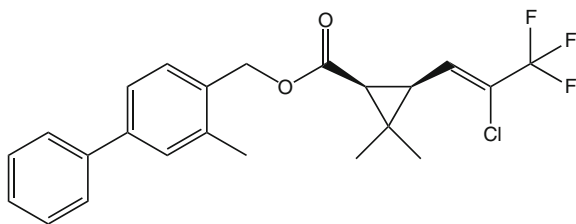
It is used to control a wide range of chewing and sucking insects. In public health, it is used to control cockroaches, mosquitoes, flies, and other insect pests. It is also used in animal health as an ectoparasiticide. The International Programme on Chemical Safety (IPCS) initially made a full evaluation of cypermethrin in 1989 and later a full evaluation of alpha-cypermethrin in 1992. IPCS concluded that when applied according to good agricultural practice, exposure of the general population to alpha-cypermethrin is low and is unlikely to present any hazards. With good work practices, hygiene measures, and safety precautions, the use of alpha-cypermethrin is unlikely to present a hazard to those occupationally exposed to it.

Evaluations of alpha-cypermethrin by the FAO/WHO and Joint FAO/WHO Expert Committee on Food

Additives JECFA (2003) are in agreement with those of IPCS. A review concluded that alpha-cypermethrin fulfils the safety requirements and that residues arising from the proposed uses with good plant protection practice, should have no harmful effects on human or animal health (EU 2004).

The main formulations available for use in public health applications (primarily IRS), are WP and SC (SC is also used for bed net treatment). The EC formulation is also used to control ectoparasites on animals. These formulations are registered and sold in Europe, South America, Africa, Australia, and Asia.

**Bifenthrin:** 2-Methyl-3-phenylphenyl methyl (1*S*,3*S*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate



In some operations in Africa and India, bifenthrin, a non alpha-cyano pyrethroid insecticide, (Talstar 80 SC, Bistar 80 SC) has been chosen even in areas where there is vector resistance to commonly used pyrethroids (permethrin and deltamethrin). Bifenthrin-impregnated bed nets offer considerable personal protection. The results presented by Chouaibou (2006) confirm that bifenthrin at 50 mg/m<sup>2</sup> could be recommended for mosquito net impregnation.

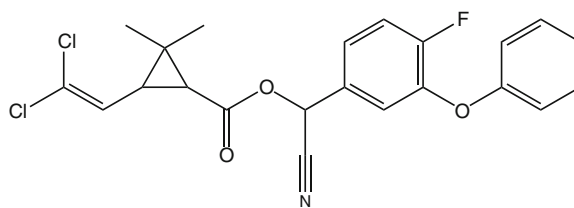
Lethal ovitraps (LOs) containing insecticide-treated ovistrips are used as a lure-and-kill device for the container-breeding dengue vectors *Ae. aegypti* and *Ae. albopictus*. Lure-and-kill strategies involve using pheromones or kairomones to attract insects to a lethal device. This ensures a targeted delivery of a minimum amount of insecticide, enhancing control efficacy while minimizing nontarget impact. Although lure-and-kill has been successfully used to control other dipterans such as tsetse flies (Brightwell et al. 1991), its application in mosquito control is in its infancy. LOs with a cloth strip treated with bifenthrin are used in north Queensland

(Ritchie 2005). Bifenthrin is chosen because of its low mammalian toxicity, strong substrate-binding properties (Lee et al. 2004), and low irritancy against mosquitoes compared with other insecticides (Hougard et al. 2002), including deltamethrin.

Successful dengue control in north Queensland was achieved using LOs in combination with source reduction and interior pyrethroid application (Ritchie 2005). The findings reported here, affirm the use of bifenthrin-treated LOs, but also demonstrate that competition with alternative breeding sites is likely to reduce field efficacy. Williams et al. (2007) confirmed that bifenthrin-treated LOs have the potential for use against *Ae. aegypti* and that they are effective in the field for at least 4 weeks. Given that untreated ovitraps were more acceptable for *Ae. aegypti* oviposition, the removal of alternative oviposition sites before deployment of LOs in the field should maximize their effectiveness.

McGinn et al. (2008) assessed the efficacy of Bistar 80 SC as a barrier treatment of Australian military tents, Northern Territory, Australia. There was a mean increase in protection of 81% against mosquitoes entering treated tents and 90.4% increase in protection against biting, predominantly by *Cx. annulirostris*.

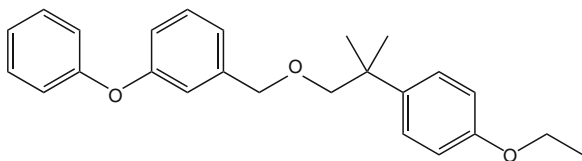
**Cyfluthrin:** [(*R*)-cyano-[4-fluoro-3-(phenoxy)phenyl]methyl] (1*R*,3*R*)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate



In response to the statement "...properly applied and timed ULV insecticide application could be effective in suppressing dengue vectors at the time of an epidemic..." (Gratz 1991), a number of field efficacy trials of ULV cyfluthrin for the rapid control of *Ae. aegypti* were conducted in Thailand. Sulaiman et al. (1998) evaluated cyfluthrin and malathion ULV applications on dengue vectors in high-rise apartment buildings in Kuala Lumpur. Both products showed

adulticidal effects, but cyfluthrin had more significant larvicidal effect than malathion. Sulaiman et al. (2000) also evaluated deltamethrin/s-bioallethrin/piperonyl butoxide and cyfluthrin against dengue vectors in Malaysia and found that both demonstrated adulticidal and larvicidal effects. According to Sulaiman et al. (2002), Solfac UL 015 (containing cyfluthrin 1.5 g/l), was diluted in diesel and applied 5 times over a 2–3 month period from a vehicle-mounted cold aerosol generator. The treatment caused adult knock-down 1 h after application outside and inside houses. There was no significant difference between cypermethrin and cyfluthrin in terms of adult mortality both inside and outside houses. These results indicate that both cypermethrin and cyfluthrin had adulticidal effects on *Ae. albopictus*. Cyfluthrin also had a slightly higher mortality effect on the larvae than cypermethrin.

**Etofenprox:** 1-ethoxy-4-[2-methyl-1-([3-(phenoxy)phenyl]methoxy)propan-2-yl]benzene

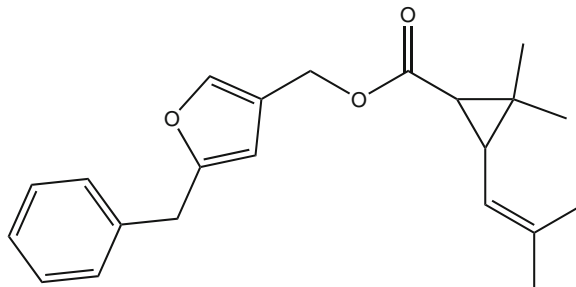


Etofenprox is a non ester pyrethroid but still it acts on the chloride channel of insect nervous systems as pyrethrins and pyrethroids do. Etofenprox disturbs insect nervous systems following direct contact or ingestion and is active against a broad range of pests. It is used in agriculture, horticulture, viticulture, forestry, animal health, and against public health pests.

In the public health sector, etofenprox is used either by direct application in infested areas or indirectly by impregnating fabrics, such as mosquito nets.

The WHO hazard classification (2004) of etofenprox (trade name Trebon) is “U”: “unlikely to present acute hazard in normal use”. The use of etofenprox in public health is mainly as EC and EW as formulations, containing 10% a.i., as, evaluated by WHOPES (1997, 1999). There are however other formulations, such as a WP, that are also used in public health and agriculture in many countries.

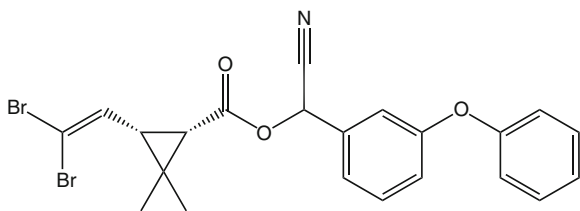
**Resmethrin:** 5-benzyl-3-[[[(3R)-2,2-dimethyl-3-(2-methylprop-1-en-1-yl) cyclopropyl] carbonyl]oxy]methyl]furan



Resmethrin is used for mosquito control primarily against urban *Culex* species, which are vectors of St. Louis Encephalitis (SLE) and West Nile Virus (WNV), applied as ULV applications in the Americas. Since some of the *Culex* species have developed resistance to compounds from other chemical groups, resmethrin has gained significance. Resmethrin is commercially available as Scourge, containing 12% a.i. and 36% synergist (PBO). It has been shown that a pair of treatments on successive evenings can increase mortality for a longer period. In addition, Reiter et al. (1990) demonstrated that resmethrin treatment resulted in an impressive reduction (74–84%) of the oviposition rate of *Culex* mosquitoes following the day of application and this impact was observable for at least 8 days following the application.

IVM strategies played an increasingly important role especially when WNV outbreaks occurred in the USA. Control measures in California relied on pyrethroids as the chosen products to suppress adult mosquito populations (Gammon 2007). These are usually applied aerially at night onto trees containing roosting birds, the primary WNV hosts. Pyrethroids are quite potent, but with generally low avian toxicity. An advantage of application at night is that they are more effective, as the temperature is reduced. Moreover, those containing a chrysanthemic acid moiety readily break down in sunlight ensuring rapid dissipation during the daylight hours. The principal pyrethroids used as adulticides in the USA are the natural pyrethrins, resmethrin, and phenothrin, which are all chrysanthemates.

**Deltamethrin:** [cyano-(3-phenoxyphenyl)-methyl] 3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate



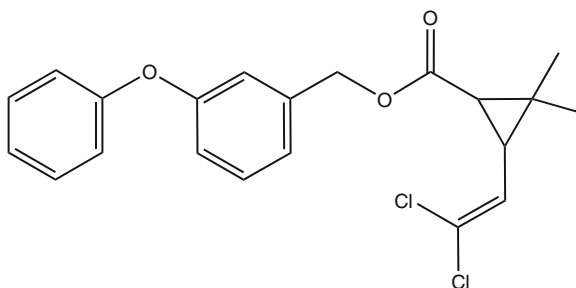
Deltamethrin, a commonly used synthetic pyrethroid in public health has been the mainstay for IRS, used to combat malaria transmission in Asia (Pothikasikorn et al. 2005).

Most pyrethroids are known to elicit behavioural responses in insects (Roberts and Andre 1994; Roberts et al. 1997; Chareonviriyaphap et al. 2001). In Thailand, deltamethrin for IRS was first launched in 1994. The extensive use of pyrethroids since that time has been a major stimulus for field evaluation of the behavioural responses of malaria vectors. One of the most important malaria vectors in Thailand, *An. minimus* remains a significant vector because of its major endophagic and anthropophagic behaviours (Chareonviriyaphap et al. 2003). However, the pattern of *An. minimus* behaviour elicited by deltamethrin is quite different from behaviour elicited by DDT. *An. minimus* females almost disappeared from the DDT-treated huts during the second half of the night, whereas they continued to bite in the deltamethrin-treated huts throughout the night (Polsomboon et al. 2008). This difference is consistent with observations on house-invading behaviour of *An. vestitipennis* after huts were treated with deltamethrin and DDT (Grieco et al. 2000). In the *An. vestitipennis* studies, there were higher numbers of mosquitoes entering the hut treated with deltamethrin than with DDT, indicating the higher spatial repellency of DDT compared to deltamethrin. Specifically, there was a 97% reduction of mosquitoes entering a hut treated with DDT, whereas there was only a 66% reduction of *An. vestitipennis* in the deltamethrin-treated hut (Grieco et al. 2000). Deltamethrin did not have as dramatic reduction on the biting population as DDT did, significantly reducing *An. minimus* biting inside huts. In conclusion, without a better understanding of the relationship between insecticide residues and mos-

quito behaviour, vector control strategies will continue to be hampered by not knowing which of several insecticide effects are actually responsible for preventing disease transmission inside homes. Das et al. (1986) conducted a study to determine the susceptibility status of multiresistant *An. culicifacies* populations using deltamethrin at a concentration of 0.025%. The strains tested showed very high resistance to DDT and dieldrin and well pronounced resistance to malathion. With deltamethrin, however, all the tests showed 100% mortality, which implies that DDT and dieldrin resistance did not confer cross-resistance to deltamethrin. By contrast, the organochlorine- and malathion-resistant *An. gambiae* in Sudan, was cross-resistant to pyrethroids (Davidson and Curtis 1979).

Deltamethrin (K-Othrine) can also be used for ground and aerial ULV applications.

**Permethrin:** 3-Phenoxybenzyl(1*RS*)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate



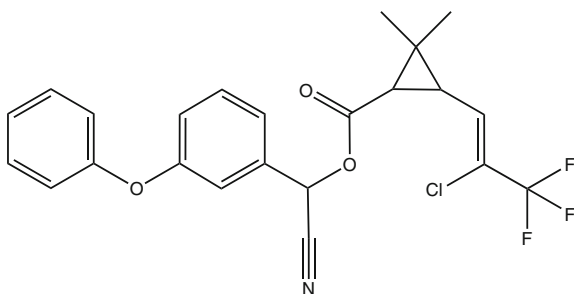
Permethrin-impregnated bed nets and clothing provide good temporary protection against mosquitoes. In tests conducted in Florida, Schreck et al. (1984) reported permethrin-treated clothing alone provided 89.1% protection from *Oc. taeniorhynchus*, compared with 94.4% protection provided by the combination of permethrin-treated uniforms and topically applied repellents. A study conducted in Alaska showed that permethrin-treated clothing alone reduced biting of *Cs. impatiens* by 93%, compared with 99% protection obtained with treated clothing and repellent (Lillie et al. 1988).

In recent publications (Munhenga et al. 2008), it was confirmed that the presence of permethrin resistance in *An. arabiensis* populations from Zimbabwe may occur at significant levels, which further emphasizes the

importance of periodic and ongoing insecticide susceptibility testing of malaria vector populations.

Synthetic pyrethroid formulations have often been based on organic solvents and carriers, but due to environmental concerns, water-based formulations to reduce the environmental impact have now been introduced. Meisch et al. (1997) conducted trials with oil-based Permanone 31–36 (permethrin 31% and 36%PBO) and water-based Aqua Reslin (permethrin 20% and 20% PBO) formulations of permethrin at rates of 2.03 and 3.93 g a.i./ha against *An. quadrimaculatus* and *Cx. quinquefasciatus*. Results indicate significantly greater control of both species at the higher application rates for both formulations.

**Lambda-cyhalothrin:** 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyano(3-phenoxyphenyl)methyl cyclopropanecarboxylate

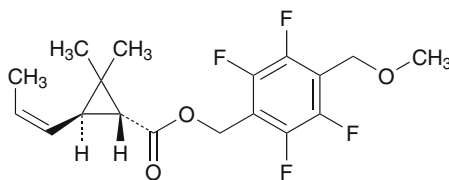


In 1989, the pyrethroid lambda-cyhalothrin (Icon) became available. Schaefer et al. (1990) conducted a laboratory and field evaluation of the compound against *Cx. tarsalis* adults and reported that lambda-cyhalothrin offered high potential for aerosol applications.

The increased threat of mosquito-borne diseases coupled with a decreased tolerance of nuisance mosquitoes in the United States, has encouraged vector management professionals to offer mosquito control services for homeowners. Lambda-cyhalothrin (0.1%) at its maximum label concentrations has been used as a barrier treatment for managing mosquito populations in suburban residential properties and its residual efficacy was evaluated in reducing adult mosquito populations. Lambda-cyhalothrin treatment significantly reduced *Aedes spp.* Treated sites had 85.1% fewer *Ae. albopictus* bites than the untreated control and residual

efficacy lasted up to 6 weeks post treatment. In contrast, *Culex spp.* were not reduced by these treatments (Trout et al. 2007). The same study indicated that lambda-cyhalothrin applied as a barrier to low-lying vegetation does not properly target adult daytime resting-sites for *Culex spp.* but they can reduce *Aedes spp.* Lambda-cyhalothrin 2.5 capsule suspension (CS), a new water-based microencapsulated formulation (2.5% a.i., w/v), is reported to have wash-resistant properties and longer persistence when applied to bed net material than other formulations (Sahu et al. 2008). The estimated protection factor based on malaria incidence was 86% for the treated nets. The results of the study showed that the use of bed nets treated with a CS formulation of lambda-cyhalothrin at 10 mg a.i./m<sup>2</sup> was acceptable to the community, and retreatment of nets at 9-month intervals can significantly reduce density and survival of *An. fluviatilis* and incidence of falciparum malaria.

**Metofluthrin:** 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (E,Z) (1R,3R)-2,2-dimethyl-3-(prop-1-enyl) cyclopropane carboxylate



Metofluthrin (SumiOne®, Eminence®) is a novel vapouractive pyrethroid discovered by Sumitomo Chemical Co. Ltd., registered in Japan in 2005 and is under worldwide development for environmental health use. Metofluthrin has extremely high knock-down activity against various insect pests, especially mosquitoes, as well as high volatility and low mammalian toxicity. It is used in mosquito mats and coils, and also various new formulations and devices such as paper emanators, fan-driven devices, and resin formulations. Metofluthrin is >40 times as active as *d*-allethrin against southern house mosquitoes (*Cx. quinquefasciatus*) when used in mosquito coils (Ujihara et al. 2008). The vapour pressure of metofluthrin ( $1.87 \times 10^{-3}$  Pa at 25 °C) is >2 times and >100 times higher than *d*-allethrin and permethrin, respectively. The product vapourizes at ambient temperature without heating,



whereas other conventional pyrethroids need heating for evaporation. High vapour pressure and insecticidal activity of metofluthrin could lead to new mosquito control devices that need no external energy for vapourization, with lower cost and longer efficacy. A significant reduction in mosquito density in metofluthrin-treated sites was maintained up to 6 weeks post treatment, depending on the feeding habits (Kawada et al. 2004). An interesting finding of this study is that exophilous species were impacted in spatial outdoor metofluthrin treatments. There are very few reports on the efficacy of antimosquito products in outdoor situations. Jensen et al. (2000) reported that only mosquito coils and MN-diethyl-3-methylbenzamide (DEET) products significantly reduced mosquito landing rates relative to untreated controls in the field.

### 18.3.5 Insect Growth Regulators (IGRs)

All compounds belonging to this group disrupt the normal growth and development of insects. They were developed as a result of rational leads from basic entomological research on metabolic disrupters, moult inhibitors, and behaviour modifiers of insects. Since the target site of action for these chemicals is known and susceptible to disruption only at certain times during their life cycle, these materials are thought to have fewer serious detrimental effects on nontarget species.

There are two major groups of IGRs, that differ in their modes of action:

- (1) The chitin synthesis inhibitors, such as diflubenzuron, cyromazine, and triflumuron, interfere with new cuticle formation, resulting in moult disruption, and
- (2) The juvenile hormone analogs, which interfere with the metamorphic processes affecting development to the adult stage.

Nine countries from the WHO regions (except the African region) reported the use of IGRs for vector control (Table 18.9). However, some countries did not submit records of their IGR use, since many national mosquito control programmes apply larvicidal IGRs to control nuisance, but not for disease vectors. Overall use of these products is substantially larger than is recorded (Table 18.8).

Benzoylurea compounds (diflubenzuron and novaluron) belong to the chitin synthesis group, while the juvenile hormone mimics include methoprene, fenoxycarb and pyriproxyfen.

#### 18.3.5.1 Benzoylphenyl Ureas (diflubenzuron and novaluron)

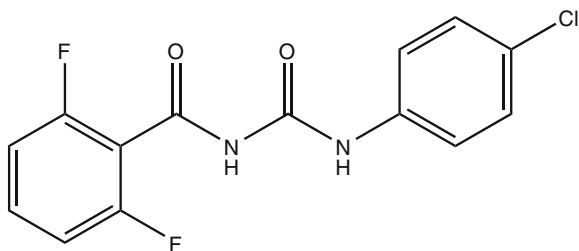
The insecticidal activity of the benzoylphenyl urea analogs was discovered in 1970 by Philips-Duphar Company in the Netherlands.

**Table 18.9** Global use of insect growth regulators for control of vector-borne diseases reported to WHOPES, in kg of active ingredient, 2003–2005, (Zaim and Jambulingam 2007)

Region	Country	Disease	Compound	Amount of active ingredient (kg)		
				2003	2004	2005
Americas	Argentina	Dengue	Triflumuron	15	NR	NR
	Brazil	Dengue	Diflubenzuron	NR	5	0
			Triflumuron	NR	<1	<1
			Methoprene	NR	2	<1
Eastern Mediterranean	Colombia	Dengue	Pyriproxyfen	NR	NR	4
	Saudi Arabia	Malaria	Pyriproxyfen	NR	45	45
	Yemen	Malaria	Pyriproxyfen	NR	NR	1
European	Turkey	Malaria	Diflubenzuron	950	966	1,250
South-East Asia	Indonesia	Malaria and dengue	Methoprene	6,043	7.5	0
			Pyriproxyfen	0	5	9
Western Pacific	New Zealand	Ross River Virus	Methoprene	1,765	837	1,766
	Singapore	Dengue	Pyriproxyfen	NR	NR	<1

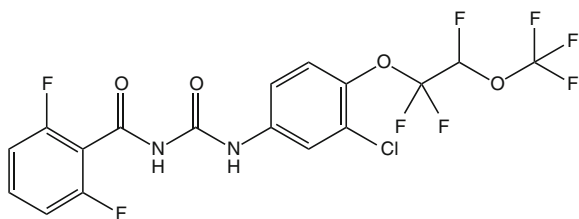
NR not reported

**Diflubenzuron:** 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea



Diflubenzuron was the first compound shown to be effective against insects, and resulted from the combination of two herbicides, dichlobenil and diuron. The resulting compound was ineffective as an herbicide, but was toxic to insects. Surprisingly, the compound's action was very different from that of other insecticides used at that time. The mortality was invariably connected with the process of moulting. Treated adults were not killed, but their reproductive capacity was strongly reduced. The chemistry and the symptoms of the benzoylphenyl urea analogs were unique and they became a new class of insecticides.

**Novaluron:** (RS)-1-[3-chloro-4-(1,1,2-trifluoro-2-trifluoromethoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea



#### 18.3.5.1.1 Mode of action

Studies on the benzoylphenyl urea mode of action, implicate the enzyme chitin synthetase as the actual biochemical moiety, which interacts with the toxicant. Post et al. (1974) showed that the last step in the chitin synthesis process, the polymerization of the

*N*-acetylglucosamine units catalyzed by the enzyme chitin synthetase, is inhibited.

After an IGR treatment, symptoms are not generally observed until the moulting process is initiated. The degree of the disruption of ecdysis (moulting of the old cuticle) is related to the dosage, and is characteristic for each benzoylphenyl urea analog and the insect species (ecdysal disruption may occur in the larval–pupal moult too). The range of effects include:

- (1) Ecdysis is prevented completely, so that the insect dies within the old cuticle.
- (2) Ecdysis is initiated, but not completed. In some cases, the exuvia splits normally but the ecdysis proceeds no further. In others, the moult proceeds until the old cuticle remains attached only to some of the last abdominal segments.
- (3) In some cases, the old cuticle is almost completely shed from the body, but remains attached to the head-capsule and mandibular region, and prevents further feeding or development.

Benzoylphenyl ureas exhibit several unique characteristics. In general, they are more toxic when they are ingested by the target organism. Application of diflubenzuron, or other IGR larvicides from the same group, in the field should therefore coincide with active feeding of the target insect. The second characteristic concerns the “activity window” of the benzoylphenyl ureas. Peak activity occurs at distinct times of the insect's development, primarily during peaks in chitin synthesis. In practical terms, susceptibility of larvae is greatest prior to each moulting. Treatments should therefore be applied either at the time of the greatest susceptibility of the insects or alternatively; the treatment should have a sufficient residual activity to span the next “activity window.” The third characteristic concerns the prolonged larval life after a toxic dose is acquired. Even after exposure at the susceptible stage, the insect may live in a moribund state for many days before dying. Tests with various mosquito species by Mulla and Darwazeh (1975) showed little or no adult emergence for 27 days after diflubenzuron treatment of larval sites. Mortality was recorded during larval and pupal stages as well as during adult eclosion.

A synchronized larval population is not essential for the effective use of these products, since all larval instars are susceptible to diflubenzuron treatment.

However, it is advisable to conduct treatments during the early larval stages of development. Field tests by Schaefer et al. (1975) revealed that several *Aedes/Ochlerotatus* spp. and *Cx. tarsalis* resistant to OPs were effectively controlled in all larval stages by diflubenzuron.

In the aquatic habitats where diflubenzuron was applied, the impact of the active ingredient on a large number of nontarget species has been assessed. In general, detrimental effects were detected as temporary, even after repeated treatments. (Ali and Mulla 1978; Miura and Takahashi 1975; Zgomba et al. 1983; Zgomba 1987).

Recent publications by Thavara et al. (2007) showed results of two diflubenzuron formulations: tablet (40 mg a.i./tablet) and granular (2% a.i.), which were evaluated for larvicidal efficacy against *Ae. aegypti* in water-storage containers under field conditions in Thailand. Each formulation was applied to 2001 clay jars at five different dosages (0.02, 0.05, 0.1, 0.5, and 1 mg/l a.i.). Another experiment was also conducted using three different dosages (0.1, 0.5, and 1 mg/l), where half the water in each treated jar and the control was removed and refilled weekly. A high degree of larvicidal efficacy (96–100% EI) was achieved with four dosages (0.05, 0.1, 0.5, and 1 mg/l) of both formulations (tablet and granular) for a period of 23 weeks posttreatment. The efficacy of the lowest dosage (0.02 mg/l) of tablet and granular formulations lasted for 21 and 22 weeks posttreatment, respectively. Under the conditions of water removal and weekly refilling, a high degree of larvicidal efficacy (96–100% EI) at the three dosages was obtained with the tablet formulation 18–21 weeks posttreatment, whereas the efficacy of the granular formulation persisted 15–23 weeks posttreatment depending on the dosage. This study clearly demonstrated a high level of residual activity with both formulations of diflubenzuron against larvae of *Ae. aegypti* in water-storage containers. Considering environmental factors and water-use conditions, it is likely that dosages of 0.05–0.1 mg a.i./l are effective dosages providing long-lasting control for 3–4 months in the field.

### 18.3.5.2 Juvenile Hormone Analogs (JHAs)

The natural juvenile hormone has two distinct biochemical effects: one during the larval stage and the

other in the adult. During the larval stage, it suppresses metamorphic changes. In the adult, juvenile hormone is required for several reproductive functions such as ovarian development, yolk synthesis, pheromone production, and accessory gland development. It is evident that upsetting the titre of juvenile hormone at certain periods during the life-history will adversely affect metamorphosis. Moreover, such an induced titre might have a domino effect and disrupt other hormonally controlled functions.

The morphogenetic effect of the JHA compounds is primarily seen during the larval–pupal transformation, and may result in various degrees of incomplete metamorphosis. They also influence the endocrine physiology of the insect, which may result in abnormal morphogenesis. Methoprene inhibits the release of an executive hormone from the corpora allata early in the last larval instar, but stimulates the glands prior to pupation. JHAs can also block embryonic development and may be ovicidal too. Various types of effects ranging from ovicidal to delayed effects during postembryonic life have been reported. Treating early larval instars will have very little effect on metamorphosis, since the requirement for larval–larval moult is a high titer of the hormone. However, if the last larval instar is treated with juvenoids, when in the natural process of metamorphosis the juvenile hormone level dramatically decreases, the result is abnormal pupation and/or incomplete adult formation.

One of the main reasons for juvenile hormone analogs being effective as control agents is their chemical structure (terpenoid), which enables them to penetrate the cuticle with great ease, and exert their effects on the target tissue, the epidermis.

The ecotoxicological effects of JHAs have been extensively investigated. The toxicity to vertebrates is extremely low, e.g. the oral LD<sub>50</sub> of methoprene for rats is over 34,500 mg/kg (Siddall 1976). However, adverse effects on nontarget organisms that receive a dose of JHA during their last larval instars could also be expected. At levels far above the dosage used for mosquito control, methoprene produces a short-term toxicity effect on the water flea, *Daphnia magna*, the side-swimmer *Hyaella azteca*, the tadpole shrimp, *Triops longicaudatus* and some other organisms (Miura and Takahashi 1975). However, high concentrations of methoprene had no adverse effects on *Dugesia dorotocephala*, the planarian that feeds on mosquito larvae and which can be used as a biological-control agent.

It has therefore been proposed that integrated use of planarians and JHA for the control of mosquitoes is viable (Levy and Miller 1978).

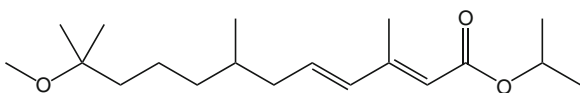
In operational studies, the most commonly used methods for evaluating the efficacy of JHAs are:

- (1) Introducing captured 3rd–4th instar larvae into the JHA-treated water, collecting pupae after an interval of time (*e.g.* 4 days), and daily observation of the adult emergence.
- (2) Collection of immature mosquitoes from the treated site, and adult emergence and mortality evaluation.

There are several alternative methods for recording and calculating efficacy, but Kline (1993) uses the following classification of emergence: dead pupae (DP); dead adults (DA); and live adults (AA “active adults”). From these data, the % emergence inhibition (% E.I. = % control) may be calculated using the formula

$$\% \text{ E.I.} = (\text{DP} + \text{DA}) / (\text{DP} + \text{DA} + \text{AA}) \times 100$$

**Methoprene:** isopropyl (*E,E*)-(*RS*)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate



Methoprene, one of the most often used JHAs was synthesized by Henrick and colleagues in 1973 (Baillie and Wright 1985). More than 30 years of field research has shown that methoprene is one of the most environmentally safe mosquito control products. It is available in liquid, pellet, and briquette formulations that allow considerable flexibility at the operational level. Liquid methoprene formulations may be applied by standard ground equipment or aerially against the 2nd, 3rd, and 4th instar larvae typically found in floodwater breeding sites, within 4 days after flooding. In areas with dense vegetation or canopy, mixtures of liquid methoprene and sand may be applied with granule application equipment. The persistence of these methoprene formulations is up to 10 days. The pellet formulation releases an effective level of methoprene for ~30 days into floodwater sites, artificial water containers, tyres, waste treatment ponds, man-made depressions, tree-holes, etc.

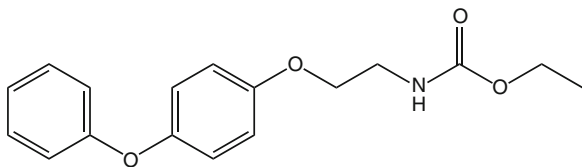
Depending on the biotic and abiotic factors of the breeding site, the application rates range from 3.0 to 11.5 kg/ha. A study by Kramer et al. (1993) demonstrated that methoprene pellets applied at 3.4 kg/ha against *Oc. dorsalis* prior to marsh inundation provided 99% control ~42 days, 86.4% ~131 days, and 66.6% control 8 months after the application. Proportions of partially emerged adults increased over the course of the study. Of all the completely emerged mosquitoes from the treated region of the marsh, 66% were found dead on the water surface, compared with only 0.7% from the untreated area.

For long-term control, methoprene briquettes are perhaps the principal choice, especially where access to the breeding site is difficult, and where the area may be flooded repeatedly during the season. The briquettes can be applied at or before the beginning of the mosquito season; *e.g.* prior to flooding, while the sites are still dry. For controlling *Aedes/Ochlerotatus* and *Psorophora* larvae in shallow depressions (up to 50–60 cm depth), one briquette/18 m<sup>2</sup> is recommended. Briquettes have to be placed at a higher rate (1 per 9 m<sup>2</sup>) for control of *Culex*, *Culiseta*, *Anopheles*, *Mansonia*, and *Coquillettidia* spp. larvae. The duration of the efficacy depends on water temperature, water quality, and the number of fluctuations. In continental and Mediterranean climates, effective suppression of mosquitoes can be expected for ~4 months. Douglas et al. (1994) reported that when solid, sustained-release methoprene formulations were applied, the highest (*S*)-methoprene residue detected was 6.0 µg/l. The majority (85%) of samples contained residues of 1.0 µg/l. Such low residues do not constitute a significant risk to nontarget organisms.

Effectiveness of a sustained-release sand granule formulation of methoprene was also established for *Oc. taeniorhynchus*, a major nuisance in coastal areas of the U.S. (Kline 1993). In the field, inhibition of emergence in mosquitoes exceeded 90% when these granules were applied post flood at 5.6 kg/ha.

Granular formulations can be prepared on-site by mixing dry sand with the liquid methoprene formulation in a rotating-type mixer (concrete mixer) for 5–10 min until the sand is uniformly coated. Silicon dioxide should be added and mixed for an additional 5 min. This will provide a dry mixture, which will flow freely through standard granule application equipment. The typical application rate of such a formulation is 11–12 kg/ha.

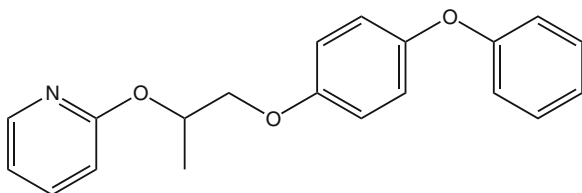
**Fenoxycarb:** ethyl N-[2-(4-phenoxyphenoxy)ethyl]carbamate



Although its mode of action is like that of juvenoids, fenoxycarb actually belongs to the carbamates. Axtell et al. (1980) and Dame et al. (1974) have shown that fenoxycarb suppresses the development of *Cx. tarsalis*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Oc. melanimon* and *Ps. columbiae*. None of the fenoxycarb formulations tested by Dorn et al. (1981) and Mulla et al. (1985) produced harmful effects on mayfly, dragonfly, or various beetle larvae. Freshwater zooplankton exposed to the treatments were also unaffected.

*Ps. columbiae* was exposed to several fenoxycarb formulations by Weathersbee III et al. (1988). The results showed that 0.5% fenoxycarb sand formulation at a rate of 22 g a.i./ha was effective against larval populations in rice fields. Excellent control (95%) was achieved against larvae introduced at up to 24 h post-treatment and residual activity remained >55% for up to 120 h posttreatment. The fenoxycarb formulation based on corn cob also offered excellent initial control (95%) at the same rate (22 g a.i./ha), but the residual activity decreased to 41% by 120 h posttreatment.

**Pyriproxyfen:** 4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether-2-[1-(4-phenoxyphenoxy)propan-2-yloxy]pyridine



Like fenoxycarb, pyriproxyfen is also structurally unrelated to the natural insect JH, but its biological activity is the same as that of the juvenoids (Schaefer et al. 1988). Through hormonal imbalance, pyriproxyfen suppress insect embryogenesis, metamorphosis, and adult

emergence. Studies with pyriproxyfen demonstrated very high activity against *Aedes/Ochlerotatus*, *Culex* and *Psorophora* species, both in laboratory and in field studies, at rates as low as 0.56 g a.i./ha (Mulla et al. 1989; Schaefer et al. 1990). When pyriproxyfen was applied in dairy waste-water lagoons at 100 g a.i./ha in single and multiple applications, it resulted in control of *Culex* spp. for a period of 7–68 days. The length of the control period appeared to be related to water quality, with greater residual activity in more polluted sources (Mulligan III and Schaefer 1990). The assumption was that the a.i. was adsorbed onto organic debris and remained highly toxic to the larvae even after the water was replenished with untreated waste-water.

Recent studies demonstrate that pyriproxyfen can be a powerful tool in controlling *Ae. aegypti*, the principal vector of dengue, the most important arboviral disease in the world today affecting an estimated 100 million people annually. It was demonstrated that a slow-release sand granule pyriproxyfen (0.1%) formulation could be effectively used as a larvicide treatments at oviposition sites, called focal treatment (Seccacini et al. 2008). Additionally, WHO (2006) has added pyriproxyfen to a group of larvicides accepted for potable water treatment.

In general, IGRs have high levels of activity and efficacy against various species of mosquitoes in a variety of habitats. Additionally, they show a good margin of safety to nontarget biota, including fish and birds. On the basis of these attributes, IGRs provide an additional tool for mosquito control, supplementing OP, carbamate, pyrethroids, and microbial larvicides.

### 18.3.6 Novel Insecticide Classes

Despite the efforts of academia, the pesticide industry, and national and international agencies, the discovery and introduction of new insecticides remains an extremely expensive and a slow process. Although several novel classes of insecticide have been recently developed for agricultural use, no new classes of insecticides have been developed for mosquito control in the last 2 decades. The Vector Control Industry Group (VCIG), which represents the major producers and marketers of vector control products used in the control of disease vectors, acknowledges that very few new products have been developed and launched in the last 20 years in the

public health and vector control market. The causes are multiple, but two main reasons can be put forward. The lack of market incentives, such as the modest public health and vector control market sizes, the small quantities of products used and the financial risk inherent in developing new public health insecticides today is a strong deterrent for the private sector to invest in research and development in this business area. The current cost of developing and obtaining registration for a new insecticide for Public Health is estimated at 100–150 million USD. The cost of developing a new product from an existing insecticide is estimated at 4–6 million USD. Not least important is that the time required for development of a new a.i. is 10–12 years, while for a new product it is 4–6 years.

The WHOPES was set-up in 1960 to collaborate with insecticide manufacturers to help identify new products, both from within existing classes and from totally novel classes. The WHO continues to encourage manufacturers to submit novel compounds for routine evaluation against mosquitoes and other pests. WHOPES promotes and coordinates the testing and evaluation of pesticides for public health. It functions through the participation of representatives of governments, manufacturers of pesticides and pesticide application equipment, WHO Collaborating Centers and research institutions, as well as other WHO programmes, notably the International Programme on Chemical Safety.

In its present form, WHOPES comprises a 4-phase evaluation and testing programme, studying the safety, efficacy, and operational acceptability of public health pesticides, and developing specifications for quality control and international trade.

WHOPES collects, consolidates, evaluates, and disseminates information on the use of pesticides for public health, facilitates the search for alternative pesticides and application methods that are safe and cost-effective. WHOPES attempts to develop and promote policies, strategies and guidelines for the selective and judicious application of pesticides for public health use, and assists and monitors their implementation by Member States.

It is hoped that a better understanding of the needs of the mosquito control community, together with new approaches to novel insecticide discovery for example, based on a better understanding of the mosquito at the genomic level, will in time facilitate the discovery of new compounds with appropriate properties. In the meantime, IMM and IVM strategies should be the mainstay for public health vector control operations.

## 18.4 Management and Monitoring of Insecticide Resistance

When mosquito populations are exposed to selection pressure from insecticides, they may become resistant. Resistance has been defined as “the developed ability in a strain of insects to tolerate doses of toxicants that would prove lethal to the majority of individuals in a normal population of the same species” (Cremlyn 1978). These strains tend to be rare in a normal population, but widespread use of an insecticide can reduce the normal susceptible population, thereby providing the resistant individuals with a competitive advantage. The resistant individuals multiply in the absence of intraspecific competition and, over a number of generations, quickly become the dominant proportion of the population. Hence, the insecticide is no longer effective and the insects are considered to be resistant. A population may even develop “cross-resistance,” which means that the population is not only resistant to one insecticide of a particular class, but also to other insecticides in the same class, even when it has never been treated with the other insecticides. More severe is the phenomenon of “multiple resistance,” where separate detoxification mechanisms for unrelated insecticides are present, resulting in an insect population that is resistant to different classes of insecticides, which makes control with insecticides extremely difficult.

Since Melander (1914) first reported insecticide resistance, the number of insect species and mites worldwide that have developed strains resistant to one or more pesticides, has increased to at least 504 and continues to rise. The number of insecticide-resistant arthropods of public health importance has risen from 2 in 1946, to 198 in 1990 (Oppenorth 1985; Georghiou 1990; Georghiou and Lagunes-Tejeda 1991).

Rodríguez et al. (2007), reports that eight Latin American strains of *Ae. aegypti* were evaluated for resistance to six OPs (temephos, malathion, fenthion, pirimiphos-methyl, fenitrothion, and chlorpirifos) and four pyrethroids (deltamethrin, lambda-cyhalothrin, betacypermethrin, and cyfluthrin). In larval bioassays, resistance to temephos, malathion, fenthion and fenitrothion was high in the majority of the strains. However, resistance to pirimiphos-methyl ranged from moderate to high in most of the strains. Adult bioassays showed that all the strains were

resistant to DDT and to the majority of pyrethroids evaluated. The use of the synergists, *S,S,S*-tributyl phosphorotrithioate and piperonyl butoxide showed that esterase and monooxygenases played an important role in the temephos, pirimiphos-methyl, and chlorpirifos resistance in some strains. Insecticide resistance in *Ae. aegypti* is a serious problem facing control operations, and integrated management strategies are recommended to help prevent or delay the temephos resistance in larvae and pyrethroids resistance in adults.

Kim et al. (2007) also demonstrated variable resistance levels in the northern house mosquito *Cx. pipiens pallens*, that have practical implications for the management of insecticide resistance. *Cx. pipiens pallens* is a nuisance-causing insect in Korea, while it is the primary vector of WNV (Bren 2003) and epidemic encephalitis in some Asian countries. This mosquito species is dominant and common in urban areas. The field-collected populations exhibited high levels of resistance to cyfluthrin and deltamethrin, but low to moderate levels of resistance were observed with bendiocarb, chlorpyrifos, lambda-cyhalothrin, *S*-bioal-lethrin, and permethrin. These results indicate that careful selection and judicious rotational use of these insecticides is required in order to maintain continued satisfactory control of field populations of *Cx. pipiens pallens*.

Many of the insecticides currently used in South Korea, have failed to control *Cx. pipiens pallens* in the field, most probably due to resistance. Widespread insecticide resistance has been a major obstacle in the IMM programme in South Korea. These problems indicate the need to establish an efficient resistance-management strategy based on detailed information on the extent and nature of resistance. Detection of changes in field resistance can facilitate the use of alternative control measures, including use of synergists, rotational use of various insecticides, reduced insecticide application, and use of environmental control measures.

#### 18.4.1 Resistance Mechanisms

Resistance mechanisms are generally dependent on single genetic factors. Species that have been under continued selection pressure with one or a range of different

insecticides, have often accumulated a number of resistance (*R*)-genes and corresponding resistance mechanisms, which may lead to cross- or multiple-resistance.

Several distinct mechanisms are responsible for the development of insecticide resistance in insects. These involve either the detoxification of the toxic compound by biochemical metabolism, or a tolerance due to a decreased sensitivity to the toxic compound at its site of action. Normally, an insecticide penetrates rapidly through the integument, reaching the site of action. The site may be a vital enzyme, nerve tissue or receptor protein. Insecticide molecules bind to the site, and when they have attained threshold concentrations, they disrupt vital processes, causing insect fatality. Resistance may be selected at each step of this pathway: the integument, where reduced permeability may occur, thus reducing the rate of entry of the insecticide; new or more abundant metabolic enzymes may be selected, causing breakdown of the insecticide more efficiently; or altered target-sites may be selected to which the insecticide no longer binds. Of these three types of mechanisms, metabolism and insensitivity at the site of action are the most important. A reduction in the rate of cuticular penetration aids both types of mechanisms in a synergistic way (Georghiou 1994). In addition to those, another form of insecticide resistance is behavioural resistance, where insect behaviour becomes modified so the insect no longer comes into sufficient contact with the insecticide deposit (Miller 1988).

#### 18.4.2 Resistance Surveillance

Resistance monitoring should be an integral part of any mosquito control programme. The susceptibility of mosquitoes should be verified before the start of control operations, to provide baseline data for insecticide selection and choice of application technique. Regular surveillance will allow early detection of resistance, so that resistance management strategies can be implemented or in the case of late detection, evidence of control failure can justify the replacement of the insecticide. The operational criterion of resistance has usually been interpreted as the survival of 20% or more of field collected individuals tested at the currently known diagnostic concentration of the particular insecticide, using WHO test kits. The diagnostic concentration of a particular insecticide is

that which has been found to reliably cause complete mortality of strains that have never before been subjected to insecticides, and are therefore assumed to be susceptible.

Practical tests for detection of specific resistance mechanisms in individual insects have been developed. These include filter paper tests for esterases (Pasteur and Georghiou 1989) and acetylcholinesterase (AChE) (Dary et al. 1991), microtitre plates for esterases (Dary et al. 1990) monooxygenases, glutathione transferase and others (Georghiou 1990).

Since malaria is the leading cause of morbidity and mortality in Africa, accounting for an estimated 360 million clinical attacks (Snow et al. 2005) and 1–2 million deaths annually (Breman et al. 2004), malaria vector control activities are crucial. Most commonly these rely on the use of effective insecticides, through IRS or on ITNs or through IVM, such as use of biological agents as larvicides in the breeding sites of the vectors. There are numerous cases of insecticide resistance reported for *Anopheles* species.

The potential development of insecticide resistance is a common threat to any insecticide-based malaria control programme. The numbers of insecticides formulated for indoor residual treatment and recommended by WHOPEs pesticide evaluation (WHO 2001a) scheme is very limited. To ensure that the insecticides used remain effective, and decisions on insecticide choice are evidence-based, monitoring and evaluation of potential resistance mechanisms within the targeted vector population need to be undertaken. The spread of pyrethroid resistance may be critical for sustainability of ITNs because this is currently the only insecticide group recommended for impregnation. Two other classes of insecticides, carbamates and OPs (Coosmans and Carnevale 1995; Walker 2000) are available for conventional malaria control programmes. Their relatively short half-lives, sometimes requiring a sequence of 2–3 IRS per year, can make these insecticides less convenient and more costly.

Increasing levels of resistance to the most commonly used insecticides have caused multiple treatments and excessive doses, raising serious human health and environmental concerns in various countries with extensive vector control programmes. Resistance monitoring can be an effective component of the resistance management approach by providing valuable information on responses of mosquito populations to currently-used insecticides. Detection of

changes in field resistance can facilitate the use of appropriate alternative control measures.

Since the 1950s, California mosquito abatement districts have used chemical control programmes with emphasis on IMM practices to reduce mosquito populations. Controlling members of the *Culex pipiens* Complex is important because of their propensity to become a nuisance. In addition to being major pest mosquitoes, *Culex pipiens* Complex members are vectors of *Wuchereria bancrofti* (Cobbold), which causes filariasis in humans in the tropics. They also transmit WNV and SLE viruses in USA, and Rift Valley Fever virus in Egypt. California populations of *Cx pipiens* complex mosquitoes developed resistance to DDT and other organochlorines soon after these chemicals were introduced. OPs replaced organochlorines and shortly thereafter, in the early 1960s, Isaak (1961) detected the first evidence of OP resistance in California *Cx. p. quinquefasciatus*. By the 1970s, OP resistance was widespread, but despite this early evidence of resistance, OPs are still used today, although pyrethroids are more widely used. Pyrethroid tolerance in members of the *Culex pipiens* Complex has been documented in countries such as Tunisia (Ben Cheikh et al. 1998), Cuba (Bisset et al. 1991), Ivory Coast and Burkina Faso (Chandre et al. 1998), Saudi Arabia (Amin and Hemingway 1998), French Polynesia (Pasteur et al. 1995), and China (Jinfu 1999). There has not been a published report of pyrethroid tolerance in North America in any member of the *Cx. pipiens* complex or, in fact, in any mosquito species. For surveillance purposes, in 2003 McAbee et al. conducted adulticide bottle bioassays on *Culex pipiens* Complex populations from various locations in California, to test them for tolerance to OP and pyrethroid compounds. It was found that the populations had a knock-down reduced resmethrin and permethrin knock-down response in the bottle bioassays relative to a standard susceptible *Cx. pipiens quinquefasciatus*. Selected larvae were shown to cross-resist to lambda-cyhalothrin as well as to DDT. However, adult knock-down time following exposure to permethrin, resmethrin, and pyrethrum was affected despite an increase in larval tolerance to pyrethroids. Partial and almost complete reversion to susceptibility as larvae was achieved with *S,S,S*-tributylphosphorotrithioate and PBO, respectively, suggesting the presence of carboxylesterase and monooxygenase-mediated resistance. Insensitive target-site



resistance (*kdr*) was also detected in some mosquitoes tested by use of an existing PCR-based diagnostic assay designed for *Cx. pipiens* mosquitoes. Carboxylesterase-mediated resistance was supported by use of newly synthesized novel pyrethroid-selective substrates in activity assays. Bottle bioassays gave underestimates of the levels of tolerance to pyrethroids of Marin County (California) mosquitoes when compared with mortality rates in field trials using registered pyrethroid adulticides with and without PBO. This study represents the first report of resistance to pyrethroids in a population of a mosquito species in the USA.

### 18.4.3 Resistance Management

According to Georghiou (1994), factors that influence the evolution of resistance can be classified into three categories: genetic, biological, and operational. Georghiou and Taylor (1976) have quantified the influence of individual factors and have shown that some are positively correlated with the development of resistance (*i.e.* gene dominance, population isolation, insecticide persistence, etc.), while others are negatively correlated (immigration of sensitive populations into areas where resistant population exist, and the presence of untreated habitats where populations without selection pressure for *R*-genes can develop). If the relative influence of each factor could be expressed quantitatively, a reliable model might be constructed to predict the risk for resistance in a given situation. That risk can then be reduced through appropriate modification of one or more of the operational factors.

In general, resistance management is intended to prevent or delay as much as possible, the development of resistance to an insecticide, while at the same time maintaining an effective level of mosquito control. On the basis of the three categorical factors that influence the development of resistance, Georghiou (1994) suggested the following approaches to resistance management: management by moderation, management by saturation, and management by multiple-attack.

*Management by moderation:* Management by moderation recognizes that susceptibility genes are a valuable resource and it attempts to preserve them by limiting the chemical selection pressure that is applied.

Measures in this category include the use of low insecticide rates, infrequent applications, non-persistent insecticides, and preservation of refugia.

While management by moderation comes close to meeting environmental standards, it may not be appealing where there is a need to control human disease vectors, or control newly introduced pests. In these cases, the saturation or multiple-attack concepts may be more appealing.

*Management by saturation:* The term “saturation” does *not* imply saturation of the environment with pesticides. It indicates saturation of the insect’s defenses by use of sufficient insecticide to leave absolutely no survivors. This approach has more merit during the early stages of selection when resistance genes are rare, and existing mainly in the heterozygous state.

Another means of suppressing the insect’s defenses is the use of synergists. PBO has been used for many years as a synergist of pyrethrins in household aerosol sprays, and more recently with pyrethroids in the control of mosquitoes and houseflies. By suppressing the insect’s mixed-function oxidase system, which is involved in the degradation of pyrethroids, PBO effectively removes the selective advantage of this mechanism. The approach would not apply where alternative pathways of detoxication are also present (Ransinghe and Georghiou 1979).

*Management by multiple-attack:* The multiple-attack strategy is based on the premise that control can be achieved through the action of several independently acting stresses, including insecticides, each exerting selection pressure that is below the level which could lead to resistance. This approach includes the application of chemicals in mixtures or in rotation (Georghiou 1983, 1990; Roush and McKenzie 1987; Tabashnik 1989).

The strategy of using mixtures assumes that the mechanisms of resistance to each member insecticide are different and that initially resistance genes exist at such low frequencies that they do not occur together in any single individual within a given population. Any insect that may survive the exposure to one of the insecticides in the mixture is nonetheless killed by the other.

The strategy of using rotation may be applied in situations where resistant mosquitoes have a lower biotic fitness than susceptible individuals, which results in a gradual decline in the frequency of resistant

genes when the selecting insecticide is withdrawn, or is replaced by a neutral insecticide that is not affected by cross-resistance.

Alternatively, different insecticides maybe applied in a mosaic pattern, with the size of the mosaic segments determined on the basis of insect movement and gene flow. The resulting mosaic of different selection pressures may effectively delay the development of resistance throughout the population.

The feasibility of using two insecticides in rotation, mixture, or sequentially for resistance-management, has been widely examined by means of cage experiments (Georghiou 1983; Cilek and Knapp 1993; Curtis et al. 1993; McKenzie and Byford 1993). However, the work has led to divergent conclusions, as the outcome of each approach will depend on many factors, including the appropriate choice of insecticides based on their mode of action, the potential mechanisms of

resistance, the prior exposure of the target population to insecticidal selection pressure, and the presence of a significant fitness differential between resistant and susceptible individuals.

In the field, the relative impact of single use, rotation and mosaic strategies on the rate of onset of insecticide resistance in Anopheline mosquitoes has been tested on a large-scale project in Mexico. Following extensive baseline studies on resistance mechanisms and gene frequencies, different villages and regions were subjected to different application regimes with different classes of insecticides over a period of several years. At present, the data are still being analyzed, but interim results indicate that although techniques such as rotation or mosaic treatment do not prevent the development of resistance, they do slow the onset as compared with repeated use of a single insecticide (WHO/IRAC 2003).

# Chapter 19

## Physical Control

### 19.1 Introduction

Physical control may be defined as suppression of insects by physical or mechanical means. This contrasts with the use of insecticides, which kill insects by chemical or biochemical means, and with the use of environmental measures which do not kill insects directly, but prevent their establishment, reproduction or impact by, for example, eliminating their larval habitat, or preventing their access to the host, for example, by better designed housing. In practice, there is some overlap between these various terms.

Physical control techniques exist for control of both mosquito larvae (*e.g.* polystyrene beads) and adults (*e.g.* mass-trapping). Physical control may be used in isolation, but is often seen as a component of integrated vector management (IVM). In general, mosquitoes are considered unlikely to develop resistance to physical control techniques.

### 19.2 Physical Control of Immature Mosquitoes

#### 19.2.1 Oil

Since the construction of the Panama Canal in the nineteenth century, to the present day, petroleum oils have frequently been used to control immature stages of mosquitoes. Along with rediscovering source reduction and water management in mosquito control programmes in recent years, oils have also regained attention as candidates for the treatment of certain types of water bodies.

A wide range of petroleum oils have been used as mosquito larvicides, although in many cases the exact

composition of the products used is unknown. Products include oils of varying paraffinic and aromatic contents, with varying carbon chain lengths, and in some cases surfactants or other additives have been included to alter the products' properties (Mulla et al. 1971; Schultz et al. 1983). Effective application rates are, in general, relatively high, typically ranging from 10 to 100 l/ha. Oils have been applied manually using conventional knapsack sprayers, by vehicles, and by aerial applications. The oils have a range of different impacts on the mosquitoes. The more paraffinic oils have a largely physical interaction with the insect's siphon, preventing effective oxygen uptake which resulting in drowning or suffocation. Oils with a higher aromatic content, particularly those with a higher volatility are considered to be toxic to mosquito larvae (Hagstrum and Mulla 1968), and so, are not strictly physical control techniques. The oil layer also inhibits mosquito oviposition on the water surface, either preventing or delaying recolonization of the treated area for up to 9 days (Beehler and Mulla 1996). Once applied, the oil film may gradually evaporate, and may also be degraded by bacterial action.

Several studies have determined the impact of oil treatments on nontarget aquatic life. Mulla and Darwazeh (1981) showed that bottom living invertebrates, such as mayfly, damselfly and dragonfly larvae, and tadpoles, were almost unaffected by treatment with mosquito oil at ca. 35 l/ha. However, diving beetle larvae and adults, together with corixids and notonectids were more severely affected, while ostracods were also affected by some oils. Phytotoxic effects were also recognised with earlier petroleum products, but are much less common with more refined products (Floore et al. 1998).

## 19.2.2 Surface Films and Polystyrene Beads

When surfactants and monomolecular organic surface films are used against mosquitoes they exert their control by physicochemical modification of the air–water interface (Garrett and White 1977). Larvae and pupae cannot penetrate the film at the water surface to obtain atmospheric oxygen, and newly emerged adults will drown on the treated water surface. Their effectiveness in controlling mosquito larvae and pupae is attributed to a reduction in water surface tension, with a subsequent wetting of tracheal structures resulting in anoxia, rather than chemical toxicity. Large numbers of film-forming surface active agents were evaluated as mosquito larvicides (Mulla 1967a,b), and some of these were found to have potential for practical mosquito control programmes.

### 19.2.2.1 Liparol

A self-spreading biodegradable surface film called “Liparol” was developed by Schnetter and Engler (1978). This substance is a mixture of soybean lecithin and paraffin, with carbon chains containing between 12 and 14 carbon atoms.

Lecithin, as a macromolecule with hydrophilic and hydrophobic ends, acts by reducing the surface tension of the water body. The hydrophilic end spreads on the water surface, whereas the hydrophobic end holds the paraffin film at the air–water interface. The mode of action is based partly on the interaction of the hydrophobic end of the lecithin molecule with the hydrophobic layers within the pupal trumpet or the larval siphon. When a pupa pierces the water surface covered by the film, the lecithin enters the trumpet and covers the hydrophobic layer of the trumpet. When the pupa dives again, the hydrophilic end of the lecithin is orientated towards the inner parts of the trumpet, so allowing water to enter the trumpet and killing the larva. In addition, the pupae and the larvae are unable to pierce the water surface and remain there, due to the reduced surface tension, and to the repulsion effect of the hydrophilic end of the lecithin molecule.

At a rate of 0.6–1.0 ml/m<sup>2</sup>, the lecithin mixture is effective against late fourth-instar larvae, and pupae. In polluted ponds with a clear surface and a low dissolved

oxygen content, all pupae and nearly all fourth-instar larvae die within 1 h of an application of 0.6 ml/m<sup>2</sup>. In polluted ponds with a higher dissolved oxygen content, an application rate of 0.8–1.0 ml/m<sup>2</sup> is necessary for 100% mortality of pupae. First-instar to early fourth-instar larvae are killed only by very high doses, since they can obtain sufficient dissolved oxygen through the integument, without access to atmospheric oxygen. In practice, the film is applied as a liquid formulation at an average application rate of 7.5 l/ha (0.75 ml/m<sup>2</sup>). To ensure maximum efficacy, application has to be very carefully timed, to coincide with the period when late fourth-instar larvae and pupae occur in the breeding sites. Depending on environmental factors such as water temperature and sunshine, the film is active for only 6–10 h.

Although the organic surface film is relatively selective, it does have some adverse effects on other surface breathing arthropods, such as water bugs (*e.g.* *Notonecta glauca*), which are highly sensitive, and some water beetles (*e.g.* hydrophilids and dytiscids). Because of the effect on surface tension of the water, arthropods which live on the water surface, such as the striders (*Gerris* spp.) are also affected (Becker and Ludwig 1981). Other aquatic organisms that live entirely within the water, such as fish, are unaffected.

### 19.2.2.2 Monomolecular Surface Films (MSF)

Monomolecular surface films consist of nonionic surfactants, that have been routinely used in other products such as detergents and cosmetics for almost 30 years. When used as mosquito larvicides, they are applied to water and spread spontaneously and rapidly over the surface to form an ultrathin film about one molecule in thickness (monomolecular layer). Their mode of action is physical rather than chemical in that they lower the water surface tension and thus disrupt normal development of mosquitoes. Mosquito larvae and pupae normally use the water surface for long periods when breathing, feeding (larvae) and/or resting, and the presence of the monomolecular layer kills them by inhibiting proper orientation at the water surface and/or by wetting and flooding tracheal structures, causing anoxia.

Monomolecular surface films for mosquito control were developed during the 1980s, but they have yet to gain wide acceptance in mosquito control programmes.

They have been sparingly used in the United States in floodwaters, brackish waters, and ponds. They have also been used in conjunction with other mosquito control agents as part of an integrated vector management (IVM) approach. MSFs are of low toxicity, and are appropriate for use on potable water.

At present, two MSF products are commercially available for use as mosquito larvicides and pupicides. These products are Arosurf<sup>®</sup> MSF (ISA-2OE or 66-E2) and Agnique<sup>®</sup> MMF. The use of Arosurf MSF (iso-stearyl alcohol 20E) as a mosquito larvicide was first reported by Garrett (1976), while Agnique was evaluated in the field in the late 1990s and first reported by Ali (2000). The two products are chemically identical (ethoxylated iso-stearyl alcohol) and are made from renewable plant oils. Arosurf MSF is a viscous, clear liquid that may be applied to the water surface without dilution. However, it contains significant amounts of polyethylene glycols (PEGs), and if mixed with water in a sprayer without high-shear agitation, it has a tendency to gel, making application difficult (Mulla et al. 1983). Agnique MMF is a more refined product, without PEGs. Again it is typically applied undiluted, but if dilution with water in the sprayer is required, high-shear agitation is still necessary. Thus, the main difference between Arosurf MSF and Agnique MMF is that the latter is chemically purer than the former. To date, the majority of published studies have involved Arosurf MSF, although it is believed that the efficacy of Agnique MMF is similar, as both products are chemically identical. Available information indicates that the physical and chemical properties, application rates, and safety of Agnique MMF are also comparable with those of Arosurf MSF (Nayar and Ali 2003).

Since their first use, the efficacy of monomolecular surface film larvicides has been demonstrated on many mosquito species in a wide variety of aquatic habitats (Levy et al. 1981, 1982; Mulla et al. 1983; Takahashi et al. 1984). These studies show that Arosurf can be applied in polluted water habitats at surface dosages as low as 0.33 ml/m<sup>2</sup>, and suppress more than 95% of the immature stages of *Culex* spp. Similar levels of control were achieved by ground and aerial applications against immature stages of *Oc. taeniorhynchus* and *Oc. infirmatus* in salt marsh habitats in Florida.

Disruption of the integrity of the film over the water surface by wind, heavy rain or by high concentration of algae and/or other emergent aquatic vegeta-

tion, is usually the cause of poor kill of larvae and pupae. However some *Culex* species are adapted to surviving in water with an extremely low oxygen concentration, Levy et al. (1982) found low efficacy against such species when they were treated in habitats with high dissolved oxygen. The poor efficacy is due to the ability of the larvae to use cuticular respiration, and to store air in their tracheal system. However, pupae are significantly more susceptible to the treatment.

Quantitative studies by Hester et al. (1989) showed that a single treatment of Arosurf MSF applied at a rate 0.94 ml a.i./m<sup>2</sup> in a 1:9 Arosurf to water mixture by volume, did not significantly affect the exposed aquatic vegetation. Effects of Arosurf MSF on a variety of aquatic nontarget organisms have been studied extensively.

In field situations, Mulla et al. (1983) tested Arosurf MSF at 4.67–7.0 l/ha and reported no apparent adverse effects on nontarget organisms, such as mayfly naiads (*Callibaetis pacificus*), diving beetle adults (*Berosus metalliceus*), ostracods, and copepods.

However, field evaluation of the same rate of Arosurf MSF by Takahashi et al. (1984) indicated that corixids, notonectids, clam shrimp, and a species of adult beetle were actually affected but all, except the clam shrimp, had recovered to pre-treatment population levels by 3 days after the treatment.

In an analysis of the available tools to control potential mosquito vectors of human and animal diseases in New Zealand, Stark (2005) emphasizes that some mosquito larvae have low susceptibility to monomolecular surface films because they have little or no surface contact (*Coquillettidia* spp., *Culex pilosus*, *Culex erraticus* and *Mansonia* spp.). However, adults of these species may still be susceptible when laying eggs or emerging to the adult stage. The same author reports that MMFs can persist in the field for up to 22 days under certain conditions, but results of most studies indicate that these products break down relatively quickly in the environment and are often undetectable 48 h after application.

A recent study by Bashir et al. (2008) shows the impact of Agnique MMF applied at 0.25 ml/m<sup>2</sup> to small pools in Sudan. One application of the product completely controlled (100%) pupae within 24 h and gave 92.6% reduction of L3–L4 larvae of *An. arabiensis* a week after treatment. In general, the susceptibility of the immature stages of mosquitoes showed the

following ranking: pupae > instars L3–L4 > instars L1–L2. Agnique MMF affected populations of anophelines faster than culicine species. However, to obtain continuous mosquito control in these breeding habitats, treatments need to be carried out at least every 7 days.

MMFs are especially useful in controlling late fourth-instar larvae and pupae because most of the other commercially available larvicides and pupicides, such as insect growth regulators (diflubenzuron, methoprene, etc.) and stomach poisons (*B.t.i.* and *B. sphaericus*) have relatively reduced larvicidal or pupicidal effects on these stages.

Monomolecular film products may be mixed with other larvicides for more immediate control of all larval instars (particularly fourth-instar) and pupae, and could also be particularly useful against strains resistant to conventional larvicides. Essentially, monomolecular films can be used for mosquito control in areas where the surface films of these products are likely to remain undisturbed (no wind action or any other interference) for sufficient time to interfere with larval and pupal suspension at the water surface, and with adult emergence.

Some disadvantages of using the monomolecular films are: (a) they are nearly invisible on the water surface and require frequent testing for their presence by placing a few drops of an indicator oil on the water surface and checking for a reaction, which is time-consuming, (b) they are less effective where emergent vegetation and floating debris are present, and (c) sustained winds tend to make the films pile up in localized downwind areas.

### 19.2.2.3 Polystyrene Beads

Mosquito larvae must penetrate the water surface to breathe air. Curtis and Minjas (1985) cited the idea of Reiter (1978) using floating layers of nonbiodegradable expanded polystyrene beads (EPB) at confined mosquito breeding sites: for example, wet pit latrines, cesspits and flooded cellars.

Unexpanded polystyrene beads contain pentane in solid solution in each bead. Heating the beads to about 100°C in boiling water softens the plastic, and causes the pentane within the beads to expand rapidly. Expansion to the required diameter can be achieved within five minutes with occasional stirring. The diameter

of the beads generally ranges between 4 and 5 mm but laboratory tests have shown that smaller beads of 2 mm give a more compact “carpet” which is less penetrable by mosquitoes of all aquatic stages (Curtis and Minjas 1985). A 2 cm thick layer of 2 mm beads is sufficient to eliminate mosquito breeding. The beads spread satisfactorily when still wet from the “cooking” process. There is no change in mass during the expansion process, so the weight of the polystyrene required per pit is the same whether it is assessed before or after expansion. The relative merits of factory or on-site expansion of the beads depend on the ease and cost of transport. About 1.5 tons of polystyrene has been shown to be sufficient for the treatment of 3,000 ventilated pit latrines (500 g/pit latrine) each with a cross-section area of approximately 1 m<sup>2</sup>. Such treatments have been routinely applied in Zimbabwe, as a precaution against mosquito breeding during the first months of life of pit latrines.

The use of expanded polystyrene beads can bring down the mosquito population within a short period of time. It does not require frequent application as with other conventional insecticides, since the beads remain on the water surface for a considerably longer duration, and are not environmentally harmful. Even if the water body dries out, the beads remain in place and are refloated when the water level rises again. They need replacing only when lost due to flooding. They can be used to control mosquito breeding in a range of sheltered stagnant water habitats such as cesspools and water storage cisterns.

Many trials on the effectiveness of expanded polystyrene beads in pit latrines and other types of habitats for the control of mosquitoes have been carried out in different parts of the world. A dramatic reduction in the emergence of *C. quinquefasciatus* was observed from pit latrines treated with expanded polystyrene beads, as compared to untreated pit latrines (Sivagnaname et al. 2005).

The effectiveness of the layers for long-term prevention of mosquito emergence from treated pits was reported by Curtis and Minjas (1985). The authors carried out community-wide trials in Zanzibar and India to control adult *Culex* populations and the filariasis that they transmit. In Zanzibar, a 1 cm-thick layer of 2-mm diameter polystyrene beads was applied to all 500 wet pit latrines in a community of 12,000 people. The treatment reduced the number of adult *Culex* that entered bedrooms by 98%. This was coupled with a single campaign of mass treatment of the community

with diethylcarbamazine (DEC). The campaign initially reduced the prevalence of microfilarial infection from 49% to 10%. During the next 4 years, the continued near-elimination of *Culex* prevented cured people from being reinfected, and prevalence declined to 3% (Maxwell et al. 1999). Another Zanzibar community used a DEC campaign but no mosquito control and showed a similar initial reduction but, in the longer term, there was resurgence towards the original level of prevalence. Comparable results were obtained in a 2-year campaign using DEC and Ivermectin mass-treatment of two South Indian communities, with or without polystyrene bead treatment of *Culex* breeding sites in bathroom water soakage-pits (Reuben et al. 2001). Again, as long as the drug treatment continued, the results were similar in the two communities. However, in the community that did not use polystyrene beads, once drug treatment was halted then filaria-infective *Culex* appeared the following year. By contrast, in the community that used polystyrene beads to suppress the mosquito population to a low level, no infective *Culex* were found. Mass drug administration coupled with the use of polystyrene beads is now considered to be a practical approach to the suppression of lymphatic filariasis. By this combination of chemotherapy and vector control, suppression of the disease may be faster than either method used alone (Curtis et al. 2002).

In several European countries, the problem of leaking sewer pipes in cellars leading to indoor breeding of human-biting *Culex pipiens molestus* mosquitoes has been reported. These mosquitoes are likely to be the main vectors of West Nile virus. Polystyrene beads work well in flooded cellars and may have a role in the control of this newly emerging disease (Curtis 2005).

### 19.3 Physical Control of Adult Mosquitoes

Mass trapping has been considered for control of mosquitoes, and in recent years has attracted more attention. Weidhaas and Haile (1978) conducted a theoretical appraisal of the potential of trapping, and estimated that around 40% of the population would need to be trapped daily to provide effective control. Kline (2007) reviewed progress and development in mass-trapping technology, and lists a number of commercially available mosquito trapping devices, using various combinations of light,

carbon dioxide, water vapour, heat, and octenol as attractants. The mosquitoes are typically killed either by desiccation within the device, or by high voltage grids. He describes operational use of mosquito traps on an isolated island surrounded by salt-marsh, in which *Oc. taeniorhynchus* was very abundant. Mosquito Magnet Pro devices were placed around the island at a rate of one device/0.44 ha. After about 2 weeks trapping, mosquito numbers were reduced by >80%, and these results were repeated over several consecutive years. Other evaluations of mass trapping have not been so successful (Henderson et al. 2006). In light of the potential for rapid increases in mosquito numbers, and the diversity of species in many areas, many of those involved in mosquito control remain skeptical about the potential and relevance of mass trapping to the majority of mosquito control situations.

#### 19.3.1 Other Technology: Novel Technology or New Tool

There have been recent reports of the development of a laser device for mosquito control (Guth 2009). The device is claimed to be able to detect individual flying female mosquitoes and destroy them with a burst of laser energy. It has been proposed that the devices could be mounted as a “fence” to protect residential areas against incoming mosquitoes.

## 19.4 Conclusions

Physical control encompasses a broad range of techniques. From the use of oil in the early days of mosquito control, through the use of mono-layers, polystyrene beads, traps, and now lasers, the technology continues to develop. Physical control techniques are attractive in that they tend not to be subject to the same regulatory constraints as pesticides, they are effective against mosquitoes resistant to conventional insecticides, and it is likely that mosquitoes will have difficulty developing resistance to most techniques. However, although physical control techniques have attracted increased attention recently as components of IVM, they remain at present, relatively restricted in application.

## Chapter 20

# Genetic Control of Mosquitoes

### 20.1 Introduction

The history of the genetic control of mosquitoes goes back about half a century, when Knippling (1959) realized that the fertility of monogamous female organisms could be readily compromised as a result of mating with a sterile male. Since that time, the development of the science and technology that supports this approach to mosquito control has accelerated greatly, in line with very rapid progress in the field of genomics in other disciplines, such as healthcare and agriculture. The recent publication of the full genome sequence for *Anopheles gambiae* (Holt 2002) has greatly enhanced the ability of scientists to elucidate the working of mosquitoes at a molecular, biochemical and genetic level, and to develop novel approaches to their management. There are yet major challenges facing the widespread use of genetic techniques for the control of mosquito vectors of disease. Nonetheless, the continued global impact of vector-borne diseases, the extent of constraints on the development and use of more conventional control techniques such as insecticides and widespread drug resistance, create a situation where investing research effort in exploring the potential of the technique, is considered worthwhile.

The prospect of practical genetic control techniques appeared sufficiently attractive and achievable for the establishment of a “genetic strategy to deplete or incapacitate a disease-transmitting insect population” to be adopted as one of the Grand Challenges in Global Health (Varmus et al. 2003).

The term “genetic control” actually covers a range of technologies and strategies. Currently, work on the genetic control of mosquitoes and mosquito-borne diseases is proceeding on two main fronts:

On the one hand, there is the objective of eliminating entire populations of a particular species of mosquito by mass releases of modified male mosquitoes

that mate with indigenous females, yet produce no fertile offspring. This approach is generally known as the Sterile Male Technique, although the most recent advances do not actually require sterilization of the males. A number of variants of Sterile Insect Techniques (SIT) have been evaluated in the field.

Alternatively, there is the approach of population replacement. The objective is to identify genes, or create genetic constructs, that render mosquitoes refractory to the selected disease (*i.e.* they cannot support development of the pathogen). These refractory genes are linked with a driver so that once released, the indigenous vector population is gradually replaced with a modified one that is unable to carry disease. This approach is not currently as close to operational use as SIT.

Genetic control is not just an alternative to conventional control techniques but offers some very specific advantages. The technique relies on the natural behaviour of the released mosquito to enable it to find and mate with members of the indigenous population. The released mosquito is unconstrained by the problems that restrict human access and as a result, the intervention is taken by the insect to innumerable places that a conventional technology may be unable to find or reach. This is important in both rural and urban environments or where transport and infrastructure are poor or where political instability or military activity prevents comprehensive access. Other advantages arise from the species specificity of the technology. Each genetic mechanism is targeted at one species only and should have no impact on related mosquito species or on other insects or animals. This specificity brings with it the hope of a very precise intervention with little or no environmental or public health repercussions. Nonetheless, as outlined below, a responsible approach to the development and use of this innovative technology requires that due consideration must still be given to the risk of unplanned consequences.



The two main approaches to the genetic control of mosquitoes, *i.e.* Population Elimination, and Population Replacement are examined below.

## 20.2 Population Elimination Via the Sterile Insect Technique (SIT)

### 20.2.1 Introduction

The potential success of the SIT hinges around the fact that female mosquitoes are monogamous, *i.e.* they mate once only, and use the sperm stored in their spermatheca to fertilize each successive batch of eggs. If that mating happens to be with a sterile male, then that female will lay only sterile eggs, and will not contribute to the next generation of mosquitoes. If sterilized male mosquitoes can be released in sufficient numbers and over a sufficiently extended period, so that they out-compete the indigenous males in terms of mating with the females, then the population should decline to extinction.

The perceived advantages of SIT are that it:

- Is highly specific to the target species.
- Works better at lower indigenous insect numbers, providing released numbers are maintained, the population declines progressively towards extinction.
- Relies on the well-developed ability of the male mosquito to locate and mate with female mosquitoes. This behaviour will take place even in areas where conventional control techniques are not easily used.
- Can if necessary, be “switched off,” *i.e.* the releases halted, after the programme is underway.

Knipling (1959) realized the potential of this technique for insect control, and practical developments then took place against a number of target pests. Probably the best known was against the New World screwworm fly (*Cochliomyia hominivorax*), which was a serious pest of cattle in southern USA and Central America. A system for mass-rearing and sterilization of the screwworm pupae was developed, together with a programme of aerial distribution. The first field trials were carried out in the early 1950s, and by 1959 Florida was declared free of screwworm. Subsequently the fly was eradicated from the rest of

the USA (Wyss 2000), and eventually from Central America as far south as the Panama Canal. The success of this programme was instrumental in creating a climate in which further research and developments in this area could take place.

The sterile insect technique involves several separate stages.

### 20.2.2 Rearing and Sexing

Given the need to numerically overwhelm the indigenous mosquito population, a mass-rearing technique needs to be developed for the target species. The technique and facility needs to provide high insect numbers of uniform quality at an acceptable cost. This inevitably requires research into handling and feeding techniques, pathogen prevention, and automation (Dame et al. 1974).

Mass release of females together with males would be completely counter-productive, because the females could enhance local disease transmission, and mate with the sterile males, reducing the impact on the indigenous population. Sterile male release programmes, therefore, require a stage in which males are separated from females.

For culicine species, the male and female pupae can often be separated mechanically, on the basis of pupal size, using calibrated screens (Bellini et al. 2007). For efficient pupal sexing using mechanical separation, the rearing technique must be sufficiently standardized to ensure a very consistent pupal size. However for anopheline mosquitoes, although there is sufficient sexual dimorphism with some species to allow mechanical separation, with most there is insufficient difference for this technique to work. Alternative techniques have therefore been developed to enable separation of the sexes with anophelines.

Genetic sexing systems have been developed by exposing batches of mosquitoes to radiation followed by crossing and selection of the resultant mosquitoes. This has resulted in the establishment of strains of mosquitoes in which resistance to propoxur (Kaiser et al. 1979, working on *An. albimanus*) or dieldrin (Lines and Curtis 1985, working on *An. arabiensis*) has been translocated onto the Y-chromosome and is, therefore, male-linked. Exposure of batches of mass-reared larvae of these strains to the appropriate insecticide results in

elimination of the females, and survival of the males. However, there is a tendency for such genetic systems to be unstable (Coleman and Alphey 2004), so that occasional purging is required to avoid the formation of resistant (and therefore surviving) females.

For *An. stephensi*, a transgenic strain has more recently been developed in which an enhanced green fluorescent protein gene is expressed in male insects throughout development. This has enabled the sexes to be separated efficiently and automatically as early as the third larval instar (Catteruccia et al. 2005). Other genetic sexing techniques are under development for other species, including *Ae. aegypti* [*St. aegypti*].

### 20.2.3 Male Sterilization

Sterilization of the male pupae has been achieved by a variety of techniques:

Chemosterilization is typically carried out by immersion of the pupae for a fixed period of time in a standard solution of an alkylating aziridiny compound, such as thiotepa or bisazir. High levels of sterility may be achieved, with minimal loss of fitness (Seawright et al. 1977). However use of large-scale chemosterilization has largely ceased now, partly due to concerns about safety to staff working with these potentially mutagenic chemicals.

Irradiation is now the most commonly used technique. It is typically the pupal stage that is exposed to the gamma rays, but work has also been carried out on adults. The gamma source used is typically cobalt-60 with a dose in the range 70–120 Gy. The exposure induces dominant lethal mutations in the sperm of the male mosquitoes. The lower doses cause partial sterilization, while higher doses induce a more complete level of sterilization but are associated with damage to other cells within the male mosquito, causing a decrease in fitness and competitiveness (Helinski and Knols 2009). It has been suggested that when using lower radiation doses, the benefits of higher fitness may outweigh the drawbacks of partial sterilization (Helinski et al. 2006).

As an alternative to sterilization, other techniques have been used or are under development to ensure the absence of female progeny:

Cytoplasmic incompatibility was one of the early and successful techniques used to eliminate female

offspring. It was known that crosses between strains of the same species from different locations were sometimes unable to produce fertile offspring. These inter-strain fertility differences were shown to be due to maternally inherited entities, and the phenomenon was termed cytoplasmic incompatibility. Releases of mass-reared mosquitoes that were incompatible with the local strain had a similar impact on sterilization achieved via chemicals or radiation (Laven 1967). These maternally inherited entities have since been recognised as being the intracellular symbiont *Wolbachia*. These symbionts are not present in anophelines, so this technique is not appropriate to control of malaria vectors.

The release of insects with a dominant lethal (RIDL) gene is a variant to SIT that is currently under consideration. In this technique, a strain of mosquitoes carrying a repressible female-specific lethal gene is developed. Removal of the repressor during mass-rearing enables male-only mosquitoes to be produced without the need for chemosterilization or irradiation and the resulting loss of fitness. The released males are not sterile, but carry a female-specific lethal gene(s). Mating between the RIDL male and wild females, therefore, result in males only. These males would carry the RIDL genetic construct in heterozygous form, and so would in turn, contribute to the elimination of females in subsequent generations (Alphey and Andreasen 2002).

### 20.2.4 SIT in Practice

SIT has been used in practice against mosquitoes on numbers of occasions (Benedict and Robinson 2003), sometimes to explore and validate aspects of the technique and sometimes to attempt to control mosquito populations. Release of the sterilized males is ideally carried out at a time when the numbers of the indigenous insects are at a minimum. This may be achieved via pre-release use of conventional insect control techniques, or release may be timed to coincide with a natural seasonal depression in numbers.

Target species have included *Ae. aegypti* and *Ae. albopictus* [*St. albopicta*], *Cx. pipiens*, *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus*, *An. albimanus*, *An. culicifacies* and *An. gambiae*. Numbers of sterile males released in individual programmes have ranged from less than 10,000, up to hundreds of millions over a 2 year period. Examples of early successful SIT

programmes include the project carried out by Patterson et al. (1970), on a small (<1 km<sup>2</sup>) isolated island in the Florida Keys. Over a 12 week release period, between 8,000 and 16,000 chemosterilized male *Cx. quinquefasciatus* were released per day. The initial ratio of released to indigenous mosquitoes was estimated to be 3:1, while towards the end of the programme, as the indigenous mosquitoes had declined, the ratio had changed to 100:1. At the end of the release period, a reduction of >99% of the indigenous mosquitoes had been obtained. Lofgren et al. (1974) released over 4 million chemosterilized male *An. albimanus* over a 22 week period into an isolated mosquito population in a 15 km<sup>2</sup> area of El Salvador. By the end of the programme, complete elimination of the indigenous mosquitoes had been obtained. Several other projects were less successful with measurable impacts being modest or nonexistent. The reasons for these disappointing outcomes were most commonly believed to be due to poor male competitiveness (as a result of the mass-rearing process and/or the effects of the sterilization process), and to a lesser extent to immigration from nearby untreated areas.

## 20.3 Population Replacement

### 20.3.1 The principle of Population Replacement

The possibility of creating and releasing a mosquito strain that is refractory to human pathogens was first suggested by Curtis (1968b). In its simplest form, this approach requires undertaking several basic steps:

- The identification or assembly of a gene or genetic construct that confers refractoriness in the chosen mosquito species to the chosen pathogen.
- The creation of a driver mechanism which will aid the dissemination and fixing of the new refractory genes within the target insect population.
- The linkage of refractory construct and driver.
- The release of the new mosquito strain.

Once the strain has been released, the dissemination and replacement process should be self-perpetuating, and in theory the release of a single modified mosquito could introduce the construct to an entire population.

In practice however there is likely to be a requirement for significant releases across regions and over time to ensure rapid dissemination and penetration.

Developing refractory strains requires full elucidation of the molecular and genetic relationship between parasite and vector. *Plasmodium* spp. undergo complex development within the mosquito, going through a number of distinct stages, from gametocyte to sporozoite, passing through two separate mosquito cellular barriers; the midgut and salivary gland epithelia. The parasite may be vulnerable to attack at each of these stages.

### 20.3.2 Refractoriness to Pathogens

#### 20.3.2.1 Natural Immunity-Based Mechanisms

In recent years, there has been intensive research on the immune system of *An. gambiae*, to improve understanding of the endogenous mechanisms behind the natural variation in susceptibility to *Plasmodium* spp. infection. Genetic and molecular tools such as microsatellite markers and microarray platforms coupled with the use of RNA interference (RNAi) have been instrumental in helping elucidate the functioning of the mosquito's immune system.

Melanization is a well-known insect immune reaction, and plays an important part in the mosquito's relationship with *Plasmodium* spp. A genetically selected strain of *An. gambiae* (L3-5) has been shown to melanize the ookinetes of several *Plasmodium* species, including allopatric *P. falciparum*, but not sympatric *P. falciparum* (Collins et al. 1986). This result indicates the role of evolution and co-adaptation in the development of the relationship between parasite and vector. Lysis of ookinetes within the midgut epithelial cells has also been shown to be an important naturally occurring defense mechanism. Intensive research has shown the complexity of the processes involved (Blandin et al. 2004; Osta et al. 2004). A number of genes involved in lysis and melanization of *Plasmodium* spp. have now been identified, as well as agonistic genes that appear to protect the parasite from these processes.

Work on mosquito resistance to dengue is also underway in a range of areas. Ramos-Castanada et al. (2008) looked at the role of nitric oxide, naturally produced in midgut epithelial cells, in inhibiting replication of dengue virus within mosquitoes. Within a

dengue-susceptible strain of *Ae. aegypti* they found no evidence of replication of dengue virus genome when a nitric oxide donor was added to the infective blood meal. Interestingly, when a nitric oxide synthesis inhibitor was added to a dengue infective blood meal provided to *An. albimanus*, then dengue virus replication did take place within the anopheline.

### 20.3.2.2 Engineered Refractory Mechanisms

In addition to identification and genetic selection of naturally occurring immunity mechanisms, a wide range of other refractory mechanisms have been created.

Ito et al. (2002) selected a short peptide (SM1) known to bind selectively to mosquito midgut and salivary gland epithelia. A transgenic strain of *An. stephensi* in which SM1 was expressed in the midgut, showed a greatly reduced ability for transmission of *P. berghei*. Antimicrobial peptides (AMPs) that attack the membrane of microorganisms causing cellular damage are also being explored. For example, transgenic over-expression of genes encoding for Cecropin A in the midgut of *An. gambiae* provided a significant reduction in the number of developing oocysts (Kim et al. 2004), although work with another endogenous AMP, Defensin A, showed that it provided protection against gram-positive bacteria but not against *Plasmodium* (Blandin et al. 2002).

However, Christophides (2005) has urged caution in the selection of immunity genes for use in transgenic control strategies, as stable over-expression of genes conferring nonspecific immunity may affect susceptibility to bacteria-based pesticides such as *Bacillus thuringiensis*, which may then render these very useful larvicides inactive.

Franz et al. (2006) used a transgenic approach to develop a strain of *Ae. aegypti* that was resistant to infection by dengue type 2 viruses. They used the natural antiviral RNAi pathway to construct an effector gene that was inserted into the genome of a strain of *Ae. aegypti* using a *mariner MosI* transformation system. The new strain then not only showed reduced viral antigen when fed an infective blood meal, but also showed diminished virus transmission when compared to control mosquitoes.

Paratransgenic strategies have also been examined as a means of establishing mosquito refractoriness to pathogens. Shiva-1, a synthetic analog of naturally

occurring cecropins, has been shown to have activity against *Plasmodium* spp. Yoshida et al. (2001) linked Shiva-1 to a single-chain variable fragment (scFv) directed against the *P. berghei* Pbs21 protein. The immunotoxin was expressed in *Escherichia coli* that were fed to *A. albimanus* which, when infected with *P. berghei*, showed significantly reduced oocyte numbers.

In general, results of research to date on refractoriness have indicated significant challenges still ahead. The efficacy of the refractory construct must be very high, in order to have a useful impact on the epidemiology of the disease (Curtis et al. 2005). Immune responses to malaria parasites in convenient laboratory models such as *P. berghei*, often differ extensively from *P. falciparum*, the human pathogenic species. It is unlikely that single genes conferring refractoriness to key pathogens will be identified. Instead, it is likely that multiple genes will have to be involved to bring about a sufficient reduction in parasite development within the mosquito to have any useful epidemiological impact. It is likely that such a combination will be associated with a fitness cost, and any improved fitness of the mosquito obtained by avoiding infection is likely to be outweighed by the cost of the refractory genes.

## 20.3.3 Genetic Drivers

For the reasons indicated above, a means has to be found to ensure that the refractory construct spreads through the target population faster than by classical Mendelian inheritance alone. A number of mechanisms have been proposed that may, if successful, drive the refractory construct into a wild mosquito population.

### 20.3.3.1 Transposable Elements

Transposable elements or transposons are sequences of DNA that have the potential to move around and propagate rapidly through a genome and are therefore of interest as gene drivers. Natural transposons have been found in *An. gambiae* (Arensburger et al. 2005). Laboratory strains of *An. stephensi*, *An. albimanus* and *An. gambiae* have now all been transformed by introduction of transposable elements carrying markers into the germ line. Research continues into the potential of these structures as practical gene drivers.

### 20.3.3.2 Meiotic Drive

A variety of meiotic drive mechanisms are naturally widespread among insects and mammals. Driver genes bias the normally random segregation of chromatids during segregation to aggressively introduce their inheritance into populations. Endogenous meiotic drive systems have been reported in *Ae. aegypti* and *Cx. pipiens*. Cage scale trials have been carried out with *Ae. aegypti*, to assess the ability of a strong meiotic drive (T37 strain) to bring about population replacement and have given encouraging results (Cha et al. 2006).

### 20.3.3.3 Intracellular and Extracellular Symbionts

Intracellular symbionts such as *Wolbachia* can be spread through populations relatively rapidly via the mechanism of cytoplasmic incompatibility. It has been suggested that by insertion of appropriate genes or constructs into the bacteria, they could become a dispersal vehicle. The genome sequence of the strain of *Wolbachia* sp. found in *Cx. quinquefasciatus* has very recently been described (Salzburg et al. 2009). Work is underway on several mosquito species with encouraging results, e.g. *Ae. albopictus* (Sinkins 2004). Although anophelines do not naturally carry *Wolbachia* sp., it has now been shown that the bacterium can be successfully transferred into *An. gambiae* cell cultures (Rasgon 2006).

### 20.3.3.4 Driver Requirements

Whatever the driver mechanism(s) used, Braig and Yan (2001) have proposed seven ground rules with which the driver mechanism must comply, in order to be acceptable:

- The driver must be sufficiently effective to compensate for the “load” (*i.e.* negative fitness) placed on it by the refractory construct.
- The driver must disseminate the construct through the natural mosquito population at an acceptable speed. Interventions that take many years to have a useful effect are unlikely to be acceptable.
- The driver must be capable of carrying a possibly substantial genetic construct consisting of numbers of genes and regulatory elements that together confer refractoriness.

- The linkage between driver and refractory construct must not be vulnerable to uncoupling through recombination. Such a change may result in the “empty” driver, once released from its refractory genetic load, spreading through the population to no useful effect.
- The driver mechanism must be capable of being reasonably easily modified in the laboratory to ensure that production of a number of different modified mosquito lines, compatible with local strains or even species, is possible. Many vectors are actually species complexes, different members of which may have different relative importance as vectors in neighboring regions, or in different seasons.
- The driver mechanism should be capable of being adapted to carry constructs that confer refractoriness to a range of different pathogens.
- Finally, the mechanism must be sufficiently robust to be able to maintain effective refractoriness levels even when faced with repeated immigration, as will happen for example around the fringes of an intervention area.

James (2005) proposed the addition of two further criteria to the above list, namely:

- The drive mechanism should prevent the construct from moving beyond the target species.
- The drive mechanism should not facilitate selection for pathogen strains that are resistant to the refractory mechanism or for more virulent pathogenic strains or make the mosquito able to act as a vector of pathogens for which it was previously not competent.

Coleman and Alphey (2004) additionally proposed that the drive system should ideally be recallable, so that in the event that the trait had unforeseen and harmful effects, the modified vectors could then be eliminated from the population.

## 20.4 Ethical, Legal and Social Implications of the Use of Genetic Techniques for Mosquito Control

Although genetic technology may appear to offer major benefits to those at risk of vector-borne diseases, a number of issues nonetheless remain to be resolved.

Suspicious about genetic technology go back a long way. In the 1970s, a valuable and substantial international scientific programme was underway in India to assess the potential of a variety of genetic techniques for mosquito control. At a time of national and political tensions, the unit was falsely accused by some local journalists and politicians of being a front for research on biological warfare sponsored by the USA. The response to the accusations on behalf of the unit was slow in forthcoming, and ultimately ineffective. At very short notice, the entire programme was terminated by the Indian Government, and the research unit closed (Curtis 2007). At the present time, concern over the use of genetic technology is still high among some sections of the international media, environmental organizations and the public. For example, the controversy over the production and use of genetically modified crop plants is widely recognised, and continues despite the best efforts of involved parties to address concerns. This debate has slowed investment in and development of such technology. With regard to new developments in the genetic control of disease vectors, it is clearly essential to work with stakeholders at an early stage in order that the benefits of the work are clearly communicated and any concerns fully addressed. Failure to be proactive in terms of involvement and communication could jeopardize much of this work. Involvement of such stakeholder groups may result in a broadening of the scope of the research necessary for the successful implementation of genetic control technology. Determination of the impact of transgenic mosquitoes on predators such as spiders or birds or of the potential for such mosquitoes to become vectors of diseases not currently carried by mosquitoes may become critical to the continuation of such work (Coleman and Alphey 2004).

The process for seeking ethical and legal approval for large-scale trials or operational use of genetic control technology will need to be examined closely. Trials on conventional technologies for vector control are typically carried out in defined areas, involving a relatively localized population. The process of obtaining ethical approval for such work therefore follows conventional guidelines. By contrast, the genetic driver mechanisms used in mosquito population replacement strategies are intended to be self-perpetuating and to have the potential for dissemination across contiguous populations of the vector. A local introduction of modified mosquitoes therefore could have international implications. The

ethics and legal issues around such releases will need very careful consideration (Knols et al. 2007).

Concerns over the immunological repercussions of a successful genetic control programme have also been raised. If the intervention is successful and is maintained, it is likely that the immunity of the local human population will decline, making them vulnerable to epidemics in the event of changes in epidemiology. Although these same general concerns have been raised in relation to other vector control techniques, such as use of ITNs (Snow and Marsh 2002), there are significant differences between genetic techniques and conventional vector control. The former tends to be very specific, creating the possibility that effective genetic control of the principal vector may result in a population-wide loss of immunity, which then allows for the possibility that a different strain of the same species or a secondary vector may later emerge to trigger an epidemic (Knols et al. 2007). Such immunological aspects of large-scale vector elimination programmes need to be considered carefully and the post-release situation monitored very closely.

#### **20.4.1 Absence of Community Participation**

One other potential criticism of the genetic approach to vector control is that it represents a move away from greater community participation (which is seen as a desirable and important component of vector management), back to a more centralized and technocratic approach. On the assumption that the genetic approach is effective, this may not be entirely negative because community participation is seldom self-sustaining, and requires continual inputs (Coleman and Alphey 2004). In addition, genetic control measures as currently envisaged are species specific, so even if one mosquito vector species is eliminated, there may still be scope for community-based programmes to address other problems such as other vector or nuisance mosquitoes, houseflies, or rodents.

#### **20.4.2 International Committee for Genetic Control Work**

The issues raised above and others, emphasize the need for establishment of an independent international coordinating body for genetic technology work in vector

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control. The overall objective would be to establish a broad coalition of all those with an interest in the application of genetic technology for vector control. The body would *inter alia* establish standards and procedures, liaise with and coordinate the work of the scientific community and countries in which genetic releases may be carried out, and involve other stakeholder groups (Knols et al. 2007).

# Chapter 21

## Personal Protection

### 21.1 Introduction

Personal protection is a key component in the prevention of mosquito nuisance and mosquito-borne diseases. Measures may include: simply staying indoors, wearing clothing that minimises exposed skin, use of personal repellents, or use of insecticides on bed nets. Insecticide treated nets (ITNs) for example play a major role in the global campaign against malaria. Even where local or regionally organised mosquito management programme are already in place, individuals may still choose to invest in additional personal protection measures. Research shows that in the USA, around one third of the population use mosquito repellents in any one year, while a recent study in India showed that as many as 99% of the urban population used personal protection products in some seasons. However such products are used not only by residents, but are widely used by tourists visiting mosquito-infested areas, and by military forces deployed to mosquito-infested areas. Given the relatively unsupervised way in which these products may be used by the individual, it is essential that they are carefully developed and evaluated, and that each country has a thorough approval system in place. Commercially available products must be properly labelled in all aspects of safe handling and use.

The following chapter reviews some of the more important personal protection measures.

### 21.2 Impregnated Bednets

#### 21.2.1 Conventional Impregnated Bednets

The use of insecticide treated bednets (ITN) for the control of malaria and other vector-borne diseases has gained much interest since the early 1990s. ITNs have

been used successfully in several regions: China, Thailand, Latin America, and some African countries. Over the last two decades, work on insecticide impregnated bednets and curtains have grown from small scale tests to the operational use of more than 36 million long-lasting insecticidal nets (LLINs) which were distributed in Africa in 2006 (RBM programme). This was almost triple the number distributed in 2004 (WHO 2008). The concept is to place a small quantity of a fast acting insecticide of low mammalian toxicity directly in the path of the host seeking mosquito. Regarding the safety and acceptability of this method of mosquito protection, it was stated by WHO that properly treated bednets should pose no hazard to those who use them. As a result of its very low mammalian acute oral toxicity, extremely low dermal toxicity, very low volatility, and high efficacy, permethrin has been widely used for bednet impregnation.

Treatment of nets or curtains may be considered the most rational form of selective insecticide treatment in the sense that the treated surfaces are those which blood-seeking mosquitoes are bound to encounter and will be attracted to, in their efforts to approach humans (Mouchet 1987). According to the costs and availability of fabrics (cotton, polyester, nylon) for bednets, and the very low dosages of the pyrethroids required for efficacy on nets, net impregnation is affordable for vector control and, if compared to DDT treatments to protect the same human population, sometimes cheaper.

Since most *Anopheles* species bite at night, it has been assumed that nets must be useful against malaria. Moreover, since the majority of malaria vectors prefer to feed on humans (anthropophily) and readily enter houses (endophily), screening is one of the essential measurements (Curtis 1990). In parts of China and in most African countries, evidence has been obtained on ITNs being effective against malaria. However, it has also been



recognised that in many parts of the world, nets may not give sufficient protection to control malaria, because of local community participation and behaviour where the bednets are used. The reasons for this are varied and may include insufficient training or education of the residents, failure by the residents to repair nets or use them appropriately, nets that are inappropriate for the beds being used, or vectors that bite the residents before they have gone to bed or after they have risen in the morning. Nonetheless, a degree of protection from biting and a decreased malaria incidence when nets are properly used, has been proven in many endemic parts of the world. The impregnation of mosquito nets with deltamethrin and permethrin was adopted for malaria control in some provinces of China and parts of Africa (Curtis 1990). In most research studies, impregnation of nets has been done by dipping them in an emulsion made by mixing an emulsifiable concentrate of the pyrethroid with water. It was also found that the amount of liquid left after dipping and wringing by hand was almost the same as if the liquid was added until the point of saturation.

Recent publications have shown that a number of compounds can be employed as effective anti-mosquito impregnation insecticides (WHO 2002).

ITNs or curtains have a profound impact on malaria transmission in sub-Saharan Africa as shown by reduction of various entomologic indices (Bögh et al. 1998; Cuzin-Ouattara et al. 1999) and more importantly, by decrease of morbidity and mortality in humans (Phillips-Howard et al. 2003). Malaria transmission in this area is intense. It is estimated that residents receive up to 300 infectious bites/person/year with peaks just after the two annual rainy seasons (Hawley WA, unpublished data in Beier et al. 1990).

The effect of permethrin-treated bed nets (ITNs) on malaria vectors was studied in western Kenya (Giming et al. 2003). Indoor resting densities of fed *An. gambiae s.l.* and *An. funestus* in intervention houses were, compared with control houses, 58.5% and 94.5% lower, respectively. The sporozoite infection rate in *An. gambiae s.l.* was 0.8% in intervention areas compared with 3.4% in control areas, whereas the sporozoite infection rates in *An. funestus* were not significantly different between the two areas. It was estimated that the overall transmission of *Plasmodium falciparum* in intervention areas was 90% lower than in control areas. As measured by densities of *An. gambiae s.l.*, the efficacy of bed nets decreased if one or more residents did not sleep under a net or if bed nets

had not been re-treated within 6 months. These results indicate that ITNs are optimally effective if used every night and if permethrin is reapplied at least biannually. There was a clear impact of ITNs upon the indoor resting densities of both *An. gambiae s.l.* and *An. funestus*. Overall, a 71.5% reduction was recorded in the indoor resting densities of fed anopheline mosquitoes in intervention areas compared with control areas. Studies in Burkina Faso, and coastal Kenya demonstrated reductions in indoor anopheline densities by more than 80% in the presence of ITNs or curtains (Bögh et al. 1998; Cuzin-Ouattara et al. 1999). The World Malaria Report 2009 shows that more African households own an ITN: 31% in 2008 compared to 17% in 2006. Household ITN ownership reached more than 50% in 13 high burden African countries that resulted also in increased ITN use in the population of children under 5 years of age (24% in 2008).

In situations when ITNs cannot provide personal protection, as in field situations and in military operations, when it is not practical for the personnel to carry mosquito nets with them, it is recommended that synthetic pyrethroid treated patches should be made available to troops operating in endemic malarial areas.

Based on the results of testing impregnated fabrics, WHO (2007) recommend that the following active ingredients may be used to treat bednets (Table 21.1).

### 21.2.2 Long-Lasting Insecticide Treated Nets

Until recently, all ITNs were typically treated with pyrethroid EC formulations, either by the user, health worker, or in local factories. These treated nets offered much improved protection against malaria, but there were still limitations such as loss of active ingredient during washing, failure by users to re-treat sufficiently often, poor insecticide uptake by coloured polyester nets, and sometimes uneven treatment of polyester nets during manufacture.

As a result, a programme to develop Long Lasting Insecticide Treated Nets (LLIN) was established. The goal was to develop a process that resulted in a single treatment of the net during manufacture remaining active for the life of the net. Two approaches were eventually established; one in which the pyrethroid is bound to the surface of polyester netting using a resin,

**Table 21.1** Suitable insecticides for the treatment of mosquito nets or curtains (WHO 2007)

Insecticide	Trade name and formulation <sup>a</sup>	Dose <sup>b</sup> (mg/m <sup>2</sup> )	Toxicity <sup>c</sup> oral LD <sub>50</sub> of a.i. <sup>d</sup> (mg/kg of body weight)
Alphacypermethrin	SC	20–40	79
Cyfluthrin	Solfac 5EW	30–50	250
Deltamethrin	K-Othrine 25 SC	15–25	135
Etofenprox	Vectron 10 EC	200	>10,000
Lambdacyhalothrin	Icon 2.5 EC Icon 2.5 CS,	10–20	56
Permethrin	Imperator 50 EC Peripel 20 EC	200–500	500

<sup>a</sup>Recommended formulations, but others may also be appropriate. *SC* suspension concentrate; *EW* oil-in-water emulsion; *CS* capsule suspension; *EC* emulsifiable concentrate

<sup>b</sup>Doses refer to synthetic netting. Higher doses may be needed for cotton netting

<sup>c</sup>Toxicity is expressed as a.i. for rats. It is not necessarily equivalent to hazard

<sup>d</sup>a.i. active ingredient

and another in which insecticide is incorporated into polyethylene fibers which then release the insecticide over several years. Evaluation of these LLINs has shown that efficacy against mosquitoes remains acceptable after 15 – 20 washes (Sreehari 2009). In practice, evaluation of these nets has shown around 3 – 5 years efficacy (Tami 2004, Kilian 2008). Currently WHOPEs has full recommendations for LLINs containing permethrin and deltamethrin. A number of other nets, including one containing alphacypermethrin, have interim recommendations.

LLINs cost around US \$5, and although initially more expensive than local treatment of nets with an EC formulation, the much longer effective life of LLINs makes them more cost effective in the long term. There are currently extensive operational programmes to introduce LLINs, primarily in Africa. Millions of LLINs have now been distributed in several countries, and these programmes are being evaluated to improve distribution and access (Hightower 2010).

In addition to their use in conventional bed nets, these long-lasting fibers have also been incorporated into plastic sheeting, which can be used by displaced people for making temporary shelters in emergencies, such as following natural disasters or conflict.

## 21.3 Repellents Against Mosquitoes

### 21.3.1 Repellents on Skin or Clothing

Repellents are the most common means of personal protection against blood seeking arthropods and for the prevention of arthropod-borne disease transmission.

Previous work concentrated mainly on simple solutions of topical repellents and the chemical treatment of clothing to prevent blood-sucking arthropods (Rutledge et al. 1978). Current protection is based on controlled release personal use of controlled-release arthropod repellent formulations, and the impregnation of fabrics with permethrin. Repellents may be applied to the skin, clothing or in some cases, to screens to prevent or deter arthropods from attacking humans.

The use of topical repellents may still play an important role in the protection of people in areas where no mosquito control methods are carried out. Several repellents have been developed for self protection against mosquitoes (benzyl benzoate, butyl ethyl propanediol, dibutyl phthalate, dimethyl phthalate, ethyl hexane-diol, butopyronoxyl, 2-chlorodiethylbenzamide, acylated 1,3 aminopropanole derivatives) among which the best known is DEET (*N,N*-diethylmethyl-3-methylbenzamide). They are used mainly in situations where individual protection by other chemical control methods or by nets and vapourizing devices is not appropriate.

Repellents are available in a variety of formulations (liquids, lotions, solid waxes, creams, foams, impregnated wipe-on towelettes, and soaps) and can be dispensed from tubes, pressurized cans, roll-ons etc. When applying a repellent, the biting habits of different mosquitoes should be considered. For general protection against mosquitoes, all exposed parts of the body, such as arms, legs, and face (except the eye area) should be treated with repellents.

Impregnating clothing with a repellent provides extra protection, and temporary protection can be achieved with a spray-on application. For longer lasting effects, clothes should be dipped in a solution of a repellent and a pyrethroid. A combination of permethrin

at a concentration of 0.65–1.0 g a.i./m<sup>2</sup> and repellents at 20 g. a.i./m<sup>2</sup>, or a total amount of 70 g repellent a.i.(s) for a jacket, trousers and socks, is often used.

Different authors have reported various durations of repellent activity of several DEET formulations. Gupta et al. (1987) demonstrated that a controlled-release cream formulation of 33% DEET was no more effective than the standard liquid formulation against several species of Australian mosquitoes. Annis (1990) presented a new extended duration repellent formulation (EDRF) of DEET which was significantly more effective than the liquid formulation. Although the EDRF provided between 6 and 12 post-application hours significantly greater protection than the liquid formulation, the degree of protection began to decline after 8 h. To provide protection against disease transmission, depending on the formulation, re-application would be necessary before significant decline in repellency occurs, which has been reported to be 6–8 h post-treatment. Hoch et al. (1995) reported a new extended duration topical insect/arthropod repellent formulation of DEET which is a multipolymer sustained-release formulation and has numerous advantages over available repellents including lower DEET concentration and extended protection time. Tests of the new formulation showed 95% or better biting protection from *Ae. aegypti* and *An. stephensi* at up to 6 and 8 h, respectively. However, questions have been raised about its safety and effectiveness against some *Anopheles* species and its aggressiveness to paint, varnish and some hard plastics.

Owing to public concerns over safety of synthetic chemicals, interest in botanical repellents has increased lately and has stimulated a re-examination of the repellent data going back as far as 1939, when MacNay published a paper on 38 botanical repellents against northern American mosquitoes. Re-analysis of the MacNay data has been carried out by Rutledge and Gupta (1996), using modern statistical techniques. According to these authors, the data showed that pyrethrum extract (1:1 and 1:3 in olive oil) was one of the most effective repellents, providing protection from *Oc. stimulans*, *Ae. vexans* and *Oc. trichurus* for about 4.5 h, together with several other materials which acted as repellents for shorter periods of time. Prior to the advent of synthetic repellents, pyrethrum and citronella oil were widely used in repellent lotions, sprays, smokes and candles. Although pine tar oil, thymol and geraniol (among others from the MacNay list) have had comparatively little use as repellents, their

protection periods (>3 h) did not differ significantly from those of pyrethrum and citronella oil in the study of Rutledge and Gupta (1996).

Several investigators have reported that repellents can act as attractants when present at low concentrations, deposits or residues. Dubitskyi (1966) found that vapours of dimethyl phthalate, benzimine, DEET and some other compounds were attractants to *An. hyrcanus*, *Ae. cinereus*, *Ae. vexans*, *Oc. caspius* and *Cx. modestus*. On the other hand, many materials that are normally thought to be attractants have been shown to be repellents at high concentrations. Smith et al. (1970) reported that lactic acid was an attractant to *Ae. aegypti* at concentrations normally present on the skin and in the breath, but repellent at a higher concentration (4 mg/cm<sup>2</sup>). Thus it is vital that the labels of commercial repellents should include instructions to wash off or re-apply the repellent when it is no longer effective.

A recent laboratory study of several different repellents conducted in Thailand by Fradin and Day (2002) demonstrates that DEET-based products provided complete protection for the longest duration. Higher concentrations of DEET provided longer-lasting protection. A formulation containing 23.8% DEET had a mean complete-protection time of 301.5 min. A soybean-oil-based repellent protected against mosquito bites for an average of 94.6 min. All other botanical repellents tested in this study provided protection for a mean duration of <20 min. Currently available non-DEET repellents do not provide protection for durations similar to those of DEET-based repellents and cannot be relied on to provide prolonged protection in environments where mosquito-borne diseases are a substantial threat.

However, the long-term usage of DEET is not recommended, particularly for children, and as a result major chemical companies are actively involved in the development of synthetic alternatives to DEET and/or improved formulations to prolong efficacy or increase consumer acceptability. For this reason, when repeated applications of a repellent are required, one of the natural products, such as Qwenling should be used. Qwenling has been derived from the lemon eucalyptus plant in China and contains an active ingredient *p*-menthane-3,8-diol (PMD). Neem oil has also shown good repellency against *Anopheles* mosquitoes, and WHO (1997c) has declared it safe for use.

DEET microencapsulation (MC) is a recent formulation development in which the repellent is gradually

released from a capsule. An experiment carried out on DEET-treated mosquito netting showed that the formulation repels, inhibits blood-feeding, and kills mosquitoes for a period of at least 6 months under laboratory conditions. N'Guessan et al. (2007) suggest that such formulations may have the potential for use on nets against pyrethroid-resistant mosquitoes or on clothing or bedding materials distributed in disaster areas, emergencies or refugee camp situations.

The history of insect repellents provides insight into some of the current scientific strategies behind newer products. Active ingredients currently available include DEET, botanicals, citronella and, the newest agent, picaridin (Katz et al. 2008). At present, the US Environmental Protection Agency's registered insect repellent ingredients approved for application to the skin include DEET, picaridin, oil of citronella, and oil of lemon eucalyptus. DEET has reigned as the most efficacious and broadly-used insect repellent for the last 6 decades with a strong safety record and excellent protection against ticks, mosquitoes, and other arthropods. Newer agents like picaridin and natural products such as oil of lemon eucalyptus are becoming increasingly popular because of their low toxicity, comparable efficacy, and public approval. Bayrepel®, (a.i. Picaridin) was tested in Burkina Faso against mosquito vectors to compare its relative efficacy and persistence profiles to DEET. Collection of >49,000 mosquitoes (95% belonging to the *An. gambiae* complex) showed that after an exposure of 10 h, picaridin based products produced the highest protection against anophelines, followed by DEET. The response of aedines was more variable. Immunoenzymatic detection of the circumsporozoite

protein (CSP) of *Plasmodium falciparum* in 842 females of *An. gambiae s.l.* showed that CSP-positive mosquitoes were equally frequent in treated and control subjects, indicating that the repellents could produce a reduction in the number of malaria infectious bites (Costantini et al. 2004).

Various characteristics and individual product advantages may lead physicians to recommend one agent over another.

### 21.3.2 Mosquito Coils

At present, the main devices used for mosquito protection are mosquito coils, electric mosquito mats and liquid vapourizers, with all these methods vapourizing insecticides into the air using heat from combustion or electricity to kill the insects.

Mosquito coils are made from a mixture of active and inert ingredients and are most commonly used in Asia, Africa and the Western Pacific region. The a.i. usually used in mosquito coils, as well as in other similar products, are pyrethroids (Table 21.2) because they provide quick knock-down action and present little hazard in use. The a.i. in the smoke released when the coil is burnt, deter mosquitoes from the immediate vicinity of the burning coil or even from entering houses. Depending on the size of the room in which the coil is used, and the type of a.i., mosquito biting rate can be reduced by up to 80% (Chavasse and Yap 1997).

Much attention has recently been directed toward the development of non-heated formulations, such as

**Table 21.2** Active ingredients suitable for personal protection in household insecticide products

Product	Active ingredient	Concentration range	Toxicity <sup>a</sup> (oral LD <sub>50</sub> ) <sup>b</sup>
Mosquito coils	<i>d</i> -allethrin <i>d</i> -trans allethrin	0.1–0.30.005–0.3	500500
	Transfluthrin	0.02–0.05	>5,000
	Prallethrin	0.03–0.08	460
Electric vapourizer mats	<i>d</i> -allethrin <i>d</i> -trans allethrin	25–60 mg/mat15–30 mg/mat	500500
	Prallethrin	6–15 mg/mat	460
	<i>S</i> -bioallethrin	15–25 mg/mat	700
	Transfluthrin	6–15 mg/mat	>5,000
Liquid vapourizers	<i>d</i> -allethrin <i>d</i> -trans allethrin	3–61.5–6.0	500500
	Prallethrin	0.6–1.5	460
	<i>S</i> -bioallethrin	1.2–2.4	700
	Transfluthrin	0.8–1.5	>5,000

<sup>a</sup>Toxicity and hazard are not necessarily equivalent

<sup>b</sup>Oral LD<sub>50</sub> of active ingredient for rats (mg/kg of body weight)

fan vapourizers, because of their increased safety and ease of use, especially during outdoor activities. However, the insecticidal activity and/or the vapour pressures of existing pyrethroids are unsatisfactory for use with such ambient-temperature devices. However, some new compounds like metofluthrin could be used both in heated and non-heated formulations. The Southern house mosquito (*Cx. quinquefasciatus*) is the most important target for mosquito coils throughout global tropical and subtropical zones. Mosquito coils containing 0.005% metofluthrin exhibited an efficacy exceeding that of burning coils containing 0.2% *d*-allethrin against this species, and its relative efficacy is estimated to exceed 40 times that of *d*-allethrin.

The unusual characteristic of metofluthrin is sufficient volatility to be bioactive at room temperatures. This activity is not seen in the majority of knock-down pyrethroids. This characteristic enables metofluthrin to be applied in non-heated formulations such as fan-type, paper and resin emanator. Formulations were evaluated by Ujihara et al. (2008). The fan-type formulation containing metofluthrin showed high efficacy. Ambient vapourization devices, where the a.i. is held in paper or resin, and is vapourized without heating or electricity, are easy to use, so there are likely to be new developments in this area of mosquito control. To confirm the efficacy of the paper device, a field experiment was conducted in Malaysia. A device containing 100 mg of metofluthrin strongly inhibited biting of *Cx. quinquefasciatus*. Tests using resin formulations were conducted in Vietnam. Resin formulations containing 1 g of metofluthrin exhibited excellent spatial repellent effects against *Cx. quinquefasciatus* and *Ae. aegypti* for at least 6 weeks.

In the study conducted by Liu et al. (2003) a comprehensive characterization of emissions was carried out for six brands of mosquito coils commonly used in China and Malaysia. The pollutants characterized included fine and ultrafine particles (<2.5 µm in diameter), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and aldehydes, with high irritation or suspected carcinogenic effects. It was found that all particles emitted from burning mosquito coils were <1 µm in diameter. In general, the pollutant emissions from the two tested Malaysian brands were substantially higher than those from the four tested Chinese brands. After comparing health-based standards and guidelines, the authors found that pollutant

concentrations resulting from burning mosquito coils could substantially exceed health-based air quality standards or guidelines and suggested that exposure to the mosquito coil smoke poses both acute and chronic health risks.

Before smoke-generating mosquito coils can be ultimately replaced with nonsmoke mosquito control devices, switching to less polluting products may bring substantial reductions in exposure and respiratory health risk.

The fifteen controlled trials identified in the systematic review by Laurance and Croft (2004) provides consistent evidence that coils inhibit nuisance mosquito biting. Different insecticide classes and different strengths of the same insecticide, provide differing levels of control. All this implies that there is a clear need for standardization and quality control in manufacture and sale of mosquito coils. Despite the clear impact of mosquito coils on mosquito biting, there is however no evidence that they reduce the risk of malaria acquisition (Laurance and Croft 2004).

### 21.3.3 Vapourizing Mats

Electric vapourizing mats have become a common and popular type of personal protection against household insect pests in all parts of the world.

They consist of a mat heater and vapourizing mat. The mat is made from fiberboard impregnated with insecticides (usually a pyrethroid), stabilizers, slow-releasing agents, perfumes and a colouring agent. The heater is plugged into an ordinary household electric socket and heats up to an optimum temperature of 110°C, depending on the type of heater and accompanying mats. When the mat is heated, insecticide vapour is released to provide a low aerial concentration of insecticide. This induces behaviour changes in flying insects through a sequence of sub-lethal effects including deterrence from entering the room, bite inhibition and knock-down. Continued exposure results in death of the insect. The advantage of using mats over coils is that, with the former, there is no unpleasant smoke. The disadvantages are that electricity is required and replacement mats are generally more expensive than coils.

Mosquito mats are usually impregnated with either *d*-allethrin (36 mg/mat) or prallethrin (15 mg/mat) although other a.i. are also used (see Table 21.2).

Research by Adanan et al. (2005) shows that the efficacy of vapourizing mats depends on both the a.i. used and the species tested. Using d-allethrin mats, the  $KT_{50}$  against *Ae. aegypti* and *Cx. quinquefasciatus* was 1.38 and 8.36 mins respectively. When d-allethrin and prallethrin mats were tested against *Ae. aegypti*, mortalities of 63.3 and 90% respectively were obtained. Longevity of *Ae. aegypti* and *Cx. quinquefasciatus* species decreased more than 80% and 45% respectively, after exposure to mosquito mats containing either d-allethrin or prallethrin. The percentage of blood-engorgement activity for *Ae. aegypti* and *Cx. quinquefasciatus* was reduced to less than 70% and 25% respectively after a 20 min exposure to a mat containing either one of the a.i. The number of eggs oviposited by the treated females, egg hatchability, pupation rate and adult emergence rate were not affected if the mosquitoes were still able to blood-engage.

Much attention has recently been directed toward the development of non-heated formulations, such as fan vapourizers, because of their increased safety and ease of use, especially during outdoor activities. By the introduction of metofluthrin as a novel pyrethroid created recently in Japan (2005) a worldwide development of this product has been carried out for environmental health use (Ujihara et al. 2008). Metofluthrin has extremely high knock-down activity against mosquitoes, as well as high volatility and low mammalian toxicity. These features make the product applicable to, not only existing mosquito-controlling devices such as mosquito mats and coils, but also to various new formulations and devices such as paper emanators, fan-driven devices, and similar formulations.

### 21.3.4 Liquid Vapourizers

The principle of these products is similar to that of the vapourizing mat. A liquid vapourizer has to be plugged into an electric socket. It consists of a heater, a wick and a bottle of liquid insecticide. The liquid, which is a mixture of a pyrethroid and a solvent, is drawn up through the wick, typically made from materials such as carbon, ceramic or fiber. The end of the wick is positioned within the heating portion of the device, and when heated, the insecticide vapourizes from the wick. A bottle of liquid insecticide with its wick is usually designed to last up to 360 h, and requires replacement of the bottle every 1 or 2 months.

Mosquito coils, vapourizing mats and liquid vapourizers are as insecticide formulations, with the pyrethroids being the most widely used a.i. The a.i. are shown in Table 21.2 (WHO 1995). There is a developing market in these devices and many different brands are monitored and approved by national authorities before introduction into a country. Worldwide surveys have shown that consumers spend a considerable amount of money each year on personal protection devices. However, due to the risk of allergic reactions and peripheral nervous system disturbances as well as the respiratory issues mentioned above these products should not be used in rooms where children sleep. Therefore, the emphasis should be on promoting the use of safe and effective wide-scale mosquito control programmes whenever possible, instead of relying on individual protection, which should be considered only as an additional tool or as an integral part of a more comprehensive mosquito control programme.

# Chapter 22

## Implementation and Integration of Mosquito Control Measures

### 22.1 Introduction

The most successful approach for controlling mosquitoes is when an Integrated Mosquito Management (IMM) strategy is implemented, in which all appropriate technological and management techniques are utilized, to bring about an effective decline of target-species populations in a cost-effective manner (WHO 2004b).

An IMM strategy could include biological, chemical, genetic, physical, environmental management, and educational components.

With the development and successful use of insecticides (e.g. DDT), most control programmes following World War II were based on the sole use of available insecticides. However, the onset of resistance to chemical insecticides, and the increasing environmental and public health concerns renewed the interest in comprehensive integrated control strategies involving environmentally safe measures.

A single method like larviciding (Table 22.1) may provide adequate control in a certain situation. However, the utilization of a range of control and management methods within an IVM approach may be more cost-effective, cause less environmental damage, be effective for a longer period of time, and be better suited at the community level (Rafatjah 1982; Srdic et al. 1986; WHO 1982a,b, 1983, 2004b).

For this reason, the IVM concept was developed, which incorporates the application of sound ecological principles to maintain mosquito populations below the nuisance and health injury threshold. It is more precisely defined as follows: "Integrated vector control is the rational combination of all available control methods in the most effective, economical, and safe manner to maintain mosquito vector populations at acceptable levels".

The most common mosquito control methods in an IVM programme are:

1. *Chemical control*: Residual applications of adulticides such as DDT, malathion, bendiocarb, permethrin, or related compounds, is the most frequently practiced method against endophilic and endophagic vector mosquitoes. Mosquitoes are repelled from entering houses, or killed when they come into contact with the insecticides which are mainly sprayed on the interior walls of the houses or used to impregnate bednets (Brown 1976; Lacey and Lacey 1990; Lengeler and Snow 1996; D'Alessandro 2001; Takken 2002). Space application against resting or flying mosquitoes can be implemented when exophilic and exophagic mosquitoes such as floodwater mosquitoes occur in masses, thereby avoiding the nuisance or vector activity by mosquitoes in a defined area. However, space application usually protects against mosquitoes only for a short time as the products used are not normally residual. This is particularly important when species of high migratory capacity are involved, which can easily re-invade the treated areas when the effect of the insecticide has declined. Chemicals can also be applied to mosquito breeding sites when they are accessible and defined. Conventional insecticides, used as adulticides or larvicides, are usually broad-spectrum control products that can affect non-target organisms, and as a result are coming under scrutiny for both health and environmental reasons. Development of more environmentally-friendly products, such as insect growth regulators (IGRs) or chitin synthesis inhibitors and microbial-control agents, can effectively reduce the ecological damage to wildlife which can result from the application of non-selective insecticides in ecologically sensitive wetlands.

**Table 22.1** Insecticides suitable for mosquito larval control (WHO 2002)

Insecticide	Type <sup>a</sup>	Dosage (a.i) <sup>b</sup>	Oral toxicity for rats(LD <sub>50</sub> mg/kg body weight) <sup>c</sup>
Temephos	OP	56–112 g/ha	8,600
Temephos	OP	1–2 mg/l drinking water	8,600
Chlorpyrifos	OP	11–25 g/ha	135
Diflubenzuron	GR	25–100 g/ha	>4,640
Diflubenzuron	GR	1–2 mg/l drinking water	>4,640
Pirimiphos-methyl	OP	50–500 g/ha	2,018
<i>B. thuringiensis</i>	MI	*	Absent
<i>B. sphaericus</i>	MI	*	Absent

<sup>a</sup> OP organophosphate; GR insect growth regulator; MI microbial insecticide

<sup>b</sup> a.i. active ingredient

<sup>c</sup> Toxicity and hazard are not necessarily equivalent

2. *Environmental control measures* include environmental management, such as physical reduction of breeding sources, water management, draining or filling of low-land areas, or vegetation management, to create conditions unfavourable for mosquito breeding. These measures range from simple actions such as cleaning street gutters to major redesigning of landscapes. Ditching in marshlands and other low areas to improve drainage is another example. The advantages of modifying breeding sites are that these methods are simple and provide long-lasting effects. However, remodelling wetlands or water-flows can disturb delicate and valuable ecosystems, which in many areas has led to controversies. Initial costs of environmental management may be high, but long-term cost-effectiveness has to be considered.
3. *Physical control measures* include:
  - a. The use of surface layers like monomolecular films or polystyrene beads prevents the immature stages of mosquitoes from breathing at the water surface and can also reduce the deposition of eggs on the water surface (Curtis et al. 1990; Reiter 1978; Webb and Russell 2009).
  - b. Reduction of human/mosquito contact based on community participation following educational efforts, e.g. use of window meshed screens, elimination of small water containers around houses, prevention of access to water surface for females to lay eggs. The use of bednets as a physical barrier could also be employed as a measure although insecticidal bednets are more frequently used now which function as baited insecticidal traps, attracting and killing anthropophilic mosquitoes (Schreck and Self 1985; MacCormack and Snow 1986; Takken 2002).
4. *Genetic control* of pest species has been used successfully in agricultural and veterinary pest-control programmes. However, so far this technique had very limited success in mosquito control. Several techniques can be considered:
  - a. The sterile-male release technique which is based on the induction of sexual sterility in males through the use of radiation or chemical sterilants, and the consequent inundating of natural populations with modified males. (Knipling 1955, 1959; Bellini 2005).
  - b. Cytoplasmic incompatibility between certain allopatric mosquito populations which has been ascribed to the presence of a rickettsial endosymbiont, *Wolbachia* spp., in the gonads (Portaro and Barr 1975; Clements 1992; Mahilum et al. 2003).
  - c. Chromosomal translocations which may cause an increase in sterility of the progeny (Curtis 1968a,b; Rai and McDonald 1971; Laven et al. 1972; Hallinan et al. 1977). Recent developments in molecular genetics such as the ability to introduce recombinant DNA constructions into the genome of organisms (transformation of organisms) offer possibilities for genetic control. It is hoped to genetically engineer mosquitoes to make them refractory to infection by parasites (e.g. *Plasmodium* spp), or perhaps render them unable to transmit the parasites (Alphey et al. 2002).
5. *Biological control* includes natural and applied biological control. Natural biological control is the reduction of mosquitoes by naturally occurring biotic agents (Woodring and Davidson 1996). Applied biological control is planned human intervention by adding biological-control agents to a breeding site (augmentation). Potential biological-control agents are pathogens (microbial-control agents) and predators. Also, aquatic plants such as ferns (e.g. *Azolla* sp.) or blue-green algae (e.g. *Anabaena* sp.) can have a negative impact upon mosquito oviposition and the survival of the developmental stages of mosquitoes.



## 22.2 Prerequisites for the Successful Implementation of a IVM programme

Most important for the implementation of an IVM programme is to build or use an appropriate infrastructure for mosquito control. This includes a well-organised personnel structure with clear responsibilities, intensive cooperative efforts with researchers from local institutes or universities, and adequate financial resources. The most important aspect is the political will to reduce nuisance and vector-mosquito populations. Despite the fact that plenty of control tools are available, most control/management programmes fail because of an inadequate infrastructure.

The following steps for implementation need to be taken:

- (1) Baseline collection of entomological and ecological data which should include the occurrence of mosquito species with special regard to their vector-capacity and/or their nuisance potential.
  - The monitoring of the population dynamics of the most important mosquito species in relation to abiotic (*e.g.* climatic factors, such as rainy and dry seasons, or temporary flooding periods) and biotic conditions (the assessment of predators of the mosquito immature stages which can assist in forecasting the development of mosquito populations), as well as defining the action thresholds for a programme.
  - Information on mosquito migration, biting, and resting habits is essential to define the intervention area.
  - Assessment of parasitological and epidemiological data when vector species are involved (*e.g.* infection or infectivity rates of vector species).
- (2) Mapping and individual numbering of each significant breeding site is essential when larvicides are used.
- (3) Selection of appropriate tools and application systems/equipment to fight the target organisms.
- (4) Effective dosage of a compound in use has to be assessed to assure a cost-effective operation.
- (5) Design of the control strategy should be based on the results obtained in pilot studies.
- (6) Training of the field staff.
- (7) Governmental application formalities, and
- (8) Intensive public relations, educational efforts and encouraging active community participation.

### 22.2.1 Entomological Research (Monitoring)

Precise knowledge of the relative abundance and phenology of the relevant mosquito species is essential to fulfil the requirements of an economical and ecologically successful control programme. Thus, a monitoring programme for mosquitoes should be conducted to determine the species composition, abundance, and phenology (population dynamics) in relation to the climatic conditions, as well as the spatial and temporal distribution related to migration. In different habitats, human bait catches (HBC) should be carried out to determine the most significant anthropophilic mosquito species. The migration behaviour of the most relevant species should be taken into account for designing the control strategy. For monitoring the adult mosquito populations, EVS CO<sub>2</sub>-baited traps are typically used to monitor adult female mosquito populations (see Chap. 4). Samples are usually taken at regular intervals from late afternoon until the next morning, during the peak of flight activity. The trap-collected mosquitoes are counted and identified. When traps have been located in an isolated mass breeding site and in concentric circles of 2, 5, and 10 or more km-diameter, the horizontal dispersal behaviour of the emerging adults can be determined in relation to wind speed, temperature, or vegetation.

In a variety of typical breeding sites within the control area, larval collections should be taken at regular intervals to assess the species composition and abundance. Usually, ten dips or more with a standard dipper are taken to obtain average and comparable data. The larval instars are recorded and a sufficient number of larvae are taken to the laboratory to determine species composition.

Monitoring of post-treatment mosquito populations can also be used to compare different control strategies, such as larviciding, adulticiding, bio-control agents, environmental modifications, etc.

#### 22.2.1.1 Action Thresholds as a Component of Integrated Mosquito Management (IMM)

The concept of Integrated Pest Management (IPM) appeared originally in agriculture during the 1970s. It was proposed as an alternative to the routine and blanket application of pesticides, that was prevalent at that

time, and which was seen as responsible for generating resistance to pesticides, and for causing environmental damage (Rabb 1972). Classical IPM has a number of key components, such as identifying pests and understanding their biology and behaviour, monitoring pest levels, establishing a level of infestation above which control measures are justified, and using a mix of appropriate control measures to reduce pest damage.

The IPM concept has been, subsequently, adjusted to suit other types of pest/vector control, including mosquito control. In nuisance vector control, most of the core components of IPM have been readily adopted, with the exception of defining a threshold infestation level. Although the threshold concept fits agriculture well, there have been difficulties in establishing and using threshold infestation levels for nuisance pests. Nonetheless, in mosquito control, as in agriculture, the fundamental need for such a threshold remains. Should nuisance mosquito control aim to reduce biting levels by 75 or 95%, or to <5/night or <1/night, or ultimately to zero? Without defining such a level, mosquito control may, on the one hand, be over-using insecticides (resulting in unnecessary expense, and possible environmental damage), or may, on the other hand, be failing to provide the standard of control expected by residents and visitors. Defining such tolerance or threshold levels should, therefore, be a central part of the planning of mosquito control programmes.

In the US, there are several examples of the use of action thresholds for mosquito control activities. For example, Headlee (1932) concluded from his research in New Jersey that the human tolerance for mosquito bites was no more than 4 bites/night. More recently, Robinson and Atkins (1983) conducted a study in Virginia on the knowledge and attitudes of residents to mosquitoes. Their data showed that 50% of the residents considered that 3 or more mosquito bites/night constituted a problem and that, in order to satisfy 50% of the residents, a mosquito control agency should aim to keep mosquito numbers below this level. By contrast, surveys of residents in Texas revealed that a mean of 5.7 bites/h at night was a “no problem,” 7.7 bites/h at night was a “mild” problem, whereas 11.5 bites/h at night was considered a “severe” problem (John et al. 1987). It should also be recognised that mosquito bites at night (e.g. *Cx. pipiens*) when residents are trying to sleep, have a different “rating” as compared to bites outdoors during daytime or dusk (e.g. bites by *Aedes/Ochlerotatus* spp.).

To date, relatively few mosquito control organizations have actively involved the public in setting standards (as opposed to the evaluation) for mosquito control. In Italy, though, there have been considerable efforts by public survey to establish their tolerance of mosquito biting and then relate this to light trap catches and timing of treatment. This process has enabled mosquito control organizations to ensure that their programmes provide the level of control expected by the community. In Germany, it was demonstrated that the flood-water mosquito *Ae. vexans* might be a nuisance problem in human settlements when more than 50 specimens were trapped in CO<sub>2</sub>-baited light traps about 2 km away from the settlements.

Experience has shown that a community’s tolerance of mosquito bites tends to drift downward over time. In the Rhine valley, for example, the level of mosquito-biting that was considered as acceptable by the community 30 years ago when the programme began is now considered unacceptable. Mosquito control efforts, therefore, have to be steadily intensified over time. These increased efforts are also likely to cost proportionately more; initially reducing mosquito bites from 20 bites/night to 5/night is likely to be less costly than subsequently reducing the biting from 5 bites/night to 1/night. Mosquito control organizations need to recognise and plan for such changes in the future.

### 22.2.1.2 Threshold for Vector Mosquitoes

All vector control measures require a high degree of coverage of the areas to be treated if human population is to be protected. The requirements will vary depending on the type of intervention concerned and the bionomics of the vector, epidemiological and ecological factors. Disease incidence and human mortality rates directly describe the disease risk for a given area. Besides these facts, parameters related to vectors are known, which could be used to determine the “threshold” limit for epidemic-risk in a particular area. Most commonly, the entomological inoculation rate (EIR) for the determination of malaria endemicity, the infectivity rate (occurrence of infective L3 larvae of the nematode; transmission risk for lymphatic filariasis) or the number of *Aedes* pupae/person or pupae/per area as transmission threshold for dengue are used.

One of the best ways to define malaria endemicity is to determine the EIR which describe the number

of infectious bites an individual is exposed to in a given time period (typically a year). The EIR allows a direct estimation of the transmission risk (Koella 1991; Killeen et al. 2000a,b). Another parameter, named parasite prevalence (parasite ratio or PR = the percentage of individuals found with a positive blood slide), is related to the intensity of transmission and is, therefore, been used to define endemicity in classical malaria epidemiology (Macdonald 1957). The levels of endemicity can be defined as follows: hypoendemic: PR in 2–9 year olds:  $\leq 10\%$ ; mesoendemic: PR in 2–9 year olds: 11–50%; hyperendemic: PR in 2–9 year olds: constantly  $> 50\%$ ; holoendemic: PR in infants constantly  $> 75\%$  (Metselaar and Van Thiel 1959).

The transmission threshold for dengue is based on the number of *Aedes* pupae/per person or pupae/per area. Risk of a dengue epidemic is considered low when the number is below the threshold; risk increases sharply above the threshold. Threshold levels were estimated to range between  $\sim 0.5$  and 1.5 *Ae. aegypti* pupae/person for a certain temperature and initial seroprevalences ranging between 0% and 67%. The ratio of *Ae. aegypti* pupae to human density has been observed in limited field studies to range between 0.3 and  $> 60$  in dengue-endemic areas in the Caribbean, Central America, and Southeast Asia.

### 22.2.2 Mapping of the Breeding Sites

Precise mapping and individual numbering of each significant breeding site enables rapid and effective communication between field staff which provides a solid basis for a successful operation when larvicides are applied. Several other studies can be carried out during mapping: (1) assessment of the diversity and abundance of mosquito species in the control area; (2) characterization (typing) of the breeding sites according to their productivity (mosquito densities) plus mosquito population dynamics; and (3) assessment of the ecological conditions of the major breeding sites, e.g. plant associations, occurrence of predators, or rare and sensitive organisms that indicate the frequency of floods.

#### 22.2.2.1 Geographic Information Systems

Geographic information systems (GIS) have now become very widely used by professionals in research, government and industry for computing spatially-related data.

A GIS consists of an organised collection of computer hardware, software, and geographic data designed to efficiently capture, store, update, manipulate, analyze, and display all forms of geographically referenced information.

Modern information technology allows the integration of GIS with database technology, and with digital mobile field data collection systems supported by a Global Positioning System (GPS).

The ability to link information about, where things are, with the information about what things are, provides the user with a better understanding of spatial phenomena and their relationships, which may not be apparent without such advanced techniques of query, selection, analysis, and display.

Special care should be taken in designing and establishing a high quality GIS to ensure that it contains all the relevant data for a specific purpose, including both geographic data (where is the feature – location and geometry) and properties (what is it like – attribute data).

Typical applications of GIS are:

- Mapping locations of certain features;
- Mapping quantities and densities;
- Finding out properties of distinct areas using database queries;
- Mapping change by comparing how features change over a period of time, in order to forecast future conditions.

An extensive overview on GIS can be found in Burrough et al. (1998).

#### 22.2.2.2 Application of GIS and Information Technology to Mosquito Control

GIS and related information technology can greatly improve survey, logistics, and documentation of mosquito control operations. The possible applications range from direct digital site-mapping using GPS assisted mobile devices to timely aggregation of operational reports.

A spatially referenced database containing all features of interest is the basis for all further data collection and analysis. This spatial element enables thematically related features (*e.g.* population densities of certain species, flooding areas, plant associations and vegetation type, zones of nuisance or disease) that can be organised in separate layers of information, which can then be analyzed and displayed in a user-defined context.

The following is an outline of possible applications in a mosquito control programme based on larviciding:

- Analysis and query of available digital maps, aerial photos or satellite imagery, and thematic maps (*e.g.* hydrology, flooding zones, wetland inventories, etc.) to determine potential larval habitats;
- Spatial analysis to determine relationships between human nuisance or disease, and breeding sites (calculation of buffer-zones, map- and database query);
- GPS-assisted field data collection and breeding site inspection (detailed habitat mapping, larval, and pupal survey). The use of hand-held systems, that synchronize data with the main data base, allows accurate and timely processing of results and database updates;
- Forecasting of time and location of appropriate control activities based on correlations between the spatial occurrence of triggering events for larval development (*e.g.* water levels and flooding areas, local weather data, the potential of larval development sites, and the results of current survey data);
- Preparation of operational maps to improve logistics, calculate the quantities of control materials and manpower required, and to calculate the duration and cost of treatment;
- Storage of historical-site profiles and related attribute data on the basis of operational maps, enables future potential larval development, resulting from dynamic triggering events to be predicted;
- GPS-assisted operations allow the tracking and direct digital documentation of field activities (*e.g.* aerial application);
- Reports and documentation of survey and control activities can be assisted by user-defined database and map queries, which give immediate access to information stored in the database. The results of the queries can then be visualized and printed in the

form of standardized thematic maps, graphics or tables.

For more detailed and up-to-date information on this fast developing technology, it is recommended to consult the webpages of commercial GIS-developers or mosquito control agencies.

### **22.2.3 Selection of Appropriate Tools**

Data are collected in the implementation phase, to ensure the appropriate choice of control tools, application techniques, correct formulations, and effective dosages, depending on the characteristics of the chosen control product, as well as on the biotic and abiotic conditions at the application site. In the laboratory and in small field tests, the effective dosages of the control compounds have to be evaluated. Then the most suitable application techniques and formulations for the various habitats and target species may be chosen.

The optimal use of the product requires homogeneous dispersal of the material in a recommended dosage over the target area in a certain period of time. For instance, the volume of water used for dilution depends on the type of equipment (size of the nozzle/s, pressure of the system, and speed of application) to obtain the desired dosage/area. The rate of emission in relation to the speed of application has to be calibrated before routine treatments, to ensure correct dosage during application. Depending upon the type of application, prevailing weather conditions must be taken into consideration to ensure accurate application.

The selected equipment must be correctly adjusted and operated. Insufficient knowledge and lack of training on how to use and maintain the application equipment and on how to supervise teams can be important factors that lead to poor application of the mosquito control products. On the basis of all available data, an intervention strategy for each individual area and situation has to be worked out. It aims to produce a comprehensive control strategy with a mosaic-like structure that takes into account ecological, epidemiological, and sociological aspects and into which various methods are integrated.

### 22.2.4 Effective Dosage Assessment

This procedure is explained when for example microbial-control agents are used. The efficacy of a microbial-control agent such as *B. thuringiensis israelensis* (*B.t.i.*) is influenced by a great variety of biotic and abiotic factors; the susceptibility of the target mosquito species, the stage of development, the feeding behaviour of mosquito larvae, the density of larval mosquito populations, the temperature and quality of water, the intensity of sunlight, and the presence of filter-feeding non-target organisms (Lacey and Oldacre 1983; Mulla et al. 1990; Becker et al. 1992; Becker and Rettich 1994). The characteristics of the formulations in use, such as potency, settling-rate, and shelf-life, can also influence the effectiveness of microbial-control agents.

It is, therefore, important to understand the impact of these factors during routine treatment, especially, as it effects the calculation of the dosage, the selection of the right formulation for each particular environmental situation, and the appropriate timing for the treatment.

#### – Assessment of the potency of the products and effective dosages

Before new formulations are used in the field, their efficacy is evaluated against *Ae. aegypti* and indigenous mosquito species in the laboratory and in the field. All bioassays are run according to World Health Organization guidelines (WHO 1981). The potency of the formulations (International Toxic Units = ITU) is determined by the formula given in Chap. 4.

#### – Assessment of the minimum effective dosage ( $LC_{99}$ )

Different instars of the indigenous mosquito species are collected in the field and their sensitivity is determined by bioassays. The procedure for bioassays has to be adapted to the specific needs of the study. Water from the larval habitat should be used instead of distilled water to avoid any dramatic change in the living conditions of the larvae which might affect the evaluation of the susceptibility of the tested species. In order to achieve mortality rates of between 10 and <100%, various amounts of a stock solution are added to test containers. Each formulation should be run at least 3 times at 5–7 different concentrations. The  $LC_{99}$  value determined for field-collected larvae is defined as the minimum effective dosage and serves as a guideline for the assessment of the field trials.

#### – Assessment of the optimum effective dosage in the field

Based on the results obtained in the laboratory, the optimum effective dosage for field application can be determined. A series of small field tests in various larval habitats should be conducted to determine the optimum effective dosage of *B.t.i.* formulations against naturally occurring larvae of the indigenous mosquito species. For the field tests, concentrations of 1, 2, 4, and even 8 times the value of the minimum effective dosage ( $LC_{99}$  value obtained in the bioassays) should be used. Each concentration should be tested in triplicate in the characteristic breeding sites, and untreated breeding sites should serve as control.

In most countries, the dosage for each mosquito control product is set by the national regulatory agency, not by the local mosquito control organization. The national regulatory agency will, in turn, typically select dosages that have been established by the manufacturer, sometimes in collaboration with WHOPES.

### 22.2.5 Design of the Control Strategy

The control strategy for large-scale operations should be determined according to several considerations:

- (1) Whether the mosquitoes are a vector or a nuisance species, the bionomics of the target organisms (e.g. are they endophilic or exophilic), and the migration behaviour of the target mosquitoes. The objective of the strategy is to keep mosquitoes away from human settlements and so the migratory behaviour of the species, in question, needs to be considered. For instance, when a nuisance species, such as *Ae. vexans* which readily migrates, has to be controlled, the breeding sites which may occur at a distance from settlements should also be controlled (*Ae. vexans* can migrate >15 km when wind conditions are favourable and when population pressures are high). Domestic mosquitoes (*Cx. pipiens*), which migrate not more than a few hundred meters, can be controlled only within the settlements and within a radius of 500 m. Snow-melt mosquitoes, such as *Oc. cantans*, usually, do not migrate further than 2 km, and prefer to stay in densely vegetated areas. These mosquitoes may be controlled in buffer zones of about 2 km in diameter around villages.

- (2) When larvicides are used, the potential mosquito productivity of a breeding site is a criterion for the relevance of that breeding site, because it allows the assessment of the mosquito threshold for the control.
- (3) The climatic conditions (changes in water level, length of rainy and dry seasons) influence the population dynamics of the mosquitoes.
- (4) The population dynamics of the target organisms determine the timing of the treatment, which will have the greatest impact on the target organisms (*e.g.* control of developmental stages of *Culex* and *Anopheles* during a dry period, when they occur in defined and limited breeding sites).
- (5) The properties of the control compound have to be considered *e.g.* the residual effect of the control agent is relevant for the sequence of re-treatments.
- (6) Adaptation of the control technique to the ecological conditions is another factor to be considered, such as water level and vegetation growth. According to the situation the most suitable formulation and application equipment should be selected (*e.g.* application of *B.t.i.* may be made manually or with the aid of helicopters).
- (7) Development of an integrated control strategy, including predators, environmental management, and community participation. The cooperation between political decision makers, public authorities, scientists, and the general public is of great importance for achieving success in a mosquito control programme.
- (8) The control strategy has to be orientated toward financial and personal resources.

### 22.2.6 Training of Field Staff

A crucial component for a successful control programme is an adequately trained staff and equally well-trained field teams. On the one hand, the control programme must be well organised and on the other, the control team must be flexible enough to respond to each individual situation with the most appropriate techniques and at the correct time, weather conditions permitting. The field staff has to be trained in the biology and ecology of the target mosquitoes and their identification, the principles of the methodology, the mode of action of the control agents, the application techniques, and

the techniques for carrying-out the most appropriate treatment for each situation. The teams should meet regularly to exchange experiences from the field and for further training.

### 22.2.7 Governmental Application Requirements

Governmental approval for the use of a mosquito control agent is a legal requirement for a planned control operation. The application for approval will contain data on the materials and formulations in use, strategy of control, dosages, application techniques, assessment of the threshold for the control operation, assessment of the mosquito density, and ecological considerations. This information is available in the material safety data sheets (MSDS) provided by the manufacturer.

### 22.2.8 Community Participation

Mosquito control can be particularly successful when the local population is well educated by the responsible agency and are encouraged to be involved in the search for solutions to mosquito problems, especially when they are related to mosquitoes breeding within dwellings (Becker 1992). Responsibility for the definition of the strategy, planning, and the regional organization of the control programme rests with the programme authorities, but even at the planning stage, the expected cooperation of the community must be taken into account (Halstead et al. 1985; Yoon 1987).

The programme authorities have to evaluate how the control strategy may be best achieved through community participation to obtain a horizontally-defined structure. The successful inter-digitation of the vertical (regional authorities) and the horizontal organizational (community) structures through community participation means that the people become "actors" instead of "spectators" (Diesfeld 1989). The programme has to enable people to contribute to the solution of their mosquito problem related to their own settlement. This can be achieved by a comprehensive campaign of

instruction dealing with the biology of the mosquitoes and with straightforward methods of control available on websites, leaflets, brochures, television, radio, daily newspapers, circulars, posters, videos, slides, and demonstrations. Thus, “help through self-help” can be achieved. It is important to keep the level of motivation high over a long period. People are frequently aware of the problem but they may take no action or there may be just short-term involvement.

Community participation can be at its best and most meaningfully achieved by controlling the immature stages of mosquitoes breeding in or close to human settlements, for example, species such as *Ae. aegypti*, *Ae. albopictus*, *Cx. p. pipiens/quinqfasciatus* or *Anopheles* in urban areas. The following methods can be used (see also 17.4.1.):

1. Source reduction by elimination of unnecessary stagnant water bodies;
2. The weekly replacement of water in bowls and jugs as well as cleaning of containers to remove and destroy mosquito eggs;
3. The careful covering of water containers, *e.g.* with tight-fitting lids or netting to prevent egg laying and larval breeding;
4. The release of larvivorous fish into water reservoirs;
5. The filling of potholes with concrete or by draining the water to avoid the collection of stagnant water;
6. The application of larvicides if none of these methods are practical (*e.g.* Culinex/Vectobac DT tablets based on *B.t.i.* or *B. sphaericus*, methoprene briquets or Abate granules). There are many arguments in favour of introducing the use of microbial-control agents with community participation. Most striking is that they are safe for humans and the environment, as well as simple to use (Table 22.1).
7. The application of adulticides, especially during epidemics, as an ultimate measure to control adults transmitting diseases, could be achieved with indoor residual house spraying (IRHS). The choice of the insecticide depends on a variety of factors including regulations of the local/state/country. The recommended products by WHO (2002) are given in Table 22.2.

### 22.2.9 Registration of Insecticides

In most countries, insecticide approval and use is regulated by law. National regulatory agencies (*e.g.* the EPA in the U.S.) will require substantial evidence that a particular pesticide is suitable for the intended use before allowing it to be sold. This evidence typically covers several categories, including the physico-chemical

**Table 22.2** Insecticides recommended for indoor residual house spraying (IRHS) (WHO 2002)

Insecticides	Dosage (g/m <sup>2</sup> )	Formulation	Duration of effectiveness	Insecticidal action	Safety class of ingredient
<i>Organochlorines</i>					
DDT	1–2	WP	6 months or more	Contact	MH
<i>Organophosphates</i>					
Malathion	2	WP	2–3 months	Contact	SH
Fenitrothion	2	WP	3–6 months	Contact, airborne	MH
Pirimiphos-methyl	1–2	WP	2–3 months or more	Contact, airborne	SH
<i>Carbamates</i>					
Bendiocarb	0.1–0.4	WP	2–6 months	Contact, airborne	MH
Propoxur	1–2	WP	3–6 months	Contact, airborne	MH
<i>Pyrethroids</i>					
Alpha-cypermethrin	0.02–0.03	WP	4–6 months	Contact	MH
Cyfluthrin	0.02–0.05	WP	3–6 months	Contact	MH
Deltamethrin	0.01–0.025	WP	3–6 months	Contact	MH
Lambda-cyhalothrin	0.02–0.03	WP	3–6 months	Contact	MH
Etofenprox	0.1–0.3	WP	3–6 months	Contact, airborne	

Note: MH moderately hazardous; SH slightly hazardous; WP wettable powder

properties of the pesticide, the impact of the material on the user, the public, the environment, and its effectiveness against the target pest. These regulations apply to manufacturers, distributors, and to the end user. They also apply to almost all pesticides, including microbial-control agents such as *B.t.i.* Pesticide users in public sector organizations and also in the private sector, are all typically obliged to comply with these regulations. Within the European Community, for example, active ingredients are regulated under the European Biocides Directive (98/8/EC, BPD), whereas individual formulations are registered at the national level.

For the end-user of insecticides, the legislation typically puts a wide range of requirements in place, and these directions *must* be followed. The requirements will, typically, be found on the product label and mosquito control staff at all levels must be familiar with the details of the label requirements. Most mosquito control organizations train their staff carefully to ensure an understanding of all aspects of the label requirements and full compliance with the regulations in safety and handling during field applications. The label directions, typically, specify a wide range of conditions including:

- Use of Personal Protective Equipment (PPE)

Staff involved in the mixing and application of insecticides are required to wear certain protective equipment, such as coveralls, gloves, respirators and face-shields, to prevent personal contamination. Such items should be provided, replaced and laundered by the employer.

- Personal Hygiene

If there is direct contamination of the eyes and/or the skin, the contaminated area must be immediately flushed/washed. In addition, it is a good practice to wash after using a product, before meals, breaks, and before leaving work at the end of the day.

- Application Equipment and Dosages

Insecticide labels will normally specify the application equipment to be used, *e.g.* granule applicator, ULV equipment, etc. The equipment should be maintained in good working condition and calibrated at regular intervals for accurate delivery. Formulations and dilutions will be explained specifically and these directions must be followed to the letter of the law. Only through careful calibration and monitoring of equipment performance, is it possible to ensure that the correct quantity of the control product is applied to

achieve high efficacy, whilst at the same time, avoiding significant environmental impact.

- Timing and Frequency of Treatments

Insecticide labels will normally provide guidance on when treatment should be applied for maximum efficacy, *e.g.* against certain developmental stages of the insect or under certain meteorological conditions. In the interest of resistance management, there may also be restrictions on the frequency of pesticide use. All the above conditions must be followed according to Label Directions. The success of the application should be evaluated after the application (pre- and post-treatment evaluations).

- Pesticide Storage

All pesticides must be stored safely and securely to avoid any possible accident resulting from unauthorized access by the public or from fire or extreme weather conditions. The storage facility must also display the appropriate warning/caution signs.

- Proper Disposal of Used Insecticide Containers

To avoid possible pollution or contamination, empty containers and washings from the application equipment should be disposed off in accordance with the local regulations. In some cases it may be possible to use “washings” as a diluent for the next batch of spray.

The MSDS can usually be obtained from the manufacturer, supplier, or the national regulatory agency. Useful information may also be obtained from a variety of other sources, for example the International Programme on Chemical Safety. Human and environmental safety during mosquito control activities have been well-regulated in recent years and it is the responsibility of all those involved in this important work to ensure that this situation is continuously maintained.

### **22.2.10 Routine Treatments**

Any control programme basically needs certain core elements, such as effective management and administration, including appropriate facilities, a personnel structure with clear responsibilities, and target-specific control tools which are used within an appropriate control strategy which the local community supports, and in which their involvement is crucial. The strategy has to be adapted to the specific needs of



each particular situation during the operation, *e.g.* selection of a suitable formulation which fits the ecological conditions and the bionomics of the target-organisms. The operation has to be guided by target surveillance and an environmental monitoring programme, as well as by goal-oriented research and a well-structured educational programme. Intensive public relations work will encourage the involvement and support of the general public.

Important prerequisites for successful routine operations are:

- (1) The planning and control operation aims at a long-lasting operation and should be based on long-term programming and encouraging long-term, responsible personnel. Control of mosquitoes needs skilled people who are familiar with the local conditions for control and have a perspective for a continuous realization of the control strategy to which they feel responsible. The sustainability of a successful programme has to be guaranteed.
- (2) Besides the governmental aspects of the activities, *e.g.* financial and logistic support, the problem should mainly be solved by utilizing local resources as this will assure a long-term operation but should not excuse the lack of governmental support.
- (3) Clear and well-defined responsibilities have to be delegated to each “actor” in the programme at each level of the operation. Usually, only limited resources and time-frames are available for control operations, therefore, these actions have to be organised in the most effective and efficient manner.
- (4) The effect of the operation has to be evaluated by entomological and epidemiological monitoring teams. Thus, failures can be quickly pin-pointed and corrected to optimize the effectiveness of the control operation.
- (5) Staff involvement and motivation should ensure the success of the programme.

### **22.2.11 Public Information Systems**

Appropriate information will increase public awareness and acceptance of the control efforts. At regular intervals, the media should be informed about the actual operations and the results of control. Improvements in understanding the actual communication networks used by community participants may alleviate problems caused by communication gaps. Carefully chosen continuing, compelling programmes with the desired “message” should be delivered to people at peak viewing hours (Curtis 1990).

Access to the Internet and computers can provide people with the tools and information to improve their knowledge about the biology of mosquitoes, mosquito-borne diseases, and the ongoing operations. Web-sites should contain information on the operations and actual matters of interest, *e.g.* descriptions of exotic and invasive species of mosquitoes. These public relations tactics might motivate the public to participate in the monitoring and control. A website could be created to announce press releases.

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# Subject Index

## A

- Abate, 457, 507
- Abdomen
- adult, 19, 52, 54, 55, 56, 57, 63, 72–76
  - larva, 16, 18, 53, 76, 80–84
  - pupa, 19, 53, 85, 86, 87
- Accessory glands, 20, 468
- Accessory seta, 85, 368
- Acetone, 21
- Acetylcholine (ACh), 452, 458
- Acetylcholinesterase (AChE), 452, 456, 458, 473
- Aciculate seta, 77
- Acrostichal rows, 69, 106
- Acrostichal stripe, 70
- Actellic. *See* Pirimiphos methyl
- Action thresholds, 501–502
- Active ingredient, 443–449, 451, 460, 466, 468, 492, 494–496, 508
- Acus, 82
- Acute pancreatitis, 36
- Acylated aminopropanole derivatives, 493
- Adenosine diphosphate (ADP), 23
- Adenosine triphosphate (ATP), 23
- Adults
- biology, 19–24
  - keys, 91–111, 318–322, 334–340, 351–356, 369–374, 387–391
  - morphology, 63–76
  - preserving, 54–55
  - rearing, 51–52, 86, 200
  - sampling, 47–51, 54
- Aedeagal setae. *See* Leaflets of aedeagus
- Aedeagus, 73–75
- Aeration, 435
- Aerenchyma, 10, 17, 19, 307, 309
- Aerial application equipment, 449, 453
- Aerial photos, 504
- Aerosol dispenser, 447
- Aerosol generator, 448–449, 453, 463
- Aerosols, 37, 446–449, 453, 459–460, 463, 465, 474
- African region, 208, 443, 450, 451, 466
- Agonistic genes, 486
- Airborn, 447, 453
- Airport malaria, 29
- Aliphatic organophosphates, 452–454
- Allethrin, 460
- Allopatric, 325, 330, 486, 500
- Alpha-cypermethrin, 460–462
- Alternative methods in mosquito control, 441
- Ammonia, 10, 45
- Anal lobe, 85
- Anal papillae, 16, 84
- Anal vein, 72
- Anautogenous, 9, 23, 24, 219, 277
- Anepisternal cleft, 70, 71
- Anepisternum, 70
- Annuli, 64, 66
- Anophelism, 29, 170
- Anoxia, 478
- Anteacrostichal patch, 70
- Anteclypeus, 65
- Antenna
- adult, 63
  - larva, 79
- Antennal seta, 79
- Antepalpmate seta, 82
- Anteprococal membrane, 70, 71
- Anteprococal patch, 71
- Antepronotum, 67, 70
- Anterior spiracular lobe, 83
- Anterolateral spiracular lobe, 81
- Anteromedian palatal brush, 79
- Anthropophilic, 24, 25, 30, 176, 180, 249, 323, 326, 329, 347, 375, 500, 501
- Anthropophily, 24, 329, 491
- Antibodies, 33, 35–39, 55
- Anticoagulant, 23, 66
- Ants (Formicidae), 409
- Anus, 73, 75, 83, 84
- Apical lobe, 73
- Apical seta, 85
- Apical spine of gonostylus, 74
- Apodous, 16
- Appetitive flight, 21
- Application equipment, 448, 449, 453, 469, 471, 504, 506, 508
- Aquatic spider, 412
- Arboreal species, 9
- Arboviruses, 30–39, 188, 196, 202, 203, 267, 277, 279, 312, 366
- Arculus, 72
- Arsenic, 443
- Arthritis, 31

- Arthropod-borne viruses (Arboviruses), 30–39, 188, 196, 202, 203, 267, 277, 279, 312, 366, 493
- Artificial mating, 52
- Asian bush mosquito. *See Ochlerotatus japonicus japonicus*
- Asian rock pool mosquito. *See Ochlerotatus japonicus japonicus*
- Asian tiger mosquito *See Aedes albopictus* in Taxonomic Index
- Aspirator, 47–49, 52, 60
- Ataxia, 451
- Attractants, 22, 45, 47–50, 60, 481, 494
- Attractant traps, 47
- Augmentation, 405, 500
- Autogenous, 10, 19, 23, 168, 170, 215, 217, 219, 279, 290, 306, 310
- Autosomes, 56
- Avian malaria, 333
- B**
- Bacteria, 25, 188, 416, 419–431, 442, 443, 487, 488
- Bait catches  
  animal bait catches, 49
- Bancroftian filariasis, 279, 323, 325, 326, 332, 333, 407
- Banding patterns, 56, 57
- Barmah Forest virus, 351, 359, 365
- Basal lobe, 73
- Basimere. *See* Gonocoxite
- Basistyle. *See* Gonocoxite
- Bats (Chiroptera), 36, 37, 409–410, 430
- Baygon. *See* Propoxur
- Bed nets, 60, 430, 434, 462, 464, 491, 492
- Behavioural resistance, 472
- Behavioural studies, 7, 45
- Behaviour changes, 496
- Bendiocarb, 444, 445, 459, 472, 499, 507
- Benign malaria, 28
- Benzoylphenyl ureas. *See* Diflubenzuron
- Benzyl benzoate, 493
- Bifenthrin, 444, 445, 462
- Bioaccumulation, 452
- Bioassay, 59, 425–428, 455, 471, 473, 474, 505
- Biochemical agents,
- Biodegradable surface film, 478
- Biodegradation, 459
- Biological control, 405–431, 468, 500
- Biorational larvicides, 424
- Birds (*Aves*), 409
- Biting midges, 38
- Blackflies *See* Simuliidae in Taxonomic Index
- Blood-meal  
  blood meal identification, 55
- Botanical repellents, 494
- Brackish water swamps, 358, 392, 394
- Branched seta, 78, 83
- Breeding site, 9–11, 13, 14, 16, 19, 21, 23, 24, 43–45, 47
- Briquettes, 424, 425, 448, 469
- Broad-spectrum insecticides, 460
- Bronchopneumonia, 39
- Browsers, 17
- Burning coil, 460, 495
- Bursa copulatrix, 20
- Butanone, 21
- Butopyronoxyl, 493
- Butyl ethyl propanediol, 493
- C**
- Caddisflies *See* Trichoptera in Taxonomic Index
- California group viruses, 31, 37
- Canalisation, 438
- Canine cardiovascular dirofilariasis, 40
- Cap, 16, 46
- n-Capric acid, 45
- Caput (larvae). *See* Head (larvae)
- Carbamates, 58, 441, 443–445, 449, 450, 452, 456, 458–459, 470, 473, 507
- Carbon dioxide, 10, 21, 22, 45, 47–51, 447, 481
- Carbon dioxide traps, 47, 49–51, 481
- Cardiac insufficiency, 40
- Carnoy's fixative, 57
- Catarrh, 39
- Catch basins, 29, 180, 435
- CDC light traps, 50, 217, 429
- Cecropins, 487
- Cemeteries, 9, 369, 434, 436, 456
- Centers for Disease Control (CDC), 29
- Cephalothorax, 19, 53, 85, 86
- Cercal lobes, 85, 86
- Cercal sclerites, 75
- Cerci, 73, 76
- Cervix  
  adult, 65  
  larva, 79
- Cesspools, 429, 480
- Chaetotaxy, 77, 84–86
- Chemical control, 441–475, 493, 499
- Chemical solubility, 450
- Chemical stability, 450, 452, 459
- Chemical type or class of insecticides, 445, 446
- Chemosterilants, 442
- Chemosterilization, 485
- Chikungunya fever, 25, 31, 332, 442
- Chitin synthesis inhibitors, 466, 499
- Chlorinated hydrocarbons, 441, 443, 445, 449–452
- Chlorinated insecticides. *See* Chlorinated hydrocarbons
- Chlorinated organics. *See* Chlorinated hydrocarbons
- Chlorodiethylbenzamide, 493
- Chloroquine, 28
- Chlorpyrifos, 444–446, 448, 449, 455, 472, 500
- Cholinesterase (ChE), 452
- Chorion, 45, 52, 171, 279
- Chromatids, 57, 488
- Cibarial pump, 23
- Cichlids (Fish), 408
- Circumsporozoite protein (CSP), 495
- Citronella oil, 494
- Cladistic trees, 3
- Clasper *See* Gonostylus
- Claspetoid, 76
- Claspette filament, 75
- Claspettes, 75, 76
- Claspette stem, 75
- Classification of insecticides, 443–446

- Claws, 71  
 Climatic, 9, 15, 24, 40, 42, 202, 248, 277, 290, 501, 506  
 Coconut growing areas, 333  
 Comb, 82  
 Comb scale, 82  
 Common carp. *See* *Cyprinus carpio* in Taxonomic Index  
 Common guppy. *See* *Poecilia reticulata*, in Taxonomic Index  
 Community participation, 412, 422, 427, 436, 438–439, 489, 492, 500, 501, 506–507  
 Community's tolerance, 502  
 Compound eyes, 22, 64, 65, 79, 80, 85  
 Compression sprayer, 448, 449  
 Conditioning, 16  
 Contact pheromones, 20  
 Contact poison, 444  
 Copulation, 9, 20, 52, 73, 196, 219, 246, 277, 310, 415  
 Cork, 54  
 Corolla, 10  
 Coronal suture, 64  
 Corpora allata, 468  
 Costa (C), 72  
 Cost effectiveness, 423, 459, 500  
 Coxa, 70, 71  
 Coxite. *See* Gonocoxite  
 Cratal setae, 84  
 Cross resistance, 455, 461, 464, 475  
 Cubitus, 72  
 Cumulative effects, 455  
 Cuticular penetration, 472  
 Cuticular respiration, 479  
 Cyanide, 54  
 Cyfluthrin, 444, 445, 449, 460, 462–463, 472, 493, 507  
 Cypermethrin, 444, 445, 449, 460, 461, 463  
 Cytodiagnostic methods, 56–57  
 Cytoplasmic incompatibility, 485, 488, 500  
 Cytoplasmic polyhedrosis virus, 431
- D**  
 Dancing flight, 20  
 Deconditioning, 16  
 DEET, 466, 494  
 Deltamethrin. *See* K-Othrine  
 Dendriiform seta, 78  
 Dengue fever (DF), 35, 436, 438, 442, 458  
 Dengue haemorrhagic fever (DHF), 35, 36, 47, 407, 436, 438, 458  
 Dermal toxicity, 445, 491  
 Design of the control strategy, 428, 501, 505–506  
 Diapause, 10, 13–16, 24, 44, 173–175, 195, 202, 241, 262, 277, 279, 401  
 Diazinon, 445, 448, 455, 456  
 Dibrom, 453  
 Dibutyl phthalate, 493  
 Dichlobenil, 467  
 Dichlorodiphenyltrichloroethane (DDT), 29, 347, 430, 441–446, 450–452, 460, 461, 464, 472, 473, 491, 499, 507  
 Dichlorvos (DDVP), 454  
 Diflubenzuron, 445, 466–468, 480, 500  
 Dilatation of the ovarian epithelium, 56  
 Dimethyl phthalate, 493  
 Dipper, 43, 46, 501  
 Dirofilariasis. *See* Canine cardiovascular dirofilariasis  
 Disease transmission, 47, 49, 315, 325, 433–436, 464, 484, 493  
 Dispersal, 21–22, 219, 325, 488, 501, 504  
 Dissolved oxygen, 14, 15, 24, 262, 478, 479  
 Distimere. *See* Gonostylus  
 Dististyle. *See* Gonostylus  
 Ditching, 433, 437, 500  
 Diuron, 466  
 DNA fingerprinting, 58  
 Dog heartworm. *See* in Taxonomic Index  
 Dormancy, 10, 24  
 Dorsal arm, (inner division), 76  
 Dorsocentral rows, 69, 70  
 Dorsocentral stripes, 70  
 Double mounted method, 54  
 Dragonflies. *See* in Taxonomic Index  
 Drainage, 168, 174, 224, 227, 288, 375, 429, 434, 435, 437, 438, 500  
 Draining, 433, 436, 500, 507  
 Driver mechanism, 486, 488, 489  
 Drop-net, 49  
 Dry ice, 50  
 Dursban. *See* Chlorpyrifos
- E**  
 Ecdysial line, 19, 85  
 Ecdysis, 467  
 Ecdysone, 18  
 Ecological data, 501  
 Economy, 32  
 Ecotoxicological effects, 468  
 Educational materials, 439  
 Effective dosage assessment, 505  
 Effector gene, 487  
 Egg batches, 10, 20, 52, 195  
 Egg burster *See* Egg tooth  
 Egg density, 44  
 Egg-laying behaviour, 10–12, 43, 45  
 Egg-laying substrate, 12  
 Egg production, 23  
 Egg raft, 10, 43, 45, 52, 276, 284, 298  
 Eggs  
   biology, 9–14, 16, 19–24  
   key, *Anopheles Maculipennis* Complex, 171  
   rearing, 45, 52  
   sampling, 43–46  
 Egg stage, 24, 31  
 Egg tooth, 16  
 Electric mosquito mats, 495  
 Electronic mosquito repellents, 47, 495–496  
 Embryogenesis, 11, 13, 16, 195, 196, 470  
 Embryonic development, 10, 13, 468  
 Emergence inhibition (EI), 468, 469  
 Empodium, 71  
 Emulsifiable concentrates (EC), 446–447, 455–457, 462, 463, 492  
 Encapsulated materials, 448

- Encephalitis, 25, 31, 32, 34, 36–38, 50  
 Encephalitis virus surveillance trap (EVS), 49–51, 429, 501  
 Endemic malaria, 28, 30, 325, 450, 456, 492  
 Endochorion, 11  
 Endogenous mechanisms, 486  
 Endogenous meiotic drive, 488  
 Endophagic, 23, 342, 347, 379, 381, 430, 464, 499  
 Endophagy, 23, 224  
 Endophilic, 23, 30, 174–176, 379, 381, 430, 450, 458, 499, 505  
 Endophily, 23, 491  
 Energy source, 23, 51  
 Entomological research (monitoring), 501–503  
 Entomological studies, 43  
 Environmental control measures, 472, 500  
 Environmental impact, 422, 429–430, 437, 465, 508  
 Environmental management, 427, 433–439, 499, 500, 506  
 Environmental manipulation, 433  
 Environmental modification, 433, 501  
 Environmental Protection Agency (EPA), 60, 422, 454–456, 495, 507  
 Environmental safety, 422, 427, 508  
 Enzootic vector, 36, 453  
 Enzyme inhibition, 452, 458  
 Epicranial plates, 78, 79  
 Epicranial suture  
   adult, 64  
   larva, 79  
 Epidemiological studies, 43  
 Epidemiology, 487, 489, 503  
 Epimeron, 70  
 Epiproct, 72, 73  
 Episternum, 70  
 Epizootic, 32, 36, 400, 442  
 Equine encephalitis viruses, 31  
 Equipment calibration, 448, 449, 508  
 Erythrocytes, 27, 28, 55  
 Erythrocytic schizogony, 27  
 Ether, 54  
 Ethyl acetate, 48, 54  
 Ethyl hexanediol, 493  
 Etofenprox, 444, 445, 449, 460, 463, 493, 507  
 European Biocides Directive, 508  
 European Centre for Disease Prevention and Control (ECDC), 31  
 Eurygamy, 20, 171  
 Exit trap, 48  
 Exophagic, 23, 178, 182, 217, 227, 306, 325, 378, 430, 499  
 Exophagy, 23, 167  
 Exophilic, 23, 167, 170, 175, 182, 224, 344, 378, 430, 499, 505  
 Exophily, 23, 166, 170, 182  
 Extended duration repellent formulation (EDRF), 494  
 Extended residual briquettes (XR), 448  
 External genitalia slide preparation, 55  
 Exuvia, 19, 467  
 Eyes  
   adult, 65  
   larva, 16, 79, 80
- F**  
 Fan-like seta, 78, 84  
 Fascicle. *See* Stylet bundle  
 Fat body, 19, 23, 24, 278, 419, 431  
 Fatty acids, 45  
 Feeders, 17, 79, 291, 298, 408  
 Feeding  
   adults, 22–24, 66  
   larvae, 17, 18, 77, 78, 426, 478, 505  
 Femur, 71  
 Fenitrothion, 444, 445, 448, 449, 454–455, 471, 507  
 Fenoxycarb, 466, 470  
 Fenthion, 444, 445, 449, 455, 457, 471  
 Ficam. *See* Bendiocarb  
 Filariasis, 25, 39–40  
 Filter feeders, 17, 79  
 Fin. *See* Ventral brush  
 Fish (Osteichthyes), 406–408  
 Flagellomere, 64  
 Flagellum, 20, 63  
 Flatworms. *See* in Taxonomic Index  
 Flight behaviour, 21  
 Flight range, 9, 21, 173, 174, 176, 183, 224, 332, 342, 349, 357, 366, 375, 376, 378, 401  
 Flood catchment areas, 438  
 Flooding method, 44  
 Floodwater mosquitoes  
   dispersal, 21  
   egg-laying behaviour, 10–12  
   embryonic development, 13  
   hatching, 13–16, 19  
   hibernation, 24  
   larval development, 15  
 Food chain, 450, 452  
 Food channel. *See* Labroepipharynx  
 Formulations, 59, 424–428, 445–449, 453, 455–459, 462, 463, 465, 468–470, 478, 493–497, 504–509  
 Frequency of treatments, 508  
 Freshwater snails, 409  
 Fringe scales, 72  
 Frons, 65  
 Frontal setae, 79, 80  
 Frontal sutures, 64, 65  
 Frontoclypeus, 78, 79  
 Fumigants, 55, 444, 446, 454  
 Fungi, 278, 416–418, 442
- G**  
 Gametocyte, 28, 29, 486  
 Gastritis, 35, 39  
 Genae, 65  
 Gene dominance, 474  
 Genetic control, 442, 483–490, 500  
 Genetic engineering, 424  
 Genetic manipulation, 29  
 Genetic mapping, 56  
 Genetic maps, 56

- Genetic markers, 26, 57, 277  
 Genetic techniques, 442, 488–490  
 Genetic translocation, 442  
 Genitalia, 55, 73–75, 85  
 Genital lobe, 85, 86  
 Genome, 31, 37, 56, 58, 325, 483, 487, 488, 500  
 Genotype, 57  
 Geographic Information Systems (GIS), 503–504  
 Glycoprotein receptors, 420  
 Gnathocephalon, 63, 65  
 Gonocoxal apodemes, 74  
 Gonocoxite, 73–75  
 Gonopod, 73  
 Gonostylus, 73, 74  
 Gonotrophic cycle, 29, 53, 55, 56, 198  
 Grid, 84  
 Grid ditching, 437  
 Gut epithelium, 40
- H**
- Haemocele, 26, 27  
 Haemolymph, 20  
 Haemorrhagic fever, 31, 35, 36, 310  
 Halophilous species, 9  
 Halteres, 67, 71  
 Hand-operated compression sprayer, 448  
 Harpagones. *See* Claspettes  
 Harpes. *See* Claspettes  
 Hatching, 9, 11, 13–16, 19, 24, 44, 52  
 Hatching in installments, 16  
 Hatching process, 13, 15  
 Hatching stimulus, 44  
 HBC. *See* Human bait catches  
 Head  
   adult, 23, 54, 63–67  
   capsule, 16, 18, 63, 64, 77, 78, 467  
   larva, 16–18, 53, 76–80  
 Heart failure, 40  
 Heartworm, 40, 203, 279, 333, 351, 365, 366, 394. *See also* Dog heartworm  
 Hepatitis, 36  
 Heterocyclic organophosphates, 455–457  
 Heteromorphic sex chromosomes, 56  
 Heterozygous, 474, 485  
 Hibernating, 24, 36, 39, 215, 224, 262  
 Hibernating shelters, 24  
 Hibernation, 19, 24, 167, 174–176, 179, 184, 191, 193, 248, 283, 285, 286, 288, 290, 294, 296, 297, 301, 303, 306, 312, 400, 411  
 Holoptic, 64  
 Homomorphic chromosomes, 56  
 Hoofprints, 400  
 Horizontal transmission, 30  
 Host odour, 21, 22, 47, 48  
 Host-seeking, 21–22, 47, 48  
 House martin, 409, 410  
 House mosquito, 278, 465, 472, 496  
 Human bait catches, 47, 501
- Humeral vein, 72  
 Humidity chamber, 54  
 Hydras, 405, 411, 423  
 Hydrophilic, 10, 478  
 Hydrophobic, 10, 17, 19, 478  
 Hydrostatic organs, 77  
 Hypnozoites, 27  
 Hypopharynx, 23, 66  
 Hypopygium, 20, 73–76  
 Hypostigmal area, 70, 71  
 Hypostigmal patch, 71  
 Hypostomal bridge, 65
- I**
- Ice granules, 424, 425  
 Imaginal disks, 19  
 Immune responses, 23, 37, 40, 487  
 Immunological repercussions, 489  
 Immunotoxin, 487  
 Implementation, 428, 430, 451, 471, 489, 499–509  
 Impregnated bed nets, 462, 464, 491–493  
 Impregnation, 460–462, 473, 491, 492  
 Incidence, 10, 28, 41, 170, 177, 198, 277, 442, 465, 492, 502  
 Indigenous malaria, 29  
 Indigenous population, 483, 484  
 Indoor catches, 47–48  
 Inert ingredients, 495  
 Infectious bites, 492, 495, 503  
 Influenza-like symptoms, 33, 38  
 Information dissemination, 439  
 Inner clypeal setae, 78, 79  
 Inner division of aedeagus, 76  
 Inoculation, 33, 405, 412, 414, 416, 502  
 Insect growth regulators (IGRs), 45, 59, 443, 445, 446, 457, 466–470, 480, 499  
 Insecticide  
   application techniques, 448–449, 472, 504, 506  
   containers–disposal, 508  
   droplet size, 447, 449, 453  
   formulations, 446–448, 497  
   label requirements, 508  
   registration, 507–508  
   resistance, 30, 41, 431, 451, 458, 471–475  
   insecticide-treated bednets (ITNs), 28, 430, 473, 489, 491, 492  
 Insemination, 20  
 Instar sensitivity, 426  
 Integrated biological control (IBC), 427  
 Integrated control strategy, 506  
 Integrated mosquito management (IMM), xxix, xxx, 406, 408, 422, 430, 442, 460, 471–473, 501–502  
 Integrated vector management (IVM), xxix, xxx, 430, 442, 463, 471, 473, 477, 479, 499, 501–509  
 Integration of mosquito control measures, 499–509  
 Internal seta, 75  
 International toxic units (ITU), 59, 423, 425, 427, 428, 505  
 Interocular space, 65  
 Interstrain fertility, 485  
 Intervention areas, 492, 501

Intracellular symbiont, 485, 488  
 Intrinsic muscles, 63, 66, 71  
 Inundation, 175, 193, 215, 231, 267, 277, 405, 406, 429, 469  
 Inversions, 57, 453  
 Invertebrate predators, 410–415  
 Iridescent virus, 431  
 Irradiation, 485  
 Isogametes, 416

## J

Japanese B encephalitis, 334, 367  
 Japanese encephalitis (JE), 34, 227, 279, 333, 334, 350, 437  
 Johnston's organ, 20, 63  
 Juvenile hormone, 441, 443, 466, 468–469  
 Juvenile hormone analogs (JHAs), 466, 468–469  
 Juvenoids. *See* Juvenile hormone analogs

## K

Kairomones, 48, 49, 462  
 Karyotype, 56, 57  
 Katepimeron, 70  
 Kerosene emulsion, 446, 454  
 Knock-down effect, 418, 423, 449, 460, 461  
 Knock-down resistance (kdr), 461  
 Knot-like dilatation. *See* Dilatation of the ovarial epithelium

## L

Labella, 23, 66  
 Labium  
   adult, 23, 63, 66  
   larva, 40, 79  
 Labral brushes, 76, 141, 190, 194. *See also* Lateral palatal brushes  
 Labroepipharynx, 23, 65, 66  
 Labro-palatum. *See* Labroepipharynx  
 Labrum, 66, 79  
 Labyrinth fish, 408  
 Lacewings. *See* in Taxonomic Index  
 Lactic acid, 21, 22, 48, 57, 494  
 Lambda-cyhalothrin, 446, 460, 465, 493  
 Landing rate counts. *See* Human bait catches  
 Larvae  
   biology, 9–11, 13–19, 24  
   key, 135–162  
   morphology, 76–84  
   preserving, 53  
   rearing, 52, 77, 484  
   sampling, 46–47  
 Larval food, 17  
 Larval indices, 436, 439  
 Larval siphon, 81–83  
 Larval stage, 19, 20, 24, 40, 46, 85, 413, 428, 468  
 Larviciding, 444, 446, 447, 449, 499, 501, 504  
 Laser leveling, 433  
 Lateral aedeagal plates, 75, 76  
 Lateral arms (outer division), 76

Lateral branched seta, 78  
 Lateral lobes, 85  
 Lateral palatal brushes, 17, 76, 79  
 Lateral plate, 82  
 Lateral stripes, 70  
 Lateral teeth. *See* Lateral arm  
 Leaflets of aedeagus, 75  
 Leaflets of palmate seta, 82  
 Lecithin, 478  
 Leeches (Hirudinea), 23  
 Legs, 10, 20, 40, 48, 54, 63, 67, 71, 85  
 Lemon eucalyptus *See* in Taxonomic Index  
 Light trap, 46, 49, 50, 217, 430, 502  
 Ligula, 66  
 Linkage maps. *See* Genetic maps  
 Liparol, 478  
 Liquid vapourizer, 495–497  
 Longevity, 25, 29, 60, 418, 497  
 Longevity estimation, 56  
 Long term control, 421, 456, 469  
 Long-term reduction, 434  
 Lower anal setae, 81, 83  
 Lower mesepimeral setae, 71  
 Lower mesepisternal setae, 71  
 Lower postpronotal patch, 71  
 Lucky bamboo (*Dracaena* sp.), 9, 203  
 Lymphatic filariasis. *See* Filariasis

## M

MacGregor's solution, 53  
 Malaria  
   quartan, 28  
   tertiana, 28  
   tropica, 28  
 Malathion, 444, 446, 449, 452–453, 455, 456, 462–464, 471, 499, 507  
 Malayan filariasis, 279, 332  
 Malignant malaria, 28  
 Malpighian tubes, 40  
 Management and monitoring of insecticide resistance, 471–475  
 Management techniques, 430, 438, 499  
 Mandibles  
   adult, 23, 66  
   larva, 79  
 Mandibular stylets, 66  
 Mapping of the breeding sites, 503–504  
 Mark-recapture, 51  
 Mass drug administration, 481  
 Mass media, 438, 439  
 Mass rearing, 410, 411, 416, 419, 484–486  
 Mating, 7, 20, 39, 52, 72, 73, 483–485  
 Matronae, 20  
 Maxillae  
   adult, 23, 66  
   larva, 79  
 Maxillary laciniae, 66  
 Maxillary palps, 63, 66, 67, 79  
 Maxillary stylets, 23, 66  
 Mayflies. *See* Ephemeroptera in Taxonomic Index  
 Median caudal lobe, 85



- Median keel, 85  
Median membrane, 73  
Median spine, 71  
Median stripe, 70  
Medical importance, 25–42, 166, 168, 170, 176–178, 180, 182, 183, 185, 186, 196, 201, 203, 211, 215, 218, 227, 229, 242, 267, 271, 275, 277, 279, 280, 282, 296, 302, 310, 312, 315, 323, 325, 326, 328, 330, 332, 340–346, 347–350, 357, 359, 360, 362, 364–367, 374–379, 381–383, 385, 386, 394, 397, 398, 400, 401  
Medio-cubital vein, 72  
Meiotic drive genes, 442, 488  
Melanization, 486  
Mendelian inheritance, 487  
Meningoencephalitis, 36  
Mentum, 79, 277  
Mermithid parasites, 415  
Meron, 70  
Merozoites, 27, 28  
Mesepimeral patch, 71  
Mesepimeron, 70, 71  
Mesepisternal patch, 71  
Mesepisternum, 70, 71  
Mesh cage, 52, 60  
Mesomeron, 70  
Mesonotum, 68  
Mesopleural suture, 70  
Mesopleuron, 67, 70  
Mesosternum, 70, 71  
Mesothoracic spiracles, 68, 70, 85  
Mesothorax, 67, 80, 85  
Mesotrochantin, 70  
Metabolic disruptors, 441, 466  
Metameron, 70  
Metamorphosis, 19, 468, 470  
Metanotum, 67, 69  
Metapneustic, 82  
Metathoracic spiracles, 70, 71  
Metathorax, 67, 70, 80  
Metepimeron, 70  
Metepisternum, 70  
Methane, 10, 45  
Methoprene, 446, 457, 466, 468, 469–470, 507  
Metofluthrin, 465–466, 496, 497  
Microarray platforms, 486  
Microbial control agents, 416, 418, 422, 426–431, 499, 500, 505, 507, 508  
Microbial larvicides, 59, 470  
Microbial pathogens, 406  
Microclimatic factors, 16  
Micro-encapsulated products, 448  
Microfilariae, 39, 40  
Microfilarial infection, 481  
Microsatellite markers, 486  
Microvilli, 27, 420  
Midges. *See* Chironomidae in Taxonomic Index  
Midgut cells, 420, 424, 426  
Midgut epithelium, 26, 420  
Midrib, 85, 87  
Migrating birds, 36  
Migration, 21, 22, 30, 43, 57, 191, 193, 196, 224, 259, 501, 505  
Migration distances. *See* Flight range  
Minimum effective dosage, 505  
Mist blower, 423, 448  
Mites, 411–412, 471  
Mode of action (insecticide), 451–452, 458, 460–461, 467–468, 470, 475  
Modification of breeding habitats, 500  
Modification of the air-water interface, 478  
Molecular genetics, 500  
Molecular methods, 4, 57–58  
Molting hormone, 18  
Monitoring, 41, 49, 58, 429–430, 451, 471–475, 501–503, 508, 509  
Monocyclic. *See* Univoltine  
Monogamous, 483, 484  
Monomolecular surface films (MSF), 478–480  
Monomorphic, 57  
Monophyletic, 3, 4  
Mosquito  
    abundance, 429, 454  
    breeder, 47, 52  
    coils, 466, 495, 496  
    fish (*Gambusia affinis*), 405, 406  
    magnet, 50, 51, 481  
    trap, 49–51, 429, 481  
Mosquito-borne diseases, 34, 41–43, 315, 408, 442, 454, 460, 465, 491, 494  
Mosquitocidal activity, 422  
Mosquitocidal bacteria, 419  
Mosquito-roofing, 47, 48, 323, 433, 435  
Moult, 441, 466–468  
Moult inhibitors, 441, 466  
Mounting, 53  
Mouth parts  
    adult, 22, 23, 63, 66  
    larva, 16–18, 40, 77, 79  
Multiple resistance, 471, 472  
Multipolymer sustained-release, 494  
Multivoltine, 14, 383, 392  
Murray Valley fever, 362  
Mutagenic, 485  
Mutations, 485  
Myocarditis, 36  
Mysids (Crustacea), 455  
Myxomatosis, 366
- N**  
Naled, 446, 449, 453–454  
Neck. *See* Cervix  
Neem oil, 494  
Net impregnation, 462, 491  
Netting, 47, 50, 52, 495, 507  
Nocturnal, 39, 301, 310, 406, 410  
Non-oriented dispersal, 21, 22  
Nontarget organisms, 420–422, 431, 442, 468, 469, 479, 499, 505  
Notched organ, 17, 163  
Notum, 67  
Novaluron, 466, 467  
Novel insecticide classes, 470–471

- Nuclear polyhedrosis virus, 431  
Nulliparity, 56  
Nutrition, 23, 191, 426  
Nuvan. *See* Dichlorvos
- O**  
Occipital foramen, 79  
Occiput  
  adult, 65  
  larvae, 79  
Octenol, 21, 51, 481  
Olfactory stimuli, 21, 48  
Oocyst, 26, 27, 29, 487  
Oocytes, 55, 56  
Oogenesis, 21  
Ookinete, 26, 27, 486  
Oospores, 417, 418  
Operational maps, 504  
Optimum effective dosage, 505  
Oral toxicity, 445, 491, 500  
Orcein, 57  
Organic phosphorous. *See* Organophosphates  
Organochlorines. *See* Chlorinated hydrocarbons  
Organophosphates, 58, 444, 452–458, 507  
Oriented host location behaviour, 21  
Ornamental pools, 400  
Ornithophilic species, 22, 24  
Ornithophily, 23  
Osmoregulation, 84  
K-Othrine, 464, 493  
Outdoor resting catches, 47  
Outer clypeal setae, 79  
Outer division of aedeagus, 76  
Ovarioles, 16, 55, 56  
Overwintering, 24, 31, 38, 176, 177, 233, 238, 250, 262, 276, 359, 392, 395  
Ovicidal effects, 468  
Oviposition  
  attractants, 45  
  behaviour, 12, 241, 246  
  oviposition traps (*See* Ovitrap)  
Ovipositor, 76  
Ovitrap, 45, 462
- P**  
Paddles, 19, 85–87  
Palatum, 65, 79  
Palmate setae, 19, 78, 82, 85  
Palpomeres, 66  
Palpi, 63, 66, 67, 79  
Parabasal setae, 75  
Paraffinic oils, 477  
Parameral apodemes, 74  
Paramere. *See* Gonocoxite  
Paraphyletic, 3  
Paraproct crown, 76  
Paraprocts, 73, 75, 76  
Parasites, 25–30, 40, 43, 55, 198, 208, 242, 290, 343, 405, 406, 415–419, 442, 486, 487, 500, 503  
Parasporal body (PSB), 419–421, 424  
Paratergite, 69  
Paratransgenic strategies, 487  
Parity, 56, 60  
Passive migration, 21  
Pathogens, 25, 26, 29, 31, 36, 41, 43, 55, 56, 198, 315, 405, 406, 416–431, 433, 441, 442, 483, 484, 486–488, 500  
PCR. *See* Polymerase chain reaction  
Pecten, 77, 81–83  
Pedicel, 20, 63  
Pellets and briquettes, 424, 448, 469  
Peptide, 487  
Permethrin, 444, 446, 448, 449, 457, 459, 460, 462, 464–465, 472, 473, 491–493  
Personal hygiene, 508  
Personal protection, 28, 60, 461, 462, 491–497  
Pesticide storage, 508  
Petroleum oils, 477  
Phagostimulants, 23  
Phallosome, 74  
Pharate, 19  
Pharyngeal pump, 23  
Phenetic clustering, 4  
Phenolic compounds, 21  
Phenotype, 57, 176  
Phenyl derivate, 454, 455  
Pheromones, 12, 20, 468  
Phosphoric acid esters. *See* Organophosphates  
Phosphorus esters. *See* Organophosphates  
Phosphorus insecticides. *See* Organophosphates  
Photoperiodic sensitivity, 9  
Phoxim, 456  
Phylogenetic trees, 3  
Physical colouration, 63  
Physical control, 477–481, 500  
Physiological stage, 21, 47, 55–56  
Piercing, 17, 22, 23, 63, 66, 83, 85  
Pigment colouration, 63  
Pink shrimp, 455  
Pinnate seta, 77, 78  
Pinning, 54  
Piperonyl butoxide (PBO), 460, 463, 465, 474  
Pirimiphos methyl, 444, 446, 449, 456–457, 472, 500, 507  
Pit latrines, 480  
Plankton net, 46  
Plasmid, 421  
Plesiomorphies, 3  
Pleurae (Pleurites), 67, 68, 70  
Pleural regions, 72  
Pleural suture, 70  
Plumose antennae, 20, 63  
Plumose seta. *See* Pinnate seta  
Polyacrylamide, 57  
Polyarthritis, 35  
Polycyclic. *See* Multivoltine  
Polycyclic aromatic hydrocarbons (PAHs), 496  
Polymerase chain reaction (PCR), 26, 58, 474  
Polymorphism, 57  
Polyporous, 54

- Polystyrene beads, 477–481, 500  
 Polytene chromosomes, 56, 57,  
   323, 344  
 Postacrostichal patch, 70  
 Postclypeal setae, 79  
 Postclypeus, 65  
 Posterolateral spiracular lobe, 81  
 Postgenital plate, 76  
 Postnotum, 69  
 Postocciput  
   adult, 65  
   larva, 79  
 Postprocoxal membrane, 70, 71  
 Postprocoxal patch, 71  
 Postpronotal setae, 69  
 Postpronotum, 67, 69–71  
 Postspiracular area, 70, 71  
 Postspiracular patch, 71  
 Postspiracular setae, 71  
 Preala, 70, 71  
 Precratal setae, 84  
 Predators, 11, 12, 15, 17, 20, 36, 405–416, 427, 430,  
   437, 442, 452, 460, 489, 503, 506  
 Prehensile setae, 79  
 Prementum, 66  
 Prescutellar area, 70  
 Prescutum, 67  
 Preserving mosquitoes, 53–55  
 Prespiracular area, 70  
 Prespiracular setae, 69  
 Pretarsus, 71  
 Primary health care concept, 427  
 Primordial compound eyes, 79, 80  
 Proboscis, 23, 39, 63–67  
 Procoxa, 70, 71  
 Proctiger, 73–75, 85  
 Progeny, 485, 500  
 Propleuron, 70, 71  
 Propoxur, 444, 446, 449, 456, 458–459,  
   484, 507  
 Prosternum, 70, 71  
 Protein crystal, 419, 420  
 Protein-electrophoresis, 57, 275  
 Prothorax, 17, 67, 70, 80  
 Public health, 29, 35, 37, 315, 422, 439,  
   442, 451, 453, 455, 458, 459, 462–464,  
   471, 483, 499  
 Public information systems, 509  
 Pulvilli, 71  
 Pupae  
   biology, 19  
   morphology, 84–87  
   preserving, 53  
   sampling, 46–47  
 Pupal trumpet, 20, 85, 478  
 Pyrethrins, 48, 446, 459, 460, 463, 474  
 Pyrethroids, 58, 441, 443–445, 449–451, 456,  
   459–466, 470, 471–474, 491–496, 507  
 Pyrethrum, 459, 473, 494  
 Pyrethrum flowers, 459  
 Pyriproxyfen, 446, 466, 470
- Q**  
 Quartan malaria, 28  
 Qwenling, 494
- R**  
 Radial sector, 72  
 Radio-medial vein, 72  
 Radius, 72  
 Rash, 31–33, 35, 36  
 Rearing mosquitoes, 51–53  
 Rearing temperature, 52, 200  
 Receptors, 21, 22, 63, 420–422, 424, 426, 472  
 Recombination, 488  
 Recycling processes, 427  
 Refractoriness, 486–488  
 Refractory, 29, 483, 486–488, 500  
 Refugia, 474  
 Registration of insecticides, 507–508  
 Regulation, 31, 84, 196, 405, 434, 436, 439, 450, 460, 507, 508  
 Repellents, 58–61, 459, 465, 491, 493–497  
 Research techniques, 43–61  
 Residual spraying, 430, 444  
 Resin formulations, 465, 496  
 Resistance, 28, 30, 41, 58, 217, 405, 421, 424, 425, 429–431,  
   433, 442, 443, 450–452, 454–458, 460–466, 471–475,  
   477, 481, 483, 484, 486, 499, 502, 508  
   management, 472–475, 508  
   mechanisms, 472, 473, 475  
   surveillance, 472–474  
 Resmethrin, 446, 460, 463, 473  
 Respiratory trumpets, 19, 85, 86, 307, 401  
 Resting behaviour, 23, 381  
 Restriction maps, 56  
 R-genes, 472, 474  
 Rice production, 436, 437  
 Rift Valley fever, 37, 282, 317, 328, 362, 473  
 Risk assessment,  
 Rockholes, 168, 392  
 Roll Back Malaria (RBM), 28, 430  
 Roof gutters, 435  
 Ross River virus, 31, 32, 332, 351, 359, 360, 362, 364–366, 466  
 Rotenone,  
 Routine treatments, 427, 504, 505, 508–509
- S**  
 Saddle seta, 81, 83  
 Saliva, 23, 26, 66  
 Salivary channel. *See* Hypopharynx  
 Salivary glands, 26, 27, 56, 57, 344, 486  
 Salt marshes, 84, 217, 227, 251, 326, 365, 392, 394, 433, 437,  
   438, 479, 481  
 Saltwater method, 44  
 Sampling, 8, 43–54, 168, 315, 409, 428  
 Sandflies. *See* in Taxonomic Index  
 Sanitation, 29, 39, 436  
 Scape, 63, 64  
 Schizogonic cycle, 28  
 Schizogony, 27, 28  
 Schizont, 27, 28

- Sclerotised plate, 78, 82, 83  
 Screening, 491  
 Scutal angle, 68, 69  
 Scutal fossa, 68  
 Scutellar setae, 69  
 Scutellum, 67, 69  
 Scutum, 54, 63, 67–69, 85  
 Selection of appropriate tools, 501, 504  
 Sensilla, 23, 66  
 Sentinel chickens, 33  
 Sentinel rabbits, 33  
 Serosal cuticle, 11  
 Serotypes, 32, 34, 35  
 Setae, 7, 17, 19, 53, 56, 63, 69–71, 73, 75–80, 82–86  
 Sewage systems, 434  
 Sewage treatment, 435  
 Sexual dimorphism, 484  
 Sexual maturity, 20, 415  
 Sibling species, 7, 8, 25, 26, 56–57, 166, 170, 171, 191, 216, 219, 220, 222, 224, 231, 240, 275, 323, 325, 347  
 Sidepiece. *See* Gonocoxite  
 Sigmoid setae, 79  
 Silicon dioxide, 469  
 Siphon, 10, 16, 17, 76, 77, 82, 83, 85  
 Siphonal index, 82  
 Siphonal setae, 83  
 Slow release systems, 447–448  
 Snow-melt mosquitoes  
   dispersal, 21  
   hatching, 13, 14  
   hibernation, 24  
   larval development, 18  
 Socio-economic aspects, 439  
 Soil samples, 16, 44  
 Soluble powders (SP), 447  
 Solution concentrates (SC), 446, 447  
 Source reduction, 433–436, 462, 477, 507  
 Southern house mosquito. *See* *Culex pipiens quinquefasciatus*  
 Space spraying, 444, 499  
 Spatial analysis, 504  
 Spermatheca, 20, 164, 187, 484  
 Spermatozoa, 20  
 Spicules, 79, 85  
 Spiders. *See* in Taxonomic Index  
 Spinosad, 419  
 Spiracular lobes, 16, 17, 81, 83  
 Spiracular plate, 82, 83  
 Spiracular teeth, 83  
 Sporogony, 26, 28  
 Sporozoite infection rate, 492  
 Sporozoites, 26, 27, 326, 486, 492, 495  
 Springtails. *See* in Taxonomic Index  
 Standard pint dipper, 46  
 Stellate seta, 78  
 Stemmata, 79, 85  
 Stenogamy, 20, 171, 173, 277  
 Sterile hybrids, 442  
 Sterile insect technique (SIT), 483–486  
 Sterile males, 483–485, 500  
 Sternum (Sternites), 67, 71–73, 85  
 Stomach poisons, 443, 444, 458, 480  
 Striders, 20, 478  
 Stripes, 63, 70, 71, 76  
 Stylet bundle, 23  
 Stylets, 23, 63, 65, 66  
 Subapical lobe, 73  
 Subbasal tooth, 71  
 Subcosta (Sc), 72  
 Subcostal-radial vein, 72  
 Submedian stripes, 70  
 Subspiracular area, 70, 71  
 Subspiracular patch, 71  
 Supraalar setae, 69  
 Surface films, 478–481  
 Surfactants, 477, 478  
 Suspension feeders. *See* Filter feeders  
 Sustained-release formulation, 494  
 Swarming, 20, 50, 171, 173, 174, 219, 233, 246, 250, 277, 310  
 Sylvatic yellow fever, 382  
 Sympatric, 168, 170, 176, 218, 486  
 Synapomorphies, 3  
 Synergists, 449, 459, 463, 472, 474  
 Synthetic repellents, 494
- T**
- Tablets, 424, 425, 429, 447–448, 468, 507  
 Tadpole shrimp. *See* *Triops cancriformis* in Taxonomic Index  
 Tarsomeres, 71  
 Tarsus, 71  
 Taxonomic techniques, 56–58  
 Technical concentrates (TC/TP), 446  
 Temephos, 444, 446, 455, 457–458, 471, 472, 500  
 Terga, 63, 73, 85  
 Tergal plate, 81, 82  
 Tergum (Tergites), 67, 72, 73, 75, 85. *See also* notum  
 Terminal spine. *See* Median spine  
 Tertian malaria, 28  
 Thermal foggers, 448, 449  
 Thermal stimuli, 21  
 Thoracic setae, 80  
 Thorax  
   adult, 67–72  
   larva, 80  
 Threshold concentration, 472  
 Threshold for vector mosquitoes, 502–503  
 Threshold infestation level, 502  
 Tibia, 71  
 Timing and frequency of treatments, 508  
 Toxicity by hazard, 445  
 Toxin genes, 421  
 Toxin proteins, 420, 424  
 Tracheal trunk, 17, 81, 85  
 Tracheoles, 56  
 Training, 28, 428, 433, 492, 501, 504, 506  
 Transformation, 9, 57, 433, 468, 487, 500  
 Transgenic, 487, 489  
 Transgenic strain, 485, 487  
 Translocations, 57, 442, 500  
 Transmission cycle, 26, 32–34, 37  
 Transovarial transmission, 30–32, 35, 36  
 Transposons, 487

- Transverse suture, 67, 68  
Traps, 21, 22, 45–51, 55, 217, 301, 387, 409, 429, 430, 436, 462, 481, 500–502  
Tree-holes, 9, 24, 29, 38, 43, 45, 46, 165, 179, 180, 197, 200, 202, 204, 206–208, 214, 248, 277, 301, 305, 310–312, 357, 381, 436, 469  
Triflumuron, 466  
Trochanter, 71  
Trochantin, 70  
Trophozoite, 28  
Trumpets. *See* Respiratory trumpets  
Tubercle, 68, 75, 80  
Tuft, 78, 83, 84  
Tularaemia. *See* *Francisella tularensis* in Taxonomic Index  
Tyres, 41, 42, 469
- U**  
Ultra-low-volume formulations (ULV), 60, 422, 434, 446–449, 453, 454, 456, 459, 463, 464, 508  
Ungues. *See* Claws  
Univoltine, 13, 14  
Upper anal setae, 81, 83  
Upper mesepimeral setae, 71  
Upper mesepisternal setae, 71  
Upper postpronotal patch, 71  
Use of personal protective equipment (PPE), 508
- V**  
Vapona. *See* Dichlorvos  
Vapourizing mats, 496  
Vapourizing strips, 447, 454  
Vector  
    vector-borne diseases, 25, 42, 442, 443, 451, 458, 466, 483, 488, 491  
    vector capacity, 4, 25, 55, 501  
    vector management, 28, 427–430, 433, 442, 443, 465, 489  
Veins. *See* Wing veins  
Venation, 72  
Ventral arm of the paraproct, 76  
Ventral brush, 78, 84  
Vertebrate predators, 406–410  
Vertex, 65
- Vertical dispersal, 22  
Vertical transmission, 30  
Viraemia, 30  
Virus amplification, 36  
Visual stimuli, 21  
Vitellogenines, 19  
Volatile organic compounds (VOCs), 496  
Volume median diameter (VMD), 453
- W**  
Water beetles. *See* Coleoptera in Taxonomic Index  
Water bugs. *See* Heteroptera in Taxonomic Index  
Water dispersible powders (WDP/WP), 447  
Water fern, 437  
Water hyacinth, 386, 435  
Water management, 174, 430, 437, 438, 477, 500  
Water mites (Hydrachnellae), 412  
Water storage, 36, 41, 433–436, 458, 468  
Water surface tension, 478  
Western Pacific Region, 35, 39, 443, 466, 495  
West Nile fever, 36, 37  
Wetland, 351, 434, 436–438, 499, 500, 504  
WHOPES. *See* WHO Pesticide Evaluation Scheme  
WHO Pesticide Evaluation Scheme, 422, 443, 444, 448, 451, 460, 463, 466, 471, 473, 505  
WHO test kits, 472  
Wind velocities, 21  
Wing beat, 20  
Wing spots in *Anopheles*, 72  
Wing veins, 63, 72  
Woodland species, 21  
Woodlice. *See* Isopoda in Taxonomic Index
- Y**  
Yellow fever, 31, 34, 47, 188, 201, 317, 382, 442  
Yolk synthesis, 468
- Z**  
Zoophily, 24, 49, 173, 182  
Zoospores, 416, 417  
Zygopteran nymphs, 412

# Taxonomic Index

## A

- Abraedes* genus, 6
- Abraedes* subgenus, 6
- Abramis brama*, 16
- Acalleomyia* subgenus, 5
- Acallyntrum* subgenus, 5
- Acanthocyclops vernalis*, 412, 416
- Acari, 422, 423
- Acartomyia* genus, 6
- Acartomyia mariae*. *See Ochlerotatus mariae*
- Acartomyia phoeniciae*. *See Ochlerotatus phoeniciae*
- Acartomyia zammitii*. *See Ochlerotatus zammitii*
- Actinotrix* subgenus, 7
- Aedeomyia* genus, 5, 79
- Aedeomyia* subgenus, 5
- Aedeomyiini tribus, 5
- Aedes aegypti*, 198–201
  - control, 407, 412, 414, 425, 431, 434–436, 438, 455, 457, 458, 461, 462, 468, 470–472, 485, 487, 488, 494–497, 503, 505, 507
  - identification, 97, 119, 142, 153, 321, 329, 333, 339, 351, 354, 369, 371, 373, 389, 390
  - medical importance, xxviii, 31, 32, 34–36
  - research, egg sampling, 45, 47, 55, 59
  - ssp. *formosus*, 201
  - var. *queenslandensis*, 201
- Aedes africanus*
  - medical importance, 34
- Aedes albocephalus*, 317
- Aedes albopictus*, 9, 201–203
  - control, 414, 435, 436, 438, 439, 454, 456–458, 462, 463, 465, 485, 488, 507
  - identification, 97, 119, 153, 154, 321, 333, 339, 351, 354, 369, 373, 387, 389, 390
  - medical importance, xiii, xxix, 31, 35, 41
  - research, egg sampling, 45
- Aedes argenteus*, 201
- Aedes bromeliae*
  - medical importance, 34
- Aedes calopus*, 201
- Aedes cinereus*, 189–191
  - biology
    - dispersal and host-seeking behaviour, 21, 22
    - feeding, 23
  - control, 494
  - identification, 101, 118, 137, 142
  - systematics, 188, 189
  - viruses carried by, 33, 38
- Aedes circumluteolus*, 317
- Aedes cooki*, 351
- Aedes cretinus*, 203–204
  - identification, 97, 119, 153
- Aedes dahuricus*, 189
- Aedes duplex*, 216
- Aedes esoensis*, 189, 192
- Aedes furcifer*, 317
- Aedes geminus*, 191–192
  - identification, 101, 118, 142
  - systematics, 189
- Aedes* genus, ix, x, xii, 187–188
  - biology, 9
    - dispersal and host-seeking behaviour, 21, 22
    - embryonic development, 13
    - hatching, 13–16
    - hibernation, 24
    - larvae, 17, 18
    - oviposition, 11
  - control, 408, 411–413, 415, 423, 426, 428, 430, 434, 437, 439, 454, 456, 457, 465, 468–470, 502, 503
  - identification, 91, 92, 95–106, 113, 118–128, 135, 136, 141–154, 318, 321, 334, 351–353, 370, 373, 388
  - medical importance, 31, 33, 35, 38–40
  - morphology, 71, 73–76, 79, 82
  - research
    - egg sampling, 44
    - rearing, 52
  - systematics, 4, 6, 7
- See also Aedes/Ochlerotatus*
- Aedes hebrideus*, 351
- Aedes hensilli*, 351
- Aedes luteocephalus*, 317
- Aedes melanimon*
  - medical importance, 37
- Aedes mubiensis*, 189
- Aedes/Ochlerotatus*
  - biology
    - dispersal and host-seeking behaviour, 21, 22
    - egg-laying behaviour, 9
    - embryonic development, 13
    - hatching, 13, 15–16

- Aedes/Ochlerotatus (cont.)*  
 hibernation, 24  
 larvae, 17  
 control, 408, 412, 413, 423, 426, 428, 430, |  
 454, 456, 457, 468–470, 502  
 identification, 92, 95–108, 113, 118–128, 136, 141–154  
 medical importance, ix, 33, 40  
 morphology, 73–75, 79  
 research  
 egg sampling, 44–45  
 rearing in laboratory, 52
- Aedes opok*, 317
- Aedes polynesiensis*, 32, 351, 412
- Aedes rossicus*, 193–194  
 biology, dispersal and host-seeking behaviour, 22  
 identification, 95, 101, 114, 118, 142  
 systematics, 188, 189
- Aedes rotumae*, 351
- Aedes sasai* systematics, 189
- Aedes scutellaris*, 351
- Aedes* subgenus, 6, 67, 76, 188, 321, 339, 353, 372, 389
- Aedes taylori*, 317
- Aedes vexans*, 194–196  
 biology  
 dispersal and host-seeking behaviour, 21  
 egg-laying behaviour, 10–12  
 embryonic development, 13  
 hatching, 13–16  
 larvae, 17–19  
 control  
 resistance, 424  
 sensitivity to *Bacillus*, 426  
 identification, 91, 95, 97, 98, 101, 118, 119, 136, 141, 142,  
 333, 387, 389  
 medical importance, xxvii, 32, 38  
 research, egg sampling, 44  
 ssp. *arabiensis*, 196  
 ssp. *nipponii*, 196  
 ssp. *vexans*, 196
- Aedes vittatus*, 196–198  
 identification, 97, 114, 119, 142, 153, 154  
 medical importance, 34  
 morphology, larva, 97
- Aedes yamadai* systematics, 189
- Aedimorphus* genus, 6
- Aedimorphus* subgenus, 6, 76, 188, 194, 196
- Aedimorphus vexans*. See *Aedes vexans*
- Aedini tribus*
- Aedinus* subgenus, 5
- Aeshna cyanea*, 411, 412
- Afrocullex* subgenus, 5
- Afrorhynchus* subgenus, 5
- Agabus* spp., 413
- Aglaonotus* subgenus, 6
- Alanstonea* genus, 6
- Alanstonea* subgenus, 6
- Albuginosus* genus, 6
- Albuginosus* subgenus, 6
- Alburnus alburnus*, 408
- Allimanta* subgenus, 5
- Alloeomyia* subgenus, 6
- Allotheobaldia* subgenus, 5, 289
- Alphavirus*, 31, 208, 229
- Amblyospora*, 418, 419
- Amphibia, 32, 285, 286, 288, 298, 314, 406, 408–409, 422,  
 423, 429, 430
- Amphicorisa, 412
- Anabaena*, 500
- Anas platyrhynchos*, 409
- Anisopodidae systematics, 3
- Ankylorhynchus* subgenus, 5
- Annelida, 422, 423
- Annulipes Group, 209–211, 214
- Anoedioporpa* subgenus, 5
- Anopheles aconitus*, 333
- Anopheles albimanus*, 376–377  
 identification, 370  
 medical importance, 30
- Anopheles albitarsis*  
 medical importance, 30
- Anopheles algeriensis*, 165–166  
 identification, 93, 115, 116, 137, 138  
 medical importance, 29  
 morphology, larvae, 79
- Anopheles amictus*  
 medical importance, 30
- Anopheles annulipes s.l.*, 351, 362
- Anopheles aquasalis*, 377–378  
 identification, 370, 371  
 medical importance, 30
- Anopheles arabiensis*, 325  
 medical importance, 25, 30
- Anopheles atroparvus*, 173  
 control, 454  
 identification, eggs, 171, 172  
 medical importance, 29
- Anopheles aztecus*, 177, 369
- Anopheles baimaii*, 344
- Anopheles bancroftii*  
 medical importance, 30
- Anopheles barberi*, 180
- Anopheles barianensis*, 180
- Anopheles beklemishevi*, 173–174  
 identification, eggs, 171, 172
- Anopheles bellator*, 369
- Anopheles bifurcatus* systematics, 168
- Anopheles bwambae*, 323, 326
- Anopheles calderoni*, 374  
 identification, 371
- Anopheles cinereus*, 180–182
- Anopheles cinereus hispaniola*  
 identification, 95, 117, 140  
 medical importance, 29
- Anopheles Claviger Complex, 82, 166, 168
- Anopheles claviger s.s.*, 166–168  
 identification, 94, 116, 138, 139  
 medical importance, 168  
 morphology, 85
- Anopheles cracens*, 344
- Anopheles crucians*, 387
- Anopheles cruzii*, 369
- Anopheles culicifacies*, 341–342  
 identification, 336  
 medical importance, 30

- Anopheles darlingi*, 378–379  
identification, 370  
medical importance, 30
- Anopheles dirus*, 342–344  
identification, 11, 12  
medical importance, 30
- Anopheles Dirus Complex systematics, 344
- Anopheles earlei*, 177
- Anopheles elegans*, 344
- Anopheles farauti*, 356–357  
identification, 351  
medical importance, 30
- Anopheles flavirostris*, 344  
identification, 337
- Anopheles fluviatilis*, 344  
identification, 336, 337  
medical importance, 30
- Anopheles freeborni*  
medical importance, 29
- Anopheles funestus*, 322–323  
identification, 318, 319  
medical importance, 30, 33
- Anopheles Gambiae Complex identification,  
25, 30, 323
- Anopheles gambiae s.s.*, 324–325  
medical importance, 25, 30, 33
- Anopheles* genus, 164–165  
biology  
hibernation, 24  
larvae, 9, 17  
oviposition, 10  
control, 408, 414, 424, 427, 428, 434, 435, 456, 457, 459,  
469, 473, 491, 494, 506, 507  
identification, 91–95, 113, 115–117, 135, 137–140, 318,  
334, 351, 352, 369, 370, 388  
medical importance, vi, vii, 25, 26, 33, 38–40  
morphology, 63, 66, 69, 72–76, 78, 79, 85  
personal protection methods, 491, 494  
research, egg sampling, 43
- Anopheles hilli*, 30
- Anopheles hyrcanus*, 169–170  
attitude at rest, 163  
identification, 94, 116, 117, 139, 334  
personal protection methods, 494  
var. *pseudopictus*, 94, 169
- Anopheles hyrcanus* group, 334
- Anopheles koliensis* systematics, 357
- Anopheles labranchiae*, 174  
identification, eggs, 171, 172
- Anopheles lesteri*, 340  
identification, 334, 335
- Anopheles maculatus*, 345–346  
identification, 335
- Anopheles Maculipennis Complex  
eggs, 52, 171, 172  
identification, 93, 116, 139  
medical importance, 29
- Anopheles maculipennis s.l.* See Anopheles Maculipennis  
Complex
- Anopheles maculipennis s.s.*, 174  
control, 409  
identification, eggs, 171, 172
- Anopheles marteri*, 177–178  
identification, 93, 116, 117, 138, 139  
var. *conquensis*, 178
- Anopheles martinius*, 177
- Anopheles melanoon*, 175  
identification, eggs, 171, 172
- Anopheles melas*, 10, 324, 326
- Anopheles merus*, 317, 323, 326
- Anopheles messeae*, 175  
identification, eggs, 171, 172
- Anopheles minimus*, 346–347  
identification, 335–337  
medical importance, 30
- Anopheles Minimus Complex  
systematics, 347
- Anopheles multicolor*, 182–183  
identification, 94, 95, 117, 140, 334, 336
- Anopheles nemophilous*, 344
- Anopheles nigerrimus*, 170
- Anopheles nuneztovari*, 380–381  
identification, 370, 371
- Anopheles occidentalis*, 177
- Anopheles paeditaeniatus*, 170
- Anopheles petragani*, 168  
identification, 94, 116, 139  
morphology, pupae, 85
- Anopheles pharoensis*, 326–328  
identification, 318  
medical importance, 30
- Anopheles plumbeus*, 178–180  
biology, hibernation, 24  
identification, 93, 94, 115, 116, 137  
medical importance, 29  
morphology, larvae, 79
- Anopheles pseudopictus*, 170
- Anopheles pseudopunctipennis*, 374–375  
identification, 370, 371
- Anopheles pulcherrimus*, 268, 333
- Anopheles punctimacula*, 375–376  
identification, 371
- Anopheles punctipennis*, 387
- Anopheles punctulatus*, 357
- Anopheles Punctulatus Complex  
systematics, 357
- Anopheles quadriannulatus s.l.*, 325
- Anopheles quadrimaculatus*, 387  
medical importance, 29
- Anopheles sacharovi*, 175–176  
control, 456  
identification, 93, 171–172  
medical importance, 30
- Anopheles scanloni*, 344
- Anopheles sergentii*, 183–185  
identification, 94, 95, 117, 140  
medical importance, 29
- Anopheles sicaulti*, 177
- Anopheles sinensis*, 340–341  
identification, 334, 335  
medical importance, 30
- Anopheles sogdianus*, 178
- Anopheles stephensi*, 347–348  
identification, 335, 336



- Anopheles subalpinus*, 176–177  
 identification, eggs, 171, 172  
 medical importance, 177
- Anopheles* subgenus, 5, 163–165  
 identification, males, 115
- Anopheles sundaicus*, 348–349  
 identification, 335, 336  
 medical importance, 30
- Anopheles superpictus*, 185–186  
 identification, 94, 95, 116, 117, 138, 140  
 medical importance, 29, 30
- Anopheles takasagoensis*, 344
- Anophelinae subfamily  
 biology, egg-laying behaviour, 10  
 morphology  
 adult, 63  
 larvae, 81  
 pupae, 86
- Antunesmyia* subgenus, 5
- Araneae, 409
- Argyroneta aquatica*, 411, 412
- Armigeres annulipalpis*, 333
- Armigeres aurolineatus*, 333
- Armigeres flavus*, 333
- Armigeres* genus, 6, 65, 66, 83, 333, 338, 414
- Armigeres malayi*, 333
- Armigeres subalbatus*, 333
- Armigeres* subgenus, 6
- Austromansonia* subgenus, 5, 307
- Austrotheobaldia* subgenus, 5
- Aves, 409
- Ayurakitia* genus, 6
- Azolla*, 500
- Aztecaedes* genus, 6
- Aztecaedes* subgenus, 6
- B**
- Babanki virus, 33
- Bacillus sphaericus* (*B.s.*), 59, 406, 421–422, 424–426,  
 428–430, 500, 507
- Bacillus thuringiensis israelensis* (*B.t.i.*), 406, 407, 418–422,  
 424–430, 480, 506, 507
- Bacillus thuringiensis kurstaki*, 424
- Baimaia* subgenus, 5, 164
- Barraudius* subgenus, 5, 264, 265
- Batai virus, 38–39
- Beauveria*, 418
- Belkinius* genus, 6
- Belkinius* subgenus, 6
- Belkinomyia* subgenus, 5
- Bibionomorpha systematics, 3
- Bifidistylus* genus, 6
- Bironella* genus, 5, 76, 78, 163
- Bironella* subgenus, 5, 67, 72
- Blastocladales, 416
- Blephariceromorpha systematics, 3
- Bohartius* subgenus, 7
- Bombina bombina*, 409
- Borichinda* genus, 6
- Bothaella* genus, 6
- Bothaella* subgenus, 6
- Bothromesostoma*, 411
- Brachycera systematics, 3
- Bradysia*, 423
- Bruceharrisonius* genus, 6
- Brugella* subgenus, 5
- Brugia malayi*, 39, 279, 332–334, 341, 367
- Brugia timori*, 39
- Bufo bufo*, 409, 423
- Bunyamwera virus, 38
- Bunyaviridae* (*Bunyavirus*), 37–39
- Buvirilia* subgenus, 6
- C**
- Cache Valley virus, 37, 39
- Caenomyiella* subgenus, 5
- California Encephalitis (CE) virus, 37, 196,  
 227, 400
- Calla*, 401
- Calovo virus. *See* Batai virus
- Cancraedes* genus, 6
- Cancraedes* subgenus, 6
- Carassius carassius*, 408
- Carex*, 230, 246, 262, 309, 401
- Carrollia* subgenus, 5
- Caspian Group, 209
- Catageomyia* genus, 6
- Catatassomyia* genus, 6
- Cellia* subgenus  
 identification, males, 180
- Ceratopogonidae, 3, 38, 415, 423, 453
- Chaetocruimyia* subgenus, 6
- Chagasia* genus, 5, 69, 76, 78, 163
- Chaoboridae  
 morphology, pupae, 87
- Chaoborus*, 415, 423
- Chikungunya virus, 31–33
- Chironomidae, 409, 410, 429  
 morphology, 63, 87
- Chiroptera (bats), 409–410
- Chlorohydra viridissima*, 411
- Christophersomyia* genus, 6
- Christophersomyia* subgenus, 6
- Chrysanthemum*, 459
- Chrysoconops* subgenus, 6
- Chytridiales, 416, 417
- Climacura* subgenus, 5
- Cloeon dipterum*, 423
- Cnidaria, 422, 423, 429
- Coelambus impressopunctatus*, 413, 423
- Coelenterata, 411
- Coelomomyces psorophorae*, 416, 417
- Coelomomyces* spp., 416
- Coenagrion puella*, 412
- Coleoptera, 409–411, 413–414, 422, 423, 457
- Collembola, 409, 457
- Collessius* genus, 6
- Collessius* subgenus, 6
- Colymbetes fuscus*, 414
- Colymbetinae, 413, 414
- Communis Group, 209
- Conopostegus* subgenus, 6

- Coquillettidia buxtoni*, 307–308  
 identification, 111, 134, 162
- Coquillettidia* genus  
 biology  
   egg-laying behaviour, 10  
   embryonic development, 13  
   emergence, 20  
   larvae, 17  
 control, 428, 469  
 identification, 92, 111, 114, 134, 135, 162,  
 352, 353, 390  
 morphology, 82, 83, 85, 86
- Coquillettidia linealis*, 351
- Coquillettidia perturbans*, 400–401  
 identification, 387, 390  
 medical importance, 32
- Coquillettidia richiardii*, 308–310  
 biology, hibernation, 24  
 identification, 111, 134, 135, 162  
 viruses carried by, 39
- Coquillettidia* subgenus, 5, 307, 469, 479
- Coquillettidia xanthogaster*, 367–368  
 identification, 352
- Corethrellidae systematics, 3
- Corixa punctata*, 412
- Corixidae, 412
- Cornetius* genus, 6
- Crustacea, 405, 406, 411, 412, 422, 423
- Cruzmyia* subgenus, 5
- Ctenogoeldia* subgenus, 5
- Ctenopharygodon idella*, 408
- Culex annulirostris*, 365–366  
 identification, 351–356
- Culex antennatus*, 328  
 identification, 318–322
- Culex apicalis*, 288
- Culex argenteus*. *See Aedes aegypti*
- Culex australicus*, 275
- Culex bifurcatus*. *See Anopheles claviger*  
 systematics, 168
- Culex brumpti*, 269–270  
 identification, 107, 129, 130, 154, 155, 158
- Culex decens*, 329
- Culex deserticola*, 282
- Culex duttoni*, 317
- Culex erythrothorax*, 387
- Culex fasciatus*. *See Aedes aegypti*
- Culex gelidus*, 334
- Culex* genus  
 biology  
   egg-laying behaviour, 10–12  
   embryonic development, 13  
   hibernation, 24  
   larvae, 16–19  
 control, 408, 414, 419, 422, 428–430, 434, 435,  
 437, 454, 456, 457, 459, 463, 465, 469, 470,  
 479–481, 506  
 identification, 106–108, 113, 128–131, 136, 154–159  
 medical importance, 33, 36, 38–40  
 morphology, 106–108  
 research, egg sampling, 43, 45, 46
- Culex globocoxitus*, 275
- Culex hortensis*  
 identification, 108, 128, 129, 136, 154, 155, 166  
 morphology, larvae, 82
- Culex impudicus*  
 identification, 107, 131, 156
- Culex inatomii*, 265
- Culex laticinctus*, 270–271  
 identification, 107, 130, 157
- Culex martinii*, 285–286  
 identification, 107, 129, 131, 155
- Culex mimeticus*, 272–273  
 identification, 106, 107, 130, 154, 156, 157
- Culex modestus*, 265–267  
 control, 494  
 identification, 106, 107, 114, 129, 157  
 medical importance, 36, 38  
 personal protection methods, 494
- Culex neavei*, 317  
 identification, 320, 329, 330
- Culex nigripalpus*, 384–385, 387  
 identification, 373, 374
- Culex perexiguus*, 273–275  
 identification, 107, 108, 130, 154, 155, 158
- Culex Pipiens Complex*, 275
- Culex pipiens erectus*, 277
- Culex pipiens pallens*, 275, 277, 472
- Culex pipiens pipiens*, 275–277  
 biology  
   dispersal and host-seeking behaviour, 21–22  
   egg-laying behaviour, 10, 13  
   embryonic development, 13, 18, 19  
   hibernation, 24  
   larvae, 19, 21  
 control, 409, 421, 422, 424, 426–429, 431, 436, 470,  
 473, 481, 485, 488, 502, 505, 507  
 identification, 106–108, 130, 131, 135, 156, 158,  
 159, 168  
 morphology, larvae, 24  
 sensitivity to *Bacillus thuringiensis israelensis*, 426  
 viruses carried by, 277, 279, 280
- Culex pipiens pipiens* biotype *molestus*, 277–278  
 biology  
   feeding, 277, 278  
   hibernation, 280  
 control, 429
- Culex pipiens quinquefasciatus*, 278–279  
 control, 406, 422, 434, 457, 459, 465, 470, 473, 480, 485,  
 488, 496, 507  
 medical importance, xxvii, 36, 39
- Culex pipiens torridus*, 277
- Culex pusillus*, 267–268  
 identification, 107, 129, 156  
 control, 411, 414, 423
- Culex quasiguiarti*, 329
- Culex restuans*, 396–397  
 identification, 391
- Culex richeti*, 265
- Culex salinarius*, 397–398  
 identification, 391
- Culex simpsoni*, 329
- Culex sitiens*, 366, 367  
 identification, 319, 320, 334, 338, 353

- Culex* subgenus, 83, 187, 268–269, 372, 390
- Culex tarsalis*, 398–400  
 control, 406, 411, 461, 465, 468, 470  
 identification, 390, 391  
 medical importance, 32  
 research, blood meal identification, 55
- Culex territans*, 287–288  
 identification, 108, 130, 131, 154–158
- Culex theileri*, 280–282  
 identification, 107, 130, 156, 157
- Culex torrentium*, 279–280  
 identification, 107, 108, 131, 158, 159  
 medical importance, 33  
 morphology, 82
- Culex tritaeniorhynchus*, 349–350  
 control, 437  
 identification, 319, 320, 329, 333
- Culex univittatus*, 329–330  
 identification, 317, 320
- Culex vishnui*, 334, 457
- Culicada* subgenus, 6
- Culicella* subgenus  
 egg-laying behaviour, 295  
 morphology, larvae, 295–296  
 research, egg sampling, 292
- Culicelsa* subgenus, 6
- Culicidae, 3–5, 40, 63, 71, 87, 409, 414, 423, 429
- Culicinae subfamily, 187–314
- Culicini tribus, 5
- Culicinomyces*, 418
- Culicinomyces clavissporus*, 418
- Culiciomyia* subgenus, 5
- Culicoides*  
 medical importance, 38
- Culicomorpha systematics, 3, 4
- Culiseta alaskaensis*, 298–300  
 identification, 110, 132–134, 160
- Culiseta alaskaensis indica*, 299
- Culiseta annulata*, 300–302  
 egg-laying behaviour, 167–168  
 identification, 109, 110, 134, 137, 159, 160, 167  
 morphology, pupae, 85
- Culiseta bergrothi*, 302–303  
 identification, 109, 110, 132, 160
- Culiseta dyary*, 296
- Culiseta fumipennis*, 291–292  
 identification, 109, 114, 133, 161  
 morphology, larvae, 83
- Culiseta* genus  
 biology  
 egg-laying behaviour, 10  
 hibernation, 24  
 control, 436, 469  
 identification, 91, 108–110, 115, 132–134, 136, 159–162  
 medical importance, 33, 38, 39  
 morphology, 69, 71–73, 79, 82, 83  
 research, egg sampling, 45
- Culiseta glaphyoptera*, 303–305  
 identification, 108–110, 115, 133, 160
- Culiseta impatiens*, 464
- Culiseta incidens*, 387
- Culiseta inornata*, 387
- Culiseta litorea*, 293–294  
 identification, 109, 132, 133, 161, 162
- Culiseta longiareolata*, 289–291  
 identification, 83, 108, 115, 132, 159
- Culiseta melanura*  
 medical importance, 32, 454
- Culiseta morsitans*, 294–296  
 biology  
 behaviour, 22  
 hatching, 14  
 hibernation, 24  
 larvae, 19  
 identification, 109, 114, 132, 133, 159, 161, 162  
 medical importance, 32, 33  
 morphology, larvae, 79
- Culiseta ochroptera*, 296–298  
 identification, 109, 133, 161
- Culiseta setivalva*, 292
- Culiseta* subgenus, 298  
 biology  
 embryonic development, 13  
 larvae, 17  
 oviposition, 10  
 egg sampling, 43  
 morphology, larvae, 79, 83
- Culiseta subochrea*, 305–306  
 identification, 110, 134, 160  
 morphology, pupae, 85
- Culisetini tribus, 5
- Cyclops*, 411, 412, 423
- Cymatia coleoprata*, 412
- Cynolebias bellottii*, 408
- Cyprinodontidae, 408
- Cyprinus carpio*, 408, 423
- D**
- Dahlia* *echinus*. See *Ochlerotatus echinus*
- Dahlia* *geniculata*. See *Ochlerotatus geniculatus*
- Dahlia* genus, 6
- Dahlia gilcolladoi*.  
 See *Ochlerotatus gilcolladoi*
- Danielsia* genus, 6
- Daphnia magna*, 423, 426, 468
- Davismyia* subgenus, 5
- Decamyia* subgenus, 5
- Deinocerites* genus, 5, 64  
 medical importance, 33
- Deinocerites* subgenus, 5
- Delichon urbica*, 409, 410
- Dendromyia* subgenus, 5
- Dendroskusea* genus, 6
- Dengue virus, 31, 34–36, 203, 486, 487
- Deuteromycetes, 416
- Dipetalonema*, 40
- Diceromyia furcifer*. See *Aedes furcifer*
- Diceromyia* genus, 6, 317

*Diceromyia* subgenus, 6, 317  
*Diceromyia taylori*. See *Aedes taylori*

## Diptera

biological control with, 409, 410, 414–416, 418, 422, 431, 457, 462  
 systematics, 3  
*Dirofilaria immitis*, 40, 203, 279, 333, 394  
*Dirofilaria repens*, 40  
 Dixidae  
 morphology, 78, 87  
*Dobrotworskyius* genus, 6  
*Dodecamyia* subgenus, 5  
 Dolichopodidae, 415  
*Downsiomyia* genus, 6, 333  
*Downsiomyia nivea*. See *Ochlerotatus niveus*  
*Dracaena*, 9, 41, 203  
*Dugesia dorotocephala*, 468  
 Dytiscidae, 413  
*Dytiscus circumflexus*, 413  
*Dytiscus marginalis*, 413

## E

Eastern Equine Encephalomyelitis virus, 32  
*Edwardsaedes* genus, 6  
*Edwardsaedes* subgenus, 6  
*Eichhornia crassipes*, 332, 386  
*Elpeytonius* genus, 6  
 Empididae, 415  
*Empihals* subgenus, 6  
*Entomophthora culicis*, 418  
 Entomophthorales, 416, 418  
 Ephemeroptera, 73, 410, 422, 423, 457  
*Eptesicus*, 410  
*Eretmapodites* genus, 6, 414  
*Etorleptomyia* subgenus, 5  
*Eucalyptus citriodora*, 494, 495  
*Eumelanomyia* subgenus, 5  
*Exallomyia* subgenus, 5

## F

*Ficalbia* genus, 5  
 Ficalbiini tribus, 5  
*Finlaya* genus, 6  
*Finlaya* subgenus, 113, 205, 354  
 morphology, larvae, 79  
 Flaviviridae, 31, 34  
*Flavivirus*, 31, 34, 208, 215  
 Formicidae, 409  
*Francisella tularensis*, 208, 218, 227  
*Fredwardsius* genus, 6, 188, 196–198  
*Fredwardsius* subgenus, 6, 188, 196–198  
*Fredwardsius vittatus*. See *Aedes vittatus*

## G

*Galindomyia*, 5, 64  
*Gambusia affinis*, 405, 406, 435  
*Gasterosteus aculeatus*, 408  
 Gastropoda, 409

*Georgecraigius fluviatilis*.  
 See *Ochlerotatus fluviatilis*

*Georgecraigius* genus, 6, 369  
*Georgecraigius* subgenus, 6  
*Geoskusea* genus, 6  
*Geoskusea* subgenus, 6  
 Gerridae, 413  
*Gerris*, 411, 413, 478  
*Gerris lacustris*, 411, 413  
*Gilesia* subgenus, 6  
*Gilesius* genus, 6  
*Gobio gobio*, 408  
*Grabhamia* subgenus, 7  
*Guignotus pusillus*, 411, 414, 423  
*Gymnometopa* genus, 6, 369  
*Gymnometopa mediovittata*.  
 See *Ochlerotatus mediovittatus*  
*Gymnometopa* subgenus, 6  
 Gyrinidae, 414

## H

*Haemagogus* genus, 6, 34, 63, 67, 371, 372, 381–382  
 medical importance, vi, 34  
*Haemagogus janthinomyis*, 381–382  
 identification, 372  
*Haemagogus* subgenus, 6  
*Halaedes* genus, 6  
*Halaedes* subgenus, 6  
 Hantavirus, 37  
*Harbachius* subgenus, 7  
*Heizmannia* genus, 6  
*Heizmannia* subgenus, 6  
*Helochares obscurus*, 414  
*Heteraspidion* subgenus, 7  
 Heteroptera, 410–414, 422, 423  
*Heterorhabditis*, 415  
*Himalaius* genus, 6  
*Hodgesia* genus, 5, 83, 307  
 Hodgesiini tribus, 5  
*Hopkinsius* genus, 6  
*Hopkinsius* subgenus, 6  
*Horsfallius* subgenus, 6  
*Howardina* genus, 6  
*Howardina* subgenus, 6  
*Huaedes* genus, 6  
*Huaedes* subgenus, 6  
*Huangmyia* subgenus, 7  
*Hulecoeteomyia* genus, 6  
*Hulecoeteomyia japonica*. See *Ochlerotatus japonicus japonicus*  
*Hyalella azteca*, 468  
*Hydra*, 411, 412, 423  
 Hydrocorisa, 412  
*Hydrometra stagnorum*, 413  
 Hydrometridae, 413  
 Hydrophilidae, 414  
*Hydrophilus caraboides*, 411, 414, 423

Hydroporinae, 413  
*Hydroporus palustris*, 413, 423  
*Hygrotus inaequalis*, 413, 423  
*Hyla arborea*, 409  
*Hyphydrus ovatus*, 413  
*Hystatamyia* subgenus, 5

**I**

*Ilyocoris cimicoides*, 412, 423  
*Indusius* genus, 6  
*Indusius* subgenus, 6  
*Ingramia* subgenus, 5  
 Inkoo virus, 37  
*Insulalutzia* subgenus, 5  
 Intrudens Group, 209  
 Iridescent virus, 431  
*Isoaedes* genus, 6  
*Isoaedes* subgenus, 6  
 Isopoda, 409  
*Isostomyia* genus, 5

**J**

Jamestown Canyon virus, 37, 38  
*Janthinosoma* subgenus, 7, 395  
 Japanese encephalitis virus, 34, 227,  
 279, 333, 350  
*Jarnellius* genus, 6  
*Jihlienius* genus, 6  
*Johnbelkinia* genus, 5  
*Juncus*, 401  
*Juppius* subgenus, 6

**K**

*Kenknightia* genus, 6  
*Kenknightia* subgenus, 6  
*Kerteszia* subgenus, 5, 164  
*Kimia* genus, 5  
*Kitzmilleria* subgenus, 5  
*Kompia* genus, 6  
*Kompia* subgenus, 6  
 Kyzylagach virus, 33

**L**

La Crosse Encephalitis virus, 37–38  
*Lagenidium giganteum*, 417, 418  
*Lasiosiphon* subgenus, 5  
 Lednice virus, 37, 39  
*Leicestera* subgenus, 6  
*Lepidokeneon* subgenus, 6  
*Lepiothauma* subgenus, 5  
*Leptosomatomyia* genus, 6  
*Leptosomatomyia* subgenus, 6  
*Lepus americanus*, 39  
*Leucaspius delineatus*, 408  
*Levua* genus, 6  
*Levua* subgenus, 6  
*Lewnielsenius* genus, 6

*Limatus* genus, 5, 71  
*Limnephilus*, 414  
 Lispe, 415  
*Lophoceraomyia* subgenus, 5  
*Lophopodomyia* subgenus, 5, 164  
*Lorrainea* genus, 6  
*Lorrainea* subgenus, 6  
*Luccophilus*, 411, 414  
*Lugenidium giganteum*, 417, 418  
*Luius* genus, 6  
*Lutzia* genus, 5, 83, 264, 307  
*Lutzia* subgenus, 5  
 morphology, larvae, 83  
*Lymnaea (Galba) palustris*, 423  
*Lynchiella* subgenus, 5

**M**

*Macleaya* genus, 6  
*Macleaya* subgenus, 6  
*Macropodus chinensis*, 408  
*Macropodus opercularis*, 408  
*Maillotia* subgenus, 5, 265, 282  
*Malaya* genus  
 morphology, adult, 63, 67  
*Mansonella*, 40  
*Mansonia africana*, 317, 330  
 identification, 321, 322  
*Mansonia annulata*, 333  
*Mansonia* genus  
 biology, pupae, 17, 19  
 medical importance, 33, 39  
*Mansonia indiana*, 333  
*Mansonia* subgenus, 372  
*Mansonia titillans*, 385–386  
 identification, 372  
*Mansonia uniformis*, 330–332, 333  
 identification, 321, 322, 339, 353  
 medical importance, 32  
 Mansoniini tribus, 4, 5, 187  
*Mansonioides* subgenus, 5, 330  
*Maorigoeldia* genus, 5  
 Mastigomycotina, 416  
*Mattinglyia* subgenus, 6  
*Megacyclops viridus*, 412  
*Melanoconion* subgenus, 5  
*Menolepis* subgenus, 5  
 Mermitbidae, 415  
*Mesocyclops aspericornis*, 412  
*Mesostoma ehrenbergii*, 411  
*Metalutzia* subgenus, 5  
*Metarhizium*, 418  
*Micraedes* subgenus, 5  
*Microculex* subgenus, 5  
 Microsporidia, 418, 419  
*Mimomyia* genus, 5  
 morphology, larvae, 83  
*Mimomyia* subgenus, 5  
*Mochlonyx culiciformis*, 414, 415  
*Mochlonyx* genus  
 larva morphology, 77–78

Mollusca, 422, 423  
*Molpemyia* genus, 6  
*Molpemyia* subgenus, 6  
*Mucidus* genus, 6, 66  
*Mucidus* subgenus, 6, 354  
*Mukwaya* subgenus, 7  
Murray Valley encephalitis virus, 364  
*Musca domestica*, 415  
Muscidae, 415  
*Myotis*, 409  
Myxoma virus, 25, 218

## N

*Natrix natrix*, 37  
Naucoridae, 412–413  
Nematocera, 3, 63, 73, 421, 423  
Nematoda (Nematodes), 25, 39, 415–416, 442, 502  
*Neobironella* subgenus, 5, 163  
*Neoculex* subgenus, 5, 265, 272, 283–284  
*Neomacleaya* subgenus, 7  
*Neomelanicion circumluteolum*.  
    *See Aedes circumluteolus*  
*Neomelanicion* genus, 6  
*Neomelanicion* subgenus, 6  
*Neotheobaldia* subgenus, 5  
*Nepa cinerea*, 413  
Nepidae, 413  
*Nicaromyia* subgenus, 5  
*Nosema*, 419  
Noterinae, 413  
*Nothokusea* subgenus, 6  
*Notonecta glauca*, 413, 423, 478  
*Notonecta undulata*, 413  
*Notonecta unifasciata*, 413  
Notonectidae, 413  
*Nunezia* subgenus, 5  
*Nymphaea*, 401  
Nymphomyiidae, 3  
*Nyssorhynchus* subgenus, 5, 75, 164, 370

## O

*Ochlerotatus* genus, ix, x, xii, 204–205, 333, 369  
    biology, 9  
        dispersal and host-seeking behaviour, 21, 22  
        embryonic development, 13  
        hatching, 13, 15, 16  
        hibernation, 24  
        larvae, 17  
        oviposition, 11  
    control, 408, 412, 413, 423, 426, 428, 430, 436,  
        454, 456, 457, 468–470, 502  
    identification, 92, 95–106, 113, 118–128, 136, 141–154,  
        318, 321, 339, 354, 372, 373, 388, 389  
    medical importance, 33, 34, 38–40  
    morphology, 71, 72–76, 79, 82–84  
    research  
        egg sampling, 44  
        rearing, 52  
        systematics, 4, 6, 7

*See also Aedes/Ochlerotatus*  
*Ochlerotatus albifasciatus*, 369, 382–383  
    identification, 373  
    medical importance, 32  
*Ochlerotatus alopnotum*, 228  
*Ochlerotatus alternans*, 357–358  
    identification, 354  
*Ochlerotatus annulipes*, 209–211  
    identification, 100, 114, 127, 128, 144, 151, 152  
*Ochlerotatus atlanticus*, 387  
*Ochlerotatus bancroftianus*, 351  
*Ochlerotatus behningi*, 211–212  
    identification, 99, 124, 127, 128, 150  
*Ochlerotatus beklemishevi*, 229  
*Ochlerotatus berlandi*, 212–214  
    identification, 95, 96, 123, 141  
*Ochlerotatus bicristatus*, 257  
*Ochlerotatus caballus*, 317  
*Ochlerotatus campestris*, 227  
*Ochlerotatus camptorhynchus*, 360  
    identification, 355  
*Ochlerotatus canadensis canadensis*, 387  
    medical importance, 32  
*Ochlerotatus cantans*, 214–215  
    biology  
        feeding, 23  
        hatching, 13, 14  
        larvae, 18, 23  
    control, 505  
    identification, 95, 98, 100, 126, 151, 152  
    medical importance, 36, 38  
*Ochlerotatus caspius*, 216–218  
    biology, egg-laying behaviour, 11  
    control, 494  
    identification, 95, 96, 121–123, 137,  
        144–146  
    morphology, larvae, 84  
*Ochlerotatus Caspius Complex*, 216  
*Ochlerotatus cataphylla*, 218–219  
    identification, 101, 103, 123–125, 143–145  
    morphology, larvae, 83  
*Ochlerotatus churchillensis*, 219  
*Ochlerotatus coluzzii*, xi, 209, 223, 224  
*Ochlerotatus communis*, 220–221, 333, 387  
    biology  
        dispersal and host-seeking behaviour, 21  
        hatching, 14  
        identification, 104, 105, 118, 124, 125, 145, 146  
        medical importance, xxvii, 39  
*Ochlerotatus Communis Complex*, 219  
*Ochlerotatus cyprius*, 221–222  
    identification, 98, 126, 127, 141  
*Ochlerotatus detritus*, 223–224, 317  
    identification, 101, 102, 125, 127, 136, 145  
*Ochlerotatus Detritus Complex*, 222  
*Ochlerotatus diantaeus*, 224–226  
    identification, 104, 121, 122, 141  
*Ochlerotatus dorsalis*, 226–228, 333, 387  
    control, 469  
    identification, 96, 122, 123, 146  
    systematic status, 218, 227–228

- Ochlerotatus eatoni*, 206
- Ochlerotatus echinus*, 205–206  
identification, 102, 120, 152, 153
- Ochlerotatus euedes*, 228–229  
identification, 100, 101, 128, 141, 149, 150  
medical importance, 34
- Ochlerotatus excrucians*, 229–231, 333  
identification, 99, 128, 149–151  
systematics, 229, 231
- Ochlerotatus Excrucians Complex*, 228
- Ochlerotatus fitchii*, 333
- Ochlerotatus flavescens*, 231–233  
identification, 98, 127, 151  
medical importance, 38
- Ochlerotatus fluviatilis*, 369
- Ochlerotatus geniculatus*, 206–208  
identification, 91, 101, 102, 120, 142, 152, 153  
morphology, larvae, 79  
systematics, 206
- Ochlerotatus hexodontus*, 233–234, 333, 387  
identification, 105, 106, 124, 141, 144  
medical importance, xxvii
- Ochlerotatus hungaricus*, 234–236  
identification, 106, 122, 147, 148
- Ochlerotatus impiger*, 236–237, 333, 387  
identification, 103, 122–124, 147
- Ochlerotatus increpitus*, 387
- Ochlerotatus infirmatus*  
medical importance, 38
- Ochlerotatus intrudens*, 237–238  
identification, 104, 120–122, 144, 145
- Ochlerotatus japonicus japonicus*, xxix, 9, 46  
medical importance, 41, 42
- Ochlerotatus krymmontanus*, xi, 257
- Ochlerotatus lepidonotus*, 257–258  
identification, 102, 121, 143
- Ochlerotatus leucomelas*, 238–240  
identification, 103, 124, 125, 146
- Ochlerotatus mariae*, 240–242  
identification, 96, 123, 148, 149  
morphology, larvae, 82
- Ochlerotatus Mariae Complex*, 240
- Ochlerotatus mediovitatus*, 369
- Ochlerotatus melanimon*, 227, 387, 470
- Ochlerotatus mercurator*, 242–243  
identification, 99, 113, 126, 127, 148, 150, 151
- Ochlerotatus nevadensis*, 219
- Ochlerotatus nigrinus*, 243–244  
identification, 106, 126, 147, 149
- Ochlerotatus nigripes*, 245–246, 333, 387  
identification, 103, 118, 120, 123, 124, 143
- Ochlerotatus nigromaculis*, 387
- Ochlerotatus niveus*, 333
- Ochlerotatus normanensis*, 360–362  
identification, 355, 356
- Ochlerotatus notoscriptus*, 351, 358–359  
identification, 354  
control, 435
- Ochlerotatus phoeniciae*, 240
- Ochlerotatus pionips*, 246–248, 333  
identification, 105, 106, 125, 146, 147
- Ochlerotatus provocans*, 257
- Ochlerotatus pulcritarsis*, 248–249  
identification, 95, 123, 152  
morphology, larvae, 79
- Ochlerotatus pullatus*, 249–250  
identification, 103, 104, 121, 146–148
- Ochlerotatus punctodes*, 250–251  
identification, 105, 124, 143, 144  
morphology, larvae, 83
- Ochlerotatus punctor*, 251–253, 333, 387  
identification, 103, 105, 124, 142–144  
medical importance, xxvii, 38
- Ochlerotatus quasirusticus*, 258–259  
identification, 102, 121, 143
- Ochlerotatus refiki*, 259–261  
identification, 102, 120, 121, 142, 143
- Ochlerotatus riparius*, 253–254  
identification, 99, 100, 114, 126, 141, 148, 149
- Ochlerotatus rusticus*, 261–263  
biology  
dispersal and host-seeking behaviour, 21, 22  
hatching, 13, 14, 24  
hibernation, 24  
larvae, 19, 24  
identification, 101, 102, 120, 142, 143
- Ochlerotatus sagax*, 362–363  
identification, 354, 355
- Ochlerotatus scapularis*, 383–384  
identification, 373
- Ochlerotatus sierrensis*, 387
- Ochlerotatus sollicitans*, 369, 391–392  
identification, 389, 390  
medical importance, 32
- Ochlerotatus sticticus*, 255–256, 333, 387  
identification, 105, 106, 125, 126, 146, 148  
medical importance, 38
- Ochlerotatus stimulans*, 387, 494
- Ochlerotatus subdiversus*, 263–264  
identification, 102, 121, 143
- Ochlerotatus* subgenus, 4, 6, 17, 45, 208–209, 354
- Ochlerotatus surcoufi*, 209, 228, 231  
identification, 101, 128, 150, 151
- Ochlerotatus taeniorhynchus*, 393–394  
identification, 390  
medical importance, 33
- Ochlerotatus tahoensis*, 219  
medical importance, 38
- Ochlerotatus theobaldi*, 363–364  
identification, 355
- Ochlerotatus togoi*, 333
- Ochlerotatus trichurus*, 494
- Ochlerotatus triseriatus*, 387  
medical importance, 38  
blood meal identification, 55
- Ochlerotatus trivittatus*, 387  
medical importance, 37, 38, 278
- Ochlerotatus vigilax*, 333, 364–365  
biology, 9  
identification, 354–356

medical importance, 32  
 control, 437  
*Ochlerotatus vittiger*, 351  
*Ochlerotatus zammitii*, 242  
   identification, 123, 149  
 Ockelbo virus, 33, 277, 280, 296  
*Oculeomyia* subgenus, 5  
 Odonata, 412, 422, 423, 457  
 Onchocercidae, 40  
*Onirion*, 5  
 O'Nyong-Nyong virus, 33  
*Opifex*, 6  
*Orechromis hornorum*, 408  
*Orechromis mossambica*, 408  
*Orthopodomyia* genus, 310  
   biology, egg-laying behaviour, 9  
   embryonic development, 13  
*Orthopodomyia pulcripalpis*, 310–312  
   biology, 9, 24  
   habitat, 9  
   hibernation, 24  
   identification, 92, 115, 136  
   morphology, larvae, 82, 83  
*Orthopodomyiini* tribus, 5  
*Oryzias latipes*, 408  
 Osteichthyes, 406–408

**P**

*Paecilomyces*, 418  
*Paraedes* genus, 6  
*Paraedes* subgenus, 6  
*Parathelohania*, 419  
*Pardomyia* subgenus, 6  
 Passeriformes, 33, 36, 202  
*Patmarksia* genus, 6  
*Pelobates fuscus*, 409  
*Petermattinglyius* genus, 6  
*Petermattinglyius* subgenus, 6  
*Peytonulus* subgenus, 5  
*Phagomyia* genus, 7  
*Phenacomyia* subgenus, 5  
*Phlebovirus*, 31, 37  
*Pholeomyia* subgenus, 6  
*Phoniomyia* subgenus, 5  
*Phragmites*, 12, 166, 262, 309,  
   314, 401  
*Phryganea*, 414  
*Phytotelmatomyia* subgenus, 5  
*Piona nodata*, 412  
 Pisces, 422, 423  
*Pistia*, 327, 386  
*Pistia stratiotes*, 330, 332, 386  
*Planipennia*, 410  
*Plasmodium*, 25–29, 242, 343, 379, 418, 442, 486, 487, 500.  
   *See also* Malaria (subject index)  
*Plasmodium falciparum*, 25, 28–30, 166, 180,  
   486, 487, 492, 495  
*Plasmodium malariae*, 25, 28, 30  
*Plasmodium ovale*, 25, 28, 30

*Plasmodium vivax*, 25, 28–30, 180, 378  
*Plea leachi*, 413, 423  
 Pleidae, 413  
*Plodia interpunctella*, 424  
*Podura aquatica*, 457  
*Poecilia reticulata*, 406, 407  
*Polylepidomyia* subgenus, 5  
*Polyleptomyia* genus, 7  
*Potamogeton*, 327  
*Prosopolepis* subgenus, 5  
*Protoculex* subgenus, 6  
*Protomacleaya* subgenus, 6  
*Pseudarmigeres* genus, 7  
*Pseudarmigeres* subgenus, 6  
*Pseudoficalbia* subgenus, 5  
*Pseudokusea* subgenus, 6  
*Psorophora columbiae*, 394–395  
   identification, 388  
   control, 406, 470  
*Psorophora ferox*, 369, 395–396  
   identification, 371, 388  
*Psorophora* genus  
   biology, larvae, 18  
   control, 412, 414, 469, 470  
   medical importance, vi, 33, 39  
   morphology, 69, 71, 75, 76  
*Psorophora* subgenus, 7  
 Psychodidae, 421–423  
 Ptychopteromorpha, 3  
 Punctor Group, 209

**R**

*Rachionotomyia* subgenus, 5  
*Rachisoura* subgenus, 5  
*Rampamyia* genus, 7  
*Rampamyia notoscripta*.  
   *See Ochlerotatus notoscriptus*  
*Rana*, 288, 409, 423  
*Ranatra linearis*, 413  
*Rhantus*, 413, 423  
*Rhinokusea* genus, 7  
*Rhinokusea* subgenus, 6  
*Rhynchotaenia* subgenus, 5, 307  
*Romanomermis*, 415, 416  
 Ross River virus, 31, 32, 332, 351, 359,  
   360, 362, 364–366, 466  
 Rotatoria, 422, 423  
*Runchomyia* genus, 5  
*Runchomyia* subgenus, 5  
*Rusticoides* subgenus, 6, 83, 205, 256–257  
*Rutilus rutilus*, 408

**S**

*Sabethes* genus, 5, 63, 67, 84  
*Sabethes* subgenus, 5  
*Sabethini* tribus, 5  
*Sabethinus* subgenus, 5  
*Sabethoides* subgenus, 5



*Saccharopolyspora spinosa*, 419  
*Sagittaria*, 401  
*Sallumia* genus, 7  
*Scardinius erythrophthalmus*, 408  
*Sciaridae*, 422, 423  
*Scutomyia* genus, 7  
*Scutomyia* subgenus, 6  
 Semliki Forest virus, 33–34  
*Shannoniana*, 5  
*Sigara striata*, 412, 423  
*Simuliidae*, 3, 7, 423, 429  
*Simulium damnosum*, 423, 424  
*Simulium vittatum*, 431  
 Sindbis virus, 31, 33, 282, 317, 330, 333, 438  
*Sirivanakarnius* subgenus, 5  
*Skusea* genus, 7  
*Skusea* subgenus, 6  
 Snowshoe Hare virus, 37, 38  
*Spercheidae*, 414  
*Spilonympha* subgenus, 5  
*Stegomyia aegypti*. See *Aedes aegypti*  
*Stegomyia africana*. See *Aedes africanus*  
*Stegomyia albopicta*. See *Aedes albopictus*  
*Stegomyia cooki*. See *Aedes cooki*  
*Stegomyia cretina*. See *Aedes cretinus*  
*Stegomyia fasciata*. See *Aedes aegypti*  
*Stegomyia* genus, 7  
*Stegomyia hebridea*. See *Aedes hebrideus*  
*Stegomyia hensilli*. See *Aedes hensilli*  
*Stegomyia luteocephala*. See *Aedes luteocephalus*  
*Stegomyia opok*. See *Aedes opok*  
*Stegomyia polynesiensis*. See *Aedes polynesiensis*  
*Stegomyia rotumae*. See *Aedes rotumae*  
*Stegomyia scutellaris*. See *Aedes scutellaris*  
*Stegomyia* subgenus, 6, 7, 188, 194, 196, 198  
     identification, 321, 339, 353, 372, 389  
     morphology, 76, 82  
     medical importance, 31, 32, 35  
*Steinernema*, 415  
*Steinernematidae*, 415  
*Stethomyia* subgenus, 5, 164  
 St. Louis encephalitis virus, 31, 34, 37, 227, 279, 385, 400, 463  
*Suaymyia* subgenus, 5  
*Syrphidae*, 409, 423

## T

Tahyna virus, 37, 38, 196, 211, 215, 218, 267, 302  
*Tanakaius* genus, 7  
*Tanakaius togoi*. See *Ochlerotatus togoi*  
*Tanichthys albonubes*, 408  
*Tewarius* genus, 7  
*Thaumaleidae* systematics, 3  
*Theobaldia* genus, 288  
*Theomyia* subgenus, 5  
*Tilapia zilli*, 408  
*Tinca tinca*, 408  
*Tinolestes* subgenus, 5  
*Tipulidae*, 63, 409, 422, 423

*Tipulomorpha*, 3  
*Tipuloidea* systematics, 3  
*Togaviridae (Alphavirus)*, 31–34  
*Tolypocladium*, 418  
*Topomyia* genus, 5  
*Topomyia* subgenus, 5  
*Toxorhynchites* genus, 5, 18, 65, 66, 69, 76, 83, 315, 414  
*Toxorhynchites* subgenus, 5  
*Toxorhynchitinae* subfamily, 3, 4  
*Toxorhynchitini* tribus, 5  
*Tricholeptomyia* subgenus, 5  
*Trichoprosopon*, 5  
*Trichoptera*, 410, 414, 422, 423  
*Triops cancrivormis*, 412, 468  
*Triops longicaudatus*, 412, 468  
*Tripteroides* genus, 5  
*Tripteroides* subgenus, 5  
*Triturus*, 408, 409, 423  
 Trivittatus virus, 37, 38  
*Tubifera*, 423  
*Turbellaria*, 411, 422, 423  
*Typha*, 230, 294, 308, 309, 374, 401

## U

*Udaya* genus, 7  
*Uranotaenia* genus, 5, 312–313  
     biology  
         egg-laying behaviour, 10  
         embryonic development, 13  
         hibernation, 24  
         research, egg sampling, 43  
*Uranotaenia* subgenus, 5  
*Uranotaenia unguiculata*, 313–314  
     identification, 91, 114, 115, 136, 137  
     morphology, 80, 82, 83  
*Uranotaeniini* tribus, 5  
*Urodela*, 408

## V

*Vansomerensis* genus, 7  
*Vavraia*, 419  
 Venezuelan Equine Encephalomyelitis virus, 32–33, 386, 394  
*Verrallina funerea*, 351  
*Verrallina* genus, 7  
*Verrallina* subgenus, 7  
*Verticillium*, 418

## W

Western Equine Encephalomyelitis virus, 32, 196, 227, 279, 369, 383, 397, 400  
 West Nile virus, 31, 34, 36–37, 41, 218, 267, 275, 277–279, 282, 310, 317, 328, 330, 333, 385, 387, 397, 398, 400, 401, 421, 428, 438, 454, 463, 472, 473, 481  
 Whataroa virus, 33

*Wolbachia*, 485, 488, 500

*Woodius* subgenus, 6

*Wuchereria bancrofti*, 39, 332, 333, 344, 346,  
347, 357, 366, 473

*Wyeomyia* genus, 5, 67, 84

*Wyeomyia* subgenus, 5

## **X**

*Xenopelopia*, 423

*Xyle* subgenus, 7

## **Y**

*Yamada* subgenus, 6

Yellow Fever virus, 31, 34, 201, 317

## **Z**

*Zavortinkius* genus, 7

*Zavortinkius* subgenus, 6

*Zeugnomyia* genus, 7

*Zinzala* subgenus, 5

*Zoromorphus* subgenus, 7