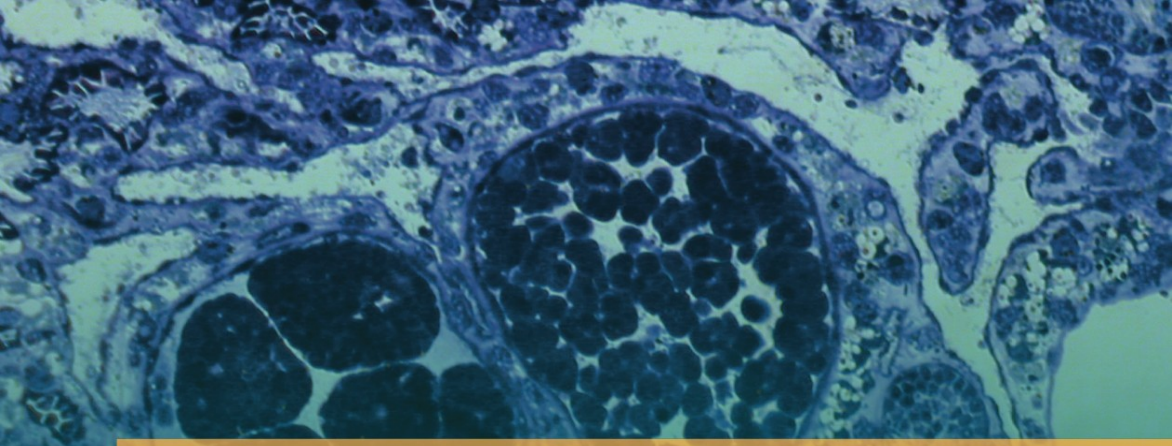

Alexander V. Ereskovsky

The Comparative Embryology of Sponges

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Preface

It is generally assumed that sponges (phylum Porifera) are the most basal metazoans (Kobayashi et al. 1993; Li et al. 1998; Mehl et al. 1998; Kim et al. 1999; Philippe et al. 2009). In this connection sponges are of a great interest for EvoDevo biologists. None of the problems of early evolution of multicellular animals and reconstruction of a natural system of their main phylogenetic clades can be discussed without considering the sponges. These animals possess the extremely low level of tissues organization, and demonstrate extremely low level of processes of gametogenesis, embryogenesis, and metamorphosis. They show also various ways of advancement of these basic mechanisms that allow us to understand processes of establishment of the latter in the early Metazoan evolution.

The position of Porifera within the animal kingdom has been problematic since the last decades of the nineteenth century. Due to the limited number of morphological/cytological characteristics, no much conclusive or plausible decision about the phylogenetic position of the sponges could be made. In the literature there were two opposite points of view on position of sponges in the system of Eukaryotes. Some researchers, since Balfour (1879) considered sponges as an independent direction in the evolution of Metazoans, which has arisen irrespective of others (Sollas 1884; Delage 1892; Minchin 1900; Livanov 1955; Hadži 1963; Fedotov 1966; Schulman 1974; Salvini-Plaven 1978; Zhuravleva and Miagkova 1987; Seravin 1997). The opposite point of view belongs to the authors considering the sponges and the true Metazoa as descendants of a common ancestor (Haeckel 1874; Lévi 1956; Beklemishev 1964; Brien 1967c; Brien 1973a; Ivanov 1968, 1971; Tuzet 1970; Hooper et al. 2002; Nielsen 2008).

Only the introduction of molecular phylogeny techniques rapidly increased our understanding of evolutionary processes, and definitively confirmed monophyly of Porifera and other Metazoa (Kobayashi et al. 1993; Müller 1997b, 1998; Kim et al. 1999; Lang et al. 2002; Lavrov et al. 2008; Philippe et al. 2009). At the same time, contradictory and often poorly supported trees have been proposed, leaving major issues such as the phylogenetic status of sponges – monophyletic (Müller 2001; Lavrov et al. 2008; Philippe et al. 2009) or paraphyletic unresolved (Cavalier-Smith et al. 1996; Zrzavy et al. 1998; Collins 1998; Adams et al. 1999; Medina et al. 2001; Borchiellini et al. 2001, 2004a; Peterson and Eernisse 2001; Manuel et al. 2003; Sperling et al. 2007; Robertson et al. 2009).

Attempts to understand the reason of an originality of the organization and biology of sponges were repeatedly done. It has been shown that specificity of different stages of sexual development of sponges, as the gametogenesis, along with the embryogenesis, larval development, and the metamorphosis are closely connected with a low level of tissues organization and their multifunctionality, with a high adaptive capability (Maas 1894; Delage 1892; Minchin 1900; Rasmont 1979; Korotkova 1981a, 1988a; Simpson 1984; Seravin 1986, 1992; Malakhov 1990; Gaino et al. 1995; Efremova 1997; Ereskovsky and Korotkova 1997, 1999; Ereskovsky 1999, 2005; Maldonado 2004; Leys and Ereskovsky 2006).

A lack of uniformity and completeness in the available data on ontogenesis of the representatives of various groups of Porifera complicates the typization of sponge development, and understanding the roots of their originality and ways of their evolution (Brien 1943, 1967a, 1972; Borojevic 1970; Korotkova 1981a, b, 1988a; Efremova 1997; Ereskovsky and Korotkova 1997, 1999; Ivanova-Kazas 1997; Ereskovsky 1999, 2004, 2007b; Leys and Ereskovsky 2006).

In one work, the evolution of sponges' ontogenesis has been represented in the form of a gradual complication of embryogenesis, correlating with complication of their aquiferous systems (Brien 1967c, 1972; Ivanova-Kazas 1997). In other works, the attention has been drawn to the balance of flagellated and amoeboid cells lines during morphogeneses in sponges (Borojevic 1970). In the third, the evolution of ontogenesis has been considered as a process of interdependent somatic and reproductive morphogeneses in evolution of life cycles of sponges (Korotkova 1981b, 1988a).

It is probable that one of the deepest causes, which affected the variety of points of view, is traditional consideration of the sponges as a homogeneous group. The results of the study of a representative of one clade (Hexactinellida, Calcarea, Demospongiae, or Homoscleromorpha) were extrapolated on all Porifera. It was not important, what kind of research has been conducted: morphological, embryological, cytological, or molecular.

Considering such a great value of Porifera for understanding the origin, evolution, and phylogeny of Metazoa, it becomes obvious an indispensability of extensive comparative investigations of the ontogenesis in these basal metazoans.

Despite having more than 150-year-old history of studies of sponges' development, their comparative embryology is not yet well developed. However, attempts of its development have been undertaken repeatedly as soon as new facts and ideas are accumulated (Delage 1892, 1899; Brien 1943; Lévi 1956; Ivanova-Kazas 1975; Korotkova 1981a; Ereskovsky 2004, Leys and Ereskovsky 2006). Accordingly, the main aim of the present book is to promote the advancements for comparative embryology of sponges at a new step of the study of this important animal group using the state-of-the-art information on their development and evolution.

The main goals here are

- To collect the up-to-date available information on development of sponges, its classification, and position according to current taxonomical structure of Porifera
- To show the heterogeneity of the morphogeneses and other peculiarities of ontogenesis in various taxonomical groups of Porifera (at a rank of order or higher),

as well as their correlation with the organization of both the adult sponges and the larvae
- To show not only the homology of the morphogeneses in Porifera and Eumetazoa, significant for understanding the general evolutionary roots of multicellular animals, but also peculiar characters of morphogeneses and ontogenesis of Porifera in general

The book consists of two parts: the special one and the theoretical one. In the 'Introduction' the general morphological characteristic of sponges is presented. A special attention is given to the anatomical–histological organization, as well as ultrastructural characteristics. Chapters 1–4 describe gametogenesis, embryonic development, larvae, metamorphosis, and asexual reproduction of representatives of various groups of Porifera. The special attention is given to the morphogeneses accompanying embryonic development and metamorphosis. Unequal volume of chapters reflects the different degree of knowledge of the sponges groups. The second part of the book is devoted to theoretical aspects of sponges' embryology.

The basis for the writing of the present book were my own research on development of sponges of various groups lead at the Department of Embryology of Saint-Petersburg State University (Russia) and in the Centre d'Océanologie de Marseille, Station Marine d'Endoume Marseille (France). Reading the course of 'Comparative Embryology of Invertebrates' for many years at Biological Faculty of Saint-Petersburg State University (Russia), gave me further insights on similarity and distinctions of morphogeneses during the development of Porifera and Eumetazoa.

I consider as a pleasant duty to express my deepest gratitude to my teacher and the first scientific supervisor to the professor of Saint-Petersburg State University, Galina P. Korotkova, who rendered a strong effect on development of my scientific interests. My comparative-embryological conceptions in many respects have been certain by impression of unforgettable lectures of the professor of Saint-Petersburg State University, Olga M. Ivanova-Kazas. I am also deeply indebted to my professors: Vladimir M. Koltun and Alexander N. Golikov from the Zoological Institute of Russian Academy of Sciences, Lev N. Seravin, Sophia M. Efremova, Archil K. Dondua, and Diana G. Polteva from Saint-Petersburg State University. I am grateful to my colleagues and friends Dr. Elizaveta L. Gonobobleva, Alexander S. Plotkin, Dr. Raisa P. Anakina, Dr. Ljudmila V. Ivanova, Dr. Ivan A. Tikhomirov, Prof. Andrey I. Granovich, and Prof. Andrey N. Ostrovsky from Saint-Petersburg State University, Prof. Vladimir V. Malakhov, Prof. Nikolay N. Marfenin, Prof. Lev V. Beloussov, and Dr. Yu. Kraus from Moskow State University, Dr. Nicole Boury-Esnault, Dr. Jean Vacelet, Dr. Carole Borchiellini, and Dr. Thierry Perez from Centre d'Oceanologie de Marseille, Station Marine d'Endoume Marseille, Dr. Philippe Willenz (Royal Belgian Institute of Natural Sciences, Bruxells) and Prof. Michael Manuel from University Paris VI for numerous discussions on a handful of problems of embryology, morphology, zoology, and developmental biology, which promoted deeper comprehension of many evolutionary problems of biology.

In preparing the book for print I have been assisted by a number of persons to whom I would like to express my gratitude on this occasion. In the first place I wish to thank Natalia Lentsman for translating my manuscript from Russia to English.

Also many thanks to all the authors and publisher who have kindly agreed to the reproduction of figures used for illustrating this book, as well as colleagues in many countries who by sending me reprints or PDF of their publications have facilitated the arduous task of keeping track of current literature. I would like to thank Elizaveta L. Gonobobleva for her help in preparing most of the drawings for the book.

I thank all my students, past and present, for their friendship and for the intellectual stimulation they have given me.

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Neither my scientific work, nor the writing of this book was possible without constant support and understanding from my wife Daria B. Tokina.

Saint-Petersburg State University, Russia Alexander V. Ereskovsky

Contents

Part II Theoretical Aspects of Sponge Embryology

Introduction

In this introduction we will discuss only those aspects of poriferan organization that have a direct bearing on the topic of the present book. Physiology, biochemistry, ecology and palaeontology are left outside the scope of our consideration. Moreover, some of the relevant molecular biological and genetic data will only be mentioned in passing. Up to now the molecular biological methods were seldom applied to the study of sponge embryonic development and gametogenesis, and the data available are insufficient for generalizations of any kind.

1 Organization and Taxonomic Structure of Sponges

Sponges (Porifera) are aquatic, mostly marine, sedentary multicellular animals, with filtration feeding and respiration. The degree of their organization is low: there is no distinct gut, no special digestive parenchyma or cell population specialized in digestion, no nervous and muscular system and no gonads. The body shape of sponges is very diverse; they may be film-like, encrusting, lumpy or spherical, tubular, branching, flabellate, etc. (Colour plates I-XIII). Correspondingly, it is difficult to speak about their polarity, except the apical-basal one. In monooscular sponges, however, regardless of the aquiferous system type, the apical-basal axis also remains the radial symmetry axis (Manuel 2009). The body size of sponges varies as much as their body shapes: from 3–10 mm to 1.5–2 m.

The rigidity of the sponge body is ensured by the collagen fibrils of the mesohyl, by the spongin fibers (in some Demospongiae orders) and by the inorganic skeleton consisting of various mineral compounds on the basis of either calcium carbonate ($CaCO_3$) (Calcarea) or silicon (SiO_2) (Hexactinellida, Demospongiae, Homoscleromorpha). Inorganic skeleton may be represented by separate spicules, connected or fused spicules, or monolithic mineral skeleton (Fig. 1). The organic, as well as the inorganic, skeleton is secreted or assembled by special cells.

Sponges are characterized by a high polymorphism. The shape, size and colour of the body are often depending directly on environmental conditions, mostly hydrodynamics. A high plasticity also characterizes the life cycles and the reproductive strategies of different populations of the same species.

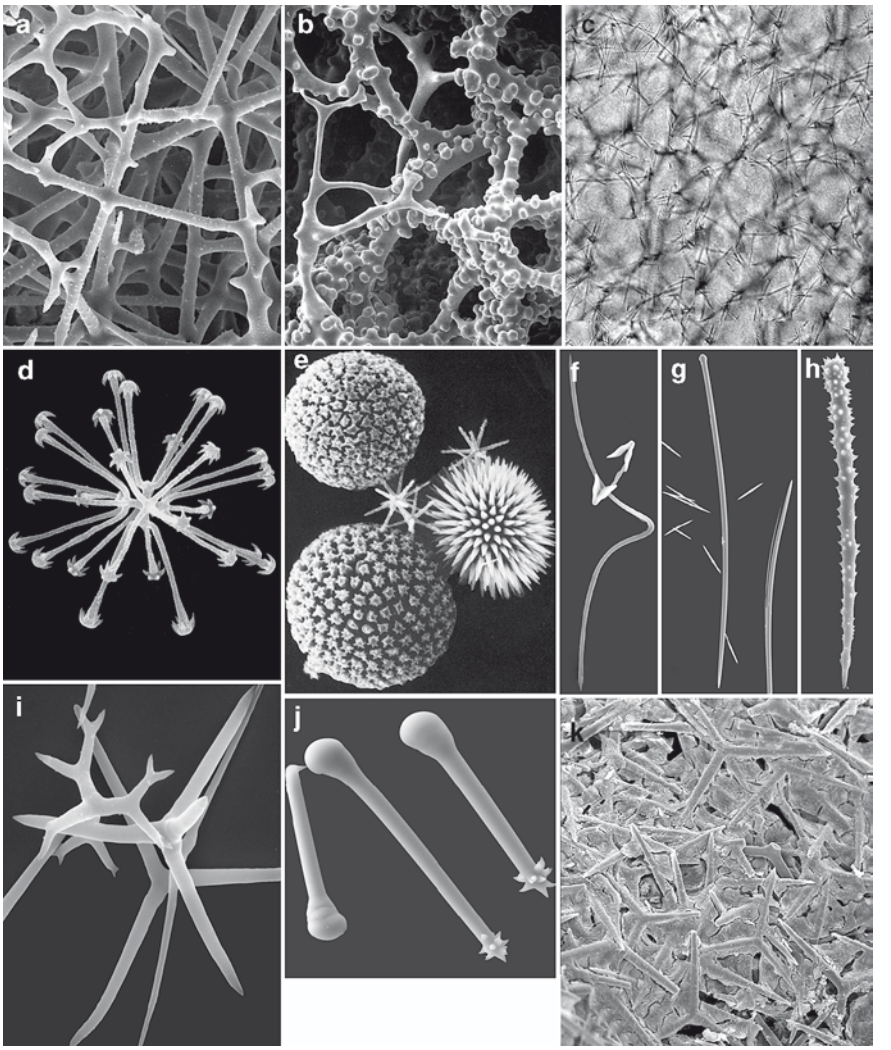

Fig. 1 Inorganic skeleton of sponges. (**a**) Scanning electron microscopy (SEM) of a skeleton of *Oopsacas minuta* (Hexactinellida). (**b**) SEM of a skeleton of *Neophrisspongia nolitangere* (Demospongiae, 'Lithistida'). (**c**) Light microscopy of a skeleton of *Plakortis* sp. (Homoscleromorpha). (**d**) SEM of the discohexaster of *O. minuta* (Hexactinellida). (**e**) SEM of the asters of *Geodia neptuni* (Demospongiae, Astrophorida). (**f**) SEM of anchorate isochela and a forceps of *Artemisina arcigera* (Demospongiae, Poecilosclerida). (**g**) SEM of the tylostyle, oxae and microrhabds of *Suberites domuncula* (Demospongiae, Hadromerida). (**h**) SEM of the acanthotylostyle of *A. arcigera* (Demospongiae, Poecilosclerida). (**i**) SEM of the diode, triode and calthrop of *Plakina* sp. (Homoscleromorpha). (**j**) SEM of echinating acanthostyles of *Hymeraphia stellifera* (Demospongiae, Poecilosclerida). (**k**) SEM of the skeleton of *Clathrina contorta* (Calcispongiae, Calcinea). (**a**, **b**, **d**, **e** – Courtesy of J. Vacelet)

Traditional systematics puts sponges in the phylum Porifera. About 8,000 sponge species are currently known, separated into four clades of the class level: Hexactinellida, Calcarea, Demospongiae and Homoscleromorpha. There is also a class of extinct sponges, Archaeocyatha, showing a close affinity with the Demospongiae. The extinct 'classes' Sphinctozoa and Stromatoporoidea, as well as Sclerospongiae are not separate taxa (Vacelet 1985; Wood 1991), but simply types of the body organization. The representatives of each clade differ in the time of origin (judging by the fossils), the skeleton structure and the spicule shape, as well as in developmental types, which are quite strikingly dissimilar.

According to the paleontological records, Porifera are the most ancient multicellular animals. The most archaic of the extant sponges are the Hexactinellida, whose fossil records date back as far as the early Proterozoic (Steiner et al. 1993; Brasier et al. 1997). Demosponges are known from the late Proterozoic (about 750 million years ago) (Reitner and Wörheide 2002). Interestingly, the first findings of the keratose, i.e. nonspicular demosponges date back to the same time (Reitner and Wörheide 2002). The calcareous sponges (Calcarea) originated somewhat later than the other Porifera, in the lower Cambrian (Reitner 1992). Judging by the paleontological data, the Homoscleromorpha are the youngest sponge group, appearing in the Early Carboniferous (Mehl-Janussen 1999).

2 Organization of 'Cellular' Sponges

The body of sponges with the cellular organization (include Demospongiae, Calcarea and Homoscleromorpha) consists of two epithelial cell layers: the pinacoderm and the choanoderm. The pinacoderm is represented by the flattened cells, the pinacocytes, which form the external cover and line the aquiferous system canals and some internal cavities. The choanoderm is formed by the flagellated collar cells, the choanocytes, lining the choanocyte chambers. The space between the external layer of pinacocytes and the aquiferous system is filled with the mesohyl. As indicated by Müller (1997a), sponge mesohyl should not be considered as an inert scaffold but as a dynamic and complex network of molecules that regulates the behaviour of cells. The mesohyl contains over ten types of highly mobile cells, as well as skeletal elements and microbial symbionts (Korotkova 1981a; Simpson 1984; Harrison and De Vos 1991).

2.1 Aquiferous System

The circulatory aquiferous system is the most characteristic feature of the poriferan organization. It comprises the following elements (Fig. 2): ostia, inhalant canals, apopyle, choanocyte chambers, prosopyle, exhalant canals and osculum.

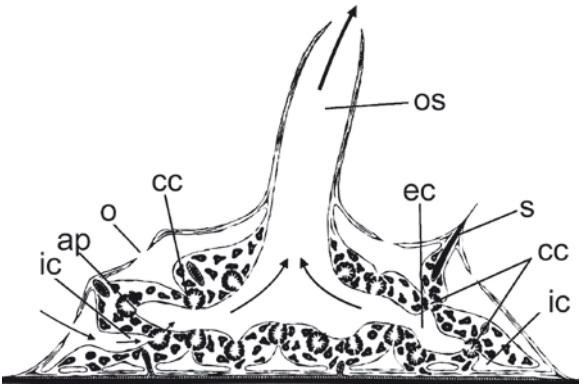

Fig. 2 Diagram of young sponge with leuconoid aquiferous system. *ap* apopyle, *cc* choanocyte chamber, *ec* exhalant canal, *ic* inhalant canal, *o* ostium, *os* osculum, *s* spicule (From Weissenfels 1975, reproduced by permission of Springer)

Water drawn into the inhalant canals via small pores called *ostia* moves to *choanocyte chambers* and then, via the system of the exhalant canals, to the large excurrent *osculum*. The unidirectional flow of water is ensured by the coordinated beating of the choanocytes' flagella. In the sponges with cellular organization, food particles and oxygen are captured from water by various cells, including choanocytes. The cells that are not included into the epithelia participate in the transport of the food particles and oxygen inside the sponge body. The aquiferous system is a modular, easily rearranged system (Gaino et al. 1995; Plotkin et al. 1999; Ereskovsky 2003). Its main physiological functions are transport and excretion of food particles, respiration and the release of the gametes and the larvae. At the same time, some representatives of the families Cladorhizidae and Esperiopsidae (Poecilosclerida, Demospongiae) lack all the elements of the aquiferous system (Vacelet 2006, 2007; Ereskovsky and Willenz 2007).

There are four main type of aquiferous system: (1) *asconoid* – the internal cavities are completely lined with choanocytes (Fig. 3a); (2); *syconoid* – the elongated choanocyte chambers pass through the whole sponge body from the cortex to the atrium (Fig. 3b); (3) *sylleibid* – the elongated choanocyte chambers are arranged radially around an invagination of the atrium cavity (Fig. 3c); (4) *leuconoid* – choanocytes are arranged into separate choanocyte chambers scattered in the mesohyl (Fig. 3d).

Choanocyte chambers are the basic elements of the aquiferous system. These chambers can be considered to be organ-like assemblies. The function of the choanocyte chambers as organs or organ-like assemblies is to orient the water flow unidirectionally from the incurrent to the excurrent to allow the extrusion of the water from the body through the exhalant oscule(s). They are divided into three types: aphodal, diplodal and eurypilous (Fig. 4). Aphodal chamber is connected directly with the inhalant canals through prosopyles and with the exhalant canal through an apopyle extended by an aphodus (Fig. 4a). Only one chamber opens into

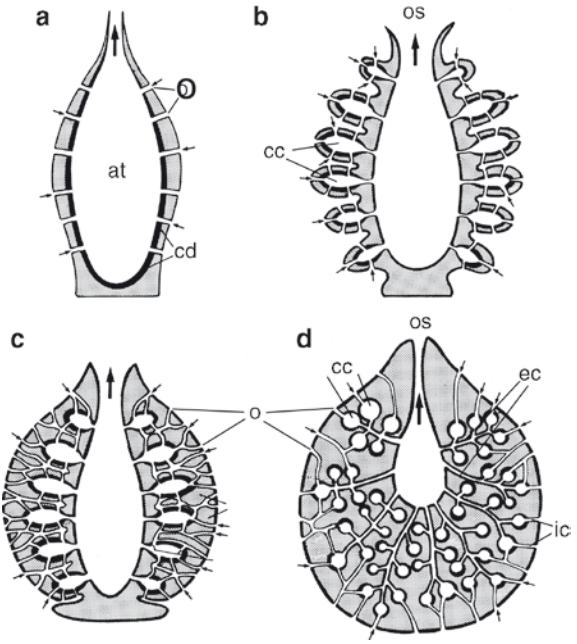

Fig. 3 Diagrams of different types of aquiferous systems in sponges. (**a**) asconoid; (**b**) syconoid; (**c**) sylleibid; (**d**) leuconoid. *at* atrium, *cc* choanocyte chamber, *cd* choanoderm, *ec* exhalant canal, *ic* inhalant canal, *o* ostium, *os* osculum (After Hyman 1940)

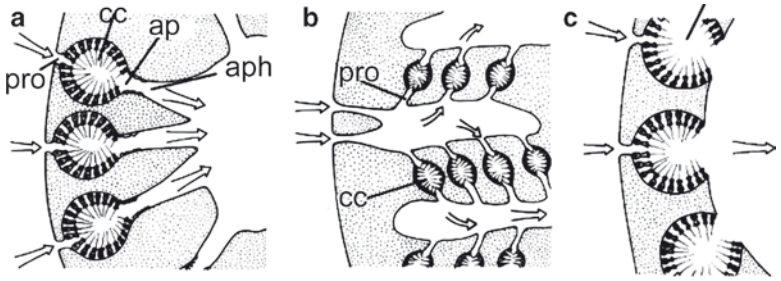

Fig. 4 Diagrams of different types of choanocyte chambers: (**a**) aphodal; (**b**) diplodal; (**c**) eurypilous. *ap* apopyle; *aph* aphodus; *cc* choanocyte chamber, *pro* prosopyle (From Boury-Esnault and Rützler 1997, reproduced by permission of Smithsonian Institution Scholarly Press)

one aphodus. Diplodal chamber connects with the inhalant canals through small canal (prosodus) and with the excurrent canal through an apopyle extended by an aphodus (Fig. 4b). Eurypylous is the type of chamber that connects directly with the inhalant canals through prosopyles and with the excurrent canal through an apopyle (Fig. 4c).

2.2 Tissue Organization

The interpretation of the sponge tissue organization is one of the most debated problems of their organization and biology (Bergquist 1978; Korotkova 1981a, 1997; Simpson 1984; Harrison and De Vos 1991; Seravin 1992; Efremova 1997; Ereskovsky 2005; Ereskovsky and Dondua 2006; Leys 2007).

Tissues of the multicellular animals are divided into two categories: the epithelial ones and the parenchymal ones. In histology, the tissue is understood as a historically formed system of elements (cells and the intercellular structures formed by them) united by a common function and structural-chemical organization (Fawcett 1994). There are four main types of tissues: (1) bordering (epithelial) tissues, (2) the tissues of the internal environment (blood, interstitial tissues, and skeletal tissues), (3) nervous system tissues and (4) muscular tissues (Fawcett 1994). Since sponges lack the latter two types, we will analyse their bordering tissues and the tissues of the internal environment.

The epithelial organization is an important characteristic of the multicellular animals, which preserve the covering epithelium throughout the life cycle (Tyler 2003). An embryonic cell layer can be considered an epithelium if their apical surface is free and non-adhesive and the baso-lateral surface contacts the embryonic cells (see Hay 1968).

An epithelium is defined as a sheet of polarized cells. The cells are joined by belt-like junctions around their apical margins, and extracellular matrix (ECM) is typically present only apically and basally due to the close apposition of cells within the epithelium (Tyler 2003). Metazoan epithelial tissues have two primary characteristics of system organization: the structural unification of the epithelial cells into uninterrupted layers or cords, functioning as integral systems, and the polarity, resulting from their bordering position (Fawcett 1994).

The tissues of the internal environment are the complex of tissues forming the internal environment of an organism and maintaining its stability (Fawcett 1994). There are three kinds of such tissues: (1) loose connective tissues (parenchyma, mesoglea and mesohyl), (2) skeletal and supportive tissues and (3) protective tissues (blood, lymph). Their main functions are the trophic function, the structural function, the maintenance of chemical and osmotic composition and the protective (immune) function.

In sponges, the bordering tissues and the tissues of the internal environment are simpler, both structurally and functionally, than in the other Metazoa. In particular, sponge tissues are always more multifunctional than their counterparts in advanced animals. Besides, the cells of sponge tissues possess a very high capacity of transdifferentiation into cells of other types (Korotkova 1981a, 1997; Gaino et al. 1995). At the same time, Porifera lacks a single category of totipotent cells, the representatives of each clad possessing its own system of stem cells: archaeocytes in the Demospongiae, choanocytes in the Calcarea, pinacocytes in the Homoscleromorpha and, apparently, archaeocytes in the Hexactinellida.

2.2.1 The Epitheliums

Pinacoderm is represented by the exo-, baso- and endopinacoderm. *Exopinacoderm* forms the external cover of the sponge. *Basopinacoderm* develops at the sponge base, attaching it to the substrate. *Endopinacoderm* forms the walls of the subdermal cavities and the aquiferous system canals. There are, correspondingly, several types of pinacocytes.

Exopinacocytes are the covering cells of the sponge. They may be spindle-shaped or T-shaped at cross section (Fig. 5). Spindle-shaped exopinacocytes are described in many Demospongiae from the orders Spongillidae and Poecilosclerida (Bagby 1970; Weissenfels 1989), in all the Homoscleromorpha (Muricy et al. 1996, 1999) and in the Calcarea (Borojevic 1969; Eerkes-Medrano and Leys 2006). T-shaped exopinacocytes are described in many Demospongiae and in the Calcarea (see Boury-Esnault 1973; Willenz and Hartman 1989).

The surface part of an exopinacocyte is polygonal in shape and covered with a mucous layer of self-secreted glycocalyx. Owing to the latter, a food particle to be phagocyted is first glued to the cell surface. Exopinacocytes lack specialized cell junctions such as desmosomes or *macula adhaerens*, but are united with a well-developed adhesive system (Blumbach et al. 1998; Müller 1982; Schütze et al. 2001). In *Hippospongia communis*, *Ephydatia fluviatilis*, *Sycon coactum, Oscarella lobularis* and *O. tuberculata* at the site of exopinacocytes' contacts there are electron-dense thickenings of the membranes resembling *zonula adhaerens* (Fig. 6) (Pavans de Ceccatty et al. 1970; Pottu-Boumendil 1975; Eerkes-Medrano and Leys, 2006; Ereskovsky and Tokina, 2007). Exopinacocytes can secrete components of the extracellular matrix and synthesize collagen (Garrone 1978; Simpson 1984; Boute et al. 1996). Exopinacocytes of the Homoscleromorpha are closely associated with the underlining dense fibrillar layer, the basal membrane, comprising collagen IV, laminin and tenascin. This basal membrane is identical to the *lamina reticulat*ain the basal lamina of the epithelia of vertebrates (Fig. 7) (Humbert-David and Garrone 1993; Boute et al. 1996).

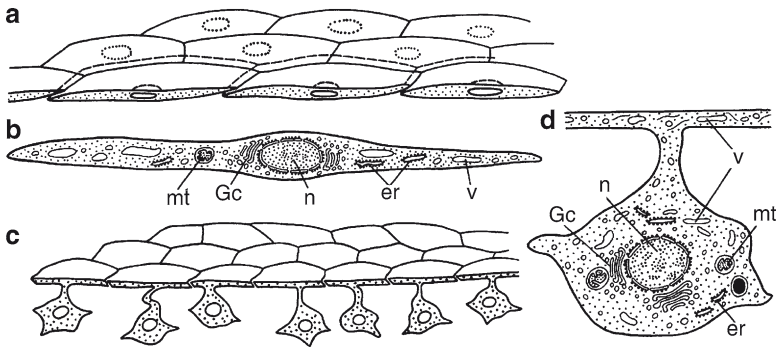

Fig. 5 Diagram of sponge's pinacoderm and pinacocytes. (**a**) pinacoderm, formed by spindle-shaped pinacocytes (**b**), (**c**) pinacoderm, formed by T-shaped pinacocytes (**d**). *er* endoplasmic reticulum, *Gc* Golgi complex, *mt* mitochondria, *n* nucleus, *v* vacuole

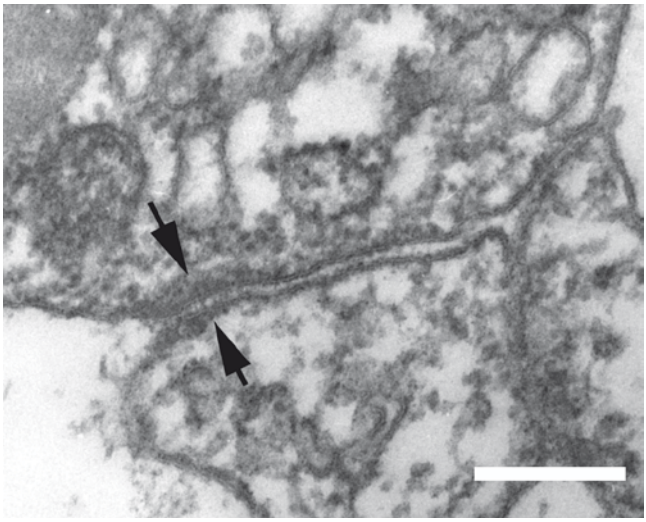

Fig. 6 Transmission electron microscopy (TEM) of cell junction (*arrows*) between the endopinacocytes of *Oscarella lobularis* (Homoscleromorpha). Scale bar 3 μm

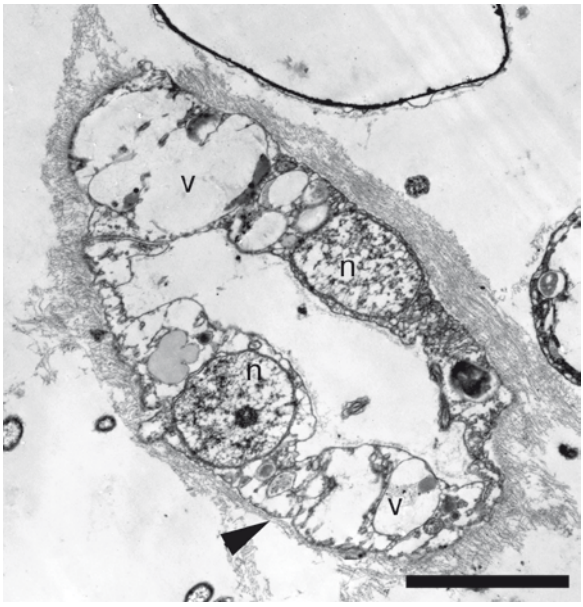

Fig. 7 TEM of basal membrane (*arrowhead*) under the endopinacocytes of *Oscarella tuberculata* (Homoscleromorpha). *n* nucleus, *v* vacuole. Scale bar 3 μm

Unique characteristics of the homoscleromorph exopinacocytes are the flagellum and the ability to synthesize spicules (Donadey 1979; Muricy et al. 1996; Ereskovsky and Tokina 2007; Maldonado and Riesgo 2007).

Exopinacoderm contains *ostia* – numerous microscopic structures which are 4-100 μm in diameter – through which the water is drawn into the aquiferous system of the sponge. In most Demospongiae and in all Homoscleromorpha ostia are intercellular (Fig. 8a). In the Calcarea, the ostia are formed inside special cylindrical tubular cells, *porocytes* (Fig. 8b) (Jones 1966; Borojevic 1969; Eerkes-Medrano and Leys 2006). In *S. coactum*, porocytes can contract in response to the mechanical stimulation and treatment with anaesthetics (Eerkes-Medrano and Leys 2006). Porocytes of some Demospongiae from order Haplosclerida are flattened cells with a central or peripheral opening, which can open and close like a sphincter (Fig. 8c) (Harrison 1972a; Weissenfels 1980; Willenz and Van de Vyver 1982; Harrison et al. 1990; Langenbruch and Scalera-Liaci 1986).

Exopinacoderm exhibits many functions characteristic of the typical eumetazoan epithelia, such as absorption, secretion, transport, excretion and protection (see Simpson 1984; Harrison and De Vos 1991; Meyer et al. 2006).

Basopinacocytes are flattened cells at the basal surface of the sponge whose main function is attachment of the sponge to the substrate. Synthesizing basal spongin and fibronectin, basopinacocytes function as spongocytes (see below) (Garrone and Pottu 1973; Garrone and Rosenfeld 1981; Labat-Robert et al. 1981). In the course of sponge growth, marginal basopinacocytes actively secrete proteins that make up spongin (Garrone 1978).

In the coralline demosponge *Acanthochaetetes wellsi* (order Hadromerida), basopinacocytes take part in the formation of the massive basal calcareous skeleton (Reitner and Gautret 1996). Basopinacocytes of other demosponges with a massive calcareous skeleton, *Ceratoporella nicholsoni* and *Stromatospongia norae* (Agelasida), also participate in its synthesis (Willenz and Hartman 1989).

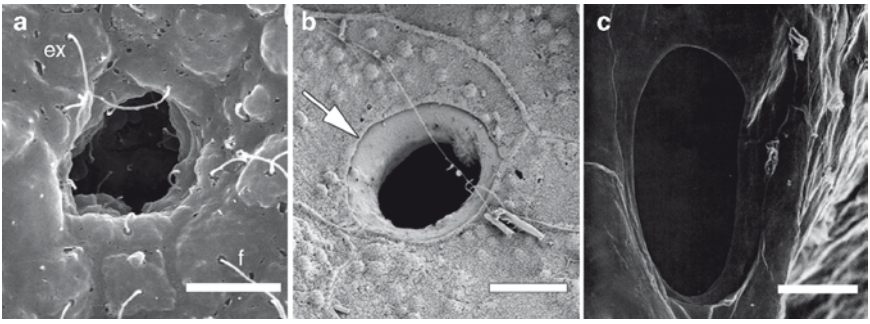

Fig. 8 Sponge's ostia. (**a**) SEM of intercellular ostium of *Oscarella lobularis* (Homoscleromorpha); (**b**) SEM of ostia formed inside of porocytes in *Sycon coactum* (Calcarea) (From Eerkes-Medrano and Leys 2006, reproduced by permission of Wiley). (**c**) SEM of porocyte of *Ephydatia fluviatilis* (Demospongiae, Haplosclerida). (From Willenz and Van de Vyver 1982, reproduced by permission of Elsevier, Ltd). *ex* exopinacocytes, *f* flagellum. Scale bars (**a**) 10 μm, (**b**) 0.5 μm, (**c**) 5 μm

As shown on the freshwater demosponges *Ephydatia muelleri* and *Spongilla lacustris*, basopinacocytes have a well-organized cytoskeleton (Pavans de Ceccatty 1986; Wachtmann et al. 1990; Wachtmann and Stockem 1992a, b; Kirfel and Stockem 1997). Actin is located in the cortical layer (directly below the plasma membrane) and in the fibrils in the cytoplasmic matrix. Microtubules radiate from the perinuclear zone, finishing at the cell periphery. At the same time, intermediate filaments have not been described (Fig. 9). In *E. muelleri*, basopinacocytes were shown to have desmosome-like junctions (Pavans de Ceccatty 1986).

Endopinacocytes are flattened, polygonal cells, spindle-shaped at cross section (Fig. 10). Endopinacocytes are divided into *prosopinacocytes*, lining the inhalant canals, and *apopinacocytes*, lining the exhalant canals. In all Homoscleromorpha (Fig. 10a, b) (Boury-Esnault et al. 1984; Vacelet et al. 1989) and in some Demospongiae endopinacocytes bear flagella. In particular, this is the case of *Tethya lyncurium* (Hadromerida) (Pavans de Ceccatty 1966) and of most studied representatives of the orders Dictyoceratida and Dendroceratida (Thiney 1972; Donadey 1982; Vacelet et al. 1989; Boury-Esnault et al. 1990). The presence of flagella appears to be associated with the involvement of endopinacocytes in the water circulation in the aquiferous system. In the sclerosponges *C. nicholsoni* and *S. norae* (Agelasida), endopinacocytes form membrane partitions perpendicular to the canals (Willenz and Hartman 1989), which are also supposed to be involved in regulation of the water passage in the sponge.

In most Demospongiae and Homoscleromorpha studied, the external surface of the endopinacocytes is covered with a glycocalyx layer (Harrison and De Vos 1991;

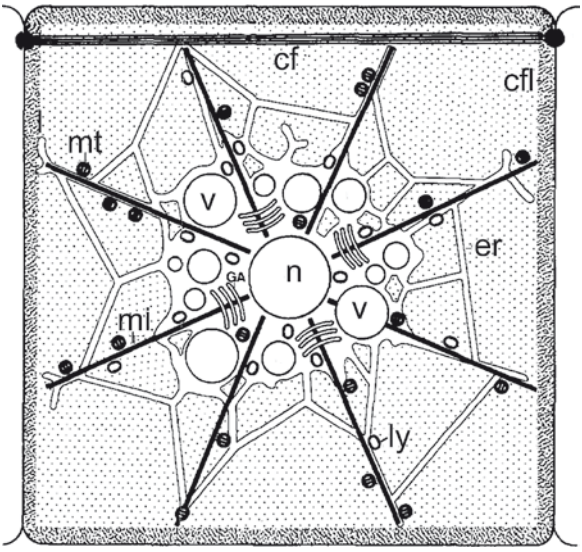

Fig. 9 Schematic drawing of the cytoplasm organization of *Spongilla lacustris* basopinacocyte (Demospongiae, Haplosclerida). *cfl* cortical filament layer, *l* lipid droplets, *ly* endosomes and lysosomes, *mi* microtubules, *mt* mitochondria, *n* nucleus, *v* vacuoles of the osmoregulating system (From Wachtmann and Stockem 1992a, reproduced by permission of Springer)

Boury-Esnault et al. 1984; Simpson 1984; Vacelet et al. 1989). The basal surface often forms numerous projections (pseudopodia) for anchoring in the extracellular matrix (Fig. 10b).

Generally, endopinacocytes contact each other by simple fitting. However, in the oscular tubes of freshwater sponges the cells of the endopinacoderm are united by desmosome-like junctions (Masuda et al. 1998). Endopinacocytes of *Oscarella* (Homoscleromorpha) are joined by *zonula adhaerens* junctions (Ereskovsky and Tokina 2007; Ereskovsky et al. 2009a).

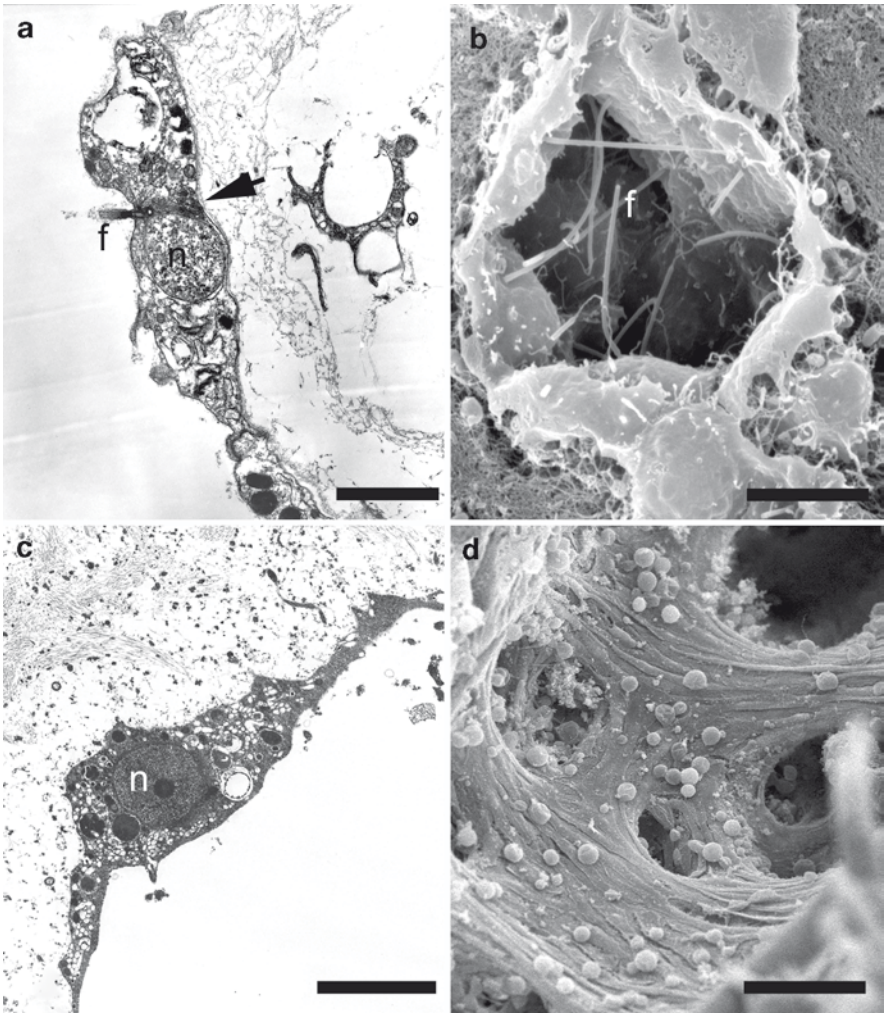

Fig. 10 Endopinacocytes. (**a**) TEM of an endopinacocyte of *Oscarella* sp. (**b**) SEM of an endopinacocytes of *Oscarella malakhovi.* (**a**, **b**) Homoscleromorpha. (**c**) TEM of an endopinacocyte of *Halisarca caerulea* (Demospongiae, Halisarcida) (Courtesy of J. Vacelet). (**d**) SEM of an endopinacoderm of *Hippospongia communis* (Demospongiae, Dictyoceratida). *Arrow* basal membrane, *f* flagellum, *n* nucleus. Scale bars (**a**, **c**) 3 μm, (**b**) 5 μm, (**d**) 50 μm

During growth of *Oscarella* (Homoscleromorpha), exopinacocytes differentiate into endopinacocytes (Gaino et al. 1987a). The latter, in their turn, can differentiate into the vacuolar cells of the mesohyl (Gaino et al. 1986a).

Endopinacocytes and choanocytes of the demosponges are thought to be functionally and ontogenetically interrelated. This is supported, in particular, by observations on *Suberites massa* (Hadromerida): its choanocytes can differentiate into endopinacocytes by reduction of the flagellum and the microvilli and the subsequent flattening of the cell (Diaz 1974).

In some Demospongiae, endopinacoderm contains special contractile cells, the *myocytes*. These long spindle-shape cells are inbuilt into the oscular tube wall of *Microciona prolifera,* the oscular sphincter of *Tedania ignis* (Poecilosclerida) and in *Aplysina cavernicola* (Verongida) (Bagby 1966; Vacelet 1966). The myocytes of *M. prolifera* contain two types of filaments: fine filaments, 50-70 nm in diameter, that form clusters around the larger ones, 150-250 nm in diameter. The myocytes of *T. ignis* have only one type of filament, 100 to 200-300 nm (Bagby 1966). Epithelial myocytes are supposed to differentiate from pinacocytes (Bagby 1970). In freshwater sponges, contractile actin filaments are present not in myocytes but in the endopinacocytes of the oscular tubes (Masuda et al. 1998).

***

Only the Homoscleromorpha have epithelium of the eumetazoan type, i.e. with a basal membrane containing collagen IV, tenascin and laminin (Humbert-David and Garrone 1993; Boute et al. 1996). The other sponges with cellular organization (Demospongiae and Calcarea) do not have a basal membrane. At the same time, the spatial organization of the cytoskeleton in the basopinacoderm of freshwater demosponges is characterized by a high degree of regularity and coordination of reaction, which are the attributes of the eumetazoan epithelia (Pavans de Ceccatty 1986; Wachtman et al. 1990). This integrated cytoskeletal organization of the basal epithelium was called the *hystoskeleton* (Pavans de Ceccatty 1986).

It is considered that typical epithelia either occupy a relatively stable surface position or line various cavities. Contrary to the Eumetazoa, the pinacocytes of sponge epithelia are contractile; they can also become amoeboid and move. For instance, the basopinacocytes of *Corvomeyenia carolinensis* become amoeboid before flattening at the new site (Harrison 1972b). It was shown that some exo- and basopinacocytes of *E. fluviatilis* can migrate (Weissenfels 1978). This unusual ability of sponge epithelia appears to be associated with the lack of true desmosomes and the presence of a well-developed network of cytoskeletal actin microfilaments associated with a system of microtubules (Pavans de Ceccatty 1986).

The pinacocytes of all types (exo-, baso, and endopinacocytes) can phagocyte. This was proved both by experiments revealing the presence of phagolysosomal acid phosphatase and by direct observations (Willenz and Van de Vyver 1984; Pottu-Blumendil 1975; Diaz 1979; Simpson 1963; Harrison 1972a). The pinacocytes are also involved in excretion.

Choanoderm consists only of choanocytes that formed the choanocyte chambers (or choanoderm in asconoid sponges) (Fig. 11). Contrary to pinacoderm, choanoderm is a cubic or palisade epithelium. Choanocytes can be cylindrical, cubic, trapezoid or slightly flattened. These cells bear a flagellum surrounded with a collar of cytoplasmic microvilli connected by glycocalyx bridges.

Choanocytes may be different both in different species and in one and the same sponge, their morphology depending on environmental conditions, physiological state or ontogenetic stage. These cells, however, do have some common characters. The nucleus occupies the apical, central or basal position, the chromatin is very condensed, the nucleolus may be present in the choanocytes of some demosponge groups (Figs. 11c, 12d) (Boury-Esnault et al. 1984; Vacelet et al. 1989), in choanoblasts

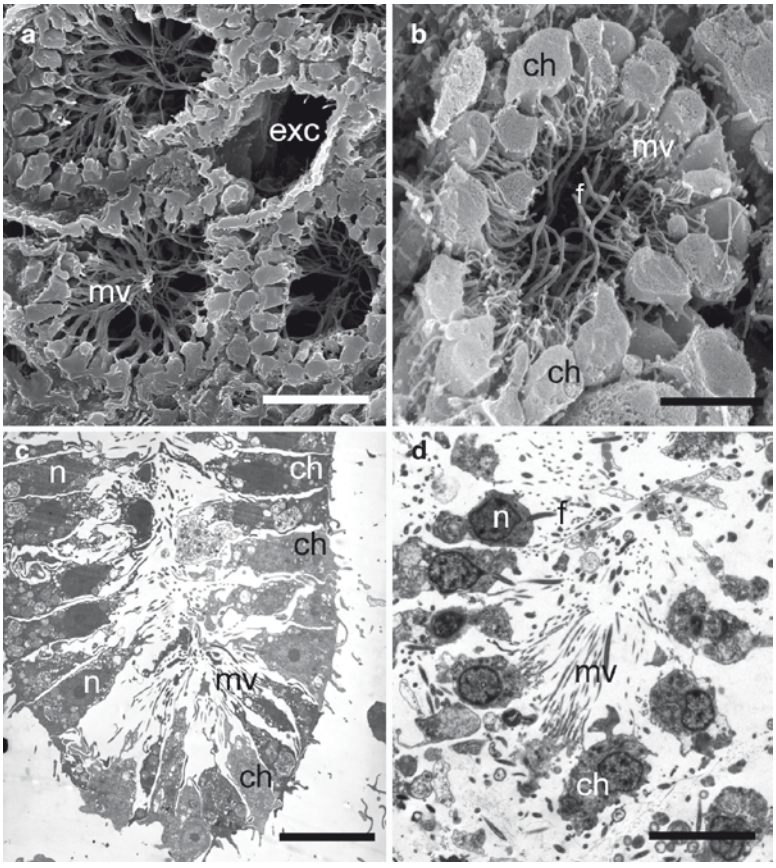

Fig. 11 Choanocyte chambers of (**a**) *Oscarella malakhovi* (Homoscleromorpha) SEM, (**b**) *Hymedesmia irregularis* (Demospongiae, Poecilosclerida) SEM, (**c**) *Halisarca dujardini* (Demospongiae, Halisarcida) TEM and (**d**) *Hymedesmia irregularis* (Demospongiae, Poecilosclerida) TEM. *ch* choanocyte, *exc* exhalant canal, *f* flagellum, *mv* microvilli, *n* nucleus. Scale bars (**a**) 15 μm, (**b**) 5 μm, (**c**) 20 μm, (**d**) 5 μm

differentiating into choanocytes in the course of asexual (Jetton et al. 1987) or sexual (Ereskovsky et al. 2007a) development and in choanocytes transforming into 'blastogenic archaeocytes' during gemmulogenesis of *Suberites domuncula* (Hadromerida) (Connes et al. 1974).

The flagellum starts from a small knob or circular depression at the apical cell surface (Fig. 12a, c-f). In some demosponges from the orders Hadromerida and Halisarcida the base of the flagellum is surrounded with a high cytoplasmic cuff (Figs. 11c, 12a, b) (Connes et al. 1971; Diaz 1979; Vacelet et al. 1989; De Vos et al. 1991; Boury-Esnault et al. 1994).

The apical cell surface within the microvilli collar, the basal part of the flagellum and the internal surface of the microvilli are covered with a thin layer of glycocalyx. In sponges from various groups the choanocyte may bear a pair of flat lateral wing-like structures (Feige 1969; Mehl and Reiswig 1991). The structure of the axoneme is typical of the Eukaryota. The basal apparatus comprises two centrioles oriented perpendicular or, as in the Halisarcida, at an angle to each other. The Golgi complex lies between the centrioles and the nucleus.

The choanocyte collar comprises 20–55 microvilli, the number correlating with the taxonomic position of the sponge (De Vos et al. 1991). The microvilli may be connected to each other by thin glycocalyx cords (Fig. 12b, e) (Boury-Esnault et al. 1984; De Vos et al. 1991; Eerkes-Medrano and Leys 2006) or cytoplasmic projections (Brill 1973; De Vos 1977; Watanabe 1978a), forming a fine honeycombed network. The microvilli are often fused at the basis (Fig. 12e). The choanocytes have a well-developed cytoskeleton, which includes F-actin and myosin; the latter are especially apparent in the dissociated cells of *Clathrina cerebrum* (Calcinea) (Burlando and Gaino 1984; Gaino and Magnino 1998).

The choanocyte chambers of the Demospongiae are usually underlined with a more or less loose layer of the extracellular matrix containing collagen, and those of the Homoscleromorpha, with a true basal membrane containing collagen IV (Humbert-David and Garrone 1993; Boute et al. 1996; Muricy et al. 1996; Ereskovsky 2006). In the basal part of the choanocytes of many demosponges, cytoplasmic projections are formed, which not only anchor the cells in the extracellular matrix, but also intertwine to lend the mechanical support to the choanoderm. Such structures are especially characteristic of the Halisarcida (Fig. 11c). The choanocytes contact by simple fitting, either in the basal or in the middle part (Boury-Esnault et al. 1984, 1990; Vacelet et al. 1990). In Homoscleromorpha (*O. tuberculata* and *O. lobularis* (Ereskovsky and Tokina 2007) and in the calcareous sponge *S. coactum* (Eerkes-Medrano and Leys 2006) the choanocytes form specialized intercellular desmosome-like junctions.

Apopylar cells are a special cell type. Intermediate between the apopinacocytes and choanocytes, these flagellated cells form the border between the exhalant canal and the choanocyte chamber. Apopylar cells are triangular at cross section; the edge facing the chamber bears a comb of microvilli (Fig. 13).

Apopylar cells are characteristic of all the Homoscleromorpha (Fig. 13a, b), Halisarcida, Dictyoceratida and Dendroceratida (Boury-Esnault et al. 1984; Vacelet et al. 1990; De Vos et al. 1990; Muricy et al. 1996; Ereskovsky 2006; Ereskovsky

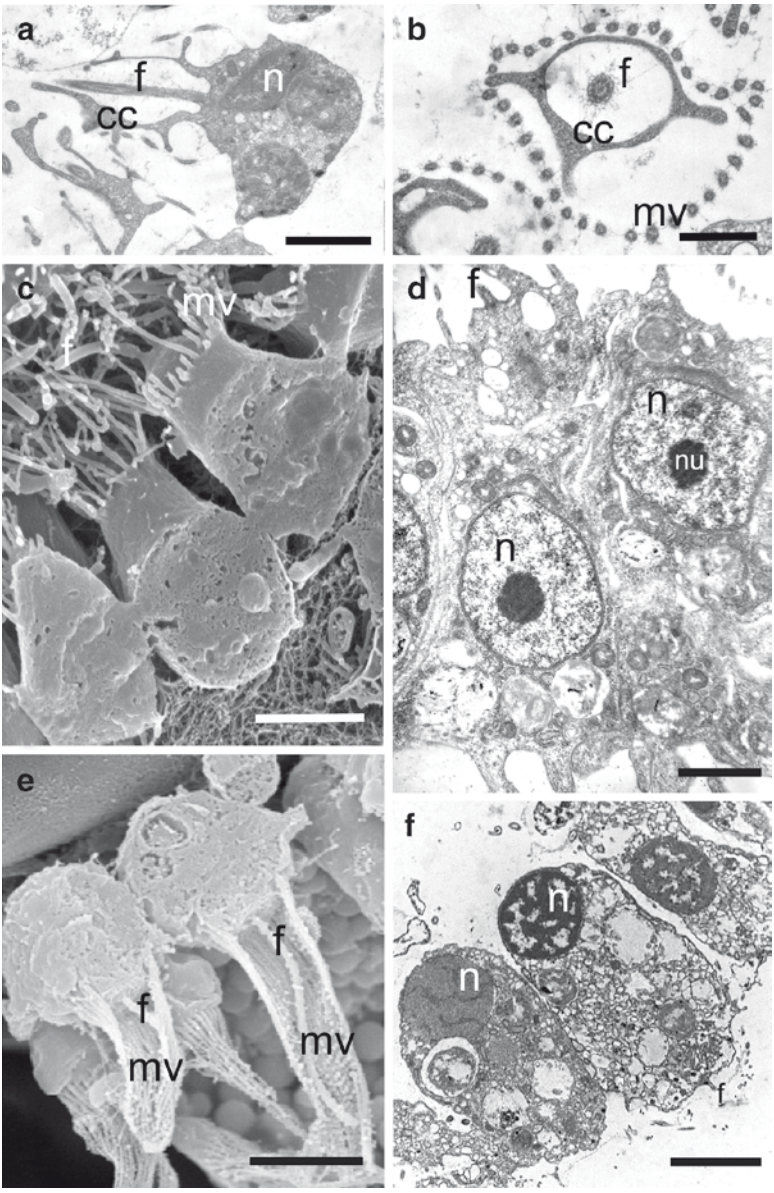

Fig. 12 Choanocytes. (**a**, **b**) Choanocytes with cytoplasm cuff (*cc*) of *Acanthochaetetes wellsi* (Demospongiae, Hadromerida) TEM longitudinal section of cell (**a**) and transversal section of the cuff (**b**), flagellum (*f*) and microvilli (*mv*). (**c**) SEM of longitudinal section of the choanocytes of *Oscarella malakhovi* (Homoscleromorpha). (**d**) TEM of longitudinal section of choanocytes of *Halisarca dujardini* (Demospongiae, Halisarcida). (**e**) SEM of choanocytes of *Clathrina cerebrum* (Calcarea, Calcinea) with the microvilli fused at the basis. (**f**) TEM of longitudinal section of choanocytes of *Clathrina clathrus* (Calcarea, Calcinea). *n* nucleus, *nu* nucleolus. (**a**, **b** – Courtesy of J. Vacelet). Scale bars (**a**, **d**, **e**) 5 μm, (**b**, **c**, **f**) 2 μm

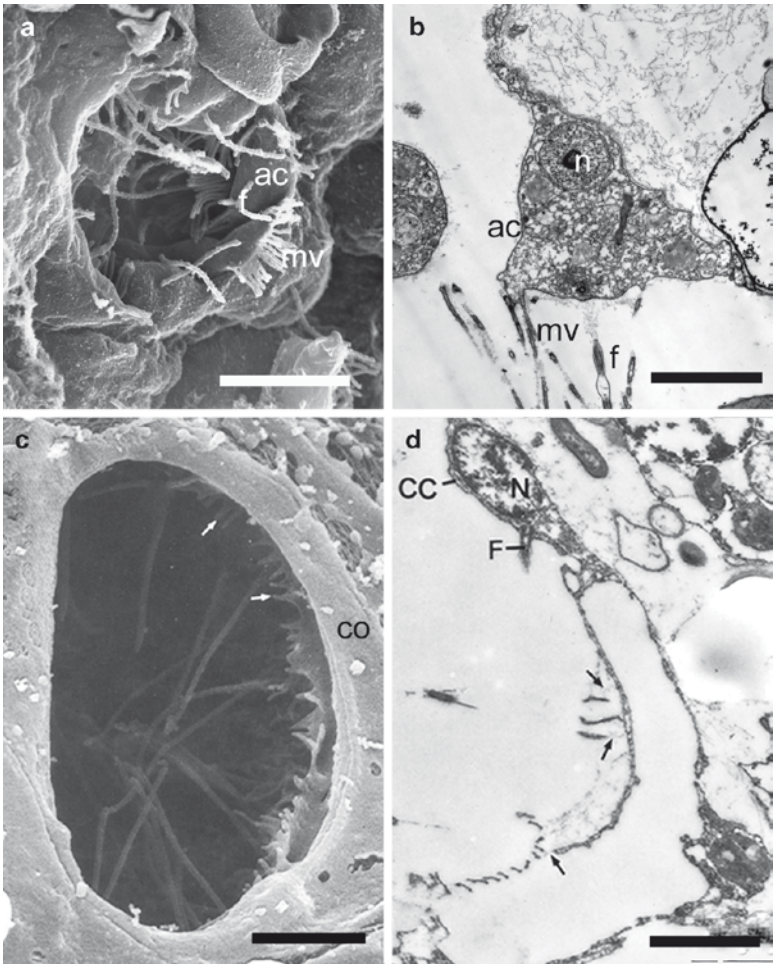

Fig. 13 Apopylar cells. (**a**) SEM of *Oscarella lobularis* (Homoscleromorpha), (**b**) TEM of *Oscarella viridis* (Homoscleromorpha), (**c**) SEM of a cone cells (*co*) of *Niphates digitalis* (Demospongiae, Haplosclerida) and (**d**) TEM of a cone cell of *Reniera sarai* (Demospongiae, Haplosclerida). *ac* apopylar cell, *cc* cone cell, *f* flagellum, *mv* microvilli, *n* nucleus. (**c**, **d** – After Langenbruch 1988, reproduced by permission of Springer). Scale bars (**a**, **b**) 4 μm, (**c**) 5 μm, (**d**) 2 μm

and Tokina 2007; Ereskovsky et al. 2009). They were also described, under the name of cone cells, in some demosponges from the orders Haplosclerida (Fig. 13c, d) (Weissenfels 1981; Langenbruch et al. 1985; Langenbruch and Scalera-Liaci 1986; Langenbruch and Weissenfels 1987; Langenbruch 1988). It is supposed that the main role of the apopylar cells is regulation of the hydrodynamics in the sponge.

Central cells are another type of cells in the choanocyte chambers. First discovered by Sollas (1888), they were repeatedly recorded at the light microscopic level (see Reiswig and Brown 1977), though only ultrastructural studies clarified their structure and function (Connes et al. 1972; Diaz 1979; Langenbruch and

Scalera-Liaci 1986; Langenbruch and Jones 1989; Sciscioli et al. 1997). In *S. massa*, the central cells have an irregular, branched shape with numerous projections and holes. The cell is perforated with a vast canal into which enter the choanocytes' flagella (Diaz 1979) (Fig. 14a). Ultrastructurally, the central cells are identical to the choanocytes. In *Pellina fistulosa* and *P. semitubulosa* (Haplosclerida), the central cells look like a perforated membrane situated at the

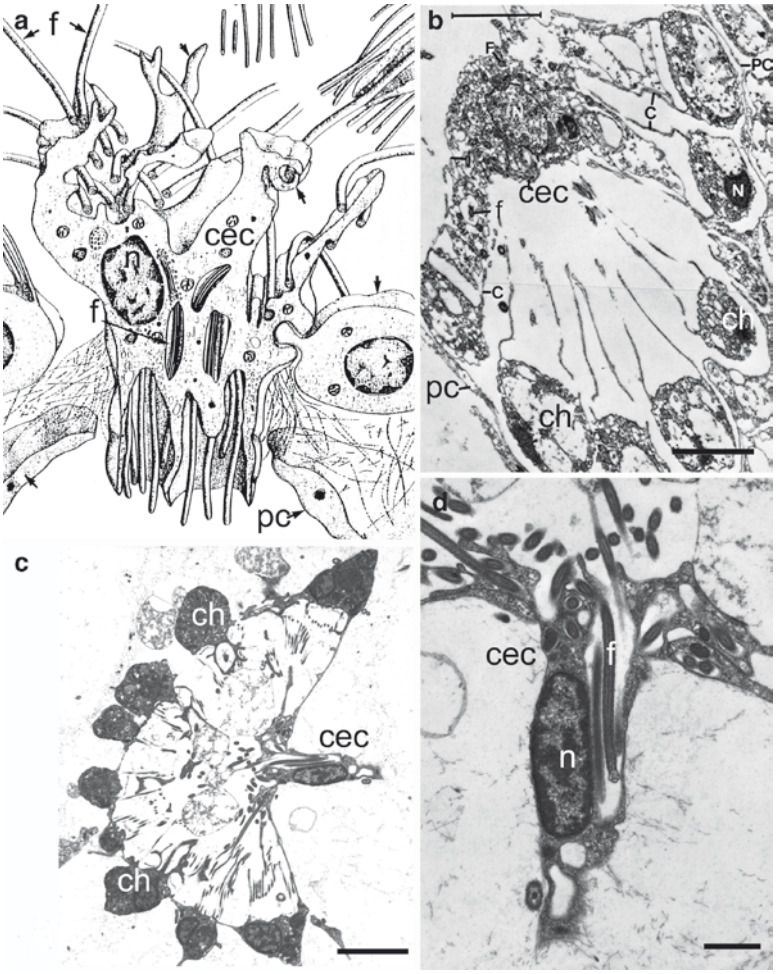

Fig. 14 Central cells of some demosponges. (**a**) Diagram of the central cell of *Suberites massa* (Demospongiae, Hadromerida). (**b**) TEM of a central cell of *Haliclona elegans* (Demospongiae, Haplosclerida) extending three processes into choanocyte *(ch)* collars (c), choanocyte flagella (*f*) penetrate the central cell it is a pinacocyte cover (*pc*) at the outer chamber surface. (**c**, **d**) TEM of a central cell of *Acanthochaetetes wellsi* (Demospongiae, Hadromerida) and its detail (**d**). *cec* central cell, *n* nucleus (**a** – Courtesy of J. Diaz; **b** – From Langenbruch and Scalera-Liaci 1986, reproduced by permission of Springer; **c**, **d** – Courtesy of J. Vacelet). Scale bars (**b**) 3 μm, (**c**) 10 μm, (**d**) 3 μm

level of the distal surface of the choanocytes' collars in each chamber (Fig. 14b) (Langenbruch and Jones 1989; Sciscioli et al. 1997). Interestingly, the central cells are present not in all the choanocyte chambers of a sponge. For instance, in *Haliclona elegans* (Haplosclerida) they are present only in 15% of the chambers (Langenbruch and Scalera-Liaci 1986).

The origin of the central cells is not quite clear: they were supposed to originate both from choanocytes (Reiswig and Brown 1977) and from endopinacocytes (Langenbruch and Scalera-Liaci 1986). The central cells participate in the regulation of beating of the choanocyte flagella within a chamber.

As noted above, sponges lack the gut epithelium and the digestive parenchyma. Food particles may be captured by almost all the cells of the covering and the internal tissues (Diaz 1979; Willenz and Van de Vyver 1982, 1984; Hahn-Keser and Stockem 1997). Therefore, neither the choanoderm nor the aquiferous system with its canals should be considered as endoderm derivatives (Ereskovsky and Dondua 2006).

2.2.2 Tissues of the Internal Environment

Taken together, the tissues of the internal environment of the sponges with cellular organization make up the *mesohyl*. There the cells, symbiotic bacteria and skeletal elements are loosely embedded in the ground matrix of mesohyl, composed primarily of collagen, galectin and glycoconjugates. Drawing an analogy with other animals, we can delimit the mesohyl of these sponges, according to the functional specialization of certain cell groups, the *supporting-connective* and the *protective-secretory* tissue (Korotkova 1981a). A characteristic feature of the sponges with cellular organization is that all the tissues of the internal environment are populations of free, mobile cells capable of transdifferentiation.

Supportive-Connective Tissue

The main function of this tissue is formation of the organic and the mineral skeleton, the supporting structures and the extracellular matrix of the mesohyl.

Collencytes, a variety of fibroblasts, are mobile cells secreting collagen. They were first described by Sollas (1888). According to the first definition, collencytes are stellate or spindle-shaped cells with branching pseudopodia and an ovoid anucleolate nucleus; their cytoplasm contains some non-specific inclusions (Borojevic 1966). It is possible, however, that the cells referred to as collencytes are actually a heterogeneous population (Simpson 1984; Harrison and De Vos 1991). Experiments on tissue transplantation demonstrate their involvement in immune reactions (Van de Vyver and Barbieux 1983; Buscema and Van de Vyver 1984a, b; Custodio et al. 2004). Interestingly, the level of the collencyte population remains rather high in allogenic pairs but drops significantly in the control. Most often, the name of collencytes has been applied to lophocytes.

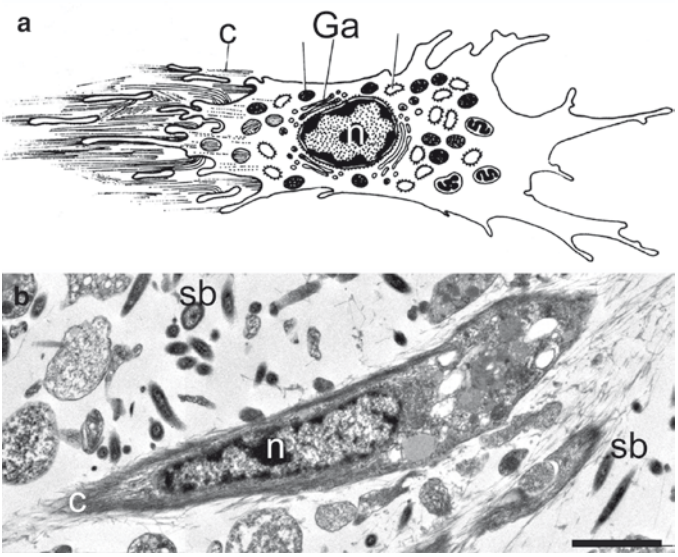

Fig. 15 Lophocytes. (**a**) Diagram of the lophocyte. (**b**) TEM of a lophocyte of *Hymedesmia irregularis* (Demospongiae, Poecilosclerida). *c* collagen, *Ga* Golgi apparatus, *n* nucleus, *sb* symbiotic bacteria. Scale bar (**b**) 2 μm

Lophocytes are considered a variety of collencytes (Lévi 1970; Boury-Esnault and Rützler 1997), thus being a variety of fibroblasts. These amoeboid cells secrete the elements of the extracellular matrix and organize it into fibrillar bundles (Fig. 15). Lophocytes gliding upon the substrate have a distinct anterior-posterior polarity. The anterior pole bears short stellate pseudopodia (Bonasoro et al. 2001), while the posterior pole leaves a trace in the form of a bundle of collagen fibrils united by the glycoprotein matrix (Garrone 1978; Bonasoro et al. 2001). The nucleus always has a nucleolus, and the cytoplasm contains well-developed RER. Microtubules and microfilaments are always present. There are temporary non-specific inclusions such as vacuoles with dense or loose fibrillar material (Bonasoro et al. 2001) but no phagosomes.

Lophocytes are thought to be the derivatives of the archaeocyte line. In *Chondrosia reniformis* (Chondrosida), however, they were shown to differentiate from endopinacocytes (Pavans de Ceccatty and Garrone 1971).

Sclerocytes secrete mineral spicules (Fig. 16). Sclerocytes of the siliceous sponges accomplish the intracellular synthesis of spicules. Depending on the spicule size, sclerocytes are divided into *megasclerocytes* and *microsclerocytes*. Megasclerocytes have a large nucleolated nucleus, an active Golgi complex, a few phagosomes and a weakly developed endoplasmic reticulum (Custodio et al. 2002; Garrone et al. 1981; Simpson 1968; Wilkinson and Garrone 1980). Silica spicule is synthesized inside a vacuole formed by the *silicalemma* (Simpson and Vaccaro 1974). This very special membrane does not contact any other membranes of the cell. It differs strikingly from the plasma membrane in the presence of unusually

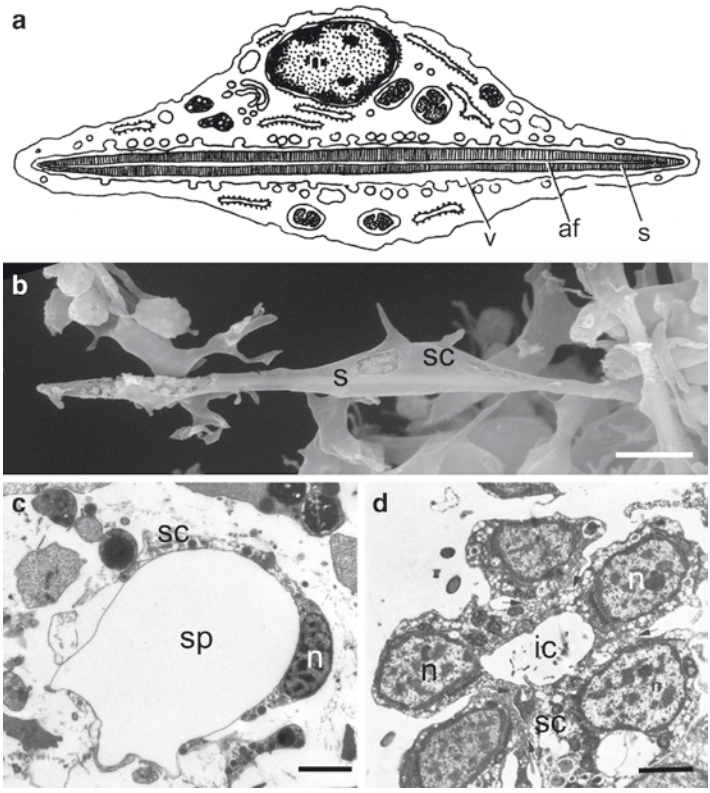

Fig. 16 Sclerocytes. (**a**) Diagram of the sclerocyte. (**b**) SEM of a macrosclerocyte of *Haliclona aquaeductus* (Demospongiae, Haplosclerida). (**c**) TEM of a microsclerocyte of *Crellomima imparidens* (Demospongiae, Poecilosclerida). (**d**) TEM of a group of sclerocytes of *Sycon ciliatum* (Calcarea, Calcaronea) and encloses an intercellular cavity *(ic)*. *Arrows* indicate the position of the septate junctions. *af* axial filament, *n* nucleus, *s* spicule, *sc* sclerocytes, *sp* spicule space, *v* perispicular vacuole. (**d** – From: Ledger and Jones 1977, reproduced by permission of Springer). Scale bars (**b**) 5 μm, (**c**, **d**) 2 μm

dense inter-membrane particles (Garrone and Lethias 1990; Uriz et al. 2000, 2003). At the early stages of spiculogenesis, sclerocytes secrete the axial filament, consisting of the silicatein protein, with a very low concentration of silica (Yourassowsky and Rastmont 1983).

Since a single megasclerocyte cannot fully form a large spicule, in sponges from the orders Astrophorida, Spirophorida, Hadromerida and Halichondrida, whose spicules are large, several sclerocytes associate for joint synthesis (see Uriz 2006). Microsclerocytes are smaller than macrosclerocytes and lack the nucleolus (Fig. 16c) (Wilkinson and Garrone 1980; Uriz et al. 2000, 2003; Uriz 2006).

In the Demospongiae, sclerocytes are the descendants of the archaeocytes.

Sclerocytes of the calcareous sponges accomplish the extracellular synthesis of spicules (Ledger and Jones 1977) and are represented only by megasclerocytes

(Simpson 1984). Cytologically, the megasclerocytes of the calcareous sponges are very similar to pinacocytes (Ledger 1976): the nucleolated nucleus is oval or pyriform; the endoplasmic reticulum is scattered throughout the cell; the Golgi complex is located close to one of the poles of the nucleus; mitochondria, phagosomes and electron-transparent vacuoles are present (Fig. 16d). The spicule is secreted by at least two sclerocytes, which tightly contact each other to form an extracellular vacuole isolated from the mesohyl matrix by septate desmosomes present between the sclerocytes (Ledger 1975; Ledger and Jones 1977). As the spicule and its rays grow, the cell shape changes, but the isolated vacuole remains until the spicule has been fully formed.

In the Calcarea, sclerocytes are supposed to differentiate from pinacocytes (Ledger 1976).

Spongocytes are responsible for secretion of the perispicular spongin. They may group and regroup in the course of spiculogenesis, forming deep finger-shaped contacts (Garrone 1978) (Fig. 17). In the demosponges without the mineral skeleton, spongocytes form the organic one (Fig. 17a-d). Spongocytes have a well-developed RER with cisterns of approximately the same size and numerous perinuclear cisterns of the Golgi complex. Spongocytes migrate towards the spicule-secreting sclerocytes or free spicules. In the course of formation of the gemmoscleres (spicules surrounding gemmules) in freshwater sponges from the family Spongillidae (Haplosclerida), spongocytes form a dense palisade epithelium surrounding the internal cells of the gemmule (De Vos 1971, 1977; Langenbruch 1981, 1982).

Spongocytes are supposed to have a positive chemotaxis to silica (Garrone 1978). They are thought to derivate from archaeocytes.

Protective-Secretory Tissue

The *protective-secretory* tissue comprises various *amoeboid cells* and various *cells with inclusions*; this division is not, however, very strict. The main functions of this tissue are protection (by phagocytal and bactericidal activity), storage, secretion of the mesohyl matrix and transfer of food particles and oxygen.

Amoeboid Cells

Archaeocytes are amoeboid cells possessing a large nucleus and a large nucleolus and capable of phagocytosis (Fig. 18). They are characteristic of the Demospongiae; in the Homoscleromorpha archaeocyte-like amoeboid cells are infrequent.

One will fail to find a clear definition of archaeocytes in the spongiological literature. At various times, such cells were called thesocytes (Sollas 1888), spherulous cells (Topsent 1892), and polyblast or hyaline cells (Tuzet and Pavans de Ceccatty 1958). Most often, however, they were referred to as amoebocytes (Müller 1911) or nucleolated amoebocytes (Wilson and Penney 1930; Faure-Fremiet 1931). Speaking of archaeocytes, the scientists may mean both totipotent (stem) cells of the kind found in other animals (such as *i*-cells of the Cnidaria and neoblasts of the

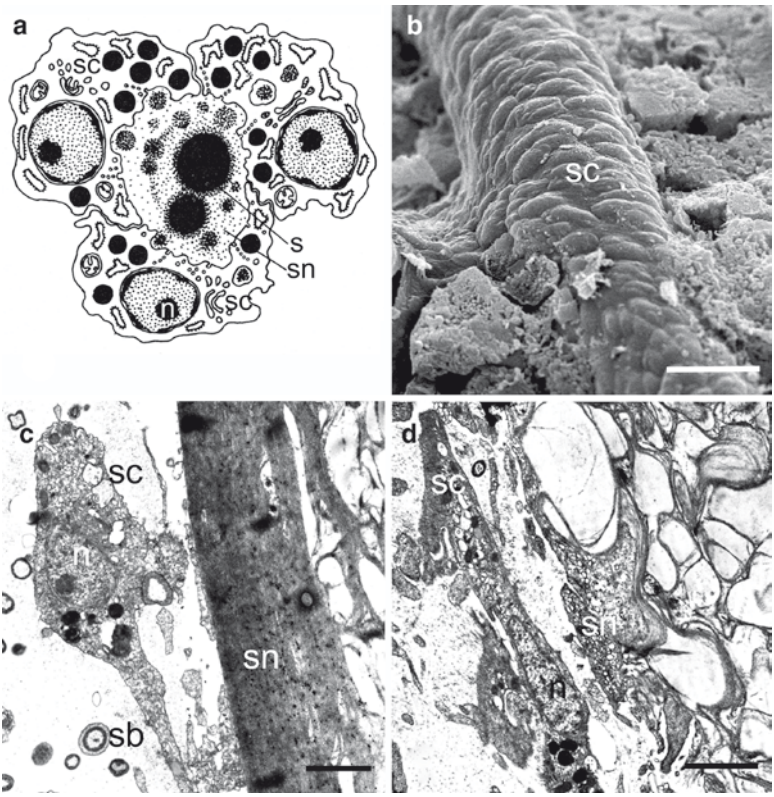

Fig. 17 Spongocytes. (**a**) Diagram of the spongocytes. (**b**) SEM of a spongocytes forming organic skeleton in *Hippospongia communis* (Demospongiae, Dictyoceratida). (**c**, **d**) TEM of a spongocytes of *Aplysina aerophoba* (Demospongiae, Verongida). (**c**, **d** – Courtesy of J. Vacelet). *n* nucleus, *s* spicule, *sc* spongocyte, *sb* symbiotic bacteria, *sn* spongin. Scale bars (**b**) 10 μm, (**c**) 2 μm, (**d**) 5 μm

Turbellaria) and any kind of amoeboid cells with a nucleolated nucleus and numerous phagosomes. The cytoplasm of archaeocytes is considered to be rich in RER, ribosomes and non-specific inclusions, in particular, phagosomes (Harrison and De Vos 1991). Archaeocytes are the most actively dividing population of free cells. In *S. massa*, archaeocytes were noted to be highly polymorphic and several morphotypes of these cells were distinguished (Diaz 1979).

Archaeocytes are traditionally considered to be pluripotent (totipotent) cells actively involved in sexual and asexual reproduction as well as regeneration (e.g. Lévi 1970; Simpson 1984; Harrison and De Vos 1991; Korotkova 1981a, 1997).

Bacteriocytes are mobile amoeboid cells with vacuoles containing prokaryotic symbionts (Fig. 19). First described in *Aplysina cavernicola* (Verongida) (Vacelet 1970), bacteriocytes have yet been found only in the demosponges. Two types of these cells could be distinguished: (1) those with a single vast bacteria-containing vacuole and (2) those with numerous small vacuoles, each containing one or several

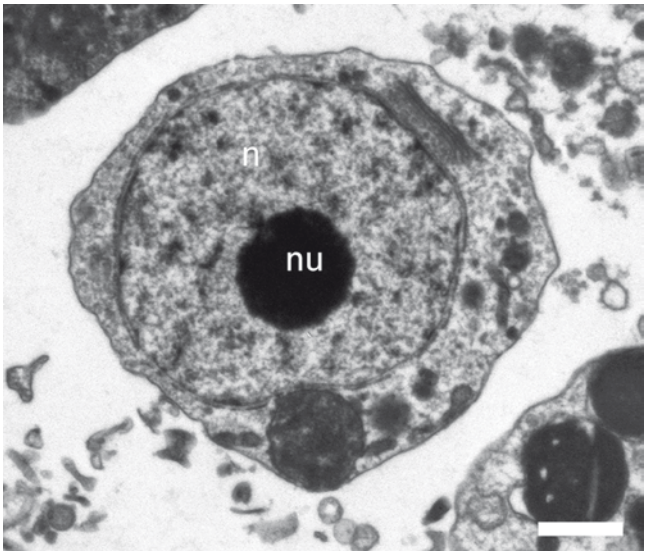

Fig. 18 TEM of an archaeocyte of *Crellomima imparidens* (Demospongiae, Poecilosclerida). *n* nucleus, *nu* nucleolus. Scale bar 1 μm

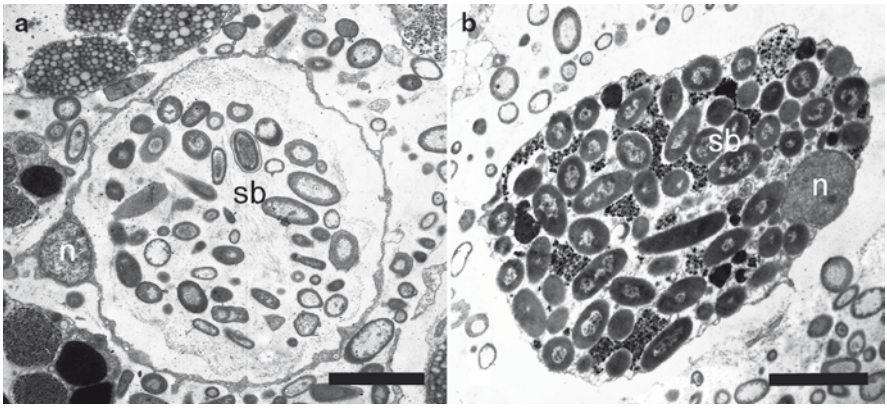

Fig. 19 TEM of a bacteriocytes of *Aplysina cavernicola* (Demospongiae, Verongida). *n* nucleus, *sb* symbiotic bacteria. Scale bars (**a**, **b**) 3 μm (Courtesy of J. Vacelet)

bacteria. Bacteriocytes of the first type were found in *A. cavernicola, Petrosia ficiformis, Biemna ehrenbergi* and *S. domuncula* (Vacelet 1970; Vacelet and Donadey 1977; Bigliardi et al. 1993; Ilan and Abelson 1995; Bohm et al. 2001; Maldonado 2007). Bacteriocytes of the second type were found in carnivorous sponge *Asbestopluma hypogea* (Poecilosclerida) (Vacelet and Boury-Esnault 1996). Bacteriocytes may be quite large: for example, in *S. domuncula* they are about 20 μm in diameter (Bohm et al. 2001). Bacteriocytes of *A. hypogea* participate in the digestion of food (Vacelet and Duport 2004).

In *C. reniformis* bacteriocytes with species-specific bacteria penetrate into the developing embryo and remain intact there until the end of the metamorphosis, thus ensuring the vertical transmission of bacteria (Lévi and Lévi 1976). Bacteriocytes were also recorded in the larva (parenchymella) of *Haliclona tubifera* (Haplosclerida) (Woollacott 1993).

Bacteriocytes are supposed to originate from archaeocytes (Simpson 1984).

Cells with Inclusions

This is a heterogeneous group of cells, characterized by the presence of specific inclusions in the cytoplasm. They differ from archaeocytes, first of all, by the reduced RER; neither acid phosphatase nor big phagosomes are revealed in them; the nucleus is anucleolate. Simpson (1984) formally divided these cells into two types according to the size of the inclusions: the cells with large inclusions and the cells with small inclusions.

Cells with Large Inclusions. ***Vacuolar cells*** are characterized by the presence of one or several large electron-transparent or light vacuoles (Fig. 20). Their cytoplasm may contain small phagosomes, mitochondria and endoplasmic reticulum.

In *Oscarella tuberculata* (Homoscleromorpha) vacuolar cells originate by transdifferentiation of endopinacocytes (Gaino et al. 1986a). They form a kind of 'hydroskeleton' of the sponge and participate in the collagen synthesis (Gaino et al. 1986a). In *Tethya citrina* and *T. aurantium* (Hadromerida) vacuolar cells are contained in the bud development during asexual reproduction (Gaino et al. 2006). Vacuolar cells can be used as a diagnostic character in closely related species of sponges without the skeleton, for example, *Oscarella* and *Halisarca* (Muricy et al. 1996; Ereskovsky 2006, 2007a).

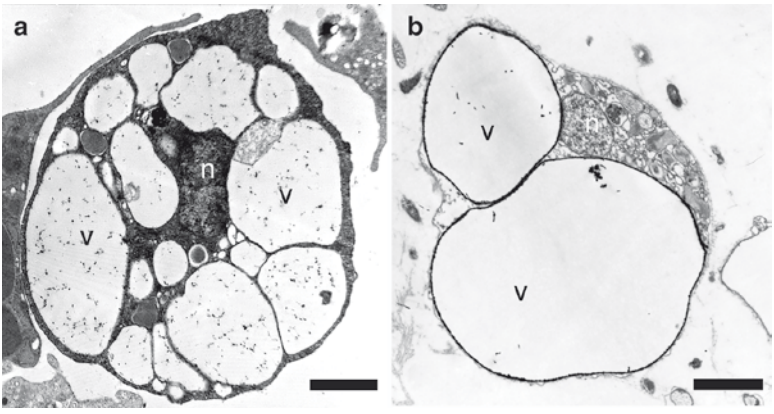

Fig. 20 TEM of vacuolar cells of *Halisarca dujardini* (**a**) (Demospongiae, Halisarcida) and *Oscarella lobularis* (**b**) (Homoscleromorpha). *n* nucleus, *v* vacuole. Scale bars (**a**, **b**) 3 μm

Cystencytes appear to be a synonym of the vacuolar cells. Most of the volume of a cystencyte is occupied by a large vacuole with an amorphous polysaccharide.

Globoferous cells are characterized by the reduction of the cytoplasm; they contain one or two large globules of various natures and chemical composition and a small deformed nucleus. Several small inclusions may also be present. In *Hymeniacidon heliophila* (Halichondrida), globoferous cells are actively involved in allogenic reconstructions, though their precise functions are unknown (Custodio et al. 2004).

Spherulous cells are packed with large rounded membrane-bounded inclusions (spherules) (Fig. 21). The small and anucleolated nucleus may be deformed by the spherules. Cytoplasmic strands contain small fragments of the endoplasmic reticulum and a few mitochondria. The content of the spherules varies in the nature and chemical composition. It is usually more or less homogeneous, but paracrystalline, fibrillar and lamellar inclusions also occur (Connes 1968; Diaz 1979; Muricy et al. 1996; Bonasoro et al. 2001). Despite this heterogeneity of the content, spherules are easily distinguishable from large phagosomes, which mean that they are not involved in digestion. Spherulous cells may be diffused in the mesohyl but usually are localized along the cortical layer or along the aquiferous system canals, as in *Halisarca dujardini, Crambe crambe, C. reniformis* and *Iophon piceum* (Lévi 1956; Uriz et al. 1996; Bonasoro et al. 2001; Ereskovsky 2006.).

The role of spherulous cells is rather diverse. In *C. crambe*, they were shown to contain toxic metabolites (Uriz et al. 1996; Becerro et al. 1997). In *Aplysina* sp. and *Plakina trilopha*, they are involved in excretion (Vacelet 1967; Donadey 1978). In *Axinella*, they accumulate lectins (Bretting et al. 1983). Spherulous cells of *Cacospongia scalaris* (Donadey 1982) and *Pleraplysilla spinifera* (Dictyoceratida) (Donadey and Vacelet 1977) are involved in the extracellular matrix maintenance. In *A. fistularis* (Verongida) brominated metabolites are stocked in spherulous cells (Thompson et al. 1983).

Spherulous cells often penetrate into the developing embryo (at the later stages of development) or into the pre-larva (Ereskovsky and Boury-Esnault 2002;

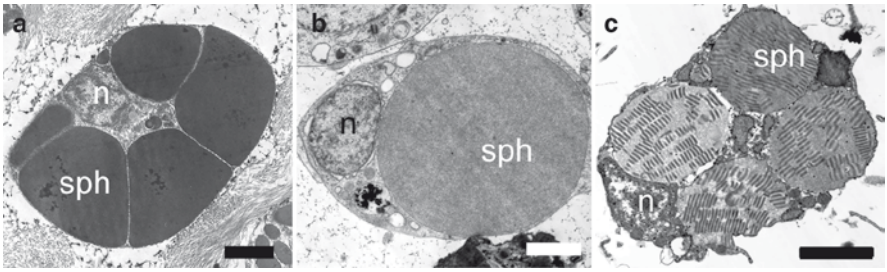

Fig. 21 TEM of a spherulous cells of *Halisarca ectofibrosa* (**a**), *Halisarca dujardini* (**b**) (Demospongiae, Halisarcida) and spherulous cell with paracrystalline inclusions of *Oscarella kamchatkensis* (**c**) (Homoscleromorpha), TEM (**a** – Courtesy of J. Vacelet). *n* nucleus, *sph* spherules. Scale bars (**a**–**c**) **2** μm

Ereskovsky and Gonobobleva 2000). They are also an important component of the development bud during asexual reproduction (Connes 1967; Gaino et al. 2006).

Spherulous cells are an obligatory component of mesohyl, since they play an important protective (immune) and secretory function. In some aspicular sponges (*Oscarella*, Halisarcida) they have a species-specific morphology and serve as an important diagnostic character (Bergquist 1996; Muricy et al. 1996; Ereskovsky 2006, 2007a).

Granular cells appear to be a variety of spherulous cells. At least, there are no essential differences between them at the ultrastructural level. If two groups of such cells, different only in the size of inclusions, are clearly present in the mesohyl, the cells with smaller inclusions are referred to as granular (Fig. 22), and the cells with larger inclusions as spherulous.

Cytoplasmic inclusions of the granular cells in the *H. dujardini* mesohyl contain cation peptides and proteins that are factors of protection from bacteria and fungi (Krilova et al. 2004). In the same species, granular cells actively penetrate into the forming larvae (Korotkova and Ermolina 1986; Ereskovsky and Gonobobleva 2000) and are retained there until the beginning of metamorphosis (Gonobobleva and Ereskovsky 2004a). Granular cells were also recorded in the parenchymellae of various demosponges (Ereskovsky 1986; Rützler et al. 2003) and the amphiblastulae of the calcareous sponges (Amano and Hori, 1992; Gallissian and Vacelet 1992).

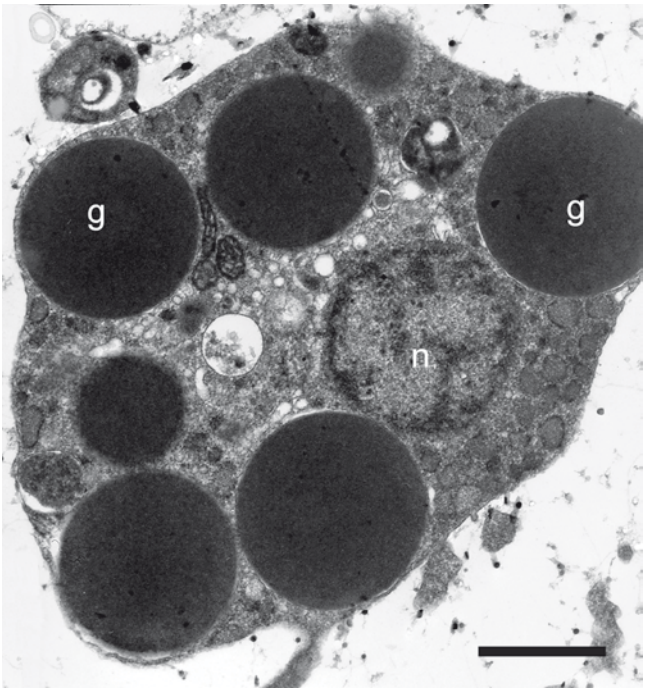

Fig. 22 TEM of a granular cell of *Halisarca dujardini* (Demospongiae, Halisarcida). *g* granules, *n* nucleus. Scale bar 2 μm

Granular cells can be used as a diagnostic character in closely related species (Pomponi 1976; Muricy et al. 1996; Bergquist 1996; Ereskovsky 2007a).

Cells with Small Inclusions ***Gray cells***, also known as ***glycocytes***, were first described in the conglomerate of living cells of *M. prolifera*, where their gray contrasted with the orange colour of other cells (Wilson and Penney 1930). Later they were found in many demosponges (Boury-Esnault 1977; Harrison and De Vos 1991). The characteristic feature of gray cells is the presence of numerous acidophilic and osmiophilic inclusions, which are small, ovoid and membrane-bounded, as well as of glycogen rosettes (Fig. 23a). Their cytoplasm contains well-developed RER and a developed Golgi complex (Boury-Esnault 1977). The nucleus usually contains a small nucleolus.

The main role of gray cells is the metabolism of glycogen and secretion of collagen (Garrone 1974; Boury-Esnault 1977; Diaz 1979). Together with the archaeocytes, gray cells are actively involved in various morphogeneses (Boury-Esnault 1977). It was suggested that during individual development of demosponges gray cells originate from flagellated cells of the parenchymella larvae (Boury-Esnault 1977; Boury-Esnault and Doumenc 1979).

Microgranular cells have the cytoplasm filled with fine electron-dense granules (Fig. 23b). The nucleus is often anucleolated (Gallissian and Vacelet 1985; Sciscioli et al. 2000; Pinheiro et al. 2004). In *Cinachyra tarentina* (Spirophorida), the granules of these cells originate from the dictyosomes of the Golgi complex (Sciscioli et al. 2000).

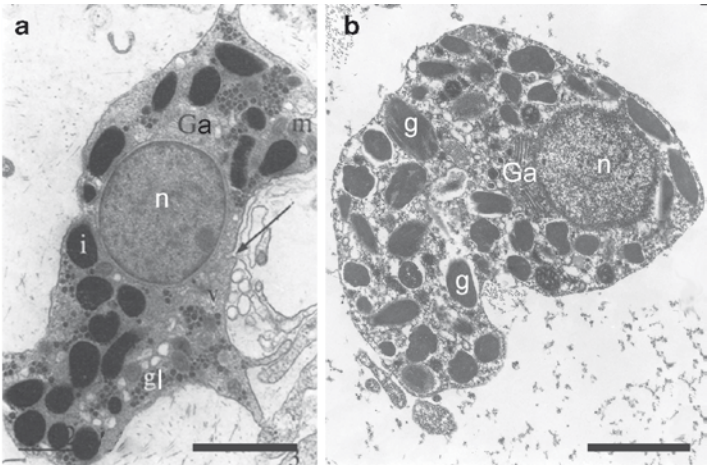

Fig. 23 Cells with small inclusions. (**a**) TEM of a gray cell of *Polymastia robusta* (Demospongiae, Hadromerida). Note the vesicles (v) suggesting pinocytosis (*arrow*). (**b**) TEM of a microgranular cell of *Halisarca dujardini* (Demospongiae, Halisarcida). *g* granules, *Ga* Golgi apparatus, *gl* glycogen, *i* osmiophilic inclusions, *m* mitochondria, *n* nucleus (**a** – From Boury-Esnault, 1977, reproduced by permission of Springer). Scale bars (**a**, **b**) 2 μm

The role and functions of the microgranular cells remain obscure. It is certain, however, that they are not involved in the collagen synthesis (Simpson 1984). At the same time, microgranular cells of *C. tarentina* participate in the synthesis of the glycoprotein component of the extracellular matrix (Sciscioli et al. 2000).

3 Organization of Syncytial Sponges (Class Hexactinellida)

Hexactinellida (glass sponges) differ from the other sponges in that their body consists mostly of a giant syncytium referred to as the trabecular syncytium. However, their tissues do include separate cells as well: choanocytes (earlier called choanosyncytium), archaeocytes, vacuolar cells and gametocytes.

The main component of the body, occupying the middle part of the body wall, is the choanosome containing *flagellated chambers*. The choanosome is limited by a network of fine walls of the *trabecular reticulum* – the *peripheral trabecular reticulum* (Reiswig and Mehl 1991; Leys 1999; Leys et al. 2007). The internal and the external trabecular reticulum occupy a comparable volume – about 5-12% – in the body walls.

3.1 Aquiferous System

Hexactinellida, in contrast to the cellular sponges, do not have a true system of canals (Reiswig 1979; Mackie and Singla 1983). The water, drawn through the pores in the *dermal membrane*, moves via the subdermal cavities penetrated by the *trabecular syncytium*. Then it seeps across the inhalant spaces (not canals!) into the syncytial trabecular reticulum and, finally, through the prosopyle into the syncytial *flagellated chambers* (Fig. 24a). Afterwards, the water is ejected via the apopyle into the exhalant trabecular syncytial system.

In *Rhabdocalyptus dawsoni*, the external peripheral trabecular reticulum forms a system of wide interconnected canals. From this area, the inhalant canals (formed by the trabecular network) enter the sponge body starting from a wide (1.25 μm) opening in the dermal membrane. Gradually narrowing to about 0.5 μm in the centre of the body wall, they give rise to numerous narrow branches ending in the flagellated chambers. The water is drawn into the chambers through hundreds of pores (prosopyles) in the trabecular reticulum. Owing to the second layer of the trabecular reticulum, bending round each collar in the flagellated chambers, the water enters the flagellated chamber freely (Fig. 24b) (Leys 1999; Bavestrello et al. 2003). Flagellated chambers have the internal diameter of 55-70 μm (in *Aphrocallistes vastus* and *R. dawsoni*) to 100 μm (in *Oopsacas minuta*) (Perez 1996; Leys 1999). The water leaves the flagellated chambers through wide openings (apopyles, 20-35 μm in diameter, directly into small gathering canals, 100-150 μm in diameter. The latter, in their turn, pass

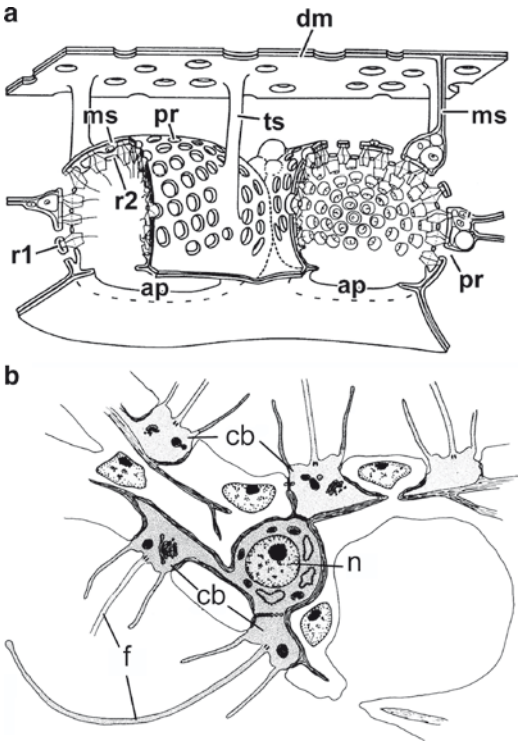

Fig. 24 (**a**) Diagrams of hexactinellid soft-tissue organization. (**b**) Aquiferous system in Hexactinellida. (**a** – From Reiswig and Mehl 1991, reproduced by permission of Springer; **b** – From Mackie and Singla 1983, reproduced by permission of The Royal Society). *ap* apopyle, *cb* collar body, *drn* dermal membrane, *f* flagellum, *ms* mesohyl space, *n* nucleus, *pr* prosopyle, *r1* primary reticulum; *r2* secondary reticulum, *ts* trabecular strands

into tubular exhalant canals, which increase in diameter from 0.3 mm in the middle part of the sponge body wall to 1.5 mm in the peripheral trabecular reticulum in the atrial surface. In the atrial area the exhalant canal fuse again to form a free network of wide interconnected canals. The water passing these canals is drawn to the sponge atrium via openings, 100-200 μm in diameter.

Food particles are captured in the glass sponges both by the primary and the secondary trabecular syncytium, as well as by the collar bodies of the flagellated chambers (Leys 1996; Perez 1996; Wyeth 1999).

3.2 Tissue Organization

Since the glass sponges have a syncytial organization, they do not possess any epithelia similar to those of the cellular sponges.

3.2.1 Trabecular (Primary) Syncytium

The trabecular reticulum of the glass sponges is a syncytium containing thousands of nuclei within a single branched cytoplasmic domain permeating the whole sponge. It forms the dermal and the atrial covering layers, as well as the trabecular fibres. The trabecular syncytium, first described by Ijima (1901) in *Euplectella marshalli*, makes up most (about 75%) of the soft tissues of the sponge. It ensures structural support of the flagellated chambers and the secondary reticulum (Figs. 24, 25a). Owing to the trabecular syncytium, the living tissues, except the choanocyte collars, are separated from the water flowing through the sponge. The trabecular

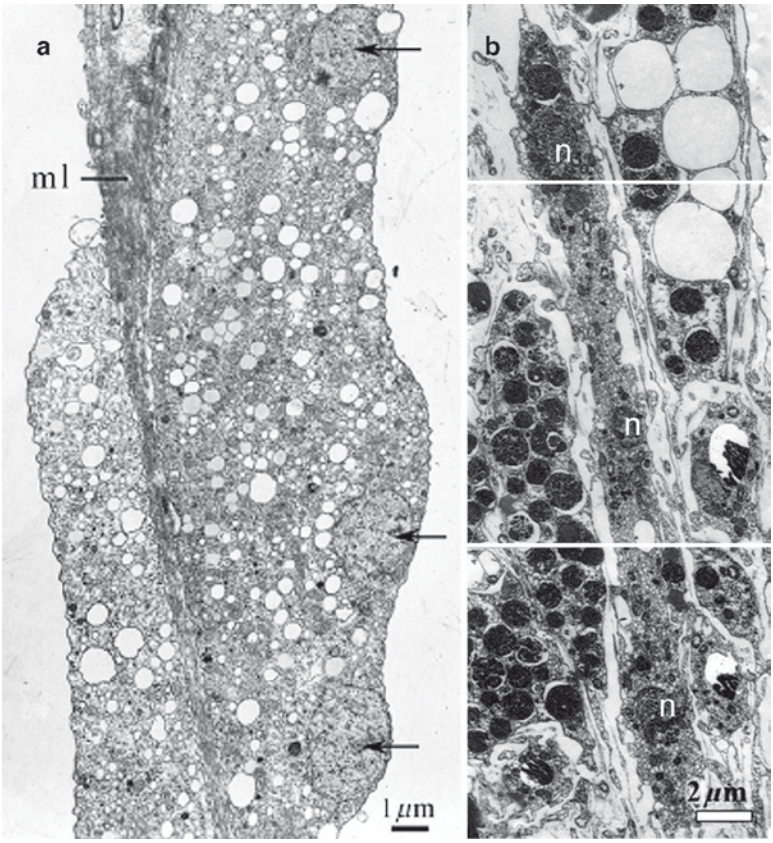

Fig. 25 (**a**) TEM of a trabecular syncytium in *Rhabdocalyptus dawsoni* (Hexactinellida) with three nuclei (*arrows*) in a continuous mass of cytoplasm. (**b**) TEM of a sclerosyncytium in *Oopsacas minuta* with three nuclei in a continuous mass of cytoplasm. (**a** – From Mackie and Singla 1983, reproduced by permission of The Royal Society; **b** – From Boury-Esnault et al. 1999, reproduced by permission of Balaban Publishers International Science Services). *ml* mesolamella, *n* nucleus. Scale bars (**a**) 1 µm, (**b**) 2 µm

tissue may be represented by flat perforated sheers, similar to the dermal membrane, or by thin trabecular ramifications, often less than 1 μm in diameter. The trabecular syncytium widens near the flagellated chambers (Fig. 25a), forming a supporting network (primary reticulum, R1) of the collar bodies of the branching choanocytes (Mackie and Singla 1983).

Almost all glass sponges, with the exception of *Caulophacus cyanae* (Boury-Esnault and De Vos 1988) and *Dactylocalx pumiceus* (Reiswig 1991), also have a secondary reticulum spreading into the cavities of the choanocyte chambers at the mid-collar level. The cytoplasm of the trabecular syncytium contains small nuclei, mitochondria, RER, the Golgi complex, the cytoskeletal elements (actin filaments and microtubules) and various vesicular inclusions, in particular, phagosomes (Leys et al. 2007). Cytoplasmic flows within the trabecular syncytium were described, with the speed of particle transport in them being about 0.33 μm/s[1] (Leys 2003a).

Interestingly, no divisions of nuclei were observed in the trabecular syncytium. Hence one may suppose that they are terminally differentiated (Leys 1996).

3.2.2 Mesohyl

The mesohyl of glass sponges, described only at the electron-microscopic level, is represented by a fine fibrillar layer, 0.05-0.10 μm wide, within the trabecular syncytium (Reiswig 1979a). This collagen 'mesolamella' is supposed to ensure internal support of the trabeculae. It is likely to be secreted not by the free cells but by the trabecular syncytium itself (Mackie and Singla 1983; Reiswig and Mehl 1991).

The mesohyl is present in most areas of the trabecular syncytium (Figs. 24, 25a), but absent in the secondary syncytium and on the internal membranes. Forming the supporting membrane within the trabeculae, the mesohyl envelopes various cells and symbiotic bacteria. There is, however, no evidence of the mesohyl being the basis for the migrating cells' movement, as is the case in the cellular sponges.

3.3 Cell Composition

Free cell elements of the glass sponges are represented by the archaeocytes, sclerocytes, choanocytes (earlier referred to as the choanosyncytium), the cells with inclusions and gametocytes.

3.3.1 Supportive-Connective Tissue

Sclerocytes and ***sclerosyncytium***. The glass sponges are considered to possess secondary syncytial issues, the sclerosyncytium, which secrete spicules (Fig. 25b)

(Boury-Esnault et al. 1999; Leys et al. 2007). The secretion of the siliceous spicules in the Hexactinellida is 'intracellular', i.e. proceeds inside the sclerosyncytium, and is thus similar to the Demospongiae (Leys et al. 2007). At the same time, the embryos of *O. minuta* have separate sclerocytes, connected by the surrounding cells by plugged cytoplasmic bridges (Leys 2003b).

3.3.2 Protective-Secretory tissue

Archaeocytes, usually grouped into clusters or congeries (Ijima 1901), are spherical or rounded. Their diameter is 3-5 μm in *A. vastus, O. minuta* and *R. dawsoni*, but about 8 μm in *Farrea occa* (Leys et al. 2007). The cytoplasm is dense, granular, with numerous mitochondria, rare phagosomes, components of the Golgi complex and RER (Fig. 26). Archaeocytes in congeries are often connected with each other and with the trabecular syncytium by the species plugged junctions. Rounded shape and lack of pseudopodia of the hexactinellid archaeocytes testify to a lack of mobility not characteristic of the archaeocytes of other sponges. It is thought that the archaeocytes of glass sponges, similarly to those of the demosponges, constitute a pool of pluripotent cells.

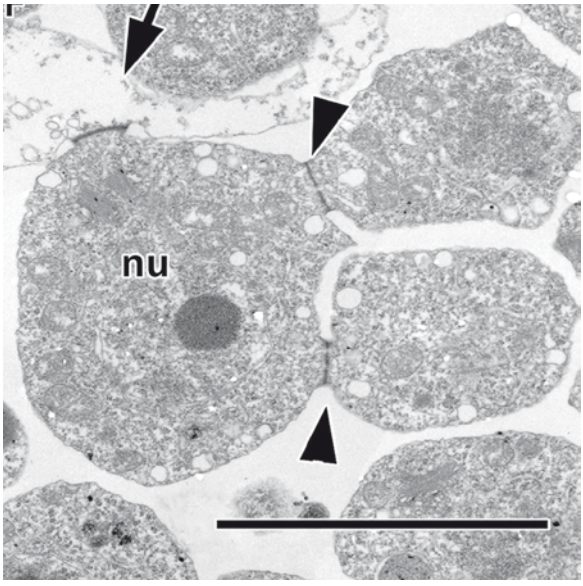

Fig. 26 TEM of an archaeocyte in *Rhabdocalyptus dawsoni* (Hexactinellida) attached to two other archaeocytes by plugged junctions (*arrowheads*) and to the trabecular syncytium (*arrow*) (From Leys 2003b, reproduced by permission of Wiley). *nu* nucleus. Scale bar 10 μm

Cells with Inclusions

Rounded cells whose cytoplasm is filled with rounded vacuoles were found in *R. dawsoni* and described as ***spherulous*** or ***vacuolar*** cells (Mackie and Singla 1983). In *O. minuta* and *Rossella nuda*, vacuolar cells contain from one to several vacuoles (Köster 1997; Boury-Esnault et al. 1999; Leys 2003a).

Thesocytes or ***cystencytes*** are oval or spherical cells from 5-7 to 7-12 μm in size, described in *A. vastus* (Reiswig 1979a). They contain one large homogeneous vacuole, which shifts the nucleus towards one of the poles into a thin cytoplasmic strand. A more correct name for these cells would be ***globular cells***. Similar cells were also described in *R. dawsoni,* but in this species they are larger (up to 17 μm) and sometimes form aggregations (Mackie and Singla 1983). In some cases the vacuoles contain crystalline bodies, testifying to their protein nature. These cells are completely surrounded by the trabecular syncytium, which forms peculiar 'pockets'.

Granular cells, first described by Schulze (1899), are oval cells about 8 μm in diameter containing numerous small (1-2 μm) electron-dense granules. Granular cells are not enclosed into the trabecular syncytium but their cytoplasmic projections pass into the mesohyl through the openings in the trabecular syncytium (Fig. 27) (Salomon and Barthel 1990; Mehl et al. 1994; Leys et al. 2007). Granular cells concentrate along the canals of the aquiferous system (Salomon and Barthel 1990; Mehl et al. 1994).

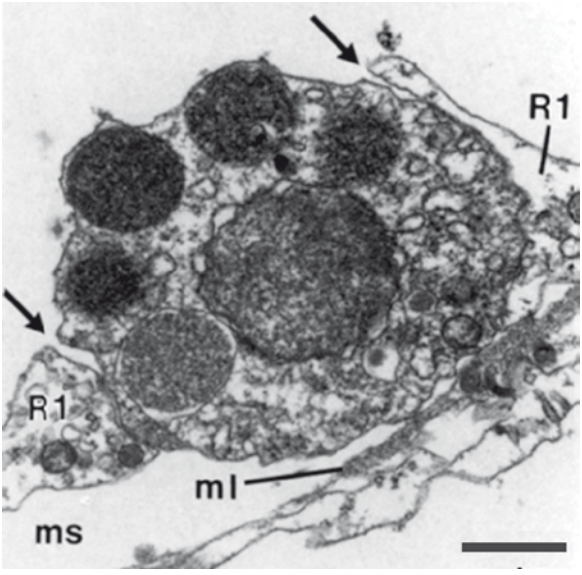

Fig. 27 TEM of a granular cell of *Farrea occa* (Hexactinellida) (From Reiswig and Mehl, 1991, reproduced by permission of Springer). *Arrows* gap, *R1* primary reticulum, *ms* mesohyl space. Scale bar 1 μm

Choanocytes

Hexactinellidalack the typical choanocytes of the sponges with cellular organization (see above). Their choanocytes are represented by branching cells consisting of the basal nucleus-containing domain whose projections finish by the collar bodies, *choanomeres*, similar to the apical part of the typical choanocytes (Figs. 24a, 28a) (Leys et al. 2007). Earlier, the term *choanosyncytium* (Reiswig 1979a) was used to denote all the tissues lining choanocyte chambers. However, it was suggested (Leys et al. 2007) to reject this term on the strength of the following facts. Although the collar bodies may be connected with the nucleated domain by an open

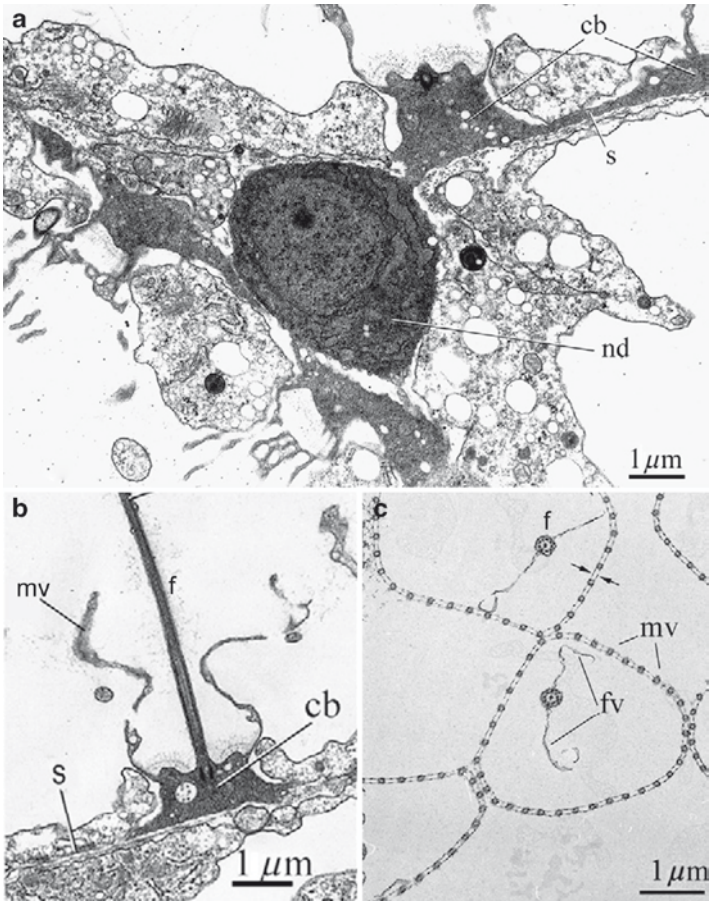

Fig. 28 TEM of branched choanocytes in *Rhabdocalyptus dawsoni* (Hexactinellida). (**a**) several collar bodies (*cb*) attached by stolons (*s*) to a single nucleated domain (*nd*). (**b**) TEM of a collar body (*cb*) in a section passing vertically through the flagellum (f). (**c**) TEM of a transverse section at the collar level shows flagellum (*f*) and collar microvilli (*mv*). (**a**, **b** – From Mackie and Singla 1983, reproduced by permission of The Royal Society, (**c**) – From Mehl and Reiswig 1991, reproduced by permission of Springer). Scale bars **a**–**c** 1 μm

cytoplasmic canal, they are usually connected with it by plugged junctions (see below). As far as it is known, the fully formed cell has only one nucleus but may have over three stolons (Fig. 28b), each with two or three collar bodies. The term choanosyncytium implies a multinucleate structure and is, therefore, misleading, since there is no evidence of more than one nucleus at any stage of the flagellated complex development. It is much more appropriate to call these complex cells *branching choanocytes* (Leys et al. 2007).

Ultrastructurally, the choanocytes of the glass sponges are identical to those of other sponges. The flagellum is usually simple, though in *A. vastus* it bears lateral wing-like projections (Fig. 28c), similarly to some demosponges (Mehl and Reiswig 1991).

3.4 Cytoskeleton

The syncytium of the glass sponges has a rather well-developed cytoskeleton. For instance, in *R. dawsoni* the actin cytoskeleton consists of rough strands radiating from the central part of the tissue (Leys 1995). In *A. vastus*, the actin strands are broader and their length is about 20 μm (Leys 1998). After aggregation, the dissociated tissues contain large bundles of microfilaments contacting each other in focal points at the periphery of the aggregates. The bundles of microfilaments dyed with rodamine-falloidine, which cross the tissues, are about 500 μm long. They also enter the giant lobopodia up to 20 μm long.

Centrosomes were found only in the archaeocytes and the branching choanocytes during aggregation of the dissociated tissues (Leys 1996). The syncytial tissue does not contain any centrioles.

4 Tissue Plasticity and Cell Transdifferentiation

As noted above, there is no single group of totipotent (‘stem’) cells in sponges from various clades.

4.1 Cellular Sponges

A characteristic feature of the Porifera, distinguishing them from the other Metazoa, is high plasticity of cellular differentiation, anatomical and tissue structures throughout the life cycle. Various differentiated cells of the sponge can move, transdifferentiate and switch functions. Adult sponges have no irreversibly differentiated cells except the gametes and highly differentiated cells. Morphogeneses and functional integration are possible only on the basis of mobility and reorganization of cells and cell populations. The direction of the differentiation depends on the needs of the organism. Owing to this feature, the sponge is constantly in the state of rearrangement

of all its structures (Efremova 1972; Pavans de Ceccatty 1979; Simpson 1984; Bond and Harris 1998; Gaino and Burlando 1990; Bond 1992; Gaino et al. 1995; Maldonado and Uriz 1999; Galera et al. 2000). Direct dependence of the Porifera on the environmental conditions and the life cycle stages, in combination with their plasticity, results in an ability to change the body shape: reduction, fragmentation, fusion and separating of individuals of the same clone are common (Burton 1949a; Johnson 1979a, b; Pansini and Pronzato 1990; Gaino et al. 1995).

This 'chronic morphogenesis' is a response not only to a change in the environmental conditions, but also to the movement of the sponge on the substrate (Maldonado and Uriz 1999; Bonasoro et al. 2001). In many oviparous[1] Demospongiae, ontogenesis is accompanied by profound reconstructions of all the anatomical and histological systems (Korotkova 1981b; Ereskovsky and Korotkova 1999), which can result in the destruction of all, or most of the aquiferous systems. This may be called for by the preparation to survival of adverse conditions (Simpson 1968; Van de Vyver and Willens 1975; Fell 1993), by the regeneration processes (Korotkova 1997), by the formation of reduction bodies (Simpson 1984), by gemmulogenesis and sexual reproduction (Ivanova 1981; Ereskovsky 1999, 2000). These characteristics of sponges are associated with their highly mobile cell differentiation.

Reproductive processes (during sexual and asexual reproduction) and the regeneration may involve various cells of covering and internal tissues.

4.2 Syncytial Sponges: Reaggregation of Dissociated Tissues of Glass Sponges

Plasticity of tissue organization was not shown in the glass sponges, and neither was free transdifferentiation of cells. At the same time, during tissue dissociation dense spherical agglomerations 5-30 μm in diameter are formed (Pavans de Ceccatty 1982). These tissue conglomerates, which have either single nuclei or no nuclei at all, consist of 'cells' united by cytoplasmic bridges and plug junctions. Several hours later the aggregates merge to form large spheres up to 1 mm in diameter. Inside them, reorganization of cellular and syncytial components starts (Pavans de Ceccatty 1982). After that, the aggregates differentiate into giant syncytial cells (Pavans de Ceccatty and Mackie 1982).

[1]*Oviparous animals* are animals that lay eggs, with little or no other embryonic development within the mother. *Ovoviviparous*, also known as *oviviparous*, animals develop within eggs that remain within the maternal body up until they hatch or are about to hatch. It is similar to *viviparity* in that the embryo develops within the maternal body. Unlike the embryos of viviparous species, ovoviviparous embryos are nourished by the egg yolk rather than by the mother's body.

5 Some Ultrastructural and Molecular-Biological Characteristics of Sponges

5.1 Intercellular Junctions

Adult cellular sponges maintain structural unity and homeostasis and regulate the flow of food particles by desmosome-like contacts between the pinacocytes (Pottu-Boumendil 1975; Pavans de Ceccatty 1986; Garrone and Lethias 1990; Harrison and De Vos 1991; Masuda et al. 1998; Ereskovsky and Tokina 2007). Tight junctions were found between pinacocytes of *Hippospongia communis* (Pavans de Ceccatty et al. 1970). Septate desmosomes are present in special cases: between the sclerocytes accumulating calcite in calcareous sponges (Ledger 1975), between choanocytes accumulating collagen (Green and Bergquist 1979) and between spongocytes secreting the envelope of the gemmules in freshwater sponges (De Vos 1977).

Specialized cell junctions have been recently found in sponge larvae as well (Fig. 29). Belt desmosomes were revealed in the apical part of the peripheral ciliated cells of the larvae of the Demospongiae (*Dysidea etheria* (Rieger 1994), *Halisarca dujardini* (Gonobobleva and Ereskovsky 2004a), *Pleraplysilla spinifera* (Ereskovsky 2005), *Ircinia oros* (Ereskovsky and Tokina 2004), *Chondrilla australiensis* (Usher and Ereskovsky 2005), the Homoscleromorpha (Boury-Esnault et al. 2003) and the Calcinea (Ereskovsky and Willenz 2008). In the larvae of the glass sponge *Oopsacas minuta* special plug junctions are present (Leys et al. 2006).

Plug junctions are considered to be an autapomorphy of the glass sponges (Leys et al. 2007). They were first described in *Rhabdocalyptus dawsoni* as 'perforated septate partitions' or 'plugs' (Mackie 1981; Mackie and Singla 1983). In *R. dawsoni*, these junctions are flat three-layered lamellae or discs inserted into narrow bridges between the cells (Fig. 30). The lamellae (about 50 nm wide) are sandwich-like. The double lipid layer was not revealed. Plasma membranes may 'fringe' the edges of the lamellae, connecting the membranes of the neighbouring cell areas. Hollow cylinders about 50 nm in diameter, interpreted as porous particles, are present within the lamellae. Plug junctions of the hexactinellids resemble the pit plugs of red algae and the plasmodesmata of the higher plants rather than the gap junctions of other animals (Leys et al. 2007). In glass sponges, plug junctions are supposed to be involved in the regulation of the intracellular transport (Leys et al. 2007).

5.2 Extracellular Matrix

Extracellular matrix (ECM) of the Demospongiae and the Homoscleromorpha was shown to possess many components characteristic of the Eumetazoa: glucoseaminеglycanes (chondroitin 4 sulfate), fibronectin, proteoglycanes, actin-binding adhesive plaques, laminin (Pavans de Ceccatty 1981; Humbert-David and Garrone 1993; Müller 1997b) and various collagens, including collagen IV – the main

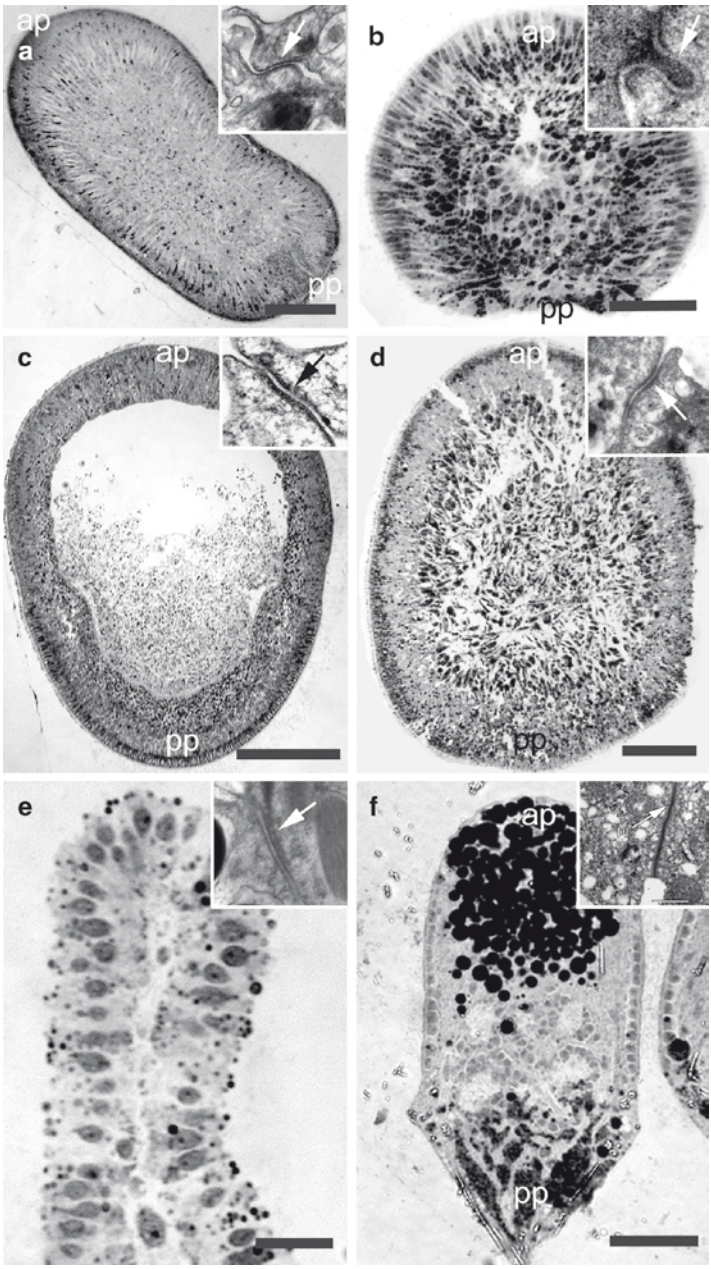

Fig. 29 TEM of specialized cell junctions in sponge larvae. (**a**) *Ircinia oros* (Demospongiae, Dictyoceratida), (**b**) *Halisarca dujardini* (Demospongiae, Halisarcida), (**c**) *Corticium candelabrum* (Homoscleromorpha), (**d**) *Pleraplisylla spinifera* (Demospongiae, Dictyoceratida), (**e**) *Guancha arnesenae* (Calcarea, Calcinea), (**f**) *Oopsacas minuta* (Hexactinellida). (*Inset* in (**f**) From Leys et al. 2006, reproduced by permission of Oxford University Press). *Arrows* cell junctions, *ap* anterior pole, *pp* posterior pole. Scale bars (**a**) 100 μm, (**b**) 30 μm, (**c**) 100 μm, (**d**) 50 μm, (**e**) 30 μm, (**f**) 25 μm

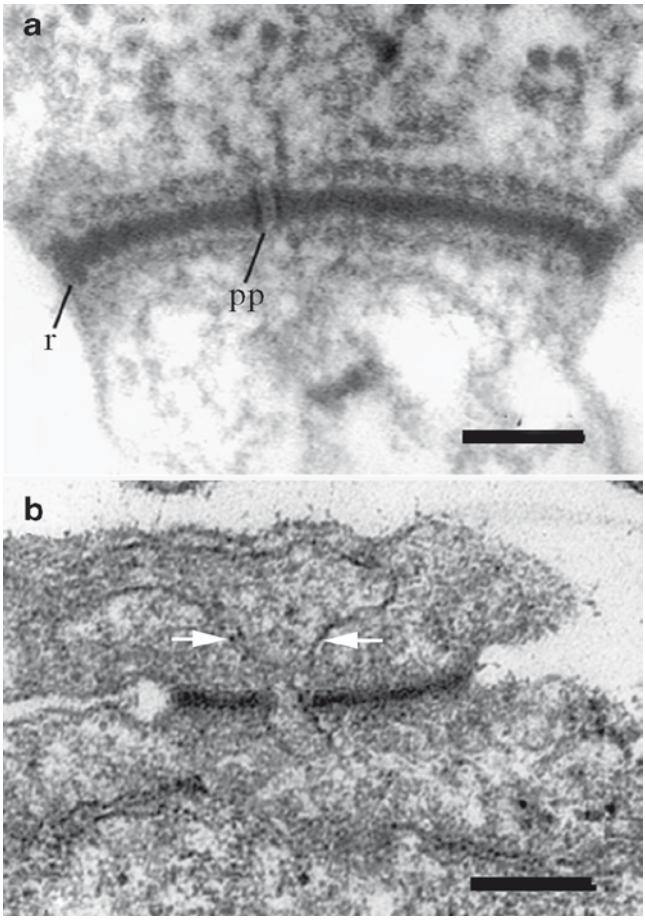

Fig. 30 TEM of plugged junctions in *Rhabdocalyptus dawsoni* (Hexactinellida). (**a**) Section through a plug showing trilaminar structure; *pp* pore particles, *r* rodlets; (**b**) section through a plugged junction showing continuity of the cell membrane (*arrows*) around the edge of the plug (From Mackie and Singla 1983, reproduced by permission of The Royal Society). Scale bars (**a**) 0.1 μm, (**b**) 0.3μm

component of the basal membrane (Garrone 1985; Pfeifer et al. 1993; Boute et al. 1996; Aouacheria et al. 2006). It is thought that the first glycoprotein to appear in the evolution of the Metazoa was fibronection, with the laminins emerging later (Pedersen 1991; Rieger 1994). Noteworthy, laminin was found only in the most evolutionary advanced sponges, the Homoscleromorpha (Humbert-David and Garrone 1993). Fibronectin was found in freshwater and marine demosponges (Labat-Robert et al. 1981; Akijama and Johnson 1983). Fibronectin III (FN_3) was extracted from *Geodia cydonium* (Pahler et al. 1998). Müller (1998) suggested that FN_3 is the most ancient fibronectin module of the Metazoa. Demosponges also possess the eumetazoan type of cell recognition and the systems responsible for

various types of cell-to-cell and cell-to-ECM adhesion, including integrins, proteoglycane aggregation factor and S-type lectins (Exposito and Garrone 1990; Müller 1998; Pancer et al. 1997; Fernandez-Busquets and Burger 1999; Fernandez-Busquets 2008), other molecules implicated in cell adhesion and also crucial members of signalling pathways (Adamska et al. 2007; Adell et al. 2003, 2007; Manuel et al. 2004a; Nichols et al. 2006; Richard et al. 2008; Lapébie et al. 2009).

Demosponges have a rather well-developed apparatus of intracellular communications. Researchers extracted from their cells tirosine-kinase receptors, enzymes involved in the production of neuromediators (monoamineoxidase, cholineesterase), as well as neuromediators, acetylcholine, epineurine, norepineurine, 5-oxytriptamine and serotonin (Lentz 1966; Thiney 1972; Schäcke et al. 1994; Weyrer et al. 1999). Moreover, the 'flask cells' and the globular cells (originally annotated as mucous cells) of the larva of *Amphimedon queenslandica* express five messengers corresponding to post-synaptic genes leading the authors to suggest that they might play neuro-sensory-like roles (Sakarya et al. 2007). Later, Richards et al. (2008) showed that these cells express three genes that are important in the nervous system patterning of Eumetazoa: *AmqbHLH1*, a gene with conserved proneural activity and its supposed (according to the eumetazoan Notch pathway) upstream regulators *AmqNotch* and *AmqDelta1*.

6 Endosymbiotic Bacteria

A very characteristic feature of the Porifera is the presence of obligatory species-specific endosymbiotic bacteria, both photosynthetic and non-autotrophic (Sarà and Vacelet 1973, Lopes et al. 1999; Hentshel et al. 2006; Taylor et al. 2007; Maldonado 2007; Vishnyakov and Ereskovsky 2009). These symbionts belong to about half of recognized bacterial phyla and both major archaeal lineages (Hentschel et al. 2006; Taylor et al. 2007). It is a distinctive feature of symbiosis in sponges in comparison to other well-studied symbiosis, in which one-host-one-symbiont variants of associations are usually recorded (Schmitt et al. 2007a). According to 16S rRNA gene analysis, there are some bacterial phyla, representatives of which are most frequently identified as sponge associated microorganisms (Hentschel et al. 2006; Taylor et al. 2007). In addition, the bacterial symbionts diversity can be divided into specialists, sponge associates and generalists depending on their occurrence in seawater and sponges as hosts (Taylor et al. 2004). The biomass of the endosymbiotic bacteria in some marine sponges may reach 60% (Vacelet and Donadey 1977; Wilkinson 1987; Fuerst et al. 1999), while the number of endosymbonts' morphotypes varies from one to eight (Fuerst et al. 1999; Muricy et al. 1999).

The most important function of the symbiotic bacteria is their participation in sponge physiology: the recycling of the insoluble proteins and the involvement into reconstructions of the connective tissues and the ECM (Wilkinson et al. 1979). Many bacteria extracted from sponges synthesize antimicrobial compounds, which testify to their involvement in protective mechanisms of the sponge (Stierle and

Stierle 1992; Shigemori et al. 1992; Jayatilake et al. 1996). Symbiotic bacteria can also serve as an additional source of food: sponges either phagocyte them or use the products of their activity (Vacelet 1975, 1979a; Gaino et al. 1977; Vacelet et al. 1996).

Bacteria may get into the sponge in two ways. First, sponges may capture the bacteria as a result of filtration activity (Reiswig 1971; Pile et al. 1996). The second, and the usual way is the vertical transmission of the species-specific symbiotic bacteria from the parent sponge to the next generation via eggs (in oviparous species) (Lévi and Lévi 1976; Sciscioli et al. 1991, 1995; Gaino and Sara, 1994; Usher and Ereskovsky, 2005; Maldonado 2007) or larvae (in ovoviviparous and viviparous species) (Amano and Hori 2001; Ereskovsky and Boury-Esnault 2002; Ereskovsky et al. 2005; Ereskovsky and Tokina 2004; Riesgo et al. 2007a). Vertical transmission of the symbiotic bacteria is an important element of the poriferan biology (Ereskovsky et al. 2005; Enticknap et al. 2006; Maldonado 2007; Sharp et al. 2007; Schmitt et al. 2007b).

7 A Brief Historical Overview

Developmental studies of sponges begin from the paper of Grant (1825) where for the first time were indicated sponges larvae and eggs in *Spongilla* sp. However the first description of larvae (by the example of *Spongilla* sp.) has been made by Lieberkühn (1856), and in 1859 he published first description of embryos of calcareous sponges. Nevertheless, the first detailed description of sponge's embryonic development has been made 10 years later by Schmidt (1866) at *Sycon* (*Sycandra*) *humboldti.*

Regular studies of sponge's development have more than 150-year-old history. It is possible to allocate three periods in history of studying of sponges embryology (Ereskovsky 2007b).

The **first** period of sponge development studies falls on the last third of the nineteenth century. It was the 'Golden Age' of sponge embryology. About 110 articles on this topic were published. Uncontested leadership in this research field belonged to German zoologists: Schulze, Maas, Schmidt, Keller (Fig. 31) and others.

The basis of sponge comparative embryology was laid at that time. Haeckel (1874) (Fig. 32a) admitted that embryological studies of calcareous sponges (Haeckel 1872) were the starting point for his ideas about the origin of Metazoa, later formalized as the Gastraea theory of ontogeny recapitulating phylogeny, in which the gastrula is viewed as the recapitulation of a gastraean ancestor that evolves via selection on a simple, planktonic hollow ball of cells to develop the capacity to feed (Haeckel 1874).

At the same time, as fairly wrote Barrois (1876), figures and descriptions of embryos and larvae of calcareous sponges made by Haeckel had more art value, than scientific. True scientific description of development of limy sponges begins with work of Metschnikoff (Fig. 32b) (1874, 1879). On the basis of comparative

Fig. 31 German spongiologs portraits. (**a**) Frans Eilhard Schulze (1840–1921) ZM B IX-608. (**b**) Otto Maas (1867–1916). (**c**) Oscar Schmidt (1823–1886). (**d**) Conrad Keller (1848–1930) ZM B I/1753 (From Ereskovsky 2007b, reproduced by permission of the Museu Nacional of Rio de Janeiro)

embryological data of some demosponges Delage (1892, 1899) (Fig. 32c) discovered that during a metamorphosis of parenchymella larvae external flagellated cells migrate inward to form the choanoderm of the adult sponge. These observations have allowed Delage to propose a hypothesis of 'inversion of the germ layers'. Being based on this hypothesis, he separated sponges from Metazoa into a special group, Enantiozoa that signified 'inside out animals'. Bidder (1898), following Minchin (1896), subdivided Calcarea into two subclasses, Calcinea and Calcaronea,

Fig. 32 (**a**) Ernst Haeckel (1834–1919) and Nicolas Miklucho-Maclay (1846–1888) during the expedition to the Red Sea in 1866 (From Ereskovsky 2007b reproduced by permission of the Museu Nacional of Rio de Janeiro). (**b**) Elias Metchnikoff (1845–1916) Odessa, 1876. (**c**) Yves Delage (1854–1920) at the Roscoff Marine laboratory, 1905 (From Ereskovsky 2007b, reproduced by permission of the Museu Nacional of Rio de Janeiro)

and distinguished deep embryological differences (e.g. coeloblastula in Calcinea, amphiblastula in Calcaronea) in the position of the nucleus in the choanocytes (with nucleus basal in choanocyte, independent of flagellum in Calcinea and with nucleus apical in choanocyte, linked to the flagellum in Calcaronea). Later Calcinea/Calcaronea division was supported by cladistic analyses of morphological and molecular characters (Manuel et al. 2003). Moreover, analyses of 18S rDNA (Manuel et al. 2003) and 28S rDNA (Manuel et al. 2004b) strongly supported the monophyly of Calcinea and Calcaronea.

Fig. 33 (**a**) Odette Tuzet (1903–1976) and Oscar Duboscq (1868–1943), Banuls-sur-Mer Marine laboratory, 1937. (**b**) Wilson Henry Van Peters (1863–1939). (**c**) Paul Brien (1894–1975), Brussels, 1968 (Courtesy of Ph. Willenz). (**d**) Claude Lévi, Paris, 2000 (Courtesy of J. Vacelet)

The **second** period falls on the first half of the twentieth century (1900-1960), when interest in sponge development declined. Almost the only active researches were made in Belgium by Brien (Fig. 33c), Meewis, Leveaux, and in France by Tuzet, Duboscq and Lévi (Fig. 33). Claude Lévi, in his famous work (1956), was the first to use embryological characters of sponges in systematics.

However, this period was marked by the emergence of a major branch in developmental biology of sponges. Wilson (Fig. 33b) (1907) pioneered the use of sponges as model animals for cell adhesion research. He described species-specific reaggregation of mechanically dissociated sponge cells. His works provided an impulse to studies of behaviour of separate cells and regeneration in sponges.

The **third** period started with the application of electron microscopy and new optical and experimental methods to sponge studies. Spermatogenesis and oogenesis, fertilization (in Calcaronea) and larval structure (in all poriferan clades) were investigated ultrastructurally. The results of these works were extensively applied to evolutionary and phylogenetic constructions concerning both Porifera and Metazoa in general. At the same time, complete development from egg to juvenile was investigated at the ultrastructural level only in some species, including *H. dujardini* (Demospongiae, Halisarcida) (see Chap. 3.6), some species of *Oscarella* and *Corticium candelabrum* (Homoscleromorpha) (see Chap. 4), some Spongillidae and *A. queenslandica* (Demospongiae: Haplosclerida) (see Chap. 3.11) and *O. minuta* (Hexactinellida) (see Chap. 3).

Looking back, we can see that out of the 570 articles on sponge embryology, only 101 are devoted to embryonic development in the strict sense. They deal with 23 species of Calcarea, 2 species of Hexactinellida and about 75 species of Demospongiae (Ereskovsky 2007b).

Part I
Special Embryology of Sponges

Chapter 1
Development of Sponges from the Class Calcarea Bowerbank, 1864

Representatives of the class Calcarea, the calcareous sponges, are characterized by a calcium carbonate mineral skeleton in the form of free diactines, triactines, tetractines, and/or multiradiate spicules. A dense basal skeleton, with the main spicules cemented together, is sometimes present. The aquiferous system may be asconoid, syconoid, sylleibid, or leuconoid. Calcareous sponges are ovoviviparous or viviparous, with hollow blastula larvae. All Calcarea are marine. The most ancient records of calcareous sponges date back to the early Cambrian (James and Klappa 1983) and the Ordovician (Kempen 1978).

Traditional morphological approach could not convincingly demonstrate the monophyletic nature of the Calcarea, but recent molecular phylogenetic investigations show this class to be monophyletic (Borchiellini et al. 2001; Manuel et al. 2003, 2004b).

The class Calcarea comprises approximately 500 species (less than 5% of all known sponges), which belong to 75 genera, 22 families, and 5 orders. The Calcarea is divided into two monophyletic subclasses: the Calcinea and the Calcaronea (Manuel et al. 2002, 2003).

The calcareous sponges have two essentially different patterns of sexual embryogenesis: the first is with the calciblastula larva (development of the "calciblastula" type), which is a characteristic of the Calcinea; the second is with the amphiblastula larva (development of the "amphiblastula" type), which is a characteristic of the Calcaronea (Borojevic 1969, 1970; Ereskovsky 2004). Differences in the embryonic development of these two subclasses were already noted by Metschnikoff (1879). Furthermore, Korotkova (1981b, 1988a) delimited four types of calcareous sponge development: *Clathrina* type, *Leucosolenia* type, *Petrobiona* type, and a last type, characteristic of other Calcaronea.

1.1 The Subclass Calcaronea Bidder, 1898

Sponges from the subclass Calcaronea are highly variable with regard to size, shape, and skeleton structure (color plate I). Their aquiferous system may be asconoid, syconoid, sylleibid, or leuconoid. The Leucosolenida is the only order in which all these types are represented. The spicules are diactines and/or sagittal triactines and

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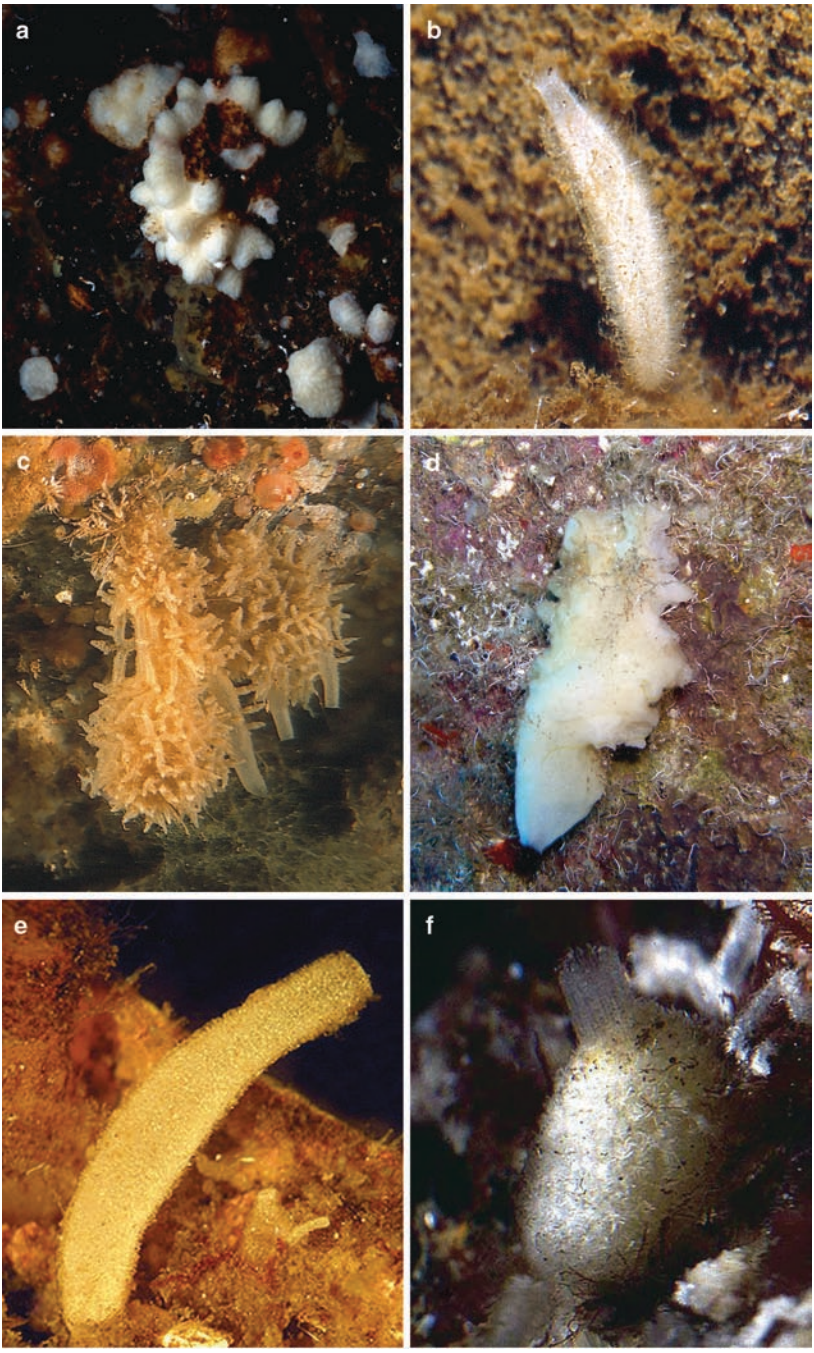

Plate I Class Calcarea, subclass Calcaronea Bidder, 1898. (**a**) *Petrobiona massiliana* Vacelet and Lévi 1958, Mediterranean Sea (Courtesy of J. Vacelet). (**b**) *Sycon ciliatum* (Fabricius 1780), White Sea. (**c**) *Leucosolenia complicata* (Montagu 1818), White Sea (**b, c** – Courtesy of M. Fedjuk). (**d**) *Paraleucilla magna* Klautau et al. 2004, Mediterranean Sea (Courtesy of T. Perez). (**e**) *Sycon* sp. (**f**) *Grantessa kuekenthali* (Breitfuss 1896). (**e**, **f**) White Sea (**e, f** – Courtesy of N. Cherviakova)

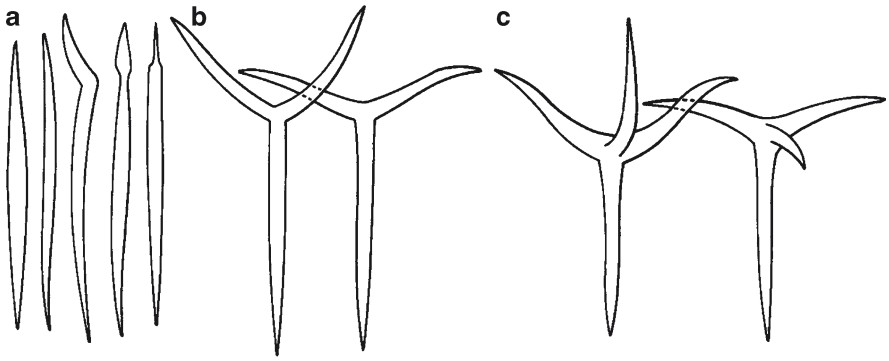

Fig. 1.1 Spicules of Calcaronea: (**a**) diactines; (**b**) triactines; (**c**) tetractines

tetractines (Fig. 1.1). Some species also have a basal skeleton made of spicules cemented together or a solid mass of calcite (*Petrobiona* – order Baerida). The first spicules to appear during ontogenesis are diactines. Choanocytes have an apical, egg-shaped, or pear-shaped nucleus; the ciliar rootlet always contacts the apical part of the nucleus. The Calcaronea sponges are viviparous and have the amphiblastula larva. In some species, asexual reproduction occurs in the form of budding.

The Calcaronea are marine, found in all oceans. The subclass comprises 4 orders, 17 families, and 59 genera.

Calcareous sponges and, in particular, representatives of the Calcaronea have been the most popular specimen of Porifera-related research for over a century. Three stages can be outlined in the development of Calcaronea studies.

The first stage embraces the last quarter of the nineteenth century and approximately the first 30 years of the twentieth century. Though researches were conducted, naturally, only at the light microscopic level, many embryological observations of that time were astonishingly accurate and are still quoted by modern scientists. Several pioneering discoveries concerning the calcareous sponges were made during this period: Lieberkühn (1859) described the larva of *Sycandra raphanus*; Schmidt (1866) illustrated the embryonic development of *S. humbodlti*; Haeckel (1871) explained the early cleavage stages in several calcareous sponges. Investigations were also devoted to oogenesis and spermatogenesis (Schulze 1875, 1878a; Polejaeff 1882; Görich 1904; Hammer 1908; Dendy 1915; Bidder 1920; Gatenby 1920a,b; 1927) and to cleavage, embryogenesis, larval structure, and metamorphosis (Metschnikoff 1874, 1879; Barrois 1876; Schulze 1875, 1878a; Polejaeff 1882; Minchin 1896, 1897, 1900; Görich 1903, 1904; Hammer 1908; Gatenby 1927).

The second stage of the Calcaronea studies falls within the period spanning from the 1930s to the 1950s. It is typically associated with Octave Joseph Duboscq and Odette Tuzet. These two French researchers worked at the Mediterranean biological station in Banyuls-sur-Mer (Fig. 33a). They thoroughly studied oogenesis, fertilization, embryonic development, larval structure, and metamorphosis in several Calcaronea sponges (Duboscq and Tuzet 1932, 1933a,b, 1935a,b, 1936, 1937, 1938, 1941, 1942, 1944; Tuzet 1947, 1948).

The third stage started in the 1980s with the advance of electron microscopy and the application of new histochemical methods. During this stage, insights into all the aspects of the Calcaronea biology were obtained (Gallissian 1980, 1981, 1983, 1988, 1989; Franzen 1988; Anakina 1981, 1988, 1997; Gaino et al. 1987b; Gallissian and Vacelet 1976, 1990, 1992; Nakamura et al. 1998; Watanabe and Okada 1998; Anakina and Korotkova 1989; Anakina and Drozdov 2000, 2001; Leys and Eerkes-Megrano 2005; Eerkes-Medrano and Leys 2006).

All Calcaronea studied are hermaphroditic, but there are different types of hermaphroditism. *Grantia compressa* and *Sycon* cf. *ciliatum* were shown to be protogynic (Sarà 1974). However, out of 100 individuals of *Sycon* cf. *ciliatum*, 99 contained only female gametes, and only one contained both oocytes and spermatozoa (Sarà and Orsi 1975). Franzen (1988) considered this species to be unisexual. In *Leucosolenia complicata* from the Barents Sea, spermatogenesis was found to occur throughout the year, while oogenesis (in the same individuals) happens only in September–October (Anakina 1981). This sponge was considered to exhibit unisexual andromonoicous hermaphorditism (Anakina 1981).

1.1.1 Gametogenesis

All sponges, including the Calcaronea, lack gonads. Spermatogenesis and oogenesis are diffused.

The origin of gametes. The origin of oocytes in sponges was, for a long time, a controversial subject. They were thought to arise from choanocytes (Haeckel 1874; Gatenby 1920b; Vacelet 1964; Anakina 1988), amoebocytes (Schulze 1875), or amoebocytes of the mesohyl, which in turn originate from choanocytes (Minchin 1896, 1897; Dendy 1915; Tuzet 1947; Sarà 1955). Finally, electron microscopic studies on several species (*G. compressa*, *Petrobiona massiliana*, *S. raphanus*, *S. sycandra*, *S. ciliatum*, *L. complicata*) supported the choanocyte origin of female gametes (Gallissian 1981; Franzen 1988; Anakina and Drozdov 2000).

Spermatogenesis. The origin of male gametes was equally controversial. They were thought to be the derivatives of mesohylar cells, mostly archaeocytes (Schulze 1878a; Polejaeff 1882; Görich 1904), or the derivatives of choanocytes (Dendy 1915). Recently, it has been proved that male gametes originate from choanocytes in *L. complicata* (Anakina and Korotkova 1989). This may well be the case for all Calcaronea, since choanocytes are their main totipotent cells (Korotkova 1970, 1997).

Despite recent advances, the origin of gametes in the Calcaronea still remains unstudied to a large extent. For instance, it is not clear what prompts a certain somatic cell to develop into a gamete or how oogenesis and spermatogenesis are regulated. To elucidate these issues, immunocytochemical and molecular research is necessary.

Spermatogenic cells of *L. complicata* were found to be diffusively distributed in the mesohyl. Single spermatocytes or their small bunches were not surrounded by any specialized cells, did not form special spermatocyte like in other sponges, and remained free under the choanoderm. Mature spermatozoa of *L. complicata* looked

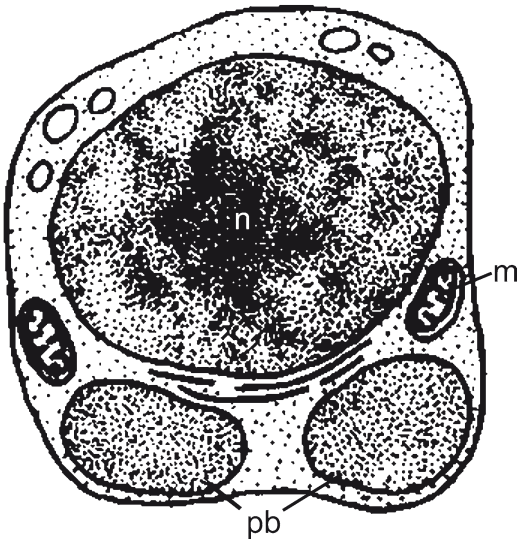

Fig. 1.2 Schematic drawing of sperm structure in *Leucosolenia complicata* (From Anakina and Drozdov 2001, Russ J Mar Biol, vol. 27, pp. 148, fig. 10c, reproduced by permission of Springer) *pb* peptide body, *m* mitochondria, *n* nucleus

like orbicular cells. Flagellum and acrosome were absent. The sperm nucleus was formed by incompletely condensed chromatin and was surrounded by a thin layer of cytoplasm. The cytoplasm comprised the ribosomes, two or three mitochondria, dictyosomes, and electron-dense protein bodies lying freely under the nucleus (Fig. 1.2) (Anakina and Drozdov 2001).

Oogenesis in the Calcaronea is found to be relatively uniform, and does not depend on the type of anatomy and aquiferous system. It follows the usual pattern, characteristic of other multicellular animals. The oocyte passes through three developmental phases:

1. The pre-meiotic phase (prophase stage)
2. The previtellogenic phase (cytoplasmic growth), when the cytoplasmic volume increases
3. The vitellogenic phase, when the oocyte synthesizes and accumulates nutrients

Oocytes of the Calcaronea lack vitelline envelope (*zonula pellucida*) and possess amoeboid motility at the pre-meiotic and pre-vitellogenic phases (Fig. 1.3a–c). The pre-meiotic phase is short and takes place beneath the choanoderm layer. At this stage, the oocyte is capable of phagocytosis, and heterophagosomes appear in its cytoplasm (Franzen 1988). Cytoplasmic growth starts when the nucleus passes into the prophase. During this stage, the oocyte migrates by amoeboid motility, through the mesohyl to peripheral or basal parts of the sponge. It forms long pseudopodia and shows phagocytic activity (Fig. 1.3d). Its size increases threefold or fourfold, and lipid droplets and fibrillar inclusions appear in the cytoplasm along with the heterophagosomes (Anakina and Drozdov 2000).

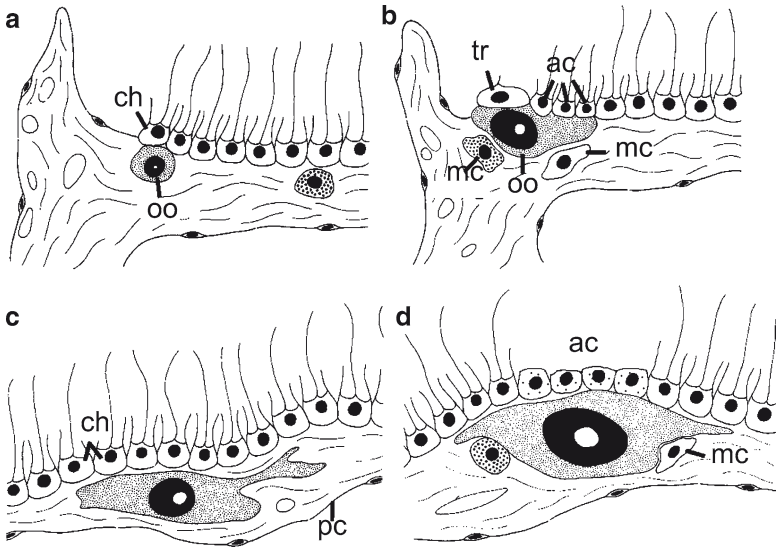

Fig. 1.3 Schematic drawing of oogenesis of Calcaronea, for example, of *Sycon ciliatum* (From Franzen 1988, Zoomorphology, vol. 107, pp. 355, fig. 10a–d, Reproduced by permission of Springer). (**a**) Oocyte at initial stage. (**b**, **c**) Oocyte at the stage of cytoplasmic growth. (**d**) Stage of vitellogenesis; *ac* accessory cells, *ch* choanocyte, *mc* mesohylar cells, *oo* oocyte, *pc* pinacocyte, *tr* trophocyte

The vitellogenic phase is the longest one. When vitellogenesis starts, oocytes lose their amoeboid motility and stop beneath the choanoderm. At the same time, *accessory* and *nurse* cells begin to form. In *L. complicata*, accessory cells, a complex of which is formed above each oocyte, are derived from choanocytes that lose the flagellum and the collar, and become much larger than the usual choanocytes. The accessory cells remain in the choanoderm cover of the oocytes. Accessory cells are thought to differentiate solely under the cytosexualizing influence of female gametes (Anakina and Drozdov 2000). The number of accessory cells does not exceed six for each oocyte in *Grantia* and *Sycon* sponges (Sarà 1974; Sarà and Orsi 1975; Franzen 1988; Gallissian 1981), but may reach 30–40 for each oocyte in *L. complicata* (Anakina and Drozdov 2000). A unique situation is observed in *P. massiliana*, the only Calcaronea with a massive calcareous skeleton (in addition to the spicules): all choanocytes of the choanocyte chamber adjacent to the vitellogenic oocyte transform into cells of the feeding complex (Fig. 1.4) (Vacelet 1964; Gallissian and Vacelet 1990, 1992).

As there are specialized contacts and partial membrane fusion between the growing oocyte and the accessory cells of *L. complicata* (Anakina and Drozdov 2000), direct transfer of nutrients from the latter to the former are theoretically possible. On the whole, phagocytic activity during oogenesis in the Calcaronea is rather high. The oocyte synthesizes mucopolysaccharide granules in the course of vitellogenesis and obtains lipid granules from the accessory cells. Accumulation of

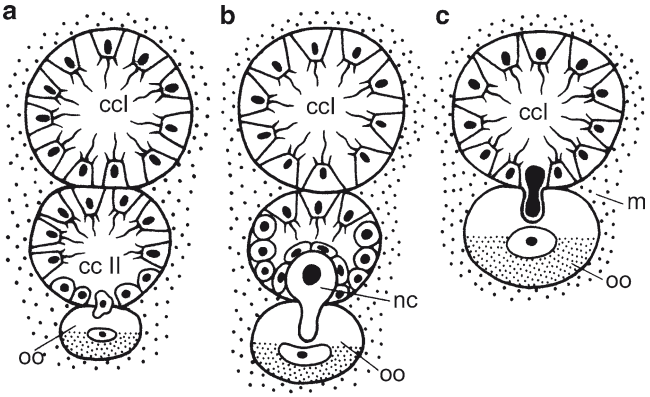

Fig. 1.4 Schematic drawing of oogenesis and interrelation between the growing oocyte and accessory cells of *Petrobiona massiliana.* (**a**) Oocyte (*oo*) situated next to choanocytes of chamber II (*cc II*). (**b**) Growing oocyte that phagocytosed the nurse cells (*nc*). (**c**) Mature oocyte with degenerated nurse cell. *ccI* choanocyte of chamber I, *m* mesohyl

ribosomal RNA results from the activity of the oocyte nucleus (Anakina and Drozdov 2000).

The mature egg is spherical or, more often, ovoid. In the latter case, the shorter axis is perpendicular to the choanoderm. During maturation divisions, the polar bodies are extracted onto the egg surface facing the choanoderm. On the basis of these characters, the egg axis perpendicular to the choanoderm is usually considered as the animal–vegetative one, with the animal pole facing the choanoderm (Tuzet 1948, 1973a; Korotkova 1981b; Ivanova-Kazas 1975, 1995). However, there is no asymmetrical distribution of inclusions along the animal–vegetative axis in any of the Calcaronea sponges studied (Franzen 1988; Gallissian and Vacelet 1990, 1992).

The size of the mature egg varies from 40 to 80 μm, and that of the nucleus varies from 14 to 35 μm. The cytoplasm contains lipid granules, heterophagosomes, mitochondria (solitary or in clusters), and fibrous inclusions. The latter are characteristic of the Calcaronea and the Calcinea, but are absent in all other sponges. In most Calcaronea (e.g., *G. compressa*, *S. raphanus*, *S. sycandra*, *S. ciliatum*, *Grantessa kuekenthali*), these inclusions are evenly distributed in the ooplasm (Gallissian 1981; Gallissian and Vacelet 1992; Franzen 1988; Ereskovsky 2005), but in *L. complicata* they are concentrated at the periphery (Anakina and Drozdov 2000).

1.1.2 Fertilization

Fertilization in the Calcaronea is unique in the animal world, as it is put into effect with the aid of the so-called *carrier cells*. This type of fertilization was

first described in *G. compressa* (Gatenby 1920a) and later studied at the light and electron microscopic level in *S. ciliatum*, *S. raphanus*, *S. elegans*, *S. sycandra*, *S. calcaravis*, *Leucandra aspesa*, *L. nivea*, *L. gossei*, *L. botrioides*, *L. complicata*, *G. compressa*, *Leucilla endoumensis*, *P. massiliana*, and *A. kuekenthali* (Duboscq and Tuzet 1932, 1935b, 1937, 1942, 1944; Gaino et al. 1987b; Gallissian 1980, 1988, 1989; Gallissian and Vacelet 1990; Watanabe and Okada 1998; Nakamura et al. 1998; Anakina and Drozdov 2000; Ereskovsky 2005) (Figs. 1.5 and 1.6).

The carrier cell had been thought to arise from a choanocyte, into which a spermatozoon penetrates (see Tuzet 1973; Korotkova 1981b). Subsequently, it was shown that the carrier cell actually originated from an accessory complex cell, which acquired the capacity to capture a spermatozoon (Gallissian 1980; Gaino et al. 1987b; Gallissian and Vacelet 1990). Special nurse cells form the feeding and fertilization complex around the carrier cell. While a spermatozoon penetrates into the carrier cell, and the latter, with the spermiocyst inside, begins to move, the oocyte continues to accumulate nutrients and to grow.

Thus, fertilization involves the following sequence of events:

1. A sperm cell penetrates into an accessory cell that differentiates into a carrier cell.
2. A spermiocyst forms inside the carrier cell.

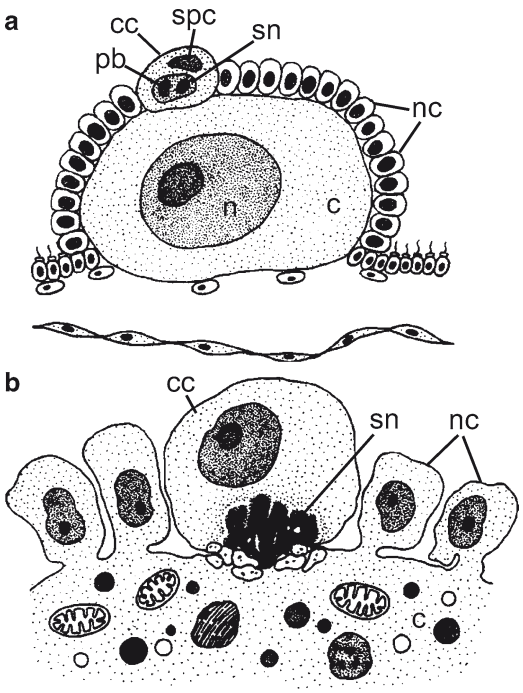

Fig. 1.5 Schematic drawing of fertilization in *Leucosolenia complicata*. (**a**) Formation of carrier cell. (**b**) Penetration of the sperm nucleus from the carrier cell into egg cytoplasm. *c* cytoplasm of oocyte, *cc* carrier cell, *ch* choanocytes, *n* oocyte nucleus, *nc* nurse cells, *pb* peptide body, *sn* nucleus of spermium, *spc* spermiocyst (From Anakina and Drozdov 2001, Russ J Mar Biol, vol. 27, pp. 148, fig. 10d, e, reproduced by permission of Springer)

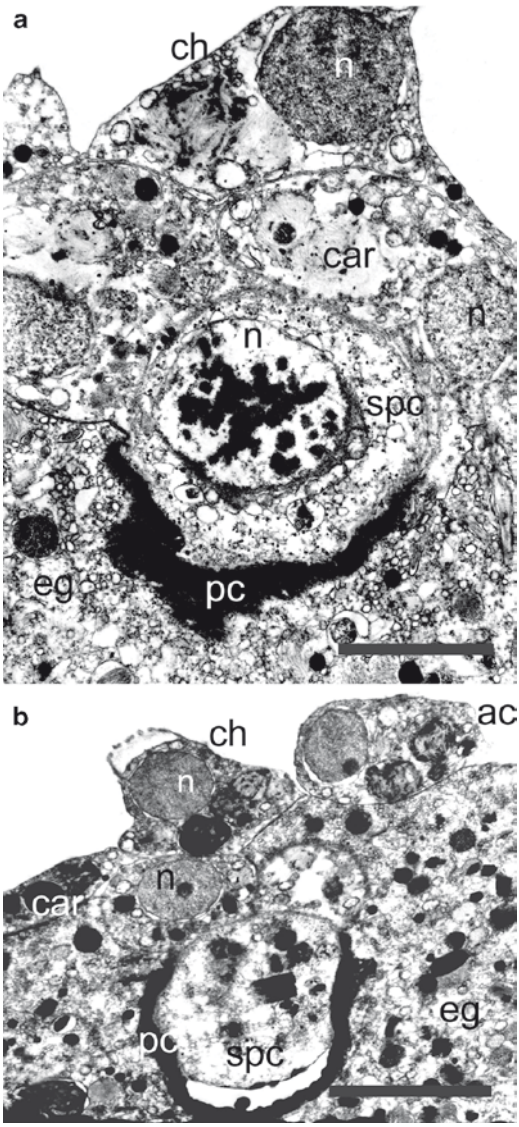

Fig. 1.6 TEM of spermiocyst penetration in the egg and the fertilization complex of *Grantessa kuekenthali*. (**a**) Early stage of penetration and (**b**) the stage of spermiocyst nuclear membrane destruction. *ac* accessory cells, *car* carrier cell, *ch* choanocyte, *eg* egg, *pc* protein capsule, *n* nucleus, *spc* spermiocyst. Scale bars (**a**, **b**) 2.5 μm

3. The spermiocyst is transferred to the oocyte.
4. Their pronuclei merge.

After capturing the sperm cell, the carrier cell enlarges two or threefold. In *L. complicata*, nurse cells directly surrounding the carrier cell probably participate in the transformation

of the latter, as, contrary to the peripheral cells of the accessory complex, they actively accumulate inclusions and grow (Fig. 1.5) (Anakina and Drozdov 2000). In *S. calcaravis*, fertilization involves two carrier cells surrounding the sperm cell (Watanabe and Okada 1998).

Once inside the carrier cell, the sperm cell undergoes certain changes and it is referred to as the *spermiocyst*. Its structure is similar in different species (Fig. 1.6). A dense protein capsule covers the spermiocyst completely or partly. The nucleus has highly condensed chromatin (Gallissian 1988). Mitochondria are either small and numerous or, as in *L. endoumensis* and *S. sycandra*, large and few in number; in the latter case, they are formed by the merging of the small ones (Gallissian 1988; Nakamura et al. 1998). Electron-transparent vesicles are situated at the spermiocyst's periphery. The spermiocyst of *S. calcaravis* contains fibrillar material (Watanabe and Okada 1998). There is no flagellum, but centriole has been observed in some species (Gaino et al. 1987b; Gallissian 1989; Nakamura et al. 1998).

The spermiocyst penetrates into the oocyte by means of one or two carrier cells, which push it into the ooplasm. Inside the oocyte, the protein capsule is destroyed and the nucleus decondenses and swells.

Spermiocyst penetration triggers maturation divisions in the oocyte. Extrusion of polar bodies always occurs at the pole oriented toward the choanoderm, though not necessarily at the site of the spermiocyst's penetration. After the chromatin in oocyte nucleus decondenses, both nuclei merge.

1.1.3 Embryonic Development

Embryonic development in Calcaronea occurs in the thin mesophyl layer between the choanoderm and the exopinacoderm.

Cleavage. A historical review of embryogenesis in Calcaronea has been recently published (Leys and Eerkes-Medrano 2005). Haeckel (1871) was the first to describe the early cleavage events in some calcareous sponges. According to him, cleavage in the syconoid Calcaronea is total and equal. The first four cleavages are meridional. Haeckel did not mention equatorial furrows, though he observed two groups of cells lying on top of each other from the stage of 32 blastomeres. Metschnikoff (1874) reported a similar early cleavage pattern in *Sycon* cf. *ciliatum* (*Sycandra raphanus*). However, according to Schulze (1875), in *S.* cf. *ciliatum,* only the first three cleavages are meridional, and the fourth is equatorial. Schulze's observations were supported by data obtained from *S. raphanus*, *G. compressa*, and *S. ciliatum* (Hammer 1908; Duboscq and Tuzet 1937, 1944). The scheme proposed by Schulze became well established in the literature and has been cited in modern embryological handbooks (Ivanova-Kazas 1975, 1995).

Cleavage has been studied thoroughly in *L. complicata* and *S. ciliatum* (Anakina 1981, 1997; Franzen 1988). Cleavage furrows are bipolar. The first two furrows pass meridionally, at right angle to each other and to choanoderm (Fig. 1.7a). The resulting four blastomeres lie in one plane and form a rhomb. The spindles of the

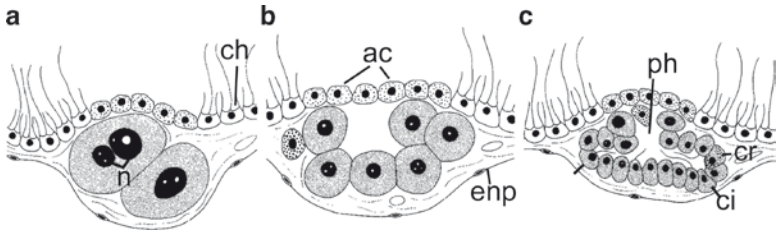

Fig. 1.7 Schematic drawing of cleavage in Calcaronea. (**a**) Two-blastomere stage. (**b**) Eight-blastomere stage. (**c**) 16-blastomere stage – early stomoblastula. *ac* accessory cells, *ci* ciliated cells, *ch* choanocytes, *cr* cross-cells, *enp* endopinacocyte, *ph* phyalopore, *n* nucleus (From Franzen 1988, Zoomorphology, vol. 107, pp. 355, fig. 10 f–h, reproduced by permission of Springer)

third-cleavage division are oblique with respect to the animal–vegetative axis. The 8-cell embryo looks like a slightly concave plate (Fig. 1.7b). The spindles of the fourth-cleavage division are positioned leiotropically to the animal–vegetative axis. The fourth-cleavage division in *Sycon* and *Grantia,* usually described as equatorial (Schulze 1878a; Duboscq and Tuzet 1937), is actually meridional. As a result of the above-mentioned ascending spiraling displacements, a 16-cell hemispherical embryo is formed, which has a broad *phyalopore* – an opening facing the choanoderm (Fig. 1.7c), which was first described by Duboscq and Tuzet (1937). During the fifth-cleavage division, the blastomeres are shifted dexiotropically. The 32-cell embryo has an almost closed phyalopore. Divisions 1–5 are synchronous, with minor deviations from synchronism. At transition to the 64-cell stage, divisions of animal blastomeres start to lag behind from those of vegetative blastomeres.

At the stage of approximately 80–100 cells, when the animal blastomeres and the vegetative ones are not yet much different in their size, the so-called cruciform cells (“cellules en croix”) (see later) are well visible. They have a refractive body in the basal part. The hemisphere facing the mesohyl divides and, as a result, the embryo curves. The macromeres are pressed back to the phyalopore and tucked inwards, being arranged in several layers. The embryo, with a vast cleavage cavity (blastocoel), consists of numerous tiny cylindrical micromeres and a few large granular macromeres. Cruciform cells are usually situated crosswise at the border between the micro- and the macromeres. This stage was first described in the development of *G. compressa* by Duboscq and Tuzet (1933b), who called it the *stomobastula* (Figs. 1.8a and 1.9a).

To sum up, cleavage in Calcaronea follows the incurvational pattern. Its main difference from typical cleavage patterns is in the incurvative formation of the cleavage cavity. Cleavage in Calcaronea is externally very similar to that of *Volvox* gonidia during asexual reproduction (Kirk 1998), and the final result in both cases is a stomoblastula-like embryo, though these phenomena, undoubtedly, originated independently.

During embryonic development, a special structure made up of flattened cells is formed. This structure is referred to as the placental membrane. It was first described

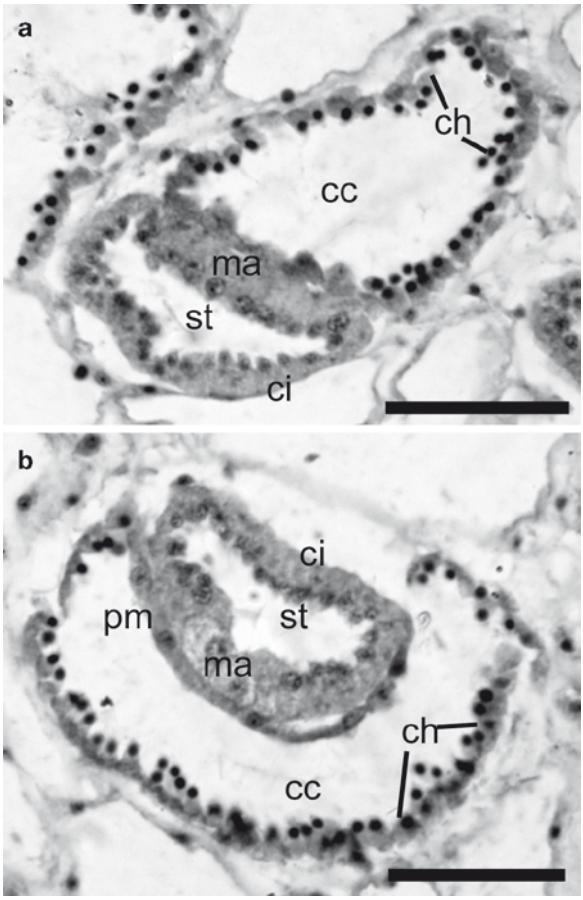

Fig. 1.8 Semi-thin micrographs of *Sycon raphanus* stomoblastula (*st*). (**a**) Stomoblastula at the beginning of the placentar membrane formation. (**b**) Stomoblastula with placentar membrane. *ci* ciliated cells, *ch* choanocytes, *ma* macromeres, *pm* placentar membrane. Scale bars (**a**, **b**) 40 μm

in *S.* cf. *ciliatum* by Schulze (1875). The placental membrane ensures the embryo's nutrition and participates in inversion (Duboscq and Tuzet 1937; Lufty 1957a).

Duboscq and Tuzet (1937) reported that the placental membrane in some calcaronean species was formed from parent choanocytes that gradually spread around the embryo (Fig. 1.8b). These observations were later confirmed at the electron microscopic level in *G. compressa* and *P. massiliana* (Gallissian 1983; Gallissian and Vacelet 1992). At the same time, no placental membrane was observed in the electron microscopic studies of *S. ciliata* (*S. ciliatum*) (Franzen 1988) and *S. calcaravis* (Yamasaki and Watanabe 1991).

The embryo gets the nutrients through the interaction of its granular cells with the cells of the placental membrane, closely associated with the embryo in the course of its development (Duboscq and Tuzet 1937; Gallissian 1983). The cells of the placental

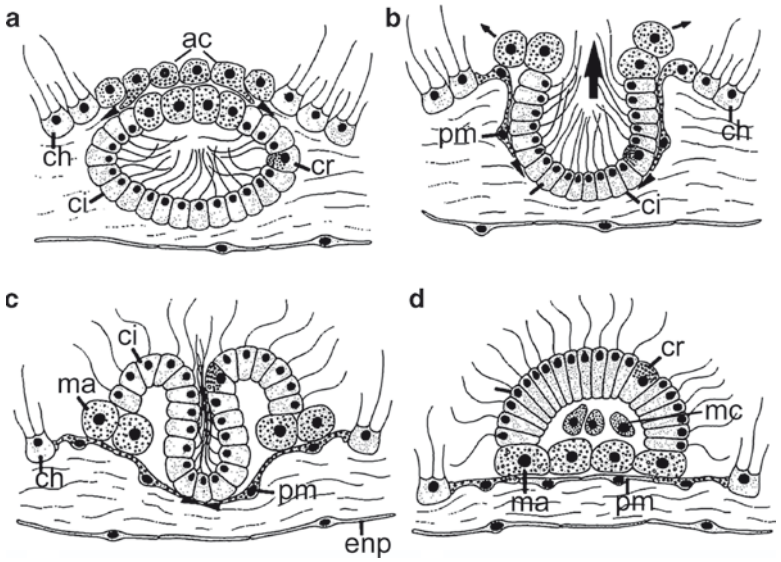

Fig. 1.9 Schematic drawing of *Sycon* stomoblastula inversion. (**a**) Stomoblastula at the beginning of placentar membrane development from the accessory-cell complex (*arrows*). (**b**) Early and middle (**c**) stages of inversion, accompanied by placentar membrane growth (*arrows*). (**d**) End of inversion. *ac* accessory cells, *ci* ciliated cells, *ch* choanocytes, *cr* cross-cells, *enp* endopinacocyte, *ma* macromeres, *mc* maternal cells, *pm* placentar membrane (From Franzen 1988, Zoomorphology, vol. 107, pp. 353, fig. 8, reproduced by permission of Springer)

membrane contain numerous food vacuoles of heterogenic nature (Gallissian 1983). In *G. compressa*, some of the placental membrane cells are phagocytosed by the granular cells and some enter the larval cavity (Gallissian 1983). Yolk inclusions of the oocytes in *G. compressa* and *S. ciliata* support the development of the embryo only until the stomoblastula stage (Gallissian 1983; Franzen 1988). With the onset of inversion, exogenous inclusions can be seen in granular cells. They result from phagocytosis of the placental membrane cells. Possibly, such an early specialization of stomoblastula macromeres for feeding is associated with the fact that, as the amount of nutrients stored in the embryo is insufficient, the embryo has to start feeding itself long before cleavage ends. This type of interaction between the embryo cells and maternal cells is known as cytotrophic viviparity (Batigina et al. 2006). The placental membrane is separated from the choanocytes of the flagellated chamber only after the formation of the amphiblastula.

Inversion and amphiblastula formation. Inversion (synonyms: incurvation and eversion) of the embryo is one of the most obscure and intriguing embryogenetic processes (Fig. 1.9). Discovered in *G. compressa* and *S. raphanus* by Duboscq and Tuzet (1935a, 1937), who called this phenomenon “inversion des surfaces,” it has been described only in the Calcaronea.

At the time of Duboscq and Tuzet, it was thought that during inversion, the cells of the placental membrane and the granular stomoblastula cells flatten and become

amoeboid. As a result, distance between the phyalopore edges increases and the stomoblastula incurvates through the broad opening formed at the animal pole, and the amphiblastula is formed. Its granular cells remain in contact with the placental membrane cells. The amphiblastula resides above the choanoderm, in the capsule formed by merging of the protective and the placental membrane. Later, the amphiblastula first breaks the capsule and then the choanoderm layer, and passes into the lumen of the radial canal. After larval release, the cells of the placental membrane are redifferentiated into choanocytes (Franzen 1988; Gallissian and Vacelet 1992; Eerkes-Medrano and Leys 2006).

Recent light and electron microscopic observations on *S. ciliata* (Franzen, 1988) and *S. calcaravis* (Yamasaki and Watanabe 1991) showed that inversion in syconoid calcaroneans results from coordinated movements of the layer of parent choanocytes and the granular cells of the stomoblastula. At the beginning of inversion in *S. ciliata*, the choanocytes lose the collar and the flagellum, and their basal parts produce long flattened processes spreading along the embryo (Fig. 1.9a, b). Such transformed choanocytes establish contact with granular stomoblastula macromeres, which, in turn, spread the filopodia towards the former. As a result of this interaction, the mass of granular cells is drawn into the choanocyte chamber, and the cells are flattened during this process. The transformed flattened choanocytes migrate along the external surface of the stomoblastula, pulling the granular cells along with them and making the stomoblastula turn inside out. The process is aided by the filopodial activity of the granular cells. The choanoderm stretches, enlarging the phyalopore (Fig. 1.9c). After inversion, the peripheral granular cells bordering the phyalopore retain the pseudopodial contact with the neighbor cells. When the inversion terminates, the phyalopore closes and the flattened choanocytes gradually become cylindrical. At the end of inversion, the larva lies within the choanocyte chamber (Fig. 1.9d). To sum up, inversion is initiated by the choanocytes of the parent sponge and is carried out by the granular cells of the stomoblastula (Yamasaki and Watanabe 1991).

Inversion in asconoid calcaroneans is somewhat different (Fig. 1.10) (Anakina 1981). In *Leucosolenia*, it starts with flattening of the accessory cells contacting the granular stomoblastula cells (Fig. 1.10a). At the same time, accessory cells diverge,

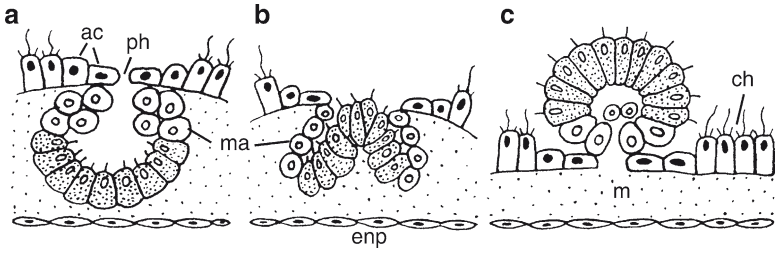

Fig. 1.10 Schematic drawing of *Leucosolenia* stomoblastula inversion. (**a**) Stomoblastula before inversion. (**b**) Middle stage of inversion. (**c**) Last stage of inversion. *ac* accessory cells, *ch* choanocytes, *enp* endopinacocyte, *m* mesohyl, *ma* macromeres, *ph* phyalopore

an opening is formed above the phyalopore, and the latter also opens up. The granular cells flatten and protrude into the spongocoel. Following them, the layer of ciliated cells incurves (Fig. 1.10). Peripheral granular cells of the stomoblastula remain in contact with the accessory cells. After inversion of the ciliated cell layer, the larva finds itself in the atrial cavity and the inversion opening begins to close (Fig. 1.10c) (Anakina 1981).

Development of the atypical leuconoid sponge *P. massiliana* is rather peculiar (Fig. 1.11) (Vacelet 1964; Gallissian and Vacelet 1992). After the formation of an early stomoblastula, choanocytes of the choanocyte chamber next to the embryo transform into nurse cells, which accumulate lipids. Some of these cells get into the stomoblastula cavity through the phyalopore (Fig. 1.11a). During inversion, granular non-ciliated cells become capable of amoeboid movement and move into the neighboring chamber, into which the stomoblastula inverses (Fig. 1.11b). As a result, the amphiblastula lies in the embryonic capsule, whose walls are made up of transformed choanocytes of the choanocyte chamber. The former choanocytes contacting

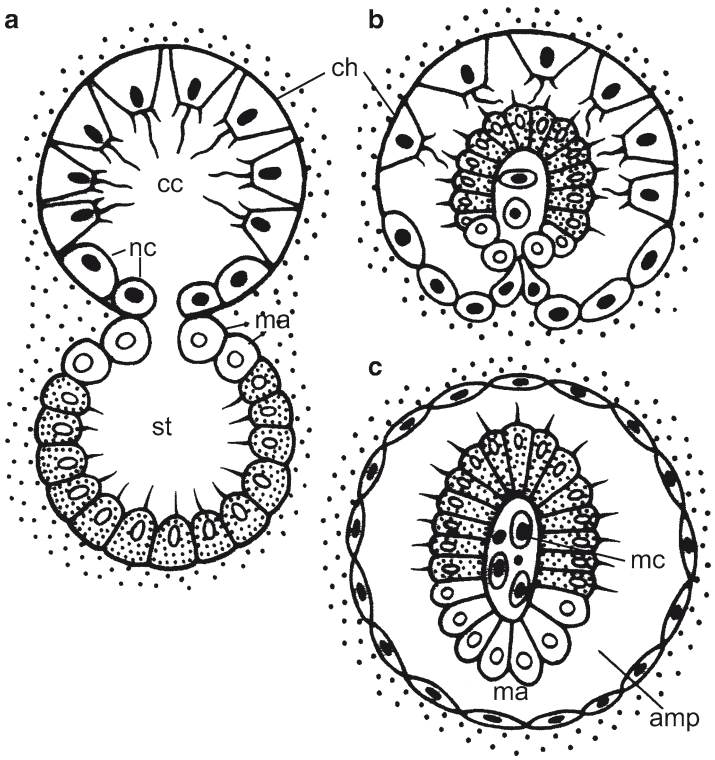

Fig. 1.11 Schematic drawing of *Petrobiona* stomoblastula inversion. (**a**) Stomoblastula, joint with choanocyte chamber. (**b**) Last stage of inversion. (**c**) Amphiblastula inside the choanocyte chamber. *amp* amphiblastula, *ch* choanocytes, *cc* choanocyte chamber, *ma* macromeres, *mc* maternal cells, *nc* nurse cells, *st* stomoblastula

the granular cells become nurse cells, some of which get into the amphiblastula cavity (Fig. 1.11c). Subsequently, the larval granular cells begin to divide and a normal amphiblastula is formed.

In all Calcaronea, different parent sponge cells, such as choanocytes and cells of the placental membrane get into the amphiblastula cavity during inversion (Lufty 1957b). Inside *G. compressa* larvae, numerous amoeboid cells with heterogeneous inclusions, rare spherule cells, and numerous symbiotic bacteria were observed (Gallissian 1983); in *S. ciliata* larvae, microgranular cells were found (Franzen 1988); and in *L. botrioides* and *L. complicata*, accessory cells were observed (Tuzet 1948; Anakina 1981).

In *S.* cf. *ciliatum,* Schulze (1875) described a temporary invagination of the layer of granular cells into the larval cavity of the amphiblastula released into the exhalant canals. He considered the granular cells as the endoderm, and the ciliated ones as the ectoderm. On the contrary, Metschnikoff (1874) described invagination of the ciliated-cell layer into the larval cavity of the free-swimming amphiblastula of the same species; consequently, he ascribed the role of the endoderm to the ciliated cells, and that of the ectoderm to the granular cells. Later, Schulze (1878a) agreed with Metschnikoff's description of the endoderm and ectoderm, and termed the temporary invagination of the cells layer into the larval cavity as *pseudogastrulation*. This process was also described in *S. raphanus* (Barrois 1876; Hammer 1908; Duboscq and Tuzet 1937). However, Hammer (1908) expressed an opinion that pseudogastrulation is due to mechanical pressure of the embryonic capsule upon the larva, and that it is of no importance for further development.

In *L. complicata*, the "pseudogastrula" stage is also present, which is the period when large, granular amphiblastula cells enter and fill the larval cavity. This stage is transitory, occurring shortly before larval release. When the descendants of macromeres enter into the amphiblastula cavity, they lose contact with the accessory cells. Consequently, the larva separates from the parent sponge (Anakina 1981). Thus, temporary invagination of the granular cells into the amphiblastula cavity has nothing to do with gastrulation. Pseudogastrulation is merely a side effect of separation of larval cells from the parent choanoderm prior to larval release.

1.1.4 Larva

In all the species of Calcaronea, amphiblastula larvae are very similar in structure and cell composition, regardless of their aquiferous system organization. The amphiblastula has a pronounced anterior–posterior axis. Ciliated cells occupy three fourth of the larval surface, while the granular cells occupy the remaining one fourth (the posterio-lateral surface) (Fig. 1.12). The larva also comprises four cruciform cells that are symmetrically positioned cells with refractive inclusions. Cruciform cells were first described in the *S. raphanus* larva (Duboscq and Tuzet 1935b).

Ciliated cells are prismatic in shape and form a regular epithelial layer. The nuclei are apical and contain nucleoli. The nucleus is usually pear-shaped, and the ciliar

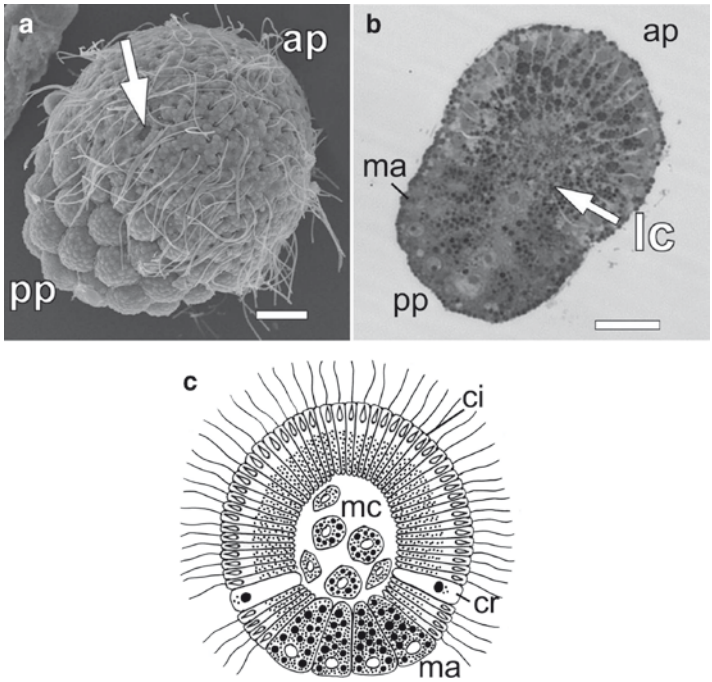

Fig. 1.12 Amphiblastula of Calcaronea. (**a**) SEM and (**b**) light micrographs of amphiblastula of *Sycon* cf. *raphanus*, and (**c**) scheme. (**a**) A 2-h-old free-swimming larva showing the anterior ciliated hemisphere (*ap*) and large globular, granular cells of the posterior hemisphere (*pp*). One of the four "cross cells" on the anterior hemisphere is shown by the *arrow*. (**b**) A longitudinal section through a free-swimming larva in the same orientation as (**a**). (**c**) Diagram of amphiblastula. (**a**, **b** From Leys and Eerkes-Megrano 2005, Integr Comp Biol, vol. 45, pp. 348, Fig. 7a, b, Reproduced by permission of Oxford University Press). *ci* ciliated cells, *ma* macromeres, *cr* cross-cell, *lc* larval cavity, *mc* maternal cells. Scale bars (**a**, **b**) 10 μm

rootlet apparatus, dictyosomes, and mitochondria are situated along the apical part. The rootlet system consists of three elements: a long rootlet running along the apical part of the nucleus and two short ones parallel to the apical cell surface (Gallissian and Vacelet 1992; Amano and Hori 1992). These rootlets are distinctly cross-striated, similar to those in *Trichoplax* and other Eumetazoa. The apical part also contains numerous small inclusions with fibrillar content, lipid drops, and glycogen granules. No specialized intercellular contacts between ciliated cells have been observed. As the larva develops, lipid and yolk inclusions gradually disappear (Gallissian 1983; Franzen 1988; Amano and Hori 1992; Gallissian and Vacelet 1992).

The posterior pole of the amphiblastula consists of non-ciliated granular cells, considered to be the descendants of macromeres. An irregularly shaped nucleus is located centrally (Gallissian 1983; Amano and Hori 1992). The granular cells contain numerous heterogeneous inclusions similar to those in the ciliated cells.

According to Franzen (1988), descendants of macromeres in *S. ciliata* larvae are differentiated into non-granular and granular cells. The author might have observed a gradual disappearance of the granules in the descendants of macromeres. This process is to be completed in the free-swimming larva, as shown, e.g., in *L. complicata* (Anakina 1981).

Large cruciform cells are usually positioned crosswise at the larval equator (Tuzet 1973; Simpson 1984). In *L. complicata,* they are situated at the border of the ciliated and non-ciliated layers (Anakina 1981). Cruciform cells have no cilium, and their nuclei have no nucleolus. In the basal part, the remains of yolk inclusions and numerous large vacuoles with lightly colored content can be observed (Gallissian 1983; Franzen 1988; Gallissian and Vacelet 1992; Amano and Hori 1992). It must be noted that cruciform cells in *P. massiliana* amphiblastulae are very weakly differentiated when compared with those in the amphiblastulae of other species (Gallissian and Vacelet 1992). Cruciform-cell determinants appear at the stage of two blastomeres as characteristic accumulations of specific granules.

The role of cruciform cells is still obscure. Since the times of Duboscq and Tuzet (1941), they have been considered to be photoreceptive. However, although *Sycon* larvae are phototactic (Elliott et al. 2004), there is still no evidence that it is the cruciform cells that are the photoreceptors. Furthermore, by the time the amphiblastula of *G. compressa* and *P. massiliana* are released into the environment, the cruciform cells begin to degenerate. Furthermore, in the free-swimming larvae of *G. compressa,* they are not found at all (Gallissian 1983; Gallissian and Vacelet 1992).

1.1.5 Metamorphosis

Postembryonic development of Calcaronea may be divided into four phases: (1) a settling larva; (2) a larva undergoing metamorphosis; (3) pupa (preolynthus); and (4) olynthus (a juvenile with asconoid aquiferous system). The term "pupa" was applied to calcareous sponges by Minchin (1900), to indicate the stage between the settling larva and the olynthus. According to Maas (1904), pupa is equivalent to preolynthus.

In the nineteenth and early twentieth centuries, some researchers described a strange phenomenon: the ciliated layer at the anterior pole of the swimming amphiblastula immersed into the larval cavity (Metschnikoff 1874; 1879; Maas 1900; Hammer 1908). Metschnikoff considered this process to be gastrulation, arguing that the posterior larval hemisphere transforms into the skeletogenous layer of the sponge, and the ciliated blastoderm invaginating into the larval cavity becomes the choanoderm. Barrois (1876), who studied the larvae of *S. raphanus* and *G. compressa*, came to a similar conclusion: ciliated cells of the larva transforms into pinacoderm, granular cells, and choanoderm. These observations and conclusions have often been reproduced in monographs and textbooks.

However, according to Jones (1971), the observations made by Metschnikoff and his followers were artifacts. Their observations were conducted in hanging drops; the larva's contact with the surface tension film could have the invagination of the ciliated layer into the cavity. Jones' idea was supported by Anakina's

experiments (1989): *L. complicata* amphiblastulae placed into a drop of water perished rather soon, developing, before they die, a series of local undulating invaginations of the ciliated layer in the larval cavity. To sum up, there are no events in the development of the Calcaronea that could be considered as manifestations of gastrulation, *sensu* Metschnikoff.

Free larval life lasts from 6 h to 3 days. Before settling, the larvae swim, still rotating, near the bottom. When an amphiblastula settles, it contacts the substrate with its ciliated (anterior) pole (Jones 1971; Amano and Hori 1993; Leys and Eerkes-Megrano 2005). The main role in the primary attachment to the substrate is played by their cilia, which seem to be an adhesive. Immediately after settlement, some of the larval granular cells flatten, become amoeboid, and spread on the substrate, attaching to the surface irregularities by pseudopodia. In this way, they increase the area of contact between the larva and the substrate. At the same time, other granular cells overgrow, dedifferentiating the cells of the ciliated layer (Fig. 1.13) (Jones 1971; Anakina 1989; Amano and Hori 1993).

According to ultrastructural data obtained from *Sycon* sp. and *Leucandra abratsbo* larvae, the first structure formed by the differentiating granular cells of the posterior pole is the exopinacoderm (Amano and Hori 1993). As shown in the experiments with colchicine, granular cells do not divide while pinacocytes are formed (Amano and Hori 1993).

For a long time, the origin of mesohylar cells was a much-debated issue. Schulze (1878a), Minchin (1896), and Anakina (1989) presumed that they originated from the larval granular cells. Duboscq and Tuzet (1937) supposed that scleroblasts differentiated from the ciliated cells, and that other mesohyl cells differentiated from the granular cells. The debate was put to an end by an electron microscopic study of metamorphic stages: all internal cells of a developing sponge were shown

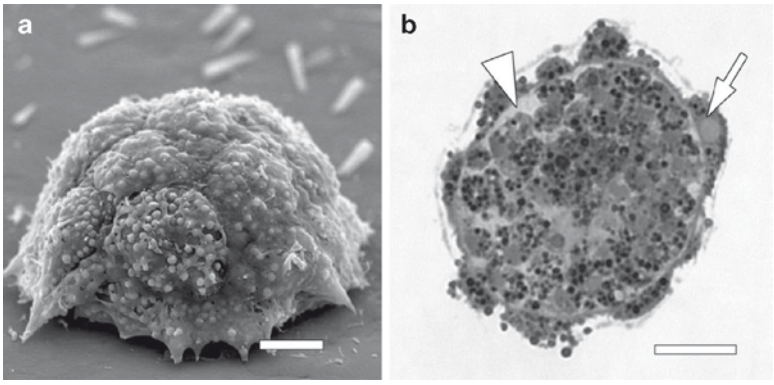

Fig. 1.13 Light and SEM of the first stage of *Sycon* cf. *raphanus* metamorphosis. (**a**) SEM of a newly adherent post-larva. (**b**) Light micrograph of a section horizontal through a plastic-embedded newly adherent and metamorphosing larva (between 12 h and 2 days after release from the parent). The post-larva has an outer layer of granular cells (*arrow*) surrounding a group of variously shaped cells in a collagenous extracellular matrix (*arrowhead*) (From Leys and Eerkes-Megrano 2005, Integr Comp Biol, vol. 45, pp. 348, Fig. 7d, e, reproduced by permission of Oxford University Press). Scale bars (**a**, **b**) 10 μm

to originate from the ciliated cells of the amphiblastula (Amano and Hori 1993). Dedifferentiating ciliated cells become rounded, lose the cilium and their rootlet, and their nucleus becomes larger. The ciliated layer does not invaginate into the larval cavity (Amano and Hori 1993). However, Leys and Eerkes-Megrano (2005), after examining over 300 settled larvae of *Sycon* cf. *raphanus*, found four larvae with an invagination of the anterior cells into the posterior half.

Thus, the loose internal mass of the pupa mostly consists of dedifferentiated ciliated cells. In *L. complicata*, a few granular cells of the posterior pole may also get inside the pupa (Anakina 1981). Scleroblasts originate from dedifferentiated ciliated cells very early, and hence, when the first spicules (diactines) are formed, pores and the osculum of the olynthus are not yet opened (Amano and Hori 1993). The fate of larval cells at metamorphosis is schematically shown in Fig. 1.14.

In a dense central mass of choanoblasts, a cavity gradually develops, and the cells organize themselves in a joint choanocyte layer. The pupa becomes the olynthus, which has a central cavity lined with a layer of choanoblasts with apical nuclei. By the time the central cavity develops, the first differentiated cells, exopinacocytes and sclerocytes would have already started functioning in the pupa. Thus, in the course of metamorphosis, all the cells of the settled amphiblastula lose the specialized larval features, and only after that, they start to transform into the definitive cells of the sponge.

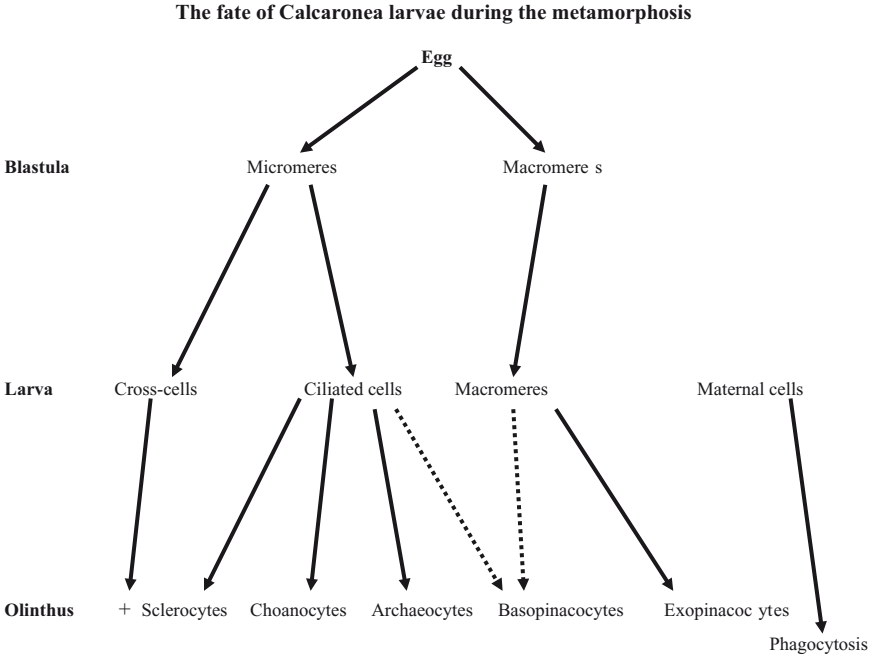

Fig. 1.14 The fate of Calcaronea larvae cells during metamorphosis

Regardless of the aquiferous system organization in the adult sponge (asconoid, syconoid, sylleibid, or leuconoid), the juvenile single-osculum sponge (olynthus) is asconoid. Olynthus has all basic cell elements of the adult sponge.

Preliminary molecular biological data indicate molecular heterogeneity between cell lineages in the Calcaronea larvae (Manuel 2001). The homeobox gene SrNkxD (NK-2 class) is expressed in the granular cells of the *S. raphanus* amphiblastula, but never in its ciliated cells or cruciform cells (Manuel 2001). In adult sponges, the gene is expressed only in the exopinacoderm.

Parent granular cells disappear at the first stages of metamorphosis, and their remains can be observed in the heterophagosomes of the pupa's internal cells (Amano and Hori 1993). However, the fate of cruciform cells has not yet been traced.

Transition of the olynthus from asconoid to syconoid organization has been studied in *S. ciliatum* (Schulze 1878a; Maas 1900; Hammer 1908). First, radiating sac-like outgrowths are formed in the olynthus wall,which gradually become the radial canals of a syconoid sponge. Concomitantly, the mass of the mesohyl increases.

1.1.6 Asexual Reproduction

Asexual reproduction in the form of budding has been described in *L. botryoides* and in some *Sycon* species (Vasseur 1979; Connes 1964, 1968). The bud is formed at the base of the parent sponge. In *Sycon*, bud development is preceded by local disintegration of choanocyte chambers (Connes 1964, 1968). Subsequently, a body-wall outgrowth is formed. It is covered with the exopinacoderm and lined inside with the choanoderm; in between, mesohyl cells can be observed. The new sponge is at first asconoid, and then active proliferation and growth follow. On the whole, morphogenesis accompanying bud formation in *Sycon* is analogous to that accompanying metamorphosis in its larvae.

Asexual reproduction has been observed in some species through basal creeping stolons on which new sponges grow, e.g., in *S. ciliatum*, *S. sycandra* (Lendenfeld 1885), and some *Leucosolenia* species (Brien 1973a). These stolons can produce terminal hollow spherical buds, which detach and form propagules with a peculiar skeleton. Alternatively, spherical propagules can be formed from the distal parts of the radial tubes through the constriction and subsequent detachment of the region just under the distal cones as in *S. frustulosum* (Borojevic and Peixinho 1976).

1.2 The Subclass Calcinea Bidder, 1898

Sponges from the subclass Calcinea are very variable (color plate II). Their aquiferous system may be asconoid, syconoid, sylleibid, or leuconoid. Their spicules generally have rays of uniform length, radiating at uniform angles. Some species also have parasagittal spicules, with one of the rays longer than the others and positioned at a different angle; some have sagittal triactines, a basal system of tetractines, or both (Fig. 1.15).

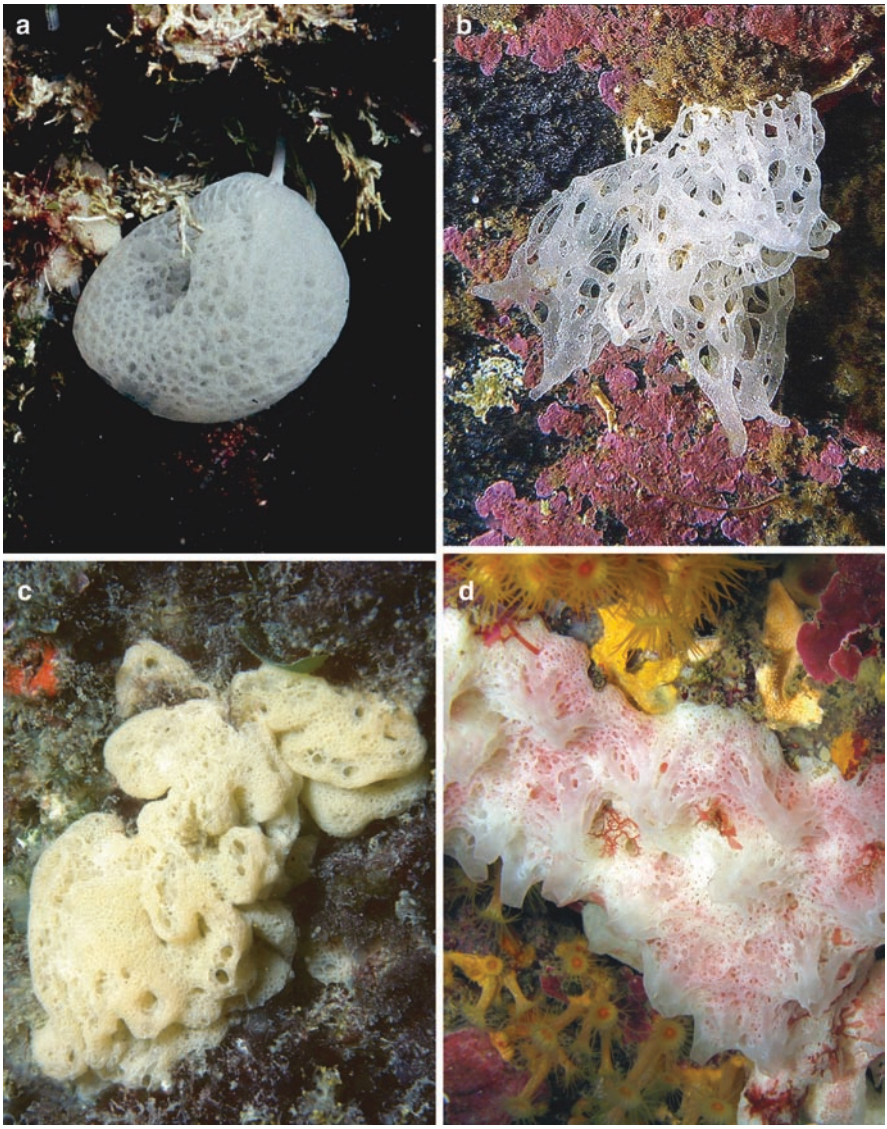

Plate II Class Calcarea, subclass Calcinea Bidder, 1898. (**a**) *Guancha lacunosa* (Johnston 1842), Mediterranean Sea (Courtesy of J. Vacelet). (**b**) *Guancha arnesenae* Rapp 2006, White Sea (Courtesy of M. Fedjuk). (**c**) *Clathrina cerebrum* (Haeckel 1872), Mediterranean Sea (Courtesy of J. Vacelet). (**d**) *Clathrina contorta* (Bowerbank 1866), Mediterranean Sea (Courtesy of J. Harmelin)

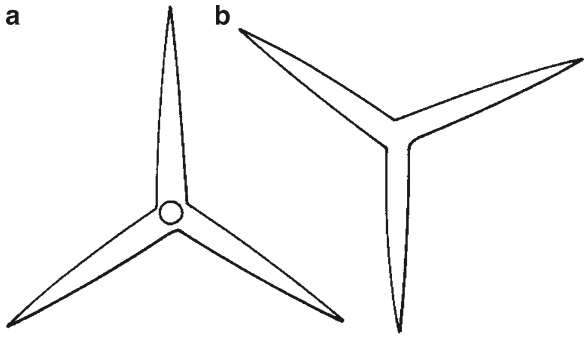

Fig. 1.15 Spicules of Calcinea. (**a**) Sagittal tetractine; (**b**) triactine

In addition to spicules, non-spicular basal skeleton is present in the genus *Murrayona*. During ontogenesis, triactines are the first spicules to develop. Choanocytes have basally located nuclei. The nucleus of the choanocyte is never associated with the flagellar basal apparatus. The Calcinea are ovoviviparous, with a calciblastula (formerly called coeloblastula) larva. Asexual reproduction occurs in some species in the form of budding.

The Calcinea are marine, distributed in all oceans. The subclass comprises two orders, Clathrinida and Murrayonida, which consists of six families and 16 genera (Manuel et al. 2002).

Research on Calcinea and Calcaronea started concomitantly. Miklucho-Maclay (1868) was the first to deal with the Calcinea embryos, briefly describing those of *Guancha blanca*. Schmidt (1877) and Metschnikoff (1879) carried out pioneer studies on Calcinea development on *Clathrina cribrata* (*Ascetta primordialis*), *G. blanca,* and *C. clathrus*.

Later, however, these sponges "fell out of favour" among the researchers, who devoted much more attention to Calcaronea. Almost all information currently available on oogenesis and embryogenesis of Calcinea was obtained from light microscopic studies (Schmidt 1877; Metschnikoff 1879; Minchin 1896, 1900; Hadži 1917; Dendy and Frederick 1924; Tuzet 1948; Sarà 1955; Borojevic 1969; Johnson 1978, 1979a, b). Only their larval structure and metamorphosis were investigated at the electron microscopic level (Borojevic 1969; Amano and Hori 2001; Ereskovsky and Willenz 2008).

Sexual differentiation in Calcinea has mostly remained unstudied, and those researchers who investigated reproductive cycles in these sponges observed only female gametes (Borojevic 1969; Johnson 1978, 1979a).

1.2.1 Gametogenesis

The origin of gametes. As no sperm cells were described in Calcinea, we can only describe the origin of female gametes. According to Borojevic (1969), oogonia in

Clathrina and *Ascandra* develop from pinacocytes (porocytes) that migrate into the mesohyl. The majority of earlier authors presumed that oogonia differentiated either from choanocytes or from amoebocytes (Minchin 1900). At present, neither of these hypotheses could be proved nor disproved.

Oogenesis. Oogonial divisions in the Calcinea have not been observed. The cytoplasmic growth of a young oocyte occurs in the thin layer of mesohyl under the choanoderm (Fig. 1.16a, b) (Borojevic 1969; Johnson 1979a). At the beginning of the vitellogenic phase, the oocytes exhibit amoeboid motility and actively phagocyte various somatic cells (Fig. 1.16c). By the end of this phase, the surface of the oocyte becomes smooth (Fig. 1.16d) and its nutrition proceeds via cytoplasmic bridges connecting it to the phagocytes. After the phagosomes have passed to the oocyte, the phagocytes degrade (Borojevic 1969).

In different Calcinea species, the oocytes may feed on different somatic cells. According to some observations, in *G. blanca* and *C. coriacea,* the oocytes readily phagocyte eosinophilic amoebocytes (Tuzet 1947; Johnson 1979a). It must be noted that the latter appear in the mesohyl only during sexual reproduction of the sponge (Johnson 1979a). According to other observations, female gametes of *G. blanca* first phagocyte choanocytes and only then phagocyte the eosinophilic

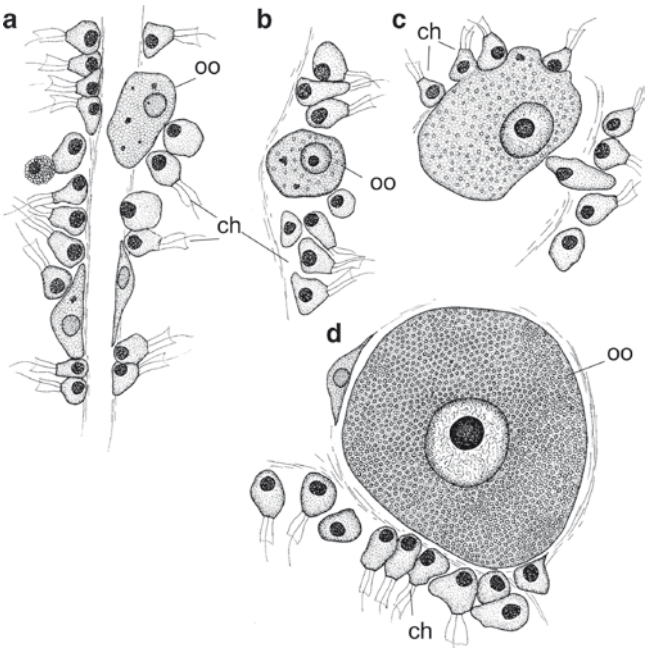

Fig. 1.16 Schematic drawing of oogenesis of *Ascandra minchini.* (**a**, **b**) early oocyte, (**c**) oocyte at the first stages of vitellogenesis, (**d**) egg (Courtesy of R. Borojevic); *oo* oocyte, *ch* choanocytes. Scale bar 10 μm

amoebocytes, whereas those of *C. coriacea* phagocyte only choanocytes (Sarà 1955). In *C. rubra*, *C. contorta,* and *Ascandra* spp., the oocytes first directly consume choanocytes, eosinophilic and hyaline amoebocytes, and then migrate to the so-called *nests* (see below), where they get nutrients from phagocytes via cytoplasmic bridges (Borojevic 1969).

Egg diameter in the Calcinea varies from 85 to 120 μm. The mature egg is not polarized, and the nucleus is located centrally. The eggs are isolecithal (the yolk granules are evenly distributed in the ooplasm) and polylecithal (rich in yolk granules).

During sexual reproduction, some Calcinea sponges (*Ascandra minchini*, *A. falcata*, *C. contorta*) develop peculiar temporary structures, referred to as "*nests"* (*nids* in French; Borojevic 1969). They are formed in the body wall, evenly throughout the sponge. A nest looks like an anarchized tissue with its own skeleton and a cylindrical cellular strand covered with pinacoderm. Its longer axis is perpendicular to the body wall. Each nest has its own developmental cycle.

Nest formation resembles the process of radial canal destruction during the development of new aquiferous system elements. Choanoderm is disorganized, and chaotically grouped choanocytes lose the flagellum and the collar. The neighboring mesohyl areas are destroyed, and their cells get dedifferentiated. The central strand is made up of amoebocytes, which actively phagocyte different somatic cells migrating into the forming nest (Borojevic 1969).

Vitellogenic oocytes migrate into the nest and group around the basis of the central strand, where oogenesis is completed. The developing eggs and embryos gradually move to the apical part of the nest, closer to the atrial surface. Through a special opening, the larvae get into the atrium and then are released into the environment via the osculum. After the last larva has been released, the nest degenerates and the normal choanoderm structure is restored.

Nests are not present in some other Calcinea sponges, such as *C. coriacea*, *G. blanca* (Johnson 1979a; Minchin 1900), and *G. arnesenae* (Ereskovsky and Willenz 2008). In *Clathrina*, each embryo is enveloped in a sheath of elongate parental cells (Johnson 1979a).

In *G. arnesenae* from the White Sea (Arctic), we found a rather unusual structure: a two-layered follicle where the embryos develop (Fig. 1.17). The external layer consists of dense extracellular matrix, while the internal layer is made up of large cells, which may be cubic, prismatic, or flattened (Fig. 1.17). Follicular cells are close to the embryo, but neither remain in contact nor produce any projections in its direction. They do, however, produce projections in the opposite direction; these projections are long and anchored in the extracellular matrix. Symbiotic bacteria often lie between the two layers of the follicle (Ereskovsky and Willenz 2008).

No special cell junctions were observed between follicular cells. Their cytoplasm is foamy, about one third of it being filled with electron-transparent vacuoles. It also contains numerous phagosomes and a few lipid droplets. Near the nucleus, there are elements of the flagellar basal apparatus, the basal body, and the accessory centriole. This indicates that the cells of the follicle originate from choanocytes.

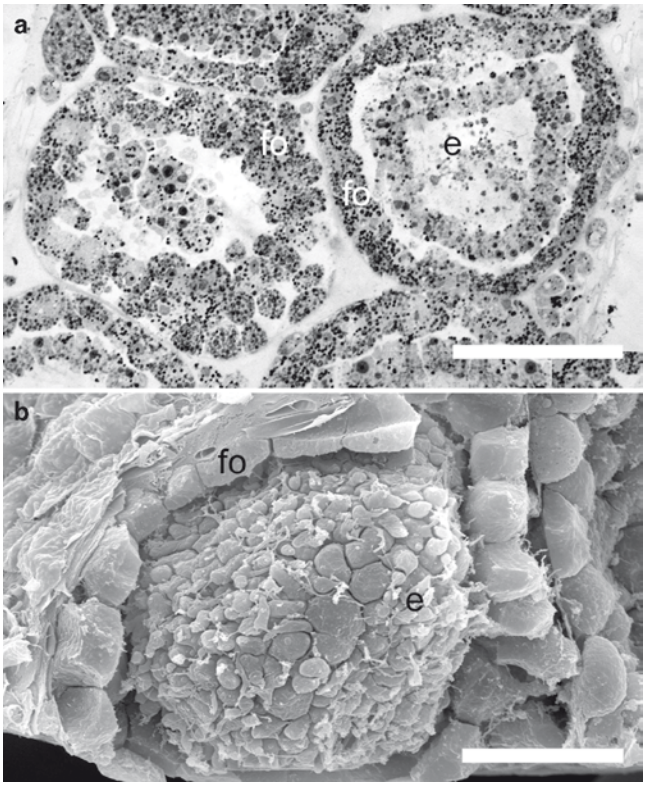

Fig. 1.17 *Guancha arnesenae*. (a) Semi-thin and (b) SEM micrographs of the follicle. *e* embryo, *fo* follicle. Scale bars (**a**) 50 μm, (**b**) 25 μm

1.2.2 Embryonic Development

Embryonic development has been described in several Calcinea species: *C. cribrata*, *G. blanca*, *C. clathrus*, *A. falcata*, *A. minchini*, *C. coriacea*, *C. cerebrum,* and *G. arnesenae* (Metschnikoff 1879; Schmidt 1877; Minchin 1900; Tuzet 1948; Borojevic 1969; Johnson 1979a; Ereskovsky and Willenz 2008).

Cleavage of the egg is total and equal. From the 8-cell stage, cleavage planes are radial and run perpendicular to the embryo's surface (Fig. 1.18) (Tuzet 1948; Borojevic 1969). As a result, a hollow, one-layered, equal, non-polarized blastula is formed and retained from the stage of eight blastomeres to the larval stage. We attributed this cleavage pattern to a new type, polyaxial cleavage, on the basic of the following characteristics (Ereskovsky 2002):

1. Cleavage planes are perpendicular to the embryo's surface from the eight-cell stage and to the end of cytodifferentiation.
2. The cleavage cavity is formed during the third cleavage cycle and retained until the end of embryogenesis.

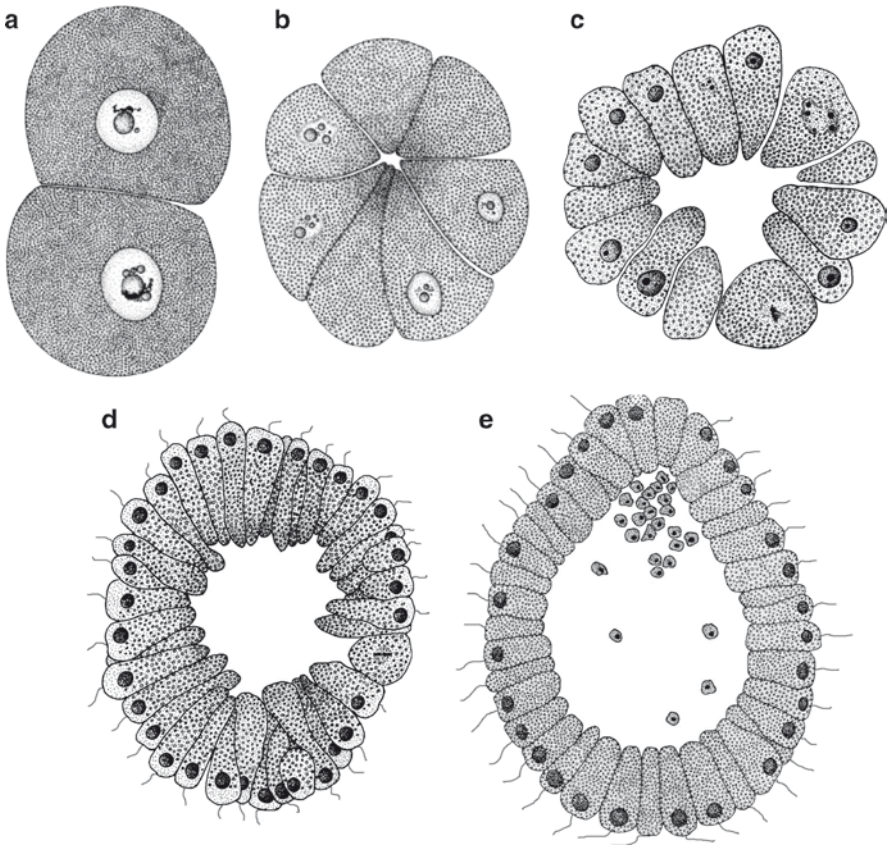

Fig. 1.18 Schematic drawing of embryonic development of Calcinea. (**a**–**c**) Polyaxial cleavage. (**d**, **e**) Larval morphogenesis. (**a**–**d**) *Ascandra minchini*, (**e**) *Leucetta chagosensis* pre-larvae (Courtesy of R. Borojevic)

3. Throughout the cleavage, the embryo has a definite number of symmetry axes (the same as the number of blastomeres), which radiate at certain angles from one point, the geometric center of the embryo.
4. The embryos do not have an anterior-posterior polarity.

Polyaxial cleavage is intermediate between the chaotic cleavage and the radial one. It differs from the former in the regular position of cleavage planes, and from the latter, in the absence of an animal–vegetative axis determining the radial symmetry of the embryo. Besides Calcinea, polyaxial cleavage is characteristic of Halisarcida (the class Demospongiae), which form a one-layered flagellated blastula (see Section 3.6). Polyaxial cleavage is associated with the lack of an animal–vegetative axis and a polarized distribution of yolk granules in the egg, and correlates with a late formation of anterior–posterior axis of the embryo during embryogenesis.

Larval morphogenesis. Larva formation in Calcinea does not involve any morphogenetic movements of the cells or their layers. The cells of the embryo

proliferate and differentiate. Active cell proliferation results in an increase in the surface area, so that the blastula becomes plicate (Fig. 1.18d, e).

The beginning of cell differentiation is marked by the shift of the nuclei from central to apical position, as described in *A. primordialis* (Metschnikoff 1879), *C. contorta*, *A. falcata*, *A. minchini* (Borojevic 1969), and *G. arnesenae* (Ereskovsky and Willenz 2008). Then a cilium is formed on the apical face of each cell (Fig. 1.18d, e). The blastula cells closely adjoin each other with their sides, but no intercellular junctions have been revealed.

In *A. minchini*, the larval cavity remains empty throughout the development. In *A. falcata*, the larva is invaded by tiny parental cells: at the four-cell stage, they start to enter into the spaces between the blastomeres, and later penetrate into the larval cavity (Borojevic 1969). Numerous tiny cells of parental origin were also noted in the cavity of *Leucetta chagosensis* blastulae (Fig. 1.18e), but they disappeared by the time of larval release (Borojevic 1969).

Endosymbiotic bacteria, characteristic of the parent sponge, are numerous both in the cavity and the intercellular spaces of the *G. arnesenae* and *C. contorta* blastula (Ereskovsky and Willenz 2008; Ereskovsky unpublished).

1.2.3 Larva

The Calcinea larva is a calciblastula, previously called coeloblastula (Maldonado and Bergquist 2002). There are numerous light microscopic descriptions of these larvae (Metschnikoff 1879; Dendy 1891; Minchin 1896, 1900; Tuzet 1948; Johnson 1979a, Borojevic 1969) and two electron microscopic studies devoted to them (Amano and Hori 2001; Ereskovsky and Willenz 2008). Calciblastulae are rounded or egg-shaped, from 50–90 to 70–200 μm in length. The longer axis is the anterior–posterior one. The body wall consists of a single layer of cells, surrounding the larval cavity. Most researchers described two types of cells: the ciliated cells and the granular cells of the posterior pole (Fig. 1.19). Ultrastructural studies of *Soleneiscus* sp. (Amano and Hori 2001; in the article, the sponge studied has been wrongly referred to as "*L. laxa* Kirk, 1895"; in fact, it belongs to *Soleneiscus* sp.) (Manuel et al. 2003), *G. arnesenae,* and *C. contorta* also revealed vacuolar cells, bottle-shaped cells, and granular cells of parent origin (Amano and Hori 2001; Ereskovsky and Willenz 2008; Ereskovsky unpublished).

Ciliated cells are the predominant cell type of the calciblastula. They have not an underlying basement membrane. All cilia are of the same length. There is a basal apparatus, consisting of the kinetosome (the basal body) and the additional centriole perpendicular to it. A double, fibrillar, cross-striated rootlet, associated with the nuclear membrane, starts from the kinetosome, and so do the radiating microtubules and a microtubular bundle parallel to the external cell surface (Borojevic 1969; Amano and Hori 2001; Ereskovsky and Willenz 2008). The microtubular bundles of all the cells remain parallel, directed towards the posterior larval pole. In this way, coordinated beating of the cilia is achieved (Fig. 1.20). In the apical

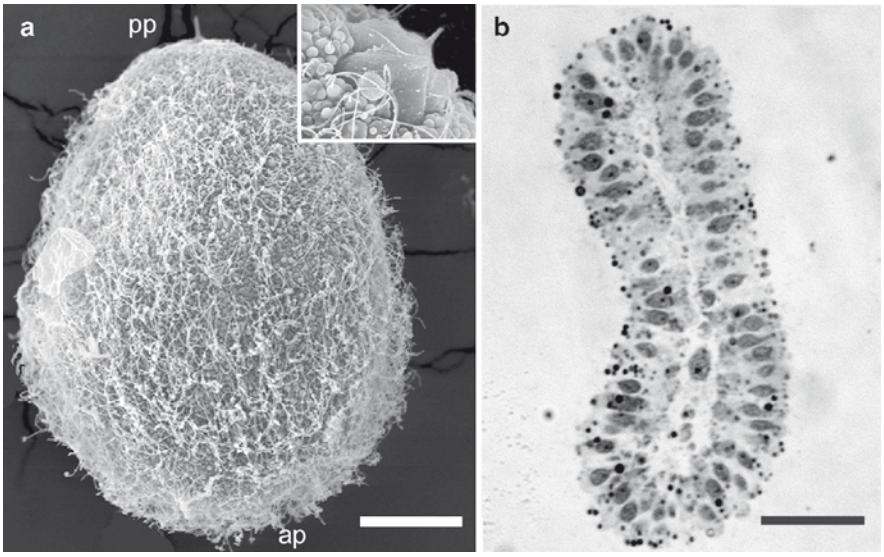

Fig. 1.19 Calciblastula larva of Calcinea. (**a**) SEM of *Clathrina contorta* larva; *inset*: big posterior pole cells. (**b**) Light micrographs of *Guancha arnesenae* larva. *ap* anterior pole, *pp* posterior pole. Scale bars (**a**) 50 μm, (**b**) 30 μm

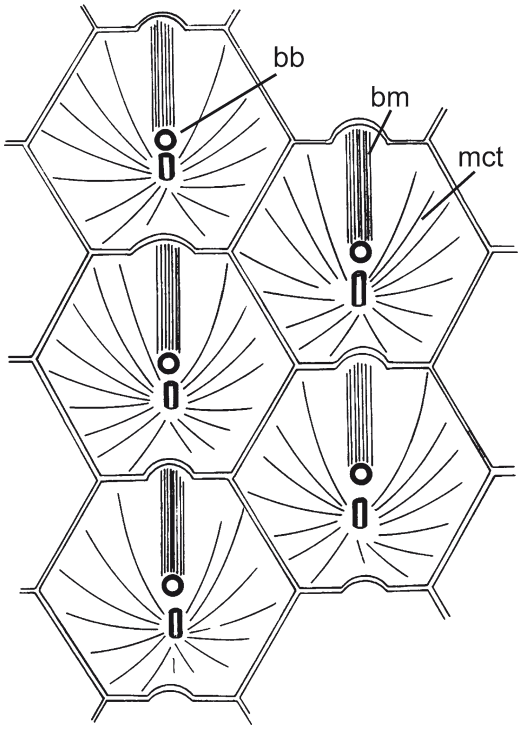

Fig. 1.20 Schematic drawing of orientation of basal ciliar apparatus of the ciliated cells of *Ascandra* calciblastula. *bb* basal body, *bm* bunch of the microtubules, *mct* microtubules (Courtesy of R. Borojevic)

regions of all ciliated larval cells of *G. arnesenae* and *C. contorta,* specialized adhesion contacts have been found (Fig. 1.21) in the form of electron-dense thickenings of filamentous material along the internal membrane (Ereskovsky and Willenz 2008; Ereskovsky unpublished).

The apical cytoplasm contains numerous electron-transparent vesicles and vacuoles with fibrillar content. Similar inclusions were observed in the egg cytoplasm. The nucleus is large, pear-shaped, with a tapered apical end adjacent to the kinetosome. The Golgi complex is situated near the apical part of the nucleus. The basal cytoplasm is filled with heterophagosomes, lipid droplets, and yolk granules.

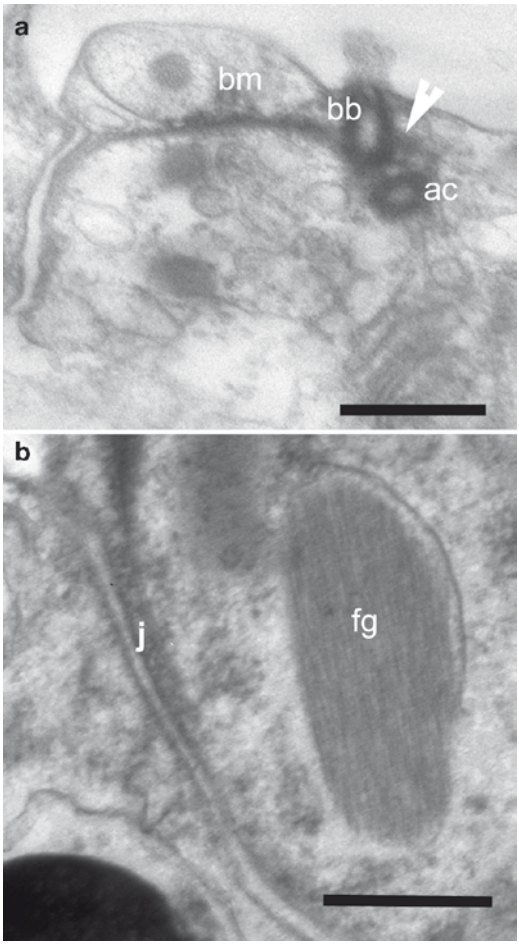

Fig. 1.21 (**a**) TEM of ciliar basal apparatus of *Guancha arnesenae* calciblastula ciliated cells. (**b**) TEM of specialized adhesion contacts between two ciliated cells (*j*), *arrowhead* basal foot, *ac* accessory centriole, *bb* basal body, *bm* bundle of microtubules, *fg* fibrous granule (From Ereskovsky and Willenz 2008, Zoomorphology, vol. 127, pp. 183, Fig 5., e.g., reproduced by permission of Springer). Scale bars (**a**) 1 μm, (**b**) 0.5 μm

In *Soleneiscus laxa*, *G. arnesenae,* and *C. contorta* larvae, bottle-shaped cells sometimes occur between the ciliated cells (Amano and Hori 2001; Ereskovsky and Willenz 2008; Ereskovsky unpublished). Bottle-shaped cells have no cilium and are larger than the ciliated cells; their cytoplasm contains numerous membranous structures (Fig. 1.21a).

At the posterior larval pole, large granular non-ciliated cells are sometimes present (Fig. 1.19a). They were first described by Metschnikoff (1879). Their number may vary from one to four; in some species, the granular cells are not found at all (Table 1.1). Tuzet (1948) thought that the presence and the number of granular cells were species-specific, whereas Minchin (1900) considered that these characters reflected individual variability.

According to Borojevic (1969), granular cells are not important for subsequent development (for metamorphosis). In his experiments, the larvae of *A. falcata* were cut across. Both the halves retained the original polarity, became rounded and formed small larvae, which started metamorphosis in 3 days and gave rise to normal pupae and olynthi (Borojevic 1969). These results seem to show that all cells of a larva are totipotent and that there is no special lineage of posterior pole cells. In the observations made by Hadži (1917), non-ciliated cells of the posterior pole disappeared in the course of metamorphosis.

In electron microscopic investigations of *S. laxa, G. arnesenae,* and *C. contorta* larvae vacuolar cells were observed (Amano and Hori 2001; Ereskovsky and Willenz 2008; Ereskovsky unpublished). In *S. laxa,* most of them were found to be concentrated in the posterior pole region, but some resided in the larval cavity. In *G. arnesenae,* these cells remained in the larval cavity, under the basal parts of the ciliated cells. Morphologically, the vacuolar cells are well-differentiated cells (Fig. 1.22b); contrary to the opinion of Borojevic (1969), they are unlikely to be retarded blastomeres. Various authors who described special non-ciliated cells might have actually described maternal vacuolar cells.

Soleneiscus laxa, *G. arnesenae,* and *C. contorta* larvae contain also tiny cells with reduced cytoplasm and underdeveloped Golgi complex in the larval cavity (Fig. 1.22c). These cells probably have parent origin (Amano and Hori 2001).

Table 1.1 The number of granular cells at the posterior pole in the larvae of different Calcinea species

Species	Number of granular cells	Reference
Clathrina coriacea	1	Minchin 1900; Tuzet 1948; Johnson 1979a
C. contorta	4	Minchin 1900; Ereskovsky unpublished
C. cerebrum	None	Borojevic 1969
C. reticulum	None	Borojevic 1969
Ascandra falcata	Four	Minchin 1900
S. laxa	None (or 10)	Amano and Hori 2001
Guancha arnesenae	None	Ereskovsky and Willenz 2008
Guancha blanca	2	Minchin 1900; Johnson 1979a

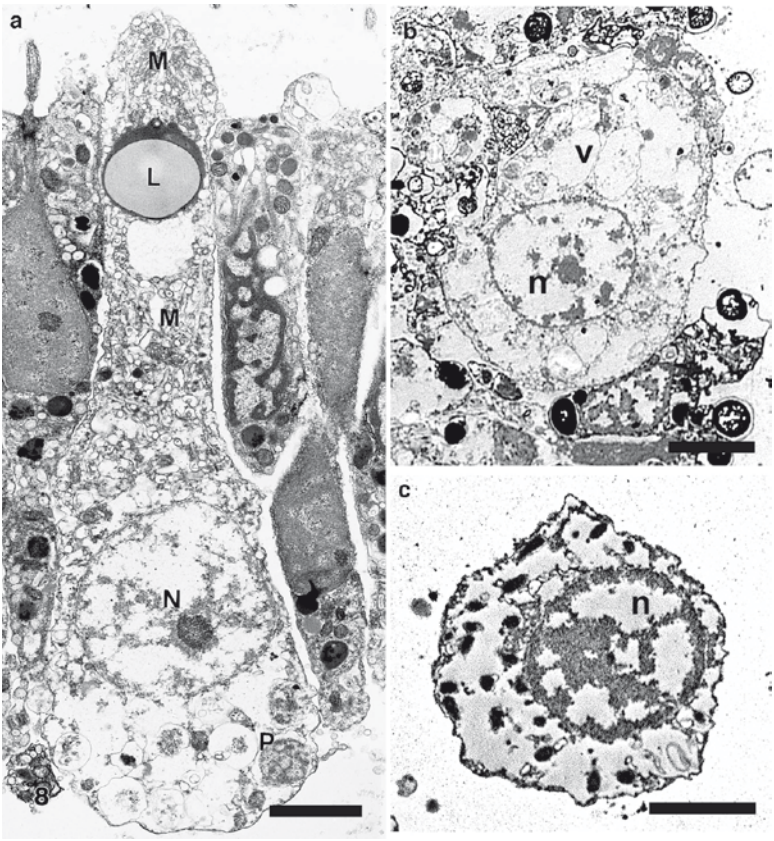

Fig. 1.22 TEM of additional types of calciblastula cells. (**a**) Bottle cell between ciliated cells in the coeloblastula of *Soleneiscus laxa* (From Amano and Hori 2001, Biol Bull, vol. 200, pp. 24, Fig. 8, Reprinted with permission from the Marine Biological Laboratory, Woods Hole, MA). (**b**) Vacuolar cells between larval ciliated cells of *Clathrina contorta.* (**c**) Free cells in a central cavity of *Clathrina contorta. L* lipid droplet, *M* membranous structure, *n* nucleus, *P* phagosome-like granule, *v* vacuole. Scale bars (**a**) 5.2 μm, (**b**, **c**) 2 μm

Finally, in the cavity of *C. contorta* calciblastulae, many big vacuolar cells of parent origin have been observed.

In all Calcinea studied at the light microscopic level, ingression of some of the ciliated cells into the larval cavity during free life of the larva was described. The cells shed the flagellum, become rounded, and migrate into the larval cavity, where they form a small loose agglomeration (Schmidt 1877; Metschnikoff 1879; Minchin 1900). Some researchers considered this process as gastrulation (Schmidt 1877; Metschnikoff 1879; Ivanov 1971; Ivanova-Kazas 1975).

Ingression in Calcinea was studied in most detail by Borojevic (1969). According to his observations, a few ciliated cells appear in the cavity of the *Ascandra* and

Clathrina larvae on the first day of free life. These cells lose the cilium and the polar position of the nucleus, but retain the typical inclusions. However, *mass* ingression occurs only on the fourth day of free life. Contrary to the cell-by-cell ingression of the first day, a large mass of cells immerses rapidly into the larval cavity. This process is accompanied by mass disorganization of the larval body wall. The metamorphosis proper starts only after the ingression is completed, which may take several hours. Borojevic (1969) considered ingression not as gastrulation, but as premature reorganization of the larva associated with metamorphosis. His experiments on larval dissociation confirm this viewpoint: cell migration into the cavity in the course of normal metamorphosis is not accompanied by cell differentiation (Borojevic 1969). The larval cavity is filled with dedifferentiating cells only when the larva ends its free life and settles.

At the same time, the only electron microscopic research of metamorphosis in Calcinea (*S. laxa*) did not reveal any cell migration prior to settlement (Amano and Hori 2001). In this species, the cells filling the calciblastula cavity are granular cells of parental origin (see above).

1.2.4 Metamorphosis

The larvae settle on the substrate anterior pole first. The external cells lose their cilia and the larva transforms into a small sphere, whose internal cells do not differ from the external ones. All the former ciliated cells undergo similar changes: they lose the ciliar apparatus, their nucleus becomes rounded, and migrates to the center. In this way, a pupa is formed (Minchin 1900). All its cells are derivatives of the ciliated larval cells and have equal morphogenetic potential. The type of cell differentiation depends on the position of the cells in the pupa.

The first structure formed in the sponge during metamorphosis is the exopinacoderm, which isolates the cell conglomerate from the environment. The surface cells flatten, lose the ciliar apparatus, and quickly differentiate into exopinacocytes. The basopinacoderm is formed somewhat later than the exopinacoderm: the pupal cells contacting the substrate remain rounded for some more time. Collagen is not yet secreted. The first lacunae, filled with mucoid substance, begin to appear (Borojevic 1969). The cells of the internal mass gradually differentiate into scleroblasts, choanoblasts, and amoeboid cells. The scleroblasts are the second cell type to differentiate (Amano and Hori 2001). Larval cells that are transdifferentiated into choanoblasts also lose the cilium and all elements of their basal apparatus (Amano and Hori 2001). The lacunae are lined with choanoblasts; 2 or 3 days after attachment, they are united into a common cavity lined with the choanoderm. Fibrillar inclusions, characteristic of the ciliated larval cells, are retained in all the pupal cells, but pigment inclusions are retained only in exopinacocytes (Borojevic 1969). Bottle-shaped cells described in *S. laxa* and *G. arnesenae* disappear during metamorphosis and evidently do not participate in olynthus formation (Amano and Hori 2001). In contrast, vacuolar cells are not lost. They are found in the mesohyl of the pupa

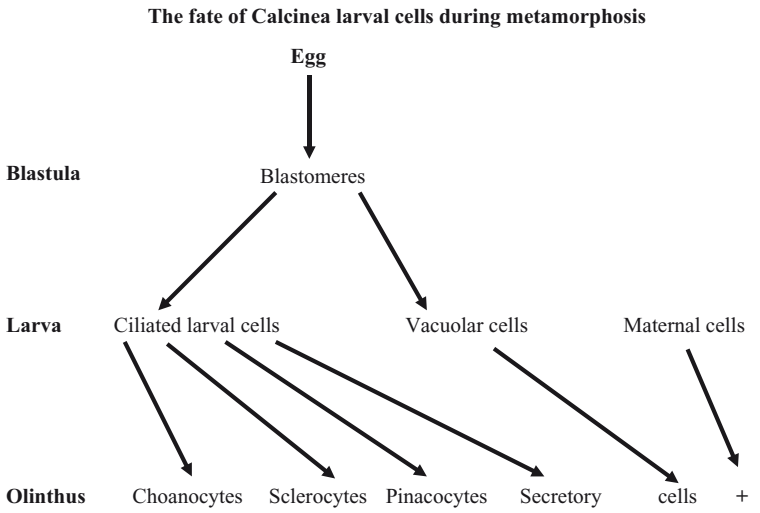

Fig. 1.23 The fate of Calcinea larval cells during metamorphosis

and juvenile sponge, and probably differentiate into eosinophilic cells. Approximately by the fifth day, the osculum and numerous pores open, and the olynthus is formed. The fate of larval cells at metamorphosis is schematically shown in Fig. 1.23.

Some earlier researchers (Metschnikoff 1879; Minchin 1900) thought that metamorphosis in Calcinea involved inversion of larval layers. According to their observations, the larval cells that had migrated inside during metamorphosis crawled outside to become pinacocytes, while ciliated cells dedifferentiated and formed a compact internal cell mass. However, the above-mentioned analysis of more recent data on metamorphosis in Calcinea showed that no such inversion takes place.

Curiously, pupae are similar in structure and cell composition to the cell conglomerates formed after the experimental dissociation of the larval cells; further development of the latter is also similar to pupal metamorphosis (Borojevic 1969). In other words, the larva is not reconstructed after dissociation of its cells; instead, a cell conglomerate is formed, similar to conglomerates of somatic cells of the adult sponge. Thus, cell differentiation is identical in the metamorphosing larva and in the adult sponge (Borojevic 1969).

1.2.5 Asexual Reproduction

Asexual reproduction in the Calcinea was described only in some *Clathrina* species (Johnson 1978). In *G. blanca,* it is a budding process. Buds form on the upper surface of the sponge by constriction of the tubes; constriction continues until only a slender thread of tissue remains between the bud and the parent sponge. Buds get separated from the tubes forming olynthi. In *C. coriacea*, fragmentation was also described (Johnson 1978).

Chapter 2
Development of Sponges from the Class Hexactinellida Schmidt, 1870

Representatives of the class Hexactinellida, commonly called glass sponges, are very variable in shape. They may be tubular, cup-shaped, lumpy, branching, or lobulate (color plate III); only encrusting forms are lacking. Hexactinellid sponges have silicate triaxial spicules or their derivatives. Typically, spicules are represented by hexactins, with three axes crossing at regular angles. A loss of one or more rays results in pentactins, tetractins (stauractins), triactins (tauactins), and diactins; rarely, monactins also occur (Fig. 2.1). The axial filament of the spicule resides in a quadrangular cavity. Spicules are divided into micro- and macroscleres; the latter, often fused together, form rigid skeletal lattices (Fig. 2.2). Dense spongin or non-spicular skeletons do not occur. Living tissues of glass sponges are syncytial and consist of the dermal and the atrial membrane, the internal trabecular reticulum enclosing cellular components of the sponge, and flagellated chambers (Figs. 24, 25). Separate nucleated cells, which are situated in syncytial pockets or capsules, may be connected by specialized contacts, porous plugs. Large eurypilous flagellated chambers are organized according to leuconoid type. All glass sponges are ovoviviparous, with the trichimella larva.

The class Hexactinellida is divided into two subclasses: Amphidiscophora and Hexasterophora. About 500 species of glass sponges (approximately 7% of all known Porifera species) are distributed across 118 genera, 17 families, and 5 orders.

Glass sponges are marine, and are found at depths of 5–6,770 m. Owing to the mostly deep-water habitats of glass sponges, their development is still poorly studied.

The Hexactinellida are generally thought to be hermaphroditic (Schulze 1880a, 1887; Boury-Esnault et al. 1999; Leys et al. 2007). Pioneering information on their sexual reproduction was obtained by Schulze (1880a, 1887) from *Euplectella aspergillum*, *Farrea occa*, and *Periphragella elisae* and by Ijima (1901, 1904) from *E. marshalli* and *Vitrollula fertilis*. In particular, Schulze (1880a, 1887) demonstrated that female gametes and spermatocytes could be present both in the same individual and in different ones. Yet, a coherent picture of embryonic development in glass sponges was lacking until Okada (1928) published a paper describing gametogenesis, embryogenesis, and larval structure in *F. sollasii*. Then, there was another long gap in developmental studies of hexactinellid sponges: they were not

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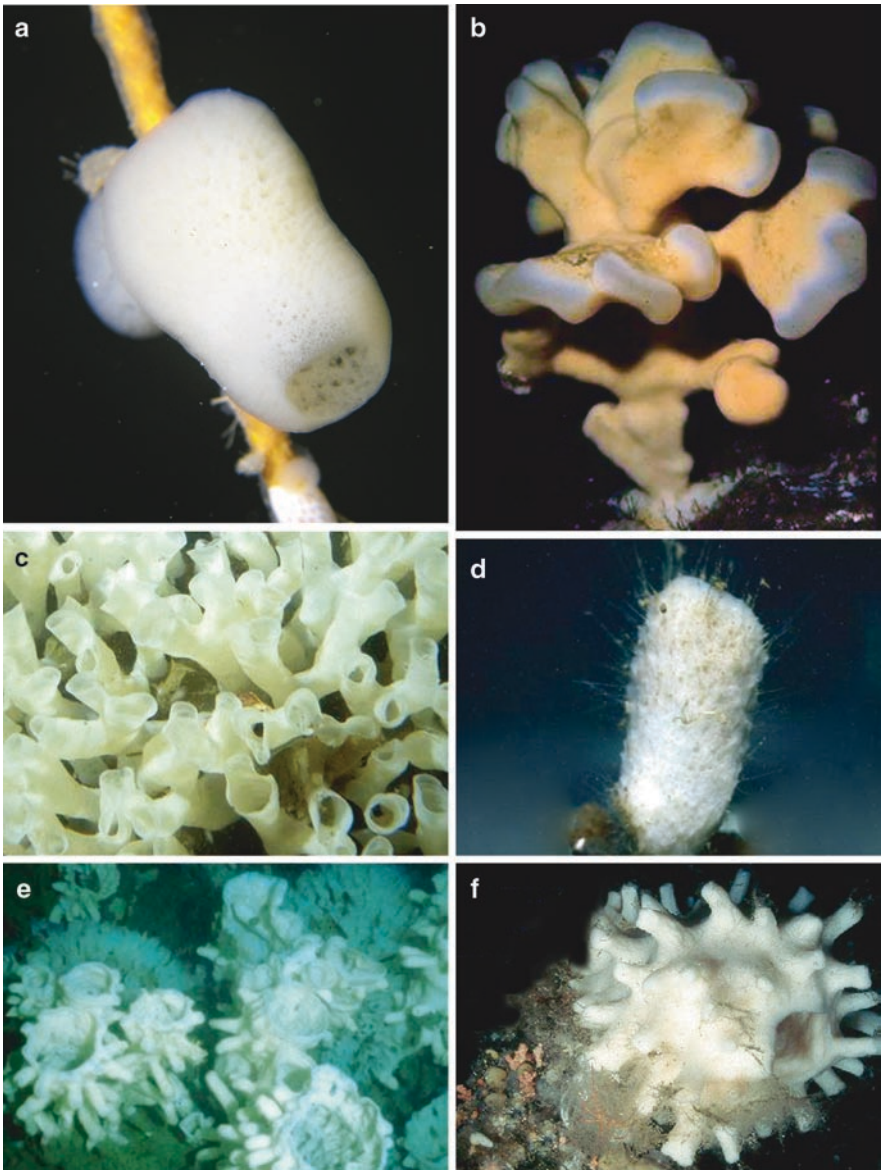

Plate III Class Hexactinellida Schmidt 1870. (**a**) *Oopsacas minuta* Topsent 1927, Mediterranean Sea (Courtesy of R. Graille). (**b**) *Aphrocallistes vastus* Schulze 1886, Okhotsk Sea. (**c**) *Farrea occa* Bowerbank 1862, Okhotsk Sea. (**d**) *Acanthascus dowlingi* Lambe 1892. (**e**) *Chonelasma calyx* Schulze 1886. Okhotsk Sea (**b–e** – Courtesy of V. Feodorov). (**f**) *Rossella nuda* (Topsent 1901) Antarctic (Courtesy of I. Gruzov)

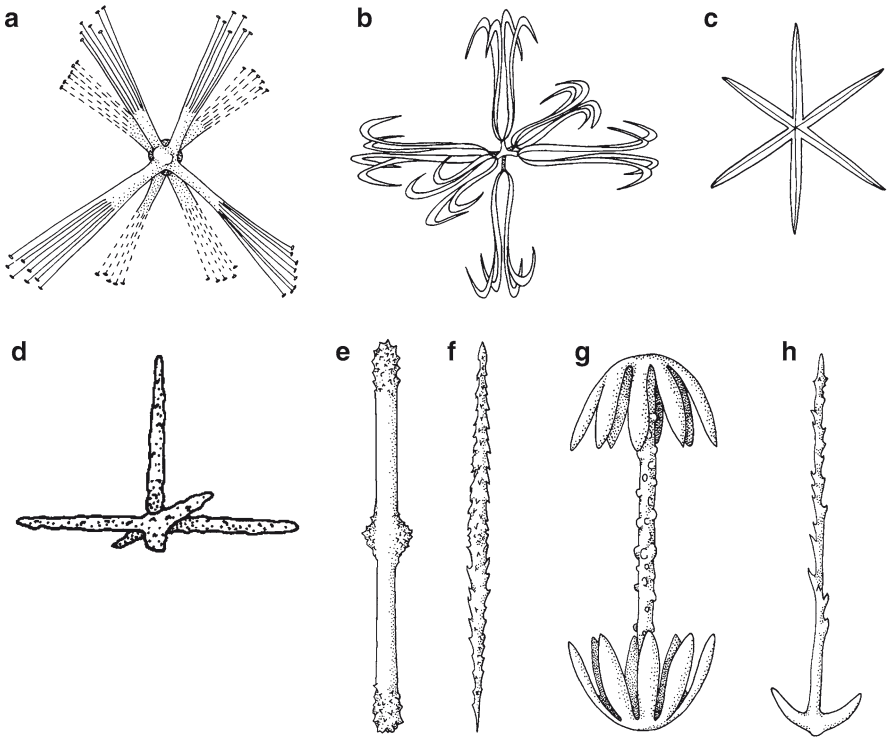

Fig. 2.1 Spicules of Hexactinellida. (**a**) Discoaster, (**b, c**) triactines, (**d**) pentactins, (**e, f**) diactins, (**g**) amphidisc, (**h**) monactin

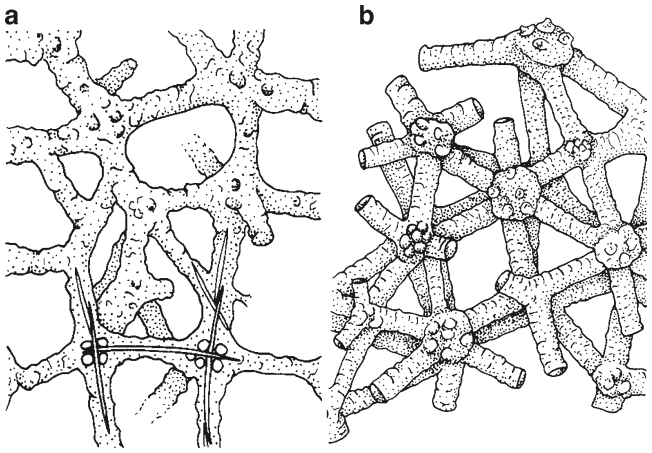

Fig. 2.2 Schematic drawings of fragments of rigid skeletons of Hexactinellida. (**a**) Dictyonal hexactin framework formed by lychnises. (**b**) Spicules fused into a rigid framework type dictyonalia (After Boury-Esnault and Rützler 1997, pp. 21, 23, Figs, 101, 113, reproduced by permission of Smithsonian Institution Scholarly Press)

mentioned in this context for 66 years. It was only in the late twentieth century that Boury-Esnault and Vacelet (1994) described, at the electron-microscopic level, the trichimella larva of a glass sponge (*Oopsacas minuta*). A detailed study of its embryogenesis followed soon (Boury-Esnault et al. 1999; Leys et al. 2006).

2.1 Gametogenesis

In Hexactinellida, both male and female gametes originate from archaeocytes, free nucleolar amoebocytes that are suspended within the trabecular reticulum between flagellated chambers (Ijima 1901; Okada 1928; Boury-Esnault et al. 1999).

Spermatogenesis. Spermatogonia arise within archaeocyte conglomerates, or *congeries* (Ijima 1901; Okada 1928; Boury-Esnault et al. 1999; Leys et al. 2006). Archaeocytes are spherical or subspherical cells with densely granular cytoplasm and numerous mitochondria; the Golgi component and endoplasmic reticulum are not prominent and phagosomes are rare. Archaeocytes in conglomerates are frequently attached to one another and to the trabecular syncytium by plugged junctions (Leys et al. 2007). Prior to differentiation, such conglomerates, as shown in *O. minuta*, are surrounded by a thin (0.5 mm) layer of the trabecular reticulum (Fig. 2.3) (Leys et al. 2007). According to light microscopic observations, spermatogenesis in the Hexactinellida follows the usual pattern. All spermatocytes are connected by plugged cytoplasmic bridges; these bridges also connect cells at the periphery of the cyst to the surrounding trabecular envelope (Leys et al. 2007). Spermatozoa are primitive; their rounded head has relatively much cytoplasm, which contains small mitochondria and dictyosomes. The acrosome has not been described.

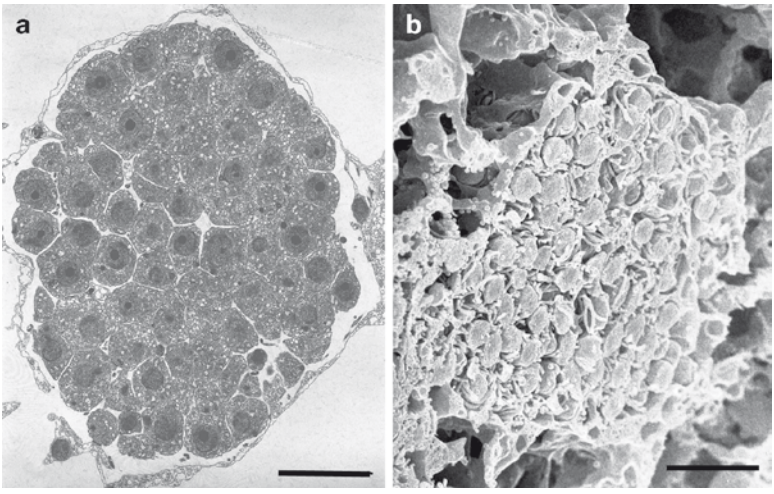

Fig. 2.3 TEM (**a**) and SEM (**b**) of a spermatocyte in *Oopsacas minuta* (Courtesy of J. Vacelet and N. Boury-Esnault). Scale bars (**a**, **b**) 10 μm

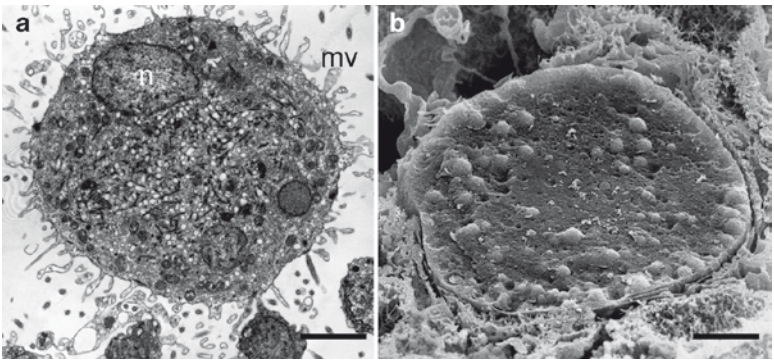

Fig. 2.4 Oogenesis in *Oopsacas minuta.* (**a**) TEM of the early oocyte during the beginning of vitellogenesis. (**b**) SEM of an egg in the sponge tissue (Courtesy of J. Vacelet and N. Boury-Esnault). *mv* microvilli, *n* nucleus. Scale bars (**a**) 5 μm, (**b**) 20 μm

Oogenesis. Oogenesis of glass sponges has not been studied in any detail. It is only known that, similar to spermatogenesis, it starts from the formation of an archaeocyte conglomerate, where the cells are connected to each other by plugged cytoplasmic bridges. Only one of the archaeocytes develops into the oocyte (Fig. 2.4a); the rest are supposed to act as nurse cells providing it with the lipid and yolk (Boury-Esnault et al. 1999). The polylecithal egg contains numerous lipid droplets and osmiophilic granules with heterogeneous content. The egg is not polarized, its nucleus is located centrally (Fig. 2.4b) (Boury-Esnault et al. 1999; Leys et al. 2006).

2.2 Embryonic Development

Embryogenesis has been described only in two hexactinellid species, *F. sollasii* and *O. minuta* (Okada 1928; Boury-Esnault et al. 1999; Leys et al. 2006).

Cleavage. In both species, cleavage is total, equal, and asynchronous up to the stage of about 32 blastomeres (Fig. 2.5a–f). The first two furrows lie in the same plane perpendicular to each other (Fig. 2.5b–d). Orientation of the first cleavage plane with respect to polar bodies varies in different embryos. The plane of the third division is perpendicular to those of the first two. In *O. minuta*, four "animal" blastomeres (situated at the pole with the polar bodies) may lie in spaces between "vegetative" ones (Boury-Esnault et al. 1999) and the authors speak about *spiral* cleavage, which is inaccurate. A displacement of interphase blastomeres is well known in the Cnidaria; this kind of cleavage should be called *pseudospiral* (Tardent 1978). Later cleavage spindles orient parallel to the embryo's surface. The result is an equal coeloblastula with a distinct blastocoel (Fig. 2.5e, f).

Larval Morphogenesis. Unequal tangential divisions of coeloblastula cells result in the formation of a two-layered hollow blastula (Fig. 2.6a). This process resembles cell delamination characteristic of the Cnidaria (Tardent 1978). Peripheral cells

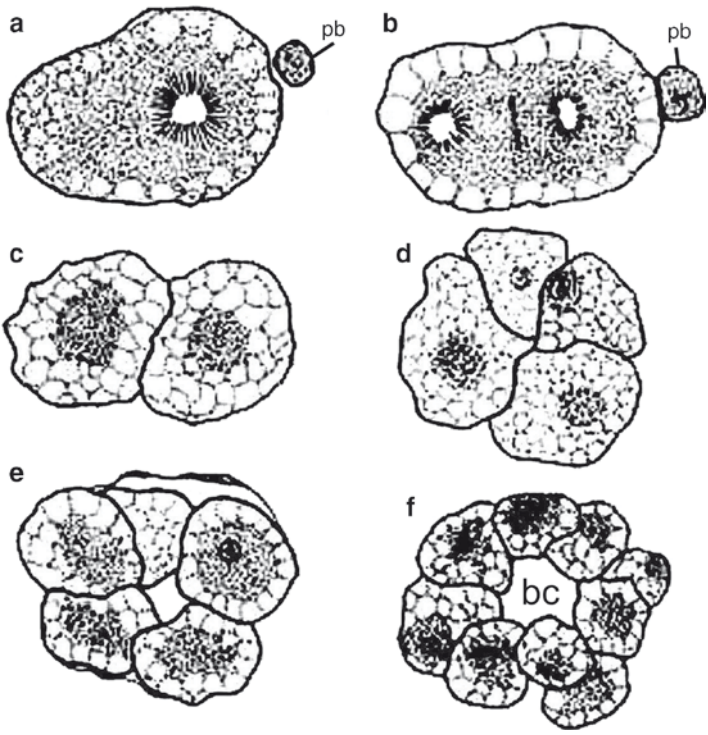

Fig. 2.5 Schematic drawing of cleavage and early stages of embryonic morphogenesis in *Oopsacas minuta*. (**a**) Egg with polar body (*pb*). (**b**) Beginning of the first cleavage division. (**c**) Two blastomeres stage. (**d**) Four blastomeres stage. (**e**) Eight blastomeres stage. (**f**) 16-cells early blastula with the blastocoel (*bc*) (From Boury-Esnault et al. 1999, p. 189, Fig 5, reproduced by permission of Balaban Publishers International Science Services and authors)

of the blastula (micromeres) are smaller than the internal cells (macromeres); during subsequent morphogenesis, they divide actively (Fig. 2.6a). As soon as they are formed, the micromeres are connected to one another by plugged cytoplasmic bridges, which may pass parallel or perpendicular to the surface of the embryo (Fig. 2.6b) (Leys et al. 2007). Internal cells, rich in yolk and lipid inclusions, also divide and fill the blastula cavity. The macromeres extend out filopodia and pseudopodia, which interact and eventually fuse with filopodia from other macromeres (Leys et al. 2007).

While the peripheral cells continue to proliferate, their derivatives differentiate and give rise to a flat syncytium, covering the whole larva, and a belt of prismatic multiciliated cells (Fig. 2.6c). Polarization of the embryo starts early in the course of differentiation. Importantly, all micromeres are interconnected by plugged cytoplasmic bridges.

The macromeres envelop the micromeres with massive filopodia (Leys et al. 2006) and then fuse to form a single multinucleated giant cell, the new trabecular syncytium (Figs. 2.6b and 2.7). This surface epithelium completely envelops the micromeres.

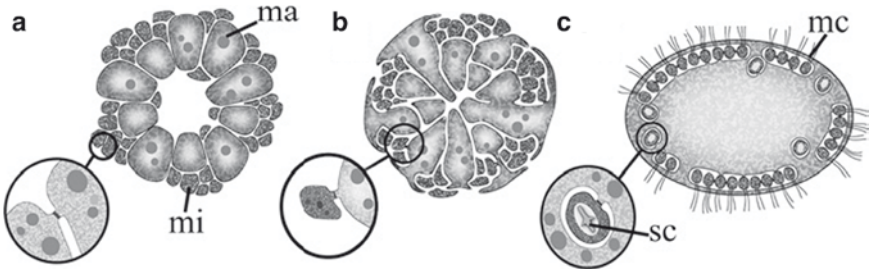

Fig. 2.6 Schematic drawing of different stages in larval morphogenesis of *Oopsacas minuta.* (**a**) Cell delamination by means of unequal cleavage to form micromeres (*mi*) and macromeres (*ma*). (**b**) Fusion of macromeres (*ma*) to form the trabecular syncytium, and envelopment of micromeres by this tissue to form the outer epithelium. (**c**) Cellular differentiation: formation of multiciliated cells (*mc*) and sclerocytes (*sc*, inset) (From Leys et al. 2007, p. 114, Fig. 54, Reproduced by permission of Elsevier Ltd.)

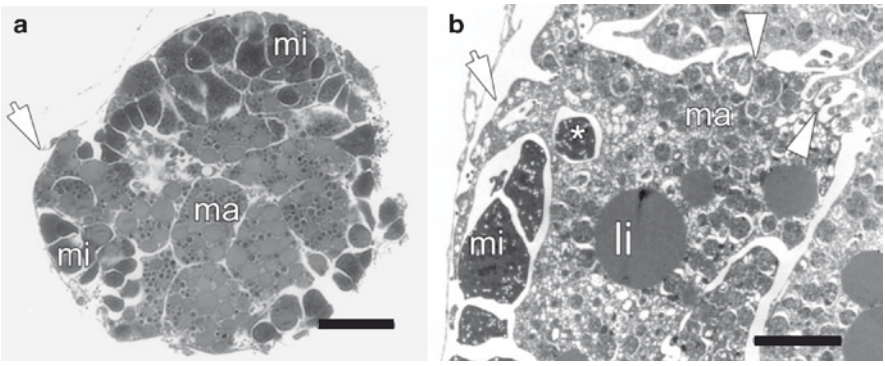

Fig. 2.7 Formation of syncytia in *Oopsacas minuta* by the fusion of macromeres. (**a**) Light microscopy of macromeres (*ma*) extended around micromeres (*mi*, *arrow*) at the surface of the embryo. (**b**) Detail of a surface region of the embryo, showing the macromere (*ma*) extending a lamellipodium (*arrow*) around micromeres (*mi*) on the *left* and forming pseudopodia that intermesh with those of another micromere at the *right* (*arrowheads*). *li* lipid droplet (From Leys et al. 2006, p. 109, Fig. 3b, c, reproduced by permission of Oxford University Press). Scale bars (**b**) 20 μm, (**c**) 2 μm

The internal cells differentiate in two ways. Large cells rich in lipid inclusions migrate toward the anterior pole, whereas cells rich in yolk inclusions and phagosomes migrate toward the posterior pole. Some of the internal cells (originating from micromeres) at the periphery of the central mass differentiate into larval sclerocytes; the latter secrete special four-rayed spicules, *strauractins*, not characteristic of adult sponges. The sclerocytes are originally uninucleate, but then become a multinucleate sclerosyncytium (Leys 2003b). After segregation of the sclerocyte line, most of the yolk-rich cells of the posterior pole merge to form a yolk syncytium, whereas amoeboid cells at the periphery of the central mass differentiate into choanoblasts (Leys et al. 2006). Choanocytes, connected to the trabecular tissue by

cytoplasmic bridges, later fuse into a choanosyncytium, consisting of several collar bodies about 30 μm in diameter in *O. minuta* (their diameter in adult sponges is 100–150 μm) (Boury-Esnault et al. 1999; Leys et al. 2006). Thus, throughout early development, this unusual embryo manages to rearrange cellular regions that are tethered to the multinucleate syncytium via cytoplasmic bridges. Membrane continuity is maintained throughout the entire embryo; there are no typical cells, or cell–cell junctions.

2.3 Larva

The name *trichimella* (from the Greek "bearing long threads") was coined for the *O. minuta* larva (Boury-Esnault and Vacelet 1994) and then extrapolated for the larvae of all hexactinellids. Several unique characters distinguish trichimellae from all other sponge larvae. They are distinctly polarized along the anterior–posterior axis and divided into three zones: the rounded anterior pole, the ciliated middle zone, and the conical posterior pole (Fig. 2.8). The major larval tissue is the syncytial trabecular reticulum, which is continuous throughout the whole larva and forms the bulk of the inner mass of both anterior and posterior poles, as well as the surface epithelium.

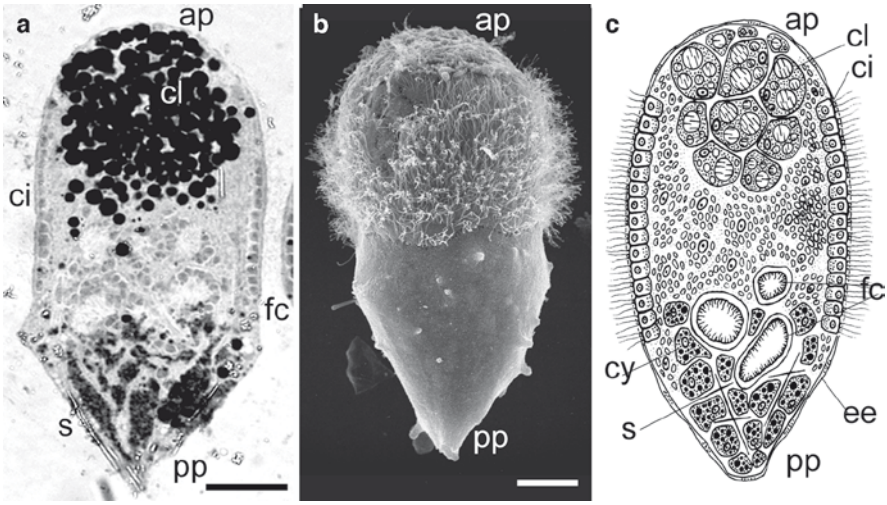

Fig. 2.8 Trichimella larva of Hexactinellida. (**a**) Semi-thin section of *Oopsacas minuta* larva. (**b**) SEM micrograph of *O. minuta* larva. (**c**) Scheme drawing. *ap* anterior pole, *ci* ciliated cells, *cl* cells of anterior pole with lipid droplets, *cy* cells of anterior pole with yolk granules; *fc* flagellated chambers, *ee* external syncytium, *pp* posterior pole; *s* stauractin spicules (**b** – Courtesy of J. Vacelet and N. Boury-Esnault). Scale bars (**a**, **b**) 25 μm

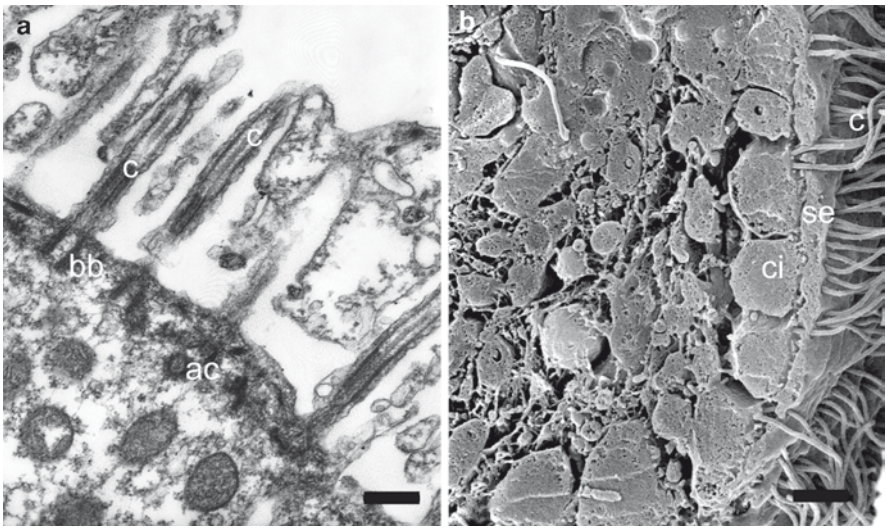

Fig. 2.9 Multiciliated cells of *Oopsacas minuta* trichimellae in the middle part of the larva. (**a**) TEM and (**b**) SEM micrographs. *ac* accessory centriole, *bb* basal body, *c* cilia, *ci* ciliated cells, *se* syncytial epithelium (**a, b** – Courtesy of J. Vacelet and N. Boury-Esnault). Scale bars (**a**) 0.5 μm, (**b**) 3 μm

Inside the larva, there are chambers formed by anucleate collar bodies. Contrary to the situation in the adult sponge, these chambers are not arranged into a reticulum. In the posterior pole, there is a unique stauractin skeleton (Fig. 2.8a, c). Larval spicules of the modern hexactinellids resemble the spicules of their ancestors, representatives of the genus *Protospongia* and *Diagoniella* from the early Palaeozoic family Protospongiidae (Mehl 1996).

Another peculiar feature of trichimellae is multiciliated cells, which form a broad belt in the middle of the larva (Fig. 2.9). Each such cell bears about 50 cilia, which have basal bodies and an accessory centriole but lack the rootlet. Each cilium passes in a special opening in the syncytial epithelium. The cell bodies are connected to one another and to the trabecular tissue above and below by plugged cytoplasmic bridges (Boury-Esnault et al. 1999; Leys et al. 2006). Similar junctions are present between the posterior pole cells, containing yolk granules.

2.4 Metamorphosis

The few available observations on metamorphosis were made on *O. minuta* (Leys et al. 2007). Most larvae settle and metamorphose into the juvenile sponge within 12–24 h after release from the parent. Larvae attach to the surface by the rounded anterior pole and flatten. After 24 h, the lipid inclusions remain at the base of the postlarva, whereas the center and former posterior pole undergo a massive change.

During morphogenesis, the reticular tissue undergoes a radical reorganization. Most of the yolk-filled inclusions disappear. The multiciliated cells resorb their cilia; their fate in the postlarva is unknown. Flagellated chambers enlarge and become enveloped by the reticular tissue, as in the adult.

Embryonic developmental studies of *F. sollasii* and *O. minuta* (Okada 1928; Boury-Esnault et al. 1999) show that syncytial organization arises during embryogenesis – more precisely, during larval histogenesis, when the sclerosyncytium, the choanocyncytium, and the cover syncytium develop. These data are an additional argument in favor of the idea that syncytial organization in the Hexactinellida is secondary (Leys 2003a). At the same time, they contradict the hypothesis that syncytial organization in the Hexactinellida arose early in the course of multicellularity formation, as a result of uncompleted cytotomy, uncompleted cleavage, or fusion of blastomeres (Rieger and Weyrer 1998).

2.5 Asexual Reproduction

Asexual reproduction in the form of external budding has often been described in Hexactinellida representatives (see Tuzet 1973b). However, we failed to find any papers especially concerned with this morphogenesis. According to some of the pictures published (Schulze 1887a; Tuzet 1973b), budding in Hexactinellida seems to be epimorphic. In *Rhabdocalyptus dawsoni*, the bud's cavity is separated from the atrial cavity of the parent sponge, but the cytoplasm of the trabecular syncytium connecting the parent with the bud is continuous (Mackie et al. 1983).

Chapter 3
Development of Sponges from the Class Demospongiae Sollas, 1885

The class Demospongiae comprises sponges, whose skeleton consists of either spongin fibers only or spongin fibers in combination with siliceous spicules (usually, macro- and microscleres). Macroscleres are mostly monoaxial and tetraxial, while microscleres are very diverse – monoaxial or polyaxial, often in complicated shape. The axial filament resides in a triangular or hexangular cavity. Fibrillar collagen has been noted in all the Demospongiae. In some groups, the reduced spicular skeleton is compensated for by a complex organic one; in some other groups, there are no skeletal elements whatsoever. In several small Demospongiae groups, a hyper-calcified basal skeleton develops in addition to other skeletal elements (Sollas 1885).

The body shape of the Demospongiae may be encrusting, massive, lobulate, tubular, filamentous and cup-shaped. Several boring sponges live in the midst of calcareous substrate. The aquiferous system is leuconoid. Sponges from the deep water family Cladorhizidae (the order Poecilosclerida) lost the aquiferous system and became carnivorous. The choanocyte chambers may be eurypilous, diploid and aphodal. The larvae are mostly parenchymellae or, in some groups, single-layer larvae. Reproductive strategies within the class are ovoviviparity, oviparity and, rarely, viviparity.

There are about 6,000 Demospongiae species, that is, 85% of all the extant Porifera. Most of the Demospongiae are marine species, but several families live in the freshwater at all the continents except the Antarctic.

Earlier researchers recognized "Keratosa", an artificial group of the superorder rank, comprising Demospongiae without silicate spicules. It has recently been divided into two monophyletic clasters: *Keratosa* (orders Dictyoceratida + Dendroceratida), and *Myxospongiae* (orders Chondrosida + Verongida + Halisarcida) (Borchiellini et al. 2004a; Boury-Esnault 2006).

The class Demospongiae includes several "living fossils": the former "Sphinctozoa", now included in the order Verticillitida, and "Sclerospongiae". The latter is an artificial group combining the demosponges from different orders, with ovoviviparity and the parenchymella larva (Vacelet 1979b).

At present, the class Demospongiae comprises 14 orders: Chondrosida, Halisarcida, Verongida, Dictyoceratida, Dendroceratida, and Agelasida, "Lithistida"

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(polyphyletic), Astrophorida, Haplosclerida, Poecilosclerida, Hadromerida, Halichondrida, Spirophorida, and Verticillitida. Several recent investigations support the polyphyly of the Halichondrida and the paraphyly of the Haplosclerida, as well as the monophyly of *Tetractinellida* (Astrophorida + Spirophorida), *Keratosa* (Dictyoceratida + Dendroceratida) and *Myxospongiae* (Chondrosida + Verongida + Halisarcida).

3.1 Reproductive Strategies and Taxonomy of the Demospongiae (Oviparous Demospongiae)

In 1956, Lévi published the results of his extensive research on the demosponges' development. Having performed a comparative analysis, he came to a conclusion that the class Demospongiae was clearly divided into two groups (subclasses) by their reproduction mode. The first group (subclass Ceractinomorpha) was characterized by the internal fertilization and the development of the embryo in the parent mesohyl (ovoviviparity and viviparity). The second group (subclass Tetractinomorpha) was characterized by the external fertilization and the development of the larva in the environment (oviparity). Differences between the subclasses also concerned morphology: the Ceractinomorpha had monoaxial macroscleres and microscleres represented by chelae and sigmas; besides, they included corneous non-spicular sponges. The Tetractinomorpha were characterized by tetra- and monoaxial macroscleres united into the radial or the axial skeleton (Lévi 1973, Bergquist 1978).

However, in the last quarter of the twentieth century, new data started to accumulate on reproduction (Scalera Liaci et al. 1973; Reiswig 1976; Hoppe and Reichert 1987; Fromont 1988, 1994, Fromont and Bergquist 1994; Lepore et al. 1995), biochemistry (Bergquist 1979; Bergquist and Wells 1983), molecular biology (Chombard 1997; Chombard and Boury-Esnault 1999) and morphology (van Soest 1990, 1991) of tetractinomorph Demospongiae. The existence of the two traditional subclasses, the Tetractinomorpha and the Ceractinomorpha, was challenged. They are now considered to be polyphyletic, and it is proposed that this division has to be abandoned (Borchiellini et al. 2004a; Boury-Esnault 2006).

At present, many spongiologists divide the class Demospongiae into orders only. Oviparous sponges belong to the following orders (the genera whose gametogenesis or embryonic development was investigated are given in brackets): Spirophorida (*Tetilla*, *Cinachyra*), Astrophorida (*Alectona*, *Geodia*, *Erylus*, *Thenea*, *Stelletta*), Hadromerida (*Polymastia*, *Suberites*, *Terpios*, *Tentorium*, *Trichastemma*, *Cliona*, *Tethya*, *Stylocordyla*, *Raspaciona*), Chondrosida (*Chondrosia*, *Chondrilla*), Verongida (*Aplysina*), Agelasida (*Agelas*), Poecilosclerida, (fam. Raspailiidae: *Raspailia*, *Hemectyon* (= *Endectyon*); fam. Desmacellidae: *Neofibularia*), "Lithistida" (*Theonella*), Haplosclerida (*Xestospongia*, *Petrosia*), Halichondrida (*Topsentia*, *Vosmaeria*, *Axinella*). At the same time, some of these typically oviparous groups may include ovoviviparous or viviparous species. For example, the order Astrophorida includes the ovoviviparous genera *Alectona* and *Thoosa,* and the

order Hadromerida includes the viviparous genus *Stylocordyla* (Vacelet 1999; Borchiellini et al. 2004b; Sarà et al. 2002).

Though oviparous sponges were attributed for a long time to a separate subclass, there are only four brief reviews of their development (Bergquist 1978; Korotkova 1981b; Ereskovsky 2004; Leys and Ereskovsky 2006). Bergquist considers their development as "alternative", while Korotkova establishes a separate "developmental type of oviparous sponges". In the latter two reviews, (ovo)viviparity and oviparity are considered as the reproductive strategies.

The majority of oviparous species are gonochoric (Sarà 1993). Thus, gonochorism is characteristic of the order Astrophorida (Sarà 1993), the order Spirophorida (in *Tetilla* sp., however, successive hermaphroditism was described by Scalera Liaci et al. 1976), the family Axinellidae and most species from the order Hadromerida. At the same time, *Polymastia mammillaris* and *P. arctica* from the latter order are hermaphroditic (Sarà 1961; our data), and so are hadromerids from the families Suberitiidae and Clionaidae (Sarà 1961; Diaz et al. 1973; Scalera-Liaci and Sciscioli 1979; Wapstra and van Soest 1987). Characteristically, male and female gametes are released synchronously in local sponge populations (Reiswig 1976).

Oviparous sponges usually have small isolecithal and oligolecithal eggs, with the central nucleus and small yolk granules at the periphery. The collagen layer, surrounding the egg at the final stages of oogenesis, is synthesized by the oocytes (Sciscioli et al. 1989, 1991, 2002). In other words, the oocytes of oviparous sponges have a true primary envelope, which is a characteristic feature of all the Eumetazoa (Aisenstadt 1984; Gilbert 2006). The released eggs retain peripheral vacuoles, which are supposed to play in important role in the attachment of the egg or the zygote to the substrate, in mechanic protection and the formation of the fertilization membrane. However, as shown on *Steletta grubii* and *Tethya citrina*, the lophocytes of the mesohyl are involved in the synthesis of the collagen capsule (Gaino et al. 1987c; Sciscioli et al. 1991). This mechanism may be considered as a prototype of the vitelline membrane in the Metazoa.

In the genus *Tetilla,* external collagen layer is supplemented with radial bundles of collagen fibers (Watanabe 1978b; Watanabe and Masuda 1990). Besides, eggs are often surrounded with layers of parent cells: granulated cells in *Cliona celata* and *Hemectyon ferox* (Warburton 1961; Reiswig 1976), bacteriocytes and granulated cells in *Chondrosia reniformis* (Lévi and Lévi 1976), spherulous and microgranular cells in *Aplysina cavernicola* (Gallissian and Vacelet 1976), and some obscure "nurse cells" in *Neofibularia nolitangere* (Hoppe and Reichert 1987).

The above variations in the envelope structure and composition of parent cells are more likely to be a species character. For instance, out of the six ovoviviparous *Oscarella* species studied by us – *O. lobularis*, *O. imperialis*, *Oscarella* sp., *O. tuberculata*, *O. microlobata* and *O. malakhovi* (Homoscleromorpha) – parent cells participate in the embryo formation only in the former three (Ereskovsky and Boury-Esnault 2002; Ereskovsky 2006).

On the strength of the above analysis of the development of oviparous Demospongiae, a conclusion can be made that all the oogenesis and early developmental characters that these sponges have in common concern only adaptations to

the development in the environment. They are an example of the parallel development of embryonic adaptations and cannot be considered as homologous.

How can we interpret the two reproduction types within the Demospongiae? There are two points of view on the matter. According to Lévi (1956), (ovo)viviparity and oviparity are phylogenetically determined reproduction types and thus have a taxonomic value. Bergquist (1980, 1985) also thought that reproduction type has taxonomic value, but only at the level of the order or below. She delimited thus the orders Verongida and Petrosida (Bergquist 1978, 1980) (the latter was reduced by Manconi and Pronzato (2002) to a suborder (Petrosina) of the order Haplosclerida). For the same reason, the order Agelasida was created (Hartman et al. 1980).

The second viewpoint was published by Reiswig (1973, 1976) and Hoppe (1988), and supported by van Soest (1991). According to them, both these reproduction types are merely different reproductive strategies, which cannot be attached phylogenetic value. Reiswig (1973) suggested that large long-living sponges dwelling in narrow econiches (i.e. generalistic species) are characterized by oviparity; while small short-living sponges with rapidly renewing populations (i.e. opportunistic species) are characterized by (ovo)viviparity. Other authors (Fromont 1994; Fromont and Bergquist 1994) also arrived at the same conclusion.

In our opinion, alternative reproduction types of the Demospongiae are elements of their reproductive strategies (Ereskovsky and Korotkova 1999; Ereskovsky 2005). It is well known that in many groups of marine invertebrates both viviparity and development in the environment occur. Moreover, different reproductions modes occur in species from the same genus, family or order. This is the case, e.g., for the Echinodermata and the Bivalvia (Strathmann 1978; Kasyanov et al. 1998; Kasyanov 2001). For instance, representatives of the genera *Patiriella* and *Cryptasterina* (Asteroidea) have four developmental types, ranging from the typical one with the planktotrophic brachiolaria larva to viviparity (Byrne 1995; Byrne et al. 2003). In all these cases, the reproduction type is not ascribed to taxonomic significance.

An important characteristic of reproduction strategy of a species is egg size. A connection between egg size and developmental type has been established for many marine invertebrates (Strathmann and Vedder 1977; Kasyanov 2001). With increasing egg diameter, a transition is observed from external fertilization and the typical planktotrophic larva to the development inside the parent organism, lecithotrophy and, finally, direct development (Strathmann 1978; Raff 1987). Though sponges lack planktotrophic larvae, the above regularity is characteristic of them, too – oligolecithal eggs of oviparous sponges are much smaller than polylecithal eggs of ovoviviparous sponges (Table 3.1).

The peculiar oogenesis structure of eggs and egg envelopes in the Demospongiae developing in the environment is the result of parallel evolution associated with fertilization features and external conditions of development, with the same reproduction types. In *Hemectyon ferox* and *Neofibularia nolitangere* (Poecilosclerida), oviparity is associated with more complex egg envelopes, characteristic of typical oviparous species, as well as with increasing of the egg diameter up to 139 and 216 μm, correspondingly (Reiswig 1976; Hoppe and Reichert 1987). At the same time, in

Table 3.1 Average egg size of sponges from different Demospongiae ovoviviparous and oviparous orders

Reproduction type	Order	Egg diameter, μm
Oviparous	Hadromerida	35–67–190
	Spirophorida	40–102–130
	Astrophorida	30–58–90
	Chondrosida	40–50–60
	Verongida	40–45
	Petrosida	80–105–160
	Halichondrida	50–100–150
	Average	75
Ovoviviparous	Dictyoceratida	300
	Dendroceratida	200
	Halisarcida	113
	Halichondrida	168
	Haplosclerida	245
	Poecilosclerida	210
	Average	206

all the other representatives of the order Poecilosclerida, the development proceeds in the parent organism, and egg envelopes are poorly developed. More complex egg envelope in species developing outside the parent organism is also noted in other invertebrates.

The structure of the egg envelope is known to have an adaptive significance and to depend greatly upon ecological conditions. The egg envelopes are the most complex in animals whose eggs develop on land (Pulmonata, Insecta, Reptilia, Aves) (Ivanova-Kazas 1995). On the contrary, in truly viviparous animals, the egg envelopes are poorly developed and are shed early in the development since they impede metabolic exchange between the embryo and the parent organism, parasitic Hymenoptera (Ivanova-Kazas 1961) and Mammalia being the example (Carlson 1981). So we support the viewpoint published by Gallissian and Vacelet (1976) and sided by van Soest (1991) that oviparity originated several times independently in different Demospongiae groups and is, therefore, of no taxonomic value.

3.2 The Order Hadromerida Topsent, 1894

Sponges from the order Hadromerida often have a massive body, but other shapes are also common: encrusting (Timeidae), boring (Clionaidae), spherical (Tethyidae), branching, etc. (colour plate IV). The skeleton is radial or subradial (Fig. 3.1a). Macroscleres in the ectosomal skeleton are smaller than those in the endosomal one and are represented by uniform monoaxial spicules: mostly tylostyles, subtylostyles and oxae or their derivatives (Fig. 3.1b, c). Microscleres, if present, are various forms of euasters, spirasters, rhabds, microstrongyles, microxea and/or raphides in trichodragmata (Fig. 3.1d–f).

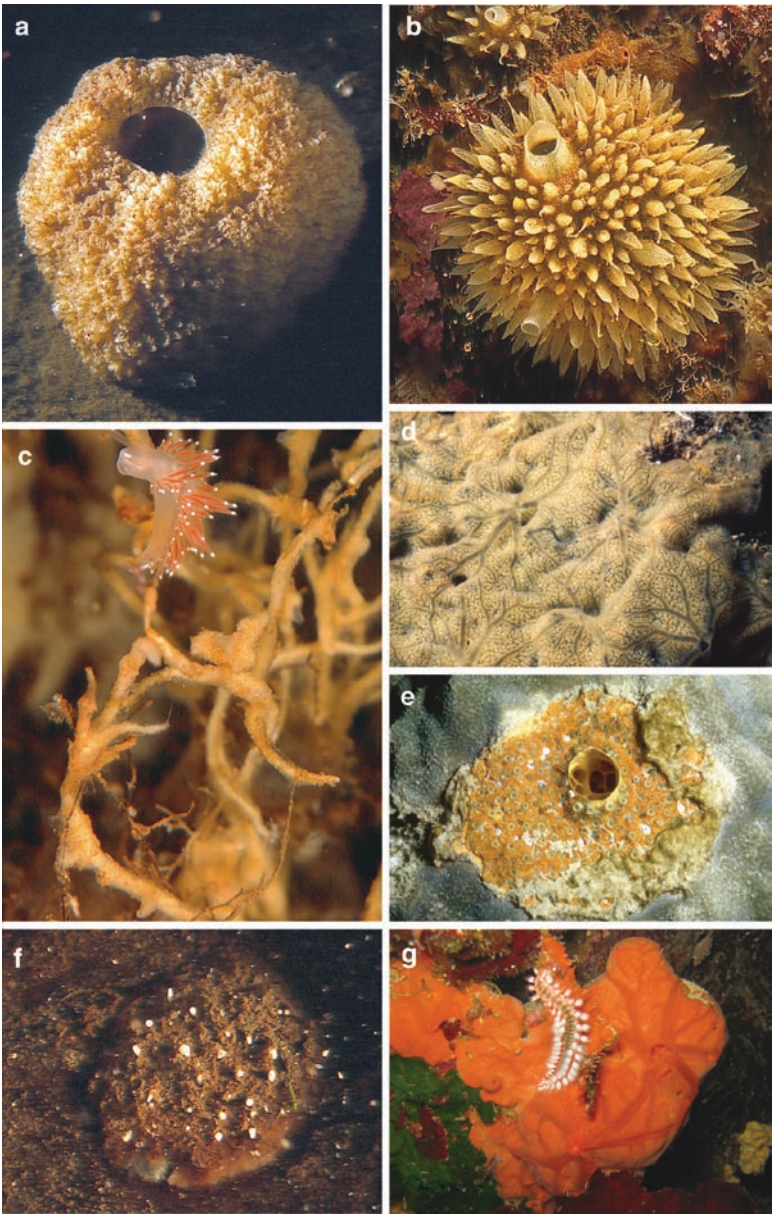

Plate IV Order Hadromerida Topsent 1894 (**a**) *Suberites domuncula* (Olivi 1792). (**b**) *Polymastia arctica* (Merejkowsky 1878). (**c**) *Suberites carnosus* (Johnston 1842). (**a**–**c**) White Sea (Courtesy of M. Fedjuk). (**d**) *Diplastrella bistellata* (Schmidt 1862) Mediterranean Sea (Courtesy of J. Vacelet). (**e**) *Cliona delitrix* Pang 1973, Caribbean Sea (Courtesy of J. Vacelet). (**f**) *Sphaerotylus borealis* (Swarczewsky 1906) White Sea (Courtesy of M. Fedjuk). (**g**) *Spirastrella cunctatrix* Schmidt 1868 Mediterranean Sea (Courtesy of T. Perez)

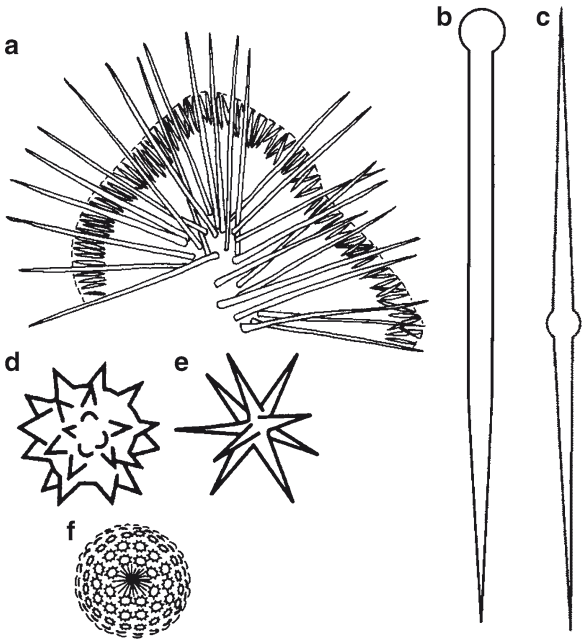

Fig. 3.1 Skeleton structure (**a**) and spicules (**b**–**f**) in the order Hadromerida. (**b**, **c**) macroscleres: tylostyle (**b**) and centrotylote oxae (**c**). (**d**–**f**) microscleres: spheraster, (**d**), oxyaster (**e**), sterraster (**f**)

Spongin fibres are weakly developed. Choanocytes have a cytoplasmic muff around the basal part of the flagellum (Fig. 3.2).

Most of the Hadromerida studied are oviparous, but in *Stylocordyla borealis,* direct development was described, with embryos developing in the parent sponge (Sarà et al. 2002). Representatives of the families Polymastiidae and Tethyidae have budding in the life cycle. Hadromerids are generally gonochoric (Sarà 1993), but *Polymastia mammillaris* and *P. arctica* (Sarà 1961, unpublished) and representatives of the families Suberitiidae and Clionaidae are hermaphroditic (Sarà 1961; Diaz et al. 1973; Scalera-Liaci and Sciscioli 1979; Wapstra and Van Soest 1987).

All the Hadromerida are marine sponges, distributed throughout the world and occurring at all depths. The order comprises 13 families.

3.2.1 Gametogenesis

3.2.1.1 The Origin of Gametes

Diaz and Connes (1980) showed that in *Suberites massa* spermatocytes developed by transdifferentiation of choanocytes. It is the only investigation devoted to the origin of male gametes in hadromerids.

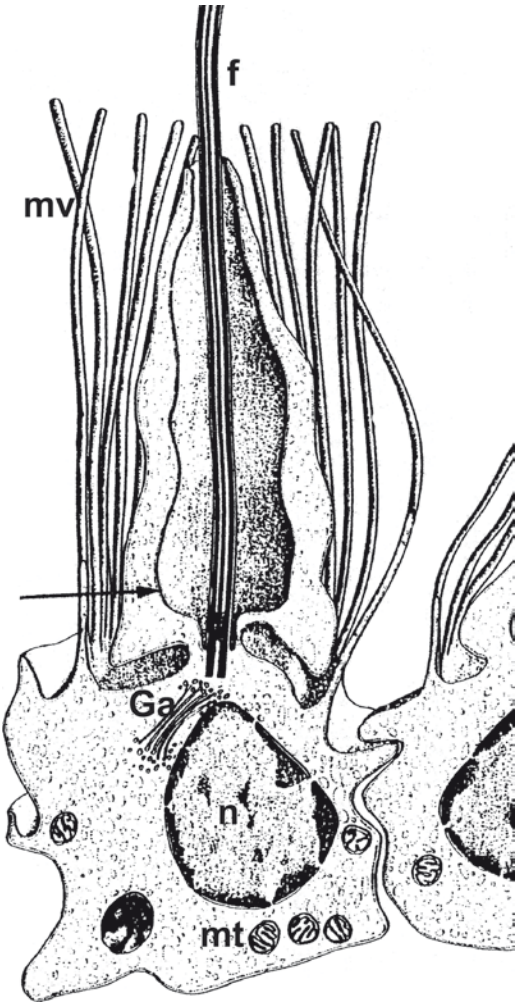

Fig. 3.2 Schematic drawing of choanocyte of *Suberites massa* with a cytoplasmic cuff (*arrow*) around the basal part of the flagellum (*f*). *Ga* Golgi apparatus, *mt* mitochondria, *mv* microvilli, *n* nucleus (After Connes et al. 1971, Compt. Rend. Acad. Sci. Paris. vol. 273, pp. 1592, Fig. 3, reproduced by permission of Elsevier Ltd)

The origin of the female gametes is more complicated. Oocytes were traditionally considered to originate from the archaeocytes of the mesohyl (see Fell 1983; Simpson 1984), but this assumption mostly relied on the similarity between the early oocytes and the archaeocytes, both being large amoeboid cells with nucleoli. These data were mostly obtained from light microscopic studies that did not focus on the female gametes' origin.

An ultrastructural investigation of the oogenesis in *S. massa* showed that female gametes originated from choanocytes (Diaz et al. 1973). The choanocyte chambers contained cells that were intermediate between choanocytes and oocytes in shape and size. They had a collar and a flagellum, but these structures were reduced and visible only at the electron-microscopic level.

Large archaeocytes taken by various authors for oogonia might have been, in fact, transdifferentiated choanocytes (Diaz et al. 1973). Choanocytes have been

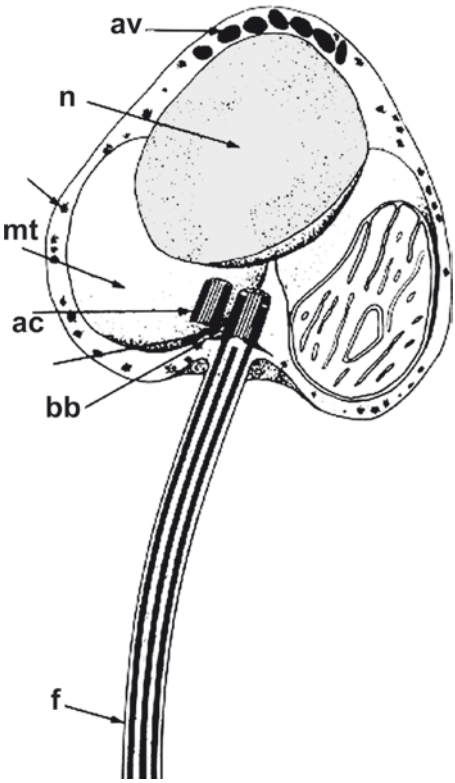

Fig. 3.3 Schematic drawing of spermatozoid of *Suberites massa*, a longitudinal section. *ac* accessory centriole, *av* acrosomal-like vesicles, *bb* basal body, *f* flagella, *mt* mitochondrion, *n* nucleus (Courtesy J. Diaz)

shown to differentiate easily into archaeocytes in other morphogeneses (Borojevic 1966; Gaino et al. 1995; Korotkova 1997).

Spermatogenesis was investigated at the TEM level only in *Suberites massa* (Suberitidae) (Diaz and Connes 1980) and in *Raspaciona aculeata* (Raspailiidae) was described spermatocytes II (Riesgo et al. 2008). Male gametes develop synchronously within a spermatocyste. The differentiated spermatozoon of *S. massa* is small, of primitive type, with a long flagellum and bilateral symmetry (Fig. 3.3). Its coneshaped head with blunted edge is closely adjacent to the intermediate piece which contains three large mitochondrial masses. Vesicles of Golgi origin are located in the acrosome's place and may play the same role. The two centrioles are arranged parallel, one to the other at the base of the nucleus. The chromatin is finely granular and denser at the periphery than at the center. Numerous granules of glycogen line the inside of the cellular membrane (Diaz and Connes 1980).

3.2.1.2 Oogenesis

We have a lot of information on oogenesis in the Hadromerida. It has been studied in *S. massa* (Diaz et al. 1973; Diaz 1979), *Tethya citrina* and *T. aurantium*

(Gaino et al. 1987c; Sciscioli et al. 2002), *Cliona trutti* (Pomponi and Meritt 1990), *Cliona viridis* (Rossel 1993), *Tethya tenuisclera* and *T. seyshellensis* (Gaino and Sarà 1994), *Tentorium semisuberites*, *Trichastemma sol* (Witte 1996), *Stylocordyla borealis* (Sarà et al. 2002) and *Raspaciona aculeata* (Riesgo and Maldonado 2009).

At the pre-meiotic phase, oocytes possess amoeboid motility and form long pseudopodia (Fig. 3.4a, c). Small yolk granules, lipids and inclusions with heterogeneous content are being accumulated at the periphery. There are clusters of mitochondria in the cytoplasm. The nucleus usually contains one large nucleolus. Mature oocytes also are irregular in shape (Fig. 3.4b). This is a characteristic feature of the Hadromerida, setting them apart from the ovoviviparous Demospongiae.

Precursors of reserve nutrients may get into the oocyte by various means: by phagocytosis of whole mesohyl cells or their fragments (*S. massa*, *S. borealis*), by phagocytosis of symbiotic bacteria (*T. citrina*), by pinocytosis and transfer of material across cytoplasmic bridges of mesohyl cells (*S. massa*), by condensation and transformation

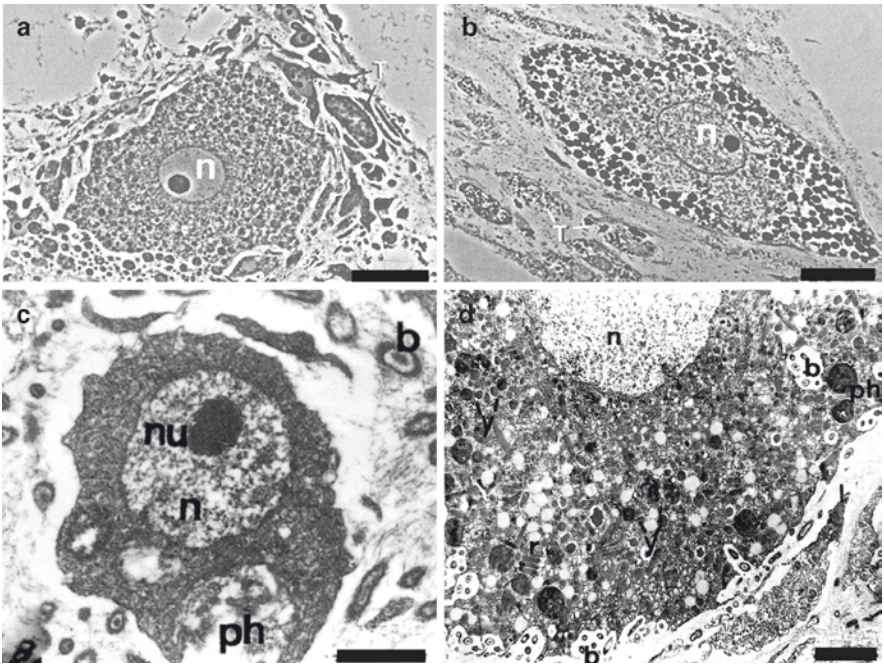

Fig. 3.4 Light micrographs of hadromerids oocytes. (**a**) Young oocyte of *Tentorium semisuberites* at the beginning of yolk accumulation. (**b**) *Trichostemma sol* stage III; oocyte, surrounded by a few nurse cells (*T*). Yolk accumulation is almost completed. (**c**, **d**) *Tethya aurantium.* (**c**) Young oocyte during phagocytosis (*ph*). (**d**) TEM of the part of mature oocyte with the ooplasme comprises different inclusions. *n* nucleus, *nu* nucleolus (**a**, **b** – From Witte 1996, Mar. Biol. vol. 124, pp. 574, Fig. 3, d, e, reproduced by permission of Springer; **c**, **d** – From Sciscioli et al. 2002, Cah. Biol. Mar. vol. 43, pp. 3, Figs. 2, 4, reproduced by permission of Cahiers de Biologie Marine). Scale bars (**a**, **b**) 20 μm, (**c**) 1 μm, (**d**) 2.5 μm

of mitochondria clusters into yolk granules (*T. serica*), and by autosynthesis (*S. massa*, *R. aculeata*) (Diaz et al. 1973, 1975; Gaino et al. 1987c; Sciscioli et al. 1991, 2002; Lepore et al. 1995; Riesgo and Maldonado 2009). The most common method is, probably, the absorption of dissolved nutrients in the mesohyl by numerous pseudopodia. In *S. borealis* and *R. aculeata* were described two potential types of nurse cells around the oocyte: the hyaline nucleolated cells and granular cells in the first species (Sarà et al. 2002); amoeboid cells that occurred in high numbers around the oocytes and less numerous small round cells filled with large vacuoles of granular content and lipid droplets in *R. aculeata* (Riesgo and Maldonado 2009). Reserve nutrients are mostly formed by endogenous synthesis.

Hadromerids have small isolecithal and oligolecithal eggs. The egg periphery is occupied by small yolk granules, the nucleus is located centrally (Fig. 3.4). The water current carries the egg through the osculum to the environment, where fertilization occurs.

As a rule, the released egg has an envelope. Usually, it is a collagen (primary) envelope. Besides, the egg is often surrounded with a layer of parent cells (Fig. 3.5a); in *C. celata*, these are granular cells (Warburton 1961).

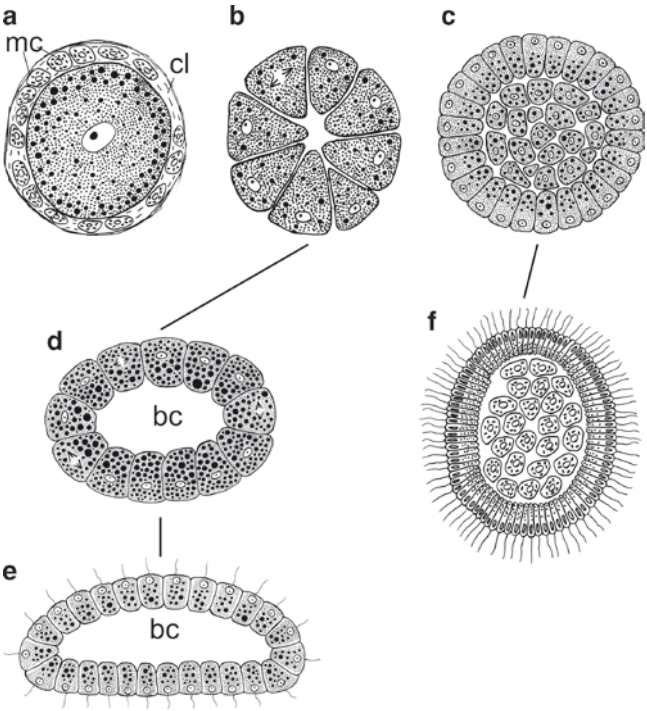

Fig. 3.5 Schematic drawing of development of the sponges in the order Hadromerida: Polymastiidae (**b, d, e**); Tethyidae, Clionaidae (**c**–**f**). (**a**) Egg, surrounding with maternal cells (*mc*) and with collagen layer (*cl*). (**b**) Radial cleavage, leading to coeloblastula. (**c**) Equal morula (stereoblastula). (**d**) Flat equal coeloblastula. (**e**) Coeloblastula larva. (**f**) Parenchymella. *bc* blastocoel

3.2.2 Embryonic Development

Though oviparous sponges are convenient research objects, development of the hadromerids has been studied very poorly. It has been traced, at light microscopic level or *in vivo*, only in *Tethya aurantium*, *Raspailia pumilla*, *Polymastia robusta* and *Stylocordyla borealis* (Lévi 1956; Borojevic 1967; Sarà et al. 2002). Cleavage has been observed *in vivo* in *Cliona celata* (Warburton 1961).

3.2.2.1 Cleavage

Cleavage in all the Hadromerida studied is total and equal (Fig. 3.5b). Furrows of the first two cleavage divisions are perpendicular to each other and lie in the same plane. The plane of the third division is perpendicular to that of the first two. As a result, the blastomeres are situated one above the other, similarly to the radial cleavage of the Metazoa. The first three cleavage divisions appear to be synchronous, which is not characteristic of the oviparous sponges (Ereskovsky and Korotkova 1999). Up to the stage of 16-cells, blastomeres (in *T. aurantium*, *P. robusta*) remain spherical, with minimal contact points (Lévi 1956; Borojevic 1967). A similar pattern of early development is characteristic of the radial cleavage in, e.g., Echinodermata or Acrania (Ivanova-Kazas 1978a, b). After the fourth cycle, the cleavage is no longer radial-like. As a result, a loose equal apolar morula is finally formed, consisting of about 64 cells (Fig. 3.5c). A characteristic feature of the cleavage in *C. celata* is penetration of parent cells into the embryo (Warburton 1961). In the viviparous *S. borealis,* the cleavage is unequal and chaotic (Sarà et al. 2002).

3.2.2.2 Larval Morphogenesis

In *Polymastia robusta* (Polymastiidae), larval development starts with elongation and flattening of the morula (Borojevic 1967). The embryo becomes a single-layer flattened equal *coeloblastula*, or "*plakula*" (Fig. 3.5d). Then the cells elongate perpendicular to the larval surface; at the apical pole, a cilium is formed, at the basal pole, yolk granules concentrate (Fig. 3.5e). At this stage, the larva, still covered with a hyaline layer, consists of 100–150 cells. Its further modifications are due to proliferation of the cells.

Development of *Tethya aurantium* (Tethyidae) follows a scheme characteristic of ovoviviparous sponges, such as the Dendroceratida or the Halichondrida (Lévi 1956). Peripheral cells proliferate more actively than the internal ones. Then they elongate perpendicular to the surface and acquire an apical–basal polarity. The nucleus migrates towards the apical pole, where a cilium develops, while the yolk granules migrate to the basal one. No differentiation of ciliar cells in connection with their position in the larva has been noted. The internal cells

proliferate and differentiate into archaeocytes. Two types of cells appear in the internal mass, the large ones and the small ones. The free-swimming larva, the parenchymella, is elongated (Fig. 3.5f).

In *Cliona viridis* (Clionaidae), the embryogenesis results in the formation of typical parenchymella, evenly covered with ciliated cells (Mariani et al. 2000).

3.2.2.3 Embryonic Morphogenesis

During the direct development (without a larva) of *Stylocordyla borealis*, cell differentiation and formation of the aquiferous system elements start in an unequal morula (Sarà et al. 2002). The young sponge, incubated after the disappearance of the nurse-cell coating directly inside the parental tissue, is characterized from the beginning by the development of an outer cortex and a spicular skeleton. In this initial phase, unorganized cell masses inside the embryo are present, and the spicules are disposed without a radial architecture. In a successive phase, choanocyte chambers are produced below the cortical zone. The young sponge shows a clear polarity. At one pole, there is a thick layer of elongated cells that move towards the surface to build the cortical layer, this zone lacks choanocyte chambers. The rest of the inner part shows choanocyte chambers, while the opposite pole is formed by the collagenous cortical layer. The young sponge is similar in its tissue structure to an adult sponge.

S. borealis is truly viviparous. The cleaving embryo has a diameter between 400 and 600 μm and is coated by a layer of nurse cells 100 μm thick. During development, the embryo reaches a diameter of 700 μm, and the layer of nurse cells can be slightly detached, reduced (about 30 μm thick) or even lacking. The young small sponge reaches a diameter of 900–1,000 μm.

Thus, in oviparous hadromerids, two different morphogeneses of the equal morula and, correspondingly, two types of larvae are possible. In the first case (*P. robusta*), the morula undergoes simple flattening and re-grouping of its cells; the result is the coeloblastula. In the second case (*T. aurantium*, *C. viridis*), the peripheral and the internal cells of the morula proliferate and differentiate at different speed, resulting in the development of two layers (in other words, morula delamination occurs), and the parenchymella is formed. In viviparous hadromerids with direct development (*S. borealis*) embryonic morphogenesis also proceeds by morula delamination.

The presence of two very different morphogeneses within the same order may be explained by two hypotheses. The first–early divergence of the Polymastiidae, with their morula flattening and the coeloblastula larva, from the main branch of the Hadromerida. Another possible explanation is the artificial status of the family Polymastiidae. Indeed, according to the molecular-biological analysis used 18S and 28S rDNA sequences, Hadromerida is paraphyletic group of demosponges (Chombard 1997; Chombard and Boury-Esnault 1999; Holmes and Blanch 2007; Kober and Nichols 2007).

Metamorphosis in the hadromerids has not been described.

3.2.3 *Asexual Reproduction*

Asexual reproduction is obligatory in the families Suberitiidae and Clionaidae, which are characterized by gemmulogenesis (Topsent 1888; Herlant-Meewis 1948; Hartman 1958; Connes 1977; Connes et al. 1978; Connes and Gil 1985) and the families Polymastiidae and Tethyidae, which are characterized by external budding (Schmidt 1868; Merejkowsky 1878, 1879; Maas 1901; Connes 1967; Battershill and Bergquist 1990; Plotkin and Ereskovsky 1997; Gaino et al. 2006).

Budding has been most extensively studied in *Tethya aurantium* (= *T. lincurium*). Indeed, it was on this species that the first studies of asexual reproduction in sponges were performed (Schmidt 1868; Merejkowsky 1878, 1879; Selenka 1879; Sollas 1888; Maas 1901; Connes 1967; Gaino et al. 2006). The bud always originates as a thin outgrowth of the sponge's cortex, supported by a spiculated stalk with few macroscleres. Separate cells or their groups migrate along the spicules to the terminal part of the outgrowth. There they form more or less spherical bodies. The main cell category is represented by cells with inclusions, which are scattered in the mesohyl and tend to concentrate along the distant surface of the bud. Containing archaeocytes, lophocytes (which secrete collagen fibres), sclerocytes, and grey cells with glycogen rosette, vacuolar cells, micro-vesicle cells morphologically resembling collencytes, and spherulous cells (Gaino et al. 2006). As the bud volume increases, lophocytes crawl onto the surface, flatten and become exopinacocytes (Connes 1967). A young bud has a radial structure due to the organized position of spicules and collagen bundles (Fig. 3.6a). Small spicules, tylostyles, cover the bud in a thick hispid layer.

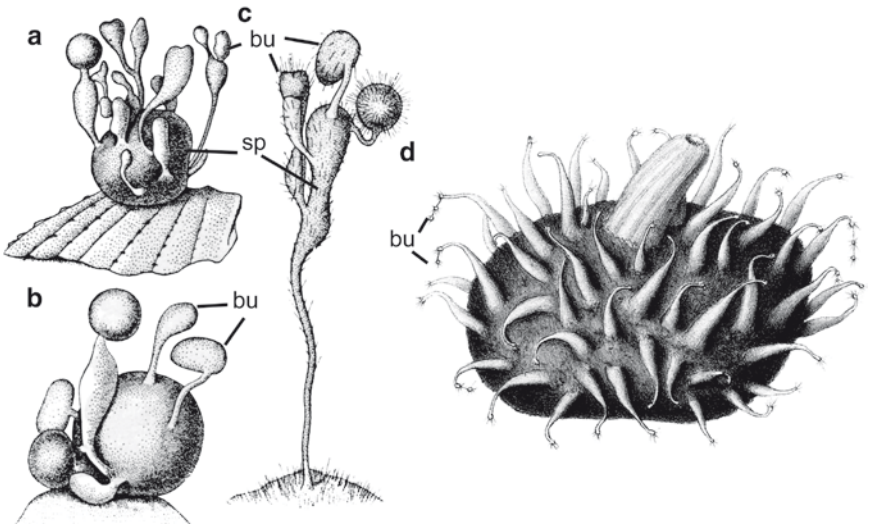

Fig. 3.6 Schematic drawing of budding in Hadromerida. (**a–c**) *Tethya aurantium* with the buds. (**d**) *Polymastia arctica* with the buds at the tips of the porous papillae. *sp* sponge, *bu* buds (From Merejkowsky 1878, 1879)

Finally the bud detaches from the parent sponge, attaches to the substrate and develops into a new sponge.

The mechanism of bud formation in *Polymastia* is similar to that in *Tethya* (Merejkowsky 1878, 1879; Arnesen 1918; Battershill and Bergquist 1990; Plotkin and Ereskovsky 1997). There is, however, one significant difference: in *Polymastia* the external buds develop at the tips of porous papillae (Fig. 3.6b). One papilla may form consecutively up to eight to ten buds in *P. arctica* and up to 17 buds in *P. granulosa* (Battershill and Bergquist 1990).

3.3 The Order Spirophorida Bergquist and Hogg, 1969

Sponges from the order Spirophorida generally have a massive, often spherical body; a few spirophorids bore calcareous substrates (colour plate XII e, f). The skeleton is radial, with bundles of macroscleres radiating from some point in the central part of the sponge (Fig. 3.7a). At the periphery there is a well-developed

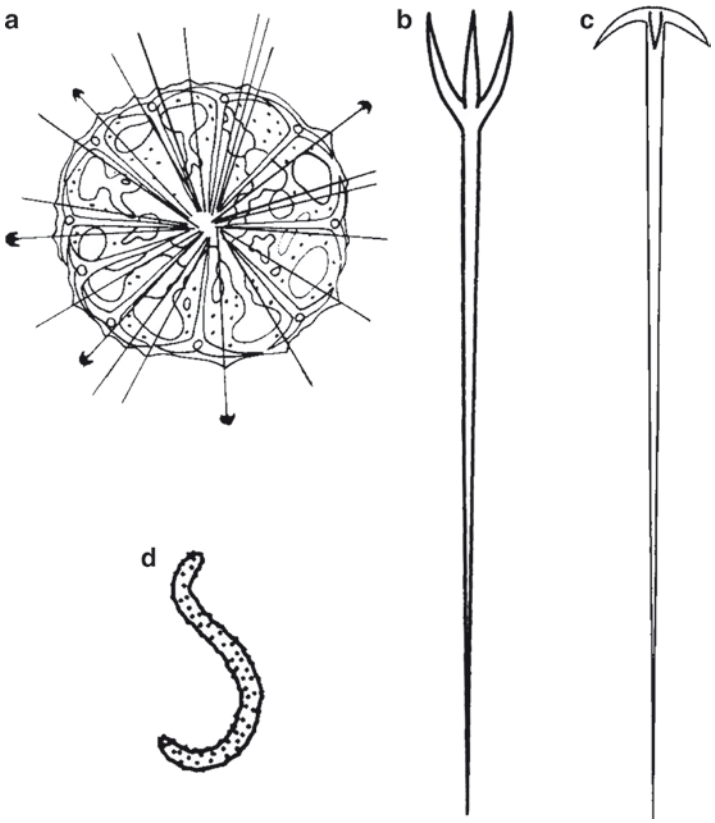

Fig. 3.7 Skeleton structure (**a**) and spicules (**b**–**d**) in sponges from the order Spirophorida: (**b**, **c**) macroscleres: protriaene and anatriaene; (**d**) microsclere sigmaspira

collagen cortical layer strengthened with special cortical oxae. Megascleres of the main skeleton are protriaenes and anatriaenes; large oxae sometimes occur (Fig. 3.7b, c). Microscleres are C- or S-shaped sigmaspires (Fig. 3.7d). Spirophorids are oviparous gonochoric sponges. The order comprises three families of marine sponges.

Embryonic development of sponges from the family Tetillidae is known that well. It was studied both at the light microscopic and electron microscopic level in *Tetilla serica* and *T. japonica* (Watanabe 1957, 1978b; Watanabe and Masuda 1990).

3.3.1 Gametogenesis

Oogenesis is synchronous both in a single sponge and in a local population. Cytologically, the development of female gametes in the Tetillidae is similar to that of the Hadromerida. The small oligolecithal non-polarized egg is formed and released through the osculum into the environment with the water flow (Watanabe 1978b; Watanabe and Masuda 1990; Lepore et al. 2000). Organization of the *Tetilla* egg is rather peculiar: first, there are long radial collagen bundles at its surface, radiating from the primary envelope (Fig. 3.8a, c, d) (Watanabe 1978b; Watanabe and Masuda 1990); second, there are no parent cells on its surface.

Spermatogenesis of the Spirophorida has not been studied.

3.3.2 Fertilization

The only investigation of fertilization in the Demospongiae was conducted on *T. serica* and *T. japonica* (Watanabe 1978b; Watanabe and Masuda 1990). In these two species, after penetration of the sperm cell into the egg a fertilization membrane develops, starting from the area of the surface where the penetration occurred. The formation of this membrane 13–18 μm thick takes about 1 min. The membrane is formed by rapid excretion of the fibrous contents of the peripheral vacuoles and probably also by delamination of the collagen envelope (Fig. 3.8b). At the beginning, radial collagen fibres still pass through the fertilization membrane, but 10 min later all of them are below it.

3.3.3 Embryonic Development

Cleavage in *T. serica* and *T. japonica* is total and equal. The furrows of the first two cleavage divisions are perpendicular to each other and lie in the same plane. The plane of the third division is perpendicular to that of the first two. As a result, groups of four blastomeres are situated on top of each other, similarly

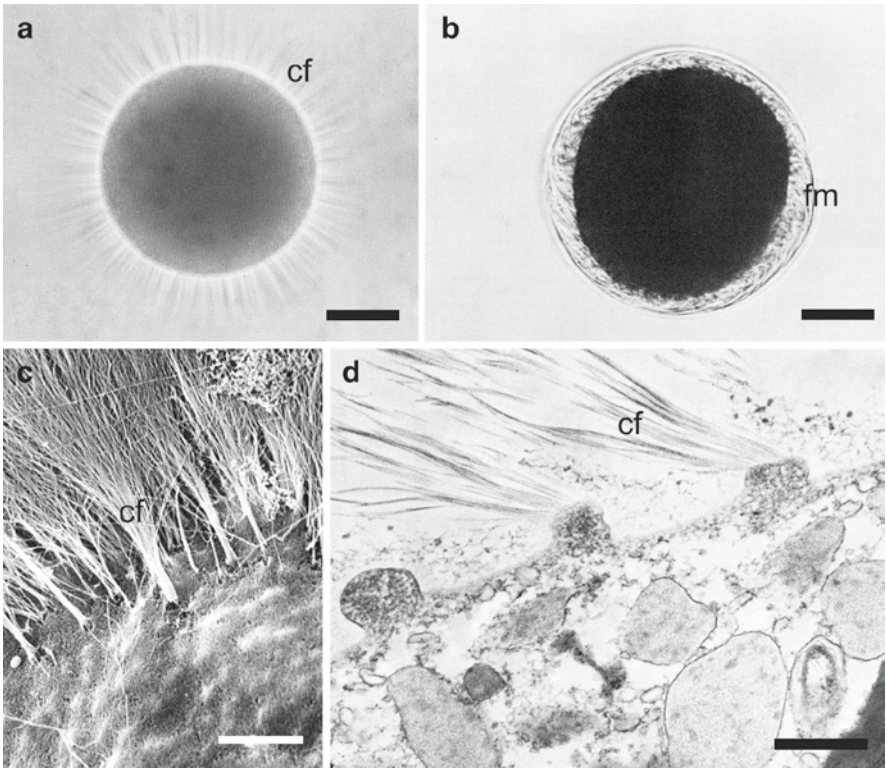

Fig. 3.8 The egg of *Tetilla japonica*. (**a**) *In vivo* image of an unfertilized egg. (**b**) *In vivo* image of an egg 10 min after fertilization with fertilized membrane (*fm*). SEM (**c**) and TEM (**d**) micrographs of collagen fibers (*cf*) of unfertilized egg, radiate from the egg's surface (From Watanabe and Masuda 1990, Figs. 1–3, reproduced by permission of Smithsonian Institution Scholarly Press). Scale bars (**a**, **b**) 50 μm, (**c**) 5 μm, (**d**) 0.5 μm

to the radial cleavage of the Metazoa (Fig. 3.9d). Until the stage of 16 cells, the blastomeres retain their spherical shape, with a minimal number of contact points (Watanabe 1957, 1978b). However, after transition to 32 cell stage, the cleavage is no longer radial. Finally, an equal, loose, apolar morula, consisting of about 64 cells, is formed.

3.3.3.1 Embryonic Morphogenesis

Tetilla development is direct, without the larva. In *T. serica* and *T. japonica* (Watanabe 1957, 1978b; Watanabe and Masuda 1990) the embryogenesis occurs outside the parent sponge, while in *T. schmidtii* (Sollas 1888), *T. australis* (Bergquist 1968) and *T. cranium* (Burton 1931) the direct development proceeds inside the parent sponges. Further details of this process are unknown.

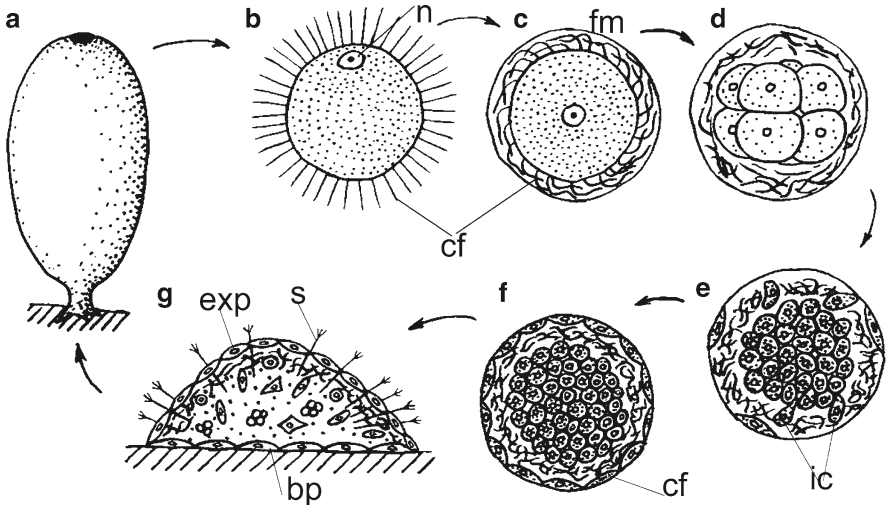

Fig. 3.9 Schematic drawing of development of *Tetilla.* (**a**) Adult sponge. (**b**) Egg. (**c**) Egg, covered by fertilization membrane (*fm*). (**d**) Radial cleavage. (**e**) Beginning of embryo morphogenesis. (**f**) Pinacoderm formation. (**g**) Rhagon-like sponge before aquiferous system development. *bp* basopinacoderm, *cf* radial collagen bunches, *exp* exopinacoderm, *ic* migrated cells, *n* nucleus, *s* spicules

The morula in *Tetilla* is covered by the fertilization membrane underlain by a dense network of collagen fibres (Watanabe 1957, 1978b; Watanabe and Masuda 1990). The morphogenesis begins with the migration of the peripheral cells of the morula into the perivitellin space (Fig. 3.9e). The cells form long pseudopodia along the collagen fibres of the perivitellin space, flatten along the internal surface of the fertilization membrane and form the pinacoderm (Fig. 3.9f). Thus, collagen fibres play an important morphogenetic role in the *Tetilla* development, determining the direction of cell migration and the character of cell differentiation (Watanabe and Masuda 1990).

Marginal cells form long pseudopodia attaching the embryo to the substrate. Then some of the cells situated in the centre of the basal surface of the embryo migrate inside by unipolar immigration. As a results, the basal surface area decreases. The internal mass of the embryo's cells separates into the ectosome and the future choanosome. The secretion of extracellular matrix starts. Within the choanosome, the cells differentiate into scleroblasts, choanoblasts and endopinacocytoblasts. The latter differentiate and form the elements of the aquiferous system of the leuconoid type. The spicules form the radial skeleton. The elements of the aquiferous system unite and their connection with the environment via external pores is established.

Thus, the *Tetilla* has a direct development without a larva and there is no talking about the fate of the larval flagellar cells or the "inversion of the germinal layers".

3.3.4 Asexual Reproduction

Sponges from the family Tetillidae have external budding. The buds are formed in the basal part of the sponge and are, similarly to other demosponges, agglomerations of totipotent cells (Chen et al. 1997).

3.4 The Order Chondrosida Boury-Esnault and Lopès, 1985

Representatives of the order Chondrosida are massive or encrusting sponges (colour plate V) with a pronounced cortex enriched with fibrillar collagen. The skeleton is often lacking; if present, it consists of nodular spongin bundles or microscleres (asters). There are no macroscleres. Chondrosida are gonochoric and oviparous.

Chondrosids are common in the shallow waters of temperate and warm seas; sometimes they are found at depths down to 750 m. The order comprises the single family Chondrillidae, three genera and about 50 species.

3.4.1 Gametogenesis

Spermatogenesis has been studied only in *Chondrilla australiensis* (Usher et al. 2004). Male gametes, presumably, derive from choanocytes. A peculiar feature of *C. australiensis* spermatogenesis is absence of spermatocystes.

Oogenesis has been studied in *Chondrilla nucula* and *C. australiensis* (Gaino 1980; Usher et al. 2004; Sidri et al. 2005) and, partly, in *Chondrosia reniformis* (Lévi and Lévi 1976). In *C. australiensis* female gametes develop from choanocytes by direct transformation (Usher et al. 2004). According to Usher et al. (2004), during vitellogenesis the oocyte is evenly surrounded by nurse cells, which are the main source of reserve nutrients (Fig. 3.10a). These "nurse cells" generally arranged in radial pattern, contain yolk inclusions and lipidic zones. Oocytes are scattered in the choanosome, but in *C. reniformis* they clustered in the choanosome in groups of 20–50, and never occurred in the very collagenous mesohyl areas (Riesgo and Maldonado 2008a). Oogenesis is synchronous within a single sponge.

The eggs are oligolecithal. In *C. reniformis* they are enclosed in a capsule of parent cells (Figs. 3.10a and 3.12a) – bacteriocytes and granular cells; this complex is covered by a collagen envelope. In *C. australiensis* and *C. nucula* the released eggs are enclosed only in a mucous capsule (Sidri et al. 2005; Usher and Ereskovsky 2005).

Plate V Order Chondrosida Boury-Esnault and Lopès 1985 (**a**) *Chondrosia reniformis* Nardo 1847. (b) *Thymosiopsis cuticulatus* Vacelet and Perez 1998. (**c**) *Chondrilla nucula* Schmidt 1862 (Courtesy of J. Vacelet). (**d**) *Thymosiopsis conglomerans* Vacelet et al. 2000. (**a**–**d**) Mediterranean Sea (**a**, **b**, **d** – Courtesy of T. Perez)

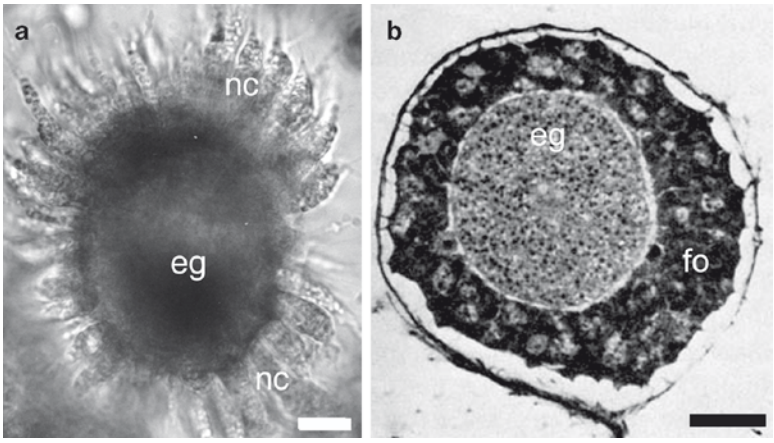

Fig. 3.10 (**a**) Mature eggs (*eg*) of *Chondrilla australiensis* with attached nurse cells (*nc*). (**b**) Fertilized egg (*eg*) of *Chondrosia reniformis* with follicle (*fo*) (**a** – Courtesy K. Usher; **b** – From Lévi and Lévi 1976, Ann. Sci. Nat. Zool. Biol. Anim. Ser. 12 vol. 18 pp. 369, Fig. 3, reproduced by permission of Elsevier Ltd). Scale bars (**a**) 10 μm, (**b**) 100 μm

3.4.2 Embryonic Development

Though representatives of the Chondrosida are traditionally considered to be oviparous, at least some of the eggs in *Chondrilla nucula* and *C. australiensis* are fertilized within the parent sponge (Sidri et al. 2005; Usher and Ereskovsky 2005).

Embryonic development of chondrosids has been studied in *Chondrosia reniformis* and *C. australiensis* (Lévi and Lévi 1976; Usher and Ereskovsky 2005), some information on *C. nucula* cleavage was also published (Sidri et al. 2005).

3.4.2.1 Cleavage and Larval Morphogenesis

Cleavage is total and equal, asynchronous from the second or the third cleavage cycle. In *C. reniformis* it is radial until the fourth cycle. Cleavage results in the formation of an apolar equal morula, without a cavity, consisting of a small number of cells (Fig. 3.11). In *C. australiensis* the morula develops into an equal blastula with a small blastocoel by the rearrangement of blastomeres; a single-layer embryo is formed.

C. reniformis has a unique morphogenesis associated with mass migration of the parent cells that have surrounded the egg and the cleaving embryo inside the morula (Fig. 3.12b). As a result, the embryonic cells of the morula are forced out to the periphery, where they proceed to proliferate, line up in a single layer. The dense internal cell mass of the embryo consists of the parent bacteriocytes and granular cells (Fig. 3.12c). Such an embryo was called “pseudoblastula” (Lévi and Lévi 1976). All the larval cells proper are cytologically similar and bear cilia. They are only different in shape: the cells of the posterior pole are flattened,

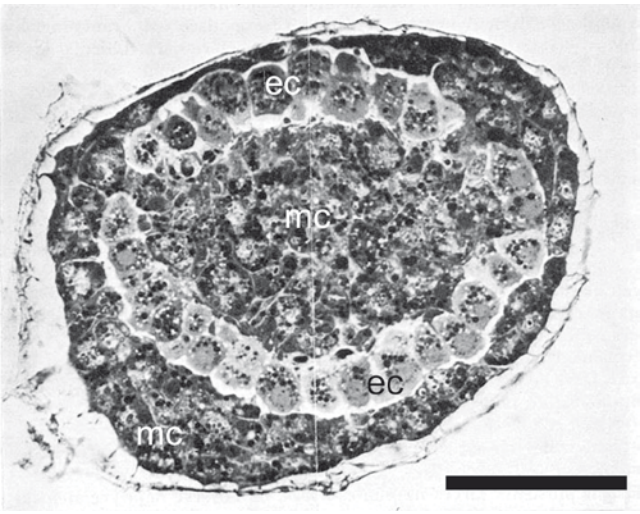

Fig. 3.11 Morula of *Chondrosia reniformis. ec* cells of the embryo, *mc* maternal cells (From Lévi and Lévi 1976, Ann. Sci. Nat. Zool. Biol. Anim. Ser. 12 vol. 18 pp. 373, Fig. 9, reproduced by permission of Elsevier Ltd). Scale bar 50 μm

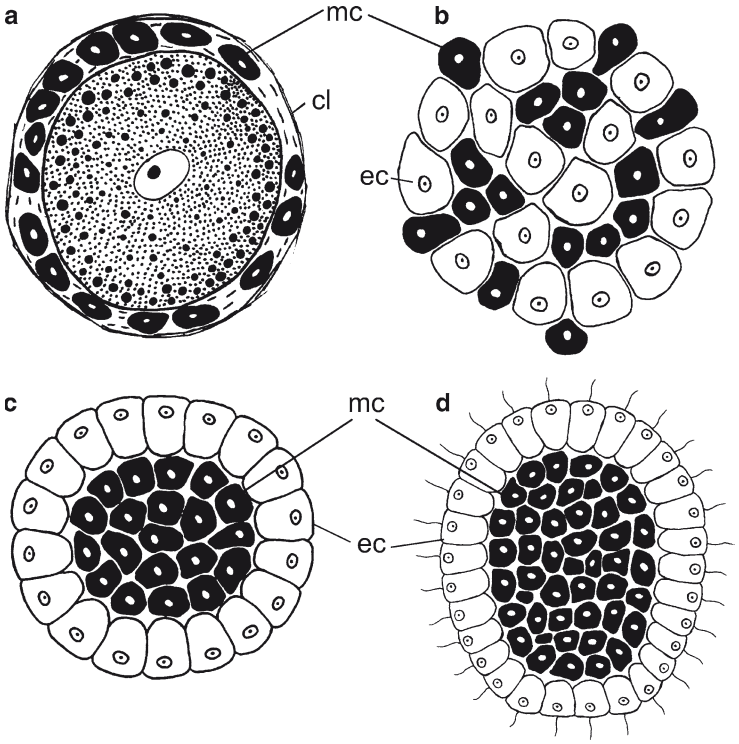

Fig. 3.12 Schematic drawing of development of *Chondrosia reniformis*: (**a**) Egg, surrounding with maternal cells layer (*mc*) and collagen layer (*cl*). (**b**) Maternal cells (*mc*) migration inside of the morula. (**c**) Bilayered morula, composed of external embryonic cells (*ec*) and internal maternal cells (*mc*). (**d**) Larva "pseudoblastula"

while all the others are cubic (Fig. 3.12d). The internal cells are not nurturing cells; they do not disintegrate during the larval life and the metamorphosis.

3.4.3 Larva

In *C. australiensis* cell division and differentiation results in an entirely ciliated coeloblastula approximately 75 μm long and 50 μm wide. The coeloblastula is spheroid or ovoid, with a flattened posterior pole (Fig. 3.13). It consists of a layer of columnar monociliated cells around a central cavity.

C. australiensis and *C. reniformis* coeloblastulae have only one cell type, the external ciliated cells. It is very unusual for Demospongiae larvae; besides these two species, only *Polymastia robusta* larvae consist of cells of a single type (Borojevic 1967).

C. australiensis larvae differ from those of *C. reniformis* (Fig. 3.13) in having an epithelium made of long columnar cells instead of cubic cells, longer cilia on the flattened posterior pole and a more clearly defined blastocoel. The blastocoel is filled with symbiotic bacteria and cyanobacteria derived from the parent sponge and a loose network of collagen fibrils. Contrary to the larvae of *C. reniformis*, there are no maternal cells in the blastocoel of *C. australiensis* larvae. No basement membrane underlies the ciliated cells lining the central cavity in chondrosid larvae, as it is the case in the cinctoblastulae of Homoscleromorpha (Boury-Esnault et al. 2003).

There is a *zonula adhaerens* (Usher and Ereskovsky 2005) in the apical region of the ciliated cells of *C. australiensis* larvae. The ciliary basal apparatus of the *C. australiensis* larval cells is not essentially different from similar structures in the other demosponges larvae. For example, the accessory centriole is situated near

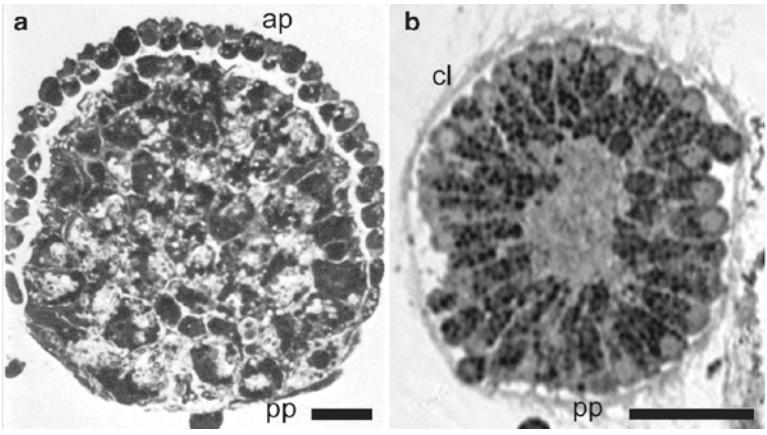

Fig. 3.13 Light micrographs of "pseudoblastula" larva of *Chondrosia reniformis* (**a**) and coeloblastula of *Chondrosia australiensis* (**b**). *ap* anterior pole, *cl* collagen layer, *pp* posterior pole (**a** – From Lévi and Lévi 1976, Ann. Sci. Nat. Zool. Biol. Anim. Ser. 12 vol. 18 pp. 375, Fig. 12, reproduced by permission of Elsevier Ltd). Scale bars (**a**, **b**) 20 μm

the kinetosome, and the single rootlet consists of non-striated fine fibrillar material, as in all Demospongiae (Woollacott and Pinto 1995). In *C. australiensis* larvae the microtubules start from the basal body opposite the basal foot and are directed to the posterior pole of the larvae. The basal foot is directed to the apical pole. A similar orientation of an analogous structure was described for the coeloblastulae of *Soleneiscus laxa*, *Guancha arnesenae* (Calcispongiae) and parenchymella of *Ircinia oros* (Demospongiae) (Amano and Hori 2001; Ereskovsky and Tokina 2004; Ereskovsky and Willenz 2008). The orientation of the transverse cytoskeletal system towards the posterior pole is probably essential for aligning the direction of the effective stroke of the cilia in the Eumetazoa (Sanderson 1984).

3.4.4 Metamorphosis

The larvae of *C. reniformis* and *C. australiensis* attach to the substrate by the anterior pole and flattened along the anterior–posterior axis (Lévi and Lévi 1976; Usher and Ereskovsky 2005). For the first 48 h after settlement, the post-larvae of *C. australiensis* consist of a homogeneous cell mass with a distinct external mucus-like coat. The internal cavity and the ciliated epithelium present in coeloblastulae are no longer visible, intercellular contacts are disrupted. There are no cilia on the cells of the settlers. The cells neither die nor proliferate during this period. Bacterial and cyanobacterial symbionts occur extracellularly throughout the post-larva.

After 2–3 days, with the beginning of cell differentiation and rhagon formation, the cells in all regions of the post-larvae change shape and size. The differentiation of larval cells into adult cells depends on their position in the post-larvae. The cells in the upper part of the post-larvae become flattened and differentiate into pinacocytes (surface cells) and basopinacocytes (basal cells). The first adult structure to develop is the exopinacoderm. The development of the aquiferous system (choanocyte chambers and canals) begins after exopinacoderm formation. The internal conglomerate of cells differentiats into choanocytes, endopinacocytes and the cells of the mesohyl. The metamorphosis of *C. australiensis* is completed in 10–12 days, when a rhagon with an osculum and a functioning aquiferous system is formed.

During metamorphosis in *C. reniformis*, granular cells of the parent origin participate in the development of the surface structures of the rhagon, while the bacteriocytes exude symbiotic bacteria into the mesohyl and then differentiate into archaeocytes (Lévi and Lévi 1976). The fate of the larval cells is unknown. They probably differentiate into both pinacocytes and choanocytes of the young sponge.

3.4.5 Asexual Reproduction

C. reniformis and *C. nucula* reproduce asexually throughout the year, mainly by fragmentation (Bavestrello et al. 1998; Bonasoro et al. 2001; Sidri et al. 2005).

In an unusual process resembling creeping, these sponges can form long outgrowths, extending downwards as if under the force of gravity. Fragmentation involves rearrangement of extracellular collagen network and change in its density (the integrity of the collagen fibers is not affected), as well as active participation of the spherulous cells containing specific granular inclusions.

3.5 The Order Halichondrida Gray, 1867

Sponges from the order Halichondrida are diverse in shape, size and colour (colour plate VI). The dermal skeleton is an unregulated "halichondroid" network; choanosomal skeleton is usually plumoreticulate, but can be modified, in different groups, to dendritic columns of megascleres or confused bundles with many loose single megascleres (Fig. 3.14a) (van Soest and Hooper 2002). Spicules are simple: oxeas, strongyles, styles (Fig. 3.14b–d). Spongin is usually well developed. Halichondrids may be ovoviviparous or oviparous; the larva is parenchymella. Asexual reproduction has been described in *Axinella*. Halichondrid sponges are marine, distributed worldwide. The order comprises five families but their monophyly has not been determined by studies using multiple methods. Different evidences support the polyphyletic nature of Halichondrida (Erpenbeck et al. 2006; Erpenbeck and Worheide 2007).

Sexual phenotype in the Halichondrida is rather labile. Some of them are gonochoric (Barthel and Detmer 1990; Witte and Barthel 1994; Tanaka-Ichihara and Watanabe 1990), some show successive hermaphroditism (Ivanova 1981; Lewandrowski and Fell 1981; Sarà 1993; Gerasimova and Ereskovsky 2007).

3.5.1 Gametogenesis

The few investigations that have been made on gametogenesis in the Halichondrida show that male and female gametes may derive from different cells. It was demonstrated at the electron-microscopic level that spermatocytes differentiated from choanocytes by direct transformation without gonial divisions (Barthel and Detmer 1990). Oocytes appear to differentiate from nucleolated amoebocytes (archaeocytes) (Ivanova 1981; Witte and Barthel 1994; Riesgo and Maldonado 2008b). Spermatogenesis and oogenesis are asynchronous both within a single sponge and in the local populations (Fell and Jacob 1979; Ivanova 1981; Tanaka-Ichihara and Watanabe 1990; Witte and Barthel 1994; Gerasimova and Ereskovsky 2007; Riesgo and Maldonado 2008a).

3.5.1.1 Spermatogenesis

Within a spermatocyste, male gametes develop synchronously (Fig 3.15). In *Halichondria panicea* choanocytes transdifferentiate into spermatogonia without

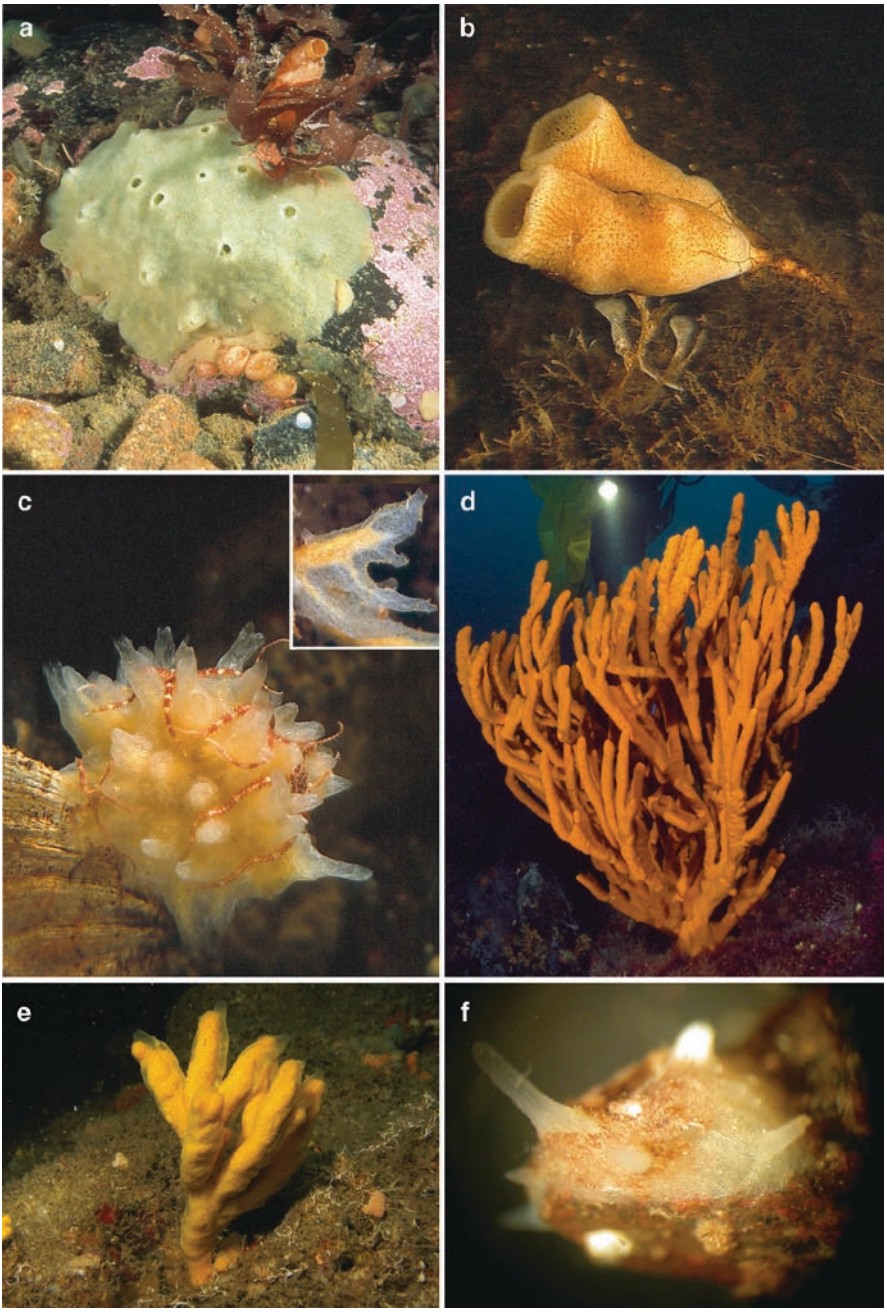

Plate VI Order Halichondrida Gray 1867 (**a**) *Halichondria panicea* (Pallas 1766), White Sea (Courtesy of N. Cherviakova). (**b**) *Phakettia cribrosa* (Miklucho-Maclay 1870), White Sea (Courtesy of M. Fedjuk). (**c**) *Halichondria (Eumastia) sitiens* (Schmidt 1870), *inset*: papillae of the sponge (Courtesy of M. Fedjuk). (**a**–**c**) White Sea. (**d**) *Axinella polypoides* Schmidt 1862, Mediterranean Sea (Courtesy of R. Graille). (**e**) *Axinella verrucosa* (Esper 1794), Mediterranean Sea (Courtesy of J. Harmelin). (**f**) *Vosmaeria crustacea* Fristedt 1885 White Sea (Courtesy of M. Fedjuk)

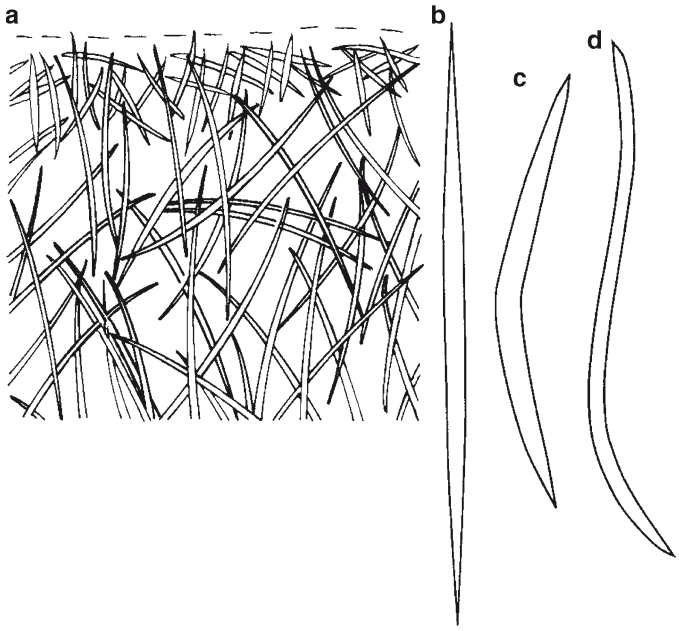

Fig. 3.14 Skeleton structure (**a**) and macroscleres – oxae (**b–d**) in sponges from the order Halichondrida

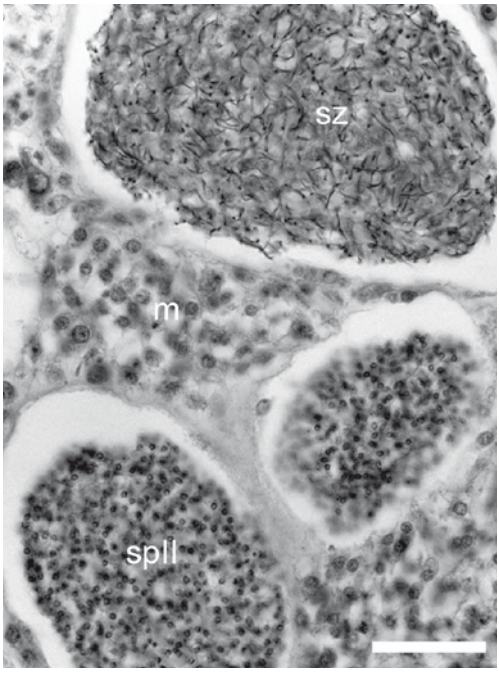

Fig. 3.15 Light micrograph of spermatocystes of *Halichondria panicea* with different stages of spermatogenesis. *m* mesohyl, *spII* spermatocystes II, *sz* spermatozoa. Scale bar 20 μm

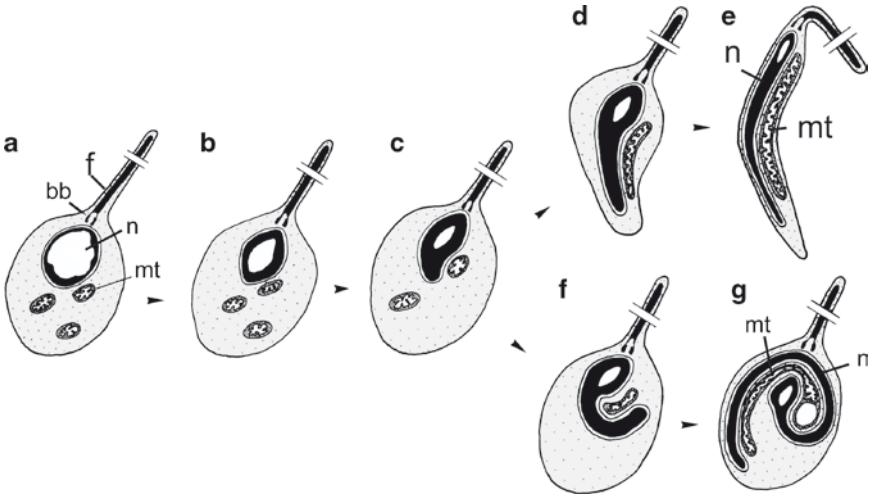

Fig. 3.16 Schematic drawing of spermiogenesis of *Halichondria panicea.* (**a**–**c**) Early stages of sperm differentiation. (**d**, **e**) Spermatids. (**f**, **g**) Spermatozoids with spiral and elongated nucleus (From Barthel and Detmer 1990, Zoomorphology vol. 110, pp. 114, Fig 16, reproduced by permission of Springer). *f* flagellum, *bb* basal body, *mt* mitochondrion, *n* nucleus

mitotic divisions. When a spermatid is transformed into a spermatozoid, the nucleus, with condensed chromatin, elongates (Fig. 3.16) (Barthel and Detmer 1990). A long mitochondrion adjoining the nucleus has been formed by fusion of smaller mitochondria in the course of spermiogenesis. An unusual feature of a *H. panicea* population from the Kiel Bay of the Baltic Sea is polymorphism of spermatozoids (Barthel and Detmer 1990): some are strongly elongated, while the others are rounded, with the spirally twisted nucleus and mitochondrion (Fig. 3.16e, g).

Oogenesis follows the general scheme characteristic of the Metazoa. In the pre-meiotic phase the oocyte, which has amoeboid motility, migrates in the mesohyl (Fig. 3.17a). In the early vitellogenic phase, nutrients start to accumulate and the yolk granules to be synthesized in the cytoplasm (Fig. 3.17b). In ovoviviparous halichondrids during the beginning of the vitellogenesis stage amoeboid cells, concentrating around the growing oocyte, form a well-developed multilayered capsule (Fig. 3.17c, d). The phagocyted cells of the capsule become yolk granules in the oocyte (Meewis 1941; Diaz 1973; Fell and Jacob 1979; Ivanova 1981; Witte and Barthel 1994; Gerasimova and Ereskovsky 2007). Though the phagocyted cells are commonly considered as specialized *nurse cells*, they have not, in fact, been described either morphologically or cytochemically. Therefore, the question whether the Halichondrida have specialized nurturing cells (similar, for instance, to those of the Haplosclerida) remains open. As its cells are phagocyted, the capsules get thinner, and all that is

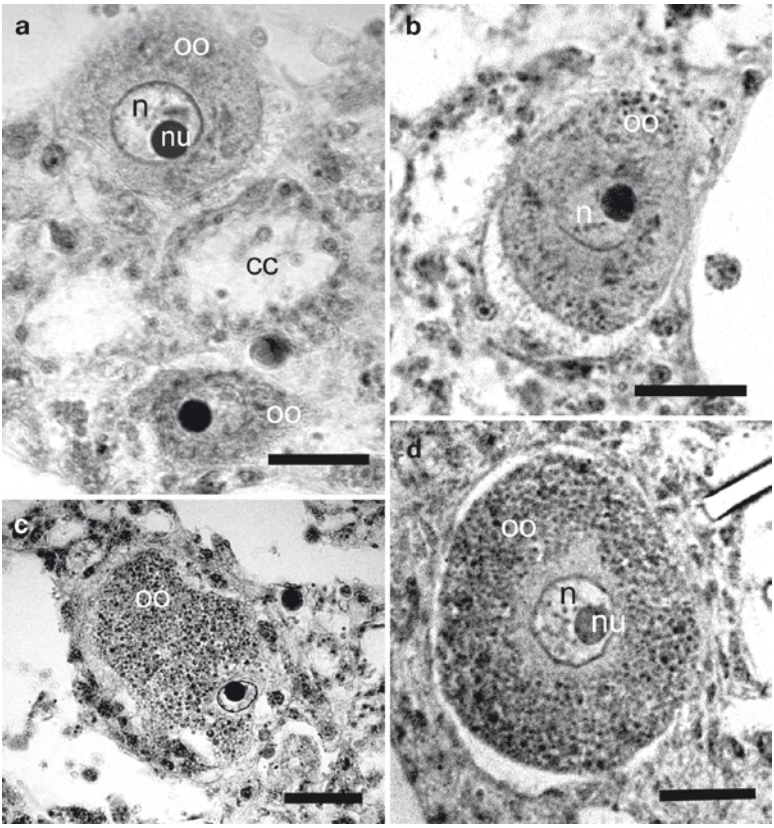

Fig. 3.17 Light micrographs of oogenesis of *Halichondria panicea*. (**a**) Early stages of oogenesis. (**b**) Beginning of vitellogenesis. (**c**, **d**) Vitellogenic oocytes. *cc* choanocyte chamber, *n* nucleus, *nu* nucleolus. *Scale bars* (**a, b**) 25 μm, (**c**) 30 μm, (**d**) 40 μm

left around the mature egg is a single-layer brood chamber consisting of flattened pinacocyte-like cells and some collagen.

In oviparous *Axinella damicornis* most yolk is elaborated by the oocytes from the phagocytosed materials, particularly, from endocytosed bacteria (Riesgo and Maldonado 2008b). It was also proposed an additional autosynthetic mechanism of vitellogenesis. Unlike ovoviviparous halichondrids, in the vitellogenesis of *A. damicornis* there are no special nurse cells, formed multilayered capsule (Riesgo and Maldonado 2008b).

Mature eggs are polylecithal and isolecithal, oval in shape; in all the halichondrids studied they have a similar size (Table 3.1). The central nucleus is surrounded with a broad area of the ooplasm free of yolk granules (Fig. 3.18a) but containing groups of mitochondria and dictyosomes of the Golgi apparatus. In the rest of the ooplasm, small and large yolk granules are evenly distributed.

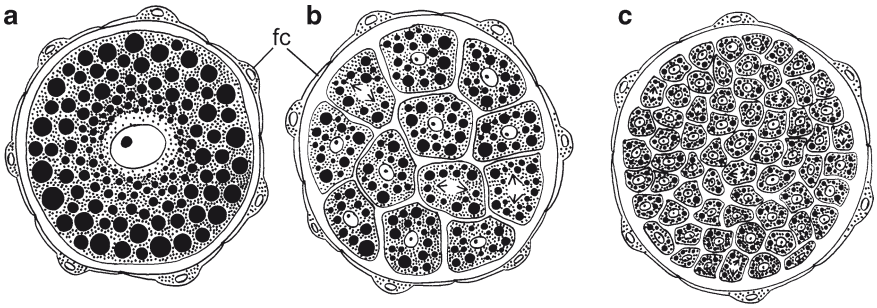

Fig. 3.18 Schematic drawing of egg (**a**), cleavage (**b**) and equal morula (**c**) of Halichondrida. *fc* follicular cells

3.5.2 Embryonic Development

Investigations of embryonic development in the Halichondrida are few. Moreover, all of them have been conducted at the light microscopic level and involved ovoviviparous species (Meewis 1941; Fell and Jacob 1979; Ivanova 1981; Gerasimova and Ereskovsky 2007). In the encrusting halichondrids embryos are situated in the basal part of the sponge (Witte and Barthel 1994). In species with other body shape they are scattered throughout the choanosome, sometimes in clusters.

3.5.2.1 Cleavage

The furrow of the first cleavage division is perpendicular to the longer egg axis (Fell and Jacob 1979; Ivanova 1981). The furrow of the second cleavage division is perpendicular to that of the first one and passes in the same direction. The first two divisions are equal and, probably, synchronous. The four-cell embryo has the shape of a tetrahedron (Meewis 1941). Later the equality of cleavage divisions may be disrupted, probably because they are asynchronous. The division spindles are non-organized, the cleavage plane is not formed (Fig. 3.18b). By the morula stage, all the cells have the same size. An apolar equal dense morula is formed (Fig. 3.18c) (Meewis 1941; Bergquist et al. 1970; Diaz 1973; Fell and Jacob 1979; Ivanova 1981; Gerasimova and Ereskovsky 2007).

Larval morphogenesis in halichondrids starts with the segregation of the cells of the equal morula into the internal conglomerate of amoeboid yolk-rich cells and the peripheral one-cell layer of the future ciliated cells. The segregation, apparently resulting from a higher proliferation rate of the peripheral morular cells, resembles the process of morula delamination in the Eumetazoa. A more active proliferation of the peripheral cells of the embryo is characteristic of most sponges. The crucial role in this process is likely to be played by positional information. In some species (e.g. *Halichondria coliata*), delamination is polarized: starting at one of the poles,

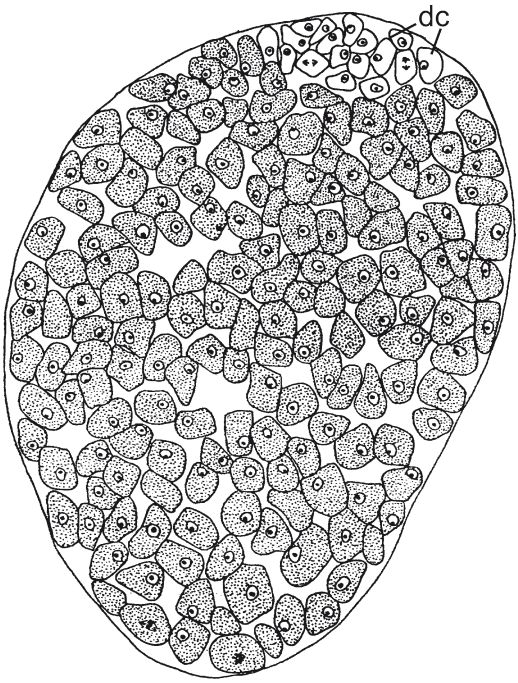

Fig. 3.19 Schematic drawing of the polarized delamination in *Halichondria coliata. dc* divided cells at one pole (From Meewis 1941)

it gradually spreads along the whole periphery of the embryo (Fig. 3.19) (Meewis 1941). This polarized delamination is in fact identical to the morphogenesis described in Poecilosclerida and Haplosclerida (see Sections 3.9 and 3.10).

At first the peripheral cells are arranged in several layers, but as the differentiation proceeds, they stretch perpendicular to the embryo's surface, forming a pseudo-multilayered columnar epithelium (Fig. 3.20) (Meewis 1941; Fell 1974a; Fell and Jacob 1979; Ivanova 1981). Noteworthy, its thickness remains the same at all the surface of the embryo from the beginning of the peripheral layer segregation and almost until the end of cell differentiation.

In *H. coliata*, while the surface layer is being formed, the larval sclerocytes differentiate (Meewis 1941). They are situated inside the embryo, stretching along the basal parts of the surface cells. Thus, in the halichondrids whose larvae have spicules, the first cells to differentiate are sclerocytes secreting the larval spicules (oxae). This advanced differentiation of sclerocytes is also characteristic of the Poecilosclerida and the Haplosclerida (see Sections 3.9 and 3.10).

The larval surface cells differentiate into the ciliated cells with a distinct apical–basal polarity (Fig. 3.20b). In the apical parts of ciliated cells of the larvae of *Halichondria moorei* and *Ulosa* sp. cells junctions (*zonula adhaerens*) were revealed (Bergquist and Green 1977). The cells of the posterior pole of the pre-larva divide slower and thus remain larger. The internal part of the pre-larva is a homogenous non-polarized cell mass, where archaeocytes, collencytes and secretory cells differentiate. No cavities or lacunae are formed during larval differentiation.

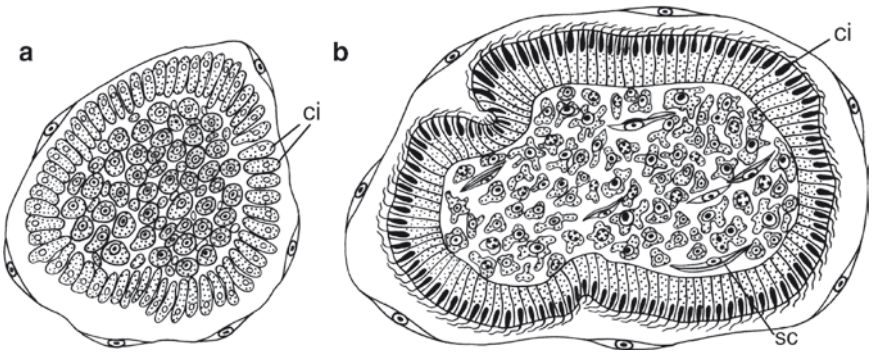

Fig. 3.20 Schematic drawing of larval morphogenesis of *Halichondria panicea* (Courtesy of L. V. Ivanova). *ci* ciliated cells; *sc* sclerocytes

As a result of intensive proliferation of the ciliated cells of the pre-larva, its surface increases and folds are formed on it (Fig. 3.20b). At the end of the larval development, the brood chamber cavity merges with that of the excurrent canal. The folds disappear; the larva elongates and is releases via the excurrent canals into the environment.

Embryonic development in halichondrids is usually accompanied by considerable modifications of the choanosome: its tissues, choanocytes chambers and aquiferous system canals are destroyed (Diaz 1973; Fell and Jacob 1979; Ivanova 1981; Barthel and Detmer 1990; Tanaka-Ichihara and Watanabe 1990; Witte and Barthel 1994; Ereskovsky, unpublished).

3.5.3 Larva

The larvae of Halichondrida are typical parenchymella. *Svenzea zeai*, a halichondrid sponge from the Caribbean Sea, has giant larvae up to 6.1 mm long (Rützler et al. 2003). The larvae are usually orange-yellow (Table 3.2), strongly elongated, cigar-shaped; all the surface cells bear cilia. In *Halichondria* sp., *H. coliata*, *H. melanadocia*, *H. bowerbanki*, *H. magnicolulosa* and *H. coerulea* the cilia are longer at the posterior pole, and the cells bearing them are less polarized and have a greater apical surface than the cells elsewhere on the larva (Fig. 3.21) (Topsent 1911; Meewis 1941; Hartman 1958; Fell and Jacob 1979; Wapstra and Van Soest 1987; Woollacott 1990, 2003; Maldonado and Young 1996). *H. panicea* larvae from the Pacific coast of Japan (Amano 1986) and *Hymeniacidon sanguinea* larvae from the Atlantic coast of France and from the Mediterranean (Lévi 1956; Uriz 1982a) have no cilia at the posterior pole. The internal layer is dense and homogeneous. Contrary to the parenchymellae of the Poecilosclerida and the Haplosclerida, halichondrid larvae have no layer of cells (collencytes or archaeocytes) underlying

Table 3.2 Characteristics of larvae of sponges from the order Halichondrida

Species	Size (diameter/ length), μm	Colour	Ciliation	Skeleton	Location	References
Halichondria coliata	216/400	Yellow	Longer at the posterior pole	Yes	Mediterranean Sea	Topsent 1911
H. coliata	120/245	Yellow	Longer at the posterior pole	Yes	North Sea	Meewis 1941
H. coliata	unknown	Yellow	Longer at the posterior pole	Unknown	Roscoff	Lévi 1956
H. panicea	176/500	Yellow	Longer at the anterior pole	Yes	Mediterranean Sea	Topsent 1911
H. panicea	128/295	Unknown	Uniform	Yes	Connecticut	Fell 1974a
H. panicea	180/600	Orange-yellow	Uniform	Unknown	Barents Sea	Ivanova 1981.
H. panicea	250/400	Yellow	Naked posterior pole	Unknown	Sea of Japan	Amano 1986
H. panicea	80/–150/340–600	Orange-yellow	Longer at the posterior pole	Unknown	North Sea	Wapstra and van Soest 1987
H. panicea	170/580	Orange-yellow	Uniform	Unknown	White Sea	Ereskovsky, unpublished
H. panicea	90–100/240–280	Yellow	Posterior pole ciliated sparsely	Unknown	British Columbia	Elliott et al. 2004
H. coerulea	130/240	Cream	Ciliar tuft at the posterior pole	Yes	Hawaii	Woollacott and Pinto 1995; Woollacott 2003
H. moorei	629/1434	Orange-yellow	Uniform	No	New Zealand	Bergquist and Sinclair 1968
H. melanodocia	200/285	Yellow	Longer at the posterior pole	No	Florida	Woollacott 1990
H. bowerbanki	unknown	Yellow	Longer at the posterior pole	Unknown	Florida	Hartman 1958
H. bowerbanki	140–240/320	Yellow	Longer at the posterior pole	Unknown	North Sea	Wapstra and van Soest 1987
H. magniconulosa	318–468	Yellow	Naked posterior pole, ring present	Unknown	Florida	Maldonado and Young 1996
H. japonica	90/207	Yellow	unknown	Yes	Sagami Bay	Hoshino et al. 2004
Halichondria sp.	150/275	Yellow	Longer at the posterior pole	Unknown	Connecticut	Fell and Jacob 1979
Hymeniacidon sanguinea	275/375	Reddish	Naked posterior pole	Yes	Roscoff	Lévi 1956

(continued)

Table 3.2 (continued)

Species	Size (diameter/ length), μm	Colour	Ciliation	Skeleton	Location	References
H. sanguinea	930/1640	Orange	Naked posterior pole	Unknown	Mediterranean Sea	Uriz 1982a
H. caruncula	185/377	Orange	Naked posterior pole	Yes	Mediterranean Sea	Topsent 1911
H. heliophila	330/400	Orange-yellow	Uniform	Unknown	Florida	Woollacott and Pinto 1995
H. heliophila	150–200/290–400	Orange	Posterior pole ciliated sparsely	Yes	Hawaii	Woollacott 2003
H. japonica	114/200	Orange	Unknown	Yes	Sagami Bay	Hoshino et al. 2004
Scopalina lophiropoda	480/1,600	Orange	Uniform	Unknown	Mediterranean Sea	Uriz 1982b
S. ruetzleri	500/1,500	Orange	Uniform	No	Caribbean Sea	Rützler et al. 2003
Ulosa sp.	90–420	Yellow	Uniform	No	New Zealand	Bergquist and Green 1977
Svenzea zeai	2,600/6,100	Cream	Uniform	No	Caribbean Sea	Rützler et al. 2003

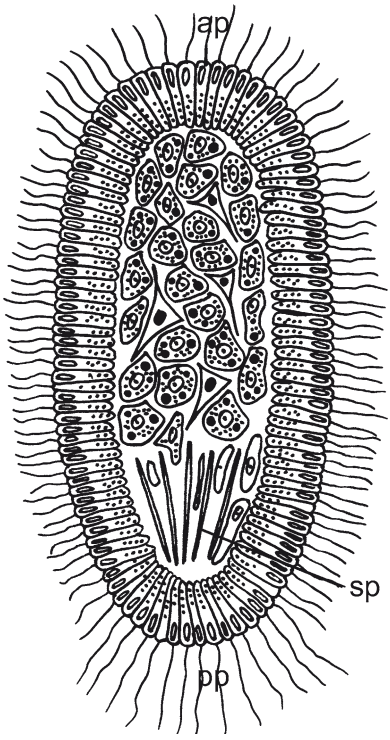

Fig. 3.21 Schematic drawing of parenchymella larva of Halichondrida. *ap* anterior pole, *pp* posterior pole, *sp* spicules

the covered ciliated layer. In the larvae of the family Halichondriidae, the space between the basal parts of the ciliated cells and the internal cell mass is loose (Bergquist and Green 1977), whereas in the family Dictyonellidae it is the densest larval layer (Rützler et al. 2003). Many halichondrid parenchymellae (*Halichondria coliata*, *H. bowerbanki*, *H. panicea*, *H. japonica*, *H. coerulea*, *Hymeniacidon sanguinea*, *H. caruncula*, *H. perlevis*, *H. japonica*, *H. heliophila*) have a skeleton; it consists of bundles of oxae parallel to the longer larval axis (Table 3.2). The covering ciliated epithelium may include granular cells (Rützler et al. 2003).

To sum up, the larval structure in the Halichondrida is very diverse (Table 3.2). Moreover, the size and shape of larvae may change considerably in the course of their free swimming life (Wapstra and van Soest 1987; Maldonado and Young 1996; Uriz et al. 1998).

The life span of the larva does not usually exceed 3 days. However, the larvae of *H. panicea* from the Barents Sea may be active for several weeks (Ivanova 1981), which might be explained by the fact that their ciliated cells may phagocyte protists and bacteria (Ivanova and Semyonov 1997). Phagosomes have also been noted in the apical part of the ciliated cells of *H. moorei* larvae (Bergquist and Green 1977).

3.5.4 Metamorphosis

There are few investigations of metamorphosis in the Halichondrida. Live observations were made on *Hymeniacydon sanguinea*, *Scopalina lophyropoda* and *Halichondria magniconulosa* (Uriz 1982a, b; Maldonado and Young 1996). The only two species in which metamorphoses has been studied in detail, both at the ultrastructural level and experimentally, are *Halichondria moorei* and *Ulosa* sp. (Bergquist and Green 1977; Evans 1977; Bergquist and Glasgow 1986).

The larvae usually settle anterior pole first and attach to the substrate by means of surface glycocalyx and cell-substrate adhesion (Bergquist and Green 1977; Evans 1977). Immediately after the settlement the larva disintegrates. The archaeocytes crawl onto the surface, while the ciliated cells migrate inside. In the surface zone archaeocytes differentiate into exopinacocytes and secrete a covering mucous layer, which covers the metamorphosing larva throughout the morphogenesis (Bergquist and Green 1977). Collencytes, migrating into the basal part, differentiate into basopinacocytes and secrete a collagen adhesive plate. Archaeocytes that remain inside the settled larva secrete layers of collagen fibres. Then they elongate along them and transform into endopinacocytes lining the canals of the aquiferous system. Collencytes concentrate along the exopinacoderm and secrete a subdermal collagen layer.

Ciliated cells of the halichondrid larvae are usually phagocyted by large internal archaeocytes (Bergquist and Green 1977; Bergquist and Glasgow 1986) and so do not participate in the formation of the new sponge. However, in *H. panicea* from the Barents Sea some of the ciliated cells are not phagocyted during metamorphosis and are involved in further developmental processes (L.V. Ivanova 2000, personal communication). The larval archaeocytes differentiate into choanoblasts (Fig. 3.22). Before the sponge starts functioning, it has several choanocyte chambers connected with canals, i.e. it is leuconoid.

Terminal differentiation of ciliated cells of the larvae of *Halichondria moorei* and *Ulosa* sp. was supported in experiments on their aggregative properties (Bergquist and Glasgow 1986). After dissociation of the larva, the cells were fractioned in percoll gradient. The ciliated cells, contrary to archaeocytes and collencytes, could not form conglomerates.

3.5.5 Asexual Reproduction

In *Axinella damicornis* buds are formed as outgrowths of the ectosome in the marginal part of the sponge (Boury-Esnault 1970). *A. damicornis* buds lack skeleton. A developing bud comprises several cell types: exopinacocytes cover it, endopinacocytes separate it from the parent sponge, collencytes are scattered throughout it, archaeocytes and spherulous cells make up the central mass. Their cell composition is similar to that of the parent sponge ectosome with one exception: the dominant cells in buds are archaeocytes.

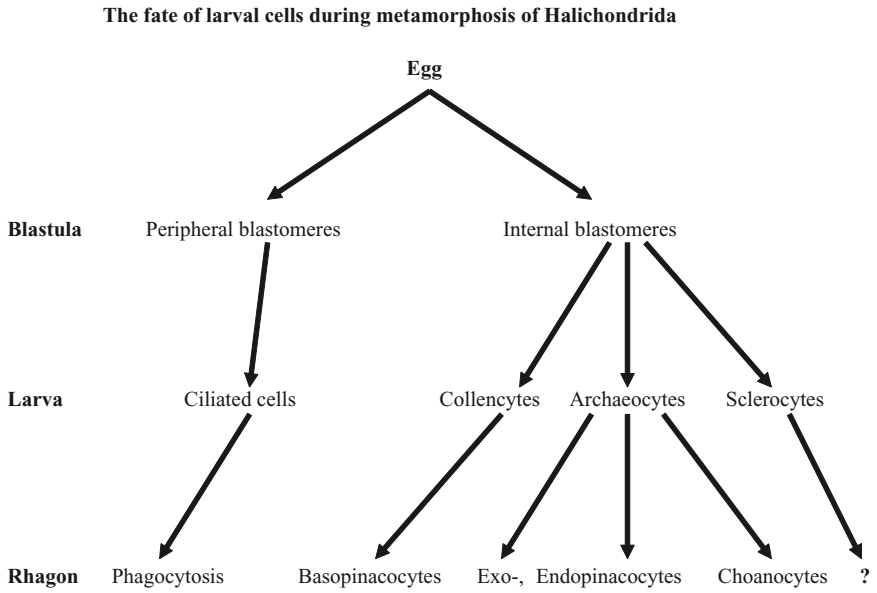

Fig. 3.22 The fate of larval cells during metamorphosis of Halichondrida

3.6 The Order Halisarcida Bergquist, 1996

Sponges from the order Halisarcida are small and encrusting (colour plate VII). The aquiferous system is, in principle, leuconoid, but inclined to the syconoid type: there are big tubular branching choanocyte chambers with broad apopyles (colour plate VII b). The weakly developed skeleton consists only of fibrillar collagen. Halisarcids have various secretory cells, the most numerous being fuchsinophilic (eosinophilic) globular cells with a unique structure. Biochemically, the order is characterized by lack of terpenoid metabolites (Bergquist and Wells 1983). All halisarcids are ovoviviparous. The larva – disphaerula or, rarely, parenchymella – is completely ciliated; its histological structure is simple and its cell diversity is low. Asexual reproduction has not been described.

The order comprises a single family Halisarcidae Schmidt 1862 and a single genus *Halisarca* Dujardin 1838. Its representatives are marine sponges and have a worldwide distribution.

For a long time the genus *Halisarca* resided in the order Dendroceratida, though its difference from dendroceratids and other non-spicular sponges was repeatedly emphasized (Lévi 1956; Bergquist et al. 1979; Bergquist and Wells 1983; Bergquist 1980, 1996; Vacelet and Donadey 1987; Vacelet et al. 1989; Ereskovsky 2000; Ereskovsky and Gonobobleva 2000; Ereskovsky 2002, 2004, 2005; Gonobobleva and Ereskovsky 2004a, b). Since halisarcids lack spicules, cytological and embryonic development characters are especially important for their taxonomy.

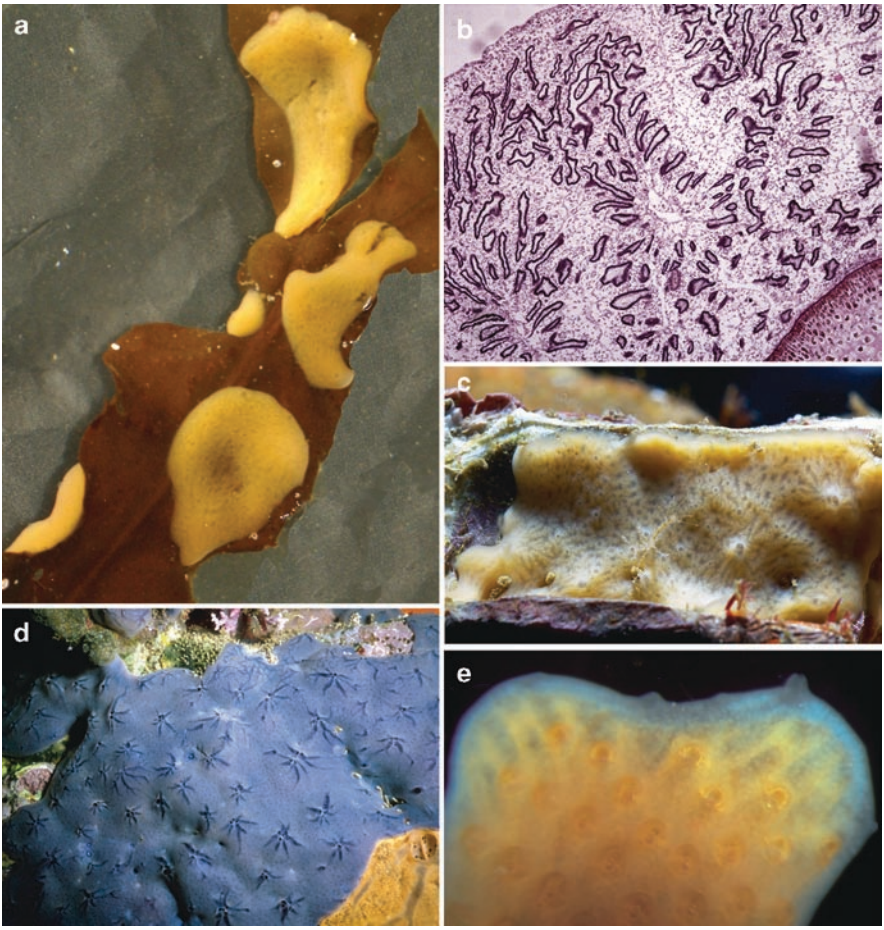

Plate VII Order Halisarcida Bergquist 1996 (**a**) *Halisarca dujardini* Johnston 1842, White Sea (Photo of A. V. Ereskovsky). (**b**) Histological micrograph of *H. dujardini* (Photo of A. V. Ereskovsky). (**c**) *H. dujardini* Bering Sea (Courtesy of K. Saramjan). (**d**) *Halisarca caerulea* Vacelet and Donadey 1987, Caribbean Sea (Courtesy of J. Vacelet). (**e**) *Halisarca* sp. Mediterranean Sea (Courtesy of J. Harmelin)

For instance, *Halisarca ectofibrosa* (Vacelet et al. 1976), *H. caerula* (Vacelet and Donadey 1987) and *Halisarca korotkovae* (Ereskovsky 2007a) were described on the basis of cytological features, and *H. metschnikovi* (Lévi 1953, 1956) and *H. nahantensis* (Chen 1976), on the basis of differences in sexual reproduction in combination with ecological features.

We investigated the type species of the order, *Halisarca dujardini* Johnston 1842. These sponges are pancake-shaped, cushion-shaped or encrusting, with a thickness of 2–6 mm and a diameter of 3–30 mm. The sponge is soft, elastic, smooth and slippery to touch. The osculum or several oscula are situated on top of

low oscular tubes. The colour varies from milky (young individuals) to beige and brown (Bergquist 1996; Ereskovsky 1993).

3.6.1 Gametogenesis

Spermatogenesis in the Halisarcida is almost unstudied. Light microscopic observations of male gametes development made by Lévi (1956) and Chen (1976) yield little information. Male gametes derive from choanocytes released into the lumen of choanocyte chambers. The latter become spermatocystes, where synchronous spermatogenesis occurs (Fig. 3.23).

Oogenesis, on the contrary, was studied rather thoroughly (Lévi 1956; Korotkova and Apalkova 1975; Korotkova and Aizenshtadt 1976; Aisenstadt and Korotkova 1976). Female gametes were shown to develop by direct transformation of choanocytes (Korotkova and Aizenshtadt 1976). At the pre-meiotic phase the oocyte has amoeboid motility and actively migrates in mesohyl. Vitellogenesis is accompanied both by the phagocytosis of somatic mesohylar cells and their fragments and by the endogenic synthesis. There are no specialized nurturing cells. The oocyte also captures symbiotic bacteria, some of which are lyzed and some retained intact in special vacuoles (Ereskovsky et al. 2005). At the end of the vitellogenic phase, the oocyte stops migrating and becomes oval; a follicle is formed around it.

Maturation divisions were studied in the oocytes of *H. metschnikovi* (Lévi 1956) and *H. dujardini* (Korotkova and Apalkova 1975). Prior to divisions, a rounded nucleus with a nucleolus is situated in the centre of the oocyte and yolk granules are evenly distributed in the cytoplasm (Fig. 3.24a). The prophase-I nucleus shifts to the

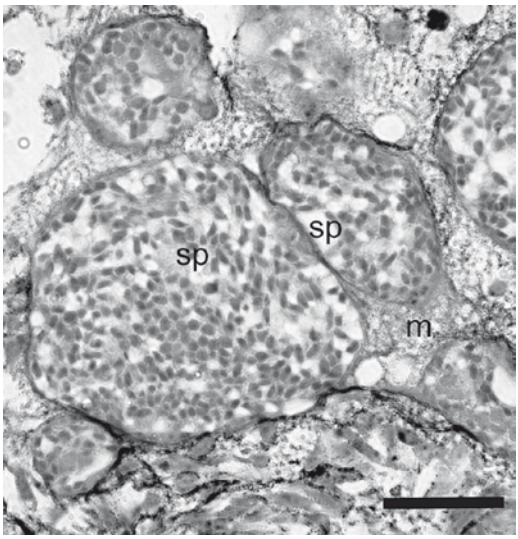

Fig. 3.23 Semi-thin micrograph of a spermatocystes (*sp*) of *Halisarca dujardini*. *m* mesohyl. Scale bar 25 μm

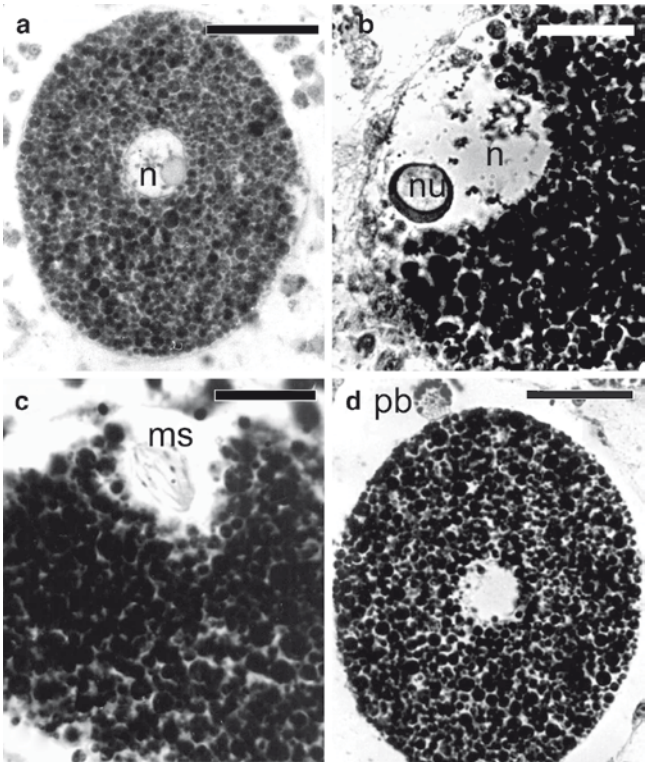

Fig. 3.24 Light micrographs of *Halisarca dujardini* oocytes during the first meiotic division. (**a**) Interphase oocyte before division. (**b**) Nucleus of an oocyte at the later stage of prophase-I. (**c**) Nucleus of an oocyte during the metaphase of the first meiotic division. (**d**) Egg and polar body. *ms* mitotic spindle, *n* nucleus, *nu* nucleolus, *pb* polar body. Scale bars (**a, d**) 50 μm, (**b, c**) 20 μm

periphery and enlarges (Fig. 3.24b). In metaphase-I chromosomes begin to condense, the nuclear envelope and the nucleolus disappear, and the division spindle of the first maturation division is formed (Fig. 3.24c). The spindle is at first oblique to the oocyte surface and then (from metaphase I to telophase I) perpendicular to it.

After the first maturation division, a large space is formed between the oocyte surface and the follicle, and the polar body gets into this space (Fig. 3.24d). The polar body is rounded, and its cytoplasm is poor in yolk granules (Fig. 3.24d). Its further fate is unclear.

3.6.2 Follicle

At the end of vitellogenesis and before the maturation divisions, the oocyte is surrounded by a temporary follicle, where embryos develop (Ereskovsky and Gonobobleva 2000). Each follicle consists of pinacocyte-like cells, which are

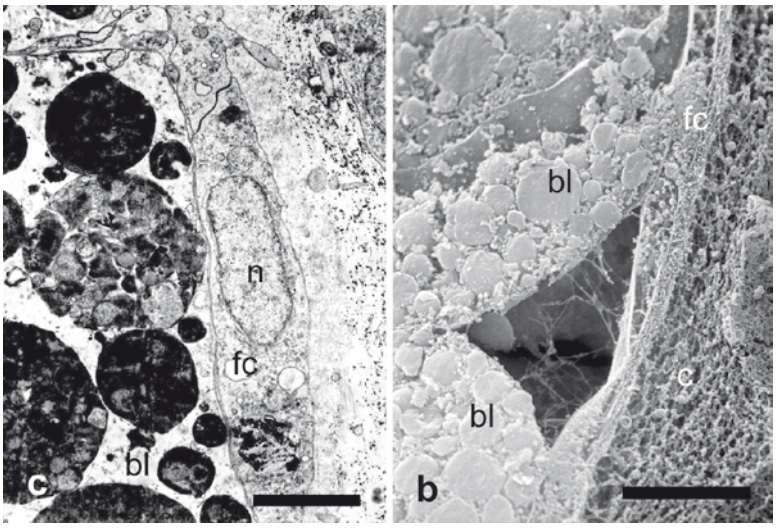

Fig. 3.25 TEM (**a**) and SEM (**b**) of a wall of a follicle in cleaving embryos of *Halisarca dujardini*. *bl* blastomere, *fc* follicular cell, *c* collagen, *n* nucleus. Scale bars (**a**) 1.5 μm, (**b**) 5 μm

dedifferentiated choanocytes (Korotkova and Apalkova 1975), and an external layer of collagen fibres parallel to the cell surface (Fig. 3.25). Similar follicles were described in *H. dujardini* from the Atlantic coast of France and the USA and in *H. nahantensis* (Lévi 1956; Chen 1976).

3.6.3 Embryonic Development

Halisarcida embryogenesis has been studied for over a century (Giard 1873; Barrois 1876; Schulze 1877; Metschnikoff 1879; Lévi 1956; Chen 1976; Korotkova and Ermolina 1982, 1986; Korotkova and Ereskovsky 1984; Sizova and Ereskovsky 1997; Ereskovsky and Gonobobleva 2000; Ereskovsky 2002; Gonobobleva and Ereskovsky 2004a; Gonobobleva 2007) and for most of that time they were regarded as one of the most primitive demosponge groups (Lévi 1956; Bergquist et al. 1979; Bergquist 1980; Korotkova 1981b; Ereskovsky and Gonobobleva 2000). However, recent molecular phylogenetic studies disprove this view. Halisarcids are now included in the clade Myxospongiae, which also comprises the Chondrosida and the Verongida with addition of *Hexadella* (Borchiellini et al. 2004a; Lavrov et al. 2008).

Cleavage in *Halisarca* is total and equal (Giard 1873). The first cleavage division in *H. dujardini* is meridional (Lévi 1956; Korotkova and Apalkova 1975; Korotkova and Ereskovsky 1984), cleavage furrows are bipolar (Fig. 3.26a).

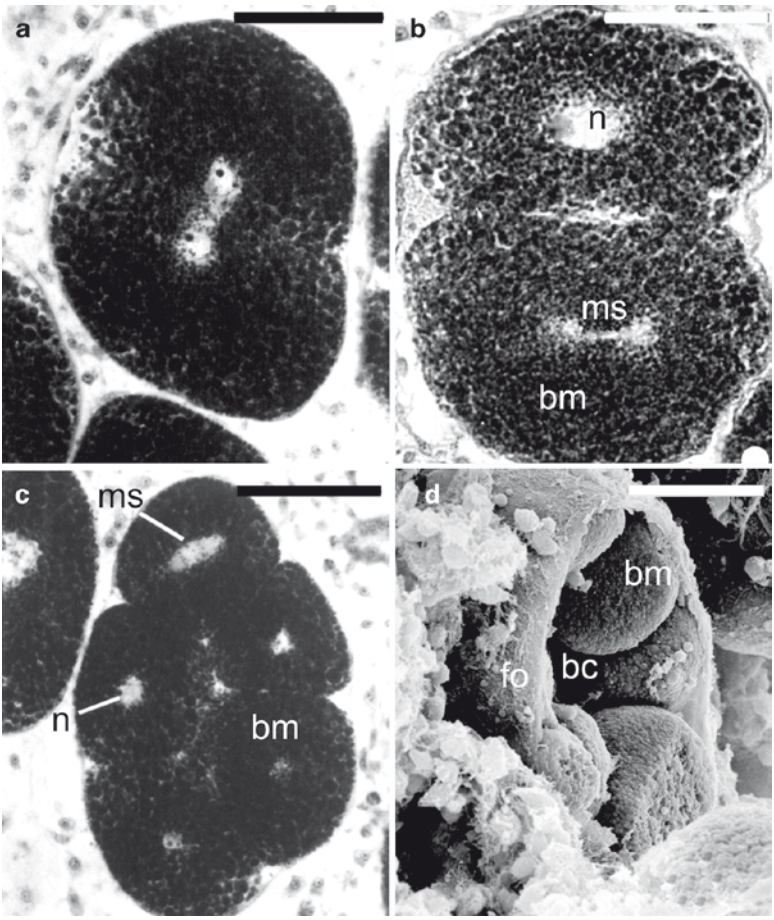

Fig. 3.26 Early cleavage of *Halisarca dujardini*. (**a**) Embryos during the first division. (**b**) Two-blastomere stage and beginning of the third cleavage division. (**c**) Eight-cell embryo at the beginning of the fourth cleavage. (**d**) Early blastula. *bl* blastomere, *bc* blastocoel, *fo* follicle, *ms* mitotic spindle, *n* nucleus. Scale bars (**a**–**b**) 50 μm, (**d**) 40 μm

The second one is often asynchronous (Fig. 3.26b). Mutual position of cleavage spindles varies considerably: from almost parallel to perpendicular. The third and all subsequent cleavage divisions are asynchronous (Fig. 3.26c). Position of the cleavage spindle varies in different embryos. Eight-cell embryo is rounded or slightly oval in cross-section; contrary to observations of Lévi (1956), it is never lamella-shaped (Fig. 3.26d). Starting from the fourth cycle, mitotic spindles are parallel to the embryo's surface, so that cleavage furrows pass perpendicular to the surface. A single-layer equal blastula with a small cavity is formed (Fig. 3.26e, f). The embryo may remain a blastula until the pre-larva stage (Korotkova and Ereskovsky 1984; Ereskovsky and Gonobobleva 2000;

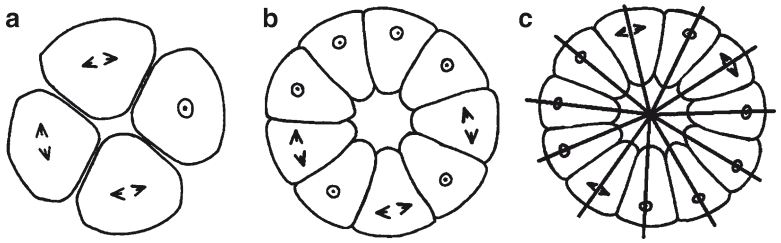

Fig. 3.27 Scheme of polyaxial cleavage in *Halisarca.* (**a**) Four blastomeres. (**b**) Early blastula. (**c**) Blastula. Straight line indicated the axis of the embryo

Ereskovsky 2002; Gonobobleva and Ereskovsky 2004a). *H. nahantensis* has a similar cleavage pattern (Chen 1976).

The cleaving embryo is not polarized (Fig. 3.26e, f). The cleavage cavity (blastocoel) is clearly delimited by the basal parts of blastomeres (Fig. 3.26d). Polarization of blastomeres starts after the fourth or the fifth cleavage cycle and is mostly expressed in the shift of the nucleus to distal position. At the stage of 48–64 blastomeres, ciliar basal apparatus start to develop on the apical cell surface (Gonobobleva 2007): an apical pocket with a finger-shaped membrane projection in the centre is formed.

The cleavage pattern in *Halisarca* was attributed to a special type, *polyaxial cleavage* (Fig. 3.27) (Ereskovsky 2002). The only other metazoans with polyaxial cleavage are the Calcinea (see Section 1.2).

Blastomeres are closely opposed to each other from the first cleavage cycles, but there are no specialized contacts between them (Fig. 3.28a). Neighboring blastomeres contact in their basal parts with filopodia (Fig. 3.28b), which gradually become more numerous, elongate and may form an elaborate network. Their presence means that *H. dujardini* has an adhesive complex of cell–cell junctions. A similar complex was described in cleaving embryos of Homoscleromorpha (Ereskovsky and Boury-Esnault 2002).

Early blastomeres of *H. dujardini* have few mitochondria; the Golgi apparatus and the RER are weakly developed. This is probably associated with their low metabolic activity. At the stage of about 16–24 cells, the number of mitochondria increases drastically and the Golgi apparatus develops (Sizova and Ereskovsky 1997). Nucleoli-like bodies in the nuclei of the early blastomeres in *H. dujardini* are not true transcriptionally active nucleoli, but RNA-containing pro-nucleolar bodies (Sizova and Ereskovsky 1997). Similar “nucleoli” in blastomeres of other sponges might also turn out to be pronuclear bodies.

In embryos at different developmental stages, spiral Gram-positive bacteria reside near the follicle wall, both in the collagen fibrillar layer and inside the follicle cells (Fig. 3.28c). Bacteria are also found below the follicle envelope, between the blastomeres and inside them (Fig. 3.28c, d). Bacteria in the blastomeres are always enclosed in vacuoles (Fig. 3.28d). From the stage of eight blastomeres, bacteria concentrate in the cleavage cavity (Ereskovsky et al. 2005).

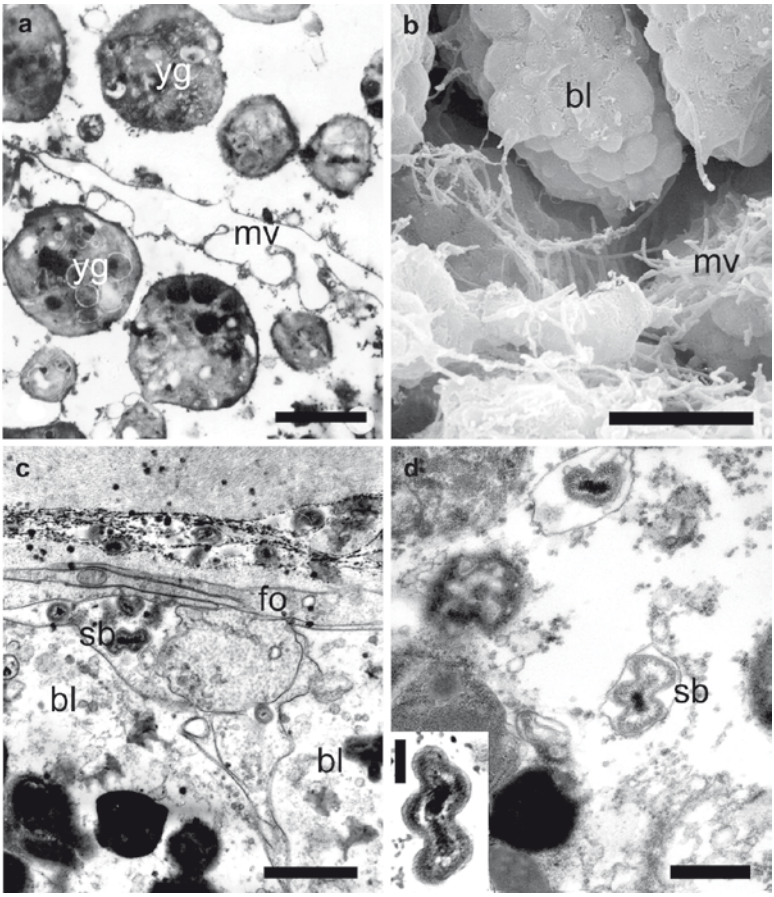

Fig. 3.28 Ultrastructural peculiarity of *Halisarca* blastula. (**a**) Boundary between the cells of early blastula. (**b**) The fragment of a blastocoel of a blastula with microfilopodia net (*fl*). (**c**) Peripheral part of a blastula with follicular cells (*fo*) and symbiotic bacteria (*sb*). (**d**) Cytoplasm of late blastula's blastomere. *bl* blastomeres, *fo* follicular cell, *yg* yolk granules, *sb* symbiotic bacteria. Scale bars (**a**) 1 μm, (**b**) 6 μm, (**c, d**) 0.1 μm, *inset* 8 nm

3.6.3.1 Larval Morphogenesis

In the course of blastulation, the area of lateral contacts between the blastomeres increases. Within the same parent sponge, embryos may become compact at different stages of early embryogenesis, after four to six cleavage divisions (16–64 cells) (Gonobobleva and Ereskovsky 2004a). Two types of blastula are formed: hollow blastulae consisting of a single layer of polarized cells (Fig. 3.29a) and blastulae with the cells in the cavity (Fig. 3.29b). These cells are the result of cell-by-cell apolar migration to the inside the early blastula; they become amoeboid in the blastula cavity.

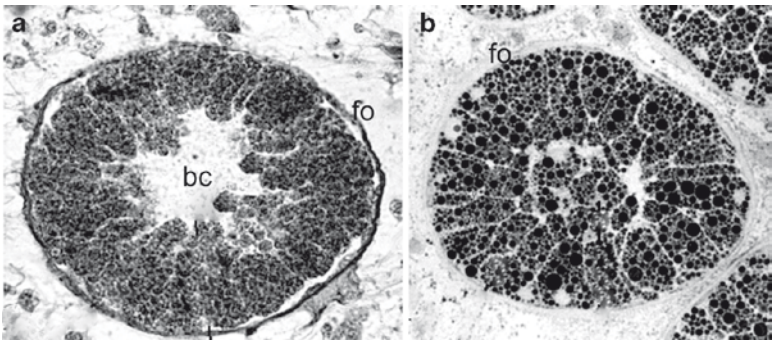

Fig. 3.29 Two blastulae types at the middle stages of embryogenesis (about 200 cells). (**a**) Coeloblastula and (**b**) morula; semi-thin micrographs. *bc* blastocoel, *fo* follicle (From Gonobobleva and Ereskovsky 2004a, Boll. Mus. Ist. Biol. Univ. Genova, vol. 68, pp. 352, Fig. 2a, b, reproduced by permission of Publisher House of University of Genova). Scale bars 50 μm

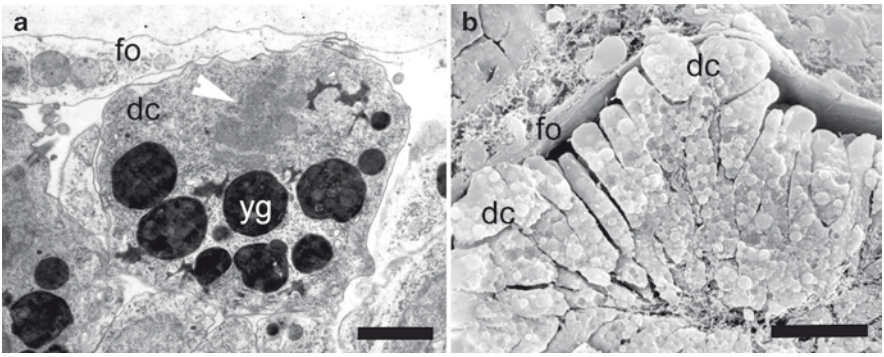

Fig. 3.30 TEM (**a**) and SEM (**b**) micrographs of divided surface ciliated cells of *Halisarca dujardini* embryos. *dc* divided cells, *fo* follicular cells, *yg* yolk granules, *arrowhead* metaphase plate. Scale bars (**a**) 2 μm, (**b**) 20 μm

In some embryos, migration of external cells into the cavity may occur throughout the embryonic development and even in the free-swimming larva (Ereskovsky and Gonobobleva 2000). This means that the pool of internal cells may be constantly replenished by dedifferentiated external cells of the embryo and the larva. Lévi (1956) described unipolar immigration in *H. dujardini*, its direction determining the future posterior larval pole. However, he was the only one to observe this morphogenesis in this species. In some hollow blastulae, only a few cells migrate into the blastocoel and such embryos develop into coeloblastula larva.

The surface ciliated cells proceed to proliferate actively. Prior to division, the cell becomes rounded and shifts to the surface of the pre-larva, where it divides. The plane of division is perpendicular to the pre-larva surface (Fig. 3.30). After the division is completed, the daughter cells elongate and make up a ciliated layer. This cell division

is characteristic of some columnar epithelia of the Metazoa. For instance, during the development of the neural tube in vertebrates, the division occurs after nucleus migration and concentration of cytoplasm at the epithelium surface (Sauer 1935). In sponges, it has been described, e.g., in *Ephydatia fluviatilis* (Brien and Meewis 1938), *Haliclona limbata* (Meewis 1939), *Spongilla lacustris* (Saller and Weissenfels 1985), some Homoscleromorpha (Boury-Esnault et al. 2003).

The anterior–posterior polarity of *H. dujardini* larva is expressed during differentiation of the covering ciliated cells. Division rates in the cells of the future posterior pole are lower than those in anterio-lateral cells and, as a result, they look like blastomeres for a longer time.

While the larval surface cells proliferate actively, the larval surface develops numerous folds and protrusions, which must be associated with an increase in the surface area (Lévi 1956; Korotkova and Ermolina 1982, 1986; Ereskovsky and Gonobobleva 2000; Gonobobleva and Ereskovsky 2004a). A pre-larva with folds has also been described in *H. nahantensis* (Chen 1976). The stage of folded pre-larvae is characteristic of many ovoviviparous Demospongiae from different orders (see Chapter 3): Halichondrida, Dictyoceratida, Poecilosclerida, Haplosclerida, and also Homoscleromorpha (see Chapter 4).

At the same time granular eosinophilic (fuchsinophilic) amoebocytes migrate from the mesohyl of the parent sponge across the follicle walls into the embryo. Proliferation gradually stops and the cells acquire the shape and size characteristic of the fully formed larva. Before the final smoothing of the surface, some pre-larvae still have one deep *invagination* of the ciliated layer, whose opening soon closes (Fig. 3.31). Importantly, invagination of the ciliated layer proceeds perpendicular

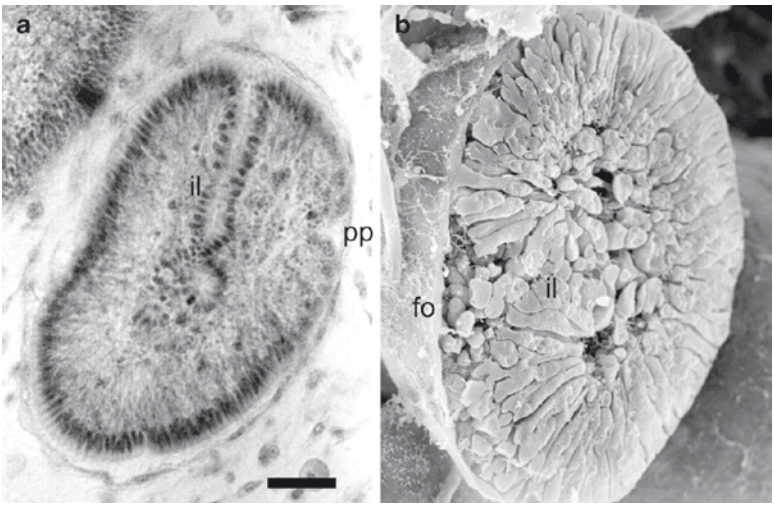

Fig. 3.31 Invagination of ciliated cells layer of pre-larva disphaerula of *Halisarca dujardini*. (**a**) Light microscopy of an initial stage. (**b**) SEM of a closing of a cavity. *pp* posterior pole of a pre-larva, *il* invaginated layer. Scale bars (**a**, **b**) 20 μm

or at an angle to the anterior–posterior axis of the larva and is not confined to any of the poles (Fig. 3.31).

Invagination results in a single-layer closed structure formed by ciliated cells, with their cilia directed inwards. The blastula cavity (blastocoel) is reduced to narrow lacunae between the layers of internal and surface ciliated cells.

Thus, the internal spherical chamber of the larva is the derivative of the surface ciliated layer. Invagination mechanism of its formation is associated with smoothing of the larval surface and is unique for Porifera. Before the larva leaves the parent sponge, the internal chamber becomes smaller and spherical, because some of the cells of the internal sphere immigrate into the remains of the blastocoel. This process is evidenced by the presence of transition cells outside epithelia. These cells are rounded and have a cilium. We call these larvae *disphaerulae* (Fig. 3.32; 3.33b) (Ereskovsky and Gonobobleva 2000).

Internal ciliated chambers were described in the larvae of *H. metschnikovi* (Lévi 1956), *H. dujardini* and *H. nahantensis* (Lévi 1956, 1963; Chen 1976; Korotkova and Ermolina 1982), but not paid any special attention to. They were considered either as temporary structures formed by migration of single cells inside the larva (Lévi 1956, 1963) or as transverse sections of deep invaginations of the external layer (Korotkova and Ermolina 1982).

Larval flagellated chambers are characteristic of the parenchymellae of freshwater sponges (Weissenfels 1989; Ereskovsky 1999) and hoplitomella larvae of astrophorids (Garrone 1974; Vacelet 1999). However, small internal chambers of larvae from these orders are absolutely different from ciliated chamber of *Halisarca* disphaerulae, since they form by differentiation of choanoblasts and are in fact larval choanocyte chambers.

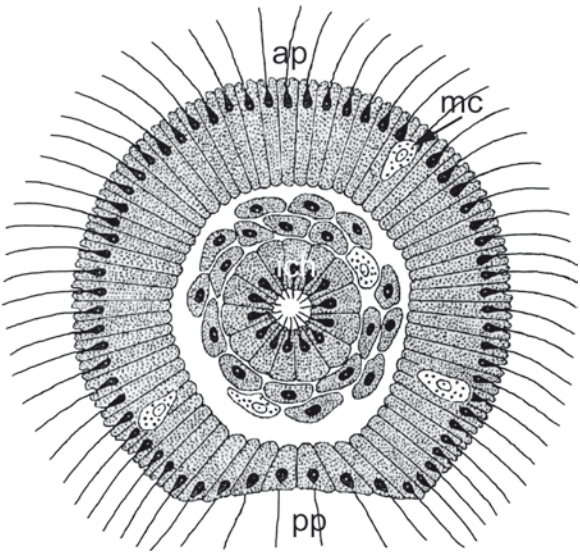

Fig. 3.32 Schematic drawing of the larva disphaerula. *ap* anterior pole, *ich* internal chamber, *mc* maternal cell, *pp* posterior pole

Thus, *H. dujardini* development is characterized by a considerable variability of embryogenesis, resulting in formation of larvae of various types. Two processes underlie the formation of different larval morphotypes: migration of single cells from the surface cell layer (parenchymella) and invagination of cell layer with subsequent closing (disphaerula). This means that larval morphotype in *H. dujardini* does not depend on environmental factors.

Larval polymorphism – both in a population and in a single sponge – is one of the most striking features of *H. dujardini* development. Coeloblastulae and parenchymellae differ from disphaerulae by lack of the internal ciliated chamber. Internal cell of coeloblastulae, if present, are few and situated along the basal part of the surface cells (Fig. 3.33c). Parenchymellae have many densely packed internal cells (Fig. 3.33d). Therefore, the larval morphotype is determined, in the first place, by the number and arrangement of internal cells.

The above larval types are not different ontogenetic stages, they can be found simultaneously within one parent sponge. After the larva has started to develop in

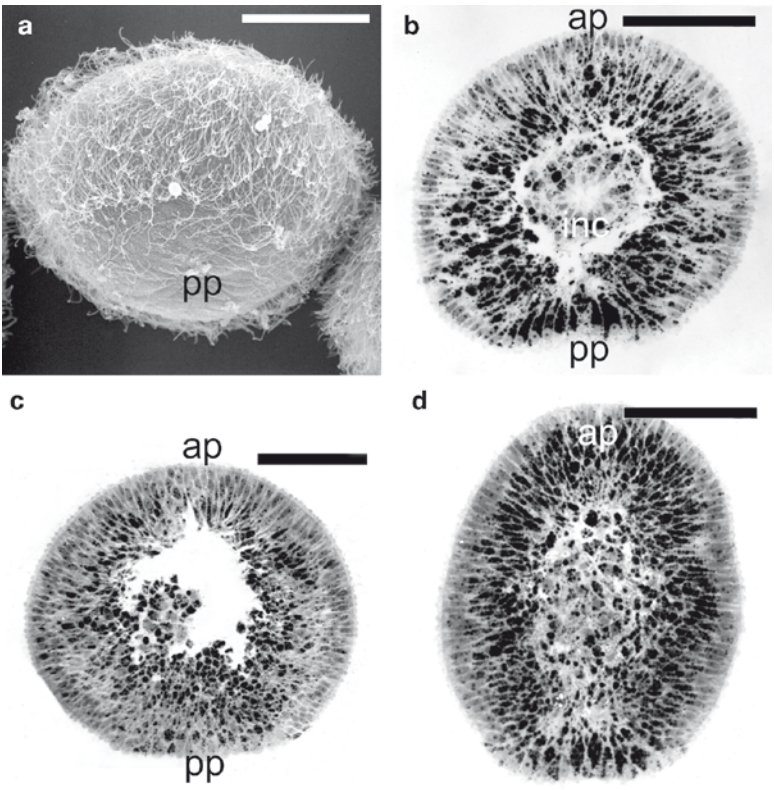

Fig. 3.33 Larval types of *Halisarca dujardini*. (**a**) SEM of external morphology of a larva. (**b**) Light microscopy of disphaerula. (**c**) Light microscopy of coeloblastula. (**d**) Light microscopy of parenchymella. *ap* anterior pole, *ich* internal chamber, *pp* posterior pole. Scale bars (**a**–**d**) 20 μm

one of the three ways opened to it, it retains its organization until the beginning of metamorphosis (Gonobobleva and Ereskovsky 2004b). Ultrastructural cell features of these three larval morphotypes are identical.

Variability of external shape of larvae in sponges of the same species is well known (Wapstra and van Soest 1987; Ivanova 1997a). However, variability of the internal larval structure has been described only for the Spongillidae and is associated with acceleration of development (Ivanova 1997a).

Finally, the walls of embryonic capsules, containing the formed larvae, fuse with the wall of exhalant canals and the larvae are released via the osculum.

3.6.4 Larvae

H. dujardini larvae swim in counter-clockwise spirals, at the same time rotating clockwise along the anterior–posterior axis. The larvae are milk white, oval or slightly flattened in anterior–posterior direction, with a diameter of 128–130 μm (Fig. 3.33a). The larva is evenly ciliated, with the exception of the posterior pole, which is ciliated less densely (Fig. 3.33a). There was an erroneous opinion that the posterior pole of *H. dujardini* larvae is not ciliated at all (Lévi 1956; Bergquist et al. 1979; Bergquist 1980).

Disphaerulae and parenchymellae contain internal cells in the cavity: nucleolated amoebocytes and dedifferentiated ciliated cells. Internal cells of *H. dujardini* larvae do not form a continuous layer underlying the covering epithelium, as they do in almost all demosponges larvae (Maldonado and Bergquist 2002). Their number varies greatly in larvae of different morphotypes of *H. dujardini*. The larva also includes granular eosinophilic cells of the parent origin, which may be situated in different parts of the larva.

A characteristic feature of the ciliated epithelium of all larval morphotypes of *Halisarca*, setting it apart from the ciliated epithelia larvae of the other Demospongiae larvae, is that it is monostratified, i.e. the nuclei of the ciliated cells are situated at the same level (Lévi 1956; Chen 1976; Korotkova and Ermolina 1982; Ereskovsky and Gonobobleva 2000; Gonobobleva and Ereskovsky 2004a, b). The only other case of monostratified epithelium in demosponges is the coeloblastulae of *Chondrilla australiensis* (Usher and Ereskovsky 2005). Besides, monostratified cell layer is characteristic of the calcinean calciblastulae (Amano and Hori 2001; Ereskovsky and Willenz 2008), calcaronean amphiblastulae (Franzen 1988; Amano and Hori 1992; Gallissian and Vacelet 1992) and the trichimellae of hexactinellid *Oopsacas minuta* (Boury-Esnault et al. 1999; Leys et al. 2006).

Surface ciliated cells of the anterior hemisphere are almost cylindrical and have a distinct apical–basal polarity (Fig. 3.34a). At the apical pole, directly above the nucleus, there is the kinetosome and an accessory centriole positioned at an acute angle to it (Gonobobleva and Ereskovsky 2004b; Gonobobleva 2007) (Fig. 3.34b). Two fine fibrillar rootlets pass from the kinetosome inside the cell. A weakly expressed horizontal bundle of microtubules also starts from the kinetosome and

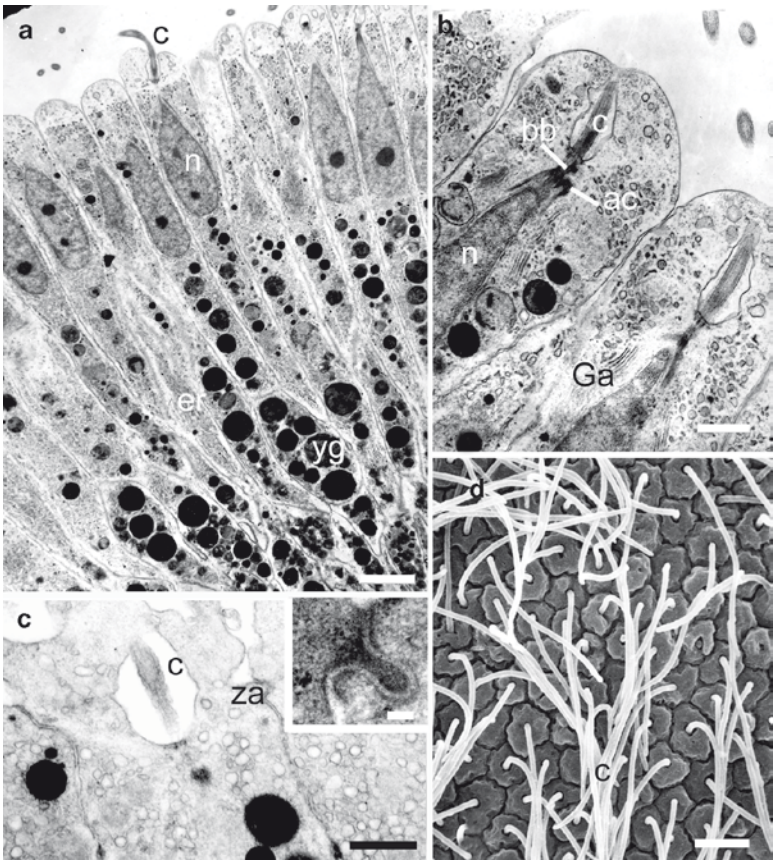

Fig. 3.34 Anterio-lateral ciliated cells of *Halisarca dujardini* larva (**a**–**c**) TEM, (**d**) SEM. (**a**) Longitudinal section of the ciliated cell with the cilium (*c*), nucleus (*n*), yolk granules (*yg*) and endoplasmic reticulum (*er*). (**b**) Apical part of cell with the nucleus (*n*), Golgi apparatus (*Ga*), accessory centriole (*ac*), cilium (*c*), basal body (*bb*). (**c**) Apical part of ciliated cells, fixed with alcian blue with a zonula adhaerens (*za*); *inset* – *zonula adhaerens*. (**d**) Surface of ciliated cells (*Inset* – From Gonobobleva and Ereskovsky 2004b, Bull. Inst. Roy. Sci. Nat. Belg. Biol. vol. 74, pp. 102, Fig. 1b, reproduced by permission of Institut Royal des Sciences Naturelles de Belgique). Scale bars (**a**) 2 μm, (**b**–**d**) 1 μm, *inset* 18 nm

reaches the side wall of the cell. The base of the cilium is in the pocket of the apical cytoplasm (Fig. 3.34a–c). In the apical part of the ciliated larval cells there are specialized cell junctions: *zonula adhaerens* or belt desmosomes (Fig. 3.34c) (Gonobobleva and Ereskovsky 2004b). These cell junctions ensure mechanical contact between cells. Below the kinetosome there is an elongated nucleus with a pointed apical part (Fig. 3.34a), near which there are two Golgi apparatus, parallel to the nuclear membrane. In the middle and the basal part of the cells there are the remains of yolk granules, numerous ribosomes and RER (Fig. 3.33a). Mitochondria with lamellate cristae are found throughout the cell.

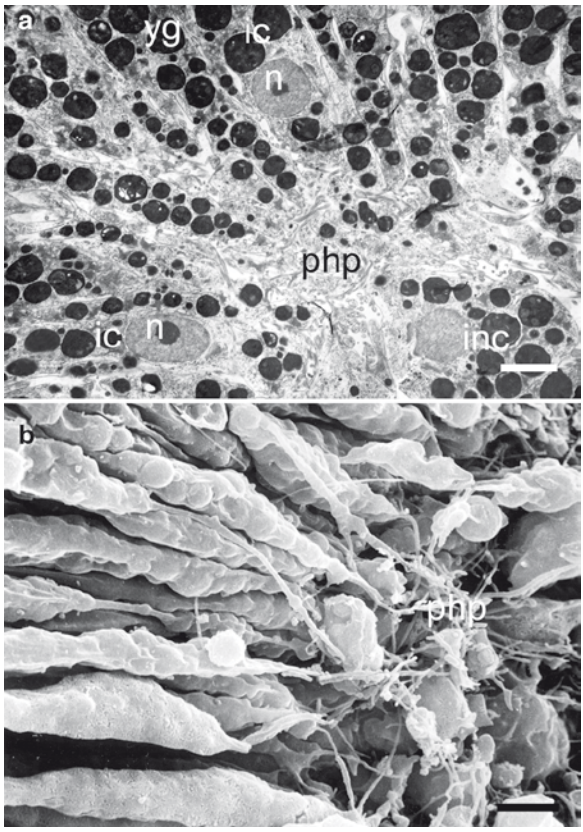

Fig. 3.35 (**a**) TEM and (**b**) SEM of the basal parts of anterior-lateral ciliated larval cells with the phylopodia (*php*) and yolk granules (*yg*). *inc* internal cell, *ic* ingressed ciliated cells with nucleus (*n*). Scale bars (**a, b**) 5 μm

In the basal parts of the cells the membrane forms a complex network of numerous filopodia (Fig. 3.35). Below the layer of the external ciliated cells there are many cells fragments separated from the basal parts of the cells (Fig. 3.35). Between the anterio-lateral ciliated larval cells, spindle-shaped or clavate cells are sometimes found on their way from the ciliated epithelium into the larval cavity.

Transitional ciliated cells are situated between the posterior pole and the lateral walls of the larva. At the longitudinal section there are not more than four to five transitional cells to the right and to the left of the posterior pole cells (Fig. 3.36a). Depending on their position in the transition zone, they may differ in the length of the apical–basal axis and the breadth in the nucleus region.

Ciliated cells of the posterior pole have different shape in different larvae (Gonobobleva and Ereskovsky 2004a). Usually they are trapezoid in cross-section, with breadth larger than length. Yolk granules may be situated in different parts of the cell. The nucleus is rounded with a small apical protrusion, there is a nucleolus. Cytoplasmic components are the same as in anterio-lateral cells. There is no cytoplasmic pocket around the basis of the cilium (Fig. 3.36b).

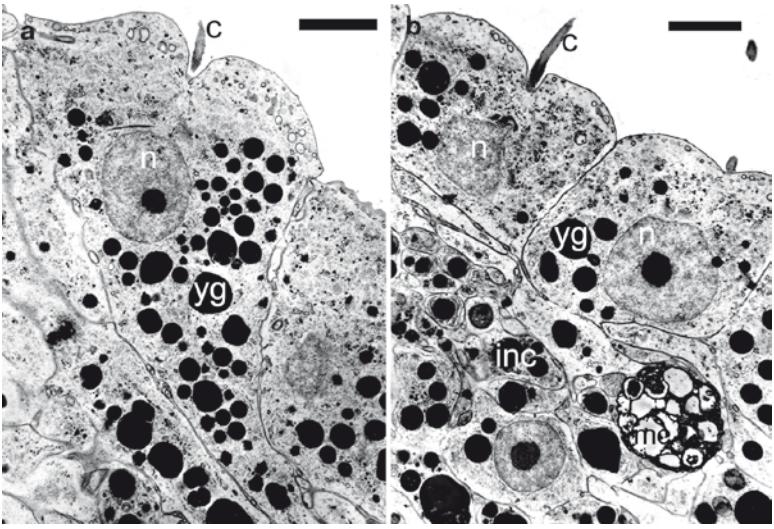

Fig. 3.36 TEM of larval posterior pole. (**a**) Intermedial cells. (**b**) Posterior pole cells. *c* cilium, *inc* internal cells, *mc* maternal cell, *n* nucleus, *yg* yolk granules. Scale bars (**a**, **b**) 2 μm

Ciliated cells of the internal chamber of disphaerula are wedge-shaped, with a broadened basal part, where yolk granules concentrate. The basal cell parts sometime separate. In the apical part there is the nucleus and the cilium, with the base in the cytoplasm pocket. Cilia are directed into a small spherical cavity.

Internal cells. Most internal cells in disphaerulae and parenchymellae of *H. dujardini* are dedifferentiated ciliated cells. There shape and ultrastructure are somewhat different, depending on the degree of dedifferentiation (Fig. 3.37). The axoneme of the cilium is sometimes retained. It may be withdrawn into the cell (Fig. 3.37a).

As in most Demospongiae, larval amoebocytes of *H. dujardini* are motile cells with a rounded nucleus containing a nucleolus, with numerous phagosomes, RER and yolk granules (Fig. 3.37b). At the same time, they are difficult to distinguish from dedifferentiated ciliated cells because of the lack of any specific inclusions. All morphological transitions occur: from newly immigrated epithelial cells with a cilium to typical amoebocytes.

Similarly to other Demospongiae, the main energy source for the lecithotrophic *H. dujardini* larvae are yolk reserves that have not been used during embryogenesis. Phagosomes in the basal parts of the ciliated cells and in internal amoebocytes testify to phagocytic activity of these cells. High level of protein synthesis is indicated by a well-developed RER.

Parent eosinophilic cells**.** These cells penetrate into the developing embryo from the mesohyl of the parent sponge (Korotkova and Ermolina, 1982, 1986; Ereskovsky and Gonobobleva 2000; Krylova et al. 2004). Eosinophilic amoebocytes, with various specific inclusions in the cytoplasm, occur in all parts of the swimming

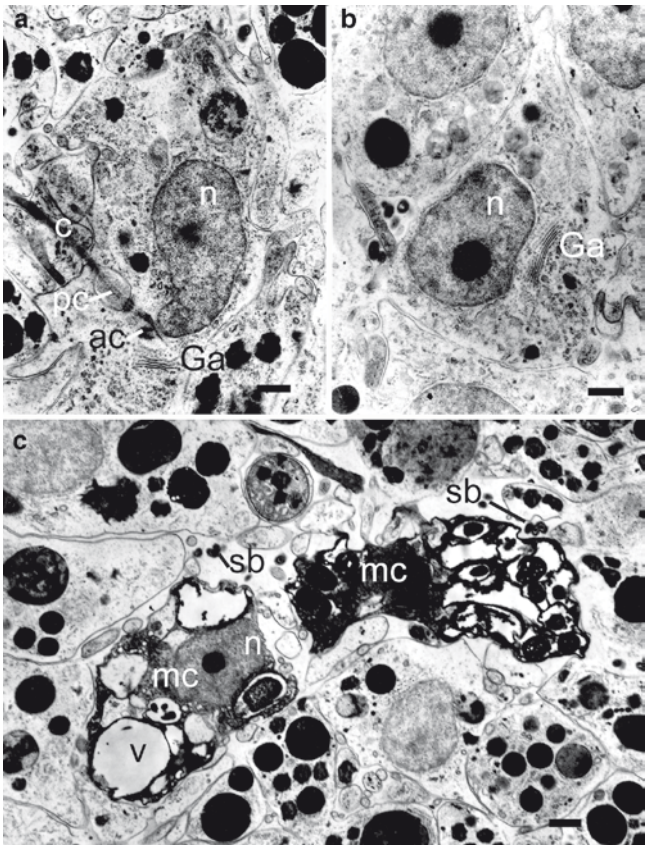

Fig. 3.37 TEM of internal cells of *Halisarca dujardini* larva. (**a**) Dedifferentiated ciliated cells with a cilium (*c*), accessory centriole (*ac*), Golgi apparatus (*Ga*), pocket of the apical cytoplasm (*pc*). (**b**) Amoebocyte. (**c**) Maternal eosinophylous amoebocytes (*mc*). *n* nucleus, *sb* symbiotic bacteria, *v* vacuole. Scale bars (**a**–**c**) 1 μm

larva (Fig. 3.37c). Some of them show signs of morphological degradation. Fragments of degrading cells are sometimes found in phagosomes in the basal parts of ciliated cells.

Parent cells of *H. dujardini* belong to the type of "cells with inclusions" (secretory cells) found in the other Demospongiae. It was suggested that parent cells in *H. dujardini* larvae may be the carriers of biologically active substances (Korotkova and Ermolina 1986). We showed that granules of eosinophilic amoebocytes contain cationic peptides and proteins with a molecular mass of 16–23 kDa. These proteins are active against bacteria *Escherichia coli* and *Listeria monocytogenes* (Krylova et al. 2002, 2004). The substances in the granules of parent eosinophilic cells may play an important role in the antibacterial protection of the larva during free life and metamorphosis. Anyway, the parent eosinophilic cells are not nurturing cells,

as Chen (1976) thought: they are retained in the *H. dujardini* larva until the first stages of metamorphosis. Eosinophilic cells appear to be physiologically active only at the first stages of metamorphosis, since there fragments are found in large numbers in the phagosomes of the ciliated and the amoeboid cells of the pupa (Gonobobleva and Ereskovsky 2004b).

Thus, the cell composition of *H. dujardini* larvae is rather poor: there are three main cell types different in origin and ultrastructure: ciliated (main cell type), amoeboid and parent eosinophilic cells, only the former two being larval cells proper. Some nucleolated amoebocytes are derivatives of the ciliated cells migrating from both the external and the internal layer. Disphaerulae have four groups of ciliated cells: cells of the anterio-lateral surface, cells of the posterior pole, transitional cells and ciliated cells of the internal chamber. Larval ciliated cells contain a specific protein with a mass of 68 kDa (Mukhina et al. 2006).

No fibrillar extracellular matrix has been noted in *H. dujardini* larvae. There are numerous symbiotic spiral Gram-positive bacteria in the intracellular spaces, mostly in the internal part of the larva (Ereskovsky et al. 2005).

3.6.5 Metamorphosis

Preparation to metamorphosis (competence) starts during the free larval life. Behavioural differences emerge: the larvae swim slower, may sink to the bottom and move slowly on the substrate. Near the substrate they proceed to rotate slowly around the axis.

At the ultrastructural level, the shape of surface ciliated cells changes first. Their lateral walls become wavy. On the apical surface of the posterior pole cells numerous lobe-shaped protrusions of the surface membrane are formed. In disphaerulae in the period of competence anarchization of the internal chamber starts (Gonobobleva and Ereskovsky 2004b). Regardless of the larval type (parenchymella, disphaerula, and coeloblastula), the character of metamorphosis and the sequence of developmental processes are the same (Fig. 3.38).

In this state the larvae settle on the substrate. Already on the substrate but before settlement they proceed to rotate slowly around the axis for about 35–40 min. The rotation stops after final attachment. Attachment is a signal to metamorphosis. As in all other sponges studied, *H. dujardini* larvae settle with anterior pole (sometimes the anterio-lateral surface) first. After attachment the larva flattens in anterior–posterior direction and spreads on the substrate as an oval or rounded disc (Figs. 3.38a and 3.39a). If several settled larvae of the same clone contact each other, they may fuse (Fig. 3.38a). There are no special secretory cells in *H. dujardini* larva responsible for attachment. When anterior pole cells contact the substrate, they excrete a mucous substance forming a thin layer between the larva and the substrate.

The surface layer of ciliated cells of the settled larva remains organized for some time. At this stage the number of parent eosinophilic amoebocytes decreases greatly.

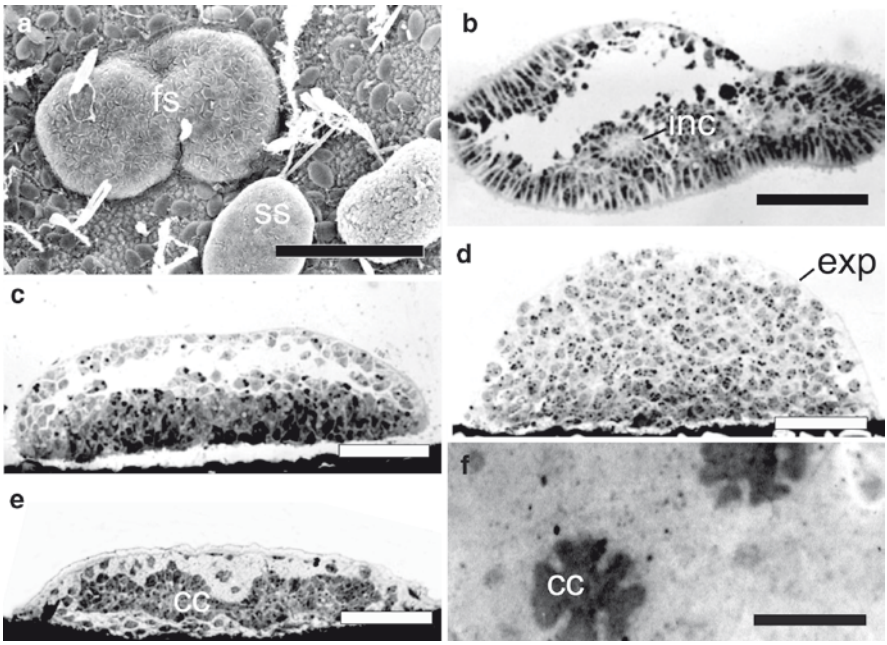

Fig. 3.38 Different stages of larval metamorphosis in *Halisarca dujardini* (**a** – SEM, **b–e** semifine sections). (**a**) Two fused settlers (*fs*) and single settler (*ss*). (**b**) First stages of metamorphosis of a disphaerula with intact internal cavity (*inc*). (**c**) Pupa with disintegrated basal layer. (**d**) Pupa with exopinacoderm layer (*exp*) at the stage of internal cells conglomerate formation. (**e**) Rhagon with choanocyte chamber (*cc*). (**f**) Rhagon *in vivo* with one central lobe-shaped choanocyte chamber (*cc*) (From Gonobobleva and Ereskovsky 2004b, Bull. Inst. R. Sci. Natl. Belg. Biol. vol. 74, pp. 103, Fig. 2, reproduced by permission of l'Institut Royal des Sciences Naturelles de Belgique). Scale bars (**a**) 100 μm, (**b–e**) 50 μm, (**f**) 200 μm

Fig. 3.39 Schematic drawing of the principal stages of *Halisarca dujardini* metamorphosis. (**a**) First stage of metamorphosis just after disphaerula attachment and internal cavity (*inc*) destruction. (**b**) Second stage: the pupa with developing exopinacoderm. (**c**) Third stage: early rhagon with one choanocyte chamber (*cc*), with the exopinacoderm (*exp*), but without the basopinacoderm (From Gonobobleva and Ereskovsky 2004b, Bull. Inst. R. Sci. Nat. Belg. Biol. vol. 74, pp. 104, Fig. 3, reproduced by permission of l'Institut Royal des Sciences Naturelles de Belgique)

In disphaerulae in the 12 h after attachment the internal chamber disintegrates completely into separate cells. About 12 h after settlement, most of anteriolateral ciliated cells move inside the pupa, where they become amoeboid.

The posterior pole cells remain on the surface and differentiate into T-shaped exopinacocytes, characteristic of the adult sponge (Fig. 3.39b). This process starts in the posterior pole region of the larva and spreads to its basis. Cell junctions between the ciliated cells of the posterior pole remain unbroken in the course of transformation into exopinacocytes (Gonobobleva and Ereskovsky 2004b). Their apical part flatten forming polygonal plates, cilia are disassembled (Fig. 3.39a–d). The nucleus-containing cell region submerges inside the pupa. Between the apical plate and the submerged nucleus-containing cell region a thin cytoplasmic bridge remains (Fig. 3.39a–c). The internal part of the pupa is a dense conglomerate of dedifferentiated amoeboid cells. At this stage, basopinacoderm is not yet formed.

The apical parts of the exopinacocytes secrete a mucous substance enveloping the pupa completely like cuticle (Fig. 3.40e). Thus, exopinacoderm and the "cuticle" secreted by it are the first definitive structures formed during the *Halisarca* metamorphosis. This was noted before for *H. dujardini* and *H. metschnikovi* (Lévi 1956). Ciliated cells differentiate in exopinacocytes also in the coeloblastulae of *Polymastia robusta*, *Ascandra*, *Clathrina* (Meewis 1938; Borojevic 1969; Amano and Hori 2001), in cinctoblastula of the Homoscleromorpha (Ereskovsky et al. 2007b), and in some Spongillidae (Ivanova 1997b). At the same time, in demosponges parenchymellae exopinacoderm usually develops from internal cells: archaeocytes and collencytes (Borojevic 1970; Fell 1989).

Further metamorphosis is associated with cell differentiation and aquiferous system formation. The pupa increases slightly in size. Most cells of the internal conglomerate differentiate into choanocytes. Several spherical cell agglomerates are formed (Fig. 3.41a), with a small cavity in the centre of each. At this stage the cells acquire apical–basal polarity; later they become wedge-shaped. In the apical part, directed into the cavity, formation of the flagellum and the microvilli starts (Fig. 3.41b, c). The flagellum usually emerged before the microvilli collar. In the basal cell parts short lobopodia are formed (Fig. 3.41b, c). New cells may be incorporated into choanoblast chambers. Neighbouring chambers gradually merge and a single large central lobe-shaped choanocyte chamber of the rhagon is formed (Figs. 3.28e, f; 3.39c).

Between the exopinacoderm and the choanocyte chamber there are a few cells with projections, secreting extracellular matrix. A layer of collagen fibres, characteristic of the definitive sponge, develops in the surface layer of the pupa. Organized accumulations of flattened cells (endopinacocytes) appear above the choanocyte chamber and later form the aquiferous system canals. Basopinacoderm is gradually being formed in the basal part of the pupa. Metamorphosis finishes with the oscular tube formation and the beginning of functioning of the rhagon aquiferous system.

At early metamorphosis stages ciliated cells of *H. dujardini* larvae possess phagocyting activity. Phagocytosis is carried out by the basal cell parts. The most usual contents of the phagosomes are basal fragments of ciliated cells, which separate during disintegration of the larval structures, as well as fragments of parent eosinophilic cells. Internal larval cells also participate in phagocytosis.

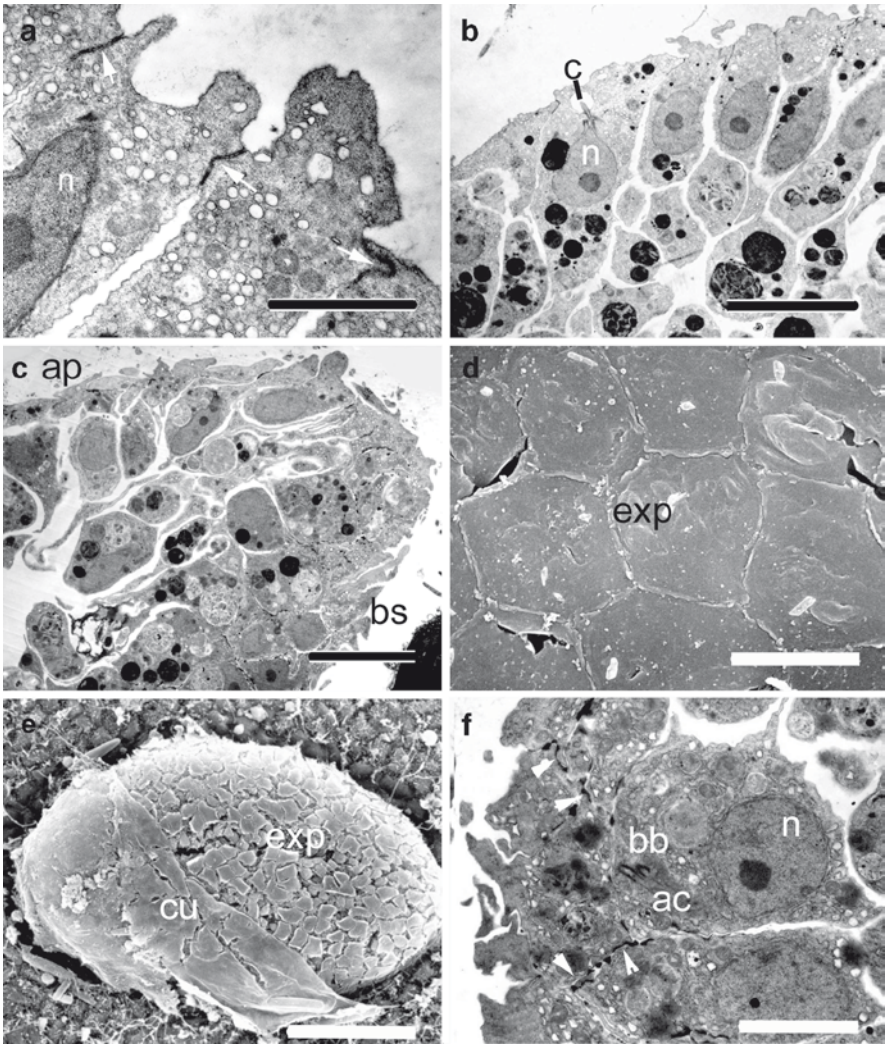

Fig. 3.40 Apical (**a, b, d, e**), marginal (**c**) and basal (**f**) fragments of metamorphosed larva of *Halisarca dujardini* (**a, b, c, f** – TEM; **d, e** – SEM). (**a**) Longitudinal section through the ciliated cells with nucleus (*n*) and cell junctions (*arrows*) at the apical part. (**b**) Former ciliated cells of larval anterior pole at the first stages of exopinacoderm development. (**c**) Marginal part of a post-larva with the basal (*bs*) and apical (*ap*) zones. (**d**) External part of developed exopinacoderm (*exp*). (**e**) Apical part of metamorphosed larva with external cuticle (*cu*). (**f**) Dedifferentiated ciliated cells from the basal part of metamorphosed larva. *arrowheads* alcian blue, *ac* accessory centriole, *bb* basal body, *c* cilium, *n* nucleus (**b–f** – From Gonobobleva and Ereskovsky 2004b, Bull. Inst. R. Sci. Nat. Belg. Biol. vol. 74, pp. 103, 105, 106, 108, Fig. 2f, 5a, c, 6, 8b, reproduced by permission of l'Institut Royal des Sciences Naturelles de Belgique). Scale bars (**a**) 1 μm, (**b**) 4 μm, (**c**) 5 μm, (**d**) 5 μm, (**e**) 50 μm, (**f**) 1.5 μm

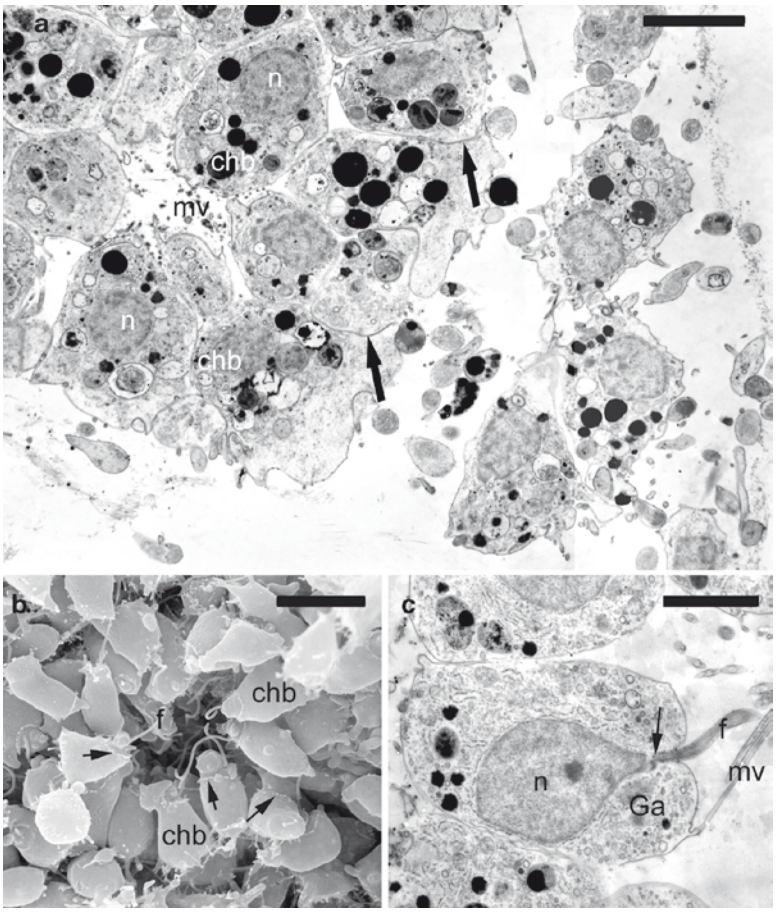

Fig. 3.41 Developing choanocyte chambers in the pupa of *Halisarca dujardini* (**a** – TEM, **b** – SEM) and larval ciliated cell that dedifferentiates in to choanoblast (**c** – TEM). (**a**) Local aggregate of the choanoblasts (*chb*) with the microvilli (*mv*); there are zones with adhesive contacts (*arrows*) of the choanoblasts. (**b**) Choanoblasts with flagella (*f*) and microvilli (*arrows*). (**c**) Ciliated larval cell with typical Golgi apparatus (*Ga*) and centrioles (*arrows*) position. *f* flagellum, *mv* microvilli, *n* nucleus (**a**, **b** – From Gonobobleva and Ereskovsky 2004b, Bull. Inst. R. Sci. Nat. Belg. Biol. vol. 74, pp. 110, Fig. 10a, b, reproduced by permission of l'Institut Royal des Sciences Naturelles de Belgique). Scale bars (**a, b**) 5 μm, (**c**) 1 μm

Phagocyting activity during metamorphosis has been described in sponges from the order Haplosclerida, Poecilosclerida, Halichondrida, Dictyoceratida (Brien and Meewis 1938; Efremova and Efremov 1979; Misevic et al. 1990; Bergquist and Glasgow 1986; Wielsputz and Saller 1990; Kaye and Reiswig 1991b; Weissenfels 1989). In these cases, larval amoebocytes phagocyte terminally differentiated ciliated cells. However, the phagocytosis described by us for *H. dujardini* is unique because it involves neither capture of food particles nor capture of whole cells.

Similar ultrastructure of dedifferentiated ciliated cells and archaeocytes, their phagocytic activity and the origin of amoebocytes by immigration of surface cells of blastula or larva may indicate the common nature of the morphogenetic potentials. This was also shown in investigations of somatic embryogenesis of *H. dujardini* (Korotkova and Movchan 1973; Volkova and Zolotarjeva 1981; Korotkova et al. 1983). In conglomerates of somatic cells choanocytes partly dedifferentiate and may phagocyte cell fragments. It was shown by marking choanocytes by Chinese ink particles that choanocytes and nucleolated amoebocytes may transform into each other and differentiate into the cells of all types of the adult sponge (Korotkova and Movchan 1973; Volkova and Zolotarjeva 1981; Korotkova et al. 1983). Chen (1976) also noted that such transdifferention in *H. dujardini* and *H. nahantensis* was possible. Direct participation of the larval ciliated cells in the development of definitive structures in *H. dujardini* was also demonstrated by immunocytochemical and experimental methods (Mukhina et al. 2006, 2007; Ereskovsky et al. 2007b).

During metamorphosis symbiotic bacteria reside in the intracellualr spaces in different parts of the sponge, they are never phagocyted. Thus, in *H. dujardini* vertical transmission of symbionts from one generation to the other occurs (Ereskovsky et al. 2005).

At the end of metamorphosis, the rhagon has all the cell types of the adult except specialized amoebocytes (fuchsinophilic and globular cells). Possibly, their differentiation only starts at the end of metamorphosis, or else is associated with the beginning of adult sponge life, that is, the functioning of the aquiferous system.

Metamorphosis in *H. dujardini* larva appears to proceed without novel cell formation, since no mitotic pictures were observed. The cytolysis of larval cells in the course of metamorphosis in *H. dujardini* and *H. metschnikovi* from Roscoff and *H. dujardini* from the White Sea did not observed (Lévi 1956; Gonobobleva and Ereskovsky 2004b). Therefore, *Halisarca* metamorphosis probably proceeds by rearrangement, sorting and transdifferentiation of ciliated cells and the homologous amoeboid larval cells (Fig. 3.42). Thus, ciliated cells are the main cell type in *H. dujardini* larvae and play the most important morphogenetic role during metamorphosis (Gonobobleva and Ereskovsky 2004b; Mukhina et al. 2006, 2007; Ereskovsky et al. 2007b).

Morphogenesis of surface structures of adult *H. dujardini* is unusual for sponges. It belongs to the type of "oblique" contact cell polarization (Figs. 3.40b and 3.43). This type is very common for Eumetazoan morphogenesis (Beloussov 1998). Here, it is possible, owing to the fact that apical regions of the ciliated cells in the posterior larval hemisphere retain specialized intercellular contacts of the *zonula adhaerens* type. We previously demonstrated that ruthenium red (a specific dye for acid mucopolysaccharides) staining makes it possible to reveal zones of adhesion in different species of sponge larvae (Gonobobleva and Ereskovsky 2004b). Preservation of intracellular junctions during exopinacocyte formation in larval metamorphosis of *H. dujardini* demonstrated their integrative function. It is known that it is through the zones of specialized cell contacts that integration of cytoskeletons of the metazoan

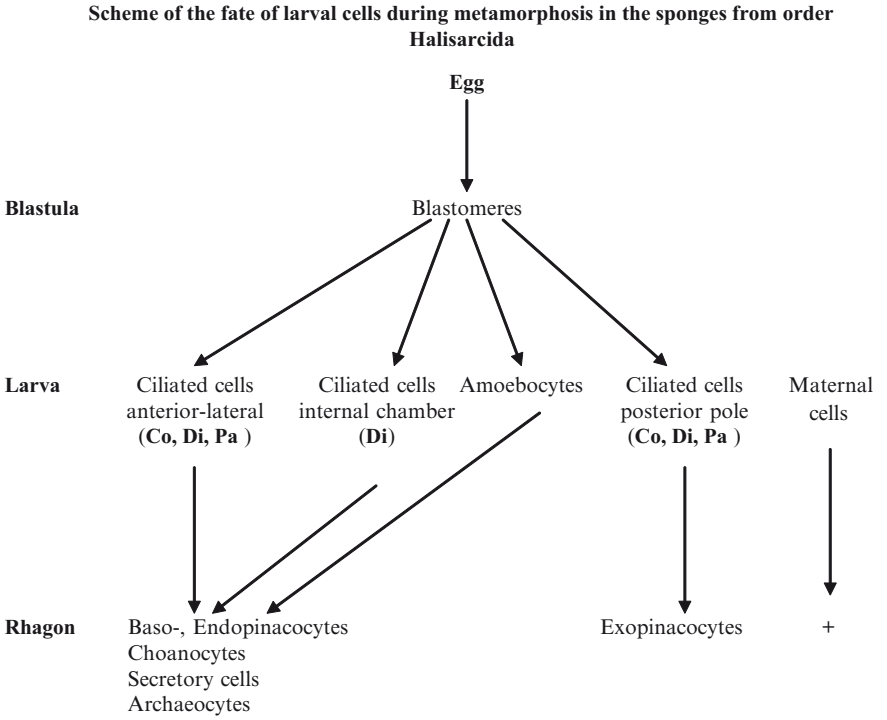

Fig. 3.42 Schematic drawing of the fate of larval cells during metamorphosis in the sponges from order Halisarcida

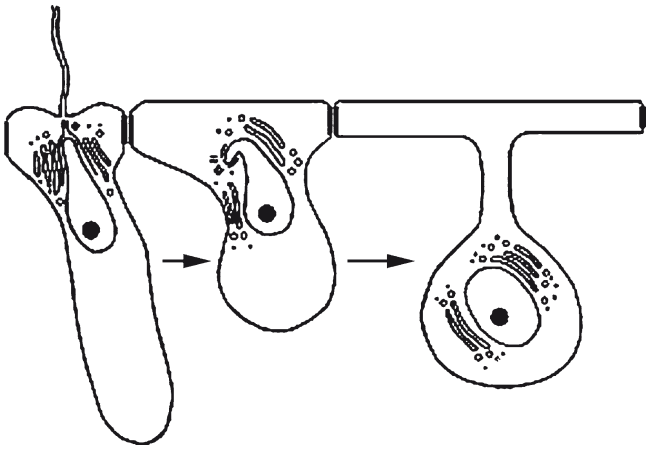

Fig. 3.43 Schematic drawing of oblique contact cell polarization of posterior pole ciliated larval cells of *H. dujardini* during metamorphosis (From Gonobobleva and Ereskovsky 2004b, Bull. Inst. R. Sci. Nat. Belg. Biol. vol. 74, pp. 104, Fig. 4, reproduced by permission of l'Institut Royal des Sciences Naturelles de Belgique)

epithelia takes place (Kolega 1986). In contrast to exopinacoderm, basopinacoderm formation in *H. dujardini* follows by means of epithelial–mesenchymal transformation (EMT) and is accompanied by the disruption of intercellular contacts. The development of elements of the aquiferous system in *H. dujardini* in the inner conglomerate of the pupa passes by means of migration of separate cells and their association followed by differentiation, i.e. it belongs to mesenchymal–epithelial transition (MET).

We showed that during *H. dujardini* metamorphosis it is a distinct succession between the layer of ciliated cells of the posterior pole and the exopinacoderm. At the same time, basopinacoderm and the internal conglomerate of the *H. dujardini* pupa and later also the elements of the mesohyl and the aquiferous system of the sponges have a mixed origin (Gonobobleva and Ereskovsky 2004b). Moreover, the larval anterior–posterior axis becomes basal–apical axis of new sponges during metamorphosis regardless of the larval type of *H. dujardini*. Thus, in this respect sponges are not very different from other attached aquatic invertebrates, e.g. Cnidaria (Martin 1997).

3.7 The Order Dendroceratida Minchin, 1900

The order Dendroceratida comprises demosponges (colour plate VIII) with a fibre skeleton: starting from the whole basal plate, spongin bundles branch dendritically and anastomose towards the apical part of the sponge (Fig. 3.44). The fibres always contain pith and are strongly laminated. Free fibre spicules are sometimes present. Choanocyte chambers are eurypilous. Matrix volume is low in relation to chamber and canal volume, and the endosomal matrix is only weakly infiltrated by collagen. Biochemically, members of this group are characterized by moderate sterol content in conjunction with the presence of terpenes, which are always diterpenes. All the Dendroceratida are ovoviviparous, the larva is parenchymella. Asexual reproduction in the form of budding has been noted. Dendroceratida are exclusively marine sponges. The order comprises two families.

There is almost no data on sexual phenotype of dentroceratids in the literature, except a mention that *Dendrilla rosea* is a simultaneous hermaphrodite (Lendenfeld 1889).

3.7.1 Gametogenesis

Investigations of gametogenesis in the Dendroceratida are scarce. In *Aplysilla rosea*, *Dictyodendrilla dendyi* and *Darwinella gardineri* male gametes derive from choanocytes by simple transformation, without proliferation stage (Tuzet et al. 1970a, b; Bergquist 1996). The phagosomes-containing posterior regions of choanocytes are cast off whereas their flagellum remains. The spermatogonia so

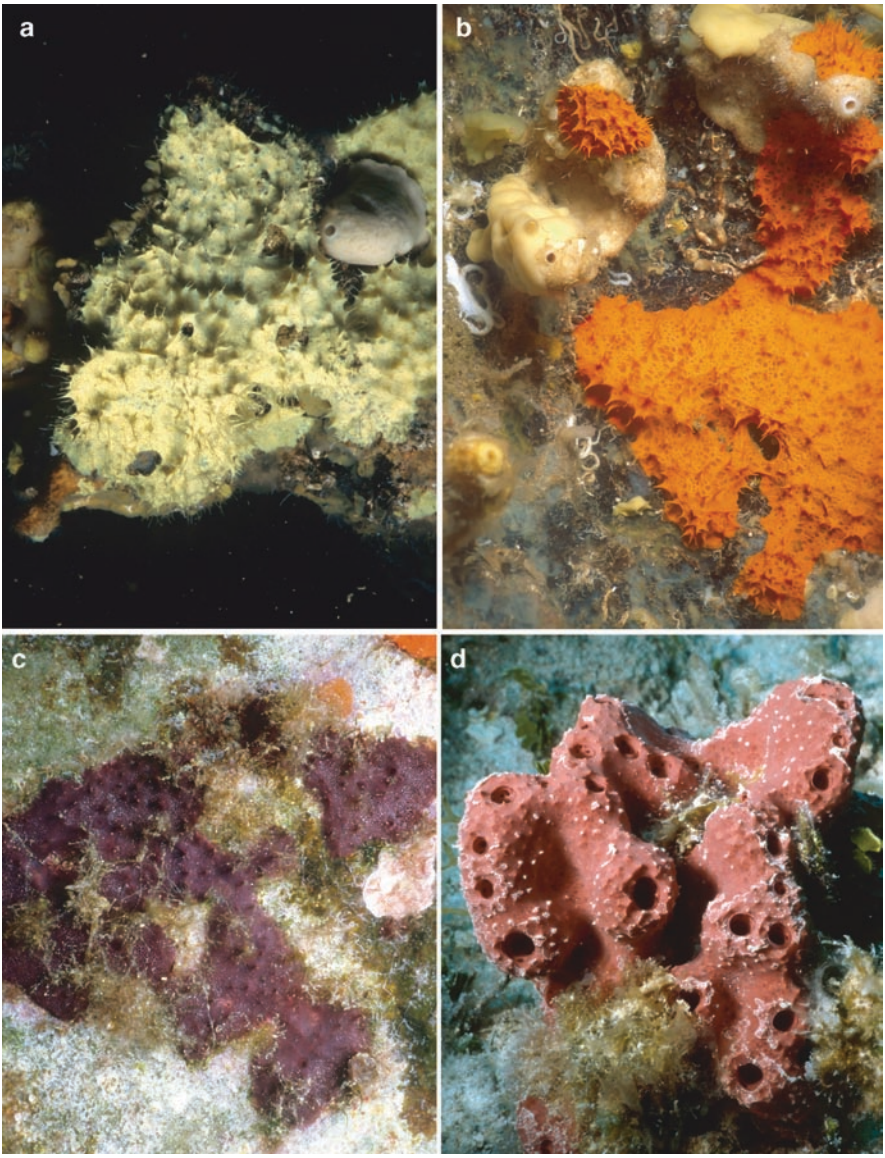

Plate VIII Order Dendroceratida Minchin 1900 (**a**) *Aplysilla sulfurea* Schulze 1878. (**b**) *Darwinella gardineri* Topsent 1905. (**c**) *Chelonaplysilla noevus* (Carter 1876). (**a**–**c**) Mediterranean Sea (Courtesy of J. Vacelet). (**d**) *Igernella notabilis* (Duchassaing and Michelotti 1864), Caribbean Sea (Courtesy of J. Vacelet)

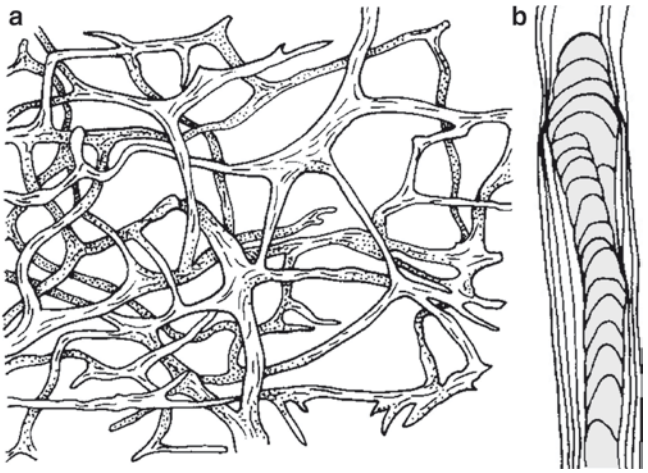

Fig. 3.44 Fibre skeleton of Dendroceratida (**a**) and the structure of one fibre (**b**) with central pith

achieved fill the whole choanocyte chambers lumen (Fig. 3.45a, b). Primary spermatocytes arise from spermatogonia which do not divide. Their flagellum and basal flagellar apparatus directly proceed from the choanocyte. Cytological spermatogenesis of *A. rosea* is identical of sperm formation in other Metazoans. The spermatozoid has an oval-shaped head with a shallow recess near of which stands the accessory centriole and basal body of the flagellum (Fig. 3.45d). Two large mitochondrial bulks surrounded the head and contained in the remaining cytoplasm. There is no acrosome. The cells forming the spermatocyste during spermatogenesis are considered to develop from archaeocytes (Tuzet et al. 1970a, b).

Oogenesis was investigated in only Mediterranean *Aplysilla sulfurea* (Ereskovsky 2005). In this species oogenesis is asynchronous and female gametes are scattered in the choanosome. Oocyte motility in the mesohyl is limited; some amoeboid motility is expressed only at the pre-meiotic phase (Fig. 3.46a). When the oocyte reaches about 16 μm in diameter, small osmiophilic inclusions start to accumulate in its perinuclear space (Fig. 3.46b). The oocyte stops near the exhalant canal and becomes enclosed in a collagen capsule synthesized by mesohylar cells and nucleolated amoebocytes elongated along the capsule wall. Groups of oocytes of different age in a common collagen capsule occur rather often (Fig. 3.46c).

At the vitellogenic phase a brood chamber is formed around the growing oocyte. At first the oocyte is surrounded with a layer of collencytes and with large spherulous cells, which appear to secrete collagen (Donadey and Vacelet 1977). Nutrients are accumulated both by endogenous synthesis and by phagocytosis of mesohylar cells (Fig. 3.46d). We did not find any specialized nurturing cells. Yolk granules and lipid droplets are evenly and loosely distributed in the ooplasm. Finally, an isolecithal oligolecithal egg with a central nucleolus develops.

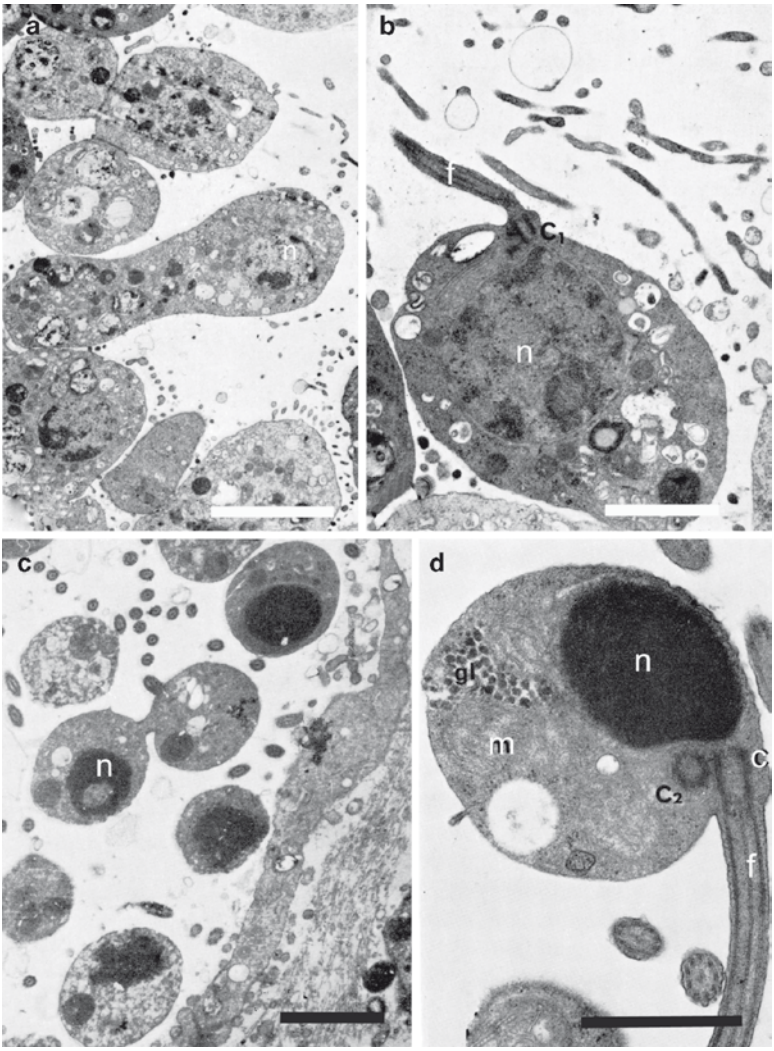

Fig. 3.45 TEM of spermatogenesis in *Aplysilla rosea*. (**a, b**) Choanocyte transformation to spermatogonia. (**c**) Part of the spermatocyste with spermatocytes II. (**d**) Spermatozoid. *c1* basal body, *c2* accessory centriole, *f* flagellum, *m* mitochondria, *n* nucleus (From Tuzet et al 1970, Annal Sci Nat vol. 12, pp. 31, 33, 43, 45, Figs. 3–5, reproduced by permission of Elsevier Ltd). Scale bars (**a**) 5 μm, (**b**) 1 μm, (**c**) 2 μm, (**d**) 1 μm

3.7.2 Embryonic Development

Development of dendroceratids is poorly studied, with only some scattered data on embryogenesis of certain species. We have studied embryonic development of in *Aplysilla sulfurea* (Ereskovsky 2005). The development is asynchronous: oocytes at

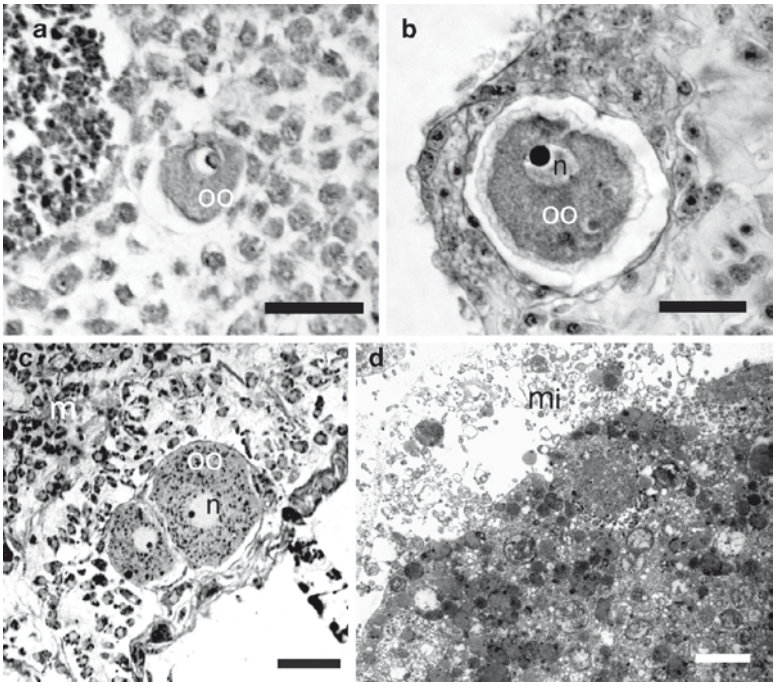

Fig. 3.46 Oocytes of *Aplysilla sulfurea* at the early stages of oogenesis. (**a**) Histological micrograph of oocyte at the pre-meiotic phase. (**b**) Histological micrograph of oocyte at the beginning of vitellogenesis. (**c**) Semifine section of two oocytes. (**d**) TEM of peripheral part of an oocyte during vitellogenesis. *m* mesohyl, *mi* microvesicles, *oo* oocyte, *n* nucleus. Scale bars (**a**) 10 μm, (**b**) 25, (**c**) 25 μm, (**d**) 1 μm

different stages, cleaving embryos and developing larvae are found simultaneously in the same sponge (Fig. 3.47). The brood chamber consists of a single layer of flattened granulated cells, spindle-shaped at cross-section. On the outside of the embryo, the brood chamber cells are covered with a collagen layer.

Cleavage in *A. sulfurea* is total, equal and asynchronous (Fig. 3.47). It is chaotic: there is no regularity in the position of the cleavage spindles and, therefore, no cleavage cavity. Blastomeres form a dense conglomerate.

An equal apolar morula formed at the stage of about 64 cells begins to segregates into the external and the internal zone in a process resembling morular delamination (Fig. 3.48); a two-layered blastula emerges. Inside the blastula there is a dense conglomerate of polygonal cells.

Larval morphogenesis. At the stage of about 128 cells, the surface cells of the blastula start to proliferate more actively than the internal ones. The peripheral zone is single-layered until the end of differentiation.

Differences between the peripheral and the internal cells of the blastula appear at the first stages of the morphogenesis. The peripheral cells diminish in size; the yolk

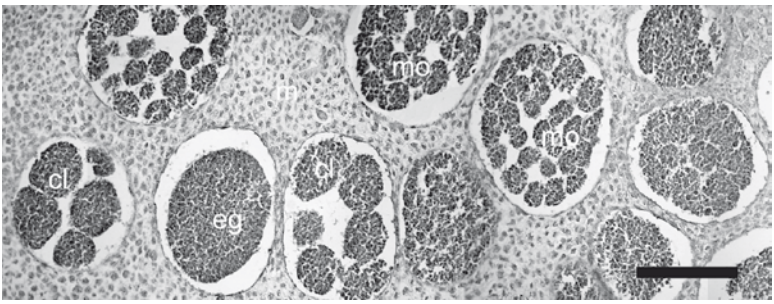

Fig. 3.47 Early embryonic development of *Aplysilla sulfurea. cl* cleaving embryo, *eg* egg, *m* mesohyl, *m* morula. Scale bar 200 μm

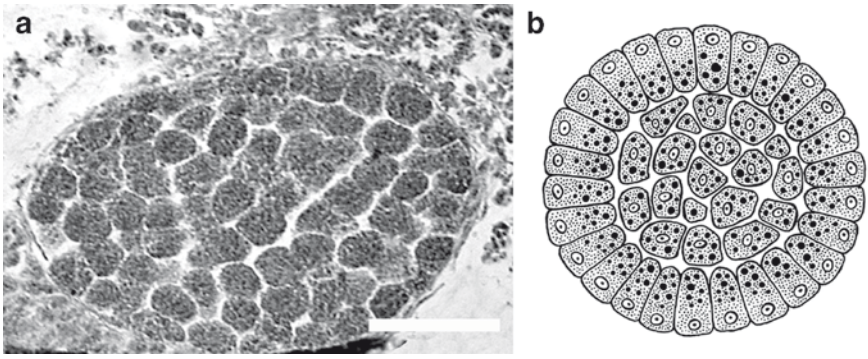

Fig. 3.48 (**a**) Light microscopy of morula delamination in *Aplysilla sulfurea.* (**b**) It scheme. Scale bar (**a**) 100 μm

granules in them become smaller than those in the internal cells due to more intense yolk utilization. The internal cells are spherical and their nuclei contain a single nucleolus; they have various sizes but are in general larger than the peripheral ones.

Later the surface cells differentiate into ciliated cells. The covered ciliated cells continue to proliferate, and numerous folds are formed on the pre-larva surface. The ciliated epithelium at this stage underlies the loose intermediate layer of amoeboid cells arranged in two to three rows. In the course of development, the cells of the intermediate layer elongate perpendicularly to the larval surface, forming long broad lobopodia inserting themselves in between the ciliated cells. These lobopodia often surround the basal part of the ciliated cells. The cells of the intermediate layer are the largest cells of the pre-larva. The vast space without cells is between the internal cell mass and the intermediate layer.

There are three types of internal cells in the pre-larva. The most numerous ones are amoeboid nucleolated amoebocytes. Their cytoplasm is filled with spherical yolk granules of various sizes. The second cell type is the cells with long branching lobopodia. In their cytoplasm there are some small osmiophilic granules and also a

few transparent vacuoles. The third cell type is rare large spherical cells with numerous rounded light vacuoles. The internal cells are loose. Symbiotic bacteria and collagen bundles occur in the space between them. The fully-formed larva, before the release from the parent sponge, no longer has surface folds.

3.7.3 Larva

Structure and ultrastructure of dendroceratid larvae was studied on *Aplysilla* sp., *A. sulfurea*, *Dendrilla cactus* (=*Aplysilla* sp.) and *Darwinella gardineri* (Schulze 1878b; Delage 1892; Bergquist et al. 1979; Woollacott and Hadfield 1989; Pinto and Woollacott 1992; Woollacott and Pinto 1995; Bergquist 1996). These investigations showed that dendroceratid larvae are rather uniform. They are oval or egg-shaped parenchymellae, with a conical anterior pole. Their size varies from 125–190 μm in *D. gardineri* to 350–600 μm in *Aplysilla* sp. Ciliation is clearly separated into three zones. The anterior pole is not ciliated at all (Delage 1892; Bergquist et al. 1979) or cilia are weakly developed (Woollacott and Hadfield 1989); the lateral surface is evenly covered with cilia of the same length; the posterior pole bears a tuft of long cilia. The border between the anterior pole and the lateral surface is rather abrupt; there is no transition zone (Fig. 3.49).

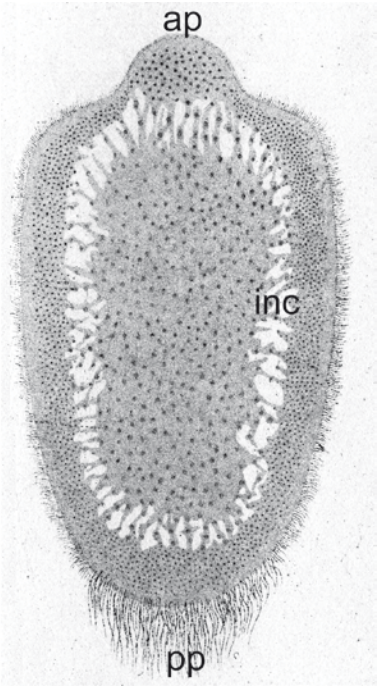

Fig. 3.49 Schematic drawing of larva of Dendroceratida. *ap* anterior pole, *inc* cells of intermedial zone, *pp* posterior pole (From Delage 1892)

The larva is covered with ciliated pseudo-multilayered epithelium. Its internal part is filled with non-polarized cell mass. The ciliated cells are strongly elongated, with nuclei at different levels. At the anterior pole these cells are almost three times broader than elsewhere (Woollacott and Pinto 1995). The ciliar rootlet is smooth, not cross-striated, as in all the Demospongiae (Woollacott and Pinto 1995). The internal cell mass lacks any structures, cavities or skeleton. According to some authors (Schulze 1878b; Delage 1892), there is no particular cell layer between the basal part of the ciliated cells and the internal mass (such a layer is present in, e.g., Haplosclerida and Poecilosclerida). However, oval nucleolated amoebocytes are loosely scattered in this space. On the whole, this zone is rather distinct, because of intercellular spaces that are greater there than in other parts of the larva. The internal cell mass consists of nucleolated amoebocytes and numerous cells with branching pseudopodia, collencytes, which form a complex three-dimensional spongin network. When the *Aplysilla* sp. larva prepares for settlement, its anterior pole cells (non-ciliated) excrete granules with a mucous substance, which is to attach the larva to the substrate (Bergquist et al. 1979).

3.7.4 Metamorphosis

Metamorphosis was described only in *A. sulfurea* (Delage 1892). The larva settles anterior pole first. Immediately after settlement it flattens strongly on the substrate, looking like a thin disc. Dome-shaped settlers, known for demosponge metamorphosis, are not characteristic. The ciliated layer is destroyed; the ciliated cells ingress inside and the archaeocytes migrate to the surface.

At the first stages of metamorphosis, the nucleolated amoebocytes that have reached the surface flatten and form exopinacoderm isolating the post-larva from the environment. The internal part of the pupa is a uniform chaotic mass of dedifferentiated ciliated cells, archaeocytes and collencytes. Later, the archaeocytes form basopinacoderm. Differentiation of adult cells and formation of the aquiferous system elements starts. However, on the basis of this investigation (Delage 1892), which was conducted at the light microscopic level, one cannot be sure about the cellular events involved in metamorphosis.

Ciliated cells that have migrated inside the post-larva assemble in groups and transdifferentiate into choanoblasts that form a series of unconnected choanocyte chambers. At this stage the pupa is still discoid (Delage 1892). Some of the archaeocytes flatten and differentiate into endopinacocytes, lining the aquiferous system canals. Later these canals form a joint network. Collencytes, situated between the pinacoderm and the choanocyte chambers, actively secrete collagen fibers. Spongocytes, synthesizing the fibers of the skeleton, differentiate (Delage 1892).

Therefore, according to Delage (1892), the ciliated cells of the larva differentiate into choanocytes; nucleolated amoebocytes (archaeocytes), into pinacocytes, and internal cells with branching pseudopodia, into spongocytes and collencytes.

3.7.5 *Asexual Reproduction*

There are no investigations especially devoted to asexual reproduction in the Dendroceratida. *Aplysina fistularis*, from the shallow waters of the Bahama Islands, was reported to reproduce asexually by budding (Maldonado and Young 1998).

3.8 The Order Dictyoceratida Minchin, 1900

The order Dictyoceratida comprises sponges (colour plate IX) with a skeleton of anastomosing spongin fibres, making up a considerable part of the body. These sponges are very resilient, easy to squeeze but difficult to tear apart because of strong collagen fibres. There are three types of skeleton fibres: primary, secondary and tertiary (Fig. 3.50); basal corneous plate is absent. The fibres are homogeneous, without the central core. There is always a marked differential pigmentation, with surface layer dark and the interior ranging from white through cream to pale brown or pale to bright yellow. Choanocyte chambers are diplodal or eurypilous. Cell diversity in the mesohyl is low. Biochemically, the order is characterized by very low sterol content and a diverse range of terpenes within the lipid fraction. All the Dictyoceratida are ovoviviparous, the larva is parenchymella with a ring of long cilia around the posterior pole. Asexual reproduction has not been described. Dictyoceratida are exclusively marine sponges. The order comprises four families.

Dictyoceratida representatives are considered to be simultaneous hermaphrodites (Scalera Liaci et al. 1971; Ayling 1980) or successive hermaphrodites (Kaye 1990). *Spongia officinalis* is predominantly gonochoric with rare individuals that showed successive hermaphrodites (Baldacconi et al. 2007), *Rhopaloeides odorabile* and unidentified species of *Ircinia* and *Strobilina* may be gonochoric (Whalan et al. 2007; Hoppe 1988).

3.8.1 *Gametogenesis*

Gametogenesis was studied at the light microscopic level in *Hippospongia communis* (Tuzet and Pavans de Ceccatty 1958); these authors considered that both male and female gametes derived from choanocytes. Development of gametes was studied at the electron microscopic level in *Spongia officinalis*, *S. barbara*, *S. graminea*, *S. cheisis*, *Hippospongia lachne* (Gaino et al. 1984; Kaye 1990, 1991; Kaye and Reiswig 1991a; Baldacconi et al. 2007). In some of these works oocytes were shown to derive from archaeocytes (Kaye 1990, 1991; Kaye and Reiswig 1991a). Choanocyte origin of spermatocytes was convincingly demonstrated in *S. officinalis* and *Hippospongia lachne* (Gaino et al. 1984; Kaye and Reiswig 1991a; Ereskovsky et al. unpublished). The whole choanocyte chamber is transformed into

Plate IX Order Dictyoceratida Minchin 1900 and family Verticillitidae Steinmann 1882 (**a**) *Ircinia dendroides* (Schmidt 1862) (Courtesy of J. Vacelet). (**b**) *Ircinia variabilis* (Schmidt 1862) (Courtesy of J. Harmelin). (**c**) *Ircinia oros* (Schmidt 1864). (**d**) *Cacospongia mollior* Schmidt 1862. (e) *Spongia officinalis* Linnaeus 1759. (**f**) *Spongia agaricina* Pallas 1766. (**g**) *Hippospongia communis* (Lamarck 1814). (**a**–**g**) Mediterranean Sea (**c**–**g** – Courtesy of T. Perez). (**h**) *Vaceletia crypta* New Caledonia (Vacelet 1977) (Courtesy of J. Vacelet)

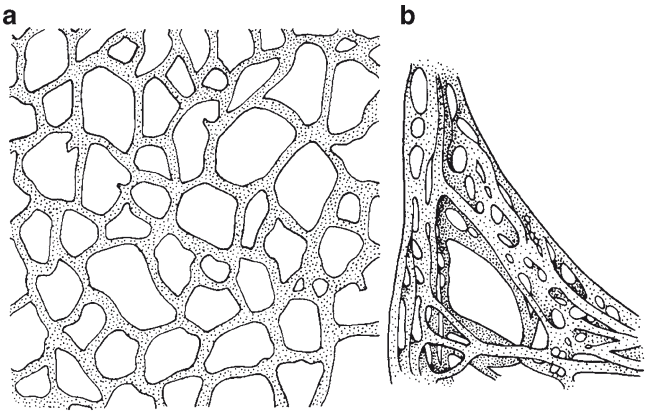

Fig. 3.50 (**a**) Fibre skeleton of Dictyoceratida and (**b**) the structure of one fibre

the spermatocyste, surrounded by flattened cells (most likely, derived from archaeocytes) (Fig. 3.51). The spermatozoid lacks the acrosome.

3.8.1.1 Oogenesis

Development of female gametes in the Dictyoceratida was studied in *Spongellia pallescens*, *H. officinalis*, *H. communis*, *Ircinia dendroides*, *I. spinulosa*, *Cacospongia scalaris*, *Spongia virgultosa*, *S. nitens*, *S. barbara*, *S. graminea*, *S. cheisis*, *S. officinalis*, *H. lachne*, *Pleraplysilla spinifera* (Schulze 1879a, b; Tuzet and Pavans de Ceccatty 1958; Scalera Liaci et al. 1971; Kaye 1990, 1991; Ereskovsky 2005; Ereskovsky et al. unpublished; Baldacconi et al. 2007).

In the pre-meiotic phase, the oocytes are weakly motile (Tuzet and Pavans de Ceccatty 1958; Scalera Liaci et al. 1971). Phagocytosis is not characteristic; they accumulate reserve nutrients (glycogen, lipids, vitelloproteins) by endogenous synthesis from macromolecules that get into the oocyte by micropinocytosis or diffusion (Kaye 1990, 1991). Before the vitellogenic phase, a brood chamber (follicle) is formed around the oocyte (Fig. 3.52a, b) from motile nucleolated mesohylar cells (Tuzet and Pavans de Ceccatty 1958; Kaye 1990, 1991). At this stage, accumulation of nutrients in the oocyte proceeds both by transport from the brood chamber cells across the cytoplasmic bridges (Kaye 1990, 1991) and by phagocytosis of some of these cells (Tuzet and Pavans de Ceccatty 1958).

Mature egg in dictyoceratids is isolecithal, oligolecithal or polylecithal. It is oval or spherical. Yolk granules are homogeneous, evenly distributed in the ooplasm; the nucleus is central (Fig. 3.52c) (Schulze 1879a, b; Tuzet and Pavans de Ceccatty 1958; Kaye 1990, 1991; Baldacconi et al. 2007).

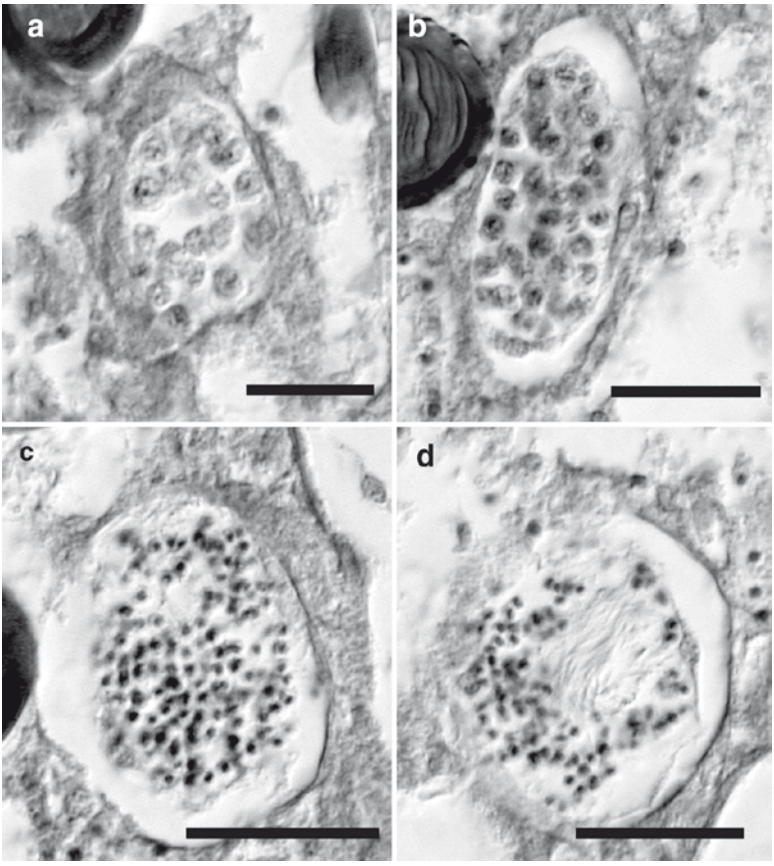

Fig. 3.51 Semifine section of spermatogenesis in *Spongia officinalis*. (**a**) Spermatocyste with the spermatogonia and spermatocytes I. (**b**) Spermatocyste with the spermatocytes I and II. (**c**) Spermatocyste with the spermatocytes I and the spermatids. (**d**) Spermatocyste with the spermatozoa. Scale bars (**a, b**) 30 μm, (**c, d**) 50 μm

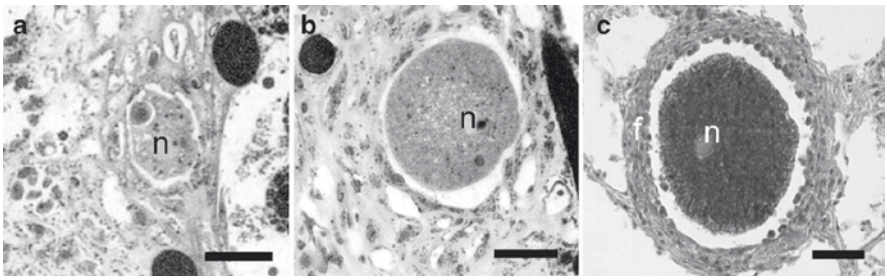

Fig. 3.52 Oogenesis *of Spongia officinalis* (**a, b**) and *Hippospongia communis* (**c**); light microscopy micrographs. (**a**) Previtellogenic oocyte and the of follicle at the beginning of formation. (**b**) The first stage of vitellogenesis. (**c**) Mature egg in a follicle (*f*). (**c** – Courtesy of S. Zarrouk). Scale bars (**a**) 10 μm, (**b**) 30 μm, (**c**) 70 μm

The developing eggs are situated, alone or in small groups (cluster), near the exhalant canals of the aquiferous system in the central or basal choanosome (Schulze 1879b; Kaye 1990, 1991; Whalan et al. 2007; Ereskovsky et al. unpublished). The choanosome disintegrates only near the developing eggs and embryos, where choanocyte chambers and canals are destroyed and the number of mesohylar cells diminishes.

3.8.2 Embryonic Development

Development of dictyoceratids is poorly studied. There are only some scattered observations of cleavage in *Spongellia pallescens* and *H. officinalis* (Schulze 1879a,b) and descriptions of embryonic development in *Hippospongia communis*, *H. lachne*, *Ircinia fasciculata*, *I. variabilis*, *Spongia barbara*, *S. graminea*, *S. cheisis*, *S. officinalis*, *Pleraplysilla spinifera* (Tuzet and Pavans de Ceccatty 1958; Scalera Liaci et al. 1971; Kaye 1991; Ereskovsky, 2005; Baldacconi et al. 2007). Development of embryos is asynchronous within a sponge.

Cleavage in dictyoceratids is total, equal and asynchronous; it follows the chaotic pattern (Schulze 1879a, b; Kaye 1990; Ereskovsky 2005). During cleavage, cytoplasmic bridges may be retained between the embryo and the brood chamber cells (follicular cells); across these bridges symbiotic bacteria are transported from the parent mesohyl into the embryo. Bacteria were shown to divide in the space between the blastomeres (Kaye 1990, 1991). Cleavage results in formation of a solid morula constituted by equal blastomeres. The polarity of the blastomeres as well as that of the morula (stereoblastula) is not expressed before the beginning of larva differentiation (Fig. 3.53a). Yolk is utilized actively during cleavage.

Larval morphogenesis starts with segregation of the morula cells into the peripheral and the internal layers. We shall consider it in some detail on the example of *P. spinifera*. At the first stages the peripheral layer of the morula differentiates by more intensive division of its cells. They become smaller and the yolk is used more intensely than in the internal cells of the embryo (Fig. 3.53b). The internal cells differentiate into amoebocytes of two types: large vacuolated cells and smaller cells (Fig. 3.53c, d). Symbiotic bacteria and collagen fibres can be seen in the space between them.

As a result, a dense two–three layered accumulation of small cells is formed at the embryo's periphery. These cells are elongated perpendicularly to the surface. Later they differentiate into the ciliated cells of the larva. Differentiation is almost equal throughout the periphery. At this stage, there are three types of internal cells in the larva. The most numerous ones are amoeboid nucleolated cells (Fig 3.54a) with the cytoplasm filled with spherical yolk granules. There are also many cells with long pseudopodia, some small osmiophilic granules and transparent vacuoles. The third cell type is rare large spherical cells filled with rounded electron-light vacuoles. The number of ciliated

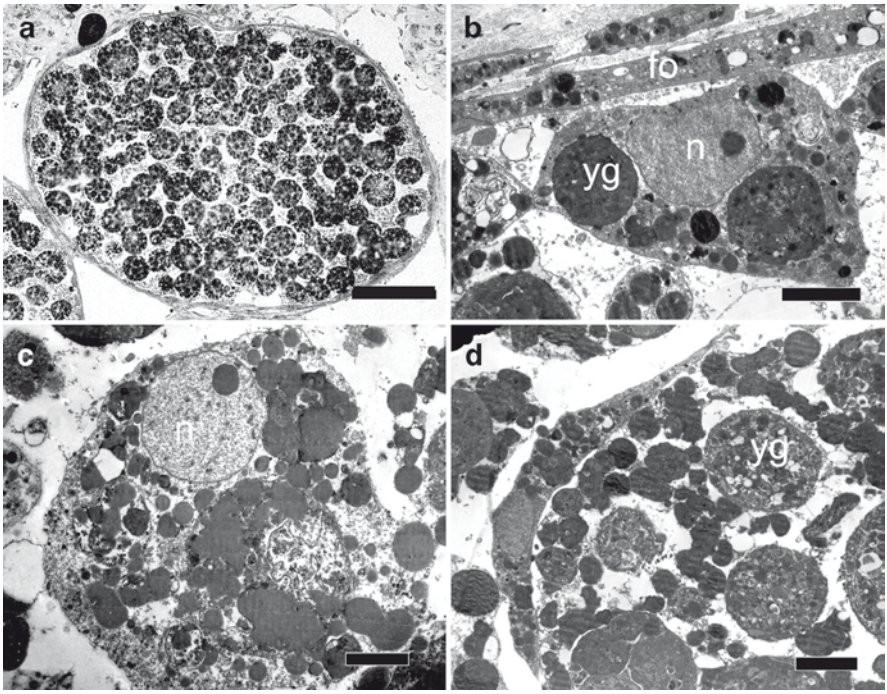

Fig. 3.53 Embryogenesis. (**a**) Semifine section of morula (stereoblastula) of *Spongia officinalis*. TEM micrographs (**b**–**d**) of cell differentiation in embryos of *Pleraplysilla spinifera*. (**b**) Differentiation of external cells. **c, d** Differentiation of internal cells. *fo* follicle, *n* nucleus, *yg* yolk granules. Scale bars (**a**) 100 μm, (**b**) 25 μm, (**c, d**) 2 μm

cells increases progressively with proliferation, so that the surface area of the pre-larva increases and numerous folds are formed (Fig. 3.54c). Similar folded pre-larvae were described in other Dictyoceratida (Scalera Liaci et al. 1971; Bergquist et al. 1970; Ereskovsky 2005; Baldacconi et al. 2007; Whalan et al. 2007).

At this stage the ciliated pseudo-epithelium of *P. spinifera* pre-larva underlies the loose intermediate layer of amoeboid cells arranged in two to three rows. During a development, the cells of the intermediate layer elongate perpendicularly to the larval surface and form long broad pseudopodia inserting themselves in between the ciliated cells. The cells of the intermediate layer are the largest cells of the pre-larva. There is a vast space without cells between the internal cell mass and the intermediate layer (Fig. 3.54d).

At the last stages of development, collagen bundles perpendicular to the larval surface are formed in the posterior third of the *H. communis* larva (Tuzet and Pavans de Ceccatty 1958). In *Ircinia oros* collagen bundles are present throughout the embryo's periphery (Ereskovsky 2005).

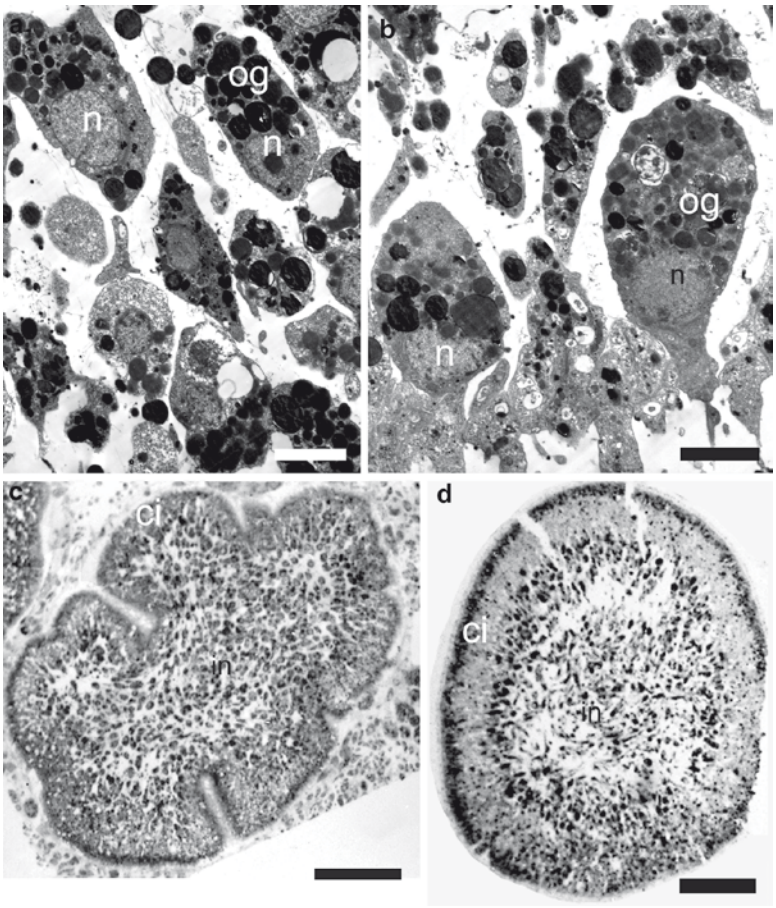

Fig. 3.54 TEM of internal cells (**a**) and pear-shaped cells of posterior pole (**b**) of pre-larvae of *Pleraplysilla spinifera.* Semifine sections of a pre-larva (**c**) and larva (**d**) of *P. spinifera. ci* ciliated cells, *ic* internal cells, *og* osmiophilic granules, *n* nucleus. Scale bars (**a**) 5 μm, (**b**) 3 μm, (**c, d**) 50 μm

3.8.3 Larva

As a rule with ovoviviparous sponges, the best studied stage in dictyoceratids is the larva. Pioneer descriptions of dictyoceratid larvae were made from *Spongellia pallescens* and *H. officinalis* (Schulze 1879a, b). Then swimming larvae were described in *Fasciospongia flagellum*, *Phyllospongia foliascens*, *Hippospongia communis*, *H. lachne*, *Ircinia variabilis*, *I. oros*, *I. felix*, *Ircinia* sp., *Spongia barbara*, *S. graminea*, *S. cheisis*, *S. officinalis*, *Spongia* sp., *Pleraplysilla spinifera*, *Cacospongia mollior* (Dendy 1889, Maas 1894, Hammer 1906, Lévi 1956, Tuzet and Pavans de Ceccatty 1958, Bergquist et al. 1979, Kaye 1990, Kaye and

Reiswig 1991b; Ereskovsky and Tokina 2004; Ereskovsky 2005; Schmitt et al. 2007b; Baldacconi et al. 2007; Uriz et al. 2008).

Dictyoceratid larvae, parenchymellae, are similar in organization and can be used as a diagnostic character in systematic at the order level (Fig. 3.40; Table 3.3). Parenchymellae are elongated or oval, covered with a layer of ciliated cells. The cilia are of the same length throughout the body except the posterior pole, where a ring of long cilia, twice as long as the lateral ones, develops. Dark, usually black pigment is concentrated at the posterior pole. The size of larvae varies in different species and, besides, changes throughout the free larval life.

The surface ciliated cells form a densely packed epithelium. In the apical part of the ciliated cells, around the axoneme, long projections of the plasma membrane, with broadened distal ends, are formed (Kaye and Reiswig 1991b; Ereskovsky and Tokina 2004; Baldacconi et al. 2007; Uriz et al. 2008). The epithelium is underlain by one to three layers of large, motile, amoeboid cells. The central mass is loose and homogeneous, consisting of archaeocytes rich in inclusions (Kaye and Reiswig 1991b; Ereskovsky and Tokina 2004; Baldacconi et al. 2007; Schmitt et al. 2007b; Uriz et al. 2008). Numerous symbiotic bacteria, extracellular matrix network and collagen fibres are also present inside the larva.

We examine the structure of *I. oros* in more detail (Ereskovsky and Tokina 2004). Anatomically, the larva is clearly separated into three zones: the surface layer of ciliated cells, the intermediate zone of loose large spindle-shaped cells oriented perpendicularly to the surface, and the loose internal mass of amoebocytes with symbiotic bacteria (Fig. 3.55).

The ciliated cells have a distinct apical–basal polarity. They are pear-shaped, with nuclei in the basal parts (Fig. 3.56a). The nuclei are situated at different levels, so that the epithelium is pseudo-stratified. The apical part is long and narrow, with the cilium and its rootlet apparatus and various organelles. To the sides of the axoneme, the cells form long cytoplasmic projections with broadened tips; the latter contain vacuoles with electron-dense or electron-transparent content (Fig. 3.56b).

In the apical cell part there is the kinetosome and the accessory centriole (Fig. 3.56e). The long rootlet passing from the kinetosome consists of fine fibrillar material and is not cross-striated; it is not connected to the nuclear membrane. In the middle part of the basal body, above the accessory centriole, the basal foot is formed (Fig. 3.56e). A well-expressed horizontal bundle of microfibrills starts from the basal body part opposite to the basal stalk and passes in the direction of the side cell wall. A similar structure was described in ciliated cells of many demosponge larvae (Woollacott and Pinto 1995). Horizontal bundles of all the cells are situated at the same level, parallel to the larval surface, and are directed towards its posterior pole.

In the apical part of all larval ciliated cells there are specialized cell junctions – belt desmosomes (Fig. 3.56d) – seen as electron-dense thickenings of the filamentous material along the internal membrane.

In the broadened basal parts of the ciliated cells there are also yolk granules pressed to the nucleus (Figs. 3.56a and 3.57a). Between the basal parts of the

Table 3.3 Characteristic of larvae of sponges from the order Dictyoceratida

Species	Size (width/ length), μm	Anterior-lateral pigment	Posterior pole pigment	A tuft of long cilia at the posterior pole	A ring of long cilia	References
Dysideidae Gray 1867						
Dysidea pallescens	210/390	Unknown	Unknown	Yes	Yes	Schulze 1879a
Spongiidae Gray 1867						
Spongia officinalis	235/330	Unknown	Unknown	Unknown		Schulze 1879b
S. officinalis	540/600	Unknown	Black	No	Yes	Baldacconi et al. 2007
Spongia sp.	500/1,000	Colourless	Dark grey	No	No	Bergquist et al. 1979.
Spongia (*Euspongia*) sp.	Unknown	White	Brown	No	Unknown	Lévi 1956
S. barbara	350/420	Grey	Black	No	Yes	Kaye 1990; Kaye and Reiswig 1991b
S. graminea	350/420	Grey	Black	No	Yes	Kaye 1990; Kaye and Reiswig 1991b
S. cheisis	350/420	Grey	Black	No	Yes	Kaye 1990; Kaye and Reiswig 1991b
Hippospongia communis	420–480/600–650	Pale yellow	Black	Yes	Unknown	Tuzet and Pavans de Ceccatty 1958
H. lachne	350/420	Grey	Black	No	Yes	Kaye 1990; Kaye and Reiswig 1991b
Hippospongia sp.	Unknown	White	Brown	Yes	Unknown	Maas 1894
Rhopaloeides odorabile	200/250–300	White	Dark	Yes	Unknown	Whalan et al. 2007
Ircinidae Gray 1867						
Ircinia sp.	460/630	White	Brown	Yes	Unknown	Maas 1894
Ircinia sp.	500/700	Colourless	Dark grey	Yes	No	Bergquist et al. 1979
I. oros	172–279/530–550	Grey	Black	No	Yes	Ereskovsky and Tokina 2004
I. variabilis	Unknown	Unknown	Unknown	Yes	Unknown	Maas 1894
I. felix	500	Light grey	Unknown	No	Yes	Schmitt et al. 2007b
Thorectidae Bergquist 1978						
Cacospongia mollior	350/600–700	Grey	Dark	No	Yes	Uriz et al. 2008
Phyllospongia foliascens	Unknown	White	Black-brown	Yes	No	Lévi 1956

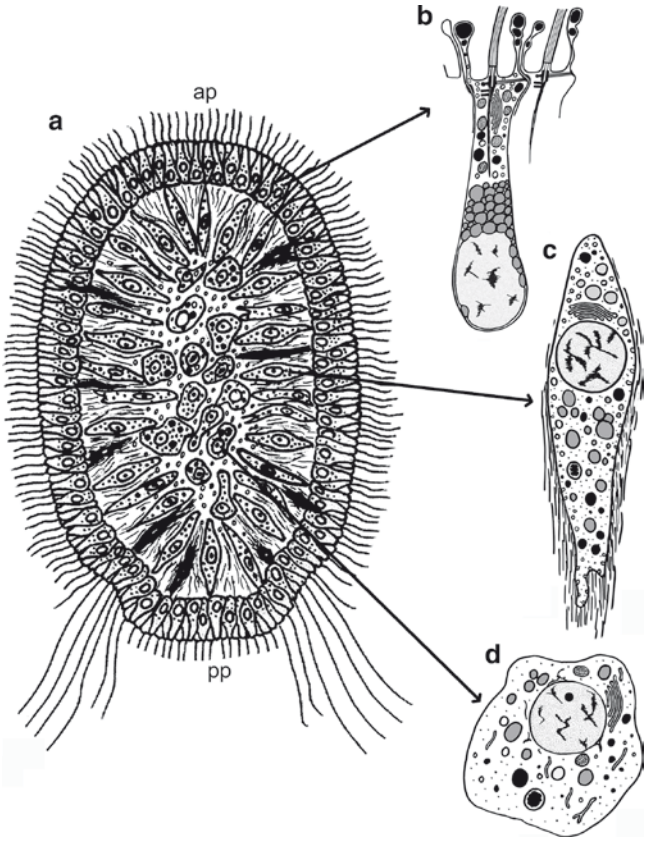

Fig. 3.55 Schematic drawing of parenchymella of Dictyoceratida by the example of *Ircinia oros*: (**a**) Free-swimming larva. (**b**) Ciliated cell. (**c**) Cell of intermedial zone. (**d**) Internal cell. *ap* anterior pole, *pp* posterior pole (From Ereskovsky and Tokina 2004, Invert. Rep. Dev. vol. 45, pp. 141, Fig. 3, reproduced by permission of Balaban Publishers International Science Services)

ciliated cells there are well-developed bundles of collagen fibres cross-striated with a period of 20 nm (Fig. 3.57a). These bundles are formed by combination of fine cross-striated fibres secreted by large cells of the intermediate zone (Fig. 3.57a). Similar collagen fibres are formed in adult *Ircinia* sponges (Garrone et al. 1973) but their period is about 60 nm.

In *P. spinifera* the ciliated layer of the posterior pole includes rare non-ciliated cells of oval or, more often, droplet shape (Fig. 3.54b). The basal cell part is broadened and contains spherical osmiophilic granules. The apical part forms a long cytoplasmic projection that is inserted into the apical layer of ciliated cells and has contact with the environment. The nucleus, with a nucleolus, is in the apical part.

The internal cells of the intermediate zone are spindle-shaped. Their longer axis is perpendicular to the larval surface (Fig. 3.57a). Intermediate zone cells are characterized by a well-developed Golgi apparatus and RER. On the membrane of

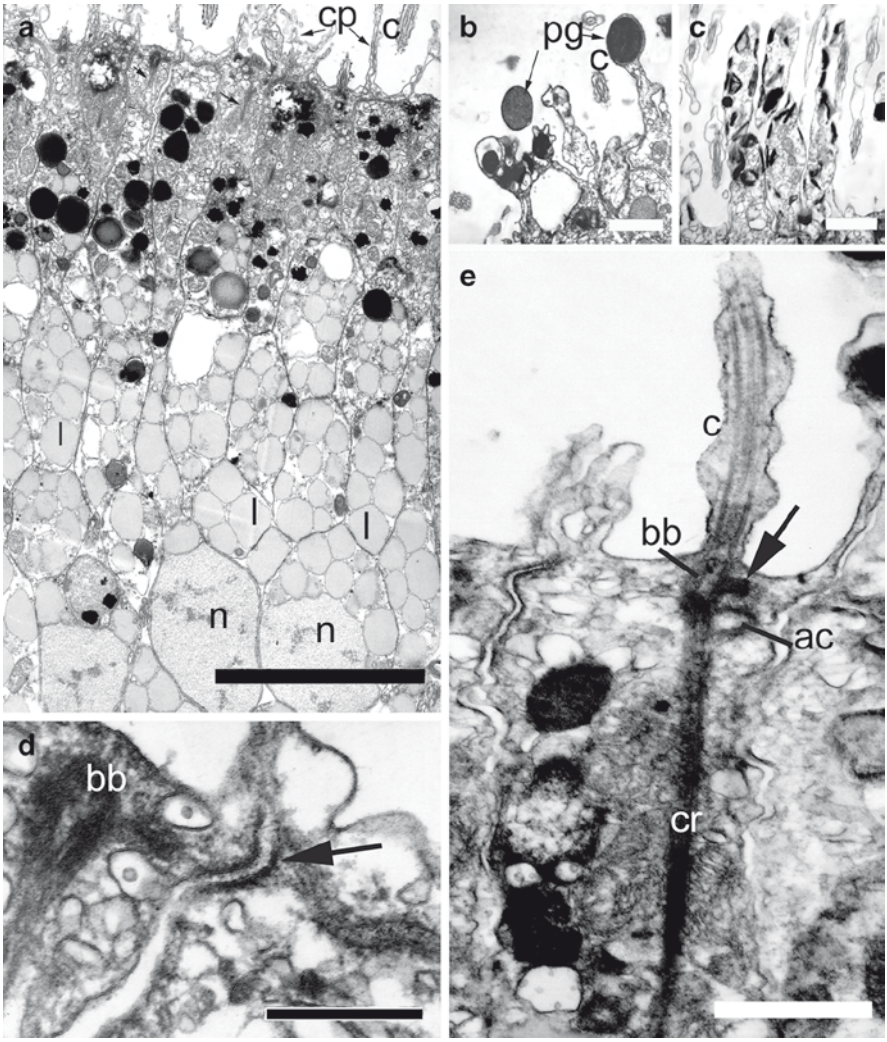

Fig. 3.56 TEM of ciliated cells of *Ircinia oros* larva. (**a**) Anterior-lateral cells (*arrows* – ciliar rootlet). (**b**) Cytoplasmic protrusions of antero-lateral cells. (**c**) Cytoplasmic protrusions of posterior pole ring cells. (**d**) Zonula adhaerens (*arrows*) at the apical part of a cell. (**e**) Basal ciliary apparatus of a ciliated cell (*arrow* – basal foot). *ac* accessory centriole, *bb* basal body, *bf* basal foot, *c* cilium, *cp* cytoplasmic protrusions, *cr* ciliar rootlet, *l* lipid droplets, *n* nucleus, *pg* pigment granules. Scale bars (**a**) 5 μm, (**b, c**) 1 μm, (**d**) 0.4 μm, (**e**) 0.3 μm (From Ereskovsky and Tokina 2004, Invert. Rep. Dev. vol. 45, pp. 140, 142, Figs. 2, 4d, reproduced by permission of Balaban Publishers International Science Services)

spindle-shaped cells collagen fibres are assembled. They are also perpendicular to the larval surface and fill almost all the space between the basal cell parts and the spindle-shaped cells. Some of the spindle-shaped cells degenerate.

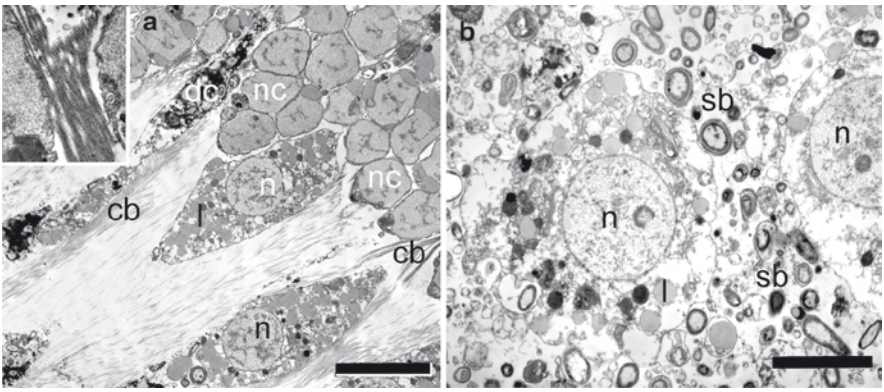

Fig. 3.57 TEM of internal cells of *Ircinia oros* larva. (**a**) Cells of intermediate zone (*inset* – collagen bunch). (**b**) Internal cells. *cb* collagen bundles, *dc* degraded cells, *l* lipid droplets, *n* nucleus, *nc* nuclea of ciliated cells, *sb* symbiotic bacteria. Scale bars (**a**) 3 μm, (**b**) 1.5 μm (From Ereskovsky and Tokina 2004, Invert. Rep. Dev. vol. 45, pp. 144, 145, Figs. 6a, 7b, reproduced by permission of Balaban Publishers International Science Services)

In the internal mass of the larva there are large amoeboid cells (Fig. 3.57b), identical in size and ultrastructure to the spindle-shaped cells of the intermediate zone. They are involved in the secretion of the extracellular matrix of the internal part of the larva, which is represented by a loose network of collagen fibres.

During preparation to settlement, cells of the anterior larval pole loose cilia. Large bubble-like vacuoles are formed in the apical part (Bergquist et al. 1979). These vacuoles contain containing mucous substance excreted when the larva contacts the substrate.

3.8.4 Metamorphosis

Metamorphosis has been studied at the electron-microscopic level in four species: *Spongia barbara*, *S. graminea* (Kaye and Reiswig 1991b), *Ircinia oros* (Ereskovsky 2005) and *Spongia officinalis* (Gaino et al. 2007).

The larva settles anterior pole first. The basal parts of the projections surrounding the cilia axoneme in the anterior pole cells are supposed to be involved in adhesion to the substrate (Kaye and Reiswig 1991b; Ereskovsky 2005; Gaino et al. 2007). Exocytose of collagen fibres to the substrate and adhesion of larval cells to these fibres is characteristic of the demosponges parenchymellae: *Mycale contarenii*, *Hamigera hamigera*, *Halichondria moorei*, *Ulosa* sp., *Microclina rubens*, four species of Dictyoceratida, as well as of Homoscleromorpha cinctoblastulae (Borojevic and Lévi 1965; Boury-Esnault 1976; Bergquist and Green 1977; Evans 1977; Kaye and Reiswig 1991b; Ereskovsky et al. 2007a).

Immediately after settlement amoebocytes, which had been below the ciliated epithelium, crawl onto the surface and the ciliated cells turn out to be inside. Cells contacting the substrate spread quickly on it, so that the post-larva flattens progressively.

In all the species studied the amoeboid cells underlying the ciliated epithelium of the posterior larval pole crawl onto the surface during the flattening of the post-larva (Fig. 3.58a), while the amoebocytes underlying the ciliated cells of the anterior pole spread upon the substrate (Fig. 3.58d). They excrete collagen fibres that form the extracellular layer isolating the conglomerate of the larval cells.

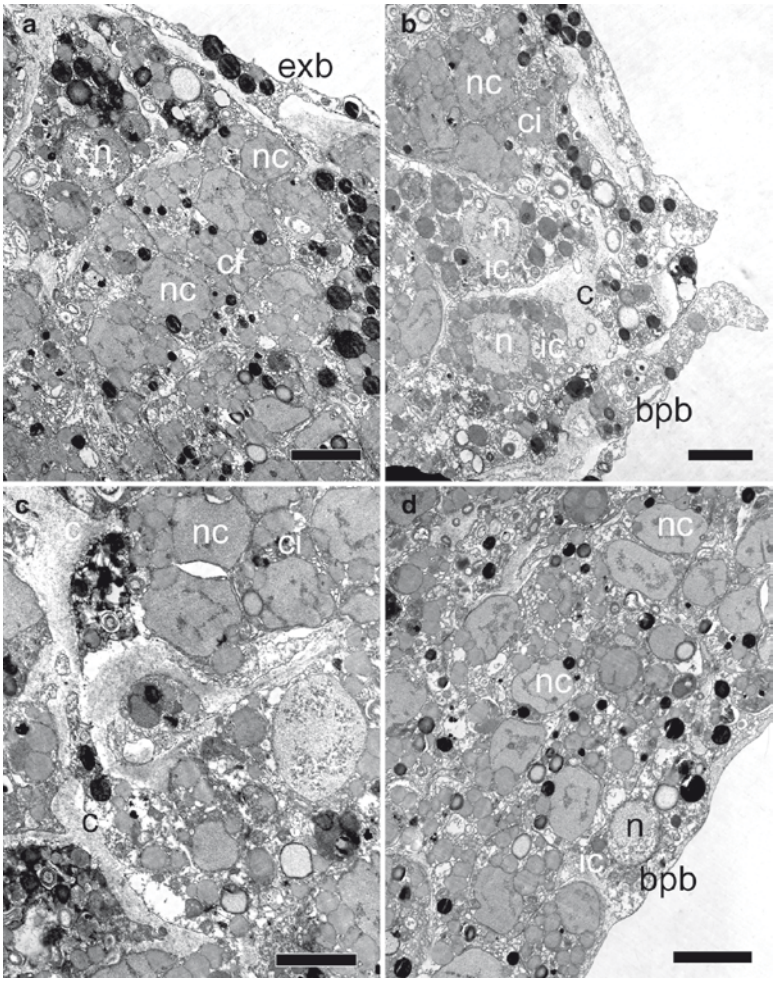

Fig. 3.58 TEM of metamorphosed larvae of *Ircinia oros* at 1 day after attachment to the substratum. (**a**) Apical part. (**b**) Marginal zone. (**c**) Internal part. (**d**) Basal part. *bpb* basopinacoblast, *c* collagen, *ci* ciliated cells, *exb* exopinacoblast, *ic* internal cell, *n* nucleus, *nc* nucleus of ciliated cells. Scale bars (**a**–**c**) 5 μm, (**d**) 3 μm

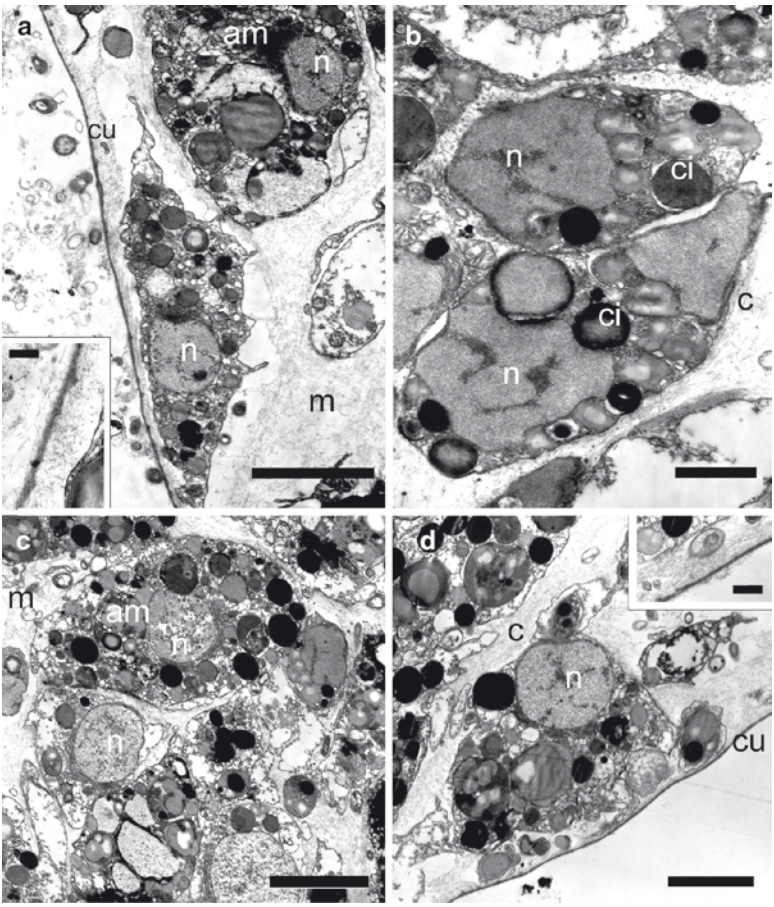

Fig. 3.59 TEM of metamorphosed larvae of *Ircinia oros* at 3 days after attachment to the substratum. (**a**) Apical part (*inset* – apical cuticle). (**b**) Former ciliated larval cells. (**c**) Internal part. (**d**) Basal part (*inset* – basal cuticle). *am* amoeboid cells, *c* collagen, *ci* ciliated cells, *cu* cuticle, *m* mesohyl, *n* nucleus. Scale bars (**a**) 5 μm, (**b**) 1 μm, (**c, d**) 3 μm, *insets* 1 μm

Collagen is also excreted on the membrane directed to the internal part of the post-larva. Later these cells differentiate into exopinacocytes. By the second to third day the external part of the extracellular matrix becomes very hardened and forms the solid cuticle plate encapsulating the developing pupa (Fig. 3.59a, b). From the internal side, the cuticle is underlain by a dense network of collagen fibres. Noteworthy, there is no difference between the structure of the apical and the basal cuticle; there are also no exo- and basopinacocytoblasts (Fig. 3.59a, d).

Ciliated cells of *Spongia barbara* and *S. graminea* larvae are phagocyted by larval archaeocytes within the first 18 h after settlement (Kaye and Reiswig 1991b). However, no indications of phagocytosis were found in the *I. oros* larvae during 10 days after settlement and in the *Spongia officinalis* larvae during 100 days after settlement (Ereskovsky 2005; Gaino et al. 2007).

The surface cells of *I. oros* lost cilia during the first 12 h after settlement. A day later the former ciliated cells became oval; most of the cell volume was occupied by a large irregular nucleus (Fig. 3.58a–d), a centriole was present near it. No traces of basal ciliar apparatus were revealed. These cells were always found in conglomerates, never singly (Fig. 3.58b, c). By the third day the shape and size of these cells remained the same but the number of inclusions decreased drastically (Fig. 3.59b). At the same time, Golgi apparatus and RER continued to develop. The cells remained in this state for the week after the beginning of metamorphosis. Importantly, we did not observe either phagocytosis or destruction of the former ciliated cells; there were also no indications of their transdifferentiation. The central mass of the pupa was very dense. Nucleolated amoebocytes synthesized collagen extracellular matrix and actively phagocyted symbiotic bacteria (Figs. 3.58c, d; 3.59c).

In *Spongia barbara* and *S. graminea* archaeocytes differentiate into choanoblasts, which assemble into separate choanocyte chambers, and also in secretory cells and endopinacocytes (Kaye and Reiswig 1991b). In *I. oros* and *S. officinalis* the source of choanocytes was not shown. To note, by the tenth day the internal amoebocytes began to differentiate into endopinacocytes and internal secretory cells, spongocytes and collencytes.

On the basis of literature data and our own observations, we have drafted a scheme of differentiation of dictyoceratid larval cells during metamorphosis (Fig. 3.60).

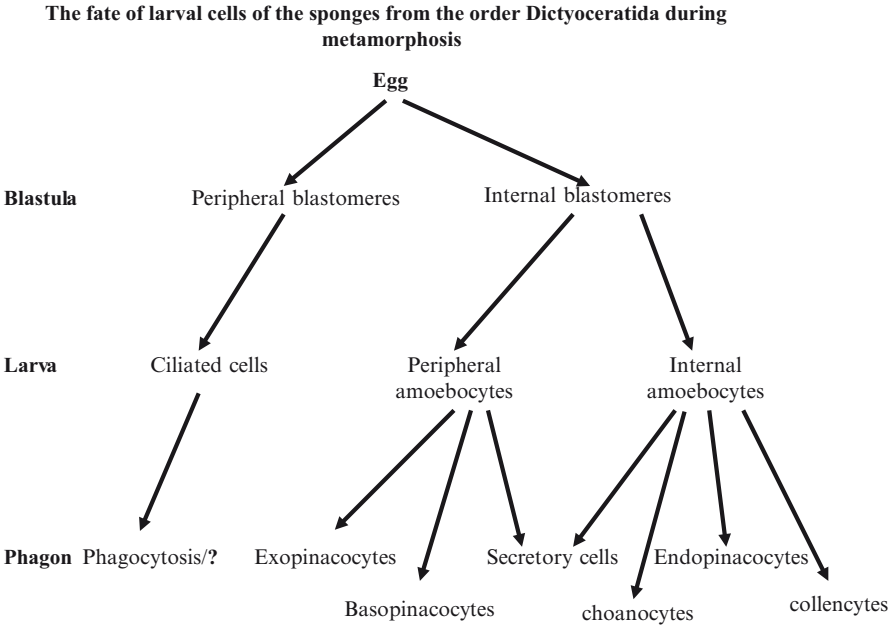

Fig. 3.60 The fate of larval cells of the sponges from the order Dictyoceratida during metamorphosis

The questions yet to be solved are the fate of surface ciliated cells and the origin of definitive choanocytes.

3.9 Recent 'Sphinctozoa', Family Verticillitidae Steinmann, 1882

The family Verticillitidae, mostly comprising extinct taxa of hypercalcified sponges, is represented by small sponges, with a subcylindrical growth form (colour plate IX h). Their chambered skeleton is an irregular arrangement of aragonite crystals; there are no spicules. This organization is characteristic for a sphinctozoan grade of organization. Inhalant openings are situated in the outer wall (of the porate type), with simple exopores provided with spines extending towards the centre of the aperture. Exowall, interwall and endowall have the same perforation pattern. Exhalant canal is siphonate, sometimes with a longitudinal dividing wall. There are regular, pillar-like filling structures in the chambers. Verticillitids are known from the Triassic.

The only extant representative of the family Verticillitidae is *Vaceletia crypta* (Vacelet 1977). Its living parts are 5–9 mm high and 3 mm in diameter; living tissue are located inside the chambers (Fig. 3.61). *V. crypta* is viviparous and hermaphroditic, with the parenchymella larva. Quite detailed information on its development is available (Vacelet 1979b). This species has a broad distribution throughout the Indo-Pacific area, inhabiting semi-closed cavities of coral reefs, front reef caves and bathyal habitats at a depth of 10–530 m. Recently, at the base on the analyses of rDNA sequences it was proposed that monophyletic taxon *Vaceletia* belongs to the Dictyoceratida, a taxon devoid of any inorganic spicules (Wörheide 2008).

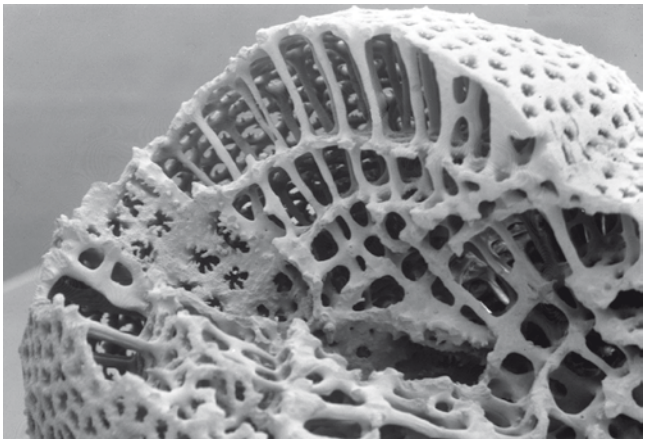

Fig. 3.61 Skeleton of *Vaceletia crypta.* (Courtesy of J. Vacelet)

3.9.1 Gametogenesis

3.9.1.1 Spermatogenesis

Male gametes of *V. crypta* develop by transdifferentiation of choanocytes. Noteworthy, all the choanocytes of a chamber transform into spermatocytes, which then multiply by mitosis (Vacelet 1979b). This process involves several neighbouring choanocyte chambers, which finally fuse to form one large spermatocyst about 70–140 μm in diameter. Each spermatocyst is enclosed into a capsule of flattened cells, endopinacocytes (Fig. 3.62a). Spermatogenesis is synchronous within a cyst. The spermatozoid is rounded, without the acrosome; the flagellum base is not enclosed in a cytoplasmic muff (Fig. 3.62c).

Oogenesis in *V. crypta* has not been studied.

3.9.2 Follicle

The embryonic development of *V. crypta* proceeds inside a complex follicle formed by bi- or trinucleate cells, referred to as nutrient cells by Vacelet (1979b), surrounded with a layer of pinacocytes. The cytoplasm of the nutrient cells is filled with electron-transparent vacuoles and small mitochondria. There is a well-developed Golgi complex around the nuclei. The presences of diffuse particles between the membranes of peripheral blastomeres and nutrient cells and of dense granules with heterogeneous inclusions in the cytoplasm of blastomeres and nutrient cells, as

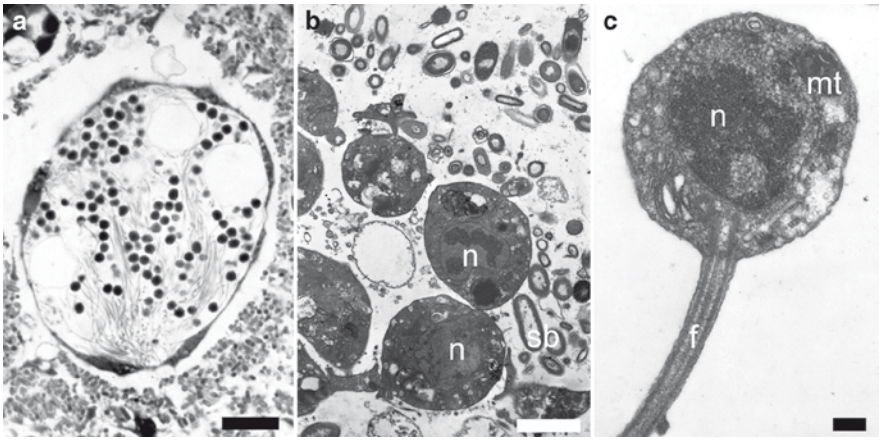

Fig. 3.62 Spermatogenesis in *Vaceletia crypta*. (**a**) Light microscopy of the spermatocyste. (**b**) TEM of spermatocyste with spermatocytes I. (**c**) TEM of spermatozoid. *f* flagellum, *mt* mitochondria, *n* nucleus, *sb* symbiotic bacteria. (Courtesy of J. Vacelet). Scale bars (**a**) 20 μm, (**b**) 2.5 μm, (**c**) 1.5 μm

well as degeneration of the nutrient cells before larval release testify to the trophic function of the follicle. Therefore, one may suppose that *V. crypta* has true viviparity, which belongs to the type of cytotrophic viviparity (Batygina et al. 2006).

3.9.3 Embryonic Development

Cleavage in *V. crypta* is total and equal. Presumably, its pattern is radial. Cleavage results in an equal, non-polarized coeloblastula 220–300 μm in diameter (Fig. 3.63a).

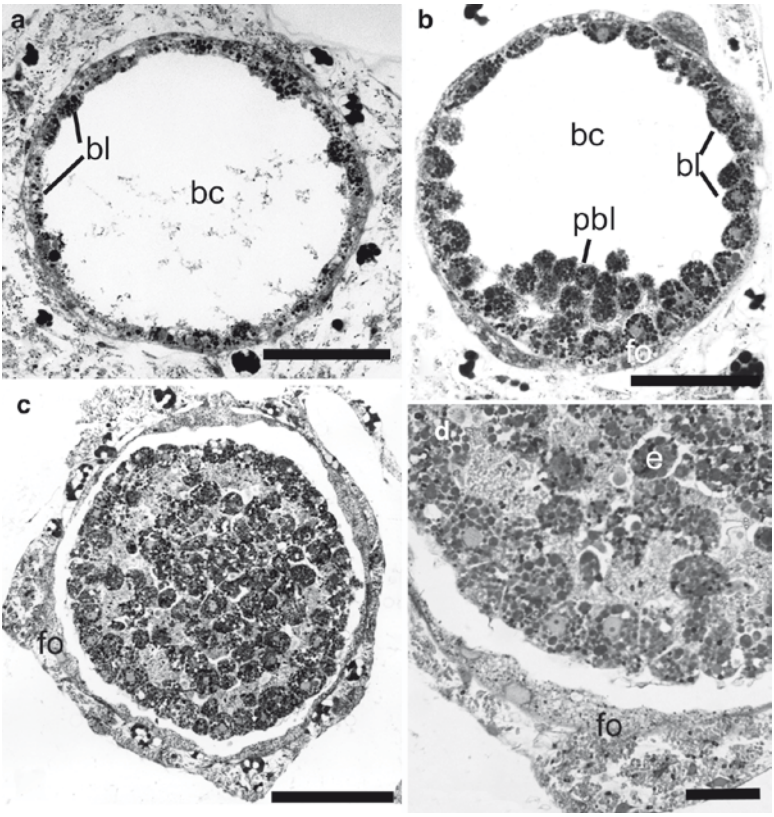

Fig. 3.63 Light microscopy of embryonic development of *Vaceletia crypta.* (**a**) Coeloblastula. (**b**) Beginning of unipolar proliferation. (**c**) Morula. (**d**) Detail of external part of the morula. *bc* blastocoel, *bl* blastomeres, *e* embryo, *fo* follicle, *pbl* proliferated blastomeres. (Courtesy of J. Vacelet). Scale bars (**a**–**c**) 50 μm, (**d**) 10 μm

3.9.3.1 Larval Morphogenesis

Vacelet (1979) described the formation of a dense morula (stereoblastula) from the coeloblastula by cell ingression from the area of one of the poles (Fig. 3.63b, c). Outwardly this process resembles unipolar immigration, well-known in many Cnidaria (Martin 1997). Careful examination of semi-thin sections kindly provided by Dr. Vacelet did not reveal any instances of cell immigration. Formation of the internal conglomeration of morula cells appears to take place due to an increased cell proliferation in the area of one of the poles of the hollow blastula. The causes of this phenomenon remain unclear. No such morphogenesis at early developmental stage has been described in other multicellular animals, which warranted its description as a new morphogenesis type, *unipolar proliferation* (Ereskovsky and Dondua 2006).

Ultrastructurally, the internal and the peripheral blastomeres of the morula (stereoblastula) are almost identical, the only difference being that in the latter the Golgi apparatus is better developed. Peripheral cells of the stereoblastula differentiate into the covering ciliated cells, and the internal ones, into collencytes, archaeocytes and granulated cells.

3.9.4 Larva

The development results in formation of the parenchymella larva about 160 × 340 μm. It is pear-shaped, with a distinct anterior–posterior polarity (Fig. 3.64a). The anterior pole has a larger diameter and a denser packaging of the internal cells; the posterior pole is narrower and contains loose cells agglomerate (Fig. 3.64a). The peripheral cells stretch perpendicularly to the surface. The cells have one cilium surrounded by cytoplasmic protrusions, which are twice as long at the posterior pole as at the anterior pole (Fig. 3.64b, c). It is interesting, that the same long projections of the plasma membrane, with broadened distal ends are characteristic only for parenchymellae of Dictyoceratida (see Section 3.8). There is one non-striated fibrillar rootlet of the cilium. The nuclea of ciliated cells situated at their basal parts.

Throughout the embryonic development the embryo and the larva comprise endosymbiotic bacteria characteristic of the mesohyl of the adult sponge (Fig. 3.64d).

Asexual reproduction in verticillitids has not been described.

3.10 The Order Poecilosclerida Topsent, 1928

The Poecilosclerida is one of the most species-rich poriferan orders, as well as the most morphologically diverse one (colour plate X). Poecilosclerids are usually leuconoid, with a well-developed ectosome and choanosome. Some representatives of the family Cladorhizidae lack all the aquiferous system elements

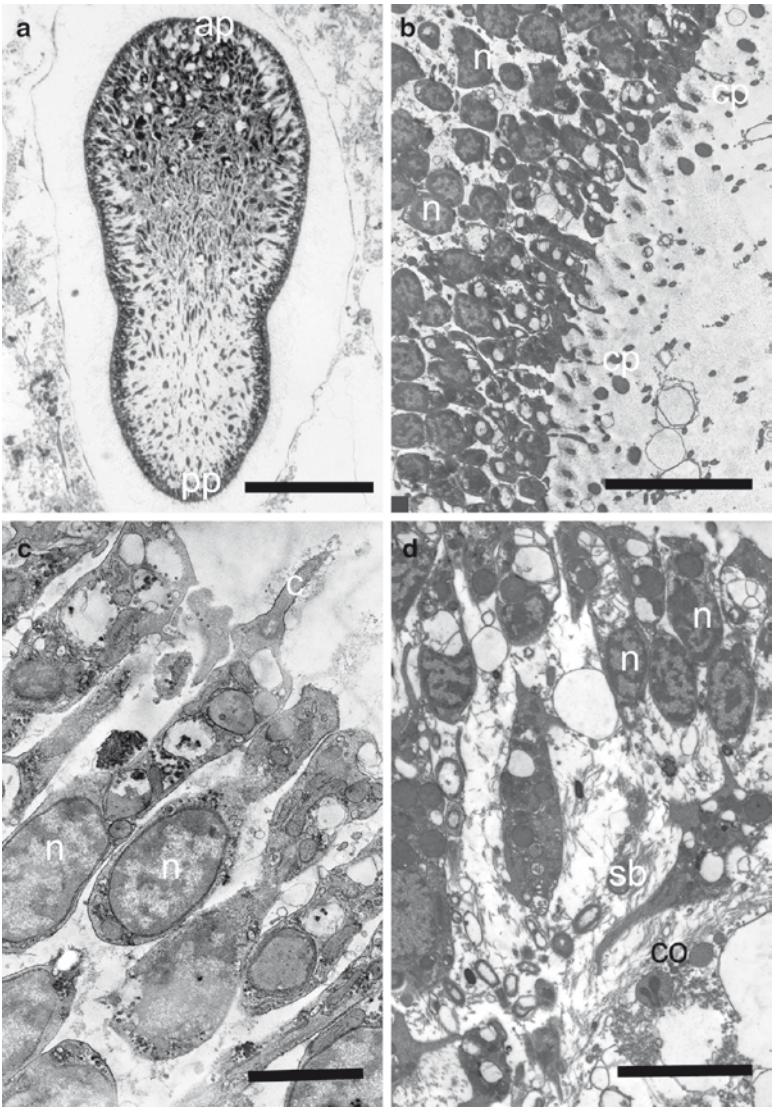

Fig. 3.64 Parenchymella of *Vaceletia crypta.* (**a**) Light microscopy of a larva. (**b**) TEM of apical parts of ciliated cells of the larva with cytoplasmic protrusions. (**c**) TEM of apical parts of ciliated cells of a larva. (**d**) TEM of internal part of a larva. *ap* anterior pole, *c* cilium, *co* collagen, *cp* cytoplasmic protrusions, *n* nucleus, *pp* posterior pole, *sb* symbiotic bacteria. (Courtesy of J. Vacelet). Scale bars (**a**) 100 μm, (**b**) 10 μm, (**c**) 2 μm, (**d**) 5 μm

(pores, canals, choanocyte chambers, choanocytes, and the osculum); they are carnivorous and also derive nutrition from symbiotic bacteria (Vacelet and Boury-Esnault 1995, 1996; Vacelet et al. 1995; Vacelet 2006, 2007). The skeleton consists of discrete spicules; the main skeleton is formed by macroscleres

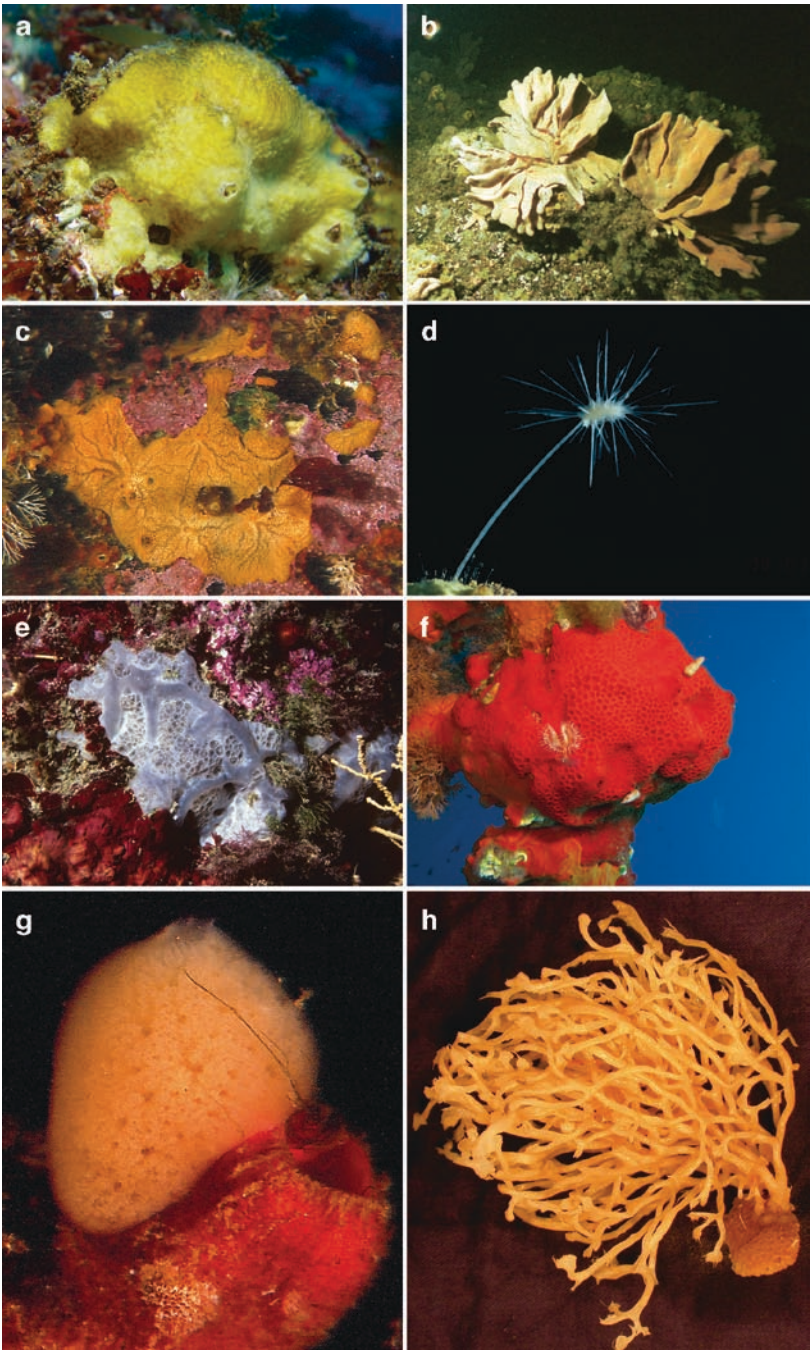

Plate X Order Poecilosclerida Topsent 1928 (**a**) *Myxilla incrustans* (Johnston 1842) (Courtesy of M. Fedjuk). (**b**) *Isodictya palmata* (Ellis and Solander 1786) (Courtesy of N. Cherviakova). (**c**) *Crellomima imparidens* Rezvoj 1925 (Courtesy of M. Fedjuk). (**a**–**c**) White Sea. (**d**) *Asbestopluma hypogea* Vacelet and Boury-Esnault 1996 (Courtesy of J. Vacelet). (**e**) *Phorbas tenacior* (Topsent 1925) (Courtesy of J. Vacelet). (**f**) *Hamigera hamigera* (Schmidt 1862) (Courtesy of J. Harmelin). (**d**–**f**) Mediterranean Sea. (**g**) *Amphilectus lobatus* (Montagu 1818) White Sea (Courtesy of M. Fedjuk). (**h**) *Esperiopsis koltuni* Ereskovsky and Willenz 2007 Okhotsk Sea (Photo of A.V. Ereskovsky)

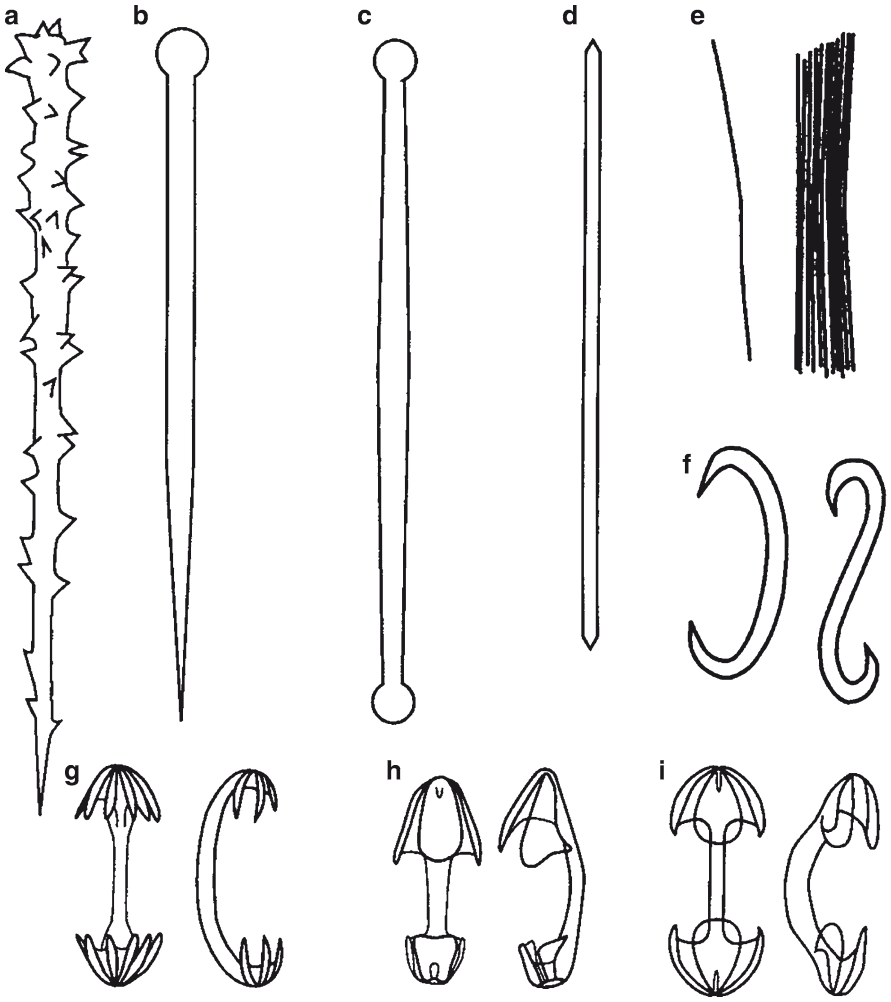

Fig. 3.65 Spicules of Poecilosclerida. Macroscleres (**a**–**d**) and microscleres (**e**–**k**). (**a**) Acanthotylostyle. (**b**) Tylostyle. (**c**) Tylote. (**d**) Strongyle. (**e**) Raphides. (**f**) Sigma. (**g**–**i**) Chelae

(monoaxial, diaxial or both) (Fig. 3.65a–d) and spongin fibres developed in different degrees. Both spongin fibres and mineral skeleton elements are always differentiated regionally. Microscleres are represented by anchorate chelae (found in this order only), sigmas and their derivates, other diverse types: toxae, raphides, microoxeas, microrhabds (Fig. 3.65e–i). In general, poecilosclerids are characterized by ovoviviparity; the larva is parenchymella, whose posterior end lacks cilia. The order also comprises two oviparous families, Raspailiidae and Rhabderemiidae.

All the Poecilosclerida are marine sponges, common in all the seas from the shallow intertidal to abyssal. The order comprises several thousand species,

currently distributed in 25 families and four suborders: Microcionina, Myxillina, Mycalina and Latrunculina incertae sedis.

Almost all poecilosclerids are simultaneous hermaphrodites. This type of hermaphoditism has been described in the families Myxillidae, Microcionidae, Biemnidae, Latrunculidae, Mycalidae, Cladorhizidae, Hymedesmiidae, Anchinoidae and Grellidae (Lévi 1956; Simpson 1968; Reiswig 1973; Ereskovsky 1986; Efremova et al. 1987a; Sarà 1993; Meroz and Ilan 1995; Ilan 1995; Fromont 1999; Riesgo et al. 2007b). In oviparous *Neofibularia nolitangere* gonochorism has been noted (Hoppe and Reichert 1987).

Developing embryos are irregularly scattered in the mesohyl of the choanosome and do not form clusters (nids). In encrusting forms reproductive elements concentrate in the basal part of the sponge. Eggs and embryos always lie very close to the exhalant canals of the aquiferous system.

3.10.1 Gametogenesis

The investigations that have been made on gametogenesis in the Poecilosclerida showed that male and female gametes may derive from different cells types. For the sponges with aquiferous system (*Myxilla incrustans*, *Iophon piceum*, *Crambe crambe*, *Crellomima imparidens*, *Hymedesmia irregularis*) it was demonstrated at the electron-microscopic level that male sexual cells differentiated from choanocytes by direct transformation without gonial divisions (Efremova et al. 1987b; Riesgo and Maldonado 2009; Ereskovsky, unpublished). However, in carnivorous sponges *Asbestopluma occidentalis* and *A. hypogea*, both gametes appear to derive from archaeocytes because of their similar size; certainly, in the absence of choanocytes in these sponges it is most likely that spermatogonia originate from archaeocytes (Riesgo et al. 2007b; Vacelet et al. unpublished).

The origin of female gametes in the Poecilosclerida is controversial. Oocytes were thought to originate from archaeocytes or nucleolated amoebocytes (Maas 1894; Simpson 1968; Meroz and Ilan 1995; Ilan 1995), but these works were conducted at the light microscopic level without special regard to oogenesis. We suggested that female gametes in *M. incrustans* and *I. piceum* developed by choanocyte dedifferentiation (Ereskovsky 1985; Tokina 1985; Efremova et al. 1987a). However, carnivorous sponges *Asbestopluma hypogea* and *A. occidentalis* (Cladorhysidae) lack choanocytes (Vacelet and Boury-Esnault 1995, 1996; Riesgo et al. 2007b) female gametes are derived from archaeocytes (Boury-Esnault and Vacelet et al. unpublished; Riesgo et al. 2007b). In any case, early oocytes always have an amoeboid stage, whether they originate from amoebocytes or choanocytes.

3.10.1.1 Spermatogenesis

Whole spermatogenesis has been investigated in *Myxilla incrustans* (Myxillina), *Iophon piceum* (Microcionina) (Efremova et al. 1987b), *Asbestopluma occidentalis*,

A. hypogea (Mycalina) and *Crambe crambe* (Myxillina) (Tripepi et al. 1984; Riesgo et al. 2007b; Vacelet et al. unpublished; Riesgo and Maldonado 2009). Moreover, we investigated the spermiogenesis in *Crellomima imparidens* and *Hymedesmia irregularis* (Myxillina) (Ereskovsky unpublished). As in all demosponges, spermatogenesis of poecilosclerids is carried out in the spermatic cystes, enveloped by a thin layer of follicle cells that became thicker and formed complex interdigitated layers as development progressed. Their external parts enveloped by extracellular matrix.

However, in carnivorous sponges without aquiferous system (*A. occidentalis* and *A. hypogea*) spermatocystes develop in the sponge body, and then migrate towards the end of the lateral filaments projecting, where the mature cysts become free. In the mature cyst, sperm cells are surrounded by two envelopes (Fig. 3.66e). The inner one unicellular, the outer one made by closely intertwined cells (Vacelet 2007; Riesgo et al. 2007b). In *A. hypogea* two tufts of forceps are diametrically protruding on the mature cyst, for flotation and/or for capture by another individual (Vacelet 2007). This structure is similar to spermatophore, characteristic for many animals (Adiyodi 1988).

Spermatogenesis in Poecilosclerida has no basic differences from similar process at other multicellular animals under cytologic characteristics. As a result of spermatogenesis the spermatozoa of "primitive" type are formed. They have a comparatively big volume of cytoplasm and several small mitochondria. Mature spermatozoa in most investigated poecilosclerids (*I. piceum*, *C. imparidens*, *A. occidentalis*, *A. hypogea*, *C. crambe*, and *H. irregularis*) are elongated cells with a citoplasmic pit that encloses basal part of the flagellum. Spermatozoid of *M. incrustans* has spherical shape (Efremova et al. 1987b).

The head of the sperm of *C. crambe*, *C. imparidens*, *A. hypogea*, *H. irregularis* is capped by acrosome (Fig. 3.67) (Tripepi et al. 1984; Ereskovsky 2005; Riesgo and Maldonado 2009; Vacelet et al. unpublished), or, in *A. occidentalis* by several proacrosomal vesicles (Riesgo et al. 2007b). In these species acrosome begins to developed in early spermatids and situated alongside Golgi apparatus. In *M. incrustans* and *I. piceum* acrosome is absent (Efremova et al. 1987b).

3.10.1.2 Oogenesis

Between choanocytes in the choanocyte chambers of *I. piceum* and *M. incrustans* there are cells with a large nucleus, a nucleolus, and a small amount of basophilic cytoplasm. These cells do not divide mitotcally, and are likely to be oocytes. At some moment they loose contact with choanocytes, migrate into the flagellated chamber cavity (Fig. 3.68a), enlarge due to an increase in cytoplasmic volume and enter the meiotic prophase (Efremova et al. 1987a). Then oocytes migrate from choanocyte chambers into mesohyl (Fig. 3.68b, c). The main source of yolk inclusions in the oocyte cytoplasm are various somatic cells (Fig. 3.68b–d), which may be engulfed both by phagocytosis and by membrane merging between the oocyte and the somatic cell.

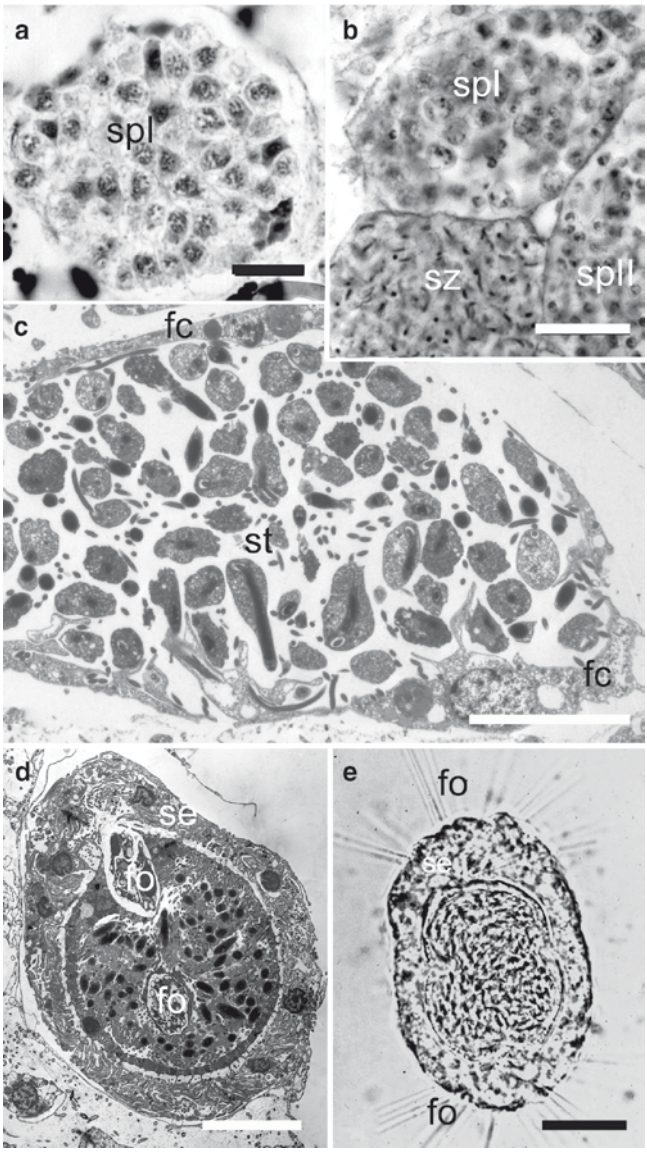

Fig. 3.66 Spermatocystes of Poecilosclerida. (**a**) Light microscopy of spermatocyste of *Myxilla incrustans* with divided spermatocytes I. (**b**) Light microscopy of three spermatocystes of *Iophon piceum* at different stages of sperm differentiation. (**c**) TEM of spermatocyste of *Crellomima imparidens*. (**d**) TEM of a spermatocyste of *Asbestopluma hypogea*. (**e**) *In vivo* photo of floating spermatophore of *Asbestopluma hypogea* with two tufts of forceps (*fo*). *fc* follicular cell, *se* spermatocystes envelop, *spI* spermatocytes I, *spII* spermatocytes II, *st* spermatidae, *sz* spermatozoa. Scale bars (**a**–**c**) 20 μm, (**d**, **e**) 10 μm (**d**, **e** – Courtesy of J. Vacelet)

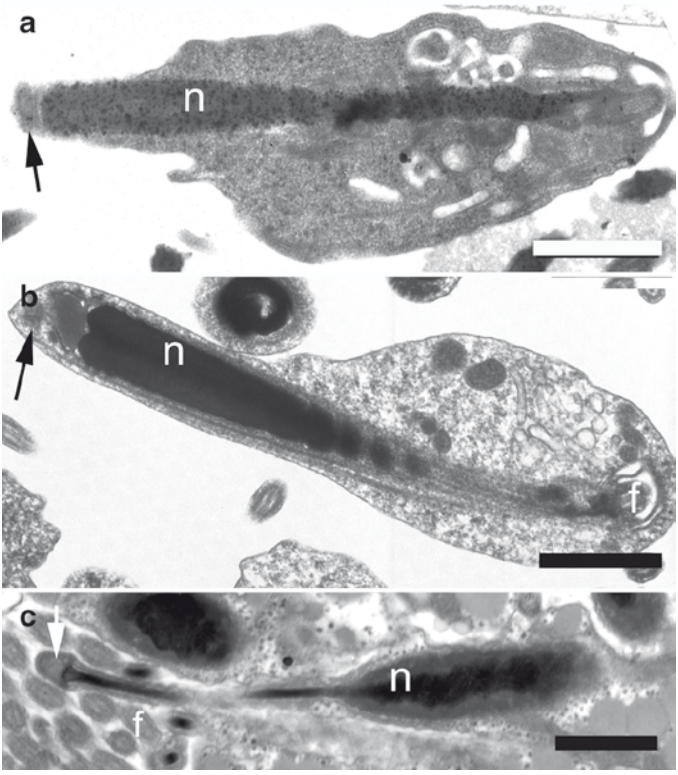

Fig. 3.67 TEM of sperm cells with acrosome of some Poecilosclerida. (**a**) Spermatida of *Hymedesmia irregularis*. (**b**) Spermatozoid of *Crellomima imparidens*. (**c**) Spermatozoid of *Asbestopluma hypogea*. *arrow* acrosome, *f* flagellum, *n* nucleus. Scale bars (**a–c**) 1 μm

Larger oocytes lie freely in the mesohyl. They contain Feulgen-positive structures – the nuclei of phagocyted mesohyl cells. Nucleolated amoebocytes, choanocytes of desorganized choanocyte chambers, collencytes and spherulous cells accumulate around the growing oocyte, surrounding it with a dense multi-layered muff (Fig. 3.68e). Thus, vitellogenic oocyte is enclosed in a capsule of chaotically arranged somatic cells (Ereskovsky 1985; Tokina 1985; Efremova et al. 1987a).

The number of cells in this capsule decreases during the vitrellogenenic phase as a result of phagocytosis. Phagocyted cells or their fragments are found in large granules of reserve substances. The oocytes phagocyte somatic cells in all the Poecilosclerida studied: *Myxilla rosacea*, *M. incrustans*, *Microciona prolifera*, *I. piceum*, *Latrunculia magnifica*, *Mycale fistulifera*, *A. lobatus*, *C. imparidens*, *Phorbas topsenti* (Maas 1894; Simpson 1968; Ereskovsky 1985; Tokina 1985; Efremova et al. 1987a; Ilan 1995; Meroz and Ilan 1995; Ereskovsky, unpublished).

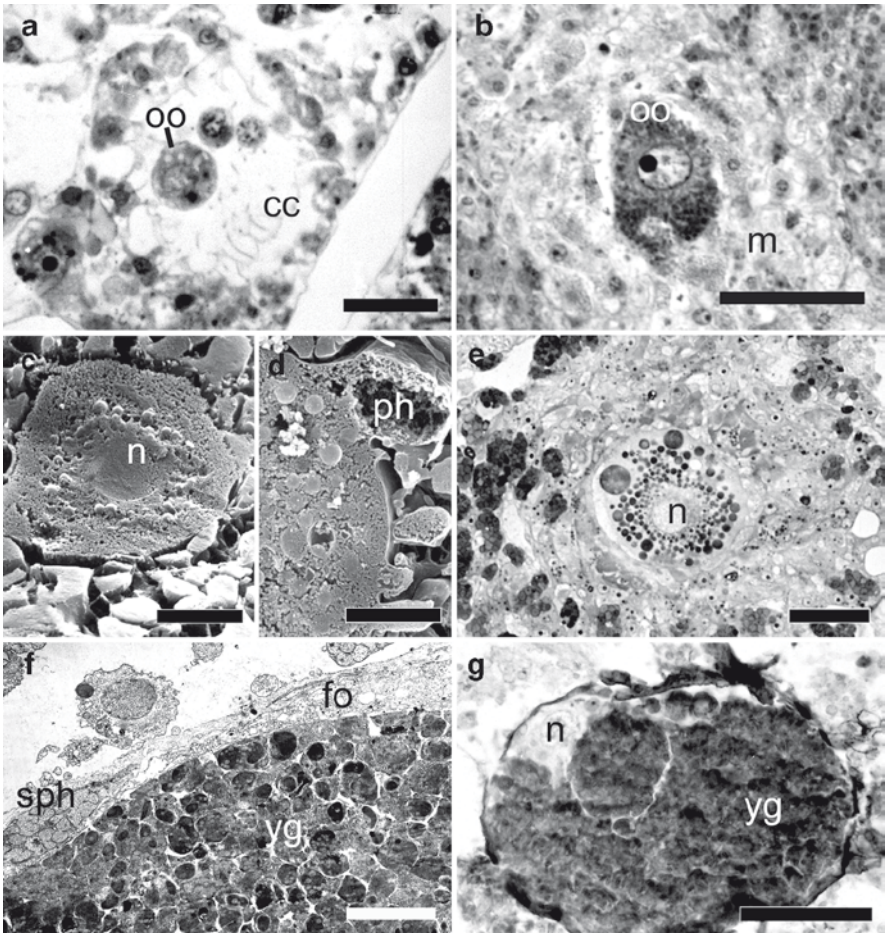

Fig. 3.68 Oogenesis in Poecilosclerida. (**a**) Light microscopy of early oocyte of *Iophon piceum* inside of a choanocyte chamber (*cc*). (**b**) Light microscopy of oocyte of *Crellomima imparidens* at the beginning of vitellogenesis. SEM of the beginning of oocyte vitellogenesis (**c**) and phagocytosis (**d**) of *Myxilla incrustans* oocyte. (**e**) Semifine section of *I. piceum* oocyte. (**f**) TEM of peripheral zone of *M. incrustans* vitellogenic oocyte. (**g**) Light microscopy of *M. incrustans* egg. *fo* follicle, *m* mesohyl, *n* nucleus, *oo* oocyte, *ph* phagocyted cell, *sph* spherulous cells, *yg* yolk granules. Scale bars (**a**) 10 μm, (**b**) 20 μm, (**c**) 10 μm, (**d**) 2.5 μm, (**e**) 20 μm, (**f**) 6 μm, (**g**) 50 μm

Judging by the presence of small granules near the nuclei of early oocytes, in their cytoplasm endogenous synthesis may also occur. Some authors who studied Poecilosclerida development mentioned specialized nurturing cells or "trophocytes" (Simpson 1968; Meroz and Ilan 1995; Ilan 1995), but we did not find any such cells.

During vitellogenesis, in the ooplasm of *M. incrustans I. piceum*, *C. imparidens*, *P. topsenti* large rounded yolk inclusions of various size are accumulated. Their content is heterogeneous: proteins, neutral mucopolysaccharides, pironinophilic

and Feulgen-positive particles are revealed. At this stage, the cells surrounding the oocyte lie loosely and their number decreases significantly. By the end of the growth period, only a layer of pinacocytes separates the oocyte from the mesohyl cells (Fig. 3.68f).

At the end of vitellogenesis, before the maturation divisions, the oocyte (oval in shape) reaches its maximal size; its nucleus shifts to one of the poles (Fig. 3.68g). The size of yolk granules in poecilosclerids varies greatly. Yolk granules are much larger at the pole opposite to the nucleus, where they are united into large yolk spherules. Rounded accumulations of yolk granules sometimes produce an illusion of a cleaving embryo, but a careful study of a section series would reveal the oocyte nucleus. Thus, the egg is telolecithal. We observed that before maturation divisions the nucleus in *I. piceum* and *M. incrustans* became vacuolated, the nucleolus disappeared and chromatin condensed into small chromosomes, but failed to observe maturation divisions themselves (Efremova et al. 1987a).

In the oviparous *Neofibularia nolitangere* a characteristic capsule formed around the eggs before their release. It consists of more than 100 parent cells and a layer of fine raphides (Hoppe and Reichert 1987). In another oviparous species, *Hemectyon ferox*, the eggs are also surrounded by parent cells, which are, in their turn, enclosed into a collagen capsule (Reiswig 1976).

3.10.2 Follicle

Similarly to the other Demospongiae, the poecilosclerid embryo develops in the cavity of the follicle. The latter is made up by a layer of flattened pinacocyte-like cells and the collagen layer around them. The follicle cells appear to originate from nucleolated amoebocytes (Ereskovsky 1986; Efremova et al. 1987a). However, the follicle wall often includes granular eosinophilic cells, which also flatten along the egg surface (Fig. 3.68f). In the carnivorous sponge *A. occidentalis* the follicle consists of two or three conical nurse cells with long extensions enveloping the oocytes. During embryo cell differentiation follicle cells extend pseudopodia both toward the mesohyl and inwards to contact the embryo (Riesgo et al. 2007b).

Noteworthy, some poecilosclerids have a peculiar follicle structure. In *Myxilla rosacea* the follicle consists of flattened cells surrounded by a layer of mesohyl with cellular and skeletal elements (Maas 1894). The follicle is “suspended” on extracellular matrix bands in the mesohyl of the sponge. The eggs of *Tedania charcoti* and *T. tenuicapitata* are enclosed in a follicle with a thick wall including numerous granular cells (Burton 1932), which disappear after the second cleavage division. By the end of cleavage, the embryo is surrounded only with a thin mesohyl layer including fine raphides. This follicle, similarly to that of *M. rosacea*, is “suspended” on extracellular matrix bands. *M. prolifera* has a multi-layer follicle consisting of numerous cells, which the author considered as nurturing (Simpson 1968). The follicle structure is retained until the larva is formed.

3.10.3 Embryonic Development

Cleavage of poecilosclerids has been studied in *Myxilla rosacea*, *M. incrustans*, *Chalinula fertilis*, *Axinella cristagalli*, *Clathria coralloides*, *Tedania charcoti*, *Microciona prolifera*, *I. piceum*, *Phorbas topsenti*, *Amphilectus lobatus*, and *Crellomima imparidens* (Maas 1894; Burton 1932; Simpson 1968; Ereskovsky 1986, 2005; Riesgo et al. 2007b). In general, poecilosclerid cleavage depends on morphological features of their eggs, which contain a great amount of unevenly distributed yolk inclusions.

If the animal–vegetative axis of the egg is considered to correspond to the gradient of yolk granules distribution, then in *I. piceum*, *M. incrustans* and *P. topsenti* (Ereskovsky 2005) and in *M. rosacea* and *C. coralloides* (Maas 1894) the furrow of the first cleavage division passes in meridional plane (Fig. 3.69a). The second division is also meridional, its plane being perpendicular to that of the first one. Blastomeres are connected with filopodia. Cleavage furrows may force some yolk granules into the space between the blastomeres or into the space between the blastomeres and the follicle. These granules, always spherical, are covered with the plasma membrane; their size varies greatly (Fig. 3.69a, d) and they are often concentrated at one of the poles. Without the serial section analyses these granules may be mistaken for small blastomeres; this happened in the study of *Mycale fistulifera* (Meroz and Ilan 1995).

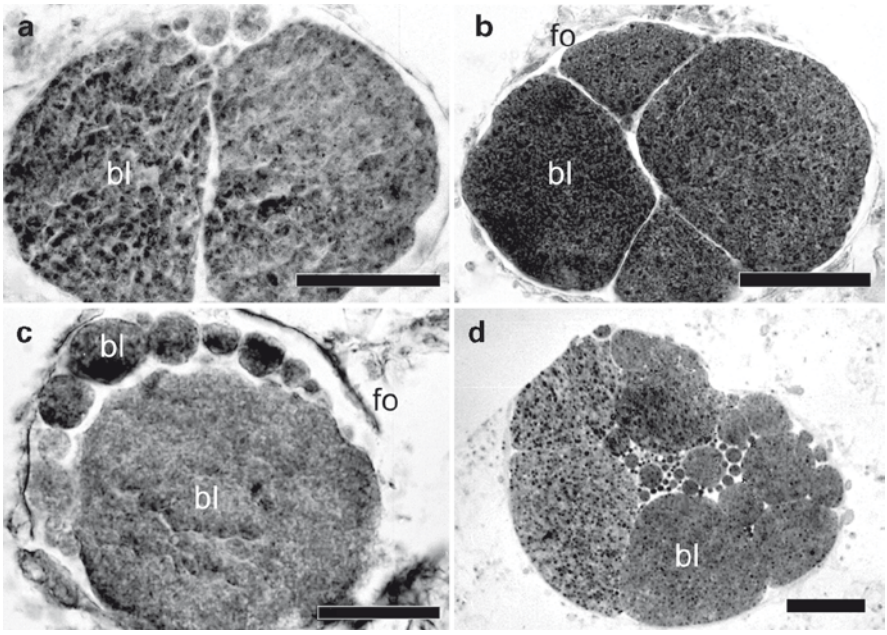

Fig. 3.69 Light microscopy of cleavage in Poecilosclerida. (**a**) Two-blastomeres stage in *Iophon piceum.* (**b**) Four-blastomeres stage in *I. piceum*. (**c**) Early stage of unequal cleavage in *I. piceum.* (**d**) Later stages of cleavage in *Phorbas topsenti. bl* blastomeres, *fo* follicle. Scale bars (**a**–**d**) 50 μm

Unequal cleavage results in formation of blastomeres of different size. There is polarization in their location (Fig. 3.69c, d): small blastomeres, dividing faster than the large ones, are situated at the periphery of the embryo. At the same time, cleaving macromeres proceed to separate micromeres. Cleavage cavity is not observed. However, in a carnivorous poecilosclerid *A. occidentalis*, which has a small egg (about 36 μm) with uniform yolk granules distribution, cleavage is holoblastic and equal (Riesgo et al. 2007b).

Cleavage results in formation of a multicellular, weakly polarized morula (stereoblastula) (Fig. 3.70a,). The cells in its internal part, which is closer to the former vegetative egg pole, are larger than those at the former animal pole or at the periphery. The cell cytoplasm is filled with rounded yolk inclusions of various sizes.

In many demosponges the cleaving embryo becomes much larger than the zygote just starting cleavage. It was explained by endocytosis of nutrients dissolved in the parent mesohyl (Simpson 1968); by penetration into the cleaving embryo of nucleolated amoebocytes (in *M. prolifera*; Simpson 1968) or of granular eosinophilic cells (in *I. piceum*; Ereskovsky 1986); by endogenous synthesizing activity or penetration of symbiotic cells into the embryo (Kaye 1991). During early cleavage in oviparous *H. ferox* and *N. nolitangere* the parent cells surrounding the egg penetrate deep into the embryo (Reiswig 1976; Hoppe and Reichert 1987).

3.10.3.1 Larval Morphogenesis

Poecilosclerid larval morphogenesis is poorly studied. In *M. rosacea* and *C. coralloides* cleavage results in a morula consisting of micro- and macromeres (Maas 1894), micromeres overgrowing macromeres during parenchymella formation. Micromeres differentiate into the surface ciliated cells, and macromeres, into amoebocytes, sclerocytes and other cells.

Embryonic development of other poecilosclerids is almost unstudied; there are only descriptions of skeleton formation during larval development in *Iophon radiatus* (Burton 1931), descriptions of some developmental stages of *Tedania charcoti* and *T. tenuicapitata* (Burton 1932), and some brief data on sexual reproduction in *M. prolifera* (Simpson 1968).

We will consider poecilosclerid development on the basis of our data on several species: *I. piceum*, *A. lobatus*, *M. incrustans*, *P. topsenti* and *Crellomima imparidens* (Ereskovsky 1986, 2005, unpublished). All morular cells have a nucleus with a nucleolus. Small peripheral cells appear to derive from micromeres; they are less densely packed with yolk granules and divide actively. As a result of cleavage divisions, the surface cells diminish progressively and loose the yolk granules (Fig. 3.70b). This process is most active at the future anterior larval pole.

A cursory glance at some sections at this stage gives an impression of epiboly. However, a more thorough examination of serial sections makes us reject this term. Epiboly is the overgrowth by the animal pole blastomeres of the vegetative ones due to an increased proliferation of the former (Fig. 3.71a, b) (Ivanova-Kazas

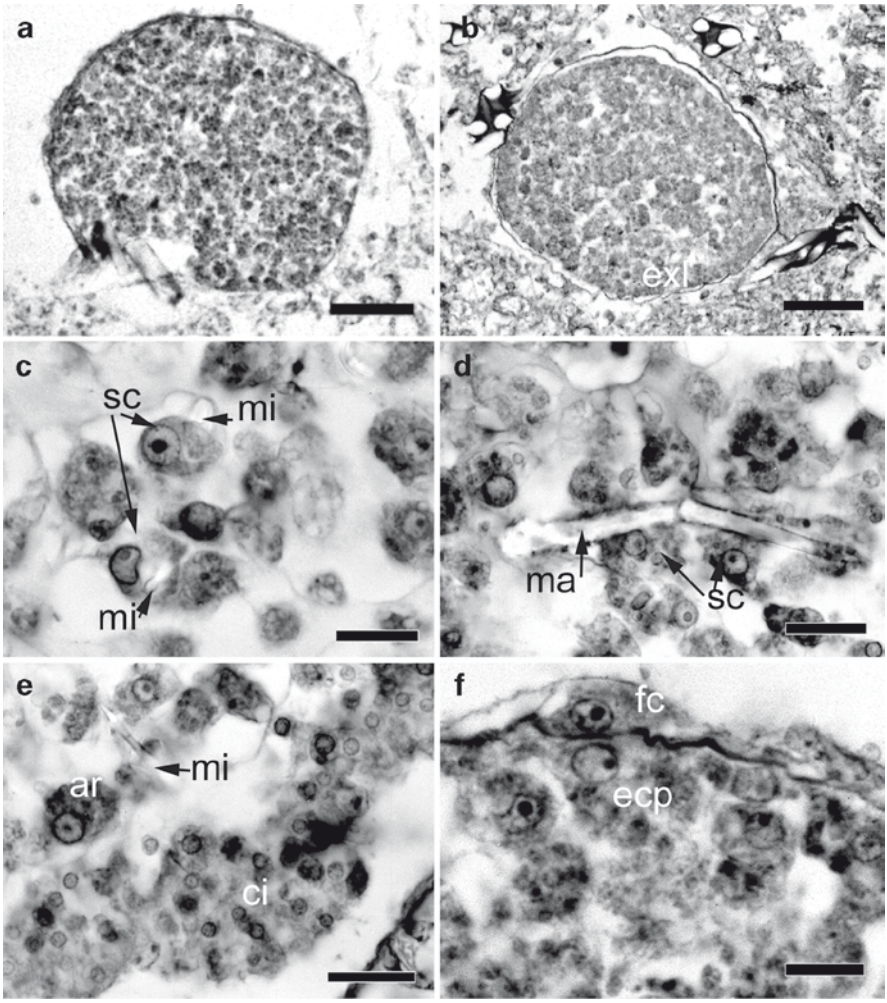

Fig. 3.70 Light microscopy of embryonic development in Poecilosclerida. (**a**) Morula of *Myxilla incrustans*. (**b**) Embryo of *Phorbas topsenti* during segregation of external larval layer (*exl*). (**c, d**) Larval sclerocytes of *Iophon piceum*, secreted a microscleres (*mi*) and a macroscleres (*ma*). (**e**) Differentiation of anterior-lateral ciliated larval cells in *I. piceum.* (**f**) Differentiation of posterior pole cells in *I. piceum. ar* archaeocytes, *ci* ciliated cells, *ecp* external cells of posterior pole, *fc* follicular cells, *sc* sclerocytes. Scale bars (**a**–**d**) 10 μm

1975, 1995). In our case, delamination occurs; it starts at one of the poles (probably, the animal one) and gradually spreads over the whole embryo. We called this process *polarized delamination* (Fig. 3.71c, d) (Ereskovsky 2005; Ereskovsky and Dondua 2006).

At early stage of larval morphogenesis in *I. piceum* and *A. lobatus* some cells of the peripheral zone differentiate into sclerocytes, which secrete microscleres. They

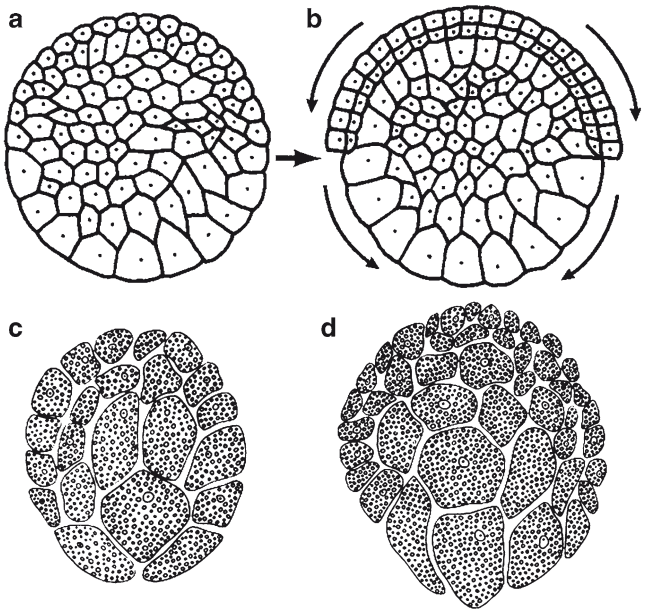

Fig. 3.71 Comparative diagram of epiboly (**a**, **b**) and polarized delamination (**c**, **d**) (**c**, **d** – From Maas 1894)

start to function before the differentiation of the surface ciliated cells. Microscleres are formed in vacuoles in the sclerocyte's cytoplasm (Fig. 3.70c), first one tip then the rest of the spicule. Macroscleres are formed as the result of secretory activity of several sclerocytes, more or less evenly arranged on their surface (Fig. 3.70d). In all poecilosclerids spicules emerge in the same order: first micro- and then macroscleres. However, we failed to establish the origin of sclerocytes exactly. The fact that they appear in the peripheral zone of the embryo, when the internal blastomeres are still filled with yolk, is an indirect evidence of their micromeric origin. The advanced differentiation of sclerocytes is also characteristic of the Halichondrida and Haplosclerida (Meewis 1941; Ereskovsky 1999).

At a more advanced stage, the surface cells are densely packed in several layers. Their cytoplasm becomes light and poor in inclusions, the nuclei diminish in size and are arranged at different levels (Fig. 3.70e, f). The cells polarize and develop a cilium. The embryo's surface enlarges due to active proliferation of surface cells. Since this process goes on in the closed space of the follicle, the surface epithelium forms numerous folds and invaginations (Fig. 3.72a, b).

During differentiation of the surface larval cells, the internal cells rich in yolk divide actively; some of them become sclerocytes, spongiocytes and collencytes. Other internal cells retain yolk inclusions and differentiate into nucleolated amoebocytes. Larval sclerocytes are motile rounded cells with a large vacuole inside which the spicule forms (Figs. 3.70c, d and 3.73). Spicule formation

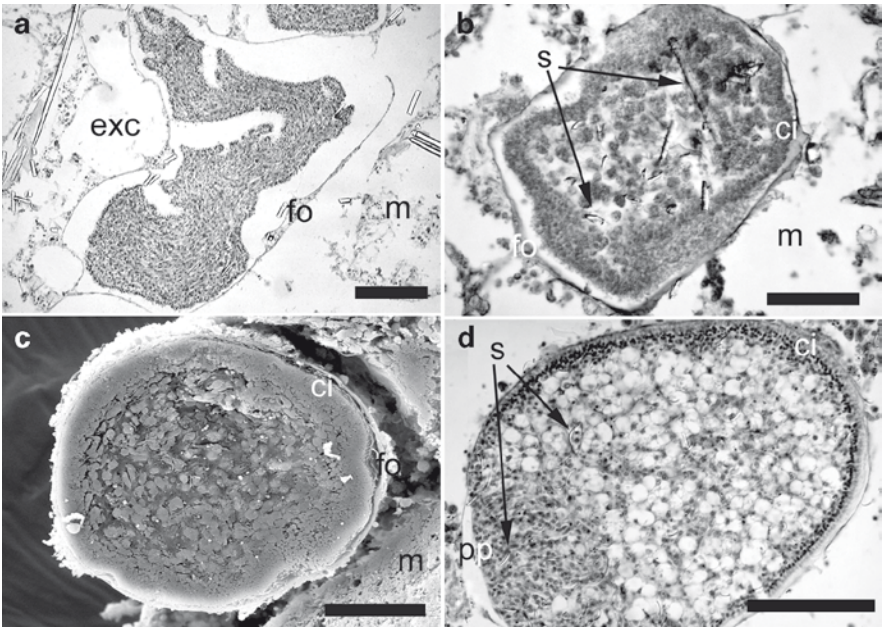

Fig. 3.72 Pre-larvae and larvae of Poecilosclerida in maternal sponge. (**a**) Light microscopy of pre-larva of *Amphilectus lobatus.* (**b**) Light microscopy of pre-larva of *Iophon piceum.* (**c**) SEM of larva of *Crellomima imparidens.* (**d**) Light microscopy of *Esperiopsis koltuni* larva. *ci* ciliated cells, *exc* exhalant canal, *fo* follicle, *m* mesohyl, *pp* posterior pole, *s* spicules. Scale bars (**a**) 50 μm, (**b**–**d**) 100 μm

sometimes results in the nucleus deformation. Spongiocytes are amoeboid cells with heterogeneous cytoplasm rich in inclusions and a large vesicular nucleus with a nucleolus. Collencytes are dendritic cells with finely granular cytoplasm and a nucleus with a small nucleolus. Nucleolated amoebocytes, or archaeocytes are large cells with amoeboid motility; they are rich in inclusions and have a rounded nucleus with a nucleolus. The cytoplasm is filled with large yolk granules and dense basophilic inclusions. By the end of morphogenesis the posterior part of the larva contains rounded cells with homogenous eosinophilic cytoplasm and rare basophilic inclusions. The nucleus lacks the nucleolus.

In the peripheral zone of the embryos of all the species studied rounded eosinophilic cells are found; their cytoplasm is reticular, sometimes with spherulous inclusions. Later these cells are traced among the ciliated surface cells or in the internal cell mass of the larva. Morphologically, these cells are identical to similar cells of the parent sponge. Granular cells were also noted in the developing larvae of *P. topsenti M. incrustans*, *I. piceum*, *Esperiopsis koltuni* and *C. imparidens* (Fig. 3.73c, d). During larval development of poecilosclerids the anterior–posterior axis of the larva appears to be the successor of the animal–vegetative axis of the egg, as suggested by Maas (1894).

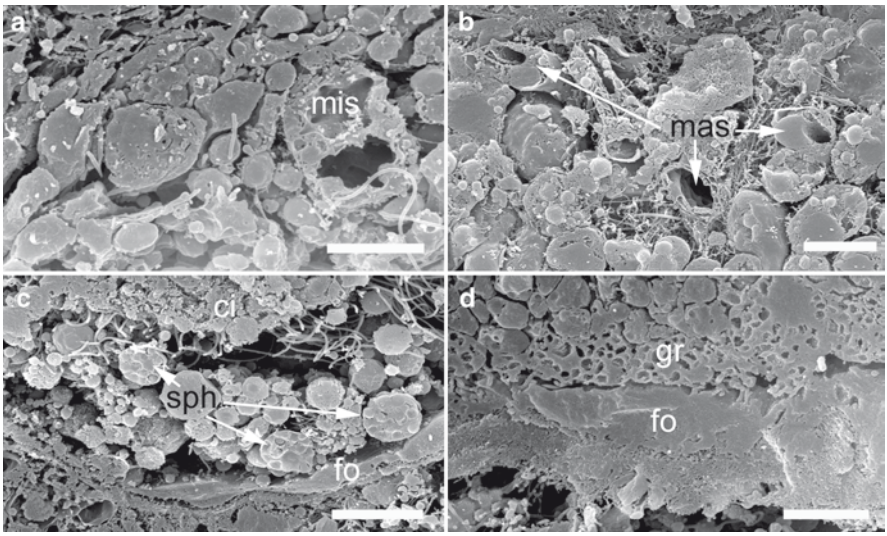

Fig. 3.73 SEM of larval cells in some Poecilosclerida. (**a**) Microsclerocyte (*mis*) of *Crellomima imparidens*. (**b**) Macrosclerocytes (*mas*) of *Hymedesmia irregularis*. (**c**) Spherulous (*sph*) and (**d**) granular (*gr*) cells of parent origin inside of the pre-larvae of *Crellomima imparidens*. *ci* ciliated cells, *fo* follicle. Scale bars **a**–**d** 5 μm

After larval formation, the cavity of the follicle and the nearest exhalant canal fuse and the parenchymella is released via the osculum into the environment.

In the carnivorous sponge *A. occidentalis* the outer layer of pre-larva is formed by multiciliated cells (Riesgo et al. 2007b). This is an unusual feature for the demosponges; multiciliated cells were known before only in the trichimella larvae of the hexactinellid *Oopsacas minuta* (Boury-Esnault et al. 1999). Contrary to the trichimellae, in *A. occidentalis* pre-larvae each cilium of the multiciliated cells has its own rootlet.

Sexual reproduction in poecilosclerids is not accompanied by a deep destruction of parent tissues (Ereskovsky 2000, 2005). This may be explained by the characteristic features of gamete development, by gametogenesis intensity, by involvement or non-involvement of choanocytes in the vitellogenesis and other aspects of interactions between the gametes and the somatic cells.

3.10.4 Larva

The larvae of poescilosclerids, parenchymellae, are very similar in appearance and structure in all the order representatives studied (Delage 1892; Maas 1894; Lundbeck 1905; Burton 1931, 1932; Lévi 1956, 1964; Backus 1964; Bergquist and

Sinclair 1968; Simpson 1968; Boury-Esnault 1976; Bergquist and Green 1977; Bergquist et al. 1979; Ereskovsky 1986, 2000; Jaeckle 1995a; Meroz and Ilan 1995; Ilan 1995; Uriz et al. 2001; Maldonado 2006; Ereskovsky and Willenz 2007). They are egg-shaped or oval, with a distended posterior hemisphere (Fig. 3.72c, d). Cilia of equal length cover the larva evenly except the posterior pole, which is formed by flattened or prismatic non-ciliated cells; there is no ring of transition cells with longer cilia. The ciliated part of the larva is brightly coloured, usually in red or yellow hues; the posterior pole is poorly pigmented or not pigmented at all; there is no larval cavity. A bundle of larval monaxon macroscleres united with spongin is present in the posterior third of the larva. It is oriented along the anterior–posterior axis (Fig. 3.74), with heads of spicules directed towards the anterior pole. Microscleres, often arranged in rosettes, are situated in the area of the posterior pole. Ciliated epithelium is pseudo-stratified. An unusual feature of carnivorous sponge *Esperiopsis koltuni* larva is the absence of megascleres (Ereskovsky and Willenz 2007). A layer of collencyte-like cells underlies the ciliated epithelium (Fig. 3.74).

The larva has most of the cells characteristic of the adult sponge except pinacocytes and choanocytes: ciliated cells, collencytes, nucleolated amoebocytes or archaeocytes, sclerocytes, vacuolated or granular cells, and spherulous cells (probably, of parent origin). *Hamigera hamigera* larvae also possess "gray cells" (Boury-Esnault 1976). Symbiotic bacteria, characteristic of the mesohyl of adult sponges, reside in the larval intercellular spaces. Near resemblance of all poecilosclerid larva is another argument in favor of the monophyletic origin of this group.

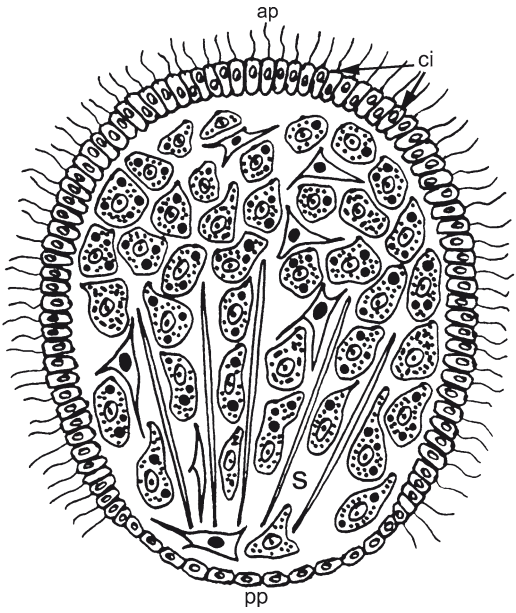

Fig. 3.74 Schematic drawing of Poecilosclerida parenchymella. *ap* anterior pole, *pp* posterior pole, *s* spicules

3.10.5 Metamorphosis

Metamorphosis in poescilosclerid larvae was first studied in *Esperella* (*Mycale*) *lovenzi* (Delage 1892), *Mycale syrinx* (Wilson 1935), *Tedania gurjanovae* (Backus 1964), *Mycale contarenii* (Borojevic, Lévi 1964, 1965; Borojevic 1966), *Microciona prolifera* (Simpson 1968; Misevic and Burger 1982, Misevic et al. 1990; Kaltenbach et al. 1999), *Hamigera hamigera* (Boury-Esnault 1976, 1977) and *Microciona rubens* (Bergquist and Green 1977).

Poecilosclerid parenchymellae, similarly to all demosponge larvae, settle anterior end first. Contacting the substrate, anterior pole cells excrete mucous substance (Bergquist and Green 1977). Immediately after settlement the ciliated epithelium and the posterior pole cells disintegrate; the ciliated cells migrate, cell by cell, inside the larva, and collencytes or archaeocytes migrate to the outside.

The cells on the outside flatten and differentiate in exopinacoderm and the substrate cells, into basopinacoderm. Exopinacocytes secrete a cuticle with high carbohydrate content (Bergquist and Green 1977). Basopinacocytes secrete a dense adhesive basal plate, which consists of proteins and has fibrillar structure (Bergquist and Green 1977). Only after that the basopinacocytes flatten on the basal plate, resembling in the process fibroblasts in a tissue culture. Thus, the boundary epithelia, the exo- and the basopinacoderm, are the first structures to be formed in the course of metamorphosis in poecilosclerids. The posterior pole larval cells inside the pupa are phagocyted by larval archaeocytes. Archaeocytes also ingest secretory cells (granular, vacuolar, spherulous ones).

What happens with the surface ciliated cells of poecilosclerid larvae during metamorphosis? At the light microscopic level, the answer seemed obvious: they transform into choanocytes (Delage 1892; Backus 1964; Simpson 1968). However, the results of electron microscopic studies cast doubt on the universality of this assumption.

In ciliated cells, migrating into post-larva, the cilium is drawn in and lyzed; the rootlet apparatus is also resorbed (Lévi 1964; Boury-Esnault 1976, 1977). Electron microscopic studies of normal metamorphosis, development of dissociated larvae and cultures of conglomerates of different cell types in *Mycale contarenii* (Mycalina) led to the conclusion that choanocytes originate from transdifferentiated ciliated cells (Borojevic and Lévi 1964, 1965; Borojevic 1966). The latter form spherical agglomerations with a cavity and later develop into choanocyte chambers (Fig. 3.75a). However, the same authors showed that in *M. contarenii* choanocytes also differentiate from archaeocytes (Borojevic and Lévi 1965).

In *H. hamigera* (Myxillina) nucleolated amoebocytes of the internal larval mass actively phagocyte migrating ciliated cells; their remains are often found in phagosomes (Boury-Esnault 1976). According to the author (Boury-Esnault 1976, 1977), the ciliated cells that have escaped phagocytosis may transform into choanoblasts and gray cells (Fig. 3.75b).

Ciliated cells of *Microciona* larvae (*M. prolifera* and *M. rubens*) (Microcionina) are a terminally differentiated provisional locomotory organ (Bergquist and Green 1977; Misevic and Burger 1982, Misevic et al. 1990; Kaltenbach et al. 1999). Experiments with ^{3}H-thymidine labeling showed that larval ciliated cells, migrating

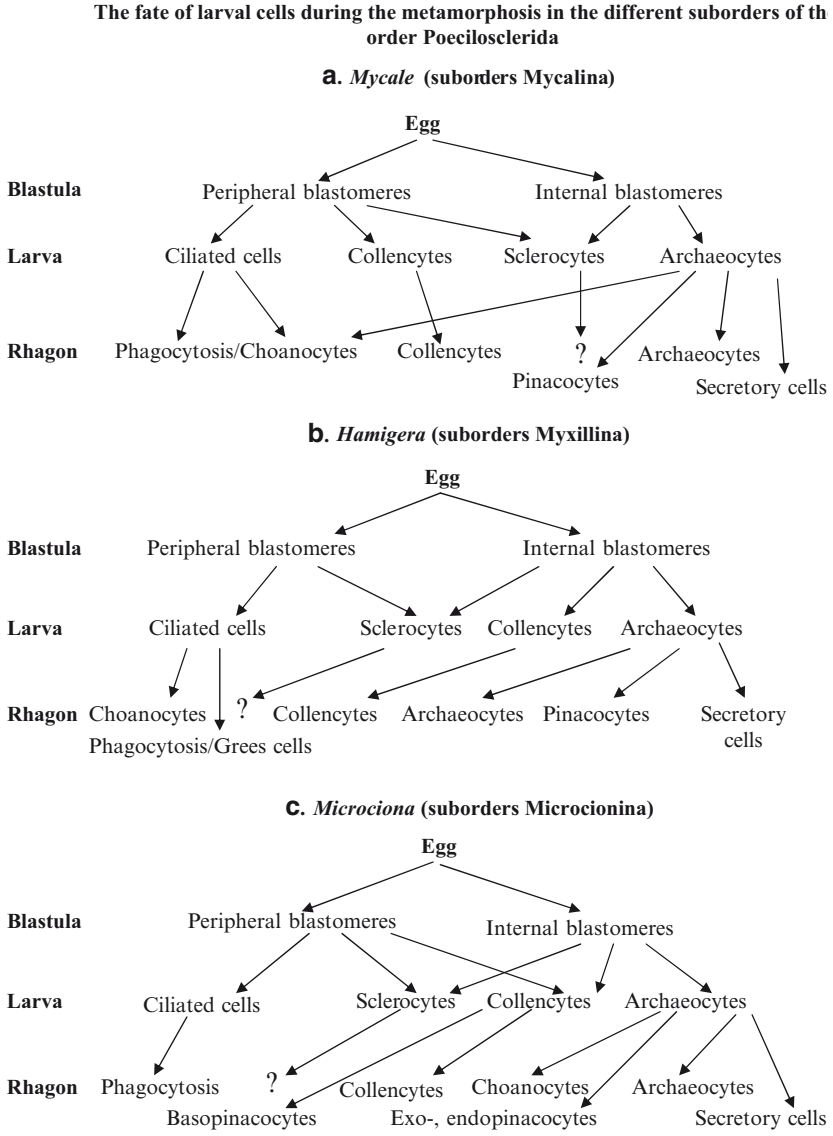

Fig. 3.75 The fate of larval cells during the metamorphosis in the different suborders of the order Poecilosclerida

into the pupa, did not differentiate into choanocytes; if this had been the case, they would have undergone a series of divisions, yet mitoses were not revealed (Misevic et al. 1990). After ^{125}I-label of ciliated cells, the tag was found either in archaeocyte phagosomes or in the mesohyl of the pupa but never in the forming choanocyte chambers (Misevic and Burger 1982, Misevic et al. 1990). It was shown

by molecular biological methods that during metamorphosis ciliated larval cells undergo apoptosis (Kaltenbach et al. 1999). Thus, in *Microciona* choanocytes develop from archaeocytes only (Fig. 3.75c).

To sum up, the general pattern of metamorphosis and its scenario are rather similar in all poecilosclerids. Phagocytosis of ciliated larval cells by archaeocytes or collencytes is another common feature. However, the sources of choanocytes may be different. In some sponges (*Mycale*, *Hamigera*) they develop both from ciliated cells and from archaeocytes, in others (*Microciona*), only from archaeocytes. In my opinion, these results are unlikely to be artifacts; neither are they contradictory. On the contrary, they prove once more the polipotency of sponge cell and a high lability of their genome at all ontogenetic stages.

3.10.6 Asexual Reproduction

In general, asexual reproduction is not characteristic of poecilosclerids (Fell 1993). It was described only in one species, *Mycale contarenii*, in the form of budding (DeVos 1965; Corriero et al. 1998). The bud is formed in the surface zone (ectosome) as a local protrusion of the body wall. Its development proceeds by migration, proliferation and differentiation of, mostly, the totipotent cells – archaeocytes. After the formation of definitive structures (the skeleton and the aquiferous system), the bud breaks from the parent sponge. On the whole, *M. contarenii* blastogenesis is epimorphic. The scenario of bud development is the same as those of larval metamorphosis and the sponge development from dissociated larvae (Borojevic and Lévi 1965, Borojevic 1966).

3.11 The Order Haplosclerida Topsent, 1928

Sponges from the order Haplosclerida are very diverse in shape and size (colour plate XI). The main skeleton is partly or completely made up by the isodictyal anisotropic or isotropic network with triangular, quadrangular or polygonal meshes (Fig. 3.76a, b). It is sometimes made up by porous reticular spongin, with or without spicules with single- or multispicule tracts. Macroscleres are represented by oxae or strongyles (Fig. 3.76c, d). Microscleres, if present, are represented by sigmas and/or smooth toxae, microoxae or microstrongyles (Fig. 3.76e–j); amphidiscs occur in one group. Spicules are not topographically differentiated.

The order Haplosclerida comprises both marine and freshwater sponges (van Soest and Hooper 2002). The order is subdivided into three suborders (Spongillina, Haplosclerina, Petrosina) and 13 families.

Most of them are ovoviviparous. Some haplosclerids are gonochoric, some are hermaphroditic; hermaphroditism may be simultaneous, successive or alternative (Sarà 1993). In the life cycle of many marine and most freshwater haplosclerids sexual reproduction is alternated with blastogenesis in the form of gemmule

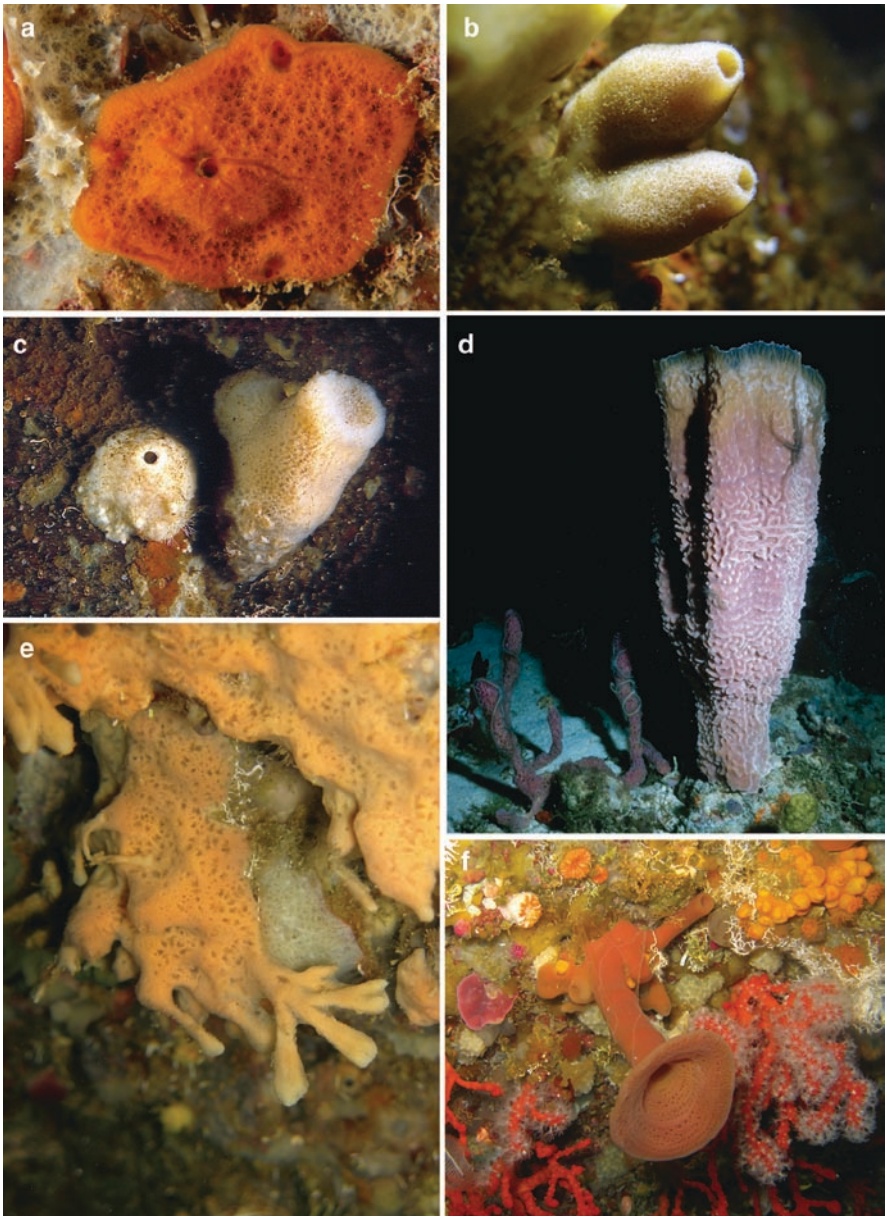

Plate XI Order Haplosclerida Topsent 1928 (**a**) *Haliclona fulva* (Topsent 1893), Mediterranean Sea (Courtesy of F. Zuberer). (**b**) *Haliclona* (*Reniera*) *aquaeductus* (Schmidt 1862), White Sea (Courtesy of N. Cherviakova). (**c**) *Gellius jugosus* (Bowerbank 1866), White Sea (Courtesy of M. Fedjuk). (**d**) *Callyspongia* (*Cladochalina*) *plicifera* (Lamarck 1814), Caribbean Sea (Courtesy of J. Vacelet). (**e**) *Haliclona (Soestella) mucosa* (Griessinger 1971), Mediterranean Sea (Courtesy of T. Perez). (**f**) *Haliclona* sp. Mediterranean Sea (Courtesy of T. Perez)

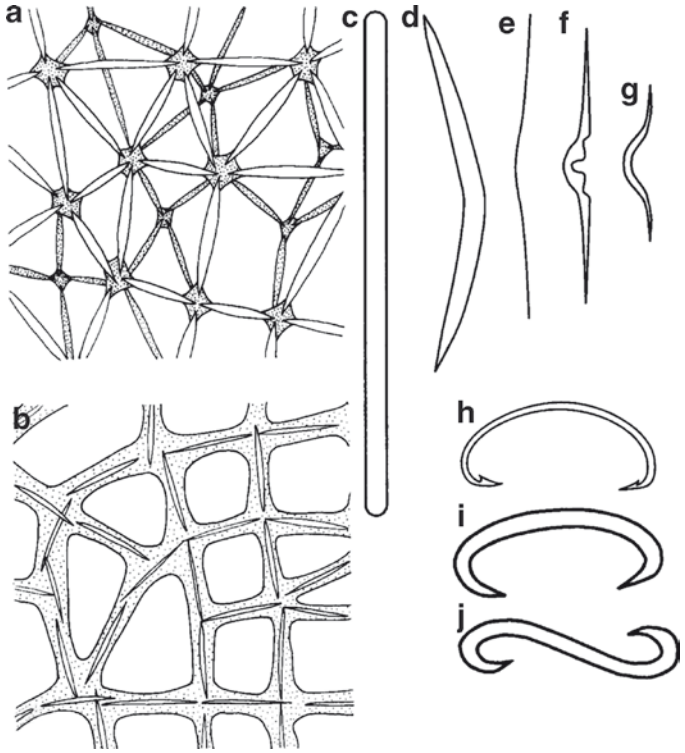

Fig. 3.76 Skeleton (**a, b**) and the spicules (**c**–**k**), typical for Haplosclerida. (**c**) Oxae. (**d**) Tylotes. (**e**–**g**) Toxae. (**h**–**k**) Sigmae

formation – the dormant stage for surviving adverse conditions such as frost or drought; they also serve for dispersion (Fell 1993).

According to the traditional morphological system, the order Haplosclerida is a sound monophyletic group (Van Soest and Hooper 2002). At the same time, molecular phylogenetic data testify to its paraphyly (McCormack et al. 2002; Borchiellini et al. 2004a; Raleigh et al. 2007; Redmond et al. 2007). In this chapter we adhere to the traditional system.

3.11.1 Gametogenesis

Spermatogenesis in Haplosclerida follows the scheme common for the Demospongiae (Reiswig 1983; Efremova 1988; Boury-Esnault and Jamiesson 1999). Male gametes develop in spermatocystes, which are agglomerations of spermatogenic cells enclosed in a single-layer capsule of flattened pinacocyte-like cells (Sukhodolskaya and Papkovskaya 1985; Paulus 1989). Size and number of spermatocystes varies both within the sponge and within the population. Spermatocystes are diffused in the

choanosome, often in its basal part. Development is usually synchronous in a spermatocyste, but gametes in neighbouring cysts may be at different stages. In freshwater haplosclerids, as in all the demosponges investigated with the exception of carnivorous poecilosclerids, male gametes are derived from choanocytes of the choanocyte chambers (Efremova and Papkovskaya 1980; Sukhodolskaya and Papkovskaya 1985; Paulus and Weissenfels 1986; Paulus 1989; Weissenfels 1989); marine haplosclerids have not been studied in this subject.

Mature spermatozoids belong to the primitive type (Reunov 2005). The cytoplasmic volume is large; there are numerous small mitochondria; the acrosome is lacking. Spermatozoids of freshwater haplosclerids (families Lubomirskiidae and Spongillidae) are constructed along a similar pattern. They differ from male gametes of other demosponges in several characteristic features: the basal part of the flagellum is situated in a narrow canal-like invagination of the cell surface; mitochondria are clustered around this canal; the nucleus and the short kinetosome are situated in the proximal part of the cell (Fig. 3.77) (Efremova and Papkovskaya 1980; Sukhodolskaya and Papkovskaya 1985; Paulus and Weissenfels 1986; Paulus 1989; Weissenfels 1989;

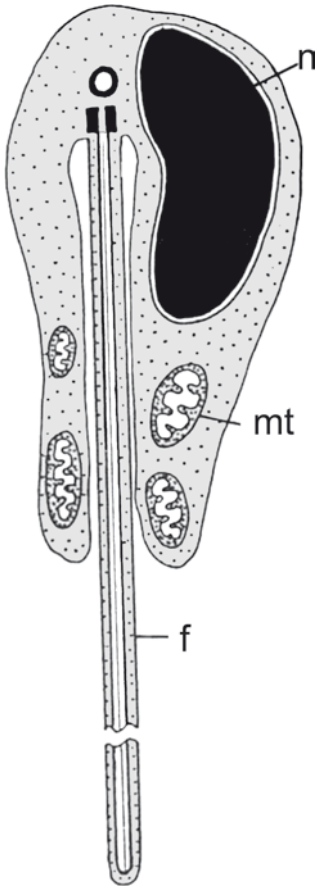

Fig. 3.77 General scheme of Haplosclerida spermatozoid. *f* flagellum, *mt* mitochondria, *n* nucleus

Ereskovsky 1999). Unfortunately, spermatogenesis in marine haplosclerids has not been studied at the ultrastructural level.

At the end of spermatogenesis, spermatocystes fuse with exhalant canals. Mature spermatozoids are released into the canals' lumens and carried out with water into the environment. Fertilization has not been investigated.

Oogenesis. It is considered that the source of female gametes in Haplosclerida is nucleolated amoebocytes with basophilic cytoplasm and a large nucleus (archaeocytes) or choanocytes. In any case, early oocytes pass the amoeboid stage. Female gametes are diffused in the choanosome, often in its basal part. Archaeocyte origin of female gametes is shown only at light microscopy in freshwater sponges from the families Spongillidae and Potamolepidae: *Spongilla lacustris*, *Ephydatia fluviatilis*, *Potamolepis stendelli* (Meewis 1936; Leveaux 1941, 1942; Brien 1967a, b; Saller and Weissenfels 1985; Saller 1988; Weissenfels 1989) and in marine sponges from the family Chalinidae: *Haliclona (Reniera) elegans* and *Haliclona simulans* (Tuzet 1932). In sponges from the Lake Baikal, *Lubomirskia baikalensis*, *Baikalospongia bacillifera* and *Swartschewskia papyracea* (Lubomirskiidae), oocytes are supposed to have choanocyte origin (Alexeeva and Efremova 1986).

A characteristic feature of both marine and freshwater haplosclerids is the appearance of "nurse cells" very early in the oogenesis. Some nucleolated amoebocytes of the mesohyl become much larger; their cytoplasm is filled with large phagosomes, numerous granules, lipid inclusions and free ribosomes (but not RER) (Fig. 3.78). These cells, gradually more and more numerous, group around

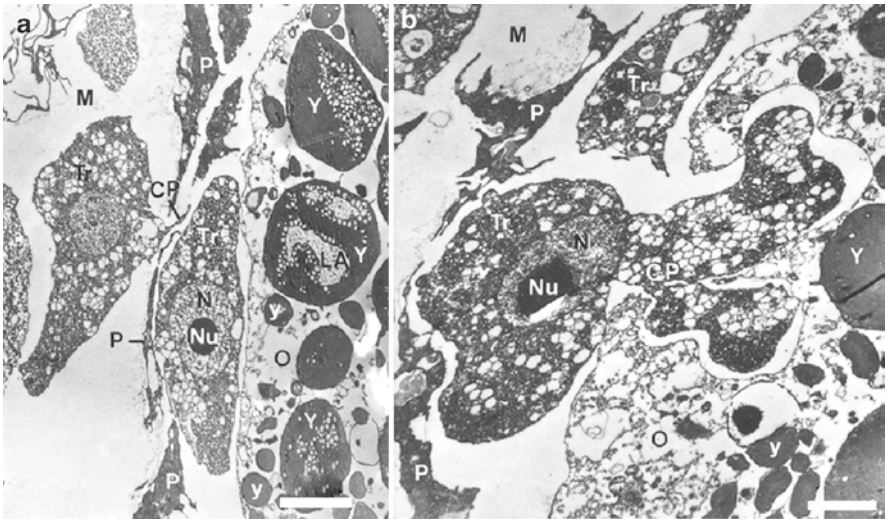

Fig. 3.78 TEM of peripheral zone of an oocyte (*O*) in the second growth phase in *Ephydatia fluviatilis*. (**a**) Trophocyte (*Tr*) between follicle and oocyte (*O*). Another trophocyte sends a cell process (*CP*) through the pinacocytes (*P*) of the follicle epithelium. (**b**) Phagocytosis of a trophocyte (*Tr*) by the oocyte (*O*). Articulated cell process (*CP*) of the trophocyte. *N* nucleus, *Nu* nucleolus, *Y* spherical yolk, *y* granular yolk, *LA* low-contrasted area, *M* mesohyl (From Saller 1988, Zoomorphology, vol. 108, pp. 24, Fig. 2, reproduced by permission of Springer). Scale bars (**a**) 4 μm, (**b**) 3 μm

the developing oocytes (Brien and Meewis 1938; Leveaux 1941; Brien 1967a, b; Fell 1969; Simpson and Gilbert 1973; Efremova 1981; Saller and Weissenfels 1985; Saller 1988; Mukai 1989; Weissenfels 1989; Ilan and Loya 1990; Leys and Degnan 2002). They are often referred to as "*nurse cells*" or "*trophocytes*" (Fell 1983; Simpson 1984; Weissenfels 1989). In this chapter we will use the term "*trophocytes*", meaning the somatic cells that are the source of yolk inclusions during oogenesis and gemmulogenesis in haplosclerids. Their presence shows a higher specialization of oogenesis in haplosclerids as compared to other demosponges orders.

A previtellogenic oocyte has amoeboid motility and move actively in the mesohyl, phagocyting trophocytes with its broad lobopodia and increasing rapidly in size (Fig. 3.79a). In haplosclerids the oocyte only phagocytes whole, intact trophocytes. Other interactions between the oocyte and the trophocytes, known in other demosponges orders, have not been described. At this stage the oocyte also actively performed endogenous synthesis, which is indicated by a considerable amount of RER and a developed Golgi apparatus (Efremova 1981; Saller and Weissenfels 1985; Saller 1988).

Before the start of vitellogenesis, the oocyte stops near an exhalant canal of the aquiferous system in the middle or basal part of the choanosome. There it is surrounded with a follicle: a layer of flattened cells of pinacocyte or collencyte origin (Fig. 3.79b). This process is known in many Spongillidae (*S. lacustris*, *E. fluviatilis*, *E. muelleri*, *Eunapius fragilis*, *Radiospongilla cerebellata*), Potamolepidae (*P. stendelli*, *Malawispongia echinata*), Chalinidae (*Haliclona ecbasis*, *H. loosanoffi*, *H. permolis*, *H. aquaeductus*, *H. gracilis*, *Chalinula* sp., *Gellius* sp.) and Lubomirskiidae (*L. baikalensis*, *B. bacillifera*, *S. papyracea*) (Leveaux 1941; Brien 1967a, b, 1973a; Fell 1969, 1974a, 1983; Efremova 1981; Saller and Weissenfels 1985; Saller 1988; Mukai 1989; Weissenfels 1989; Ilan and Loya 1990; Gaino et al. 2003; Ereskovsky 2005). Trophocytes increase in number, penetrate the follicle wall and are incorporated into the ooplasm.

In some marine haplosclerids the trophocytes incorporated into the ooplasm almost do not undergo cytolysis (Fig. 3.79c). For instance, in *H. ecbasis* and *H. loosanoffi* the nuclei of such cells degenerate, but their cytoplasm remains almost unchanged (Fell 1969, 1974a, 1976). In *H. aquaeductus* and *H. gracilis* the membranes of the captured cells are destroyed and the cytoplasm is transformed into large granules, yet nuclei with nucleoli remain intact in these granules for a long time (Ereskovsky 2005).

By the end of vitellogenesis the egg of marine haplosclerids is completely filled with partly digested trophocytes (Fig. 3.79e) and the egg of freshwater ones, with yolk inclusions of various size and content. Yolk inclusions are divided into granular ones in the central, perinuclear space and larger spherical ones in the periphery (Saller and Weissenfels 1985; Saller 1988; Mukai 1989) (Fig. 3.79d). By that time only the follicle remains around the egg (Fell 1983). If the trophocytes are almost undestroyed, the egg looks like a morula-like cell mass Fell (1974a, 1983) thought that trophocytes were still metabolically active even in the egg.

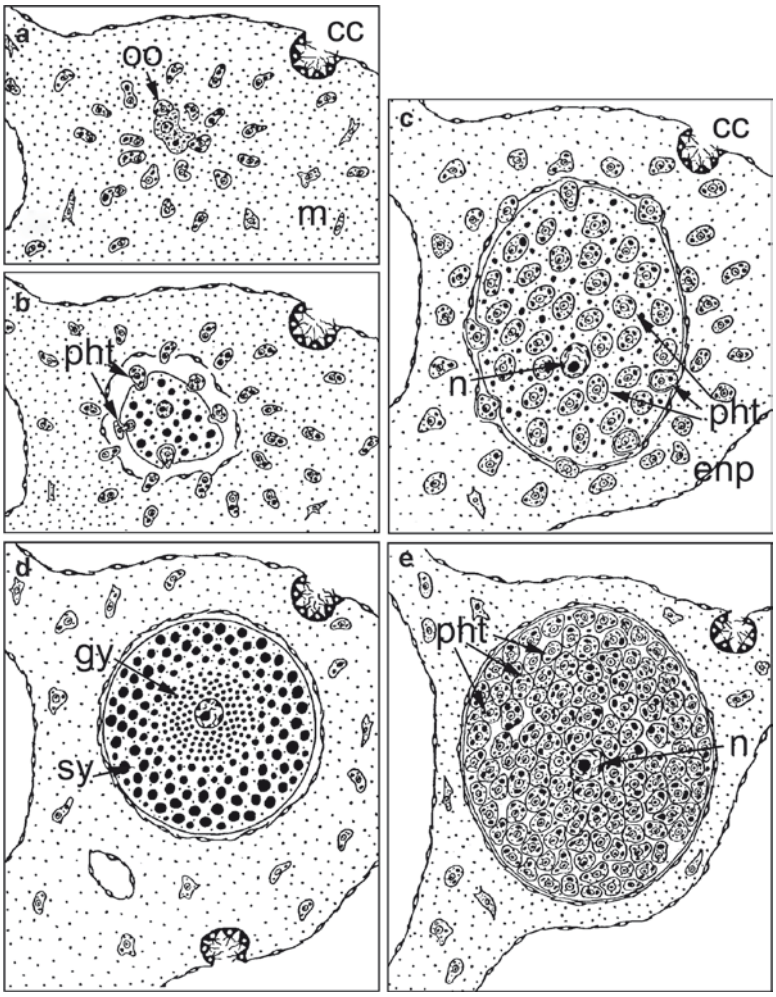

Fig. 3.79 Generalized scheme of freshwater and marine Haplosclerida oogenesis. (**a**) Stage of cytoplasmic growth. (**b**) Beginning of the vitellogenesis. (**c**) Vitellogenesis. (**d**) Egg of freshwater Haplosclerida. (**e**) Egg of marine Haplosclerida. *cc* choanocyte chamber, *enp* endopinacoderm, *gy* granular yolk inclusions, *m* mesohyl, *n* nucleus, *oo* oocyte, *pht* phagocyted trophocyte, *sy* spherular yolk inclusions, *yg* yolk granules (From Ereskovsky 1999, Russ. J. Mar. Biol. vol. 25, pp. 363, Fig. 2, reproduced by permission of Springer)

3.11.2 Embryonic Development

3.11.2.1 Cleavage

In general, haplosclerids, similarly to all the demosponges, have a total, unequal and asynchronous cleavage (Brien 1973a; Fell 1974a, 1989; Korotkova 1981b; Simpson 1984; Ereskovsky 1999). However, cleavage is unequal in *Chalinula fertilis*,

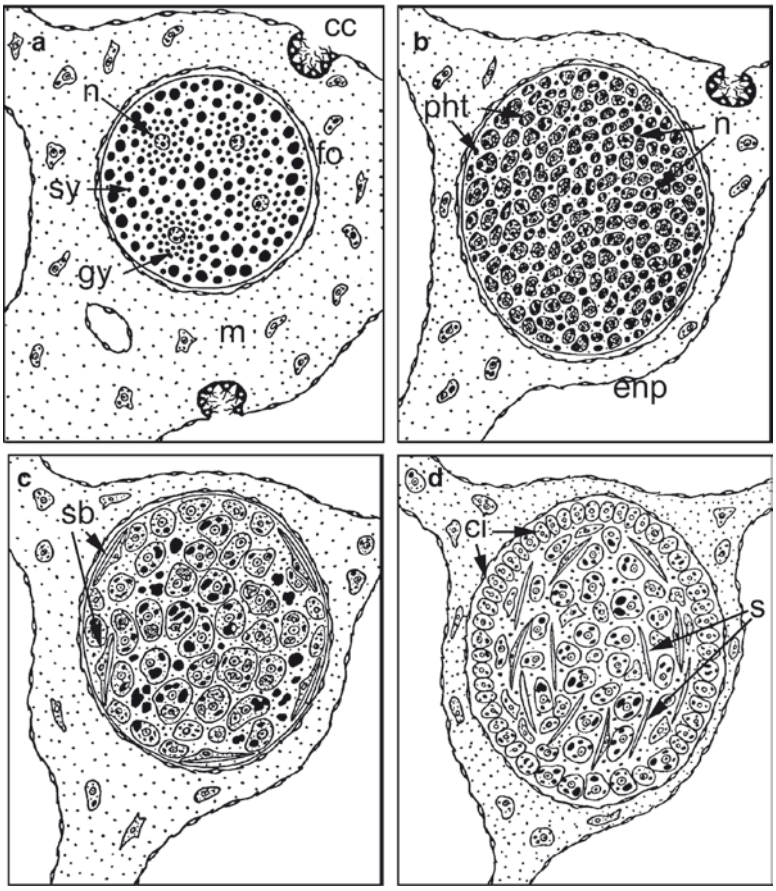

Fig. 3.80 Generalized scheme of freshwater and marine Haplosclerida embryogenesis. (**a**) Early cleavage of freshwater Haplosclerida, family Spongillidae. (**b**) Cleavage of marine Haplosclerida. (**c**) Morula, beginning of embryo cytodifferentiation; larval sclerocyte differentiation. (**d**) Beginning of differentiation of larval ciliated cells. *bl* blastomeres, *cc* choanocyte chamber, *ci* differentiated ciliated cells, *enp* endopinacocyte, *fo* follicle, *gy* granular yolk inclusions, *m* mesohyl, *n* nucleus, *pht* phagocyted trophocyte, *s* larval spicules, *sb* scleroblast, *sy* spherular yolk inclusions (From Ereskovsky 1999, Russ. J. Mar. Biol. vol. 25, pp. 365, Fig. 3, reproduced by permission of Springer)

some African freshwater sponges (*P. stendelli*, *M. echinata*) and in freshwater sponges from the Lake Baikal (Keller 1880; Maas 1894; Brien 1967b, 1973b; Ropshtorp and Reitner 1994). In marine haplosclerids cleavage is difficult to study because the egg and then the blastomeres are filled with large yolk granules or trophocytes at various stages of digestion (Fig. 3.80a, b). These inclusions mask the dividing nuclei and the borders between the blastomeres. For instance, the early cleaving embryo of *H. ecbasis* is morphologically indistinguishable from the mature egg (Fell 1969); cleavage nuclei become visible only when there are 15–20

of them and cells borders, only in the morula consisting of about 1,000 cells (Fell 1969). In *H. aquaeductus* cleavage nuclei and borders between blastomeres can be distinguished only at the morula stage (Ereskovsky 2005).

Early cleavage in some freshwater haplosclerids (*E. fluviatilis*, *S. lacustris*) is not accompanied by cytotomy. The egg nucleus, situated centrally, undergoes one or two fissions and several nuclei are formed; they have equal size and are surrounded with small granular inclusions (Fig. 3.80a). Cytotomy occurs later and the resulting blastomeres differ in size (Fig. 3.80b) (Brien and Meewis 1938; Saller and Weissenfels 1985; Saller 1988; Weissenfels 1989).

3.11.2.2 Larval Morphogenesis

Cleavage results in a morula (Fig. 3.80c). At this stage active utilization of the trophocytes fragments or phagosomes and the formation of yolk granules starts in marine haplosclerids. The first indications of cytodifferentiations can be seen (Fig. 3.80c, d).

The morula is the only stage where any homologies between the embryogenesis of sponges and other multicellular animals can be traced (see Chapter 6). Instead of germ layers' formation, differentiation of cell forming provisory larval structures starts. The surface cells of haplosclerids embryo, proliferating actively, progressively diminish in size and loose yolk inclusions; the nucleolus disappears in the nucleus. Finally, the embryo segregates into two layers, the external and the internal one (Fig. 3.80d). This process resembles *morula delamination*. In the case of an unequal morula, *polarized delamination* is observed: starting at one of the pole, it spreads to the other one (Maas 1894; Brien and Meewis 1938; Meewis 1941). Though this process is outwardly similar to the epiboly, cells do not overgrow other cells.

In many haplosclerid embryos the first cells to differentiate and start functioning are sclerocytes, the cells secreting larval spicules (Fig. 3.81c). Their differentiation starts immediately after digestion of the trophocytes fragments or yolk granules by the morula cells (Brien and Meewis 1938; Meewis 1941; Brien 1967a, b, 1969, 1973a, b; Fell 1969; Saller 1988; Leys and Degnan 2002; Leys 2003b). The first spicules may be located in the periphery of the embryo (*Haliclona cinerea*, *H. ecbasis*, *H. aquaeductus*) (Fig. 3.80c, d), in its centre (*E. fluviatilis*, *S. lacustris*) or throughout it (*P. stendelli*, *Corvospongilla thysi*). Future ciliated cells also begin to differentiate at this stage (Fig. 3.81a, b).

However, Leys and Degnan (2002) consider that in *Amphimedon queenslandica* the first differentiation is associated with the formation of a lineage of surface cell, "micromeres", which later become the surface ciliated cells and the flattened cells underlying the ciliated epithelium. Noteworthy, pigmented ciliated cells migrate towards the further posterior pole, revealing the anterior–posterior polarity of the larva (Leys and Degnan 2002). In *A. queenslandica* an extensive range of metazoan transcription factor genes, including members of the ANTP class (outside Hox, ParaHox, and extended-Hox clades), Pax, POU, LIM-HD, Sox, nuclear receptor (NR),

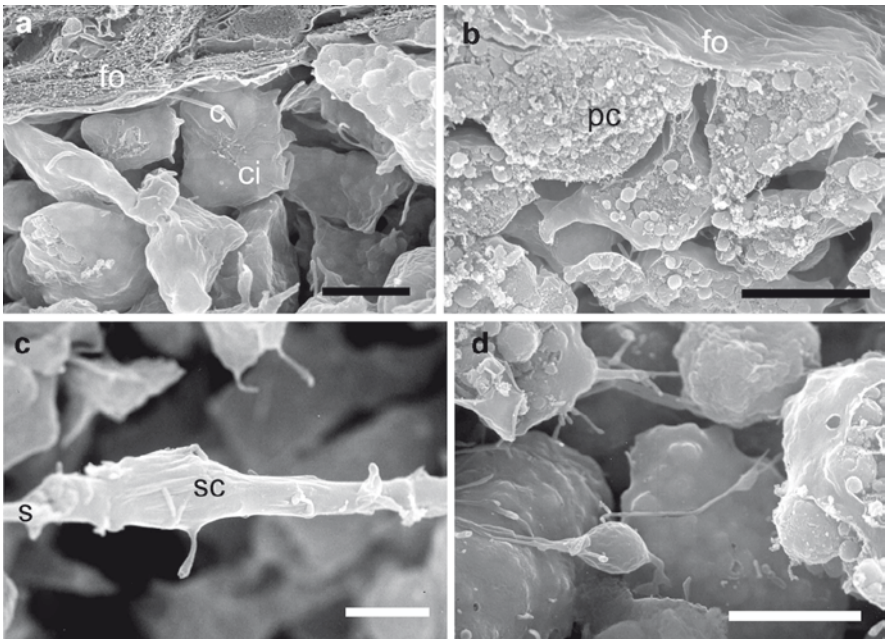

Fig. 3.81 SEM of differentiated larval cells in the embryo of *Haliclona aquaeductus*. (**a**) Ciliated cells differentiation from lateral larval zone. (**b**) Ciliated cells differentiation from larval posterior pole. (**c**) Larval sclerocyte. (**d**) Internal cells. *c* cilium, *ci* ciliated cell, *fo* follicle, *pc* peripheral cells, *s* spicule, *sc* sclerocyte. Scale bars (**a**) 50 μm, (**b**, **d**) 5 μm, (**c**) 10 μm

Fox (forkhead), T-box, Mef2, and Ets gene classes, is expressed during larval morphogenesis, sometimes in a cell-lineage-restricted manner (Larroux et al. 2006).

In the course of proliferation of the ciliated cells, the surface area of the prelarva increases and folds are formed.

3.11.3 Larva

Haplosclerid larva is parenchymella. In the larvae of marine haplosclerids the non-ciliated posterior pole is surrounded with a ring of cells with longer cilia than elsewhere at the larval surface; the pigment concentrates either in non-ciliated posterior pole cells or in the ring of cells surrounding the posterior pole; the skeleton in the posterior part of the larva is represented by a dense bundle of oxae (Fig. 3.82b; 3.83a). These characters have a diagnostic value in sponge systematic (Keller 1880; Maas 1894; Bergquist et al. 1979; Simpson 1984; Wapstra and van Soest 1987; Woollacott 1993; Fromont 1994; Ereskovsky 1999, 2005; Leys and Degnan 2001).

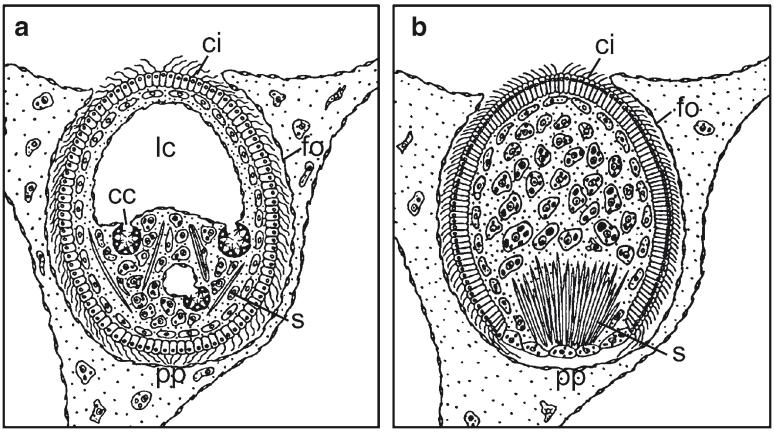

Fig. 3.82 Comparative diagram of freshwater (**a**) and marine (**b**) Haplosclerida larvae in a follicle. *cc* larval choanocyte chamber, *ci* ciliated cells, *fo* follicle, *lc* larval cavity, pp posterior pole, *s* larval spicules. (From: Ereskovsky 1999, Russ. J. Mar. Biol. vol. 25, pp. 366, Fig. 4, reproduced by permission of Springer)

Parenchymellae of freshwater sponges from the suborder Spongillina have a number of characters (Fig. 3.82a) that make them different from the parenchymellae of both marine haplosclerids (Fig. 3.82b) and all other demosponges. First of all, their surface, including the posterior pole, is completely and uniformly ciliated. Secondly, their larval skeleton is fan-shaped at cross-section. Thirdly, they have a vast cavity lined with larval pinacoderm in their anterior part, as well as larval choanocyte chambers (Fig. 3.82a). These larval structures are described in *E. fragilis*, *C. thysi*, *Ochridaspongia rotunda*, *S. lacustris*, *E. fluviatilis*, *E. muelleri*, *R. cerebellata*, *S. moorei*, *P. stendelli*, *M. echinata* (Spongillidae) (Brien and Meewis 1938; Brien 1970, 1973a, b; Harrison and Cowden 1975; Gilbert and Hadzisci 1977; Saller and Weissenfels 1985; Saller 1988; Sukhodolskaya and Ivanova 1988; Mukai 1989; Ivanova 1997a; Gaino et al. 2003), *L. baikalensis*, *B. bacillifera*, *S. papyracea* (Lubomirskiidae) (Efremova and Efremov 1979; Alexeeva 1980; Efremova 1981). Among marine haplosclerids larval choanocyte chambers are known only in *Chalinula* sp. (Ilan and Loya 1990). In the larvae of African freshwater sponges from the family Potamolepidae there are no choanocyte chambers but cavities do occur (Brien 1967a, b, 1970).

Cavities are sometimes considered to be a flotation organ (Maldonado 2006), but there is no direct evidence of that. Since cavities are present only in freshwater sponges, they are more likely to be an organ of osmoregulation. The larval cavity is shown to contain neutral mucin and basic proteins (Harrison and Cowden 1975), which also points to an osmoregulatory function.

Cell composition of the haplosclerid parenchymellae, especially in freshwater species, appears to be the most diverse in comparison with the parenchymellae from other demosponges orders. Their surface epithelium consists of ciliated cells

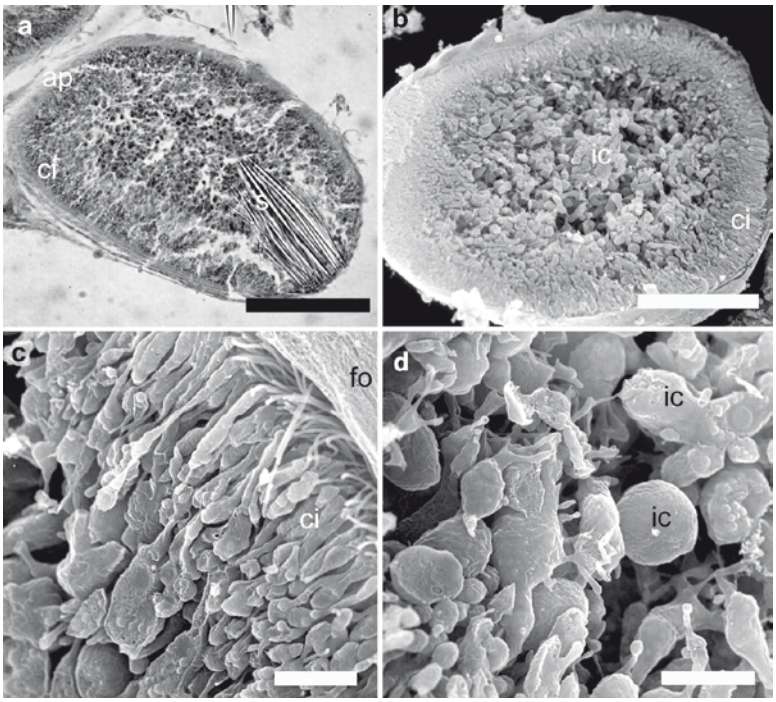

Fig. 3.83 Light microscopy (**a**) and SEM (**b**) of *Haliclona aquaeductus* larvae during leaving of follicle to exhalant canal (**a**, **b**). (**c**) SEM of ciliated larval cells. (**d**) SEM of internal larval cells. *ap* anterior pole, *ci* ciliated cells, *fo* follicle, *ic* internal cells, *s* spicules. Scale bars (**a**, **b**) 50 μm, (**c**, **d**) 5 μm

(Fig. 3.83a,b, c). In marine haplosclerids it also includes secretory globular ciliated cells (Woollacott 1993), large mucous cells and flask-shaped ciliated cells (Leys and Degnan 2001). The globular cells (originally annotated as mucous cells) and flask cells of *A. queenslandica* larva have molecular characteristics strongly suggestive of a role in sensing the environment. In particular they express the postsynaptic orthologs and a gene *AmqbHLH1* with conserved proneural activity (Sakarya et al. 2007; Richards et al. 2008). As the environmental sensing these cells, require membrane specializations and perhaps are a cell type that served as a starting point for the evolution of neurons (Sakarya et al. 2007).

The surface epithelium is underlain with a collencyte layer, which in marine haploclerids often incorporates special vesicular cells. The posterior pole consists of non-ciliated archaeocyte-like cells. Inside the larva there are many archaeocytes; there are also sclerocytes, granular eosinophilic cells (Fig. 3.83d) and gray cells, as well as choanocytes and endopinacocytes lining cavities and canals (Sukhodolskaya and Ivanova 1988; Woollacott 1993; Amano and Hori 1994; Weissenfels 1989; Ivanova 1997a; Leys and Degnan 2001). Parenchymellae cells are strikingly similar to the cells of the definitive sponge of the same species.

Variability of the internal larval structure is described only in Spongillidae, where it is associated with acceleration of development and preparation to metamorphosis (Ivanova 1997a). Spongillid larval morphological types differ in the number of internal cells and the degree of their differentiation.

Parenchymellae before settlement are sometimes considered as non-attached definitive sponges (Simpson 1984). In our opinion, this viewpoint is unjustified. Parenchymellae of haplosclerids, even of freshwater ones, are true larvae, with all their characteristic features. Firstly, they possess all the typical provisory organs, disappearing during metamorphosis: terminally differentiated ciliated epithelium (locomotory organ), larval skeleton of provisory spicules and larval choanocyte chambers; freshwater sponges also have an osmoregulatory organ lined with pinacoderm. Secondly, parenchymellae are free-swimming organisms, occupying a different ecological niche from adult sponges. Parenchymellae have an almost complete set of specialized cells, some of which function already in the larva: spicules and collagen fibres are secreted.

To remind, sponges with such parenchymellae inhabit fresh or brackish waters. These advanced larvae may have formed as a result of heterochrony and may be considered as an adaptation to a rapid metamorphosis and a fast "switching on" of the aquiferous system.

3.11.4 Metamorphosis

Metamorphosis has been most thoroughly studied in freshwater haplosclerids from the suborder Spongillina, but there are also are two detailed investigations of marine suborder Haplosclerina (Amano and Hori 1996; Leys and Degnan 2002). As in most investigated demosponges, the larvae settle anterior pole first (Fig. 3.84a) and attach to the substrate by means of cell-substrate adhesion and surface glycocalyx. Immediately after the settlement the larva disintegrates.

Metamorphosis of freshwater larvae is rather fast. It is often accompanied by a partial or complete elimination of provisory ciliated cells, which are phagocyted by the collencytes or amoebocytes underlining them (Fig. 3.85a). The amoebocytes later develop into exopinacocytes. Larval choanocyte chambers continue functioning. Adult choanocytes develop by active proliferation of larval archaeocytes (Fig. 3.84b), which subsequently aggregate into clusters. Archaeocytes in them differentiate into choanoblasts, and the clusters themselves become choanocyte chambers. New populations of sclerocytes secrete definitive spicules (Fig. 3.84c). Other definitive cells differentiate from larval archaeocytes (Brien and Meewis 1938; Meewis 1939; Harrison and Cowden 1975; Efremova and Efremov 1979; Efremova 1981; Weissenfels 1989). However, in *Haliclona permolis*, *Amphimedon queenslandica* and some Spongillidae choanocytes of the young sponges form partly by transformation of larval ciliated cells (Amano and Hori 1996; Ivanova 1997b; Leys and Degnan 2002). Metamorphosis is completed by the formation of a common aquiferous system. As the sponge grows, new modules of this system are formed (Fig. 3.85b).

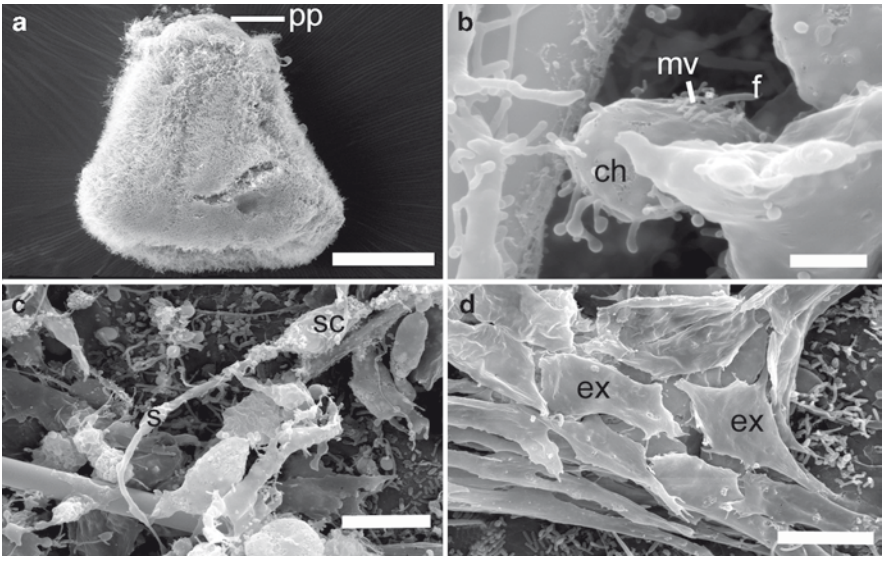

Fig. 3.84 SEM of metamorphosis in *Haliclona aqueductus*. (**a**) Larva attached to the substrate by anterior pole. (**b**–**d**) Cell differentiation in metamorphosed larva. (**b**) Differentiated choanoblast (*ch*). (**c**) Sclerocyte (*sc*) during spicule (*s*) formation. (**d**) Differentiation of the exopinacocytes (*ex*). *f* flagellum, *mv* microvilli, *pp* posterior pole. Scale bars (**a**) 100 μm, (**b**) 2 μm, (**c**, **d**) 10 μm

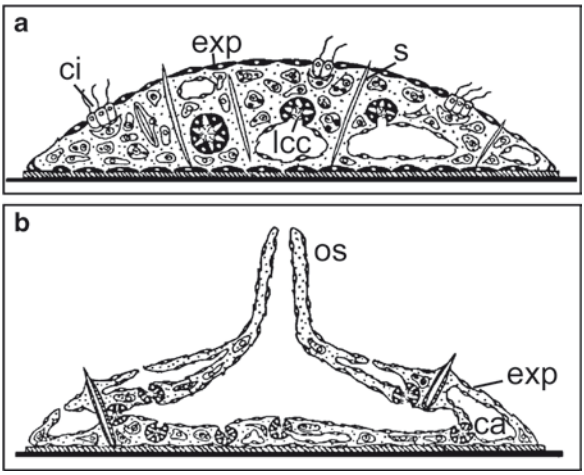

Fig. 3.85 Diagram of metamorphosed parenchymella of Haplosclerida at the early stage (**a**) and at the rhagon stage (**b**). *ca* canals of aquiferous system, *ci* larval ciliated cells, *exp* exopinacoderm, *lcc* larval choanocyte chamber, *os* osculum, *s* larval spicules (From Ereskovsky 1999, Russ. J. Mar. Biol. vol. 25, pp. 366, Fig. 5, reproduced by permission of Springer)

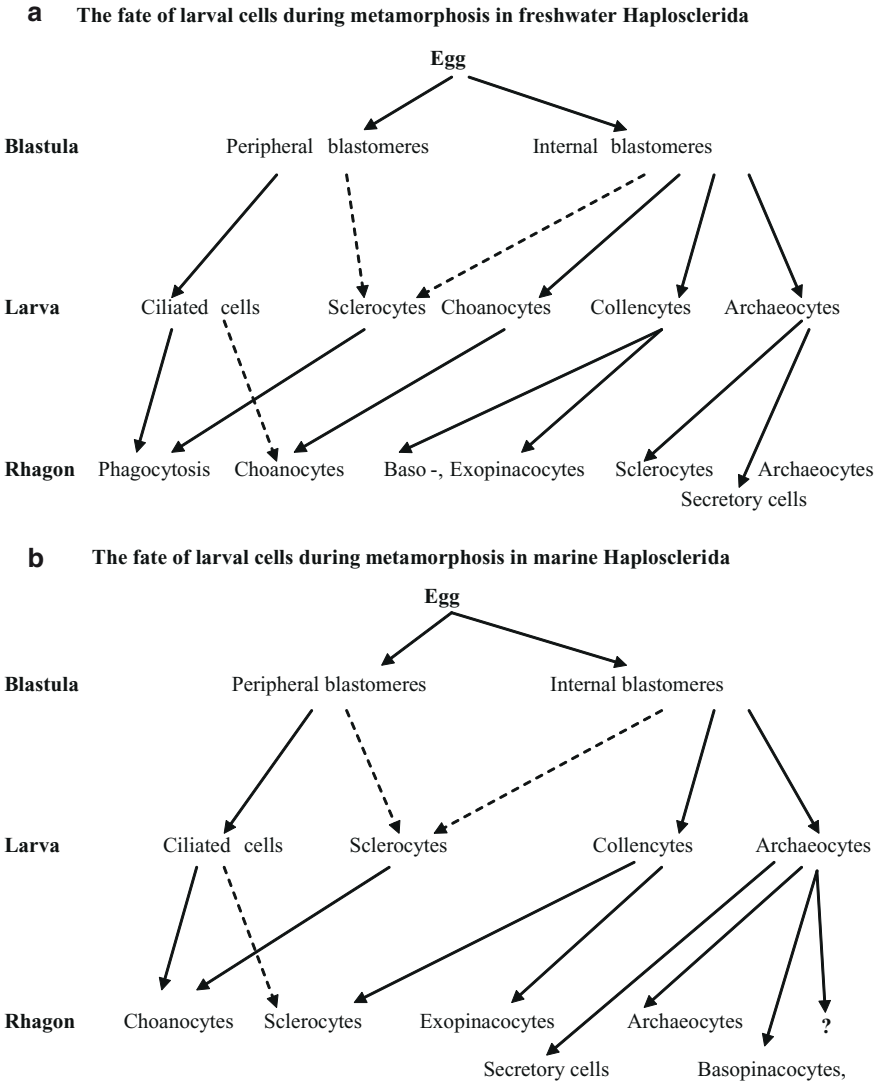

Fig. 3.86 The fate of larval cells during metamorphosis in freshwater (**a**) and marine (**b**) Haplosclerida

On the basis on the literature data, the schemes of cell differentiation during metamorphosis in freshwater (Fig. 3.86a) and marine (Fig. 3.86b) haplosclerids were compiled.

3.11.5 Asexual Reproduction

Freshwater haplosclerids from the families Spongillidae, Potamolepidae, Metaniidae, Palaeospongillidae and some marine Chalinidae form gemmules. It usually occurs after completion of sexual reproduction or, more rarely, during embryogenesis (Brien 1973a; Fell 1974a; Simpson 1984; Ereskovsky 1999; Manconi and Pronzato 2002). Gemmulogenesis consists of several stages (Fig. 3.87). It starts with directed migration of gemmule trophocytes, archaeocytes and spongocytes into certain parts of the mesohyl, where they form dense aggregated 250–500 μm in diameter (Fig. 3.87a). These three types of cells have some common features: a large nucleus with a nucleolus, numerous Golgi apparatuses, ribosomes, mitochondria and inclusions of various sizes (De Vos 1971; Simpson 1984). Archaeocytes in these aggregates actively phagocyte trophocytes, so that characteristic *yolk plates* are accumulated in them (De Vos 1971; Simpson and Fell 1974). In sponges with symbiotic zoochlorellae, archaeocytes also capture them; these symbionts remain intact

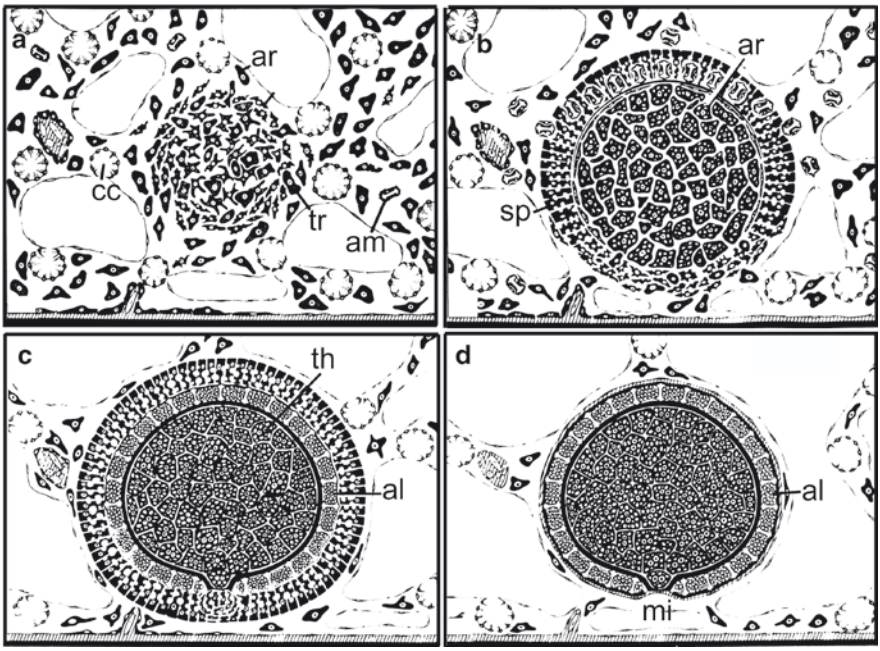

Fig. 3.87 Schematic drawing of gemmulogenesis in freshwater Haplosclerida. (**a**) Beginning of the gemmulogenesis: concentration of archaeocytes, trophocytes and spongoblasts in the mesohyl. (**b**) Gemmule-envelope formation. (**c**) Development of alveolar layer, micropyle, and differentiation of thesocytes. (**d**) Developing gemmule. *ar* archeocytes and archaeocytes with yolk plates, *am* amphidiscoblast with a young amphidisc, *al* alveolar layer, *cc* choanocyte chamber, *mi* micropyle, *sp* spongocytes, *th* thesocytes, *tr* trophocytes (From Langenbruch 1981, Zoomorphology vol. 97, pp. 279, Fig. 16, reproduced by permission of Springer)

in *thesocytes* (Williamson 1979; Kanayama and Kamishima 1990). Symbiotic algae are essential for normal hatching and germination of gemmules (Kanayama and Kamishima 1990).

As yolk plates develop, two layers are formed around the cell aggregates: the internal one, consisting of flattened archaeocytes, and the external, columnar one, consisting of flattened spongiocytes (Fig. 3.87b). Mesohylar microslerocytes with the formed microscleres, amphidiscs or rhabds, migrate towards the developing gemmule and incorporate into the columnar layer (Fig. 3.87c). Gemmule envelope formation starts at one of the poles and gradually spreads to the other one, where a *micropyle*, a spicule-free area, is later formed (Langenbruch 1981).

A fully-formed gemmule consists of a dense non-cellular envelope and the homogenous internal mass of thesocytes (Fig. 3.87d) (De Vos 1971; Langenbruch 1981, 1984; Simpson 1984; Weissenfels 1989). The latter have numerous yolk plates (in freshwater species) or yolk granules (in marine species). Thesocytes of Spongillidae are binucleate, while those of marine haplosclerids are uninucleate (Brien 1973a; Fell 1974a; Simpson 1984). On the whole, gemmulogenesis proceeds similarly in marine and freshwater haplosclerids. In both cases, blastogenesis is accompanied by a considerable or complete disorganization of the aquiferous system and the mesohyl of the parent sponge.

Gemmules are well-protected reproductive elements, resistant to numerous unfavorable factors. For instance, they can germinate after 2 months of exposure at −80°C and even −100°C (Barbeau et al. 1989). Twenty-five per cent of gemmules have been shown to survive 4 months out of water at +5°C (Fell and Bazer 1990).

Germination is usually triggered by external factors: certain values of temperature, illumination, humidity, ionic composition of water and so on. However, in cold climate gemmules in a population need a diapause; they can germinate only after a lapse of some time. In northern latitudes, gemmules, in order to germinate, have to be exposed to frost (Fell 1993). It was showed that stress protein Hsp70 presumably allows gemmules to stabilize their proteins and membranes during dormancy from autumn to spring when water temperatures change (Schill et al. 2006).

Experiments show that gemmule germination in some sponges is triggered by photosynthetic activity of symbiotic algae (Kanayama and Kamishima 1990).

Shortly before hatching, intense collagen synthesis starts in thesocytes, this substance being necessary for attachment of basopinacocytes to the substrate (Mizoguchi and Watanabe 1990). After hatching, the gemmule attaches to the substrate and differentiation of totipotent thesocytes starts. Pinacocytes and sclerocytes are the first to differentiate; mesohylar cells follow. Pinacoderm grows fast. Spicules are secreted, choanocyte chambers differentiate and aquiferous system is formed. In the course of these processes, three peaks of nuclear DNA synthesis are recorded: the first two before hatching and the third one at the time of preparation for differential divisions of archaeocytes and choanocytes (Rozenfeld 1974).

At the first stages of gemmule germination, homeobox-containing genes *Em*H-3 are actively expressed in archaeocytes (Richelle-Maurer and Van de Vyver 1999;

Richelle-Maurer et al. 2004, 2006). This indicates their involvement in cell specification and differentiation.

In the course of the first 2 days after hatching intensive autolysis proceeds and proteolytic enzymes are activated. As a result, certain high-molecular fractions of summary proteins are lost (Avenirova and Sukhodolskaya 1983, 1984).

Researchers have often pointed out similarities between certain stages of gemmulogenesis and oogenesis in Spongillidae. For instance, in species that have both reproduction in the life cycle gemmules and female gametes develop from nucleolated amoebocytes (archaeocytes) (Meewis 1936; Brien and Meewis 1938; Leveaux 1941; Saller and Weissenfels 1985; Saller 1988). To note, in Lubomirskiidae, which do not reproduce asexually, oocytes probably have choanocyte origin (Alexeeva and Efremova 1986). The way in which yolk inclusions are formed is also similar during oocyte vitellogenesis and gemmulogenesis, in marine as well as in freshwater sponges; in both cases whole trophocytes or their parts are phagocytes (De Vos 1971; Simpson 1984). In *S. lacustris*, *E. fluviatilis*, *E. muelleri* and some other species gemmulogenesis may begin before the completion of sexual reproduction, which indicates the involvement of the same trophocytes into these two processes (Saller and Weissenfels 1985; Saller 1988; Mukai 1989). Directed movement of trophocytes towards the centre of gemmule formation is thought to be based on chemotaxis (Rasmont and De Vos 1974; Rosenfeld et al. 1979; Simpson 1984). Chemotaxis of these cells towards to the substances incorporated into oocyte's membrane also occurs during oogenesis (Fell 1983). Some Spongillidae have both multinuclear blastomeres and multinuclear thesocytes, which is unlikely to be a mere coincidence (Langenbruch 1981; Saller and Weissenfels 1985; Saller 1988).

In the development of various haplosclerids a correlation between the sites of embryo- and gemmulogenesis can be revealed. For instance, in species with gemmules oogenesis and embryogenesis are confined to the basal choanosome part; in case of sexual reproduction disintegration of parent tissues is only local. This is the case with both marine haplosclerids (*H. permolis*, *H. ecbasis*, *H. loosanoffi*, *H. occulta*) (Fell 1969, 1974b; Elvin 1976) and freshwater haplosclerids from the families Spongillidae and Potamolepidae (Brien and Meewis 1938; Brien 1970b, 1973a; Simpson 1984; Weissenfels 1989). In marine and freshwater (long-lived) sponges that always or occasionally lack gemmulogenesis in the life cycle spatial distribution of reproductive elements varies, depending on environmental conditions and the reproductive strategy of the population (Fromont 1994; Ereskovsky and Korotkova 1997, 1999).

Haplosclerids are distributed worldwide, not only in all the seas but also in the freshwater of all the continents. Moreover, it is these sponges that are most often found in highly unstable habitats: circumlittoral zone, brackish-water lagoons and estuaries, drying freshwater bodies (in tropics and subtropics) and freezing freshwater bodies (in temperate and high latitudes). To colonize these unstable habitats, haplosclerids have evolved protective mechanisms, one of them being gemmulogenesis; their gemmules can withstand long-term drying and freezing and other unfavorable conditions (see Simpson 1984; Weissenfels 1989;

Fell 1993; Manconi and Pronzato 2007). Freshwater haplosclerids from the family Spongillidae have evolved another adaptive strategy: gametogenesis is lost from the life cycle in populations under unstable conditions (Pronzato and Manconi 1991), though the potential ability to revert to sexual reproduction is retained.

It is the inclusion of gemmulogenesis into the life cycle of shallow-water marine ancestors of haplosclerids that may have made it possible colonization of intertidal, estuarial and freshwater habitats (Manconi and Pronzato 1994, 2007; Pronzato and Manconi 1994). Therefore, the life cycle with alternation of sexual and asexual reproduction should be considered the typical life cycle of haplosclerids, and the initial one for the family Spongillidae.

In general, budding is not characteristic of haplosclerids. However, it was described in the freshwater sponge *Radiospongilla cerebellata* (Spongillidae) (Saller 1990). The organization of the bud is identical to that of the adult sponge. Beginning as an outgrowth of the body surface, the bud finally breaks off and, yet unattached, forms an oscular tube and starts normal functioning. Long spicules protrude over its surface. Choanocyte chambers are small but increase in size progressively by means of active proliferation of choanocytes. In this aspect, *R. cerebellata* is different from other sponges, whose choanocytes originate from archaeocytes. Noteworthy, exopinacocytes of the oscular tube of the bud bear flagella, lacking in the adult sponge. Another unusual feature of the bud is a large number of large vacuolated cells in the mesohyl.

3.12 Order Astrophorida Sollas, 1888

The order Astrophorida comprises demosponges with, usually, a coarse texture emphasizing silica content over spongin in the skeleton. Megascleres are tetractinal, usually triaenes, calthrops, or short-shafted triaenes, together with oxae (Fig. 3.88a–f), always with some radial skeletal architecture obvious at least in the peripheral skeleton; the architecture is often more confused towards the centre of the body. Microscleres are asters in one or more categories, sometimes accompanied by microxea and microrhabds (Fig. 3.88g–j). According to the latest investigations, the order also comprises sponges boring calcareous substrates (family Thoosidae) (Borchiellini et al. 2004b). Most astrophorids are oviparous and gonochoric, but the Thoosidae are ovoviviparous. Astrophorids are known from all oceans and at all depths (colour plate XII). Five families, with about 40 genera and two subgenera, are currently included into the order.

Despite the broad distribution and the large number of species, there have been few investigations of astrophorid development and they are mostly confined to the description of oogenesis.

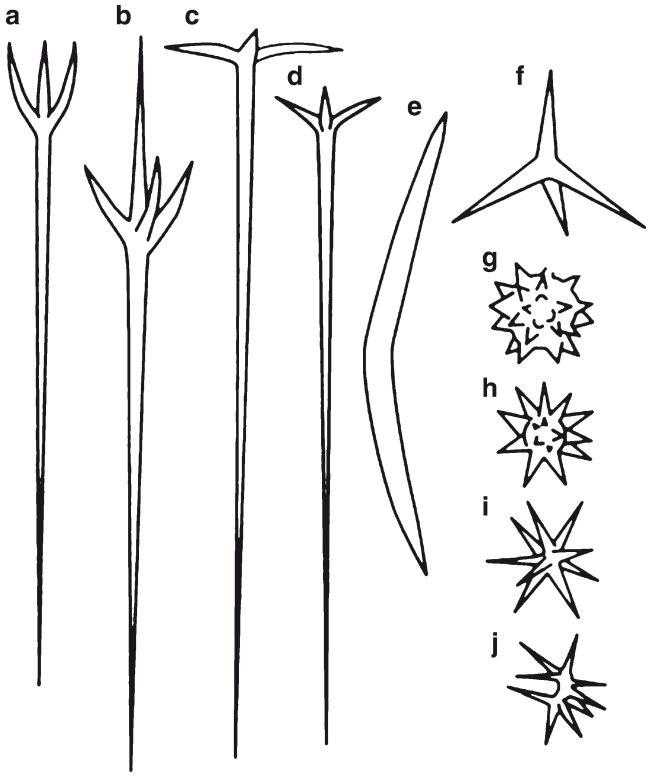

Fig. 3.88 Spicules of Astrophorida. (**a**–**d**) Triaenes. (**e**) Oxae. (**f**) Calthrop. (**g**) Spheraster. (**h**) Spheroxyaster. (**i**) Oxyaster. (**j**) Metaster

3.12.1 Gametogenesis

3.12.1.1 Spermatogenesis

Information on spermatogenesis is based on light microscopic research on two *Alectona* species and on *Geodia barretti* (Vacelet 1999; Spetland et al. 2007). As in all demosponges, spermatogenesis proceeds in small spherical spermatocystes, surrounded with pinacocyte-like cells. Spermatogenesis synchronous within a spermatocyste but asynchronous within a sponge, results in formation of flagellated spermatozoa with an elongated head.

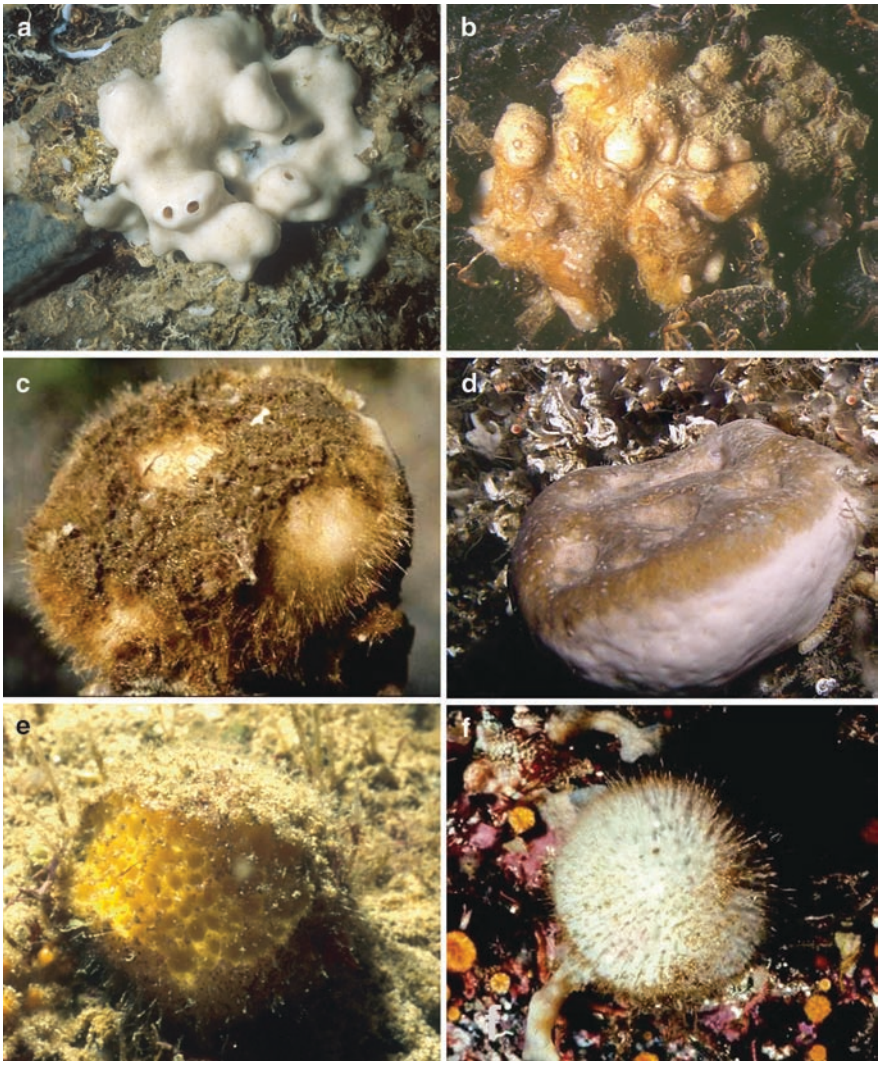

Plate XII Order Astrophorida Sollas 1888 (a–d) and order Spirophorida Bergquist and Hogg 1969 (**e**, **f**) (**a**) *Penares helleri* (Schmidt 1864), Mediterranean Sea (Courtesy of J. Vacelet). (**b**) *Calthropella* sp. Mediterranean Sea (Courtesy of J. Vacelet). (**c**) *Geodia mesotriaena* Lendenfeld 1910, (**d**) *Geodia barretti* Bowerbank 1858. (**c**, **d**) Barents Sea (Courtesy of M. Fedjuk). (**e**) *Cinachyrella levantinensis* Vacelet et al. 2007, Mediterranean Sea, Liban (Courtesy of T. Perez). (**f**) *Craniella* sp. Okhotsk Sea (Courtesy of V. Feodorov)

3.12.1.2 Oogenesis

The origin of gametes, both female and male, remains obscure. According to light microscopic observations on *Thenea abyssorum*, they stem from archaeocytes (Witte 1996).

Oogenesis has been studied at the electron microscopic level in *Erylus discophorus* and *Geodia cydonium* (Geodiidae), *Stelletta grubii* (Ancorinidae) and at the light microscopic level in *Thenea abyssorum* (Theneidae), two species of *Alectona* (Thoosidae) and *Geodia barretti* (Geodiidae) (Sciscioli et al. 1989, 1991, 1994; Witte 1996; Vacelet 1999; Spetland et al. 2007).

The oocytes usually dispersed uniformly in the mesohyl, form pseudopodia permitting the capture of the numerous bacteria present in the mesohyl (Fig. 3.89c). The eggs of the oviparous astrophorids are small (30–120 μm), isolecithal and oligolecithal. The nucleolated nucleus is surrounded by dictyosomes containing small vesicles, which contribute to formation of the reserve material. Vesicles, numerous food vacuoles and groups of mitochondria are observed in the granular cytoplasm. Electron-dense yolk inclusions and lipids are found peripherally. In the cortical portion of the egg cytoplasm there are vacuoles with fibrillar content (Fig. 3.89). Ultrastructural analysis of the *S. grubii* eggs has demonstrated both the autosynthetic activity (the presence of nutrient vesicles) and the phagocytosis mechanisms (the capture of bacteria by pseudopods) (Sciscioli 1991).

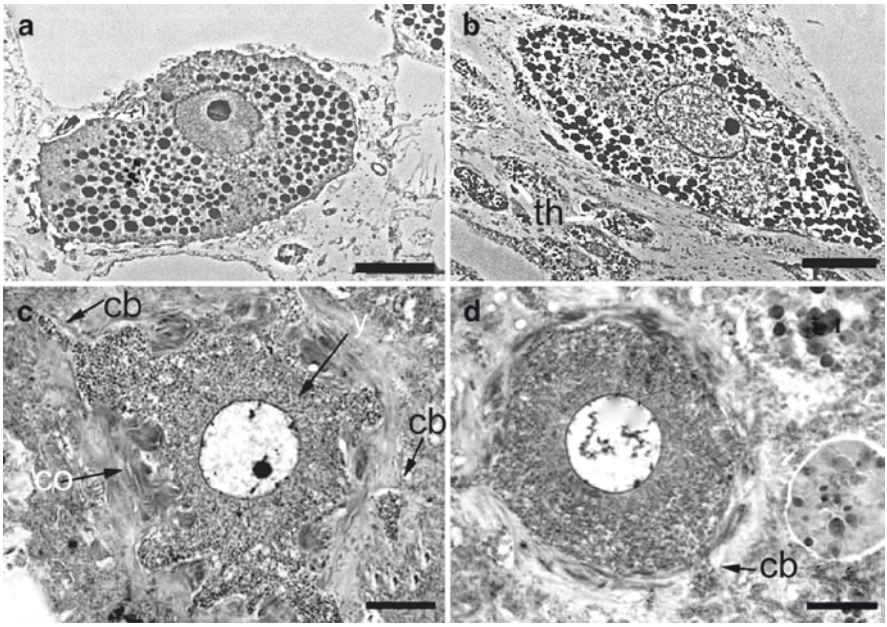

Fig. 3.89 Light microscopy of oogenesis in *Thenea abyssorum* (**a, b**) and *Geodia barretti* (**c, d**). (**a**) Oocyte in middle stage of vitellogenesis. (**b**) Almost mature oocyte; only few nurse cells (*th*) are found in the vicinity. (**c**) An almost full-grown oocyte; some cytoplasmic bridges (*cb*) are still present and yolk is still being transported into the oocyte. (**d**) The mature oocyte. *co* collagen (**a**, **b** – From Witte 1996, Mar. Biol. vol. 124, pp. 574, Fig 3, reproduced by permission of Springer; (**c**, **d**) – From Spetland et al. 2007, Museu Nacional vol. 28, pp. 618, Fig. 4, reproduced by permission of the Museu Nacional of Rio de Janeiro). Scale bars (**a**–**d**) 20 μm

The oocytes of the oviparous astrophorids can synthesize the collagen layer, surrounding the egg at the final oogenetic stages, by excreting the fibrillar contents of the peripheral vacuoles (Fig. 3.89d) (Sciscioli et al. 1989, 1991, 1994; Spetland et al. 2007). In other words, they have a true primary envelope, the product of egg synthesis characteristic of all the Eumetazoa (Gilbert 2007). Moreover, the collagen capsule in *S. grubii* is synthesized with participation of the mesohyl lophocytes (Sciscioli et al. 1991) and so in this case the capsule may be considered as the prototype of the metazoan vitelline envelope.

In all the oviparous astrophorids studied, symbiotic bacteria get from the mesohyl into the developing oocyte and are then transferred to the individuals of the new generations. Large numbers of bacteria are phagocyted by the oocytes by means of pseudopodia.

At the final stages of vitellogenesis of the ovoviviparous sponges of the genus *Alectona* the egg becomes enclosed in a follicle consisting of flattened cells and a peripheral collagen layer (Vacelet 1999).

3.12.2 Embryonic Development

The embryonic development of oviparous astrophorids has not been studied; their larva is unknown. Some information is available on the embryogenesis of two ovoviviparous astrophorids species: *Alectona wallichii* and *A. mesatlantica* (Vacelet 1999).

Cleavage in *Alectona* is total, equal and, probably, chaotic (Fig. 3.90a). It results in formation of an equal, apolar morula (stereoblastula); 200–320 μm in diameter in *A. wallichii* and 200–230 μm in diameter in *A. mesatlantica* (Fig. 3.90b). There is no cleavage cavity. An unusual feature of *Alectona* development is the presence of a large number of collagen fibers between the blastomeres and between the blastomeres and the follicle (Vacelet 1999). These collagen fibrils are secreted by the blastomeres, unique features in sponges.

The type of larval morphogenesis has not been described. It is known that larval sclerocytes are among the first differentiated and functioning cells of the embryo. Their secretory activity results in simultaneous formation of three types of larval spicules: small and very thin styles, discotriaenes and amphiasters. Discotriaenes are exclusively larval spicules, absent in adult sponges. In advanced stages, the embryo is surrounded by a single layer of discotriaenes in which the shaft is inwardly directed. Styles are disposed in three fascicles made up of two parallel spicules. These fascicles penetrate the surface of the late embryo. Amphiasters are mostly located near the discotriaene cover. At later stages of *A. millari* development larval choanocytes and granulated cells are formed (Garrone 1974).

Symbiotic bacteria are present inside the embryo from the first cleavage stages and are retained in the intercellular spaces of the larva.

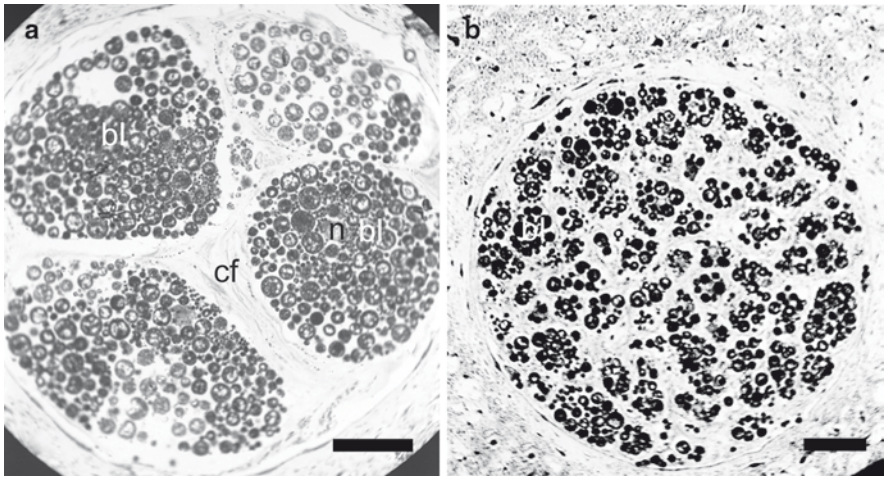

Fig. 3.90 Light microscopy of early embryonic development in *Alectona wallichii.* (**a**) Early cleavage. (**b**) Morula. *b* blastomeres, *cf* collagen fibrils, *n* nucleus (Courtesy of J. Vacelet). Scale bars (**a**, **b**) 50 μm

3.12.3 Larva

The nature of armoured planktonic propagules of the excavating sponges *Alectona* and *Thoosa* has long remained obscure (Fig. 3.91). First described as planktonic radiolaria (Karawaiew 1896, 1897), they were then noted in the mesohyl of *Alectona* and *Thoosa* sponges (Topsent 1903) and identified as larvae or gemmules (Tregouboff 1939, 1942).

It also took a long time to determine whether these "armoured gemmules" were sexual or asexual. At first they were interpreted as sexual embryos (Topsent 1903, 1904), but later their unusual morphology inclined spongiologists to the opinion that they were asexual (Topsent 1920). The name "armoured gemmules" stuck firmly in the literature reviews. Garrone (1974), having studied the structure of these propagules cytologically, decided that they were neither larvae nor gemmules and suggested the term "armoured buds". Finally, Vacelet (1999) proved convincingly the sexual nature of these armoured planktonic propagules in *Alectona* and *Thoosa* and described them as hoplitomella, a new type of larvae.

Our knowledge of hoplitomella ultrastructure is based on the study by Garrone (1974). The larva is spherical, about 300 μm in diameter, without any signs of the anterior–posterior polarity. It is covered by the flat parts of the larval spicules, discotriaenes, in which the shaft is inwardly directed, without any ciliated cells. Long thin protrusions on the larval surface are pinacoderm-covered fascicles of styles.

Anatomically, three larval layers can be distinguished. The external layer contains covering pinacocytes and spherulous cells below the discotriaene plates. The intermediate layer is conspicuous by a dense packaging of archaeocytes,

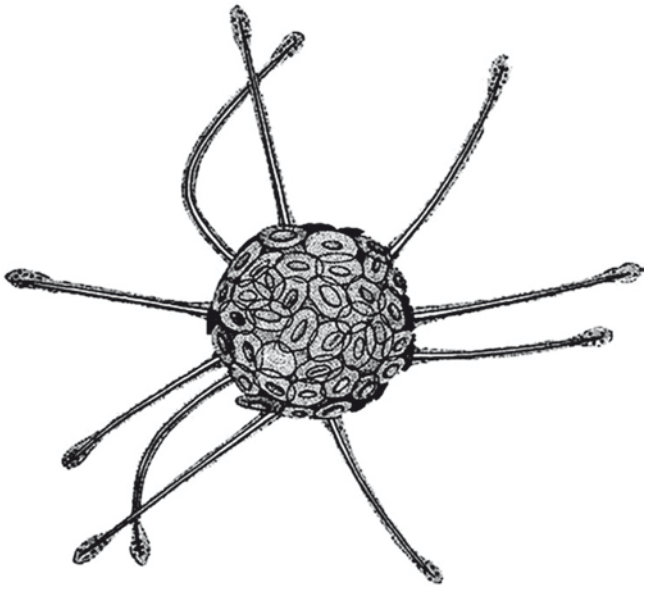

Fig. 3.91 Schematic drawing of hoplitomella larva of Alectonidae (From Treguboff 1942)

bacteriocytes, gray cells and granular cells. In the internal layer uncertain cells and the choanocyte chambers are loosely distributed. Contrary to all other sponge larvae, hoplitomellae have no covering ciliated cells. The intercellular spaces are filled with collagen fibrils, secreted, mostly, by the gray cells. Symbiotic bacteria are present both in the intercellular spaces and in the vacuoles of bacteriocytes.

To sum up, the hoplitomella of *Alectona* and *Thoosa* is unique among poriferan larvae. Its characteristic features are the lack of anterior–posterior polarity and cilia, the presence of the larval spicules absent in the adult, a strong development of collagen fibrils and a long planktonic life with special flotation devices (Vacelet 1999).

3.12.4 Asexual Reproduction

Some astrophorids have asexual reproduction in the form of external budding. Buds were observed in *Thenea muricata* (Vosmaer 1882; Sollas 1888; Burton 1959) and in *T. abyssorum* (Babic 1916; Barthel and Tendal 1993; Witte 1996). The latter species was found to produce a considerable number of buds less than 8 mm in diameter. All of them were situated in the lower half of the individual, i.e. *in situ* below the sediment surface. In *Geodia barretti* bud formation occurs by cell-by-cell migration into the certain parts of the sponge surface (Burton 1949b), which indicates that the budding follows the epimorphic type.

Chapter 4
Development of Homoscleromorpha of the Order Homosclerophorida Dendy, 1905

The Homoscleromorpha is a very distinct group of Porifera. Homoscleromorphs are small sponges, encrusting or lumpy with a smooth surface (color plate XIII). The body consists of two layers, the pinacoderm and the choanoderm, with a thin mesohyl layer between them. The skeleton, if present, consists of small calthrops and/or their derivatives (diodes and triodes) (Fig. 4.1) evenly distributed in the sponge body. The spicule rays may branch. The aquiferous system is sylleibid or leuconoid, often with a vast basal exhalant chamber. Choanocyte chambers are large, usually eurypilous (*Plakortis*, *Plakinastrella*, *Plakina*, and *Oscarella*), and aphodal or diploid (*Corticium, Pseudocorticium*). The apopyle is always surrounded with a ring of flattened apopylar cells (Fig. 4.2a, b) having both the flagellum and the microvilli collar. The basal membrane underlies the choanoderm and the pinacoderm (Fig. 4.2c, d); exo- and endopinacocytes are flagellated (Fig. 4.2e, f). Homoscleromorphs are marine sponges, usually occurring at low depth. The group is composed of one family Plakinidae Schluze 1880 and seven genera.

All the Homoscleromorpha are ovoviviparous sponges with the cinctoblastula larva. They can be both gonochoric and simultaneous hermaphrodites, i.e., male and female gametes mature in a sponge at the same time (Schulze 1877b; Tuzet and Paris 1964; Ereskovsky and Boury-Esnault 2002; Riesgo et al. 2007a; Ereskovsky et al. 2009a).

Homoscleromorpha was earlier considered to be a subclass of the class Demospongiae (Lévi 1973). Recently, molecular studies have challenging phylogenetic relationships of Homoscleromorpha within Demospongiae and alternative scenarios have been proposed as follows: (i) they are closer to eumetazoan than to the other sponges in sponge paraphyletic hypothesis (Borchiellini et al. 2001, 2004a; Peterson and Eernisse 2001; Medina et al. 2001; Manuel et al. 2003; Sperling et al. 2007), sponge para- or polyphyly for the rest has been occasionally discussed by spongologists or (ii) they are considered as the sister group of the calcareous sponges (Dohrmann et al. 2008; Philippe et al. 2009) in agreement with monophyletic view, as already postulated by some authors based on an incorrect interpretation of the larval type (van Soest 1984; Grothe and Reitner 1988; Grothe 1989). However, although the phylogenetic relationships within Porifera and between sponge clades and basal metazoans are still unresolved, it seems to emerge that Homoscleromorpha are not Demospongiae and constitute a fourth high-level sponge taxon.

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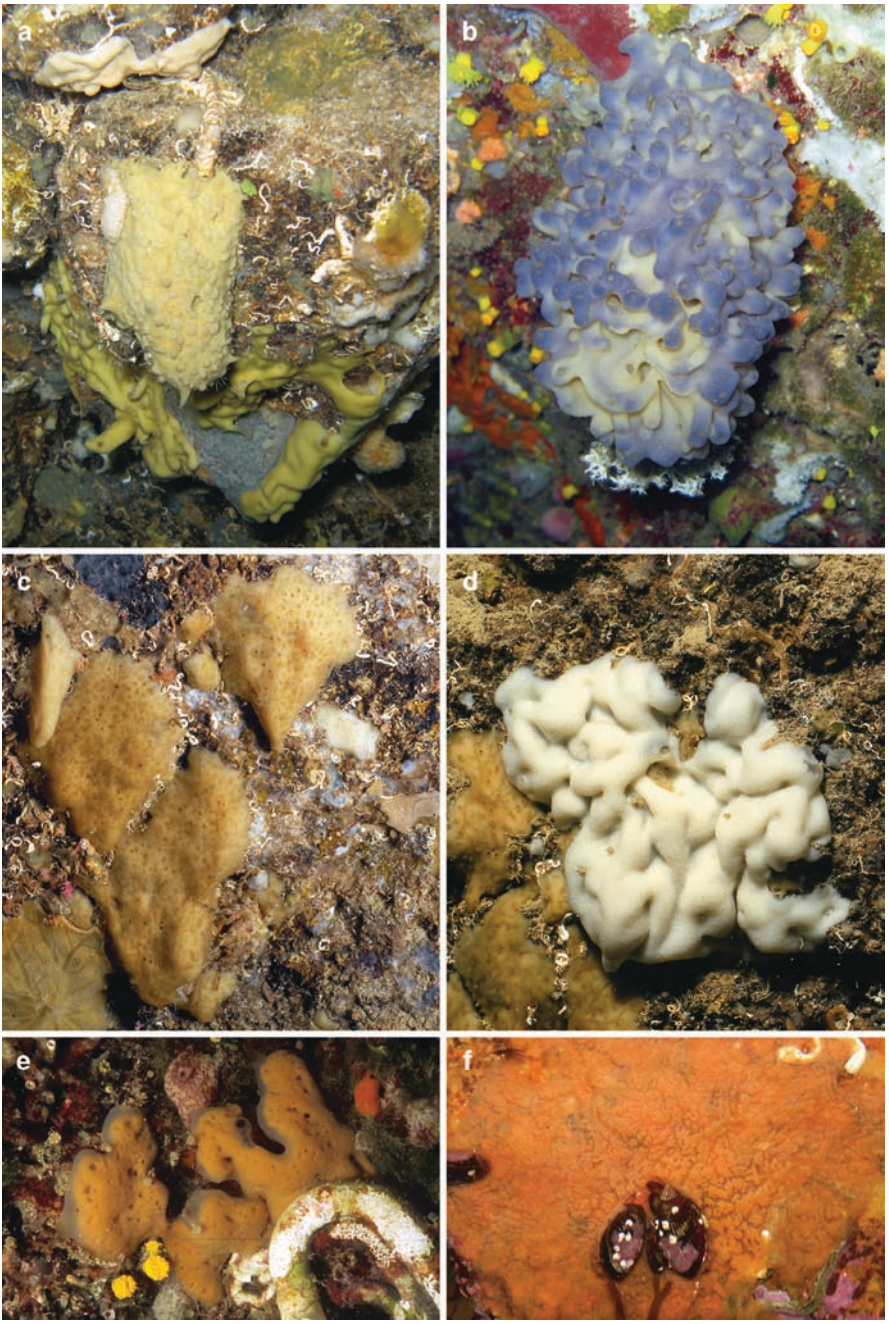

Plate XIII Homoscleromorpha, order Homosclerophorida Dendy 1905. (**a**) *Plakortis simplex* Schulze 1880 (Photo of A. V. Ereskovsky). (**b**) *Oscarella lobularis* (Schmidt 1862) (Photo of A. V. Ereskovsky). (**c**) *Plakina weinbergi* Muricy et al. 1998 (Courtesy of T. Perez). (**d**) *Plakina trilopha* Schulze 1880 (Photo of A. V. Ereskovsky). (**e**) *Corticium candelabrum* Schmidt 1862 (Courtesy of J. Vacelet). (**a**–**e**) – Mediterranean Sea. (**f**) *Oscarella malakhovi* Ereskovsky 2006, Japan Sea (Photo of A. V. Ereskovsky)

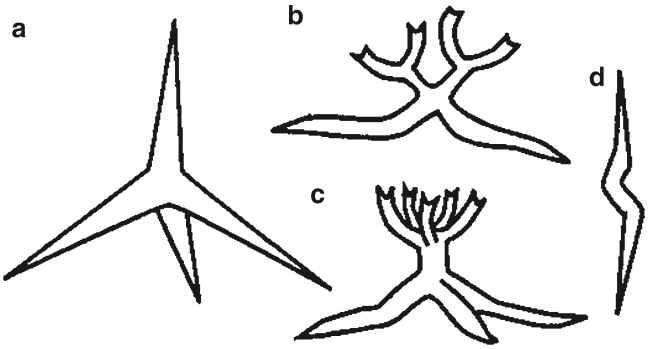

Fig. 4.1 Spicules of Homoscleromorpha. (**a**) Calthrop. (**b**, **c**) Lophocalthrop. (**d**) Diod

An important morphological difference of the Homoscleromorpha from the Demospongiae is a scarcity of cell types. They have no collencytes, spongocytes, and lophocytes, common in the Demospongiae. The mesohyl of homoscleromorphs contains mostly cells with specialized inclusions (spherulous, vacuolated, and granular cells) and rare nucleolated amoebocytes (archaeocytes); representatives of the genera *Plakina*, *Corticium*, *Plakortis*, *Placinolopha*, and *Plakinastrella* also have sclerocytes. Homoscleromorphs choanocytes are larger than those of the demosponges, being similar to those of the Calcarea.

Homoscleromorph's simplicity tempted some authors to consider them as "an almost ideal prototype of poriferan organization" (Lévi and Porte 1962). Indeed, according to the anatomy and aquiferous system structure, the Homoscleromorpha can be considered as the simplest poriferans. However, simplicity of organization does not always mean that the group is primitive. The latest research questions the traditional views on the primitiveness of the Homoscleromorpha. First and foremost, homoscleromorphs are the only sponges to have a true basal membrane with collagen IV, tenascin, and laminin (Humbert-David and Garrone 1993; Boute et al. 1996). In nonreproducing sponges, the basal membrane underlines choanoderm and pinacoderm (endo-, exo-, and basopinacoderm) (Boury-Esnault et al. 1984, 1995; Muricy et al. 1996; Ereskovsky 2006, Ereskovsky et al. 2009a), whereas in reproducing ones, it covers the spermatocytes, egg, and embryo follicles (Ereskovsky and Boury-Esnault 2002; Riesgo et al. 2007a).

On the other hand, homoscleromorph epithelium is an ancient, primitive one. The main indication is its polyfunctional nature: the epithelial cells are not terminally differentiated but totipotent. For instance, exopinacocytes can transdifferentiate into endopinacocytes; the latter, into choanocytes, apopylar, and vacuolated cells as well as the cells of the follicle (Gaino et al. 1987a; Ereskovsky and Boury-Esnault 2002). Choanocytes can transform into oocytes and spermatocytes (Gaino et al. 1986b,c).

To sum up, it is still unclear whether homoscleromorphs' simplicity is primary or secondary. Moreover, a seemingly simple organization may not be an indication of primitiveness at all.

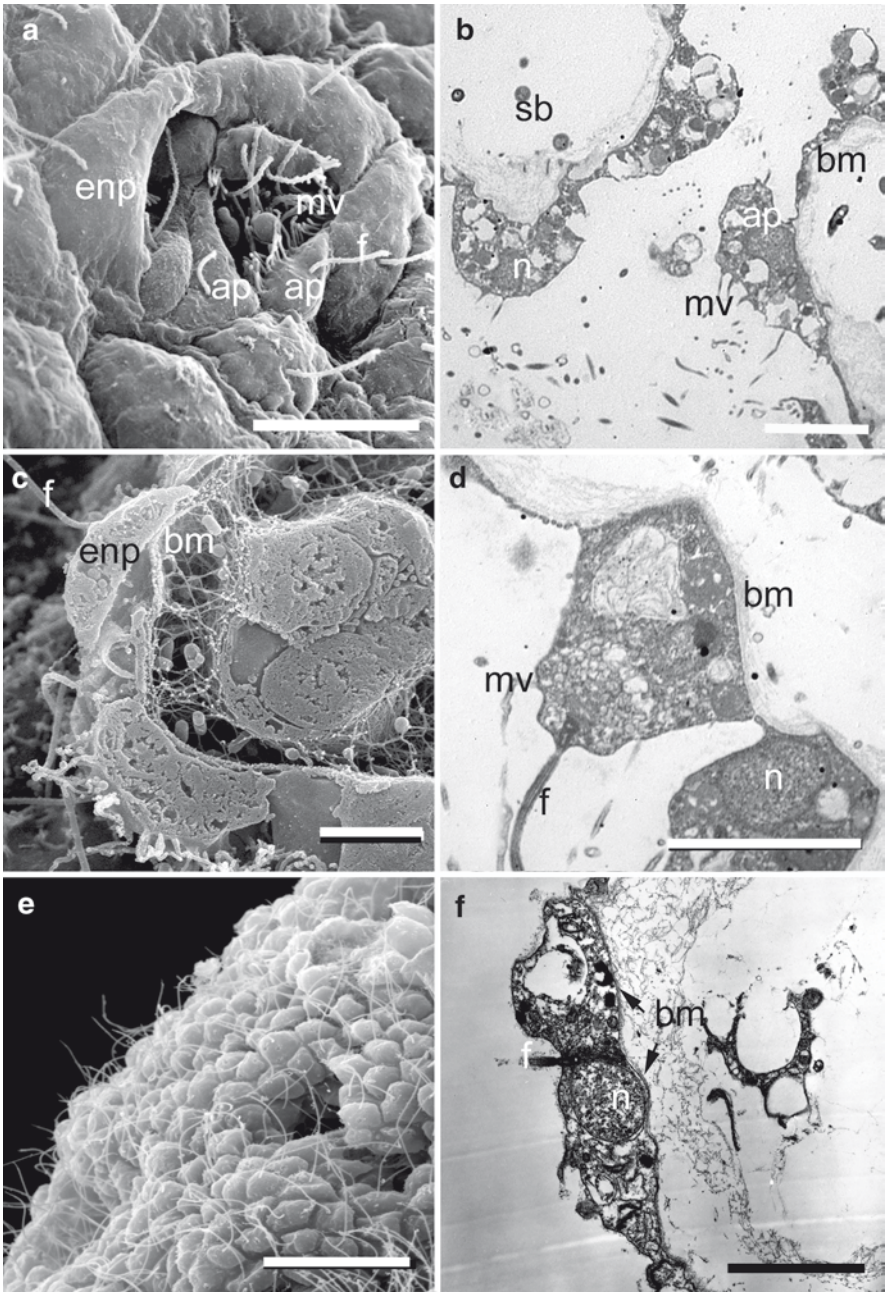

Fig. 4.2 (**a**) SEM and (**b**) TEM of apopyle of *Oscarella lobularis.* (**c**) SEM of endopinacocytes with underlining basement membrane in *O. tuberculata.* (**d**) TEM of choanocytes with underlining basement membrane in *O. lobularis.* (**e**) SEM of flagellated exopinacocytes of *O. malakhovi.* (**f**) TEM of flagellated endopinacocyte of *Oscarella* sp. *ap* apopylar cell, *bm* basement membrane, *f* flagellum, *enp* endopinacocyte, *mv* microvilli, *n* nucleus, *sb* symbiotic bacteria. Scale bars (**a**) 10 μm, (**b**–**d**), (**f**) 5 μm, (**e**) 20 μm

Embryonic development of Homoscleromorpha was in the focus of the researchers' attention since the late nineteenth century (Carter 1874; Barrois 1876; Schulze 1877, 1880b, 1881; Heider 1886; Sollas 1884; Meewis 1938; Lévi 1956; Tuzet and Paris 1964; Ereskovsky and Boury-Esnault 2002; Boury-Esnault et al. 2003; Ereskovsky et al. 2007a; de Caralt et al. 2007a; Riesgo et al. 2007a; Maldonado and Riesgo 2008), and by the early twentieth century, they had been quite thoroughly studied. This particular interest in homoscleromorph development is associated with the importance of sponges without a skeleton for the morphology of the lower animals (Metschnikoff 1879), as well as with the search for the development prototype of both Porifera and Metazoa in general.

Of the early research on homoscleromorphs, noteworthy are certain investigations on the development of *Oscarella lobularis* (=*tuberculata*) (Carter 1874; Barrois 1876; Schulze 1877; Sollas 1884; Heider 1886; Maas 1898) and the works of Schulze (1880b, 1881) on the development of *Plakina* and *Corticium*. As for the structure and origin of different larval cells, there was only one truly detailed investigation by Heider (1886), whose brilliant observations were later supported by Meewis (1938). However, all this research was conducted at the light microscopic level and called for further verification.

4.1 Gametogenesis

The origin of the gametes. The sole sources of both male and female gametes in most of Homoscleromorpha are specialized somatic cells – choanocytes (Gaino et al. 1986b, c; Ereskovsky 2005; Efremova et al. 2006; de Caralt et al. 2007a). But for *Corticium candelabrum,* it was supposed that oocytes' origin was the result of transdifferentiation of migrating archaeocytes (Riesgo et al. 2007a).

Spermatogenesis is similar in all the homoscleromorphs studied. Male gametes develop inside the spermatocyte, a modified choanocyte chamber isolated from the environment for the period of spermatogenesis (Fig. 4.3a, b).

In the course of spermatogenesis, the choanocyte loses the flagellum, microvilli, and basal filopodia, becomes rounded, and enters the lumen of the choanocyte chamber. Spermatocytes I are similar to choanocytes in size and the composition of the cytoplasmic inclusions. During early leptotene and zygotene, they retain contact with the basal membrane of the choanocyte chamber (Fig. 4.3c), which means that the transformation of choanocytes into spermatocytes is direct. After the first maturation division, spermatocytes I may lose this connection (Fig. 4.3c). During spermiogenesis, dictyosomes of the Golgi apparatus transform into electron-dense granules, considered as acrosomal ones (Gaino et al. 1986c; Riesgo et al. 2007a), which after shedding out from the residual cytoplasm become closely associated with the nuclear envelope.

The acrosome is present in the spermatozoa of all the homoscleromorph species studied: *O. tuberculata*, *O. imperialis*, *O. lobularis*, and *O. microlobata* (Baccetti et al. 1986; Gaino et al. 1986c; Ereskovsky 2005; Efremova et al. 2006), *Pseudocorticium jarrei* (Boury-Esnault and Jamieson 1999), and *C. candelabrum*

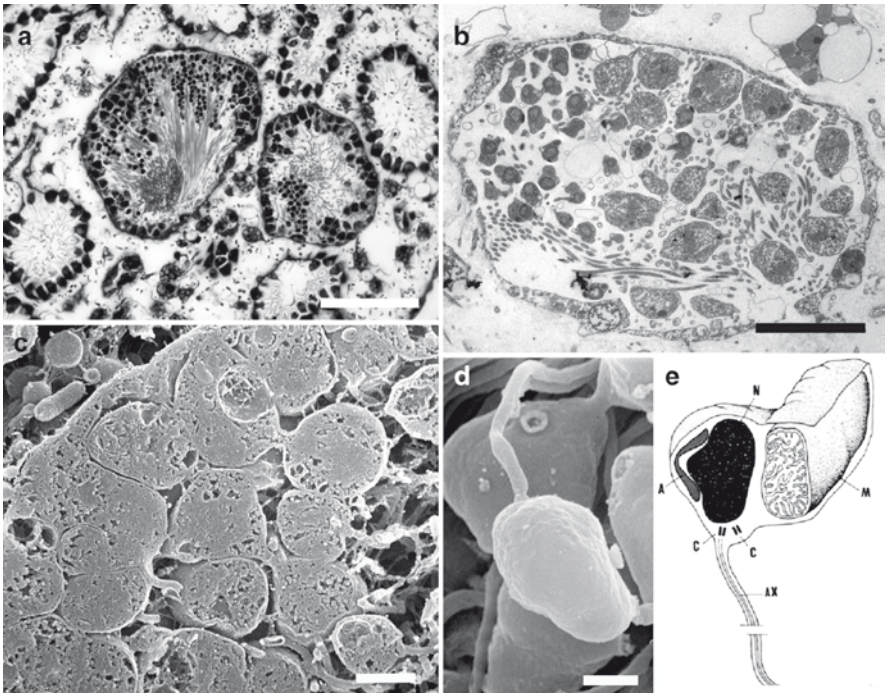

Fig. 4.3 Spermatogenesis in Homoscleromorpha. (**a**) Light microscopy of *Oscarella lobularis* spermatocyste. (**b**) TEM of spermatocyte of *Oscarella* sp. (**c**) SEM of a part of the spermatocytes of *O. tuberculata*: spermatocytes I (*scI*), keeping direct contact with the basal membrane of the choanocyte chamber. (**d**) SEM of a spermatozoid of *O. viridis*. (**e**) Scheme of mature spermatozoid of *O. lobularis* (From Baccetti et al. 1986, p 187, Fig. 4, reproduced by permission of Elsevier Ltd.). Scale bars (**a**) 30 μm, (**b**) 20 μm, (**c**) 2 μm, (**d**) 1 μm

(de Caralt et al. 2007a; Riesgo et al. 2007a). The head contains the nucleus with a densely packed chromatin and a large mitochondrion located near the distal centriole (Fig. 4.3d). The flagellum, with a typical structure, lies basally from the head (Fig. 4.3e).

Development of different clusters of male gametes within the spermatocyte is asynchronous, i.e., there is a developmental gradient. This means that in the Homoscleromorpha, local impulses triggering this process arise several times in the course of the spermatogenesis. To remind, in the Demospongiae, gametogenesis is synchronous within a cyst (Tuzet et al. 1970a,b; Efremova et al. 1987a; Paulus 1989). Since asynchronous spermatogenesis is characteristic of many Eumetazoa, spermatogenesis in the Homoscleromorpha may be considered as the most evolutionary progressive one within the Porifera.

Oogenesis. In *O. lobularis*, oogenesis starts with the choanocyte losing the flagellum, microvilli, and basal filopodia (Gaino et al. 1986b). It becomes rounded, leaves

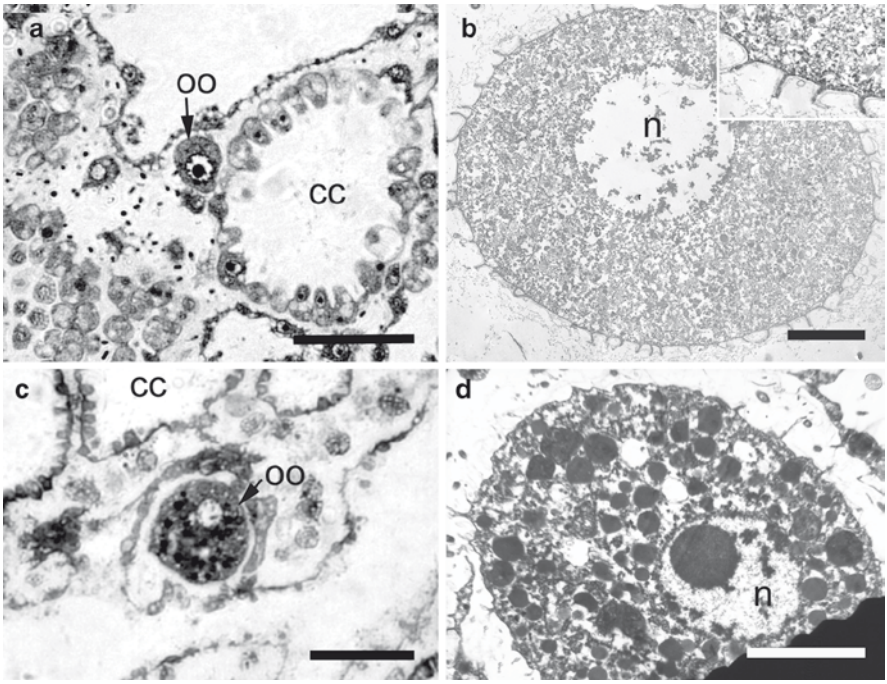

Fig. 4.4 Oogenesis in Homoscleromorpha. (**a**) Light microscopy of previtellogenic oocyte of *Oscarella kamchatkiensis* in maternal mesohyl. (**b**) TEM of previtellogenic oocyte of *Oscarella* sp. *Inset*: detail of oocyte surface with the microvilli. (**c**) Light microscopy of vitellogenic oocyte of *O. viridis*. (**d**) TEM of vitellogenic oocyte of *Oscarella* sp. *cc* choanocyte chambers, *oo* oocyte, *n* nucleus. Scale bars (**a**) 25 μm, (**b**) 5 μm, (**c**) 35 μm, (**d**) 5 μm

the choanocyte chamber, and enters the mesohyl, where it migrates actively. At the early stages, the activity of the oocyte surface layer is high – numerous microvilli and pseudopodia are formed (Fig. 4.4a, b).

During the vitellogenesis of *Oscarella*, the oocyte retains microvilli (Fig. 4.4c, d). Numerous invaginations of the plasmalemma between them are an indication of pinocytosis, whereas phagocytosis by the growing oocyte has not been shown. Thus, the reserve substances during the oogenesis in *Oscarella* are mainly formed by pinocytosis and endogenous synthesis. This type of vitellogenesis is characteristic of many oviparous Demospongiae (Diaz et al. 1973; Gallissian and Vacelet 1976; Watanabe 1978b; Gaino and Sarà 1994; Lepore et al. 1995). Unlike in other homosclerophorids, oocytes of *C. candelabrum* lacked both interdigitations and microvilli at any stage (Riesgo et al. 2007a). The same time, during its oogenesis, abundant amoeboid nurse cells migrated toward the growing oocytes. There are two types of nurse cells: one containing bacteria in cytoplasmic vacuoles, and the other mostly carrying putative proteinaceous and lipid precursors of yolk (Riesgo et al. 2007a).

Mature eggs of the Homoscleromorpha are rounded or oval, with an average size of 140 × 170 μm. They are polylecithal and isolecithal. The central nucleus contains one to two nucleoli.

Between the mature egg and the follicle cells, there is a complex network of microvilli and collagen fibers. Parent spherulous and granular cells often adhere to the egg surface, and so do numerous symbiotic bacteria, which are accumulated below the follicle cover during this period. In *C. candelabrum*, symbiotic bacteria seem to be transferred directly during oocyte growth, together with the nurse cells from the adult (Riesgo et al. 2007a; de Caralt et al. 2007b).

4.2 Follicle

Homoscleromorph embryos and larvae develop in a closed follicle, formed at the late vitellogenetic stages by the closing of a continuous endopinacoderm layer around the egg (Fig. 4.5a, b). The follicle-forming pinacocytes remain densely associated with each other. In the process of follicle closure in *Oscarella*, granular cells and symbiotic bacteria get into the space between the follicle and the egg surface. The size of the follicle remains the same throughout the embryogenesis.

To sum up, homoscleromorph follicle consists of an internal endopinacocyte layer underlain by the basal membrane and an external collagen layer. This kind of follicle is unknown in other sponges.

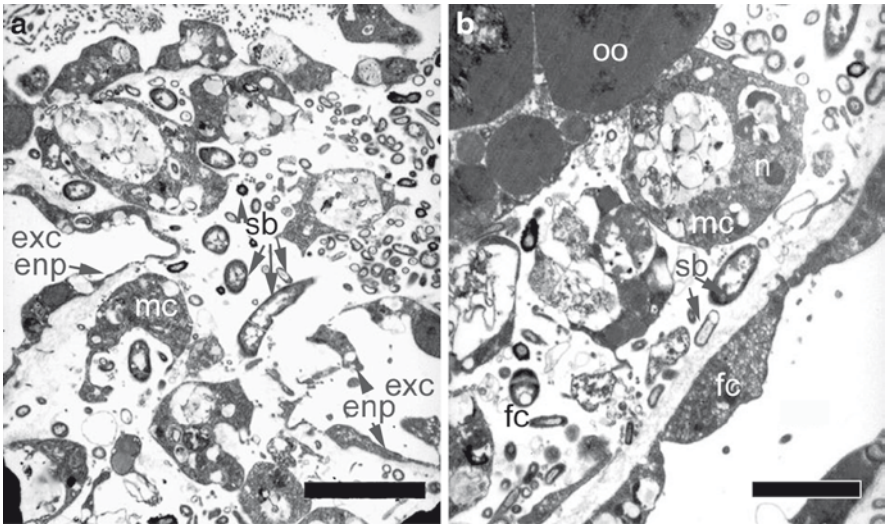

Fig. 4.5 (**a**) TEM of follicle closing in *Oscarella* sp. (**b**) TEM of follicle structure in *Oscarella* sp. *enp* endopinacocyte, *exc* exhalant canal, *fc* follicle cavity, *fo* follicular cell, *mc* maternal cell, *n* nucleus, *oo* oocyte, *sb* symbiotic bacteria. Scale bars (**a**) 5 μm, (**b**) 2.5 μm

4.3 Embryonic Development

Cleavage is total, equal, and asynchronous (Fig. 4.6). The first two furrows pass in the same direction but are perpendicular to each other. Four blastomeres always form a tetrahedron. They are opposed to each other in pairs; one pair is shifted to one side of the egg, and the other pair, to the other side (Fig. 4.6a). This position of blastomeres is characteristic of the lower Metazoa, mostly Cnidaria (Tardent 1978).

Cleavage becomes asynchronous from the third cycle (Fig. 4.6b). It remains equal until later stages, but the mitotic spindles are positioned chaotically. The result is a typical equal stereoblastula (morula), characteristic of all the Homoscleromorpha (Fig. 4.6c). There are no indications of the embryo's polarization.

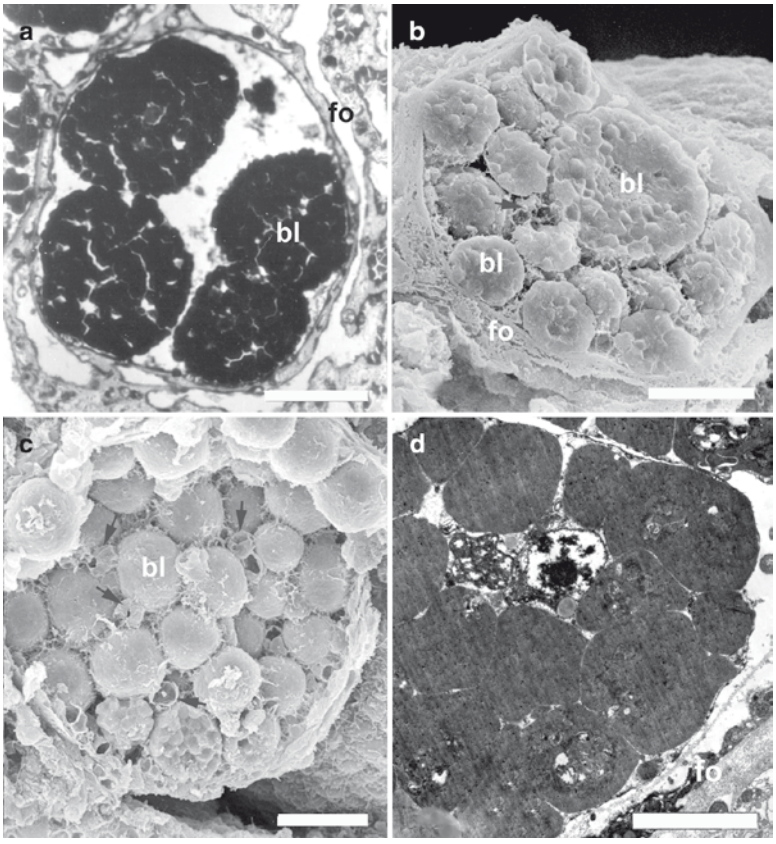

Fig. 4.6 Cleavage in Homoscleromorpha. (**a**) Light microscopy of four-blastomere embryo of *Oscarella lobularis*. (**b**) SEM of total equal asynchronic cleavage of *O. lobularis* at the stage of approximately 36 blastomeres. (**c**) SEM of cleaving embryo of *O. lobularis* at the stage of approximately 48 blastomeres. (**d**) TEM of blastomere embryo of *O. imperialis* at the stage of approximately 16 blastomere. *Arrows* maternal cell, *bl* blastomere, *n* nucleus. Scale bars (**a**–**c**) 50 μm, (**d**) 10 μm

Blastomeres are spherical and rich in yolk granules; the nuclei are central and contain one nucleolus each (Fig. 4.6d). There is no cleavage cavity.

The mode of cleavage in the Homoscleromorpha appears to be associated with the fact that the mature egg is structurally homogeneous. The nucleus is central both in the egg and in the blastomeres. The neighboring blastomeres are connected by numerous microvilli and fine filopodia formed on their lateral surfaces. These filopodia make up a network incorporating symbiotic bacteria and parent spherulous cells. Cell-to-cell adhesive complexes in filopodia were described in the cleaving embryos of the sponge *Halisarca dujardini* (Ereskovsky and Gonobobleva 2000), the hydra (Martin et al. 1997), some Octocorallia (Anthozoa) (Benayahu et al. 1989; Dahan and Betanayahu 1998), the sea urchin (Schroeder 1978), the lancelet (Hirakow and Kajita 1990), and the mouse (Calarco 1975).

Intercellular spaces of the morulae of *O. imperialis, O. lobularis, Oscarella* sp. 1, *Oscarella* sp. 2, and *P. jarrei* contain spherulous cells – specialized parent cells with inclusions (Ereskovsky and Boury-Esnault 2002; Ereskovsky 2005). Unexpectedly, there are no such cells in the embryos and larva of *O. tuberculata* and *O. malakhovi*. Therefore, it is likely that some authors who studied the development of *Oscarella* (*Halisarca*) *lobularis* (Giard 1873; Carter 1874; Barrois 1876; Schulze 1877b; Heider 1886; Meewis 1938; Lévi and Porte 1962) were in fact dealing with the species *O. tuberculata*, since they did not note any large spherulous cells. Maternal cells are also absent in the embryos of *Plakina trilopha, P. jani*, and *C. candelabrum* (Boury-Esnault et al. 2003; de Caralt et al. 2007a; Maldonado and Riesgo 2008).

From the very beginning of cleavage, embryos of all the Homoscleromorpha contain species-specific symbiotic bacteria. They are always found only in the intercellular spaces and never in the cytoplasm of the blastomeres. Most of these bacteria are Gram-negative (Maldonado 2007; Vishnyakov and Ereskovsky 2009). They can actively reproduce inside of the embryo (Ereskovsky and Boury-Esnault 2002).

In all the homoscleromorphs studied, differentiation of blastomeres starts at the stage of about 64 cells, as noted already by Meewis (1938). This is associated with the formation of the surface-ciliated layer of the larva. Thus, one can say that after the sixth cycle, cleavage ends and larval morphogenesis begins.

Larval morphogenesis has been studied in eight homoscleromorph species from the Mediterranean Sea: *O. tuberculata*, *O. lobularis*, *O. imperialis*, *O. microlobata*, *P. jarrei*, *P. trilopha, P. jani*, and *C. candelabrum* (Boury-Esnault et al. 2003; de Caralt et al. 2007a; Maldonado and Riesgo 2008), and one from Japan Sea: *O. malakhovi* (Ereskovsky 2007, unpublished).

Peripheral blastomeres of the morula start to divide more actively. They diminish in size and their nuclei shift apically (Fig. 4.7a). Both small peripheral and large internal blastomeres can integrate into the forming surface layer of the embryo (Fig. 4.7b). The cell size becomes more or less uniform after some time of proliferation. The cells themselves become oval, with the longer axis parallel to the embryo's surface (Fig. 4.7c).

Internal morula blastomeres migrate toward the periphery of the embryo. This centrifugal migration started simultaneously at several points (Fig. 4.8).

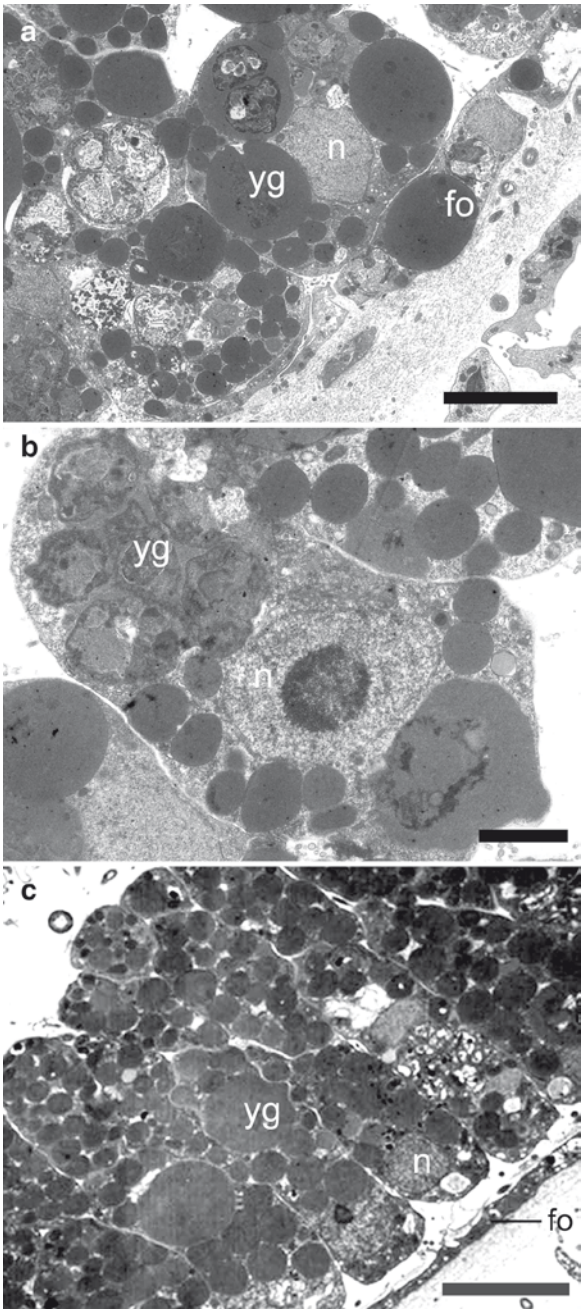

Fig. 4.7 TEM of successive stages of peripheral morular blastomeres differentiation to ciliated larval cells in Homoscleromorpha. (**a**, **b**) *Oscarella malakhovi*, (**c**) *O. imperialis*. *fo* follicular cell, *n* nucleus, *yg* yolk granules. Scale bars (**a**) 5 μm, (**b**) 2 μm, (**c**) 10 μm

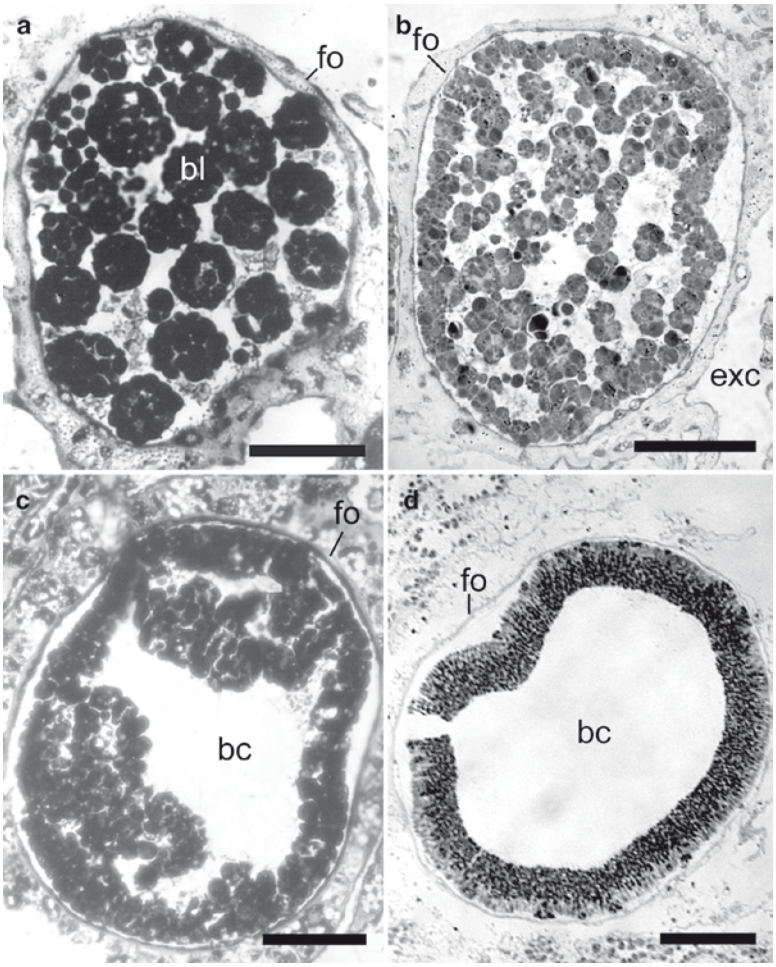

Fig. 4.8 Light microscopy of multipolar egression in Homoscleromorpha. (**a**) Morula of *Oscarella lobularis* at the stage about 64 blastomeres. Morula of *O. lobularis* (**b**) and *O. tuberculata* (**c**) at successive stages of internal cells migration to the surface of the embryos. (**d**) Coeloblastula-like prelarva of *Oscarella* sp. *bc* blastocoel, *bl* blastomeres, *fo* follicle, *exc* exhalant canal. Scale bars (**a**–**d**) 50 μm

A rather large cavity – the future larval cavity – is formed in the embryo. It contains symbiotic bacteria and, in *O. lobularis*, *O. imperialis, Oscarella* sp., and *P. jarrei*, parental cells as well. Formation of the surface layer is apolar, i.e., it precedes simultaneously at all the embryo's surface.

Though the postmorula stage in homoscleromorph development is usually referred to as the coeloblastula, the term prelarva is more correct. The blastula is the last cleavage stage of the more complexly organized multicellular animals. It is followed by embryonic or larval morphogenesis, associated with mass migration of

cells or cell complexes, and then by cytodifferentiation and organogenesis. Embryonic morphogenesis cannot result in a blastula (coeloblastula). A hollow coeloblastula-like prelarva of the Homoscleromorpha and the coeloblastula of other animals, e.g., Echinodermata, are only superficially similar (Ereskovsky and Dondua 2006). In the former case, this stage results from the morphogenesis, after the cinctoblastula pattern has been formed. In the latter case, this stage results from cleavage and is, therefore, a true blastula.

Meewis (1938) thought that transformation of the morula into the prelarva in *O. lobularis* (=*O. tuberculata*) is accompanied by the lysis of the large central blastomeres, with only the small ones integrating into the surface layer. Tuzet and Paris (1964) did not note any lysis during the development of the *Octavella galangaui* (=*O. tuberculata*) prelarva. We did not observe any mass lysis of the morula cells, either. Single disintegrated blastomeres could be seen only in the embryos of *O. lobularis*, at their periphery. The main mechanism of formation of the single-layer hollow homoscleromorph prelarva is the centrifugal migration of the embryo's cells. To distinguish this process from "multipolar ingression," we coined for it the term "*multipolar egression"* (Fig. 4.8) (Ereskovsky and Boury-Esnault 2002). This morphogenesis is unique both for Porifera and Metazoa in general. Indeed, the embryo's development from the morula is accompanied, as a rule, by morula delamination or epiboly (Ivanova-Kazas 1995; Brusca et al. 1997), whereas the coeloblastula usually develops directly, without any intermediate stages, as a result of radial (Echinodermata), spiral (Nemertini), or chaotic (Cnidaria) cleavage (Henry and Martindale 1997; Martin 1997; Wray 1997).

Multipolar egression as the means of prelarva formation rules out the possibility of gastrulation. Should this disappearance of gastrulation from the ontogenesis be considered as secondary? The author does not think so. In his opinion, the sponges never had this morphogenetic process in the first place (Ereskovsky and Dondua 2006; Ereskovsky 2007b; see Chapter 6).

At the beginning of the ciliated epithelium formation, the prelarval cells are rather loose. As they increase in number, they become more compact and stretch in the apical–basal direction, acquiring prismatic shape (Boury-Esnault et al. 2003; Maldonado and Riesgo 2008). The cilium is formed in the apical part, and collagen synthesis starts in the basal part (Fig. 4.9a, b); yolk utilization proceeds. The surface cell layer now looks like palisade epithelium, with more or less the same thickness. The fully formed prelarva has no morphological axes.

As polarization of the surface cells proceeds, specialized intercellular contacts, belt desmosomes are formed in the apical zone (see below). In the basal zone, collagen molecules are assembled at the plasma membrane facing the prelarval cavity, and finally a basal membrane lining the cavity is formed.

Embryonic cells continue to proliferate actively. Prior to division, they become rounded and crawl out onto the embryo's surface, into the space between the ciliated epithelium and the follicle. Division plane is perpendicular to the embryo's surface. After mitosis, new cells are again incorporated into the ciliated layer, acquiring a characteristic polarization. The increasing number of epithelium cells results in the formation of folds (Fig. 4.10). Sometimes, the embryo flattens and becomes cup-like.

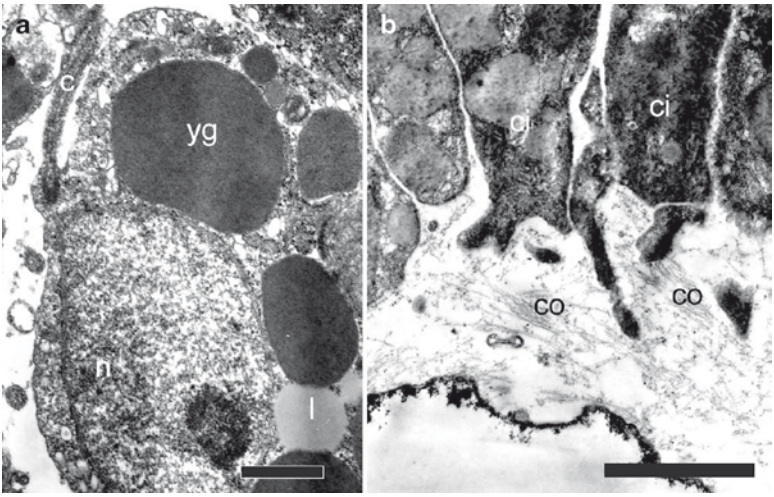

Fig. 4.9 TEM of differentiated larval ciliated cells of *Oscarella tuberculata.* (**a**) Cilium formation at the apical part of a cell. (**b**) Collagen synthesis in the basal part of ciliated cell. *c* cilium, *ci* ciliated cell, *yg* yolk granule, *co* collagen, *l* lipid droplet, *n* nucleus. Scale bars (**a**, **b**) 2 μm

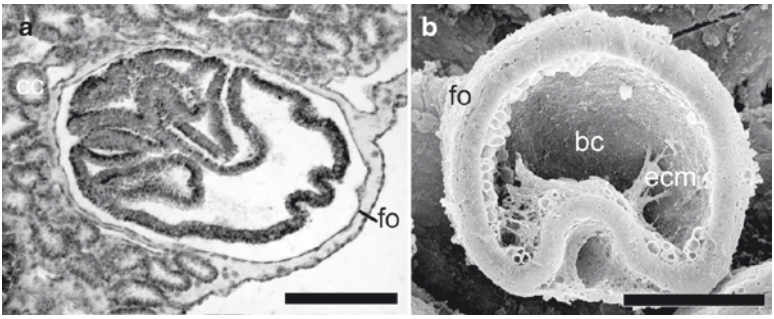

Fig. 4.10 Light (**a**) and SEM (**b**) micrographs of folded coeloblastulae of Homoscleromorpha. (**a**) *Oscarella malakhovi.* (**b**) *O. lobularis. bc* blastocoel, *cc* choanocyte chamber, *ecm* extracellular matrix, *fo* follicle. Scale bars (**a**, **b**) 100 μm

In the areas with folds, bundles of extracellular matrix are formed between the basal ends of the ciliated cells of the deep fold's "roof" and the opposite wall of the embryo; if the folds are numerous, extracellular matrix bundles fill the whole cavity.

Folded blastula and prelarvae are very common in sponges (see Ereskovsky 2005) and echinoderms (Byrne 1995), whereas in most other animals blastulae are smooth. In the Eumetazoa embryogenesis, epithelial invaginations or folds are the main morphogenetic mechanism ensuring the formation of tubular structures: archenteron, the nerve tube, etc. The key role in these morphogeneses is played by interactions between the cells and the extracellular matrix. The latter is very

likely to be equally important in the case of formation of prelarva folds in the Homoscleromorpha.

Later in the development, ciliated larval cells become regionally differentiated. Differentiation proceeds in two stages: first, the cells differentiate into ciliated and nonciliated ones; second, the ciliated cells differentiate into anterio-lateral cells, posterio-lateral cells, and the cells of the posterior pole (Boury-Esnault et al. 2003). These three categories have been repeatedly described in *O. lobularis* (=*tuberculata*) larvae (Heider 1886; Meewis 1938; Lévi and Porte 1962).

At the end of the development, the follicle wall merges with that of the exhalant canal, and the larvae are released via the osculum.

4.4 Larva

The larva of *Oscarella* was discovered by Barrois (1876). At first, it was thought that the cells of its posterior pole are much larger than those of the anterior pole (Barrois 1876; Maas 1898) and so the *Oscarella* larva was attributed to the amphiblastula type (Heider 1886). Meewis (1938) showed that the above assumption about the relative size of larval cells in *Oscarella* was based on a fixation artifact, but since according to her own investigation the posterior pole cells were indeed somewhat larger, she did not reject the name of amphiblastula as applied to the *Oscarella* larva.

So, for over a century, the Homoscleromorpha were thought to have the amphiblastula larva (Heider 1886; Meewis 1938; Lévi and Porte 1962; Tuzet and Paris 1964; Brien 1973a). On the basis of this assumption, the Homoscleromorpha were sometimes considered a transition group between the Calcarea and the Demospongiae (Grothe and Reitner 1988; Grothe 1989; van Soest 1991).

In fact, the homoscleromorph larva has nothing in common with the typical calcaronean amphiblastula; their morphogenesis and cell ultrastructure are entirely different (see Section 1.1). The homoscleromorph larvae cannot be considered coeloblastulae, despite the fact that they are single-layered. The typical coeloblastula larvae – those of the Calcinea (see Section 1.2) and some oviparous Demospongiae (see Sections 3.2 and 3.4) — do not have the regional differentiation of ciliated cells and the basal membrane characteristic of the Homoscleromorpha. To avoid misinterpretations of the homoscleromorph phylogenetic relations, a special name of *cinctoblastula* (“a larva with a belt”) was coined for their larvae (Boury-Esnault et al. 1995; Boury-Esnault and Rützler 1997).

The larvae of all the Homoscleromorpha are completely and evenly ciliated, with all the cilia having the same length. The larvae have a pronounced polarity expressed in their shape, color, and distribution of symbiotic bacteria and parent cells (Fig. 4.11). The anterior pole is distended, whereas the posterior pole is tapered and pigmented. Most of the symbiotic bacteria (in *Oscarella* sp., *O. lobularis, O. imperialis,* and *P. jarrei*, and also most of the parent cells) concentrate in the posterior pole region (Fig. 4.11c, d). The size of the larvae is more or less stable within the species, but varies considerably from species to species; the color is species-specific

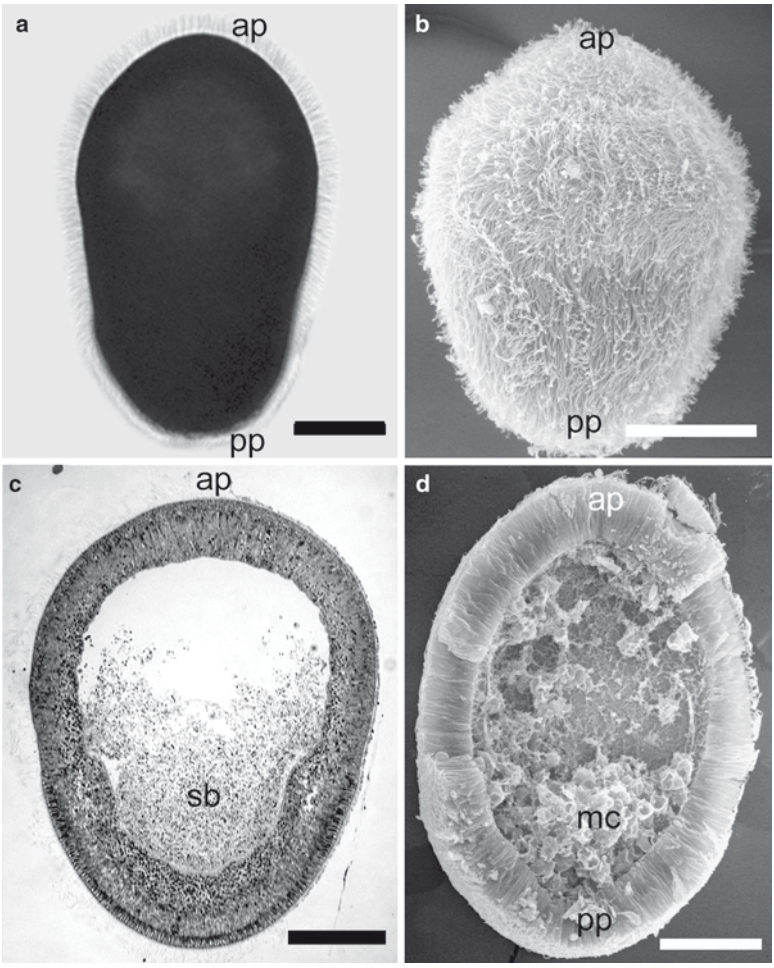

Fig. 4.11 Cinctoblastulae of Homoscleromorpha. (**a**) *In vivo* photo of *Pseudocorticium jarrei* larva. (**b**) SEM of *Plakina trilopha* larva. (**c**) Semifine section of *Corticium candelabrum* larva. (**d**) SEM of longitudinal freeze-fracture of *Oscarella lobularis* larva. *ap* anterior pole, *mc* maternal cells, *pp* posterior pole, *sb* symbiotic bacteria. Scale bars (**a**–**d**) 100 μm

(Boury-Esnault et al. 2003). The larvae swim in spirals, anterior pole first, rotating clockwise along the anterior–posterior axis. The larvae of different species may exhibit both positive and negative phototaxis.

Homoscleromorph larvae are differentiated into three morphological regions: the anterio-lateral region, occupying about 60% of the surface; the narrow posterio-lateral region; the region of the posterior pole. The ciliated cells of each region differ as to the size and shape, the nature and distribution of the cytoplasmic inclusions, the shape and position of the nucleus, and some other features (Fig. 4.12).

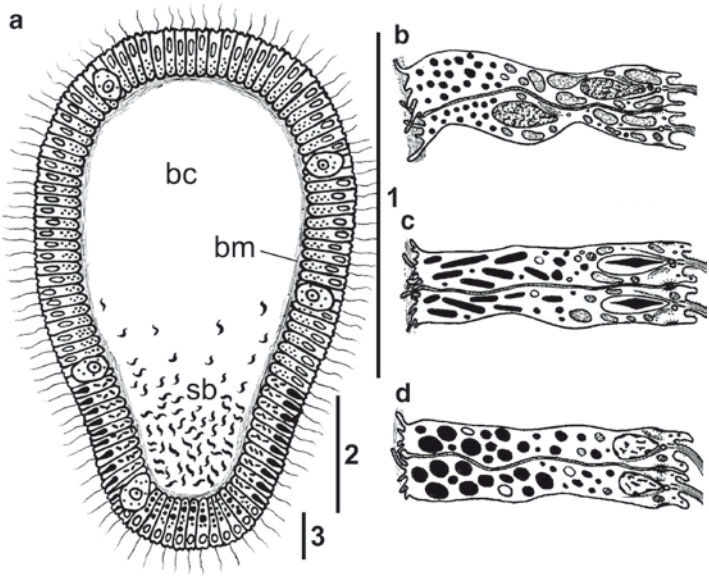

Fig. 4.12 Schematic drawing of homoscleromorphs cinctoblastula (**a**) and its different ciliated cells: (**b**) anterio-lateral; (**c**) intermedial zone; (**d**) posterior pole. 1 – anterio-lateral zone, 2 – intermedial zone, 3 – posterior pole zone, *bc* blastocoel, *bm* basement membrane, *sb* symbiotic bacteria (From Boury-Esnault et al. 2003, p. 193, Fig. 16 with modifications, reproduced by permission of Wiley)

The cells are polarized along the apical–basal axis, so that three zones can be delimited: the apical zone, the middle zone, and the basal zone (Boury-Esnault et al. 2003; de Caralt et al. 2007; Maldonado and Riesgo 2008; Ereskovsky et al. 2009b).

The apical zone contains the ciliated apparatus. The cilium base is surrounded by a collar of cytoplasmic projections, the outgrowths of the apical membrane (Fig. 4.13a, b). The central pair of microtubules radiates from the basal axoneme plate (Fig. 4.13c). At the basis of the ciliar transition zone, below the basal plate, there are alar sheets radiating from the external microtubular doublets (Fig. 4.13c–f). The basal body (the kinetosome) has a similar size in all the species studied. The basal foot, shaped as a champagne cork, is not cross-striated and radiates from the central kinetosome part. Microtubular network, starting from the kinetosome, forms a dense unpaired bundle orthogonal to the basal foot (Fig. 4.13d). The bundle is parallel to the plasma membrane and associated with the belt desmosome. The additional (nonciliar) centriole is situated below the kinetosome at the same axis with the axoneme (Fig. 4.13c, d). A short bundle of microtubules radiates from the centriole toward the basal foot. A single rootlet is cross-striated and closely associated with the nuclear membrane (Fig. 4.13e) (Boury-Esnault et al. 2003; Maldonado and Riesgo 2008).

The apical cytoplasm is usually free from yolk and lipid inclusions. It contains the Golgi apparatus and small rounded mitochondria with lamellate cristae

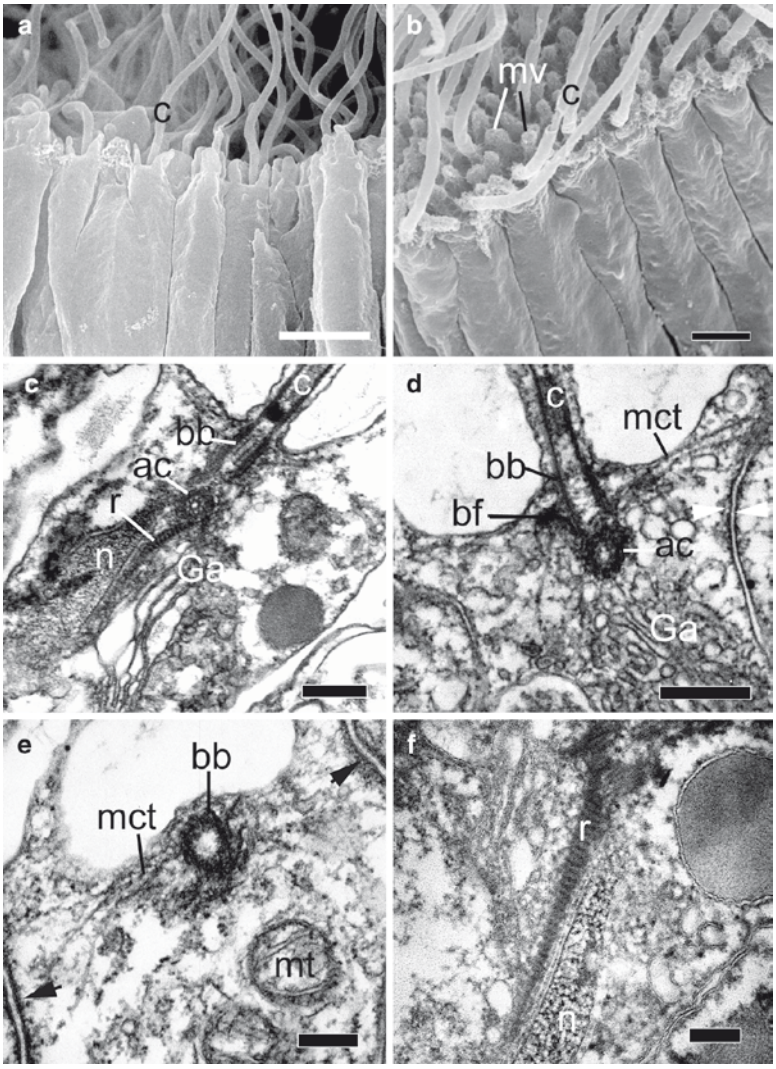

Fig. 4.13 SEM of apical parts of larval ciliated cells in *Oscarella lobularis* (**a**) and *Plakina trilopha* (**b**). TEM of a basal ciliar apparatus of larval cells in *P. trilopha* (**c**, **e**), *O. tuberculata* (**d**), *O. microlobata* (**f**). *ac* accessory centriole, *arrowhead* belt desmosome, *bb* basal body, *c* cilia, *Ga* Golgi complex, *mct* microtubules, *mv* microvilli, *mt* mitochondria, *n* nucleus. *r* ciliar rootlet. Scale bars (**a**, **b**) 3 μm, (**c**, **d**) 1 μm, (**e**) 0.25 μm, (**f**) 0.5 μm. (**c**–**e** – From Boury-Esnault et al. 2003, p. 194, Figs. 17–20, reproduced by permission of Wiley)

(Fig. 4.13c–f). The nucleus is rounded or, more often, oval; it may be situated in the apical, middle, or basal cell part.

Specialized cell junctions (belt desmosome or *zonula adhaerens*) are present between the apical cell parts. These junctions have regularly spanned septae in the intercellular space (Fig. 4.14a) (Boury-Esnault et al. 2003; Maldonado and Riesgo 2008).

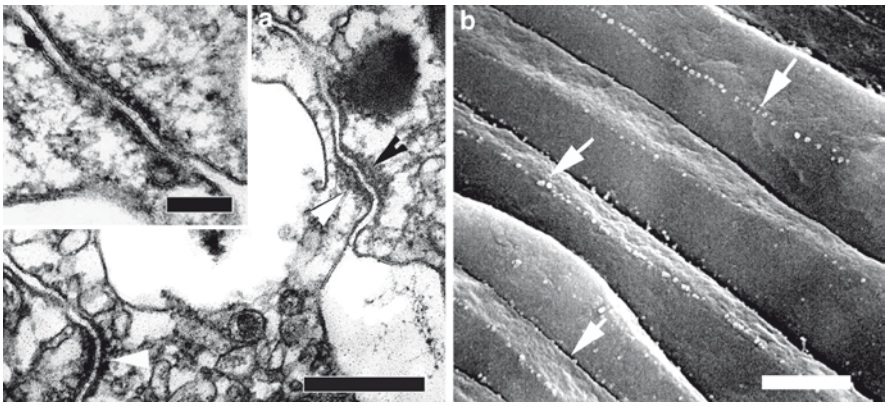

Fig. 4.14 Cell junctions in ciliated larval cells in Homoscleromorpha. (**a**) TEM of a belt desmosome (*arrowhead*) in *O. microlobata*; *inset* – belt desmosome. (**b**) SEM of other junctions by regular rows of oval structures (*arrows*) in *Plakina trilopha.* Scale bars (**a**) 1.1 μm, *inset* 0.25 μm, (**b**) 1.4 μm (From Boury-Esnault et al. 2003, p. 194, Figs. 21, 22, reproduced by permission of Wiley)

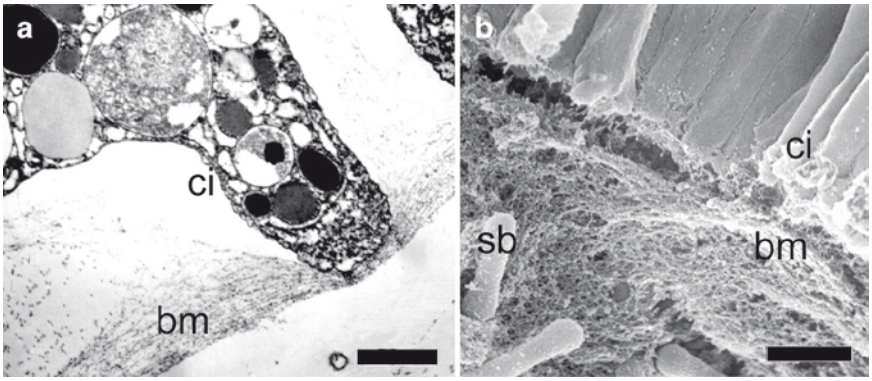

Fig. 4.15 Basal part of ciliated larval cells with a basement membrane. (**a**) TEM in *Oscarella microlobata.* (**b**) SEM in *Plakina trilopha. bm* basement membrane, *ci* ciliated cells, *sb* symbiotic bacteria. Scale bars (**a**, **b**) 1.7 μm

Below the belt desmosome, from the apical third and down to the basal part, there are cell junctions of a different type: regular rows of oval structures parallel to the longer cell axis (Fig. 4.14b) (Boury-Esnault et al. 2003).

The basal cytoplasm is filled with numerous yolk and lipid granules and, in some cells, large phagosomes. Inclusions-free areas contain mitochondria, ribosomes, rare small electron-transparent vacuoles, and EPR elements. Long branching filopodia stretch parallel to the internal larval surface. Membranes of filopodia and basal cell areas are the assemblage sites of collagen molecules, which form the basal membrane underlining the inside of the larva (Fig. 4.15).

To sum up, homoscleromorph larvae possess a typical eumetazoan palisade epithelium (Tyler 2003). In its apical part, the cells are connected by belt desmosomes; in the lateral areas, with amazingly regular membrane projections resembling septate contacts. The basal areas form a complex filopodial network underlain by the basal membrane made up of the basal lamina and the extracellular matrix. Among the Porifera, a true basal membrane is present only in the Homoscleromorpha (Boute et al. 1996). It can be expected that collagen IV would be found in the basal lamina of the homoscleromorph prelarvae and larvae. Basal membranes, with collagen IV as their main component, have been found in all metazoans (Morris 1995), including the coeloblastulae of the Echinodermata (Cherr et al. 1992).

The cells of the posterior-lateral zone were first described by Meewis (1938), who thought them to be some kind of light-sensitive organ. These cells form a belt around the posterior pole (Figs. 4.12c, 4.16). Their nuclei contain an osmiophilic crystalloid parallel to the longer cell axis (Fig. 4.16a). The basal third of the cell contains osmiophilic rod-like inclusions parallel to the longer cell axis.

The posterior pole cells (Fig. 4.12d) are characterized by the apical position of a rounded nucleus with clearly visible heterochromatin. In *Oscarella* larvae, the nuclei of these cells are positioned in one to three rows, whereas in *Plakina* and *Corticium*, they are at approximately the same level. The basal cell parts contain numerous oval or spherical osmiophilic inclusions – probably, pigment granules.

The larval epithelium includes randomly distributed single nonciliated cells (Fig. 4.17). Their shape varies from spherical to pear-shaped to oval. They do not form either filopodia or lobopodia and are connected to the neighboring cells by belt desmosomes. Their rounded, usually central nucleus contains one nucleolus.

These cells were first described in the swimming larva of *O. tuberculata* by Heider (1886) as secretory. Later, they were studied in the same species at the light microscopic level (Meewis 1938) and at the electron microscopic level (Lévi and Porte 1962; Boury-Esnault et al. 2003; de Caralt et al. 2007a).

Competence and settlement. Similar to the larvae of other invertebrates, homoscleromorph larvae have a period of competence, i.e., preparation to settlement and metamorphosis (Ereskovsky et al. 2007a, 2009b). During this period, excretory vacuoles with mucous contents in the anterior pole cells shift to the apical surface and merge, forming vast irregular cisterns (Fig. 4.18). When the larva settles down, the mucous substance is excreted, attaching it to the substrate. During the same period, the basal parts separate from the anterior pole and the posterio-lateral cells. The posterior pole cells of the competent larva demonstrate pseudopodial activity. Just before the settlement, the larva changes its shape from the typical egg-like one to trapezoid or pentagonal at cross-section.

The larvae settle 12–48 h after their release from the parent sponge. The settlement occurs at the anterior end first. In the initial stage of attachment, the anterior larval pole flattens and the posterior one becomes nipple-shaped, with a species-specific color. The edges of the marginal zone (the former anterior pole) are uneven and wavy. During attachment and in the beginning of the metamorphosis, the larvae that left the same sponge can fuse. The settled larva (postlarva) is bell-shaped or flattened (Fig. 4.19a, c).

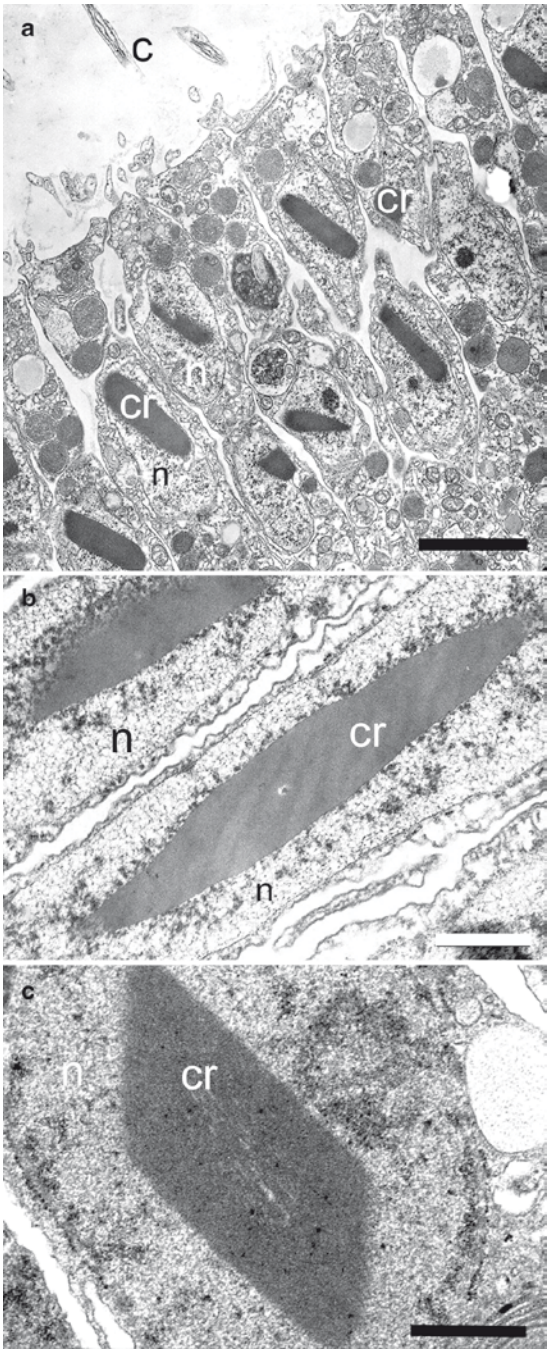

Fig. 4.16 TEM of cells of intermediate zone in cinctoblastula of *Oscarella tuberculata* (**a**) and longitudinal section of intranuclear paracrystalline inclusion in *Plakina trilopha* (**b**) and *O. malakhovi* (**c**). *c* cilium, *cr* intranuclear paracrystalline inclusion, *n* nucleus. Scale bars (**a**) 3 μm, (**b**) 1 μm, (**c**) 0.5 μm

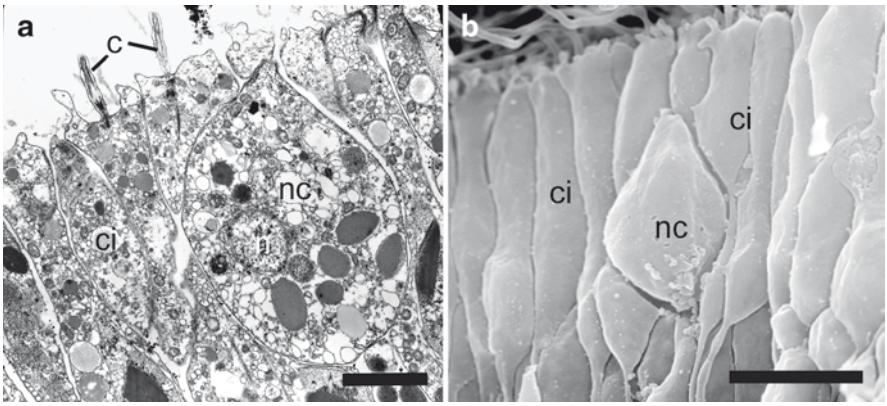

Fig. 4.17 Nonciliated cells in the ciliated epithelium of *Oscarella tuberculata* cinctoblastula (**a** TEM, **b** SEM). *c* cilium, *ci* ciliated cell, *n* nucleus, *nc* nonciliated cell. Scale bars (**a**) 2 μm, **(b**) 5 μm

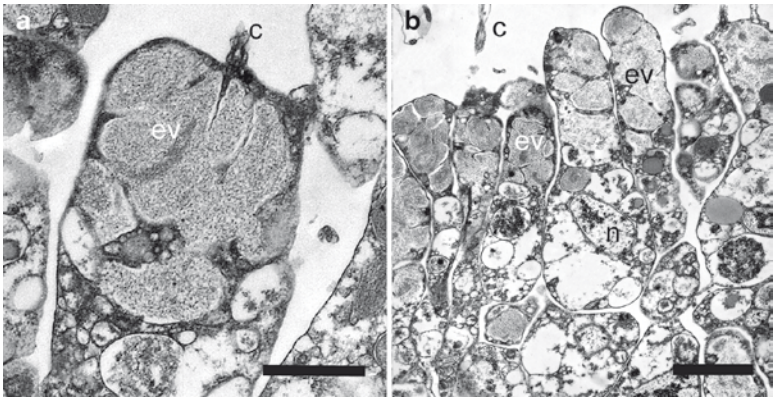

Fig. 4.18 TEM of excretory vacuoles with mucous contents in an anterior pole ciliated cells of the larva of *Oscarella tuberculata* during the competention stage. *c* cilium, *ev* excretory vacuole, *n* nucleus. Scale bars (**a**) 1 μm, (**b**) 2.5 μm

4.5 Metamorphosis

In the beginning of the metamorphosis, the cells in various parts of the postlarva change their shape. At the former posterior pole, the apical cell parts flatten and form numerous lobopodia and filopodia. The fate of apical cells of the metamorphosing larva (postlarva) depends on the direction of invagination during the aquiferous system formation. If this morphogenesis proceeds in the apical direction, the cells differentiate into choanocytes or endopinacocytes; otherwise, into exopinacocytes. This is true of all the homoscleromorph species studied (Ereskovsky et al. 2007a, 2009b). The cells differentiating into choanocytes

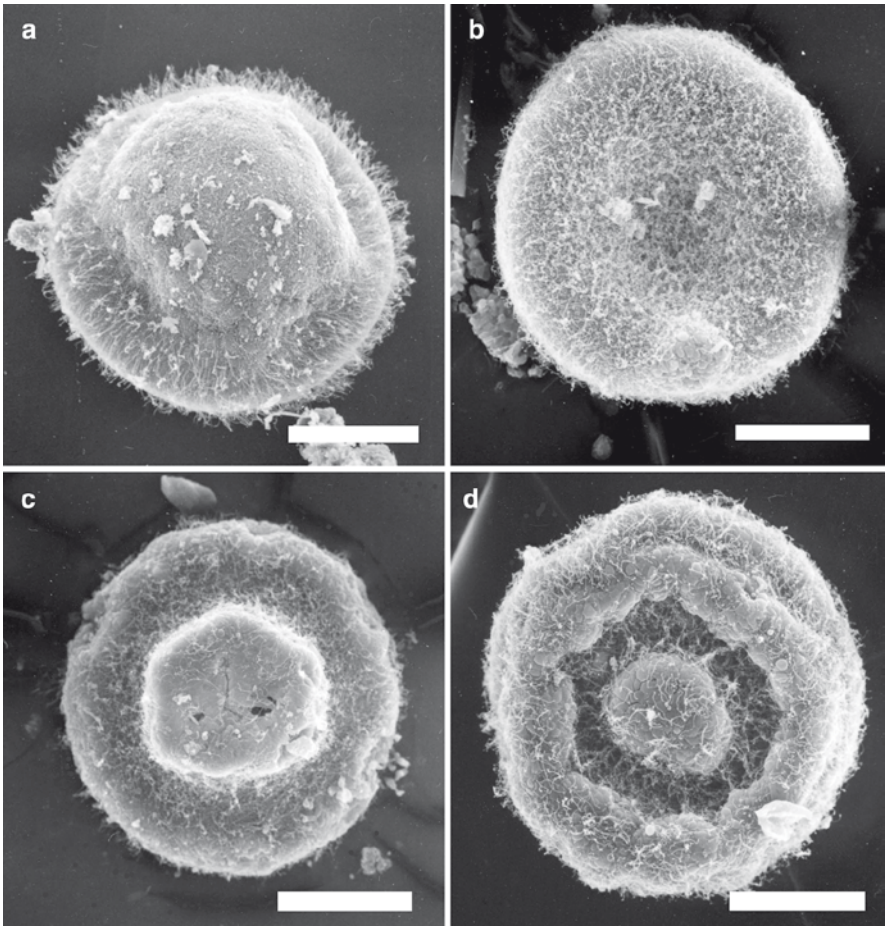

Fig. 4.19 SEM of larvae of Homoscleromorpha at the first stages of metamorphosis. Apical pole (**a**) and basal pole (**b**) of settlers of *Plakina trilopha.* Apical pole (**c**) and basal pole (**d**) of settlers of *Oscarella tuberculata.* Scale bars (**a**–**d**) 100 μm. (**a**, **d** – From Ereskovsky et al. 2007a, p. 520, 524, Figs. 3a, 7b, reproduced by permission of Wiley)

remain prismatic, whereas those differentiating into pinacocytes become first cubic and then flattened.

Lateral cells of the postlarva are more active than the basal and the apical ones. Some of them ingress into the larval cavity (Fig. 4.20a, b). Their shape changes from prismatic to club-like because of the nucleus shifting to the basal part (Fig. 4.20a). In the basal part, lobopodia are also formed. The basal membrane may partially degenerate (Fig. 4.20a) or be retained. Specialized cell junctions are disrupted and a broader space is formed between the cells. During ingression, the cilium is withdrawn and reduced. The noningressing cells become smaller, partly because of the separation of their basal parts.

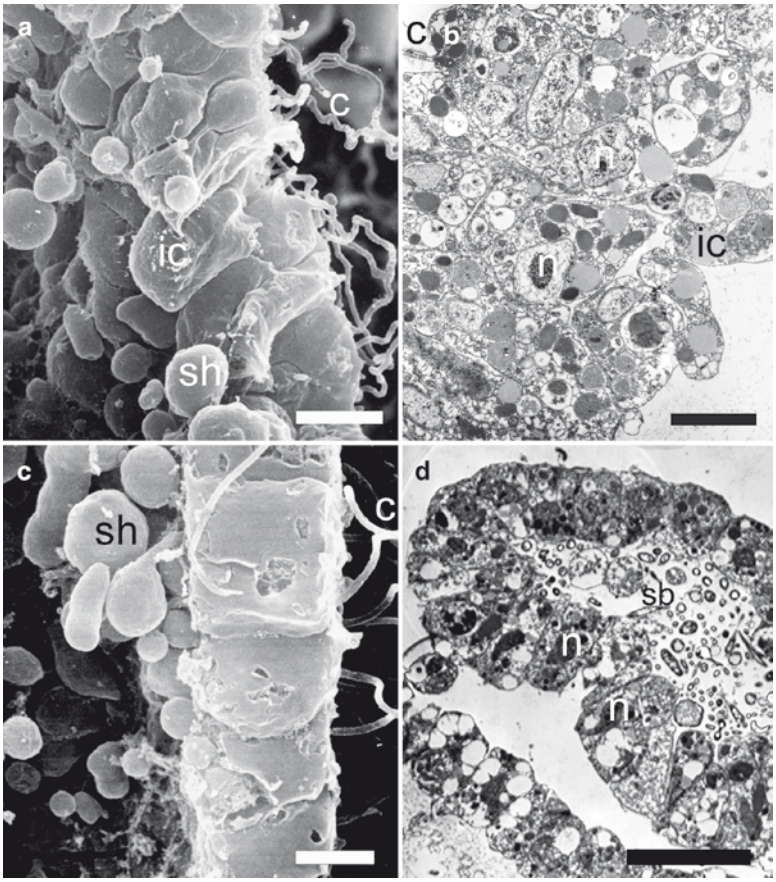

Fig. 4.20 Larval metamorphosis in Homoscleromorpha (**a**, **c** SEM, **b**, **d** TEM). (**a**, **b**) Antero-lateral cells ingression in *Oscarella microlobata*. (**c**) Shedding of basal parts of the posterior pole cells in *Plakina trilopha.* (**d**) Marginal zone with intermedial cells in *P. trilopha. c* cilium, *in* ingressed cell, *sb* symbiotic bacteria, *sh* shedded cell parts, *n* nucleus. Scale bars (**a**–**d**) 5 μm

The marginal zone cells flatten and form numerous pseudopodia and cytoplasmic projections. Invaginating cells retain their cilia. The marginal zone mostly contains larval posterio-lateral cells with a gradually disappearing intranuclear crystalloid (Fig. 4.20d).

As early as the competence period, the former anterior pole cells (now the basal cells of the postlarva) start to separate their basal parts with fragments of the cytoplasm, sometimes containing yolk granules (Fig. 4.20a) (Ereskovsky et al. 2009b). These spherical or oval fragments are positioned along the basal membrane of the postlarva. We have called this phenomenon "*cytoplasmic shedding*" (Ereskovsky et al. 2009b). Cytoplasmic shedding appears to play an important role in the change of epithelial cell shape and volume in metamorphosing cinctoblastula. This mechanism has never been described during early morphogenesis of any other poriferan

species. The vesicles that have been shed are phagocytized later by the cells of the developing rhagon.

During the early metamorphic stages, the basal cells change their shape from prismatic to cubic (Fig. 4.20c); they retain cilia, the specialized cell junctions (in the apical parts) and the basal membrane (Fig. 4.20c). There is no ingression of the basal cells. Some of them differentiate into basopinacocytes, which excrete the mucus attaching the rhagon to the substrate.

The invagination of the ciliated cells layer into the postlarva is the major morphogenetic event during the metamorphosis of the homoscleromorph cinctoblastulae. However, this event is polymorphic (Fig. 4.21) (Ereskovsky et al. 2007a,

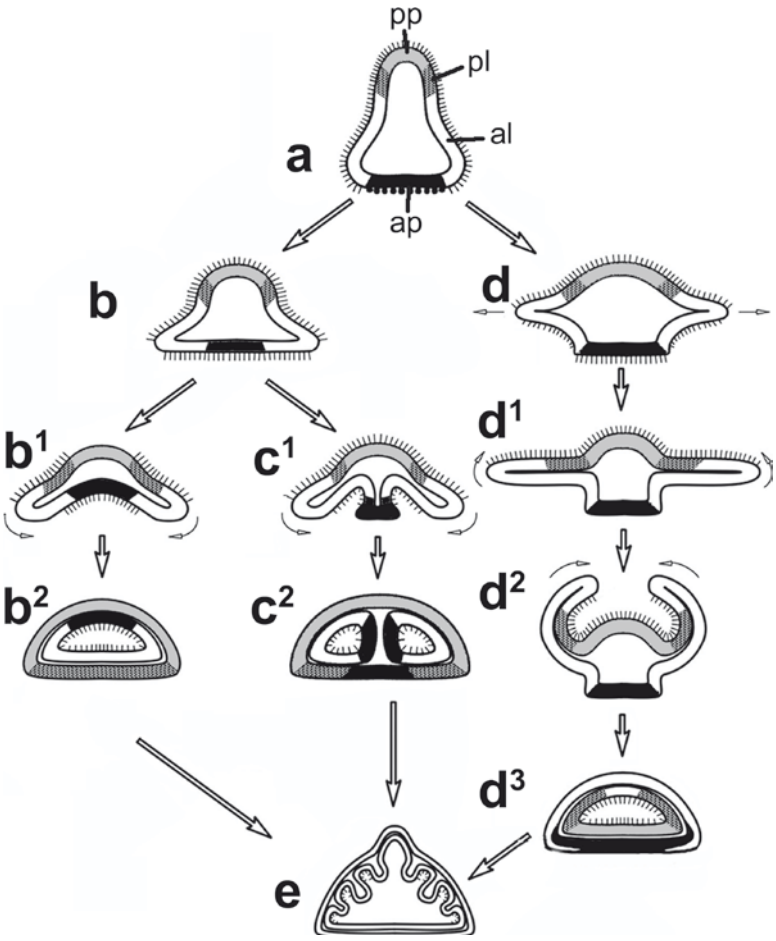

Fig. 4.21 Schematic drawing of the different paths of rhagon formation during the metamorphosis of homoscleromorph larvae. (**a**) Cinctoblastula. (**b**–**b**^{2}) Basal invagination. (**c**^{1},**c**^{2}) Basal ring like invagination. (**d**–**d**^{3}) Extension and folding up of lateral sides of postlarva. (**e**) Rhagon. *al* anterio-lateral cells, *ap* anterior pole cells, *pl* posterio-lateral cells with intranuclear paracrystalline inclusions, *pp* posterior pole cells (From Ereskovsky et al. 2007a, p. 520, Fig. 2, reproduced by permission of Wiley)

2009b). As a rule, this epithelial morphogenesis proceeds as a dome-shaped invagination of the postlarval basal layer (Figs. 4.19b; 4.21b–b^2). In some postlarvae of *O. tuberculata*, it is the ring of cells around the central attached area that invaginates (Figs. 4.19d; 4.21c^1–c^2). Finally, there may be numerous local invaginations of the basal surface epithelium.

In many postlarvae, the formation of the rhagon's aquiferous system is based on the apical ciliated epithelium: lateral flattened areas overgrow the apical surface and close there (Fig. 4.21, d–d^3). In other postlarvae, the aquiferous system is formed by a combination of basal invagination and apical overgrowth. Finally, in some postlarvae, central or circular invagination of the basal surface is accompanied by the formation of local invagination areas. In the course of epithelial morphogeneses, single cells of the lateral parts of the marginal zone may migrate into the postlarval cavity.

After the closure of exopinacoderm, formation of the rhagon's aquiferous system starts. The development of the rhagon's internal structures (choanocyte chambers and canals) is also based on the epithelial morphogenesis. Terminal parts of the internal folds transform into choanocyte chambers (Fig. 4.22a), whereas proximal ones transform into canals. The rhagon is originally syconoid, but there is no radial symmetry in the position of the aquiferous system elements (Fig. 4.21e).

The larval cells are differentiated according to their position in the postlarva. During differentiation of pinacocytes and choanocytes, the larval cilium is retained (Fig. 4.22b–d), but the ciliar basal apparatus undergoes changes. The rootlet dissociates; the additional centriole of pinacoblasts, orthogonal in the larvae, shifts to the kinetosome edge opposed to the basal foot. The differentiating endopinacocyte is greatly flattened in the apical–basal direction (Fig. 4.22b). The differentiating choanocytes become cubic or prismatic; microvilli develop in the apical part and the nucleus shifts to the basal part (Fig. 4.22c, d).

The most considerable changes occur to the cells that have migrated into the larval cavity. They lose entirely the ciliar apparatus, become amoeboid or rounded (Fig. 4.22e, f), and later differentiate into various mesohyl cells: archaeocytes, secretory cells, and in sponges with spicules, sclerocytes.

Symbiotic bacteria, early in the metamorphosis densely concentrated in the apical part of the postlarva, gradually spread evenly in the rhagon. During the metamorphosis, they continue to divide actively in the intercellular space (Figs. 4.20d; 4.22f).

We did not observe in metamorphosing homoscleromorph larvae either cell destruction or phagocytosis of certain cells by others, the phenomena characteristic of parenchymella larvae of many Demospongiae during this process. There was also no proliferation of postlarval cells.

To sum up, during the metamorphosis of the homoscleromorph cinctoblastulae, all the rhagon structures develop from the ciliated larval cells (Fig. 4.23). The main metamorphic role belongs to the anterio-lateral cells, which are the most numerous. The fate of nonciliated larval cells was not traced.

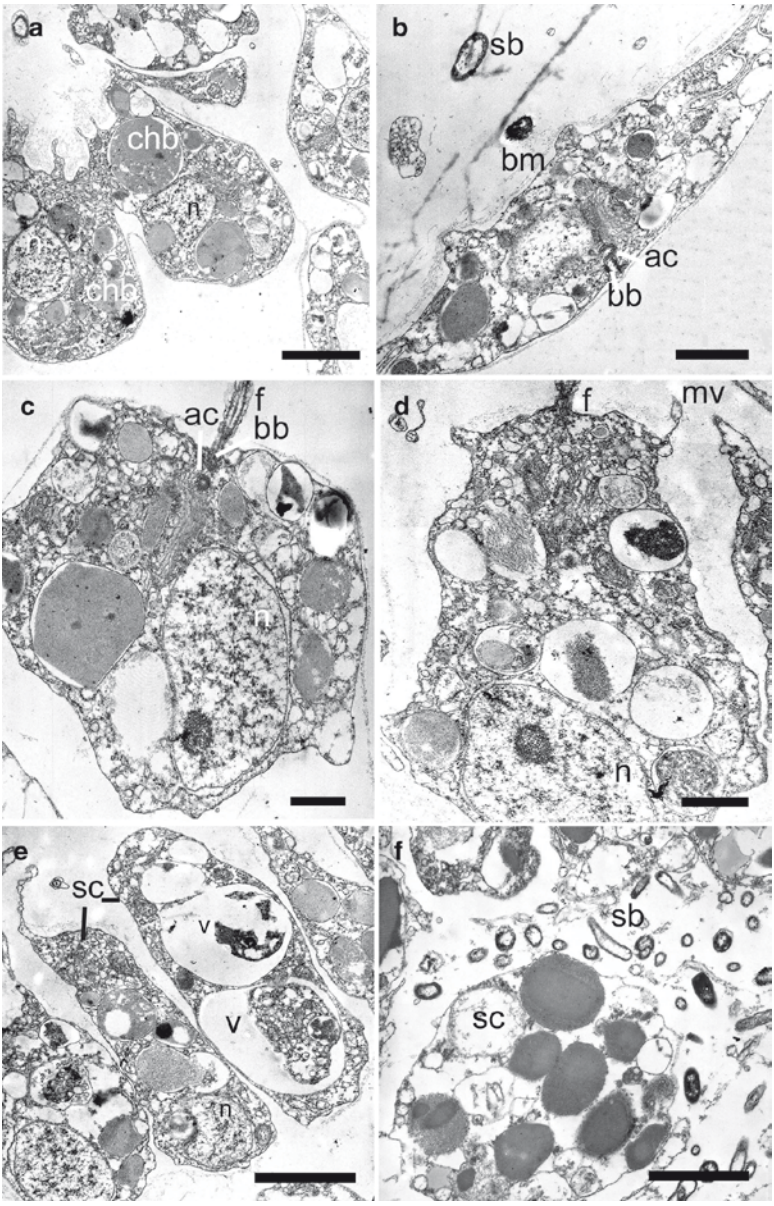

Fig. 4.22 TEM of adult cells differentiation in the rhagon of *Oscarella tuberculata.* (**a**) Developing choanocyte chamber. (**b**) Endopinacocyte differentiation. (**c**, **d**) Differentiation of choanocytes. (**e**, **f**) Differentiation of mesohylar secretory cells. *ac* accessory centriole, *bb* basal body, *bm* basement membrane, *chb* choanoblast, *f* flagellum, *mv* microvilli, *n* nucleus, *sb* symbiotic bacteria, *sc* secretory cell, *v* vacuole. Scale bars (**a**, **f**) 5 μm, (**b**) 2 μm, (**c**, **d**) 1 μm, (**e**) 3 μm. (**b**–**d**) – From Ereskovsky et al. 2007a, p. 526, Fig. 9, reproduced by permission of Wiley)

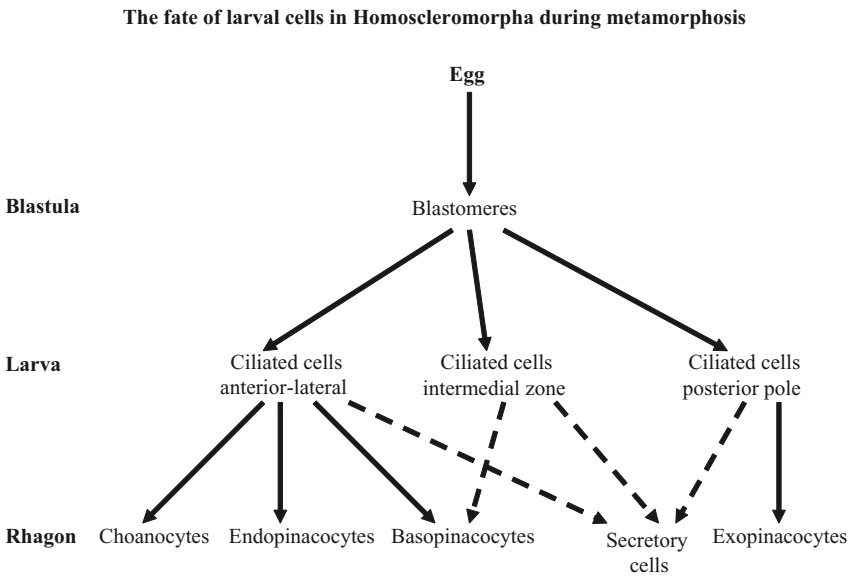

Fig. 4.23 The fate of larval cells in Homoscleromorpha during metamorphosis

4.6 Asexual Reproduction

Homoscleromorphs are characterized by asexual reproduction via simple fission and budding. Budding in *O. tuberculata* was first mentioned in a short communication bySchulze (1879a). We studied in detail the budding in four homoscleromorph species: *O. lobularis, O. tuberculata, O. malakhovi*, and *C. candelabrum* (Fig. 4.24) (Ereskovsky 2006; Ereskovsky and Tokina 2007; unpublished).

External budding in *Oscarella* can be divided into three stages:

1. Numerous small nipple-like projections form in the depressions between the lobes in the lower part of the sponge (Fig. 4.25a, b). These projections are made up of the parent ectosome; they comprise the exopinacoderm and a thin mesohyl layer.
2. The projections elongate and increase in diameter. They are tubular at cross-section, the internal cavity being an outgrowth of the exhalant canal (Fig. 2.25c). Their walls are three-layered: the external layer consists of exopinacoderm and the internal one, of endopinacoderm. The intermediate layer is mesohylar, with small choanocyte chambers, short stretches of the aquiferous system canals, and cells with inclusions.
3. Bud formation is completed. The apical part of the projection becomes a bubble, and the basal part becomes the stalk of the bud (Fig. 4.24; 4.25d). Short conical papillae develop on the bud surface. Finally, the buds separate from the parent sponge.

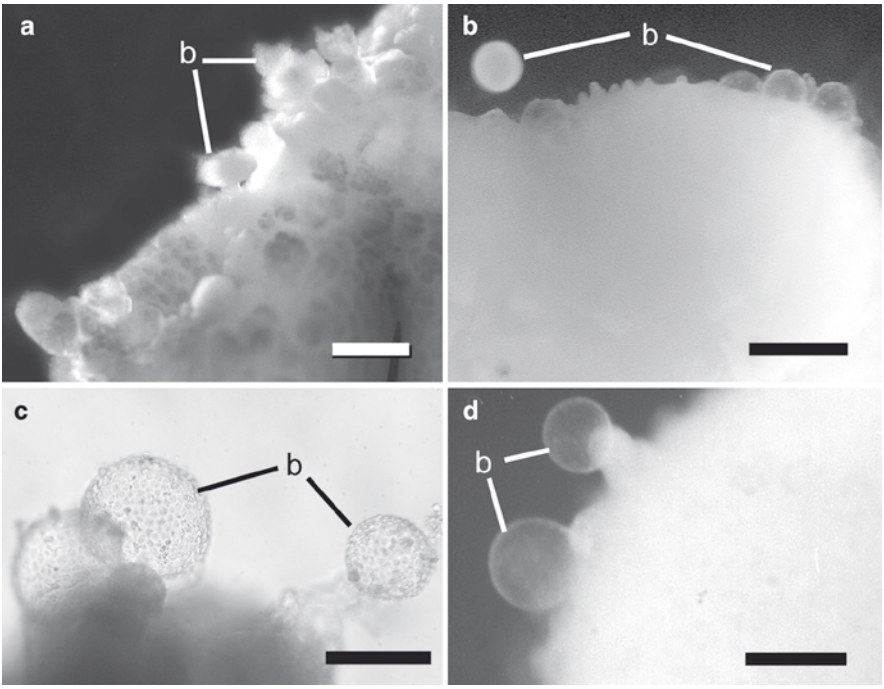

Fig. 4.24 Budding in Homoscleromorpha. *In vivo* photos of the buds (*b*) in *Oscarella tuberculata* (**a**), *Plakina trilopha* (**b**), *Oscarella malakhovi* (**c**), and *O. lobularis* (**d**). Scale bars (**a**, **b** 200) μm, (**c**, **d**) 100 μm

Since their buoyancy is close to zero, the buds may float with water currents for a long time, but sooner or later they attach, with the help of the papillae, to the substrate. In 2 or 3 days, the attached bud develops into a small adult sponge, which lacks the papillae but has the oscular tube. Structurally, the new sponge is a rhagon.

To sum up, external budding in the Homoscleromorpha is essentially different from that in other sponges, the differences concerning both the morphogenesis and the bud structure. The bud develops by an epithelial morphogenesis, evagination, i.e., the forming is just an outgrowth of the parent body wall. The cells at budding sites do not migrate to the periphery, do not form accumulations, and do not proliferate. Cell composition of the bud is identical to that of the resulting definitive sponge.

Homoscleromorph blastogenesis, unique among sponges, is likely to be possible due to the epithelial structure of their body. It is similar to morphallaxis during regeneration, since the bud area transforms completely into a new individual, which, though small, has the proportions of the adult sponge. Morphallactic blastogenesis is widespread in Scyphozoa and Hydrozoa (Cnidaria) (Webster and Hamilton 1972; Hofmann and Honegger 1990) and sometimes occurs during palleal, stolonial, and vascular budding of Ascidiacea (Kawamura and Nakauchi 1986).

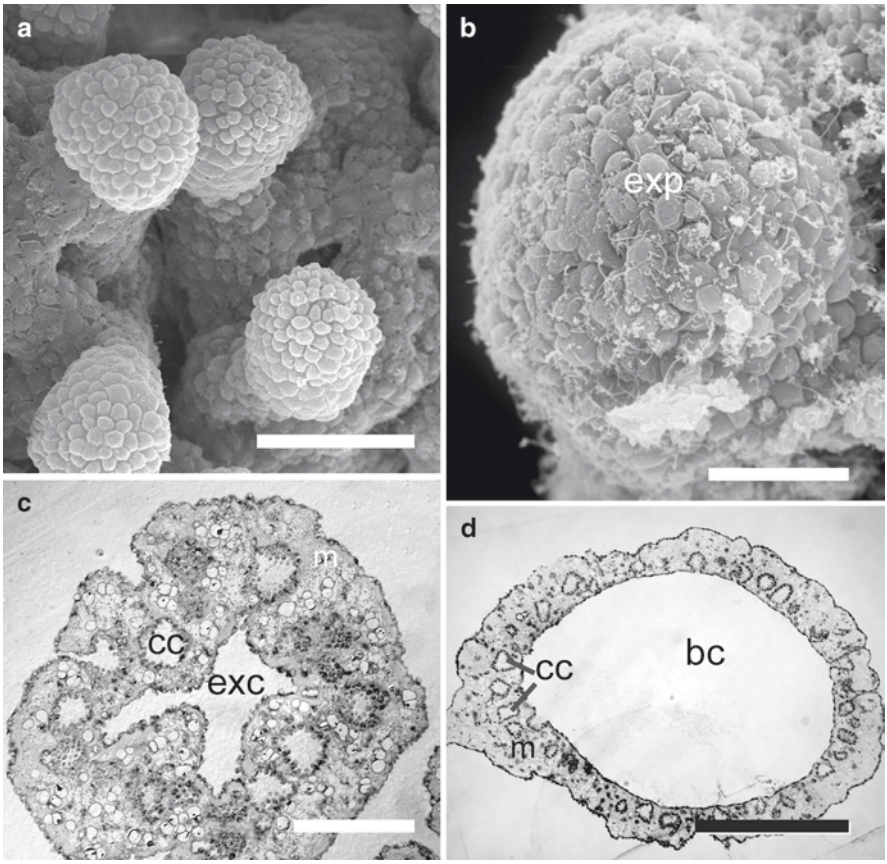

Fig. 4.25 Budding in Homoscleromorpha. SEM of the first stage of buds formation in *Oscarella lobularis* (**a**) and *Corticium candelabrum* (**b**). (**c**) Light microscopy of perpendicular section of the protuberance at the second stage of budding in *Oscarella tuberculata.* (**d**) Light microscopy of floating bud of *O. lobularis. bc* buds' cavity, *cc* choanocyte chambers, *exc* exhalant canal, *exp* exopinacocytes, *m* mesohyl. Scale bars (**a**) 100 μm, (**b**) 50 μm, (**c**) 10 μm, (**d**) 150 μm

Part II
Theoretical Aspects of Sponge Embryology

Chapter 5
Typization of Sponge Development and Its Significance for Phylogeny

Though the morphology of sponges is highly plastic and diverse, we can always find a set of morphological characters typical of a species, genus, family, and order. In sponges, these specific features are associated not only with the adult individuals, as is usually the case in taxonomy, but also with earlier ontogenetic stages. Therefore, to reveal all the structures characterizing a taxon, a comparative study of all the life-cycle stages is necessary.

Each sponge structure is the result of a preceding morphogenesis. The challenging task of systematizing the diversity of morphogeneses accompanying sexual and asexual reproduction in sponges has attracted many researchers (Lévi 1956; Brien 1973a; Borojevic 1970; Korotkova 1981b; Fell 1989; Ereskovsky 2004, 2005; Leys and Ereskovsky 2006). Some of them saw the evolution of sponge ontogenesis as a gradual series of complications of the embryogenesis, correlating with the emergence of a more complex aquiferous system (Brien 1967c, 1973a; Ivanova-Kazas 1997). The others represented it as a result of the interactions between the amoeboid and the flagellated cell line during morphogenetic processes (Borojevic 1970). However, almost all these studies took into account only the morphogeneses accompanying sexual reproduction. The only exception was an attempt at typization of sponge development made by Korotkova (1981b, 1988), who considered both the sexual and the somatic morphogeneses and the possibility of their alteration in the life cycle as well as in the phylogenesis.

One of the reasons that precluded the classification of sponge development and its evolutionary interpretations was the insufficiency of material. Only scarce data, often obtained at the light microscopic level, were available on many taxa.

For the purpose of the present research, a comparative embryological study of 35 species from the ten orders of Demospongiae and Calcarea (Table 5.1) was undertaken. The results obtained were supplemented with the data from the literature (see Chapters 1–4). Only literature data were used for most representatives of the Calcarea and the Hexactinellida. The orders and the suborder of the Demospongiae with only one species studied were left out of the analysis ("Lithistida," Verongida, Agelasida, and Petrosina from Haplosclerida).

The aim of this chapter is to describe the developmental types found in sponges, to ascribe them to sponge high taxons, and to find out whether they can be applied

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Table 5.1 Sponge species studied within the present investigation

Order	Species	Collection area	Depth (m)	Collection time
Demospongiae				
Haplosclerida	*Haliclona aquaeductus* (Schmidt 1862)	White Sea	3–9	1983–2003
	Haliclona sp.	White Sea	3–9	1991–2002
	H. mediterannea Griessinger 1973	Mediterranean Sea	7–12	1999
	Gellius angulatus Bowerbank 1866	White Sea	8–12	2006
	Ephydatia muelleri (Lieberkuhn 1855)	Rivers and lakes of Leningrad Region	0.2–0.5	1997–1998
Halichondrida	*Halichondria panicea* (Pallas 1766)	White Sea	1–9	1993–2002, 2005
	H. panicea (Pallas 1766)	Barents Sea	1–3	1987–1989
	H. sitiens (Schmidt 1870)	White Sea	4–12	2002–2006
Halisarcida	*Halisarca dujardini* Johnston 1842	White Sea	3–8	1982–2004
	H. korotkovae Ereskovsky 2007	Okhotsk Sea	500–600	1984
Poecilosclerida	*Myxilla incrustans* (Johnston 1842)	White Sea	8–20	1985–2000
	Iophon piceum (Vosmaer 1881)	White Sea	8–20	1985–2000
	Crellomima imparidens Rezvoy 1925	White Sea	5–15	1995–2000, 2003
	Amphilectus lobatus (Montagu 1818)	White Sea	3–12	1989–1996, 2000
	Hymedesmia irregularis Lundbeck 1910	White Sea	9–14	2003
	Esperiopsis koltuni Ereskovsky and Willenz 2007	Okhotsk Sea	600–800	1984
	Phorbas topsenti Vacelet and Perez 2008	Mediterranean Sea	4–15	1999–2001
Dictyoceratida	*Ircinia oros* (Schmidt 1864)	Mediterranean Sea	14–17	2000, 2007
	Pleraplysilla spinifera (Schulze 1878b)	Mediterranean Sea	7–15	1999–2001, 2007
	Spongia officinalis (Linnaeus 1759)	Mediterranean Sea	9–20	2006–2008
	Hippospongia communis (Lamarck 1814)	Mediterranean Sea	5–15	2006–2008
Dendroceratida	*Aplysilla sulfurea* Schulze 1878b	Mediterranean Sea	14–20	1999–2001
Chondrosida	*Chondrilla australiensis* Carter 1873	Western Australia, reef at Fremantle	8–12	2002–2004

Homoscleromorpha				
Homosclerophorida	*Oscarella microlobata* Muricy et al. 1996	Mediterranean Sea	13–15	1999–2007
	O. imperialis Muricy et al. 1996	Mediterranean Sea	22–25	1999, 2007
	O. lobularis (Schmidt 1862)	Mediterranean Sea	15–27	1999–2008
	O. tuberculata (Schmidt 1868)	Mediterranean Sea	15–17	1999–2008
	O. viridis Muricy et al. 1996	Mediterranean Sea	13–15	1999–2008
	Oscarella sp.1	Mediterranean Sea	16–18	1999
	O. malakhovi Ereskovsky 2006	Sea of Japan	1–3	2001, 2006
	Corticium candelabrum Schmidt 1862	Mediterranean Sea	8–16	1999–2008
	Plakina trilopha Schulze 1880	Mediterranean Sea	13–15	1999–2008
	P. jani Muricy et al. 1999	Mediterranean Sea	13–15	1999–2008
	Pseudocorticum jarrei Boury-Esnault et al. 1995	Mediterranean Sea	13–17	1999–2008
Calcarea				
Clathrinida	*Guancha arnesenae* Rapp 2006	White Sea	9–12	2003
	Clathrina contorta Bowerbank 1866	Mediterranean Sea	12–17	2008
Leucosolenida	*Sycon raphanus* Schmidt 1862	Mediterranean Sea	1–3	2005–2008

for the construction of phylogenetic relationships within the Porifera. A developmental type is understood here as a sequence, established during the evolution, of invariant stages characteristic of the development of animals within high taxonomic groups (above the order). Taxa that have the same developmental type can be considered monophyletic.

5.1 Developmental Types of Modern Sponges

The original hypothesis was that certain reproductive features of sponges may turn out to be good synapomorphies at a high taxonomic level. The comparison was conducted along the following main characteristics:

1. Egg type
2. Cleavage pattern
3. Blastula type
4. Mode of movement of cells or cell layers in the developing larva
5. The time of anterior–posterior polarity formation
6. Larval type
7. Ultrastructural features of the larva
8. Mode of morphogenesis during metamorphosis
9. Type of asexual reproduction

Comparative analysis of the data on sexual and asexual morphogeneses revealed eight types of development of recent sponges (Fig. 5.1) (Ereskovsky 2004). They are named by the types of larva characteristic of each group.

5.1.1 Amphiblastula Type of Development (Calcarea, Calcaronea)

Viviparous sponges (Fig. 5.2; see Section 1.1). The egg is isolecithal or oligolecithal. Its short axis is regarded as an animal-vegetative axis. A complex of specialized nurse cells is present. Cleavage is total, asynchronous, unequal, and incurvational (table palyntomy). The embryo is organized as a hollow blastula throughout the development, but this is an inverted blastula (a stomoblastula). The formation of the amphiblastula larva is accompanied by inversion (turning inside out) of the stomoblastula. Anterior–posterior polarity is evident from the first cleavage cycles. The anterior larval pole, corresponding to the vegetative egg pole, is formed by ciliated cells; the posterior pole is composed of non-ciliated granular cells. Four translucent cells (known as “cellules en croix”) are characteristic of the larva. The ciliar rootlet is cross-striated. No specialized cell junctions in larva are seen. Morphogenesis accompanying metamorphosis is of a mixed character: exopinacoderm develops according to the epithelial type from the granular cells

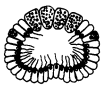
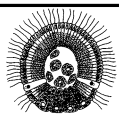
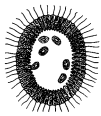
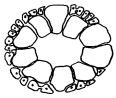
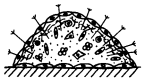
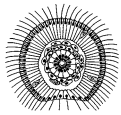
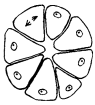
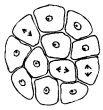
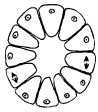
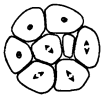
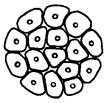
Type of development	Cleavage	Blastula	Morphogenesis	Larva	Taxon
1. Amphiblastula				 Amphiblastula	s/c Calcaronea
2. Calciblastula				 Calciblastula	s/c Calcinea
3. Trichimella				 Trichimella	Hexactinellida
4. Direct development				No larva	Demospongiae, o. Spirophorida o. Hadromerida
5. Disphaerula				 Disphaerula	Demospongiae, o. Halisarcida
6. Parenchymella				 Parenchymella	Demospongiae, o.Haplosclerida o. Halichondrida o. Hadromerida o. Dendroceratida o. Dictyoceratida
7. Coeloblastula				 Coeloblastula	Demospongiae, Chondrosida, Hadromerida
8. Cinctoblastula				 Cinctoblastula	Homosclero-morpha

Fig. 5.1 Diagram of the developmental types found in the Porifera and their resultant larvae (From Ereskovsky 2004, p. 305, Fig. 1 with modifications, reproduced by permission of Publisher House of University of Genova)

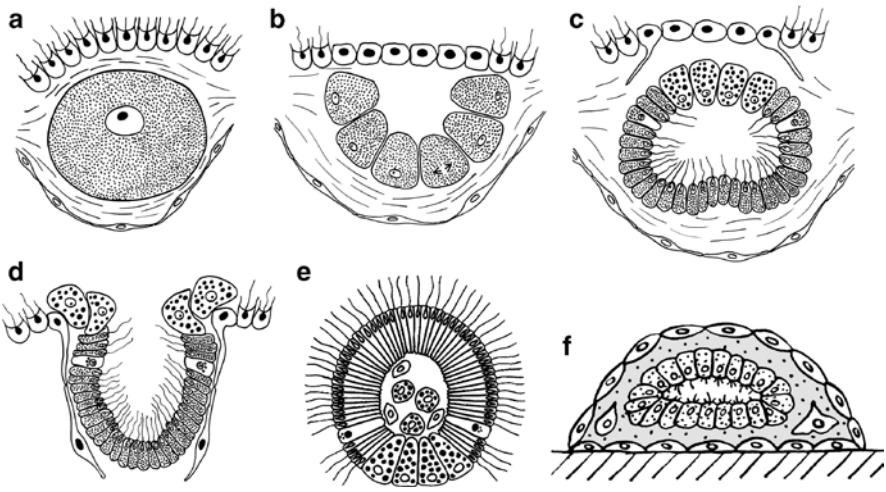


Fig. 5.2 Schematic drawing of Amphiblastula type of development (Calcarea: Calcaronea). (**a**) Egg. (**b**) Incurvational cleavage. (**c**) Stomoblastula. (**d**) Incurvation. (**e**) Amphiblastula larva. (**f**) Olynthus

of the posterior larval pole, whereas basopinacoderm, endopinacoderm, choanoderm, and mesohyl cells develop according to the epithelial–mesenchymal transition (EMT). Ciliated larval cells play the main role during metamorphosis. The anterior–posterior axis of the larva becomes the basal–apical axis of the olynthus. Facultative asexual reproduction is in the form of epimorphic budding.

5.1.2 Calciblastula Type of Development (Calcarea, Calcinea)

Ovoviviparous sponges (Fig. 5.3; see Section 1.2). The egg is isolecithal, oligolecithal, and nonpolarized. No special nurse cells are seen. Cleavage is total, equal, polyaxial; a cleavage cavity is present. The embryo is organized as a coeloblastula throughout the development. The larva, calciblastula, is formed by direct transformation of the hollow blastula. Anterior–posterior polarity becomes expressed toward the end of larva formation. Ciliated cells are the predominant larval cell type; bottle-shaped and vacuolar cells are also present. The double ciliar rootlet is cross-striated. There are specialized cell junctions. Metamorphosis is mesenchymal by EMT, accompanied by the migration of some of the ciliated cells into the blastocoel and their transdifferentiation into endopinacocytes, choanocytes, amoebocytes, and other internal cells; the cells remaining on the surface transform into exopinacocytes. Ciliated larval cells play the main role during metamorphosis. The anterior–posterior axis of the larva becomes the basal–apical axis of the olynthus. Facultative asexual reproduction is in the form of epimorphic budding.

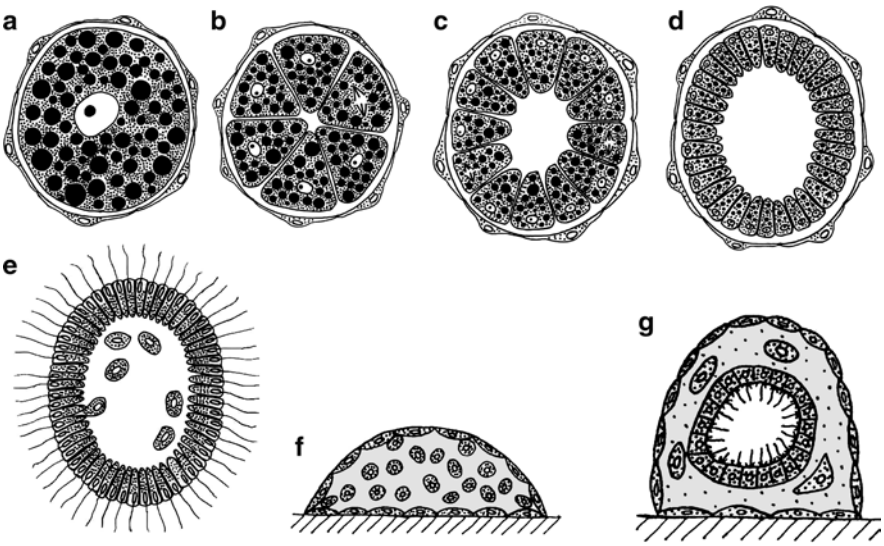


Fig. 5.3 Schematic drawing of Calciblastula type of development (Calcarea: Calcinea). (**a**) Egg. (**b**, **c**) Polyaxial cleavage. (**d**) Coeloblastula. (**e**) Calciblastula larva. (**f**) Beginning of metamorphosis. (**g**) Olynthus

5.1.3 Trichimella Type of Development (Hexactinellida)

Ovoviparous sponges (Fig. 5.4; see Chapter 2). The egg is isolecithal, oligolecithal, and nonpolarized. No specialized nurse cells. Cleavage is total, equal, and asynchronous, with elements of pseudospirality. The embryo is organized as a hollow blastula throughout the early development. Cleavage is followed by cellular delamination, and a two-layered morula is formed. Anterior–posterior polarity of the larva becomes expressed in the course of its cytodifferentiation. Development results in a trichimella larva, with a belt of multiciliary cells and syncytial structures. No ciliary rootlets. Perforate plugged junctions are present. Metamorphosis has not been described. Facultative asexual reproduction is in the form of epimorphic budding.

5.1.4 Direct Development (Demospongiae, Spirophorida, Tetilla)

Oviparous dioecious sponges (Fig. 5.5; see Section 3.3). Eggs are small, isolecithal, and nonpolarized. No special nurse cells. Collagen bundles are present on the egg surface. Sperm penetration into the egg is followed by fertilization coat formation. Cleavage is total, equal, asynchronous, and radial up to the fourth cycle. Cleavage results in a loose equal apolar morula. Morphogenesis proceeds by morula delamination.

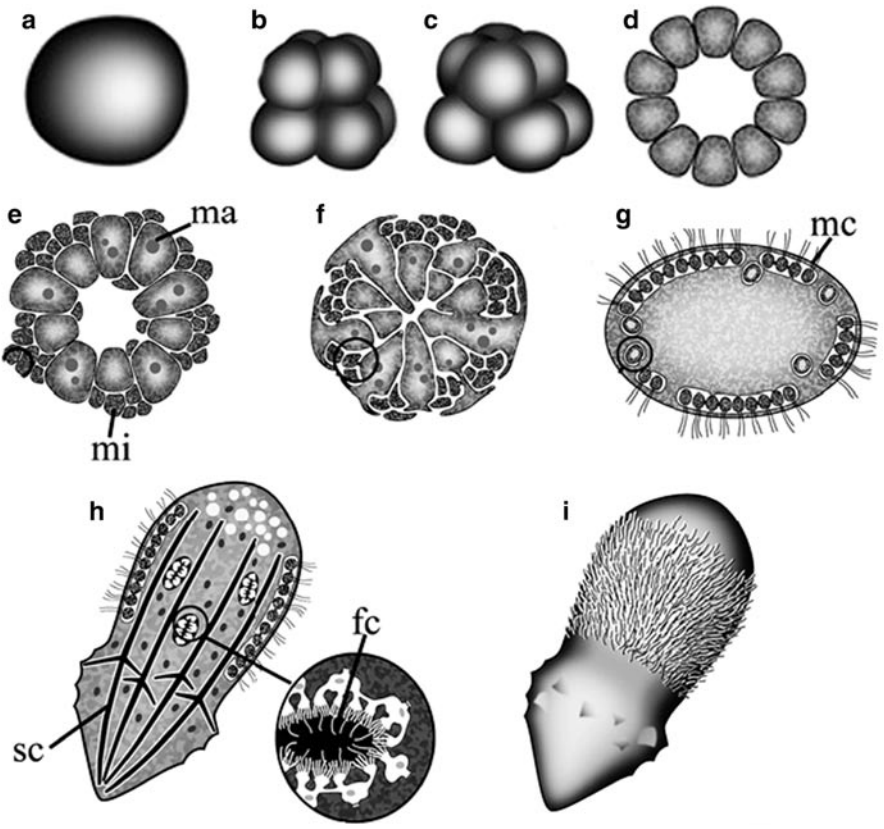


Fig. 5.4 Schematic drawing of Trichimella type of development (Hexactinellida). (**a**) Egg. (**b**, **c**) Radial cleavage – eight cells. (**d**) Hollow blastula. (**e**) Unequal cell division to form micromeres (*mi*) and macromeres (*ma*) – cell delamination. (**f**) Fusion of macromeres (*ma*) to form the trabecular syncytium, and envelopment of micromeres by this tissue to form the outer epithelium. (**g**) Cellular differentiation: formation of multiciliated cells (*mc*) and sclerocytes (*sc*). (**h**) Larva in longitudinal section with sclerocytes (*sc*) and flagellated chambers (*fc*, *inset*). (**i**) External view of trichimella (After Leys et al. 2007, p. 114, Fig. 54 with modifications, Reproduced by permission of Elsevier, Ltd.)

The embryo develops directly into a young sponge, without a larval stage. Asexual reproduction is in the form of epimorphic budding.

5.1.5 Disphaerula Type of Development (Demospongiae, Halisarcida)

Ovoviviparous sponges (Fig. 5.6; see Section 3.6). The egg is isolecithal, polylecithal, and nonpolarized. No specialized nurse cells. Cleavage is total, equal, asynchronous, polyaxial; a cleavage cavity is present. Cleavage results in the formation of

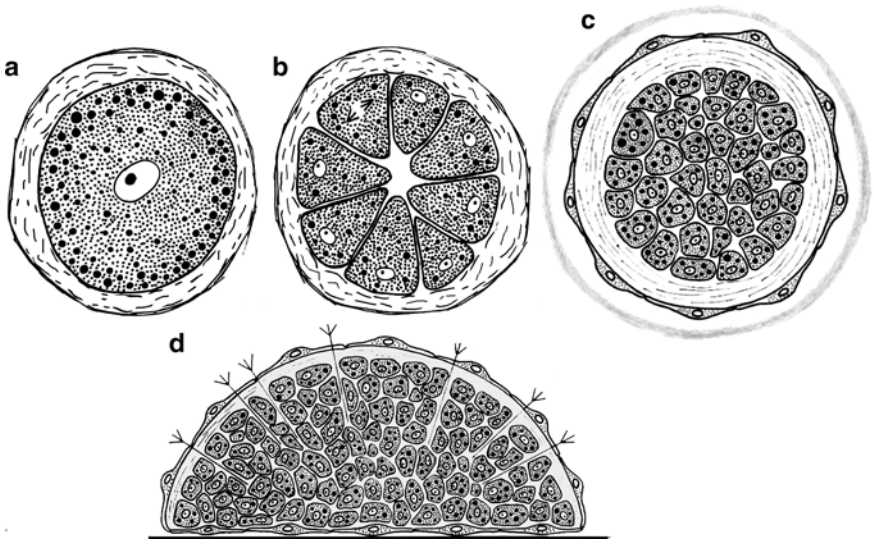


Fig. 5.5 Schematic drawing of direct development (Demospongiae: Spirophorida, *Tetilla*). (**a**) Zygote inside of the fertilized membrane. (**b**) Radial cleavage. (**c**) Exopinacoderm formation. (**d**) Young sponge

either a coeloblastula or a morula. The latter develops by means of multipolar ingression of single cells. Anterior–posterior polarity of the embryo is expressed late, during cytodifferentiation of the surface cell layer. Larval polymorphism is characteristic: disphaerula, coeloblastula, and parenchymella larvae are present. The internal cell layer of the disphaerulae is formed by an invagination of the lateral surface of the prelarva. The apical–junctional complexes similar to *zonula adhaerens* are present. Two non-striated ciliar rootlets are present. Morphogenesis accompanying metamorphosis is of a mixed character: exopinacoderm develops according to the epithelial type from the ciliated cells of the posterior larval pole, whereas basopinacoderm, endopinacoderm, choanoderm, and the cells of mesohyl develop according to the EMT. Ciliated larval cells play the main role during metamorphosis. The anterior–posterior larval axis becomes the basal–apical axis of the rhagon. Asexual reproduction is unknown.

5.1.6 Parenchymella Type of Development (Most of Demospongiae)

This type occurs both in viviparous, oviparous, and in ovoviviparous demosponges with total, chaotic, asynchronous cleavage, usually resulting in the formation of a morula. Further development results in the parenchymella larva, with diagnostic

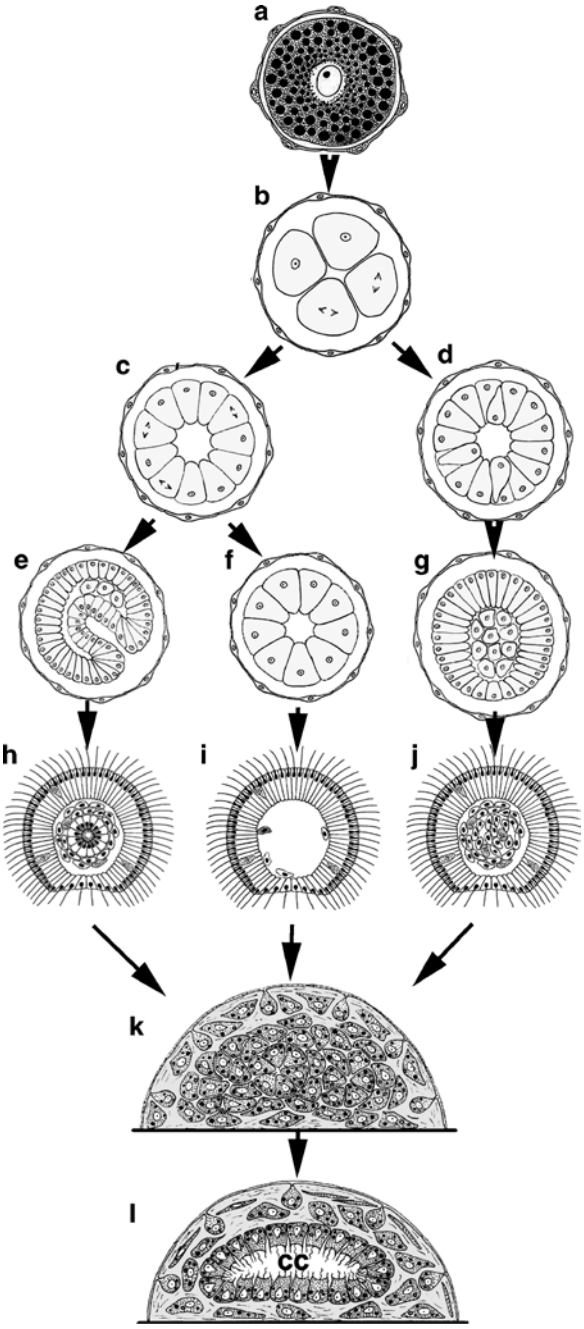


Fig. 5.6 Schematic drawing of Disphaerula type of development (Demospongiae: Halisarcida). (**a**) Egg. (**b**, **c**) Polyaxial cleavage. (**d**) Multipolar ingression. (**e**) Invagination. (**f**) Coeloblastula. (**g**) Bilayered embryo. (**h**) Disphaerula larva. (**i**) Coeloblastula larva. (**j**) Parenchymella larva. (**k**) Pupa. (**l**) Young rhagon

features at the order level. The ciliar rootlet is non-striated. Specialized cell junctions may be present. Metamorphosis may follow both the EMT and mesenchymal–epithelial transformation (MET), and is accompanied by exchange of external and internal larval cells. The larval ciliated cells sometimes are partially or completely phagocyted during the metamorphosis. Four subtypes may be outlined within this type of development.

The first subtype (Halichondrida, Dendroceratida, marine Haplosclerida, Dictyoceratida, and, partly, Hadromerida) is characterized by the following features (Fig. 5.7; see Sections 3.2, 3.5, 3.7, 3.8, 3.11): (1) the egg is isolecithal, oligolecithal (in oviparous species), or polylecithal (in ovoviviparous species), without any features of polarization; (2) an equal apolar morula is formed; (3) the morula segregates into the surface layer and the internal cell mass by means of morular or polarized delamination; (4) anterior–posterior polarity of the larva is expressed during its cytodifferentiation; and (5) asexual reproduction, if any, is by epimorphic budding or epimorphic gemmulogenesis.

The second subtype (Hadromerida: *Stylocordyla borealis*) (see Section 3.2) is associated with the direct development (without a larva). Other features of this subtype are as follows: (1) two types of nurse cells, (2) embryonic morphogenesis

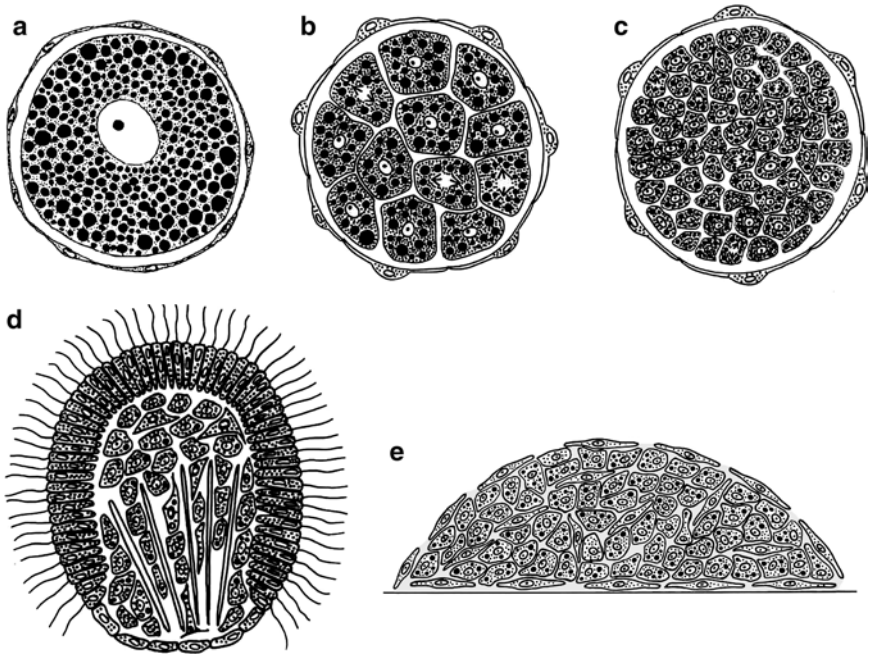


Fig. 5.7 Schematic drawing of the first subtype of Parenchymella type of development (Demospongiae: Halichondrida, Dendroceratida, Dictyoceratida, marine Haplosclerida, and, partly, Hadromerida). (**a**) Egg. (**b**) Chaotic cleavage. (**c**) Morula. (**d**) Parenchymella. (**e**) Metamorphosis

by morula delamination, (3) formation of the aquiferous system elements in an unequal morula during cell differentiation, and (4) true viviparity.

The third subtype (Poecilosclerida) (Fig. 5.8; see Section 3.10) is determined by the structure of the egg, which is telolecithal, polarized, and polylecithal. Other specific features are (1) total, unequal, asynchronous, chaotic cleavage; (2) dense, polarized, non-equal morula; (3) morula segregation into the surface layer and the internal cell mass by polarized delamination; and (4) animal–vegetative polarity of an egg morphologically corresponds to the larval anterior–posterior polarity.

The fourth subtype (freshwater Haplosclerida) is characterized by the following features (Fig. 5.9; see Section 3.11): (1) specialized trophocytes, (2) segregation of morula into the surface layer and the internal cell mass by morular or polarized delamination, (3) parenchymella in the freshwater species with highly differentiated cells and with specialized structures (a spacious cavity lined with pinacocytes, choanocyte chambers), and (4) regular alteration of sexual and asexual (gemmulogenesis) reproduction in the life cycle of many freshwater species.

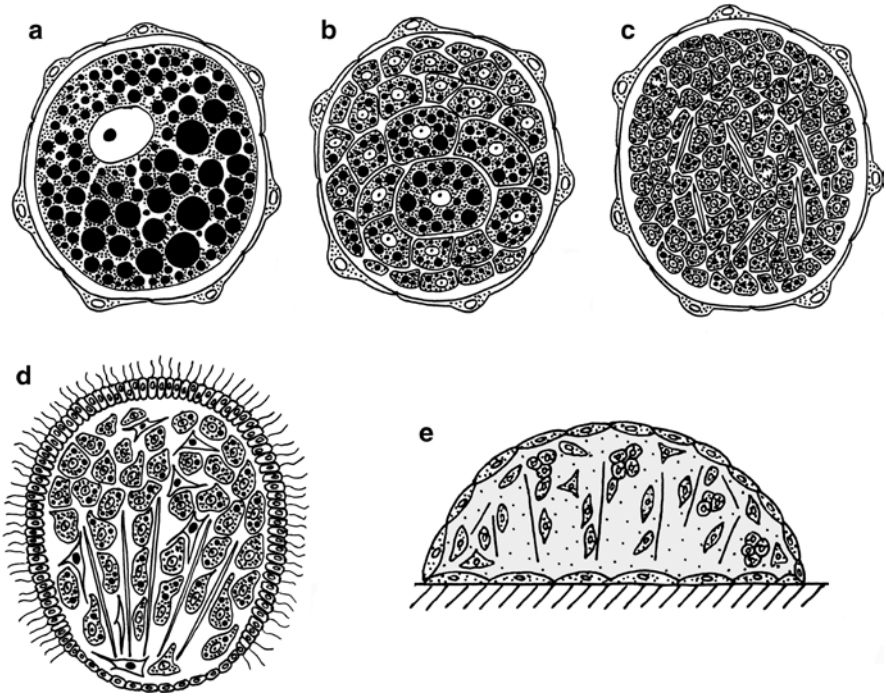


Fig. 5.8 Schematic drawing of the third subtype of Parenchymella type of development (Demospongiae: Poecilosclerida). (**a**) Egg. (**b**) Chaotic cleavage. (**c**) Morula. (**d**) Parenchymella. (**e**) Metamorphosis

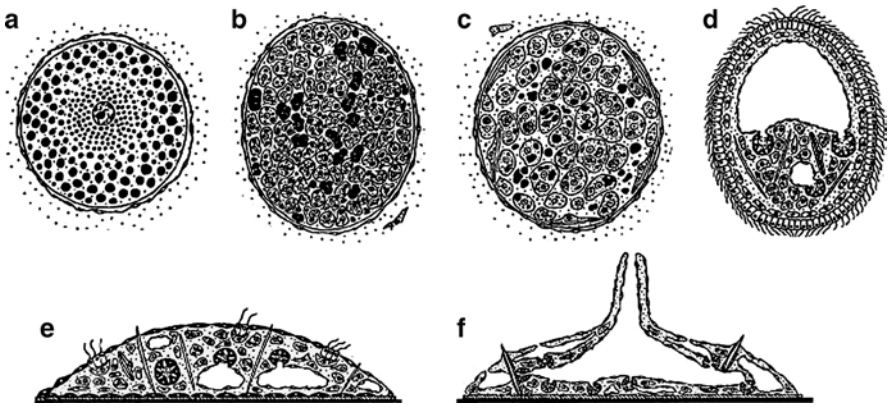


Fig. 5.9 Schematic drawing of the fourth subtype of Parenchymella type of development (Demospongiae: freshwater Haplosclerida). (**a**) Egg. (**b**) Morula. (**c**) Cell differentiation. (**d**) Parenchymella. (**e**) Metamorphosis. (**f**) Rhagon

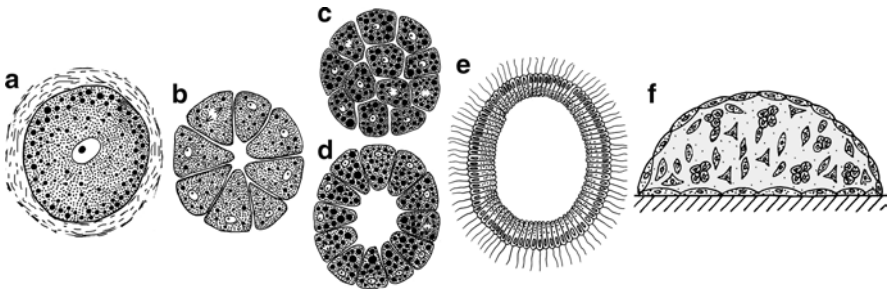


Fig. 5.10 Schematic drawing of Coeloblastula type of development (Demospongiae: Chondrosida; Hadromerida – Polymastiidae). (**a**) Egg. (**b**) Equal, radial cleavage. (**c**) Equal morula. (**d**) Equal coeloblastula. (**e**) Coeloblastula larva. (**f**) Metamorphosis

5.1.7 Coeloblastula Type of Development (Demospongiae: Chondrosida; Hadromerida – Polymastiidae)

Oviparous dioecious sponges (Fig. 5.10; see Sections 3.2 and 3.4). No follicular envelope around the mature oocyte; the egg is surrounded by collagen fibrils and mucus. The egg is isolecithal, oligolecithal, and nonpolarized. Cleavage is total and equal, may be radial at the early stages, resulting in an equal apolar morula. The morula develops into an equal blastula with a small blastocoel by passing the rearrangement of blastomeres to form a single-layer embryo. The anterior–posterior polarity of the larva becomes evident during cytodifferentiation. The coeloblastula larva is composed of one-layered ciliated epithelium. In *Chondrosia reniformis*, the dense internal cell mass of the larva consists of the parent bacteriocytes and granular

cells. The ciliar rootlet is non-striated. *Zonula adhaerens* may be present. Metamorphosis is according to EMT and is accompanied by the migration of some of the ciliated cells into the blastocoel and their transformation into choanocytes and amoebocytes; the cells remaining on the surface transform into pinacocytes. Ciliated larval cells play the main role during metamorphosis. The anterior–posterior larval axis becomes the basal–apical axis of the rhagon. Asexual reproduction is in the form of epimorphic budding.

5.1.8 Cinctoblastula Type of Development (Homoscleromorpha)

The egg is isolecithal, polylecithal, and nonpolarized. No specialized nurse cells (Fig. 5.11; see Chapter 4). Cleavage is total, equal, asynchronous, chaotic, resulting in the formation of an equal morula; no cleavage cavity. Coeloblastula develops by multipolar egression (centrifugal migration of the morula cells to the periphery). Anterior–posterior polarity becomes evident during cytodifferentiation. The cinctoblastula larva is composed of one-layered ciliated epithelium, with rare non-ciliated cells scattered in it. A belt of ciliated cells, each with an intranuclear crystalloid, is situated around the posterior pole. A basement membrane and belt desmosomes are present. The ciliar rootlet is cross-striated. Metamorphosis follows the epithelial type: the ciliated epithelium of the anterior hemisphere of the cinctoblastula

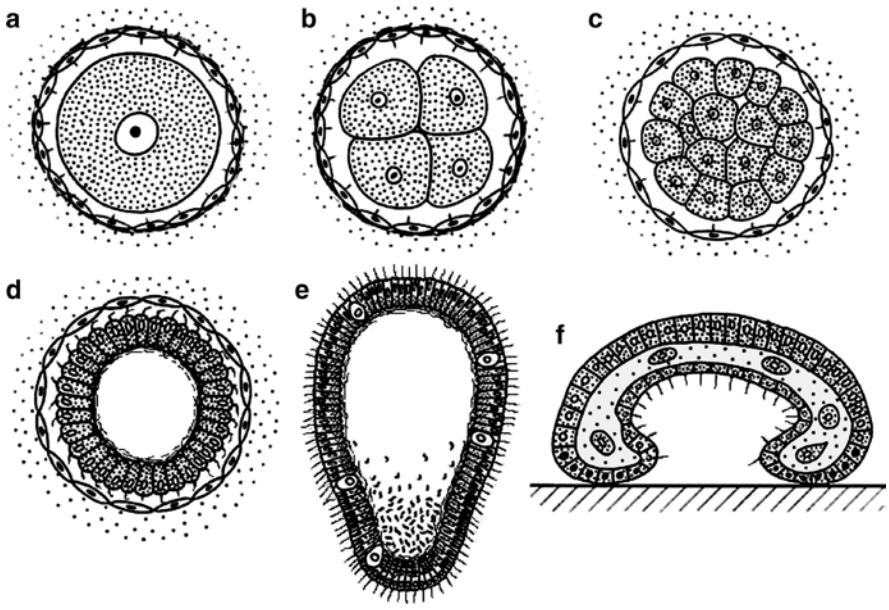


Fig. 5.11 Schematic drawing of Cinctoblastula type of development (Homoscleromorpha). (**a**) Egg. (**b**) Cleavage. (**c**) Morula. (**d**) Coeloblastula. (**e**) Cinctoblastula larva. (**f**) Metamorphosis

invaginates to form the choanoderm and the endopinacoderm. All sponge cells are derived from ciliated larval cells. The anterior–posterior larval axis becomes the basal–apical axis of the rhagon. Asexual reproduction is in the form of facultative budding, which follows the morphallactic type.

5.2 Phylogenetic Interpretation of Sponge Developmental Types

The above comparative embryological analysis shows that most clades of Porifera are characterized by distinct types of development. Some of the orders are not distinguished by a single type, which calls for a revision of phylogenetic relationships within them.

Information about the embryology of Hexactinellida is insufficient and the phylogenetical relationships inside of this group cannot be discussed from embryological point of view.

Calcareous sponges (Calcarea) demonstrate two distinct developmental types, which reveals profound differences in their development (Borojevic 1970; Ereskovsky 2004, 2005; Leys and Ereskovsky 2006; Ereskovsky and Willenz 2008). Unfortunately, such elements of sexual reproduction as spermatogenesis, fecundation, and ultrastructural features of oogenesis remain unknown in the Calcinea. We do not know whether fertilization of the Calcinea involves the unique cells participating in fertilization of the Calcaronea: the cells of the fertilization complex and the carrier cells. However, the differences between these developmental types do not call into question the monophyly of the Calcarea, which is strongly supported by molecular and biochemical data (Manuel et al. 2003, 2004b; Dohrmann et al. 2006; Schreiber et al. 2006), but indicate the early divergence of its two subclasses, the Calcinea and the Calcaronea.

The cinctoblastula type of development (Homoscleromorpha) has a number of apomorphic characters supporting the monophyly of this group. The characters unusual for demosponges are yet another reason for the exclusion of Homoscleromorpha from Demospongiae (Boury-Esnault et al. 2003; Borchiellini et al. 2004a). However, certain characters show the affinity of Homoscleromorpha with Eumetazoa: cross-striated ciliar rootlet in the larva, the presence of collagen IV, the basal membrane in the larva and the adult sponge, the presence of specialized cell junctions in the epithelia at all ontogenetic stages, expressed core components of the *Wnt* signaling pathways, transforming growth factor β, receptor tyrosine kinase, *Notch*, *Hedgehog*, and *Jak/Stat* signaling pathways (Boury-Esnault et al. 2003; Ereskovsky 2004; Nichols et al. 2006; Ereskovsky and Tokina 2007; Gazave et al. 2008; Lapébie et al. 2009).

Disphaerula development type (Halisarcida) also has some of apomorphies and is very different from the developmental types of the other Demospongiae (Borojevic 1970; Korotkova 1981b; Ereskovsky and Gonobobleva 2000; Ereskovsky 2004). These data, in combination with the organization features, testify to the monophyly of the order Halisarcida.

Phylogenetic relationships within the parenchymella type are unclear. Cleavage pattern, the mode of blastula formation, the type of embryonic morphogenesis, the larval morphology, the ultrastructure of the ciliar basal apparatus, and general mode of metamorphosis are very similar in all the species studied within Haplosclerida, Dendroceratida, Dictyoceratida, Poecilosclerida, Halichondrida, and partly Hadromerida. Characteristic features of the Poecilosclerida type of development are associated with the complication of the egg structure, which is certainly a later acquisition in the evolution of the demosponge ontogenesis. Three developmental variants within the Poecilosclerida correspond to the three suborders: Mycalina, Myxillina, and Microcionina. They are different as to the fate of the larval cells during metamorphosis (see Section 3.10), which is determined by the degree of their differentiation and by their significance for the development of the larva and the adult sponge. These differences, however, do not warrant our reconsidering the Poecilosclerida as a paraphyletic group. In other words, at present we do not have enough comparative embryological data to doubt the monophyly of this order.

The development of Haplosclerida, especially of the freshwater families Spongillidae, Potamolepidae, and Lubomirskiidae, was surely influenced by adaptation to life in brackish water and freshwater habitats. Differences in the development of marine and freshwater haplosclerids testify to an early divergence of these ecological groups. They may even indicate the polyphyly of the order, since, as shown by the molecular phylogeny data, the freshwater haplosclerids appear to be more closely related to other demosponges than to the marine haplosclerids (Itskovich et al. 2007; Raleigh et al. 2007; Redmond et al. 2007; Redmond and McCormack 2008). On the other hand, structural similarities in spermatozoa, eggs, development, and larvae support the monophyly of the freshwater suborder Spongillina (Ereskovsky 1999).

Within the vast order Hadromerida, the development has been studied only in representatives of the four genera: *Tethya*, *Polymastia, Cliona,* and *Stylocordyla* (Lévi 1956; Borojevic 1967; Mariani et al. 2000; Sarà et al. 2002). However, even this scarce material shows that within this order one comes across both direct development (without a larva) and indirect development (with a larva). The development of *S. borealis* is identical with the first subtype of the parenchymella development until the beginning of embryonic morphogenesis. Then, the stage of larval morphogenesis falls out, and embryonic cytodifferentiation results in the formation of a young sponge, the homologue of the rhagon formed during parenchymella development. Moreover, direct development (*S. borealis*) is accompanied by true viviparity (Sarà et al. 2002). Indirect development of Hadromerida may proceed by one of the two morphogenesis of an equal morula, resulting into two types of larva. In the first case (*Polymastia robusta* – Polymastiidae), the morula is transformed into the coeloblastula by simple flattening and regrouping of cells (Borojevic 1967). In the second case (*Tethya aurantium* – Tethyidae and *Cliona viridis* – Clionaidae), the morula is differentiated into the surface and the internal layers as a result of morula delamination, and a parenchymella larva is formed (Lévi 1956; Mariani et al. 2000). These different developmental patterns may reveal the paraphyly of the order or its early divergence (Chombard 1997; Kober and Nichols 2007).

The order Halichondrida is one of the pivotal demosponge taxa (Erpenbeck and van Soest 2002). Larval structure in this order is very diverse both between different species and within the same species. This diversity is expressed, first of all, in the larval skeleton and the ciliation (Topsent 1911; Lévi 1956; Wapstra and van Soest 1987; Woollacott 2003; Hoshino et al. 2004), as well as in the fate of larval cells during metamorphosis. Developmental heterogeneity within the Halichondrida may be due to a high polymorphism of their development, the paraphyly of the group, or the taxonomic confusion. A recent study, showing that the order is non-monophyletic and that the most halichondrid families cluster are polyphyletic (Erpenbeck et al. 2005; Erpenbeck and Worheide 2007), appears to support the second option.

It is difficult to say whether the absence of the larva in the case of direct development of Spirophorida is primary or secondary. Whatever the case, this type of development is closely related to the parenchymella type.

Difficulties in phylogenetic interpretation of the comparative-embryological data on Porifera are associated with a high polymorphism and the phenomenon of equifinality of their development, for example, development of Halisarcida (see Section 3.6). The same cleavage pattern and the same blastula type may result in the development of different types of larvae. For instance, radial cleavage and an equal morula are characteristic both of *Polymastia* (Hadromerida), with its coeloblastula larva, and of *Tetilla* (Spirophorida), which has no larva (Borojevic 1967; Watanabe 1978b). On the other hand, larvae of the same type may result from different cleavage patterns and larval morphogeneses. For instance, the parenchymella of *Amphimedon* (Haplosclerida) develops by chaotic cleavage and morula delamination (Leys and Degnan 2002), whereas the parenchymella of *Halisarca* (Halisarcida) develops by polyaxial cleavage and multipolar ingression (Gonobobleva and Ereskovsky 2004a).

5.3 Embryonic Development and Early Radiation of Sponges

Comparative analysis of embryonic development reveals four groups of poriferan taxa of orders and subclasses levels, corresponding to four cleavage patterns: the incurvational cleavage (table palyntomy), polyaxial cleavage, radial cleavage, and chaotic cleavage (Fig. 5.12, *1–4*).

5.3.1 Development of Sponges with Incurvational Cleavage (the Subclass Calcaronea, Calcarea)

Cleavage is total and unequal (Fig. 5.12, *1*). The embryo retains hollow blastula organization throughout the development, but the blastula is inverted (stomoblastula) (Fig. 5.12, *5*). Formation of the amphiblastula larva (Fig. 5.12, *32*) is accompanied by its inversion (Fig. 5.12, *10*).

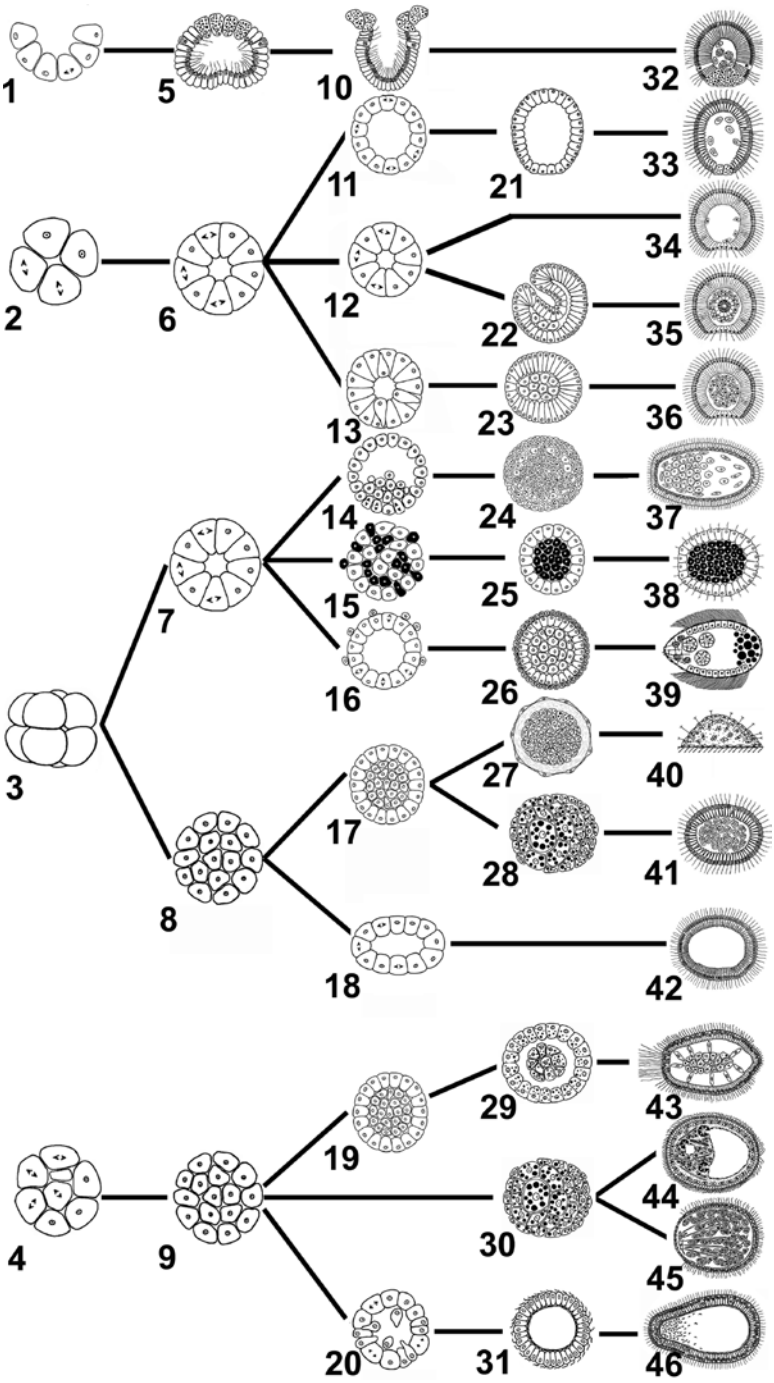


Fig. 5.12 Diagram of sponge's cleavage and morphogenesis, leading to the larvae. 1–4 – Cleavage patterns in sponges: incurvational (1), polyaxial (2), radial (3), and chaotic (4). Three main form of sponges blastula: stomoblastula (5), coeloblastula (6, 7), and stereoblastula (morula) (8, 9). 10–31 – morphogenesis and prelarva. 32–46 – larvae. (10) incurvation; (11, 12) the coeloblastula

5.3.2 Development of Sponges with Polyaxial Cleavage

Cleavage planes are perpendicular to the embryo's surface until the end of cytodifferentiation; throughout cleavage, the embryo has a certain number of symmetry axes radiating at certain angles from the geometric center of the embryo (Fig. 5.12, *2*). Starting with the stage of 64 cells, the fate of the embryo and the mechanisms of its transformation become different in different sponges.

2.1. The coeloblastula organization is retained until the larval stage only in the subclass Calcinea (Calcarea) (Fig. 5.12, *6–11–21–33*) (Borojevic 1969; Ereskovsky and Willenz 2008) and, as one of the variants of larval morphogenesis, in *Halisarca dujardini* (Halisarcida, Demospongiae) (Fig. 5.12, *6–12–34*) (Gonobobleva and Ereskovsky 2004a).

2.2. In *H. dujardini* (Halisarcida, Demospongiae), two other larval morphotypes may develop from the coeloblastula:

Disphaerula, formed by invagination (Fig. 5.12, *6–12–22*) with subsequent separation of the invaginated layer (Fig. 5.12, *35*) (Ereskovsky and Gonobobleva 2000; Gonobobleva and Ereskovsky 2004a)

Parenchymella (Fig. 5.12, *23–36*), formed by multipolar ingression (Fig. 5.12, *13–23*) (Gonobobleva and Ereskovsky 2004a) or by unipolar ingression from the future posterior larval pole (Lévi 1956)

5.3.3 Development of Sponges with Radial Cleavage

Cleavage planes are perpendicular to the embryo's surface; there is one symmetry axis (Fig. 5.12, *3*). Approximately until the stage of 16 cells, the embryo has a coeloblastula organization; further development may proceed in two directions.

3.1. Cleavage planes remain radial until the formation of the coeloblastula, consisting approximately of 64 cells (Fig. 5.12, *7*).

Fig. 5.12 (continued) organization is retained until the larval stage; (13) multipolar ingression; (14) unipolar proliferation; (15, 25) ingression maternal cells (*black*) inside of morula; (16) cell delamination; (17) morula delamination; (18) flatten of morula; (19) morula delamination; (20) multipolar emigration; (21) coeloblastula of Calcinea without basement membrane; (22) invagination; (23) preparenchymella; (24, 27, 28, 29, 30) morulae; (25) prepseudoblastula; (26) bilayered stereoblastula; (31) coeloblastula of Homoscleromorpha with basement membrane; (32) amphiblastula of Calcaronea; (33) calciblastula of Calcinea; (34) coeloblastula of Halisarcida; (35) disphaerula of Halisarcida; (36) parenchymella of Halisarcida; (37) parenchymella of Verticillitida; (38) pseudoblastula of Chondrosida; (39) trichimella of Hexactinellida; (40) young sponge of *Tetilla*; (41) parenchymella of Hadromerida; (42) coeloblastula of *Polymastia* and *Chondrilla*; 43–45 – parenchymellae of Demospongiae: Dendroceratida (43), Haplosclerida (44), Poecilosclerida (45); 46 – cinctoblastula of Homoscleromorpha (From Ereskovsky 2007b, p. 49, Fig. 11 with modifications, reproduced by permission of the Museu Nacional of Rio de Janeiro)

3.1.1. In *Vaceletia* (Verticillitidae, Demospongiae), unipolar proliferation (Fig. 5.12, *14*) results in a stereoblastula, which develops into a parenchymella larva (Fig. 5.12, *24–37*) (Vacelet 1979b).

3.1.2. In *Chondrosia reniformis* (Chondrosida, Demospondiae), a single-layered blastula (pseudoblastula) (Fig. 5.12, *38*) is formed by a unique mechanism of ingression of the parent cells of the brood chamber into embryos' blastocoel (Fig. 5.12, *15–25*) (Lévi and Lévi 1976).

3.1.3. In *Oopsacas minuta* (Hexactinellida), cell delamination (Fig. 5.12, *7–16*) results in a two-layered stereoblastula (Fig. 5.12, *26*), which develops into a trichimella larva (Fig. 5.12, *39*) (Boury-Esnault et al. 1999; Leys et al. 2006).

3.2. After the fourth cycle (16 blastomeres), cleavage is no longer radial but chaotic, and an equal morula is formed (Fig. 5.12, *8*). This type of early development is characteristic exclusively of the oviparous demosponges, which have small oligolecithal eggs (see Section 3.1). Further transformations of the morula may involve two morphogeneses.

3.2.1. Segregation of the surface cell layer of the morula occurs owing to their increased proliferation. This morphogenesis resembles morula delamination in Eumetazoa. In *Tetilla* (Spirophorida, Demospongiae) (Fig. 5.12, *17–27*), further development is direct and results in a young sponge (Fig. 5.12, *40*) (Watanabe 1978b); in *Tethya aurantium* and *Cliona viridis* (Hadromerida, Demospongiae) (Fig. 5.12, *17, 28*), further development results in a parenchymella (Fig. 5.12, *41*) (Lévi 1956; Mariani et al. 2000).

3.2.2. The morula develops into an equal blastula with a small blastocoel by passing the rearrangement of blastomeres to form a single-layered ciliated coeloblastula larva (Fig. 5.12, *8–18–42*), as in *Polymastia robusta* (Hadromerida, Demospongiae) or *Chondrilla australiensis* (Borojevic 1967; Usher and Ereskovsky 2005).

5.3.4 Development of Sponges with Chaotic Cleavage

This results in the formation of an equal apolar (except in Poecilosclerida) morula (Fig. 5.12, 4, *9*). After the stage of 64 cells, the following variants of larval development are possible:

4.1. In all ovoviviparous demosponges except Halisarcida, Verticillitida, and *S. borealis* (Hadromerida), the morula is transformed into the parenchymella larva (Fig. 6.12, *43–45*) mainly by morula delamination (Fig. 5.12, *19*) or polarized delamination (Fig. 5.12, *30*).

4.2. In Homoscleromorpha, coeloblastula (Fig. 5.12, *31*) is formed by multipolar egression of morula cells (Fig. 5.12, *20*); further development results in a cinctoblastula larva (Fig. 5.12, *46*) (Ereskovsky and Boury-Esnault 2002; Boury-Esnault et al. 2003).

The comparative analysis of cleavage and larval morphogenesis in sponges shows that these characters, taken separately, cannot be used for establishing

phylogenetic relations within Porifera. For instance, the embryogenesis of the Calcinea (Calcarea) (Fig. 5.12, *2–6–11–21–33*) is much closer to the development of Halisarcida with coeloblastula larva (Demospongiae) (Fig. 5.12, *2–6–12–34*) than to that of the related Calcaronea (Fig. 5.12, *1–5–10–32*). Attempts at reconstructing the evolution of sponge ontogenesis based only on cleavage patterns or larval morphogeneses are unlikely to be productive.

The question about the evolutionarily primary cleavage type is often discussed in the literature. Unfortunately, the analysis usually starts with Cnidaria, whereas Porifera, the most ancient Metazoa, are ignored. Most authors consider radial cleavage as the initial one (Siewing 1979; Valentine 1997). Ivanova-Kazas (1995) considers the incurvational cleavage (table palyntomy) of the Calcaronea (Calcarea) as primary, but in the author's opinion, this unique and, in many aspects, aberrant cleavage type (as well as the Calcaronea development in general) cannot be considered as ancestral. A more likely scenario of the evolution of early metazoan embryogenesis involves chaotic or polyaxial cleavage as the most primitive type. Radial cleavage may have emerged on the basis of both these cleavage types.

The four main cleavage patterns of sponges – table palyntomy, polyaxial, radial, and chaotic – result in three main blastula types: stomoblastula, coeloblastula, and morula (stereoblastula). On the other hand, the latter two blastula types emerge as a result of different cleavage patterns (Fig. 5.12).

Embryonic morphogeneses involved in larva formation in sponges are very diverse. They are based on morphogenetic movements of cells and their layers. Different forms of embryonic morphogenesis evolved many times in sponges that raise the question: what form of morphogenesis was ancestral in Porifera? Let us have a look at the phylogenetic distribution of different forms of embryonic morphogenesis and recent molecular phylogeny of Porifera published by Borchiellini et al. (2001, 2004a). To determine the ancestral kind of morphogenesis, we mapped on the tree its different modes.

More forms of morphogenesis occur in demosponges than in other Porifera. The morula delamination is characteristic for most demosponge groups (Fig. 5.13). This form of morphogenesis has been acquired once in all branches. Nevertheless, morula delamination disappears in the branch leading to group "Myxospongiae" (Verongida, Chondrosida and Halisarcida) by Borchiellini et al. (2004a). In the branch leading to orders Haplosclerida, Hadromerida, Halichondrida, Poecilosclerida, Agelasida, and Spirophorida, morula delamination occurs together with polar delamination, and in some Hadromerida, with direct hollow blastula formation. These results allow proposing a hypothesis that the ancestral form of embryonic morphogenesis was morula delamination in Demospongiae.

The investigations of ancestral mode of embryonic morphogenesis in Homoscleromorpha and Hexactinellida are impossible because only one mode – multipolar egression – was shown in the former, and the developments have been described in detail in only one species – *O. minuta* with cell delamination in the latter.

For two subclasses of Calcarea, Calcinea and Calcaronea, only two modes of embryonic morphogenesis have been described – direct hollow blastula formation

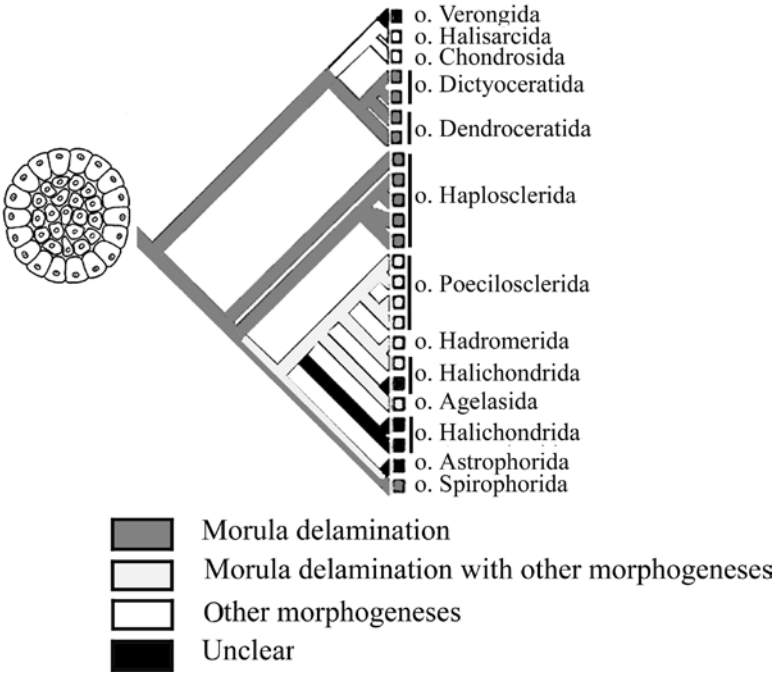


Fig. 5.13 Evolution of embryonic morphogeneses in Demospongiae

and inversion, respectively. We cannot reveal the ancestral mode of morphogenesis in Calcarea. Probably, these two modes evolved independently during the divergence of Calcinea and Calcaronea from their common ancestor. Unfortunately, from our results we cannot display ancestral mode of embryonic morphogenesis for all sponges.

To sum up, the diversity of cleavage patterns, blastula types, and morphogenesis types involved in the formation of sponge larvae does not allow one to come to a conclusion about certain linear ways of poriferan evolution. This diversity rather testifies to an early divergence of sponge clades and their long parallel evolution.

Chapter 6
Comparative Analysis of Individual Development in Sponges

The preceding chapters demonstrated the variability of stages and processes within the sponge taxa. At the same time, they revealed certain developmental features characteristic of sponges only and not found among other metazoans. In this chapter, these features are treated in ontogenetic rather than taxonomic perspective.

6.1 Gametogenesis

Sponges lack gonads; the gametogenesis is usually diffuse. A characteristic feature of this process in sponges is the origin of gametes by direct transformation from the somatic cells – archaeocytes or choanocytes. Otherwise, the stages and cytological features of gamete development in sponges are similar to those in other animals (Fell 1983; Reiswig 1983; Simpson 1984; Boury-Esnault and Jamieson 1999).

In Hexactinellida, Demospongiae and Homoscleromorpha spermatogenesis proceed in spermatocytes – temporary spherical structures bounded by flattened somatic cells (for review, see Reiswig 1983; Boury-Esnault and Jamieson 1999). Calcaronea have no spermatocystes (Reiswig 1983; Simpson 1984; Anakina and Korotkova 1989; Anakina and Drozdov 2001). Development of male gametes within a cyst is usually synchronous. In Homoscleromorpha, there is a gradient of male gamete maturation within a cyst, a feature they have in common with the Eumetazoa (Gaino et al. 1986c; Ereskovsky 2005; Efremova et al. 2006; Riesgo et al. 2007a; Chapter 4).

Calcaronea have aberrant spermatozoa: spherical and non-flagellated (Fig. 6.1a). (To note, the term 'aberrant' is applied to spermatozoa that are very diverse as concerns their external morphology (Reunov 2005). Spermatozoa of most other sponges belong to the primitive type (ancestral-primitive type according to Reunov 2005): they have a large cytoplasmic volume and several mitochondria and usually lack the acrosome (Fig. 6.1b). Electron microscopic studies of the last 20 years revealed that sponge species whose spermatozoa have an acrosome – a feature earlier thought to be associated with more advanced animals. In particular, acrosomes were revealed in the spermatozoa of all the homoscleromorph species

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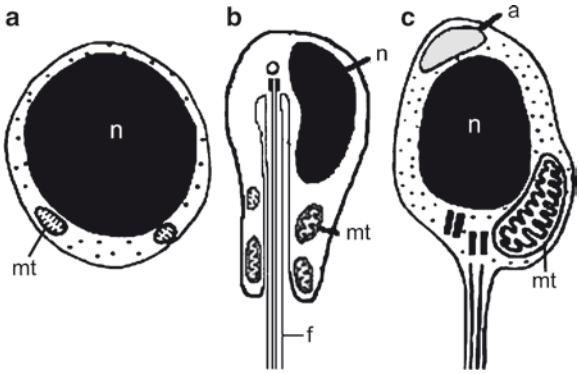

Fig. 6.1 Schematic drawings of sponge's spermatozoa. (**a**) 'Aberrant' sperm type of Calcaronea. (**b**) 'Primitive' sperm type of Demospongiae. (**c**) Sperm with acrosome of Homoscleromorpha and some Poecilosclerida (Demospongiae). *a* acrosome, *f* flagellum, *mt* mitochondria, *n* nucleus

studied (Fig. 6.1c; see Chapter 4) and in one species of the Calcarea, *Sycon calcaravis* (Nakamura et al. 1998). However, among the demosponges, the most species-rich sponge taxon, acrosomal spermatozoa, were found only in poecilosclerid species *Crambe crambe*, *Crellomima imparidens*, *Asbestopluma hypogea* and *Hymedesmia irregularis* (Fig. 3.67) (Tripepi et al. 1984; Ereskovsky 2005; Riesgo and Maldonado 2009; Vacelet et al. unpublished).

Although organization of spermatozoa is considered to be a sound taxonomic and phylogenetic character (see Afzelius 1979; Franzén 1970), the presence of acrosome in spermatozoa of Poecilosclerida and Calcaronea is at variance with this idea. For instance, *C. crambe, C. imparidens* and *Hymedesmia irregularis* (suborder Myxillina; Poecilosclerida) have an acrosome (Tripepi et al. 1984; Ereskovsky 2005; Riesgo and Maldonado 2009; see Section 3.10), but another sponge from the same suborder, *Myxilla incrustans*, does not (Efremova et al. 1987b; see Section 3.10). The acrosome is likely to have originated several times in the course of evolution. Its emergence is associated with the insemination and fertilization character, with the structure of the vitelline envelope and its accessibility for sperm penetration rather than with the taxonomic affiliation or phylogenetic position of the species.

Female gametes develop in the choanosome diffusely or, sometimes, in small clusters. This is true for both oviparous and ovoviviparous sponges (Korotkova 1981b; Fell 1983; Simpson 1984; Ereskovsky and Korotkova 1999). In the oogenesis of most ovoviviparous sponges, yolk does not form in the typical way but rather by various kinds of nutrients transfer from the nurse cells to the oocytes. These nutrients are the source of secondary yolk. Whole cells or their fragments are often phagocyted to become phagolysosomes (Fig. 6.2).

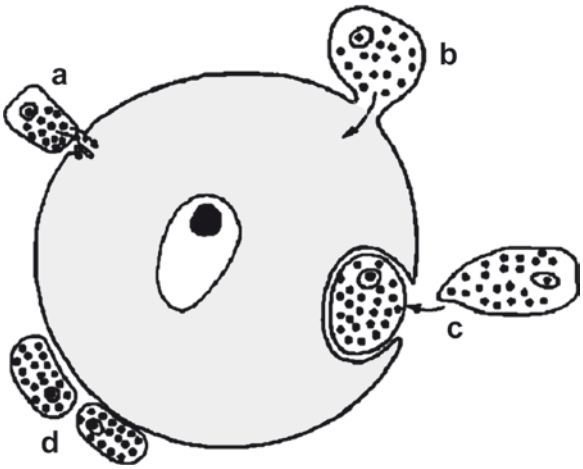

Fig. 6.2 Schematic drawings of interrelations of sponges' oocytes with auxiliary cells during vitellogenesis. (**a**) Exocytose of nutritive materials by auxiliary cells in the perioocyte space and its subsequent absorption by the oocyte. (**b**) Transmission of nutritive materials from auxiliary cells to oocyte by cytoplasmic bridges. (**c**) Phagocytosis of auxiliary cells by oocyte. (**d**) Auxiliary cells remain to be tight associated with oocyte; transmission of nutritive materials will be realized during embryogenesis (After Fell 1983, p. 5 Fig. 2 with modifications, reproduced by permission of Wiley)

The somatic cells ingested (choanocytes, mesohyl cells) are often referred to as trophocytes or nurse cells. However, strictly speaking, sponges cannot be said to possess true trophocytes. First, trophocytes are cells developing from oogonia in the course of oogenesis. Second, oocytes and trophocytes are connected by cytoplasmic bridges and surrounded by follicular epithelium (Aizenstadt 1984; de Cuevas et al. 1997), which is not the characteristic of sponges. In haplosclerids, however, vitellogenesis is accompanied by the emergence of a special population of phagosome-rich nucleolated amoebocytes that migrate towards the oocyte and are phagocyted by it. This is an indication of a higher specialization of haplosclerids oogenesis as compared to other demosponges orders (see Section 3.11). In oviparous demosponges and homoslceromorphs, vitellogenesis is predominantly by endogenous synthesis (see Section 3.1, Chapter 4).

A primitive feature of oogenesis and oocyte structure in sponges is the lack of ooplasm segregation and, apparently, of cytoplasmic determinants. Consequently, also lacking are mechanisms of determination of the axes of the future larva or, in the case of direct development, of the young sponge. In ovoviviparous sponges, another primitive feature of oogenesis is the lack of specialized vitelline envelope (Brien 1973a; Bergquist 1978; Korotkova 1981b; Fell 1983; Ereskovsky and Korotkova 1997). Oviparous demosponges do have a primitive vitelline envelope (see Sections 3.1 and 3.3).

6.2 Embryogenesis

Embryonic development of ovoviviparous and viviparous sponges proceeds in temporary brood chambers, often referred to as follicles. They are formed at the end of vitellogenesis from flattened cells originating from choanocytes (e.g. in *Halisarca dujardini* (Demospongiae) and calcareous sponges), amoebocytes (e.g. in Haplosclerida (Fell 1983; Simpson 1984), or endopinacocytes (Homoscleromorpha) (Ereskovsky and Boury-Esnault 2002). The follicular epithelium later participates in the formation of aquiferous system canals.

6.2.1 Cleavage

Four main cleavage patterns in sponges are the following: chaotic cleavage – Homoscleromorpha, most ovoviviparous Demospongiae; radial-like cleavage – oviparous Demospongiae, Hexactinellida; polyaxial cleavage – Halisarcida (Demospongiae), Calcinea (Calcarea); incurvational cleavage (table palintomy type) – Calcaronea (Calcarea) (Fig. 6.3). Primitive features of cleavage in sponges are

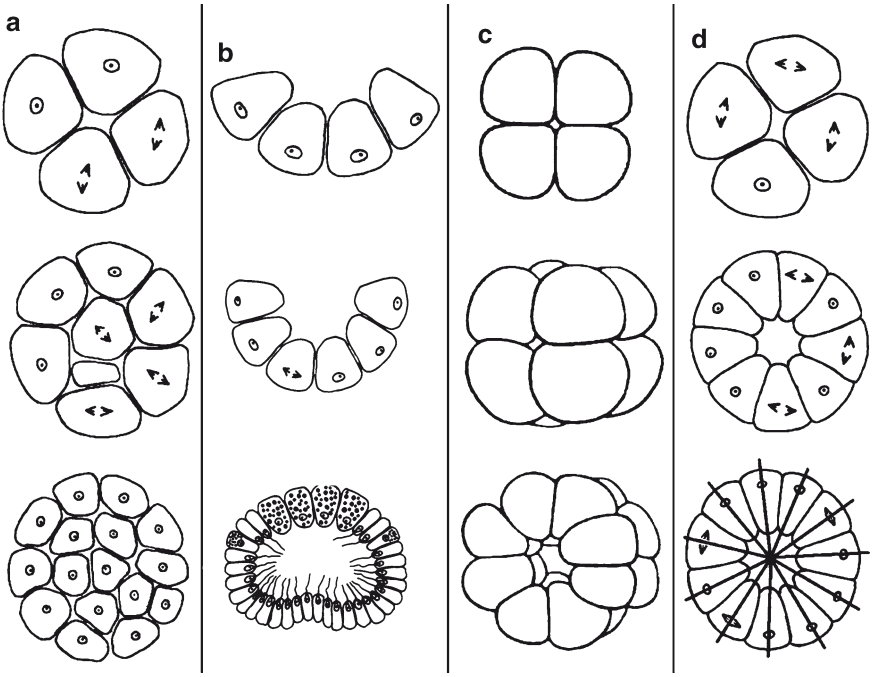

Fig. 6.3 Schematic drawings of main types of the cleavage in sponges with resulting blastula. (**a**) Chaotic cleavage. (**b**) Incurvational cleavage. (**c**) Radial cleavage. (**d**) Polyaxial cleavage

associated, first of all, with the low specialization of the oocyte. The spatial position of the blastomeres is usually unstable; divisions either become asynchronous very early or are not synchronous at all from the very beginning (see Chapter 3). Exceptions to this rule are calcareous sponges with the amphiblastula larva, the Calcaronea. Their cleavage is less chaotic and more synchronous, due to the early specialization of the macromeres, which acquire the nurturing function, and to the peculiar incurvational cleavage pattern (see Section 1.1).

A distinctive feature of early embryogenesis in many poriferan species is penetration of endosymbiotic bacteria from mesohyl into the embryo (for review see Ereskovsky et al. 2005). Another noteworthy feature is the integration into the embryo of somatic parent cells. In Homoscleromorpha, parent cells penetrate into the space between the egg and the follicle during the closing of the latter; later they are positioned in the embryo's cavity (Ereskovsky and Boury-Esnault 2002). In some ovoviviparous demosponges (*Microciona prolifera*, *Halisarca nahantensis*, *H. dujardini*, and *Iophon piceum*), parent granular cells with eosinophilic inclusions get into the forming larva via the follicle and the larval epithelium (Simpson 1968; Chen 1976; Korotkova and Ermolina 1982; Ereskovsky 1986; Ereskovsky and Gonobobleva 2000). In oviparous demosponges, e.g. *Cliona celata* (Warburton 1961), *Hemectyon ferox* (Reiswig 1976), *Chondrosia reniformis* (Lévi and Lévi 1976), there are the parent cells from the surface of the released oocytes that penetrate into the embryo. Finally, in Calcaronea, various somatic cells get into the amphiblastula cavity during inversion: nurse cells in *Leucosolenia botrioides* and *L. complicata* (Tuzet 1948; Anakina 1981), amoebocytes with heterogeneous inclusions and spherulous cells in *Grantia compressa* (Gallissian 1983), amoeboid microgranular cells in *Scypha ciliata* (Franzen 1988). The function of parent cells in the larva is still to be investigated, and so is their role in the course of metamorphosis.

In oviparous demosponges from the genera *Tetilla* and *Stylocordyla*, which have direct development, cleavage followed by cell differentiation and the emergence of the young sponge (Watanabe 1978b; Watanabe and Masuda 1990; Sarà et al. 2002).

6.2.2 *Embryonic Morphogenesis and the Question of Whether Early Morphogenetic Movements in Sponges are Gastrulation*

Applicability of the terms 'gastrulation' and 'germ layers' to sponges is the vexed question of their developmental biology. This problem is aggravated by the extreme variability of analogous developmental processes in sponges from various taxonomic groups and by the so-called 'inversion of germ layers' – transformation of larval ciliated cells into choanocytes in the course of metamorphosis. The notion of 'inversion of germ layers' in sponges, voiced by Schulze (1878a), was in such a stark contrast with the classical theory of germ layers that it has been attracting heated discussions for over a century (see below). Many authors tried to reconcile

the peculiarities of sponge development with the theory of germ layers by proving that the layers are formed in sponges only during metamorphosis (Korschelt and Heider 1936; Duboscq and Tuzet 1937; Lévi 1963; Brien 1967c, 1973a; Fioroni 1979, and others). Other investigators, while accepting the fact of inversion, supposed that cleavage in sponges is immediately followed by gastrulation (Ivanov 1968, 1971; Ivanova-Kazas 1975, 1995, 1997; Efremova 1997; Boury-Esnault et al. 1999; Leys and Degnan 2002; Maldonado 2004; Leys 2004). Finally, some researchers think that at the poriferan evolutionary stage the germ layers are not yet formed, and so there is no speaking about their 'inversion' (Borojevic 1969, 1970; Salvini-Plawen and Splechtna 1979; Korotkova 1979, 1981a; Seravin 1986, 1992; Gaino and Burlando 1990; Ereskovsky and Korotkova 1997, 1999; Ereskovsky 1999, Ereskovsky 2007b; Ereskovsky and Dondua 2006; Coutinho and Maia 2007).

The terms 'gastrula' and 'gastrulation' were introduced into scientific usage by Haeckel in his monograph 'Biology of Calcareous Sponges' (1872). According to Haeckel (1872), the sponge gastrula is 'a spherical, egg-like or elongated body, which has an inner cavity with an opening (the primordial mouth). The wall of this cavity is made up of two different cell layers – the first is the ectoderm or the outer (animal, sensory or dermal) layer and the second is the endoderm or the inner (vegetative, nutritive or gastral) layer in higher animals' (Haeckel 1872, p. 333). Haeckel elaborated that 'this theory is based on the recognition of the true homology between the formation of the primary anlage of the gut (i.e. gastrulation – A.E.) and both primary germ layers in all animals' (Haeckel 1874). In the same place Haeckel admits an assumption that invagination could be a primary mechanism of gastrulation.

The two current definitions of the term 'gastrulation' are, in fact, contradictory. The first is used by most developmental biologists: gastrulation is the process in embryonic development in the course of which two or three primary germ layers are formed and the gut is formed through complex cell migrations (Technau and Scholz 2003; Keller 2005; Martindale 2005; Leptin 2005). The second, rare definition is used only by some spongiologists: gastrulation is the process that results in a multilayered organism during embryonic development (Efremova 1997; Leys and Degnan 2002; Maldonado 2004; Leys 2004). According to these authors, the formation of a multilayered embryo during embryogenesis in sponges should be considered as gastrulation, since mechanisms of cell reorganization in the blastula are similar with those recognized as gastrulation in cnidarians. This contradiction stems from the absence of a generally accepted point of view on the homology of embryonic processes and their derivatives in sponges and other animals.

In more complex animals, embryogenesis comprises distinct stages of cleavage, blastulation, gastrulation, histogenesis and organogenesis. In sponges, these stages are not so distinct. First, they lack the final phase of blastulation. At the end of cleavage, at the stage usually referred to as that of the blastula or the morula, there begins the differentiation of larval cells (external ciliated cells and sclerocytes). Second, they actually lack the gastrula stage in its classic meaning, i.e. the stage when germ layers, ectoderm and endoderm, are being formed (Ivanova-Kazas 1995; Gilbert 2006). The fate of the specialized larval cells (cover cells, skeletogenic cells, etc.) in sponges may be different; it is not homologous to the fate of germ

layers in Eumetazoa, since Porifera do not have body parts, organs and tissues homologous to those of other metazoans. Besides, there are no mechanisms of early determination of cells in the border and internal tissues of the definitive sponge. This is the reason behind the transdifferentiation of cells from one type into another and the constant rearrangement of histological structures. Also, sponges do not and cannot have embryonic anlagen (such as ectoderm, endoderm or homologous cell groups) determined for the development of the gut and the cutis, since they have no such tissue systems (Salvini-Plawen and Splechtna 1979; Rasmont 1979; Korotkova 1981a, b, 1988a, 1997; Anakina 1981; Seravin 1986, 1992; Malakhov 1990; Gaino and Burlando 1990; Ereskovsky and Korotkova 1997, 1999; Peterson and Davidson 2000; Brusca and Brusca 2003; Ruppert et al. 2004; Ereskovsky 2005; Ereskovsky and Dondua 2006; Coutinho and Maia 2007).

Modern developmental biology holds that morphogenetic movements and the formation of structures referred to as germ layers are two different things (Gilbert 2006). During the development of Eumetazoa, gastrulation, being a morphogenetic process, is usually not associated with cell differentiation processes (Leptin 2005).

Almost all types of cell movements characteristic of eumetazoan gastrulation are also found in the poriferan larval morphogenesis: morular delamination (Fig. 6.4a; demosponge orders Dendroceratida, Dictyoceratida, Halichondrida, Haplosclerida; see Chapter 3), cell delamination (Fig. 6.4b; *Oopsacas minuta* – Hexactinellida; see Chapter 2), invagination (Fig. 6.4c) and multipolar ingression (Fig. 6.4d; *H. dujardini* – Demospongiae, Halisarcida; see Section 3.6). At the same time, some poriferan morphogeneses are not found in other multicellular animals: inversion in Calcaronea (Fig. 6.4e; see Section 1.1), formation of blastula (pseudoblastula) by means of ingression of maternal cells into the cleaving embryo *C. reniformis* (Fig. 6.4f; Demospongiae, Chondrosida; see Section 3.4), polarized delamination (earlier considered as epiboly) (Fig. 6.4g, *M. rosacea* – Demospongiae, Poecilosclerida; see Section 3.10), unipolar proliferation (considered by some authors as a variety of unipolar immigration) (Fig. 6.4h; *Vaceletia cripta* – Demospongiae, Verticillitida; see Section 3.9) and multipolar egression in Homoscleromorpha (Fig. 6.4i; see Chapter 4).

The above morphogenetic movements – of separate cells or their layers – may precede the differentiation of larval cells, coincide with it or follow it (Table 6.1). It can be seen from the table that only epithelial morphogeneses follow morphologically noticeable cell differentiation – first of all, that of the cover ciliated larval cells.

Special attention should be paid to the morphogeneses that occur only in sponges: polarized delamination, unipolar proliferation and multipolar emigration.

Polarized delamination is outwardly similar to epiboly (Fig. 6.4g). However, in the case of epiboly small animal pole blastomeres overgrow the vegetative ones due to an increased proliferation of the former, whereas delamination starts at one of the poles (probably, the animal one) and gradually spreads over the whole embryo.

In *V. cripta*, a relic sphinctozoan, Vacelet (1979b) described the formation of a dense morula from the coeloblastula by means of ingression of cells from the area of one of the poles. Outwardly this process resembles unipolar immigration, well-known in many Cnidaria (Martin 1997). However, immigration means that single cells immigrate from the blastula wall into the blastocoel. After examining

Table 6.1 Character of embryonic morphogeneses in sponges

Morphogenesis	Epithelial (E) or mesenchymal (M) types	Precedes differentiation	Coincides with differentiation	Follows differentiation	Example	References
Morular delamination	M	Yes	No	No	Dendroceratida, Dictyoceratida, Halichondrida, Haplosclerida	Brien and Meewis 1938; Meewis 1941; Fell and Jacob 1979; Leys and Degnan 2002; Ereskovsky 2005
Cell delamination	M	Yes	No	No	Hexactinellida	Boury-Esnault et al. 1999
Multipolar ingression	M	Yes	No	No	Halisarcida	Ereskovsky and Gonobobleva 2000
Unipolar ingression	M	No	Yes	No	Halisarcida	Lévi 1956
Invagination	E	No	No	Yes	Halisarcida	Ereskovsky and Gonobobleva 2000
Inversion	E	No	No	Yes	Calcaronea	Duboscq and Tuzet 1935a, b, 1937
Ingression of maternal cells	M	Yes	No	No	Chondrosida	Lévi and Lévi 1976
Polarized delamination	M	No	Yes	No	Poecilosclerida	Ereskovsky 2005
Unipolar proliferation	M	No	Yes	No	Verticillitida	Vacelet 1979b; Ereskovsky 2005
Multipolar egression	M	Yes	No	No	Homoscleromorpha	Ereskovsky and Boury-Esnault 2002

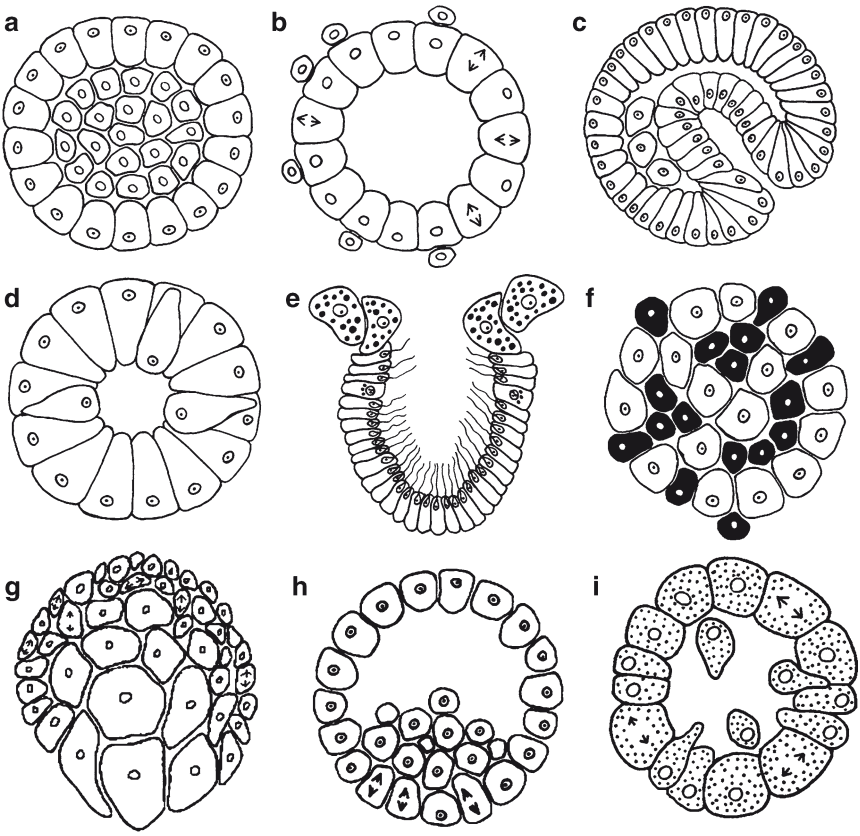

Fig. 6.4 Schematic drawings of different types of embryonic morphogenesis in sponges, leading to larvae formation. (**a**) Morula delamination (Demospongiae: Dendroceratida, Dictyoceratida, Halichondrida, Haplosclerida). (**b**) Cell delamination (Hexactinellida: *Oopsacas minuta*). (**c**) Invagination (Demospongiae: Halisarcida). (**d**) Multipolar ingression (Demospongiae: Halisarcida). (**e**) Incurvation (Calcarea: Calcaronea). (**f**) Pseudoblastula (blastula) formation by ingression of maternal cells (*black*) inside of the morula (Demospongiae: Chondrosida, *Chondrosia reniformis*). (**g**) Polarized delamination (Demospongiae: Halichondrida, Poecilosclerida). (**h**) Unipolar proliferation (Demospongiae: Verticillitidae). (**i**) Multipolar egression (Homoscleromorpha) (From Ereskovsky and Dondua 2006, p. 68, Fig. 2, reproduced by permission of Elsevier, Ltd.)

carefully the semi-thin sections kindly provided by Dr. Vacelet, I did not reveal any instances of cell immigration similar to those occurring, for instance, during embryogenesis of *H. dujardini*. Formation of the internal conglomeration of morula cells appears to take place due to an increased proliferation of cells in the area of one of the poles of the hollow blastula. The causes of this phenomenon remain unclear. No such morphogenesis at early developmental stage has been described in other multicellular animals, which warranted its description as a new morphogenesis type, unipolar proliferation (Fig. 6.4h).

The mechanism of multipolar egression (Homoscleromorpha) is similar to that of *schizocoely*, a process widespread among the Eumetazoa (Fig. 6.4i). The essential difference is that multipolar egression is associated with the formation of the coeloblastula from the morula. In other words, transformation of the whole developing organism takes place before its axialization and cell differentiation. On the contrary, schisocoely is usually associated with the divergence of the differentiated cells in local agglomerations in the polarized embryo. This is the case during formation of the endodermal lining in cnidarian planulae and formation of coelomic cavities in annelids. Thus, multipolar egression (and, as a matter of fact, invagination) results in formation of the archicoel, while schisocoely, in formation of the coelom or some cavities in the organs.

The other important functions of gastrulation in the broad sense of the word are morphological formation of the main axis (axialization) and the formation of the *bauplan*. Let us consider early morphogenesis of sponges from this angle.

It was shown above that sponges possess almost all morphogenetic movements that occur in the course of gastrulation in Eumetazoa. Only four of them are polarized: inversion, polarized delamination, invagination and unipolar proliferation.

Inversion is a rigorously polarized process. Inversion of the stomoblastula occurs along the anterior–posterior axis but does not determine it, since the axis is formed during the first cleavage cycles.

Polarized delamination is closely associated with the orientation of the embryo's main axis: it starts at one of the poles and spreads towards the other. However, as in the case of inversion, polarized delamination does not result in the morphological manifestation of the anterior–posterior axis of the larva. This axis is morphologically expressed as early as in the zygote because the egg is telolecithal.

Our special investigations demonstrated that invagination resulting in the formation of the disphaerula larva in *H. dujardini* never occurs at the future posterior larval pole (Ereskovsky and Gonobobleva 2000; Gonobobleva and Ereskovsky 2004a). It is always confined to the lateral part of the pre-larva. Therefore, it is not involved in the establishment of the anterior–posterior larval axis.

There is no information about whether polarized proliferation in *V. cripta* is in any way associated with the future main axis of the parenchymella.

All morphogeneses in Metazoa belong to one of the two types: epithelial and epithelial–mesenchymal (Keller et al. 2003; Hay 2005; Gilbert 2006). As shown above, both these types occur in poriferan embryogenesis. Different types of morphogenetic movements lie at the basis of germ layers' formation in Eumetazoa. This does not allow one to consider this process as recapitulation of events that occurred in the common ancestral form. Stable appearance of germ layers at a certain stage of development may be interpreted as a necessary element of morphogenesis, taking into account that separation of cells into a layer is a universal event of epithelial formation. Gastrulation as a morphogenetic process does not utilize some unique specific mechanism. Migrations of separate cells or cell sheets characteristic of gastrulation are a particular manifestation of general morphogenetic principles.

Despite the long history of the discussion about presence or absence of gastrulation in sponges, investigations on revealing cell lines in developing sponge embryos

with the use of molecular-biological and immunocytochemical methods are only starting (for review see Coutinho and Maia 2007). Studies of the cell lines in sponges by traditional morphological methods are complicated by transdifferentiation of sponge cells on the basis of their high polipotency, which was demonstrated best in experiments on development of sponges from conglomerates of dissociated somatic cells (for reference see Simpson 1984; Weissenfels 1989; Korotkova 1997). The results obtained at the morphological level require verification by molecular-biological and immunocytochemical methods.

The presence of metazoan developmental genes in Demospongiae and Homoscleromorpha genomes has been shown (e.g. Degnan et al. 1995; Coutinho et al. 1994, 2003; Seimya et al. 1994, 1997; Hoshiyama et al. 1998; Richelle-Maurer et al. 1998, 2006; Manuel and Le Parco 2000; Adell et al. 2003, 2007; Perovic et al. 2003; Wiens et al. 2003; Adell and Müller 2004; Hill et al. 2004; Manuel et al. 2004a; Larroux et al. 2006, 2007, 2008; Adamska et al. 2007; Coutinho and Maia 2007; Gazave et al. 2008; Lapébie et al. 2009). However, their roles in embryogenesis and metamorphosis are insufficiently investigated. To date, our understanding of sponge gene expression is restricted chiefly to asexual reproductive processes, such as gemmule germination, cell aggregation and primorphs formation.

Recent growing interest in molecular repertoires of developmental regulator genes in sponges has led to the identification of Wnt pathway components in two demosponges (Adell et al. 2003, 2007; Adamska et al. 2007) and in two homoscleromorphs (Nichols et al. 2006; Lapébie et al. 2009). It appears that sponges have a much poorer genomic Wnt repertoire than bilaterians and cnidarians, since only three *Wnt* genes would be retrieved from the *Amphimedon* genome sequence (compared with 12–15 bilaterian and cnidarian families).

6.3 Postembryonic Development

Postembryonic development starts after larval release from the parent body (in the case of viviparity and ovoviviparity) or from the egg envelopes (in the case of oviparity). Animal development may be direct or indirect. In the former case, the organism emerging from the egg – the juvenile – possesses the main features of the adult's *bauplan*. Its postembryonic development means the formation of the tissue and organ systems and the growth. In the case of indirect development, the organism emerging from the egg or the parent body – the larva – is essentially different from the adult. The differences may be represented only by a greater simplicity and 'incompleteness' of organization as compared to the adult (blastula-like larvae) or by the presence of special larval organs. Indirect development involves metamorphosis and results in a young individual with functioning systems (in sponges, the aquiferous systems). All sponges except the representatives of the genus *Tetilla* (Spirophorida, Demospongiae) and the genus *Stylocordyla* (Hadromerida, Demospongiae) have indirect, i.e. larval development.

6.3.1 *Larvae*

Eight types of sponge larvae have been described: coeloblastula, calciblastula, cinctoblastula, amphiblastula, disphaerula, hoplitomella, parenchymella and trichimella (Fig. 6.5) (Ereskovsky and Korotkova 1999; Maldonado and Bergquist 2002; Maldonado 2006). In general, there are two principal larval constructions in sponges: hollow single-layered larvae (coeloblastula, calciblastula, cinctoblastula, amphiblastula) and two-layered larvae without cavity (parenchymella, hoplitomella, trichimella). Disphaerula occupies a morphologically intermediate position.

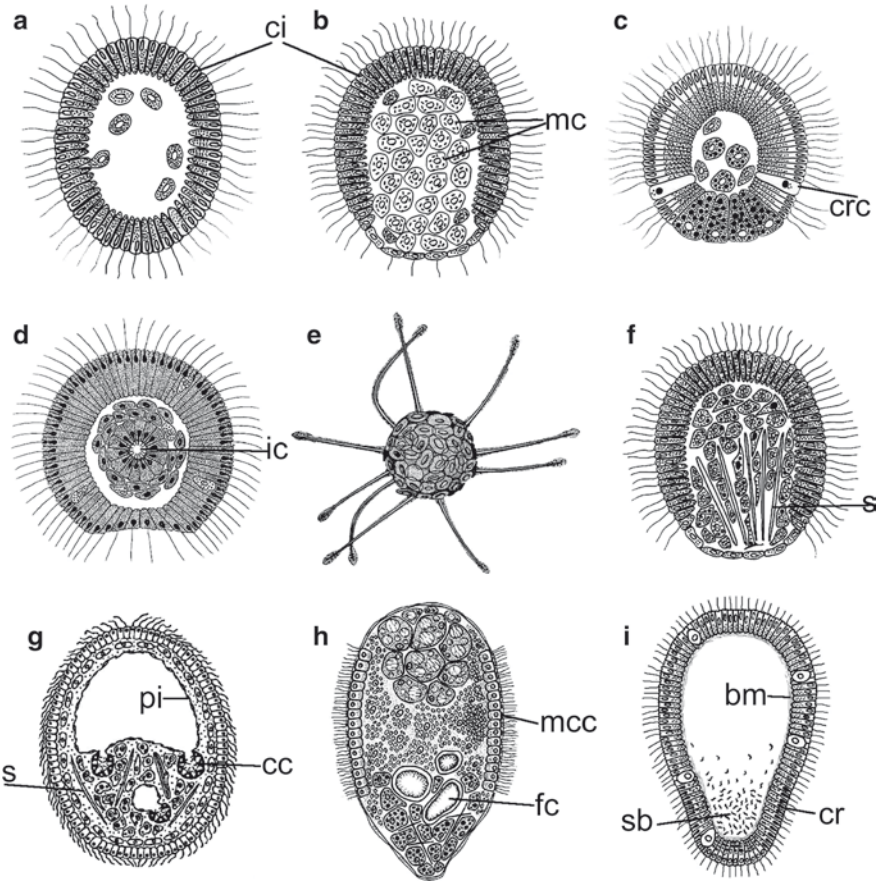

Fig. 6.5 Schematic drawings of larval types in sponges. (**a**) Calciblastula. (**b**) Pseudoblastula. (**c**) Amphiblastula. (**d**) Disphaerula. (**e**) Hoplitomella. (**f**) Parenchymella (example of Poecilosclerida). (**g**) Parenchymella of freshwater Haplosclerida. (**h**) Trichimella. (**i**) Cinctoblastula. *bm* basement membrane, *cc* larval choanocyte chamber, *ci* ciliated cells, *cr* cells with intranuclear crystalloids, *crc* cross cells, *fc* flagellated chamber, *mc* maternal cells, *ic* internal chamber, *mcc* multiciliar cells, *pi* larval pinacoderme, *s* larval spicules, *sb* symbiotic bacteria

The most simply organized larval types are coeloblastula and calciblastula. They have a one-row ciliation cover and a vast internal cavity (blastocoel) containing unstructured extracellular matrix, single somatic cells of parent origin and symbiotic bacteria. Coeloblastula is known in some oviparous demosponges (Borojevic 1967; Usher and Ereskovsky 2005; Maldonado 2009). Calciblastula, the larva of the sponges from the subclass Calcinea, is very similar to the coeloblastula type (Fig. 6.5a), but its epithelium may include various kinds of non-ciliated cells (see Section 1.2; Amano and Hori 2001; Ereskovsky and Willenz 2008).

Pseudoblastula is the larva of the oviparous demosponge *C. reniformis* (see Section 3.4) (Lévi and Lévi 1976). All the larval proper cells are ciliated and arranged in a one-row layer (Fig. 6.5b). This larva might have been attributed to the coeloblastula type, if not for the dense conglomerate of parent cells inside it.

Cinctoblastula is the larva of the Homoscleromorpha (see Chapter 4) (Boury-Esnault et al. 2003). It is single-layered, hollow and completely ciliated. Cinctoblastula, too, could be formally considered to belong to the coeloblastula type, but it has a unique feature – a belt of cells with intranuclear paracrystal bodies in the posterior pole region (Fig. 6.5i). Moreover, these larvae have a true epithelial structure, with belt desmosomes in the apical cell part and basal membrane in the basal cell part.

Amphiblastula is also single-layered: its anterior hemisphere is formed by small ciliated cells and the posterior one, by large non-ciliated cells with granular cytoplasm (Fig. 6.5c). There are four ‘cross cells’ in the equatorial part. The internal cavity is small and contains various parent cells and symbiotic bacteria. Amphiblastula larvae are characteristic of the subclass Calcaronea (see Section 1.1) (Duboscq and Tuzet 1933).

Disphaerula is one of the three larval morphotypes of *Halisarca* (Halisarcida, Demospongiae) (see Section 3.6) (Ereskovsky and Gonobobleva 2000). Its characteristic feature is the presence of a spherical ciliated cell layer in the internal part. The layer results from invagination of the surface-ciliated epithelium at the last stages of larval morphogenesis (Fig. 6.5d).

Hoplitomella is a unique planktonic larva of the boring sponges *Alectona* and *Thoosa* (Astrophorida, Demospongiae) (Garrone 1974; Vacelet 1999) (Fig. 6.5e). Its most striking feature is the absence of covering ciliated cells. Inside, there are larval choanocyte chambers, various cells, collagen fibers and discotriaenes – spicules are absent in adult sponges. These larvae were earlier taken for swimming ‘armoured gemmules’ (Garrone 1974; Fell 1993) or ‘armoured buds’ (Simpson 1984).

The most complex sponge larvae are parenchymellae. Their organization may vary even within a genus (Fig. 6.5f, g). They have a single-layered or pseudo-multilayered ciliated epithelium consisting of uniciliated cells covering most or the entire larval surface. Inside, there are sclerocytes, archaeocytes, secretory cells and collencytes; if adults have the skeleton, the larvae have it, too. Parenchymellae of all freshwater and some shallow-water marine haplosclerids have a vast endopinacocyte-lined chamber in the anterior pole region, choanocyte chambers and canals in the posterior pole region (Fig. 6.5g; see Section 3.11). Parenchymellae are

released into the environment with various degrees of development of larval and, sometimes, definitive cells. This larval type is characteristic of most demosponges (see Chapter 3).

Trichimella is the larva of the glass sponge *O. minuta* (see Chapter 2; Fig. 6.5h). It is somewhat similar to the parenchymella, but there are several essential differences. In trichimella, there is a broad belt of cells bearing up about to 50 cilia each. This belt, covered with a special layer of pinacoderm, occupies most of the larval surface; only the poles are free from it. Inside the larva there are chambers, formed by anucleate collar bodies, which, contrary to the chambers of the definitive sponge, are not arranged in the reticulum (Boury-Esnault et al. 1999; Leys et al. 2006). Trichimellae have special spicules, stauroactines, absent in the adult sponges.

All the larval types except hoplitomella are characterized by a distinct anterior–posterior polarity. Externally, it may be expressed in the body shape, the ciliation character at different poles and the unevenness of colour. At the histological level, the polarity is expressed in the distribution of various cell types, in orientation and position of spicules (parenchymella) and in uneven concentration of endosymbiotic bacteria (cinctoblastula).

Provisory structures of sponge larvae are the layer of ciliated cells and the larval skeleton. The functions of the former are locomotion (during swimming), attachment (during settling) and, probably, feeding. Two hypotheses have been discussed about the role of spicules in larvae of demosponges (Woollacott 1993, 2003). First, the presence of the spicules in larvae is the result of a heterohronic event and they may not have a function in larval life. Second, they have a direct role in the larval biology; in particular, they can participate in orientation to gravity (Maldonado et al. 1997). The larval skeleton may also play the role of the supporting carcass during early metamorphosis.

Belt desmosome-like (*zonula adhaerens*) cell junctions, ensuring mechanic contact between cells, have been shown in the ciliated layer of parenchymellae of the demosponges *Dysidea etheria*, *Ircinia oros*, *Pleraplisylla spinifera*, *Cacospongia mollior*, *Scopalina lophyropoda* (Rieger 1994; Ereskovsky and Tokina 2004; Ereskovsky 2005; Uriz et al. 2008), in disphaerula of *Halisarca dujardini* (Gonobobleva and Ereskovsky 2004b), and in coeloblastula of *Chondrilla australiensis* and *Aplysina aerophoba* (Usher and Ereskovsky 2005; Maldonado 2009), in the cinctoblastulae of the Homoscleromorpha (Boury-Esnault et al. 2003; Ereskovsky et al. 2007a; de Caralt et al. 2007a, b; Maldonado and Riesgo 2008) and in the calciblastula of *Guancha arnesenae* and *Clathrina contorta* (Calcinea) (Ereskovsky and Willenz 2008; Ereskovsky 2008, unpublished data). The posterior pole cells and the cell bodies in *Oopsacas minuta* trichimella are connected to one another and to the trabecular tissue by plugged cytoplasmic bridges (Boury-Esnault et al. 1999; Leys et al. 2006).

A characteristic feature of the sponge larvae is the presence of inclusions-containing cells of parent origin in the internal cavity. Spherulous cells – cells of parent origin found in various demosponge larvae – were shown to contain toxic metabolites (Uriz et al. 1996; Becerro et al. 1997) and metabolites involved

in sexual reproduction (Simpson 1984). They also participate in excretion and the extracellular matrix maintenance (Vacelet 1967; Donadey and Vacelet 1977; Donadey 1982; Bretting et al. 1983). In *Geodia cydonium*, spherulous cells synthesize and contain lectin, one of the key compounds responsible for cell-to-cell adhesion (Müller 1998).

Another type of parent origin cells are granular cells with eosinophilic (fuchsinophilic) inclusions. They were found in the larvae of Demospongiae (*Halisarca metschnikovi, H. dujardini*, *Mycale contarenii*, *Hamigera hamigera*, *Haliclona* sp., *Spongilla lacustris*) and Homoscleromorpha (*Oscarella lobularis*, *O. imperialis*, *O. malakhovi*, *Pseudocorticium jarrei*) (Lévi 1956, 1964; Boury-Esnault 1976; Sukhodolskaya and Ivanova 1988; Woollacott 1993; Amano and Hori 1994; Ereskovsky and Gonobobleva 2000; Ereskovsky and Boury-Esnault 2002). Ultrastructural features of granular cells testify to the synthesizing activity, but, judging by the diversity of inclusions, their specialization may be different. Granular cells in the mesohyl of the adult *H. dujardini* contain cation peptides and proteins that are factors of protection from bacteria and lower fungi (Krylova et al. 2004), but the role of granular cells in the larvae remains completely unknown.

All sponge's larvae are lecithotrophic. Their source of energy is yolk granules and lipid globules that were not used during embryogenesis. The granules usually concentrate in the basal parts of the ciliated cells or in the internal larval cells. However, ciliated cells of some demosponge parenchymellae (*Halichondria panicea* and *S. lacustris*) were shown to phagocyte organic particles from the environment (Ivanova and Semyonov 1997).

6.3.2 Metamorphosis

Metamorphosis is a short stage of post-embryonic development during which the larva undergoes radical morphological and physiological changes related to its transition to the attached adult stage. Some structures, usually the larval or provisional ones, disappear, but the others, that were rudimentary or imaginal, rapidly complete their development. Metamorphosis involves both progressive and regressive processes, but their relative importance varies. The more specialized is the larva, the deeper is its reorganization during metamorphosis. Metamorphosis can be understood as the transformation of structures and functions indispensable for an organism adapted successively to more than one biotope.

Metamorphosis occurs in an overwhelming majority of the members of the phylum Porifera. The knowledge of metamorphosis mechanisms in sponges will enable us to approach the understanding of the first stages of life cycles and morphogenesis evolution, the formation of cell lines in ontogenesis and the mechanisms of cells transdifferentiation in multicellular animals.

The characteristic features of metamorphosis in different sponge groups are discussed in the preceding chapters. This chapter tackles the general issues of sponge metamorphosis.

The main feature of the metamorphosis of sponge larvae is the acquisition of the sponge *Bauplan*, which is mainly represented by the aquiferous system. The first adult structure to be formed de novo is the exopinacoderm, which isolates the young sponge from the milieu. Later steps include organization of the choanocyte chambers and the water current channels, the opening of the ostia and osculum and the acquisition of the elements of the adult skeleton. The detailed sequence of these events has been described only in a few species (Brien and Meewis 1938; Lévi 1956; Boury-Esnault 1976; Evans 1977; Ilan and Loya 1990; Kaye and Reiswig 1991b; Amano and Hori 1993, 1996, 2001; Leys and Degnan 2002; Gonobobleva and Ereskovsky 2004a; Ereskovsky et al. 2007a, b, 2009b; Gaino et al. 2007).

Sponge larvae attach to the substrate with their anterior pole. In my opinion, evidence of attachment by the posterior pole or the lateral surface is based on artefacts. Primary adhesion is affected by the mucous substance secreted by the external ciliated cells of the anterior pole or by their cilia, which seems to be adhesive. Final attachment occurs after differentiation of collagen-secreting cells (Borojevic and Lévi 1965; Boury-Esnault 1976; Bergquist et al. 1979; Kaye and Reiswig 1991b; Leys and Degnan 2002; Gonobobleva and Ereskovsky 2004b; Usher and Ereskovsky 2005; Ereskovsky et al. 2007a). The substance secreted by larval cells during primary adhesion was shown to contain carbohydrates (acid mucopolysaccharides) and collagen fibrils (Borojevic and Lévi 1965; Boury-Esnault 1976; Evans 1977; Bergquist and Green 1977). Thus, sponges possess the universal mechanism of primary adhesion found in many larvae of marine benthic invertebrates such as Cnidaria and Bryozoa (Woollacott and Zimmer 1978; Stricker 1985).

The apical adhesion area (*zonula adhaerens*) present in the larvae of some Demospongiae disappears during the metamorphosis and is not reconstructed in the adult sponges. The loss of apical cell junctions appears to be associated with the basic principle of demosponge organization and function – cells mobility, or 'chronic morphogenesis' (Pavans de Ceccatty 1986). However, in homoscleromorphs larvae cell junctions and basement membrane are retained throughout the metamorphosis (Ereskovsky et al. 2007a, 2009b).

Although the sequence of the steps of metamorphosis is generally stable, there are certain particularities depending on the larval type. These variations are often associated with the morphogenetic processes involved in the formation of the pinacoderm and the choanocyte chambers.

For example, in the homoscleromorph cinctoblastulae the outer larval epithelium is not destroyed. All the main morphogeneses occur according to the epithelial type, with the elements of epithelial–mesenchymal transition (EMT). During exo- and basopinacoderm formation, the ciliated larval cells transdifferentiate according to their position in the settler. Aquiferous system elements develop by invagination and involution of larval epithelium. Mesohylar cells differentiate from the cells ingressing from the larval epithelium (Ereskovsky et al. 2007a).

The metamorphosis of the Halisarcid disphaerulae and the Calcarean amphiblastulae is accompanied by a combination of epithelial morphogenesis and EMT. The outer epithelium of the larva is destroyed only in the antero-lateral zone, where the

cells form an inner conglomerate. The exopinacoderm develops from the cover cells of the posterior larval pole, while the aquiferous system elements and mesohylar cells differentiate from the inner cells mass (Gonobobleva and Ereskovsky 2004a; Mukhina et al. 2006; Anakina 1989; Amano and Hori 1993; Leys and Eerkes-Megrano 2005).

The metamorphosis of all the other larval types investigated (parenchymella, calciblastula and coeloblastula) occurs by EMT with the subsequent mesenchymal–epithelial transformation (MET). A common feature of the metamorphosis of these larvae is the destruction of the larval covering epithelium and the formation of surface structures of the juvenile by the internal cells of the pupa. Exo- and basopinacoderm, aquiferous system elements and mesohylar cells differentiate from the inner cell mass (Bergquist and Green 1977; Kaye and Reiswig 1991b; Amano and Hori 1996; 2001; Leys and Degnan 2002; Usher and Ereskovsky 2005).

6.3.2.1 The Fate of Ciliated Larval Cells During Metamorphosis

When studying the metamorphosis in sponges, the authors often attempt to find out whether there is a link between the ciliated cells of the larva and the choanocytes of the adult specimens. This has a direct bearing on the so-called problem of 'the inversion of germinal layers' in sponges (Delage 1892; Tuzet 1963; Brien 1967c; Efremova 1997; Maldonado 2004; Leys 2004; Ereskovsky and Dondua 2006). According to this hypothesis, during metamorphosis the germ layers invert in such a way that the cell layers of adult sponges are reversed as compared to those of other metazoans. Based on this hypothesis, Delage (1892, 1899) separated sponges from the other Metazoa into a special group, Enantiozoa ('inside out animals'). Some authors think that this succession does exist (Delage 1892; Tuzet 1963; Brien 1967c; Lévi 1963; Amano and Hori 1993, 1996; Mukhina et al. 2006, 2007). According to the others, the ciliated cells of the larva are differentiated terminally (Wilson 1935; Efremova and Efremov 1979; Misevic and Burger 1982; Bergquist and Glasgow 1986; Misevic et al. 1990; Weissenfels 1989; Kaltenbach et al. 1999).

However, no unequivocal answer has been given to the question of their lineage. Above we demonstrated that the fate of larval ciliated cells depends on the larva organization (Table 6.2). Therefore, it appears unwarranted to search for a single interpretation of larval ciliated cells fate during the metamorphosis in Porifera.

In hollow single-layered entirely ciliated larvae (coeloblastula, calciblastula, cinctoblastula) there is no other source of choanocytes and other cells of the adults than the ciliated larval cells. For example, during rhagon development of the Homoscleromorpha all the structures are formed by direct transformation of the ciliated cells, and therefore ciliated cells are the sole source of choanocytes (Ereskovsky et al. 2007a, 2009b).

At the same time, the fate of larval cells depends on their position. For example, in the homoscleromorph cinctoblastula during the metamorphosis by basal invagination antero-lateral cells of the larvae play the leading role in the formation

Table 6.2 Larval cells fate during the metamorphosis in different sponge's larvae

Larval type	Ciliated cells of anterior-lateral zone	Cells of posterior pole	Internal amoebocytes	Internal secretory cells	Underlining cell layer	Larval choanocytes	References
Coeloblastula	Participate in differentiation of all adult cells	Participate in differentiation of all adult cells	No	No	No	No	Usher and Ereskovsky 2005
Calciblastula	Participate in differentiation of all adult cells	Unknown	No	Eosinophilic cells	No	No	Amano and Hori 2001
Amphiblastula	Participate in differentiation of all internal adult cells and basopinacoderm	Exopinacoderm formation	No	Disintegrated during metamorphosis	No	No	Amano and Hori 1993; Leys and Eerkes-Megrano 2005
Disphaerula	Participate in differentiation of all internal adult cells and basopinacoderm	Exopinacoderm formation	Participate in differentiation of all internal adult cells and basopinacoderm	Disintegrated during metamorphosis	No	No	Gonobobleva and Ereskovsky 2004; Mukhina et al. 2006; Ereskovsky et al 2007b.
Parenchymella (Poecilosclerida)	Partly phagocytosis by larval archaeocytes or apoptosis (Microcionina), partly transdifferentiate in choanocytes and grey cells (Mycalina, Myxillina)	Phagocytosis by larval archaeocytes	At the surface – differentiate in exopinacoderm, and basopinacoderm; internal population differentiate in different mesohylar cells. In Microcionina differentiate in choanocytes	Phagocytosis by larval archaeocytes	Migrated at the settler's surface	No	Borojevic and Lévi 1964, 1965; Bergquist and Green 1977; Boury-Esnault 1976; Misevic et al. 1990; Kaltenbach et al. 1999

Parenchymella (Dictyoceratida)	Partly phagocytosis by larval archaeocytes, partly transdifferentiate in choanocytes	Phagocytosis by larval archaeocytes	At the surface – differentiate in exopinacoderm, and basopinacoderm; internal population differentiate in different mesohylar cells	Unknown	Migrated at the settler's surface	No	Kaye and Reiswig 1991b; Ereskovsky 2005; Gaino et al. 2007
Parenchymella (Haplosclerida. Spongillina)	Partly phagocytosis by larval archaeocytes, partly transdifferentiate in choanocytes	Phagocytosis by larval archaeocytes	At the surface – differentiate in exopinacoderm, and basopinacoderm; internal population differentiate in choanocytes and other mesohylar cells	Unknown	Migrated at the settler's surface	Larval choanocyte chambers continue functioning	Brien and Meewis 1938; Meewis 1939; Harrison and Cowden 1975; Efremova and Efremov 1979; Weissenfels 1989.
Cinctoblastula	Participate in differentiation of all adult cells	Mostly exopinacoderm formation	No	Disintegrated during metamorphosis	No	No	Meewis 1938; Ereskovsky et al. 2007a.

of the aquiferous system of the rhagon (Fig. 4.21b–b^2, c–c^2). In the case of apical involution, anterio-lateral cells differentiate into baso- and exopinacoderm, whereas posterio-lateral and posterior cells give rise to the aquiferous system elements (Fig. 4.21d–d^3). Mesohylar cells differentiate from the ingressed ciliated cells of anterio-lateral and posterio-lateral zones. Posterior pole cells generate the exopinacoderm and posterio-lateral cells, the basopinacoderm (Ereskovsky et al. 2007a, 2009b).

A similar sequence has been described for calcaronean amphiblastulae (Amano and Hori 1993) and for halisarcid larvae (Gonobobleva and Ereskovsky 2004a). After attachment of calcinean calciblastula and chondrosids coeloblastula the settler becomes a mass of dedifferentiated cells. The cells at the surface of the cell mass differentiate into pinacocytes. The cells of the inner cell mass differentiate into choanocytes (arranged in the choanoderm), scleroblasts and other mesohylar cells (Borojevic 1969; Amano and Hori 2001; Usher and Ereskovsky 2005).

A more intricate situation is observed in two-layered larvae without a cavity. The metamorphosis of hoplitomella and trichimella was not investigated, but there are many descriptions of metamorphosis in parenchymella. In parenchymellae larvae of Demospongiae, the basic sources of choanocytes are either the internal cells (Wilson 1935; Bergquist and Green 1977; Misevic and Burger 1982; Bergquist and Glasgow 1986; Misevic et al. 1990; Weissenfels 1989; Kaltenbach et al. 1999) or the external ciliated cells (Borojevic and Lévi 1965; Amano and Hori 1996; Ivanova 1997b; Leys and Degnan 2002).

The fate of ciliated cells in parenchymella larvae may vary significantly within a taxon (see Chapter 3). For example, phagocytosis of the ciliated larval cells by archaeocytes is a common feature in all the investigated larvae of the order Poecilosclerida. The sources of choanocytes, however, may be different. In some sponges (*Mycale*, *Hamigera*) they develop both from ciliated cells and from archaeocytes (Borojevic and Lévi 1964, 1965; Borojevic 1966; Boury-Esnault 1976, 1977), in others (*Microciona*), only from archaeocytes (Bergquist and Green 1977; Misevic and Burger 1982; Misevic et al. 1990; Kaltenbach et al. 1999). In my opinion, these results are unlikely to be artefacts; they are not contradictory. On the contrary, they prove once more the polipotency of sponge cell and a high lability of their genome at all ontogenetic stages.

Differences in the morphogenetic potential between the external ciliated larval cells and the internal larval cells demonstrate their diverse differentiation abilities. These two groups of cells are not simply derived from different cell lines or different germinal layers but initially have a different degree of specialization. The restricted morphogenetic potential of the surface-ciliated larval cells of demosponge parenchymellae may be due to their early specialization. During metamorphosis, such ciliated larval cells frequently degenerate and are phagocyted by other cells (Wilson 1935; Bergquist and Green 1977; Misevic and Burger 1982; Bergquist and Glasgow 1986; Misevic et al. 1990; Weissenfels 1989; Kaltenbach et al. 1999). More rarely, these cells are transformed, after a dedifferentiation stage, either into choanocytes or into pinacocytes (Borojevic and Lévi 1965; Amano and Hori 1996; Ivanova 1997b; Leys and Degnan 2002).

Evidence against the applicability of the hypothesis about the 'inversion of layers' to all the Porifera are data on the development of *Tetilla* (Spirophorida), *Stylocordyla* (Hadromerida), *Alectona* and *Thoosa* (Family Thoosidae – Astrophorida). *Tetilla* and *Stylocordyla* have direct development; they lack any ciliated cells analogous to the covering ciliated cells of other larvae (see Sections 3.2 and 3.3). Representatives of the Thoosidae have the hoplitomella larva, which lacks the external ciliated cells (Garrone 1974; Vacelet 1999). In all these cases, there is no inversion of layers.

The reason behind these differences in the fate of the ciliated cells is the fact, ignored in many papers of sponge metamorphosis, that the larvae in various poriferan taxa are essentially different as to their structure and cell composition. Consequently, morphogeneses involved in metamorphosis are different.

The analysis of our results and the literature data allowed us to classify all the diversity of metamorphoses into two types on the basis of general features and the fate of cells.

The first type of metamorphosis is observed in parenchymella larvae of Demospongiae, which are characterized by the presence of different cells in the internal cavity. A common feature of the metamorphosis of parenchymellae is the degeneration of the covering epithelium of the larva, and the formation of surface structures of the juvenile by internal cells. The main role during metamorphosis belongs to the inner larval cells. The surface layers of the rhagon are formed by archaeocytes, underlining external ciliated cells; their position relatively to the larval anterior–posterior axis corresponds to the basal–apical position in the juvenile sponge.

The second type of metamorphosis is characteristic of coeloblastulae, calciblastulae, amphiblastulae, halisarcids larvae and cinctoblastulae. A common feature of these larvae is the differentiation of only external cells during their embryogenesis. These cells play the main role in metamorphosis. External larval cells form cover epithelium and their localization relatively to the larval anterior–posterior axis corresponds to the basal–apical position in the juvenile sponge.

At the same time, based on morphogenetic events, one can distinguish three types of metamorphoses.

1. The morphogenesis during metamorphosis of calciblastula, coeloblastula and parenchymella begins as epithelial–mesenchymal transition (EMT) with entire destruction of the covering epithelium. In the parenchymella it proceeds then by rotation of external and internal settler's cells. After that, in all the three larvae types the morphogenesis proceeds as mesenchymal–epithelial transformation (MET) with the formation of covering pinacoderm. The formation of the inner cell layers (choanoderm and endopinacoderm) involves the MET (Fig. 6.6).
2. The morphogenesis during metamorphosis of the amphiblastula and the halisarcids larvae (disphaerula, parenchymella-like and coeloblastula) involves the epithelization of the apical pole of the settler and the EMT at basal–lateral zone, with the subsequent destruction of the latter. The formation of the inner cell layers (choanoderm and endopinacoderm) involves the MET (Fig. 6.7).

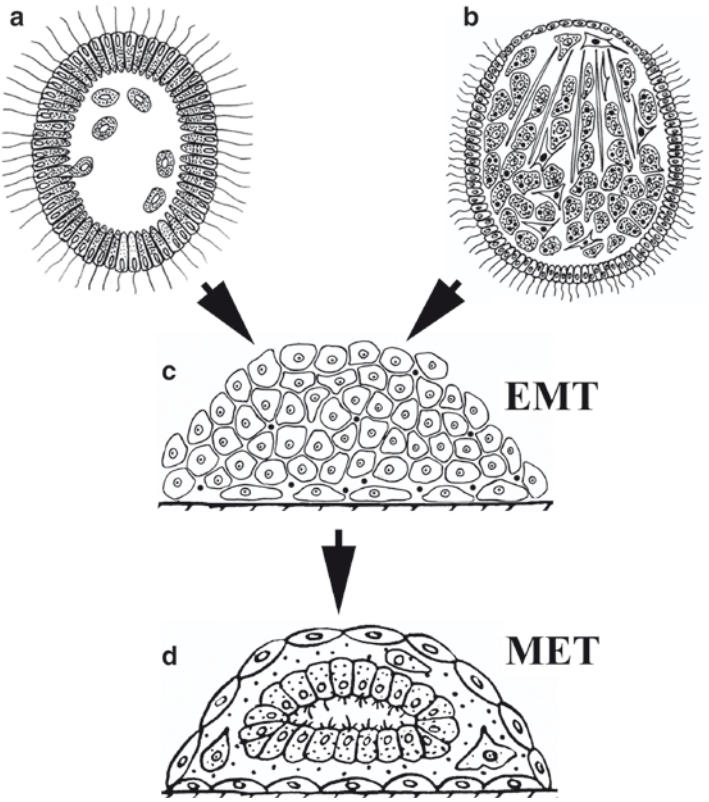

Fig. 6.6 First type of metamorphosis, based on morphogenetic events, characteristic for calciblastula, coeloblastula and parenchymella. (**a**) Calciblastula larva. (**b**) Parenchymella larva. (**c**) Settler with entire destruction of the covering epithelium. (**d**) Pre-rhagon. EMT – epithelial–mesenchymal transition, MET – mesenchymal–epithelial transformation

3. The morphogenesis during metamorphosis of the cinctoblastula passes mainly by epithelization, with the elements of the EMT (Fig. 4.21).

To sum up, no universal classification of sponge metamorphosis can be proposed. Neither taxonomy, nor larval morphology, nor morphogeneses, used as classification criteria, yield a satisfactory result. This could testify to the independent evolution of the metamorphosis in different sponge groups.

6.3.2.2 Formation of the Main Body Axis of the Definitive Sponge in the Course of Metamorphosis and Its Correlation with the Larval Axis

Recently, a special attention has been paid to the problem of formation of the main axis of the animal body and its fate in ontogenesis and phylogenesis (see Davidson 2001;

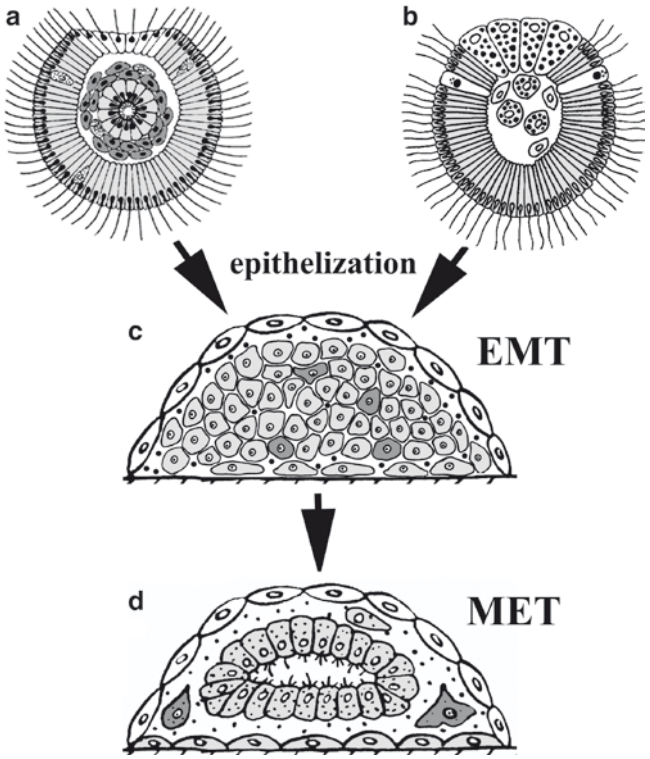

Fig. 6.7 Second type of metamorphosis based on morphogenetic events, characteristic for amphiblastula and the halisarcids larvae (disphaerula, parenchymella-like and coeloblastula). (**a**) Disphaerula. (**b**) Amphiblastula. (**c**) Settler with epithelized of the apical pole. (**d**) Pre-rhagon. EMT – epithelial–mesenchymal transition, MET – mesenchymal–epithelial transformation

Gilbert 2006). The body plans of multicellular organisms can be defined in terms of symmetry properties and axes of polarity. Bilaterian species have two principal body axes, dorso–ventral and anterior–posterior, while cnidarians, ctenophores and sponges all have a single main axis of polarity (clear in sponge larvae, but not always in adults). The relationships between the polarities of bilaterian and non-bilaterian animals remain a fundamental unresolved issue in evolution and developmental biology.

Sponges, which are at the basis of the phylogenetic tree of Metazoa, are of paramount interest in this respect. All young (rhagon or olynthus) and monooscular sponges have a single overt axis (apical–basal) defined by the presence of an osculum at one end (Fig. 6.8). At the same time, all sponge larvae possess a clear anterior–posterior polarity (Fig. 6.8) (Ereskovsky 2005, 2007b; Maldonado 2006). The results of our studies of metamorphosis in *H. dujardini*, *C. australiensis* and eight species of Homoscleromorpha, as well as the data of other authors, testify to the fact that larval anterior–posterior axis becomes the baso–apical axis of the adult regardless of the larval type (Gonobobleva and Ereskovsky 2004a; Usher and Ereskovsky 2005; Ereskovsky et al. 2007b). Analysis of the metamorphosis of the

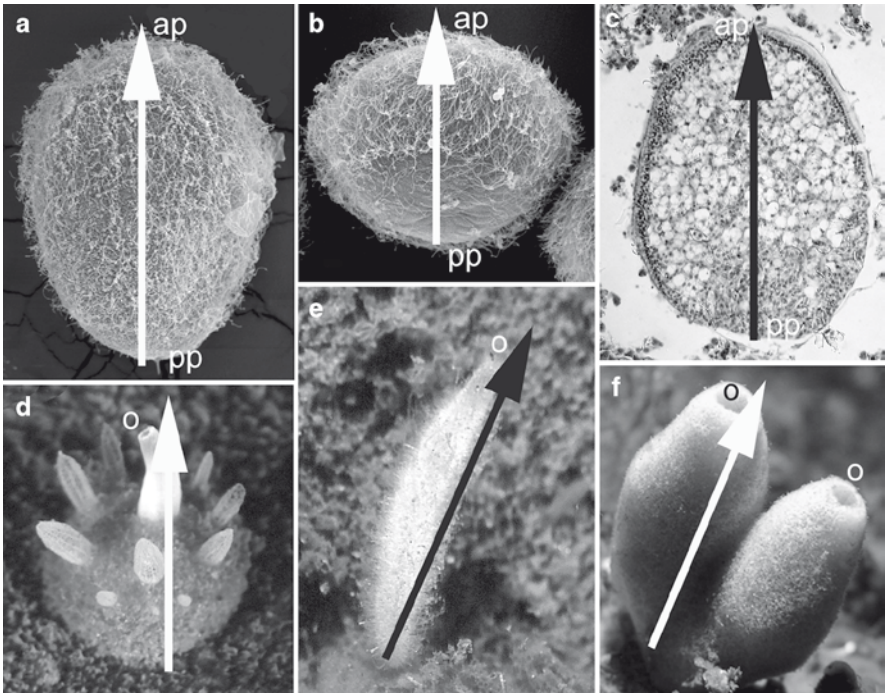

Fig. 6.8 The basal–apical and posterior–anterior axis of sponge's larvae and adult sponges. (**a**) SEM of *Clathrina reticulata* (Calcinea) larva. (**b**) SEM of *Halisarca dujardini* larva. (**c**) Light microscopy of *Esperiopsis koltuni* parenchymella. (**d**) *Polymastia arctica* from White Sea (Courtesy of M. Fedjuk). (**e**) *Sycon* sp. White Sea (Courtesy of M. Fedjuk). (**f**) *Haliclona aquaeductus* from White Sea (Courtesy of M. Fedjuk). The *arrows* indicate the posterior–anterior (**a**–**c**) and basal–apical (**d**–**f**) axis. *ap* anterior pole, *pp* posterior pole, *o* osculum

Calcinea calciblastulae (see Section 1.2) and the Calcaronea amphiblastulae (see Section 1.1) allows one to suppose that in these sponges, too, there is succession between the posterior pole cells and the exopinacoderm.

In the parenchymella larvae of the Demospongiae, the external covers (exopinacoderm and basopinacoderm) form during the metamorphosis from the archaeocytes underlining the external layer of the ciliated cells. The archaeocytes of the anterior hemisphere differentiate into basopinacocytes and participate in the adhesion of the larva to the substrate, while the archaeocytes situated in the posterior hemisphere differentiate into exopinacocytes. Regionalization of the internal conglomerate of the pupa and the development of the aquiferous system elements of the rhagon mostly result from differentiation of the larval archaeocytes, but participation of the other larval cells is also possible.

As noted above, regardless of the larval type, the anterior–posterior axis of the larva becomes the baso–apical axis of the adult sponge. Thus, sponges do not differ in this respect from other benthic water invertebrates, e.g. Cnidaria (see Gilbert and Raunio 1997).

6.3.2.3 Similarities Between the Larval Metamorphosis and the Development of Sponges from Cell Conglomerates

Numerous experiments showed that the metamorphosis of the settling larva and the development of a sponge from a conglomerate of somatic cells involve similar morphogeneses (see Simpson 1984; Korotkova 1997). This is associated with the similarity in cell movements and the characteristics of differentiation and transdifferentiation of cell groups in these two instances. Therefore, the regularities revealed in the studies of sponge development from conglomerates of somatic or larval cells, of the same kind or of different kinds, can be, with a certain caution, used for explanation of processes involved in the larval metamorphosis.

It has been known for a long time that dissociated sponge cells reaggregate and potentially reorganize into a fully functional and structured sponge (Wilson 1907). This experimental model has been extensively used to study sponge cell differentiation and morphogenesis (Curtis 1962; Korotkova 1962, 1972; Borojevic and Lévi 1964; Efremova 1969, 1970, 1972). The dissociated cells are either phagocyted by archaeocytes or reaggregate and subsequently sort out following adhesion and motility gradients. This leads to the formation of a compact spherical body, contrasting with the rather loose irregular cellular contacts during the aggregation. The formation of pinacoderm, either from pre-existing pinacocytes or from archaeocytes (reviewed in Borojevic 1970; Korotkova 1972, 1997), represents the first step in reorganization of tissue-like structures. This stage, termed 'primmorphs' (Custodio et al. 1998) represents the end of the aggregation of cellular material and the separation of the internal milieu from the external environment by a continuous pinacoderm. After adherence and stable fixation onto the solid substrate, this stage will lead to morphogenetic processes ending in the full reorganization of the sponge body.

The first essential similarity between larval metamorphosis and the development of primmorphs or tissue fragments is the important role of attachment to the substrate. To remind, attachment is a crucial stage for triggering metamorphosis. The motile free-swimming larva becomes the immobile attached individual – a transition accompanied by a change in physiology, gradients, axial relations, etc.

The period preceding attachment is a crucial one for primmorphs as well. Numerous experiments were made on development of sponges of different species after tissue dissociation and from small body fragments (Curtis 1962; Korotkova 1962, 1972, 1997; Borojevic and Lévi 1964; Connes 1966, 1968; Korotkova and Nikitin 1969a, b; Efremova 1969, 1970, 1972; Buscema et al. 1980; Custodio et al. 1998; Sipkema et al. 2003; Zhang et al. 2003). Similarly to the settled larva, the transition to apical–basal polarization and the accompanying establishment of definitive relations between various cell complexes and the aquiferous system begins in primmorphs only after attachment to the substrate. The establishment of new apical–basal axis is an indication of the normal development of the new sponge.

Deep destructive processes in morphogeneses are usually associated with reorganizations resulting in a considerable transformation of the general or local symmetry type. In many species, when the free-swimming parenchymella

transform into the rhagon, the surface cells migrate inside and the internal cells move simultaneously outside. The change in the position of the cells is accompanied by their transdifferentiation (see Chapter 3). Analogous processes occur during the development of the primmorphs. Small tissue fragments cut out of the sponge *Halichondria panicea* (Halichondrida) before the attachment become rounded, radially symmetrical and epithelized. Their canal system consists of partially disconnected endopinacoderm-lined cavities and old degenerating choanocyte chambers and canals and cannot function properly (Korotkova and Nikitin 1969a, b). The final development of the exo-, baso- and endopinacoderm happens after attachment to the substrate. Amoebocytes regroup and the spicule synthesis starts. The aquiferous system is reorganized and starts functioning (Korotkova and Nikitin 1969a, b).

At the same time, non-attached fragments eventually degenerate, regardless of the level of their development. In non-attached *H. panicea* primmorphs, contrary to the attached ones, there was no sorting of cells resulting in the apical–basal polarization (Korotkova 1972). On the other hand, when the attached and flattened small sponges were experimentally separated from the substrate and kept in suspension state, they lost the typical organization and polarization and became rounded (Korotkova 1972). These experiments highlight the close dependence of the morphogeneses on the contact with the substrate, both in the case of primmorphs and the metamorphosing larva.

Attachment to the substrate, however, is not an obligatory condition for the realization of some late morphogenetic stages of the *Halisarca dujardini* primmorphs (Halisarcida). In suspended state, they developed the system of canals and choanocyte chambers, though the osculum was never formed (Volkova and Zolotarjeva 1981). Noteworthy, after such sponges attached to the substrate, the establishment of the apical–basal axis caused the reconstruction of the aquiferous system formed during suspended state (Volkova and Zolotarjeva 1981).

6.3.2.4 The Aquiferous System Development During Metamorphosis

Larval metamorphosis usually results in a mono-oscular individual, whose aquiferous system is different from that of the adult sponge. In the Calcarea such a young individual has the aquiferous system of the asconoid type and is called the *olynthus* (Minchin 1900); in the Demospongiae and the Homoscleromorpha it has the aquiferous system of the leuconoid or syconoid type and is called the *rhagon* (Fig. 6.9) (Sollas 1888).

Development of the inhalant canals was in part considered in some early investigations (Brien 1932; Fauré-Fremiet 1932; Wintermann 1951; Lévi 1956; Mergner 1970). It was shown that in young leuconoid sponge subdermal cavities and inhalant canals develop from the lacunae forming below the exopinacoderm and in the mesohyl. There is no direct connection between the emergence of pores and the formation of the inhalant canals, though exopinacoderm always develops before the pores and the canals (Wintermann 1951).

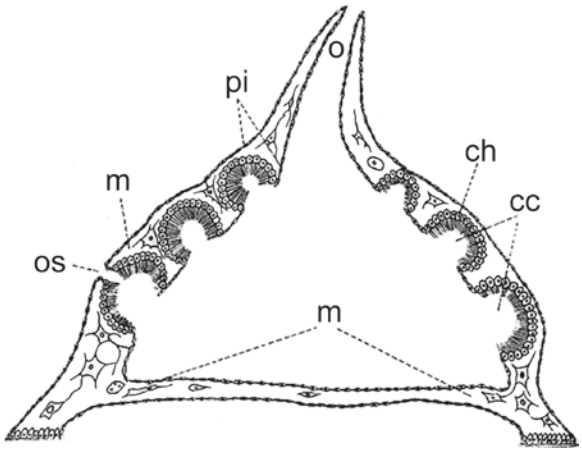

Fig. 6.9 Sponges' rhagon. *cc* choanocyte chamber, *ch* choanocytes, *m* mesohyl, *pi* pinacocytes, *o* osculum, *os* ostium (After Sollas 1888)

The flagella of the choanocytes may function actively even in the early choanocyte chambers not connected to the exhalant canal (Fauré-Fremiet 1932; Wintermann 1951; Weissenfels 1981). Flagellar activity creates internal micro-currents, which may initiate the development of the exhalant canals (Fauré-Fremiet 1932). The contact of choanocytes with the exhalant canal stimulates or even induces the formation of the inhalant canal (Weissenfels 1981). According to this author, the exhalant system, which is the first to arise, is the controlling factor of the further development of the aquiferous system. This viewpoint is supported by the experiments on development of sponges from primmorphs or tissue fragments. For instance, in *H. dujardini* primmorphs the formation of choanocyte chambers is closely associated with the presence of large exhalant canals or other cavities (Volkova and Zolotarjeva 1981). The primordia of choanocyte chambers arise as groups of various cells, mostly amoebocytes, localized around large canals. Later these groups transform into elongated chambers consisting of choanoblasts.

In primmorphs of *Suberites domuncula* evidence has been obtained that for the induction of a canal system, higher local water current is required (Perovic et al. 2003). After incubation of the primmorphs in the current chamber, the *Iroquois*-positive cells surround canal-like pores. The formation of canal-like pores, which are surrounded by *Iroquois*-expressing cells, might indicate that under *in vitro* culture conditions primmorphs can form a canal-like system (Perovic et al. 2003). The homeobox gene *Iroquois* expression may be supposed to be involved in the formation of the aquiferous system in sponges during metamorphosis and of the canal-like structures in primmorphs (Perovic et al. 2003).

The initial formative stages of the exhalant canals are also poorly studied but appear to be similar to those of the inhalant ones. The cavities emerging in the mesohyl fuse to form a star-like structure, with an atrium developing in its centre (Fauré-Fremiet 1932; Brien 1937; Wintermann 1951; Gonobobleva and Ereskovsky

2004b). The newly formed cavities of the exhalant canals are not connected to the choanocyte chambers (Wintermann 1951). At this stage the canals do not connect to the environment yet, since the oscula are not fully formed and are closed (Fauré-Fremiet 1932; Brien 1932). Similar to the development of the inhalant canals, weak water currents may induce the development of the exhalant canals (Fauré-Fremiet 1932). Other factors inducing the development of the exhalant canals are periodic contractions and relaxations of a small area of the sponge body or of the whole sponge (Kilian 1952; Rasmont 1963; Pottu-Boumendil and Pavans de Ceccatty 1976; Kilian and Wintermann-Kilian 1979; De Vos and van de Vyver 1981).

6.3.3 Growth

The growth period of the definitive sponge starts with the beginning of the aquiferous system functioning and finishes with the sexual or asexual reproduction. It is the period of increase in size, weight and the number of the aquiferous system modules, the module being an osculum with the adjoining canals, choanocyte chambers and tissue.

The duration of the growth period – that is, the increase in the body size of the sponge before the sexual reproduction – may vary considerably even within a species. For instance, young *H. panicea* individuals from the Barents Sea intertidal zone start active growth in the middle or at the end of the hydrological winter (February–April). Merging with each other, they cover an area of up to 1–1.5 m^2. At the end of the hydrological spring – the beginning of the hydrological summer (June) – these sponges separate into fragments, which start sexual reproduction (Ereskovsky 1994). In *H. panicea* from the Kiel Bay of the Baltic Sea, the growth period lasts for about a year. The shape and size of the sponges (which strictly depend on local hydrological conditions) are not different during the growth period and before the sexual reproduction (Barthel 1986; Witte and Barthel 1994). In the estuarine population of the *Halichondria* sp. from the Atlantic Coast of the USA, the reproductive cycle consists of two sexual generations. The first is represented by the overwintered sponges that have completed the growth period and reached the body size normal for the population. The second one comprises young post-larval individual that almost completely lack the growth period (Fell and Jacob 1979; Fell et al. 1979).

6.3.4 Asexual Reproduction

Asexual reproduction in sponges is an essentially heterogeneous phenomenon, since some kinds of *blastogeneses* (morphogeneses involved in asexual reproduction) are closer to regeneration, while others are closer to reduction events, in particular, somatic embryogenesis (for review, see Ivanova-Kazas 1977).

Asexual reproduction occurs in all poriferan clades. It may proceed by fragmentation, gemmulogenesis and budding (for review, see Fell 1974a, 1993; Simpson 1984; Ereskovsky 2005). Some *Leucosolenia* sp., *Sycon ciliatum* and *S. sycandra* (Calcaronea) (Lendenfeld 1885; Brien 1973a; Ivanova-Kazas 1977) were reported to have stolonial budding.

The earliest fossil evidence of asexual reproduction in sponges dates back to the early Vendian, about 580 million years ago. In phosphate deposits of the Guizhou province (South China), Li et al. (1998) found tiny (150–750 μm) monaxonic demosponges with small clavate projections – presumably, buds.

Obligatory asexual reproduction was noted only in two Demospongiae orders: Haplosclerida and Hadromerida. Within the former, gemmulogenesis occurs in the freshwater sponges from the families Spongillidae, Potamolepidae, Metaniidae and Palaeospongillidae and in some marine Chalinidae (for review, see Brien 1973a; Fell 1974a; Simpson 1984; Ereskovsky 1999; Manconi and Pronzato 2002). Within Hadromerida, gemmulogenesis occurs in the families Suberitidae and Clionaidae (Topsent 1888; Herlant-Meewis 1948; Hartman 1958; Connes 1977; Connes et al. 1978) and the budding occurs in the families Polymastiidae and Tethyidae (Merejkowsky 1878, 1879; Maas 1901; Connes 1967; Battershill and Bergquist 1990; Plotkin and Ereskovsky 1997; Gaino et al. 2006).

The simplest mode of asexual reproduction is fragmentation. It occurs accidentally, and the separation of the fragment from the parent sponge is not preceded by any changes in the cells composition. Fragmentation is characteristic of attached animals with a low degree of integration and high regeneration capacities. It is widespread in Demospongiae and Anthozoa (Cnidaria) (for review, see Ivanova-Kazas 1977).

Fragmentation mostly occurs in the demosponges, whose body branches or bears projections. Fragmentation may be triggered by various factors: wave impact during storms (Wulff 1990), the activities of predators such as fishes and turtles (Kelly-Borges and Bergquist 1988; Wulff 1990), certain infections, ruptures and splits of the substrate (Frost et al. 1982), and, mostly in the case of encrusting sponges, growth of the sponge body (colour plate XIVa, b) (Bond and Harris 1988; Bonasoro et al. 2001).

Asexual reproduction in the form of budding occasionally occurs in almost all sponges, regardless of the taxonomic position and habitat (colour plate XIVa, c–g) (Fell 1974a, 1993; Simpson 1984; Ereskovsky and Korotkova 1999; Gaino et al. 2006; Ereskovsky and Tokina 2007). However, in these cases blastogenesis is an accidental, and thus a facultative phenomenon.

A general characteristic of the buds formed in all the sponges except *Radiospongilla cerebellata* (Haplosclerida) (Saller 1990) and *Oscarella* (Homoscleromorpha) (Ereskovsky and Tokina 2007) is that at the initial stages of development they are represented by a dense conglomerate of cells, mostly totipotent archaeocytes, at the parent sponge surface. Such a bud does not have choanocyte chambers, canals, or an osculum (for review, see Brien 1973a; Fell 1974a, 1993). Separated buds settle on the substrate and attach to it, after which the formation of the aquiferous system and growth start. Thus, the buds of all the sponges resemble the pupae, which form after

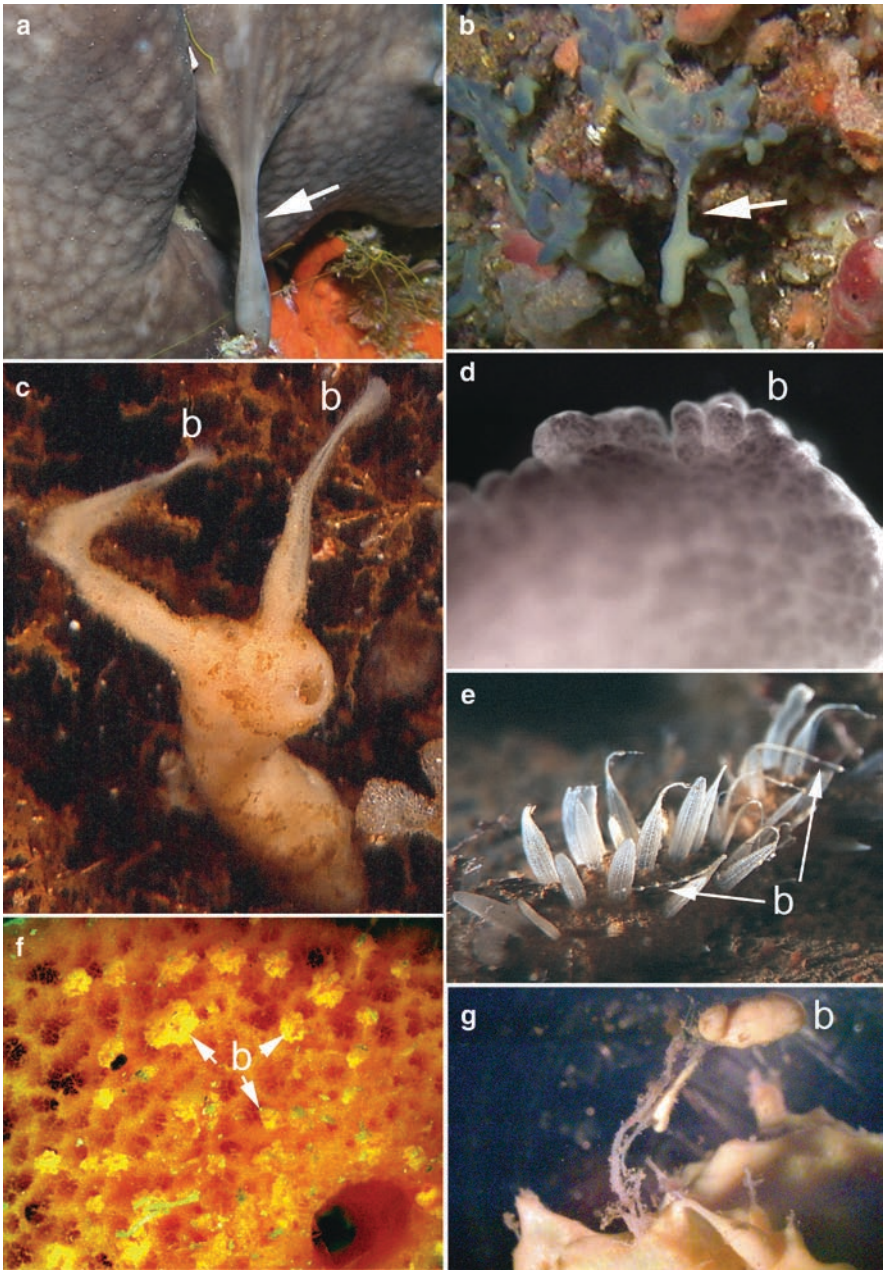

Plate XIV *In vivo* photos of asexual reproduction in sponges. (**a**) Fragmentation (*arrow*) in *Chondrosia reniformis* (Courtesy of T. Perez). (**b**) Fragmentation (*arrow*) in *Oscarella tuberculata* (Courtesy of R. Graille). (**c**) Budding in *Haliclona* sp. (Photo of A. Ereskovsky). (**d**) Budding in *Oscarella lobularis* (Photo of A. Ereskovsky). (**e**) Papillar budding in *Polymastia arctica* (Courtesy of M. Fedjuk). (**f**) Budding of *Reniera fulva*. (**g**) Budding in *Aplysilla cavernicola* (Photo of A. Ereskovsky). *b* buds

larval settlement. During attachment to the substrate, the buds may merge to form larger sponges (Connes 1967; Battershill and Bergquist 1990; Saller 1990).

While bud development in the Homoscleromorpha is based on epithelial morphogesesis (see Chapter 4), budding in the above demosponges proceeds by epimorphosis and is based on migration of totipotent cells, with subsequent differentiation into definitive cells.

Gemmulogenesis was discussed in detail earlier (see Section 3.11). To remind, in all the sponges studied gemmules develop from the same cell sources – totipotent archaeocytes (Simpson 1984; Weissenfels 1989).

What is the origin of such an unusual form of asexual reproduction as gemmulogenesis? Some light can be shed upon this issue by studying the so-called coralline demosponges. In the Paleozoic and the Mesozoic, these marine sponges were among the most common filtrators and reef-builders; now there are 14 species left. They have a massive calcareous skeleton and siliceous spicules lying freely in the fine living tissue. In three extant species, the 'reduction bodies' were found – accumulations of totipotent or 'storage'cells localized in each basal cavity of the aragonite skeleton (Vacelet 1990). Slight freshening of the water brings about degenerative changes in these sponges: the aquiferous system and the mesohyl are reduced and cells of various types disappear (Vacelet 1990). When the salinity returns back to normal, totipotent cells proliferate actively and differentiate into cells of all types. The similarity of the reduction bodies with the gemmules of marine sponges is apparent. Although the reduction bodies lack an envelope, their morphogenesis and reaction to change in environmental conditions are the same (Vacelet 1990).

In the marine sponges inhabiting shallow-water areas with periodical freshening, such reduction bodies might have evolved into gemmules. In fact, gemmules are nothing but the reduction bodies with a spongin envelope. Indeed, the three families of marine sponges that have gemmulogenesis – Suberitidae, Clionaidae (Hadromerida) and Chalinidae (Haplosclerida) – occur in shallow-water areas and in estuaries. For them, gemmulogenesis is the only way of surviving adverse conditions (Connes and Gil 1985; Fell 1993).

Colonization of fresh waters called for a further improvement of the gemmule covers, which were to protect the internal cells both from the osmotic shock and from a prolonged drying. So, a pneumatic layer and a dense spongin theca strengthened with special spicules gradually evolved.

The Demospongiae with the asexual reproduction in the life cycle often have a regular alternation of the sexual and the asexual reproduction (Ayling 1980; Pomponi and Meritt 1990; Fell 1993; Corriero et al. 1996; Plotkin and Ereskovsky 1997). For instance, gemmulogenesis in a sponge does not start until the vitellogenesis of the oocytes has been completed. Budding in the *Polymastia arctica* population from the White Sea circumlittoral zone (15–20 m) is the most intensive in spring and summer, when gametogenesis occurs, and becomes less intensive during the period of the oocytes' vitellogenesis (Plotkin and Ereskovsky 1997). The cause of this phenomenon is competition for cells – in particular, for the totipotent cells, archaeocytes. Cessation or a considerable decrease in the intensity of the asexual

reproduction during sexual reproduction is associated with the involvement of archaeocytes both in the oogenesis and embryogenesis of the sponge. This agrees with the hypothesis of Korotkova (1988a, b) about the incompatibility of sexual and somatic morphogeneses involving simultaneously the same cells or structures.

Asexual reproduction usually intensifies after the sexual one, completing the active phase of the life cycle of sponge populations from habitats with unstable conditions. It prepares them for surviving the periods when the conditions are stressful or lethal for the adult sponge. In the northern latitudes these periods are autumn and winter, with the below-zero temperature and ice cover. In the south latitudes, these are the periods of drying up, autumn storms or tropical showers.

The reproductive elements formed as the result of asexual reproduction (gemmules and buds) pass through the phases analogous to the metamorphosis and growth of the sponge. However, blastogenesis is never accompanied by cleavage or larval formation, since it always starts from a multicellular primordium consisting of various somatic cells.

6.4 Life Cycle Structure and Environmental Conditions

Reproductive and life history patterns of marine invertebrates are affected by several biotic and abiotic factors. These factors may vary locally or geographically, and result in concomitant variation in reproduction and life history on the same spatial scales. Many environmental factors are known to affect reproductive and developmental processes in sponges, including temperature, food and its availability, ocean hydrodynamics, and biomechanics (for review, see Fell 1989). Water temperature has been frequently assumed to be a major environmental factor regulating the reproduction of sponges (Wells et al. 1964; Simpson 1968; Fell 1976; Elvin 1976; Fell and Jacob 1979; Kaye and Reiswig 1991a, b; Witte 1996; Ereskovsky 2000; Ettinger-Epstein et al. 2007; Riesgo and Maldonado 2008a).

Let us consider some examples of interdependent variability of different ontogenetic stages in sponges from the same species during their adaptation to different environmental conditions. In the life cycle of *Halichondria panicea* (Demospongiae, Halichondrida), the reproductive effort is different depending on environmental conditions, and so is the degree of degradation of the parent sponge tissues after larval release. In *H. panicea* populations from the intertidal zone of the Barents Sea and the shallow waters of the White and the Northern Sea, after intensive gametogenesis and embryogenesis the mesohyl transforms almost completely into a ‘gonad’ filled with brood chambers containing larvae (Ivanova 1978, 1981; Barthel 1986; Witte and Barthel 1994; Ereskovsky 1994; Gerasimova and Ereskovsky 2007). During this life cycle period the somatic cells are intensively used for formation of the yolk inclusions in the oocytes and the follicle walls around the eggs. These processes, occurring at the end of the hydrological summer, result in the larval release and the subsequent degradation of the parent sponge. The latter is often accompanied by fragmentation, with the small fragments formed resembling the reduction

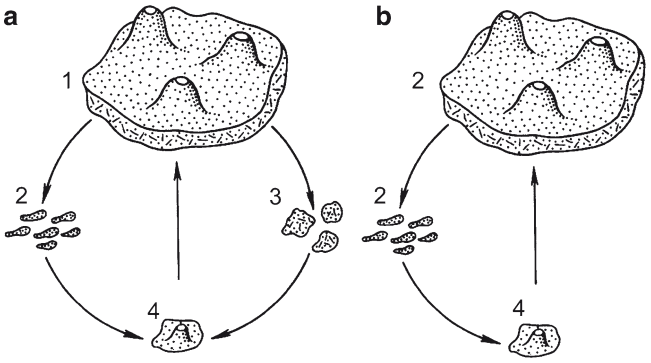

Fig. 6.10 Life cycles of *Halichondria panicea*, living in different ecological conditions of high-latitude seas: (**a**) in the littoral and upper sublittoral zones; (**b**, **c**) in circumlittoral zone. 1 – stages of gametogenesis and embryogenesis, 2 – free larval life, metamorphosis and development of young sponges, 3 – post-reproductive restorative morphogenesis of the parent sponge or its fragments, 4 – the growth period preceding the start of gametogenesis

bodies. Young sponges developing from the metamorphized larvae or from the fragments grow during hydrological spring, and a new reproductive cycle starts.

To sum up, the life cycle in the *H. panicea* populations from the shallow waters of high-latitude seas consists of four periods (Fig. 6.10a):

1. Gametogenesis and sexual reproduction, concluded by larval release from the parent sponge
2. Free larval life, metamorphosis and development of young sponges
3. Post-reproductive restorative morphogenesis of the parent sponge or its fragments
4. Growth period preceding the start of gametogenesis

The second and the third periods are parallel.

A different life cycle structure is observed in *H. panicea* under less demanding climatic conditions – in the shallow waters of the Northern Sea (South-Western coast of the Netherlands) and the sublittoral (10–18 m) of the White Sea (Vethaak et al. 1982; Wapstra and van Soest 1987; Gerasimova and Ereskovsky 2007). In sponges from these regions, gametogenesis and embryogenesis are less intensive, and so the parent tissues are reconstructed only locally, near the few embryos. As a result, the sponge functions normally during the reproductive period and does not undergo any deep destructive changes. The life cycle of *H. panicea* from these regions consists of three periods: gametogenesis and embryogenesis, larval development and metamorphosis, and growth. The period of post-reproductive restorative morphogenesis of the parent sponge is lacking from the life cycle (Fig. 6.10b).

A similar variability of the life cycle is observed in the freshwater cosmopolite species *Ephydatia fluviatilis* (Demospongiae, Haplosclerida). The most complicated life cycle structure in this species comprises the following periods:

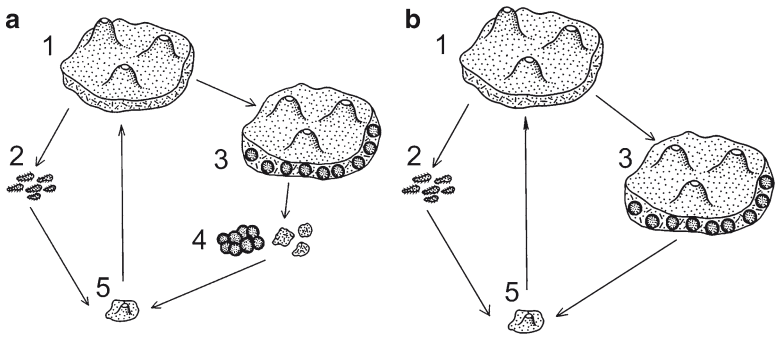

Fig. 6.11 Life cycles of *Ephydatia fluviatilis*, living in different climatic conditions. (**a**) Typical life cycle, characteristic for sponges from the regions with instable climate. (**b**) Life cycle of sponges from the regions with stable all-the-year-round climate. 1 – stages of gametogenesis and embryogenesis, 2 – free larval life, metamorphosis and development of young sponges, 3 – gemmulogenesis and degeneration of the parent sponge, 4 – survival of adverse conditions in the state of gemmules or reduction bodies, 5 – germination of gemmules, growth and formation of the sexually mature sponge

1. Gametogenesis and sexual reproduction
2. Free larval life, metamorphosis and development of young sponges
3. Gemmulogenesis and degeneration of the parent sponge
4. Survival of adverse conditions in the state of gemmules or reduction bodies
5. Germination of gemmules, growth and formation of the sexually mature sponge (Fig. 6.11a)

The second and the third periods may occur parallely. This life cycle structure is sometimes considered to be the typical one of *E. fluviatilis* and other Spongillidae (Pronzato and Manconi 1994).

This life cycle may change depending on climatic or ecological conditions. For instance, in the north of Italy, where the climate is cool and humid all year round, *E. fluviatilis* sponges are physiologically active in all seasons and no destructive processes are observed in them. However, alongside with the rather inert sexual reproduction, some gemmules are constantly being formed. The gemmulogenesis becomes more intense in winter and less intense in autumn. Both sexual cells or larvae and gemmules are present in the mesohyl of these sponges all year round (Corriero et al. 1994). Thus, the fourth period is absent from the life cycle (Fig. 6.11b).

E. fluviatilis from Lake Piediluco (Central Italy) lacks gemmulation (Gaino et al. 2003). This lake is artificially regulated and the variation in water level is about 52 cm/day. The water temperature varies throughout the year, on average from 20°C to 9°. A rise in the water temperature by about 6°C from April to May has been shown to play a role in controlling gamete differentiation in sponges from this lake. However, this difference is not so crucial as to provide an insight into this asexual reproduction. In winter, the sponges are reduced in size but physiologically active (Gaino et al. 2003). Thus, the population of *E. fluviatilis* from Lake Piediluco lacks the third, the fourth and the fifth periods in the life cycle.

Another *E. fluviatilis* population inhabits a canal in Sicily. The climate there is warm and dry, with a short rainy period and high summer and autumn temperatures, which results in the drying up of the water bodies. During the dry period, gemmules are actively formed, while sponge tissues degenerate. Correspondingly, the life cycle is complete, with all the above-mentioned periods being present. Gametogenesis and larval development occur – very rapidly – in spring and early autumn, and then gemmules are formed (Pronzato and Manconi 1994; Corriero et al. 1994).

In *E. fluviatilis* from northern Europe, the life cycle is also complete, with gemmulogenesis occurring in autumn and winter, before the water freezes. However, it may change in warm winters: the tissues of the parent sponges do not degenerate and gemmules lie at the base of functioning sponges. Gametogenesis and embryogenesis occur in spring (van de Vyver and Willenz 1975).

To sum up, different populations of the same sponge species may have a different structure of the life cycle depending on whether the environmental conditions are stable or unstable. Comparison of the varying life cycles reveals distinct competitiveness between the intensity of the morphogeneses accompanying development at sexual reproduction and the possibility of realization of the somatic ones (growth processes, formation of gemmules, restorative morphogeneses after fragmentation or reduction). A comparatively easy switching of the morphogenesis from one pattern (e.g. gametogenesis and embryogenesis) to another (e.g. restorative morphogenesis or gemmulogenesis) can be explained by the presence of totipotent cells in the sponge body capable of participating in these morphogenesis, as well as by the ability of some somatic cells to dedifferentiate and transdifferentiate.

6.5 Vertical Transmission of Bacteria

Vertical transmission of symbiotic bacteria – from the parent sponge to the next generation – is another specific trait of the poriferan individual development. The bacteria are transmitted through eggs (in oviparous species) or through larvae (in ovoviviparous ones). The first observations of bacteria in the sponge larvae were made on *Oscarella tuberculata* (Homoscleromorpha) (Lévi and Porte 1962). The evidence of vertical transmission was first obtained from the oviparous sponge *Condrosia reniformis* (Lévi and Lévi 1976).

Symbiotic bacteria may be transmitted from the parent sponge into eggs or embryos at various stages and by various means (Ereskovsky et al. 2005). Five main ways of penetration may be outlined (Fig. 6.12):

1. The bacteria are phagocyted by the oocytes directly from the mesohyl (Fig. 6.12a) as shown for *Halisarca dujardini* (Demospongiae, Halisarcida) (Ereskovsky et al. 2005), for oviparous Demospongiae *Aplysina cavernicola* (Gallissian and Vacelet 1976), *Erylus discophorus* (Sciscioli et al. 1989), *Stelletta grubii* (Sciscioli et al. 1991), *Geodia cydonium* (Sciscioli et al. 1994), *Tethya tenuisclera* and *T. seychellensis* (Gaino and Sarà 1994), and for homoscleromorph *Corticium candelabrum* (de Caralt et al. 2007b; Riesgo et al. 2007a).

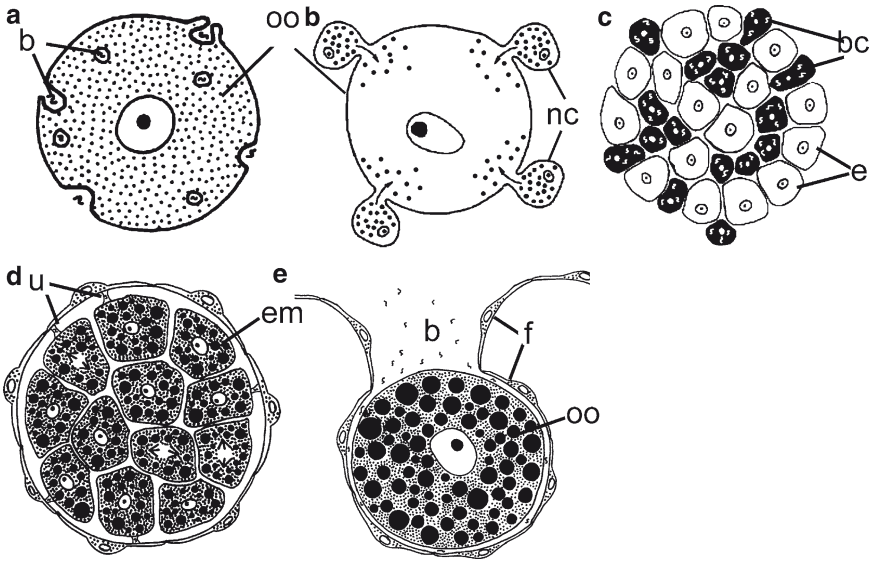

Fig. 6.12 Schematic drawings of different incorporation modes of symbiotic bacteria from maternal mesohyl into egg or embryos of sponges. (**a**) Phagocytosis of bacteria (*b*) by oocyte (*oo*) directly from the adult mesohyl. (**b**) Transfer of bacteria (*b*) from parent nurse cells to growing oocyte (*oo*). (**c**) Incorporation of bacteria from maternal bacteriocytes (*bc*) in the embryo (*e*) of *Chondrosia reniformis*. |(**d**) Transfer of bacteria (*b*) from parent to embryo (*e*) along a mucous umbilicus (*u*) in some Dictyoceratida. (**e**) Penetration of symbiotic bacteria (*b*) in the space between follicle (*f*) and egg before closing of the follicle in Homoscleromorpha (After Ereskovsky et al. 2005, p. 873, Fig. 5 with modifications, reproduced by permission of Springer). *b* bacteria, *bc* bacteriocytes, *e* embryo, *f* follicle, *oo* oocyte, *u* mucous umbilicus

2. The bacteria transferred to the cytoplasm of growing oocytes by parent nurse cells as described for *Asbestopluma occidentalis* (Riesgo et al. 2007b), *Chondrilla australiensis* (Usher et al. 2005) and *C. nucula* (Maldonado 2007) (Fig. 6.12b).
3. The bacteria penetrate into the embryo with follicular bacteriocytes (Fig. 6.12c) as shown for *C. reniformis* (Lévi and Lévi 1976) and *Svenzea zeai* (Rützler et al. 2003).
4. The bacteria appear to pass from the parent mesohyl into the embryo by mucous bridges in the course of early embryogenesis (Fig. 6.12d) as shown for four Dictyoceratida sp.: *Spongia barbara*, *S. cheisis, S. graminea*, *Hippospongia lachne* (Kaye 1990, Kaye and Reiswig 1991b).
5. The bacteria get into the space between the egg and the follicle before its closure and then penetrate between the blastomeres (Fig. 6.12e) as shown for the Homoscleromorpha with the exception of *Corticium candelabrum* (Ereskovsky and Boury-Esnault 2002).

Symbiotic bacteria were found in the embryos or larvae of the representatives of all poriferan groups: Demospongiae – *Alectona millary, A. wallichii* and *A. mesatlantica*

(Garrone 1974; Vacelet 1999), *H. hamigera* (Boury-Esnault 1976), *A. occidentalis* (Riesgo et al. 2007b), *A. cavernicola*, *A. aerophoba* (Gallissian and Vacelet 1976; Maldonado 2009), *H. ferox* (Reiswig 1976), *C. reniformis* (Lévi and Lévi 1976), *V. crypta* (Vacelet 1979b), *S. barbara*, *S. graminea*, *Hippospongia lachne* (Kaye 1990; Kaye and Reiswig 1991), *I. oros* (Ereskovsky and Tokina 2004), *I. felix* (Schmitt et al. 2007a, b), *C. mollior* (Uriz et al. 2008), *Haliclona tubifera* (Woollacott 1993), *H. aquaeductus* (Ereskovsky 2005), *H. caerulea* (Maldonado 2007), *Cladorhiza* sp. (Vacelet et al. 1995; 1996), *Astrosclera willeyana* (Wörheide 1998), *H. dujardini* (Ereskovsky et al. 2005), *Svenzea zeai* (Rützler et al. 2003) ; in all the investigated Homoscleromorpha (Ereskovsky and Boury-Esnault 2002; Boury-Esnault et al. 2003; de Caralt et al. 2007a, b; Riesgo et al. 2007a); Calcarea, Calcaronea – *G. compressa* (Lufty 1957a; Gallissian 1983), *S. ciliatum* (Franzen 1988), *Leucandra abratsbo*, *Sycon* sp. (Amano and Hori 1992, 1993), Calcinea – *C. laxa* (Amano and Hori 2001), *G. arnesenae* (Ereskovsky and Willenz 2008), *C. contorta* (Ereskovsky 2008, unpublished data); Hexactinellida – *O. minuta* (Boury-Esnault et al. 1999). Symbiotic bacteria in sponge embryos and larvae may play the trophic role, but its importance is insignificant.

6.6 The Causes of the Peculiarities of Sponge Ontogenesis

Finishing the brief analysis of the modern data on sponge reproduction and life cycles, one may formulate three main causes of the peculiarities of sponge ontogenesis, in particular, of the fact that the share of sexual and asexual morphogeneses in the life cycle of the same sponge species may be variable (Ereskovsky and Korotkova 1999).

1. The lack in the sponge bodies of tissues fully homologous to the ectodermal covers, the gut epithelium, the nervous system and the mesoderm of the Eumetazoa. The simple border tissues (pinacoderm and choanoderm), as well as the tissues of the sponge mesohyl, are more multifunctional than in the Eumetazoa. In representatives of different systematic groups, analogous tissues have a different organization and a different formative potential, which is reflected in the features of gametogenesis, embryogenesis and somatic morphogeneses, as well as their relations to each other.
2. The plasticity of cell differentiation and tissue systems in sponges ensures a comparatively fast morphogenetic reaction to change in the environment. This reaction is reflected, first of all, in transformation of the aquiferous system, an increase in migrations of the totipotent cells and their regrouping in the sponge body. This, in turn, facilitates a rapid change in the directivity of the morphogenetic processes, e.g. increase or decrease in the intensity of gametogenesis and embryogenesis or even complete stoppage of these processes. At the same time, other morphogeneses, such as blastogenesis or regeneration ones can be stimulated.

3. A weak determination of both somatic and, importantly, sexual cells of sponges have an effect on a low specialization of the gametogenesis and embryogenesis. As a result, not only the somatic cells, but also the gametocytes, embryonic cells and larval cells can, under the influence of certain factors, dedifferentiate and integrate into other, non-sexual morphogeneses.

The main criterion of the complexity of the animal body organization is the level of differentiation and specialization of the body parts, organ, tissue and cell systems, as well as the presence of specialized integrative systems. To estimate the complexity of developmental processes, one should apply similar criteria: the level of specialization of morphogeneses at various ontogenetic stages, the corresponding level of specialization of the cells involved in these processes, the degree of stability of the reproduction process and the degree of its determination.

The criteria of evolutionary simplicity or complexity of morphogeneses during sexual and asexual reproduction within the Porifera are evident. The greater the number of anatomic, tissue and cell systems in the sponge body and the greater their differentiation, the higher is the probability that these sponges possess stable reproduction forms. Besides, it is also more probable that they would have specialized forms of blastogenesis and embryogenesis.

Modern sponges are the result of a long parallel adaptive evolution. That is why the search for a single basic sponge prototype is futile. There are simpler and more complicated organizations of species' representatives within each large taxonomic group. Moreover, developmental processes of sponges from the same group may reveal both relatively specialized and relatively primitive traits.

Chapter 7
Evolution and Individual Development of Sponges: Regularities and Directions

The origin and early evolution of multicellular animals is discussed in numerous works (see Nielsen 2008). This chapter briefly addresses the origin and early evolution of sponges, with the focus mainly on the morphological and developmental aspect.

Most of the classical and modern hypotheses of the origin of sponges and multicellular animals in general are based on the assumption that the hypothetical ancestors could possess only one type of organization. Candidates nominated for the role of the last common ancestor of Metazoa were the Gastrea (Haeckel 1874; Wolpert 1994), the Phagocytella (Metschnikoff 1886; Remane 1963; Ivanov 1968, 1971; Buss 1987), the Plakula (Bütchli 1884), the Genitogastrula (Salensky 1886) the Synzoospora (Zakhvatkin 1949; Mikhailov et al. 2009), the Bilaterogastraea (Jägersten 1955) and the Hallertoid (Grasshoff and Gudo 2002). Authors and adherents of these hypotheses do not recognize the possibility of polymorphism in the ancestral forms, implicitly admitting that their development was rather determined (Ivanov 1968; Willmer 1990; Nielsen 1998, 2001, 2008; Rieger and Weyrer 1998; Dewell 2000). However, there were researchers who developed an idea about multiple emergence of the multicellular organization (Shulman 1974; Korotkova 1979), which seems reasonable, since the transformations involved in this process occurred not only at the level of the organism, but also at the ontogenetic, population and coenotic levels. In my opinion, transformations involved in the early poriferan evolution also occurred at various levels. An illustration of this idea is the normal polymorphism found in larvae of some recent sponges (Ivanova 1997a; Gonobobleva and Ereskovsky 2004a, b).

Some of the characteristic features of the poriferan organization and ontogenesis are permanent cell transdifferentiation, non-determined development and a comparatively easy transition of the morphogenesis into each other. Therefore, one can suppose that the hypothetical ancestors were also polymorphic and could also change easily the body shape and organization. At the same time, the diversity of ancestral sponges could probably be reduced to two basic forms: the coeloblastula-like organism resembling the Blastea (Haeckel 1874) and the parenchymella-like organism resembling the Phagocytella (Metschnikoff 1886).

Based on this concept, as well as on Porifera monophyly hypothesis, I will try to reconstruct the possible ways of the origin of sponges and the early evolution of

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their development until their divergence into clades. Each thesis of the hypothesis will be illustrated by the data on structure and morphogenesis of concrete representatives of recent Porifera.

7.1 Early Evolution of Sponges

One can suppose that the last common poriferan ancestors were non-attached organisms, multicellular but consisting of few cells. In the case of hollow blastula organization, all the cells were surface flagellated with a microvilli collar; they formed pseudoepithelium (Fig. 7.1a). In the case of parenchymella-like organization, there were two types of cells: the surface-flagellated cells and the internal cells organized into a loose mass (Fig. 7.1b). The main role of the surface-flagellated cells was separation of the internal environment from the external one, maintenance of the homeostasis, locomotion and capture of food particles. The internal cells were amoeboid totipotent cells resembling nucleolated amoebocytes. Some of them could be secretory cells, producing, for example, extracellular matrix (ECM) for structuring the internal environment or collagen for supporting structures.

The essential conditions for the existence of the first multicellular organisms, regardless of the level of their organization, were continuity of the structural integrity and isolation of the internal environment from the external one. Erwin (1993), postulating the hypothesis of key innovations of the metazoan origin, cites a well-known idea (Zavarzin 1945) about the necessity of isolation of the organism's internal environment for homeostasis formation. Correspondingly, one of the first developmental mechanisms was the formation of the covering layer isolating the primitive organism. Two variants of such isolation are possible: a non-cellular cover

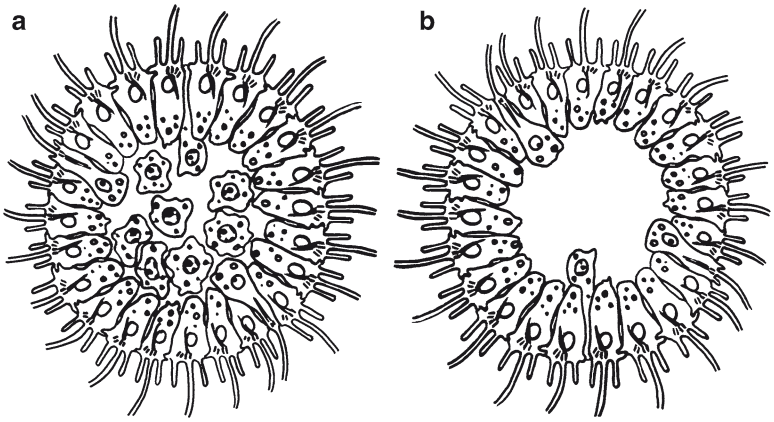

Fig. 7.1 Schematic drawing of hypothetical sponges ancestors. (**a**) The coeloblastula-like organism resembling the blastea. (**b**) The parenchymella-like organism resembling the phagocytella

(the cuticle) and a cellular cover (external epithelium). Therefore, the development of mechanisms of differential synthesis of the ECM appears to be initial in the origin and the evolution of the sponge tissue organization. One type of the ECM should be synthesized at the apical surface of cells, with subsequent formation of the cuticle, and another type, in the basal part, with subsequent transformation into basal lamina. Formation of the non-cellular cuticle surrounding the cell conglomerate means that there are mechanisms of synthesis of its components (proteins and glycoproteins), mechanisms of intracellular transport and excretion, as well as mechanisms controlling the direction of this transport (to the outside). In recent sponges, formation of the non-cellular cuticle covering the cell conglomerate takes place at the first stages of the larval metamorphosis in some Demospongiae (e.g. *Halisarca dujardini, Ircinia oros*) and in the Homoscleromorpha (Fig. 7.2) (Gonobobleva and Ereskovsky 2004a; Ereskovsky 2005; Ereskovsky et al. 2007a). In these species, cytodifferentiation and rhagon development start only after the isolation of the cell aggregate by the cuticle.

ECM of the ancestral sponges could include not only the fibrillar collagen components, but also various classes of macromolecules: glycoproteins, glycosaminoglycanes and proteoglycanes. It is thought that one of the first glycoproteins in the metazoan evolution was fibronectin, whereas laminines emerged later (Pedersen 1991; Rieger 1994; Exposito et al. 2002, 2008). Noteworthy, laminin has been found only in the sponges with basement membrane – the Homoscleromorpha (Humbert-David and Garrone 1993), while fibronectin was found in different freshwater and marine sponges (Labat-Robert et al. 1981). Fibronectin III (FN_3),

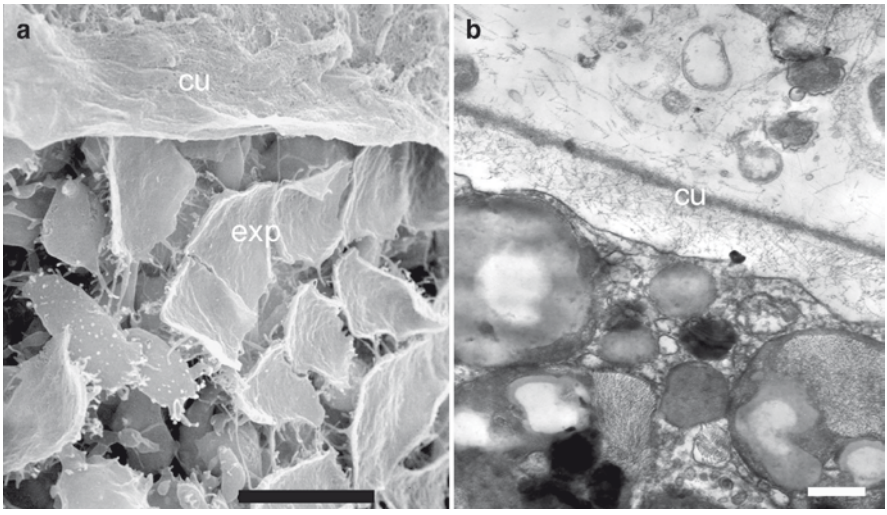

Fig. 7.2 Non-cellular cuticle at the surface of metamorphosed larvae of *Halisarca dujardini* (**a**) and *Ircinia oros* (**b**). (**a**) SEM, (**b**) TEM. *cu* cuticle, *exp* exopinacocytes. Scale bars (**a**) 10 μm, (**b**) 5 μm

extracted from *Geodia cydonium* (Pahler et al. 1998), is supposed to be the most phylogenetically ancient fibronectin (Müller 1998).

The first sponges are likely to have been very tiny living things. The most ancient fossil sponges (580 million years old; found in central Guizhou, South China) were spherical or cylindrical organisms ranging from 150 to 750 μm (Li et al. 1998). It was possibly due to their small size that the first sponges lacked the aquiferous system, a seemingly indispensable attribute of the Porifera. The above-mentioned fossil sponges are proof of that: they lack any of the aquiferous system elements, such as pores, canals, choanocyte chambers and the osculum (Li et al. 1998). The aquiferous system is also lacking in some recent carnivorous sponges, namely, some species of the family Cladorhizidae (order Poecilosclerida, Demospongiae), which live under stable environmental conditions (Vacelet 2006, 2007).

Ancestral sponges were probably fed by micropinocytosis of the dissolved organic matter and by phagocytosis of bacteria, protists and organic particles. Since these organisms were small, every cell could capture food particles independently: the cells were either situated at the surface or could easily migrate there from the interior. Present-day sponges also lack specialized digestive cells or systems; food particles are captured and digested by all the cells (Willenz 1984; Hahn-Keser and Stockem 1997; Ereskovsky and Dondua 2006). Some of the food probably got inside the ancestral sponges across the intercellular space; even in modern sponges, with the exception of Homoscleromorpha (Ereskovsky and Tokina 2007), there are no constant specialized cell junctions. Besides, food could be transferred from the surface cells to the internal ones by intracellular transport, a mechanism well developed in modern sponges (Diaz 1979; Willenz 1982; Weissenfels 1989).

Some of the bacteria that got inside the sponge by phagocytosis or across the intercellular space could, if the endogenous conditions were favourable, remain there as endosymbionts. The first endosymbionts are likely to have been facultative and also extracellular, since intracellular symbiosis required special cellular mechanisms. Originally, the symbionts were the source of nutrients. Gradually, however, they could become obligatory, with their metabolic products becoming involved into the metabolism of individual cells as well as the whole sponge. All the modern sponges investigated were shown to contain bacterial endosymbionts (Fig. 7.3) (for review see Hentschel et al. 2006; Taylor et al. 2007).

Endosymbionts could be both photosynthetic (in shallow water populations) and non-photosynthetic or metanotrophic (in populations from deep water or from the aphotic zone) (Taylor et al. 2007). Therefore, bacterial symbiosis may be supposed to have facilitated adaptive radiation of sponges under various ecological conditions and their fast dispersal in bottom biotopes.

A prerequisite of an increase in the body size and of the diversification of the body shape was the origin of internal skeleton. There are two types of skeletons: organic and inorganic. In the case of the organic skeleton its most primitive variant is the network of fibrillar collagen. Such organic (spongin) skeleton is found in some modern Demospongiae (orders Verongida, Dictyoceratida, Dendroceratida) (Fig. 7.4). Since skeleton of this type is poorly preserved in paleontological record, the time of its origin is difficult to determine. However, three finds of corneous

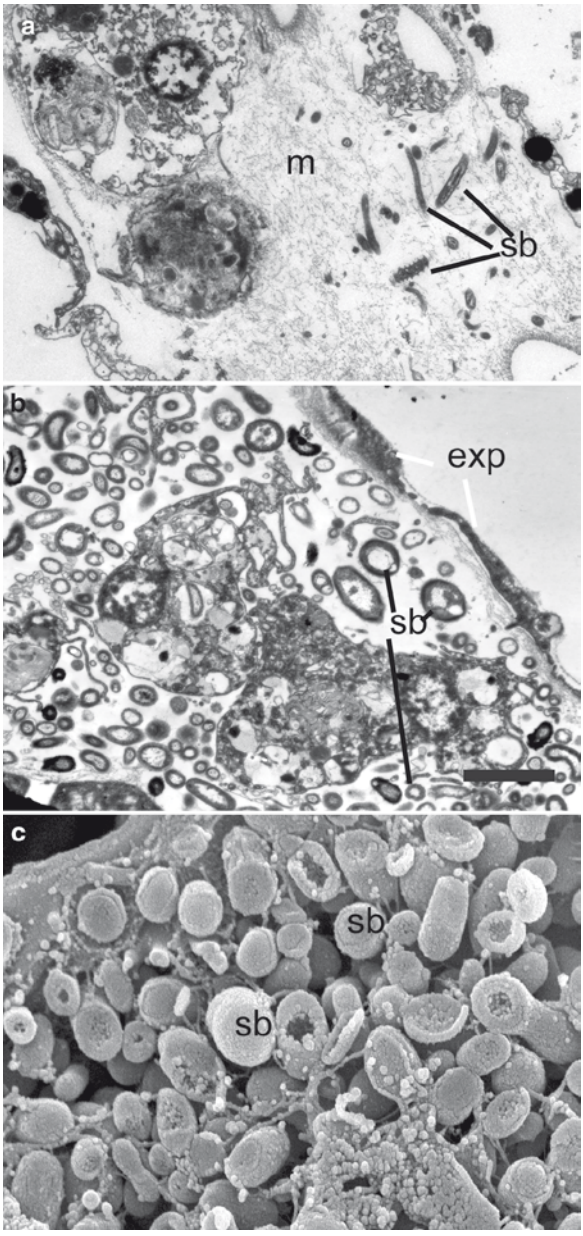

Fig. 7.3 (**a**) TEM of endobiotic bacteria in the mesohyl of *Guancha arnesenae* (Calcinea). (**b**) TEM of endobiotic bacteria in the mesohyl of *Oscarella* sp. (Homoscleromorpha). (**c**) SEM of endobiotic bacteria in the mesohyl of *Ircinia oros* (Demospongiae). *exp* exopinacocytes, *m* mesohyl, *sb* symbiotic bacteria. Scale bars (**a**–**c**) 2 μm

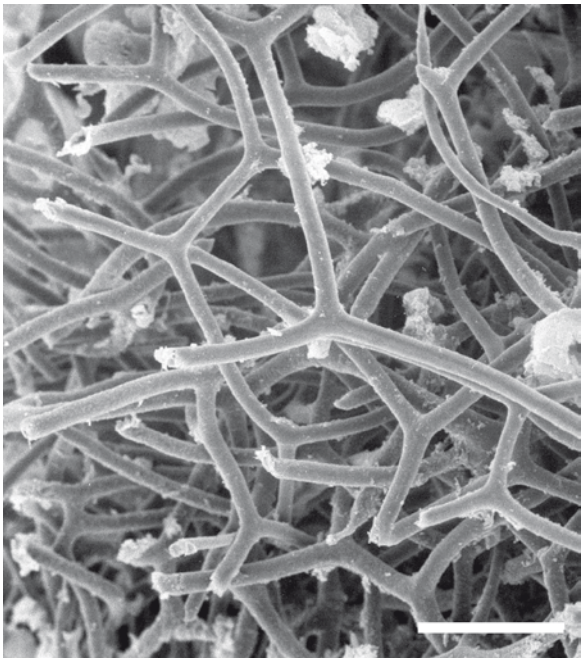

Fig. 7.4 SEM of spongin skeleton of *Spongia officinalis* (Dictyoceratida). Scale bar 200 μm

sponges from the lower Cambrian (ca. 580 million years ago) seem to testify that the corneous skeleton is a rather ancient one (Rigby 1986; Li et al. 1998; Reitner and Wörheide 2002).

Inorganic skeleton is presented by mineralized spicules containing amorphous silica (SiO_2) (Demospongiae, Hexactinellida and Homoscleromorpha) or calcium carbonate ($CaCO_3$) (Calcarea). Recent sponges have two basic mechanisms of spiculogenesis: intracellular and extracellular. In the case of the intracellular mechanism siliceous spicules are formed (Fig. 1.1; see Introduction). In the case of the extracellular mechanism, known only in the Calcarea, calcareous spicules are formed.

A gradually increasing body size was accompanied by the formation of the aquiferous system. In modern sponges it is represented by four main types: asconoid, syconoid, sylleibid and leuconoid (Fig. 7.5). One of the possible mechanisms of the aquiferous system formation in ancestral sponges was submergence of the uninterrupted layer of the surface-flagellated cells (invagination), with the layer retaining connection to the outside (Fig. 7.6a). In this way, the asconoid and the syconoid systems could have formed. This mechanism of invagination accompanies the metamorphosis of the Homoscleromorpha larvae (Meewis 1938; Ereskovsky et al. 2007a, 2009b), some *Sycon* cf. *raphanus* larvae (Leys and Eerkes-Megrano 2005) and the disphaerula larva development of *Halisarca dujardini* (Demospongiae) (Ereskovsky and Gonobobleva 2000; Gonobobleva and Ereskovsky 2004b).

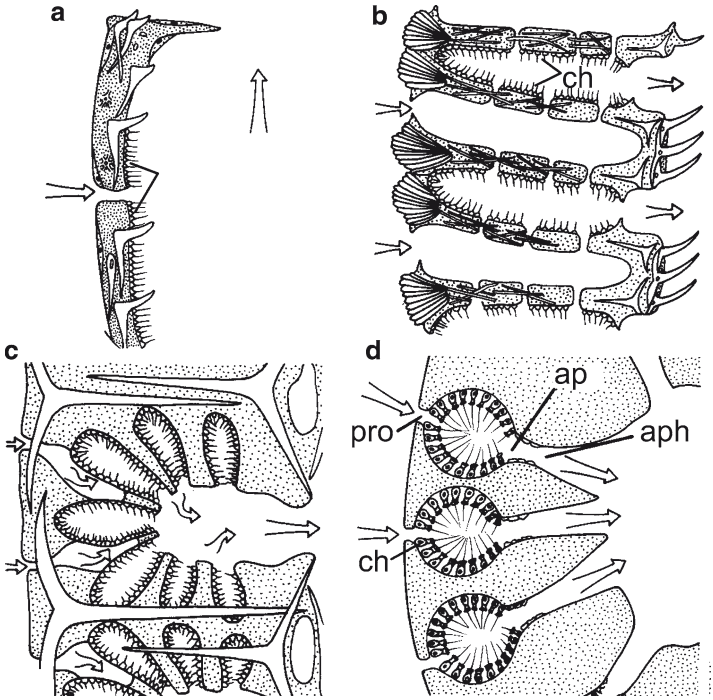

Fig. 7.5 Schematic drawing of different types of sponge's aquiferous systems. (**a**) Asconoid. (**b**) Syconoid. (**c**) Sylleibid. (**d**) Leuconoid. *ap* apopyle, *aph* aphodus, *cc* choanocyte chamber, *ch* choanocytes, *pro* prosopyle (From Boury-Esnault and Rützler 1997, p9, Figs. 37, 39, 47, 48, reproduced by permission of Smithsonian Institution Scholarly Press)

The sylleibid and the leuconoid systems could have formed by multiple invaginations of the flagellated layer (Fig. 7.6b), as observed during metamorphosis of the Homoscleromorpha cinctoblastulae (Ereskovsky et al. 2007a) (see Chapter 4).

For invagination to be possible, at least one of the two conditions should be fulfilled: the presence of specialized cell junctions in the apical part of the epithelia and the basement membrane in the basal part. In the case of the *H. dujardini* disphaerulae, the former condition is fulfilled, while in the case of the Homoscleromorpha cinctoblastulae both conditions are fulfilled: these larvae have belt desmosomes as well as the basal membrane (Boury-Esnault et al. 2003).

At the same time, we cannot rule out the possibility that the formation of the choanocyte chambers in the ancestral sponges proceeded by migration of the individual flagellated cells inside the sponge and their subsequent aggregation (Fig. 7.6c). High aggregative capacities of the flagellated cells were often shown in experiment on dissociation of the larvae (Borojevic and Lévi 1964, 1965; Ereskovsky et al. 2007b) and the definitive sponges (see Simpson 1984; Korotkova 1997). This morphogenesis is often observed during metamorphosis of the Demospongiae and the Calcarea larvae (see Chapters 1 and 3).

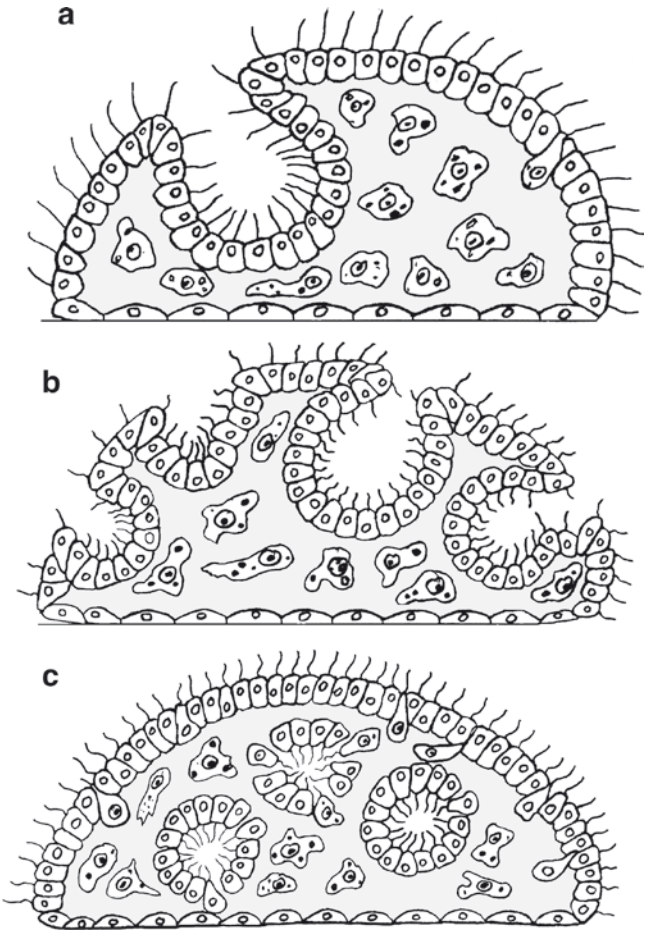

Fig. 7.6 Hypothetical scheme of aquiferous systems formation during evolution of sponges by: (**a**) submergence (invagination) of the surface-flagellated cells layer; (**b**) multiple invaginations of external flagellated layer; and (**c**) migration of the individual flagellated cells inside the sponge and their subsequent aggregation

The two mechanisms of the aquiferous system development discussed above are associated with different organization of the surface epithelium and so, probably, emerged independently several times. Therefore, it appears unreasonable to consider one of them as an evolutionary initial variant and the other as an evolutionary derived one.

There are two contradictory views on the evolutionary relations between the different constructions of the aquiferous system. According to the view coming from Haeckel (1871), the evolution of the aquiferous system was quite straightforward: from the most simple asconoid system via the syconoid one to the most complex leuconoid one. However, recent paleontological discoveries testify that the leuconoid

aquiferous system was the initial one (Mehl et al. 1998). The asconoid and the syconoid organization could have formed on the basis of the leuconoid one as adaptations to shallow water conditions (Vacelet 1985, 1999).

Another traditional argument in favour of the hypothesis that the asconoid system was the evolutionary initial one is that the rhagon and the olynthus are asconoid. However, this argument is also dubious. In fact, metamorphizing sponge larva may be asconoid (e.g. *Halisarca* (Demospongiae, see Section 3.6), leuconoid (e.g. Dictyoceratida, Poecilosclerida, Haplosclerida (Demospongiae, see Sections 3.7–3.12) and syconoid (some Homoscleromorpha, see Chapter 4).

Taking into account the early and, at the geological time scale, almost simultaneous radiation of the major poriferan groups, one can suppose that different types of the aquiferous systems also originated simultaneously and independently. In the long run, the whole morphological diversity of sponges, the number and size of their aquiferous modules are determined by the body volume, which, in its turn, correlates with the environmental conditions, in particular, hydrodynamic ones (Plotkin et al. 1999; Ereskovsky 2003).

The emergence of the aquiferous system was a major event in poriferan evolution. Water-propelling cells, immersed in the body but connected to the environment by numerous ostia and canals, enabled an entirely new mode of feeding – filtration. Sponges, possessing diverse aquiferous systems, rapidly occupied the so far empty ecological niches of bottom filter-feeders, capturing organic particles up to 2 μm in size. The next evolutionary step appears to have been the emergence of modular growth: multiplication of functional units (the aquiferous modules) made it possible for sponges to considerably increase their body size.

According to Rieger and Weyrer (1998), the syncytial organization of the Hexactinellida originated early in the course of multicellularity origin as the result of an unfinished cytotomy. However, it is much more likely that it arose by fusion of uni- or multinuclear cells. For example, in embryogenesis, the syncytium emerges only after the multicellular blastula formation (Boury-Esnault et al. 1999; Leys et al. 2006). The evolutionary significance of syncytial organization could be resolution of the contradiction between the large size of the glass sponges and the necessity of coordination. At least, it is the syncytium that is optimal for signal transfer in a large organism lacking the nervous system (Mackie and Singla 1983; Leys 1998).

7.2 Evolution of the Development Accompanying Sexual Reproduction in Sponges

It is broadly considered that one of the first differentiations in the evolution of multicellular animals was the separation of the sexual and the somatic cell lines (Metschnikoff 1886; Denis and Mignot 1994): the cells that immigrated inside gave rise to the sexual line, whereas the cells that remained at the surface, to the somatic one. However, modern data on sponge development do not support this hypothesis. As shown in the preceding chapters, sponge gametes can develop from choanocytes

and archaeocytes. Male gametes, at least in sponges with cellular organization, always develop from choanocytes (see Boury-Esnault and Jamieson 1999). In some Demospongiae (*Suberites massa*, *Halisarca dujardini*, *Iophon piceum*, *Myxilla incrustans*) and in all the Calcaronea oocytes also originate from choanocytes (Gallissian 1981; Diaz et al. 1975; Korotkova and Aizenshtadt 1976; Efremova et al. 1987a, b; Gallissian and Vacelet 1992). Moreover, in the course of larval metamorphosis, choanocytes (as the precursors of sexual cell line) result from either differentiation of larval archaeocytes or transdifferentiation of the larval ciliated cells. Characteristically, during metamorphosis of the single-layered larvae (coeloblastulae, calciblastulae and cinctoblastulae) all the cells of the adult sponge develop from the ciliated cells of the larva (Borojevic 1969; Lévi and Lévi 1976; Amano and Hori 2001; Gonobobleva and Ereskovsky 2004a; Usher and Ereskovsky 2005; Ereskovsky et al. 2007a; Chapter 6). In many demosponges with the parenchymella larva the larval ciliated cells are terminally differentiated and undergo apoptosis during metamorphosis (Bergquist and Green 1977; Evans 1977; Efremova and Efremov 1979; Misevic and Burger 1982; Bergquist and Glasgow 1986; Weissenfels 1989; Misevic et al. 1990; Kaltenbach et al. 1999). This means that in these cases the internal amoeboid cells of the parenchymella give rise to both the epithelial and the mesenchymal lines of the adult sponge cells. High mobility of the adult sponge cells and their capacity to transdifferentiation (Efremova 1972; Gaino et al. 1995; Gaino and Burlando 1990; Korotkova 1997) also do not back up the hypothesis of early segregation of the sexual and the somatic lines in the sponge embryogenesis.

The spermatozoa of the sponges' ancestors were probably of the primitive type: they had a rather large cytoplasmic volume and small mitochondria and did not have an acrosome. Such spermatozoa are characteristic of most modern Demospongiae (Boury-Esnault and Jamieson 1999).

The oogenesis of sponges has a number of distinct protozoan traits. The oocyte is capable of amoeboid movement, as well as of active phagocytosis of somatic cells and symbiotic bacteria (see Chapter 6). At the same time, some sponge groups have evolved more advanced features of oogenesis. In oviparous demosponges and homoslceromorphs the oocyte can synthesize reserve nutrients itself (see Chapter 6). In several groups (e.g. Haplosclerida and Halichondrida), specialized nurturing cells (trophocytes) and the follicular envelope cells have developed, though the follicle structure is never strictly determined, being formed in different species by different cells (archaeocytes, endopinacocytes, choanocytes) (see Chapter 6). The oviparous sponges have evolved the capacity to synthesize the vitelline envelope, protecting the oocyte and probably facilitating species-specific recognition of the spermatozoa.

Since the first sponges were probably small, they could neither provide their eggs with enough nutrients during the previtellogenic phase, nor bear embryos and larvae. Therefore, they were oviparous, with external fertilization. This hypothesis is supported by the aforementioned fossil sponges from the early Cambrian (Li et al. 1998). The authors studied hundreds of specimens and not a single one of them contained any sexual elements. The gametes must have been released by the

parent sponges either one by one across the intercellular spaces or via the body wall ruptures. Incidentally, it is the oviparous sponges that usually have small eggs with little yolk (see Section 3.1).

One of the most important synapomorphies of the Metazoa is their complex embryonic development, which comprises certain obligatory elements (cell differentiation, pattern formation, change in shape) (Wolpert 1990) and certain obligatory stages (cleavage, morphogenesis, histogenesis and organogenesis). According to Wolpert (1990), the problem of the origin of the Metazoa is, in fact, that of the origin of embryonic development.

Cleavage in the ancestral sponges was probably total, equal, asynchronous and chaotic, since this is the cleavage type found in the most primitive lower metazoans (Gilbert and Raunio 1997). Depending on the organization of the adult, cleavage resulted in the formation of either stereoblastula (morula) or coeloblastula. Further development consisted in differentiation of cells according to their position in the embryo – a process based on positional information and inductive specification. It is very likely that the ancestral sponges had no particular morphogenesis, i.e. organized movement of cells or cell complexes depending on the axial pattern, since there was no axial pattern as such. This type of development accompanying sexual reproduction is characteristic, as shown above, of many Demospongiae and Calcinea sponges.

The life cycle of the ancestral sponges (and ancestral Metazoa in general) was, in my opinion, a single-phase one, i.e. without the larva. Among recent sponges, such a life cycle is found in the genus *Tetilla* (order Spirophorida) (see Section 3.3). Indeed, larval development is an intercalary morphogenesis. It is difficult to imagine that primitive Metazoa – which lacked tissues and distinct cell lines and whose genetic mechanisms were imperfect – could have had such specialized forms as larvae. In fact, sponge larvae sometimes possess more evolutionary advanced structures than the adult sponges (Maldonado 2006; Chapter 6). For instance, homoscleromorphs cinctoblastulae have a true columnar ciliated epithelium with the basal membrane and with the belt desmosomes in the apical part (Boury-Esnault et al. 2003; Chapter 4). In the larvae of Halisarcida, Dictyoceratida and Dendroceratida the ciliated cells are connected with belt desmosomes, which are absent in the adults (Fig. 29 – Introduction; Chapters 3 and 4). The larvae of *Tedania ignis* (Poecilosclerida) are shown to have cells synthesizing serotonin-like substances (Weyrer et al. 1999). The parenchymellae of *Amphimedon queenslandica* are supposed to have flavin or karotenoid as the photosensory pigment (Leys et al. 2002). Sponge larvae are mobile and exhibit, like those of most eumetazoans, rapid responses to external stimuli such as light, gravity and current. Geotaxis, phototaxis and rheotaxis have all been documented in sponge larvae (Maldonado 2006). Moreover, globular and flask cells of the larva of *A. queenslandica* express post-synaptic and proneural genes (Richards et al. 2008; Sakarya et al. 2007).

The direct development requires a lesser expenditure of matter and energy than the larval one (Jaeckle 1995b). Besides, it is simpler from the genetic and the morphogenetic viewpoints. Since the larvae usually have provisory organs disappearing

after metamorphosis, there should have formed, as early as in the embryogenesis or oogenesis, the genetic mechanisms ensuring the development of the provisory organs and the formation of the gene complexes to be expressed only during metamorphosis.

To sum up, it is most probable that the ancestral sponges had direct development. The intercalation of the motile pelagic larva into the ontogenesis of the benthic sponges appears to have been associated with the necessity of expansion into new areas and econiches. The fossil sponges from the lower Cambrian (Li et al. 1998) seem to have been exactly at this evolutionary stage. The bottom sediments they were found in also contained hundreds of parenchymella-like larvae, very similar in size (100 × 300 μm) and structure with the modern parenchymellae. Incidentally, comparison of their size with that of the adult sponges (maximum 700 μm) yields yet another confirmation of the hypothesis that the ancestral sponges were oviparous.

So, sponges with the direct development at some moment gave a branch with the larval development. Two variants are possible here: the larvae could develop either in the environment or in the parent body. Ovoviviparity probably developed later.

Ovoviviparity appears to be more evolutionary advanced than the development in the environment (Batigina et al. 2006). Firstly, this follows from the morphogenetic considerations discussed above. Secondly, ovoviviparity calls for a larger size of the parent body, a higher degree of its integration and the development of mechanisms of internal fertilization, larval development and larval release. Ovoviviparity is likely to have originated simultaneously with the formation of the aquiferous system and the differentiation of the internal cells into endopinacocytes.

On the strength of our hypothesis postulating early radiation of the major poriferan branches and the secondary nature of the larval development, we can suppose that the larvae of the ancestral sponges were different. Depending on the cleavage and the blastula types, they could be either single-layered (coeloblastulae) or double-layered (parenchymellae). Both these larvae appear to have had a simple structure and weakly differentiated cells.

The first parenchymellae were probably equal. The number of their cell types probably did not exceed two or three: there were the covering ciliated cells responsible for locomotion and the internal polypotent cells. It is very likely that the ciliated cells had in their apical parts long microvilli-like cytoplasmic projections, which captured food particles driven towards them by the undulating cilium. Such microvilli, forming a sort of collar around the cilium, are present in the covering cells of the larvae of many recent sponge species. They are especially long in the parenchymellae of Dendroceratida, Dictyoceratida and Verticillitidae (see Sections 3.7, 3.8, 3.9; Fig. 7.7). These projections increase the surface area of the cells, facilitating more effective intake of the dissolved macromolecules (Jaeckle 1995a). To note, choanocytes have never been found among the covering cells of the sponge larvae.

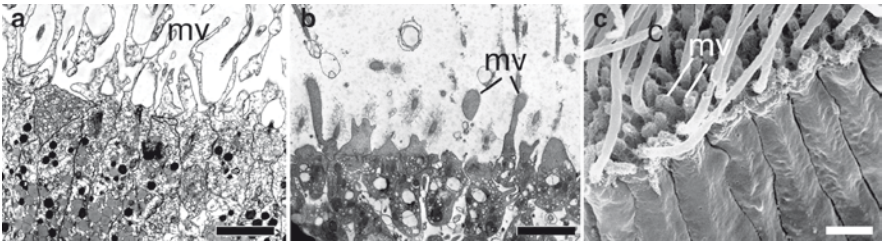

Fig. 7.7 Long microvilli-like cytoplasmic projections in apical parts of larval ciliated cells. (**a**) TEM of parenchymella of *Ircinia oros* (Demospongiae, Dictyoceratida). (**b**) TEM of parenchymella of *Vaceletia crypta* (Demospongiae, Verticillitidae). (**c**) SEM of cinctoblastula of *Plakina trilopha* (Homoscleromorpha). *c* cilium, *mv* microvilli. Scale bars (**a**) 5 μm, (**b**) 3 μm, (**c**) 2 μm

The ancestral larvae had no specialized cell junctions, and the cells were connected only by overlapping membranes. Therefore, the cells could crawl inside the larvae after the capture of large food particles and crawl to the surface for feeding. This mechanism, first suggested by Metschnikoff (1886) for his hypothetical Phagocytella, was later considered in detail by his followers (see Nielsen 2008). Migration of the ciliated cells inside the larvae is shown at the electron-microscopic level in the parenchymellae of *Ephydatia fluviatilis* (Ivanova 1997a) and the larvae of *H. dujardini* (see Section 3.6).

The traditional view holds that sponge larvae cannot feed. However, ciliated cells of some demosponge parenchymellae are shown to be capable of feeding both by phagocytosis (Ivanova and Semyonov 1997) and by micropinocytosis of the dissolved macromolecules (Jaeckle 1995a). Besides, the ciliated cells of the *Halisarca* larvae and the homoscleromorph larvae can phagocyte fragments of the internal cells by their basal parts (see Sections 3.6 and Chapter 4).

Intercalation of the larva into sponge life cycle entailed the emergence of the metamorphosis, which is considered to be an evolutionary reaction to the structural divergence between the pelagic larva and the bottom adult animal (Ivanov 1937).

Since the Porifera are situated at the base of the phylogenetic tree of multicellular animals, our version of their early evolution could also be viewed as that of the early metazoan evolution.

Chapter 8
In Place of Conclusion: *Bauplan* and Phylotypic Stage in Porifera

Bauplan (construction plan) is a key notion of developmental biology and evolutionary morphology, applied for the establishment of new taxonomic phyla and for the construction of the high-level classification. It is the bauplan, or morphological type, that had been assumed by Cuvier (1817) as a basis for the division of animals into four large groups (vertebrates, mollusks, articulates, and radiates). Bauplan is understood as the type of the organism's construction formed within a certain group and characterized by an original architectonics.

There are two main concepts of the bauplan. The first one, stemming from Owen's ideas about the archetype (Owen 1848), is based on the comparison of the structure of the adult animals, without considering the preceding stages of development. It is well known; however, that similar developmental types may result in very different adult animals, while different developmental types may produce similar adults (see for references Ivanova-Kazas 1995; Gilbert and Raunio 1997). The second concept is based on the comparison of the structure of the larvae, which are rather conservative in the evolution (see Raff 1996), and also does not take into account the embryonic development.

At the same time with Cuvier, the notion of the "developmental plan" was introduced by von Baer (1828). According to the latter, each body plan was seen to be created by a particular kind of developmental organization – the type. Therefore, the developmental plan *sensu* von Baer is the bauplan in the period of its ontogenetic formation.

So, each animal phylum has its own bauplan and, consequently, has to have its own developmental plan. There are, however, groups of phyla or classes, which, while differing in the development, possess a certain common stage. This is the case of the coelomic Spiralia, comprising the phyla Annelida, Mollusca, Echiurida, and Sipunculida. The adult representatives of these phyla are essentially different, and so is their morphogenesis, but most of them having a common stage – the trochophore. At this stage, the bauplan of the whole big animals group reveals itself. Seidel (1960), who was the first to pay attention to this stage, called it the *Korpergrundgestalt*, while Sander (1983) termed it the *phylotypic stage*. Both these authors considered such a stage to be decisive in the development of an animal group. They did not attach any phylogenetic significance to the intermediate processes and stages such as cleavage, gastrulation, and morphogenesis.

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The phylotypic stage was back in the spotlight after the investigation by Slack et al. (1993), with the discussions based, this time, also on the molecular–biological data. The phylotypic stage was characterized as the one when the main morphogenetic movements have been concluded and all the anlages have occupied their places, i.e. the axial complex of anlages has been formed. In other words, the phylotypic stage is the embryological stage during which the phylum-level characters appear. Phylotypic stages were revealed in many animals: the tailbud stage (pharyngula) in vertebrates, germ band stage in arthropods, the fully segmented, ventrally closed leech embryo, etc. (Slack et al. 1993; Minelli and Schram 1994; Hall 1998; Gilbert 2006).

Phylotypic stages are not the earliest stages in the embryogenesis. Moreover, they may occupy a different place in the ontogenesis in different representatives of the group, which may be associated with the adaptations of early stages, various reproductive strategies and tactics, the nurturing needs of the embryo, etc. At the same time, phylotypic stages themselves are the least subjected to adaptive modifications (Slack et al. 1993). Conservative phylotypic stages are sandwiched between the preceding and the following variable ones.

It should be noted, however, that the validity of the phylotypic stage has been questioned, both on the basis of comparative studies showing that the uniformity of the putative phylotypic stages is in fact absent, and on the basis of considerations about the typological connotations of this concept (e.g. Richardson et al. 1997, 1998; Fèlix 1999; Scholtz 2004, 2005).

The formation of the complex body organization in multicellular animals during ontogenesis is controlled by a sophisticated cascade of the complexes of regulatory genes, whose expression is spatially and temporally ordered (Davidson 2001). Although the investigations of the role of regulatory genes in the embryonic development of Porifera are few, the existing ones show that sponges have a genetic mechanism of specification of the regional morphological differentiation along the body axis of the larva and the adult sponge (e.g. Larroux et al. 2006). Indeed, all monooscular and radially symmetrical sponges with a secondary osculum have a clear radial symmetry around the apical–basal axis (Fig. 6.8). In almost all the sponges, the body is regionalized into the ectosome and the choanosome, with the corresponding differences in the structure of the skeleton and the aquiferous system.

On the basis of the analysis of the sponge development during sexual and asexual reproduction, presented in this book, a hypothetical variant of their phylotypic stage (*spongotype*) can be suggested. It is the rhagon, the organization type of the Demospongiae (Fig. 8.1), with the olinthus corresponding to it in the Calcarea.

The rhagon, emerging after the larval metamorphosis, is the ontogenetic stage shared by all the Porifera. It is small (up to 1–2 mm); its surface is formed by flattened epithelial cells (pinacocytes), excreting the extracellular matrix (the cuticle). It is characterized by radial symmetry and apical–basal polarity.

The major feature, which allows one to consider the rhagon as the phylotypic stage of the Porifera, is the final, definitive position of all the cellular and anatomical

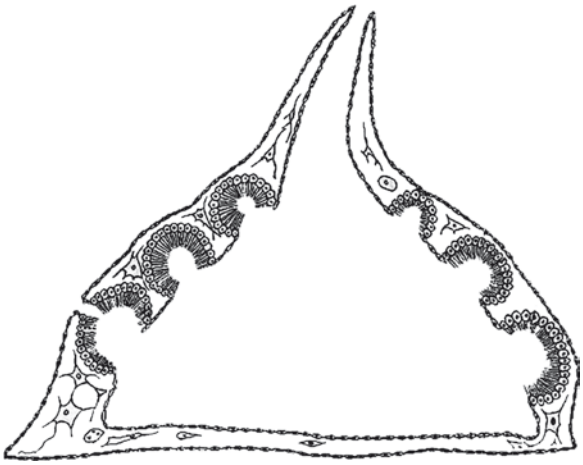

Fig. 8.1 Sponges' rhagon – hypothetical spongotype (After Sollas 1888)

elements of the future adult sponge. One may say that at the rhagon stage the pattern of the axial complex of anlages is formed; growth and organogenesis follow. Moreover, a rhagon-like structure is formed after embryogenesis in sponges with direct development (see Section 3.3), and immediately after germination of gemmules and buds (DeVos 1965; Connes 1967; Simpson 1984; Weissenfels 1989). This stage is obligatory in the case of the sponges forming from the cell conglomerates of the dissociated larvae or adult sponges.

References

Adams CL, McInerney JO, Kelly M (1999) Indications of relationships between poriferan classes using full-length 18S rRNA gene sequences. Mem Queensl Mus 44:33–43

Adamska M, Degnan SM, Green KM, Adamski M, Craigie A, Larroux C, Degnan BM (2007) Wnt and TGF-b expression in the sponge *Amphimedon queenslandica* and the Origin of Metazoan Embryonic Patterning. PLoS ONE 2(10 e1031): doi: 10.1371/journal.pone.0001031

Adell T, Nefkens I, Müller WEG (2003) Polarity factor 'frizzled' in the demosponge *Suberites domuncula*: identification, expression and localization of the receptor in the epithelium/pinacoderm. FEBS Lett 554:363–368

Adell T, Müller WEG (2004) Cloning and characterization of T-box and forkhead transcription factors from Porifera. Boll Mus Ist Biol Univ Genova 68:163–173

Adell T, Thakur AN, Müller WEG (2007) Isolation and characterization of Wnt pathway-related genes from Porifera. Cell Biol Int 31:939–949

Adiyodi KG, Adiyodi R.G (eds) (1988) Reproductive biology of invertebrates: accessory sex glands. Wiley, New York

Afzelius B (1979) Sperm structure in relation to phylogeny in lower metazoa. In: Fawcett DW, Bedford JM (eds) The spermatozoon. Urban & Schwarzenberg Inc, Baltimore-Munich

Aisenstadt TB (1984) Cytology of the oogenesis. Nauka, Moscow

Aisenstadt TB, Korotkova GP (1976) A study of oogenesis in the marine sponge *Halisarca dujardini.* II Phagocytic activity of the oocytes and vitellogenesis. Tsitologia 18:818–823

Akijama SK, Johnson MD (1983) Fibronectin in evolution: presence in invertebrates and isolation from *Microciona prolifera*. Comp Biochem Physiol 76:687–693

Alekseeva NP (1980) Organization of parenchymella of an endemic Baikal Sponge *Swartchewskia papyracea* (Dyb.). Arch Anat Histol Embryol 79:74–80

Alekseeva NP, Efremova SM (1986) Oogenesis of Baikal sponges. In: Zakonomernosti individual'nogo razvitiya zhivykh organizmov. Nauka, Moscow

Amano S (1986) Larval release in response to a light signal by the intertidal sponge *Halichondria panacea*. Biol Bull 171:371–370

Amano S, Hori I (1992) Metamorphosis of calcareous sponges. I. Ultrastructure of free-swimming larvae. Invert Rep Dev 21:81–90

Amano S, Hori I (1993) Metamorphosis of calcareous sponges. II. Cell rearrangement and differentiation in metamorfosis. Invert Rep Dev 24:13–26

Amano S, Hori I (1994) Metamorphosis of a demosponge. I. Cells and structure of swimming larva. Invert Rep Dev 25:193–204

Amano S, Hori I (1996) Transdifferentiation of larval flagellated cells to choanocytes in the metamorphosis of the Demosponge *Haliclona permolis*. Biol Bull 190:161–172

Amano S, Hori I (2001) Metamorphosis of coeloblastula performed by multipotential larval flagellated cells in the calcareous sponge *Leucosolenia laxa*. Biol Bull 200:20–32

Anakina RP (1981) The embryological development of Barents Sea sponge *Leucosolenia complicata* Mont. (Calcarea). In: Korotkova GP (ed) Morphogenesis in sponges. Leningrad University Press, Leningrad

Anakina RP (1988) The early stages of fertilization of sponge *Leucosolenia complicata* Montagu (Calcispongia, Leucosoleniida) from the Barents Sea. In: Koltun VM, Stepanjants SD (eds) Porifera and Cnidaria. Modern and perspective investigations. Zoological Institute USSR Academy of Science Press, Leningrad

Anakina RP (1989) Experimental-morphological investigations of the embryogenesis in Barents Sea calcareous sponge *Leucosolenia complicata* Montagu. Ph.D. thesis, Leningrad University, Leningrad

Anakina RP (1997) The cleavage of Barents Sea Sponge *Leucosolenia complicate*. In: Ereskovsky AV, Keupp H, Kohring HR (eds) Modern problems of Poriferan biology. Berliner Geowiss Abh, Freie University, Berlin

Anakina RP, Drozdov AL (2000) Peculiarities of oogenesis in the Barents Sea sponge *Leucosolenia complicata* Montagu (Calcispongiae, Calcaronea). Tsitologia 42:128–135

Anakina RP, Drozdov AL (2001) Gamete structure and fertilization in the Barents Sea sponge *Leucosolenia complicata*. Russ J Mar Biol 27:143–150

Anakina RP, Korotkova GP (1989) Spermatogenesis in *Leucosolenia complicata* Mont., Barents Sea sponge. Ontogenesis 20:77–86

Aouacheria A, Geourjon C, Aghajari N, Navratil V, Deleage G, Lethias C, Exposito J-Y (2006) Insights into early extracellular matrix evolution: spongin short chain collagen-related proteins are homologous to basement membrane type iv collagens and form a novel family widely distributed in invertebrates. Mol Biol Evol 23:2288–2302

Arnesen E (1918) Brutknospenbildung bei *Polymastia mammilaris* (O.F. Müll.) Bow. (*Rinalda arctica* Merej.). Det Kgl Norska Videnskabers selskabs skribter 1:1–24

Avenirova EL, Sukhodolskaya AN (1983) Biochemical peculiarities of the development of gemmules in freshwater sponges. 1. Investigation of proteins by electrophoresis in polyacrylamide gel. Vestnik Leningr Gos Univ (Biol) 3:5–11

Avenirova EL, Sukhodolskaya AN (1984) Biochemistry peculiarities of the development of freshwater sponges from the gemmules. 2.The effect of puromycin on the autolysis of *Spongilla lacustris*. Vestnik Leningr Gos Univ 21:80–81

Ayling AL (1980) Patterns of sexuality, asexual reproduction and recruitment in some subtidal marine Demospongiae. Biol Bull 158:271–282

Babic K (1916) Zur Kenntnis der Theneen. Zool Jahrb 40:389–408

Baccetti B, Gaino E, Sarà M (1986) A sponge with acrosome: *Oscarella lobularis*. J Ultrastruct Mol Struct Res 94:195–198

Backus GJ (1964) Morphogenesis of *Tedania gurianovae* Koltun (Porifera). Pac Sci 18:58–63

Baer KE von (1828) Über Entwicklungsgeschichte der Thiere. Bornträger, Koningsberg

Bagby RM (1966) The fine structure of myocytes in the sponges *Microciona prolifera* (Ellis and Sollander) and *Tedania ignis* (Duchassaing and Michelotti). J Morphol 118:167–182

Bagby RM (1970) The fine structure of pinacocytes in the marine sponge *Microciona prolifera* (Ellis and Solander). Zeitsch Zell 105:579–594

Baldacconi R, Nonnis-Marzano C, Gaino E, Corriero G (2007) Sexual reproduction, larval development and release in *Spongia offcinalis* L. (Porifera, Demospongiae) from the Apulian coast. Mar Biol 152:969–979

Balfour FM (1879) On the morphology and systematic position of the Spongida. Quart J Microsc Sci 19:103–109

Barbeau MA, Reiswig HM, Rath LC (1989) Hatching of freshwater sponge gemmules after low temperature exposure: *Ephydatia muelleri* (Porifera: Spongillidae). J Therm Biol 14:225–231

Barrois C (1876) Mémoire sur l'embryologie de quelques éponges de la Manche. Ann Sci Nat 11:1–84

Barthel D (1986) On the ecophysiology of the sponge *Halichondria panicea* in Kiel Bight. I. Substrate specificity, growth and reproduction. Mar Ecol Prog Ser 32:291–298

Barthel D, Detmer A (1990) The spermatogenesis of *Halichondria panicea* (Porifera, Demospongiae). Zoomorphology 110:9–15
Barthel D, Tendal OS (1993) The sponge association of the abyssal Norwegian-Greenland-Sea - species composition, substrate relationships and distribution. Sarsia 78:83–96
Batigina TB, Bragina EA, Ereskovsky AV, Ostrovsky AN (2006) Viviparity in plants and invertebrate animals. St. Petersburg University Press, St. Petersburg
Battershill CN, Bergquist PR (1990) The influence of storms on asexual reproduction, recruitment, and survivorship of sponges. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
Bavestrello G, Benatti U, Calcinai B, Catianeo-Vietti R, Cerrano C, Favre A, Giovine M, Lanza S, Pronzato R, Sarà M (1998) Body polarity and mineral selectivity in the demosponge *Chondrosia reniformis*. Biol Bull 195:120–125
Bavestrello G, Attilio A, Calcinai B, Cerrano C (2003) The aquiferous system casts of *Scolymastra joubini* (Porifera, Hexactinellida) studied by corrosion. Zoomorphology 122:119–123
Becerro MA, Uriz MJ, Turon X (1997) Chemically-mediated interactions in benthic organisms – the chemical ecology of *Crambe crambe* (Porifera, Poecilosclerida). Hydrobiologia 355:77–89
Beklemishev WN (1969) Promorphology, principles of comparative anatomy of invertebrates, vol 1. Oliver & Boyd, Edinburgh
Beloussov LV (1998) The dynamic architecture of a developing organism, Kluwer Acad. Publishers
Benayahu Y, Berner T, Achituv Y (1989) Development of planulae within a mesogleal coat in the soft coral *Heteroxenia fascescens*. Mar Biol 100:203–210
Bergquist PR (1968) The marine fauna of New Zeland. Porifera, Demospongiae. Part 1. (Tetractinomorpha and Lithistida). N Z Dep Sci Ind Res Bull 188:1–106
Bergquist PR (1978) Sponges. University of California Press, Los Angeles, CA
Bergquist PR (1979) Sponge chemistry – a review. In: Lévi C, Boury-Esnault N (eds) Biologie des spongiaires. Colloques Internationaux. CNRS 291, Paris
Bergquist PR (1980) A revision of the supraspecific classification of the orders Dictyoceratida, Dendroceratida, and Verongida (class Demospongiae). N Z J Zool 7:443–503
Bergquist PR (1985) Porifera relationships. In: Morris SC, George JD, Gibson R, Platt HM (eds) The origin and relationships of lower invertebrates. Clarendron, Oxford
Bergquist PR (1996) The marine fauna of New Zealand: Porifera, class Demospongiae. Part 5. Dendroceratida and Halisarcida. N Z Oceanogr Inst Mem 107:1–53
Bergquist PR, Glasgow K (1986) Developmental potential of ciliated cell of ceractinomorph sponge larvae. Exp Biol 45:111–122
Bergquist PR, Green CG (1977) An ultrastructural study of settlement and metamorphosis in sponge larvae. Cah Biol Mar18:289–302
Bergquist PR, Sinclair ME (1968) The morphology and behaviour of larvae of some intertidal sponges. N Z J Mar Freshwater Res 2:426–437
Bergquist PR, Wells RJ (1983) Chemotaxonomy of the Porifera. The development and current status of the field. In: Scheuer PJ (ed) Marine natural products chemical and biological perspectives. Academic, New York
Bergquist PR, Sinclair MR, Hogg JJ (1970) Adaptation to intertidal existence: reproductive cycles and larval behaviour in Demospongiae. Symp Zool Soc Lond 25:247–271
Bergquist PR, Sinclair ME, Green CR, Silyn-Roberts H (1979) Comparative morphology and behavior of larvae of Demospongiae. In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris
Bidder GP (1898) The skeleton and classification of calcareous sponge. Proc Roy Soc Lond 64:61–76
Bidder GP (1920) The fragrance of calcinean sponges and the spermatozoa of *Guancha* and *Sycon*. Linn Soc J Zool 34:299–327
Bigliardi E, Sciscioli M, Lepore E (1993) Interactions between prokaryotic and eukaryotic cells in sponge endocytobiosis. Endocyt Cell Res 9:215–221
Blumbach B, Pancer Z, Diehl-Seifert B, Steffen R, Munkner J, Müller I, Müller WEG (1998) The putative sponge aggregation receptor. J Cell Sci 111:2635–2644

Bohm M, Hentschel U, Friedrich AB, Fieseler L, Steffen R, Gamulin V, Müller I, Müller WEG (2001) Molecular response of the sponge *Suberites domuncula* to bacterial infection. Mar Biol 139:1037–1045

Bonasoro F, Wilkie IC, Bavestrello G, Cerrano C, Candia Carnavali MD (2001) Dynamic structure of the mesophyl in the sponge *Chondrosia reniformis* (Porifera, Demospongiae). Zoomorphology 121:109–121

Bond C (1992) Continuous cell movements rearrange anatomical structures in intact sponges. J Exp Zool 263:284–302

Bond C, Harris A (1988) Locomotion of sponges and its physical mechanism. J Exp Zool 246:271–284

Borchiellini C, Manuel M, Alivon E, Boury-Esnault N, Vacelet J, Le Parco Y (2001) Sponge paraphyly and the origin of Metazoa. J Evol Biol 244:171–179

Borchiellini C, Chombard C, Manuel M, Alivon E, Vacelet J, Boury-Esnault N (2004a) Molecular phylogeny of Demospongiae: implications for classification and scenarios of character evolution. Mol Phylog Evol 32:823–837

Borchiellini C, Alivon E, Vacelet J (2004b) The systematic position of *Alectona* (Porifera, Demosppongiae): a Tetractinellid sponge.Boll. Mus Ist Biol Univ Genova 68:209–217

Borojevic R (1966) Ètude expérimentale de la différenciation des cellules de l'éponge au cours de son développement. Dev Biol 14:130–153

Borojevic R (1967) La ponte et le développement de *Polymastia robusta* (Demosponges). Cah Biol Mar 8:1–6

Borojevic R (1969) Etude du développement et dé la différentiation cellulaire d'éponges calcaires Calcinées (genres *Clathrina* et *Ascandra*). Ann Embryol Morphol 2:15–36

Borojevic R (1970) Différenciation cellulaires dans l'émbryogenèse et la morphogenèse chez les Spongiaires. In: Fry WG (ed) The biology of the Porifera, vol 25. Symposium of the Zoological Society, London

Borojevic R, Lévi C (1964) Etude au microscope électronique des cellules de l'éponge: *Ophlitaspongia seriata* (Grant), au cours de la réorganization après dissociation. Z Zellforsch 64:708–725

Borojevic R, Lévi C (1965) Morphogenése expérimentale d'une éponge a partir de cellules de la larve negeante dissociee. Z Zellforsch Mikrosk Anat 68:57–69

Borojevic R, Peixinho S (1976) Eponges calcaires du nord-nord-est du Brésil. Bull Mus Nat Hist Nat Paris 402:987–1036

Boury-Esnault N (1970) Un phénomène de bourgeonnement externe chez l'éponge *Axinella damicornis* (Esper). Cah Biol Mar 11:491–496

Boury-Esnault N (1973) L'exopinacoderme des spongiaires. Bull Mus Nat Hist Nat Paris 178:1193–1206

Boury-Esnault N (1976) Ultrastructure de la larve parenchymella d'*Hamigera hamigera* (Shmidt) (Démosponge, Poecilosclerida). Origine des cellules grises. Cah Biol Mar 17:9–20

Boury-Esnault N (1977) A cell type in sponges involved in the metabolism of glycogen. Cell Tissue Res 175:523–539

Boury-Esnault N (2006) Systematics and evolution of Demospongiae. Can J Zool 84:205–224

Boury-Esnault N, De Vos L (1988) *Caulophacus cyanae* n. sp., une éponge hexactinellide des sources hydrothermales. Biogéographie du genre *Caulophacus* Schulze, 1887. Oceanol Acta 8:51–60

Boury-Esnault N, Doumenc D (1979) Glycogen storage and transfer in primitive invertebrates: Demospongea and Actiniaria. In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris

Boury-Esnault N, Jamieson BGM (1999) Porifera. In: Adiyodi KG, Adiyodi RG (eds) Reproductive biology of invertebrates. 9: Pt A Progress in male gamete biology. Oxford/IBH Publishing, New Delhi/Calcutta

Boury-Esnault N, Rützler K (eds) (1997) Thesaurus of sponge morphology. Smiths Contrib Zool 596:1–55

Boury-Esnault N, Vacelet J (1994) Preliminary studies on the organisation and development of a hexactinellid sponge from a Mediterranean cave, *Oopsacas minuta*. In: van Soest RWM, van

Kempen TMG, Braekman JC (eds) Sponges in time and space. Balkema, Rotterdam/ Amsterdam
Boury-Esnault N, De Vos L, Donadey C, Vacelet J (1984) Comparative study of the choanosome of Porifera: I The Homoscleromorpha. J Morphol 180:3–17
Boury-Esnault N, De Vos L, Donadey C, Vacelet J (1990) Ultrastructure of choanosome and sponge classification. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
Boury-Esnault N, Hajdu E, Klautau M, Custodio M, Borojevic R (1994) The value of cytological criteria in distinguishing sponges at the species level: the example of the genus *Polymastia*. Can J Zool 72:795–804
Boury-Esnault N, Muricy G, Gallissian MF, Vacelet J (1995) Sponges without skeleton: a new Mediterranean genus of Homoscleromorpha (Porifera, Demospongiae). Ophelia 43:25–43
Boury-Esnault N, Efremova SM, Bézak C, Vacelet J (1999) Reproduction of a hexactinellid sponge: first description of gastrulation by cellular delamination in the Porifera. Invert Rep Dev 35:187–201
Boury-Esnault N, Ereskovsky AV, Bezac C, Tokina DB (2003) Larval development in Homoscleromorpha (Porifera, Demospongiae) first evidence of basal membrane in sponge larvae. Invert Biol 122:187–202
Boute N, Exposito JY, Boury-Esnault N, Vacelet J, Noro K, Miyazaki K, Yoshigato K, Garrone R (1996) Type IV collagen in sponges, the missing link in basement membrane ubiquity. Biol Cell 88:37–44
Brasier M, Green O, Shiels G (1997) Ediacarian sponge spicule clusters from southwestern Mongolia and the origins of the Cambrian fauna. Geology 25:303–306
Bretting H, Jacobs G, Donadey C, Vacelet J (1983) Immunohistochemical studies on the occurrence and the function of the D-galactose-specific lectins in the tissue of the sponge *Axinella polypoides* Schmidt. Cell Tissue Res 229:551–572
Brien P (1932) Contribution à l'étude de la régénération naturelle chez les Spongillidae *Spongilla lacustris* (L.), *Ephydatia fluviatilis* (L.). Arch Zool exp gén 74:461–506
Brien P (1937) La réorganisation de l'éponge après la dissociation par filtration et phénomènes d'involution chez *Ephydatia fluviatilis*. Arch Biol Liège 48:185–268
Brien P (1943) L'embryologie des éponges. Bull Acad Roy Belg (Classe des Sci) 19:1–20
Brien P (1967a) Embryogenése de *Potamolepis stendelli* et *Spongilla moori.* Polyphylétisme des éponges d'eau douce. Bull Acad Roy Belg 53:752–757
Brien P (1967b) Eponges du Luapula et du lac Moero. Exp Hydrobiol Bangweolo-Luapula 11:1–53
Brien P (1967c) Les éponges: leur nature métazoaire – leur gastrulation – leur état colonial. Ann Soc Roy Zool Belg 97:197–235
Brien P (1969) A propos de deux éponges du Cameroun appartenant au genre *Corvospongilla*, embryogenése. Parenchymula. Gemmule. Rev Zool Afr 80:121–156
Brien P (1970) Les potamolepides africaines nouvelles du Luapula et du lac Moero. In: Fry WG (ed) The biology of the Porifera, vol 25. Symposium of the Zoological Society, London 25
Brien P (1972) Les feuillets embryonaires des Eponges. Bull Acad R Belg 5(Series 53):715–732
Brien P (1973a) Les Démosponges. In: Grasse PP (ed) Traite de Zoologie, 1:3, Maison Cie Paris
Brien P (1973b) *Malavispongia echinoides* Brien. Etudes complementaires. Histologie – sexualite – embryologie. Affinites systematiques. Rev Zool Bot Afr 87:50–76
Brien P, Meewis H (1938) Contribution à l'étude de l'embryogenèse des Spongillidae. Arch Biol Liège 49:177–250
Brill B (1973) Untersuchungen zur Ultrastruktur der Choanocyte von *Ephydatia fluviatilis* L. Z Zelforsch 144:231–245
Brusca GJ, Brusca RC, Gilbert SF (1997) Characteristics of metazoan development. In: Gilbert SF, Raunio AM (eds) Embryology. Constructing the organism. Sinauer, Sunderland, MA
Brusca RC and Brusca GJ (2003) Invertebrates. 2nd edition. Sinauer Associates, Sunderland, MA
Burlando B, Gaino E (1984) Actin and tubulin in dissociated sponge cells. Evidence for peculiar actin- containing microextensions. Eur J Cell Biol 35:317–321

Burton M (1931) The interpretation of the embryonic and post-larval characters of certain tetraxonid sponges with observations on post-larval growth stages in some species. Proc Zool Soc Lond 2:511–525
Burton M (1932) Sponges. Discov Rep 6:237–392
Burton M (1949a) Observation on the littoral sponges, including the supposed swarming of larvae, movement and coalescence in mature individuals, longivety and death. Proc Zool Soc Lond 118:893–915
Burton M (1949b) Non-sexual reproduction in sponges, with special reference to a collection of young *Geodia*. Proc Linn Soc Lond 160:163–178
Burton M (1959) Spongia. In: Friëriksson A, Tuxen SL (eds) The zoology of Iceland. Ejnar Munksgaard, Copenhagen/Reykjavik
Buscema M, de Sutter D, Van de Vyver G (1980) Ultrastructural study of differentiation processes during aggregation of purified sponge archaeocytes. Wilhelm Roux Arch Dev Biol 188:45–53
Buscema M, Van de Vyver G (1984a) Cellular aspects of alloimune reactionsin sponges of the genus *Axinella.* I. Axinella polypoides. J Exp Zool 229:7–17
Buscema M, Van de Vyver G (1984b) Cellular aspects of alloimune reactions in Sponges of the genus *Axinella.* II. *Axinella verrucosa* and *Axinella damicornis*. J Exp Zool 229:19–32
Buss L (1987) The Evolution of Individuality. Princeton University Press, Princeton, NJ
Bütchli O (1884) Bemerkungen zur Gastraea-Theorie. Morphol Jahrb 9:415–427
Byrne M (1995) Changes in larval morphology in the evolution of benthic development by *Patiriella exigua* (Asteroidea: Asterinidae), a comparison with the larvae of *Patiriella* species with planktonic development. Biol Bull 188:293–305
Byrne M, Hart MW, Cerra A, Cisternas P (2003) Reproduction and larval morphology of broadcasting and viviparous species in the *Cryptasterina* species complex. Biol Bull 205:285–294
Calarco PG (1975) Clevage (mouse). In: Hafez ES (ed) Scanning electron microscopic atlas of Mammalian reproduction. Igaku-Shoin, Tokyo
Carlson BM (1981) Patten's foundations of embryology, 4th edn. McGraw-Hill, New York
Carter HJ (1874) Development of the marine sponges from the earliest recognizable appearance of the ovum to the perfected individual. Ann Mag Nat Hist 4(321–337):389–406
Cavalier-Smith T, Allsopp MT, Chao EE, Boury-Esnault N, Vacelet J (1996) Sponge phylogeny, animal morphology, and the origin of the nervous system: 18S rRNA evidence. Can J Zool 74:2031–2045
Chen WT (1976) Reproduction and speciation in *Halisarca*. In: Harrison FW, Cowden RR (eds) Aspects of sponge biology. Academic, New York/London
Chen YH, Chen CP, Chang KH (1997) Budding cycle and bud morphology of the globe-shaped sponge *Cinachyra australiensis*. Zool Stud 36:194–200
Cherr G, Summers R, Baldwin J, Morrill J (1992) Preservation and visualisation of the Sea Urchin embryo blastocoelic extracellular matrix. Microsc Res Tech 22:11–22
Chombard C (1997) Les Démospongiae a asters: phylogénie moléculaire et homologie morphologique, Thèse doctor, Marseille
Chombard C, Boury-Esnault N (1999) Good congruence between morphology and molecular phylogeny of Hadromerida, or how to bother sponge taxonomists. Mem Queens Mus 44:100
Collins AG (1998) Evaluating multiple alternative hypotheses for the origin of bilateria: an analysis of 18S rRNA molecular evidence. Proc Natl Acad Sci USA 95:15458–15463
Connes R (1964) Contribution de l'étude de la prolifération par voie asexuée chez le *Sycon*. Bull Soc Zool France 89:188–195
Connes R (1966) Contribution a l'étude histologique des premiers stades d'embryogenése somatique chez *Tethya lyncurium* Lamarck. Bull Soc Zool France 91:639–645
Connes R (1967) Structure et développement des bourgeons chez l'Eponge siliceuse *Tethya lincurium* Lamarck. Arch Zool Exp Gen 108:157–195
Connes R (1968) Etude histologique, cytologique et expérimentale de la régénération et de la reproduction asexuée chez *Tethya lyncurium* Lamarck (= *T. aurantium* Pallas (Démosponges). Thesis University, Montpelliar, France

Connes R (1977) Contribution a l'Etude de la gemmulogenése chez la d'Eponge marine *Suberites domuncula* (Olivi) Nardo. Arch Zool Exp Gen 118:391–407
Connes R, Gil M (1985) Influence de facteurs externes sur le développement gemmulaire d'une démosponge marine. Vie et Milieu 35:49–55
Connes R, Diaz JP, Paris J (1971) Choanocytes et cellule centrale chez la Démosponge marine *Suberites massa* Nardo. C R Acad Sci Paris 273:1590–1593
Connes R, Diaz JP, Paris J (1972) Variations saisonnières des populations cellulaires de l'éponge *Suberites massa* Nardo. I.- Etude histologique et cytologique. Bull Mus Nat Hist Nat Paris 84:1013–1039
Connes R, Paris J, Artiges JM (1974) L'origine des cellules blastogénétiques chez *Suberites domuncula* (Olivi) Nardo. L'équilibre choanocytes-archeocytes chez les Spongiaires. Ann Sci Nat Biol Anim 16:111–118
Connes R, Carriere D, Paris J (1978) Etude du développement des gemmules chez la démosponge marine *Suberites domuncula* (Olivi) Nardo. Ann Sci Nat Zool Paris 20:357–387
Corriero G, Manconi R, Vacarro P, Pronzato R (1994) Life strategies of *Ephydatia fluviatilis* (L., 1758) in two different environments. In: van Soest RWM, van Kempen TMG, Braekman JC (eds) Sponges in time and space. Balkema, Rotterdam/Amsterdam
Corriero G, Sara M, Vaccaro P (1996) Sexual and asexual reproduction in two species of *Tethya* (Porifera, Demospongiae) from a Mediterranean coastal lagoon. Mar Biol 126:175–181
Corriero G, Scalera Liaci L, Marzano N, Gaino E (1998) Reproductive strategies of *Mycale contarenii* (Porifera: Demospongia). Mar Biol 131:319–328
Coutinho CC, Vissers S, Van de Vyver G (1994) Evidence of homeobox genes in the freshwater sponge *Ephydatia fluviatilis*. In: van Soest RWM, van Kempen TMG, Braekman JC (eds) Sponges in time and space. Balkema, Rotterdam/Amsterdam
Coutinho CC, Fonseca RN, Mansurea JJC, Borojevic R (2003) Early steps in the evolution of multicellularity: deep structural and functional homologies among homeobox genes in sponges and higher metazoans. Mech Dev 120:429–440
Coutinho CC, Maia AG (2007) Mesenchymal cells in ancestral spongiomorph urmetazoa could be the mesodermal precursor before gastrulation origin. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro 28
Curtis ASG (1962) Pattern and mechanism in the reaggregation of sponges. Nature 196:245–248
Custodio MR, Prokic I, Steffen R, Koziol C, Borojevic R, Brummer F, Nickel M, Müller WEG (1998) Primmorphs generated from dissociated cells of the sponge *Suberites domuncula*: a model system for studies of cell proliferation and cell death. Mech Ageing Dev 105:45–59
Custodio MR, Hajdu E, Muricy G (2002) *In vivo* study of microsclere formation in sponges of the genus *Mycale* (Demospongiae, Poecilosclerida). Zoomorphology 121:203–211
Custodio MR, Hajdu E, Muricu G (2004) Cellular dynamics of *in vitro* allogeneic reactions of *Hymeniacidon heliophila* (Demospongiae: Halichondrida). Mar Biol 144:999–1010
Cuvier G (1817) Le règne animal distributé d'après son organisation pour servir de base à l'histoire naturelle des animaux et d'introduction à l'anatomie comparée, vol. 2. Deterville, Paris, 540 p
Dahan M, Benayahu Y (1998) Embryogenesis, planulae longevity, and competence in the octocoral *Dendronephthya hemprichi*. Invert Biol 117:271–280
Davidson EH (2001) Genomic regulatory systems: development and evolution. Academic, San Diego, CA
de Caralt S, Uriz MJ, Ereskovsky AV, Wijffels RH (2007a) Embryo development of *Corticium candelabrum* (Demospongiae: Homosclerophorida). Invert Biol 126:211–219
de Caralt S, Uriz MJ, Wijffels RH (2007b) Vertical transmission and successive location of symbiotic bacteria during embryo development and larva formation in *Corticium candelabrum* (Porifera: Demospongiae). J Mar Biol Ass UK 87:1693–1699
de Cuevas M, Lilly MA, Spradling AC (1997) Germline cyst formation in *Drosophila*. Annu Rev Genet 31:405–428

Degnan BM, Degnan SM, Giusti A, Morse DE (1995) A hox hom homeobox gene in sponges. Gene 155:175–177
Degnan BM, Leys SP, Larroux C (2005) Sponge development and antiquity of animal pattern formation. Integr Comp Biol 45:335–341
Delage Y (1892) Embryogenése des éponges silicieuses. Arch Z Exp Gen 10:345–498
Delage Y (1899) On the position of sponges in the animal kingdom. In: Sedgwick (ed) Proceedings of the fourth International Congress of Zoology. Clay &Sons, Cambridge
Dendy A (1889) Preliminary notes on the structure and development of a horny sponge (*Stelospongus flabelliformis*). Proc Roy Soc Victoria 1:1–11
Dendy A (1891) Studies on the comparative anatomy of sponges. III. On the anatomy of *Grantia labyrinthica*, and the so-called family Teichonidae. Quart J Microsc Sci 32:1–39
Dendy A (1915) Observations on the gametogenesis of *Grantia compressa*. Quart J Microsc Sci 60:313–376
Dendy A, Frederick LM (1924) On a collection of sponges from the Abrolhos Islands, Western Australia. J Linn Soc Lond Zool 35:477–519
Denis H, Mignot J-P (1994) L'origine des métazoaires. Méd Sci 10:551–563
De Vos L (1965) Le bougeonnement externe de l'Eponge *Mycale contarenii* Martens. Bull Mus Hist Nat Paris 37:534–555
De Vos L (1971) Etude ultrastructurale de la gemmulogenese chez *Ephydatia fluviatilis*. J Microsc 10:283–304
De Vos L (1977) Etude au microscope électronique a balayage des cellules de l'éponge *Ephydatia fluviatilis*. Arch Biol 88:1–14
De Vos L, Van de Vyver G (1981) Etude de la contraction spontanée chez l'éponge d'eau douce *Ephydatia fluviatilis* cultivée *in vitro*. Ann Soc Roy Zool Belg 111:21–31
De Vos L, Boury-Esnault N, Vacelet J (1990) The apopylar cell of sponges. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
De Vos L, Rutzler K, Boury-Esnault N, Donadey C, Vacelet J (1991) Atlas de morphologie des Eponges – atlas of sponge morphology. Smithsonian Institution Press, Washington, DC
Dewell RA (2000) Colonial origin for Eumetazoa: major morphological transitions and the origin of bilaterian complexity. J Morphol 243:35–74
Diaz JP (1973) Cycle sexuel de deux démosponges de l'étang de Thau: *Suberites massa* Nardo et *Hymeniacidon caruncula* Bowerbank. Bull Soc Zool France 98:145–156
Diaz JP (1974) De l'origine de certains endopinacocytes à partir de choanocytes chez la démosponge *Suberites massa* Nardo. Bull Soc Zool France 99:687–696
Diaz JP (1979) Variations, différentiations et functions des categories cellulaires de la démosponge d'eaux saumatres, *Suberites massa*, Nardo, au cours du cycle biologique annuel et dans des conditions expérimentales. Thèse doct. Acad Montpellier
Diaz JP, Connes R, Paris J (1973) Etude ultrastructurale de l'ovogenèse d'une Démosponge: *Suberites massa* Nardo. J Microsc 24:105–116
Diaz JP, Connes R, Paris J (1975) Etude ultrastructurale de l'ovogenèse d'une Démosponge: *Suberite massa* Nardo. J Microsc 24:105–116
Diaz JP, Connes R (1980) Etude ultrastructuralè de la spermatogenèse d'une Démosponge. Biol Cell 38:225–230
Dohrmann M, Voigt O, Erpenbeck D, Wörheide G (2006) Non-monophyly of most supraspecific taxa of calcareous sponges (Porifera, Calcarea) revealed by increased taxon sampling and partitioned Bayesian analysis of ribosomal DNA. Mol Phyl Evol 40:830–843
Dohrmann M, Janussen D, Reitner J, Collins AG, Wörheide G (2008) Phylogeny and evolution of glass sponges (Porifera, Hexactinellida). Syst Biol 57:388–405
Donadey C (1978) Origine choanocytaire des cellules a inclusions de l'éponge *Plakina trilopha* Schulze (Démosponge Homosclérophoride). C R Acad Sci Paris 286:519–521
Donadey C (1979) Contribution a l'étude cytologique de deux Démosponges Homosclérophorides: *Oscarella lobularis* (Schmidt) et *Plakina trilopha* Schulze. In: Lévi C, Boury-Esnault N (eds) Biologie des spongiaires. Colloques Internationaux. CNRS 291, Paris

Donadey C (1982) Les cellules pigmentaires et les cellules à inclusions de l'éponge *Cacospongia scalaris* (Demosponge Dictyoceratide). Vie Mar 4:67–74
Donadey C, Vacelet J (1977) Les cellules â inclusions de l'Eponge *Pleraplysilla spinifera* (Schulze) (Demospongiae, Dendroceratides). Arch Zool Exp Gén 118:273–284
Duboscq O, Tuzet O (1932) Sur la fécondation de *Sycon ciliatum* Lieberkühn. C R Acad Sci Paris 109:829
Duboscq O, Tuzet O (1933a) Sur l'ovogenèse et la fécondation des éponges calcaires: *Grantia compressa pennigera* Haeckel et *Sycon cilatum* Lieberkhün. Arch Zool Exp Gén 73:45–56
Duboscq O, Tuzet O (1933) Quelques structures des amphiblastules d'éponges calcaires. C R Acad Sci Paris 197:561–563
Duboscq O, Tuzet O (1935a) Un nouveau stade du développement des éponges calcaires. C R Acad Sci Paris 200:1788
Duboscq O, Tuzet O (1935b) Sur l'accroissement des ovocytes de *Sycon raphanus* O. S.: la signification des «dolly-cells». Arch Zool Exp Gén 77:71–78
Duboscq O, Tuzet O (1936) Sur quelques points de l'ovogenèse et du développement des éponges calcaires. Comptes rendus du XIIe Congrès International de Zoologie, Lisbonne, 1935. Casa Portuguesa, Lisboa
Duboscq O, Tuzet O (1937) L'ovogenèse, la fécondation et les premiers stades du développement des éponges calcaires. Arch Zool Exp Gén 79:157–316
Duboscq O, Tuzet O (1938) L'origine et l'évolution des cellules en croix des éponges calcaires. Trav Stat Zool Wimereux 13:267–277
Duboscq O, Tuzet O (1941) Sur les cellules en croix des *Sycon* (*Sycon ciliatum* Fabr., *Sycon coronatum* Ellis et Sol., *Sycon elegans* Bower.) et leur signification. Arch Zool Exp Gén 81:151–163
Duboscq O, Tuzet O (1942) Recherches complémentaires sur l'ovogenèse, la fécondation et les premiers stades du développement des éponges calcaires. Arch Zool Exp Gén 81:395–466
Duboscq O, Tuzet O (1944) L'ovogenèse, la fécondation et les premiers stades du développement de *Sycon elegans* Bower. Arch Zool Exp Gén 83:445–459
Eerkes-Medrano D, Leys SP (2006) Ultrastructure and embryonic development of a syconoid calcareous sponge. Invert Biol 125:177–194
Efremova SM (1969) The morphological analysis of the development of the sponge *Ephydatia fluviatilis* from the dissociated cells. Vest Leningr Univ 9:39–53
Efremova SM (1970) Proliferation activity and synthesis of protein in the cells of fresh-water sponges during development after dissociation. In: Fry WG (ed) The biology of the Porifera, vol 25. Symposium of the Zoological Society, London
Efremova SM (1972) Morphophysiological analysis of the development of freshwater sponges *Ephydatia fluviatilis* and *Spongilla lacustris* from the cells after Dissociation. In: Tokin BP (ed) Asexual reproduction, somatic embryogenesis and regeneration. Leningrad State University, Leningrad
Efremova SM (1981) The structure and embryogenesis of the Baikal sponge *Lubomirskia baicalensis* (Pallas) and relations of Lubomirskiidae with other sponges. In: Korotkova GP (ed) Morphogenesis in sponges. Leningrad University Press, Leningrad
Efremova SM (1997) Once more on the position among Metazoa – Gastrulation and germinal layers of sponges. In: Ereskovsky AV, Keupp H, Kohring R (eds) Modern problems of Poriferan biology. Eds Freie University, Berlin
Efremova SM, Efremov VI (1979) Proliferation cellulaire dans l'embryogenese de *Baikalospongia bacillifera.* In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris
Efremova SM, Papkovskaya MV (1980) Spermatogenesis of the sponge *Lubomirskia baikalensis* (Pallas) ultrastructural investigation. Arch Anat Histol Embryol 79:88–95
Efremova SM, Ereskovsky AV, Tokina DB (1987a) Gametogenesis in sponges of the family Myxillidae from the White Sea. 1. Oogenesis in *Myxilla incrustans* and *Iophon piceus* (Demospongiae, Poecilosclerida). Ontogenez 3:257–262

Efremova SM, Ereskovsky AV, Tokina DB (1987b) Gametogenesis in sponges of the family Myxillidae from the White Sea. 2. Spermatogenesis in *Myxilla incrustans* and *Iophon piceus* (Demospongia, Poecilosclerida). Ontogenez 3:263–268

Efremova SM, Ereskovsky AV, Mukhina YI, Willenz P, Boury-Esnault N (2006) The peculiarities of spermatogenesis in the Homoscleromorpha. In: Hajdu E (ed) 7th International Sponge Symposium. Biodiversity, Innovation, Sustainability. Rio de Janeiro. Ser. Livros 16, Buzios Brazil

Elliott GRD, Macdonald TA, Leys SP (2004) Sponge larval phototaxis: a comparative study. In: Pansini M, Pronzato R, Bavestrello G, Manconi R (eds) Sponge science in the new millennium. Boll Mus Ist Biol Univ Genova, 68

Elvin DV (1976) Seasonal growth and reproduction of an intertidal sponge *Haliclona permolis* (Bow.). Biol Bull 151:108–125

Enticknap JJ, Kelly M, Peraud O, Hill RT (2006) Characterization of a culturable alphaproteobacterial symbiont common to many marine sponges and evidence for vertical transmission via sponge larvae. Appl Environ Microb 72:3724–3732

Ereskovsky AV (1985) Oogenesis of white sea sponge *Iophon piceus* (Demospongiae, Poecilosclerida). In: Cherepanov G (ed) The organism in onto- and phylogenesis. VINITI, Moscow

Ereskovsky AV (1986) Larval formation in *Iophon piceus* (Demospongiae, Poecilosclerida). Zool Zh 65:1614–1621

Ereskovsky AV (1993) Materials to the faunistic study of the White Sea and Barents Sea sponges. I Taxonomic composition. Vestnik St Petersburg Univ 3(17):19–28

Ereskovsky AV (1994) On the population structure and distribution of sponges in the tidal zone of Eastern Murman. Zool Zh 73:5–17

Ereskovsky AV (1999) Development of sponges of the order Haplosclerida. Russ J Mar Biol 25:361–371

Ereskovsky AV (2000) Reproduction cycles and strategies of cold-water sponges *Halisarca dujardini* (Demospongiae, Dendroceratida), *Myxilla incrustans* and *Iophon piceus* (Demospongiae, Poecilosclerida) from the White Sea. Biol Bull 198:77–87

Ereskovsky AV (2002) Polyaxial cleavage in sponges (Porifera): a new pattern of metazoan cleavage. Doklady Biol Sci 386:472–474

Ereskovsky AV (2003) Problems of coloniality, modularity, and Individuality in sponges and special features of their morphogeneses during growth and asexual reproduction. Russ J Mar Biol 19:46–56

Ereskovsky AV (2004) Comparative embryology of sponges and its application for sponge phylogeny. In: Pansini M, Pronzato R, Bavestrello G, Manconi R (eds) Sponge science in the new millennium. Boll Mus Ist Biol Univ Genova, 68

Ereskovsky AV (2005) Comparative embryology of sponges (Porifera). St. Petersburg University Press, St. Petersburg

Ereskovsky AV (2006) A new species of *Oscarella* (Demospongiae: Plakinidae) from the Western Sea of Japan. Zootaxa 1376:37–51

Ereskovsky AV (2007a) A new species of *Halisarca* (Demospongiae: Halisarcida) from the Sea of Okhotsk, North Pacific. Zootaxa 1432:57–66

Ereskovsky AV (2007b) Sponge embryology: the past, the present and the future. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro 28

Ereskovsky AV, Boury-Esnault N (2002) Cleavage pattern in *Oscarella* species (Porifera, Demospongiae, Homoscleromorpha), transmission of maternal cells and symbiotic bacteria. J Nat Hist 36:1761–1775

Ereskovsky AV, Dondua AK (2006) The problem of germ layers in sponges (Porifera) and some issues concerning early metazoan evolution. Zool Anzeig 245:65–76

Ereskovsky AV, Gonobobleva EL (2000) New data on embryonic development of *Halisarca dujardini* Johnston, 1842 (Demospongiae: Halisarcida). Zoosystema 22:355–368

Ereskovsky AV, Korotkova GP (1997) The reasons of sponge sexual morphogenesis peculiarities. In: Ereskovsky AV, Keupp H, Kohring HR (eds) Modern problems of Poriferan biology. Berliner Geowiss Abh, Freie University, Berlin

Ereskovsky AV, Korotkova GP (1999) On the reasons of onthogenesis pecularity in sponges. Zh Obsh Biol 60:318–332

Ereskovsky AV, Tokina DB (2004) Morphology and fine structure of the swimming larvae of *Ircinia oros* (Porifera, Demospongiae, Dictyoceratida). Invert Rep Dev 45:137–150

Ereskovsky AV, Tokina DB (2007) Asexual reproduction in homoscleromorph sponges (Porifera; Homoscleromorpha). Mar Biol 151:425–434

Ereskovsky AV, Willenz P (2007) *Esperiopsis koltuni* sp. nov. (Demospongiae: Poecilosclerida: Esperiopsidae), a carnivorous sponge from deep water of the Sea of Okhotsk (North Pacific). J Mar Biol Ass UK 87:1379–1386

Ereskovsky AV, Willenz P (2008) Larval development in *Guancha arnesenae* (Porifera, Calcispongiae, Calcinea). Zoomorphology 127:175–187

Ereskovsky AV, Gonobobleva EL, Vishnyakov AE (2005) Morphological evidence for vertical transmission of symbiotic bacteria in the viviparous sponge *Halisarca dujardini* Johnston (Porifera, Demospongiae, Halisarcida). Mar Biol 146:869–875

Ereskovsky AV, Tokina DB, Bézac C, Boury-Esnault N (2007a) Metamorphosis of cinctoblastula larvae (Homoscleromorpha, Porifera). J Morphol 268:518–528

Ereskovsky AV, Konjukov P, Willenz P (2007b) Experimental metamorphosis of *Halisarca dujardini* larvae (Demospongiae, Halisarcida): evidence of flagellated cell totipotentiality. J Morphol 268:529–536

Ereskovsky AV, Borchiellini C, Gazave E, Ivanisevic J, Lapebie P, Perez T, Renard E, Vacelet J (2009a) The Homoscleromorph sponge *Oscarella lobularis*, a promising sponge model in evolutionary and developmental biology. BioEssays 31:89–97

Ereskovsky AV, Konyukov PY, Tokina DB (2009b) Morphogenesis accompanying larval metamorphosis in *Plakina trilopha* (Porifera, Homoscleromorpha). Zoomorphology 129:21–31

Erpenbeck D, van Soest RWM (2002) Family Halichondriidae Gray, 1867. Hooper JNA, van Soest RWM (eds) Systema Porifera: a guide to the classification of sponges. Kluwer/Plenum, New York

Erpenbeck D, Worheide G (2007) On the molecular phylogeny of sponges (Porifera). Zootaxa 1668:107–126

Erpenbeck D, Breeuwer JAJ, van Soest RWM (2005) Implications from a 28S rRNA gene fragment for the phylogenetic relationships of halichondrid sponges (Porifera: Demospongiae). J Zool Syst 43:93–99

Erpenbeck D, Breeuwer JAJ, Parra FJ, van Soest RWM (2006) Speculation with spiculation? – three independent gene fragments and biochemical characters versus morphology in demosponge higher classification. Mol Phyl Evol 38:293–305

Erwin DH (1993) The origin of metazoan development: a palaeobiological perspective. Biol J Linn Soc 50:255–274

Ettinger-Epstein P, Whalan SW, Battershill CN, de Nys R (2007) Temperature cues gametogenesis and larval release in a tropical sponge. Mar Biol 153:171–178

Evans CW (1977) The ultrastructure of larvae from the marine sponge *Halichondria moorei* Bergquist (Porifera, Demospongiae). Cah Biol Mar 18:427–433

Exposito JY, Garrone R (1990) Characterization of a fibrillar collagen gene in sponges reveals the early evolutionary appearance of two collagen gene families. PNAS 87:6669–6673

Exposito JY, Cluzel C, Garrone R, Lethias C (2002) Evolution of collagens. Anat Rec 268:302–316

Exposito JY, Larroux C, Cluzel C, Valcourt U, Lethias C, Degnan BM (2008) Demosponge and sea anemone fibrillar collagen diversity reveals the early emergence of A/C clades and the maintenance of the modular structure of type V/XI collagens from sponge to human. J Biol Chem 283:28226–28235

Faure-Fremiet ME (1931) Etude histologique de *Ficulina ficus* L. (Demospongia). Arch Anat Microsc Morph Exp 27:421–448

Fauré-Fremiet ME (1932) Morphogénèse expérimentale (reconstitution) chez *Ficulina ficus* L. Arch Anat Microsc Morph Exp 28:1–80

Fawcett DW (1994) Bloom and Fawcett, a textbook of histology, 12th edn. Chapman & Hall, New York

Fedotov DM (1966) Evolution and phylogeny of invertebrates. Nauka, Moscow
Feige W (1969) Die Feinstruktur der epithelien von *Ephydatia fluviatilis*. Zool Jahrb 86:177–237
Fèlix MA (1999) Evolution of developmental mechanisms in nematodes. J Exp Zool (Mol Dev Evol) 285:3–18
Fell PE (1969) The involvement of nurse cells in oogenesis and embryonic development in the marine sponge *Haliclona ecbasis*. Morphology 127:133–150
Fell PE (1974a) Porifera. In: Giese AC, Pearse JS (eds) Reproduction of marine invertibrates. Academic, New York
Fell PE (1974b) Diapause in the gemmules of the marine sponge *Haliclona loosanoffi*, with a note on the gemmules of *Haliclona oculata*. Biol Bull 147:333–351
Fell PE (1976) The reproduction of *Haliclona loosanoffi* and its apparent relationschip to water temperature. Biol Bull 150:200–210
Fell PE (1983) Porifera. In: Adiyodi KG, Adiyodi RG (eds) Reproductive biology of Invertebrates, vol 1. Oogenesis, oviposition and oosorption. Wiley, Chichester
Fell PE (1989) Porifera. In: Adiyodi KG, Adiyodi RG (eds) Reproductive biology of Invertebrates, vol 4. Part A: fertilization, development and parental care. Oxford/IBH Publishing, Calcutta
Fell PE (1993) Porifera. In: Adiyodi KG, Adiyodi RG (eds) Reproductive biology of invertebrates, vol. 6. Asexual propagation and reproductive strategies. Wiley, Chichester
Fell PE, Bazer LJ (1990) Survival of the gemmules of *Anheteromeyenia ryderi* (Potts) following aerial exposure during winter in New England. Hydrobiology 190:241–246
Fell PE, Jacob WF (1979) Reproduction and development of *Halichondria* sp. in the Mystic Estuary, Connecticut. Biol Bull 155:62–75
Fell PE, Lewandrowski KB, Lovice M (1979) Postlarval reproduction and reproductive strategy in *Haliclona loosanoffi* and *Halichondria* sp. In: Lévi C, Boury-Esnault N (eds) Biologie des spongiaires. Colloques Internationaux. CNRS 291, Paris
Fernandez-Busquets X (2008) The sponge as a model of cellular recognition. In: Conn PM (ed) Source book of models for biomedical research. Humana Press, Totowa, NJ
Fernandez-Busquets X, Burger MM (1999) Cell adhesion and histocompatibility in sponges. Microsc Res Tech 44:204–218
Fioroni P (1979) Abanderungen des gastrulationsverlauf und ihre phylogenetische Bedeutung. Ztschr Zool Syst Evol 1:101–119
Franzén Å (1970) Phylogenetic aspects of the morphology of spermatozoa and spermiogenesis. In: Baccetti B (ed) Comparative spermatology. Academic, New York
Franzen W (1988) Oogenesis and larval development of *Scypha ciliata* (Porifera, Calcarea). Zoomorphology 107:349–357
Fromont J (1988) Aspects of the reproductive biology of *Xestospongia testidinaria* (Great Barrier Reef). In: Choat JH, Barnes D, Borowitzka MA (eds) Proceedings of the 6th International Coral Reef Symposium, vol 2, pp 685–691. James Cook Univ North Queensl, Townsville, Australia
Fromont J (1994) Reproductive development and timing of tropical sponges (Order Haplosclerida) from the Great Barrier Reef, Australia. Coral Reefs 13:127–133
Fromont J (1999) Reproduction of some demosponges in a temperate Australian shallow water habitat. Mem Queensl Mus 44:185–192
Fromont J, Bergquist PR (1994) Reproductive biology of three sponges species of the genus *Xestospongia* (Porifera: Demospongiae: Petrosiida) from the Great Barrier Reef. Coral Reefs 13:119–126
Frost TM, DeNagy GS, Gilbert JJ (1982) Population dynamics and standing biomass of the freshwater sponge *Spongilla lacustris*. Ecology 63:1203–1210
Fuerst JA, Webb RI, Garson MJ, Hardy L, Reiswig HM (1999) Membrane-bounded nuclear bodies in a diverse range of microbial symbionts of Great Barrier Reef sponges. Mem Queensl Mus 44:193–203
Gaino E (1980) Indagine ultrastrutturale sugli ovociti maturi di *Chondrilla nucula* Schmidt (Porifera, Demospongiae). Cah Biol Mar 21:11–22

Gaino E, Burlando B (1990) Sponge cell motility: a model system for the study of morphogenetic processes. Boll Zool 57:109–118

Gaino E, Magnino G (1998) Exogenous cyclic AMP (cAMP) and dibutyryl-cyclic AMP (DB-cAMP) induce in vitro morphological variations in the choanocytes of *Clathrina cerebrum* (Porifera, Calcarea). In: Watanabe Y, Fusetani N (eds) Sponge sciences. Multidisciplinary perspectives. Springer, Tokyo

Gaino E, Sarà M (1994) An ultrastructural comparative study of the eggs of two species of *Tethya* (Porifera, Demospongiae). Invert Rep Dev 26:99–106

Gaino E, Pansini M, Pronzato R (1977) Aspetti dell' associazione tra *Chondrilla nucula* (Demospongiae) e microorganismi simbionti (Batteri e Cianoficee) in condizioni naturali e sperimentali. Cah Biol Mar 18:303–310

Gaino E, Burlando B, Zunino L, Pansini M, Buffa P (1984) Origin of maile gametes from choanocytes in *Spongia officinalis* (Porifera, Demospongiae). Int J Invert Rep Dev 7:83–93

Gaino E, Burlando B, Buffa P (1986a) The vacuolar cells of *Oscarella lobularis* (Porifera, Demospongiae): ultrastructural organisation, origin and function. J Morphol 188:29–37

Gaino E, Burlando B, Buffa P (1986b) Contribution to the study of egg development and derivation in *Oscarella lobularis* (Porifera, Demospongiae). Int J Invert Rep Dev 91:297–305

Gaino E, Burlando B, Buffa P, Sarà M (1986c) Ultrastructural study of spermatogenesis in *Oscarella lobularis* (Porifera, Demospongiae). Int J Invert Rep Dev 10:297–305

Gaino E, Burlando B, Buffa P (1987a) Structural and ultrastructural aspects of growth in *Oscarella lobularis* (Porifera, Demospongiae). Growth 51:451–460

Gaino E, Burlando B, Buffa P (1987b) Ultrastructural study of oogenesis and fertilization in *Sycon ciliatum* (Porifera, Calcispongiae). Int J Invert Rep Dev 12:73–81

Gaino E, Burlando B, Buffa P, Sarà M (1987c) Ultrastructural study of the mature egg of *Tethya citrina* Sarà and Melone (Porifera, Demospongiae). Gamet Res 16:259–265

Gaino E, Manconi R, Pronzato R (1995) Organizational plasticity as a successful conservative tactics in sponges. Anim Biol 4:31–43

Gaino E, Rebora M, Corallini C, Lancioni T (2003) The life-cycle of the sponge *Ephydatia fluviatilis* (L.) living on the reed *Phragmites australis* in an artificially regulated lake. Hydrobiologia 495:127–142

Gaino E, Scalera Liaci L, Sciscioli M, Corriero G (2006) Investigation of the budding process in *Tethya citrina* and *Tethya aurantium* (Porifera, Demospongiae). Zoomorphology 125:87–97

Gaino E, Baldacconi R, Corriero G (2007) Post-larval development of the commercial sponge *Spongia officinalis* L. (Porifera, Demospongiae). Tissue Cell 39:325–334

Galera J, Turton X, Uriz MJ, Becerro M (2000) Microstructure variation in sponges sharing growth form: the encrusting demosponges *Dysidea fragilis* and *Crambe crambe*. Acta Zool 81:93–107

Gallissian MF (1980) Ultrastructural study of fertilization in *Grantia compressa* F. Int J Invert Rep 2:321–329

Gallissian MF (1981) Etude ultrastructurale de l'ovogenèse chez quelques éponges calcaires (Porifera, Calcarea). Arch Zool Exp Gén 122:329–340

Gallissian MF (1983) Etude ultrastructurale du développement embryonaire chez *Grantia compressa* F. (Porifera, Calcarea). Arch Anat Microsc Morph Exp 1:59–75

Gallissian MF (1988) Etude ultrastructurale de l'ovogénèse et de la fécondation chez *Leucilla endoumensis* (Spongiaire, Calcarea). C R Acad Sci Paris 306:245–252

Gallissian MF (1989) Le spermiokyste de *Sycon sycandra* (Porifera, Calcarea): l'étude ultrastructurale. C R Acad Sci Paris 309:251–258

Gallissian MF, Vacelet J (1976) Ultrastructure de quelques stades de l'ovogenése de spongiaires du genre *Verongia* (Dictyoceratida). Ann Sci Nat Zool Biol Anim Ser 12 18:381–404

Gallissian MF, Vacelet J (1990) Fertilization and nutrition of the oocyte in the calcified sponge *Petrobiona massiliana*. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC

Gallissian MF, Vacelet J (1992) Ultrastructure of the oocyte and embryo of the calcified sponge, *Petrobiona massiliana* (Porifera, Calcarea). Zoomorphology 112:133–141

Garrone R (1974) Ultrastructure d'une «gemmule armee» planctonique d'Eponge clionidae. Arch Anat Micros Morph Exp 63:163–182

Garrone R (1978) Phylogenesis of connective tissue. Morphological aspects and biosynthesis of sponge intercellular matrix. Front Matrix Biol 5:1–250

Garrone R (1985) The collagen of the Porifera. In: Bairati A, Garrone R (eds) Biology of invertebrate and lower vertebrate collagens. Plenum, New York/London

Garrone R, Lethias C (1990) Freeze-fracture study of sponge cells. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC

Garrone R, Pottu J (1973) Collagen biosynthesis in sponges: elaboration of spongin by spongocytes. J Submicros Cytol 5:199–218

Garrone R, Rozenfeld F (1981) Electron microscope study of cell differentiation and collagen synthesis in hydroxyurea-treated fresh-water sponges. J Submicrosc Cytol 13:127–134

Garrone R, Vacelet J, Pavans de Ceccatty M, Junqua S, Robert L (1973) Une formation collagene particuliere: les filaments des Eponges cornées *Ircinia*. Etude ultrastructurale, physicochimique et biochimique. J Microsc 17:241–260

Garrone R, Simpson TL, Pottu-Boumendil J (1981) Ultrastructure and deposition of silica in sponges. In: Simpson TL, Volcani BE (eds) Silicon and siliceous structures in biological systems. Springer, Berlin

Gatenby JB (1920a) Further notes on the oogenesis and fertilization of *Grantia compressa.* J R Microsc Soc 40:277–282

Gatenby JB (1920b) The germ-cells, fertilization, and early development of *Grantia* (*Sycon*) *compressa.* J Linn Soc Zool 34:291–297

Gatenby JB (1927) Further notes on the gametogenesis and fertilization of sponges. Quart J Microsc Sci 71:173–188

Gazave E, Lapébie P, Renard E, Bézac C, Boury-Esnault N, Vacelet J, Pérez T, Manuel M, Borchiellini C (2008) NK homeobox genes with choanocyte-specific expression in homoscleromorph sponges. Dev Genes Evol 218:479–489

Gerasimova EI, Ereskovsky AV (2007) Reproduction of two species of *Halichondria* (Demospongiae: Halichondriidae) in the White Sea. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro 28

Giard A (1873) Contribution à l`histoire naturelle des synascidies. Arch Zool Exper 2:401–514

Gilbert SF (2006) Developmental biology. Sinauer, Sunderland, MA

Gilbert JJ, Hadzisce S (1977) Life cycle of the freshwater sponge *Ochridaspongia rotunda* Arndt. Arch Hydrobiol 79:285–318

Gilbert SF, Raunio AM (eds) (1997) Embryology. Constructing the organism. Sinauer, Sunderland, MA

Gonobobleva EL (2007) Basal apparatus formation in external flagellated cells of *Halisarca dujardini* larvae (Demospongiae: Halisarcida) in the course of embryonic development. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro 28

Gonobobleva EL, Ereskovsky AV (2004a) Metamorphosis of the larva of *Halisarca dujardini* (Demospongiae, Halisarcida). Bull Inst Roy Sci Nat Belg Biol 74:101–115

Gonobobleva EL, Ereskovsky AV (2004b) Polymorphism in free-swimming larvae of *Halisarca dujardini* (Demospongiae, Halisarcida). In: Pansini M, Pronzato R, Bavestrello G, Manconi R (eds) Sponge science in the new millennium. Boll Mus Ist Biol Univ Genova, 68

Görich W (1903) Zur Kenntniss der Spermatogenese bei den Poriferen und Coelenteraten. Zool Anz 27:64–70

Görich W (1904) Zur Kenntniss der Spermatogenese bei den Poriferen und Coelenteraten nebst Bemerkungen uber die Oogenese der ersteren. Z Wiss Zool 76:522–543
Grant RE (1825) Observations and experiments on the structure and functions of the sponge. Edinb Phil J 13(94–107):333–346
Grasshoff M, Gudo M (2002) The origin of the Metazoa and the main evolutionary lineages of the animal kingdom: the Gallertoid hypothesis in the light of modern research. Senckenberg Lethaea 82:295–314
Green CR, Bergquist P (1979) Cell membrane specializations in the Porifera. In: Lévi C, Boury-Esnault N (eds) Biologie des spongiaires. Colloques Internationaux. CNRS 291, Paris
Grothe F (1989) On the Phylogeny of the Homoscleromorpha. Berliner Geowiss Abh (A) 106:155–164
Grothe F, Reitner J (1988) Discussion on the phylogenetic position of the Homoscleromorpha (Porifera). Berliner Geowiss Abh 100:16–17
Gureeva MA (1972) "Sorites" and oogenesis in the Baikal endemic sponges. Tsitologia 14:32–45
Hadzi J (1917) Rezultati bioloskih istrazivanja Jadranskog Mora. Porifera. Calcarea I. *Clathrina blanca* (Miklucho-Maclay). Prirod Istraziv Jugoslav Akad Znaon Umjet Zagreb 9–10:1–164
Hadži J (1963) The evolution of the Metazoa. Pergamon, London
Haeckel E (1871) Ueber die sexuelle fortpflanzung und das naturliche system der Schwämme. Jena Zer 6:641–651
Haeckel E (1872) Die Kalksschwämme, Verlag von Georg Reimer, Berlin
Haeckel E (1874) Die Gastrae Theorie, die phylogenetische Classification des Thierreichs und die Homologie der Keimblatter. Jiena Ztschr Natur 8:1–55
Hahn-Keser B, Stockem W (1997) Detection of distinct endocytotic and phagocytotic activities in epithelial cells (pinacocytes) of freshwater sponges (Porifera, Spongillidae). Zoomorphology 117:121–134
Hall BK (1998) Evolutionary developmental biology, 2nd edn. Kluwer, Amsterdam
Hammer E (1906) Zur Kenntnis von *Hircinia variabilis*. Naturforsch Freunde 6:1–6
Hammer E (1908) Neue beiträge zur Kenntnis der Histologie und Entwicklung von *Sycon raphanus*. R. Friedländer & Sohn, Berlin
Harrison FW (1972a) Phase contrast photomicrography of cellular behaviour in spongillid porocytes (Porifera: Spongillidae). Hydrobiologia 40:513–517
Harrison FW (1972b) The nature and role of the basal pinacoderm of *Corvomeyenia carolinensis* Harrison (Porifera: Spongillidae). A histochemical and developmental study. Hydrobiologia 39:495–508
Harrison FW, Cowden RR (1975) Cytochemical observations of larval development in *Eunapius fragilis* (Leidy): Porifera; Spongillidae. J Morphol 145:125–142
Harrison FW, DeVos L (1991) Porifera. In: Harrison FW, Westfall JA (eds) Microscopic anatomy of invertebrates, vol 2. Placozoa, Porifera, Cnidaria, Ctenophora. Wiley, New York
Harrison FW, Kay NW, Kaye GW (1990) The dermal membrane of *Eunapius fragilis*. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
Hartman W (1958) Natural history of the marine sponges of southern New England. Bull Peabody Mus Nat Hist 12:1–155
Hartman WD, Wendt JW, Wiedenmayer F (1980) Living and fossil sponges. (Notes for a short course). University of Miami, Miami
Hay ED (1968) Organization and fine structure of epithelium and mesenchyme in the developing chick embryo. In: Fleischmajer R, Billingham RE (eds) Epithelial–mesenchymal interactions. Williams & Wilkins, Baltimore, MD
Hay ED (2005) The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create It. Dev Dyn 233:706–720
Heider K (1886) Zur metamorphose der *Oscarella lobularis* O. Schm Arbeit Zool Inst Wien 6:175–236
Henry J, Martindale MQ (1997) Nemerteans, the ribbon worms. In: Gilbert SF, Raunio AM (eds) Embryology. Constructing the organism. Sinauer, Sunderland, MA

Hentshel U, Uscher KM, Taylor MW (2006) Marine sponges as microbial fermenters. FEMS Microbiol Ecol 55:167–177

Herlant-Meewis H (1948) La gemmulation chez *Suberites domuncula*. Arch Anat Micros 37:289–322

Hill A, Tetrault J, Hill M (2004) Isolation and expression analysis of a poriferan Antp-class Bar-/Bsh-like homeobox gene. Dev Genes Evol 214:515–523

Hirakow R, Kajita N (1990) An electron microscopic study of the development of amphioxus, *Branchiostoma belcheri tsingtauense*: cleavage. J Morphol 203:331–344

Hofmann DK, Honegger TG (1990) Bud formation and metamorphosis in *Cassiopea andromeda* (Cnidaria, Sciphozoa): a developmental and ultrastructural study. Mar Biol 105:509–518

Holmes B, Blanch H (2007) Genus-specific associations of marine sponges with group I crenarchaeotes. Mar Biol 150:759–772

Hoppe WF (1988) Reproductive patterns in three species of large coral reef sponges. Coral Reefs 7:45–50

Hoppe WF, Reichert MJ (1987) Predicable annual mass release of gametes by the coral reef sponge *Neofibularia nolitangere* (Porifera, Demospongiae). Mar Biol 94:277–285

Hooper JNA, van Soest RWM, Debrenne F (2002) Phylum Porifera Grant, 1836. In: Hooper JNA, van Soest RWM (eds) Systema Porifera: a guide to the classification of sponges. Kluwer/Plenum, New York

Hoshino S, Takeda M, Watanabe Y (2004) Systematic status of *Halichondria japonica* (Kadota) (Demospongiae, Halichondriida) from Japan. Boll Mus Ist Biol Univ Genova 68:373–379

Hoshiyama D, Suga H, Iwabe N, Koyanagi M, Nikoh N, Kuma K, Matsuda F, Honjo T, Miyata T (1998) Sponge pax cDNA related to pax-2/5/8 and ancient gene duplications in the pax family. J Mol Evol 47:640–648

Humbert-David N, Garrone R (1993) Six-armed, tenascin-like protein extracted in the Porifera *Oscarella tuberculata* (Homoscleromorpha). Eur J Biochem 216:255–260

Hyman LH (1940) The invertebrates. 1. Protozoa through Ctenophora. McGraw-Hill, New York

Ijima I (1901) Studies on the Hexactinelida, Contribution I. (Euplectellidae). J Coll Sci Imper Univ Tokyo 15:1–299

Ijima I (1904) Studies on the Hexactinellida. Contribution IV. (Rossellidae). J Coll Sci Imper Univ Tokyo 28:13–307

Ilan M (1995) Reproductive biology, taxonomy, and aspects of chemical ecology of Latrunculiidae (Porifera). Biol Bull 188:306–312

Ilan M, Abelson A (1995) The life of a sponge in a sandy lagoon. Biol Bull 189:363–369

Ilan M, Loya Y (1990) Sexual reproduction and settlement of the coral reef sponge *Chalinula sp*. from the Red Sea. Mar Biol 105:25–31

Itskovich V, Belikov S, Efremova S, Masuda Y, Perez T, Alivon E, Borchiellini C, Boury-Esnault N (2007) Phylogenetic relationships between freshwater and marine Haplosclerida (Porifera, Demospongiae) based on the full length 18S rRNA and partial COXI gene sequences. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro 28

Ivanov AV (1968) The origin of the multicellular animals. Nauka, Leningrad

Ivanov AV (1971) Embryogenesis of sponges and their position in the system of animal kingdom. Zh Obsch Biol 32:557–572

Ivanov PP (1937) General and comparative embryology. Nauka, Moscow/Leningrad

Ivanova LV (1978) Morphogenetic processes and seasonal changes in the anatomic and histological organisation of the Barents sea sponge *Halichondria panicea* (Pallas). Arch Anat Hist Emb 75:62–72

Ivanova LV (1981) The life cycle of *Halichondria panicea* (Pallas) In: Korotkova GP (ed) Morphogenesis in sponges. Leningrad University Press, Leningrad

Ivanova LV (1997a) New data about morphology and metamorphosis of the spongillid larvae (Porifera, Spongillidae). 1. Morphology of the free-swimming larvae. In: Ereskovsky AV, Keupp H, Kohring HR (eds) Modern problems of Poriferan biology. Berliner Geowiss Abh, Freie University, Berlin

Ivanova LV (1997b) New data about morphology and metamorphosis of the spongillid larvae (Porifera, Spongillidae). 2. The metamorphosis of the spongillid larvae. In: Ereskovsky AV, Keupp H, Kohring HR (eds) Modern problems of Poriferan biology. Berliner Geowiss Abh, Freie University, Berlin

Ivanova LV, Semyonov VV (1997) Feeding habits of the larvae of sponges. In: Ereskovsky AV, Keupp H, Kohring HR (eds) Modern problems of Poriferan biology. Berliner Geowiss Abh, Freie University, Berlin

Ivanova-Kazas OM (1961) Stadies of comparative embryology of Hymenoptera. Leningrad University Press, Leningrad

Ivanova-Kazas OM (1975) Comparative embryology of Invertebrates: protista and lower invertebrates. Nauka, Novosibirsk

Ivanova-Kazas OM (1977) AAsexual reproduction of animals. Leningrad University Press, Leningrad

Ivanova-Kazas OM (1978a) Comparative embryology of invertebrates. Echinodermata and Hemichordata, Nauka, Moscow

Ivanova-Kazas OM (1978b) Comparative embryology of Invertebrates. Lower Chordata. Nauka, Moscow

Ivanova-Kazas OM (1995) Evolutionary embryology of animals. Nauka, St. Petersburg

Ivanova-Kazas OM (1997) Analysis of sponges ontogeny at sexual reproduction. In: Ereskovsky AV, Keupp H, Kohring HR (eds) Modern problems of Poriferan biology. Berliner Geowiss Abh, Freie University, Berlin

Jaeckle WB (1995a) Transport and metabolism of alanine and palmitic acid by field-collected larvae of *Tedania ignis* (Porifera, Demospongiae): estimated consequences of limited label translocation. Biol Bull 189:159–167

Jaeckle WB (1995b) Variation in the size, energy content, and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: McEdward LR (ed) Ecology of marine invertebrate larvae. CRC Press, New York/London/Tokyo

Jägersten G (1955) On the early phylogeny of the Metazoa. The Bilaterogastraea-theory. Zool Bidr Uppsala 30:321–354

James NP, Klappa CF (1983) Petrogenesis of Early Cambrian Reef Limestones, Labrador, Canada. J Sediment Petrol 53:1051–1096

Jayatilake GS, Thornton MP, Leonard AC, Grimwade JE, Baker BJ (1996) Metabolites from an Antarctic sponge-associated bacterium, *Pseudomonas aeruginosa*. J Nat Prod 59:293–296

Jetton TL, Fell PE, Harrison FW (1987) Cytological and cytochemical investigations of development from dormant gemmules of the marine sponge, *Haliclona loosanoffi*. J Morphol 193:99–116

Johnson MF (1978) Studies on the reproductive cycles of the calcareous sponges *Clathrina coriacea* and *C. blanca*. Mar Biol 50:73–79

Johnson MF (1979a) Gametogenesis and embryonic development in the calcareous sponges *Clathrina coriacea* and *C. blanca* from Santa Catalina Island, California. Bull S Calif Acad Sci 78:183–191

Johnson MF (1979b) Recruitment, growth and seasonal variations in the cal careous sponges *Clathrina coriacea* (Montagu) and *Clathrina blanca* (Miklucho-Maclay) from Santa Catalina Island, California. In: Lévi C, Boury-Esnault N (eds) Biologie des spongiaires. Colloques Internationaux. CNRS 291, Paris

Jones WC (1966) The structure of the porocytes in the calcareous sponge *Leucosolenia complicata* (Montagu). J R Microsc Soc 85:53–62

Jones WC (1971)Spicule formation and corrosion in recently metamorphosed *Sycon ciliatum* (O. Fabricius). In: Crisp DJ (ed) Fourth European Marine Biology Symposium. Cambridge University Press, Cambridge

Kaltenbach JC, Kuhns WJ, Simpson TL, Burger MM (1999) Intense concanavalin A staining and apoptosis of periferal flagellated cells in larvae of the marine sponge *Microciona prolifera*: significance in relation to morphogenesis. Biol Bull 197:271–273

Kanayama Y, Kamishima N (1990) Role of symbiotic algae in hatching of gemmules of the freshwater sponge *Radiospongilla cerebellata*. Zool Sci 7:649–655
Karawaiew W (1896) Über ein neues Radiolar aus Villafranca. Zool Anz 19:501
Karawaiew W (1897) Nachtrag zu meinem Artikel: über ein neues Radiolar aus Villafranca. Zool Anz 20:193
Kasyanov VL (2001) Reproductive strategy of marine bivalves and echinoderms. Science Publishers, Enfield, NH
Kasyanov VL, Kyuchkova GA, Kulikova VA, Medvedeva LA (1998) Larvae of marine bivalves and echinoderms. Oxonian Press, New Delhi
Kawamura K, Nakauchi M (1986) Mitosis and body patterning during morphallactic development of palleal buds in ascidians. Dev Biol 116:39–50
Kaye HR (1990) Reproduction in West Indian commercial sponges: oogenesis, larval development and behavior. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
Kaye HR (1991) Sexual reproduction in four Caribbean commercial sponges. II. Oogenesis and transfer of bacterial symbionts. Invert Reprod Dev 19:13–24
Kaye HR, Reiswig HM (1991a) Sexual reproduction in four Carribean commercial sponges. I. Reproductive cycles and spermatogenesis. Invert Reprod Dev 19:1–11
Kaye HR, Reiswig HM (1991b) Sexual reproduction in four Carribean commercial sponges. III. Larval behaviour, settlement and metamorphosis. Invert Reprod Dev 19:25–35
Keller C (1880) Studien uber organisation und Entwicklung der Chaliniden. Z Wiss Zool 33:317–349
Keller R (2005) Cell migration during gastrulation. Cur Opin Cell Biol 17:533–541
Keller R, Lance A, Davidson D, Shook R (2003) How we are shaped: the biomechanics of gastrulation. Differentiation 71:171–205
Kelly-Borges MK, Bergquist PR (1988) Success in a shallow reef environment: sponge recruitment by fragmentation through predation. In: Choat JH et al. (eds) Proceedings of the 6th International Coral Reef Symposium, vol 2, pp 757–762
Kempen TMG van (1978) Anthaspidellid sponges from the Early Paleozoic of Europe and Australia. Neues Jahrb Geol Palaont Abh 156:305–337
Kilian EF (1952) Wassersrömung und Nahrungsaufnahme beim Süßwasserschwamm *Ephydatia fluviatilis*. Z Vergl Physiol 34:407–447
Kilian EF, Wintermann-Kilian G (1979) Mouvement cellulaire et contraction chez *Spongilla lacustris* et *Ephydatia fluviatilis*. In: Lévi C, Boury-Esnault N (eds) Biologie des spongiaires. Colloques Internationaux. CNRS 291, Paris
Kim JH, Kim W, Cunningham CW (1999) A new perspective on lower metazoan relationships from 18S rDNA sequences. J Mol Evol 16:423–427
Kirfel G, Stockem W (1997) Detection and cytoplasmic localization of two different microtubule motor proteins in basal epithelial cells of freshwater sponges. Protoplasma 196:167–180
Kirk DL (1998) *Volvox*: molecular genetic origins of multicellularity and cellular differentiation. Cambridge University Press, New York
Kobayashi M, Takahashi M, Wada H, Satoh N (1993) Molecular phylogeny inferred from sequences of small subunit ribosomal DNA, supports the monophyly of the Metazoa. Zool Sci 10:827–833
Kober KM, Nichols SA (2007) On the phylogenetic relationships of hadromerid and poecilosclerid sponges. J Mar Biol Ass UK 87:1585–1598
Kolega J (1986) Effect of mechanical tension on protrusive activity and microfilament and intermediate filament organization in an epidermal epithelium moving in culture. J Cell Biol 102:1400–1411
Korotkova GP (1962) Regeneration and somatic embryogenesis in colonial cornacusponge *Halichondria panicea* Pallas. Vest Leningr State Univ 15:33–44
Korotkova GP (1970) Regeneration and somatic embryogenesis in sponges. The biology of the Porifera. In: Fry WG (ed) The biology of the Porifera, vol 25. Symposium of the Zoological Society, London

Korotkova GP (1972) Comparative morphological investigations of development of sponges from dissociated cells. Trans Lening Soc Nat 78:74–109
Korotkova GP (1979) The origin and evolution of ontogenesis. Leningrad Univ Press, Leningrad
Korotkova GP (1981a) Origin and evolution of ontogenesis. Leningrad University Press, Leningrad
Korotkova GP (1981b) General characteristics of sponge organization. In: Korotkova GP (ed) Morphogenesis in sponges. Leningrad University Press, Leningrad
Korotkova GP (1981c) Sexual embryogenesis in Porifera and regularities in their evolution. In: Korotkova GP (ed) Morphogenesis in sponges. Leningrad University Press, Leningrad
Korotkova GP (1988a) Peculiarities of organization and types of the development in sponges. In: Koltun VM, Stepanjants SD (eds) Porifera and Cnidaria: modern and perspective investigations. Zool Inst USSR Acad Sci, Leningrad
Korotkova GP (1988b) Integrative mechanisms and morphogenesis (to problem of the evolution of ontogenesis). Zh Obshey Biol 69:464–475
Korotkova GP (1997) Regeneration in animals. St. Petersburg University Press, St. Petersburg
Korotkova GP, Aizenshtadt TB (1976) A study of the oogenesis of the marine sponge *Halisarca dujardini*. Part 1. The origin of the oogonia and early stages of oocyte development. Tsitologiya 18:549–555
Korotkova GP, Apalkova LV (1975) Oogenesis of the Barents Sea sponge *Halisarca dujardini* Johnston. In: Tokin BP (ed) Comparative and experimental morphology of the sea organisms. Kola Fil Acad Sci USSR, Murmansk
Korotkova GP, Ereskovsky AV (1984) Peculiarities of the egg cleavage of White Sea sponge *Halisarca dujardini* Johnston. Vestnik Leningr Univ 21:36–42
Korotkova GP, Ermolina NO (1982) The larval development of *Halisarca dujardini* (Demospongiae). Zool Zh 61:1472–1480
Korotkova GP, Ermolina NO (1986) Destruction of embryos during the reproductive period in the White Sea sponge *Halisarca dujardini* Johnston (Demospongiae). Vestnik Leningr Univ 4:104–106
Korotkova GP, Movchan NA (1973) The peculiarities of defensive-regenerational processes of the sponge *Halisarca dujardini*. Vestnik Leningr Univ 21:16–25
Korotkova GP, Nikitin NS (1969a) The comparativ morphological analysis of regeneration and somatic embryogenesis of the cornacusponge *Halichondria panicea*. Reconstructional processes and immunological reactions. In: Tokin BP (ed) Morphological investigations of different stages of development of the marin organisms. Nauka, Leningrad
Korotkova GP, Nikitin NS (1969b) The peculiarities of the morphogenesis during the development of cornacusponge *Halichondria panicea* from the small part of the body. Reconstructional processes and immunological reactions. In: Tokin BP (ed) Morphological investigations of different stages of development of the marin organisms. Nauka, Leningrad
Korotkova GP, Suchodolskaya AN, Krasukevitch TN (1983) The pecularities of morphogenesis of the development of *Halisarca dujardini* from the small part of the body. Vestnik Leningr Univ 9:41–46
Korschelt E, Heider K (1936) Vergleichende Entwicklungsgeschichte der Tiere. Verlag vonGustav Fischer, Jena
Köster J (1997) Untersuchungen zur Ultrastuktur antarktischer Hexactinelliden (Porifera) Diploma thesis, Institut Meereskunde, Christian-Albrechts-Universitat, Kiel
Krylova DD, Aleshina GM, Kokrjakov VN, Ereskovsky AV (2002) The study of secretory maternal cell functions in the sponge *Halisarca dujardini* (Demospongiae, Halisarcida). Boll Mus Ist Biol Univ Genova 66–67:84–85
Krylova DD, Aleshina GM, Kokrjakov VN, Ereskovsky AV (2004) Antimicrobal properties of mesohylar granular cells of *Halisarca dujardini* Johnston, 1842 (Demospongiae, Halisarcida). In: Pansini M, Pronzato R, Bavestrello G, Manconi R (eds) Sponge science in the new millennium. Boll Mus Ist Biol Univ Genova, 68
Labat-Robert J, Robert L, Auger C, Lethias C, Garrone R (1981) Fibronectin-like protein in Porifera: its role in cell aggregation. Proc Natl Acad Sci USA 78:6261–6265
Lang BF, O'Kelly C, Nerad T, Gray MW, Burger G (2002) The closest unicellular relatives of animals. Curr Biol 12:1773–1778

Langenbruch PF (1981) Zur enstehung der gemmulae bei *Ephydatia fluviatilis* L. (Porifera). Zoomorphol 97:221–284
Langenbruch PF (1982) Die Entstehung der Gemmula-Schalen bei *Spongilla fragilis* Leidy (Porifera). Zoomorphology 99:221–234
Langenbruch PF (1984) Vergleichende rasterelektronenmikroskopische Darstellung der Gemmulaschalen von *Ephydatia fluviatilis*, *E. muelleri* und *Spongilla fragilis* (Porifera). Zoomorphology 104:79–85
Langenbruch PF (1988) Body structure of marine sponges. V. Structure of choanocyte chambers in some Mediterranean and Caribbean haplosclerid sponges (Porifera). Zoomorphology 108:13–21
Langenbruch PF, Jones WC (1989) A new type of central cell in the choanocyte chambers of *Pellina fistulosa* (Porifera, Demospongiae). Zoomorphology 109:11–14
Langenbruch PF, Scalera-Liaci L (1986) Body structure of marine sponges. IV. Aquiferous system and choanocyte chambers in *Haliclona elegans* (Porifera, Demospongiae). Zoomorphology 106:205–211
Langenbruch PF, Weissenfels N (1987) Canal systems and choanocyte chambers in freshwater sponges (Porifera, Spongillidae). Zoomorphology 107:11–16
Langenbruch PF, Simpson TL, Scalera-Liaci L (1985) Body structure of marine sponges. III. The structure of choanocyte chambers in *Petrosia ficiformis* (Porifera, Demospongiae). Zoomorphology 105:383–387
Lapébie P, Gazave E, Ereskovsky AV, Derelle R, Bézac C, Houliston E, Borchiellini C (2009) Conserved roles for WNT signalling in metazoan epithelial morphogenesis:Insights from a homoscleromorph sponge. Plos ONE 4(6):e5823. doi:10.1371/journal.pone.0005823
Larroux C, Fahey B, Liubicich D, Hinman VF, Gauthier M, Gongora M, Green K, Wörheide G, Leys SP, Degnan BM (2006) Developmental expression of transcription factor genes in a demosponge: insights into the origin of metazoan multicellularity. Evol Dev 8:150–173
Larroux C, Fahey B, Degnan SM, Adamski M, Rokhsar DS, Degnan BM (2007) The NK homeobox gene cluster predates the origin of hox genes. Curr Biol 17:1–5
Larroux C, Luke GN, Koopman P, Rokhsar DS, Shimeld SM, Degnan BM (2008) Genesis and expansion of metazoan transcription factor gene classes. Mol Biol Evol 25:980–996
Lavrov DV, Wanga X, Kelly M (2008) Reconstructing ordinal relationships in the Demospongiae using mitochondrial genomic data. Mol Phyl Evol 49:111–124
Ledger PW (1975) Septate junctions in the Calcareous sponge *Sycon ciliatum*. Tissue Cell 7:13–18
Ledger PW (1976) Aspect of the secretion and structure of Calcareous sponge spicules. Departement de Zoology, University College of North Wales, Bangor
Ledger PW, Jones WC (1977) Spicule formation in the calcareous sponge *Sycon ciliatum*. Cell Tissue Res 181:553–567
Lendenfeld R Von (1885) A monograph of the Australian sponges. III. The Calcispongiae. Proc Linn Soc N S W 9:1083–1150
Von LR (1889) A monograph of the horny sponges. Trebner & Co, Ludgate Hill, EC, London
Lentz TL (1966) Hystochemical localization of neurohumors in a sponges. J Exp Zool 162:171–180
Lepore E, Sciscioli M, Gherardi M, Scalera-Liaci L (1995) The ultrastructure of the mature oocyte and the nurse cells of the ceractinomorpha *Petrosia ficiformis*. Cah Biol Mar 36:15–20
Lepore E, Sciscioli M, Scalera Liaci L, Santarelli G, Gaino E (2000) Sexual reproduction of *Cinachyra tarentina* (Porifera, Demospongiae). Ital J Zool 67:153–158
Leptin M (2005) Gastrulation movements: the logic and the nuts and bolts. Dev Cell 8:305–320
Leveaux M (1941) Contribution à l'étude histologique de l'ovogenèse et de la spermatogenèse des Spongillidae. Ann Soc R Zool Belg 72:251–269
Leveaux M (1942) Contribution à l'étude histologique de l'ovogenèse et de la spermatogenèse des Spongillidae. Ann Soc R Zool Belg 73:33–50
Lévi C (1953) Structure de la larve et du rhagon de l'éponge *Halisarca dujardini* (Johnston). C R Acad Sci Paris 234:1710–1712

Lévi C (1956) Ètude des *Halisarca* de Roscoff. Embryologie et systematique des Démosponges. Trav Stat Biol Roscoff N.S 7:3–181
Lévi C (1963) Gastrulation and larval phylogeny in sponges. In: Dougherty EC (ed) The lower Metazoa: comparative biology and phylogeny. California University Press, Berkeley, CA
Lévi C (1964) Ultrastructure de la larve parenchymella de Démosponge. I. *Mycale contarenii* (Martens). Cah Biol Mar 5:97–104
Lévi C (1970) Les cellules des éponges. In: Fry WG (ed) The biology of the Porifera, vol 25. Symposium of the Zoological Society, London
Lévi C (1973) Systematique de la class des Démospongiaria (Demosponges). In: Grasse PP (ed) Traité de Zoologie, T. 1. N 3. Maison Cie, Paris
Lévi C, Lévi P (1976) Embryogenése de *Chondrosia reniformis* (Nardo), démosponge vipare, et transmission des bactéries symbiotiques. Ann Sci Nat Zool Biol Anim Ser 12(18):367–380
Lévi C, Porte A (1962) Etude au microscope électronique de l'éponge *Oscarella lobularis* Schmidt et de sa larve amphiblastula. Cah Biol Mar 3:307–315
Lewandrowski KB, Fell PE (1981) Sequential reproduction by different types of specimens of the estuarine sponge, *Halichondria* sp., with an emphasis on reproduction of postlarval specimens. Inter J Invert Reprod 3:227–236
Leys SP (1995) Cytosceletal architecture and organelle transport in geant syncytia formed by fusion of hexactinellid sponge tissues. Biol Bull 188:241–254
Leys SP (1996) Cytoskeletal architecture, organelle transport, and impulse conduction in hexactinellid sponge syncytia. Doctoral Dissertation. University of Victoria, Victoria
Leys SP (1998) Fusion and cytoplasmic streaming are characteristics of a least two hexactinellids: examination of cultured tissue from *Aphrocallistes vastus*. In: Watanabe Y, Fusetani N (eds) Sponge sciences. Multidisciplinary perspectives. Springer, Tokyo
Leys SP (1999) The choanosme of hexactinellid sponges. Inver Biol 118:221–235
Leys SP (2003a) The significance of syncytial tissues for the position of the Hexactinellida in the Metazoa. Integr Comp Biol 43:19–27
Leys SP (2003b) Comparative study of Spiculogenesis in Demosponge and Hexactinellid Larvae. Microsc Res Tech 62:300–311
Leys SP (2004) Gastrulation in Sponges. In: Stern CD (ed) Gastrulation. From cell to embryon. Cold Spring Harbor Lab Press, New York
Leys SP (2007) Sponge coordination, tissues, and the evolution of gastrulation. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro 28
Leys SP, Degnan BM (2001) Cytological basis of photoresponsive behavior in a sponge larva. Biol Bull 201:323–338
Leys SP, Degnan BM (2002) Embryogenesis and metamorphosis in a haplosclerid demosponge: gastrulation and transdifferentiation of larval ciliated cells to choanocytes. Invert Biol 121:171–189
Leys SP, Eerkes-Megrano D (2005) Gastrulation in Calcareous sponges: in search of Haeckel's gastraea. Integr Comp Biol 45:342–351
Leys SP, Ereskovsky AV (2006) Embryogenesis and larval differentiation in sponges. Can J Zool 84:262–287
Leys SP, Cronin TW, Degnan BM, Marshall JN (2002) Spectral sensitivity in a sponge larva. J Comp Physiol [A]. 188:199–202
Leys SP, Cheung E, Boury-Esnaulty N (2006) Embryogenesis in the glass sponge *Oopsacas minuta*: formation of syncytia by fusion of blastomeres. Integr Comp Biol 46:104–117
Leys SP, Mackie GO, Reiswig HM (2007) The biology of glass sponges. Adv Mar Biol 52:1–145
Li CW, Chen JY, Hua TE (1998) Precambrian sponges with cellular structures. Science 279:879–882
Lieberkühn N (1856) Zusatze zur Entwickelungsgeschichte der Sponilliden. Arch Anat Physiol 5:496–514
Lieberkühn N (1859) Neue Beitrage zur Anatomie der Spongien. Arch Anat Physiol Wiss Med 353–382. Taf. IX, X, XI

Livanov NA (1955) Pathways of evolution od animals. Nauka, Moscow
Lopes JV, McCarthy PJ, Janda KE, Willoughby R, Pomponi S (1999) Molecular techniques reveal wide phyletic diversity of heterotrophic microbes associated with *Discodermia* spp. (Porifera, Demospongiae). Mem Queensl Mus 44:329–341
Lufty RG (1957a) On the placental membrane of calcareous sponges. Cellule (Belgique) 58:239–246
Lufty RG (1957b) On the origin of the so-called mesoblast cells in the amphiblastula larvae of calcareous sponges. Cellule (Belgique) 58:231–236
Lundbeck W (1905) Porifera. Part II. Desmacidonidce (pars). Danish Ingolf Exp 6:1–219
Maas O (1894) Die Embryonal-Entwincklung und Metamorphose der Cornacuspongien. Zool Jehrb Abt Morphol 7:331–448
Maas O (1898) Die Keimblätter der Spongien und die Metamorphose von *Oscarella* (*Halisarca*). Z Wiss Zool 63:665–679
Maas O (1900) Die Weiterentwicklung der Syconen nach der Metamorphose. Zeitschr Wiss Zool LXVII: 215–240, Pl. IX–XII
Maas O (1901) Die Knospenentwicklung der *Tethya* und ihr Vergleich mit der geschlechtlichen Fortpflanzung der Schwämme. Z Wiss Zool 70:263–288
Maas O (1904) Ueber die Wirkung der Kalkentzeihung auf die Entwicklung der Kalkschwamme. Sitz Ber Ges Morph Physiol Munchen XX:4–21
Mackie GO (1981) Plugged syncytial interconnections in hexactinellid sponges. J Cell Biol 91:103a
Mackie GO, Lawn ID, Pavans de Ceccatty M (1983) Studies on hexactinellid sponges. II. Excitability, conduction and coordination of responses in *Rhabdocalyptus dawsoni* (Lambe 1873). Phil Trans Roy Soc Lond Ser B 301:401–418
Mackie GO, Singla CL (1983) Studies on hexactinellid sponges. I. Histology of *Rhabdocalyptus dawsoni* (Lambe, 1873). Phil Trans Roy Soc Lond 301:365–400
Malakhov VV (1990) Enigmatic groups of marine invertebrates: Trichoplax, Orthonectida, Diciemida, Porifera. Moscow State University Press, Moscow
Maldonado M (2004) Choanoflagellates, choanocytes, and animal multicellularity. Invert Biol 123:1–22
Maldonado M (2006) The ecology of the sponge larva. Can J Zool 84:175–194
Maldonado M (2007) Intergenerational transmission of symbiotic bacteria in oviparous and viviparous demosponges, with emphasis on intracytoplasmicallycompartmented bacterial types. J Mar Biol Ass UK 87:1701–1713
Maldonado M (2009) Embryonic development of verongid demosponges supports the independent acquisition of sponging skeletons as an alternative to the siliceous skeleton of sponges. Biol J Linn Soc 97:427–447
Maldonado M, Bergquist PR (2002) Phylum Porifera. In: Young CM, Sewel MA, Rice ME (eds) Atlas of marine invertebrate larvae. Academic, Barcelona
Maldonado M, Riesgo A (2007) Intra-epithelial spicules in a homosclerophorid sponge. Cell Tissue Res 328:639–650
Maldonado M, Riesgo A (2008) Reproductive output in a Mediterranean population of the homosclerophorid *Corticium candelabrum* (Porifera, Demospongiae), with notes on the ultrastructure and behavior of the larva. Mar Ecol 29:298–316
Maldonado M, Uriz MJ (1999) An experimental approach to the ecological significance of microhabitat-scale movement in an encrusting sponge. Mar Ecol Prog Ser 185:239–255
Maldonado M, Young CM (1996) Effects of physical factors on larval behavior, settlement and recruitment of four tropical demosponges. Mar Ecol Prog Ser 138:169–180
Maldonado M and Young C (1998) Limits on the bathymetric distribution of keratose sponges: a field test in deep-water. Mar Ecol Progr Ser 174:123–139
Maldonado M, George SB, Young CM, Vaquerizo I (1997) Depth regulation in parenchymella larvae of a demosponge – relative roles of skeletogenesis, biochemical changes and behavior. Mar Ecol Progr Ser 148:115–124
Manconi R, Pronzato R (1994) Spongillids of Mediterranean Islands In: Soest RWM van, Kempen TMG van, Braekman JC (eds) Sponges in time and space. Balkema, Rotterdam/ Amsterdam

Manconi R, Pronzato R (2002) Suborder Spongillina subord. nov.: freshwater sponges. In: Hooper JNA, van Soest RWM (eds) Systema Porifera: a guide to the classification of sponges. Kluwer/Plenum, New York

Manconi R, Pronzato R (2007) Gemmules as a key structure for the adaptive radiation of freshwater sponges: a morphofunctional and biogeographical study. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro 28

Manuel M (2001) Origine et évolution des mécanismes moléculaires controlant la morphogenèse chez les Métazoaires: un nouveau modéle spongiaire, *Sycon raphanus* (Calcispongia, Calcaronea). Universite Paris XI Orsay

Manuel M (2006) Phylogeny and evolution of calcareous sponges. Can J Zool 84:225–241

Manuel M (2009) Early evolution of symmetry and polarity in metazoan body plans. CR Biol 332: 184–209

Manuel M, Le Parco Y (2000) Homeobox gene diversification in the calcareous sponge, *Sycon raphanus*. Mol Phyl Evol 17:97–107

Manuel M, Borojevic R, Boury-Esnault N, Vacelet J (2002) Class Calcarea. In: Hooper JNA, van Soest RWM (eds) Systema Porifera: a guide to the classification of sponges. Kluwer/Plenum, New York

Manuel M, Borchiellini C, Alivon E, Le Parco Y, Vacelet J, Boury-Esnauly N (2003) Phylogeny and evolution of Calcareous sponges: monophyly of Calcinea and Calcaronea, high level of morphological homoplasy, and the primitive nature of axis symmetry. Syst Biol 52:311–333

Manuel M, Le Parco Y, Borchiellini C (2004a) Comparative analysis of Brachyury T-domains, with the characterization of two new sponge sequences, from a hexactinellid and a calcisponge. Gene 340:291–301

Manuel M, Borchiellini C, Alivon E, Boury-Esnauly N (2004b) Molecular phylogeny of calcareous sponges using 18S rRNA and 28S rRNA sequences. In: Pansini M, Pronzato R, Bavestrello G, Manconi R (eds) Sponge science in the new millennium. Boll Mus Ist Biol Univ Genova 68

Mariani S, Uriz MJ, Turton X (2000) Larval bloom of the oviparous sponge *Cliona viridis*: coupling of larval abundance and adult distribution. Mar Biol 137:783–790

Martin VJ (1997) Cnidarians, the jellyfish and hydras. In: Gilbert SF, Raunio AM (eds) Embryology. Constructing the organism. Sinauer, Sunderland, MA

Martin VJ, Littlefield CL, Archer WE, Bode HR (1997) Embryogenesis in *Hydra*. Biol Bull 192:345–363

Martindale MQ (2005) The evolution of metazoan axial properties. Nat Rev Genet 6:917–927

Masuda Y, Kuroda M, Matsuno A (1998) An ultrastructural study of the contractile filament in the pinacocyte of a freshwater sponge. In: Watanabe Y, Fusetani N (eds) Sponge sciences. Multidisciplinary perspectives. Springer, Tokyo

McCormack GP, Erpenbeck D, van Soest RWM (2002) Major discrepancy between phylogenetic hypotheses based on molecular and morphological criteria within the Order Haplosclerida (Phylum Porifera: Class Demospongiae). Zool Syst Evol Res 40:237–240

Medina MN, Collins AG, Silberman JD, Sogin ML (2001) Evaluating hypothesis of basal animal phylogeny using complete sequences of large and small subunit rRNA. Proc Natl Acad Sci USA 98:9707–9712

Meewis H (1936) Contribution a l'étude histologique des éponges d'eau douce. *Spongilla lacustris, Ephydatia fluviatilis*. Mem Mus R Hist Nat Belg 3:519–537

Meewis H (1938) Contribution à l'étude de l'embryogénèse des Myxospongiae: *Halisarca lobularis* (Schmidt). Arch Biol Liége 59:1–66

Meewis H (1939) Contribution a l'étude de l'embryogénese de Chalinulidae: *Haliclona limbata*. Ann Soc R Zool Belg 70:201–243

Meewis H (1941) Contribution a l'étude de l'embryogénese des éponges siliceuse. Ann Soc R Zool Belg 72:126–149

Mehl D (1996) Phylogenie und Evolutionsökologie der Hexactinellida (Porifera) im Paläozoikum. Geol Paläontol Mit Innsbruck 4:1–55

Mehl-Janussen D (1999) Die frühe evolution der Porifera. Münchiner Geowiss Abh (A) 37:1–72

Mehl D, Reiswig HM (1991) The presence of flagellar vanes in choanomeres of Porifera and their possible phylogenetic implications. Zool Syst Evol 29:312–319
Mehl D, Reitner J, Reiswig HM (1994) Soft tissue organization of the deep water hexactinellid *Schaudinnia arctica* Schulze, 1900 from the Arctic Seamount Vesterisbanken (Central Greenland Sea). Berliner Geowis Abh 13:301–313
Mehl D, Müller I, Müller WEG (1998) Molecular biological and paleontological evidence that Eumetazoa, including Porifera (Sponges), are monophyletic origin. In: Watanabe Y, Fusetani N (eds) Sponge sciences. Multidisciplinary perspectives. Springer, Tokyo
Merejkowsky CS (1878) Les Eponges de la mer Blanche. Mem Acad Imper Sci 26:1–51
Merejkowsky CS (1879) Reproduction des éponges par bourgeonnement extérieur. Arch Zool Expér Gén 8:417–433
Mergner H (1970) Ergebnisse der entwicklungsphysiologie bei Spongilliden. In: Fry WG (ed) The biology of the Porifera, vol 25. Symposium of the Zoological Society, London
Meroz E, Ilan M (1995) Life history characteristics of a coral reef sponge. Mar Biol 124:443–451
Metschnikoff E (1874) Zur Entwickelungsgeschichte der Kalkschwamme. Z Wiss Zool 24:1–14
Metschnikoff E (1879) Spongiologische Studien. Z Wiss Zool 32:349–387
Metschnikoff E (1886) Embryologische Studien an Medusen. Ein Beitrag zur Genealogie der Primitiv-Organe. Wien, Alfred Holder
Meyer W, Sidri M, Brümmer F (2006) Glycohistochemistry of a marine sponge, *Chondrilla nucula* (Porifera, Desmospongiae), with remarks on a possibly related antimicrobial defense strategy and a note on exopinacoderm function. Mar Biol 150:313–319
Mikhailov KV, Konstantinova AV, Nikitin MA, Troshin PV, Rusin LY, Lyubetsky VA, Panchin YV, Mylnikov AP, Moroz LL, Kumar S, Aleoshin VV (2009) The origin of Metazoa: a transition from temporal to spatial cell differentiation. Dedicated to the 60th year of A. A. Zakhvatkin's Synzoospore hypothesis. BioEssays 31:758–768
Miklucho-Maclay N (1868) Beiträge zur Kenntniss der Spongien 1. Jen Zeitschr 4:221–240
Minchin EA (1896) Note on the larva and the postlarval development of *Leucosolenia variabilis* n. sp., with remarks on the development of other Asconidae. Proc Roy Soc Lond 60:43–52
Minchin EA (1897) *Ascandra* or *Homandra*? A test-case for the rules of zoological nomenclature. Zool Anz 20:49–50
Minchin EA (1900) Sponges – phylum Porifera. In: Ray Lankaster E (ed) Treatise on zoology, vol 2. The Porifera and Coelenterata. Adam & Charles Black, London
Minelli A, Schram FR (1994) Owen revisited: a reappraisal of morphology in evolutionary biology. Bijdr Dierkunde 64:65–74
Misevic GN, Burger MM (1982) The molecular basis of species specific cell-cell recognition in marine sponges, and a study on organogenesis during metamorphosis. In: Liss A (ed) Embryonic development. Part B: cellular aspects. Allan R. Liss, New York
Misevic GN, Schlup V, Burger MM (1990) Larval metamorfosis of *Microciona prolifera*: evidens against the reversal of layers. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
Mizoguchi H, Watanabe Y (1990) Collagen synthesis in *Ephydatia fluviatilis* during its development. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
Morris P (1995) The developmental role of the extracellular matrix suggests a monophyletic origin of the kingdom Animalia. Evolution 47:152–165
Mukai H (1989) Growth and reproduction in four species of freshwater sponges cultured in their natural surrounding. Sci Rep Educ Gunma Univ 38:25–47
Mukhina YI, Kumeiko VV, Podgornaya OI, Efremova SM (2006) The fate of larval flagellated cells during metamorphosis of the sponge *Halisarca dujardini*. Int J Dev Biol 50:533–541
Mukhina YI, Kumeiko VV, Podgornaya OI, Efremova SM (2007) The events of metamorphosis in the demosponge *Halisarca dujardini* Johnston, 1842, studied with immunocytochemical method. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro 28

Müller K (1911) Das Regenerationsverm gen der Süßwassershwämme, insbesondere, Untersuchungen Über die bei ihnen vorkmmende Regeneration nach dissociation und Reunition. Arch Entwick 32:397–446
Müller WEG (1982) Cell membrane in sponges. Int Rev Cyt 77:129–181
Müller WEG (1997a) Origin of metazoan adhesion molecules and adhesion receptors as deduced from cDNA analyses in the marine sponge *Geodia cydonium*: a review. Cell Tissue Res 289:383–395
Müller WEG (1997b) Molecular phylogeny of Metazoa (animals): evidence for monophyly of animals based on genes in sponges (Porifera). Progr Mol Subcell Biol 19:89–132
Müller WEG (1998) Molecular phylogeny of Eumetazoa: genes in sponges (Porifera) give evidence for monophyly of animals. In: Müller WEG (ed) Molecular evolution: evidence for monophyly of metazoa. Springer, Berlin
Müller WEG (2001) Review: how was metazoan threshold crossed? The hypothetical Urmetazoa. Comp Biochem Physiol A Mol Integr Physiol 129:433–460
Muricy G, Boury-Esnault N, Bezac C, Vacelet J (1996) Cytological evidence for cryptic speciation in Mediterranean *Oscarella* species (Porifera, Homoscleromorpha). Can J Zool 74:881–896
Muricy G, Bezac C, Gallissian MF, Boury-Esnault N (1999) Anatomy, cytology and symbiotic bacteria of four Mediterranean species of *Plakina* Schulze, 1880 (Demospongiae, Homosclerophorida). J Nat Hist 33:159–176
Nakamura Y, Okada K, Watanabe Y (1998) The ultrastructure of spermatozoa and its ultrastructural change in the choanocyte of *Sycon calcaravis* Hozawa. In: Watanabe Y, Fusetani N (eds) Sponge sciences. Multidisciplinary perspectives. Springer, Tokyo
Nichols SA (2005) An evaluation of support for order-level monophyly and interrelationships within the class Demospongiae using partial data from the large subunit rDNA and cytochrome oxidase subunit I. Mol Phyl Evol 34:81–96
Nichols SA, Dirks W, Pearse JS, King N (2006) Early evolution of animal cell signaling and adhesion genes. Proc Natl Acad Sci USA 103:12451–12456
Nielsen C (1998) Origin and evolution of animal life cycles. Biol Rev 73:125–155
Nielsen C (2001) Animal evolution: interrelationships of the living phyla (2nd edition). Oxford University Press, New York
Nielsen C (2008) Six major steps in animal evolution: are we derived sponge larvae? Evol Dev 10:241–257
Okada Y (1928) On the development of a hexactinellid sponge, *Farrea sollasii*. J Fac Sci Imper Univ Tokyo 2:1–27
Owen R (1848) On the archetype and homologies of the vertebrate skeleton. John van Voorst, Paternoster Row, London
Pahler S, Blumbach B, Müller I, Müller WEG (1998) A putitative multiadhesive basal lamina protein from the marine sponge *Geodia cydonium*: cloning of the cDNA encoding a fibronectin-, an SRCR- as well as a complement control protein module. J Exp Zool 282:332–343
Pancer Z, Kruse M, Müller I, Müller WEG (1997) On the origin of metazoan adhesion receptors: cloning of integrin‘ subunit from the sponge *Geodia cydonium*. Mol Biol Evol 14:391–398
Pansini M, Pronzato R (1990) Observations on the dynamics of a Mediterrane an sponge community. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
Paulus W (1989) Ultrastructural investigation of spermatogenesis in *Spongilla lacustris* and *Ephydatia fluviatilis* (Porifera, Spongillidae). Zoomorphology 109:123–130
Paulus W, Weissenfels N (1986) The spermatogenesis of *Ephydatia fluviatilis* (Porifera). Zoomorphology 106:155–162
Pavans de Ceccatty M (1966) Ultrastructures et rapports des cellules mésenchymateuses de type nerveux de l’éponge *Tethya lyncurium* Lmk. Ann Sci Nat Biol Anim 8:577–614
Pavans de Ceccatty M (1979) Cell correlation and integration in sponges. In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris
Pavans de Ceccatty M (1981) Demonstration of actin filaments in sponge cells. Cell Biol Int Rep 5:945–952

Pavans de Ceccatty M (1982) *In vitro* aggregation of syncytia and cells of a hexactinellid sponge. Dev Comp Immun 6:15–22

Pavans de Ceccatty M (1986) Cytosceletal organisation and tissue patterns of epithelia in the sponge *Ephydatia muelleri*. J Morphol 189:45–65

Pavans de Ceccatty M, Garrone R (1971) Fibrogenèse du collagène chez l'éponge *Chondrosia reniformis* Nardo (Démosponge Tétractinellide). Origine et évolution des lophocytes. C R Acad Sci Paris 273:1957–1959

Pavans de Ceccatty M, Mackie G (1982) Génèse et évolution des interconnexions syncytiales et cellulaires chez une éponge Hexactinellide en cours de réagrégation après dissociation *in vitro*. C R Acad Sci Paris 294:939–940

Pavans de Ceccatty M, Thiney Y, Garrone R (1970) Les bases ultrastructurales des communications intercellulaires dans les oscules de quelques éponges. In: Fry WG (ed) The biology of the Porifera, vol 25. Symposium of the Zoological Society, London

Pedersen KJ (1991) Invited review: structure and composition of basement membranes and ather basal matrix systems in selected invertebrates. Acta Zool 72:181–201

Perez T (1996) Particle uptake by a hexactinellid sponge, *Oopsacas minuta* (Leucopsacasidae): the role of the reticulum. C R Acad Sci Paris 319:385–391

Perovic S, Schröder HC, Sudek S, Grebenjuk VA, Batel R, Maurotifani IMM, Müller WEG (2003) Expression of one sponge *Iroquois* homeobox gene in primmorphs from *Suberites domuncula* during canal formation. Evol Dev 5:240–250

Peterson KJ, Davidson EH (2000) Regulatory evolution and the origin of the bilateriens. Proc Natl Acad Sci USA 97:4430–4433

Peterson KJ, Eernisse DJ (2001) Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA genes sequences. Evol Dev 3:170–205

Pfeifer K, Haasemann M, Gamulin V, Bretting H, Fahrenholtz F, Müller WEG (1993) S-type lectins occur also in invertebrates: high conservation of the carbohydrate recognition domain in the lectin genes from the marine sponge *Geodia cydonium*. Glycobiology 3:179–184

Philippe H, Derelle R, Lopez P, Pick K, Borchiellini K, Boury-Esnault N, Vacelet J, Renard E, Houliston E, Queinnec E, Da Silva C, Wincker P, Le Guyader H, Leys S, Jackson DJ, Schreiber F, Erpenbeck D, Morgenstern B, Worheide G, Manuel M (2009) Phylogenomics revives traditional views on deep animal relationships. Curr Biol 19:706–712

Pinheiro UDS, Hajdu E, Costodio MR (2004) Cell types as taxonomic characters in *Aplysina* (Aplysinidae, Verongida). Boll Mus Ist Biol Univ Genova 68:527–533

Pile AJ, Patterson MR, Witman JD (1996) *In situ* grazing on plancton <10 μm by the boreal sponge *Mycale lingua*. Mar Ecol Progr Ser 141:95–102

Pinto R, Woollacott RM (1992) Ultrastructure of the basal body apparatus in epidermal cells of a sponge larva (*Aplysilla*. sp: Demospongiae). In: Baley GW, Bentky J, Small JA (eds) Proceedings of 50th Annual Meeting of the Electron Microscopy Society of America. San Francisco Press, San Francisco, CA

Plotkin AS, Ereskovsky AV (1997) Ecological aspects of asexual reproduction of the White Sea Sponge *Polymastia mammillaris* (Demospongiae, Tetractinomorpha) in Kandalaksha Bay. In: Ereskovsky AV, Keupp H, Kohring HR (eds) Modern problems of Poriferan biology. Berliner Geowiss Abh, Freie University, Berlin

Plotkin AS, Ereskovsky AV, Khalaman VV (1999) The analysis of modular organization of Porifera using *Polymastia mammillaris* (Müller, 1806) as a model. Zh Obshchei Biol 60:18–28

Polejaeff N (1882) Uber des Sperma und die Spermatogenesis bei *Sycandra raphanus* Haeckel. Anz K Akad Wissensch Wien 86:276–298

Pomponi SA (1976) A cytological study of the Haliclonidae and the Callyspongiidae (Porifera, Demospongiae, Haplosclerida). In: Harrison FW, Cowden RR (eds) Aspects of sponge biology. Academic, New York

Pomponi SA, Meritt DW (1990) Distribution and life history of the boring sponge *Cliona trutti* in the Upper Chesapeake Bay. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC

Pottu-Boumendil J (1975) Ultrastructure, cytochimie, et comportements morphogenétiques des vellules de l'éponge *Ephydatia muelleri.* These, University, Claude Bernard, Lyon
Pottu-Boumendil J, Pavans de Ceccatty M (1976) Mouvements cellulaires et morphogenèse de l'éponge *Ephydatia Mülleri* Lieb. Bull Soc Zool Fr 101:31–38
Pronzato R, Manconi R (1991) Colonization, life cycles and competition in a freshwater sponge association. Reitner J, Keupp H (eds) Fossil and recent sponges. Springer, Berlin/ Heidelberg
Pronzato R, Manconi R (1994) Life history of *Ephydatia fluviatilis* a model for adaptive strategies in discontinuous habitats. In: van Soest RWM, van Kempen TMG, Braekman JC (eds) Sponges in time and space. Balkema, Rotterdam/Amsterdam
Raff RA (1987) Constraint, flexibility, and phylogenetic change in the evolution of direct development sea urchins. J Evol Biol 1:27–44
Raff R (1996) The shape of life: genes, development, and the evolution of animal form. University of Chicago Press, Chicago/London
Raleigh J, Redmond NE, Delahan E, Torpey S, van Soest RWM, Kelly M, McCormack GP (2007) Mitochondrial Cytochrome oxidase 1 phylogeny supports alternative taxonomic scheme for the marine Haplosclerida. J Mar Biol Ass UK 87:1577–1584
Rasmont R (1963) Le rôle de la taille et de la nutrition dans le déterminisme de la gemmulation chez les spongillides. Dev Biol 8:243–271
Rasmont R (1979) Les Eponges: des Métazoaires et des sociétés de cellules. In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris
Rasmont R, De Vos L (1974) Etude cinématographique de la gemmulation d'une éponge d'eau douce: *Ephydatia fluviatilis*. Arch Biol 85:329–341
Redmond NE, van Soest RWM, Kelly M, Raleigh M, Travers J, McCormack GP (2007) Reassessment of the classification of the Order Haplosclerida (Class Demospongiae, Phylum Porifera) using 18S rRNA gene sequence data. Mol Phyl Evol 43:344–352
Redmond NE, McCormack GP (2008) Large expansion segments in 18S rDNA support a new sponge clade (Class Demospongiae, Order Haplosclerida). Mol Phyl Evol 47:1090–1099
Reiswig HM (1971) *In situ* pumping activites in tropical Demospongiae. Mar Biol 9:38–50
Reiswig HM (1973) Population dynamics of three Jamaican Demospongiae. Bull Mar 23:191–226
Reiswig HM (1976) Natural gamete release and oviparity in Caribbean Demospongiae. In: Harrison FM, Cowden RR (eds) Aspects of sponge biology. Academic, New York
Reiswig HM (1979a) Histology of Hexactinellida (Porifera). In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris
Reiswig HM (1983) Porifera. In: Adiyodi KG, Adiyodi RG (eds) Reproductive biology of Invertebrates, vol 2. Spermatogenesis. Wiley, Chichester
Reiswig HM, Brown MJ (1977) The central cells of sponges. Their distribution, form, and function. Zoomorphologie 88:81–94
Reiswig HM, Mehl D (1991) Tissue organization of *Farrea occa* (Porifera, Hexactinellida). Zoomorphology 110:301–311
Reitner J (1992) Coralline Spongien. Der Versuch einer phylogenetisch-taxonomischen Analyse. Berliner Geowis Abh 1:1–352
Reitner J, Gautret P (1996) Skeletal formation in the modern but ultraconservative chaetetid sponge *Spirastrella* (*Acanthochaetetes*) *wellsi* (Demospongiae, Porifera). Facies 34:193–207
Reitner J, Wörheide G (2002) Non-Lithistid Fossil Demospongiae – origins of their palaeobiodiversity and highlights in history of preservation. In: Hooper JNA, van Soest RWM (eds) Systema Porifera: a guide to the classification of sponges. Kluwer/Plenum, New York
Remane A (1963) The evolution of the Metazoa from colonial flagellates vs plasmodial ciliates. In: Dougherty CE (ed) The lower metazoa. Comparative biology and phylogeny. University of California Press, Berkeley, CA
Reunov AA (2005) Problem of terminology in characteristics of spermatozoa of metazoa. Russ J Dev Biol 36:335–351

Richards GS, Simionato E, Perron M, Adamska M, Vervoort M, Degnan BM (2008) Sponge genes provide new insight into the evolutionary origin of the neurogenic circuit. Curr Biol 18:1156–1161

Richardson MK, Hanken J, Gooneratne ML, Pieau C, Raynaud A, Selwood L, Wright GM (1997) There is no highly conserved embryonic stage in the vertebrates, implications for current theories of evolution and development. Anat Embryol 196:91–106

Richardson MK, Allen SP, Wright GM, Raynaud A, Hanken J (1998) Somite number and vertebrate evolution. Development 125:151–160

Richelle-Maurer E, van de Vyver G (1999) Expression of homeobox-containing genes in freshwater sponges. Mem Queensl Mus 44:509–514

Richelle-Maurer E, van de Vyver G, Vissers S, Coutinho CC (1998) Homeobox-containing genes in freshwater sponges: characterization, expression, and phylogeny. In: Müller WEG (ed) Molecular evolution: evidence for monophyly of Metazoa. Springer, Berlin

Richelle-Maurer E, Sonet G, van de Vyver G (2004) Identification of homologues of the EmH-3 homeobox-containing gene in demosponges. In: Pansini M, Pronzato R, Bavestrello G, Manconi R (eds) Sponge science in the new millennium. Boll Mus Ist Biol Univ Genova, 68

Richelle-Maurer E, Boury-Esnault N, Itskovich VB, ManuelM PSA, van de Vyver CBJ (2006) Conservation and phylogeny of a novel family of non-hox genes of the Antp class in Demospongiae (Porifera). J Mol Evol 63:222–230

Rieger RM (1994) Evolution of the "lower" Metazoa. In: Bengdson S (ed) Early life of Earth. Nobel symposium N 84. Columbia UP, New York

Rieger R, Weyrer S (1998) The evolution of the lower Metazoa: evidence from the phenotype. In: Müller WEG (ed) Progress in molecular and subcellular biology, vol 21. Springer, Berlin

Riesgo A, Maldonado M (2008a) Differences in reproductive timing among sponges sharing habitat and thermal regime. Invert Biol 127:357–367

Riesgo A, Maldonado M (2008b) Ultrastructure of oogenesis of two oviparous demosponges: *Axinella damicornis* and *Raspaciona aculeata* (Porifera). Tissue Cell 41:51–65

Riesgo A, Maldonado M (2009) An unexpectedly sophisticated, V-shaped spermatozoon in Demospongiae (Porifera): reproductive and evolutionary implications. Biol J Linn Soc 97:413–426

Riesgo A, Maldonado M, Durfort M (2007a) Dynamics of gametogenesis, embryogenesis, and larval release in a Mediterranean homosclerophorid demosponge. Mar Fresh Res 58:398–417

Riesgo A, Taylor C, Leys SP (2007b) Reproduction in a carnivorous sponge: the significance of the absence of an aquiferous system to the sponge body plan. Evol Dev 9:618–631

Riesgo A, Maldonado M, Durfort M (2008) Occurrence of somatic cells within the spermatic cysts of demosponges: a discussion of their role. Tissue Cell 40:387–496

Rigby JK (1986) Cambrian and Silurian sponges from Nothern Greenland. Rapp Groenlands Geol Unders 132:51–63

Robertson AJ, Larroux C, Degnan BM, Coffman JA (2009) The evolution of Runx genes II. The C-terminal Groucho recruitment motif is present in both eumetazoans and homoscleromorphs but absent in a haplosclerid demosponge. BMC Res Not2:59. doi:10.1186/1756-0500-2-59

Ropstorp P, Reitner J (1994) Morphologie einiger Subwasser Porifera (*Baikalospongia bacillifera, Lubumirskia baikalensis, Swartschewskia papyracea*) des Baikal-Sees (Sibirien, Rusland). Berliner Geowiss Abh E 13:507–525

Rossel D (1993) Effects of reproduction in *Cliona viridis* (Hadromerida) on zooxantellae. Sci Mar 57:405–413

Rozenfeld F (1974) Biochemical control of fresh-water sponge development: effect on DNA, RNA and protein synthesis of an inhibitor secreted by the sponge. J Emb Exp Morph 32:287–295

Rosenfeld F, Masson H, Rasmont R (1979) Analyse statistique du mouvement des cellules amiboide au cours de la gemmulation d'une éponge d'eau douce. In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris

Ruppert EE, Fox RS, Barnes RD (2004) Invertebrate zoology: a functional evolutionary approach, 7th edn. Brooks/Cole, Thomson Learning Inc., Belmont, MA

Rützler K, van Soest RWM, Alvarez B (2003) *Swenzea zeai*, a Caribbean reef sponge with a giant larva, and *Scopalina ruetzleri*: a comparative fine-structural approach to classification (Demospongiae, Halichondrida, Dictyonellidae). Invert Biol 122:203–222
Sakarya O, Armstrong KA, Adamska M, Adamski M, Wang IF, Tidor B, Degnan BM, Oakley TH, Kosik KS (2007) A post-synaptic scaffold at the origin of the animal kingdom. PLoS ONE 2(6):e506
Salensky W (1886) Die Urform der Heteroplastiden. Biol Centralbl 6:514–525
Saller U (1988) Oogenesis and larval development of *Ephydatia fluviatilis* (Porifera, Spongillidae). Zoomorphology 108:23–28
Saller U (1990) Formation and construction of asexual buds of the freshwater sponge *Radiospongilla cerebellata* (Porifera, Spongillidae). Zoomorphology 109:295–301
Saller U, Weissenfels N (1985) The development of *Spongilla lacustris* from the oocyte to the free larva (Porifera, Spongillidae). Zoomorphology 105:367–374
Salomon D, Barthel D (1990) External choanosome morphology of the hexactinellid sponge *Aulorossella vanhoeffeni* Schulze and Kirkpatrick (1910) A preliminary report. Sencken Mar 21:87–99
Salvini-Plaven LV (1978) On the origin and evolution of the lower Metazoa. Z Zool Syst Evol 16:479–490
Salvini-Plawen L, Splechtna H (1979) Zur Homologie der Keimblatter. Z Zool Syst Evol 17:77–81
Sander K (1983) The evolution of patterning mechanisms: gleanings from insect embryogenesis and spermatogenesis. In: Goodwin BC, Holder N, Wylie CG (eds) Development and evolution. Cambridge University Press, Cambridge
Sanderson MJ (1984) Cilia. In: Bereiter-Hahn MAG, Richards J, Sylvia K (eds) Biology of the integument. Springer-Verlag, Berlin
Sarà M (1955) La nutrizione dell'ovocita in Calcispongie Omoceli. Ann Istit Mus Zool Univ Napoli 7:1–30
Sarà M (1961) Ricerche sui gonochorismo ed ermafroditismo nei poriferi. Boll Zool 28:47–59
Sarà M (1974) Sexuality in the Porifera. Boll Zool 41:327–348
Sarà M (1993) Porifera. In: Adiyodi KG, Adiyodi RG (eds) Reproductive biology of invertebrates, vol 5. Sexual differentiation and behaviour. Wiley, Chichester
Sarà M, Vacelet J (1973) Écologie des Demosponges. In: Grasse PP (ed) Traite de Zoologie. T. 1. N 3, Maison Cie, Paris
Sarà M, Relini Orsi LR (1975) Sex differentiation in *Sycon* (Porifera, Calcispongiae). Pub Staz Zool Napoli I: Mar Ecol 39:618–634
Sarà A, Cerrano C, Sarà M (2002) Viviparous development in the Antarctic sponge *Stylocordyla borealis* Loven, 1868. Polar Biol 25:425–431
Sauer FC (1935) Mitosis in the neural tube. J Comp Neurol 62:377–405
Scalera Liaci L, Sciscioli M (1970) The sexual cycle of *Erylus discophorus* (Schmidt) (Porifera Tetractinellida). Riv Biol 63:255–270
Scalera-Liaci L, Sciscioli M (1979) La riproduzione sessuale in *Suberites carnosus* (Johnston) (Porifera). In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris
Scalera Liaci L, Sciscioli M, Matarrese A, Giove C (1971) Osservazioni sui cicli sessuali di alcune Keratosa (Porifera) e loro interesse negli studi filogenetici. Atti Soc Pelorit Sci Fisiche Mat E Natur 17:33–52
Scalera Liaci L, Sciscioli M, Matarrese A (1973) Raffronto tra il comportamento sussuale di alcune Ceractinomorpha. Riv Biol 66:135–162
Scalera Liaci L, Sciscioli M, Piscitelli G (1976) The sexual reproduction of *Tetilla* sp. (Tetractinellida – Porifera). Riv Biol 69:331–342
Schäcke H, Rinkevitch B, Gamulin V, Müller I, Müller WEG (1994) Immunoglobulin-like domain is present in the extracellular part of the receptor tyrosin kinase from the marine sponge *Geodia cydonium*. J Mol Rec 7:272–276
Schill RO, Pfannkuchen M, Fritz G, Kohler H, Brummer F (2006) Quiescent Gemmules of the Freshwater Sponge, *Spongilla lacustris* (Linnaeus, 1759), contain remarkably high levels of Hsp70 stress protein and hsp70 stress gene mRNA. J Exp Zool 305A:449–457

Schreiber A, Wörheide G, Thiel V (2006) The fatty acids of calcareous sponges (Calcarea, Porifera). Chem Phys Lipids 143:29–37
Sharp KH, Eam B, Faulkner DJ, Haygood MG (2007) Vertical transmission of diverse microbes in the tropical sponge *Corticium* sp. Appl Environ Microbiol 73:622–629
Schmidt O (1866) Zweites Supplement der Spongien des Adriatischen Meeres enthaltend die Vergleichung der Adriatischen und Britischen Spongiengattungen. Verlag von Wilhelm Engelmann, Leipzig
Schmidt O (1868) Die Spongien der Küste von Algier mit Nachträgen zu den Spongien des Adriatischen Meeres (drittes Supplement). Verlag Wilhelm Engel, Leipzig
Schmidt O (1877) Das Larvenstadium von *Ascetta primordialis* und *Ascetta clathrus*. Arch Mikr Anat 14:249–263
Schmitt S, Wehrl M, Bayer K, Siegl A, Hentschel U (2007a) Marine sponges as models for commensal microbe–host interactions. Symbiosus 44:43–50
Schmitt S, Weisz JB, Lindquist N, Hentschel U (2007b) Vertical transmission of a phylogenetically complex microbial consortium in the viviparous sponge *Ircinia felix*. Appl Environ Microb 73:2067–2078
Scholtz G (2004) Baupläne versus ground patterns, phyla versus monophyla: aspects of patterns and processes in evolutionary developmental biology. In: Scholtz G (ed) Evolutionary developmental biology of Crustacea. Balkema, Lisse
Scholtz G (2005) Homology and ontogeny: pattern and process in comparative developmental biology. Theory Biosci 124:121–143
Schroeder TE (1978) Microvilli on sea urchin eggs: a second burst of elongation. Dev Biol 64:342–346
Schulman SS (1974) The problem of the Metazoa origin. In: Ivanov AV (ed) Theoretical problems of the taxonomy and phylogeny of animals. Nauka, Leningrad
Schulze FE (1875) Uber den bau und Entwicklung von *Sycandra raphanus* Haeckel. Z Wis Zoll 25:247–280
Schulze FE (1877) Untersuchungen über den bau und Entwicklung der Spongien. Die Gattung *Halisarca*. Z Wiss Zool 28:1–48
Schulze FE (1878a) Recherches sur l'anatomie et le développement des éponges. Les métamorphoses de *Sycandra raphanus.* Arch Zool Exp Gén 1: XXI–XXV
Schulze FE (1878b) Untersuchungen über den Bau und die Entwicklung der Spongien. IV. Die Familie der Aplysinidae. Z Wiss Zool 30:379–420
Schulze FE (1879a) Untersuchungen über den bau und Entwicklung der Spongien. Die Gattung *Spongelia*. Z Wiss Zool 32:117–157
Schulze FE (1879b) Untersuchungen über den bau und Entwicklung der Spongien. Die Familie der Spongiidae. Z Wiss Zool 32:593–660
Schulze FE (1879c) Uber die Bildung freischwebender Brutknospen bei einer Spongie, *Halisarca lobularis*. Zool Anz 2:636–641
Schulze FE (1880a) On the structure and arrangement of the soft parts in *Euplectella aspergillum.* Trans R Soc Edinb 29:661–673
Schulze FE (1880) Untersuchungen über den bau und Entwicklung der Spongien. Die Plakiniden. Z Wiss Zool 34:407–451
Schulze FE (1881) Untersuchungen über den bau und Entwicklung der Spongien. Zehnte Mittheilung *Corticium candelabrum* O. Schmidt. Z Wiss Zool 35:410–430
Schulze FE (1887) Report on the Hexactinellida collected by H.M.S. Challenger during the years 1873–1876. Majesty's stationery Office, London
Schulze FE (1899) Zur Histologie der Hexactinelliden. Sitz Ber K Pr Akad Wiss Berlin 14:198–209
Schütze J, Krasko A, Diehl-Seifert B, Müller WEG (2001) Cloning and expression of the putative aggregation factor from the marine sponge *Geodia cydonium*. J Cell Sci 114:3189–3198
Sciscioli M, Lepore E, Scalera-Liaci L, Gherardi M (1989) Indagine ultrastrutturale sugli ovociti di *Erylus discophorus* (Schmidt) (Porifera, Tetractinellida). Oebalia 15:939–941
Sciscioli M, Scalera-Liaci L, Lepore E, Gherardi M, Simpson TL (1991) Ultrastructural study of the mature egg of the marine sponge *Stelletta grubii* (Porifera Demospongiae). Mol Rep Dev 28:346–350

Sciscioli M, Lepore E, Gherardi M, Scalera-Liaci L (1994) Transfer of symbiotic bacteria in the mature oocyte of *Geodia cydonium* (Porifera, Demospongiae): an ultrastructural study. Cah Biol Mar 35:471–478

Sciscioli M, Lepore E, Corriero G, Scalera-Liaci L, Gaino E (1997) Ultrastructural organization of choanocyte chambers in the haplosclerid *Pellina semitubulosa* (Porifera, Demospongiae): a cue for water flow into the sponge body. Ital J Zool 64:291–296

Sciscioli M, Ferri D, Liquori GE, Lepore E, Santarelli G (2000) Lectin histochemistry and ultrastructure of microgranular cells in *Cinachyra tarentina* (Porifera, Demospongiae). Acta Histochem 102:219–230

Sciscioli M, Lepore E, Mastrodonato M, Scalera-Liaci L, Gaino E (2002) Ultrastructural study of the mature oocyte of *Tethya aurantium* (Porifera: Demospongiae). Cah Biol Mar 43:1–7

Seimya M, Ishiguro H, Miura K, Watanabe Y, Kurosawa Y (1994) Homeobox-containing genes in the most primitive metazoa, the sponges. Eur J Biochem 221:219–225

Seimiya M, Watanabe Y, Kurosawa YAD (1997) Identification of POU-class homeobox genes in a freshwater sponge and the specific expression of these genes during differentiation. Eur J Biochem 243:27–31

Selenka E (1879) Ueber einen Kieselschwamm von achtstrahligen Bau und über Entwicklung der Schwammknospen. Zeitschr Wiss Zool 3:467–476

Seravin LN (1986) The nature and the origin of the Spongia. In: Krylov MV (ed) Systematics of protozoa and their phylogenetic links with lower eukariotes, vol 144. Proceedings of the Zoological Institute, USSR Academy of Science, Leningrad

Seravin LN (1992) Peculiarities of organization and embrional [sic] development in Spongia. Vestnik Saint-Peterburg Univ 2:13–28

Seravin LN (1997) The modern notions on the place of sponges in the system of eukaryotes. Vestnik St Petersburg Univ 3:34–41

Shigemori H, Myung-Ae B, Yazawa K, Sasaki T, Kobayashi J (1992) Alteramide A, a new tetracyclic alkaloid from a bacterium *Alteromonas* sp. associated with the marine sponge *Halichondria okadai*. J Org Chem 57:4317–4320

Seidel F (1960) Körpergrundgestalt und Keimstruktur: eine Erörterung über die Grundlagen der vergleichenden und experimentellen Embryologie und deren Gültigkeit bei phylogenetischen Überlegungen. Zool Anz 164:245–305

Sidri M, Milanese M, Bummer F (2005) First observations on egg release in the oviparous sponge *Chondrilla nucula* (Demospongiae, Chondrosida, Chondrillidae) in the Mediterranean Sea. Invert Biol 124:91–97

Siewing R (1979) Homology of cleavage-types? Z Zool Syst Evol 1:7–18

Simpson TL (1963) The biology of the marine sponge *Microciona prolifera* (Ellis and Solander). I. A study of cellular function and differentiation. J Exp Zool 154:135–152

Simpson TL (1968) The biology of the marine sponge *Microciona prolifera* (Ellis and Sollander). II. Temperature-related annual chamges in functional and reproductive elements with a discription of larval metamorphosis. J Exp Mar Biol Ecol 2:252–277

Simpson TL (1984) The cell biology of sponges. Springer, New York

Simpson TL, Fell PE (1974) Dormancy among the Porifera: gemmule formation and germination in fresh-water and marine sponges. Trans Am Microsc Soc 93:544–577

Simpson TL, Gilbert JJ (1973) Gemmulation, gemmule hatching and sexual reproduction in freshwater sponges. I. The life cycle of *Spongilla lacustris* and *Tubella pennsylvanica*. Trans Am Microsc Soc 92:422–433

Simpson TL, Vaccaro CA (1974) An ultrastructural study of silica deposition in the freshwater sponge *Spongilla lacustris*. J Ultra Res 47:296–309

Sipkema D, van Wielink R, van Lammeren AAM, Tramper J, Osinga R, Wijffels RH (2003) Primmorphs from seven marine sponges: formation and structure. J Biotech 100:127–139

Sizova NA, Ereskovsky AV (1997) Ultrastructural peculiarities of the early embryogenesis in a White Sea Sponge *Halisarca dujardini* (Demosponge, Dendroceratida). In: Ereskovsky AV, Keupp H, Kohring HR (eds) Modern problems of Poriferan biology. Berliner Geowiss Abh, Freie University, Berlin

Slack JMW, Holland PMH, Graham CF (1993) The zootype and the zootypic stage. Nature 361:490–492
Sollas WJ (1884) On the development of *Halisarca lobularis* (O.Schmidt). Quart J Microsc Sci 24:603–621
Sollas WJ (1885) A classification of the sponges. Ann Mag Nat Hist 16:395
Sollas WJ (1888) Report on the Tetractinellida collected by H.S.M. Challenger during the years 1873–1876. Rep Sci Res Voyage Challenger Zool 25:1–458
Sperling EA, Pisani D, Peterson KJ (2007) Poriferan paraphyly and its implications for Precambrian palaeobiology. Geol Soc Lond Spec Publ 286:355–368
Spetland F, Rapp HT, Hoffmann F, Tendal OS (2007) Sexual reproduction of *Geodia barretti* Bowerbank, 1858 (Porifera, Astrophorida) in two Scandinavian fjords. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera research: biodiversity, innovation and sustainability. Museu Nacional, Rio de Janeiro 28
Steiner M, Mehl D, Reitner J, Erdtmann BD (1993) Oldest entirely preserved sponges and other fossils from the lowermost Cambrian and new facies reconstruction of the Yangtze platform (China). Berliner Geowiss Abh 9:293–329
Stierle DB, Stierle AA (1992) Pseudomonic acid derivatives from a marine bacterium. Experientia 48:1165–1169
Strathmann RR (1978) The evolution and loss of feeding larvae stages of marine invertebrates. Study of larval settlement in the sea. Evolution 32:894–906
Strathmann RR, Vedder K (1977) Size and organic content of eggs of echinoderms and other invertebrates as related to developmental strategies and egg eating. Mar Biol 39:305–309
Stricker SA (1985) An ultrastructural anemone *Urticina crassicornis* (Cnidaria, Actiniaria). J Morphol 186:237–253
Sukhodolskaya AN, Ivanova LV (1988) Fine structural investigation of the fresh-water sponge *Spongilla lacustris* swimmmg larva. Tsitologiya 30:1409–1417
Sukhodolskaya AN, Papkovskaya MV (1985) Electron microscopical study of spermatogenesis in the fresh-water sponges, *Ephydatia fluviatilis* and *Spongilla lacustris*. Tsitologiya 27:297–302
Tanaka-Ichihara K, Watanabe Y (1990) Gametogenic cycle in *Halichondria okadaii*. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
Tardent P (1978) Coelenterata, Cnidaria. In: Seidel F (ed) Morphogenese der Tiere, erste Reihe, Deskriptive Morphogenese. Lieferung 1, A-I. Gustav Fischer Verlag, Jena
Taylor MW, Schupp PJ, Dahllöf I, Kjelleberg S, Steinberg PD (2004) Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. Environ Microbiol 6:121–130
Taylor MW, Radax R, Steger D, Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol Mol Biol Rev 71:295–347
Technau U, Scholz CB (2003) Origin and evolution of endoderm and mesoderm. Int J Dev Biol 47:531–539
Thiney Y (1972) Morphologie et cytochimie ultrastructurale de l'oscule *d'Hippospongia communis* Lmk et de sa regeneration: These doctorate, Lyon
Thompson JE, Barrow KD, Faulkner DJ (1983) Localization of 2 Br-metabolites, aerothionin and homoaerothionin, in spherulous cells of marine sponge *Aplysina fistularis* (=*Verongia thiona*). Acta Zool 64:199–210
Tokina DB (1985) Gametogenesis of the white sea sponge *Myxilla Incrustans* (Demospongiae, Poecilosclerida). In: Cherepanov G (ed) The organism in onto- and phylogenesis. VINITI, Moscow
Topsent E (1888) Sur les gemmules de quelques silicisponges marines. C R Acad Sci Paris 106:1298–1300
Topsent E (1892) Notes histologiques au sujet de *Leucosolenia coriacea* (Mont.) Bwk. Bull Soc Zool Fr 17:125–129
Topsent E (1903) Sur les larves cuirassées de *Thoosa armata*. Arch Zool Exp Gén 1 (Notes et Revues 1):1–3
Topsent E (1904) Spongiaires des Açores. Résultats des Campagnes Scientifiques accomplies par le du Prince Albert Ier de. Monaco 25:1–280

Topsent E (1911) Sur les affinités des *Halichondria* et la classification des Halichondrines d'après leurs formes larvaires. Arch Zool Exp Gén 7:1–15
Topsent E (1920) Caractères et affinités des *Thoosa* Hanc. et des *Alectona* Cart. Considérations sur leurs germes à armure. Bull Soc Zool Fr 45:89–97
Tregouboff G (1939) Sur les larves planctoniques d'éponges. C R Acad Sci Paris 208:1245–1246
Tregouboff G (1942) Contribution à la connaissance des larves planctoniques d'éponges. Arch Zool Exp Gén 82:357–399
Tripepi SM, Longo O, La Camera R (1984) A new pattern of spermiogenesis in the sponge *Crambe crambe*: preliminary observations in electron microscopy. In: Csanady A, Rehlich P, Szaby E (eds) Eight European Congress on Electron Microscopy. Programme Committee, Budapest
Tuzet O (1932) Recherches sur l'histologie des éponges *Reniera elegans* (Bow) et *Reniera simulans* (Johnston). Arch Zool Exp Gèn 74:169–192
Tuzet O (1947) L'ovogenése et la fécondation de l'éponge calcaire *Leucosolenia coriacea* et de l'éponge siliceuse *Reniera elegans*. Arch Zool Exp Gén 85:127–148
Tuzet O (1948) Les premieres stades du développement de *Leucosolenia botrioides* Ellis et Sollander et de *Clathrina* (*Leucosolenia*) *coriacea* Mont. Ann Sci Nat 10:103–114
Tuzet O (1963) The phylogeny of sponges according to embryological, histological, and serological data, and their affinities with the Protozoa and the Cnidaria. In: Dougherty E (ed) The lower metazoa. Comparative biology and phylogeny. University of California Press, Berkeley
Tuzet O (1970) La polarité de l'oeuf et la symétrie de la larve des éponges calcaires. In: Fry WG (ed) The biology of the Porifera, vol 25. Symposium of the Zoological Society, London
Tuzet O (1973a) Eponges calcaires. Spongiaires. In: Grass PP (ed) Traite de Zoologie. T. 1. N 3, Maison Cie, Paris
Tuzet O (1973b) Hexactinellides ou hyalosponges. Spongiaires In: Grass PP (ed) Traite de Zoologie. T. 1. N 3, Maison Cie, Paris
Tuzet O, Paris J (1964) La spermatogenése, l'ovogenése, la fécondation et les premiers stades du développement chez *Octavella galangaui*. Vie et Milieu 15:309–327
Tuzet O, Pavans de Ceccatty M (1958) La spermatogenèse, l'ovogenèse, la fécondation et les premiers stades du développement d'*Hippospongia communis* LMK. (= *H. equina* O.S.). Bull Biol Fr Belg 92:1–18
Tuzet O, Garrone R, Pavans de Ceccatty M (1970a) Origine choanocytaire de la lignèe germinale male chez la Démosponge *Aplysilla rosea* Sch. (Dendroceratides). C R Acad Sci Paris 270:955–957
Tuzet O, Garrone R, Pavans de Ceccatty M (1970b) Observations ultrastructurales sur la spermatogenése chez la démosponge *Aplysilla rosea* Sch. (Dendroceratide): une métaplaise exemplaire. Ann Sci Nat 12:27–50
Tyler S (2003) Epithelium – the primary building block for metazoan complexity. Integr Comp Biol 43:55–63
Uriz MJ (1982a) Reproduccion en *Hymeniacidon sanguinea* (Grant, 1926): Biologia de la larva y primeros estadios postlarvarios. Invest Pesquera 46:29–39
Uriz MJ (1982b) Morfologia y comportamiento de la larva parenquimula de *Scopalina lophyropoda* Schmidt 1982 (Demospongia, Halichondrida) y formacion del rhagon. Invest Pesquera 46:313–322
Uriz MJ (2006) Mineral skeletogenesis in sponges. Can J Zool 84:322–356
Uriz MJ, Becerro MA, Tur JM, Turton X (1996) Location of toxicity within the Mediterranean sponge *Crambe crambe* (Demospongiae, Poecilosclerida). Mar Biol 124:583–590
Uriz MJ, Maldonado M, Turon X, Marti R (1998) How do reproductive output, larval behaviour, and recruitment contribute to adult spatial patterns in Mediterranean encrusting sponges? Mar Ecol Progr Ser 167:137–148
Uriz MJ, Turon X, Becerro MA (2000) Silica deposition in Demosponges: spiculogenesis in *Crambe crambe*. Cell Tissue Res 301:299–309
Uriz MJ, Turon X, Becerro MA (2001) Morphology and ultrastructure of the swimming larvae of *Crambe crambe* (Demospongiae, Poecilosclerida). Invert Biol 120:295–307

Uriz MJ, Turon X, Becerro M (2003) Silica deposition in Demosponges. In: Müller WEG (ed) Silicon biomineralisation. Springer, Berlin/Heidelberg

Uriz MJ, Turon X, Mariani S (2008) Ultrastructure and dispersal potential of sponge larvae: tufted versus evenly ciliated parenchymellae. Mar Ecol 29:280–297

Usher KM, Kuo J, Fromont J, Sutton DC (2001) Vertical transmission of cyanobactreial symbionts in the marine sponge *Chondrilla australiensis* (Demospongiae). Hydrobiologia 461:15–23

Usher K, Ereskovsky AV (2005) Larval development, ultrastructure and metamorphosis in *Chondrilla australiensis* Carter, 1873 (Demospongiae, Chondrosida, Chondrillidae). Invert Rep Dev 47:51–62

Usher KM, Sutton DC, Toze S, Kuoc J, Fromont J (2004) Sexual reproduction in *Chondrilla australiensis* (Porifera: Demospongiae). Mar Fresh Res 55:123–134

Usher KM, Sutton DC, Toze S, Kuoc J, Fromon J (2005) Inter-generational transmission of microbial symbionts in the marine sponge *Chondrilla australiensis* (Demospongiae). Mar Fresh Res 56:125–131

Vacelet J (1964) Ètude monographique de l'éponge calcaire Pharétronide de Méditerranée. *Petrobiona massiliana* Vacelet et Lévi. Les Pharétronides actuelles et fossils. Rec Trav Stat Mar Endoume 34:1–125

Vacelet J (1967) Les cellules à inclusions de l'éponge cornée *Verongia cavernicola* Vacelet. J Microsc (Paris) 6:237–240

Vacelet J (1970) Description de cellules a bactéries intranucleaires chez des éponges *Verongia*. J Microsc (Paris) 9:333–346

Vacelet J (1975) Étude en microscopie électronique de l'association entre bactéries et spongiaires du genre *Verongia* (Dictyoceratida). J Microsc Biol Cell 23:271–288

Vacelet J (1977) Une nouvelle relique du Secondaire: un représentant actuel des Eponges fossiles Sphinctozoaires. C R Acad Sci Paris 285:509–511

Vacelet J (1979a) La place des Spongiaires dans les systèmes trophiques marins. In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris

Vacelet J. (1979b) Quelques stades de la reproduction sexuée d'une éponge sphinctozoaire actuelle. In: Lévi C, Boury-Esnault N (eds) Biologie des spongiaures. Colloques Internationaux. CNRS 291, Paris

Vacelet J (1985) Coralline sponges and the evolution of Porifera. In: Conway Morris S, George JD, Gibson R, Platt H M (eds) The origins and relationships of lower invertebrates. The Systematics Association, vol 28. Clarendon Press, Oxford

Vacelet J (1990) Storage cells of calcified relict sponges. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC

Vacelet J (1999) Planktonic armoured propagules of the excavating sponge *Alectona* (Porifera: Demospongiae) are larvae: evidence from *Alectona wallichii* and *A. mesatlantica* sp. nov. Mem Queensl Mus 44:627–642

Vacelet J (2006) New carnivorous sponges (Porifera, Poecilosclerida) collected from manned submersibles in the deep Pacific. Zool J Linn Soc 148:553–584

Vacelet J (2007) Diversity and evolution of deep-sea carnivorous sponges. In: Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G (eds) Porifera Research: Biodiversity, Innovation and Sustainability. Museu Nacional, Rio de Janeiro 28

Vacelet J, Boury-Esnault N (1995) Carnivorous sponges. Nature 373:333–335

Vacelet J, Boury-Esnault N (1996) A new species of carnivorous sponge (Demospongiae, Cladorhizidae) from a Mediterranean cave. Bull Inst Sci Nat Belg Biol 66:123–140

Vacelet J, Donadey C (1977) Electron microscope study of the association between some sponges and bacteria. J Exp Mar Biol Ecol 30:301–314

Vacelet J, Donadey C (1987) A new species of *Halisarca* (Porifera, Demospongiae) from the Caribbean with remarks on the cytology and affinities of the genus. In: Jones WC (ed) European contribution to the taxonomy of sponges. Litho Press Co, Middleton

Vacelet J, Duport E (2004) Prey capture and digestion in the carnivorous sponge *Asbestopluma hypogea* (Porifera: Demospongiae). Zoomorphology 123:179–190

Vacelet J, Boury-Esnault N, De Vos L, Donadey C (1989) Comparative study of the choanosome of Porifera: II The Keratose sponges. J Morphol 201:119–129

Vacelet J, Boury-Esnault N, Fiala-Médioni A, Fischer CR (1995) A methanotrophic carnivorous sponge. Nature 377:296

Vacelet J, Fiala-Médioni A, Fisher CR, Boury-Esnault N (1996) Symbiosis between methane-oxidising bacteria and a deep-sea carnivorous cladorhizid sponge. Mar Ecol Progr Ser 145:77–85

Vacelet J, Vasseur P, Lévi C (1976) Spongiaires de la pente externe des récifs coralliens de Tuléar (Sud-Ouest de Madagascar). Mém Mus Nat Hist A Zool 49:1–116

Valentine JW (1997) Cleavage patterns and the topology of the metazoan tree of life. Proc Natl Acad Sci USA 94:8001–8005

van de Vyver G, Barbieux B (1983) Cellular aspects of allograft rejection in marine sponges of the genus *Polymastia*. J Exp Zool 227:1–7

van de Vyver G, Willenz P (1975) An experimental study of the life cycle of the fresh-water sponge *Ephydatia fluviatilis* in its natural surroundings. Wilhelm Roux's Arch 177:41–52

van Soest RWM (1984) Deficient *Merlia normani* Kirkpatrick, 1908, from the Curaçao Reefs, with a discussion of the phylogenetic interpretation of Sclerosponges. Bijdr Dierkd 53:211–219

van Soest RWM (1990) Toward a phylogenetic classification of sponges. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC

van Soest RWM (1991) Demosponge higher taxa classification re-examined. In: Reitne J, Keupp H (eds) Fossil and recent sponges. Springer, Berlin

van Soest RWM and Hooper JNA (2002) Order Halichondrida Gray, 1867. In: Hooper JNA, van Soest RWM (eds) Systema Porifera: a guide to the classification of sponges. Kluwer/ Plenum, New York

Vasseur G (1879) Reproduction asexuelle de la *Leucosolenia botryoides* (*Ascandra variabilis*). Archs Zool Exp Gén 8:59–66

Vethaak AD, Cronie RJ, van Soest RWM (1982) Ecology and distribution of two sympatric, clousely related sponge species, *Halichondria panicea* (Pallas, Van Honten, 1990, 1766) and *H. bowerbanki* Burton, 1930 (Porifera, Demospongiae), with remarks on their speciati on. Bijdrag Dierkunde 52:82–102

Vishnyakov AE, Ereskovsky AV (2009) Bacterial symbionts as an additional cytological marker for identification of sponges without a skeleton. Mar Biol 156:1625–1632

Volkova MA, Zolotarjeva GA (1981) The development of *Halisarca dujardini* Johnston from conglomerates of somatic cells. In: Korotkhova GP (ed) Morphogenesis in sponges. Leningrad University Press, Leningrad

Vosmaer GCJ (1882) Report on the sponges dredged up in the Arctic Sea by the "Willem Barents" in the years 1878 & 1879. Niederl Arch Zool Suppl 1:1–58

Wachtmann D, Stockem W (1992a) Significance of the cytoskeleton for cytoplasmic organization and cell organelle dynamics in epithelial cells of fresh-water sponges. Protoplasma 169:107–119

Wachtmann D, Stockem W (1992b) Microtubule based and microfilament based dynamic activities of the endoplasmic-reticulum and the cell-surface in epithelial-cells of *Spongilla lacustris* (Porifera, Spongillidae). Zoomorphology 112:117–124

Wachtmann D, Stockem W, Weissenfels N (1990) Cytoskeletal organisation and cell organelle transport in basal epithelial cells of the freshwater sponge *Spongilla lacustris*. Cell Tiss Res 261:145–154

Wapstra M, van Soest RWM (1987) Sexual reproduction, larval morphology and bechaviour in Demosponges from the southwest of the Netherlands. In: Boury-Esnault N, Vacelet J (eds) Taxonomy of Porifera. NATO ASI Ser vol 13, Springer, Berlin

Warburton F (1961) Inclusion of parental somatic cells in sponge larvae. Nature 191:1317

Watanabe Y (1957) Development of *Tetilla serica* Lebwohl, a tetraxonian sponge. 1. Observations on external changes. Nat Sci Rep Ochanomizu Univ 8:97–104

Watanabe Y (1978a) Structure and formation of the collar in choanocytes of *Tetilla serica* (Lebwohl), Demosponge. Dev Growth Diff 20:79–91

Watanabe Y (1978b) The development of two species of *Tetilla* (Demosponge). Nat Sci Rep Ochanomizu Univ 29:71–106

Watanabe Y, Masuda Y (1990) Structure of fiber bundles in the egg of *Tetilla japonica* and their possible function in development. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC

Watanabe Y, Okada K (1998) The involvment of two carrier cells in fertilization and the ultrastructure of the spermiocyst in *Sycon calcaravis*. In: Watanabe Y, Fusetani N (eds) Sponge sciences. Multidisciplinary perspectives. Springer, Tokyo

Webster G, Hamilton S (1972) Budding in *Hydra*: the role of cell multiplication and cell movement in bud initiation. J Embryol Exp Morphol 27:301–316

Weissenfels N (1978) Bau und Funktion des Süßawasserschwamms *Ephydatia fluvialis* (Porifera). V. Das Nadelskelet und seine Entstchung. Zool Jb Anat 99:211–223

Weissenfels N (1980) Bau und Funktion des Süßawasserschwamms *Ephydatia fluvialis* (Porifera). VII. Die Porocyten. Zoomorphologie 95:27–40

Weissenfels N (1981) Bau und Funktion des Süßawasserschwamms *Ephydatia fluvialis* (Porifera). VIII. Die Entstehung und Entwicklung der Kragengeißelkammern und ihre Verbindung mit dem ausführenden Kanalsystem. Zoomorphology 98:35–45

Weissenfels N (1989) Biologie und microscopishe Anayomie der Süßwassershwämme (Spongillidae). Fisher, Stuttgart, New York

Wells HW, Wells MJ, Gray IE (1964) Ecology of sponges in Hatteras harbor, North Carolina. Ecology 45:752–767

Weyrer S, Rützler K, Rieger R (1999) Serotonin in Porifera? Evidence from developing *Tedania ignis*, the Caribbean fire sponge (Demospongiae). Mem Queensl Mus 44:659–665

Whalan S, Battershill C, de Nys R (2007) Sexual reproduction of the brooding sponge *Rhopaloeides odorabile*. Coral Reefs 26:655–663

Wielsputz C, Saller U (1990) The metamorphosis of the parenchymula-larva of *Ephydatia fluviatilis* (Porifera, Spongillidae). Zoomorphology 109:173–177

Wiens M, Mangoni A, D'Esposito M, Fattorusso E, Korchagina N, Schroder HC, Grebenjuk VC, Krasko A, Batel R, Müller IM, Müller WEG (2003) The molecular basis for the evolution of the metazoan bodyplan: extracellular matrix-mediated morphogenesis in marine Demosponges. J Mol Evol 57:S60–S75

Wilkinson CR (1987) Interocean differences in size and nutrition of coral reef sponge population. Science 236:1654–1657

Wilkinson CR, Garrone R (1980) Ultrastructure of siliceous spicules and microsclerocytes in the marine sponge *Neofibularia irata* n. sp. J Morphol 166:51–64

Wilkinson C, Garrone R, Herbage D (1979) Sponge collagen degradation *in vitro* by sponge-specific bacteria. In: Lévi C, Boury-Esnault N (eds) Biologie des Spongiaires. Colloques Internationaux. CNRS 291, Paris

Willenz P (1982) Exocytose chez l'éponge d'eau douce *Ephydatia fluviatilis* et chez l'éponge marine *Hemimycale columella*. Biol Cell 45:23–34

Willenz P (1984) Ultrastructural localization of lysosomal digestion in the freshwater sponge *Ephydatia fluviatilis*. J Ultrastruct Res 87:13–22

Willenz P, Hartman WD (1989) Micromorphology and ultrastructure of Caribbean sclerosponges. I. *Ceratoporella nicholsoni* and *Stromatospongia norae* (Ceratoporellidae: Porifera). Mar Biol 103:387–401

Willenz P, van de Vyver G (1982) Endocytosis of latex beads by the exopinacoderm in the fresh water sponge *Ephydatia fluviatilis*: an *in vitro* and *in situ* study in SEM and TEM. J Ultrastruct Res 79:294–306

Willenz P, van de Vyver G (1984) Ultrastructural localization of lysosomal digestion in the freshwater sponge *Ephydatia fluviatilis*. J Ultrastruct Res 87:13–22

Williamson CE (1979) An ultrastructural investigation of algal symbiosis in white and green *Spongilla lacustris* (L.) (Porifera: Spongillidae). Trans Am Microsc Soc 98:59–77

Willmer P (1990) Invertebrate relationships: patterns in animal evolution. Cambridge University Press, Cambridge

Wilson HV (1907) On some phenomena of coalescence and regeneration in sponges. J Exp Zool 5:245–258

Wilson HV (1935) Some critical points in the metamorphosis of the halichondrine sponge larva. J Morphol 58:285–353
Wilson HV, Penney JT (1930) A new variety of the Hexactinellid sponge *Rhabdocalyptus dawsoni* (Lambe) and the species of *Rhabdocalyptus*. Proc US Natl Mus 76:1–9
Wintermann G (1951) Entwicklungsphysiologishe Untersuchungen an Susswassershwammen. Zool Jarhb Abt Anat 71:427–486
Witte U (1996) Seasonal reproduction in deep-sea sponges – triggered by vertical particle flux? Mar Biol 124:571–586
Witte U, Barthel D (1994) Reproductive cycle and oogenesis of *Halichondria panicea* (Pallas) in Kiel Bight. In: van Soest RWM, van Kempen TMG, Braekman JC (eds) Sponges in time and space. Balkema, Rotterdam/Amsterdam
Wolpert L (1990) The evolution of development. Biol J Linn Soc 39:109–124
Wolpert L (1994) The evolutionary origin of development: cycles, pattering, privilege and continuity. Dev Suppl:79–84
Wood RA (1991) Problematic reef-building sponges. In: Simonetta AM, Conway Morris S (eds) The early evolution of Metazoa and the significance of problematic taxa. Cambridge University Press, Cambridge
Woollacott RM (1990) Structure and swimming behavior of the larva of of *Halichondria melanadocia* (Porifera, Demospongiae). J Morphol 205:135–145
Woollacott RM (1993) Structure and swimming behavior of the larva of *Haliclona tubifera* (Porifera: Demospongiae). J Morphol 218:301–321
Woollacott RM (2003) Spicule content in larvae of two species of Demosponge (Porifera). Spec Divers 8:203–217
Woollacott RM, Hadfield MG (1989) Larva of the Sponge *Dendrilla cactus* (Demospongiae: Dendroceratida). Trans Am Microsc Soc 108:410–413
Woollacott RM, Pinto RL (1995) Flagellar basal apparatus and its utility in phylogenetic analyses of the Porifera. J Morphol 226:247–265
Woollacott RM, Zimmer RL (1978) Metamorphosis of cellularioid bryozoans. In: Chia FS, Rice ME (eds) Settlement and metamorphosis of marine invertebrate larvae. Elsevier, New York
Wörheide G (1998) The reef cave dwelling ultraconservative coralline demosponge *Astrosclera willeyana* Lister 1900 from the Indo-Pacific. Facies 38:1–88
Wörheide G (2008) A hypercalcified sponge with soft relatives: *Vaceletia* is a keratose demosponge. Mol Phyl Evol 47:433–438
Wray GA (1997) Echinoderms. In: Gilbert SF, Raunio AM (eds) Embryology. Constructing the organism. Sinauer, Sunderland, MA
Wulff JL (1990) Patterns and processes of size change in Caribbean demosponges of branching morphology. In: Rützler K (ed) New perspectives in sponge biology. Smithsonian Institution Press, Washington, DC
Wyeth RC (1999) Video and electron microscopy of particle feeding in sandwich cultures of the hexactinellid sponge, *Rhabdocalyptus dawsoni*. Invert Biol 118:236–242
Yamasaki A, Watanabe Y (1991) Involvement of maternal choanocyte during embryogenesis in *Sycon calcar-avis*, Calcarea, Porifera. Zool Sci 8:1107
Yourassowsky C, Rasmont R (1983) The differentiation of sclerocytes in fresh-water sponges grown in a silica- poor medium. Differentiation 25:5–9
Zakhvatkin AA (1949) Comparative embryology of lower invertebrates: sources and pathways of the evolution of individual development of metazoans. Nauka, Moscow
Zavarzin AA (1945) Essays of evolutionary histology of blood and connective tissues. Nauka, Moskow/Leningrad
Zhang X, Cao X, Zhang W, Yu X, Jin M (2003) Primmorphs from archaeocytes- dominant cell population of the sponge *Hymeniacidon perleve*: improved cell proliferation and spiculogenesis. Biotech Bioeng 84:583–585
Zhuravleva IT, Miagkova EI (1987) Phanerozoic primitive multicellulars. Nauka, Moscow
Zrzavy J, Mihulka S, Kepka P, Bezdek A (1998) Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. Cladistics 14:49–285

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