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Larval development in the Homoscleromorpha (Porifera, Demospongiae)

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Abstract. Embryonic development from coeloblastula to fully developed larva was investigated in 8 Mediterranean homoscleromorph species: *Oscarella lobularis, O. tuberculata, O. microlobata, O. imperialis, Plakina trilopha, P. jani, Corticium candelabrum,* and *Pseudocorticium jarrei.* Morphogenesis of the larva is similar in all these species; however, cell proliferation is more active in species of *Oscarella* than in *Plakina* and *C. candelabrum.* The result of cell division is a wrinkled, flagellated larva, called a cinctoblastula. It is composed of a columnar epithelium of polarized, monoflagellated cells among which are scattered a few non-flagellated ovoid cells. The central cavity always contains symbiotic bacteria. Maternal cells are also present in *O. lobularis, O. imperialis,* and *P. jarrei.* In the fully developed larva, cell shape and dimensions are constant for each species. The cells of the anterior pole have large vacuoles with heterogeneous material; those of the postero-lateral zone have an intranuclear paracrystalline inclusion; and the flagellated cells of the posterior pole have large osmiophilic inclusions. Intercellular junctions join the apical parts of the cells, beneath which are other specialized cell junctions. A basement membrane underlying the flagellated cells lines the larval cavity. This is the first observation of a basement membrane in a poriferan larva. The basal apparatus of flagellated cells is characterized by an accessory centriole located exactly beneath the basal body. The single basal rootlet is cross striated. The presence of a basement membrane and a true epithelium in the larva of Homoscleromorpha—unique among poriferan clades and shared with Eumetazoa—suggests that Demospongiae could be paraphyletic.

Additional key words: reproduction, cell differentiation, cinctoblastula, Mediterranean Sea, sponges

Homoscleromorpha, a key subclass within Demospongiae (Porifera), contains only 7 genera; it is considered monophyletic but has no clear phylogenetic relationship with other demosponges. Homoscleromorph species share many morphological, anatomical, cytological, biochemical, and embryological characters (Boury-Esnault et al. 1984, 1992, 1995; Solé-Cava et al. 1992; Muricy et al. 1996a,b, 1999; Ereskovsky & Boury-Esnault 2002). For example, they possess both an acrosome in the spermatozoa (Baccetti et al. 1986; Boury-Esnault & Jamieson 1999) and a basement membrane with type IV collagen underlying the pinacoderm and the choanoderm (Boute et al. 1996), characters which are common to eumetazoans and absent in other poriferan clades.

The role of embryological studies in establishing the relationships between animals has been paramount since Haeckel (1866) and what became known as the "biogenetic law." The basal phylogenetic position of Porifera among Metazoa and its suggested paraphyly (Borchiellini et al. 2001) makes new investigations on embryology and larvae especially timely (Leys & Degnan 2002).

We have already described (Ereskovsky & Boury-Esnault 2002) the early development from the egg to the coeloblastula stage in 5 species of *Oscarella* (Demospongiae, Homoscleromorpha). The most distinctive feature of early development in homoscleromorphs is the formation of a coeloblastula from a stereoblastula by "multipolar egression," the progressive migration of the internal cells to the periphery. Penetration of symbiotic bacteria into the embryos of all species investigated, and maternal cells in *Oscarella lobularis, O. imperialis,* and *Oscarella* sp., have been documented. Symbiotic bacteria and maternal

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Table 1. Stations and depth (m) of homoscleromorph species collected in the northwest Mediterranean. Numbers refer to the map (Fig. 1).

Station	*Oscarella lobularis*	*O. tuberculata*	*O. microlobata*	*O. imperialis*	*Plakina trilopha*	*P. jani*	*Corticium candelabrum*	*Pseudocorticium jarrei*
(1) Endoume Cave	—	5–7	—	—	—	—	—	—
(2) Riou Archipelago	22–25	8–10	—	—	—	—	—	—
(3) Jarre Island	20–24	7–9	—	22–25	—	—	12–15	—
(4) Jarre Cave	—	—	10–12	—	10–12	10–12	—	10–12
(5) La Vesse	10–15	8–13	—	—	—	—	10–12	—
(6) Cap Fauconniéres	13–15	8–10	—	—	—	—	—	—
(7) 3PP Cave	—	—	—	—	12–15	13–15	—	—
Medes Islands	15–17	13–17	—	—	—	—	15–18	—

cells migrate from the mesohyl into the space between eggs and follicular cells before the closing of the follicles (Ereskovsky & Boury-Esnault 2002). The coeloblastula is composed of a monolayer of undifferentiated flagellated cells surrounding a blastocoel.

Development from the egg to the coeloblastula to the fully developed larva (cinctoblastula[1]), including differentiation of larval cells, occurs within the maternal tissue. Although several histological studies have described the embryogenesis and larva of *Oscarella lobularis* (= *tuberculata*) (Meewis 1938; Lévi 1956; Tuzet & Paris 1964), only one ultrastructural study has been devoted to describing of the larva of *O. lobularis* (Lévi & Porte 1962).

We describe here the embryogenesis from coeloblastula to cinctoblastula in 8 species of Homoscleromorpha using light, transmission, and scanning electron microscopy. We will try to provide evidence on phylogenetic characters to answer the questions: (1) Do the embryological characters support the monophyly of Homoscleromorpha? (2) What embryological characters are shared with other taxa of Demospongiae, Calcarea, and/or Eumetazoa?

Methods

Larval development in 8 Mediterranean species of homoscleromorph sponges was investigated, *Oscarella tuberculata* (SCHMIDT 1868), *O. lobularis* (SCHMIDT 1862), *O. imperialis* MURICY ET AL. 1996a, *O. microlobata* MURICY ET AL. 1996a, *Corticium candelabrum* SCHMIDT 1862, *Pseudocorticium jarrei* BOURY-ESNAULT ET AL. 1995, *Plakina trilopha* SCHULZE 1880, and *P. jani* MURICY ET AL. 1998.

The sponges were collected by SCUBA diving from June to August 1999 and August to September 2000, in the western Mediterranean Sea, at depths of 5–25 m (Fig. 1, Table 1). Pieces of each specimen were fixed *in situ* or immediately after collection.

For scanning electron microscopy (SEM), the fixative used was a 5:1 mixture of 2% OsO_4 and saturated mercury chloride (Johnston & Hildemann 1982).

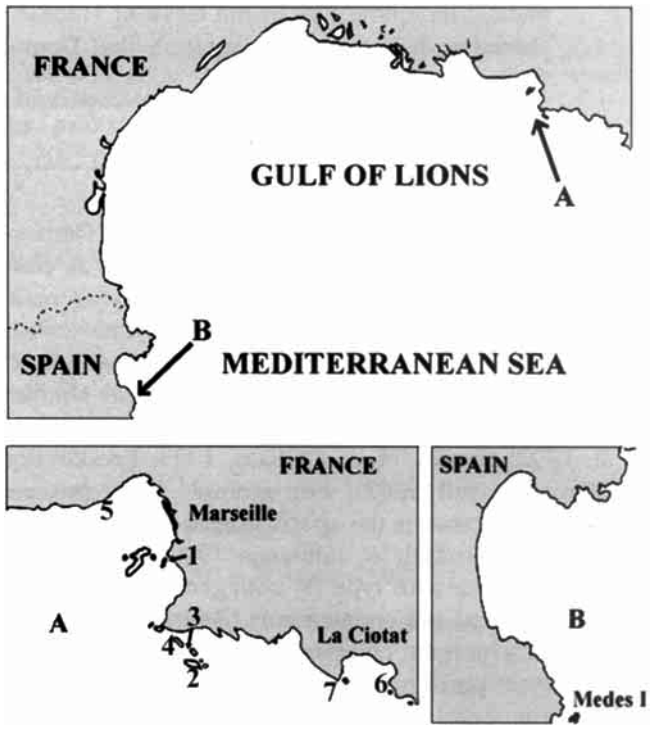
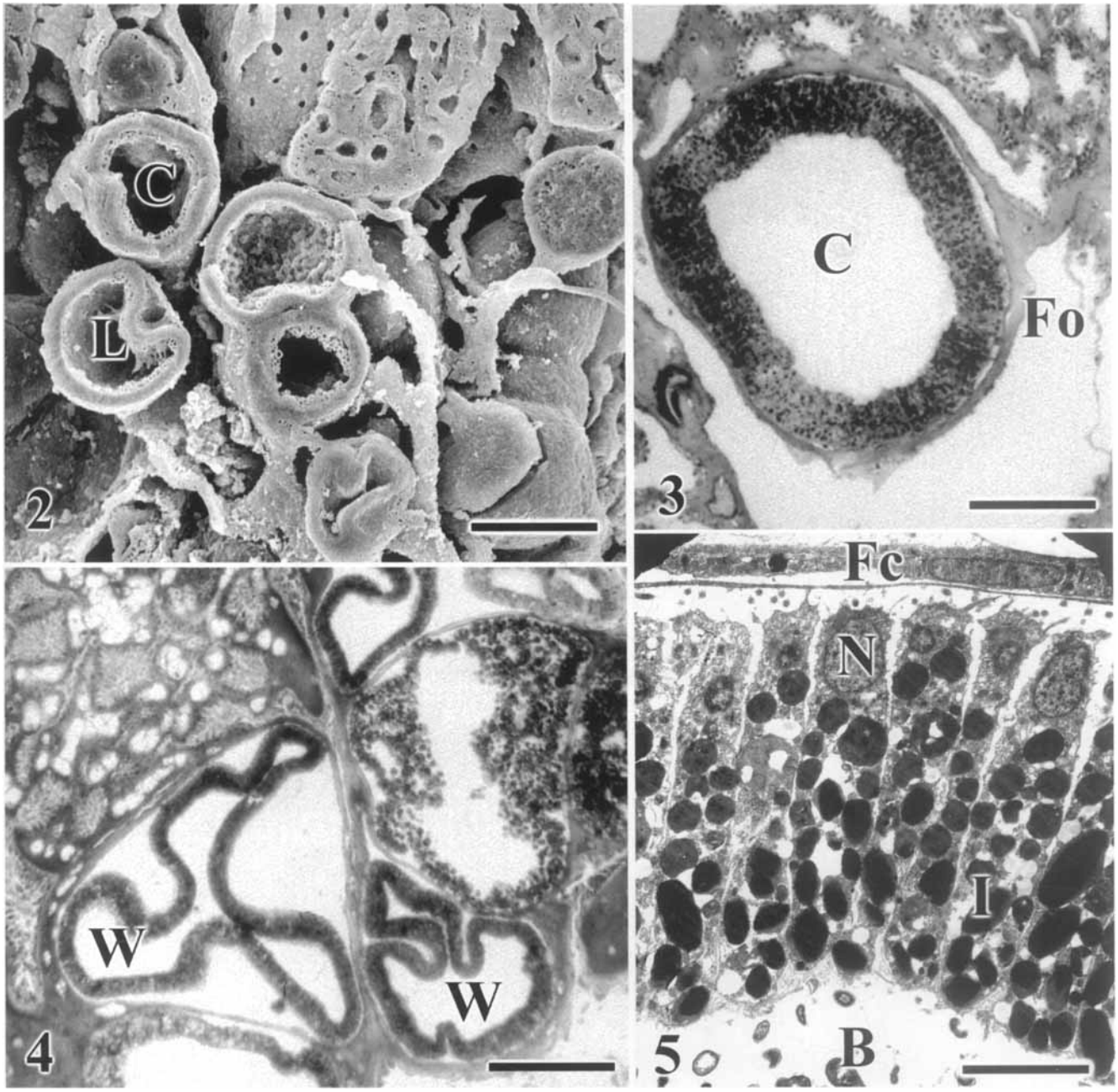


Fig. 1. Map of the northwest Mediterranean coast showing collection sites. **A.** Details of the Provence coast. **B.** Detail of the Catalan coast with the Medes Islands.

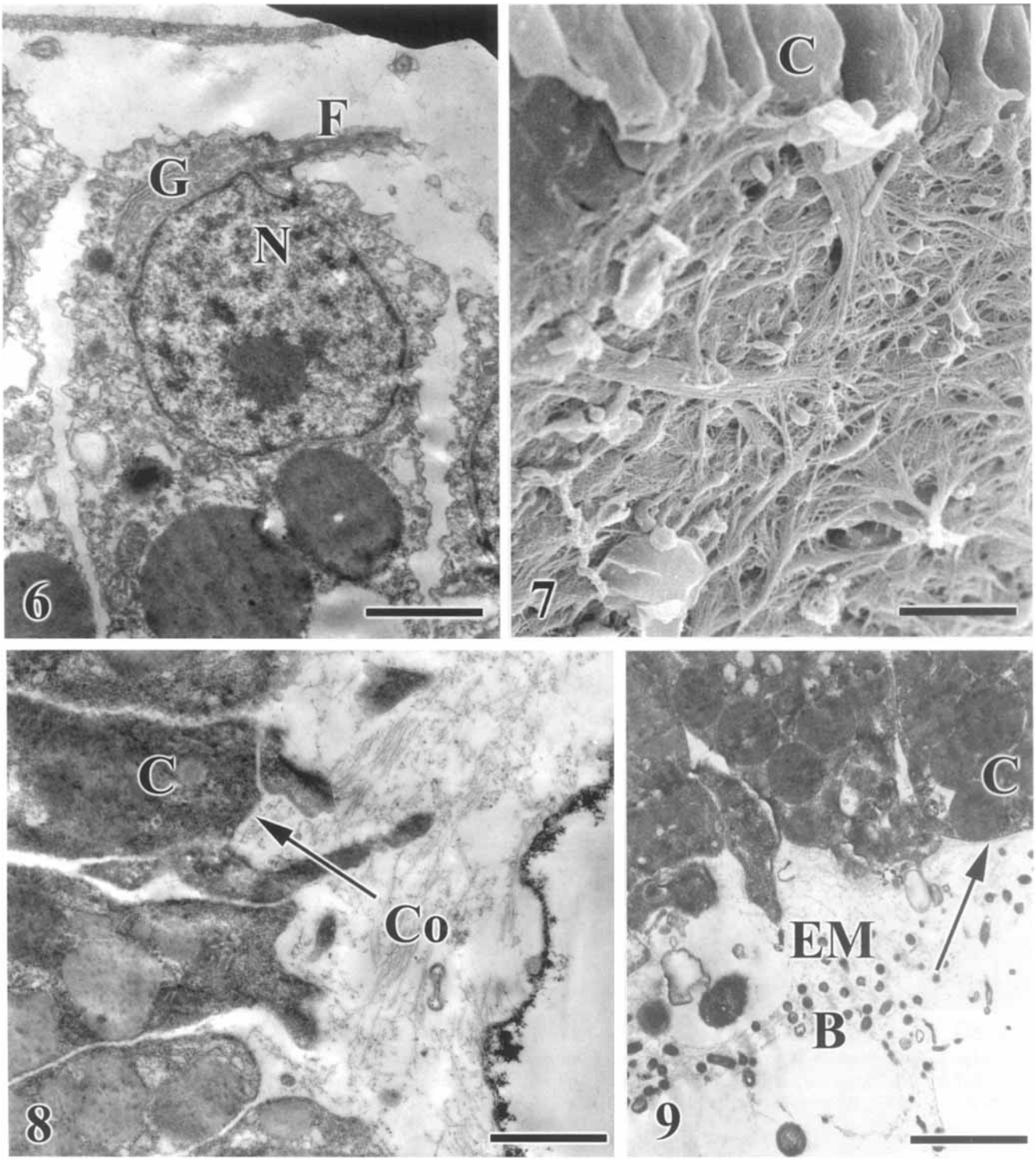
[1] The larva of Homoscleromorpha and that of Calcaronea (Calcispongia) have both been called "amphiblastulae," creating an invalid impression of homology. In fact, larval embryogenesis differs in these 2 taxa. To avoid confusion, we use the name cinctoblastula to refer to the fully developed larva of homoscleromorphs. The cinctoblastula is characterized by regional differentiation of the flagellated cells and a belt of flagellated cells with an intranuclear paracrystalline inclusion.



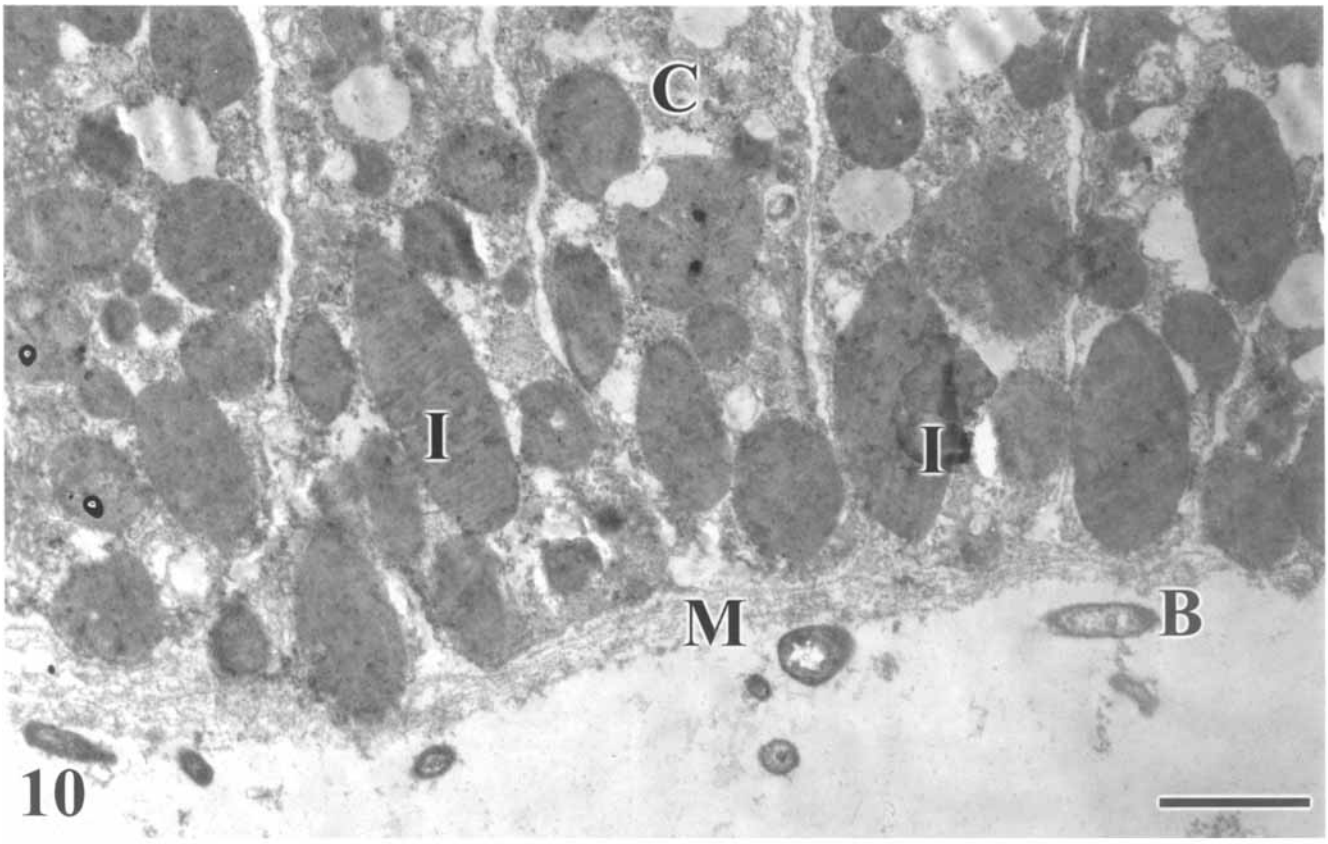
Figs. 2–5. Fig. 2. Coeloblastula (C) and developed larva (L) (cinctoblastula) of *Oscarella lobularis*. SEM. Scale bar, 200 μm. **Fig. 3.** Coeloblastula (C) of *Corticium candelabrum* within the follicle (Fo). Semi-thin. Scale bar, 175 μm. **Fig. 4.** Wrinkled coeloblastulae (W) of *Oscarella tuberculata*. Semi-thin. Scale bar, 67 μm. **Fig. 5.** Flagellated cells of coeloblastula of *Plakina trilopha*. Bacteria (B), follicular cells (Fc), osmiophilic inclusion (I), nucleus (N). TEM. Scale bar, 8.2 μm.

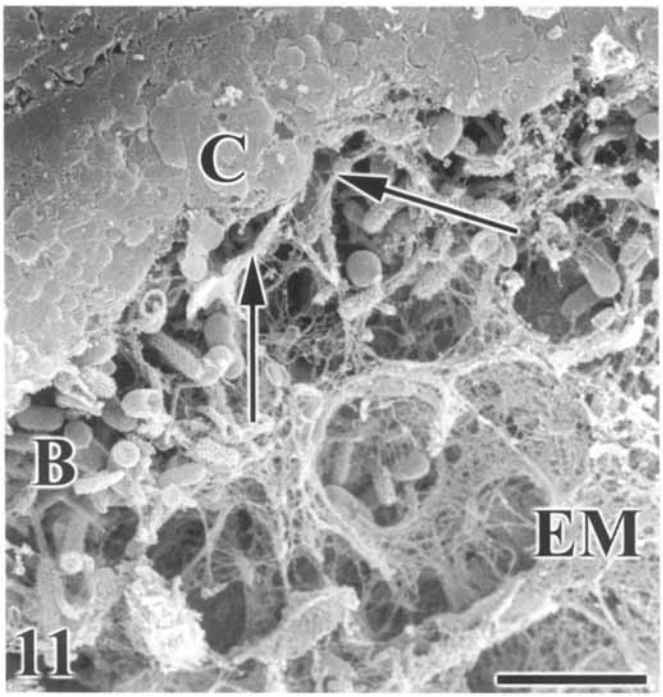
Specimens were fractured in liquid nitrogen, critical-point-dried, sputter-coated with gold-palladium, and observed under a Hitachi S570 SEM.

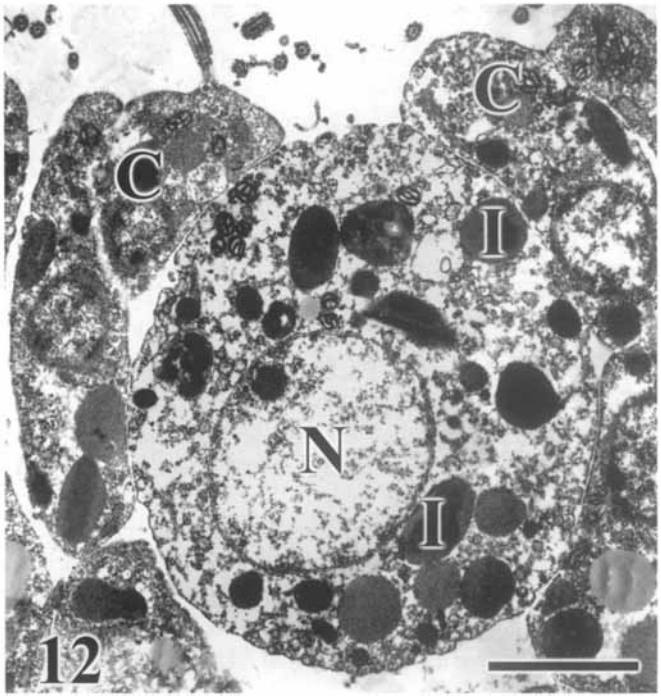
For transmission electron microscopy (TEM), 2 fixation methods were used: (1) 2.5% glutaraldehyde in a mixture of 0.4 M cacodylate buffer and seawater (1: 4:5; 1120 mOsm) and post-fixation in 2% OsO_4 in seawater (Boury-Esnault et al. 1984), and (2) samples were prefixed in 1% OsO_4 in 0.1 M phosphate buffer for 10 min and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (1120 mOsm) at room temperature for 1 h. After fixation, samples were washed in 0.1 M phosphate buffer and postfixed in 1% OsO_4 in 0.1 M phosphate buffer for 1 h, dehydrated through a graded ethanol series, and embedded in Araldite. For light microscopy, specimens were fixed in Bouin's fixative and



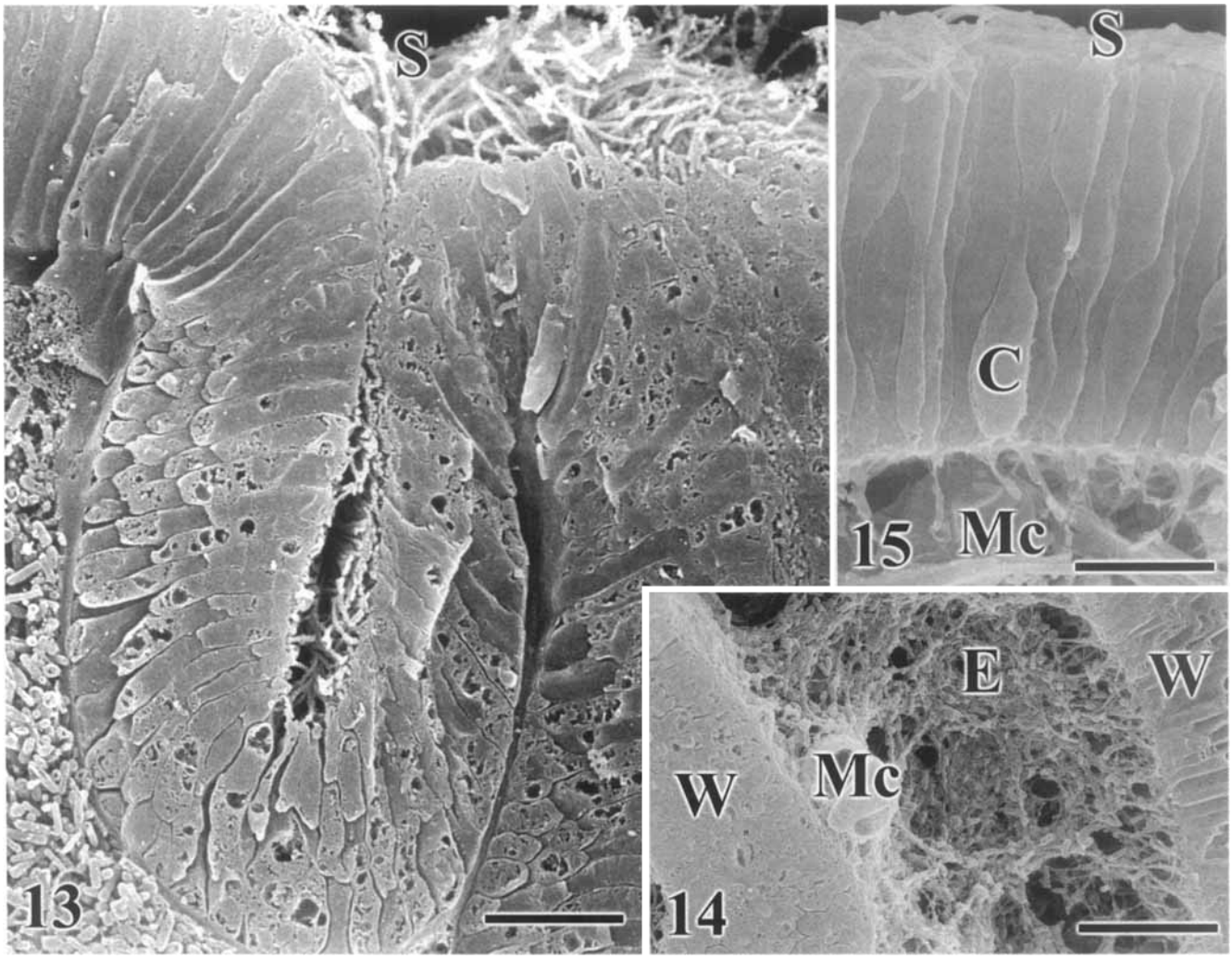
Figs. 6–9. Fig. 6. Apical end of flagellated cells of coeloblastula of *Plakina trilopha.* Flagellum (F), Golgi apparatus (G), nucleus (N). TEM. Scale bar, 2.7 μm. **Fig. 7.** Basal end of flagellated cells (C) of coeloblastula of *Oscarella lobularis.* SEM. Scale bar, 4.4 μm. **Fig. 8.** Early stage of basement membrane formation in coeloblastula of *Oscarella tuberculata.* Note collagen fibrils (Co) in the basal parts of flagellated cells (C). TEM. Scale bar, 0.94 μm. **Fig. 9.** Basement membrane of larva of *Oscarella tuberculata* consists of a thin layer of collagen (arrow) and an extracellular matrix (EM). Bacteria (B), flagellated cells (C). TEM. Scale bar, 1.4 μm.







Figs. 10–12. Fig. 10. Basement membrane (M) of larva of *Plakina trilopha.* Bacteria (B), flagellated cells (C), osmiophilic inclusion (I). TEM. Scale bar, 1.8 μm. **Fig. 11.** Basement membrane of larva of *Oscarella microlobata.* Note the tough mat of collagen (arrows) and extracellular matrix (EM). Bacteria (B), flagellated cells (C). SEM. Scale bar, 6.1 μm. **Fig. 12.** Non-flagellated cell of a larva of *Oscarella tuberculata.* Flagellated cells (C), nucleus (N), osmiophilic inclusion (I). TEM. Scale bar, 1.75 μm.



Figs. 13–15. Fig. 13. Wrinkles of a larva of *Plakina trilopha.* Surface (S). SEM. Scale bar, 13.5 μm. **Fig. 14.** Extracellular matrix (E) between wrinkles (W) of a larva of *Oscarella lobularis.* Maternal cell (Mc). SEM. Scale bar, 14 μm. **Fig. 15.** Flagellated cells (C) of lateral part of a larva of *Oscarella lobularis.* Surface (S), maternal cell (Mc). SEM. Scale bar, 7.3 μm.

embedded in paraffin. Paraffin sections were stained with Mayer's hematoxylin and semi-thin sections were stained with toluidine blue. Thin sections, stained with uranyl acetate and lead citrate, were observed under a JEM-7 and Zeiss-1000 TEM.

All dimensions given in the text are an average of ≥25 measurements made on 10 larvae of each species.

For the definition of terms for sponge morphology and embryology, see Boury-Esnault & Rützler (1997). For definitions of larvae, see also Maldonado & Bergquist (2002).

Results

Early stage

Morphogenesis of the larva takes place within the maternal tissue, in the follicle where cleavage has occurred (Figs. 2–4). This follicle remains the same size throughout cleavage and larval development (Fig. 2). The coeloblastula has a columnar epithelium of flagellated cells closely apposed to each other. The thickness of this epithelium is relatively homogeneous, ranging from ~18 μm for *Oscarella* to 25 μm for *Plakina* (Fig. 5). The beginning of larval morphogenesis is indicated by the differentiation of a flagellum at the summit of a small cone on the apical face of each cell. The flagella lie parallel to the surface of the coeloblastula (Fig. 6). In some species (*Plakina trilopha* and *P. jani*), the apical end of the cell displays small irregular villi (Fig. 5). The flagellar basal apparatus is composed of a basal body and several associated structures—an accessory centriole, a rootlet, and alar sheets. The pear-shaped to spherical nucleus, located just below the flagellar basal apparatus in the

apical region of the cell, contains 1 or 2 nucleoli (Fig. 6). A very active Golgi apparatus, ribosomes, and numerous spherical or ovoid mitochondria surround the nucleus (Fig. 6). The cytoplasm is filled with many osmiophilic yolk inclusions of various sizes (0.4–4.1 μm in diameter), including some lipid droplets (0.5–1.1 μm in diameter), and also 1–5 large spherules (4.3–9.8 μm in diameter) in the central part of the cell (Fig. 5).

A complex network of filopodia, pseudopodia, and collagen fibrils is formed at the basal end of the cells (Fig. 7). The synthesis of collagen fibrils (Fig. 8) coincides with the differentiation of the flagella. These collagen fibrils constitute most of the extracellular matrix, which fills most of the internal cavity of the developing larva. Close to the cell membrane of the basal part of the cell, the extracellular matrix is arranged in a tough mat of collagen fibrils about 0.3–0.6 μm thick (Figs. 9–11). Beneath this layer, there is a loose net of collagen fibrils (Figs. 9, 11). These 2 collagen layers constitute a basement membrane similar to the one found below the pinacoderm and choanoderm of the adult.

Scattered among the flagellated cells are ovoid or spherical cells (6.1–12.2 μm in diameter) without flagella (Fig. 12).

Within the central cavity are symbiotic bacteria, present since the beginning of cleavage (Figs. 5, 10). They are identical to those present in the mesohyl of the parent. During development, they divide frequently and increase in numbers. The coeloblastulae of *O. lobularis, O. imperialis,* and *Pseudocorticium jarrei* also contain maternal cells within the central cavity (Figs. 14, 15). These cells have a structure similar to those of adults and correspond, in *O. lobularis,* to the vacuolar cell type I (Boury-Esnault et al. 1992), in *O. imperialis,* to spherulous cell type II (Muricy et al. 1996a), and in *P. jarrei,* to cell type 1 with paracrystalline inclusions (Boury-Esnault et al. 1995).

Throughout larval morphogenesis, cells actively proliferate. Before dividing, the cell becomes spherical and is situated at the surface of the embryo. The plane of cell division is perpendicular to the surface. After mitosis, the 2 new cells are again incorporated into the columnar flagellated epithelium. The multiplication of cells expands the larval surface, and the epithelium becomes folded, while the space inside the follicle remains constant (Fig. 4). The folds or wrinkles can be very numerous and extensive, giving larvae of *Oscarella,* for example, a convoluted appearance (Fig. 4). The wrinkles in *O. imperialis, Corticium candelabrum,* and *Plakina trilopha* can be very close to each other with a space of only 1–3 μm between them (Fig. 13). In the other species, the folding is less marked.

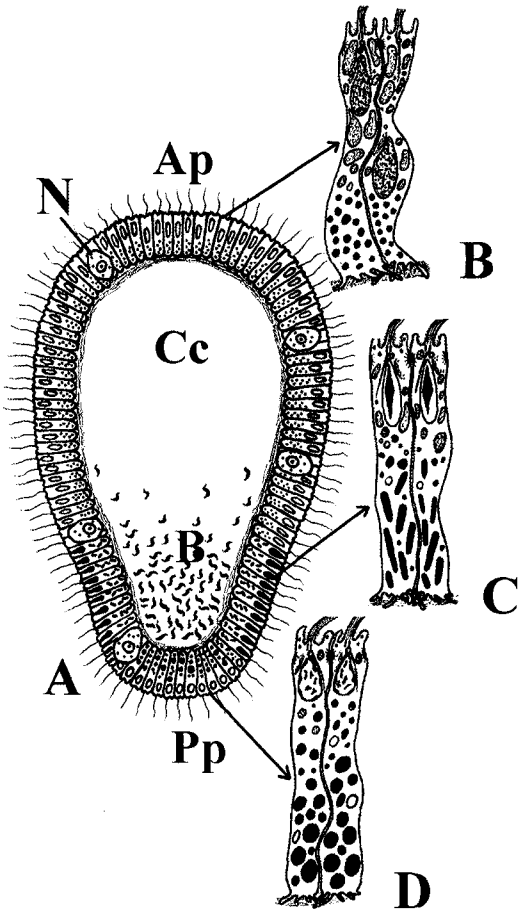
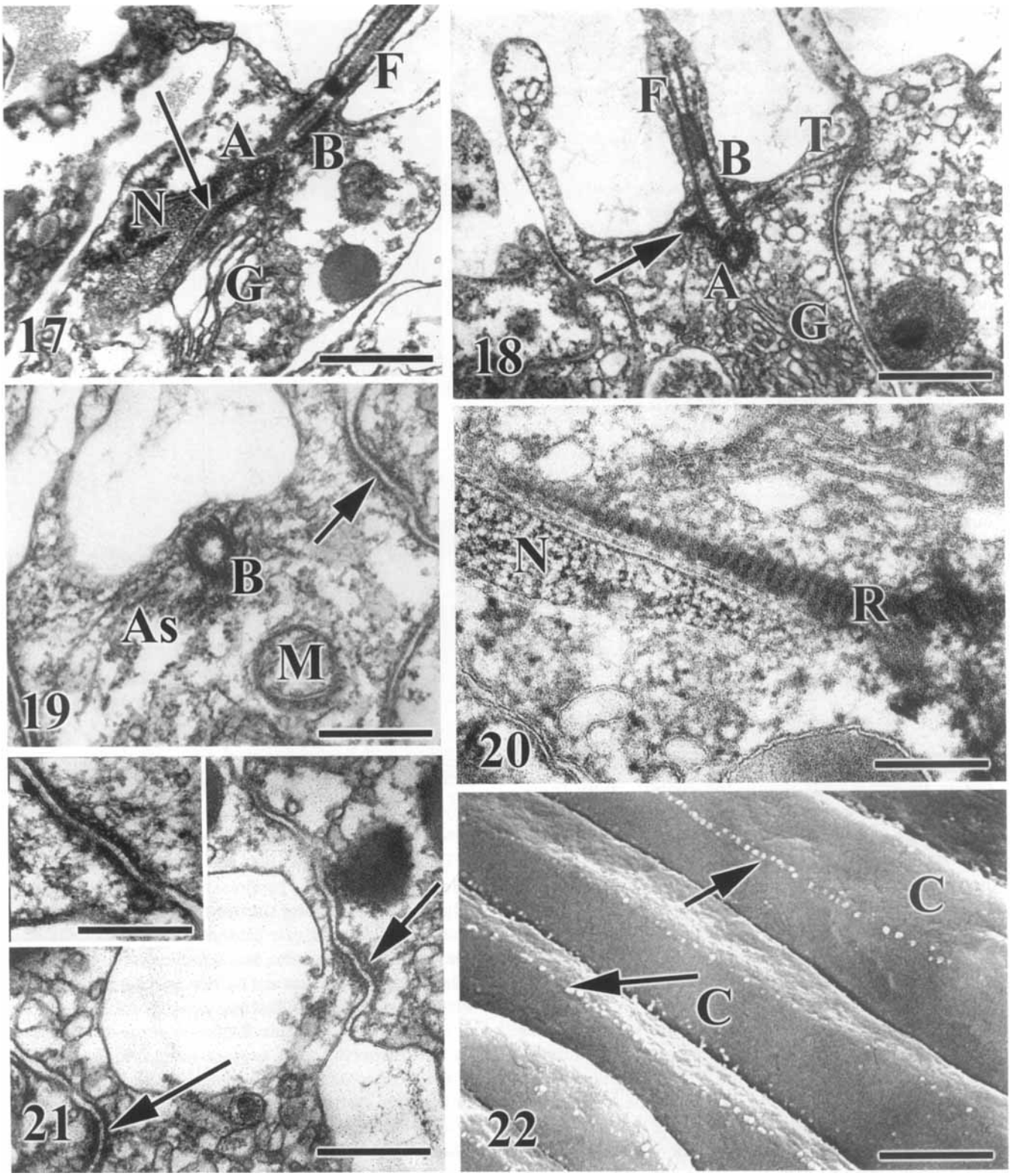


Fig. 16. Diagram of homoscleromorph cinctoblastula. **A.** Free-swimming larva. **B.** Flagellated cell of anterior pole. **C.** Flagellated cell of postero-lateral zone. **D.** Flagellated cell of posterior pole. Anterior pole (Ap), bacteria (B), central cavity (Cc), non-flagellated cells (N), posterior pole (Pp).

The internal space between the folds, as well as the whole central cavity, is progressively filled by collagen fibrils (Fig. 14). During the folding of the epithelium, as the space between the maternal follicular cells and the larval cells increases, the flagella became perpendicular to the outer surface of the developing larva.

Flagellated cells of *Plakina, Corticium,* and *Pseudocorticium* are elongated and columnar, whereas those of *Oscarella* have a more irregular shape (Fig. 15). In *Plakina* and *Corticium,* the flagellum is surrounded by 5 or 6 irregular cytoplasmic villi, whereas in *Oscarella,* it is situated in a depression of the apical plasmalemma, a pit ~0.9 μm deep.

The flagellar basal apparatus is composed of a basal body ~0.38–0.46 μm long and 0.31–0.35 μm in diameter (Figs. 17, 18). The accessory centriole, 0.41–0.65 μm long and 0.3–0.4 μm in diameter, is always located exactly beneath the basal body and oriented orthogonally to its long axis (Figs. 17, 18). A bundle



Figs. 17–22. Fig. 17. Flagellar apparatus of a flagellated cell of a cinctoblastula of *Plakina trilopha.* Accessory centriole (A), basal body (B), flagellum (F), cross-striated rootlet (arrow), Golgi apparatus (G), nucleus (N). TEM. Scale bar, 1.2 μm. **Fig. 18.** Apical part and flagellar apparatus of a flagellated cell of a cinctoblastula of *Oscarella tuberculata.* Accessory centriole (A) perpendicular to the basal body (B), flagellum (F), basal foot (arrow), Golgi apparatus (G), bundle of microtubules (T). TEM. Scale bar, 1 μm. **Fig. 19.** Transverse section through a basal body (B) of a flagellar apparatus of a cell

of microtubules links the basal body to the lateral plasmalemma at the level of the intercellular junctions (Fig. 18). The basal foot (0.27–0.3 μm long) has a champagne cork shape and originates proximo-laterally on the basal body (Fig. 18). Alar sheets link the base of the flagellum to the apical plasmalemma (Fig. 19). A single cross-striated rootlet originates close to the end of the basal body. It surrounds the accessory centriole and its distal end is associated with the nuclear membrane (Fig. 20).

Desmosomes develop between the apical parts of these long columnar cells (Fig. 21). The intercellular space of these junctions is ~14–22 nm wide and contains electron-opaque material. The cytoplasmic side of the cell membrane (15–25 nm thick) is covered by electron-opaque filamentous material. Below these apical junctions, in the middle portion of the flagellated cells, other specialized cell junctions are observed along the long axis of the cells. These appear in SEM as regular lines of spherical globules ~0.05–0.13 μm in diameter and separated by spaces of ~0.02–0.2 μm (Fig. 22).

Later stage

At a later stage of morphogenesis of the developing larva, the epithelium becomes differentiated regionally. It consists of 3 types of flagellated cells distributed according to their position in 3 different regions of the larva: the antero-lateral, postero-lateral, and posterior regions (Fig. 16, Table 2). The nonflagellated cells are always present but without specific regional localization.

In antero-lateral flagellated cells, the nuclei have an irregular shape, being distorted by the numerous cytoplasmic inclusions, and are located at various levels in the columnar cells (Figs. 16B, 23). The nuclear region of the cell is generally wider. The apical region contains numerous mitochondria, large vacuoles (1.1–5.9 μm in diameter) with a fibrillar content, and others with a heterogeneous content. The basal region contains numerous small osmiophilic inclusions (0.4–2.8 μm in diameter) and lipid droplets (Fig. 23).

In the postero-lateral zone, flagellated cells with a paracrystalline intranuclear inclusion form a narrow belt, 57–70 μm wide (Figs. 16C, 24). The ovoid nucleus (5.8–7.7 × 2.3–3.6 μm) contains a paracrystalline inclusion (~3.8–4.3 × 0.6–0.7 μm) oriented parallel to the long axis of the nucleus and of the cell. This inclusion is lens-shaped or rod-shaped in longitudinal section (Fig. 24) and rhomboid or square in transverse section. The nucleus is always localized in the apical part of the cell where numerous mitochondria and some vacuoles are also present. The middle part of the cell is filled with vacuoles (~0.6–2.0 μm in diameter) containing heterogeneous material, and the basal part contains ovoid osmiophilic electron-opaque inclusions (about 4.4–6.7 × 0.8–1.0 μm) (Figs. 16C, 24).

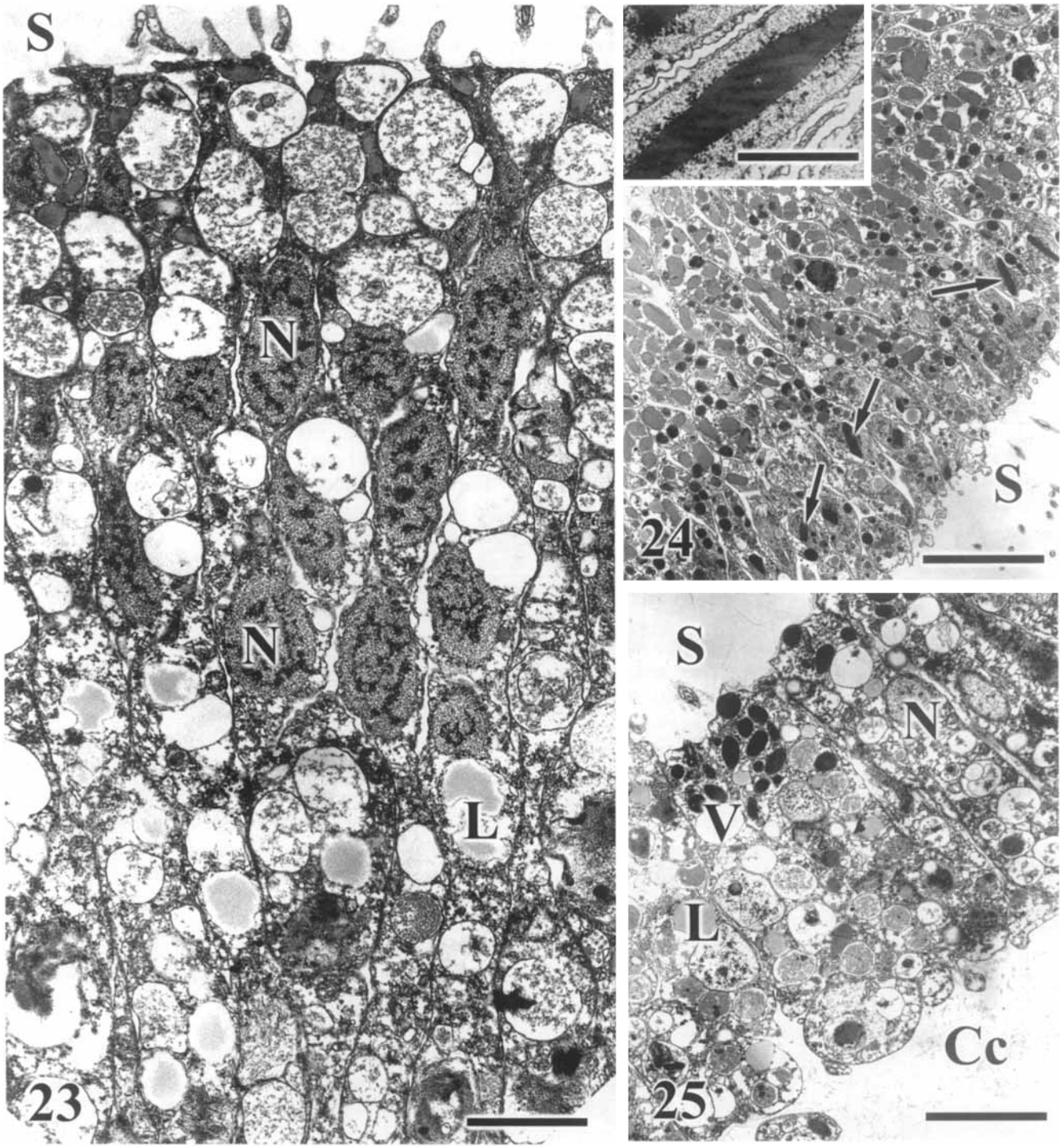
The flagellated cells of the posterior pole have a spherical or ovoid, nucleolated nucleus situated in the apical part below a vacuolated area (Figs. 16D, 25). Many lipid droplets and large electron-opaque inclusions (0.7–3.6 μm) fill the cytoplasm, and these may be responsible for the pigmentation of these cells.

The nonflagellated cells are spherical (diameter ~5.5 μm in *Oscarella*) or pear-shaped (~7.9 × 4.9 μm in *Oscarella* and 32 × 9.2 μm in *Corticium*). They are inserted between flagellated cells in the upper part of the larval epithelium (Fig. 26) and never reach the base of the epithelium. Flagella and filopodia are absent. The spherical nucleus is central and has a single nucleolus. The cytoplasm contains lipid droplets, osmiophilic inclusions, vacuoles, and vesicles of various sizes.

When the larvae have become fully developed, the follicle and the apopinacoderm of the excurrent canal of the adult merge together. The larvae unfold during release from the maternal sponge. The free-swimming cinctoblastula is ovoid or pear-shaped, the anterior pole being larger than the posterior one, and the whole surface is flagellated (Figs. 16A, 27–29). Species of *Oscarella* have the smallest larvae, from ~208 × 148 μm in *O. tuberculata* to ~235 × 136 μm in *O. imperialis*; *Plakina trilopha* has the largest larvae, ~276 × 207 μm; whereas *Corticium candelabrum* and *P. jani* have larvae about equal in size, ~257 × 212 and 258 × 180 μm, respectively. The pigmentation of the posterior pole varies according to species (Table 2). The columnar, flagellated epithelium of the fully developed larvae lies on a basement membrane. A large central cavity containing extracellular matrix and symbiotic bacteria, more abundant in the posterior region,

←

of a cinctoblastula of *Oscarella tuberculata*. Note alar sheets (As), mitochondria (M), cell junctions (arrows). TEM. Scale bar, 0.7 μm. **Fig. 20.** Cross-striated flagellar rootlet (R) of a cell of a cinctoblastula of *Plakina trilopha*. Nucleus (N). TEM. Scale bar, 0.3 μm. **Fig. 21.** Apical parts of flagellated cells of a cinctoblastula of *Oscarella microlobata* with 2 desmosomes (arrows). TEM. Scale bar, 1.1 μm. Inset, detail of a desmosome. Scale bar, 0.25 μm. **Fig. 22.** Cell junctions (arrows) in the middle part of flagellated cells (C) of a cinctoblastula of *Plakina trilopha*. SEM. Scale bar, 1.4 μm.



Figs. 23–25. Fig. 23. Flagellated cells of the anterior pole of a cinctoblastula of *Plakina trilopha.* Surface (S), nucleus (N), lipid droplet (L). TEM. Scale bar, 3.2 μm. **Fig. 24.** Cells of the postero-lateral zone, each with an intranuclear paracrystalline inclusion (arrows) of a cinctoblastula of *Oscarella tuberculata.* Surface (S). Scale bar, 5.4 μm. Inset, detail of a paracrystalline inclusion in a nucleus. TEM. Scale bar, 1.4 μm. **Fig. 25.** Flagellated cells of the posterior pole of a cinctoblastula of *Plakina trilopha.* Surface (S), central cavity (Cc), lipid droplet (L), nucleus (N), vacuole (V). TEM. Scale bar, 8 μm.

is always present. All larvae for which observations are available (Table 2) rotate clockwise when swimming forward and viewed from the anterior pole.

Discussion

Epithelial systems are characterized by cell polarization, apical cell junctions, and a basement membrane (Hay 1981). In Porifera, the first 2 characteristics have been found in larvae of Calcispongia (*Ascandra falcata,* Borojevic 1969), Hexactinellida (*Oopsacas minuta,* Boury-Esnault et al. 1999), and Demospongiae (*Dysidea etheria,* Rieger 1994; *Halisarca dujardini,* Ereskovsky, unpubl. data). The cinctoblastula of the Homoscleromorpha also displays the first 2 characteristics. This larva is characterized by an epithelium of highly polarized flagellated cells. Desmosomes are present in the apical parts (Fig. 21). Beneath the desmosomes, we observed another specialized cell junction that we cannot relate to other junctions described so far in metazoans (Fig. 22). Moreover, a tough mat of collagen fibrils underlying the basal ends of the cells is very similar to what has been described lining the pinacoderm and choanoderm of the homoscleromorph adults (Donadey 1979; Garonne 1985) and the epithelia of cnidarians and ctenophores (Franc 1985). This is the first time that all 3 characteristics have been found in sponge larvae. The larval epithelium of homoscleromorphs, being a true columnar epithelium, could be considered homologous to the eumetazoan epithelium: the feltwork of collagen fibrils underlying the cells is interpreted here as a basal lamina, and the loose net of collagen fibrils below as a lamina reticularis.

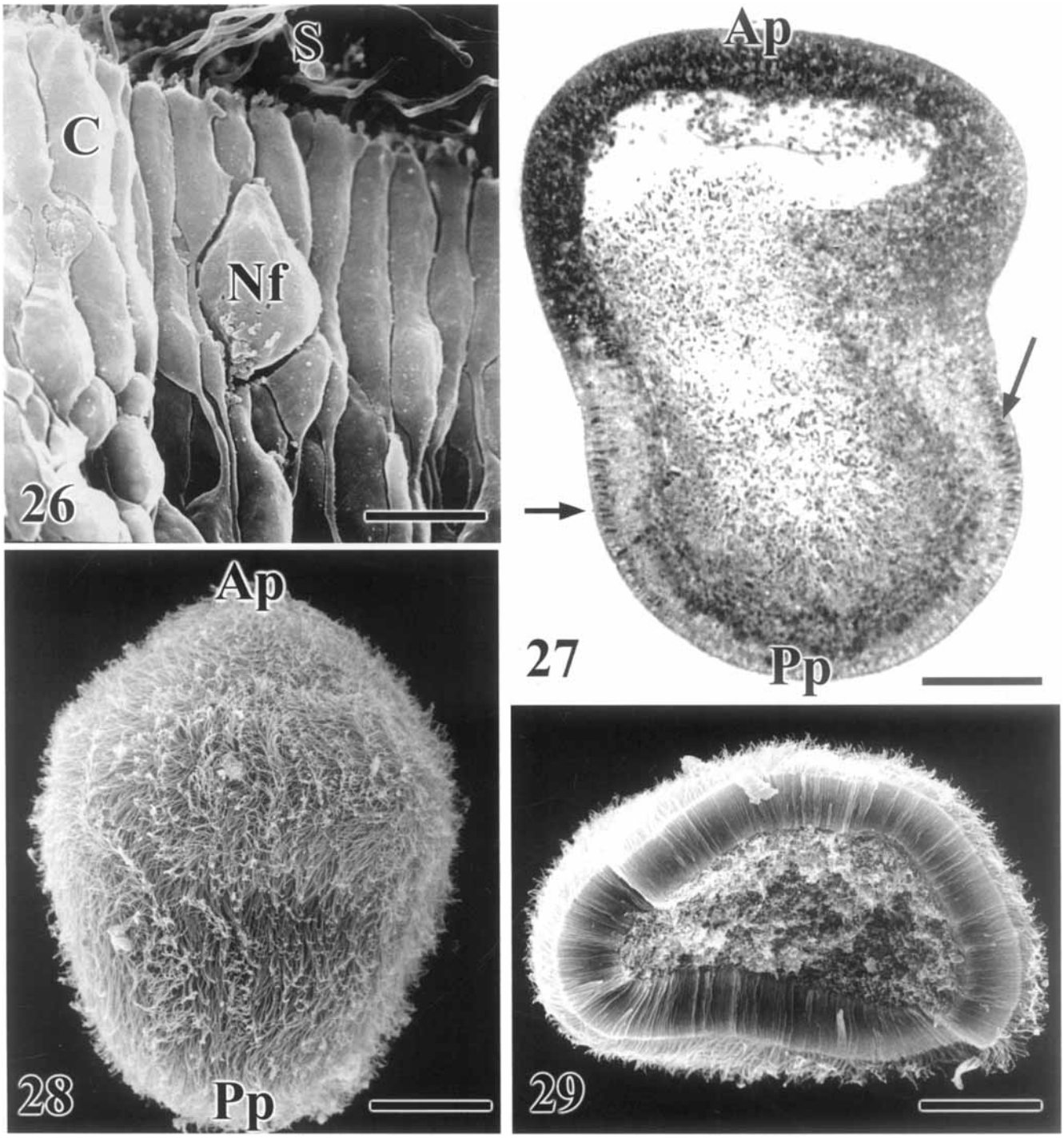
The main component of basement membrane—type IV collagen—has been observed in all eumetazoans (Morris 1993). In Porifera, it has been sequenced in the Homoscleromorpha, as well as tenascin- and laminin-like proteins (Humbert-David & Garrone 1993; Boute et al. 1996). We expect that type IV collagen found in adults, lining the pinacoderm and the choanoderm, will also be found to constitute the basement membrane of the epithelium of the larvae.

In all homoscleromorph species that we have investigated, the larvae developing in the maternal tissue are wrinkled. Wrinkled larvae have also been described in Demospongiae: *Hippospongia communis* (Tuzet & Pavans de Ceccatty 1958), *Spongia reticulata* (Bergquist et al. 1970), *Halisarca nahantensis* and *H. dujardini* (Chen 1976; Ereskovsky & Gonobobleva 2000), *Halichondria panicea* (Ivanova 1981), *Ochridaspongia rotunda* (Gilbert & Hadziscie 1977), *Baikalospongia bacillifera* (Ropstorp & Reitner 1994; S.M. Efremova, pers. comm.), *Iophon piceus* (Ereskovsky 1986), *Mycale lobata, Myxilla incrustans, Haliclona aqueductus,* and *Pleraplysilla spinifera* (Ereskovsky, unpubl. data). We assume that wrinkling of the growing pre-larva is due to the limited space within the follicles.

Flagellated and non-flagellated cells are distinguishable early in cell differentiation of the pre-larva. Non-flagellated cells were scattered in the larval epithelium, recognizable by their ovoid shape and smaller size. These cells have been described in fully developed larvae of *O. tuberculata* (*lobularis*) by previous authors who considered that they could be secretory cells (Heider 1886; Meewis 1938; Lévi & Porte 1962). Non-flagellated cells scattered within the layer of flagellated cells have been observed in the parenchymella of *Hamigera hamigera* (Demospongiae, Poecilosclerida) (Boury-Esnault 1976), in *Haliclona tubifera* (Demospongiae, Haplosclerida) (Woollacott 1993), and in the coeloblastula of a calcinean species (mis-identified as *Leucosolenia*) (Calcispongiae, Calcinea) (Amano & Hori 2001).

The differentiation of flagellated larval cells into antero-lateral, postero-lateral, and posterior cells occurs later in development. These 3 cell types were previously described for the larva of *O. tuberculata* (*lobularis*) (Heider 1886; Meewis 1938; Lévi & Porte 1962), and are present in all homoscleromorph genera we investigated: *Oscarella, Plakina, Corticium,* and *Pseudocorticium.* The characteristic structures of the cells of the anterior pole are large vacuoles with mucus-like material. For the postero-lateral flagellated cells, the most important feature is the intranuclear paracrystalline inclusion. This structure was described by Meewis (1938), who considered it to be a light sensitive organ. Among all described larvae, this belt of cells is unique to homoscleromorphs. The flagellated cells of the posterior pole have electron-opaque granules, which probably support the pigmentation.

The flagellar basal apparatus is similar in all species we investigated. It is characterized by an accessory centriole located exactly beneath the basal body and by a cross-striated rootlet. In other sponge larvae, the accessory centriole is arranged side by side with the basal body (Woollacott & Pinto 1995). However, an accessory centriole with an orthogonal and proximal orientation with regard to the basal body has been observed in the spermatozoa of *Spongilla lacustris* and of many invertebrates and vertebrates (Paulus 1989; Jamieson et al. 1995). Cross-striated rootlets are known in the flagellated cells of amphiblastula and coeloblastula larvae of Calcispongiae (Amano & Hori 1992, 2001; Gallissian & Vacelet 1992), in placozoans, and in all eumetazoans (Nielsen 2001). In Demospongiae (except for Homoscleromorpha) and in Hexacti-



Figs. 26–29. Fig. 26. Non-flagellated cell (Nf) of a cinctoblastula of *Oscarella tuberculata.* Flagellated cells (C), surface (S). SEM. Scale bar, 6.1 μm. **Fig. 27.** Longitudinal section through a cinctoblastula of *Corticium candelabrum,* free-swimming stage. Arrows indicate the position of the postero-lateral belt of cells with intranuclear paracrystalline inclusions. Anterior pole (Ap), posterior pole (Pp). Semi-thin. Scale bar, 49 μm. **Fig. 28.** External view of a released, free-swimming cinctoblastula of *Plakina trilopha.* Anterior pole (Ap), posterior pole (Pp). SEM. Scale bar, 48 μm. **Fig. 29.** Longitudinal section through a released, free-swimming cinctoblastula of *Plakina trilopha.* SEM. Scale bar, 46.3 μm.

Table 2. Characteristics of homoscleromorph cinctoblastula larvae. Dimensions (μm) are an average of ≥25 measurements made on 10 larvae of each species.

	Oscarella lobularis	*O. tuberculata*	*O. microlobata*	*O. imperialis*	*Plakina trilopha*	*P. jani*	*Corticium candelabrum*	*Pseudo-corticium jarrei*
Swims anterior pole first	yes	yes	yes	yes	yes	?	yes	?
Rotation around anterior-posterior axis	Clockwise	Clockwise	Clockwise	Clockwise	Clockwise	?	Clockwise	?
Egg-shaped	yes	yes	yes	yes	yes	yes	yes	yes
Dimensions	217 × 134	208 × 148	228 × 165	235 × 136	276 × 207	258 × 180	257 × 212	?
Color of posterior pole	Pink	Pink	Gray-brown	Milk-white	Orange	?	Pink-yellow	?
Ciliation	Complete, equal	Complete, equal	Complete, equal	Complete, equal	Complete, equal	Complete, equal	Complete, equal	Complete, equal
Presence of maternal cells	yes	no	no	yes	no	no	no	yes
Types of symbiotic bacteria	2	1	6	2	8	8	?	5
Cells of anterior pole	23.5 × 2.4	17.3 × 2	17.3 × 2.8	17 × 2.2	28 × 2.0	15.6 × 2.2	38 × 3.1	32 × 3.4
Cells of intermediate zone	22 × 2.8	19.3 × 2.2	24.6 × 2.1	16.4 × 2.5	30.8 × 2.5	15.7 × 2.3	38 × 3.2	33 × 3.7
Cells of posterior pole	26 × 2.3	21 × 2.5	16.1 × 3.5	17.3 × 2.3	31.6 × 2.7	14.8 × 2.4	39.5 × 3.7	?
Non-flagellated cells	9.2 × 6.1	7.3 × 5.5	?	?	?	?	32.7 × 9.2	?
Basal layer of collagen	yes	yes	yes	yes	yes	yes	yes	yes
Basal network of filopodia	yes	yes	yes	yes	yes	yes	yes	yes
Striated rootlet	yes	yes	yes	yes	yes	yes	yes	yes
Villi at cell's apical end	no	no	no	no	yes	?	yes	yes

nellida, cross-striated rootlets are absent. Cross-striated rootlets are also present in *Monosiga* (Choanoflagellata), *Naegleria* (Percolozoa), *Noctiluca* (Dinophyta), and *Tritrichomonas* (Parabasalia) (Dillon 1981; Vickerman et al. 1991; Nielsen 2001). If the cross-striated rootlet is homologous between metazoans and these protistan lineages, then this character is plesiomorphic for Metazoa and has been lost in the Demospongiae (except for Homoscleromorpha) and Hexactinellida.

We conclude that embryological and larval ultrastructural characters support the monophyly of Homoscleromorpha. The formation of the coeloblastula, the morphogenesis and morphology of cinctoblastula larvae, the presence of a basement membrane underlying the larval epithelium, and the characteristics of the basal apparatus of flagellum are similar in all the homoscleromorph species investigated.

The phylogenetic relationships of Homoscleromorpha within Demospongiae are controversial. Many authors hypothesize that it occupies a basal position within Demospongiae (Lévi 1970; Brien 1973; Simpson 1984). Other authors have suggested that Homoscleromorpha is the sister group of Calcarea, based on an incorrect interpretation of the larval type (van Soest 1984; Reitner & Grothe 1988; Grothe 1989). The recent findings of a basement membrane (Boute et al. 1996) and an acrosome in the spermatozoa (Baccetti et al. 1986; Gaino et al. 1986; Boury-Esnault & Jamieson 1999), and of multipolar egression (Ereskovsky & Boury-Esnault 2002), are indications that the phylogenetic position of Homoscleromorpha needs to be reassessed.

The presence of an epithelium with a basement membrane in the larva of the Homoscleromorpha is unique among poriferan clades and is shared with Eumetazoa. This result suggests that Demospongiae could be paraphyletic, and emphasizes the need for reassessment of the phylogenetic relationships between poriferan clades and Eumetazoa, as already suggested for Calcispongia (Zrzavy et al 1998; Borchiellini et al. 2001; Collins & Valentine 2001; Medina et al. 2001; Peterson & Eernisse 2001).

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