Invertebrate Reproduction and Development, 45:2 (2004) 137–150
Balaban, Philadelphia/Rehovot
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Morphology and fine structure of the swimming larvae of *Ircinia oros* (Porifera, Demospongiae, Dictyoceratida)

ALEXANDER V. ERESKOVSKY[1*] and DARIA B. TOKINA[2]
[1]*Department of Embryology, Faculty of Biology and Soils, Saint-Petersburg State University, Universitetskaja nab. 7/9, St.-Petersburg 199034, Russia; and Centre d'Océanologie de Marseille, Station marine d'Endoume, Université de la Méditerranée, UMR-CNRS 6540, rue de la Batterie des Lions, 13007-Marseille, France*
Tel. +7 (812) 328-9453; Fax +7 (812) 328-9530; email: aereskovsky@hotmail.com
[2]*Zoological Institute, Russian Academy of Sciences, Universitetskaja nab. 1, St. Petersburg 199034 Russia*

Received 24 June 2003; Accepted 4 May 2004

Summary

The fine structure of the free-swimming larvae of the sponge *Ircinia oros* (Dictyoceratida, Demospongiae) from the northern Mediterranean was studied using light and electron microscopy. Larvae are of the parenchymella type, oval. The larva is extensively ciliated. A band of long cilia and pigment-filled protrusions separates the posterior region from the antero-lateral region. The parenchymella is three-layered, with a ciliated epithelium, an intermediate layer and an internal zone. The surface of the larva is made of pear-shaped monociliated cells, which form a pseudo-stratified columnar epithelium. Zonula adhaerens, which join the apical parts of the ciliated cells, can be observed. The single basal ciliary rootlet is fibrillar and not associated with nucleus. A network of microtubules extends from the side of the basal body and forms electron-dense bundles. These bundles are opposite to the basal foot, run parallel to apical part of the plasmalemma and are oriented towards the posterior pole of the larva. A nucleus without a nucleolus is situated in the basal part of the cell. Above the nucleus there is a complex of large lipid droplets. The basal part of the cells are anchored by thick bundles of collagen fibrils with a regular transverse banding pattern of 25 nm periodicity. A prominent spindle-shaped sheath of subepidermal cells separates the epithelium from the central region of the larva. These cells are situated perpendicular to the epithelium and synthesize abundant fibrils of collagen. A part of these cells degrades. In the internal part of the larva there are multiple intercellular endosymbiotic bacteria and amoeboid nucleolated cells. Our data show that the study of sponge larvae is a source of diagnostic characters at the order level.

Key words: Larva, *Ircinia*, ultrastructure, collagen, Mediterranean Sea

Introduction

The larva, the free-living postembryonic stage of many aquatic animals, plays an important role in the geographical distribution of the populations and the colonization of new habitats. The presence of a larval stage in the life-cycle is characteristic of most representatives of the Porifera. Nine types of larvae have been described in sponges: calciblastula (Calcinea, Calcarea), coeloblastula (Hadromerida, Demospon-

*Corresponding author.

giae), pseudoblastula (Chondrosida, Demospongiae), amphiblastula (Calcaronea, Calcarea), cinctoblastula (Homoscleromorpha), parenchymella (the many of Demospongiae), trichimella (Hexactinellida), hoplitomella (*Alectona* and *Thoosa,* Demospongiae) and disphaerula (Halisarcida, Demospongiae) (Maldonado and Bergquist, 2002). Parenchymellae may be are the most ancient sponges larvae (Li et al., 1998). They are considered to be characteristic of the demosponges (Lévi, 1956; Simpson, 1984; Fell, 1989; Maldonado and Bergquist, 2002). They have a single layer of ciliated cells. The inner part of the larva is filled with differentiated cells — archaeocytes, granular cells, sclerocytes, and collencytes. In some haplosclerid species (freshwater sponges, order Haplosclerida, Demospongiae), choanocytes and endopinacocytes are also differentiated.

In ovoviviparous Demospongiae, the larva is the most well studied ontogenetic stage. Sponges of the order Dictyoceratida Minchin 1900 are tough and flexible; they are hard if debris infiltrates the matrix, or the skeletal mesh is condensed and soft when the density of the fiber skeleton in relation to soft tissue volume is reduced. Fibers are anastomosing and, except in two genera, have a hierarchical arrangement with respect to diameter and surface orientation. Fiber composition is homogenous with growth laminae just apparent, or pithed and strongly laminated with bark and pith elements (Cook and Bergquist, 2002). The description of larvae of order Dictyoceratida (Demospongiae) started with the works of Schulze on *Dysidea pallescens* and *Spongia officinalis* (Schulze 1879a, 1879b). Later, the swimming larvae were studied by light microscopy in *Ircinia variabilis, Spongia officinalis* (Maas, 1894), *Phyllospongia foliascens* (Lévi, 1956), *Hippospongia communis* (Tuzet and Pavans de Ceccatty, 1958), and electron microscopy in *H. lachne, Spongia barbara, S. graminea*, *S. cheisis* (Kaye, 1990; Kaye and Reiswig, 1991). The ciliated epithelium was studied using the scanning electron microscope in *Spongia* sp. and *Ircinia* sp. (Bergquist et al., 1979).

Though sponges lack a nervous system, their larvae possess a rather complex behavior and a quick reaction to external stimuli (light, gravity, current) (Wapstra and Van Soest, 1987; Maldonado and Young, 1996, 1999; Leys and Degnan, 2001). To understand the cellular mechanisms involved in the complex behavior of the sponge, larvae ultrastructural investigations are necessary, with precise descriptions of cells and their relations. Unfortunately, works of this kind are insufficient, to say the least (Boury-Esnault, 1976; Evans, 1977; Saller and Weissenfels, 1985; Woollacott, 1993; Amano and Hori, 1992, 1994, 2001; Ivanova, 1997; Leys and Degnan, 2001; Uriz et al., 2001; Boury-Esnault et al., 2003).

In the present study we describe for the first time the fine structure of the parenchymella in *Ircinia oros* (Demospongiae, Dictyoceratida) from the Mediterranean Sea on the basis of light and electron microscopy to answer the question: are morphological features of larvae an important characteristic in the classification of sponges?

Materials and Methods

Reproducing specimens of *I. oros* (Schmidt, 1864) (Irciniidae, Dictyoceratida) were collected by SCUBA diving in August 2000 in the western Mediterranean Sea (Golf of Lion), at a depth of 12 m. Material for light microscopy was fixed in Bouin's fixatives. Tissue fragments were dehydrated through an ethanol series and embedded into paraffin, 6-µm-thick sections were stained with Mayer's hematoxylin, eosin and Heidenhein ferric hematoxylin.

For scanning electron microscopy (SEM), the fixative used was a 5:1 mixture of 2% OsO_4 and saturated mercury chloride (Johnston and Hildemann, 1982). 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) samples were prefixed in 1% OsO_4 for 10 min and fixed in 2.5 % glutaraldehyde in phosphate buffer (pH 7.4) at room temperature for 1 h. After fixation, larvae were washed in phosphate buffer (pH 7.4) and postfixed in 1% OsO_4 in phosphate buffer for 1 h. Samples were dehydrated through a graded ethanol series and embedded in araldite. For investigation of the cell contacts, methods with alcian blue were used. The larvae were fixed for 1.5 h in 1% OsO_4 buffered with phosphate buffer (pH 7.4) at room temperature. After fixation, larvae were washed in phosphate buffer (pH 7.4) and postfixed in 2.5% glutaraldehyde to which alcian blue was added at a final concentration of 1%. Semi-thin sections were stained with methylene blue-borax. Ultrathin sections were contrasted with uranyl acetate and lead citrate. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under a JEM-100 CX.

Results

Behavior

The larvae of *Ircinia oros* are characterized by a rather complex behavior. They can swim with both

anterior or posterior pole first. In the latter case, their speed is approximately two times less than during normal swimming. While moving, the larvae may turn quickly and change the direction of movement by 90°. A ring of long cilia surrounding the posterior pole acts as a paddle. Viewed from the anterior pole, the larva moving anterior pole first, rotates around its axis clockwise, whereas the larvae moving posterior pole first, anti-clockwise. The larvae of *I. oros* have a clearly pronounced positive phototaxis.

Morphology

The larvae have an oval shape elongated in the anterior–posterior direction (about 172–279 × 530–550 μm in size), with a slightly pointed anterior pole (Fig. 1a). The larvae are evenly covered with cilia (Fig. 1a). Cilia have equal length on all larval surfaces (about 30.7 μm in length) excepted the posterior pole, where they are shorter (about 7.1 μm), and the ring of cells, surrounding the posterior pole, where the cilia are the longest (Fig. 1c and d). The cilia show a

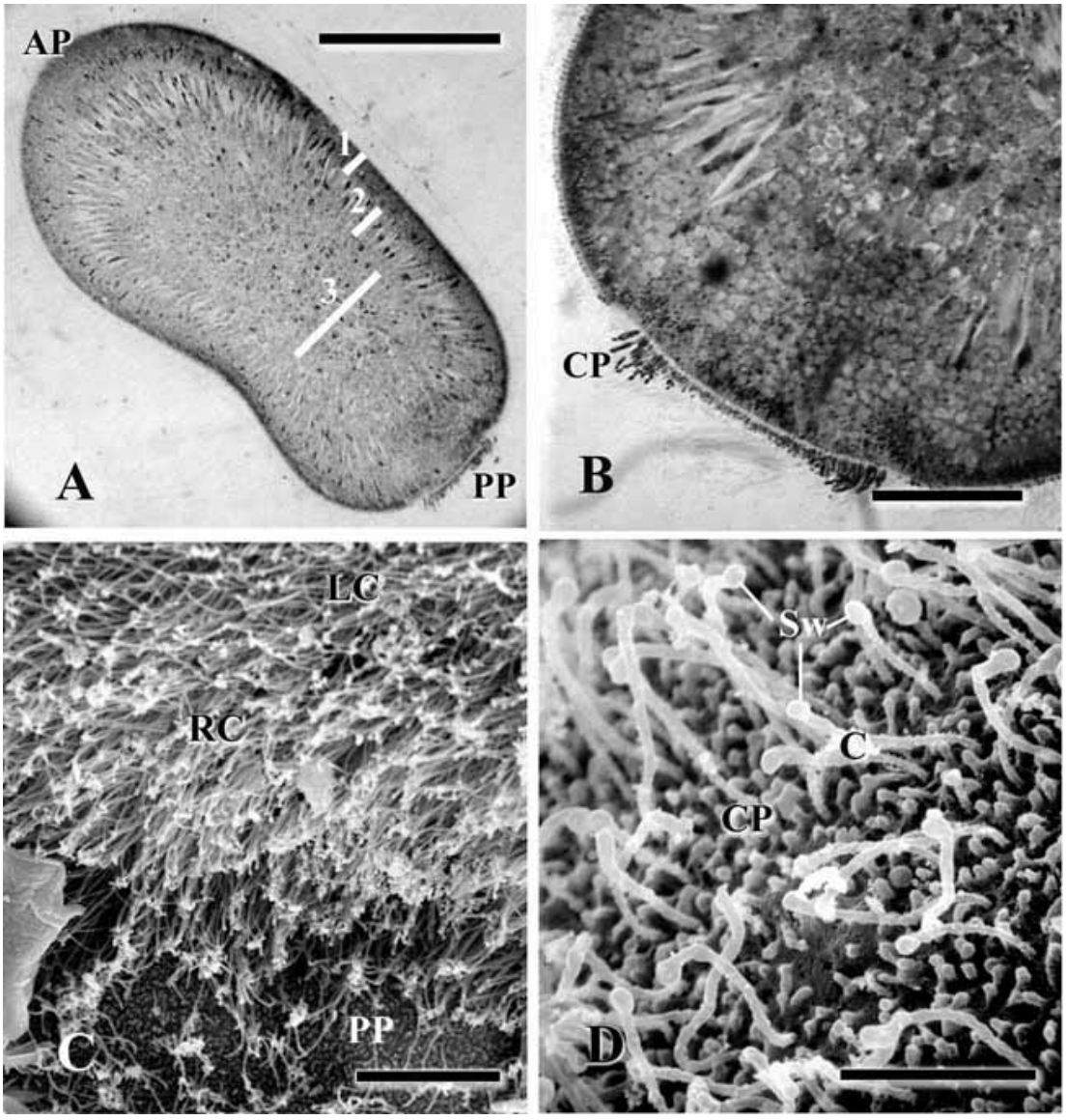

Fig. 1. Parenchymella of *Ircinia oros* A: Longitudinal semi-thin section showing three zones: 1, external ciliated cells; 2, intermediated layer of secretic cells; 3, internal part. B: Semi-thin section of posterior pole with long cytoplasmic protrusions (CP). C: Scanning electron micrograph of posterior pole ring of cells with long cilia (RC), lateral cilia (LC) and posterior pole ciliated cells (PP). D: Scanning electron micrograph of posterior pole ciliated cells with cytoplasmic protrusions (CP) and cilia (C) with the terminal swelling (Sw). AP, anterior pole; PP, posterior pole. Scale bars: A: 170 μm; B: 40 μm; C: 20 μm; D: 5 μm.

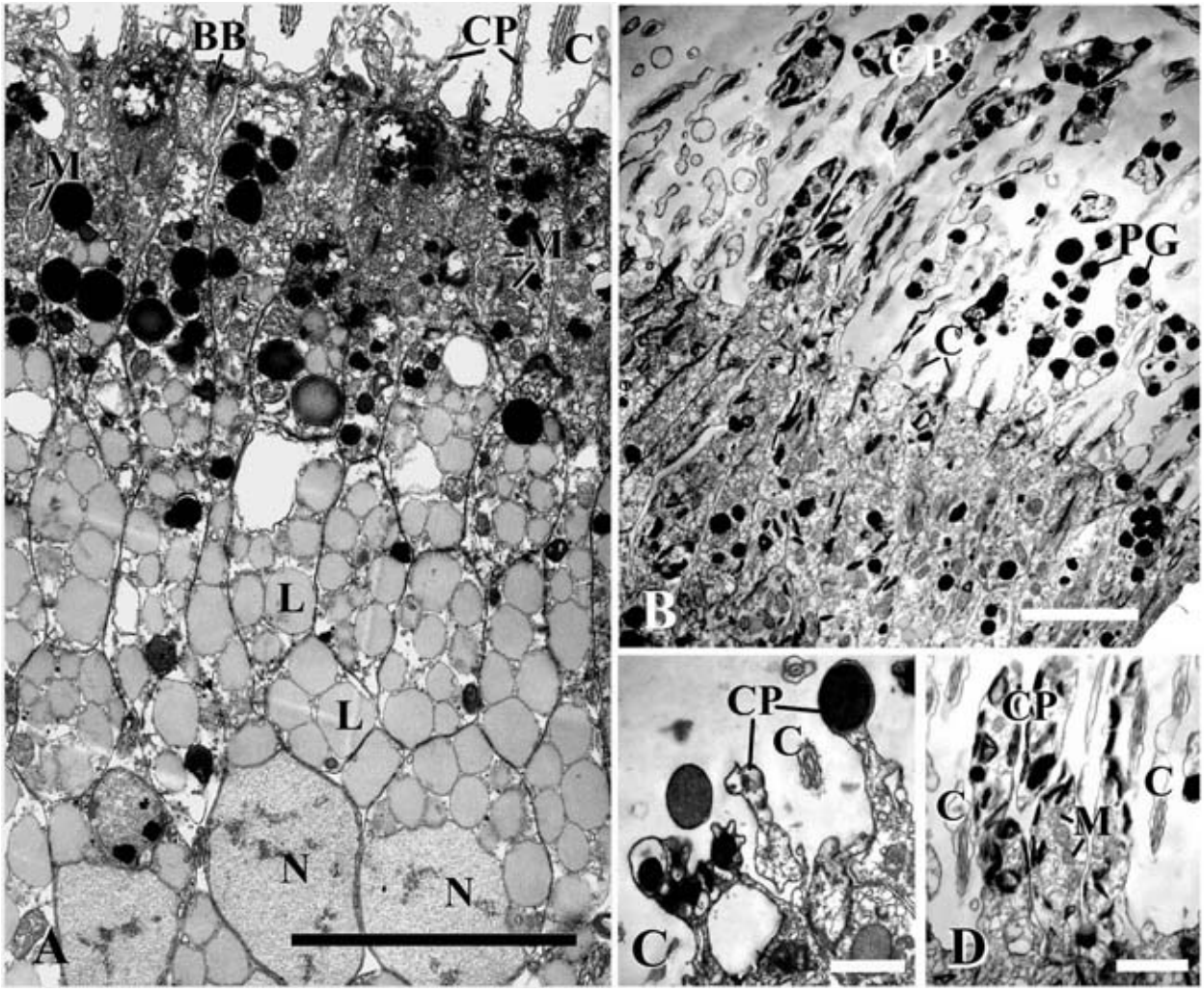

Fig. 2. Ciliated cells of parenchymella of *Ircinia oros*: A: Ciliated cells. B: Apical parts of the posterior pole ring cells. C: Cytoplasmic protrusions of antero-lateral cells. D: Cytoplasmic protrusions of posterior pole ring cells. (TEM). CP, cytoplasmic protrusions; C, cilia; L, lipid droplets; M, mitochondria; N, nucleus; PG, pigment granules. Scale bars: A: 5μm; B: 3 μm; C: 1 μm; D: 1 μm.

terminal swelling (Fig. 1d). The ring cells are also characterized by a dark-gray pigmentation (Fig. 2b). The larvae are gray, but the posterior pole contains black pigment.

Anatomically, *I. oros* larvae can be subdivided into three zones: (1) external ciliated epithelium, (2) intermediate layer consisting of large loose spindle-shaped cells oriented perpendicular to the surface, (3) the loose inner mass of amoebocytes and symbiotic bacteria (Figs. 1a and 3a).

Ciliated cells are characterized by a clear apical-basal polarity expressed in the localization of cellular organelles and inclusions. The cells are pear-shaped, with the nuclei in their basal parts (Figs. 2a and 3b). The cell length is from 9.5 to 12.8 μm, the width in the nucleus area is 2.5–2.7 μm. The nuclei are 1.25–1.9 to 2.3–2.5 μm in size, of irregular shape. They are situated at different levels, and thus the epithelium is pseudostratified. There are no nucleoli. Within the nuclei there are islands of heterochromatin (Fig. 2a). Two zones may be delimited in the ciliated cells: the apical and the basal.

There are long cytoplasmic processes around the cilia, forming a kind of collar (about 3 μm long and 0.3–0.9 μm in diameter). Within them, vacuoles with electron-dense or electron-transparent granules can be often found (Fig. 2d). They often have spherical swellings at the end (Fig. 2c). The ciliated cells of the posterior pole ring possess the longest and the thickest processes (Figs. 1b; 2b and d). They include many organelles characteristic of apical cytoplasm: mitochondria, electron-dense and electron-transparent vacuoles (Fig. 2b and d).

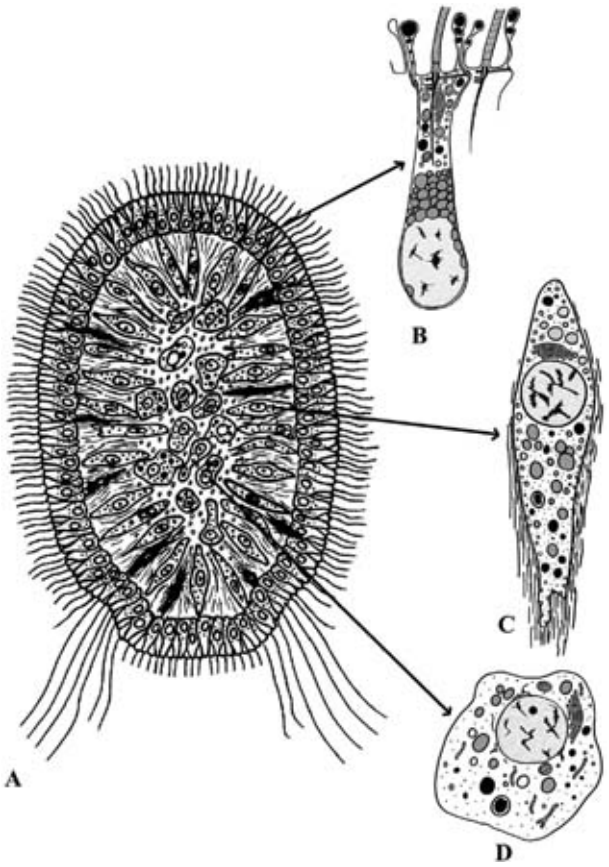

Fig. 3. Diagram of *I. oros* parenchymella. A: Free-swimming larva. B: Flagellated cell of antero-lateral part. C: The spindle-shaped cells of the intermediate layer. D: Cell of inner part of the larva.

The apical region of the cell is narrow and long. The cilia with the rootlet apparatus and the organelles are situated in this part (Figs. 2a, b; 3b; 4a). The rootlet apparatus includes the kinetosome (0.34 μm long, 0.28 μm in diameter), an accessory centriole, oriented perpendicular to it, and a long unpaired rootlet, leaving the centriole, which consists of non-striated fine fibrillar material (Fig. 4a). In all larval ciliated cells except those at the posterior pole, the accessory centriole is situated laterally to the kinetosome (Fig. 4a). At the posterior pole, it is located beneath the basal body and oriented orthogonally to its long axis (Fig. 4b). The rootlet is not associated with the nuclear membrane. Approximately in the middle of the basal body, a basal foot is formed (0.14 μm long, 0.17 μm wide). It is always located above the accessory centriole (Fig. 4a). The basal foot is ovoid and lacks a pedicle. From the side of the basal body, opposite to the basal foot, a well-pronounced horizontal bundle of microtubules starts (4.1 μm long, 0.09 μm wide), which goes to the lateral cell wall (Fig. 4c). Horizontal bundles of all cells are situated at the same level. They are parallel to each other and directed towards the posterior larval pole. The external ciliary membrane is covered with a loose layer of glycocalyx at their basis.

In the apical regions of all ciliated larval cells specialized adhesion contacts have been found (Fig. 4a–d) — electron-dense thickenings of filamentous material along the internal membrane.

In the cytoplasm of the apical part of the cell there are numerous electron-transparent vesicles 0.2–0.5 μm in diameter, oval mitochondria (0.8–1.6 μm in diameter) with flattened cristae, small (0.9–1.7 μm) yolk granules and spherical, possibly pigment, granules (1.2–1.7 μm in diameter) (Fig. 4a–c). In this cell part the Golgi apparatus is also situated (Fig. 4a).

The basal part of ciliated cells is enlarged, which are not in direct contact with the inner cells (Figs. 5a and 6a). It contains the nucleus adjacent to the plasma membrane or sometimes surrounded by a thin cytoplasmic rim. Above the nucleus a complex of densely packed lipid droplets and yolk granules is situated. They are often closely pressed against the nucleus (Fig. 5a and b). There are no cytoplasmic processes like filopodia or pseudopodia. The basal parts of the ciliated cells are interspersed by thick bundles of collagen fibrils with a regular transverse banding pattern of about 25 nm periodicity (Fig. 5c and d). One fibril is about 1.5 μm in width. These fibrils are formed from joined thin microfibrils with a transverse banding pattern (Fig. 5b–d), secreted by the large cells of the intermediate layer.

The cells of the intermediate layer are spindle-shaped (2.2–3.0×12–16 μm). Their long axis is oriented perpendicular to the larval surface (Fig. 6a and b). The cells often form apical branches, penetrating between the basal parts of ciliated cells. The nucleolated nucleus is spherical (about 2.4 μm in diameter) and contains a lot of heterochromatin. The cytoplasm contains numerous lipid droplets (from 0.2 to 0.8 μm), small homogenous osmophilic inclusions and vacuoles with symbiotic bacteria. In addition, the intermediate layer cells contain rounded mitochondria, a well-developed Golgi apparatus and rough endoplasmic reticulum. The membranes of the spindle-shaped cells are the assemblage sites of the collagen fibrils. Collagen bundles are also oriented perpendicular to the larval surface and fill almost all space between the basal parts of the ciliated cells and spindle-shaped cells (Fig. 6b). Some of the spindle-shaped cells degrades: the cytoplasm becomes dense, the osmophilic inclusions merge to form shapeless electron-dense bodies, the nucleus and specialized

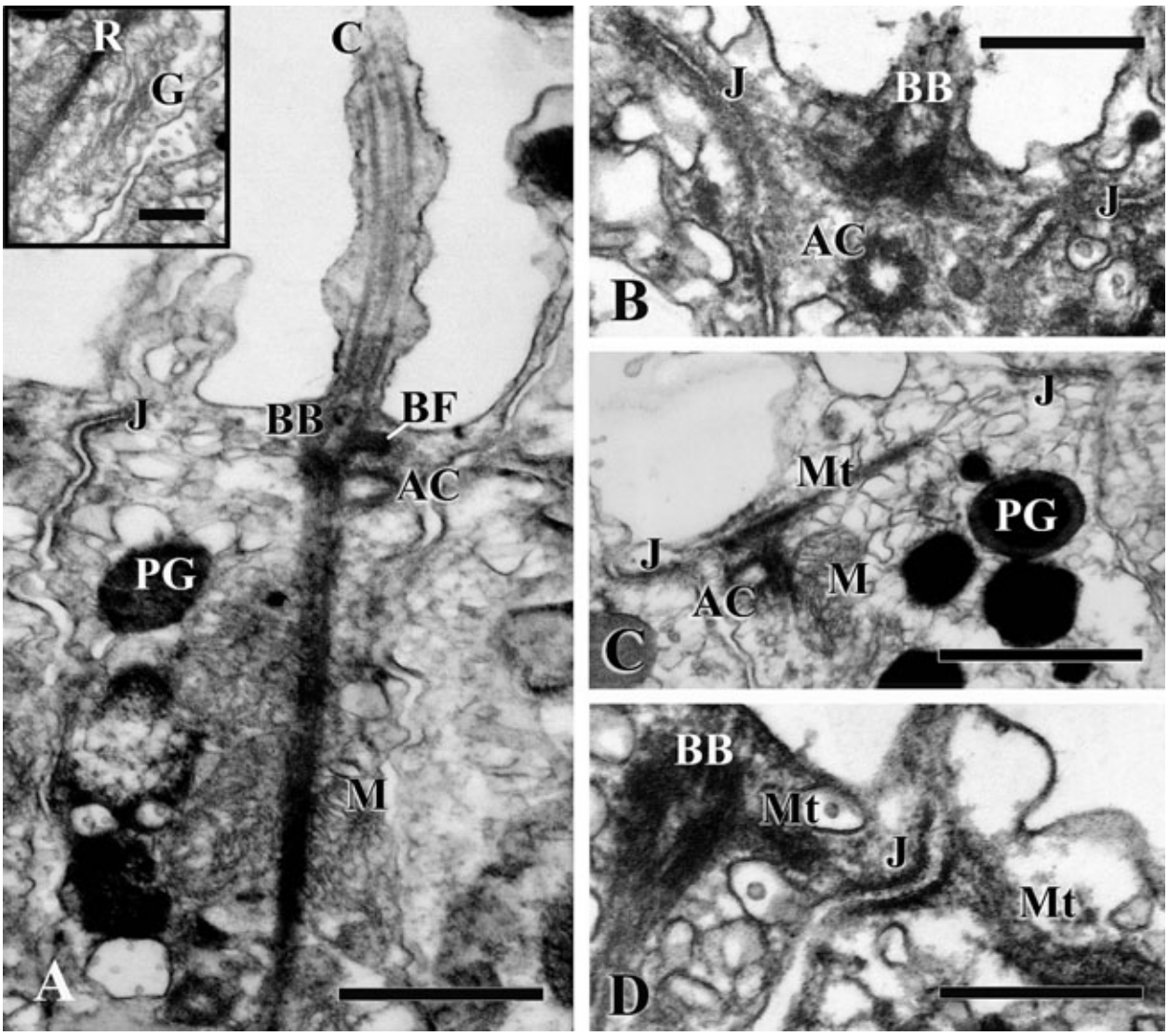

Fig. 4. Apical part and ciliary apparatus of a ciliated cell of parenchymella of *Ircinia oros* (TEM). A: Antero-lateral cells with cilia (C), basal body (BB), accessory centriole (AC) and basal foot (BF) from the basal body arrive a ciliar rootlet (R). Insert: Golgi system (G) near the ciliar rootlet (R). B: Apical part of the posterior pole cells characterized by position of accessory centriole (AC) below the basal body (BB) at the same axis with it. C: Transverse section through an accessory centriole (AC) of a ciliar apparatus with the bundle of microtubules (Mt). D: Zonula adhaerens (J) in the apical part of ciliated cells. C, cilia; CP, cytoplasmic protrusions; G, Golgi system; J, zonula adhaerens; M, mitochondria; PG, pigment granules. Scale bars: A: 1 μm; Insert: 0.3 μm; B: 0.3 μm; C: 1 μm; D: 0.4 μm.

organelles degrade and the plasma membrane disappears (Fig. 6a and b). However, these cells are surrounded by a trail of collagen fibrils, which testifies to their role in the extracellular matrix synthesis. Such cells often penetrate into the middle and even apical part of the ciliated epithelium. Symbiotic bacteria are rarely encountered in the intermediate layer, concentrating mostly in the basal part of the spindle-shaped cells.

The inner part of the larva contains the products of cell exocytosis and numerous symbiotic bacteria which are identical to those present in the mesohyl of the parent (Vacelet, 1975). A skeleton and any kind of cavity are lacking. The inner mass also contains characteristic large amoeboid cells with a large spherical nucleus and a nucleolus (Fig. 7). These cells are similar to the spindle-shaped cells of the intermediate layer in their ultrastructural characteristics. The extracellular matrix is represented by a loose network of collagen fibers.

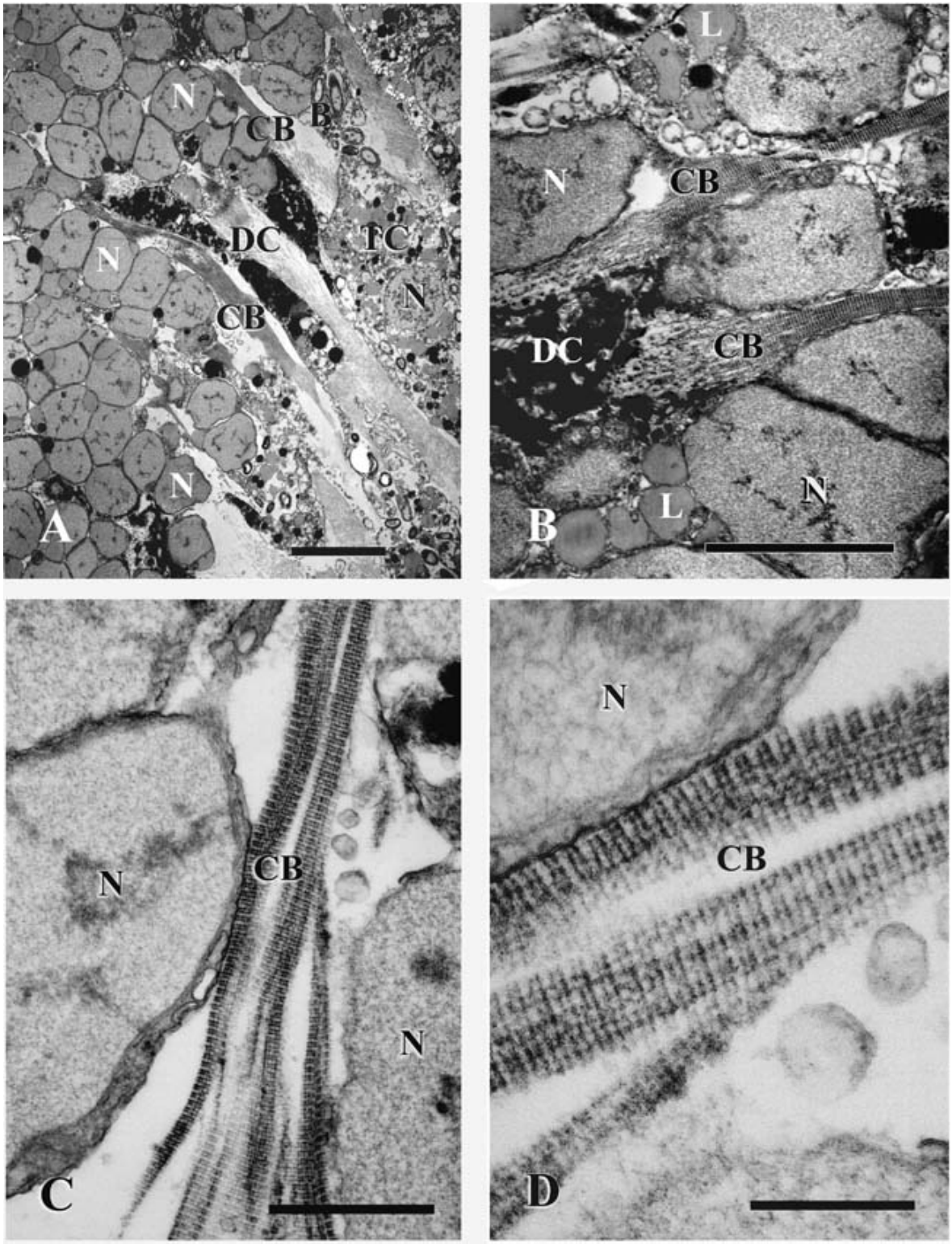

Fig. 5. Basal parts of the ciliated cells of larva *Ircinia oros* (TEM). A: Longitudinal section with the bundles of collagen (CB), secreted by intermediate zone cells (IC), part of them are degraded (DC). Symbiotic bacteria (B) are dispersed between a collagen bundles. B: Transverse section. The complex of lipid droplets (L) there is above the nucleus. C, D: Detail of bundles of collagen fibrils with a regular transverse banding pattern (CB). N, nucleus. Scale bars: A: 3 μm; B: 1 μm; C: 1 μm; D: 0.3 μm.

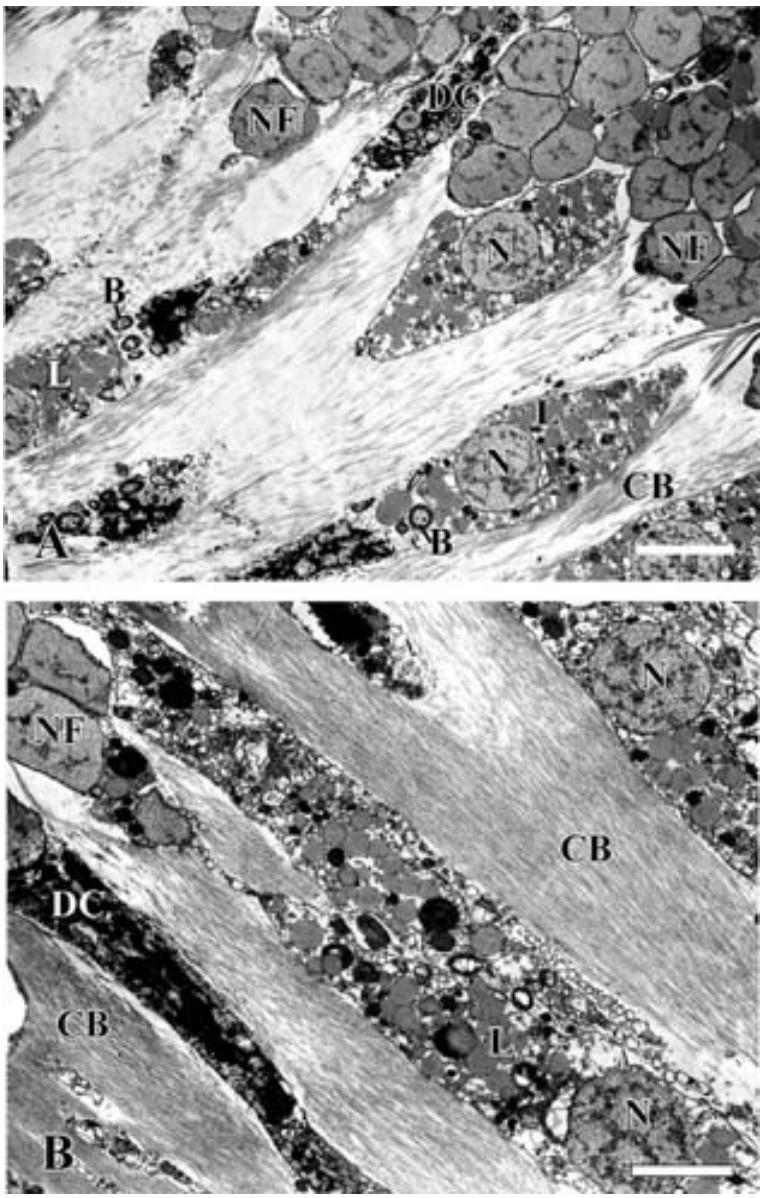

Fig. 6. The spindle-shaped cells of the intermediate layer of parenchymella with a spherical nucleus (N), lipid droplets (L) and symbiotic bacteria (B) inside of its cytoplasm. Parts of the cells are degraded (DC). Between the cells there are collagen bundles (CB) are situated. NF, nucleus of ciliated cells. Scale bars: A: 3 μm; B: 2 μm.

Discussion

The morphology and ultrastructure of the larva of *I. oros* described above have features both similar to and different from those found in other members of the order Dictyoceratida. The cilia are of equal length on all larval surfaces except the posterior pole and posterior pole ring. In some other species of Dictyoceratida (according to several authors), there are bunches of long cilia at the posterior larval pole, which are more than two times longer than lateral ones (Table 1). However, in the *I. oros* larva investigated here, the cilia at the posterior pole are, on the contrary, much shorter than in other areas. This kind of ciliature has been described for a number of dictyoceratids: *Spongia officinalis*, *Hippospongia lachne*, *Spongia barbara*, *S. graminea*, *S. cheisis*, *Cacospongia mollior* (Schulze, 1879a, 1879b; Kaye, 1990; Kaye and Reiswig, 1991; Uriz et al., 2002). It is not to be ruled out that the authors who have described a bunch of long cilia at the posterior pole erroneously interpreted in this way the cilia of the posterior pole ring. A posterior

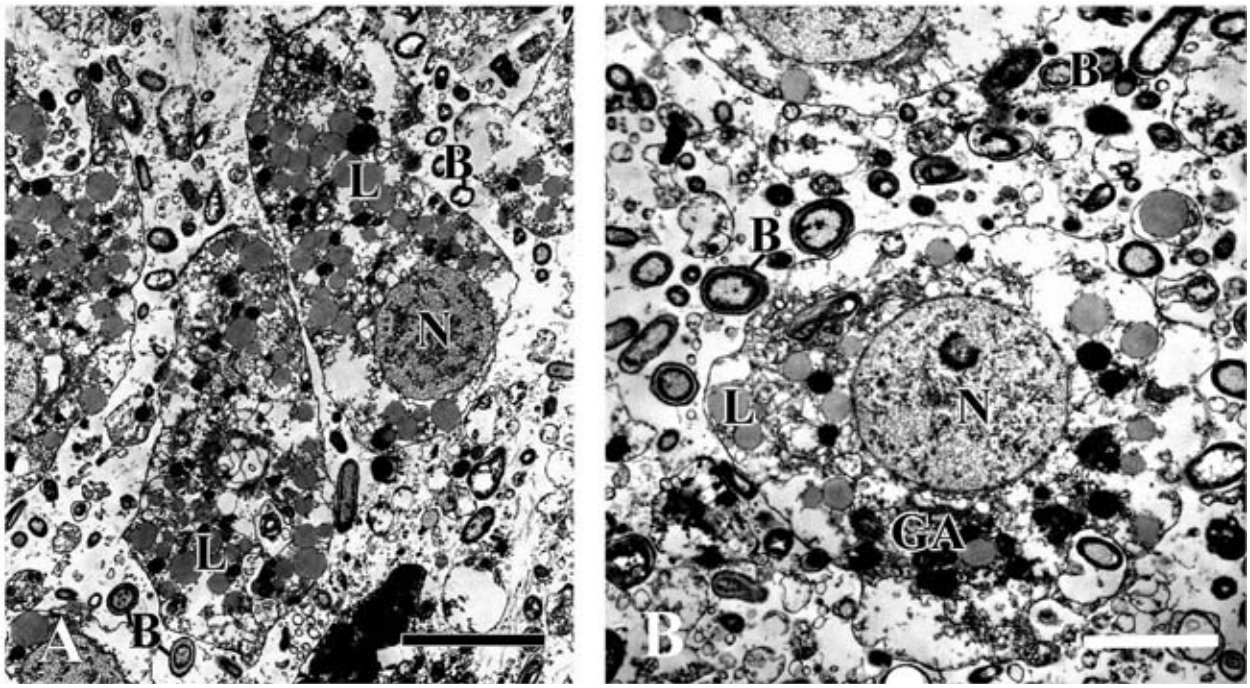

Fig. 7. Inner part of the larva with anucleolated (A) and nucleolated (B) amoeboid cells. B, symbiotic bacteria; GA, Golgi apparatus; L, lipid droplets; N, nucleus. Scale bars: A: 2.5; B: 1.5 μm.

pole ring of cells with long cilia is rather common in the parenchymellae of a number of marine Haplosclerida, but the posterior pole of these larvae is bare (Bergquist et al., 1979; Woollacott, 1993; Leys and Degnan, 2001).

As in all Dictyoceratida studied, the ciliated larval cells of *I. oros* are densely packed in a pseudostratified epithelium. But morphologically they are pear-shaped, with the nucleus in the distended basal part which is rather unusual for parenchymellae. The cells of the covering ciliated epithelium in the sponges are commonly columnar in shape with the nuclei situated at three quarters of the height of the epithelium. It is also unusual that there be a thin cytoplasmic layer left between the nucleus and the plasma membrane.

The nuclei of the ciliated cells of *I. oros* possess a homogenous structure. Heterochromatin is usually concentrated in 1–2 places in the central nucleus part. These cells may be supposed to be terminally differentiated and are to be subject to phagocytosis in the course of metamorphosis, as suggested by Kaye and Reiswig (1991).

The ciliated cells of *I. oros*, as well as those of other Demospongiae, have long cytoplasmic processes in their apical part, forming a kind of collar. Contrarily to parenchymellae of other sponges groups, these cytoplasmic processes in dictyoceratids larvae are much longer and often bear large electron-dense inclusions in their distended apical part that seem to be characteristic of the order Dictyoceratida (Kaye, 1990; Kaye and Reiswig, 1991; Uriz et al., 2002). These cytoplasmic processes are supposed to increase the surface area in contact of the cells with the environment, thus facilitating a more effective intake of macromolecules dissolved in the water (Oschman, 1978; Jaeckle, 1995).

Apical processes of the cytoplasm are most developed in the ciliated cells of the posterior pole ring in *I. oros* larvae. It is interesting that only in these cells the gray pigment is contained.

The ciliary basal apparatus of the ciliated cells of *I. oros* parenchymella is not essentially different from similar structures in other demosponges. One long rootlet leaves the kinetosome. It consists of fine fibrillar material and is non-striated, as in all Demospongiae (Woollacott and Pinto, 1995). A similar structure was described for the larvae of *Aplysilla* sp. (Dendroceratida) (Woollacott and Pinto, 1995). As in many parenchymellae of Demospongiae (Woollacott and Pinto, 1995), in *I. oros* a well-developed horizontal bundle of microtubules, directed towards the lateral cell wall, starts from the basal body part opposite to the basal foot. Woollacott and Pinto (1995) referred to these structure as a transverse cytoskeletal system.

Table 1. Characteristics of the larvae of the sponges from the different families of the order Dictyoceratida

Species	Size, µm	Anterior-lateral pigment	Posterior pole pigment	Posterior pole tuft of cilia	Posterior pole ring of long cilia	Author
			Dysideidae Gray, 1867			
Dysidea pallescens	210–390	?	?	Present	Present	Schulze 1879a
			Spongiidae Gray, 1867			
Spongia officinalis	235–330	?	?	?	Present	Schulze 1879 b
Spongia sp.	500–1000	Colourless	Dark gray	Present	Absent	Bergquist et al., 1979
Spongia (*Euspongia*) *sp.*	?	White	Brown	Present	?	Lévi, 1956
Spongia barbara	350–420	Gray	Black	Absent	Present	Kaye, 1990; Kaye and Reiswig, 1991
S. graminea	350–420	Gray	Black	Absent	Present	Kaye, 1990; Kaye and Reiswig, 1991
S. cheisis	350–420	Gray	Black	Absent	Present	Kaye, 1990; Kaye and Reiswig, 1991
Hippospongia communis	420–480 600–650	White-yellow	Black	Present	?	Tuzet and Pavans de Ceccatty, 1958a
H. lachne	350–420	Gray	Black	Absent	Present	Kaye ,1990; Kaye and Reiswig, 1991
Hippospongia sp.	?	White	Brown	Present	?	Maas, 1894
			Irciniidae Gray, 1867			
Ircinia sp.	460×630	White	Brown	Present	?	Maas, 1894
Ircinia sp.	500–1000	Colourless	Dark gray	Present	Absent	Bergquist et al., 1979
Ircinia oros	172–279× 530–550	Gray	Black	Absent	Present	Original
Ircinia variabilis	?	?	?	Present	?	Maas, 1894
			Thorectidae Bergquist, 1978			
Cacospongia mollior	?	Greyish	Dark	Absent	Present	Uriz et al., 2002
Phyllospongia foliascens	?	White	Black-brown	Present	Absent	Lévi, 1956

We observed a clear orientation of elements of the ciliar basal apparatus of the *I. oros* parenchymellae. They are expressed in the parallel orientation of the horizontal bundle of microtubules, directed to the posterior larval pole and the basal foot directed to the apical pole. A similar orientation of an analogous structure was described for the coeloblastula of *Clathrina* (*Leucosolenia*) *laxa* (Amano and Hori, 2001). Such an orientation of a transverse cytoskeletal system in Eumetazoa is generally directed posterior and probably essential for aligning the direction of the effective stroke of the cilia (Rieger, 1976; Sanderson, 1984).

There are specialized intercellular contacts (of the zonula adhaerens or the belt desmosomes type) in Dictyoceratida larvae in the apical regions of all ciliated larval cells. They are electron-dense thickenings of filamentous material along the internal membrane. Belt desmosomes have also been shown in the larvae of the demosponges *Dysidea etheria* (Dendroceratida) (Rieger, 1994), *Halisarca dujardini* (Halisarcida), *Pleraplysilla spinifera* (Dictyoceratida) (Gonobobleva and Ereskovsky, unpubl.), in the trichimella of the hexactinellid sponge *Oopsacas minuta* (Boury-Esnault et al., 1999), and between the ciliated cells of the cinctoblastulae, the larvae of Homoscleromorpha (Boury-Esnault et al., 2003). This type of cell contact ensures mechanical connection between the cells and is viewed by some authors as the first evolutionary stage of desmosomes (Boury-Esnault et al., 1999).

A large amount of lipid may be considered another feature characteristic of *I. oros* larvae. Lipid inclusions are characteristic of almost all sponge larvae (Boury-Esnault, 1976; Woollacott, 1990, 1993; Amano and Hori, 1994, 2001; Ivanova, 1997; Boury-Esnault et al., 2003). However, a large amount of lipid inclusions has

Table 2. Comparative characteristics of the larvae of the sponges from the different orders of "Keratosa"

Character/Order	Dendroceratida	Dictyoceratida	Halisarcida
Type of larva	Parenchymella	Parenchymella	Disphaerula or parenchymella
Shape	Oval	Oval or ellipsoid	Oval or slightly flattened in the anterior/posterior direction
Cone-shaped anterior pole	Yes	No	No
Size, μm:			
Length	350–500–600	330–500–1000	100–110
Diameter	125–190	172–350–580	125–130
Ciliation pattern:			
Anterior pole	Absent or short cilia	Equal length	Equal length
Lateral zone	Equal length	Equal length	Equal length
Posterior pole	Tuft of long cilia	Posterior pole cells have short cilia or tufted	Sparse on the posterior pole
Posterior pole ring of long cilia	No	Yes	No
Anatomy:			
Anatomical division	Two layers: ciliated epithelium and loose inner part	Two layers: ciliated epithelium and loose inner part	Two layers: ciliated epithelium and central part
Internal part of larva	Loosely	Loosely	Spherical chamber formed by flagellated cells or compact cells conglomerate
Cells of internal part	Archaeocytes and collencytes	Archaeocytes	Nucleolated amoebocytes, ciliated cells
Subepidermal layer of spindle-shaped cells situated perpendicular to the epithelium	Absent	Present	Absent
Pigmentation :			
General pigmentation	Uniformly	Differently	Uniformly
Anterior-lateral pigment	Pink, yellow	White-gray	Milky
Posterior pole pigment	Pink, yellow	Dark	Milky
Ciliated cells:			
Ciliated cells	Elongated with nuclea on different levels in apical 1/3	Pear-like form with basal nuclea	Elongated, nearly prismatic with nuclei on the one level in apical 1/3
Apical parts of the ciliated cells	Short cytoplasmic processes	Long cytoplasmic processes (about 27% from the length of cell) with large electron-dense inclusions	Short cytoplasmic processes (about 2% from the length of cell)
Reference	Delage, 1892; Bergquist et al., 1979; Woollacott and Hadfield, 1989	See Table 1	Lévi, 1956; Ereskovsky and Gonobobleva, 2000; Gonobobleva and Ereskovsky, 2004

been shown only for the parenchymellae of two poecilosclerid species *Hamigera hamigera* and *Crambe crambe*, and in the trichimella of hexactinellid *Oopsacas minuta* (Boury-Esnault 1976; Uriz et al. 2001; Boury-Esnault et al. 1999). These reserve substances ensure the long pelagic life of the larva, as well as its successful survival during the critical metamorphosis period.

Contrarily to most of the Demospongiae larvae, in *I. oros* there are no cytoplasmic processes in the basal part of the ciliated cells. At the same time, the basal parts of the ciliated cells are tightly connected by longitudinal collagen bundles. The presence of a powerful complex of spindle-shaped cells of the intermediate layer and of the collagen fibrils secreted by them is a unique feature of *I. oros* parenchymellae. It

has not been described for any other Porifera larva. Characteristically, as they penetrate into intercellular spaces of the ciliated epithelium, collagen fibrils are assembled into powerful bundles with a clear regular transverse banding pattern. This layer of parallel collagen fibrils is distinct from the basal lamina, characteristic for the cinctoblastulae of Homoscleromorpha (Boury-Esnault et al., 2003).

All investigations of sponge collagen have been made on adult animals with the exception of a hoplitomella larva in *Alectona* sp., in cinctoblastulae of Homoscleromorpha and in *Cacospongia mollior* (Garrone, 1974; Boury-Esnault et al., 2003; Uriz et al., 2002). Sponge collagen fibrils are about 20 nm in diameter with a banding patterns (Garrone, 1978, 1985). The diameter of collagen fibers in the larva of *I. oros* is about 17 nm. The same type of collagen fibers, forming a bundle in the filaments of a skeleton, presents in adult sponges of genus *Ircinia* (Garrone et al., 1973). However, the transverse banding pattern of collagen fibers of adult *Ircinia* is about 60 nm periodicity, while the periodicity of the fibers of a larva is about 25 nm, which may reflect probable mechanical constraint.

Thus, the ciliated epithelium of *I. oros* larvae and the underlying layer of secretory spindle-shaped cells together with collagen fibrils seem to be an integrated complex. Taking into account the presence of zonula adhaerens in the apical parts of the ciliated cells, it may be supposed that *I. oros* larvae possess a special type of columnar epithelium among the sponges.

Another feature of the intermediate layer, unique for Dictyoceratida, is a large number of degrading spindle-shaped cells with a characteristic electron-dense cytoplasm and a large number of electron-transparent microvesicles. Such cells often penetrate into the middle, and even the apical part of the ciliated epithelium. The role of these cells and the causes of their degradation are unknown.

The central mass of the larval cells is loose and homogenous. Denser or looser zones cannot be distinguished there. The central mass consists of archaeocytes, rich in inclusions. Our investigations confirm the electron-microscopic observations made on four species of Dictyoceratida (Kaye and Reiswig, 1991) that the archaeocytes are the only cell type in the internal mass of the larva. Furthermore, inside the larva of *I. oros* there is a network of extracellular matrix and collagen fibrils, and there are numerous symbiotic bacteria, similar to those in the adult sponges (Vacelet, 1975).

Orders Dictyoceratida, Dendroceratida, Verongida and Halisarcida form a well-defined group — "Keratosa", distinct from other Demospongiae on the basis of the absence of spicules and the development of the pithed sponging fiber skeleton (Bergquist, 1980, 1996; Van Soest, 1991; Bergquist et al., 1998). Morphology of the spongin fibre skeleton, anatomy, differential pigmentation, cytology, biochemistry and molecular phylogeny of adults are similar in different dictyoceratids families and distinct Dictyoceratida from the other "Keratosa" (Bergquist, 1996; Bergquist et al., 1998). Our comparison of morphological features of the dictyoceratids larvae from different families (Table 1) may testify to the presence of some peculiarity that also confirm the distinction of this order from other "Keratosa" (Table 2). At the same time some larval characters such as posterior pole ciliation are variable within the Dictyoceratida. The phenomenon of polymorphism of larval morphology has been also showed in other sponge groups (Wapstra and Van Soest, 1987; Ivanova, 1997; Gonobobleva and Ereskovsky 2004).

To answer the question of which larval characters could be proposed as diagnostic characters of Dictyoceratida, we have carried out a comparison of larval features between orders of "Keratosa" (Table 2). Unfortunately, larvae are unknown in Verongida. From Table 2 it can be seen that the larvae of each order have some of the specific morphological features.

The parenchymella of the order Dictyoceratida have the following diagnostic characters: (1) the posterior pole, as a rule, contains a dark pigment; (2) the cilia of external ciliated columnar epithelium are of equal length on all larval surfaces except the posterior pole ring with more longer cilia; (3) in the apical parts of the ciliated cells there are long cytoplasmic processes with large electron-dense inclusions; (4) archaeocytes are the only cell type in the internal part of the larva.

Larval morphological features are important characteristics in the classification of sponges (Lévi, 1956; Bergquist et al., 1979; Wapstra and Van Soest, 1987; Maldonado and Bergquist, 2002; Ereskovsky, 2002; Boury-Esnault et al., 2003). The results obtained on the structure of *I. oros* larvae and their comparison with literature data show that the morphology of sponge larvae may provide a number of useful characters at the order level.

Acknowledgments

The authors thank Nicole Boury-Esnault, Jean Vacelet and Maria Uriz for helpful discussions. This work was funded by the programme "Universities of Russia", No. 3248, RFBR N 03-04-49773. Alexander Ereskovsky received a special grant from the French

"Ministère de l'Education nationale et de la Recherche" and the position of Invited Professor from the "Université de la Méditerranée".

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