

Choanocyte Ultrastructure in *Halisarca dujardini* (Demospongiae, Halisarcida)

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ABSTRACT Understanding poriferan choanocyte ultrastructure is crucial if we are to unravel the steps of a putative evolutionary transition between choanoflagellate protists and early metazoans. Surprisingly, some aspects of choanocyte cytology still remain little investigated. This study of choanocyte ultrastructure in the halisarcid demosponge *Halisarca dujardini* revealed a combination of minor and major distinctive traits, some of them unknown in Porifera so far. Most significant features were 1) an asymmetrical periflagellar sleeve, 2) a battery of specialized intercellular junctions at the lateral cell surface complemented with an array of lateral interdigitations between adjacent choanocytes that provides a particular sealing system of the choanoderm, and 3) a unique, unexpectedly complex, basal apparatus. The basal apparatus consists of a basal body provided with a small basal foot and an intricate transverse skeleton of microtubules. An accessory centriole, which is not perpendicular to the basal body, is about 45°. In addition, a system of short striated rootlets (periodicity = 50–60 nm) arises from the proximal edge of the basal body and runs longitudinally to contact the nuclear apex. This is the first flagellar rootlet system ever found in a choanocyte. The accessory centriole, the rootlet system, and the nuclear apex are all encircled by a large Golgi apparatus, adding another distinctive feature to the choanocyte cytology. The set of distinct features discovered in the choanocyte of *H. dujardini* indicates that the ultrastructure of the poriferan choanocyte may vary substantially between sponge groups. It is necessary to improve understanding of such variation, as the cytological features of choanocytes are often coded as characters both for formulation of hypotheses on the origin of animals and inference of phylogenetic relationships at the base of the metazoan tree. *J. Morphol.* 270:615–627, 2009. © 2008 Wiley-Liss, Inc.

KEY WORDS: sponges; basal apparatus; striated rootlet; periflagellar sleeve; cell junctions

INTRODUCTION

Choanocytes are distinctive cells of sponges that are characterized by a distal beating flagellum surrounded by a collar of microvilli with an internal contractile cytoskeleton. Choanocytes usually line discrete cavities (choanocyte chambers) occurring in the canal system that runs through the sponge body. Flagellar beating is thought to create a water

flow throughout the sponge body, whereas the microvilli of the collar appear to be responsible for retaining bacteria and pikoplankton suspended in the inflow, redirecting somehow these particles towards the distal cell surface for eventual phagocytosis (Harrison and De Vos, 1991). However, choanocytes not only function in feeding but also during sexual reproduction, and choanocytes of many sponges transdifferentiate into male and/or female gonial cells. Likewise, in many sponges characterized by internal fertilization, choanocytes are responsible for capturing swimming spermatozoa from the inflow, transdifferentiating into amoeboid cells that carry the spermatozoon to the oocytes located in the sponge mesohyl (e.g., Nakamura and Okada, 1998; Riesgo et al., 2007a).

The embryonic origin of poriferan choanocytes appears to be diverse. In some cases, the choanocytes have been interpreted to derive directly from the monoflagellated cells of larval epithelia, which reabsorb their axonemes and internalize during larval metamorphosis to transdifferentiate into choanocytes (e.g., Delage, 1892; Lévi, 1956; Borojevic and Levi, 1965; Amano and Hori, 1993, 1996, 2001; Leys and Degnan, 2002; Gonobobleva and Ereskovsky, 2004a; Maldonado, 2004; Ereskovsky et al., 2007). In other cases, the monoflagellated larval cells have been considered as a terminal lineage, and choanocytes are interpreted to derive from internal totipotent archeocytes (e.g., Meewis, 1939; Bergquist and Green, 1977; Bergquist and Glasgow, 1986; Misevic et al., 1990; Wielspütz and Saller, 1990; Kaye and Reisinger, 1991; Kaltenbach et al., 1999). Additionally, some authors have expressed suspicions that in some sponges choanocytes

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could derive from both flagellated larval cells and larval archaeocytes (e.g., Ivanova, 1997; Gonobleva and Ereskovsky, 2004a). More intriguing is the origin of the choanocytes in those species in which their free-swimming larva already contains choanocytes organized in chambers—though not functional (Harrison and Cowden, 1975; Wielspütz and Saller, 1990; Jaeckel, 1995).

The choanocyte is also regarded as a crucial cell type in animal evolution. The long-standing hypothesis that the first animals (presumably porifera-like organisms) evolved from a choanoflagellate-like protist ancestor (James-Clark, 1868) was inspired by the striking similarity in cell structure and function between choanoflagellate protists and poriferan choanocytes. Arguments supporting and refuting a putative homology between the poriferan choanocyte and collar cells of other metazoans have been discussed several times (e.g., Rieger, 1976; Willmer, 1991; King, 2004; Maldonado, 2004).

Although choanocyte cytology is at the heart of these relevant phylogenetic and developmental debates, many aspects of poriferan choanocyte ultrastructure remain little investigated. The fine structure of the choanocyte was first described by Kilian (1954) and Rasmont (1959). Ever since, cytological research on the choanocyte has been conducted using techniques of increasing resolution (e.g., Garrone, 1969; Brill, 1973; Watanabe, 1978; Boury-Esnault et al., 1984; Vacelet et al., 1989; Hartman and Willenz, 1990; Mehl and Reisinger, 1991; Eerkes-Medrano and Leys, 2006, etc). However, some ultrastructural aspects appear to have been neglected. For instance, only two studies have specifically investigated the ultrastructure of the basal apparatus (Brill, 1973; Karpov and Efremova, 1994), despite the potential phylogenetic signal contained in this complex organelle system (Woollacott and Pinto, 1995). More importantly, both studies were conducted on the same freshwater haplosclerid demosponge, *Ephydatia fluviatilis*. Because of this scarcity in ultrastructural descriptions, the variability affecting some of the characters used for phylogenetic inference is not appropriately incorporated into the analyses.

We have investigated the choanocyte of the halisarcid demosponge *Halisarca dujardini* (Johnston, 1842) devoting particular attention to its basal apparatus. Although the cytology of embryos and metamorphic stages of this sponge have been studied extensively (Lévi, 1956; Ereskovsky and Gonobleva, 2000; Gonobleva and Ereskovsky, 2004a,b; Mukhina et al., 2006; Gonobleva, 2007), its choanocyte fine structure remains poorly known (Vacelet et al., 1989; Bergquist, 1996).

MATERIALS AND METHODS

Adult specimens of *H. dujardini* (Johnston, 1842) (Demospongiae, Halisarcida) were collected from 1.5 to 5 m depths by

snorkeling in the Chupa Inlet, near the Sredny Island (Kandalaksha bay, White Sea) in June–July 2001.

For transmission electron microscopy (TEM), tissue fragments and larvae were prefixed in 1% OsO₄ in 0.2 M phosphate buffer (PBS) for 10 min, rinsed in PBS (pH = 7.4), fixed in 2.5% glutaraldehyde in PBS at room temperature for 1 h, rinsed in PBS again, and postfixed in 1% OsO₄ in PBS for 1 h. Subsequently, samples were dehydrated through a graded ethanol series and embedded in Epon-Araldite. By using an LKB-Nova ultramicrotome provided with diamond knives, we obtained serial and nonserial ultrathin sections, which were later mounted on formvar-coated blends and contrasted with 2% aqueous uranyl acetate and Reynolds lead citrate. Blends were viewed using a JEM-100CX TEM. Because the microscope was not equipped with a module for digital image acquisition, photographic negatives were scanned for elaboration of digital figures.

RESULTS

General Cytology of the Choanocyte

Choanocytes of *H. dujardini* are irregularly pseudocylindrical to ovate cells, strongly polarized, with a distal flagellum and collar microvilli consistently oriented towards the lumen of the choanocyte chambers (Fig. 1A,B,D,E). The cell body is 7–9.4 μm long and 3.7–5 μm wide. The collar consists of 38 to 40 microvilli (Fig. 1C), which arise directly from either the distal cell surface or from a distal-lateral “neck” area (Figs. 1B,D,E and 2A,B). The external side of the plasmalemma at the distal cell surface and the axoneme basal region are covered by a 100 nm-thick glycocalyx (Figs. 1A,E and 2A,B). The flagellum emerges from the center of the distal cell surface, at the bottom of a cavity formed by an irregular, ring-like protrusion of the cell surface around the base of the axoneme (Figs. 1D,E and 2A,B). This structure is a periflagellar sleeve similar to that described from the choanocyte of some hadromerid demosponges, but markedly asymmetrical (Fig. 1D).

Within the chambers, choanocytes make a cohesive cell layer. The lateral cell surfaces show an irregular contour at their distal and medial regions, leaving a narrow intercellular space of about 60 nm (Fig. 1A,B,D,E). In these surface regions, there are short contact zones at which the plasmalemma of the contacting cells and the very narrow intercellular space becomes more electron dense, revealing occurrence of special junctions (Figs. 1A,B and 2A–E). Although junctions apparently look like “zonula adhaerens,” deficient fixation of materials in their intercellular space prevents a definitive identification (Fig. 2E). At the proximal region of the lateral cell surface, numerous deep interdigitations between adjacent choanocytes occur (Fig. 2C–E). In this area, cells are so tightly juxtaposed that the intercellular space becomes as narrow as ~12 nm. All together, this set of distal–lateral structures provides a complex sealing system for the choanoderm. The proximal cell surface of choanocytes is characterized by abundant long pseudopodia that extend into the

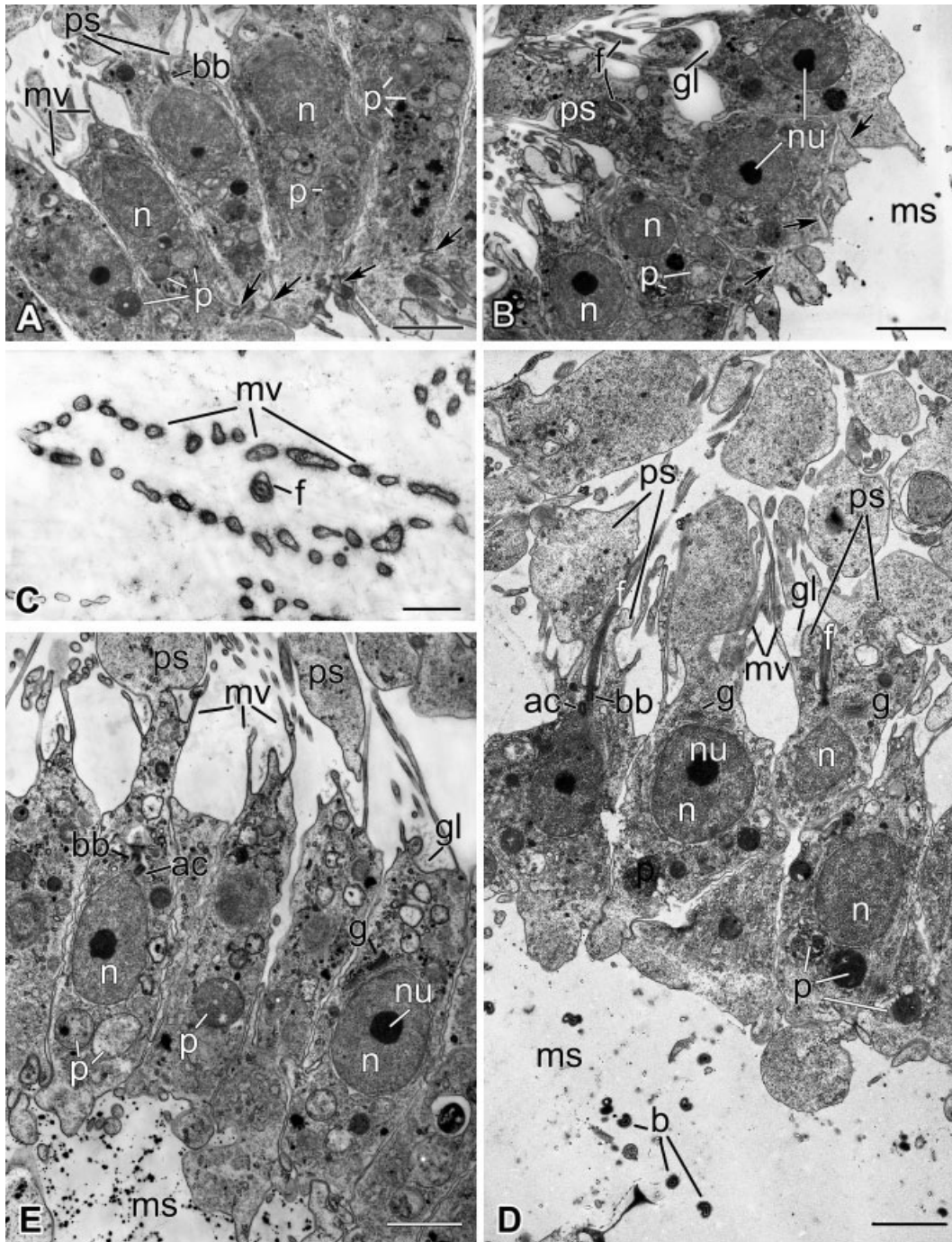


Fig. 1. *Halisarca dujardini*. TEM. **A, B:** The general morphology of choanocytes in the chambers. Arrows indicate apparent special cell junctions. **C:** Cross section of the collar microvilli encircling the flagellum. **D, E:** General view of the choanocytes, showing the asymmetrical periflagellar sleeve (ps) sectioned at different angles. Scale bars: A–B = 2 μ m, C = 0.3 μ m, D = 2 μ m, E = 2 μ m. ac, accessory centriole; b, symbiotic bacteria; bb, basal body; f, flagellum; g, Golgi apparatus; gl, glycocalyx; m, mitochondrion; ms, mesohyl; mv, microvilli; n, nucleus; nu, nucleolus; p, phagosome; ps, periflagellar sleeve.

underlying mesohyl (Figs. 1E and 2C,D). Neither a basement membrane nor other condensation type of collagen fibrils are evident (Figs. 1D,E and 2C,D). Choanocyte cytoplasm exhibits a marked distal–proximal gradient in organelle distribution. The nucleus is located distally and is large ($4.5 \times$

2.6μ m) and pyriform, showing a distal beak-like protrusion (apex) that consistently occurs below the flagellum insertion. Nuclear chromatin is visualized as a combination of granular and fibrous material, with heterochromatin often associated with the inner side of the nuclear membrane

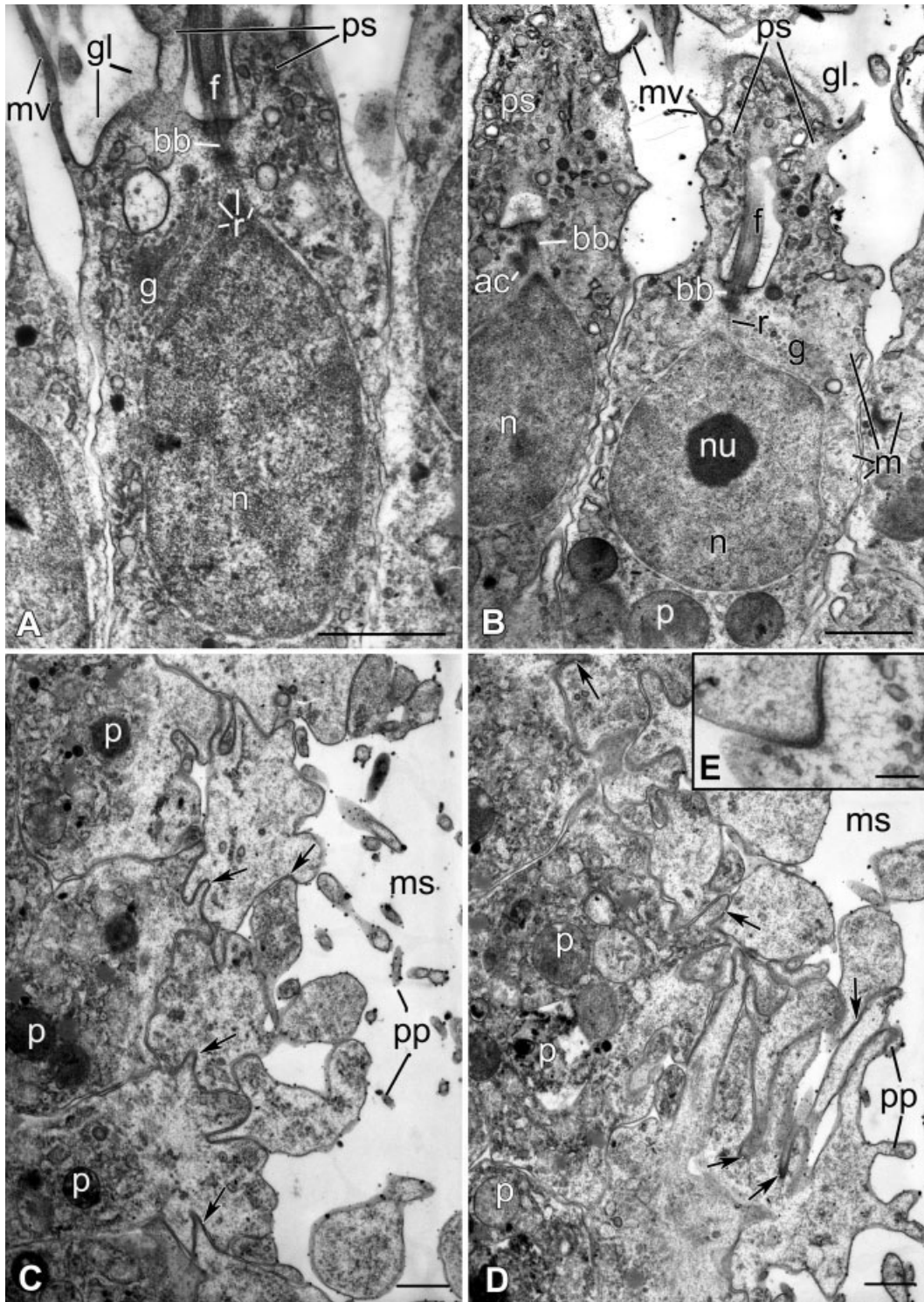


Fig. 2. *Halisarca dujardini*. TEM. **A, B**: Longitudinal sections of the distal and mid region of choanocytes, showing the periflagellar sleeve (ps), part of the basal apparatus, the collar arising either directly from the cell body (A) or from a "neck" region (B), and the glycocalyx on the distal cell surface. **C, D**: Cross sections of the proximal region of choanocytes showing numerous interdigitations, pseudopodia, and specialized cell junctions (arrows). **E**: Detail of the electron-dense differentiation of the choanocyte plasmalemma at the intercellular junctions, which are of an unidentified type. Scale bars: A–D = 1 μ m; E = 0.2 μ m. ac, accessory centriole; bb, basal body; f, flagellum; g, Golgi apparatus; gl, glycocalyx; m, mitochondrion; ms, mesohyl; mv, microvilli; n, nucleus; nu, nucleolus; p, phagosome; pp, pseudopodia; ps, periflagellar sleeve; r, rootlet.

(Fig. 2A,B) and one or two very evident, 80 nm nucleoli (Figs. 1A,B,D,E and 2B). A large Golgi apparatus located atop the nucleus (Figs. 2A,B and 3B-F) encircles the nuclear apex. (Fig. 3L). Some sections revealed duplication of this Golgi apparatus (not shown), probably for choanocyte mitosis. The distal cytoplasm is also characterized by numerous, small vesicles with either electron-clear or electron-dense content (Figs. 1D and 2A-B). Only a few large vesicles and phagosomes occur in this cell region. Some small, globate mitochondria with lamellar cristae occur both in the distal and mid regions of the cytoplasm (Figs. 1A and 2A,B). The mid part of the choanocyte cytoplasm, immediately below the nucleus, contains most of the numerous phagosomes and lysosome-like vesicles found in these cells (Figs. 1A,B,D,E and 2B). In contrast, the proximal-most part of the choanocyte, that is, that at the level of lateral interdigitations, is virtually devoid of organelles, containing just sparse fibrous material and microvesicles (Figs. 1A,B,D,E and 2C,D).

The Flagellum and Its Basal Apparatus

The ultrastructure of the axoneme and its basal apparatus was revealed using a combination of serial and nonserial ultrathin sections (Figs. 3–5). The axoneme has a typical 9 + 2 microtubule organization (Fig. 3G) and, apparently, a regular membrane with no special modification. Nevertheless, some cross sections at the axoneme distal region revealed occasional unpaired expansions of the membrane (Fig. 3G), vaguely resembling vanes (see Discussion). The portion of the axoneme within the periflagellar sleeve is externally covered by a glycocalyx (Figs. 2B and 3A-E,H) similar to that found on the plasmalemma of the distal cell surface (Figs. 1A and 2A).

The transition region between the axoneme and the cell body is relatively short. The central microtubules terminate above the level of the cell plasmalemma (Figs. 2A and 3C), as corresponding to a type II model (*sensu*, Pitelka, 1974). At this level, the lumen of the flagellar tube formed by the nine doublets becomes darker, because of the presence of subtle electron-dense bands of unidentified material oriented in parallel to the longitudinal axis of microtubules (Figs. 3C and 5B). Cross sections at this level revealed a membrane-like structure lining the internal side of the flagellar tube and enveloping the electron-dense material (Figs. 3H and 5B,D). The proximal edge of the central pair of microtubules is embedded in this cylinder-like portion of electron-dense material that fills the lumen of the flagellar tube at the transition region (see schematic interpretation in Fig. 5B,D). Immediately below, a 60-nm globular condensation of undetermined electron-dense material also occurs in the lumen of the tube (Fig. 3C,D; see also

Fig. 5B). Slightly below that level, the axonemal microtubule doublets contact the triplets of the basal body, also known as principal centriole (Figs. 3C,D and 5B,D,E). Nine 160 nm-long alar sheets project radially (Fig. 3I) from the external surface of the proximal edge of the basal body. Alar sheets contact the cell membrane at their corresponding anchor points (Fig. 3C,I). An electron-dense globular basal foot (50 nm) is attached laterally to the basal body (Fig. 3B,K). Numerous microtubules radiate from the basal foot, extending deep into the peripheral cytoplasm and even reaching the cell membrane of the disto-lateral region of the choanocyte (Fig. 3C,J,K). Cross sections revealed that microtubules also radiate from the side of the basal body that is opposite to that of the basal foot (Fig. 3J,K), producing a quite complex transverse cytoskeleton at this cell level (see diagram in Fig. 5B,F). The principal centriole (*i.e.*, basal body) of the basal apparatus is 360 nm in length and 180 nm in diameter, located about 300 nm above the nucleus (Figs. 3B-E and 5B). An accessory centriole occurs adjacent to the basal body (Figs. 3A-C and 5B) but at a slightly deeper level and angling about 45° relative to the longest axis of the basal body.

Five to six, short, fibrous rootlets project from the proximal edge of the basal body to contact the distal beak of the nucleus (Fig. 3C-E). More importantly, they all show striations, with band periodicity ranging from 50 to 60 nm (Figs. 3C-E and 4). Longitudinal serial sections (Fig. 3B-E) revealed that two of the rootlets arising from the basal body edge zone opposite to the side of the basal foot are longer (about 600 nm) than the three or four rootlets (200–350 nm in length) that arise from the remaining edge. The rootlet system and the nuclear beak are encircled by the *cis*-side of the large Golgi apparatus (Fig. 3L).

DISCUSSION

General Cytology of the Choanocyte

Choanocytes of different sponge species or groups can be distinguished by differences in shape and size, relative position of the nucleus and the Golgi apparatus, and structure of the basal apparatus (Table 1). Choanocytes can be flattened, cubical or truncated, cylindrical or vase-shaped cells. Additionally, their shape may vary with not only species or taxonomic group but also depending on their location in the chamber (*e.g.*, Boury-Esnault *et al.*, 1984; Eerkes-Medrano and Leys, 2006) and physiological cell stage (*e.g.*, Vacelet, 1964; Simpson, 1984). The general cytological organization of the choanocyte of *H. dujardini* is similar to that known from a number of other sponges in both Demospongiae and Calcarea. We found, in agreement with previous general descriptions of *H. dujardini* choanocytes by Vacelet *et al.*

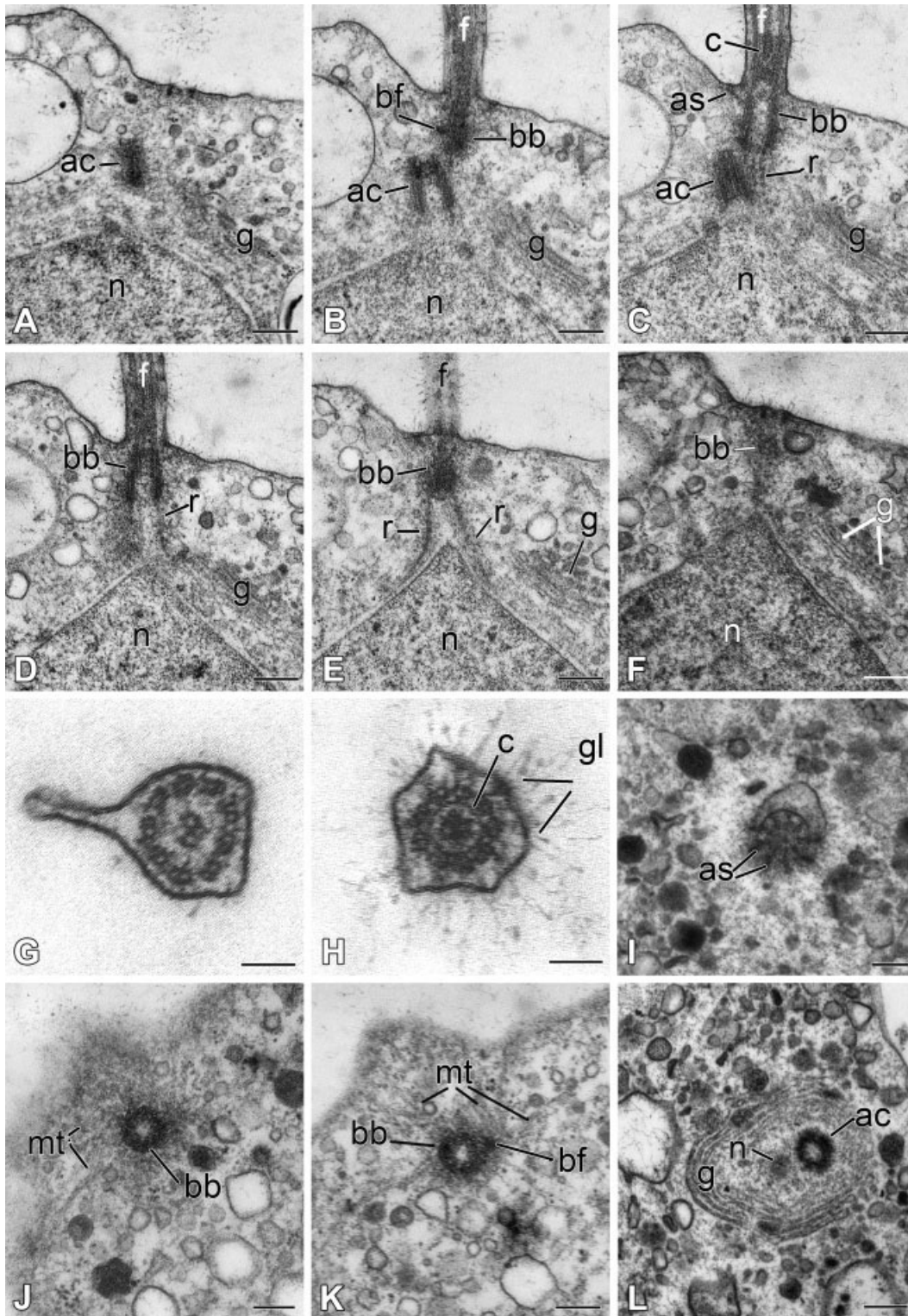


Fig. 3. *Halisarca dujardini*. TEM. **A-F**: Longitudinal serial sections of the choanocyte basal apparatus showing its relation with the Golgi apparatus and the nucleus. **G-L**: Cross sections of the choanocyte at the level of the distal region of the axoneme (G), the transition region between the axoneme and the cell membrane (H), the transition region between the proximal portion of axoneme and the basal body (I), the basal body (two serial sections: J, K), and the accessory centriole (L). Scale bars: A-F = 0.2 μm , G = 0.1 μm , H = 0.15 μm , I-L = 0.18 μm . ac, accessory centriole; as, alar sheet; bb, basal body; bf, basal foot; c, transitional cylinder; f, flagellum; g, Golgi apparatus; gl, glycocalyx; mt, microtubules; n, nucleus; r, rootlet.

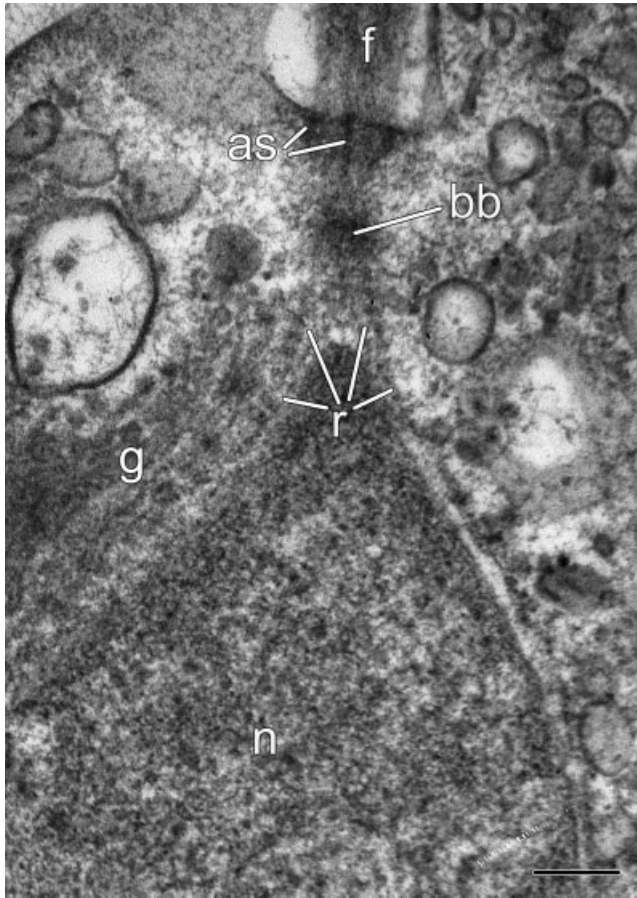


Fig. 4. *Halisarca dujardini*. Longitudinal section of the basal apparatus of the choanocyte. TEM. Note the system of striated rootlets (r) arising from the basal body (bb) to surround the apex of the nucleus (n). A Golgi apparatus (g) is seen adjacent to the nucleus. as, alar sheet; f, flagellum. Scale bar: 0.2 μ m.

(1989) and Bergquist (1996), that this choanocyte has an irregularly cylindrical to ovate shape, a distal microvilli collar surrounding the flagellum, and a marked distal–proximal gradient in the distribution of organelles within the cytoplasm. Yet our ultrastructural study revealed that the choanocyte of *H. dujardini* is characterized by a combination of both minor and major distinct traits, some of them described for a poriferan choanocyte for the first time.

Minor distinctive traits involve aspects of size and shape. In *H. dujardini*, the size of choanocytes and their nucleus is approximately equal to those of archaeocytes/amoebocytes. Nevertheless, in most demosponges, choanocytes are clearly smaller than archaeocytes/amoebocytes; relatively large choanocytes are also characteristic of Calcarea sponges. Unlike most demosponges, the choanocyte of *H. dujardini* and other *Halisarca* species is nucleolated. Such a condition is typical of Calcarea

and a minority of demosponges, such as homosclerophorids (Boury-Esnault et al., 1984), some “sclerosponges” (Hartmann and Willenz, 1990), and some chondrosids (Maldonado, 2004). It is also likely that cytological data published in the literature as corresponding to the cosmopolitan species *H. dujardini* actually belong to cryptic sister species, as it is suggested by the fact that Vacelet et al. (1989) reported a collar consisting of 25–30 microvilli in *H. dujardini* populations from N. Wales (northeastern Atlantic), whereas we find 38–43 microvilli in the studied individuals from the White Sea. Our cross sections of the choanocyte axoneme occasionally revealed an expansion of the axoneme membrane (Fig. 3G). We were unable to elucidate whether such unpaired expansions correspond to a vane-like flagellar differentiation or are artifactual. The latter option emerges as more probable because no consistent distribution pattern was noticed for these expansions and previous SEM observations of the flagellum of two halisarcid sponges revealed smooth flagellar outline (Vacelet et al., 1989). Paired rather than unpaired vanes have been described from the flagellum of both choanoblasts of the hexactinellids (e.g., Mehl and Reiswig, 1991) and choanocytes of *Ephydatia fluviatilis* (Willenz, 1983).

More interesting cytological features were the many lateral interdigitations between adjacent choanocytes and the system of specialized electron-dense junctions, whose nature could not be resolved from our descriptive approach. Specialized junctions between choanocytes had previously been described in the chondrosid demosponge *Thymosia guernei* (Alves de Matos et al., 2002) and in the calcareous sponges *Clathrina* sp. (Green and Bergquist, 1979) and *Sycon coactum* (Eerkes-Medrano and Leys, 2006). In these three cases, junctions showed septate structure. Septate junctions have also been described between the collar bodies and the trabecular tissue of hexactinellids (Mackie and Singla, 1983). It is worth noting that the membrane interdigitations, which are not a conventional feature in the choanocytes of other sponges, also occur at the distal–lateral surface of adjacent monoflagellated cells of the larval epithelium of *H. dujardini* (Gonobobleva and Ereskovsky, 2004a). The set of specialized intercellular junctions in the choanocytes of *H. dujardini*, along with the proximal array of membrane interdigitations, provide a system for either sealing the choanoderm or enhancing its integrity. The occurrence of long pseudopodia at the proximal cell surface, along with the absence of both phagosomes in the proximal-most cytoplasm and collagen fibrils in the underlying mesohyl, suggest exocytotic activity through the proximal free surface of choanocytes.

An unexpected, remarkable feature of the choanocyte of *H. dujardini* is the occurrence of an asymmetrical periflagellar sleeve. So far, the peri-

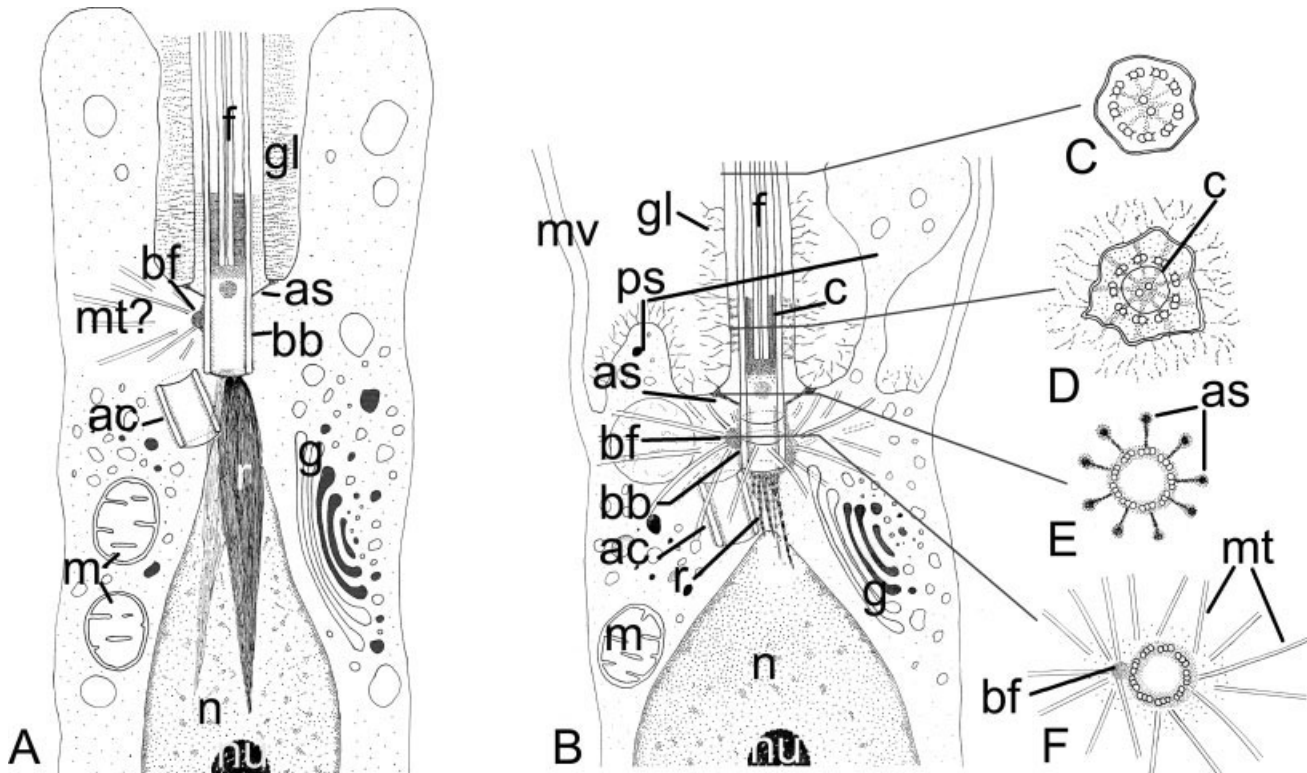


Fig. 5. Schematic drawing summarizing and comparing the structure of the basal apparatus of the monoflagellated cell of the larva of *H. dujardini* (A) and the choanocyte of the adult (B). C–F: Cross sections of the choanocyte at the distal region of the axoneme (C), the transition region between the axoneme and the cell membrane (D), the transition region between the proximal portion of axoneme and the basal body (E), and the basal body (F). ac, accessory centriole; as, alar sheets; bb, basal body; bf, basal foot; c, transitional cylinder; f, flagellum; g, Golgi apparatus; mt, microtubules; n, nucleus; nu, nucleolus; r, rootlet.

flagellar sleeve had only been reported from hadromerid demosponges. It was described as a symmetrical ring-like, permanent protrusion of the distal choanocyte surface inside the collar that forms a conical cavity around the flagellum base (Connes et al., 1971; De Vos et al., 1991). Whether the shared presence of a periflagellar sleeve indicates a certain level of phylogenetic relationship between the genus *Halisarca* and the hadromerids is a possibility that deserves further investigation. Currently, the genus *Halisarca*, which contains around 10 valid species, is the only representative of its own taxonomic order, that is, Halisarcida (Bergquist, 2002). This order remains relatively stranded in the classification of Demospongiae, except for recently suggested affinities with Chondrosiida and Verongida based on 18S rDNA (Borchiellini et al., 2004; Boury-Esnault, 2006). On the other hand, the periflagellar sleeve of *H. dujardini* is reminiscent of the asymmetrical sleeve reported around the flagellum of photoreceptor cells in the tufted parenchymella larva of the demosponges *Haliclona (Gellius) caerulea* (formerly *Sigmatocia*) and *Ircinia oros* (Maldonado et al., 2003).

The Basal Apparatus

Information on the ultrastructure of the basal apparatus of the poriferan choanocyte is scarce (Table 1), with the detailed studies only available for the freshwater haplosclerid demosponge *Ephydatia fluviatilis* (Brill, 1973; Karpov and Efremova, 1994). A transition zone similar to the one found in the choanocyte of *H. dujardini* and characterized by a central cylinder-like body of electron-dense material peripherally limited by a membrane had previously been described in the flagellated larval cells of the haplosclerid demosponge *Haliclona caerulea* (Maldonado et al., 2003). There was, however, a difference: the membrane enveloping the cylinder was apparently bi-layered in *H. caerulea*. In the only other sponge, that is, *E. fluviatilis*, in which the choanocyte transition zone has been investigated in detail, a quite different organization was found, with the occurrence of a transition helix above a transversal plate (Karpov and Efremova, 1994). The variability in the organization of the transition zone revealed by just the few poriferan choanocytes in which this region is well described contrasts with its relative uniformity in choanoflagellates. In nearly all investigated

TABLE 1. Summary of traits of the basal apparatus and associated organelles for sponge choanocytes

Species and bibliographic source	Transition zone	Accessory centriole	Basal foot	Transverse cytoskeleton from bf	Transverse cytoskeleton from bb	Rootlets	Golgi apparatus	Nucleus
<i>Sycon coactum</i> (C: Calcaronea) 1	Long*	Absent*	?	?	?	?	Single*, adjacent to the distal region of nucleus	Distal, subjacent to bb, nucleolate
<i>Corticium candelabrum</i> (D: Homoscleromorpha) 2	?	Present, perpendicular to the bb	Present, simple*	?	Microtubules	Absent*	Single, adjacent to the distal region of nucleus	Distal, subjacent to bb, nucleolate
<i>Calcifibrosporgia actinostomarioides</i> (D: Sclerosponge) 3	Short*	Absent*	?	?	?	Absent*	Single, subjacent to the bb	Proximal, nucleolate
<i>Haliclona rosea</i> (D: Haplosclerida) 4	Short	Absent*	Present, simple	Microtubules*	Microtubules	Absent*	Single, subjacent to the bb	Proximal, anucleolate
<i>Ephydatia fluviatilis</i> (D: Haplosclerida) 5,6	Short	Present, perpendicular to the bb	Absent	Absent	Microtubules	Absent	Single, subjacent to the bb	Proximal, anucleolate
<i>Baicalospongia bacillifera</i> (D: Haplosclerida) 7	?	?	?	?	Microtubules	Absent	Single, subjacent to the bb	Proximal, anucleolate
<i>Halisarca dujardini</i> (D: Halisarcida)	Short	Present, 45° relative to the bb	Present, simple	Microtubules	Microtubules	Striated rootlets	Single, encircling nucleus and rootlets	Distal, subjacent to bb, nucleolate

(C, Calcareia; D, Demospongiae; bb, basal body; bf, basal foot; ?, data not available; *, data absent from the text and inconclusive from the pictures). Numbers refer to bibliographic sources (1, Eerkes-Medrano and Leys; 2006; 2, Boury-Esnault et al., 1984; 3, Hartmann and Willenz, 1990; 4, Garrone, 1969; 5, Brill, 1973; 6, Karpov and Efremova, 1994; 7, Efremova et al., 1988).

TABLE 2. Summary of features of the basal apparatus and associated organelles of embryonic flagellated cells, flagellated cells of the free-swimming larva and, choanocytes of *Halisarca dujardini adults*

Cell type	Transition zone	Alar sheets	Basal foot	Stalked*	Transverse cytoskeleton from bf	Transverse cytoskeleton from bb	Position of ac relative to bb	Rootlets	Golgi apparatus	Nucleus
Embryonic flagellated cell (Gonoboleva, 2007)	Short*	Present	Present	Stalked*	Present	Absent*	Variable	Short, cross-striated	One to several units, location depending on embryogenetic stage	Distal, shape depending on stage of embryogenesis, nucleolate
Larval flagellated cell (Gonoboleva, 2007)	Short*	Present	Present	Simple	Present*	Absent*	Lateral, oblique	Long, fibrous, non-striated	Single, encircling the nuclear apex and rootlets	Distal, pyriform, nucleolate
Choanocyte	Short	Present	Present	Simple	Present	Present	Lateral, oblique	Short, cross-striated	Single, encircling nuclear apex and rootlets	Distal, pyriform, nucleolate

*Preliminary data, because the structure of the basal apparatus of larval and embryonic flagellated cells was not reconstructed from serial sections; bb, basal body; bf, basal foot.

choanoflagellates, the central doublet of microtubules is replaced by a single, thin filament of considerable length (Karpov and Leadbeater, 1998). To our knowledge, such a peculiar transition zone appears to be exclusive of choanoflagellates.

The most distinctive feature of the *H. dujardini* choanocyte is the unexpectedly complex structure of the basal apparatus. The basal body is provided with a small basal foot and an intricate transverse skeleton of long microtubules. The accessory centriole is at 45°. In addition, a system of short striated rootlets arises from the proximal edge of the basal body and runs longitudinally to contact the nucleus apex. The accessory centriole, the rootlets, and the nucleus apex are encircled by a large Golgi apparatus. This is the first report of a rootlet system in a poriferan choanocyte. An assumed shared absence of rootlets in choanoflagellates and sponge choanocytes versus the presence of striated rootlets in choanocyte-like cells of other metazoans had traditionally been regarded as a trait suggesting occurrence of two nonhomologous types of collar cells and used as a relevant character for phylogenetic inference (reviewed in Maldonado, 2004). It is also noteworthy that the rootlets of the *H. dujardini* choanocyte are cross-striated. Such a condition is common in larval flagella of calcareous sponges (e.g., Amano and Hori, 1992, 2001). In most ciliated or flagellated larval cells of Demospongiae, the rootlets have been reported to lack striation. Nevertheless, there are a few instances of cross-striated rootlets, such as in the monoflagellated larval cells of homosclerophorids (e.g., Boury-Esnault et al., 2003; Maldonado and Riesgo, 2008) and the poecilosclerid *Mycale contarenii* (Lévi, 1964), monoflagellated embryonic cells—but not larval cells—of the halisarcid *H. dujardini* (Gonoboleva, 2007), and multiciliated larval cells of the poecilosclerid *Asbestopluma occidentalis* (Riesgo et al., 2007b). Band periodicity in the rootlets of the *H. dujardini* choanocyte was about 60 nm, a value identical to that reported from the larva of *M. contarenii* (Lévi, 1964), but higher than most others reported from embryonic or larval cells of other sponges, that is, 21 nm in *Corticium candelabrum* (Maldonado and Riesgo, 2008), 29 nm in *A. occidentalis*, about 30 nm in *Petrobiona massiliana* (Gallissian and Vacelet, 1992), 33 nm in *Plakina trilopha* (Boury-Esnault et al., 2003), and about 40 nm in *Leucosolenia laxa* (Amano and Hori, 2001). Collar cells from metazoans other than sponges often have striated rootlets originating from the basal body, with striation periodicity of about 60 nm in Nemertini (Cantel et al., 1982), 65 nm in Nematoda (Hope and Gardiner, 1982), and 68 nm in Cnidaria (Lyons, 1973). Most choanoflagellates have no obvious rootlet. Nevertheless, a small structure derived from the accessory centriole has been described in the choanoflagellate *Monosiga ovata* (Karpov and Leadbeater, 1998).

Several types of basal foot structure can be found in cells of Porifera (Woollacott and Pinto, 1995; Maldonado et al., 2003). The one in the choanocytes of *H. dujardini* belongs to the simplest type, being similar to that reported from the choanocyte of both the homosclerophorid *Corticium candelabrum* (Boury-Esnault et al., 1984; Riesgo et al., 2007a) and the haplosclerid *Haliclona rosea* (Garrone, 1969). In several cytological studies of choanocytes, explicit description of the pattern of microtubules arising from the basal body is either scarce or lacking in the text, though noted in the figures, for example, in micrograph 17 by Boury-Esnault et al. (1984) on *C. candelabrum* and micrograph 3 by Garrone (1969) in *Haliclona rosea*, etc. This leads to the suspicion that this transversal cytoskeleton may be a conventional feature of choanocytes. In *H. dujardini*, transverse microtubules radiate from both the basal foot and the basal body. In other sponges, microtubules radiate directly from the whole periphery of the basal body and appear to enter each of the microvilli of the collar, such as in *H. rosea* (Garrone, 1969). In another sponges, it has been reported that microtubules arise from a system of electron-dense granules located around the basal body (De Saedeleer, 1929; Karpov and Efremova, 1994) and that has been interpreted as part of a distinctive type of basal foot (Woollacott and Pinto, 1995; Maldonado, 2004). The basal body of choanoflagellates is surrounded by an electron-dense annulus or composite arc, from which a complex array of microtubules radiates towards the surrounding cytoplasm (Laval, 1971; Leadbeater and Morton, 1974; Hibberd, 1975; Karpov, 1982; Leadbeater, 1994; Karpov and Leadbeater, 1998). This transverse skeleton of microtubules supports the actin microfilament bundle that runs within each of the tentacles of the collar (e.g., Karpov and Leadbeater, 1998).

The angling orientation of the accessory centriole is also a distinct feature of the *H. dujardini* choanocyte. A similar orientation is only known from the flagellated cells of the larva of this sponge (Gonoboleva, 2007). In nearly all flagellated cells described in Porifera, the accessory centriole, if present, lies perpendicular to the basal body, though both centrioles position in parallel in the spermatozoon of the hadromerid demosponge *Suberites massa* (Diaz and Connes, 1980). In choanoflagellates, the accessory centriole is also reported to lie perpendicularly to the basal body (Karpov and Leadbeater, 1998).

A comparison between the organization of the basal apparatus in the choanocyte and the monoflagellated larval cell of *H. dujardini* may shed some light on the phylogenetic versus ontogenetic signal contained by this multiorganelle structure (Table 2). During the life cycle of this sponge, three types of flagellated cells can be found: choa-

nocytes, monoflagellated larval cells, and spermatozoa (which remain to be described). The flagellated cells of the free-swimming larva start differentiating at the surface of early embryos while still proliferating by mitosis (Ereskovsky and Gonobobleva, 2000; Gonobobleva and Ereskovsky, 2004b). During metamorphosis, flagellated cells from the internal and external larval epithelia internalize to the nascent mesohyl and transdifferentiate into choanocytes (Gonobobleva and Ereskovsky, 2004a; Mukhina et al., 2006). Most observations indicate that the distal–proximal polarity of the flagellated larval cells is preserved in the choanocytes: the basal apparatus of the larval cells becomes the basal apparatus of the choanocytes (Gonobobleva and Ereskovsky, 2004a). In both the cell types, the general organization of the basal apparatus and its links with the nucleus and the Golgi apparatus are nearly identical (Table 2, Fig. 5), except for the fact that flagellated larval cells possess long, non-striated rootlets (Gonobobleva, 2007) whereas choanocytes have short, cross-striated rootlets. It is remarkable that embryonic flagellated cells also have short cross-striated rootlets (Gonobobleva, 2007) as do choanocytes. This ontogenetic sequence, that is, short striated rootlets in embryonic cells that become long non-striated rootlets in larval cells and revert to the condition of short striated rootlets in the choanocytes—suggests that the structure and organization of the flagellar rootlet system depend upon the function carried out by the cell at each stage of the life cycle. Therefore, the phylogenetic signal provided by this structure may be weaker than previously thought and should be used with caution.

The cytological study of the *H. dujardini* choanocyte has uncovered a significant set of unexpected features. These results suggest that the ultrastructure of the poriferan choanocyte may vary substantially between sponge groups and strongly signal the need of devoting further research effort to choanocytes if we are to completely understand the functional and evolutionary roles of this important metazoan cell.

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