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# Reproduction in a carnivorous sponge: the significance of the absence of an aquiferous system to the sponge body plan

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**SUMMARY** Sponges usually produce, release, and capture gametes via the aquiferous system, and so the absence of both choanocytes and an aquiferous system in the carnivorous sponge *Asbestopluma occidentalis* has led to unusual characteristics of development for this Phylum. Sperm are highly specialized elongate cells tightly packed into spermatic cysts in the peripheral tissue of the sponge. Mature spermatozoa have proacrosomal vesicles at the anterior end and a ciliary pit surrounding the flagellum. Clusters of four to five oocytes are in synchronous stages of cleavage, suggesting that fertilization is synchronous. All stages of embryos occur in the same individual. Early cleavage was holoblastic and equal; blastomeres in two-, four- and eight-cell embryos were compact and 16-cell stage embryos were bi-layered. Late-stage embryos show three cellular regions along the anterior-posterior axis: the anterior hemisphere with heterogeneous cells, a mid-region with cells lying perpendicular to the A-P axis in a collagenous matrix, and small cells at the posterior pole. Unusually for Porifera, multiciliated cells cover all but the posterior pole. It is inferred that fertilization occurs by capture of intact spermatic cysts whose surrounding forceps spicules become trapped in the anisochelae of neighboring sponges. The elongate shape of sperm may be designed to penetrate the loose collagenous mesohyl, such that the arrival of a packet of sperm would lead to simultaneous fertilization of oocytes in a cluster. Loss of the water canal system in carnivorous sponges has allowed the evolution of features that are highly specialized for the habitat of this animal, but such modifications were not necessarily a prerequisite for the subsequent evolution of metazoans. Given the extremely versatile mechanisms of gametogenesis, embryogenesis, and tissue/body structure in sponges, generalizations regarding basal metazoan reproduction, development, and structure must be approached with caution.

## INTRODUCTION

Sponges are generally characterized as simple filter-feeding animals that use flagellated cells to pump water through canals and chambers where food (primarily bacteria) is extracted and wastes are excreted (Bergquist 1978). In fact, the sponge body plan seems to be well maintained through evolution, displaying a great variety of morphologies (tube, vase, and encrusting) but always having an aquiferous system for feeding, *except* in cladorhizids in which flagellated filtering chambers are absent or modified (Vacelet and Boury-Esnault 1995, 1996; Vacelet et al. 1995, 1996; Kübler and Barthel 1999; Vacelet 2006). The higher taxonomy of sponges starkly reflects the conundrum of one body plan one phylum. Although some studies defend the monophyly of Porifera (Cavalier-Smith et al. 1996), there is increasing evidence that sponges may be paraphyletic (Kruse et al. 1998; Zrzavy et al. 1998; Borchiellini et al. 2001; Medina et al. 2001). If the hypothesis of sponge paraphyly is confirmed, this implies that metazoans share a common ancestor that had a poriferan body plan, that is, either the water canal system (WCS) is a feature shared with other metazoans or loss of canals and chambers is implied.

Cladorhizids are deep sea poecilosclerid demosponges, a group well defined by its skeletal composition and design (Hajdu and Vacelet 2002), whose unusual carnivorous habits were discovered with in situ experiments carried out when a cladorhizid was first found in a Mediterranean cave (Vacelet and Boury-Esnault 1995). The family Cladorhizidae comprises four genera: *Abyssocladia* Lévi, 1964, *Asbestopluma* Topsent, 1901, *Chondrocladia* Thompson, 1873, and *Cladorhiza* Sars, 1872, all of which include species that derive at least some of their nutrition from carnivory. In addition, it is suggested that some members of two other poecilosclerid families (Guitarridae and Esperiopsidae) may also be carnivorous (Vacelet 2006). This unique feeding habit among sponges is likely due to the low nutrient levels present in abyssal depths (Vacelet and Boury-Esnault 1995, 1996), a modification

shared with some deep-water tunicates (Monniot 1984) and several species of clams (Morton 1987, 2003). Carnivorous sponges usually feed on small crustaceans (Vacelet and Duport 2004) or have developed symbiotic relationships with chemotrophic bacteria. For example, *Asbestopluma hypogea* feeds solely on small crustaceans (Vacelet and Duport 2004), whereas *Chondrocladia gigantea* retains a modified but functional aquiferous system that it uses to inflate massive spheres covered with spicules that trap prey (Kübler and Barthel 1999). Another genus, *Cladorhiza*, is found near hydrothermal vents and, like many vent invertebrates, it harbors symbiotic extracellular methanotrophic bacteria to supplement its diet of crustaceans (Vacelet et al. 1995, 1996). Perhaps the most remarkable species is *Cladorhiza pteron*, a 40-cm-long bilaterally symmetrical sponge that can capture 4–7000 prey per individual where it lives at 1500 m depth on the San Juan Seamount off Southern California (Reiswig and Lee 2006).

*Asbestopluma occidentalis* was first described by Lambe (1893) as *Esperella occidentalis* from samples collected in the Strait of Georgia, British Columbia. Lambe provided a good description of spicule types, their arrangement and the general appearance of the sponge, but gave no details on the cytology. Collection of specimens from 100 to 200 m depths using a remote-operated vehicle and dredging has revealed that specimens collected in July and August have been fecund, with multiple stages of gametogenesis and embryogenesis in a single animal. Although embryogenesis was briefly documented by Lundbeck (1905), only spermatocytes have been reported from *A. hypogea*; curiously, in that species, oocytes and embryos are rarely found (Vacelet 1996; Vacelet and Boury-Esnault 1996). In conventional sponges, sperm are thought to arise from the flagellated cell population of choanocytes or from amoebocytes, and at maturity are released via the aquiferous canals and are subsequently captured by choanocytes of other individuals (Fell 1983; Reiswig 1983; Gaino et al. 1984; Paulus and Weissenfels 1986; Paulus 1989; Boury-Esnault and Jamieson 1999). In theory, such sperm do not need to be highly specialized for penetrating the egg, because the choanocyte acts as the intermediary carrier cell, transferring the male pronucleus to the egg. In the absence of an aquiferous system, how are male gametes formed, released, and captured?

Gametogenesis and embryogenesis are both unusual in *Asbestopluma*. Sperm originate from amoeboid cells and have a modified ("derived") spermatid morphology. Here, we show that in *A. occidentalis*, clusters of embryos cleave synchronously, which supports the hypothesis of Vacelet and Boury-Esnault (1996) and Vacelet (1996) that sperm packets are released and subsequently captured intact, thereby ensuring simultaneous fertilization of a group of oocytes. Embryogenesis in *A. occidentalis* involves the differentiation of multiciliated cells only otherwise known in the Hexactinellida (glass sponges) (Boury-Esnault and Vacelet 1994; Boury-Esnault et al. 1999; Leys et al. 2006). The versatility of the ciliated/flagellated cell lineage (multiciliated cells in larvae and uniflagellated sperm, but no choanocytes) in these sponges forces us to consider whether early sponges might indeed have lacked a filter-feeding habit (e.g., Li et al. 1998; Vacelet 1999). However, the fact that all molecular data place poecilosclerids firmly among the demosponges means the most parsimonious hypothesis is that *Asbestopluma* has lost choanocytes and early sponges most likely possessed a WCS.

## MATERIALS AND METHODS

Approximately 45 specimens attached to rock and glass sponge skeletons were collected by the remote-operated vehicle ROPOS from fjord walls at 120 m depth in Barkley Sound (48°53′54″N, 125°03′9″W) in July 2003, and by dredge at 100 m depth near Tahsis Inlet, Vancouver Island, in August 2004. For light microscopy, specimens were fixed immediately in 70% ethanol, 10% formalin, or Bouin's fixative; other specimens were maintained in running seawater tanks at the Bamfield Marine Sciences Center for 3–5 days before fixation. Spicules were prepared for scanning electron microscopy (SEM) by digestion with nitric acid and ethanol washes directly on a round coverslip (Hooper 2000). For histology, specimens were dehydrated through a graded ethanol series, embedded in paraffin, and 6-μm sections were stained in Mallory's (Humason 1979). For transmission and scanning electron microscopy (TEM and SEM), specimens were fixed and prepared as described by Leys and Degnan (2002), except that all specimens were fractured in liquid nitrogen before embedding in epoxy for TEM or critical point drying for SEM. Specimens were viewed in a Phillips (FEI Company, Hillsboro, OR, USA) transmission electron microscope at 75 KV and a Joel 6301 Field emission scanning electron microscope (JOEL, Peabody, MA, USA) at 5 KV. Descriptions follow the terminology used by Boury-Esnault and Ruetzler (1997), Hajdu and Vacelet (2002), and Vacelet (2006).

## RESULTS

### General description of the adult

The basic structure of the adult sponge was described by Lambe (1893). Briefly, mature adults consist of a slender cylindrical trunk up to 6 cm long and 1.5 mm wide, from which long filaments arise (Fig. 1A). Embryos were visible through the transparent outer layers of the trunk (Fig. 1B), which is anchored onto the substrate by a roughly spherical base about twice the diameter of the trunk. Histological sections through the sponge body (Fig. 1, C and D) showed two distinctive regions: an inner region of densely packed cells, spicules, oocytes, spermatic cysts, and embryos (hereafter termed the core), and an outer region with few cells, but rich in collagen (hereafter termed the subpinacoderm). The subpinacoderm was reduced in the base of the sponge, where collagen was much denser.

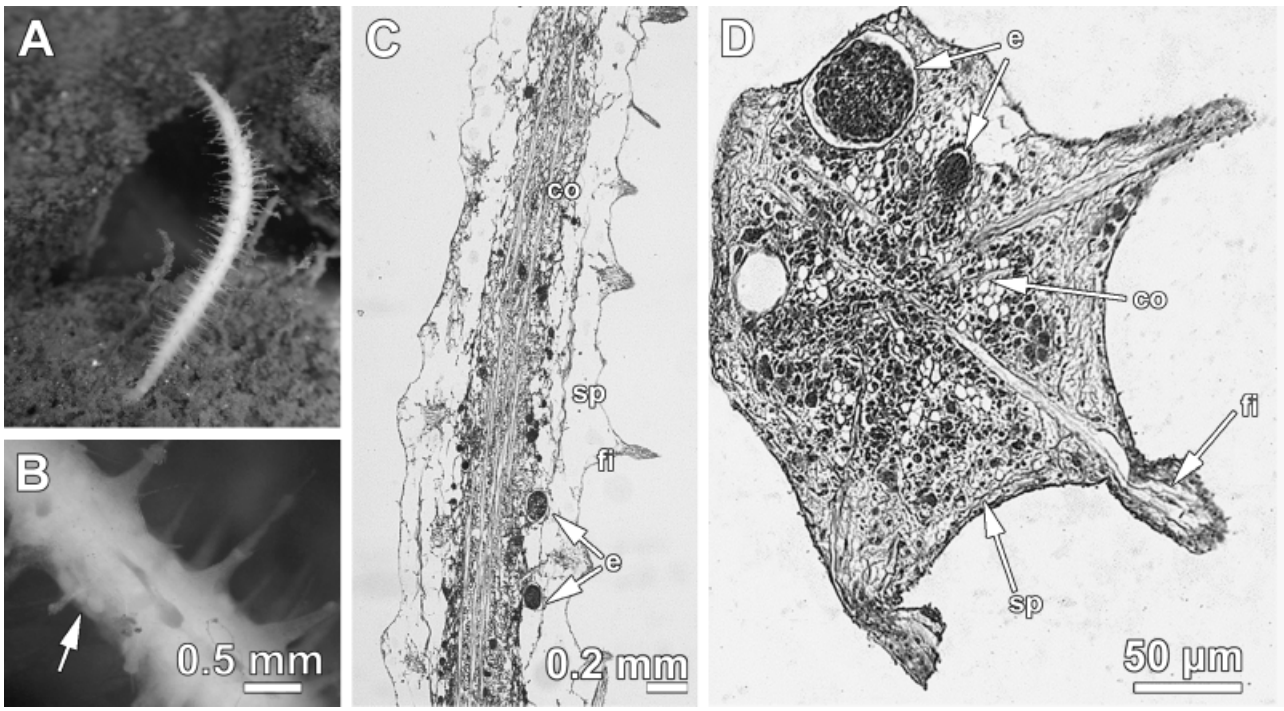



**Fig. 1.** Structure of the adult sponge. (A) Live *Asbestopluma occidentalis* attached to the skeleton of a glass sponge in an aquarium after collection; the specimen is approximately 2 cm tall. (B) Embryos (arrow) can been seen through the transparent outer layer of the stalk. Longitudinal (C) and cross (D) sections of the sponge showing the core (co), subpinacoderm (sp), filaments (fi), and embryos (e).

The skeleton (Fig. 2) was organized around a central axis of styles ($731.8 \pm 275.1$ µm, $n = 50$ long and $18.3 \pm 4.7$ µm, $n = 50$ wide) enveloped in a dense collagen sheath; styles also formed an internal support for each filament. Subtylostyles and tylostyles ($245.6 \pm 123.9$ µm long and $9.2 \pm 4.1$ µm, $n = 50$ wide) were more common in the base, arranged transversally to the longitudinal styles. Palmate anisochelae ($11.4 \pm 0.8$ µm, $n = 50$ long) lay at the sponge surface, except at the most basal part of the trunk and on the base. Forceps spicules ($38.3 \pm 3.9$ µm, $n = 7$) were associated with the spermatic cysts.

The surface of the sponge was slightly hispid due to protruding anisochelae (Fig. 3), the spicules thought to trap setae of prey. Sclerocytes that contained anisochelae had a remarkable shape with a root-like base anchored in the collagen matrix below the pinacocytes (Fig. 3). Cell density was highest in the core, where five types of cells were reliably identified: Type I bacteriocytes (Vacelet and Boury-Esnault 1996) were numerous; Type II bacteriocytes were slightly less common; "stellate" cells with extensions up to 20 µm long lay throughout the collagenous matrix; and archaeocytes (spherical amoeboid cells), and sclerocytes (with a triangular axial filament in cross-section) were found throughout the core. Bacteria were common in the extracellular matrix among the cells, but were most concentrated in the filaments.

## Gametogenesis

*A. occidentalis* is a contemporaneous hermaphrodite, with oocytes, spermatic cysts, and embryos simultaneously present in the tissue.

*Spermatogenesis* Spermatic cysts were round to oval, about 30–60 µm in diameter, and were enveloped by a thin layer of follicle cells that became thicker and formed complex interdigitated layers as development progressed (Fig. 4). The youngest sperm cells (primary spermatocytes) found in the core were in loose congeries of archaeocyte-like cells partially surrounded by follicle cells (Fig. 5A). A few of these cells had a basal body indicating formation of the flagellum (Fig. 5B). Early-stage spermatic cysts were densely packed with round cells (4.5–5 µm diameter)—either spermatogonia or primary spermatocytes (no clear synaptonemal complexes were seen) (Fig. 4A). Secondary spermatocytes were much smaller (2.5 µm diameter) flagellated cells with numerous pseudopodia (Figs. 4B and 5C-E). In contrast, spermatids in late-stage spermatocytes were elongate cells (approximately 8 µm long) with a very long anterior extension containing the nucleus (Fig. 6, A–C). The flagellum was inserted into the middle of the cell body and the proximal portion of the free flagellum was enclosed by a 1-µm-long cytoplasmic channel (a ciliary pit) (Fig. 6B, inset). The membrane of spermatocytes appeared smooth in early stages, ridged in spermatids and

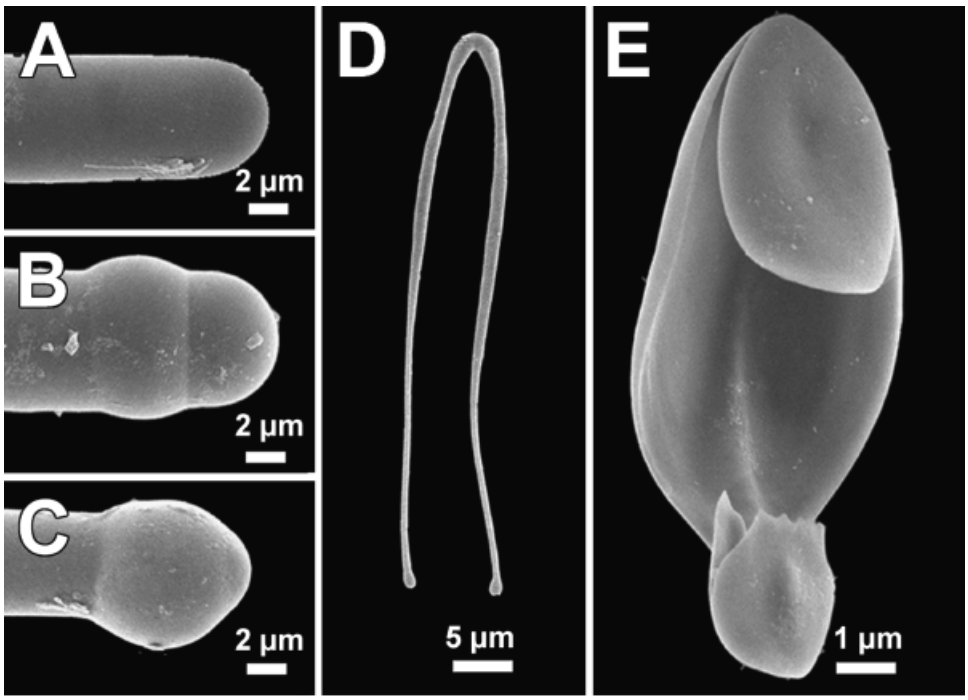



**Fig. 2.** Distinctive spicule skeleton (scanning electron microscopy). (A) Style head. (B) Subtylostyle head. (C) Tylostyle head. (D) Forceps. (E) Palmate anisochelae, which are responsible for trapping prey.

was smooth again in mature sperm (Figs. 5D and 6, C and E). All sperm cells were connected by cytoplasmic bridges during the entire process of spermatogenesis (Figs. 5E and 6A). The anterior region of the nucleus of mature spermatozoa appeared swollen, but sections showed that the anterior-most end of nucleus was flared at either side, like a hammerhead (Fig. 6, D–G). At the very tip of the nucleus, three to six proacrosomal vesicles were located (Fig. 6G). Bundles of longitudinal microtubules appeared in mature spermatozoa, parallel to the nucleus (Fig. 6D). Several layers of closely juxtaposed cells with highly entwined membranes surrounded all late-stage spermatic cysts (Fig. 6, H–I).

The youngest spermatic cysts were found in the inner part of the sponge core, adjacent to the bundles of the spicules. More advanced cysts were in the subpinacoderm, and mature spermatic cysts were mostly located in the fine filaments projecting from the sponge stalk. In live sponges, bulbous structures, presumably spermatic cysts, were often seen on filaments.

*Oogenesis* Most oocytes occurred in small clusters of four or five in the outer part of the core (Fig. 7, A–B). Oocytes were 6–24 µm in diameter ($n = 38$), with few inclusions and little yolk (Fig. 7B), but each had two to three conical nurse cells with long extensions that enveloped the oocyte. Mature oocytes, corresponding to the smallest size recorded for two-cell stage embryos, usually contained intracellular bacteria, transferred by the nurse cells from the parent sponge (Fig. 7B, inset).

## Embryogenesis

All embryos were located in the outermost edge of the core. Clusters of two-cell embryos suggested that fertilization was synchronous (Fig. 7C). Early cleavage was holoblastic and equal, and four- and eight-cell stage embryos were compact, with cells tightly juxtaposed against one another (Fig. 7D). In 32-cell embryos, two layers were already evident, the external layer flatter than the internal (Fig. 7E). As development continued, cellular differentiation was more obvious (Fig. 8A), and after this stage each cell of the external layer began to form multiple cilia (45–55 µm) (Fig. 8, A–C). During all stages, follicle cells surrounded the embryo (Fig. 8D) separated from it by only a thin layer of collagen. Follicle cells (nurse cells) extended pseudopodia both toward the mesohyl, and inwards to contact the embryo (Fig. 8, A and B). Cells in the inner region of early embryos were loosely arranged among filamentous bacteria and collagen (Fig. 8, A and C). At this stage, the outer layer of the embryo was mostly formed by multiciliated cells whose now long cilia were bent over within the follicular epithelium (Fig. 8, E–H); each cilium possessed a striated rootlet with a 29 nm periodicity between striations (Fig. 8I and inset). At one pole, there was a single nonciliated cell with long extensions that reached the whole length of the embryo, lying between, but joined to, the multiciliated cells (Fig. 8, E–F).

The most fully differentiated embryos (pre-larvae) lay at the periphery of the subpinacoderm (Fig. 9A, inset). The pre-larva was differentiated into three regions: cells in the anterior hemisphere were heterogeneous; cells in the mid-region were

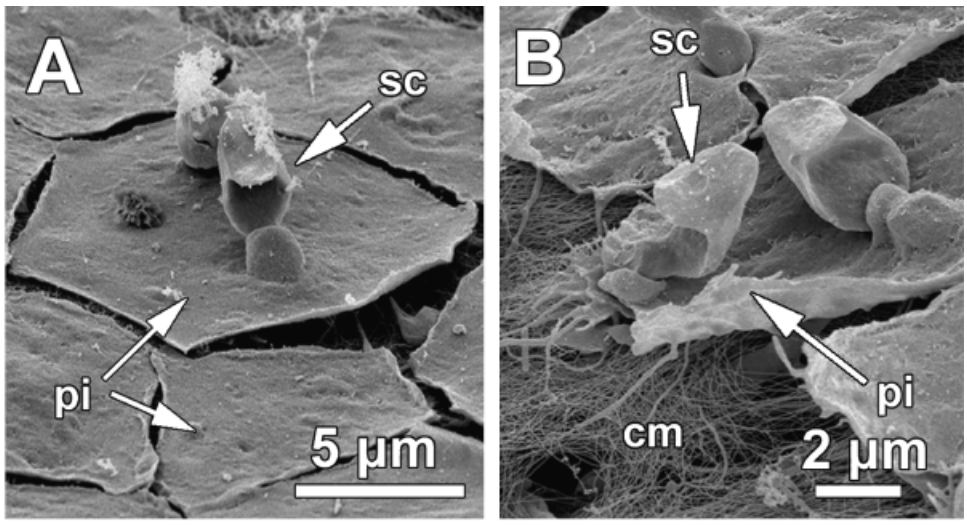



**Fig. 3.** Surface pinacoderm viewed by SEM. (A) Pinacocytes (pi) are pierced by anisochelae-containing sclerocytes (sc). (B) Protruding sclerocytes (sc) that contain anisochelae are anchored in the collagenous matrix (cm) below the pinacocytes (pi) by root-like extensions.

aligned perpendicular to the A–P axis and were associated with dense bundles of collagen; and cells at the posterior pole were small and contained numerous vesicles (Fig. 9A). All but the posterior pole was ciliated (Fig. 9, A and D). Multiciliated cells were juxtaposed with one another *and* with a type of nonciliated cell that protruded slightly among the bases of the cilia (Fig. 9, A–C).

## DISCUSSION

### Relevance to sponge body plan evolution

Sponge gametogenesis and embryogenesis is poorly understood—there are developmental data on only some 100 species (Leys and Ereskovsky 2006) and even less is known on the structure of gametes, in particular sperm (Boury-Esnault and Jamieson 1999). Part of the reason is that most sponges brood embryos cryptically, and reproductive seasons are often brief. The dearth of evidence has led to a number of not-so-well-founded generalizations regarding the origin of gametes from choanocytes, the primitive nature of sponge sperm, the capture of sperm by choanocytes, and use of a carrier cell to transfer sperm for fertilization. *A. occidentalis* is quite unusual, not only for its carnivorous habit, but because it contains all stages of gametes and embryos during summer months, although this last feature can also be observed in other demosponges (Simpson 1984). Not only is ready access to all stages of gametes for many months not very common in poriferans, but production of gametes and embryos in sponges that lack an aquiferous system shows features that have important implications for the body plan of the phylum. These include the finding that oocytes occur in small clusters in a well-defined tissue area where they appear to be simultaneously fertilized; the modified elongate mature sperm with proacrosomal vesicles and a cytoplasmic channel that harbors the flagellum, a structure described in only a few other demosponges (Efremova and Papkovskaya 1980; Paulus 1989; De Vos et al. 1991; Riesgo and Maldonado 2007); and, most unexpectedly, the discovery that embryos have multiciliated cells, only previously described in hexactinellids (Boury-Esnault and Vacelet 1994; Boury-Esnault et al. 1999; Leys et al. 2006). Sponge body plans are relatively homogenous across a great phylogenetic range, from hexactinellids to homoscleromorphs, demosponges and calcareous sponges, and so the unusual characteristics of cladorhizid demosponges are particularly useful in pointing out the developmental potential of sponges.

### Origin and "derived" structure of gametes

The origin of gametes in sponges is controversial—some studies suggest that gametes arise from archaeocytes, and others, from choanocytes, both considered to be multipotent stem cells; however, interpretations are based on static images of fixed tissue, and until experimental studies are conducted in sponges, conclusions about the origin of gametes remain equivocal (Fell 1983, 1997; Reiswig 1983; Simpson 1984). Maternal segregation of RNA for the germ lineage is thought to have derived from an ancestral epigenetic mechanism (induction by neighboring tissues) (Extavour and Akam 2003). In cnidarians, the closest metazoan relatives of sponges, germ cells have traditionally been considered to arise from multipotent stem cells by epigenesis; however, use of RNA markers for germ-cell lineage indicates an early separation of the somatic and germ lineage in *Hydra* (Mochizuki et al. 2000), the jellyfish *Podocoryne carnea* (Torras and González-Crespo 2005), and in the anthozoan *Nematostella vectensis* (Torras et al. 2004). Nothing is yet known of homologs of these genes in sponges, but it has been suggested that there is an early separation of germ and somatic cell lineages in calcinean calcar-

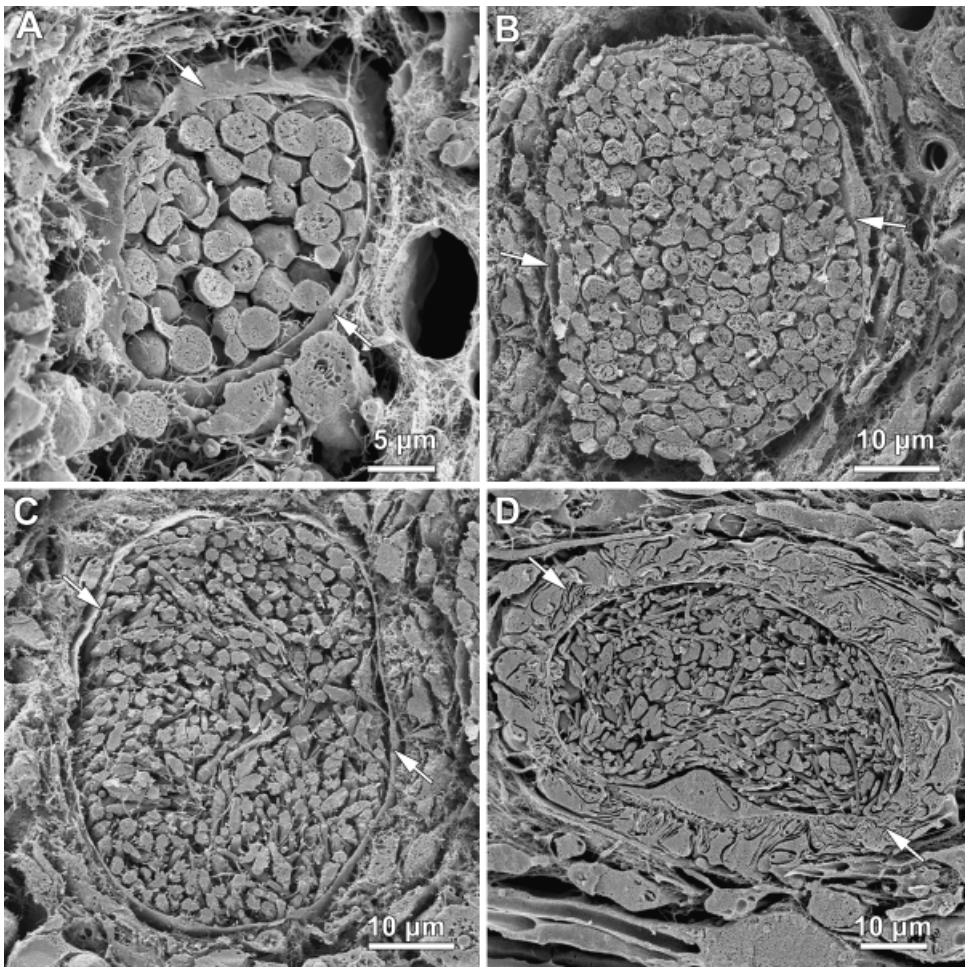



**Fig. 4.** Developmental progression of spermatic cysts. (A) Primary spermatocytes, surrounded by a thin layer of follicle cells (arrows). (B) Spermatic cyst containing secondary spermatocytes, enveloped by a thin layer of cells (arrows). (C) Spermatids surrounded by a slightly thicker layer of cells (arrows). (D) Mature spermatozoa in a late-stage spermatic cyst in one of the filaments. The envelope is now a complex layer of tightly interwoven cells (arrows).

eous sponges (e.g., Borojevic 1969); whether this difference can be detected at the gene level remains to be seen.

In *A. occidentalis*, both gametes appear to derive from archaeocytes because of their similar size and appearance; certainly, in the absence of flagellated cells in *Asbestopluma* it is most likely that spermatogonia originate from archaeocytes, as occurs in Hexactinellida (Boury-Esnault et al. 1999) and in some other demosponges (Fell 1974; Reiswig 1983; Simpson 1984). The flagellum is the first obvious marker of primary spermatocytes, but these round cells are also joined by cytoplasmic bridges. Mature spermatozoa are elongated cells with a ciliary pit that encloses part of the flagellum, a feature that has only been observed in a few sponges but is known from other invertebrates (Hinsch 1974; Efremova and Papkovskaya 1980; Paulus 1989; De Vos et al. 1991; Reunov and Klepal 2004; Riesgo and Maldonado 2007), and the head of the sperm is capped by several proacrosomal vesicles. Proacrosomal vesicles have so far only been reported in *Suberites massa* (Diaz and Connes 1980) and *Spongia officinalis* (Gaino et al. 1984), but a true acrosome has been observed both in Homosclerophorida (Gaino et al. 1986; Boury-Esnault and Jamieson 1999; Riesgo et al. 2007) and in Poecilosclerida (Tripepi et al. 1984; Riesgo and Maldonado 2007). The occurrence of proacrosomal vesicles in the sperm of *A. occidentalis* suggests that acrosomal structures (i.e., true acrosomes and proacrosomal vesicles) could be more widespread across Porifera than is thought, and reinforces the need for additional ultrastructural studies to clarify the issue in the phylum.

Both the elongated shape and the cytoplasmic sheath for the flagellum unequivocally categorize this spermatozoon as "modified" (Reunov 2005), and although modified sperm are known among Demospongiae (Tripepi et al. 1984; Barthel and Detmer 1990), "primitive" sperm are more common (Reiswig 1983; Boury-Esnault and Jamieson 1999). In calcareous sponges, spermatozoa were characterized as primitive by Gatenby (1920), but a recent report suggests that *Leucosolenia complicata* has apyrene (nonflagellated) sperm (Anakina and Drozdov 2001). In hexactinellids, primitive (round) sperm are described in two species (Okada 1928; Mackie and Singla 1983; Boury-Esnault and Vacelet 1994; Leys et al. 2007). Thus, spermatogenesis in *A. occidentalis* comprises the basic features described for most animals:

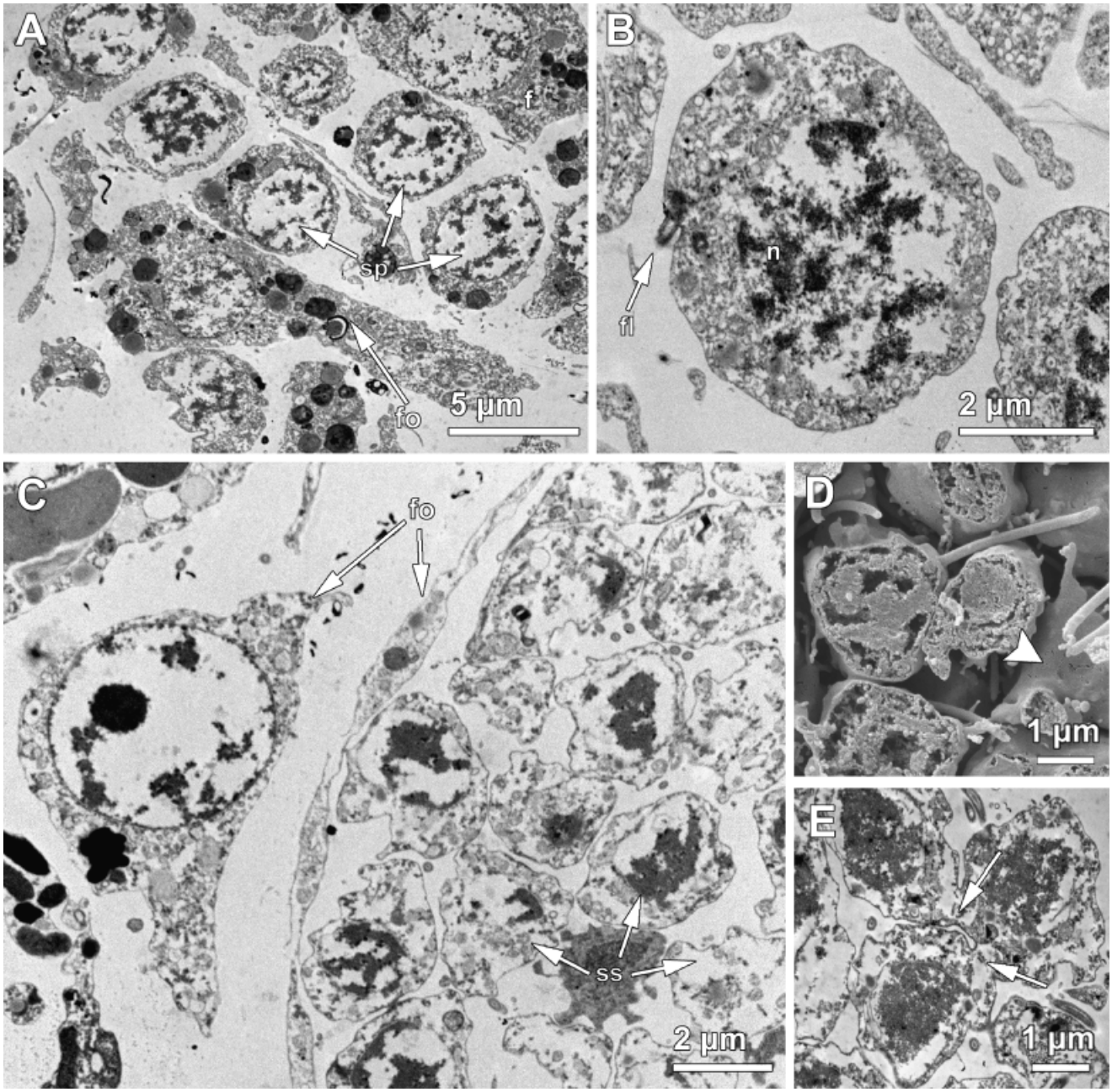
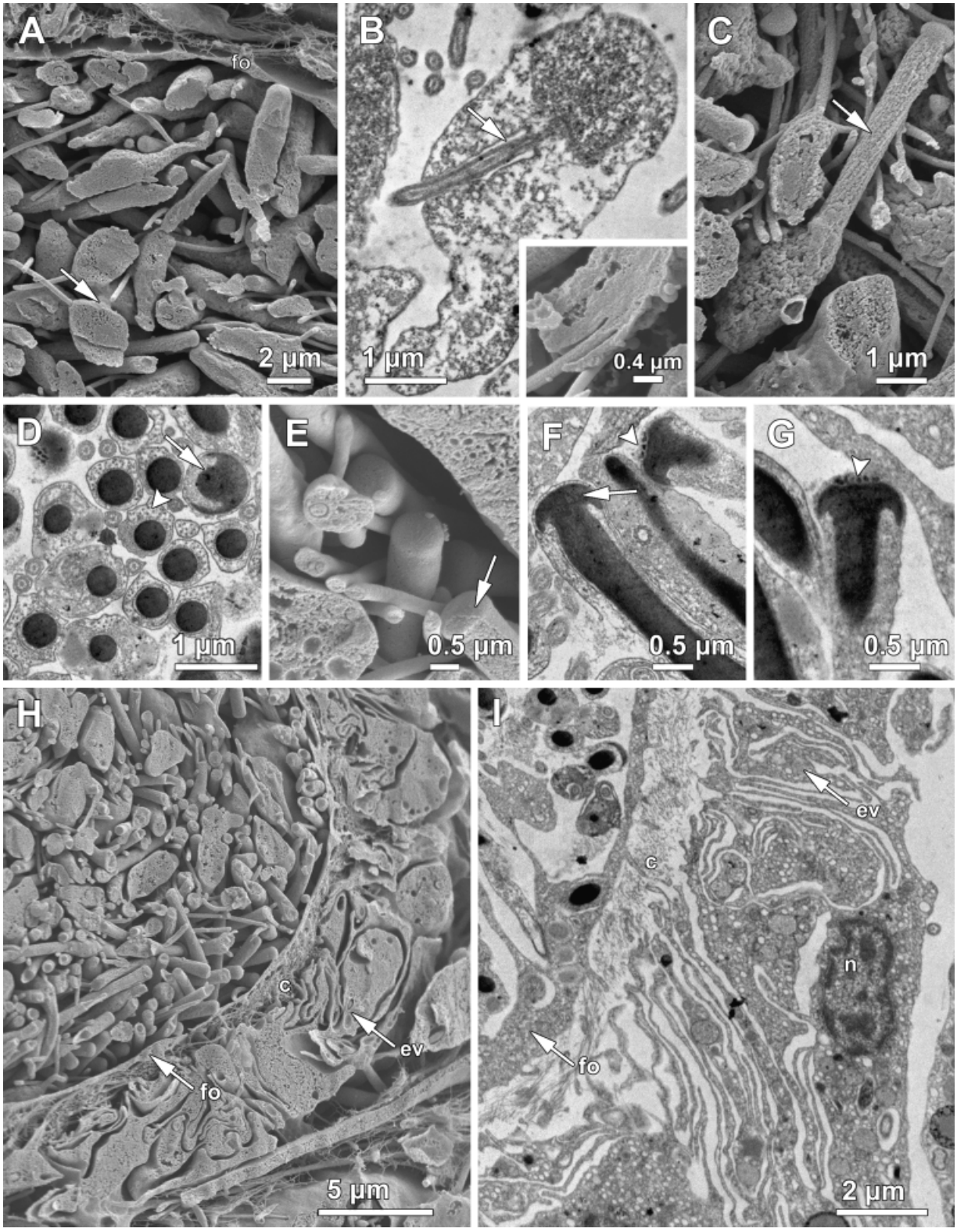



**Fig. 5.** Early spermatogenesis. (A) The earliest groupings of primary spermatocytes (sp) adjacent to presumed future enveloping (follicle) cells (fo). (B) A primary spermatocyte with a flagellum (fl) from the group shown in (A). (C) A spermatic cyst containing irregular secondary spermatocytes (ss) and surrounded by two layers of follicle cells (fo). (D, E) Secondary spermatocytes shown by SEM (D) and TEM (E) are connected by cytoplasmic bridges (arrowhead and arrows).

**Fig. 6.** Spermiogenesis. (A) Sister spermatids connected by cytoplasmic bridges (arrows). (fo), follicle cells enclosing the cyst. (B) and inset. Spermatids showing the cytoplasmic channel harboring the flagellum. (C) An elongated spermatid showing the swelling of the head and the ridged surface typical of that stage (arrow). (D) Cross section of a spermatic cyst containing mature spermatozoa. Note the hammer-shaped nucleus (arrow), and the several bundles of longitudinal microtubules present in the cytoplasm (arrow head). (E) Fracture plane through the outer membrane of a mature spermatozoan (arrow) at the edge of a spermatic cyst, imaged by scanning electron microscopy. Note the smooth surface, distinctive of that stage. (F) Longitudinal section of two mature spermatozoa showing the hammer-shaped nucleus (arrow), and the proacrosomal vesicles (arrowhead). (G) Higher magnification of the spermatozoon head containing the proacrosomal vesicles (arrowhead). (H, I) Fracture and section of a spermatic cyst containing both spermatids and mature spermatozoa. Note the follicle cell layer (fo, arrow), the collagen envelope (c), and the thick enveloping layer of juxtaposed cells enclosing the cyst (ev); nucleus (n).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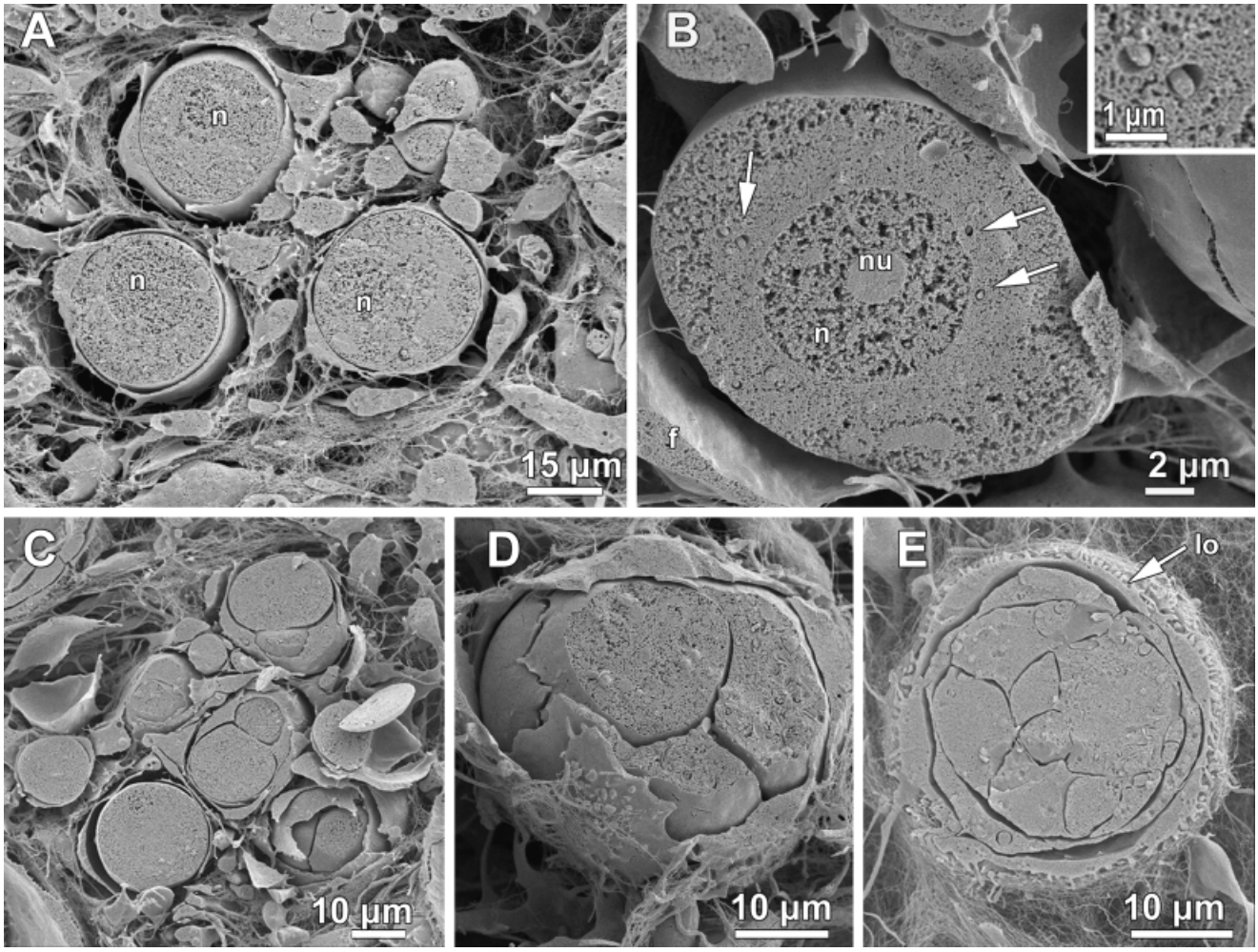


**Fig. 7.** Oogenesis and cleavage. (A) Oocyte cluster. Note the round nucleus (n) in all oocytes. (B) An oocyte with a nucleolated (nu) nucleus (n) and with bacterial symbionts (arrows and inset) in the cytoplasm. (C) A cluster of two-cell embryos. (D) 4-cell, and (E), 32-cell embryos. In the latter, two distinct layers have formed, and the follicle cells have numerous lobopodia (lo).

reduction in size, cytoplasmic bridges, and occurrence of pro-acrosomal vesicles (Alberts et al. 1994).

## Significance of sperm shape for fertilization

What does the shape of sperm signify? It has been assumed that round sperm in sponges are "primitive," but presumably the shape has to do with the mechanism of locomotion and not phylogenetic position (Franzén 1956; Fawcett 1970). Modified sperm (elongated and acrosome bearing) are regarded to be adapted for movement in dense media in which fertilization takes place (Tuzet 1950; Franzén 1970), and has arisen independently and convergently in many metazoan groups (Reunov 2005). The elongated shape of the sperm, the lack of choanocytes (and thus "normal" carrier cells; Watanabe and Okada 1996; Boury-Esnault and Jamieson 1999), and the synchronous development of cohorts of embryos prompted us to consider how fertilization might occur in the absence of carrier cells. The complex cellular envelope is likely responsible for transporting the cysts toward the surface of the sponge, where they are released. If spermatic cysts are released intact as suggested previously by Vacelet (1996), forceps spicules could serve two functions: they could, by projecting from the cyst, decrease the sinking rate (Uriz 2006) and they would enhance capture in the anisochelae of neighboring sponges. Thus, entire cysts would be incorporated into other sponges like prey. Upon release from the cyst, sperm would enter the subpinacoderm, and in the dense collagenous mesohyl, the elongated shape of the sperm would better allow spermatozoa to burrow toward the oocyte clusters. Release of an entire packet (spermatocyst) of sperm into the sponge body at once would also explain the simultaneous fertilization and subsequent synchronous development of clusters of oocytes.

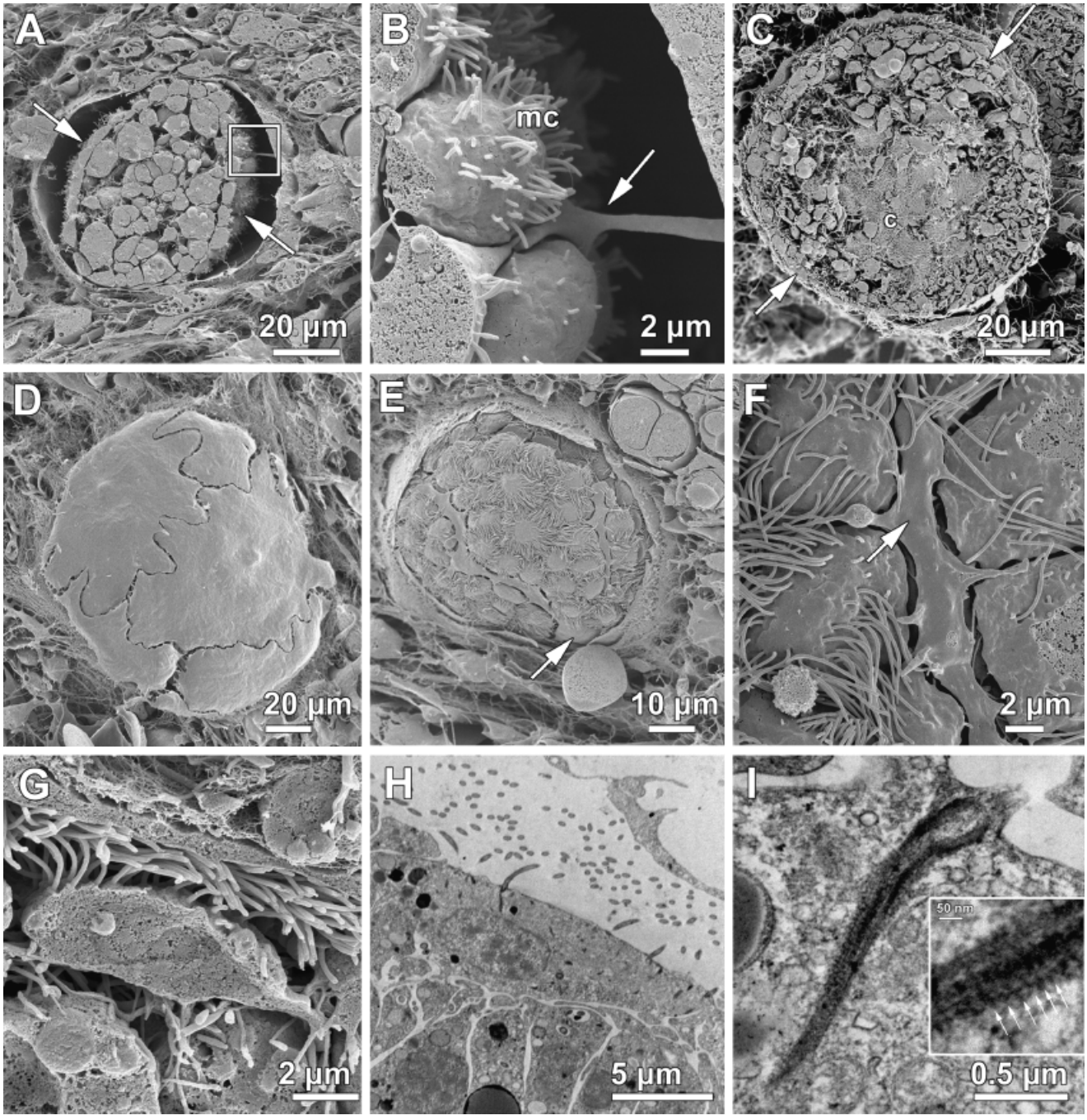


**Fig. 8.** Differentiation of the embryo. (A) A late-stage embryo showing the multiciliated cells (arrows) in the outer layer. (B) An enlargement of multiciliated cells (mc) in the outer layer of the embryo. At this early stage, cilia are relatively short. Note the pseudopod (arrow) extended by the follicle cells toward the embryo. (C) Late-stage embryo with multiciliated cells (arrows); collagen (c) has been secreted in the center of the embryo. (D–I) Aspects of the outer surface of the late embryo. (D) Follicle cells surrounding the late-stage embryo. (E, F) Multiciliated cells lie directly under the follicle cells; at one pole, there is an unusual nonciliated cell (arrow) that sends long extensions out between the ciliated cells. (G, H) A fracture and section through a multiciliated cell at the surface of the embryo. (I) The rootlet of the cilia in multiciliated cells has cross striations. (Inset: high magnification that shows the striations have a periodicity of 29 nm.)

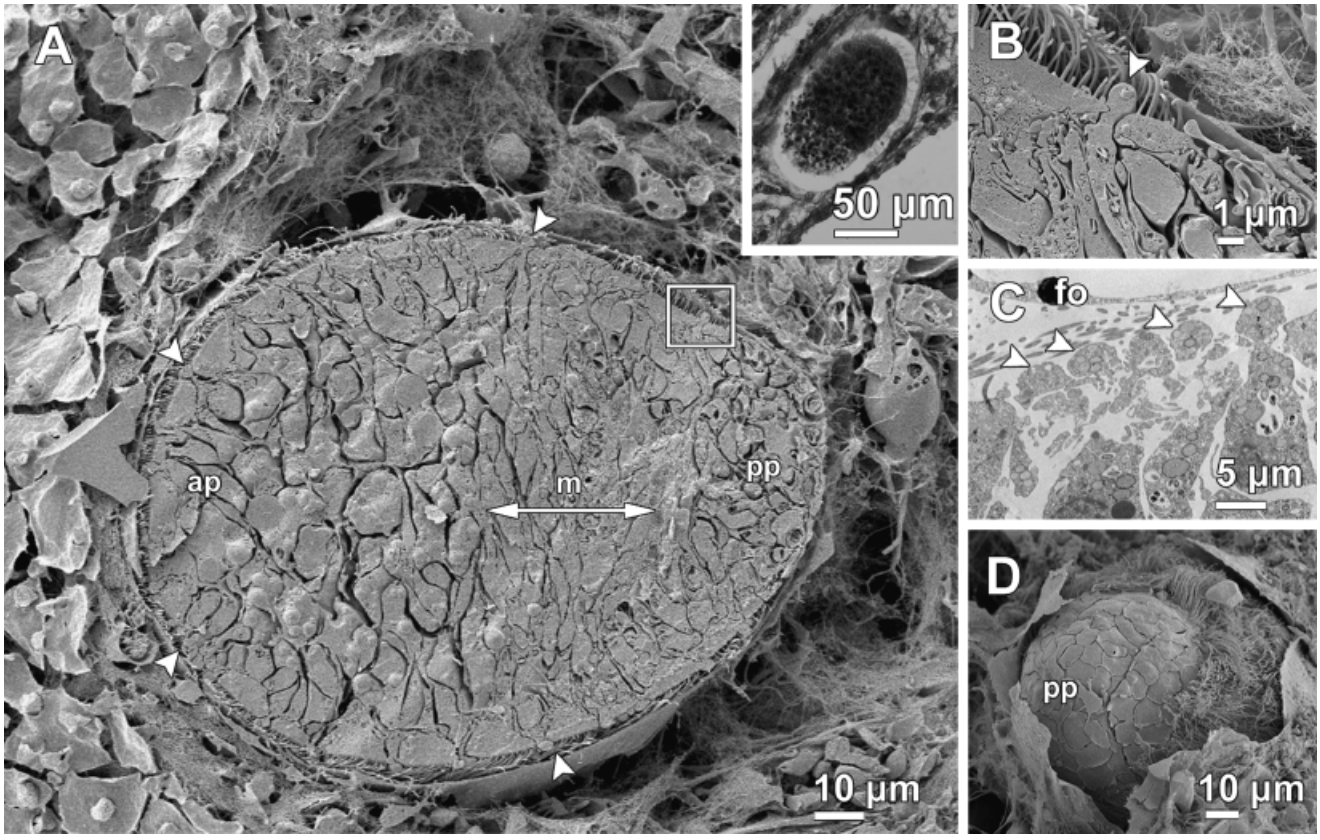


**Fig. 9.** Larval structure. (A) A fracture of the sponge surface showing a pre-larva close to the pinacoderm. The larva shows three distinctive regions: the anterior pole (ap) with large cells, the mid-region (m and double arrow) with cells aligned perpendicular to the (A–P) axis and associated with dense bundles of collagen, and the posterior pole (pp) with small and vesiculated cells. Multiciliated cells cover all but the posterior pole and occasional non-ciliated cells project through to the surface between the multiciliated cells (arrowheads). The boxed region is shown in (B). (The inset shows a longitudinal section of a larva viewed by light microscopy.) (B, C) Fracture and section, respectively, showing the bulbous projection of the nonciliated cell (arrowheads) under the follicular envelope, fo. (D) View of the nonciliated posterior pole (pp) of the larva.

## Tissue organization and cellular differentiation

Where oocytes occur in *Asbestopluma* must greatly affect fertilization success. In many sponges, oocytes arise throughout the body (Fell 1983, 1997), but frequently close to a canal or a choanocyte chamber. Congeries of oocytes, like the oocyte clusters found in *A. occidentalis*, are less common, but have been observed in some demosponges (Halisarcida, Lévi 1956; Poecilosclerida, Diaz 1973; Halichondrida, Fell and Jacob 1979; Dictyoceratida, Kaye 1991; Fell 1997; and Haplosclerida, Leys and Degnan 2002), and in hexactinellids (Mackie et al. 1983; Leys et al. 2006). Oocyte clusters are often adjacent to choanocyte chambers presumably because of the need to transfer the sperm pronucleus to the mature egg, assuming transfer by a carrier cell. In a few sponges, oocytes are not necessarily in clusters but are located in particular regions of the sponge body, often closest to the substratum in encrusting species (Ereskovsky and Boury-Esnault 2002). The clustered arrangement and particular localization of gametes in *A. occidentalis*, as in these few other cases, may be considered in the light of being the first step in developing a particular place where the gametes are always located (i.e., gonads). The tissue regionalization is more obvious in *A. occidentalis* because of the absence of canals and chambers. Oocyte clusters ("almost gonads") are in the outer part of the core, approximately 2–300 µm from the pinacoderm surface. By grouping oocytes together sperm–egg encounters may be increased if sperm enter as a packet. Hence, instances of oocyte clusters in other sponges may be suggestive of a similar mechanism of sperm transfer.

## Significance of cilia and ciliary structure

Development in *A. occidentalis* also sheds light on the question of the homology of multiciliated cells in metazoans. Ciliary structure—composition of the basal apparatus, existence of basal body, rootlets, etc.—in eukaryotes presumably reflects functional differences of the cells, but in many cases has

also been given phylogenetic significance where convergence is considered unlikely (Woollacott and Pinto 1995). For example, it has long been considered that the monociliated condition is primitive (Nielsen 1987, 2001), reflecting the origin of ciliated cells from a choanoflagellate ancestor; hence, it was with some surprise that Hexactinellid sponge larvae were found to have multiciliated cells (Boury-Esnault and Vacelet 1994). Here, we show that cladorhizid larvae also have multiciliated cells, and that each cilium possesses a cross-striated rootlet (most often simply referred to as a "striated rootlet"), a feature previously only known from calcareous sponges and homosclerophorids (Amano and Hori 1992, 2001; Boury-Esnault et al. 2003; Maldonado 2004).

The presence of cross-striated rootlets in these groups is frequently held up as an indication of the closer phylogenetic association between Calcarea, Homoscleromorpha, and other metazoans (which largely have cross-striated rootlets), but the fact that ciliary rootlets can also be striated in protists and plants (Pitelka 1974) and now also in other sponges suggests that its structure carries little phylogenetic signal, and that rather functionality is the primary driver of rootlet morphology. In metazoans, striated rootlets and basal feet are thought to dissipate the stresses on the cytoplasm (Pitelka 1974), or in instances where there is a close association of the rootlet with mitochondria the striations may act as a "trapping system" for receiving energy for extremely active cilia (Olsson 1962). Thus, striated rootlets might be expected to be found where stresses are particularly great and recent observations of striated rootlets in the sperm of *Crambe crambe* (Riesgo and Maldonado 2007) suggest that this is the case.

The presence of multiciliated cells in larvae of both hexactinellids and cladorhizids—two quite divergent lineages of siliceous sponges—implies that the ancestral condition presumably had the potential to have both mono- and multiciliated cells, that is, the monociliated condition seen in choanocytes and choanoflagellates probably also reflects a functional similarity rather than a shared ancestral trait.

## Significance of loss of the WCS

In 1998, Li et al. published images of putative fossil sponges in which there is no evidence of canals or chambers. This finding has led to the speculation that ancestral sponges may have lacked a WCS, and it has been suggested that loss of choanocyte chambers in carnivorous sponges indicates that the potential to lose the WCS may have existed in an earlier group of sponges, which may have then given rise to stem cnidarians (Vacelet 1999; E. Sperling and K. Peterson, personal communication). However, evidence that the sponge WCS shares physiological characteristics of a peristaltic contractile system with the cnidarian gastrovascular cavity (Leys and Meech 2006; Elliott and Leys in press) shows that the WCS may have more in common with the body plans of other metazoans than is often thought. Although the sponge WCS presumably provided an effective mechanism for feeding on the bacterial- and pico-plankton of NeoProterozoic oceans, it required only the acquisition of synaptic transmission—the protein scaffold of which has been recently revealed in sponges (Sakarya et al. 2007)—and muscle to modify the spongeocoel into a gut for feeding on more active prey (Cavalier-Smith 2006); in fact, the same elements of the epithelial-lined peristaltic tubes may be seen in most metazoans today (Leys in press). The loss of the WCS in modern cladorhizids is nevertheless informative of a later ability to colonize deep oceans where such plankton is limited (Vacelet 1999), and illustrates a plasticity of body plan like that seen in deepwater ascidians and bivalves (Monniot 1984; Morton 1987, 2003). What we can learn from cladorhizids today is that the potential for phenotypic plasticity exists within the genotype of an animal specialized for feeding by phagocytosis.

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