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Sexual reproduction in demosponges: ecological and evolutive implications

Reproducción sexual en demosponjas: implicaciones ecológicas y evolutivas

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Chapter 4:

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, encrusting) but always having an aquiferous system for feeding, *except* in cladorhizids in which flagellated filtering chambers are absent (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. While 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 which had a poriferan body plan. Loss of canals and chambers and the filtering habit is implied, and would seem a massive and intangible macroevolutionary event, if not for the example of the cladorhizids.

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 the sponge 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 Mycalidae) 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; Vacelet and Boury-Esnault 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), while *Chondrocladia gigantea* retains a modified but functional aquiferous system which 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 40cm-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 Straight 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 100-200m depths using 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 (Reiswig, 1983; Fell 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). Molecular data indicates poecilosclerids are definitely demosponges, and thus the most parsimonious hypothesis is that *Asbestopluma* has lost choanocytes. However, 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 places poecilosclerids firmly amongst the demosponges means the most parsimonious hypothesis is that *Asbestopluma* has lost choanocytes and early sponges most likely possessed a water canal system

Material and methods

Specimens were collected by the remote operated vehicle ROPOS from fjord walls at 120 m depth in Barkley Sound (48º53'54'' N, 125º03'9'' 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 Centre for 3-5 days prior to fixation. Spicules were prepared for scanning electron microscopy (SEM) by digestion with nitric acid and ethanol washes directly on a round coverslip (Hooper 1998). 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 prior to embedding in epoxy for TEM or critical point drying for SEM. Specimens were viewed in a Phillips (FEI) transmission electron microscope at 75KV and a Joel 6301 Field emission scanning electron microscope at 5KV.

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 (Figs. 1C, 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.

The skeleton (Fig 2) was organized around a central axis of styles (731.8 μm ± 275.1, n=50 long and 18.3 μm ± 4.7, n=50 wide) enveloped in a dense collagen sheath; styles also formed an internal support for each filament. Subtylostyles and tylostyles (245.6 μm ± 123.9 long and 9.2 μm ± 4.1, n=50 wide) were more common in the base, arranged transversally to the longitudinal styles. Palmate anisochelae (11.4 μm ± 0.8, 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 μm ± 3.9, n=7) were associated with the spermatic cysts.

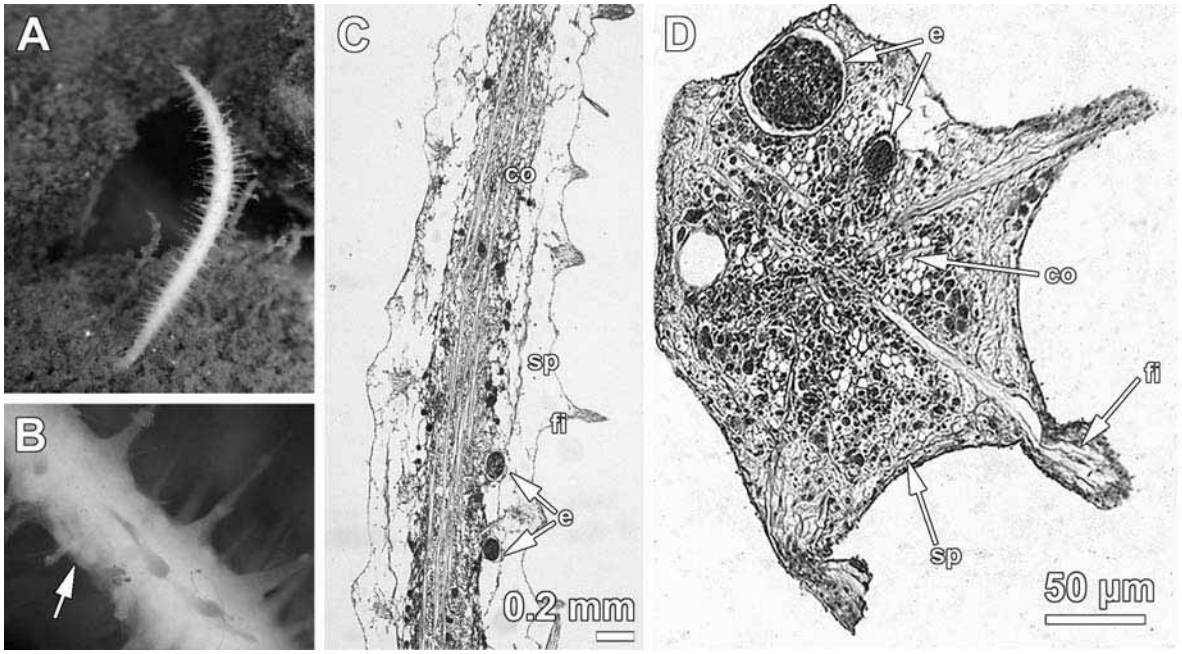

Figure 1. Structure of the adult sponge. **(A)** Live *Asbestopluma occidentalis* attached to the skeleton of a glass sponge in an aquarium after collection; the specimen is approximately 2cm tall. **(B)** Embryos (arrow) can been seen trough the transparent outer layer of the stalk. Longitudinal **(C)**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

The 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 that projected through T-shaped pinacocytes to the collagen matrix below (Fig. 3). Cell density was highest in the core, where 5 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; 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.

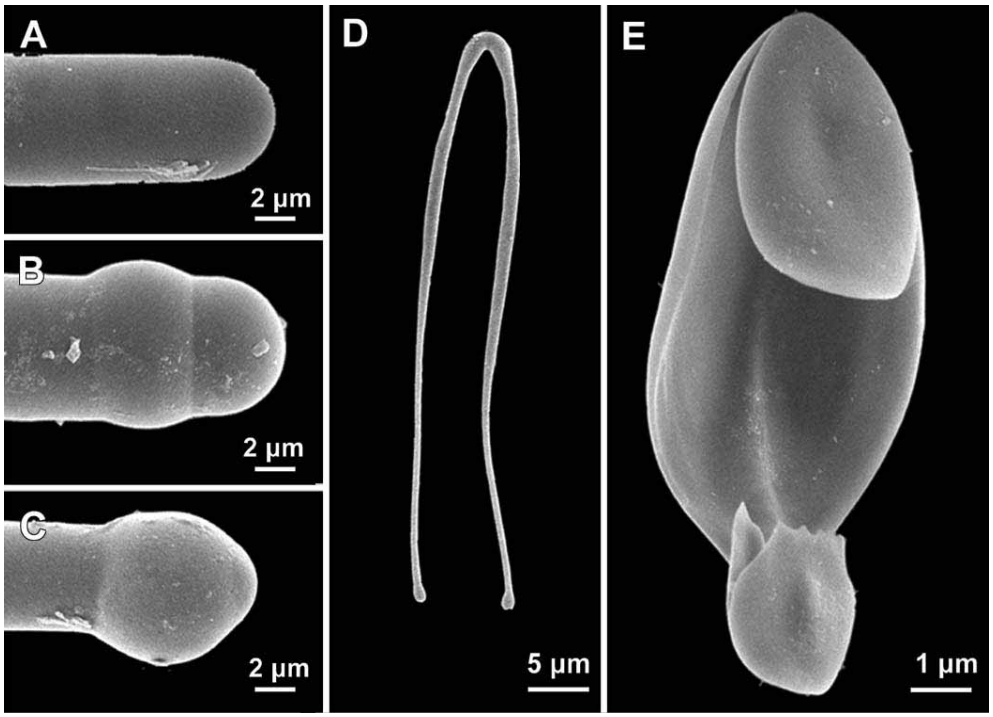

Figure 2. Distinctive spicule skeleton of *Asbestopluma occidentalis* (SEM). **(A)** Style head. **(B)** Subtylostyle head. **(C)** Tylostyle head. **(D)** Forceps. **(E)** Palmate anisochelae, which are responsible for trapping prey.

Gametogenesis

Asbestopluma 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 (spermatogonia) 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).

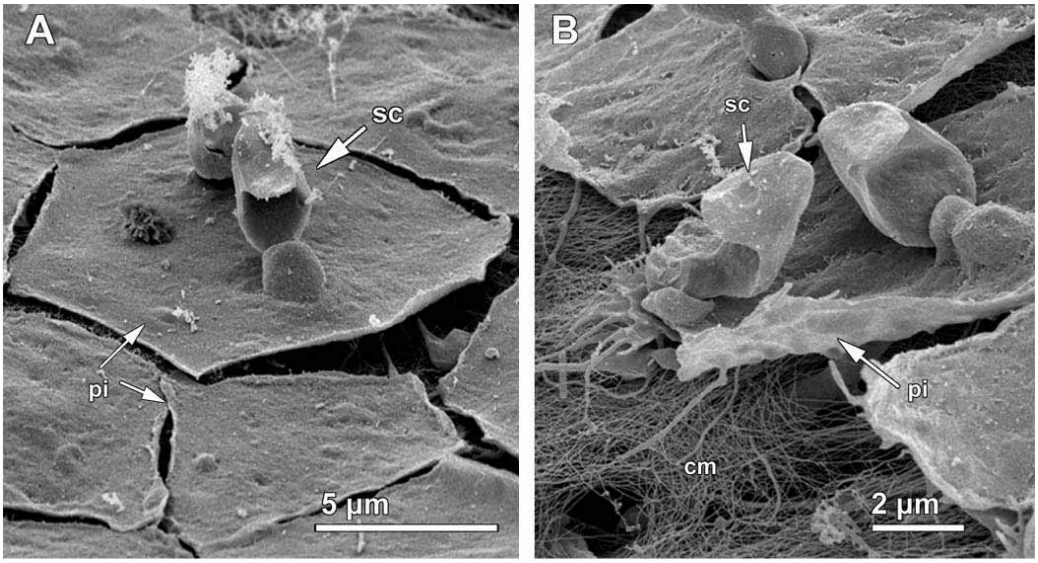

Figure 3. Surface pinacoderm viewed by scanning electron microscopy. **(A)** Pinacocytes (pi) are pierced by anisochalae-containing sclerocytes (sc). **(B)** The sclerocytes (sc) are anchored by root-like extensions in the collagenous matrix below the pinacocyte (pi).

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, 5A). Secondary spermatocytes were much smaller (2.5 µm diameter) flagellated cells with numerous pseudopodia (Fig. 4B, 5C-E). In contrast, spermatids in late-stage spermatocytes were elongate cells (~8 µm long) with a very long anterior extension containing the nucleus (Fig. 6A-C). The flagellum was inserted in 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 was smooth again in mature sperm (Figs. 5D, 6C, E). All stages of spermatogenesis were connected by cytoplasmic bridges (Figs. 5E, 6A). In late stage cysts mature spermatozoa were densely packed (Fig. 6D). Externally, the anterior region of the nucleus of mature spermatozoa appeared swollen, but sections showed the anterior-most end of nucleus was flared at either side, like a hammerhead (Fig. 6D-G). At the very tip of the nucleus, 3-6 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. 6H-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. 7A-B). Oocytes were 6-24 μm in diameter (n=38), with few inclusions and little yolk (Fig. 7C), but each had 2-3 conical nurse cells with long extensions that enveloped the oocyte. Mature oocytes, corresponding to the smallest size recorded for two-cell stage embryos (see below), usually contained intracellular bacteria, transferred by the nurse cells from the parent sponge (Fig. 7D).

Embryogenesis All embryos were located in the outermost edge of the core. Clusters of two-cell embryos suggested that fertilization was synchronous (Fig. 7E). Early cleavage was holoblastic and equal, and 4 and 8 cell stage embryos were compact, with cells tightly juxtaposed against one another (Fig. 7F). In 16-cell embryos two layers were already evident, the external layer flatter than the internal (Fig. 7G). At the 5^{th} cleavage (32-cell stage) 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. 8A-C). Cells in the inner region of early embryos were loosely arranged among filamentous bacteria and collagen (Fig. 8A,C).

During all stages follicle cells surrounded the embryo (Fig. 8D) separated from it by only a thin layer of collagen. The nurse cells extended pseudopodia both towards the mesohyl, and inwards to contact the embryo (Fig 8A,B).

Later stage embryos (60-100 μm) were more compact, and cells containing inclusions and bacteria appeared to secrete collagen (Fig. 8C). At this stage, the outer layer of the embryo was mostly formed by multiciliated cells whose now long cilia were bent

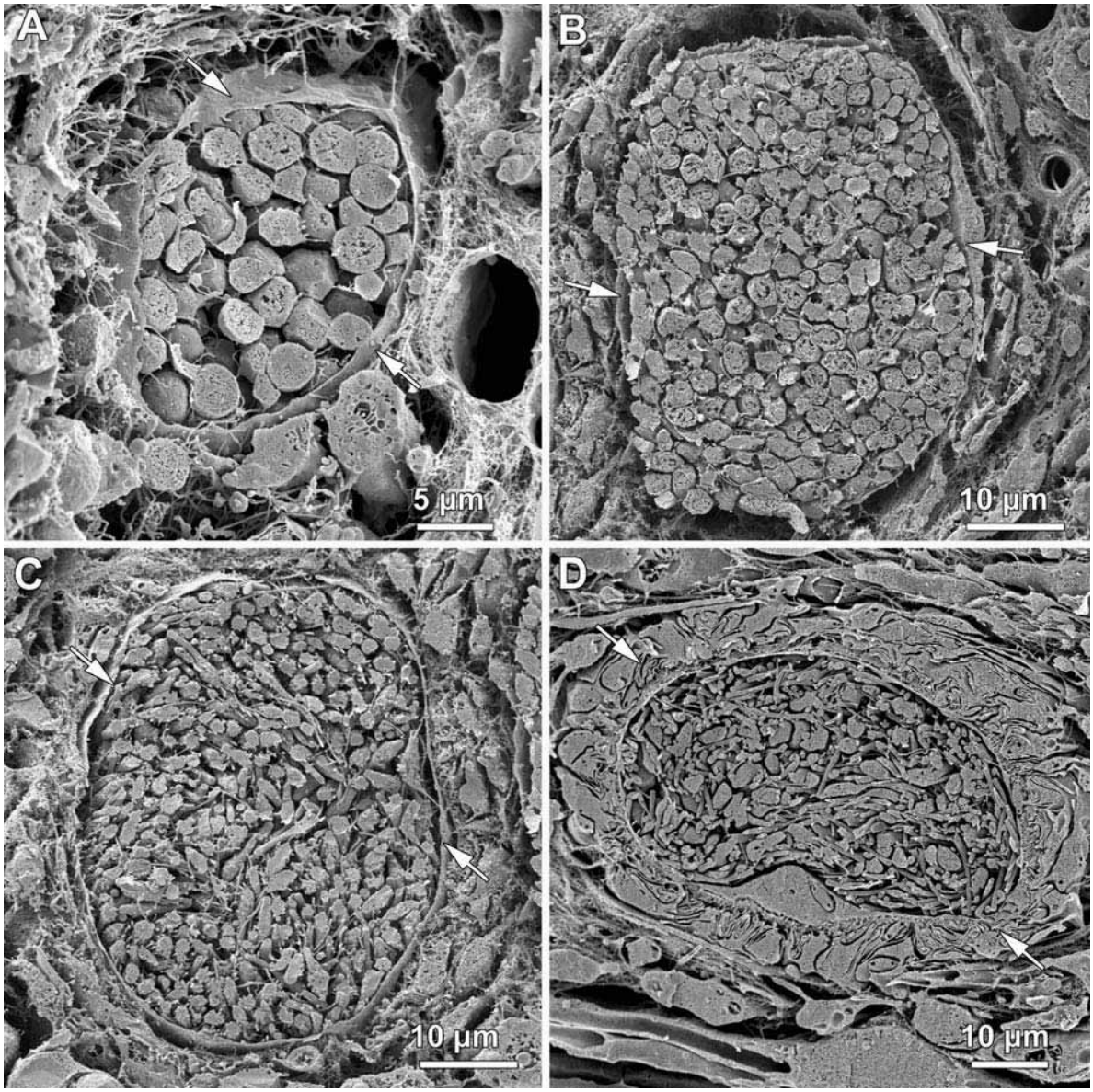

Figure 4. Developmental progression of spermatic cysts. **(A)** Primary spermatocytes, surrounded by a thin layer of follicle cells (arrows). **(B)** Spermatic cyst containing secondary spermatocytes, enveloped by a thin layer of cells (arrows). **(C)** Spermatids surrounded by a slightly thicker layer 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).

over within the follicular epithelium (Fig. 8E-H); each cilium possessed a striated rootlet (Fig. 8I). At one pole there was a single non-ciliated cell with long extensions that reached the whole length of the embryo, lying between, but joined to, the multiciliated cells (Fig. 8E-F).

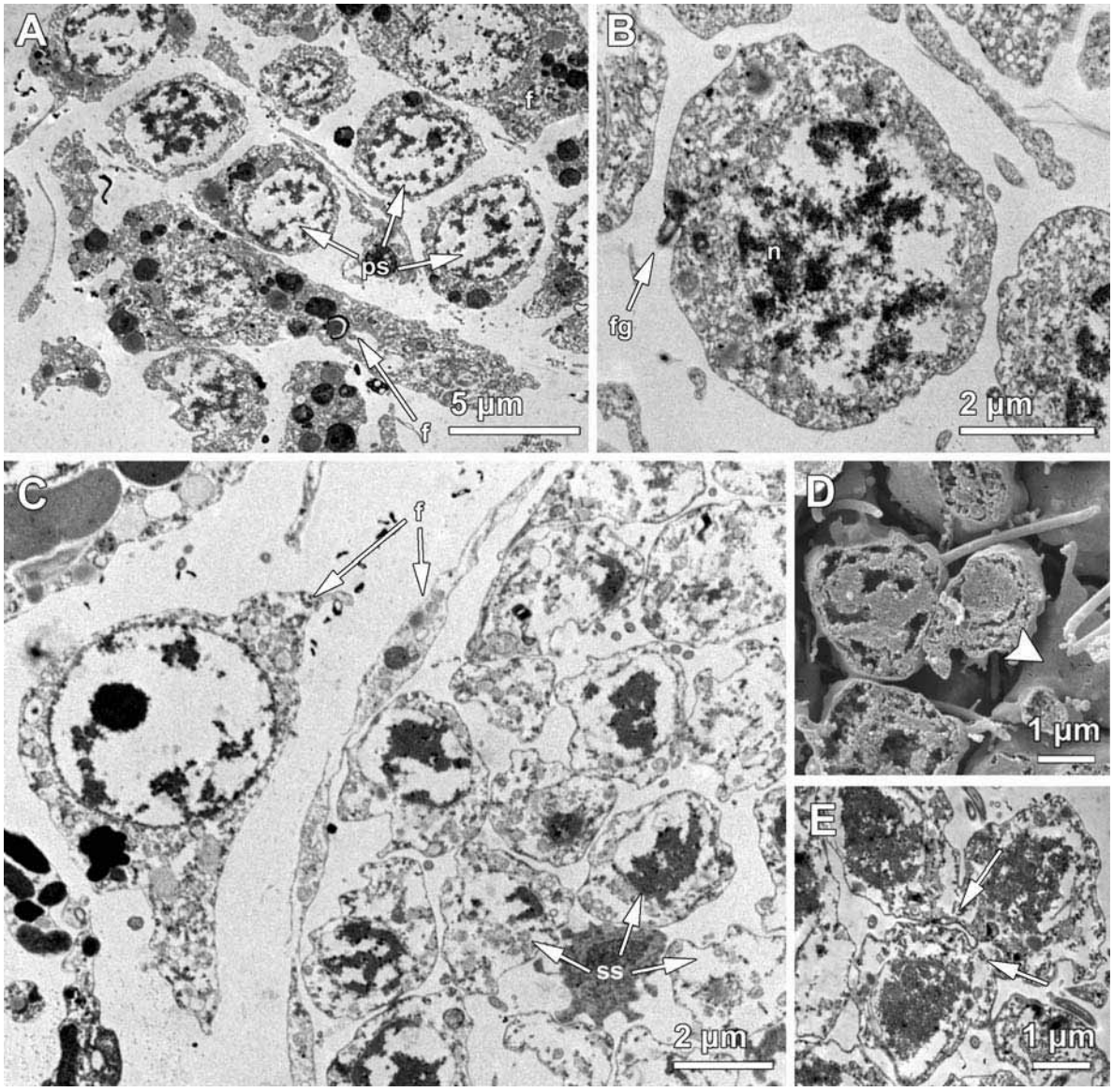

Figure 5. Early spermatogenesis. **(A)** Congeries of amoeboid primary spermatocytes (ps) and future enveloping cells (f). **(B)** Primary spermatocyte with flagellum (fg). Note the incipient chromatin coiling in the nucleus (n). **(C)** Spermatic cyst containing irregular secondary spermatocytes, and surrounded by two layers of follicle cells (f). **(D)** Secondary spermatocytes with a smooth surface (arrow head). **(E)** Sister spermatocytes connected by cytoplasmic bridges (arrows).

The most differentiated embryos (pre-larvae) lay at the periphery of the subpinacoderm (Fig. 9A, insert). The pre-larva was differentiated into three regions: cells in the anterior hemisphere were heterogeneous; cells in the mid-region were 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. 9A, D). Multiciliated cells were juxtaposed with one- another and with a type of non-ciliated cell that protruded slightly among the bases of the cilia (Fig. 9A-C).

Discussion

Relevance to sponge body plan evolution

Sponge gametogenesis and embryogenesis is poorly understood – there is 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. *Asbestopluma 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 be observed also 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 acrosomal 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 in press); and, most unexpected, 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).

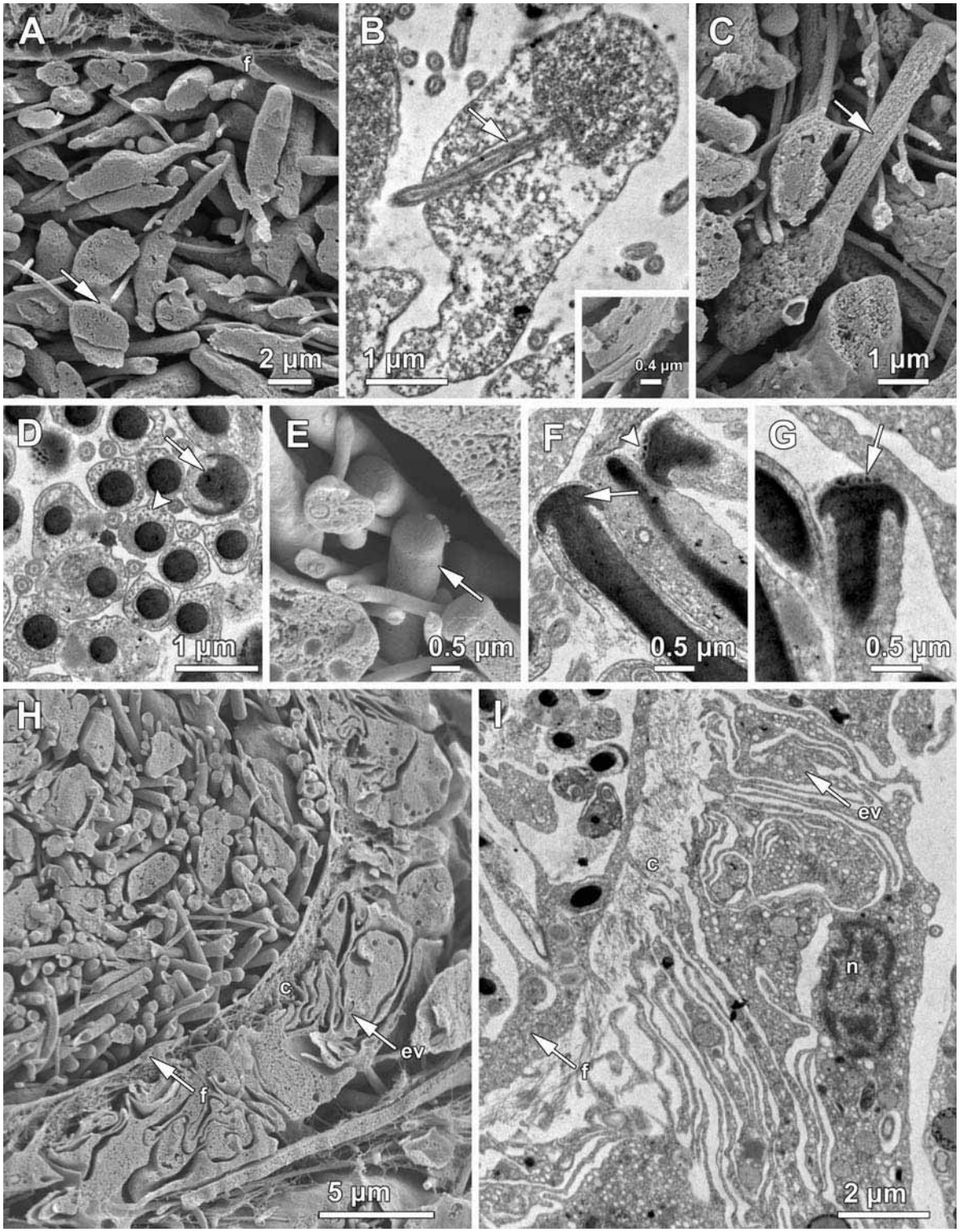
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Figure 6. Spermiogenesis. **(A)** Sister spermatids connected by cytoplasmic bridges (arrows). (f), follicle cells enclosing the cyst. **(B and inset)** Spermatids showing the pit harboring the flagellum. (C) An elongated spermatid showing the swelling of the head and the ridged surface typical of that stage. **(D)** Cross section of a spermatic cyst containing mature spermatozoa. Note the hammer-shaped nucleus (arrow), and the bundles of microtubules present in the cytoplasm (arrow head). **(E)** Mature spermatozoa (arrow) at the edge of a spermatic cyst. Note the smooth surface distinctive of that stage. **(F)** Longitudinal section of two mature spermatozoa showing the hammer-shaped nucleus (arrow) and the acrosomal vesicles (av). **(G)** Higher magnification of the spermatozoon head containing the acrosomal vesicles (arrow). **(H-I)** Fracture and section of a spermatic cyst containing both spermatids and mature spermatozoa. Note the follicle cell layer (f, arrow), the collagen envelope (c), and the thick layer of juxtaposed cells enclosing the cyst; nucleus (n).

Sponge body plans are relatively homogenous across a great phylogenetic range, from hexctinellids to homoscleromorphs, demosponges and calcareous sponges, 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 gametes arise from amoebocytes, and others, from choanocytes, both considered to be multipotent stem cells; however, interpretations are based upon static images of fixed tissue, and until experimental studies are conducted in sponges, conclusions about the origin of gametes remain equivocal (Reiswig 1983; Simpson 1984; Fell 1983, 1997). 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 indicate an early separation of the somatic and germ lineage in *Hydra* (Mochizuki et al. 2000), the jellyfish *Podocoryne*

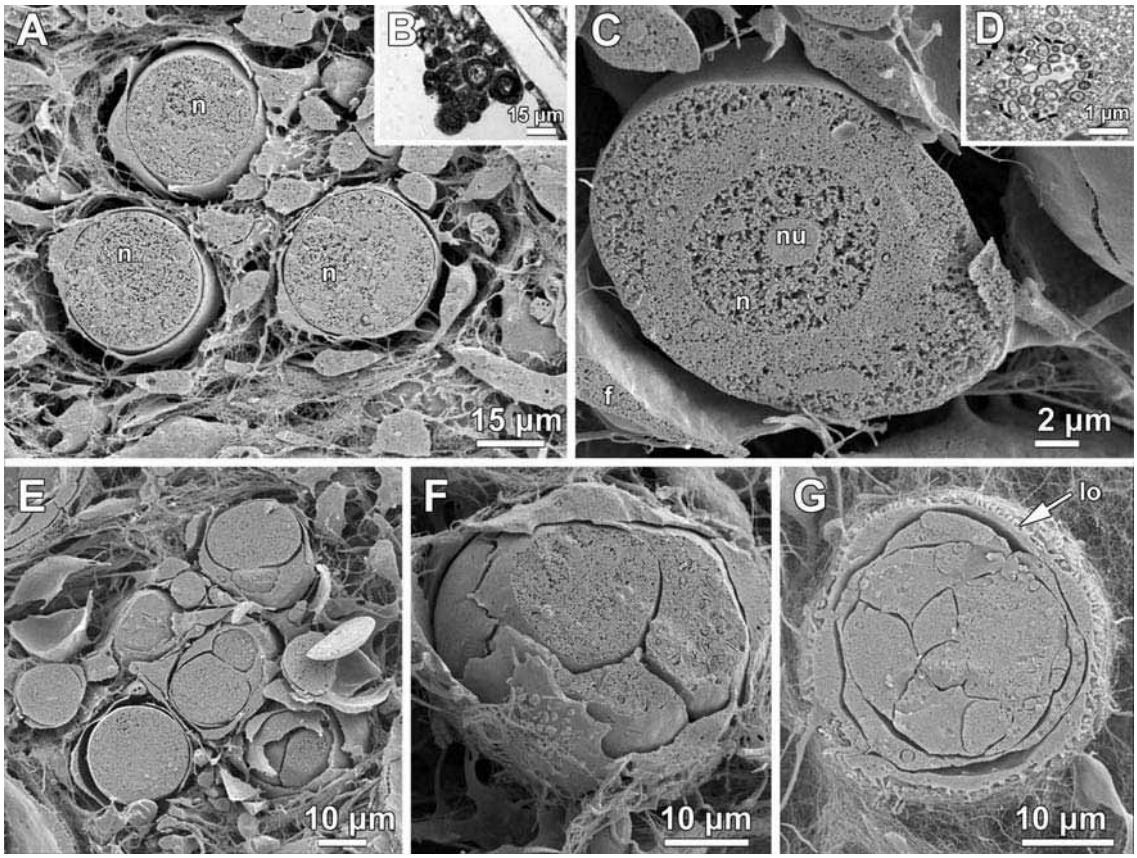

Figure 7. Oogenesis and cleavage. **(A)** Oocyte cluster. Note the round nucleus (n) in all oocytes. **(B)** Light micrograph of an oocyte cluster. **(C)** An oocyte with a nucleolated (nu) nucleus (n). **(D)** Intracellular bacteria found in the cytoplasm of an oocyte. **(E)** A cluster of 2-cell embryos. F. 8-cell, and G, 32-cell embryos. In the latter two distinct layers have formed, and the follicle cells have numerous lobopodia (lo).

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 calcareous 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)

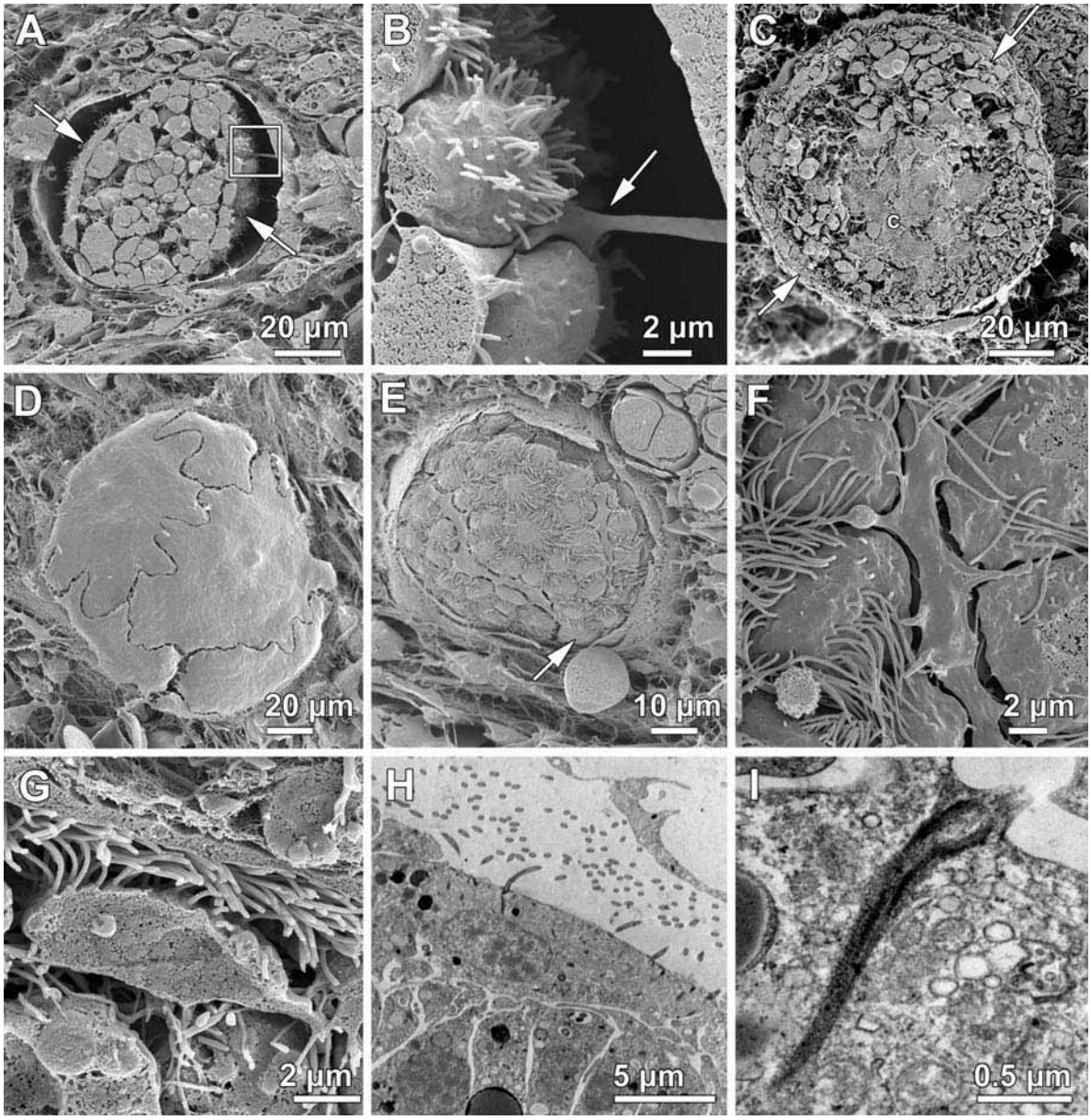

Figure 8. Differentiation of the embryo. **(A)** Embryo with multiciliated cells (arrows) in the outer layer. **(B)** Magnification of A, showing a pseudopodium (arrow) extended by the follicle cells towards the embryo. **(C)** Late stage embryo with well-developed multiciliated cells (arrow) on the outer surface; 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 cell 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)** Striated rootlet of the cilium insertion in the multiciliated cells.

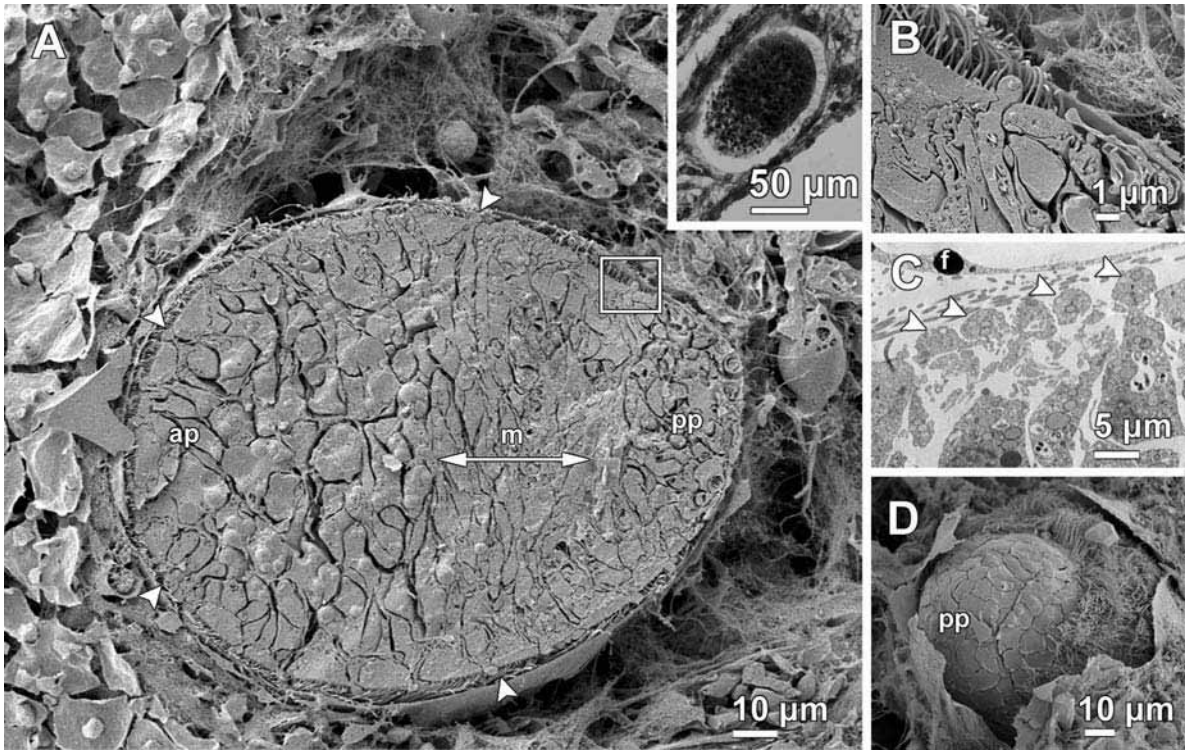

Figure 9. Larval structure. **(A)** A fracture of the sponge surface showing a mature larva close to the pinacoderm. Multiciliated cells cover all but the posterior pole (arrow), and occasional non-ciliated cells project through to the surface between the multiciliated cells (arrowheads). The boxed region is shown in B. (Inset shows a longitudinal section of a larva viewed by light microscopy.) **(B-C)** Fracture and section showing the bulbous projection of the non-ciliated cell (arrowheads). **(D)** View of the non-ciliated posterior pole of the larva.

and in some other demosponges (Fell 1974; Reiswig 1983; Simpson 1984). The flagellum is the first obvious marker of spermatogonia, 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 few sponges but is known from other invertebrates (Hinsch 1974; Efremova and Parkovskaya 1980; Paulus 1989; De Vos et al. 1991; Reunov and Klepal 2004; Riesgo and Maldonado in press), 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. 1986b; Boury-Esnault and Jamieson

1999; Riesgo et al. 2007) and Poecilosclerida (Tripepi et al. 1984; Riesgo and Maldonado in press). The occurrence of acrosomal vesicles in the sperm of *Asbestopluma 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 *Leucosolenia complicata* has apyrene (non-flagellated) 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: reduction in size, cytoplasmic bridges, occurrence of proacrosomal vesicles, and uniform orientation of spermatozoa in mature cysts (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 to 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; Hodgson 1986), 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 carrier cells. The complex cellular envelope is likely responsible for transporting the cysts towards the surface of the sponge, where

they are released. If spermatic cysts are released intact as previously suggested by Vacelet (1996), forceps spicules could serve two functions: they could, by projecting from the cyst, decrease 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 the dense collagenous mesohyl, the elongated shape of the sperm would better allow spermatozoa to burrow towards 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.

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 Haplosclerina, 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 *Asbestopluma 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 its structure. In metazoans striated rootlets and basal feet are thought to dissipate the stresses on the cytoplasm (Pitelka 1974), or in instances where there is 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 in press) suggest 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 mono-ciliated condition seen in choanocytes and choanoflagellates probably also reflects a functional similarity rather than a shared ancestral trait.

The significance of loss of the water canal system

It has been suggested that loss of choanocyte chambers in carnivorous sponges indicates that the potential to loose the water canal system (WCS) may have existed in an earlier group of sponges, which may have then given rise to stem cnidarians (Vacelet 1999). 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 review) 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 shown to be already present in sponges (Sakarya et al. 2007) – and muscle to modify the spongeocoel into a gut for feeding on more active prey (Cavalier-Smith 2006); the retention of 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, 1993). 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.