Sexual elements and major cell types of the freshwater sponge, *Spongilla lacustris*

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Abstract

Spongilla lacustris is a freshwater sponge (Phylum Porifera, Family Spongillidae) that is present in the lakes of Western Montana. In an effort to understand more about its sexual reproductive cycle and about its microscopic anatomy, sections prepared from samples obtained from Salmon Lake (T15N/R14/S8) between May-August 1994 were examined using light microscopy. Spermatogenic cysts, eggs, and larvae were found in samples obtained. The structures of pinacocytes, choanocytes, and archaeocytes are described.

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Introduction and Literature Review

Spongilla lacustris is a freshwater sponge belonging to the Phylum Porifera, Class Demospongiae, Order Haplosclerida, Family Spongillidae (Bergquist, 1978). As with all other sponges, *S. lacustris* is a sedentary filter-feeder which lacks true organ systems. The tissue level of organization is the highest attained, and as a result the cells retain a certain degree of independence (Bergquist, 1978).

Overview of Structure and Function

S. lacustris typically grows attached to rocks or logs in less than 2 m of water. It sometimes appears as low encrusting growth, but it can also be found extending finger-like projections for several cm from an attached surface. The projections may have a diameter between 1-8 mm (Gilbert and Simpson, 1973). *S. lacustris* ranges in color from green to light brown. The green color is due to the presence of algal symbionts. The symbionts supply the sponge with photosynthetically fixed carbon, while the sponge provides the symbiont with nitrogen, phosphorous, and carbon dioxide (Frost, 1991). Contributions from the symbiont seem to account for 50-80% of the growth of *S. lacustris*, without which the sponge would not be able to maintain normal life-functioning properties (Frost 1991).

S. lacustris exhibits a leucon body plan. Extensive folding of the epithelium (pinacoderm) exists in this type. This series of folding results in the formation of

choanocyte chambers and canals, both exhalant and inhalant (Bergquist, 1978). There is also a thickening of the mesohyl (the layer between the two epithelial layers) (Bergquist, 1978), with a great diversification of the cellular and skeletal components within the mesohyl (Bergquist, 1978).

Essential to the survival of a sedentary filter feeder is the flow of water through the canals that is brought about by the activity of the choanocytes. Through the movement of the choanocytes' flagella, a current of water is circulated through the sponge. The water enters through the ostia and into a large subdermal cavity. From here, it moves into the incurrent canals. The incurrent canals carry the water and small suspended particles into a choanocyte chamber where it enters through small openings called prosopyles. The chamber is a spherical structure with choanocytes composing the outer wall. Each choanocyte has an associated collar surrounding its flagellum. The beating flagella drive the water out of the chamber and into the excurrent canal through another type of opening, the apopyle. The apopyle acts as a valve that will not let water re-enter the chamber (Weissenfels, 1992). Eventually the water enters the atrium and finally exits the sponge via the osculum (Frost, 1991). The series of canals and chambers acts as a filter, allowing only select particles to proceed. The incurrent canals branch repeatedly and decrease in diameter each time. The cells lining the canals, endopinacocytes, have cellular extensions that form net-like structures to prevent large particles from going through (Weissenfels, 1991). The finest filtration device in the sponge is the collar on the choanocyte (Frost, 1991).

Food particles are taken up by phagocytosis. Many cells, including the pinacocytes, porocytes (components of the inhalant system) (Bergquist, 1978), and choanocytes are capable of phagocytosis. Particles too large to be taken in by the ostia are engulfed directly by the epithelium, while those particles that do advance through the canals will be picked up by the choanocytes and endopinacocytes (Frost, 1991). After particles are taken up by choanocytes, they are transferred to digestive cells (typically archaeocytes) (Frost, 1991). The archaeocytes digest the material and then migrate throughout the sponge body, transferring the nutrients to other cells (Frost, 1991). The material that is not digested is expelled into the excurrent canals and then released to the outside of the sponge (Frost, 1991).

In *S. lacustris*, both asexual and sexual reproduction occur. It has been reported that this species is one of the few organisms that can potentially change from one sex to another each year (Gilbert and Simpson, 1976). Thus, one year it will be a male and produce male gametes, whereas the next year it may be female and produce female gametes (Gilbert and Simpson, 1976). Specialized cells are responsible for sexual reproduction in *S. lacustris*. Spermatogenesis occurs inside of spermatogenic cysts which are derived from choanocytes and cyst-building cells (Frost, 1991). When spermatogenesis begins, choanocytes enlarge and lose their collars and become spermatogonia (Paulus, 1989). Cyst-building cells lie in close proximity to the developing spermatogonia and surround them with pseuodopodia-like processes (Paulus, 1989). Many of these cysts will fuse. Spermatozoa will be formed from the spermatogonia within these cysts (Paulus, 1989). When they are mature, the sperm are released into the excurrent canals of the sponge. They are eventually picked up by a female sponge through the canal system and taken to the egg where fertilization occurs (Frost, 1991).

Eggs (oocytes) appear to be derived from archaeocytes (Saller and Weissenfels, 1985). The archaeocytes grow in size and develop into oocytes. They then engulf (via phagocytosis) trophocytes that are rich in nutrients. The oocytes will grow dramatically in size, increasing their volume by 64-100 times (Saller and Weissenfels, 1985). A follicular membrane of flattened cells develops around one oocyte (Saller and Weissenfels, 1985). After fertilization, fertilized eggs undergo developmental changes on their way to forming larvae. *S. lacustris* has "solid" parenchymula larvae (Frost, 1991). The early larvae consist of many yolk filled cells with a follicular epithelium surrounding the entire structure. In later stages, a larval cavity develops and some scleroblasts will also be included in the larvae (Saller and Weissenfels, 1985). After the larvae have freed themselves from the protective mesenchyme surrounding them, they will pass through the endopinacocyte wall and proceed into the excurrent canal. The larvae have flagellated epithelial cells allowing them to swim upon release from the mother sponge. The larvae will swim for hours or days until they reach a suitable substrate, attaching there and metamorphosizing into adult sponges (Frost, 1991).

Asexual reproduction occurs by means of gemmules. These are formed in a process of dedifferentiation in which the archaeocytes and fibers come together and become surrounded by a resistant membrane (Frost, 1991). This occurs when the environment becomes unfavorable, such as with the onset of winter. These gemmules germinate when the favorable conditions return. Ungerminated gemmules have a very low metabolic rate (Frost, 1991). Once germination occurs, a new sponge will develop, and sexual reproduction may also proceed when the sponge reaches maturity. In *S. lacustris,* gemmulation may occur at two different intervals in a given year. One is in early July, while the other is in the late fall months of November and October (Gilbert and Simpson, 1973).

Epithelial Cell Structure

The following descriptions are adapted from Bergquist (1978).

The cells that line the surfaces of *S. lacustris* are the pinacocytes, which make up the pinacoderm. The fundamental role of these cells is to protect and separate the sponge from the environment. Three types of pinacocytes exist: exopinacocytes, endopinacocytes, and basopinacocytes. The exopinaocytes line the external surface of the sponge. In *S. lacustris,* they have a fusiform shape. In sponges in general, there exists some degree of overlap between adjacent exopinacocytes. The endopinocytes line the aquiferous canals (incurrent and excurrent) that are in the sponge. These cells also have a fusiform or subspherical shape, yet there is no overlap between adjacent cells. Often a thin cellular extension protrudes toward the lumen of the canal. The basopinacocytes form the basal epithelium of the sponge. These cells are active in secreting fibrillar collagen and a polysaccharide complex, together which account for the attachment site for the sponge. Other roles of the basopinacocytes include feeding via phagocytosis, and gathering bacteria with the use of filipodia.

As mentioned above, the choanocyte is the cell that is responsible for the production of the water current in the sponge. This flagellated cell exists as a single layer of epithelium forming a choanocyte chamber in *S. lacustris*. Choanocytes are irregular, subspherical cells, in which there exists a centrally located nucleus (lacking a nucleolus). The flagellum is also centrally located, and has surrounding it about 30-40 microvilli that form a collar-like structure. Many vacuoles exist in the cells since pinocytosis and phagocytosis are very active processes in the choanocyte. The cell sits directly on the mesohyl, thus no basal membrane exist.

Cells and Inclusions of the Mesohyl

The following descriptions are again adapted from Bergquist (1978) unless otherwise noted.

The mesohyl of the sponge is the part of the sponge that exists between the layers of epithelium. Here, the cells can move about freely. It consists of an organic matrix and a number of cells that interact with the epithelial cells to perform certain activities such as digestion, gas exchange, and water processing for food. It also provides structural support. The mesohyl contains numerous cell types.

The archaeocyte is capable of differentiating into any type of cell that exists in the sponge. The main function of the archaeocyte appears to be that of digestion. This cell is responsible for phagocytosis of many different things such as bacteria and expended cells. The cell carries out the major portion of digestion in the sponge, as well as playing a large role in excretion. They also play a part in the formation of a sponge since they are one of the main components of a gemmule.

The skeleton that makes up the sponge is secreted by a number of cells in the mesohyl, all working together to provide support for the sponge. The cells included in this group are the collencytes, lophocytes, spongocytes, and the sclerocytes. They are all amoeboid cells that are free to move about in the mesohyl. The collencytes have the function of secreting the main component of the skeleton, fibrillar collagen. They are stellate or fusiform in shape and often lack a nucleolus. Lophocytes are also cells which secrete fibrillar collagen. They are very large cells often seen with a tail-like structure. This "tail" is a band of collagen that remains behind the cell as it is being secreted out of the cell. Lophocytes possess a nucleolus, are extremely irregular in shape, and sometimes have a number of filipodia extending off the cell. Spongocytes produce spongin, fiber-forming collagen. They are usually seen in groups or aggregates, clumping around fibers or spicules. The sclerocytes contribute to the formation of spicules. The spicule is a component of the framework and it may or may not have spines protruding from it. After spicule formation is complete, the sclerocytes disappear through a process of disintegration.

Objectives

This research project began as an attempt to describe the sexual reproductive structures (sexual elements) in a population of *S. lacustris*. Due to the scarcity of such structures, however, the focus was expanded to the major cell types of this sponge. Thus the goals of the project were: (1) to identify and describe the sexual elements and major cell types of *S. lacustris*, and (2) to determine when sexual reproduction occurred.

Materials and Methods

Samples of *Spongilla lacustris* were collected from Salmon Lake, a lake located about 35 mi east of Missoula, MT (T15N/R14W/S8) at 2-wk intervals between May-August 1994. The samples were taken up from the excurrent end of the lake, which is the southernmost portion. All collected sponges were found growing on the undersides or sides of rocks. The depth of the water was between 0.25-1.0 m.

The specimens of *S. lacustris* that were collected were of various sizes. Those selected in the early weeks usually had no finger-like projections, which is characteristic of *S. lacustris* picked at later stages of growth (Fig. 1). The sponges were either green because of algal symbionts, or a light brown. Portions of the sponge (about 1 cm by 1 cm) were removed with a razor blade and transferred into a container filled with lake water. The samples were transferred within 15 min into vials containing Bouin's fixative and were fixed for between 3-24 hr.

Once in the laboratory, the specimens were washed six times with a 0.2 M sodium phosphate buffer, pH 7.2/6.9% sucrose (wash buffer) (see Appendix A). Each wash lasted 10 min. At this stage, identification was confirmed by examining spicules. A tiny section of the sponge was removed and treated with a mixture of half water and half bleach before viewing with a light microscope. *S.*

lacustris can be distinguished from other sponges in Salmon Lake by its spiny microscleres (small spicules) (Frost, 1991).

The tissue was then placed into a 5% hydrofluoric acid solution for 2 hr in an attempt to rid the sponge of the spicules that made sectioning difficult. Two more washes of 10 min each with the wash buffer removed the hydrofluoric acid. The tissue was dehydrated with a graded series of alcohol solutions ranging from 50%-100% and finally xylene (Appendix A). On occasion, the process was interupted at the 70% alcohol stage and left overnight. Specimens were next infiltrated and embedded with Paraplast and sectioned. Embedding involved pouring a small amount of Paraplast into a metal mold, positioning the specimen in the mold, and filling the mold with Paraplast. When cool, the blocks of embedded tissue were sectioned with a microtome.

Sections between 6-8 μm were cut. A "ribbon"of sections (usually five or six) was placed into a few drops of water on a gelatin coated slide and dried on a warming tray.

The slides were then stained with hematoxylin (Delafield's) and eosin (eosin y) in a modified version of the protocol described by Sheeham and Hrapchak (1973) and Raphael (1976) (Appendix B). Sections were examined and photographed with a Nikon Optiphot microscope equipped with a Nikon AFX camera.

Results and Discussion

Cells Lining Surfaces (Epithelia)

The cells that line the external surface of the sponge, the exopinacocytes, can be seen in Figs. 2 and 3. The cells form a single layer in which some overlap between adjacent cells is evident (Fig. 3). The cells are flattened compared to cells on the inside of the sponge. The nuclei of the exopinacocytes are oval in shape, lack a nucleolus, are fairly centrally located, and appear to span nearly the entire height of the cells. In Fig. 2 there is also seen an eosinophilic, fibrous, non-cellular layer external to the exopinacoderm. This may act as a cuticle in protecting the sponge from its environment (Bergquist, 1978). The cells appear to be about 3-5 μm x 15-20 μm.

The endopinacocytes are evident in Fig. 4. They are long, flattened cells with a centrally located nucleus. Some cellular extensions are present. Overlap between adjacent cells is minimal. The cells dimensions look to be 5-7 μm x 20 μm. Probable spongin is also seen in Fig. 4.

As shown in Fig. 5, choanocyte chambers in *S. lacustris* consist of a ring of choanocytes. The flagella are seen extending from each choanocyte into the middle of the chamber. A collar composed of microvilli which cannot be resolved (Bergquist, 1978) can be seen on some choanocytes where the flagellum comes off of the individual choanocyte. The nuclei of the choanocytes, although somewhat difficult to discern, appear to be spherical in shape and near the outside of the chamber. An apopyle and prosopyle are seen in Fig. 6. The apopyle is evident by the large opening in the chamber, with a cone cell on either side of the opening. The prosopyle is merely a gap between adjacent choanocytes. The chamber seen in Fig. 7 has canals on either side of it. One is probably the incurrent canal and the other the excurrent canal, but a positive identification is not possible in this case. Fig. 7 shows that many of these such chambers exist in the sponge.

Cells and Inclusions of the Mesohyl

The mesohyl of *S. lacustris* contains many different types of cells. The most obvious is the archaeocyte (Fig. 8). The cell is quite large (about 7-13 μm) with a somewhat irregular shape and a prominent nucleolus. The nucleus is located centrally with eosinophilic granules surrounding it in the cytoplasm.

The lophocyte, a collagen-secreting cell in the mesohyl, is a large cell (about 7-12 μm) in comparison to other cells in the sponge (Fig. 9). The cell is spherical in shape, with a granular cytoplasm. The nucleolus is also extremely easy to make out. The tail of collagen that the cell is secreting is shown in Figs. 9 and 10.

Spongocytes can be distinguished in the sections by the fact that they exist in aggregates (Fig. 11). These particular cells are aggregated around a spicule. The cells are irregular in shape (diameter 4-5 μm), and their cytoplasms contains many dark elements believed to be precursors of the fibrous material they secrete (Bergquist, 1978). There are also extensions coming from these cells (Bergquist, 1978). One might confuse them with sclerocytes since they too cluster around spicules, but sclerocytes are spherical and lack the extensions coming from the cell (Bergquist, 1978).

The fibers in the mesohyl that make up the skeleton are evident in Figs. 12 and 13. They are usually long and "stringy" and are found in many parts of the sponge.

Sexual Elements

Fig. 14 shows a structure repeatedly found in sponges collected between June 25 and July 17 that appears to be a spermatogenic cyst. The cysts are composed of a large number of small cells with diameters of about 1-2μm each (Fig. 14). The nuclei are much smaller than the nuclei of any of the cells that are seen around the cysts, suggesting that they are developing sperm (Fig. 15). Flagella extending from some of the spermatids can be seen in Fig. 14. As is seen in both Figs. 14 and 15, there are 4 cysts fused together. Although a large number of sponges collected during this time frame contained such spermatogenic cysts, the cysts were rare in sponges collected before and after this period. This finding suggests that in *S. lacustris*, spermatogenesis occurs mainly between mid-June and mid-July.

An egg was observed in a sponge collected on June 8 (Fig. 16). This cell is extremely large (40-50 μm in diameter) compared to any other cell. The egg stained intensely with eosin, most likely due to the presence of many granules and yolk existing in the cytoplasm. The cell looks like an oversized archaeocyte with the nucleus set off to one side. An indistinct follicular membrane surrounds the egg. No spermatogenic cysts were present in this particular sponge.

Three unusual structures in a sponge collected on June 25 appear to be developing larvae. Fig. 17 shows one such embryo in which a portion of the epithelium is torn. A larval cavity can be seen, as well as ciliated epithelium surrounding the larva. This layer of cells appears to be a simple cuboidal epithelial layer. No follicular epithelium is evident in this micrograph, however. Trophocytes that are associated with the developing larva are not present, although others have recorded their presence (Saller and Weissenfels, 1985). It appears as if this larva is still in the mesohyl. The structure in the lower portion of Fig. 17 also appears to be a larva. The cavity in the middle may be a larval cavity.

A possible larva at an earlier stage of development can be seen in Fig. 18. The specimen from which it was found also was collected on June 25. The structure consists of a mass of yolk-filled cells surrounded by a membrane. Each irregularly shaped cell consists of a yolk-filled cytoplasm and a nucleus. At first glance, this mass of cells appears to resemble a spermatogenic cyst, but the cells in the larva are distinctly larger then the cells of the spermatogenic cyst and are also more eosinophilic then cysts.

The findings of this research are in agreement with the timings of gametogenesis and larval development of the works done by Gilbert and Simpson (1973), Paulus (1989), and Saller and Weissenfels (1985).

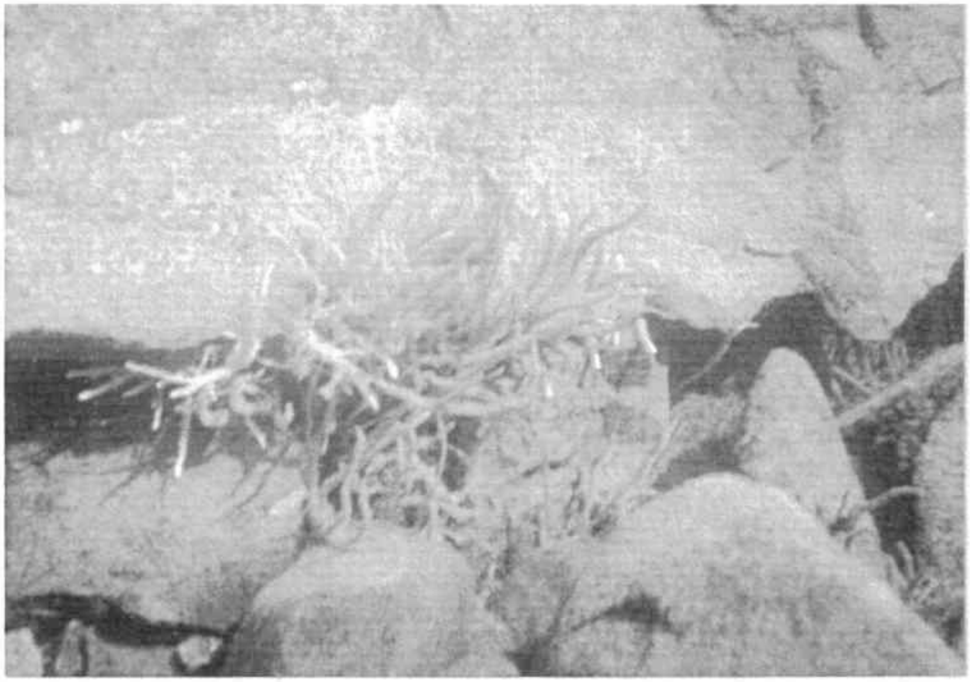

Fig. 1. ***S. lacustris*** **in Salmon Lake exhibiting finger-like growth patterns.**

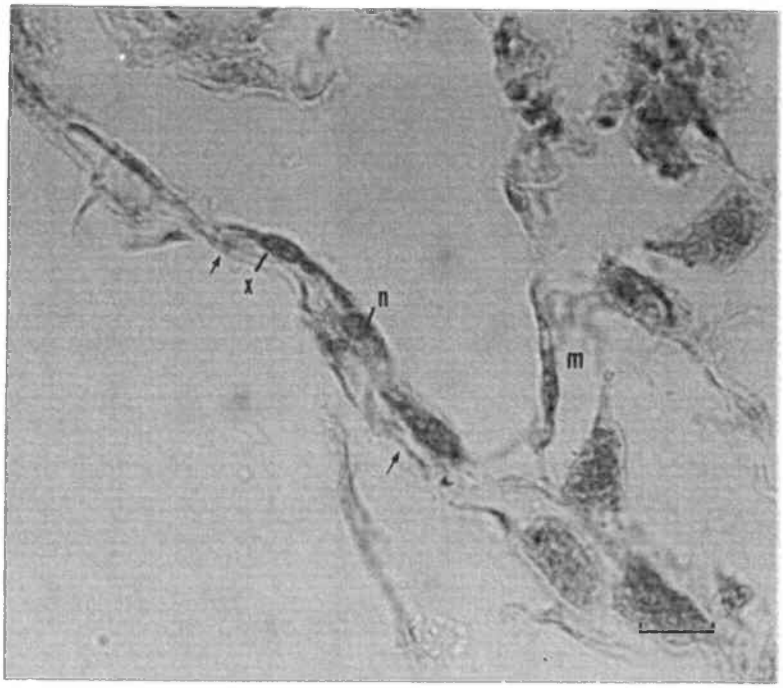

Fig. 2. **Exopinacocytes and cuticle of the exopinacoderm.** x, exopinacocyte; arrows, cuticle; m, mesohyl; n, nucleus. Bar = 10 μm

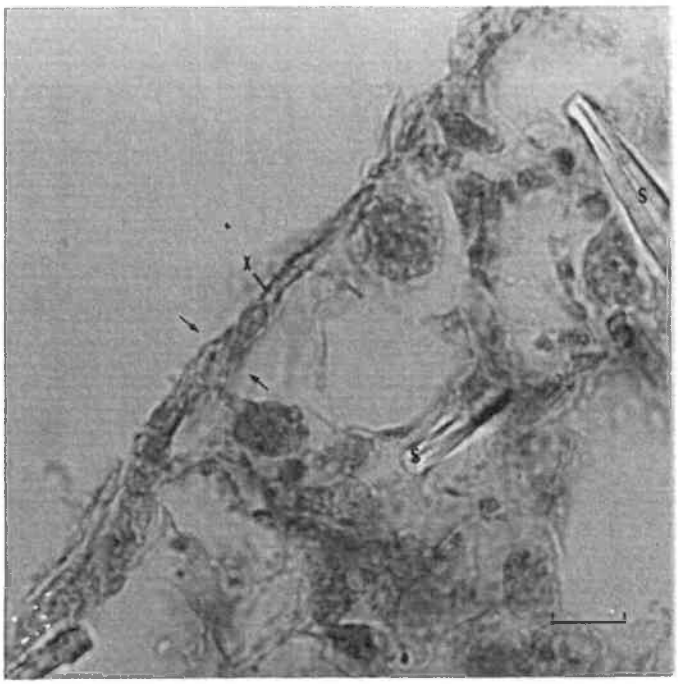

Fig. 3. **Overlapping exopinacocyte of the exopinacoderm.** x, exopinacocyte; arrows, zone of overlap; s, spicules in the mesohyl. Bar = 10 μm.

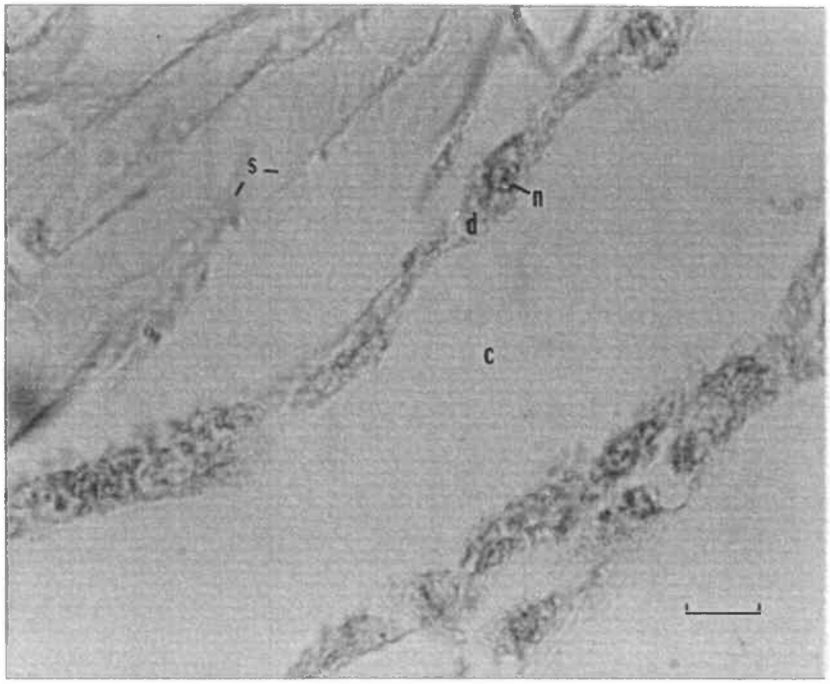

Fig. 4. Endopinacocytes. d, endopinacocyte; c, canal; n, nucleus; s, probable spongin. Bar = 10 μm.

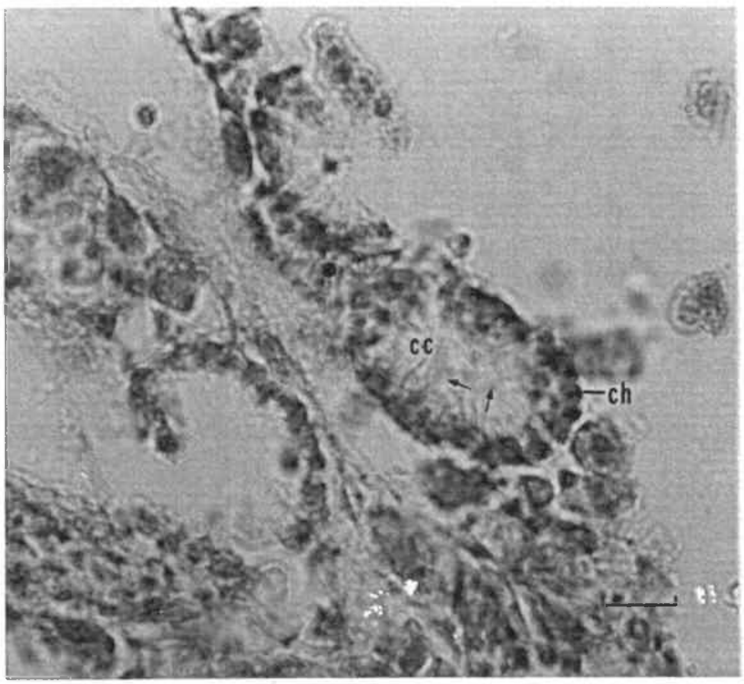

Fig. 5. Choanocyte chamber. cc, choanocyte chamber; arrows, flagella; ch, choanocyte. Bar = 10 μm.

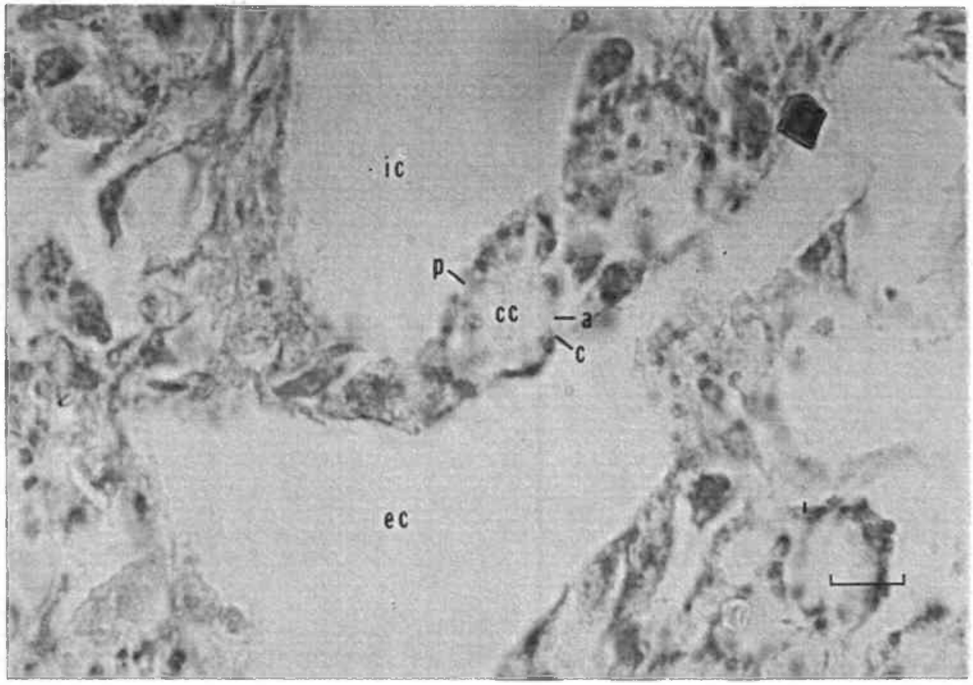

Fig. 6. Apopyle and prosopyle of a choanocyte chamber. cc, choanocyte chamber; a, apopyle; p, prosopyle; ic, incurrent canal; ec, excurrent canal; c, cone cell. Bar = 10 μm.

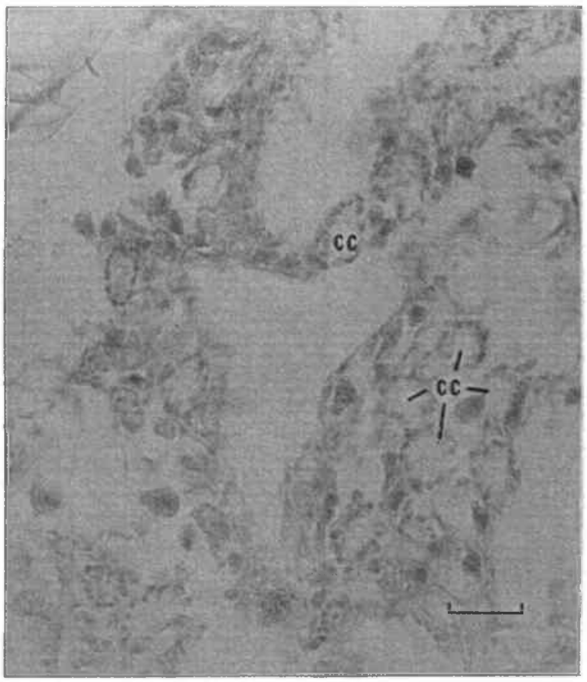

Fig. 7. Choanocyte chambers of *S. lacustris*. cc, choanocyte chambers. Bar = 50 μm.

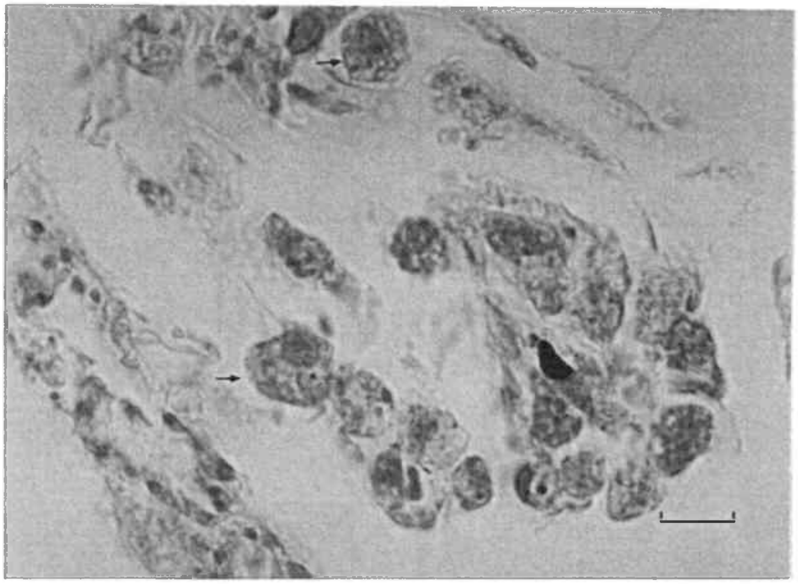

Fig. 8. Archaeocyte in the mesohyl. Arrow, archaeocyte. Bar = 10 μm.

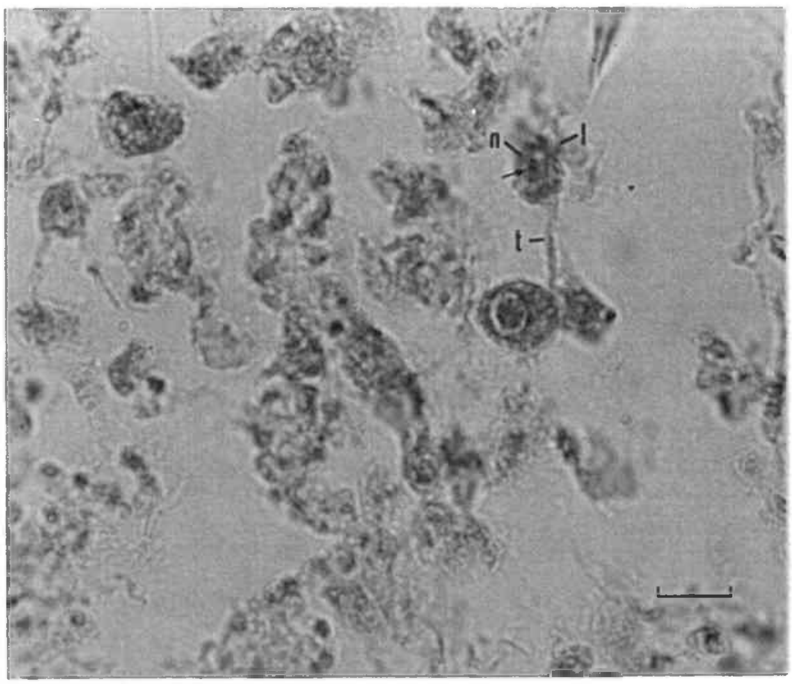

Fig. 9. Lophocyte in the mesohyl. l, lophocyte; n, nucleus; arrow, nucleolus; t, tail. Bar = 10 μm

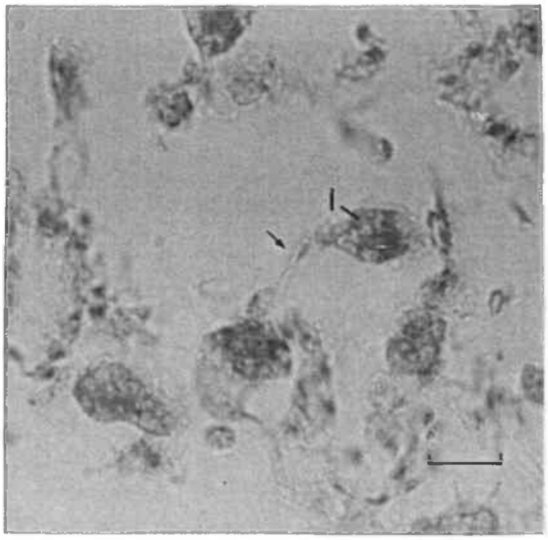

Fig. 10. Lophocyte with associated tail. l, lophocyte; arrow, tail. Bar = 10 μm.

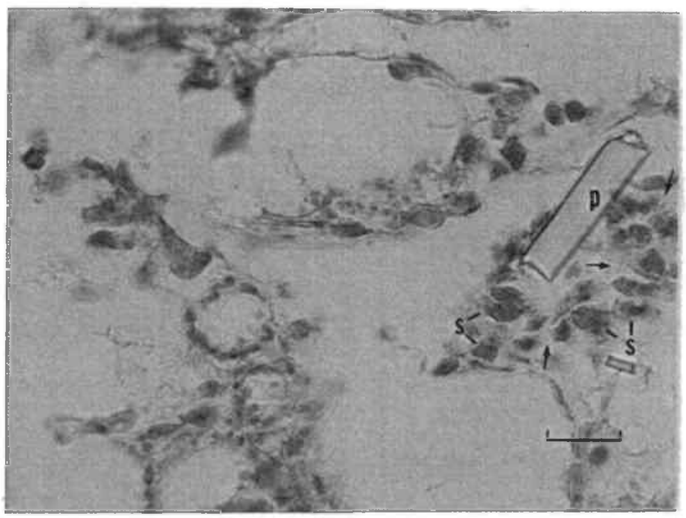

Fig. 11. Aggregate of spongocytes surrounding spicule. s, spongocyte; arrows, cellular extensions; p, spicule. Bar = 10 μm.

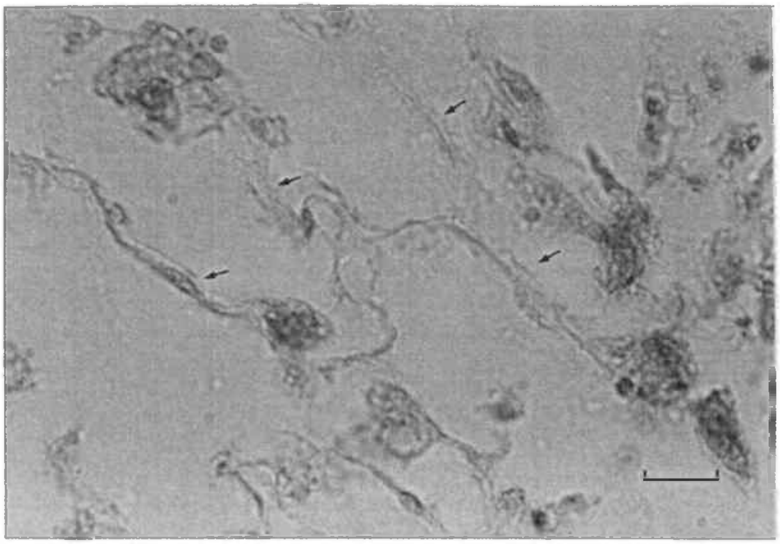

Fig. 12. Fibers of the mesohyl. Arrows, fibers. Bar=10 μm.

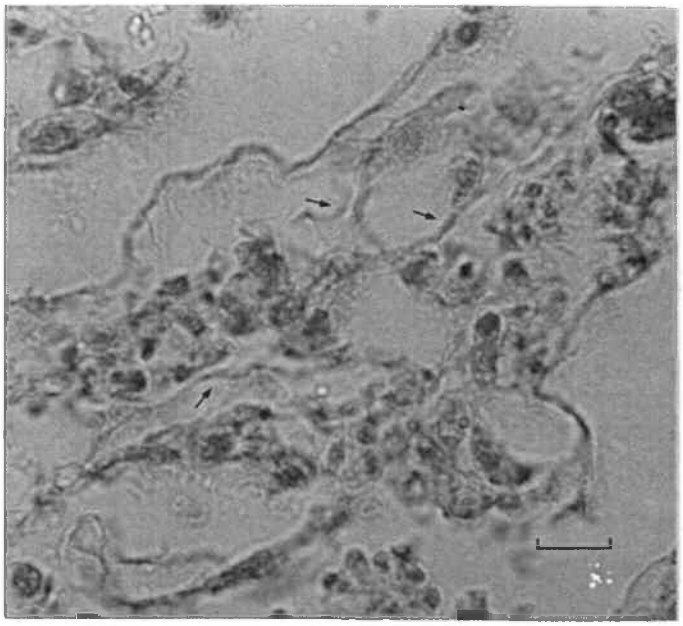

Fig. 13. Fibers of the mesohyl. Arrows, fibers. Bar=10 μm.

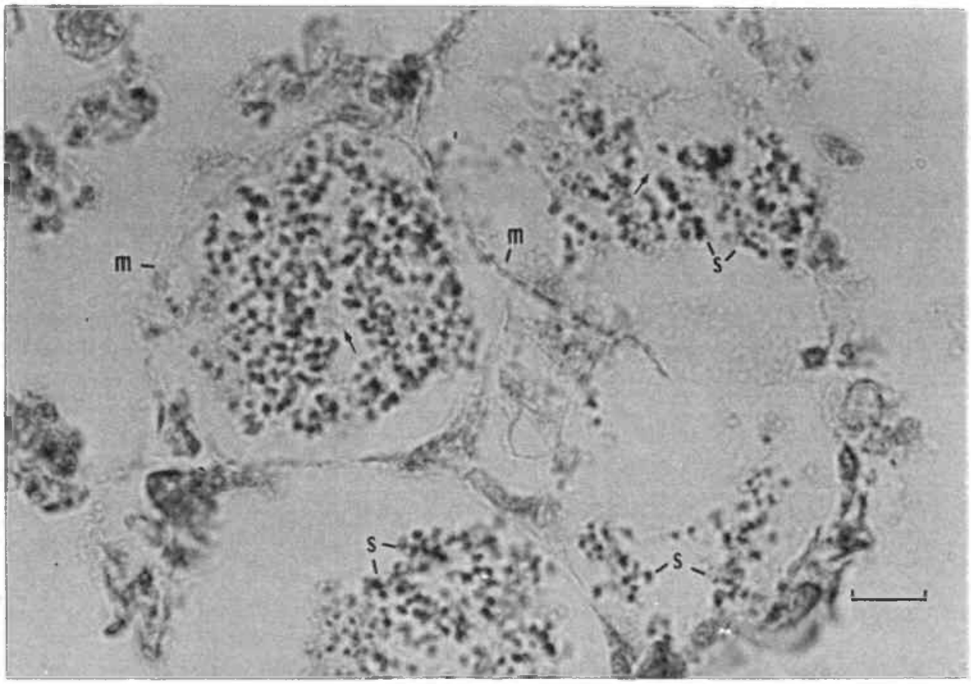

Fig. 14. Four partially fused spermatogenic cysts (June 25). m, membrane; arrows, flagella; s, spermatid. Bar = 10 μm.

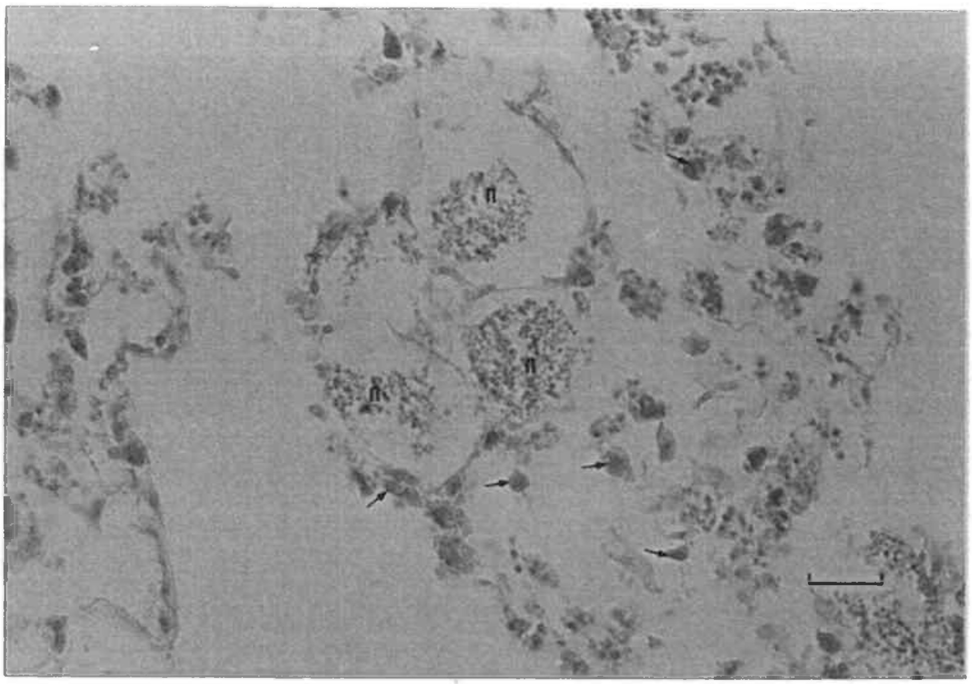

Fig. 15. Comparison of nuclei between cysts and other cells. n, nuclei of spermatogenic cyst; arrows, nuclei of cells. Bar = 50 μm.

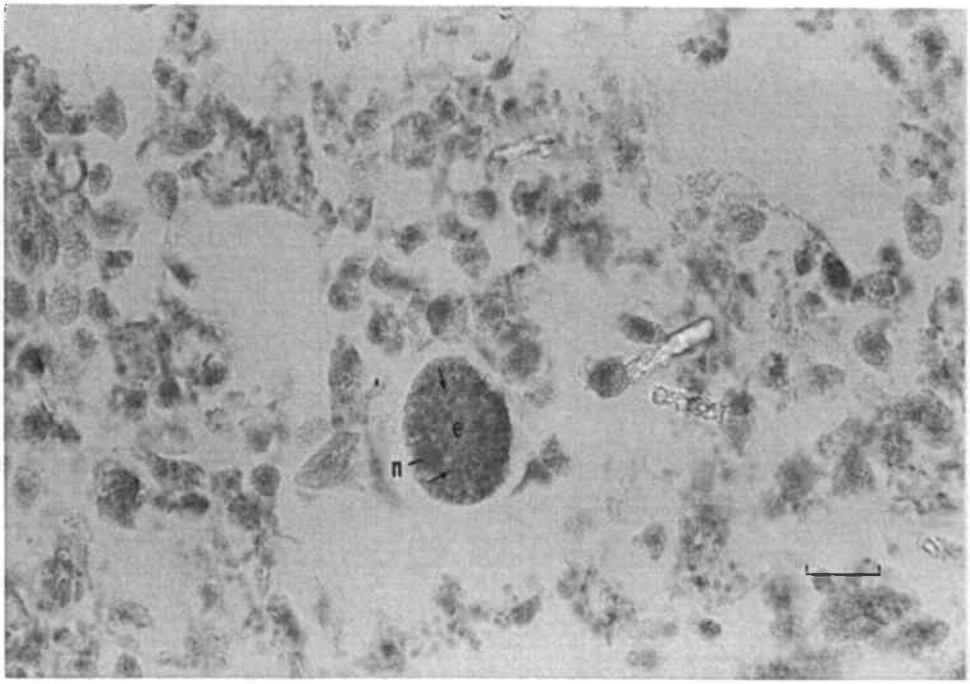

Fig. 16. Immature egg. (Sponge collected on June 25). e, egg; n, nucleus; arrows, granules. Bar=25 μm.

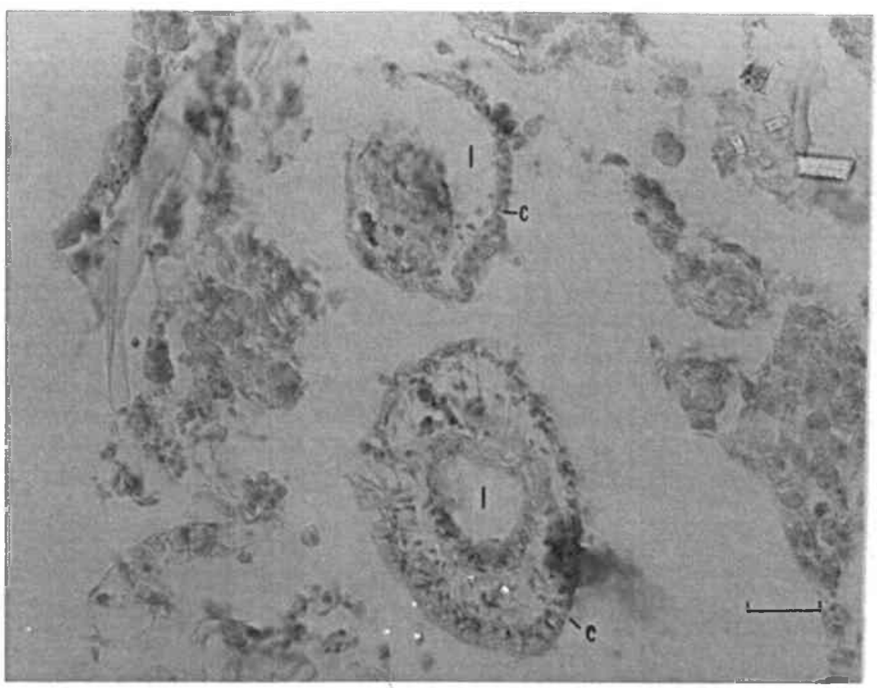

Fig. 17. Larva. (Sponge collected on June 25). l, larval cavity; c, ciliated epithelium. Bar=25 μm.

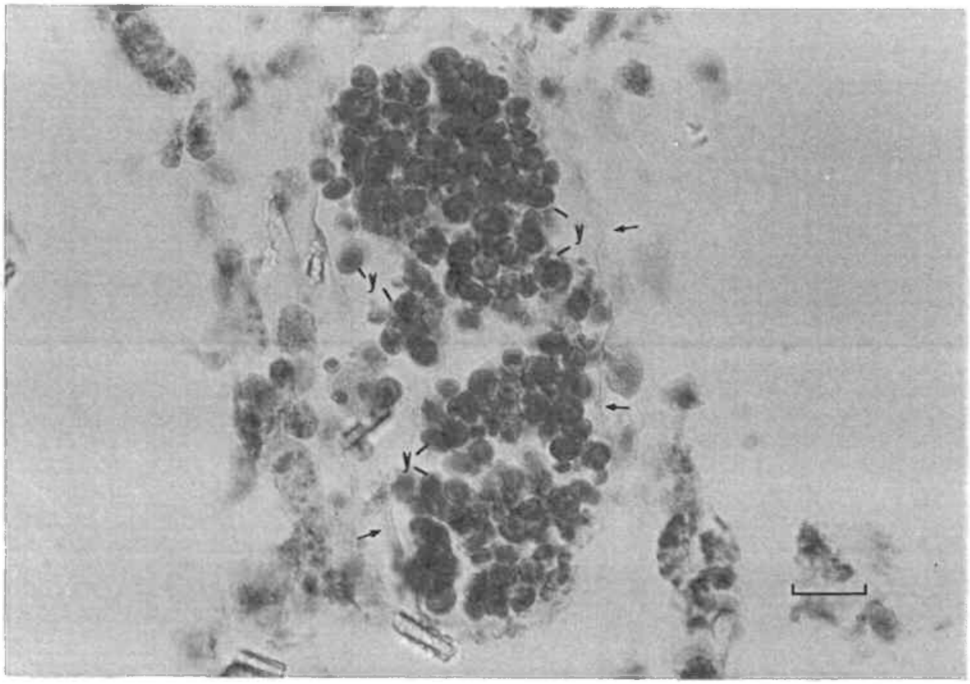

Fig. 18. Developing embryo. (Sponge collected on June 25). y, yolk-filled cells; arrow, membrane. Bar = 10 μm

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Appendix A

Procedure for dehydrating, clearing, and embedding in Paraplast (from Humanson, 1962.)

1. **After fixation with Bouin's fixative, wash with a 0.2 M sodium phosphate buffer, pH 7.2/6.9% sucrose, six times, 10 min each.**

2. **Place in 5% hydrofluoric acid for 2 hr.**

3. **Wash with 0.2 M sodium phosphate buffer pH 7.2/6.9% sucrose, two times, 10 min each.**

4. **Dehydrate with ethanol at room temperature in the following manner:**

   50%, 0.5-1 hr
   70%, 1 hr
   95%, 1-1.5 hr
   95%, 1-1.5 hr
   100% 0.5-1 hr
   100% 0.5-1 hr

5. **Clear with xylene, two changes, 0.5-1 hr each.**

6. **Infiltrate with liquid Paraplast, two changes, 0.5-1 hr each.**

7. **Embed in Paraplast:**
   A. **Pour small amount of molten paraplast into embedding mold and allow to harden**
   B. **Transfer specimen into mold**
   C. **Fill mold with molten paraplast and allow to cool and harden**

Note: Do not leave the tissue in any of the reagents for longer then the time specified with the sole exception of the 70% EtOH, in which the tissue can be allowed to set overnight but not longer.

Appendix B

Procedure for the hematoxylin and eosin staining (from Sheehan and Hrapchak, 1973, and Raphael et al., 1976)

1. **Deparaffinize in three changes of xylene, 3 min each.**
2. **Hydrate through graded alcohols as follows:**
   A. **100% ethanol, 2 min**
   B. **95% ethanol, 2 min**
   C. **80% ethanol, 2 min**
   D. **70% ethanol saturated with lithium carbonate, 2 min**
   E. **70% ethanol, 2 min**
   F. **Gently running tap water, 1 min**
   G. **Distilled water, 1 min**
3. **Stain in Delafield's Hematoxylin, 10 min.**
4. **Dip in tap water, 10 sec.**
5. **Decolorize in 1% concentrated hydrochloric acid-ethanol (1% conc. hydrochloric acid in 80% ethanol) mixture, dip two-three times, 10 sec each.**
6. **Rinse in two changes of tap water, 20 sec each.**
7. **Intensify blue color by dipping in 1% lithium carbonate, 30 sec.**
8. **Wash in tap water, two changes, 3 min each.**
9. **Counterstain in 1% aqueous eosin Y, 1-2 min.**
10. **Rinse in tap water, 30 sec.**
11. **Dehydrate in absolute ethanol, three changes, 30 sec each.**
12. **Pass through two changes of xylene, 20 sec each.**
13. **Mount in Permount.**

Note: In step 9, dipping for 2 min provided the best results.