Comparative Study of Spiculogenesis in Demosponge and Hexactinellid Larvae

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KEY WORDS spicules; hexactinellid sponges; skeleton; silica deposition; sclerocytes *Reniera; Oopsacas*

ABSTRACT Spicule deposition was studied by electron microscopy in fixed embryos and larvae of the haplosclerid sponge *Reniera* sp. and the hexactinellid *Oopsacas minuta*. Spicules form in centrally located vacuoles within cells and within syncytia, as in the adult sponges. In *Reniera*, scleroblasts differentiate from micromeres prior to gastrulation. At gastrulation the scleroblasts migrate to the periphery of the embryo and commence spicule deposition around a hexagonal axial filament. Sclerocytes have numerous pseudopodia and migrate to the posterior pole where they become aligned along the antero-posterior axis in the free-swimming larva. *Reniera* larvae possess some 40–50 oxeas, each 70–75 μm long and 1 μm wide. Mature *Oopsacas* larvae have up to 14 stauractin spicules, which are produced on a rectangular axial filament in tissues that lie under the smooth epithelium at the posterior pole of the larva. Young sclerocytes have many pseudopodia. The 4-rayed spicules elongate along both the antero-posterior and medial axes, until the longitudinal rays become anchored in a lipid-filled cytoplasm at the anterior of the larva and the lateral rays intersect around the midline. The length of the transverse rays of the stauractins in free-swimming larvae are 27–45 μm each, while the length of the two longitudinal rays is 40–80 μm. Although spicule deposition begins in cells with a similar morphology in both cellular and syncytial sponges, the elaboration and organization of the spicules differ markedly in cellular and syncytial sponges and appear to be an outcome of the very distinct cellular differentiation and larval morphogenesis that occur in each of these groups. *Microsc. Res. Tech. 62:300–311, 2003.*

INTRODUCTION

Despite their unusual body plans and lack of discrete tissues, sponges are remarkably capable of secreting vast quantities of mineral and organic skeletons. The sponge skeleton is generally considered to be a genetically heritable feature, and as such has long been used as the primary taxonomic character in sponge systematics, even though spicules can show quite a range of sizes and abundance in the same species in different environments (Palumbi, 1986) and over a geographical range (e.g., Kennedy, 1999). In the search for additional taxonomic tools, some spongologists have turned to larval characteristics (e.g., Wapstra and van Soest, 1987), but surprisingly, the development and morphology of larval spicules is rarely considered.

The term skeleton implies a role in support of the adult organism, and although some sponges lack either a mineral or organic skeleton, a relationship between environmental stress and strength of sponge skeletons has been shown (Wilkinson, 1979). The same assumptions of genetic heritability of spicule size, morphology, and arrangement presumably also apply to larvae; however, the role of spicules in these reproductive propagules is not so clear. First, although many sponge larvae possess spicules, there are almost as many species that only secrete spicules at metamorphosis. A summary of studies reporting spicule occurrence in sponge larvae is presented in Table 1. Notably, spicules have not been reported in any calcareous sponge embryos or larvae (Tuzet, 1973). Second, of those sponge larvae that have spicules, some develop them during embryogenesis and larval development, while others only develop spicules after release from the parent sponge. Finally, all histological studies of larval spiculogenesis note that larval spicules typically differ from those of the adult. Spicules may have a different morphology and tend to be up to half the size of the adult spicules. In sponges with a variety of spicule types, larvae often lack some of the adult spicules; in particular, microscleres are frequently absent. Furthermore, in only a few larvae are the spicules already embedded in spongin, a secondary organization of the skeleton that is a permanent structure in the juvenile (e.g., Saller and Weissenfels, 1985). In most larvae the spicules are either randomly distributed or are aggregated at the posterior pole, and in no way illustrate the characteristic architecture of the skeleton of the adult sponge.

Historically, sponge larvae have been grouped into four general morphological categories (Fig. 1): the amphiblastula, the coeloblastula, the parenchymella, and the trichimella of hexactinellid sponges. Recently, ad-

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TABLE 1. Histological studies documenting the occurrence, kinds, and position of spicules in sponge larvae

Sponge[1]	Spicules present: In embryo	Spicules present: In larva	Spicule size/type compared to adult	Position of spicules in embryo and larva	Reference
Demospongiae					
Subcl. Ceractinomorpha					
Halichondrida					
Halichondria bowerbanki	Y	Y	Oxeas, 120–150μm (half the length of adult spics)	Mid-posterior; bundled; lie parallel to longitudinal axis	Hartman, 1958
H. bowerbanki[2] *[coalita]*	Y	Y		Form in macromeres under outer layer of ciliated cells Cent/Post location in larva	Meewis, 1941
H. moori	None	None			Bergquist and Green, 1977
H. melanodocia	None	None			Woollacott, 1990
H. panicea		Y			Wapstra & Van Soest, 1987
Axinella crista-galli		Y	'needle-like'	Dispersed, but more at PP.	Maas, 1894
Poecilosclerida					
Myxilla rosacea	Y	Y	Large oxeas; some microscleres	At periphery under ciliated layer; later bundled at PP.	Maas, 1894
Mycale sp.	Y	Y	Both macroscleres and microscleres	Form in outer layer	Reiswig, 1971
M. macilenta		Y	All adult macroscleres, same size as in adults. No sigmas	Subtylostyles - central, aggregated Anisochelae-PP., peripheral Toxas - central; ant. to subtylostyles	Bergquist and Sinclair, 1968, 1973
M. sordida[3] *[Esperella sordida]*		Y	Very thin	PP, aggregated	Delage, 1892
M. lorenzi [Esperia lorenzi]		Y	Both macroscleres and microscleres	PP, aggregated along A-P axis	Brien, 1973
M. contarenii		Y		PP, in a ring	Lévi, 1964
Clathria coccinea[3] *[Microciona coccinea]*		Y	All macroscleres, except styles; toxas and subtylostyles very rare; great size variation	PP, aggregated	Bergquist and Sinclair, 1968, 1973
C. prolifera[3] *[Microciona prolifera]*		Y	Styles - one size; Lumpy styles, no acanthostyles; some lack toxas	Center, dispersed; some at periphery	Simpson, 1968
Desmacidon fructicosa		Y	Same size and type as adult: oxeas, sigmas and chelas	Not stated	Lévi, 1956
Pronax plumos		Y	Acanthostyles, some already in spongin	PP, dispersed	Lévi, 1956
Haplosclerida					
Haliclona sp.		Y	Oxeas (≅60μm)	PP, aggregated	Bergquist and Sinclair, 1968
H. (Haliclona) ecbasis[3] *[H. ecbasis]*	Y	Y	Oxeas (≅ 60μm)	First at periphery; then aggregated at PP,	Fell, 1969
H. (H.) limbata[3] *H. limbata*	N	Y	Smaller than H. ecbasis	PP, dispersed	Meewis, 1939
H. (H.) xena[3] *[H. xena]*		Y			Wapstra and van Soest, 1987
H. (H.) cinerea[3] *[Adocia cinerea]*	Y	Y	Less "developed" than maternal spicules	First in micromeres at periphery Later lie under the columnar epithelium at PP	Meewis, 1941
H. (H.) simulans[3] *[Adocia simulans]*	Y	Y	'small'	PP	Lévi, 1956
H. (Gellius) caerulea[3] *[Sigmadocia caerulea]*	Y	Y	Styles - one type Oxeas - shorter and thinner Lumpy styles, no acanthostyles	PP, aggregated	Maldonado et al., 1997
H. (Gellius) varius[3] *Gellius varius*	Y	Y	Only macroscleres	PP, aggregated, but not bundled	Maas, 1894
H. (Reniera) densa[2] *Reniera densa*		Y	Few, very small spicules	PP	Delage, 1892
Chalinula sp.[3] *[Haliclona loosanoffi]*	Y	Y		At periphery; then move to PP	Fell, 1976
C. fertilis	Y	Y		At periphery, toward PP	Maas, 1894
Ephydatia fluviatilis	Y	Y	≅ 100μm	Central, dispersed	Brien and Meewis 1938; Saller and Weissenfels, 1985
Spongilla lacustris	Y	Y			Saller, 1988
Hexactinellida					
Farrea sollasii	Y	Y	Stauractins Discohexasters without terminal ends	At periphery; full length of larva At dermal and gastral surfaces	Okada, 1928
Oopsacas minuta	Y	Y	Stauractins	At periphery; full length of larva	Boury-Esnault et al., 1999
Vitrollula fertilis	Y	Y			Ijiima 1901

[1]Revised name; name originally published under is given in parentheses.
[2]Revised name as published by Wapstra and van Soest, 1987.
[3]Revised name: JNA Hooper, Queensland Museum, 2002 pers. commun.

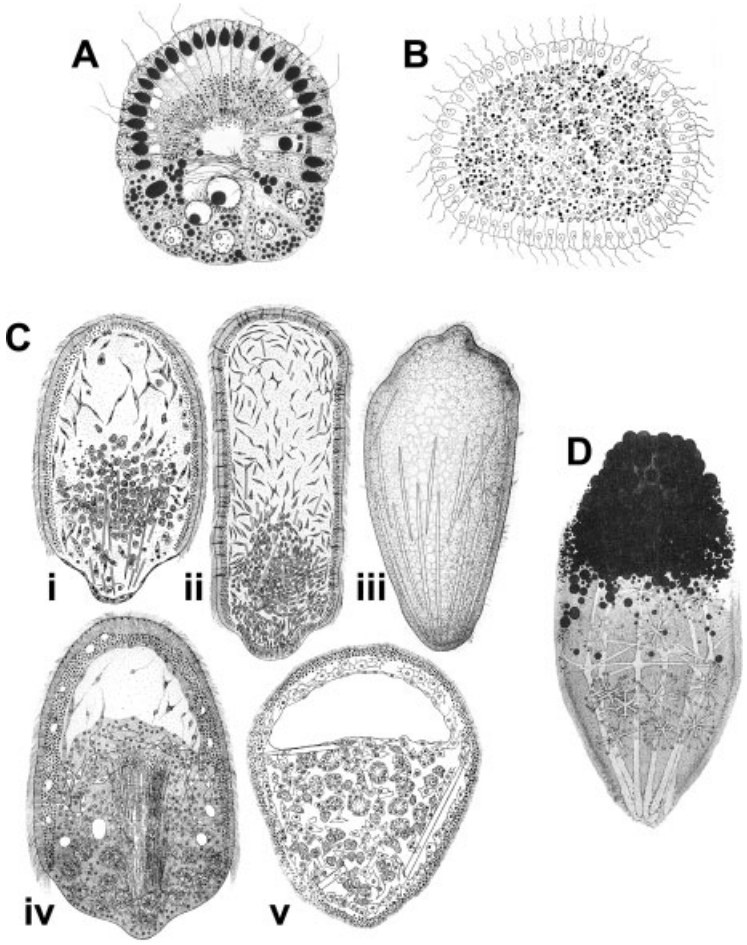

Fig. 1. General morphologies of sponge larvae (**A–C:** after Brien, 1973; **D:** after Okada, 1928). **A:** Amphiblastula (*Sycon raphanus*). **B:** Coeloblastula (*Tethya aurantium*). **C:** Parenchymella; i. *Myxilla rosacea*; ii. *Axinella crista-galli*; iii. *Corvospongilla thysi*; iv. *Mycale* (=*Esperia*) *lorenzi*; v. *Spongilla lacustris*. **D:** Trichimella(*Oopsacas minuta*).

ditional morphologies of larvae have been described (e.g., the cinctoblastula of homoscleromorph sponges (see Maldonado and Bergquist, 2002), and the hoplitomella of the Alectonidae (Vacelet, 1999)). Spicules are only known from parenchymella and trichimella larvae and the unusual, gemmule-like, armored hoplitomella.

As most sponge larvae are brooded, embryogenesis must be interpreted from static images of embryos fixed and sectioned at various, uncertain, stages of development. Details regarding cellular differentiation—for example of sclerocytes—in sponge larvae are therefore the subjective interpretations of each author. In the late nineteenth and early twentieth century, numerous excellent histological studies of sponge embryogenesis were conducted. Relatively few of these results have been confirmed with electron microscopy, and remarkably there are no ultrastructural studies that have examined spiculogenesis in sponge larvae. The present article provides the first ultrastructural examination of spiculogenesis in the larvae of two siliceous sponges, the parenchymella of the demosponge *Reniera* sp., and the trichimella larva of the hexactinellid *Oopsacas minuta*. The results are discussed with respect to earlier descriptions of spicule formation and of their proposed function in sponge larvae.

MATERIALS AND METHODS

Sexually mature individuals of *Reniera* sp. (Chalinidae, Haplosclerida, Demospongiae) were collected in the intertidal zone, from Heron Island Reef, Australia; mature individuals of *Oopsacas minuta* Topsent, 1927 (Leucopsacasidae, Lyssacinosida, Hexasterophora, Hexactinellida) were collected by scuba from a submarine cave near Marseille, France. The North Atlantic genus *Reniera* has previously been called both *Haliclona* and *Adocia*, and although taxonomists have transferred the genus *Reniera* to *Haliclona* (de Weerdt, 1986), the Pacific species of these genera have not been revised recently.

Larvae were released from all sponges when sponge pieces were left in still seawater at room temperature for 30 minutes. Sponge pieces—containing developmental stages of embryos and larvae—and larvae were fixed and processed for light and electron microscopy as described in Leys and Degnan (2002). Preparations of larval spicules were made following the procedure of Hooper (1998). The larva was placed on a round glass coverslip and the tissue was dissolved in HNO_3 over an alcohol flame. The coverslip was mounted on an electron microscope stub, coated with gold in an Edwards S150B sputter coater, and viewed in a Hitachi S-3500N

Scanning Electron Microscope at the University of Victoria, British Columbia.

RESULTS

Observations of Spicule Formation in *Reniera* sp. Larvae

Spicules are produced during early embryogenesis in *Reniera*. Spicule production is first evident in small cells that lie just below the smooth plate-like cells of the maternal follicle in the blastula (Fig. 2A, inset). Other cells at this stage are fairly uniform in size and have many yolky inclusions. Sclerocytes appear to be among the earliest cells to differentiate in the embryo and are identified by the presence of a small vacuole containing a hexagonal proteinaceous filament, 0.5 μm in diameter (Fig. 2B). Spicule deposition appears to be rapid. In embryos that have initiated gastrulation (ciliated micromeres dominate the periphery of the embryo), sclerocytes lie among the ciliated micromeres and have a vast central vacuole, which contains a large amount of osmiophilic amorphous material and a hexagonal axial filament (Fig. 2C–F). There is only one cell associated with each central vacuole. As the cells of the interior of the embryo continue to differentiate, a thick collagenous extracellular matrix (ECM) is formed. The sclerocytes develop numerous pseudopodia and migrate towards the posterior pole of the larva (Fig. 2E,F).

Prior to its release from the brood chamber, the embryo elongates along the antero-posterior axis. In the free-swimming larva the spicules remain intracellular and occupy a very small region at the posterior pole (Fig. 3A). Longitudinal sections of the *Reniera* larva show three cell layers. Uni-ciliated columnar epithelial cells and scattered mucous-like cells form the outer layer. Underlying the epithelial layer is a layer of cells, termed subepithelial cells, which is arranged perpendicular to the columnar epithelial cells. This layer forms a sheath around the larva except at the posterior end, where sclerocytes and other cells of the interior are in contact with large mucous-like cells that form the posterior pole. In addition to sclerocytes, there are three differentiated cell types in the interior of the larva, all of which are surrounded by a thick layer of collagen fibers. The anterior pole of the larva is bare and is formed by large cells filled with small vesicles. At the posterior pole of the larva the sclerocytes become elongate (Fig. 3B,C). Some sclerocytes at this stage stain darkly (Fig. 3B); all contain a large axial filament, 0.5 μm in diameter within the narrow 1 μm wide space left by the spicule after desilicification (Fig. 3C); and all sclerocytes still have numerous pseudopodia. The larval spicules are long and thin in *Reniera*: the oxeas range in length from 70–75 μm and are 1 μm wide (Fig. 3D).

Observations on Spicule Formation in *Oopsacas* Larvae

The early stages of spicule formation in *Oopsacas* larvae are not easily detected. The first evidence of sclerocyte differentiation is the presence of a vacuole containing a rectangular axial filament in what appear to be discrete cells with many mitochondria and clear vesicles at the posterior pole of the embryo (Fig. 4A,B,E,F). These cells lie between the outer syncytial epithelial layer and the multinucleated yolk-filled central tissue. The cells have numerous pseudopodial extensions (Fig. 4C,D). The axial filament measures 0.3–0.5 μm in diameter.

In late embryos, spaces left by desilicification of spicules are much larger (Fig. 5A). In some regions the cytoplasm of the sclerocyte appears to have open bridges to other tissues (e.g., Fig. 5A, black arrowhead), and in other regions the sclerocyte is connected to other tissues by perforate plugged junctions (Fig. 5B,C). Embryos at this stage already have several types of multinucleate tissues, a skirt of multiciliated cells, and flagellated chambers (Fig. 5D). Although sections of these cells show only one nucleus, because the embryos are only 50–70 μm in diameter at this stage, it is possible that additional nuclei are present but not in the same plane of section. In late embryos and free-swimming larvae, four-rayed spicules—stauractins—are well developed and lie in multinucleate tissues just under the multiciliated cells and syncytial epidermis (Fig. 5D).

Only one larva survived the treatment with HNO_3 intact. In that mature larva there were 14 four-rayed stauractin spicules (Fig. 6A). The long rays are oriented along the antero-posterior axis of the larva with the short, or transverse, rays overlapping each other in the posterior third of the larva. The two transverse rays are nearly equal in length, each measuring 27–51 μm (average length, 45.8 μm, n = 7). In the single larva in which the anterior–posterior polarity was evident by the yolk remnants, the anterior longitudinal rays of some spicules appear shorter than the posterior rays. The rays at the posterior end of the larva are 60–81.5 μm long (mean, 70 μm, n = 11), while the one measurable anterior ray is 40 μm long (Fig. 5A). As the anterior ends of most rays were embedded in the yolk mass, their length could not be determined. However, the anterior and posterior rays of stauractins from other embryos and larvae were the about same length (45 and 50 μm each). Unless the two rays grow at unequal rates in late embryos and larvae, it is likely that all the rays are more or less of equal lengths. All of the spicule rays are only 1 μm wide. The edges of the oxystauractins have very small spines along their length (Fig. 6B), but are smooth at their tips (Fig. 6C). Together, the spicules appear to form a scaffold for the syncytial tissues in which they lie, with one end of the longitudinal rays embedded in the yolk-filled cytoplasm at the anterior pole, the other ends meeting at the posterior pole of the larva. No microscleres are evident either in thin section or in spicule preparations examined by scanning electron microscopy.

DISCUSSION

Despite rudimentary similarities in spiculogenesis between the two representative siliceous sponge larvae studied here, there are several fundamental differences between demosponges and hexactinellids with respect to differentiation of scleroblasts, morphology of the axial filament, and organization of the spicule skeleton in the embryo and larva.

Differentiation of Scleroblasts

Sclerocytes are among the earliest cells or tissues to differentiate in *Reniera*. Precocious differentiation of

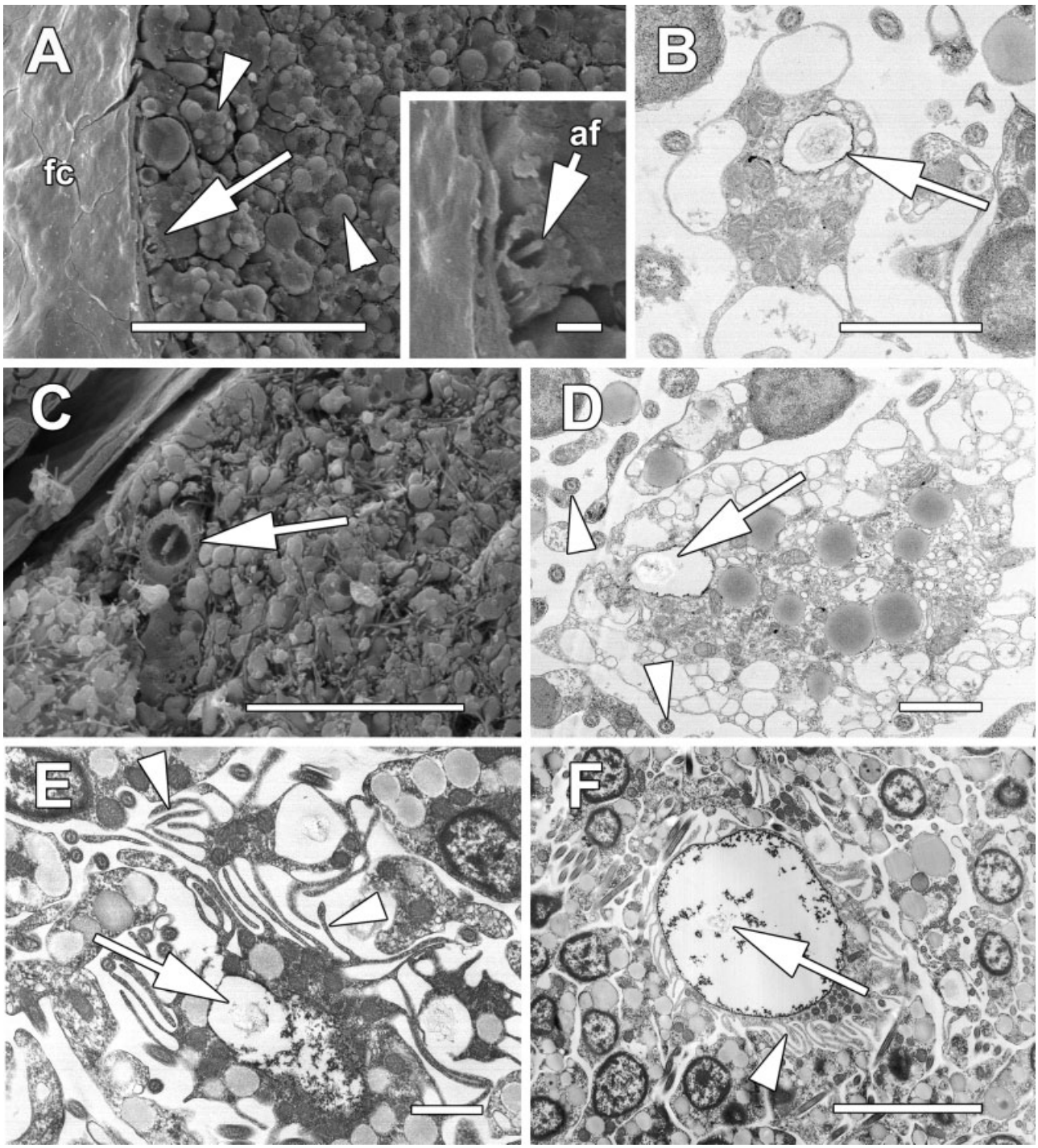

Fig. 2. Spicule formation in *Reniera* sp. **A:** Scanning electron micrograph (SEM) of a blastula-stage embryo that has been desilicified and fractured in liquid nitrogen. A small sclerocyte (arrow) lies at the periphery of the embryo; all other cells have yolk inclusions (arrowheads). Fc, maternal follicle cells. Inset: magnification of the sclerocyte, vacuole, and axial filament (af, arrow). **B:** Transmission electron micrograph (TEM) of a young sclerocyte in an embryo at the late blastula stage that can be identified by the darkly staining silicalemma (arrow) and a hexagonal axial filament. **C:** SEM of a sclerocyte (arrow) surrounded by ciliated micromeres in a gastrula-stage embryo. **D:** TEM of a sclerocyte in a gastrula-stage embryo showing the hexagonal axial filament (arrow), dark-staining silicalemma, and numerous clear vesicles. The sclerocyte is surrounded by the cilia of micromeres (arrowheads) in late gastrula-stage embryos. **E,F:** Sclerocytes with numerous pseudopodia (arrowheads) in a late stage gastrula. The spicule space now occupies most of the cell and is considerably larger than the axial filament (arrow). Scale bars: **A**, 50 μm, inset, 10 μm; **B,D,E,** 1 μm; **C**, 20 μm; **F**, 5 μm.

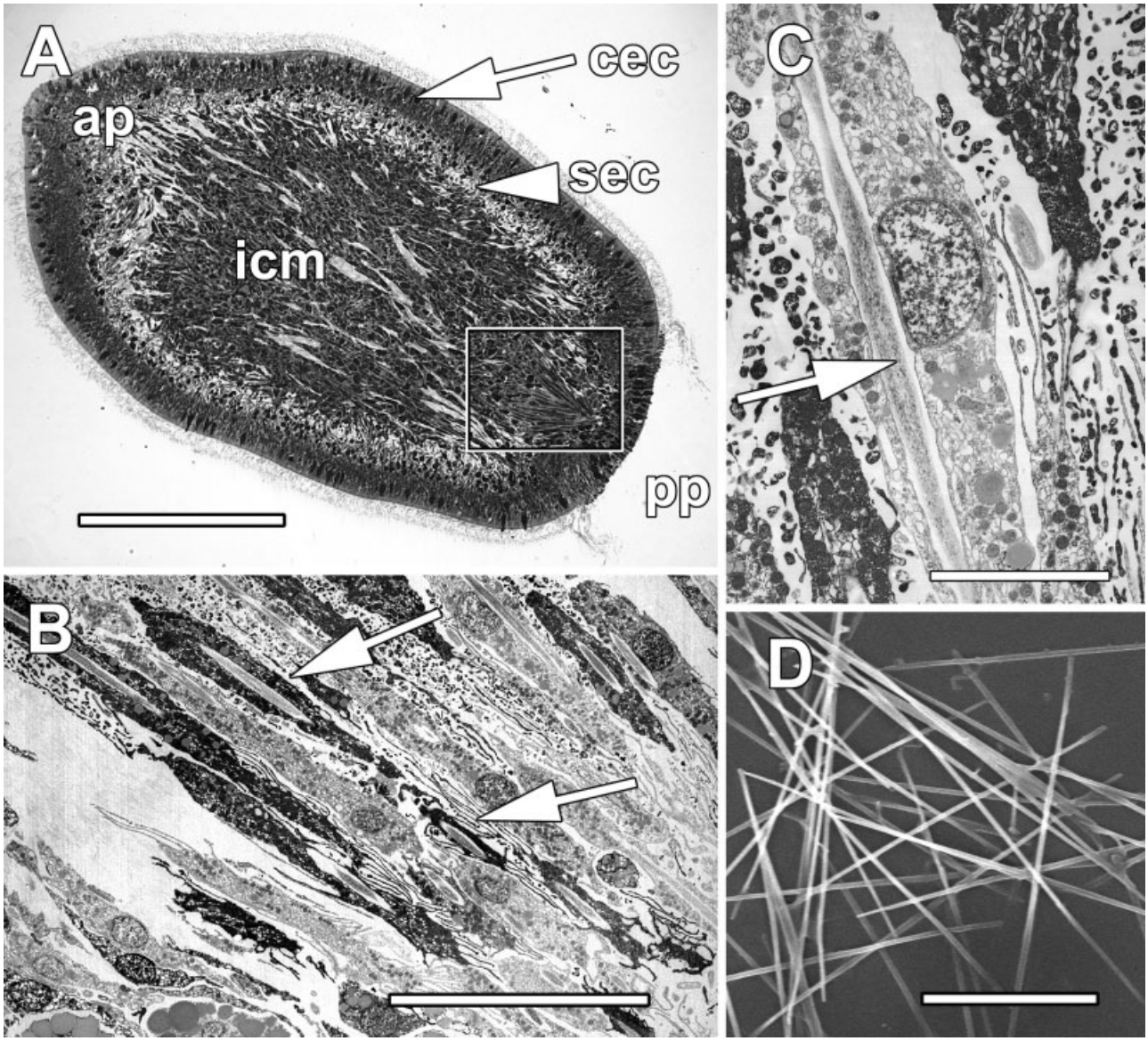

Fig. 3. Spicules in the free-swimming larva of *Reniera*. **A:** The larva has three cell layers: a layer of monociliated columnar epithelial cells (cec); a sheath of subepithelial cells (sec) and the cells of the inner cell mass (icm). Sclerocytes (box) are one of at least four cell types that occupy a small region at the posterior of the larva. Pp, posterior pole; ap, anterior pole. **B:** TEM of sclerocytes at the posterior pole of the larva. All sclerocytes (arrows) are elongate and have numerous pseudopodia. **C:** Longitudinal section of a sclerocyte at the posterior pole of the larva showing a large proteinaceous axial filament (arrow) within a vacuole, or space, that is enveloped by the sclerocyte. **D:** SEM of spicules from a *Reniera* larva. Scale bars: **A**, 100 μm; **B**, 10 μm; **C**, 2 μm; **D**, 25 μm.

sclerocytes during embryogenesis appears to be common among demosponges. Larval development is known in four Atlantic haplosclerid sponges. In three of these, *Haliclona (=Adocia) cinerea* (Meewis, 1941), *H. ecbasis* (Fell, 1969), and *H. tubifera* (Woollacott, 1993), spicules are already present when the larva is released. In *H. cinerea* and *H. ecbasis,* sclerocytes arise from the first micromeres formed (Meewis, 1941; Fell, 1969). At gastrulation—which is defined here as the migration cells resulting in a two layered embryo—as these micromeres migrate to the periphery of the embryo, a vacuole appears at the edge of the cell and elongates as the spicule is produced within. Spicule growth in *H. cinerea* continues while the sclerocytes lie at the periphery of the embryo just under the ciliated cells that form the outer epithelium. It is not until the embryo begins to elongate along the antero-posterior axis shortly prior to release of the larva that the sclerocytes migrate toward the center and posterior pole, as found in *Reniera* sp. larvae. In *H. limbata,* spicules develop in small cells with nucleoli, at the posterior pole during the free-swimming life of the larva (Meewis, 1939).

In the freshwater spongillids, sclerocytes and pinacocytes are the first cells to differentiate in the embryo (Saller and Weissenfels, 1985; Saller, 1988). In *Ephydatia fluviatilis,* spicules form before the larval cavity

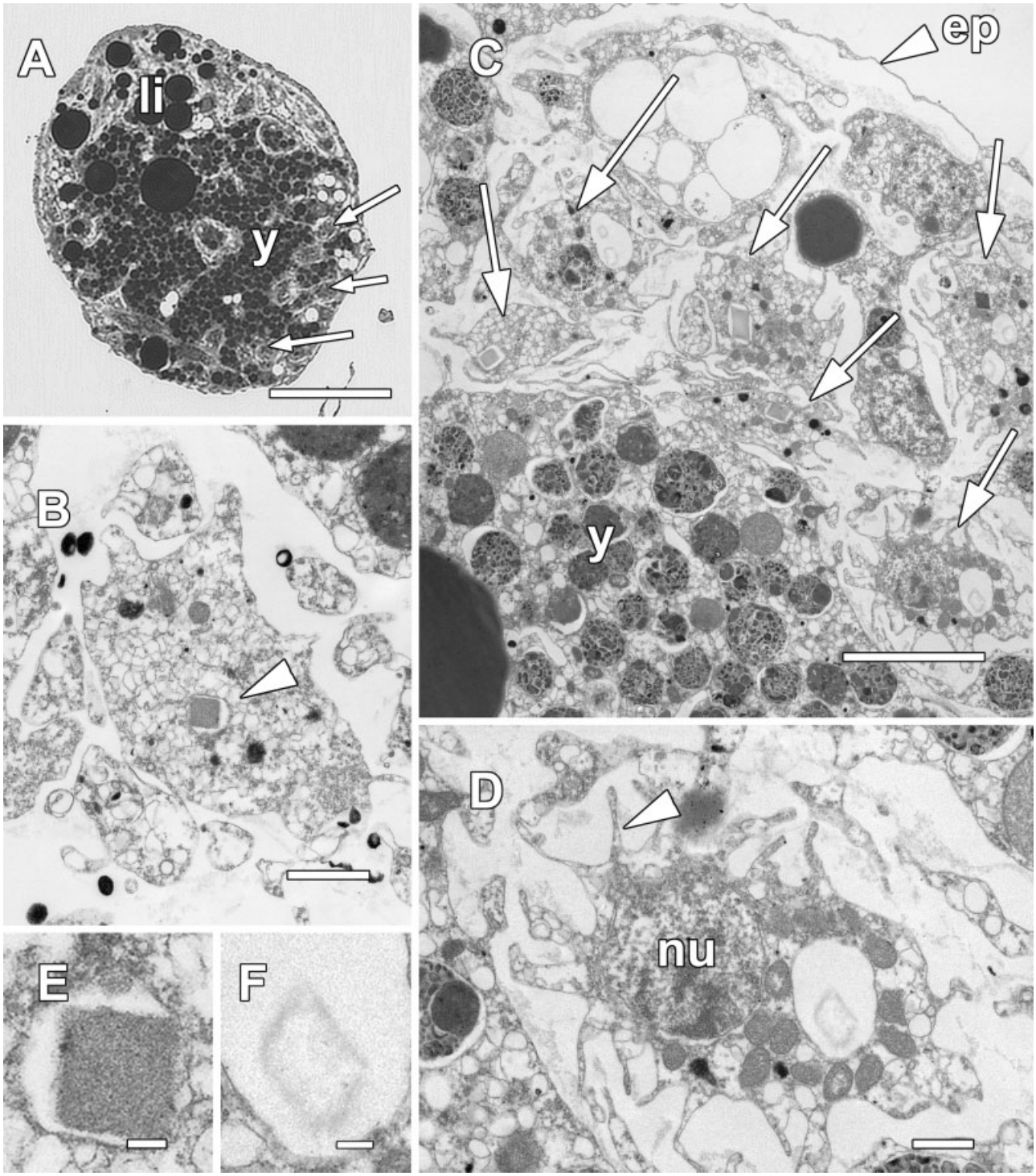

Fig. 4. Sclerocyte development in early *Oopsacas* embryos. **A:** Epon section of an embryo when sclerocytes (arrows) are first evident. Li, lipid inclusions; y, yolk inclusions. **B:** TEM of a young sclerocyte. The earliest stages of sclerocytes can be identified by the presence of a rectangular axial filament (arrowhead) lying within a small vacuole. **C:** Several sclerocytes (arrows) from the embryo shown in **A**. All sclerocytes are located just under the epithelial tissue (ep), and all have numerous pseudopodia. Y. yolk. **D:** Magnification of one of the sclerocytes shown in **C** showing the pseudopodia (arrowhead). Nu, nucleus. **E,F:** Magnification of the axial filaments from sclerocytes shown in **B** and **D**. Scale bars: **A**, 25 μm; **B,D**, 1 μm; **C**, 5 μm; **E,F**, 0.1 μm.

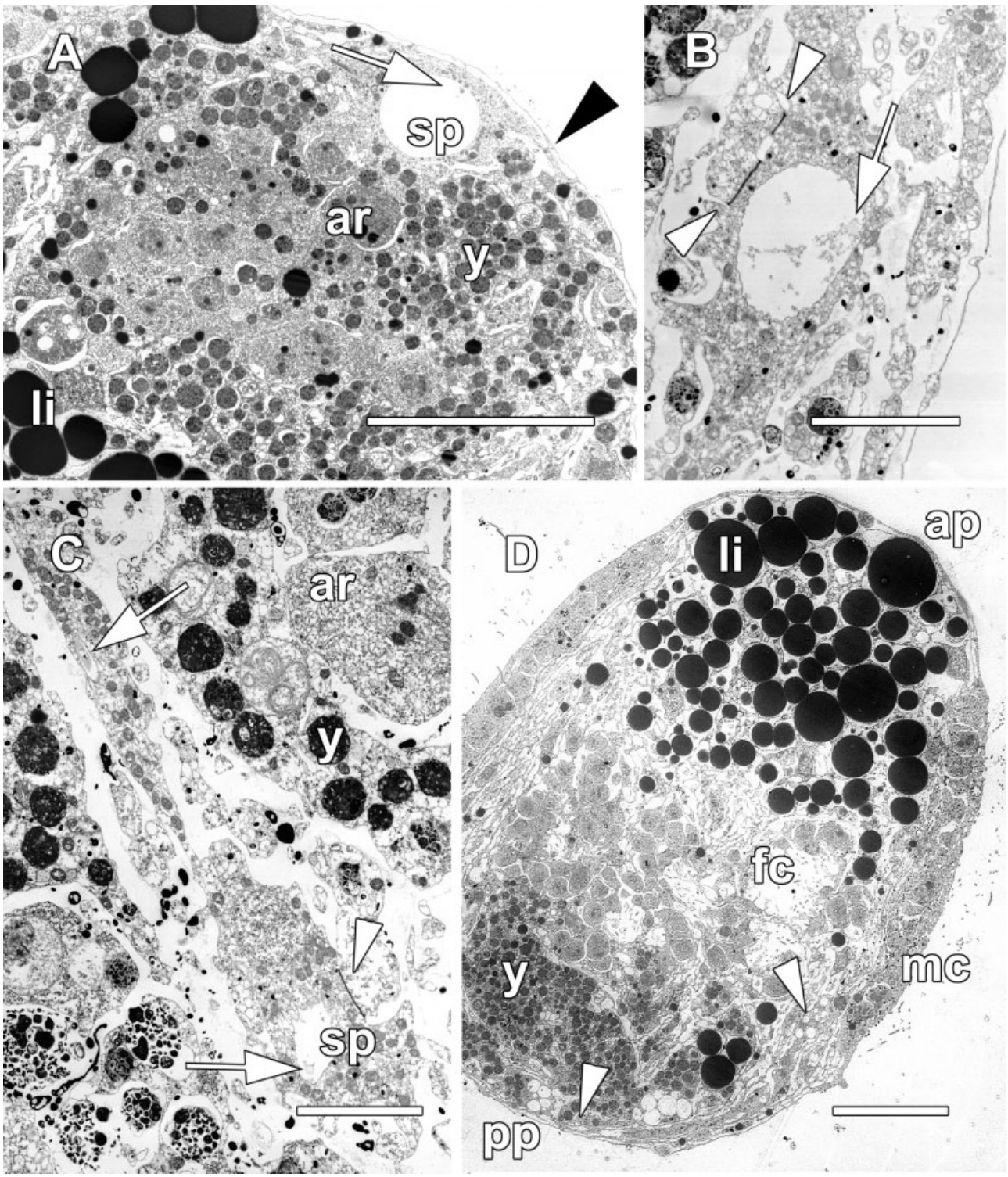

Fig. 5. Spicule formation in late embryos and larvae of *Oopsacas*. **A:** TEM of the posterior pole of a late embryo with a spicule space (sp) containing a rectangular axial filament (arrow). Although the cytoplasm appears continuous with the outer epithelial layer (black arrowhead), the membranes are not continuous. **B,C:** Sclerocytes are connected to other tissue by plugged cytoplasmic bridges. **B:** TEM showing a spicule space containing a rectangular axial filament (arrow) and amorphous material in a sclerocyte that is connected by two plugged cytoplasmic bridges (arrowheads) to other tissues. **C:** A sclerocyte in a late embryo with two spicules (arrows indicate spicule space and axial filament). The sclerocyte is also connected to other tissues by a cytoplasmic bridge with a perforate plugged junction (arrowhead). Ar, archaeocyte; y, yolk inclusions. **D:** A low magnification TEM of a late embryo. Spicule spaces (arrowheads) are only at the posterior pole (pp). Li, lipid inclusions; y, yolk inclusions; fc flagellated chambers; mc, multiciliated cells. Scale bars: **A**, 10 μm; **B,C**, 5 μm; **D**, 20 μm.

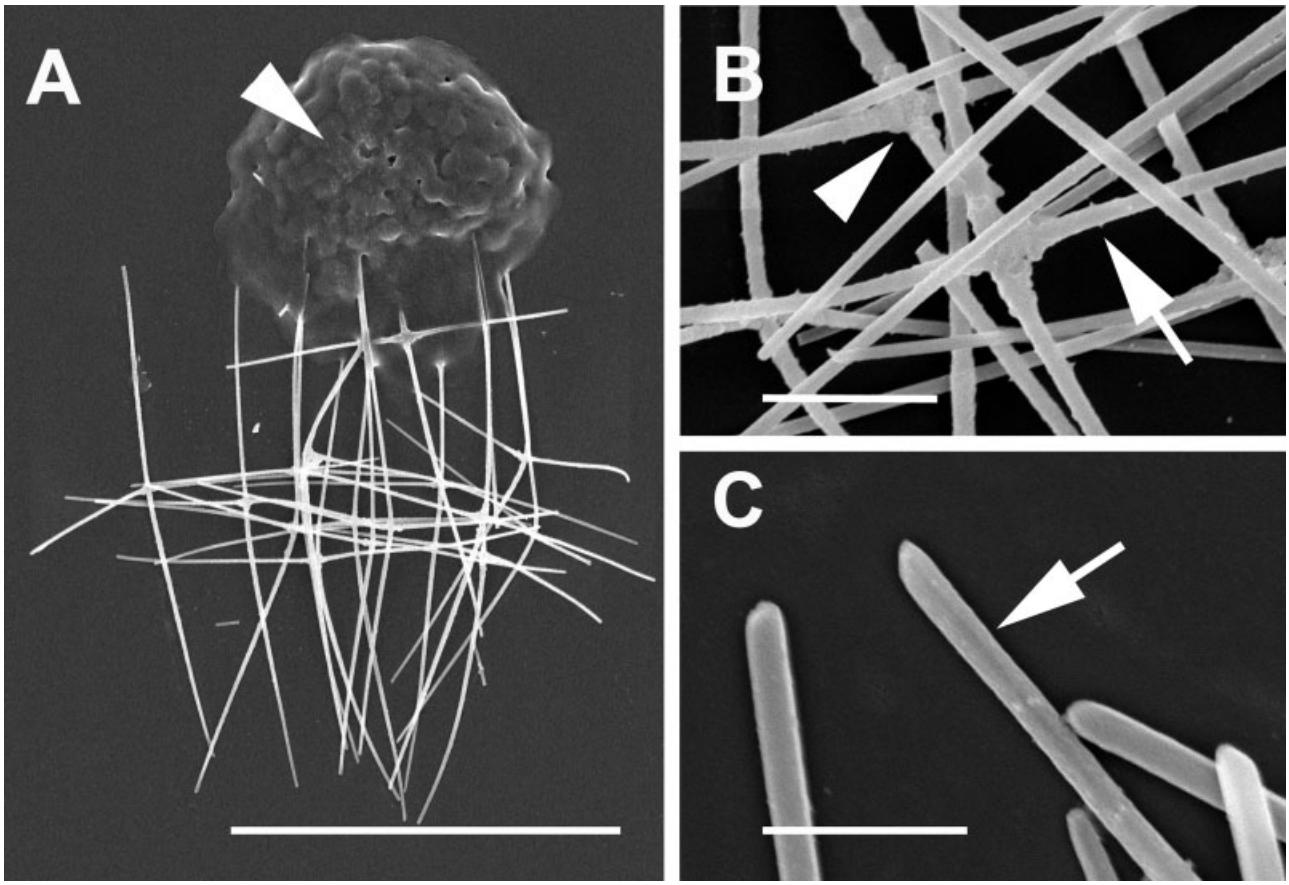

Fig. 6. Spicules in the free-swimming larva of *Oopsacas minuta*. **A:** Stauractin spicules with their longitudinal rays still embedded in the anterior lipid mass (arrowhead). There are 14 spicules in this larva. **B:** At the intersection of the longitudinal and transverse rays the spicules have an uneven surface (arrowhead). Spines occur along the length of the spicules (arrow). **C:** The ends of all rays are smooth and rounded; there are no spines at the terminals of any of the rays. Scale bars: **A**, 100 μm; **B**, 10 μm; **C**, 5 μm.

develops, and remain scattered throughout the embryo under the ciliated epithelial layer. In *Spongilla lacustris*, the sclerocytes aggregate towards the posterior pole of the embryo and become embedded in spongin in the free-swimming larva (Saller and Weissenfels, 1985).

Although many larvae in the Halichondrida appear to lack spicules, in *H. coalita (=bowerbanki)* spicules are formed in the macromeres, rather than micromeres, and at a much later stage than in *Haliclona* (Meewis, 1941). However, subsequent positioning of the spicules, first at the periphery, and then at the posterior pole, is similar in most aspects to that described in haplosclerids. Poecilosclerid larvae are known to possess both adult macroscleres and occasionally also microscleres (Simpson, 1968; Bergquist and Sinclair, 1968), but the mechanism of spicule formation has not been studied in these larvae.

The difficulty of obtaining and preserving hexactinellid sponges in good condition explains the paucity of studies on embryogenesis and larval spicule development in this group. Furthermore, the small size of embryos and larvae of hexactinellids has meant that light microscope observations of the early stages are difficult to interpret. The largest larvae found in numerous collections of *Farrea sollasii* by Okada (1928) were 250 μm long and 100–200 μm wide; the largest larvae of *Oopsacas minuta* are 150–180 μm long (Boury-Esnault et al., 1999; and present study).

According to Okada (1928), scleroblasts are the first syncytial tissues to develop in the embryo of the hexactinellid *Farrea sollasii*. These tissues are said to arise from uninucleate micromeres, which are derived from the so-called "columnar epithelium" of the gastrula-stage embryo, which migrate into the inner cell mass and undergo nuclear division without cytokinesis to become multinucleate. However, in his figures, Okada (plate 3, figs. 3, 8, 9; 1928) suggests that in embryonic stages that have not yet formed spicules, both the inner cell mass and the outer epithelium are already syncytial. Certainly in *Oopsacas* embryos, multinucleate tissues are formed well before spicule development is evident (Boury-Esnault et al., 1999). After gastrulation the macromeres form two "cell types" that occupy the inner cell mass and the anterior and posterior hemispheres of the embryo. Micromeres differentiate into the multinucleate epithelial layer and the cuboidal multiciliated cells. Intrasyncytial plugged junctions are found between all of these tissues.

In the development of both hexactinellids, macroscleres—four-rayed stauractins—are formed in separate multinucleated tissues that lie beneath the syncytial epithelium near the posterior pole of the embryo (Okada, 1928; Boury-Esnault et al., 1999). In *Farrea*, microscleres—discohexasters—develop at the conical posterior pole of the larva in multinucleate cells that also arise from the early micromeres in the postgastrula (Okada, 1928). [Prior to the work of Boury-Esnault and Vacelet (1994), the swimming direction of the larva was not known, and the conical end was incorrectly named the anterior pole.] No microscleres were found in the free-swimming larva of *Oopsacas*.

Silica Deposition

Although few studies have examined silica deposition in demosponge larval scleroblasts, the process is said to be similar to the secretion of microscleres in freshwater gemmules and adult spongillids (Lévi, 1964; reviewed in Garrone et al., 1981). According to Meewis (1941), a vesicle forms at the edge of the scleroblast cytoplasm, is gradually distended at either extremity as the axial filament is laid down, and then disappears as silica is deposited around the filament. In *Reniera* larvae, scleroblasts have numerous clear vacuoles that appear to fuse with a large central vacuole. Although the involvement of vesicles budding off the Golgi apparatus has been demonstrated in silica deposition in some testate amoebae (Anderson, 1994), choanoflagellates (Leadbeater, 1981), and in silica deposition in adult haplosclerid sponges (see Simpson, 1984:181), the Golgi apparatus was not apparent in any sections of sclerocytes in *Reniera*.

The small size and central location of the vacuole in early sclerocytes suggests that spicule secretion is intracellular in *Reniera*, rather than extracellular as demonstrated in *Crambe crambe* (Uriz et al., 2000). However, the possibility that the silicalemma is a continuation of the plasma membrane, as discussed by Simpson (1984:173), cannot be ruled out without extensive serial sectioning and 3D reconstruction of a sclerocyte and spicule. In all micrographs of *Reniera* larval sclerocytes, the silicalemma was darkly stained with osmium, uranyl acetate, or lead citrate during fixation and processing, obscuring the structure of the membrane. Nonetheless, SEMs of fractured embryos show the axial filament lying in a large intracellular vacuole (e.g., Fig. 2C), and the development of numerous pseudopodia in sclerocytes suggests that this cell alone is responsible for moving the spicule from its peripheral position in the embryo to the posterior-central position in the larva.

The proteinaceous axial filament appears hexagonal in all sclerocytes found in *Reniera* larvae and rectangular in cross section in *Oopsacas* larvae. Both hexagonal and triangular axial filaments have been reported in demospongiae (e.g., Garrone, 1969; Reiswig, 1971; Simpson et al., 1985), although Uriz et al. (2000) have suggested that the process of desilicification of tissues can alter the shape of the axial filament, and that a hexagonal shape can result from the rounding off of a triangular filament. However, as many micrographs of haplosclerid spicules that have not been desilicified show a hexagonal axial filament (e.g., Garrone, 1969), it is quite probable that two shapes of axial filament exist in demosponges. In addition to the clearly defined rectangular axial filament, an amorphous osmiophilic material was found in all spaces left by desilicified spicules in *Oopsacas* embryos. A similar amorphous organic material was found in hexactinellid spicules by Travis et al. (1967) and in sections of adult sponges of *Rhabdocalyptus dawsoni* (Mackie and Singla, 1983), so it is possible that there is an additional organic matrix involved in spicule deposition. While the function of the proteinaceous axial filament in spicule formation is not known, an organic matrix appears to be required for silica deposition in all siliceous sponges, but not in deposition of silica in siliceous ameba or choanoflagellates (Harrison et al., 1981; Leadbeater, 1981). No organic matrix exists in the spicules in calcareous sponges (Ledger and Jones, 1977).

Organization of Spicules in Sponge Larvae

In the majority of demosponge larvae that possess a spicule skeleton, the macroscleres are oriented along the antero-posterior axis as shown in the illustrations of parenchymella larval types in Figure 1. With few exceptions, both macroscleres and microscleres are aggregated at the posterior pole of the larva.

Early sclerocytes in the pregastrula embryo of *Reniera* are only distinguishable from other micromeres by the presence of a central vacuole containing a hexagonal axial filament. After gastrulation, the sclerocytes develop numerous pseudopodia, which suggests that they are highly motile. Presumably they require these extensions to navigate to the posterior pole of the embryo. The pseudopodia remain at either end of the elongate sclerocytes when they are at the posterior pole of free-swimming larvae, indicating that the extensions might also play a role in anchoring the cells in the otherwise fluid milieu of the ECM.

The mature *Oopsacas* larva possesses up to 14 stauractins that are positioned around the periphery of the larva, with the long ray oriented along the antero-posterior axis of the larva. A similar number of stauractins (12) was described in *Farrea* (Okada, 1928). The spicules differ slightly between the two genera: stauractins in *Oopsacas* possess small spines along their length, but lack spines at the ends. In *Farrea* larvae, Okada (1928) describes the reverse. Rectangular axial filaments are first apparent in cells at the posterior pole of *Oopsacas*. The unequal distribution of spicules in the larva continues through much of its development; even when flagellated chambers are formed and all other aspects of the larva appear fully developed, spicule spaces are only seen at the posterior pole of the larva (Fig. 4D). It is only when the larva begins to elongate that spicule spaces are found at other locations in the larva. It appears that spicules in *Oopsacas* form at the posterior end and then grow—rather than migrate—intrasyncytially towards the anterior end of the larva. Sclerocytes in *Oopsacas* embryos, like sclerocytes in *Reniera* embryos, have many pseudopodia. The collagenous mesohyl of hexactinellid sponges has been considered too thin to support cell motility. Certainly, observations of live tissues suggest that none of the cells or syncytia in aggregates or in adult tissues migrate; nutrient transport, growth, and regeneration occur by extension of the syncytial membranes and cytoplasmic streaming (Leys, 1995, 1998).

If the pseudopodia seen on the sclerocytes in young *Oopsacas* embryos are not used for motility, it is possible that these extensions fuse with one another, as do the pseudopodia of skeletogenic cells in echinoderm embryos (Decker and Lennarz, 1988). In this case, spicule growth would continue as the sclerocyte cytoplasm extended. The presence of spines at the intersection of the longitudinal and horizontal rays, and lack of spines at the tips of the rays implies that, as suggested by Okada (1928), growth of the spicule occurs at the ends of the rays such that the lateral rays eventually intersect, and the posterior ends of the long rays cluster together, giving the larva a conical appearance.

According to Okada (1928), the multinucleate scleroblasts remain separate from the rest of the syncytial tissue in the sponge until secretion of the spicule is complete, at which point the scleroblast cytoplasm joins with the trabecular syncytium. Sections of mature *Oopsacas* larvae show that spicule spaces are in cytoplasm that is both continuous with the smooth syncytial epithelium and is connected to other tissues by perforate plugged junctions. Nevertheless, whether the connection is to other sclerocytes or to the trabecular syncytium cannot be conclusively determined. The lack of cytoplasmic continuity of sclerocytes with the trabecular syncytium in adult sponges is taken to imply that silica deposition requires a separate cytoplasmic environment (Mackie and Singla, 1983). If the sclerocyte cytoplasm is continuous with the trabecular syncytium in the free-swimming larva, then these spicules must be considered to be completely formed larval spicules rather than underdeveloped adult spicules, as is the case in demosponges. It is extremely unlikely that a fifth ray is added to the stauractin at metamorphosis to produce the pentactin found immediately after metamorphosis in the juveniles (Okada, 1928; Leys, unpubl. obs.), since all true rays in hexactinellids emanate from the spicule central cross at inception. If silica deposition requires a separate cytoplasmic environment and continues after settlement and metamorphosis, then it is probable that the scleroblasts remain separate from the rest of the trabecular syncytium, as seen in at least one adult hexactinellid (Mackie and Singla, 1983). Further work on silica deposition in hexactinellids is definitely warranted.

Role of Larval Spicules

Considering that there is so little known about the fate of larval cells at metamorphosis, it is very difficult to speculate whether spicules serve a role in the larva or in the juvenile. In other invertebrate larvae the skeleton—shell, carapace, or endoskeleton—is suggested to function in buoyancy regulation (Pennington and Strathmann, 1990; Vacelet, 1999), as an antagonist for larval muscles (Strathmann, 1971), and in defense (Emlet, 1983; Hickman, 1999).

The fact that most sponge larval spicules are macroscleres—the spicules used in structural support in the adult—has prompted the suggestion that the primary role of sponge larval spicules may be during postlarval life (Woollacott, 1993). If the development of larval spicules, like the development of choanocyte chambers in many larvae, is a heterochronic event, the spicules could provide early metamorphs and juveniles with the advantage of protection and support before the adult skeleton has formed. However, Maldonado et al. (1997) have argued that the presence of discarded spicules around newly metamorphosed sponges is evidence that larval spicules are not used by the juvenile. They suggest instead that larval spicules, like yolk reserves, are important during larval life as an aid for orientation to gravity. Warburton (1966) first proposed that differential weighting by spicules at the posterior pole of sponge larvae could be responsible for the fact that larvae often swim upwards, whether illuminated from above or below. In a detailed study of the physical characteristics of haplosclerid larvae, Maldonado et al. (1997) were able to demonstrate that sinking rates of larvae increase with age, and that the increased density of larvae is primarily due to a greater number and mass of spicules. However, formaldehyde-killed larvae do not sink in a preferred position (Warburton, 1966), and several published observations indicate that older larvae with spicules concentrated at the posterior end tend to rotate on the substrate on their anterior end, not their posterior end (e.g., Maldonado and Young, 1996; Leys and Degnan, 2001). The picture that is emerging is complex. Perhaps one role of the larval skeleton is to minimally assist the larva to orient during its initial free-swimming phase, as has been demonstrated in echinoderm larvae (Pennington and Strathmann, 1990).

As to the possible role of spicules as a defense against predation, Woollacott (1993) has suggested they may act either as a mechanical barrier (e.g., Young and Chia, 1987) or in assisting to introduce toxins into a predator. It has been demonstrated that some sponge larvae are unpalatable to fishes (Lindquist and Hay, 1996), although this is not always the case (see Uriz et al., 1996), but it has not determined whether the toxicity is greater in species that have a spicule skeleton. As Hickman (1999) has demonstrated with gastropod larval shells, the impact of the larval skeleton on increased survivorship may be very discrete, and certainly difficult to assess in such small organisms. Clearly the role of sponge larval spicules in larval and postlarval life requires further investigation.

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