Invertebrate Biology 122(3): 203–222.

Svenzea zeai, a Caribbean reef sponge with a giant larva, and *Scopalina ruetzleri:* a comparative fine-structural approach to classification (Demospongiae, Halichondrida, Dictyonellidae)

Klaus Rützler,[1,a] Rob W. M. van Soest,[2] and Belinda Alvarez[3]

[1] Department of Systematic Biology, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560-0163, USA
[2] Zoologisch Museum, University of Amsterdam, PO Box 94766, 1090 GT Amsterdam, The Netherlands
[3] Museum and Art Gallery of the Northern Territory, PO Box 4646, Darwin NT 0801, Australia

Abstract. *Svenzea zeai,* abundant on many deep Caribbean fore-reef habitats but of uncertain systematic position within the Demospongiae, is closely examined histologically and cytologically for evidence of its phylogenetic relationship beyond the traditional analysis of gross morphology and skeletal structure. We document that *S. zeai* is a bacteriosponge containing substantial quantities of unicellular photosynthetic and autotrophic microbes; that the most abundant cell type is an unusual cell with refractile granules that only few species share and whose composition and function are still enigmatic; and that it produces the largest—by a factor of 3—embryos and larvae recorded in the phylum Porifera. A combination of characters such as the granular cells, ciliary pattern, and aspects of larval shape and behavior are comparable with those of *Scopalina ruetzleri,* family Dictyonellidae, a prominent member of the Caribbean mangrove community. These results support our earlier decision to establish *Svenzea* as a new genus in Dictyonellidae to accommodate its unprecedented skeletal structure, styles in isodictyal reticulation.

Additional key words: Porifera, microbial symbiosis

The common Caribbean coral-reef sponge *Svenzea zeai* (ALVAREZ, VAN SOEST, & RÜTZLER 1998) forms sprawling thick, crumbly crusts or masses with volcano- or chimney-like oscular elevations, purplish brown in color and often associated with maroon parazoanthid polyps. Specimens are abundant on exposed coral rock on many off-shore reefs, from Florida to Colombia and from Trinidad and Tobago to Belize; they favor a zone below the reach of strong wave action, but with high light intensity, at 10–30 m depth. This species had sponge workers puzzled since it was first noted by scuba divers in the late 1960s. Problems with classification derived from the presence of monaxon skeletal spicules (styles) similar to those found in Hymeniacidonidae (now part of Halichondriidae) and Axinellidae but arranged in a paucispicular reticulation held together by small patches of spongin, a combination which lends a crumbly soft consistency that is characteristic of Haplosclerida. Accordingly, the few published records of this sponge placed it in *Hymeniacidon amphilecta* DE LAUBENFELS 1936 (Hymeniacidonidae) (Pulitzer-Finali 1986) and *Calyx podatypa* (DE LAUBENFELS 1934) (Haliclonidae) (Humann 1992), both taxonomic misinterpretations of valid species (Wiedenmayer 1977). A popular field guide (Humann 1992), however, included a good color picture of the live animal and coined a suitable common name, dark volcano sponge.

Realizing that there was no valid scientific name for the volcano sponge, we described it as *Pseudaxinella(?) zeai* ALVAREZ, VAN SOEST, & RÜTZLER 1998 and signaled the questionable generic assignment (Alvarez et al. 1998). In subsequent treatments, the species was assigned to a new genus, *Svenzea,* and transferred (with some hesitation) to the family Dictyonellidae (van Soest et al. 2002; Alvarez et al. 2002).

During recent fieldwork off Carrie Bow and Pelican Cays on the Belize barrier reef (Rützler et al. 2000), we re-examined *Svenzea zeai* and its conspicuous embryos and managed to observe for the first time a fully developed, swimming larva, the largest ever recorded from a sponge. This discovery stimulated additional research on the histology, embryogenesis, and larval

[a] Author for correspondence.
E-mail: ruetzler.klaus@nmnh.si.edu

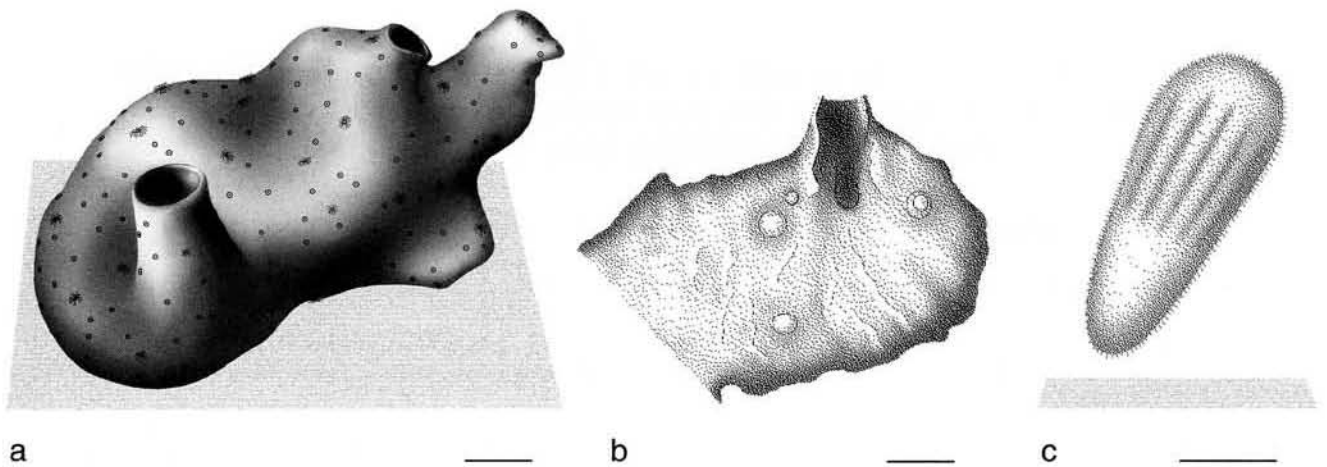


Fig. 1. *Svenzea zeai.* **a.** Drawing of the live sponge. Scale bar, 10 mm. **b.** Specimen cut open to display the large embryos. Scale bar, 10 mm. **c.** Larva with longitudinal furrows along its sides, in characteristic inclined position swimming-drifting in the direction of the larger anterior pole. Scale bar, 2 mm.

anatomy of this species. Hoping to shed more light on the enigmatic systematic position of the genus *Svenzea,* we made comparative studies on a common lagoon sponge in the same area, a typical representative of the Dictyonellidae, *Scopalina ruetzleri* (WIEDENMAYER 1977).

Methods

Specimens examined for this study came from the following locations. *Svenzea zeai*: USNM (uncatalogued), Scotts Head Bay, Dominica, West Indies (15°12.4′ N, 61°22.8′ W), 10 m, on coral rock, collected by K. Rützler, 12 June 1966; USNM (uncatalogued), Sunken Reef, south of La Parguera, Puerto Rico (17°53.1′ N, 67°04.0′ W), 33 m, on coral rock, collected by K. Rützler, 9 April 1967. *Scopalina ruetzleri*: USNM 24459 (holotype), Bimini Lagoon, Bimini, Bahamas (25°45.0′ N, 78°17.0′ W), 1 m, on rock, collected by F. Wiedenmayer, 3 July 1967; USNM (uncatalogued), Walsingham Pond, Bermuda (32°20.64′ N, 64°42.4′ W), 0.3 m, on mangrove root, collected by K. Rützler, 26 December 1966. Material of *S. zeai* for histological and larval study was collected from the fore-reef east of Carrie Bow Cay, Belize (16°48.2′ N, 88°04.9′ W), 12–25 m depth (low spur-and-groove zone, outer ridge; Rützler & Macintyre 1982). Specimens of *Scopalina ruetzleri* came from submerged red-mangrove stilt roots at nearby Twin Cays, Belize (16°50.0′ N, 88°06.3′ W; Rützler 1995). To improve chances of obtaining mature embryos, sponge fragments were collected from multiple specimens and taken to the field laboratory in sealed plastic bags with ambient water. Care was taken to keep samples cool (ambient sea temperature, 28°C) and not to expose them to air.

To obtain larvae, broken-up sponges and liberated embryos were observed in slowly running seawater (overflow tube closed by 0.5-mm plastic screen) for up to several days. Free larvae were observed by stereomicroscope and magnifying glass (10×) and movements recorded using a stopwatch and 10-mm-mesh reference grid.

For histological processing, thin (2–3 mm) slices of ectosomal and choanosomal tissue and whole larvae were fixed in cold (4°C) buffered glutaraldehyde (1.5% in 0.2 M cacodylate with 0.1 M sodium chloride and 0.4 M sucrose, pH 7.2) and stored in the same solution (cold, one change) for up to 2 weeks. Subsequently, samples for light microscopy (LM) and scanning electron microscopy (SEM) were rinsed with distilled water and dehydrated in a graded ethanol series (30–100%). SEM samples were critical-point dried after immersing tissue in HMDS (hexamethyldisilazane) (Ted Pella, Inc., Redding, CA, USA). Squash preparations were made to isolate cells and granules and to test solubility in sodium hypochlorite or optical property under polarized light. The tissue was not decalcified or desilicified. For SEM, granules were isolated by grinding tissues (using a mortar and pestle) and separating them from cell debris by gravity in a series of alcohol suspensions. For transmission electron microscopy (TEM), small subsamples were postfixed in 2% osmium tetroxide in the same cacodylate buffer, then dehydrated. Embedding medium for thick (LM) and thin (TEM) sections was Spurr low-viscosity epoxy (Polysciences, Inc.), mixed either for "hard" (grinding-polishing) or for "firm" (microtome section-

ing). Sections for LM were ground, polished, and stained with toluidine blue or safranin O followed by crystal violet (all aqueous 1% at 60°C), or they were thick-sectioned (1 μm) on a microtome and stained with methylene blue and azur A (with borax) or with toluidine blue. TEM stain was saturated alcoholic uranyl acetate with 0.25% lead citrate. SEM photomicrographs were taken on a Leica Stereoscan 440 W microscope (150–5000×, primary magnification) and TEM images on a JEOL 1200 EX electron microscope (3000–30000×).

Numerous studies of inclusion cells of sponges by workers with different native-language backgrounds have created a colorful terminology which, unlike that for other cellular elements, is not yet entirely resolved (Simpson 1984; Boury-Esnault & Rützler 1997). One complication is that cellular components undergo developmental stages, as during the synthesis of nutrients or the formation of skeletal elements, which are reflected by changes in size, structure, or reaction to staining. Not even the presence of a nucleolus can be relied upon as an identifying character (see below). Here, we refer mainly to granular, microvesicular, and spherulous cells. Granular cells contain strongly staining (e.g., with toluidine blue), moderately to strongly electron-opaque granules or angular pellets averaging 3 μm in diameter; the granules have no substructure except for crystalline patterns in some. Microvesicular cells appear dark gray in transmitted light (stain poorly) and are densely packed with minute vesicles and highly electron-translucent grains <1 μm; these cells differ from microgranular and "gray cells" (glycocytes), which have minute but highly osmiophilic granules. Spherulous cells contain medium to large lipid globules or spherules (3–30 μm) which occupy most of the cell, leaving only thin sheets of cytoplasm.

Results

Svenzea zeai, adult and larva

Adult macroscopic morphology

This species is found in Caribbean fore-reef habitats at 10–30 m depths. The sponges thickly encrust their dead-coral substrate, and the sponge's mass may cover areas as large as 0.1–0.25 m^2. Many specimens have maroon-colored parazoanthids (*Parazoanthus puertoricense* WEST 1979) dotting the surface. The oscula, raised on volcano-shaped or tubular projections (Fig. 1a), are 3–10 mm in diameter, often with thin oscular tissue collars (growth extensions), not yet reinforced by skeleton, which collapse when the sponge is handled. Ostia (incurrent pores, 0.2–1 mm) are spread densely over the entire surface, rendering it finely porous. The surface is dark reddish to purplish brown. A cut perpendicular to the surface reveals a pigmented (reddish brown) outer zone, 0.5–3 mm thick (average 1 mm), followed by a cream to light gray, bread-like, porous interior mass. The pigmented zone extends into the interior, where it lines the incurrent and excurrent canals. Large oscular canals may be pigmented to more than 30 mm into the sponge. The consistency is firm but crumbly. Remarkably, no signs of decay develop for weeks if the sponge is broken up into fragments and kept in good-quality running seawater; broken or cut surfaces heal readily. Up to 80% or more of the specimens collected throughout the year are full of yellow or cream embryos. These appear as yellowish circular stains (early stages) or solid balls 1–4 mm in diameter (average 3 mm) (Fig. 1b), occasionally fused to irregular 4–5-mm masses. Embryos occur throughout the choanosome, on average nearly 1 per cm^3, but up to 7 per cm^3 where they are close to large excurrent canals.

Adult histology and fine-structure

Ectosome. This part of the body, extending from a typical exopinacoderm (single layer of thin flat cells) to the choanosome, is ~600 μm thick; it is thinner than the reddish- to purplish-brown outer zone of the sponge, which owes its color to the density of extracellular cyanobacteria crowding the mesohyle. Here and there, spicules of the skeletal framework protrude from the surface, at least in fixed material. Canals 80–130 μm in diameter traverse the ectosome, but there are also larger, irregular lacunae.

Four principal cell types are present here: pinacocytes, skeleton-forming cells (spongo- and sclerocytes), bacteriocytes, and granular cells (Figs. 2, 3). Pinacocytes are flat cells (2 μm thick, tapering to <0.1 μm), with spherical nuclei (1.5–2 μm) and without conspicuous inclusions, that form the outermost cell layer and line the incurrent canals. The skeleton-forming cells are triangular or elongate, typically 10–35 μm in cross-section with a 5–6.5-μm nucleus; many are wrapped tightly around spicules and spongin nodes. Their presence indicates an active growth zone. Bacteriocytes are typically spherical, 18–30 μm in diameter with a 3.5–5.5-μm nucleus. Most of the volume is occupied by a vesicle filled with oval bacteria. A few bacteriocytes are much larger, with a triangular outline 60 × 40 μm, with 2 or 3 extensive vesicles containing bacteria, nucleus up to 8.5 × 6 μm, and a 1-μm nucleolus (where present). In LM sections, there are from ~20 to >100 bacteria per cell cross-section. Closer to the choanosome, larger and more irregularly

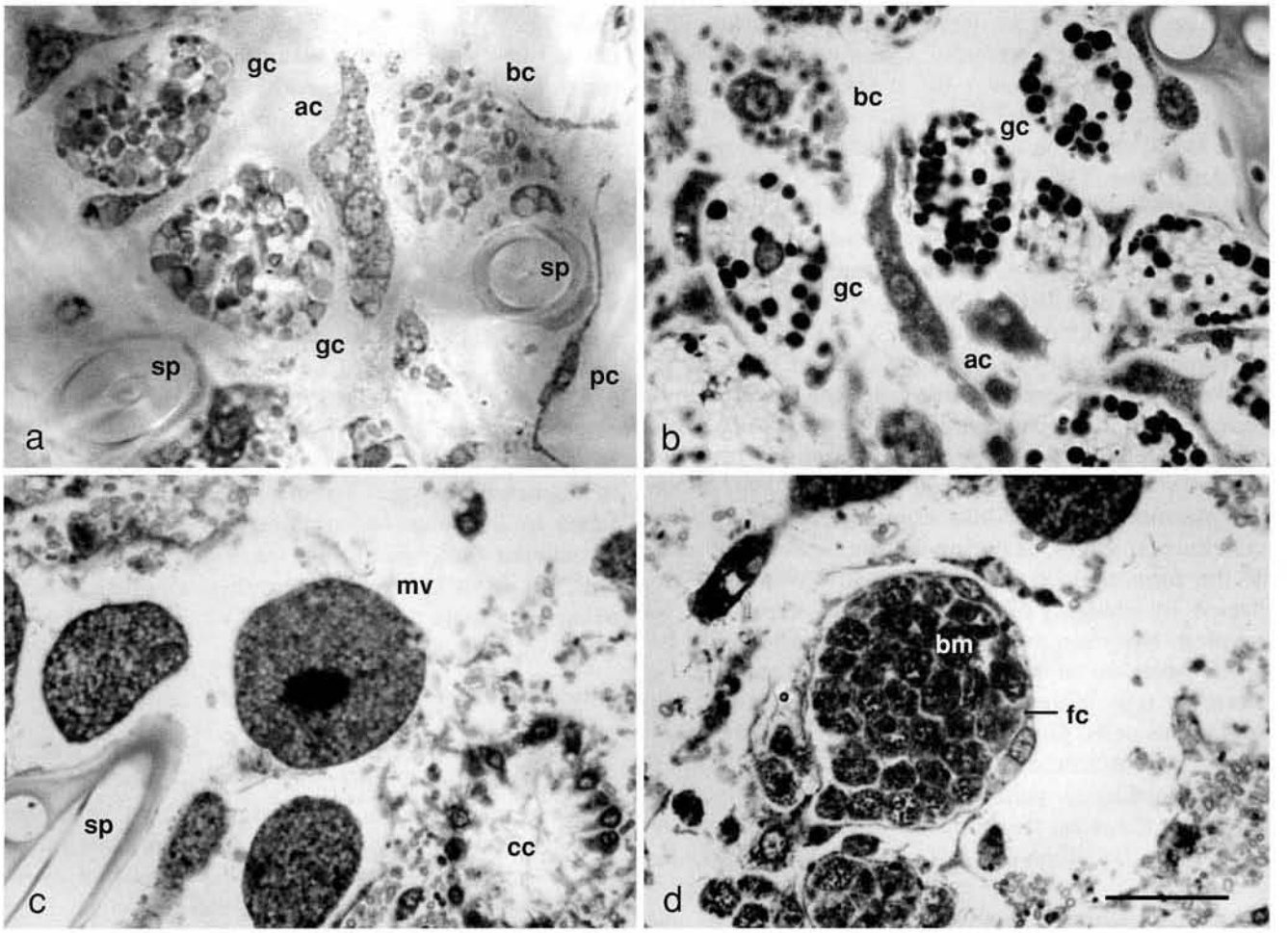


Fig. 2. *Svenzea zeai,* micro-anatomy of an adult specimen. LM. **a.** Ectosome, polished thick-section with granular cells (gc) stained in toluidine blue. Archaeocyte (ac), bacteriocyte (bc), pinacocyte (pc), spicules (sp; on right, with sclerocyte). **b.** Ectosome, semi-thin section showing granular cells (gc) stained in methylene blue. Archaeocyte (ac), bacteriocyte (bc). **c.** Choanosome, polished thick-section stained in toluidine blue, with microvesicular cells (mv). Choanocyte chamber (cc), parts of spicules (sp) cemented by spongin. **d.** Embryo in the choanosome, polished thick-section stained in toluidine blue, showing blastomeres (bm) and follicle cell (fc). Scale bars, 10 μm.

shaped bacteriocytes are found, with multiple vesicles containing microorganisms.

The most abundant cell type here is a spherical granular cell 18–35 μm in diameter and with a simple anucleolate nucleus (4–6-μm), quite similar in size and shape to many bacteriocytes, but filled, even overstuffed, with characteristic angular granules (Figs. 2, 3b, 4). These granular cells are denser along the skeletal strands than within the meshes; their abundance in sections was calculated in sections at 8–80 cells per 0.1 mm^2. The inclusions, 1–6 μm (average, 2.6 μm) in diameter, have a glassy translucent appearance in transmitted light; their shape is irregular with sharp edges or with a trapezoid or hexagonal outline; they are not birefringent in polarized light. They can be freed from the cell by exposure to sodium hypochlorite (5%), but appear to dissolve after extended exposure. The inclusions take up stain (toluidine blue) in 1 μm or thicker LM sections where some of the granules may be missing; only membrane-bound spaces indicate their previous presence. Larger inclusions drop out of the cells in TEM, leaving behind holes in the embedding medium. Those remaining are generally less than 2 μm in diameter, moderately electron-opaque, uniform in fine-granular structure, and bound by a thin membrane. Their shape is basically spherical, but granules in close proximity flatten where they meet, giving them an angular appearance. Although most of these inclusions fill the membrane-bound space, some are surrounded by a small percentage (<10%) of flocculent material, a few are 40–100% flocculent material, possibly the precursor substance

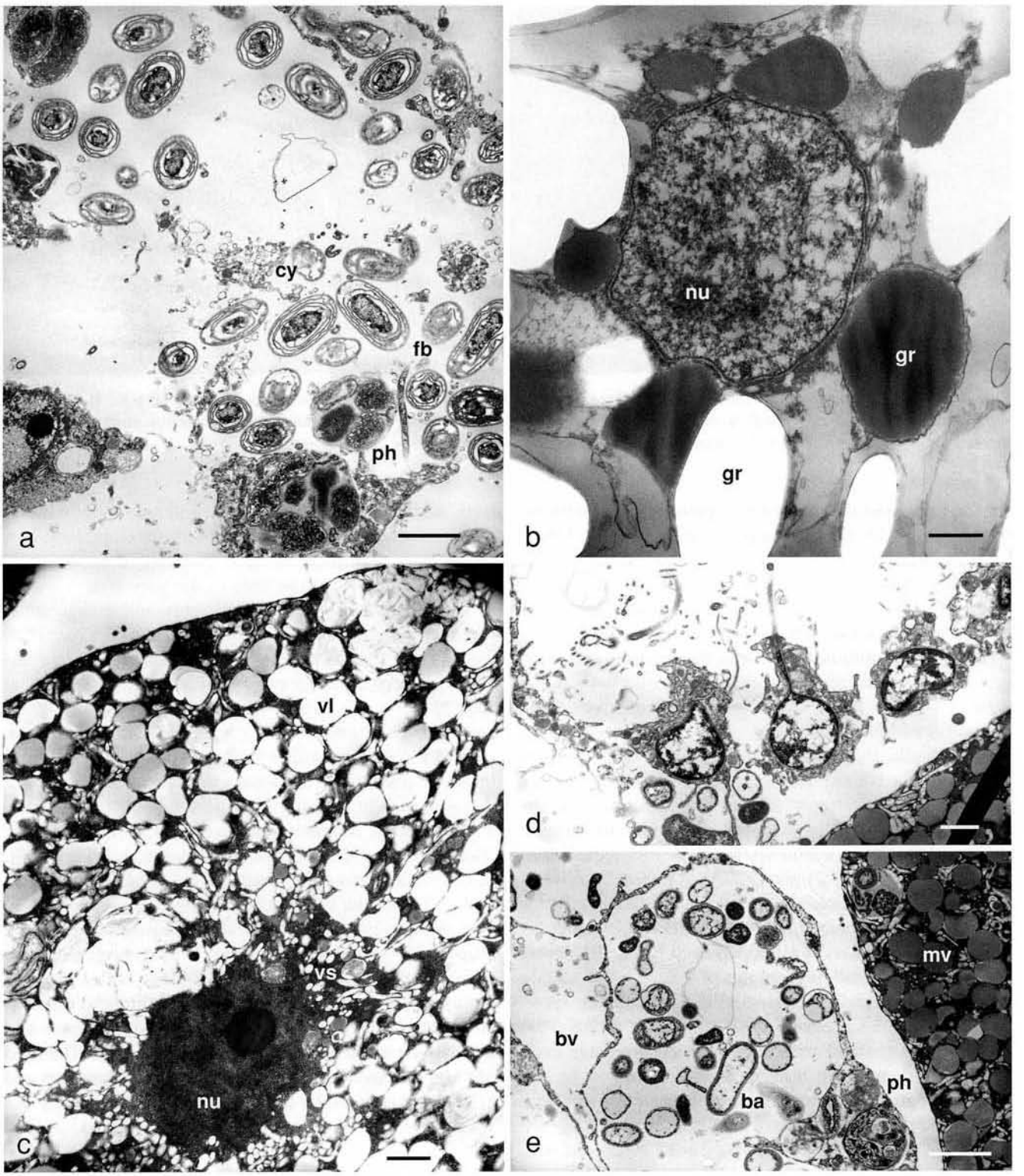


Fig. 3. *Svenzea zeai,* cellular components in an adult specimen. TEM. **a.** Cyanobacteria (cy) free in the mesohyle of ectosome. Filamentous bacterium (fb), phagosomes (ph) in an archaeocyte. Scale bar, 2 μm. **b.** Granular cell in the ectosome showing a nucleus (nu) and granules (gr); some granules are in place, others (clear oval spaces) disintegrated during processing. Scale bar, 0.5 μm. **c.** Choanosome, microvesicular cell displaying nucleolate nucleus (nu), and larger (vl) and smaller (vs) vesicles. Scale bar, 1 μm. **d.** Part of a choanocyte chamber. Scale bar, 2 μm. **e.** Choanosomal bacteriocyte with bacteria (ba) incubated in a vesicle (bv) and being broken down inside a phagosome (ph); part of a microvesicular cell (mv) is on the right. Scale bar, 2 μm.

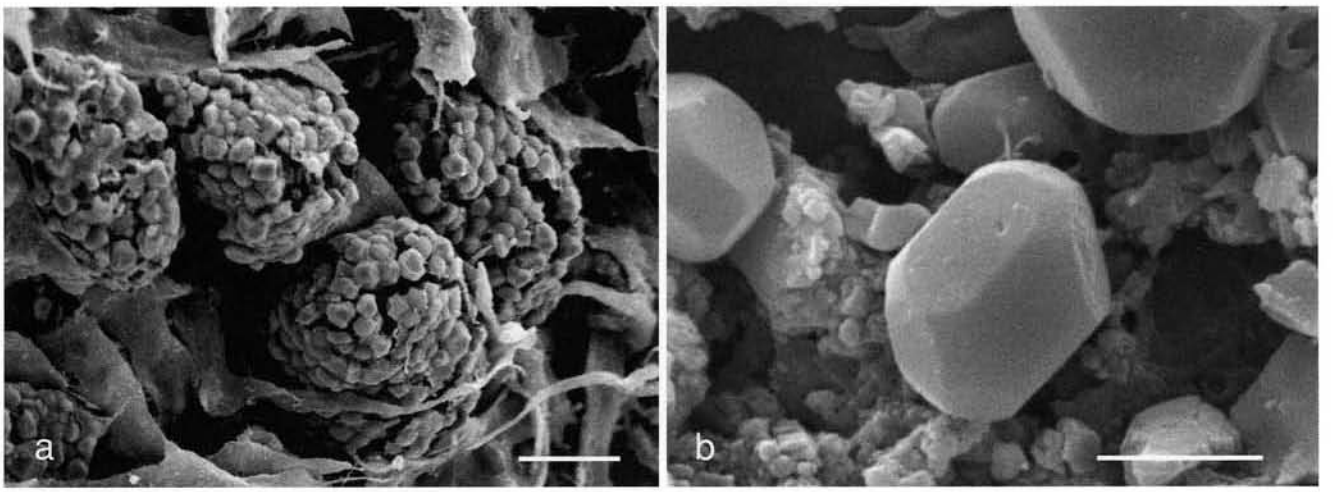


Fig. 4. *Svenzea zeai,* granular cells and granules. SEM. **a.** Cells in choanosomal tissue. Scale bar, 10 μm. **b.** Granules separated by mechanical grinding of choanosomal tissue and specific-weight separation in an alcohol suspension; note faceted shape maintained in this organic structure despite processing stress. Scale bar, 2 μm.

for the final granules. Rare loose granules can be found in the mesohyle, possibly a preparation artifact. Granules isolated from their cells by mechanical means (grinding) show under SEM the distinctive angular shape (Fig. 4b). Unless coated by carbon, they disintegrate readily under the electron beam.

The most common bacteria in the ectosome are single, oval cyanobacteria of the *Aphanocapsa feldmanni*-type, 2.4 × 1.4 μm (mean length × width from near-median TEM sections) (Fig. 3a). The thylacoid is a spiral lamella surrounding the nucleoid. Cell division is by median constriction perpendicular to the longer axis (resembling a figure-8 before separation of daughter cells). Many cyanobacteria are found in various stages of digestion inside sponge-cell vacuoles. There are also a few (<1%) extracellular coccoid and filiform heterotrophic bacteria.

Choanosome. A substantial part of the body is composed of the isodictyal reticulate skeleton made up by styles, with a small percentage of oxeas with acerate tips (<10%) mixed in, cemented at the nodes by spongin. The spicules measure 190–290 (253) × 3.8–10.0 (7.0) μm (range and means of 25 measurements each for 3 specimens); the mesh size is determined by the spicule length. Granular cells are just as common here as they are in the ectosome; in this region they are joined by choanocyte chambers, 23–35 × 20–30 μm. The chambers are circular to oval, commonly flattened on the side of the apopyle, and contain few choanocytes, only 8–17 per cross-section (Figs. 2c, 3d). Other prominent cells in the choanosome are bacteriocytes, loose bacteria, and grayish (weakly-staining; LM) cells. Bacteriocytes are like those of the ectosome but they are filled with heterotrophic (non-photosynthetic) bacteria, similar in shape and mode of division, but without thylacoid and smaller, 1.8 × 0.9 μm (average length × width from near-median TEM sections) (Fig. 3e). The same bacteria also occur free in the mesohyle.

The "gray" cells appear dark gray and grainy under LM and are irregularly lobate in outline (Fig. 2c). They have dense, microgranular content and a nucleolate nucleus (some with 2 nucleoli). In LM sections they measure 20–30 × 15–28 μm, with a 4–5 × 4–8-μm nucleus and a 1.5–2-μm nucleolus. Under TEM, these cells are packed throughout with weakly to moderately electron-opaque, spherical or bean-shaped inclusions 0.4–1.2 μm in cross-section, leaving space only for a few slightly larger vesicles containing bacteria, minute elongate vesicles, and the nucleus packed in between the grains (Fig. 3c). Vesicles containing bacteria are spherical to ovoid (1.4–2.3 μm); the bacteria are in stages of disintegration. The small vesicles between the inclusions are worm-shaped, 70 × 650 nm (diameter × maximum length in section; inflated parts are up to 150 nm wide), clear to highly electron-translucent, with membranous and fibrous structures inside. They are particularly dense and interwoven in the area surrounding the nucleus. The ratio of these microvesicular cells to choanocyte chambers and to granular cells in the choanosome is approximately 30:10:10 (average of 10 counts of 0.1 mm^2 areas).

Larval development, behavior, and fine-structure

Embryonic stages. Early embryos can be noted in the choanosome, where they appear as cell clusters surrounded by a cellular envelope (Fig. 2d). More developed embryos are conspicuous inside the maternal sponge, even to the naked eye, because of their large

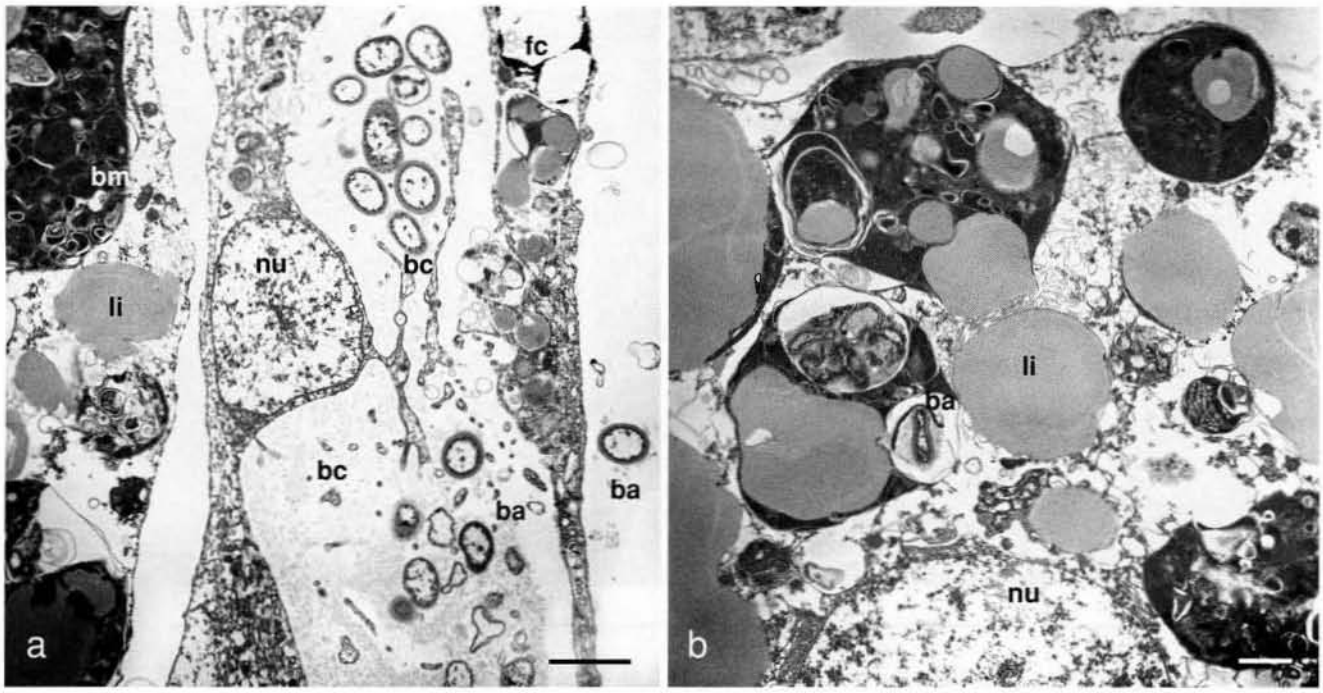


Fig. 5. Embryo of *Svenzea zeai*. TEM. **a.** Bacteriocyte (bc) with nucleus (nu) and bacteria (bc) inside a follicle cell (fc); part of a blastomere (bm) with lipid inclusion (li) is also visible. Scale bar, 2 μm. **b.** Part of a blastomere showing nucleus (nu) and inclusions such as phagosomes containing disintegrating bacteria (ba) and lipid droplets (li). Scale bar, 1 μm.

size (>1 mm) and distinctive color. Embryos in most sponges are yellow to orange-yellow; in a few parental specimens, they are cream, but otherwise macroscopically similar. Large, well-defined embryonic spherules seem to have severed cellular ties with the maternal sponge and readily fall out of the bounding tissue. They may survive for weeks in running seawater in the laboratory without attaching to substrate or developing into larvae. Liberated embryonic balls have a smooth outer surface and break up into a milky cell suspension when slightly squeezed under a cover slip. Excised embryos show fine knobs and filamentous extensions which penetrated the maternal tissues where they were anchored.

Embryos are encapsulated in a pinacoderm-like cell layer (Fig. 5). These follicular cells are flat (0.4–1.0 μm thick) except for the area of the anucleolate nucleus (3.5 μm) and other bulges (to 5 μm) from which arise clusters of stout pseudopodia and thread-like filapodia, extending into the surrounding maternal tissue and into the embryo. The cell content includes phagosomes and moderately electron-opaque granules (1.0–1.5 μm). The embryonic cell mass within the follicle is composed mainly of blastomeres with a few bacteriocytes. The blastomeres (30–40 × 20–30 μm) are technically archaeocytes, each with a nucleolate nucleus (6–8-μm nucleus, 1-μm nucleolus), and are filled with phagosomes (5–10 μm). The phagosomes contain bacteria in various stages of digestion, strongly electron-opaque areas with fibrous structure suggesting residual bacterial membranes, and unstructured, moderately electron-opaque granules. These granular inclusions can be seen protruding from the phagosome and as granules free in the cytoplasm.

Cream-colored embryo-like globules are very similar in histological structure to the more common yellow ones, but in addition to archaeocytes and bacteriocytes, they contain the same kind of granular cells described for the adult tissue above. Many granular cells can be seen engulfed by archaeocytes and apparently being digested by them.

Observations on live larvae. Seemingly fully developed embryos, spheres ~4 mm in diameter, readily released by breaking sponges apart by hand, were closely observed for 1–3 weeks at a time during the months of January and February (1997), April (2002), July (1995), August (1997), and November (2000). Of several hundred specimens, only 4 transformed into swimming larvae, 1 in August and 3 in November, all during mid-morning, although observations started at sunrise. The spherical embryo changes its shape and becomes an elongate, swimming-floating larva with the larger, anterior end flattened and the smaller, posterior end rounded. Although its cross-section is basically circular, it may change to flat or irregular and back again without obvious cause, but possibly in re-

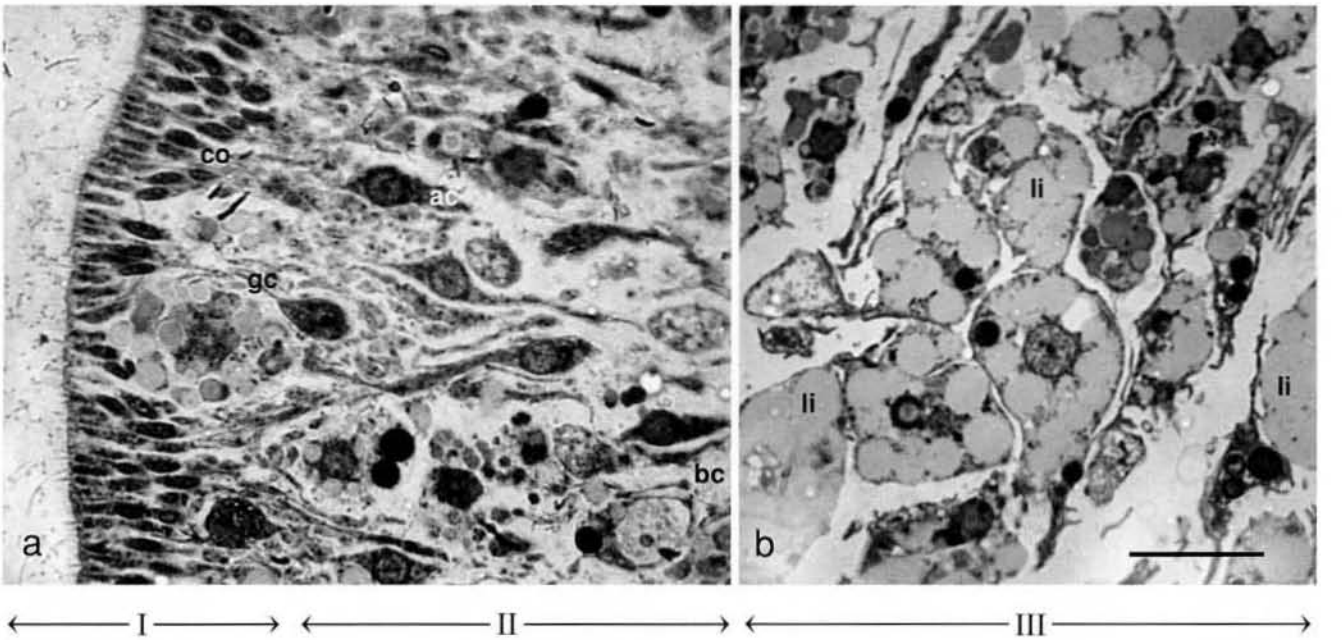


Fig. 6. Larva of *Svenzea zeai,* showing the 3 body zones: I, ectosome; II, subectosomal sheath; III, core. Longitudinal sections (ground, stained in toluidine blue). LM. **a.** Layer I and distal part of II with columnar epithelium (co), granular cells (gc), archaeocytes (ac), and bacteriocytes (bc). **b.** Core (III) with inclusion cells full of lipid (li) globules. Scale bars, 10 μm.

sponse to the light and warming from the stereomicroscope illuminator. Sizes of the 4 larvae ranged 6.0–6.3 mm long and from 2.1 mm (circular) to 3.0 × 1.5 mm (flattened) in diameter. The surface bears ~14 longitudinal grooves (Fig. 1c) that run from near the flat top toward the narrow end and stop two-thirds of the distance down the body length. The ridges between the grooves may develop smaller secondary grooves. Cilia are not visible under a dissecting microscope, but were made discernable by gently squeezing a larva in a depression slide under phase-contrast optics; they cover the entire body. Ciliary length could not be measured exactly in this way, but was estimated at 25 μm near the ends, and somewhat shorter (~20 μm) along the rest of the larva. Unlike other known tropical sponge larvae, those of *Svenzea zeai* float nearly motionless near the bottom or at the water surface of a dish (Fig. 1c). In the laboratory, active motion by the larvae could be detected only after stopping all water and air flow. The position of the larva floating through the water column is oblique, typically angled 10–20° from a vertical line, with the larger end towards the water surface. There is a slow, barely perceptible right-handed rotation (counter-clockwise, as seen facing the larger, anterior end), 1 turn per second or less. Settlement was not observed, owing to the scarcity of fully developed larvae.

Larval anatomy. Only a single larva remained intact through all fixation steps for LM and retained its general shape; it shrank ~5% in length (to 5.8 mm) and 10% in diameter (to 1.9 mm). A median longitudinal section in transmitted light (Fig. 6) shows 3 zones, a central region surrounded by 2 concentric layers. The outermost layer (layer I) is 50–300 μm thick (~50 μm at the larger end of the larva, 150 μm along the sides, and increasing toward the smaller end) and uniformly covered by cilia of nearly equal length, 15 μm on average (a few extremes range 8–24 μm, the result of various degrees of contraction during fixation). Optically, it is moderately dense. Medial to this layer is a fairly sharply defined layer (II) of densely packed, dark inclusion cells. It appears optically dense, almost opaque in transmitted light. Layer II is almost 400 μm thick at the larger, anterior larval pole, 300 μm in the middle part of the larva, and 900 μm at the narrow, posterior pole. The central mass (III) is optically the most transparent, composed of loosely arranged cells containing large, light gray inclusions. There is no larval cavity or larval spicules. Shrinkage during fixation caused some tearing in layer II and between layer II and the core (III).

Layer I is an epithelium of long, slender, ciliated cells oriented perpendicular to the surface (Figs. 6–8). Near their bases, some 25–30 μm below the surface, the ciliated cells are interlaced with much larger but also radially stretched archaeocytes and granular cells.

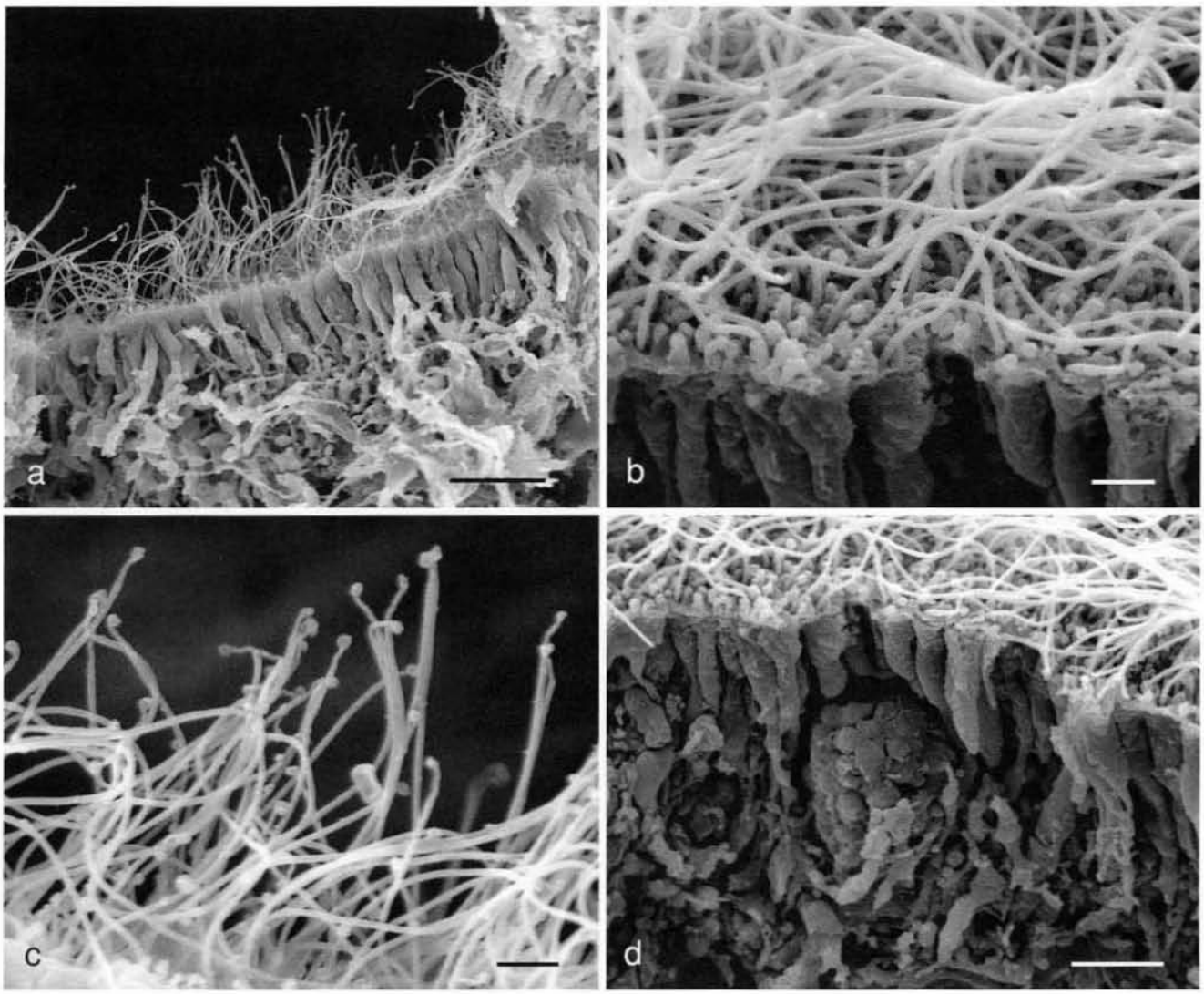


Fig. 7. Larva of *Svenzea zeai*. SEM. **a.** Fracture showing columnar epithelium of ciliated cells. Scale bar, 10 μm. **b.** Ciliate cells from above showing protoplasmic extensions from the cells between the cilia. Scale bar, 1 μm. **c.** Cilia close up, with knobbed ends. Scale bar, 1 μm. **d.** Fracture exposing granular cell wedged between the lower parts (nuclear region) of the ciliated cells. Scale bar, 10 μm.

Together they form what may be called a columnar larval ectosome. Most ciliated cells are long, thin, and twisted (Fig. 8a,b) and show an oval to spindle-shaped basal nucleus (2.5 × 1.0 μm, dense heterochromatin); because they are never entirely located in the sectioning plane, they are difficult to measure, but SEM images indicate that they may be as long as 30–50 μm, with a diameter of 0.5–2.5 μm. These cells are barely wider than the nucleus at the base where the cell bodies taper out thinly toward the larval interior. Distal to the nuclei, the cell bodies first slim and then increase in diameter toward the top. Their distal ends form ciliary cups (crypts), 1.4–2.5 μm wide (from SEM), which are flattened on the sides where they are attached to their neighbors by way of desmosome-like junctions (30 nm wide). The larval surface between the cilia, on both SEM- and TEM-fixed material, bears wart-like or bulbous protuberances. There is one cilium (200 nm diameter) per cell, originating from a basal body and anchored by a fibrillar but non-striated rootlet that extends toward the nucleus, ending in a knob-like expansion. The ciliated cells contain numerous spherical mitochondria (300–400 nm, many with a single electron-opaque granule) and elongate vesicles (800 × 200 nm average) containing moderately electron-opaque granules which become strongly electron-opaque at the rounded extremities (Fig. 8b insert).

The archaeocytes in this layer are spindle-shaped

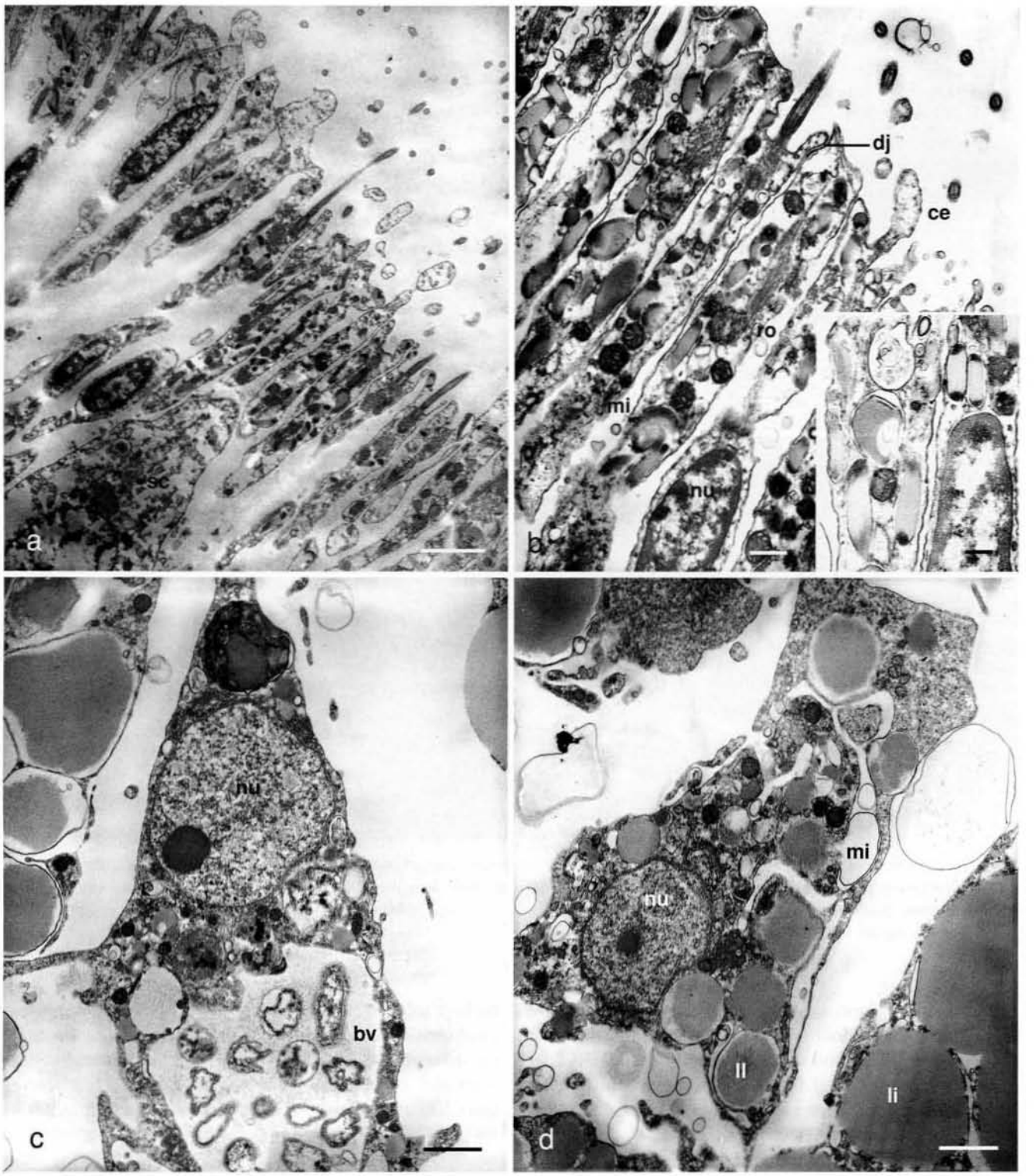


Fig. 8. Larva of *Svenzea zeai*. Sections through the larval ectosome perpendicular to the ciliated surface. TEM. **a.** Columnar epithelium of ciliated cells with part of a spherulous cell (sc) in radial orientation. Scale bar, 2 μm. **b.** Columnar epithelium enlarged showing cytoplasmic extensions (ce), desmosome-like junctions (dj), mitochondria (mi), nuclei (nu), and rootlets (ro); inset: enlarged elongate granules. Scale bar, 0.5 μm; inset, 0.2 μm. **c.** Bacteriocyte with large vesicle filled with bacteria (bv) and nucleolate nucleus (nu). Scale bar, 1 μm. **d.** Granular cell with nucleolate nucleus (nu), lipid-like granules (ll), and spherical mitochondra (mi); a neighboring spherulous cell contains a lipid inclusion (li). Scale bar, 1 μm.

and are oriented perpendicular to the larval surface. The main cell body averages 12 × 5 μm, but gradually tapering extensions increase the total length to 26 μm; it contains a spherical nucleus (3–4 μm) with a nucleolus (0.5 μm) and numerous phagosomes. Granular cells are also radially oriented in this layer (Fig. 8d), but they are less stretched out (14 × 8 μm), have a nucleolate nucleus (3.5 × 2.5 μm), conspicuous Golgi body, and spherical mitochondria (300–400 nm); they are packed with globular to irregularly shaped inclusions (1–3 μm) that are moderately electron-opaque and stain strongly in toluidine blue. Many granular cells contain electron-opaque, structured phagosomes, some of which contain moderately opaque areas (10–90% of the cross-section), suggesting active synthesis of nutrients and deposit in the form of lipid granules.

In the transitional region between layer I and layer II, the radial orientation becomes less pronounced and finally disappears. There is an abundance of large bacteriocytes (13–27 × 5–9 μm) in this region (Fig. 8c), each containing a spherical nucleolate nucleus (3–4 μm), phagosomes, and 1 or 2 large vesicles, filled with bacteria (up to 20 per cross-section), that make up most of the cell volume. Some of the bacteriocytes are spindle-shaped with long, filamentous cell extensions, but most are nearly spherical. A number of sections show phagosomes and a few moderately electron-opaque spherules.

The subectosomal sheath (layer II; beginning 50–300 μm below the larval surface) contains more of the bacteriocytes, granular cells, and archaeocytes, but is primarily made up by crowded spherulous cells with large lipid globules (Figs. 6b, 8d). The spherical cell bodies measure ~30 μm in diameter, contain a 4–6-μm nucleus (without nucleolus), an occasional phagosome containing bacteria, and very little cytoplasm that separates large, moderately electron-opaque globules (3–15 μm). These inclusions, which stain light grayish blue in toluidine blue, are so densely packed that the nucleus appears indented by the pressure from one or more.

The central mass, or core (layer III; Fig. 6b) contains more bacteriocytes and the same type of spherulous cells described from the subectosomal sheath, but the cells and granules are much less crowded together, leaving extended electron-translucent spaces between them, without structure detectable by TEM. On the other hand, lipid globules reach diameters of nearly 30 μm and in many TEM sections, the granules seem to be merely tied together by cytoplasmic threads or free among the cells.

Estimates of volumes taken up by the different larval tissue zones (after adjusting measurements to an idealized ellipsoid bodyshape) are 5% for the columnar ectosome (I), 71% for the subectosomal sheath (II), and 24% for the central mass (III).

Scopalina ruetzleri, adult sponge and larva

Adult macroscopic morphology

This sponge is found in Caribbean lagoon habitats where it is conspicuous on semi-shaded substrates, such as cave walls and mangrove roots at 1–3 m depths (Fig. 9a). It is encrusting to massive, with a soft, limp consistency. Most specimens are brilliant orange, but a few are ochre. The surface is covered by distinctive slender conules, 1–5 mm (average 2 mm) tall and 1–4 mm apart, which mark the endings of skeletal fibers. Sparse oscula are flush with the sponge surface, ~2 mm in diameter; ostia are concentrated in porous areas. The interior is ochre and cavernous. Some specimens, when cut open, display clusters of orange balls, ~1 mm in diameter, which are almost fully developed embryos (Fig. 9b).

This species was originally described in the genus *Ulosa* (Wiedenmayer 1977) and transferred to *Scopalina* by van Soest (1993). De Laubenfels (1950) provided a detailed description under the name *Dysidea crawshayi* de Laubenfels, a mistaken identify.

Adult histology and fine-structure

The skeleton of *Scopalina ruetzleri* consists of dendritic spongin fibers (15–85 μm in diameter) connected by secondary branches. The main fibers radiate toward the surface where they lift the ectosome to form the characteristic conules; each has a core of long, slender spicules, styles with oxeote modifications, 180–530 (388) × 5.0–7.5 (6.1) μm (range and mean, 3 specimens).

The ectosome is a thin zone, 90–110 μm thick, rich in layered spongin and associated collencytes. Granular cells (Fig. 10) are abundant, with anucleolate nuclei (3–5 μm) and glassy, faceted inclusions (1–3 μm), stained red where exposed to the dye safranin O. Cytoplasmic inclusions are phagosomes and single bacteria in vesicles. In TEM preparations, most larger granules dropped out of the cells leaving behind holes in the embedding medium, which apparently did not penetrate the granules as well as the rest of the cytoplasm.

Choanocyte chambers are near-circular in outline, crowded throughout the choanosome. Excluding areas with major channels, their density in sections is 50–70 per 0.1 mm^2. The larger cross-sections (likely to be through the center) measure 30–36 μm in diameter and are bordered by 19–22 choanocytes. Oocytes (30 μm diameter, 9-μm nucleus), spermatic cysts (18–20 μm),

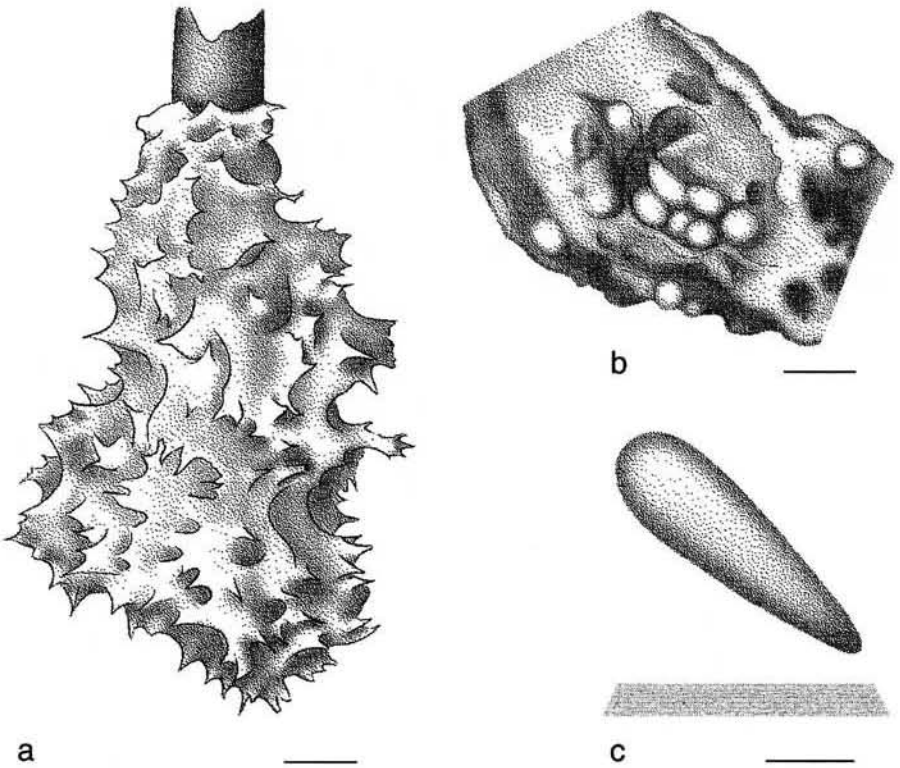


Fig. 9. *Scopalina ruetzleri.* **a.** Drawing of the live sponge covering a red-mangrove stilt root. Scale bar, 10 mm. **b.** Specimen cut open to display a cluster of embryos. Scale bar, 2 mm. **c.** Larva swimming with the larger, anterior pole first. It will come to rest in an oblique position, contacting the substrate and attaching by the narrow, posterior end. Scale bar, 0.5 mm.

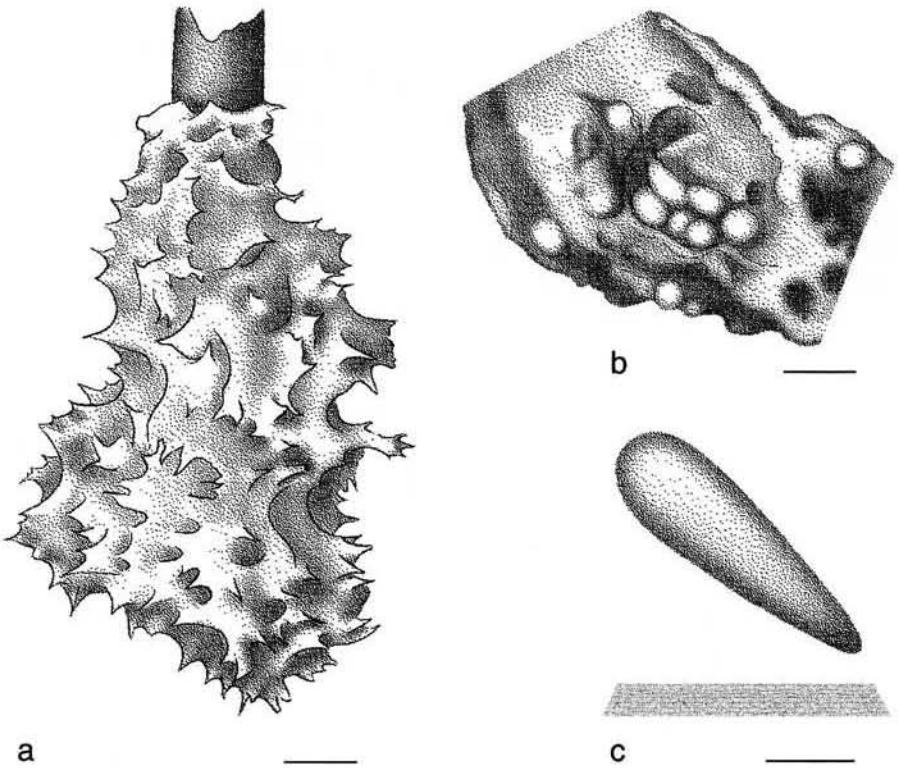
and embryos of various stages (blastomeres surrounded by a pinacoderm-like follicular envelope) occur throughout the choanosome of reproductive specimens.

Larval morphology, behavior, and fine-structure

The live, freshly released larva (Fig. 9c; obtained during August 2001) is deep orange, like most of the adult sponges, and measures 1.2–1.8 × 0.4–0.5 mm. It is elongate, circular in cross-section, with a larger, rounded anterior pole and a thinner, more pointed posterior. This basic shape may change slightly by gradual contraction and expansion. The entire body is densely covered by short cilia (~20 μm average length), with slightly longer cilia (22 μm) around the poles. Swimming a straight course is quite fast, 4–5.5 mm/sec (based on 10 larvae, 5 from each of 2 parent sponges), and involves slow, right-handed rotation (counterclockwise as seen facing the larger, anterior pole), but no spiraling. If an obstacle is encountered, such as the wall of an observation dish, the larva tends to come to rest in an oblique position, larger (anterior) pole upward, at the air-water interface. Likewise, when settlement time approaches after 1–3 days of swimming (in laboratory dishes), the larva assumes an oblique position toward the substrate, which it touches with the narrow (posterior) pole until it becomes attached and flattens.

Shape of the chemically fixed larva (size 1.25 × 0.4 mm), ciliary arrangement, and ciliary terminal knobs (possibly an artifact) are revealed by SEM examination (Fig. 11). Viewed in cross-section, there are short cilia densely covering the surface and originating from the columnar epithelium of the ectosomal layer I (25 μm thick), a subectosomal sheath II (50 μm thick), and a central mass III (Fig. 12). The cilia are 10–12 μm long and 200 nm thick; a few reach 18 μm in length. Cilia emerge from slim, elongate cells (11–30 × 1.0–1.6 μm), each with an oval nucleus marked by dark heterochromatin (2.5 × 1.5 μm) and located at the base of the cell (Fig. 13a,b). The ciliated cells are widest at the levels of crypt and nucleus, tapering to a fine point below the nucleus; they contain a basal body and filamentous rootlet connecting to the single cilium, and numerous spherical mitochondria (300 nm). The distal ciliary crypts connect to their neighbors by desmosome-like junctions, 30 nm wide.

Archaeocytes and granular cells are also part of the columnar ectosome. The archaeocytes, 10 μm below the surface, are oval to spindle-shaped, in radial orientation, 12–15 × 5–7 μm, with a 3.5 μm nucleolate nucleus and a few phagosomes. Just below, 20–50 μm from the surface, are granular cells wedged in among and displacing the bodies of the ciliated cells (Fig. 13c). These cells are droplet-shaped, narrow end pointing out, 12–20 × 6–10 μm, with a 2.5 μm spherical



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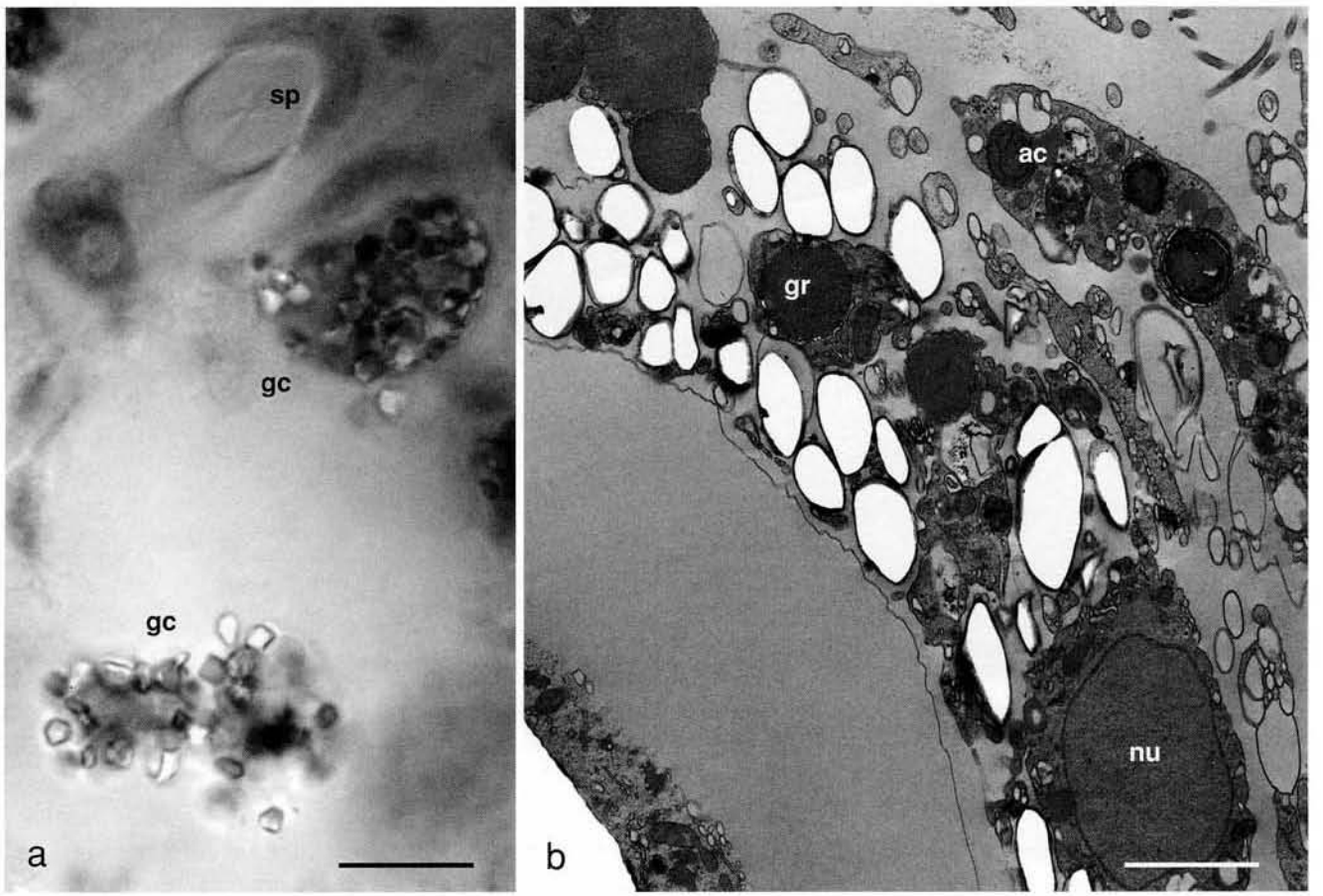

Fig. 10. *Scopalina ruetzleri,* micro-anatomy of an adult specimen. **a.** Ectosome, polished thick-section showing granular cells (gc) stained in toluidine blue; a cross-section of a spicule (sp) is near the top. LM. Scale bar, 10 μm. **b.** Granular cell with nucleus (nu) and granules (gr); many granules are missing from their vesicles (white spaces); adjacent cell is an archaeocyte (ac). TEM. Scale bar, 5 μm.

(often nucleolate) nucleus, and filled with granules (0.8–1.5 μm). Golgi bodies are often distinct and there are numerous spherical mitochondria (250–275 nm). The inclusions stain deeply blue (toluidine blue), are highly electron-opaque, and are distinctively structured in TEM. Each granule is located in a vesicle with a well-defined 25-nm wall and consists of flocculent, moderately electron-opaque material that appears more electron-dense toward the center. At higher magnification, the dense part shows a striation of 30 nm periodicity, bars ~18 nm wide separated by lighter, 12 nm spaces. In some granules, the striation appears in cross section revealing rows of octagons intersecting at 30° angles (Fig. 13d).

The spherulous cells are more abundant at the base of the ectosomal layer (I) where archaeocytes co-occur, both with some radial orientation. In this zone, the archaeocytes are angular, 12 × 6 μm, with pseudopodia extending to 19 μm, and a nucleolate nucleus (3.5 μm). Some contain moderately electron-opaque lipid droplets (1–2 μm). Similar cells, but larger (18 × 12 μm) with a nucleolate nucleus (4.5 μm) and large inclusions (5 μm), occur in this zone and make up the entire central mass, where they are loosely dispersed. The lipid globules are moderately electron-opaque, lack structure under TEM, and stain light bluish gray with toluidine blue; many appear to be free (outside cells), and/or in clumps.

A quantitative estimate of each zone, based on the volume of an idealized ellipsoid larva, is 28% columnar ectosome, 39% subectosomal sheath, and 33% central mass.

Discussion

General morphology

Svenzea zeai has the same appearance as many massive coral reef sponges, without unusual features that would raise the attention of an observant diver (Alvarez et al. 1998). Only careful examination of ana-

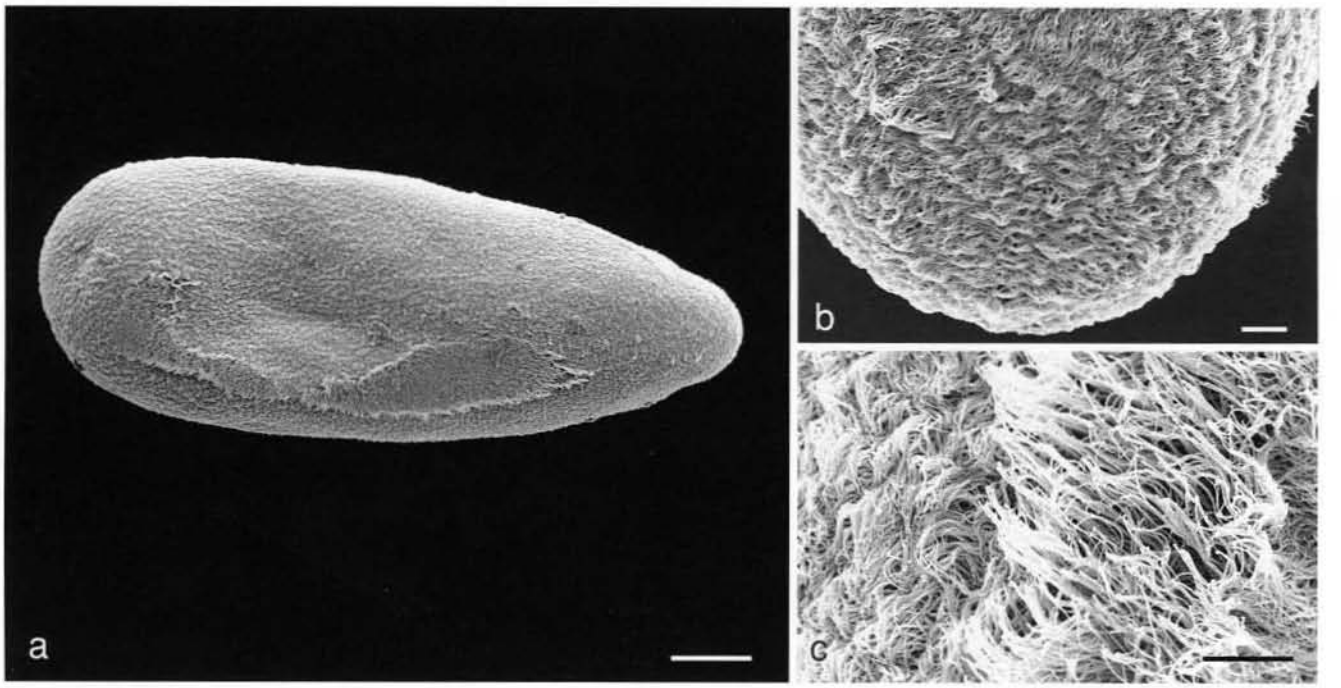

Fig. 11. Larva of *Scopalina ruetzleri.* SEM. **a.** Whole larva, larger, anterior pole (determined from the swimming direction) to the left. Scale bar, 100 μm. **b.** Close-up of anterior pole. Scale bar, 10 μm. **c.** Cilia with knobbed ends. Scale bar, 10 μm.

tomical details reveal that this is a bacteriosponge containing substantial populations of auto- and heterotrophic prokaryotic symbionts; that the skeleton consists mainly of short stout styles that are arranged in isodictyal reticulation (a unique combination); that histological features include unusual granular cells; that abundant and exceptionally large embryos occur year-round, although mature larvae are rarely ob-

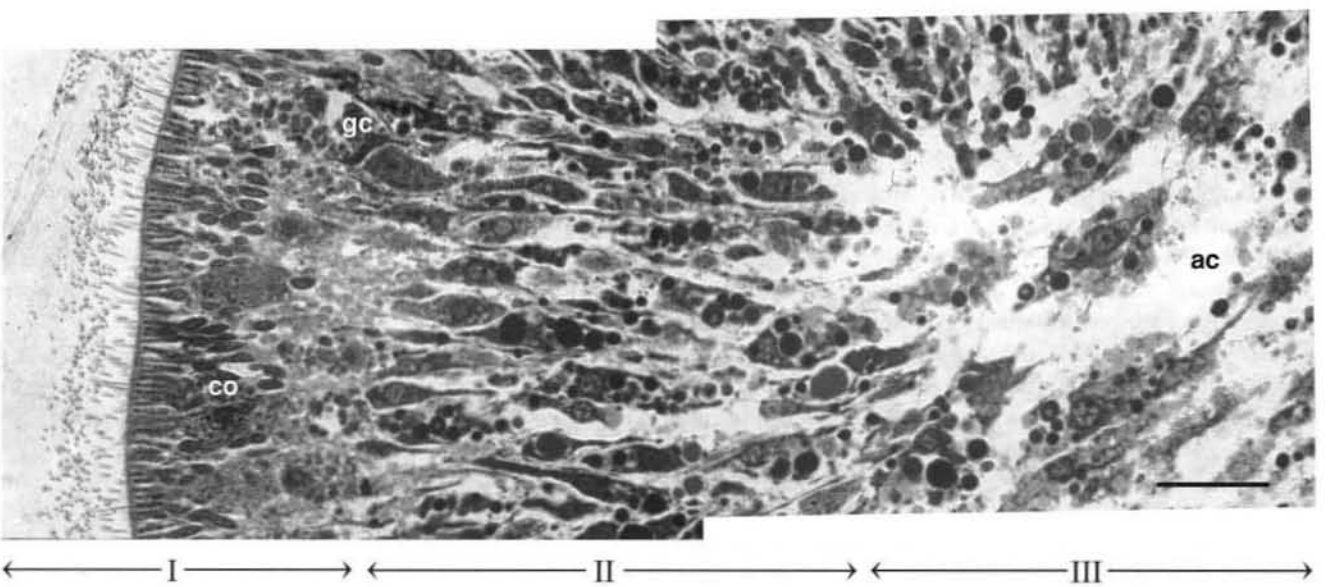

Fig. 12. Larva of *Scopalina ruetzleri,* showing the 3 body layers: I, larval ectosome; II, subectosomal sheath; III, core. Cross-section (semi-thin section stained in methylene blue). Layer I includes the columnar epithelium (co) of ciliated cells, layer II shows granular cells (gc), and layer III is composed of archaeocytes (ac) containing lipid droplets. LM. Scale bar, 2 μm.

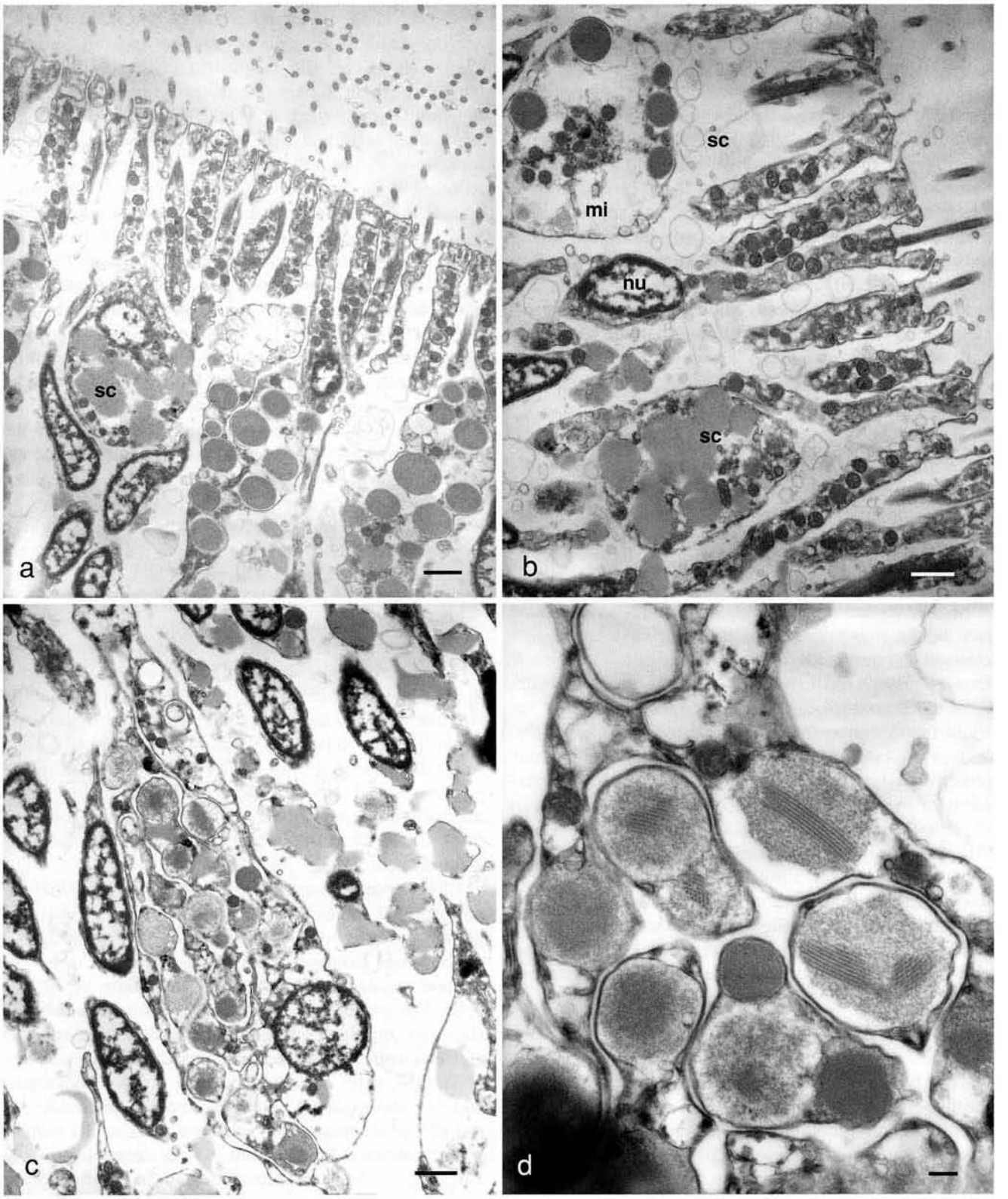

Fig. 13. Larva of *Scopalina ruetzleri,* sections through the larval ectosome perpendicular to the ciliated surface. TEM. **a.** Columnar epithelium of ciliated cells with a spherulous cell (sc) wedged in at the level of the ciliated-cell nuclei. Scale bar, 1 μm. **b.** Ciliated cells with elongate nuclei (nu) and spherulous cells (sc) showing mitochondria (mi). Scale bar, 1 μm. **c.** Granular cell near nuclear region of ciliated cells. Scale bar, 1 μm. **d.** Detail of granules with crystalline structures. Scale bar, 0.2 μm.

served; and that the 6-mm-long larvae are 3–5× larger than the largest known from sponges of any kind, but barely swim, mostly rotate and float.

Scopalina ruetzleri is ecologically adapted to lagoonal water, where it tolerates short-term exposure to air during low tides (Rützler 1995). It lacks constant populations of bacterial symbionts and its skeleton consists of long slender styles at the core of dendritic spongin fibers, but it possesses the same unusual granular cells as seen in *Svenzea zeai.* Embryos appear only seasonally (late spring to summer), shortly before they are released as large larvae (up to 2 mm and among the largest hitherto known), which are active swimmers, but take a straight rather than the corkscrew path of many other sponge larvae.

Bacterial symbionts

Svenzea zeai is a bacteriosponge because nearly half its biomass consists of microbial symbionts (Reiswig 1981). Its reddish- to purplish-brown color reflects the presence of small unicellular ("*Aphanocapsa feldmanni*-type") cyanobacteria in the ectosomal region penetrated by light (Sarà 1971; Wilkinson 1980; Vacelet 1981; Rützler 1990). Deeper tissue regions are densely populated by heterotrophic unicellular bacteria located free in the mesohyle or inside large vesicles of specialized bacteriocytes in the choanosome (Vacelet & Donaday 1977; Wilkinson 1978). Both bacterial types have nutritional roles, either by phagocytosis or by nutrient translocation from symbiont to host (Diaz 1979; Wilkinson 1980; Rützler 1981). Bacteriosponges may contribute significantly to the process of nitrogen fixation in the sea (Wilkinson & Fay 1979; Corredor et al. 1988; Diaz & Ward 1997). Symbiotic bacteria in this sponge are engulfed and digested by cells, mostly archaeocytes, of the adult sponge and are transferred live to the embryo. Bacteria are passed to the next generation mainly by migration of bacteriocytes (Gallissian & Vacelet 1976; Lévi & Lévi 1976), but this may also be accomplished by extracellular pathways (Kaye 1991).

Granular cells

The function of the granular cells that abound throughout the 2 sponges studied and the nature of their characteristic angular inclusions is not yet understood. Very similar-looking cells were previously described as chromocytes, present in sponges of the genera *Cyamon* and *Trikentrion* (Raspailiidae) (Smith 1968). The cells and granules are both in the same size class as those of *Svenzea zeai* and *Scopalina ruetzleri*; the nucleus is anucleolate; and the inclusions are angular, stain strongly in semithin sections, are electron-dense in TEM, and tend to drop out from the cell leaving behind a similarly shaped space ("lucent" area). However, the raspailiid chromocytes were named for their content of carotenoid pigments, absent in granular cells of *S. zeai* and *S. ruetzleri,* which, if not stained artificially, have glassy colorless, refractile inclusions. A possible function in nutrient storage and as nurse cells has been suggested for cells of similar appearance in species of the dictyoceratid genus *Aplysina* (as *Verongia;* Gallissian & Vacelet 1976). Spherulous cells described by these authors are 6–7 μm in diameter, with an anucleolate nucleus, and with 1–2-μm spherules (up to 10 μm with 1–3-μm inclusions on their plates IIIe, VIc) that are angular in outline. The main difference from our dictyonellid granular cells is that aplysinid granules have a substructure of osmiophilic microspheres (100 nm). The cells in *Aplysina* were seen incorporated inside the follicle of the maturing oocyte of these oviparous sponges, along with symbiotic bacteria that are embedded in the outer cytoplasm.

Until we can determine the chemical composition of these unusual inclusions, we assume that it is protein- or lipid-based because the granules lack mineral properties, absorb histological stain, evaporate under the electron beam (unless carbon coated), and are phagocytized and digested during the formation of embryos. In TEM, they look similar to the moderately opaque lipid globules, except that the granules are generally smaller and have an angular shape which is maintained when they are separated from the cell (Fig. 4b). They resist dissolution during brief exposure to sodium hypochlorite and larger granules are not readily penetrated by embedding resin, hence the holes left in sections where they dropped out or evaporated under the electron beam (Figs. 3b, 10b).

Close examination of both sponges suggests that developmental stages of these granular cells occur as amphora-shaped bodies (comparable to urn cells described by Boury-Esnault 1976) wedged into the nuclear region of the columnar epithelium of ciliated cells. The granules in larval cells are small but already show an angular outline and have the same staining and electron-density properties as their adult counterparts. The cells are often but not always nucleolate, and they have conspicuous Golgi bodies and numerous mitochondria, many with one or a few minute osmiophilic granules. One feature, so far seen only in developing granules of *Scopalina ruetzleri,* is the denser areas inside the moderately electron-dense granules that, at high magnification, are seen to be crystalline in structure. Similar patterns were reported for the inclusions of spherulous cells in adults of *Verongia* (Dictyoceratida) (Vacelet 1967) and in yolk-like granules

in larval "gray cells" of *Haliclona* sp. (Haplosclerida) (Amano & Hori 1994).

Archaeocytes, microvesicular cells, and reproductive cells

Two additional inclusion cells are commonly seen in sections of the choanosome of *Svenzea zeai*: archaeocytes and gray (in transmitted light) microvesicular cells. The archaeocytes are characterized by their large size, ameboid shape, nucleolate nucleus, and load of phagosomes (De Vos et al. 1991). The phagosomes contain single-celled bacteria that range from intact or only slightly deformed cells to coils of undigested membranes and droplets of lipids. Microvesicular cells appear colorless and dark under the light microscope (they accept stain poorly) and have a microgranular cytoplasm. They are different from the "gray cells" that have been described in many sponge species and are more appropriately termed glycocytes (Boury-Esnault 1976; Simpson 1984; Boury-Esnault & Rützler 1997). Unlike the glycocytes, the microvesicular cells are unusually large and have a nucleolate nucleus, abundant moderately electron-opaque granules alternating with minute elongate vesicles, and a few larger vesicles containing bacteria in various stages of disintegration. They lack conspicuous Golgi bodies and glycogen rosettes. They are rather similar in vesicular structure to cruciform cells in the amphiblastula larva of Calcarea (Amano & Hori 1992), but the latter are lost in the adults and are attributed a light-sensory function, unlikely in our present material.

Oocytes were rarely seen but are distinguished by spherical shape, large size, a nucleolate nucleus, and lipid droplets. Sperm cysts were not encountered in the sectioned specimens. Sections of *Scopalina ruetzleri* show archaeocytes, oocytes, and occasional sperm cysts but lack weakly staining grayish cells.

Embryos

Various stages of embryos are common year-round in the choanosome of *Svenzea zeai,* from a few blastomeres to 4-mm-diameter balls, all surrounded by flat follicle cells. Bacteriocytes are already incorporated in young embryos and contribute to the production of storage material (yolk) through phagosome activity, but cytoplasmic extensions from the follicle cells suggest the transfer of additional nutrients from the mother. Embryos of most specimens of *S. zeai* are deep yellow and rich in yolk, but in a few they are cream, similar to the color of the sponge interior. The presence of granular cells in addition to archaeocytes and bacteriocytes may indicate that the cream-colored "embryos" are asexually derived buds, not uncommon in sponges, but at times wrongly interpreted as sexual offspring (Wilson 1894, 1902; Simpson 1984). It is unknown how the embryo develops into a ciliated larva and whether in nature this is a more dynamic and wide-spread event than in the laboratory. An attempt to catch larvae *in situ* on a Curaçao reef by placing nets above adult sponges (February 2000) resulted in only 6 trapped specimens, but suggests that larvae might swim or float upward before settling (R. Gomez, pers. comm.) In *Scopalina ruetzleri,* embryonic stages are most common during spring (March–May) and are histologically similar to those of *S. zeai,* but embryos are bright orange-red, occur in clusters, and reach only 1 mm in diameter.

Parenchymella larvae

The larva of *Svenzea zeai* is uniformly ciliated over the entire body and is elongate, with rounded ends and a larger anterior pole. It lacks spicules, cavity, and choanocyte chambers. Despite its unusually large size—at least 3 times the length of any previously known sponge larva—and its longitudinal grooving, it closely resembles larvae of Halichondrida as classified and described by Bergquist & Green (1977), Uriz (1982), and Wapstra & van Soest (1987), several of which are also known for poor swimming ability—they float as they rotate around the long axis. As in *S. zeai,* the larva of *Scopalina ruetzleri* is cylindro-conical and uniformly ciliated; it can reach 2 × 0.5 mm. It swims with the larger pole forward, rotating but not on a spiral path, and settles on its smaller, posterior pole after coming to rest, obliquely oriented to the substrate. Similar dimensions and behavior have been reported for a related, Mediterranean species, *Scopalina lophyropoda* (Uriz 1982).

One feature common to all parenchymellae is the 3 distinctive anatomical zones (Bergquist & Greene 1977; Woollacott 1993): (I) an outer envelope composed of a columnar ciliated epithelium with associated cells (larval ectosome), (II) an intermediate layer or subectosomal sheath, and (III) a core. Cell composition varies with larval type and species but there are many parallels. The ciliated cells of the larva of *Svenzea zeai* are of only one type. They are very long and thin and can be measured only on SEM images of fractured specimens because they do not show in their entirety in sections. Similar lengths, reaching 50 μm, have been reported for other species as well (Woollacott 1990; Uriz et al. 2001). An abundance of mitochondria (many with single granules), a single cylindrical basal granule, filamentous rootlet, and nucleus with distinctive heterochromatin have also been reported before for parenchymellae (Amano & Hori

1994; Uriz et al. 2001). A conspicuous feature of larval ciliated cells of *S. zeai* is the abundance of elongate vesicles with moderately electron-opaque granules which abruptly become denser at both rounded extremities. Comparable structures of similar size (up to 500 × 200 nm) were reported for *Haliclona permollis* and used as markers to trace the development of ciliated cells into choanocytes (Amano & Hori 1998); the more elipsoid granules of *H. permollis* are, however, uniform in electron density and have a crystalloid substructure. Chemical composition and function of these granules remain unknown.

The proportions by volume of the body layers I (ectosome) and III (core) are not very different in the 2 species, but layer II (subectosome) in *Svenzea zeai* is nearly double that in *Scopalina ruetzleri,* owing primarily to a large number of bacteriocytes and cells with lipid reserves; this may be advantageous to a possibly long larval life.

SEMs of the larva of *Svenzea zeai* show wart-like or bulbous protrusions extending from the free distal surface of the ciliated cell. TEM confirms that they are cell extensions and not attached bacteria or other extraneous matter. Similar, but much less pronounced and rather filiform processes appear also on ciliated cells of *Scopalina ruetzleri.* Comparable cytoplasmic expansions are known from *Crambe crambe* (Poecilosclerida; Uriz et al. 2001) and *Haliclona* spp. (Chalinidae; Amano & Hori 1994, 1998), but seem to be absent from *Halichondria melanadocia* (Halichondrida; Woollacott 1990) and *Haliclona tubifera* (Chalinidae; Woollacott 1993). Cytoplasmic extensions could serve in uptake of dissolved organic matter, but it should be confirmed that they are not an artifact, e.g., the result of osmotic stress of chemical fixation.

Cilia of both *Svenzea zeai* and *Scopalina ruetzleri* end in terminal knobs, but not nearly as pronouncedly clubbed or spatulate as described from some other halichondrids (*Halichondria moorei, Ulosa* sp.; Bergquist et al. 1977). We assume that these features are the result of shrinkage during fixation.

Inclusion cells in both adult and larval sponges have important functions that are not easy to interpret. Their formation starts with archaeocytes ingesting food, primarily unicellular bacteria that reach the choanocyte chambers from the outside, or are incubated in the mesohyle or cultivated in specialized bacteriocytes within the adult or larval sponge. The possible connection between adult and larval granular cells is discussed above. Both species investigated have larvae with large amounts of lipid reserves stored in spherulous cells with bulbous vesicles in a large range of sizes. Cells with small lipid inclusions occur as part of the larval ectosome (layer I); with small and medium droplets in the subectosomal sheath (layer II); and with very large globules in the core (layer III). In the core the globules reach a diameter of 30 μm and overwhelm the carrier cells; many appear to be located outside cellular bounds. Large lipid reserves in parenchymellae are not common but have been found in a few other species of Demospongiae (Boury-Esnault 1976; Uriz et al. 2001).

Conclusions

In the absence to date of molecular evidence, we have tested our notion that the genus *Svenzea* is a member of the family Dictyonellidae (order Halichondrida) by comparing *S. zeai* to an undoubted representative of this family that is also abundant in Belize, *Scopalina ruetzleri* (Rützler 1995). Although this common lagoon and mangrove species is morphologically quite different from *S. zeai,* the 2 species are similar in having styles bound by spongin (albeit in *S. ruetzleri* in the core of fibers rather than cemented in reticulate fashion), the same enigmatic granular cells, and exceptionally large larvae of similar shape and structure, including the uniform ciliation, cell-type composition, lipid distribution, and mode of swimming. The principal histological difference between the 2 taxa is the lack of a massive population of symbiotic bacteria in *S. ruetzleri,* not considered to be of systematic significance.

Acknowledgments. We thank Jennifer Dorton, Michael Lang, Michelle Nestlerode, Cheryl Thacker, Martha Robbart, and Sven Zea for their enthusiasm in collecting sponges used in our study. Rachel Gomez provided useful field observations. This work could not have been accomplished without the expertise in electron microscopy offered by Andrea Brothers (sectioning, TEM operation) and Kathleen McGinley, Michelle Nestlerode, and Scott Whittaker (SEM operation). Molly K. Ryan contributed the original artwork and helped prepare the micrographs. Comments and suggestions by Sally Leys and by one anonymous reviewer substantially improved the final version of this paper. This is contribution number 641 of the Caribbean Coral Reef Ecosystems Program (CCRE), Marine Science Network, Smithsonian Institution.

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