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Morphology and ultrastructure of the swimming larvae of *Crambe crambe* (Demospongiae, Poecilosclerida)

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Abstract. We describe the morphology and ultrastructure of the free-swimming larvae of the sponge *Crambe crambe,* one of the most abundant encrusting sponges on shallow rocky bottoms of the western Mediterranean. Larvae of *C. crambe* are released in July and August. The larva is uniformly flagellated except at the posterior zone. Flagellated cells are extraordinarily slender, elongate, and sinuous and form a pseudo-stratified layer. Their distal zone contains abundant mitochondria, some small vesicles, a Golgi complex, and the basal apparatus of the flagellum. Abundant lipid droplets are present throughout the cell. The nucleus is most often in a basal position. The flagellum projects from the bottom of an asymmetrical socket formed by cytoplasmic expansions. The basal body extends in a conical tuft and a laminar rootlet in close association with the Golgi system. The cells at the posterior pole are flat and polygonal on the surface, with long overlapping pseudopodia in the typical shape of a pinacoderm. Sparse collagen is present throughout the whole larva including the flagellated layer. Archeocytes and sclerocytes are abundant in the posterior region. Typical collencytes and spherulous cells seem to be absent. Intracellular and extracellular rod-like bacteria with conspicuous fimbria occur exclusively in the posterior region of the larva. The asymmetrical cytoplasmic prolongations, which surround the flagellum, and the basal apparatus of the flagellum are suggested as the sites of stimulus reception and triggering of locomotor responses, respectively. This ultrastructural study of the larva of *C. crambe* has shown features directly linked to its behavior and ecology.

Additional key words: behavior, lipid reserves, Mediterranean Sea, Porifera

Features of larval behavior and metamorphosis decisively account for settlement success in marine invertebrates (e.g., Burke 1986; Bonar et al. 1990; Bingham & Young 1991; Maida et al. 1994; Stoner 1994; Davis & Moreno 1995; Boettcher & Targett 1996), which strongly influences the abundance and distribution of species (e.g., Carlon & Olson 1993; Eckman 1996; Uriz et al. 1998). As in many other aspects of sponge biology, larval behavior must rely on cellular features because sponges lack organs or true tissues (e.g., Simpson 1984). However, despite their apparent simplicity, sponge larvae respond to environmental signals and settle in particular habitats suitable for survival (e.g., Bergquist & Sinclair 1968; Bergquist & Green 1977; Bergquist et al. 1979; Uriz 1982a,b; Kaye & Reiswig 1991; Uriz et al. 1998).

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Ultrastructural observation is essential to investigating the cell mechanisms underlying larval behavior in sponges. Particularly, the way in which larval cells receive, process, and transmit external stimuli to the flagella remains enigmatic despite the exhaustive description of the flagellum parabasal apparatus by Woollacott & Pinto (1995). Studies on the ultrastructure of sponge larvae using scanning and transmission microscopes have revealed different larval types (Lévi & Porte 1962; Lévi 1964; Vacelet 1964; Boury-Esnault 1976; Evans 1977; Misevic et al. 1990; Woollacott 1990, 1993; Kaye & Reiswig 1991; Amano & Hori 1992, 1994; Gallissian & Vacelet 1992; Meroz & Ilan 1995; Boury-Esnault et al. 1999; Ereskovsky & Gonobobleva 2000). Yet much remains to be learned. Sponge larvae are believed to provide phylogenetic (evolutionary) information (Lévi 1956, 1957; Bergquist & Sinclair 1968) and thus taxonomic arrangements based on larval characteristics have been proposed (Lévi 1973). A better understanding of the ultrastruc-

ture of larvae may contribute to those fields as well as helping to identify the cell processes that underlie larval behavior.

We present a SEM and TEM description of the free larva of *Crambe crambe* (SCHMIDT 1862), one of the most abundant encrusting sponges in the western Mediterranean (Uriz et al. 1992). Larval behavior of *C. crambe* has been studied both in the laboratory and in the field (Uriz et al. 1998). During July and August, larvae are released and can be observed in the water column near the surface. They have the potential to disperse far from the parental population in currents (Uriz et al. 1998). Larvae of *C. crambe* are not toxic and do not deter predators (Uriz et al. 1996a). The non-toxic nature of these larvae has been attributed to the absence of the spherulous cells responsible for the strong toxicity found in adults (Becerro 1994; Uriz et al. 1996b). However, only now is there available a detailed ultrastructural study of the different cell types that are the basis of the larva's biology.

Methods

Recently released larvae were collected underwater with a syringe by SCUBA diving at the Blanes sublittoral (western Mediterranean, Iberian Peninsula, 41°40.4′N 2°48.2′E) in the summers of 1992 and 1994. Larvae were fixed underwater using different procedures (detailed below). In addition, brooding adults as well as free larvae were collected and maintained in the laboratory for morphological and behavioral observations.

For scanning electron microscopy (SEM), the fixative used was a mixture of 2% OsO_4 and saturated $HgCl_2$ (6/1) for 90 min (Johnston & Hildemann 1982). Some fixed larvae were frozen in liquid nitrogen and freeze-fractured. Larvae were then dehydrated in an ethanol series, critical-point dried, mounted and sputter-coated with gold-palladium following standard procedures, and examined through a Hitachi-2300 scanning electron microscope (Institute of Marine Sciences of Barcelona, CSIC).

For transmission electron microscopy (TEM), fixation was for 2 h in 2.5% glutaraldehyde in 0.4 M cacodylate buffer (osmolarity adjusted to 980 mOsm with saccharose) at 4°C. Larvae were post-fixed for 90 min with OsO_4 (2%) in the same buffer (Boury-Esnault et al. 1984). Between each step the material was washed 3 times in buffer for 10 min each. The samples were then dehydrated in an acetone series and embedded in Spurr's resin. Semi-thin sections were stained with methylene blue. Ultrathin sections were stained with uranyl acetate and lead citrate and observed under a Hitachi H-600 transmission electron microscope (Microscopy Unit of the Scientific and Technical Services of the University of Barcelona).

Results

Morphology and behavior

Spontaneously released larvae of *Crambe crambe* obtained from the field were dark-orange to red in color. They were spherical, oblong, or elongate, 350–1200 × 350–600 μm in size, and uniformly flagellated except at the posterior pole, which remained bare (Figs. 1–4). The bare zone became larger and more prominent as the larva got older (Fig. 2). However, two larval types were obtained in the laboratory when brooding individuals were shaken and torn to provoke larval release. Some of the larvae were small (420 ± 12.5 μm, mean diameter ± SE), spherical or slightly conical, and completely flagellated (including the posterior zone), while others were similar in shape, size (850 ± 29 μm), and arrangement of flagella to those spontaneously released in the field. The larvae had a surface layer formed by flagellated cells except at the posterior zone. The inner part of the larva (mesohyl) was largely composed of ground substance with cells present at low densities. Cell density was greater in the posterior region of the larva (Fig. 1). This high density of cells was reflected in SEM by the solid aspect of this zone in contrast to the grooved pattern of the rest of the larva (Fig. 2).

Larvae were competent to settle immediately after release. We observed in the field that some larvae, if

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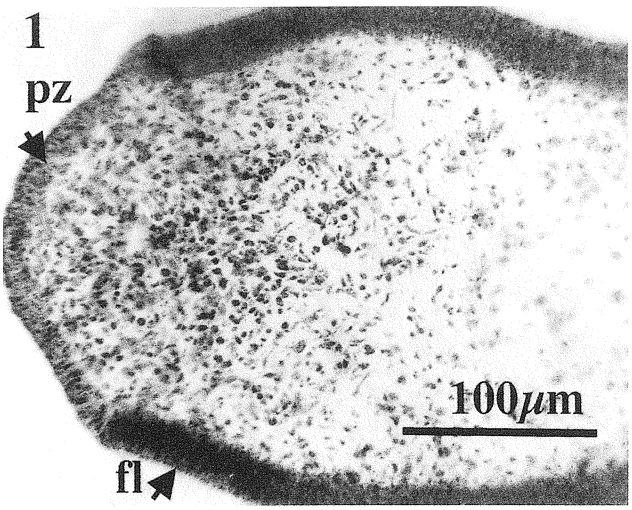
Figs. 1–6. Fig. 1. Semithin section of parenchymella of *Crambe crambe* stained with methylene blue. At left, the posterior zone (pz) with a high cell density. The pseudo-stratified flagellated layer (fl) is absent from the posterior zone. Note that cell density decreases toward the anterior zone (at right). LM. **Fig. 2.** A larva at settlement once the flagella are lost. Note the different aspect of the posterior zone (pz), which appears solid, and the rest of the larva (fz) with ridges (arrows) indicating a low cell density. SEM. **Fig. 3.** Junction between the bare zone (bz) formed by flattened cells and the flagellated zone (fz). SEM. **Fig. 4.** Flagella of the flagellated layer. Note their uniform width and the absence of a terminal swelling (arrow). SEM. **Fig. 5.** Two settlers (24 h after settling) coming from larvae from different sponges. Note the "non-confluence" line (arrows) between them. LM. **Fig. 6.** Rhagon 10 days after larval settlement. Note the typical oscular chimney (o), the incurrent zones (iz), and the excurrent canals (ec). LM.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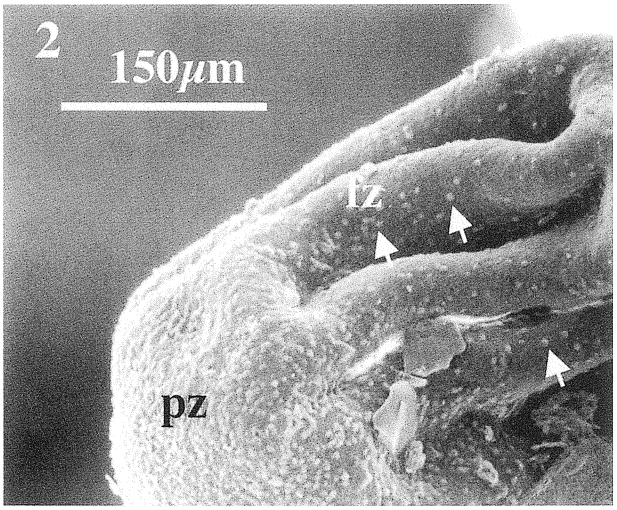
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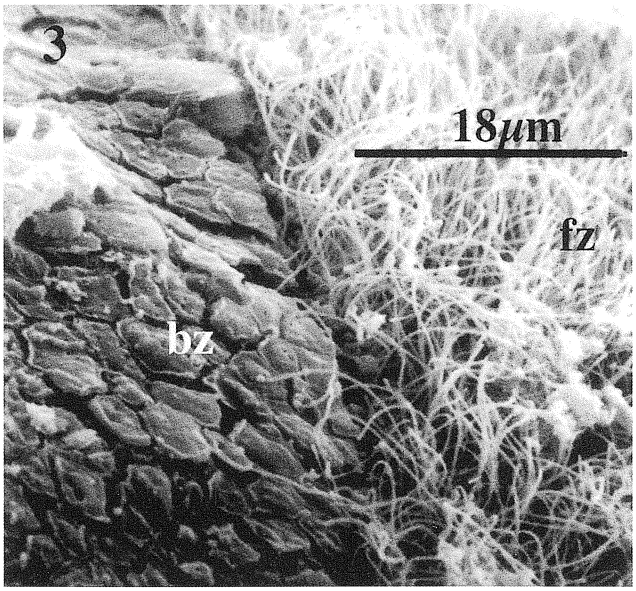
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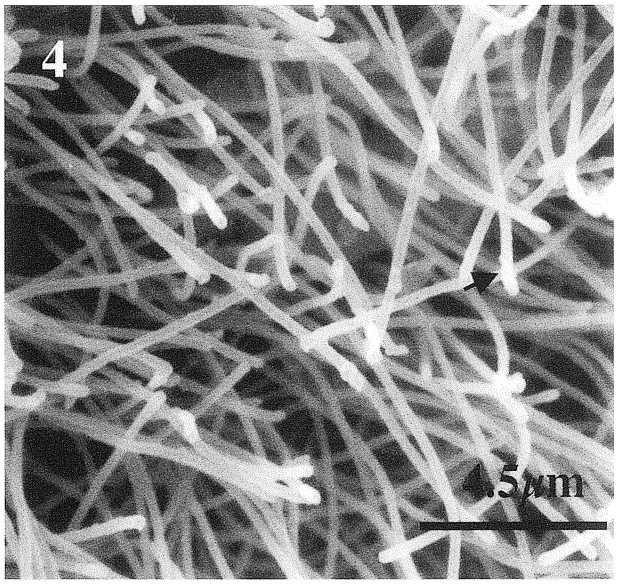
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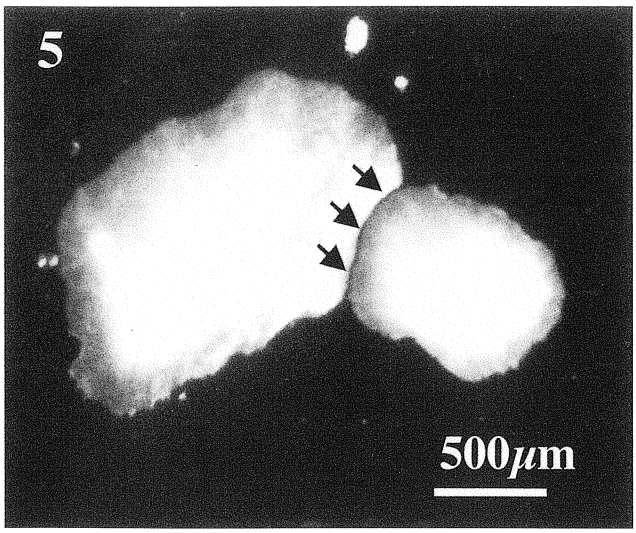
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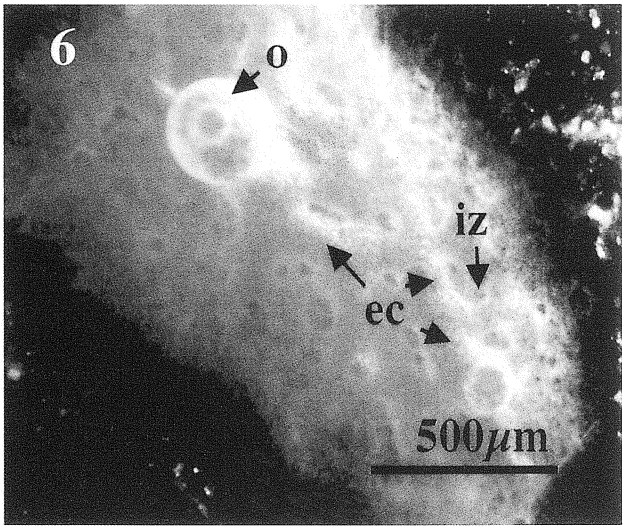
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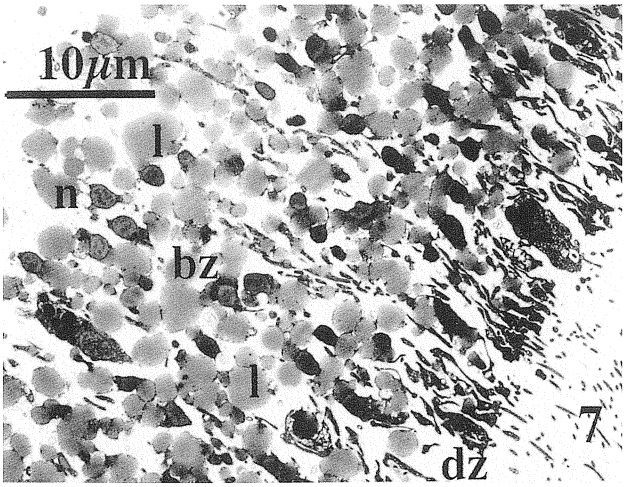
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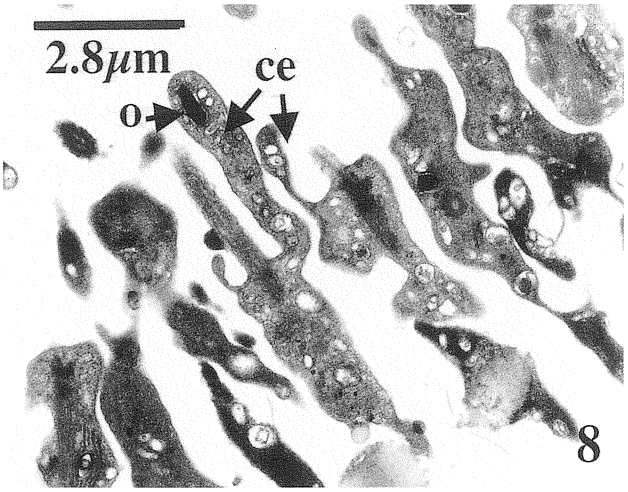
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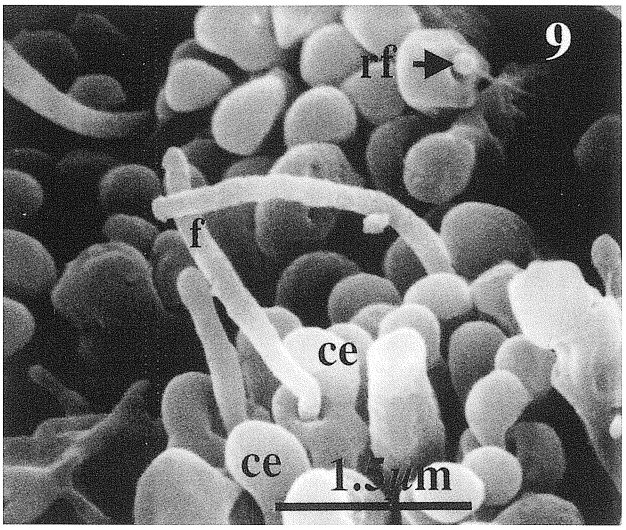
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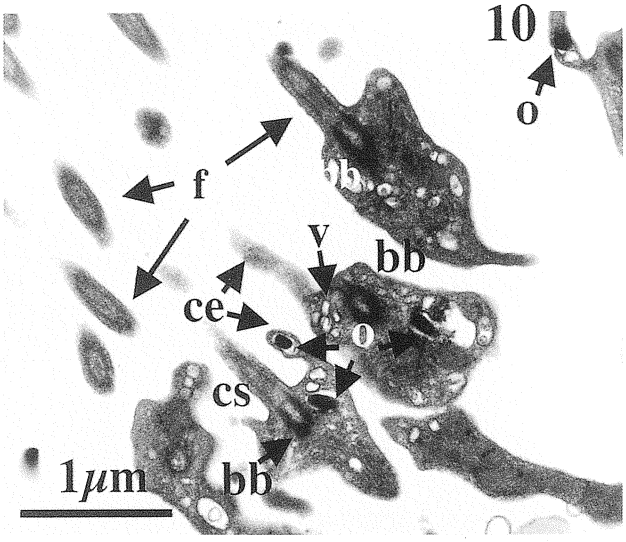
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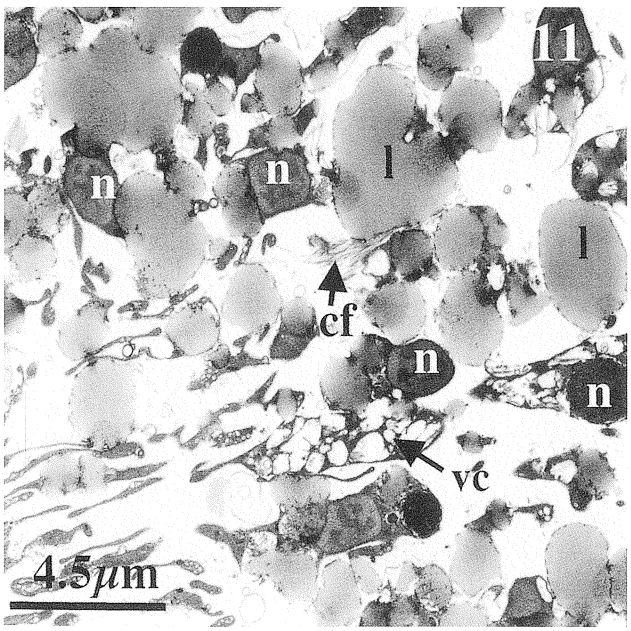
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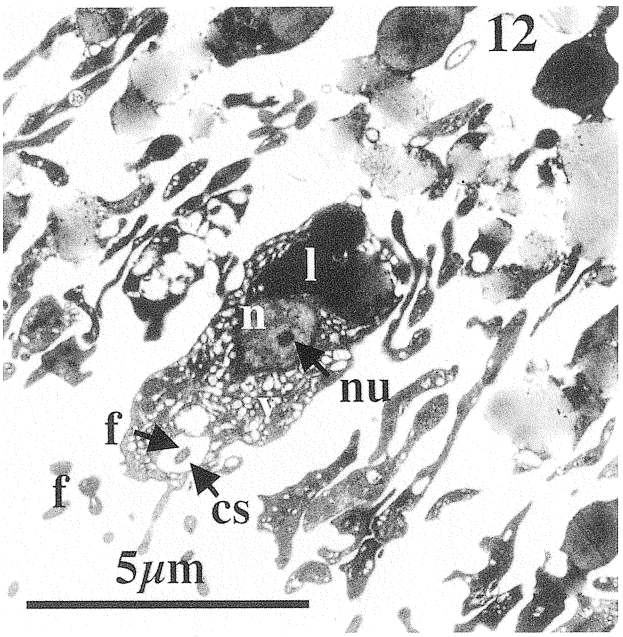
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entrapped by any irregularity of the substratum on release, settled immediately. It is impracticable for divers to track sponge larvae underwater for sufficient time to assess the duration of larval life in the field. Thus, the maximum length of larval life was estimated from laboratory observations. In the laboratory, most larvae settled on plastic Petri dishes with smooth surfaces within 2 days of release. In contrast, some, generally smaller, larvae swam for up to 7 days before settling. Larvae attached to the substratum through either the lateral or posterior zones. They subsequently lost their flagella, starting at the zone in contact with the substratum. Consequently, settling larvae with some of their flagella still moving were observed. After settling, larvae spread on the substratum forming a sponge patch ~1.5–2 mm in diameter (Fig. 5). Functional rhagons (Fig. 6) were completed within a week after settling. They had 1 to 3 chimney-like oscula and some excurrent canals, which converged radially into the oscula (Fig. 6).

Ultrastructure

As in most parenchymella larvae, two superficial zones were observed in the larva of *C. crambe:* the flagellated and the bare zones. The surface is composed of flagellated cells except for an area 200–300 μm in diameter at the posterior zone, which is covered by flat pinacocyte-like cells.

Flagellated cells form a pseudo-stratified columnar layer 30–40 μm in thickness (Figs. 1, 7). These cells are slender (0.5–1.5 μm in width) and elongate (Figs. 7, 8). Their length is difficult to measure because they appeared fragmented in the thin sections due to their tortuous shape (Figs. 7–12), but they presumably span the thickness of the layer. The distal zone of these cells contains abundant mitochondria, small clear vesicles, and the flagellar basal apparatus. A well-developed Golgi system is always close to the flagellar parabasal structures (Figs. 13–16). Abundant lipid droplets are present throughout the cell (Figs. 7, 11). The nucleus, showing heterochromatin masses, is in most cases in a basal position (Figs. 11, 12, 17) but some instances of nuclei close to the flagellar base were also observed (Fig. 14). The flagellum (20–25 × 0.15–0.2 μm) is uniform in diameter along its length (Fig. 4). It does not show the terminal swelling described by Bergquist et al. (1977). It projects from the bottom of a flagellar socket formed by asymmetrical cytoplasmic expansions (Figs. 8–10, 15). The longer cytoplasmic prolongation (up to 3.4 μm in length) often contains one or more vacuoles with osmiophilic inclusions (Figs. 8, 10). The basal body of the flagellum is cylindrical (2 × 1.5 μm), and extends in a conical tuft (1.5 μm in length) and a laminar rootlet (up to 2 μm in length), which is in close association with the Golgi system (Figs. 10, 13–16). An accessory centriole, perpendicular to the basal body, was observed in some images (Fig. 13). Connections between the basal body and the cellular membrane, termed alar sheets and anchor points (Woollacott & Pinto 1995), were occasionally visible (Fig. 15).

Interspersed among the distal ends of the typical flagellated cells are some flagellated, vacuolated cells about 6 × 3 μm (Fig. 12). They have numerous vesicles with electron-lucent contents. A nucleolated nucleus in a central position may be an indication of their origin from archeocytes (Fig. 12). The morphology of these cells matches that described by Amano & Hori (1994) in *Haliclona* sp. and may also correspond to the globular cells of *Haliclona tubifera* (Woollacott 1993), which are also flagellated and have been assigned a secretory function. The characteristic spherulous cells of the species (Uriz et al. 1996b) were not differentiated in any of the larvae examined.

The epithelial cells of the posterior pole appear flat and polygonal in SEM (Fig. 3). They adhere closely to each other and project thin overlapping pseudopodia (Figs. 18–22) in the typical shape of the pinacoderm of adult sponges (e.g., Uriz et al. 1996a; Galera et al. 2000). There are some membrane pores about 0.1–0.2

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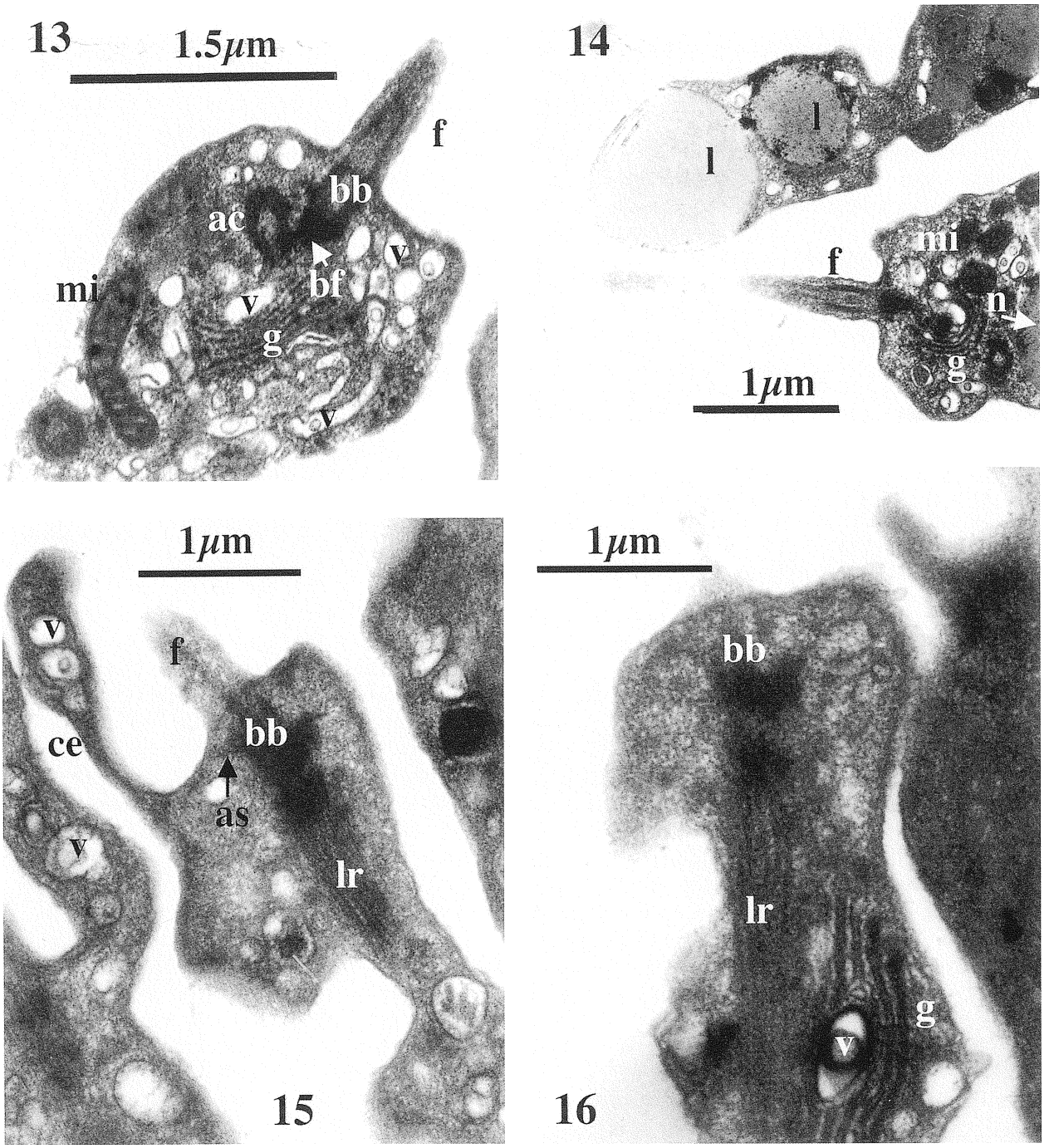
Figs. 7–12. Fig. 7. Pseudo-stratified flagellated layer. Note the accumulation of lipid inclusions (l) except at the distal zone of cells (dz). The nuclei (n) are at the basal zone (bz). TEM. **Fig. 8.** Detail of the distal zone of the flagellated layer showing cytoplasmic expansions (ce) containing osmiophilic inclusions (o) at the outer surface of the larva. TEM. **Fig. 9.** Flagellated zone of a 6-day old larva. Some flagella are almost reabsorbed (rf). The cytoplasmic expansions (ce) are also visible; flagellum (f). SEM. **Fig. 10.** Detail of the distal zone of the flagellated cells. Note the insertion of the flagella (f) within a cytoplasmic asymmetrical socket (cs) and the flagellar basal bodies (bb). Osmiophilic inclusions (o) are contained close to the flagellar bases and in the cytoplasmic expansions (ce). TEM. **Fig. 11.** Vesicular cells (vc) at the basal zone of the flagellated cells. Note the lipid inclusions (l), the nuclei (n) with heterochromatin masses of the flagellated cells, and some collagen fibrils (cf) in the mesohyl. TEM. **Fig. 12.** A vesicular flagellated cell, which forms part of the flagellated layer. Note the lipid inclusions (l), the nucleolated (nu) nucleus (n), and the flagellum (f) arising from a cytoplasmic socket (cs). TEM.



Figs. 13–16. Fig. 13. Enlarged view of the distal part of a flagellated cell showing the flagellar basal apparatus. Accessory centriole (ac); basal body (bb); basal foot (bf); flagellum (f); Golgi system (g); mitochondrion (mi); vesicle (v). TEM. **Fig. 14.** A flagellated distal end of a cell in the outer layer of a larva intermingled with a vacuolated portion of another cell in the same layer. Lipid inclusion (l); nucleus (n). TEM. **Fig. 15.** Distal part of a flagellated cell. The alar sheets (as) and laminar rootlet (lr) are visible in the basal apparatus, as well as several vesicles in the cytoplasmic expansions (ce). TEM. **Fig. 16.** Distal part of another flagellated cell showing the relationship between the laminar rootlet and the Golgi system, in which a vesicle is visible. TEM.

μm in diameter, visible in SEM (Fig. 22), which may correspond to sites of exocytosis (Fig. 18). The epithelial cells are T-shaped with the part of the cytoplasm containing the nucleus, organelles, and inclusions immersed in the mesohyl. Their nuclei possess heterochromatin masses, but no nucleolus was visible. The distal, flattened zone of the cytoplasm contains abundant, large vesicles with a fibrillar, collagen-like content (Fig. 21). The morphology of these vacuoles is similar to that observed in the collencytes of *Dysidea avara* (Galera et al. 2000). These cells, aside from their epithelial function, may be involved in the secretion of collagen necessary for larvae to attach to the substratum (see Simpson 1984).

As for the cell types found in the interior of the larva, archeocytes up to 10 μm in cross-section are particularly numerous in the posterior region. They have a large nucleus (3 μm in diameter) with a conspicuous nucleolus of about 1 μm, and heterochromatin masses. A well-developed Golgi system is always visible in the peri-nuclear region (Fig. 23). Archeocytes contain numerous phagosomes, small vesicles, osmiophilic granules, and some lipid inclusions (Fig. 23). These are the larval cells that contain the fewest lipid inclusions. Cells undergoing degeneration are found close to healthy cells in some parts of the larva (Fig. 24). In the degenerating areas, a mixture of vesicles, membranes, and nuclei appear within the mesohyl.

Spicules were not visible in the larvae examined but sclerocytes containing an axial filament were abundant in the posterior region (Figs. 25, 26). Sclerocytes show the typical structure of these cells in adult individuals (Uriz et al. 2000), but they can be recognized as embryonic cells by the presence of lipid reserves (Figs. 25, 26). They contain abundant mitochondria, small vesicles, a relatively large nucleus with chromatin granules, and a Golgi system close to the nucleus. Axial filaments are completely intracellular. The silicalemma membrane was visible surrounding the axial filament in some instances (Fig. 25).

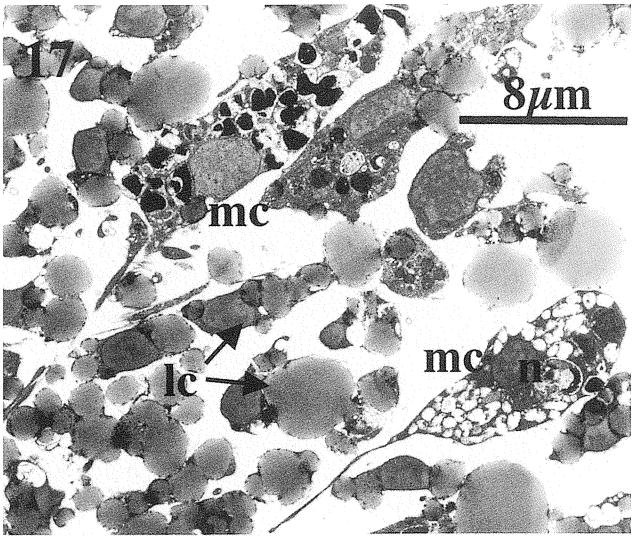
Rod-like bacteria, 0.1–1 μm × 0.025–0.2 μm, with conspicuous, 20-nm wide fimbria were occasionally found in the posterior of the larvae, both within the epithelial cells (Fig. 27) and in the mesohyl (Fig. 28). No other cell types were found to contain bacteria.

Discussion

Two types of larvae were obtained by forcing larval release from brooding individuals of *Crambe crambe* in the laboratory. Some larvae were small and completely flagellated; the others were large, with a bare posterior zone. Small, completely flagellated larvae were found to spend more time swimming before settling than larger larvae. These smaller larvae are probably early developmental stages (although competent) that may not be released under natural conditions. It seems, therefore, that the posterior bare zone of these larvae is formed as larval development proceeds, possibly through cell migration. This phenomenon may be quite general, because two larval types (one smaller and completely flagellated and the other larger, with the posterior zone bare) were also described in *Hymeniacidon sanguinea* (Uriz 1982a). No substantial differences in the cell types and general ultrastructure are evident among the parenchymella larvae so far studied, despite the fact that they belong to Haplosclerida, Poecilosclerida, and Dictyoceratida. Indeed, some recorded differences appear to result from a different interpretation of structures by the respective researchers. Differences lie mainly in external anatomy, arrangement of flagella, swimming behavior (Bergquist & Sinclair 1968; Bergquist et al. 1979; Wapstra & Soest 1987), and structure of the flagellar basal apparatus (Woollacott & Pinto 1995).

The larva of *C. crambe* corresponds, judging from the arrangement of flagella, external morphology, and structure of the flagellar basal apparatus, to the typical parenchymella of poecilosclerid sponges but is considerably larger than those described for other, closely related poecilosclerid species (Boury-Esnault 1976; Bergquist et al. 1979). Moreover, it is uniformly pigmented in contrast to the larva reported for *Phorbas* sp., which had a colorless posterior region (Bergquist et al. 1979). The cytoplasmatic expansions are reminiscent of those described for the larva of *Vaceletia crypta* (Vacelet 1979). In contrast, cell density was higher in the posterior region of the larva of *C. crambe,* whereas that of *V. crypta* had the highest cell density in the anterior zone.

The most remarkable feature of larvae of *C. crambe* from an ultrastructural perspective is the large amount of lipid in all cell types, but particularly in the flagellated cells. Lipids are reported to a lesser or greater extent in all the parenchymellae described to date (e.g., Boury-Esnault 1976; Woollacott 1990, 1993; Amano & Hori 1994; Ivanova 1997) but only in the case of *Hamigera hamigera* were lipid inclusions present in large amounts (Boury-Esnault 1976). Larvae of *H. hamigera* and *C. crambe* have similar external morphology. Lacking a basal ring of long flagella, both larvae appear to be less capable of autonomous swimming than larvae of Haplosclerida, Dictyoceratida, or Dendroceratida with a posterior ring of long flagella (Kaye & Reiswig 1991; Woollacott 1993; Amano & Hori 1994). Lipids constitute an energy source that may allow the larva of *C. crambe* to lengthen its pe-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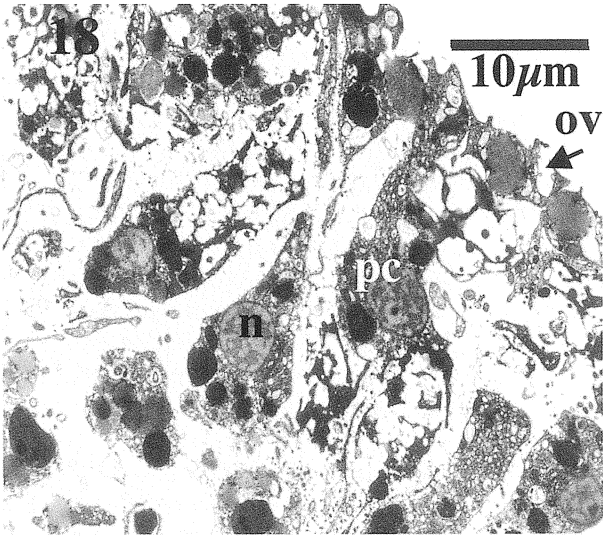
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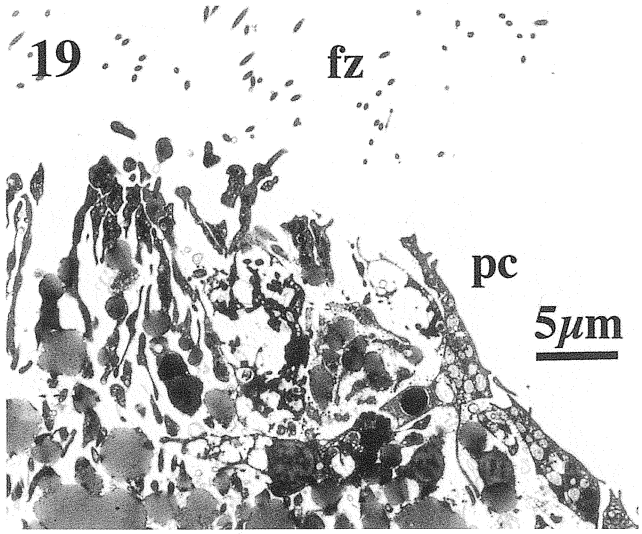
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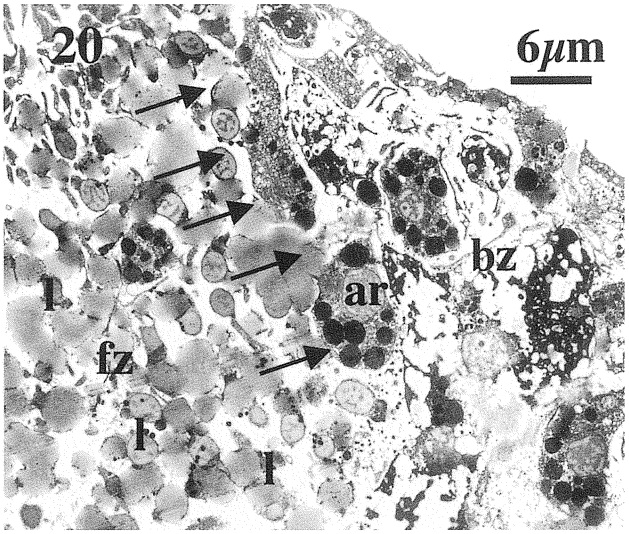
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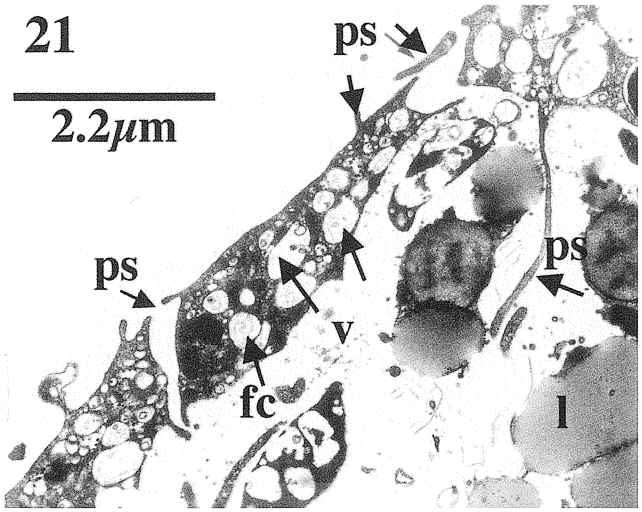
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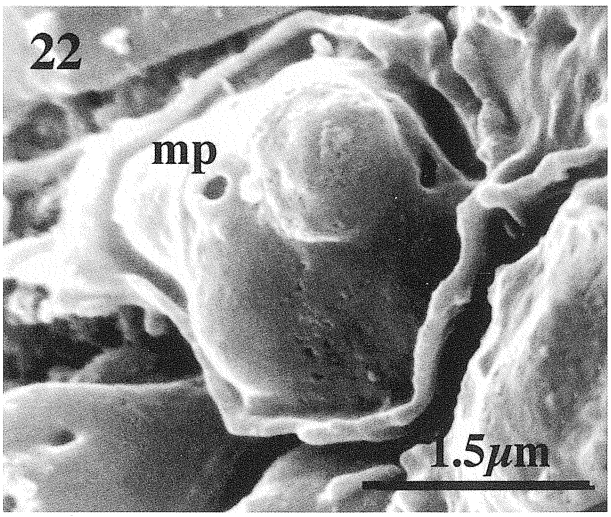
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lagic life when conditions are unfavorable for settlement. Some larvae settled in the laboratory within 48 h, whereas smaller siblings maintained in the same conditions swam for 168 h before settling. This observation indicates a long free-swimming period in laboratory conditions as compared to 26–56 h reported for dictyoceratid larvae with negligible lipid content (Kaye & Reiswig 1991). Furthermore, although it is difficult to ascertain the duration of the larval free-swimming period at sea, larvae may stay longer in the water column under natural conditions than in the laboratory (Uriz 1982b). Lipid reserves may also enable early settlers to survive during the critical first days of metamorphosis before the juvenile begins feeding. Consequently, these large lipid reserves may contribute to survival of this relatively slow growing species (Turon et al. 1998).

Some authors have reported the presence of a cellular layer under the flagellated cells (e.g., Meewis 1939; Bergquist & Green 1977; Woollacott 1993; Amano & Hori 1994). No such layer was clearly differentiated in *C. crambe,* although globular multivesicular cells were found at the base of the pseudostratified layer, sometimes interspersed among the proximal ends of the flagellated cells (Fig. 17). They have elongate pseudopodia and the images suggest that they tend to extend mostly towards the outer surface as reported in larvae of *Halichondria moorei* (Bergquist & Green 1977). These cells may have a secretory function as judged by their multivesicular aspect. Amano & Hori (1994) called the cells under the flagellated layer collencytes. In our case, there was no evidence of collagen secretion by the multivesicular cells, although sparse collagen was seen throughout the whole larva, including the flagellated layer, so it seems likely that some cells besides those of the posterior region (see below) produce collagen.

Bacteria were found in the posterior region of larvae of *C. crambe,* in the mesohyl and within the cells of the surface. Adults, however, had an axenic surface (Becerro et al. 1994) and no bacteria were found in an ultrastructural study of adult specimens (Galera et al. 2000). The axenic surface was attributed to antibacterial metabolites that are produced by the spherulous cells, which accumulate below the pinacoderm both at the external sponge surface and at the internal canals (Uriz et al. 1996b). Here we document that larvae lack spherulous cells, which is consistent with the finding that larvae, in contrast to adult individuals, did not deter predatory fishes (Uriz et al. 1996a) and had no antibacterial properties (Uriz et al. 1996b). The nontoxic larvae may benefit from these bacterial symbionts during their pre-feeding development. Incorporation of dissolved organic molecules and small particles has been reported (Manahan 1990; Jaeckle 1995; Ivanova & Semenov 1997) in swimming sponge larvae and assimilation of the dissolved molecules might be achieved through association with bacteria. These bacteria may be eliminated once the spherulous cells are differentiated and the toxic properties of the sponge are established.

Cell degeneration occurs in free larvae (Woollacott 1993; this study), and archeocytes presumably digest the remnants of degenerate cells, as can be inferred from the abundant phagosomes in the archeocytes of such non-feeding larvae. As Woollacott (1993) suggested, larval components are transitory and, upon resorption, may provide nutrient materials for morphogenesis. Cell disintegration has also been described in adults (Diaz 1979; Turon et al. 1999; Galera et al. 2000) and seems to be a part of the reorganization processes that take place regularly during the sponge life cycle.

Spicules were not observed in the larvae we examined but sclerocytes with an axial filament were present in the posterior of these larvae. Consequently, spicules are probably present in larvae older than those studied. It has been suggested that spicules may increase larval density and thus cause larvae to sink to the bottom (Maldonado et al. 1997). The location of spicules at the posterior of the larva has been reported repeatedly in poecilosclerid and haplosclerid species

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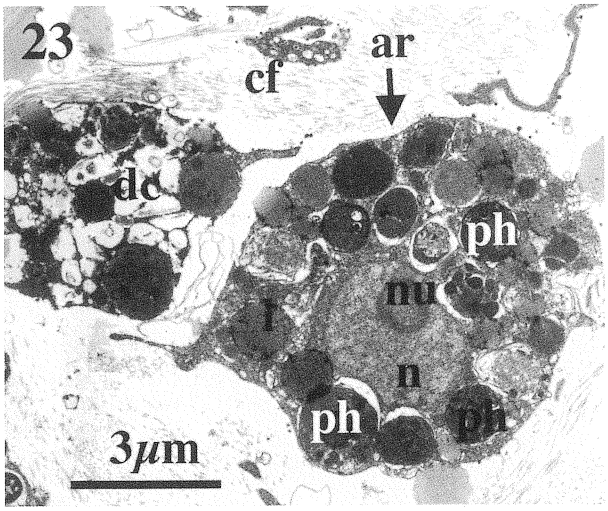
Figs. 17–22. Fig. 17. Basal zone of the flagellated layer showing ameboid migrating cells (mc) interspersed among the flagellated (lipid-rich) cells (lc). Their nuclei (n) show heterochromatin masses. TEM. **Fig. 18.** Posterior zone of a larva. Note that the nucleus and dark inclusions of the epithelial cells are immersed in the mesohyl, whereas the vesicular cytoplasm flattens to form the surface covering (see Fig. 21). The posterior cells (pc) show vesicles open to the exterior (ov) and a nucleus (n) with heterochromatin masses. TEM. **Fig. 19.** Outer junction zone between the flagellated and the bare zones. Note the "T" shape of some non-flagellated epithelial cells of the posterior zone (pc) and the flagellar sections (fz). TEM. **Fig. 20.** Inner junction zone (arrows) between the flagellated zone (fz) and bare zone (bz) at the posterior pole of the larva. Note the difference in lipid content (l) between cells from the two zones and the presence of archeocytes (ar). TEM. **Fig. 21.** Enlarged image of the bare posterior pole. Note the paved aspect of the cells, the numerous pseudopodia (ps), and the multiple vesicles (v) with a fibrillar content (fc). TEM. **Fig. 22.** Cell from the bare zone of the larva with a membrane pore (mp). SEM.


23
cf
ar
dc
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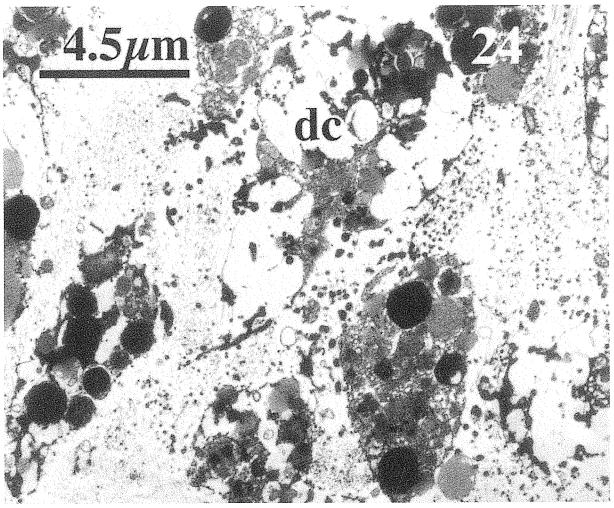
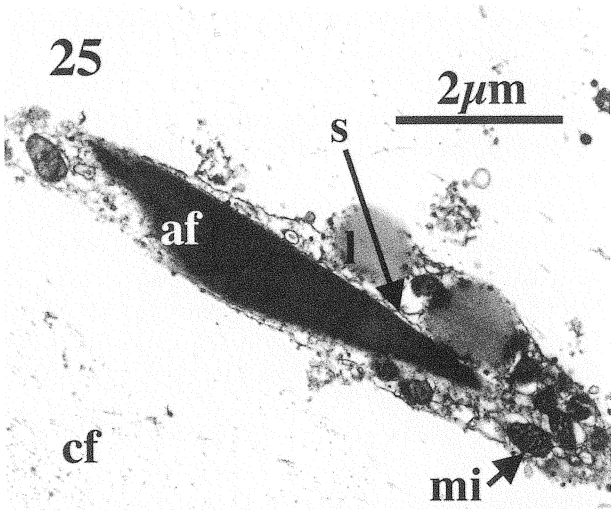
3μm


4.5μm
24
dc


25
2μm
s
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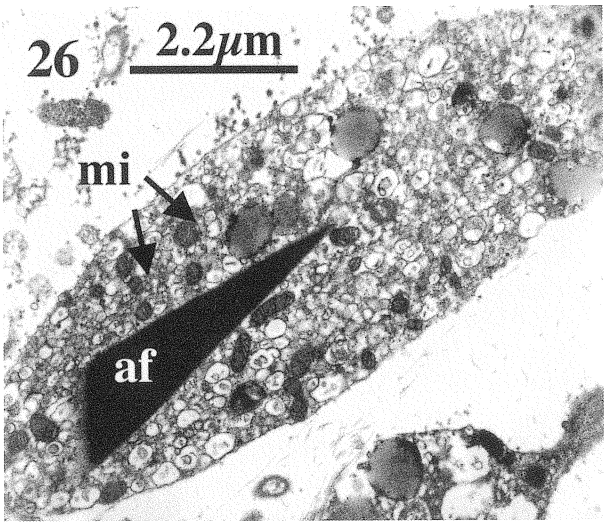
26
2.2μm
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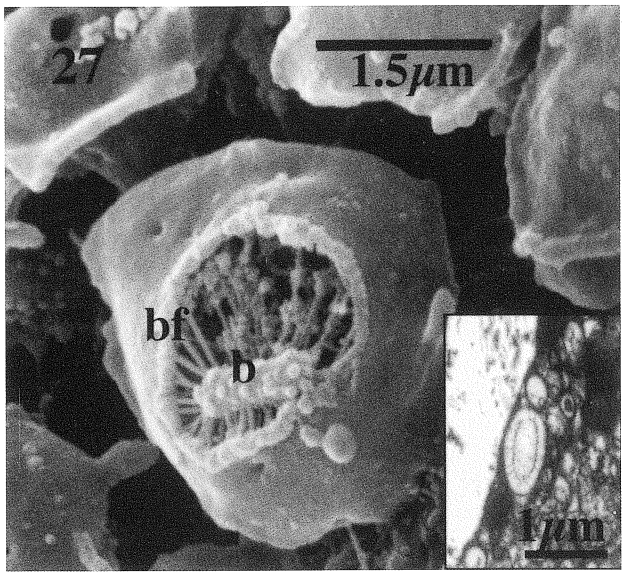
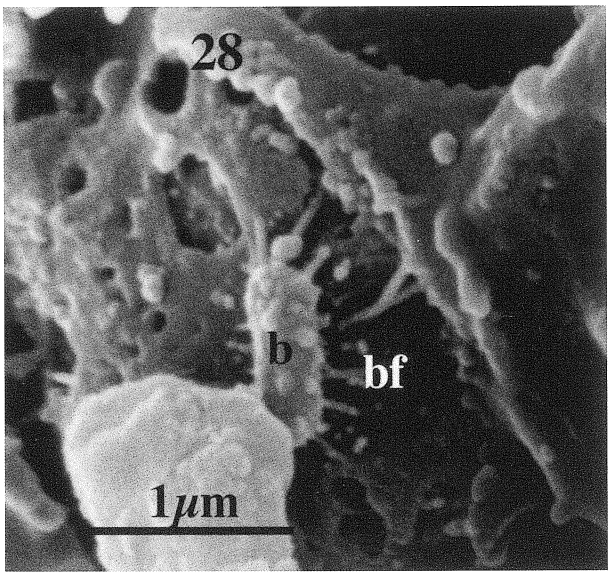

27
1.5μm
bf
b
1μm


28
b
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1μm

(e.g., Bergquist & Sinclair 1968; Woollacott 1993; Meroz & Ilan 1995). Vertical sinking due to a displacement of the larval center of gravity towards the posterior end might increase the chances of larvae contacting the substratum via the posterior cells. In larvae of *C. crambe,* these cells appeared to produce collagen-like material and may form the basal collagenous layer necessary for settlement. However, settlement on the anterior end has also been observed in the laboratory in other species (e.g., Bergquist & Sinclair 1968; Uriz et al. 1982a). Settlement, therefore, can occur irrespective of the part of the larva that contacts the substratum. However, basopinacocyte-like cells (see Simpson 1984) are always necessary to form the basal attachment membrane. In the cases of settlement on the lateral surface or anterior end, this could be achieved through migration of collagen-producing cells across the flagellated layer. Migration of vesicular cells, sometimes called collencytes, towards the larval periphery has been documented for this and other sponge larvae (Borojevic 1966; Amano & Hori 1994; this work).

Larvae of *C. crambe* are uniformly pigmented and did not display any particular phototaxis. However, larvae of other sponge species (i.e., *Reniera* sp.) proved photonegative. These larvae have been reported to possess a certain polarity represented by a pigmented ring of cells lacking flagella at the posterior zone. The larval polarity was also indicated by the expression of Pax 2/5/8 and Bar-type genes, which have been suggested to play a role in photoreception (Leys et al. 1999).

Larvae effect locomotion by modifying flagellar motion. However, the mechanism that controls flagellar beating remains unknown. It has been repeatedly described that flagella arise from an asymmetrical socket formed by cytoplasmic expansions (e.g., Amano & Hori 1994). The asymmetry of these expansions (up to 3.5 μm in length on the longer side vs. no projection on the opposite side, see Figs. 8, 9) was remarkable and suggests that some directionality is possible either in the reception of stimuli or in the control of flagellar movement.

Bergquist & Green (1977) suggested that the cell body expansions surrounding the flagellum could exert a control over flagellar activity by contraction or expansion of the socket walls. The longer cytoplasmic prolongation was particularly exposed to external stimuli such as mechanical pressure produced by microcurrents or light variations. Furthermore, it had one or more vacuoles, containing osmiophilic inclusions (Figs. 8, 10, 15) which might correspond to pigment granules involved in the reception of light signals. A signal received at the membrane level could be transmitted through the well-developed vacuolar system towards the Golgi system, which is always associated with the laminar rootlet and the two centrioles. The basal apparatus may control the flagellar (effector) movement. The intracellular membranes of the Golgi system and the abundant small vacuoles may provide ionic gradients, favoring the formation of electric potentials and thus the transmission of signals. Moreover, coordination of flagellar movement is necessary for directional swimming, although no particular structures for intercellular communication have been found in the flagellated layer (Amano & Hori 1994; this study). If the asymmetries in the flagellar sockets were all oriented in the same direction (a point that needs further confirmation), these may determine the preferential direction of beating and hence the directionality of the larval movements.

This ultrastructural study of the larvae of *C. crambe* has documented features directly linked to behavior and ecology (e.g., absence of spherulous cells and hence of toxicity, ability to delay settlement due to abundant lipid reserves), and has revealed presumptive cellular sites where signal reception and effector mechanisms may be located. Sponges, because of their cellular construction, are particularly suited for studies on the influence of microarchitectural and cytological features at the organismic and ecological levels (Galera et al. 2000). More species spanning different orders and families and featuring different larval behaviors must be examined before a general picture of the relationships between sponge larval features and larval biology can be drawn.

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Figs. 23–28. Fig. 23. Archeocyte (ar) containing a nucleus (n) with nucleolus (nu), and abundant phagosomes (ph), close to a degenerating cell (dc). Note the abundant collagen (cf) in the larval mesohyl. TEM. **Fig. 24.** TEM image of a zone with degenerating cells (dc). **Figs. 25, 26.** Sclerocytes, containing an axial filament (af). Note the silicalemma membrane (s) surrounding the filament, the abundant mitochondria (mi), and some lipid droplets (l). TEM. **Fig. 27.** Cryo-fractured cell from the posterior larval zone showing a rod-like symbiotic bacterium (b) with fimbria (bf). SEM. Insert: view of an intracellular bacterium. TEM. **Fig. 28.** An extracellular bacterium (b) similar to that in Fig. 27. SEM.

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