Scanning electron microscopic observations of the larval development of the reef-building polychaete *Phragmatopoma lapidosa*

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The scanning electron microscope (SEM) was used in a study of the larval development of the reef-building sabellariid polychaete *Phragmatopoma lapidosa*. Mature sperm are modified from the 'primitive' polychaete plan by possessing a tapered and bent acrosome. The outer surface of the irregularly shaped, freshly spawned egg envelope is granular in appearance. SEM observations of various stages in development from the early trochophore through larval metamorphosis and the early juvenile stages suggest that the egg envelope serves as the cuticle through the trochophore stage but is then replaced by another cuticle penetrated by microvilli. SEM has revealed the presence of 'sensory tufts' on the dorsal surface of the larval tentacles which may play a role in larval substrate selection.

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Le microscope électronique à balayage a permis d'étudier le développement larvaire de la polychète sabellariide constructrice de récifs, *Phragmatopoma lapidosa*. Les spermatozoïdes à maturité constituent une modification du spermatozoïde 'primitif' des polychètes à cause de leur acrosome à l'extrémité aplatie et recourbée. Chez l'œuf frais, de forme irrégulière, la surface externe de l'enveloppe est d'apparence granulaire. L'observation au microscope électronique à balayage de plusieurs stades de développement, de la jeune trochophore à la métamorphose larvaire et aux premiers stades juvéniles, démontre que l'enveloppe de l'œuf sert peut-être de cuticule durant tout le stade trochophore, mais est remplacée par la suite par une cuticule pénétrée de microvillosités. Le microscope a révélé également la présence de 'touffes sensorielles' à la surface dorsale des tentacules larvaires; ces touffes pourraient jouer un rôle lors du choix d'un substrat par la larve.

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Introduction

The scanning electron microscope (SEM), with its high resolution and great depth of focus, is an effective tool in studying minute specimens such as marine larvae. It can provide fine details of larval structures which were not possible previously with light microscopy. As far as we are aware, no SEM investigations of polychaete development have been reported in the literature although scanning electron micrographs were published by Holborow *et al.* (1969) and Holborow (1971) on the trochophore of the polynoid *Harmothoe imbricata* and by Gustus and Cloney (1973) on an early larval stage of the nereid *Nereis vexillosa*. In this paper, we describe the morphology of the gametes, selected larval stages, and the metamorphosed young of the reef-building polychaete *Phragmatopoma lapidosa* Kinberg, as revealed by SEM; it is largely a reconfirmation of what has been described at the light microscope level (Eckelbarger, 1976). However, some aspects of this report are new findings and other aspects are described with greater detail and clarity.

Materials and Methods

Sexually mature males and females of *Phragmatopoma lapidosa* were collected in July 1975 at Seminole Shores, Hutchinson Island, Martin County, Florida, and kept at the Harbor Branch Foundation laboratory.

Mature sperm from males were fixed for 30 min at room temperature (21–24 °C) in 2% glutaraldehyde buffered to pH 7.4 with 0.4 *M* Millonig's phosphate buffer. Sperm were then pipetted into Teflon 'Flo-thru Specimen Capsules' (Sargent–Welch Scientific Co.) containing Nucleopore membranes 25 mm in diameter with a 1.0-μm

pore size (Sargent–Welch Scientific Co.), and rapidly dehydrated in ascending concentrations of amyl acetate to 100%. After critical-point drying with carbon dioxide, the capsules were opened and the Nucleopore filters were removed and mounted on aluminum stubs. Sperm were then coated with carbon and gold to a total thickness of 7.5–12.5 nm and examined with a Cambridge Stereoscan S-4 scanning electron microscope.

Larval and juvenile stages of *Phragmatopoma lapidosa* were obtained from artificially fertilized eggs in the laboratory following the procedures of Eckelbarger (1975). Eggs, larvae, and juvenile worms were fixed at room temperature (21–24 °C) for 1 h in the following mixture: one part 50% glutaraldehyde (Fisher Scientific Company) were diluted with nine parts 0.3 *M* NaCl and then diluted 1:1 with 0.4 *M* Millonig's phosphate buffer to give a final concentration of 2.5% glutaraldehyde. Specimens were then washed for 1 h in three changes of an equal mixture of 0.4 *M* Millonig's phosphate buffer and 0.6 *M* sodium chloride and postfixed at 4 °C for 1 h in a mixture of one part 4% osmium tetroxide, two parts 0.75 *M* NaCl and one part 0.4 *M* Millonig's phosphate buffer. Specimens were then rapidly dehydrated in ascending concentrations of ethanol and passed through ascending concentrations of amyl acetate to 100%. Specimens were placed in glass vials, critical-point dried in carbon dioxide and individually mounted with fine needles on double-sided tape. After coating with carbon and gold, the specimens were examined with a Cambridge Stereoscan S-4 scanning electron microscope.

Observations

Gametes

Mature sperm are shed directly into seawater and possess an unusual tapered and bent acrosome (Fig. 1). The number of mitochondria in the midpiece vary from five to eight, with five being the most common. The midpiece and head total about 6 μm in length while the tail is about 32 μm in length.

Freshly spawned unfertilized eggs are characteristically irregular in shape. A few minutes after contacting seawater, the egg rounds up. SEM examination of the freshly spawned egg reveals regularly spaced granules over the surface of the outer egg envelope. In Fig. 2, the egg envelope has been partially removed, revealing the oolemma, which bears scattered microvilli.

Larvae

The trochophore, 17 h after fertilization, is actively swimming and possesses a single row of long cilia forming the prototroch and a small apical tuft of cilia (Fig. 3). The entire cuticle of the trochophore appears to be granulated (Fig. 4), resembling the egg envelope shown in Fig. 2.

Figure 5 is a lateral view of a 19-day-old larva just before metamorphosis. At this stage, the larva is alternately swimming and crawling over the culture bottom, stopping periodically to test the substrate with its tentacles and ciliated mouth region. The body of the larva has elongated with the formation of three parathoracic and three abdominal segments. The pair of larval tentacles are about one-half the length of the body and possess ventral ciliated food grooves. The prototroch is visible as a complete ring except dorsally, where it is broken by the dorsal hump. The opercular cirrus and associated barbed, provisional setae are clearly observed, although the underlying paddle-shaped opercular paleae are obscured from view. The three parathoracic segments with their associated capillary setae and the first abdominal segment bearing a dorsal lobe with uncinigerous tori are also visible.

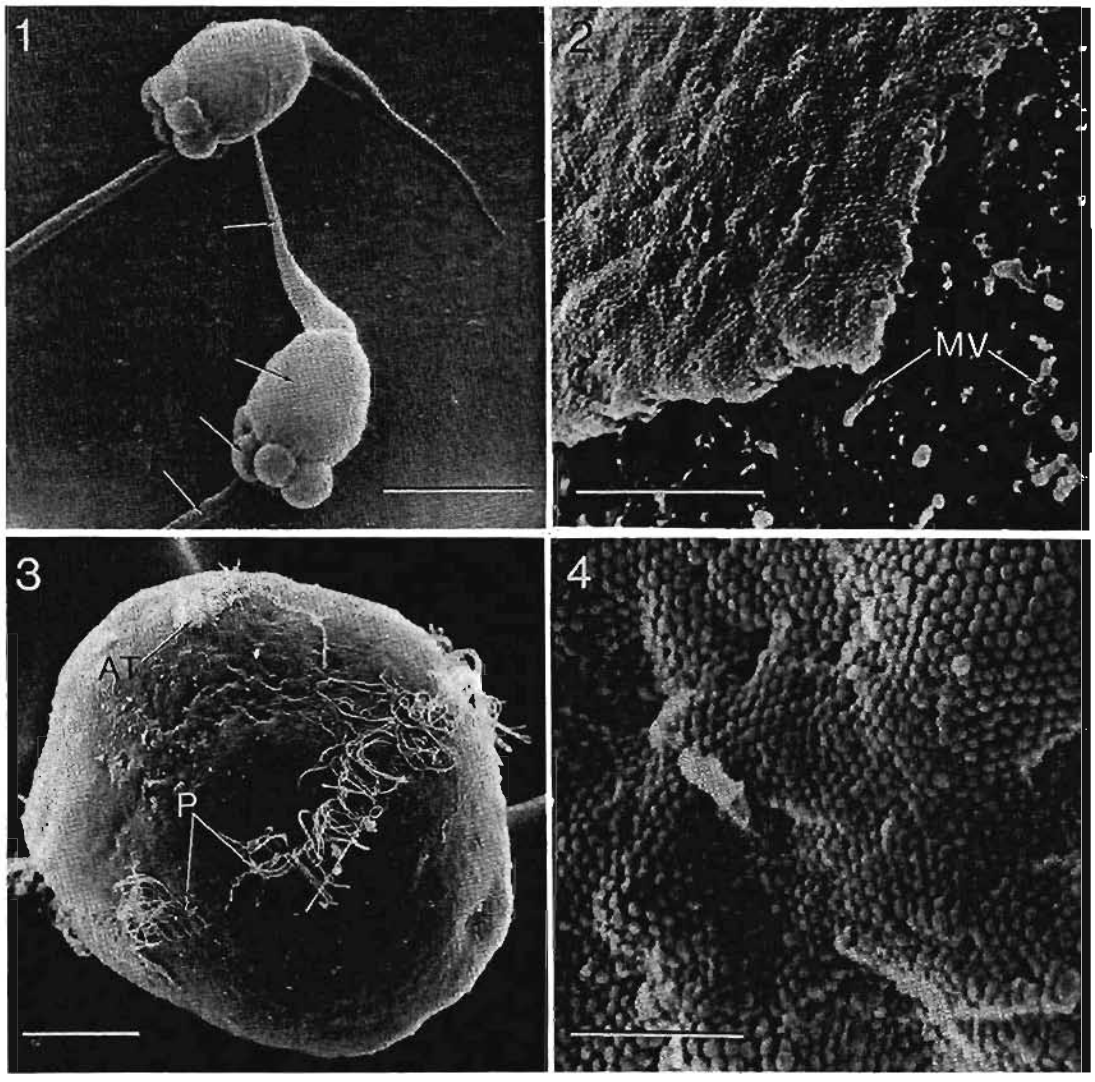
Each larval tentacle at this stage bears a number of 'sensory tufts,' forming two distinct rows on the dorsal surface along its length (Fig. 6). Each tuft is composed of a variable number of stiff 'hairs,' which form a characteristic radiating pattern. The tufts have been observed in a widely scattered random pattern over the entire body surface of the larva. However, only on the tentacles are the tufts symmetrically arranged.

Figure 7 shows the ventral side of a 19-day-old larva revealing a prominent neurotroch, ciliated building organ, mouth, and a series of gland pores on the first parathoracic segment. Figure 8 shows scattered cilia and microvilli over the surface of the building organ. The microvilli appear to cover the entire surface of the larva and penetrate a thin cuticle that has been observed with the transmission electron microscope (TEM).

Hidden among each bundle of provisional setae are a pair of small barbed opercular spines (Fig. 9), which are useful in the identification of sabellariid larvae in the late larval stages. The number of rows of denticles on the spines appears to be constant but they are difficult to resolve with the light microscope. Figures 10 and 11 show uncini from the dorsal uncinigerous lobe of the third abdominal segment and capillary setae from the ventral parapodium of the first parathoracic segment, respectively. The morphology of the setae are easily resolved with the light microscope but the denticles on the uncini are not.

Metamorphosing and Postmetamorphic Stages

Larval metamorphosis occurs 14 to 30 days after fertilization. Metamorphosis involves an elongation of the body of the larva and extensive

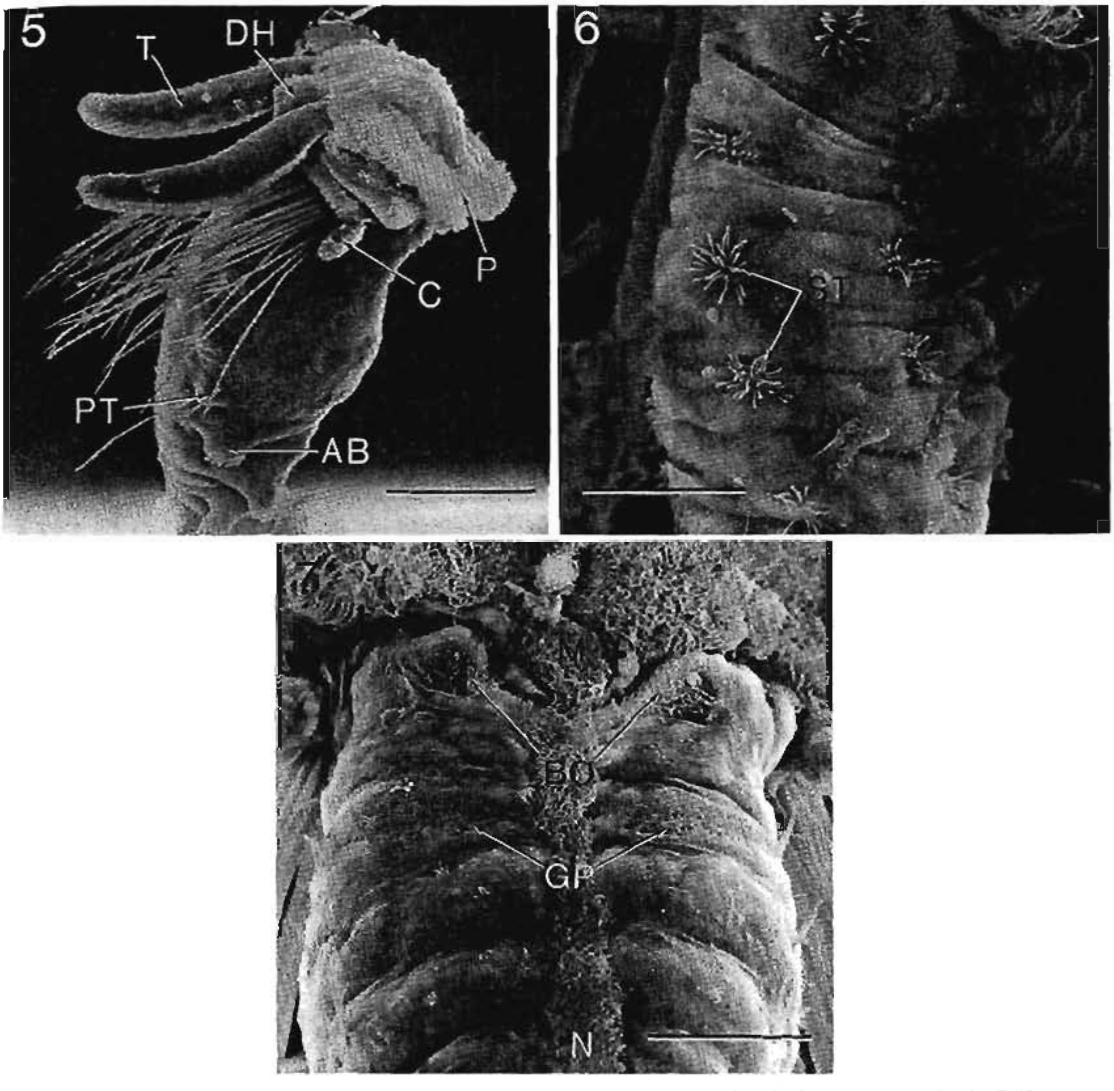


FIGS. 1–4. *Phragmatopoma lapidosa*. Fig. 1. Freshly spawned mature sperm. A, acrosome; N, nucleus; M, mitochondrion; F, flagellum. Scale = 2 μm. Fig. 2. Egg surface 10 min after spawning into seawater, showing granulated, outer envelope at upper left. The envelope has been removed from the egg (lower right) revealing the microvillous (MV) oolemma. Scale = 2 μm. Fig. 3. Trochophore larva, 17 h old. AT, apical tuft; P, prototroch. Scale = 20 μm. Fig. 4. Higher magnification of specimen in Fig. 3, showing granular cuticle. Scale = 1.5 μm.

changes in the head region. The larva in Fig. 12 has taken up a benthic existence and is in the early stages of metamorphosis. The provisional setae and prototroch have been lost and the tentacles and primary paleae have rotated anteriorly. The tentacles are hidden from view in this micrograph by the paleae. The nototrochal bands of cilia are observed along the posterior borders of the dorsal parathoracic segments.

Figure 13 shows a ventral view of a newly metamorphosed worm removed from its tube, with fully rotated tentacles bearing ciliated food grooves and paleae-bearing opercular lobes. The body has elongated somewhat and tentacle buds, which will give rise to the first of many feeding tentacles, have appeared lateral to the mouth. The telotroch is still present. The neurotroch meets the ciliated building organ just posterior to the heavily ciliated mouth region.

Figure 14 is a ventral view of a juvenile worm

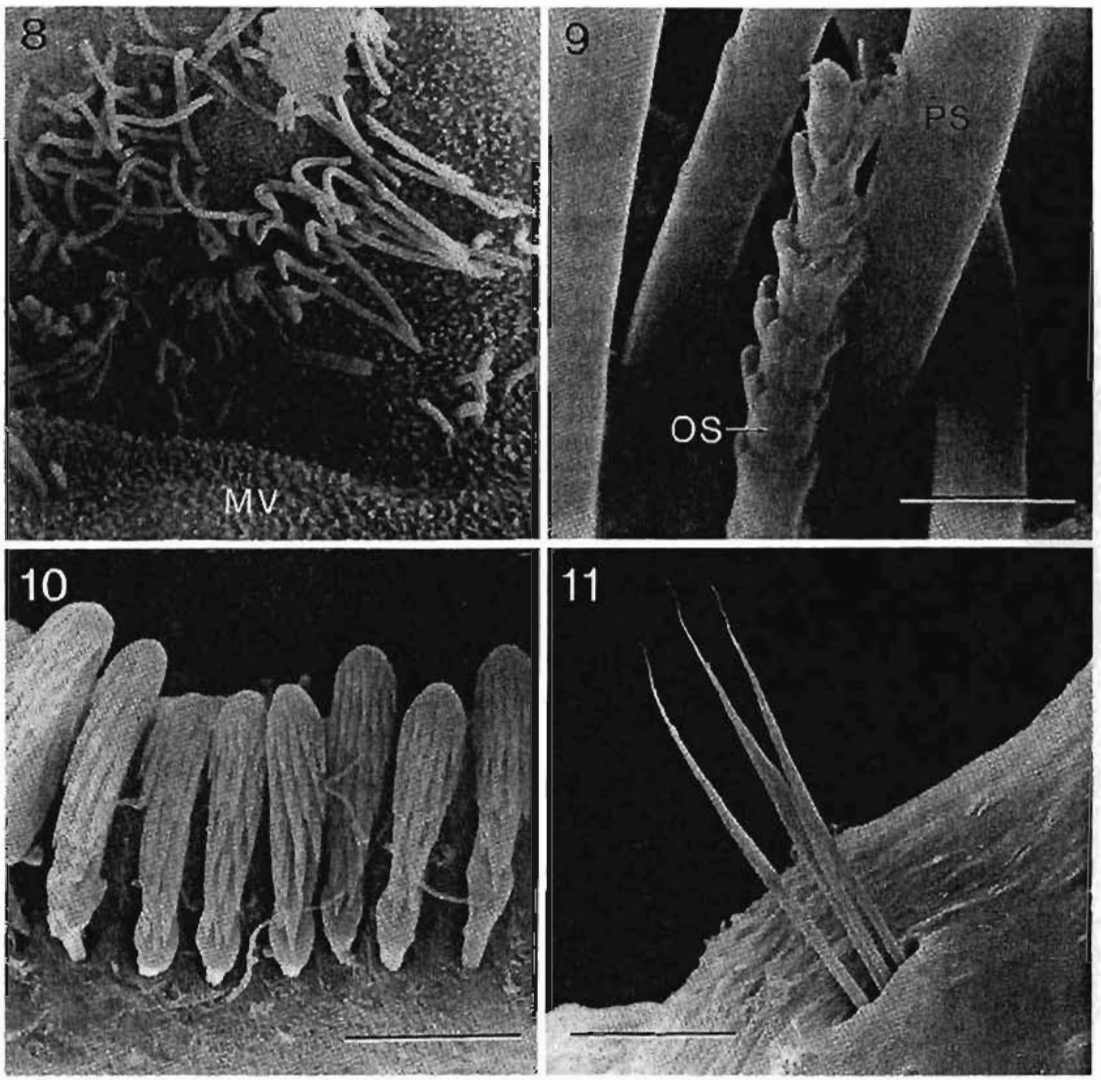


FIGS. 5–7. *Phragmatopoma lapidosa*. Fig. 5. Nineteen-day-old larva just before metamorphosis. DH, dorsal hump; T, tentacle; P, prototroch; C, opercular cirrus; PT, third parathoracic segment; AB, first abdominal segment. Scale = 225 μm. Fig. 6. Higher magnification of dorsal surface of larval tentacle showing rows of 'sensory tufts' (ST). Scale = 20 μm. Fig. 7. Ventral view of specimen shown in Fig. 5. M, mouth; BO, tube-building organ; GP, gland pores; N, neurotroch. Scale = 60 μm.

about 30 days after metamorphosis (50 days from fertilization). The body has greatly elongated and the opercular lobes have fused into a single operculum characteristic of the adult. The primary paleae have been lost and three new types of adultlike paleae have replaced them. Additional feeding tentacles have formed and the caudal appendage, which bears the anus, has developed. The neurotroch has become a broken band through the parathoracic segments and now forms two bands over the abdominal segments. Figure 15 shows a lateral view of the head region of the specimen shown in Fig. 14. The ring of opercular paleae, the cirri at their base, and the tentacle buds are all visible. Clusters of evenly spaced stiff cilia along the lateral edges of each feeding tentacle and the setae-bearing rudimentary parapodium of the first thoracic segment are indicated in this figure.

Discussion

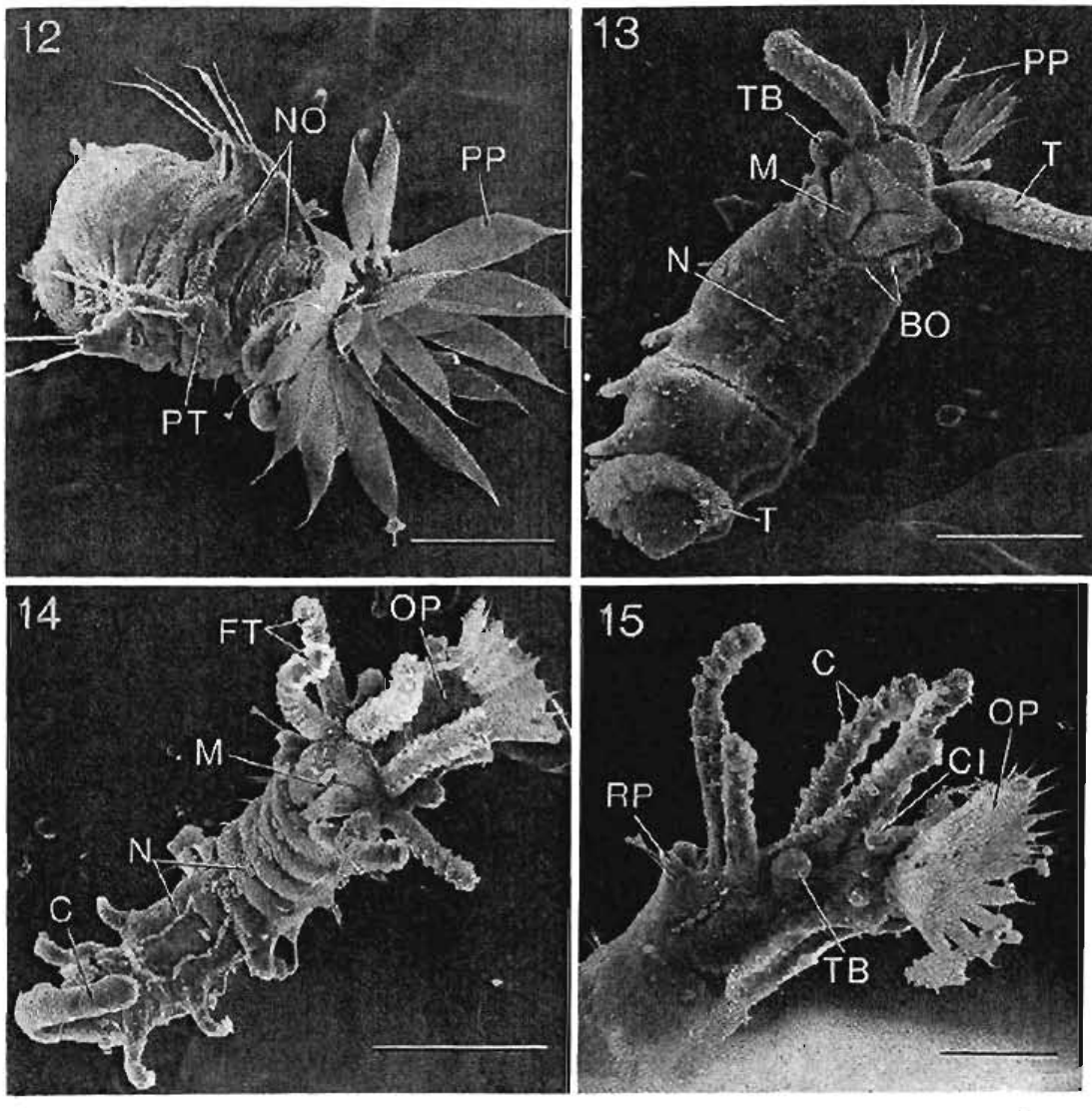
The unusual acrosome of *Phragmatopoma*



FIGS. 8–11. *Phragmatopoma lapidosa*. Fig. 8. Surface of building organ of larva in Fig. 5, showing scattered cilia and microvilli (MV). Scale is the same as Fig. 9. Fig. 9. Larval opercular spine (OS) surrounded by smoother provisional setae (PS) of same larva. Scale = 6 μm. Fig. 10. Face-on view of uncini from dorsal lobe of third abdominal segment of premetamorphosed larva. Scale = 5 μm. Fig. 11. Capillary setae from ventral parapodium of first parathoracic segment. Scale = 12 μm.

lapidosa sperm is not the result of an acrosomal reaction or fixation artifact since living sperm in seawater and coelomic fluid have the same morphology when observed with the light microscope. The acrosome superficially resembles the bent and elongated acrosome of the archiannelid *Protodrilus rubropharyngeus* figured by Franzén (1956), a worm whose biology of fertilization is modified from the general plan (Jagerston 1952). *P. lapidosa*, however, shed sperm freely into seawater where fertilization is external.

TEM studies by Franklin (1966) on the oocytes of the sabellariid *Sabellaria vulgaris* and Pasteels (1965*a*, 1965*b*) on those of *S. alveolata* demonstrated the presence of a monolayer of closely packed vesicles over the surface of the egg envelope. Recent preliminary TEM studies of *Phragmatopoma lapidosa* and *P. californica* oocytes have revealed identical structures (unpublished observations). SEM observations of the egg and trochophore of *P. lapidosa* in the present study suggest that the egg envelope is



FIGS. 12–15. *Phragmatopoma lapidosa*. Fig. 12. Larva in process of metamorphosing, with newly rotated primary paleae (PP), prominent parathoracic parapodia (PT), and dorsal nototrochs (NO). Scale = 15 μm. Fig. 13. Newly metamorphosed larva (ventral view) with anteriorly rotated larval tentacles (T) and primary paleae (PP). Note tentacle bud (TB) representing first pair of feeding tentacles in adult. BO, building organ; N, neurotroch; T, telotroch; M, mouth. Scale = 200 μm. Fig. 14. Juvenile worm 30 days after metamorphosis with fused operculum (OP), feeding tentacles (FT), and newly formed caudal appendage (C). M, mouth; N, neurotroch. Scale = 200 μm. Fig. 15. Lateral view of head region of juvenile in Fig. 14, showing opercular paleae of crown (OP), opercular cirri (CI) around base of crown, newly formed tentacle buds (TB), clusters of lateral cilia in feeding tentacles (C), and the rudimentary parapodium of the first thoracic segment (RP). Scale = 100 μm.

retained as the cuticle through the trochophore stage since both have identical surface granules. The absence of granules and presence of microvilli penetrating the cuticle in the later larval stages indicate that a new cuticle is formed after the early larval stages. However, a light microscope study of Wilson (1929) on *Sobellaria alveolata* suggested that the egg envelope was retained as the cuticle throughout larval development. Novikoff (1938) also provided evidence that the "vitelline membrane" serves as the cuticle in the very early larval stages of *S. vulgaris*. Wilson (1929) did observe the loss of the egg envelope from some *S. alveolata* larvae in

the late trochophore stage but considered it an exceptional occurrence. A TEM study of the development of the larval cuticle of *Phragmatopoma lapidosa* is presently in progress and will certainly clarify this issue.

'Sensory hairs' as described in this paper have not been previously reported from sabellariid larvae although Wilson (1929) referred to paired "stout cilia" on each wrinkle of the tentacle and at the tentacle tips of young postmetamorphic *Sabellaria alveolata*, which probably are not comparable. The sensory tufts observed in *P. lapidosa* larvae superficially resemble some of the epidermal "diffuse sense organs" figured by Langdon (1900) in adult *Nereis virens*, which consisted of one or more distal processes penetrating the cuticle from basally located bipolar nerve cells. These structures were particularly numerous around the mouth and over the distal portions of cephalic appendages such as the palps, cirri, and tentacles. The fine structure of the tufts in *P. lapidosa* larvae is currently under investigation.

Wilson (1929) described in detail the settlement behavior of *Sabellaria alveolata*, which is essentially identical to that of *Phragmatopoma lapidosa* larvae, recently described by Eckelbarger (1976). Both are highly gregarious rock-adherent species that form massive colonies intertidally. In both species, larvae approaching metamorphosis crawl head downwards over the bottom of laboratory cultures, applying their mouths to the substratum. The lips of the mouth are spread out and flattened to allow maximum contact of their inside ciliated surfaces to the substratum. The tentacles are turned forward and their ventrally ciliated surfaces are alternately raised and lowered onto the substrate in a manner suggesting 'feeling' or testing. Although the tentacular 'sensory tufts' of *P. lapidosa* larvae are primarily dorsal in position, additional tufts are located on the lateral tentacle surfaces. Other tufts may be present within the ventral food groove but are obscured by the dense cilia within them. In any event, the dorsal tentacle surface undoubtedly contacts the substrate as the larvae wander over and through the sand grains and shell hash. The concentration of tufts on the larval tentacles suggests that they may play a role in substrate selection.

In general, SEM observations of the later larval stages of *Phragmatopoma lapidosa* have confirmed morphological interpretations previously reported from light microscope studies. However, although the 'sensory tufts' can be resolved with careful light microscopic technique, SEM provides a more satisfactory impression of their morphology and distribution. The surface morphology of the cuticle in the trochophore larva and the later larval stages cannot be resolved with light microscopy and is most easily discerned with SEM.

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