Observations on Oceanic Pelagosphera Larvae (Sipuncula): Morphology, Behavior, and Metamorphosis

Mary E. Rice,[1] Julianne Piraino,[2] Hugh F. Reichardt,[2] and Michael J. Boyle[3]*

ABSTRACT. Pelagosphera larvae are common components of warm-water currents throughout oceanic waters. Because of this wide distribution, it has been presumed that the larvae could serve to disperse species across ocean basins and to maintain genetic connectivity between isolated populations. However, without specific identification of larvae and a known distribution of corresponding adults, the role of oceanic pelagospheras has yet to be confirmed. As part of our longtime studies of the life histories of sipunculans, we have accumulated observations on the morphology, behavior, and metamorphosis of a diversity of oceanic pelagosphera larvae, with the general intent of determining their adult affiliations and their potential role in dispersal and genetic exchange. Here, we review our observations of the larvae, primarily from plankton collections in the Florida Current, defining the distinguishing characters of the diverse larval types and, when possible, their juveniles, thereby providing the morphological basis for relating larval types to their corresponding adults. We focus on 10 larval types that occur most commonly in our collections. Of these 10, specific adult affiliations were proposed for six, and generic status was proposed for the other four. Among the larval types, three larval groups were recognized by morphological and behavioral characters, as well as by sequential changes at metamorphosis. These three groups correspond to the recently recognized families in a taxonomic revision of the phylum. The morphologic data provided in our studies also complement and confirm molecular identification of these same larval types.

INTRODUCTION

The term pelagosphera was first used by Mingazzini (1905), who erroneously described a planktonic larval form as an adult sipunculan, creating a new genus and species, *Pelagosphaera aloysii* (Figure 1). His description, in which he mistook larval glands as gonads, was of a single preserved and contracted specimen from plankton collections at a depth of 500 m by the Italian ship *Liguria* in the Pacific between New Caledonia and New Zealand. His mistake was soon realized by Senna (1906), who examined sectioned material of similar specimens from collections of the same expedition but from other locations in Ceylonese and Indonesian waters. Later, Spengel (1907), referring to developmental studies of *Sipunculus nudus* by Hatschek (1883) and others, proposed the planktonic larval form described by Mingazzini to be a species of *Sipunculus*. Over the next several years the term pelagosphera continued to be used in reports of similar large oceanic larvae of the genus *Sipunculus* (ranging 6–10 mm in diameter) collected from plankton in the Atlantic and Pacific Oceans (Heath, 1910; Dawydoff, 1930; Stephen, 1941; Fisher, 1947; Åkesson, 1961; Damas, 1962). However, the specimens were from preserved collections and usually contracted, and the morphology of the retracted head was not properly understood. In a study of live oceanic pelagosphera larvae during expeditions of the Russian ship *Akademik Kovalensky*, Murina (1965) demonstrated that the spherical larval body, when extended, consisted of a well-formed head and a band of cilia, both of which could

[1] Senior Research Scientist Emeritus, Life Histories Program, Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949, USA.

[2] Retired from Life Histories Program, Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949, USA.

[3] Life Histories Program, Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, Florida 34949, USA.

* Correspondence: rice@si.edu

Manuscript received 28 March 2016; accepted 1 November 2017.

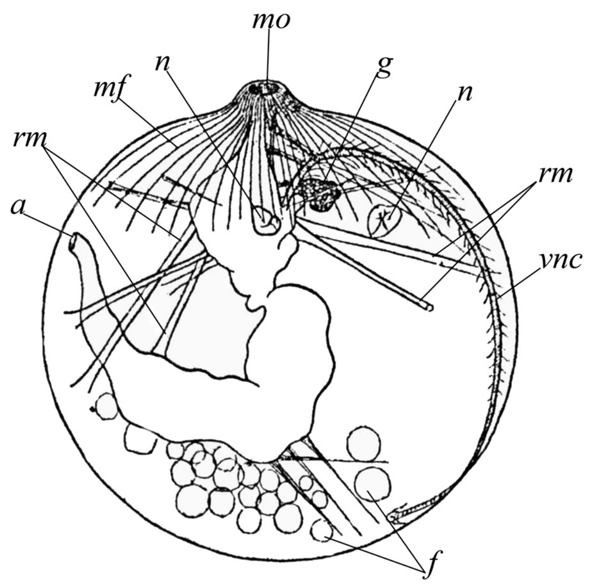


FIGURE 1. *Pelagosphaera aloysii*, modified from Mingazzini (1905). Abbreviations: a = anus, f = fat globules, g = glands attached to esophagus, mf = muscle fibers, mo = mouth, n = nephridium, rm = retractor muscles, vnc = ventral nerve cord.

be retracted into a posterior trunk. She was able to make specific identifications based on their morphology, identifying them as *Sipunculus aequabilis* from the Gulf of Aden and *Sipunculus norvegicus* from the Northwest Pacific; the former species was later synonymized with *S. norvegicus* (Cutler, 1994).

In more recent studies the definition of the term pelagosphera has been broadened. Current usage defines the pelagosphera as the developmental stage in sipunculans that succeeds the trochophore and is characterized by the loss or reduction of the prototroch and the development of a well-defined band of metatrochal cilia that serves as a locomotory organ (Rice, 1967). Usually, but not always, there is a terminal organ by which the larva may attach temporarily to the substratum. Within the various developmental patterns of sipunculans there are both lecithotrophic and planktotrophic pelagospheras (Rice, 1967, 1975, 1981). The pelagosphera larvae of the open ocean are planktotrophic (i.e., feeding larvae) and are well adapted for a prolonged planktonic existence. There is a great diversity among the oceanic pelagospheras, including the large planktonic larvae of the genus *Sipunculus*. Häcker (1898), in his report for the Plankton Expedition of the Humboldt Foundation, noted four sipunculan larval types: one similar to that of *Sipunculus* and three for which he designated a genus *Baccaria*, because of the "berry-like" configuration of the cuticle. Later, Jägersten (1963) reviewed the general morphology and behavioral traits common to 12 pelagosphera larvae from the Florida Current off Key Biscayne, Florida, and Bimini, the Bahamas. He recognized two groups on the basis of the texture of the body surface: one "smooth" as in the *Sipunculus*-like larvae and one "rough" or papillated as in the *Baccaria* larvae of Häcker. Hall and Scheltema (1975) were the first to give complete descriptions of the oceanic pelagospheras, distinguishing eight larval types, including those reported by Häcker (1898) and Mingazzini (1905). Scheltema and colleagues also reported the broad distribution of the various pelagospheras in the North and South Atlantic and Pacific Oceans, suggesting the potential role of oceanic pelagosphera larvae in the dispersal of species over great distances (Scheltema, 1963; Hall and Scheltema, 1975; Scheltema, 1975; Scheltema and Hall, 1975; Scheltema, 1986; Scheltema and Rice, 1990).

The present chapter is an overview of observations compiled over the past 40 years on oceanic pelagosphera larvae, primarily from the Florida Current, including basic morphological features, behavior, and metamorphosis of larvae and, when possible, morphological features of juveniles. The intent is to further define the larval types by confirming and extending previous studies on morphology of both living and preserved specimens and by providing the basis for relating larvae to their adult specific affiliations.

MATERIALS AND METHODS

Pelagosphera larvae were generally collected in the surface plankton of the Florida Current 32 to 40 km east of the Fort Pierce Inlet over bottom depths of 200 to 270 m with a net 0.75 m in diameter and a mesh of 200 μm. The tows, each lasting 15 to 20 min, were sorted on return to the laboratory. Larvae were placed in seawater in covered glass containers of 350 mL capacity and maintained at approximately 25°C. Specimens to be used for scanning electron microscopy (SEM) or to be retained for reference were fixed in 2.5% glutaraldehyde buffered with Millonig's phosphate buffer and adjusted to an osmolality of 1,000 milliosmoles by the addition of sodium chloride. Prior to fixation, larvae or juveniles were anesthetized in 5%–10% ethanol in seawater for approximately 5 min or until their heads remained extended. Larvae to be maintained alive were fed by the occasional addition of a mixture of algal/diatom cultures such as *Thalassiosira, Chlorella, Isochrysis,* and *Dunaliella*. Substratum, provided for studies of metamorphosis and rearing larvae to identifiable juveniles or adults, consisted of sediment from habitats of known adult sipunculans or from aquariums in which adult sipunculans had been maintained. Seawater in all containers was changed periodically, usually twice or more weekly. For longtime rearing, containers were placed in recirculating seawater systems. Two of the larval types (identified as *Apionsoma misakianum* and *Siphonosoma cumanense*) were reared through all stages of their life cycles (see Discussion).

RESULTS

Common Features of Oceanic Pelagosphera Larvae

The body of a pelagosphera is characterized by three regions: anterior head, midregion or thorax, and posterior trunk

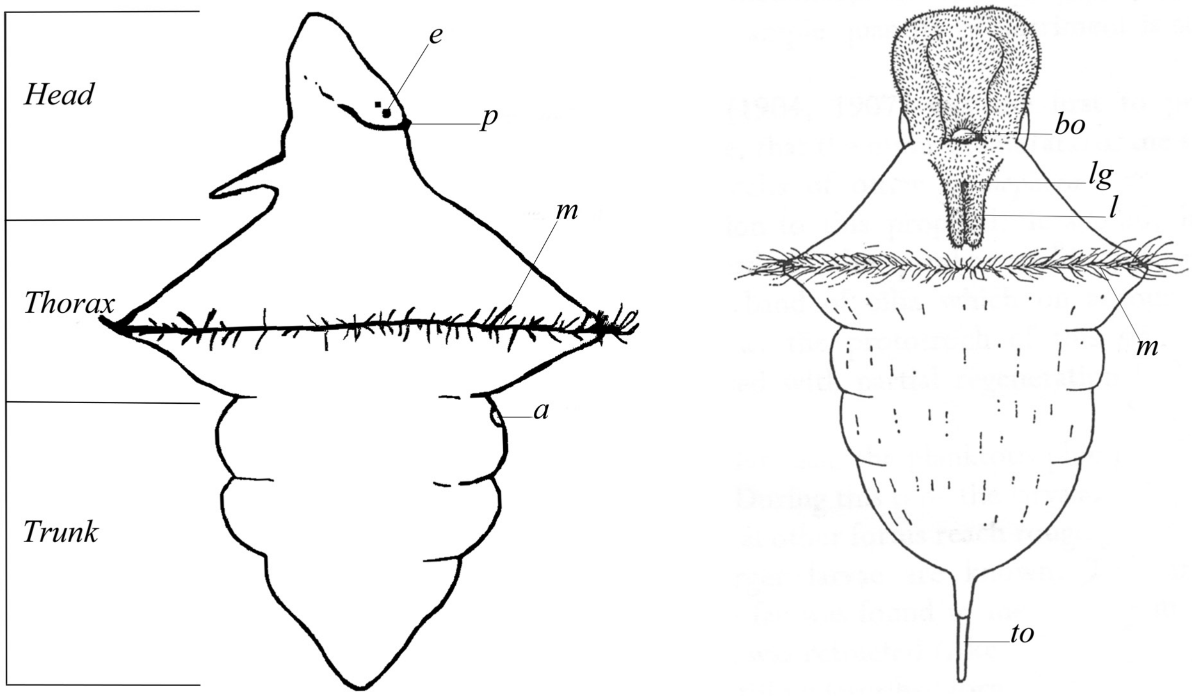


FIGURE 2. Diagrams of a swimming pelagosphera larva. Left, lateral view showing regions of the body. Modified from Jägersten (1972). Right, ventral view. Modified from Jägersten (1963). Abbreviations: a = anus, bo = buccal organ, e = eye, l = lip, lg = pore of the lip gland, m = metatroch, p = prototroch, to = terminal organ.

(Figure 2). The ventral ciliated head is bilobed with a central groove leading to the mouth. Posterior to the mouth, a ciliated lower lip extends outward, bearing a pore that opens to the duct of an internal two-lobed or four-lobed pendular gland, the lip gland. At the base of the lip and posterior to the mouth is a slit through which a muscular bulb, the buccal organ, can be everted. The dorsal head is marked by a U-shaped ciliated band, designated as the prototroch, above which is located a pair of dorsolateral eyespots. The thorax bears the metatroch, a prominent band of cilia that serves a locomotory function. Both the head and thorax can be retracted into the trunk. The elongated posterior trunk is delineated from the mid-region by the postmetatrochal sphincter. The trunk is usually terminated by a retractable terminal organ.

Internally, the digestive tract consists of an elongate esophagus leading to a bulbous stomach and a recurved intestine that opens through an anus on the mid-dorsal trunk (Figure 3). Two nephridia open through nephridiopores situated ventrolaterally on the mid- to anterior trunk. Traversing the spacious undivided coelom are retractor muscles, functioning in the withdrawal of the head and thorax. The coelom encloses a variety of freely floating coelomocytes.

In observations of living larvae in the laboratory, either by video or with the aid of a dissecting microscope, several common behavioral traits are revealed. When the larva is swimming, the metatroch is fully extended, the entire thorax is inflated, and the posterior trunk is contracted, pushing coelomic fluid and internal organs (e.g., nephridia and stomach) into the expanded region of the thorax. A common behavior is retraction of the head and thorax into the trunk. Various other degrees of contraction have also been noted: head only, metatroch only, or metatroch partially retracted. When both the head and metatroch are retracted, the body may assume a spherical or near-spherical shape closed anteriorly by the contraction of the postmetatrochal sphincter. When the head is retracted, the lower lip is flattened against the ventral head; otherwise, it is extended outward perpendicularly from the head. In some larvae, the terminal organ may serve as a temporary attachment to the substratum. A common behavior is bending of the body along the dorsoventral axis and the periodic placement of the terminal organ in the region of the mouth. The larva also may glide along a substratum with the ventral head applied to the substratum and lower lip flattened against the substratum while periodically extending the buccal organ. Larval types may show distinctive behavior in the extensibility of the body and the assumption of a variety of body shapes. When exposed to appropriate sediment, larvae will usually burrow and subsequently undergo metamorphosis. Prior to burrowing, the larva may move along with its ventral ciliated head applied to the sediment, passing particles through the ventral groove and over the ventral lip and leaving a trail of mucus-cemented sediment.

FIGURE 3. Color renderings showing the morphology of major groups of oceanic pelagosphera larvae: from left to right, group 1, Smooth (represented by larval type smooth yellow-green); group 2, annulated (represented by larval type transverse groove); and group 3, papillated (represented by larval type white blackhead). Colors indicate larval structures: blue = brain and ventral nerve cord, green = buccal organ, light orange = nephridia, pink = glands, red orange = muscles, white = metatroch, yellow = cuticle and body wall. Illustrations by Carolyn B. Gast.

Another behavior is the curling of the body into a doughnut shape with the head touching the tail, then rolling over so that all parts of the body are exposed to the sediment and again leaving trails of aggregated particles of sediment.

Major Larval Groups and Distinct Larval Types

Here, on the basis of cuticular properties, we follow, in part, the classification of Scheltema and Rice (1990) and recognize three major larval groups (Figure 3): (1) smooth (cuticular structures absent), (2) annulated (body wall in transverse grooves encircling the body), and (3) papillated (body covered with cuticular papillae). Among the major larval groups, 10 larval types are described: four smooth, two annulated, and four papillated. To distinguish larval types within major larval groups, morphologic characters include cuticular elaborations, body shape and size, the form of the terminal organ (when present), eyespots (color and number), the shape of the head and ventral lip, the relative positions of the anus and nephridiopores, and color. Although color can be an important character, it must be used with caution and with a consideration of the range of variation. In the following descriptions, color is noted as observed with a dissecting microscope and reflected light. Among the papillated larvae, additional distinctions are shape and structure of the cuticular papillae. These characters are most readily distinguished in fixed specimens observed with scanning electron microscopy. Internal structures, used when visible through a transparent or translucent body wall, are the presence and number of longitudinal muscle bundles, attachments of retractor muscles, and relative length and attachment of nephridia. Distinctive behavioral traits include the use of the terminal organ, that is, whether it is used for attachment. Certain larval types may also be distinguished by the plasticity of the body and the assumption of a variety of body shapes and patterns of movement.

Terminology

In lieu of known specific identity, the descriptive designations assigned to larval types in this paper (e.g., large transparent, yellow pap) are those that have been adopted in our laboratory over the years for ease of communication. Table 4 in Schulze et al. (this volume) compares our designations with those of Hall and Scheltema (1975) for these same or similar larval types. The present study will propose specific identifications for six of the larval types on the basis of morphological characters of adults and juveniles reared from larvae in the laboratory. Schulze et al. (this volume) confirm these specific adult affiliations by matching DNA of larvae and adults.

Description of Individual Larval Types

Of the approximately 30 larval types that have been distinguished in the Florida Current (Rice, 1981), we have selected for this review the 10 types that occur commonly in our plankton samples. The descriptions that follow are unequal in detailed information because more specimens were available for some larval types or some metamorphosed more readily.

Large Transparent

Usually 4–5 mm in extended length, this is the largest of the pelagospheras of the Florida Current (Figures 4A,B, 5A,B). The cuticle is smooth, lacking papillae, with a bluish or iridescent cast. The body wall is transparent, and the internal organs are readily visible. Two pairs of retractor muscles extend from the metatrochal collar to attach at a level on the trunk approximately one-third the distance along the length of the trunk, with ventral retractors attaching slightly anterior to the dorsal retractors. In addition, eight thin and short retractors of the metatroch extend from the metatrochal collar to the anterior trunk. There are two nephridia, each opening in a ventrolateral position anterior to the anus. Extending from the ventral mouth, a pharyngeal area leads to a yellowish pigmented esophagus and bulbous stomach, from which the elongate intestine descends posteriorly, then recurves and ascends anteriorly to open at a dorsal anus in the anterior third of the trunk. One fixing muscle attaches the descending intestine to the anterior body wall, and a second fixing muscle attaches the ascending intestine to the posterior body wall. The ventral nerve cord is clearly visible as a broad strand extending from the base of the ventral lip to the posterior tip of the larva, where it ends in a small loop at the point of its attachment; circumesophageal connectives between the anterior nerve cord and dorsal brain have not been identified. A pair of lip glands is connected by a common duct (pigmented orange) to a pore that opens on the tip of the bifurcated lip. Extending outward from the base of the ventral head, the lip is relatively small and pointed. The rounded ventral head and the median groove leading to the mouth are covered with short cilia, as is the upper surface of the lip. Bordering the lip are somewhat longer cilia, and on the under surface of the distal end of the lip there is a tuft of longer cilia. The dorsal head is marked by a band of short cilia that extends laterally and is presumed to represent the prototroch. A pair of small red/black dorsolateral eyespots is located anterior to the prototroch. A smaller reddish eyespot is located above each of the larger eyes, and between the eyes there may be flecks of reddish or yellowish pigment. The base of the metatrochal band, also pigmented, is marked by a reddish-orange coloration. A typical terminal organ is lacking, although a shallow depression, sometimes everted as a small knob, is apparent at the posterior tip. The posterior loop of the ventral nerve cord ends at this terminal depression. The intersecting longitudinal and circular muscle bands of the body wall are clearly visible in the live larva, with the longitudinal bands numbering 54 to 55.

Within 1 week after metamorphosis the metatroch is lost, and the body consists of a short introvert and elongated trunk; the introvert to trunk ratio is 1:6. The introvert, formed from the larval thorax, is encircled by rows of triangular papillae (typical of the genus *Sipunculus*). Four tentacular lobes surround the mouth. The dorsal head shows a distinctive lobe, presumably the site of the underlying brain. The dorsal area above and lateral to the brain is covered with balls of cilia of unknown origin (Figures 4C,D, 5C).

This larva is identified as *Sipunculus polymyotus* Fisher, 1947, primarily on the basis of the number of longitudinal muscle bands (54–55) that fall within the range diagnostic for this species. First recognized by Fisher (1947) in plankton collections off Cape Hatteras, North Carolina, it was later described more fully from living specimens by Hall and Scheltema (1975), who reported its common occurrence in the Gulf Stream.

Smooth Small Transparent

The approximate length of the body when extended is 1 mm. The body wall is transparent, and the surface iridescent. Visible through the body wall, the posterior gut is usually yellowish, as is the groove of the ventral head and the duct to the lip gland. The rim of the metatrochal band sometimes shows an orange pigmentation. A pair of ventrolateral nephridia is located anterior to the anus. The tubular nephridia are tannish, with characteristic red pigment at their proximal attachments to the body wall. The two eyespots are brownish/dark red, each with an anterior smaller orange/red spot. Intermediate pigment, when present, is yellowish. Although there is a slight invagination or extended bulbous structure at the posterior extremity of the larva, there is no definitive terminal organ (Figures 6A, 7A,B).

At 1 week, the metamorphosed larva has elongated; the mouth is terminal and surrounded by four tentacular lobes, two longer dorsals, and two shorter ventrals. The brain with eyespots is now subdermal, entirely within the coelomic cavity. Ventrally, there is a red/yellow remnant of the lip gland. The nephridia are distended tannish clear spheres with a persistent red spot. At 6 weeks the extended length ranges from 10 to 14 mm; the ratio of the introvert to trunk is approximately 1:6. The introvert is clear and bulbous, covered by triangular, scalelike papillae, and terminated by four tentacular lobes. Visible through the transparent body wall are clear, elongated, and tubular nephridia, opening anterior to the anus, and a highly coiled gut, which may be packed

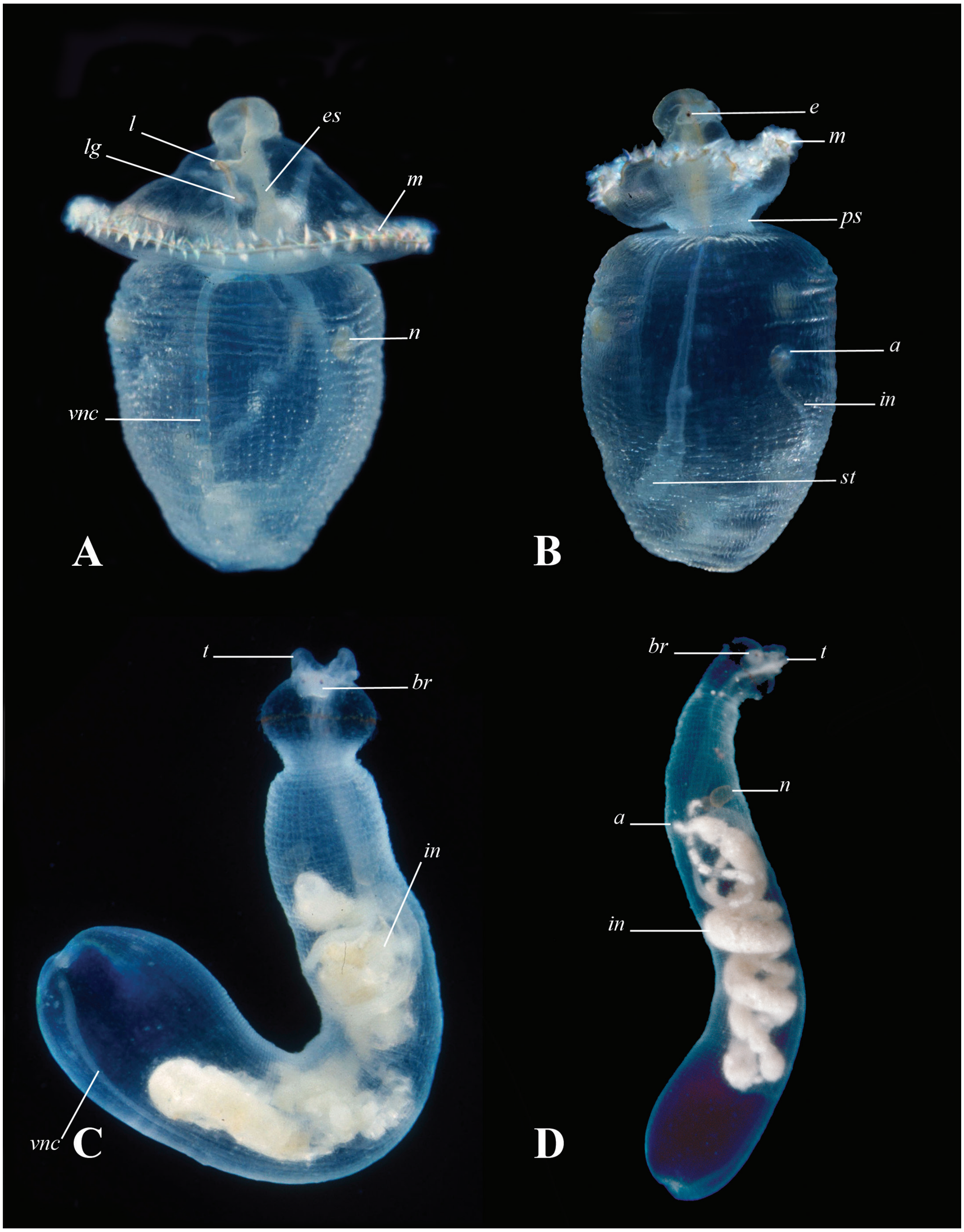


FIGURE 4. Photomacrographs of living large transparent larvae and early juveniles. (A) Ventrolateral view of larva. Modified from Jaeckle and Rice (2002). (B) Dorsolateral view of larva. (C) Juvenile, 7 days. (D) Juvenile, 18 days. Abbreviations: a = anus, br = brain, e = eye, es = esophagus, in = intestine, l = lip, lg = lip gland, m = metatroch, n = nephridium, ps = postmetatrochal sphincter, st = stomach, t = tentacles, vnc = ventral nerve cord.

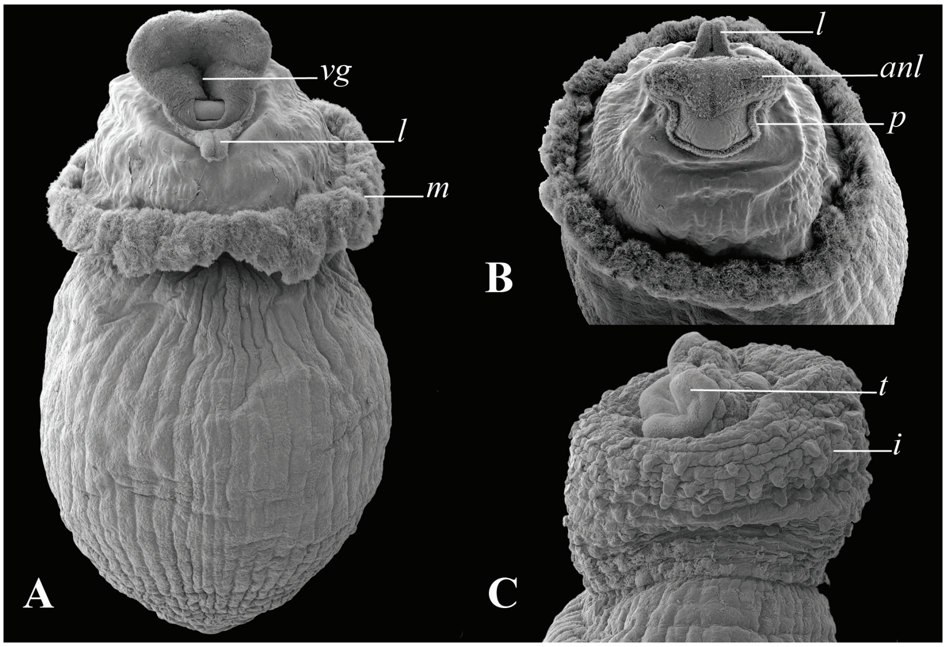


FIGURE 5. Scanning electron micrographs of large transparent larva and early juvenile. (A) Ventral view of larva. (B) Dorsoapical view of anterior head and metatroch of larva. (C) Juvenile, 1 week. Lateral view of head with tentacular lobes and introvert. Note characteristic scalelike papillae on introvert. Abbreviations: anl = anterior lobe of ventral head, i = introvert, l = lip, m = metatroch, p = prototroch, t = tentacular lobes, vg = ventral groove of head.

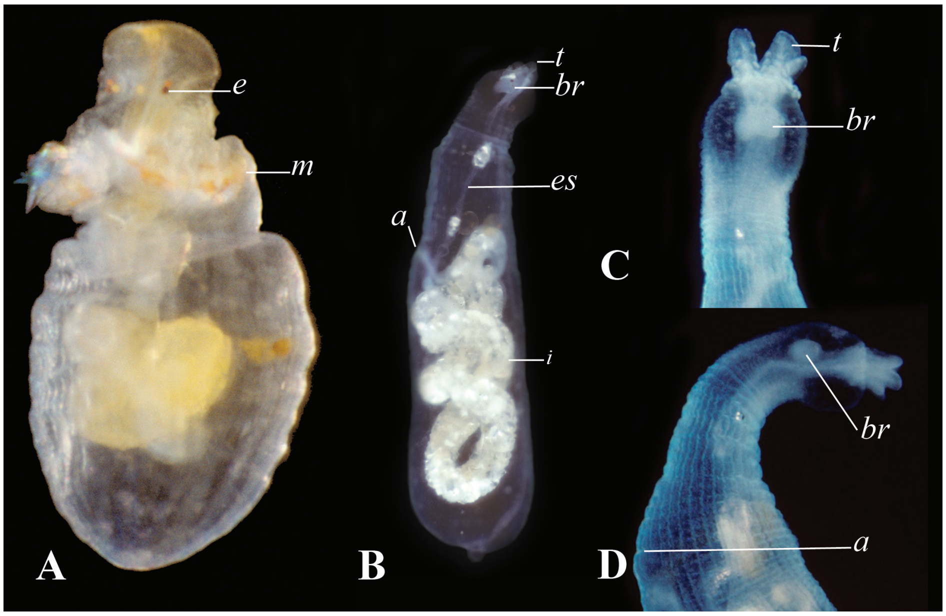


FIGURE 6. Photomacrographs of living smooth small transparent larva and juveniles. (A) Dorsolateral view of larva. (B) Juvenile, 2 weeks. Lateral view. Note sand grains in the esophagus and intestine. (C) Juvenile, 6 weeks. Ventral view of head and introvert showing bilobed brain and bulbous shape of introvert. (D) Juvenile, 6 weeks. Note subdermal location of brain. Abbreviations: a = anus, br = brain, e = eye, es = esophagus, i = intestine, m = metatroch, t = tentacles.

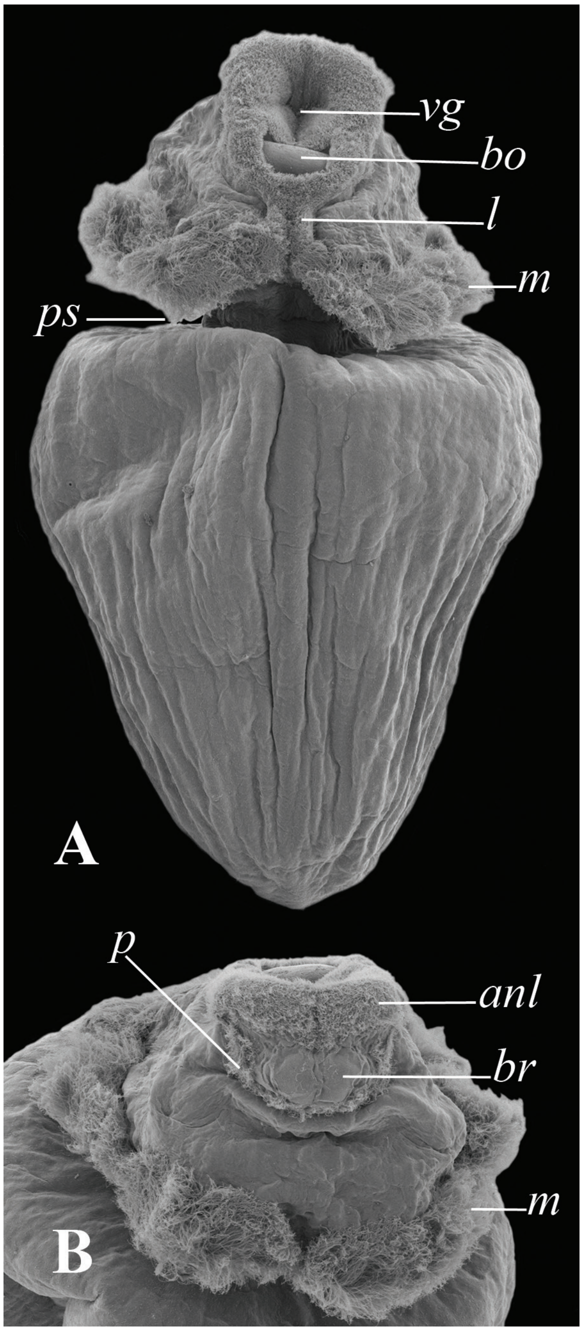


FIGURE 7. Scanning electron micrographs of smooth small transparent larva. (A) Ventral view. (B) Dorsoapical view of head and metatroch. Abbreviations: anl = anterior lobe of ventral head, bo = buccal organ, br = area of the brain, l = lip (with median bifurcation), m = metatroch, p = prototroch, ps = postmetatrochal sphincter, vg = ventral groove of head.

with sand grains. The well-developed longitudinal and circular muscles are apparent as intersecting bands (Figure 6B–D).

Several different types of smooth small transparent larvae occur in the plankton of the Florida Current, varying in relative opacity of the body wall, pigmentation, and characteristics of the eyespots. The description here is one of the more common types and is the one selected for molecular studies (Schulze et al., this volume).

The larval and early juvenile forms are characteristic of the genus *Sipunculus* as described in developmental studies (Hatschek, 1883; Rice, 1988). However, the available morphological information is insufficient for a specific diagnosis.

Smooth Orange

Ranging from 1 to 2 mm in extended length, this larva is readily recognized by its external iridescence and bright orange-yellow coloration (Figure 8A–C). As observed through the relatively opaque body wall, the gut is orange brown, and the nephridia brownish. In some specimens, reddish-brown spots are scattered over the region of the thorax posterior to the metatroch and occasionally over the entire trunk. Above the prototroch are two large reddish-black eyespots, each accompanied by a small anterolateral ancillary eyespot; between the large eyespots are patches of red pigment. The ciliated ventral head, as viewed by SEM, is bilobed by the median groove leading to the mouth (Figure 9A,B).

Posteriorly the lateral lobes form a smaller secondary lobe on either side of the region of the mouth and ciliated lower lip. The lip is narrow and bisected by a groove in which the pore of the underlying lip gland is located. Brownish pigment lines the lip groove as well as the median groove of the ventral lip. The body wall of the thorax is relatively thin, transparent, and greatly distended during swimming when the metatrochal band of cilia is fully extended. The body wall of the posterior trunk is characterized by three to four folds or grooves, which are more pronounced when the body is contracted. Opening onto the trunk are the mid-dorsal anus and the ventrolateral nephridiopores posterior to the anus.

The longitudinal musculature is arranged in 34 to 37 bands. The terminal organ has two components that are apparent when the organ is fully extended: a tubular extension of the posterior body from which a slender, relatively transparent rod can be extended or retracted. The latter is flexible and can move independently of the tube from which it is extended.

Within 1 week after the beginning of metamorphosis, the larval body has elongated, and the body wall has lost its pigmentation (Figure 8D). Clumps of orange or brownish pigment are visible in the coelomic cavity, presumably remnants of pigment released from the body wall. The body is clearly divided by the larval postmetatrochal sphincter into an elongated posterior trunk and a shorter bulbous anterior introvert, a transformation of the larval thorax (Figure 9C). The ratio of introvert to trunk is approximately 1:5. The metatroch has been lost, and at the anterior extremity of the introvert there are four tentacular lobes, the more dorsal being broader and longer than the ventral. The lower lip has regressed, and the mouth is in a more terminal position within the tentacular lobes. Eyespots and patches of intermediate pigment are still visible on the brain, which is now detached from the body wall and enclosed within the coelom. The

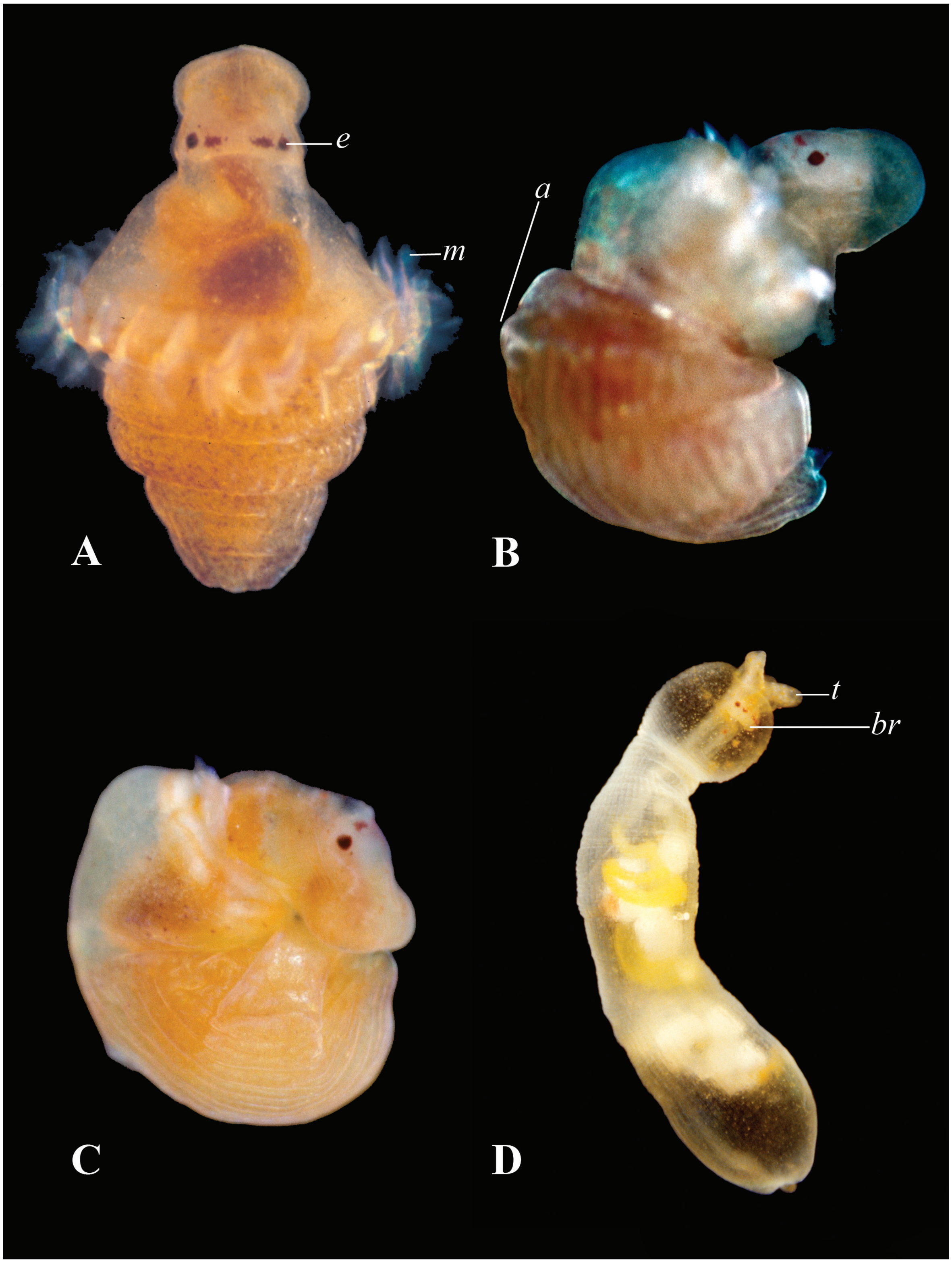


FIGURE 8. Photomacrographs of living smooth orange larvae and juvenile. (A) Dorsal view of swimming larva with metatroch extended. Note lateral eyespots on head with intermediate pigment. (B) Lateral view of larva showing bending behavior and partial retraction of metatroch. (C) Lateral view of larva with terminal organ in mouth. (D) Juvenile, 1 week with tentacular lobes. Note bulbous introvert and bilobed brain with eyespots. Abbreviations: a = anus, br = brain, e = eye, m = metatroch, t = tentacles.

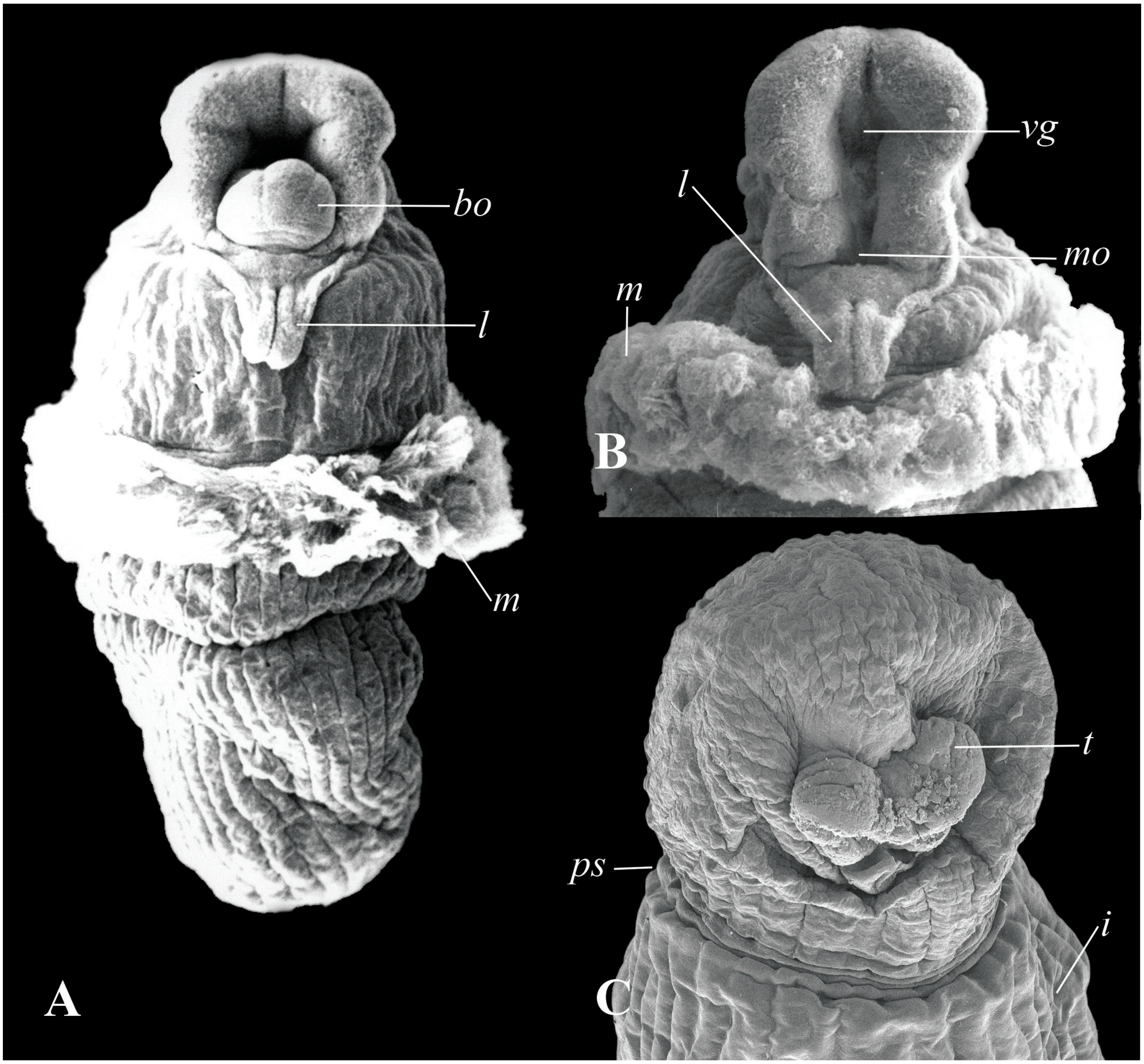


FIGURE 9. Scanning electron micrographs of smooth orange larva and beginning metamorphosis. (A) Ventral view of larva with buccal organ extended. (B) Ventral view of head and metatroch with buccal organ retracted. (C) Recently metamorphosed specimen. Ventral view of head and introvert. Note tentacular "buds," loss of metatroch, and constriction in the position of the larval postmetatrochal sphincter. Abbreviations: bo = buccal organ, i = introvert, l = lip, m = metatroch, mo = mouth, ps = postmetatrochal sphincter, t = tentacular lobes, vg = ventral groove of head.

ventral lip has regressed, and the remnant of the lip gland and duct are apparent as a clump of orange pigment at the anterior extremity of the ventral nerve cord, which extends as a broad uninterrupted band along the length of the body. The external surface of the trunk is smooth (nonpapillated), but scattered over the anterior bulbous introvert are small brownish spots that mark the beginnings of the scalelike papillae of later juveniles. Longitudinal muscle bundles extend the length of the trunk, and circular muscles are apparent at the anterior region of the trunk. At the posterior extremity, a small knob is in the position of the larval terminal organ. Internal structures visible through the transparent body wall include a pair of short, rounded nephridia,

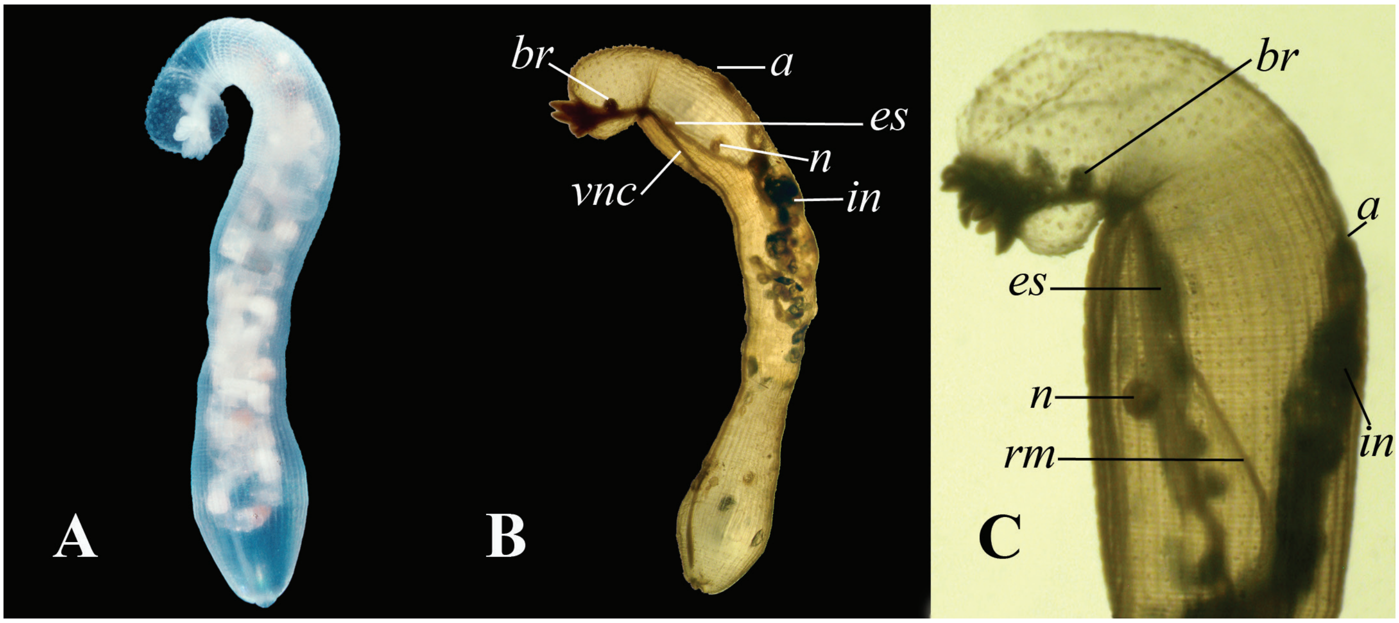


FIGURE 10. Photographs of smooth orange juveniles. (A) Photomacrograph of living juvenile, 4 months. (B) Juvenile, 5 weeks. Fixed specimen in alcohol, lateral view. (C) Juvenile, 6 months. Fixed specimen in alcohol, lateral view of anterior one-fourth of body showing the relative position of the rounded nephridium and anus. Abbreviations: a = anus, br = brain, es = esophagus, in = intestine, n = nephridium, rm = retractor muscle, vnc = ventral nerve cord.

attached at the nephridiopores just posterior to the level of the dorsal anus. Four retractor muscles are attached to the body wall of the trunk posterior to the level of the nephridiopores. The gut, with approximately 12 coils, may be packed with sand particles, indicating that the juvenile is actively feeding on the surrounding sediment (Figure 10A–C).

Observations and fixations of juveniles were made at various intervals from early metamorphosis to an age of 6 months (Figures 8D, 10A–C). At 5 weeks the total body length was 8 mm, with an introvert to trunk ratio of 1:7 and a crown of six to seven digitiform tentacles surrounding the terminal mouth. By 6 months, the length was 18 mm, the introvert to trunk ratio was 1:8, and the number of tentacles was approximately 16. Scalelike papillae covered the introvert of all juveniles. Scanning electron microscopy revealed pores on the papillae, as well as on the body wall of the introvert surrounding the papillae and over the surface of the trunk (Figure 11A–F). Ciliary processes extended from the larger pores, suggesting a sensory function. A remnant of the terminal organ was observed at 2 months.

Several morphological features of the larva and juvenile correspond to those of the genus *Xenosiphon*. They include the position of the nephridia posterior to the anus, tentacular arrangement, scalelike papillae on the introvert, and the number of longitudinal muscle bands. Other characters that are diagnostic for the genus (Cutler, 1994) but not possible to assess in larvae or juveniles are the presence of protractor muscles, the absence of a postesophageal loop in the gut, and the arrangement of coelomic canals in the body wall. Although there are three species of *Sipunculus* (*S. indicus, S. mundanus,* and *S. longipapillosus*) in which the nephridia are posterior to the anus (Cutler, 1994), the possibility of an association of smooth orange with this genus seems unlikely because of the differences in larval form and function with known *Sipunculus* larvae as reported in this chapter.

Smooth Yellow-Green

This larval type is distinguished from the smooth orange type primarily by its coloration (Figure 12A–D). The overall body commonly has a light greenish tinge. The head and metatrochal band are a golden yellow. Visible through the body wall, the gut is green, and the nephridia are brown. Two prominent black eyespots are present on either side of the dorsal brain. Above each is a small reddish spot, and between the two are splotches of red pigment. The extended length of the body is approximately 1 mm. The nephridiopores are posterior to the prominent dorsal anus. The longitudinal musculature of the body wall is divided into 33 to 40 muscle bands.

The morphology of the larval head is essentially the same as that of the smooth orange larva. On the dorsal head the U-shaped prototrochal band forms a lateral loop around either side of the head, continuing ventrally around the border of the lower lip (Figures 12C, 13A–D). As in all larvae in the smooth group, the ventral lower lip is narrow, completely ciliated, and bisected by a median groove. The shape of the trunk may be

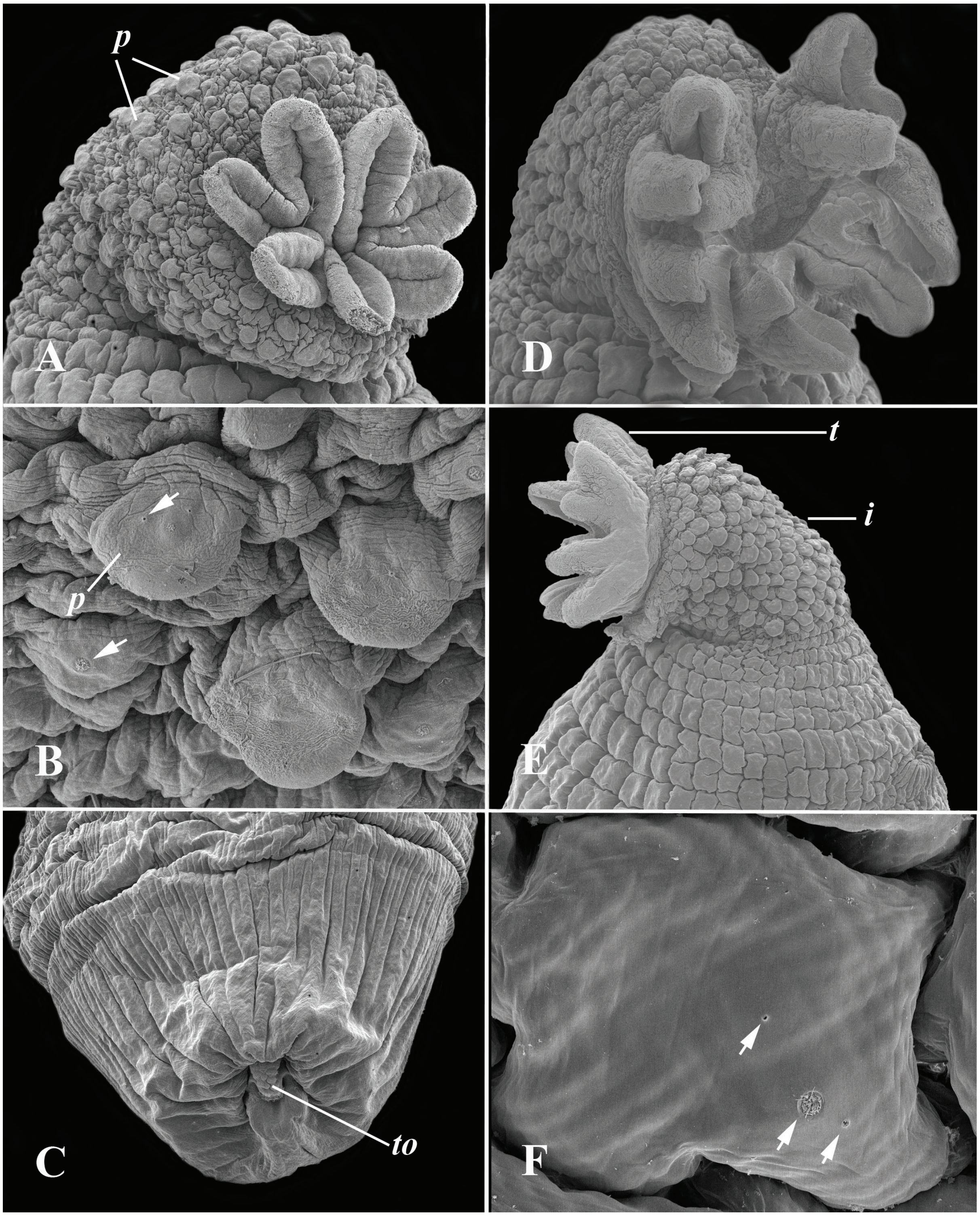


FIGURE 11. Scanning electron micrographs of smooth orange juveniles. (A–C) A 5-week juvenile. (A) Ventral view of tentacles and introvert. (B) Higher magnification of introvert papillae. Note scattered small pores and larger pores with cilia (arrows). (C) Terminal body showing remnant of terminal organ. (D–F) A 2-month juvenile. (D) Ventrolateral view of tentacles and introvert. (E) Lateral view of tentacles, introvert showing papillae, and anterior trunk. (F) Body wall showing small and larger, ciliated pores at arrows. Abbreviations: i = introvert, p = introvert papillae, t = tentacles, to = terminal organ.

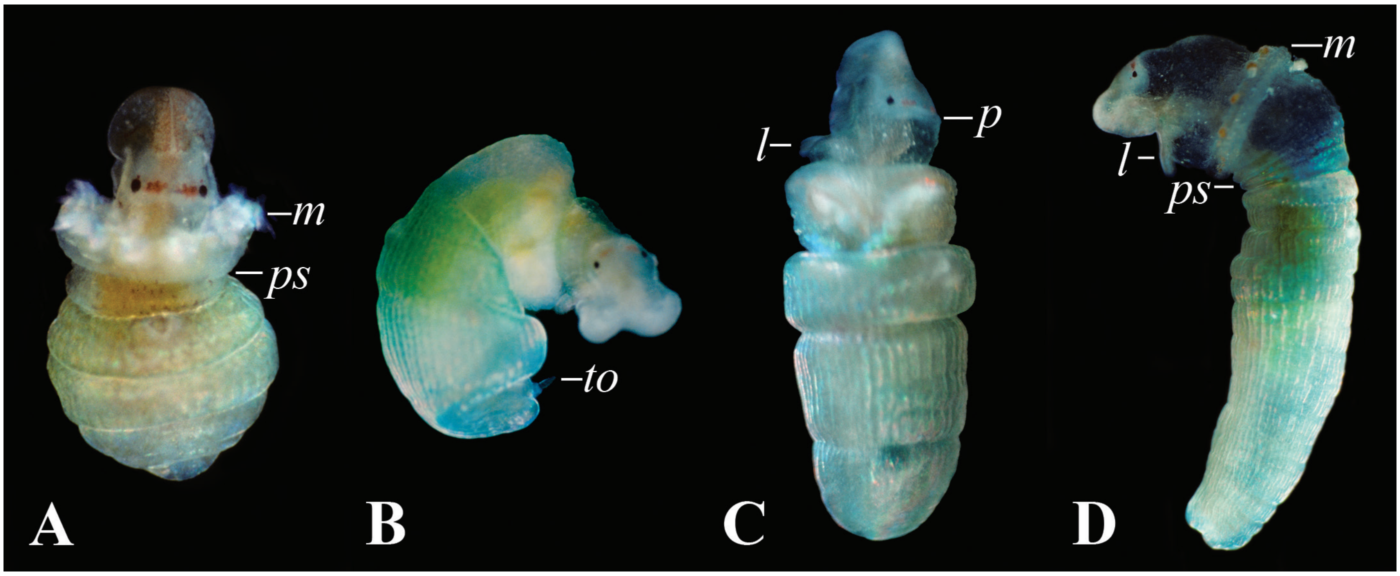


FIGURE 12. Photomacrographs of smooth yellow-green larvae and beginning metamorphosis. (A) Larva swimming with metatroch extended, dorsal view. (B) Larva bending, metatroch retracted, terminal organ extended, lateral view. (C) Larva "resting," metatroch retracted, lip and head extended, lateral view. (D) Larva beginning metamorphosis, partial loss of metatroch, lateral view. Abbreviations: l = lip, m = metatroch, p = prototroch, ps = postmetatrochal sphincter, to = terminal organ.

pyramidal, especially during swimming, and is characterized by three to four transverse grooves.

Postlarval stages have been observed over 2 to 3 month intervals from beginning metamorphosis to 1 year (Figures 12D, 14A–F, 15A–E). The first indication of metamorphosis is the loss of the metatrochal cilia. At this stage, the position of the former band is marked by clumps of bright orange pigment. Within 1 to 2 days the ciliated lip and lip gland have regressed, and tentacular lobes have formed at the anterior and lateral margins of the ventral head, the more anterior being longer than the lateral.

Within 2 days the body is elongate and transparent; the larval length has more than doubled, and the terminal organ has been lost. Visible through the body wall are coils of the ascending and descending gut that may be packed with sand grains. A pair of brownish-green nephridia is attached to the body wall at the nephridiopores. At 1 week after metamorphosis, the tentacular crown consists of three pairs of tentacles surrounding the mouth, and it has moved from a ventral to a more terminal position. The brain, still with a pair of eyespots, is subdermal, within the coelom. The introvert is clearly defined by a posterior constriction (the larval postmetatrochal sphincter) and often assumes a bulbous shape. A characteristic movement of the newly metamorphosed stage is a rapid thrusting of the head and bulbous introvert, a movement utilized in burrowing.

At 4 months, the total length of the larva is approximately 12 mm, the introvert to trunk ratio is 1:5, and the number of tentacles is 16. Along the length of the trunk, transverse and longitudinal striations of the cuticle mark the underlying musculature of the body wall. Visible through the transparent body wall are two pairs of retractor muscles, dorsal and ventral, that attach at the same level on the anterior one-quarter of the trunk posterior to the nephridiopores. Two elongate, slender, brownish nephridia attach at the nephridiopores on either side of the ventral nerve cord at the sixth longitudinal muscle band on either side of the ventral nerve cord. Extending approximately one-fifth the length of the trunk, they are completely attached to the body wall (Figure 14D). At 1 year the total body length has reached 30 mm, the ratio of introvert to trunk is 1:10, and the tentacular crown has added numerous digitiform tentacles apparently arranged in three rows (Figure 14E–F).

The basic morphology of this larval type is similar to that of the smooth orange larva and is also closely aligned with the genus *Xenosiphon*. However, differences between the two larval types in coloration of the larvae and in the form and relative length of juvenile nephridia suggest that they may represent two species (compare Figures 10C and 14D). Neither larval type displays the external "gills" reported by Cutler (1994) in one of the two species of *Xenosiphon* that he recognized.

Transverse Groove

A common larva in the Florida Current, it is typically 2 mm in extended length with an overall light greenish-yellow hue. Readily distinguished by marked transverse grooves in the body wall of the trunk, it is also characterized by behavioral changes in shape ranging from longitudinal extensions to a series of circular,

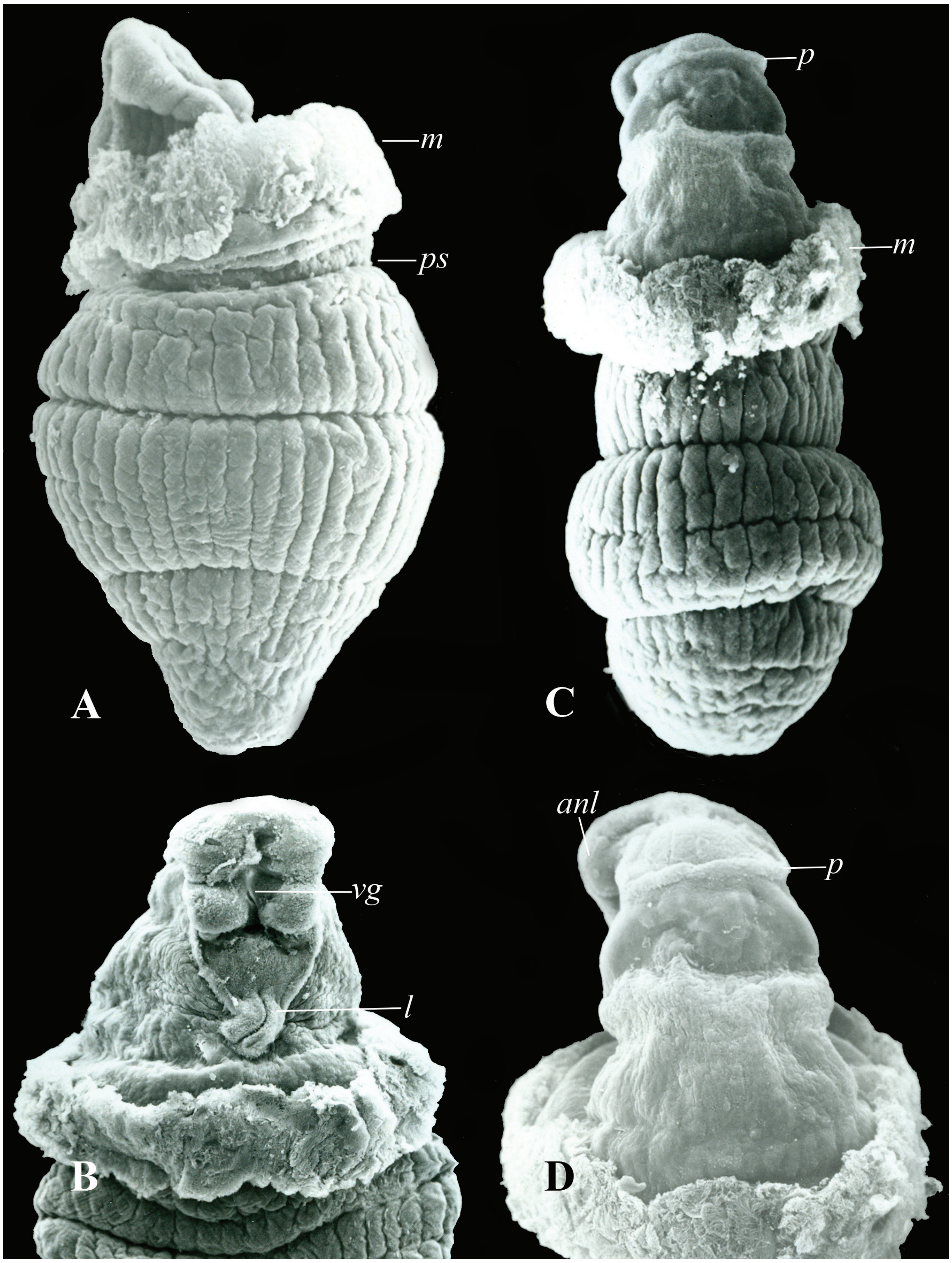


FIGURE 13. Scanning electron micrographs of smooth yellow-green larvae. (A) Lateral view. Note transverse folds in body wall of trunk and bands of longitudinal muscles. From Rice (1981). (B) Ventral view, higher magnification of ventral head and lip. (C) Dorsal view. (D) Dorsal view, higher magnification of dorsal head and prototroch. Abbreviations: anl = anterior lobe of ventral head, l = lip, m = metatroch, p = prototroch, ps = posttrochal sphincter, vg = ventral groove of head.

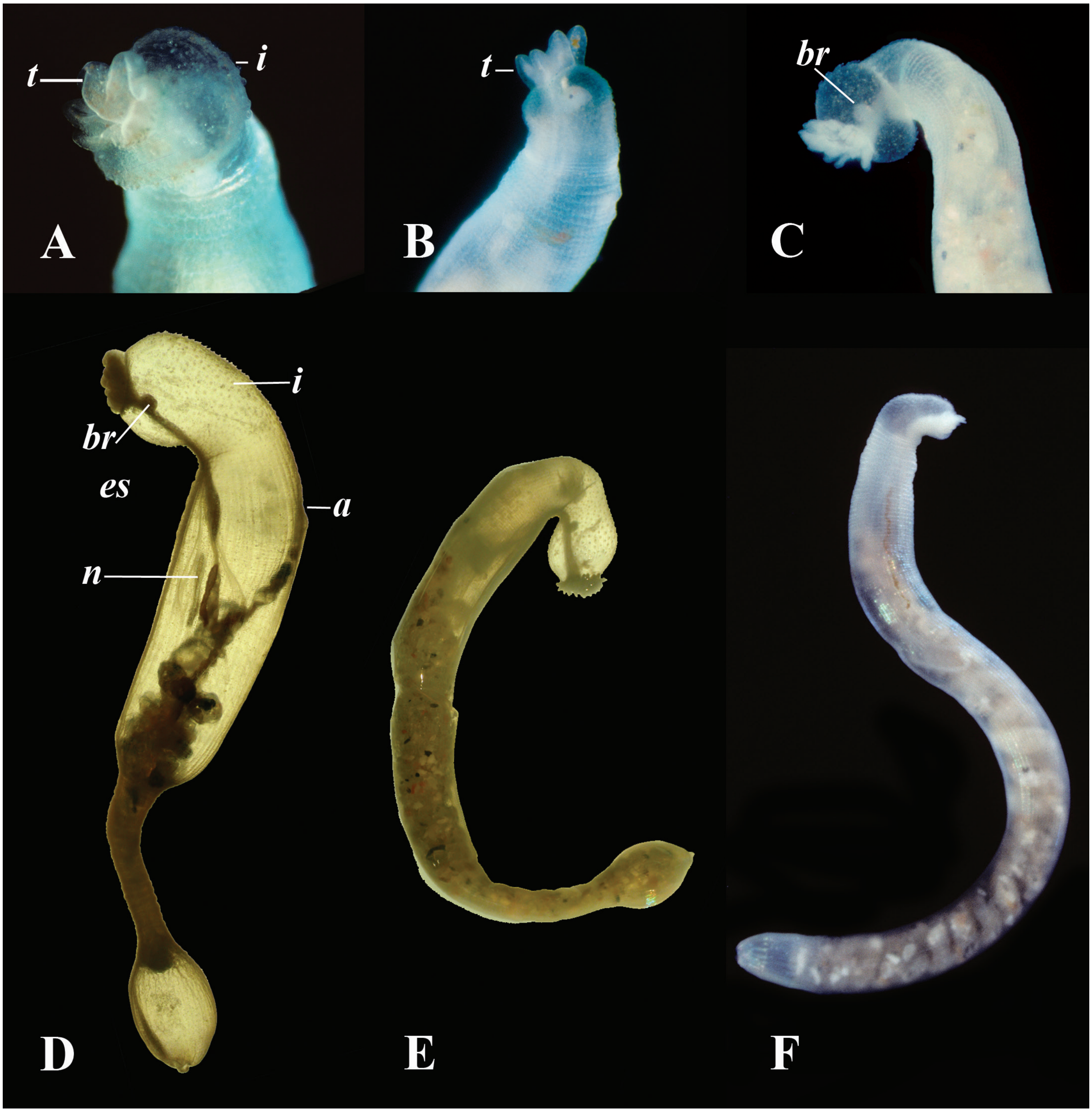


FIGURE 14. Smooth yellow-green juveniles. (A–C) Photomacrographs of living juveniles. Lateral views of head and anterior trunk. (A) Beginning metamorphosis. Note loss of metatroch, bulbous introvert, tentacular lobes. (B) Juvenile, 1 month with three pairs of elongate tentacles, subdermal brain. (C) Juvenile, 4 months. Numerous tentacles (16), subdermal brain. (D) Juvenile, 4 months. Fixed specimen in alcohol, lateral view. Note elongate nephridium and relative position of nephridium posterior to anus. (E, F). Adult, 1 year. The same specimen is shown alive in (F) and in alcohol after fixation in (E). Abbreviations: a = anus, br = brain, es = esophagus, i = introvert, n = nephridium, t = tentacles.

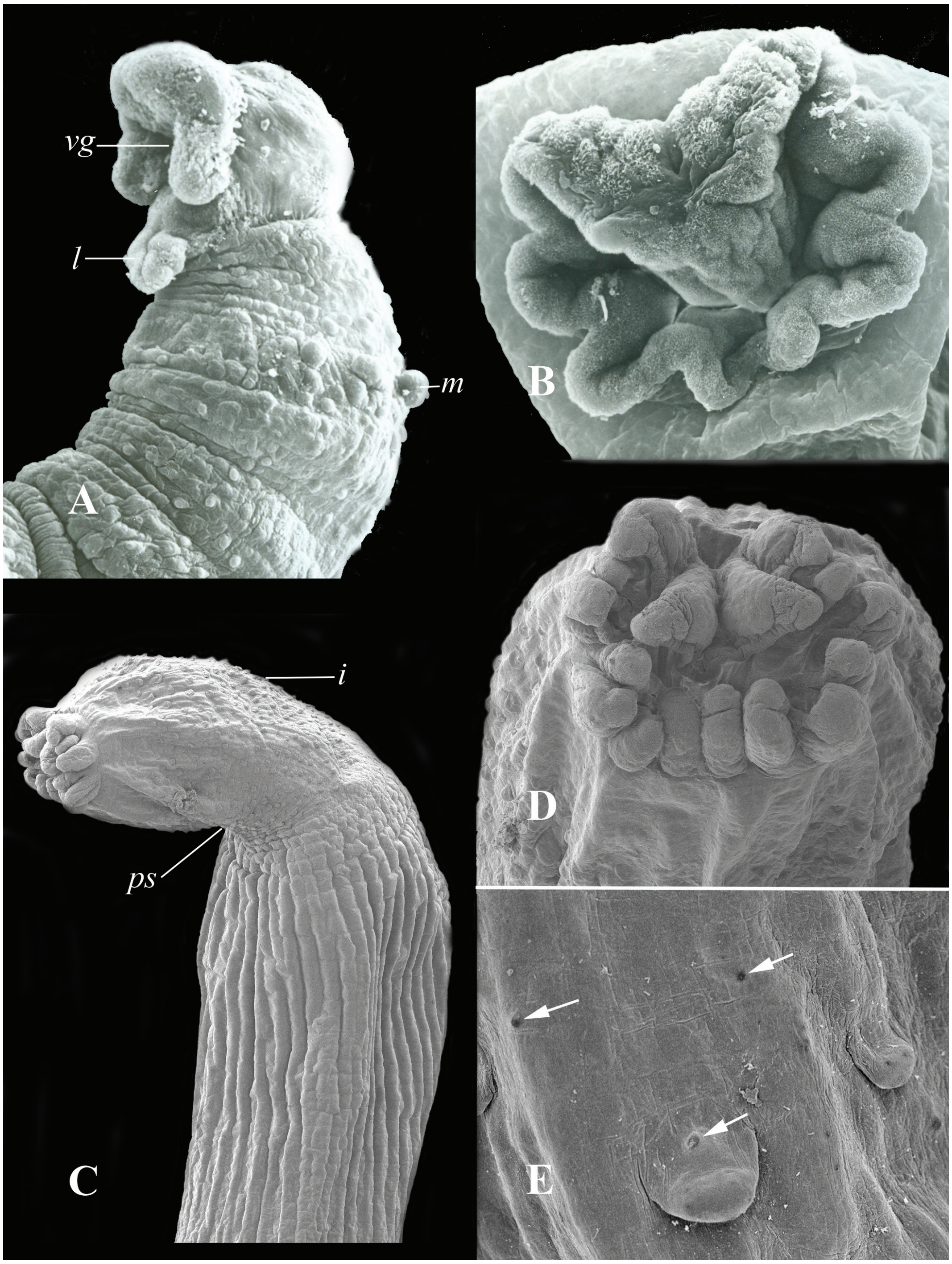


FIGURE 15. Scanning electron micrographs of smooth yellow-green juveniles. (A) Beginning metamorphosis showing the head and remnants of the metatroch, ventrolateral view. Compare with living specimen in Figure 12D. (B) Early metamorphosis, ventral view of tentacles. Note dorsal tentacles more developed than ventral. (C–E) Juvenile, 4 months. (C) Lateral view of head, introvert, and anterior trunk. The posterior boundary of the introvert is in the position of the former larval postmetatrochal sphincter. Longitudinal muscle bands are evident as longitudinal striations in the body wall of the trunk. (D) Ventral view of tentacles and introvert papillae. (E) Higher magnification of introvert and papillae showing scattered small pores and larger pore with cilia (arrows). Abbreviations: i = introvert, l = lip, m = metatroch (remnant), ps = postmetatrochal sphincter, vg = ventral groove of head.

peristaltic-like contractions along the length of the trunk (Figure 16A–E). As in other pelagospheras, the trunk may be contracted and shortened while the metatrochal collar is expanded. In a typical larva, the color of the ventral groove of the head and lower lip, as well as the anterior and posterior boundaries of the metatrochal band, is dark green. The stomach is also green, and the intestine is a lighter green/yellow; occasional specimens have a strikingly deep-blue stomach and aqua intestine. There is a single pair of small black eyespots on the dorsal head. The ventral head is ciliated and markedly bilobed (Figures 17A, 18A). Each lobe is constricted laterally to form a larger anterior or primary lobe and a smaller posterior or secondary lateral lobe. Posterior to the mouth a rounded flap of tissue, continuous with the ventral head, extends outward as the lower lip. Ciliation of the ventral head continues onto the lip as a central raised band, ending at the middle of the lip to surround the pore of the lip glands. The outer edge of the lip is rimmed by cilia. The trunk has distinctive transverse grooves and, when viewed with the dissecting microscope, a relatively smooth cuticular surface. However, examination at high magnifications with scanning electron microscopy reveals irregularly spaced longitudinal grooves, more superficial than the prominent and regular transverse grooves (Figure 17A–D). These superficial longitudinal grooves give the cuticle a wrinkled or puckered appearance. There are, in addition, superficial and irregular circular or transverse grooves.

A pair of light green nephridia, visible through the semitransparent body wall, open ventrolaterally on the trunk just below the postmetatrochal sphincter and anterior to the anus. Between the nephridia and the anus there are several internal transparent, round glandular structures of unknown function that are best observed in sectioned larvae. A long esophagus leads to a wide and sometimes bulbous stomach, usually pigmented dark green. The intestine, a lighter green, descends posteriorly, then loops anteriorly to the anus. Four retractor muscles attach anteriorly on the premetatrochal collar, but their posterior attachment has not been observed.

This larva is capable of an extraordinary degree of contraction and extension, the extended length sometimes twice the contracted. Also, the trunk may undergo tight circular contractions, occurring anywhere along its length in the form of peristaltic movements (Figure 16C–E). Other than the knobby type, no other larva exhibits this same kind of circular contractile behavior.

Initial metamorphosis consists of the formation of tentacles and the beginning regression of the ventral lip (Figures 18B,C, 19A). Six tentacles are formed from the lobes of the ventral head. The two anterior lobes give rise to two dorsal and two lateral tentacles, and the more posterior lobes give rise to two ventral tentacles. Within 1 to 2 days the metatrochal cilia are lost, and the mouth, surrounded by tentacles, has moved to a terminal position. The lower lip and lip glands are lost, and the trunk has elongated.

Within 1 week after initial metamorphosis, the extended length of the young juvenile, at approximately 4 mm, is double that of the larva (Figure 19B). The introvert, now clearly distinguished from the trunk, is transformed from the thorax, anterior to the postmetatrochal sphincter, and in a living larva can assume a bulbous shape. It is covered by what appear to be small papillae. A characteristic behavior is the rapid expulsion and retraction of the introvert, a behavior utilized in burrowing. The body wall is clear, and both longitudinal and circular muscle bands are evident. The gut has formed six to eight coils. The nephridia, greenish or tan, are attached to the body wall at the nephridial opening, anterior to the anus. The brain, with two small black eyespots, is now attached to the anterior esophagus and surrounded by coelom. By 6 weeks the number of tentacles has increased to 10 or 12 (Figure 18D).

Juveniles reared in the laboratory for 3 months have an extended length of 25 mm (Figure 19C). Tentacles are numerous, long, and filiform, and the introvert is covered with small papillae. Internal organs visible through the clear body wall include four retractor muscles attached to the body wall at the same level at a distance one-third the length of the body from the anterior end. The brownish tubular nephridia open ventrolaterally slightly anterior to the anus. The numerous coils of the gut may be packed with large fragments of shell and sand and extend the length of the body. A spindle muscle is attached anterior to the dorsal anus and also at the posterior extremity of the body. Wing muscles attach the rectum to the body wall. Four internal glands are located at the base of the introvert, and the longitudinal musculature is present in bands.

This larval type has been reared in the laboratory to an age of 3 years and has been identified as *Siphonosoma cumanense* (Keferstein, 1867; Figure 19D). In a separate study, larvae reared from spawnings of adult *Siphonosoma cumanense* appeared identical to those collected from oceanic plankton samples (Rice, 1988).

Knobby

This larva is characterized by rounded knobs or protrusions of the body wall. The protrusions appear in annular rows, marked by circular constrictions or transverse grooves along the length of the trunk. Irregular in shape and closely apposed in the contracted larva, the knobs become more distinctive when the larva is extended (Figure 20A–C). The overall coloration of the body is tan to yellowish with darker tan or brownish knobs. The base of the metatrochal band is brownish or green, as are the ventral groove and mouth region. Visible through the relatively opaque body wall, the gut is greenish. Scanning electron microscopy reveals minute cuticular papillae scattered over the surface of the trunk. Among the papillae are minute pores of unknown significance.

Knobby is a relatively large larva, ranging from 2 to 4 mm in extended length. The head is bifurcated, each lobe constricted laterally to form an anterolateral lobe and a smaller posterolateral lobe (Figure 21A–D). As in the transverse groove larva, the ciliation of the ventral head continues around the mouth to

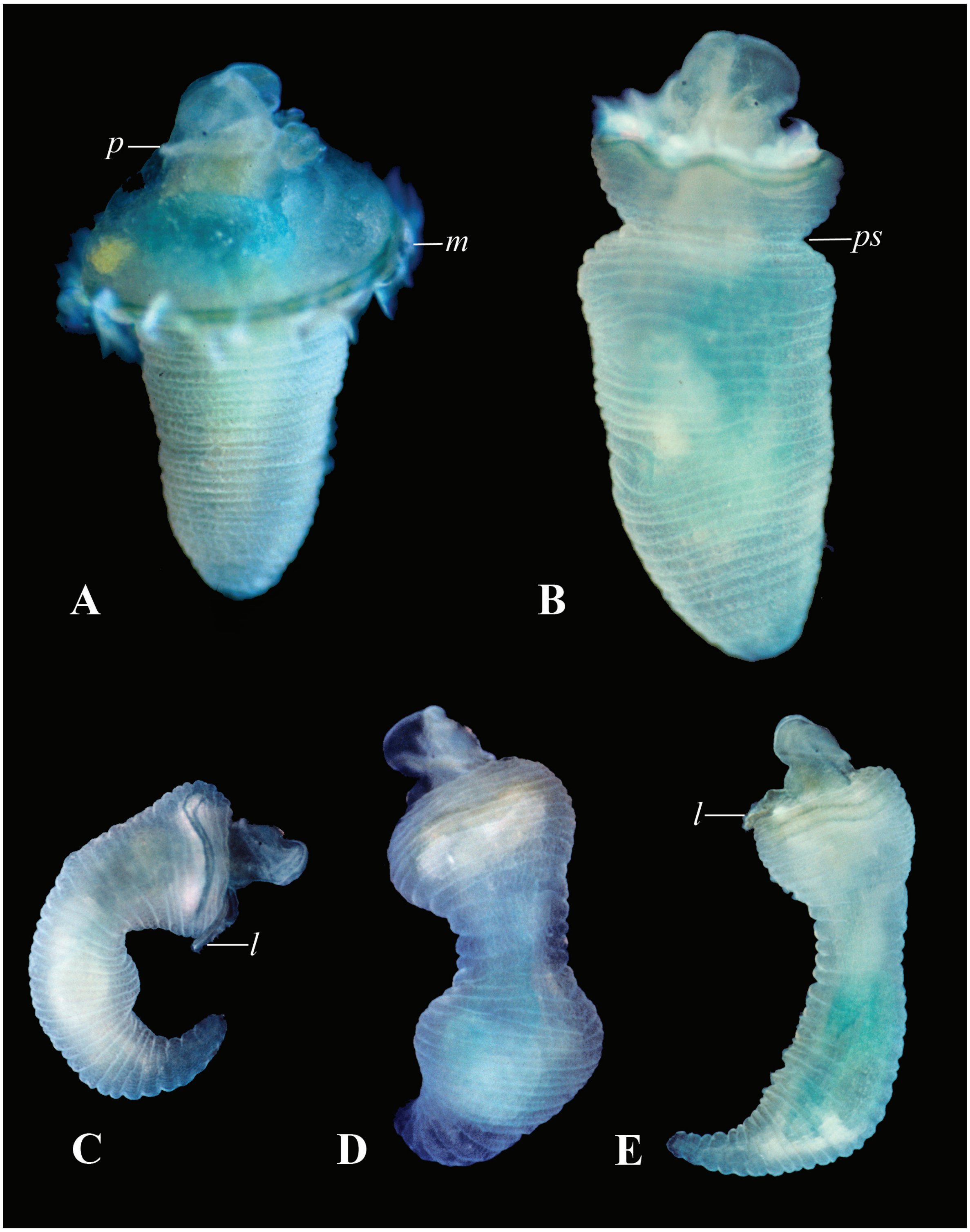


FIGURE 16. Photomacrographs of living transverse groove larvae illustrating a variety of behavioral patterns. (A) Larva swimming, lateral view, metatroch fully extended. (B) Larva at rest, dorsal view, metatroch partially extended. (C) Larval body curved ventrally, head toward tail, lateral view, lip extended. (D) Circular contractions along length of body, peristaltic-like contractions, metatroch completely retracted, lateral view. (E) Larval body extended, anterior body contraction, lateral view, lip extended, metatroch mostly retracted. Abbreviations: l = lip, m = metatroch, p = prototroch, ps = postmetatrochal sphincter.

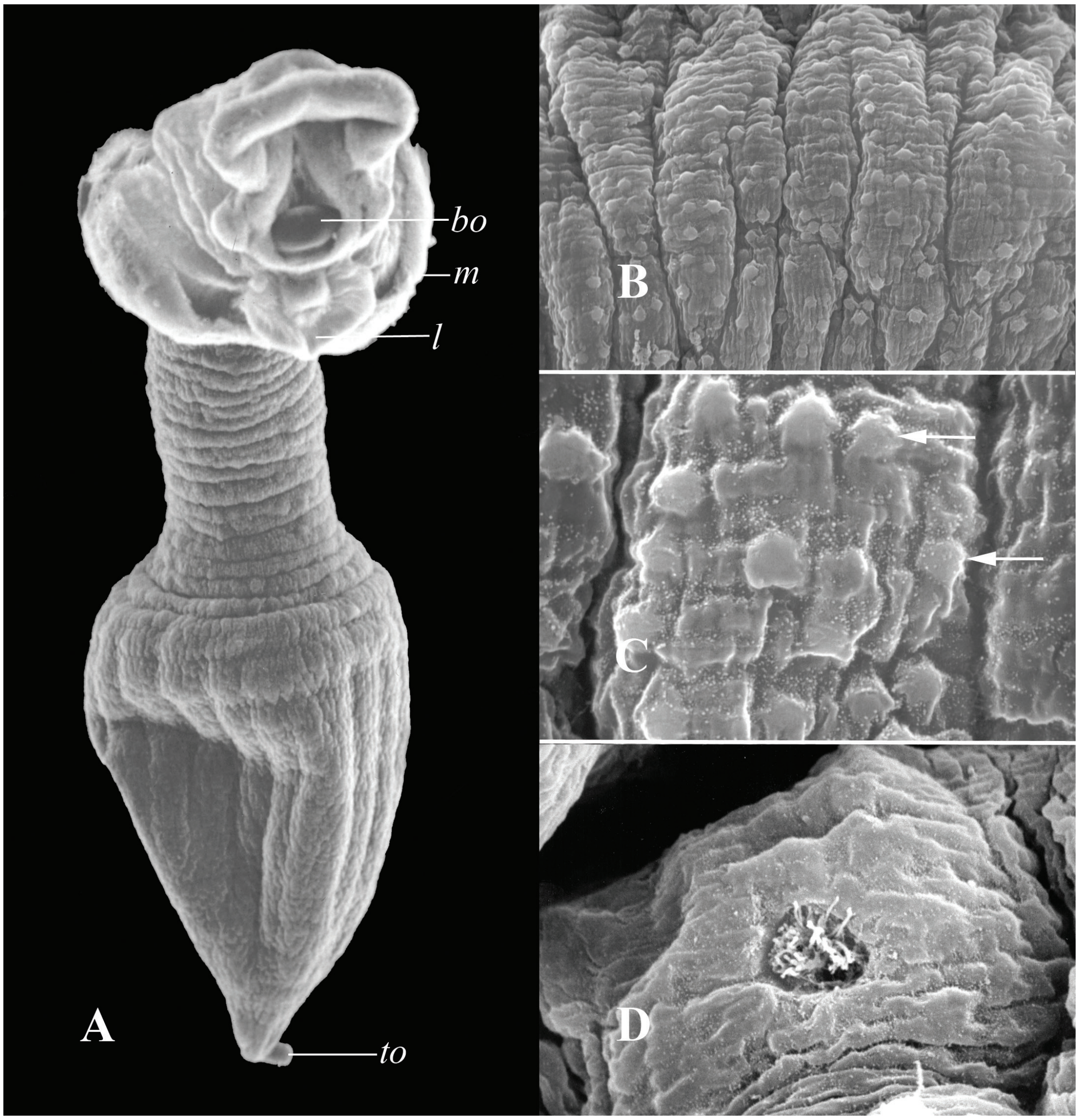


FIGURE 17. Scanning electron micrographs of transverse groove larva. (A) Ventral view. (B) Higher magnification of grooves and folds in mid-trunk region (orientation anterior-posterior = left to right), showing small papillae. (C) Higher magnification of a fold with papillae (arrows). (D) Pore with cilia from anterior trunk. Abbreviations: bo = buccal organ, l = lip, m = metatroch, to = terminal organ.

the lower lip, where it forms a raised median band, surrounding the distal pore to the lip gland. The outer portion of the lower lip, surrounding the median ciliated band, is rounded and bordered by a rim of longer cilia. Located on the dorsal head, the U-shaped prototroch continues laterally around the head to join the ventral ciliation. A pair of small black eyespots is anterior to the dorsal prototroch. The terminal organ is a single slender retractable rod with no apparent telescoping component.

Behavior characteristic of both knobby and transverse groove larvae is their exceptional contractibility, not only in lengthening and shortening but also in circular contractions along the length of the body (Figure 20B,C). As in other larvae, placing the terminal organ in the mouth is a common behavior.

A metamorphosed specimen was observed in the laboratory on one occasion, after 18 days in substratum (Figure 20D,E). Ten filiform tentacles surrounded the terminal mouth. Small brown pigment spots were scattered on the introvert. The larval knobs were still apparent on the trunk. The introvert to trunk ratio was about 1:3. Unfortunately, this juvenile did not survive, and no fixations were made of this stage.

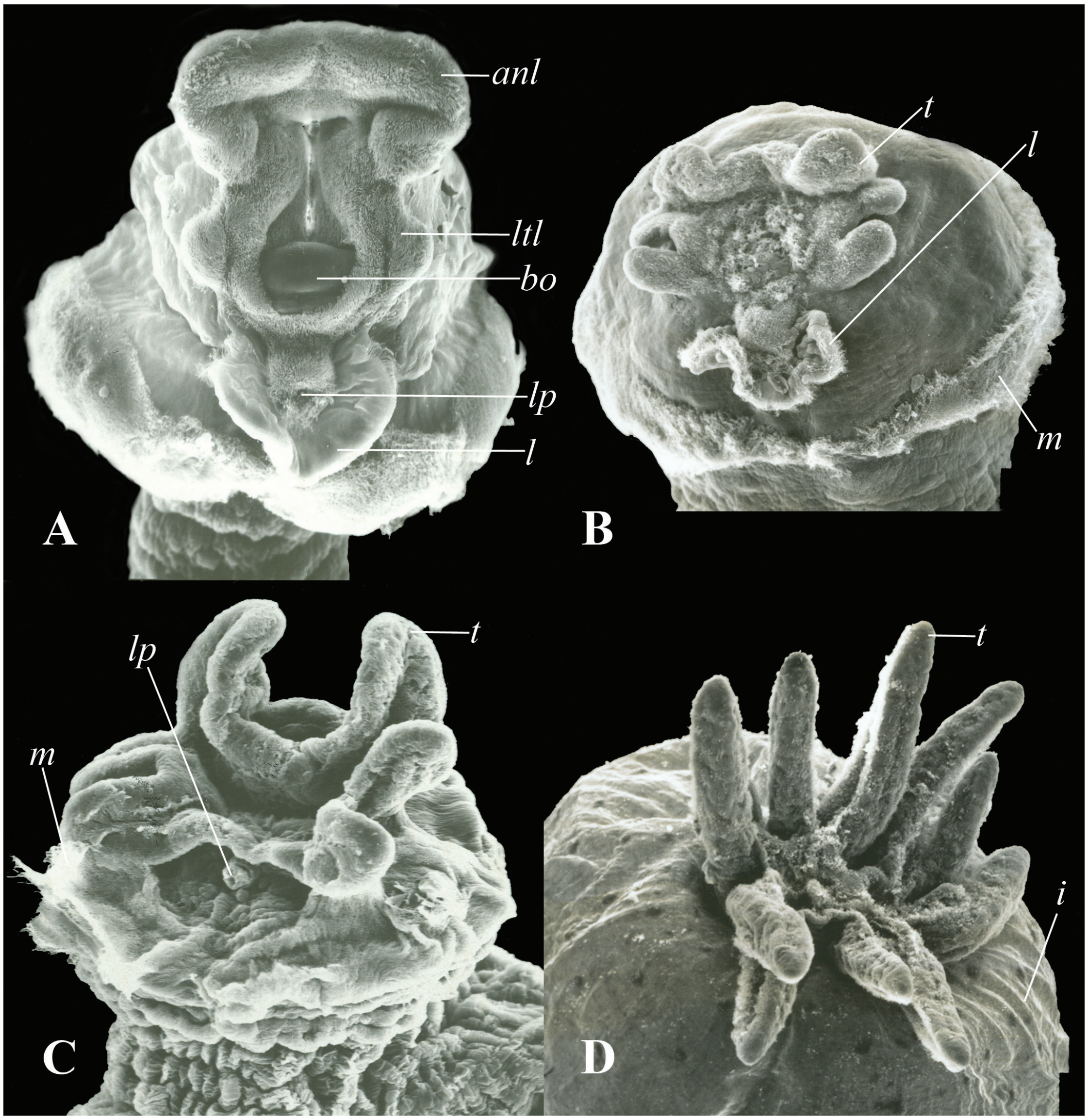


FIGURE 18. Scanning electron micrographs of metamorphosis in transverse groove. (A) Head of larva. (B) Beginning metamorphosis. Tentacular lobes forming from anterior and lateral lobes of the ventral larval head, lip regressing, metatroch still present. (C) Beginning metamorphosis. Lip is lost, pore to lip gland is still evident, remnants of metatroch remain. (D) Juvenile, 6 weeks. Tentacular crown fully formed, small papillae present on introvert. Images (A) and (C) are from Rice (1976); (B) is from Jaeckle and Rice (2002). Abbreviations: anl = anterior lobe of ventral head, bo = buccal organ, i = introvert, l = lip, lp = lip pore, ltl = lateral lobe of ventral head, m = metatroch, t = tentacles.

Spotted Velvet

The overall coloration of the body varies from pink to light yellow (Figure 22A,B). A green gut and a pair of elongate green nephridia are visible through the relatively opaque body wall. Covered with papillae, the trunk has a "velvety" appearance that is interrupted by numerous clear spots that have not been defined by scanning electron microscopy. The entire head may have a greenish cast; the ventral groove and proximal lower lip are pigmented dark green/black. The lower lip is rounded, with a median raised ciliated band that is continuous with ciliation of the ventral head (Figure 23A,B).

The dorsal prototrochal band extends laterally to connect with the ciliated border of the ventral head. There are two small black eyespots. The terminal organ is small. Nephridiopores are anterior to the anus. The extended length of the larvae averages

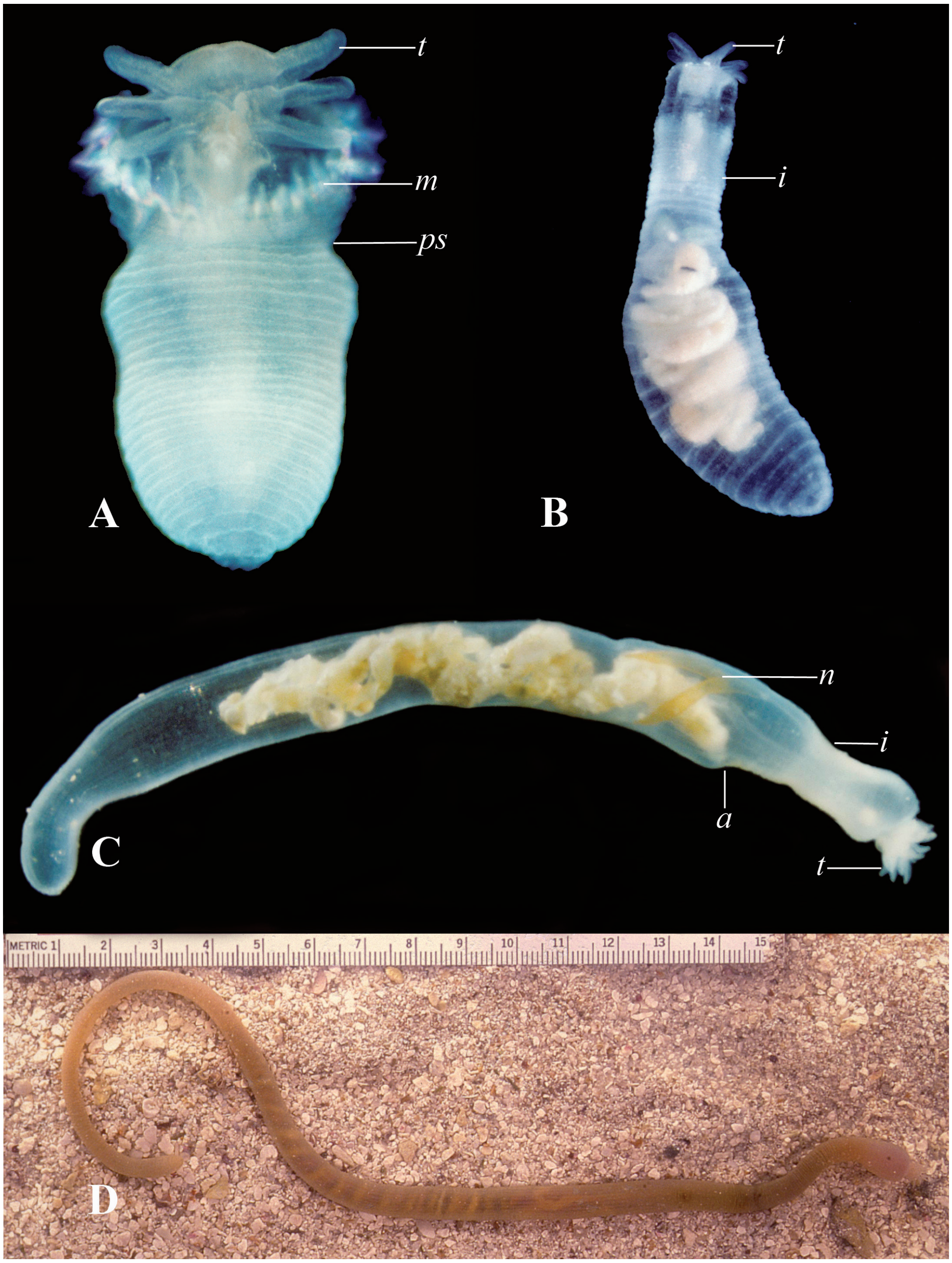


FIGURE 19. Photomicrographs of living transverse groove beginning metamorphosis and juveniles. (A) Beginning metamorphosis. Note tentacles and presence of metatroch. (B) Juvenile, 1 week. Transverse grooves of body wall are apparent. (C) Juvenile, 3 months. Lateral view. Note relative position of nephridiopores and anus and well-developed tentacular crown. (D) Adult on sediment, 3 years. *Siphonosoma cumanense*. Reared in laboratory from transverse groove larva. The scale is in millimeters. Abbreviations: a = anus, i = introvert, m = metatroch, n = nephridium, ps = postmetatrochal sphincter, t = tentacles.

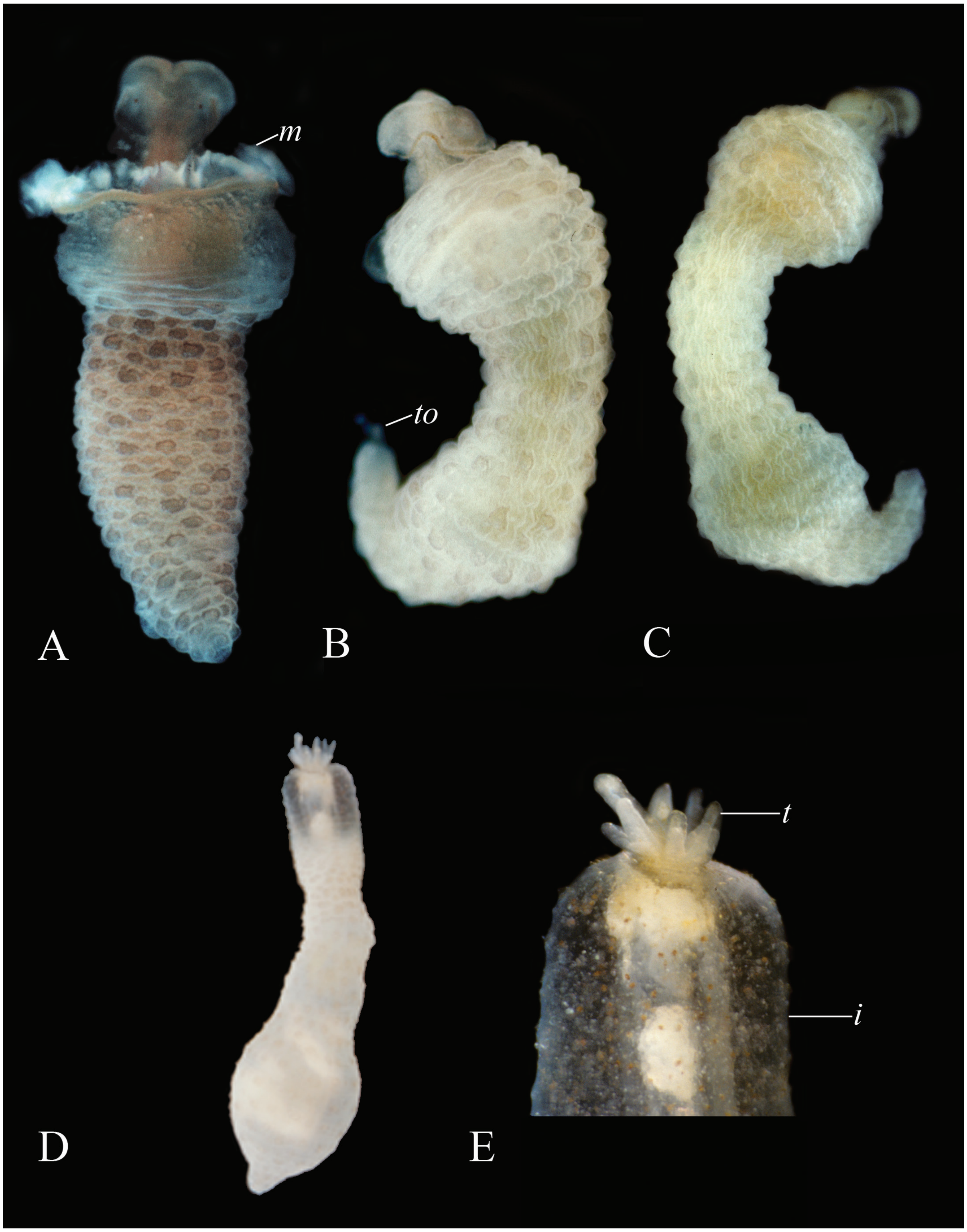


FIGURE 20. Photomacrographs of living knobby larvae and juvenile. (A–C) Larvae illustrating the variety of morphological shapes and behavior. (A) Dorsal view, metatroch extended. (B, C) Lateral views, circular contractions of trunk along length of body. Compare with transverse groove larva (Figure 16C–E). (D) Juvenile, 18 days. (E) Juvenile, 18 days. Higher magnification of head and introvert with papillae. Abbreviations: i = introvert, m = metatroch, t = tentacles, to = terminal organ.

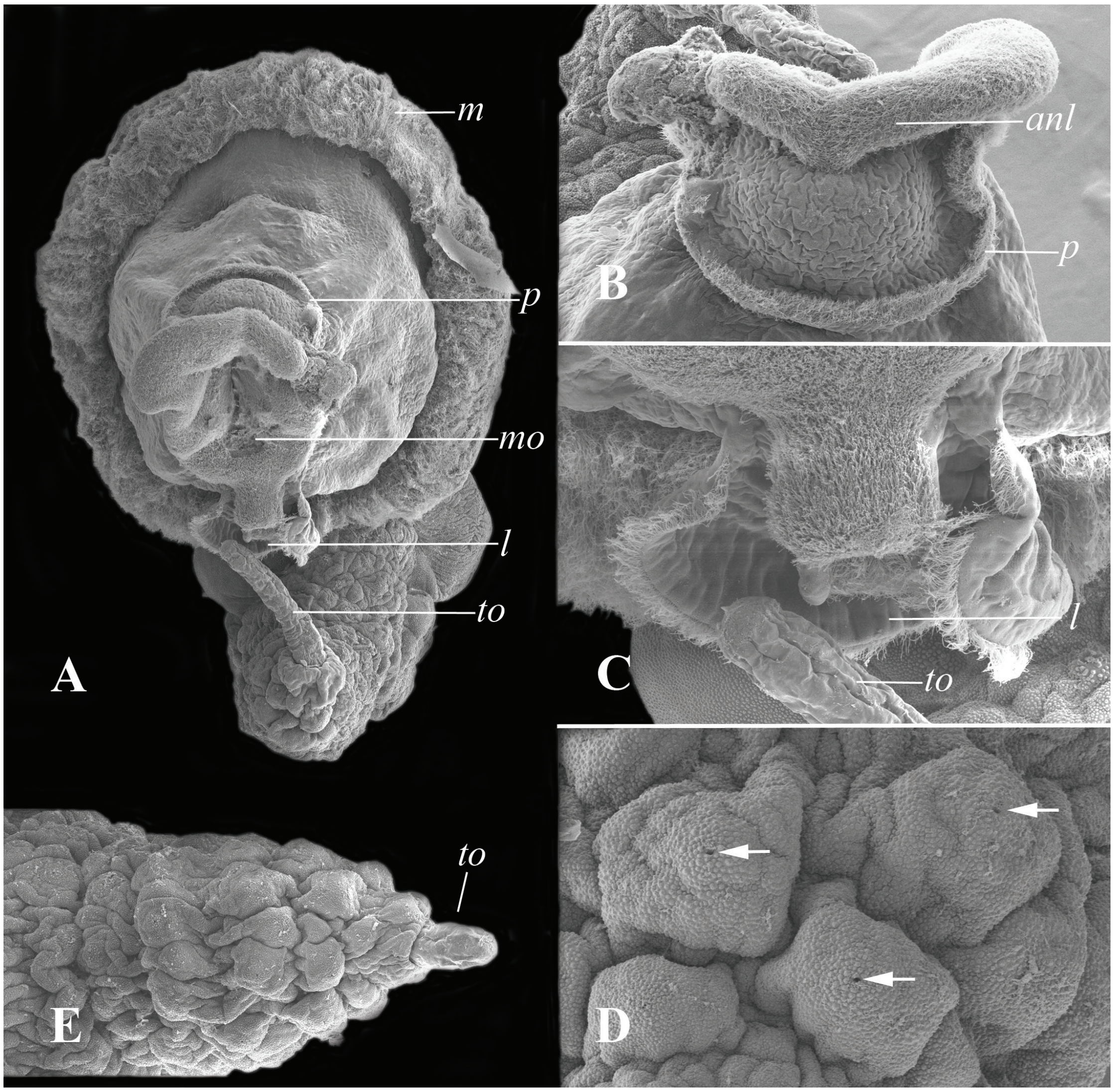


FIGURE 21. Scanning electron micrographs of knobby larvae. (A) Apical view of larval head. Body is bent in ventral curvature with extended terminal organ near the lip of the ventral head. (B) Dorsal view of head showing prototroch. (C) Higher magnification of lip and tip of terminal organ. (D, E) Posterior trunk of different larva. (D) Higher magnification of outpocketings of body wall. Note small pores (arrows). (E) Posterior trunk of larva with partially extended terminal organ. Abbreviations: anl = anterior lobe of ventral head, l = lip, m = metatroch, mo = mouth, p = prototroch, to = terminal organ.

about 1 mm. Papillae, as viewed by scanning electron microscopy, are cone shaped with a broad base and a rounded cap. Just below the cap, the papilla is encircled by two or three ridges (Figure 23C).

Metamorphosis is initiated with the loss of metatrochal cilia and the retraction of the head into the trunk (Figure 22C,D). Within 1 month the extended introvert is fully formed and has reached a length five times that of the trunk. Small hooks are present on the anteriormost introvert, and papillae cover the trunk. As seen through the relatively opaque body wall, the longitudinal musculature is continuous, and a pair of elongate dark green nephridia, one-half the length of the trunk, hangs freely in the coelom from their attachment to the body wall anterior to the anus.

The tentacular crown of a 3-month juvenile, as observed in scanning electron micrographs, consists of a circle of short tentacles dorsal to the mouth, partially enclosing a dorsal ciliated nuchal organ (Figure 24A–D). A ciliated ridge, broadest ventrally, forms the ventral rim of the mouth. Below the head a prominent elongate unciliated “collar” forms the anteriormost

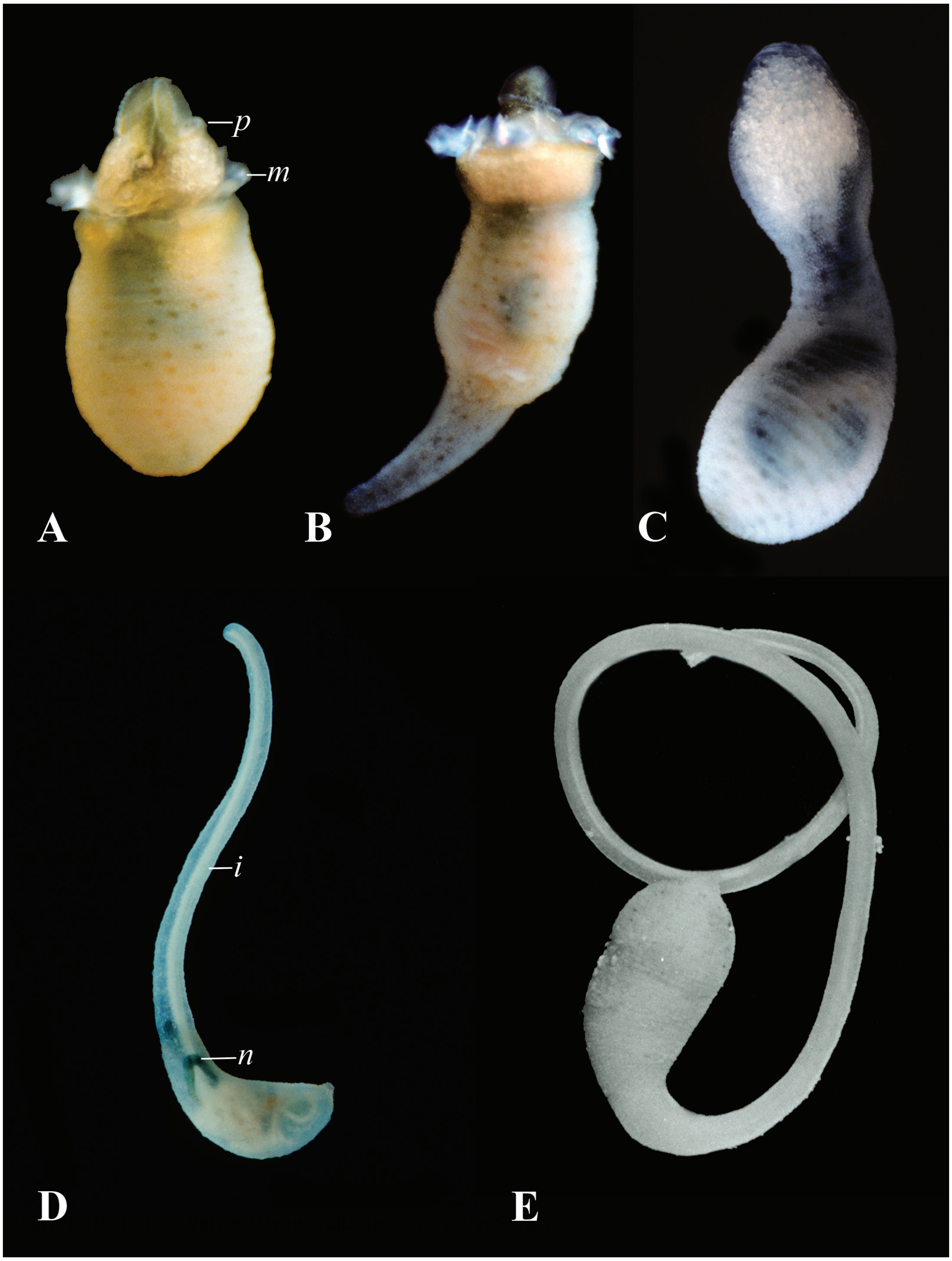


FIGURE 22. Photomacrographs of spotted velvet larvae, beginning metamorphosis, juvenile and adult. (A, B) Larvae, ventrolateral views with metatroch extended. (C) Bulbous stage of metamorphosis, head retracted. (D) Juvenile, 2 months. (E) Adult, 1 year 9 months. Reared in laboratory from oceanic spotted velvet larva. Identified as *Apionsoma misakianum*. Abbreviations: i = introvert, m = metatroch, n = nephridium, p = prototroch.

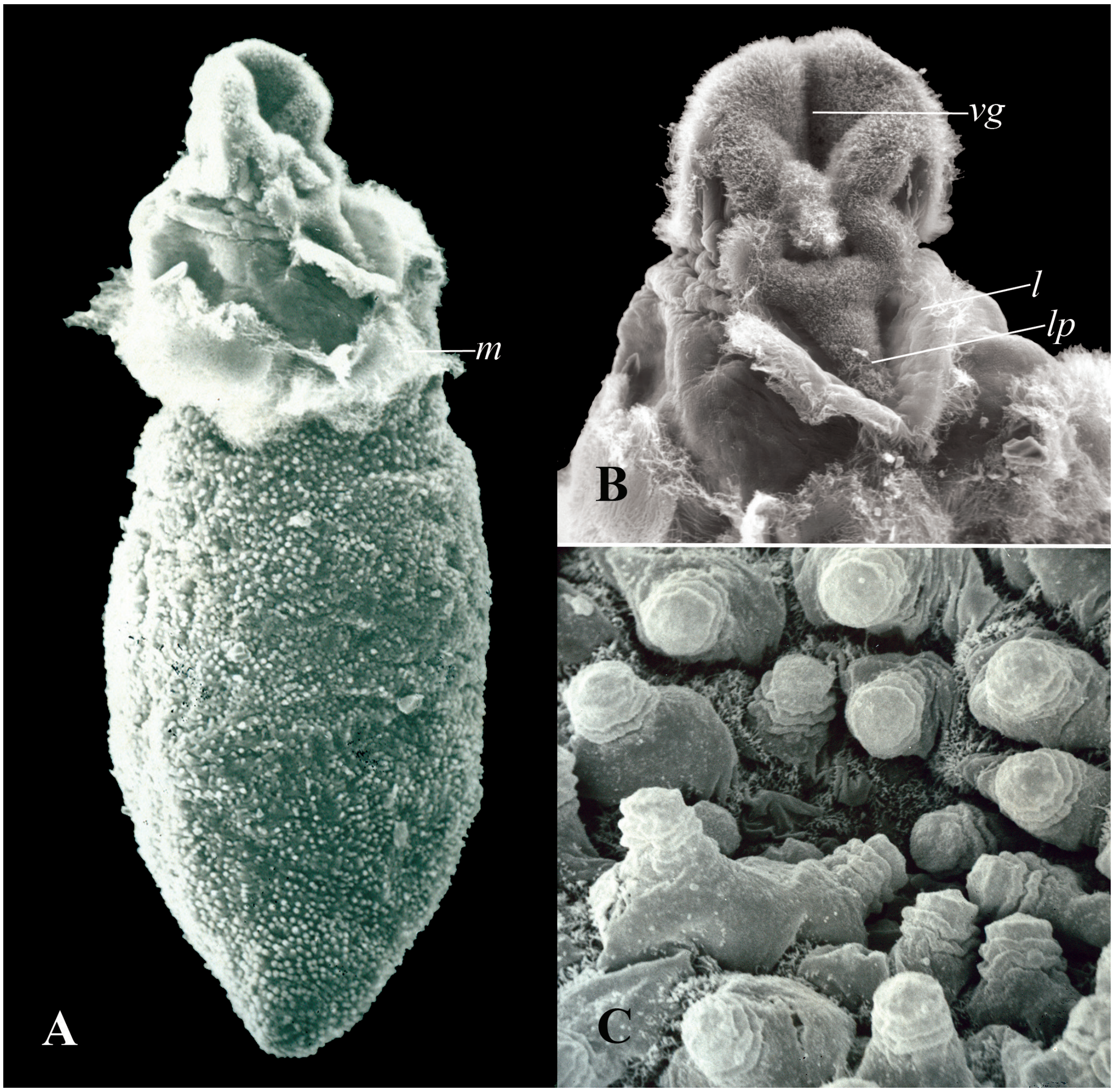


FIGURE 23. Scanning electron micrographs of spotted velvet larva. (A) Ventrolateral view. (B) Head, ventral view. (C) Cuticular papillae. Abbreviations: l = lip, lp = lip pore, m = metatroch, vg = ventral groove of head.

introvert. Posterior to the collar, rows of hooks encircle the anterior half of the introvert. The most-anterior hooks are closely apposed in tight circles; individual hooks are sharply curved and have three to four basal spinelets. Posteriorly, the hooks are more scattered and may lack spinelets. The introvert is four to five times the length of the trunk. Prominent papillae are present at the junction of introvert and trunk, as well as at the posterior extremity of the trunk. In a single specimen, reared in the laboratory for 21 months, the introvert to trunk ratio was approximately 6:1 (Figure 22E).

Morphological features of the juveniles, especially hook characteristics, tentacular arrangement, and ratio of introvert to trunk, resemble the diagnostic characters of *Apionsoma misakianum* (Ikeda, 1904) (see Discussion).

White Blackhead

This is one of the most common pelagospheras in the Florida Current, occurring most abundantly in the winter months, December through February. The body is white, sometimes with a pinkish cast, and covered by small papillae (Figure 25A,B). The ventral head is marked by black to dark green pigmentation that extends around the mouth to include the central area of the lip and the distal lip pore. Long cilia extend out beyond

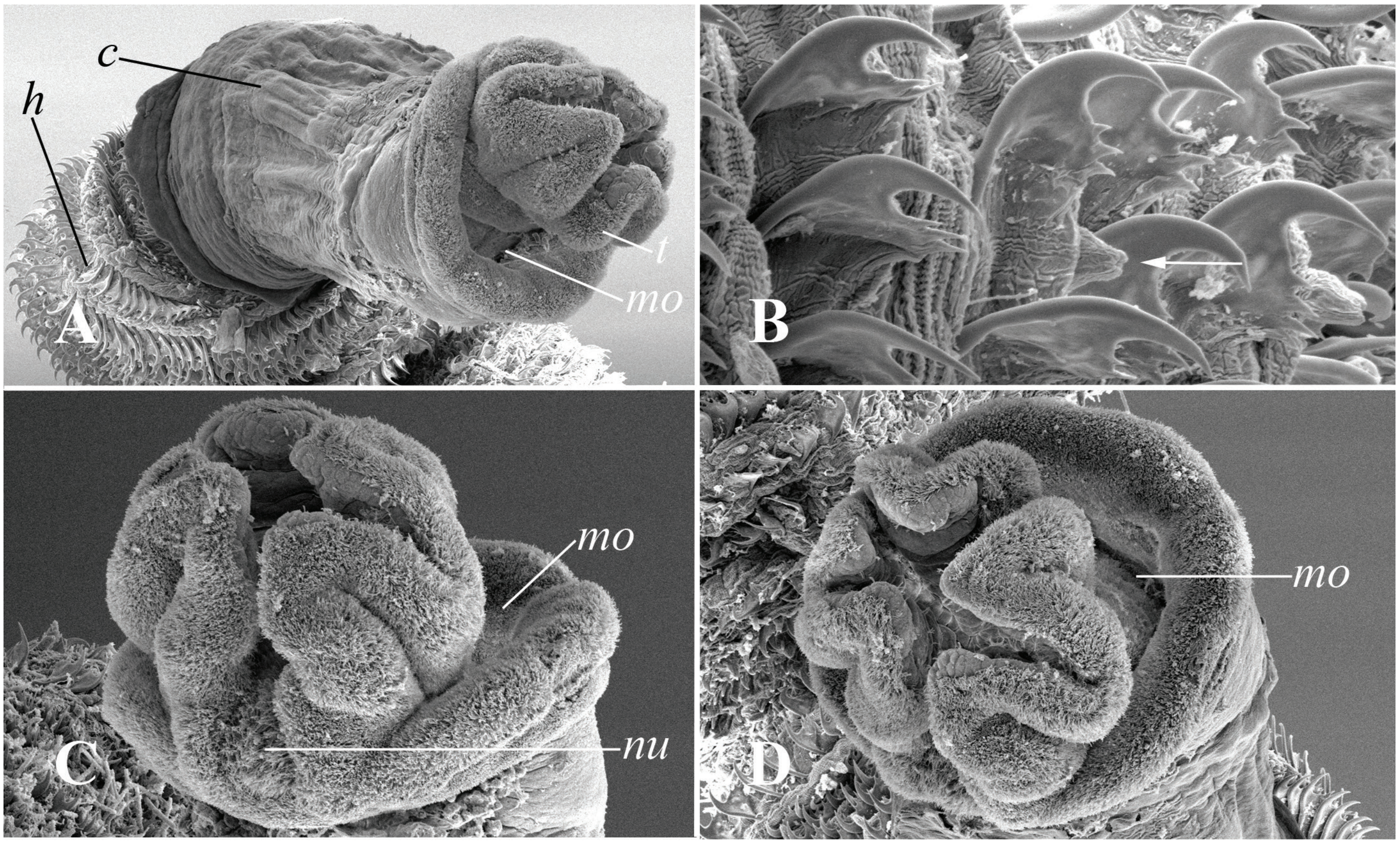


FIGURE 24. Scanning electron micrographs of spotted velvet 3-month juvenile. (A) Ventral view of head and anterior introvert. Note tentacles dorsal to mouth, broad ciliated ridge forming ventral rim of mouth, and elongated unciliated collar followed by closely apposed rows of hooks. (B) Hooks with basal spinelets, or "teeth," and sensory organ (arrow). (C) Dorsal view of head and nuchal organ. (D) Apical view of tentacles and mouth. Abbreviations: c = collar, h = hooks, mo = mouth, nu = nuchal organ, t = tentacles.

the pore; the surrounding lip is rounded and bordered by long cilia (Figure 26D). Visible through the opaque body wall, the esophagus, stomach, proximal intestine, and nephridia are dark green to blackish. The nephridia hang freely in the coelom from their ventrolateral attachments slightly anterior to the anus. When the larva is actively swimming, the nephridia may be pushed forward into the expanded mid-region above the metatrochal band, along with stomach and coelomocytes (Figure 25B). In the fully extended metatroch there is a ventral medial gap below the lower lip. A pair of small black/dark red eyespots lies above the prototroch on the dorsal head. This larva is one of the smaller oceanic pelagospheras, ranging in extended length from 0.5 to 0.8 mm. The larva may assume several shapes during swimming. Most commonly, the metatroch is completely extended, and the posterior trunk is rounded. Or the postmetatrochal sphincter may be tightly constricted, and the trunk may be more pyramidal in shape. The terminal organ is rarely extended. Occasionally, the body may be curved so that the head approaches the posterior extremity, but actual contact of the terminal organ with the mouth has not been observed. Further, the use of the terminal organ for attachment is not known for this larva.

The cuticular papillae, similar to those of spotted velvet, are elongate with a rounded cap (Figure 26A–C). The base may be cylindrical or broad. Just below the cap, the papilla is encircled by two or three ridges. Scattered among the papillae are epidermal organs, each consisting of a central pore surrounded by approximately six cuticular lobes. Cilia extend outward from the pore. Transmission electron micrographs reveal both secretory and sensory cells (M. E. Rice, personal observation).

Metamorphosis begins by a loss of metatrochal cilia, a narrowing of the head, and regression of the lower lip (Figures 25C, 26D–M). Within 3 days the entire head region, including the thorax, is retracted, and the postmetatrochal sphincter is tightly constricted, preventing further extension of the anterior body (Figure 25D,E). Within 2 weeks a fully developed elongate introvert with terminal tentacles is extended from the trunk. Four tentacular lobes are dorsal to the mouth. The introvert at this stage is two to three times the length of the trunk, and rows of hooks encircle the anterior introvert. The most-anterior hooks have four

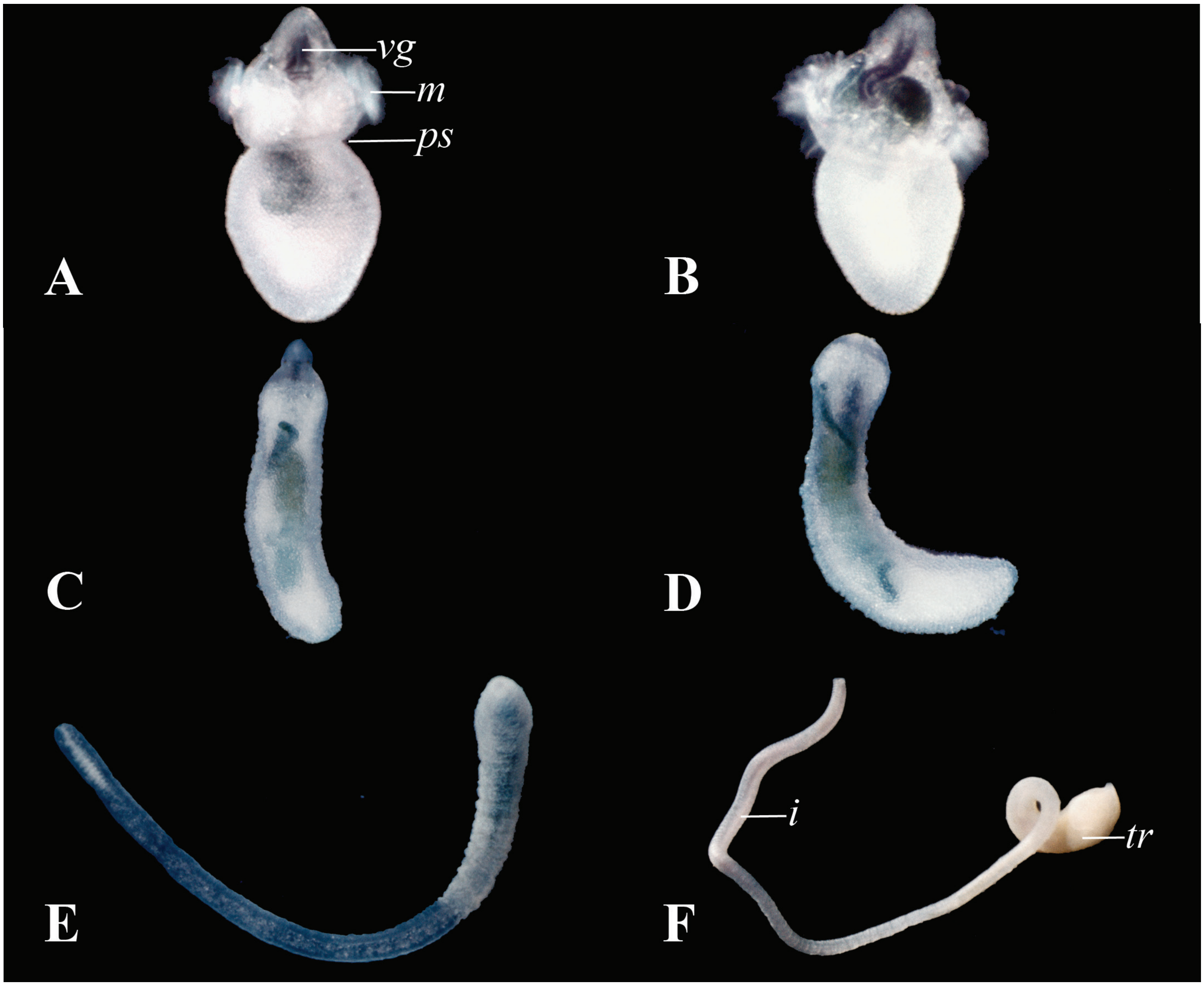


FIGURE 25. Photographs of living white blackhead from larva to adult. (A) Swimming larva, ventral view. Ventral head and stomach have black pigmentation. (B) Swimming larva, ventral view. Stomach is pushed into anterior thorax when metatroch is fully extended. (C) Beginning metamorphosis, 1 day, ventral view. The metatroch is lost, and the shape of the head becomes pointed. (D) Beginning metamorphosis, 1 day, bulbous stage. The head is withdrawn into the trunk; anterior constriction prevents extension. (E) Juvenile, 2 weeks. (F) Adult, 1 year. Reared from white blackhead larva in the laboratory. Identified as *Apionsoma misakianum.* Abbreviations: i = introvert, m = metatroch, ps = postmetatrochal sphincter, tr = trunk, vg = ventral groove of head.

to five basal spinelets and are arranged tightly in rows. As in the juveniles of spotted velvet, the more posterior hooks may be scattered and lack basal spinelets (Figure 26J–L). Juveniles have been reared in the laboratory to adults, attaining maturity at 9 months following metamorphosis. Morphological characters of an adult (e.g., papillae, hooks, length of introvert) that is reared in the laboratory from the white blackhead larva are consistent with those described for *Apionsoma misakianum* (Figure 25F).

White White

The trunk is translucent white with a bluish cast (Figure 27A). It is covered with evenly dispersed small papillae that reflect the light, giving the body a silvery or jewel-like appearance. The metatrochal collar and head are usually clear. On the dorsal head a pair of medium-sized red eyespots is anterior to the prototroch. The ciliated ventral head and rounded lower lip are

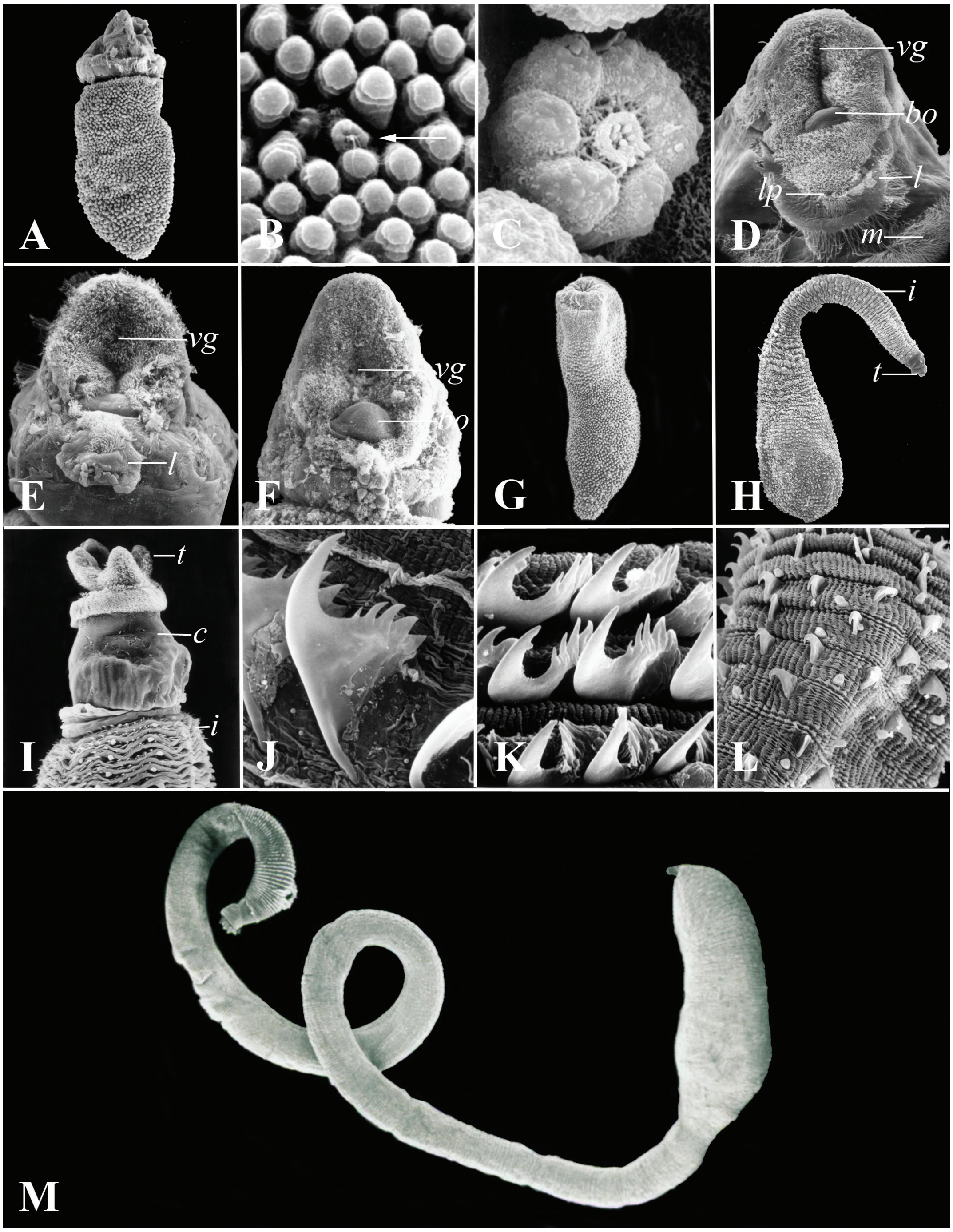


FIGURE 26. Scanning electron micrographs of white blackhead from larva to adult. (A) Larva, ventrolateral view. (B) Larval cuticular papillae. (C) Sensory-secretory organ (see arrow in B). (D) Larval ventral head. (E) Ventral head at beginning of metamorphosis. Note regressing lip and loss of metatroch. (F) One day after initiation of metamorphosis, loss of lip, change in shape of head. (G) Five days after beginning of metamorphosis. Head is retained within trunk. (H) Juvenile, 2 weeks. (I) Juvenile, 11 months. Lateral view of head, ventral on left. (J, K) Anterior hooks on introvert, in rows with basal spinelets. (L) Posterior hooks, more scattered, lacking basal spinelets. (M) Adult, 2 years. Reared from white blackhead larva in the laboratory. Note rows of hooks on anterior introvert. Panels (D), (E), (F), (K) from Rice (1978); panels (G), (H) from Rice (1981). Abbreviations: bo = buccal organ, c = collar, i = introvert, l = lip, lp = lip pore, m = metatroch, t = tentacles, vg = ventral groove of head.

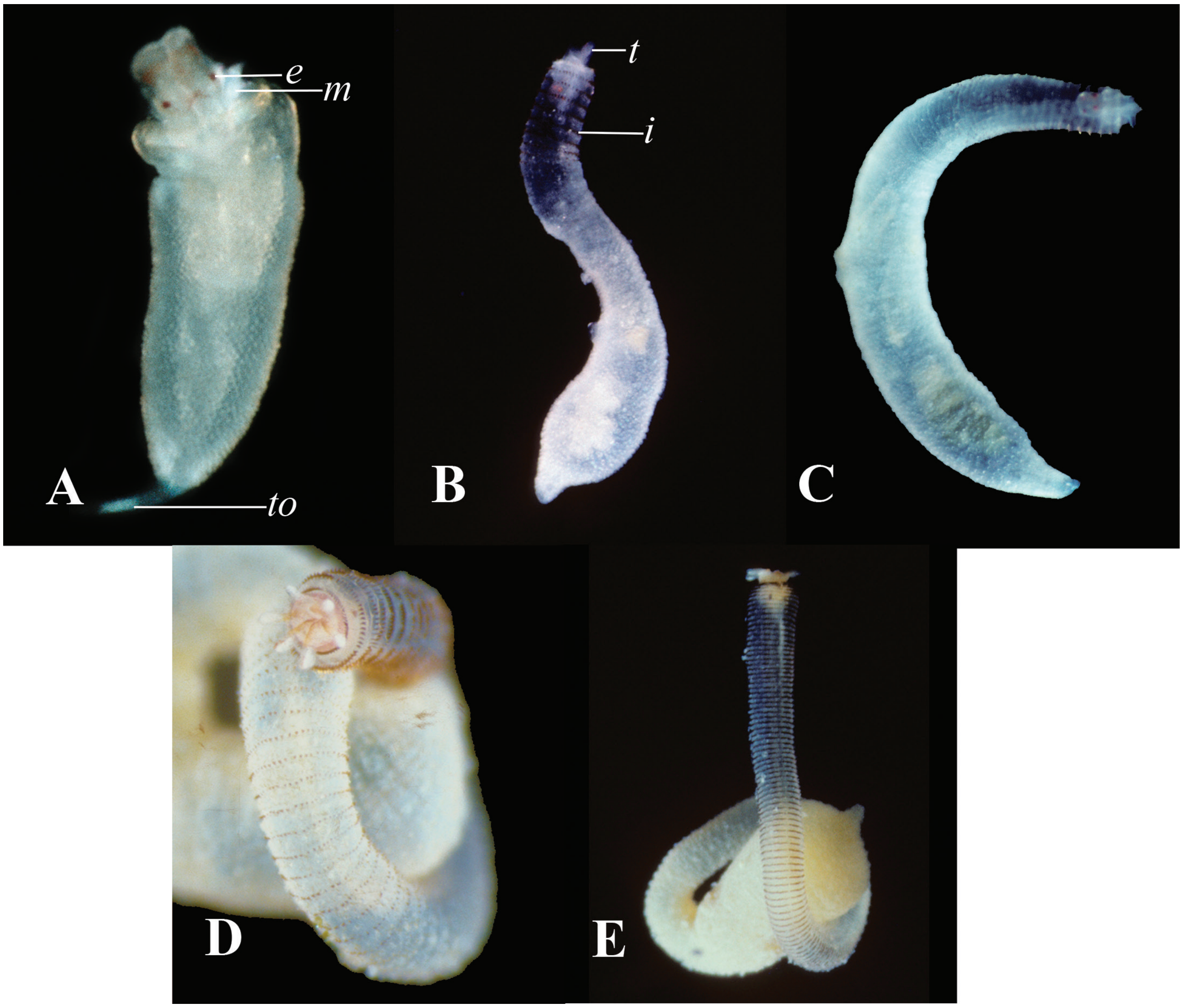


FIGURE 27. Photomacrographs of living white white larvae and juveniles. (A) Larva, dorsal view. Note large red eyespots, partly retracted metatroch, and extended terminal organ. (B) Juvenile, 2 weeks. Eyes present on brain, tentacular lobes, and introvert with rows of hooks. (C) Juvenile, 2 months. (D) Juvenile, 4.5 months. Apical view of tentacular crown. On the anterior introvert the rows of hooks are closely apposed, but they are more widely separated on the posterior introvert. (E) One year, total body length = 10 mm. Abbreviations: e = eye, i = introvert, m = metatroch, t = tentacles, to = terminal organ.

similar to those of white blackhead. The internal organs are usually colorless, although occasionally, the gut may show a light maroon pigmentation. The extended length is approximately 1 mm. A prominent terminal organ is frequently extended. When fully extended, two components are obvious: an outer tubular continuation of the terminal body wall from which an elongate rod can be extended (Figure 28A,D).

The papillae covering the trunk are well separated and have broad, low bases that are capped by two or three petallike tiers, the lower having a greater diameter than the upper (Figure 28B,C). The surface of the apical tier is marked by irregular curved ridges of varying lengths and curvatures. Scattered among the papillae are epidermal organs, consisting of a funnel-shaped cuticular structure with a central pore surrounded by several smaller, irregularly arranged cuticular projections (Rice, 1976). These organs are presumed to have sensory and secretory components similar to those of white blackhead. On the posterior thorax, immediately anterior to the postmetatrochal sphincter, there is a region of small rounded papillae.

This larval type is more active than others in the extension and use of the terminal organ. In the laboratory it frequently attaches by its terminal organ to the bottom of glass containers.

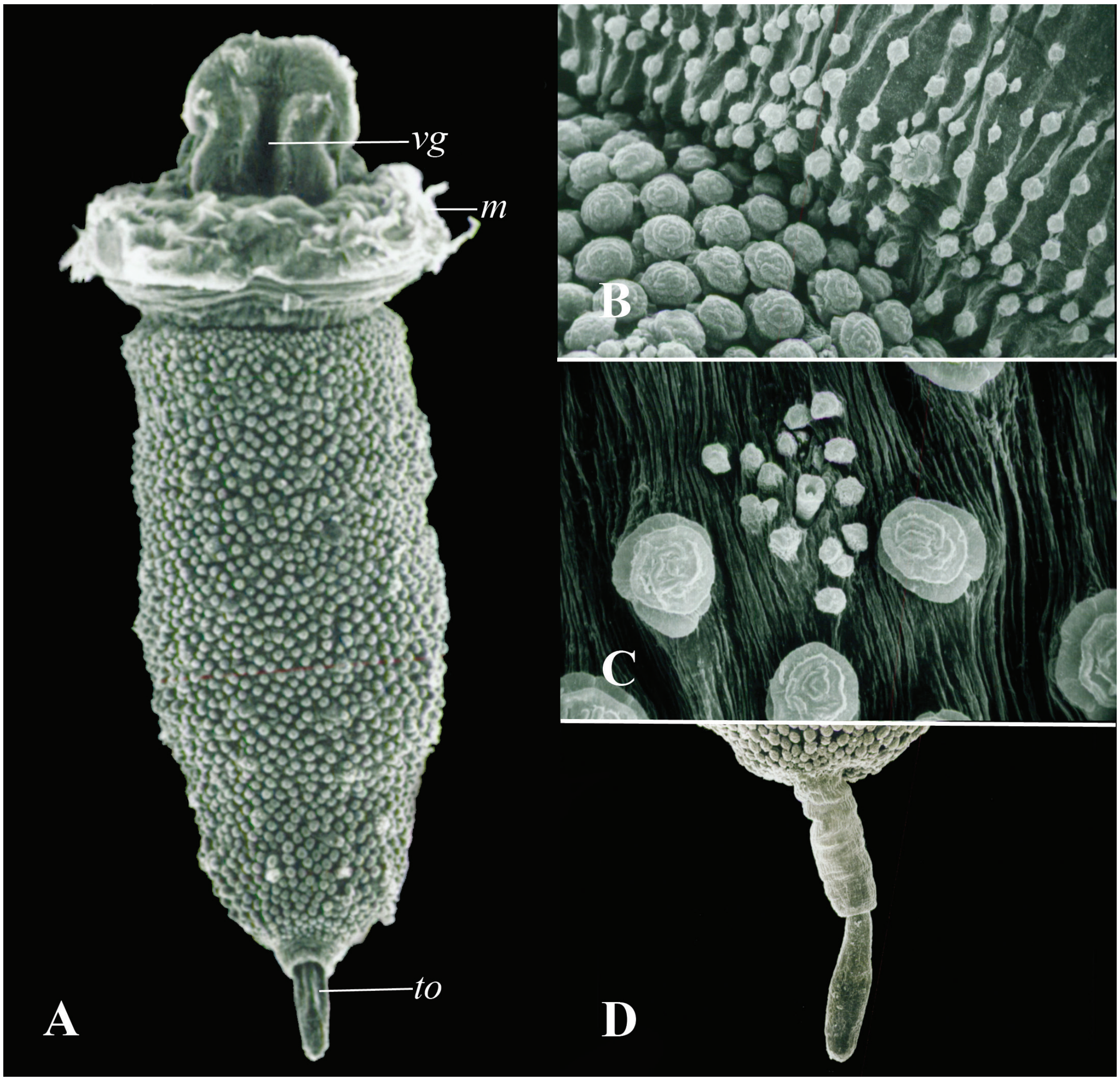


FIGURE 28. Scanning electron micrographs of white white larva. (A) Larva. (B) Cuticular papillae at intersection between posterior thorax (right) and anterior trunk (left). (C) Cuticular papillae and sensory-secretory organ complex. (D) Terminal organ, fully extended. Images (A), (D) from Jaeckle and Rice (2002). Abbreviations: m = metatroch, to = terminal organ, vg = ventral groove of head.

From the point of attachment it may extend its body upward, with the metatroch fully extended, or downward, with the ventral head applied to the substratum. In this latter position the buccal organ may be protruded to make contact with the substratum. It may also turn on the point of attachment so that the body is extended at different angles from the attachment. Or it may release itself from the attachment and, with metatroch fully extended and terminal organ retracted, swim rapidly, as do other larvae, in a spinning top-like movement through the water. Another behavior, characteristic of most pelagosphera larvae, is placement of the elongate terminal organ in the region of the ventral mouth.

Larvae were reared from metamorphosis through juvenile stages to the age of 1 year, at which time the extended length had reached approximately 10 mm, with an introvert to trunk ratio of approximately 2:1. At the beginning of metamorphosis the metatroch is lost, the head is retracted into the trunk, and hooks appear on the anterior thorax. One or two weeks later, a fully formed head emerges bearing four tentacular lobes or buds (Figures 27B–E, 29A–F).

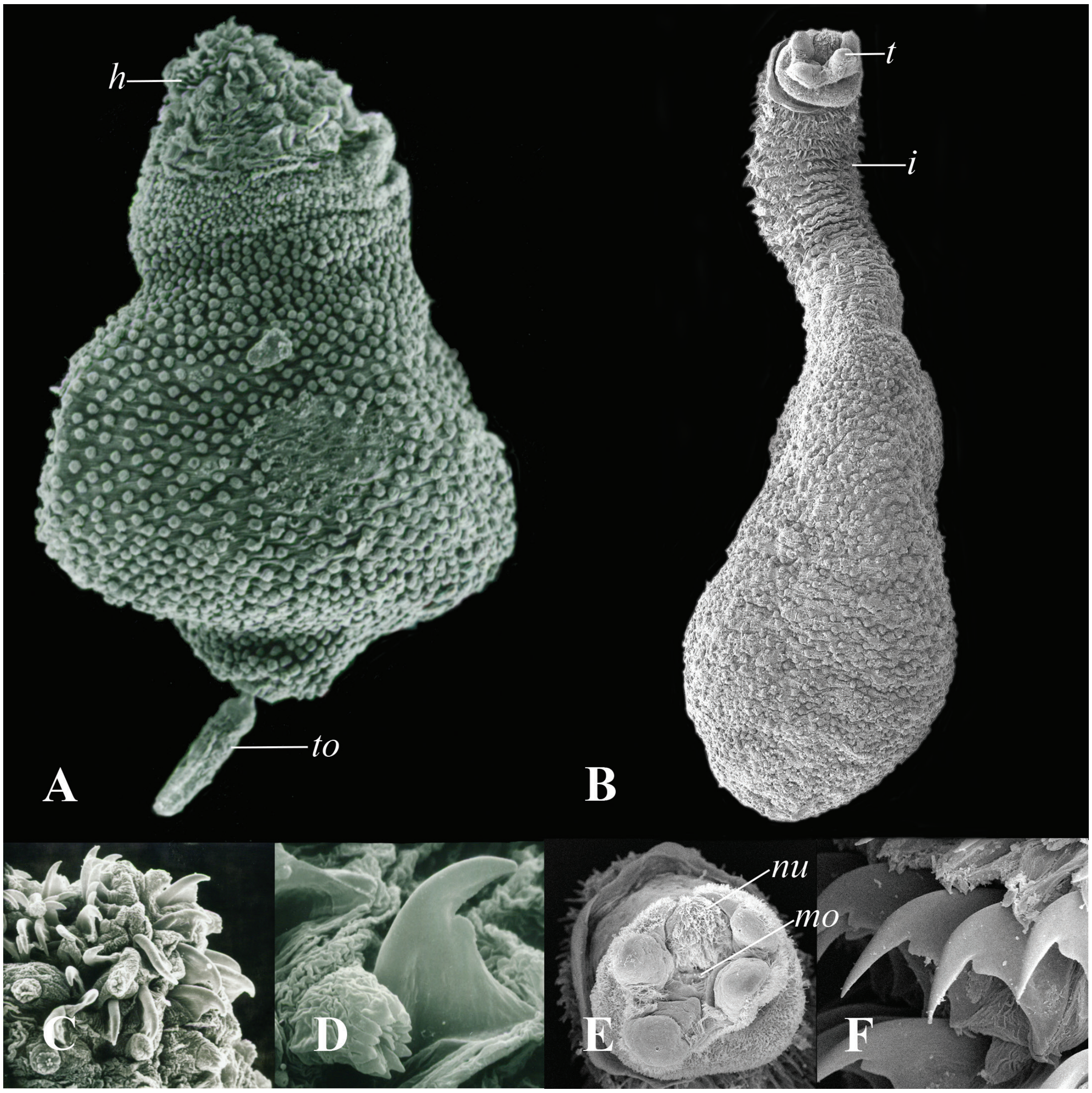


FIGURE 29. Scanning electron micrographs of white white, early metamorphosis stage and juvenile. (A) Beginning metamorphosis. The metatroch has been lost, and hooks of the developing introvert are protruding from the anterior thorax. (B) Juvenile, age between 2 and 4 weeks, ventral view. (C) Hooks from beginning metamorphosis in (A). (D) Hook and epidermal organ from juvenile in (B). (E) Apical view of head and tentacles from juvenile in (B). Note that tentacles are dorsal to the mouth and the nuchal organ is between two dorsal tentacles. (F) Introvert hooks from juvenile in (B). Abbreviations: h = hooks, i = introvert, mo = mouth, nu = nuchal organ, t = tentacles, to = terminal organ.

In a 2-week juvenile the distinctive dark brown hooks are arranged in seven transverse rows on the anteriormost one-third of the introvert (Figures 27B, 29B,D,E). At this stage the body is somewhat elongated, the introvert to trunk ratio is about 1:1, and the trunk is still covered by larval cuticular papillae. The terminal organ has been lost or is in the process of atrophy and detachment. By 4 months the juvenile has continued to elongate, with the introvert to trunk ratio being about 1.5:1. The rows of hooks have increased to 30 to 40, with the more posterior consisting of smaller, well-separated hooks. The number of tentacles has increased to six (Figure 27C,D). At 1 year the tentacular crown consists of eight tentacles dorsal to the mouth and 80 rows of dark hooks extending over one-half of the introvert (Figure 27E). Larval cuticular papillae are lost, and conical epidermal papillae cover the trunk, most numerous at the anterior and posterior ends. The hooks of the introvert are broadly based, with a light curvature and usually a bump on the concave side. The hooks, distribution of papillae, and body proportions are characteristic of the species *Phascolosoma nigrescens* (Keferstein, 1865; Stephen and Edmonds, 1972; as defined by Cutler, 1994).

Yellow Pap

This larval type is characterized by its dark pink to orange coloration, which often ranges to various shades of yellow (Figure 30A). The color pattern varies over the surface of the larva. In the region of the trunk posterior to the anus the color is darker, whereas anterior to the anus and posterior to the postmetatrochal sphincter the color is absent or pale. Although the mid-region is relatively transparent, the rim of the metatrochal band is orange, as are the margins of the ventral lobes of the head and the mid-ventral groove. The postmetatrochal sphincter is distinctive as a brownish band. At the posterior tip of the trunk, the area surrounding the region of the retracted terminal organ is colorless. When extended, the terminal organ is seen to be a thin transparent rod. The cuticular papillae covering the trunk are set closely together in a regular pattern (Figure 31A–C). Their shape, distinctive for this larval type, is rounded and mammiform with an apical nipple. Scattered among the papillae are epidermal sensory-secretory organs (Figure 31B,C). Immediately posterior to the postmetatrochal sphincter, the papillae are more widely spaced, quadrangular, and flattened. On the posterior thorax the papillae are small, dark brown, and widely spaced.

The prototroch on the dorsal head forms a U-shaped band of shortened cilia that is continuous with the ciliation along the outer rims of the lobes of the ventral head (Figure 31A). Just anterior to the prototroch is a laterally placed pair of small dark red eyespots. On the ventral head, which is covered with short cilia, a median groove leads to the mouth, posterior to which is a rounded lower lip, similar in structure to that of other papillated larvae. The gut, as observed through the rather opaque body wall, is commonly bright orange. Nephridia, often a brownish coloration, open ventrolaterally on the trunk anterior to the prominent anus. They hang free in the body cavity and are sometimes seen to extend anteriorly into the transparent thorax.

The general shape of this larval form is relatively thickened or robust. The extended length averages 1.4 mm with a range of 1.0–1.8 mm, and the ratio of length of trunk to width is 1:0.67.

When the larva is swimming, the posterior trunk is contracted into a pyramidal shape, and the thorax is greatly inflated. Stomach, nephridia, and coelomocytes are pushed anteriorly into the thoracic coelom; the lower lip is flattened downward, and the actively beating ciliary band is fully distended. As in other larval types, the body is flexible; however, it does not have the same degree of extensibility as the white white and the annulated larval types. It is occasionally observed to bend so that the posterior end is placed in the region of the mouth. The terminal organ is extended only rarely and has not been observed to function as an organ of attachment. Typical of the behavior of most of the other pelagospheras, this larval type can move along a substratum with the ventral head applied to the bottom and the posterior end directed upward.

Within 4 to 6 days after the larva burrows into sediment, the metatroch is lost, and the posterior thorax has elongated (Figures 30B–F, 32A,B). The anterior thorax and head are retracted, and it is in this retracted position within the posterior body (trunk plus posterior thorax) that metamorphosis of the head and introvert takes place. The region of the metatroch contracts, forming a constriction that prevents the extension of the premetatrochal thorax and head. Whereas the premetatrochal thorax will give rise to the anterior introvert, the posttrochal thorax (not inverted) forms the shorter posterior introvert. Within 2 weeks (Figure 32C–E) the newly formed anterior introvert and the recently metamorphosed head are completely extended from the posterior body. The anterior introvert is transparent and covered with rings of widely spaced, unidentate, blunt-pointed hooks, and the mouth is now in a terminal position on the introvert. Dorsal to the mouth are four tentacular lobes, two longer ventral and two shorter dorsal. Between the two dorsal tentacles is the ciliated nuchal organ; the brain with two eyespots is visible through the transparent body wall. At 4 weeks (Figure 32F–I) the number of tentacles has increased to six. Hooks cover about half of the anterior introvert, and the length of the introvert is 1–1.5 times the length of the trunk, depending on the degree of extension. The shape of the trunk and the cuticular papillae still resemble those of the larva. At 6 weeks (Figure 32J–M), the number of tentacles and body shape remain essentially the same, although the introvert is more defined and the body wall is thicker. Hooks are curved, broadly based, and unidentate, similar in both anterior and posterior rows. At 6 months (Figure 32N–T) there is an anal shield, typical of the genus *Aspidosiphon*. The anal shield has about 14 longitudinal grooves, and the caudal shield has about 20 radial grooves. The number of tentacles is eight, and the hooks are unidentate. One larva was reared in the laboratory to an age of 9 years. Unfortunately, the specimen died before details of hook structure and internal morphology were recorded. Morphological characteristics of this larval type and its juveniles correspond to those of *Aspidosiphon laevis*, as defined by Cutler (1994).

DISCUSSION

Comparative Features of Larval Groups

The three major larval groups distinguished here on the basis of cuticular properties (e.g., smooth, annulated, and papillated) are further defined by certain morphological and behavioral characteristics, as well as events of metamorphosis. The group designated as smooth is distinguished by the shape and ciliation of the lower lip. As a relatively narrow projection posterior to

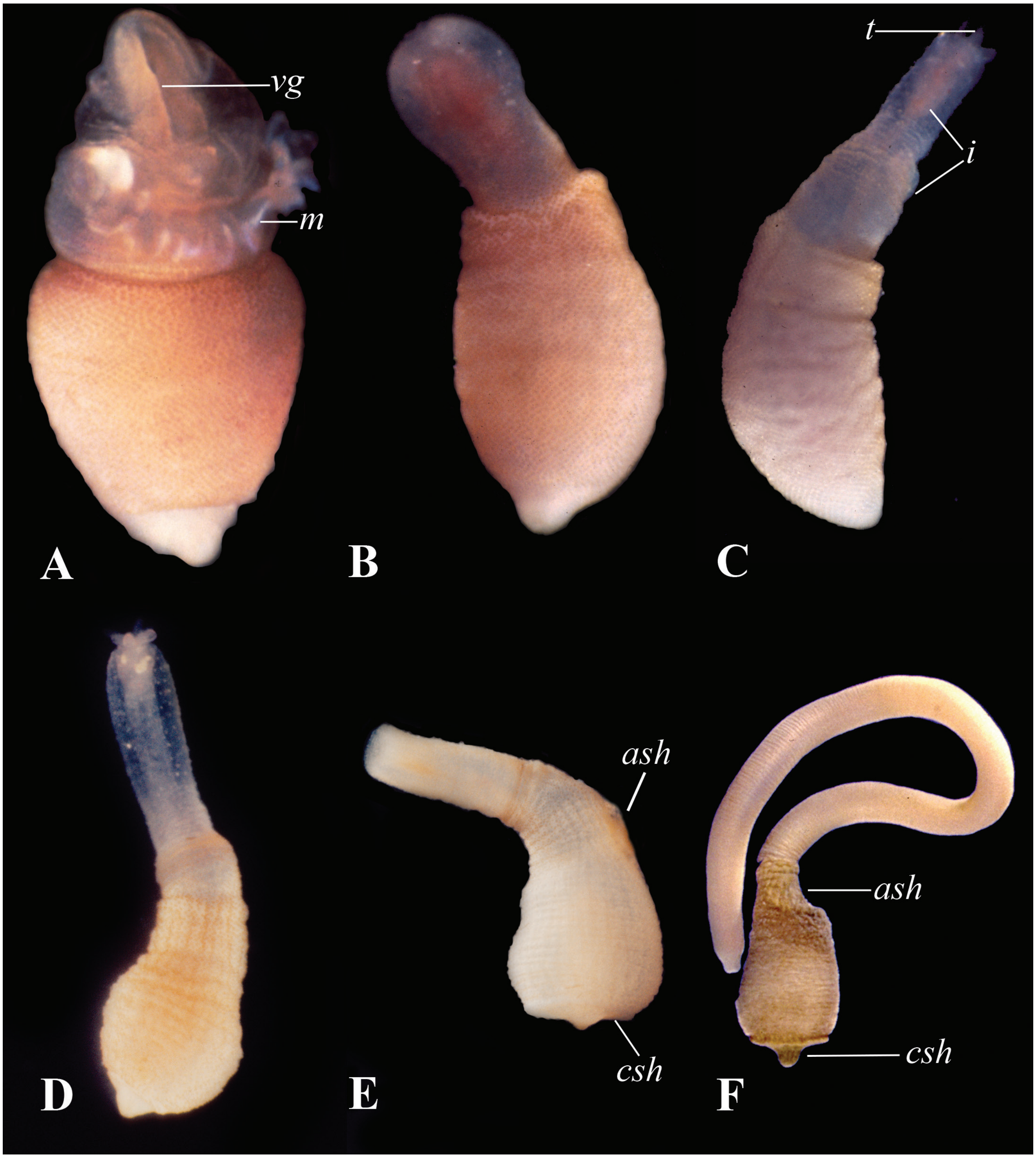


FIGURE 30. Photomacrographs of living yellow pap larva and juveniles. (A) Ventrolateral view of swimming larva. (B) Bulbous stage of metamorphosis, 7 days following initiation of metamorphosis. The head is retracted into the trunk, and anterior constriction prevents extension. (C) Juvenile, 2 weeks. Tentacles and introvert formed and extended. (D) Juvenile, 4 weeks. Longitudinal muscle bundles of body wall visible. Beginning formation of caudal shield. (E) Juvenile, 6 months. Anal shield and caudal shield fully formed. In this specimen the introvert is mostly retracted. (F) Adult, 9 years. Reared in the laboratory from yellow pap larva. Identified as *Aspidosiphon laevis*. Abbreviations: ash = anal shield, csh = caudal shield, i = introvert, m = metatroch, t = tentacles, vg = ventral groove of head.

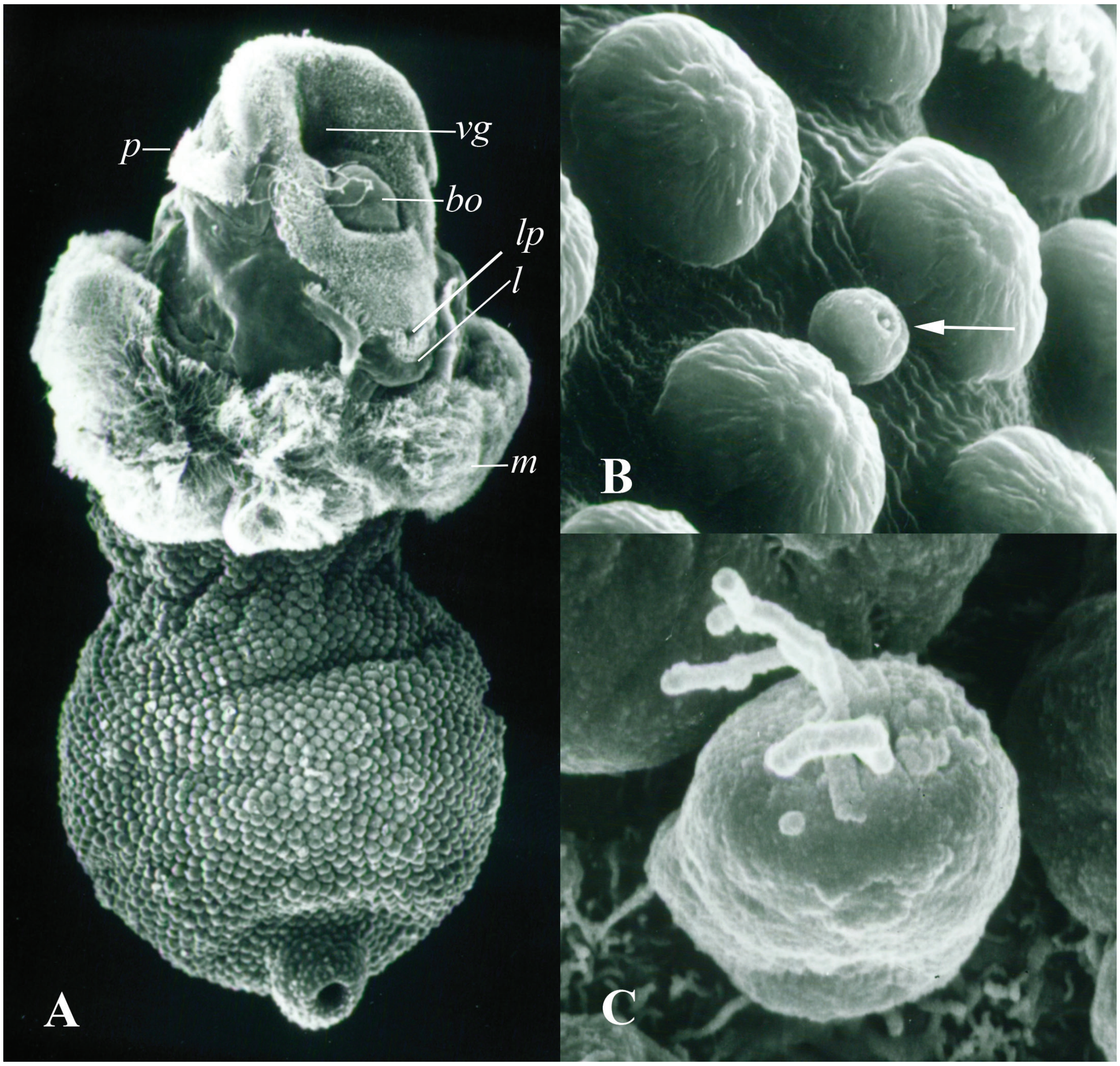
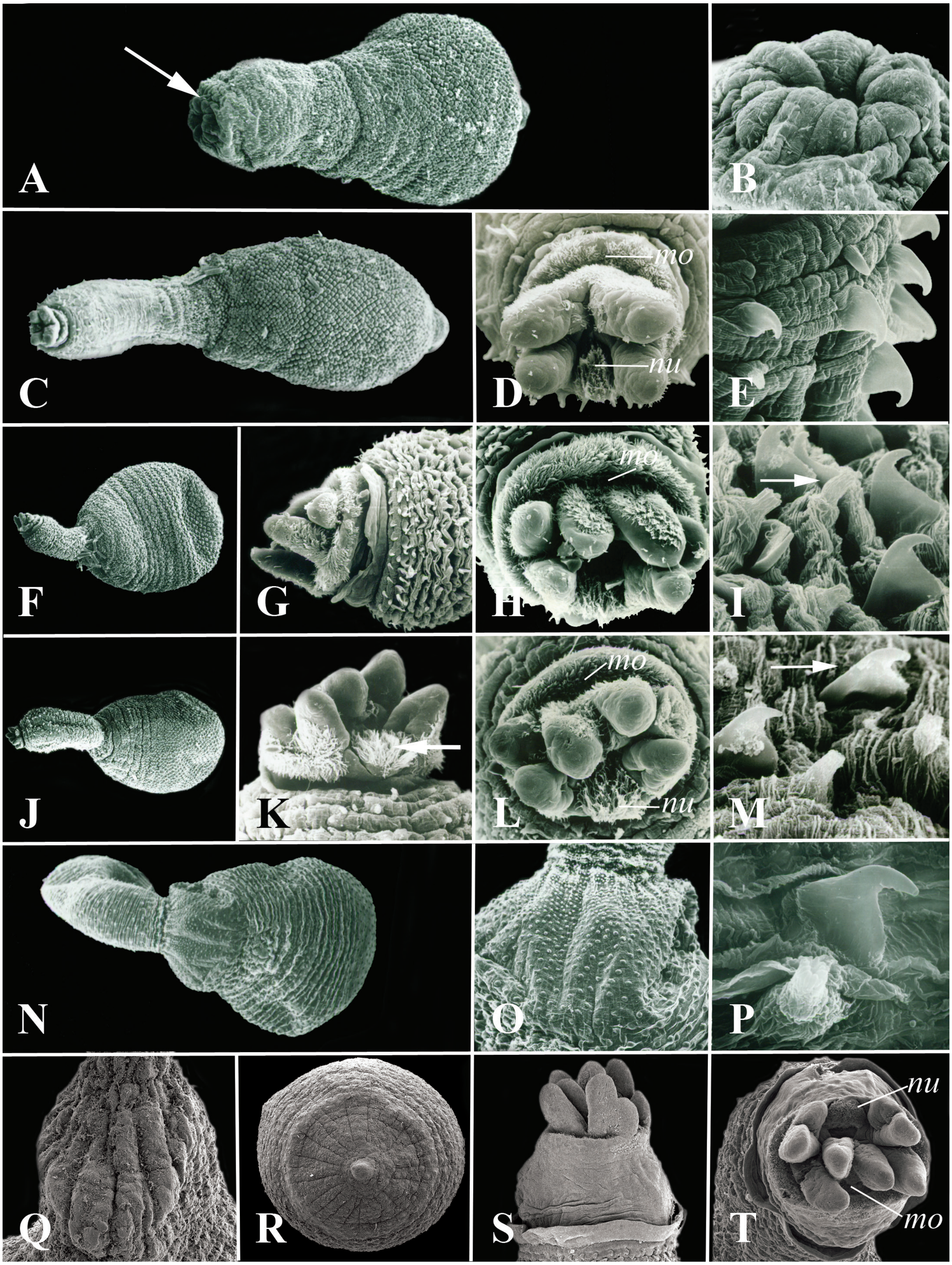


FIGURE 31. Scanning electron micrographs of yellow pap larva. (A) Larva with buccal organ, lip, and metatroch extended, terminal organ retracted. Ventrolateral view. From Rice (1981). (B) Cuticular papillae including sensory-secretory organ. From Rice (1976). Arrow points to sensory-secretory organ. (C) Higher magnification of a sensory-secretory organ with extending cilia. Abbreviations: bo = buccal organ, l = lip, lp = lip pore, m = metatroch, p = prototroch, vg = ventral groove of head.

FIGURE 32. (*Opposite page*) Scanning electron micrographs of yellow pap early metamorphosis and juveniles. (A) One week after initiation of metamorphosis. Head and anterior thorax are retracted; constriction (arrow) prevents extension. (B) Enlargement of constriction (see A). (C–E) Juvenile, 2 weeks. (C) Ventral view showing tentacles and introvert. (D) Apical view of head; tentacles are dorsal to the mouth, and the ciliated nuchal organ is between two dorsal tentacles. (E) Unidentate hooks on anterior introvert. (F–I) Juvenile, 4 weeks. (F) Lateral view. (G) Enlargement of head and anterior introvert with hooks. (H) Apical view of head showing six tentacles dorsal to mouth; ventral tentacles are longer. (I) Anterior hooks and introvert sensory organs (arrow). (J–M) Juvenile, 6 weeks. (J) Dorsolateral view. (K) Dorsolateral view of tentacles with ciliated nuchal organ (arrow) between the two most dorsal tentacles. (L) Apical view of tentacles. Note ventral mouth and dorsal nuchal organ. (M) Hooks (arrow) and sensory organs of introvert. (N–P) Juvenile, 6 months. (N) Dorsal view showing anal shield. (O) Higher magnification of shield showing grooves and small papillae. (P) Unidentate hook and sensory organ of anterior introvert. (Q–T) Juvenile, 6 months. Different specimen from (N)–(P) to show more advanced development. (Q) Anal shield with 14 longitudinal grooves. (R) Caudal shield with about 20 radial grooves. (S) Lateral view of head (ventral on left). Extended collar between tentacles and introvert. (T) Apical view of head. Note relative positions of tentacles, nuchal organ, and mouth. Abbreviations: mo = mouth, nu = nuchal organ.


A
B
C
mo
nu
D
E
F
G
mo
H
I
J
K
mo
nu
L
M
N
O
P
Q
R
S
nu
mo
T

the mouth, the lip is covered with short cilia continuous with the ciliation of the lateral lobes of the ventral head and is bifurcated by a prominent groove. The pore of the lip gland is located at the proximal end of the bifurcation. By comparison, in both the annulated and papillated larvae the lower lip is a rounded projection of the ventral body wall with a rim of long cilia and a central raised area of short cilia continuous with the ciliation surrounding the mouth. The lip gland pore is within the raised area of ciliation. The annulated group is distinguished from the other two by the well-developed lobes of the ventral head and by behavioral characteristics of the larvae, exemplified by the exceptional extensibility of the larval body and the circular contractibility along the length of the larva.

Details of the sequence of metamorphic events vary among the three groups of larval types. In all larvae, metamorphosis from larval to juvenile form consists basically of a loss of metatrochal cilia, formation of tentacles, movement of the mouth from the ventral to terminal position, extension of the mid-region (thorax) to form the introvert, and elongation of the trunk. In the group of smooth larvae the first events are loss of the metatroch and regression of the lower lip, followed by the formation of tentacles, whereas in the annulated group, the tentacles are formed prior to the loss of the metatroch. Metamorphosis in the papillated larvae begins with a narrowing of the head and loss of metatroch, followed by a withdrawal of the head and anterior thorax into the trunk. The constriction of the postmetatrochal sphincter prevents extension of the anterior larval body for a period as long as 1 to 2 weeks, during which time the head undergoes a complete reorganization, and the tentacles and anterior introvert are formed.

Identification of Larval Types

Of the 10 larval types described here, specific affiliations can be attributed to six, and generic status can be attributed to four. These designations are based on a combination of morphological characters of larvae and juveniles reared in the laboratory. It should be noted that the morphological characters are those available from external examination of specimens and include internal features only when visible through a transparent body wall or in a specimen large enough for dissection.

The large transparent larval type described in this study is identified as *Sipunculus polymyotus*, primarily on the basis of the number of longitudinal muscle bands (54–55). Fisher (1947) was the first to describe this larval type in plankton collections from the Straits of Florida to Cape Hatteras and to recognize its specific affiliation as *S. polymyotus*. It was later reported by Jägersten (1963) from collections off Miami and Bimini and by Hall and Scheltema (1975) from plankton tows throughout the temperate and tropical North Atlantic. The number of longitudinal muscle bands (42–55) is a defining character of this species (Cutler, 1994).

The transverse groove larva, described by Hall and Scheltema (1975) as type E, was tentatively identified as *Siphonosoma cumanense* by Rice (1976, 1981). Confirmation was provided in a later study of the development of *S. cumanense* in which larvae, reared from spawnings of adults, were found to resemble oceanic larvae collected from the Florida Current (Rice, 1988). Further, as noted earlier in this chapter, a postlarval stage of transverse groove was reared in the laboratory to an identifiable adult, 3 years of age.

The white white larva was first reported in 1976 and was tentatively placed in the genus *Phascolosoma* (Rice, 1976). In a further assessment of morphological features of juveniles in this chapter, it is assigned to the species *Phascolosoma nigrescens*. The distribution and morphology of hooks, the body proportions, and the arrangement of tentacles in juveniles of 2 weeks, 4 months, and 6 months and a 1-year adult are consistent with the definition of this species by Cutler (1994).

The yellow pap larva is identified as *Aspidosiphon laevis*. This larval type was initially reported by Häcker (1898) as *Baccaria citrinella* and later described as type A by Hall and Scheltema (1975), who reared the postlarval stage to an age of 1 year. Even though their specimen developed shields typical of *Aspidosiphon,* the authors were unable to make a specific determination. In this study morphological features observed in a 6-month juvenile that concur with Cutler's (1994) definition of *Aspidosiphon laevis* are as follows: the presence of longitudinal muscle bands, unidentate blunt hooks arranged in rings, a solid anal shield with 14 longitudinal grooves, a posterior shield (about 20 radial grooves), and tentacles that enclose the nuchal organ but not the mouth.

The white blackhead larva is considered identical to that described by Häcker (1898) as *Baccaria oliva* and by Hall and Scheltema (1975) as type C. The latter authors gave a detailed description of the distinctive larval papillae, and although they were able to rear larvae through metamorphosis to juveniles, they did not propose an adult affinity. Subsequently, there have been numerous studies of this larval type, including detailed descriptions of morphology, behavior, and metamorphosis, as well as factors inducing metamorphosis and the rearing of juveniles in the laboratory (Rice, 1976, 1978, 1981). Postlarval stages have been reared to sexual maturity within 9 months and maintained in the laboratory as long as 26 years. These studies have demonstrated similarities in the morphology of the adult of the white blackhead larva and the defining characters of *Apionsoma misakianum*. In a comparison of external and internal anatomy of 20 white blackhead adults reared from larvae collected in the Florida Current with 20 adults of *Apionsoma misakianum* from the continental shelf off the central east coast of Florida, the following taxonomic characters were found to be similar: average total body length of about 14 mm, introvert four to five times the length of the trunk, six to eight tentacles dorsal to the mouth, nuchal organ within the tentacular crown between the shorter dorsal tentacles, rings of hooks on the anterior introvert with four to five basal spinelets, a pair of ventral retractor muscles attached near the level of the anus, a pair of dorsal retractors attached slightly posterior to bilobed nephridia, continuous longitudinal musculature of the body wall, and spindle muscle attached posteriorly (Rice, 1981).

A larva similar to the spotted velvet larva was first described by Hall and Scheltema (1975) as type J. Differing from the description reported in this study, the longitudinal musculature of the body wall was described as divided into bundles, whereas in our specimens the muscle layer was continuous. Otherwise, coloration and general appearance of the external body surface were the same in the two descriptions. As observed in this study, the cuticular papillae of spotted velvet resemble those of the white blackhead in structure, although the papillae are in a more closely configured pattern in the latter. Morphological features of juveniles of spotted velvet, as well as those of white blackhead, are consistent with defining characters of the species *Apionsoma misakianum*, that is, the relative length of the introvert, structure of hooks with basal spinelets, and arrangement of the tentacular crown. The probability of two cryptic species of *Apionsoma misakianum* has been suggested by studies of Staton and Rice (1999) in an allozyme analysis that distinguished two species, one occurring off the east central coast of Florida (Sebastian Pinnacles) and another from the Florida Keys and the Bahamas. Further evidence from Schulze et al. (this volume) using DNA bar coding supports the concept of cryptic species, aligning the spotted velvet larva with the population of *Apionsoma misakianum* from the coast of central Florida and the white blackhead with the more southern population. Further evidence of differences between the two populations is found in comparative developmental studies from spawnings of adult white blackheads, reared from oceanic larvae, and spawnings from field-collected *Apionsoma misakianum* from the Sebastian Pinnacles. Differences were reported in the egg size, position of the first meiotic metaphase spindle, pigmentation of the gut in the trochophore, and developmental time (Rice, 1981).

It is of interest to note how the larval groups and their larval types, as identified above, relate to the latest classification and phylogeny of the Sipuncula by Kawauchi et al. (2012) in which a novel classification is proposed, recognizing six families, two of which are new. The smooth larvae of larval group 1, which includes larval types of the genera *Sipunculus* and *Xenosiphon*, would fall within the family Sipunculidae, and the annulated larvae of larval group 2, with larvae identified as *Siphonosoma*, would be in the newly created family Siphonosomatidae. Of the papillated larvae of larval group 3 the white blackhead and spotted velvet (designated as *Apionsoma*) and the white white (*Phascolosoma*) would fall in the family Phascolosomatidae. The remaining papillated larva in group 3, yellow pap (*Aspidosiphon*), would be in the family Aspidosiphonidae. Thus, of the six families recognized in this latest phylogeny, the oceanic pelagospheras, as identified in this study, would be represented in four.

Functional Morphology of Larval Organs and Behavior

The pelagosphera larva has a unique combination of highly specialized larval features that serve the larva in species dispersal. They include the metatroch, the ventral head and its associated buccal organ, the lip and lip gland, and the posterior terminal organ. These organs are lost in metamorphosis to the adult.

Metatroch

The metatroch is a band of strongly active and exceedingly long cilia that encircles the mid-thorax of the pelagosphera larva. Its primary function is as a locomotory organ. The term metatroch is used in reference to its position posterior to the mouth. Of unknown cell lineage, its homology is uncertain. The metatroch of sipunculans is not involved in feeding; the ciliary beat is downward, away from the mouth.

When the larva is swimming, the metatrochal band is fully extended, its circumference exceeding that of the head and trunk. The entire thorax, bearing the metatrochal band, is inflated, and the posterior trunk is contracted, pushing the coelomic fluid, stomach, and nephridia anteriorly into the coelomic region of the thorax. The metatrochal cilia beat in a metachronal pattern. A rapidly swimming larva moves forward while turning in a spinning movement resembling that of a spinning top. When the metatroch is retracted either completely or partially, the larva, as observed in the laboratory, falls to the bottom of the container.

Organs Associated with the Ventral Head

The ventral head and its associated organs, the buccal organ, the lip, and lip gland, form the major feeding apparatus of the pelagosphera larva. The short cilia of the ventral bilobed head move particulate matter through a median groove into the mouth. The buccal organ pushes particles into the mouth and, on occasion, may eject larger particulate matter. The lip gland appears to have a secretory function in association with larval feeding and possibly locomotion.

The buccal organ is a muscular sac that hangs down into the coelom from the esophagus and is protrusible to the exterior by evagination through a groove at the base of the mouth. It is covered by a thick cuticle overlying elongate epithelial cells. At least two internal cavities are separated by extensions of the epithelial layer. Details of the histology and ultrastructure of the organ in an early pelagosphera larva of *Phascolosoma agassizii* are reported by Rice (1973) and Tzetlin and Purschke (2006).

The buccal organ was first described in the larva of *Sipunculus nudus* by Hatschek (1883), who referred to it as the "Schlundkopf" or buccal mass of enigmatic or "rathselhaft" function. Damas (1962), examining preserved specimens of large oceanic *Pelagosphaera*, proposed the term "machoire," or jaw. He described it as a plug at the entrance of the digestive tube and suggested that it might act as a rocker, pushing food into the esophagus. Jägersten (1963), in his report on a variety of living oceanic pelagospheras, presumed that the organ might break up large pieces of food into smaller ones through a kneading (in and out) action. The buccal organ has been reported also to be used in feeding when the larva is swimming as well as when it is on a substratum (Jägersten, 1963; Rice, 1973, 1985; Adrianov et al., 2011). A common behavior observed in the laboratory is movement of a larva along the bottom of a laboratory container with the posterior end directed upward and the ventral surface of the head flattened against the substratum. In this position the buccal organ is frequently protruded, presumably scraping material from the bottom, as the larva moves forward. Another larval behavioral pattern, more common among larvae in early stages of development reared from spawnings in the laboratory, is temporary attachment to a substratum by the well-developed terminal organ. From the point of attachment the larval body is bent, so that the ventral head and mouth region are applied to the substratum. In this position the protrusion of the buccal organ serves to scour the area around the attachment, scraping and breaking up material for ingestion into the mouth.

The lip gland is an elongate, usually bilobed secretory organ that is suspended in the coelom from an attachment to the lower lip of the ventral head. Glandular lobes lead to a duct that opens through a pore on the ciliated area of the lip. The secretory product is presumably adhesive and has been considered to contribute to mucus-ciliary feeding of the larva (Jägersten, 1963). It has also been suggested that the secretion is utilized during larval grazing when the ventral head is applied to a substratum (Jägersten, 1963; Rice, 1985).

The organ was given the name lip gland by Jägersten (1963), who noted its association with a pore on the ventral lip of oceanic pelagosphera larvae. It had been recognized earlier in the developing larva of *Sipunculus nudus* by Hatschek (1883), who named it "Anhangsdruse" and described it as an evagination of the esophagus. Mingazzini (1905) mistakenly identified the lip gland as a gonad in a large planktonic larva that he described as a new species, *Pelagosphaera aloysii*. His mistake was soon noticed by Senna (1906) and others, who recognized the organ as the glandular structure previously described by Hatschek.

Terminal Organ

The terminal organ is a retractable appendage at the posterior extremity of the trunk found in all pelagosphera larvae, both lecithotrophic and planktotrophic, with the exception of the lecithotrophic larva of *Themiste alutacea*. It is formed in early development during transformation (metamorphosis) of the trochophore to pelagosphera, appearing as a knob joined to the posterior body by a short, flexible stalk. A pair of muscles extending within the body from the posterior knob to an attachment on either side of the dorsal anus functions to retract the organ into the trunk. As reported in an ultrastructual study of a 5.5-day larva of *Apionsoma misakianum* (=*Golfingia misakiana*), a central apical pore of the terminal organ is lined by mucus-secreting cells, and sensory cells are terminated by ciliary processes (Ruppert and Rice, 1983). The organ is commonly well developed and prominent in young larvae reared from spawning in the laboratory. These larvae tend to remain near the bottom of laboratory dishes, and the primary function of the terminal organ appears to be attachment of the larva. However, in the larger and presumably older oceanic larvae the terminal organ is proportionately reduced and differs in relative size and function among the various larval types described in this review. In oceanic larvae of the genus *Sipunculus* (large transparent and smooth small transparent) the terminal organ appears as an inconspicuous eversible knob, usually retracted and of unknown function. In contrast, the terminal organ of the white white larva (*Phascolosoma nigrescens*) is a long telescoping rod that can extend to a length one-fifth that of the larval body and is frequently used in attachment of the larva to a substratum. In larvae of the annulated group (Siphonosomatidae) the terminal organ is slender and occasionally used for attachment, whereas in the larvae of the genera *Apionsoma* (white blackhead and spotted velvet) and *Aspidosiphon* (yellow pap), the terminal organ is relatively small, rarely extended, and not used for attachment. As noted previously, a characteristic behavior of all sipunculan larvae (with the exception of *Sipunculus*) is bending of the body so that the terminal organ is placed in or around the mouth. Although the significance of this activity has not been documented, it has been suggested that it could serve to discharge a secretion into the mouth as a means of feeding by transferring debris accumulated in mucus secretions to the mouth. Another possibility is the communication between the head and terminal organ in testing the substratum for possible settlement or attachment (Ruppert and Rice, 1983).

Behavior on Sediment and Burrowing

When an oceanic pelagosphera larva is exposed to sediment, behavior changes markedly from that on a solid substratum, as observed in the laboratory. After an initial period of apparent exploration of the surface, lasting from a few minutes to a few hours, the larva burrows within the sediment, where it undergoes metamorphosis. During initial contact with the sediment, the larva commonly moves over the surface in the manner of an inchworm by repeated extension of the head followed by contraction of the trunk. As the larva moves forward with the ventral head applied to the substratum, it leaves behind trails of aggregated sand grains, presumably formed by mucus secretions as the sediment passes through the ciliated ventral groove of the head and over the buccal organ and lower lip. At the edge of the lip, the agglutinated sand grains are moved away from the body by ciliary activity and protrusion of the buccal organ. There is no evidence that particulate matter is taken into the gut during this activity. Another behavioral pattern is curling of the larval body with the head approaching the tail and the repeated rolling of the curled body over the substratum, resulting in the adherence of sand grains over the surface of the larva. These behavioral patterns have been interpreted as larval sensing or testing the substratum prior to burrowing and metamorphosis (Rice, 1978, 1986). As noted in descriptions of larval cuticular structures, sensory-secretory epidermal organs are scattered over the surface of the larval body. Both secretory glands and sensory cells have been previously described in the ventral head of early planktotrophic pelagosphera larvae of *Phascolosoma agassizii* (Rice, 1973).

Burrowing is initiated by a thrusting of the head into the sediment, followed by a series of rapid peristaltic-like movements of contraction and extension along the length of the body. Once burrowed, the larva undergoes initial metamorphosis within a few minutes to several hours. Completion of metamorphosis occurs over 2 to 3 days but may vary among different larval types. A small percentage of larvae will metamorphose without substratum when maintained in the laboratory over a period of several months in glass dishes with frequent changes of seawater and the addition of phytoplankton for food (Hall and Scheltema, 1975; Rice, 1978). A laboratory investigation of factors influencing metamorphosis in the white blackhead pelagosphera revealed that the highest percentage of metamorphosis was attained when larvae were placed in dishes with substratum and with seawater previously occupied by adults of the same or cryptic species (Rice, 1981, 1986). This study established the procedure for inducing metamorphosis in large numbers of larvae for studies of metamorphosis, while at the same time, it posed many questions regarding not only the nature of the inducing factor but also the mechanism of larval response.

Role of the Oceanic Pelagosphera Larva in the Life Histories of Sipuncula

The planktotrophic pelagosphera larval stage consists of two phases, an early precompetent phase and a later competent phase. The early phase is primarily a benthic-pelagic period of growth and differentiation, whereas a larva in the later competent phase has reached a definitive size and, having attained the characteristic features of the oceanic larva, is well adapted for prolonged life in the surface waters of the open ocean.

The precompetent phase has been described for two oceanic pelagosphera larval types: transverse groove and white blackhead, identified respectively as *Siphonosoma cumanense* and *Apionsoma misakianum*. In studies of the complete life history of these two species, from spawning and fertilization through larval stages to juvenile and adult, the precompetent phase was found to last as long as 2 to 3 months.

In studies of *Siphonosoma cumanense* the pelagosphera larva, obtained from spawnings of field-collected adults, was reared in the laboratory for 8 weeks (Rice, 1988). During this time it increased approximately 10 times in extended length and changed markedly in relative body proportions and behavior, finally attaining the characteristic features of the oceanic transverse groove larva. The early pelagosphera, with a well-developed terminal organ, was frequently attached to the bottom of the laboratory container, feeding on both bottom detritus and suspended phytoplankton. By 6 weeks the larvae commonly rested on the bottom, usually unattached, with the proportionately smaller terminal organ retracted. Metamorphic competence was tested at 18, 45, and 58 days by placing larvae on substratum previously occupied by adult *Siphonosoma cumanense*. At 58 days larvae responded by undergoing metamorphosis.

The development of *Apionsoma misakianum* was described from spawnings of adults reared in the laboratory from oceanic white blackhead larvae (Rice, 1978, 1981). Pelagosphera larvae, formed at metamorphosis of the trochophore, were reared in the laboratory for 3 months, during which time they increased in length approximately five times, changing relative body proportions and manifesting most of the defining characters of the oceanic larva. During the 3-month period, the prominent terminal organ of the young pelagosphera was considerably reduced in size and no longer used for attachment. Cuticular papillae appeared but did not attain the complexity of structure or density of those in the oceanic larva. Larvae were tested every 2 weeks at ages from 4 to 12 weeks for metamorphic competence by subjecting them to combinations of sediment and water previously exposed to adults. Results at all ages were negative, although this same procedure induced metamorphosis of the oceanic white blackhead larva (Rice, 1978, 1981, 1986).

Each phase of larval development has a distinctive role in the overall life history of a species. As observed in the laboratory, the morphological and behavioral features of the early precompetent larva indicate that the larval stage is well adapted for a benthipelagic existence. The larva swims and feeds on or near the bottom of the laboratory dish and is frequently attached to the dish by means of a prominent adhesive terminal organ. If we extrapolate from behavior in the laboratory to that in the field, we can assume that the young larva remains near the substratum, where it may attach to such structures as rock, rubble, or seagrass. As the larva differentiates and loses its capacity for strong attachments, it is subject to water movements such as currents and tidal flows that could move it away from its benthic habitat. If, when the larva attains competence, it is still in the vicinity of its population of origin or of other populations of similar species, it will metamorphose without delay. If, however, it has moved from the coastal benthic environment to surface waters of the open ocean, metamorphosis will be delayed.

The pelagosphera of the open ocean is a competent larva that has moved beyond the boundary of the continental shelf to drift with the surface waters of the oceanic currents. With a highly developed locomotory band of metatrochal cilia, it is well adapted for prolonged existence in the open ocean. In extensive studies of long-distance dispersal by larvae of marine invertebrates, Scheltema has reported that pelagosphera larvae are widely distributed and occur frequently throughout all trans-Atlantic currents of the warm temperate and tropical North Atlantic Ocean (Scheltema, 1971, 1975; Hall and Scheltema, 1975; Scheltema and Hall, 1975). From a comparison of current velocities and an estimated length of larval life, as determined by maintenance of larvae in the laboratory, Scheltema and Hall (1975) concluded that the duration of the larval stage is sufficient for transport across the ocean. They proposed further that the pelagosphera larva may serve to increase the geographic range of a species and maintain genetic continuity between widely isolated populations.

Support for the proposed genetic exchange is currently lacking. It requires the specific identification of larval types, as well as an amphi-Atlantic distribution of the corresponding adults. Presented in this chapter is morphological and behavioral evidence for identification of several of the larval types, and another chapter in these proceedings (Schulze et al., this volume) presents molecular evidence that confirms these specific identifications. However, distribution of corresponding amphi-Atlantic adult populations has yet to be documented. Although there have been surveys of sipunculan fauna from both the west coast of Africa and the subtropical western coast of the North Atlantic, including the Caribbean, the specific identifications are currently uncertain because of the changing status of sipunculan taxonomy. For example, in a recent taxonomic revision of the phylum, Cutler (1994), through specific synonymies, reduced the number of recognized species in the phylum from approximately 350 to 149. In addition, more recent molecular studies have recognized numerous cryptic species, questioning the validity of cosmopolitan species in sipunculans (Kawauchi and Giribet, 2010, 2013; Schulze et al., this volume). Thus, previous reports of widespread distribution of adult populations of sipunculan species in West Africa and the Caribbean (Wesenberg-Lund, 1959; Stephen, 1960; Murina, 1967a, 1967b; Scheltema, 1971) must be reevaluated in view of the more recent systematic revisions and the changing concept of cosmopolitan species.

There are many questions that remain to be explored for a better understanding of the role of the oceanic pelagosphera in life histories of the Sipuncula. From what geographical populations do the larvae originate, and to what populations do they contribute? What are the local hydrographic conditions responsible for the transport of larvae from and eventual return to coastal waters? How are larvae retained to maintain local populations in their region of origin? What are the factors in the field that induce larvae to settle, and are they similar to those reported in laboratory experiments? Is there a seasonality of currents relative to reproductive seasonality? Resolution of the central question concerning the role of the oceanic pelagosphera in transoceanic dispersal of species requires additional information on the amphioceanic distribution and the taxonomy of adult populations. Definitive evidence for such genetic connectivity could include molecular comparisons of a specific larval type collected from oceanic currents with an adult from either side of the ocean of a presumed specific identity corresponding to that of the larva.

CONCLUSIONS

Over the past several decades we have observed and documented the morphology and behavior of oceanic pelagosphera larvae and juveniles, primarily from the Florida Current, with the intent of determining their adult affiliations and, more generally, contributing to an understanding of their role in the life histories of the Sipuncula. In this review, we have focused on descriptions of 10 of the larvae that occur most commonly in our collections and for which we have the most information. Specific adult affiliations have been proposed for six of the 10 larvae, and generic status has been proposed for the remaining four.

Our studies have shown that there is considerable morphological diversity among the oceanic larvae and that this diversity can be similar to or greater than that of adults. For example, the larvae spotted velvet and white blackhead have distinctive morphologies, whereas adults reared from these two larval types are not morphologically distinguishable and are currently recognized as cryptic species of *Apionsoma misakianum*. Thus, larval forms should be considered in addressing the larger questions of cosmopolitan and cryptic species.

Through studies of larval morphology, we have also defined distinguishing larval characters that—if utilized to supplement specific characters of corresponding adults—will provide for a more accurate taxonomy in the Sipuncula, now limited by the relatively conserved morphology of the adults. The addition of larval characters could contribute also to an expanded morphological data set and, consequently, more meaningful phylogenetic analyses of the Sipuncula.

The morphological data compiled in our studies complement and confirm molecular identification of these same larval types (Schulze et al., this volume) and further provide the basis for an integrative morphological and molecular approach to the phylogeny of the Sipuncula that is currently a goal in systematic and phylogenetic research (Kawauchi and Giribet, 2013).

The ability to identify larvae to species level provides an unprecedented opportunity for addressing current questions not only in phylogeny and biodiversity but also in dispersal, species range, and population connectivity. Documentation of genetic exchange between populations and across ocean basins requires the specific identification of larval types as well as an accurate taxonomy of adult species. Sipunculans have a worldwide distribution, and additional types of pelagosphera larvae exist throughout oceanic waters (Scheltema, 1986; Scheltema and Rice, 1990), awaiting further examination. This review provides a reference and basis for future comparisons.

ACKNOWLEDGMENTS

Many individuals, too numerous to mention individually, have made valuable contributions to different aspects of this study, such as collecting, sorting, and maintaining specimens. They include staff of the Smithsonian Marine Station, visiting scientists, and postdoctoral fellows. We wish to recognize the crews of the research vessels *Tursiops*, *Gosnold*, *Aquarius*, *Edwin Link*, *Chain*, and *Morning Watch* and especially of the Smithsonian Marine Station's vessel, the R/V *Sunburst*, for the offshore plankton sampling. We thank Anja Schulze for her review of the manuscript and many helpful suggestions. Carolyn Gast is gratefully acknowledged for the color renderings of larvae in Figure 3. This paper is dedicated to Rudolph S. Scheltema, who introduced us to the larvae of the open ocean and whose research has inspired and encouraged us throughout our studies. This is Smithsonian Marine Station contribution number 1058.

REFERENCES

Adrianov, A. V., A. S. Maiorova, and V. V. Malakhov. 2011. Embryonic and Larval Development of the Peanut Worm *Phascolosoma agassizii* (Keferstein 1867) from the Sea of Japan (Sipuncula: Phascolosomatidea). *Invertebrate Reproduction and Development*, 55(1):22–29. https://doi.org/10.1080/07924259.2010.548638.

Åkesson, B. 1961. Some Observations on Pelagosphaera Larva (Sipunculoidea). *Galathea Report*, 5:7–17.

Cutler, E. B. 1994. *The Sipuncula: Their Systematics, Biology and Evolution*. Ithaca, N.Y.: Cornell University Press.

Damas, H. 1962. La Collection de *Pelagosphaera* du "Dana." *The Carlsberg Foundation's Oceanographical Expedition Round the World 1928–30 and Previous "Dana" Expeditions*, 59:1–22.

Dawydoff, C. N. 1930. Quelques Observations sur *Pelagosphaera*, Larve de Sipunculide des Côtes d'Annam. *Bulletin de la Société Zoologique de France*, 55:88–90.

Fisher, W. K. 1947. New Genera and Species of Echiuroid and Sipunculoid Worms. *Proceedings of the United States National Museum*, 97(3218):351–372. https://doi.org/10.5479/si.00963801.97-3218.351.

Häcker, V. 1898. *Die pelagischen Polychaeten- und Achaetenlarven der Plankton-Expedition*. Ergebnisse der Plankton-Expedition der Humboldt-Stiftung 2. Kiel, Germany: Lipsius and Tischer.

Hall, J. R., and R. S. Scheltema. 1975. "Comparative Morphology of Open-Ocean Pelagosphaera." In *Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura*, ed. M. E. Rice and M. Todorović, pp. 183–197. Belgrade: Naučno Delo Press.

Hatschek, B. 1883. Ueber Entwicklung von *Sipunculus nudus*. *Arbeiten aus dem Zoologischen Institute der Universitat Wien und der Zoologischen Station in Triest*, 5(2):61–140.

Heath, H. 1910. *Pelagosphaera*, a Larval Gephyrean. *Biological Bulletin*, 18:281–284. https://doi.org/10.2307/1536063.

Ikeda, I. 1904. The Gephyrea of Japan. *Journal of the College of Science, Imperial University of Tokyo*, 20(4): 1-87.

Jaeckle, W. D. and M. E. Rice. 2002. "Sipuncula." In *Atlas of Marine Invertebrate Larvae*, ed. C. M. Young, M. A. Sewell, and M. E. Rice, 375–396, 606–607. London: Academic Press.

Jägersten, G. 1963. On the Morphology and Behaviour of Pelagosphaera Larvae (Sipunculoidea). *Zoologiska Bidrag fran Uppsala*, 36(1):27–36.

Jägersten, G. 1972. "Sipunculida." In *Evolution of the Metazoan Life Cycle*, pp. 145–156. London: Academic Press.

Kawauchi, G. Y., and G. Giribet. 2010. Are There True Cosmopolitan Sipunculan Worms? A Genetic Variation Study within *Phascolosoma perlucens* (Sipuncula, Phascolosomatidae). *Marine Biology*, 157:1417–1431. https://doi.org/10.1007/s00227-010-1402-z.

Kawauchi, G. Y., and G. Giribet. 2013. *Sipunculus nudus* Linnaeus, 1766 (Sipuncula): Cosmopolitan or a Group of Cryptic Species? An Integrated Molecular and Morphological Approach. *Marine Ecology*, 35(4):478–491. https://doi.org/10.1111/maec.12104.

Kawauchi, G. Y., P. P. Sharma, and G. Giribet. 2012. Sipunculan Phylogeny Based on Six Genes, with a New Classification and the Descriptions of Two New Families. *Zoologica Scripta*, 41:186–210. https://doi.org/10.1111/j.1463-6409.2011.00507.x.

Keferstein, W. 1865. Beitrage zur anatomischen und systematischen Kenntniss der Sipunculiden. *Zeitschrift fur Wissenschaftliche Zoologie*, 15:404-445.

Keferstein, W. 1867. Untersuchungen uber einige amerikanische Sipunculiden. *Zeitschrift fur Wissenschaftliche Zoologie*, 17:44-55.

Mingazzini, P. 1905. Un Gefireo Pelagico: *Pelagosphaera aloysii* n. gen., n. sp. *Rendiconti delle Sedute Solenni della R. Accademia Nacionale dei Lincei*, 5(14):713–720.

Murina, V. V. 1965. Some Data on the Structure of Pelagospheres-Sipunculid Larvae. *Zoologicheskii Zhurnal*, 44:1610–1619.

Murina, V. V. 1967a. On the Sipunculid Fauna the Littoral of Cuba [in Russian]. *Zoologicheskii Zhurnal*, 46(1):35–46.

Murina, V. V. 1967b. Report on the Sipunculid Worms from the Sublittoral Zone of Cuba and the Mexican Gulf. *Zoologicheskii Zhurnal*, 46(9):1329–1339.

Rice, M. E. 1967. A Comparative Study of the Development of *Phascolosoma agassizii*, *Golfingia pugettensis*, and *Themiste pyroides* with a Discussion of Developmental Patterns in the Sipuncula. *Ophelia*, 4:143–171. https://doi.org/10.1080/00785326.1967.10409618.

Rice, M. E. 1973. *Morphology, Behavior, and Histogenesis of the Pelagosphera Larva of* Phascolosoma agassizii *(Sipuncula)*. Smithsonian Contributions to Zoology 132. Washington, D.C.: Smithsonian Institution Press. https://doi.org/10.5479/si.00810282.132.

Rice, M. E. 1975. "Observations on the Development of Six Species of Caribbean Sipuncula with a Review of Development in the Phylum." In *Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura*, ed. M. E. Rice and M. Todorović, pp. 141–160. Belgrade: Naučno Delo Press.

Rice, M. E. 1976. Larval Development and Metamorphosis in Sipuncula. *American Zoologist*, 16:563–571. https://doi.org/10.1093/icb/16.3.563.

Rice, M. E. 1978. "Morphological and Behavioral Changes at Metamorphosis in the Sipuncula." In *Settlement and Metamorphosis of Marine Invertebrate Larvae*, ed. F.-S. Chia and M. E. Rice, pp. 83–102. New York: Elsevier/North-Holland Biomedical Press.

Rice, M. E. 1981. Larvae Adrift: Patterns and Problems in Life Histories of Sipunculans. *American Zoologist*, 21:605–619. https://doi.org/10.1093/icb/21.3.605.

Rice, M. E. 1985. "Sipuncula: Developmental Evidence for Phylogenetic Inference." In *The Origins and Relationships of the Lower Invertebrates*, ed. S. C. Morris, J. D. George, R. Gibson, and H. M. Platt, pp. 274–296. Oxford: Oxford University Press.

Rice, M. E. 1986. Factors Influencing Larval Metamorphosis in *Golfingia misakiana* (Sipuncula). *Bulletin of Marine Science*, 39(2):362–375.

Rice, M. E. 1988. Observations on Development and Metamorphosis of *Siphonosoma cumanense* with Comparative Remarks on *Sipunculus nudus* (Sipuncula, Sipunculidae). *Bulletin of Marine Science*, 42(1):1–15.

Ruppert, E. E., and M. E. Rice. 1983. Structure, Ultrastructure, and Function of the Terminal Organ of a Pelagosphera Larva (Sipuncula). *Zoomorphology*, 102:143–163. https://doi.org/10.1007/BF00363806.

Scheltema, R. S. 1963. Larvae in the Open Sea. *Oceanus*, 9(3):3–10.

Scheltema, R. S. 1971. "The Dispersal of the Larvae of Shoal-Water Benthic Invertebrate Species over Long Distances by Ocean Currents." In *Proceedings of the Fourth Marine Biology European Symposium*, ed. D. Crisp, pp. 7–28. Cambridge, UK: Cambridge University Press.

Scheltema, R. S. 1975. "The Frequency of Long-Distance Larval Dispersal Rate of Gene-Flow between Widely Separated Populations of Sipunculans." In *Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura*, ed. M. E. Rice and M. Todorović, pp. 199–210. Belgrade: Naučno Delo Press.

Scheltema, R. S. 1986. Long-Distance Dispersal by Planktonic Larvae of Shoal-Water Benthic Invertebrates among Central Pacific Islands. *Bulletin of Marine Science*, 39(2):241–256.

Scheltema, R. S., and J. R. Hall. 1975. "The Dispersal of Pelagosphera Larvae by Ocean Currents and Its Relationship to Geographical Distribution of Sipunculans." In *Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura*, ed. M. E. Rice and M. Todorović, pp. 103–116. Belgrade: Naučno Delo Press.

Scheltema, R. S., and M. E. Rice. 1990. Occurrence of Teleplanic Pelagosphera Larvae of Sipunculans in Tropical Regions of the Pacific and Indian Oceans. *Bulletin of Marine Science*, 47(1):159–181.

Senna, A. 1906. Sulla Struttura di alcune Larve (Pelagosphaera) di Sipunculidi. *Pubblicazioni del (R.) Instituto di Studio Superiori Practici e di Perfezionamento in Firenze; Sezione de Scienze Fisichee Naturale*, Racc. Planct. 1, Fasc. II, 29:50–78.

Spengel, J. W. 1907. Eine verkannte *Sipunculus*-Larve. *Zoologischer Anzeiger*, 31(4):97–99.

Staton, J., and M. E. Rice. 1999. Genetic Differentiation Despite Teleplanic Larval Dispersal: Allozyme Variation in Sipunculans of the *Apionsoma misakianum* Species Complex. *Bulletin of Marine Science*, 65(2):467–480.

Stephen, A. C. 1941. The Echiuridae, Sipunculidae and Priapulidae Collected by the Ships of the Discovery Committee during the Years 1926 to 1937. *Discovery Reports*, 21:235–260. https://doi.org/10.5962/bhl.part.21627.

Stephen, A. C. 1960. Echiuroidea and Sipunculoidea from Senegal, West Africa. *Bulletin de l'Institut Fondamental d'Afrique Noire*, 22:512–520.

Stephen, A. C., and S. J. Edmonds. 1972. *The Phyla Sipuncula and Echiura*. London: Trustees of the British Museum (Natural History).

Tzetlin, A. B., and G. Purschke. 2006. Fine Structure of the Pharyngeal Apparatus of the Pelagosphera Larva in *Phascolosoma agassizii* (Sipuncula) and Its Phylogenetic Significance. *Zoomorphology*, 125:109–117. https://doi.org/10.1007/s00435-006-0025-x.

Wesenberg-Lund E. 1959. Sipunculoidea and Echiuroidea from Tropical West Africa. *Atlantide Report*, 5:177–210.