BULLETIN OF MARINE SCIENCE, 42(1): 1–15, 1988

OBSERVATIONS ON DEVELOPMENT AND METAMORPHOSIS OF *SIPHONOSOMA CUMANENSE* WITH COMPARATIVE REMARKS ON *SIPUNCULUS NUDUS* (SIPUNCULA, SIPUNCULIDAE)

Mary E. Rice

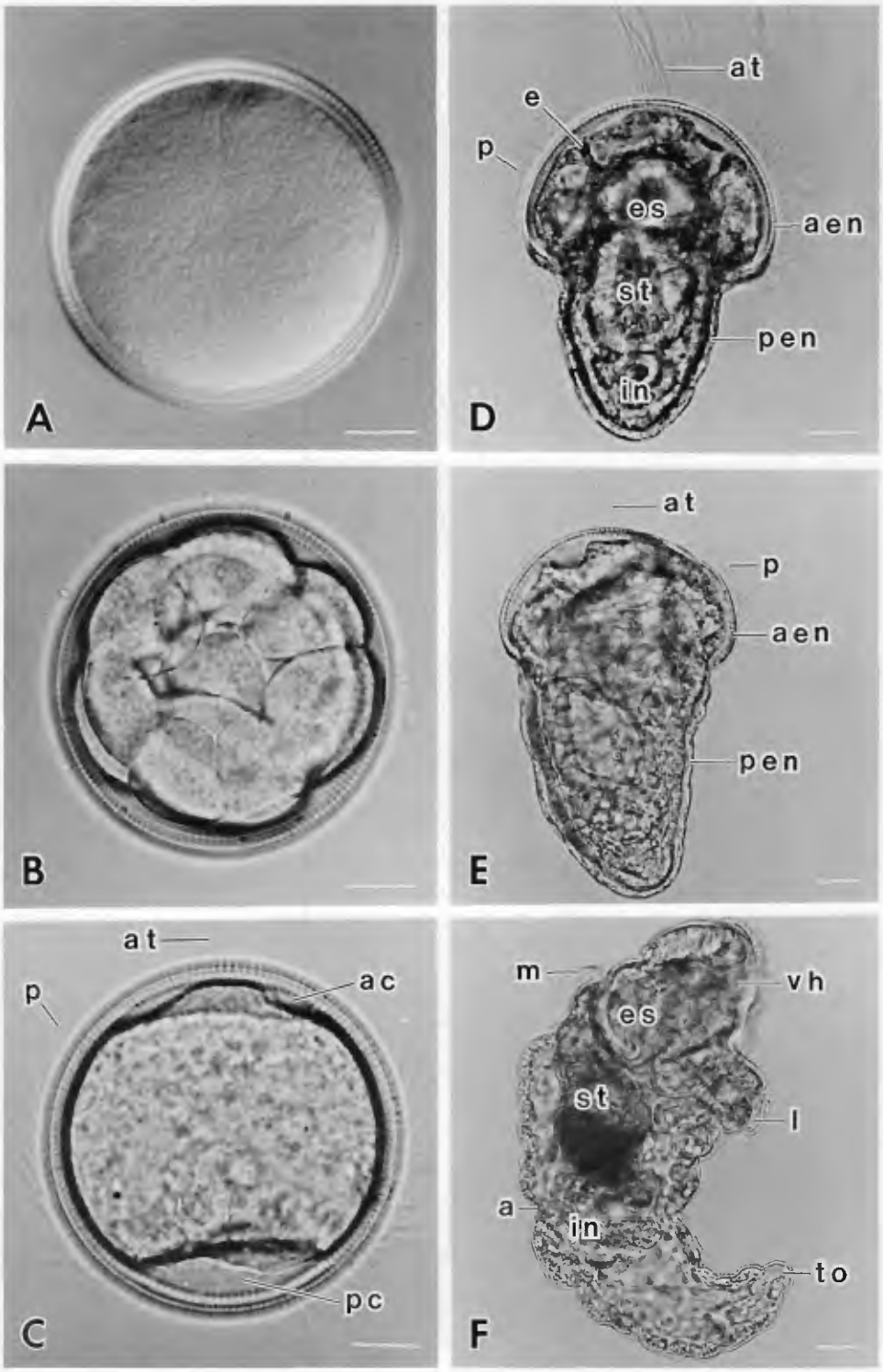
ABSTRACT

The development of *Siphonosoma cumanense* (Sipuncula, Sipunculidae) is described from fertilization through trochophore and planktotrophic pelagosphera larval stages and metamorphosis of the pelagosphera to the juvenile. The planktotrophic pelagosphera larva becomes competent to metamorphose at 8 weeks of age, at which time metamorphosis can be induced by exposure to sediment previously occupied by adults of this species. The resemblance of this pelagosphera larva, reared in the laboratory from known adults, to that of an oceanic larva frequently collected in the Gulf Stream System, confirms the previously tentative identification of the oceanic larva as *Siphonosoma cumanense*. This is the first study in which a sipunculan species known to possess a planktotrophic oceanic larva has been reared in the laboratory from fertilized egg to juvenile. Comparisons of the development of *Siphonosoma cumanense* and *Sipunculus nudus*, both in the family Sipunculidae, show marked differences. Whereas in *S. cumanense* the developmental features are similar to those of species in other families of the Sipuncula which have planktotrophic development, in *S. nudus* the embryo differs in that it is enclosed by ciliated cells which are presumed homologues to the prototroch cells of other species, and, at transformation to the pelagosphera, it casts off these cells as well as the entire overlying egg envelope. It is concluded that the peculiar developmental features of *S. nudus* are not characteristic of the family Sipunculidae and can be considered as highly modified within the phylum.

Previous studies of development in the phylum Sipuncula have concentrated on three of the four families recognized in the classification of Stephen and Edmonds (1972): Golfingiidae, Aspidosiphonidae, and Phascolosomatidae (Gerould, 1906; Akesson, 1958; 1961; Rice, 1967; 1975a; 1978). Knowledge of the development of the fourth family, Sipunculidae, has been limited previously to one study (Hatschek, 1883) on the species *Sipunculus nudus*. The development of this species is markedly different from that of any other in the phylum. Rather than having a distinctive band of prototrochal cilia, the embryo is covered by a "serosa," consisting of ciliated cells, presumably homologous to prototroch cells, and an overlying egg envelope (Gerould, 1903). Unlike other species, at the time of transformation into the pelagosphera larva, the entire egg envelope as well as the ciliated cells are cast off. In the present study, I examined the development of another species of the family Sipunculidae, *Siphonosoma cumanense* (Keferstein, 1867), and re-examined the development of *Sipunculus nudus* Linnaeus, 1766 to determine whether the development of the latter is representative of the family and to assess further the intra- and inter-phyletic relationships of the phylum.

MATERIALS AND METHODS

Collections of *Siphonosoma cumanense* and *Sipunculus nudus* were made in July and September 1983 and in August 1984 at two localities off the southwest coast of Puerto Rico near La Parguera. In these localities specimens occurred in the upper 30 cm of intertidal sediment, consisting of coarse calcareous sand mixed with *Porites* rubble. After collection, the specimens were placed in sediment and, within 1 day, transported by air to the laboratory in Fort Pierce, Florida where they were placed in aerated 15 gallon tanks containing sea water (35 ppt) and sediment, 8 cm in depth, composed of


A
B
C
at
p
ac
pc
D
at
e
p
es
aen
st
pen
in
E
at
p
aen
pen
F
m
vh
es
st
l
a
in
to

quartz sand grains mixed with shell fragments. The maximum density of animals was 21 per tank. The tanks were checked once or twice daily for spawnings over a 6-week period, during which time the temperature of the water varied from 23 to 25°C.

For developmental studies and records of spawning, the tanks were checked daily for the presence of gametes or embryos. Water samples, taken from the surface of the sediment with a plastic baster, were examined and, when eggs were found, the water was siphoned from the tank and passed through a 63 μm sieve. The eggs and embryos retained by the sieve were rinsed into culture dishes for later observation or fixation. Male spawnings were recorded when fertilized eggs were recovered or when microscopic examination of cloudy water masses in the tanks revealed the presence of sperm. Occasionally, actual spawnings of individual males and females were observed.

Developmental stages were cultured in tall covered petri dishes, 100 × 80 mm, having a capacity of 500 ml. Dishes were filled to approximately one-quarter of their capacity with sea water filtered through a 0.2 μm Millipore filter and the water was changed several times weekly during early development and less frequently during later development. Thirty-four cultures of developmental stages of *Siphonosoma cumanense* and six of *Sipunculus nudus* were observed. Success in rearing larvae through metamorphosis to juveniles was realized in only one culture of *Siphonosoma cumanense*. Planktotrophic pelagospheras were fed a mixture of algal and diatom cultures, most commonly consisting of species of *Thalassiosira, Chlorella, Isochrysis,* and *Dunaliella.*

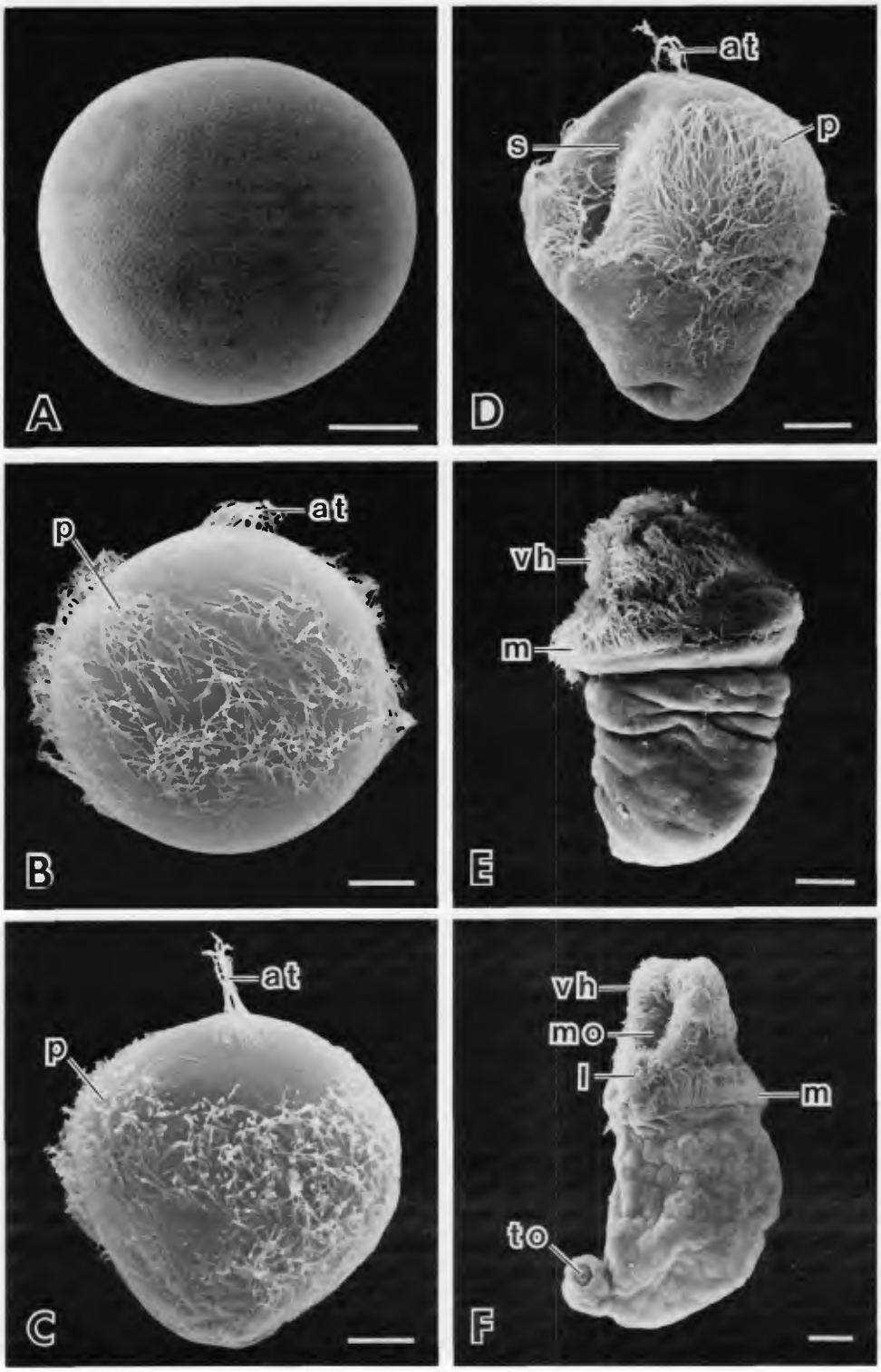
For scanning electron microscopy, material was fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer adjusted to 1,000 milliosmoles by the addition of sodium chloride. Prior to fixation, larvae or juveniles were relaxed in 10% alcohol in sea water for approximately 5 min or until the head remained extended. Specimens were dehydrated in an alcohol and acetone series, dried in a critical point dryer with liquid carbon dioxide, coated in a sputtering unit with gold-palladium, and viewed with a NovaScan microscope.

Observations on *Siphonosoma cumanense*

Spawning.—Eleven individuals, six females and five males, were observed in the laboratory in the act of spawning in 1983 and 1984 during the months of September and October. Females spawned by extending the anterior body from 5 to 20 cm above the sand, exposing the nephridiopores and expelling oocytes simultaneously from both nephridiopores. The forceful ejection at spawning dispersed the eggs throughout the tank. Males extended the anterior end from the burrow to the level of the surface of the sand or 1 to 2 cm above the surface, usually releasing two clouds of sperm, one on either side of the burrow. Release from both nephridiopores was thus indicated in the male, even though the region of the nephridiopores was within the burrow and hidden from view. Typically, but not consistently, tentacles of males were retracted at the time of sperm release, and, following sperm release, they were briefly extended, then again retracted. Whereas in females eggs were released by a single spawning, in males a spawning involved the repeated release of sperm in numerous small bursts over a prolonged period. In the five male spawnings observed, the duration of the spawning period ranged from 10 min to 2 h. During a single spawning period one male was observed to release sperm 55 times over 60 min and another male 43 times during 61.4

←

Figure 1. Light photomicrographs of living developmental stages of *Siphonosoma cumanense*. A. Recently spawned unfertilized egg. Note spindle of first meiotic metaphase and thick egg envelope perforated by fine pores. B. Early cleavage, 2 h and 20 min after fertilization. Sperm are attached to the egg envelope. C. Swimming embryo about one day of age. D. Trochophore, 59 h after fertilization. E. Premetamorphosis stage, transforming from trochophore to pelagosphera, 61 h after fertilization. The post-trochal body has elongated and the anterior egg envelope (aen) is separating from the head region. F. Young pelagosphera, 6 to 7 days of age. Metatroch (m) and terminal attachment organ (to) have formed, the anterior egg envelope has been sloughed off, and the gut is completed and functional. a, anus; ac, anterior cavity; aen, anterior egg envelope; at, apical tuft; e, eye; es, esophagus; in, intestine; l, lower lip; m, metatroch; p, prototroch; pc, posterior cavity; pen, posterior or posttrochal egg envelope; st, stomach; to, terminal organ; vh, ventral head. Scale bar equals 25 μm.


A
at
s
p
D
at
p
B
vh
m
E
at
p
C
vh
mo
l
m
to
F

min. Intervals between sperm releases averaged 1.09 (±0.36) min and 1.43 (±0.56) min, respectively.

Gametes, Maturation, and Fertilization.—The unfertilized egg of *Siphonosoma cumanense* is spherical and clear, having a bluish cast under the direct light of a dissecting microscope. Egg size, as determined by measurements of 50 eggs from each of seven females, averaged 122 μm. The egg envelope, 5 to 6 μm in thickness, is marked by prominent well-spaced pores (Figs. 1A, 2A). The spermatozoan is of the primitive type (Fransen, 1956), having rounded head, basal mitochondrial spheres and elongate tail.

In the recently spawned, unfertilized egg, the metaphase plate of the first meiotic metaphase is located at the animal pole (Fig. 1A). Following sperm penetration, maturation is completed within 22 min when two polar bodies have been formed. Within 40 min male and female pronuclei are joined. The first cleavage is complete within 60 min after sperm penetration.

Development.—Cleavage is spiral, holoblastic, and unequal (Fig. 1B). At the 8-cell stage macromere D is the largest cell and the remaining macromeres and micromeres are all approximately the same size. The 8-cell stage occurs at 1 h and 50 min after fertilization. Within 4 h development has proceeded to the blastula stage, characterized by a few elongate apical cilia and a discrete equatorial band of short prototrochal cilia. For the next 3 h the embryo rotates slowly near the bottom, but by 7 h most embryos are swimming actively throughout the dish. At 11 h they have become positively phototropic, concentrating on the side of the dish nearest the light. By this time the cytoplasm encircling the apical tuft has retracted from the egg envelope forming an apical groove. The posttrochal cytoplasm has also withdrawn from the egg envelope, leaving a space designated as the posterior cavity (Fig. 1C). Thus, only the ciliary cells of the apical tuft and prototrochal band, which has increased considerably in breadth, remain in close contact with the egg envelope, the cilia of these cells projecting through the pores of the envelope (Fig. 2B, C). The ventral stomodaeal invagination is obvious just below the prototroch at 28 h and by 48 h the gut is differentiated into three parts (esophagus, stomach, and intestine), filling the space of the posterior cavity (Fig. 1D). Other characteristic features of this stage, now designated as the trochophore, are a pair of dorsal eyespots in the pretrochal hemisphere, elongation of the posterior end, dorsal expansion of the apical groove, and the presence of cavities to the interior of the prototroch cells. By 58 h the larvae have secreted mucous strands from their posterior ends by which they attach to one another (Fig. 3A). As the trochophore develops, the posterior body continues to elongate, stretching the egg envelope to form the larval cuticle. The posterior egg envelope is distinguished by a loss of porosity and lamellation, whereas the pretrochal envelope

←

Figure 2. Scanning electron micrographs of developmental stages of *Siphonosoma cumanense*. A. Recently spawned, unfertilized egg. B. Embryo, 25 h after fertilization. Note broad band of prototrochal cilia (p) and apical tuft (at). C. Trochophore at 58.5 h. Dorsal view. D. Trochophore at 58.5 h. Ventrolateral view showing stomodaeum (s) and a posterior depression marking the site of the future terminal organ. E. Recently metamorphosed pelagosphera larva, 72 h. Lateral view. Metatroch (m) is present, but terminal organ is not yet well formed. F. Pelagosphera larva, 9 days. Ventrolateral view, showing mouth region (mo), lower ciliated lip (l), and partially retracted terminal attachment organ (to). at, apical tuft; l, lower lip; m, metatroch; mo, mouth region; p, prototroch; s, stomodaeum; to, terminal organ; vh, ventral ciliated head. Scale bar equals 20 μm.

retains both pores and the layered structure (Figs. 1D, E; 4A–C). Metamorphosis of the trochophore to the pelagosphera larva occurs between 60 and 70 h. Metamorphic changes, most of which are completed within a 5-h period, are marked by a rupture of the ventral pretrochal egg envelope and opening of the mouth, followed by a sloughing off of the entire pretrochal egg envelope (Figs. 1D–F, 2D–F, 3B–D). The egg envelope overlying the anus also ruptures, completing the functional gut. The coelomic cavity is expanded, the body wall becomes more transparent, and a small but definitive terminal organ is formed posteriorly. The prototrochal cilia are lost or reduced and a new ciliary band, the metatroch, is formed (Figs. 1F; 2E, F). Behavioral changes include an increased extensibility, retractability of the head into the trunk, a ventral flexing of the body so that mouth and terminal organ make contact, and the protrusion of the muscular buccal organ from the mouth region. For 1 to 2 days after metamorphosis into the pelagosphera, the larvae remain in clumps; then they disperse, swimming independently or forming temporary attachments to the substratum. At this stage the posterior half of the larval stomach is marked by a white pigmentation which, by the time the larva reaches 14 days, has changed to a yellowish green. The 14-day larva feeds on phytoplankton or bottom detritus and produces abundant fecal clumps. The terminal attachment organ is well formed with a pair of prominent glands near the posterior tip and several protruding bristles. Attached larvae feed, as has been described for other species (Rice, 1978) by applying the head to the substratum and ingesting detritus which is moved into the mouth through the activity of the oral cilia or the buccal organ. Another method of feeding, not previously noted, has been observed at 4 weeks when many of the larvae are suspended in the water, drifting in an extended state. Particles of suspended debris and feces have been observed to move, as if tethered to the larvae, directly over distances several times the length of the larvae, into the larval mouth. Because of the directed manner in which they move, it is assumed that the particles are attached to fine mucous threads, not visible with the dissecting microscope, which are emitted from the larvae.

At 6 weeks the larvae have attained an average extended length of 1 mm and width of 0.4 mm (N = 10). The color of trunk, body and metatroch is yellowish. The single pair of small, dorsal eyespots is black and the nephridia, as viewed through the body wall, are green or clear with a green posterior tip. The trunk is marked by numerous transverse grooves. Some of the larvae are swimming, but more commonly they are resting on the bottom, usually unattached.

Settlement and Metamorphosis.—Attempts were made to induce metamorphosis of the pelagosphera at the ages of 18, 45, and 58 days by presenting them with substratum previously occupied by adults of *Siphonosoma cumanense.* At 18 days, 10 larvae, averaging 0.8 mm in length, were placed on substratum previously occupied by adults and, failing to metamorphose, they died within 12 days. At 45 days, when larvae had reached an average extended length of 1.0 mm, 10 specimens were again placed on substratum that had been occupied by adults; they did not metamorphose and within 4 days 6 were dead. At 58 days 6 larvae, ranging in extended length from 1.2 to 1.6 mm, were placed on adult substratum and within 2 days three of the larvae had burrowed and undergone initial metamorphic changes. Morphological changes occurring over a period of 2 to 3 days included loss of metatrochal cilia and lower lip, change of the mouth from a ventral to a terminal position, formation of three pairs of tentacular lobes around the mouth, and the elongation of the body anterior to the postmetatrochal sphincter to become the introvert (Fig. 5A, B).

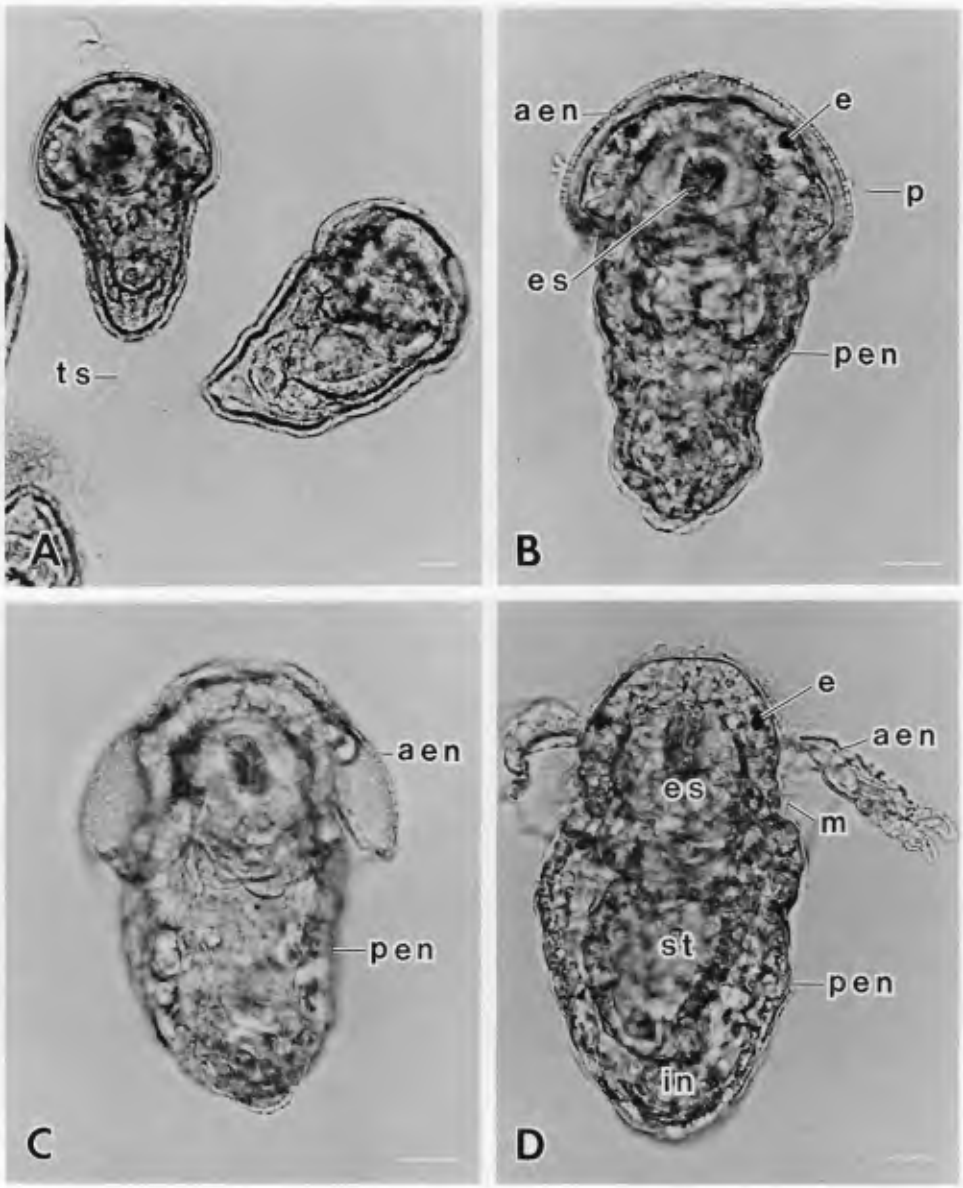
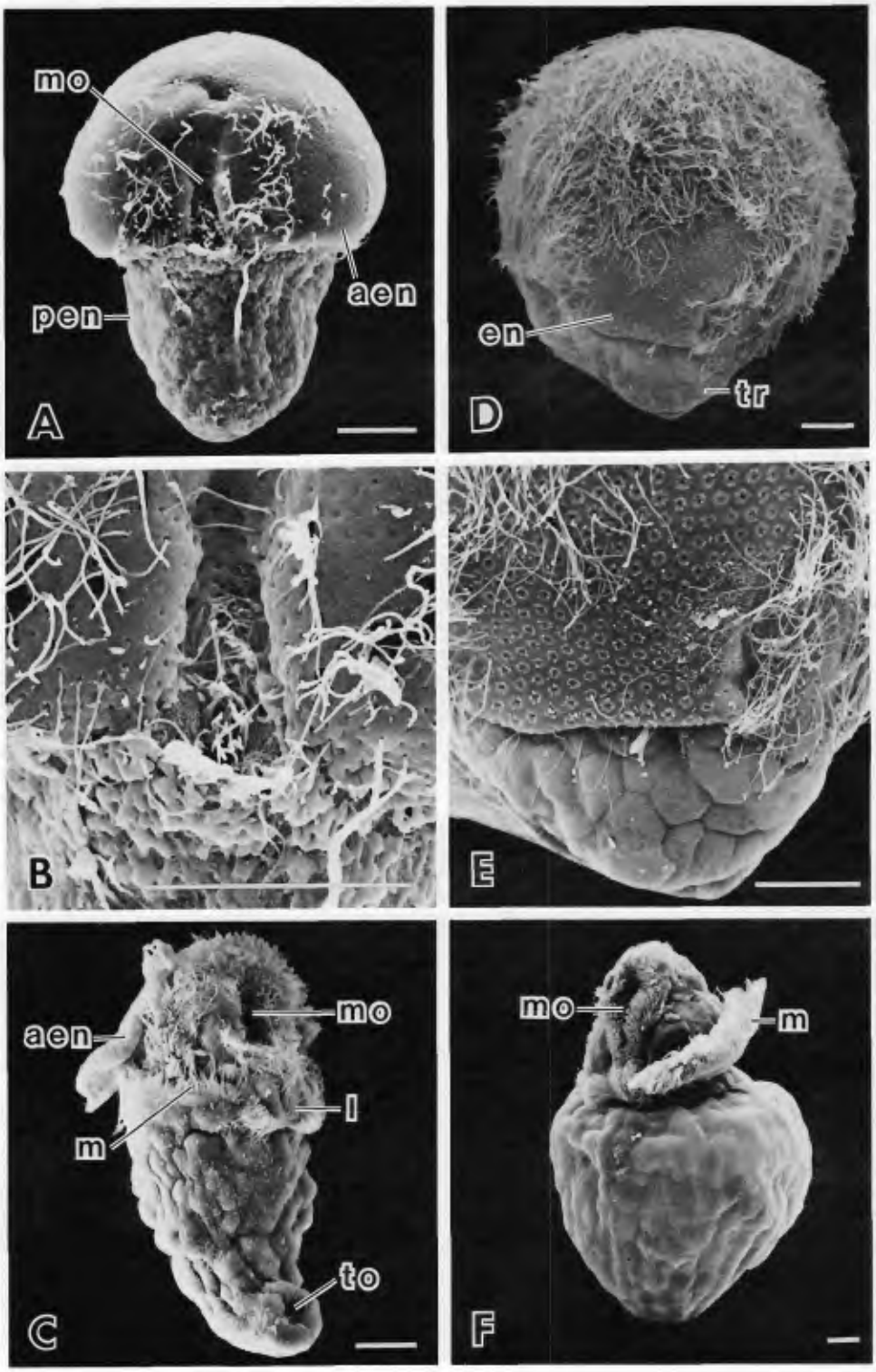


Figure 3. Light photomicrographs of live larvae of *Siphonosoma cumanense*, showing loss of anterior egg envelope during metamorphosis from trochophore to pelagosphera. A. Late trochophore, 59 h. Note terminal attachment strands (ts). B. Premetamorphosis stage, between 2 and 3 days of age. Dorsal view. Anterior egg envelope (aen) is beginning to separate from the larval head. Posterior egg envelope (pen) is stretched to form the posttrochal larval cuticle. C. Metamorphosing larva, between 2 and 3 days of age. Ventral view. Anterior egg envelope (aen) has ruptured below the prototroch and separated further from the larva. D. Metamorphosing larva between 2 and 3 days of age. Anterior egg envelope (aen) in a late stage of detachment. aen, anterior egg envelope; e, eye; es, esophagus; in, intestine; m, metatroch; p, prototroch; pen, posterior egg envelope; st, stomach; ts, terminal attachment strand. Scale bar equals 25 μm.


mo
aen
pen
A
B
C
aen
mo
l
m
to
D
en
tr
E
F
mo
m

OBSERVATIONS ON *SIPUNCULUS NUDUS*

Observations of four female spawnings, two in July 1983 and two in September 1984, revealed that eggs are expelled from the nephridiopores in long strands, which settle in the aquarium as clumps. Eggs are spherical, clear, and 178 μm in diameter (N = 200, 50 eggs measured for each of four females). The germinal vesicle was intact in the recently spawned, unfertilized eggs of the four spawnings noted above, but was observed to break down after 2 to 4 h in sea water. The egg envelope is 5 μm thick, perforated by prominent pores, and covered by a thin jelly coat (Figs. 6A, 7A).

Early developmental stages were collected on six occasions from laboratory tanks containing adults, and reared in culture for 12 days. Neither early cleavage nor fertilization was observed in this species. From observations of the six cultures, it was determined that late cleavages show the typical spiral pattern and at 1 day of age the embryos are positively phototropic and entirely covered by cilia with the apical end distinguished by the more elongate cilia of the apical tuft (Figs. 6B, C; 7B). As in *Siphonosoma cumanense,* apical and posterior cavities are present at this stage, but unlike *Siphonosoma cumanense,* the outer wall of the cavity is bounded by ciliated cells and overlying egg envelope rather than egg envelope alone. As the embryo develops, the cavities are overgrown and disappear, a pair of eyespots is formed and the gut is differentiated. Within the egg envelope, movement of the embryo, as well as the beating of the cilia of the newly formed metatroch, can be detected. At 2 days hatching from the egg envelope and covering of ciliated cells begins and may extend over a period of 7 to 8 h (Figs. 4D–F, 6D–F, 7C–F). The first changes are noted in the posteriormost region of the egg envelope where there is a loss of cilia and a blebing of the envelope (Figs. 4D, E; 6D, E; 7C). These changes are followed by a rupture of the posterior egg envelope and the emergence of the posterior end of the larva. Emergence of the larva from the egg envelope is gradual, and even after the postrochal body is completely free and extended, the egg envelope may remain attached anteriorly for 3 h (Figs. 6E, 7D). A large cavity separates the envelope from the pretrochal body, but the head is attached by cytoplasmic strands within the cavity. When the larva is completely separated from the envelope (about 8 h after the beginning of hatching) there are still large ciliated cells, presumably prototroch cells, adhered to the head in two rows just above the metatroch and a long, rigid tuft of cilia on the anterior head (Fig. 6F). Within 12 h both the adherent cells and the ciliary tuft have been lost (Figs. 4F; 7E, F). The cast-off ciliated cells may be seen swimming independently in the surrounding water either individually or in clumps. The hatched larvae do not attach to the bottom or to each other as most young pelagosphera larvae do,

←

Figure 4. Scanning electron micrographs comparing loss of egg envelope in *Siphonosoma cumanense* and *Sipunculus nudus.* A. Premetamorphosis stage of *S. cumanense,* 2 to 3 days. Ventral view. Note that the porous nature of the anterior egg envelope (aen) remains unchanged, whereas the pores are lost in the transformation and elongation of the posterior egg envelope (pen). B. Higher magnification of A above shows differences in the anterior and posterior envelopes and the loss of the envelope in the region of the mouth at the posterior of the ventral groove. C. Pelagosphera larva of *S. cumanense* at a late stage of detachment of the anterior envelope. Ventrolateral view, 2 to 3 days. A remnant of the anterior egg envelope (aen) remains attached to the dorsal head. D. Beginning hatching in embryo of *S. nudus,* about 50 h of age. The posterior of the developing larva is emerging from the rupture in the egg envelope (en). E. Higher magnification of D above, showing pores of the egg envelope and loss of cilia in the region of the rupture. F. Hatched pelagosphera of *S. nudus,* 9 days of age. Ventrolateral view. aen, anterior egg envelope; en, egg envelope; l, lip; m, metatroch; mo, mouth region; pen, posterior egg envelope; to, terminal organ; tr, posterior trunk. Scale bar equals 20 μm.

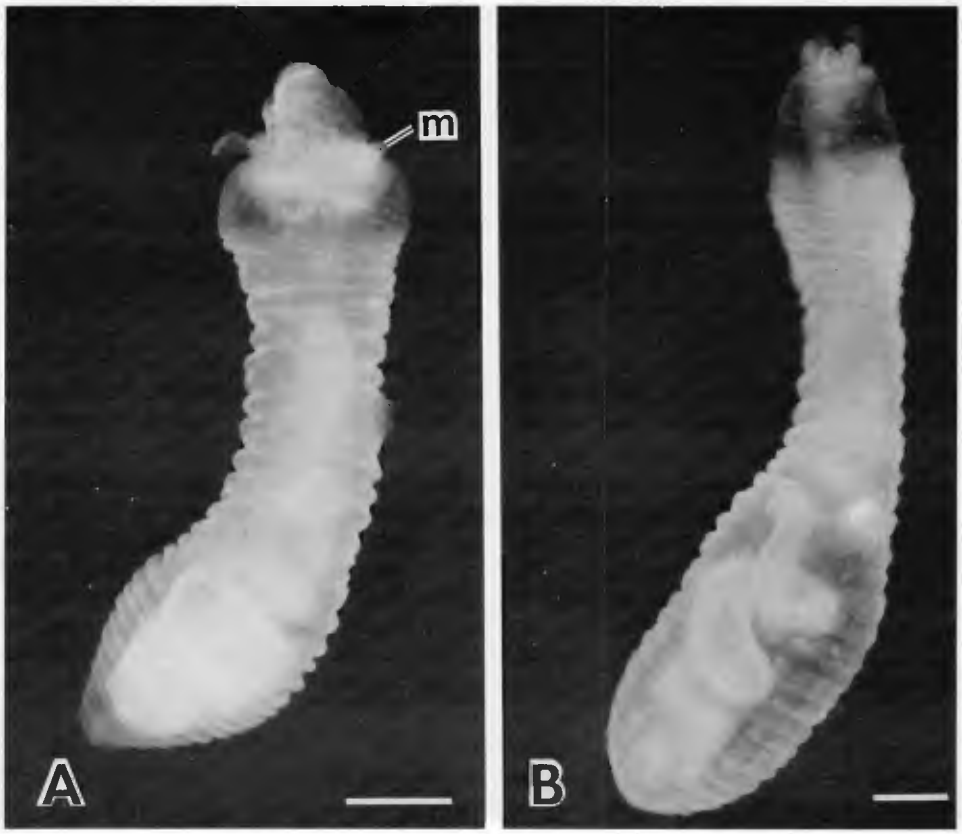
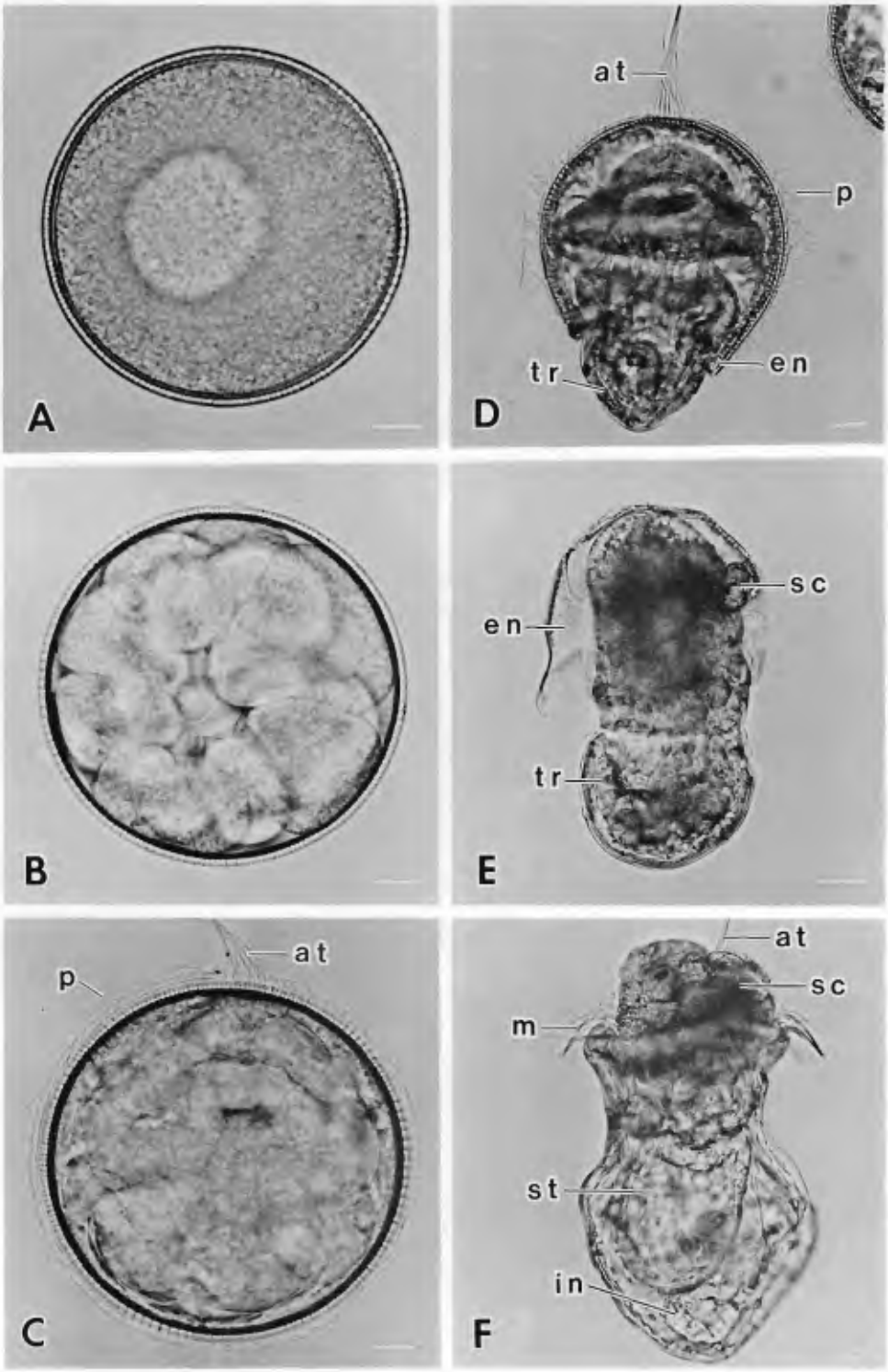


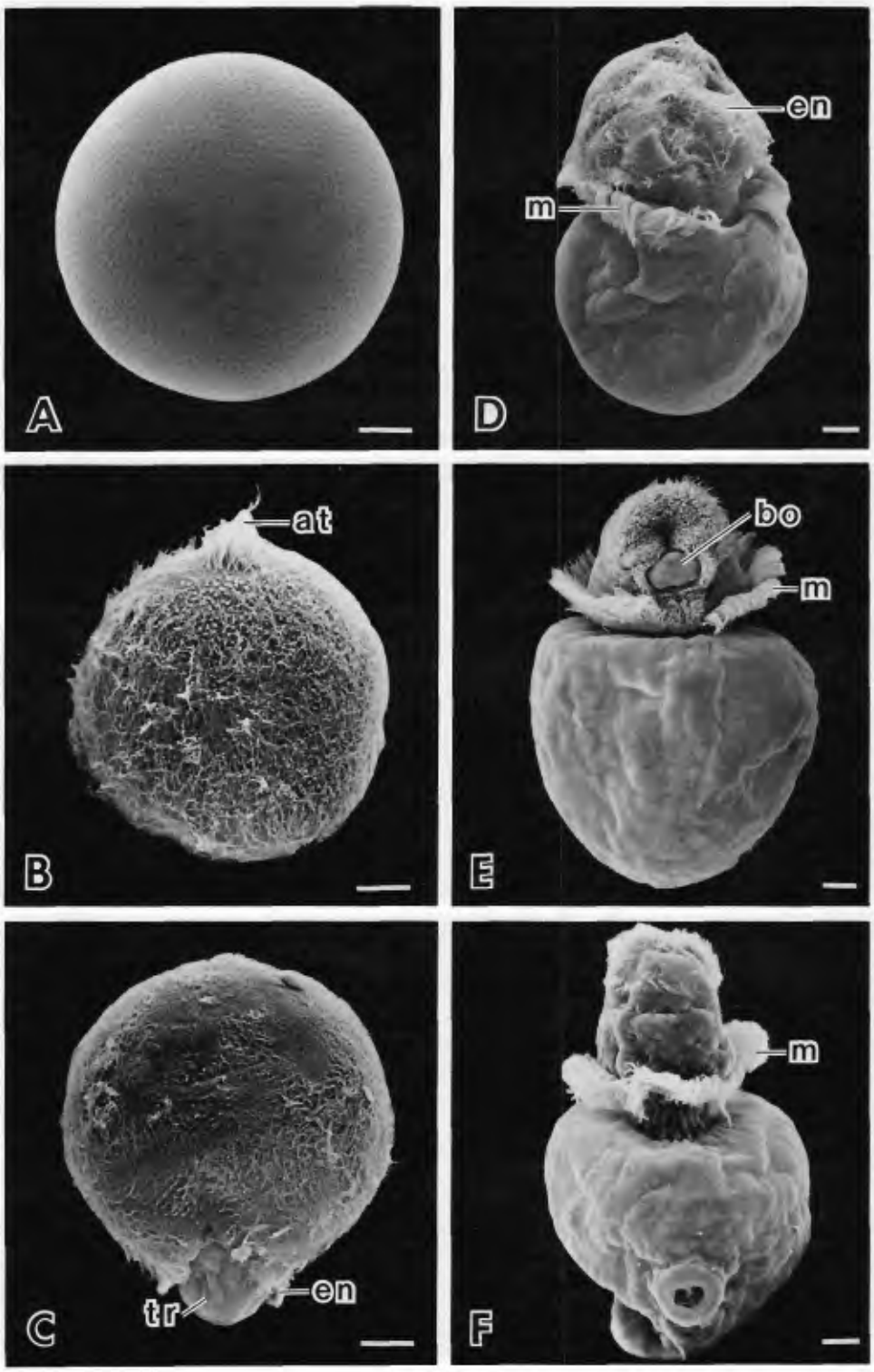
Figure 5. Photomacrographs of living larva and juvenile of *Siphonosoma cumanense,* reared in the laboratory from spawned gametes. A. Larva, 8 weeks old. Lateral view with lower lip projecting from head on left. Metatroch (m) is retracted in this specimen. B. Juvenile, 8 weeks old, 1 day after metamorphosis. Metatrochal cilia are lost and 3 pairs of terminal tentacles have formed. Scale bar equals 200 μm.

although they may occur in loose clumps, swimming in and out of the masses. Even 4 days after hatching they are still positively phototropic. Differing from most other pelagosphera larvae, the larva of *Sipunculus nudus* lacks a terminal attachment organ. However, there is in older larvae a posterior thickening, often retracted, with no apparent adhesive properties.

→

Figure 6. Light photomicrographs of living developmental stages of *Sipunculus nudus.* A. Recently spawned, unfertilized egg with germinal vesicle. B. Mid- to late cleavage stage. C. Completely ciliated embryo, approximately 28 h. D. Hatching embryo, approximately 50 h. The posterior egg envelope has ruptured and the posterior trunk of the developing larva is emerging. E. Late hatching stage. Egg envelope remains attached only at anterior point. Ciliated "serosa" cells (sc) are still adherent beneath the egg envelope (en). F. Recently hatched pelagosphera, approximately 57 h. The envelope is entirely detached, but the ciliated cells of the "serosa" remain adhered to the larval head. at, apical tuft; en, egg envelope; in, intestine; m, metatroch; p, prototroch; sc, "serosa" cells; st, stomach; tr, trunk. Scale bar equals 25 μm.


A
B
C
D
at
p
tr
en
E
sc
en
tr
C
p
at
F
at
sc
m
st
in


A
B
at
C
tr
en
D
en
m
E
bo
m
F
m

Discussion

In previous studies, sipunculan larvae of the open ocean, collected from the surface waters of the Florida Current and reared in the laboratory through metamorphosis to the juvenile, have been tentatively identified as *Siphonosoma cumanense* (Rice, 1976; 1981). This identification was based on characteristics of the gross morphology of juveniles, which were reared up to an age of 4 months. The present study, in which larvae and juveniles were reared from spawnings of known adults of *Siphonosoma cumanense,* provides data for comparisons of laboratory-reared larvae with field-collected oceanic larvae. Such comparisons support the accuracy of the previous identification of oceanic larvae by showing that laboratory-reared larvae of 4 to 6 weeks of age resemble oceanic larvae in form, presence of characteristic transverse grooves over the body, size (1–2 mm), and pigmentation of the nephridia, gut, metatrochal collar and body. Changes at metamorphosis, which include the formation of three tentacle-pairs in the early juvenile, also were found to be the same in both laboratory-reared larvae and those collected from the open ocean. This is the first study in which a species known to possess a planktotrophic, oceanic larva has been reared in the laboratory from fertilized egg to juvenile.

The observations reported in this paper confirm some of the peculiar features of development of *Sipunculus nudus,* particularly the ciliated covering of the embryo and the mode of larval hatching described by Hatschek in 1883, and demonstrate that these features are not found in the development of *Siphonosoma cumanense.* Thus, we can conclude that the unusual developmental features of *S. nudus* are not characteristic of the entire family of large sand-burrowing species, the Sipunculidae, which includes both the genera *Sipunculus* and *Siphonosoma.* The development of *Siphonsoma cumanense,* although unlike *Sipunculus nudus,* was found to be similar to that of other species of sipunculans known to have planktotrophic larval stages in the families Aspidosiphonidae, Golfingiidae, and Phascolosomatidae (Rice, 1967; 1975a; 1978; 1981; 1985) in that there is a typical prototroch, transformation of the egg envelope into the posttrochal larval cuticle, and a functional terminal attachment organ.

A comparison of development of *Siphonosoma cumanense* and *Sipunculus nudus,* based on observations from this study and, in the case of the latter species, also from the literature, shows that the trochophore of *S. cumanense* has a distinctive equatorial band of prototrochal cilia, whereas the embryo and trochophore of *S. nudus* are completely covered by cilia. Gerould (1903), reviewing Hatschek's work, proposed that the ciliated cells of the embryo of *S. nudus,* which are reported to spread anteriorly and posteriorly from the equator to enclose the embryo, are homologous to prototroch cells of other protostomes. As Hatschek (1883) also reported and this study substantiates, the covering of ciliated cells and the entire overlying egg envelope in *S. nudus* are discarded at metamorphosis and all of the

←

Figure 7. Scanning electron micrographs of developmental stages of *Sipunculus nudus.* A. Recently spawned, unfertilized egg. Note pores of the egg envelope. B. Embryo at about 50 h. Completely ciliated with apical tuft (at). C. Beginning hatching, about 50 h. D. Advanced stage of hatching, about 58 h. The posterior larva is free of the egg envelope, but the envelope (en) is still attached to the larval head. E. Pelagosphera larva at 9 days of age. Ventral view showing well developed metatroch (m) and extended buccal organ (bo). F. Pelagosphera larva at 9 days of age. Dorsal view. Retraction of posteriormost trunk is seen as rounded depression. at, apical tuft; bo, buccal organ; en, egg envelope; m, metatroch; tr, trunk. Scale bar equals 20 μm.

larval cuticle is a new formation. In *Siphonosoma cumanense,* on the other hand, only the anterior pretrochal envelope is discarded and the posttrochal envelope is stretched to form the larval cuticle of the elongating larva. A striking difference in the morphology of the pelagosphera larvae of the two species is found in the well developed terminal attachment organ of *S. cumanense.* Another difference is in the nuclear state of the egg of the two species at the time of spawning. The egg of *S. cumanense,* like that of all sipunculans for which information is known, is arrested in the first meiotic metaphase at spawning (Rice, 1975b). However, in the four spawnings of *S. nudus* in which unfertilized eggs were observed, the eggs possessed an intact germinal vesicle. Because of a lack of simultaneous spawnings of sperm, it was not possible to determine whether these eggs were fertilizable and whether these spawnings could be assumed to be normal.

Information is now available, including that presented in this paper, on the development of 21 species of sipunculans, 2 of which, *Sipunculus nudus* and *Siphonosoma cumanense,* belong to the family Sipunculidae (Rice, 1985). The fact that the development of *Sipunculus nudus* is strikingly different from all other species, including the species *Siphonosoma cumanense* in the same family, is evidence that this developmental pattern is highly modified within the phylum. The development of *Siphonosoma cumanense* is closer to the proposed primitive pattern for the phylum, as discussed by Rice (1985), and, with its more typical band of prototrochal cilia, it has a greater similarity to that of other protostomous invertebrates.

Acknowledgments

This work was made possible through the exceptionally fine technical support provided by J. Piraino and H. Reichardt, research assistants at the Smithsonian Marine Station at Link Port. Their expert assistance in every aspect of this study is acknowledged with sincere gratitude. The help given by C. Cutress, University of Puerto Rico at Mayaguez, in obtaining the animals, is very much appreciated. This is contribution number 193 of the Smithsonian Marine Station at Link Port.

Literature Cited

Akesson, B. 1958. A study of the nervous system of the Sipunculoideae, with some remarks on the development of the two species *Phascolion strombi* (Montagu) and *Golfingia minuta* (Keferstein). Undersökningnar över Öresund. 38: 1–149.

———. 1961. The development of *Golfingia elongata* Keferstein (Sipunculidae), with some remarks on the development of the neurosecretory cells in sipunculids. Ark. Zool. 13: 511–531.

Fransen, A. 1956. On spermiogenesis, morphology of the spermatozoan, and biology of fertilization among invertebrates. Zool. Bidr. Uppsala 31: 355–482.

Gerould, J. H. 1903. Studies on the embryology of the Sipunculidae, I. The embryonal envelope and its homologue. Mark Anniversary Volume, pp. 439–452. Henry Holt and Co., New York.

———. 1906. Studies on the embryology of the Sipunculidae, II. The development of *Phascolosoma.* Zool. Jahb. Abt. Anat. Ontog. Thiere 23: 77–162.

Hatschek, B. 1883. Ueber Entwicklung von *Sipunculus nudus.* Arb. Zool. Inst. Univ. Wien Zool. Stat. Triest 5: 61–140.

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.

———. 1975a. Observations on the development of six species of Caribbean Sipuncula with a review of development in the phylum. Pages 141–160 *in* M. E. Rice and M. Todorovic, eds. Proceedings of the international symposium on the biology of the Sipuncula and Echiura. Vol. I. Naucno Delo, Belgrade.

———. 1975b. Sipuncula. Pages 67–127 *in* A. Giese and J. Pearse, eds. Reproduction of marine invertebrates. Volume II, Chapter 4. Academic Press, New York.

———. 1976. Larval development and metamorphosis in Sipuncula. Amer. Zool. 16: 563–571.

———. 1978. Morphological and behavioral changes at metamorphosis in the Sipuncula. Pages 83–

102 *in* F. S. Chia and M. E. Rice, eds. Settlement and metamorphosis of marine invertebrate larvae. Elsevier North-Holland Biomedical Press, New York.

———. 1981. Larvae adrift: patterns and problems in life histories of sipunculans. Amer. Zool. 21: 605–619.

———. 1985. Sipuncula: developmental evidence for phylogenetic inference. Pages 274–296 *in* S. Conway Morris, J. D. George, R. Gibson, and H. M. Platt, eds. The origins and relationships of lower invertebrates. Clarendon Press, Oxford.

Stephen, A. C. and S. J. Edmonds. 1972. The Phyla Sipuncula and Echiura. The British Museum (Natural History), London. 527 pp.

DATE ACCEPTED: February 16, 1987.

ADDRESS: *Smithsonian Marine Station at Link Port, 5612 Old Dixie Highway, Fort Pierce, Florida 34946.*