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Larval Development and Metamorphosis in Sipuncula

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SYNOPSIS. In a brief review of development of the phylum Sipuncula, four patterns of development are recognized: (1) direct with no pelagic stage; (2) one larval stage, a lecithotrophic trochophore; (3) two larval stages, a lecithotrophic trochophore and a lecithotrophic pelagosphera; (4) two larval stages, a lecithotrophic trochophore and a planktotrophic pelagosphera. Larval types and their metamorphoses are described, with special attention to the development and morphology of the larval cuticle. In the majority of species studied, the egg envelope is transformed into the larval cuticle at metamorphosis of the trochophore. The cuticle of many planktotrophic pelagosphera larvae is characterized by surface papillae of diverse form and pattern. The underlying cuticle in some species is composed of layers of fibers at right angles to one another.

INTRODUCTION

The first description of spiral cleavage in the Sipuncula appeared in a publication by Gerould (1907) on cell lineage and larval development in *Phascolopsis gouldi* and *Golfingia vulgaris.* In earlier studies Selenka (1875) gave an abbreviated account of the development of *Golfingia elongata,* and Hatschek (1883) reported in detail the embryogenesis and organogenesis of *Sipunculus nudus.* Although these earlier reports on sipunculans did not include studies of early cleavage, the authors recognized certain developmental similarities to annelids, echiurans and molluscs, and thus established the basis for the consideration of sipunculans as members of the Spiralia.

Following the reports of Gerould (1903, 1907), there were no studies on sipunculan development until 1958 when Åkesson published a treatise on the nervous system of sipunculans in which he considered the development of two species, *Phascolion strombi* and *Golfingia minuta.* Later he repeated Selenka's observations on *Golfingia elongata* and investigated the development of neurosecretory cells in this species (Åkesson, 1961*a*). More recently, development of additional species has been described, bringing the total number of species studied to 18 (Rice, 1967, 1973, 1975*a,b*).

In this paper a brief resume will be presented of current knowledge on larval development and metamorphosis in the Sipuncula, including some previously unpublished information on morphology, cuticle structure and metamorphosis of open-ocean planktotrophic larvae. For more detailed accounts of development and complete bibliographies, the reader is referred to several recent reviews (Hall and Scheltema, 1975; Rice, 1967, 1975*a,b*).

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DEVELOPMENTAL PATTERNS

Four patterns of development are now recognized in the Sipuncula (Table 1). Three species develop directly with no pelagic larval stage. Two species exhibit a short lecithotrophic trochophore stage which gradually transforms into a vermiform stage, then into the juvenile form. In a third developmental pattern, characteristic of four species, there are two larval stages, a lecithotrophic trochophore and a lecithotrophic pelagosphera. One species, *Themiste lageniformis,* listed in category III in Table 1 does not completely fit the definition in that it lacks a swimming trochophore stage, developing directly into a lecithotrophic pelagosphera (Williams, 1972).

TABLE 1. *Patterns of development in Sipuncula.*

Direct Development

I. Egg → Vermiform Stage → Juvenile

Golfingia minuta[a]
Phascolion cryptus[f]
Themiste pyroides[e]

Indirect Development

II. Egg →Trochophore → Vermiform Stage → Juvenile

Phascolion strombi[a]
Phascolopsis gouldi[c]

III. Egg → Trochophore → Lecithotrophic Pelagosphera → Vermiform Stage → Juvenile

Golfingia elongata[h, b]
Golfingia pugettensis[e]
Golfingia vulgaris[c]
Themiste alutacea[f]
Themiste lageniformis[i, g, j]

IV. Egg → Trochophore → Planktotrophic Pelagosphera → Juvenile

Aspidosiphon parvulus[g]
Golfingia pellucida[g]
Paraspidosiphon fischeri[f]
Phascolosoma agassizii[e]
Phascolosoma antillarum[f]
Phascolosoma perlucens[f]
Phascolosoma varians[f]
Sipunculus nudus[d]

[a] Åkesson, 1958.
[b] Åkesson, 1961*a*.
[c] Gerould, 1907.
[d] Hatschek, 1883.
[e] Rice, 1967.
[f] Rice, 1975*b*.
[g] Rice, Unpublished.
[h] Selenka, 1875.
[i] Williams, 1972.
[j] Develops directly from egg to lecithotrophic pelagosphera, without a trochophore stage.

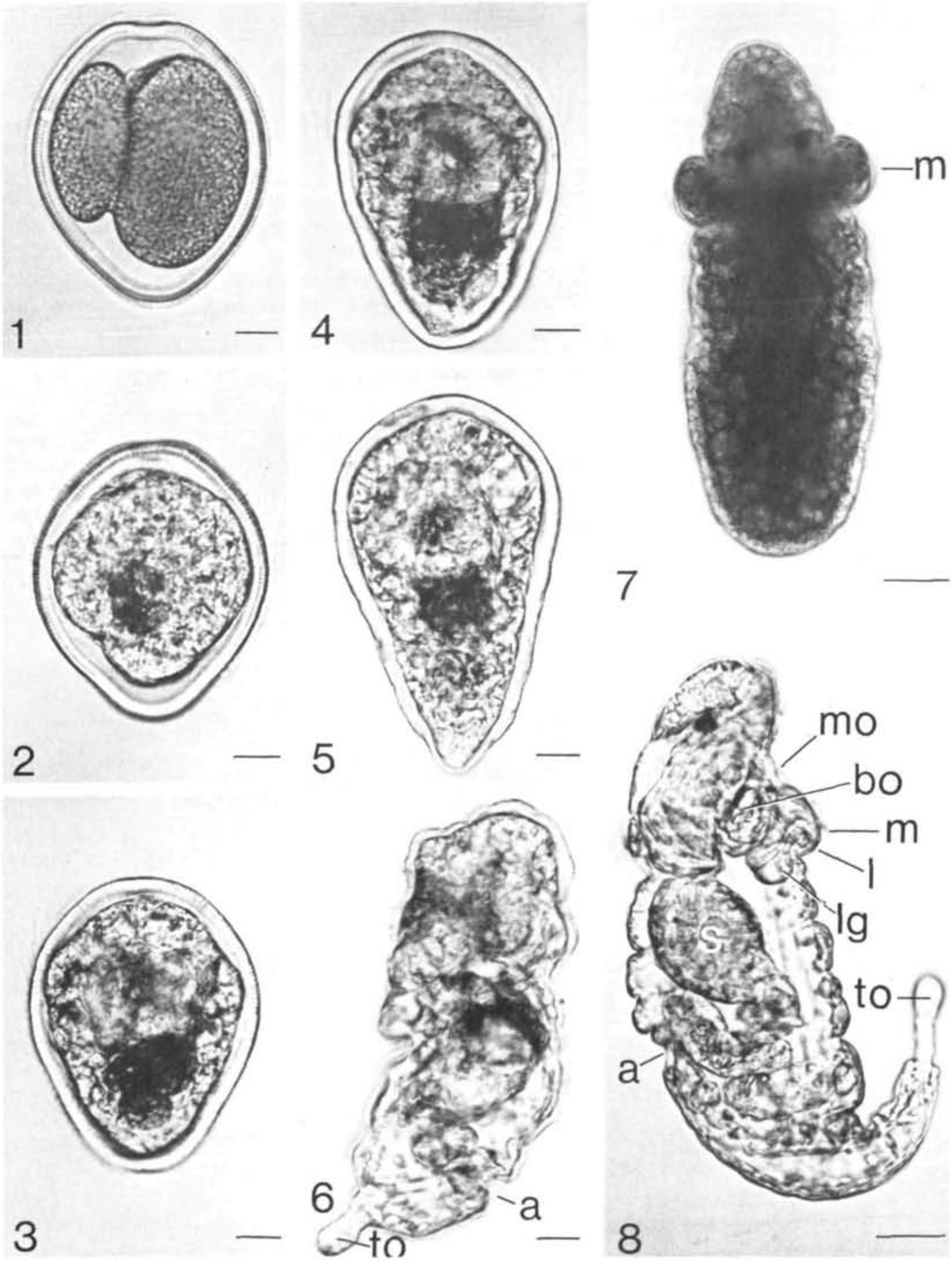
The majority of species fall into a fourth developmental classification similar to the third with two pelagic stages, but differing by having a planktotrophic pelagosphera larva which often remains in the plankton for several months. Numerous reports of planktotrophic pelagospheras of unknown species from oceanic plankton are found in the literature (see reviews of Hall and Scheltema, 1975; Rice 1975*a*).

A close interrelationship is apparent between the yolk content of an egg and its ensuing developmental pattern. Species exhibiting the first three developmental patterns have eggs relatively higher in yolk content than those species in the fourth developmental category, as judged by opacity in the living egg and concentration of yolk granules in sectioned material. All species in the first three categories pass through a vermiform stage, *i.e.,* a crawling lecithotrophic form, which undergoes a gradual transformation into a feeding juvenile. The only clearly metamorphic change in the first three categories is from the lecithotrophic trochophore to the lecithotrophic pelagosphera. The latter larval form swims for a relatively short time before undergoing a gradual transformation into the vermiform stage. In the fourth developmental category the vermiform stage is absent and there are two distinct metamorphoses, one from the trochophore to the planktotrophic pelagosphera and the other from the pelagosphera to the juvenile. It has not been possible thus far to rear larvae in category 4 in the laboratory from the egg through two metamorphoses. In most studies of development, observations have been made through metamorphosis of the trochophore and resulting pelagospheras have been maintained in some instances for several months in culture without undergoing a second metamorphosis (Rice, 1967). Metamorphosis from the pelagosphera to the juvenile has been studied only in oceanic pelagospheras collected in plankton samples (Hall and Scheltema, 1975).

The term pelagosphera was first used by Mingazinni (1905) to designate what he believed to be a new genus and species of Sipunculan, *"Pelagosphaera aloysii".* This was later shown to be a larval rather than adult form; however, the name pelagosphera persisted in the literature in reference to certain oceanic sipunculan larvae. In light of recent studies it has been redefined as a larval stage unique to the Sipuncula which succeeds the trochophore and is distinguished by a prominent metatrochal ciliary band and a loss or reduction of the prototroch (Rice, 1967).

CLEAVAGE

Cleavage in all sipunculan eggs is spiral, holoblastic, and unequal (Fig. 1). In species



FIGS. 1-6. Developmental stages of *Phascolosoma varians.* Scale, 25 μm.
1. Two-cell stage. 2-4. Trochophores. Note developing gut, prototroch, and apical tuft. 5. Beginning metamorphosis. Four days. 6. Recently metamorphosed. Planktotrophic pelagosphera, 5 days.

FIG. 7. Lecithotrophic pelagosphera of *Themiste alutacea* 2 days. Dorsal view. Head partially retracted. Scale, 25 μm.

FIG. 8. Planktotrophic pelagosphera of *Golfingia pellucida,* 7 days. Lateral view. Scale, 25 μm.
a, anus; bo, buccal organ; l, lower lip; lg, lip gland; m, metatroch; mo, mouth; s, stomach; to, terminal organ

with lecithotrophic development and yolk-rich eggs, the micromeres in the A, B, and C quadrants at the 8-cell stage may be larger than the macromeres. The greater size of the micromeres is reflected in the exceptionally large prototroch cells which serve as a source of nutrition to the developing larva by releasing yolk granules into the coelom usually at the time of metamorphosis of the trochophore.

The apical plate at the 48-cell stage in *Golfingia vulgaris*, as reported by Gerould (1907), consists of rosette cells ($1q^{111}$), cross cells ($lq^{121,122}$), and intermediate cells (lq^{112}). The cross cells are in the frontal and sagittal planes, the radial position of the molluscan cross. There are 19 cells in the prototrochal band of *G. vulgaris;* 16 are derived from the trochoblasts (lq^2) and 3 are secondary, later additions probably from divisions of intermediate cells. In the posterior hemisphere the descendants of the 2d cells give rise to the somatic plate and most of the ectoderm of the trunk. Micromeres of the third quartette also form ectoderm and the macromeres 3A–3D give rise to both endoderm and mesoderm. Mesoderm of the trochophore is derived from descendants of the 4d cell formed by a laeotropic division of the 3D cell at the 64-cell stage.

TROCHOPHORE LARVA

The trochophore larva of sipunculans is lecithotrophic, enclosed by the thick envelope of the egg (Figs. 2, 3). It is characterized by an apical tuft and an equatorial band of prototrochal cilia. A pair of red eyespots is present in a dorsolateral position in the pretrochal hemisphere and in the posttrochal hemisphere the ventral stomodaeum occurs just below the prototroch. Differing from many polychaetes and some molluscs, the sipunculan trochophore does not possess a protonephridium. The trochophore of one species, *Sipunculus nudus*, is unique in that it is entirely enclosed by a layer of ciliated cells lying just beneath the egg envelope (Hatschek, 1883). Gerould (1903) has interpreted these cells to be homologous to the prototroch of other species.

Metamorphosis of the trochophore may result in either a lecithotrophic or a planktotrophic larva (Figs. 4-6, 7-9). In both cases metamorphic alterations consist of elongation of the posttrochal body, formation or expansion of the coelom, reduction in the prototroch and formation or enlargement of the metatroch as the primary locomotory organ. Usually a terminal attachment organ is formed at the posterior extremity. At the time of metamorphosis the egg envelope is ruptured in the region of the stomodaeum, the outer portion of which is everted to form the ventral ciliated surface of the head. Part of the prototroch immediately above the stomodaeum is lost and the remainder is usually retained to form a horseshoe-shaped band on the dorsal head. Retractor muscles become functional at metamorphosis and the anterior body, including head and metatroch, is retractable into the posterior trunk.

PELAGOSPHERA LARVA

In the lecithotrophic pelagosphera (Fig. 7), the lumen of the gut is not complete, the anus not open and, even though the mouth may be formed, the gut is not functional. Larvae, as observed in the laboratory, may swim through the water, or they may remain quiescent on the bottom or attached to the substratum. Attachment is accomplished by the terminal organ, or in the absence of that organ, as in *Themiste alutacea* (Fig. 7), the larva attaches presumably through an adhesive secretion from glands, not yet described, in the posterior body. After a pelagic or benthopelagic period of a few days to two weeks, the larva loses its metatrochal cilia and gradually transforms into the vermiform stage, finally assuming the form of the juvenile.

At the time of metamorphosis of the trochophore to the planktotrophic pelagosphera, the mouth and anus are opened with the rupture of the overlying egg envelope, and the gut, with lumen completed in initial metamorphic stages, is then fully developed (Figs. 8, 9). A ventral ciliated groove extends from the anterior end of the head to the mouth, resulting in a bilobate ventral head. Ventral to the mouth

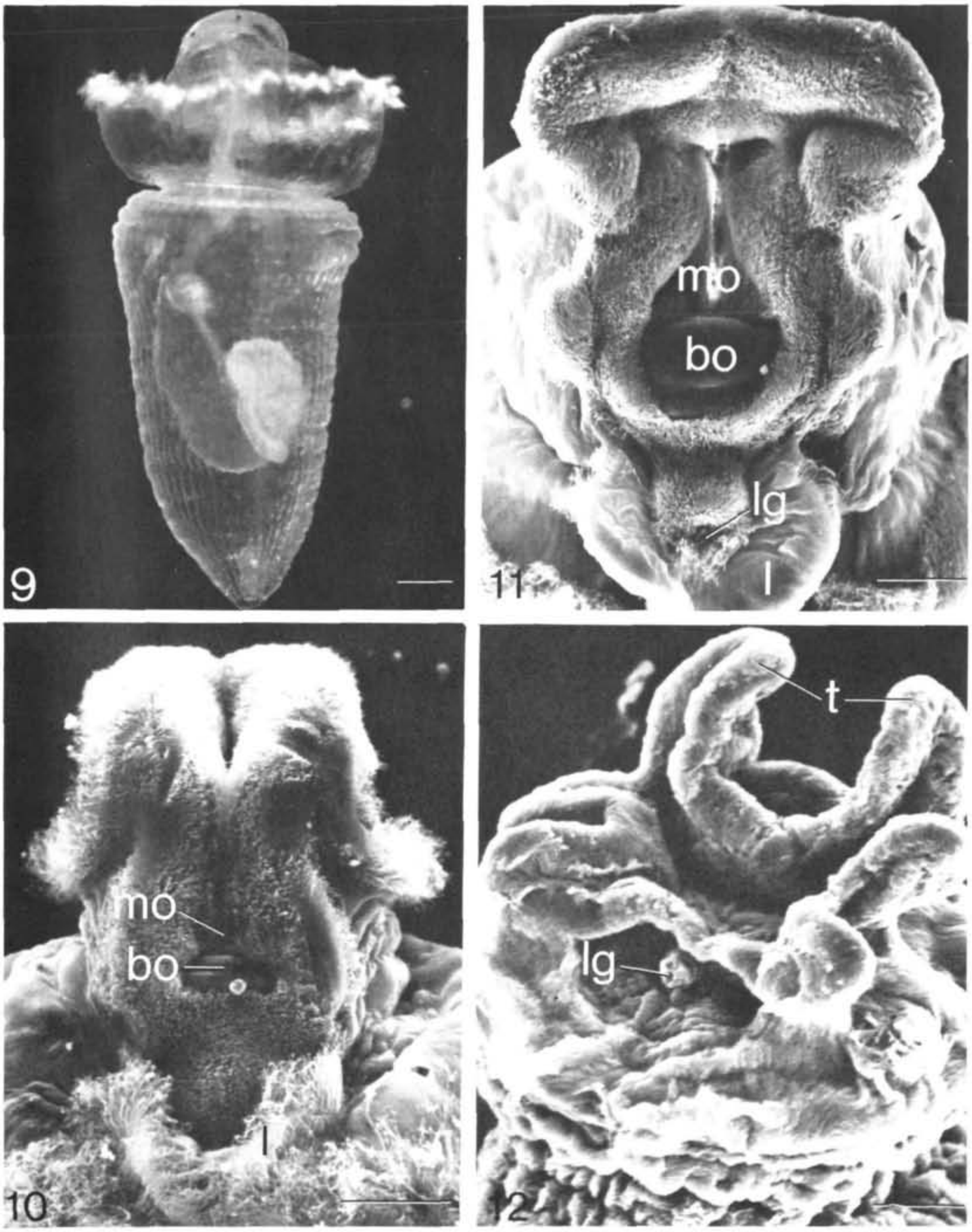


FIG. 9. Planktotrophic pelagosphera larva from open-ocean plankton. *Sipunculus sp.* Live specimen. Note extended head and expanded metatroch. Scale, 3.0 mm.

FIG. 10. Scanning electron micrograph of ventral head of planktotrophic pelagosphera from open-ocean plankton. *Aspidosiphon sp.* Scale, 50 μm.

FIG. 11. Scanning electron micrograph of ventral head of planktotrophic pelagosphera from open-ocean plankton, tentatively identified as belonging to the genus *Siphonosoma.* Scale, 50 μm.

FIG. 12. Scanning electron micrograph of head of recently metamorphosed larva of same type as in Figure 8. Tentacles have developed from the ciliated larval lobes surrounding the mouth region. Scale, 50 μm.
bo, buccal organ; l, lip; lg, pore of lip gland; mo, mouth; t, tentacles.

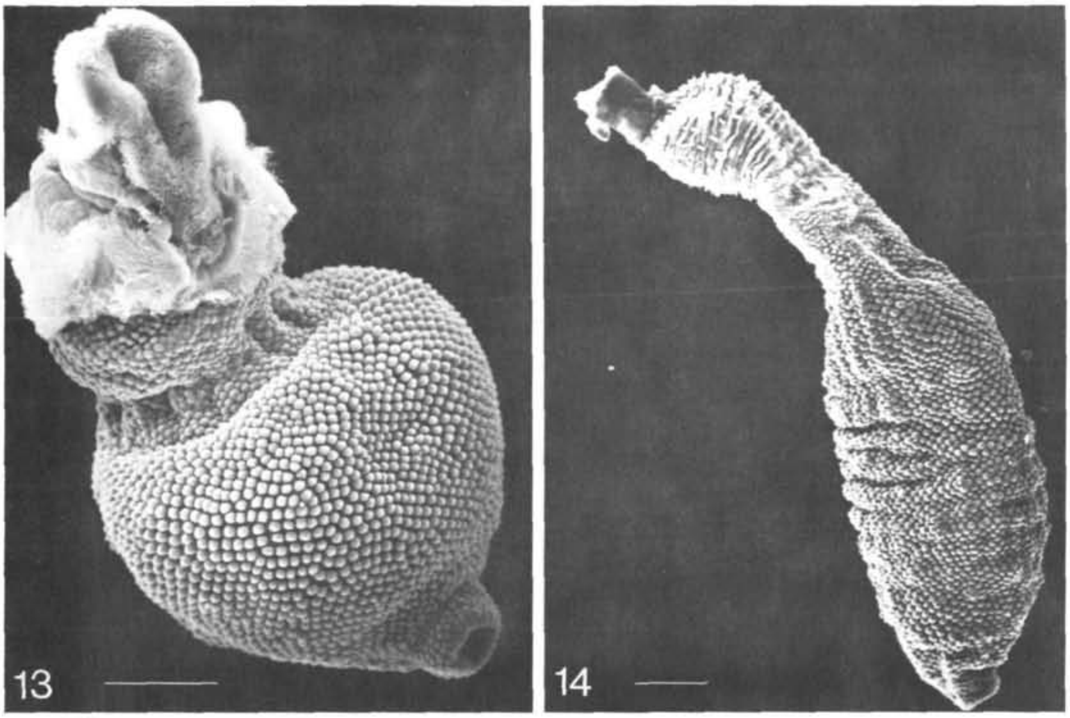
is a protruding lower lip which is usually extended at right angles to the head (Figs. 8, 10, 11). Two organs associated with the mouth are the buccal organ and the lip glands (Figs. 8, 11). The lip glands consist of 2 to 4 pendulous lobes, suspended within the coelom by a duct opening to the surface of the distal lip through a pore. A ciliated groove between the pore and the mouth bifurcates the ventral lip. Secretion of the lip gland has not been characterized. The buccal organ, a protrusible muscular structure located on the floor of the mouth, has been implicated in swallowing of food as well as in rejection of unwanted particles and in loosening of food material from the substratum prior to ingestion (Jägersten, 1963; Rice, 1973). A well-defined, bulbous stomach is unique to the planktotrophic pelagosphera. The intestine is looped, descending posteriorly from the stomach, then anteriorly to the dorsal anus. In some larvae a pair of sacciform glands of unknown function opens on either side of the anus. There is a pair of pigmented nephridia with openings to the exterior by way of ventrolateral nephridiopores and to the coelom through ciliated coelomic funnels. A single unpaired ventral nerve cord divides in the region of the lip glands to form the circumesophageal connectives which continue dorsally to unite with the supraesophageal ganglion. Retractor muscles, usually four in number, extend from the head region to the body wall of the trunk. A metatrochal collar, when fully expanded as in swimming, is the widest part of the larval body; posteriorly it is bounded by a postmetatrochal sphincter (Fig. 9). A retractable terminal organ, usually serving for temporary attachment to the substratum, is present in most larvae. Planktotrophic pelagosphera larvae have been reared in the laboratory as long as 7 months at which time they have attained the size and form of oceanic larvae. Metamorphosis, however, has not been observed in laboratory-reared planktotrophic larvae, but only in those collected from open-ocean plankton (Fig. 12).

Of the numerous reports on oceanic larvae, only two have attempted to assign specific designations (See literature reviews by Hall and Scheltema, 1975; Rice, 1975*a*). Fisher (1947), using number of muscle bands as a taxonomic character, identified a large, transparent pelagosphera from the Gulf Stream off Cape Hatteras, North Carolina as *Sipunculus polymyotus*. Later Murina (1965) identified two pelagospheras, one from the Gulf of Aden as *Sipunculus aequabilis* and one from the Northwest Pacific as *S. norvegicus*. In a comparative study of the morphology of open-ocean pelagospheras, Hall and Scheltema (1975) described 10 larvae, but did not make specific identifications. Characters by which larval forms may be distinguished are size, shape, behavioral patterns, pigmentation patterns, number of eyespots, arrangement of body wall musculature, number of retractor muscles, and the presence and form of cuticular papillae. For specific identification larvae must usually be reared to the post-juvenile stage.

FORMATION AND MORPHOLOGY OF LARVAL CUTICLE

Oceanic pelagosphera larvae have been grouped by Jägersten (1963) as "rough" or "smooth" depending on the presence or absence of cuticular elevations (Figs. 9, 13, 14). In "rough" larvae cuticular papillae are found in a variety of forms and patterns, each characteristic of a specific larval type (Figs. 13, 15, 16). The form may be dome-shaped and smooth or mammiform with apical nipples, or papillae may consist of two or more tiers with flattened, rounded or tapered apices, with either smooth or rugose surfaces.

In laboratory studies of sipunculan development it has been found that in 15 of a total of 18 species the cuticle of the larva or vermiform stage is formed all, or at least in part, from transformation of the egg envelope. Two of the exceptions are *Golfingia vulgaris* and *Phascolopsis gouldi*, reported to shed the egg envelope at the time of metamorphosis of the trochophore (Gerould, 1907). In *Phascolion cryptus*, a species which develops directly, the pretrochal egg envelope is lost as the embryo transforms into the vermiform stage while the posttrochal envelope is retained as the cuticle (Rice, 1975*b*). With the exception of

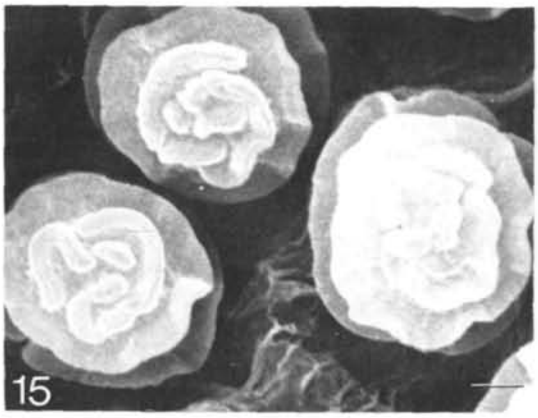


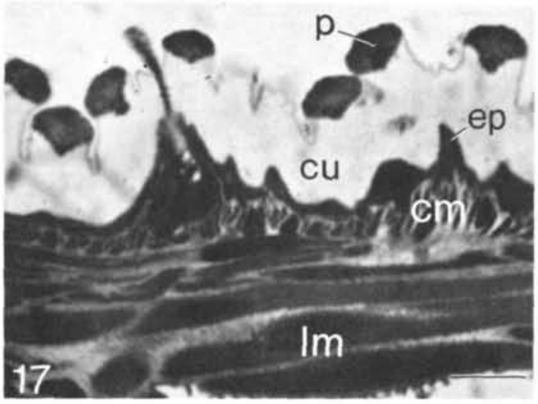
FIGS. 13-14. Scanning electron micrographs of planktotrophic pelagosphera larva and recently metamorphosed juvenile of *Aspidosiphon sp.* From open-ocean plankton. Metamorphosis occurred in the laboratory. Scale, 100 μm.

Sipunculus nudus, all planktotrophic pelagospheras retain the egg envelope as the larval cuticle. *Sipunculus nudus* is unique in that the trochoblast cells spread anteriorly and posteriorly on the inside of the egg envelope to surround the embryo with a ciliated covering or "serosa" (Hatschek, 1883; Gerould, 1903). At trochophoral metamorphosis the entire serosa, cells and egg envelope, is cast off.

The egg envelope of all sipunculans is comprised of two or more layers and perforated by pores. When retained at metamorphosis of the trochophore, it undergoes a posttrochal elongation, losing its lamellation and porosity; from the thick rigid covering, with the characteristic shape of the egg, it is transformed into the exceedingly extensible and flexible cuticle of the early pelagosphera (Figs. 1-6). In species of *Phascolosoma* studied in early development from laboratory spawnings, the cuticle in larvae of 3 to 4 weeks of age develops surface elevations in the form of papillae similar to those of later pelagosphera larvae of the open-ocean (Rice, 1973).

In sectioned material papillae appear different in composition from the underlying cuticle, as indicated by differences in staining and microstructure (Fig. 17). The underlying cuticle of two oceanic larvae identified by rearing through metamorphosis as species of *Aspidosiphon* and *Paraspidosiphon,* when examined by transmission and scanning electron microscopy revealed an arrangement of fibers in layers perpendicular to one another (Fig. 18). A similar arrangement has been reported for the cuticle of the adult sipunculan, *Phascolion strombi* (Moritz and Storch, 1970). In a third oceanic larva, tentatively identified as a species of *Golfingia,* fibers are scattered irregularly throughout the underlying cuticle in no obvious pattern. Functional significance of cuticular form and structure remains to be investigated.





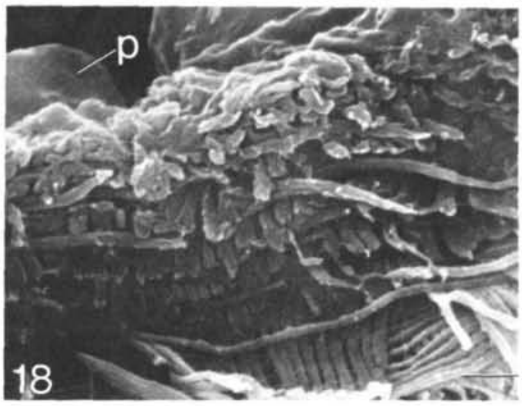


FIG. 15. Scanning electron micrograph of surface cuticular papillae of planktotrophic pelagosphera from open-ocean plankton tentatively identified as belonging to the genus *Phascolosoma*. Scale, 2 μm.

FIG. 16. Scanning electron micrograph of cuticular papillae of open-ocean pelagosphera larva of *Aspidosiphon sp.* The smaller papilla contains the duct of an epidermal organ, similar to that shown in Figure 17. Scale, 5 μm.

FIG. 17. Light micrograph of a one-micron section of the body wall of an open-ocean pelagosphera larva, *Paraspidosiphon sp.* Embedded in Epon; stained in methyene blue and Azure II. Note longitudinal muscles (lm), circular muscles (cm), epidermis (ep) with epidermal organ extending to the surface and lightly staining cuticle (cu) with darkly staining surface cuticular papillae (p). Scale, 10 μm.

FIG. 18. Scanning electron micrograph of a section, cut with razor, through the cuticle of an open-ocean pelagosphera larva, *Aspidosiphon sp.* (same type as in Fig. 16). Note surface papillae (p) and underlying cuticle composed of layers of crossing fibers. Scale, 2 μm.

METAMORPHOSIS OF THE PELAGOSPHERA

Metamorphosis of the planktotrophic pelagosphera to the juvenile sipunculan is marked by a loss of metatrochal cilia and an elongation and growth of the body anterior to the postmetatrochal sphincter to become the retractable introvert which in many species develops characteristic spines and hooks (Figs. 13, 14). The mouth assumes a terminal rather than ventral position and tentacular lobes are formed, usually around the rim of the mouth (Figs. 11, 12). The lower lip regresses and larval organs such as terminal organ, buccal organ, and lip glands disappear, their fate not determined. It has been suggested that the duct for the lip glands may become the canal of the ventral sensory organ found in the adult of many species, but this remains to be verified (Åkesson, 1961*b*).

The period of time over which these metamorphic changes take place may vary from 1 to 5 days. The specific or generic characters of the adult may not develop fully, under laboratory conditions, for several weeks to several months after metamorphosis. For example, the anterior and posterior horny shields characteristic of the genera of *Aspidosiphon* and *Paraspidosiphon* may require months to develop (Scheltema and Hall, 1965). And many species in which the absence of hooks is used as a taxonomic character in the adult possess hooks on the introvert in the juvenile stage.

CONCLUDING REMARKS

Typical of other Spiralia, the Sipuncula exhibit spiral cleavage followed by a trochophore larva. In yolk-rich eggs the micromeres may be larger than the macromeres in the A, B, and C quadrants, the larger size being related in later development to the nutritive function of yolk-filled prototroch cells. Unique to Sipuncula is a second larval form which follows the trochophore in two of four developmental patterns in the group, including the majority of species studied. This larva is the pelagosphera and may be either lecithotrophic or planktotrophic. Planktotrophic pelagosphera larvae may live in the open ocean for prolonged periods. Some of these larvae are characterized by cuticular papillae of distinctive form and pattern. In the development of species with planktotrophic pelagosphera larvae, there are two distinct metamorphoses, one from the trochophore to the pelagosphera and the second from the pelagosphera to the juvenile.

REFERENCES

Åkesson, B. 1958. A study of the nervous system of the Sipunculoideae with some remarks on the development of two species *Phascolion strombi* Montagu and *Golfingia minuta* Keferstein. Undersökningar över Öresund 38: 1-249.

Åkesson, B. 1961*a*. The development of *Golfingia elongata* Keferstein (Sipunculidea) with some remarks on the development of neurosecretory cells in sipunculids. Ark. Zool. (Ser. 2) 13 (2): 511-531.

Åkesson, B. 1961*b*. Some observations on *Pelagosphaera* larva. Galathea Report 5:7-17.

Fisher, W. K. 1947. New genera and species of echiuroid and sipunculoid worms. Proc. U.S. Nat. Mus. 97 (3218):351-372.

Gerould, J. H. 1903. Studies on the embryology of the Sipunculidae, I. The embryonal envelope and its homologue. Mark Anniversary Volume, 439-452.

Gerould, J. H. 1907. Studies on the embryology of the Sipunculidae, II. The development of *Phascolosoma*. Zool. Jahrb. Abt. Anat. Ontog. Thiere 23:77-162.

Hall, J. R. and R. S. Scheltema. 1975. Comparative morphology of open-ocean pelagosphaera. *In* M. E. Rice and M. Todorovic (eds.), *Proceedings of the international symposium on the biology of the Sipuncula and Echiura*, Vol. I, pp. 183-197. Naučno Delo, Belgrade.

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

Jägersten, G. 1963. On the morphology and behavior of *Pelagosphaera* larvae (Sipunculoidea). Zool. Bid. Fran Uppsala 36: 27-35.

Mingazinni, P. 1905. Un Gefireo pelagico. *Pelagosphaera Aloysii* n. gen., n. sp. Rendiconti delle sedute solenni della R. Academia Nacionale dei Lincei 14: 713-720.

Moritz, K. and V. Storch. 1970. Uber den Aufbau des Integumentes der Priapuliden und der Sipunculiden. Zeit. Zellforsch. mikrosk. Anat. 105: 55-64.

Murina, V. V. 1965. Some data on the structure of pelagospheres—sipunculid larvae. Zool. Zhurnal. 44(11):1610-1619. (In Russian)

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.

Rice, M. E. 1973. Morphology, behavior, and histogenesis of the pelagosphera larva of *Phascolosoma agassizii* (Sipuncula). Smithsonian Contributions to Zoology, No. 132:1-51.

Rice, M. E. 1975*a*. Sipuncula. *In* A. C. Giese and J. S. Pearse (eds.), *Reproduction of marine invertebrates*, Vol. II, Chapter 4, pp. 67-127. Academic Press, Inc., New York.

Rice, M. E. 1975*b*. Observations on the development of six species of Caribbean Sipuncula with a review of development in the phylum. *In* M. E. Rice and M. Todorovic (eds.), *Proceedings of the international symposium on the biology of the Sipuncula and Echiura*, Vol. I, pp. 141-160. Naučno Delo, Belgrade.

Scheltema, R. S. and J. R. Hall. 1965. Trans-oceanic transport of sipunculid larvae belonging to the genus *Phascolosoma*. Amer. Zool. 5(2):100. (Abstr.)

Selenka, E. 1875. Eifurchung und larvenbildung von *Phascolosoma elongatum* Kef. Zeit. wiss. Zool. 25:442-449.

Williams, J. 1972. Development of a rock burrowing sipunculid inhabiting stony coral. Amer. Zool. 12(4):723. (Abstr.)