

18. Sipuncula: developmental evidence for phylogenetic inference

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Abstract

Sipunculans are marine worms characterized by a unique combination of features which set them apart as a distinct coelomate phylum. A review of developmental features demonstrates clearly their similarities to annelids and molluscs. Cleavage is spiral, giving rise to a trochophore larva. Like molluscs, the cross cells of the apical plate are in the radial position. Close affinities to annelids are apparent in the prototrochal and metatrochal ciliary bands, the formation of the larval cuticle by transformation of the egg envelope, and especially in the development of the nervous system. They differ from annelids in that they lack segmentation. Thus, it is proposed that sipunculans are a primitive group which arose from a molluscan-annelidan stem.

Within the phylum the primitive developmental pattern is presumed to have included a moderately yolky egg covered by a thick egg envelope, a non-feeding trochophore encircled by the persistent egg envelope, and a planktotrophic pelagosphaera larva. This developmental pattern is assumed to have arisen from an ancestral pattern with a planktotrophic trochophore and an egg having a simple envelope and low yolk content. Planktotrophy in sipunculans, apparently retarded by the development of the thick egg envelope and an associated moderate increase in yolk, was accordingly shifted to the specialized larval form, the pelagosphaera. Based on these hypotheses two evolutionary sequences can be defined in extant developmental patterns of sipunculans: one toward an increase in yolk and decrease in the pelagic stage and the other toward a reduction in yolk and the prolongation of the pelagic



stage as represented in long-lived planktotrophic pelagosphaera larvae of the open ocean.

Introduction

Sipunculans are unsegmented, coelomate marine worms, characterized by a unique combination of features which have led to their recognition as a separate phylum (Sedgwick 1898; Baltzer 1931; Pickford 1947; Fisher 1952; Stephen and Edmonds 1972). The currently accepted name, *Sipuncula*, was proposed by Stephen (1964). Characters of the group include division of the body into two regions: a posterior thickened trunk and a narrow anterior retractable introvert usually terminating in a mouth surrounded by tentacles. Other definitive features include a spacious, undivided coelomic cavity, a recurved gut ending in a dorsal anus on the anterior trunk, a median unpaired ventral nerve cord, and usually two metanephridia located ventrolaterally in the anterior trunk.

The classification of Stephen and Edmonds (1972) recognized 4 families, 17 genera, and approximately 320 species. The genus *Golfingia*, containing by far the greatest number of species, has been presumed by many authors (Selenka, de Man, and Bülow 1883; Hérubel 1907; Gerould 1913; Åkesson 1958; Murina 1977) to represent the most primitive adult morphology. Primitive features are considered to be a ring of simple tentacles surrounding the mouth, four retractor muscles, a continuous layer of body wall muscles, two nephridia and a supra-oesophageal ganglion in a superficial position (cf. Clark, 1969). Other genera can be related to *Golfingia* on the basis of varying combinations of *Golfingia* characters or by specializations of these characters.

Although sipunculans are a homogeneous group, their phylogenetic position has long been an enigma, as shown by the widely divergent views that have been proposed for their relationships. In the early 1800s sipunculans were placed close to the holothurian echinoderms by Lamarck (1816) and Cuvier (1830). Subsequently, Quatrefages (1847) recognized their similarities to annelids and considered sipunculans as a link between holothurians and annelids. Thus he created the group 'Gephyrea' (from the Greek word, *gephyros*, meaning bridge) in which he included sipunculans, echiurans and priapulids. The Gephyrea became a convenient taxon for groups of uncertain affinities, and even after the relationships of sipunculans were better understood, the term persisted in the literature. Most frequently it was used to indicate a class of Annelida which included only sipunculans and echiurans. Another view which received considerable recognition was that of Lang (1888) who erected a group *Prosopygii* to contain sipunculans, phoronids,

bryozoans, and brachiopods. This grouping was based on resemblances in the crown of tentacles, position of anus, unsegmented body, and the presence of one or two pairs of nephridia. A more recent proposal for the relationships of sipunculans, while retaining the accepted classification, has revived the ideas of Lamarck (1816), Cuvier (1830) and Meyer (1904), by again supposing an alliance between sipunculans and holothurians (Nichols 1967). Nichols has pointed to the similarities in general bodyplan, noting particularly the resemblance in structure and function of the water vascular system of holothurians and the coelomic tentacular system of sipunculans. Many other affinities for sipunculans have been proposed in the past (for review see Hyman 1959); however, all of these phylogenies have been based on morphological similarities with no reference to developmental biology.

Beginning in the late nineteenth century, studies on development of sipunculans suggested a relationship with annelids and to a lesser extent with molluscs (Selenka 1875; Hatschek 1883; Gerould 1903, 1906; Åkesson 1958, 1961a; Rice 1967, 1973, 1975a, b). The typical pattern of spiral cleavage found in sipunculan development and the formation of the trochophore larva have placed the sipunculans, along with the annelids and molluscs, in the Protostomia.

This paper briefly reviews the available information on development of sipunculans and compares it, where relevant, to that in other invertebrates, and considers its possible implications for phylogenetic interpretations, including evolutionary sequences within the phylum and affinities to other groups.

General developmental patterns

A wide diversity of developmental patterns is found within the Sipuncula, ranging from direct development with no pelagic stages to planktotrophic development with a long-lived larva which exists in the plankton for several months before undergoing settlement and metamorphosis into a juvenile worm. Table 18.1 gives information on the development of 20 species of sipunculans, representing all four families recognized in the classification of Stephen and Edmonds (1972). Based on this information, developmental patterns of sipunculans can be classified into four categories (Rice 1967, 1975a). Three are entirely lecithotrophic and the fourth includes a planktotrophic larva. Of the three lecithotrophic patterns, one is direct development, a second includes one larval stage, the trochophore, which transforms into a crawling vermiform stage, and the third includes a trochophore which metamorphoses into a second larval stage, the pelagosphera. The pelagosphera is a larval stage, unique to the Sipuncula, in which a prominent metatroch

develops as the primary locomotory organ, and the prototroch is reduced or lost (Rice 1967). The fourth category is characterized by a lecithotrophic trochophore followed by a planktotrophic pelagosphera. The latter may live for a prolonged period in the plankton, attaining a length of 5-10 mm in some species (Åkesson 1961b; Damas 1962; Jägersten 1963). They are commonly found near the surface in warm oceanic current streams (Scheltema 1975).

Gametes, cleavage, and early development

Sipunculans, usually dioecious, spawn directly into the sea where fertilization occurs. This mechanism of discharging gametes is considered primitive among invertebrates (Jägersten 1972).

As in many other groups in which free spawning occurs, spermatozoa are of the primitive type (Franzén 1956).

All sipunculan eggs possess a thick egg envelope, comprising several layers and perforated by pores, but they show considerable variation in size, shape, pigmentation, and yolk content (Table 18.1). Although extremes in egg size represent two extremes in developmental pattern (i.e. direct and planktotrophic development) there is considerable overlap in size range within the various developmental patterns and no clear-cut correlation emerges.

Cleavage in sipunculans is spiral, holoblastic, and unequal. In a study of cell lineage of *Golfingia vulgaris* Gerould (1906) reported an alternating direction of spindles up to 48 cells and further in some regions of the egg. Differing from typical spiral cleavage, at the eight-cell stage the micromeres in the A, B, and C quadrants are larger than the macromeres—a size difference which is indicative of yolk content and is later reflected in the enormous yolk-laden cells of the prototroch. This peculiar feature of cleavage is found in the terebellid *Amphitrite* and some other polychaetes (Mead 1897; Gerould 1906) and in both cases the primary cells of the prototroch arise from cells $1a^2-1d^2$. The 2d cell or somatoblast gives rise to the somatic plate and is the largest cell of the 16-cell stage. At the 48-cell stage in *Golfingia vulgaris* a radial division of the $1q^{12}$ cells results in the formation of an apical cross. The cross cells, as described by Gerould, extend out from the tips of the rosette cells and lie in the sagittal and frontal planes of the future embryo. The arms of the cross are thus in the radial position of molluscs rather than the interradial of annelids. Some authors (Åkesson 1958; Hyman 1959) have mistakenly reported an annelidan cross in sipunculans because of confusion over Gerould's terminology. However, his description and illustration (Fig. 18.1) clearly indicate a molluscan cross. As in many other spiralian the third set of micromeres forms the

Table 18.1. A summary of developmental patterns of the Sipuncula*

Species	Egg size: diameter or length x width (μm)	Eight-cell stage relative size of micro- and macromeres in quadrants A, B, C	Length of pelagic stage			
			Gastrulation	Trochophore	Lecithotrophic	Planktotrophic [†]
Category I						
<i>Golfingia minuta</i> ¹	260-280 x 214-230	?	Epiboly	0	0	0
<i>Themiste pyroides</i> ⁶	190	Micromeres > macromeres	Epiboly	0	0	0
<i>Phascolion cryptus</i> ⁷	136	Micromeres > macromeres	Epiboly	0	0	0
Category II						
<i>Phascolion strombi</i> ¹	125	Micromeres > macromeres	Epiboly	8 days	0	0
<i>Phascolopsis gouldi</i> ⁴	150-180	Micromeres > macromeres	Epiboly	3 days	0	0
Category III						
<i>Golfingia vulgaris</i> ⁴	150-180	Micromeres > macromeres	Epiboly	3 days	2 days	0
<i>Golfingia elongata</i> ²	125	?	Epiboly + invagination	2 days	4 days	0
<i>Golfingia pugelensis</i> ⁶	160	Micromeres = macromeres	Epiboly	8 days	13 days	0

<i>Themiste alutacea</i> ⁷	138	?	Epiboly	2 days	6 days	0
<i>Themiste lageniformis</i> ¹¹	145	Micromeres > macromeres	Epiboly	0	8-12 days	0
<i>Themiste petricola</i> ³	156	Micromeres > macromeres	Epiboly	2 days	5 days	0
Category IV						
<i>Aspidosiphon parvulus</i> ¹⁰	139 × 107	Micromeres = macromeres	?	3 days	0	1 month
<i>Golfingia mixakiana</i> ⁹	108 × 77	Micromeres = macromeres	?	5 days	0	1 month
<i>Golfingia pellucida</i> ⁸	105	Micromeres = macromeres	?	3 days	0	1 month
<i>Paraspidosiphon fischeri</i> ⁷	103 × 94	?	?	2 days	0	1 month
<i>Phascolosoma agassizii</i> ⁶	140 × 110	Micromeres = macromeres	Epiboly + invagination	8-10 days	0	1 month
<i>Phascolosoma antillarum</i> ⁷	127 × 97	?	?	3 days	0	1 month
<i>Phascolosoma perlucens</i> ⁷	112 × 91	Micromeres = macromeres	Epiboly + invagination	3 days	0	1 month
<i>Phascolosoma varians</i> ⁷	104 × 90	?	?	3 days	0	1 month
<i>Sipunculus nudus</i> ⁵	186	Micromeres < macromeres	Invagination	3 days	0	1 month

References: 1. Akesson 1958; 2. Akesson 1961a; 3. Amor 1975; 4. Gerould 1906; 5. Hatschek 1883; 6. Rice 1967; 7. Rice 1975a; 8. Rice 1975b; 9. Rice 1981; 10. Rice unpublished; 11. Williams 1972.

* Modified from Rice 1975b

† Time indicated is minimal period of survival in the laboratory. Metamorphosis to juvenile was not observed.

ectoderm, 3A-3D form endoderm and mesoderm. The 3D gives rise to 4d by a laeotropic division which in turn produces two daughter cells, the teloblasts of the mesoderm.

A survey of what is known of early cleavage in species other than *Golfingia vulgaris* reveals that the relative size of micromeres and macromeres at the eight-cell stage is related to the yolk content of the egg. Micromeres exceed the macromeres in size only in eggs with a high yolk content and in which the development is lecithotrophic. Those species with microlecithal eggs have micromeres equal to or smaller than the macromeres (Table 18.1).

Blastulation and gastrulation in sipunculans follow the pattern typical for other spiralian. Modifications are correlated with yolk content of the egg. A small blastocoel occurs in species with planktotrophic development. Gastrulation is accomplished entirely by epiboly in sipunculans having macrolecithal eggs, with the exception of *Golfingia elongata*, in which invagination plays a minor role (Åkesson 1958). Species which have planktotrophic development achieve gastrulation by a combination of epiboly and emboly. In *Phascolosoma perlucens* and *P. agassizi* gastrulation occurs mainly by epiboly, although a narrow archenteron is indicative of some invagination (Rice 1967, 1975a,b). In *Sipunculus nudus* gastrulation is accomplished primarily through invagination (Hatschek 1883).

The trochophore

Trochophores are essentially similar to those of polychaetes and molluscs. Typically the trochophore is top-shaped and characterized by pretrochal and post-trochal hemispheres, separated by a prominent

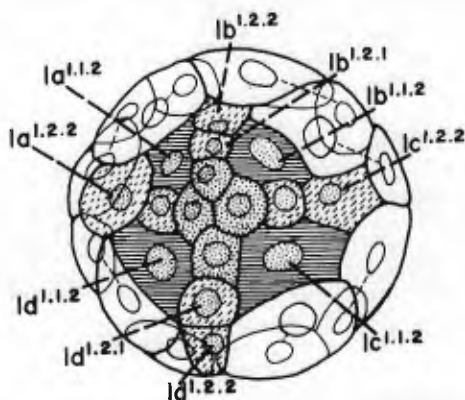


Fig 18.1 Apical view of the 48-cell stage of *Golfingia vulgaris*, showing molluscan cross and intermediate cells. Rosette cells are dotted, cross cells dashed, and intermediate cells barred. (Redrawn from Gerould 1906, after Rice 1975b).

band of ciliated prototrochal cells. Anteriorly an apical circle of rosette cells, encircled by the apical groove, bears the long cilia of the apical tuft. A pair of pigmented eyespots is located dorsolaterally in the pre-trochal hemisphere, and in a midventral position just posterior to the prototroch, there is a ciliated stomodaeum. The trochophore of sipunculans is always completely enclosed by the thick egg envelope and thus is lecithotrophic or non-feeding, regardless of the content of yolk. Rudiments of the gut are present, and in the late trochophore of species with planktotrophic pelagospheras (Table 18.1) the gut may be fully formed, though not functional. The trochophore of sipunculans never develops a pronephridium, thus differing from many annelids and some molluscs.

Diversity among sipunculan trochophores is expressed in the pattern and degree of ciliation, the relative development of organs and the concentration of yolk (Fig. 18.2). In addition to the prototroch, pre-trochal and post-trochal (metatrochal) cilia occur in trochophores of *Golfingia elongata* (Åkesson 1961a) and *G. vulgaris* (Gerould 1906). *Sipunculus nudus* differs in that it is completely ciliated (Hatschek 1883; Gerould 1903). An equatorial band of ciliated cells, presumed to be homologous to the prototroch, grows posteriorly and anteriorly beneath the egg envelope, completely enclosing the embryo. The egg covering, thus composed of both prototroch cells and egg envelope, was referred to by Hatschek (1883) as the 'serosa'. Metatrochal cilia are present in the trochophore of *Sipunculus nudus*, but because they are enclosed by the egg envelope are non-functional. The embryos of directly developing species lack ciliation, but have a broad equatorial band of large cells which are homologous to ciliated prototroch cells of other species. In *Golfingia minuta*, Åkesson (1958) reports rudimentary non-locomotory cilia on marginal cells above and below the prototroch cells. He interprets this condition as evidence that ancestors of this species were forms with a pelagic stage and that direct development is therefore secondary.

The end of the trochophore stage may occur in one of several ways, depending on the developmental category (Table 18.1). In categories 1 and 2 the non-swimming trochophore (or comparable stage) elongates, transforming into a small crawling vermiform stage. In other sipunculans the end of the trochophore stage results in a metamorphosis to a pelagosphera larva—a process in which the prototroch is lost or reduced and the metatroch becomes the primary locomotory organ. In developmental category 3 the larva is lecithotrophic and in category 4 it is planktotrophic. The lecithotrophic larva swims for a short period before transforming into the vermiform stage. The planktotrophic larva, after a planktonic existence from one to several months, undergoes a second metamorphosis into the juvenile form.

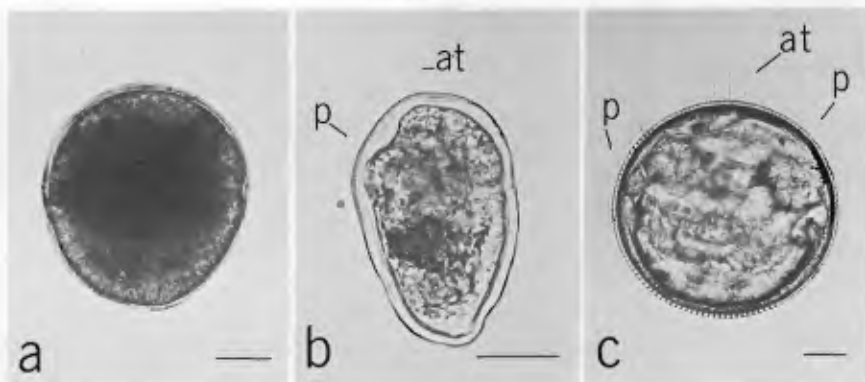


Fig. 18.2. Trochophores of sipunculans, illustrating diversity of ciliation patterns. (a) Trochophore of *Themiste lageniformis*, 2 days, lacking prototrochal ciliation (occasionally prototrochal cilia are weakly developed but non-functional in this species; the succeeding pelagosphera has a well-developed metatroch). (b) Late trochophore of *Phuscolosoma varians*, 4 days. Note apical tuft and band of prototrochal cilia. (c) Trochophore of *Sipuncululus* sp. collected from plankton. In this highly modified trochophore, prototrochal cilia surround the larva. An apical tuft is prominent anteriorly. Light micrographs of living larvae. Abbreviations: at, apical tuft; p, prototrochal cilia. Scale bars 40 μ m.

Developmental processes associated with the termination of the trochophore stage, regardless of developmental category, are formation or expansion of the coelom, dissolution of the prototroch, and formation of the larval cuticle either by transformation of the egg envelope or hatching from the egg coverings. An additional process, formation of the metatrochal band of cilia, occurs when metamorphosis results in a pelagosphera larva.

The coelom is formed in sipunculans, as in other protostomes, by splitting of mesoderm bands (Hatschek 1883; Gerould 1906; Rice 1967). The time of formation relative to other developmental events may vary among species, differences being related to the degree of yolk development. Coelom formation occurs in directly developing species at the time of dissolution of non-ciliated prototroch cells and elongation. In species with macrolecithal eggs and a lecithotrophic pelagosphera, coelom formation takes place simultaneously with the other events of trochophoral metamorphosis. In species with planktotrophic development the coelom is formed during the trochophore stage and is expanded at the time of metamorphosis of the trochophore to the pelagosphera stage.

Dissolution of the prototroch cells in species with macrolecithal eggs coincides with transformation of the trochophore into either the pelagosphera or the vermiform stage at the time of development of the coelom. In these species with relatively large micromeres at the eight-cell stage and large, yolk-rich prototroch cells, yolk material is released

into the coelom and, when prototroch cells break down, it provides an important source of nutrition for the developing larva. Even in species with microlecithal eggs, the prototroch cells, although smaller, are relatively yolky and serve a nutritive function for the developing embryo. Granules are released into the prototrochal cavities of *Phascolosoma agassizi* and into the 'amniotic' cavity of *Sipunculus nudus* before metamorphosis of the trochophore. Thus it is evident that specialization of the prototroch to serve a nutritive function is characteristic of the phylum and is found throughout all of the developmental patterns, being most highly developed in species with lecithotrophic development.

At metamorphosis of the trochophore to the pelagosphera larva or transformation of the embryo to the vermiform stage, characteristic changes occur in the egg envelope as the cuticle of the larva or young worm is formed. The changes may involve shedding of the egg coverings or transformation of the egg envelope. Whether the egg envelope is shed or transformed has little relevance to the developmental pattern or amount of yolk. Trochophores of *Sipunculus nudus*, a species with planktotrophic development, shed the entire egg envelope along with the prototroch cells at metamorphosis of the trochophore (Hatschek 1883). Other species with lecithotrophic development, *Golfingia vulgaris* and *Phascolopsis gouldi*, shed only the egg envelope and develop a new larval cuticle (Gerould 1906). *Phascolion cryptus*, a species lacking a pelagic larval stage, loses the prototrochal and pretrochal portions and retains the post-trochal egg envelope (Rice 1975b). Egg envelopes of other species appear to be transformed into the larval cuticle either entirely or in part (Rice 1967, 1973, 1975a,b). The process includes a loss of porosity and lamellation of the envelope and, as the larva elongates, the envelope increases in elasticity. A similar transformation has been reported in many polychaetes; for example in *Phragmatopoma lapidosa* by Eckelbarger and Chia (1978).

Pelagosphera larvae

Pelagosphera larvae, whether planktotrophic or lecithotrophic share basic features (Figs 18.3, 18.4). Three regions of the body are clearly distinguished: head, metatrochal collar, and posterior trunk. Dorsally the head bears a U-shaped prototroch comprised a short, weakly-developed cilia. On the inner side of the prototroch there is a pair of dorsolateral pigmented eye spots. Ventrally the head is ciliated, both above and below the region of the mouth and may be bifurcated by a median channel leading to the mouth. Formation of the ventral head occurs at trochophoral metamorphosis when the egg envelope overlying the stomodaeum ruptures and the latter opens outward to the exterior

to form the entire ventral ciliated surface of the head. At the same time the prototrochal band is modified by loss of the ventral portion and a reduction in size of cells and length of cilia. In planktotrophic pelagospheras the ciliated portion of the ventral head posterior to the mouth is expanded into the ventral lip, a lobe which usually extends out perpendicularly to the head, or, when the larva is feeding, can be flattened against a substratum. Organs associated with the mouth and presumed to be used in feeding are the buccal organ and lip gland. The buccal organ is a protrusible muscular sac which can be extended through a transverse slit just below the mouth. The lip glands (one or two pairs) are pendulous lobes hanging down into the anterior coelom and opening to the exterior through a pore on the lip.

The metatrochal collar or 'thorax' (Jägersten 1963) bears a prominent band of long, active cilia which serve as a means of locomotion. The metatrochal collar may be considerably expanded during swimming. Both head and metatroch can be retracted into the trunk.

The trunk is by far the largest region of the larval body and encloses a spacious coelom which in lecithotrophic larvae is filled with yolk granules originating from prototrochal and other cells. A terminal attachment organ is found at the posterior tip of most lecithotrophic and planktotrophic larvae although the degree of development may vary. When highly developed it is used for temporary attachment to a

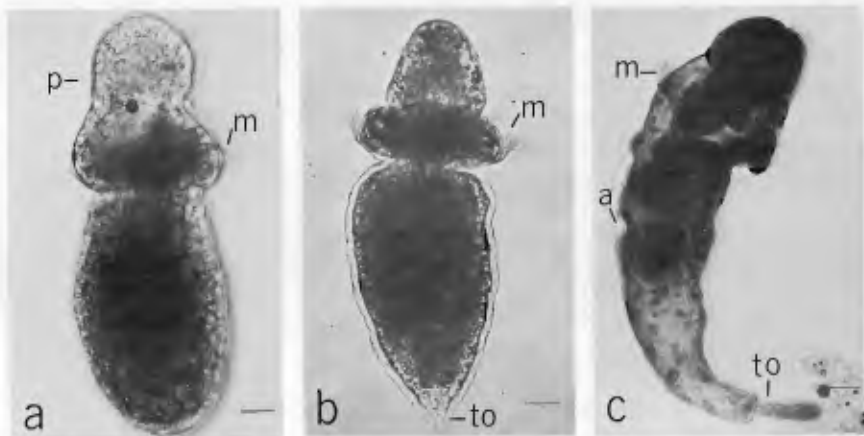


Fig. 18.3. Young pelagosphera larvae, reared from eggs in the laboratory. (a) *Themiste alutacea*, 3 days; a lecithotrophic larva lacking a terminal organ. (b) *Golfingia pugettensis*, 13 days; a lecithotrophic larva with a terminal organ. (c) *Phascolosoma perlucens*, about 7 days; a planktotrophic larva showing a prominent terminal organ. Light micrographs of living larvae. Abbreviations: a, anus; m, metatroch; p, prototroch; to, terminal organ. Scale bars 25 μ m. (From Rice 1975a, b).

substratum, but if relatively small may be primarily sensory (Hatschek 1883).

The internal organs of a pelagosphera larva consist of a central nervous system including brain, circumoesophageal connectives and ventral nerve cord; digestive system comprised in planktotrophic larvae of oesophagus, stomach, intestine, and rectum; a pair of nephridia with internal and external openings; retractor muscles which serve to withdraw the head and metatrochal region into the trunk.

Metamorphosis of the pelagosphera to the juvenile form is marked by a loss of metatrochal cilia in both lecithotrophic and planktotrophic forms. In the former there is a transition through a vermiform stage which may last for several weeks during which time the gut is completed, yolk absorbed, introvert and body elongated, and the tentacular lobes formed. Metamorphosis of planktotrophic larvae is more rapid, the adult habitus being acquired usually within 1-3 days of loss of the metatroch (Rice 1978). The major changes occur in the region of head and metatroch and involve movement of the mouth from a ventral to a terminal position, formation of the tentacles and elongation of the introvert. Most of the organ systems of the larva are retained in the adult, including central nervous system, digestive system, retractor muscles and nephridia. During growth and differentiation of the juvenile, however, there may be some loss or specialization of organs.

Some authors have suggested resemblances of the pelagosphera larva of sipunculans to larvae of molluscs and entoprocts. Gerould (1906) pointed to the similarity of the buccal organ and lip glands of planktotrophic pelagospheres to the rudiments of the radular sac and pedal gland in chiton larvae. Jägersten (1972) further compared the lip of sipunculans to the ventral creeping lobe or foot that occurs between mouth and anus in larvae of molluscs and entoprocts. Other authors (Åkesson 1958; Rice 1973) have presumed that these organs of the mouth of planktotrophic pelagospheres may represent adaptations for feeding activity with little phylogenetic significance.

The pelagosphera of sipunculans represents a later developmental stage than that of the trochophore of polychaetes. Features considered to be more advanced in the pelagosphera are the well developed trunk, spacious coelom, and metanephridia. Evidence of segment rudiments is entirely lacking in the pelagosphera. Papillae, bearing bristles, and sometimes arranged in transverse rows, are frequently found in young sipunculan larvae, but they are generally not considered as evidence of metamerism, nor are the bristles homologous to polychaete chaetae (Åkesson 1958, 1961a; Clark 1969). The pelagosphera is seen as a specialized form without demonstrable parallels in other groups.

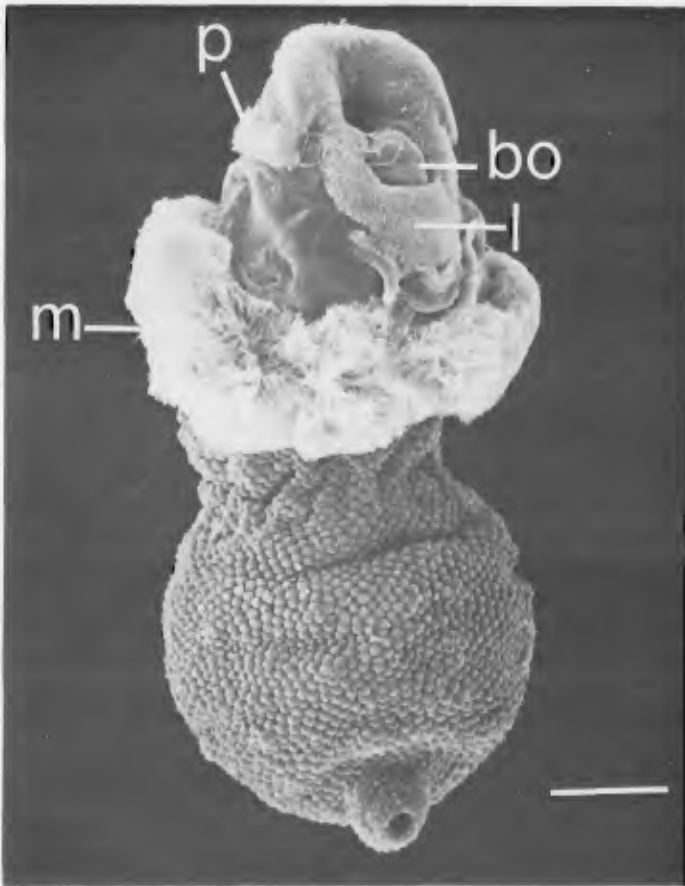


Fig. 18.4. Scanning electron micrograph of an oceanic planktrophic pelagosphaera larva, *Aspidosiphon* sp. The terminal organ at the posterior extremity is retracted. Abbreviations: bo, buccal organ; l, lip; m, metatroch; p, prototroch. Scale bar 150 μm . (After Rice 1981).

Organogenesis

Development of the nervous system and the nephridia in sipunculans is of particular concern when making phylogenetic comparisons. The central nervous system in sipunculans and polychaetes has many striking similarities both in anatomy and development. The ventral nerve cord develops in both as a ventral ectodermal thickening which is typically double and segmented in annelids, but in sipunculans is generally single, median, and unsegmented. An exception is found in the development of the nerve cord in *Phascolosoma agassizi* which, although unsegmented, is double at its inception but unites to form a

single cord in the older larva of two months (Rice 1973). Similarly the nerve cord in the young larva of *Golfingia pellucida* throughout much of its length, though not completely divided, is distinguished by two rows of nerve cell nuclei joined medially by the neuropile. In the nerve cords of all other larval species examined, the nerve cell nuclei form a continuous band on the ventral side of a median cord and the fibres are dorsal and central. Gerould (1906) reported a transitory metamerism in the nerve cord of developing larvae, but later repudiated this statement (Hyman 1959).

The phylogenetic significance of the development of the nerve cord has been interpreted variously by different authors. Gerould (1906) suggested that the single, unpaired nerve cord in the development of species which he studied related sipunculans to annelids such as *Polygordius*. Åkesson (1958), on the other hand, took the position that the orthogonal nervous system of turbellarians should be considered primitive for molluscs and annelids, and that the single nerve cord of sipunculans was therefore a derived feature. He considered this as evidence that sipunculans split off from the annelids after the orthogonal system was reduced. The rudimentary paired nerve cord found in the development of *Phascolosoma agassizi* is interpreted by Rice (1973) to be reminiscent of a double cord that occurred in the ancestors of extant sipunculans, thus relating sipunculans more closely to annelids.

A protonephridium, found in the trochophores of many annelids and some molluscs, is absent in sipunculans. However, it has been noted by Anderson (1966) and others that protonephridia in annelids are characteristic of planktotrophic trochophores, but not yolky larvae. As the trochophore of sipunculans is lecithotrophic or non-feeding, the significance of the lack of a protonephridium may not be as great as supposed earlier (Gerould 1906; Åkesson 1958; Hyman 1959). The larval nephridia of sipunculans are U-shaped structures opening at one end into the coelom and at the other to the exterior. Usually occurring as a pair, they are located ventrolaterally in the trunk posterior to the metatroch. These are also the nephridia of the adult sipunculan, undergoing little or no change at metamorphosis of the pelagospheara into the juvenile. Goodrich (1945) classified the paired sipunculan nephridia as mixonephridia, similar to those found in most polychaetes, but not in molluscs.

Functional morphology and behaviour of larvae

Ciliary bands of sipunculan larvae show considerable diversity in degree of development and function, even though, at least in the case of the prototroch, they are homologous. Information is not sufficient to deter-

mine whether the metatroch has the same lineage (Gerould 1906). If we compare the function of the cilia in the planktotrophic trochophore of a polychaete such as *Pomatoceros* (Segrove 1941) or *Eupomatus* (Shearer 1911) with that in the planktotrophic pelagosphera of a sipunculan we find some striking differences. In the polychaete the prototroch produces a downward current for both swimming and feeding whereas the more weakly developed metatroch evokes an upward current. The latter moves particles upward to an intermediate band of cilia (adoral) continuous with the mouth which directs food particles toward the mouth. This method of feeding, known as the opposed-band mechanism (Jägersten 1972; Strathmann 1978) does not occur in sipunculans in which the function of the cilia is quite different. Here the strongly beating metatroch functions primarily in locomotion, and the prototroch, reduced to a weak partial band on the head, is of unknown function. Feeding may be accomplished in swimming or resting pelagospheras by the movement of suspended particulate matter into the mouth by means of the short but numerous cilia on the ventral head and median ventral groove. Presumably the lip glands participate by secreting an adhesive substance for trapping food particles.

Another more specialized mode of feeding, not known to occur in polychaete larvae, involves feeding on a substratum by application of the ventral head to a surface. With the head in this position, particles can be moved into the mouth through activity of the ventral cilia alone or with the assistance of the protrusible buccal organ. This organ may also aid in swallowing larger particles, scraping material from the substratum or ejection of particles from the mouth. Feeding on a substratum can be accomplished either while the larva moves along with posterior end directed upward or, if attached by the terminal organ, the larva can bend over and graze the surrounding surfaces. This latter method of feeding is particularly characteristic of young benthopelagic pelagospheras.

The functional significance of the terminal organ may vary with age in planktotrophic pelagospheras (Rice 1978, 1981; Ruppert and Rice 1983). All young pelagospheras that have been studied in the laboratory spend much of their time near the bottom of culture dishes, either attached, resting or swimming. Yet older pelagospheras are found in the surface waters of the open ocean, presumed to survive there for many months (Scheltema and Hall 1975). In the older larvae the terminal organ may be proportionately reduced, and even when in contact with a substratum under laboratory conditions is rarely used for attachment. Young larvae, adapted for a benthic existence, do not appear able to metamorphose, but seem to represent a stage of growth and differentiation. Older larvae, on the other hand, are highly adapted for a planktonic existence, and are able to delay metamorphosis until a sub-

stratum is contacted. They serve as a highly specialized means of dispersal for the species (Rice 1978, 1981).

Evolutionary sequences of development within the phylum

Previous papers on sipunculan development have presented arguments in favour of the primitiveness of lecithotrophic development in the phylum (Gerould 1906; Åkesson 1958; Rice 1967, 1975a). But if we assume, along with other authors (Jägersten 1972; Strathmann 1978; Chia 1974; Hermans 1979) that planktotrophy is the primitive mode of development among marine invertebrates, then it would follow that planktotrophy in sipunculans must be a derived condition and the planktotrophic pelagosphera must have re-acquired the feeding habit. In view of studies made since the previous hypotheses another interpretation is now suggested.

The previous rationale for the primitiveness of lecithotrophy was based on the premise that the genus *Golfingia*, having the most primitive adult features and being the largest genus with characters from which the other genera could be derived (see Introduction) would include the most primitive developmental patterns. At the time these arguments for lecithotrophy were developed, all species of *Golfingia* for which developmental studies had been made did indeed exhibit lecithotrophic development. Now, in view of additional information on other species of *Golfingia* we know that nearly every developmental category is represented in the genus *Golfingia* (as defined by Stephen and Edmonds 1972). A directly developing species, *Golfingia minuta*, has exceptionally large, yolky eggs which it broods within its tube, epibolic gastrulation, and a lecithotrophic developmental period of 8 weeks. *Golfingia vulgaris*, *G. elongata*, and *G. pugettensis* have a lecithotrophic development which includes a lecithotrophic pelagosphera of a relatively short pelagic existence of 5, 6, and 21 days respectively. Two other species studied more recently, *G. pellucida* and *G. misakiana*, have a planktotrophic pelagosphera which in the former species lives for 1 to 2 months in the laboratory before metamorphosis of the pelagosphera to the juvenile worm (Rice 1975b, 1978, 1981). The pelagosphera of *G. misakiana* has a much more prolonged pelagic existence and is commonly collected in the surface waters of the component currents of the Gulf Stream System where they are believed to live for several months (Hall and Scheltema 1975; Rice 1978).

If we assume with Jägersten (1972) and others that a planktotrophic pelagic larva is common to the development of the ancestors of metazoans, and that a planktotrophic development similar to that found in extant *Golfingia pellucida* is primitive for sipunculans, we can then

speculate on the derivation of sipunculan developmental patterns. We can hypothesize that the ancestral larva common to sipunculans, annelids, and molluscs was a feeding trochophore with prototrochal and metatrochal ciliary bands and an opposed-band mechanism of feeding, and that this larva metamorphosed by processes of elongation and reduction of cilia to a benthic adult (cf. Jägersten 1972). The ancestral egg which gave rise to this larva was presumably low in yolk and covered by a thin egg envelope. In contrast, in the presumably primitive development of *G. pellucida* the eggs are moderately yolky and covered by a thick egg envelope which persists in the trochophore, so precluding feeding. The non-feeding trochophore is followed by a feeding larva, the pelagosphera, which then metamorphoses into the benthic adult. To derive the supposed primitive development of sipunculans from that of an ancestor, we can propose an increase in yolk of the egg associated with an increase in the thickness of the egg envelope. The additional yolk, mostly stored in the prototroch cells, served to provide nutriment for the developing trochophore while enclosed by the envelope. The feeding stage, thus retarded, occurred later in development when the envelope overlying the anus and stomodaeum ruptured and the gut became functional. The prototroch of the ancestral trochophore was retained in the trochophore of sipunculans for locomotion. The metatroch, used for feeding in the ancestral trochophore, did not develop in the non-feeding sipunculan trochophore, but appeared in the pelagosphera in which it assumed the function of locomotion rather than feeding. The prototroch was reduced in the pelagosphera and a new method of feeding adopted. The ciliated ventral head and lower lip, derived from the stomodaeum, along with the accessory organs of the mouth, buccal organ, and lip gland, took over the process of feeding. According to Jägersten (1972) the ciliation of the head and buccal organ were ancient adult characters, used by the adult for crawling and feeding, and were shifted to the larval stage.

If then, we assume that a development similar to that of *Golfingia pellucida*, characterized by a moderately yolky egg and a relatively short-lived planktotrophic pelagosphera, is primitive for the phylum, then two evolutionary sequences become apparent. One sequence would diverge toward a direct development with no pelagic stage, and the other toward a development with a planktotrophic larva specialized for a prolonged existence in the plankton (Fig. 18.5). The first sequence would evolve towards development such as that exhibited by *Themiste pyroides* and *Phascolion cryptus* in which the increase in yolk correlated with the large size of the non-ciliated prototroch cells and an increased significance in their nutritive function. Beginning this sequence would be a development such as that of *Golfingia vulgaris*, *G. pugettensis*, and *Themiste alutacea*, in which there is a trochophore as well as a lecitho-

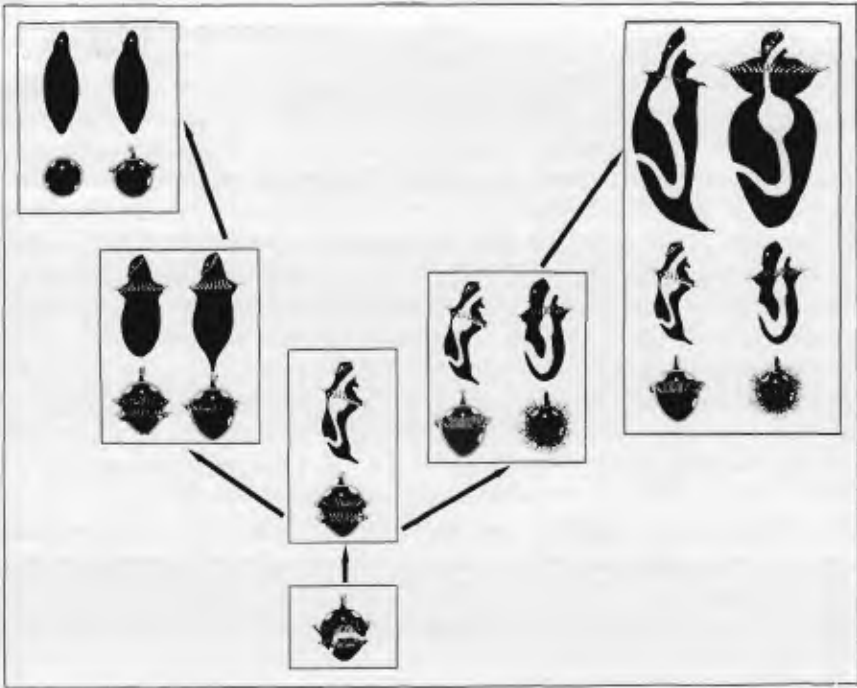


Fig. 18.5. Hypothetical scheme for evolution of developmental patterns within the Sipuncula. The ancestral development, represented at the bottom, is presumed to have had an egg with simple envelope and a planktotrophic trochophore with prototrochial and metatrochal bands of cilia. Derived from this, the primitive development of sipunculans probably had an egg with the thick egg envelope characteristic of all present-day species, a non-feeding trochophore and a newly evolved, feeding pelagosphera similar to that of *Golfingia pellucida*. From this, two evolutionary sequences are proposed, based on extant developmental patterns. The left branch shows a tendency to an increase in yolk and a reduction in pelagic stages via an intermediate lecithotrophic pelagosphera. On the right the sequence is towards a reduction in yolk and a prolongation of the pelagic phase by the large pelagospheras of the open ocean. See text for detailed explanation.

trophic pelagosphera. Intermediate in this sequence is the development of species such as *Phascolion strombi* and *Phascolopsis gouldi* which show a pelagic trochophore but no pelagosphera stage. The highly modified development of *Golfingia minuta*, occurring within the protective tube of the parent, is considered to be derived from this line. The second sequence would evolve towards a reduction in yolk in the egg, a highly modified trochophore as occurs in *Sipunculus nudus*, and the long-lived planktotrophic pelagosphera of the open ocean. The greatest modification in this sequence is found in the trochophore of *S. nudus* in which the prototroch grows over the entire larva.

All species of planktotrophic larvae studied thus far have terminal attachment organs, except those of species of *Sipunculus*. Assuming *Sipunculus* to have the most modified development, we can conclude that the terminal organ is a primitive feature for sipunculan pelagospheras. In the presumably primitive development of *Golfingia pellucida*, the terminal organ remains prominent and functional until metamorphosis into the juvenile. However, in *G. misakiana*, a species with a prolonged planktonic larva, the terminal organ is highly developed in the young pelagosphera as an organ of attachment, whereas in the older oceanic pelagosphera it is considerably reduced and rarely extended. Based on these observations, and on the adaptations previously mentioned for bottom feeding in pelagosphera larvae, it can be assumed that primitively the pelagosphera was 'bentho-pelagic', i.e. it remained close to the substratum, whereas the long-lived planktotrophic pelagosphera evolved more recently. Terminal organs are also present in some but not all lecithotrophic pelagospheras. In species in which the terminal organ is missing, such as *Themiste alutacea*, terminal glands produce a substance by which the lecithotrophic larva may adhere to a substratum. Thus, in lecithotrophic larvae the presence of the terminal organ is considered to be a derived feature.

Phylogenetic affinities

Developmental evidence for phylogenetic affinities of sipunculans has been discussed and reviewed by many authors (Hatschek 1883; Gerould 1906; Åkesson 1958; Rice 1967, 1975a; Clark 1969; Jägersten 1972). The typical spiral cleavage in sipunculans relates them by definition to annelids and molluscs as members of the Protostomia. As in other protostomes, the stomodaeum forms at the site of the blastopore and the coelom develops by schizocoely. Cleavage, gastrulation, and closure of the blastopore in sipunculans, polychaetes and many molluscs result in a trochophore larva characterized by an apical plate bearing a tuft of cilia, an equatorial band of ciliated prototroch cells, a ventral median stomodaeum below the prototroch, and lateral bands of mesoderm on either side of the gut. The anus of sipunculans does not form at the posterior blastopore as in annelids, but arises later as a new formation in a dorsal position. Another difference is that sipunculan trochophores, being enclosed by a thick egg envelope, are always non-feeding, even in species with low yolk content. A protonephridium, found in the trochophores of many annelids and some molluscs, is lacking in sipunculans, but this is true also in non-feeding larvae of polychaetes.

Resemblances of sipunculan development to that of polychaetes in particular include the similarity of prototrochal and metatrochal bands of cilia, the retention of the egg envelope in some species to form the larval cuticle, and the development of the nervous system. Although the ventral nerve cord, usually double in polychaetes, is single in the development of most sipunculans, exceptions have been noted for *Phascolosoma agassizi* and *Golfingia pellucida* which relate the sipunculans more closely to the annelids. The former has a double nerve cord in early larval stages and the latter shows an incipient splitting in the young pelagosphera larva.

Differing from annelids, sipunculans lack segmentation during both developmental and adult phases. Larval epidermal papillae appearing in pairs or transverse rows in some species are not considered indicative of segmentation as it occurs in polychaetes nor are their bristles homologous to polychaete chaetae (Clark 1969; Rice 1975a).

As in molluscs, the apical cross at the 48-cell stage of sipunculans is in the radial position, representing the sagittal and frontal planes of the future embryo rather than in the interradial position of polychaetes. The highly modified and hypertrophied prototroch of *Sipunculus nudus* has been proposed as homologous to the velum of molluscs. Gerould (1906) noted that the prototroch is shed in this species as is the velum of molluscs, but as pointed out by Åkesson (1958) the velum is shed at the end of the pelagic stage and the prototroch of *Sipunculus* at an early stage of development after which another ciliary band, the metatroch, takes over the function of locomotion for the larva. Less convincing is the comparison of the lip of the planktotrophic pelagospheras to the foot of veliger larvae and the supposed homology of the lip gland and buccal organ of pelagospheras to the pedal gland and radular sac of veligers of chitons.

Trochophores of sipunculans have been compared to those of both polychaetes and molluscs. Gerould (1906) noted the resemblance of the trochophore of *Golfingia vulgaris* to that of the polychaete *Amphitrite* and also the likeness to trochophores of *Chiton*, *Patella*, and other molluscs. However, there is no larval stage of either polychaetes or molluscs that is comparable to the pelagosphera—a specialized larval form unique to the Sipuncula.

The differences in developmental features from both molluscs and annelids suggest that the Sipuncula is a distinctive group within the Protostomia. The lack of segmentation and the retention of larval organs in the adult as well as the simple organization of the adult are assumed to be primitive features. Thus it is concluded that sipunculans are a primitive group derived from the annelidan-molluscan stem and closely related to the common ancestor of annelids and molluscs.

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