Reprinted from:
REPRODUCTION OF MARINE INVERTEBRATES, VOL. II

ACADEMIC PRESS, INC.
New York San Francisco London

Chapter 4

SIPUNCULA

Mary E. Rice

4.1 Introduction

The phylum Sipuncula is a small group of unsegmented coelomate marine worms noted for its lack of morphological diversity. Comprised of 17 recognized genera and approximately 320 species, the group is distributed throughout the polar, temperate, and tropical oceans (Stephen and Edmonds, 1972). The worms dwell in benthic habitats, ranging from intertidal shores to abyssal depths. Commonly they burrow into sand, mud, or gravel and, in the tropics, are frequently found in burrows within dead coral or other calcareous rock. Some species inhabit discarded mollusc shells and others live under rocks or wedge themselves into crevices.

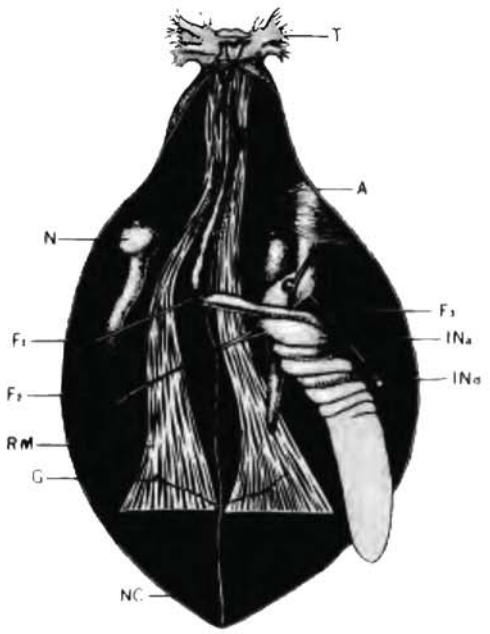


FIG. 1. Dissected specimen of *Themiste lissum*. A, anus; F_1, F_2, F_3, fixing muscles of the digestive tract; G, gonad; N, nephridium; NC, nerve cord; RM, retractor muscle; T, tentacles; INd, descending intestine; INa, ascending intestine. (Redrawn from Fisher, 1952.)

The adult body consists of an elongated cylindrical trunk with slender anterior introvert which may be withdrawn into the trunk by the contraction of one or more pairs of retractor muscles. The introvert is usually terminated by tentacles surrounding or dorsal to the mouth. The essential features of sipunculan anatomy are illustrated in Fig. 1, a dissected specimen of *Themiste lissum*. A long narrow esophagus, extending the length of the introvert, is continuous with the intestinal spiral in the trunk. Recurved, with descending and ascending limbs typically coiled about one another, the intestine opens to the exterior in a dorsal anus commonly located on the anterior trunk. Two nephridia, reduced in some species to one, open through ventrolateral pores usually at the approximate level of the anus. The nervous system includes a supraesophageal ganglion, circumesophageal connectives, and a ventral, unpaired and unsegmented median nerve cord. The gonad is commonly located at the base of the ventral retractor muscles.

The Sipuncula are classified as coelomate protostomes. Cleavage is spiral, the mouth forms in the position of the blastopore, and the coelom

is formed by schizocoely. Characteristically there is a trochophore larva which in some species may be followed by a second larval type, the pelagosphera. A protonephridium, found in developmental stages of many protostomes, is absent in sipunculans. The complete lack of segmentation either during development or in the adult, distinguishes the Sipuncula from the Annelida and, in the opinion of most contemporary zoologists, justifies the recognition of the group as a separate phylum.

4.2 Asexual Reproduction

Asexual reproduction is known to occur in two species of sipunculans. A small, rock-boring species from the Caribbean Sea, *Aspidosiphon brocki,* reproduces asexually, dividing into two unequal parts (Rice, 1970). A constriction separates the smaller posterior daughter portion from the larger parent at a distance approximately one-fifth of the total length of the trunk from the posterior extremity (Fig. 2a). Before the completion of fission, the parent portion and the daughter portion each regenerates the structures essential to the formation of a new individual. In the case of the daughter the regenerated structures include the entire anterior body and introvert, anterior gut, retractor muscles, and nephridia. Adult structures contributed by the parent and incorporated into the daughter are one or two coils of the posterior intestinal spiral, posterior parts of the spindle muscle and ventral nerve cord, and coelomocytes (Fig. 3). The formation of the juvenile is completed at the time of detachment from the adult or shortly thereafter when the newly regenerated introvert is everted (Fig. 2c). The parent regenerates only the posterior body wall which is formed as an invagination anterior to the constriction and is everted at the time the daughter individual is detached. Asexual reproduction is a naturally occurring phenomenon in *Aspidosiphon brocki* and has been found in 15% of the specimens collected in a population in Key Largo, Florida. Sexual reproduction has not been observed in this species, although gonadal tissue is present in most individuals at the base of the retractor muscles.

A second asexually reproducing species, *Sipunculus robustus,* has been reported to reproduce both by transverse fission and lateral budding (Rajulu and Krishnan, 1969; Rajulu, 1975). The posterior third of the animal may constrict off by transverse fission to form a new individual or the posterior one-half or two-thirds may give rise to as many as 5 individuals simultaneously. The newly formed organs and structures of the daughter develop from a blastema composed of coelomocytes and pro-

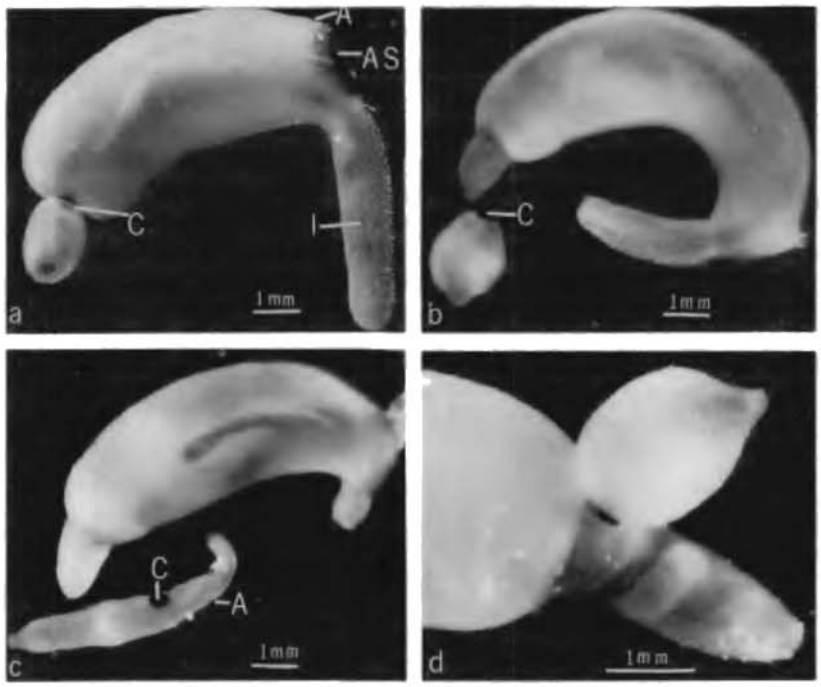


FIG. 2. Asexual reproduction in *Aspidosiphon brocki*. (a) Animal showing posterior constriction. (b) Recently separated parent and daughter. Note everted, newly regenerated posterior end of parent and black anterior cap of daughter. (c) Parent and juvenile, 2 days after separation. Regenerated anterior end of juvenile has been everted. (d) Posterior end of parent and daughter individual in process of separation. Regenerated posterior of parent has been everted and daughter remains attached only by fragments of the black material of the collar. A, anus; AS, anterior shield; C, collar on constricted animal or cap on daughter individual and juvenile; I, introvert. (From Rice, 1970.)

liferations of epidermal and gut cells of the parent. Most of the internal organs are formed before fission, but tentacles, introvert, and anus develop after detachment. Lateral buds may be formed in the region posterior to the anus, and at the time of separation from the parent the daughter individual is completely formed. Asexual reproduction in *Sipunculus robustus* has been observed only under conditions of stress such as maintenance of animals in stale sea water in the laboratory.

Although asexual reproduction has been only recently reported in sipunculans, their regenerative capabilities have been recognized for some time. In experimentally induced regeneration of the introvert of several species, formation of anterior gut, retractor muscles, and brain have been

FIG. 3. Diagram of dissected posterior end of constricted *Aspidosiphon brocki* illustrating internal structures of daughter and posterior portion of parent. C, collar; E, esophagus; EP, epidermal invagination; G, gonad; INa, ascending intestine; INd, descending intestine; N, nephridium; NC, nerve cord; R, rectum; RM, retractor muscle; S, internal noncellular sheet of constriction; SM, spindle muscle. (From Rice, 1970.)

observed (Schleip, 1934; Wegener, 1938). Ectodermal elements have been presumed to form from a strand of regenerative cells in the nerve cord and mesodermal elements from coelomocytes. The literature on regeneration in sipunculans is reviewed by Hyman (1959).

4.3 Sexual Reproduction

4.3.1 Sexual Dimorphism

As a rule sipunculans are dioecious; however, external signs of sexual dimorphism are entirely lacking. Only in those species in which the body wall is translucent and the oocytes are of a distinctive pigmentation is it possible to ascertain by gross examination whether an individual is male or female. For example, in mature specimens of *Phascolosoma perlucens*

the female will appear bright red in coloration due to the pigmentation of the oocytes within the coelom, whereas the males, because of concentrations of coelomic sperms, will be pale yellow or white. In many sipunculans it is possible through most of the year to determine sex without permanent injury to the animal by extracting a small sample of coelomic gametes with hypodermic syringe and needle and examining the sample microscopically for developing oocytes or spermatocytes.

4.3.2 Hermaphroditism

Hermaphroditism has been documented in only one species of sipunculan, *Golfingia minuta* (Åkesson, 1958). In this species, reported to be a protandrous hermaphrodite, coelomic oocytes and spermatocytes may occur simultaneously or only oocytes may be present. Coelomic oocytes of those animals with gametes of both sexes are immature. As the breeding season progresses, the percentage of animals with both male and female coelomic gametes decreases, and the percentage with oocytes only increases. It has been assumed, therefore, that the spermatocytes require a shorter time than the oocytes to mature and that an animal functions as a male before the definitive maturation of the oocytes is completed. The gonad is divided into specialized regions, the more median section near the ventral nerve cord producing only oocytes and the more lateral parts giving rise to both oocytes and spermatocytes.

An unexplained prevalence of females has been recorded in populations of some species. In a collection of 200 specimens of three different species of *Golfingia* (*G. elongata*, *G. vulgaris*, and *G. minuta*), Keferstein (1863) found only females. Claparède (1863) examined hundreds of specimens of *Golfingia elongata* and found only one or two males. In an Indian population of *Themiste signifer*, Awati and Pradhan (1936) reported a ratio of 1 male to 60 females. Cole (1952), in a study of the morphology of *Golfingia pugettensis*, found only 2 males in 100 specimens. In a later study on the development of this same population, the male to female ratio was 50:37 with 13% of undetermined sex (Rice, 1966).

4.3.3 Anatomy of the Reproductive System

The gonad of most sipunculans extends as a narrow digitate band of tissue along the base of the two ventral retractor muscles, extending from or near the lateral edge of one muscle, under the ventral nerve cord to the lateral edge of the other muscle (Fig. 1). In species such as *Phascolion strombi*, in which the number of ventral retractors has

been reduced to one, the gonad may be asymmetric, extending from the ridge on the base of the muscle anteriorly for a short distance along one side of the ventral nerve cord. Male and female gonads are similar in form, although in the male the gonadal digitations are frequently more numerous and thinner. The length of the digitations may vary from 0.1 to 0.5 mm and in those species with a definite breeding season, the gonad may disappear or be considerably reduced in size during part of the year.

Enclosed by a peritoneal sheath and suspended by a peritoneal mesentery, the gonad has been presumed to originate from peritoneal cells which are transformed into gonia in the proximal region of the organ (Andrews, 1889; Hérubel, 1908). Within the gonad the gonocytes are found in a gradient of progressively advanced stages from proximal to distal ends of the digitations. At the distal border of the gonad the gonocytes are released into the coelom where, as freely floating cells bathed in coelomic fluid, they continue to grow and differentiate until the time of spawning when they are accumulated from the coelom into the nephridia.

The nephridium of a sipunculan is an elongated tubular structure with two openings, one external, the nephridiopore, and one internal, often referred to as the nephrostome (Fig. 4). Both openings are located

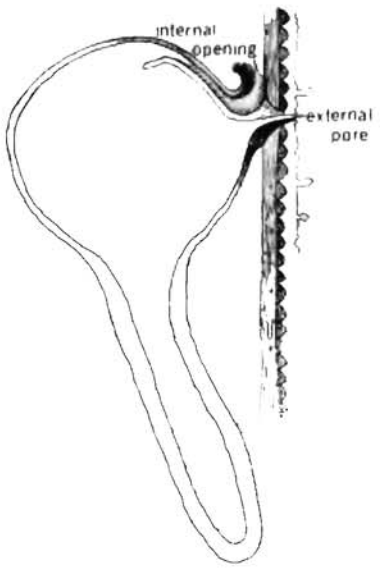


FIG. 4. Diagram of longitudinal section through nephridium and body wall of *Phascolosoma varians*. (From Shipley, 1890.)

at or near the anterior point of attachment of the nephridium to the body wall. The internal opening is ciliated and funnel-shaped and frequently bordered by a crescentic lip. It joins the nephridial and coelomic cavities by way of a narrow ciliated canal, and it is through this canal that coelomic gametes are directed into the nephridium before spawning by some still unknown mechanism. After a short period of storage in the nephridium the gametes are discharged to the exterior through the nephridiopore.

4.3.4 Origin of the Germ Cells

There is no evidence concerning the origin of the germ cells in sipunculans. The similarity in cytology of peritoneal cells and oogonia has led to the assumption in the past that germ cells arise from the peritoneum surrounding the base of the gonads. Andrews (1889) proposed that ". . . the nuclei of the peritoneum multiplied rapidly to form a mass of germ nuclei." Later he amended his proposal to suggest that germ cells might be retroperitoneal rather than peritoneal in origin (Andrews, 1890). Hérubel (1908), in an extensive treatise on the biology of sipunculans, stated that the oocytes ". . . sont certaines cellules péritonéales qui se différencient."* Although Gonse (1956a) did not refer to the source of the oogonia, he noted that the cells in the proximal region of the gonad resembled those of the peritoneum. A similarity between peritoneal and oogonial cells as well as an apparent elaboration or thickening of the peritoneum during the period of gonadal growth has been reported (Rice, 1973).

The assumption that germ cells are derived from peritoneum does not take into account the possibility of segregation of the germ line in early developmental stages. The only observation of germ cells during development was made by Gerould (1907). He found what he termed "reproductive cells" at the base of the ventral retractor muscles in larvae of *Golfingia vulgaris* and *Phascolopsis gouldi* at 2 to 3 weeks of age, but he did not follow the embryological derivation of these cells. Until more information is available any consideration of the origin of the germ cells in sipunculans remains speculative.

4.3.5 Cytodifferentiation of the Gametes

4.3.5.1 Differentiation of Ovarian Oocytes

In the ovary of sipunculans, cytodifferentiation occurs in sequential stages from proximal to distal ends, beginning with the oogonia or mitoti-

* Oocytes "are certain peritoneal cells which are differentiated."

cally dividing cells at the proximal end and progressing distally through the successive nuclear states of the meiotic prophase of the primary oocyte. At the distal border the cells have progressed to the diplotene stage with notable increases in nuclear and cytoplasmic volumes. It is at this stage that the oocytes are liberated into the coelom to undergo the remainder of their growth. Based on the successive nuclear changes, Gonse (1956a) has recognized seven regions in the ovary of *Golfingia vulgaris* (Fig. 5). In the first and most proximal region, oogonia are characterized by small nuclei similar to peritoneal cells and the divisions are mitotic. Cells of the second region are transitional from oogonia to the leptotene stage of the first meiotic prophase. The chromosomes are first condensed into balls in the telophase of the last mitotic division, then despiralized and grouped at the thin end of the nucleus. A nucleolus appears in this stage and the nucleus is increased in size. Region 3 consists of the leptotene stage. The chromosomes are strongly despiralized and the zone is narrow, indicating rapid change. The fourth and fifth regions are, respectively, the zygotene stage of chromosomal synapsis and the pachytene stage with thickened and elongated chromosomes in the bouquet form. The diplotene stage marks the sixth region; chromosomes are spiralized and tetrads are formed. In the seventh and most distal region the prophase is completed. Chromosomes are despiralized, nucleoli appear, and the nuclear and cytoplasmic volumes increase. Other authors have mentioned similar nuclear gradients in ovaries of *Phascolion strombi*, *Golfingia minuta* (Åkesson, 1958), *Phascolosoma agassizi*, *Golfingia pugettensis* (Rice, 1974), and *Phascolosoma arcuatum* (Green, 1975).

Gonse (1956a) noted, in addition to the oocytes in the ovary of *G. vulgaris*, small cells which he designated as "annex cells" and assumed to be abortive oocytes. These cells, later to become the follicle cells of the coelomic oocytes, are first distinguishable in region 2 and undergo the same meiotic changes as the developing oocytes. In the ovaries of other species, similar small cells, assumed to become the follicle cells of later stages, have been found among the oocytes; however, meiotic changes have not been observed and they have been interpreted as infolded peritoneal cells rather than as abortive oocytes (Åkesson, 1958; Rice, 1974).

4.3.5.2 Differentiation of Coelomic Oocytes

After liberation from the ovary, oocytes of sipunculans undergo vitellogenesis and the major portion of their growth as freely floating cells suspended in coelomic fluid and surrounded by coelomocytes. Commonly the oocytes break off from the ovary in clumps of 10–20 cells, interspersed

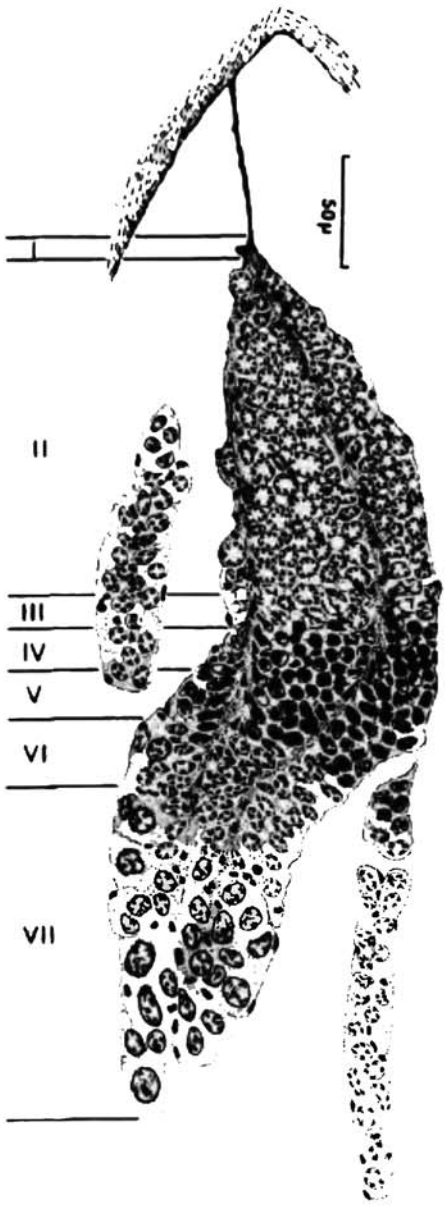


FIG. 5. Diagram of longitudinal section of ovary of *Golfingia vulgaris*. Ovary is suspended by peritoneal mesentery from base of retractor muscle. Seven regions of successive nuclear changes are designated. I, oogonia; II, transitional from oogonia to first meiotic prophase; III, leptotene stage; IV, zygotene stage; V, pachytene stage; VI, diplotene stage; VII, completion of first meiotic prophase; chromosomes despiralized, nuclear and cytoplasmic volumes increased. (From Gonse, 1956a.)

with smaller "annex" or peritoneal cells. Once in the coelom the clumps soon disperse into single oocytes, the smaller cells arranging themselves around the periphery of the oocytes to become the follicle cells. An exception is found in oocytes of *Phascolosoma*, which are usually detached from the ovary as single cells and lack a covering of follicle cells. During the period of coelomic growth the volume of an oocyte increases as much as 200 or more times.

Coelomic oogenesis has been studied in four species: *Golfingia vulgaris* (Gonse, 1956a,b, 1957a,b), *Golfingia ikedai* (Sawada *et al.*, 1968), *Golfingia pugettensis*, and *Phascolosoma agassizi* (Rice, 1974). Incidental observations on oogenesis in additional species are found in various studies on development or reproductive cycles (Gerould, 1907; Åkesson, 1958; Green, 1975). In the most comprehensive of the studies on oogenesis, Gonse has investigated cytological, cytochemical, and physiological properties of coelomic oocytes of *Golfingia vulgaris*. He distinguished six stages of coelomic oocytes, which he referred to as 0, 1, T, 2, 3, and M. Stage 0 is represented by clumps of oocytes and "annex" cells recently detached from the ovary and stage M is the final or mature stage which precedes nephridial accumulation and spawning. Stages 0, T, and M are considered to be transitory. Each stage is defined cytologically by size, appearance of nucleus, and kind and localization of cytoplasmic inclusions. Characteristics of the stages are summarized in Fig. 6. Similar stages of growth have been demonstrated in coelomic oocytes of other species, although specific variations occur in the persistence of follicle cells, size and shape of oocytes, structure of egg envelope, and localization and relative time of appearance of cell inclusions.

Follicle cells, when present, are detached during coelomic oogenesis, but the relative time of detachment varies in different species. Detachment occurs in *Golfingia vulgaris* (stage T) and *G. ikedai* when the oocytes reach a diameter of 60 μm. In *G. pugettensis* it is much later; in this species at the time the oocytes attain a diameter of 90 μm the follicle cells are raised up from the surface of the oocyte, standing out as blebs over the egg's surface, but they are not detached until the final stage of coelomic oogenesis when the oocytes are approximately 150 μm in diameter (Fig. 7a–d). In coelomic oocytes of *Themiste pyroides*, ranging in diameter from 30 to 190 μm, follicle cells are elevated from the surface of oocytes at a diameter of 100 μm and are retained to a diameter of 186 μm at which stage they are replaced by a jelly layer 50 μm in thickess.

While in the coelom as freely suspended cells, oocytes may manifest their first indication of polarity. Oocytes of species of *Phascolosoma*, released from the ovary as spherical cells usually 20 μm in diameter,

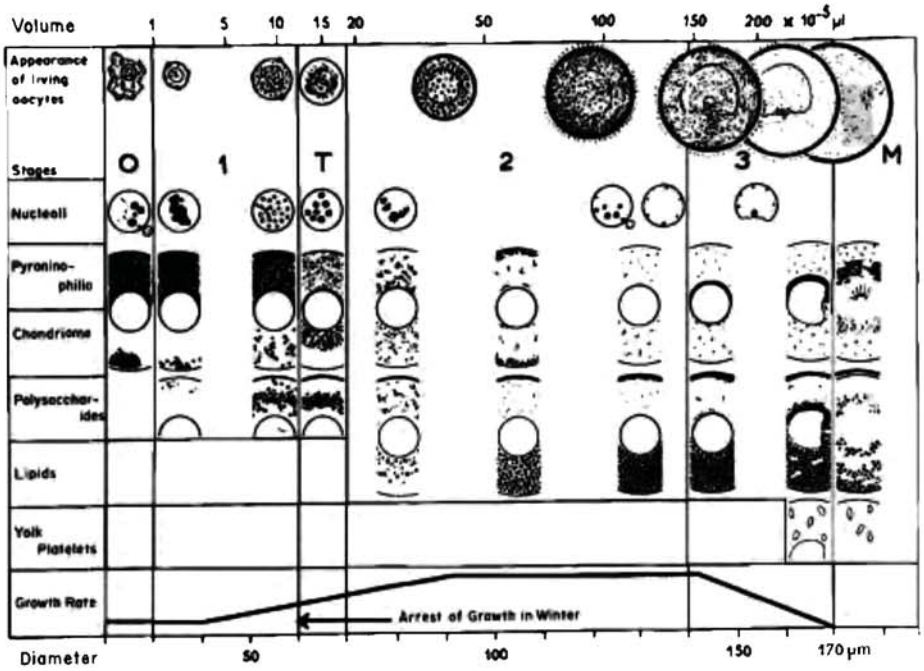


FIG. 6. Pictorial summary of differentiation of coelomic oocytes of *Golfingia vulgaris*. Six stages of coelomic differentiation are designated: O, oocytes in clumps, recently detached from ovary along with annex cells; 1, clumps dispersed as single oocytes with surrounding follicle cells, growth begins; T, transitory stage, follicle cells lost; 2, main phase of growth; 3, growth decelerated, burst of RNA production, appearance of yolk platelets; M, final or "mature" stage preceding nephridial accumulation, dissolution of germinal vesicle. (From Gonse, 1956a, p. 222.)

are changed during coelomic oogenesis to slightly flattened ellipsoids (Fig. 7c–h) which in *P. agassizi* measure 140 × 110 × 90 μm. The polarity and bilateral symmetry exhibited at this phase of oogenesis are retained in the developing embryo. Polarity in coelomic oocytes of *Golfingia vulgaris* first becomes apparent when the nucleus undergoes an asymmetric flattening in stage 3. Oocytes of most other species of *Golfingia* and *Themiste* are spherical throughout coelomic oogenesis with little or no manifestation of polarity.

The egg envelope of the fully differentiated coelomic oocyte of sipunculans is comprised of several layers and perforated by pore canals. Cytoplasmic extensions or microvilli extend through the pore canals and in a few species the entire egg is covered by an outer jelly coat. The egg envelope has been characterized cytochemically as a mucoprotein

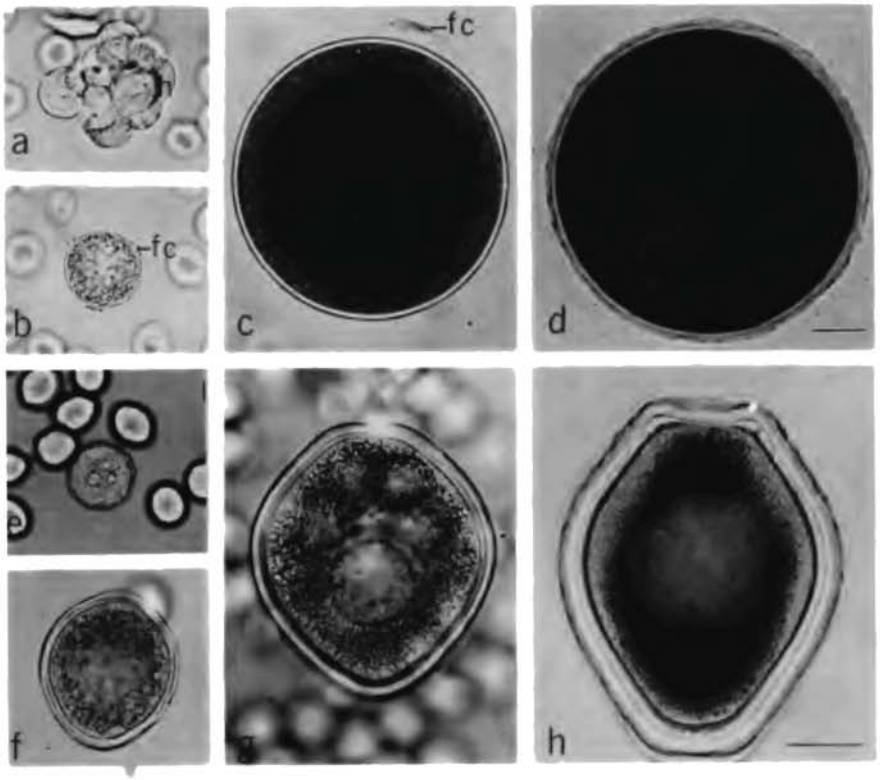


FIG. 7. Photographs of living coelomic oocytes. (a–d) *Golfingia pugettensis*; (e–h) *Phascolosoma agassizi*. Coelomocytes are seen out of focus in background. Scale, 25 μm. fc, Follicle cells. [From (in part) Rice, 1974.]

which is secreted by the oocyte during the period of coelomic growth (Gonse, 1956a; Rice, 1974).

The envelope of oocytes of *Phascolosoma agassizi* reaches a thickness of 10 μm and fully developed envelopes of *Golfingia pugettensis* and *G. ikedai* are 5 and 2 μm, respectively. In these latter species the number of layers may be as many as 14. In *P. agassizi*, three layers are distinguishable. As shown in electron micrographs, microvilli pass through pores in the inner and middle layers, branching into fan-shaped structures in the outer layer (Fig. 8). Tips of the microvilli are marked by an amorphous material or fuzz which consolidates in the latest oocytes as a homogeneous fringe around the egg and is expanded in the regions of the animal and vegetal poles. The inner layer gives a strongly positive reaction for mucoprotein and a weak response for protein whereas the

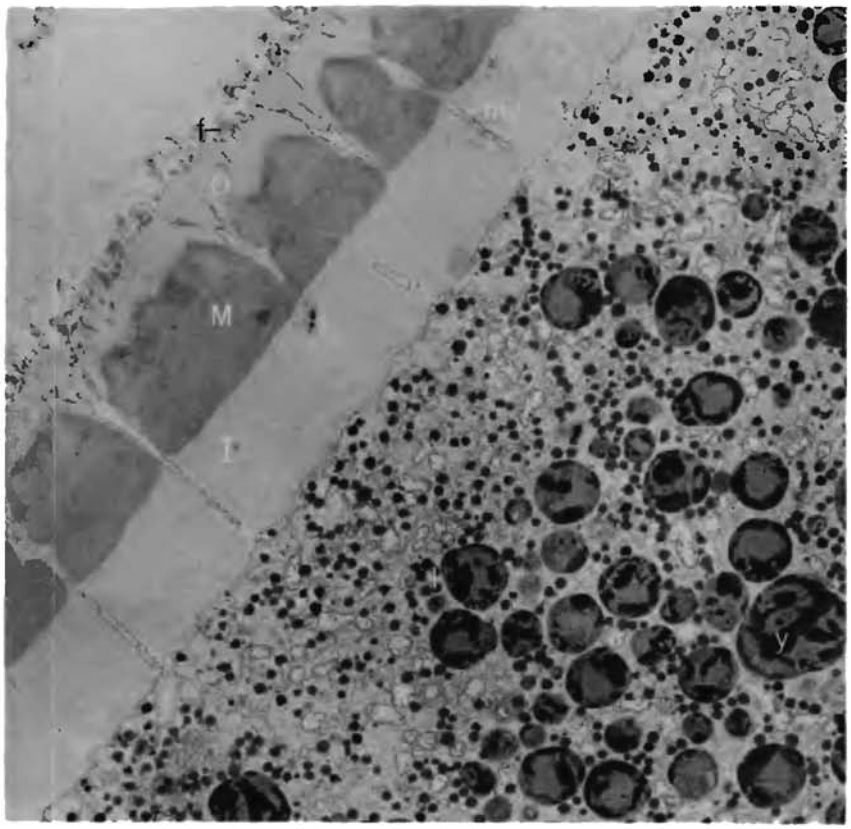


FIG. 8. Electron micrograph of a coelomic oocyte of *Phascolosoma agassizi*, showing egg envelope. O, M, I, Outer, middle, and inner layers of egg envelope; f, surrounding fuzz; l, lipid; mv, microvillus; y, yolk granule. Scale, approximately 1 μm.

reverse is true of the middle layer. The outer layer has been identified as an acid mucopolysaccharide and the surrounding fuzz as a mucoprotein.

On the basis of their electron microscopic studies on coelomic oocytes of *Golfingia ikedai*, Sawada *et al.* (1968) have proposed a scheme of envelope formation (Fig. 9). The completed envelope of this species is composed of a fibrous outer layer, a multilamellate middle layer, and a diffuse inner layer. The earliest oocytes show no indication of an envelope, but projecting from the surface are numerous microvilli with pinocytotic vesicles at their bases. As the oocyte grows, the diffuse material of the inner layer, presumed to be precursor substances extruded from the cell surface, condenses to form in succession the 14 middle

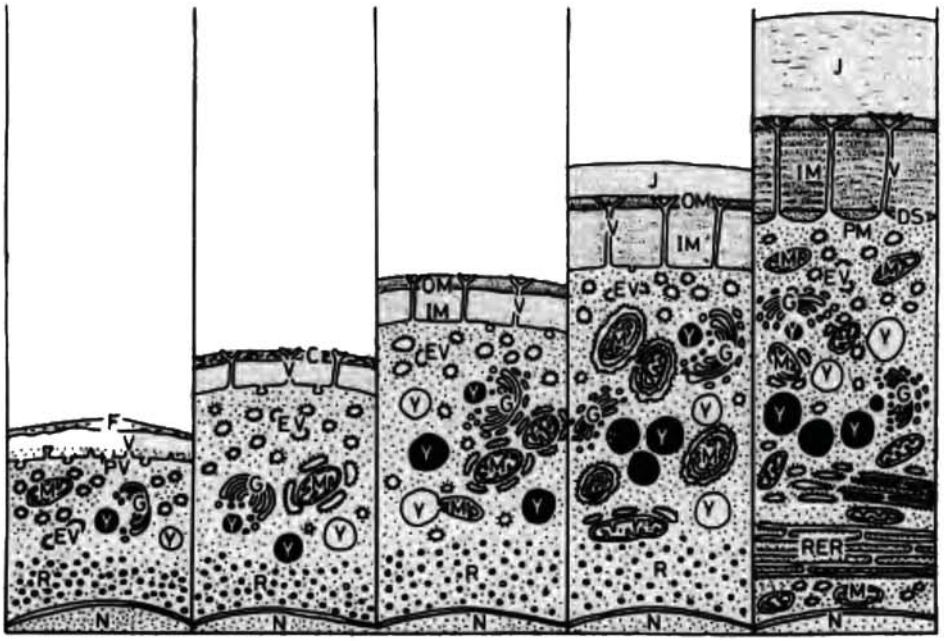


FIG. 9. Schematic representation of growth of coelomic oocytes of *Golfingia ikedai*, based on electron microscopic observations. C, chorion; DS, diffused substances; EV, endoplasmic vesicles; F, follicle; G, golgi body; IM, inner membrane; J, jelly layer; M, mitochondria; N, nucleus; OM, outer membrane; PM, plasma membrane; PV, pinocytotic vacuole; R, ribosome cluster; RER, rough-surfaced endoplasmic reticulum; V, microvillus; Y, yolk granule. (From Sawada *et al.*, 1968, p. 37.)

layers. The origin of the fibrous outer layer is uncertain, but the microvillar tips have been implicated. A jelly layer surrounding the oocyte in the latest stages may also be a secretion of the microvilli.

The nucleus in early oocytes is always rounded, occupying a central position in the cell. Later, but at characteristic times in different species, the nucleus is marked by numerous peripheral infoldings. In *Golfingia vulgaris* and *Phascolosoma agassizi* this occurs in the final stage of oogenesis; in the former the folds occur on only one side of the nucleus. The nucleus of *G. pugettensis* becomes scalloped much earlier, when the diameter of the oocyte is only one-half of its mature dimension. The nucleoli appear as numerous fragments in the early oocytes of *G. pugettensis* and *G. vulgaris*, later diminishing in *G. pugettensis* to two or three discrete nucleoli with few fragments and in *G. vulgaris* to several discrete spherical structures arranged around the periphery, one of which in the final stage dominates the others in size. The oocytes of *P. agassizi*

have 2–5 nucleoli throughout oogenesis, but in younger stages one is larger than the others.

Mitochondria, as observed in two species of *Golfingia* (Gonse, 1956a; Sawada *et al.*, 1968), are localized in early oocytes near the periphery of the cell, but as their numbers increase in later stages they move throughout the cytoplasm. In electron microscopic studies of *G. ikedai* the mitochondria are found to be fused with peripheral endoplasmic vesicles in early coelomic oocytes; in later stages they are associated with one of two kinds of yolk granules.

Pyroninophilia, interpreted as a probable indication of the presence of ribonucleic acid, has been demonstrated in the cytoplasm of coelomic oocytes of *Golfingia vulgaris*. Strong perinuclear concentrations occur in the earliest stages and again in the latest stage. Electron microscopy of the oocytes of *G. ikedai* has similarly revealed an early concentration of rough endoplasmic reticulum.

Vitellogenesis in *Golfingia vulgaris* is initiated soon after detachment of oocytes from the ovary when carbohydrate yolk granules, presumed to be galactogen, make their appearance at the periphery of the cells. As the oocytes develop the granules move toward the nucleus, then are dispersed toward the cortex where, in the final stage (stage M), they form a layer of cortical granules. A second polysaccharide, recognized histochemically as glycogen, appears as a diffuse substance in a perinuclear position late in coelomic oogenesis. Lipid does not appear until stage 2 when it is found in association with a mitochondrial mass in the perinuclear zone; later it disperses through the cytoplasm. At the time of germinal vesicle breakdown (stage M) the lipid is concentrated in the peripheral cytoplasm. Prominent elongate bodies or yolk platelets, not defined histochemically, but presumed to be proteinaceous yolk, occur in well advanced oocytes of stage 3 (Fig. 6). From investigations of physiological properties of the oocytes of this species, two peaks of exogenous respiration have been demonstrated during coelomic oogenesis, the earlier corresponding to the beginning of carbohydrate synthesis and both corresponding to periods of high concentrations of ribonucleic acid. The peaks of respiration were found to be coupled with metabolism of hexoses and pentoses (Gonse, 1957a,b).

In the elaboration of yolk there is little consistency among different species in the sequence in which carbohydrate, protein, and lipid make their appearance in the developing oocytes, or in the form and localization of the yolk granules within the cells. The carbohydrate yolk of *Phascolosoma agassizi* differs from that of *Golfingia vulgaris* in that it is associated with protein as a carbohydrate–protein complex in the form of distinctive yolk granules of irregular staining pattern. The gran-

ules are visible first in the perinuclear zone, rather than at the periphery as in *G. vulgaris,* and after dispersal through the cytoplasm they move in later stages away from the periphery leaving a clear cortical area. The earliest oocytes of *G. pugettensis* that have been examined show carbohydrate yolk granules midway between nucleus and periphery; no tests for protein have been made. Lipid in this species is present as large droplets at the periphery in the early oocytes, appearing relatively sooner than in *G. vulgaris.* It is then dispersed and finally, in late oocytes, as in *G. vulgaris,* it is localized in the peripheral cytoplasm. The large and rather rare lipid droplets of *P. agassizi* have not been seen until late in oogenesis, but very small granules, suspected to be lipid, are distributed through the cytoplasm in the earliest stages of coelomic oocytes. Characteristic of lipid, these small granules appear after centrifugation in the centripetal half of the egg and in electron micrographs they are seen as homogenous spheres without surrounding membranes (Fig. 8).

Recently spawned, unfertilized eggs of many species of sipunculans have been described in studies on development. Sizes of the eggs of 17 species can be found in Table II (see Section 4.4.2). All sipunculan eggs are encompassed by a thick characteristic envelope which is composed of several layers and perforated by pores. There is considerable variation among species in the thickness of the envelope and the number of its layers as well as in the size, shape, pigmentation, and yolk content of the eggs (Fig. 10 a–e). The eggs of most sipunculans are spherical, but those of a few species, such as *Paraspidosiphon fischeri* and *Golfingia minuta* are oval and those of the genus *Phascolosoma* are typically flattened ellipsoids, frequently with depressed apexes. Sipunculan eggs are commonly various shades of red or yellow. Large eggs with high yolk content, such as eggs of *Themiste,* may be white or grayish and eggs low in yolk, as found in species of *Sipunculus* and *Siphonosoma,* are transparent. At the time of spawning, the germinal vesicle of the eggs has broken down and meiosis is arrested in the first meiotic metaphase (see Sections 4.3.8, 4.4.1).

4.3.5.3 Differentiation of Male Gametes

Little is known about cytodifferentiation of male gametes. The cytology of the male gonad of *Phascolosoma arcuatum* has been reported to resemble that of the ovary, as described by Gonse (1956a) for *Golfingia vulgaris,* with the exception that the cytoplasmic volume of the distal spermatocytes is less than that of the oocytes in that position (Green, 1975). Spermatocytes break off from the testis as loosely associated clumps of cells which undergo two meiotic divisions and differentiate into spermatids while floating as morulae in the coelomic fluid. The cells within

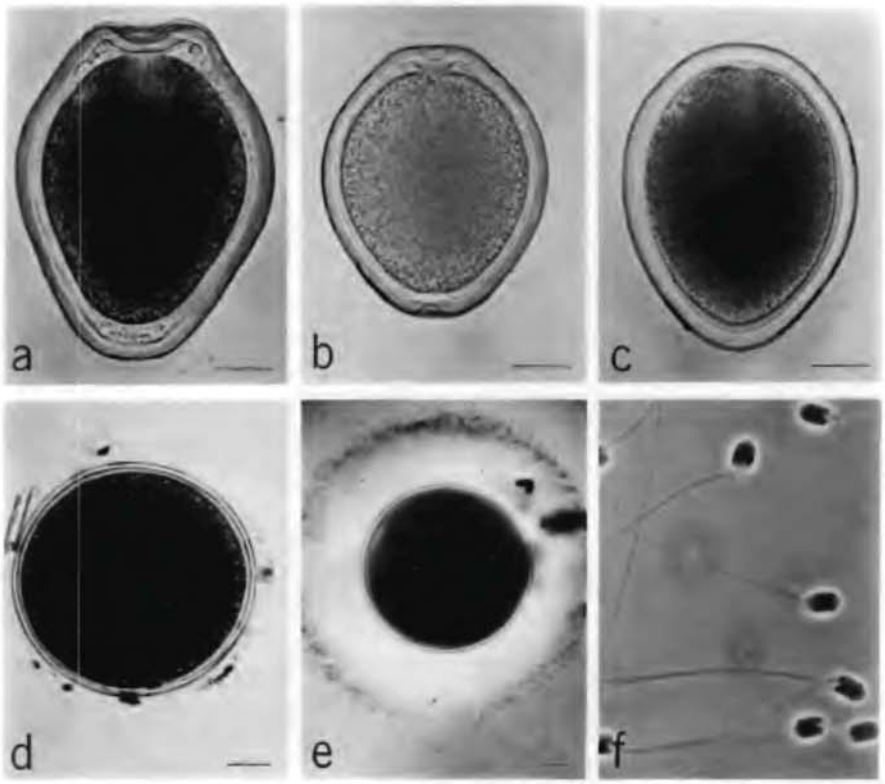


FIG. 10. Recently spawned gametes of sipunculans. Photographs of living eggs and sperms. Eggs are unfertilized. (a) Egg of *Phascolosoma agassizi*. (b) Egg of *Phascolosoma perlucens*. (c) Egg of *Phascolosoma antillarum*. (d) Egg of *Phascolion cryptus*. (e) Egg of *Themiste pyroides*. Note thick jelly layer. (f) Spermatozoa of *Themiste pyroides*. Scale a–e, 25 μm. (a and c from Rice, 1967. b, c, and d from Rice, 1975).

a clump increase in number as divisions occur and in each cell the cytoplasmic volume decreases and a flagellum develops. The clumps of differentiated spermatids break up into free spermatozoa in the coeloms of *G. pugettensis* and *Themiste pyroides* where they may be seen through most of the year. In *P. agassizi* a few spermatozoa are free in the coelom at the time of the breeding season, but the majority remain in clusters. In *P. arcuatum*, spermatids and free spermatozoa occur in the coelomic fluid only a short time before spawning.

Spermatozoa of sipunculans have the typical morphology of the primitive sperm as defined by Franzén (1956). Similar to those of other species which discharge their gametes freely into the seawater, they

are comprised of low acrosomal caps, midpieces each with four mitochondrial spheres, and long filamentous tails. The sperms of *Themiste pyroides* are somewhat modified in that they possess a highly developed acrosomal cap with pointed tip and swollen basal rim (Fig. 10f). It is probable that this increased complexity is associated with the thick jelly coat of the egg through which the sperms must pass (Rice, 1974).

4.3.6 Gametogenic Cycles

Annual reproductive cycles are known for four species of sipunculans: *Golfingia vulgaris* (Gonse, 1956b), *G. pugettensis* (Rice, 1966), and two populations of *Phascolosoma agassizi* (Rice, 1966; Towle and Giese, 1967), all from temperate waters, and one tropical species, *Phascolosoma arcuatum* (Green, 1975). Cycles have been defined in these studies by estimations of breeding seasons from observations on spawning, cytological examination of gonads, and measurements of coelomic oocytes throughout the year. Oocyte measurements have been expressed in relative frequencies and no absolute estimations are available of the various sizes of cells.

Golfingia vulgaris has a limited breeding season lasting from June to September in Roscoff, France. The gonad, however, is continuously active, releasing small oocytes into the coelom throughout the year, with reduced activity in the winter. Monthly measurements of coelomic oocytes over a year show that although oocytes are always present in the coelom their growth is arrested during part of the year (Figs. 6 and 11). In the winter all of the oocytes are small, i.e., less than 61 μm, and the size-frequency curve is unimodal, indicating arrested growth. Growth commences in the spring and with the appearance of a slightly larger group of cells the size-frequency curve becomes bimodal, the second mode at 66 μm, close to the first. In the summer the latter mode disappears and all sizes of oocytes are found: the smallest oocytes persist as a prominent mode, intermediate oocytes are present but in low frequencies, and a population of large oocytes makes its appearance, forming a second mode at 154 μm. In the autumn, at the conclusion of the breeding season, growth of small oocytes is again arrested and oocytes of intermediate and large size gradually disappear. Low frequencies are interpreted as indicative of a rapid rate of growth and high frequencies as slow growth. Thus, those populations that form prominent modes, that is, the small and large cells, grow more slowly than the cells of intermediate size (Fig. 6).

In the annual cycle of *Golfingia pugettensis*, endemic to the Northwest Pacific Coast of the state of Washington, the breeding season occurs

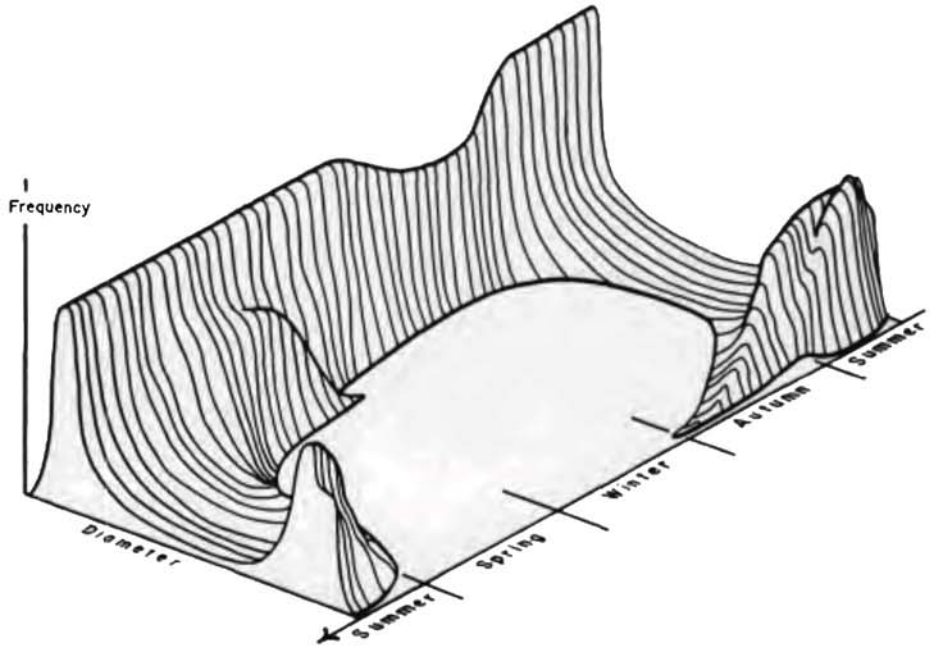


FIG. 11. Three-dimensional diagram of size-frequency curves of coelomic oocytes of *Golfingia vulgaris* over a period of 1 year. The absolute value of the frequency of the small oocytes is arbitrarily kept constant except in the autumn when the ovary is less active and the production of coelomic oocytes is decreased. (From Gonse, 1956b, p. 232.)

in October, November, and December. During this period size-frequency curves show, as for *G. vulgaris*, two major populations of coelomic oocytes, one of small oocytes and one of large, with only a small proportion of oocytes of intermediate size (Fig. 12). In January, after the breeding season, large oocytes are no longer present but the population of small oocytes persists and a new population of slightly larger cells becomes evident. This exists through the spring but disappears in the summer at the same time several small populations of cells of intermediate size are formed.

Although the breeding periods of *Golfingia pugettensis* and *G. vulgaris* are at different seasons, the latter spawning during the summer months, there is, nevertheless, the same sequence of oocyte growth with similar phases of apparent arrest and acceleration. However, the relative time of appearance of the various phases differs. In *G. pugettensis* there is an apparent arrest of growth of small oocytes during the breeding season, and after the large eggs are discharged by spawning, the small

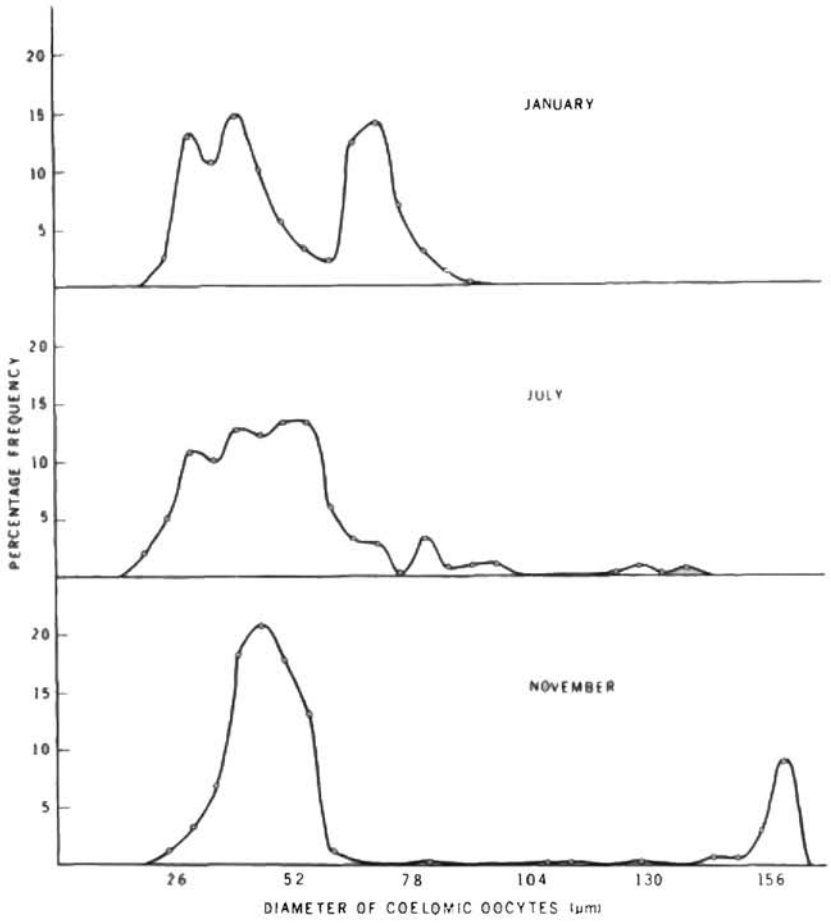


FIG. 12. Size-frequency curves of coelomic oocytes of *Golfingia pugettensis* (1962–63). In January 450 eggs were measured, 50 from each of 9 animals. In both July and November, 300 eggs were measured, 100 from each of 3 animals. (From Rice, 1966.)

eggs begin to grow. In *G. vulgaris* the frequency of small eggs passing into the intermediate stage is much higher; growth of small oocytes continues throughout the breeding season and is arrested only after spawning, the period of arrest lasting for several months. Thus during the period of spawning in *G. vulgaris* intermediate oocytes are prevalent. Those remaining after spawning are presumably resorbed. In *G. puget-tensis* by the time of spawning most of the intermediate cells have already given rise to mature cells and the growth of the oocytes has ceased or been considerably reduced.

The oogenic cycle of a population of *Phascolosoma agassizi* in the San Juan Archipelago off the Northwest Pacific Coast of Washington, differs from that of both *Golfingia pugettensis* and *G. vulgaris* in that oocytes of all sizes, small, intermediate, and large, are present throughout the year (Rice, 1966). An examination of the frequency polygons reveals that there are always two principal size groups of cells, the largest oocytes usually predominating except immediately after spawning (Fig. 13). This population of *P. agassizi* spawns from early June through August.

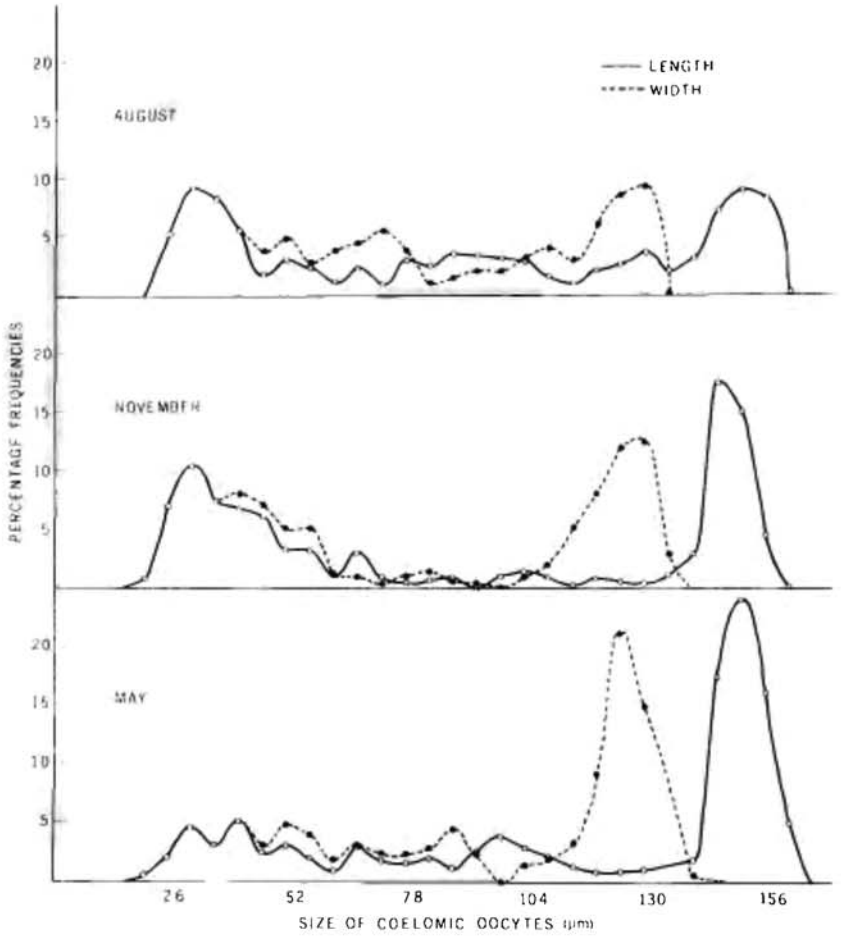


FIG. 13. Size-frequency curves of coelomic oocytes of *Phascolosoma agassizi* from the San Juan Archipelago, Washington (1964–1965). Measurements each month represent a total of 500 oocytes, 100 from each of 5 animals. (From Rice, 1966.)

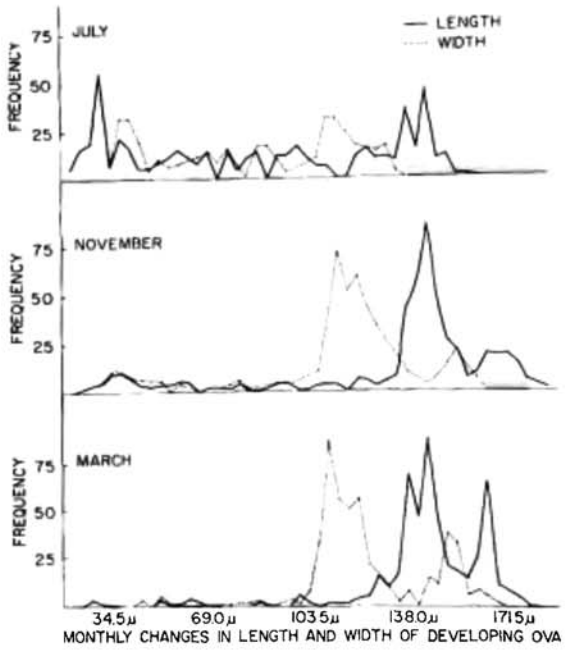


FIG. 14. Monthly changes in size of coelomic oocytes of *Phascolosoma agassizi* from Monterey Bay, California (1960–1961). Frequency distribution each month represents measurements of 500 oocytes, 20 from each of 25 females. [From Towle and Giese, 1967, fig. 1 (in part), p. 232.]

A population of the same species at Monterey, California spawns three months earlier, in March, and for a 3-month period following spawning both male and female gametes are absent in the majority of animals (Towle and Giese, 1967). By the fourth month, July, all sizes of coelomic oocytes are found, including high proportions of both small and large oocytes (Fig. 14). During the fall and winter the frequency of the small oocytes diminishes while the large oocytes proportionately increase until a month before the March spawning, when approximately 90% of the coelomic oocytes are large. By contrast, the month before the breeding season in the population from the San Juan Archipelago only 55% of the coelomic oocytes are in the large size group. An important difference in the oogenic cycles of the two populations seems to be the degree and duration of reduction in gonadal activity. The gonad of the Monterey animals is active for only a limited time during the year and after a temporary disappearance, again develops following the spawning season. In the animals from San Juan Island the gonad

is continually active but partially reduced before and during the breeding season.

In summary, the growth of the coelomic oocytes of *Golfingia pugettensis* and *G. vulgaris* is not continuous, but is characterized by phases of arrest and acceleration. In contrast, the coelomic oocytes of *Phascolosoma agassizi*, including both the population from Monterey and San Juan Island, appear to undergo continual growth, although not necessarily at a continuous rate, until the definitive size is attained.

A tropical species, *Phascolosoma arcuatum* from Queensland, Australia, breeds from December through February and, like the population of *P. agassizi* from Monterey, California, but unlike other species from higher latitudes, it lacks coelomic oocytes entirely for a 2-month period following the breeding season (Green, 1975). Moreover, in *P. arcuatum*, spermatocytes are released from the testis only a short time before breeding begins in December and development into spermatozoa is rapid. This is in contrast to other species of sipunculans in which male gametes are present in the coelom much of the year (Section 4.3.5.3).

4.3.7 Factors Influencing Gametogenesis

Little is known of the factors controlling the growth of gametes in sipunculans. It has been suggested (Åkesson, 1961a) that neurosecretory products may play some role in the reproductive cycle since Carlisle (1959), in studies of *Sipunculus nudus*, found a greater amount of neurosecretory material in animals before the breeding season than following it. Neurosecretory material has been demonstrated in other sipunculans (Gabe, 1953; Åkesson, 1961a), but its significance in reproduction has not been investigated.

The possible influence of exogenous factors such as temperature on gametogenesis of sipunculans is suggested by the difference in the duration of breeding seasons of some tropical and temperate species. Observations on spawning in certain tropical or subtropical species, e.g., *Phascolosoma perlucens, Themiste alutacea*, and *T. lageniformis*, indicate that breeding may occur throughout the year (Section 4.3.8). However, no information is available on gametogenesis in these species, and in other tropical species, such as *P. arcuatum*, the breeding season is known to be restricted, as it is in all species from temperate waters. In species with well-defined breeding seasons differences may be found in the time of initiation of breeding in the same species at different latitudes. At Monterey, California, *Phascolosoma agassizi* breeds 3 months earlier than at 11° farther north in the San Juan Archipelago, Washington (Rice, 1966; Towle and Giese, 1967). However, in two populations of *P. arcua-*

tum in Australia, separated by 8° in latitude, correlation between temperature and spawning is the reverse, that is, the population at the higher latitude spawns first (Green, 1975).

4.3.8 Spawning and Breeding Periods

Gametes are spawned from the nephridia by forceful ejection through the nephridiopores into the seawater where fertilization occurs. When spawning is imminent the nephridiopores are often swollen and the animals may become quite active, frequently extending and retracting the introverts. *Phascolosoma agassizi*, when maintained in an aquarium with sand and gravel, has been observed to extend the anterior end above the surface of the substratum so that the nephridia are well exposed, and at the time gametes are released the body is extended and turgid (Rice, 1966). Although usually in sipunculans all of the gametes from both nephridia are spawned at once, variations of this process have been observed. A male specimen of *Paraspidosiphon fischeri* spawned 7 times in the laboratory over a period of 40 minutes, first several times from the right nephridium, then from the left. *Phascolion cryptus,* with only a single nephridium, was observed to spawn short intermittent spurts of sperm over a period of 15 minutes (Rice, 1975).

Since sipunculans, along with the majority of marine invertebrates, spawn their gametes into the seawater, synchronization of spawning is essential to assure fertilization and the survival of the species. Synchronization in sipunculans is dependent on the regulation of two separate events: (1) uptake of coelomic gametes by nephridia; (2) release of gametes from the nephridia into the seawater. Factors controlling these events have not been analyzed, but hypotheses have been proposed for nephridial uptake and data have been accumulated on such aspects of spawning as sequence of male and female activity, time of day that spawning occurs, and intervals between spawnings within a breeding season.

Before spawning, gametes are accumulated into the nephridium from the coelom by way of the internal funnels or nephrostomes. The process of nephridial selectivity by which the most mature gametes, either oocytes or sperm, are taken into the nephridium, while the immature gametes and coelomocytes are rejected, is poorly understood. Since oocytes are known to enter the nephridium soon after breakdown of the germinal vesicle (Gerould, 1907; Åkesson, 1958; Rice, 1966) it can be assumed that nephridial selectivity is related to the initiation of maturation of the gametes. (It should be noted, however, that maturation divisions do not occur until after sperm entry; see Section 4.4.1.) Gerould (1907) noted in *Golfingia vulgaris* that before spawning the nephridia

became greatly distended with fluid, which he presumed to be seawater taken in through the nephridiopores. Assuming that the eggs selected by the nephridium were hydrotropic because of their lower specific gravity, Gerould proposed that these eggs were accumulated in the region of the nephrostome and directed by ciliary currents into the nephridia where they resorbed water. Åkesson (1958) further suggested that a chemical change occurring in the oocyte at breakdown of the germinal vesicle might alter the direction of nephridial cilia which would then move these eggs into the nephridium. In explaining nephridial uptake of mature sperm, Åkesson noted that seawater induced motility of coelomic sperm. He then hypothesized that as the nephridia filled with seawater, some of the water passed into the coelom through the nephrostomes and the mature sperm were activated to swim toward the increasing concentration of seawater to the nephridium.

Breakdown of the germinal vesicle in the coelom and dispersal of coelomic sperm clusters are apparent prerequisites for nephridial uptake. In many species oocytes of maximum size with intact nuclei are present in the coelom through much of the year (Section 4.3.6), but only during the breeding season shortly before spawning does dissolution of the germinal vesicle occur. Few studies have been made of the mechanisms regulating the maturation of gametes. Pasteels (1935) carried out *in vitro* studies on the breakdown of the germinal vesicle in coelomic oocytes of *Phascolion strombi*. He found that the addition of increasing quantities of calcium ions induced maturation of the eggs. In preliminary studies (Rice, 1966) it was found that crude extracts of coelomic sperm, coelomic oocytes, coelomocytes, brain, and muscle all induced germinal vesicle breakdown *in vitro* of coelomic oocytes of *Phascolosoma agassizi*. The majority of coelomic sperm in *P. agassizi* remain in the form of clusters until a short time before nephridial uptake when the clusters break down into free spermatozoa. Experimental dispersal of spermatid clusters was accomplished *in vitro* by exposure to hypertonic seawater. The resultant free spermatozoa became motile within 24 hours after transfer to seawater.

After nephridial uptake gametes remain in the nephridium only a brief period before spawning occurs. Gerould (1907) noted in *Golfingia vulgaris* and *Phascolopsis gouldi* that the nephridia filled with gametes a few hours before spawning. Åkesson (1958) reported that oocytes of *Phascolion strombi* underwent germinal vesicle breakdown 12 hours before spawning. In small transparent specimens of *Paraspidosiphon fischeri* in which nephridia were distended with gametes at the time of collection, spawning occurred within periods varying from 1 to 24 hours.

The sequence of male and female spawning is variable in different species. At least two species of sipunculans, *Golfingia vulgaris* and *Phascolion strombi* (Gerould, 1907, Åkesson, 1958), follow Thorson's rule of epidemic spawning in which the males spawn first, stimulating the spawning of the females (Thorson, 1946). In populations of *Phascolosoma agassizi* from Monterey, California (Towle and Giese, 1967) and from the San Juan Archipelago (Rice, 1967), spawning of females has been observed to precede that of males. In the latter population the female spawning may be followed by the male or either sex may spawn independently. Only a small proportion of the animals placed in one container spawned at the same time. It was found that spawning can often be triggered in the laboratory, if gametes are present in the nephridia, by a change to freshly aerated water or a sudden change in temperature. No observations have been made on the spawning of sipunculans under natural conditions in the field.

Gerould (1907), in his studies of *Golfingia vulgaris* and *Phascolopsis gouldi*, reported that spawning is confined to hours of darkness between 2000 and 0400 or 0500. He stated further that when animals were maintained continuously in a dark aquarium the rhythm was interrupted and spawning sometimes occurred during the day. His explanation for spawning at night was that the sipunculan's body relaxes in darkness, resulting in distension of the nephridia with consequent nephridial uptake of surrounding seawater and accumulation of mature hydrotropic eggs from the coelom. Spawning at night has also been reported for *Phascolion strombi* and *Golfingia elongata* (Åkesson, 1958, 1961a). However, in *Phascolosoma agassizi* spawning did not occur exclusively at night. Of a total of 89 spawnings, 9% spawned during daylight hours, 38% at night but in the artificial illumination of the laboratory, and 53% in darkness. Thus Gerould's explanation for night spawning would not be applicable in 47% of these cases. Again, in *Themiste pyroides*, of the spawnings of 20 animals recorded over a 3-year period, 55% occurred overnight, the remainder during daylight hours (Rice, 1967).

The only suggestion for periodicity in spawning in sipunculans is found in records for *Phascolosoma agassizi* (Rice, 1967). A total of 99 spawnings were recorded in the laboratory over a period of 3 years. The data, summarized in Fig. 15, reveal a peak of maximum spawning each year as well as a certain periodicity throughout the breeding season. In 1962 and 1963 the periodicity between spawning peaks ranged from 21 to 29 days. There was no obvious correlation of spawning periods with either tides or phases of the moon. Since these observations were made under uncontrolled conditions in the laboratory it cannot be assumed that the same periodicity exists in the natural habitat.

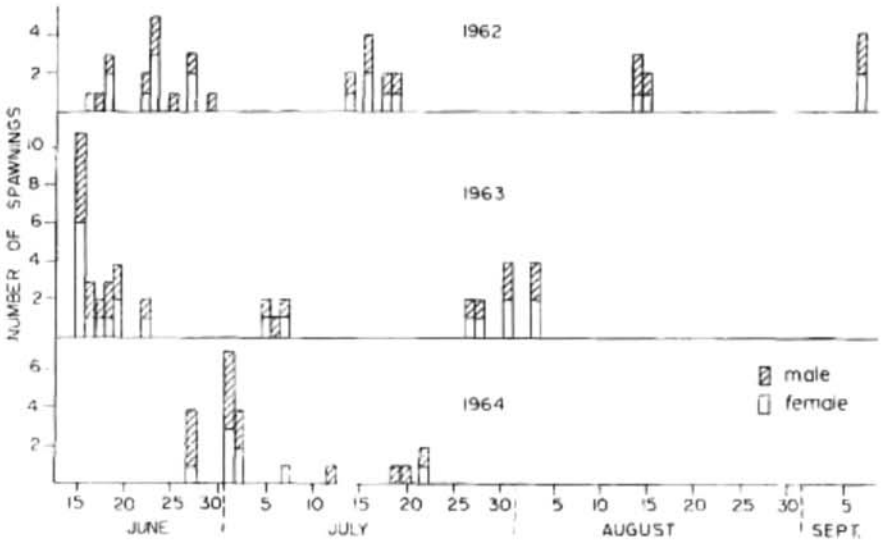


FIG. 15. Spawning dates and number of spawnings of *Phascolosoma agassizi* from the San Juan Archipelago, Washington. Spawnings were recorded in the laboratory from 1962 to 1964. (From Rice, 1967, p. 147.)

Although there may be a periodicity in spawning during the breeding season there is not a complete gametogenic cycle between each spawning in sipunculans. A single animal may spawn repeatedly during a breeding season as evidenced in the six recorded spawnings of a single female of *Themiste pyroides* during an 8-week period (Rice, 1967). It seems probable that each spawning is preceded by a maturation of only a certain percentage of the largest oocytes in the coelom which are then collected by the nephridium.

Estimations of breeding periods have been made for 10 species of sipunculans by one or more of the following methods of study: observations on spawning in the laboratory over a period of a year, annual studies of gametogenic cycles with periodic examination of coelomic oocytes and gonads, or from studies concerned primarily with development. Additional observations on spawning of 9 species have been made at sporadic intervals, but not carried out over a period of a year. It is important to note, therefore, that in these instances the times of recorded spawning are not indicative of the range or duration of breeding seasons, but show only the months in which spawning was observed and can be expected to occur. The information is included for its possible value to persons who may wish to carry out further investigations in the localities named. The data are summarized in Table I.

TABLE I
BREEDING PERIODS OF SIPUNCULANS

Species	Locality	Reference	Breeding period of months of observed spawning
Golfingia elongata	Roscoff, France	Åkesson, 1961a	July, Aug.
Golfingia minuta	Kristineberg, Sweden	Åkesson, 1958	Sept.–Nov.[a]
Golfingia pellucida	Fort Pierce, Florida	Rice, unpublished	Feb.–May, Aug., Sept. Nov.
Golfingia pugettensis	San Juan Islands, Washington	Rice, 1967	Oct.–Jan.[a]
Golfingia vulgaris	Roscoff, France	Gerould, 1907; Gonse, 1956b	June–Sept.[a]
Paraspidosiphon steenstrupi	British Honduras	Rice, unpublished	June
Paraspidosiphon fischeri	Isla Margarita, Venezuela	Rice, 1975	Oct.–Dec.
	Isla Perico, Panama	Rice, unpublished	July
	Galeta, Panama	Rice, unpublished	June, July
	Key Largo, Florida	Rice, unpublished	Oct.
Phascolion cryptus	Virginia Key, Florida	Rice, 1975; Rice, unpublished	July–Nov.
Phascolion strombi	Kristineberg, Sweden	Åkesson, 1958	Sept., Oct., Nov.[a]
Phascolopsis gouldi	Newport, Rhode Island	Gerould, 1907	June–Aug.
	Woods Hole, Massachusetts	Gerould, 1907	Aug.–Sept.
Phascolosoma agassizi	Monterey, California	Towle and Giese, 1967	Mar.–May[a]
	San Juan Islands, Washington	Rice, 1967	June–Sept.[a]
Phascolosoma antillarum	Key Biscayne, Florida	Rice, 1975; Rice, unpublished	July–Sept.
Phascolosoma arcuatum	Ross River, Queensland, Australia	Green, 1975	Dec.–Feb.[a]
Phascolosoma perlucens	La Parguera, Puerto Rico	Rice, 1975	April
	Barbados	Rice, 1975	Feb., Mar.
	Isla Margarita, Venezuela	Rice, 1975	Oct.–Feb.
	Key Biscayne, Florida	Rice, 1975, unpublished	Nov.–Apr., June–Sept.[a]
Themiste alutacea	Isla Margarita, Venezuela	Rice, 1975	Oct.–Dec.
	Fort Pierce, Florida	Rice, unpublished	Mar.–Aug., Oct.[a]
Themiste lageniformis	Fort Pierce, Florida	Rice, unpublished	Mar.–Aug., Oct., Dec.[a]
Themiste pyroides	San Juan Islands, Washington	Rice, 1967	March–August[a]
	Vancouver Island, British Columbia		
Siphonosoma cumanense	Tampa Bay, Florida	Rice, unpublished	June
Sipunculus nudus	Tampa Bay, Florida	Rice, unpublished	June
	Naples, Italy	Hatschek, 1883	July

[a] Indicates duration of breeding season; population observed during all seasons of the year.

Of the 10 species for which the breeding season has been determined with some certainty, 6 are temperate and 4 tropical or subtropical. All of the temperate species and one of the tropical species (*Phascolosoma arcuatum*) have well-defined breeding seasons. Three of the tropical or subtropical species, *Themiste lageniformis, T. alutacea,* and *Phascolosoma perlucens,* appear to spawn throughout much of the year.

4.4 Development

4.4.1 Fertilization

Sipunculan eggs when spawned into the seawater are arrested in the first meiotic metaphase. The process of fertilization, beginning with the entrance of the sperm into the egg, includes the extrusion of the first and second polar bodies and enlargement of the sperm nucleus to form the male pronucleus, and, finally, the fusion of the female and male pronuclei.

Gerould (1907) described fertilization in the eggs of *Golfingia vulgaris* and *Phascolopsis gouldi,* but he was unable to observe sperm penetration. Both Gerould (1907) and Åkesson (1958) supposed the sperm to enter through a pore of the egg membrane. However, the width of the sperm head exceeds that of the pore several times and, in more recent studies (Rice, 1966), it has been demonstrated that sperm penetration is effected by the formation of a hole in the egg envelope. Penetrating sperm or the resultant sperm entry holes have been observed in living and/or sectioned eggs of the following species: *Phascolosoma agassizi, P. perlucens, P. varians, Golfingia pugettensis, G. pellucida,* and *Themiste pyroides.* In addition to the sperm entry hole in the egg envelope, the penetrating sperm of *Themiste pyroides* also leaves behind it a clearly discernible track in the thick jelly coat of the egg. Before penetration this sperm extends an acrosomal filament 50 μm in length from the edge of the jelly to attach to the egg envelope. The sperm entry hole may persist for 2 to 3 days in the developing embryo.

Following sperm penetration, maturation of the egg is completed with the formation of the two polar bodies. In the elliptical eggs of *Phascolosoma* the cytoplasm rounds up, pulling away from the egg envelope at the two poles, and the polar bodies are formed at the animal pole. In spherical eggs such as those of most *Golfingia* and *Themiste,* a space between egg envelope and cytoplasm forms at the animal pole at the time or shortly before extrusion of the polar bodies.

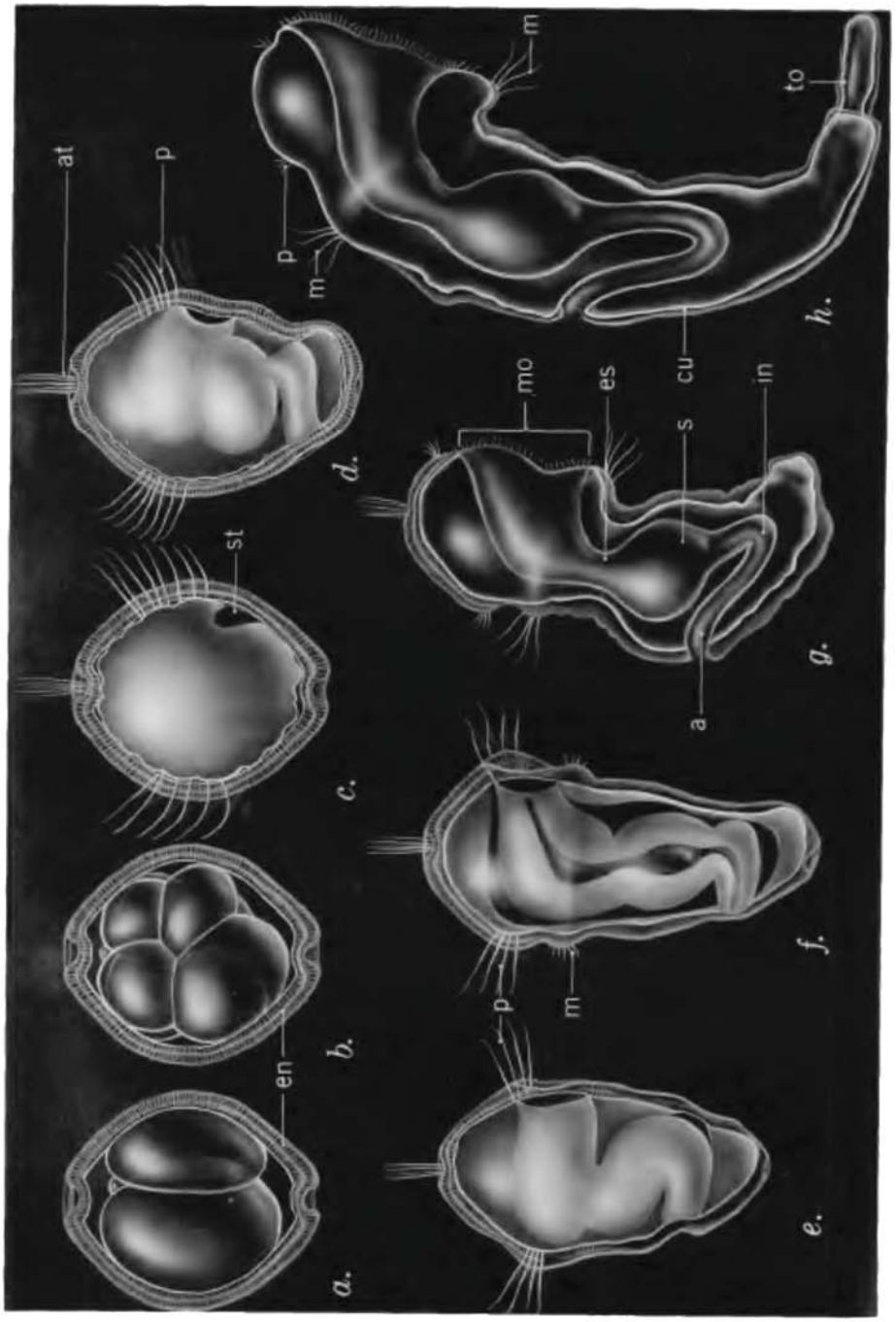
Details of sperm migration and zygote formation have been studied in *Golfingia vulgaris* and *Phascolopsis gouldi* (Gerould, 1907). In these species, the sperm, after entrance into the egg, rotates until its long axis is parallel to the surface of the egg. An aster with centrosome appears at the base of the nucleus and within about 10 minutes the sperm, led by the aster, migrates to the center of the egg where it increases in size and the chromatin becomes separated into a loose network. At the same time the astrosphere enlarges and from it prominent fibers radiate throughout the cytoplasm except in the direction of the animal pole.

During this period of sperm enlargement the two polar bodies are given off by the egg. According to Gerould's account, the reduction or transverse division occurs at the first polar body division and the equational or longitudinal division takes place at the second division, the reverse of the usual process. During maturation divisions the number of chromosomes is reduced from 20 to 10, the haploid number. After extrusion of the second polar body the nucleus of the egg is broken up into 10 chromatic vesicles which soon fuse to form the female pronucleus. A centrosome, from which astral fibers radiate, lies in a fold of the pronucleus toward the center of the egg. As the two pronuclei move toward each other, the astral rays of the female pronucleus become less prominent and the centrosome moves to one side, about 90° from that point which will first contact the sperm nucleus. As the sperm nucleus moves toward the animal pole, its astrosphere moves to one side and decreases in size. At the time the two pronuclei unite, their astrospheres are of approximately the same size, but the astral fibers of the male pronucleus, are much more prominent. As the first cleavage approaches, the two asters are equal in size.

4.4.2 Embryonic Development

4.4.2.1 Cleavage

Cleavage of the sipunculan egg is spiral, unequal, and holoblastic (Fig. 16a,b). Cell lineage of one species, *Golfingia vulgaris*, was studied through the 48-cell stage by Gerould in 1907. Starting with a dexiotropic cleavage at the third division, the cleavage planes alternate in direction to the 48-cell stage, after which the spiral pattern continues only in certain areas of the egg. A characteristic feature of the cleavage of *Golfingia vulgaris* is the relatively large size of the micromeres at the 8-cell stage. At the 16-cell stage, the 8 micromeres of the animal hemisphere, all of approximately equal volume, exceed in size all of the cells of


a.
b.
c.
d.
e.
f.
g.
h.
en
st
at
p
p
m
m
to
mo
es
s
cu
in
a
p
m

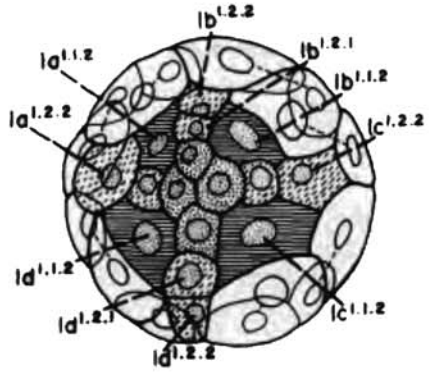


FIG. 17. Forty eight-cell stage of *Golfingia vulgaris*, anterior hemisphere, showing the molluscan cross and intermediate cells. Rosette cells are dotted, cross cells are dashed, and intermediate cells barred. Prototroch cells are around the periphery. (Redrawn from Gerould, 1907, fig. D, p. 99.)

the vegetal hemisphere except those of the D quadrant; the 2d cell or somatoblast is the largest cell at this stage and the second largest is the 2D. The relative size of the micromeres in early cleavage is reflected in the extraordinarily large size of the prototroch cells in the embryo. At the 48-cell stage the animal hemisphere consists of the 32 cells which make up the apical plate and the prototroch. The spiral pattern is interrupted at the 48-cell stage by the radial division of the $1q^{1^2}$, the result of which is the formation of the apical cross. The cross cells are in the position of the molluscan cross, that is, the arms extend out from the rosette cells along the future sagittal and frontal planes of the embryo in a radial direction rather than in the interradial direction characteristic of the annelidan cross (Fig. 17). This feature has been misunderstood by many authors probably because of Gerould's adoption of terminology

---

FIG. 16. Diagrammatic representation of developmental stages of *Phascolosoma perlucens*, illustrating events in the metamorphosis of the trochophore. (a) Two-cell stage. (b) Eight-cell stage. (c) Early trochophore. (d) Late trochophore. (e–f) Premetamorphosis stages. (g) Planktotrophic pelagosphera larva, immediately after trochophoral metamorphosis, 2½–3 days of age. (h) Pelagosphera larva, about 1 week old. As the trochophore elongates during metamorphosis (e,f) the egg envelope loses its porosity and lamellation and is transformed into the cuticle of the pelagosphera larva (g,h). Immediately before metamorphosis (f) the gut cavity and coelomic cavity are formed and metatrochal cilia appear. At the time of metamorphosis the anus and mouth are opened to the exterior, the prototroch reduced and terminal organ everted (g). a, anus; at, apical tuft; cu, cuticle; en, egg envelope; es, esophagus; in, intestine; m, metatroch; mo, mouth; p, prototroch; s, stomach; st, stomodeum; to, terminal organ.

previously used for polychaetes (Clark, 1969); however, when using these terms he places them within quotation marks. Thus he labels the cross cells "intermediate" cells, apparently referring to the intermediate cells of annelids, not of sipunculans. In his figure of the apical region of the 48-cell stage, reproduced here (Fig. 17), the prominent cross cell are clearly seen to be in the radial position. The 4 cells of the rosette and the 16 cells of the prototroch are notable for their large size. The prototroch, derived from the trochoblasts, $1q^2$ cells, consists of a girdle of 16 primary cells at the 48-cell stage; later three secondary cells, probably derived from intermediate cells, are added to complete the prototrochal girdle of the trochophore. A gap persists in the prototroch between cells of the C and D quadrants, joining apical and somatic plates, and has been designated as the dorsal cord. The division of 3D gives rise to 4d which immediately divides to form two equal cells which sink below the surface to form the mesodermal teloblasts.

Additional information available on early cleavage of other species indicates that the relatively large micromeres are a feature of yolky eggs and lecithotrophic development, whereas in eggs with relatively small amounts of yolk, micromeres are smaller than, or in some cases equal to, the macromeres. For example, in the 8-cell stages of *Phascolosoma agassizi* and *P. perlucens*, species with eggs low in yolk content, the micromeres and macromeres in the A, B, and C quadrants are all approximately the same size; 1d is slightly larger and 1D is by far the largest of the 8 cells. The largest cell of the 16-cell stage of *P. agassizi* is the somatoblast 2d which is nearly twice the size of 2D. The relative size of micromeres and macromeres at the 8-cell stage of 10 species of sipunculans is found in Table II.

4.4.2.2 General Developmental Patterns

Several patterns of development are known to occur in the Sipuncula. One is direct development, defined here as nonpelagic development in which ciliated stages are absent and the embryo transforms directly into a small, crawling worm, usually hatching from one or more egg coats. The remaining patterns are classified as indirect development and include either one or both of the following swimming larval stages: (1) trochophore, characterized by ciliated prototroch and apical tuft, and (2) pelagosphera with prominent ciliated metatrochal band, lacking a prototroch or with considerably reduced prototroch.

Information is now available on the development of 17 species of sipunculans, including 5 species of *Golfingia*, 4 species of *Phascolosoma*, 3 of *Themiste*, 2 of *Phascolion*, and one each of *Phascolopsis, Paraspidosiphon,* and *Sipunculus*. Table II lists the species according to devel-

opmental patterns. Four common categories are designated: I, direct development; II, indirect development with one larval stage, the trochophore, which transforms into the vermiform stage; III, indirect development with two larval stages, the trochophore and lecithotrophic pelagosphera; and IV, indirect development with two larval stages, the trochophore and planktotrophic pelagosphera. The species listed range in distribution from the cold waters of the Northeastern Pacific and North Atlantic to the tropical waters of the Atlantic.

Three species are known to undergo direct development: *Golfingia minuta* from Kristineberg, Sweden (Akesson, 1958), *Phascolion cryptus* from Virginia Key, Florida (Rice, 1975), and *Themiste pyroides* from San Juan Archipelago, Washington (Rice, 1967). The eggs are rich in yolk, blastulae are solid, and gastrulation occurs by epiboly. In two of the species in which 8-cell stages have been examined (*Themiste pyroides* and *Phascolion cryptus*), the macromeres in the A, B, and C quadrants are smaller than their respective micromeres. Derivatives of the large micromeres, the prototroch cells, form a prominent band of nonciliated cells of extraordinary size. At this stage, comparable to the trochophore although lacking cilia, the cytoplasm is retracted from the egg envelope in the pretrochal area around the apex of the embryo, forming an apical groove which delimits the cells of the nonciliated rosette. Joining pretrochal and posttrochal hemispheres a dorsal groove interrupts the prototrochal band and immediately posterior to the prototroch a space between cytoplasm and egg envelope marks the position of the stomodeum.

At the age of 2 days (25°C), *Phascolion cryptus* hatches as a crawling worm from the anterior portion of the egg envelope and the surrounding jelly coat (Fig. 18). The embryo first elongates posttrochally, rupturing the jelly coat posteriorly and then the pretrochal region, followed by the prototrochal, separates from the egg envelope resulting in a large anterior cavity. As the retractor muscles become functional the head is extended and withdrawn and through this activity the anterior egg envelope is severed from its posttrochal connection to the embryo. Thus the jelly coat and anterior egg envelope are discarded, a new cuticle is formed to cover the pretrochal and prototrochal regions, and the posttrochal egg envelope is transformed into the posterior cuticle of the vermiform stage.

In *Themiste pyroides*, the entire egg envelope appears to be retained as the cuticle of the vermiform stage. The embryo develops within the envelope and surrounding jelly coat until the age of 8 to 9 days (12°–13°C) when, by a series of movements extending and contracting the body, it hatches out of the jelly coat as a small, crawling worm.

TABLE II

A SUMMARY OF DEVELOPMENTAL CHARACTERISTICS OF THE SIPUNCULA

Species[a]	Egg size diameter or length × width (μm)	8-Cell stage relative size of micro- and macromeres in quadrants A, B, C	Gastrulation	Length of pelagic stage		
					Pelagosphera	
				Trochophore	Lecitho-trophic	Plankto-trophic
Category I						
Golfingia minuta[1]	260–280 × 215–230	?	Epiboly	0	0	0
Themiste pyroides[5]	190	Micromeres > Macromeres	Epiboly	0	0	0
Phascolion cryptus[6]	136	Micromeres > Macromeres	Epiboly	0	0	0
Category II						
Phascolion strombi[1]	125	Micromeres > Macromeres	Epiboly	8 Days	0	0
Phascolopsis gouldi[3]	150–180	Micromeres > Macromeres	Epiboly	3 Days	0	0
Category III						
Golfingia vulgaris[3]	150–180	Micromeres > Macromeres	Epiboly	3 Days	2 Days	0
Golfingia elongata[2]	125	?	Epiboly + invagination	2 Days	4 Days	0
Golfingia pugettensis[5]	160	Micromeres = Macromeres	Epiboly	8 Days	13 Days	0
Themiste alutacea[6]	138	?	Epiboly	2 Days	6 Days	0
Themiste lageniformis[8]	145	Micromeres > Macromeres	Epiboly	0	8–12 Days	0

Category IV						
Golfingia pellucida[7]	165	?	?	3 Days	0	1 Month
Paraspidosiphon fischeri[6]	103 × 94	?	?	2 Days	0	1 Month
Phascolosoma agassizi[6]	140 × 110	Micromeres = Macromeres	Epiboly + invagination	8–10 Days	0	1 Month
Phascolosoma antillarum[6]	127 × 97	?	?	3 Days	0	1 Month
Phascolosoma perlucens[6]	112 × 91	Micromeres = Macromeres	Epiboly + invagination	3 Days	0	1 Month
Phascolosoma varians[6]	104 × 90	?	?	3 Days	0	1 Month
Sipunculus nudus[4]	120	Micromeres < Macromeres	Invagination	3 Days	0	1 Month

[a] References: 1. Åkesson, 1958; 2. Åkesson, 1961a; 3. Gerould, 1907; 4. Hatschek, 1883; 5. Rice, 1967; 6. Rice, 1975; 7. Rice, unpublished; 8. Williams, 1972.

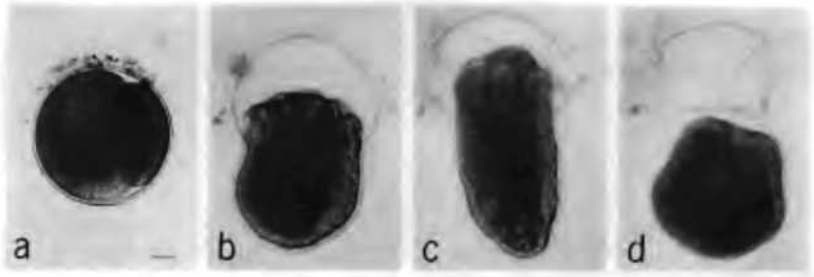


FIG. 18. Developmental stages of *Phascolion cryptus*, depicting direct development and partial hatching from egg envelope. Photographs of living embryos. Scale, 25 μm. (a) Two-cell stage. Presence of adhesive jelly layer is indicated by attached debris. (b–c) Egg envelope has detached in pretrochal and prototrochal regions. Anterior space develops between envelope and embryo. Anterior end can be retracted as in (b) or extended as in (c), and by such movements the envelope is eventually ruptured at the posttrochal junction. (d) Hatched vermiform stage. Head is retracted. Anterior egg envelope is completely detached and posterior egg envelope has been transformed into cuticle. [From Rice, 1975 (in part).]

The sticky jelly coats of the eggs of *Themiste pyroides* and *Phascolion cryptus* cause adhesion to any surface contacted; thus these embryos develop attached to the substratum in the area of spawning. The direct development of *Golfingia minuta*, exemplifying a form of primitive brood protection, takes place over a period of 3 to 4 weeks within the burrows occupied by the females. The embryos do not develop prototrochal cilia, but cytoplasmic blebs, similar to those noted in other species before formation of cilia, have been interpreted by Åkesson to indicate that lack of prototrochal cilia in this species is secondary rather than primitive. An extremely weak and temporary ciliation with no locomotory function forms on marginal and rosette cells. The egg envelope transforms into the cuticle of the juvenile, but beneath it a definitive cuticle is apparent. Development is slow in this species; young worms begin to migrate from the burrow at 1 month, but heads are not retractable until the age of 6 weeks.

The development of both *Phascolion strombi* from Kristineberg, Sweden (Akesson, 1958) and *Phascolopsis gouldi* from Newport, Rhode Island and Woods Hole, Massachusetts (Gerould, 1907) is marked by a single pelagic larval stage, a lecithotrophic trochophore (Category II, Table II). Eggs of the two species are macrolecithal with micromeres exceeding macromeres in size in the A, B, and C quadrants at the 8-cell stage, the blastulae are solid, and gastrulation occurs by epiboly. Trochophores are characterized by rosette cells with long cilia and a band of ciliated prototroch cells (Fig. 19a). Marginal cells on either side of

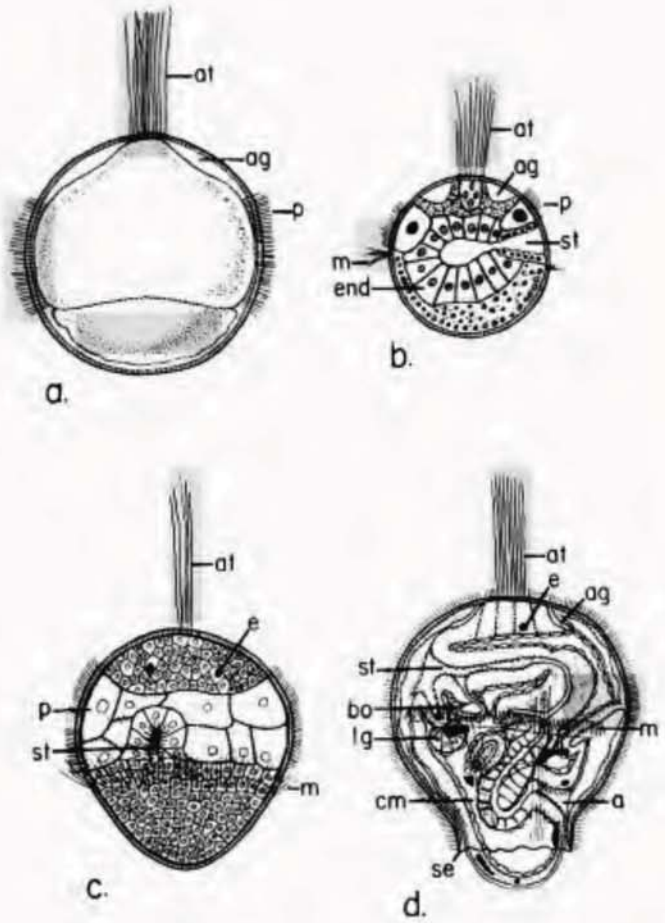


FIG. 19. Trochophores of sipunculans. (a) Trochophore of *Phascolopsis gouldi*. Surface view; 20 hours. (Redrawn from Gerould, 1907, plate 8, fig. 59.) (b) Trochophore of *Golfingia elongata*. Diagram of sagittal section. (Redrawn from Åkesson, 1961a, p. 516.) (c) Trochophore of *Golfingia vulgaris*. Ventral view, indicating cell outlines; about 40 hours. (Redrawn from Gerould, 1907, plate 7, fig. 50.) (d) Trochophore of *Sipunculus nudus*. Lateral view. Egg envelope an underlying cell layer (serosa) have split at posterior end, preceding shedding of envelope. (Redrawn from Hatschek, 1883, plate 3, figure 45.) a, anus; ag, apical groove; at, apical tuft; bo, buccal organ; cm, coelom; e, eye; end, endoderm; lg, lip gland; m, metatroch; p, prototroch; se, serosa; st, stomodeum.

the prototroch of *Phascolion strombi* are weakly ciliated and anterior to the prototroch of *Phascolopsis gouldi* is a band of well-developed preoral cilia. At metamorphosis the prototroch cells degenerate and the

trochophore is transformed into an elongate worm, capable of extending and retracting the anterior portion of the body. The egg envelope of *Phascolopsis gouldi* is shed at metamorphosis, but in *Phascolion strombi* it is retained as the cuticle.

A developmental pattern consisting of two pelagic larval stages, a lecithotrophic trochophore and a lecithotrophic pelagosphera, is found in the following species: *Golfingia elongata, G. pugettensis, G. vulgaris,* and *Themiste alutacea* (Category III, Table II). All are temperate or cold-water species except *T. alutacea* which ranges from the temperate water off North Carolina and Brazil to the tropical waters of the Caribbean Sea. In these species a pelagic lecithotrophic trochophore metamorphoses into a lecithotrophic pelagosphera which swims for a short time, often near the bottom, undergoes a gradual loss of cilia, and transforms into the veriform stage. The 4 species within this pattern show a gradient in development from least to most highly modified from that of the preceding pattern. The development of *Golfingia vulgaris* most nearly resembles that previously described, whereas that of *G. pugettensis* is probably the most highly modified. In *G. vulgaris* the egg is rich in yolk and gastrulation is epibolic (Fig. 20a). There is no abrupt formation of metatrochal cilia since these are developed in the trochophore stage (Fig. 19c). Metamorphosis of the trochophore occurs at 2 days when the egg envelope is ruptured and cast off and the anterior end of the body becomes retractable (Fig. 21c,d). Prototrochal cilia appear often to be destroyed, but metatrochal cilia slip through the envelope without injury. The larva continues to twirl, usually near the bottom, by means of the metatroch until the cilia are lost at 5 days of age and the young vermiform stage assumes an elongate shape and begins to creep along the bottom (Gerould, 1907).

Development of *Golfingia elongata* is similar, except that during gastrulation an archenteron is formed. The trochophore is spherical with a long apical tuft, short prototrochal cilia, and long metatrochal cilia (Fig. 19b). Metamorphosis consists of elongation, beginning at 48 hours (17°C), and disintegration of the prototrochal cells. The egg envelope is transformed into the cuticle of the vermiform stage.

The trochophore of *Themiste alutacea* changes from the spherical shape of the blastula to an oval shape with a prominent equatorial band of prototrochal cilia and a long apical tuft. Metamorphosis, beginning at 28 hours (25°C) with elongation of the trochophore, results at 32 hours in a lecithotrophic pelagosphera. During metamorphosis a well-developed circlet of metatrochal cilia and a postmetatrochal sphincter appear, the stomodeum opens through the egg envelope to form the mouth and ventral ciliated surface of the head, and the egg envelope

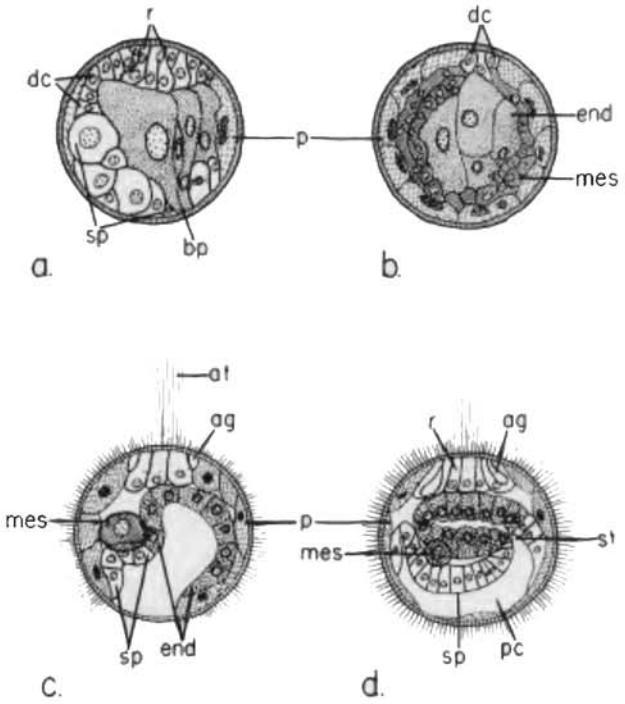


FIG. 20. Gastrulation and mesoderm formation. Prototroch cells are marked by dashes, other ectoderm cells are clear; endoderm is dotted; mesoderm is barred. (a) Sagittal section of embryo of *Golfingia vulgaris* showing blastopore. Gastrulation is epibolic; 14½ hours. (Redrawn from Gerould, 1907, plate 6, fig. 40b.) (b) Cross section of embryo of *Golfingia vulgaris*; 24 hours. Mesodermal bands have split into splanchnic and somatic layers. (Redrawn from Gerould, 1907, plate 6, fig. 45. Prototrochal cilia, although described in text, are not shown in Gerould's figures.) (c) Optical median section of embryo of *Sipunculus nudus* showing formation of ectodermal somatic plate and embolic gastrulation. (Redrawn from Hatschek, 1883, plate 2, fig. 15.) (d) Optical median section of embryo of *Sipunculus nudus*, later than (c). Prototroch cells have surrounded embryo. (Redrawn from Hatschek, 1883, plate 2, fig. 23.) ag, apical groove; at, apical tuft; bp, blastopore; dc, dorsal cord; end, endoderm; mes, mesoderm; p, prototroch; pc, posterior cavity; r, rosette cells; sp, somatic plate; st, stomodeum.

is transformed into the larval cuticle. Transformation into the juvenile may begin as early as 7 to 8 days with loss of metatrochal cilia, and by 2 weeks the coelomic yolk has been absorbed and the gut completed. Within 1 month it has assumed the shape of the juvenile with elongated introvert and 4 ciliated tentacular lobes.

The trochophore of *Golfingia pugettensis* resembles that of *Themiste alutacea*, but a characteristic feature in this species is the presence

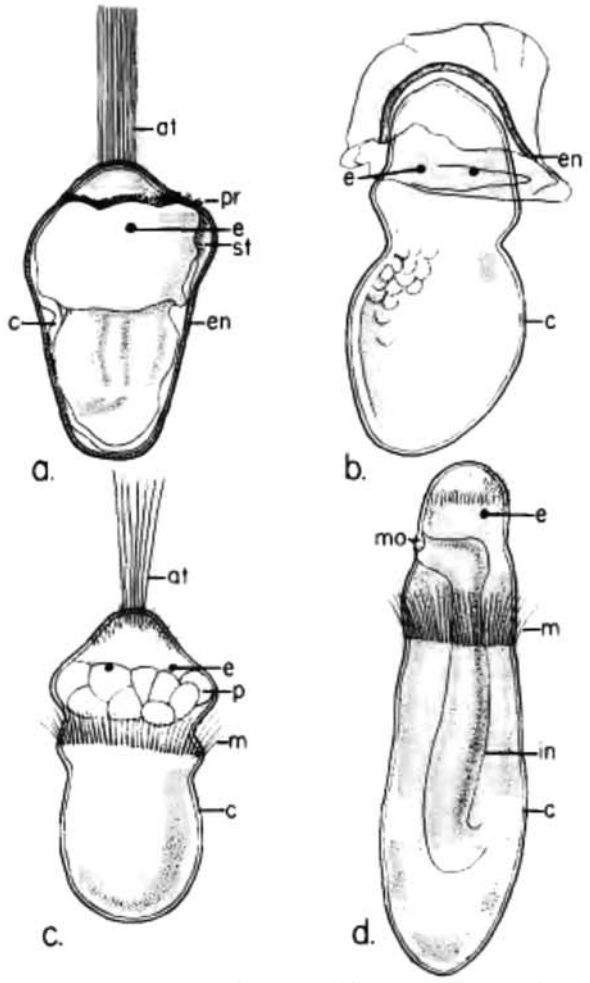


FIG. 21. Metamorphosis of *Phascolopsis gouldi* and *Golfingia vulgaris*. (a) Trochophore of *P. gouldi*, 48 hours, showing circlet of preoral cilia and developing cuticle beneath egg envelope. Lateral view. (Redrawn from Gerould, 1907, plate 8, fig. 62.) (b) Metamorphosing trochophore of *P. gouldi* in process of shedding egg envelope; 43 hours. Dorsal view. (Redrawn from Gerould, 1907, plate 9, fig. 68.) (c) Metamorphosing trochophore of *G. vulgaris*. Egg envelope has been cast off, prototrochal cilia lost, and prototrochal cells are in process of degeneration. Dorsal view. (Redrawn from Gerould, 1907, plate 7, fig. 52.) (d) Lecithotrophic pelagosphera larva of *G. vulgaris;* 60 hours. Lateral view. Gut is incomplete. (Redrawn from Gerould, 1907, plate 7, fig. 54.) at, apical tuft; c, cuticle; e, eye; en, egg envelope; in, intestine; m, metatroch; mo, mouth; p, prototroch; pr, preoral cilia; st, stomodeum.

of conspicuous lipid droplets in the prototroch cells. Metamorphosis of the trochophore occurs at 8 days (8°–9°C). Differing from others with this developmental pattern, the lecithotrophic pelagosphera of *Golfingia pugettensis* has a well-developed terminal organ by which it attaches to the substratum.

A species with an apparently highly modified development, similar to that described above but not readily classified in any of the developmental categories commonly found in sipunculans, is *Themiste lageniformis*. Its early development is essentially the same as that of directly developing species in that it lacks a pelagic trochophore stage. However, the nonpelagic stage, comparable to a trochophore, metamorphoses to a lecithotrophic pelagosphera with metatroch and terminal attachment organ (Williams, 1972).

The final developmental pattern known to occur in the phylum includes two larval stages: a pelagic lecithotrophic trochophore and a planktotrophic pelagosphera (Category IV, Table II). The development of 7 species falls within this classification. Four belong to the genus *Phascolosoma: P. agassizi, P. antillarum, P. perlucens,* and *P. varians*. Other species are *Golfingia pellucida, Paraspidosiphon fischeri,* and *Sipunculus nudus*. *Phascolosoma agassizi* is a cold- to temperate-water species; others are either tropical or range from temperate to tropical waters. With the exception of *Sipunculus nudus* in which the trochophore is uniquely modified, the development is very similar in all species.

Some of the characteristic features of this developmental pattern are summarized in Fig. 16, a diagrammatic representation of development through metamorphosis to the pelagosphera larva in *Phascolosoma perlucens*. The egg of this species is relatively low in yolk content, the blastula has a small blastocoel in the anterior hemisphere, and gastrulation, although mostly epibolic, is in part achieved by invagination with the formation of a small archenteron. During the trochophore stage the gut and mesodermal bands are differentiated and posttrochal elongation begins (Fig. 16c–e). Long prototrochal cilia, extending through the pores in the egg envelope function to propel the larva in a spiral swimming movement while the cilia of the apical tuft are directed forward. Metamorphosis of the trochophore into the planktotrophic pelagosphera takes place at 2½ to 3 days of age (25°C) and lasts over a period of several hours. Metamorphosis is marked by formation of a new ciliary band, the metatroch, reduction of the prototroch, loss of the apical tuft, posttrochal elongation, expansion of coelom, rupture of the egg envelope in the region of the stomodeum to give rise to the ventral ciliated surface of the head and mouth, opening of the anus to complete the gut, and formation of the adhesive terminal organ (Fig. 16f–h). Pelago-

sphera larvae of *P. perlucens* have been maintained in the laboratory as long as 6 months, but a second metamorphosis into the juvenile form has not been observed.

Other species included within this pattern, except *Sipunculus nudus,* show essentially the same developmental features. The early development of *S. nudus* is unique, particularly in the elaboration of prototroch and embryonic cavities. Blastomeres are nearly equal in size, but slightly larger at the vegetal pole. In the blastula a nearly central blastocoel is displaced slightly toward the animal pole. Gastrulation occurs by invagination of the endoderm cells which pull away from the egg envelope at the vegetal pole, leaving a posterior cavity (Fig. 20c,d). At the same time the cells of the apical plate surrounding the rosette cells sink from the envelope forming an apical groove or cavity of the head around the central rosette. The posterior cavity and the apical groove are connected by a narrow dorsal canal which results from the sinking of a double row of small ectoderm cells. Ciliated cells, assumed to be homologs of trochoblasts (Gerould, 1903), then spread out to surround the embryo and form in conjunction with the egg envelope a structure which has been termed a "serosa" (Hatschek, 1883). The trochophore develops within the serosa and at the time of metamorphosis to the planktotrophic pelagosphera the entire serosa is shed (Figs. 19d and 22a,b). The pelagosphera has a ciliated metatrochal band and is similar to other such larvae in this developmental category. After 1 month in the laboratory a second metamorphosis occurs into the juvenile form (Fig. 22c).

4.4.2.3 Gastrulation and Formation of Mesoderm

In species with macrolecithal eggs, as in the first three developmental categories, gastrulation occurs entirely by epibolic movements with the exception of *Golfingia elongata* in which a small archenteron is formed. This mode of gastrulation, best described for *Golfingia vulgaris* and *Phascolopsis gouldi* (Gerould, 1907), occurs by an overgrowth of the cells of the dorsal somatic plate ventrally and laterally to enclose the solid mass of large endoderm cells. The blastopore is represented by the narrow ends of the club-shaped endoderm cells which maintain contact with the egg envelope until closed over by the somatic plate (Fig. 20a,b). Descendants of the stomatoblasts divide rapidly and invaginate in the region of the trochoblasts at the anterior end of the blastopore to form the stomodeum.

In species with less yolky eggs and planktotrophic larvae, as found in the fourth developmental category, gastrulation is accomplished in part by invagination, but the major role is usually played by epiboly.

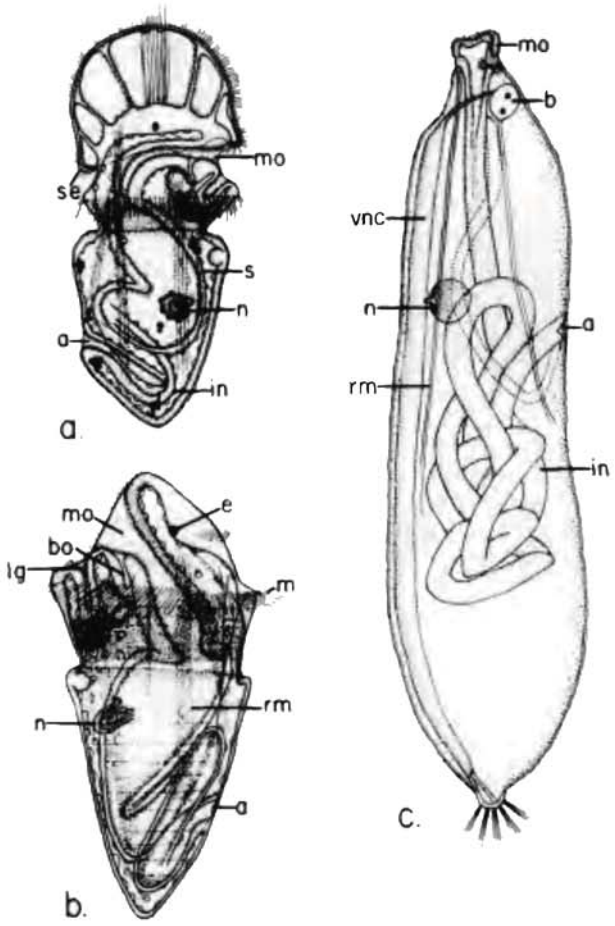


FIG. 22. Metamorphosis of *Sipunculus nudus*. (a) Trochophore hatching from "serosa," i.e., egg envelope plus prototroch cells. Serosa is still attached in head region. (Redrawn from Hatschek, 1883, plate 5, fig. 49.) (b) Pelagosphera larva, 2 days after shedding of serosa. (Redrawn from Hatschek, 1883, plate 5, fig. 51.) (c) Juvenile, after metamorphosis of pelagosphera. (Redrawn from Hatschek, 1883, plate 6, fig. 71.) a, anus; b, brain; bo, buccal organ; e, eye; in, intestine; lg, lip gland; m, metatroch; mo, mouth; n, nephridium; rm, retractor muscle; s, stomach; se, serosa; vnc, ventral nerve cord.

Endoderm cells in blastulae of species of *Phascolosoma*, such as *P. agassizi* and *P. perlucens*, are separated from the egg envelope by a large space around the vegetal pole. During gastrulation, cells of the somatic plate grow down over the entomeres filling the space in an epibolic

gastrulation. A narrow slit in the region of the blastopore marks an invagination leading into a narrow archenteron. Endodermal invagination is the primary process of gastrulation in *Sipunculus nudus* (Fig. 20c,d). Closure of the blastopore in this species is achieved by a forward and ventral growth of ectoderm of the dorsal lip; this ectodermal growth at the same time gives rise to the median somatic plate.

Mesoderm is derived from a pair of mesodermal teloblasts located on either side of the endoderm cells. Proliferation of these cells, observed in *Golfingia vulgaris*, *Phascolopsis gouldi* (Gerould, 1907), *Sipunculus nudus* (Hatschek, 1883), and *Phascolosoma agassizi* (Rice, 1967), results in the formation of the lateral bands of mesoderm (Fig. 20b).

4.4.3 Larvae

4.4.3.1 Trochophore Larva

A trochophore stage is a characteristic phase in the development of all sipunculans, although in some species it may be highly modified. Trochophores of several species are illustrated in Fig. 19. The apical plate of the pretrochal hemisphere consists of numerous small ectoderm cells which later give rise to the head ectoderm and brain and of a central circle of large rosette cells, usually bearing the long cilia of the apical tuft. The apical groove around the rosette cells may persist in the early trochophore, but later it is filled by a growth of ectoderm cells. Embedded in the apical plate in a dorsal position just anterior to the prototroch is a pair of small eyespots. The somatic plate covers the posterior hemisphere and is the source of the ectoderm of the trunk. The stomodeum is in a medioventral position, extending anteriorly into the region of the prototroch. The large cells of the prototroch (19 in *Golfingia vulgaris*) form a prominent equatorial band which spreads out over a large proportion of the surface of the larva. Prototroch cells are usually ciliated and always concentrated with yolk granules. The early trochophore retains the shape of the egg, but then an oval shape is assumed and in later stages the length increases as posttrochal elongation begins. Enclosed by the overlying egg envelope, trochophores of all species of sipunculans are lecithotrophic. Rudiments of most internal adult organs are present at this stage. Three regions of the gut (esophagus, stomach, and intestine) are differentiated and mesodermal bands, two cell layers in thickness, are present on either side of the gut.

The pattern and functional significance of ciliation varies among different species. Directly developing species, such as *Phascolion cryptus* and

Themiste pyroides, lack cilia entirely; *Golfingia minuta* shows a weak and temporary ciliation on pretrochal, posttrochal, and rosette cells, but not on prototrochal cells. All other species possess ciliated prototroch cells and an apical tuft of cilia. Prototrochal cilia are well developed and serve as the means of locomotion in species of *Phascolosoma* and in *Golfingia pugettensis, G. pellucida,* and *Paraspidosiphon fischeri.* In trochophores of *G. elongata, G. vulgaris,* and *Phascolopsis gouldi,* pretrochal and metatrochal bands of cilia are also present; metatrochal cilia are by far the longest in the two former species and most significant for larval locomotion, whereas in the latter the pretrochal cilia are the longest and the metatrochal vestigial. Metatrochal cilia are present in the trochophore of *Sipunculus nudus* but, enclosed by the serosa, are not functional (Fig. 19d).

4.4.3.2 Metamorphosis of the Trochophore

Metamorphosis of the trochophore may result in one of several developmental stages. In those species with only one pelagic stage, it may end the pelagic phase of development, the trochophore metamorphosing into a vermiform stage which gradually transforms into the juvenile form. It may result in a second pelagic larval stage, the lecithotrophic pelagosphera, which swims for a short time in the plankton or near the bottom before transforming into the vermiform stage. Finally, it may give rise to a planktotrophic pelagosphera which swims in the plankton for a prolonged period before a second metamorphosis into a juvenile form. Main events of metamorphosis into a planktotrophic pelagosphera are illustrated in the diagram of development of *Phascolosoma perlucens* (Fig. 16).

Metamorphosis is characterized by reduction or loss of the prototroch, formation or expansion of the coelom, transformation or shedding of the egg envelope, and, in those species with pelagosphera larvae, by the elaboration of the metatroch as the principal locomotory organ. Muscular activity is initiated either at metamorphosis or shortly thereafter with the introversion and extrusion of the introvert.

In species which undergo direct development without a pelagic trochophore stage, changes occur at the time of transformation into the vermiform stage which are comparable to those of the metamorphosis of the trochophore, e.g., elongation, formation of the coelom, dissolution of prototroch cells, and the beginning of muscular activity.

The coelom is formed by schizocoely or a splitting of the mesodermal bands into two layers, one of which forms the inner layer of the body wall and the other an outer covering of the gut. In species

with relatively microlecithal eggs and planktotrophic pelagosphera the coelom is formed before metamorphosis of the trochophore, but undergoes a great expansion at this time. The coelom of species with yolky eggs forms simultaneously with other events of metamorphosis.

The egg envelope of most species appears to be transformed at the time of metamorphosis into the larval cuticle. Transformation is accompanied by a loss of porosity and lamellation, first immediately posterior to the prototroch in the region of initial elongation, and later throughout. At the same time, as the larva stretches and the muscles become functional, the previously rather rigid egg envelope becomes elastic and flexible. Two species, *Phascolopsis gouldi* and *Golfingia vulgaris,* have been reported to shed the egg envelope at metamorphosis and another species, *Phascolion cryptus,* loses the prototrochal and pretrochal portions (Figs. 18b–d and 21a,b). *Sipunculus nudus* sheds not only the egg envelope, but also the underlying ciliated cells (Figs. 19d, and 22a).

Prototroch cells, usually heavily laden with yolk, release their yolk granules during metamorphosis into the coelomic cavity, thus contributing a substantial source of nutrition for the developing larva. The significance of this contribution is greatest in species with macrolecithal eggs and lecithotrophic development. Entire cells of the prototroch of *Golfingia vulgaris* and *Phascolopsis gouldi,* including nuclei and cytoplasm, are cast into the coelom at metamorphosis and the region of the prototroch is overgrown by ectoderm cells. In *Golfingia elongata* and *Phascolion strombi* the prototroch cells degenerate, releasing lipid and yolk granules into the coelom, after which they are replaced with ectoderm. Prototroch cells of *G. pugettensis* are marked by characteristic large lipid globules which at the time of metamorphosis are released into the coelom where they are readily recognizable; the cells, although retained as a prototrochal band on the head of the pelagosphera, are much reduced in size. The large yolk-laden prototroch cells of *G. minuta* and *Themiste pyroides* begin to degenerate at the time of coelom formation releasing their granules into the coelom. In species of *Phascolosoma,* release of yolk from the prototroch cells begins at an early stage of development when granules are passed from the cells into cavities formed along the inner sides of the prototroch. As the prototrochal cells decrease in size, the size of the "prototrochal cavities" becomes larger. The granules appear to break down in the cavities where presumably they provide nutrition for the developing embryo. At metamorphosis the prototrochal cells, reduced in size, persist in the pelagosphera as a weakly developed dorsal band of cilia on the head. Before metamorphosis in *Sipunculus nudus,* the size of the cells of the serosa is diminished as they gradually

liberate nutrients into the anterior and posterior cavities (Figs. 19 and 20). By the time of metamorphosis of *P. agassizi* and *S. nudus* all of the prototrochal yolk has been discharged into embryonic cavities.

At metamorphosis of the trochophore the metatroch assumes the role of the principal organ of locomotion. In *Golfingia vulgaris* and *G. elongata* the metatroch is present and well developed in the trochophore stage along with the prototroch, but at metamorphosis the prototroch diminishes and the metatroch remains as the sole locomotory organ. In *G. pugettensis* the metatroch is established late in the trochophore stage, but is elaborated at the time of metamorphosis when the prototroch regresses. In species of *Phascolosoma* metatrochal cilia are developed at the time of metamorphosis. In *Sipunculus nudus* metatrochal cilia appear before metamorphosis but become functional only when the serosa is shed.

4.4.3.3 Pelagosphera Larva

Following the stage of trochophore, the pelagosphera larva, whether lecithotrophic or planktotrophic, is characterized by a prominent band of metatrochal cilia and a regionalization of the body into head, metatrochal region or "thorax," and elongated trunk (Figs. 23–25). The head and thorax are retractable into the trunk and later become the introvert of the adult. Dorsally the head bears at least one pair of eyespots. Posterior to the eyespots and along either side of the head, a prototrochal ridge forms a U-shaped band of weakly developed cilia. The ventral head is ciliated and divided into two lobes by a median groove which continues into the mouth. Posterior to the head the metatrochal region or thorax is greatly distended when the larva is swimming and at its maximum extension is by far the widest portion of the larva (Fig. 25a). The posterior boundary of the thorax is marked by the metatrochal sphincter muscle, which is usually contracted when the larva is swimming and when the anterior end is completely retracted (Figs. 23–25). The elongated trunk may vary somewhat in shape depending on the degree of extension or contraction of the flexible larval body. At the posterior tip of most larvae there is a terminal attachment organ. Terminal organs are absent in a few planktotrophic larvae and in the lecithotrophic larvae of *Golfingia vulgaris* and *Themiste alutacea* (Figs. 21d and 24a), although the posterior end of the latter species does possess adhesive properties. Terminal organs of planktotrophic pelagospheras, in contrast to those of lecithotrophic larvae, are retractile, being withdrawn into the body by the contraction of a single pair of retractor muscles, originating from the dorsal body wall near the anus (Fig. 24b,c). In lecitho-

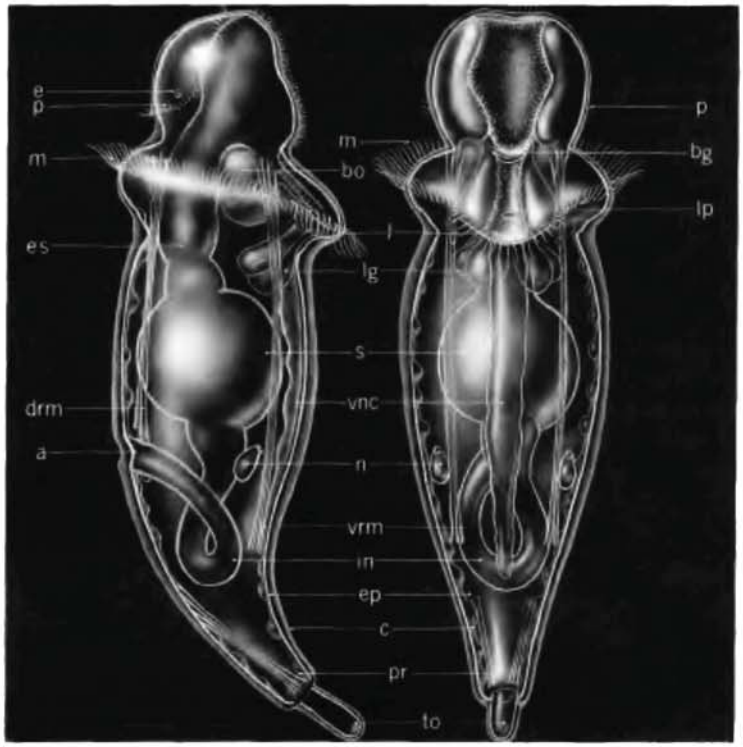


FIG. 23. Pelagosphera larva of *Phascolosoma perlucens*. Diagram showing internal structures at 1 week of age. Left, lateral view; right, ventral view; a, anus; bg, buccal groove; bo, buccal organ; c, cuticle; drm, dorsal retractor muscle; e, eye; ep, epidermis; es, esophagus; in, intestine; l, lip; lg, lip gland; lp, lip pore; m, metatroch; n, nephridium; p, prototroch; pr, posterior retractors; s, stomach; to, terminal organ; vnc, ventral nerve cord; vrm, ventral retractor muscle. (From Rice, 1975.)

trophic pelagospheras the gut cavity is incomplete and, although the mouth is usually open, the anus has not broken through the larval cuticle. The gut of planktotrophic larvae is complete, the anus opening dorsally on the middle or the anterior region of the trunk and the larvae are independent feeders. Associated with the feeding process are structures not present in lecithotrophic larvae: the lower lip, a projection forming the posterior boundary of the mouth, and two organs associated with the lip, the buccal organ and lip glands. The buccal organ is extrusible through a transverse slit or buccal groove at the base of the lip and the lip glands open through a pore on the lip.

In addition to the 7 species of planktotrophic larvae known from developmental studies (Table II), numerous larvae of uncertain species

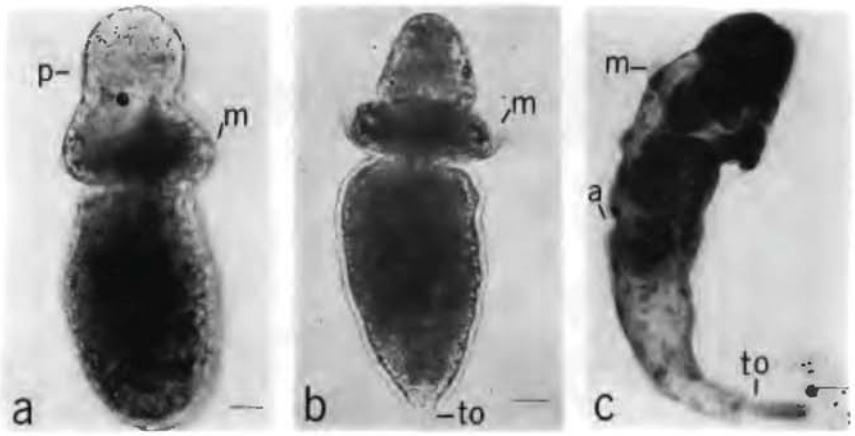


FIG. 24. Photographs of living pelagosphera larvae, reared from spawnings in the laboratory. (a) Lecithotrophic pelagosphera of *Themiste alutacea;* 3 days. Lateral view. Terminal organ is absent. (From Rice, 1975.) (b) Lecithotrophic pelagosphera of *Golfingia pugettensis*; 13 days. Dorsal view. Note presence of terminal organ. (From Rice, 1967.) (c) Planktotrophic pelagosphera of *Phascolosoma perlucens*; about 7 days. Lateral view. (From Rice, 1975.) Scale, 25 μm. a, Anus; m, metatroch; p, prototroch; to, terminal organ.

have been reported from the oceanic plankton. Such larvae were first recognized as belonging to the Sipuncula by Häcker (1898) from plankton collections in the North and South Atlantic Ocean. Comparing them with *Sipunculus nudus* known from Hatschek's studies, he described three larval types which he named "Baccaria oliva," "Baccaria citrinella," and "Baccaria pirum," distinguishing them by body shape and form of cuticular papillae. These larvae are less spectacular in size and form than the large, transparent planktonic larvae described by later workers.

Unaware of earlier work, Mingazinni (1905) described a larva from one preserved and contracted specimen collected in the plankton from a depth of 500 m between New Caledonia and New Zealand. He erroneously assumed the larva to be an adult, creating a new genus and species, *Pelagosphaera aloysii*. Senna (1906), working with material from the same expedition but from waters off India and Ceylon, suggested that structures identified by Mingazinni as gonads were instead the glandular appendages of the esophagus which are now referred to as lip glands (Jägersten, 1963). Although Mingazinni's error was soon realized, the name pelagosphera remains entrenched in the literature and is today used to designate the larval stage of sipunculans which succeeds the stage of trochophore (Rice, 1967).

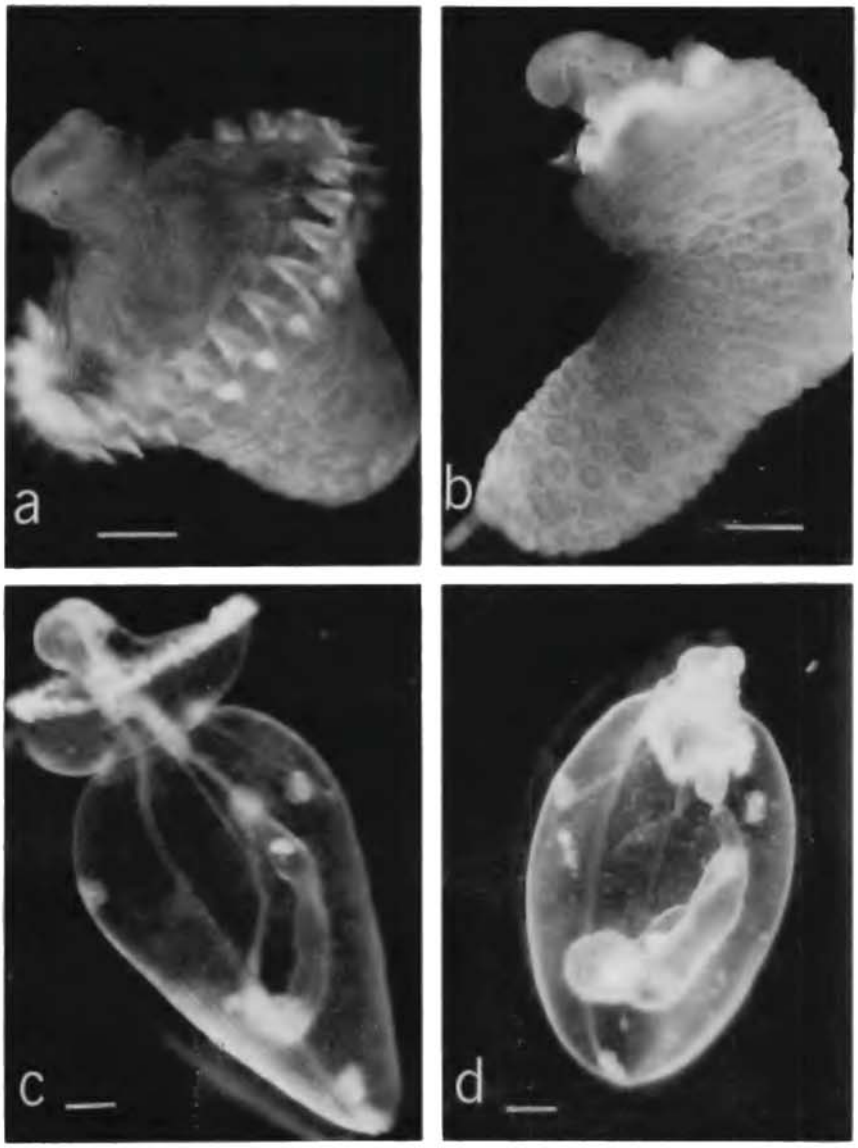


FIG. 25. Photographs of living planktotrophic pelagosphera larvae from oceanic plankton. (a) Larva of unknown adult. From plankton of Florida Current. Antero-dorsal view of swimming larva with extended metatroch. (b) Same specimen as (a) in quiescent position with terminal organ extended. Metatroch partially extended. (c) Larva of *Sipunculus polymyotus*. From plankton of Florida Current. Larva swimming with metatroch extended. (d) Same specimen as (c). Larva quiescent; metatroch withdrawn. Scale, 250 μm.

Other reports of oceanic planktotrophic larvae of sipunculans have been made by Heath (1910), Dawydoff (1930), Stephen (1941), Fisher (1947), Åkesson (1961b), Damas (1962), Jägersten (1963), Murina (1965), Scheltema and Hall (1965), and Hall and Scheltema (1975). Most of the earlier observations were made on preserved and contracted specimens, and only in more recent studies on living material (Jägersten, 1963; Murina, 1965; Hall and Scheltema, 1975) has the morphology of oceanic larvae been correctly interpreted. Jägersten gave the first accurate description of the head region, pointing out the relationships between lip, lip glands, and buccal organ. He examined several larval types which he divided into two groups, those with a "smooth" body surface and those with a "rough" or papillated body cuticle.

Characters generally used to distinguish pelagosphera larvae externally are texture of cuticle, presence and form of cuticular papillae, pigmentation, shape of head and lip, number and color of eyespots, general body form, and presence or shape of the terminal organ (Fig. 25). Some distinctive internal characters are number of lobes of lip gland, number of retractor muscles, shape and pigmentation of nephridia, and presence and number of longitudinal muscle fibers. Pigmentation in different larval forms may vary from brilliant orange to various shades of pink, yellow, green, and brown. The body may be white or transparent with pigmented nephridia and buccal groove. Eyespots are either red or black and usually two in number, but in some species there are accessory spots lateral or anterior to the larger ones. The retractable terminal organ may be a thick elongated cylinder or thin narrow rod, or in some instances no more than a rounded knob. The terminal organ in some species may be extended frequently, rarely in others, and in a few no terminal organ has been observed (Hall and Scheltema, 1975).

Specific affinities have been tentatively assigned to three of the oceanic planktotrophic larvae. Fisher (1947), basing his identification on the number of muscle bands, designated a larva collected off Cape Hatteras, North Carolina as *Sipunculus polymyotus*. Murina (1965) identified larvae from the Gulf of Aden as *S. aequabilis* and from the Northwest Pacific as *S. norvegicus*. Hall and Scheltema (1975) described external and internal morphology of 10 larval types from the North Atlantic. Of these, 7 were previously undescribed and the following 3 were redescribed: *Sipunculus polymyotus*, "Baccaria oliva," redescribed as Type C, and "Baccaria citrinella," redescribed as Type A. The latter was assigned to the genus *Aspidosiphon*, but no other adult affinities were determined.

The pelagosphera larva of *Sipunculus polymyotus* is large and transparent (Fig. 25c,d), often 4–5 mm in contracted length, commonly found

in the Gulf Stream (Fisher, 1947; Hall and Scheltema, 1975). The head is simple with a single pair of black eyespots and accessory pigment spots. The lip is bifurcated by a groove marked by a yellowish-green pigment. Similarly pigmented are the duct of the lip gland, the metatrochal band at the base of the cilia, the entire recurved gut, and the small rounded nephridia. The cuticle is smooth and iridescent. Internally the body wall musculature is divided into approximately 50 longitudinal muscle bundles. There are three pairs of retractor muscles of the introvert, two dorsal and one ventral.

The most commonly occurring larva in the North Atlantic is "Baccaria citrinella" or Type A (Hall and Scheltema, 1975). The cuticle is "rough" with numerous cuticular elevations arranged in a characteristic pattern of regularly crossing rows on the trunk. Each elevation or cuticular papilla is rounded and surmounted by a smaller cap. Pigmentation of the opaque body wall varies in living specimens from dark to light pink or pinkish white and in preserved specimens the color is changed to yellow. The metatrochal region and head are pale yellow and on the dorsal head there is a single pair of small red eyes. The lip is a simple thin lobe, slightly heart-shaped and flattened, but with no bifurcating ciliated groove. The body, when contracted, is "lemon-shaped" and from the blunt posterior end a thin, rod-shaped terminal attachment organ can be extended.

Planktotrophic pelagospheras may swim throughout the water by means of the prominent metatrochal collar (Fig. 25a–c), but most frequently under laboratory conditions their activity is carried out on or near the bottom. Young larvae of *Phascolosoma agassizi*, reared in the laboratory, have been reported to be chiefly bottom feeders although they are also able to feed on planktonic microorganisms (Rice, 1973). The larva may attach by the terminal organ and extend out from this point of attachment either parallel to the substratum or upward at any angle. With head upward, food may be directed into the mouth by the cilia of the ventral surface of the head, or, still attached, the larva may bend downward, applying the ventral surface of the head to the substratum with lip extended posteriorly, grazing over the bottom. The larva is able to release itself from its terminal attachment and glide along with terminal organ directed upward and head flattened against the bottom. Frequently the larvae lie in a quiescent state or they may crawl in the manner of an inchworm, presumably scraping material from the bottom. The continual eversion of the buccal organ during feeding probably aids in the removal of food from the substratum. This tough muscular organ is believed to function in breaking up material into small particles for feeding and to aid also in the rejection of un-

wanted material or in swallowing. The secretion of the lip glands is presumed to have some function in feeding, but the nature of the secretory product is unknown (Rice, 1973). A peculiar behavior pattern of uncertain significance is the insertion of the terminal organ or posterior tip of the larva into the mouth. Jägersten (1963) has suggested that by this action the larva may discharge some secretion from the terminal organ into the mouth.

The pelagosphera larva is a significant agent in the dispersal of species over wide geographical areas. Planktotrophic pelagospheras have been found in warm and temperate waters around the world. Studies in the North Atlantic Ocean of at least 5 species of pelagosphera larvae show that the larvae occur along the entire length of the major east-west currents and that, because of their long larval life (Section 4.4.3.5), may be transported between continents (Scheltema and Hall, 1965, 1975; Scheltema, 1975).

4.4.3.4 Larval Organogenesis

Derivation of tissues and organs has been studied in 7 species of sipunculans, representing all developmental patterns: *Golfingia minuta*, *Phascolion strombi* (Åkesson, 1958), *Phascolopsis gouldi*, *Golfingia vulgaris* (Gerould, 1907), *G. elongata* (Åkesson, 1961a), *Phascolosoma agassizi* (Rice, 1973), and *Sipunculus nudus* (Hatschek, 1883).

As in all protostomous coelomates, the endomesoderm is derived from the 4d cell and the coelom originates by a splitting of the mesodermal bands of the trochophore into splanchnic and somatic layers. The coelom forms before trochophoral metamorphosis in species with planktotrophic development and relatively later in species with more yolky eggs and lecithotrophic development (Section 4.4.3.2). In all species yolk granules are released from the regressing prototroch cells into the developing coelom where they furnish an important source of nutrition for the larva.

The stomodeum develops as an ectodermal invagination at the anterior site of the closed blastopore and pushes farther anteriorly into the region of the prototroch. At the time of metamorphosis of the trochophore, the stomodeum ruptures through the egg envelope to form the mouth and the ventral ciliated surface of the head. The esophagus is derived from the stomodeum and, in planktotrophic species, the buccal organ (in part) and the lip gland, both appendages of the mouth, are also derived from the stomodeum. Entomeres, frequently marked by distinctive pigmentation, give rise to the stomach and intestine. A stomach is present only in planktotrophic larvae and disappears at metamorphosis of the pelagosphera. The anus and rectum are derived from

a proctodeal invagination which is located in a dorsal position in the middle or posterior trunk.

Larval retractor muscles, retained as the muscles of the adult, are reported to arise from ectomesoderm in all species except *Sipunculus nudus*. Hatschek (1883) proposed that in the latter species the retractors originate from somatic mesoderm, although he was unable to observe their early development. Reports of the origin of body wall musculature are conflicting. In *S. nudus* both circular and longitudinal muscles are supposed to form from endomesoderm (Hatschek, 1883). Circular muscles of *Golfingia vulgaris* and *Phascolopsis gouldi* are presumed to be derived from ectomesoderm, but no information is available on the source of the longitudinal muscles in these species (Gerould, 1907). Circular musculature of *G. minuta* and *Phascolion strombi* is believed to originate from ectomesoderm and longitudinal musculature from endomesoderm (Åkesson, 1958).

Descriptions of the derivation of the nephridia vary for different species. Observations on development in *Phascolion strombi, Golfingia minuta, G. vulgaris,* and *Phascolopsis gouldi* (Gerould, 1907; Åkesson, 1958) indicate that the nephridia are composite structures, conforming to the concept of mixonephridia by Goodrich (1945). In these species the nephridium proper has been reported to arise from an ectodermal invagination and the internal funnel from peritoneum. In *Sipunculus nudus* and *Phascolosoma agassizi*, on the other hand, a purely mesodermal origin of the nephridia has been suggested (Hatschek, 1883; Rice, 1973).

The larval cuticle in most species is formed by a transformation of the egg envelope at metamorphosis of the trochophore. However, in three species, *Phascolopsis gouldi, Golfingia vulgaris,* and *Sipunculus nudus*, the egg envelope is shed at the time of metamorphosis and the cuticle of the larva is a newly formed secretion of the epidermis (Section 4.4.3.2).

4.4.3.5 Metamorphosis of the Pelagosphera

The end of the pelagosphera stage is marked by the loss of metatrochal cilia in both lecithotrophic and planktotrophic pelagospheras. Lecithotrophic larvae, which live in the plankton for only a short time, change to the adult form gradually, passing through a vermiform stage and sometimes requiring several weeks to attain the form of the juvenile. During this time the coelomic yolk is entirely absorbed, the gut is completed, and that portion of the body anterior to the postmetatrochal sphincter is elongated to form the introvert with terminal ciliated tentacu-

lar lobes. In *Golfingia pugettensis* 7 weeks may elapse before these changes are completed; in *Themiste alutacea* the change may take 3–4 weeks.

Planktotrophic pelagospheras, after a prolonged period in the plankton, undergo a relatively rapid metamorphosis into the juvenile form. After a month in the plankton, the larva of *Sipunculus nudus* elongates and sinks to the bottom where metamorphosis takes place over a period of 1 or 2 days (Fig. 22c). The metatroch and associated organs of the mouth are lost, the mouth moves anteriorly, a tentacular lobe forms on either side of the mouth, and the head becomes proportionately smaller (Hatschek, 1883). Unidentified planktotrophic pelagospheras from the oceanic plankton have been reported to undergo a similar metamorphosis with regression of the lip and formation of tentacles on the rim of the mouth (Jägersten, 1963). Metamorphosis of "Baccaria citrinella" or Type A larvae is marked by an elongation of the introvert to a length three times that of the trunk with 35 rings of hooks at its anterior end. Over a period of several weeks, the juvenile develops anterior and posterior shields which characterize it as belonging to the genus *Aspidosiphon* (Hall and Scheltema, 1975).

Metamorphosis of oceanic pelagospheras, of unknown adult affinities, has been observed in the laboratory from 11 to 129 days after collection. Maximum duration of larval life has been calculated from approximations of age at the time of collection to be 254 days (Scheltema and Hall, 1975). Metamporphosis of planktotrophic pelagospheras reared from spawnings of known adults in the laboratory has not been observed, although larvae of *Phascolosoma agassizi* have been maintained in culture to an age of 7 months.

4.4.4 Summary of Developmental Patterns

The most common developmental patterns in the phylum Sipuncula are summarized in the schematic diagram (Fig. 26). As shown for Category I, the embryo may develop directly within the egg coats with no ciliated stage, hatching out as a small, crawling worm which gradually transforms into a juvenile, or it may develop into a lecithotrophic trochophore with ciliated prototroch and apical tuft which then develops further along one of three pathways, represented by Categories II, III, and IV. In Category II the trochophore transforms directly into the vermiform stage, ending the pelagic phase, or the trochophore may metamorphose, as in Categories III and IV, into a second larval stage, the pelagosphera with metatrochal band of cilia. The pelagosphera of Category III is lecithotrophic, remaining in the plankton only a short time

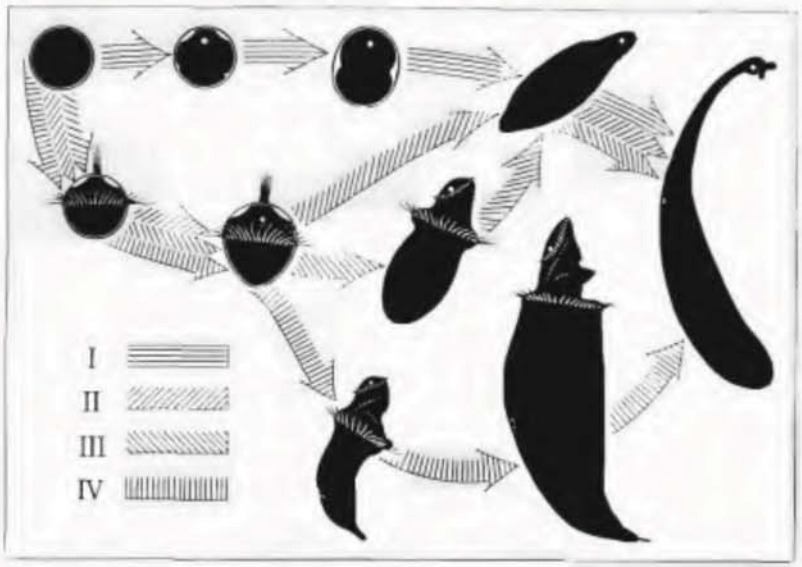


FIG. 26. Diagrammatic summary of chief developmental patterns in the Sipuncula. Category I. Direct development with no pelagic stages. Category II. Pelagic, lecithotrophic trochophore which transforms into vermiform stage. Category III. Pelagic, lecithotrophic trochophore metamorphoses into second larval stage, lecithotrophic pelagosphera, which then transforms into vermiform stage. Category IV. Pelagic, lecithotrophic trochophore metamorphoses into second larval stage, planktotrophic pelagosphera. After a prolonged period in the plankton the larva, having increased in size, undergoes a second metamorphosis into the juvenile form. (From Rice, 1975.)

before transforming into the vermiform stage, then gradually assuming the features of the juvenile. The planktotrophic pelagosphera of Category IV lives for a prolonged period in the plankton, increasing considerably in size, before undergoing a second metamorphosis directly into the juvenile form.

Acknowledgments

I am grateful to Harbor Branch Foundation Laboratory, Fort Pierce, Florida, where much of this chapter was written, for use of their facilities, and the cooperation of their staff in preparation of the figures. Mary Goldizen, Harbor Branch Foundation Laboratory, drew Figs. 17, 19, 20, 21, and 22. Douglas Putnam and William Davenport assisted in the photographic efforts.

Carolyn B. Gast, scientific illustrator in the Department of Invertebrate Zoology, National Museum of Natural History, Smithsonian Institution, is gratefully acknowledged for her illustrations which appear as Figs. 1, 3, 16, 23, and 26.

4.5 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. (1961a). 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. (1961b). Some observations on pelagosphaera larvae. *Galathea Rept.* 5, 7–17.

Andrews, E. A. (1889). The reproductive organs of *Phascolosoma gouldii*. *Zool. Anz.* 12, 140–142.

Andrews, E. A. (1890). Notes on the anatomy of *Sipunculus gouldii* Pourtalès. *Johns Hopkins Univ. Biol. Lab. Stud.* 4, 390–430.

Awati, P. R. and Pradhan, L. B. (1936). The anatomy of *Dendrostoma signifer* Selenka and de Man. *J. Univ. Bombay* 4(5), 114–131.

Carlisle, D. B. (1959). On the neurosecretory system of the brain and associated structures in *Sipunculus nudus* with a note on the cuticle. *Gunma J. Med. Sci.* 8, 183–194.

Claparède, É. (1863). "Anatomie und Entwicklungsgeschicte wirbelloser Thiere an der Küste von Normandie angestellt." vii + 120 pp. Leipzig.

Clark, R. B. (1969). Systematics and Phylogeny: Annelida, Echiura, Sipuncula. In "Chemical Zoology" (M. Florkin and B. T. Scheer, eds.) Vol. 4, Chapter 1, pp. 1–68. Academic Press, New York and London.

Cole, J. B. (1952). The morphology of *Golfingia pugettensis*: A sipunculid worm. M. S. Thesis, 78 pp. University of Washington, Seattle.

Damas, H. (1962). La collection de *Pelagosphaera* du "Dana". *Dana Rept.* No. 59, 22 pp.

Dawydoff, C. N. (1930). Quelques observations sur *Pelagosphaera*, larve de sipunculide des côtes d'Annam. *Bull. Soc. Zool. Fr.* 55, 1–22.

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

Fisher, W. K. (1952). The sipunculid worms of California and Baja, California. *Proc. U.S. Nat. Mus.* 102(3306), 371–450.

Franzén, Å. (1956). On spermiogenesis, morphology of the spermatozoan, and biology of fertilization among invertebrates. *Zool. Bidr. Uppsala* 31, 355–482.

Gabe, M. (1953). Données histologiques sur la neurosécrétion chez quelques Sipunculiens. *Bull. Lab. Dinard* 38, 3–15.

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.

Gerould, B. (1907). Studies on the embryology of the Sipunculidae, II. The development of *Phascolosoma*. *Zool. Jahrb. Abt. Anat. Ontog. Thiere* 23, 77–162.

Gonse, P. (1956a). L'ovogenèse chez *Phascolosoma vulgare*. I. Définition cytologique des stades de croissance des ovocytes. *Acta Zool.* 37, 193–224.

Gonse, P. (1956b). L'ovogenèse chez *Phascolosoma vulgare*. II. Recherches biométriques sur les ovocytes. *Acta Zool.* 37, 225–233.

Gonse, P. (1957a). L'ovogenèse chez *Phascolosoma vulgare*. III. Respiration exogène et endogène de l'ovocyte. Effet de l'eau de mer. *Biochim. Biophys. Acta* **24**, 267–278.

Gonse, P. (1957b). L'ovogenèse chez *Phascolosoma vulgare*. IV. Etude chromatique des sucres du plasma, action de différents substrats et du malonate sur la respiration de l'ovocyte. *Biochim. Biophys. Acta* 24, 520–531.

Goodrich, E. S. (1945). The study of the nephridia and genital ducts since 1895. *Quart. J. Microsc. Sci.* 86 (342–344), 113–392.

Green, W. (1975). The annual reproductive cycle of *Phascolosoma lurco* (Sipuncula). *Proc. Intern. Symp. Biol. Sipuncula and Echiura*, in press. Naučno Delo Press, Belgrade.

Häcker, V. (1898). Die pelagischen Polychaeten- und Achaetenlarven der Plankton-Expedition. *Ergeb. Plankton-Exped. Humboldt-Stiftung* **2**, 1–50.

Hall, J. R. and Scheltema, R. S. (1975). Comparative morphology of open-ocean pelagosphaera. *Proc. Intern. Symp. Biol. Sipuncula and Echiura*, in press. Naučno Delo Press, Belgrade.

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

Heath, H. (1910). *Pelagosphaera*, a larval Gephyrean. *Biol. Bull.* **18**, 61–140.

Hérubel, M. A. (1908). Récherches sur les sipunculides. *Mém. Soc. Zool. Fr.* **20**, 107–419.

Hyman, L. H. (1959). "The Invertebrates: Smaller Coelomate Groups," Vol. 5. McGraw-Hill, New York.

Jägersten, G. (1963). On the morphology and behaviour of *Pelagosphaera* larvae (Sipunculoidea). *Zool. Bidr. Uppsala* 36, 27–35.

Keferstein, W. (1863). Beiträge zur Kenntnis der Gattung *Phascolosoma*. *Z. Wiss. Zool.* **12**, 35–51.

Mingazinni, P. (1905). Un Gefireo pelagico: *Pelagosphaera Aloysii* n. gen., n. sp. *Rend. Acad. Naz. Lincei* **14**, 713–720.

Murina, V. V. (1965). Some data on the structure of pelagospheres—sipunculid larvae. *Zool. Zh.* **44**(11), 1610–1619 (in Russian).

Pasteels, J. J. (1935). Recherches sur le déterminisme de l'entrée en maturation de l'oeuf chez divers Invertébrés marins. *Arch. Biol.* **46**, 229–262.

Rajulu, G. S. (1975). Asexual reproduction by budding in the Sipuncula. *Proc. Intern. Symp. Biol. Sipuncula and Echiura*, in press. Naučno Delo Press, Belgrade.

Rajulu, G. S. and Krishnan, N. (1969). Occurrence of asexual reproduction by budding in Sipunculida. *Nature (London)* **223**, 186–187.

Rice, M. E. (1966). Reproductive biology and development in Sipuncula. Doctoral Dissertation, University of Washington. Seattle. 322 pp.

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. (1970). Asexual reproduction in a sipunculan worm. *Science* **167**, 1618–1620.

Rice, M. E. (1973). Morphology, behavior, and histogenesis of the pelagosphera larva of *Phascolosoma agassizii* (Sipuncula). *Smithson. Contrib. Zool. No.* 132, 1–51.

Rice, M. E. (1974). Gametogenesis in three species of Sipuncula: *Phascolosoma agassizii*, *Golfingia pugettensis*, and *Themiste pyroides*. *La Cellule*, **70**(2), 295–313.

Rice, M. E. (1975). Observations on the development of six species of Caribbean Sipuncula with a review of development in the phylum. *Proc. Intern. Symp. Biol. Sipuncula and Echiura*, in press. Naucno Delo Press, Belgrade.

Sawada, N., Noda, Y., and Ochi, O. (1968). An electron microscope study on the oogenesis of *Golfingia ikedai*. *Mem. Ehime Univ. Sci. Ser. B*6(1), 25–39.

Scheltema, R. S. (1975). The frequency of long-distance larval dispersal and the rate of gene-flow between widely separated populations of sipunculans. *Proc. Intern. Symp. Biol. Sipuncula and Echiura*, in press. Naucno Delo Press, Belgrade.

Scheltema, R. S., and Hall, J. R. (1965). Trans-oceanic transport of sipunculid larvae belonging to the genus *Phascolosoma*. *Amer. Zool.* 5(2), 216 (Abstr.).

Scheltema, R. S. and Hall, J. R. (1975). The dispersal of pelagosphera larvae by ocean currents and its relationship to geographical distribution of sipunculans. *Proc. Intern. Symp. Biol. Sipuncula and Echiura*, in press. Naučno Delo Press, Belgrade.

Schleip, W. (1934). Die Regeneration des Rüssels *Phascolion strombi* Mont. (Sipunculidae). *Z. Wiss. Zool.* **145**, 462–496.

Senna, A. (1906). Sulla struttura di alcune larve (Pelagosphaera) di Sipunculidi. *Pubbl. Inst. Stud. Superiori Pract. Perfezionamento Firenae; Sez. Sci. Fis. Nat.*, pp. 50–78.

Shipley, A. E. (1890). On *Phymosoma varians*. *Quart. J. Microsc. Sci.* **31**, (1), 1–27.

Stephen, A. C. (1941). The Echiuridae, Sipunculidae and Priapulidae collected by the ships of the "Discovery" Committee during the years 1926 to 1937. *Discovery Rep.* **21**, 237–260.

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

Thorson, G. (1946). Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund). *Medd. Kommn Danm. Fisk. Havunders. Plankton* 4, 1–523.

Towle, A. and Giese, A. C. (1967). The annual reproductive cycle of the sipunculid *Phascolosoma agassizii*. *Physiol. Zool.* **40**, 229–237.

Wegener, F. (1938). Beitrag zur Kenntnis der Russelregeneration der Sipunculiden. *Z. Wiss. Zool.* **150**, 527–565.

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