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# MORPHOLOGICAL AND BEHAVIORAL CHANGES AT METAMORPHOSIS IN THE SIPUNCULA

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Pelagosphera larvae, collected from open-ocean plankton, were reared in the laboratory to sexually mature adults and identified as *Golfingia misakiana* (Ikeda, 1904). From spawnings of these adults, embryos were reared through the trochophore stage to the young pelagosphera. Metamorphosis is described from the trochophore to the pelagosphera and from the pelagosphera to the juvenile. Behavioral changes at metamorphosis are noted and a procedure is reported for inducing metamorphosis of the pelagosphera in the laboratory. Literature on metamorphosis in the Sipuncula is reviewed.

## INTRODUCTION

Developmental patterns of sipunculans, now known for 20 species, fall into four basic patterns (Table 1). One is direct development in which the egg develops into the juvenile without passing through a swimming larval stage. Usually the embryo hatches from one or more enveloping egg coats into a vermiform stage—a crawling, nonfeeding form with plastic body shape—which undergoes a gradual transition into the juvenile. More frequently development of sipunculans is indirect, including one or two swimming larval stages. Some species have only one pelagic larva, a lecithotrophic trochophore which swims for a short time before settling and transforming into the juvenile. The majority of species have two larval stages, a trochophore and a pelagosphera. The trochophore stage, which is always lecithotrophic, is followed by the pelagosphera, which may be either lecithotrophic or planktotrophic. The lecithotrophic pelagosphera swims for a short time, from 2 to 9 days in different species, before transforming to the crawling, vermiform stage.[1] The planktotrophic larva, on the other hand, may remain in the plankton for as long as 6 to 7 months,[1,2] during which time it increases in size considerably before a final metamorphosis into the juvenile form.

Two metamorphoses are recognized in sipunculan development. The first is metamorphosis of the trochophore. In those species in which the trochophore is the only larval stage, this metamorphosis ends pelagic existence. In other species metamorphosis of the trochophore results in another larval stage, the pelagosphera. The second metamorphosis is from the pelagosphera larva to the bottom-dwelling juvenile.

Of the two larval types in the Sipuncula, the trochophore, has the same essential features as that of other Protostomia.[1,3,4,5,6,7,8,9] An equatorial band of ciliated prototroch cells divides the larva into anterior and posterior hemispheres, the former bearing a pair of dorsal eyespots and an apical rosette with long apical cilia. In the posterior hemisphere a ventral stomodaeum is located just beneath the prototroch and a lateral band of mesoderm lies on either side of the rudiment of the gut. There is no protonephridium.

The second larval form, the pelagosphera, is unique to the Sipuncula.[1,10] Characteristic features of the pelagosphera are a prominent band of metatrochal cilia and a reduced prototroch. The body is regionated into head, "thorax"or metatrochal collar, trunk, and, frequently, a terminal attachment organ.[1,7,10] In the planktotrophic pelagosphera, there is a completed gut, consisting of esophagus, bulbous stomach, and looped intestine opening into a dorsal anus

TABLE 1

Patterns of Development in the Sipuncula[a]

DIRECT DEVELOPMENT

I. EGG → WORM

*Golfingia minuta*[5]
*Phascolion cryptus*[11]
*Themiste pyroides*[1]

INDIRECT DEVELOPMENT

II. EGG → TROCHOPHORE → WORM

*Phascolion strombi*[5]
*Phascolopsis gouldi*[4]

III. EGG → TROCHOPHORE → LECITHOTROPHIC PELAGOSPHERA → WORM

*Golfingia elongata*[6]
*Golfingia pugettensis*[1]
*Golfingia vulgaris*[4]
*Themiste alutacea*[11]
*Themiste lageniformis*[b12]
*Themiste petricola*[13]

IV. EGG → TROCHOPHORE → PLANKTOTROPHIC PELAGOSPHERA → WORM

*Aspidosiphon parvulus*[9]
*Golfingia misakiana*
*Golfingia pellucida*[9]
*Paraspidosiphon fischeri*[11]
*Phascolosoma agassizii*[1]
*Phascolosoma antillarum*[11]
*Phascolosoma perlucens*[11]
*Phascolosoma varians*[11]
*Sipunculus nudus*[14]

[a] Modified from Rice 1976.[9]
[b] A pelagic trochophore stage is lacking.

in the middle of the trunk. Two organs associated with the mouth and presumably used in feeding are the protrusible buccal organ and the lip glands, which open through a pore on the lower lip at the base of the mouth. A prominent ventral nerve cord extends from the posterior trunk to the lip, and a dorsal, bilobed brain is present in the head. A pair of nephridia opens ventrolaterally in the anterior or middle trunk. Two pairs of retractor muscles, which function to withdraw the head into the trunk, traverse a spacious coelom containing numerous coelomocytes.

Metamorphosis of the trochophore to the pelagosphera usually results in a loss or reduction of the prototroch; a rupture in the egg envelope overlying the stomodaeum which opens to form the ventral ciliated surface of the head; an elongation of the post-prototrochal body; expansion of the coelom; formation of the metatroch as the functional locomotory organ; and frequently, the formation of a terminal attachment organ. When the resulting pelagosphera is planktotrophic, the gut is completed at metamorphosis with the opening of the mouth and anus.

The events of larval development and metamorphosis in the Sipuncula have been enumerated and reviewed in a series of previous articles.[1,7,8,9] Much of the previous information on metamorphosis has been concerned with metamorphosis of the trochophore. Only a few studies have considered metamorphosis of the planktotrophic pelagosphera to the juvenile.[7,10,14,15,16]

In this paper recent studies will be reported on the metamorphosis of an open-ocean pelagosphera, reared in the laboratory and identified as *Golfingia misakiana* (Ikeda 1904).* This larva, previously designated as *Baccaria oliva* by Häcker in 1898,[17] and later as Type C by Hall and Scheltema in 1975,[15] has been reared through metamorphosis to sexually mature adults. From spawnings of these adults, embryos have been reared through the trochophore stage to the young pelagosphera. This is the first time that breeding adults of Sipuncula have been reared from planktotrophic larvae and, with the exception of Hatschek's observations on *Sipunculus nudus*,[14] that metamorphosis has been observed in both trochophore and pelagosphera larva in a single species with planktotrophic larval development. Efforts to rear the young pelagosphera to the larger and older larval form found in the plankton have been unsuccessful. Observations have been made on behavioral changes of this species at metamorphosis and a technique devised for inducing metamorphosis in the laboratory. The final discussion in the paper will review the literature on metamorphosis in the Sipuncula, including a consideration of recent observations on *Golfingia misakiana.*

## MATERIALS AND METHODS

Pelagosphera larvae were collected in surface plankton tows in the Florida Current 20 to 25 miles offshore from Fort Pierce, Florida over bottom depths of 200 to 270 m, with a net 3/4 m in diameter and mesh of 125 μm. The tows, each lasting from 15 to 20 minutes were made from the RV *Gosnold* of the Harbor Branch Foundation, Inc. or, weather permitting, from a smaller 22-ft boat. Sipunculan larvae were sorted immediately on return to the laboratory.

Substratum, when provided, consisted of a silty-mud collected from offshore stations in areas known to be inhabited by *Golfingia misakiana.* It was sieved through a mesh of 100 to 200 μm and placed in plastic dishes of 500 to 1000 ml capacity to a depth of 1 cm. Being larger than substratum particles, specimens could be easily removed for observation or fixation by sieving the substratum through a screen of appropriate mesh size. An inlet tube inserted into the dish above the level of the substratum and an outlet tube at a higher level allowed passage of fresh sea water through the dish without disturbing the substratum. Water was run through the dishes for 10 to 20 minutes each day, usually 6 days a week.

Specimens to be used for light microscopy and scanning electron microscopy were fixed in 2.5% glutaraldehyde, buffered with Millonig's phosphate buffer adjusted to an osmolality of 1000 milliosmols by the addition of sodium chloride. Prior to fixation, larvae or juveniles were anesthetized in 10% ethanol in sea water for approximately 5 minutes or until their heads remained extended. If not extended, heads could sometimes be forced to protrude by gentle pressure on the trunk with an applicator stick, broken to form a fine point. Specimens to be used for light microscopy were embedded in polyester, sectioned at 4 to 6 μm and stained in Mallory's stain, or post-fixed in 2% osmium tetroxide with Millonig's phosphate buffer, embedded in Epon for sectioning at 1 μm and stained with Richardson's stain.[18] For scanning electron microscopy, specimens were dried in a critical point dryer with liquid $CO_2$ and coated in a sputtering unit with gold-palladium.

Adults, reared from larvae, were removed periodically from the substratum and examined for coelomic gametes which, when present, are visible through the thin body wall of the

*Identification as *Golfingia misakiana* is based on the following characters of the adult: average ratio of introvert length to trunk, 4 to 1; usually 6 to 8 tentacles dorsal to mouth; introvert hooks usually with 5 basal spinelets; 4 retractor muscles, dorsals usually attached near level of nephridiopores, ventrals slightly posterior to anus; bilobed nephridia attached anterior to anus; spindle muscle attached posteriorly.

introvert. Specimens with gametes ranged in age from 9 months to two and one-half years. They were maintained in fingerbowls without substratum until spawning occurred. Fertilized eggs were transferred to tall covered petri dishes for culturing of embryos and larvae.

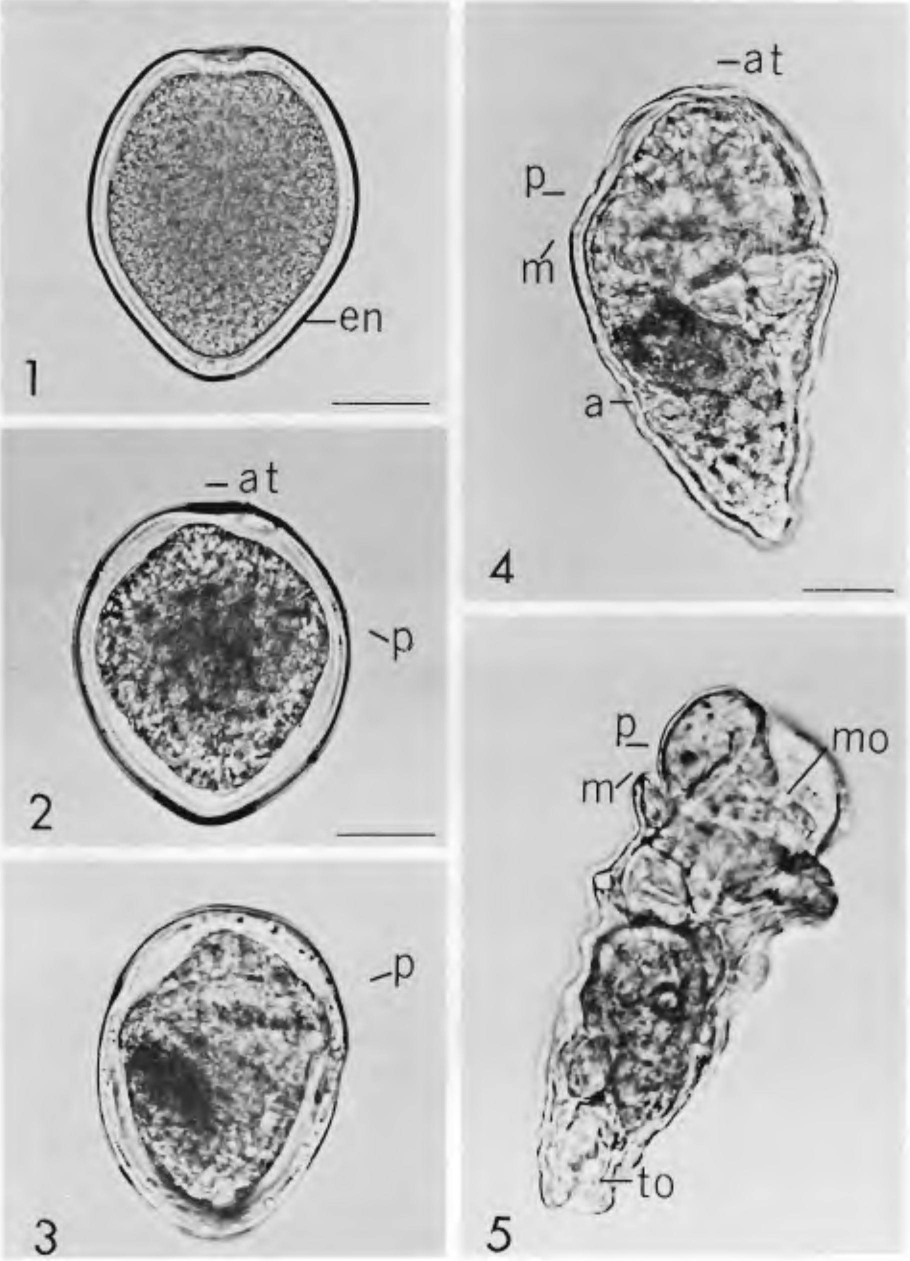
## LARVAL DEVELOPMENT AND METAMORPHOSIS OF THE TROCHOPHORE

Development from the fertilized egg through metamorphosis of the trochophore to the young pelagosphera larva of *Golfingia misakiana* is illustrated in photographs of living embryos and larvae in Figures 1 to 5. At fertilization the egg is ovoid in shape with a central apical depression and measures 70 x 88 μm. There is a thick egg envelope composed of 3 distinct layers which are perforated by pores. Within 8 hours, at 23°C, prototrochal cilia and the apical tuft make their appearance. The embryo begins to swim, at first remaining near the bottom but later swimming throughout the water column. By 24 hours the features of the trochophore are fully developed. Enclosed by the egg envelope, the trochophore has about the same size and shape as the egg. A pair of red eyespots is located dorsolaterally above the prototroch, and an apical groove, separating cytoplasm from egg envelope surrounds the cells bearing the apical tuft. The prominent equatorial prototroch with its numerous long cilia is expanded ventrally to form a lobe, beneath which lies the ciliated stomodaeal opening leading to the esophagus. Rudiments of stomach and intestine are distinguished in living larvae by a white pigmentation.

Certain changes preliminary to metamorphosis are apparent at 3 to 3½ days of age. The trochophore begins to elongate posterior to the prototroch. A small bulge at the posterior extremity marks the position of the future terminal organ and depressions in the egg envelope at the level of stomodaeum and rectum indicate the sites where the envelope will rupture to form the mouth and anus. The three areas of the gut are well defined and the lumen has formed. The buccal organ is visible and actively protrusible beneath the egg envelope. The coelom, which will undergo later expansion at metamorphosis, is visible as a narrow slit traversed by long retractor muscles.

Metamorphosis of the trochophore to the pelagosphera larva occurs on the fourth day after fertilization and takes place over a period of 6 to 8 hours (Fig. 6). At metamorphosis the trochophore elongates, attaining a length of 150 μm when fully extended. The mouth and anus are opened to the exterior by rupture of the overlying egg envelope, thus completing the gut, and the terminal attachment organ is formed. The eversion of the entire stomodaeal area results in the formation of the ventral ciliated surface of the head and the disruption of the ventral portion of the prototroch. The head is rotated backward, the former apex assuming a more dorsal position and the apical tuft is soon lost. At the same time the retractor muscles become functional, so that the head and terminal organ can be withdrawn into the trunk. The postprototrochal egg envelope is transformed into the larval cuticle, losing its porosity and lamellation. The pretrochal envelope of the head is sloughed off gradually over a period of several days and replaced by a thin underlying cuticle. The entire body becomes quite extensible, with the capacity for considerable elongation and contraction. A metatrochal lobe is formed, delimited posteriorly by the sphincter muscle, and metatrochal cilia appear on the inner side of the lobe nearest the head. Metatrochal cilia are apparent in the sectioned material, although they are difficult to distinguish from prototrochal cilia in living larvae. Unlike young pelagosphera of other species, the dorsal and lateral prototroch is not reduced and metatrochal cilia combine with prototrochal cilia to perform a locomotory function.

At the time of metamorphosis the larva descends to the bottom of the dish, at first swimming along the bottom, and making temporary attachments with the terminal organ. Although most frequently attached to the substratum, the larva may swim or crawl along the bottom or it may move along the bottom on its head, with ventral surface of the head applied to the substratum and posterior extremity directed upward.




Figs. 1-5. Early development and metamorphosis of the trochophore of *Golfingia misakiana*. 1. Recently spawned, unfertilized egg. 2. Early trochophore, approximately 24 hours. 3. Trochophore, 30 hours. 4. Premetamorphosis, 3½ days. Regions of future mouth are indicated by depressions on larval cuticle. 5. Young pelagosphera, 5½ days. Terminal organ retracted. Scale, 25 μm.

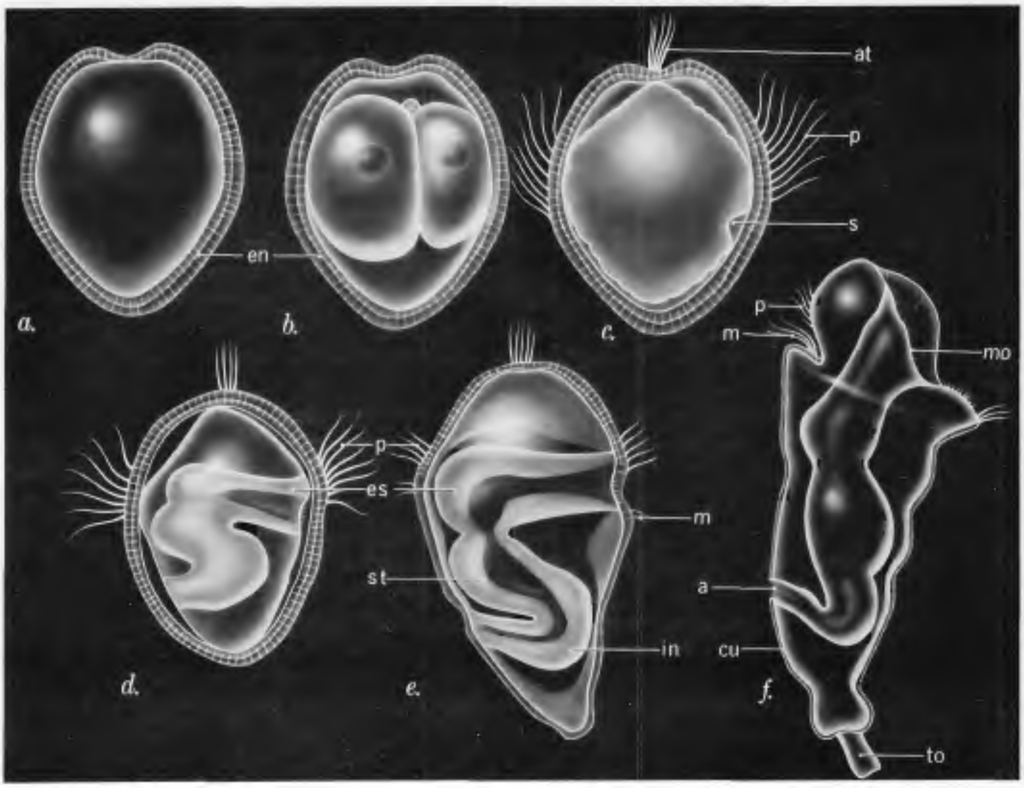



Fig. 6. Diagrammatic representation of metamorphosis of the trochophore of *Golfingia misakiana,* showing opening of gut; formation of coelom, terminal organ, metatroch, and ventral ciliated surface of head; and transformation of egg envelope to larval cuticle. a. Unfertilized egg. b. Two-cell stage. c. Early trochophore, one day. d. Trochophore, 3 days. e. Premetamorphosis, 4 days. f. Young pelagosphera, 5 days.

Cultures of early pelagosphera larvae, reared from the fertilized egg, have survived in the laboratory as long as 30 days. The oldest larvae reached a maximum length of 170 $\mu$m and were either attached or swimming close to the bottom of the dish.

## MORPHOLOGY OF THE PELAGOSPHERA LARVA

Although they have the same basic morphological features, older pelagosphera larvae, collected from the oceanic plankton, are strikingly different from the younger larvae reared in the laboratory (Figs. 7a, 7b, 14, 20). Oceanic larvae range in size from 0.5 to 0.8 mm and the body is generally white or pinkish in color. The green nephridia and the black esophagus, stomach, and intestine are readily visible through the body wall. The shape and proportions of the older larva differ in that the head is relatively smaller and the thorax or metatrochal collar is distinctly delimited by the postmetatrochal sphincter and is more extensible. The trunk is less attenuated posteriorly, being either cylindrical or ovoid, depending on the state of contraction, and the terminal organ is proportionally reduced. Details of the external and internal morphology of the oceanic pelagosphera larva are outlined below, with notations regarding other differences from the younger larva.

The ciliated ventral head of the oceanic pelagosphera is bisected by a black-pigmented median groove. This groove broadens basally to include the mouth opening and the transverse groove through which the buccal organ is protruded (Fig. 10). The pattern of ciliation of the ventral

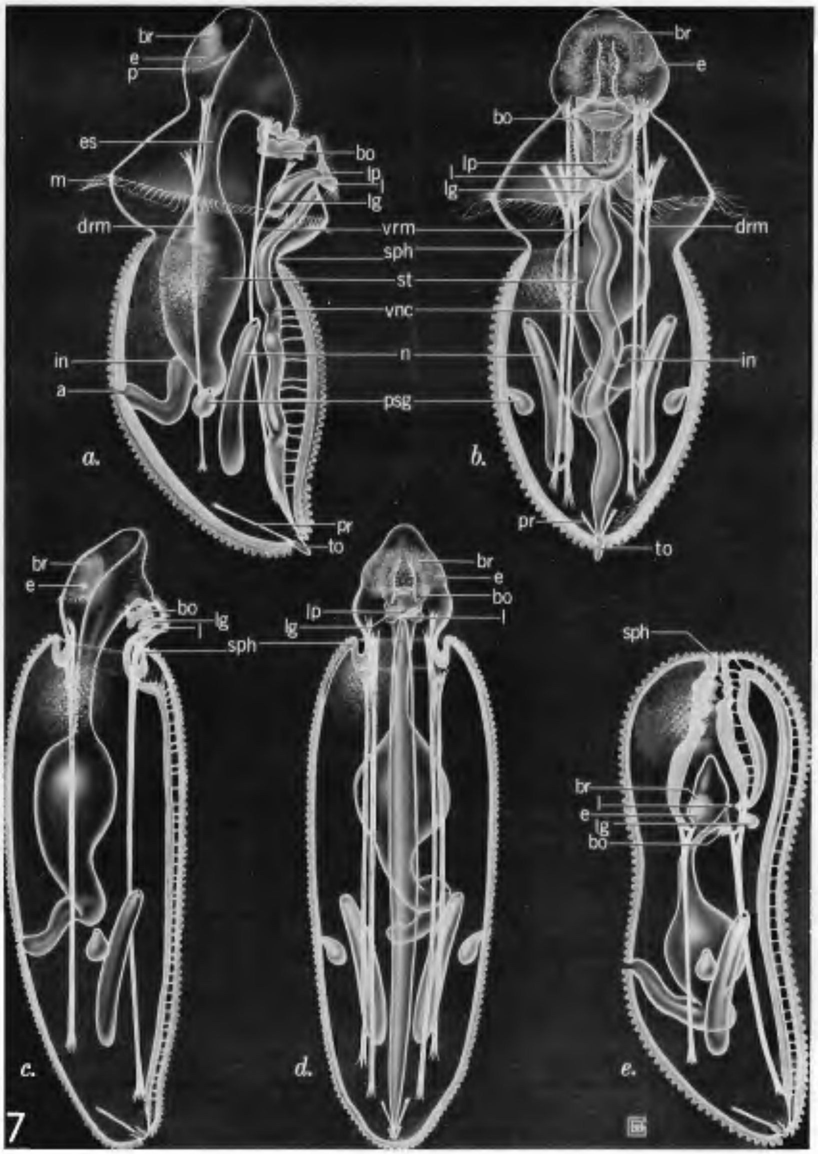



Fig. 7. Illustrations showing initial metamorphosis of the pelagosphera larva of *Golfingia misakiana* from open-ocean plankton. a. Lateral view of larva. b. Frontal view of larva. c. Two days in substratum, lateral view. d. Two days in substratum, frontal view. e. Three days in substratum, lateral view. Head retracted.

head continues around the buccal groove to surround the lip pore of the lower lip, covering about one-half of the surface of the lip. The lower lip is more clearly delimited from the thorax than in the younger larva. The ciliated portion of the lip is raised and separated from the remainder of the lip by a semicircular groove. The remainder of the crescent-shaped lip is devoid of cilia, except at the margin where the cilia are longer and less dense. A horseshoe-shaped band of short cilia on the dorsal head, presumably a remnant of the prototroch of the larva, merges anterolaterally with the ventral cilia of the head. The two small eyespots of the oceanic larva, dark red to black in color, are located in the same position on the head as the larger eyespots of the young pelagosphera.

The thorax, defined as the region of the body between the head and postmetatrochal sphincter, includes the metatrochal band[10] (Figs. 7a, 7b, 8, 14). Unlike the weakly ciliated band of the early pelagosphera of *Golfingia misakiana,* the metatroch of the older larva is formed of dense cilia and is the primary locomotory organ of the larva. The entire thorax is capable of great distension and may be fully distended when the larva is swimming. When the larva is contracted, both head and thorax may be withdrawn within the trunk.

The anterior margin of the trunk is formed by the postmetatrochal sphincter. Unlike the cuticle of the remainder of the body and that of the early pelagosphera, the cuticle of the trunk is covered with small papillae (Fig. 19). They are approximately 10 μm in height and 8 μm wide at the base, tapering to a rounded apex of 5 μm in diameter. Beneath the rounded cap of the papilla, the column characteristically bears two or three projecting ridges. Sensory-secretory organs, observed with the scanning electron microscope, are scattered among the papillae. On the anterior trunk, immediately posterior to the sphincter, the papillae are smaller and spaced farther apart.

In contrast to its prominence in the early pelagosphera, the terminal organ in the oceanic larva is relatively small and usually retracted. Even when extended, it is used for attachment only infrequently, and even then the attachment is weak and the animal easily dislodged.

Major features of the internal morphology of the oceanic larva of *Golfingia misakiana* observed in sectioned material are illustrated in Figures 7a, 7b, and 8. There is a long esophagus, a large, bulbous stomach, and an intestine. The intestine forms two or more loose loops before ascending anteriorly to the short rectum and anus in the mid-dorsal trunk. Two organs associated with the mouth and common to all planktotrophic pelagosphera larvae are the buccal organ, a protrusible muscular organ at the base of the lower lip, and a pair of lip glands opening through a common pore on the lip. Two tubular nephridia, approximately 1/3 the length of the trunk, are suspended in the coelom from ventrolateral attachments anterior to the anus. A posterior sacciform gland is present in a lateral position on either side of the anus. There are four retractor muscles, two dorsal and two ventral. Posteriorly the dorsal retractors attach to the body wall somewhat below the anus while the ventral retractors attach to the body wall farther posteriorly. Anteriorly both dorsal and ventral retractors split, each muscle with one branch attaching to the mid-premetatrochal body wall. The dorsal retractors continue into the head, attaching just posterior to the brain. The anteriormost attachments of the ventral retractors are on either side of the mouth region, at the base of the lobes of the ventral head. Two short retractors of the terminal organ attach posteriorly to the dorsal body wall. A median, unsegmented ventral nerve cord is connected to the body wall along its length by numerous nerves. The cord extends from the posterior extremity of the body anteriorly and attaches to the

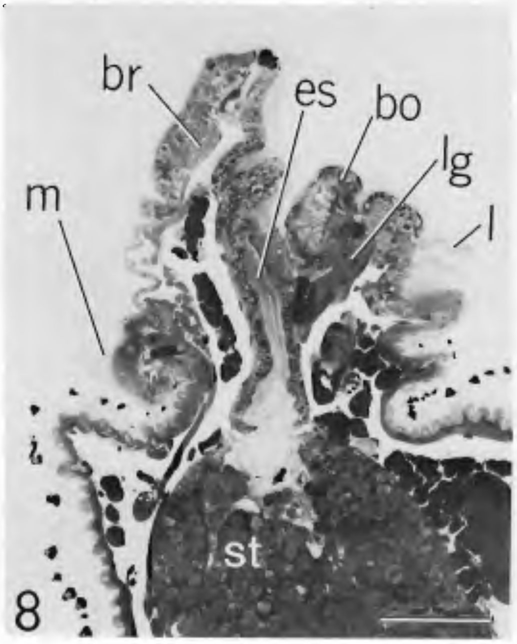


Fig. 8. Sagittal section through anterior body of oceanic pelagosphera of *Golfingia misakiana.* 1 μm, Epon-embedded, Richardson's stain. Scale, 50 μm.

body just beneath the lower lip. Circumesophageal connectives are difficult to follow, but presumably are basiepidermal, extending along the ventral lobes of the head to the dorsal brain. The brain is subepidermal and bilobed with a prominent central neuropile. The lateral eyespots occur within the outermost posterior portion of the brain.

## MORPHOLOGICAL CHANGES AT METAMORPHOSIS OF THE PELAGOSPHERA

Complete metamorphosis of the pelagosphera of *Golfingia misakiana* from larva to juvenile requires approximately 16 days at 23°C under laboratory conditions. It begins with retraction of the larval head and ends with complete extension of the newly formed introvert and juvenile head, bearing terminal tentacles (Figs. 10-18, 20-25). Whereas changes in external morphology have been studied for the entire period of metamorphosis, internal changes were noted only during the first three days.

Metamorphosis begins with construction of the postmetatrochal sphincter, preventing the extension of the retracted head. This may occur after the larva burrows into substratum, or, in the absence of substratum, after a prolonged period in laboratory containers (see **Behavioral Changes**). Approximately two days after construction of the sphincter, several major morphological changes are apparent. The body becomes more elongate and the head, which at this time may still occasionally be extruded past the tightened sphincter of the anterior trunk, is narrower and more pointed (Figs. 7c, 7d, 11, 12, 15). The lobes of the ventral head are reduced, the head is more flattened dorsoventrally and the lower lip has regressed (Figs. 11, 12). Sectioned material shows the head coelom to be diminished (Fig. 9). Although the prototroch is still present, the metatroch has undergone considerable change. The entire metatrochal area or thorax is narrower and the metatrochal cilia are reduced in size and patchy in distribution. In some individuals the cilia are entirely lost and in others they occur only in a limited mid-dorsal region. The epidermis on either side of the metatrochal band has thickened and the premetatrochal body appears shortened.


Fig. 9. Sagittal section through anterior body of metamorphosing pelagosphera of *Golfingia misakiana* after 3 days in substratum. Head is retracted. 1 μm, Epon-embedded, Richardson's stain. Scale, 50 μm.

At three days the larval head can no longer be extended from its retracted position (Figs. 7e, 9, 16). The sphincter of the anterior trunk is tightly contracted and the two pairs of thickened retractor muscles extend to the posterior trunk from their attachments at the base of the head. Anteriorly the dorsal retractors attach to the body wall at the base of the brain and the ventral retractors attach on either side of the esophageal opening. The branch of each retractor which formerly attached above the metatroch in the larva now appears fused with the inverted premetatrochal body wall and, in part, with the main muscles at the base of the head.

The head, as seen in sectioned material, is further reduced in size and more pointed in shape. The head coelom is completely obliterated, effecting the apposition of epidermis of the ventral head and the brain. The brain is in a more posterior position in the head and its two lobes, now closer together, enclose the central neuropile. The ventral nerve cord, attached to the body wall by numerous nerves, extends anteriorly to the closed sphincter, then posteriorly to follow the inverted body wall, ending, as in the larva, in a subepidermal attachment beneath the lip pore. At this 3-day stage the lip pore is still present but the lip glands and buccal organ are reduced in size. The epidermis of the inverted thorax has inreased in thickness and, along with its overlying cuticle, has become greatly elongated with numerous infoldings.

At 5 days the newly forming tubular introvert can be extruded for a short distance beyond the constricted sphincter. By relatively rapid growth, the inverted larval thorax gives rise to the introvert of the juvenile and, as the new introvert is pushed outward, the epidermis is unfolded and stretched. (Fig. 17).

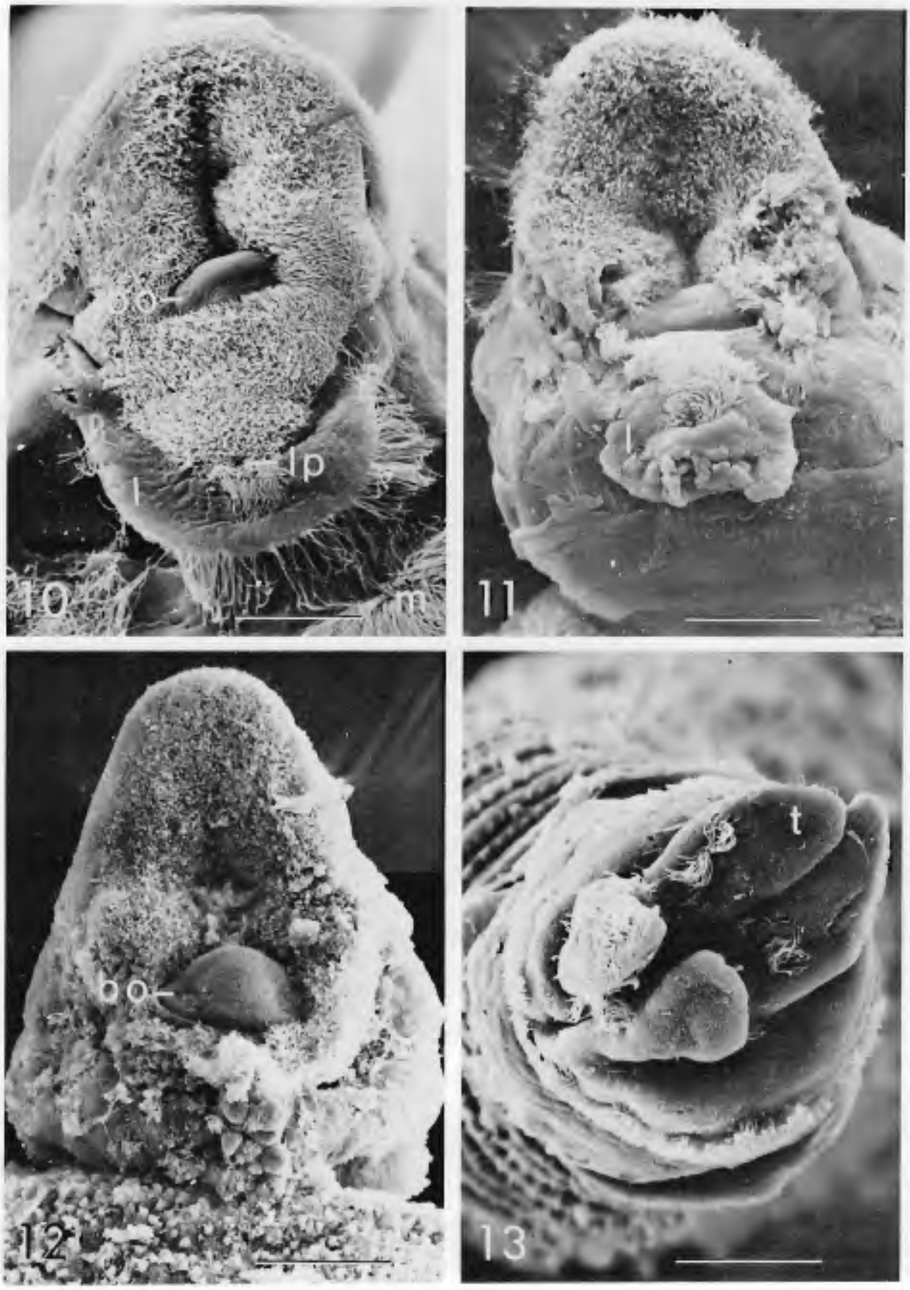
At 7 days the body shape is similar to that of juvenile and adult (Fig. 18). The anterior trunk is slender, tapering into the narrow introvert. The anterior swelling of the trunk has disappeared, although the position of the sphincter can still be detected by a slight constriction. The newly formed introvert that is extended beyond the sphincter has a thin and transparent body wall and lacks the cuticular papillae characteristic of the trunk. The terminal organ is lost within the first few days, but internal organs of the trunk remain relatively unchanged during the initial phases of metamorphosis.

Although the introvert continues to grow it is not fully extended until two to three weeks after metamorphosis begins (Figs. 22, 23, 24). At this time the transformed head, bearing 4 short terminal tentacles, is finally extruded and the extended introvert is two to three times the length of the trunk. With extension of the head, transformation to the juvenile is complete.

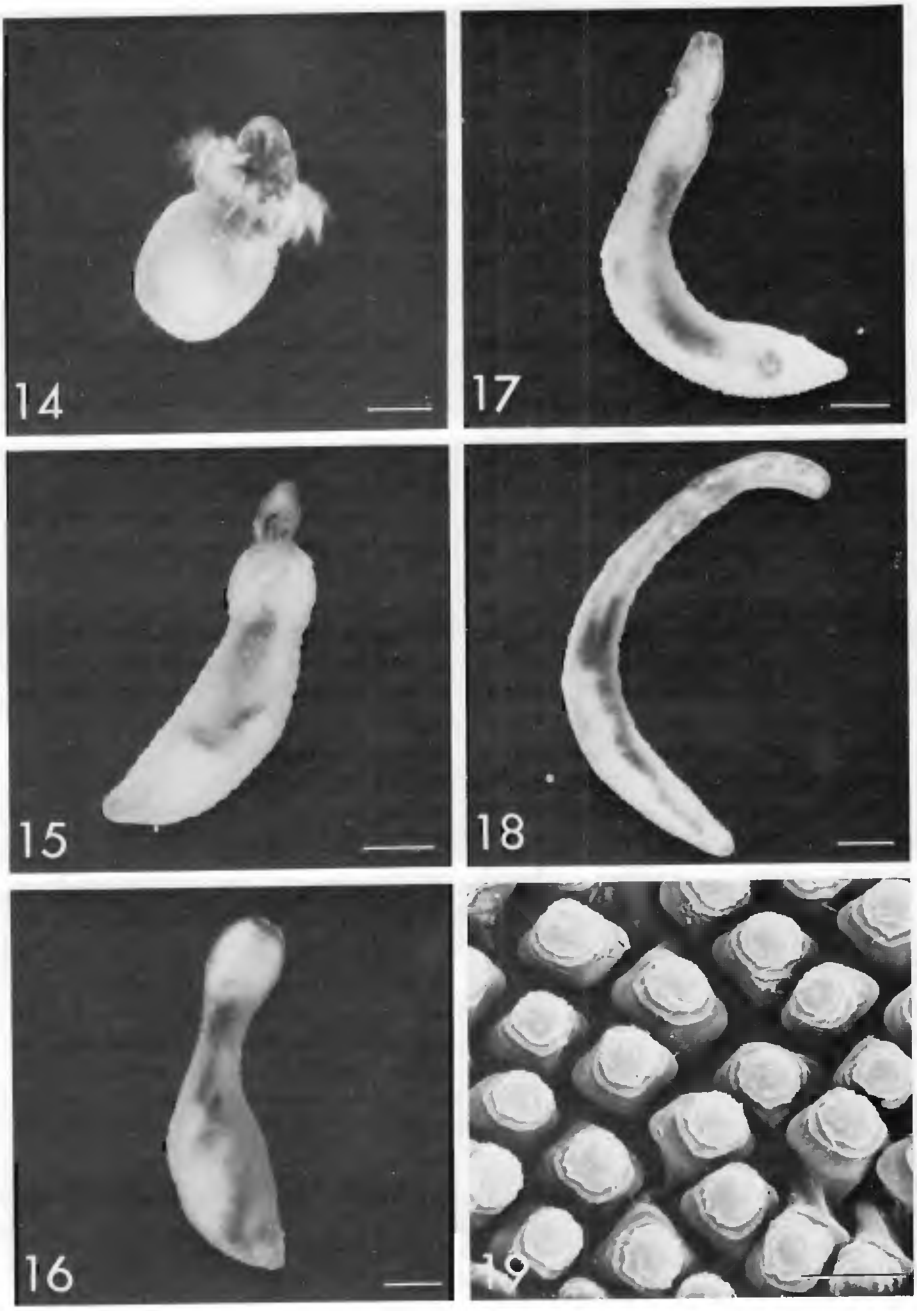
As in the adult, the tentacles are dorsal to the mouth and the dorsal tentacles are always shorter than the ventral tentacles (Figs. 13, 26). Outer surfaces of the tentacles are ciliated and on the inner surfaces there are scattered clumps of cilia. A ciliary band surrounds the base of the tentacular crown and the mouth opening (Fig. 26). Posterior to the ciliary band the cuticle is smooth and thin, and forms a distinctive neck region which is set apart from the remainder of the introvert by a cuticular fold. The most anterior introvert bears 6 rows of hooks, each hook usually with 5 basal spinelets (Fig. 27). The rows of hooks alternate with rows of small introvert papillae, quite different from the cuticular papillae which are still present on the trunk. Scattered hooks, simple in form and lacking the basal spinelets, occur more posteriorly on the introvert. Minute cuticular folds or ridges give the surface of the cuticle a finely striated appearance (Fig. 24). The brain, visible through the wall of the introvert, bears 4 eyespots. Two eyespots are lateral, posterior and black, and two are red, smaller, more median and anterior; the former are the larval eyespots and the latter the newly formed eyes of the adult. The long esophagus, stretching the length of the introvert, has lost its pigmentation. Many juveniles of this age, but not all, show a partial loss of pigmentation in the gut. The expanded stomach is no longer obvious and the intestine forms 2 to 3 loose coils, recurving to the mid-dorsal anus. With an expansion of the coelom of the trunk, the body wall appears thinner than that of the larva and the cuticular papillae are farther apart. The nephridia are lighter green in color, but otherwise not noticeably changed. Because of the great extensibility and changes in shape, absolute size measurements are difficult. The extended body is estimated to be 1.5 mm in length, about 2/3 of which is introvert. In the adult the ratio of introvert to trunk is 4 to 1, the number of tentacles has increased to 6 or 8, and there are many more rows of hooks.

## BEHAVIORAL CHANGES AT METAMORPHOSIS OF THE PELAGOSPHERA

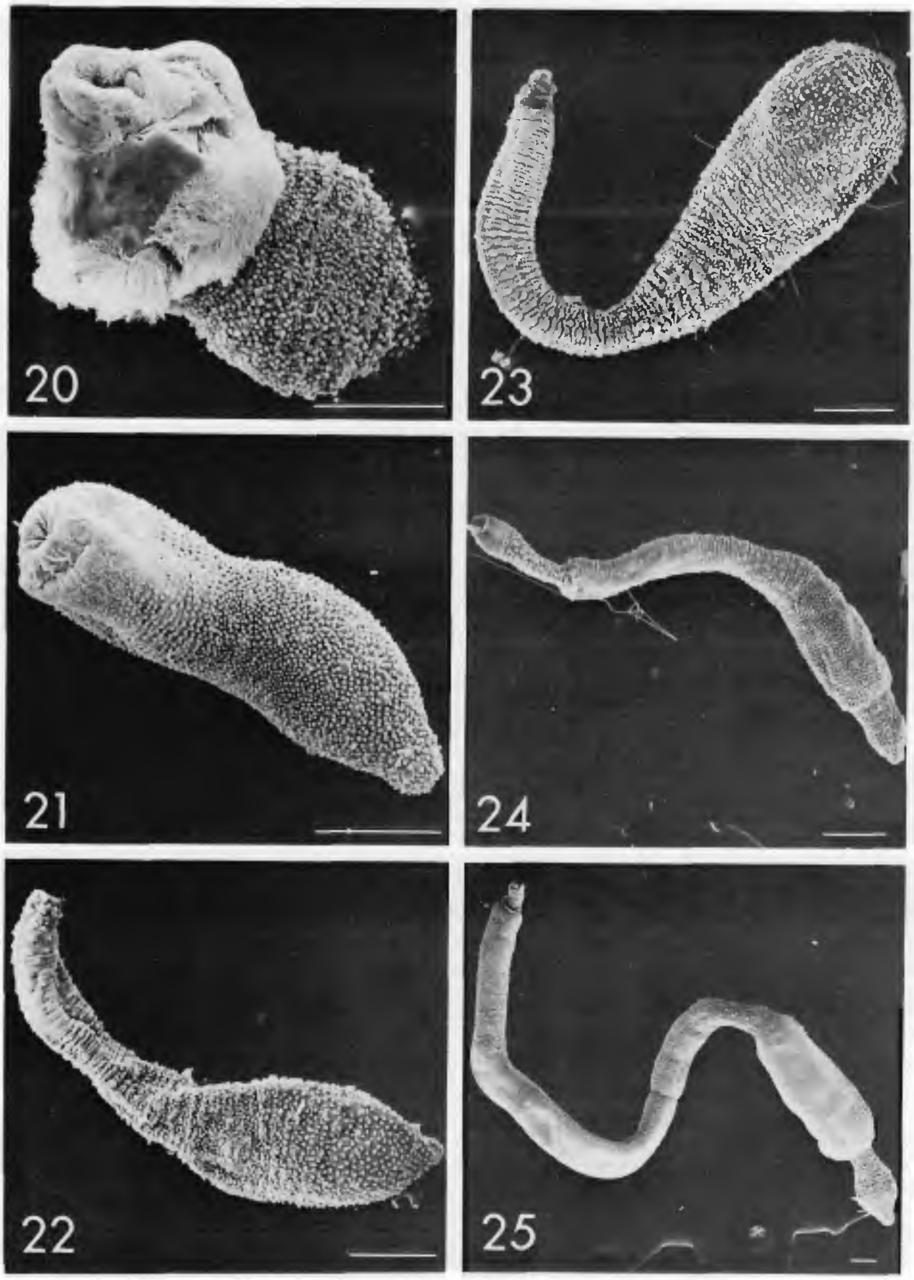
Changes in behavior precede the morphological changes of metamorphosis in the pelagosphera larva of *Golfingia misakiana*. When metamorphosis is about to begin, the larva elongates and contracts, stretching to its full length in a long cylindrical form. Usually the metatroch and frequently the head are retracted during elongation. Such elongation can be a normal behavioral




Figs. 10-13. Scanning electron micrographs showing changes in the head during metamorphosis of the pelagosphera of *Golfingia misakiana*. 10. Head of larva. Ventral view. 11. Head of metamorphosing larva after two days in substratum. Ventral view. Note beginning regressions of lip. 12. Head of metamorphosing larva after two days in substratum. Ventral view. Metamorphosis has progressed beyond that specimen in 11; head is narrower and pointed, ventral lobes are reduced, lip has completely regressed. 13. Head of juvenile, 8 weeks. Apical view showing four tentacles. Scale, 50 $\mu$m.




Figs. 14-19. Metamorphosis of the oceanic pelagosphera of *Golfingia misakiana.* Photographs of live specimens. Scale, 200 μm. 14. Larva swimming with metatroch extended. 15. Beginning metamorphosis after two days in substratum. Head is narrower, metatroch retracted. 16. "Bulbous" stage after three days in substratum. Postmetatrochal sphincter is constricted and head permanently retracted. 17. Five days in substratum. Note eversion of newly formed introvert beyond constricted sphincter. 18. Seven days in substratum. Shape resembles juvenile. Head is retracted. 19. Scanning electron micrograph of cuticular papillae of the oceanic pelagosphera larva of *Golfingia misakiana.* Scale, 10 μm.




Figs. 20-25. Scanning electron micrographs showing metamorphosis of the pelagosphera of *Golfingia misakiana* and juvenile development to the adult. Scale, 200 μm. 20. Larva. 21. Five days in substratum. 22. Ten days in substratum. 23. Sixteen-day juveniles. Head extended. 24. Three-week juvenile. 25. Adult, 9 months after metamorphosis.

pattern of all larvae; however, it occurs with greater frequency when metamorphosis is imminent. Once this behavior has begun, the larva can still revert to the more typical larval activities of extending the head and metatroch and of swimming. The actual onset of metamorphosis is marked by constriction of the postmetatrochal sphincter which prevents extrusion of the retracted head. For convenience this can be termed bulbous behavior because of the anterior swelling which results as the head is pressed forward against the closed sphincter. Once this behavioral change has been established, the process of metamorphosis is initiated and cannot be reversed. A series of morphological changes over a period of two to three weeks, described in the previous section, then give rise to the definitive juvenile form.

When placed on a substratum of fine sand and silt (see **Materials and Methods**) larvae begin what appear to be exploratory movements over the surface. They elongate and stretch with the head extended and the metatroch usually but not always retracted. They may also progress over the substratum in the manner of an inchworm, or with the ventral head applied to the substratum and the posterior end pointed upward. When the ventral surface of the head is on the substratum, sand grains may be passed by a ciliary current through the ventral groove to the lower lip. During this passage they are adhered together by secretions, presumably from the ventral head and lip glands. At the region of the lip pore the agglutinated sand grains are directed away from the larva by ciliary action and the occasional protrusion of the buccal organ. Tracks of curled strands of adhering sand grains are left wherever the larva has moved over the surface. There is no evidence that the larva ingests this sand; thus the behavior could be interpreted as a testing of the substratum. Another characteristic behavioral pattern is the approach of anterior and posterior ends, the larva assuming the shape of a C or doughnut, usually with the head retracted. The body then rotates around a central axis, remaining in place on the substratum. This movement allows the many sensory-secretory organs scattered over the surface of the trunk to contact the substratum, and could serve as another means for testing. If conditions are favorable, the larvae will burrow, either soon after contact or after a period of several hours of activity on the surface. If conditions are not appropriate, larvae may continue exploratory activities or, more commonly, remain quiescent on the substratum, periodically leaving the bottom to swim through the water. Larvae may show similar exploratory movements on the bottom of a glass dish, except that the metatroch is more frequently extended and rotating behavior is not usually observed.

Metamorphosis of a small percentage of larvae will occur in glass dishes over a one- to two-month period after collection, with no treatment other than frequent changes of sea water and the addition of phytoplankton for food. In an attempt to increase the number of metamorphosed individuals available for study, larvae were provided with substratum collected from the same station as adults of this species. When the substratum was meticulously selected from that immediately surrounding burrows of adults of *Golfingia misakiana,* larvae responded to it by burrowing and subsequent metamorphosis. This observation suggested that adults might produce a metamorphosis-inducing factor and led to the series of 6 tests described below and summarized in Table 2. The tests were designed to establish a procedure for inducing metamorphosis of large numbers of larvae for rearing and studies of metamorphosis.

The first 4 tests were intended to ascertain the effect of the presence of adults in the substratum on larval metamorphosis (Table 2). The substratum used in these and other tests was obtained as described in **Materials and Methods**, sieved through a 100 $\mu$m screen, and added to plastic dishes of 1000 ml capacity to a depth of 1 cm. Fifty larvae were introduced to each dish and at the end of 3 or 4 days they were removed by sieving and the number of metamorphosed specimens counted. The number of dishes in any one test was limited by the availability of larvae. The relatively low percentage recovered in some preliminary tests indicated the desirability of using no less than 50 larvae for each dish. The results of the first 4 tests showed that when

TABLE 2

Influence of Adults on Metamorphosis of Pelagosphera Larva of *Golfingia misakiana*

| With Substratum | | Percent Metamorphosis (3-4 days)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment of Substratum | Treatment of Sea Water | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 | Test 6 | $\bar{X}$ | $\sigma$ |
| Without adults | None | 24.0 | 46.8 | 69.7 | 30.4 | 17.8 | 25.0 | 35.6 | ±19.4 |
| Adults present (added with larvae) | None | 94.0 | 93.8 | 87.2 | 54.8 | | | 82.5 | ±18.7 |
| Adults present (added 3 days prior to larvae) | None | 91.8 | 87.0 | 88.9 | 57.9 | | | 81.4 | ±15.8 |
| Adults removed (added 3 days prior to larvae, then removed) | None | | | 84.4 | 78.3 | | | 81.4 | ±4.3 |
| Without Adults | Exposed to Adults[b] | | | 93.5 | 86.6 | 63.6 | 93.3 | 84.3 | ±14.1 |
| Without Substratum | | | | | | | | | |
| – | None | | | | | | 3.2 | | |
| – | Exposed to Adults[b] | | | | | | 0.0 | | |

[a]Figures listed for the 6 separate tests represent the percent of metamorphosed larvae of the total live specimens recovered 3 to 4 days after placement in test conditions.

[b]Adults were placed in sea water without substratum for a minimum of 3 days and removed prior to use of the water in the experiments.

adults were present in the substratum, whether introduced simultaneously with the larvae or 3 days prior, the percentage of metamorphosed larvae was considerably increased over the controls.

The effect of the past presence of adults in the larval medium, either substratum or sea water, was investigated in tests 3 through 6. When adults were placed in substratum for 3 days, then removed before introduction of larvae, the percentage of metamorphosed larvae again increased (Tests 3, 4, Table 2). Similarly, when sea water, previously inhabited by adults, was added to untreated substratum, the number of metamorphosed larvae far exceeded that in the controls (Tests 3-6, Table 2). The water was prepared by placing adults (20 animals per 500 ml sea water) in a dish without substratum at least 3 days prior to the test. The high percentage of metamorphosed larvae in media previously inhabited by adults suggests that adults may release some factor into the sea water which is effective in inducing larval metamorphosis. That substratum makes some significant contribution to metamorphosis is indicated by the absence or low rate of metamorphosis in the absence of substratum.

## DISCUSSION

### Review of Metamorphosis in the Phylum

*Metamorphosis of the Trochophore.* Features common to metamorphosis of the trochophore, regardless of the form that results, are formation or expansion of the coelom, elongation of the body, shedding or transformation of the egg envelope, and regression of the prototroch. When, as in the majority of species, the resultant form is a pelagosphera, metamorphic changes also include elaboration of the metatroch and usually the formation of a terminal attachment organ. The retractor muscles begin to function either just before or soon after metamorphosis. Metamorphosis of *Golfingia misakiana* differs from other known species in that the prototroch



Fig. 26. Head of *Golfingia misakiana* 7 months after metamorphosis. Apical view showing tentacles. Scanning electron micrograph. Scale, 20 μm.

Fig. 27. Introvert hooks of juvenile of *Golfingia misakiana.* Unknown age. Scanning electron micrograph. Scale, 5 μm.

is retained at metamorphosis and continues to function in locomotion along with the newly formed metatroch.

The cells of the prototroch generally undergo a marked regression at trochophoral metamorphosis. In *Golfingia vulgaris* and *Phascolopsis gouldi* the entire cytoplasm and nuclei of the prototroch cells are passed into the coelom.[4] In *Golfingia pugettensis* the cells release lipid and yolk granules into the coelom, then degenerate.[1] The prototroch thus serves an apparent nutritive function, although its significance for the total nutrition of the developing larva may vary in different species. In species with planktotrophic larvae the prototroch cells contain relatively less yolk and release it prior to trochophoral metamorphosis. In *Phascolosoma agassizii* it is released into a prototrochal cavity beneath the prototroch, and by the time the trochophore metamorphoses into a planktotrophic larva the yolk has been entirely utilized.[1] The trochophore of *Sipunculus nudus* lacks an equatorial band of prototrochal cilia, but a ciliated "serosa" surrounds the entire embryo and is considered homologous to the prototroch of other sipunculans.[3] Granules are discharged from the cells of the serosa into an inner "amniotic cavity."[14]

In all species with planktotrophic pelagosphera larvae, with the exception of *Sipunculus nudus,* the egg envelope of the posttrochal trochophore is retained as the cuticle of the pelagosphera. At metamorphosis of the trochophore the egg envelope is stretched as the larva elongates, losing its porosity and lamellation. The pretrochal egg envelope is sloughed off and replaced by a thin underlying layer which forms the cuticle of the head. The ventral, pretrochal egg envelope ruptures and the ciliated stomodaeal area opens outward to form the ventral ciliated surface of the head. In *Sipunculus nudus* the egg envelope is cast off at metamorphosis along with the underlying layer of ciliated cells.[14] The egg envelope of *Golfingia vulgaris* and *Phascolopsis gouldi* is also shed at metamorphosis.[4] In all other species studied having both lecithotrophic larvae and direct development, the posttrochal egg envelope is transformed into the cuticle of the larva or vermiform stage.

At metamorphosis of the trochophore into the pelagosphera larva, the metatroch generally becomes the functional locomotory organ. Although present prior to metamorphosis in *Golfingia elongata,*[6] *G. pugettensis,*[1] and *G. vulgaris,*[4] it becomes more fully developed as the prototroch cells regress, and assumes a primary role as the locomotory apparatus. The metatroch of *Sipunculus nudus* is present shortly before metamorphosis, but not functional until the egg envelope is shed.[14] In other species with planktotrophic development, it is usual for the metatroch to become ciliated at metamorphosis.

*Metamorphosis of the Pelagosphera.* The end of the pelagosphera stage in both lecithotrophic and planktotrophic larvae is marked by the loss of metatrochal cilia. The lecithotrophic pelagosphera then passes through what has been termed a "vermiform stage,"[1] a transition period during which the yolk is absorbed, the gut completed, the body and introvert elongated to assume the adult habitus, and tentacular lobes formed.[1,8,9] The duration of the stage varies among species, lasting only 10 to 11 days in *Themiste lageniformis,*[16] and as long as 5 to 7 weeks in *Golfingia pugettensis.*[1] Initially the shape of the small worms is generally plastic; they crawl in the manner of an inchworm, but later become less mobile, their major activity being extension and retraction of the anterior end.

In contrast to the lecithotrophic pelagosphera which is pelagic for only a few days, the planktotrophic pelagosphera may remain in the plankton from one month, in the case of *Sipunculus nudus,*[14] up to as long as 6 to 7 months in other species.[1,2] In studies of *Sipunculus nudus,* Hatschek[14] reported that larvae at the age of one month sank to the bottom and, over a period of one to two days lost the metatroch, buccal organ and lip glands. At the same time the head became proportionately smaller, the mouth moved anteriorly and, on either side of the mouth, a tentacular lobe was formed. In an unidentified pelagosphera, observed in the laboratory, Jagersten[10] reported a similar metamorphosis, noting the regression of the lip and the formation of three pairs of tentacles from the rim of the mouth. Hall and Scheltema[15] reared 4 pelagosphera larvae from open-ocean plankton samples through metamorphosis to juveniles, assigning one to the genus *Aspidosiphon,* but were unable to identify the others. They described the larvae and juveniles, but gave no details of metamorphic events.

The most detailed observations of metamorphosis of pelagosphera larvae are reported in a recent study by Williams[16] comparing the lecithotrophic pelagosphera of *Themiste lageniformis* with three oceanic planktotrophic larvae of unknown species. In all 4 larvae it was noted that the head is permanently retracted during metamorphosis and head epidermis fused to the wall of the introvert. Initial retraction in *Themiste lageniformis,* the species described in greatest detail, is attributed to a constriction of the postmetatrochal sphincter, which prevents extension of the head. Permanent retraction is then assured by dorsal and lateral fusion of the epidermis of the head and introvert with the dissolution of intervening cuticular layers. Lack of ventral fusion results in a tube which becomes the anterior portion of the esophagus. Four lobes grow out around the anterior opening of the tube, or the mouth, and later give rise to the tentacles. The process of head fusion was found to be similar in oceanic pelagosphera larvae except that in two of the three larval types the anterior esophagus and anteriorly directed mouth are formed by a fusion of the lobes of the ventral head. Dorsally a slit resulting from incomplete fusion was proposed to be an ocular tube. Less information was available on the third planktotrophic pelagosphera, labeled Type P, which, in contrast with the other two larvae with smooth cuticles, had a rough or papillated cuticle, similar to that of *Golfingia misakiana.* Fusion of the ventral lobes of the head of the Type P larva was not observed.

*Metamorphosis of Golfingia misakiana.* With the information reported here on *Golfingia misakiana,* it has been possible for the first time to compare the morphology and behavior of early planktotrophic larvae with that of older oceanic larvae of a single species. Previously, young planktotrophic larvae of various species reared in the laboratory have been described as benthi-pelagic or demersal, usually attached to the substratum by a prominent terminal organ.[1,7,11] However, such larvae had never been reared to the fully grown larval form and metamorphosis had not been observed. For *Golfingia misakiana* we now know that the young pelagosphera has a relatively prominent terminal organ and behaves as other young pelagosphera larvae by remaining close and usually attached to the bottom of dishes in the laboratory. In the later pelagic larva occurring in the surface plankton of the open ocean, benthic features such as the terminal organ have been reduced, and the metatroch, a highly specialized swimming structure, has been greatly elaborated. Thus the older pelagosphera is well adapted for a long planktonic existence in the ocean. Scheltema[2,19] has referred to such larvae as teleplanic and has hypothesized trans-Atlantic transport of pelagosphera species. He emphasized their importance in the widespread dispersal of species and their significance as genetic carriers between widely separated populations. Because young demersal pelagosphera larvae of *Golfingia misakiana* have not been reared in the laboratory to the older form, it is not known whether the young larvae, if exposed to an appropriate substratum, would metamorphose without undergoing the more prolonged planktonic phase.

Metamorphosis of the trochophore of *Golfingia misakiana* is similar to that of other species with planktotrophic larvae except that the prototroch is not reduced. However, in older pelagospheras it is diminished and must therefore regress at some point during the growth of the larva.

Morphological changes during metamoprhosis of the pelagosphera larva of *Golfingia misakiana* are similar in many respects to those described by Williams[16] for *Themiste lageniformis* and three unidentified planktotrophic pelagospheras, in that the postmetatrochal sphincter contracts, preventing extension of the head. However, not reported by Williams are the reduced size of the head, obliteration of the coelomic cavity of the head, and regression of the lobes of the ventral head. Also, unlike the other species, no fusion of head epidermis with introvert, or of the ventral lobes of the head with one another has occurred at the end of three days, the oldest stage critically examined in sectioned material of *Golfingia misakiana.* Both the pre- and postmetatrochal epidermis of the thorax thickens, and the thorax grows rapidly to form the long introvert characteristic of this species. Observations of living specimens show that at 5 days the newly forming introvert presses outward beyond the sphincter. It increases daily in its extensible length, as the constriction of the sphincter becomes less obvious. The retention of the head and the most anterior introvert within the body for a period of at least two weeks may also be related to a delayed development in length and extensibility of the retractor muscles. Finally, when the head is fully extended the brain is attached to the wall of the esophagus and maintains contact with the head epidermis within the circle of tentacles, presumably the location of the cerebral pit of the adult. The various processes by which these changes are achieved have not been defined at this time. More definitive explanations will be forthcoming with ultrastructural studies. Differences in metamorphic changes in larvae studied by Williams and those of *Golfingia misakiana* might be attributed to differing morphologies of the adults; such correlations must await the determination of adult affinities for all of the larvae under consideration.

Tests designed to induce metamorphosis of the pelagosphera larva of *Golfingia misakiana* for purposes of rearing revealed that metamorphosis was increased by the presence of adults, or by media previously occupied by adults. While successful in establishing a procedure for rearing larvae through metamorphosis, these tests at the same time raised a number of questions regarding a possible metamorphosis-inducing factor. Is a chemical factor released by the adults to which the larvae respond, or does the response depend on some associated microfloral assemblage, as has been demonstrated for certain other infaunal organisms?[20] Is there a speciesspecific inducing factor, or will the larvae respond equally well to other species, particularly those occupying the same habitat? What role does the substratum play in enhancing metamorphosis? Does it provide only an inert medium which facilitates burrowing, or are organic components and the presence of microorganisms important? What is the ecological significance, if any, of these laboratory responses?

Considerable information has been accumulated on factors influencing larval settlement and metamorphosis for several groups of marine invertebrates. This literature has been the subject of recent, intensive reviews by Crisp[21,22] and Scheltema.[20] No previous studies, however, have been made on sipunculans. Whether the response of larvae of *Golfingia misakiana* to the presence of adults, as suggested in the present study, can be considered a true gregarious response, as defined by Crisp[22] and Scheltema[20] for other invertebrates, must await definitive proof of species specificity. Invertebrates for which gregarious responses have been demonstrated in the past are sessile organisms, such as oysters,[23,24] barnacles[25,26] and serpulid worms,[27] whose larvae settle on solid substrata, or tube-dwelling sabellariid polychates whose larvae settle on previously constructed tubes.[28,29] Studies of settlement-inducing factors of infaunal organisms, particularly polychaetes, have focused on physical attributes of the substratum and on bacterial-algal films on substratum particles.[20] In burrowing organisms the effect of adult factors on the substratum or on the surrounding water column generally has not been considered. The nature of the adult-related factor demonstrated in this study to promote metamorphosis of *Golfingia misakiana*, and other interacting factors influencing settlement and metamorphosis, will be pursued in future experimental investigations.

## ABBREVIATONS

a, anus; at, apical tuft; bo, buccal organ; br, brain; cu, cuticle; drm, dorsal retractor muscle; e, eye; en, egg envelope; es, esophagus; in, intestine; l, lip; lg, lip gland; lp, lip pore; m, metatroch; mo, mouth; n, nephridium; p, prototroch; pr, posterior retractor muscle; psg, posterior sacciform gland; sph, postmetatrochal sphincter; s, stomodaeum; st, stomach; t, tentacle; to, terminal organ; vnc, ventral nerve cord; vrm, ventral retractor muscle.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## REFERENCES


1. Rice, M. E. (1967) Ophelia, 4, 143-171.
2. Scheltema, R. S. and Hall, J. R. (1975) in Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura, Vol. I, Rice, M. E. and Todorovic, M. eds., Naučno Delo Press, Belgrade, pp. 103-115.
3. Gerould, J. H. (1903) Mark Anniversary Volume, 439-452.
4. Gerould, J. H. (1907) Zool. Jahrb., Anat., 23, 77-162.
5. Åkesson, B. (1958) C.W.K. Gleerup, Lund, Unders. över Öresund 38, 1-249.
6. Åkesson, B. (1961) Ark. Zool., 13, 511-531.
7. Rice, M. E. (1973) Smithson. Contrib. Zool., No. 132, 1-51.
8. Rice, M. E. (1975) in Reproduction of Marine Invertebrates, Vol. II, Giese, A. and Pearse, J. eds., Academic Press, New York, pp. 67-127.
9. Rice, M. E. (1976) Amer. Zool., 16, 563-571.
10. Jägersten, G. (1963) Zool. Bidr. Uppsala, 36, 27-35.

11. Rice, M. E. (1975) in Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura, Rice, M. E. and Todorovic, M. eds., Naučno Delo Press, Belgrade, pp. 141-160.
12. Williams, J. (1972) Amer. Zool., 12, 723.
13. Amor, A. (1975) Physis, Sec. A, 34, 359-370.
14. Hatschek, B. (1883) Arb. Zool. Inst. Univ. Wien Zool. Sta. Triest, 5, 61-140.
15. Hall, J. R. and Scheltema, R. S. (1975) in Proceedings of the International Symposium on the Biology of the Sipuncula and Echiura, Vol. I, Rice, M. E. and Todorovic, M. eds., Naučno Delo Press, Belgrade, pp. 183-197.
16. Williams, J. A. (1977) Functional Development in Four Species of the Sipuncula. Doctoral Dissertation, Univ. of Hawaii, pp. 1-218.
17. Häcker, V. (1898) Ergeb. Plankton-Exped. Humboldt-Stiftung, 2, 1-50.
18. Richardson, K. C., Jarett, L., and Finke, E. H. (1960) Stain. Technol. 35, 313-323.
19. Scheltema, R. S. (1975) in Proceedings of the International Symposium on the Biology or the Sipuncula and Echiura, Vol. I, Rice, M. E. and Todorovic, M. eds., Naučno Delo Press, Belgrade, pp. 199-210.
20. Scheltema, R. S. (1974) Thalassia Jugoslavica, 10, 263-296.
21. Crisp, D. J. (1974) in Chemoreception in Marine Organisms, Grant, P. T. and Mackie, A. M. eds., Academic Press, New York, pp. 177-265.
22. Crisp, D. J. (1976) in Adaptation to Environment: essays on the physiology of marine animals, Newell, R. C. ed., Butterworths, London, pp. 83-124.
23. Cole, H. A. and Knight-Jones, E. W. (1949) Minis. Agric. and Fish., Fish. Invest., Ser. 2, 17, 1-39.
24. Bayne, B. L. (1969) J. mar. biol. Assoc. U.K., 49, 327-356.
25. Daniel, A. (1955) J. Madras Univ., Sect. B, 25, 97-107.
26. Knight-Jones, E. W. (1955) Nature, 175, 266.
27. Knight-Jones, E. W. (1951) J. mar. biol. Assoc. U.K. 30, 201-222.
28. Wilson, D. P. (1968) J. mar. biol. Assoc. U.K., 48, 387-435.
29. Wilson, D. P. (1970) J. mar. biol. Assoc. U.K., 50, 33-52.