INVERTEBRATE
ZOOLOGY

Embryonic Development of the Phoronid Phoronis ijimai

V. V. Malakhov and E. N. Temereva
Biology Department, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia
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Abstract—We studied the embryonic development of the phoronid Phoronis ijimai Oka, 1897. The egg cleavage is radial. The fourth and fifth cleavage furrows extend along the meridian of the egg. The blastula is flattened. Gastrulation occurs by a combination of epiboly, bending, and invagination. The mesoderm originates from two sources. The anterior mesoderm arises through immigration and gives rise to the first and second coeloms. The third coelomic mesoderm originates enterocoelically from the hindgut. The newly hatched larva has preoral and postoral ciliary bands, which can be compared with the corresponding ciliary bands of diploula and with the prototroch and metatroch of trochophore larvae.

Keywords: phoronids, embryonic development.

The phoronids are a poorly studied group of marine invertebrates comprising no more than 10–11 species. Peter the Great Bay of the Sea of Japan is inhabited by 5 species of phoronids [5, 6]; i.e., it hosts about 50% of the world fauna. Some species of phoronids here have a high biomass, reaching 100 g/m² [4]. Therefore, Peter the Great Bay is an ideal area in which to study the embryology of phoronids. The development of this group of animals is also of particular interest in comparative anatomy.

In phoronids, two major types of egg cleavage known for Bilateria have been described: spiral cleavage [17, 25] and radial cleavage [7, 13, 15, 20, 42]. Both the enteroconic development of the coelomic mesoderm [8, 17, 20] and the immigration of mesoderm cells from the archenteron and schizocoelic development of the coelom are characteristic of this group [10, 15, 30, 31, 34, 35, 42, 43]. The combination of these traits allows the phoronids to occupy an intermediate position between the protostomes and the deuterostomes. Studies on this group of animals allowed Masterman to develop the archicoelomat concept [18–20], which became one of the major generalizations in comparative anatomy of the 20th century [25–27, 32, 33, 37–39]. Proceeding from this concept, the phoronids form the central group of archicoelic animals and are similar in their organization to the ancestors of Bilateria [9, 11, 12, 14, 34, 36]. Therefore, studies of the embryonic development of the phoronids are of particular importance.

MATERIALS AND METHODS

As the object of this study, we used the phoronid Phoronis ijimai Oka, 1897. Adult specimens of this species burrow into the shells of the mollusk Acmea pallida. The mollusks were collected in June–July 1997 in Peter the Great Bay, Sea of Japan, around the Vostok Biological Station of the Institute of Marine Biology (Far East Branch, Russian Academy of Sciences), from stones at a depth of 1.5–10 m. The head portions of the phoronid bodies containing developing embryos were cut off. Early stages of cleavage and external morphology were studied in live embryos using reflected light, in a hanging drop preparation. Early larvae were reared in the laboratory from embryos.

To study the microscopic anatomy of the animals using light microscopy, we fixed the larvae of Ph. ijimai in a 4% solution of formaldehyde or paraform in seawater and, after rinsing them in water, we stored them in 70% alcohol. Later, these specimens were dehydrated in ascending alcohol series and embedded in paraffin. The 5- to 7-μm-thick sections were stained with Carazzi hematoxylin.

The following designations are used in Figs. 1–6: a, anus; ap, apical plate; B, blastopore; bl, blastocele; c1, first coelom (proctocoel); c3, third coelom (metacoel); c3p, primordium of the third coelom; ect, ectoderm; egg, egg; ent, entoderm; fp, female pronucleus; hg, hindgut; hgp, hindgut primordium; lph, lophophore; m, mouth; mc, muscle cells; mes, mesoderm; met, metatroch; mp, male pronucleus; n, nephridium; neg, nerve ganglion; ng, nidamental glands; np, nephridium primordium; eso, esophagus; pb, polar body; pb1, first polar body; pl, preoral lobe; plm, paired lophophoral masses; pm, proctophore; pro, prototroch; s, stomach; sh, shell; tr, tentacular ridge; v, vestibulum.

RESULTS

Phoronis ijimai is a hermaphroditic species. As in other phoronids, egg fertilization takes place after the transfer of spermatothores from one specimen to another and the penetration of spermatozoa (probably
from the tentacular wall into the coelom, see Zimmer [43]). Egg cells are fertilized in the coelom, then rise and are released through the nephridia. Chains of white fertilized eggs move upward, toward the funnels of the nephridia, and are usually visible through the semitransparent pink body wall of the phoronids (Fig. 1).

The released fertilized egg cells are bound together by a secretion of the nidamental glands so that two aggregations of embryos are formed, anchored to the bottom of the lophophoral concavity, and surrounded by lophophoral tentacles (Fig. 1). The number of embryos in an aggregation ranges from 7 to 30. The newly deposited eggs are fastened to the bottom of the lophophoral concavity, pushing the previously deposited eggs upward. Thus, in the course of development, the embryos gradually move upward, and the developed larvae finally appear at the top of the aggregations, where they later open up and swim away.

Fertilized egg cells are usually irregular in shape, due to the pressure of neighboring embryos (Fig. 2a). Yolk-rich egg cells are usually about 100 μm in diameter. In transmitted light, the eggs are almost opaque, while in reflected light, they look milk-white. In studying both live eggs and sections, we failed to find any gradient in yolk distribution.

Maturation Divisions

The earliest stage of development that we were able to find in sections through aggregations of embryos was the metaphase of the first maturation division (Fig. 2a). At this stage, the female pronucleus is located at the periphery of the egg cell, while the male one is in the yolk column. The division spindle in the female pronucleus is oriented perpendicularly to the egg-cell surface. The male pronucleus looks like a group of small chromosomes.
The sections through egg cells at the stage of the second maturation division show the female pronucleus surrounded by an area of cytoplasm lacking yolk granules (Fig. 2b). In the center of this area, in a light zone, there are the small chromosomes of the female pronucleus. The first polar body is attached to the border of this area from the outside. When the maturation divisions are complete, the first and second polar bodies are arranged close to each other, causing the thin envelope surrounding the egg to bulge slightly (Fig. 3a). As in all cases, we observed only two polar bodies, one larger than the other (see Fig. 2c). One may conclude that the first polar body undergoes no more divisions.

**Cleavage**

Considering that the polar bodies generally (although not always) mark the animal pole, the first cleavage division proceeds along the meridional plane (Fig. 2c; 3b, c), beginning at the animal pole (i.e., under the polar bodies) as a unipolar furrow. The first cleavage divides the egg into two approximately equal blastomeres.

The embryos of *Ph. iijimai* only develop normally within the aggregations located inside the lophophore of the maternal organism. Embryos isolated from the aggregations and placed into a suspended preparation underwent no more than a single cleavage and then died. This allowed us to trace the cleavage from stage to stage; however, we failed to determine the time intervals between successive cleavage divisions. Judging from the fact that 1 to 3 hours passed from the moment the embryo was placed in the hanging drop preparation to the next division, the interval between successive divisions is several hours.

The furrows of the second cleavage division are also located meridionally (Fig. 3d). Sometimes, the beginning of the second division does not coincide in the two blastomeres and there is even a brief stage of three blastomeres. The four blastomeres are approximately equal in size (Fig. 3e).

The furrows of the third cleavage division run equatorially; therefore, the embryo consists of eight blastomeres almost equal in size (Figs. 3f, g). The arrangement of the blastomeres corresponds to the rule of
radial cleavage: the animal blastomeres lie exactly above the vegetal ones. The nuclei are visible in interphase blastomeres at the eight-cell stage under reflected light.

The furrows of the fourth cleavage division are located meridionally, almost parallel to each other (Figs. 3h, i). As a result of this, the embryo is stretched perpendicularly to the animal–vegetal axis and to the plane of the fourth division and acquires biradial symmetry (Fig. 3i). At the 16-cell stage the blastomeres are almost equal in size.

The furrows of the fifth cleavage division are oriented meridionally. They are almost parallel to each other and perpendicular to the furrows of the fourth division (Fig. 3j). The 32-cell embryo looks like a two-layered plate consisting of two halves, one animal and one vegetal, each comprising 16 cells (Fig. 3j). The gross shape of the 32-cell embryo is similar to that of the 16-cell embryo; the biradial symmetry is retained.

The fates of certain cells in the course of the following cleavage have never been traced. We can only say that the compressed shape of the embryo in the animal–vegetal biradial direction is retained at the later stages of cleavage, at least up to the stages of 128–264 cells (Fig. 3k). Up to the late stages of cleavage, the blastomeres are almost equal in size. This allows the cleavage in Ph. iijmai to be characterized as equal.

**Blastula**

At no stage of cleavage are there noticeable gaps between the blastomeres. Even at the stages of several hundred blastomeres, the embryos mostly retain their compressed shape (Fig. 2e). The embryos lack blastocoel (or, in more exact terms, the latter is represented by a slitlike space between the animal and vegetal cell plates); therefore, this stage has to be characterized as a sterroblastula.

**Gastrula**

At the initial stages of gastrulation, the rate of cell division at the vegetal pole is lower than at the animal pole. Therefore, the cells of the vegetal pole appear to be somewhat inserted into the embryo (Fig. 2f). Actively penetrating into the embryo, the larger cells of the vegetal pole acquire a flaslike shape. At later stages, an important role is played by the bending of the embryo, which consists of a convex ectoderm layer and a concave entoderm layer (Fig. 2g, h). At later stages of gastrulation, a deep entodermal invagination arises (Fig. 4a); the formation of the latter probably has to be considered a result of the invagination process. Thus, gastrulation involves several processes: the ingrowth (or, more precisely, the insertion) of entodermal cells, the bending of the flattened embryo, and invagination.

The blastopore is located in the center of the vegetal half of the embryo. First, it has a rounded shape.
Fig. 4. Origin of mesoderm and organogenesis. (a) Transverse section through an embryo at the slitlike blastopore stage; (b) immigration of cells of the anterior mesoderm (sagittal section); (c) formation of the anterior coelomic primordium (sagittal section); (d, e) frontal sections of the same embryo at the stage of formation of the anterior coelomic primordium (d) closer to the dorsal side and (e) closer to the ventral side; (f) sagittal section of a late embryo at the stage of the formation of the late primordium of the coelomic mesoderm; (g) parasagittal section of a late embryo. Scale bar: 20 μm.

The Origin of the Mesoderm

The mesoderm in a Ph. ijimai embryo is formed from two primordia: one anterior and one posterior. The anterior mesoderm develops through cell migration from the anterior portion of the archenteron. This process begins during the development of the slit-shaped blastopore and continues throughout the closing of the blastopore. Some cells of the anterior portion of the archenteron divide and migrate into the blastocoel of the anterior portion of the embryo (Fig. 4b). They settle on the inner surface of the basal membranes of the ectoderm and entoderm, and, as a result, a more-or-less complete coelomic lining appears in the developing head lobe of the larva (Fig. 4c). These cells seem to
migrate between the ectoderm and the entoderm backward from the anterior end of the embryo, because on transverse sections through the middle part of the embryo one can see isolated mesodermal cells in the narrow lumen between the ectoderm and entoderm (Fig. 4a).

The head portion of the embryo enlarges and a spacious coelomocavity appears inside (Fig. 4e, f). The posterior extremities of the coelomic primordium extend along the right and left sides of the body between the ectoderm and entoderm, approximately up to the midpoint of the embryo (Fig. 4d, e).

The primordium of the posterior mesoderm first appears in the almost fully developed larva. The posterior part of the intestine (where the latter fuses with the ectoderm of the posterior end of the body) forms an unpaired expansion (Fig. 4f), which retains its epithelial structure and is an outgrowth of the dorsal intestine wall. This is similar to the enterocoelic type of mesoderm development.

The Development of the Larva

The changes in the shape of the embryo during the development of the larva are accompanied by uneven growth in different parts of the embryo. The preoral portion expands and gives rise to the head lobe, which, in the course of development, bulges more and more above the mouth (Fig. 4f, g). In the center of the head lobe, an expansion of the mesoderm arises, the primordium of the apical plate, which also moves forward as the head lobe grows upwards. The coelom of the anterior part of the larva, which is lined with anterior mesoderm cells, is subdivided into a preoral portion located in front of the apical plate primordium and a postoral portion located behind the latter.

In late embryos, a primordium of the tentacular ridge appears as an ectoderm expansion. From the inside, the cavity of the tentacular ridge is lined with mesoderm cells that probably originate from the cells of the anterior mesoderm that migrated into this part of the embryo (Fig. 4g).

As mentioned above, the posterior part of the archenteron is fastened to the ectoderm of the posterior body end, where a primordium of the hindgut arises (Fig. 4g). Prior to the completion of embryonic development, the posterior part of the intestine has no connection with the outside. The anus appears only in the swimming larva.

The parasagittal sections show protonephridia primordia located in the posterior part of the embryo. The primordia arise as paired ectoderm invaginations located under the tentacular ridge (Fig. 4g).

Early Larvae

Larvae that have just detached from the aggregations of embryos are 180–220 μm long. The diameter of the episphere (up to 180 μm) is only slightly smaller than the whole length of the larva, so the latter acquires characteristic T-shaped outlines (Fig. 6). The entire surface of the larva is covered with cilia; in certain parts of the body, two ciliary bands arise consisting of especially long and densely arranged cilia: a preoral band running along the edge of the head lobe and a postoral band running along the ventral and lateral sides of the body behind the mouth. Both the bands run toward the same point, an area on the dorsal side, where the head lobe is connected with the body. In this area, both bands are open (Fig. 6).

The postoral ciliary band is located on the surface of the tentacular ridge, where expansions are noticeable that correspond to two pairs of larval tentacles. Among the latter, the ventral pair is more clearly pronounced than the lateral pair (Fig. 6).

The larva shows characteristic coloration due to black pigmentation on the ventral surface, from the mouth to the tentacular ridge (Fig. 6).

The larva has an apical tuft of cilia underlined on the dorsal side of the head lobe by an expanded ectoderm (Fig. 6). This expansion is a primordium of the aboral nerve ganglion.

The thin, transparent body wall allows the anatomical organization of the larva to be seen. Under the bulging head lobe, there is a spacious vestibulum narrowing toward the mouth opening. The mouth has no clearly visible borders and continues into the narrowing esophagus, separated from the stomach by a valve. The thickened walls of the stomach (the midgut) contain yolk inclusions. A short hindgut occupies a terminal position at the posterior end of the larva (Fig. 6). All parts of the intestinal tract are lined with cilia.
Fig. 6. A larva just detached from the aggregation of embryos (a drawing from a live specimen). Scale bar: 25 μm.

The inner surface of the head lobe both in front and behind the primordium of the aboral ganglion and the postoral portion of the larva up to the tentacular ridge are lined with coelomic epithelium. The cells of the latter (at least, some of them) have an epithelio-muscular nature and form a system of muscle fibers running along the body of the larva and allowing it to contract in response to outer stimuli (Fig. 6).

A primordial body coelom (the metacoel) is visible in the posterior part of the larva, under the tentacular ridge. It is a horseshoe-shaped vesicle enveloping the hindgut from the dorsal and lateral sides. The wall of the metacoel is made of branched cells, the pseudopodia of which run toward the body wall (Fig. 6).

The transparent body wall of the larva allows the protonephridia to be observed as paired organs located ventrolaterally on each side of the intestine (Fig. 6).

The apical portion of the protonephridium is formed of flask-shaped cells (solenocytes?) fused together to form an excretory duct that opens to the outside under the tentacular ridge.

DISCUSSION

The embryonic development of phoronids has been described in the literature in different ways; the most important features of development in this group of animals are the subject of controversy. One of the points of contention is the phoronid cleavage pattern. In the published papers, two major hypotheses have been presented. According to the first one, phoronid cleavage shows a spiral pattern [1, 2, 17, 25]. Other authors consider phoronid cleavage to be typically radial [7, 13, 15, 20, 42]. Some scientists describe phoronid cleavage as
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The initial stages of gastrulation take place in a different way in different phoronid species, but in all cases a deep archenteron develops, connected to the outside via the blastopore on the ventral side of the embryo. It is shown that, in species whose embryos develop in the water column, uneven growth takes place in different sectors of the gastrula in the course of gastrulation; therefore, the relative positions of the blastopore and animal pole are prone to change [11, 15, 25]. This process is difficult to record in Ph. jirimai, as the shape of the embryo is determined by the pressure of neighboring specimens while the later development of ciliary structures does not allow researchers to locate the apical ciliary tuft that indicates the position of the animal pole. By the way, in Ph. jirimai, the blastopore also corresponds to the ventral side of the larva. As in most phoronids, the blastopore in the studied species closes from the posterior to the anterior end, taking the shape of a slit or teardrop, and the mouth of the larva is the anterior portion of the blastopore [7, 11, 16, 20, 30, 31].

The most difficult problem in the development of the phoronids concerns the origin of the mesoderm. There are two hypotheses that address this question. The first describes the origin of the coelom as a result of the enterocoelic process [8, 17, 20]. The second, advanced by later scientists [11, 15, 30, 31, 34, 35, 42–44], interprets the origin of the mesoderm as the immigration of cells from the anterior and lateral walls of the archenteron.

The authors that describe the immigration of mesoderm cells from the wall of the archenteron consider these cells to provide the coelomic lining not only of the first and second coeloms, but also of the third coelom. The formation of the first and second coeloms has been traced many times, but the development of the third coelom (the most remote from the point at which the immigration of mesoderm cells begins) has only been speculated about.

Our observations show that, in Ph. jirimai, there are two sources of mesoderm. The first one is the anterior portion of the archenteron: the mesenchyme cells immigrate from the wall of the latter in the early stages of gastrulation (see Fig. 4b, c). These cells provide the lining of the protocoel and mesocoel. The other source is the posterior portion of the archenteron: from these, a mesoderm vesicle bulges out and gives rise to the metacoel of the larva (Fig. 6). Thus, in Ph. jirimai, mesoderm cells both immigrate and originate in the enterocoel. However, it is pertinent to note that the enterocoel revealed in Ph. jirimai has nothing to do with the enterocoelic origin of the coelom described earlier by Caldwell [8] and Masterman [20]. These authors observed the enterocoelic origin of protocoel, whereas the Ph. jirimai protocol develops from mesenchyme cells [11, 15, 30, 31, 34, 35, 42–44]. As for the enterocoelic form of development, it is characteristic of the metacoel, and this peculiarity has not been recorded in phoronids thus far.

As a feature of spiral cleavage in the development of phoronids, we may consider the displacement of one quarter of the blastomeres relative to another one by an angle of 0° to 45°, which, as shown in a special study by Herrmann [15], occurs in certain embryos within the framework of individual variability of cleavage. However, as particularly emphasized by the cited author (see Herrmann, 1986 [15], p. 447), no crosses, rosettes, four groups of blastomeres, or other blastomere figures characteristic of true spiral cleavage have ever been observed. On the other hand, no figures typical of spiral cleavage have been described, not even by Rattenbury [25], whose paper is usually cited as the best description of spiral cleavage in phoronids. As regards small deviations from the typical radial arrangement of blastomeres, these might also be observed in Ph. jirimai (see, for instance, Fig. 3j); at later stages of cleavage, the illusion of a spiral arrangement of the blastomeres might appear (Fig. 3k).

Both the published data and our observations allow us to conclude that the cleavage in phoronids is a kind of radial cleavage.
Let us mention here that both the enterocoeal origin of the mesoderm of the third coelom and the immigration of mesoderm cells providing the first and second coeloms are multicellular mesoderm origins. They are significantly different from the teloblastic origin of coelomic mesoderm in true spiralia, where the mesoderm arises from only two cells. Thus, the origin of the mesoderm clearly separates the phoronids from the typical spiralia and brings them closer to the deutero- somes.

The early larvae of Ph. iijimai differ from typical actinotroch larvae due to the absence of developed tentacles. The anlagen of the tentacles at this stage might only be imagined in the expansions of the tentacular ridge indicated by the postoral ciliary band in the larva. The edge of the head lobe bears the preoral ciliary band. According to Nielsen [21-23], the preoral ciliary band of the actinotroch larva might be homologous to the prototroch of the Spiralia larva, while the ciliary band on the tentacles corresponds to the metatroch. Even more probable is that these structures are homologous to the preoral and postoral portions of the ciliary band in the tornaria larvae of the pterobranchs and diploleuroids of echinoderms, as is cautiously proposed by Ivanova-Kazas [3]. The ciliary bands of an early larva of Ph. iijimai, which still does not have tentacles, correspond rather precisely to the preoral and postoral bands of the early tornaria and diplopelura and are somewhat similar to the prototroch and metatroch in the larvae of Spiralia.

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