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A brief examination of the development of *Phoronis* sp

Introduction

Phoronida is a small phylum associated with the lophophorates. The phylum consists of two genera (*Phoronis* and *Phoronopsis*) defined by the presence or absence of a fold of epidermis at the base of the lophophore (Emig 1974). There are at least ten species the number is likely slightly higher (Emig 1977). The adults are worm-like creatures that usually inhabit sand tubes in intertidal mudflats. All known species, except one, have a type of planktonic larvae called the actinotroch. Initially, the larvae and adults were not connected as the same group of organisms, but were assigned different names, so the systematics of phylum has long been a source of debate (Santagata and Zimmer 2002).

Phoronids have internal fertilization, but three different patterns for early development occur (Zimmer 1987). Firstly, there may be a large number of small, non-yolky eggs which are released into the water column. Second, there may be a smaller number of larger, yolkier eggs which are brooded until the actinotroch stage, then released via the nephridia (Emig 1977). Thirdly, the eggs may be larger still and possess greater quantities of yolk which feed the growing larvae. This third group is represented by only one species which has a slug-like juvenile which crawls away from the maternal tube before metamorphosis (Zimmer 1987).

In *Phoronis*, development proceeds via radial cleavage (Emig 1977, Malakhov and Temereva 2000). In some cases, the second cleavage is often non-synchronized, so there is a brief three-cell stage (Emig 1977). The blastula is ciliated and gastrulation is

initiated by a flattening of the vegetal pole into the gastral plate (Emig 1977). Gastrulation proceeds via a combination of invagination, bending of the gastrula and epiboly (Malakhov and Temereva 2000). The anterior portion of the gastrula becomes the oral hood of the actinotroch larva via growth of the ectoderm (Caldwell 1882, Freeman 1991). These phases of development occur on different timelines depending on the species.

The actinotroch larva is made of three body regions, the hood, the collar and the trunk. The hood is separated from the collar by the mouth and the collar is separated from the trunk by the ring of larval tentacles (Santagata 2004). The larva does not start to feed until it can swim, at which point, the gut is completed (Malakhov and Temereva 2000). Feeding is accomplished by a muscular contraction of the oral hood after food particles have come in contact with the tentacles (Strathmann and Bone 1997). It is likely that cilia also have a role in food capture. Different species of Phoronids have differing numbers of larval tentacles. *Phoronis pallida* has 10 to 12 larval tentacles when it is competent to metamorphose (Santagata 2004), and *Phoronis muelleri* as been found with 16 tentacles still living in the plankton (Bartolomeaus 2001). The tentacles of the adult do not necessarily come from the larval tentacles, though that is occasionally the source (Santagata 2002). Adult tentacles also arise from thickenings of the tissue at the base of the larval tentacles, or are formed as an entirely new set prior to metamorphosis (Santagata 2002). The actinotroch is competent to metamorphose when a particular number of larval tentacles have developed; the number varies between species (Santagata 2002). Additionally, enervation and development of the hood sense organ indicates

competence to settle and metamorphose (Santagata 2002, Santagata and Zimmer 2002). The time to settlement varies between species.

Methods

An unknown species of Phoronid from the genus *Phoronis* was collected in the mudflats near the Portside restaurant in Charleston, Oregon. Specimens were collected in their sand tubes and brought into the laboratory for dissection. The sand tubes of the adult organisms were broken in multiple places and the worms were gently pulled out of the broken tube. Once removed from the tube, specimens were placed under a dissecting scope in a dish of seawater and the lophophore was cut off using a razor blade. The cut tip of the body was gently squeezed to expel any eggs or sperm residing within that portion of the animal. Sperm and eggs were combined in a small custard bowl with approximately 75 ml of 0.45 micron filtered sea water. Cultures were kept in flowing sea water tables to keep the temperature in the range of 12 to 14 degrees Celsius.

Cultures were fed a combination of two types of algae, *Rhodomonas lens* and *Dunaliella tertiolecta*. Both species were raised in nutrient growth medium in one liter Erlenmeyer flasks and bubbled to encourage growth. Algae was prepared for the culture by centrifuging and removing the supernatant, then mixing with a small amount of filtered sea water.

The culture was changed every two to three days via reverse filtration to remove most of the water. The larvae were then transferred to a new bowl, new water was added and they were fed on a combination of *R. lens* and *D. tertiolecta*. Cultures were fed with

every water change. Development of the larvae was monitored through drawings and pictures over the course of three weeks.

Results

Eggs and sperm were combined in culture dishes. Initial cleavage was not observed due to time constraints. A blastula had developed by the end of the first day post fertilization (Figure 1). Cilia and flattening of the gastral plate were both evident at 36 hours post fertilization, indicating that gastrulation had begun (Figure 2). The characteristic bent shape of the early actinotroch larvae was observed at approximately one week (Figure 3). At this stage, the larva has a complete gut and the oral hood is beginning to become differentiated. There is no evidence of tentacle budding. The animal moves rapidly in spinning motions using cilia.

Just a few hours later, tentacle buds appear, but the animal is still mostly symmetrically bent (Figure 4, Figure 5). After eleven days, the buds of the first pair of tentacles are evident (Figure 6, Figure 7). At this stage, the hood is more differentiated and the body is beginning to elongate. The swimming now has orientation and direction as the animal swims with its hood first. After two weeks, there are three pairs of larval tentacles. The trunk of the larva has extended considerably and there is now a band of cilia around the base of the trunk called the telotroch (Figure 8, Figure 9, Figure 10). At the conclusion of the observation period (week three) the larvae were still actively swimming, but metamorphosis had not yet occurred. Contamination of the culture at this point made further observation impossible due to death of the culture.

Discussion

It is difficult to compare the developmental timeline of this organism with any known timelines for this group because the species studied was an unknown. It is, undoubtedly, one of the ten or so species already described for the phylum, but it is difficult to tell them apart. In some respects, the development of the study *Phoronis* sp. is similar to the development of *Phoronis pallida*. That species develops a bent gastrula at approximately 30 hours, and the telotroch develops cilia at the six-tentacle stage (Santagata 2004). Both of these are consistent with the studied species. Other species have different timelines. For instance, *Phoronis vancouverensis* does not initiate gastrulation for approximately 66 hours and nine days pass before the first tentacle buds appear (Freeman 1991). It is impossible to determine when the study species may have become competent to settle. *Phoronis pallida* is competent when it has ten or twelve larval tentacles (Santagata 2004), but *P. muelleri* persists in the plankton with 16 tentacles (Bartolomeaus 2001) and *P. ijimai* develops without any (Malakhov and Temereva 2000). It seems likely, therefore, that the study species would have developed at least one more set of tentacles prior to the initiation of settlement and metamorphosis.

This group of organisms has an intriguing larval form which deserves further examination. Though these animals are somewhat difficult to culture in the laboratory, it is well worth the effort to discover more about the early stages of their development which were not observed over the course of this study. It would also be prudent to examine the larvae in shorter time intervals so developmental steps could be placed into a more specific timeline.

Literature Cited

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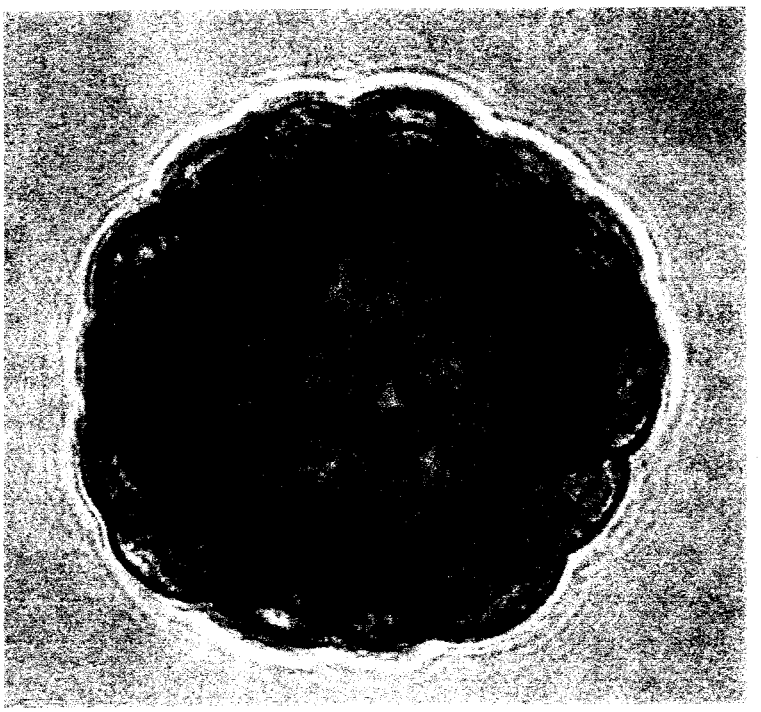

Figure 1. Blastula of *Phoronis* sp.

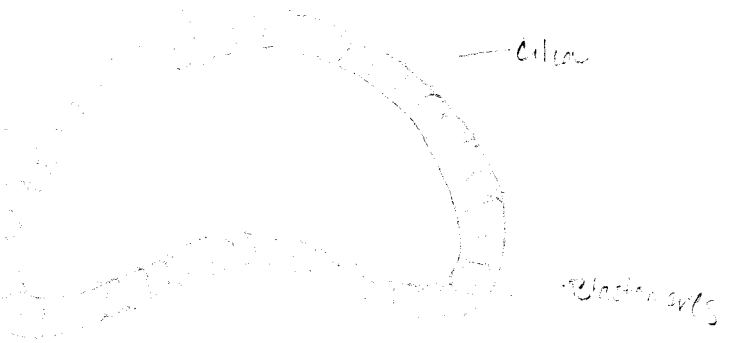

Figure 2. Ciliated blastula with flattened gastral plate.

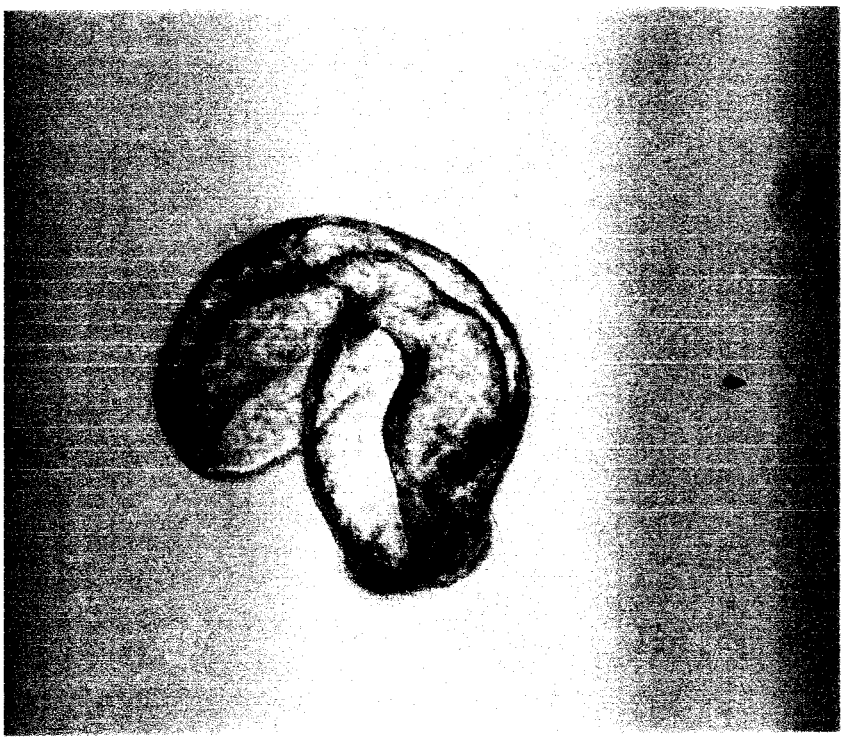

Figure 3. Side view, slightly dorsal (Anterior up, posterior down) of early actinotroch larva. The gut is complete, but the hood and trunk are still the same size.

Figure 4. Side view of early actinotroch larva (hood at top). Complete gut is evident, and tentacle buds are just beginning to be visible.

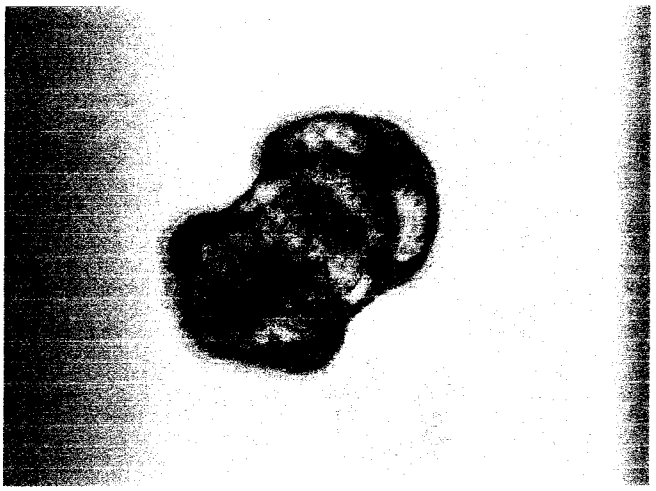

Figure 5. Ventral view of early actinotroch larva. Mouth is located toward the top and right of the larva. The cavity under the oral hood is evident.

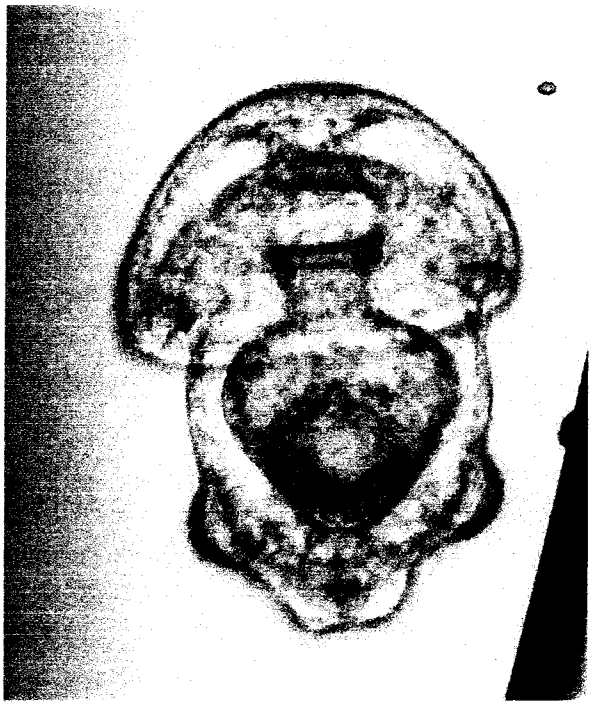

Figure 6. Front view of later actinotroch (11 days). First pair of tentacles is evident on either side of the lower portion of the body. Mouth and stomach can both clearly be seen.

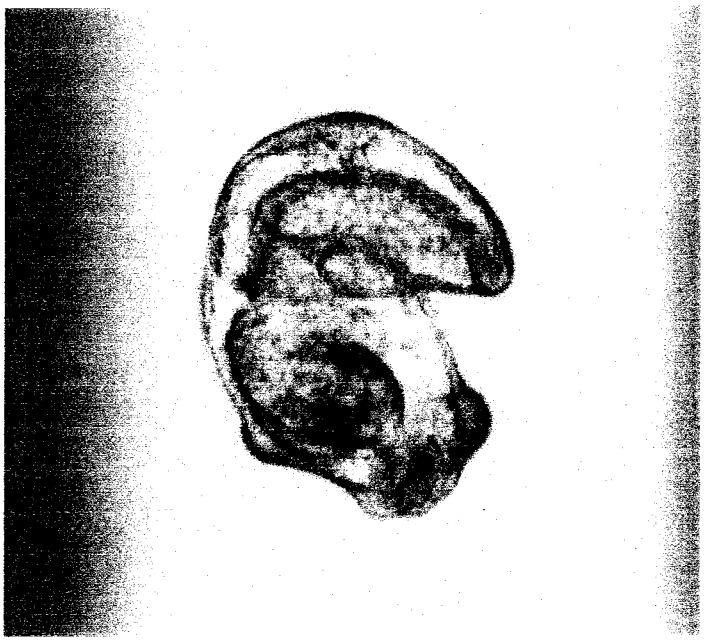

Figure 7. Side view of day 11 actinotroch. Cavity under oral hood is evident along with the first pair of larval tentacles.

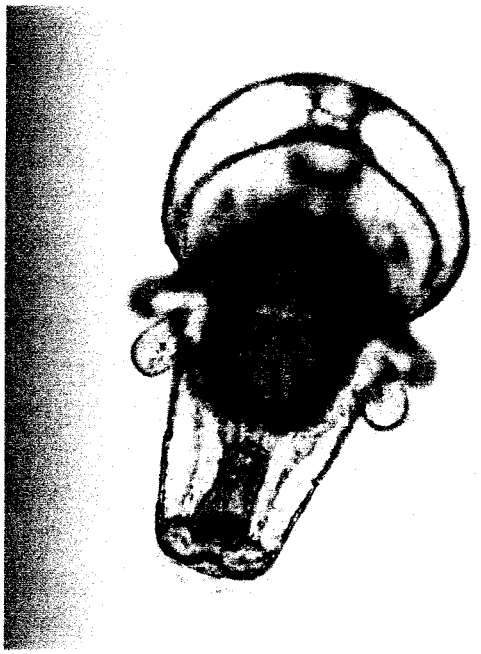

Figure 8. Two week actinotroch. Two sets of larval tentacles are clearly visible as is the telotroch.

Figure 9. Two week actinotroch with long larval tentacles evident.

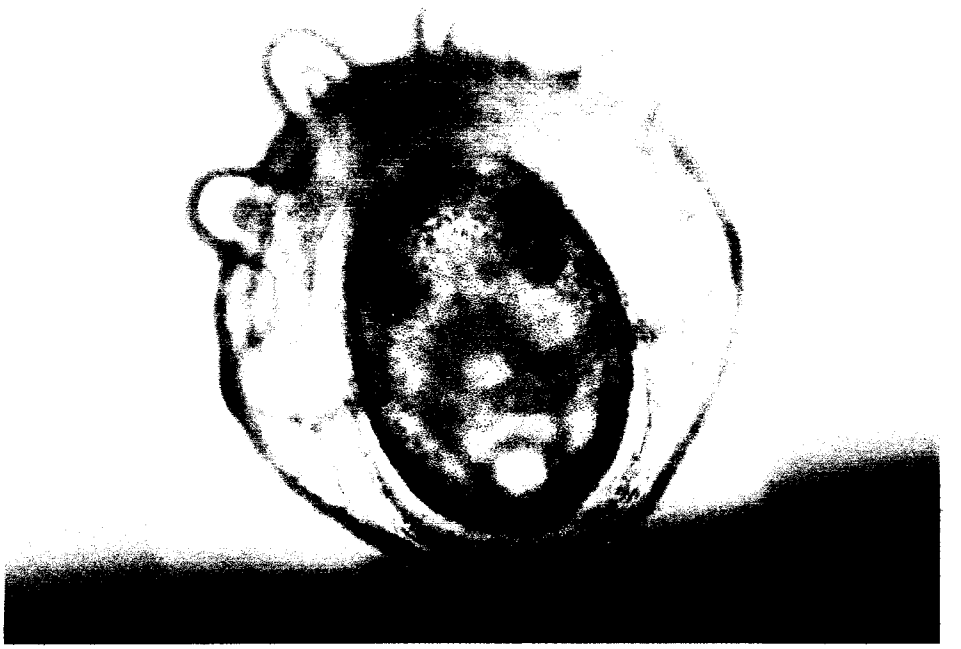

Figure 10. Two week actinotroch from posterior looking toward oral hood. Three pairs of larval tentacles are evident.