A Non-Feeding Pilidium With Apparent Prototroch and Telotroch

SVETLANA A. MASLAKOVA*
AND GEORGE VON DASSOW
Oregon Institute of Marine Biology, University of Oregon, Charleston, Oregon

The concept of the trochophore larva dominates comparative embryology of spiralian larvae. Some consider an elaborate trochophore, feeding with opposed prototroch and metatroch, as an archetype of spiralian larvae, which many groups have simplified, lost, or greatly elaborated (Nielsen, '87, 2001). However, the ancestral trochophore may have been no more than a swimming gastrula in which a prototroch, composed of cleavage-arrested multiciliated cells descended from specific blastomeres, separates episphere from hyposphere (Rouse, '99). Either hypothesis promotes speculation about how novel forms—for example, the mitraria larva of the polychaete Owenia, the bryozoan cyphonautes, or the nemertean pilidium—might really be transformations of the trochophore (Nielsen, '87, 2004, 2005), even while others argue that these forms converge upon common design traits (Salvini-Plawen, '80; Ivanova-Kazas, '87).

Here, we report sighting a novel type of non-feeding nemertean pilidium which superficially resembles a trochophore with two transverse ciliary bands—an equatorial prototroch and a posterior telotroch. Since the canonical pilidium larva is itself a derived form within a phylum whose basal members have been inferred to possess a modified trochophore (Maslakova, 2010a), this new larval form exemplifies the perennial mystery about trochophores in general: whether the arrangement of ciliated bands is a functional trait which arises convergently, or whether such an arrangement is evidence of a shared embryological history.

In a plankton sample taken from the Charleston marina in Coos Bay, Oregon during the first week of December 2011, we each collected an individual larva which seemed to be a trochophore of an unfamiliar type. These were opaque, pale brown, about 300 μm long and not quite so wide, possessed of a prominent blade-like apical tuft, and swim rapidly with the aid of two clearly visible ciliated bands, one at the equator and one at the posterior (Fig. 1A). The blade-like bearing of the apical tuft, and a certain rhythm to the swimming motion, suggested to each of us independently that this might be a nemertean.

Indeed, when compressed slightly under a coverslip, the larva appeared, despite its near-opacity, to contain a worm-like internal body (Fig. 1A). Both equatorial and posterior ciliated bands appeared to be circumferential. In addition to the ciliated bands and apical organ, the rest of the surface was also densely ciliated. Epidermal cells outside the ciliated bands contained evenly distributed refractile spheres 5–10 μm in diameter (Fig. 1B). A singular, distinctive ciliary cirrus projected from one side just behind the “prototroch” (Fig. 1A, arrowhead). Tellingly, these trochophores both exhibited several characteristic behaviors...
Figure 1. External appearance and metamorphosis of a novel type of nemertean larva. (A) This non-feeding trochophore-like pilidium is characterized by a blade-like apical tuft (ap), two circumferential ciliary bands—the equatorial "prototroch" (pt) and the posterior "telotroch" (tt)—and a ciliary larval cirrus (arrowhead) located between the two ciliary bands. Scale 100 µm. (B) A close-up focused near the surface of larval epidermis to show the characteristic refractile spheres. Scale 50 µm. (C) Newly metamorphosed juvenile with larval body in its gut (gt), juvenile caudal cirrus (jc), and a constriction (double arrowhead) separating head and trunk. Scale 100 µm. (D1–D4) Selected frames from Supplementary Movie 2 illustrating the sequence of events during metamorphosis. Scale 100 µm. Time-stamp (min:sec) at upper right. (D1) Intact, slightly contorted larva at the onset of metamorphosis. (D2) Juvenile posterior end just breaking through the thinned larval epidermis in the vicinity of the larval cirrus. (D3) Juvenile posterior end emerged from the larva. (D4) The larval body is disappearing into the juvenile mouth (asterisk) during the final stage of metamorphosis.
sequences from both loci matched almost exactly (>99% sequence similarity) to those we obtained from a nemertean worm we collected in North Cove of Cape Arago, Oregon in May 2009 (GenBank accession numbers JQ430741–JQ430746). This worm was a few centimeters long and 2–3 mm wide, uniformly reddish-brown, lacked ocelli, possessed longitudinal ciliated slits, and a prominent caudal cirrus. It does not match any description of the pilidiophoran species known to occur in the region (Roe et al., 2007) or elsewhere along the Pacific coast of North America, and likely belongs to an undescribed species possibly within the genus Micrura.

While the manuscript was in review we found several other larval individuals of this species in the plankton (as late as end of January 2012), at various stages of development (including a gastrula), which further reinforces our conviction that the adults must be nearby.

Several species of Micrura have been reported to possess reduced non-feeding pilidia—for example, the Iwata’s larva of M. akkeshiensis (Iwata, ’58), the larvae of M. verrilli (Schwartz, 2009) and M. rubramaculosa (Schwartz and Norenburg, 2005), and at least two other (undescribed) species (Schwartz, 2009; Schwartz and Norenburg, 2010). Most of these are oviparous and uniformly ciliated, except for the larva of M. rubramaculosa, which possesses a prototroch-like equatorial circumferential band of longer cilia (Schwartz and Norenburg, 2005). Lecithotrophic encapsulated forms, known as Schmidt’s and Desor’s larva are also described in the lineiform species Lineus ruber and L. viridis (Schmidt, ’64). None of these lecithotrophic forms are known to possess both a prototroch and telotroch, and metamorphosis of such a pilidium is documented here for the first time.

A larval telotroch has been reported in two unusual planktotrophic pilidial forms. Pilidium recurvaturn originally described by Fewkes (1883) from Newport, RI, and later reported by Cantell (’66b) from Gullmarfjord, Sweden possesses a prominent ciliary band encircling the posterior end. Likewise does a very similar larva Pilidium incurvaturn described by Dawydoß (’40) from Vietnam. It is not currently known which species (genus or family) these forms belong to, and where they might be placed on the phylogeny of the Pilidiophora. It appears likely, however, that the telotrochs of these forms are not homologous to that of the lecithotrophic pilidium described here.

We do not pretend to report more than the existence of this novel larval form, which we nickname Pilidium nieseni in appreciation of Claus Nielsen’s provocative speculations about the evolution of larval forms and the trochophore in particular. Thanks to DNA sequences we know that the adult lives in the intertidal nearby, and we eagerly anticipate the day when we find reproductive individuals of both sexes and can investigate the development of this non-feeding pilidium.

Even without knowing how it develops, however, the mere fact that such a larva exists is significant. Some basal nemerteans have a trochophore, albeit a cryptic one: fate mapping was required to
reveal the presence of a prototroch derived from the canonical trochoblast lineages in the palaeonemertean Carinoma tremaphoros (Maslakova et al., 2004a,b). Cryptic or not, this suggests that the trochophore sensu Rouse (‘99) was ancestral to nemerteans, and was either abandoned or highly modified in derived groups.

The pilidium larva characterizes a monophyletic clade among the nemerteans—the Pilidiophora (Thollesson and Norenburg, 2003)—and, although it retains certain characteristics of the spiralian fate map (Henry and Martindale, ’98), it is difficult to relate to the trochophore since the configuration of ciliated bands and the development is wholly different (Maslakova, 2010a,b). The conventional feeding pilidium is ancestral to the Pilidiophora, and lecithotrophic larval forms, such as those mentioned above for some species of Micrura and Lineus, evolved secondarily within this clade (Thollesson and Norenburg, 2003; Schwartz, 2009). Pilidium nielseni must likewise be derived from a conventional feeding pilidium.

If, despite the difficulties, one is inclined to see the conventional pilidium as a highly derived trochophore (Nielsen, 2005), then Pilidium nielseni might seem like a reversion to the archetype. If, on the other hand, one views the conventional pilidium as a completely novel larval form which has departed from the trochophore plan entirely (Maslakova, 2010a), then Pilidium nielseni represents an instance of convergence upon, rather than retention of, the ciliated band arrangement characteristic of the trochophore. The detailed cell lineage of the pilidial ciliated bands, in both conventional forms and P. nielseni especially, would help distinguish between these alternatives.

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LITERATURE CITED