RAPID COMMUNICATION

A Non-Feeding Pilidium With Apparent Prototroch and Telotroch

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ABSTRACT The nemertean pilidium larva is a long-lived planktotrophic form which is challenging to homologize to other invertebrate larval forms. Here we report a reduced, lecithotrophic pilidium which superficially resembles a trochophore. We document the pilidium-like catastrophic metamorphosis of this larva, including devouring of the larval body. Sequences of COI and 16S rRNA show that this larva belongs to an undescribed lineiform species. This novel larval form highlights the long-standing question, is the trochophore a conserved larval ground-plan or a functional design arrived at by convergence? *J. Exp. Zool. (Mol. Dev. Evol.) 318B:586–590, 2012.* © 2012 Wiley Periodicals, Inc.

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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 just 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 swam 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

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All the authors have read the paper and have agreed to having their names listed as authors. The authors have no conflict of interest to declare.

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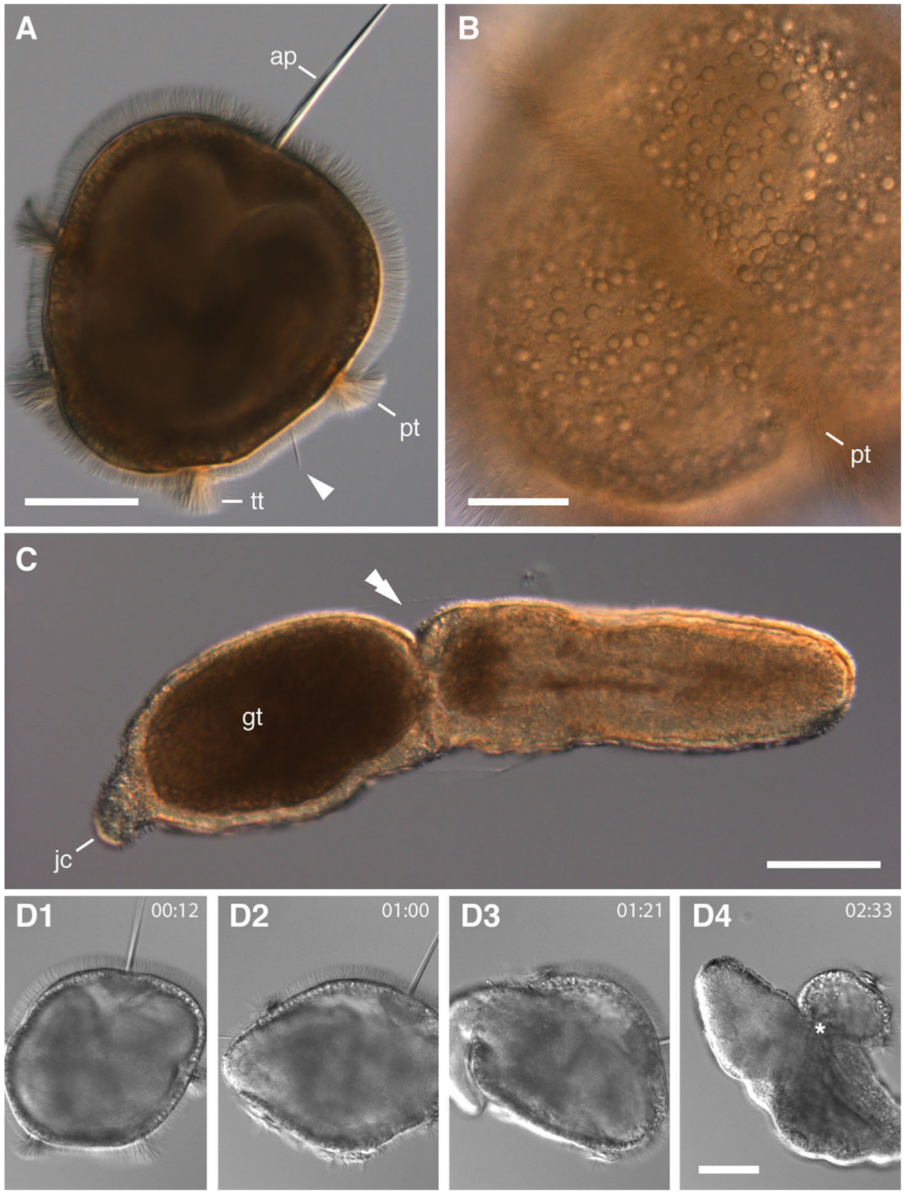

Figure 1. External appearance and metamorphosis of a novel type of nemertean larva. (A) This non-feeding trochophore-like pilidium is characterized by a blade-like apical tuft (ap), two circumferential ciliary bands—the equatorial "prototroch" (pt) and the posterior "telotroch" (tt)—and a ciliary larval cirrus (arrowhead) located between the two ciliary bands. Scale 100 µm. (B) A close-up focussed 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.

(Supplementary Video 1): when constrained, but not trapped, under a coverslip they alternated active swimming with a complete stall of all ciliary beating; they regularly depressed the apical organ deep into the episphere; and they intermittently contracted the prototroch, cinching in the waist; and upon such occasions, the dim outline of the worm inside could be seen to squirm, seemingly in response.

These larvae were isolated in small bowls of filtered seawater. Over night following collection, one of them underwent metamorphosis; in the morning, we found a young nemertean which possessed cephalic slits, a posterior cirrus, and a slight but consistent constriction between head and trunk (Fig. 1C, double arrowhead). It had a full stomach and was very sticky, owing to the thin transparent sheath it secreted around itself. Based on these traits we surmised that these larvae must be reduced pilidia of some species from the heteronemertean family Lineidae, and that, as reported for conventional (i.e., feeding) pilidia (Fewkes, 1883; Cantell, '66a, '69; Lacalli, 2005; Maslakova, 2010b), the juvenile worm had made its first meal of its own larval body.

We therefore tried to prompt metamorphosis in the second individual by trapping it under a coverslip, which stimulus is often effective for competent pilidium larvae of *Micrura alaskensis* (Maslakova, 2010b) and other species (Maslakova and von Dassow, unpublished data). This was unsuccessful for 3 days, but on the 4th day the juvenile had evidently had enough: initially, the worm inside was arranged in a pronounced S-shape with apparent head on one side of the apical organ and with apparent juvenile cirrus right underneath the larval cirrus (as in Fig. 1A); as it began its emergence, the juvenile straightened, distending the larval body greatly (Fig. 1D, panel 1; Supplementary Video 2). It repeatedly thrust its tail against the larval epidermis in the region of the larval cirrus, as if using the juvenile cirrus as an egg tooth, and the larval epidermis visibly thinned before the juvenile tail broke through (Fig. 1D, panel 2).

Shortly after the juvenile's tail broke through, larval material could be seen to stream into the juvenile mouth just anterior to the prototroch (Fig. 1D, panel 3). Within a little over 2½ min, the entire larval body had been devoured; the telotroch was the last part to be swallowed (Fig. 1D, panel 4).

Because the newly metamorphosed juveniles possessed both the longitudinal cephalic slits and the caudal cirrus we speculated that they belong to a local species of *Cerebratulus* or *Micrura*. To attempt to relate the new larval type to a morphologically identifiable adult, we froze one of the juveniles at −80°C in a small volume of sea water, extracted DNA using Instagene Matrix (Biorad, Life Science Research, 2000 Alfred Nobel Drive, Hercules, CA, USA), PCR-amplified and sequenced the 658 bp "barcoding" region of COI using universal primers LCO1419 and HCO2198 (Folmer et al., '94) and a 460 bp region of 16S rRNA using 16S-arL and 16S-brH primers from Palumbi et al. ('91). We compared these to a reference database of sequences from nemerteans collected by us in Oregon and Washington assembled by SAM over the years. 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 cephalic 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 ovoid 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 recurvatum* 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 incurvatum* described by Dawydoff ('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 nielseni* 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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