

Article

## Larvae of the pycnogonids *Ammothea gigantea* Gordon, 1932 and *Achelia cuneatis* Child, 1999 described from archived specimens

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Received 30 July 2012; Accepted 2 September 2012; Published online 1 December 2012

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### Abstract

The larvae of two species of Pycnogonida are described from archived collections. *Achelia cuneatis* Child, 1999 is an example of a typical protonymphon larva in having three pairs of three segmented appendages, cheliphores, palps and ovigerous appendages. *Ammothea gigantea* Gordon, 1932 is an example of a lecithotrophic protonymphon larva, having the three pair of appendages plus buds of the first walking legs. *Ammothea gigantea* is the third species known to have this larval type. The *Achelia cuneatis* protonymphon larvae is oval, 150  $\mu\text{m}$  long and characterized by a relatively long spinneret spine arising at the distal end of the first segment of each cheliphore. Spines also arise from the distal end of the first segment of the second and third limbs. This typical protonymphon larva of *Achelia cuneatis* has few yolk granules. The lecithotrophic protonymphon of *Ammothea gigantea* lacks a spinneret spine on the cheliphore; the larva is oval in shape, but much larger than *Achelia cuneatis* with a length of 700  $\mu\text{m}$ . Its second limb is much larger than the third limb on the first post-embryonic stage, and the third limb is further reduced to a spine-like structure on the second post-embryonic stage. This lecithotrophic protonymphon larva has a large number of yolk granules.

**Keywords** Pycnogonids; larvae; specimen; *Ammothea gigantea*; *Achelia cuneatis*.

### 1 Introduction

Pycnogonida is a class in the phylum Arthropoda usually considered the sister group of the remaining chelicerates or less often the sister group of chelicerates plus mandibulates (see Machner and Scholtz, 2010). The first post-embryonic stage of development is termed a protonymphon larva and is characterized by: an unsegmented body with the anterior three pairs of limbs developed, the most anterior of which forms a chela, and with a mouth at the end of a proboscis (Hoek, 1881; Morgan, 1891 and Hodgson, 1907). The earliest account of the larval stages of the Pycnogonida appear in the nineteenth century (Hoek, 1881), and the first complete description of the post-embryonic stages of development from hatching to adult form of a pycnogonid, *Tanystylum orbiculare*, was published by Morgan (1891). Among approximately twelve hundred known species, larvae of about 70 species (about 6%) have been described, and complete development from hatching to adult has been described for only a small number of species (Bogomolova and Malakhov, 2006).

## 2 Materials and Methods

The source of the protonymphon larvae of *Achelia cuneatis* Child, 1999 (USNM 234738) and *Ammothea gigantea* Gordon, 1932 (USNM 1122796 and 1122798) for this study was archived specimens from the collections of the National Museum of Natural History, Smithsonian Institution identified by Dr. Colby Allan Child. Larvae were recovered from the ovigers of adult male specimens. A single stage, presumably the first post-embryonic stage, was available for *Achelia cuneatis*. *Ammothea gigantea* protonymphon larvae were present as two stages, presumably the first and second post-embryonic stages. The length of each larva was measured from the base of the cheliphores to the posterior end of the animal. Larvae were critical point dried and coated with gold alloy for the scanning electron micrographs; a Phillips Electron Microscope was used in this study.

## 3 Results

The first post-embryonic stage of *Achelia cuneatis* is oval in shape, 150  $\mu\text{m}$  long and 140  $\mu\text{m}$  wide. The cheliphores arise at the base of the proboscis and have a well developed spinneret which is located on the lateral side at the distal end of the proximal segment. The spinneret is as long as the proximal segment, 50  $\mu\text{m}$ , and extends beyond the distal edge of the middle segment. An attenuation of the middle segment forms the chela with the distal segment (Fig. 3). The chela is smooth, lacking denticles, but distal segment of the chela has small bristles on the lateral surface. The second and third limbs are also 3-segmented. On both limbs, the proximal segment is 20  $\mu\text{m}$  long, about one-fourth the length of the second segment, and is armed with lateral spine which is twice as long as the segment (Fig. 1). The middle segment is 50  $\mu\text{m}$  long and unarmed. The distal segment is as long as the second but much thinner and is curved to form a subchela with the middle segment. The proboscis extends ventrally terminating in three flaps that cover the mouth (See Fig. 2). There is no anus at this stage (Figs. 1, 3).

The first post-embryonic stage, a protonymphon larva, of *Ammothea gigantea* is 700  $\mu\text{m}$  in length and 660  $\mu\text{m}$  in width (Figs. 4, 5 and 8). The first segment of the cheliphore is 430  $\mu\text{m}$  in length by 200  $\mu\text{m}$  in width, and lacks a spinneret. The second segment which forms the non-moving portion of the chela is 400  $\mu\text{m}$  in length. The third segment is 260  $\mu\text{m}$  in length. There are no denticles on the chela (Fig. 8). The second limb is 122  $\mu\text{m}$  in total length; its first segment is 61  $\mu\text{m}$  in length, and its second segment is 28  $\mu\text{m}$  in length. The third segment is 33  $\mu\text{m}$  in length. The third limb is much smaller, 72  $\mu\text{m}$  in total length; its first segment is 39  $\mu\text{m}$  in length. The second segment is 22  $\mu\text{m}$  in length, and the third segment is 11  $\mu\text{m}$  in length (Fig. 6). There are two sensilla on the ventral side of the larva just posterior to the base of walking leg 1 (Fig. 8) and an anus is present (Fig. 4). The mouth, located on the end of the proboscis is a three part structure (Fig. 7). The paired buds of walking leg 1, the fourth limb, bear a seta (Fig. 8).

The second post-embryonic stage of *Ammothea gigantea* has a segmented walking leg 1 and unsegmented buds of walking leg 2. A rudimentary the abdomen appears at this stage (Figs. 10, 11 & 12).

## 4 Discussion

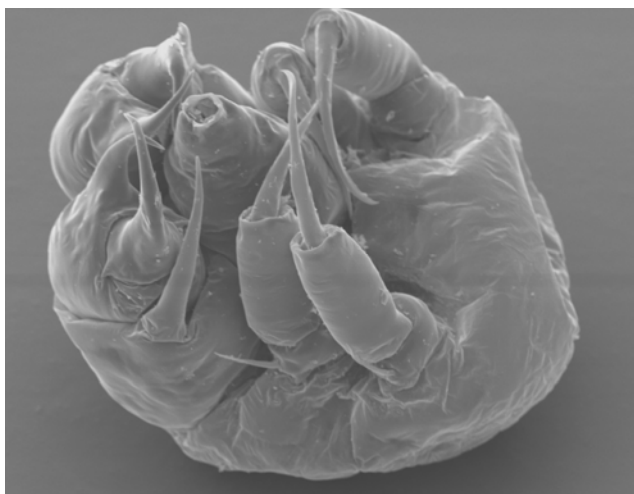
Bain (2003) recognized four distinct types of protonymphon larvae: typical protonymphon; encysted larva; atypical protonymphon; and attaching larva. These types are based on the number of limbs present on the first post-embryonic stage, and the developmental sequence of limbs during successive stages. The typical protonymphon larva hatches from the egg with the anterior three pairs of appendages. As development proceeds, one pair of walking legs is added at each molt and the anterior three pairs of limbs are transformed

into the adult cheliphores, palps and ovigerous limbs. The morphology of the encysted larva is similar to the typical protonymphon but soon after hatching the second and third pair of limbs fails to develop as the larva enters a parasitic life phase within the gastrocoel of cnidarians. While in the host, three pairs of walking legs are added simultaneously, and a fourth pair is added later. In the atypical protonymphon larvae, buds of all four pairs of walking legs are present at the first stage as well as the anterior three appendages. Further development of the atypical protonymphon larva takes place in the mantle cavity of mollusks or on sedentary polychaetes. The last larval type recognized by Bain is the attaching larva which hatches without the second and third pair of larval limbs, but with buds of the first and second pairs of walking legs (the fourth and fifth limbs). The attaching larva does not feed until it matures and leaves the body of the adult male parent at which time it has added two more pairs of walking legs. Bogomolova et al. (2004) recently identified a fifth larval type, the lecithotrophic protonymphon. This larva hatches with the anterior three limbs present, all 3-segmented, and the first pair of walking legs present as unsegmented buds. This larval form has been reported for *Nymphon grossipes* Fabricius, 1794 (Bogomolova, 2007) and *Ammothea glacialis* Hodgson, 1907 (Cano et al., 2009; Ferrari et al., 2011).

These five different larval types appear to have little phylogenetic significance. Some families have only a single larval type, as in the Phoxichilidiidae with only encysted larvae, or Callipallenidae with only attaching larvae. In contrast, the family Ammotheidae has been shown to have four of the five larval types (Bain, 2003; Bogomolova et al., 2004).

The lecithotrophic protonymphon larva of *Ammothea gigantea* closely resembles that of *Ammothea glacialis*. It differs in that the third appendage of *Ammothea gigantea* is reduced in size compared to second. By the second stage, the third appendage is reduced further to a large spine (Fig. 12). The first description of the larva of *Ammothea glacialis* was provided by Cano et al. (2009). They believed that a first stage of development was passed within the egg, and they attributed the first post-embryonic stage as comparable to a second stage. Ferrari et al. (2011) proposed the first post-embryonic stage was the first stage of development and that there was no earlier stage within the egg of this species.

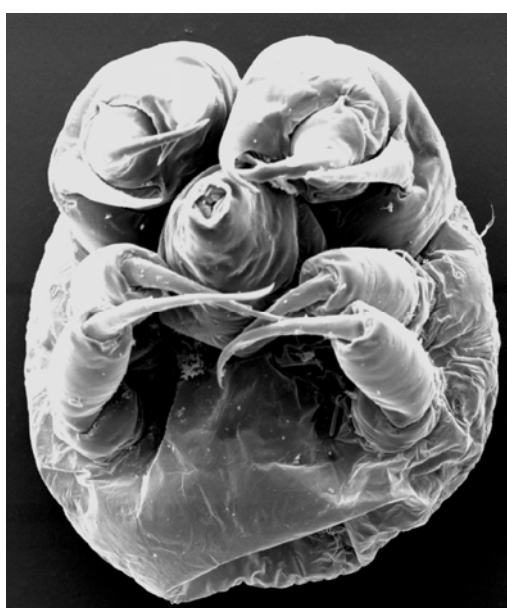
The protonymphon larva of *Achelia cuneatis* conforms to the first post-embryonic stage of a typical protonymphon larva based on Bain's (2003) classification. The first post-embryonic stage of *Achelia simplissima* described by Burris (2010) and *Achelia assimilis* described by Lehmann et al (2011) and *Achelia borealis* described by Bogomolova, et al., (2004) are all similar in many aspects. All four larvae have a spinneret spine on the proximal segment of the cheliphore, and this spinneret is as long as the appendage. The body is dorsoventrally flattened and an eye tubercle is lacking on the larva. Sensilla, one pair located ventrally on the body of the larva posterior to third appendage and three pair dorsally posterior to the cheliphores are present on *Achelia borealis*, *Achelia simplissima* and *Achelia assimilis*. Only one set of sensilla were observed on our specimens of *Achelia cuneatis* (Figs. 2, 3).



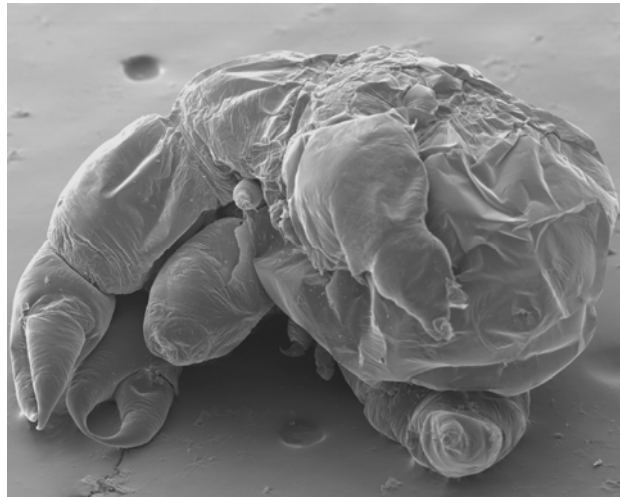
**Fig. 1** *Achelia cuneatis* Child, 1999 first stage, protonymphon larva showing the three pairs of limbs; spinneret on the cheliphores arises at the distal end of the first segment of the cheliphore; smaller spines arise from the first segments of second and third limbs; proboscis extends ventrally between the cheliphores.



**Fig. 2** *Achelia cuneatis* Child, 1999 first stage limbs and proboscis showing the three flaps over mouth, and setules and spinneret on the cheliphore.



**Fig. 3** Ventral view of habitus of *Achelia cuneatis* Child, 1999 larva.



**Fig. 4** Ventral view of first stage of *Ammonothea gigantea* Gordon, 1932 unsegmented buds of the first pair of walking legs.



**Fig. 5** *Ammonothea gigantea* Gordon, 1932 protonymphon at time of hatching from the egg; reduced limb 3 is near the margin of the egg shell.



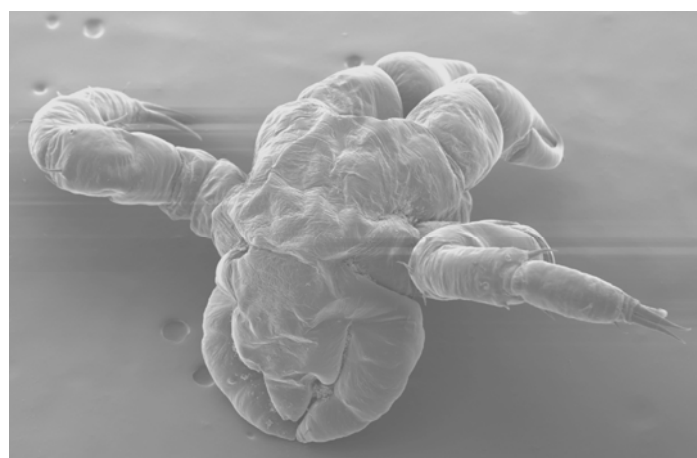
**Fig. 6** Second and third limbs of first stage *Ammonothea gigantea* Gordon, 1932 protonymphon.



**Fig. 7** Mouth of first stage larva of *Ammothea gigantea* Gordon, 1932 protonymphon.



**Fig. 8** Ventral view of *Ammothea gigantea* Gordon, 1932 protonymphon larva showing limb buds of first walking legs and two sensilla between limb buds; analage of the abdomen posteriorly.



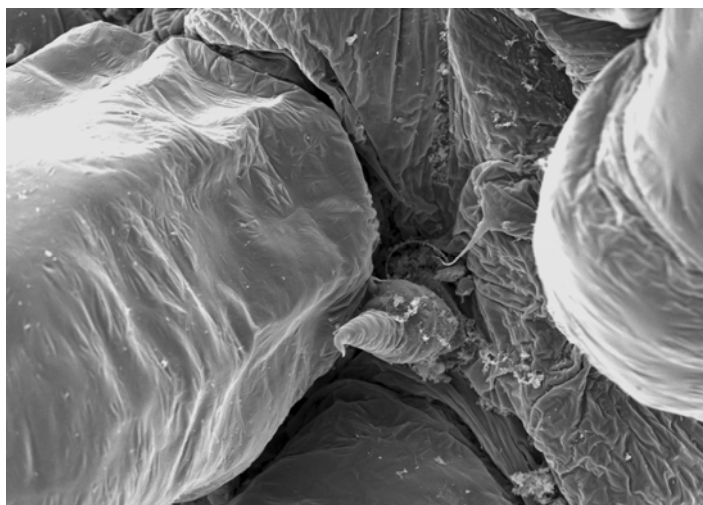
**Fig. 9** Dorsal view of second stage of *Ammothea gigantea* Gordon, 1932 showing first walking legs with seven segments and the buds of the second walking legs are present.



**Fig. 10** Segments of first walking leg of second stage of *Ammothea gigantea* Gordon, 1932.



**Fig. 11** Ventral view of the second stage of *Ammothea gigantea* Gordon, 1932 protonymph larva.



**Fig. 12** Second and third limbs second stage of *Ammothea gigantea* Gordon, 1932 protonymph larva; third limb reduced to a curved spine.

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