Article

Early post hatching stages of *Nymphon australe* Hodgson

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Abstract
Using scanning electron microscopy, we describe the first and second post hatching stages of *Nymphon australe* Hodgson 1902. In the first post hatching stage the animals have a pair of three segmented cheliphores and a pair of three segmented larval appendages either II or III. Three undeveloped buds of walking legs, 1, 2 and 3 are also present. The digestive system is still incomplete, the back ectodermal part of an alimentary canal or proctodeum has not appeared at the first post hatching stage. The second post hatching stage has seven-segmented walking legs 1 and 2 and a four-segmented walking leg 3. The fourth walking leg is present as an undeveloped bud in this stage. The eye tubercle has appeared by the second post hatching stage. The back ectodermal part of an alimentary canal or proctodeum has appeared as a slit at the posterior end of the opistosome. In addition to *N. australe* the early post hatching stages of several other species of pycnogonids have developmental traits typically associated with embryonic development. The paired primordia of the ventral nerve chord ganglia are present on the epidermis. The eye tubercle is not present until one or more post hatching molts. The digestive tract is incomplete as indicated by the absence of the back ectodermal part of an alimentary canal or proctodeum. The undeveloped limb buds of the walking legs are morphologically analogous to those in chelicerate embryos. We propose that in the Pycnogonida at the time of hatching embryonic development is incomplete.

Keywords *Nymphon australe*; embryonic development; hatching; developmental biology; larvae.

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1 Introduction

Pycnogonida, sea spiders, have an unusual reproductive strategy for arthropods, the males care for the eggs and early post hatching developmental stages. When sexually competent adults encounter members of the opposite sex, the female crawls over the male and release her eggs into the water. At the same time, the male releases his sperm into the water and fertilization occurs externally. In most species, after mating, the male collects and carries the eggs on modified legs called ovigerous appendages. Depending on the developmental pattern of the species, when the eggs hatch the male may continue to carry the post-hatching stages until they

reach a stage resembling the adults (King, 1973; Bain, 2003). In this study, the early post hatching stages of *Nymphon australe* Hodgson 1902 in the archived collections of the National Museum of Natural History (NMNH) are described. The Antarctic pycnogonid, *N. australe* displays great phenotypic variability and morphological variability (Hodgson, 1902; Mahon et al., 2008).

Multiple developmental patterns are described in the literature for the members of the genus, *Nymphon* (Bain, 2003; Bogomolova and Malakhov, 2006; Bogomolova, 2007; Burris 2011; Fornshell, 2014, 2015, 2017; Alexeeva, et al., 2017; Brenneis, et al., 2017). The early post hatching stages of 13 species of the genus *Nymphon*, *N. gallicum* Hoek, 1881, *N. gracile* Leach, 1814, *N. grossipes* (Fabricius, 1780), *N. longitarse* Kreyer, 1845, *N. leptocheles* Sars, 1891, *N. macronyx* Sars, 1877, *N. maculatum* Carpenter, 1910, *N. ortmanni* Helfer, 1938, *N. parasiticum* Merton, 1906, *N. stromii* Kreyer, 1845, *N. brevirostre* Hodge, 1863, *N. micronyx* Sars, 1888, and *N. Tenellum* (Sars, 1888) have been described in the literature (Hoek, 1881; Bain, 2003; Bogomolova and Malakhov, 2006; Bogomolova, 2007; Burris, 2011; Fornshell, 2014, 2015, 2017; Alexeeva et al., 2017; Brenneis et al., 2017).

2 Materials and Methods

Archived specimens of the protonymphon larvae of *Nymphon australe* Hodgson 1902 (USNM 87361) from the collections of the National Museum of Natural History, Smithsonian Institution identified by Dr. Colby Allan Child were used in this study. Larvae were recovered from the ovigers of adult male specimens. *Nymphon australe* eggs and two post-hatching instars were present. The specimens were critical point dried and coated with gold alloy for the scanning electron micrographs. A Phillips Electron Microscope was used in this study. The length of each specimen was measured from the base of the cheliphores to the posterior end of the animal. A total of 48 images of 25 specimens were analyzed in this study (Fornshell, 2012, 2014, 2015, 2017).

3 Results

The eggs (See Fig. 1) are oval in shape 506µm (range 442 µm - 667 µm) X 428 µm (range 423 µm - 433 µm) n=eight.

The first post hatching stage has an average length of 583 µm (571 µm-633 µm) n = seven. The first post hatching stage has three undeveloped walking legs. Larval appendage, either II or III, is present as a three-segmented appendage with a terminal spine. The cheliphores are longer than the proboscis and have a short spinneret spine on the first segment. The back ectodermal part of an alimentary canal or proctodeum is lacking in this stage, as is the eye tubercle (See Fig. 2).

The second post-hatching instar has an average length of 810 µm (628 µm - 961 µm) n = ten. The Cheliphores, which have a short spinneret spine and are longer than the Proboscis. They are longer than in the cheliphores of the first post hatching stage. Larval appendage, either II or III, is present and longer than in the first post hatching stage with four short spines on the end. The eye tubercle is present on the second post-hatching stage (See Fig. 3). Appendages IV and V are seven segmented. The third walking leg, appendage VI, is four segmented in the second post-hatching stage. The fourth walking leg, appendage VII, is present as an undeveloped bud, and the opisthosoma is present in the second post-hatching sage. The back ectodermal part of an alimentary canal or proctodeum is visible as a slit at the terminal end of the opisthosoma. (Figs. 4 and 5).

The adult male has three segmented cheliphores, which are longer than the proboscis and five-segmented palps in addition to the ovigerous appendages. The four pairs of walking legs each have eight segments.

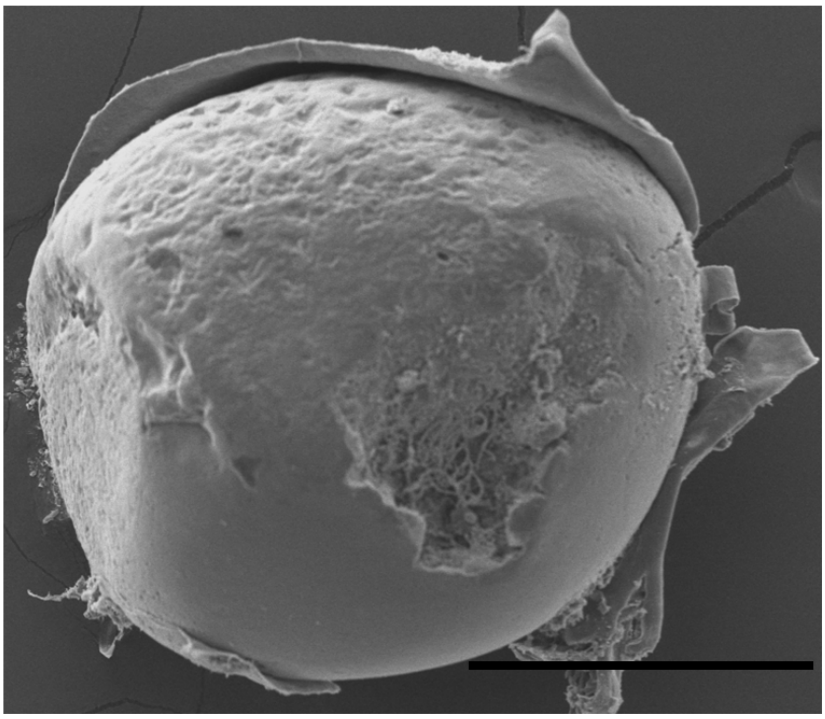

Fig. 1 *Nymphon australe* egg. The scale bar is 200 µm.

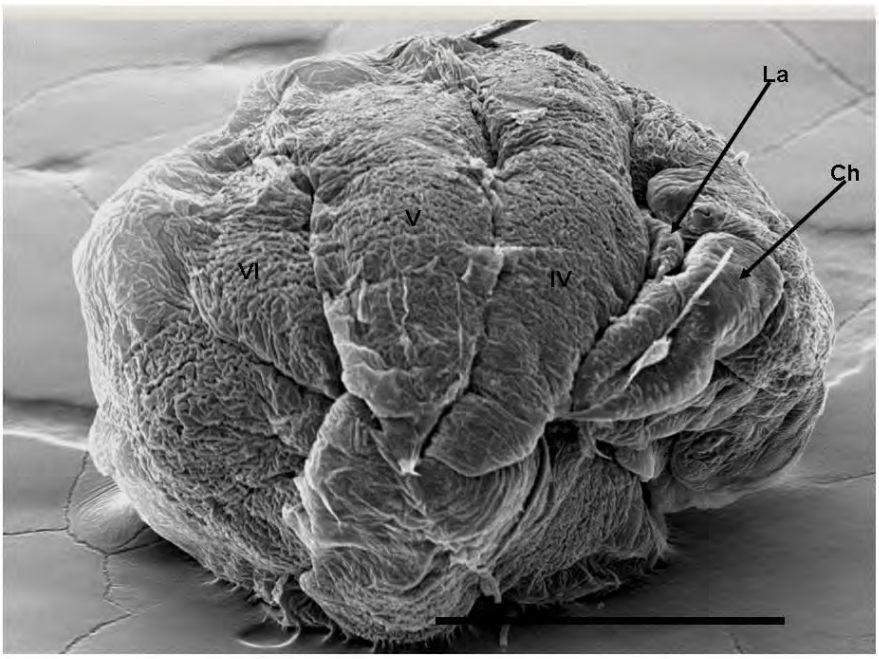

Fig. 2 First post hatching stage of *Nymphon australe*. Ch = Cheliphores(s); La = larval appendage II or III; IV-VI= walking legs one through three. The scale bar is 200 µm.

4 Discussion

The first two post hatching stages of *N. australe* Hodgson display the following characteristics of embryonic development: (1) The paired primordia of the ventral nerve chord ganglia are present on the epidermis; (2) The

digestive tract is incomplete as indicated by the absence of the back ectodermal part of an alimentary canal or proctodeum; (3) The undeveloped limb buds of the walking legs are morphologically analogous to those in chelicerate embryos, and (4) The eye tubercle is absent at the time of hatching.

In addition to the first two post hatching stages of *N. australe*, the early post hatching stages of other pycnogonids display many developmental processes normally associated with embryonic development as described for the Chelicerata. The tubercle appears after several molts in typical *N. grossipes* and the anus and proctodeum are absent in the first hatching stage (Bogomolova and Malakhov, 2006). In *Ammothella biunguiculata* (Dohrn, 1881), the anus appears in the sixth post hatching instar and the tubercle appears in the fifth instar (Mochizuki and Myazaki, 2017). In the Encysting *Anoplodactylus eroticus* Stock, 1968, the tubercle appears in the sixth instar and the anus and proctodeum in the seventh instar (Maxmen, 2013). The first post-hatching stage of *N.brevirostre* lacks an anus or proctodeum (Bogomolova, 2007). In the first post-hatching stage of *Achelia cuneatis* Child, 1999 and *Ammothea gigantea* Gordon, 1932 the eye tubercle is absent as is the anus and proctodeum (Fornshell and Ferrari, 2012; Fornshell, 2014, 2015, 2017). In *Achelia borealis* (Schimkewitsch, 1895) and *N. brevirostre* the anus and proctodeum are absent in the first post hatching stage, and the segmental ventral ganglia are present (Brenneis, 2017). In *Pseudopallene, sp.* Wilson 1878 the ectodermal invaginations of early post hatching stages of Pycnogonida are interpreted as neurogenic niches of late embryonic and post-embryonic nervous system development by Brenneis et al. (2013) and Brenneis and Sholts (2014). Non-larval, i.e. walking legs always appear first in the form of unsegmented structures similar to the development of these appendages in the embryos of other chelicerates (Grbic, et al, 2007; Hilbrant, et al., 2012; Brenneis et al. 2013; Schwager, et al., 2015). The central nervous system is still developing via invaginations of the ectoderm to form presumptive segmental ganglia of the ventral nerve cords (Hoek, 1881; Korschelt and Heider, 1899; Brenneis, et al., 2013). Based on these observations, we propose that in the Pycnogonida at the time of hatching embryonic development is incomplete.

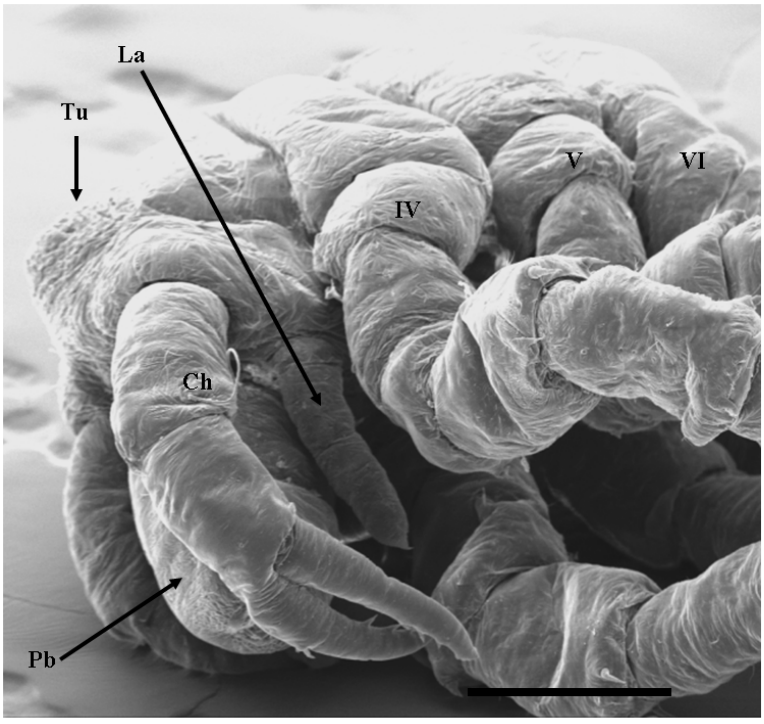

Fig. 3 Second post hatching stage of *Nymphon australe* showing the anterior region of the animal. Ch = Cheliphore(s); Pb = Proboscis; La = larval appendage II or III; IV-VI= walking legs one through three; Tu= eye tubercle. The scale bars are 200 µm.

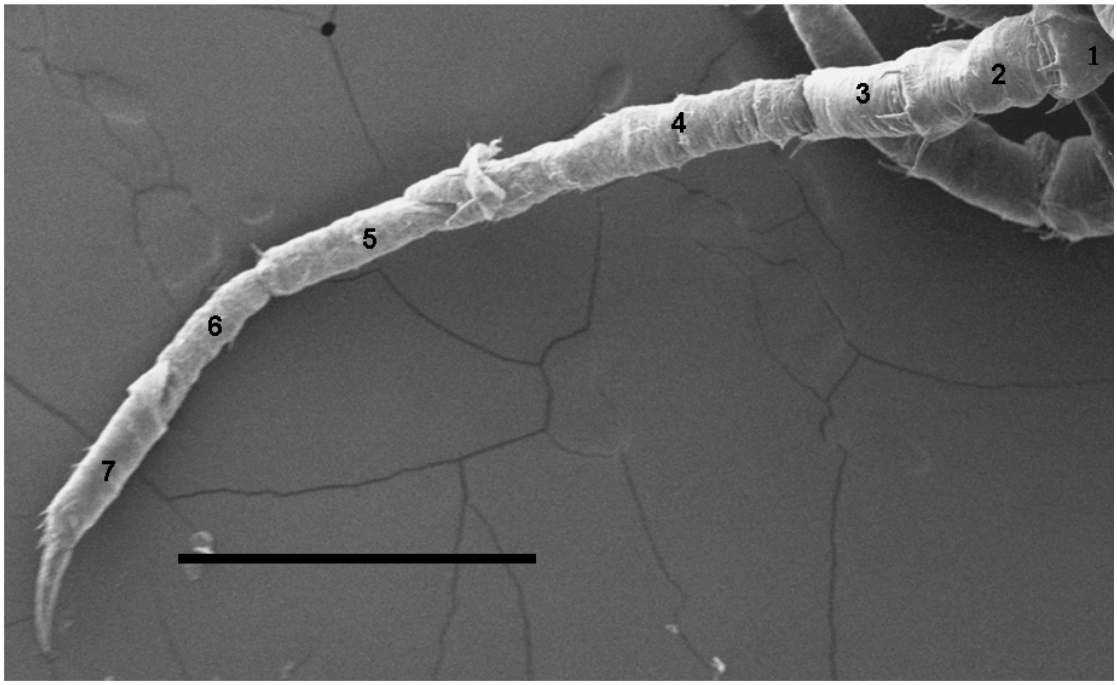

Fig. 4 The first walking leg of the second post hatching stage of *Nymphon australe* showing the seven segments and terminal spine. Walking leg segments: 1 - coxa 1; 2 - coxa 2; 3 - coxa 3; 4 - femur/tibia I; 5 - tibia II; 6 - tarsus; 7 - propodus. The scale bar is 500 µm.

Fig. 5 Second post hatching stage of *Nymphon australe* showing the posterior region of the animal. IV-VII=walking legs one through four; Op=Opisthosoma; Pr=proctodeum. The scale bar is 200 µm.

In the Arthropoda the completion of embryonic development and the transition from the larvae to juveniles or adults is not universally synchronized with hatching and metamorphosis. In fact, hatching, molting, and the degree of morphological differentiation of the hatchling and body segmentation are not correlated universally (Minelli, et al., 2006). This lack of synchronization of the termination of embryonic development, first molting and hatching is found in the chelicerates. In *Limulus polyphemus* Linnaeus, 1758, the horseshoe crab, before the egg hatches the developing embryo molts four times within the egg (Packard, 1880; Botton et al., 2010). Also in the scorpion, *Centruroides vittatus* (Say, 1821), hatching occurs before embryonic development is complete (Farley, 2005).

Truman et al. (1999) described the following four character traits observed in crustaceans and insects displaying a larval stage in their life cycle: (I) The presence of organs and/or tissues characteristic of the larva which are not found in the adult; (II) Metamorphosis which is characterized by the loss of tissues, organs and/or limbs involving apoptosis; (III) Hormonally mediated changes in growth and development; (IV) A fundamental change in life style, habitat, nutrition and or behavior from larva to adult.

The four larval traits listed above are absent from post hatching pycnogonids in all currently described species. There are on purely larval organs or tissues described in early post hatching pycnogonids. While apoptosis occurs in the second and third larval appendages, in many species, these appendages are not universally lost from the adult morphology. They also may reappear in subsequent instars (Maruzzo et al., 2005; Minelli et al., 2006; Fornshell, 2014). There is no evidence of hormonally mediated metamorphosis in the Pycnogonida. Characteristic IV occurs gradually as the juvenile matures into the adult form. There is no evidence of metamorphosis from a larval form to an adult form in a single molt (Bain, 2003; Bogomolova and Malakhov, 2006; Bogomolova, 2007; Burris, 2011; Fornshell, 2012, 2015; Hilton, 1916; Brenneis et al., 2017; Alexeeva et al., 2017).

Earlier workers have opined that larval types are the result of a change in nutrition resulting in the evolutionary loss and or gain of morphological features characterizing the larva (Smith et al., 2007; Reitzel, 2006; Raff, 2008; Burris, 2011). Alternatively, we may view the hatching of the pycnogonids as not being the end of embryonic development. The subsequent appearance of the proctodeum one or more molts after hatching indicates embryonic development is still in progress. The development of the walking legs is analogous to that seen in the embryonic development of the walking legs in other chelicerates (Packard, 1880; Hilbrant and McGregor, 2012). In addition, the formation of the ventral nerve chord ganglia in post hatching instars is an indication of continued embryonic development. The lack of larval-like characteristics associated with larval development and metamorphosis are also an indication that the Pycnogonida post-hatchings stages represent embryonic developmental stages.

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