Article

Larval stages Nymphon charcoti Bouvier 1911

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Abstract

The protonymphon larva and next four instars of the pycnogonids *Nymphon charcoti* are described. The developmental pattern is that of a "Typical Protonymphon" according to Bain's 2003 classification. The description is based on archived specimens from the National Museum of Natural History collections. The walking legs appear initially as underived buds with three pseudo-segments beginning with walking leg one in the second instar. In the subsequent instars the walking legs appear as six segmented appendages and then eight segmented appendages. In the genus *Nymphon* there are four different post embryonic developmental patterns, "Typical Protonymphon", "Attaching Larva", "Lecithotrophic Larva" and "Elvie's Larva". This diversity of developmental patterns within the same genus which is not restricted to the Nymphonidae indicates that the developmental patterns as currently defined in the literature have little phylogenetic relevance.

Key words Nymphon charcoti; Protonymphon; Pycnogonida.

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1 Introduction

The first post-embryonic stage of development is a Protonymphon larva which is characterized by an unsegmented body with a tripartite mouth at the end of a proboscis and the anterior three pairs of limbs developed. The first pair of larval appendages is the three segmented chelicerae which remain three segmented throughout development. The second and third pairs of appendages present in the protonymphon larva, appendages II and III are larval appendages for walking and (rarely) swimming (Hoek, 1881; Morgan, 1891; Hodgson, 1907).

The larva of 20 species of the genus *Nymphon*has have been described in the literature. *Nymphon gallicum* Hoek, 1881, *N. gracile* Leach, 1814, *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 and *N. micronyx* Sars, 1888 are described as possessing a Typical Protonymphon Larva developmental pattern (Bain, 2003 and Bogomolova and Malakhov, 2006). *Nymphon gracilipes* Sars, 1877, *N. hiripes* Bell, 1855, *N. hirium* Fabricius, 1794, *N. sluiteri* Hoek, 1881 and *N. spinosum* Goodsir, 1842 are described as possessing an Attaching Larva developmental pattern (Bain, 2003). *Nymphon grossipes* (Fabricius, 1780) is described as possessing a Lecithotrophic Larva developmental pattern (Bogomolova and Malakhov, 2006). The larvae of *N. brevicaudatum* were described by Hoek (1902) as having 3 pairs of legs besides 3 cephalic pairs of appendages. This same developmental pattern was also described by Bogomolova (2014) in *N. austral* Hodgson 1902 and by Fornshell (2015) in *N. tenellum* (Sars, 1888) giving it the name Elvie's Larva.

There are approximately 1300 described species of the class *Pycnogonida*. The number of species for which one or more larval stage has been described is less than 100. In this paper the post embryonic development of *Nymphon charcoti* Bouvier 1911 from the protonymphon larva to the fifth instar is described from electron micrographs.

2 Methods

Larval stages of the pycnogonids *Nymphon charcoti* (National Museum of Natural History accession number 342105) were collected from ovigerous males. At the time of collection the specimens were fixed in a formaldehyde solution and, after species determination at the Natural History Museum, stored in a 70% ethyl alcohol solution. The larvae were dehydrated in progressively more concentrated ethanol solutions and critical point dried and coated with gold alloy for the scanning electron micrographs using a Philips XL30 ESEM LaB6 Electron Microscope. The length of each larva was measured from the electron micrographs from the base of the chelicerae to the posterior end of the animal.

The following abbreviations are used in the electron micrographs: AB — abdomen; CH — chelicera; CL — terminal claw with two auxiliary claws present; PC — proctodeum; PR — proboscis; SI — sensilla; SP — spines; TM —tripartite mouth; II — second larval appendages; III — third larval appendages; IV — walking leg one; V — walking leg two; VI — walking leg three; VII — walking leg four; 1 — coxa 1; 2 — coxa 2; 3 — coxa 3; 4/5 — femur-tibia I; 6 — tibia II; 7 — tarsus; 8 —propodus. The eight segments of a pycnogonid walking leg are coxa 1, coxa 2, coxa 3, femur, tibia I tibia II tarsus and propodus. The articulated terminal claw is not considered to be a segment (King, 1973)

3 Results

The Protonymphon larva is 600 μ m to 680 μ m long and 460 μ m wide. The chelicerae are three segmented with a 50 μ m long spinneret spine on the first segment and smaller spines on the first and second segments. In the protonymphon stage there are no teeth on the chelicerae. The chelicerae are 280 μ m long. The proboscis is 100 μ m, less than half the length of the chelicerae. Larval appendages II and III are three segmented and 220 μ m long (Table 1). The first segments of appendages II and III have thin spines. There is no indication of an anus or tubercle with eyes. Note: this is a Typical Protonymphon Larva according to Bain's (2003) classification (Figs. 1a and 1b).

The second instar is 440 μ m to 510 μ m long and 340 μ m to 400 μ m wide. The three larval appendages are unchanged from the protonymphon stage. A pair of underived buds with three articles or pseudo segments representing the first pair of walking legs appears at this stage. The first walking leg buds are 100 μ m wide and 150 μ m long (Figs 1c and 1d).

walking legs as they appear during development.							
	Ι	II	III	1	2	3	4
Protonympho	3	3	3				
Second Instar	3	3	3	X'			
Third Instar	3	3	3	6	X'		
Fourth Instar	3	3	3	8	6	X'	
Fifth Instar	3	3	3	8	8	6	X'

 Table 1
 Summarizing the number of segments observed in the three larval appendages, I, II and III and the four walking legs as they appear during development

I - chelicera; II - second larval appendages; III - third larval appendages; walking legs - 1, 2, 3 and 4; X' - underived walking leg bud with three articles.



Fig. 1a Ventral view of the protonymphon larva with larval appendages I - chelicera, II - second larval appendage and III - third larval appendage. Scale bar = $100 \mu m$. **1b**. Dorsal view of the protonymphon larva. Scale bar = $200 \mu m$. **1c**. Ventral-lateral view of second instar larva with larval appendages I = chelicera, II - second larval appendage and III - third larval appendage and showing the underived bud of the first walking leg = IV. Scale bar = $100 \mu m$. **1d**. An enlargement of the first three larval appendages of a second instar larva. The Spinneret spine = Ss is visible with a thread of attaching thread extending from the end of the Spinneret spine.

The third instar is 670 μ m long by 470 μ m wide. The chelicerae and larval appendages II and III have the same form as in the second instar. The proboscis is still less than half the length of the chelicera. The first pair of walking legs is present as a six segmented appendage, coxa 1, coxa 2 and coxa 3, femur/tibia 1, tibia 2,

tarsus/propodus. The second pair of walking legs is present as underived buds with three articles or pseudo segments (Figs. 2a and 2b). In Fig. 1d, the spinneret spine with attaching thread can be seen.

The fourth instar is 690 μ m long and 290 μ m wide. The chelicerae have eight teeth on the movable and stationary segments of the claw (Fig. 3b). The Chelicerae are more than twice as long as the proboscis. There are three spines at the distal end of the first segment of the chelicera and two spines on the second non-movable segment of the chelicera. The proboscis is 125 μ m long. Larval appendages II and III are three segmented and unchanged in form from the protonymphon stage. The first pair of walking legs is now eight segmented, coxa 1, coxa 2 and coxa 3, femur, tibia 1, tibia 2, tarsus, and propodus and the second pair of walking legs is six segmented, coxa 1, coxa 2 and coxa 3, femur/tibia 1, tibia 2, tarsus/propodus. The third pair of walking legs is represented by underived buds with three articles (Fig. 3a).



Fig. 2 Dorsal view of the third instar larva with larval appendages I - chelicera, II - second larval appendage and III - third larval appendage IV - six segmented first walking leg, 1 - coxa 1; 2 - coxa 2; 3 - coxa 3; 4 - Femur/Tibia I; 5 - Tibia II; 6 - Tarsus/propodus. V - underived bud of walking leg 2. The articles are clearly visible. Scale bar = $200 \,\mu m$.

The fifth instar is 1,125 μ m long and 750 μ m wide. The chelicerae have eight teeth on both the stationary and movable segments of the claw. Larval appendages II are 400 μ m long with 10 spines at the distal end. Larval appendages III are three segmented and much smaller than larval appendages II. Walking legs one and two are eight segmented, coxa 1, coxa 2 and coxa 3, femur, tibia 1, tibia 2, tarsus, and propodus. Walking legs three are six segmented, coxa 1, coxa 2 and coxa 3, femur/tibia 1, tibia 2, tarsus/propodus. The fourth pair of walking legs is represented by underived buds with three articles (Fig. 4).



Fig. 3a Dorsal view of the fourth instar larva with larval appendages I - chelicera, IV - eight segmented first walking leg, V - six segmented second walking leg, VI - underived bud of walking leg 3. Scale bar = $200 \,\mu$ m. **3b**. The chelicera showing the teeth on the palm and movable segment. Scale bar = $200 \,\mu$ m.



Fig. 4 Ventral view of fifth instar larva: I = chelicera, II = second larval appendage and III = third larval appendage IV = eight segmented first walking leg, V = eight segmented second walking leg, VI = six segment third walking leg, VII = underived bud of walking leg 4. Scale bar = $500 \,\mu$ m.

4 Discussion

There are at present six recognized development patterns in the Pycnogonida, Typical Protonymphon, Atypical Protonymphon, Attaching Larva, Encysted Larva (Bain, 2003), Lecithotrophic Larva (Bogomolova and Malakov, 2006) and Elvie's Larva (Fornshell, 2015). These developmental patterns, which are in part based on nutrition and behavior, and only secondarily on morphology, do not reflect phylogenetic relationships. They do, however, remain useful as a framework for describing the post embryonic development of pycnogonids. Four of these types, Typical Protonymphon, Attaching Larva, Lecithotrophic Larva and Elvie's Larva are found in the single genus *Nymphon* family *Nymphonidae*.

The Lecithotrophic larval type hatches as a protonymphon stage. The larva is large about 500 μ m long and filled with yolk. Walking legs are added sequentially as in the Typical Protonymphon larval developmental pattern. The larva remains attached to the male until the end of larval development. The first species known to display this is *N. grossipes* (Bogomolova and Malakov, 2006).

The Attaching Larva undergoes its first molt before hatching. The larva that hatches has chelicera and the buds of the first and second walking legs (Nakamura, 1981; Bain, 2003). Species of *Nymphon* known to have this larval type include *N. gracilipes; N. hiripes; N. hirium; N. sluiteri;* and *N. spinosum.* The complete development from protonymphon to adult has not been achieved for any of these species.

Elvie's Larva, has larval appendages I, II and III plus the underived buds of the first three pair of walking legs. A single late larval example *N. tenellum* was described as possessing Elvie's larval developmental type (Fornshell, 2015). A complete description of development from hatching to adult was not possible with the data available (Fornshell, 2015). This type of larval developmental pattern was described for *N. australe in an abstract* (Bogomolova, 2014). Hoek (1902) described *N. brevicaudatum* embryos as having the three larval appendages and buds of the first three walking legs in a pre-hatching stage. These are figured in Plate XIX figures 9 - 11 (Hoek, 1902).

The Typical Protonymphon developmental pattern hatches as a protonymphon larva and in successive molts adds segments to the trunk each with a pair of walking legs until the adult number, usually four pairs is achieved (Bain, 2003). Species of *Nymphon* known to have this larval type include *N. gallicum; N. gracile; N. longitarse; N. leptocheles;N. macronyx; N. maculatum; N. ortmanni; N. parasiticum; N. stromii; N. brevirostre* and *N. micronyx* (Bain, 2003; Bogomolova and Malakov, 2006). The development of the post protonymphon larva varies somewhat between species. The buds of the first pair of walking legs typically first appear the second instar, first post protonymphon stage. In some cases the buds of the first pair of walking legs, 6, 7 or 8 depending on the stage also varies between members of this genus (Fornshell and Ferrari, 2012; Fornshell, 2015; Cano and López-González, 2009 & 2013; King, 1973; Morgan, 1891). The larval stages of *N. charcoti* described in this study clearly place it in the Typical Protonymphon developmental pattern. The development of teeth on the chelicerae in the fifth instar is different from the other described species of Nymphon.

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