*Article*

# Early developmental stage of *Pentapycnon charcoti* Bouvier

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**Abstract**

The first post hatching stage of *Pentapycnon charcoti* Bouvier is described for the first time. The eggs and first hatching stage were recovered from a cocoon carried on the ovigerous appendages of an adult male specimen archived in the collections of the National Museum of Natural History. Several different morphologies of the first hatching stage of the family Pycnogonidae have been described by Bain (2003) and Brenneis et al. (2017). The first hatching stage of *P. charcoti* was analogous to Bain's typical protonymphon and Brenneis' et al. type one protonymphon. This stage displays morphological characteristics of an embryo.

**Keywords** *Pentapycnon charcoti* Bouvier; developmental stage; pycnogonidae.

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## 1 Introduction

The Pycnogonida are unique in that the male animals carry the fertilized eggs until they hatch and provide, if any, post hatching parental care. Most males have specialized appendages, termed ovigerous appendages, anterior to the first pair of walking legs which are modified to carry the fertilized eggs until they hatch. In some pycnogonids, the eggs are carried in a cocoon attached to the ovigerous appendages. There are many descriptions of pycnogonid larval stages in the literature extending back for over 130 years (Korschelt and Heider, 1889; Hedgpeth, 1947; Bain, 2003; Bogomolova and Malakhov, 2006; Bogomolova, 2007; Burris, 2011; Fornshell, 2014; 2015; 2017; Fornshell and Ferrari, 2012; Alexeeva et al., 2017; Brenneis et al., 2017; Mochizuki and Myazaki, 2017).

The first post hatching stage, of *Pentapycnon charcoti* Bouvier 1910, is described for the first time using material archived in the US National Museum of Natural History. The presence of five pairs of walking legs characterize the members of this genus. Historically this trait was sufficient to create a separate family (King, 1973). The genus *Pentapycnon* is placed in the family Pycnogonidae in more recent taxonomic systems (Clarke and Johnston, 2003).

## 2 Materials and Methods

Early developmental stages of the pycnogonid *P. Charcoti* (National Museum of Natural History accession number 397470) were collected from ovigerous males. At the time of collection the specimens were fixed in a formaldehyde solution and, after species determination by C. Allen Child 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 cheliphores to the posterior end of the animal. The photomicrographs were produced using a ScopePhoto MI-DC1300 digital camera Home Science Tools 665 Carbon Street, Billings MT59102 USA. The specimens in these images were in the original preservative, 70 ethyl alcohol.

## 3 Results

The eggs and first post hatching stage of *P. charcoti* are present in a cocoon attached to theovigerous appendages of the male. Each cocoon contains several hundred eggs and first post hatching stage animals (Fig. 1). When broken open, the cocoon contained a mixture of eggs and first post hatching stage animals.

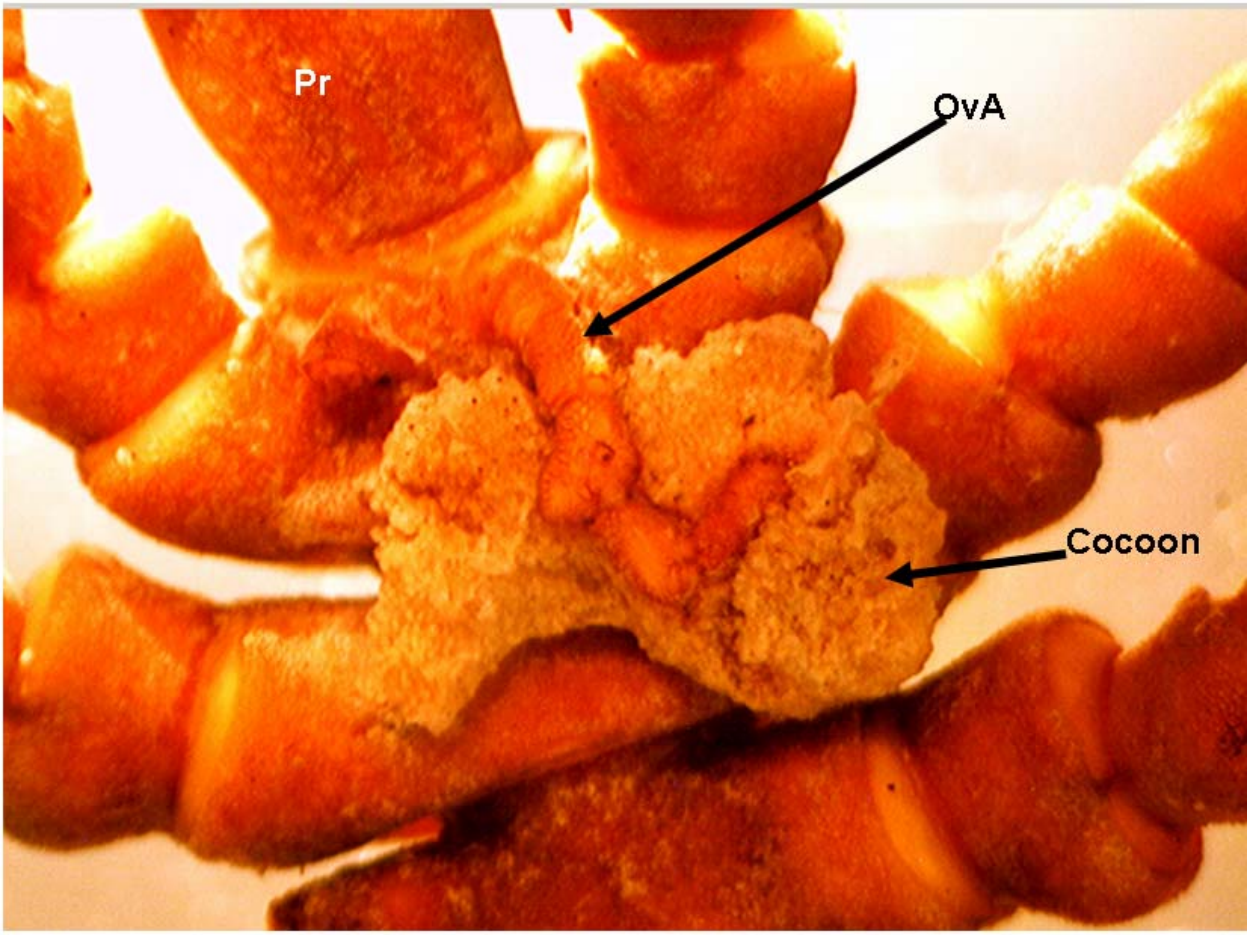


**Fig. 1** A ventral view of an adult male sea spider showing cocooncontaining the eggs and first post hatching stage. Pr = proboscis; OvA = ovigerous appendage

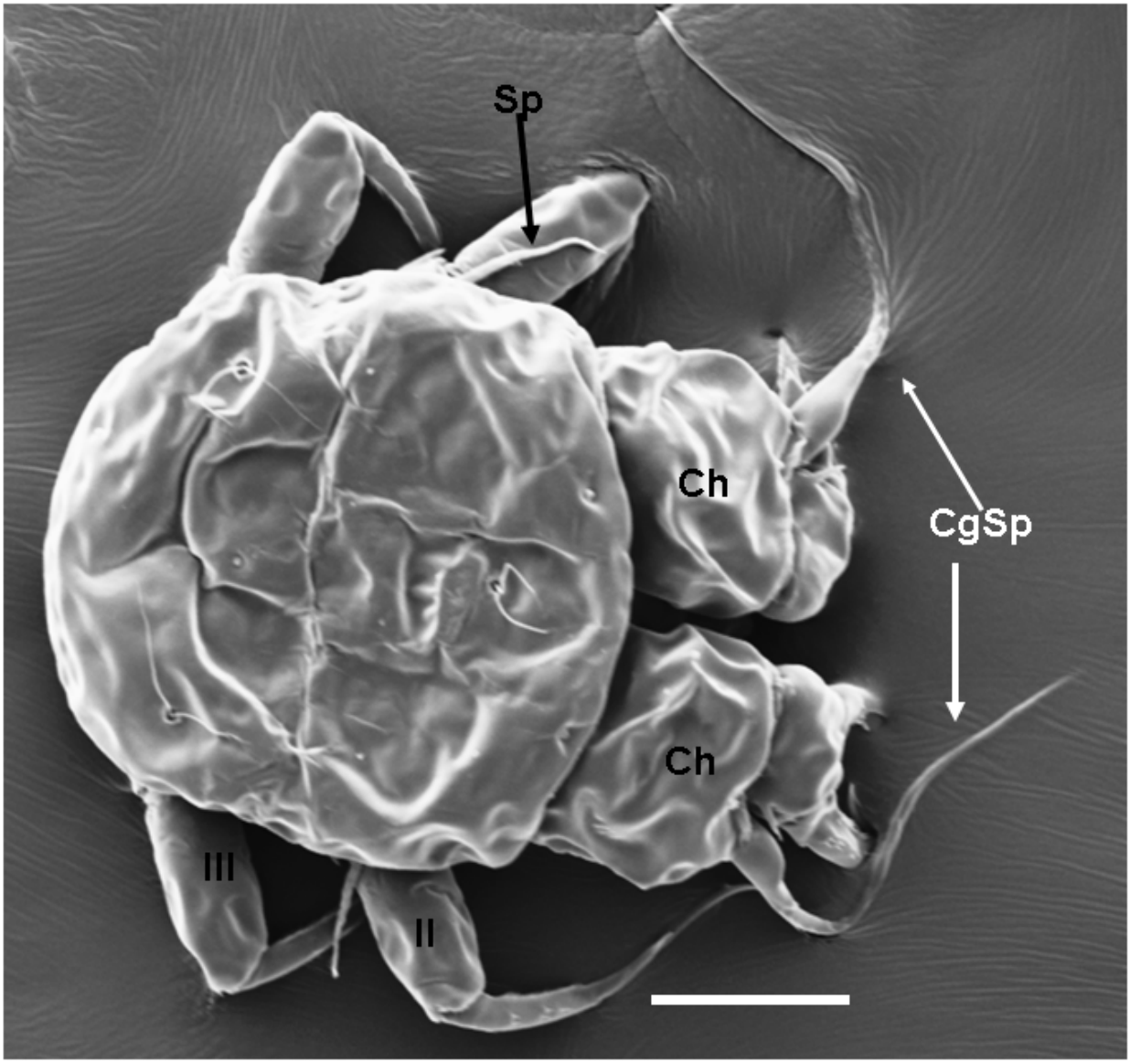


**Fig. 2** A dorsal view of the first post hatching stage of *Pentapycnon charcoti*. CgSp = Cement gland spine; Ch=cheliphores; II = second larval appendage or larval palps; III = third larval appendage or larval ovigerous appendage; Sp j-shaped spine on the second larval appendage. Scale Bar=100 µm.

The oval eggs measured 172 µm by 138 µm. The first post hatching stage is 141 µm long from the posterior edge to the base of the cheliphores. Dorsal and ventral views of the first post hatching stage are shown in Figs. 2 and 3 respectively. The body is also 141µm wide. The cheliphores are 60 µm long. A long curved spine arises from the distal end of the first segment of the cheliphores. In many pycnogonids this spine is associated with a cement gland which produces an attachment thread extending from a pore at the end of the spine. This attachment thread was not observed in the specimens used in this study.There is also a small spine arising from the base of the second segment of the cheliphores. The cheliphores are longer than the proboscis in the first stage. Three segmented larval appendages II and III are present. The first segment on each appendages II and III bears a fish-hook shaped spine at its distal end. The third segment is spine-like in shape and bears several short spines. The ventral organs interpreted as neurogenic niches of late embryonic and post-embryonic nervous system development are present (Brenneis et al., 2017).

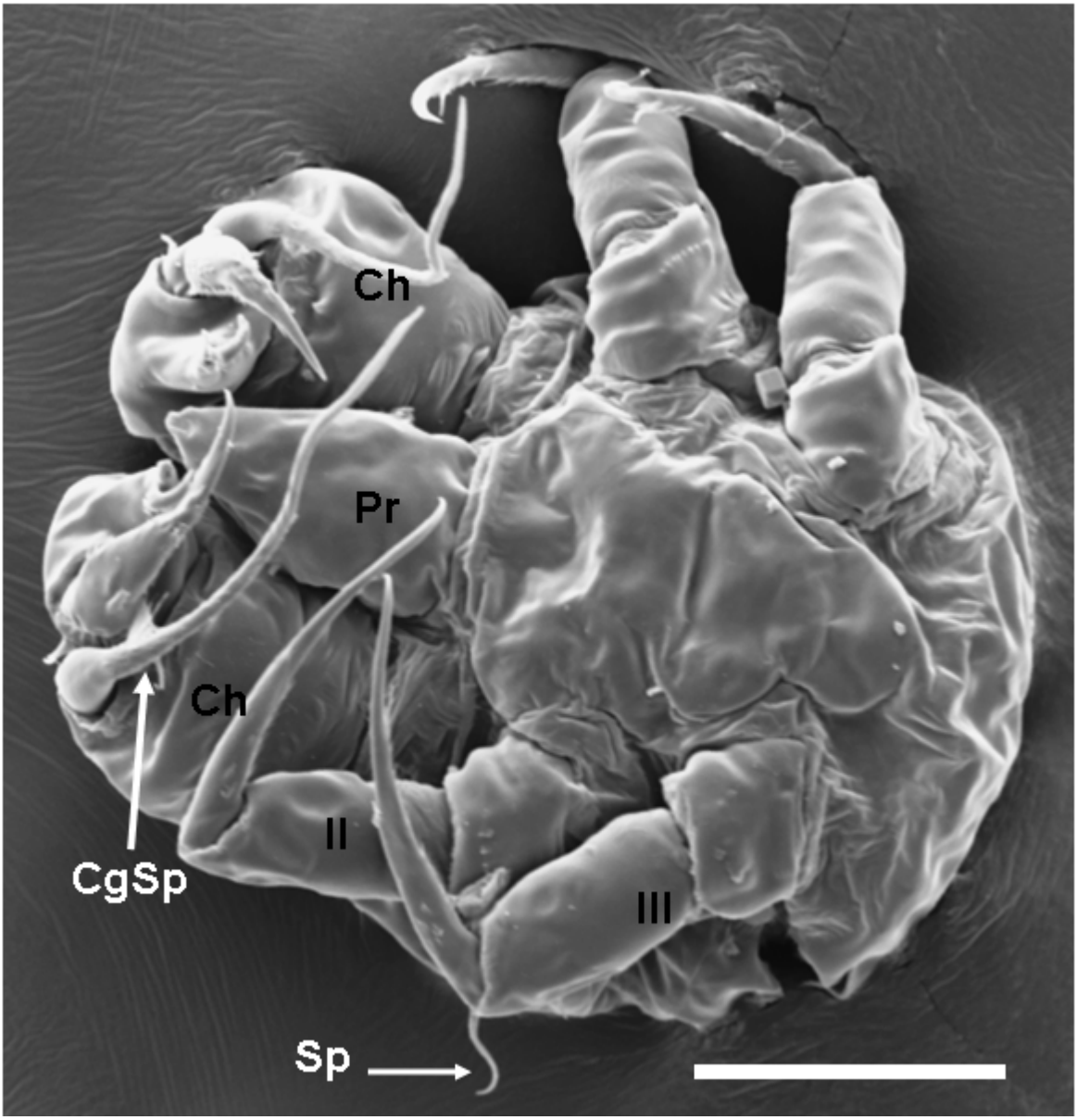


**Fig. 3** Ventral view of the first post hatching stage of *Pentapycnon charcoti*. CgSp=Cement gland spine; Pr-Proboscis; Ch = cheliphores; II = second larval appendage or larval palps; III = third larval appendage or larval ovigerous appendage; Sp j-shaped spine on the second larval appendage. Scale Bar=100 µm.

## 4 Discussion

Bain (2003) proposed a classification of pycnogonid development taking into account the following criteria: (1) the first postembryonic stage (a protonymphon or a so-called attaching larva); (2) the larvae's way of life; (3) the order of the initiation of the walking legs (sequentially one pair after another or two, three, or all four pairs simultaneously). Using these criteria, she described four developmental patterns: (1) The typical protonymphon, which hatches from the egg with the anterior three pairs of appendages. As development proceeds, one pair of walking legs are added at each molt and the anterior three pairs of limbs are transformed into the adult cheliphores, palps and ovigerous limbs. (2) The Encysted larva whose morphology 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. (3) The typical protonymphon in which buds of all four pairs of walking legs are present at the first stage as well as the anterior three pairs of appendages. Further development of the Atypical protonymphon larva takes place in the mantle cavity of mollusks or on sedentary polychaetes. (4) 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.

Brenneis et al. (2017) using three criteria, (1) type of the hatching stage, (2) developmental-morphological features during postembryonic development and (3) selected life history characteristics, described five developmental pathways. These five pathways are: Type 1: parasitic development with sequential differentiation of walking legs and less than 200 µm, Type 2: Lecithotrophic development with sequential differentiation of walking legs, Type 3: Ectoparasitic development with synchronous differentiation of walking legs, Type 4: Endoparasitic development with partially synchronous differentiation of walking legs, Type 5: Postembryonic development with hatching of an advanced post larva.

The first post hatching stage of *P. charcoti* is less than 200µm in length. The Cheliphores have secreting cells and a long spine on the distal end of the first segment. Larval appendages II and III are three-segmented with a spine on the proximal segment while the terminal segment is claw-like. The limb buds of the walking legs are not present. The first post hatching stage of *P. charcoti* corresponds to Bain's (2003) Typical Protonymphon and Brenneis' et al. (2017) Type 1 parasitic development.

Minelli et al. (2006) have presented evidence that in the Arthropoda the completion of embryonic development and the transition from the larval stage to the juvenile or adult stage may not be coincident with hatching and metamorphosis. This lack of synchronization between completion of embryonic development and hatching is seen in other chelicerates such as the horseshoe crab, *Limulus polyphemus* Linnaeus 1758, which molts four times before hatching (Packard, 1880; Botton et al., 2010). Also in the scorpion, *Centruroides vittatus* (Say, 1821), hatching occurs before embryonic development is complete (Farley, 2005). The first post hatching stage of *N. austral* has several traits resembling an embryo. These are as follows: (1) The absence of an eye tubercle; (2) The absence of an anus and proctodeum, indicating that the digestive tract is still in a rudimentary stage of development; (3) The central nervous system is still developing via invaginations of the ectoderm to form presumptive segmental ganglia of the ventral nerve cords. The first post hatching stage of *N. austral* may then represent a late embryonic stage in the development of this species.

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