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Larval development of the symbiotic pea crab *Pinnaxodes chilensis* (H. Milne Edwards, 1837) (Decapoda, Brachyura, Pinnotheridae) reared in laboratory

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ABSTRACT

The complete larval development of *Pinnaxodes chilensis* (including four zoeal stages and a megalopa stage) is described and illustrated in detail for the first time. The descriptions are based on laboratory-reared larvae obtained from ovigerous females found inside specimens of the sea urchin *Loxechinus albus* collected in the coast of Valparaíso, Chile. In order to allow the correct differentiation of specimens from plankton samples, the larval stages of *P. chilensis* are compared with those from other Pinnotheridae species, whose larval development is known for the Chilean continental waters (*Calyptraeotheres politus*). The morphological characters described for *P. chilensis* larvae, as well as the comparison with the remaining larval development descriptions available for the genus *Pinnaxodes*, are used to discuss the heterogeneity within this genus.

1. Introduction

The brachyuran family Pinnotheridae De Haan, 1833, comprises about 300 species (updated from Ng et al., 2008 by Palacios-Theil et al., 2016) ascribed to 51 recognized genera. However, only 16.7% of the Pinnotheridae species have been studied regarding their larval development (Clark and Cuesta, 2015). In the Chilean coastal waters there are 7 species belonging to this family (Retamal and Moyano, 2010): *Calyptraeotheres politus* (Smith, 1870), *Holothuriophilus pacificus* (Poeppig, 1836), *Pinnaxodes chilensis* (H. Milne Edwards, 1837), *Pinnixa bahamondei* Garth, 1957, *Pinnixa chiloensis* Garth, 1957, *Pinnixa transversalis* (H. Milne Edwards and Lucas, 1842), and *Pinnixa valdiviensis* Rathbun, 1907. *Pinnotherelia laevigata* H. Milne Edwards and Lucas, 1843, was removed from the previous list due to its exclusion from the Superfamily Pinnotheroidea by Palacios-Theil et al. (2016). Despite the number of Pinnotheridae species described for Chilean waters, the description of the complete larval series is known for just one species: *Calyptraeotheres politus* (described as *Pinnotheres politus* by Saelzer and Hapette (1986)). Besides some descriptions of the adult biology and ecology, and some notes on morphological data of the first zoeal stage, the larval morphology of *P. chilensis* is almost unknown (Schwabe, 1936; Gutierrez-Martinez, 1971; Lardies and Castilla, 2001; Vasquez and Bay-Schmith, 2011; Runil, 2014).

Pinnaxodes chilensis is a symbiotic pinnotherid crab of the sea urchins *Loxechinus albus* (Molina, 1782), *Caenocentrotus gibbosus* (Agassiz and Desor, 1846) and *Tetrapygus niger* (Molina, 1782) (Garth, 1948; Haüsserman and Försterra, 2009). Its geographical distribution spans from coastal waters of Ecuador to Chile, including adjacent islands (Lardies and Castilla, 2001). The female, with a size up to 20 mm of cephalothorax length, lives alone inside the echinoderm, more specifically in the rectum. In contrast, the male, smaller in size (7 mm), moves freely in the benthos and, during the reproductive season, enters through the anal orifice of the sea urchin to visit the female (Gutierrez-Martinez, 1971). In the Chilean coast the proportion of *L. albus* that houses a female of *P. chilensis* living in solitude reaches 90% (Schwabe, 1936). Both, sea urchin and crab, are of economic interest as they are considered a delicacy by the consumers (Vásquez and Bay-Schmith, 2011).

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In this paper we thoroughly describe and illustrate the complete larval development (four zoeal stages and the megalopa) of the pinnotherid crab *P. chilensis*. We also perform a comparison with larval descriptions published for other species of the genus for which the complete larval development has been described, namely *Pinnaxodes mutuensis* Sakai, 1939 and *Pinnaxodes major* Ortmann, 1894 (Hong, 1974; Konishi, 1981). An additional comparison is made with *C. politus*, the single species recorded in the area for which the larval development is known. As proposed by Palacios-Theil et al. (2016), and given the lack of phylogenetic knowledge based on DNA analysis of the genus *Pinnaxodes*, we also performed a comparison between species related to the subclade Pinnotherinae II.

2. Materials and methods

Ovigerous females of *Pinnaxodes chilensis* were recovered from the rectum of specimens of the sea urchin *Loxechinus albus* collected in El Quisco (33°23′S, 71°42′W), Valparaíso (central coast of Chile) in September 2013. The animals were placed in 1 L plastic bowls containing filtered seawater without aeration, maintained at 16 °C. Females were kept in dark conditions due to their sensitivity to light, and under starvation until the eggs hatched. A total of 171 zoeae hatched a month and a half later from one of the females. Larvae showed low activity, and were transferred to several bowls with soft aeration for mass culture. They were fed *ad libitum* with a mix of the rotifer *Brachyonus plicatilis* (only for first zoea), *Artemia* sp. nauplii, *Algamac 200*® and sea urchin embryos. Cultures were checked daily for exuviae and mortality control and seawater was changed every two days. Exuviae and specimens of all stages were fixed in formaldehyde (4%) for further examination.

Dissections, drawings and measurements were made following the methodology described in previous studies by the authors (for details see Marco-Herrero et al., 2012, 2014). The morphological measurements performed in zoeal stages (Z I–Z IV) were: rostro-dorsal length (RDL) measured from the tip of the rostral spine to the tip of the dorsal spine; cephalothorax length (CL) measured from frontal margin (between the eyes) to the posterolateral cephalothoracic margin; cephalothoracic rostral spine length (RL) measured as the distance from the base to the tip of the rostral spine; and cephalothoracic dorsal spine length (DL) as the distance from the base to the tip of the dorsal spine. For the megalopa (M), morphological measurements included: cephalothorax length (CL) measured from the frontal to the posterior margin of the cephalothorax; and cephalothorax width (CW) as the cephalothorax maximum width.

The first zoeal stage was fully and thoroughly described following the taxonomic guidelines of Clark and Cuesta (2015), so only the main differences observed in subsequent stages were noted.

Parental females and the full series of laboratory-reared larval stages of *P. chilensis* were deposited at the *Museo Nacional de Ciencias Naturales* (Madrid, Spain) (number pending).

3. Results

3.1. Culture results

From the 171 larvae that emerged from an ovigerous *P. chilensis* female, 8 were taken for collection, and the remaining 163 larvae were used to perform the culture. The different larval stages appeared in the following order from hatching: Z II, between 5 and 13 days; Z III, between 11 and 23 days; Z IV, between 16 and 27 days; megalopa (M), between 22 and 34 days. The mean duration of each stage was: Z I, 6.5 days; Z II, 7 days; Z III, 8.5 days; Z IV, 8 days; M, 10.5 days. The culture ended with the first crab stage, which occurred 31 days after hatching (Fig. 1). The main loss of individuals (63%) was recorded for the first zoea. Approximately 7% of the initial amount of larvae (first zoea) reached the first crab stage.

3.2. Larval descriptions

***Pinnaxodes chilensis* (H. Milne Edwards, 1837)**

Zoea I (Figs. 2A, B, 3A, D, I, 4A, D, 5A, C, 6A)

Size: RDL = 1.628 ± 0.039 mm; CL = 0.556 ± 0.012 mm; RL = 0.315 ± 0.010; DL = 0.521 ± 0.013; $n = 10$.

Cephalothorax (Fig. 2A, B): Spherical, wrinkled, without tubercles. Dorsal spine straight and well developed, a bit longer and thick than rostral and lateral spines, with small parallel tubercles. Rostral spine slightly curved towards the ventral margin. Lateral spines directed downwards near the posterior angle of the ventral margin. Three pairs of spiniform setae on the cephalothorax: 1 pair located at the base of the dorsal spine, 1 seta at the base of each lateral spine and 1 pair behind the dorsal spine, on the posterior margin, all of them simple setae. Three small setae at the postero-ventral margin. Eyes sessile.

Antennule (Fig. 3A): Unsegmented and conical. Endopod absent. Exopod with 2 aesthetascs and 1 short simple seta.

Antenna (Fig. 3D): Protopodal process serrated. Endopod absent. In most cases one serrulate seta located at the base of the protopodal process.

Mandible (Fig. 3I): Well-developed, incisor and molar process developed. Palp absent.

Maxillule (Fig. 4A): Coxal endite with 3 plumodenticulate setae and microtrichia setae at the ventral margin. Basial endite with 5 plumodenticulate setae. Endopod 2-segmented, proximal segment without setae and with 4 terminals (2 + 2) sparso-plumose setae on distal segment. Epipod and exopod setae absent.

Maxilla (Fig. 4D): Coxal endite single-lobed, with 5 setae (2 simple + 3 plumodenticulate) and microtrichia setae at the dorsal margin. Basial endite bilobed, with 9 plumodenticulate (5 + 4) setae. Unsegmented endopod bilobed, with 1 long slightly plumodenticulated seta on proximal lobe, and 2 long slightly plumodenticulated setae on distal lobe, microtrichia on both lobes. Exopod (scaphognathite) with 4 plumose setae plus one stout plumose process.

First maxilliped (Fig. 5A): Coxa without setae, although occasionally one of the maxillipeds presents 1 simple seta. Basis with 10 simple setae (2 + 2+3 + 3). Endopod 5-segmented, with 2,2,1,2,5 (1 subterminal + 4 terminal) setae. Exopod unsegmented, with 4 terminal plumose natatory setae.

Second maxilliped (Fig. 5C): Coxa without setae. Basis with 4 simple setae (1 + 1+1 + 1). Endopod two-segmented, with 0, 5 (1 serrated subterminal + 4 simple terminal) setae. Exopod unsegmented, with 4 terminal plumose natatory setae.

Third maxilliped: Absent.

Pereiopods: Absent, or not clearly defined under the cephalothorax.

Pleon (Fig. 6A): Composed by 5 pleonites. Pleonite I without seta. Pleonite II–V with pair of spiniform setae on posterodorsal margin. Pleonite II with a pair of forwardly directed dorsolateral knobs. Pleonite III with a pair of dorsolateral knobs laterally directed.

Pleopods: Absent.

Telson (Fig. 6A): Usually its length doubles the length of pleonite V. Bifurcated, with 3 pairs of serrulate setae on furcal arch margin. Furcal arch with a middle notch, and a minute distal spine at dorsal margin on each furca. Furcas covered with setulae distally.

Zoea II (Figs. 2C, 3B, E, 4B, E, 6B)

Size: RDL = 1.935 ± 0.076 mm; CL = 0.568 ± 0.021 mm; RL = 0.429 ± 0.013; DL = 0.675 ± 0.034; $n = 10$.

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