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ORIGINAL ARTICLE

Larval development of *Calyptraeotheres garthi* (Fenucci, 1975) (Brachyura, Pinnotheridae) described from laboratory-reared material, with notes of larval character use on Pinnotheridae systematic

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Abstract *Calyptraeotheres garthi* (Brachyura, Pinnotheridae) is a pinnotherid crab that lives in association with some species of *Crepidula* sp. (Calyptraeidae). The larvae of *C. garthi* were cultured under laboratory conditions (20°C and 35 PSU). Larval development from hatchling to megalopa took 30 days. Five zoeal stages and the megalopa are described and fully illustrated. The genus *Calyptraeotheres* consists only of four species. Larval characters were compared with the previous description of larvae from the southeastern Pacific species *C. politus* and with *Tumidotheres maculatus* a closely related pinnotherid crab. In addition, in light of recent molecular phylogeny of the family Pinnotheridae, we present a discussion about the *Calyptraeotheres* and its close related genera.

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Introduction

The family Pinnotheridae is composed of approximately 304 species of small-sized crabs (De Grave et al. 2009) most of them living in association with many benthic invertebrates like mollusks, ascidians, annelids, other crustaceans, echiurans, or echinoderms (Schmitt et al. 1973). With the exception of *Tunicotheres moseri* (Bolaños et al. 2004), the larvae of pinnotherids are free-living and the number of zoeal stages ranges from 2 to 5. The genus *Calyptraeotheres* Campos, 1990 consists of four species: *Calyptraeotheres garthi* Fenucci, 1975 from southwestern Atlantic, *C. hernandezi* Hernández-Ávila and Campos, 2006 from Cubagua Island, Venezuela, *C. granti* Glassell, 1933, and *C. politus* Smith, 1870 from the eastern and southeastern Pacific, respectively. All of these species are obligatory symbiont of slipper limpets of the molluscan family Calyptraeidae. Up to day, only the larval development of *C. politus* has been formally studied (Saelzer and Hapette 1986).

Phylogeny and classification of the pinnotherids have been the aim of many revisions based on adults and larvae morphology and DNA sequences (Ahyong and Ng 2007; Campos 1996a, b, 2009; Griffith 1987; Marques and Pohle 1995; Palacios-Theil et al. 2009; Pohle and Marques 1998). Particularly, the genera *Calyptraeotheres* and *Tumidotheres* have been the focus of debate. Upon larval morphological characters, Marques and Pohle (1995) suggested the species *Pinnotheres politus* (now *Calyptraeotheres politus*) should be included in the genus *Tumidotheres*. Then, throughout morphology of adult crabs, Campos

(1999) transferred the pinnotherid species, which lives as obligatory symbiont of slipper limpet (family Calyptraeidae), to the genus *Calyptraeotheres*. Finally, the phylogeny made on the basis of mitochondrial genes shows a closer relationship between the genera *Tumidotheres* and *Calyptraeotheres* (Palacios-Theil et al. 2009).

In the present study, the larval development of *Calyptraeotheres garthi* from Las Grutas, Río Negro (Argentina), is described and illustrated. In addition, an exhaustive comparison is made with not only *C. politus* but also with *Tumidotheres maculatus* in order to discuss the systematic position of these genera. Moreover, in light of recent molecular phylogeny of the family Pinnotheridae (Palacios-Theil et al. 2009), we presented larval remarks about *Calyptraeotheres* and its close related genera.

Materials and methods

Individuals of the host *Crepidula* sp. were obtained from the commercial fleet near to Piedras Coloradas (40°57′ S, 65°04′ W), Río Negro, Argentina, on March 23, 2009. The limpets were transported to IBMP (Instituto de Biología Marina y Pesquera, Almirante Storni), and 12 of them were placed individually in aquaria with 1-μm filtered seawater. In San Matías Gulf, the prevalence of *C. garthi* is around 80%, so we preferred to cultivate hosts in order to avoid the stress that crabs would suffer when taken off. The larvae of four incubating females started to hatch on March 29 and were transferred to individual beakers of 25 ml capacity. Natural seawater was used at a temperature of 20°C and salinity of 35 PSU. Larvae were cultured under artificial light regime: 12/12 h (L/D). The first, second, and third zoeae were fed with a mixed culture of algae *Nannochlorophis oculata/Tethraselmis suecica* and rotifers *Brachionus plicatilis* ad libitum, and the fourth and fifth zoeae were fed with a mix of rotifers and nauplii of *Artemia salina*. Water and food were changed every day. In addition, the *Artemia salina* was reared with antibiotic-treated seawater (Cloranfenicol, 50 μg/ml). Several individuals of each stage were preserved in 4% formaldehyde.

Specimens were dissected under a Zeiss Axio Scope 2 stereomicroscope. Measurements and drawings were made using an Olympus SZ-SC compound microscope equipped with a *camera lucida*. Drawings in zoea were based on 5 larvae per stage and measurements on 10 larvae. Drawings and measurements in megalopa were based on 5 individuals. In zoea, rostrodorsal length (RDL) was measured from the rostral spine tip to the dorsal spine tip; carapace length (CL) from the base of the rostrum to the posterior margin; carapace width (CW) as the distance between the tips of the lateral spines. In megalopa, carapace CL was measured from of rostrum to the posterior margin and the CW as the maximum width of the carapace. In addition, for the first and fourth zoeae, two illustrations have been shaded in order to show the animals aspect as natural as possible.

Descriptions were arranged according to the standard proposed by Clark et al. (1998). The different kinds of setae were named *sensu* Bookhout and Costlow (1974, 1977) and Pohle and Telford (1981). The zoea I and megalopa were completely described here, and for the rest of zoeae, only differences with precedents stages were detailed. Samples of larvae and the adult female were deposited in the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" under the catalog number MACN-IN 37722.

Results

The larvae development of *Calyptraeotheres garthi* took place through five zoeal and one megalopa stages. Six zoeae from a single female reached the megalopal stage, in at least 30 days after hatching. Larvae of the three other females failed to reach the megalopa. In the Table 1 are represented the mean sizes and first day of appearance of each stage. These data were based on the larvae of the hatch, which reached the megalopa.

Zoea I

Carapace (Fig. 1a, b) Globose and smooth, without tubercles. Rostral and dorsal spines well developed. Lateral spines prominent and slightly curved downwards. One pair of simple posterodorsal setae flanking dorsal spine. Eyes sessile. Each ventroposterior margin without setae.

Antennule (Fig. 1c) Uniramous. Endopod absent. Exopod unsegmented with 2 aesthetascs (1 long, 1 shorter and thinner than the first one) and 1 seta.

Table 1 Mean (±SD) rostrodorsal length (RDL), carapace width (CW), carapace length (CL) in mm, and first day appearance of the larval stages of *Calyptraeotheres garthi* reared in the laboratory

Estadio	RDL	CW	CL	1st day to appear
Zoea I	1.03 ± 0.07	0.51 ± 0.05	0.43 ± 0.01	0
Zoea II	1.33 ± 0.05	0.61 ± 0.07	0.48 ± 0.02	5
Zoea III	1.74 ± 0.11	0.71 ± 0.10	0.59 ± 0.03	10
Zoea IV	2.03 ± 0.15	0.91 ± 0.11	0.68 ± 0.04	14
Zoea V	2.24 ± 0.25	0.99 ± 0.13	0.73 ± 0.09	22
Megalopa	–	0.73 ± 0.03	0.80 ± 0.03	30

All measures were taken from the larvae of the hatch that reached the megalopa

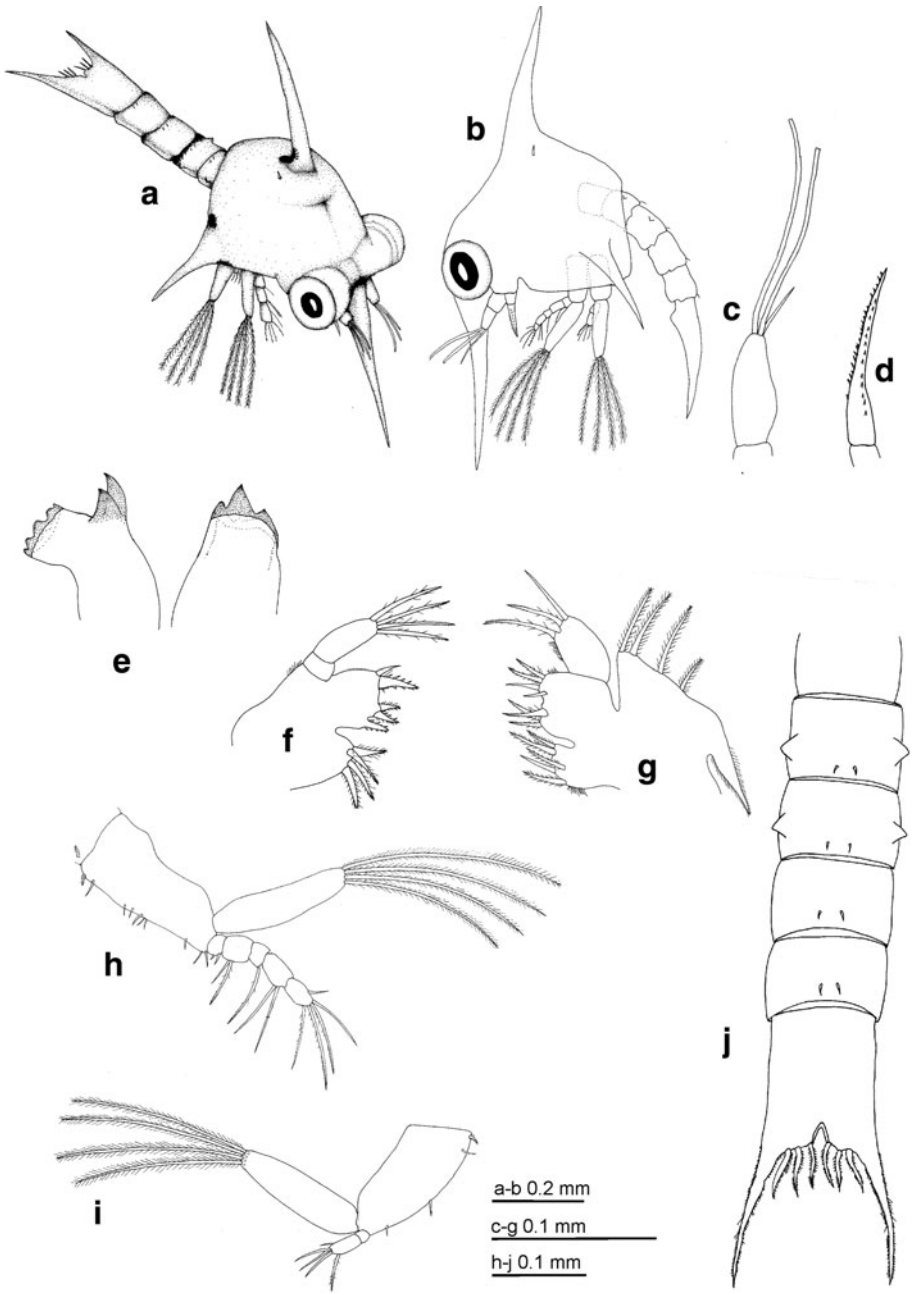

Fig. 1 *Calyptraeotheres garthi*. Zoea I. **a** General view, **b** lateral view, **c** antennule, **d** antenna, **e** mandibles, **f** maxillule, **g** maxilla, **h** first maxilliped, **i** second maxilliped, **j** abdomen, dorsal view

Antenna (Fig. 1d) Uniramous. Protopod with spinous process armed with spines arranged in two lines. Endopod and exopod absent.

Mandible (Fig. 1e) With circular serrated molar region and one incisor processes with 2–3 flanking lateral tooth. Cuticle brown distally. Palp absent.

Maxillule (Fig. 1f) Coxal endite with 4 plumodenticulate setae. Basial endite with 5 setae, 1 simple, 1 plumodenticulate and 3 plumodenticulate cuspidate. Endopod 2-segmented, proximal segment without setae and distal segment with 4 terminal plumodenticulate setae. Exopod setae absent.

Maxilla (Fig. 1g) Coxal endite simple lobed with five setae (4 plumose and 1 simple). Basial endite weakly bilobed with 3 plumodenticulate setae + 4 (2 plumodenticulate and 2 simple) setae. Endopod slightly bilobed with 3 setae, 1 of them simple and the others 2 plumodenticulate. Scaphognathite with 4 marginal densely plumose setae and a long posterior process.

First maxilliped (Fig. 1i) Coxa with 1 plumose seta. Basis with 10 setae arranged in four groups: 2 (1 plumose, 1 simple), 2 simple, 2 simple, and 3 simple. Endopod 5-segmented with 2 simple, 2 (1 simple, 1 plumodenticulate), 1 plumodenticulate, 2 simple, 5 (1 lateral simple, 3

terminal plumodenticulate, and 1 terminal simple) setae. Exopod unsegmented, with 4 long terminal natatory plumose setae.

Second maxilliped (Fig. 1h) Coxa without setae. Basis with 4 setae: 2 proximal (1 simple, 1 sparcely plumose), 1 medial simple and 1 distal simple setae. Endopod 2-segmented, proximal segment without setae, distal segment with 1 subterminal plumodenticulate, 1 short subterminal simple, and 3 terminal simple setae. Exopod unsegmented with 4 long terminal natatory plumose setae.

Third maxilliped Absent.

Pereiopods Absent.

Abdomen (Fig. 1j) Five abdominal somites. Somites 2–5 with pair of posterodorsal simple setae. Somites 2–3 with pair of dorsolateral processes.

Telson (Fig. 1j) Smooth, bifurcated, and elongate. Furcal arms with small spines on lateral margins. Furcal arch with three plumodenticulate setae on either side of distinct median depression.

Zoea II

Carapace (Fig. 2a) Two pairs of simple setae on dorsolateral border of the eyes and halfway between eyes and dorsal spine. Each ventroposterior margin with 1 pair of

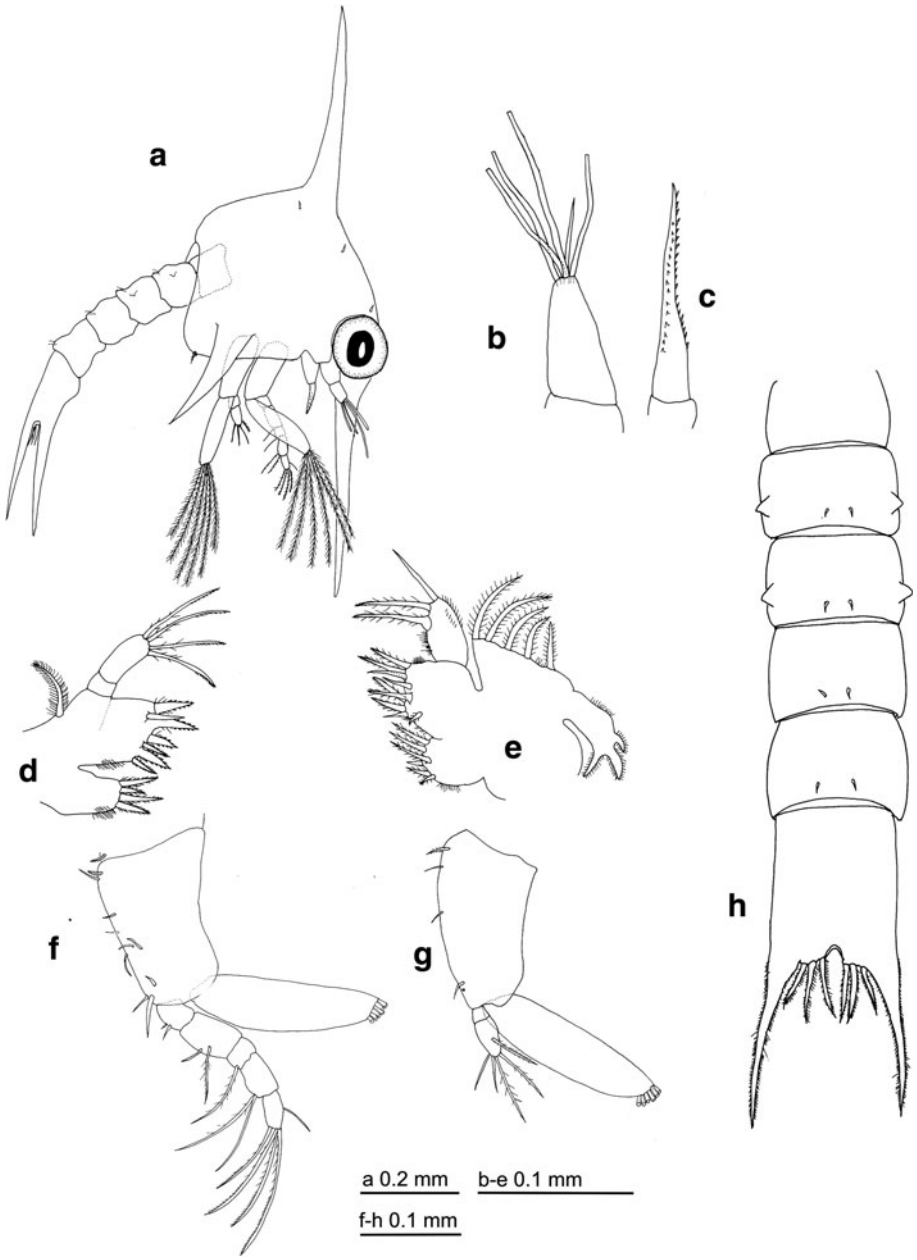

Fig. 2 *Calyptraeotheres garthi*. Zoea II. **a** Lateral view, **b** antennule, **c** antenna, **d** maxillule, **e** maxilla, **f** first maxilliped, **g** second maxilliped, **h** abdomen, dorsal view

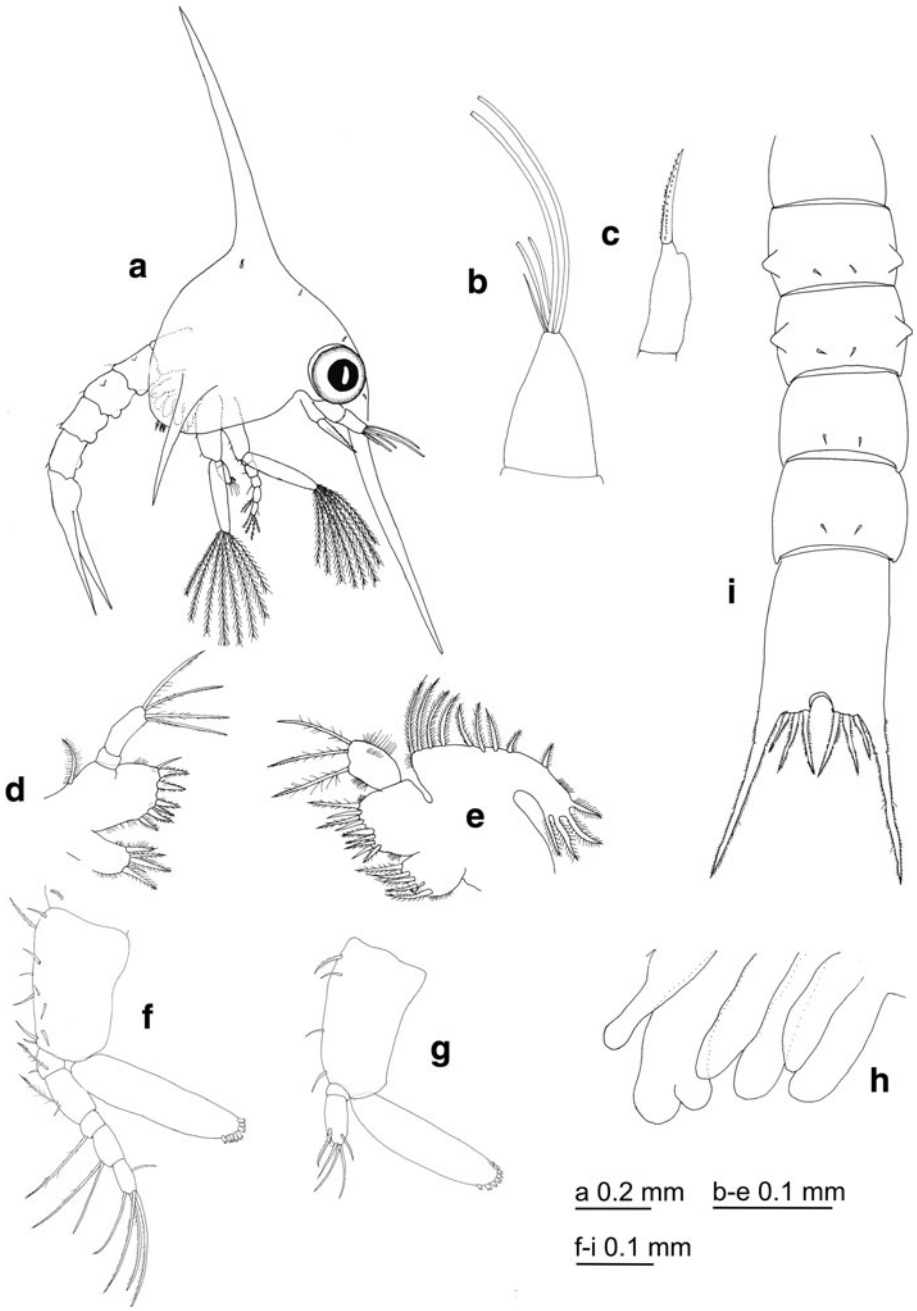

Fig. 3 *Calyptraeotheres garthi*. Zoea III. **a** Lateral view, **b** antennule, **c** antenna, **d** maxillule, **e** maxilla, **f** first maxilliped, **g** second maxilliped, **h** developing third maxilliped and pereiopods, **i** abdomen, dorsal view

plumodenticulate setae. Eyes stalked. *Antennule* (Fig. 2b). Exopod now with 4 terminal aesthetascs (1 slightly longer than the others).

Maxillule (Fig. 2d) Basial endite now with 7 setae: 1 simple, 2 plumodenticulate and 4 plumodenticulate cuspidate. Exopod seta now present.

Maxilla (Fig. 2e) Coxal endite with 5 plumose and 1 short simple setae. Basial endite with 5 (4 plumodenticulate and 1 short simple) and 4 (2 plumodenticulate, 2 simple) setae. Scaphognathite bearing 8 marginal densely plumose setae.

First maxilliped (Fig. 2f) Endopod now with 2 (1 simple, 1 sparsely plumose), 5 (1 lateral simple, 4 terminal plumodenticulate) setae on the segments 4 and 5, respectively. Exopod now with 6 long terminal natatory plumose setae.

Second maxilliped (Fig. 2g) Exopod bearing 6 long terminal natatory plumose setae.

Third maxilliped Absent.

Pereiopods Absent.

Zoea III

Carapace (Fig. 3a) Additional pair of simple setae on the basis of rostral spine. Three plumodenticulate setae on ventroposterior margin.

Antennule (Fig. 3b) Exopod with 2 long and 2 thin and short aesthetascs.

Antenna (Fig. 3c) With rounded, undifferentiated endopodite bud reaching of the distal portion of protopodite.

Maxillule (Fig. 3d) Coxal endite with 4 plumodenticulate and 1 small simple setae.

Maxilla (Fig. 3e) Scaphognathite now with 13 marginal densely plumose setae.

First maxilliped (Fig. 3f) Endopod now bearing 1 simple and 1 plumodenticulate setae on the first segment. Exopod now with 8 long terminal natatory plumose setae.

Second maxilliped (Fig. 3g) Exopod with 8 long terminal natatory plumose setae.

Third maxilliped (Fig. 3h) Present as undifferentiated bud.

Pereiopods (Fig. 3h) Present as undifferentiated buds. Chela slightly bilobed.

Abdomen (Fig. 3i) Pleopod buds present but without endopods.

Zoea IV

Carapace (Fig. 4a, b) 6 plumodenticulate setae on ventroposterior margin.

Antennule (Fig. 4c) Biramous. Small endopod bud. Exopod with 4 aesthetascs (3 long and 1 thin and short) and 2 setae. Optionally some aesthetascs bear minute spines on the tip.

Antenna (Fig. 4d) Endopodite bud about half length of the protopodal process.

Maxillule (Fig. 4e) Coxal endite with 5 plumodenticulate setae. Basial endite with: 2 simple, 3 plumodenticulate and 4 plumodenticulate cuspidate setae. A short plumose seta is present on the lateral margin.

Maxilla (Fig. 4f) Basial endite with 5 (4 plumodenticulate, 1 simple), 5 (3 plumodenticulate, 2 simple) setae. Scaphognathite with 17 marginal densely plumose setae.

Second maxilliped (Fig. 4h) Exopod with 10 long terminal natatory plumose setae.

Third maxilliped (Fig. 4i) Biramous. Exopod and endopod present as undifferentiated buds.

Pereiopods (Fig. 4i) Longer. Chela differentiated.

Abdomen (Fig. 4j) Pleopods longer, unsegmented and uniramous.

Zoea V

Carapace (Fig. 5a, b). Additional pair of simple setae between the eyes. 7 plumodenticulate setae on ventroposterior margin.

Antennule (Fig. 5c) Biramous. Endopod bud developing. Exopod now with 6 aesthetascs, 5 terminal and 1 subterminal. 1 aesthetasc thin and short. Minute sipines on tip of aesthetascs. 3 subterminal setae.

Antenna (Fig. 5d) Endopod now as long as protopodite.

Maxillule (Fig. 5f) Basial endite now with 2 simple, 4 plumodenticulate and 4 plumodenticulate cuspidate setae. On the lateral margin, 2 small setae (1 plumose and 1 simple).

Maxilla (Fig. 5g) Coxal endite with 7 setae (6 plumose and 1 simple). Basial endite with 6 and 6 setae, 4 plumodenticulate and 2 simple in each lobe. Scaphognathite now with 19-21 marginal densely plumose setae.

First maxilliped (Fig. 5h) Coxa with 1 additional plumose setae. Exopod with 9 long terminal natatory plumose setae.

Third maxilliped (Fig. 5j) Elongated. Now epipodite bud present.

Pereiopods (Fig. 5j) Chelipeds and pereiopods slightly segmented.

Abdomen (Fig. 5k) Pleopod 2-segmented and elongated, with rudimentary endopods.

Megalopa

Carapace (Fig. 6a) Slightly longer than broad. Rostrum ventrally deflected (approximately 60°). Each lateral margin with 9 plumodenticulate setae. Disposition of simple setae as figured. Eyes stalked.

Antennule (Fig. 6b) Peduncle 3-segmented with 0, 1 and 2 simple setae, respectively. Endopod 2-segmented with 1 subterminal and 3 terminal simple setae on distal segment. Exopod 4-segmented with 0, 2, 7 and 3 (1 terminal and 2 subterminal) aesthetascs, respectively.

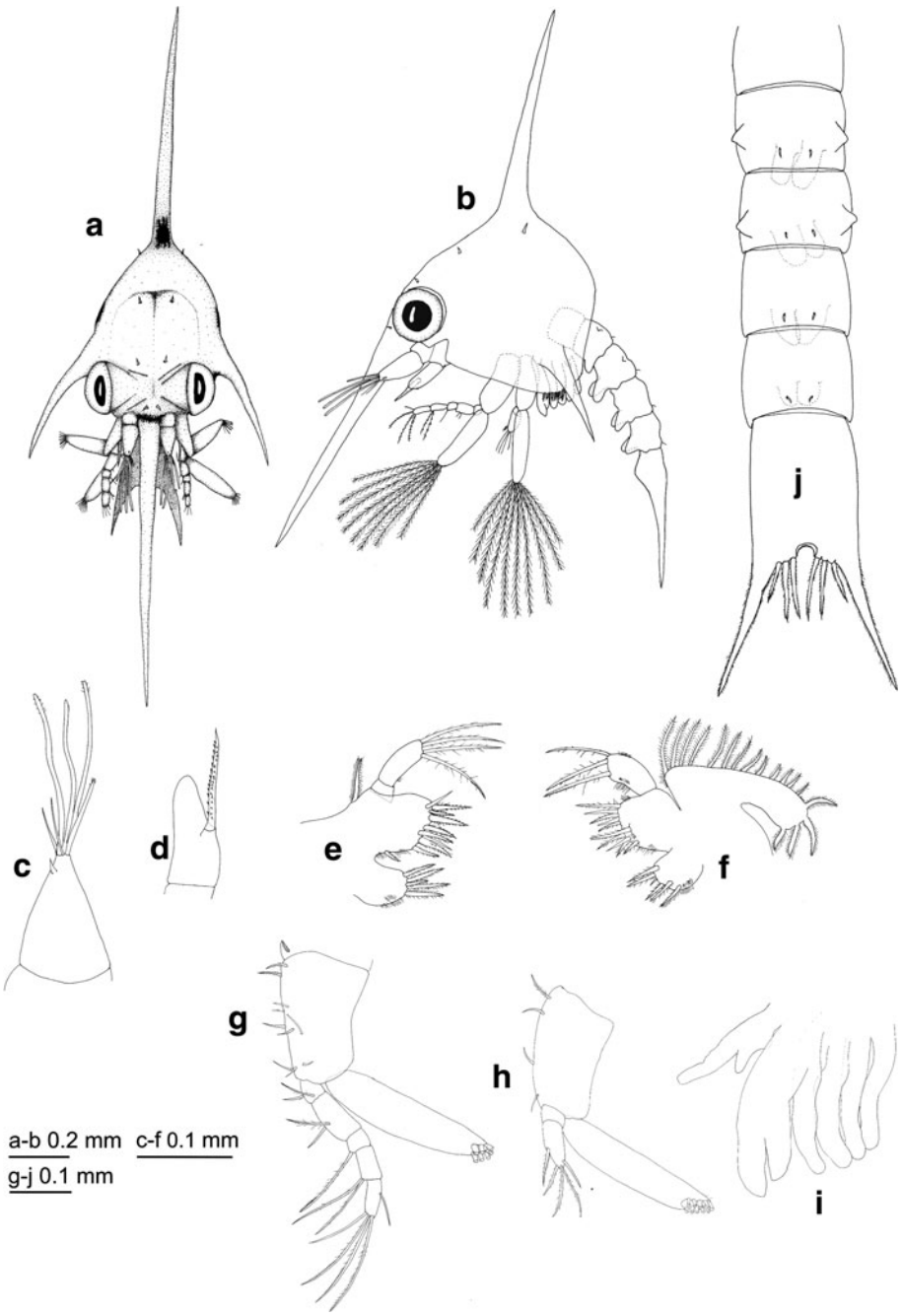

Fig. 4 *Calyptraeotheres garthi*. Zoea IV. **a** Frontal view, **b** lateral view, **c** antennule, **d** antenna, **e** maxillule, **f** maxilla, **g** first maxilliped, **h** second maxilliped, **i** developing third maxilliped and pereiopods, **j** abdomen, dorsal view

Antenna (Fig. 6c) 6-segmented with 3 long setae on subdistal segment and 2 setae (1 long and 1 reaching at the middle of this) on terminal segment. Basal segment with 1 small seta.

Mandible (Fig. 6d) Unsegmented palp present. Scoop-shaped, smooth incisor process with thin cutting edge. Molar process with 1 well-developed and 3 flanking lateral minor tooth.

Maxillule (Fig. 6e) Coxal endite with 11–12 plumodenticulate setae, 1 basally on lateral margin. Basial endite with: 7–8 denticulate, 8 denticulate cuspidate setae and, on the lateral margin, 2 short plumose setae. Basally to the exopod seta, appear 1 plumodenticulate seta (may be the epipodal seta). Endopod 2-segmented without setae.

Maxilla (Fig. 6f) Coxal endite unlobed with 8 setae, 7 plumose and 1 simple. Basial endite slightly bilobed with 5 and 5 plumodenticulate setae. Endopodite reduced and without setae. Scaphognathite with 29-31 marginal densely plumose setae.

First maxilliped (Fig. 7a) Coxal endite with 1 plumose and 3 plumodenticulate setae. Basial endite with 3 plumodenticulate setae. Endopod unsegmented ending in a

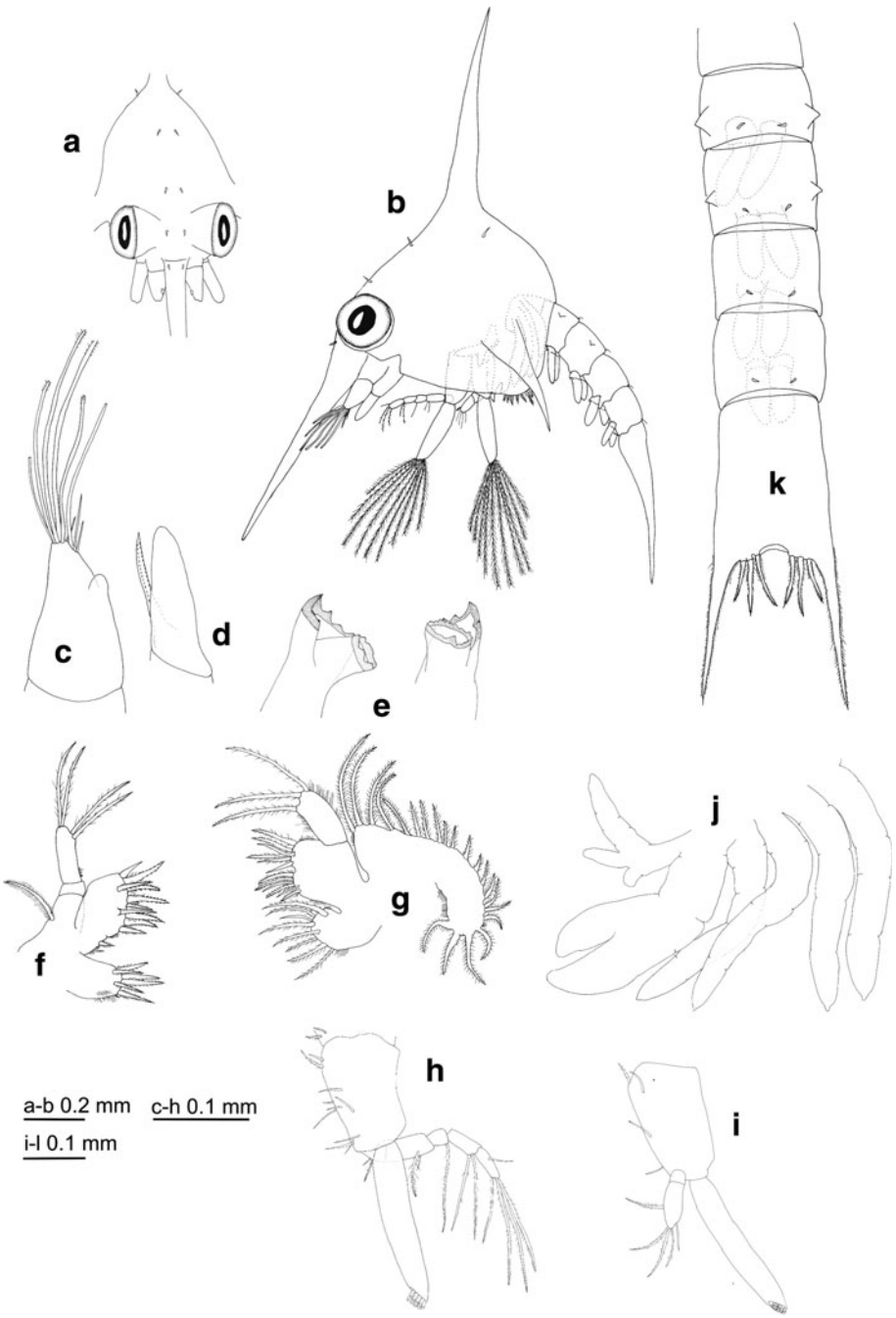

Fig. 5 *Calyptraeotheres garthi*. Zoea V. **a** Frontal view, **b** lateral view, **c** antennule, **d** antenna, **e** mandibles, **f** maxillule, **g** maxilla, **h** first maxilliped, **i** second maxilliped, **j** developing third maxilliped and pereiopods, **k** abdomen, dorsal view

triangular shape, with 1 subterminal simple seta. Exopod 2-segmented, proximal segment with 3 plumose setae; distal segment ending in sharper shape with 3 terminal plumose setae. Epipod subtriangular with 2 medial sparsely plumose setae and 3 subterminal and 1 terminal sparsely plumose setae.

Second maxilliped (Fig. 7b) Epipodite absent. Coxa and basis not differentiated, without setae. Endopod 4-segmented, with 0, 1 long plumodenticulate, 4 plumodenticulate and 4 (3 plumodenticulate, 1 simple) setae, respectively. Exopod 2-segmented with 1 medial simple and 1 distal plumose setae on proximal segment; the distal segment bear 3 long terminal simple setae.

Third maxilliped (Fig. 7c) Coxa and basis not differentiated, without setae. Endopod 4-segmented, fused ischium-merus with 2 small proximal simple, 3 medial (1 plumose and 2 simple), 2 distal (1 sparsely plumose and 1 small simple) setae; carpus with 3 (1 small simple, 1 small plumodenticulate and 1 plumodenticulate) setae; propodus with 3 plumodenticulate (1 subterminal) and 1 small simple

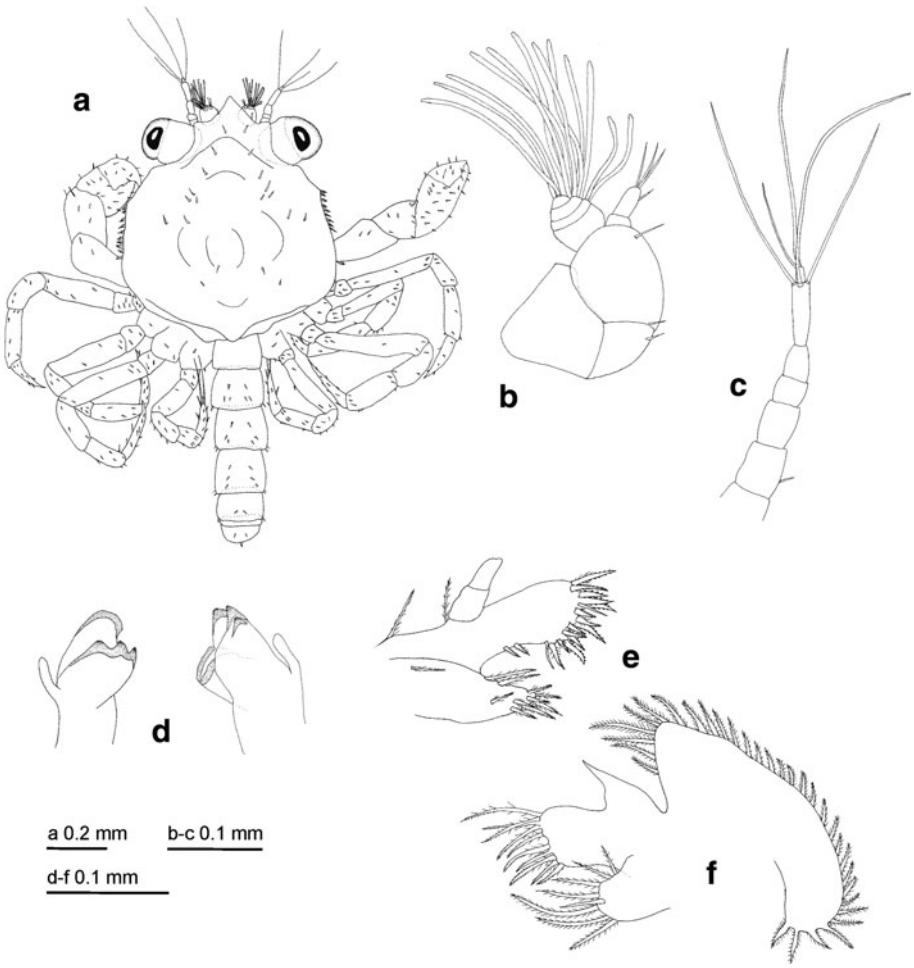

Fig. 6 *Calyptraeotheres garthi*. Megalopa. **a** Dorsal view, **b** antennule, **c** antenna, **d** mandibles, **e** maxillule, **f** maxilla

setae; dactylus with 2 plumodenticulate setae. Exopod 2-segmented, proximal segment without setae, distal segment with 3 terminal (2 plumose and 1 short simple) setae. Epipod elongated with 5 medial (4 plumodenticulate and 1 short simple) and 4 terminal plumodenticulate setae.

Pereiopods (Fig. 7d, e) All segments well differentiated and with setae as figured. Dactylus of pereiopods 2–5 with a long proximal inner spine. Dactylus of fifth pereiopod with 3 terminal simple setae (2 long and 1 shorter than these). Coxa of cheliped with 1 plumose seta.

Abdomen (Fig. 7f) Somite sixth now present, without setae. Somites bear simple setae dorsally and laterally as shown. Somites 2–5 with a pair of biramous pleopods, each one with unsegmented and without setae endopod and 2-segmented exopod. Pleopods 1-4 bearing 8 long terminal plumose natatory setae and 1 subterminal plumose seta, pleopods on somite fifth with 6 long terminal natatory plumose setae and 1 subterminal plumose seta. Uropods absent.

Telson (Fig. 7f) Square-shaped, rounded posterior margin, with 3 dorsal setae.

Discussion

The pea crab *Calyptraeotheres garthi* is one of nine species of the family Pinnotheridae reported from Argentinean marine waters (Fenucci 1975; Torres 2006). Fenucci (1971) described briefly some aspects of the first zoea of this species. The observed general morphology of this stage coincides with the report by Fenucci (1971), but the carapace setae and coxal setae of the first maxilliped are described in the present work for the first time, as well as the other larval stages.

The *Calyptraeotheres* genus grouped four species, with only two larval developments described formally, *C. garthi* (present work) and *C. politus* (Saelzer and Hapette, 1986). *Calyptraeotheres garthi* zoeae and megalopa described here are very similar to the previously reported for *C. politus* (Saelzer and Hapette 1986). The larvae of both species may be distinguished only by the setation arrangement of the maxilla and the number of aesthetascs of the antennule of fifth zoea (Table 2). A third difference, the appearance of the sixth abdominal segment in the

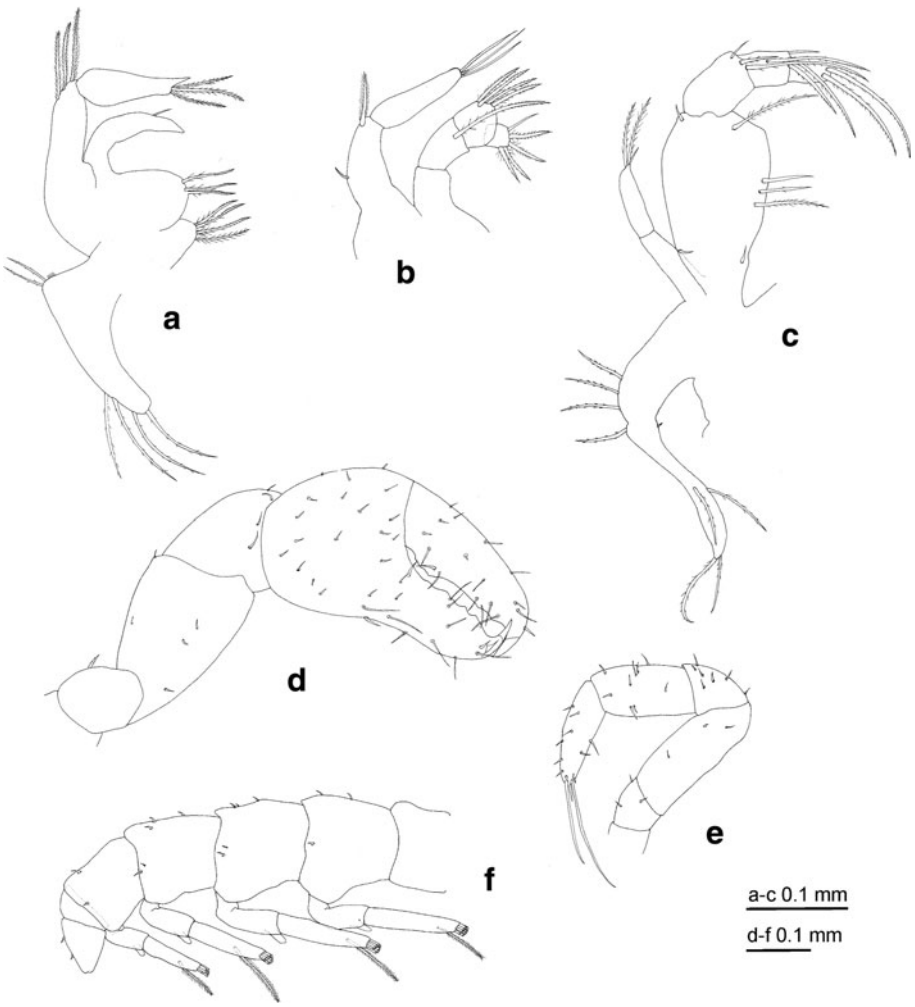

Fig. 7 *Calyptraeotheres garthi*. Megalopa. **a** First maxilliped, **b** second maxilliped, **c** third maxilliped, **d** first pereiopod, **e** fifth pereiopod, **f** abdomen and pleopods, lateral view

fourth zoea of *C. politus* and in the megalopa of *C. gathi,* will be discussed later.

The systematic position of the genera *Calyptraeotheres* and *Tumidotheres* has been focus of discussion (Campos 1999; Marques and Pohle 1995; Palacios-Theil et al. 2009). Due to this, an exhaustive comparison among *C. garthi* and reported characters of *C. politus* (Saelzer and Hapette 1986) and *T. maculatus* (Costlow and Bookhout 1966) was carried out here (Table 2).

The larval development of *T. maculatus* (Costlow and Bookhout 1966) has similarities with *Calyptraeotheres*. These three species have five zoeal stages in their development. Moreover, the morphology of the telson, the setation of zoeal mouthparts, and the segmentation of the megalopal antenna are almost equal. However, there are several differences, some of them referring to the time of appearance of certain characters throughout the development (Table 2). The appearance of pleopods bud and the endopod bud of antennule occurs earlier in *Calyptraeotheres* species than *T. maculatus*. In *Calyptraeotheres,* the marginal seta of the basial endite (maxillule) appears later than in *T. maculatus*. In addition, the carapace of *T. maculatus* bears three prominent spines, character shared with other members of Pinnotheridae, but absent in *Calyptraeotheres*.

Two additional differences between *C. garthi* and *T. maculatus,* but unfortunately not described in *C. politus* (Saelzer and Hapette 1986)*,* have been found. One of them is the appearance of rudimentary pereipods in the third zoea of *C. garthi* but latter in the fourth zoea in *T. maculatus.* The second character is the mandible palp, which is present in fifth zoea of *T. maculatus* but in the megalopa of *C. garthi*.

As mentioned above, in *C. garthi,* the sixth abdominal segment appears in the megalopa while *C. politus* and *T. maculatus* develop this segment in the fourth zoea. Up to now, variations in the number of the abdominal segment among congeneric species, such as those found here, were not reported in Pinnotheridae. Thus, this striking particularity of *Calyptraeotheres* may lead to an erroneous interpretation of the phylogeny of the genus.

In this sense, the larval phylogeny postulated by Marques and Pohle (1995) shows a group which encompasses

Table 2 Differences among the larval morphology of *Calyptraeotheres garthi*, *C. politus* (described by Saelzer and Hapette 1986), and *Tumidotheres maculatus* (described by Costlow and Bookhout 1966)

	Calyptraeotheres		*Tumidotheres maculatus*
	C. garthi	*C. politus*	
Antennule			
Endopod bud (appearance)	ZIV	ZIV	ZV
Exopod aesthetascs + setae	3-5-5-7-9	3-5-5-7-11	3-5-6-6-10
Appearance of mandibular palp	Megalopa	Not described	ZV
Maxillule			
Appearance setae on the lateral margin of the basial endite	ZIV	ZIV	ZIII
Coxal endite setae	4-4-4-5-6	4-4-4-5-6	4-6-6-6-9
Basial endite setae	5-7-7-9-10	5-7-7-9-10	5-7-7-12-12
Maxilla			
Coxal endite setae	5-6-6-6-7	5-5-5-6-9	4-6-6-7-8
Basial endite setae	7-9-9-10-12	8-9-9-10-10	9-9-9-11-16
Scaphognathite marginal setae	4-8-13-17-21	4-8-13-19-30	4-8-14-20-25
First maxilliped			
Basis setae	10	9	3?
Second maxilliped			
Basis setae	4	4	0?
Appearance of pereiopods bud	ZIII	Not described	ZIV
Abdomen			
Appearance 6th segment	Megalopa	ZIV	ZIV
Appearance pleopod buds	ZIII	ZIII	ZIV
Megalopa			
Carapace spines	0	0	3

P. politus (now *Calyptraeotheres politus*) and *T. maculatus,* but excludes *C. granti*. In our opinion, the hypothesis that each genus is monophyletic is strongly supported by the following facts: (1) Campos (1999) pointed out the differences between crab adult morphology of *Tumidotheres* and *Calyptraeotheres* (2) *Tumidotheres* and *Calyptraeotheres* live within bivalve species and in slipper limpets, respectively. (3) At least the known *Calyptraeotheres* larvae (see table 2) show differences when compared to the larvae description of *Tumidotheres*. Thus, the contrary results found by Marques and Pohle (1995) would be due to the fact that the authors used the number of abdominal segments in their analysis and, for this character, assigned different conditions for *C. politus* and *T. maculatus* than for *C. granti* (see methodological section of Marques and Pohle 1995). Since the segmentation of the abdomen is variable within same genus, this character should be analyzed with care in future phylogenetic studies including *Calyptraeotheres.*

According to the DNA phylogeny postulated by Palacios-Theil et al. (2009), the genus *Tumidotheres* is neighbor of *Calyptraeotheres* within the clade IIC, while *Dissodactylus-Clypeasterophilus* and *Tunicotheres* (see Fig. 1 of Palacios-Theil et al. 2009) are positioned in other branch of the same clade.

Although a formal cladistic analysis is necessary in order to demonstrate whether there is correspondence between larval morphology of these genera and the DNA phylogeny, some remarkable features were discussed here.

In the Table 3, the larval differences among some species of the clade IIC are listed. The species *Pinnaxodes major,* which is positioned close to *Holothuriophilus* within another clade (IIB) *sensu* Palacios-Theil et al. (2009), was also included in such comparison because this species share important features with *T. maculatus*.

For *Tunicotheres* and *Dissodactylus-Clypeasterophilus*, the larval morphology seems to be consistent with the molecular arrangement mentioned above, since both genera share five segments in the megalopal antenna, two dorsal spines on the zoeal telson and a seta on the first somite of the zoeal abdomen.

Simultaneously, several larval similarities between *Calyptraeotheres* and *Tumidotheres* have been found in our comparison (Tables 2, 3). However, *Tumidotheres* shares important characteristics with *Pinnaxodes major* (described by Hong 1974). Both genera, *Tumidotheres* and

Table 3 Larval comparison among *Calyptraeotheres* (present work and Saelzer and Hapette 1986), *Tumidotheres maculatus* (described by Costlow and Bookhout 1966), some species of *Dissodactylus-Clypeasterophilus* group [*Dissodactylus mellitae, D. crinitichelis* and *Clypeasterophilus stebbingi* described by Marques and Pohle (1996a), Pohle and Telford (1981) and Marques and Pohle (1996b), respectively], *Tunicotheres moseri* (described by Bolaños et al. 2004) and *Pinnaxodes major* (described by Hong 1974)

	Calyptraeotheres species	*Tumidotheres maculatus*	*Pinnaxodes major*	*Tunicotheres moseri*	*Dissodactylus-Clypeasterophilus*
Zoea stages	5	5	5	2	3–4
Antennule endopod bud (appearance)	ZIV	ZV	ZV	ZI	ZIII–ZIV or not develop
Antenna endopodite bud (appearance)	ZIII	ZIII	ZIV	ZI	ZII–ZIII
Appearance of mandibular palp	Megalopa but not described in *C. politus*	ZV	ZV	–	Megalopa or not develop
Maxillule					
Appearance setae on the lateral margin of the basial endite	ZIV	ZIII	ZIV	ZII	ZIII
First maxilliped basis setae	10–9	3?	7–10	5–6	10
Second maxilliped basis setae	4	0?	3–4	0–1	4
Appearance of pereiopods bud	ZIII but not described in *C. politus*	ZIV	Not described	ZI	ZII
Abdomen					
Appearance pleopod buds	ZIII	ZIV	ZIV	ZI	ZII–ZIII
Mid-dorsal setae on first somite	Absent	Absent	Absent	Present	Present
Telson					
Dorsal spine on furcate	Smooth	Smooth	Smooth	Covered with spinules	Covered with spinules
	Absent	Absent	Absent	Present	Present
Megalopa					
Carapace spines	0	3	3	0	0
Antenna segmentation	6	6	7	5	5

Pinnaxodes, bear three carapace spines in the megalopa, a segmented mandibular palp with setae, and develop the mandibular palp in the fifth zoea (Table 3).

Thus, from the point of view of the larval morphology, if *Tumidotheres* is the sister clade of *Calyptraeotheres* or, by the contrary, is the neighbor group of *Pinnaxodes* remains unclear. This matter will be resolved with future full comparative taxonomic analysis.

Here, we present the description of the complete larval development of *C. garthi.* In addition, we pointed out morphological traits that will improve the understanding of phylogeny of *Calyptraeotheres* and its close related species. Further investigation on the larval morphology of the Pinnotheridae is crucial in order to complete the understanding of the systematic of this group.

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