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Morphology of the first zoeal stage of the commensal southwestern Atlantic crab *Austinixa aidae* (Righi, 1967) (Brachyura: Pinnotheridae), hatched in the laboratory

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Running head First zoeal stage of *Austinixa aidae*

Abstract The first zoeal stage of the endemic southern Atlantic pinnotherid crab *Austinixa aidae* is described and illustrated based on laboratory-hatched material from ovigerous females collected from the upper burrows of the thallassinidean shrimp *Callichirus major* at Ubatuba, São Paulo, Brazil. The zoeae of *Austinixa* species can be distinguished from other pinnotherids and especially from zoeae of the closely related species of *Pinnixa* by the telson structure.

Keywords Crustacea, Decapoda, Larval development, Southern Atlantic, Zoea

Introduction

In recent decades, a combination of different tools has helped to elucidate life histories, taxonomy and systematics of decapod crustaceans. One of these tools is the morphological characterization of larvae. Larvae are recognized as a significant source of independent information for phylogenetic analyses. Considering the large number of species described worldwide by their adult morphologies, much effort is still needed to describe larval morphologies. This is particularly evident in the families of the Brachyura which represent almost half the known decapod species, because analyses of their systematic relationships are partly based on zoeal characters (Rice 1980; Ng and Clark 2000; Marques and Pohle 2003; Anger 2001, 2006).

Crabs of the family Pinnotheridae De Haan, 1833, with currently more than 300 species distributed among about 52 genera (Ng et al. 2008), are one of the little known groups in terms of larval morphology. This probably relates to the small size of these crabs and their intriguing life cycle. They typically show complex symbiotic relationships with various invertebrate hosts. In addition, the phylogenetic position of some members is still unclear and under active discussion (Palacios-Theil et al. 2009).

Members of the polyphyletic genus *Austinixa* Heard and Manning, 1997 (*sensu* Palacios-Theil et al. 2009) currently comprise 9 described and 2 still undescribed species, most of which occurring in the western Atlantic and the Caribbean; only *Austinixa felipensis* (Glassel 1935) is found on the Pacific coast (Heard and Manning 1997; Coelho 1997, 2005; Harrison 2004; Palacios-Theil et al. 2009). In only 3 of these species have the larval stages been completely or partially been described (Table 1).

In the present study, we describe and illustrate the morphology of the zoea I of *Austinixia aidae* (Righi 1967) from laboratory-hatched material. The results are compared with those from larvae of other species of Pinnotheridae (*sensu* Ng et al. 2008) previously described for the South Atlantic, in order to offer data for future studies on the phylogeny and biogeography of the group as well as for plankton analyses.

Material and Methods

Ovigerous females of *Austinixa aidae* were collected in November 2004 and July 2009 in the intertidal of a semi-protected and dissipative beach composed by fine sands at Perequê-Açu, Ubatuba Bay, State of São Paulo, Brazil (23°24’59.99”S, 45°03’17.13”W). Crabs were collected with suction pumps from galleries of *Callichirus major* and separated from the sand with a 1-mm mesh sieve.

Species identification was confirmed on the basis of morphological characters from available references (Manning and Felder 1989; Heard and Manning 1997). Additionally, and because of the complex taxonomy of this genus, tissue samples were taken from the animals for molecular analysis of a partial fragment of the 16S rDNA gene, in order to confirm the species identification. DNA extraction, amplification, sequencing protocols, and phylogenetic analysis followed Schubart et al. (2000), with modifications as in Mantelatto et al. (2007, 2009) and Palacios-Theil et al. (2009).

Ovigerous females were transported to the laboratory in an insulated box containing water from the site of collection. In the laboratory, the animals were isolated in aquaria with oxygenated sea water at a salinity of 34 and constant temperature (24 ± 1°C) until hatching. Newly hatched zoeae were fixed in a 1:1 mixture of 70% ethyl alcohol and glycerin.

The first zoeae were dissected for detailed examination under a stereoscope and mounted on semi-permanent slides. Morphological characters were studied with Leica DM 1000® and Zeiss Axioskop® compound microscopes attached to a personal computer using an Axiovision® image analysis system and a drawing tube, respectively. A minimum of 10 specimens was used in the descriptions and measurements. The sequence of the zoeal description is based on the malacostracan somite plan, from anterior to posterior, following literature recommendations (see Clark et al. 1998 and Pohle et al. 1999). Setae terminology follows Garm (2004). Long natatory setae on the first and second maxilliped are drawn truncated in Figure 2. Dimensions measured on each zoea were: rostro-dorsal length (rdl) as the distance between the tips of the dorsal and rostral spines; carapace length (cl), measured from the base of the rostral spine (between the eyes) to the most posterior margin of the carapace; dorsal spine length (dsl), from the base to the tip of the dorsal spine; rostral spine length (rsl), from the base (between the eyes) to the tip of the rostral spine; and lateral spine length (lsl), from the base to the tip of the lateral spine.

The females and zoeal stages of *Austinixa aidae* were deposited as voucher specimens in the Crustacean Collection of the Department of Biology (CCDB), Faculty of

Philosophy, Science and Letters of Ribeirão Preto (FFCLRP), University of São Paulo (USP), and allocated registration numbers CCDB 2643 to 2648, 2657, and 2658.

Results

The mtDNA obtained from ovigerous females matched 100% with the sequence from the nucleotide region of the 16S rDNA that was studied previously (Genbank EU934966) by Palacios-Theil et al. (2009), confirming the species' correct identification. During the culture, we obtained two different hatches from a single female (on 10 Nov 2004 and 8 Dec 2004), showing a pattern of multiple hatching without additional copula.

Austinixa aidae (Righi, 1967)

(Figs 1 and 2)

Size.— rdl: 0.95 ± 0.002 mm; cl: 0.036 ± 0.003 mm; dsl: 0.023 ± 0.003 mm; rsl: 0.036 ± 0.002 mm; lsl: 0.016 ± 0.002 mm.

Morphology.—Carapace (Fig. 1A-B): Globose, smooth, without tubercles. Dorsal spine long, slightly curved. Rostral spine present and straight, longer than dorsal spines. Lateral spines well developed, long, ventrally deflected. One pair of posterodorsal simple setae, posterior and ventral margins without setae. Eyes sessile.

Antennule (Fig. 1D): Uniramous; endopod absent; exopod unsegmented, with 2 long stout aesthetascs and 1 simple seta, all terminal.

Antenna (Fig. 1E): Protopod well developed, length less than one-third of that of the rostral spine, with 2 rows of minute spines along most of protopod length except the base. Exopod present as a small bud with a terminal simple seta.

Mandibles (Fig. 1C): Right molar with short teeth, and left molar with 1 tooth, confluent with incisor process. Endopod palp absent.

Maxillule (Fig. 2A): Coxal endite with 3 plumodenticulate setae and 1 plumose seta. Basial endite with 2 plumodenticulate and 2 cuspidate setae. Endopod 2-segmented, with 4 plumodenticulate setae (2 subterminal + 2 terminal) on distal segment.

Maxilla (Fig. 2B): Coxal endite slightly bilobed, with 4 + 1 plumose setae. Basial endite bilobed, with 4 + 4 plumodenticulate setae. Endopod not bilobed, unsegmented, with 3 (2+1) plumodenticulate terminal setae and microtrichia on both proximal and distal margins. Exopod (scaphognathite) margin with 4 plumose setae and a long setose posterior process.

First maxilliped (Fig. 2C): Coxa with one simple setae. Basis with 10 simple setae arranged 2, 2, 3, 3. Endopod 5-segmented with 2, 2, 1, 2, 5 (1 subterminal + 4 terminal) plumose setae, respectively. Exopod unsegmented, with 4 long terminal plumose natatory setae.

Second maxilliped (Fig. 2D): Coxa without setae. Basis with 4 plumose setae arranged 1, 1, 1, 1. Endopod 2-segmented, with 0, 5 (1 subterminal + 4 terminal) plumose setae. Exopod unsegmented, with 4 long terminal plumose natatory setae.

Third maxilliped: Absent.

Pereiopods: Absent.

Pleon (Fig. 1F): Five somites present. Somites 2-3 with 1 pair of lateral processes. Somite 5 laterally expanded, overlapping the telson. Somites 2-5 with 1 pair of posterodorsal setae. Pleopods absent.

Telson (Fig. 1F): Bifurcated, with 3 pairs of stout spinulate setae on posterior margin separated by a prominent median subtriangular lobe. Each furca long, with a small lateral spine, and with two rows of spinules.

Discussion

In the western Atlantic, the family Pinnotheridae encompasses more than 30 named species (Melo 1996; Coelho 1997, 2005), but to date the larval stages have been described completely or partially for only 16 pinnotherids (see Table 1). From 1996 to the present, the rate of description of new larval stages of pinnotherids lagged behind that of other brachyuran groups, probably due to the difficulties in collecting ovigerous females and in rearing their small zoeae. We are probably far from knowing the real diversity of larval forms that this family may present.

Taking into account the few descriptions of pinnotherid larvae available, the morphological characters of the zoea I of *A. aidae* are compared with those of previously described zoeae of the genera *Austinixa* and *Pinnixa* (Table 1), assuming the hypothesis of a close phylogenetic proximity of the two genera (Palacios-Theil et al. 2009).

Although the zoeae of the eight species of *Austinixa* and *Pinnixa* are basically similar in morphology, zoeae of *Austinixa* can be easily distinguished from those of *Pinnixa* by the telson structure. However, there is one exception: *Pinnixa chaetopterana* has the posterior median lobe on the telson that characterizes *Austinixia* zoeae and is absent in all other known species of *Pinnixa*. This interesting relationship of *P. chaetopterana* with *Austinixa* was also detected in a recent molecular phylogeny of the group, where *P. chaetopterana* together with *P. sayana* and *P. rapax* occupied a basal position in the *Austinixa* clades (Palacios-Theil et al. 2009: Fig. 1, clades IA, IB, IC, p. 464). To date, there are no data available on larvae of *P. rapax*, but *P. sayana* larvae lack the median lobe like all other known larvae of *Pinnixa* except for *P. chaetopterana*. Therefore, at this point the interpretation of this feature with respect to the phylogenetic position of these species is unclear, although the polyphyly of *Pinnixa sensu lato* has been clearly pointed out recently (Palacios-Theil et al. 2009). In any case, the known zoea stages of the congeneric species of *Pinnixa* of the western and eastern Pacific, *P. tumida*, *P. rathbuni*, and *P. longipes* (Konishi et al. 1988; Sekiguchi 1978; Bousquette 1980) do not have the median lobe on the posterior margin of telson either. Therefore this character seems to be appropriate to distinguish the zoeae of *Pinnixa* from the rest of the Pinnothereliinae.

A comparison between larvae of *A. aidae* and the previously described zoeae I of other *Austinixa* species must remain restricted to *A. cristata* and *A. bragantina*. The published data on *A. patagoniensis* is but a small lateral view of the zoea II which only allows us to confirm the presence of the median lobe on the posterior margin of the telson (Boschi 1981).

The setation pattern of the mouthparts seems to be constant through the complete zoeal phase in all these species: 2, 2, 3, 3 and 1, 1, 1, 1 for the first and second maxilliped, respectively. Where deviations from this pattern were reported (such as 2, 3, 1, 2 and 1, 1, 1, respectively, for *A. bragantina*; Lima 2009), these findings require confirmation. The same applies to another observation by Lima (2009), the absence of lateral spines on the telson of the zoea I in *A. bragantina*.

Therefore, differences between the zoea I of *Austinixa* larvae are probably only evident in the cephalothorax and the pleon armature. *Austinixa cristata* zoea I (Dowds 1980) can be differentiated by the similar lengths of the dorsal and rostral spines; in *A. bragantina* and *A. aidae*, the rostral spine is clearly longer than the dorsal. Regarding the pleon differences, we found that *A. aidae* can be separated from *A. bragantina* and *A. cristata* by the presence of lateral spines on the telson. However, in *A. bragantina* these spines have been reported for the zoea II and subsequent stages (Lima 2009), and thus might have been overlooked in the zoea I. We also found that *A. cristata* has the longest furcal arms (from the telson base) compared with *A. aidae* and *A. bragantina*.

Adult morphological characters are particularly difficult to use in inferring evolutionary relationships among species of *Austinixa* (Harrison 2004). In addition, apparent convergent evolution and/or stabilizing selection due to commensal lifestyles makes it difficult to find ‘‘good’’ morphological characters for phylogenetic studies (Zmarzly 1992).

Unfortunately, the larvae of *A. bragantina* were not archived in a zoological collection, and no additional material is available to double-check the analysis (J. Lima, pers. comm.). Thus, the possibility remains that there are no real morphological differences between the zoea I of *A. bragantina* and *A. aidae*. Addition analyses of the morphology and DNA of adults and larvae of *A. bragantina* would be welcomed and necessary to reassess the treatment of *A. bragantina* as a valid species.

Our study evidences some important differences in the morphology of *Austinixa* larvae, which may reflect a high morphological plasticity in this genus. The outcome of the present study should encourage future studies of the larval morphology in congeners. Moreover, our findings confirm the need for a revised classification based on both molecular analyses and re-evaluations of the larval and adult morphology (Bolaños et al. 2004).

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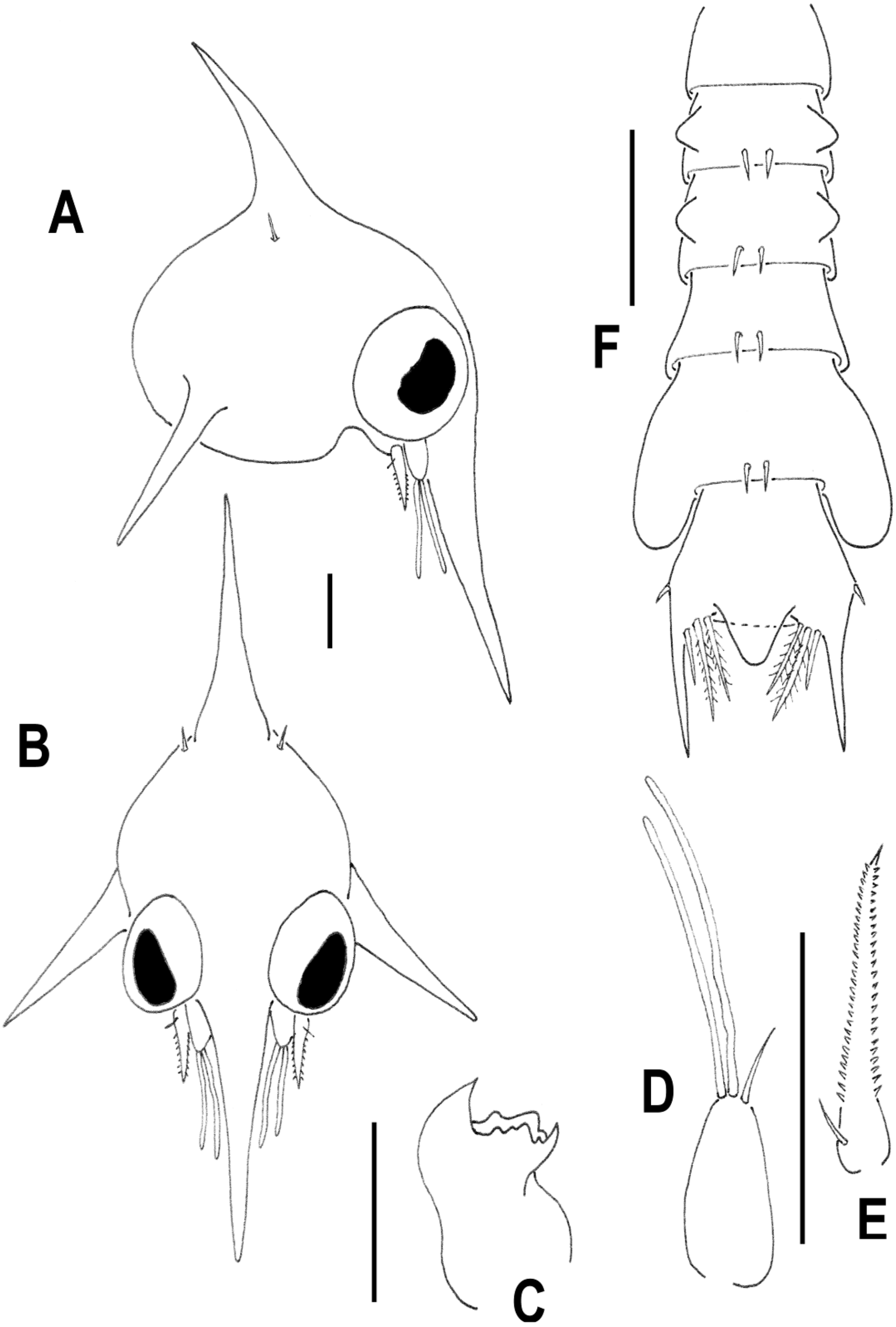
Table 1 Species of pinnotherid crabs from the western Atlantic Ocean with known larval stages, and respective references. Z, zoeal stages; M, megalopa stage; (?) possible error.

Species	Larval stages	Reference
Austinixa aidae (Righi, 1967)	ZI	Present study
Austinixa bragantina Coelho, 2005	ZI-V+M	Lima (2009)
Austinixa cristata (Rathbun, 1900)	ZI	Dowds (1980)
Austinixa patagoniensis (Rathbun, 1918)	ZI-V(?)+M?	Boschi (1981)
Clypeasterophilus stebbingi (Rathbun, 1918)	ZI-IV+M	Marques and Pohle (1996)
Dissodactylus crinitichelis Moreira, 1901	ZI-III+M	Pohle and Telford (1981)
Dissodactylus mellitae (Rathbun, 1900)	ZI	Sandifer (1972)
Gemmotheres chamae (Roberts, 1975)	ZI-III+M	Roberts (1975)
Orthotheres barbatus (Desbonne, 1867)	ZI-II+M	Bolaños et al. (2005)
Pinnaxodes chilensis (H. Milne Edwards, 1837)	ZI	Gutiérrez-Martinez (1971)
Pinnixa chaetopterana Stimpson, 1860	ZI-V+M	Hyman (1925), Sandifer (1972)
Pinnixa cylindrica (Say, 1818)	ZI	Hyman (1925), Sandifer (1972)
Pinnixa gracilipes Coelho, 1997	ZI-V+M	Lima et al. (2006)
Pinnixa sayana Stimpson, 1860	ZI-V+M	Hyman (1925), Sandifer (1972)
Tumidotheres maculatus (Say, 1818)	ZI-V+M	Costlow and Bookhout (1966)
Tunicotheres moseri (Rathbun, 1918)	ZI-II + M	Bolaños et al. (2004)
Zaops ostreum Say, 1817	ZI-IV +M	Hyman (1925), Sandifer (1972)

Figure Captions

Fig. 1 *Austinixa aidae* (Righi, 1967) zoea I. A, lateral view of cephalothorax; B, frontal view of cephalothorax; C, mandible; D, antennule; E, antenna; F, dorsal view of pleon. Scale bars = 0.1 mm.

Fig. 2 *Austinixa aidae* (Righi, 1967) zoea I. A, maxillule; B, maxilla; C, first maxilliped; D, second maxilliped. Scale bars = 0.05 mm.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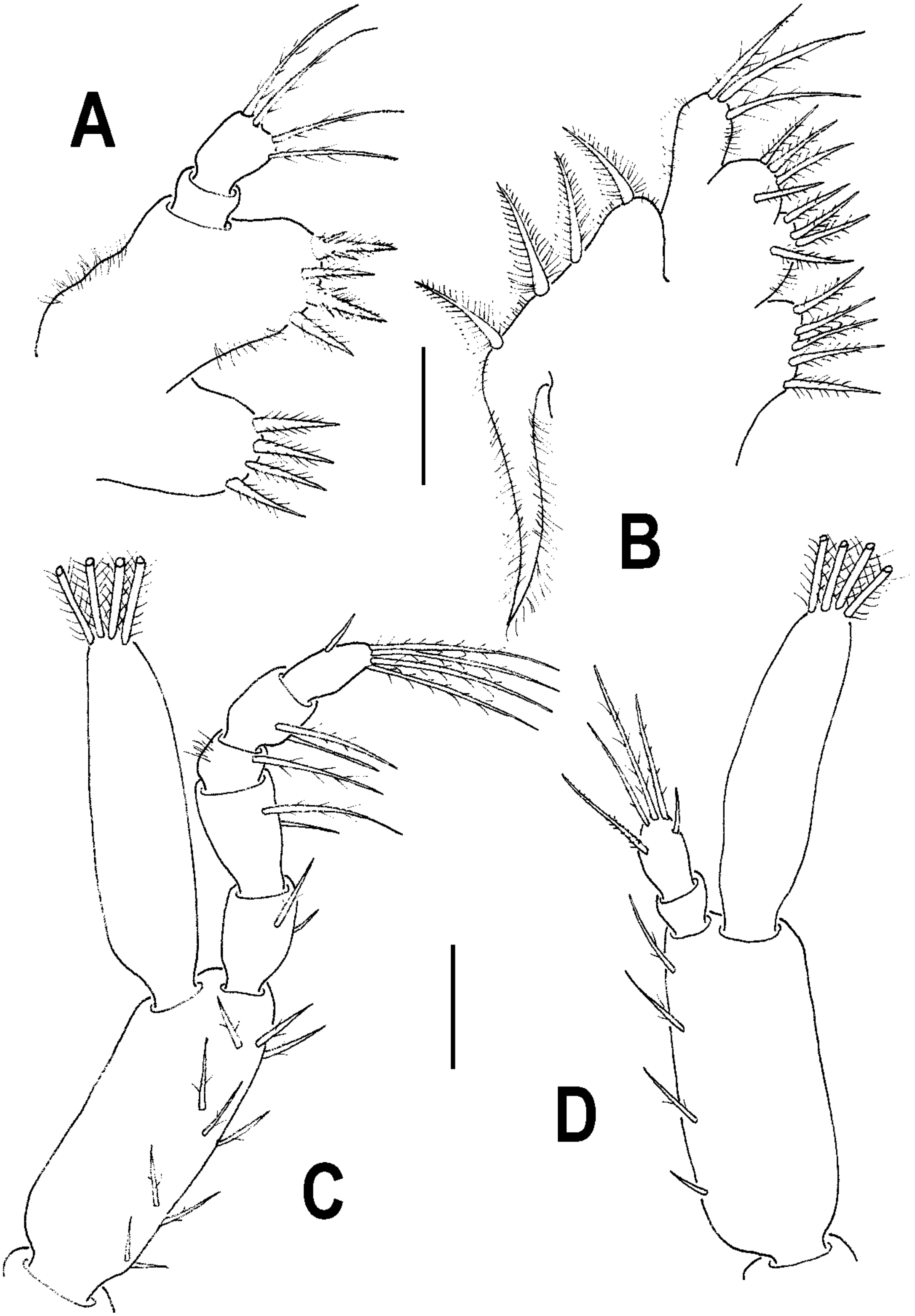
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