Larval development of *Austinixa bragantina* (Crustacea: Brachyura: Pinnotheridae) reared in the laboratory

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ABSTRACT. The zoeal and megalopal stages of *Austinixa bragantina* Coelho, 2005, a small pinnotherid crab found in association with ghost shrimps *Callichirus major* (Say, 1818) and *Lepidophthalmus siriboia* Felder & Rodrigues, 1993 in the northeastern region of the state of Pará, Brazil, were reared in the laboratory from hatching to the megalopal stage. The duration of the larval period from hatching to megalopa was 28 days and the mean of duration for each larval stage was 6, 5, 5, 6, and 6 days, respectively. In the present study, the zoeal and megalopal stages are described and illustrated in detail.

KEY WORDS. Larval stages; larval description; Pará; Pinnotherid crab.

Members of Pinnotheridae are known to spend most or all of their post-planktonic life in symbiosis with other invertebrates, such as mollusks, polychaetes, annelids, ascidians, echinoderms, and other crustaceans (Williams 1984). According to Ng *et al*. (2008) the family is currently subdivided in two subfamilies: Pinnotheriliinae and Pinnotherinae, as well as several *insertae sedis* species. According to Bezerra *et al*. (2006), in the coast of Brazil, this family is represented by 22 species distributed across the 11 genera.

Austinixa Heard & Manning (1997) is represented by *A. aidae* (Righi, 1967) from Amapá to Rio Grande do Sul, *A. leptodactyla* (Coelho, 1997) from Pará to Sergipe, *A. patagoniensis* (Rathbun, 1918) from Rio de Janeiro to Rio Grande do Sul, and *A. bragantina* Coelho, 2005 from Pará to Ceará, always living in symbiosis with both thallassinidean shrimps of the *Callichirus major* (Say, 1818) complex, and a few ecologically equivalent thallassinidean species. In addition, it has been reported in association with the genus *Pinnixa* White, 1846 by Lima *et al*. (2006).

Larval descriptions of Pinnotheridae crabs are available for 46 species among 15 genera. However, descriptions of the complete larval development has been published for 26 species. The remaining publications provide only partial information, being mostly restricted to the first zoea (Marques & Pohle 1996a). Currently, most studies focus on *Dissodactylus* Smith, 1870, *Pinnixa* and *Tunicotheres* Campos, 1996 (Faxon 1879, Sekiguchi 1978, Bousquette 1980, Marques & Pohle 1996a,b, Lima *et al*. 2006). No larval description has hitherto been provided for *Austinixa*.

In the present paper, the larval stages of *A. bragantina,* a small pinnotherid crab found in association with ghost shrimps *Callichirus major* (Say, 1818) and *Lepidophthalmus siriboia* Felder & Rodrigues, 1993 are described and illustrated in detail from larvae reared in the laboratory. These results are briefly compared with other descriptions reported on pinnotherid larvae from Brazil.

MATERIAL AND METHODS

Six egg-bearing females were obtained from Ajuruteua beaches, northeastern state of Pará, Brazil. In the laboratory, all females were cleaned and monitored until hatching occurred in a five liters aquarium with constant aeration.

After hatching, larvae were transferred to glass containers of 500 ml capacity (approximately 30 larvae per container) filled with filtered seawater and kept at room temperature in the laboratory (~27ºC). Salinity was maintained at 30‰ and pH 8.1. Water and food were changed daily. The larval and postlarval stages were fed with rotifers (*Brachionus* sp.). Cultured diatoms (*Thalassiosira* sp.) were added to the culture. Survival was recorded daily and intermolt periods were inferred using mean of occurrence of exuviae for each stage.

Exuviae, some zoeal larvae, and a megalopa were preserved in ethanol 70% + glycerine 10% (1:1) solution. The larvae were dissected with fine needles, measured and illustrated using an ocular micrometer coupled with binocular microscope. Carapace length was measured using the distance from the anterior portion of ocular orbit to posterodorsal margin of the carapace.

The terminology used in the description followed Sekiguchi (1978), Bousquette (1980), Pereyra Lago (1987, 1989), Marques & Pohle (1996a,b), Magalhães & Medeiros (1998), Bolaños *et al*. (2004, 2005), Lima & Abrunhosa (2006), Lima *et al*. (2006).

RESULTS

The larval development of *A. bragantina* consisted of five zoeal stages and one megalopa. The intermolt period of each larval stage and survival rate are presented in the table I. Morphological differences between zoea I of *A. bragantina* and Brazilian pinnotherids are provided in the table II. The first stage

of *A. bragantina* is described in detail. Only main morphological changes were described for stages following the first zoea.

Table I. Survival rate, intermolt period and cumulative days of Austinixa bragantina reared in the laboratory.

Larval stages	Intermolt period (days)	Cumulative (days)	Survival rate (%)
Zoea I	6	6	70
Zoea II	5	11	60
Zoea III	5	16	30
Zoea IV	6	22	20
Zoea V	6	28	10
Megalopa	*	*	1

* Not recorded.

Zoea I

Carapace length 0.26 mm (0.25-0.28 mm), bearing one dorsal, one rostral, and two lateral spines (Fig.1). Lateral margin with one small simple setae. Eyes sessile. Antennule uniramous, unsegmented, smooth and conical, with two long and one short terminal aesthetascs (Fig. 7). Antenna uniramous, elongate, presented antennal flagellum spinulose with one simple (Fig. 8) Basal seta. Mandible symmetrical, with two distal incisor process (Fig. 9). Molar region circular, more or less serrated. Cuticle distally brown. Palp absent. Maxillule coxal endite supporting four plumose setae (Fig. 10). Basial endite with three cuspidate and two plumose setae. Endopod 2-segmented, proximal segment without setae, distal segment with four long plumose setae. Protopod absent. Exopod and epipod seta absent. Maxilla coxal endite with proximal and distal lobes almost fused with five plumodenticulate setae (Fig. 11). Basial endite with proximal and distal lobes fused bearing eight plumodenticulate setae. Endopod unsegmented with 2+1 long plumose setae. Scaphognathite with three or four plumose setae along anterior margin and one conical terminal process supporting microtrichia in the posterior margin. First Maxilliped basipod with internal margin bearing 2, 3, 1, 2 setae (Fig. 12). Endopod 5-segmented with 2, 2, 1, 2, 4 setae, respectively. Exopod unsegmented, with four plumo-natatory setae. Second maxilliped basipod with three to four setae (Fig. 13). Endopod 2-segmented with 0 and (4+1) simple setae, respectively. Exopod unsegmented with four plumo-natatory setae. Third maxilliped absent. Pereiopods absent. Abdomen and telsonwith five abdominal somites, 2th and 3th with a pair of acute spines projecting sidelong, somite 5 expanded laterally into two lobes, which extend backward on each side of the terminal segment overlap the telson (Fig. 14). Telson bifurcated with two long lateral plumose spines. Internal margin with six (3+3) plumose setae of the asymmetric size separated for a triangular median projection.

Zoea II

Carapace length 0.35 mm (0.33-0.36 mm) (Fig. 2). Lateral margin with three simple setae. Eyes stalked. Antennule uniramous, unsegmented and conical with four long and one short terminal aesthetascs (Fig. 15). Antenna as illustrated (Fig. 16). Mandible as illustrated (Fig. 17). Maxillule coxal endite as illustrated (Fig. 18). Basial endite with two additional cuspidate setae. Protopodite with one long plumose seta. Maxilla coxal endite with proximal and distal lobes almost fused with six plumose setae (Fig. 19). Basial endite with 5+3 plumose setae. Scaphognathite with 4+3 marginal densely plumose setae. First maxillipedbasipod and endopod as illustrated (Fig. 20). Distal portion of the endopod with five simple setae. Exopod with six plumo-natatory setae. Second maxillipedexopod with six plumo-natatory setae (Fig. 21). Third maxilliped absent. Pereiopods absent. Pleopods absent. Abdomen and telson as illustrated (Fig. 22). External margin of telson with a pair of small spines projecting laterally.

Zoea III

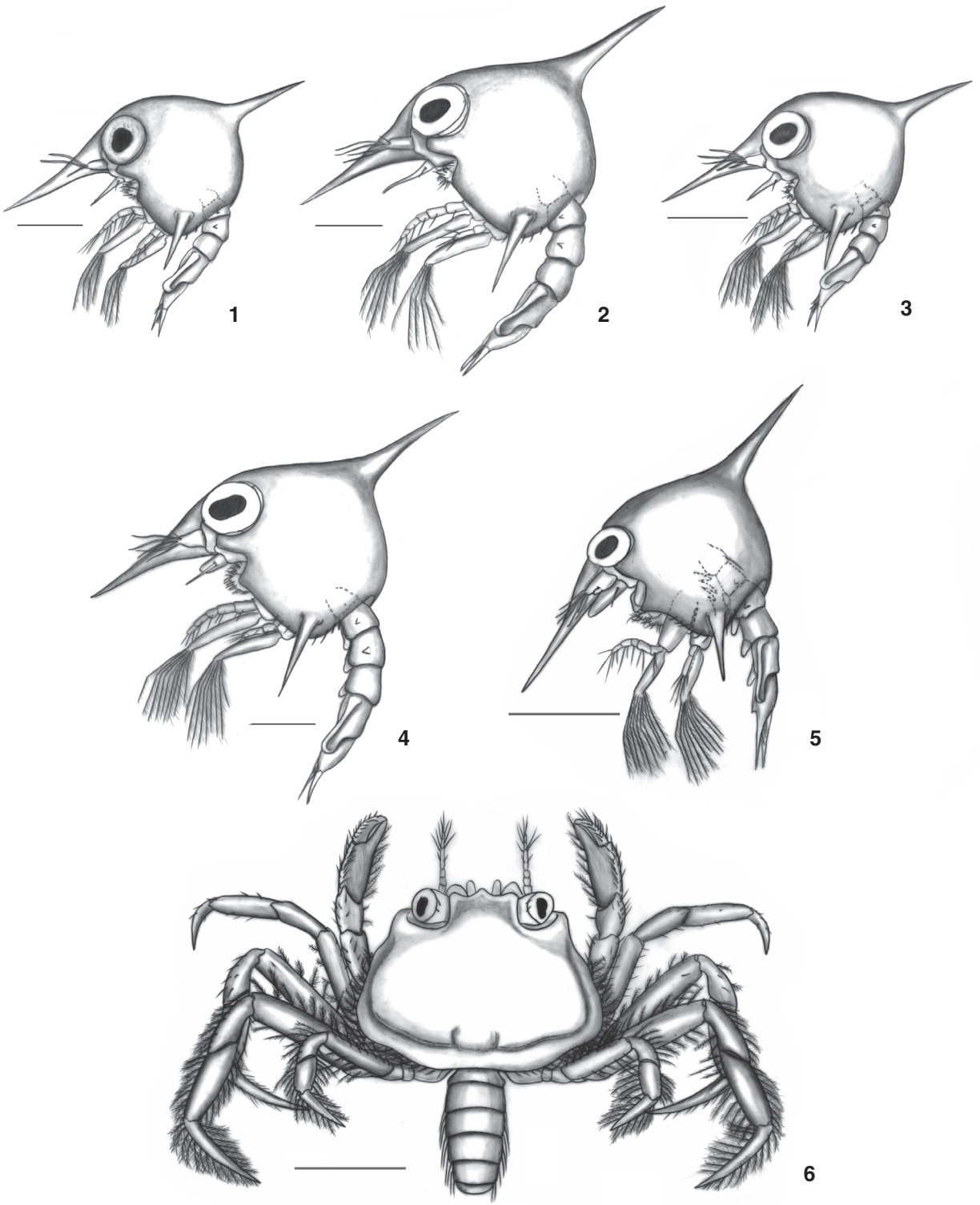
Carapace length 0.42 mm (0.40-0.44 mm) (Fig. 3). Lateral margin with four small simple setae. Eyes peduncled and mobile. Antennule as illustrated (Fig. 23). Antenna showing endopod bud reaching in the medial portion of protopodite (Fig. 24). Basial seta present. Mandible as illustrated (Fig. 25). Maxillule coxal endite with five plumodenticulate setae (Fig. 26). Basial endite with five cuspidate and two simple setae. Protopodite as illustrated. Maxilla coxal endite with six plumose setae (Fig. 27). Basial endite with 5+5 plumodenticulate setae. caphognathite with 7+6 densely plumose setae. First maxilliped basipod with 1, 2, 1, 2, 1 and 3 simple setae, respectively (Fig. 28). Exopod with eight plumo-natatory setae. Second maxilliped exopod with eight plumo-natatory setae (Fig. 29). Third maxilliped absent. Pereiopods absent. Pleopods absent. Abdomen and telson as illustrated (Fig. 30).

Zoea IV

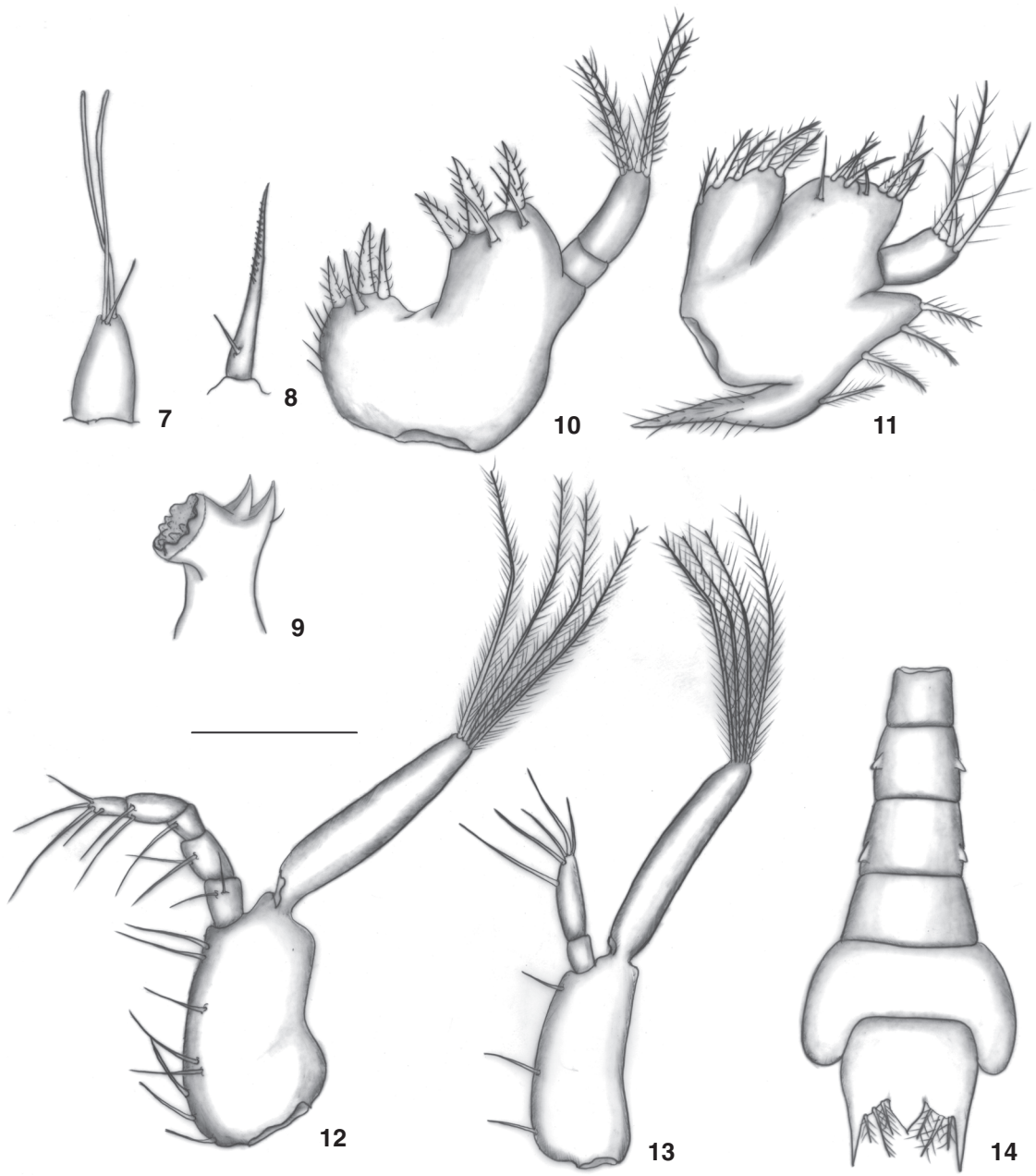
Carapace length 0.52 mm (0.52-0.53 mm) (Fig. 4). Lateral margin with six small simple setae. Antennule showing four terminal and one subterminal aesthetascs and one simple terminal seta (Fig. 31). Endopod bud present. Antenna endopod bud well developed with one small basial seta, flagellum as illustrated (Fig. 32). Mandible – with three distal incisor process. Molar region circular serrated with two small denticulettes (Fig. 33). Palp present as small bud. Maxillule coxal endite with one simple and five plumodenticulate setae (Fig. 34). Basial endite with eight plumodenticulate and three plumose setae. Protopodite with two plumose setae in external border. Maxilla coxal endite with proximal and distal lobes fused with eight plumodenticulate setae and one simple seta (Fig. 35). Basial endite with proximal and distal lobes fused showing 16 to 17 plumodenticulate setae. Scaphognathite with 14 + 7 densely plumose setae. First Maxilliped– basipod with 2, 1, 1, 3, 3 simple setae, as illustrated (Fig. 36). Exopod with 10 plumo-natatory

Table II. Morphological features of zoeae I of the Pinnotheridae from the Brazilian coast. Data were obtained from previous descriptions, illustrations, and the present study. (1) *Austinixa bragantina* in this paper, (2) *Clypeasterophilus stebbing* (Marques & Pohle 1996b), (3) *Dissodactylus crinitichelis* (Pohle & Telford 1981), (4) *Pinnixa gacilipes* (Lima *et al.* 2006), (5) *Tumidotheres maculatus* (Costlow & Bookhout 1966), (6) *Zaops ostreum* (Sandoz & Hopkins 1947).

Appendages	1	2	3	4	5	6
Carapace						
Setation	absent	2	2	absent	absent	absent
Dorsal spines	present	present	present	present	present	absent
Lateral spines	present	present	present	present	present	absent
Adomen						
Dorsal spines	2	2	2	2	2	2
2-somite	2	absent	2	2	2	2
3-somite	modified	normal	normal	modified	normal	normal
Antennule						
Exopod aesthetascs	2	2	2	2	2	3
Setation	1	1	1	1	1	0
Antenna	uniramous	uniramous	uniramous	uniramous	uniramous	small bud
Setation	1	absent	absent	1	absent	
Maxillule						
Endopod segment	2	2	2	2	2	2
Setation	4	4	4	4	4	4
Protopodite						
Basial endite setation	3+2	3+2	3+2	4+1	2+3	3+2
Coxal endite setation	4	3+1	3+1	3+1	4	4
Maxilla						
Scaphognathite setation	4	4	4	4	4	4
Endopod segment	uniramous	uniramous	uniramous	uniramous	uniramous	uniramous
Basial endite setation	8	8	8	5+1	9	9
Coxal endite setation	5	5	5	4+1	4	6
Terminal process	sharp	sharp	sharp	rounded	sharp	sharp
Maxilliped I						
Exopod setation	4	4	4	4	4	4
Endopod setation	11	11	12	11	12	11
Basipod setation	3	10	10	7	3	8
Maxilliped II						
Exopod setation	4	4	4	4	4	4
Endopod setation	5	5	5	5	5	4
Basipod setation	3	4	4	6	0	4
Telson						
Setation	6	6	6	6	6	6
Triangular lobe	present	absent	absent	absent	absent	present



Figurses 1-6. *Austinixa bragantina*. (1-4) Zoea I-IV lateral view: (1) zoea I; (2) Zoea II; (3) zoea III; (4) zoea IV; 5-6. Zoea V and megalopa: (5) zoea V lateral view; (6) megalopa dorsal view. Scale bar: 1-2 = 0.2 mm, 3-4 = 0.4 mm, 5-6 = 0.6 mm.

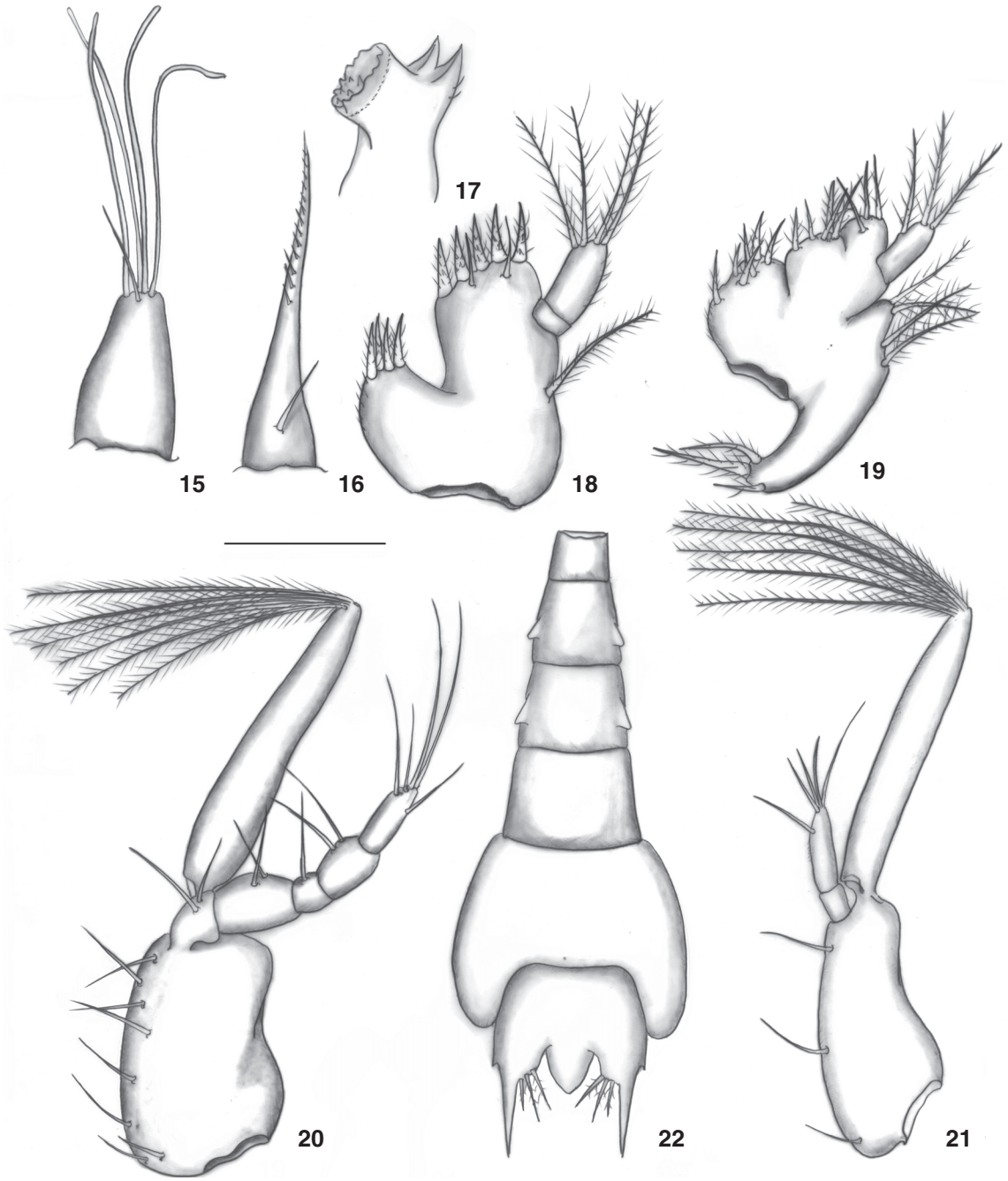


Figures 7-14. Appendages of zoea I of *Austinixa bragantina*: (7) antennule; (8) antenna; (9) mandible; (10) maxillule; (11) maxilla; (12) first maxilliped; (13) second maxilliped; (14) abdomen and telson. Scale bar: 7-10 = 0.05 mm; 11-13 = 0.075 mm; 14 = 0.15 mm.

setae. Second Maxilliped– Exopod with 10 plumo-natory setae (Fig. 37). Third maxilliped present as small bud. Pereiopods present as small bud. Pleopods present as four small bud (Fig. 4). Abdomen and telson as illustrated (Fig. 38).

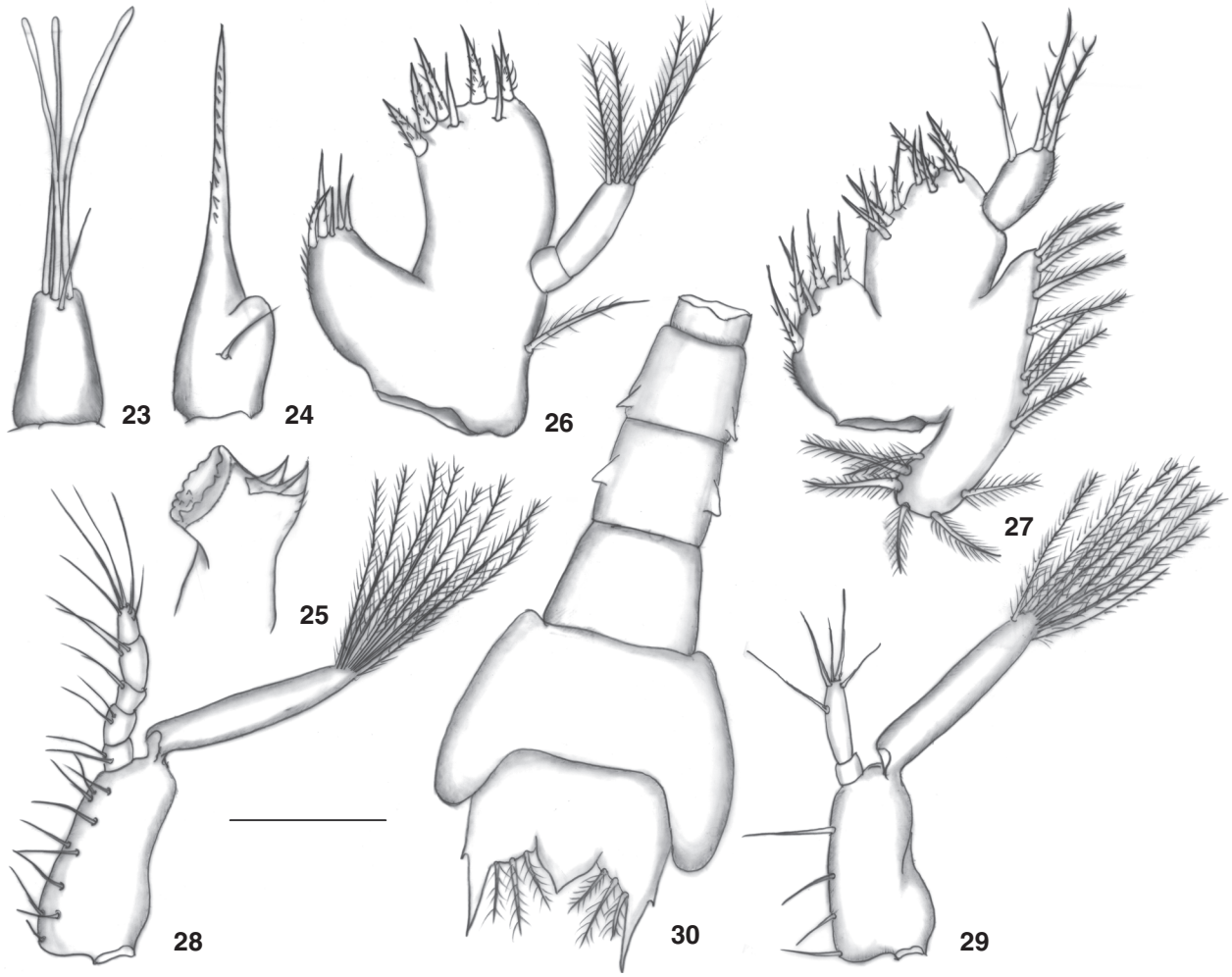
Zoea V

Carapace length 0.73 mm (0.72-0.74 mm) (Fig. 5). Lateral margin with 10 small setae as illustrated. Antennule well developed with eight aesthetascs and one simple seta (Fig. 39). Endopod bud present. Antenna endopod well developed with size twice of the protopodite (Fig. 40). Mandible as illustrated (Fig. 41). Maxillule coxal endite with five plumodenticulate and three simple setae (Fig. 42). Basial endite with 30 plumodenticulate and five simple setae. Maxilla coxal endite with eleven plumodenticulate setae (Fig. 43). Basial endite with 14



Figures 15-22. Appendages of zoea II of *Austinixa bragantina*: (15) antennule; (16) antenna; (17) mandible; (18) maxillule; (19) maxilla; (20) first maxilliped; (21) second maxilliped; (22) abdomen and telson. Scale bar: 15-16 = 0.037 mm; 17 = 0.080; 18-19 = 0.085 mm; 20-21 = 0.075 mm; 22 = 0.15 mm.

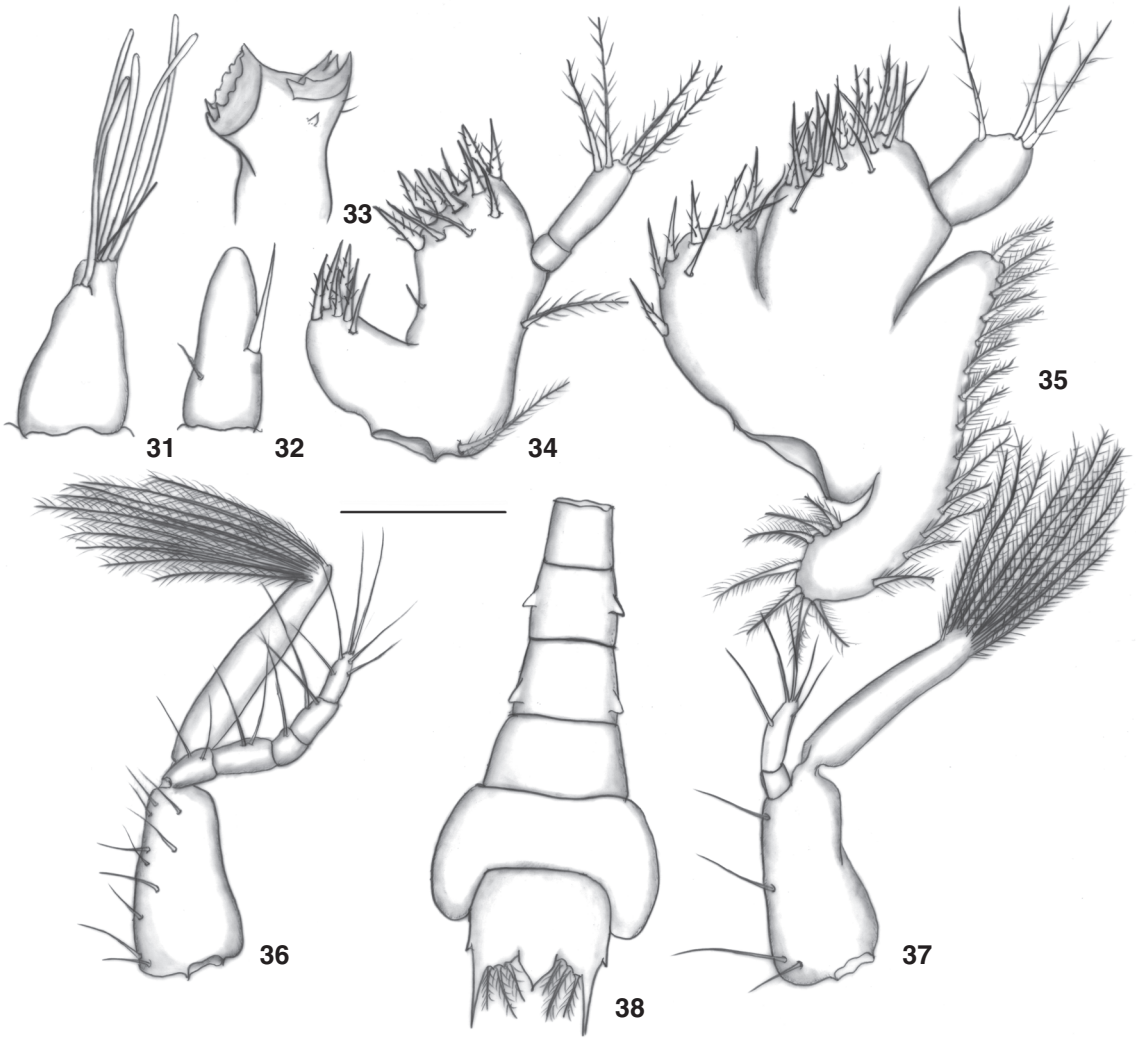
plumodenticulate and six plumose setae. Scaphognathite with 33 plumose setae. First maxilliped as illustrated (Fig. 44). Second maxilliped as illustarted (Fig. 45).Third maxilliped with endopod and exopod buds visible (Fig. 46). Pereiopods longer, with sharply ponted tips, segmentation apparent and bifurcation of chelae clearly visible (Fig. 5). Pleopods longer, not yet segmented nor biramous (Fig. 5). Abdomen and telson as illustrated (Figs 5 and 47).



Figures 23-30. Appendages of zoea III of *Austinixa bragantina*: (23) antennule; (24) antenna; (25) mandible; (26) maxillule; (27) maxilla; (28) first maxilliped; (29) second maxilliped; (30) abdomen and telson. Scale bar: 23 = 0.04 mm; 24 = 0.038 mm; 25-27 = 0.085 mm; 28-29 = 0.22 mm; 30 = 0.18 mm.

Megalopa

Carapace length 0.70 mm, almost hexagonal, wider than longer, base of rostrum rectangular, terminal portion short and triangular (Fig. 6). Eyes deeply lodged in the orbit. The cardiac area shows a small high carina almost straight line, extending without interruption. The lateral margin with numerous plumose setae. Antennule basial segment inflated showing three simple setae (Fig. 48). Peduncle 2-segmented without setae. Endopod uniramous with 3+1 setae. Exopod 4-segmented with 10 aesthetascs and one simple seta. Antenna antennal peduncle 3-segmented with each segment showing one simple seta (Fig. 49). Antennal flagellum 4-segmented. Segments 2 with four long terminal setae and two small simple setae. Segment 3 with one terminal simple seta. Last segment with three terminal setae. Mandible scoop-shaped process with smooth, thin cutting edge distally; with five to seven small denticulattes (Figs 55 and 56). Palp 2-segmented, proximal segment smooth, distal segment with 20 small simple and one plumose seta. Maxillule coxal endite with three plumodenticulate and five plumose setae (Fig. 50). Basial endite with 23 plumodenticulate setae. Endopod 2-segmented, proximal with two and distal segments with one simple terminal seta. Protopodite present. Maxilla coxal endite with 21 plumose setae (Fig. 51). Basial endite with 49 plumodenticulate setae. Endopod as illustrated. Scaphognathite with 71 plumose setae marginally and one simple seta subdistally in the anterior border. First Maxilliped basial and coxal endite with approximately 30 setae (Fig. 52). Endopod 2-segmented with two simple setae in each segment. Exopod 2-segmented, proximal and distal segment with one and five simple setae, respectively. Second maxilliped basial and coxal endites lacking

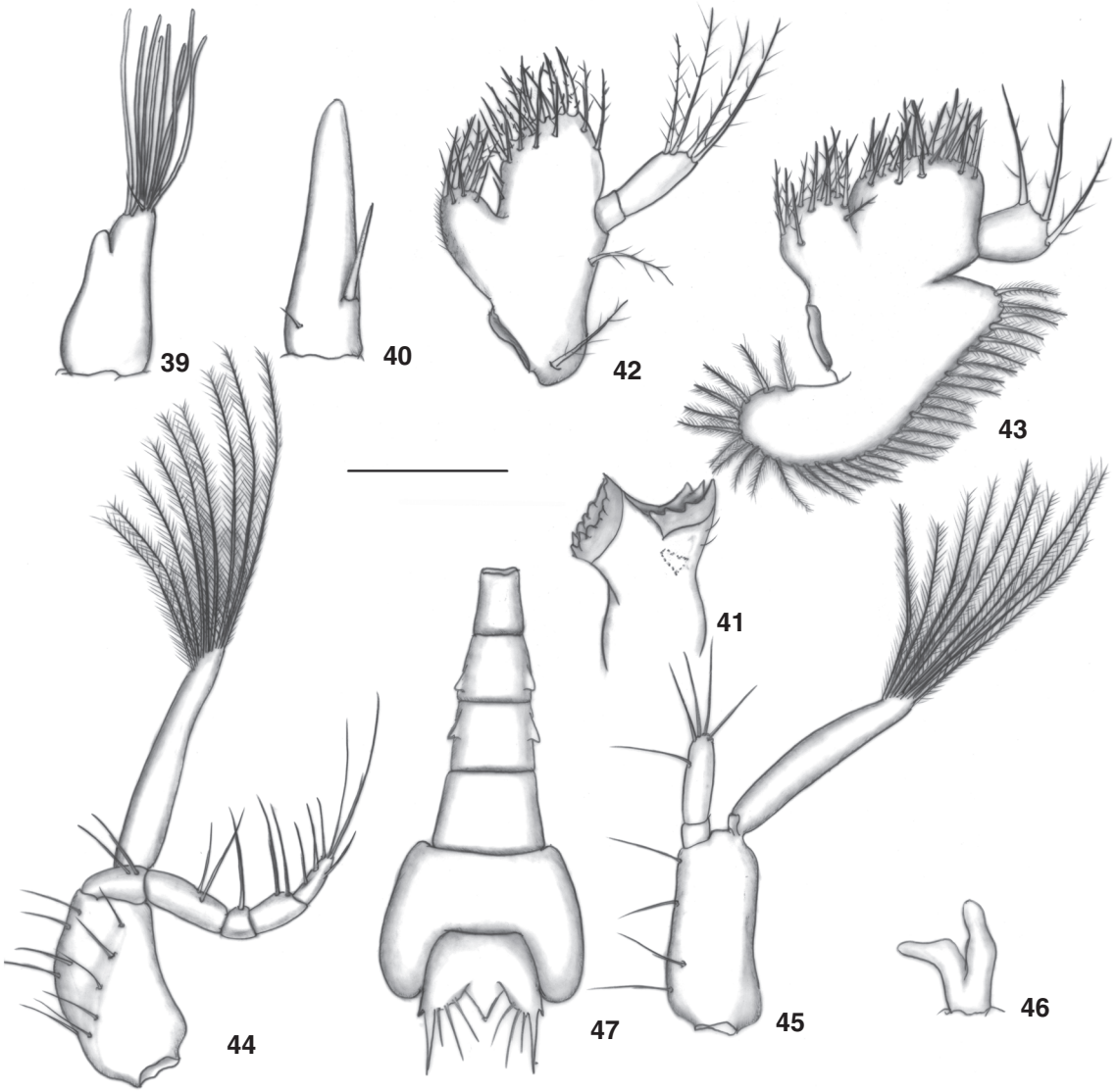


Figures 31-38. Appendages of zoea IV of *Austinixa bragantina*: (31) antennule; (32) antenna; (33) mandible; (34) maxillule; (35) maxilla; (36) first maxilliped; (37) second maxilliped; (38) abdomen and telson. Scale bar: 31-32 = 0.1 mm; 33-35 = 0.15 mm; 36-37 = 0.24 mm; 38 = 0.3 mm.

setae (Fig. 53). Endopod 5-segmented, with 2, 4, 0, 14, 10 simple setae, respectively. Exopod 2-segmented, proximal segment with two spines and one simple seta, distal segment with five plumose setae. Third maxilliped well developd (Fig. 54). Endopod 4-segmented: basipod showing two small plumose setae, ischiomerus with six plumose setae and two simple setae, carpus with six small simple setae, propodus with 12 long plumose setae, dactylus with 12 long plumose setae. Exopod 2-segmented, proximal segment with five simple setae, distal segment with four plumose setae. Epipodite with 22 small basial and seven long terminal setae. Pereiopods developed and functional for walking, covered with numerous long plumose setae (Figs 59 and 63). Chelipeds symmetric bearing small setae with propodus longer than other segments. Pereiopods 2 and 3 similar in structure. Pereiopod 4 longer and stronger than others with numerous long plumose setae, as illustrated. Pereiopod 5 short with dorsal and ventral margin showing numerous long plumose setae. Pleopods progressing distally, pair 1-4 usually with 10, 8, 8 and 6 long plumose setae, respectively (Fig. 57). Abdomen and telson additional somite 6th bearing 12 small simple setae (Figs 57 and 58). Telson semi-circular, wider than longer, with four small simple setae.

DISCUSSION

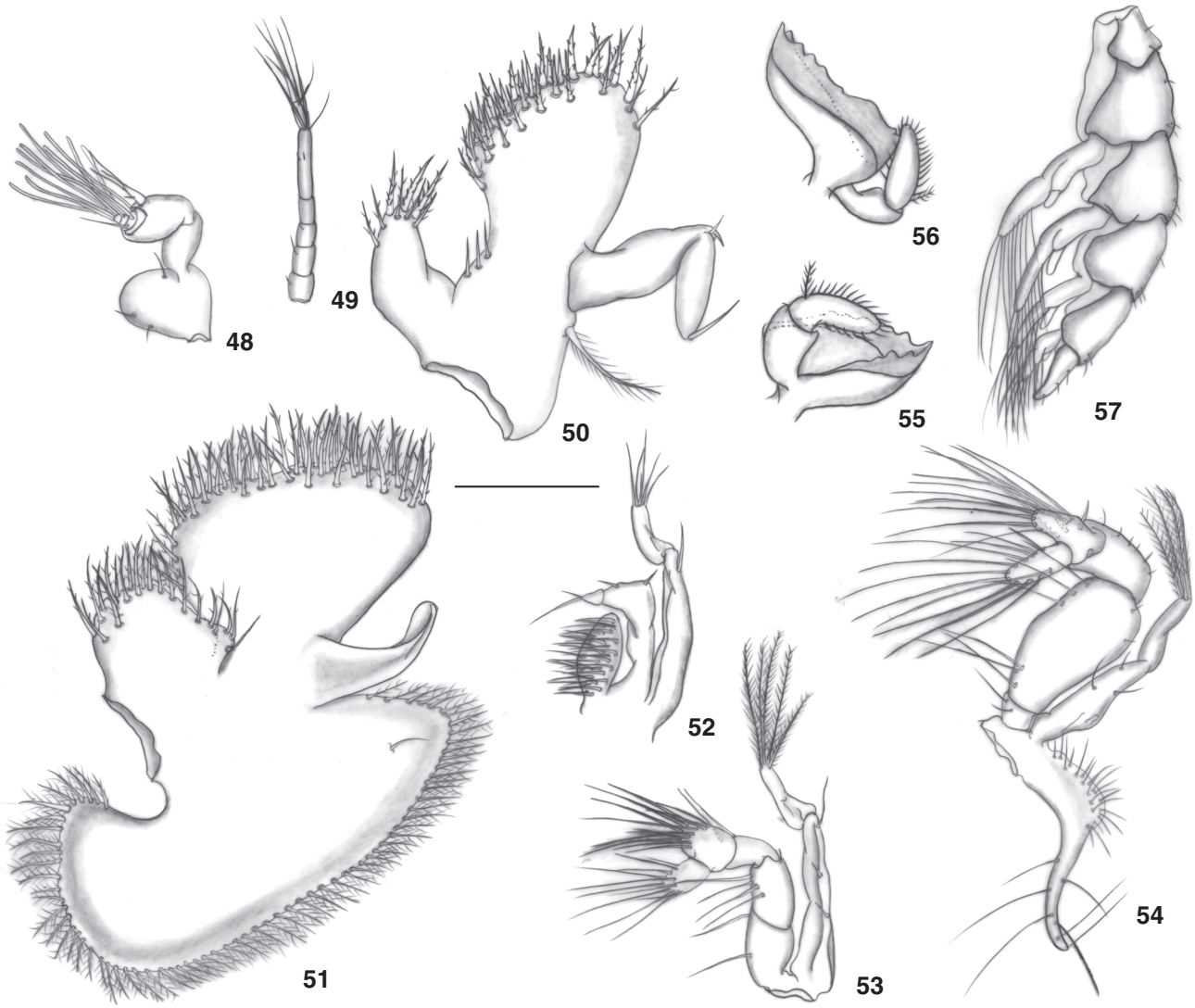
Descriptive studies of pinnotherid larvae have been carried out in recent yearsthus improving the classification of Pinnotheridae, mainly in morphological aspects. The number of zoeal stages within the Pinnotheridae varies from only one,



Figures 39-47. Appendages of zoea V of *Austinixa bragantina*: (39) antennule; (40) antenna; (41) mandible; (42) maxillule; (43) maxilla; (44) first maxilliped; (45) second maxilliped; (46) third maxilliped; (47) abdomen and telson. Scale bar: 39-40 = 0.15 mm; 41 = 0.18 mm; 42-43 = 0.2 mm; 44-46 = 0.35 mm; 47 = 0,55 mm.

in *Epulotheres* Manning, 1993 (Goodbody 1960) to five stages in some genera, such as *Asthenognathus* Stimpson, 1858, *Pinnaxodes* Heller, 1865, *Pinnixa* White, 1846, *Pinnotheres* Latreille, 1802, *Tumidotheres* Campos, 1989 as reported by Marques & Pohle (1996a) and *A. bragantina* in the present study.

Based on morphological characters, it is straightforward to discriminate zoeae of *Austinixa* from the other Pinnotherid genera. Similar to what is observed in species of *Fabia* Dana, 1851, *Pinnixa* , and *Parapinixa* Holmes, 1894, the zoeal stages of *A. bragantina* showed a structural enlargement in the fifth abdominal segment. This appears to be a distinct characteristic of *Fabia*, *Parapinixa*, *Pinnixa*, and *Austinixa*, as observed in *Fabia subquadrata* (Dana, 1851) (Irvine & Coffin 1960), *Parapinnixa affinis* Holmes, 1900 (Glassell 1933), *P. longipes* (Lockington, 1877) (Bousquette 1980), *P. rathbuni* Sakai 1934 (Sekiguchi 1978), *P. Gracilipes* Coelho, 1997 (Lima *et al*. 2006), *P. chaetopterana* Stimpson, 1860 (Hyman 1925), as well as *A. bragantina*, in the present study. In agreement with Hyman (1924), the first to fifth stage of *P. chaetopterana* showed an elongate deltoid extending in the middle of the furcal arch of the telson. This characteristic was also found in *A. bragantina* in the present study, *Zaops ostreum* (Say, 1817) by Sandoz &

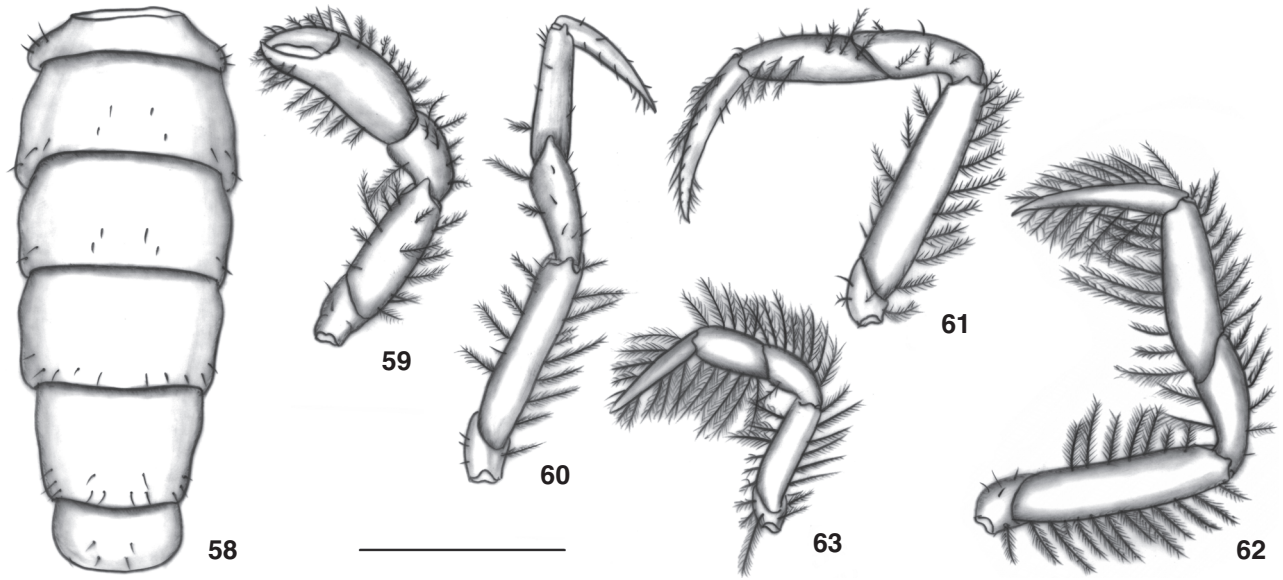


Figures 48-57. Appendages megalopa of *Austinixa bragantina*: (48) antennule; (49) antenna; (50) maxillule; (51) maxilla; (52) first maxilliped; (53) second maxilliped; (54) third maxilliped; (55-56) mandible; (57) abdomen and telson lateral view. Scale bar: 48-49 = 0.3 mm; 50-51 = 0.24 mm; 52-54 = 0.40 mm; 55-56 = 0.24 mm; 57 = 0.6 mm.

Hopkins (1947), *Gemmotheres chamae* Campos, 1996 by Roberts (1975) and absent in *P. gracilipes* by Lima *et al*. (2006), *P. longipes* by Bousquette (1980) and *P. rathbuni* by Sekiguchi (1978). Other morphological difference among zoea I of *A. bragantina* and Brazilian pinnotherids are provided in the table II.

Few differences were observed in abdomen and carapace of *A. bragantina* during zoeal stages. The first zoea stage shows telson with spines absent. However, similar to what is observed in *P. gracilipes* by Lima *et al*. (2006), the 2th to 5th zoeal stages show a pair of small spines projected laterally on lateral margin of telson (Figs 22, 30, 38 and 47). These spines appear to be unique for *A. bragantina* and *P. gracilipes*, as they are not present in closely-related species, such as *P. longipes* by Bousquette (1980) and *P. rathbuni* by Sekiguchi (1978). Other changes include the increase in the number of setae in the feeding appendages, the appearance of the endopod in the antenna and antennule and appearance of the pleopods and pereiopods starting from the 4th zoea, which are similar to those observed in other pinnotherids larvae (Sandoz & Hopkins 1947, Sekiguchi 1978, Bousquette 1980, Lima *et al*. 2006). The number of zoeal stages was similar in *A. bragantina*, *P. rathbuni*, *P. longipes* and *P. gracilipes*.

The megalopa of *A. bragantina* can be easily distinguished from the other already described pinnotherids species known in Brazilian coast through the morphological features. The cara-



Figures 58-63. Appendages megalopa of *Austinixa bragantina*: (58) abdomen and telson dorsal view; (59) first pereiopod; (60) second pereiopod; (61) third pereiopod; (62) fourth pereiopod, (63) fifth pereiopod. Scale bar: 58 = 0.6 mm; 59-63 = 0.84 mm.

pace in *A. bragantina* is smooth and semi-rounded similar to *C. stebbingi* (Rathbun, 1918) , *Pinnixa gacilipes* Coelho, 1997 and *D. crinitichelis* Moreira, 1901, *Z. ostreum* (Sandoz & Hopkins 1947) and *Tumidotheres maculatus* (Say, 1818) (Costlow & Bookhout 1966). Carapace is wider than long in *A. bragantina*, *P. gracilipes* and *T. maculatus* contrary to what is observed in *C. stebbingi* and *D. crinitichelis*, in which the carapace is nearly as wide as long, and *Z. ostreum* by Sandoz & Hopkins (1947) with carapace longer than wide. The small triangular rostrum observed in *A. bragantina* was similar to the reported for *P. gracilipes* (Lima *et al*. 2006) and clearly different of *C. stebbingi* (Marques & Pohle 1996b) with long and spine form rostrum, *D. crinitichelis* (Pohle & Telford 1981) with small and sub-triangular rostrum, *T. maculatus* (Costlow & Bookhout 1966) with rostrum terminates as two rounded lateral and one slightly larger, median projection. The rostrum in *Z. ostreum* (Sandoz & Hopkins 1947) is represented by two small lobes, covered by small setae.

The megalopal stage of *A. bragantina* and *T. maculatus* are more easily identified among pinnotherids crabs from Brazil. *Austinixa bragantina* is distinct due to the presence of a fine carina that cuts every cardiac area, whereas *T. maculatus* shows dorsal to the rostrum; a pair of spines projecting anteriorly and laterally over the eyestalks and one dorsal spine arises from the carapace on the median line. The general morphological differences among megalopal stages of *A. bragantina* and Brazilian pinnotherids are summarized in the table III.

The descriptions in the present study are sufficient to distinguish *A. bragantina* from the described pinnotherid larvae from the Brazilian coast. However, further larval studies are needed to increase the understanding of this taxonomic group.

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