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IMMATURE STAGES OF NEOTROPICAL *BEROSUS* (COLEOPTERA, HYDROPHILIDAE): *B. TOXACANTHUS* OLIVA, 1989, *B. COPTOGONUS* JENSEN-HAARUP, 1910, *B. CORNICINUS* KNISCH, 1922 AND *B. AURICEPS* BOHEMAN, 1859

Archangelsky, M., 2002. Larvae of Neotropical *Berosus* (Coleoptera, Hydrophilidae): *B. toxacanthus* Oliva, 1989, *B. coptogonus* Jensen-Haarup, 1910, *B. cornicinus* Knisch, 1922 and *B. auriceps* Boheman, 1859. – Tijdschrift voor Entomologie 145: 19-28, figs. 1-27, table 1. [ISSN 0040-7496]. Published 1 June 2002.

The larval stages of three Neotropical *Berosus* are described and illustrated for the first time: *B. toxacanthus* Oliva, 1989, *B. coptogonus* Jensen-Haarup, 1910 and *B. cornicinus* Knisch, 1922; the pupa of *B. auriceps* is also described. Comparative notes on first and third instar larvae, and information on their biology is included. Morphological characters that differentiate third instar larvae of these three species from other known Argentine *Berosus* larvae are tabulated.

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With over 260 species, *Berosus* Leach, 1817 is the largest genus of hydrophilids (Hansen 1999). About half of the known species are from the New World, and in South America alone there are over 80 species. Regardless of this diversity, very few descriptions of Neotropical *Berosus* larvae are available, with only three species having described larvae. Spangler (1966) published the description of an unidentified South American larva from Peru; Archangelsky (1999) described the larvae of two Argentine species: *B. aulus* Orchymont, 1941 and *B. auriceps* Boheman, 1859. The remaining larval descriptions of New World *Berosus* are from the Nearctic region (Richmond 1920, Wilson 1923, Van Tassell 1966, Archangelsky 1994, 1997).

In this paper the larvae and egg cases of *B. toxacanthus* Oliva, 1989, *B. coptogonus* Jensen-Haarup, 1910 and *B. cornicinus* Knisch, 1922, are described; the pupa of *B. auriceps*, not described by Archangelsky (1999), is included in this paper. Brief notes on the biology of these species are also provided.

The genus *Berosus* is subdivided in three subgenera: *B. (Berosus)*, *B. (Enoplurus)*, and the Australian *B.* (*Phelerosus)* (Hansen 1999). This subgeneric subdivision, still applied, is based mostly on European species, but it does not work satisfactorily for the Neotropical species. For this reason Oliva (1989, 1993), based on adult characters, proposed the subdivision of the Neotropical species in several complexes, her classification is followed here. *B. coptogonus* and *B. toxacanthus* belong to the *chalcocephalus*-complex, *B. cornicinus* to the *adustus*-complex, and *B. auriceps* to the *auriceps*-complex, the characters defining these groups are discussed in Oliva (1989, 1993). The *chalcocephalus*-complex is formed by seven Neotropical species: *B. chalcocephalus* Germain, 1865, *B. pallipes* Brullé, 1841, *B. dehiscens* Jensen-Haarup, 1910, *B. coptogonus*, *B. toxacanthus*, and *B. navatus* Orchymont, 1940.

The *adustus*-complex includes four Neotropical species: *B. cornicinus*, *B. adustus* Knisch, 1922, *B. asphaltinus* Knisch, 1922, and *B. bruchianus* Knisch, 1924.

The *auriceps*-complex is formed by only three Neotropical species: *B. aulus* Orchymont, 1940, *B. auriceps*, and *B. ethmonotus* Oliva, 1989.

B. coptogonus is endemic to central and northern Argentina (provinces of Mendoza, Córdoba, Santiago del Estero, Chaco, La Rioja and Salta). *B. toxacanthus* is found in northwestern Argentina (provinces of Salta

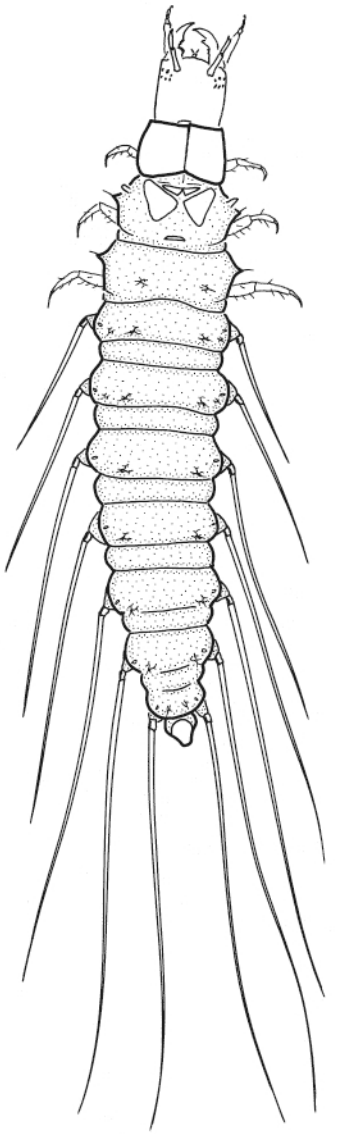


Fig. 1. *Berosus toxacanthus*, third instar larva.

and La Rioja), so this is the first record of this species from central Argentina, Córdoba province. *B. cornicinus* is also endemic to central and northwestern Argentina (Salta and Córdoba provinces). *B. auriceps* has a more widespread distribution, both northwestern and northern (parts of Brasil and Paraguay, and Argentina: provinces of Catamarca, Córdoba, La Rioja, Salta, Jujuy, Tucumán and Misiones).

MATERIAL AND METHODS

All larvae and pupae were reared from adults collected in the field and brought alive to the laboratory. Adults were kept in small plastic aquaria (20 cm long x 10 cm wide x 8 cm high) with gravel, small pieces of wood, and some aquatic plants or algae found in collecting sites. Adults fed on both plant material and commercial fish food which was provided twice a week (this added some protein to the diet).

Egg cases were constructed below the water's surface either on small rocks or on the vegetation. After hatching, larvae were transferred to small, individual containers (in order to avoid cannibalism); tissue culture plates with 12 cells (for first and second instars) and six cells (for third instars) were used. Each cell had some sand and a pair of small sticks (for the larvae to cling on); the depth of the water was kept between 5 and 8 mm, and was changed every other day. Larvae were fed chironomid larvae, small oligochaetes, ostracods and other small invertebrates. Pupae could be obtained only for *B. auriceps*; to accomplish this prepupae were placed in small, inclined, petri dishes with sand on the upper half and water on lower one. Prepupae were left there for several days until they dug a pupal chamber, water was added every other day in order to prevent the sand from drying.

The specimens were fixed with boiling water and stored in 75% ethyl alcohol. Larvae were cleared in lactic acid, dissected and mounted on slides for observation and description; the medium used was Hoyer's. Pupae were punctured under the wing pads after fixation in order to avoid swelling. Drawings were done using a Leica DML compound microscope with camera lucida; the drawings were scanned and the plates were put together with the use of Adobe Photoshop(r) and Adobe Illustrator(r).

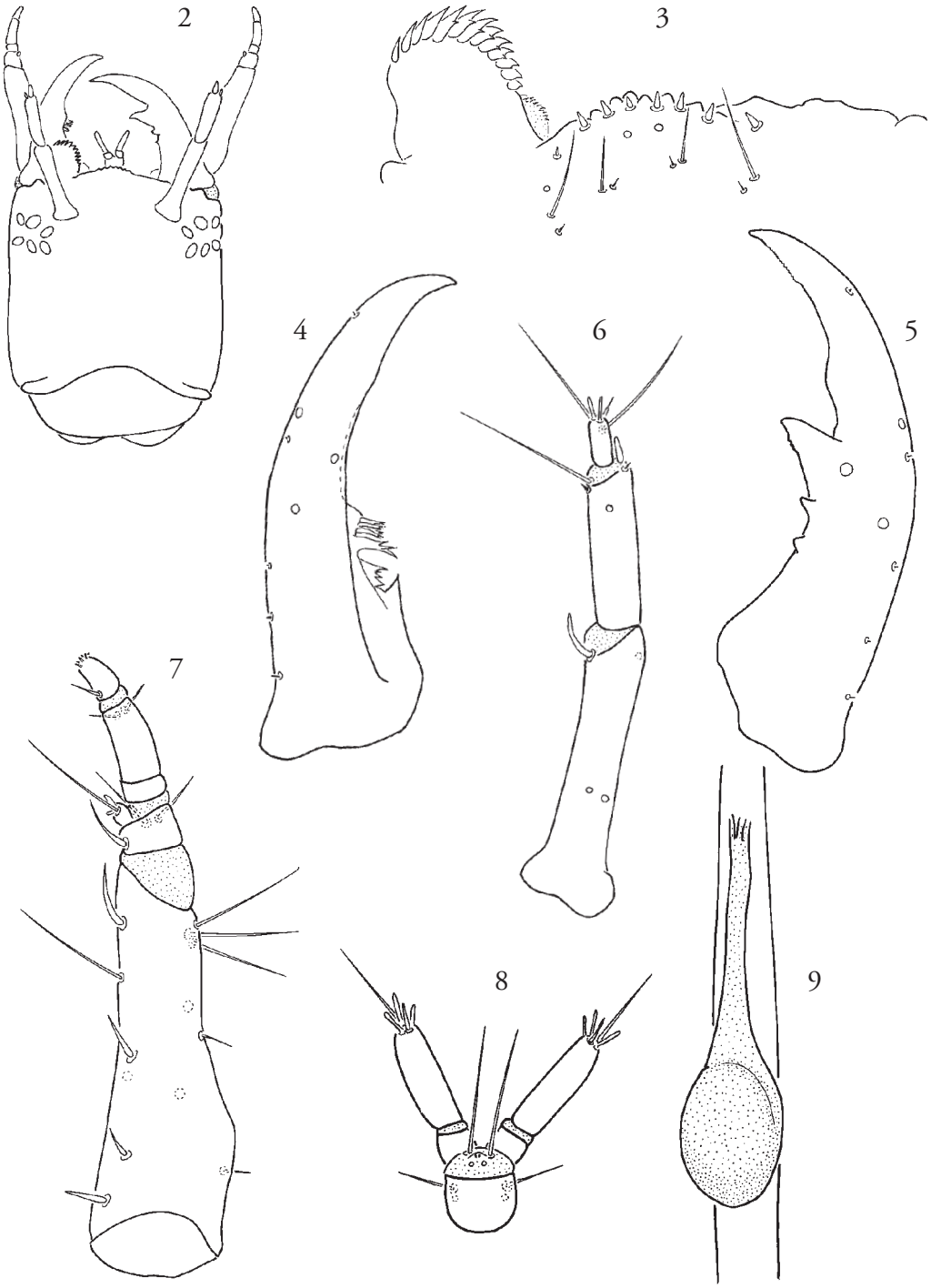
Adult specimens were identified using the keys by Oliva (1989, 1993).

DESCRIPTIONS

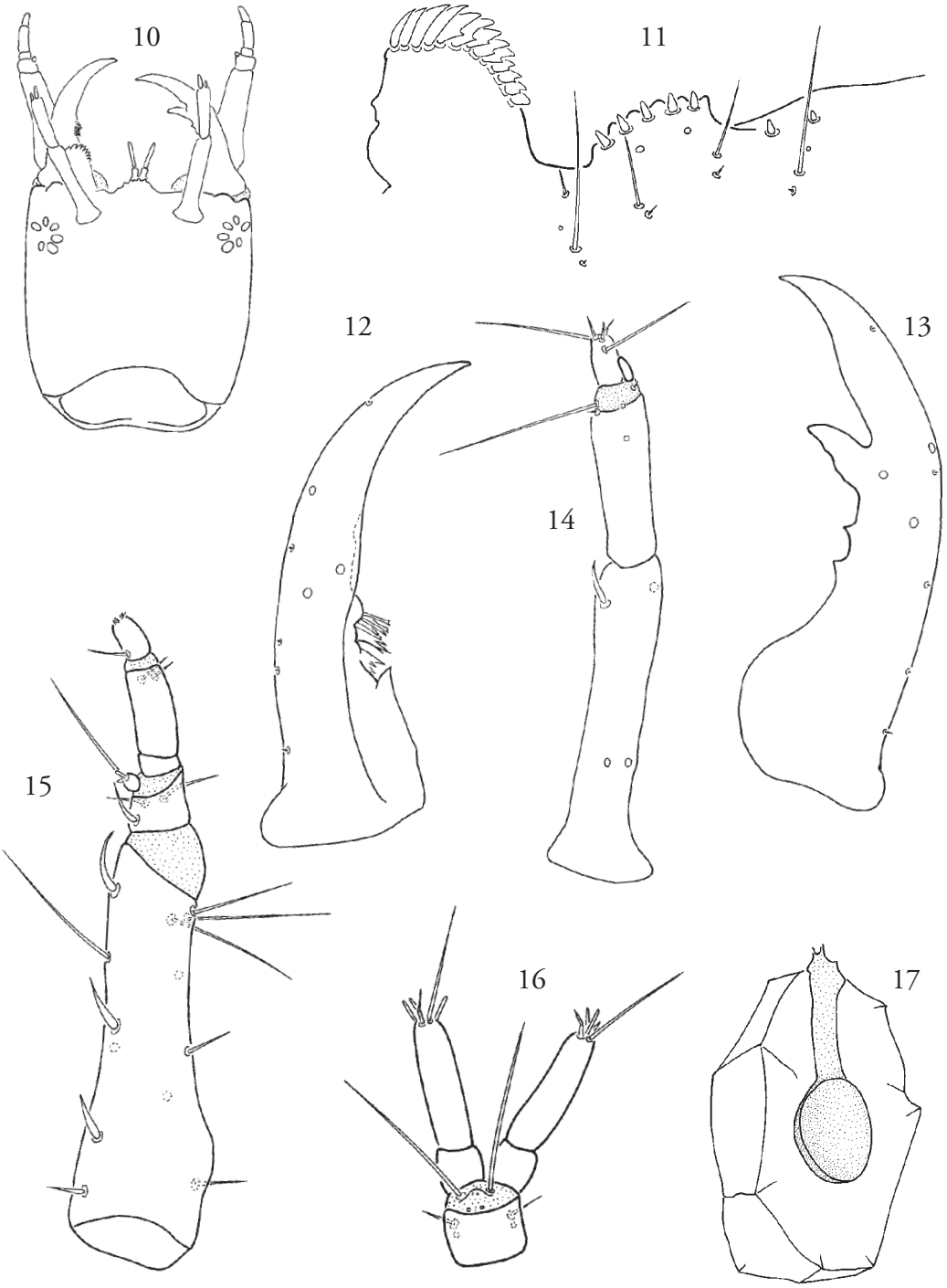
Berosus toxacanthus Oliva, 1989
(figs. 1-9)

Material studied. – Argentina, Córdoba province, Salinas Grandes, ditch at intersection of Road 60 and dirt road to Totoralejos, 185 m, 29° 37' 26" S, 64° 50' 23" W, pH 9.5-9.9, 13.III.1999.

Egg case. – Size: 1.15-1.30 mm long, without mast (n= 3). Whitish, attached to algae or stems of aquatic plants in the aquarium, rarely at the bottom. Made of two layers, first one laid on substrate, second one covering eggs, light brown in color. Final shape elliptical (fig. 9), length of mast variable, one to two times the



Figs. 2-9. *Berosus toxacanthus*, third instar larva. – 2, Head, dorsal view; 3, labroclypeus, dorsal view; 4, left mandible; 5, right mandible, dorsal view; 6, right antenna, dorsal view; 7, right maxilla, dorsal view; 8, labium, dorsal view; 9, egg case.



Figs. 10-17. *Berosus coptogonus*, third instar larva. – 10, Head, dorsal view; 11, labroclypeus, dorsal view; 12, left mandible; 13, right mandible, dorsal view; 14, right antenna, dorsal view; 15, right maxilla, dorsal view; 16, labium, dorsal view; 17, egg case.

length of the egg case. Eggs easily seen through the silk cover, two eggs per case, always side by side; eggs separated by thin layer of silk.

Third instar larva. – Size: 5.0-7.2 mm long (n= 8). Color creamy-yellow, sclerotized areas light brown. Abdomen bearing seven pairs of tracheal gills (fig. 1); body with sparse and fine hairs.

Head capsule subquadrangular (fig. 2), epicranial sulcus not defined; cervical sclerites absent. Ocular areas with six stemmata on each side, arranged as in two groups of three.

Labroclypeus asymmetrical (fig. 3), with short nasale. Nasale with six, poorly defined, small blunt teeth arranged in convex semicircle; six short setae intercalated with nasale teeth, two more setae on each side of nasale, towards epistomal lobes. Lateral lobes of epistome strongly asymmetrical; right one not developed, lacking setae; left one large, covering base of mandible, projecting further than nasale, with 12-13 strong and curved spines, becoming shorter towards inner margin, four or five outer ones simple, remaining ones bearing small median tooth.

Mandibles (figs. 4-5) strongly asymmetrical. Right mandible minutely serrated near apex, with three teeth on inner margin, distal one large and rudimentarily serrated, remaining two small and blunt. Left mandible with four teeth, basal one sharp and pointing upwards; second tooth from base pointing mediad, with sharp points on inner edge; third one with three or four sharp distal points; distal tooth triangular, pointing mediad, bearing a group of stout spines with branched apices.

Antenna three-segmented (fig. 6). Basal segment longer than other two combined, with strong, curved and blunt subapical seta on inner margin. Second segment bearing three subapical setae, one short and one long on inner margin, one short on outer margin; also carrying sensory appendage, half as long as last antennal segment. Third segment the shortest, with four apical setae (three short and one long) and one long subapical seta projecting mediad.

Maxilla (fig. 7) five-segmented, with short, irregularly shaped cardo. Stipes stout and long, with five setae on inner margin and five on outer margin, ventral face with three campaniform sensilla. Palp four-segmented; first segment subquadrate, bearing one curved seta on inner margin and two slender setae on ventral side, also with small, sclerotized appendage bearing three apical setae on inner margin. Second segment short, lacking setae; third segment the longest of palp, with two subapical setae, one on ventral side; last segment with one long basal seta, projecting mediad, and six or seven short apical setae.

Labium slender (fig 8). Prementum small and subquadrate, with reduced ligula on dorsal side; one long seta on each side of ligula; ventral side of prementum with one long seta at base of each palp. Palpi two-segmented, basal segment short, distal segment three times as long as basal one, bearing five apical setae, one long and four short.

Pronotum strongly sclerotized, subdivided by fine sagittal line. Mesonotum with two pairs of subtriangular sclerites, inner pair much narrower and smaller than outer one; metanotum lacking sclerites, bearing one pair of small setiferous lobes on each side of midline. Prosternum with large subrectangular sclerite, divided by sagittal line; meso- and metasterna without sclerites. Meso- and metathoracic segments each with lateral tubercle.

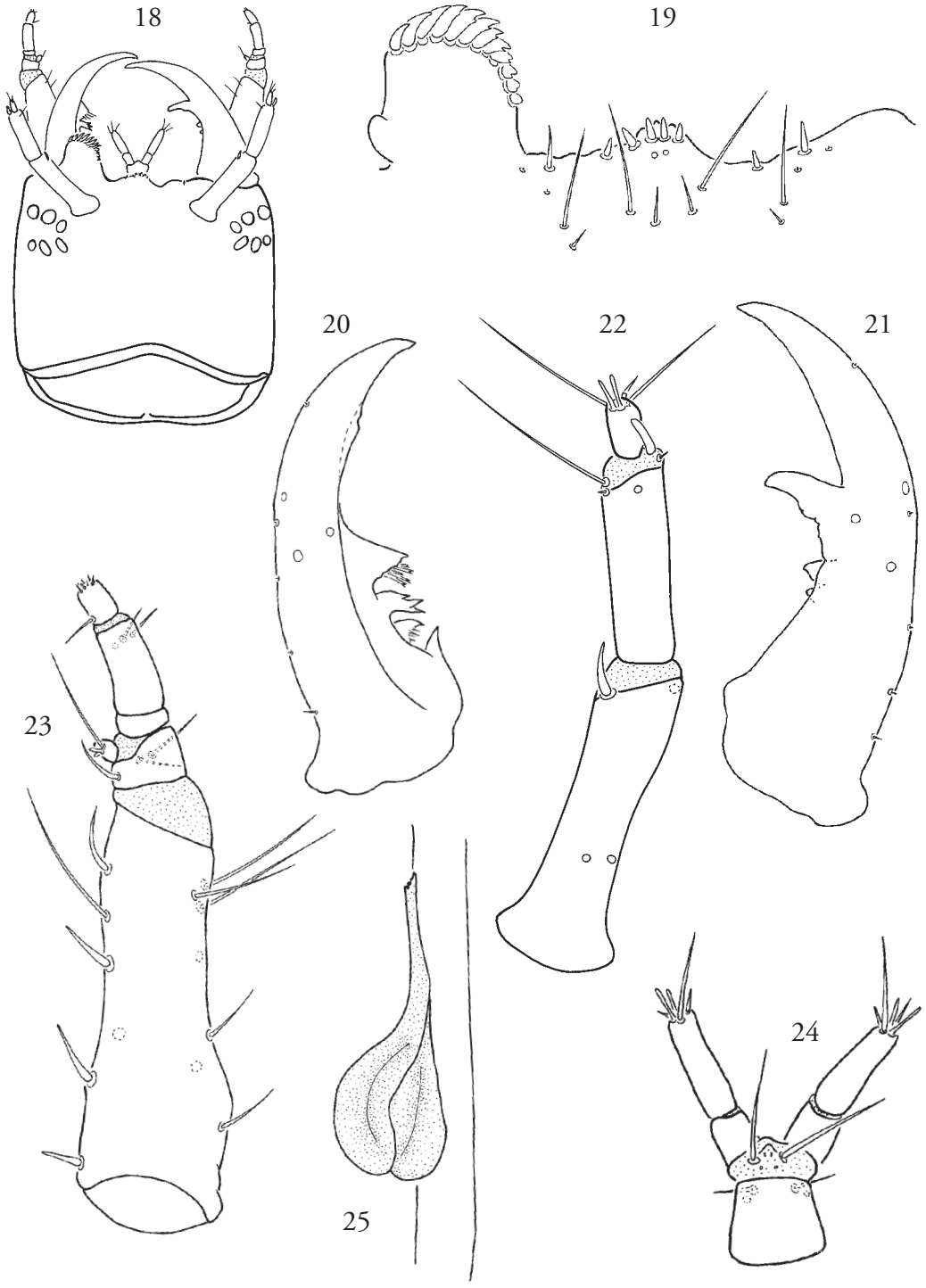
Legs five-segmented, long, easily seen in dorsal view (fig. 1). First pair the shortest, third pair the longest. Coxae widely separated and elongate; trochanters small, subtriangular; femora longer and slender than coxae; tibiotarsi slender, as long as coxae; pretarsal claw long and slender, curved inwards and bearing strong subbasal spine.

Abdomen ten-segmented, with segments IX and X strongly reduced; segment VIII small, subtriangular, with a subcircular tergite. Abdominal cuticle covered by sparse and fine hairs, without patches of cuticular spines. Segments I to VII similar in shape, tapering towards distal end, each segment subdivided by transverse fold and bearing a long, slender tracheal gill on each side, base of gill with a sclerotized ring; small conical tubercle present at base of each tracheal gill, carrying non-functional spiracle. Terga of segments I and II with two pairs of setiferous tubercles on dorsal view; segments III to VII with only one pair.

Comparative notes on first instar larvae. – Epicranial suture shaped as an inverse bell, coronal suture absent; stemmata smaller. Labroclypeus less prominent, nasale with six to seven small teeth. Antennae proportionally wider, especially basal segment, sensorium about ⅔ as long a third antennal segment. Maxillae similar to third instars, except for stouter stipes. Left mandible with coarse serration; right mandible with distal end slightly serrated, distal tooth also serrated. Ligula of labium larger, projecting past base of palpi; palpi longer, reaching distal teeth of mandibles. Thorax and abdomen similar, sclerotization less developed; tracheal gills longer in relation to body length, base of gills without sclerotized ring.

Size: first instar larvae 1.30-1.80 mm (n= 7); second instar larvae 4.10-4.25 mm (n= 2).

Bionomical notes. – *B. toxacanthus* adults were collected in pools of saline temporary water, living among the aquatic vegetation. In the laboratory adults were very active swimmers. The egg cases were constructed on algae or stems of aquatic plants. De-



Figs. 18-25. *Berosus cornicinus*, third instar larva. – 18, Head, dorsal view; 19, labroclypeus, dorsal view; 20, left mandible; 21, right mandible, dorsal view; 22, right antenna, dorsal view; 23, right maxilla, dorsal view; 24, labium, dorsal view; 25, egg case.

velopmental time of *B. toxacanthus* larvae was the fastest of the three species studied (from egg to moult into third instar larvae 24-30 days). The duration of the different stages was as follows: four to five days for the eggs to hatch, nine to 11 days for first instar larvae to moult into second instars, and nine to 14 days for these to moult into third instars. Pupae were not obtained. Cannibalism was observed among first and second instar larvae, but was not common; third instars were not tested for cannibalism.

The larvae were fed mostly with small oligochaetes and chironomid larvae.

Berosus coptogonus Jensen-Haarup, 1910
(figs. 10-17)

Material studied. – Argentina, Córdoba province, Salinas Grandes, ditch at intersection of Road 60 and dirt road to Totoralejos, 185 m, 29° 37' 26" S, 64° 50' 23" W, pH 9.5-9.9, 13.III.1999.

Egg case. – Size: 1.60-1.73 mm long, without mast (n= 4). Attached to pieces of wood, small rocks or stems at the bottom of the aquarium (fig. 17). Similar to that of *B. toxacanthus*, darker in color, eggs not easily seen through silk cover. Mast wider than that of *B. toxacanthus*, also variable in length, one to two times length of egg case. One or two egg per case.

Third instar larva. – Size: 5.8-7.9 mm long (n= 11). Head capsule (fig. 10) subrectangular, similar to that of *B. toxacanthus*, stemmata arranged in two groups of three. Labroclypeus (fig. 11) with more prominent nasale, nasale with six blunt teeth and only five short setae intercalated with teeth, two more short setae to the right of nasale and one to the left; left lobe of epistome bearing larger spines, outer four simple, remaining with an inner tooth. Mandibles asymmetrical (figs. 12-13), right one minutely serrated towards distal end; left mandible with branched setae of distal inner tooth covering part of third tooth. Antenna (fig. 14) similar to that of *B. toxacanthus*. Maxilla (fig. 15) as that of *B. toxacanthus* except for position of one sensorium on outer margin. Labium (fig. 16) with subquadrate prementum, similar to that of *B. toxacanthus*. Abdominal segments I-VII with two patches of dorsal cuticular microspines. Setiferous lobes of metathorax and abdomen as those of *B. toxacanthus*.

Comparative notes on first instar larvae. – Epicranial suture shaped as an inverse bell, coronal suture absent; stemmata smaller. Labroclypeus less prominent, nasale with five small teeth. Antennae proportionally wider, especially basal segment, sensorium about ⅔ as long a third antennal segment. Maxillae similar to third instars, except for stouter stipes. Left mandible with coarse serration; right mandible with distal end slightly serrated, distal tooth also serrated. Ligula of labium larger, projecting past base of palpi, palpi longer, reaching distal teeth of mandibles. Thorax and abdomen less sclerotized; tracheal gills longer in relation to body length, base of gills without sclerotized ring.

Size: first instar larvae 1.25-1.75 mm (n= 8); second instar larvae 3.75-4.60 mm (n= 5).

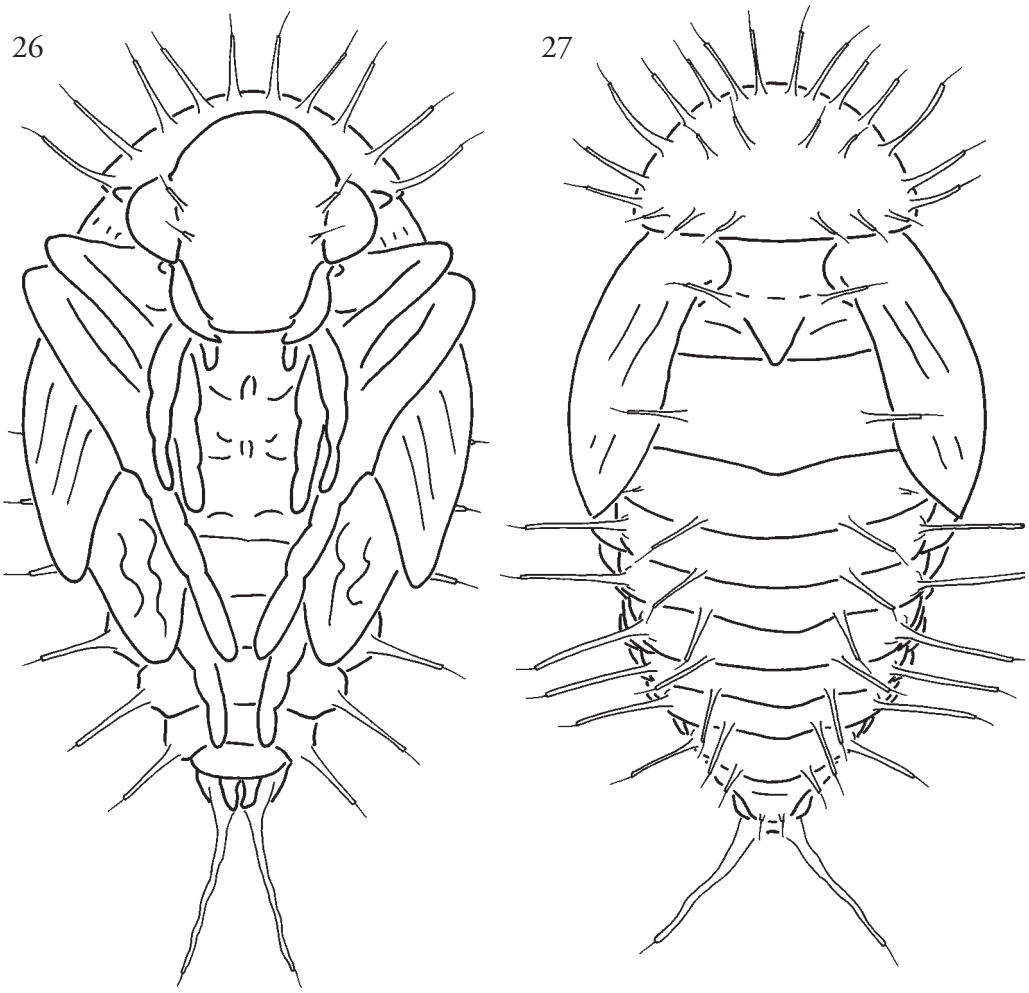
Bionomical notes. – *B. coptogonus* adults were collected in the same habitat as those of *B. toxacanthus*. Adults were very active swimmers. The egg cases were constructed on pieces of wood, small rocks or stems at the bottom. Developmental time of *B. coptogonus* larvae was slower than that of *B. toxacanthus* (from egg to moult into third instar larvae 30-35 days). The duration of the different stages was as follows: six to seven days for the eggs to hatch, 11 to 13 days for first instar larvae to moult into second instars, and 11 to 15 days for these to moult into third instars. Pupae were not obtained. Small oligochaetes and chironomid larvae were used as food.

Berosus cornicinus Knisch, 1922
(figs. 18-25)

Material studied. – Argentina, Córdoba, Las Mojarras Creek, North of San Roque Lake, 650 m, 3.IV.1999.

Egg case. – Size: 1.70-1.75 mm long, without mast (n= 2). Attached to algae or rocks, in very shallow water (fig. 25). Suboval in shape, lower layer attached to substrate, upper layer united to lower one only at middle, therefore forming narrow flaps at sides and bottom. Mast variable in size, one to two times the length of egg case. Two eggs per case.

Third instar larva. – Size: 5.8-7.8 mm long (n= 7). Head capsule (fig. 18) subrectangular, similar to that of other two species, stemmata arranged in two groups of three. Labroclypeus (fig. 19) with small nasale, which bears three blunt teeth and four short setae intercalated with teeth of nasale, two more short setae on either side of nasale; left lobe of epistome with 12 stout spines, outer four simple, remaining ones with inner tooth. Mandibles asymmetrical (figs. 20-21), with very finely serrated distal area on right mandible, two small basal teeth closer than in other two species; left mandible with third inner tooth trifurcate, slightly curved downwards. Antenna (fig. 22) similar to that of the other two species, distal sensory appendage of second segment longer. Maxilla (fig. 23) as that of other two species except for position of sensorium on inner margin, placed closer to second inner seta. Labium



Figs. 26-27. *Berosus auriceps*, pupa. – 26, Ventral view; 27, dorsal view.

(fig. 24) with subquadrate prementum, basal palpal segment proportionally longer than that of the other two species. Tergal areas of abdomen, and membranous parts of metathorax, completely covered by fine cuticular microspines. Base of gills without sclerotized ring. Abdominal segment III with two pairs of setiferous lobes instead of one.

Comparative notes on first instar larvae. – Epicranial suture shaped as an inverse bell but with arms more separated than in other two species, coronal suture absent; stemmata smaller. Labroclypeus less prominent, nasale with three small teeth. Antennae proportionally wider, especially basal segment, sensorium almost as long as third antennal segment. Maxillae with stouter stipes. Left mandible with coarse serration; right mandible not serrated, basal tooth of right mandible very small, distance between first and second teeth greater than in third instar larvae. Ligula of labium poorly developed, palpi not reaching distal teeth of mandibles. Thorax and abdomen less sclerotized; tracheal gills longer in relation to body length, base of gills as in third instars, without sclerotized ring.

Size: first instar larvae 1.75-1.83 mm (n= 5); second instar larvae 4.20 mm (n= 1).

Bionomical notes. – *B. cornicinus* adults were collected in a small and shallow creek running along a narrow valley. This creek is temporary and carries water during the rainy season (usually the summer and autumn), the waters are cool and clear. Adults were collected in those segments of the creek where it had a rocky bottom and filamentous algae (*Chara* sp.) grew on the margins; deeper areas with a muddy bottom of the same creek were inhabited by *B. auriceps* and other hydrophilids. Larvae reared in the laboratory were sluggish and covered by dirt, suggesting that in nature they must live among the algae. Larval development was slow (from egg to moult into third instar 42 to 46 days). First instars emerged from the egg cases 10-11 days after the cases were constructed; moult to second instar took place 15-18 days later; moult to third instar took place 14-17 days later. No pupae were obtained. The larvae were fed with small oligochaetes and chironomid larvae. First instar larvae emerged from the egg case by chewing a small hole on one side of the mast, to aid themselves in the process they produced slow dorsoventral movements until they got their legs out of the case, then they used the legs to pull out the rest of the body.

Berosus auriceps Boheman, 1859
(figs. 26-27)

Material studied. – Argentina, Córdoba, Las Mojarras Creek, North of San Roque Lake, 650 m, 2.II.2001.

Pupa: Size: 4.7-4.9 mm long, not counting cerci (n=2). Color white, eyes of pharate adult red to brown. Antennae almost completely covered by head and pronotum(fig. 26); mouthparts visible in ventral view, maxillary palps long, projecting as far as base of mesotarsi. Pro- and mesothoracic legs visible in ventral view, metathoracic legs covered by wingpads, only distal segments of tarsi visible, reaching seventh abdominal segment.

Styli on head, thorax and abdomen as follows (figs. 26-27). Head with two pairs of supraorbital styli on inner margin of each eye, upper pair twice as long than basal one. Pronotum with 24 styli, five pairs on anterior margin, five pairs on posterior margin, two pairs on disc; pairs of anterior margin longer than remaining styli. Mesonotum with one stylus on each side of scutellum; metanotum also with one pair. Abdominal segments I to VII with two pairs of styli, one

Table 1. Comparison between third instar larvae of *B. toxacanthus, B. coptogonus, B. cornicinus, B. aulus,* and *B. auriceps.*

Character	*B. toxacanthus*	*B. coptogonus*	*B. cornicinus*	*B. aulus*	*B. auriceps*
Size (mm)	5.0-7.2	5.8-7.9	5.8-7.8	8.5-11.0	9.1-10.0
Cervical sclerites	Absent	Absent	Absent	Present, narrow	Absent
Nasale, number of teeth	6	5	3	5	3
Nasale, number of setae intercalated with teeth	6	5	4	4	4
Antenna, length of sensorium compared to third antennite	About half	About half	More than half	More than half	About half
Right mandible, distance between inner teeth	Three inner teeth equidistant	Three inner teeth equidistant	Basal two closer than to distal tooth	Three inner teeth equidistant	Three inner teeth equidistant
Left mandible, distance between 3rd and 4th inner teeth	Close to each other	Close to each other	2nd, 3rd and 4th inner teeth equidistant	Close to each other	Close to each other
Maxilla, outer camp. sensillum	Close to 2nd outer seta	Equidistant between 2nd and 3rd outer setae	Equidistant between 2nd and 3rd outer setae	Equidistant between 2nd and 3rd outer setae	Equidistant between 2nd and 3rd outer setae
Maxilla, inner camp. sensillum	Close to 3rd inner seta	Close to 3rd inner seta	Close to 2nd inner seta	Close to 3rd inner seta	Close to 3rd inner seta
Cuticular microspines on tergal areas of abdomen	Absent	Restricted to central areas	All over tergal areas	All over tergal areas	All over tergal areas
Gills, sclerotized ring at base	Present	Present	Absent	Present	Present

on each side of midline of terga, pleural areas lacking styli; segment eight with one distal pair of short styli; segment nine bearing two long cerci.

Comparative notes with larvae of other Berosus species from Argentina

There are few descriptions of South American *Berosus* larvae, one unidentified larva from Perú, described by Spangler (1966), and two species described by Archangelsky (1999), *B. aulus* and *B. auriceps*.

Most known *Berosus* larvae are very homogeneous in their morphology, and so far the most distinct morphological characters that seem useful to tell species apart are found in the labroclypeus (shape and size of nasale), the mandibles (number and shape of inner teeth), the antennae (size of the sensorium), the maxillae (position of some ventral campaniform sensilla, and the abdomen (gills and cuticular microspines).

The differences among third instar larvae of the five known Argentine species (*B. toxacanthus, B. coptogonus, B. cornicinus, B. aulus* and *B. auriceps*) are summarized in table 1.

Acknowledgements

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