

Description of immatures of *Berosus decolor* Knisch, 1924 (Coleoptera: Hydrophilidae: Berosini), with emphasis on chaetotaxy and morphometry

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Abstract

The preimaginal stages of *Berosus decolor* Knisch, 1924 are described for the first time, including illustrations of the egg case and all larval instars. Larval morphology of this species is studied emphasizing chaetotactic and morphometric analyses. The egg case lacks a mast and contains 2–3 eggs; all larval instars are characterized by the following features: presence of 6 teeth on anterior margin of nasale; left epistomal lobe carrying 14–16 strong and curved setae (gFR2); seta FR1 elongate, inserted posteriorly to seta PA7; pore FR14 absent; parietale with spinulae in the area between setae PA5 and PA12; mandibles asymmetrical; seta MN1 minute; first antenommere with a digitiform projection on distal inner margin; antennal sensilla AN8 and AN9 absent; maxillary sensilla MX10 hair-like, MX11 and MX16 with an intermediate shape between hair-like and spine-like; labial sensilla LA4 and LA15 absent; seta LA2 present, minute; abdomen with 7 pairs of tracheal gills. The morphology of the egg cases and larvae within the genus *Berosus* are compared and discussed.

Key words: Water scavenger beetles, Berosini, egg case, larvae, sensilla

Introduction

The cosmopolitan genus *Berosus* Leach 1817, includes 287 species (Hansen 1999; Short & Fikáček 2011, Oliva & Short 2012), of which 144 occur in the New World. Members of this genus are common inhabitants in a wide range of aquatic habitats throughout South America, where they have radiated considerably resulting in almost 100 described Neotropical species (Archangelsky 1999, Oliva & Short 2012). In spite of this high diversity, few descriptions of Neotropical *Berosus* larvae are available, and studies including detailed chaetotactic analyses are lacking. Spangler (1966) described an unidentified species from Perú, Archangelsky (1999, 2002) described *B. aulus* Orchymont, 1941; *B. auriceps* Boheman, 1859; *B. toxacanthus* Oliva, 1989; *B. coptogonus* Jensen-Haarup, 1910 and *B. cornicinus* Knisch, 1922. Also, Fernández & Campos (2002) described the immatures of *B. alternans* Brullé, 1841. The remaining larval descriptions of New World *Berosus* are from the Nearctic region (Richmond 1920, Wilson 1923, Van Tassell 1966, Archangelsky 1994, 1997).

The current classification of *Berosus* is based mainly on adult morphology (Oliva, 1989). As with most holometabolous insects, the different life stages within Hydrophiloidea are very dissimilar morphologically, and the characters provided by immatures have proven to be very useful in the development of more comprehensive classificatory systems (e.g., Archangelsky 2004, 2008; Fikáček *et al.* 2013; Minoshima *et al.* 2013; Fikáček & Vondráček 2014). In the past recent years, the study of the preimaginal stages of Hydrophilidae has improved considerably with the incorporation of detail morphometric and chaetotactic analyses (e.g., Fikáček *et al.* 2008; Torres *et al.* 2008, 2011, 2014; Minoshima & Hayashi 2011, 2012, 2015; Minoshima *et al.* 2012, 2013). In particular, larval chaetotaxy is recognized as a source of characters potentially useful both in the establishment of diagnostic differences among species and in the development of phylogenetic hypotheses (Bousquet & Goulet 1984; Alarie 1997, 1998; Fikáček 2006). Archangelsky (1999) suggested that a worldwide revision of *Berosus* is needed, and that larval and pupal characters may support the subdivision of the genus into natural groups.

Berosus decolor Knisch, 1924 is a pioneer species, abundant in the basins of the great South American rivers with a wide distribution in Argentina, Paraguay and Brazil. It is currently included in the *reticulatus*-group, defined by Oliva (1989) based on adult characters such as the presence of adhesive discs on the two basal segments of the protarsus, and the shape of the male genitalia. This group includes nine Neotropical species: *B. decolor*, *B. reticulatus* Knisch, 1921; *B. ghanicoides* Orchymont, 1941; *B. ghanicus* Orchymont, 1941; *B. borelli* Knisch, 1925; *B. festai* Knisch, 1925; *B. erraticus* Mouchamps, 1921; *B. ambogynus* Mouchamps, 1963 and *B. brevibasis* Oliva, 1989.

In this paper, the larvae and egg case of *B. decolor* are described and illustrated for the first time, with particular emphasis on morphometric and chaetotaxic analyses of the cephalic capsule, head appendages and legs. This represents the first morphometric treatment for larvae of the genus *Berosus*. Also, a detailed comparison of the morphology and chaetotaxy of the known species of the genus is performed, and significant differences are discussed.

Material and methods

Source of material. Adults of *B. decolor* were collected in the field and brought alive to the laboratory in order to obtain egg cases and larvae. Breeding adults were collected in Mburucuyá National Park (Corrientes Province, Argentina) in December 2012. Twenty-four egg cases, 20 larvae of instar I, 8 of instar II and 4 of instar III were used for the descriptions. No pupae were obtained.

Methods. Larvae were fixed with boiling water and then transferred to 96% ethyl alcohol. Egg cases were fixed and preserved in 96% ethyl alcohol. Larval specimens were cleared in lactic acid for several days, dissected and mounted on glass slides with polyvinyl-lacto-glycerol. Observation (at magnifications up to 1000x) and drawings of larvae were made using an Olympus CX41 compound microscope equipped with a drawing tube. Egg cases were drawn with a Leica MZ6 stereoscopic microscope equipped with a drawing tube. The material studied is held in the larval collection of the Laboratory of Entomology, Buenos Aires University, Argentina. Adults were identified using the key of Oliva *et al.* (2002).

Taxonomic descriptions. The methods and terms used in the present paper follow those employed in recent papers dealing with the larval morphology and chaetotaxy of members of Hydrophilidae. The reader is referred to Byttebier & Torres (2009), Torres *et al.* (2011) and Minoshima & Hayashi (2011) for additional explanations of the terms used here.

Morphometric analysis. Several individual measurements were used to calculate ratios, which characterize body shape. Larval structures to be measured were adjusted as parallel as possible to the plane of the objective. The following measurements were taken (with abbreviations shown in parentheses): total body length (TL); maximum body width measured at level of metathorax (MW); head length measured dorsomedially from anterior margin of labroclypeus to occipital foramen (HL); maximum head width measured at level of stemmata (HW); length of antenna (AL) derived by adding the lengths of the first (A1), second (A2) and third (A3) antennomere; length of antennal sensorium (SEL); length of stipes (SL); length of maxillary palpus (MPL) derived by adding the lengths of the first (MP1), second (MP2), third (MP3) and fourth (MP4) palpomere; length of maxilla (ML) derived by adding SL and MPL (cardo omitted); length of labial palpus (LPL) derived by adding the lengths of the first (LP1) and second (LP2) palpomere; length of prementum (PrmtL) measured from its base to the base of LP1; maximum width of prementum (PrmtW); length and width of mentum (MtL, MtW) could not be measured due to the soft and retractile nature of the structure, which rendered unreliable values; therefore, these measures are not shown in the table, and the PrmtW/MtW ratio could not be calculated; lengths of pro- (Leg 1 L), meso- (Leg 2 L) and metathoracic (Leg 3 L) leg derived by adding the lengths of coxa (CO), trochanter (TR), femur (FE), tibiotarsus (TITA) and claw (CL). The length of trochanter includes only the proximal portion, the length of distal portion is included in the femoral length.

Chaetotaxy. Primary (present in first-instar larva) and secondary (arising in later instars) setae and pores were distinguished in the cephalic capsule and head appendages. Sensilla present in first-instar larvae of *B. decolor* were labeled by comparison with the ground plan of chaetotaxy of the family Hydrophilidae (Fikáček *et al.*, 2008; Byttebier & Torres, 2009). Homologies were established using the criterion of similarity of position (Wiley, 1981). Sensilla were coded with two capital letters, in most cases corresponding to the first two letters of the name of the

structure on which they are located, and a number. The following abbreviations were used: AN = antenna, FR = frontale, LA = labium, MN = mandible, MX = maxilla, PA = parietale. Setae located at the apices of maxillary and labial palpi were extremely difficult to distinguish due to their position and small size. Accordingly, they are not well represented in the drawings. Sensillar series on the legs were named considering their position on each segment, using the following abbreviations (alone or in combinations), according to Torres *et al.* (2011): A = anterior, D = dorsal, Di = distal, Me = medial, P = posterior, Pr = proximal, V = ventral.

Results

Diagnosis of the immatures of the genus *Berosus*

Egg case: whitish, oval-shape, sometimes elongate, usually flat. With or without mast; when present up to two or three times the length of the egg case. Bearing 1–25 eggs depending on the species. **Larvae:** Head capsule subquadrangular; epicraneal sulcus present; frontal sulci inverse bell shaped, defined or not in instar III; coronal sulcus absent; cervical sclerites absent or present, when present, narrow; labroclypeus strongly asymmetrical, nasale short, usually projecting forward, with 3–7 teeth; epistomal lobes asymmetrical, left one strongly projecting anteriorly; first antennomere with a distal membranous projection on inner margin, length of the sensorium half the length of third antennomere; mandibles strongly asymmetrical, right mandible with 1–3 inner teeth, inner ventral margin serrate or not; left mandible with a groove and usually with four teeth, basal tooth sharper and pointing to the apex, second tooth pointing mediad, third tooth trifid or tetrafurcate, distal tooth triangular, pointing mediad, with a group of long bifid projections; distal inner margin serrate or not; ligula reduced; pronotum strongly sclerotized, with sagittal line; mesonotum with two pairs of subtriangular sclerites, inner pair much narrower and smaller than outer pair; metanotum lacking sclerites; meso- and metathorax with a pair of lateral tubercles each one bearing a long seta; abdominal segments with four or seven pairs of lateral tracheal gills. **Chaetotaxy:** FR1 long, inserted posteriorly to PA7; seta FR3 short, displaced anteriorly, close to seta FR8; seta FR12 similar in length to medial gFR1 setae; pore FR14 absent; pore PA27 located between setae PA26 and PA28; chaetotaxy of epistomal lobes asymmetrical, left one with 11–18 strong and curved setae (gFR2), right one lacking setae; setae AN8 and AN9 absent; seta MN1 minute; setae MX7–MX11 unifid, setae MX10, MX11 and MX16 setiform; labial sensilla LA4 and LA15 absent, LA3 short.

Berosus decolor Knisch, 1924

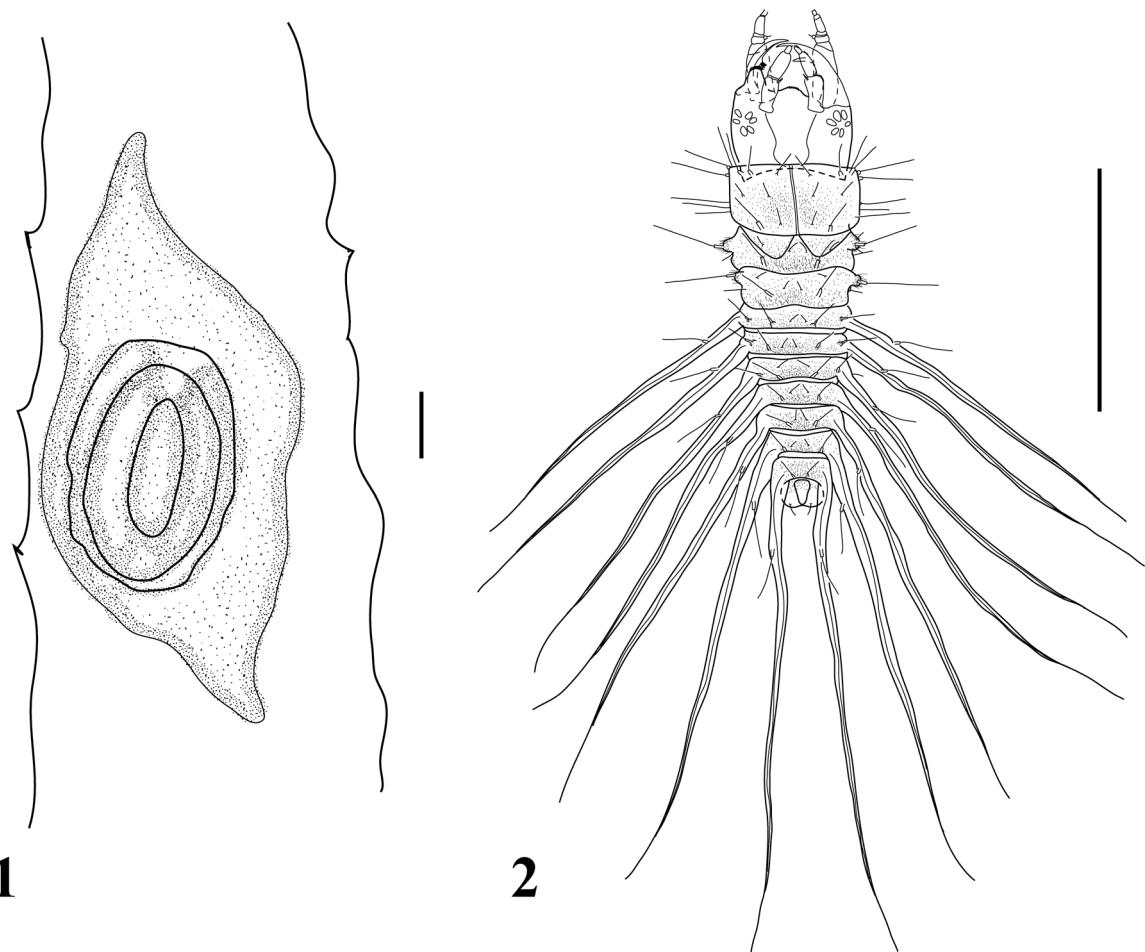
(Figs. 1–14)

Diagnosis. **Egg case:** oval-shape, mast lacking and bearing 2–3 eggs. **Larvae:** Frontal sulcus well defined in all instars; cervical sclerites absent, anterior margin of the nasale with six teeth; left epistomal lobe with 14–16 strong and curved setae (gFR2) on anterior margin except for first outer seta that is short and stout, inner eight setae bearing a small tooth; first antennomere with a digitiform membranous projection on distal inner margin; right mandible with three teeth; abdominal segments I–VII with a pair of tracheal gills, lacking sclerotized basal ring.

Description, egg case (Fig. 1). Whitish, oval-shape, sometimes elongate. Attached to stems and leaves of aquatic plants. Made of two layers: inferior layer very thin, weaved onto the substrate, superior layer thicker, covering the eggs. Mast lacking. Two eggs per case, occasionally three. Egg case length: 1.60–3.65 mm, width: 1.00–1.55 mm.

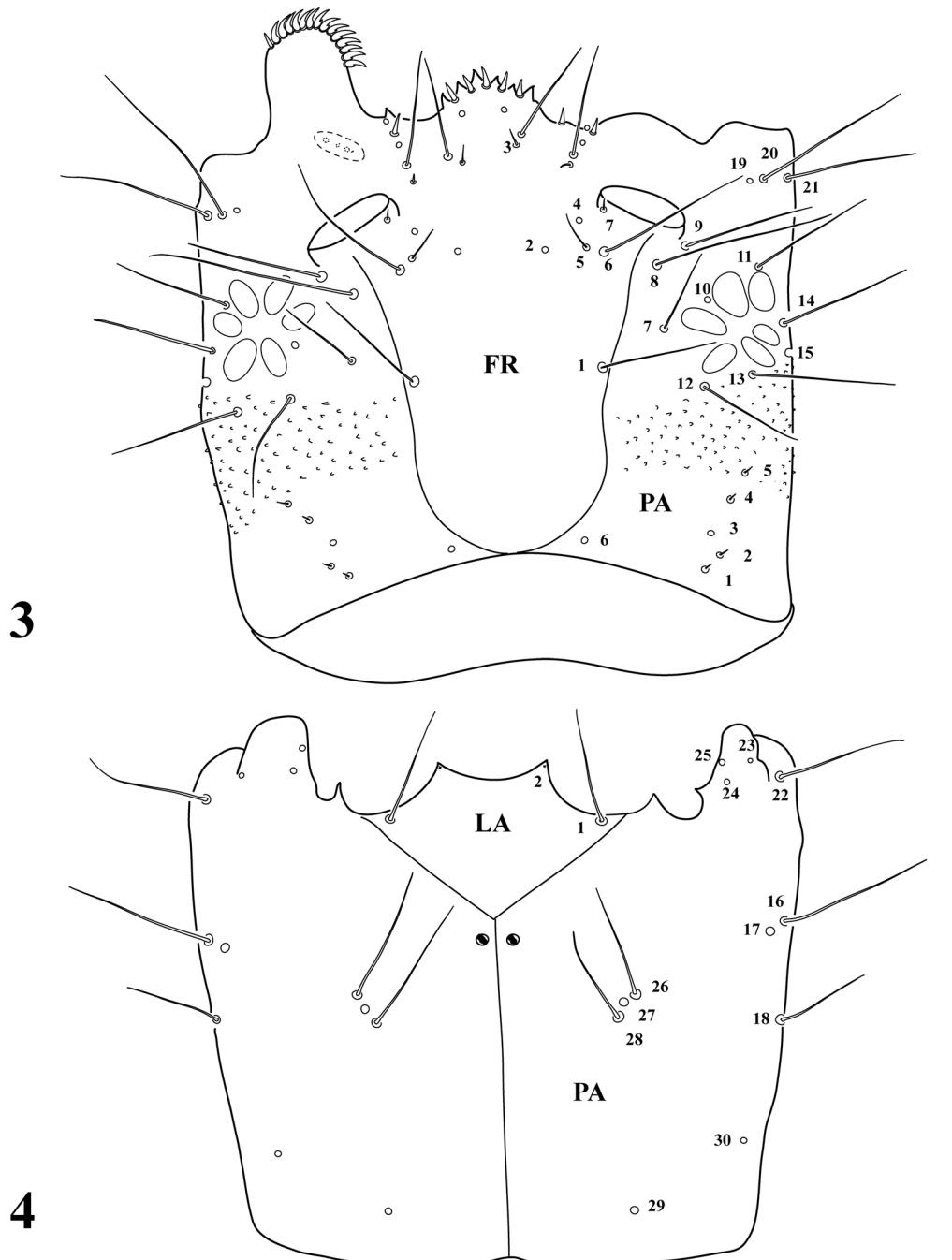
Description, instar I (Figs. 2–14). Color: creamy-yellow, sclerotized areas light brown, lacking color pattern.

Body (Fig. 2): morphometric measurements and ratios shown in Table 1. **Head capsule (Figs. 3–5):** subquadrangular, epicraneal sulcus well developed, frontal sulci inverse bell shaped, coronal sulcus absent; cervical sclerites absent; area between setae PA5 and PA12 entirely covered by spinulae. **Labroclypeus (Fig. 5):** asymmetrical, nasale slightly projected forward with 6 anterior teeth; lateral lobes of the epistome strongly asymmetrical, right one weakly developed, left one large, covering the base of the mandible; one small tooth present between left epistomal lobe and nasale. **Antenna (Fig. 8):** composed of three antennomeres, A1 shorter than A2+A3, with a membranous digitiform projection on distal inner margin; sensorium located on outer apical margin of A2, slightly shorter than A3. **Mandibles (Figs. 6–7):** asymmetrical, right mandible with three teeth on



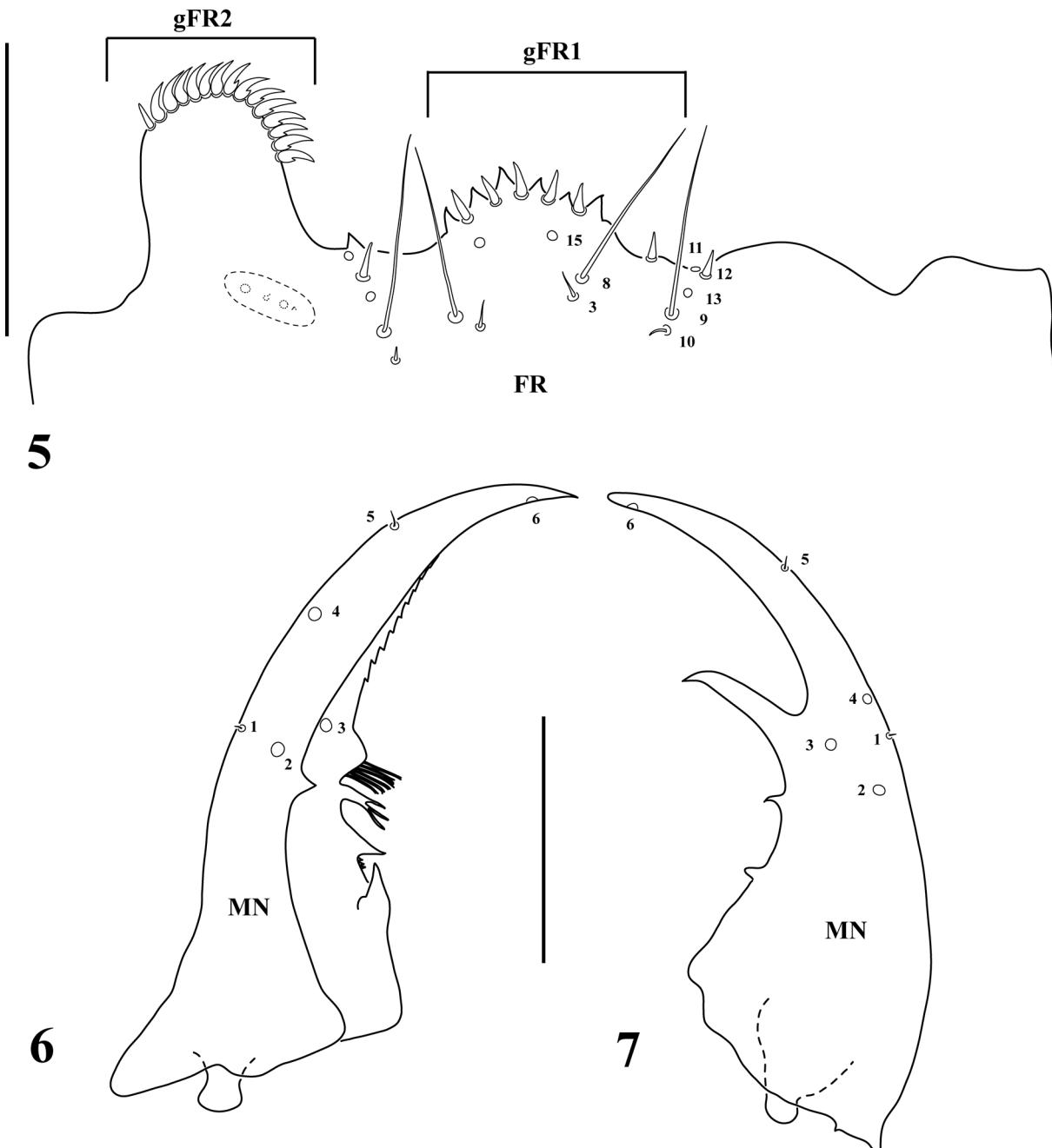
FIGURES 1–2. *Berosus decolor*, (1) egg case, dorsal view, built over leaves and stems of *Elodea*. sp; (2) first-instar larva: habitus, dorsal view. Scale bars = 1.00 mm (Fig. 1) and 0.50 mm (Fig. 2).

inner margin, distal one much larger than the other two; left mandible with a shallow groove and four teeth, basal tooth sharp and pointing to the apex, second tooth pointing mediad with three sharp points on base, third tooth trifid, distal tooth triangular, pointing mediad, with six long bifid projections; distal inner margin serrate. **Maxilla** (Figs. 9–10): longer than the antenna, composed of three articles. Cardo small, irregular. Stipes slightly longer than palpus. Palpus composed of four palpomeres, MP1 and MP4 subequal in length, MP2 shortest, MP3 longest. MP1 with a short projection on distal inner margin, MP4 subconical. **Labium** (Figs. 4, 11–12): submentum wide, subtrapezoidal. Mentum reduced, subpentagonal. Prementum subrectangular, wider than long. Ligula reduced to a membranous lobe between palpi. Palpus composed of two palpomeres, LP2 much longer than LP1. **Thorax** (Fig. 2): pro-, meso- and metathorax covered with setae and fine spinulae. Pronotal plate large, covering most of prothorax, subrectangular with posterior margin rounded, with fine sagittal line. Mesonotum with one pair of subtriangular sclerites on anterior margin (we were unable to find the inner pair, although we cannot confirm if it is really absent). Metanotum without sclerites. Meso- and metathorax shorter than prothorax, with a pair of lateral tubercles bearing one long and several short spinulae. **Legs** (Figs. 13–14): five-segmented, long, visible in dorsal view. Metathoracic leg longest, prothoracic leg shortest. Coxa large, cylindrical; trochanter short; femur slender, slightly longer than coxa; tibiotarsus subcylindrical, slender, almost as long as femur; claw long, slender, curved, about 3/4 times as long as tibiotarsus. **Abdomen** (Fig. 2): Ten-segmented, covered by a dense layer of fine spinulae. Segments I–VII similar in shape, tapering towards posterior end, subdivided by a transverse fold, each with a pair of tracheal gills, segments I–VII with two pairs of tubercles bearing one long seta and several spinulae (one pair dorsal, one pair at the base of each tracheal gill). Segment VIII small, with two posterior lobes and a subcircular sclerite dorsally. Segments IX and X strongly reduced. Tracheal gills filiform, as long as total length of body, with a long seta near the base. Sclerotized ring at the base of gills absent. Nine pairs of non-functional



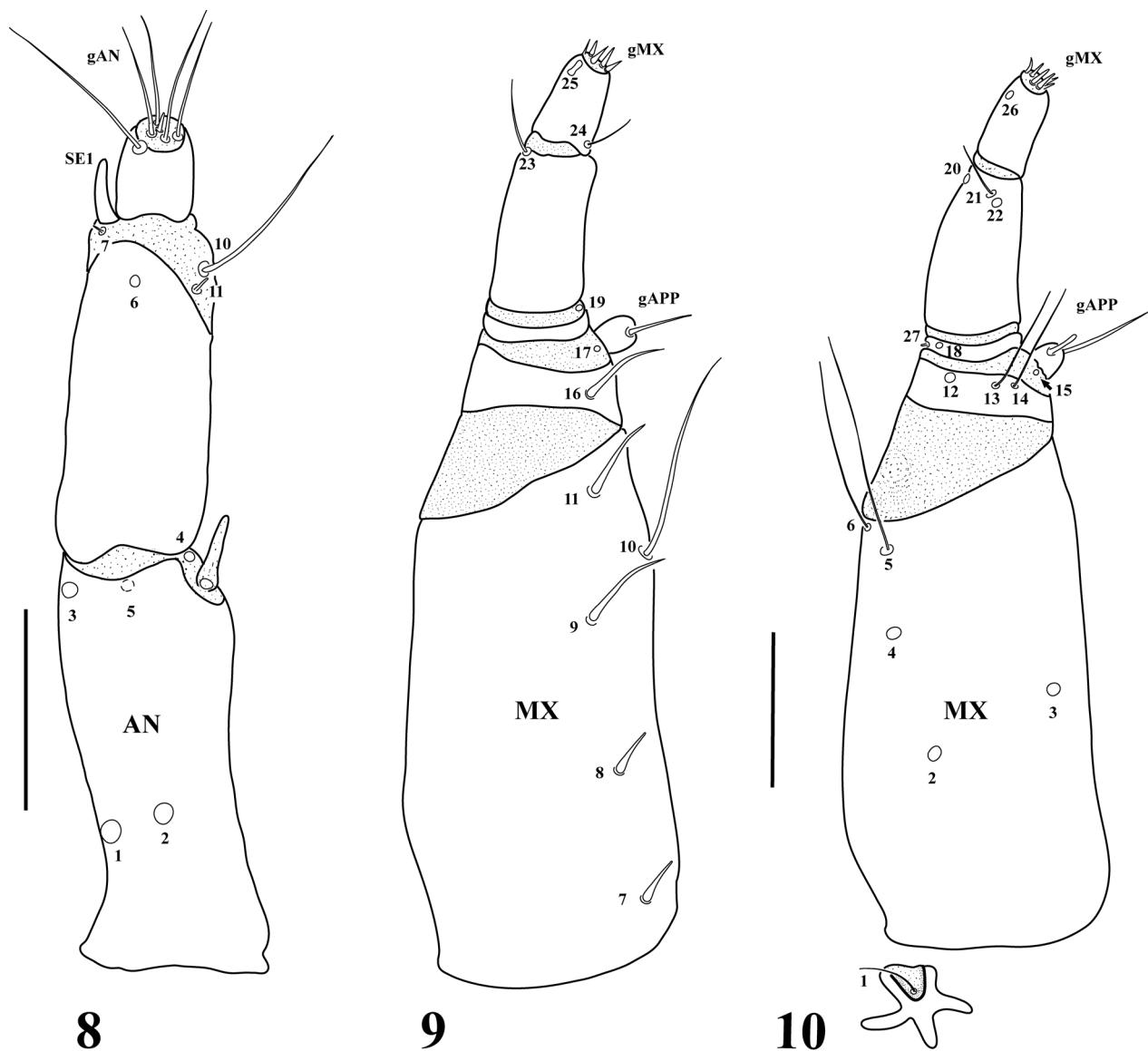
FIGURES 3–4. *Berosus decolor*, first-instar larva: (3) head capsule, dorsal view; (4) head capsule, ventral view. Scale bars = 0.10 mm.

spiracles, one mesothoracic and eight abdominal. Spiracular atrium absent. **Chaetotaxy. Head capsule (Figs. 3–5):** Frontale with 49 dorsal sensilla. Central part with 2 long setae (FR1) near frontal sulci and 2 pores (FR2) situated more anteriorly and mesally, seta FR3 short and displaced anteriorly close to seta FR8. Two setae (FR5 long, FR6 short) and 1 pore (FR4) near the base of each antenna. One short seta (FR7) close to the inner margin of the antennal socket. Nasale with a group of 6 stout and short setae (gFR1) on anterior margin, 5 setae between nasale teeth and 1 seta displaced between right epistomal lobe and nasale; 1 pore (FR15) and 1 long seta (FR8) on median portion of the nasale. Right epistomal lobe lacking sensilla; left epistomal lobe with 14–16 strong and curved setae (gFR2) on anterior margin except for first outer seta which is straight and somewhat shorter, inner 8 setae bearing a small tooth. A group of 5 sensilla placed in a row in the area between nasale and epistomal lobes; 1 pore (FR11) on anterior margin, 1 stout short seta (FR12) and 1 pore (FR13) followed by 1 long seta (FR9) and 1 short seta (FR10) on posterior margin of the nasale; pore FR14 absent. Ventral surface of left epistomal lobe with a



FIGURES 5–7. *Berosus decolor*, first-instar larva: (5) labroclypeus, dorsal view; (6) left mandible, dorsal view; (7) right mandible, dorsal view. Scale bars = 0.10 mm.

suboval sclerotized area bearing 1 minute seta, 1 spinula and 2 pores that could be easily confused with FR14. Each parietale with 30 sensilla; dorsal surface with a group of 4 short setae (PA1, PA2, PA4, PA5) and 1 pore (PA3) placed in a row posteriorly; 1 pore (PA6) near frontal sulci, close to posterior margin of cephalic capsule; 7 long setae (PA7, PA8, PA9, PA11, PA12, PA13, PA14) and 2 pores (PA10, PA15) on surrounding ocular area; anterolateral corner of epicranium with 2 long setae (PA20, PA21) and 1 pore (PA19) on anterolateral position. Ventral surface with 1 long seta (PA22) and 3 pores (PA23, PA24, PA25) near mandibular acetabulum; 2 long setae (PA16, PA18) and 1 pore (PA17) on lateral surface at mid-length; 2 long setae (PA26, PA28) and 1 pore (PA27) near midline, posterior to the tentorial pit; 2 pores (PA29, PA30) on basal third of parietal. **Antenna (Fig. 8):** A1 with 2 dorsal pores (AN1, AN2) at basal third, 2 lateral pores (AN3, AN4) and 1 ventral pore (AN5) at the tip; A2 with 1 dorsal pore (AN6) on distal margin, 1 long seta (AN10) and 1 short seta (AN11) apically on inner margin, and 1 minute seta (AN7) and the sensorium (SE1) apically on outer margin; setae AN8 and AN9 absent; A3 with at



FIGURES 8–10. *Berosus decolor*, first-instar larva: (8) left antenna, dorsal view; (9) left maxilla, dorsal view; (10) right maxilla, ventral view. Scale bars = 0.05 mm.

least 5 long and 1 short setae apically (gAN). **Mandibles (Figs. 6–7):** with 1 minute seta (MN1) and 3 pores (MN2, MN3, MN4) at mid-length, MN3 on inner side of the groove and MN4 displaced anteriorly on left mandible, MN4 closer to MN1 in right mandible; 1 minute seta (MN5) subapically and 1 pore (MN6) distally. **Maxilla (Figs. 9–10):** Cardo with 1 long and thin seta (MX1) on a membranous area. Stipes with 10 sensilla: 5 unifid setae (MX7–MX11) on inner margin, seta MX10 hair-like and MX11 with an intermediate shape between hair-like and spine-like, the others short and stout; 2 long distal setae (MX5, MX6) on outer lateral surface and 3 ventral pores (MX2–MX4). MP1 with 1 long seta (MX16) on inner basal margin, similar to MX11, 1 pore (MX12) and 2 long setae (MX13, MX14) ventrally along apical margin of sclerite; inner process of MP1 with 2 pores at the base (MX15 ventral, MX17 dorsal), and 2 long and 1 short setae at the tip (gAPP). MP2 with 2 pores (MX18 ventral, MX19 dorsal) and 1 minute lateral seta (MX27). MP3 with 2 subapical setae (MX21 ventral, MX23 dorsal) and 2 distal pores (MX20, MX22). MP4 with 1 basal seta (MX24) on inner margin, 1 digitiform sensillum (MX25) and 1 pore (MX26) subapically on outer margin, and several short sensilla on apex (gMX). **Labium (Figs. 4, 11–12):** Submentum with 1 long (LA1) and 1 minute (LA2) seta on each side. Mentum with 1 short seta (LA3) at distal third of ventral surface; pore LA4 absent. Prementum with 2 pores: LA8 at mid-length and LA9 distally, and 1 long seta (LA10) on dorsal surface; 1 minute seta (LA5), 1 long seta (LA6) and 1 pore (LA7) on ventral surface; ligula

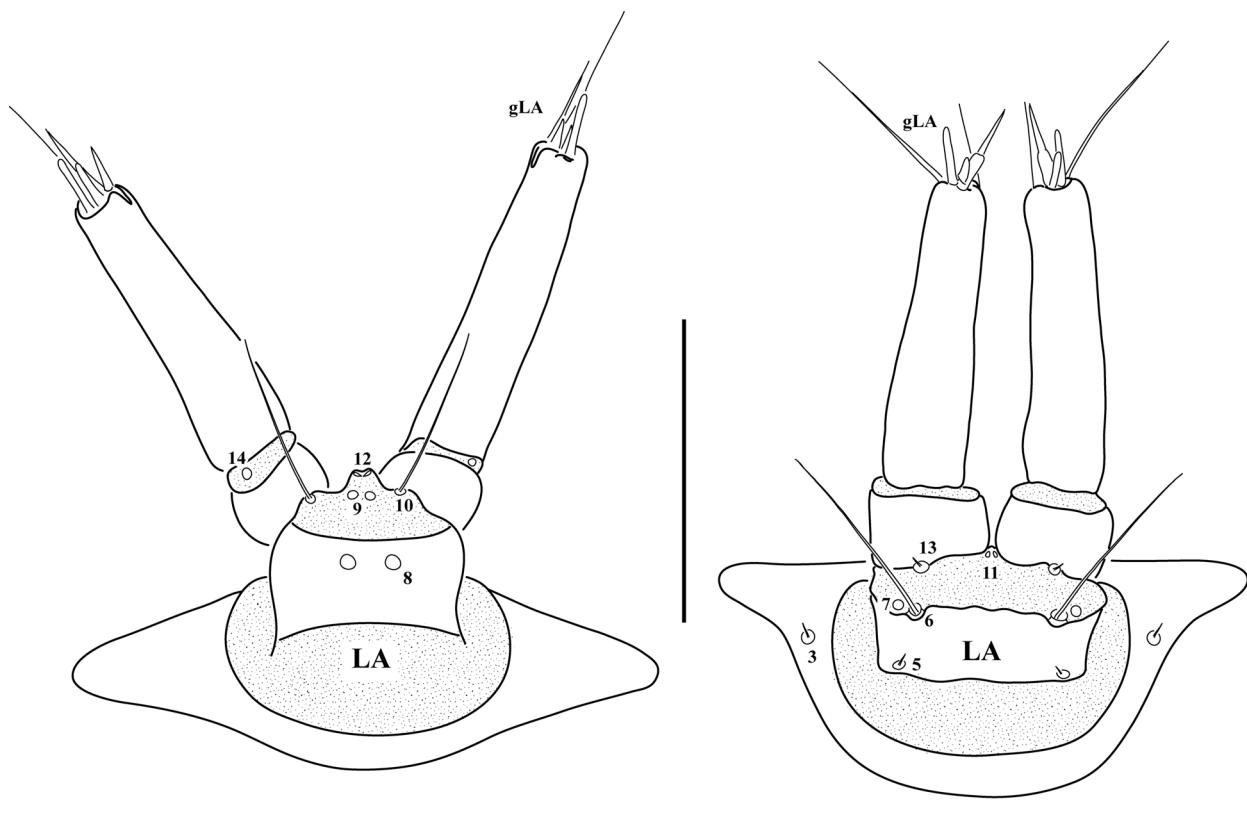
with 2 pores (LA11, LA12) apically. LP1 with 1 pore (LA14) dorsally on distal margin and 1 minute seta (LA13) at the base on ventral surface; LP2 with several apical setae (gLA), LA15 absent. **Legs** (Figs. 13–14): The number and position of pores are the same in pro- meso- and metathoracic legs. Coxa with 2 pores (1 anterodorsal, 1 posterior), trochanter with 8 pores (6 anterior, 2 posterior), femur with 2 pores (1 anteroproximal, 1 posterodorsal), tibiotarsus with 2 pores (1 anterodorsal, 1 posterodorsal). Tibiotarsus and femur with a ventral patch of spinulae on anterior and posterior surfaces. For setae distribution see Table 2.

Description, instar II. As instar I except for the following features.

Body. Morphometric measurements and ratios shown in Table 1. **Chaetotaxy.** **Head capsule:** each parietale with 5 secondary setae, 1 short seta between PA8 and PA9, 1 close to frontal sulcus at mid-length between PA1 and PA6; and a group of 3 setae distributed between PA13 and PA14, PA14 and PA16 and ventrally near PA16. **Mandibles:** with 3 minute secondary setae distributed on basal half along outer margin. **Antenna:** ventral surface of A2 with 1 minute secondary seta at the base. **Maxilla:** cardo with 1 secondary seta close to MX1; stipes with 1 long secondary seta near MX5 and 2 minute secondary setae on outer margin (extremely difficult to observe in instar II but clearly visible in instar III). **Labium:** Mentum with 1 long secondary seta at each side on dorsal surface. **Legs:** secondary sensilla absent (Table 2).

Description, instar III. As instar II except for the following features.

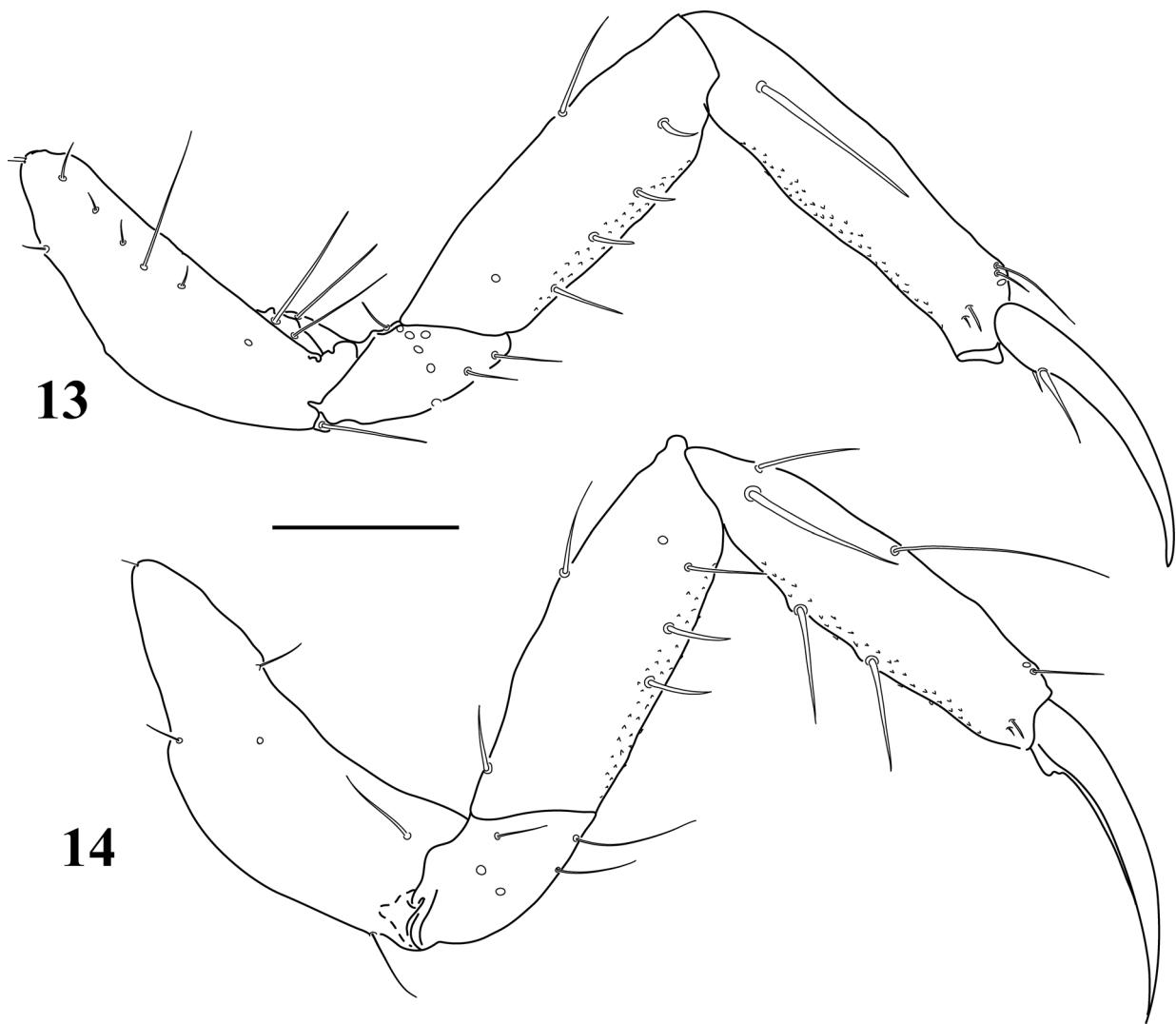
Body. Morphometric measurements and ratios shown in Table 1. **Chaetotaxy.** **Head capsule:** each parietale with 1 long secondary seta close to PA19. **Legs:** distribution of secondary setae shown in Table 2.



FIGURES 11–12. *Berosus decolor*, first-instar larva: (11) labium, dorsal view; (12) labium, ventral view. Scale bar =0.05 mm.

TABLE 1. Measurements (in mm) and ratios for the three larval instars of *Berosus decolor*.

Measure	Instar I	Instar II	Instar III
TL	1.02–1.05	2.40–2.90	4.10–4.20
MW	0.25–0.27	0.40–0.60	0.60–0.70
HL	0.27–0.29	0.40–0.42	0.43–0.45
HW	0.37–0.39	0.50–0.55	0.60–0.62
HL/HW	0.72–0.75	0.76–0.80	0.69–0.73
AL	0.22–0.25	0.32–0.33	0.35–0.37
A1L	0.11–0.13	0.17–0.18	0.17–0.18
A2L	0.07–0.09	0.11–0.12	0.12–0.13
A3L	0.02–0.03	0.03–0.04	0.03–0.04
SEL	0.02	0.01–0.02	0.02–0.03
SEL/A3L	0.64–0.73	0.50–0.75	0.50–0.71
A1L/A2L	1.32–1.48	1.39–1.56	1.40–1.50
A1L/(A2L+A3L)	1.00–1.09	1.09–1.20	1.06–1.14
HL/AL	1.18–1.25	1.23–1.27	1.20–1.25
HW/AL	1.60–1.70	1.54–1.67	1.65–1.73
SL	0.18–0.19	0.25–0.27	0.27–0.28
MPL	0.12–0.14	0.14–0.17	0.15–0.16
SL/MPL	1.34–1.48	1.59–1.72	1.68–1.80
MP1L	0.02–0.03	0.03–0.04	0.03–0.04
MP2L	0.01	0.01	0.01
MP3L	0.05–0.06	0.06–0.07	0.07
MP4L	0.03	0.03–0.04	0.03–0.04
ML	0.30–0.32	0.39–0.43	0.43–0.45
LPL	0.06	0.07–0.08	0.09
LP1L	0.01–0.02	0.02	0.02
LP2L	0.05	0.05–0.06	0.07
LP2L/LP1L	3.33–4.20	2.88–3.00	3.37–3.50
PrmtW	0.04	0.04–0.05	0.05
PrmtL	0.02	0.02–0.03	0.02–0.03
LEG 1 L	0.82–0.86	0.83–1.08	1.20
LEG 2 L	0.86–0.88	0.89–1.22	1.34–1.38
LEG 3 L	1.02–1.04	0.97–1.38	1.50–1.51
LEG 3/LEG 1	1.20–1.24	1.16–1.27	1.25–1.26
LEG 3/LEG 2	1.18	1.08–1.13	1.09–1.12
LEG 1 (TITA/FE)	0.95–1.00	0.90–0.97	0.89–0.90
LEG 2 (TITA/FE)	0.97–1.02	0.86–1.02	0.88–0.91
LEG 3 (TITA/FE)	0.96–1.01	0.93–1.07	0.92–0.96
LEG 1 (CL/TITA)	0.77–0.80	0.61–0.69	0.62–0.65
LEG 2 (CL/TITA)	0.71–0.79	0.60–0.79	0.60
LEG 3 (CL/TITA)	0.76–0.77	0.57–0.64	0.55–0.57



FIGURES 13–14. *Berosus decolor*, first-instar larva: (13) left prothoracic leg, anterior view; (14) right prothoracic leg, posterior view. Scale bar = 0.10 mm.

Discussion

A number of descriptions of immature *Berosus* are available in the literature (Archangelsky 1994, 1997, 1999, 2002; Fernández & Campos, 2002; Watts, 2002), focused mainly on the description of third-instar morphology. Larvae of *Berosus* are quite homogeneous and exhibit few morphological differences. Therefore, characters derived from primary chaetotaxy are important to differentiate species. The following characters can be used to distinguish species within *Berosus*.

Egg case morphology. The majority of the species of *Berosus* build egg cases with a broad mast. Those of *B. decolor* and *B. corrini* Wooldridge, 1964, however, lack this structure (Archangelsky, 1994). Egg case of *B. decolor* contain 2–3 eggs as most *Berosus* species; *B. pugnax* LeConte, 1863 (Archangelsky, 1994) lays egg cases with 10–25 eggs, the maximum number recorded for the genus.

Larval morphology. The head capsule of *Berosus* larvae typically presents the frontal sulci shaped as an inverse bell and the coronal sulcus is absent. In *B. decolor*, the frontal sulcus is well defined in all instars, in contrast to all other species where it is not visible in the third instar. Cervical sclerites in *B. decolor* are absent as in most *Berosus* except *B. japonicus* Sharp, 1873 (Minoshima & Hayashi, 2015), *B. australiae* Mulsant & Rey, 1858 (Watts, 2002), *B. aulus* (Archangelsky, 1999) and *B. peregrinus* Herbst, 1797 (Richmond 1920, Wilson 1923). On

TABLE 2. Number and position of setae on the legs of larvae of *Berosus decolor*. Numbers between slash marks refer to pro-, meso-, and metathoracic leg, respectively.

Segment	Position	Instar I	Instar II	Instar III
Coxa	AD	8/8/8	8/8/8	8/8/8
	DPr	3/3/3	3/3/3	3/3/3
	P	1/1/1	1/1/1	1/1/1
	PD	1/1/1	1/1/1	1/1/1
	PPr	2/2/2	2/2/2	3/3/3
	VDi	2/2/2	2/2/2	2/2/2
Trochanter	Total	17/17/17	17/17/17	18/18/18
	Di	5/5/5	5/5/5	5/5/5
Femur	Pr	1/1/1	1/1/1	1/1/1
	Total	6/6/6	6/6/6	6/6/6
Tibiotarsus	AV	4/4/4	4/4/4	4/4/4
	D	3/3/3	3/3/3	3/3/3
	PV	3/3/3	3/3/3	3/3/3
	Total	10/10/10	10/10/10	10/10/10
Pretarsus	ADI	2/2/2	2/2/2	2/2/2
	AME	1/1/1	1/1/1	1/1/1
	Pme	1/1/1	1/1/1	1/1/1
	D	5/5/5	5/5/5	5/5/5
	PDi	2/2/2	2/2/2	2/2/2
	V	2/2/2	2/2/2	2/2/2
Total	Total	13/13/13	13/13/13	13/13/13
	V	2/2/2	2/2/2	2/2/2
Total	Total	2/2/2	2/2/2	2/2/2

the other hand, the number of teeth on the anterior margin of the nasale is variable within the genus: *B. peregrinus* bears seven teeth whereas *B. decolor* bears six teeth as well as *B. toxacanthus* (Archangelsky, 2002) and *B. sayi* Hansen, 1999 (Richmond 1920, Wilson 1923 (under *B. striatus* Melsheimer, 1806)); other known larvae bear five teeth (*B. corrini* (Archangelsky, 1994), *B. aulus* (Archangelsky, 1999), *B. coptogonus* (Archangelsky, 1999) and *B. alternans* (Fernández & Campos, 2002)) or three teeth (*B. pugnax* (Archangelsky, 1994), *B. auriceps* (Archangelsky, 1999) and *B. cornicinus* (Archangelsky, 2002)). A particular case is that of *B. hoplites* Sharp, 1887 which has a straight nasale not projected forward and with numerous irregular teeth (Archangelsky, 1994). Also, Richmond (1920) mentioned that the labroclypeus of *B. signaticollis* (Charpentier, 1825) is untoothed, rounded and short, although a more detailed study (Fikáček, 2006) showed that it is projected forward and bears six teeth. Another important feature is the strong asymmetry in the mandibles. The right mandible bears three teeth in *B. decolor* and in the majority of the known species whereas *B. corrini* and *B. spinosus* (Steven, 1808) bear two teeth and *B. hoplites* only one (Böving & Henriksen, 1938). Left mandibles are similar in most species. A particular case is that of *B. pantherinus* Leconte, 1855 in which the left mandible instead of having four teeth has only one large tooth with apical projections and a deep groove on the inner margin (from the teeth to the base of the mandible) with a row of irregular teeth projecting from the bottom (Wilson, 1923). According to Archangelsky (2008) in most Berosini the ligula is well developed, although variable in length. However, in *Berosus* the ligula is strongly reduced. The mesonotum of *Berosus* species exhibits two pairs of subtriangular sclerites. We were unable to find the inner pair in *B. decolor*, although we cannot confirm if it is really absent. All known larvae exhibit seven pairs of tracheal gills on the abdomen with the exception of *B. pugnax* which has four pairs. According to Karaouzas & Incekara (2011), *B. affinis* Brullé, 1835 has four pairs of tracheal gills; however, seven pairs are observed in their illustrations. Another character useful in recognizing *Berosus* larvae is the presence or absence of a sclerotized ring at the base of the tracheal gill. This structure is typically present except in *B. decolor* and *B. cornicinus*.

Chaetotaxy. The primary chaetotaxy of the genus *Berosus* is poorly known. Therefore, the following discussion is based mostly on the comparison of *B. decolor* with the two species of *Berosus* with known primary chaetotaxy (*B. signaticollis* (Fikáček *et al.*, 2008) and *B. japonicus* (Minoshima & Hayashi, 2015)) and with the ground plan of chaetotaxy of the family Hydrophilidae (Fikáček *et al.*, 2008; Byttebier & Torres, 2009). The head capsule and, in particular, the epistomal lobes are responsible for most of the variability observed in chaetotaxic characters within larval Hydrophilidae (Fikáček *et al.*, 2008). Usually, the anterior margin of the nasale bears six setae (gFR1), although this number may vary among taxa. In *Berosus*, gFR1 is composed of six stout short setae in *B. decolor* (Fig. 5) and *B. signaticollis* (Fikáček *et al.*, 2008). This character is conserved as well in the third instars of *B. aulus*, *B. toxacanthus*, *B. corrini*, *B. coptogonus*, *B. alternans*, *B. auriceps*, *B. cornicinus*, *B. hoplites* and *B. pugnax* (Archangelsky 1994, 1999, 2002). The chaetotaxy of the labroclypeus is asymmetrical, most likely as a consequence of its also asymmetrical shape (Fig. 5): the right epistomal lobe is poorly developed and lacks sensilla whereas the left epistomal lobe is projected forward and bears a variable number of sensilla (gFR2), ranging from 11 to 18 setae depending on the species. Also, within gFR2, the number of outer simple setae and the rest of inner setae with a minute tooth vary among species. They are usually alike with the exception of *B. hoplites*, *B. coptogonus* and *B. cornicinus* which exhibit smaller and blunt setae on the inner side and *B. decolor* in which the first outer seta is short and stout (Fig. 5). The remaining sensilla of the head capsule only vary mainly in their relative size and position. The absence of pore FR14 distinguishes *Berosus* from other hydrophilid genera. It is interesting to mention that a group of sensilla (two pores and one minute seta) is present on the ventral surface of the left epistomal lobe (Fig. 5), which could be mistaken with FR14 (see Fikáček 2006, Fikáček *et al.*, 2008). On the other hand, Fikáček *et al.* (2008) regarded pore FR11 as absent in *B. signaticollis*. This pore, however, is present in *B. decolor* and *B. japonicus* (Minoshima & Hayashi, 2015). The marginal position of FR11 on the labroclypeus makes it difficult to observe. With respect to the antenna, the sensillum AN8 is absent in *Berosus*, a character state only known to be present in *Tropisternus sahlbergi* (Sharp, 1883) (Hydrophilini) (Torres *et al.*, 2008), Spercheidae and Hydrochidae (Fikáček *et al.* 2008), and the seta AN9 is also absent. On the other hand, pore AN6 is located apically and pore AN2 is displaced near AN1 on the basal half. Fikáček *et al.* (2008) found an additional pore on the apical inner margin of A1 in *B. signaticollis*, which stands in contrast with *B. decolor* where no additional sensilla were found. A noteworthy feature of *Berosus*, however, is the presence of a membranous digitiform projection on the distal inner margin of the first antennomere, a character only known to occur in *Hemiosus multimaculatus* (Jensen-Haarup, 1910) and *Derallus paranensis* Oliva, 1981 (personal observations) although likely present in all *Hemiosus* and *Derallus* species. In the case of *D. paranensis*, this membranous projection is much complex, apically bifurcate and is located inside a cuticular pouch. It is worth mentioning that the base of this membranous projection (Fig. 8) could be confused with an additional pore, and may have cause that the additional pore mentioned by Fikáček *et al.* (2008) for *B. signaticollis* and be mistaken for pore AN4 for *B. japonicus* (Minoshima & Hayashi, 2015) is in fact the base of this structure (see also Fikáček, 2006). In the Berosini genera *Allocotocerus* Kraatz, 1883 and *Regimbartia* Zaitzev, 1908 this structure is regarded as a cuticular projection (see character 13 in Archangelsky, 2008). Several descriptions of *Berosus* larvae mention the presence of a seta or spine in the same position as the membranous finger-like projection of *B. decolor*, *B. japonicus* and *B. signaticollis*, all most likely being homologous structures. The presence of this projection in larval Berosini is unique within Hydrophilidae. With regards to the mandibles, few differences were found: in *Berosus* the seta MN1 is minute and the pore MN2 is located proximally to MN1 (Figs. 6–7). The stipes of hydrophilid larvae generally bears a row of five spiniform setae (MX7-MX11). In *Berosus*, the seta MX10 is hair-like and MX11 and MX16 exhibit an intermediate shape between hair-like and spine-like (Fig. 9). Chaetotaxy of the labium varies considerably among taxa, but this variability is caused mainly by the reduction of labial structures (Fikáček *et al.* 2008). The ligula in *Berosus* is strongly reduced, however, sensilla LA11 and LA12 are present in *B. decolor*, *B. pallipes* Brullé, 1841 (personal observation) and *B. japonicus*. This stands in contrast with *B. signaticollis* in which sensilla LA11 and LA12 are absent (Fikáček *et al.* 2008). The sensilla LA4 and LA15 are lacking, although prementum and palpus have not undergone visible reduction. Makarov (1996) mentioned that it is difficult to define the reasons for reduction of sensilla and that minimization of body size does not lead directly to chaetome reduction. The majority of secondary setae are restricted to the head capsule and mouth appendages whereas in the legs only one secondary sensillum was observed. Only few secondary sensilla are useful to distinguish between larval instars. The main differences between second- and third-instar larvae are the presence of a single secondary seta dorsally on the cephalic capsule, near pore FR19 and of a proximal seta on the posterior surface of the coxa.

Also, the size of the cephalic capsule (Table 1) is useful to separate instars of a given species. However, in absence of detailed morphometric analyses for most species, it is not possible to recognize useful morphometric characters to distinguish taxa within the genus. Most descriptions of preimaginal *Berosus* have taken into account only the total length of the body, especially that of the third-instar larva.

Conclusion

The traditional definition of *Berosus* subgenera, based on adult morphology of European species exhibit certain problems when applied to the Neotropical species. Oliva (1989), based also in adult characters, proposed a subdivision of the Neotropical species based on adult characters into several species groups. In the future, when more detailed descriptions (including morphometric and chaetotoxic analyses) of immature *Berosus* are available, a more comprehensive classification, taking into consideration both preimaginal and imaginal characters, can be attempted. To summarize, larvae of *Berosus* are characterized by the following features: labroclypeus asymmetrical, nasale projected forward with variable number of teeth; left epistomal lobe strongly projecting forward with several stout and curved setae (gFR2); parietale with spinulae between setae PA5 and PA12; seta FR1 elongate, pore FR14 absent; mandibles strongly asymmetrical, with seta MN1 minute; first antennomere with a digitiform projection on distal inner margin, sensilla AN8 and AN9 absent; sensilla MX10 hair-like, MX11 and MX16 with an intermediate shape between hair-like and spine-like; ligula strongly reduced, labial sensilla LA4 and LA15 absent; abdominal segments bearing lateral tracheal gills.

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