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IMMATURE STAGES OF NEOTROPICAL HYDROPHILIDAE (COLEOPTERA): *HYDRAMARA ARGENTINA* (KNISCH, 1925) AND *HEMIOSUS BRUCHI* KNISCH, 1924

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Abstract.—The egg case and pupa of *Hydramara argentina* (Knisch 1925) and the larva, egg case and pupa of *Hemiosus bruchi* Knisch 1924 are described and illustrated for the first time. Information on the biology of these two species is provided. Couplets are included to interpolate larvae of *Hemiosus* and first instars of *Hydramara* in the recent key to the immature stages of hydrophiloid genera of the New World.

Resumen.—Los géneros *Hydramara* Knisch y *Hemiosus* Sharp son endémicos de la región Neotropical (con la excepción de un par de especies de *Hemiosus* que llegan al sur de la región Neártica). *Hemiosus* tiene unas 30 especies descriptas, y hasta ahora era uno de los pocos géneros de Hydrophilinae del Nuevo Mundo cuyos estados preimaginales eran desconocidos. Por su parte *Hydramara* es un género monotípico, siendo *H. argentina* (Knisch 1925) la única especie conocida. En este trabajo se describen e ilustran por primera vez las larvas, pupa y desoves de *Hemiosus bruchi* Knisch 1924, y la pupa y desoves de *Hydramara argentina.* También se provee información sobre la biología de estas dos especies y se actualizan las claves genéricas existentes para incluir las larvas de *Hemiosus* y las larvas I de *Hydramara* en la clave genérica de hydrofiloideos del Nuevo Mundo. Finalmente, se incluyen notas comparativas contrastando las larvas de estos dos géneros con las larvas de géneros relacionados o similares.

Key Words: Hydrophilidae, *Hemiosus bruchi, Hydramara argentina,* larva, pupa, morphology, biology, Neotropical

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With the exception of two species of *Hemiosus* that reach the south of the Nearctic Region, the genera *Hydramara* Knisch and *Hemiosus* Sharp are endemic to the Neotropical Region. Until now, *Hemiosus* was one of the few New World Hydrophilinae whose larval stages were unknown, the remaining three being *Beralitra* Orchymont, *Troglochares* Spangler, and *Dieroxenus* Spangler (Archangelsky 1997).

Hydramara is restricted to montane regions of central and northwestern Argentina; it is a monotypic genus, *H. argentina* (Knisch 1925) being the only described species. Up to now it has been reported from the provinces of Mendoza, Córdoba, San Luis, Salta, and Tucumán; this is the first time it is reported from La Rioja. Spangler (1979) described a third instar larva collected in association with three adults from Salta Province. This association is now confirmed as correct since adults of this species were collected and reared in the laboratory. All the preimaginal stages were obtained and I find it valuable to describe those that were previously unknown (egg case and pupa). It is also important to add some information on the biology and life

history of this interesting species as well as a few comparative notes between *Hydramara* and *Hydrobius* Leach larvae, and between the first and third instar larvae of *Hydramara.*

The genus *Hemiosus* has about 30 described species in South America (Oliva 1991, 1994a, b) and, until now, larvae of this genus were unknown. Spangler (1966) gave a brief description of a larva from Perú that, according to him, did not agree with any other known hydrophilid larva; he placed it within the Berosini since it had some characters relating it to *Berosus* Leach. Recently, in a small montane creek in La Rioja Province, I had the opportunity to collect several adults of *H. bruchi* Knisch 1924, associated with larvae that resembled the one described by Spangler. They were reared in the laboratory and pupae and adults were obtained, confirming the association as correct. *Hemiosus* was the last Neotropical Berosini genus whose immatures were unknown.

Materials and Methods

About 25 adults of *Hydramara argentina* were carried alive to the laboratory in a plastic container with a perforated lid to allow air circulation. Algae and a little water were used to keep them moist and protected. In the laboratory they were placed in two larger clear plastic containers (20 cm long by 8 cm wide by 9 cm high); sand, small rocks and algae from the collection site were used as the substrate. The containers were slightly inclined in order to provide an artificial littoral habitat, on one half water and algae were placed, and on the other sand and rocks. The egg cases were transferred to smaller containers (tissue culture plates with six cells). After the eggs hatched, the larvae were placed in small, clean cells with filter paper, some algae, and water (2–3 mm deep); to prevent cannibalism only one larva was placed in each cell. Larvae were fed twice a day and were moved to clean cells every other day. When they stopped eating, the prepupae were placed in deeper cells, with moist sand, and kept there until pupation. After the prepupae were in the pupal chamber, the top part of the sand was removed and replaced with a piece of filter paper covered with sand; in that way it was possible to check the cells every day in order to know the exact time of pupation and moult to adult stage.

Hemiosus bruchi third instar larvae were collected in the field and carried alive to the laboratory in small containers with wet tissue paper. In the laboratory they were placed in tissue culture plates with six cells. Each cell contained sand from the collection site, water about 7 mm deep, and one larva. Water was changed every other day in order to keep it clean. Larvae were fed chironomid larvae and, in a few cases Trichoptera larvae. Prepupae were treated in the same way as those of *Hydramara.*

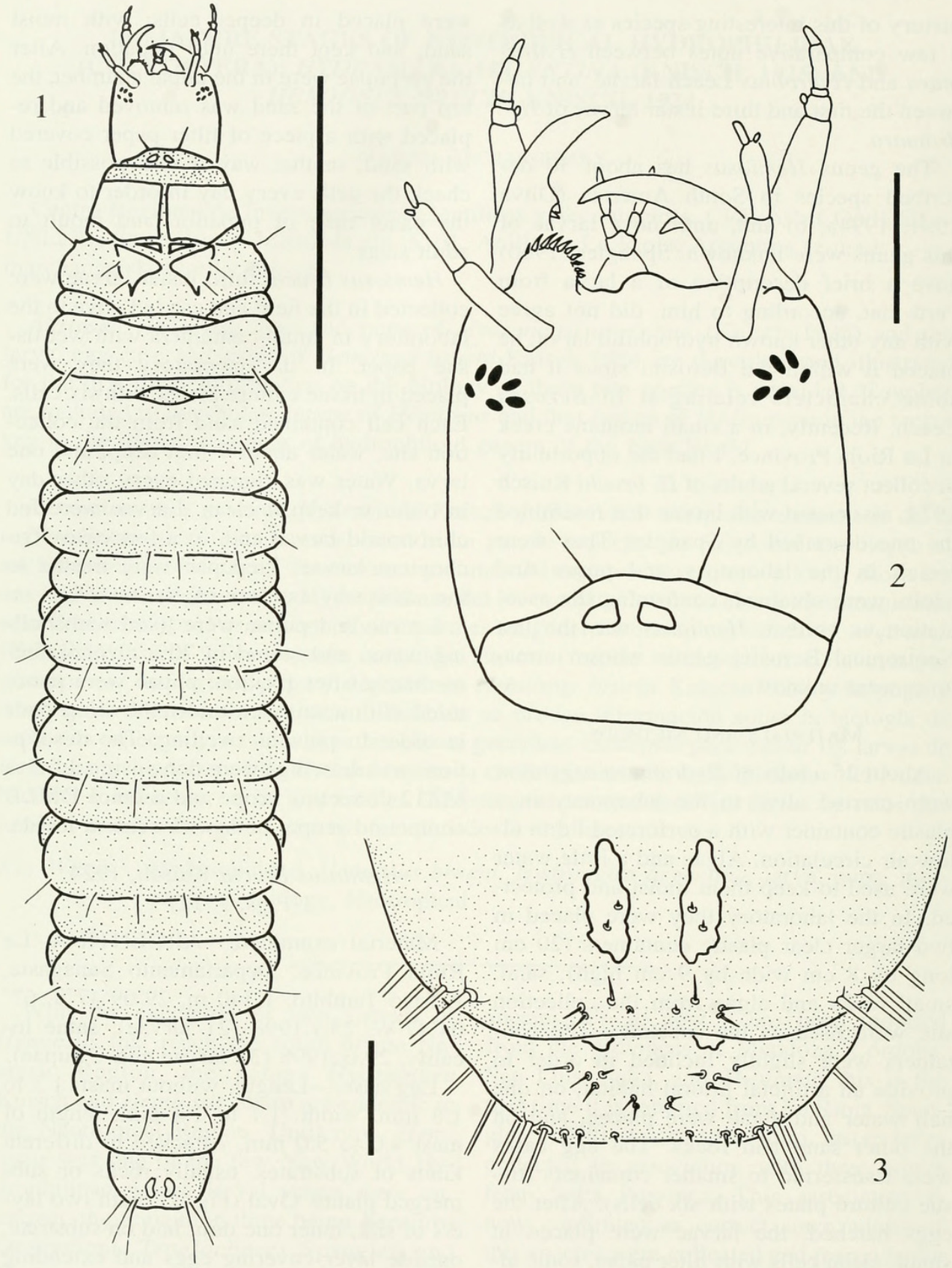
Larvae and pupae were fixed with boiling water, and stored in 70% alcohol. Immediately after fixation, pupae were punctured with a minuten under the wing pads in order to prevent swelling. The descriptions and drawings were done using a Leica MZ12 dissecting scope and a Leica DMLB compound scope, both with camera lucida.

Hemiosus bruchi Knisch, 1924
(Figs. 1–12)

Material examined.—ARGENTINA, La Rioja Province: Departamento Sanagasta, Arroyo Tambito, 1,340 m, 29°09′47″S, 67° 04′47″W, 23.v.1998 (21 larvae); same locality, 29.ix.1998 (33 larvae and 7 pupae).

Egg case.—Length, without mast: 1.3 to 1.8 mm, width: 1.1 to 1.4 mm; length of mast 4.0 to 5.0 mm. Attached to different kinds of substrates, usually rocks or submerged plants. Oval (Fig. 7), with two layers of silk, inner one thin, laid on substrate, outside layer covering eggs and extending into long and narrow mast, up to three or four times the length of case. Two or three eggs in each case, easily seen through silk covering. Eggs 0.60 to 0.65 mm long.

Third instar larva.—Length: 6.2 to 8.1



Figs. 1–3. *Hemiosus bruchi*. 1, Habitus. 2, Head capsule, dorsal view. 3, Abdominal segments 8 and 9, dorsal view. Scale bars: 1 = 2.0 mm, 2–3 = 0.25 mm.

mm. Color whitish, with sclerotized parts light brown; non-sclerotized integument covered by microspines. Habitus as in Fig. 1.

Head capsule subquadrate (Fig. 2); occipital foramen wide, dorsal part of cervix with two small subtriangular cervical sclerites. Frontoantennal sutures not defined in third instar larva, present in first and second instars, subparallel, extending from base of antennae to occipital foramen. Six stemmata on each side of head, close to base of antenna.

Labroclypeus strongly asymmetrical (Fig. 4), with four or five very blunt marginal teeth; six short setae present along outer margin. Right epistomal lobe poorly developed, lacking setae or spines; left lobe strongly developed, with row of 12 stout spines projecting mediad, eight of those spines with small inner tooth.

Antenna three-segmented (Fig. 6); basal segment as long as next two combined, bearing stout and blunt subapical seta on inner margin. Second segment more slender, with three subapical setae, one short on each side, and long one on inner margin; distal sensory appendage present on apical margin. Third segment smallest, with group of five apical setae, two long and three short ones, one of those articulated.

Maxilla five-segmented (Fig. 5), longer than antenna; cardo small, irregularly shaped, with long outer seta. Stipes long and slender, bearing row of five inner setae, distal two longer than basal three; outer margin with row of five long setae. Palp four-segmented, third segment the longest; basal segment widest, bearing three slender setae and inner process with three setae; second segment very short, lacking any setae; third segment with two subapical setae, one dorsal and one ventral; last segment bearing one long basal seta, projecting mediad, and six short distal setae.

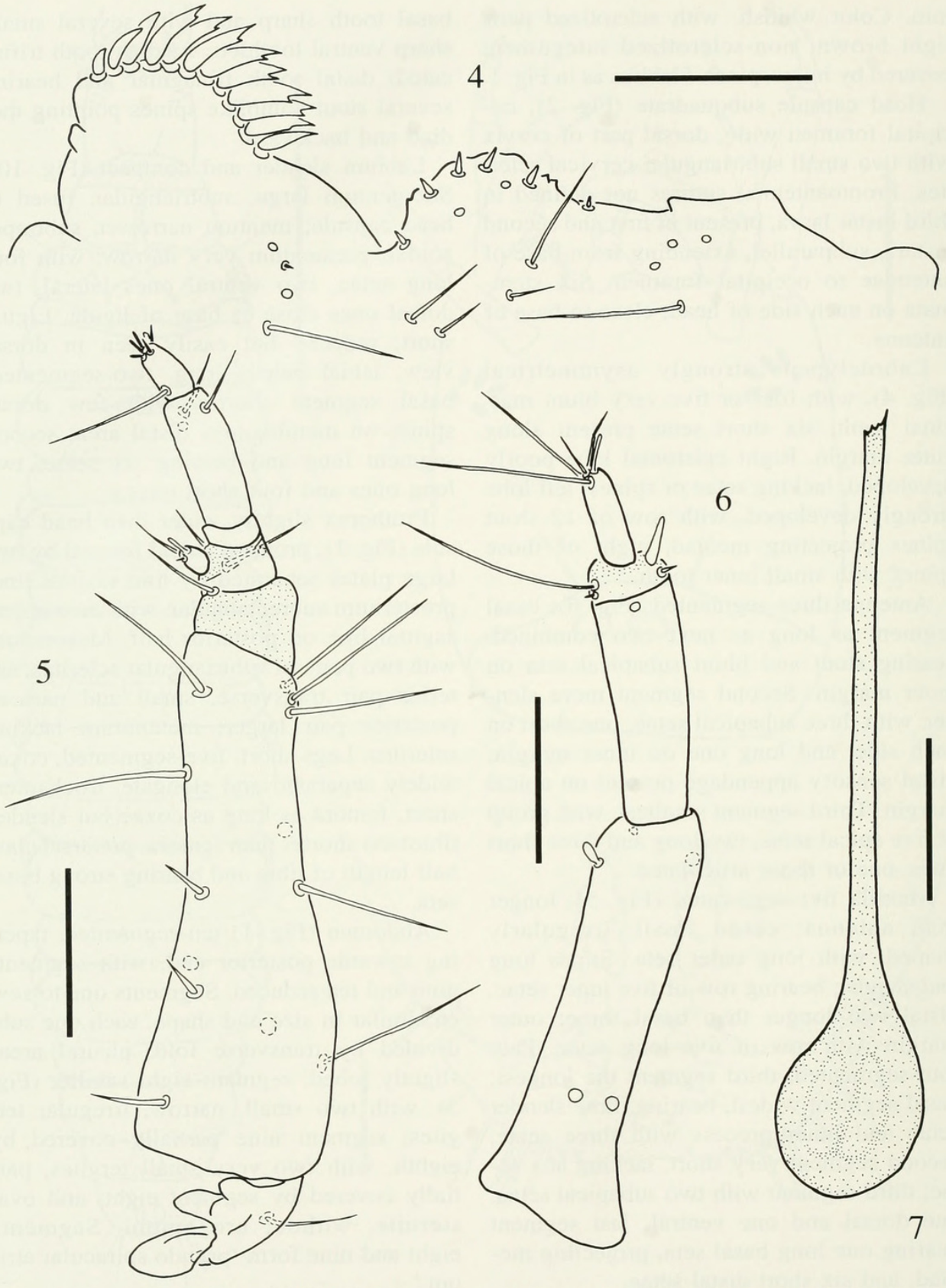
Mandibles strongly asymmetrical (Figs. 8–9). Right mandible with one large inner tooth and two or three more blunt and short teeth on basal half. Left mandible with a group of three inner teeth on basal half; basal tooth sharp and with several small, sharp ventral toothlets; median tooth trifurcated; distal tooth triangular and bearing several stout comblike spines pointing mediad and backward.

Labium slender and compact (Fig. 10). Submentum large, subtriangular, fused to head capsule; mentum narrower, subtrapezoidal; prementum very narrow, with four long setae, two ventral ones lateral, two dorsal ones close to base of ligula. Ligula short, reduced but easily seen in dorsal view; labial palpus long, two-segmented; basal segment shorter, with few dorsal spines on membranous distal area; second segment long and bearing six setae, two long ones and four short ones.

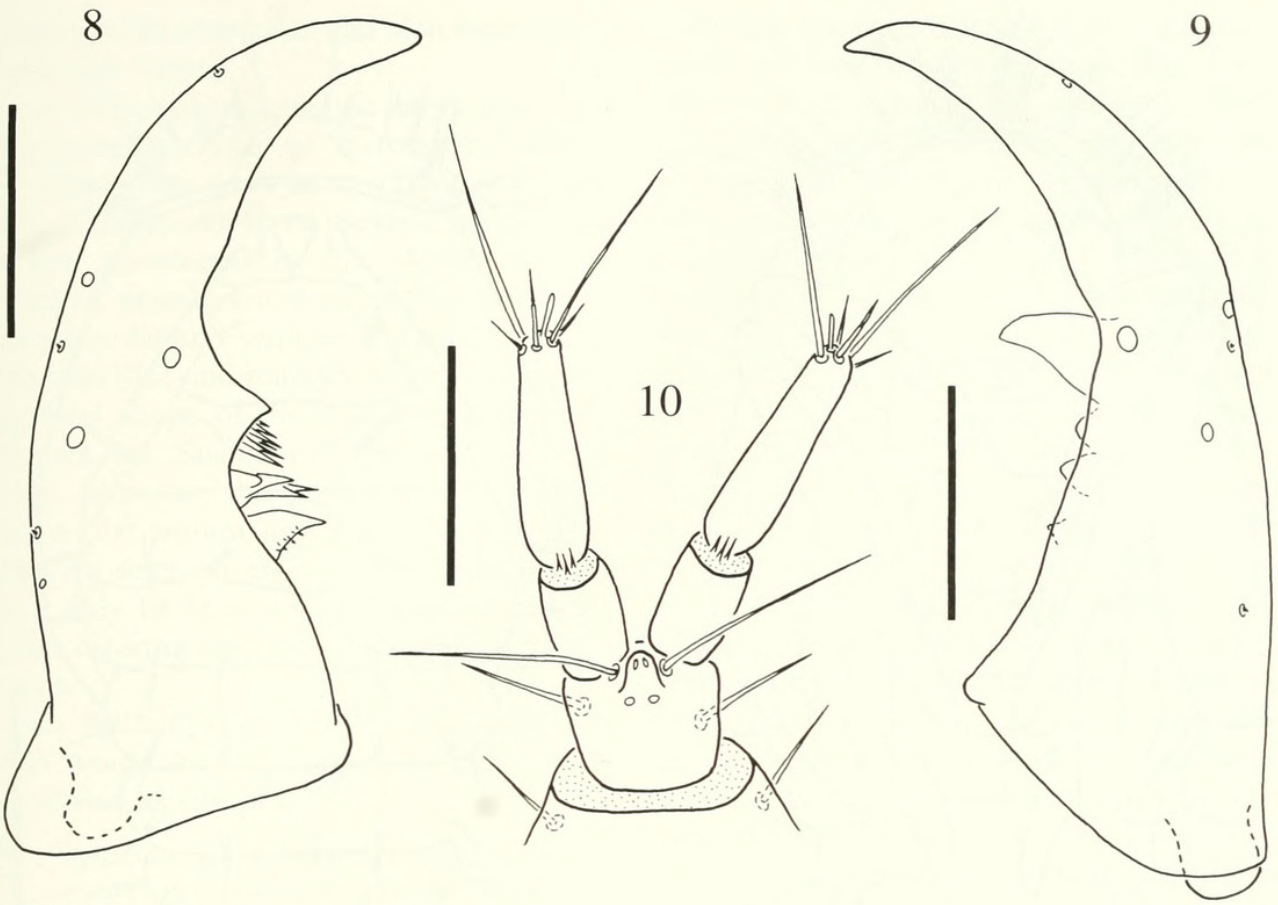
Prothorax slightly wider than head capsule (Fig. 1); pronotal shield formed by two large plates separated by fine sagittal line; prosternum subrectangular, with incomplete sagittal line on posterior half. Mesonotum with two pairs of subtriangular sclerites, anterior pair transverse, small and narrow, posterior pair larger; metanotum lacking sclerites. Legs short, five-segmented, coxae widely separated and elongate, trochanters short, femora as long as coxae but slender, tibiotarsi shorter than femora, pretarsal claw half length of tibia and bearing strong basal seta.

Abdomen (Fig. 1) ten-segmented, tapering towards posterior end, with segments nine and ten reduced. Segments one to seven similar in size and shape, each one subdivided by transverse fold, pleural areas slightly lobed; segment eight smaller (Fig. 3), with two small, narrow, irregular tergites; segment nine partially covered by eighth, with two very small tergites, partially covered by segment eight, and oval sternite, without urogomphi. Segments eight and nine form 'pseudo spiracular atrium.'

Nine pairs of spiracles, one on mesothorax and eight abdominal. Thoracic and first seven abdominal pairs of spiracles nonfunctional. Last abdominal pair enclosed within shallow spiracular atrium, different



Figs. 4–7. *Hemiosus bruchi*. 4, Labroclypeus, dorsal view. 5, Right maxilla, dorsal view. 6, Right antenna, dorsal view. 7, Egg case. Scale bars: 4–6 = 0.1 mm, 7 = 1.0 mm.

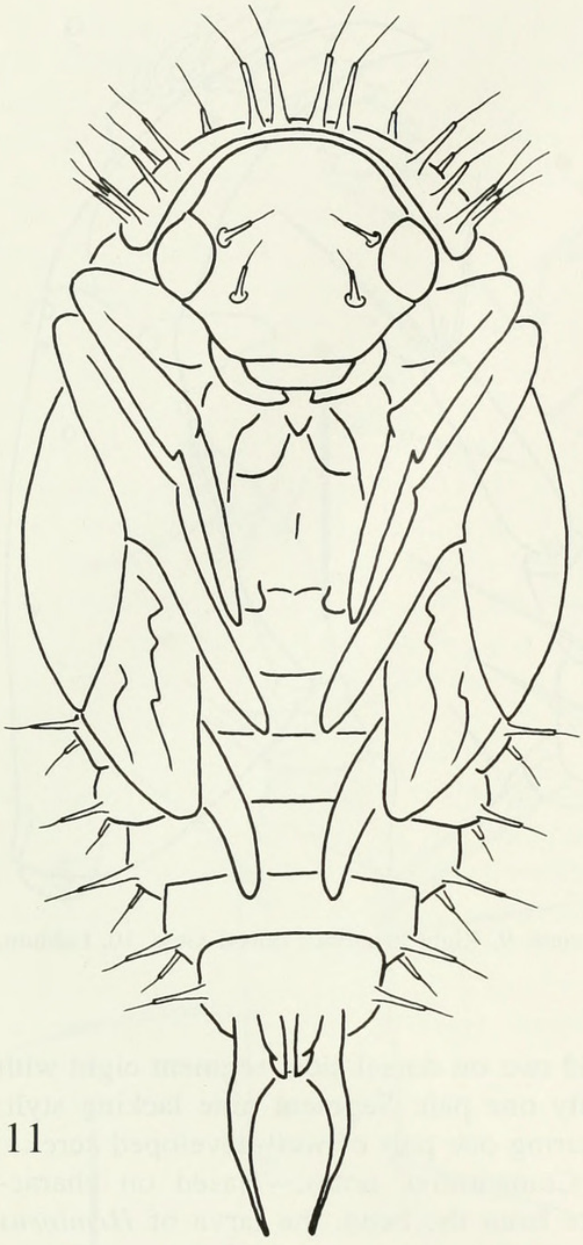


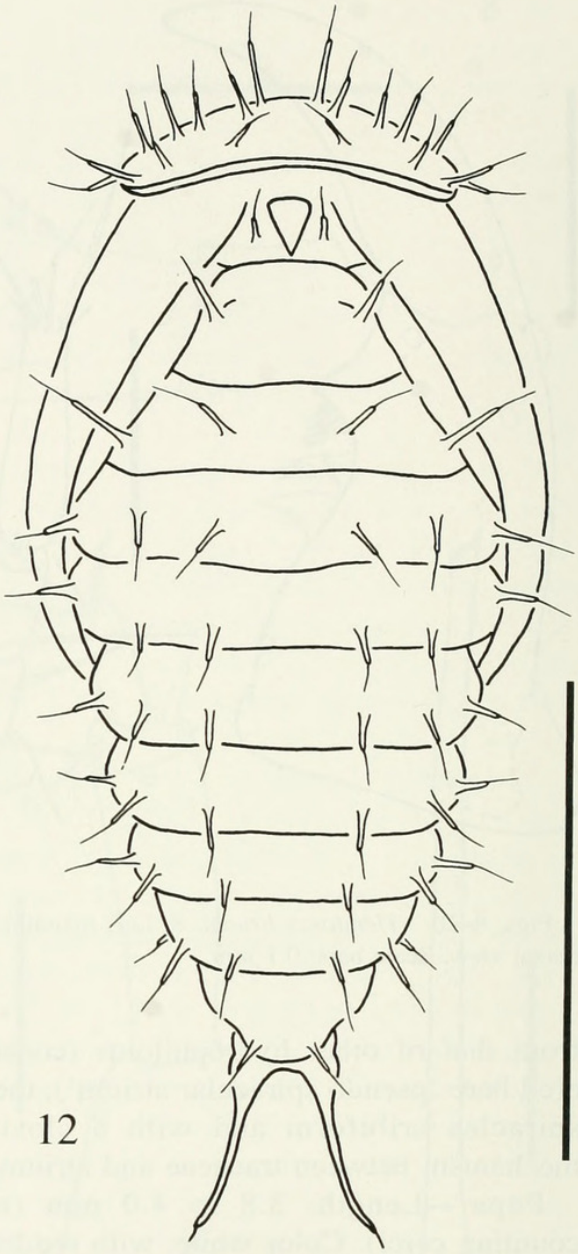
Figs. 8–10. *Hemiosus bruchi.* 8, Left mandible, dorsal view. 9, Right mandible, dorsal view. 10, Labium, dorsal view. Scale bars: 0.1 mm.

from that of other hydrophiloids (considered here 'pseudo spiracular atrium'); these spiracles cribiform and with a closing mechanism between tracheae and atrium.

Pupa.—Length: 3.8 to 4.0 mm (not counting cerci). Color white, with reddish-brown eyes in older pupae. Mandible small, covered in part by head capsule and labrum (Fig. 11), antenna not visible. Pro- and mesotibiae short, folded against femora, never extending past wing pads; metathoracic leg almost completely covered by wings, distal part of tarsus visible, extending past wing pads. Head with two pairs of supraorbital styli. Pronotum small, with 12 pairs of styli (Figs. 11–12), five pairs on anterior margin, one on lateral margin, four on posterior margin, and remaining pairs on disc. Meso- and metanota each with one pair of styli. First abdominal segment with two pairs of styli on dorsal side; segments two to seven each with three pairs, one on pleural area and two on dorsal side; segment eight with only one pair. Segment nine lacking styli, bearing one pair of well-developed cerci.

Comparative notes.—Based on characters from the head, the larva of *Hemiosus* is clearly related to that of *Berosus,* but at the same time it easy to tell apart from those of *Berosus* and *Derallus,* the other two Berosini genera with known immature stages (Archangelsky 1997, 1999). *Derallus* larvae have numerous thoracic and abdominal setiferous projections, their labroclypeus and mandibles are symmetrical, they have a well developed spiracular atrium with the eighth segment bearing a large tergal plate and the ninth abdominal segment subdivided in three lobes. The main difference separating *Hemiosus* larvae from those of *Berosus* is the presence of several pairs of abdominal gills in all known *Berosus.* There are other minor differences, which include: the presence of one oval tergal





Figs. 11–12. *Hemiosus bruchi*, pupa. 11, Ventral view. 12, Dorsal view. Scale bar: 2.0 mm.

plate on segment eight in *Berosus* (*Hemiosus* has two narrow elongated plates), the presence of a 'pseudo spiracular atrium' in *Hemiosus* larvae (absent in *Berosus*), and the presence of cervical sclerites in *Hemiosus* (absent in *Berosus*).

Other genera, outside the Berosini, that resemble larvae of *Hemiosus* are *Laccobius* Erichson and *Oocyclus* Sharp. Both these genera have asymmetrical mandibles and labroclypeus (with a left epistomal lobe bearing a row of stout spines), as well as a labium with reduced ligula. The following characters, shared by *Laccobius* and *Oocyclus,* will differentiate their larvae from those of *Hemiosus.* First, *Laccobius* and *Oocyclus* have a well developed spiracular atrium, segment eight with a large oval tergite and a pair of annular spiracles. They also have a trilobed ninth segment, with a pair of one-segmented urogomphi. The right lobe of the epistome of *Laccobius* and *Oocyclus* is well developed and projects farther than the nasale. Also the second antennal segment in these two genera is longer than the first, and the stipes, is wider

than that of *Hemiosus* and also bears small cuticular spines.

A comparison with the larva described by Spangler (1966) as 'Berosinae-? Genus-?' shows that what he described was actually a *Hemiosus* larva. Several of the characters mentioned in that description are similar: asymmetrical mandibles and labroclypeus, labium without a ligula, shape of the maxillae and number of setae on stipes, general shape of the thorax, legs and abdomen, etc. Spangler (1966) does not mention, however, the presence of a reduced spiracular atrium and the cribiform spiracles on segment eight, but these characters can only be seen with a compound scope, after clearing and slide mounting the specimen.

In Archangelsky (1997), *Hemiosus* larvae would key to couplet 6, this can be modified as follows:

6. Spiracular atrium absent or reduced (when reduced, with cribiform spiracles), urogomphi absent; usually some of the abdominal segments with long, slender lateral tracheal gills 6a

6. Spiracular atrium present (with annular spiracles), urogomphi present; projections from the thoracic and abdominal segments present or absent, but never functioning as tracheal gills 7

6a. At least some of the abdominal segments with long, slender, lateral gills; spiracular atrium absent; cervical sclerites absent; one oval tergal plate on segment eight . . . *Berosus*

6a′. No lateral tracheal gills present; 'pseudo spiracular atrium' present; cervical sclerites present; abdominal segment eight with one pair of narrow tergal plates . . . . . *Hemiosus*

Biological notes.—Both adults and larvae were collected in a small and shallow montane creek flowing from Sierra de Velasco. The adults were active and could be seen swimming close to the bottom of the creek. The larvae were collected by stirring the sandy bottom of the creek; they were found in the top layer of the creek's bottom which was a mixture of sand, fine silt and algae, about 2 cm deep. Most of the collected larvae were third instars, but a couple of second instars were also found.

The larvae were not very active, burrowing in the sand most of the time. They usually remained inactive, and started to move when they detected a prey with the long sensory setae they have on the head and other parts of the body. Most of the prey consumed were chironomid larvae, which were also found burrowing in the same habitat; some additional prey were Hydroptilidae larvae. The larvae were green, but the contents of the digestive track were dark, usually reddish from the chironomids they ate. Because of their low activity, *Hemiosus* larvae were able to survive several days (up to a month) without feeding; these low levels of activity are probably correlated with their respiratory system, since they lack the tracheal gills present in *Berosus* larvae, their sister group. The last pair of spiracles of *Hemiosus* larvae is cribiform; apparently gas exchange occurs either through the cuticle, through the last abdominal pair of spiracles, or a combination of both. Cribiform spiracles are known to occur in some terrestrial beetle larvae and the cribose plate covering the spiracles prevents the penetration of water into the atrium; in the case of *Hemiosus* this cribose plate could allow for gas exchange. This may also explain why *Hemiosus* larvae are usually found in well oxygenated bodies of water while those of *Berosus* can live in a wider range of habitats.

On the first collecting date (23.v.98) both adult and larvae were abundant, but on the second (29.ix.98) only third instar larvae were abundant, and only two adults were found. The first date coincides with the beginning of Autumn, and the second with that of Spring. This suggests that the main overwintering stage may be the third larval instar, and that only a few adults are able to survive the winter.

Hydramara argentina (Knisch 1925) (Figs. 13–17)

Material examined.—ARGENTINA, La Rioja Province: Departamento General La Madrid, Río del Peñón, 2,925 m, 5.iv.1998

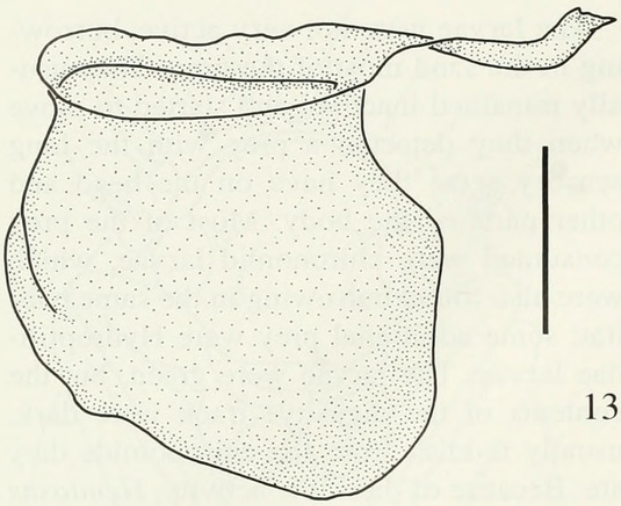
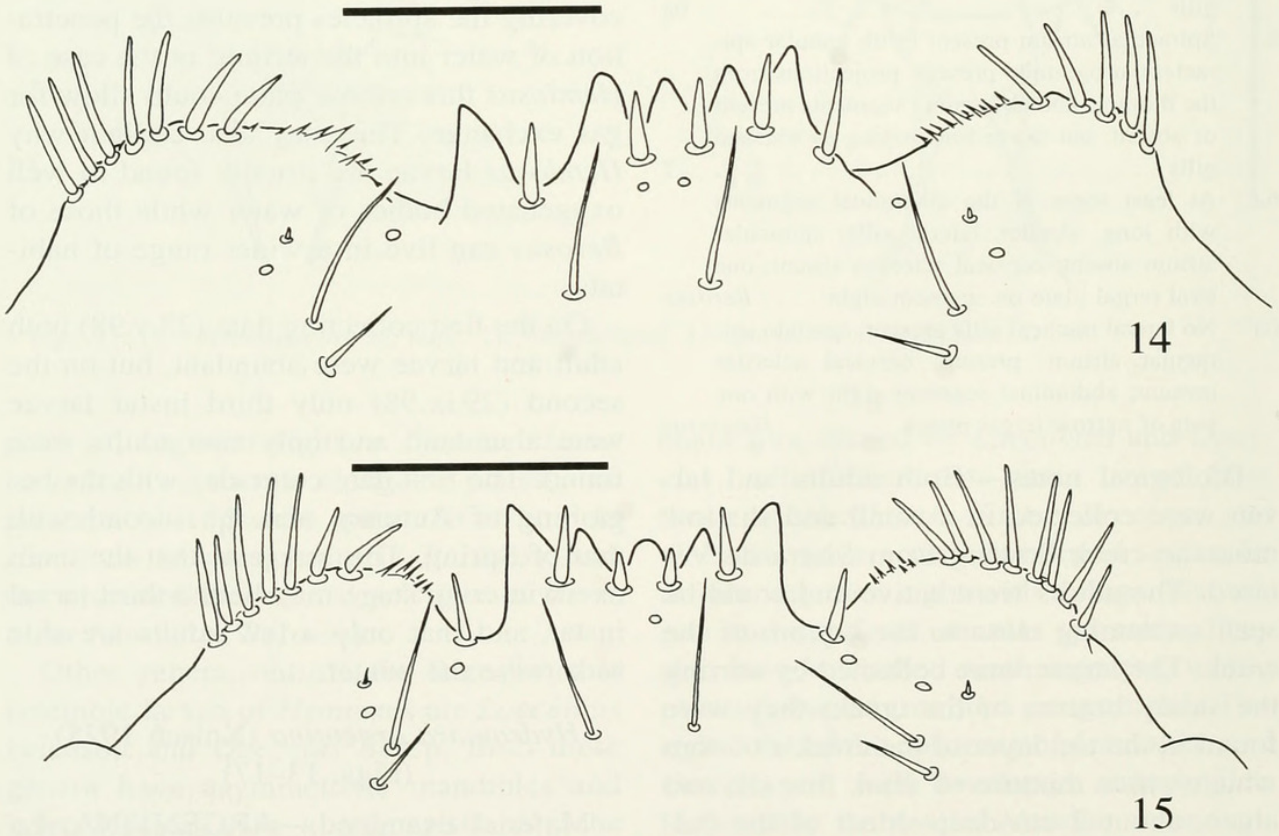


Fig. 13. *Hydramara argentina*, egg case. Scale bar: 2.0 mm.

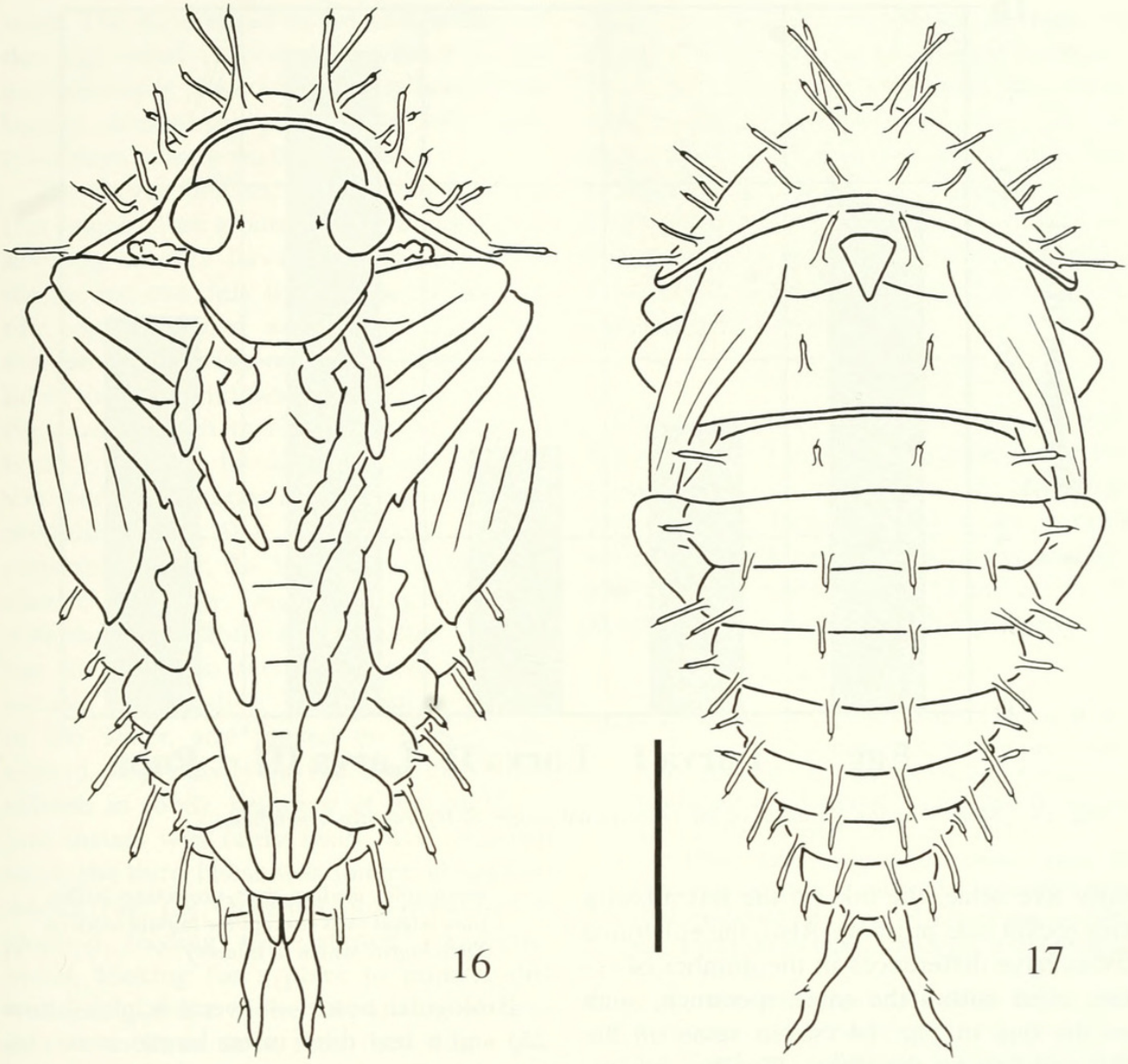
(28 larvae, 2 pupae, 4 egg cases); Departamento Sanagasta, Arroyo Tambito, 1,340 m, 29°09′47″S, 67°04′47″, 23.v.1998 (1 larva); Departamento Chilecito, road to Mina de Oro, 1990 m, 6.xii.1998, (1 larval skin, 2nd instar).

Egg case.—Length: 4.5 mm, width: 3.0 mm, height: 4.0 mm. Bag-shaped (Fig. 13), white, with two silk layers, outside one forms the receptacle for the eggs, inside one forms cap and short mast (in some cases). Number of eggs 5 to 27 per case; length of eggs 1.2 to 1.4 mm.

Pupa.—Length: 8.0 to 8.8 mm (not counting cerci). White, with reddish-brown eyes in mature pupae. Mandibles partially covered by head (Fig. 16); maxillary palp long, reaching distal end of mesotibia; antenna visible, covered in part by head and profemur. Pro- and mesothoracic legs with tibiae folded against femora and tarsi extending backwards, subparallel; mesotarsus reaching farther than wing pads; metathoracic leg covered by wing pads, only end of tarsus visible, reaching end of abdomen. Head with one pair of short supraorbital styli (all styli bearing very short setae). Pronotum with 11 pairs of styli (Figs. 16–17), five pairs on anterior margin, four on posterior margin, and two pairs on disc.



Figs. 14–15. *Hydramara argentina*. 14, Labroclypeus, third instar larva, dorsal view. 15, Labroclypeus, first instar larva, dorsal view. Scale bars: 14 = 0.2 mm, 15 = 0.1 mm.



Figs. 16–17. *Hydramara argentina*, pupa. 16, Ventral view. 17, Dorsal view. Scale bar: 2.0 mm.

Meso- and metanota each with one pair of styli. Abdominal segment one with two pairs of styli; segments two to seven with transverse row of six styli, one pair on pleural areas and two pairs on dorsal side; segment eight with two pairs of short styli; segment nine bearing one pair of cerci, each cercus with a terminal and median projection, each bearing short seta.

Comparative notes.—*Hydrobius* and *Hydramara* are the only two genera of Hydrobiina in the New World. Although they do not overlap biogeographically, it seems useful to describe the differences between their pupae. *Hydrobius* pupae have 20 styli on the pronotum, while those of *Hydramara* have 22, the length of the setae at the end of the styli are also different, short in *Hydramara* and long in *Hydrobius*; the legs of *Hydrobius* pupae are short, not reaching the end of the abdomen (metathoracic legs) and not extending farther that the wing pads (mesothoracic legs).

A comparison among third instar larvae of *H. argentina* shows variation in the number and position of the labroclypeal setae. The larva described and illustrated in Archangelsky (1997) shows six short setae intercalated with the five labroclypeal teeth, and the one illustrated here (Fig. 14) has

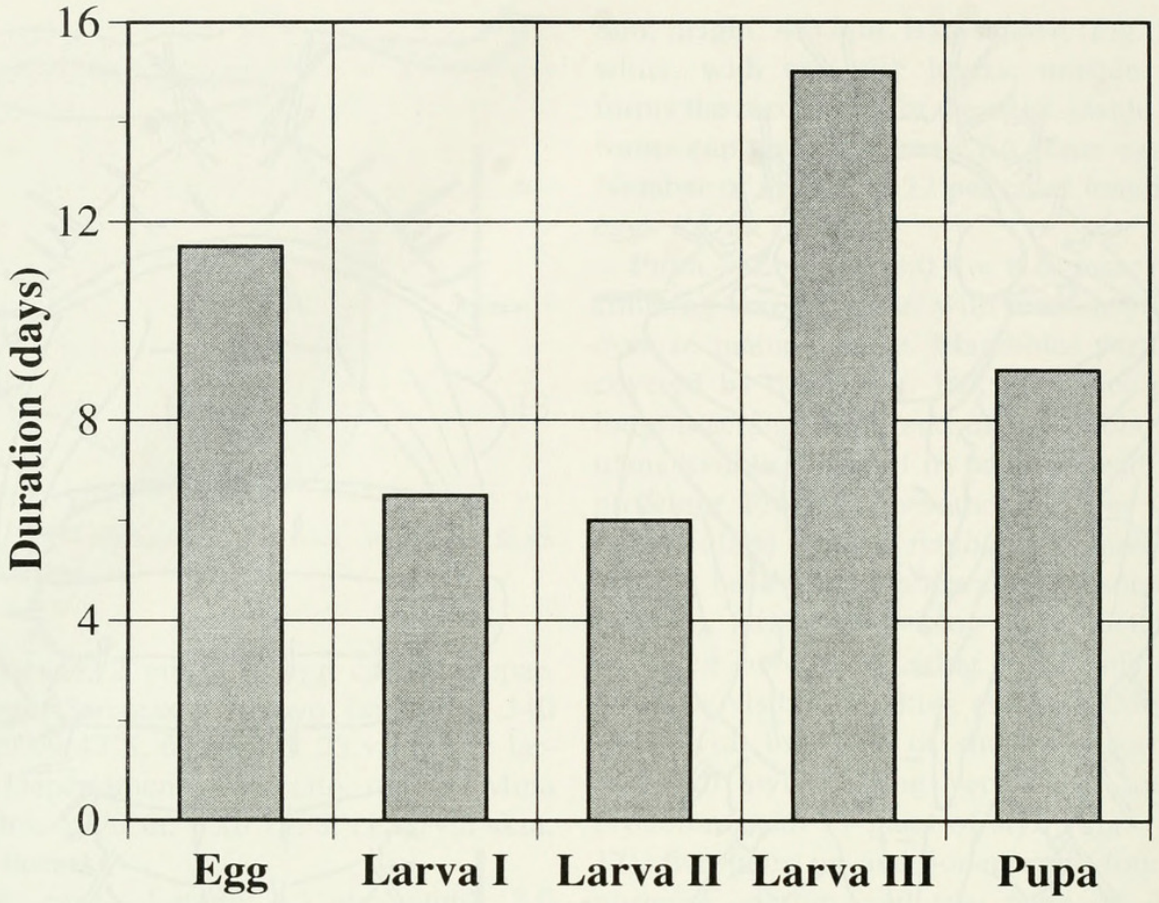


Fig. 18. Mean duration (in days) of the preimaginal stages of *Hydramara argentina*.

only five setae, the one on the left missing (no socket was present). Also, the epistomal lobes have differences in the number of setae, even within the same specimen, such as the one in Fig. 14 (seven setae on the left and five on the right). Finally, one important difference was observed between first and third instar larvae, the shape of the labroclypeus, which is asymmetrical in third instar larvae (also in second instars), but it is symmetrical in first instars (Fig. 15). In Archangelsky (1997) a first instar larva of *Hydramara* would run to couplet 31 instead of 32, this key can be modified as follows:

30. Labroclypeus symmetrical or nearly so, with teeth arranged in a semicircle (concave or convex) 30a
30.′ Labroclypeus distinctly asymmetrical, right side projecting farther than left side 32
30a. Teeth of labroclypeus arranged in a concave semicircle, lateral ones projecting farther than median ones . . . . *Hydramara* (1st instar)
30a.′ Teeth of labroclypeus arranged in a convex semicircle, median ones projecting farther than lateral ones (except for middle tooth of *Sperchopsis* which is minute) 31

Biological notes.—Several adults (about 25) and a few third instar larvae were collected in Río del Peñón, a small creek that originates in the high altitude lagoon Laguna Brava. The adults were found on the underside of rocks lying in the water, close to the shore, and surrounded by algae. The adults were taken alive to the laboratory and during the trip they constructed two egg cases. Two more egg cases were built in the laboratory, but these contained no eggs. The first two egg cases were constructed on the underside of algae that were in the container (to avoid any damage to the beetles the container had no sand or rocks); the egg cases built in the laboratory were partially dug within the sandy substrate, against a small rock, and close to the waterline. The first two egg cases had no mast or ribbon, while the two built in the lab had a flat

mast. The differences in the architecture of the egg cases is probably related to the movements in the container that housed the beetles during the trip to the laboratory (two days mostly on dirt roads).

The larvae hatched after 11 or 12 days (lab temperature around 20–22°C); from the first egg case 27 larvae hatched, and from the second one only five (about 20 non-fertile eggs remained within the case). The first larva to emerge cut a small hole on the lid of the case, and the remaining larvae left the case through that hole too. It took 24 hours for all the larvae to emerge. The larvae were predaceous as those of other hydrophilids and also cannibalistic. While consuming prey, the larvae raised the head almost vertically, manipulating the food with their mandibles and maxillae, following the common feeding pattern of other aquatic hydrophilids. They went in and out of the water, and preyed on chironomid, culicid, and mayfly larvae (the only ones offered as food). Duration of first and second instars was fairly short, five to seven days; the third instar was longer, about two weeks (Fig. 18). Once the third instar larvae stopped feeding they started wandering about, looking for a place to pupate; this wandering phase of the prepupal stage lasted two to three days, then they dug a pupation chamber in the sandy soil used as substrate. The pupation chamber was 1 to 2 cm below the surface, and it measured 1.5 cm wide by 2 cm long by 1 cm deep. Once in this chamber the prepupa took 5 to 6 days to pupate. The pupal stage lasted about eight days, and after emerging, the adults remained for two days in the chamber.

The adults fed on the algae, and also on flakes of fish food that were provided to add some protein to the diet. They were rather active in the laboratory, but mostly at night, and could move fairly well in the water. Stridulation by the adults was heard during the two months they were kept alive in the laboratory, and also when disturbed. The collection date of the adults and larvae suggests that the overwintering stage could be either the adult or the last larval stage, or perhaps both since one more third instar larva of this species was collected later (from a different locality, together with some of *Hemiosus bruchi*) well into the autumn, but no adults could be found at that time. Other Hydrophilinae such as *Hydrobius melaenus* (Germar 1824) have been reported to overwinter both as adults and third instar larvae (Archangelsky 1997).

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