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First record and revised description of *Herpetocypris helenae* G. W. Müller, 1908 from the Neotropics (central-west Argentina)

(Crustacea, Ostracoda)

D. Sabina D'Ambrosio, Analía R. Díaz, Adriana García & María Cristina Claps

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The genus *Herpetocypris* Brady & Norman, 1889 has a wide distribution in most of the zoogeographical regions except in the Neotropical Region, where only three species have so far been found – these being *H. reptans* (Baird, 1835), *H. chevreuxi* (Sars, 1895) and *H. pectinata* Brehm, 1934. We here report the first record of *Herpetocypris helenae* G. W. Müller, 1908 from the Neotropical Region, and provide a complete revised description and illustration of the valves and soft parts of this species, as well as information on ecological factors affecting its distribution and aspects of morphological variability of its valves.

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Introduction

At present, c. 2000 non-marine ostracod species have recently been described representing c. 200 genera; but many more taxa are to be expected in the Southern Hemisphere, and especially in South America (Martens et al. 2008, Martens & Savaternalinton 2011).

The ostracod fauna, particularly in Argentina, is still poorly known, although the study of this group has greatly increased in the last decades (Ramírez 1967, Schwalb et al. 2002, Cusminsky et al. 2005, Fontana & Ballent 2005, Laprida 2006, Laprida et al. 2006, Díaz & Lopretto 2009, 2011a, 2011b, Ballent & Díaz 2011, Liberto et al. 2012, Ramón Mercau et al. 2012,

2014, Díaz & Martens 2014, and D'Ambrosio et al. 2015), with approximately 23 genera and 50 species recorded. According to Martens & Savaternalinton (2011), areas in Argentina with high ostracod species richness could possibly still remain unknown.

The subfamily Herpetocypridinae Kaufmann, 1900 comprises some of the larger species of ostracods (length 1.80–3.00 mm) and is one of the most diverse within the family Cyprididae, the latter involving half of the known non-marine ostracod fauna (Higuti et al. 2009, Karanovic 2012). *Herpetocypris helenae* G. W. Müller, 1908, belongs to the tribe Herpetocypridini Kaufmann, 1900 along with the genera *Candonocypris* Sars, 1894 and *Ilyodromus*

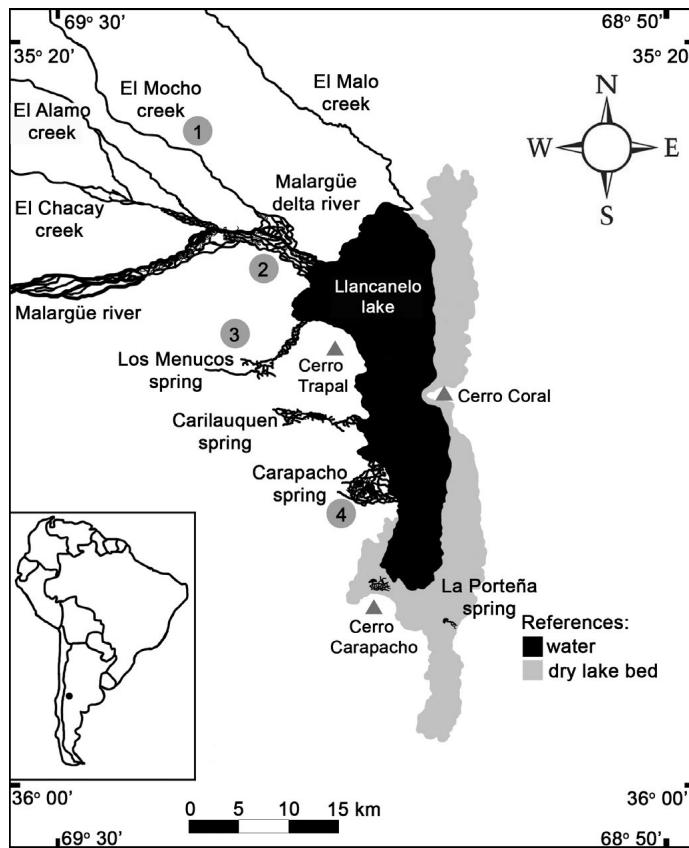


Fig. 1. Location of sampling sites in Llancanelo basin (Mendoza province, Argentina). 1. El Mocho stream; 2. Malargüe River delta; 3. Los Menucos Spring; 4. Carapacho Spring.

Sars, 1894. The genus *Herpetocypris* Brady & Norman, 1889 is widely distributed and highly diverse in most of the zoogeographical regions, excepting the Neotropical ecozone, where the species found until now – according to Martens & Behen (1994) and Martens & Savaternalinton (2011) – were exclusively *H. pectinata* Brehm, 1934 in Chile; *H. chevreuxi* (Sars, 1896) in Ecuador; and *H. reptans* (Baird, 1835) in Chile, Perú, and Argentina (Wierzejski 1892 in Ramírez 1967).

The original description of *H. helena*, published in 1908 by Müller, was based on the morphology of the valves and certain appendages: a description of the second antenna along with large natatory setae that extended farther than the tips of the terminal claws, the maxilla with the serrated seta on the endites, and a strong caudal ramus with spines on its distal margins. In 1996, González Mozo et al. performed a taxonomic revision of the European *Herpetocypris* on the basis of *H. helena* material from Spain, Africa, Israel, and England and at the same

time made a complete material revision of *H. helena* itself with good illustrations and photographs of the valves and soft parts, but without a description of those features. Since, until now, *H. helena* has been found in only the Afrotropical and Palearctic Regions (Balanás & García-Avilés 1993, González Mozo et al. 1996, Mezquita et al. 1999, Martens & Savaternalinton 2011, Rasouli et al. 2014), this present report extends the distribution of the species to the Neotropical Region.

According to González Mozo et al. (1996) the species of *Herpetocypris* have been determined on the basis of the valves (shape, size), the length of the natatory setae on A2, the spines (size and number) on the ventral margin of the furcal rami, and the length of the setae on the T1 palp. *Herpetocypris helena* was considered to be synonymous with *H. chevreuxi* until 1996, when González Mozo et al. provided four characters to maintain the individuality of both species (Meisch 2000). However, the taxonomic position of several *Herpetocypris* species remains

uncertain, especially that of *H. helenae*, because of the incompleteness of its description. For this reason, a complete delineation of the soft parts and valves of *H. helenae* along with the details of its morphologic variability within different regions will constitute essential information for both, the description of the species and for the clarification of the taxonomic status of the members of the genus.

Materials and methods

The area studied is the Llancanelo basin, located in an arid zone c. 70 km east of the Andes mountain range (Mendoza Province, Argentina; 35°00'-36°30'S, 68°30'-70°00'W; 1330 m above sea level). Sixteen sites were selected for the seasonal collection within that endorheic basin. The main water source in the basin is from spring snowmelt, with the Malargüe River being the principal tributary. Each environmental site was sampled at a single location during each season, but *H. helenae* was found only in the El Mocho stream, the delta of the Malargüe River, and the Los Menucos and Carapacho springs.

The El Mocho stream (Fig. 1, Site 1) originates in the Andes and is a tributary to the Malargüe River joining the latter in the lower section of the river's course. This stream is temporary with a marked seasonal fluctuation in its flow.

The Malargüe River originates in the Andes Mountains at 2500 m above sea level; it is fed by numerous tributaries and after receiving water from the El Chacay and El Mocho streams, it forms a delta in the northwest corner of Llancanelo Lake. This delta (Fig. 1, Site 2) develops in a lowland area and can be considered as temporary.

The Carapacho spring is fed by groundwater and forms large wetlands, usually connected to the lake by small streams. The Los Menucos rheocrene spring (Fig. 1, Site 3) is located in the northwest sector of Llancanelo Lake and has a mean water depth of 0.3 m. The Carapacho heleocrene spring (Fig. 1, Site 4), located on the southwestern side of the lake, is the only warm spring in the area and remains at a constant temperature of around 19–20 °C during all four seasons. This spring, having a depth of as much as 4 m, also constitutes the deepest wetland in the area, though the mean depth is 1 m. The samples there were accordingly collected close to the shore.

The physical and chemical parameters (pH, temperature, dissolved oxygen, and conductivity) were obtained seasonally at the time of ostracod collection through the use of a Horiba U-10 multiprobe. The material was collected from surface sediments (upper 3 cm) and then rinsed through sieve of 100 µm mesh size. The specimens were fixed in 70 % aqueous ethanol for permanent storage. The ostracods were sorted under a binocular microscope and dissected. The valves were stored dry on micropaleontological slides and the soft parts in glycerine on sealed slides. Line drawings were

made using a camera lucida, while the valves were illustrated and measured by means of scanning electron microscopy (JEOL JSM 6360 LV, Museo de La Plata; Philips XL 30 Museo Argentino de Ciencias Naturales, MACN, Buenos Aires, Argentina). The material was deposited in the Invertebrate Collection of the Museo de La Plata, Argentina.

The nomenclature of the limb chaetotaxy follows Broodbakker & Danielopol (1982), of the second antenna the revised model proposed by Martens (1987), and of the second and third thoracopods Meisch's nomenclature (2000). Higher taxonomy of the Ostracoda follows the synopsis by Horne et al. (2002). The following abbreviations were used: Cp = carapace; valves: H = height of valves, L = length of valves, LV = left valve, RV = right valve; limbs: A1 = antennula, A2 = antenna, CR = caudal ramus, CRa = caudal ramus attachment, db = dorsal branch, Lo = concave lobe, Md = mandibula, Mx = maxillula, Rlo = rake-like organ, T1 = first thoracopod, T2 = second thoracopod, T3 = third thoracopod, vb = ventral branch. Measurements are expressed as the arithmetic mean ± the standard deviation (with the minimum and maximum values shown between brackets). Size measurements are given in millimetres; N = number of individuals.

Taxonomy

- Class Ostracoda Latreille, 1802
- Order Podocopida G. O. Sars, 1866
- Superfamily Cypridoidea Baird, 1845
- Family Cyprididae Baird, 1845
- Subfamily Herpetocypridinae Kaufmann, 1900
- Tribe Herpetocypridini Kaufmann, 1900
- Genus *Herpetocypris* Brady & Norman, 1889

Herpetocypris helenae G. W. Müller, 1908

Material examined. 30 of a total of 48 ♀ adults; 10 ♂ adults were deposited in the Colección de Carcinología del Museo de La Plata, Argentina (MLP 26929; MLP 26930; MLP 26931).

Diagnosis

(after González Mozo et al. (1996), emended)

Large species (1.5–2.5 mm long). Carapace elongated, narrow in dorsal view. Dorsal margin straight. Postero-ventral corners in both valves well produced. RV anteriorly and posteriorly with a clear broad inner list. LV anteriorly and posteriorly with a clearly inwardly displaced selvage. Marginal zone well developed. LV overlaps RV, anterior overlap pronounced. Surface of valves smooth with numerous marginal setae. Third segment of A1 with two unequal apical setae. Natatory setae of A2 slightly beyond tips of terminal claws. Palp of Mx spatula-like. T2 with seta d1 and seta d2, seta d2 longer.

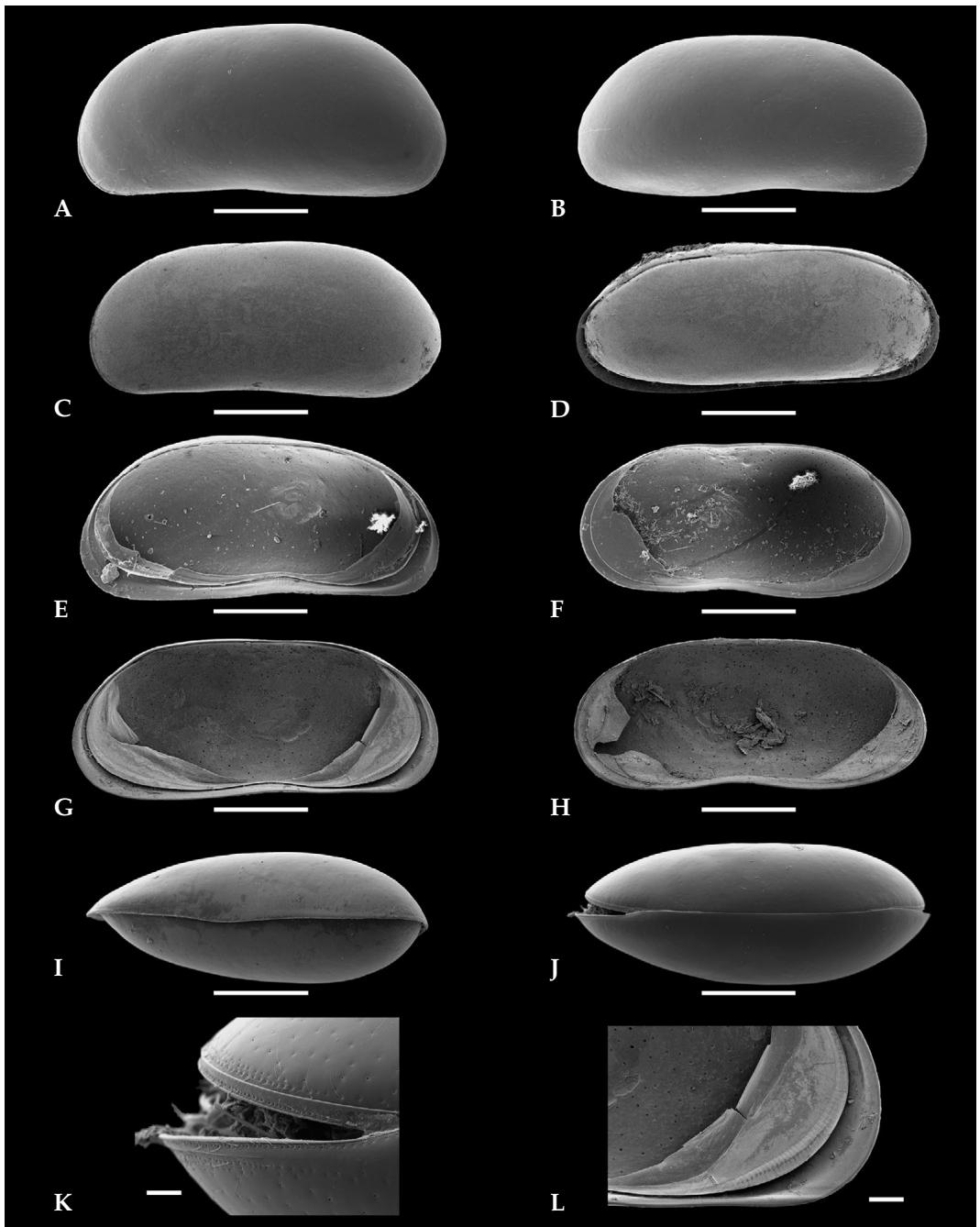


Fig. 2. Scanning electron micrographs of *Herpetocypris helenae*. A. LV, external view of the higher valve (MLP 26929); B. RV, external view of the higher valve (MLP 26929); C. LV, external view of the elongated valve (MLP 26930); D. RV, external view of the elongated valve (MLP 26930); E. LV, internal view of the higher valve (MLP 26929); F. RV, internal view of the higher valve (MLP 26929); G. LV, internal view of the elongated valve (MLP 26930); H. RV, internal view of the elongated valve (MLP 26930); I. Carapace, ventral view (MLP 26929); J. Carapace, dorsal view (MLP 26929); K. Carapace dorsal view, detail of the anterior part; L. LV, internal view, detail of the anterior part. Scale bars: A-J=5 mm, K=0.5 mm, L=1 mm.

Anatomy of valves (Fig. 2A,B,E,F,I,J,K)

Carapace elongated in lateral view (1.6–2.2 mm long). Dorsal margin straight. Anterior and posterior margins rounded. Postero-ventral corners in both valves well produced. Ventral margin with a slight concavity near the middle. Valves sub-equal and laterally compressed. Inner lamella well developed in both anterior and posterior margins. Large anterior and posterior vestibula. LV with wide selvage along anterior and posterior margin, narrow in ventral margin. Selvage inwardly displaced from the outer margin. RV with a clear anteriorly and posteriorly broad inner list. Marginal zone well developed. External valve surface smooth with numerous marginal setae, internal valve surface with large pores. Central muscle scars typical of the subfamily Herpetocypriidae. Anterior overlap LV/RV pronounced.

Remarks. Among the specimens collected in the Llano Cañuelo basin there is a marked morphological variability that permits a differentiation of specimens collected in streams (Fig. 2C,D,G,H) from those obtained in springs (Fig. 2A,B,E,F), to which the above description refers. The specimens from streams are more elongated and lower ($N=21$; LV: $L=1.96 \pm 0.05$ mm, $H=0.84 \pm 0.02$ mm; RV: $L=1.90 \pm 0.07$ mm, $H=0.81 \pm 0.06$ mm) than specimens from springs ($N=9$; LV: $L=1.89 \pm 0.08$ mm, $H=0.89 \pm 0.03$ mm; RV: $L=1.77 \pm 0.08$ mm, $H=0.82 \pm 0.02$ mm). The left valves of specimens from flowing waters are thus 3.5 % longer and 5.5 % less high than those collected in standing waters. Additionally, in the specimens from streams the posterior margin of LV is not evenly rounded, and the inner lamella is well developed in both anterior and posterior margin and the selvage presents conspicuous striations. The selvage and the inner lamella are absent in the middle of ventral margin, although, these variations are within the range recorded in literature for *H. helenae*.

Anatomy of soft parts

A1 (Fig. 3A) with seven segments. First segment with one long dorsal seta. Second segment with one short apical-ventral seta. Rome organ present, ventrally inserted. Two apical-dorsal setae and one short ventral seta on third segment, two long apical-dorsal and two long apical-ventral setae on both fourth and fifth segments, four long apical setae and one short seta on sixth segment. Terminal segment with two long setae, one short seta, and one aesthetasc y_a of approximately the same length as the setae on the terminal segment.

A2 (Fig. 3B) Second segment of protopodite with two short and one long ventral setae. Exopodite with one long and two short setae. Aesthetasc Y ventrally

inserted. With five natatory setae extending just to tips of terminal claws, 6th seta smooth and minute, one long plumose ventral seta subapically inserted, three subequal ventral t-setae. The external side has three short subapical dorsal z-setae, and two medium-sized (G1, G3) plus one small (G2) claws. Terminal segment with one medium apical-dorsal serrated claw (GM), a small (Gm) claw, a smooth seta (g), and an aesthetasc (y3). The claws G1–G3, GM, Gm armed with a row of small denticles.

Md-coxa (Fig. 3C) with 5–6 cuspatate teeth, the first being bicuspatate with a small pappose seta inserted at the inner angle. One spiny and two plumose setae are inserted on the external surface with one smooth seta between the first and second tooth. Two plumose setae are inserted at the inner angle. Exopodite is with five apical plumose setae. Mandibular palp with four segments: the first with two plumose S1 and S2 setae, one long smooth seta, and a smooth α -seta with a relatively broad base; the second with four long and one short internal setae and three dorsal apical setae; the third with four internal setae, four external subapical setae, and a plumose γ -seta with a swollen base. Apical segment with 4 claw-like setae and two smooth apical setae.

Rlo (Fig. 3D) T-shaped with many teeth.

Mx (Fig. 3E) with terminal segment of palp spatula-like. First segment with 1 plumose subapical and five plumose apical setae, second segment with six smooth setae. Third endite with 2 serrated Zahnborsten, and 5 apical setae, two of which are plumose. Second and third endite with many setae. First endite with two subapical smooth setae. Respiratory plate (Fig. 3F) with c. 16 rays.

T1 (Fig. 3G) with endite bearing 10 plumose setae of subequal lengths and four plumose setae subapically inserted. Both d and b setae present and the two a-setae smooth. Exopodite (Fig. 4A) with three setae, h1, h2, and h3; the second plumose and almost three times longer than the other two.

T2 (Fig. 4B) with both d1 and d2 setae present, d2 longer. First, second, and third segments of endopodite with one apical seta. Terminal segment with one large apical claw, flanked by 2 apical setae.

T3 (Fig. 4C) is a cleaning limb. The first segment of endopodite with three plumose setae, the second segment with one apical seta. Third and fourth segments form the pincer-shaped organ. The last segment has two setae – the shorter strongly bent, serrate; the longer claw-like (CL) – along with a serrate concave lobe (Lo) with fine pseudochaetae.

Seta pz1 is also present.

CR (Fig. 4D) symmetrical, relatively straight with spines on the posterior margin. Posterior seta claw-like, anterior and posterior claws strongly serrated. Anterior seta long and fairly close to claws.

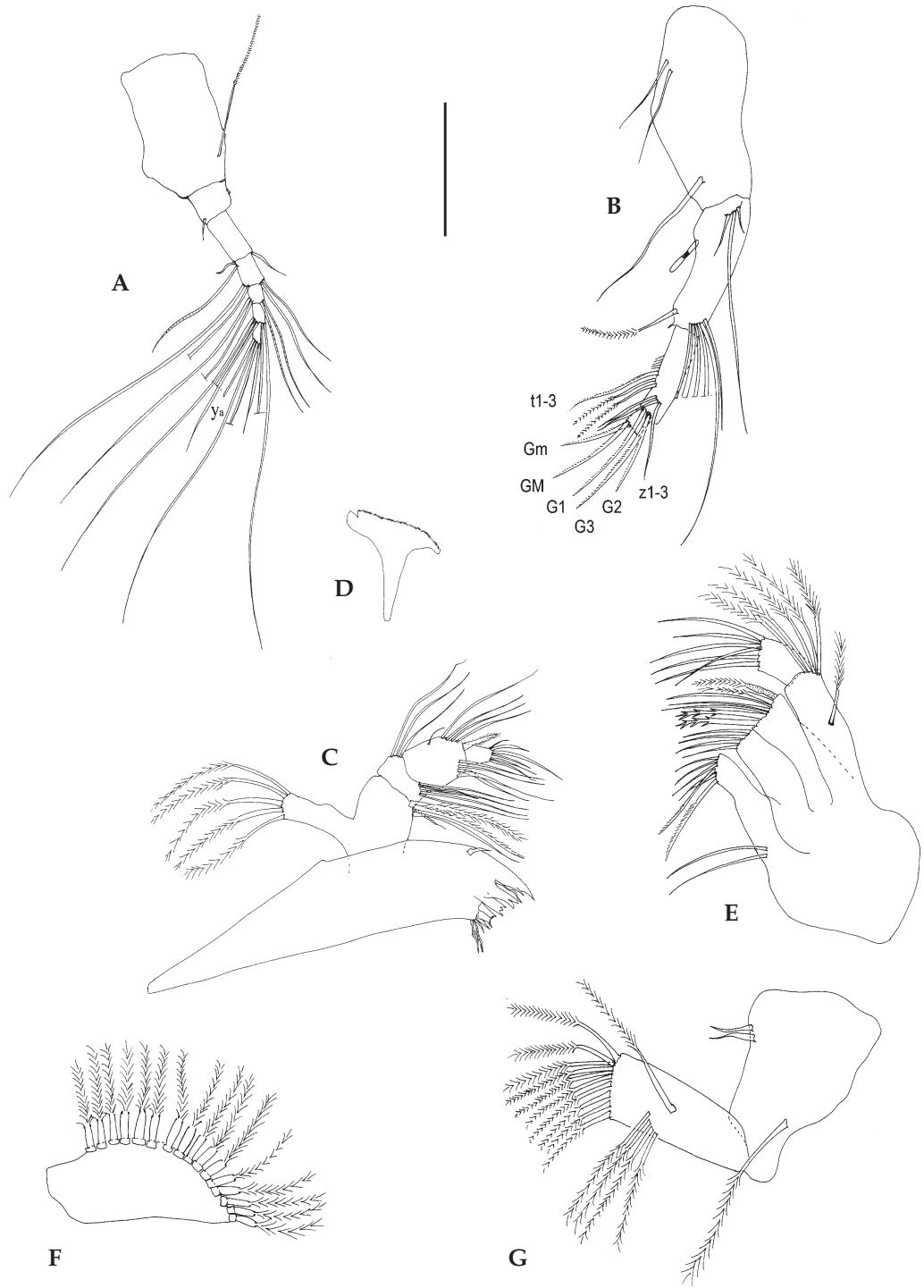


Fig. 3. *Herpetocypris helenae*. **A.** A1 (MLP 26931); **B.** A2 (MLP 26931); **C.** Md (MLP 26931); **D.** Rlo (MLP 26931); **E.** Mx (MLP 26931); **F.** Prmx (MLP 26931); **G.** T1 (MLP 26931). Abbreviations: ya, aesthetasc; G1, G2, G3, GM, Gm, claws; t1-3, setae; z1-3, zeta setae. Scale bar: A-G = 1 mm.

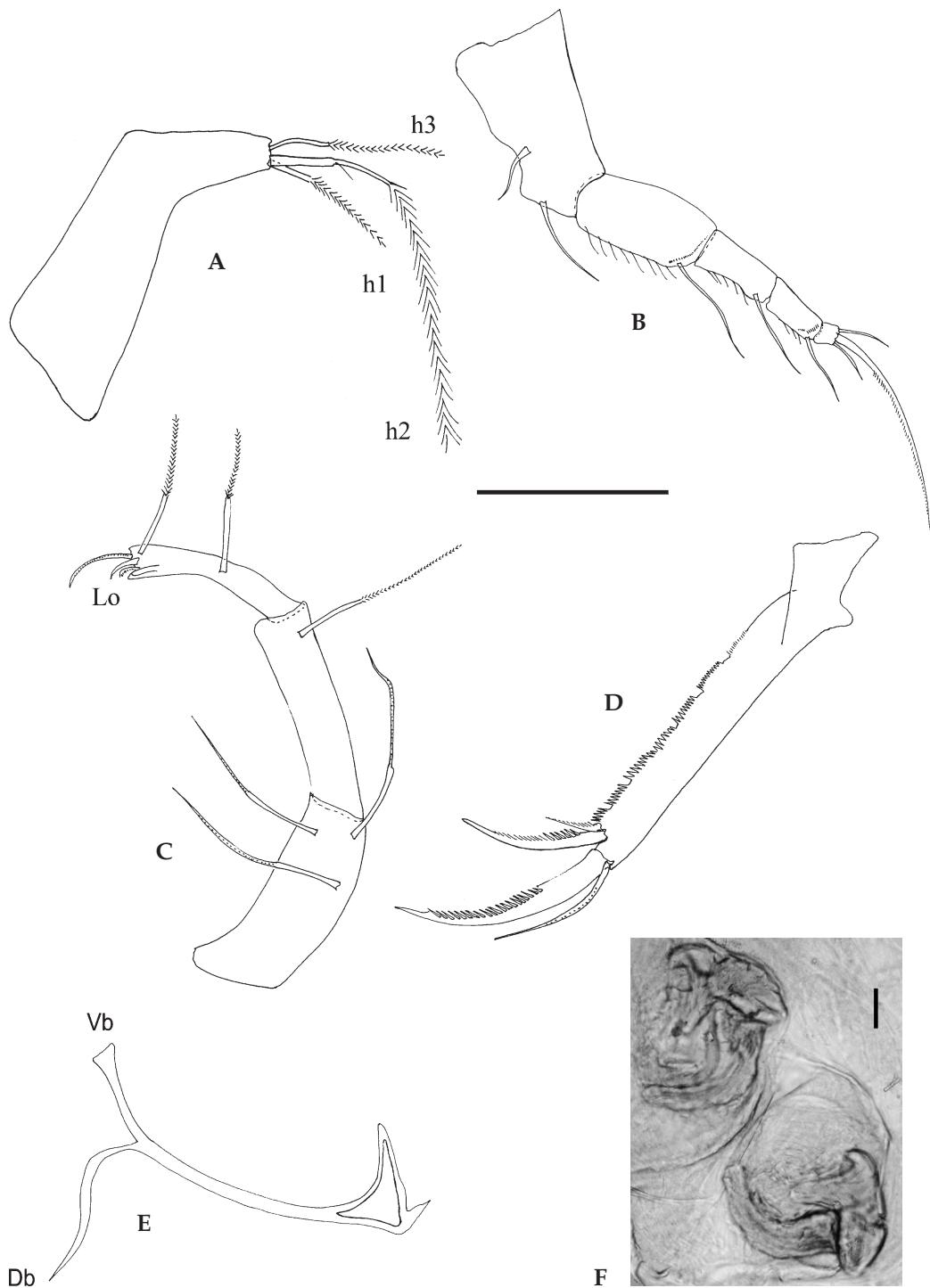


Fig. 4. *Herpetocypris helenae*. A. Exopodite T1 (MLP 26931); B. T2 (MLP 26931); C. T3 (MLP 26931); D. CR (MLP 26931); E. CRA (MLP 26931); F. Light-microscopical image of genital lobe (MLP 26931). Abbreviations: h1, h2, h3, setae; Lo, concave lobe; Db, Vb, dorsal and ventral branches. Scale bars: A-E = 1 mm, F = 0.4 mm.

CRa (Fig. 4E) proximal with a well sclerotized triangle, distally Y-shaped with the dorsal branch longer than the ventral.

Genital lobe as in Figure 4F.

Males. Unknown.

Ecology

Herpetocypris helenae has been found in fresh to sub-saline waters (salinity range: 0.2–1.4 g·L⁻¹). The environments are characterized by alkaline pH and temperatures between 19.8 and 29.5 °C with values of dissolved oxygen ranging between 19 and 84 % in lentic environments and from 100–108 % in the lotic environments (Table 1).

Discussion

As stated above, the genus *Herpetocypris* (Cyprididae) was proposed by Brady & Norman, 1889, with *Cypris reptans* Baird, 1835 as the type species. Of all the species in this genus, *H. helenae* is the closest to *H. chevreuxi* (Sars, 1896) and *H. intermedia* Kaufmann, 1900. *Herpetocypris helenae* and the latter two species belong to the *chevreuxi-helenae-intermedia* group, characterized by long natatory setae on A2 (González Mozo et al. 1996). However, the appendages of *H. helenae* have not been described and illustrated completely. Here we filled this descriptive gap to enable for a fundamental understanding of this group, and the required delineation of species.

Herpetocypris helenae differs from *H. chevreuxi* in the valve morphology, while the postero-ventral corners of both valves are not produced in *H. chevreuxi* (in contrast to those well produced in *H. helenae*). In *H. chevreuxi* the length of the natatory setae of A1 are very extensive, longer than three times that of the 5 terminal segments combined (in *H. helenae*, the natatory setae of A1 are 2–2.5 times the length of 5 terminal segments combined); the length of the natatory setae of A2 extends beyond the tips of the claws and are plumose (in *H. helenae* the natatory setae of

A2 reach to or slightly beyond tips of the claws). Other length differences are found in the z-setae (in *H. helenae* the external side of A2 has three short sub-apical dorsal z-setae), the h2 in the palp of T1 – with this latter seta also being smooth, not plumose as in *H. helenae* – d1 and d2 in T2 (in *H. helenae* d2 is longer than d1 in T2), and the dorsal branch of CRa (in *H. helenae* the dorsal branch is longer than ventral branch of CRa). Finally, the CR is slender with a unique type of spines in *H. chevreuxi*.

Herpetocypris helenae differs from *H. intermedia* in the valve morphology. The dorsal margin is straight in *H. helenae* in contrast to *H. intermedia*, where the margin is smoothly rounded; in the latter the RV has a clear broad inner list anteriorly inward from the margin (in *H. helenae* the inner list is not inwardly displaced from the margin). Furthermore, in *H. intermedia* the length of the natatory setae of A1 are 2.5–3 times the length of the 5 terminal segments combined, and the length of the natatory setae of A2 reach the distal half of the claws plus the length of h2 in the palp of T1, which is smooth. In *H. helenae* the length of natatory setae is 2–2.5 times the length of 5 terminal segments combined, reaching to or slightly beyond tips of claws. The palp of T1 of *H. helenae* has a long and plumose seta h2.

The characterization of the genus *Herpetocypris* has recently undergone repeated morphological alterations (González Mozo et al. 1996) with respect to both the valves and the soft parts. Müller's (1908) original description is quite incomplete, with a good drawing of the valves, but with only a few appendages of *H. helenae*. Then, González Mozo et al. (1996) made good and complete drawings and photographs of the valves and the soft parts, but did not include a description of both: for that reason, the species has been redescribed. Certain differences are also evident in the anatomy of the soft parts of the Argentine *H. helenae* compared to the descriptions provided by Müller (1908) and González Mozo et al. (1996). Nevertheless, we consider the following differences within the range of variability of *H. helenae* as a species.

Table 1. Seasonal measurements of five environmental variables at four localities in the Llancanelo basin.

Site sampled	Season sampled	Conductivity (mS·cm ⁻¹)	pH	Temperature (°C)	Dissolved oxygen (%)	Salinity (g·L ⁻¹)
El Mocho Creek (1)	Summer	2.37	9.2	26.1	108	1.1
Malargüe Delta River (2)	Summer	0.83	9.0	26.8	100	0.2
Los Menucos (3)	Spring	0.98	8.5	29.5	39	0.3
	Summer	1.10	8.4	22.0	84	0.3
Carapacho (4)	Spring	1.29	8.8	19.8	43	0.5
	Autumn	2.90	8.6	19.5	19	1.4

Müller's specimens exhibit differences in the length of the natatory setae on A2, which exceed the tips of the terminal claw (extending just to the tips of the claws in the Argentinian specimens). The specimens of González Mozo et al. (1996) principally present the following differences: The number of setae present in A1 differs, with the second segment having two apical ventral setae (one short ventral-apical seta in the Argentinian specimens). In A2 the sixth natatory setae is plumose and minute (smooth and minute in the Argentinian specimens). The apical segment of the mandibular palp has three claw-like setae and two smooth apical setae (in the Argentinian specimens there are 4 claw-like setae and two smooth apical setae). In the maxilla, the first segment of the palp has two plumose and three smooth apical setae and the second segment four claw-like and two smooth setae (the first segment of the palp of the maxilla has one plumose subapical and five plumose apical setae, the second segment has six smooth setae in the Argentinian specimens). The first endite of the maxilla has two subapical plumose setae (the same endite has two smooth subapical setae in the Argentinian specimens).

The specimens of *H. helenae* in this study were found in two of the four freshwater lentic environments sampled (the Los Menucos and Carapacho springs); the absence of these specimens in the La Porteña spring can be tentatively related to a different ion content, and to the relatively higher conductivity values (3.6 to $5.1\text{ mS}\cdot\text{cm}^{-1}$) record in this spring. In addition, the species was found in only two of the four lotic environments surveyed, the El Mocho stream and Malargüe River delta, both of them freshwater bodies with slow flows. Finally, the absence of *H. helenae* in the El Chacay stream as well as the main course of the Malargüe River could be related to the rheonomic conditions of those bodies, as such characterized by turbulence and a high flow rate.

We therefore consider *H. helenae* having an affinity to freshwater conditions, higher water temperatures and higher pH values. The morphologic variations between the specimens from other biogeographical regions and those here described indicate that this species has not developed a conservative phenotype. In the sampling sites we found two morphologic variants in differing habitats. We here postulate the differing morphologies representing an ecophenotypic response to different rheological conditions in the water bodies. The morphotype found in streams is possibly formed more streamlined as an adaptation to life in flowing water, while the higher and shorter carapaces of specimens collected in standing waters may represent a response to lentic environments.

Referring to experiments with *Mytilocypris henricae* (Chapman, 1966) in Martens et al. (1985), Rossetti & Martens (1996) attribute carapace size differences in different populations of *Darwinula stevensoni* (Brady & Robertson, 1870) to epigenetically active environmental effects during larval development, such as water temperature, food and salinity. Given that in both variants of carapace morphology in Argentinian specimens of *H. helenae* the soft part morphology does not differ, we here postulate that flow rate may also influence carapace shape and size.

To conclude, the here presented complete description of *H. helenae*, in conjunction with comparisons between congeners, confirms that the relative length of the natatory setae in A1 is a distinguishing character of this species. It supports the decision by González Mozo et al. (1996) to retain *H. helenae* and *H. chevreuxi* as valid species.

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