



First description of the soft part anatomy of *Ilyocypris ramirezi* Cusminsky & Whatley (Crustacea, Ostracoda) from Argentina, South America

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Abstract

The anatomy of the soft parts of *Ilyocypris ramirezi* Cusminsky & Whatley, 1996 is described and illustrated for the first time, based on findings of this species from water bodies in the shallow areas of the Llanquanelo basin, south-west of Mendoza Province, Argentina. This species is common in Quaternary and extant environments of the Pampa and Patagonian regions. Its distribution is now extending in Argentina to the Central-West area, locally named “Cuyo region”. *Ilyocypris ramirezi* is a good environmental indicator and constitutes a useful tool in paleolimnological studies.

Key words: soft parts anatomy, ecology, Llanquanelo basin, *Ilyocypris* key

Introduction

The family Ilyocypridae Kaufmann, 1900 belongs to the superfamily Cypridoidea Baird, 1845. According to the most recent view, the subfamily Ilyocyprinae Kaufmann, 1900 is the only one in this family. The subfamily Pelocyprinae has been transferred to the family Cyprididae (Karanovic 2012; Martens & Savatnalinton 2011). The subfamily Ilyocyprinae has only two extant genera: *Ilyocypris* Brady & Norman, 1889 and *Indiacypris* Hartmann, 1964.

Ilyocypris is a widespread freshwater genus including between 25 (Karanovic 2012) and 37 (Martens & Savatnalinton 2011) living species; however, no author has yet included *Ilyocypris ramirezi* in the species list.

The specific identification of the living *Ilyocypris* relies mainly on the appendages (length of the swimming setae on the antenna, A2; division of the penultimate segment of the second thoracic limb, T2; and the number of setae on the penultimate segment of the third thoracic limb, T3). According to Mazzini *et al.* (2014) the morphology of soft parts is considered to be more conservative than that of valves and can be used to derive phylogenetic and taxonomic relationships. For several non-marine species, intraspecific variability in valve size, shape, ornamentation (e.g. reticulation and nodding) depends on genetic factors, reproductive modes (Rossi *et al.* 2007), environmental conditions or a combination of these and several other factors. Such morphological plasticity may hamper taxonomic identification and lead to the questionable erection of new species and genera. In addition, the “classical” morphological approach to species identification is further complicated because of the presence of cryptic diversity, where species cannot be differentiated by morphological but only by genetic characters (Mazzini *et al.* 2014).

Brady & Norman (1889) designated *Ilyocypris gibba* (Ramdohr, 1808) as the type species. It was collected from a water-body near the entrance to the botanical garden at Neumarkt, Halle (Germany). Many species of this genus can be found in small and shallow permanent water bodies as well as in larger ones such as lakes. Some species are also recorded from springs and brooks and can tolerate a slightly higher salinity. Most of the species have a wide distribution; according to Karanovic (2012), the genus has been recorded from all continents, except Antarctica and South America. However, there are several records of the genus since 1893 in Argentina. Wierzejski

(1893) recorded *Ilyocypris gibba* Ramdohr, var. *repens* in Mendoza Province. *Ilyocypris gibba* was also recorded by Ramírez (1967) in extant environments from Buenos Aires Province, in Quaternary sediments from Chaco (Zabert 1981), Entre Ríos (Zabert & Herbst 1986), Buenos Aires and San Luis Provinces (García 1996). In 1996, Cusminsky & Whatley described *Ilyocypris ramirezi* obtained from Northern Patagonian Quaternary sediments. However, it was also recorded in recent environments in Patagonia, Argentina (Cusminsky *et al.* 2005; Cusminsky *et al.* 2011; Ramón Mercau *et al.* 2012; Schwalb *et al.* 2002) and in Buenos Aires province (Laprida 2006).

Material and methods

Samples were taken seasonally during the year 2010 from different water bodies in the shallow areas of the Llacanelo basin, south-west of Mendoza Province, Argentina (35° 00'– 36° 30' S, 68° 30'– 70° 00' W; 1,330 m asl, 70 km east from Cordillera de los Andes). From water bodies related to Laguna Llacanelo eighteen localities were selected for sampling, but since two sites were consistently dry (Arroyo El Alamo and El Malo), sixteen sites were sampled for ostracods, including lotic and lentic, fresh to mesosaline ecosystems (Fig. 1). The basin is endorheic, and developed within the Huarpes depression in the northern section of the Payenia Volcanic Province (Ramos & Folguera 2011).

The material was collected from surface sediments (upper 3 cm), which were rinsed with a sieve of 100 µm mesh size. The ostracods were then transferred to 70% ethanol for permanent storage. The limb morphology was studied under the light microscope and line drawings were made with the help of a Zeiss Standard 25 stereomicroscope with camera lucida.

Valves were photographed in a JEOL JSM 6360 LV (Museo de La Plata) and Philips XL30 (Royal Belgian Institute of Natural Sciences) scanning electron microscope. The material was deposited in the Invertebrate Collection at Museo de La Plata, Argentina. The nomenclature of the limb chaetotaxy follows Broodbakker & Danielopol (1982) and Boxshall *et al.* (2010) for the first antenna, the revised model proposed by Martens (1987) for the second antenna, and Meisch's nomenclature (2000) for the second and third thoracopods. Higher taxonomy of the Ostracoda follows the synopsis by Horne *et al.* (2002).

Abbreviations used in text and figures: Cp = carapace; Valves: H = height, L = length; W = width; LV = left valve; RV = right valve; CIL = calcified inner lamella. Limbs: A1 = antennula; A2 = antenna; Md = mandible; Mx = maxillula; T1 = first thoracic limb; T2 = second thoracic limb; T3 = third thoracic limb; CR = caudal ramus; CRa = attachment of caudal ramus. Values are expressed as the arithmetic mean ± the standard deviation (with the minimum and maximum values shown between brackets); n = number of individuals; MLP = Museo de La Plata.

Results

Class Ostracoda Latreille, 1802

Subclass Podocopa G. O. Sars, 1866

Order Podocopida G. O. Sars, 1866

Family Ilyocyprididae Kaufmann, 1900

Subfamily Ilyocypridinae Kaufmann, 1900

Genus *Ilyocypris* Brady & Norman, 1889

[Syn.: *Ilyocyprilla* Daday, 1900; *Ilyocyprois* Masi, 1906]

Type species: *Ilyocypris gibba* (Ramdohr, 1808)

Diagnosis (after Karanovic 2012). Medium sized species. Valves subquadrate in lateral view, not highly asymmetrical. LV overlapping RV in all sides, except in postero dorsal margin. CIL narrow. Swimming setae on

both A1 and A2 usually long, sometimes reduced on A2. Mxl palp stout, with first segment distally not distinctly dilated, and second segment short and broad. Endopodite of T1 small with only one segment with a small constriction on one side.

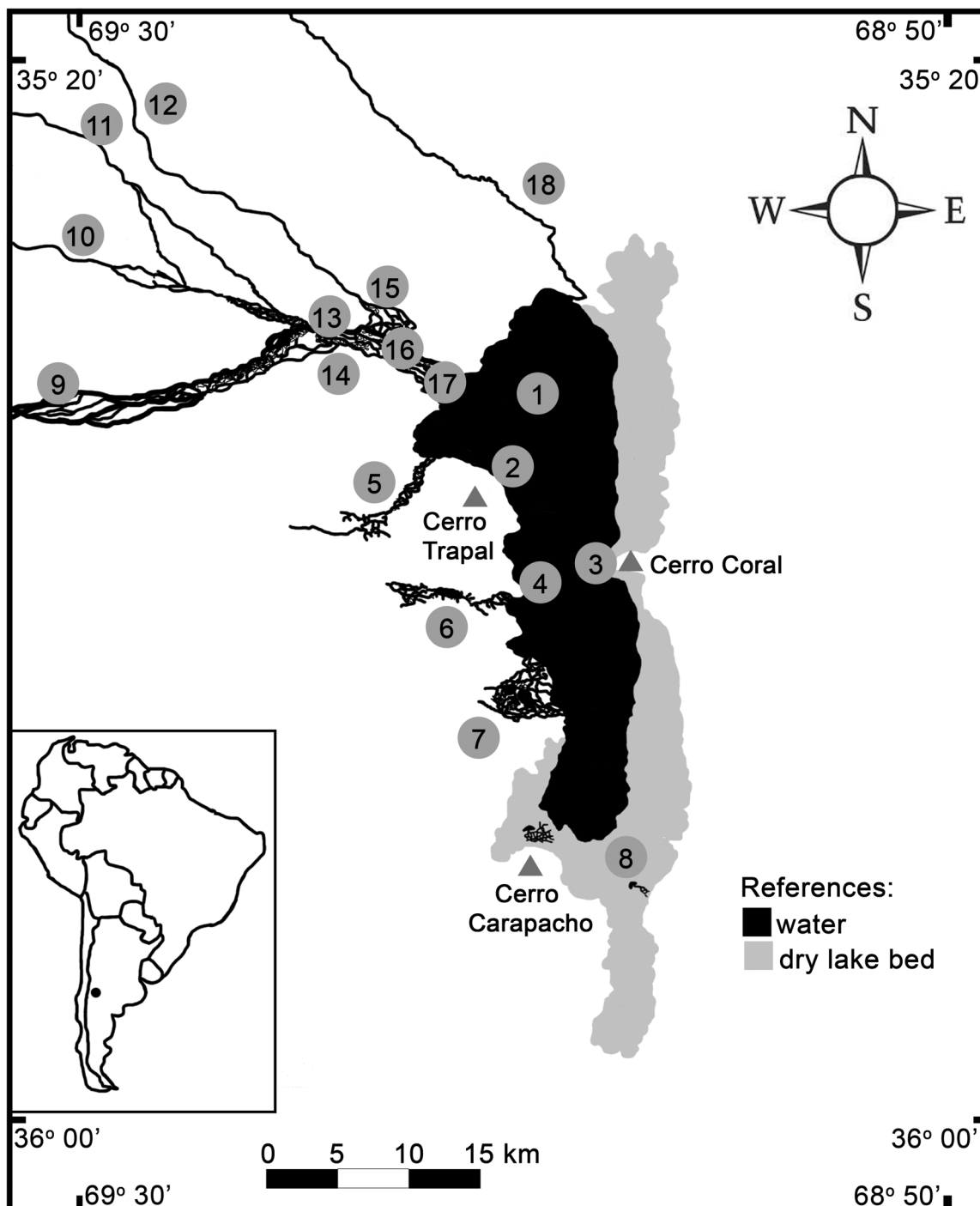


FIGURE 1. Location of sampling sites. 1–4 Llanqueto Lake, 5 Los Menucos spring, 6 Carilauquen spring, 7 Carapacho spring, 8 La Porteña spring, 9 Malargüe River, 10 El Chacay stream, 11 El Alamo stream, 12 El Mocho stream, 13–17 Malargüe Delta River, 18 El Malo stream.

Ilyocypris ramirezi Cusminsky & Whatley, 1996

Material examined. 17 of a total of 818 ♀♀ adults and juveniles; 3 ♀♀ adults deposited in the Invertebrate collection of the Museo de La Plata, Argentina (MLP26900).

Diagnosis. *Ilyocypris* species with dorsal margin inclined and rather sinuous. Ornamented regularly with small pits, mainly concentrically arranged as a sieve. Marginal spines present in posterior margin, arranged in a conspicuous row of six to eight spines situated at little distance from it. Natatory setae of A2 short. Endopodite of T1 small and with only one segment with a small constriction on one side. Protopodite of T2 with only one seta d_1 . Terminal segment cylindrical with three terminal setae. Males unknown.

Measurements of adults.

RV (n=11), L = 728–901 μm ($816 \pm 46 \mu\text{m}$); H = 358–506 μm ($426 \pm 38 \mu\text{m}$);

LV (n=11), L = 765–876 μm ($845 \pm 34 \mu\text{m}$); H = 407–494 μm ($444 \pm 25 \mu\text{m}$);

Cp (n= 6), W = 259–370 μm ($321 \pm 44 \mu\text{m}$); LV, L = 728–900 μm ($820 \pm 75 \mu\text{m}$), H = 370–494 μm ($422 \pm 49 \mu\text{m}$); RV, L = 728–901 μm ($832 \pm 73 \mu\text{m}$) 898 μm , H = 395–506 μm ($434 \pm 44 \mu\text{m}$)

Anatomy of valves (after Cusminsky & Whatley 1996) (Fig. 2A–K):

Medium sized. Valves subrectangular in lateral view (Fig. 2A–C). Anterior margin rounded. Posterior margin rounded in ventral part and straighter dorsally. Dorsal margin inclined and rather sinuous. Ventral margin concave. Maximum height situated approximately at anterior third of maximum length. Carapace elongated in dorsal view (Fig. 2D). Regularly ornamented with small pits, mainly concentrically arranged as a sieve. Three lateral tubercles present, low, rounded and ornamented, the posterior one well developed. Two medio-dorsal transverse sulci. Marginal spines present and pointed, numerous in anterior margin and in posterior margin bigger and arranged in a conspicuous row of six to eight spines situated at a little distance from it (Fig. 2G–I). Normal pore canal with few, perforated small conuli. Internal features as for genus. Inner lamella relatively narrow (Fig. 2E–F, J–K) but well developed. Adductor scars a tightly compact group of seven scars in two rows. LV overlapping RV on all sides, except in postero-dorsal margin.

In juveniles, the dorsal margin of the carapace is straight, and the ornamentation, tubercles and sulci are more prominent.

Anatomy of soft parts (Fig. 3A–I). A1 (Fig. 3A) with seven segments. First segment with one short ventral and two long dorsal setae, all of them plumose. Second segment with one ventral seta. Third segment with one short aesthetasc and one short seta ventrally, same segment with one short dorsal seta. Fourth segment with two long subequal setae ventrally, and with one aesthetasc and one long seta dorsally inserted. Fifth segment with two long sub-equal ventral setae and two unequal dorsal setae. Sixth segment with four long apical natatory setae. Terminal segment with one long aesthetasc y_a , one long seta and two claws.

A2 (Fig. 3B) second segment of protopodite with one ventral seta. Exopodite with one long and two short setae. First segment of endopodite with a ventral aesthetasc Y, five sub-equally short natatory setae and one long seta inserted on the anteroventral corner of the segment. Second segment of endopodite with one dorsal and one ventral setae, one mid-ventral aesthetasc y_1 and one apical aesthetasc y_2 , same segment with three medium-sized apical claws G_1 , G_2 , G_3 . Terminal segment with two strong claws GM and Gm and aesthetasc y_3 fused with a short seta.

Md (Fig. 3C) Md-coxa slender with 6–7 cusped teeth. Mandibular palp with four segments. First segment with two plumose and one serrate seta S1. Second segment with four long setae, one short ventral β -setae, and one long dorsal seta. Third segment with two sub-equal ventral setae and three dorsal setae. Terminal segment with three apical claw-like setae. Branchial plate (exopodite) with three setae that arise dorsally from the palp.

Mx (Fig. 3D) with terminal segment of palp sub-quadrate. Second segment of palp cylindrical, c , twice as long as basal width, with two dorso-apical setae. Terminal segment with three apical claw-like setae and two setae. Zahnborsten on third endite smooth. Branchial plate with c . 20 rays.

T1 (Fig. 4E) with masticatory process bearing 12 mostly smooth setae of unequal length, d and b setae plumose. Branchial plate (exopodite) with 5 plumose setae. Endopodite small with only one segment with a small constriction on one side. Three smooth setae present.

T2 (Fig. 3F) a walking leg, with one seta d_1 . First segment of endopodite with one short apical e seta, second segment with one apical d seta, third segment with one apical f seta. Terminal segment with one long apical claw and two smooth short setae.

T3 (Fig. 3G) a cleaning limb; first segment of endopodite with one apical seta. Second segment with one ventral and one dorsal setae. Terminal segment cylindrical with three terminal setae.

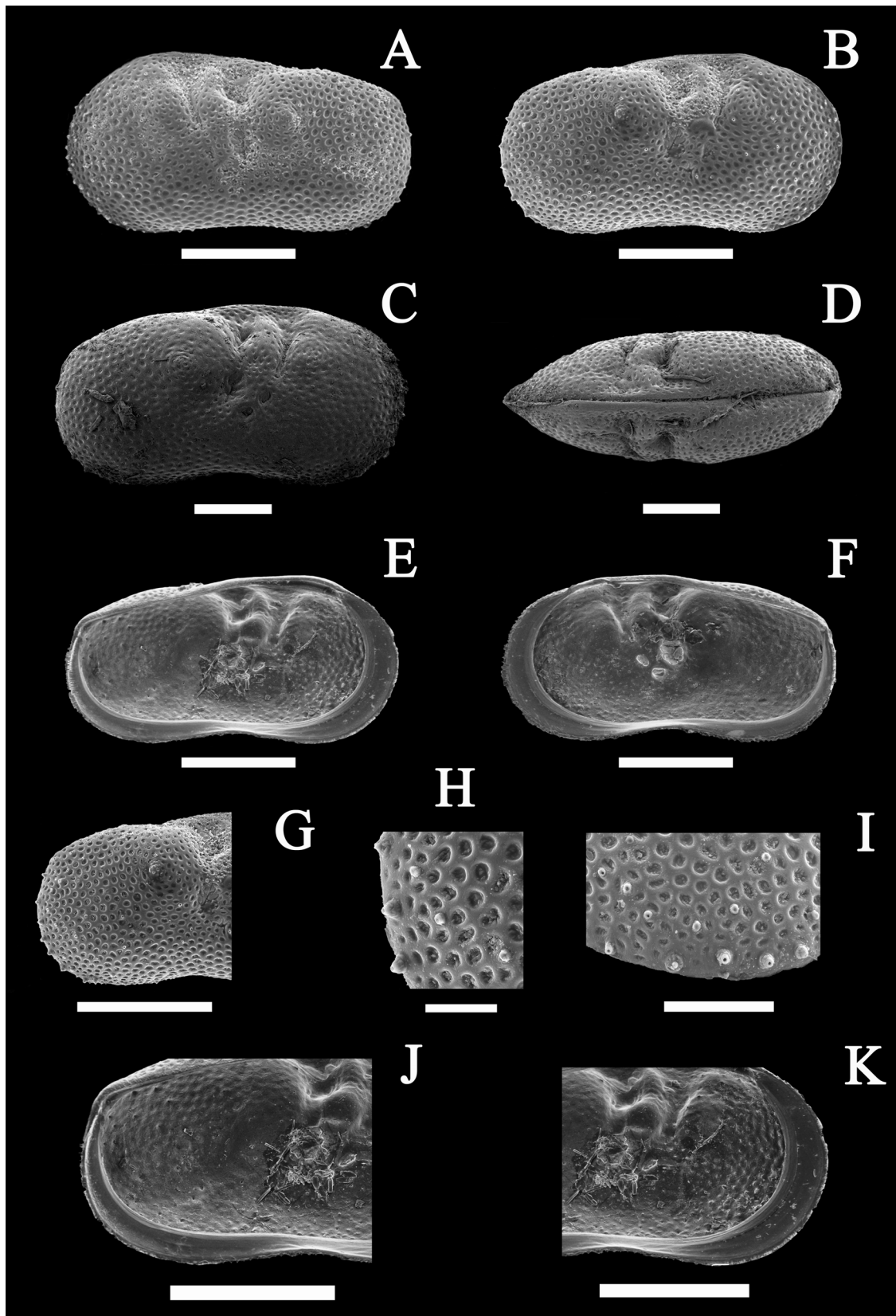


FIGURE 2. *Ilyocypris ramirezi* ♀: A. LV, external view (MLP26900) B. RV, external view (MLP26900); C. RV, external view (MLP26900); D. Cp. dorsal view (MLP26900); E. LV, internal view (MLP26900); F. RV, internal view (MLP26900); G. Detail of pores of posterior part of RV; H. Detail of pores of posterior part of RV; I. Detail of pores of ventral part of RV; J. Detail of postero ventral margin of LV (postero-ventral marginal ripples position); K. Detail of antero ventral margin of LV (antero-ventral marginal ripples positions). Scale bars in μm : 250 for A, B, E, F, J and K, 200 for C and D, 300 for G, 50 for H and 100 for I.

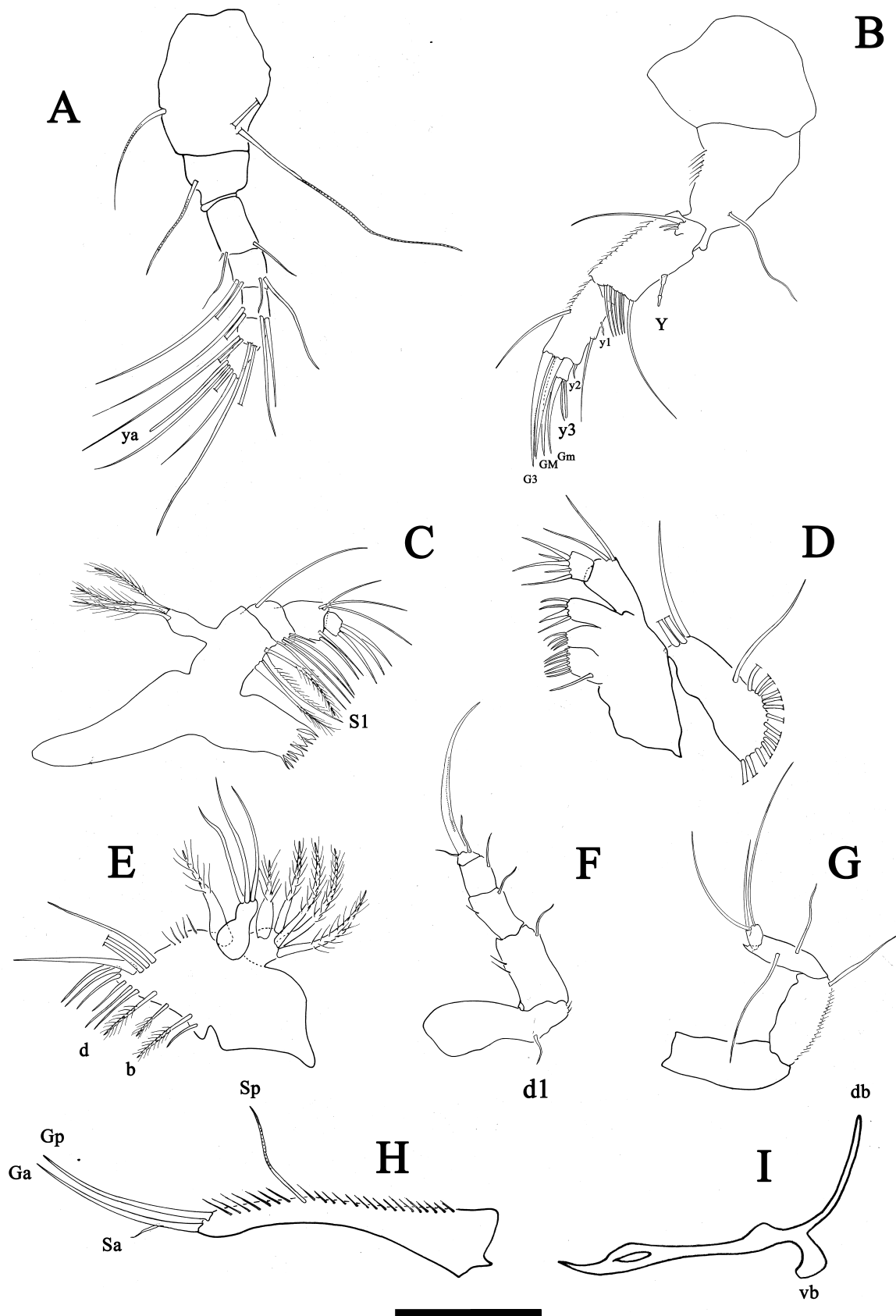


FIGURE 3. *Ilyocypris ramirezi* ♀: A. A1 (MLP26900); B. A2 (MLP26900); C. Md (MLP26900); D. Mx (MLP26900); E. T1 (MLP26900); F. T2 (MLP26900); G. T3 (MLP26900); H. CR (MLP26900); I. CRa (MLP26900). y_a , Y, y_1 , y_2 , y_3 : aesthetascs, G_1 , G_2 , G_3 , GM, Gm, Gp, Ga: claws, S_1 , d, b, d_1 , d_2 , Sp, Sa: setae, db: dorsal branch, vb: ventral branch. Scale bar: 100 μ m.

CR (Fig. 3H) of normal structure, posterior seta long and plumose. Anterior and posterior claws smooth. Posterior seta (Sp) longer than anterior seta (Sa).

CRA (Fig. 3I) robust but short, with simple triangular structure at basis. Dorsal branch (db) long and ventral branch (vb) short.

Ecology. *Ilyocypris ramirezi* has been found in freshwater to sub-saline (salinity range: 0.2–2.7 g/L) environments. The species was most abundant in Malargüe Delta River in summer and in Los Menucos rheocene spring in autumn and spring, but the specimens were found in all seasons. In creeks and La Porteña limnocene spring specimens of *I. ramirezi* were less abundant, but present in all seasons. The environments were characterized by alkaline pH values, temperature from 0.1 to 29.5 °C, conductivity from 0.8 to 5.1 mS/cm. Dissolved oxygen saturation varied between 20 and 170 % (Table 1).

Discussion

As stated above, the morphology of hard parts of non-marine ostracod species depends on a combination of factors. In *Ilyocypris* species the environmental conditions induce different degrees of ornamentations and tubercles development. According to Mazzini *et al.* (2014) the marginal ripples are not considered as a diagnostic character, thus, and according to Meisch (2000) and Mazzini *et al.* 2014 the identification of *Ilyocypris* species based on valves only is very difficult. The genus *Ilyocypris* (Ilyocypridae) was erected by Brady & Norman 1889, with *I. gibba* (Ramdohr, 1808) as the type species. Of all the species in this genus, *I. ramirezi* is closest to *Ilyocypris bradyi* Sars 1890 and *I. gibba*.

Ilyocypris ramirezi is more similar to *I. bradyi* but differs in the morphology of T1 (4-segmented in *I. ramirezi*, while 5-segmented in *I. bradyi*), as well as in the valve morphology while the dorsal margin is sinuously curved in *I. ramirezi*, is rectilinear in *I. bradyi*.

Ilyocypris ramirezi differs from *I. gibba* mainly in the morphology of the natatory setae on the A2, which are short, reaching the middle of the penultimate segment in *I. ramirezi*, but very long, conspicuously extending beyond tips of terminal claws in *I. gibba*. As in *I. bradyi*, the dorsal margin is rectilinear in *I. gibba*, in contrast to the sinuous dorsal margin in *I. ramirezi*.

Updated key to the species of the genus *Ilyocypris* (modified after Karanovic 2012)

1	T3 with no (?) setae on penultimate segment	<i>I. nagamalaiensis</i> Victor & Michael, 1975
-	T3 with one or more setae on the penultimate segment	2
2	T3 with one seta on the penultimate segment	3
-	T3 with two or three setae on the penultimate segment	4
3	Second segment of male prehensile palp without any seta	<i>I. fallax</i> Brehm, 1929
-	Second segment of male prehensile palp with one dorsal seta	<i>I. haterumensis</i> Okubo, 1992 (in Okubo & Terauchi 1992)
4	T3 with two (third sometimes present, but very small) setae on the penultimate segment	5
-	T3 with three (all long) setae on the penultimate segment	18
5	Swimming setae on An2 reaching the basis of the terminal claws	6
-	Swimming setae on An2 exceeding basis of the terminal claws	8
6	Swimming setae on An2 very short	7
-	Swimming setae on An2 reaching middle of the penultimate segment	<i>I. bradyi</i> Sars, 1890
7.	Swimming setae on An2 barely visible.	<i>I. inermis</i> Kaufmann, 1900 and <i>I. nitida</i> Lerner-Seggev, 1968
-	Swimming setae on An2 short but reaching at least the middle of the segment.	<i>I. ramirezi</i> Cusminsky & Whatley, 1996
8	T1 palp in females non-segmented	<i>I. perigundi</i> De Deckker, 1981
-	T1 palp in females 2- or 3-segmented	9
9	Swimming setae on An2 reaching the tip of terminal claws	<i>I. australiensis</i> Sars, 1889
-	Swimming setae on An2 extending beyond the tip of terminal claws	10
10	Penultimate segment of T1 palp in female with one seta	11
-	Penultimate segment of T1 in female without any seta	12
11	In dorsal view posterior end of female with triangular alae	<i>I. dentifera</i> Sars, 1903
-	In dorsal view posterior end of female with rounded extensions of carapace	<i>I. carinata</i> Kovalenko, 1970
12	T1 palp in female 3-segmented	<i>I. brehmi</i> Schäfer, 1952
-	T1 palp in female 2-segmented	13
13	Swimming setae on An2 reaching and slightly exceeding the tip of terminal claws	14

-	Swimming setae on An2 by far exceeding the tip of terminal claws	15
14	Valve margins strongly denticulated	<i>I. hartmanni</i> Lerner-Seggev, 1968
-	No denticulation along margins	<i>I. decipiens</i> Masi, 1905
15	Dorsal margin sloping down gradually toward posterior end	16
-	Dorsal margin straight	17
16	T2 5-segmented	<i>I. monstifica</i> (Norman, 1862)
-	T2 4-segmented	<i>I. gibba</i> (Ramdohr, 1808)
17	Posterior seta on the CR very long, by far exceeding distal end of posterior margin	<i>I. mongolica</i> Martens, 1991
-	Posterior seta on the CR short, reaching half way to the end of posterior margin	<i>I. divisa</i> Klie, 1926 and <i>I. botniensis</i> Kovalenko, 1972
18	T2 4-segmented	<i>I. lacustris</i> Kaufmann, 1900
-	T2 5-segmented	<i>I. getica</i> Masi, 1906

Ecology and distribution. The genus *Ilyocypris* has a ubiquitous distribution, and many species have been reported from a worldwide area (Karanovic & Lee 2013).

So far, *Ilyocypris ramirezi* has been recorded only from sites in Argentina. Here, it was sampled from running waters and is also considered the most conspicuous species in springs in Argentinean Patagonia (Cusminsky *et al.* 2005, Schwalb *et al.* 2002). While Schwalb *et al.* (2002) report *I. ramirezi* being the only ostracod species collected in high energy environments of running water in the investigated area, it has also been found in ephemeral lakes such as Cari-Laufquen and permanent lakes such as Cardiel and Ñeluan (Cusminsky *et al.* 2005). Other sites of occurrence have been reported from Los Juncos Lake (Cusminsky & Whatley 1996), La Salina Lake, and in the area of Cari-Laufquen Lake in the Río Negro province (Cusminsky & Whatley 1996; Whatley & Cusminsky 1999).

Previous studies showed that *Ilyocypris ramirezi* prefers fresh to low salinity environments (Laprida 2006, Ramón Mercau *et al.* 2012, Markgraf *et al.* 2003, Schwalb *et al.* 2002), but is capable of inhabiting water with a wide range of total dissolved solids (TDS) concentration, from fresh water TDS = 0.035 g L⁻¹ to polyhaline TDS = 23.760 g L⁻¹ (Ramón Mercau *et al.* 2012).

In the present study, *Ilyocypris ramirezi* was found with greater frequency in the creeks and the delta of the Malargüe River, showing an affinity for freshwater conditions and lotic environments. The fact that it is found in these springs supports the findings of Cusminsky *et al.* (2005) with records predominantly in streams and springs.

The most relevant information was the absence of the specimens in the hot springs in Carapacho and in the shallow lake Llananelo. In the Carapacho hot springs the temperature fluctuated slightly between 19.5 °C–21.6 °C in the sampling period, with a mean temperature of 20 °C; these constantly high temperatures, which are considerably higher than mean air temperature, do not favour the development of this species. In the shallow Llananelo lake the salinity fluctuated greatly between 9.3 g/L–44.3 g/L in the sampling period, with salinity mean 25.8 g/L. These results allow us to suggest that the species cannot live in high constant temperatures and in salinity levels higher than 9 g/L, but we have not information about the range between 2.7 to 9 g/L. Although Cusminsky & Whatley (1996) reported the presence of males in quaternary sediments from Patagonia, it was not possible to find them in the sampled site. However, Cusminsky & Whatley (1996) based the determination on hard parts, which could be considered a mistake with regard to the morphological plasticity present in the *Ilyocypris* species.

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