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Two new species of *Chilina* Gray from Cuyo Malacological Province, Argentina (Gastropoda: Hygrophila: Chilinidae)

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The family Chilinidae in Argentina has been thought to include 19 species. We provide new information on the shells, radulae, reproductive and nervous systems, and the molecular genetics of the chilinid species from the Cuyo Malacological Province (as defined by Núñez, V., Gutiérrez Gregoric, D.E. & Rumi, A. (2010) Freshwater gastropod provinces from Argentina. *Malacologia* 53, 47–60), along with the description of two new species. *Chilina sanjuanina* n. sp. is characterized by a small shell with a conical and low spire and with one columellar tooth, a radula with an asymmetric central bicuspid tooth, and a penis sheath with an inner sculpture with regular conical pustules over the entire surface. *Chilina cuyana* n. sp. has a small shell with a low spire and two columellar teeth and a radula with an asymmetric tricuspid central tooth with serrated edges. The other two species in this province, *Chilina mendozana* and *Chilina parchappii*, are redescribed. Mitochondrial gene cytochrome c oxidase subunit I sequences between *C. sanjuanina* and *C. mendozana* differ by 4%.

Keywords: taxonomy; *Chilina sanjuanina* n. sp.; *Chilina cuyana* n. sp.; molecular genetics; anatomy

Introduction

The freshwater gastropod fauna of Australasia and South America uniquely share the Glacidorbidae and Chilinoidea. Glacidorbidae is represented by 19 species in Australia and one species in South America (Ponder and Avern 2000). Chilinoidea is represented by the genera *Chilina* Gray, 1828 (Chilinidae) only to South America and *Latia* Gray, 1828 (Latiidae) of New Zealand (Marshall 2011).

Chilinidae (Gastropoda, Hygrophila) is one of the oldest freshwater families currently known (Duncan 1960). This extensive history is reflected in primitive characteristics for 'pulmonates', such as the streptoneurous nervous system, horizontal lamellar tentacles, a noncontractile pneumostome, and an incomplete division of male and female ducts (Haeckel 1911; Hubendick 1945, 1978; Harry 1964). Dayrat *et al.* (2001) published a molecular phylogeny of Euthyneura that argued for the monophyly of Hygrophila and proposed the Chilinidae as a basal group. Klussmann-Kolb *et al.* (2008) and Jörger *et al.* (2010) distinguished two clades within the Hygrophila, the first including *Chilina* and *Latia* and the second comprising higher limnic Basommatophora. More recently, on the basis of molecular-genetics analyses Dayrat *et al.* (2011) concluded that the Hygrophila was, in fact, not a monophyletic group and proposed a relationship between the Chilinoidea and the Amphiboiloidea, although this contention was not stated with certainty.

Of the 19 species of Chilina found in Argentina, 13 are endemic and seven vulnerable (Rumi *et al.* 2006; Núñez *et al.* 2010). In addition, 10 of these species are only present in a malacological province (Núñez *et al.* 2010). Most of the Argentine species of *Chilina* have been originally described on the basis of shell characters alone. Recently two new species, *Chilina iguazuensis* Gutiérrez Gregoric & Rumi, 2008 and *Chilina lilloi* Ovando & Gutiérrez Gregoric, 2012, were reported from Misiones and Tucumán Provinces in Argentina along with descriptions of the shell, radula, and reproductive and nervous systems (Gutiérrez Gregoric and Rumi 2008; Ovando and Gutiérrez Gregoric 2012).

The central western portion of Argentina—extending between latitudes 28° and 37°S and longitudes 65° and 71°W—is an ecological region comprised of six of the 18 eco-regions of Argentina described by Burkart *et al.* (1999): Puna, Altos Andes, Monte de Sierras y Bolsones, Chaco seco, Monte de Llanuras y Mesetas, and Estepa Patagónica. Most of this region lies within the dominion of the South American Arid Diagonal, a region considered to have been climatically sensitive to the latitudinal shift

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of the Pacific and Atlantic anticyclonic centres during the late Pleistocene and the Holocene (Abraham de Vazquez *et al.* 2000). The dominant climate is semiarid with a mean annual rainfall of 250 mm in the eastern piedmont (Capitanelli 2005). With respect to freshwater gastropods, this region constitutes the 'Cuyo Malacological Province' (Núñez *et al.* 2010) and covers an area of approximately 280,000 km^2. The gastropod diversity of this province is one of the lowest in the country and comprises only 16 known species within the families Ampullariidae (one species), Cochliopidae (5), Physidae (4), Planorbidae (2), Lymnaeidae (2), and Chilinidae (2) (Núñez *et al.* 2010). The two species of *Chilina* reported are: *Chilina parchappii* (d'Orbigny, 1835) and *Chilina mendozana* Strobel,1874. The former was originally described from Buenos Aires Province and associated with lotic, mesohaline environments of the southern Pampa (Tietze and De Francesco 2010). *C. mendozana* is endemic to Cuyo Malacological Province and was originally described as *Chilina tehuelcha var. mendozana*. Castellanos and Gaillard (1981) elevated this variety to the rank of species on the basis of the characteristics of the shell. The species in this malacological province were traditionally identified as *C. mendozana* (De Francesco and Dieguez 2006; Rumi *et al.* 2006, 2008; Ciocco and Scheibler 2008; De Francesco and Hassan 2009), however, no anatomical studies had been carried out to corroborate these determinations.

The aim of the present study was to provide information on the anatomy and genetics of the species of *Chilina* present in the Cuyo Malacological Province of Argentina and to review their taxonomic status.

Materials and methods

The specimens studied come from malacological collections at the Museo de La Plata (MLP), Buenos Aires Province and Museo Argentino de Ciencias Naturales (MACN) of Buenos Aires city. Additional material was collected in western Argentina. The adult specimens obtained were first relaxed in menthol for 12 hours, then immersed in hot water (70°C), and finally stored in 96% (v/v) aqueous ethanol. Ten shell measurements were taken (Fig. 1): total length (TL), penultimate whorl length (PWL), length of the last whorl (LWL), aperture length (AL), total width (TW), initial penultimate whorl width (IPW), final penultimate whorl width (FPW), aperture width (AW), aperture projection (AP), and spire length (SL). For anatomical studies of the reproductive and pallial systems, the methodology proposed by Cuezzo (1997) was followed. Dissections were made under a Leica MZ6 stereoscopic microscope and anatomical systems drawn with the help of a *camera lucida*. Figures were made only for characters that showed specific differences. The terminology used for the anatomical descriptions follows that of

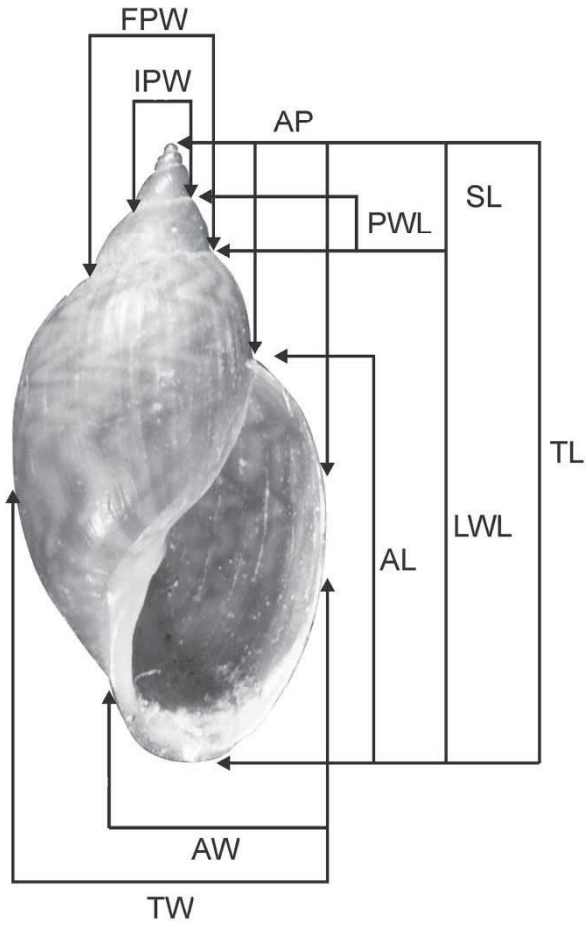

Figure 1. Shell measurements used for Chilinidae. Abbreviations: AL — aperture length; AP — aperture projection; AW — aperture width; FPW — final penultimate whorl width; IPW — initial penultimate whorl width; LWL — last whorl length; PWL — penultimate whorl length; SL — spire length; TL — total length; TW — total width.

Harry (1964) and for the details of the nervous system that of Ituarte (1997). In order to obtain size-free variables to facilitate comparisons between different individuals and species, anatomical measurements were expressed as a proportion of the length of the last whorl.

The radulae were separated from the buccal mass, cleaned by immersion in a sodium-hypochlorite solution (Clorox™) and mounted for scanning electron microscopy. The radular-dentition formula gives the number of teeth per row: [(number of left and right teeth)/(number of cusps) + (number of central teeth)/(number of cusps)] plus the number of transverse rows or their lower and maximum number.

Total DNA was extracted from 2 mm^3 samples from the foot of recently collected specimens (2011). The tissue was rinsed in distilled water; ground in 100 mM EDTA and 20 mM Tris, and digested overnight in CTAB (cetyl trimethyl ammonium bromide) buffer containing proteinase K. DNA was purified by a threefold extraction with

chloroform-isoamyl alcohol (24:1) followed by precipitation with isopropanol. The DNA was then resuspended in Tris-EDTA (TE) buffer. A 655-bp fragment of the gene encoding the mitochondrial cytochrome *c* oxidase subunit I (COI) was amplified by means of the primers of Folmer *et al.* (1994). Amplification by the polymerase chain reaction (PCR) was performed in a final volume of 50µl, following the protocol utilized by Gutiérrez Gregoric *et al.* (2013), containing: 50–100 ng of template DNA, 0.1µM of each primer, 1X PCR buffer, 50µM dNTPs, 2.5 mM $MgCl_2$, and 1.2 U Fermentas Taq polymerase. The thermocycling profile consisted of 3 min at 94°C, 5 cycles of 30 s at 94°C; 40 s at 45°C; 1 min at 72°C; followed by 35 cycles of 30 s at 94°C; 40 s at 51°C; 1 min at 72°C; with a final extension for 10 min at 72°C. The PCR products were purified using AxyPrep PCR Clean-up Kit (Axygen Biosciences, Union City, California) and both DNA strands were then directly sequenced (Macrogen Inc., Seoul, Korea). The resulting sequences were trimmed to remove the primers, and the consensus sequences of the individuals were compared to reference sequences deposited in GenBank. Only four sequences for *Chilina* sp. from Chile were available on this database. From these, one sequence was not employed in further analysis because it was too short (i.e., EF489383, 448 bp). The sequence alignment was performed with the Clustal X 2.0.12 software (Larkin *et al.* 2007), optimized by visual inspection, and edited with a word processor. The total length of the matrix analysed was 569 bp and the genetic distances among individuals were estimated in MEGA 5.05 software by using the Kimura 2 parameter model as evolutionary paradigm.

Systematics

Family Chilinidae Dall, 1870

Genus *Chilina* Gray, 1828

Type species: *Auricula* (*Chilina*) *fluctuosa* Gray, 1828 (subsequent designation of Gray 1847).

Diagnosis

The genus and family have a oval (oblong to ventricose) shell with expanded last whorl. Nervous system with vestigial chiastoneury. Pulmonary roof pigmented with kidney occupying almost entire length. Kidney inner wall with numerous transverse trabeculae of irregular contour. Rectum on right side of mantle cavity, anus near pneumostome. Incomplete division of male and female ducts; common duct opens to hermaphrodite duct, with irregular contours on both sides. Proximal portion of uterus with glandular walls. Calcareous granules in vaginal lumen and secondary bursa copulatrix or accessory seminal receptacle present. Penial terminal portion with cuticularized teeth-like structures.

Remarks

The Chilinidae contains only the genus *Chilina* with 34 nominal species; 19 of which are found in Argentina (Núñez *et al.* 2010; Ovando and Gutiérrez Gregoric 2012) and the remainder in Chile and Brazil (Castellanos and Gaillard 1981; Simone 2006; Valdovinos Zarges 2006).

Chilina sanjuanina n. sp.

Type material and type locality: Aguas Negras, Jachal, San Juan Province, Argentina (30°18′S; 68°43′W), coll. N. Ciocco & E. Koch, Nov. 2011.

Holotype. MLP 13545.

Paratypes. MLP 13646, same data (four preserved specimens in alcohol).

Material examined

Type material. *Other material examined*. MLP 13750: Aguas Negras, Jachal, San Juan Province, Argentina (30°18′; 68°43′W), coll. N. Ciocco & E. Koch, Nov. 2011 (20 preserved specimens in ethanol); MLP 13648: Gualcamayo river, El Chepical, Jachal, San Juan Province, Argentina, 29°45′S; 68°45′W, coll. J. Contreras, 11 Feb. 1976 (199 preserved specimens in ethanol, material not relaxed); MLP 13649: Jachal river, Jachal, San Juan Province, Argentina, 30°35′S; 68°35′W, 27 Apr. 1999 (3 preserved specimens in ethanol).

Diagnosis

Shell small, one columellar tooth; radula with central tooth bicuspid and asymmetric with weak serrated edges; penis sheath inner sculpture with regular conic pustules over entire surface.

Description

Shell (Fig. 2). Oval. Four or five convex whorls. Spire conical, low, and whitish. Small length of penultimate whorl. Last whorl globose and large (88% of the total length). Aperture 70% of the total length, not expanded, with translucent white callus and strong columellar tooth. Periostracum reddish brown with tenuous dark brown zigzag bands, mainly in the last whorl.

Dimensions. see Table 1.

Reproductive system (Fig. 3).

Female genital system: Bursa copulatrix duct long (mean = 4.40 mm, DS = 1.21, $N = 2$) five times bursa sac diameter. Bursa copulatrix sac spherical, located on left side of cephalopedal haemocoel between pericardial cavity and columellar base. Secondary bursa copulatrix

Figure 2. Shell of *Chilina sanjuanina* from Aguas Negras, San Juan. Total length = 10.80 mm.

Table 1. Mean and range of 10 measurements for *Chilina* spp. from Cuyo Malacological Province, Argentina.

	TL	LWL	AL	TW	AW	AP	SL	FPW	PWL	IPW
Chilina sanjuanina ($n = 13$)										
Holotype	10.80	9.30	7.40	6.50	4.20	1.50	1.50	2.90	0.85	1.30
Mean	10.96	9.63	7.74	6.67	4.43	1.41	1.29	2.85	0.83	1.29
Max	14.67	12.2	9.80	8.70	5.2	1.80	2.20	4.20	1.30	1.80
Min	8.30	7.15	5.80	5.00	3.40	1.00	0.70	2.89	0.60	1.00
Chilina cuyana ($n = 15$)										
Holotype	11.50	10.30	8.20	7.30	5.00	2.00	1.20	2.40	0.50	1.20
Mean	10.10	8.94	7.15	6.52	4.33	1.69	1.16	2.31	0.62	1.24
Max	11.50	10.30	8.50	7.60	5.00	2.00	1.70	3.10	0.80	1.60
Min	8.40	7.20	6.00	6.00	4.00	1.50	0.70	1.70	0.50	1.00
Chilina mendozana ($n = 62$)										
Mean	14.34	12.82	10.61	8.74	6.12	2.07	1.51	3.48	1.27	1.59
Max	19.48	18.33	15.58	13.09	8.93	3.24	4.87	5.30	2.34	2.50
Min	6.00	5.20	4.50	4.00	2.50	1.05	0.44	1.25	0.50	0.60
Chilina parchappii ($n = 32$)										
Mean	14.85	12.52	10.20	8.17	5.69	1.84	2.33	3.34	1.40	1.69
Max	19.08	16.13	13.29	10.54	6.93	2.50	4.87	4.90	2.00	2.50
Min	10.07	8.92	7.28	5.65	3.72	1.40	1.36	2.00	0.70	1.00

Note: Measurements in mm.
Abbreviations: AL — aperture length; AP — aperture projection; AW — aperture width; FPW — final penultimate whorl width; IPW — initial penultimate whorl width; LWL — last whorl length; PWL — penultimate whorl length; SL — spire length; TL — total length; TW — total width.

short, emerging from base of uterus, spherical (17% the length of bursa copulatrix duct), expanded in its distal portion. Vagina shortest and narrowest portion between free oviduct and female genital atrium. Vagina cylindrical, longer than wide, folded over free oviduct, and entering female atrium. Female genital atrium opening outward through female pore on right side of head above male pore.

Male genital system: Prostate gland extending to lower half of uterus and consisting of variable size and cylindrical acini. Vas deferens coiled twice, overlapping vagina. At level of penis complex, vas deferens bent back on itself. Penis sheath muscular, 2 1/2 length of prepuce, with slight convexity on right side. Penis elongated, slightly longer than the penis sheath. Penis-sheath inner sculpture with regular pustules over entire surface. Pustules conical, raised at centre. Prepuce cylindrical, thin, with constriction marked by oblique lines arranged in V making connection with penis sheath (Fig. 3).

Radula (Fig. 4). Mean number of rows 44 ($n = 2$; range = 41–48). Mean number of teeth per half row 35 ($n = 2$; range = 34–36). Central tooth asymmetrical, bicuspid, elongated base higher than wide, right cusp more developed and saw-like weak edges in the two cusps. First lateral tooth tricuspid, with mesocone more developed, base of tooth narrower than the apical part (cusp area).

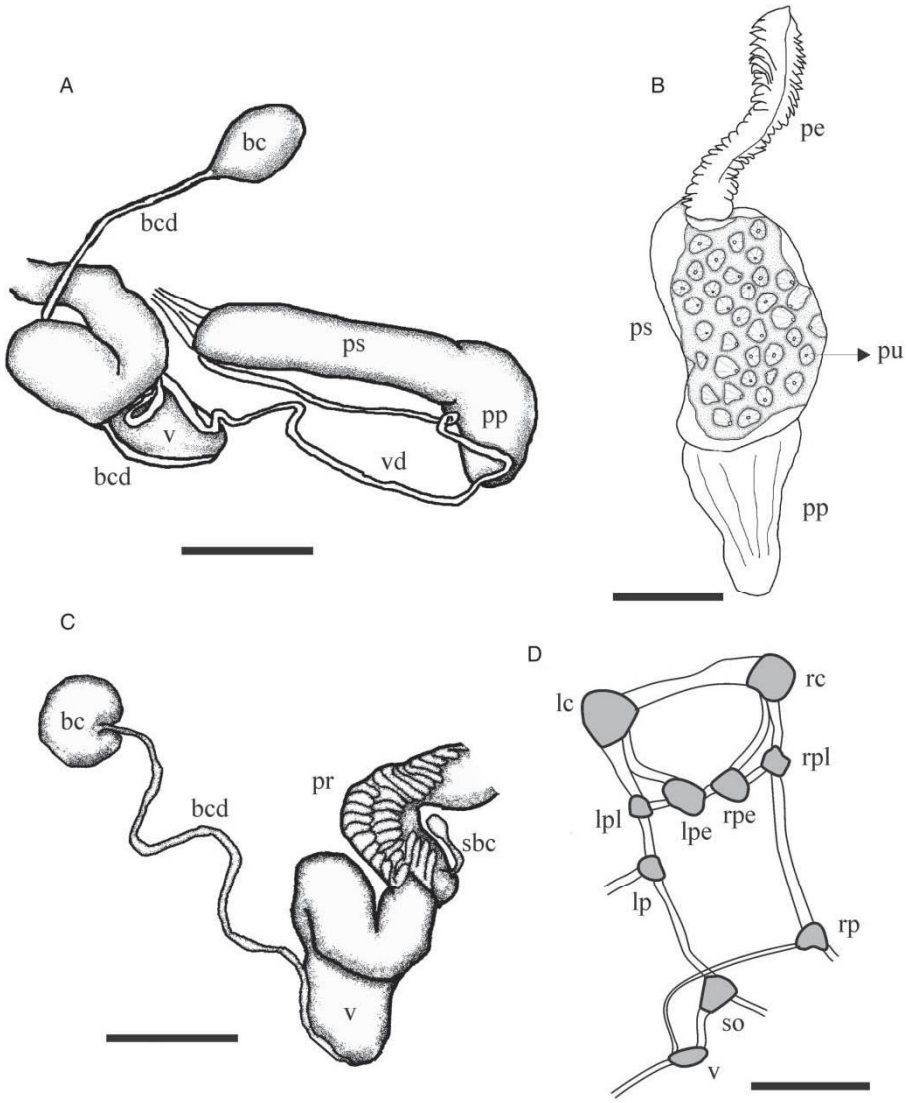

Figure 3. *Chilina sanjuanina*. **A,** distal part of reproductive system in dorsal view; **B,** penis inner wall; **C,** distal part of female reproductive system in ventral view.
Abbreviations: bc — bursa copulatrix; bcd — bursa copulatrix duct; pe— penis; pp — preputium; pr — prostate; ps — penis sheath; pu — pustules; sbc — secondary bursa copulatrix; v — vagina; vd — vas deferent. **D,** Diagram of nervous system. Abbreviations: lc — left cerebral; lpe — left pedal; lp — left parietal; lpl — left pleural; rc — right cerebral; rpe — right pedal; rp — right parietal; rpl — right pleural; so — suboesphageal; v — visceral. Scale bar = 1 mm.

Second lateral tooth tricuspid (mainly) or tetracuspid, with mesocone (of the tricuspid) or the inner second cusp (in the tetracuspid) more developed, base of tooth narrower than apical part. Unlike previous tooth, base is more notable. Last teeth with thin base, even with four to five cusps with similar development. Radular formula: $[35/(3-5)+1/2]44$.

Nervous system (Fig. 3, Table 2). All connectives between ganglia relatively thin compared to size of both ganglia and central nervous system in general. Similar lengths of cerebral-pleural connective (6.57% and 6.23% of LWL). Right pleuroparietal connective passing over the penis complex. Length of pleuroparietal connective left smaller than right (4.00 *vs.* 14.57% of LWL). Parietal-suboesophageal connective shorter than parietal-visceral connective (11.52 *vs.* 15.31 of LWL). One very short connective (ratio: 4.95 of LWL) linking suboesophageal ganglion to visceral ganglion and closing posterior nerve ring.

Distribution and habitat (Fig. 5)

Abundant on rocky bottoms from headwaters of Aguas Negras stream and shallow, moderately lotic (current velocity: 0.5 m s^{-1}, range: 0.47–0.62 m s^{-1}) and vegetated streams with transparent water and mean levels of

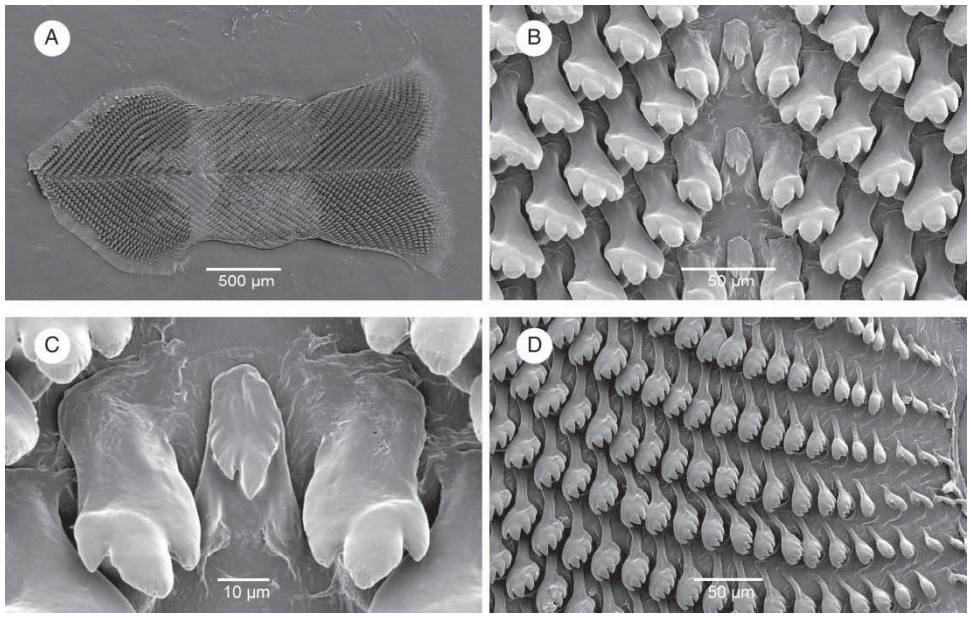

Figure 4. Radula of *Chilina sanjuanina* from Aguas Negras, San Juan. **A,** general view. Scale bar = 500 µm. **B,** central tooth and first lateral teeth. Scale bar = 50 µm. **C,** central tooth. Scale bar = 10 µm. **D,** lateral teeth. Scale bar = 50 µm.

Table 2. Ratio between lengths of ganglia and last whorl in *Chilina sanjuanina* ($n = 4$) and *Chilina mendozana* ($n = 7$).

	C. sanjuanina			*C. mendozana*		
	Ratio	Mean	SD	Ratio	Mean	SD
lc-rc	12.87	1.19	0.31	11.88	1.47	0.41
lpe-rpe	4.07	0.38	–	4.38	0.54	0.20
lc-lpl	6.57	0.61	0.29	5.90	0.73	0.27
rc-rpl	6.23	0.58	0.28	7.68	0.95	0.16
c-p	7.99	0.74	0.43	10.02	1.24	0.33
rpl-rp	14.57	1.34	0.61	16.32	2.02	0.69
lpl-lp	4.00	0.37	0.20	5.09	0.63	0.09
lp-so	11.52	1.06	0.76	16.32	2.02	0.38
rp-v	15.31	1.41	0.66	16.97	2.10	0.38
so-v	4.95	0.46	0.23	7.07	0.88	0.17

Notes: Measurements in mm.
Abbreviations for each ganglion: c — cerebral; lc — left cerebral; lp — left parietal; lpe — left pedal; lpl — left pleural; p — pedal; rc — right cerebral; rp — right parietal; rpe — right pedal; rpl — right pleural; so — suboesphageal; v — visceral.

conductivity, dissolved oxygen, and pH of 2.6 mS cm^{-1}, 8.1 mg l^{-1}, and 7.4, respectively.

Etymology

sanjuanina – named after the San Juan Province

Available genetic data: COI of 655 bp from holotype material was deposited in the GenBank under the number KC347574.

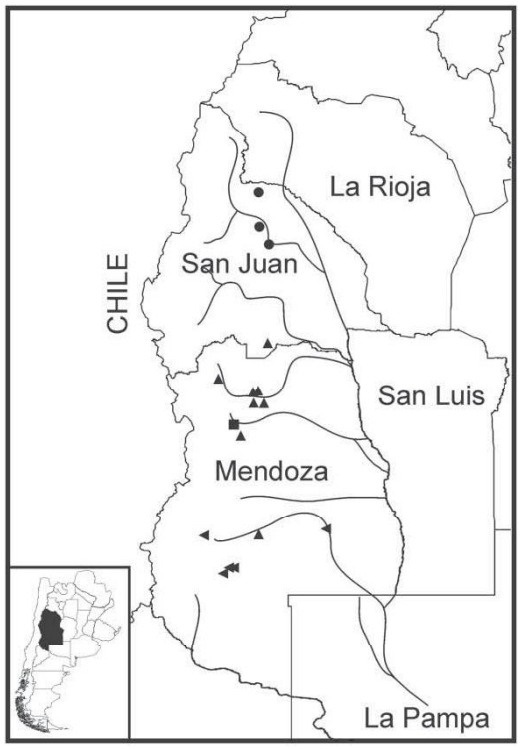

Figure 5. Species distribution of Chilinidae in Cuyo Malacological Province. ●, *Chilina sanjuanina*; ■, *Chilina cuyana*; ▲, *Chilina mendozana*; ◀, *Chilina parchappii*.

Remarks

This new species is distinctive in its small size and most closely resembles *Chilina portillensis* Hidalgo, 1880 from

Figure 6. Shell of *Chilina cuyana* from Vila Stream, Mendoza. Total length = 11.15 mm.

the Tucumán and Jujuy geographic provinces. It differs mainly in being smaller and more globose and the inner wall of the penis sheath of *C. sanjuanina* has conical pustules over the entire surface, whereas in *C. portillensis* the inner-wall sculpture has star-shaped pustule arrangements on the left and right margins around a smooth middle portion (Ovando and Gutiérrez Gregoric 2012). *C. sanjuanina* has a bicuspid central tooth, like *C. portillensis*, but the base of that tooth has a greater length than width. Comparisons with the other species in the Cuyo Malacological Province are made in the remarks under those species below.

Chilina cuyana n. sp.

Type material and type locality. Vila stream, Tupungato, Mendoza Province, Argentina (33°22′S; 69°07′W), coll. L. Serra, 13 Jan. 1952.

Holotype. MLP 13660.

Paratypes. MLP 3636, same data (15 preserved specimens in ethanol).

Material examined

Type material

Diagnosis. Shell small, spire low, two columellar teeth; radula with tricuspid central tooth, asymmetric with serrated edges.

Description

Shell (Fig. 6). Small and oval, of four whorls. Spire low, conical, and whitish. Small length of penultimate whorl. Last whorl large (88.5% of the total length). Aperture not expanded, of 70% of the total length, with strong white callus. Two columellar teeth, lower tooth more prominent and developed than upper. Light brown periostracum without zigzag bands. Fine growth striations visible in all whorls.

Dimensions. see Table 1.

Radula (Fig. 7). Mean number of rows 48 ($n = 2$; range = 48). Mean number of teeth per half row (except for central tooth) 38 ($n = 2$; range = 38). Central tooth asymmetrical, tricuspid, elongated base higher than wide, mesocone more developed and saw-like edges in all three cusps. First lateral tooth tricuspid (mainly) or tetracuspid, with mesocone (of the tricuspid) or the inner second cusp (in the tetracuspid) more developed, base of tooth narrower than apical part (cusp area). Second lateral tooth tricuspid, with mesocone more developed, base of tooth narrower than apical part of tooth. Last teeth with thin base, having five cusps with similar development. Radular formula: $[38/(3-5)+1/3]48$.

Etymology

cuyana—named after the Cuyo Malacological Province

Remarks

Because the material from the MLP collection had not been properly maintained, the dissections failed to reveal the internal anatomy except for the radula, which organ became rehydrated. The specimens were contracted within the shell with signs of losing the liquid in which they were conserved. *C. sanjuanina* and *C. cuyana* are similar at the shell level. Both have lower lengths of the penultimate whorl (7.6% and 6.53% of the TL, respectively), but *C. cuyana* contains two teeth on the inner edge of the aperture where *C. sanjuanina* has only one. *C. cuyana* has a greater width-to-length ratio (64.5%), giving a more globose appearance to the shell. The aperture projection (AP) is also higher in *C. cuyana* (16.73% of TL *vs.* 12.86% of TL in *C. sanjuanina*). *C. cuyana* is

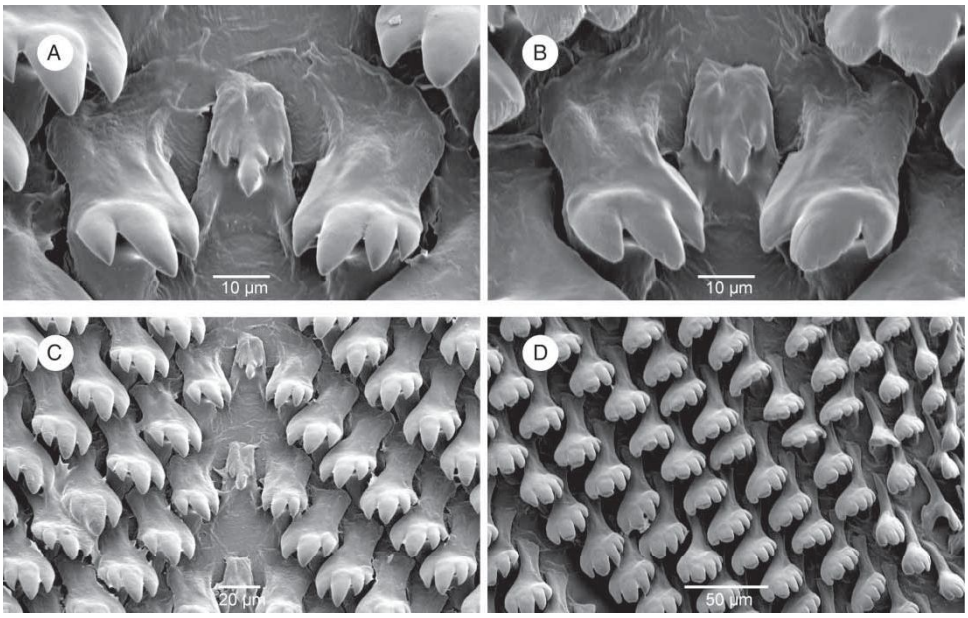

Figure 7. Radula of *Chilina cuyana* from Vila Stream, Mendoza. **A, B,** central tooth. Scale bar = 10 µm. **C,** central tooth and first lateral teeth. Scale bar = 20 µm. **D,** lateral teeth. Scale bar = 50 µm.

also similar to *C. portillensis*, which species also has two teeth in the aperture, but differs from *C. cuyana* in having a shell with dark bands (Ovando and Gutiérrez Gregoric 2012). Other species with two teeth in the aperture are *Chilina fluminea* (Maton, 1809), *Chilina rushii* Pilsbry, 1896, *Chilina gallardoi* Castellanos & Gaillard, 1981 and *Chilina megastoma* Hylton Scott, 1958, all present in the Del Plate basin. All these species differ from *C. cuyana* because the upper tooth is larger. Furthermore, these species have a shell with dark bands (Gutiérrez Gregoric 2010). *C. cuyana* has an asymmetric tricuspid central tooth like the other species recorded in Argentina, such as *C. fluminea*, *C. lilloi*, and *Chilina tucumanensis* Castellanos & Miquel, 1980 (Gutiérrez Gregoric 2010; Ovando and Gutiérrez Gregoric 2012). The cusps present in *C. cuyana*, however, are less developed.

Chilina mendozana Strobel, 1874

Synonymy

Chilina tehuelcha var mendozana—Strobel 1874: 43, Fig 4.

Chilina mendozana—Castellanos and Gaillard 1981: 39; Rumi *et al.* 2006: 206; Rumi *et al.* 2008: 82; De Francesco and Hassan 2009: 107 in part; Núñez *et al.* 2010: 51.

Original description (from Strobel 1874: 43)

Original diagnosis

'*a. Testa maxima: alta 18 mill.; — Testa minima: alta 14 mill. b. Testa elongata: alta 17, lata 10 mill.; — testa ovata: alta 14 1/2, lata 10 mill. — c. Testa corrosa; — testa apice demum corroso; — testa omnino intacta. — d. Columella uniplicata; — columella subbiplicata; — plica columellari obsoleta; — e. anfractu ultimo valde crasso, albescente, fasciis obsoletis; — Anfractu ultimo subtenui, lutescente aut oleee coloris, fasciis distinctis.*'

Type material and type locality. Arroyo del Rosario, Mendoza Province, Argentina.

Holotype. MSNU (Natural History Museum of the University of Parma) 2679.

Material examined

Type material. Only photographs. *Other material examined.* Mendoza Province: MSNU 2685: San Carlos, 34°04′S; 69°08′W (15 dry shells); MLP 8260: Mendoza River, Mendoza, 33°03′S; 68°40′W (3 dry shells and 4 preserved specimens in ethanol); MLP 8268: El Infiernillo Stream, El Sauce, Tunuyán, 32°53′S; 68°40′W, Jan. 1950, coll. A. Caballero (5 dry shells); MLP 7391: water treatment plant from Mendoza city, 32°53′S; 68°49′W, 2004, coll. D. Zelaya (6 preserved specimens in ethanol); MLP 5385: Las Pintadas, Tunuyán, 33°34′S; 69°01′W, 1998, coll. M.C. Damborenea (12 preserved specimens in ethanol); MLP 13647: Uspallata stream, 32°40′S; 69°21′W, Dec. 2011, coll. N. Ciocco (2 preserved specimens in ethanol); MLP 13651: Atuel River, El Nihuil dam, 35°05′S; 68°45′W, 1999, coll. C. Quiroga (10 dry shells); MLP 13652: Mendoza, 33°03′S; 68°40′W, 1 May 2004, coll. V. Flores (13 preserved specimens in ethanol); MLP 3639: Mendoza River, Lunlunta, 33°03′S; 68°40′W, 12 Feb. 1950, coll. L. Serra (4 preserved specimens in

Figure 8. Shell of *Chilina mendozana*: water treatment plant of Mendoza city. Total length = 15.15 mm.

ethanol); San Juan Province: MLP 8261: Acequión River, Sarmiento, 32°07′S; 68°36′W, Nov. 1941, coll. Orlando (1 dry shell).

Diagnosis

Globose shell parallel-sided, low spire, with single columellar tooth; radula with central tooth bicuspid and asymmetric with strong serrated edges; penis-sheath inner sculpture differentiated into two regions, one with pustules and the other with longitudinal folds.

Description

Shell (Fig. 8). Oval. Five to six strong whorls, little convex or not at all. Penultimate whorl domed and low. Spire short. Last whorl large but not dilated (89% of the total length), being sometimes angular with almost parallel edges. Aperture 74% of the total length, with white callus expanded. One weak columellar tooth and, rarely, with an outline of parietal tooth. Periostracum olive-reddish-brown, usually smooth, although sometimes with dark-reddish-brown tenuous bands. Visible tight growth striations in the first whorls and irregular ones in the last. Individuals found in smaller-sized high-altitude rivers and are of globose appearance.

Dimensions. See Table 1.

Reproductive system. Female genital system: Bursa copulatrix duct large (mean = 5.6 mm, DS = 1.28, $N = 4$). Bursa copulatrix sac oval. Secondary bursa copulatrix short, (14% bursa copulatrix duct length), cylindrical, expanded in its distal portion.

Male genital system (Fig. 9): Muscular penis sheath, $3^2/_3$ length of prepuce. Penis-sheath inner sculpture differentiated into two regions, one with pustules and the other with longitudinal folds. Penis long (82% length of penis

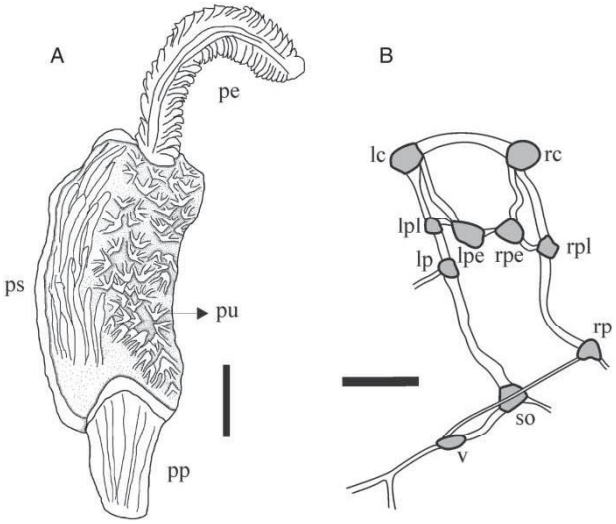

Figure 9. *Chilina mendozana*. **A,** penis inner wall. Abbreviations: pe — penis; pp— preputium; ps — penis sheath; pu — pustules. **B,** diagram of nervous system. Abbreviations: lc — left cerebral; lpe — left pedal; lp— left parietal; lpl — left pleural; rc — right cerebral; rpe — right pedal; rp — right parietal; rpl — right pleural; so — suboesphageal; v — visceral. Scale bar = 1 mm.

sheath), robust, with outer surface cut by transverse lamellae, triangular in cross section. Prepuce inner sculpture with numerous smooth, very tight longitudinal folds.

Radula (Fig. 10). Mean number of rows 41 ($n = 4$; range = 39–43). Mean number of teeth per half row (except for central tooth) 40 ($n = 4$; range=37–43). Central tooth asymmetrical, bicuspid, elongated base higher than wide, right cusp more developed, and saw-like strong edges in the two cusps. First lateral tooth tricuspid or tetracuspid, with mesocone (of the tricuspid) or the inner second cusp (in the tetracuspid) more developed, base of tooth narrower than the apical part (cusp area). Second

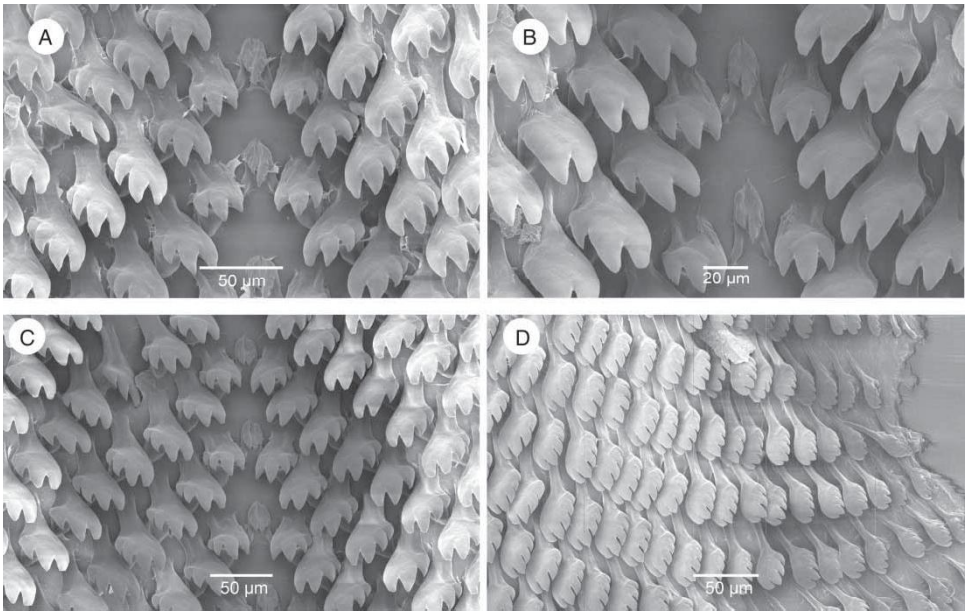

Figure 10. Radula of *Chilina mendozana* from Mendoza, Argentina. **A, C,** central tooth and first lateral teeth. Scale bar = 50 µm. **B,** central tooth and first lateral teeth. Scale bar = 20 µm. **D,** lateral teeth. Scale bar = 50 µm.

lateral tooth tetracuspid, with two inner cusps more developed, base of tooth narrower than apical part of tooth. Last teeth with thin base, even with four to five cusps of similar development. Radular formula: $[40/(3-5)+1/2]41$.

Nervous system (Fig. 9, Table 2). All connectives between ganglia relatively thin compared to both size of ganglia and system in general. Similar length of cerebral-pleural connective right and left (7.68% and 5.9% of LWL). Right pleuroparietal connective passing over the penis complex. Length of pleuroparietal connective left smaller than right one (5.09 *vs.* 16.29% of LWL). Long connective (ratio: 16.32 of LWL) linking left parietal ganglion to suboesophageal ganglion, located above posterior half of columellar muscle. Long connective (ratio: 16.97 of LWL) linking right parietal ganglion to visceral ganglion. One very short connective (ratio: 7.07 of LWL) linking suboesphageal ganglion to visceral ganglion and closing posterior nerve ring.

Distribution and habitat (Fig. 5)

Chilina mendozana was detected in two geographic provinces (Mendoza and San Juan). The species was collected in reservoirs and mostly on the rocky bottoms of the shallow margins of moderately lotic rivers and streams fed by glacial melting waters of conductivity <1 mS s^{-1} and oxygen saturation above 50% (Uspallata stream).

Available genetic data

COI of 655 bp from one specimen from Uspallata stream (MLP 13647) was deposited at GenBank with the number KC347575.

Remarks

Chilina gibbosa Sowerby, 1841 is a larger species (reaches 35 mm TL) and presents a greater angularity in the aperture and last whorl. The juveniles of the species described here bear a striking resemblance to the juveniles of *C. gibbosa*. De Francesco and Dieguez (2006) and De Francesco and Hassan (2009) recorded fossils identified as *C. mendozana* from the study area, but their illustrations of Quaternary and Holocene specimens, do not belong to *C. mendozana*, rather, to another species linked to *C. parchappii*.

Chilina mendozana differs from the other three species studied here and from those in the province of Tucumán (Ovando and Gutiérrez Gregoric 2012) in having a more angular and globose shell. Shells of that type link *C. mendozana* to species of the so-called gibbous group (grupo gibbosa) present in Patagonia. In *C. mendozana* the last whorl length is relatively larger than it is in the other three species studied here (89.40% of TL). The same applies to aperture length with respect to last whorl length (82% of LWL).

Chilina mendozana has a bicuspid central radular tooth as in *C. sanjuanina*, but the cusps are more elongated and with more strongly serrated edges.

The penis complex in *C. mendozana* is curved and U-shaped, like that of *C. lilloi*, whereas that organ in *C. sanjuanina* n. sp. is cylindrical and elongated but never curved, such as in *C. tucumanensis* (Ovando and Gutiérrez Gregoric 2012). In the inner wall of the penis sheath of *C. mendozana* two different regions are presented, one with pustules and the other with longitudinal folds. In contrast, in *C. lilloi*, this sculpture is divided into three

Figure 11. Shell of *Chilina parchappii*: Llancanelo Lake, Malargüe, Mendoza. Total length = 14.05 mm.

well-delimited portions (Ovando and Gutiérrez Gregoric 2012). In *C. tucumanensis* the inner wall exhibits only one type of sculpture, consisting in polyhedral pustules in an orderly array.

Chilina mendozana and *C. sanjuanina* have the same general pattern of the nervous system. *C. mendozana* exhibited a greater difference between the lengths of the pleuro-cerebral connective compared to *C. sanjuanina*. In *C. mendozana*, the ratios of the parietal-visceral and parietal-suboesophageal connectives have a similar length, while in *C. sanjuanina*, parietal-suboesophageal connective was shorter.

Chilina parchappii (d′Orbigny, 1835)

Synonymy

Linneus parchappii d'Orbigny, 1835: 24.

Chilina parchappii.—d'Orbigny, 1835–1846: 338; Smith, 1881: 843; Pilsbry, 1911: 544; Castellanos & Gaillard, 1981: 32 in part; Rumi *et al.*, 2008: 82 in part.

Chilina mendozana.—De Francesco and Dieguez, 2006; 75; Ciocco and Scheibler, 2008; De Francesco and Hassan, 2009: 107 in part.

Original description. d'Orbigny (1835): Synopsis terrestrium et fluviatilum Molluscorum in suo per Americam Meridionalem itinere collectorum. *Magasin de Zoologie* 5 (61):1–44.

Original diagnosis: Testa oblongo—conica, elongata, longitudinaliter et irregulariter striata, fuscente, fasciis quatuor transversis fusco-maculata; spira conica, elongata; apice acuto; sex anfractibus , ultimo brevi; apertura oblonga, albida; columella crassa, umbilicata; labro acuto; umbilico subaperto.

Type material and type locality

Pampas Region (Argentina), MNHN (Muséum National d'Histoire Naturelle, Paris) 23104.

Material examined

Type material. Photographs only. *Other material.* Mendoza Province: MACN 15449: Llancanelo Lake, Malargüe, 35°35′S; 69°11′W, coll. Culis Múcula (4 dry shells); MLP 8327: Llancanelo Lake, Malargüe, 35°35′S; 69°11′W, 10 May 1985, coll. Aramburu & Darrieu (13 dry shells); MLP 13650: Bañado Carilauquen, 35°39′S; 69°15′W, Autumn 2001, coll. Scheibler (3 specimens preserved in ethanol); MLP 8274, 663 and 767, General Alvear, 34°59′S; 67°42′W, Jan. 1942, coll. Umana (6 dry shells and 34 specimens preserved in ethanol); MLP 29403: El Sosneado, 35°05′S; 69°03′W, 4 Jan. 1921, coll. Cabette (8 dry shells).

Diagnosis

Slender shell, elongated and conical spire, with single columellar tooth; radula with central tooth tetracuspid and asymmetric with serrated edges in main cusp.

Description

Shell (Fig. 11). Oval, slender, thin, weak, transparent, furrowed with irregular longitudinal striations. Spire often

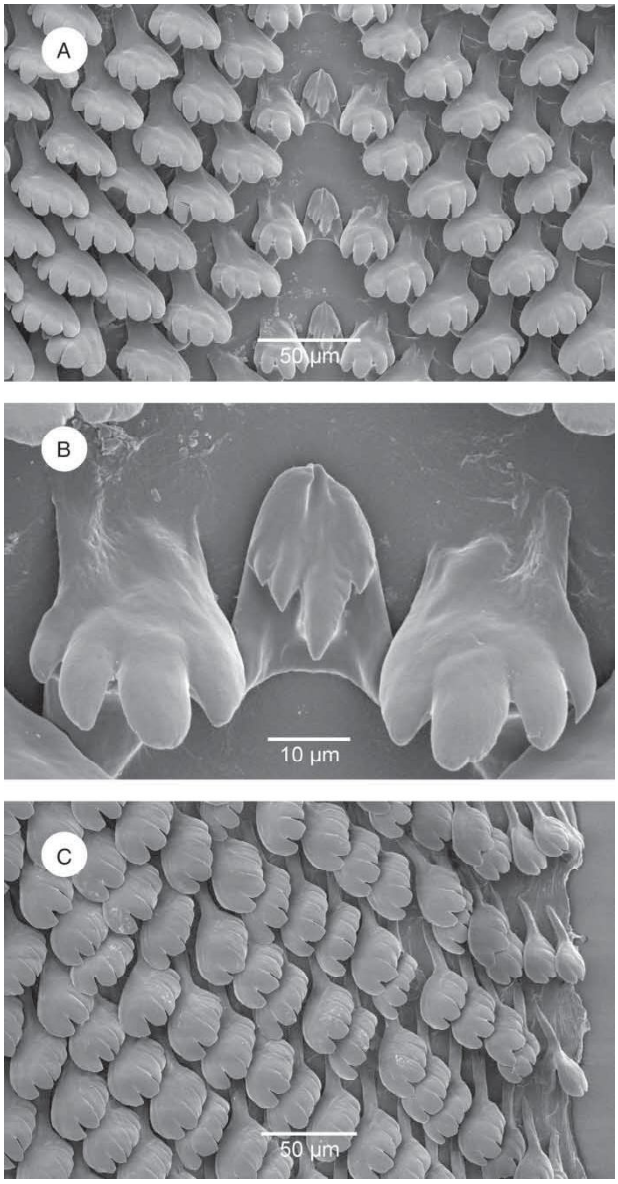

Figure 12. Radula of *Chilina parchappii* from Carilauquen, Mendoza, Argentina. **A,** central tooth and first lateral teeth. Scale bar = 50 µm. **B,** central tooth. Scale bar = 10 µm. **C,** lateral teeth. Scale bar = 50 µm.

very elongated, conical apex pointed; with four to five whorls. Last whorl 84% of the total length. Unexpanded aperture. Aperture 69% of total length, narrow, thin edges, sharp, and almost parallel. Penultimate whorl high. Columella thin, white, and with weak tooth. Periostracum uniform light brown. Reddish-brown zigzag bands on all whorls. Apex brown or pink.

Dimensions. See Table 1.

Radula (Fig. 12). Mean number of rows 48 ($n = 3$; range = 46–49). Mean number of teeth per half row (except for central tooth) 37 ($n = 4$; range = 31–39). Central tooth asymmetrical, tetracuspid, elongated base higher than wide, with a main cusp, slightly displaced to the right, and three minor cusps. Saw-like edges in the major cusp. First lateral tooth tricuspid or tetracuspid, with mesocone (of the tricuspid) or second inner cusp (in the tetracuspid) more developed; base of tooth narrower than the apical part (cusp area). Second lateral tooth tri- or tetracuspid, with two inner cusps more developed, base of tooth narrower than apical part of tooth. Last teeth with thin base, with five cusps with similar development. Radular formula: $[37/(3-5)+1/4]48$.

Distribution and habitat (Fig. 5)

Pampean littoral area (east of the Buenos Aires Province), with some records in the inner Pampas and northern coast of Patagonia. Castellanos and Miquel (1980) located in Cachi, Salta Province, although not re-registered (Ovando and Gutiérrez Gregoric 2012). Lives in small streams and ponds. The records in the Mendoza Province are in the most southwardly and westerly parts.

Remarks

Since the body of the available material of this species from the Mendoza Province was very dehydrated, we could not perform dissections to analyse the different systems (reproductive and nervous). In the description of Castellanos and Gaillard (1981) the radular formula is given for individuals present in the south of the Buenos Aires Province: $[42-45/(4-6)+1/4]55-60$. The description here coincides with the shape and number of cusps of the central tooth, but not in the total number of rows and the number of teeth per row. The radula of *C. parchappii* is the only one that contains a central tooth with four cusps. *C. parchappii* exhibits the greatest length of the spire (more than 15% of TL) and the narrowest shell (TW least than 55% of TL), of the four species studied here, while notably the penultimate whorl length (at 9.4% of the TL) is the longest of the four as well. *C. parchappii* has the shape of the aperture (AW *vs*. AL) slimmer (55% of AL) of species here studied.

Molecular analyses

The DNA sequences obtained here for *C. sanjuanina* n. sp. (KC347574) and *C. mendozana* (KC347575), were both 655 bp in length, differing around 4%. The other three sequences available in GenBank were unfortunately shorter than those obtained (i.e., JN051382: 626 bp; EF489382: 630 bp; and HQ660030: 591 bp). For comparison, our sequences had to be shortened to 569 bp. The genetic distance matrix showed than *C. sanjuanina* has a

Table 3. Genetic distances for the COI gene within Chilinidae.

	Chilina sanjuanina	*Chilina* sp. JN051382	*Chilina* sp. EF489382	*Chilina mendozana*
Chilina sp. JN051382	0.022			
Chilina sp. EF489382	0.018	0.018		
C. mendozana	0.033	0.033	0.025	
Chilina sp. HQ660030	0.033	0.033	0.025	0.025

Note: Sequences length: 569 bp. GenBank # JN051382, EF489382, and HQ660030: *Chilina* spp. from Chile.

smaller genetic distance with two of the sequences from Chile, than with *C. mendozana* (Table 3).

Discussion

This report provides anatomical, molecular-genetic, and distributional information on the species of *Chilina* within the Argentinean Cuyo Malacological Province, increasing the known species from two to four species. *C. parchappii* is the only species of this malacological province that is recorded in other parts of Argentina, the Transition Zone (located between Central and Cuyo Malacological Provinces) and Central Malacological Province (Núñez *et al.* 2010). In all, 21 species of *Chilina* are now known from Argentina, of which 14 are endemic.

The organization of the pallial system was similar in all the species analysed, and this finding is in agreement with previous descriptions provided by Harry (1964) and Brace (1983) for other *Chilina* species. At the level of the nervous system, in the species studied here, as in those studied by Gutiérrez Gregoric and Rumi (2008), Gutiérrez Gregoric (2010) and Ovando and Gutiérrez Gregoric (2012), the right pleuroparietal connective runs either above or behind the penis complex, and not in front of that complex as in *C. lilloi*.

The characters of the radula, shell, reproductive and nervous systems, and molecular genetics that were analysed in this study, are allowing more complete descriptions of the species present in Argentina. In recent years (in addition to this work), four new species were described and six were redescribed (Gutiérrez Gregoric and Rumi 2008; Gutiérrez Gregoric 2010; Ovando and Gutiérrez Gregoric 2012).

The molecular-genetic studies provided here are the first to be used for taxonomic purposes in this family. The comparison with sequences deposited in GenBank can be considered as preliminary, given that only four other shorter sequences were available, and without a specific determination. The molecular data here presented and from other mitochondrial genes that are being studied, as 16S and cytochrome b, combined with anatomical studies, represent one more tool to assist in defining the validity of the relationships among the many species of Chilinidae.

Acknowledgements

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