REDESCRIPTION OF TWO ENDEMIC SPECIES OF CHILINIDAE (GASTROPODA: HYGROPHILA) FROM DEL PLATA BASIN (SOUTH AMERICA)

DIEGO EDUARDO GUTIÉRREZ GREGORIC

CONICET, División Zoología Invertebrados, Museo de La Plata, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/nº, 1900, La Plata, Buenos Aires, Argentina.

Abstract *The Chilinidae comprises 17 species currently cited for Argentina, mostly distributed in Patagonia. All original descriptions of these species have been based on shell characters, and their internal anatomy is poorly known. Characters of the shell, radula and nervous system are described here for two endemic species of Chilinidae from Del Plata basin (South America),* Chilina rushii *Pilsbry and* C. gallardoi *Castellanos & Gaillard. Materials were collected during the years 2000 and 2005 in the rivers Uruguay and Río de La Plata from Del Plata basin, Argentina. Shell:* Chilina gallardoi *shows, sometimes, the keel in the last whorl, and the most significant difference between these species are the length of the aperture and the size of the last whorl. Radula: it has 58 rows in* C. gallardoi, *whereas in* C. rushii *it has 48 rows; first lateral is tricuspid in* C. gallardoi, *whereas in* C. rushii *is tetracuspid; last teeth have up to five cusps in* C. gallardoi, *whereas in* C. rushii *they have up to seven. Nervous system: differences between the species are based in the distances between the ganglions, and the one that shows the most significant difference is the distance between the left pleural and the parietal. As a conclusion, some measures of shells (mainly the ones that are relationed with the aperture) as well as the length between the nervous ganglia and characteristics of radula, can be used to differentiate species of Chilindae in the Del Plata basin*

Key words *Argentina, freshwater molluscs,* Chilina gallardoi, Chilina rushii

INTRODUCTION

The family Chilinidae Dall 1870 (Gastropoda, Hygrophila) is exclusive to South America, occurring from the Tropic of Capricorn to Cape Horn and the Falkland islands. The family comprises a single genus, *Chilina* Gray 1828, with about 33 species, 17 of which have been recorded in Argentina (Castellanos & Miquel, 1980, 1991; Castellanos & Gaillard, 1981; Rumi *et al.*, 2006, 2008; Gutiérrez Gregoric & Rumi, 2008) while the rest are distributed in Chile. Original species descriptions are restricted to shell characters (Hidalgo, 1880; Marshall, 1924, 1933; Hylton Scott, 1958, among others), and very few subsequent works have dealt with internal anatomy (Haeckel, 1911; Duncan, 1960a, b, 1975; Harry, 1964; Brace, 1983; Ituarte, 1997). Recently a new species has been described, *Chilina iguazuensis* Gutiérrez Gregoric & Rumi 2008, for the Iguazú river, Misiones province, Argentina, with descriptions of the shell, radula and nervous system. Miquel (1984, 1987) analyzed the penial complex of eight species from Argentina, Brazil, Chile and Uruguay, and concluded that no microanatomical differences could be found at this level. Based on characteristics of Chilean species, Valdovinos and Stuardo (1995) rearranged the family systematics and proposed the nervous system as the only reliable character for the distinction of supraspecific taxonomic units.

From an evolutionary perspective, the family Chilinidae is among the most primitive of pulmonate gastropods, and one of the first that entered the aquatic environment. This is reflected in primitive characters such as the streptoneurous nervous system, horizontal lamellar tentacles, non-contractile pneumostome, and incomplete division of male and female ducts (Hubendick, 1947, 1978; Duncan, 1960a; Harry, 1964; Brace, 1983). More recently, and based on analyses of 28S rRNA sequences, Dayrat *et al.* (2001) confirmed the monophyly of the Hygrophyla, including the Chilinidae which is at the base of this clade.

Characters of the shell, radula and nervous system, are described here for two endemic species of Chilinidae from Uruguay and Río de la Plata rivers, *Chilina rushii* Pilsbry 1896 and *Chilina gallardoi* Castellanos & Gaillard 1981, since their reproductive systems are very similar.

MATERIAL AND METHODS

Materials were collected during 2000 and 2005 in the Uruguay and the Río de la Plata rivers (see material for each species). Soft parts were separated from the shell after relaxation in 10%

Contact author : dieguty@fcnym.unlp.edu.ar

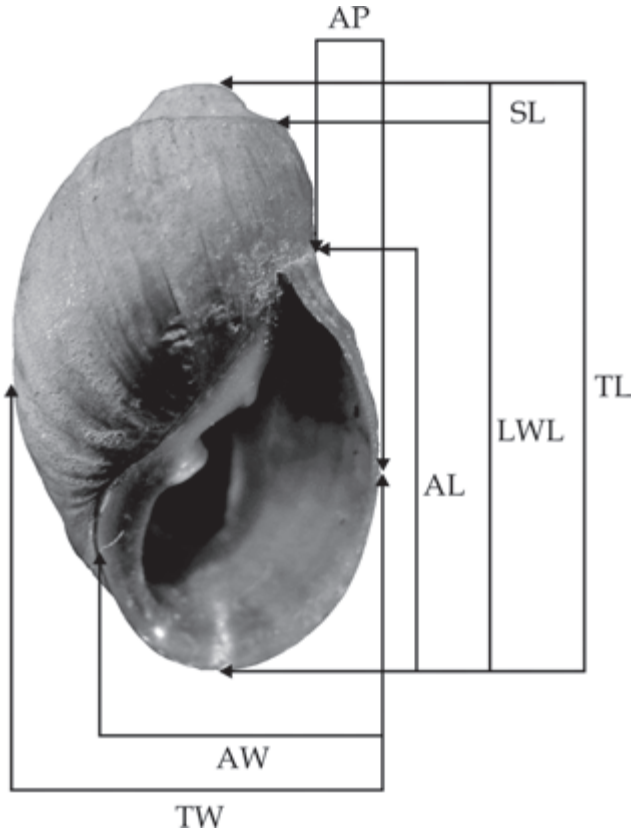

Figure 1 Shell measurements of *Chilina spp.* All measurements are in mm. TL total length; LWL last whorl length; SL spire length; AL aperture length; TW total width; AW aperture width; AP aperture reflection.

Nembutal solution for 12 h, and then fixed in modified Raillet-Henry solution for freshwater animals (93% distilled water, 2% glacial acetic acid, 5% formaldehyde and 6 g sodium chloride per litre of solution). Radulae were separated from the buccal mass and then cleaned with sodium hypochlorite (Clorox). Specimens are deposited at Museo de La Plata (MLP), Buenos Aires province, Argentina.

Six shell measurements were taken (Fig. 1): total length (TL); last whorl length (LWL); aperture length (AL); total width (TW); aperture width (AW); aperture reflection or projection (AP); and number of teeth.

Radulae were observed using JEOL 6360 scanning elecytron microscope at Museo de La Plata (Facultad de Ciencias Naturales y Museo – Universidad Nacional de La Plata). Internal anatomy was analyzed using a Leica MZ6 with *camera lucida*. For the descriptions of the nervous system Ituarte (1997) is followed, also Gutiérrez Gregoric & Rumi (2008). In some cases a Student *t* test was applied measurements of the length of the right and left nervous conectives. To study the soft parts of *C. gallardoi*, one specimen from Monte Caseros was used.

Average measurements of soft parts were divided by length of the last whorl in order to obtain size-free variables that facilitate comparisons among different individuals and species. Length of last whorl was preferred over dimensions of soft parts because it was the most consistent measurement among individuals, and it was not affected by relaxation techniques. In addition, a comparison was made between these species, *Chilina megastoma* Hylton Scott 1958 studied by Ituarte (1997), *C. iguazuensis* described by Gutiérrez Gregoric & Rumi (2008) and *C. fluminea* (Maton 1809) studied by Gutiérrez Gregoric (2008).

Principal Components Analysis (PCA) was used to compare standardized measurements of the shells (log(x)). Forty-five specimens of *C. gallardoi* from San Javier, Misiones province, Argentina were used, and 44 of *C. rushii* taken from various sites (14 from Gualeguaychú, Entre Ríos, Argentina, 13 from Martín García Island, Buenos Aires Argentina, and 17 from Fray Bentos, Uruguay). Later, ANOVA was performed to examine differences between the species.

REDESCRIPTION

CHILINIDAE Dall 1870
Chilina Gray 1828

Diplicaria Rafinisque, 1833: 165
Linneus d'Orbigny, 1835: 24
Dombeia d'Orbigny, 1843:
Pseudochilina Dall, 1870: 357.
Acyrogonia Rochebrunne & Mabille, 1889: 6

Type species *Auricula (Chilina) fluctuosa* Gray 1828 *Specil. Zool.* 5.

The genus *Chilina* is characterized by an oval (oblong to ventricose) shell with last whorl expanded, spire erect or immersed (sunken), whorls generally convex (sometimes carinate or angulose), aperture oval with columellar and parietal margins callous, with up to two oblique tooth-like folds. Periostracum always present, olive-yellow with dark brown longitudinal bands in zigzag pattern. Lacks operculum. Head

with large labial tentacles and cephalic tentacles are short triangular. Foot wide and short. Wide opening of the lungs, protected by a well-developed mantle lobe.

Digestive System Salivary glands slightly lobed, oesophagus rather narrow, muscular stomach forming a caecum, anus opening to a large mantle lobe. Radula with numerous V-shaped rows of teeth, with one central tooth and numerous lateral and marginal teeth to the side and behind the central one.

Reproductive System This family is characterised by a genital system that is primitive because of the incomplete separation of male and female reproductive ducts, absence of a fertilisation chamber, and the presence of numerous penial spines. There is an accessory seminal receptacle and calcareous granules in the vaginal lumen.

Nervous System Has well developed ganglia, cerebral commissure is broad and pedal commissure is short.

Differences have not been found in genital structures between the studied species (Haeckel, 1911; Duncan 1960a, b; Harry 1964; Castellanos & Gaillard, 1981; Miquel, 1984; Ituarte, 1997; Gutiérrez Gregoric & Rumi, 2008).

Chilina rushii Pilsbry 1896

Pilsbry, 1896: 561
Pilsbry & Rush, 1896: 78
Formica Corsi, 1900: 88
Pilsbry, 1911: 547;
Barattini, 1951: 218
Olazarri, 1961: 10
Figueriras, 1964: 186
Castellanos & Gaillard, 1981: 30
Castellanos & Landoni, 1986: 776–777
Rumi *et al.*, 1996: 8
Sacarabino, 2004: 353
Rumi *et al*., 2006: 206
Gutiérrez Gregoric *et al*., 2006: 57
Rumi *et al*., 2008: 82
Gutiérrez Gregoric, 2008: 48–57.

Original description Pilsbry (1896 New species of freshwater mollusks from South America Proceedings of the Academy of Natural Science of Philadelphia **48**: 561–565).

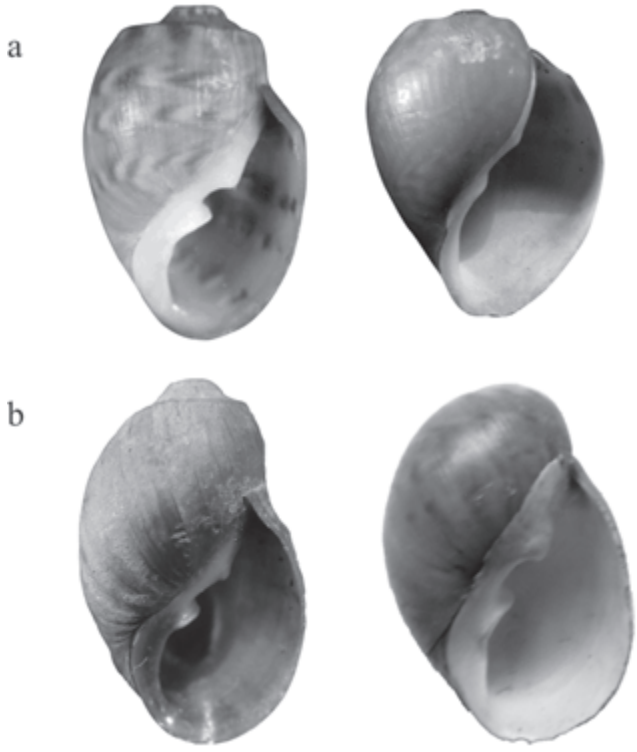

Figure 2 a *Chilina rushii*. Left: from Fray Bentos, Uruguay (TL: 11.8 mm); Rigth: from Federación, Entre Ríos province, Argentina (TL: 11 mm). b *Chilina gallardoi* from San Javier, Misiones province, Argentina.

Type locality Uruguay River, Fray Bentos, República Oriental del Uruguay.

Material examined Martín García island (MLP 12589–12591), Federación (MLP 12609) and Gualeguaychú (MACN-In 9341; MLP 8276, 8277) Argentina, and Fray Bentos (MLP 5274; MLP 12588), República Oriental del Uruguay.

Shell The shell is strong, oval, yellow to olive, with 4 to 5 bands in zigzag reddish brown varying in intensity (Fig. 2a). In some specimens these bands are absent. Up to 5 angular or subangular whorls with a very noticeable keel. Spire very depressed, almost involute. Occasionally the keel is missing or insignificant because of erosion of the spire and last whorl. Aperture is oval and about 81% of length of last whorl. Callus extends to the umbilical region, carrying two strong teeth, one parietal and one columellar, and an inconspicuous basal columellar ridge.

Dimensions of specimens from Martín García island (13), Gualeguaychú (14) and Fray Bentos (17) are given in Table 1.

Table 1 Shell measurements (mm) of *Chilina rushii*, from Fray Bentos. TL total length; LWL last whorl length; AL aperture length; TW total width; AW aperture width; AP aperture reflection; SL spire length. w/d without data.

TL	LWL	AL	TW	AW	AP	SL
15.01	14.66	11.81	10.94	7.12	3.55	0.35
13.61	13.15	10.71	9.07	6.38	2.85	0.46
13.57	12.78	9.80	9.79	6.24	3.17	0.79
12.81	12.10	9.72	8.92	5.91	2.89	0.71
12.02	11.67	9.38	8.43	5.59	2.73	0.35
11.88	11.39	9.69	8.54	6.34	2.11	0.49
10.96	10.69	8.56	7.26	4.99	2.35	0.27
10.60	10.34	8.61	7.16	5.25	2.39	0.26
10.57	10.21	8.19	7.9	5.57	2.56	0.36
10.46	9.82	7.98	7.46	5.14	1.89	0.64
8.52	8.40	7.27	6.44	4.7	2.09	0.12
6.5	6.37	6.11	4.89	3.61	1.58	0.13
w/d	9.32	7.87	7.31	5.51	2.37	w/d
w/d	11.9	9.48	8.8	5.89	2.85	w/d
w/d	12.55	10.35	9.56	6.68	3.11	w/d
w/d	14.85	12.04	10.6	6.82	3.44	w/d
w/d	10.60	9.50	8.06	6.06	2.21	w/d

Radula Five radulae were analyzed (Fig. 3). The central tooth in each file has two different cusps, a large triangular cusp placed nearly centrally, and a smaller cusp placed on the right side. From both cusps up to the base it is possible to see a contouring rim. The base of the tooth is triangular and the upper side shows a low groove, which divides the tooth. The principal cusp is central, the lower cusp is in the right side and cusp with a serrate margin is on the left side. First lateral tooth is tricuspid with a major development of the mesocone. In one of the radulae, the first lateral of the left side had a trace of a fourth cusp outside the ectocone (Fig. 3b). As well as the cusps, teeth are short. Second lateral tooth is tetracuspid, it has a lower ectocone and a fourth cusp in its external side. From this tooth on, the cusps are less developed producing a more rounded shape. The last teeth of each row have five cusps, and they are round and wide (in one specimen, seven cusps were found).

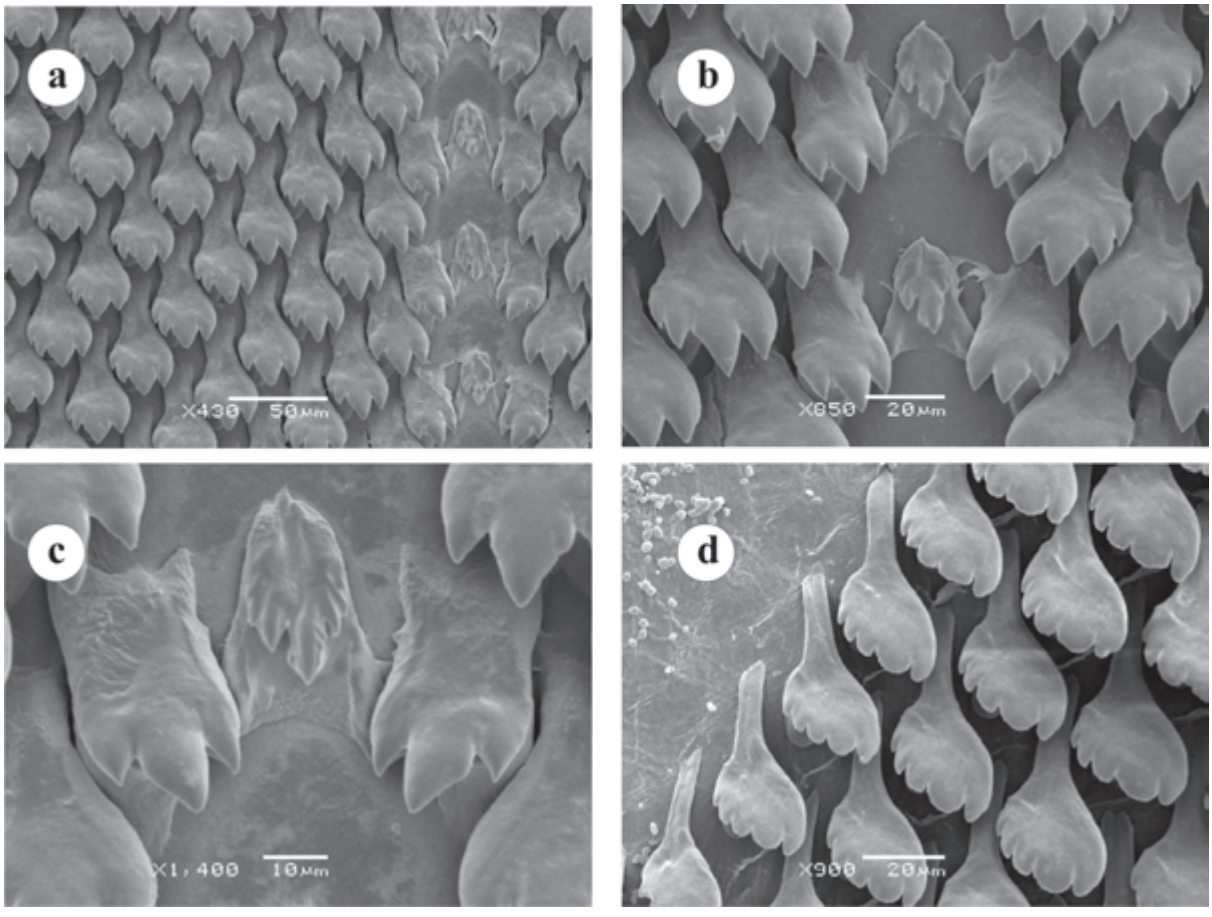

Figure 3 Radula of *Chilina rushii*. a general view of anterior part; b central view; c and d central tooth and first lateral teeth; e marginal teeth. Scale bars: **a** 50 µm; **b** and **d** 20 µm; **c** 10 µm.

Radular formula: [35/(3–7) + 1/2] 48 [= number of right and left teeth/(number of cusps) + number of central teeth/number of cusps] extreme numbers for transverse rows.

Reproductive System The Chilinidae are hermaphroditic, with an ovotestis (hermaphroditic gonad) and a common duct anterior to the separation of both systems. Female and male reproductive elements (Fig. 4) are described separately for reasons of organization.

Female Genital System Bursa copulatrix duct emerging from anterodorsal vagina and extending alongside intestine, then passing under vagina and over uterus-vagina complex toward visceral mass; finally opening into flat oval bursa copulatrix next to ventricle. Secondary bursa copulatrix arising at base of uterus (oviduct) and becoming distally enlarged, pear-shaped.

Male Genital System Vas deferens emerging from prostate, passing under uterus and vagina, then turning on itself and passing over vagina. Vas deferens turns around itself about three times above the vagina and then penetrates the body wall. From the wall it emerges near the penial complex, then crosses over the prepuce and turns back before entering the penial complex adjacent to the buccal mass. Prepuce length 46% of penis sheath length.

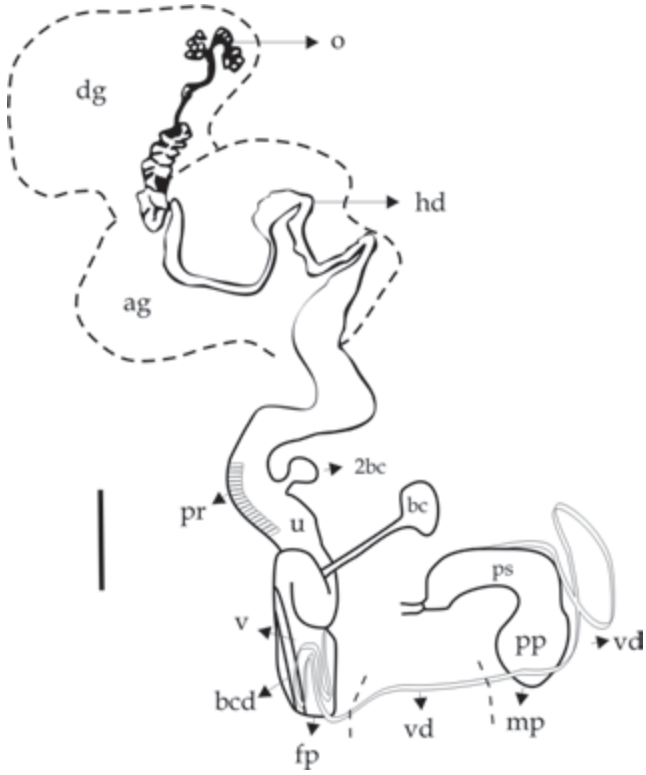

Figure 4 Diagram of reproductive system of *Chilina rushii*: ag: albumen gland; vd: vas deferent; dg: digestive gland; bc: bursa copulatrix; bcd: bursa copulatrix duct; fp: female pore; hd: hermaphrodite duct; mp: male pore; o: ovotestis; pp: prepuce; pr: prostate; ps: penis sheath; u: uterus; v: vagina; 2bc: secondary bursa copulatrix. Dotted line: wal of the body. Scale bar: 2 cm.

Nervous system (Fig. 5a; Table 2) Pedal and cerebral ganglia connected by commissures and connectives, forming anterior nerve ring located at anterior half of buccal mass, only slightly posterior to beginning of oesophagus. The left connective which joins the cerebral ganglion with the pleural ganglion is larger than the right one, but this does not reach statistic significance difference (t_{24}; $p > 0.05$) (7.12 vs 6.65% of LWL). Right parietal ganglion generates two nerves, one to the osphradium and a long very thin connective to the visceral ganglion behind the posterior nerve ring. Two connectives on the left side of anterior nerve ring, are longer than those on right side, connecting the left cerebral and pedal ganglia to the pleural ganglion. Length of the left pleuroparietal connectives is significantly greater than the right pleuroparietal connectives (t_{24}; $p < 0.0001$; 12.56% vs 4.92% of LWL). Long connective (ratio: 18.20 of last whorl length) linking left parietal ganglion to subintestinal ganglion, located above posterior half of columellar muscle. One very short connective (ratio: 7.58 of last whorl length) linking subintestinal ganglion to visceral ganglion and closing posterior nerve ring. Two nerves arising from visceral ganglion toward visceral mass. One large nerve extending to right from subintestinal ganglion through columellar muscle to innervate distalmost vagina and accesory pneumostome. Pleuro-visceral connectives with incomplete torsion characteristic of the genus.

Chilina gallardoi Castellanos & Gaillard 1981

Castellanos & Gaillard, 1981: 30–31
Castellanos & Landoni, 1986: 777
Rumi *et al*., 2006: 206
Gutiérrez Gregoric *et al*., 2006: 57
Rumi *et al*., 2008: 82
Gutiérrez Gregoric, 2008: 66–74.

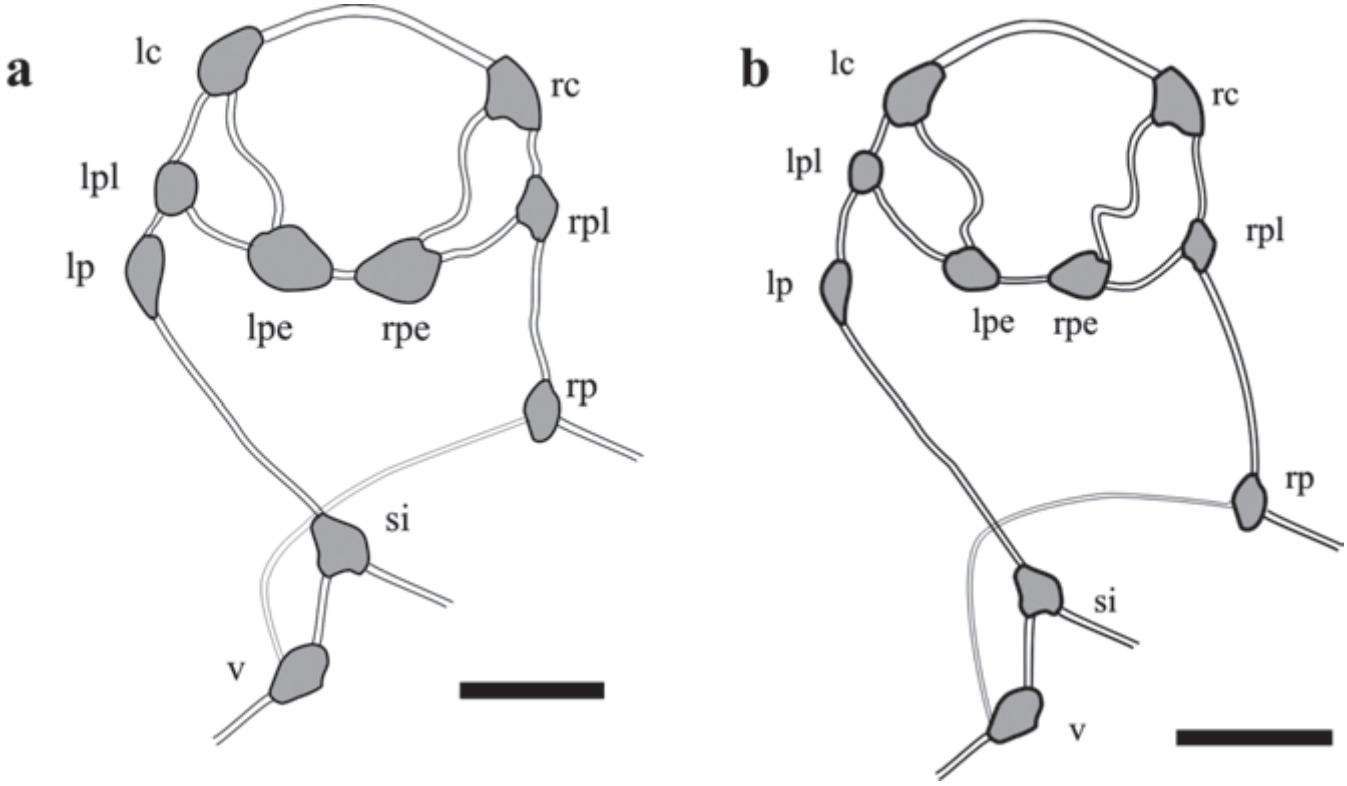

Figure 5 Diagram of nervous system. a *Chilina rushii*; b *Chilina gallardoi.* Abbreviations for ganglia: lc left cerebral; lpe left pedal; lp left parietal; lpl left pleural; rc right cerebral; rpe right pedal; rp right parietal; rpl right pleural; si subintestinal; v visceral. Scale bar: 1 mm.

Table 2 Ratio between ganglion length and last whorl length in *Chilina rushii* and *C. gallardoi.* Abbreviations for each ganglion: c cerebral; lc left cerebral; lp left parietal; lpl left pleural; p pedal; rc right cerebral; rp right parietal; rpl right pleural; si subintestinal; v visceral.

	C. rushii			*C. gallardoi*	
	Ratio	mean (mm)	SD	Ratio	mm
Cerebrals ganglion	17.27	1.65	0.32	12.8	1.60
Pedals ganglion	5.02	0.48	0.11	5.2	0.65
lc–lpl	7.12	0.68	0.27	3.6	0.45
rc–rpl	6.65	0.64	0.19	7.2	0.90
c–p	9.91	0.95	0.37	12.0	1.50
rpl–rp	12.56	1.2	0.35	12.0	1.50
lpl–lp	4.92	0.47	0.19	5.6	2.10
lp–si	18.20	1.74	0.49	16.8	2.10
rp–v	20.15	1.93	0.37	19.6	2.45
si–v	7.58	0.72	0.10	5.6	0.70

Original description Castellanos & Gaillard (1981 Mollusca Gasterópoda: Chilinidae *Fauna de Agua Dulce de la República Argentina*, **15**(4): 23–51. PROFADU (CONICET), Buenos Aires).

Type locality Santa María island, San Javier, Misiones, Argentina.

Paratypes Five shells, Santa María island, San Javier, Misiones, Argentina (MLP 4096, 11067).

Material examined Martín García island (MLP 12607), Federación (MLP 12608), Concordia (MLP 4451–1; MACN-In 17345), Monte Caseros (MLP 12592); Santa María island, San Javier (MLP 4096, 11067; MACN-In 8241, 25801), San Javier (MLP s/n°), Itacaruaré stream, Itacaruaré (MACN 17196), San Lucas island, Barra Concepción (MACN-In 17103), Cumanda–í rapids, San Javier (MACN-In 17104), Argentina; Conchilla (MACN-In 13011), República Oriental del Uruguay.

Shell Shell strong, solid, oval (Fig. 2b). Spire flat or eroded (most specimens), making measurement of total length difficult. Last whorl expanded and convex, with a keel. Keel not evident in the aperture as in the *C. rushii*. Some slight spiral striae below the suture. Penultimate whorl angular, bearing a strong keel. Aperture

Tabla 3 Shell measurements (mm) of *Chilina gallardoi* from San Javier, Misiones. TL total length; LWL last whorl length; AL aperture length; TW total width; AW aperture width; AP aperture reflection; SL spire length. w/d without data.

TL	LWL	AL	TW	AW	AP	SL
w/d	13.23	10.30	7.99	6.40	2.18	w/d
w/d	14.81	10.98	10.60	7.41	3.32	w/d
w/d	13.88	10.13	9.65	6.62	2.91	w/d
14.67	13.66	10.79	9.65	6.52	2.94	1.01
w/d	13.13	10.51	9.32	6.55	2.82	w/d
w/d	14.20	10.42	9.18	6.32	1.70	w/d
w/d	14.55	11.27	9.50	6.53	2.14	w/d
w/d	13.80	10.60	9.44	6.81	2.64	w/d
w/d	14.89	11.85	9.57	6.77	2.75	w/d
w/d	13.89	10.61	9.91	6.65	2.56	w/d
w/d	15.25	11.29	10.49	7.40	3.25	w/d
w/d	15.26	10.48	8.78	6.24	2.53	w/d
w/d	15.61	11.80	9.96	7.43	2.65	w/d
w/d	14.00	10.53	9.46	6.60	2.64	w/d
w/d	15.21	11.35	10.25	7.04	2.94	w/d
w/d	13.55	10.81	9.53	6.49	3.34	w/d
w/d	10.56	8.41	7.85	5.46	2.31	w/d
12.90	12.52	10.05	9.30	6.58	2.05	1.50
13.24	12.80	10.10	9.17	6.23	2.10	1.36
15.92	15.44	12.26	10.74	8.24	3.51	1.60

is oval and about 78% of length of last whorl. Callus spreading in the umbilical region, carrying two strong teeth, one parietal and one columellar. Periostracum dark brown with faint dark bands, although the bands may be reduced or absent. Bands often joined lengthwise. Shell marked with growth lines.

Dimensions of fifty specimens of *Chilina gallardoi* taken from the collection of MLP, including the paratype, were analysed (Table 3). Sites are: Santa María island, (Paratype); San Javier; Monte Caseros; Salto Grande and Martín García island.

Radula Central tooth of the radula (Fig. 6) asymmetric and showing two cusps, the left a little bigger than the right. On the external side of cusps are three protuberances of different size, producing a serrate edge. On the anteroposterior side, each tooth has a central groove. Biggest cusp is in the middle. Base of cusp is small and triangular, with the back and sides wider. First lateral tooth tricuspid with a small protuberance on the external side of the ectocone. Biggest cusp is the mesocone. The tooth is straight and lacks a groove. Beyond the second lateral tooth all are tetracuspids, with the small protuberances enlarged to form a new cusp, lower than the other three. Mesocone is the biggest cusp but the difference in length compared to the endocone is less than with the first lateral tooth. Second lateral tooth shows an expansion in the zone of the cusps, like a paddle, as in other species of the family. Second lateral teeth of the left side has a small protuberance on the external side of the ectocone, just as in the first lateral (Fig. 6c). This condition does not appear in all the rows or near the external rim of the radula. The protubernace never develops into a full cusp. Most of the last teeth of each row have four poorly developed cusps (sometimes five), giving the appearance of serrate paddles.

Radular formula: [44/(3–5) + 1/2] 58.

Reproductive System (Fig. 7).

Female Genital System Duct of the spermatheca emerges from the vagina, running adjacent to the intestine. Secondary bursa copulatrix arises at base of uterus becomes distally enlarged, pear-shaped.

Male Genital System Vas deferens runs along top of prostate, passing beneath uterus and vagina, turning back on itself and running above the vagina. Vas deferens turns around itself about twice above the vagina and turns into the body wall. Inside wall, the tube emerges near the penial complex, then crosses over the prepuce with out turning back. It then enters the penial complex adjacent to the buccal mass. Prepuce length 62% of penis sheath length.

Nervous system (Fig. 5b) Presents a pattern typical for family Chilinidae. Compared to *C. rushii*, length of right connective joining the cerebral and pleural ganglions is longer than the left one (7.2 vs. 3.6% of LWL) (Table 2). The length difference between the left and right pleuroparietal conectives is large, with the left roughly half the length of the right one.

PRINCIPAL COMPONENTS ANALYSIS

Total length and spire lengths were not used in the analysis, due to shell erosion in some

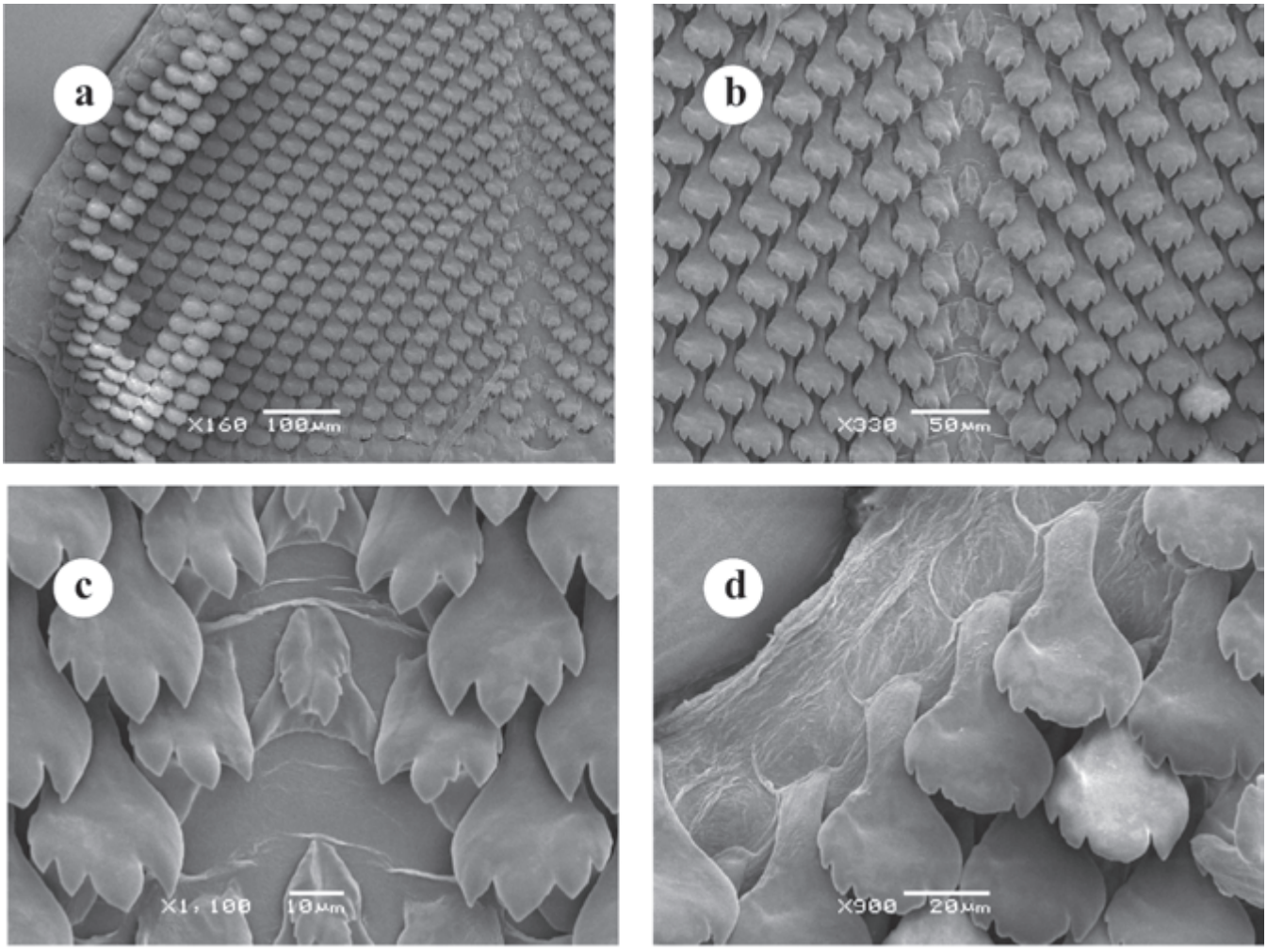

Figure 6 Radula of *Chilina gallardoi*. a general view of anterior part; b central view; c central tooth and first lateral teeth; d marginal teeth; e central tooth. Scale bars: **a** 100µm; **b** 50µm; **c** and **e** 10µm; **d** 20µm.

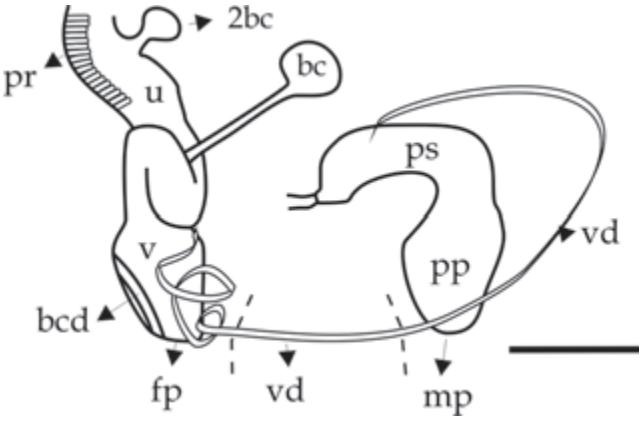

Figure 7 Part of diagram of reproductive system of *Chilina gallardoi*: vd: vas deferent; bc: bursa copulatrix; bcd: bursa copulatrix duct; fp: female pore; mp: male pore; pp: prepuce; pr: prostate; ps: penis sheath; u: uterus; v: vagina; 2bc: secondary bursa copulatrix. Dotted line: wal of the body. Scale bar: 2 cm.

Table 4 Results of PCA based on characters of the shell among the four studied populations. Abbreviations see Table 1

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigen valor	4.263	0.496	0.165	0.046	0.029
% variance	85,266	9,913	3,308	0,923	0,589
LWL	**0.475**	0.021	**–0.314**	–0.172	**0.803**
AL	**0.472**	0.044	**–0.376**	**–0.575**	**–0.551**
TW	**0.472**	–0.078	**–0.300**	**0.794**	–0.225
AW	**0.403**	**0.714**	**0.571**	0.038	–0.026
AP	**0.406**	**–0.694**	**0.587**	–0.092	–0.012

samples. The PCA explains 95.1% of variance in the first two components (Table 4, Fig. 8). In the first principal component (PC 1) all the measures

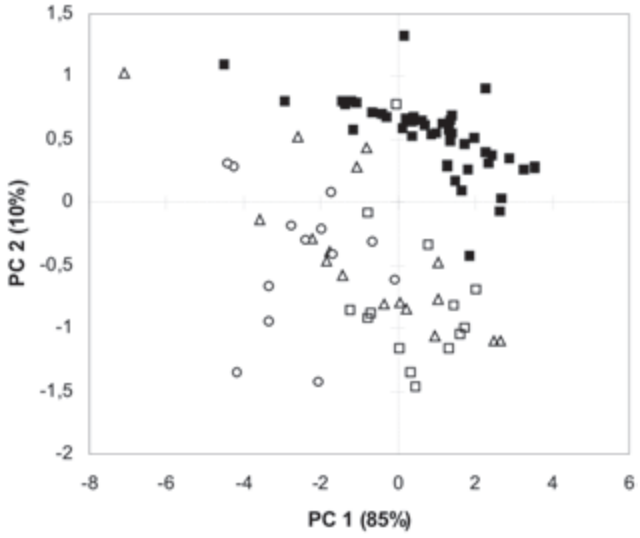

Figure 8 Results of Principal Components Analysis (PC 1 vs PC 2) based on characters of the shell among the four studied populations. Full squares: *Chilina gallardoi* from San Javier city. Empty symbols *Chilina rushii* (squares: from Gualeguaychú; circles: from Martín García island; triangles: from Fray Bentos).

contribute positively in similar proportions and this component provides information about shell size. In the second component (PC 2) comparing apertural shapes (apertural width and degree of reflection) differences are seen between the species. The third component, comparing the measures mentioned in PC 2 plus the rest of the measurements, also shows differences. Fourth and fifth components are more related to the shape of the shell. Morphological characters of the shell can separate all populations of *Chilina gallardoi* from three populations of *C. rushii* (Fig. 8).

Significant differences were found between the four populations analysed (Table 5) for components 1 to 4. A Tukey test showed the first component (related to the shell size) showed a relationship between the San Javier population of *Chilina gallardoi* and the Gualeguaychú population of *C. rushii*. The latter has the largest shells in the study. The Martín García population of *C. rushii*, by contrast, is separated from the rest because of its small shell size. Significant differences were also found using the second component, related to size and reflection of the aperture, between populations of the two species. There were no differences between the two species using components three and four.

Conclusion and Discussion

Chilina gallardoi shells sometimes show a keel on the last whorl, and the most significant difference between the species relate to ratio of length of the aperture and length of the last whorl. In *C. rushii* that ratio is lower than in *C. gallardoi* (0.77 vs 0.82). Using PCA analysis, differences about the degree of reflection and width of the aperture were found among these species.

Chilina gallardoi and *C. rushii* typically possess keels. The closest species morphologically is *C. fluminea*, common in the Río de la Plata river, but this species lacks a keel, and its size is usually smaller. *Chilina fluminea* reaches the city of Gualeguaychú (Entre Ríos province), although at lower densities than those recorded for *C. rushii* (Gutiérrez Gregoric, 2008). Furthermore, *C. iguazuensis* and *C. megastoma* are very different from the taxa studied here, lacking a keel, having globular shells with little development of the spire (it is intorted in adults of *C. iguazuensis*) and only very faint zigzag bands, if present

Table 5 ANOVA test on principal component análisis scores from the four populations. M.S.: mean square. $F_{3,\ 88}$ significance test for homogeneity of means (ns: $p > 0.05$ *: $p < 0.05$; **: $p < 0.001$). Populations means (SJ: San Javier; GU: Gualeguaychú; FB: Fray Bentos; MG: Martín García island) are ordered for each component; subsets not significantly different by the Tukey test ($p > 0.05$) are enclosed between parenthes or square brackets.

Principal Component	M.S. between populations	M.S. within populations	$F_{3,\ 88}$	*Tukey test*
PC 1	44.634	2.889	15.451**	(SJ [GU) FB] MG
PC 2	8.607	0.215	39.99**	SJ (GU FB MG)
PC 3	1.377	0.125	11.053**	(SJ [MG) (FB] GU)
PC 4	0.139	0.043	3.195*	(FB [SJ MG) GU]
PC 5	0.031	0.030	1.041 n.s.	

Table 6 Radulae of five species of Chilinidae.

Species	Formula	1er lateral	2do lateral	Last laterals
C. iguazuensis	[51/(3–5)+1/2] 57–65	Tricuspid	Tetracuspid	5 cusps
C. megastoma	[42/(3–4) + 1/2] 49	Tricuspid	Tricuspid	4 cusps
C. fluminea	[32(4–7) + 1(3)] 49	Tri/Tetracuspid	Tetracuspid	5–7 cusps
C. rushii	[35/(3–7) + 1/2] 48	Tricuspid	Tetracuspid	5–7 cusps
C. gallardoi	[44/(3–5) + 1/2] 58	Tricuspid	Tetracuspid	4–5 cusps

at all. *Chilina fluminea, C. rushii* and *C. gallardoi* have two well-developed teeth on the inner lip, but in *C. megastoma* these teeth are small and in *C. iguazuensis* only one is present and this is poorly developed.

Organization of the radular teeth is fairly typical for the family as a whole with oblique rows of teeth joining in the middle at an angle of about 90 (Castellanos & Gaillard, 1981; Ituarte, 1997; Gutiérrez Gregoric & Rumi, 2008). All of the teeth, except for the central tooth, have two bases that join the tooth to the radular membrane, one going to the back and the other to the head of the tooth, meeting together at the bases of the cusps, as described for *C. iguazuensis* (Gutiérrez Gregoric & Rumi, 2008). The only studied radula of *C. gallardoi* showed more rows (58) than radulae of *C. rushii* with 48. The first lateral is tricuspid in *C. gallardoi* (tetracuspid in *C. rushii*) and the last teeth have up to five cusps (up to seven in *C. rushii*). On average, in *C. gallardoi* there are nine teeth more than in *C. rushii* within each half row (44 vs 35). The radular formulae of both species and of *C. iguazuensis*, *C. megastoma* and *C. fluminea*, are shown in Table 6.

The female genital systems of the two species are very similar to that of *C. fluminea,* but in both the spermathecal duct is located laterally to the intestine, whereas in *C. fluminea* it is located above the vagina. The secondary bursa copulatrix of both species shows a pear-shaped enlargement which in *C. iguazuensis* has the same width along its length, and in *C. megastoma* widening only towards the end.

In the male genital system the route of the vas deferens within the body wall is similar in both species unlike that of *C. megastoma* and *C. iguazuensis* where the vas deferens runs along a contoured shape. However *C. gallardoi* differs from *C. fluminea* and *C. rushii* because the vas deferens, when it emerges from the body wall above the penial complex, does not turn back on itself. This layout is similar to that of *C. iguazuensis* and *C. megasoma.*

Regarding the nervous system, all *Chilina* species appear to be based on a similar model. In *C. megastoma* there is a slight swelling between the left pleural ganglion and the subintestinal ganglion (but this does not form a real ganglion). The swelling does not appear in *C. rushii* and *C. gallardoi* nor in *C. iguazuensis.* Other differences between the species relate to the distance between the left pleural and the parietal ganglion (Table 7).

In conclusion, shell measurements relating to aperture size and shell height, distance between nerve ganglia and certain characteristics of the radula, can be used to differentiate species of Chilinidae from the Del Plata basin.

Table 7 Ratio between the longitude of three ganglion and last whorl length in five species of Chilinidae.

	Parietal – Pleural right		Parietal left – subintestinal		Parietal right -visceral	
Species	mm	(% LWL)	mm	(% LWL)	mm	(% LWL)
C. iguazuensis	2.59	13.53	3.57	18.43	4.05	21.11
C. megastoma	1.82	15.3	2.77	23.22	3.4	28.9
C. fluminea	1.91	18.82	1.95	19.23	1.78	17.52
C. rushii	1.2	12.56	1.74	18.24	1.93	20.15
C. gallardoi	1.5	12	2.1	16.8	2.45	19.6

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