

## The genus *Potamolithus* Pilsbry, 1896 (Gastropoda: Tateidae) on the Somuncurá Plateau, Patagonia, Argentina

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### ABSTRACT

The genus *Potamolithus* (Gastropoda: Tateidae) is endemic to South America where it is distributed mainly in the Ribeira, Itajaí-açú and Jacuhy Rivers in southern Brazil and the Uruguay River, part of the Paraná and Río de la Plata drainage systems. An exception to the pattern of distribution is *Potamolithus valchetensis* Miquel, 1998, which is endemic to the Valcheta Stream, Somuncurá Plateau, Patagonia, Argentina. Here we provide a review of *Potamolithus* from this plateau based on new samples and review of material deposited in collections. A new species, *Potamolithus elenae*, is named. The shell, head-foot, penis, pallial organs and radula are described. Differences between the new species and *P. valchetensis* include details in the shell, radula, body pigmentation and penis. A phylogeny using mitochondrial gene cytochrome c oxidase subunit I is provided which shows that *Potamolithus* is related to species in New Caledonia (Australasia), suggesting that the Tateidae has a Gondwanan distribution.

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### Introduction

At the southern end of South America freshwater gastropods are mostly represented by two families, Chiliniidae (with about 50 species) and Tateidae (38 species) (Pons Da Silva 2003; Valdovinos Zarges 2006; Gutiérrez Gregoric and de Lucía 2016). The latter family is represented by the endemic genus *Potamolithus* Pilsbry, 1896 (Gastropoda: Tateidae) mainly present in the rivers of the Del Plata basin, Parana, Uruguay and Río de la Plata rivers in southern Brazil, Uruguay, Paraguay and Argentina (López Armengol 1985). Studies of species of the genus *Potamolithus* have been neglected despite their presence in the Uruguay and Río de la Plata rivers being largely responsible for these areas being considered biodiversity hotspots for freshwater gastropods (Strong et al. 2008). Most of the species of *Potamolithus* are known only from their shells. The few which have been studied anatomically are: *P. agapetus* Pilsbry, 1911; *P. buschii* (Frauenfeld, 1865); *P. catharinae* Pilsbry, 1911; *P. karsticus* Simone & Moracchioli, 1994; *P. lapidum* (d'Orbigny, 1835); *P. paranensis* (Pilsbry, 1911); *P. ribeirensis* Pilsbry, 1911; *P. rushii* Pilsbry, 1896; *P. simplex* (Pilsbry, 1911); *P. troglobius* Simone & Moracchioli, 1994 and *P. valchetensis* Miquel, 1998 (Pilsbry 1911; Davis and Pons Da Silva 1984; López Armengol 1985, 1996; Simone and Moracchioli 1994; Miquel 1998; Pons Da Silva and Veitenheimer-Mendes 2004; Núñez 2016).

Concerning their distribution, only two species have been mentioned outside the Del Plata basin:

*Potamolithus australis* Biese, 1944 in southern Chile, which is considered a dubious species as the description is based only on the holotype (shell and operculum) which was found in a lentic environment (López Armengol 1985); and *P. valchetensis*, which is endemic to the Valcheta Stream of Somuncurá Plateau, Río Negro province, Patagonia, Argentina. The latter species was based on samples recovered from the stomach of *Gymnocharacinus bergi* Steindachner, 1903 (Pisces: Characidae), and has not been subsequently collected. The description of *P. valchetensis* is based on shell and radular characters and a brief account of some pallial organs.

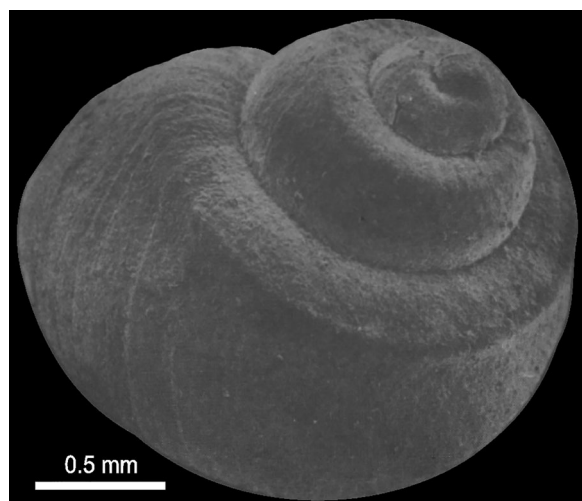
Somuncurá Plateau is a basaltic plain with an approximate area of 29,000 km<sup>2</sup>, and is located in the central region of the provinces of Río Negro and Chubut (Muzón et al. 2005). On the surface there are numerous aquatic environments (streams and lakes) some permanent and others temporary, only present following rainfall. In the province of Río Negro this area is a Provincial Reserve, mainly protecting the Valcheta Stream. This stream is an internal drainage basin formed after the union of two branches, known as 'Cold' and 'Hot', because the temperature of its waters ranges from 21 °C to 22 °C and 22 °C to 26 °C, respectively (Wegrzyn et al. 1992). The Valcheta Stream follows a SO–NE direction, flowing through the villages of Valcheta and Chanquín, with a total length of c. 75 km culminating in the low Valcheta forming the Curicó Lagoon, which is an internal

drainage system. This area is of great importance due to the existence of endemic species of vertebrates such as: *Gymnocharacinus bergii*, *Pleurodema somuncurensis* (Cei, 1969); *Atelognathus reverberii* (Cei, 1969); *Lagidium viscacia somuncurensis* (Crespo, 1963); *Phymaturus somuncurensis* (Cei & Castro, 1973); *Liolaemus rui-zleali* (Donoso Barros & Cei, 1971); *L. elongatus petrophilus* (Donoso Barros & Cei, 1971); and the caenogastropods *Heleobia rionegrina* (Gaillard, 1974) and *Potamolithus valchetensis* (Cei 1969; Bettinelli and Chebez 1986; Ortubay and Cussac 2000; Rumi et al. 2008).

Based on new samples in the basin of the Valcheta Stream, the objective of this work is to comment on the description of *Potamolithus valchetensis* and to describe a new species, *Potamolithus elenae*, based on shell, anatomy, and radula and some molecular data.

## Materials and methods

*Potamolithus valchetensis* type material, deposited in the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (MACN 34011, 34012 and 34013) was examined. Samples from the Valcheta Stream were obtained in 2015 and were deposited in the Malacological Collection at the Museo de La Plata of the La Plata National University, Buenos Aires Province, Argentina (MLP-Ma). These specimens were examined under a Leica MZ6 stereoscopic microscope and drawn with the aid of *camera lucida*. Shell analysis was based on Hershler and Landye (1988) and Núñez (2016). The number of whorls was counted according to Parodiz (1951). The shells were drawn and photographed in apical, umbilical, ventral, lateral and dorsal views in order to make further measurements. All measurements given were taken from the drawings and represented in millimetres. Two specimens were placed



**Figure 1.** *Potamolithus valchetensis*. From Miquel (1998) with permission.

in Railliet-Henry solution (distilled water 93%, acetic acid 2%, formalin 5% and 6 g of sodium chloride per L of solution) (Paraense 1976), to dissolve the shell. Length of the body, head, penis, ctenidium and osphradium were measured, and the colour described, according to Davis et al. (1986), Hershler and Landye (1988) and Hershler and Ponder (1998). We followed Davis et al. (1986) for radular measurements and Hershler and Ponder (1998) for descriptions of the teeth. A radula, two opercula and a shell were photographed using a scanning electron microscope (SEM) (JEOL/JSMT 6360 LV, Tokyo, Japan) at MLP. In addition, DNA was extracted from two specimens and mitochondrial gene cytochrome c oxidase subunit I (COI) was amplified. A partial sequence of COI was amplified by the polymerase chain reaction (PCR) with the universal primers of Folmer et al. (1994). Amplification was performed in a final volume of 50  $\mu$ L, following Gutiérrez Gregoric et al. (2013, 2014). The PCR products were purified with a Highway ADN PuriPrep-GP Kit (INBIO, Tandil City, Argentina) and both DNA strands were then directly cycle-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 in GenBank. The sequence alignment was performed with Clustal X 2.0.12 (Larkin et al. 2007), optimised by visual inspection and edited with a word processor. Phylogenetic analyses were undertaken comparing gene sequences from this study and related sequences in GenBank. *Ascorhis tasmanica* Martens, 1858 was used as the outgroup. The phylogenetic analyses were performed by using maximum likelihood (ML) and Bayesian inference (BI). A phylogenetic tree was constructed using the ML method based on the Tamura-Nei model (Tamura and Nei 1993). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed (Felsenstein 1985). The initial tree for the heuristic search was obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach. The analysis involved 34 nucleotide sequences. There was a total of 639 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). The BI was carried out with the MrBayes 3.2 software (Ronquist et al. 2012). Two runs were performed simultaneously with four Markov chains that went for 1,000,000 generations, sampling every 100 generations.

## Results

### *Potamolithus valchetensis* Miquel, 1998

(Figure 1)

*Potamolithus valchetensis* Miquel 1998: 145–157, figs 2–9.

*Type material and type locality.* Zoilo Chico stream, largest tributary of Valcheta, Río Negro province, Argentina, 1993, coll. S. Ortubay.

### Material examined

*Type material.* Holotype: MACN 34011 (one specimen preserved in ethanol). Paratypes: MACN 34012: Zoilo Chico stream, largest tributary of Valcheta, Río Negro province, 1993, coll. S. Ortubay (two specimens in ethanol) and MACN 34013: Asconape stream, minor tributary of Valcheta, Río Negro province, 1993, coll. S. Ortubay (10 specimens in ethanol). The radula could not be revised as it is not deposited in the collection.

### Diagnosis

Very small, shell trochoid, thick, light brown. Four whorls convex, with strong spiral keel at shoulder in upper part of whorl. Gill filaments triangular, few in number and poorly developed. Osphradium ovoid, small. Penis lacking lobe.

### Description

Total length 2.00–2.53 mm; total width 1.87–2.13 mm; aperture length 1.20–1.47 mm; aperture width 0.80–1.07 mm. Shell trochoid, thick, whorls convex; periostracum light brown; with 3.50 to 4.25 whorls. Last whorl 80% of total length, narrow shoulder with strong keel. Spire low, with blunt apex. Sculptured with distinct axial ribs and growth striations. Sutures deeply impressed. Aperture oval, with continuous peristome. Outer lip thin, not reflected, with moderate basal expansion; columella thick, curved, dark brown and excavated with deep furrow. Umbilicus absent. Operculum corneous, paucispiral. Cephalic tentacles digitiform. Gill with few (number not specified and could not be determined from type material) small, triangular filaments, osphradium small. Penis long, cylindrical, with sharp end, surrounded by preputial ring, attached to the left of the midline of the neck. Females with nuchal node. Radula with central tooth having 9 (4–1–4) upper and 3 basal cusps on each side; lateral tooth with 9 (4–1–4) cusps; inner marginal teeth with 25 cusps and outer marginal with 30 cusps.

### Remarks

The material deposited in the MACN, including the type material, was originally preserved in formaldehyde and then transferred to ethanol. Unfortunately, the shells were decalcified, the radula was not available for examination and the body was in poor condition. Thus it was not possible to obtain new data to expand the original description. The length and width of the median cusp on the central tooth (longitude 4.37 µm, width 1.87 µm) were obtained using the published figures of the radula in the original work. The penis is attached

to the left of the midline of the neck, similar to the New Caledonian tateid genera *Hemistomia* Crosse, 1872 and *Leiorhagium* Haase & Bouchet, 1998 (Haase and Zielske 2015).

*Potamolithus elenae* de Lucía & Gutiérrez Gregoric n. sp.

(Figures 2–4)

*Type material and type locality.* Thermal spring at Chipauquil, Valcheta Stream, Río Negro Province, Argentina, (40°59'26.59" S, 66°40'44.33" W), coll. M. Velasco, 2015.

*Holotype.* MLP-Ma 14202.

*Paratypes.* MLP-Ma 14191, same data (three dry specimens—two radulae and one shell, two specimens in Railliet-Henry solution and one in ethanol).

### Diagnosis

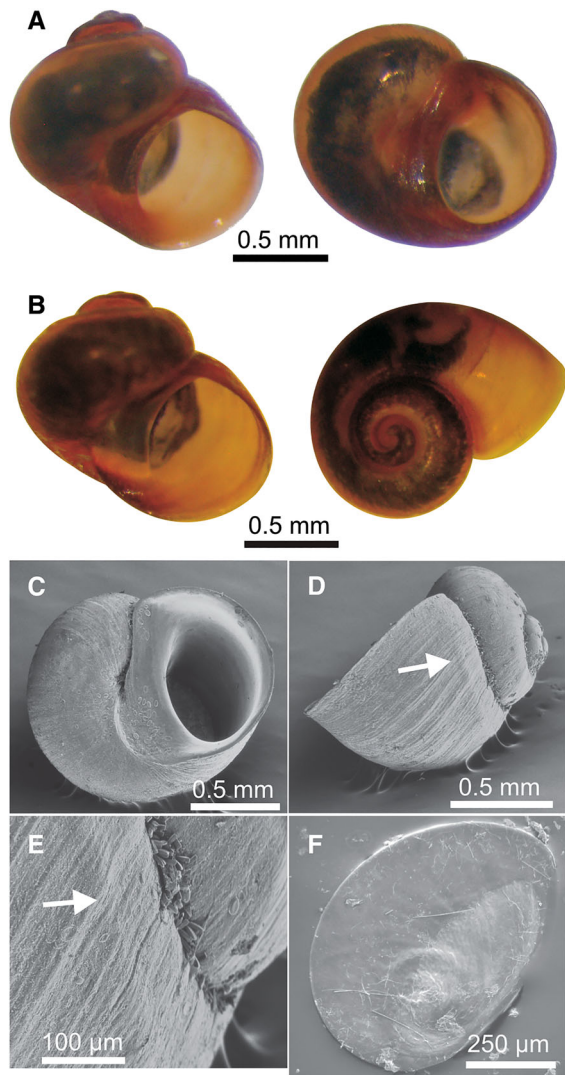
Shell up to 1.2 mm size, trochoid, thin, light brown, with weak spiral keel on shoulder, visible only on last whorl; 5–8 gill filaments; mid-ventral lobe on penis.

### Description

Shell trochoid, thin, light brown, very small, wider than long (length 0.89–1.18 mm, width 0.92–1.39 mm), with 3.09 to 3.61 whorls (Figure 2A–E; Table 1). Last whorl large (91% of total length), with weak spiral keel bordering narrow shoulder; spire low (9% of total length), with blunt apex. Sculpture of rather weak axial ribs. Suture moderately impressed. Aperture oval, large (69% of total length), with continuous peristome; outer lip simple. Columella thick, flat, light brown, width 0.21–0.29 mm. Aperture prosocline, inclined 29°. Operculum corneous, light brown, elliptical, spiral, with submarginal nucleus, paucispiral, fills aperture when retracted, about 3 whorls (Figure 2F).

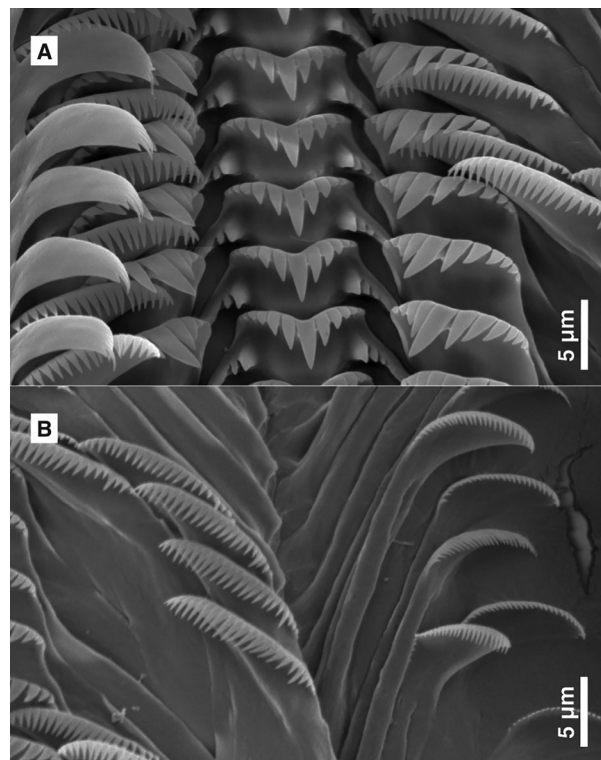
Radula of medium size (33% of total shell length), length 0.375 mm, width 0.05 mm, total rows teeth 80, 4 rows teeth forming. Central tooth trapezoidal, dorsally concave, basal cusps arise from tooth face and larger than those on cutting edge. Width of base of central tooth 0.013 mm, top width of central tooth 0.009 mm. Central tooth with median cusp well-developed (length 3.45 µm, width 1.25 µm), pointed, 2/3 longer than other lateral cusps (Figure 3A). Lateral teeth with main cusp well-developed (length 5 µm, width 1.56 µm), pointed. Marginal teeth long, slightly curved, with pointed ends. Cusps on inner marginal teeth longer and wider than those on outer marginals (Figure 3B). Radular formula: central teeth (5–6)–1–(5–6), 2 left and 3 right basal cusps; lateral teeth with 10 to 11 cusps; inner marginal teeth with 20 to 25 cusps; outer marginal teeth with 25 to 29 (Table 2).

Head-foot with heavily pigmented head and snout, bluish-black, except on lips; snout wider than long



**Figure 2.** *Potamolithus elenae* n. sp. **A**, Holotype MLP-Ma 14202; **B–E**, Paratypes MLP-Ma 14191; **F**, operculum. The arrow indicates the keel at the edge of the shoulder.

with strong distal lobes (Figure 4A–B). Tentacles with unpigmented band distal to eyes, darkly pigmented with unpigmented distal ends. Above eyes pigmented



**Figure 3.** Radula of *Potamolithus elenae* n. sp. (MLP-Ma 14191 specimen 1). **A**, Detail of central, lateral and inner marginal teeth; **B**, inner and outer marginal teeth.

but below eyes small unpigmented area. Eye lobes weakly developed. Mantle roof and visceral coil bluish-black. Mantle cavity with reduced ctenidium comprised of about 5 to 8 filaments, and occupies 21% of visceral mass length. Osphradium oval, opposite posterior part of ctenidium, occupies 32% of ctenidium length. Anus near the edge of mantle on right side (Figure 4C). Penis occupies 21% of visceral mass length, with pigmentation in the mid-dorsal region, lobe in mid-ventral region and terminal papilla surrounded by the preputial ring (Figure 4D–E); distal end blunt. Distance between base of penis and eye

**Table 1.** Range (R), mean and standard deviation (SD) of shell measurements (mm) and ratios of *Potamolithus elenae*.

	Holotype	Paratypes				Total (Holotype + Paratypes)		
		No. of snails analysed	R	Mean	SD	R	Mean	SD
NW	3.35	6	3.09–3.61	3.34	0.21	3.09–3.61	3.34	0.19
TL	1.18	6	0.89–1.18	1.08	0.11	0.89–1.18	1.09	0.11
LS	0.13	6	0.08–0.13	0.10	0.02	0.08–0.13	0.10	0.02
LBW	1.05	6	0.79–1.10	0.98	0.12	0.79–1.10	0.99	0.11
SB	0.39	6	0.32–0.4	0.35	0.03	0.32–0.40	0.36	0.03
TW	1.16	6	0.92–1.39	1.18	0.15	0.92–1.39	1.18	0.14
MWBW	1.05	6	0.84–1.29	1.12	0.16	0.84–1.29	1.11	0.15
LA	0.79	5	0.71–0.87	0.77	0.06	0.71–0.87	0.77	0.06
WA	0.58	5	0.55–0.63	0.58	0.03	0.55–0.63	0.58	0.03
CW	0.18	5	0.21–0.29	0.24	0.03	0.21–0.29	0.23	0.04
IA	34	2	20–34	27.00	9.90	20–34	29.33	8.08
LS/TL	0.11	6	0.07–0.12	0.09	0.02	0.07–0.12	0.09	0.02
SB/LS	3.00	6	2.92–4.25	3.80	0.53	2.92–4.25	3.69	0.57
SB/TW	0.33	6	0.27–0.45	0.33	0.07	0.27–0.45	0.33	0.06
TL/TW	1.02	6	0.85–0.98	0.91	0.05	0.58–1.02	0.93	0.06
LA/TL	0.67	5	0.63–0.74	0.69	0.04	0.63–0.74	0.69	0.04
LBW/TL	0.89	6	0.88–0.93	0.91	0.02	0.88–0.93	0.91	0.02

SB—spire base; CW—columella width; IA—inclination of the aperture; LA—length of aperture; LBW—length of body whorl; LS—length of spire; MWBW—medium width of body whorl; NW—no. of whorls; SB—spire base; TL—total length; TW—total width; WA—width of aperture.

**Table 2.** Radular formula of one radula of *Potamolithus elenae*.

Tooth	No. of teeth analysed	Formula (%)
Central	14	<u>5-1-5</u> (57.14); <u>6-1-5</u> (28.57); <u>6-1-6</u> (14.30) 2-3 2-3 2-3
Lateral		
Left	4	5-1-4
Right	12	4-1-5 (66.67); 4-1-6 (33.33)
Inner	14	20-25
marginal		
Outer	6	25-29
marginal		

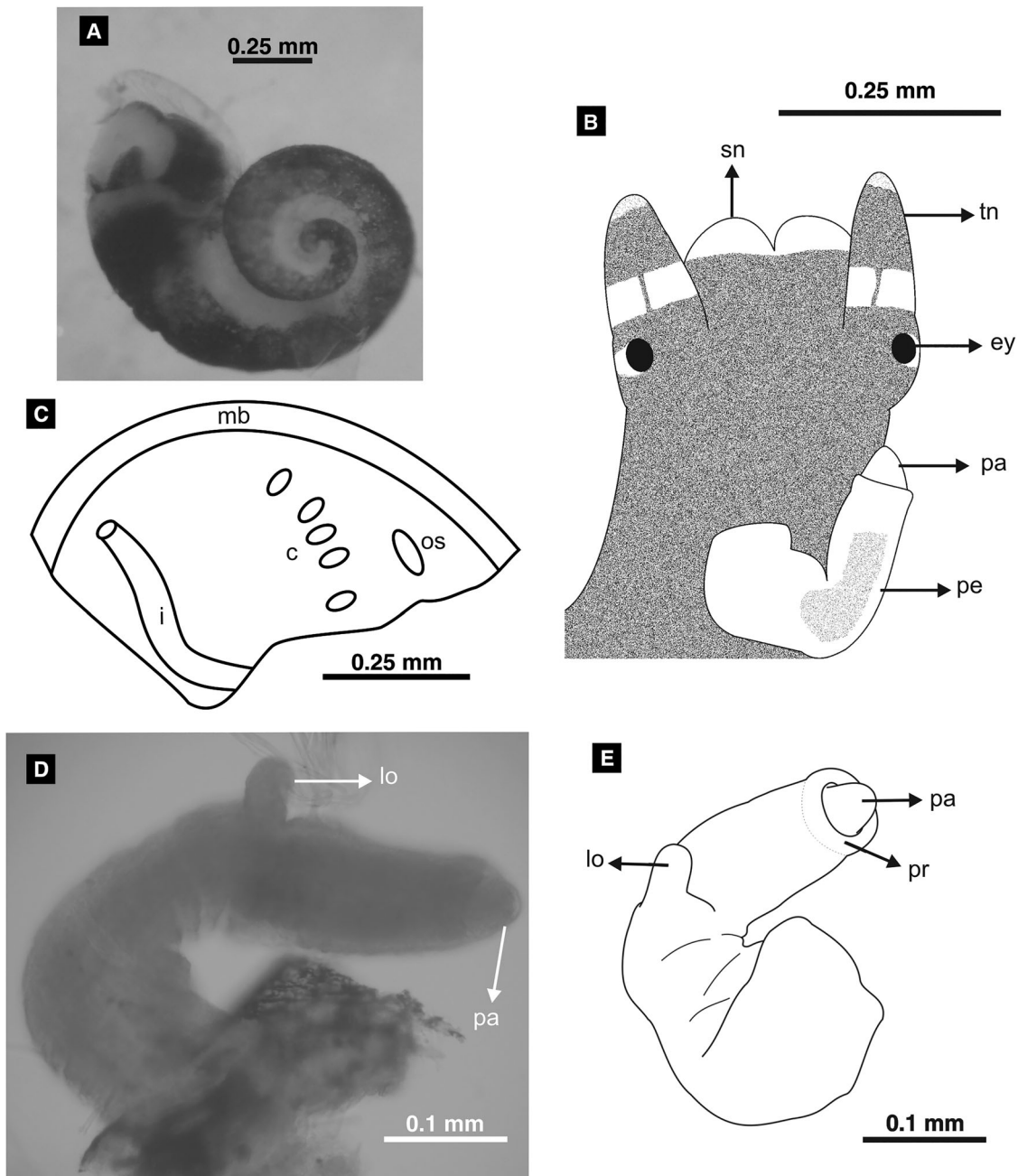
lobes 0.21 mm. Angle of base of penis (with respect to mid-line of neck) 60° (see Table 3 for measurements).

#### Genetic information

Both sequences of COI had a length of 659 bp for paratypes (MLP-Ma 14191, specimens 1 and 2) were deposited in GenBank (Nos. KX397599 and KX397600).

#### Distribution (Figure 5)

Only known from the type locality.



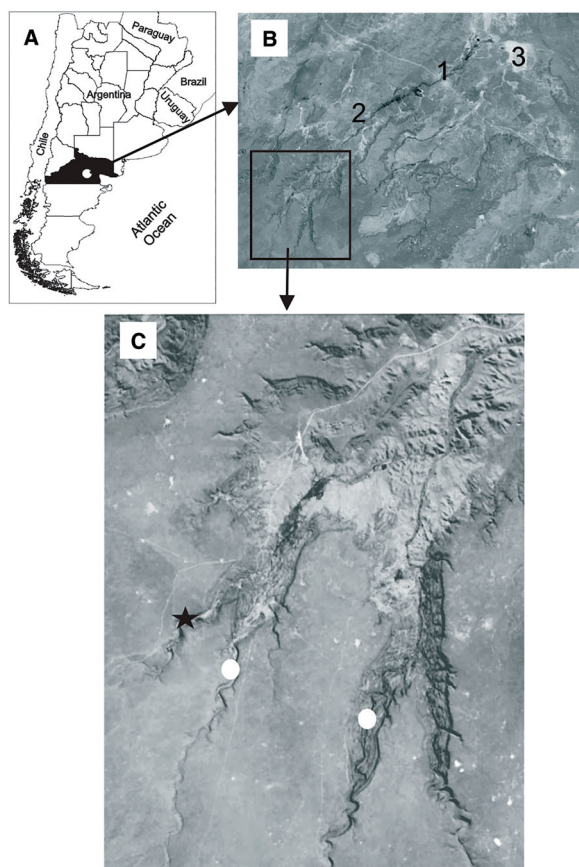
**Figure 4.** *Potamolithus elenae* n. sp. **A**, Pigmentation of the body; **B**, pigmentation pattern of the head and location of the penis; **C**, schematic diagram of mantle cavity structures; **D**, penis detail in ventral view; **E**, schematic diagram of penis detail in right ventral-lateral view. c—ctenidium; ey—eye; i—intestine; lo—lobe mb—mantle border; os—osphradium; pa—terminal papilla; pe—penis; pr—preputial ring; sn—snout; tn—tentacle.

**Table 3.** Range (R), mean and standard deviation (SD) of organs of the mantle cavity and penis measurements (mm) of *Potamolithus elenae* ( $n = 2$ ).

	R	Mean	SD
Ctenidium length (CL)	0.33–0.50	0.42	0.12
Ctenidium width	0.04–0.07	0.07	0.06
Osphradium length (OL)	0.09–0.18	0.14	0.06
Osphradium width	0.03–0.10	0.07	0.05
Width of mantle border (WMB)	0.03–0.04	0.04	0.01
Gill filament No.	5.00–8.00	6.50	2.12
Mantle cavity length (MCL)	0.34–0.57	0.46	0.16
Visceral mass length (VML)	2.39–2.55	2.47	0.11
WMB/MCL (%)	4.56–11.76	8.16	5.09
OL/CL (%)	30.00–32.73	31.37	1.93
CL/VML (%)	12.94–20.92	16.93	5.64
Penis length (PL)	0.50–0.53	0.52	0.02
Medium width of penis	0.10–0.11	0.10	0.004
Width of the terminal end of penis	0.05–0.09	0.07	0.028
Width of the base of penis	0.18–0.23	0.21	0.04
Maximum width of penis (MWP)	0.18–0.23	0.21	0.04
Papilla length (PaL)	0.079	0.08	0.00
MWP/PL (%)	36.00–43.39	39.70	5.23
PL/VML (%)	20.78–20.92	20.85	0.10
PaL/PL (%)	14.90–15.80	15.35	0.64

**Remarks**

This species is smaller in size than *P. valchetensis*, and are mature adults given the well-developed penis

**Figure 5.** Map showing the type locality of *Potamolithus elenae* n. sp. and the locality of *Potamolithus valchetensis*. **A**, Map of southern South America; **B**, Valcheta Stream basin; **C**, collection sites. **1**, Valcheta city; **2**, Valcheta Stream; **3**, Curicó lagoon. ★—*Potamolithus elenae*; ○—*Potamolithus valchetensis*.**Table 4.** Taxon, collection locality data, reference and GenBank Accession Numbers for specimens analysed in this study.

Taxon	Locality	Reference	GenBank
<i>Ascorhis tasmanica</i> (Martens, 1858)	Australia	Hershler et al. (1999)	AF129329
<i>Beddomeia krybetes</i>	Australia	Zielske et al. (2017)	KT313292
<i>Beddomeia paludinella</i> (Reeve, 1857)	Australia	Wilke et al. (2013)	JX970603
<i>Crosseana fallax</i> (Haase & Bouchet, 1998)	New Caledonia	Zielske and Haase (2015)	KJ490909
<i>Fluviopupa daunivucu</i> Haase, Ponder & Bouchet, 2006	Fiji	Zielske and Haase (2014)	KF939813
<i>Halopyrgus pupoides</i> (Hutton, 1882)	New Zealand	Haase (2005)	AY631118
' <i>Heleobia</i> ' <i>hatcheri</i> (Pilsbry, 1911)	San Juan, Argentina	Koch et al. (2015)	KM220905
<i>Heleobia kuesteri</i> (Strobel, 1874)	Mendoza, Argentina	Koch et al. (2015)	KM220904
<i>Heleobia parchappii</i> (d'Orbigny, 1835)	Mar Chiquita, Argentina	Kroll et al. (2012)	JQ972709
<i>Heleobia piscium</i> (d'Orbigny, 1835)	Buenos Aires, Argentina	Koch et al. (2015)	KM220906
' <i>Heleobia</i> ' sp.	Mendoza, Argentina	Koch et al. (2015)	KM220908
<i>Heleobia umbiculata</i> (Haas, 1955)	Bolivia	Kroll et al. (2012)	JQ973053
<i>Hemistomia andreae</i> Haase & Zielske, 2015	New Caledonia	Zielske and Haase (2015)	KJ490851
<i>Hemistomia eclima</i> Haase & Bouchet, 1998	New Caledonia	Zielske and Haase (2015)	KJ490859
<i>Hemistomia fabrorum</i> Haase & Bouchet, 1998	New Caledonia	Zielske and Haase (2015)	KJ490841
<i>Hemistomia minor</i> Haase & Bouchet, 1998	New Caledonia	Zielske and Haase (2015)	KJ490831
<i>Hemistomia oxychila</i> Haase & Bouchet, 1998	New Caledonia	Zielske and Haase (2015)	KJ490894
<i>Leiorhagium implicatum</i> Haase & Bouchet, 1998	New Caledonia	Zielske and Haase (2015)	KJ490845
<i>Leptopyrgus tainui</i> Haase, 2008	New Zealand	Haase (2005)	AY631078
<i>Meridiopyrgus muaukau</i> Haase, 2008	New Zealand	Haase (2005)	AY631083
<i>Meridiopyrgus murihiku</i> Haase, 2008	New Zealand	Haase (2005)	AY631084
<i>Obtusopyrgus alpinus</i> Haase, 2008	New Zealand	Haase (2005)	AY631089
<i>Opacuincola johannstraussi</i> Haase, 2008	New Zealand	Haase (2005)	AY631094
<i>Opacuincola josefstraussi</i> Haase, 2008	New Zealand	Haase (2005)	AY631096
<i>Opacuincola permutata</i> Haase, 2008	New Zealand	Haase (2005)	AY631100
<i>Potamolithus agapetus</i> Pilsbry, 1911	Buenos Aires, Argentina	Koch et al. (2015)	KM220910
<i>Potamolithus buschii</i> (Frauenfeld, 1865)	Buenos Aires, Argentina	Koch et al. (2015)	KM220909
<i>Potamolithus elenae</i> de Lucía & Gutiérrez Gregoric, 2016	Valcheta Stream, Argentina	This study	KX397599; KX397600
<i>Potamolithus lapidum supersulcatus</i> Pilsbry, 1896	Uruguay River, Argentina.	This study	KX158843
<i>Potamolithus ribeirensis</i> Pilsbry, 1911	Sao Paulo, Brazil	Wilke et al. (2013)	JX970618
<i>Potampopyrgus antipodarum</i> (Gray, 1843)	New Zealand	Neiman et al. (2010)	GQ996429
<i>Tatea huonensis</i> (Tenison-Woods, 1876)	Australia	Colgan & da Costa (*)	FJ619824
<i>Tatea rufflabris</i> (Adams, 1862)	Australia	Golding (*)	KC439802

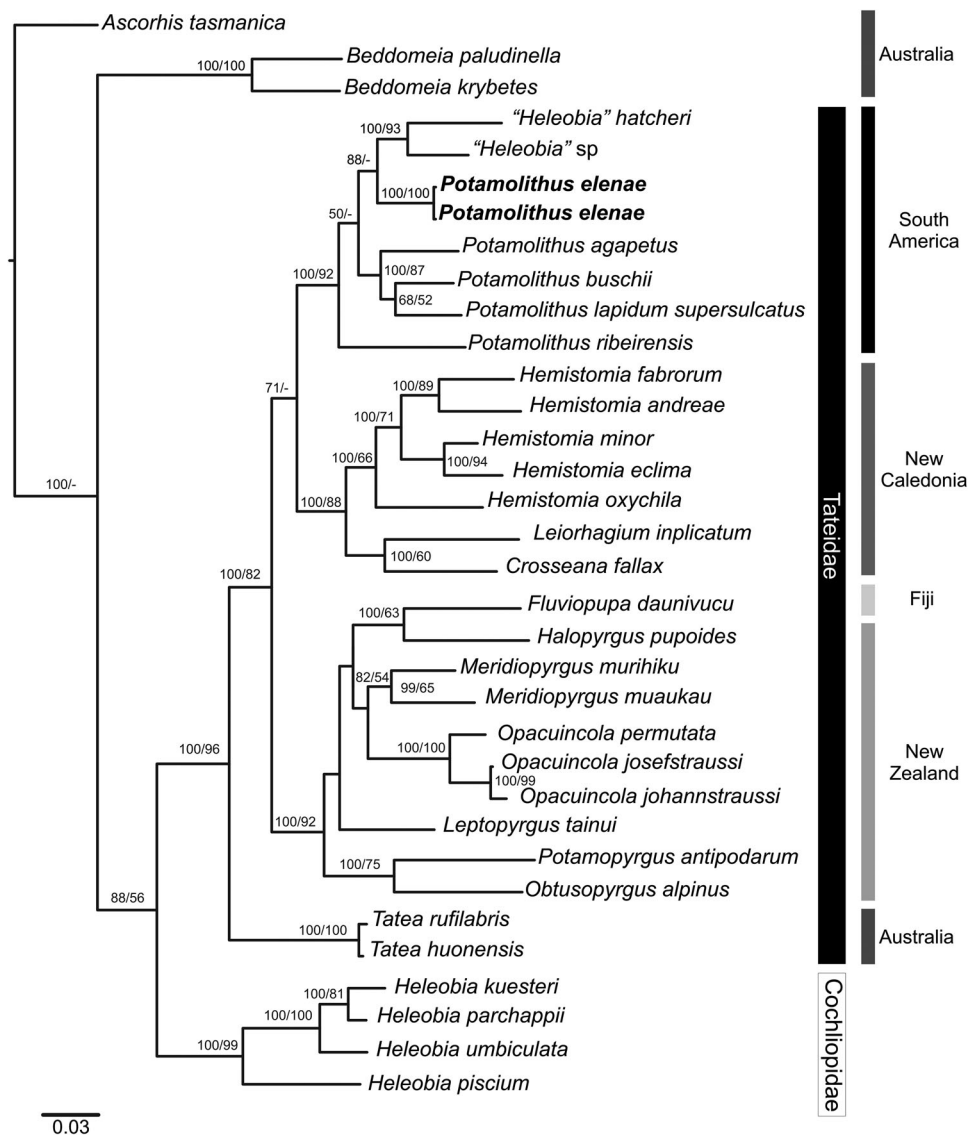
\*Unpublished data.

**Table 5.** Pairwise genetic divergence (Kimura two-parameter, %) among species of *Potamolithus* and two species of '*Heleobia*' assessed by means of COI gene sequences. The new species is highlighted in bold.

	1	2	3	4	5	6
1. ' <i>Heleobia</i> ' <i>hatcheri</i>						
2. ' <i>Heleobia</i> ' sp.	8.53					
<b>3. <i>Potamolithus elenae</i></b>	<b>9.62</b>	<b>7.85</b>				
4. <i>Potamolithus buschii</i>	12.08	10.24	<b>7.34</b>			
5. <i>Potamolithus lapidum supersulcatus</i>	12.26	8.93	<b>8.97</b>	6.81		
6. <i>Potamolithus agapetus</i>	12.91	9.88	<b>9.16</b>	7.91	8.08	
7. <i>Potamolithus ribeirensis</i>	13.77	11.16	<b>10.61</b>	11.72	12.09	11.70

and apertural development. The new species differs from *P. valchetensis* in the following additional characters. Thin shell (vs. thick), fewer number of whorls (3.34 vs. 4), LT/TW ratio smaller (93 vs. 110) and LBW/LT larger (91 vs. 80), shoulder keel weaker; columella flat

without groove (with groove in *P. valchetensis*); the radula has more cusps on the central teeth (12 vs. 9), basal cusps asymmetrical (2–3) vs. symmetrical (3–3), and the median cusp is triangular with a pointed end (vs. parallel edges and blunt end) and is smaller (length 3.45  $\mu\text{m}$  vs. 4.37  $\mu\text{m}$ , width 1.25  $\mu\text{m}$  vs. 1.87  $\mu\text{m}$ ); the lateral teeth have more cusps (10–11 vs. 9) and the main cusp is shaped like an isosceles triangle (vs. parallel edges and blunt end). In the penis of the material examined there is a lobe in the mid-ventral region (not present in *P. valchetensis*), and there is a terminal papilla protruding from the blunt distal end (pointed distal end in *P. valchetensis*). The penis is pigmented in the mid-dorsal region (this feature is not mentioned in *P. valchetensis*). Also, the penis is triangular in appearance, with wide base and attached to the middle of the right neck area while in *P. valchetensis* it is cylindrical, maintaining a uniform thickness from its base to the prepuce ring. No females were observed in the material analysed. Compared to the



**Figure 6.** Bayesian consensus tree based on a 639 pb fragment of the COI gene. Bayesian posterior probabilities and bootstrap values (%) are shown (BI/ML). Values lower than 50% are not shown. The new species is highlighted in bold.

cavernicolous Brazilian species, *P. karsticus* and *P. troglobius*, described by Simone and Moracchioli (1994), which are also of small size, *P. elenae* is smaller (1.03–1.18 mm vs. 1.9–2.5 mm), with a wider shell (vs. shells longer than wide) and has fewer gill filaments (5–8 vs. 10–12). The head is heavily pigmented, while *P. karsticus* has a brown pigmentation with dark spots behind the eyes and around the dorsal sagittal line, and *P. troglobius* is light yellow in colour. In addition, *P. elenae* has well-developed eyes and eye lobes are weakly developed, while *P. karsticus* has eyes without eye lobes and *P. troglobius* has no eyes or eye lobes. *Potamolithus paranensis* (Pilsbry, 1911), redescribed by Núñez (2016), has a small lateral lobe at the junction of the base and the terminal part (2.94% of penis length), while the lobe in *P. elenae* is at 15% of the penis length and is located mid-ventrally. *Potamolithus elenae* has a terminal penial papilla, as do *P. agapetus*, *P. buschii*, *P. lapidum supersulcatus* Pilsbry, 1896, *P. ribeirensis* and *P. valchetensis* (Davis and Pons Da Silva 1984; López Armengol 1996; Miquel 1998; de Lucía, pers. obs.). This terminal papilla is surrounded by a preputial ring as observed in *P. lapidum supersulcatus*, *P. ribeirensis* and *P. valchetensis* (Davis and Pons Da Silva 1984; Miquel 1998; de Lucía, pers. obs.).

### Etymology

Dedicated to the grandmother, Elena, of the first author of this paper.

### Molecular analysis

The length of the COI sequences for *P. elenae* was 659 bp while *P. lapidum supersulcatus* was 658 pb and *P. agapetus*, *P. buschii*, *P. ribeirensis*, '*Heleobia*' *hatcheri* (Pilsbry, 1911) and '*Heleobia*' sp. were all 638 pb (Table 4). There is a gap in both sequences of *P. elenae* in location 629. The genetic distance in the COI gene with the other species found in South America is higher than 6.8% (Table 5). The phylogenetic trees obtained by ML and BI methods show a similar topology differing only in minor details (Figure 6). *Potamolithus elenae* is more closely related to '*Heleobia*' sp. and '*Heleobia*' *hatcheri*, both present in south central Argentina. On the other hand, the species in the Del Plata basin constitute another clade within the genus *Potamolithus*. *Potamolithus ribeirensis* from the Iporanga River basin, Brazil, is basal in *Potamolithus* in our analysis. Of the taxa in the analysis, the South American tateid clade is more closely related to the New Caledonian genera than those found in Fiji and New Zealand, and even less related to the few Australian taxa included.

### Discussion

Phylogenetic analyses confirm that the South American taxa included are members of the Tateidae, namely the

species of *Potamolithus* and two species attributed to the cochliopid genus *Heleobia* Stimpson, 1865 ('*Heleobia*' sp. and '*Heleobia*' *hatcheri*), already mentioned by Koch et al. (2015). '*Heleobia*' *hatcheri* is a parthenogenetic species and has similarities in radular morphology (two or three pairs of basal cusps in the central tooth) as in species of *Potamolithus* and which are absent in typical species of *Heleobia* (Cochliopidae) in Argentina (Cazzaniga 2011; Koch et al. 2015). '*Heleobia*' sp. and '*Heleobia*' *hatcheri* are known only from females, but in our study of *Potamolithus elenae* we did not find females for comparison. The two taxa previously attributed to *Heleobia* clearly do not belong to Cochliopidae, and the generic status of these species will be discussed elsewhere (E. Koch, pers. comm., 2016). The Cochliopidae family is a well-defined clade being quite distinct from Tateidae (Wilke et al. 2013; Koch et al. 2015). Reproductive characters (morphology of the penis, prostate gland, as well as female characters) are considered of importance in defining tateid genera, but due to the small size of the samples, and because the specimens were not relaxed prior to fixation, it was not possible to make a more detailed anatomical study of the reproductive system.

The Valcheta Stream has distinctive habitat and faunistic characteristics compared to other Patagonian basins, so it was excluded from the malacological provinces of North and South Patagonia defined by Núñez et al. (2010). This new species, *Potamolithus elenae*, enhances the conservation status of the Somuncurá Plateau, now a reserve (Protected Natural Area, Somuncurá Plateau). However, the level of protection is insufficient, mainly due to overgrazing, poaching, removal of individuals, modification of waterways, water pollution and introduced species (Muzón et al. 2005; Anonymous 2007).

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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