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Systematic evaluation of cryptic freshwater snails from central Chile, including the enigmatic *Littoridina santiagensis* (Gastropoda, Truncatelloidea)

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Abstract. Walter Biese described *Littoridina santiagensis* Biese, 1944 (Cochliopidae) from Estero Dehesa in 1944 based exclusively on external shell features and a second allopatric population in Yeso Spring three years later. Since 2011 different samplings have been carried out at the type locality and have only provided specimens of the morphologically similar invasive mudsnail *Potamopyrgus antipodarum* Gray, 1843 (Tateidae), raising doubts about the identity of the species. The recent finding of two snail morphotypes in Yeso Spring, a thick shelled form congruent with type specimens of *L. santiagensis* and a slender one morphologically associable to *P. antipodarum*, allowed comparative studies, including the taxonomic analysis of additional populations with similar shell morphology occurring in central Chile. A DNA barcoding (COI) approach identified the slender form from Yeso Spring in Maipo Basin and a second population from the contiguous Rapel Basin indeed as the invasive *P. antipodarum*; however, *L. santiagensis* was recovered among species of *Potamolithus* Pilsbry, 1896 (Tateidae), justifying the *Potamolithus santiagensis* (Biese, 1944) comb. nov. Besides recognition of three other populations as belonging to *Potamolithus*, the molecular analysis also suggests trans-Andean dispersal of this group of snails in the Southern Cone of South America.

Keywords. Cryptic species, freshwater snails, Cochliopidae, new combination, Tateidae.

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Introduction

The taxonomic placement of minute Chilean freshwater snails solely described based on morphological characters, such as *Littoridina santiagensis* Biese, 1944, is rather controversial. This species was described in the genus *Littoridina* Souleyet, 1852 by Walter Biese from Estero Dehesa, a small tributary of the Mapocho River located east of Santiago in the Maipo Basin, based on characteristics of the shell and for which only a single drawing is available from the original description (Biese 1944) (Fig. 1). Along with similar forms, Biese related *L. santiagensis* to the *Littoridina hatcheri* Pilsbry, 1911 group, a species subsequently transferred to the genus *Heleobia* Stimpson, 1865 within the family Cochliopidae (Hershler & Thompson 1992), but now recognized as member of the family Tateidae, closely related to the genus *Potamolithus* Pilsbry, 1896 (Koch *et al.* 2015). Shortly after the original publication, Biese (1947) discovered a second allopatric population in a spring tributary of the Yeso River, 55 km south of the type locality, which he assigned to *L. santiagensis* using the same typological criteria. Subsequent finds of the species have not been made, but it was cited by Stuardo (1961) in one of the most important checklists of Chilean freshwater mollusks of the 20th century. Later, Hershler & Thompson (1992), in their influential work on Cochliopinae, placed all the Chilean species of *Littoridina,* including *L. santiagensis,* in the genus *Heleobia*, but without providing arguments for this translocation. This was perhaps one of the reasons why several authors continued to consider these species as members of *Littoridina* (e.g., Sielfeld 2001; Valdovinos 1999, 2006, 2008).

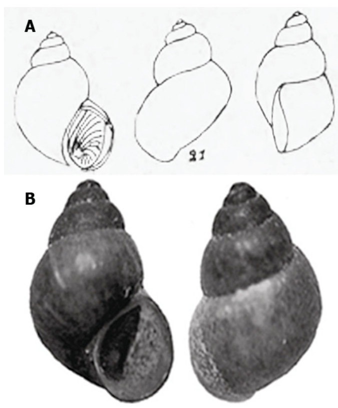

Fig. 1. "*Littoridina*" *santiagensis* Biese, 1944. **A**. The only drawings of a specimen of the species taken from the original description by Biese (1944). **B**. Lectotype housed at the Museo Nacional de Historia Natural, Santiago, Chile (MNHNCL) (after Collado *et al.* 2011).

During a study of Chilean freshwater snails performed by the first author in 2011, specimens of *L. santiagensis* were not found at its type locality but specimens attributable to the species were found in Yeso Spring (Collado *et al.* 2011). Based on anatomical characters of the male copulatory organ, the authors moved the Chilean species of *Littoridina* to the genus *Heleobia*, although in the case of *L. santiagensis* this was not justified because no males were found among the specimens from that locality. More recently, a molecular phylogenetic analysis based on DNA sequences of the mitochondrial gene cytochrome oxidase subunit I (COI) obtained from snails collected from Estero Dehesa identified these specimens as the New Zealand mudsnail *Potamopyrgus antipodarum* (Gray, 1843) in the family Tateidae (Collado 2014), which is morphologically similar to *L. santiagensis*, suggesting either species displacement or an early misidentification of these snails as members of the genus *Littoridina*. The absence of males in snails examined from Yeso Spring (Collado *et al.* 2011) also contributed to raise doubts about the identity of this species, since the genera *Littoridina* and *Heleobia* contain dioecious species (e.g. Hubendick 1955; Gaillard & de Castellanos 1976; Cazzaniga 1980, 1982a, 1982b; Hershler & Thompson 1992; Collado *et al.* 2011; Ovando & De Francesco 2011; Collado 2012, 2015).

New collections at the type locality (Estero Dehesa) of *L. santiagensis* in 2014, 2015 and 2017 have only been successful for *P. antipodarum* despite intensive sampling effort performed in different microhabitats (pers. obs.). On the other hand, two morphotypes were found in Yeso Spring, a slender form conferred to *P. antipodarum* and another thicker one provisionally assigned to *L. santiagensis,* taking into account the previous record by Biese and its morphological similarity regarding the type specimens of this species studied previously (Fig. 1) (Collado *et al.* 2011). Given the new material, in the present study we performed comparative studies between these snails and evaluated their systematic position based on morphological and molecular data, including other morphologically similar snail populations of uncertain taxonomic status found in central Chile.

Material and methods

The snails were collected from Estero Dehesa (33°21′58.49″ S, 70°31′15.70″ W) and Yeso Spring (33°45′29.36″ S, 70°10′00.41″ W), Maipo Basin, Región Metropolitana, Chile. Collections were also performed at three other localities harboring populations with a similar shell morphology, the irrigation canal Lo Carreño (34°36′45″ S, 70°55′47.8″ W) in the Región de O'Higgins, located about 143 km south of Estero Dehesa, two populations from the Región del Maule, Viña Casas del Maule (35°33′04.37″ S, 71°40′12.25″ W), located about 265 km south of Estero Dehesa, and El Colorado, located about 50 km southeast of Viña Casas del Maule. To study the gross morphology, specimens were photographed using a Motic SMZ–168 stereo microscope provided with a Moticam 2000 digital camera and then compared with standard specimens of known taxonomic allocation (Collado 2014, 2016), type specimens or drawings of them (Pilsbry 1911; Biese 1944, 1947). The following measurements were taken on 95 snails: shell length (SL), shell width (SW), aperture length (AL), aperture width (AW), body whorl length (BWL) and spire length (SPL). Shell variables were logarithmically transformed for statistical analyses. Since variables were partly non-normally distributed and had inhomogeneous variances, we performed non-parametric Kruskal-Wallis tests and post-hoc pairwise Mann-Whitney U-tests for comparisons in STATISTICA ver. 7.0 (StatSoft Inc. 2004). A principal component analysis (PCA) was performed to visualize the morphological variation of different populations. Protoconchs, radulae and opercula were isolated and cleaned in a diluted sodium hypochlorite solution and then observed using a Hitachi SU3500 scanning electron microscope (SEM). Voucher specimens were deposited in the Museo de Ciencias Naturales Profesor Pedro Ramírez Fuentes (MCNPPRF 139–11 to 139–21), Chillán, Chile.

DNA extraction and amplification of partial sequences of the COI gene were performed following Collado (2014) using the new pair of primers ATCTGGTTTAGTRGGRACA (forward) and CCTGCYAAAACAGGYAAAG (reverse). The new sequences were deposited in GenBank (MH536524–MH536536). Genetic uncorrected p-distances were estimated in MEGA v.7.0 (Kumar *et al.* 2016). An initial BLASTn-analysis against the data base of GenBank confirmed that the species in question belonged to Tateidae. We also performed a Bayesian tree-based barcoding approach (Erickson & Driskell 2012) to assess the familial/generic allocation of the species analyzed together with representative species of these families retrieved from GenBank (Hershler *et al.* 1999; Haase 2005; Kroll *et al.* 2012; Hamada *et al.* 2013; Wilke *et al.* 2013; Koch *et al.* 2015; Collado *et al.* 2016a; de Lucía & Gutiérrez Gregoric 2017; Colgan & da Costa, unpublished data). As COI was highly saturated across both families as shown by the test of Xia *et al.* (2003), our analysis was not supposed to reflect accurate relationships, but it allowed us to assign the Chilean species to their respective families and genera. We constructed a Bayesian tree using MrBayes ver.3.2.2 (Ronquist *et al.* 2011) based on the HKY + I + Γ substitution model identified as best fitting by jModelTest ver.2.1.4 (Darriba *et al.* 2012) according to all four criteria implemented in this program. MrBayes was run for 2 Mio generations with a burn-in of 25%. In order not to aggravate the saturation problem by introducing an outgroup, the tree was midpoint rooted in FigTree ver.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). For further comparison, we also obtained sequences of two specimens of *Potamolithus* from Puerto Chico, Llanquihue Lake, Región de Los Lagos (41°19′37.06″ S; 72°57′26.64″ W), the type locality of *P. australis* Biese, 1944, the only species of the genus reported from Chile. However, as it has been considered a *nomen dubium* (López Armengol 1985), we treat these snails as *Potamolithus* sp. until a formal redescription may be established.

Results

Species delimitation

The classification of specimens based on external shell features was difficult to perform in the field. Under magnification, two snail morphotypes could be recognized in Yeso Spring and Lo Carreño, a slender form morphologically associable to *P. antipodarum* and a thick-shelled one congruent with the type specimens of *L. santiagensis*, here transferred to *Potamolithus* based on anatomical characters and DNA barcode analysis (see below). The thicker-shelled specimens from El Colorado and Viña Casas del Maule were assigned to *Potamolithus* as well (Fig. 2).

Superfamily Truncatelloidea Gray, 1840
Family Tateidae Thiele, 1925
Genus *Potamolithus* Pilsbry, 1896

Type species: *Paludina lapidum* d'Orbigny, 1835, by subsequent designation.

Potamolithus santiagensis (Biese, 1944) comb. nov.
Figs 1–5

Littoridina santiagensis Biese, 1944: 187–188, fig. 21 (Estero Dehesa, Cerro Manquehue, Barnechea, east to Santiago, Chile, type locality).

Littoridina santiagensis–Stuardo, 1961: 17. —Valdovinos, 1999: 128, 2006: 90. —Sielfeld, 2001: 3.
Heleobia santiagensis (Biese, 1944). Hershler &Thompson, 1992: 55. — Collado *et al.*, 2011: 51, 54–56, fig. 2R–S.

Type material

Lectotype
CHILE • Santiago; 200611, MNHNCL (Collado *et al.* 2011).

Description

SHELL. Ovate-conic, dark brown, relatively thick, with about 5.5 whorls (Fig. 3A–D). Shell length about 3.0 mm (Table 1). Periostracum brown. Protoconch smooth (Fig. 3E), with about 1.3 whorls and 400 µm length (± 10 µm of standard deviation, n = 9). Aperture ovate, slightly angled adapically, lip thickened. Umbilicus imperforate or absent. Operculum ovate, thin, multispiral, light brown-transparent, with eccentric nucleus (Fig. 3F–G); attachment scar occupying almost half of the internal surface (Fig. 3G). Mantle black with a conspicuous gray band on anterior margin, head black, somewhat depigmented in center (Fig. 3H–I). Foot black.

FEMALES. With nuchal node, white lips, tentacles grayish or black (Fig. 3H–I). Some specimens with a white band at base of tentacles where eyes are located. Males were not observed.

RADULA. Taenioglossate (formula 3-1-3), with two marginal teeth and a lateral tooth placed on each side of the central tooth (Fig. 3J–K). Central tooth trapezoidal, dorsally concave, ca. 20 µm wide; basal tongue U-shaped; with 5–6 lateral cusps on each side of median cusp and 3–4 pairs of basal cusps, first basal cusps arise from tooth face and are larger than those on cutting edge. Median cusp of central tooth

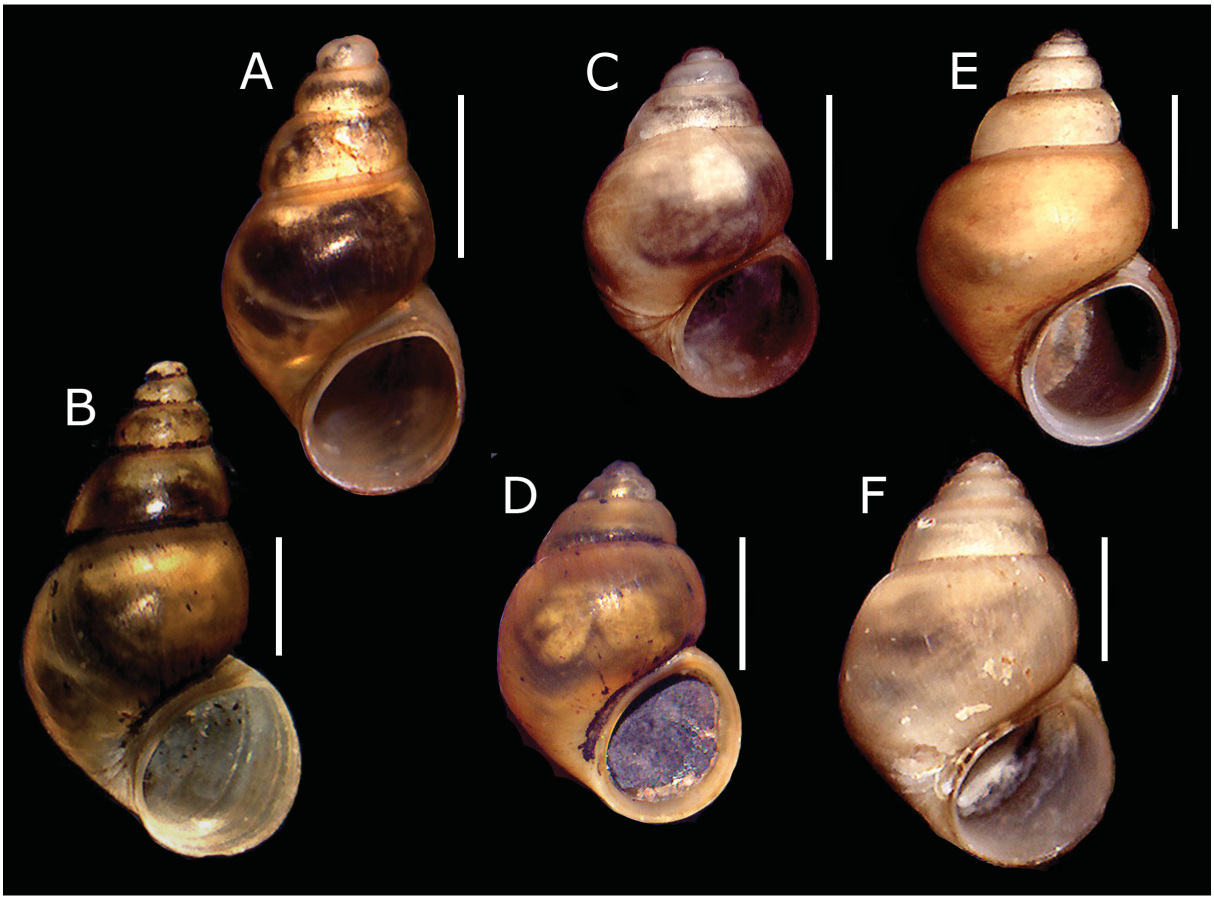

Fig. 2. Shells of truncatelloidean freshwater snails observed in the present study. **A–B**. Slender morphotype from El Yeso Spring (A) and Lo Carreño (B) assigned to *Potamopyrgus antipodarum* (Gray, 1843). **C–E**. Thicker morphotype from El Yeso Spring (C), Lo Carreño (D) and El Colorado (E) assigned to *Potamolithus santiagensis* (Biese, 1944) comb. nov. **F**. Thicker morphotype from Viña Casas del Maule assigned to *Potamolithus* sp. Scale bar = 1 mm.

well-developed and pointed. Lateral tooth with 11 cusps and median cusp well-developed and pointed. Inner marginal teeth with about 30 cusps (Fig. 3K).

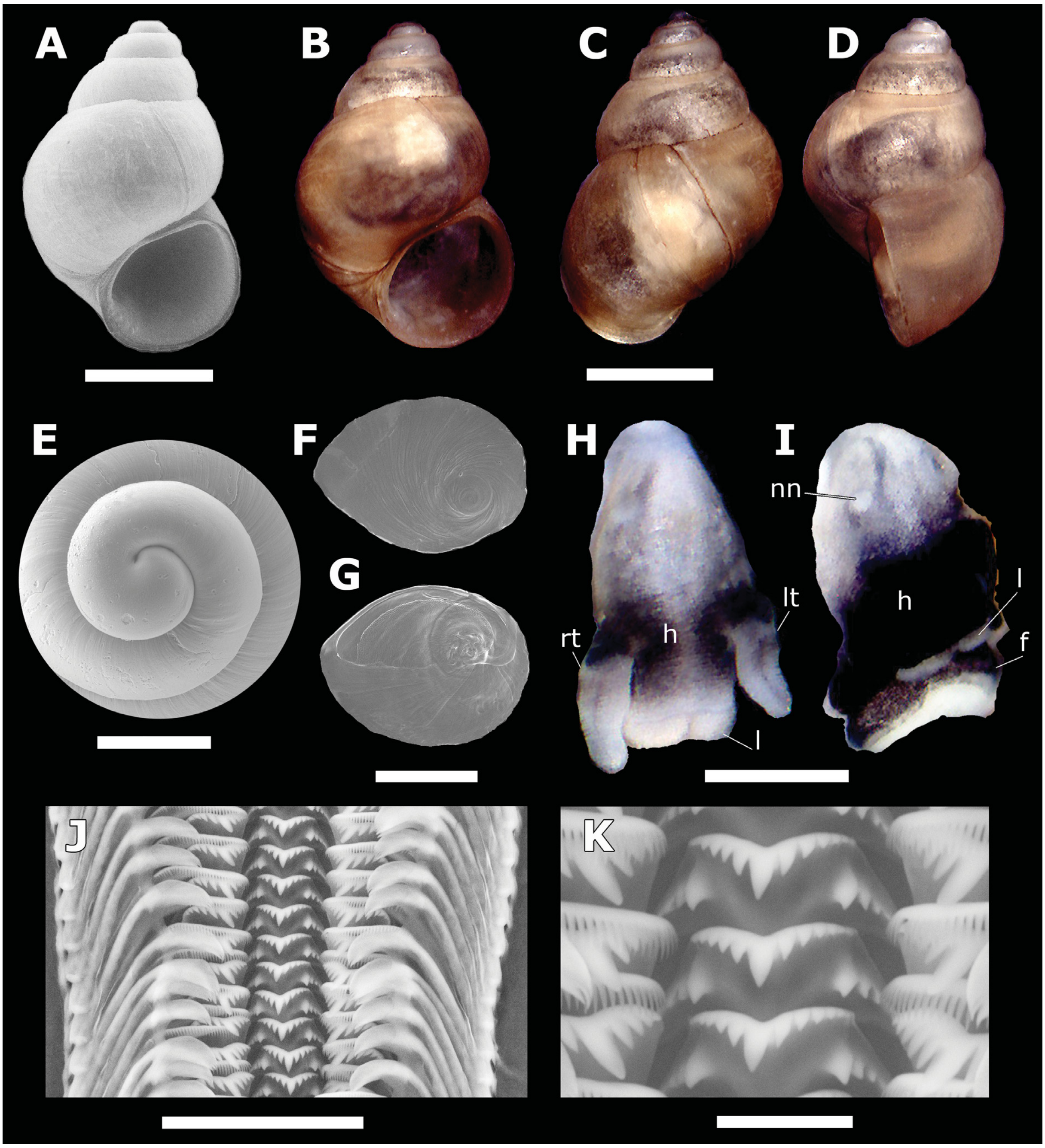

Fig. 3. *Potamolithus santiagensis* (Biese, 1944) comb. nov., Yeso Spring, Chile. **A**. Shell imaged using SEM. **B**–**D**. Shell of the same specimen photographed under a stereo microscope (frontal, dorsal, lateral views). **E**. Protoconch. **F**–**G**. Opercula of two specimens (outer, inner sides, respectively). **H**. Head of a female. **I**. Head of another female having a nuchal node. **J**. Anterior-central section of radular ribbon. **K**. Central teeth. Abbreviations: f = foot; h = head; l = lip; lt = left tentacle; nn = nuchal node; rt = right tentacle. Scale bars: A–D = 1.0 mm; E = 250 µm; F–G = 500 µm; H–I = 0.5 mm; J = 50 µm; K = 10 µm.

Ecology

Potamolithus santiagensis comb. nov. is a herbivorous-detritivorous species that inhabits small water bodies like springs and small streams.

Distribution

Yeso Spring in Cajón del Maipo, Región Metropolitana, Central Chile (Biese 1947; Collado *et al*. 2011; present study). This spring is a small tributary of the Yeso River, which empties in the Maipo River. The species also inhabits small irrigation canals in the Región de O'Higgins (Lo Carreño) and Región del Maule (El Colorado). Snail collections made in 2011, 2014, 2015 and 2017 at the type locality Estero Dehesa have not provided specimens of the species. This ecosystem has been invaded by *P. antipodarum* (Collado 2014) and *Physa* sp. (unpublished data). In Yeso Spring, *P. santiagensis* comb. nov. coexists with *P. antipodarum* and snails of the genus *Chilina* Gray, 1828.

Morphometric analysis

The mean values of the shell variables obtained for the freshwater snails inhabiting central Chile are shown in Table 1. Although the Kruskal-Wallis tests considering the six variables analyzed were statistically significant, only 23 of 60 pairwise post-hoc comparisons among native *Potamolithus* populations and *P. antipodarum* were significant, providing evidence of the difficulties in distinguishing these snails (Table 2). However, in the PCA the *Potamolithus* populations were well separated from *P. antipodarum*

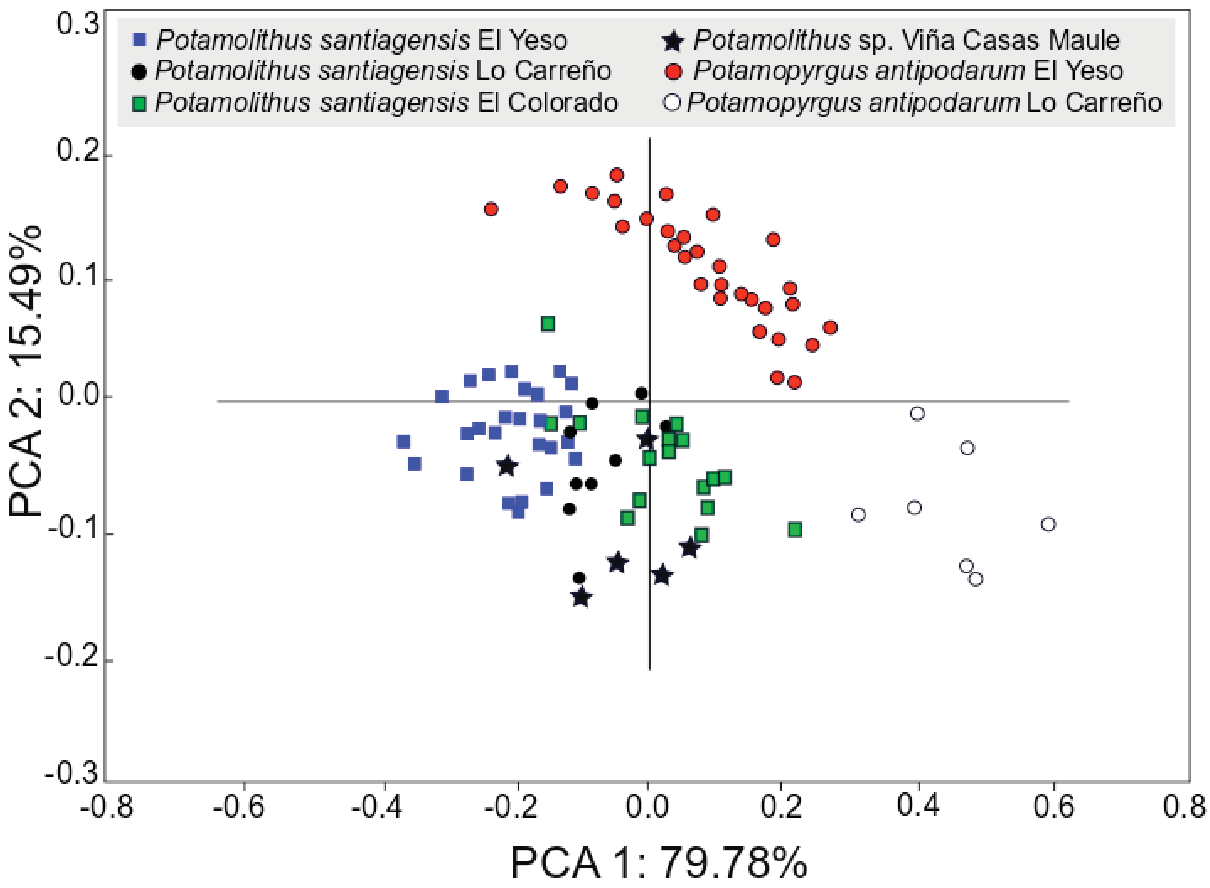

Fig. 4. PCA of *Potamolithus* populations and *Potamopyrgus antipodarum* (Gray, 1843) collected from central Chile.

Table 1. Average shell dimensions (± standard deviation) of six linear variables used in the comparative study of *Potamolithus* populations and *Potamopyrgus antipodarum* (Gray, 1843) from central Chile.

Shell variable	n	SW	AL	AW	BWL	SPL
P. santiagensis comb. nov. El Yeso	26	1.7 (± 0.1)	1.3 (± 0.1)	0.9 (± 0.1)	2.1 (± 0.1)	0.6 (± 0.1)
P. santiagensis comb. nov. El Colorado	17	1.9 (± 0.1)	1.6 (± 0.1)	1.1 (± 0.1)	2.4 (± 0.2)	0.9 (± 0.1)
P. santiagensis comb. nov. Lo Carreño	9	1.7 (± 0.1)	1.3 (± 0.1)	1.2 (± 0.1)	2.1 (± 0.1)	0.8 (± 0.1)
Potamolithus sp. Viña Casas del Maule	6	2.0 (± 0.1)	1.6 (± 0.2)	1.1 (± 0.1)	2.4 (± 0.2)	0.7 (± 0.1)
P. antipodarum El Yeso	30	1.8 (± 0.2)	1.4 (± 0.1)	1.0 (± 0.1)	2.2 (± 0.2)	1.2 (± 0.1)
P. antipodarum Lo Carreño	7	2.6 (± 0.2)	2.1 (± 0.2)	1.4 (± 0.1)	3.2 (± 0.2)	1.7 (± 0.3)

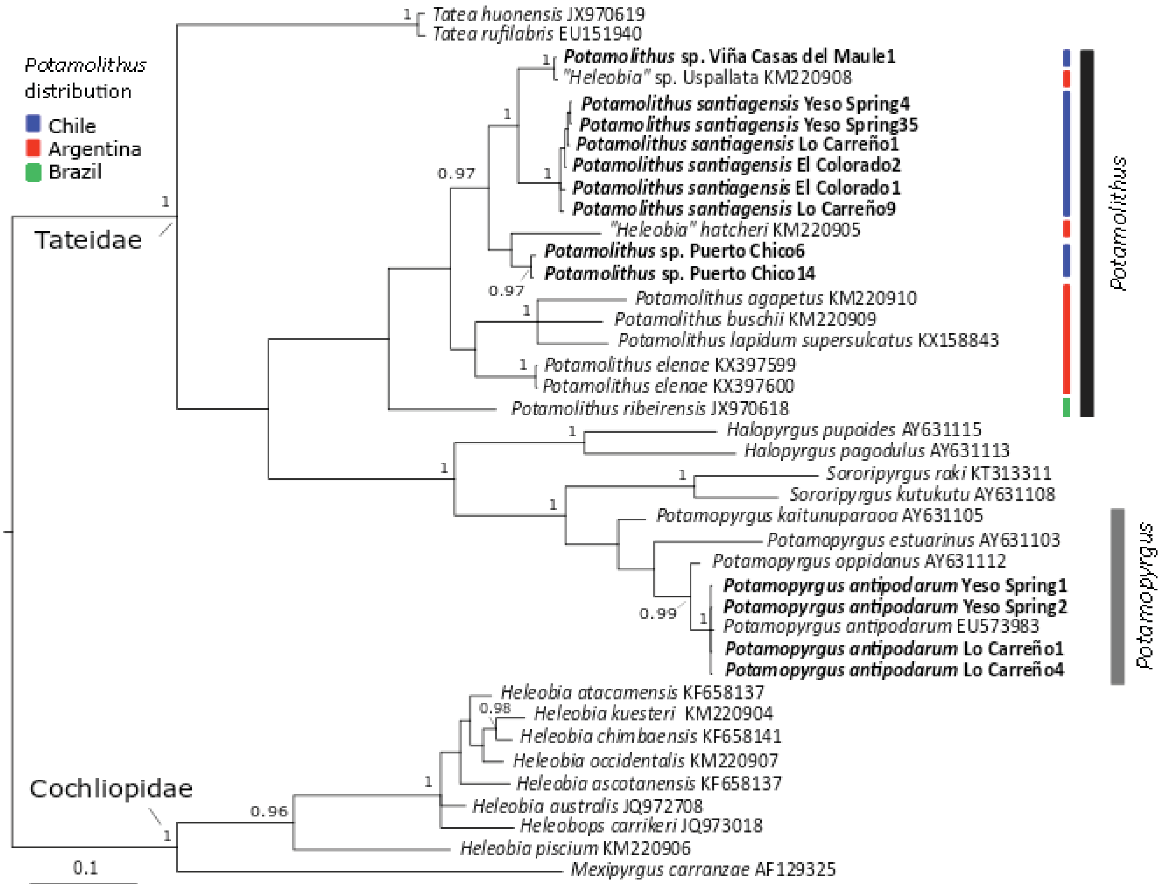

Fig. 5. Bayesian tree based on COI sequences. Numbers at nodes indicate posterior probability values (only given if ≥ 0.95). Names in bold refer to new sequences reported in this paper. Numbers following taxa refer to GenBank sequences.

in the morphometric space (Fig. 4). In this analysis, the first two components accounted cumulatively for 95.27% of the variance (PC1: 79.78%; PC2: 15.49%).

Molecular analysis

The COI phylogenetic analysis grouped the original sequences and those downloaded from GenBank into two main clades, Tateidae and Cochliopidae, respectively, both supported by posterior probabilities (p.p.) of 1.00 (Fig. 5). Our original sequences clustered in the first clade, either in the genus *Potamolithus* or *Potamopyrgus*, both groups inferred with high node support (0.97 and 1.00 p.p., respectively). The sequences of the slender morphotype from El Yeso Spring and Lo Carreño were identified as *P. antipodarum*, and those of the thicker one as representatives of *Potamolithus* (Fig. 5). The snails from El Colorado, Viña Casas del Maule, Puerto Chico, "*Heleobia*" sp. from Uspallata and "*Heleobia*" *hatcheri* from Aguas Negras in Argentina retrieved from GenBank were also recovered among *Potamolithus* species. The COI genetic distances among Tateidae species/populations from South America ranged between 0.2% and 17.0% (Table 3). The genetic distances between *P. santiagensis* comb. nov. and snails from Puerto Chico were estimated at 6.7–7.2%. In the Bayesian tree, they were placed in different subclades. The population from Viña Casas del Maule grouped with "*Heleobia*" sp. from Uspallata (1.00 p.p.); they differed genetically from *P. santiagensis* comb. nov. by about 5.0%. On the other hand, the sequence divergence between the population from El Colorado and *P. santiagensis* comb. nov. from Yeso Spring and Lo Carreño was low (0.0–0.6%).

Discussion

The minute native gilled freshwater snails in Chile, at present placed in the genera *Littoridina*, *Heleobia* or *Potamolithus*, have had a complicated taxonomic history, as they have been ascribed to the families Amnicolidae, Hydrobiidae, Littoridinidae and Cochliopidae (e.g., Pilsbry 1911; Biese 1944, 1947; Hershler & Thompson 1992; Valdovinos 1999, 2006; Figueroa *et al.* 1999; Sielfeld 2001). In Argentina, the taxonomic position of some species traditionally recognized as representatives of the genus *Heleobia* (Cochliopidae) as members of the family Tateidae has recently been discussed by different authors (Koch *et al.* 2015; de Lucía & Gutiérrez Gregoric 2017). In the present study, the tree-based barcoding analysis provided evidence to transfer *Littoridina/Heleobia santiagensis* to the genus *Potamolithus* (Tateidae). The presence of a nuchal node, also observed in females of several species of this genus (Davis & Pons da Silva 1984; López Armengol 1996; Pons da Silva & Veitenheimer-Mendes 2004a), absent in *Heleobia*, is a morphological character that also supports this change, although it is not a diagnostic character of the genus (Núñez 2017). The three or four pairs of basal cusps on the rachidian tooth characteristic of the tateid radula also distinguish this group from *Heleobia* species (Pons da Silva & Veitenheimer-Mendes 2004a; Cazzaniga 2011; Koch *et al.* 2015; de Lucía & Gutiérrez Gregoric 2017), which usually have only one pair of basal cusps, rarely two (Gaillard & de Castellanos 1976; Pons da Silva & Veitenheimer-Mendes 2004b; Collado *et al.* 2016b).

The Bayesian analysis revealed that part of the material from Lo Carreño and the population from El Colorado grouped with sequences of *P. santiagensis* comb. nov. from El Yeso Spring, so we assigned these snails to this species. In Lo Carreño we also identified the New Zealand mudsnail *P. antipodarum,* extending its invasive range to a new hydrological basin further south of the previously known distribution in the country (Collado 2014, 2016). Morphometric data subjected to PCA also separated *Potamolithus* populations and the New Zealand mudsnail. Taking into account that the presence of the New Zealand mudsnail has also been reported at several type localities of endemic species of *Heleobia* in the Región de Coquimbo (Biese 1944, 1947; Collado 2014), where they were previously confused (Collado *et al.* 2011), these cryptic snails could potentially also be discriminated using this simple methodology. Single shell dimensions may or may not be operational, though, since a number of comparisons were statistically undifferentiable. The Bayesian analysis also inferred the sequences of "*Heleobia*" sp. Uspallata and

Table 2. P-values of Kruskal-Wallis (in column Shell variable) and pairwise post-hoc Mann-Whitney U-tests of six linear variables.

Species/population	Shell variable	*P. santiagensis* (El Yeso)	*P. antipodarum* (El Yeso)	*P. santiagensis* (El Colorado)	*Potamolithus* sp. (VCM)
P. antipodarum (El Yeso)	SL<0.001	< 0.001			
P. santiagensis comb. nov. (El Colorado)		< 0.001	1.000		
Potamolithus sp. (Viña Casas Maule)		0.068	1.000	1.000	
P. santiagensis comb. nov. (Lo Carreño)		0.379	0.236	1.000	1.000
P. antipodarum (El Yeso)	SW<0.001	1.000			
P. santiagensis comb. nov. (El Colorado)		0.001	0.003		
Potamolithus sp. (Viña Casas Maule)		0.016	0.044	1.000	
P. santiagensis comb. nov. (Lo Carreño)		1.000	1.000	0.019	0.054
P. antipodarum (El Yeso)	AL<0.001	0.374			
P. santiagensis comb. nov. (El Colorado)		< 0.001	< 0.001		
Potamolithus sp. (Viña Casas Maule)		< 0.001	0.029	1.000	
P. santiagensis comb. nov. (Lo Carreño)		1.000	1.000	0.022	0.151
P. antipodarum (El Yeso)	AW<0.001	1.000			
P. santiagensis comb. nov. (El Colorado)		< 0.001	0.008		
Potamolithus sp. (Viña Casas Maule)		0.058	0.271	1.000	
P. santiagensis comb. nov. (Lo Carreño)		< 0.001	< 0.001	0.848	1.000
P. antipodarum (El Yeso)	BWL <0.016	0.511			
P. santiagensis comb. nov. (El Colorado)		0.002	0.361		
Potamolithus sp. (Viña Casas Maule)		0.013	0.371	1.000	
P. santiagensis comb. nov. (Lo Carreño)		1.000	1.000	1.000	0.872
P. antipodarum (El Yeso)	SPL<0.001	< 0.001			
P. santiagensis comb. nov. (El Colorado)		< 0.001	0.038		
Potamolithus sp. (Viña Casas Maule)		1.000	0.004	1.000	
P. santiagensis comb. nov. (Lo Carreño)		0.382	0.003	1.000	1.000

“*Heleobia*” *hatcheri* from Argentina as members of the genus *Potamolithus*, representing other cases of familial and generic misidentification.

In the Southern Cone of South America, the close phylogenetic relationships found between samples of “*Heleobia*” *hatcheri* from the Central-West region of Argentina and *Potamolithus* sp. from Llanquihue Lake in the Chilean Patagonia suggests that dispersal across the Andes may have played a role in the distribution of these species. The same may be inferred regarding the Chilean *Potamolithus* populations from Viña Casas del Maule and “*Heleobia*” sp. from Uspallata, Argentina, and the lowland populations of *P. santiagensis* comb. nov., which exhibit disjunct distributions in Chile.

The New Zealand mudsnail has been considered as a highly invasive species worldwide (Son 2008; Alonso & Castro-Díez 2012; Butkus *et al.* 2012), potentially able to seriously affect the native snail fauna in invaded aquatic ecosystems (Richards 2002). After this species arrived in the Snake River drainage in North America in 1987 (Bowler 1991), five native species were recorded as either “threatened” or “endangered”, in part due to the introduction of the species (Richards 2002). It is possible that something similar has happened in Estero Dehesa considering the failure to find specimens of *P. santiagensis*

Table 3. Percent of mean sequence variation (based on p-distance) of COI data among South American species/populations belonging to the Tateidae.

Species/population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *Potamopyrgus antipodarum* El Yeso Spring 1	0.0															
2. *Potamopyrgus antipodarum* Lo Carreño 1	0.0	0.0														
3. *"Heleobia*" *hatcheri*	17.0	17.0	0.0													
4. *"Heleobia"* sp. Uspallata	14.5	14.5	8.2	0.0												
5. *Potamolithus agapetus*	15.1	15.1	11.4	9.2	0.0											
6. *Potamolithus ribeirensis*	17.0	17.0	12.1	9.6	11.0	0.0										
7. *Potamolithus buschii*	15.1	15.1	10.6	9.2	7.6	10.2	0.0									
8. *Potamolithus elenae* 14191	15.3	15.3	9.0	7.0	8.8	9.4	6.8	0.0								
9. *Potamolithus santiagensis* El Yeso Spring 4	15.3	15.3	9.0	5.3	10.2	10.8	10.4	9.0	0.0							
10. *Potamolithus santiagensis* El Yeso Spring 35	15.3	15.3	9.0	5.3	10.2	10.8	10.4	9.0	0.0	0.0						
11. *Potamolithus santiagensis* El Colorado 1	15.3	15.3	8.6	5.1	10.0	10.4	10.4	8.6	0.6	0.6	0.0					
12. *Potamolithus santiagensis* El Colorado 2	15.3	15.3	9.0	5.3	10.2	10.8	10.4	9.0	0.0	0.0	0.6	0.0				
13. *Potamolithus santiagensis* Lo Carreño 9	15.1	15.1	8.6	5.1	9.8	10.4	10.4	8.6	0.6	0.6	0.4	0.6	0.0			
14. *Potamolithus santiagensis* Lo Carreño 1	15.3	15.3	9.0	5.3	10.2	10.8	10.4	9.0	0.0	0.0	0.6	0.0	0.6	0.0		
15. *Potamolithus* sp. Viña Casas del Maule 1	14.3	14.3	8.0	0.2	9.0	9.4	9.0	6.8	5.1	5.1	4.9	5.1	4.9	5.1	0.0	
16. *Potamolithus* sp. Puerto Chico 6	15.5	15.5	5.7	6.1	9.2	10.0	8.2	6.5	7.2	7.2	7.2	7.2	6.8	7.2	5.9	0.0
17. *Potamolithus* sp. Puerto Chico 14	15.3	15.3	5.5	5.9	9.0	9.8	8.0	6.3	7.0	7.0	7.0	7.0	6.7	7.0	5.7	0.2

comb. nov. and the current occurrence of New Zealand mudsnails at this locality. Although without being alarmist, perhaps a "chronicle of a death foretold" could be written for the El Yeso Spring population based on the noticeable abundance of New Zealand mudsnails in this habitat compared to *P. santiagensis* comb. nov. In this context, the finding of the new populations of this species in El Colorado, where the New Zealand mudsnail has not been introduced, acquires real significance giving a light of hope for the preservation of the species.

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