

A molecular contribution to the controversial taxonomical status of some freshwater snails (Caenogastropoda: Risssooidea, Cochliopidae) from the Central Andes desert to Patagonia

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ABSTRACT. For over 40 years malacologists have been discussing the taxonomical status of *Heleobia* species, an enigmatic genus from Cochliopidae family (Caenogastropoda: Risssooidea). As with other risssooidean families, the considerable character convergence and the paucity of anatomical synapomorphies has proved to be a problem in resolving cochliopid phylogenetic relations and establishing the validity of several nominal cochliopid species. Here we present a molecular contribution to solve the taxonomical status of one of the most abundant Southern South America cochliopid genera which has many endemic species. We report molecular evidence that supports three of the four *Heleobia* groups described for this region, the “*australis*”, “*parchappii*” and “*piscium*” groups. The fourth, the “*hatcheri*” group, belongs not to *Heleobia* but to a different genus which itself should not be considered as part of the family Cochliopidae but closely related to genus *Potamolithus* Pilsbry & Rush, 1896.

KEYWORDS. Cochliopidae, *Heleobia*, taxonomical status, Arid diagonal, South America.

RESUMEN. Una contribución molecular al controvertido estatus taxonómico de un grupo de caracoles dulceacuicolas (Caenogastropoda: Risssooidea, Cochliopidae) distribuidos desde el desierto de los Andes centrales hasta la Patagonia. Durante más de 40 años se ha discutido el estatus taxonómico de diversas especies del enigmático género *Heleobia* de la Familia Cochliopidae (Caenogastropoda: Risssooidea). Como sucede con otras familias de risssooideos, la abundancia de caracteres convergentes y la escasez de sinapomorfias anatómicas han representado un problema para resolver las relaciones filogenéticas de Cochliopidae y definir la validez de varias de las especies nominales de esta Familia. Presentamos aquí una contribución molecular tendiente a resolver el estatus taxonómico de uno de los más abundantes géneros de la porción meridional de Sudamérica que incluye varias especies endémicas. Nuestra evidencia molecular reconfirma tres de los cuatro grupos de *Heleobia* en los que se han agrupado las especies del género en esta región: “*australis*”, “*parchappii*” y “*piscium*”. El cuarto, el grupo “*hatcheri*”, no pertenece a *Heleobia* sino a un género diferente que no debería ser considerado como integrante de la Familia Cochliopidae, sino estrechamente relacionado al género *Potamolithus* Pilsbry y Rush, 1896.

PALABRAS CLAVE. Cochliopidae, *Heleobia*, estatus taxonómico, Diagonal Árida, Sudamérica.

Cochliopidae is a family of risssooidean snails composed of more than 30 genera and more than 260 species that mainly inhabit freshwaters in tropical and temperate regions of America and several regions of Eurasia (HERSHLER & THOMPSON, 1992). The status of this enigmatic family remained unstable during many years, until WILKE *et al.* (2001), using molecular tools, confirmed that Cochliopidae is a family distinct from Hydrobiidae as it is accepted by BOUCHET & ROCROI (2005). The monophyly of the family, the consistency of molecular and anatomical characters (mainly closed spermathecal duct and oviduct jointed directly to the albumen gland), and its phylogenetic relationships have been assessed and discussed by LIU *et al.*, 2001 (as Cochliopinae) and WILKE *et al.* (2001) who mainly utilized DNA sequences of mitochondrial genes. Previous attempts to resolve systematic and/or phylogeny of Hydrobiids based only on morphological data (e.g. KABAT & HERSHLER, 1993; FALNIOWSKY & SZAROWSKA, 2000) poorly resolved the uncertainty due to considerable character convergence and the scarcity of anatomical synapomorphies.

Cochliopidae are abundant snails worldwide. However, sequenced taxa from South America are

underrepresented, as was pointed out by LIU *et al.* (2001) discussing biogeography. Recent molecular characterization of endemic gastropod fauna from Titicaca Lake (KROLL *et al.*, 2012) and Northern of Chile (COLLADO *et al.*, 2013) appear to be the only available cochliopid information for this subcontinent.

The Argentinian Cochliopidae were studied originally by M. C. Gaillard (unpublished data) and GAILLARD & CASTELLANOS (1976) both as family Hydrobiidae and genus *Littoridina*. They listed 18 nominal species, gathered in 4 groups (“*australis*”: South Atlantic littoral waters; “*piscium*”: subtropical freshwaters; “*parchappei*”: Pampean region, North Patagonia and Centre-West of Argentina; “*hatcheri*”: Patagonian continental region) using morphological characters of the shell, operculum, radula and penis. A compilation of gastropod snails from the freshwater of Argentina (RUMI *et al.*, 2008) reported 16 species of Cochliopidae (all belonging to the genus *Heleobia* and 10 of them endemics). A review of the taxonomy of *Heleobia* with emphasis on Argentina was reported by CAZZANIGA (2011); none of these previous studies for the Argentinian cochliopidae species included molecular data with the exception of KROLL *et al.* (2012), where two

sequences of *Heleobia* are mentioned, both collected from Laguna Mar Chiquita by C. G. de Francesco and identified by R. Hershler (Museum records) as *H. australis* and *H. parchappii* (both preserved in the Smithsonian Natural History Museum).

The Centre-West of Argentina, extending between 28° and 37°S and 65° and 71°W, lies within the dominion of the South American Arid Diagonal, which is considered to have been climatically sensitive to the latitudinal shift of the Pacific and Atlantic anticyclone centers 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 foothills of the Andes (CAPITANELLI, 2005). With respect to freshwater gastropods, this region constitutes the 'Cuyo Malacological Province' (CMP) (NÚÑEZ *et al.*, 2010) and covers approximately 280,000 km². The gastropod diversity of this region is one of the lowest in the country and comprises only 18 known species spread over the families Ampullariidae (1 species), Cochliopidae (5), Physidae (4), Planorbidae (2), Lymnaeidae (2), and Chilinidae (4) (NÚÑEZ *et al.*, 2010; GUTIÉRREZ GREGORIC *et al.*, 2014). Despite this low biodiversity, the cochliopid species from the CMP are interesting issue due to their confused taxonomical status. The five Cochliopidae species originally described for the CMP belong to the genus *Heleobia*; one included in the "hatcheri group" *H. hatcheri* (Pilsbry, 1911) and four in the "parchappii group": *H. parchappii* (d'Orbigny, 1835), *H. kuesteri* (Strobel, 1874), *H. occidentalis* (Doering, 1885), and *H. vianai* (Parodiz, 1960). According to CAZZANIGA (1980), this last name is a synonym of *H. occidentalis* (as *Littoridina occidentalis*). GAILLARD & CASTELLANOS (1976) proposed *H. occidentalis* as a geographical variation of *H. parchappii* for saline waters, while CAZZANIGA (1980) considered *H. occidentalis* a valid species after examining penis morphology. DE FRANCESCO (2007) suggested *H. occidentalis* as synonym of *H. parchappii* based on conchological characters, criteria adopted in recent ecological studies in saline areas from the CMP by CIOCCO & SCHEIBLER (2008) and DE FRANCESCO & HASSAN (2009).

Heleobia kuesteri also remain enigmatic. Based on geographic distribution and the original conchological description of Strobel, M. C. Gaillard (unpublished data) considered this species within the "parchappii group". CAZZANIGA (1981) proposed *H. kuesteri* as *species inquirenda* and CIOCCO (2011) suggested that it could be a valid taxon related to the "parchappii group".

Heleobia hatcheri, abundant in Patagonian waters, differs from the other *Heleobia* species from the CMP in, among other characters, the presence of a so called *nuchal papilla* in all females studied (pseudohermaphroditism or natural imposex, MARTÍN, 2002), the only reported sex in CMP populations where sex ratios have been studied (Uspallata River; MARTÍN, 2002; CIOCCO, 2011). This organ was previously mistakenly interpreted as a reduced and functional penis from hypothetical *H. hatcheri* males (GAILLARD & CASTELLANOS, 1976; CAZZANIGA, 1981), to

the point that a new genus was proposed (*Strobiliella*; CAZZANIGA, 1981).

Additionally, a new morphotype with similar shell features to *H. hatcheri* (ovate-conic shell), but discontinuous peristome, was recently found in several localities of the CMP (*Heleobia* sp.; CIOCCO & KOCH, unpublished data).

The goal of this study is to develop a molecular approach to solve the taxonomical status of *Heleobia* species from the Centre-West of Argentina as a first step towards an integrated phylogenetic study of the Southern South America cochliopids. Considering: i) the protein-coding mitochondrial citochrome oxidase I (COI) gene does not show insertions or deletions in the superfamily Rissosoidea; ii) the vast information on COI gene sequence available in NCBI GeneBank for the Gastropoda in general and iii) that this sequence shows good phylogenetic signals from population to family levels (WILKE *et al.*, 2001), we analyze COI sequences from 7 taxa (5 cochliopid and 2 non cochliopid ones) without previous data in GenBank, in an attempt to provide new considerations tending to solve a long term controversial issue.

MATERIALS AND METHODS

Specimens. Individuals from CMP were collected in Aguas Negras (30°18'6.72"S, 68°43'46.62"W, San Juan Province), Uspallata stream (32°40'11.1"S, 69°21'52.8"W, Mendoza Province), and Laguna Bebedero (33°39'S, 66°34'W, San Luis Province) during expeditions to the CMP between 2011 and 2013. *Heleobia piscium* (d'Orbigny, 1835) and *Potamolithus* spp. specimens were collected in 2014 from Martín García Island, upper portion from de la Plata River basin (34°11'S, 58°15'W, Buenos Aires Province). In all cases, animals were alcohol preserved following previous menthol relaxation. Voucher specimens for all studied taxa were deposited in the Museo de La Plata collection under voucher numbers: MLP MA 13806 to 13812.

DNA isolation, PCR and sequencing. The total DNA was extracted from the foot of dissected snails. Tissues were rinsed in TE buffer (10mM Tris 1mM EDTA, pH 8) and digested overnight in CTAB (Cetyl trimethylammonium bromide) buffer containing proteinase K (0.14 mg at 60°C) and 2-Mercaptoethanol. DNA was purified by a threefold extraction with chloroform-isoamyl alcohol (24:1) followed by precipitation with ethanol. The DNA was then resuspended in DNase/RNase free distilled water. A 655-bp fragment of the COI gene 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 50 µl containing: 50–100 ng of template DNA, 0.1 µM of each primer, 1X PCR buffer, 50 µM dNTPs, 2.5 mM MgCl₂, and 1 U Taq polymerase (Invitrogen, Brazil). The thermocycling sequence was conducted at 94°C for 3 min; with 5 cycles at 94°C for 30 s, 42°C for 30 s, and 72°C for 1 min 30 s; followed by 34 cycles at 94°C for 30 s, 45°C for 30 s, and 72°C for 1 min 30 s; with a final chain

extension at 72°C for 5 min. 5 µl of each PCR product was tested on a 1% (w/v) agarose gel electrophoresis. The remainders (45µL) of reactions with the expected PCR product were purified with AccuPrep® PCR purification Kit (Bioneer Corporation, Korea), then sequenced in both directions (Instituto de Biotecnología, Unidad de Genómica, INTA Castelar, Argentina). The resulting sequences were analyzed with BioEdit (HALL, 1999) to obtain consensus sequences for each individual.

Sequence alignment. The COI sequences were unambiguously aligned in MEGA6 (TAMURA *et al.*, 2013) and trimmed to a total length of 638bp. Phylogenetic analysis was undertaken comparing gene sequences from this study and related sequences in GenBank (Tab. I). A phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model (TAMURA & NEI, 1993). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (FELSENSTEIN, 1985). Branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates are collapsed.

The initial tree for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with the best log likelihood value. The analysis involved 38 nucleotide sequences. All codon positions were included. All positions containing gaps and missing data were eliminated. There were a total of 636 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (TAMURA *et al.*, 2013).

RESULTS

The bootstrap consensus tree yielded from the ML analysis comprises the outgroup taxon *Pomatiopsis lapidaria* (Say, 1817), 4 dominant clades and several subclades (from top to bottom; Fig 1.):

1. Clade Cochliopidae was represented by 4 genera (*Heleobia*, *Semisalsa*, *Heleobops* and *Tryonia*), 3 subclades and 4 lineages:

Tab. I. Taxon, collection locality data, reference and GenBank accession numbers for specimens analyzed in this study.

Taxon	Locality	Reference	GenBank accession number
<i>Benedictia baicalensis</i>	Lake Baika, Russia	WILKE <i>et al.</i> , 2013	HQ623171
<i>Heleobia andicola andicola</i>	Patapatini Island, Lake Titicaca, Bolivia	KROLL <i>et al.</i> , 2012	JQ973028
<i>Heleobia andicola culminea</i> (Peru)	Umayo Island, Lake Umayo, Peru	KROLL <i>et al.</i> , 2012	JQ973034
<i>Heleobia andicola culminea</i> (Bolivia)	Huarina, Lake Titicaca, Bolivia	KROLL <i>et al.</i> , 2012	JQ973030
<i>Heleobia andicola neveui</i>	Vilque Chico, Lake Titicaca, Peru	KROLL <i>et al.</i> , 2012	JQ973035
<i>Heleobia aperta</i>	Chua, Lake Titicaca, Bolivia	KROLL <i>et al.</i> , 2012	JQ973036
<i>Heleobia australis</i>	Mar Chiquita, Argentina	KROLL <i>et al.</i> , 2012	JQ972708
<i>Heleobia hatcheri</i>	Aguas Negras, San Juan, Argentina	this study	KM220905
<i>Heleobia kuesteri</i>	Uspallata	this study	KM220904
<i>Heleobia languiensis</i>	Lake Langui Layo, Peru	KROLL <i>et al.</i> , 2012	JQ973042
<i>Heleobia limariensis</i>	Huasco River, Vallendar Chile	KROLL <i>et al.</i> , 2012	JQ973043
<i>Heleobia loaensis</i>	Loa River, Quillagua Chila	KROLL <i>et al.</i> , 2012	JQ973044
<i>Heleobia mirum</i>	Ajilata, Lake Titicaca, Bolivia	KROLL <i>et al.</i> , 2012	JQ973046
<i>Heleobia occidentalis</i>	Laguna Bededro, San Luis Argentina	this study	KM220907
<i>Heleobia ortoni</i>	Chucuito, Lake Titicaca, Peru	KROLL <i>et al.</i> , 2012	JQ973049
<i>Heleobia parchappii</i>	Mar Chiquita, Argentina	KROLL <i>et al.</i> , 2012	JQ972709
<i>Heleobia piscium</i>	Isla Martín García	this study	KM220906
<i>Heleobia poopoensis</i>	Laca Jahuirá River, Bolivia	KROLL <i>et al.</i> , 2012	JQ973050
<i>Heleobia saracocha</i>	Lake Saracocha, Peru	KROLL <i>et al.</i> , 2012	JQ973051
<i>Heleobia</i> sp.	Uspallata	this study	KM220908
<i>Heleobia umbiculata</i>	Sol Island, Lake Titicaca, Bolivia	KROLL <i>et al.</i> , 2012	JQ973053
<i>Heleobops carrikeri</i>	Oyster Pond, Falmouth, USA	KROLL <i>et al.</i> , 2012	JQ973019
<i>Hydrobia acuta acuta</i>	Étang du Prévost, Hérault, France	WILKE <i>et al.</i> , 2000	AF278808
<i>Hydrobia acuta neglecta</i>		DAVIS <i>et al.</i> , 1998	AF253079
<i>Leptopyrgus tainui</i>	Kawhia, New Zealand	HAASE, 2005	AY631078
<i>Lithoglyphus naticoides</i>	Narew River, Poland	WILKE <i>et al.</i> , 2001	AF367642
<i>Meridiopyrgus murihiku</i>	Browns, New Zealand	HAASE, 2005	AY631084
<i>Opacuincola permutata</i>	Inangahua, New Zealand	HAASE, 2005	AY631100
<i>Pomatiopsis lapidaria</i> (outgroup)	Cruger Island, Hudson River, USA	LIU <i>et al.</i> , 2001	AF354774
<i>Potamolithus agapetus</i>	Isla Martín García	this study	KM220910
<i>Potamolithus buschii</i>	Isla Martín García	this study	KM220909
<i>Potamolithus ribeirensis</i>	Iporanga River, Sao Paulo, Brazil	WILKE <i>et al.</i> , 2013	JX970618
<i>Potamopyrgus antipodarum</i>	New Zealand	NEIMAN <i>et al.</i> , 2010	GQ996429
<i>Semisalsa dalamatica</i>	Pirovac Spring, Croatia	WILKE <i>et al.</i> , 2001	AF367631
<i>Semisalsa foxianensis</i>	Thermal Springs, Torretta, Montecatini Terme, Italy	KROLL <i>et al.</i> , 2012	JQ973023
<i>Semisalsa scamandri</i>	Étang du Charnier, Saint Gilles, France	KROLL <i>et al.</i> , 2012	JQ973025
<i>Semisalsa stagnorum</i>	Kaaskenswaters, Zierikzee, The Netherlands	KROLL <i>et al.</i> , 2012	JQ973024
<i>Tryonia imitator</i>	Peñasquitos Lagoon, California, USA	HERSHLER <i>et al.</i> , 1999	AF061769

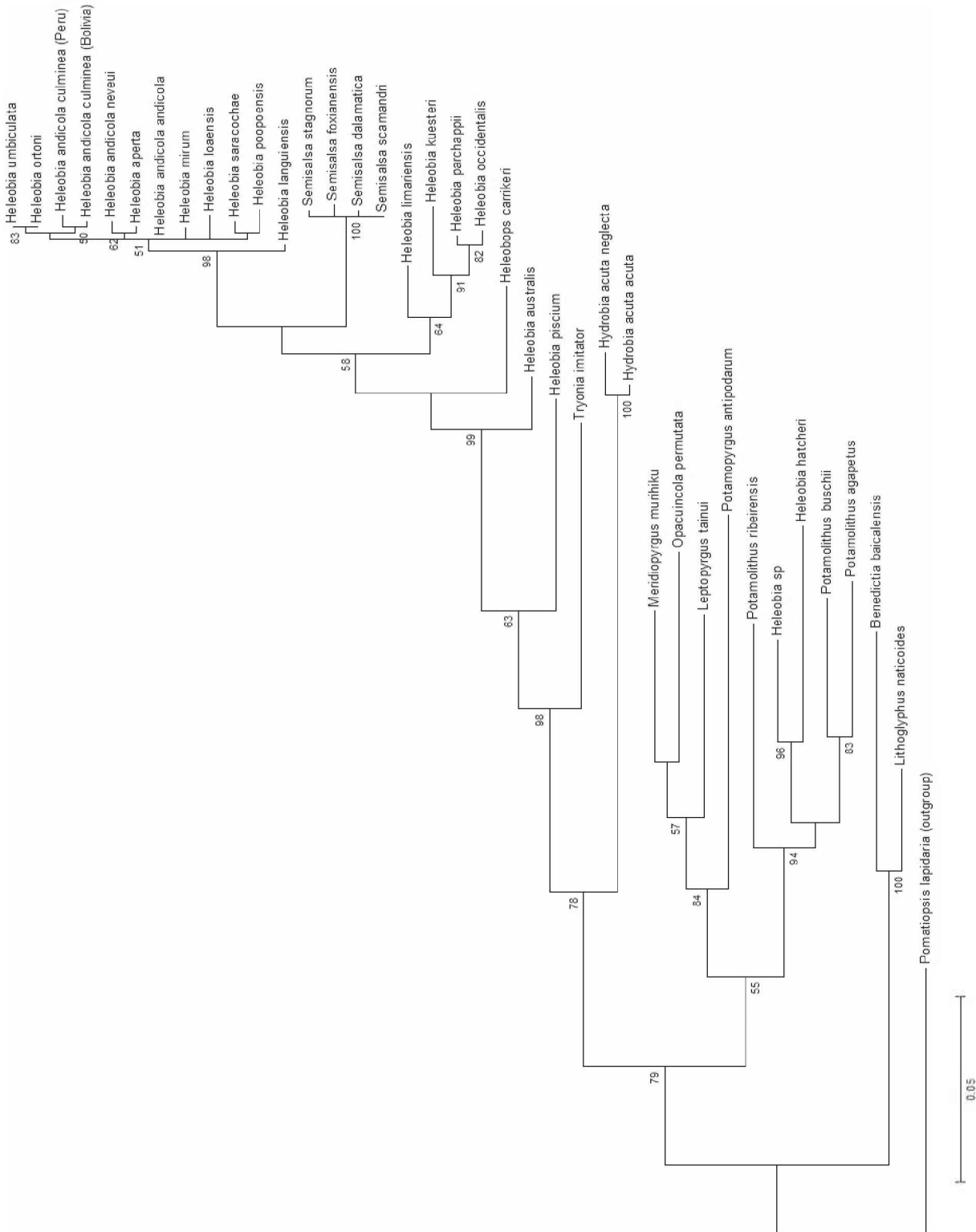


Fig. 1. Molecular Phylogenetic analysis by Maximum Likelihood method Bootstrap consensus tree inferred from 1000 replicates. All codon positions were included. All positions containing gaps and missing data were eliminated. There were a total of 636 positions in the final dataset. Only bootstrap values greater than 50 are indicated.

1.1. An Altiplano Lakes subclade containing 11 species and subspecies of *Heleobia* genus;

1.2. An European *Semisalsa* species subclade including 4 taxa;

1.3. A subclade containing 4 *Heleobia* spp.

Four basal lineages [*Heleobops carrikeri* (Davies and McKee, 1989), *Heleobia australis*, *Heleobia piscium* and *Tryonia imitator* (Pilsbry, 1899)] not belonging to Subclades 1.1- 1.3.

2. Clade Hydrobiidae represented by 2 species of the genus *Hydrobia*

3. Clade Tateidae containing the genera *Meridyopirgus*, *Opacuicola*, *Leptopyrgus*, *Potamopyrgus* and *Heleobia*, grouped in two subclades:

3.1. With *Meridyopirgus*, *Opacuicola*, *Leptopyrgus*, *Potamopyrgus*.

3.2. Containing 3 species of *Potamolithus*, *Heleobia hatcheri* and morphotype *Heleobia* sp.

4. Clade Litoglyphidae with *Benedicta* and *Lithoglyphus* genera.

Three of the *Heleobia* spp. from Subclade 1.3 correspond to taxa recorded from the CMP: *H. parchappii*, *H. occidentalis* and *H. kuesteri*. The topology of this subclade showed that two first species are very close, reinforcing that *H. occidentalis* is synonym of *H. parchappii*. Also, analysis of this subclade confirmed that *Heleobia kuesteri* belongs to “*parchappii* group” and suggested that it should be accepted as a valid species. *Heleobia limariensis*, from Huasco River Basin from northern Chile and the three CMP species of this subclade, share an arid Andean environment.

The four basal lineages of Cochliopidae (*Heleobops carrikeri*, *Heleobia australis*, *Heleobia piscium* and *Tryonia imitator*) correspond to saline or euryhaline taxa.

Heleobia hatcheri (and the very similar morphotype *Heleobia* sp.) from CMP, resolved outside Cochliopidae. Both were integrated in the well-defined Tateidae subclade 3.2 composed of 3 *Potamolithus* species: *P. agapetus* (Pilsbry, 1911) and *P. buschii* (Fraunfeld, 1865), sympatric taxa from de la Plata River, and *P. ribeirensis* (Pilsbry, 1911, *sensu* DAVIS & PONS DA SILVA, 1984), from Iporanga River, Southern Brazil, part of the Paraná and La Plata River drainage systems. “*Heleobia hatcheri*” and “*Heleobia* sp.” were closely-related to the three *Potamolithus* species studied in this work and these three taxa are more closely linked to Tateidae family than to Lythoglyphidae, as was pointed out by WILKE *et al.* (2013) for *P. ribeirensis*.

DISCUSSION

Our results suggest that only two Cochliopidae species of the “*parchappii* group” should be recognized in the Centre-West of Argentina: *H. parchappii* and *H. kuesteri*. *Heleobia parchappii* is a elongate-conic shell species abundant in oligohaline waters from the Pampean Region, able to develop populations in estuaries (DE FRANCESCO & ISLA 2004) or in hard continental waters such as those of Desaguadero, Llancanelo and Bebebero

saline’s from the CMP (CIOCCO & SCHEIBLER, 2008; DE FRANCESCO & HASSAN, 2009, and this work). *Heleobia vianai* (cited from only one CMP locality, M. C. Gaillard, unpublished data) and *H. occidentalis* from Bebebero and Llancanelo saline areas, should be considered as synonymus of *H. parchappii* as was suggested by CAZZANIGA (1980) and DE FRANCESCO (2007), respectively. We were unable to obtain COI sequences for preserved *H. vianai* material studied (Lote MLP 9224 Invertebrates Collection of Museo de La Plata, Argentina), or collect any *H. vianai* specimen from the same area as the type locality where saline waters are predominant. CAZZANIGA (1980), based in penial morphology, did not detect significant differences between *H. vianai* and *H. occidentalis*, and proposed that the former is a synonym of *H. occidentalis* which, according to the COI sequences studied in this work, should itself be considered as a synonym of *H. parchappii*.

Heleobia kuesteri, meanwhile, is an elongate-conic shell endemic species from the Centre-West of Argentina abundant in relatively soft waters of the subandean foothills of the CMP. Despite this species needs to be redescribed including soft parts. The shell features of the scarce available material in malacological collections identified as *H. kuesteri* (Lote 20997/1 Invertebrates Collection of Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina) appear identical to the numerous specimens we collected in the Centre and North of the CMP. Although shell morphology of this species is different from typical *H. parchappii*, DE FRANCESCO & HASSAN (2009) cited for the South of the CMP another abundant batch of individuals, with identical conchological features to those we found in the Centre and North of the CMP, as *Heleobia aff. parchappii* based on similarity of penial complex. However, these authors recognized difficulties in identifying this material and considered the possibility that it could be an undescribed species or *H. kuesteri*.

Preliminary studies using scanning electron microscopy (SEM) performed on shell, penis and radula of many Cochliopidae from Argentina indicated that i) the radulae of *H. parchappii* and *H. kuesteri* are similar, ii) although both species penial complexes are similar, there are small differences in the shape and the porosity porous of the papillae, and distal end shape, iii) the whorls of *H. kuesteri* are less convex than those of *H. parchappii*, supporting the possibility that they are different species. The COI sequences analyzed here seemed to reinforce this possibility. However, more detailed anatomical studies, including female genitalia and use of other molecular markers are necessary to solve the question definitively.

Our molecular results would validate the “*australis*” and “*piscium*” groups. *H. australis* is an elongated-conic shell species of marine and littoral waters that, along with *Tryonia imitator* and *Heleobops carrikeri* (both from hard USA waters; HERSHLER *et al.*, 1999 and WILKE *et al.*, 2000, respectively) shares with *H. piscium* (an oligo- to euryhaline species) its condition of basal lineage of the subclades

from clade Cochliopidae (*i.e.* Altiplano Lakes, European *Semisalsa* and CMP *Heleobia* spp.; Fig. 1 tree). Besides COI sequences differences reported in this work and differences in penis morphology and shell form (elongate-conic in *H. australis* and conic in *H. piscium*; GAILLARD & CASTELLANOS, 1976), both species differs in development mode: indirect in *H. australis* (MARCUS & MARCUS, 1963, 1965; NEVES *et al.*, 2010) and direct in *H. piscium* (Stella M. Martin, pers. observ.).

Our results suggest that *Heleobia hatcheri* and the morphologically similar *Heleobia* sp. should not be included among the family Cochliopidae, and that they would be closely-related to the three studied *Potamolithus* species. The latter has two novel and significant implications: i) the conspicuous group “*hatcheri*”, traditional component of the Cochliopidae from Chile (BIESE, 1944) and Argentina (GAILLARD & CASTELLANOS, 1976), would disappear as part of this family; ii) as was recently suggested by WILKE *et al.* (2013), the *Potamolithus* genus endemic from South America would not be Lithoglyphidae as was proposed originally by DAVIS & PONS DA SILVA (1984).

Several other morphological features of *H. hatcheri* in addition to its ovate-conic and small shells have previously suggested that this taxon departs from typical cochliopid characters. These include the absence of a penis and the parthenogenetic (and pseudohermaphroditic) condition of the species shown by MARTÍN (2002). This reproductive mode is infrequent in Cochliopidae. Moreover, unlike other parthenogenetic rissoidaeans such as *Potamopyrgus*, *H. hatcheri* is oviparous (CAZZANIGA, 2011). Although mistaken, the original interpretation of the species nuchal papillae as a reduced penis, also suggested that *H. hatcheri* was different from the remaining Cochliopidae, to the point that a new genus (*Strobiliella*) was proposed for *H. hatcheri* (CAZZANIGA, 1981). In the same sense, the two or three cusps present in the basis of rachidean teeth of *H. hatcheri* radula are absent in all other Cochliopidae from the southern end of South American (CAZZANIGA, 2011). Although HERSHLER & THOMPSON (1992) maintained the synonymy of *Strobiliella* with *Heleobia*, assuming that the new proposed genus was based on gerontic specimens having a degenerate penis, our molecular data reinstate the requirement for a distinct generic name for *H. hatcheri*.

Interestingly, there is no fossil record of *H. hatcheri* from the Centre-West of Argentina although the Holocene aquatic malacofauna of the region is, with the exception of this species and the exotic *Physa acuta* Draparnaud, 1805, identical to the current gastropod and bivalve assemblages (DE FRANCESCO & HASSAN, 2009). These observations suggest that this enigmatic taxon may have colonized the Centre-West of Argentina in the last *ca.* 11,000 years.

Heleobia sp., as previously mentioned, must undoubtedly be considered as very close to *H. hatcheri*, a taxon with which it shares in sympatry the relatively soft waters of the CMP. Morphological studies indicate that the species have an identical radula, similar pigmentation in the

proboscis and tentacles together with nuchal papillae and absence of males in all the examined populations. The only notable difference from *H. hatcheri* is that the *Heleobia* sp. shell has a discontinuous peristome, a character that could be interpreted as an intraspecific variation, as also appears to be the case in *H. kuesteri*. Nevertheless, a detailed morphological description of this morphotype and the eventual incorporation of other molecular markers should be taken into account before considering it as a new species or a *H. hatcheri* variation.

With respect to the phylogenetic proximity of *H. hatcheri* to the South American genus *Potamolithus* and the suggestion that the latter belong not to Lithoglyphidae but to Tateidae (WILKE *et al.*, 2013), these authors indicated that “We do not know of any unique characters defining this group”. Nevertheless, the diagnosis of the Palearctic-Nearctic Lithoglyphidae is made by the closed ventral wall of the female capsule gland and the blade-like penis lacking large appendages and specialized glands, remarking finally that the genus *Potamolithus* was resolved as a member of the Tateidae Clade in all their molecular analysis (WILKE *et al.*, 2013).

The *Potamolithus* species incorporated in this study, *P. buschii* and *P. agapetus*, are sympatric in the La Plata River basin. *Potamolithus agapetus* presents a marked secondary sexual dimorphism on shell shape and size (LÓPEZ ARMENGOL, 1996). Females of both taxa show a nuchal node on the right side of the neck as was described by DAVIS & PONS DA SILVA (1984) for *P. ribeirensis*. This fleshy protuberance is situated where the base of the simple, and without appendages, penis is located (DAVIS & PONS DA SILVA, 1984; LÓPEZ ARMENGOL, 1996) in the three mentioned *Potamolithus* species, also coinciding with the position of the nuchal papilla of the parthenogenetic *H. hatcheri* females (MARTÍN, 2002). Unfortunately, the female genitalia of *P. buschii* and *P. agapetus* have not been described.

The only description of female genitalia available for the genus corresponds to that of *P. ribeirensis* (DAVIS & PONS DA SILVA, 1984). While it has served as the basis to define the “typical” idealized anatomical ground plan of the Lithoglyphidae (WILKE *et al.*, 2001), it is not incompatible with the characterization of the Tateidae female genitalia as “simple, usually with one distal seminal receptacle and a *bursa copulatrix*; ventral channel occasionally separated to form a vestibule”, (WILKE *et al.*, 2013). In *H. hatcheri* the spermathecal tube seems not be separated from the albumen gland, which would distinguish it from the Cochliopidae. However a deeper anatomical study of *H. hatcheri*, with emphasis on the female genitalia, and the incorporation of other mitochondrial markers is necessary to determine the genus and, more importantly, the family to which *H. hatcheri* belongs.

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REFERENCES

- ABRAHAM DE VAZQUEZ, E.; GARLEFF, K.; LIEBRICHT, H.; REIGARAZ, A.; SCHÄBITZ, F.; SQUEO, F.; STINGL, H.; VEIT, H. & VILLAGRAN, C. 2000. Geomorphology and paleoecology of the arid diagonal in southern South America. In: MILLER, H. & HERVE, F. eds. **Geodesy, Geomorphology and Soil Science**. Sonderheft, ZAG, p. 55-61.
- BIESE, W. A. 1944. Revisión de los moluscos terrestres y de agua dulce provistos de concha de Chile. Parte I, Familia Amnicolidae. **Boletín del Museo de Historia Natural, Chile** 22:169-190.
- BOUCHET, P. & ROCROU, J. P. 2005. Classification and nomenclator of gastropod families. **Malacologia** 47(1/2):1-397.
- CAPTANELLI, R. G. 2005. **Climatología de Mendoza**. Mendoza, Editorial de la Facultad de Filosofía y Letras de la Universidad Nacional de Cuyo.
- CAZZANIGA, N. J. 1980. Notas sobre hidróbidos argentinos (Gastropoda Rissoidea). I. Acerca de *Littoridina occidentalis* (Doering, 1884). **Neotropica** 26(76):187-191.
- _____. 1981. Notas sobre hidróbidos argentinos (Gastropoda Rissoidea). *Strobeliella*, un nuevo género de la Patagonia. **Neotropica** 27(77):3-10.
- _____. 2011. *Heleobia* Stimpson, 1865: Taxonomía. In: CAZZANIGA, N. J. ed. El género *Heleobia* (Caenogastropoda: Cochliopidae) en América del Sur. **Amici Molluscarum** (número especial):12-17.
- CIOCCO, N. F. 2011. Diversidad, biología y ecología de especies del género *Heleobia* de la Provincia Malacológica de Cuyo, Argentina. In: CAZZANIGA, N. J. ed. El género *Heleobia* (Caenogastropoda: Cochliopidae) en América del Sur. **Amici Molluscarum** (número especial):20-22.
- CIOCCO, N. F. & SCHEIBLER, E. E. 2008. Malacofauna of the littoral benthos of a saline lake in southern Mendoza, Argentina. **Fundamental and Applied Limnology** 172(2):87-98.
- COLLADO, G. A.; VALLADARES, M. A. & MENDEZ, M. A. 2013. Hidden diversity in spring snails from the Andean Altiplano, the second highest plateau on Earth, and the Atacama Desert, the driest place in the world. **Zoological Studies** 52:1-13.
- DAVIS, G. M. & PONS DA SILVA, M. C. P. 1984. *Potamolithus*: morphology, convergence, and relationships among hydrobioid snails. **Malacologia** 25(1):73-108.
- DE FRANCESCO, C. G. 2007. Las limitaciones a la identificación de especies de *Heleobia* Stimpson, 1856 (Gastropoda, Rissooidea) en el registro fósil del Cuaternario Tardío sus implicancias paleoambientales. **Ameghiniana** 44(3):631-635.
- DE FRANCESCO, C. G. & HASSAN, G. S. 2009. The significance of molluscs as paleoecological indicators of freshwater systems in central-western Argentina. **Palaeogeography, Palaeoclimatology, Palaeoecology** 274(1-2):105-113.
- DE FRANCESCO, C. G. & ISLA, F. I. 2004. Reproductive period and growth rate of the fresh-water snail *Heleobia parchappii* (d'Orbigny, 1835) (Gastropoda: Rissooidea) in a shallow brackish habitat (Buenos Aires Province, Argentina). **Malacologia** 45:443-450.
- FALNIOWSKI, A. & SZAROWSKA, M. 2000. A new species of *Daphniola* Radoman, 1973 (Gastropoda: Hydrobiidae) from Greece. **Folia Malacologica** 8:181-188.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. **Evolution** 39:783-791.
- FOLMER, O.; BACK, M.; HOEH, W.; LUTZ, R. & VRIJENHOEK, R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. **Molecular Marine Biology and Biotechnology** 3:294-299.
- GAILLARD, M. C. & DE CASTELLANOS, Z. A. 1976. Mollusca, Gasteropoda, Hydrobiidae. In: RINGUELET, R. A. ed. **Fauna de agua dulce de la República Argentina**, 15(2). Buenos Aires, Fundación para la Educación, la Ciencia y la Cultura (FECIC), p. 1-39.
- UTIERREZ GREGORIC, D. E.; CIOCCO, N. F. & RUMI, A. 2014. Two new species of *Chilina* Gray from Cuyo Malacological Province, Argentina (Gastropoda: Hygrophila: Chiliniidae). **Molluscan Research** 34(2):84-97.
- HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. **Nucleic Acids Symposium Series** 41:95-98.
- HERSHLER, R.; MULVEY, M. & LIU, H. P. 1999. Biogeography in the Death Valley region: evidence from spring snails (Hydrobiidae: Tryonia). **Zoological Journal of the Linnean Society** 126(3):335-354.
- HERSHLER, R. & THOMPSON, F. G. 1992. A review of the aquatic gastropod subfamily Cochliopinae (Prosobranchia: Hydrobiidae). **Malacological Review** 5:1-140.
- KABAT, A. R. & HERSHLER, R. 1993. The prosobranch snail family Hydrobiidae (Gastropoda: Rissooidea): review of classification and supraspecific taxa. **Smithsonian Contributions to Zoology** 547:1-94.
- KROLL, O.; HERSHLER, R.; ALBRECHT, C.; TERRAZAS, E. M.; APAZA, R.; FUENTEALBA, C. & WILKE, T. 2012. The endemic gastropod fauna of Lake Titicaca: correlation between molecular evolution and hydrographic history. **Ecology and Evolution** 2(7):1517-1530.
- LIU, H. P.; HERSHLER, R. & THOMPSON, F. G. 2001. Phylogenetic relationships of the Cochliopinae (Rissooidea: Hydrobiidae): an enigmatic group of aquatic gastropods. **Molecular Phylogenetics and Evolution** 21:17-25.
- LÓPEZ ARMEGOL, M. F. 1996. Taxonomic revision of *Potamolithus agapetus* (Pilsbry, 1911) and *Potamolithus buschii* (Frauenfeld, 1865) (Gastropoda: Hydrobiidae). **Malacologia** 38(1/2):1-17.
- MARCUS, E. & MARCUS, E. 1963. Mesogastropoden von der Küste São Paulos. **Abhandlungen der Mathematisch-Naturwissenschaftlichen Klasse. Akademie der Wissenschaften und der Literatur in Mainz** 1:32-105.
- _____. 1965. On Brazilian supra-tidal and estuarine snails. **Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo, Zoologia** 25:18-103.
- MARTÍN, P. R. 2002. Evidence for parthenogenesis and natural imposex in the Patagonian freshwater snail *Heleobia hatcheri* (Gastropoda: Hydrobiidae). **Journal of Molluscan Studies** 68:291-295.
- NEVES, R. A. F.; VALENTIN, J. L. & FIGUEROA, G. M. 2010. Morphological description of the gastropod *Heleobia australis* (Hydrobiidae) from egg to hatching. **Brazilian Journal of Oceanography** 58(3):247-250.
- NÚÑEZ, V.; GUTIERREZ GREGORIC, D. E. & RUMI, A. 2010. Freshwater gastropod provinces from Argentina. **Malacologia** 53:47-60.
- RUMI, A.; GUTIERREZ GREGORIC, D. E.; NÚÑEZ, V. & DARRIGRAN, G. A. 2008. Malacología latinoamericana. Moluscos de agua dulce de Argentina. **Revista de Biología Tropical** 56:77-111.
- TAMURA, K. & NEI, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. **Molecular Biology and Evolution** 10:512-526.
- TAMURA, K.; STECHER, G.; PETERSON, D.; FILIPSKI, A. & KUMAR, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. **Molecular Biology and Evolution** 30:2725-2729.
- WILKE, T.; DAVIS, G. M.; FALNIOWSKI, A.; GIUSTI, F.; BODON, M. & SZAROWSKA, M. 2001. Molecular systematics of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships. **Proceedings of the Academy of Natural Sciences of Philadelphia** 151:1-21.
- WILKE, T.; HAASE, M.; HERSHLER, R.; LIU, H. P.; MISOF, B. & PONDER, W. 2013. Pushing short DNA fragments to the limit: phylogenetic relationships of 'hydrobioid' gastropods (Caenogastropoda: Rissooidea). **Molecular Phylogenetics and Evolution** 66(3):715-736.
- WILKE, T.; ROLÁN, E. & DAVIS, G. M. 2000. The mudsnail genus *Hydrobia* ss in the northern Atlantic and western Mediterranean: a phylogenetic hypothesis. **Marine Biology** 137(5-6):827-833.