A molecular contribution to the controversial taxonomical status of some freshwater snails (Caenogastropoda: Rissooidea, 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: Rissooidea). As with other rissooidean 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 Pilbsry & Rush, 1896.

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

RESUMEN. Una contribución molecular al controvertido estatus taxonómico de un grupo de caracoles dulceacuicos (Caenogastropoda: Rissooidea, 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: Rissooidea). Como sucede con otras familias de rissooideos, la abundancia de caracteres convergentes y la escasez de sinapomorfías 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 Pilbsry y Rush, 1896.

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

Cochliopidae is a family of rissooidean 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 Bouche & 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. Kabay & Hershler, 1993; Falniowski & 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 Tittica 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; “parchappii”: 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), Cochliophoridae (5), Physidae (4), Planorbidae (2), Lymnaeidae (2), and Chilinidae (4) (*Núñez et al.*, 2010; *Gutierrez Gregoric et al.*, 2014). Despite this low biodiversity, the cochliopid species from the CMP are interesting issues due to their confused taxonomical status.

The five Cochliophoridae 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 & Scheibling* (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 *mucal papilla* in all females studied (pseudohermaphroditism or natural imposex, *Martin*, 2002), the only reported sex in CMP populations where sex ratios have been studied (*Uspallata River, Martin*, 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 (*Strobeliella; 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 superfamilies *Rissooidea*; 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 Agüas 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 the 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 (10 mM Tris 1 mM EDTA, pH 8) and digested overnight in CTAB (Cetyltrimethylammonium bromide) buffer containing protease 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/RNAase 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. 1). 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 Pomatotopsis lapidaria (Sav, 1817), 4 dominant clades and several subclades (from top to bottom; Fig 1):

1. Clade Coeliliopidae was represented by 4 genera (Heleobia, Semisalsa, Heleobus and Tryonia), 3 subclades and 4 lineages:

Tab. 1. Taxon, collection locality data, reference and GenBank accession numbers for specimens analyzed in this study.

| Taxon                                      | Locality                  | Reference | GenBank accession number |
|--------------------------------------------|----------------------------|-----------|--------------------------|
| Benedictia baicalensis                     | Lake Baikal, Russia        | Wilke et al., 2013 | HQ623171                |
| Heleobia andicola andicola                | Patapati Island, Lake Titicaca, Bolivia | Kroll et al., 2012 | JQ973028                |
| Heleobia andicola culminae (Peru)          | Umayo Island, Lake Umayo, Peru | Kroll et al., 2012 | JQ973034                |
| Heleobia andicola culminae (Bolivia)       | Huairina, Lake Titicaca, Bolivia | Kroll et al., 2012 | JQ973030                |
| Heleobia andicola nevowi                  | Vilque Chico, Lake Titicaca, Peru | Kroll et al., 2012 | JQ973035                |
| Heleobia aperta                            | Chua, Lake Titicaca, Bolivia | Kroll et al., 2012 | JQ973036                |
| Heleobia australis                         | Mar Chiquita, Argentina    | Kroll et al., 2012 | JQ972708                |
| Heleobia hatcheri                          | Aguas Negras, San Juan, Argentina | this study | KM220905                |
| Heleobia kuesteri                          | Uspallata                  | this study | KM220904                |
| Heleobia lenguinus                         | Lake Langua Layo, Peru     | Kroll et al., 2012 | JQ973042                |
| Heleobia limariensis                       | Huasco River, Valdivia Chile | Kroll et al., 2012 | JQ973043                |
| Heleobia loensis                          | Lea River, Quilina Quila Chila | Kroll et al., 2012 | JQ973044                |
| Heleobia mirum                            | Ajilata, Lake Titicaca, Bolivia | Kroll et al., 2012 | JQ973046                |
| Heleobia occidentalis                      | Laguna Bededero, San Luis Argentina | this study | KM220907                |
| Heleobia ornata                           | Chincuta, Lake Titicaca, Peru | Kroll et al., 2012 | JQ973049                |
| Heleobia parchappai                       | Mar Chiquita, Argentina    | Kroll et al., 2012 | JQ972709                |
| Heleobia piscivum                         | Isla Martin Garcia         | this study | KM220906                |
| Heleobia poopoensis                       | Laca Jahuira River, Bolivia | Kroll et al., 2012 | JQ973050                |
| Heleobia saracochaes                      | Lake Saracocha, Peru       | Kroll et al., 2012 | JQ973051                |
| Heleobia sp.                              | Uspallata                  | this study | KM220908                |
| Heleobia umbicalata                       | Sol Island, Lake Titicaca, Bolivia | Kroll et al., 2012 | JQ973053                |
| Heleobus carrikeri                        | Oyster Pond, Falmouth, USA | Kroll et al., 2012 | JQ973019                |
| Hydrobia acuta acuta                      | Etang du Prévost, Hérault, France | Wilke et al., 2000 | AF278808                |
| Hydrobia acuta neglecta                   |                              | Davis et al., 1998 | AF253079                |
| Leptopyrgus taimui                        | Kawhin, New Zealand        | Haase, 2005 | AY361078                |
| Lithophyllum natricoides                  | Narow River, Poland        | Wilke et al., 2001 | AF367642                |
| Maridopyrgus murihula                     | Brown, New Zealand         | Haase, 2005 | AY361084                |
| Oxyauncola perimitata                     | Inagahua, New Zealand      | Haase, 2005 | AY361100                |
| Pomatotopsis lapidaria (outgroup)          | Cougar Island, Hudson River, USA | Liu et al., 2001 | AF354774                |
| Potamobius aguapatia                      | Isla Martin Garcia         | this study | KM220909                |
| Potamobius buschii                        | Isla Martin Garcia         | this study | KM220909                |
| Potamobius ribeirensis                    | Iporanga River, Sao Paulo, Brazil | Wilke et al., 2013 | JX970618                |
| Potamobius rieppopodarum                  | New Zealand                | Neiman et al., 2010 | GQ996429                |
| Semisalsa clamator                       | Pirovac Spring, Croatia    | Wilke et al., 2001 | AF367631                |
| Semisalsa foaziensis                      | Thermal Springs, Torretta, Montecatini Terme, Italy | Kroll et al., 2012 | JQ973023                |
| Semisalsa scamandri                      | Etang du Charnier, Saint Gilles, France | Kroll et al., 2012 | JQ973025                |
| Semisalsa staggerum                      | Kaakkenswaters, Zieriksee, The Netherlands | Kroll et al., 2012 | JQ973024                |
| Tryonia imitator                          | Peñasquitos Lagoon, California, USA | Herseifer et al., 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 [Heleobopsis carrieri (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, Opacuncula, Leptopyrgus, Potamopyrgus and Heleobia, grouped in two subclades:
   3.1. With Meridyopirgus, Opacuncula, Leptopyrgus, Potamopyrgus and
   3.2. Containing 3 species of Potamolithus, Heleobia hatcheri and morphotype Heleobia sp.

4. Clade Litophylidae 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 Cochliodiaceae (Heleobopsis carrieri, 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 Cochliodiaceae. 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 Cochliodiaceae species of the “parchappii group” should be recognized in the Centre-West of Argentina: H. parchappii and H. kuesteri. Heleobia parchappii is an 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, Llananelo and Bebebero saline’s from the CMP (Cioccoli & Schebeler, 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 Llananelo saline areas, should be considered as synonym of H. parchappii as was suggested by Cazzania (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. Cazzania (1980), based on 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 subandeau 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 Cochliodiaceae 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 Heleobopsis carrieri (both from hard USA waters; Hershley 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 Semisalsas 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 differ 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 (Biesi, 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 Martin (2002). This reproductive mode is infrequent in Cochliopidae. Moreover, unlike other parthenogenetic rissooideans such as Potamopyrgus, H. hatcheri is oviparous (Cazzangia, 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 (Strobeliella) was proposed for H. hatcheri (Cazzangia, 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 (Cazzangia, 2011). Although Hershler & Thompson (1992) maintained the synonymy of Strobeliella 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 (Dr. 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 sympathy the relatively soft waters of the CMP. Morphological studies indicate that the species have an identical radula, similar pigmentation in the prosbsciss 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. kwesteri. 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 Paleartic-Neartic 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 protubernance 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 (Martin, 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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