The South-American distribution, southernmost record, and genetic variability of Biomphalaria peregrina - a potential intermediate host of schistosomiasis

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Schistosomiasis remains a major parasitic disease, endemic in large parts of South America. Five neotropical species of *Biomphalaria* have been found to act as intermediate hosts of *Schistosoma mansoni* in natural populations, while others have been shown to be susceptible in experimental infections, although not found in the field. Among these potential intermediate hosts, *Biomphalaria peregrina* represents the most widespread species in South America, with confirmed occurrence records from Venezuela to northern Patagonia. In this study, we report the southernmost record for the species at the Pinturas River, in southern Patagonia, which finding implies a southward displacement of the limit for the known species of this genus. The identities of the individuals from this population were confirmed through morphological examination, and by means of two mitochondrial genes, *cytochrome oxidase subunit I* (*COI*) and *16S-rRNA*. With both markers, phylogenetic analyses were conducted to assess the pattern of genetic variation of *B. peregrina*, and to explore evolutionary relationships of these southernmost individuals from the Pinturas River through available DNA sequences for the species from various locations. In addition, we produced a potential distribution model of *B. peregrina* in South America and identified the environmental variables that best predict that distribution. The model was estimated through a maximum entropy algorithm and run with occurrence points obtained from several sources, including the scientific literature and international databases, along with climatic and hydrographic variables. Different phylogenetic analyses with either the *COI* or *16S-rRNA* sequences did not conflict, but rather gave very similar topological organizations. Two major groups were identified, with sequences from the Pinturas River grouping together with haplotypes from subtropical and temperate regions. The model

developed had a satisfactory performance for the study area. We observed that the areas with higher habitat suitability were found to be mainly linked to subtropical and temperate regions of South America between 15° and 45° south latitude, with different moderate- and low-suitability areas outside this range. We also identified the coldest temperatures as the main predictors of the potential distribution of this snail, which thermal driver could act as a climatic barrier for the spread of schistosomiasis into temperate regions. Nonetheless, susceptibility surveys would be required to evaluate if southern populations of *B. peregrina* still retain their potential as intermediate hosts of *S. mansoni*.

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Short title: *Biomphalaria peregrina*, potential distribution and genetic variability

ABSTRACT

Schistosomiasis remains a major parasitic disease, endemic in large parts of South America. Five neotropical species of *Biomphalaria* have been found to act as intermediate hosts of *Schistosoma mansoni* in natural populations, while others have been shown to be susceptible in experimental infections, although not found in the field. Among these potential intermediate hosts, *Biomphalaria peregrina* represents the most widespread species in South America, with confirmed occurrence records from Venezuela to northern Patagonia. In this study, we report the southernmost record for the species at the Pinturas River, in southern Patagonia, which finding implies a southward displacement of the limit for the known species of this genus. The identities of the individuals from this population were confirmed through morphological examination, and by means of two mitochondrial genes, *cytochrome oxidase subunit I* (*COI*) and *16S-rRNA*. With both markers, phylogenetic analyses were conducted to assess the pattern of genetic variation of *B. peregrina*, and to explore evolutionary relationships of these southernmost individuals from the Pinturas River through available DNA sequences for the species from various locations. In addition, we produced a potential distribution model of *B. peregrina* in South America and identified the environmental variables that best predict that distribution. The model was estimated through a maximum entropy algorithm and run with occurrence points obtained from several sources, including the scientific literature and international databases, along with climatic and hydrographic variables. Different phylogenetic analyses with either the *COI* or *16S-rRNA* sequences did not conflict, but rather gave very similar topological organizations. Two major groups were identified, with sequences from the Pinturas River grouping together with haplotypes from subtropical and temperate regions. The model developed had a satisfactory performance for the study area. We observed that the areas with higher habitat suitability were found to be mainly linked to subtropical and temperate regions of South America between 15° and 45° south latitude, with different moderate- and low-suitability areas outside this range. We also identified the coldest temperatures as the main predictors of the potential distribution of this snail, which thermal driver could act as a climatic barrier for the spread of schistosomiasis into temperate regions. Nonetheless, susceptibility surveys would be required to evaluate if southern populations of *B. peregrina* still retain their potential as intermediate hosts of *S. mansoni*.

Key words: Gastropoda, Genetic variation, Planorbidae, Potential distribution, South America

INTRODUCTION

Schistososomiasis is an acute and chronic parasitic disease that affects at least 258 million people worldwide. Seventy-eight countries are considered endemic for schistosomiasis, with the populations of 52 countries requiring preventive chemotherapy (World Health Organization, 2015). The disease is frequent in tropical and subtropical regions, particularly in poor communities, and in the Americas is caused by the trematode *Schistosoma mansoni* (Sambon, 1907) (Digenea); which species is transmitted by the freshwater snails of the genus *Biomphalaria* Preston, 1910; e.g. *Biomphalaria glabrata* (Say, 1818), *Biomphalaria tenagophila* (d'Orbigny, 1835), and *Biomphalaria straminea* (Dunker, 1848). Other snail species, such as *Biomphalaria peregrina* (d'Orbigny 1835), have been infected experimentally and are thus considered as potential hosts of *S. mansoni* (Paraense & Côrrea, 1973).

In the Americas, schistosomiasis currently occurs in Brazil, Venezuela, Surinam, Puerto Rico, the Dominican Republic, and on several islands of the Lesser Antilles, with recent evidence indicating a spread from northeastern Brazil southward. An expansion of the current disease-distribution area can be expected, since the geographical range of snails that can act as intermedial hosts (IHs) is wider than that of the pathogen (Pan American Health Organization, 2010). The southern area with the highest risk of establishing an endemic and a new focus of disease is located in the northeast of Argentina (the NEA Region), where the majority of the *Biomphalaria* species inhabit the major rivers of the Del-Plata basin. Five of those species are listed as potential IHs of schistosomiasis: *B. tenagophila*, *B. straminea*, *B. peregrina*, *B. orbignyi*, and *B. oligoza* (Rumi, 1991; Rumi & Vogler, 2014).

The identification and recognition of *Biomphalaria* species thus far has mainly relied on features of shell morphology and the reproductive system (*cf.* Paraense, 1966, 1975, 2003; Rumi, 1991). Character similarity among the species, however, has in fact hampered classification (Paraense, 1988; Estrada et al., 2006). Within the historical context, several of the South-American species were described in genera of doubtful taxonomic position, as the example of *Taphius* Adams & Adams, 1855; *Biomphalaria* Preston, 1910; *Tropicorbis* Brown & Pilsbry, 1914; *Platytaphius* Pilsbry, 1924; and *Australorbis* Pilsbry, 1934. In addition, the original diagnoses were mostly made based only on shell characters. Both situations facilitated the generation of species of doubtful validity. Subsequently, the anatomical evidence has demonstrated that no differences really existed between the genera and the various taxa all

belonging to the same genus (Paraense, 1958). Although the oldest name was *Taphius*, in 1965 the International Committee of Zoological Nomenclature imposed the sole name *Biomphalaria*, considering that one to be the most widespread in the world (Barbosa et al., 1961; Paraense, 2008). More recently, though, difficulties concerning morphological identification have been overcome through the use of molecular-genetic techniques that have contributed to delimiting species, mainly those occurring in the Neotropics (e.g. Caldeira et al., 1998, 2000; Vidigal et al., 1998, 2000, 2001, 2002, 2004; Spatz et al., 1999, 2000; Velázquez et al., 2002; Estrada et al., 2006; Standley et al., 2011; Collado & Méndez, 2012).

Among the *Biomphalaria* species in South America, *B. peregrina* exhibits one of the most widespread distributions –and one involving a great diversity of hydrologic systems– with that species thus far having been recorded from Venezuela to northern Patagonia, Argentina. In the study reported here, the presence of *B. peregrina* in southern Patagonia is now documented for the first time, that location being the southernmost record for the species –and the genus as well– worldwide. In order to confirm the identity of this most southerly population, we assessed the main conchologic and anatomical diagnostic characters (e.g. shell, genitalia, and radula) and obtained DNA sequences of the mitochondrial *cytochrome oxidase subunit I* (*COI*) and the *16S-rRNA* genes. Based on both markers, we examined the phylogenetic position of the recently discovered population and assessed the pattern of genetic variation by comparison with available data for *B. peregrina* from GenBank. In addition, upon consideration that *B. peregrina* represents a potential host for schistosomiasis, we produced a predictive model of the species's spatial distribution in South America and identified the environmental variables that best predict its location. The resulting model indicating the likely whereabouts of that potential host will hopefully provide further guidance for future efforts aimed at schistosomiasis surveillance and control.

MATERIALS AND METHODS

The material analyzed (19 specimens) came from the Pinturas River, Santa Cruz province, Argentina (Gatherer: Hugo Merlo Álvarez, col. Date: 15-XI-2013, geographical coordinates: 46° 50' 6' S; 70° 27' 38" W; 355 m above sea level). The specimens studied were deposited in the malacological collections at the Museo de La Plata (DZI-MLP-Ma), Buenos Aires province (MLP-Ma N° 14186).

112

113 *Morphological examination*

114 The adult specimens were analyzed according to Paraense (1966, 1975) and Rumi (1991).
115 The morphology of the shell, the radula, and the other anatomical features (certain aspects of the
116 reproductive system) were analyzed. The soft parts were separated from the shell for subsequent
117 processing and fixed in Railleit-Henry or 90% (v/v) aqueous alcohol. Shell measurements
118 (maximum and minimum diameter and height) were obtained with a Mitutoyo digital calipter.
119 The dissection was done under a stereoscopic binocular microscope (LEICA MZ6).

120 The radula and jaw were cleaned following Holznagel (1998): the structures were
121 separated from the mass of tissue and placed in 1.5-ml microtubes containing 500 µl NET buffer
122 (1 ml 1M Tris pH 8.0, 2 ml 0.5 M ethylenediaminetetraacetic acid, 1 ml 5 M NaCl, 20 ml 10%
123 [w/v] sodium dodecyl sulfate, 76 ml water) and 10 µl of Proteinase K (20 mg/ml) were added.
124 The samples were then incubated at 37 ºC with a subsequent renewal of the NET buffer and
125 Proteinase K to verify the absence of tissue. After two washes with distilled water, 25% (v/v)
126 aqueous ethanol was added for preservation. Finally the radula and jaw were examined by
127 scanning electron microscopy (JEOL 6360) in the Museum of La Plata. The radular formula
128 gives the number of teeth per row: [(number of left teeth) + (number of central teeth) + (number
129 of right teeth)] plus the number of transverse rows.

130

131 *DNA extraction, polymerase chain reaction (PCR) and DNA sequencing*

132 Total genomic DNA was extracted from the foot muscle of five individual snails through
133 the use of the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) according to the
134 manufacturer's protocol. Partial sequences of the mitochondrial *cytochrome oxidase subunit I*
135 (*COI*) and the *16S-rRNA* genes were amplified by means of the primers LCO1490 (5'–GGT CAA
136 CAA ATC ATA AAG ATA TTG G–3') and HCO2198 (5'–TAA ACT TCA GGG TGA CCA
137 AAA AAT CA–3') for *COI* (Folmer et al., 1994), and 16SF-104 (5'–GAC TGT GCT AAG GTA
138 GCA TAA T–3') and 16SR-472 (5'–TCG TAG TCC AAC ATC GAG GTC A–3') for *16S-rRNA*
139 (Ramírez & Ramírez, 2010). The amplification of the *COI* region was conducted following
140 Vogler et al. (2014). The amplification of *16S-rRNA* was performed in a total volume of 30 µl
141 containing 30–50 ng of template DNA, 0.2 µM of each primer, 1X PCR green buffer, 0.2 mM
142 dNTPs, and 1 U Dream *Taq* DNA Polymerase (Thermo Scientific). The thermocycling profile

143 was 35 cycles of 30 s at 94 °C, 30 s at 48 °C, 1 min at 72 °C followed by a final extension of 1
144 min at 72 °C. The amplifications were run on a T18 thermocycler (Ivema Desarrollos). The PCR
145 products were purified by means of a ADN PuriPrep-GP kit (InBio-Highway, Tandil, Buenos
146 Aires). After purification, both DNA strands for each gene were then directly cycle-sequenced
147 (Macrogen Inc., Seoul, Korea). The resulting sequences were trimmed to remove the primers, and
148 the consensus sequences were visually edited by means of the BioEdit 7.2.5 software (Hall,
149 1999).
150
151 *Phylogenetic analysis*
152 Phylogenetic analyses were conducted in order to confirm the morphology-based
153 identification and to explore possible evolutionary relationships of the genetic sequences from the
154 southernmost individuals from the Pinturas River to those of other *B. peregrina* individuals from
155 various locations available in GenBank (Table 1). The phylogenetic analyses were carried out
156 separately for each mitochondrial region as follows: the sequence alignment was performed with
157 Clustal X 2.1 (Larkin et al., 2007), and optimized by visual inspection. The total lengths of the
158 matrices analyzed were 546 bp for the *COI* gene, and 269 bp for the *16S-rRNA* locus. The data
159 were subjected to four different phylogenetic analyses by the methods of neighbor joining (NJ),
160 maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). The NJ
161 analysis was conducted with MEGA 6.06 (Tamura et al., 2013) through the use of the Kimura's
162 two-parameter (K2P) substitution model. The MP and ML analyses were carried out with
163 PAUP*4.0b10 (Swofford, 2002). The MP was conducted by means of a heuristic search with the
164 characters equally weighted, tree bisection and reconnection, branch-swapping, and 10 random
165 stepwise additions. The optimal model of nucleotide substitution for ML inference was evaluated
166 by the likelihood-ratio test and selected by means of the corrected Akaike Information Criterion
167 with Jmodeltest 2.1.7 (Darriba et al., 2012). The TVM+I (for *COI*), and the TIM1+I (for *16S-*
168 *rRNA*) substitution models were used as evolutionary paradigms. Statistical support for the
169 resulting phylogenies was assessed by the bootstrap method with 1,000 replicates (Felsenstein,
170 1985). BI was performed with Mr. Bayes 3.2.6 (Ronquist et al., 2012). Two runs were conducted
171 simultaneously with 4 Markov chains that went for 10^6 generations, sampling every 100
172 generations. The first 10,001 generations of each run were discarded as burn-in, and the
173 remaining 18,000 trees were used to estimate posterior probabilities. In addition, the number of

haplotypes in the dataset was explored with DnaSP 5.10 (Librado & Rozas, 2009) and the genetic distances estimated in MEGA 6.06 through the use of the number of differences (*p*) and the K2P-substitution model. Since we obtained shorter *16S-rRNA* sequences than previously reported for *B. peregrina* from Argentina that did not include previously characterized molecular diversity (Standley et al., 2011), the *COI* data were employed only for estimating the number of haplotypes and genetic distances.

Species-distribution model

The study area comprised the South-American countries: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, Uruguay and Venezuela. The occurrence data for *B. peregrina* were retrieved from the scientific literature, and from malacological collections and international databases (Table 2). All together, 689 spatially unique records were used. When the coordinates of localities were lacking, those data were derived secondarily following Wieczorek et al. (2004). Twenty-three environmental variables were used as predictors; comprising 19 climatic, 3 hydrologic, and 1 topographical (Table 3). The variables were downloaded from WorldClim (http://www.worldclim.org) and HydroSHEDS (http://hydrosheds.cr.usgs.gov) at a spatial resolution of 30 arc seconds (~1 km^2). WorldClim and HydroSHEDS provide climatic information derived from weather stations spanning 1950–2000 and hydrographic data obtained from a STRM digital-elevation model, respectively (Hijmans et al., 2005; Lehner et al., 2008). These variables have been commonly employed for generating distribution models in gastropods, including freshwater members, such as those belonging to the genus *Biomphalaria* (Scholte et al., 2012; Vogler et al., 2013; Pedersen et al., 2014; Beltramino et al., 2015; Martín et al., 2016). All environmental layers were trimmed to the study area. The potential-distribution model was estimated by using a maximum entropy algorithm in MaxEnt 3.3.3k (Phillips et al., 2006; Phillips & Dudík, 2008). The data were randomly divided into the training data (75% of occurrences) and the model-testing data (the remaining 25%). The output was computed as *logistic*, which setting returns a map with an estimated probability ranging between 0 (no probability of the species presence) and 1 (high probability of presence). The resulting model was assessed by estimating the area under the receiver-operating-characteristic curve (ROC-curve analyses; Fielding & Bell, 1997). The relative contribution of variables to the

development of the model was evaluated by means of a jackknife test and through the response curves obtained in MaxEnt following Meichtry de Zaurlín et al. (2016).

RESULTS

Morphological examination

Shell (Fig. 1, panel A). The empty shell is fundamentally light brown in color, with the growth lines clearly visible. The whorls, up to 5¾ in number, increase slowly and display a rounded surface on both sides. The shells exhibit a more or less marked deflection of the outer whorl to the left. The larger diameter was 13.5 mm (mean = 9.77 mm, SD = 1.54 mm, n = 12); the smaller 8.98 mm (mean = 7.53 mm, SD = 1.40 mm, n = 11). The greatest height has been 4.6 mm (mean = 3.86 mm, SD = 0.64 mm, n = 12).

Radula (Fig 1, panels B to E). Central tooth rather asymmetrical, bicuspid, with or without accessory cups, the base without special features. Number of rows is 106 and of teeth per half row (except for the central tooth) 20, of which 8 are lateral and 12 marginal. The first lateral tooth is tricuspid, with the mesocone more developed, the border free from the rounded, mesocone below or in the shape of a sword point. A crest with a central depression toward the posterior part of the tooth is evident. Finally, the marginal teeth are without special features. Radular formula: [20-1-20] 106.

Jaw. The features correspond to the standard description of the species.

Genital system. The specimens showed the typology described for *B. peregrina*, without any special variations.

Phylogenetic analysis

The molecular-genetic characterization of the specimens from the Pinturas River confirmed their identity as *B. peregrina*. Partial DNA sequences consisted of 655 bp for *COI* and 265 bp for *16S-rRNA*. Both markers contained no variation among the five individuals sequenced, resulting in the existence of only one haplotype per marker. After the inclusion of GenBank sequences from other locations with subsequent alignment, six haplotypes were identified within the *COI* dataset (Fig. 2). The sequence divergence among haplotypes is presented in Table 4. Different phylogenetic analyses with either the *COI* or *16S-rRNA* marker did not conflict; rather, both loci gave very similar topological organizations for the NJ, MP, and

BI trees with minor differences in the ML-tree organization. In all instances, two major groups were identified, referred to as the *tropical* and *temperate* clades (Figs. 2 and 3). In terms of that subdivision, the specimens from the Pinturas River were placed within the temperate group.

Species-distribution model

Fig. 4 illustrates the potential distribution area for *B. peregrina*. The model conformed well to expectations, with values for the area under the curve of 0.927 for the training data and 0.905 for the test data, with a standard deviation of 0.009. The areas with higher probability of the snail's presence were found to be mainly linked to subtropical and temperate regions of South America between 15° and 45° south latitude, comprising central and northeastern Argentina, central Chile, eastern Paraguay, southeastern Brazil, and southern Bolivia and Uruguay. In addition, regions with a moderate to high habitat suitability were predicted for Peru, Ecuador, Colombia, and Venezuela. The areas of lower habitat suitability were located in French Guiana, Guyana, Suriname, Venezuela, and a large area of Brazil (Fig. 4, panel B). The jackknife test showed that the mean temperature of the coldest quarter (bio11), the minimum temperature of coldest month (bio6) and the annual mean temperature (bio1) were the variables that most greatly influenced the model development when used in isolation (Fig. 5, panel A). The flow accumulation produced a reduction in training gain when removed from the model, thus indicating that that variable contained information necessary for the model. The remaining predictors contributed less to the modelling. Fig. 5, panel B contains the marginal-response curves for the four strongest environmental predictors – i.e. the mean temperature of the coldest quarter, the minimum temperature of the coldest month, the annual mean temperature, and the flow accumulation.

DISCUSSION

The conchology and anatomy of the reproductive system of the specimens from the Pinturas River were consistent with the descriptions of Paraense & Deslandes (1956) and Paraense (1966) for *B. peregrina*. The shells of the individuals from that river recall the original descriptions by the first authors for *Australorbis inflexus* (nowadays considered synonymous with *B. peregrina*; Paraense, 1966) from Pouso Alegre, Mina Gerais, Brazil because of the strong inflection of the aperture located toward the left side. Nevertheless, the specimens from the

Pinturas River have much wider and rounder shells on both sides than those described for *A. inflexus*. The radulae of the individuals from south Patagonia have a folding and a groove between the cuspids of the central tooth that has not been described for other populations of this species.

In addition, the specimens from the Pinturas River were confirmed genetically as being *B. peregrina* and no intrapopulational sequence variation was detected in the mitochondrial-marker loci examined. Likewise, Standley et al. (2011) reported the absence of variation in the *COI* marker for the population studied from Agua Escondida, Mendoza, Argentina. Those authors suggested that the lack of variation in that population could be owing to a founding event in recent years since the species of *Biomphalaria* are hermaphroditic and capable of rapidly colonizing a new locality, even from a sole individual founder. This explanation would appear to be also valid for the lack of genetic variation evidenced in the *B. peregrina* population from the Pinturas River, although studies based on a greater number of specimens would be required to confirm this hypothesis. Nevertheless, the phylogenetic trees revealed that the species can be considered as divided into two clades, here referred to as the tropical and the temperate. For the *COI* marker, the first clade comprised exclusively *B. peregrina* individuals from Brazil, whereas the second included specimens from southern Brazil and Argentina. For the *16S-rRNA*, the sequences from Brazil all grouped together within the tropical clade, whereas the temperate clade included only sequences from Argentina and Uruguay. These clades could be linked to the biogeography and ecologic history of *B. peregrina* in terms of the colonization of freshwater environments. Nonetheless, of pertinence to emphasize here is that although *B. peregrina* possesses a wide distribution in South America, at the present time only few DNA sequences are available in Genbank. Therefore, investigations focussing on the phylogeography of this species are required to acquire a comprehensive understanding of its evolutionary history, similar to those carried out for *B. glabrata* (Mavárez et al., 2002; DeJong et al., 2003), especially since *B. peregrina* has been shown to occupy a basal position in the phylogenetic analyses of the genus (DeJong et al., 2001; Jarne et al., 2011).

Biomphalaria peregrina –as its name might suggest– is the species most widely distributed in South America within the genus. The occurrence of representative individuals from Venezuela down to one of the most southern areas of Patagonia in the Province of Santa Cruz demonstrates that the species possesses an ample range of environmental tolerance. Even so, the

PeerJ reviewing PDF | (2016:12:15126:0:1:NEW 18 Dec 2016)

historical registration of *B. peregrina* and the area of its potential distribution described here nevertheless indicate that the species's greatest abundance and dispersion are within the Atlantic corridor in the south of Brazil as well as in the northeast and pampean region of Argentina. According to the results obtained here, and in agreement with the findings of Rumi (1991), the distribution of *B. peregrina* involves the most southerly areas of the Great-Del-Plata basin, then spreads to the west through the endorrheic basins of Córdoba –the ancient beds of the Paraná River– toward the Andes region, and finally, remaining east of the Andes, extends to the north of the continent up to Venezuela, where the registers become quite scarce. To the south, the distribution stretches from the pampas of the Buenos Aires province, which area corresponds to the central-Argentine malacological region V (Núñez et al., 2010), to the west and to the south, occupying areas on both sides of the Andes range. On the Argentine side, *B. peregrina* inhabits the malacologic regions VI in Cuyo, VII in northern Patagonia, and to the south reaches the region VIII in southern Patagonia (Núñez et al., 2010). On the Chilean side of the Andes, the species is dispersed from the region IX to IV.

As to the distribution of *Biomphalaria* in South America (*cf.* Teles et al., 1991; Teles, 1996; Scholte et al., 2012), and especially with reference to *B. glabrata*, *B. tenagophila*, and *B. straminea* –natural propagators of schistosomiasis– along with *B. peregrina*, various authors have proposed that the patterns observed could be attributed to, among other causes, a competitive exclusion or displacement among themselves. For this reason, their ecological functionality in the face of certain adverse aspects of their local environment could be considered critical for their development at a given site, such as their inherent resistance to draught and their tolerance to extremes in temperature (Tuan et al., 2012). This same argument may be advanced for *B. peregrina*. When the results of studies along those lines worldwide are analyzed (*cf.* Barbosa, 1973, 1987; Michelson & Dubois, 1979; Monteiro & Ferreira Dias, 1980; Luz et al., 1982; Olazarri, 1984, Barbosa et al., 1984, 1985, 1993), the conclusion can be drawn that, in general, *B. straminea* and *B. tenagophila* prove to be species that are more competitively aggressive than *B. glabrata* (better IH and also more susceptible to parasitosis by *S. mansoni*). On the contrary, *B. peregrina* –it only a potential host– could be competitively displaced by all three of the other species. If, however, we restrict the analysis along these lines to the degree of competitiveness and aggressiveness that the four species could manifest with respect to each other within the Argentine southern cone, we could infer the possibility that *B. tenagophila* and *B. straminea* have

obstructed the southern dispersion of *B. glabrata*, along with other influences. In the same way, those two more aggressive species have been able to displace *B. peregrina* toward a southward direction since the latter would apparently be a species better adapted to subtropical or temperate climates – just as these present results would suggest, where the environmental variables that most greatly influenced the potential-distribution model were the coldest temperatures.

Each species of *Biomphalaria* that is a natural or potential host for *S. mansoni* consists in populations that exhibit varying degrees of susceptibility to different local strains of the parasite (Paraense & Côrrea, 1973, 1978, 1985; Coelho et al., 2004; Simões et al., 2013; Marques et al., 2014). The susceptibility to *S. mansoni* has been shown to be heritable and linked to the gene pool of the IHs, each of which –according to its capability for reproduction through cross- or self-fertilization– produces progeny with differing degrees of parasitotrophic susceptibility (Newton, 1953; Richards & Merritt, 1972; Richards, 1973, 1975; Coelho et al., 2004). As mentioned above in Results under the investigation of the genetic variability of *B. peregrina*, the haplotypes were found to be bifurcated into two, those from tropical areas and those inhabithing the southern cone of South America, where the climate goes from subtropical to temperate in the Patagonian region to the south. These results are highly relevant since Paraense & Côrrea (1973) demonstrated experimentally that populations of *B. peregrina* from Lapa of Paraná (in Brazil), and Chillogallo (in Ecuador) are markedly susceptible to infection with the BH (Belo-Horizonte) and SJ (San-Jose) strains of *S. mansoni*, though these *B. peregrina* strains have not yet been found to be infected in the wild. Although the susceptibility to different strains of *S. mansoni* of populations of *B. peregrina* that inhabit subtropical or temperate areas have still not been evaluated, other *Biomphalaria* species such as *B. tenagophila* and *B. straminea* in the northeast of Argentina have indeed been found to serve as IHs (Borda & Rea, 1997; Simões et al., 2013). Moreover, as has been demonstrated for other species of snails, variable rates of infection exist among populations that do not depend on the species' geographical origin, but rather on their genetic diversity (Mohamed et al., 2012 and references therein). In fact, Carvalho et al. (2001) had observed that the genetics of the snail host may be even more influential on the epidemiology of schistosomiasis than those of the parasite itself. Because of this possibility, new studies on the susceptibility and genetic variation of diverse variants and morphotypes of *B. peregrina* are needed involving populations that contain the haplotypes identified here as subtropical and temperate. For example, those individuals characterized within the snails at the Pinturas River,

for their part, presented morphologic similarities to a morphotype of *B. peregrina* registered in Mina Gerais, Brazil; but that Brazilian variant, in contrast, was demonstrated experimentally to not be susceptible to parasitism by *S. mansoni* (Paraense & Deslandes, 1956). Thus, determinations of this nature are fundamental for delineating the potential area of occupation of *B. peregrina* and obtaining a more realistic approximation of the corresponding potential zone of occupation of populations susceptible to parasitism, which information could be associated with the possible appearance of foci of infection and a southward dispersion of the endemium.

With respect to region, *B. peregrina* has a relatively high incidence of parasitism by digenean species, i.e. at least 25 taxa from 7 families (Flores et al., 2010 and references therein). If we consider the current distribution of the Schistosomatidae in South America (Fernández et al., 2013; Ostrowski de Núñez & Hamann, 2013), in general the majority of the schistosome cercariae have thus far been restricted to the northeast of Argentina, which region presents hydrologic and climatic conditions similar to those of the south of Brazil. The registers of these parasites have, however, reached more southern locations; namely, in the Quequén River (38° 32' S; 58° 42' W) in the southeast of the Buenos Aires province between the Tandilia and Ventania mountains, where the mean annual temperature registered between 1961 and 1990 was 14 ºC (Kruse et al., 1997). Along these lines, schistosome cercariae have been described for Argentina that use *B. peregrina* as an IH: for example, *Cercaria quequeni* Szidat, 1951 and *Cercaria planorbicola* Szidat & C. de Szidat, 1960 (Fernández et al., 2013). Moreover, *C. planorbicola* is similar to *Furcocercaria* sp. XVIII, but uses *B. straminea* as an IH (Fernández et al., 2013). Specimens of this *Furcocercaria* sp. XVIII were collected in a rice field in the Corrientes province and were found to be rare species; being present only in March (during a survey period from December 2010 through May 2011), which monthly temperatures ranged from 19.5 to 30.5 °C (mean value, 24.3 ± 4.5 ºC). In accordance with the results from our model, the mean temperature of the coldest quarter (bio11), the minimum temperature of the coldest month (bio6), and the annual mean temperature (bio1) were the variables that most strongly influenced the development of the potential-distribution model for *B. peregrina*. Thus, the low temperatures of southern Patagonia very likely acted as a climatic barrier that functioned negatively in the survival of the free-living schistosome stages, the miracidia and cercariae; but not in the dispersion of the IHs, and especially *B. peregrina*.

Finally, as mentioned above, further reserch is still needed for a better understanding of the evolutionary history, ecology, parasitic susceptibility, and genetic variarion among the potential IHs of *S. mansoni* in South America, such as *B. peregrina*; which species is comparatively underrepresented in current research on planorbid snails despite its wide distribution in South America, as indicated by the evidence of the record of the new population described here in southern Patagonia.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Monika Hamann (Centro de Ecología Aplicada del Litoral, CONICET) for providing valuable comments on an early version of the manuscript, and Patricia Sarmiento (Museo de La Plata) who obtained the scanning photographs. Finally, the authors are grateful to Dr. Donald F. Haggerty, a retired academic career investigator and native English speaker, for translating the manuscript from the original Spanish and editing the final version.

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TABLES

Table 1. Information on the samples used in the phylogenetic reconstruction of *Biomphalaria peregrina*.

Sample	Geographical origin	Voucher #	GenBank accession #		Reference
			COI	*16S-rRNA*	
*B. tenagophila***	Mogi das Cruzes, São Paulo, Brazil	LBMSU547	KF926202	KF892001	Tuan et al. (2013)* Tuan & Palasio (2013)*
*B. pfeifferi***	Abu Usher, Sudan	–	DQ084835	DQ084857	Jørgensen et al. (2007)
B. peregrina	Pinturas River, Santa Cruz, Argentina	MLP-Ma14186	KY124272	KY124273	This work
	Agua Escondida, Mendoza, Argentina	–	GU168593	GU168591 GU168592	Standley et al. (2011)
	La Plata, Buenos Aires, Argentina	UCH La Plata1	JN621901	JF309030	Collado et al. (2011)
		UCH La Plata2	JN621902	JF309031	Collado & Méndez (2012)
		UCH La Plata3	JN621903	JF309032	
	Rancharia, São Paulo, Brazil	LBMSU584	KF926176	–	Tuan et al. (2013)*
	Bagé, Rio Grande do Sul, Brazil	LBMSU663	KX354439	–	Palasio & Tuan (2016)*
	Ipaussu, São Paulo, Brazil	LBMSU761	KX354440	–	Palasio & Tuan (2016)*
		LBMSU756	KX354441	–	Palasio & Tuan (2016)*
		LBMSU755	KX354442	–	Palasio & Tuan (2016)*
		LBMSU338	–	KF892035	Tuan & Palasio (2013)*
	Ourinhos, São Paulo, Brazil	LBMSU747	KX354443	–	Palasio & Tuan (2016)*
		LBMSU739	KX354444	–	Palasio & Tuan (2016)*
		LBMSU300	–	KF892034	Tuan & Palasio (2013)*
	Martinópolis, São Paulo, Brazil	LBMSU582	KF926180	–	Tuan et al. (2013)*
		LBMSU581	KX354445	KF892036	Palasio & Tuan (2016)* Tuan & Palasio (2013)*
	Nova Lima, Minas Gerais, Brazil	–	–	AY030232	DeJong et al. (2001)
	San Antonio, Uruguay	–	–	AY030231	DeJong et al. (2001)

*GenBank unpublished sequences: the sequence author and submission year are indicated. **Outgroup species. LBMSU, Laboratório de Bioquímica e Biologia Molecular, Superintendência de Controle de Endemias do Estado de São Paulo, Brazil; MLP, Museo de La Plata, Argentina; UCH, Universidad de Chile, Chile.

748 **Table 2**. Sources of *Biomphalaria peregrina* occurrences in South America used in the distribution model.

Country	Occurrences	Sources consulted*
Argentina	343	d'Orbigny (1835); Paraense (1966); Bonetto et al. (1990); Castellanos & Miquel (1991); Rumi (1991); Rumi et al. (1996); Rumi et al (1997); Flores & Brugni (2005); Rumi et al. (2006, 2008); Ciocco & Scheibler (2008); Standley et al. (2011) Malacological collections: CECOAL; IFML; FIOCRUZ; MACN; MLP
Bolivia	8	Paraense (1966) *Malacological collections*: MACN
Brazil	241	Paraense (1966); Teles et al. (1991); Prando & Bacha (1995); De Jong et al. (2001); Pepe et al. (2009) *Malacological collections*: FIOCRUZ *Websites*: GenBank; WMSDB
Chile	24	Dunker (1848); Biese (1951); Barbosa et al. (1956) *Malacological collections*: FIOCRUZ; MACN
Colombia	1	*Website*: WMSDB
Ecuador	12	d'Orbigny (1835); Cousin (1887); Paraense (1966, 2004) *Malacological collections*: FIOCRUZ *Website*: WMSDB
Paraguay	29	In Quintana (1982): Paravicini (1894); Bertoni (1925); Schade (1965); Russel (1972); Moreno González (1981) *Malacological collections*: MACN
Peru	6	Paraense (2003) *Malacological collections*: FIOCRUZ *Website*: WMSDB
Uruguay	23	Paraense (1966); De Jong et al. (2001); Scarabino (2004) *Malacological collections*: FIOCRUZ; MACN *Website*: GanBank; WMSDB
Venezuela	2	*Website*: WMSDB

749

750 *CECOAL, Centro de Ecología Aplicada del Litoral; FIOCRUZ, Fundação Oswaldo Cruz; IFML, Instituto Fundación Miguel
751 Lillo; MACN, Museo Argentino de Ciencias Naturales; MLP, Museo de La Plata; WMSDB, Worldwide Mollusc Species Data
752 Base

753 **Table 3.** Variables used in the model development. Temperatures are expressed in ºC*10, precipitations in
754 mm, elevation above sea level in m, and flow accumulation in number of cells.

Variable	Description
alt	Altitude
bio1	Annual mean temperature
bio2	Mean diurnal range (monthly mean, T° max-T° min)
bio3	Isothermality (bio2/bio7) x 100
bio4	Temperature seasonality (standard deviation x 100)
bio5	Maximum temperature of warmest month
bio6	Minimum temperature of coldest month
bio7	Temperature annual range (bio5-bio6)
bio8	Mean temperature of wettest quarter
bio9	Mean temperature of driest quarter
bio10	Mean temperature of the warmest quarter
bio11	Mean temperature of coldest quarter
bio12	Annual precipitation
bio13	Precipitation of wettest month
bio14	Precipitation of driest month
bio15	Precipitation seasonality (coefficient of variation)
bio16	Precipitation of wettest quarter
bio17	Precipitation of driest quarter
bio18	Precipitation of the warmest quarter
bio19	Precipitation of the coldest quarter
acc	Flow accumulation
dir	Flow direction
con	Hydrologically conditioned elevation

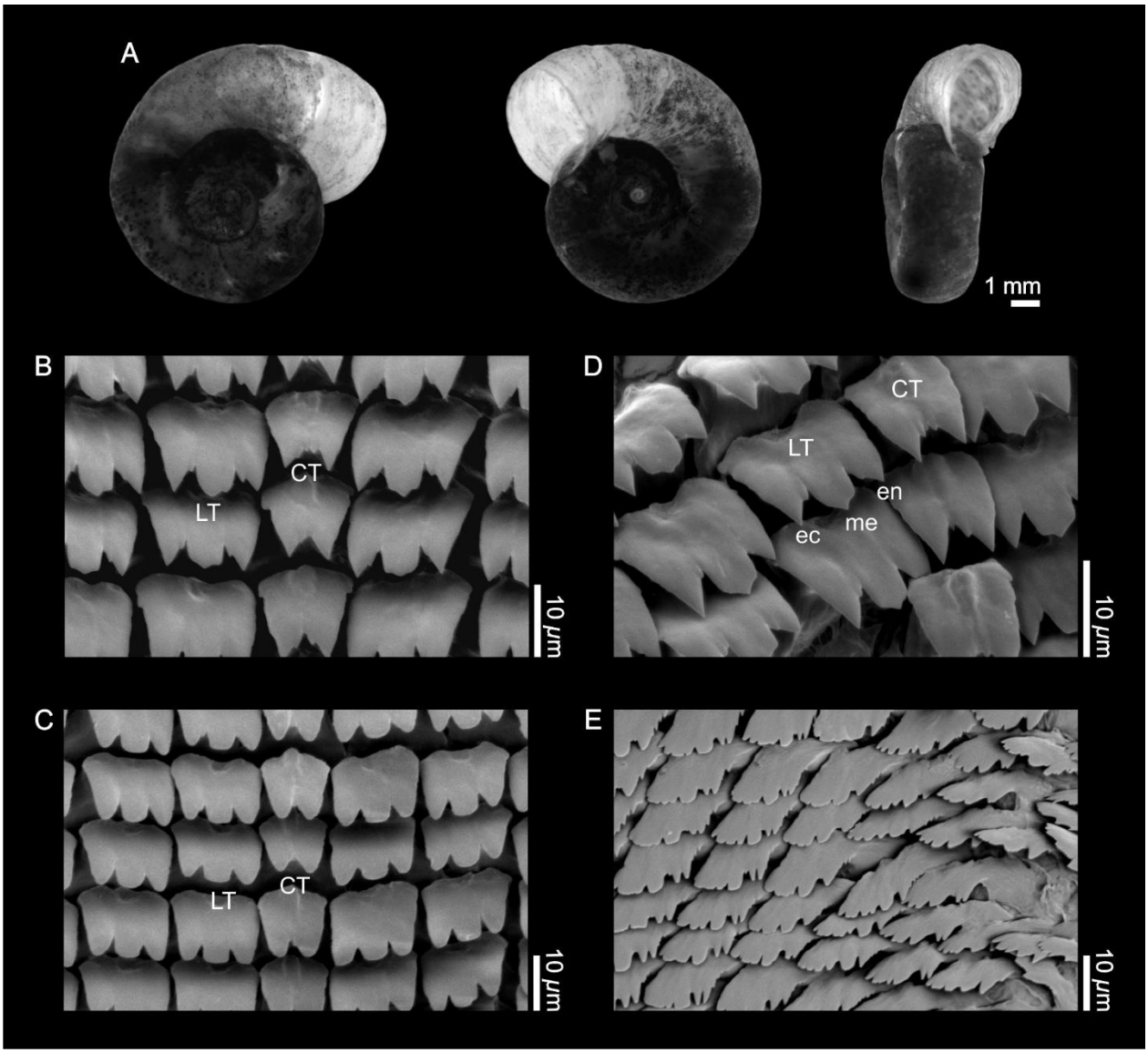
755

Table 4. Genetic distances among *COI* haplotypes of *Biomphalaria peregrina.* The distances are listed as uncorrected (below the diagonal) and corrected by the Kimura's two parameter substitution model (above the diagonal).

	H_1	H_2	H_3	H_4	H_5	H_6	GenBank accession numbers*
H_1	–	0.001834	0.003676	0.026321	0.032147	0.032147	H_1: KY124272; GU168593
H_2	0.001831	–	0.005525	0.028256	0.034104	0.034104	H_2: JN621901; JN621902; JN621903
H_3	0.003663	0.005494	–	0.030198	0.036068	0.036068	H_3: KX354439
H_4	0.025641	0.027472	0.029304	–	0.012987	0.012987	H_4: KF926176; KF926180; KX354445
H_5	0.031135	0.032967	0.034798	0.012820	–	0.011116	H_5: KX354441
H_6	0.031135	0.032967	0.034798	0.012820	0.010989	–	H_6: KX354443; KX354444

*References to the sequences are provided in Table 1.

761 **FIGURES**



762

763 **Figure 1. External shell morphology and radula of *Biomphalaria peregrina* from the**
764 **Pinturas River, Argentina.** Panel A: Right, left, and ventral views. Panels B–D: Detail of the
765 rachidian or central (CT) and lateral teeth (LT); ec, ectocone; en, endocone; me, mesocone. Panel
766 E: Detail of marginal teeth.

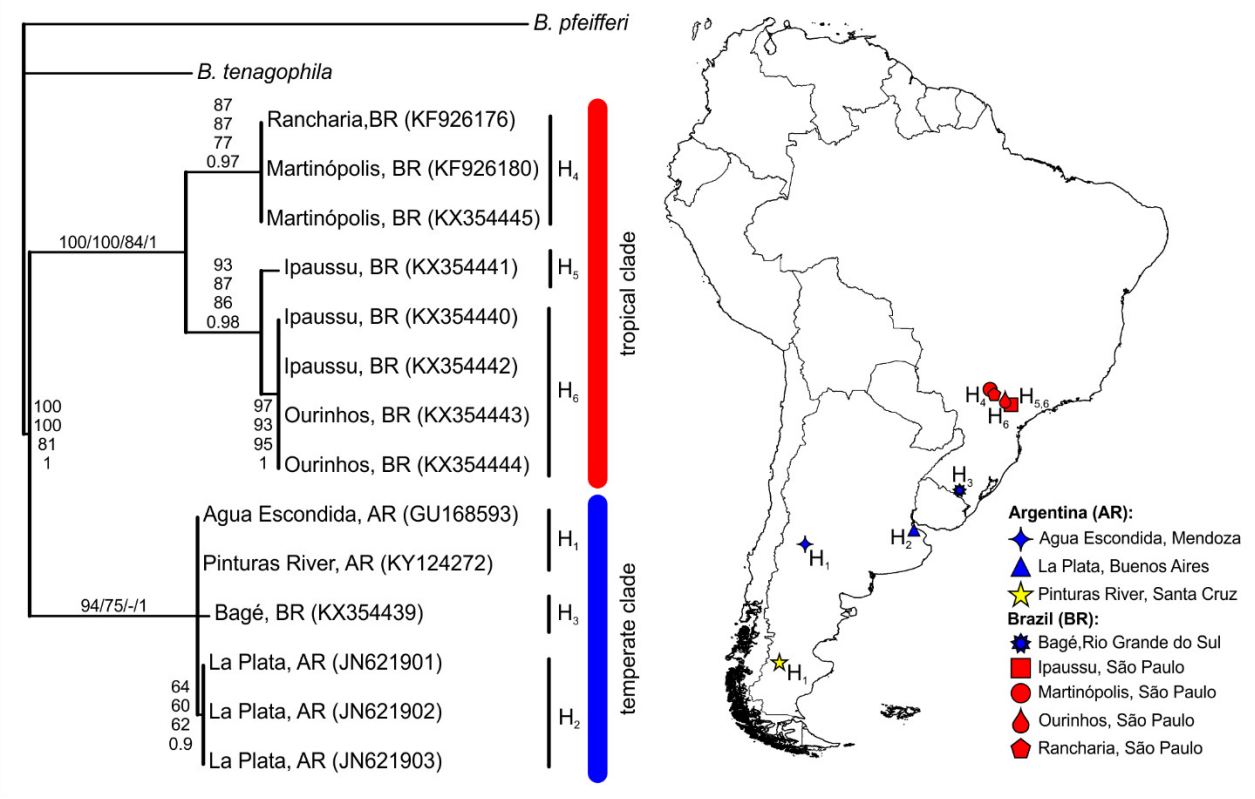


Figure 2. Bayesian tree of *Biomphalaria peregrina* based on the partial COI gene. The bootstrap values for the NJ, MP, ML trees and posterior-probability values for BI are shown above and below the branches. The numbers within parentheses are GenBank-accession numbers. The geographical distribution of the localities sampled and the haplotypes (H) is shown. The literature references to the sequences are given in Table 1.

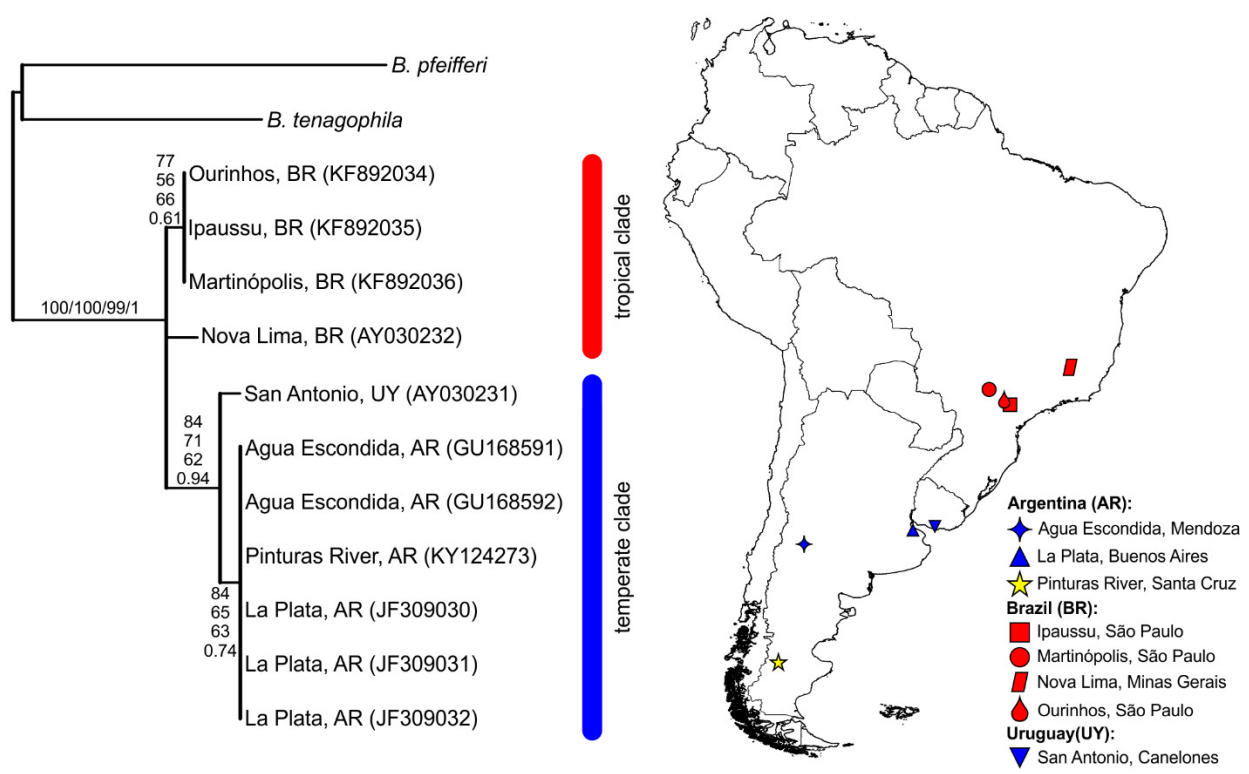


Figure 3. Bayesian tree of *Biomphalaria peregrina* based on the partial *16S-rRNA* gene. The bootstrap values for the NJ, MP, ML trees and posterior-probability values for BI are shown above and below the branches. The numbers within parentheses are GenBank-accession numbers. The geographical distribution of the localities sampled is shown. The literature references to the sequences are given in Table 1.

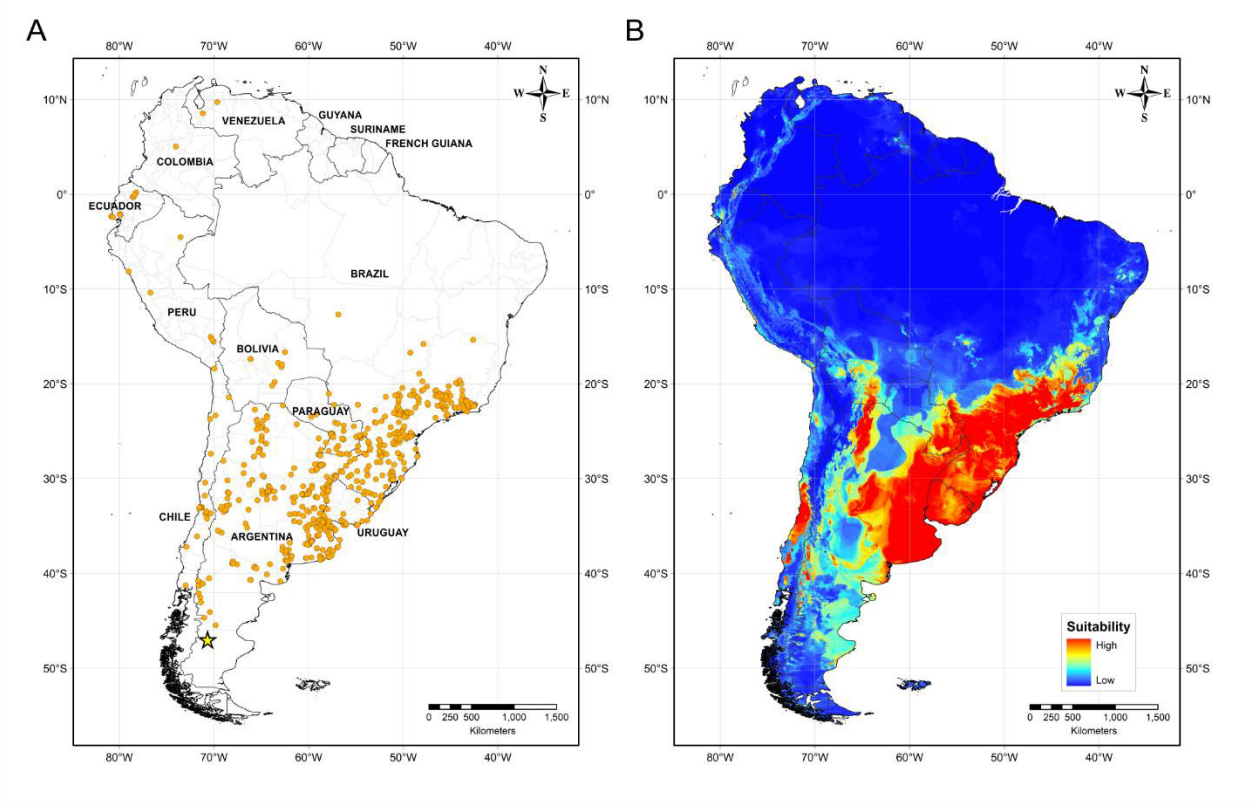
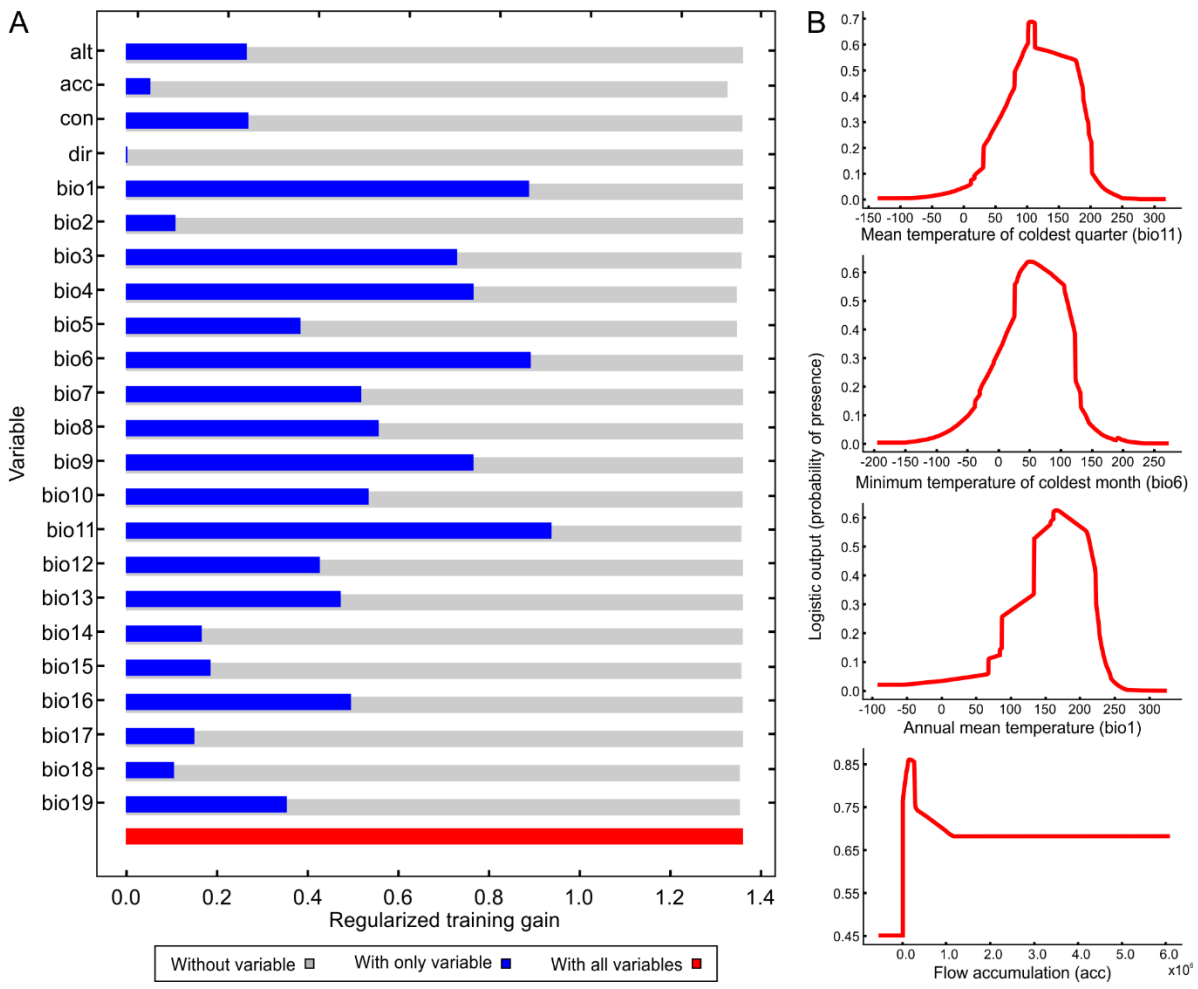


Figure 4. Distribution of *Biomphalaria peregrina* in South America. Panel A: Records of the snail's presence used in the modelling approach, with the southernmost record being from the Pinturas River, Argentina, as indicated by a yellow star. Panel B: Potential distribution in logistic format. The color code for location suitability and thus probability of the snail's presence: red, very high; yellow, high; azure, moderate; blue, low.



785

786 **Figure 5. Relative influence of the environmental variables for the potential distribution of**
787 ***Biomphalaria peregrina* in South America.** Panel A: Jackknife test determining the contribution
788 of each environmental variable to the development of the model. In the figure, the regularized
789 training gain is plotted on the abscissa for each of the variables indicated on the ordinate. Color
790 code: gray, without a variable; blue, with only a single variable; red, with all variables. Panel B:
791 Marginal-response curves for the four strongest environmental predictors. In each of the figures,
792 the logistic output, a measure of the probability of presence, is plotted on the ordinate for—from
793 the upper to the lower figure – the mean temperature of the coldest quarter (bio11), the minimum
794 temperature of the coldest month (bio6), the annual mean temperature (bio1), and the flow
795 accumulation (acc).