The South-American distribution and southernmost record 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 infected 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 reassessment 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 in order to compare the genetic background of individuals from the Pinturas River with previously genetically characterized strains of B. peregrina from various South American 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. Susceptibility surveys would be required to evaluate if southern populations of B. peregrina still retain their potential as intermediate hosts of S. mansoni.

1 **The South-American distribution and southernmost record of *Biomphalaria peregrina* - a**

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18 **Short title:** *Biomphalaria peregrina*, potential distribution and southernmost record

19   **ABSTRACT**

20   Schistosomiasis remains a major parasitic disease, endemic in large parts of South

21   America. Five neotropical species of *Biomphalaria* have been found to act as intermediate hosts

22   of *Schistosoma mansoni* in natural populations, while others have been shown to be susceptible

23   in experimental infections, although not found infected in the field. Among these potential

24   intermediate hosts, *Biomphalaria peregrina* represents the most widespread species in South

25   America, with confirmed occurrence records from Venezuela to northern Patagonia. In this

26   study, we report the southernmost record for the species at the Pinturas River, in southern

27   Patagonia, which finding implies a southward reassessment of the limit for the known species of

28   this genus. The identities of the individuals from this population were confirmed through

29   morphological examination, and by means of two mitochondrial genes, *cytochrome oxidase*

30   *subunit I* (*COI*) and *16S-rRNA*. With both markers, phylogenetic analyses were conducted in

31   order to compare the genetic background of individuals from the Pinturas River with previously

32   genetically characterized strains of *B. peregrina* from various South American locations. In

33   addition, we produced a potential distribution model of *B. peregrina* in South America and

34   identified the environmental variables that best predict that distribution. The model was

35   estimated through a maximum entropy algorithm and run with occurrence points obtained from

36   several sources, including the scientific literature and international databases, along with climatic

37   and hydrographic variables. Different phylogenetic analyses with either the *COI* or *16S-rRNA*

38   sequences did not conflict, but rather gave very similar topological organizations. Two major

39   groups were identified, with sequences from the Pinturas River grouping together with

40   haplotypes from subtropical and temperate regions. The model developed had a satisfactory

41   performance for the study area. We observed that the areas with higher habitat suitability were

42   found to be mainly linked to subtropical and temperate regions of South America between 15°

43   and 45° south latitude, with different moderate- and low-suitability areas outside this range. We

44   also identified the coldest temperatures as the main predictors of the potential distribution of this

45   snail. Susceptibility surveys would be required to evaluate if southern populations of *B.*

46   *peregrina* still retain their potential as intermediate hosts of *S. mansoni*.

47

48   **INTRODUCTION**

49   Schistosomiasis is an acute and chronic parasitic disease that affects at least 258 million

50   people worldwide. Seventy-eight countries are considered endemic for schistosomiasis, with the

51   populations of 52 countries requiring preventive chemotherapy (World Health Organization,

52   2015). There are two major forms of schistosomiasis –intestinal and urogenital– caused by five

53   main species of trematodes, and the disease is frequent in tropical and subtropical regions,

54   particularly in poor communities (World Health Organization, 2015). In the Americas, the only

55   species present is *Schistosoma mansoni* (Sambon, 1907) (Digenea); which species is associated

56   with intestinal schistosomiasisis, and is transmitted by the freshwater snails of the genus

57   *Biomphalaria* Preston, 1910; e.g. *Biomphalaria glabrata* (Say, 1818), *Biomphalaria tenagophila*

58   (d'Orbigny, 1835), and *Biomphalaria straminea* (Dunker, 1848). Other snail species, such as

59   *Biomphalaria peregrina* (d'Orbigny 1835), have been infected experimentally and are thus

60   considered as potential hosts of *S. mansoni* (Paraense & Côrrea, 1973).

61   In the Americas, schistosomiasis currently occurs in Brazil, Venezuela, Surinam, Puerto

62   Rico, the Dominican Republic, and on several islands of the Lesser Antilles, with recent

63   evidence indicating a spread from northeastern Brazil southward (Spatz et al., 2000; Kloos et al.,

64   2008; Noya et al., 2015). An expansion of the current disease-distribution area can be expected,

65   since the geographical range of snails that can act as intermedial hosts (IHs) is wider than that of

66   the pathogen (Pan American Health Organization, 2010). The southern area with the highest risk

67   of establishing an endemic and a new focus of disease is located in the northeast of Argentina

68   (the NEA Region), where the majority of the *Biomphalaria* species inhabit the major rivers of

69   the Del-Plata basin. Five of those species are listed as potential IHs of schistosomiasis: *B.*

70   *tenagophila*, *B. straminea*, *B. peregrina*, *B. orbignyi* Paraense, 1975, and *B. oligoza* Paraense,

71   1974 (Rumi, 1991; Rumi & Vogler, 2014).

72   The identification and recognition of *Biomphalaria* species thus far has mainly relied on

73   features of shell morphology, radula, jaw and the reproductive system (*cf*. Paraense, 1966,

74   1975a, 2003; Rumi, 1991). Character similarity among the species, however, has in fact

75   hampered classification, as the case of *B. peregrina, B. orbignyi* and *B. oligoza* found in

76   Argentina whose morphological similarities difficult correct identification (Paraense, 1988;

77   Spatz et al., 2000; Vidigal et al., 2000a; Estrada et al., 2006). Within the historical context,

78   several of the South-American species were described in genera of doubtful taxonomic position,

79 as the example of *Taphius* Adams & Adams, 1855; *Biomphalaria* Preston, 1910; *Tropicorbis*

80 Brown & Pilsbry, 1914; *Platytaphius* Pilsbry, 1924; and *Australorbis* Pilsbry, 1934. In addition,

81 the original diagnoses were mostly made based only on shell characters. Both situations

82 facilitated the generation of species of doubtful validity. Subsequently, the anatomical evidence

83 has demonstrated that no differences really existed between the genera and the various taxa all

84 belonging to the same genus (Paraense, 1958). Although the oldest name was *Taphius*, in 1965

85 the International Committee of Zoological Nomenclature imposed the sole name *Biomphalaria*,

86 considering that one to be the most widespread in the world (Barbosa et al., 1961; Paraense,

87 2008). More recently, though, difficulties concerning morphological identification have been

88 overcome through the use of molecular-genetic techniques that have contributed to delimiting

89 species, mainly those occurring in the Neotropics (e.g. Caldeira et al., 1998; Vidigal et al., 1998,

90 2004; Spatz et al., 2000).

91   Among the *Biomphalaria* species in South America, *B. peregrina* exhibits one of the

92 most widespread distributions –and one involving a great diversity of hydrologic systems– with

93 that species thus far having been recorded from Venezuela to northern Patagonia, Argentina

94 (Paraense, 1966, 2003, 2004; Rumi, 1991; Spatz et al., 2000; Núñez et al., 2010; Standley et al.,

95 2011). In the study reported here, the presence of *B. peregrina* in southern Patagonia is now

96 documented for the first time, that location being the southernmost record for the species –and

97 the genus as well– worldwide. In order to confirm the identity of this most southerly population,

98 we assessed the main conchological and anatomical diagnostic characters (e.g. shell, genitalia,

99 and radula) and obtained DNA sequences of the mitochondrial *cytochrome oxidase subunit I*

100 (*COI*) and the *16S-rRNA* genes. Based on both markers, we compared the genetic background of

101 individuals from the Pinturas River with previously genetically characterized strains of *B.*

102 *peregrina* from various South American locations and examined the intra-specific phylogenetic

103 position of the recently discovered population. In addition, upon consideration that *B. peregrina*

104 represents a potential host for schistosomiasis, we produced a predictive model of the species's

105 spatial distribution in South America and identified the environmental variables that best predict

106 its location. The resulting model indicating the likely whereabouts of that potential host will

107 hopefully provide further guidance for future efforts aimed at schistosomiasis surveillance and

108 control.

109

110 **MATERIALS AND METHODS**

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

116

117 *Morphological examination*

118   The adult specimens (n = 12; MLP-Ma N° 14186/1 to 14186/12) were analyzed
119 according to Paraense & Deslandes (1956), Paraense (1966, 1974, 1975a,b) and Rumi (1991).
120 The morphology of the shell, the radula, and the reproductive system were analyzed. The soft
121 parts were separated from the shell for subsequent processing and fixed in Railliet-Henry –93%
122 (v/v) distilled water, 2% (v/v) glacial acetic acid, 5% (v/v) formaldehyde, and 6 g sodium
123 chloride per liter– or 90% (v/v) aqueous alcohol. Shell measurements (maximum and minimum
124 diameter and height) were obtained with a Mitutoyo digital calipter. The dissection was done
125 under a stereoscopic binocular microscope (LEICA MZ6) (individuals MLP-Ma N° 14186/1 to
126 14186/8).

127   The radula and jaw were cleaned following a modification of the non-destructive method
128 described by Holznagel (1998) and Vogler et al. (2016): the structures were separated from the
129 mass of tissue and placed in 1.5-ml microtubes containing 500 µl NET buffer (1 ml 1M Tris pH
130 8.0, 2 ml 0.5 M ethylenediaminetetraacetic acid, 1 ml 5 M NaCl, 20 ml 10% [w/v] sodium
131 dodecyl sulfate, 76 ml water) and 10 µl of Proteinase K (20 mg/ml) were added. The samples
132 were then incubated at 37 ºC with a subsequent renewal of the NET buffer and Proteinase K to
133 verify the absence of tissue. After two washes with distilled water, 25% (v/v) aqueous ethanol
134 was added for preservation. Finally the radula and jaw were examined by scanning electron
135 microscopy (JEOL 6360) in the Museum of La Plata (individuals MLP-Ma N° 14186/1, MLP-
136 Ma N° 14186/2, MLP-Ma N° 14186/4 and MLP-Ma N° 14186/6). The radular formula gives the
137 number of teeth per row: [(number of left teeth) + (number of central teeth) + (number of right
138 teeth)] plus the number of transverse rows (Paraense & Deslandes, 1956).

139

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

141   Total genomic DNA was extracted from the foot muscle of five individual snails (MLP-
142   Ma N° 14186/1 to 14186/5) through the use of the DNeasy Blood & Tissue kit (Qiagen,
143   Valencia, CA) according to the manufacturer's protocol. Partial sequences of the mitochondrial
144   *cytochrome oxidase subunit I* (*COI*) and the *16S-rRNA* genes were amplified by means of the
145   primers LCO1490 (5'–GGT CAA CAA ATC ATA AAG ATA TTG G–3') and HCO2198 (5'–
146   TAA ACT TCA GGG TGA CCA AAA AAT CA–3') for *COI* (Folmer et al., 1994), and 16SF-
147   104 (5'–GAC TGT GCT AAG GTA GCA TAA T–3') and 16SR-472 (5'–TCG TAG TCC AAC
148   ATC GAG GTC A–3') for *16S-rRNA* (Ramírez & Ramírez, 2010). The amplification of the *COI*
149   region was conducted following Vogler et al. (2014). The amplification of *16S-rRNA* was
150   performed in a total volume of 30 µl containing 30–50 ng of template DNA, 0.2 µM of each
151   primer, 1X PCR green buffer, 0.2 mM dNTPs, and 1 U Dream *Taq* DNA Polymerase (Thermo
152   Scientific). The thermocycling profile was 35 cycles of 30 s at 94 °C, 30 s at 48 °C, 1 min at 72
153   °C followed by a final extension of 1 min at 72 °C. The amplifications were run on a T18
154   thermocycler (Ivema Desarrollos). The PCR products were purified by means of a ADN
155   PuriPrep-GP kit (InBio-Highway, Tandil, Buenos Aires). After purification, both DNA strands
156   for each gene were then directly cycle-sequenced (Macrogen Inc., Seoul, Korea). The resulting
157   sequences were trimmed to remove the primers, and the consensus sequences between forward
158   and reverse sequencing were obtained by means of the BioEdit 7.2.5 software (Hall, 1999).

159

160   *Phylogenetic analysis*

161   The consensus sequences of the individuals were compared to reference sequences in
162   GenBank through the use of the BLASTN algorithm (Altschul et al., 1990) to identify similar
163   sequences in order to confirm the morphology-based identification. Subsequently, phylogenetic
164   analyses were conducted to explore the possible intra-specific phylogenetic relationships of the
165   genetic sequences from the southernmost individuals from the Pinturas River to those of other *B.*
166   *peregrina* individuals from various locations available in GenBank (Table 1). Sequences of other
167   similar species to *B. peregrina* were not used in the analysis. The phylogenetic analyses were
168   carried out separately for each mitochondrial region as follows: the sequence alignment was
169   performed with Clustal X 2.1 (Larkin et al., 2007), and optimized by visual inspection. The total
170   lengths of the matrices analyzed were 546 bp for the *COI* gene, and 269 bp for the *16S-rRNA*
171   locus. The data were subjected to four different phylogenetic analyses by the methods of

172 neighbor joining (NJ), maximum parsimony (MP), maximum likelihood (ML), and Bayesian
173 inference (BI). The NJ analysis was conducted with MEGA 6.06 (Tamura et al., 2013) through
174 the use of the Kimura's two-parameter (K2P) substitution model. The MP and ML analyses were
175 carried out with PAUP*4.0b10 (Swofford, 2002). The MP was conducted by means of a
176 heuristic search with the characters equally weighted, tree bisection and reconnection branch-
177 swapping, and 10 random stepwise additions. The optimal model of nucleotide substitution for
178 ML inference was evaluated by the likelihood-ratio test and selected by means of the corrected
179 Akaike Information Criterion with Jmodeltest 2.1.7 (Darriba et al., 2012). The TVM+I (for *COI*),
180 and the TIM1+I (for *16S-rRNA*) substitution models were used as evolutionary paradigms.
181 Statistical support for the resulting phylogenies was assessed by the bootstrap method with 1,000
182 replicates (Felsenstein, 1985). BI was performed with Mr. Bayes 3.2.6 (Ronquist et al., 2012).
183 Two runs were conducted simultaneously with 4 Markov chains that went for 10^6 generations,
184 sampling every 100 generations. The first 10,001 generations of each run were discarded as
185 burn-in, and the remaining 18,000 trees were used to estimate posterior probabilities. In addition,
186 the number of haplotypes in the dataset was explored with DnaSP 5.10 (Librado & Rozas, 2009)
187 and the genetic distances estimated in MEGA 6.06 through the use of the number of differences
188 (*p*) and the K2P-substitution model. Since we obtained shorter *16S-rRNA* sequences than
189 previously reported for *B. peregrina* from Argentina that did not include previously
190 characterized molecular diversity (Standley et al., 2011), the *COI* data were employed only for
191 estimating the number of haplotypes and genetic distances.

192

193 *Species-distribution model*

194   The study area comprised all of the South-American countries. The occurrence data for *B.*
195 *peregrina* were retrieved from the scientific literature, and from malacological collections and
196 international databases (Table 2). All together, 689 spatially unique records were used. When the
197 coordinates of localities were lacking, those data were derived secondarily following Wieczorek
198 et al. (2004). Twenty-three environmental variables were used as predictors; comprising 19
199 climatic, 3 hydrologic, and 1 topographical (Table 3). The variables were downloaded from
200 WorldClim (http://www.worldclim.org) and HydroSHEDS (http://hydrosheds.cr.usgs.gov) at a
201 spatial resolution of 30 arc seconds (~1 km^2). WorldClim and HydroSHEDS provide climatic
202 information derived from weather stations spanning 1950–2000 and hydrographic data obtained

203   from a STRM digital-elevation model, respectively (Hijmans et al., 2005; Lehner et al., 2008).

204   These variables have been commonly employed for generating distribution models in gastropods,

205   including freshwater members, such as those belonging to the genus *Biomphalaria* (Scholte et

206   al., 2012; Vogler et al., 2013; Pedersen et al., 2014; Beltramino et al., 2015; Martín et al., 2016).

207   All environmental layers were trimmed to the study area. The potential-distribution model was

208   estimated by using a maximum entropy algorithm in MaxEnt 3.3.3k (Phillips et al., 2006;

209   Phillips & Dudík, 2008). The data were randomly divided into the training data (75% of

210   occurrences) and the model-testing data (the remaining 25%). The output was computed as

211   *logistic*, which setting returns a map with an estimated probability ranging between 0 (no

212   probability of the species presence) and 1 (high probability of presence). The resulting model

213   was assessed by estimating the area under the receiver-operating-characteristic curve (ROC-

214   curve analyses; Fielding & Bell, 1997). The relative contribution of variables to the development

215   of the model was evaluated by means of a jackknife test and through the response curves

216   obtained in MaxEnt following Meichtry de Zaurlín et al. (2016).

217

218   **RESULTS**

219   *Morphological examination*

220   **Shell** (Fig. 1, panel A). The empty shell is fundamentally light brown in color, with the

221   growth lines clearly visible. The whorls, up to 5¾ in number, increase slowly and display a

222   rounded surface on both sides. The shells exhibit a more or less marked deflection of the outer

223   whorl to the left. The maximum value for the larger diameter was 13.5 mm (mean = 9.77 mm,

224   SD = 1.54 mm, n = 12); the maximum value for the smaller diameter was 8.98 mm (mean = 7.53

225   mm, SD = 1.40 mm, n = 11). The greatest height has been 4.6 mm (mean = 3.86 mm, SD = 0.64

226   mm, n = 12).

227   **Radula** (Fig 1, panels B to E). The central tooth is rather asymmetrical, bicuspid, with or

228   without accessory cusps, with the base without special features. There are 106 rows of teeth;

229   with 8 laterals and 12 marginals per half row. The first lateral tooth is tricuspid with the

230   mesocone more developed, and with the free border rounded or in the shape of a sword point. A

231   crest with a central depression toward the posterior part of the tooth is evident. Finally, the

232   marginal teeth are without special features. Radular formula: [20-1-20] 106.

233   **Jaw.** The jaw is T-shaped, with the dorsal part composed of a single crescent-shaped

234 piece. The features correspond to the standard description of the species and do not differ from

235 those of other *Biomphalaria* species.

236   **Genital system.** The specimens showed the typology described for *B. peregrina*, without

237 any special variations as ilustrated in Rumi (1991). The following distinctive characters are

238 highlighted: in the male genital system, the vas deferens was wider distally and narrower than the

239 the middle portion of the penis sheath; the number of the prostatic diverticula was 12 to 14. In

240 the female system, the apical portion of the spermathecal body was almost always covered by an

241 anterior and long prostatic diverticulum; the vaginal pouch was well developed.

242

243 *Phylogenetic analysis*

244   The BLASTN search results, with the obtained partial *COI* and *16S-rRNA* sequences

245 from the Pinturas River as the query sequences, showed top-ranking scores and a 100% sequence

246 identity with sequences available in the GenBank from Agua Escondida, Mendoza (Argentina)

247 and confirmed their identity as *B. peregrina*. Partial DNA sequences consisted of 655 bp for *COI*

248 and 265 bp for *16S-rRNA*. Both markers contained no variation among the five individuals

249 sequenced, resulting in the existence of only one haplotype per marker. After the inclusion of

250 GenBank sequences from other locations with subsequent alignment, six unique haplotypes were

251 identified within the *COI* dataset (Fig. 2). The sequence divergence among haplotypes is

252 presented in Table 4. Different phylogenetic analyses with either the *COI* or *16S-rRNA* marker

253 did not conflict; rather, both loci gave very similar topological organizations for the NJ, MP, and

254 BI trees with minor differences in the ML-tree organization. In all instances, two major groups

255 were identified, referred to as the *tropical* and *temperate* clades (Figs. 2 and 3). In terms of that

256 subdivision, the specimens from the Pinturas River were placed within the temperate group.

257

258 *Species-distribution model*

259   Fig. 4 illustrates the potential distribution area for *B. peregrina*. The model conformed

260 well to expectations, with values for the area under the curve of 0.927 for the training data and

261 0.905 for the test data, with a standard deviation of 0.009. The areas with higher probability of

262 the snail's presence were found to be mainly linked to subtropical and temperate regions of

263 South America between 15° and 45° south latitude, comprising central and northeastern

264   Argentina, central Chile, eastern Paraguay, southeastern Brazil, and southern Bolivia and
265   Uruguay. In addition, regions with a moderate to high habitat suitability were predicted for Peru,
266   Ecuador, Colombia, and Venezuela. The areas of lower habitat suitability were located in French
267   Guiana, Guyana, Suriname, Venezuela, and a large area of Brazil (Fig. 4, panel B). The jackknife
268   test showed that the mean temperature of the coldest quarter (bio11), the minimum temperature
269   of coldest month (bio6) and the annual mean temperature (bio1) were the variables that most
270   greatly influenced the model development when used in isolation (Fig. 5, panel A). The flow
271   accumulation produced a reduction in training gain when removed from the model, thus
272   indicating that that variable contained information necessary for the model. The remaining
273   predictors contributed less to the modelling. Fig. 5, panel B contains the marginal-response
274   curves for the four strongest environmental predictors – i.e. the mean temperature of the coldest
275   quarter, the minimum temperature of the coldest month, the annual mean temperature, and the
276   flow accumulation.

277

278   **DISCUSSION**

279   The conchology and anatomy of the reproductive system of the specimens from the
280   Pinturas River were consistent with the descriptions of Paraense & Deslandes (1956) and
281   Paraense (1966) for *B. peregrina*. The shells of the individuals from that river recall the original
282   descriptions by the first authors for *Australorbis inflexus* (nowadays considered synonymous
283   with *B. peregrina*; Paraense, 1966) from Pouso Alegre, Minas Gerais, Brazil because of the
284   strong inflection of the aperture located toward the left side. Nevertheless, the specimens from
285   the Pinturas River have much wider and rounder shells on both sides than those described for *A.*
286   *inflexus*. The radulae of the individuals from south Patagonia have a folding and a groove
287   between the cuspids of the central tooth that has not been described for other populations of this
288   species. As mentioned in the Results section, the anatomy of the male genitalia of the specimens
289   examined here exhibited a vas deferens wider distally and narrower than the middle portion of
290   the penis sheath, which is typical of *B. peregrina*. This feature allowed distinguishing the
291   individuals of the Pinturas River from the similar species *B. orbignyi*, whose widest portion of
292   the distal segment of the vas deferens is nearly equal in width to the penis sheath (Paraense,
293   1975b). On the other hand, the number of prostatic diverticula in the material examined allowed
294   distinguishing from *B. oligoza*, whose diverticula number is much smaller (tipically 1 to 6, rarely

295 7), and are also poorly developed. Other morphological features which confirmed the specific

296 identity of the specimens studied as *B. peregrina* were the apical portion of the spermathecal

297 body –almost always covered by an anterior and long prostatic diverticulum–, and the vaginal

298 pouch well developed (Paraense 1966, 1975a). When analyzing individuals collected at Agua

299 Escondida, Mendoza, Argentina, Standley et al. (2011) found a conchology and reproductive

300 organ morphology consistent with that of typical *B. peregrina*, as found here for the Pinturas

301 River individuals. However, those authors stated that "the internal morphology of *B. peregrina*

302 has been shown to distinguish it even from closely related species, but not from *B. orbignyi*

303 (Spatz et al., 2000)", and consequently they did not rule out *B. orbignyi* as an alternative for the

304 identity of the individuals from that location. This is not the case for the individuals of the

305 Pinturas River, given that the statment in Standley et al. (2011) is not necessarily true, as the

306 differentiation of *B. peregrina* and *B. orbignyi* rest on differences in the wider portion of the

307 distal segment of the vas deferens (Paraense, 1975b).

308   In addition, the morphologically identified specimens from the Pinturas River were

309 confirmed genetically as being *B. peregrina* and a single haplotype was found among the five

310 individuals analysed for each mitochondrial-marker loci examined. Likewise, Standley et al.

311 (2011) reported the absence of variation in the *COI* marker for the population studied from Agua

312 Escondida, Mendoza, Argentina. As mentioned above, despite having found the typical internal

313 morphology of *B. peregrina*, those authors did not rule out *B. orbignyi* as a possible alternative,

314 although the similar species was not included in that genetic approach. Those authors also

315 suggested that the lack of variation in that population could be owing to a founding event in

316 recent years since the species of *Biomphalaria* are hermaphroditic and capable of rapidly

317 colonizing a new locality, even from a sole individual founder. Strikingly, a single *COI*

318 haplotype seems be shared between the populations of Agua Escondida and the Pinturas River,

319 which are geographically distant (roughly around 1200 km). Two main (non-exclusive)

320 alternatives might explain such pattern: i- the resolution of the *COI* sequence used is not

321 resolutive enough to detect genetic variability and discriminate these samples, and ii- these

322 populations share a close common evolutionary history. Further studies based on a greater

323 number of specimens and molecular markers would be required to test these alternatives.

324   The phylogenetic trees revealed that the *B. peregrina* can be considered as divided into

325 two clades, here referred to as the tropical and the temperate. For the *COI* marker, the first clade

326   comprised exclusively individuals from Brazil, whereas the second included specimens from

327   southern Brazil and Argentina. For the *16S-rRNA*, the sequences from Brazil all grouped

328   together within the tropical clade, whereas the temperate clade included only sequences from

329   Argentina and Uruguay. These clades could be linked to the biogeography and ecologic history

330   of *B. peregrina* in terms of the colonization of freshwater environments. Nonetheless, of

331   pertinence to emphasize here is that although *B. peregrina* possesses a wide distribution in South

332   America, at the present time only few DNA sequences are available in Genbank for this snail, as

333   well as for the morphologically similar species *B. oligoza* and *B. orbignyi.* Early molecular

334   studies based on the internal transcribed spacer region of the ribosomal DNA sequences (*ITS*)

335   demonstrated a closer relationship between *B. peregrina* and *B. oligoza* (Spatz et al., 2000;

336   Vidigal et al., 2000b), but still further research is required to fully understand the relationship

337   among the similar species (*B. peregrina*, *B. oligoza*, and *B. orbignyi*) and to verify whether

338   others molecular markers such as *COI* –currently unavailable for *B. oligoza* and *B. orbignyi*–

339   corroborates the classical morphological identification. Therefore, investigations focussing on

340   the phylogeography of *B. peregrina* are required to acquire a comprehensive understanding of its

341   evolutionary history, similar to those carried out for *B. glabrata* (Mavárez et al., 2002; DeJong et

342   al., 2003), especially since *B. peregrina* has been shown to occupy a basal position in the

343   phylogenetic analyses of the genus (DeJong et al., 2001; Jarne et al., 2011).

344   *Biomphalaria peregrina,* as its name might suggest –from the latin *peregrinus,* meaning

345   wanderer, in reference to its wide geographical distribution (Paraense, 1975a)– is the species

346   most widely distributed in South America within the genus. The occurrence of representative

347   individuals from Venezuela down to one of the most southern areas of Patagonia in the Province

348   of Santa Cruz demonstrates that the species possesses an ample range of environmental

349   tolerance. Even so, the historical registration of *B. peregrina* and the area of its potential

350   distribution described here nevertheless indicate that the species's greatest abundance and

351   dispersion are within the Atlantic corridor in the south of Brazil as well as in the northeast and

352   pampean region of Argentina. According to the results obtained here, and in agreement with the

353   findings of Rumi (1991), the potential distribution of *B. peregrina* involves the most southerly

354   areas of the Great-Del-Plata basin, then spreads to the west through the endorrheic basins of

355   Córdoba –the ancient beds of the Paraná River– toward the Andes region, and finally, remaining

356   east of the Andes, extends to the north of the continent through regions which correspond to

specific altitude areas within the Andes, 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 malacological 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.

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 the Results section under the investigation of the genetic background 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 experimentally as IHs of several strains of *S. mansoni* (Borda & Rea, 1997; Simões et al., 2013). In this context, 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 Minas Gerais, Brazil; but that Brazilian variant, in contrast, was demonstrated

388 experimentally to be not susceptible to parasitism by *S. mansoni* (Paraense & Deslandes, 1956).

389 Thus, determinations of this nature are fundamental for delineating the potential area of

390 occupation of *B. peregrina* and obtaining a more realistic approximation of the corresponding

391 potential zone of occupation of populations susceptible to parasitism, which information could

392 be associated with the possible appearance of foci of infection and a southward dispersion of the

393 endemium. In addition, regions with a moderate to high habitat suitability for *B. peregrina* were

394 predicted within the Andes (Peru, Ecuador, Colombia, and Venezuela), which indicates a

395 preference for relatively cold climatic ranges in that area and could have also implications on the

396 possible risks of schistosomiasis in some specific altitudinal regions in the Andes.

397 Finally, as mentioned above, further research is still needed for a better understanding of

398 the evolutionary history, ecology, parasitic susceptibility, and genetic variarion among the

399 potential IHs of *S. mansoni* in South America, such as *B. peregrina*; which species is

400 comparatively underrepresented in current research on planorbid snails despite its wide

401 distribution in South America, as indicated by the evidence of the record of the new population

402 described here in southern Patagonia.

403

404 **ACKNOWLEDGEMENTS**

405 The authors would like to thank Dr. Monika Hamann (Centro de Ecología Aplicada del

406 Litoral, CONICET) and three anonymous reviewers for providing valuable comments on an

407 early version of the manuscript, and Patricia Sarmiento (Museo de La Plata) who obtained the

408 scanning photographs. Finally, the authors are grateful to Dr. Donald F. Haggerty, a retired

409 academic career investigator and native English speaker, for translating the manuscript from the

410 original Spanish and editing the final version.

411

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Figure 1

External shell morphology and radula of *Biomphalaria peregrina* from the Pinturas River, Argentina.

Panel A: Right, left, and ventral views. Panels B–D: Detail of the rachidian or central (CT) and lateral teeth (LT); ec, ectocone; en, endocone; me, mesocone. Panel E: Detail of marginal teeth.

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.

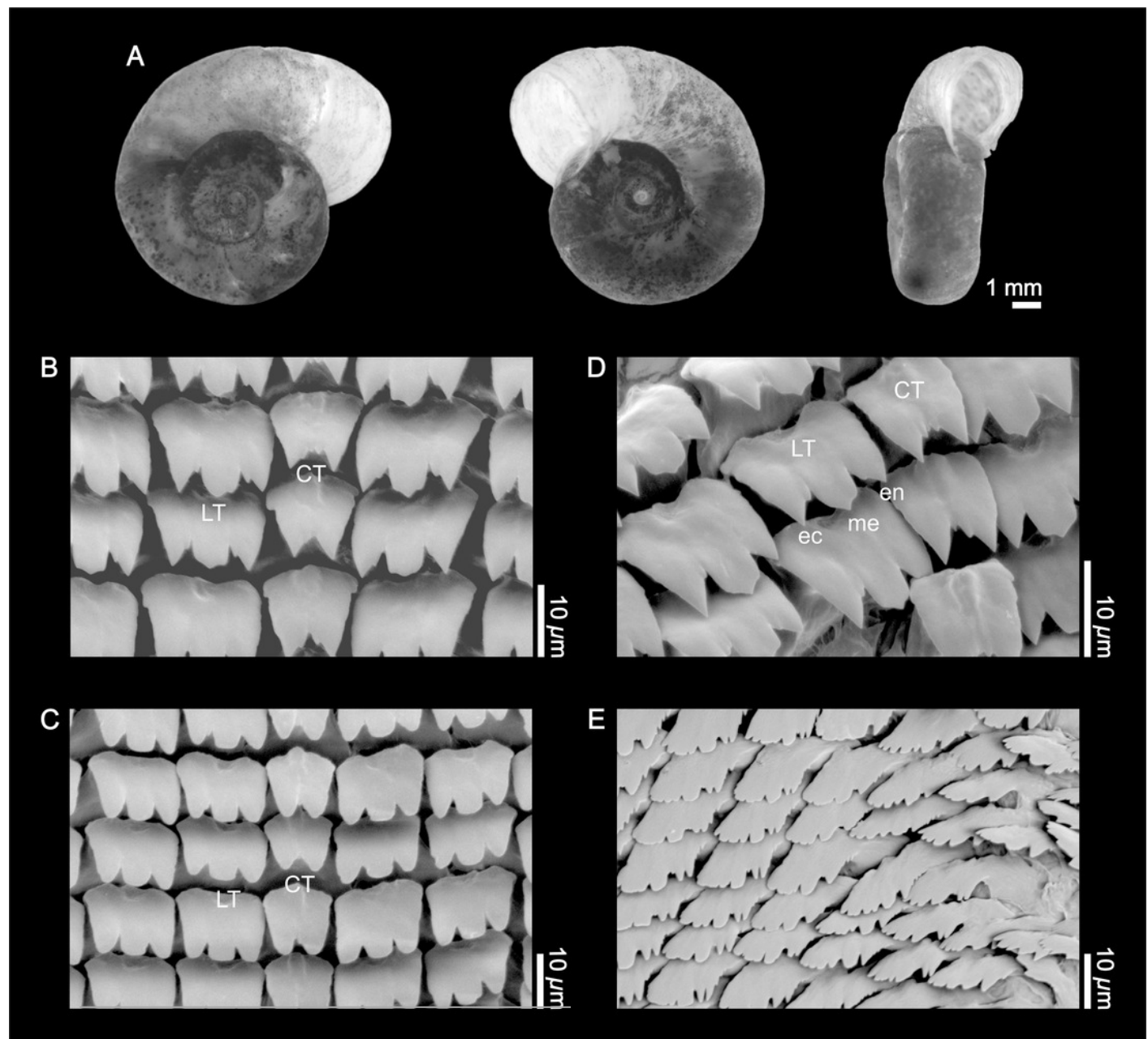



Figure 2

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.

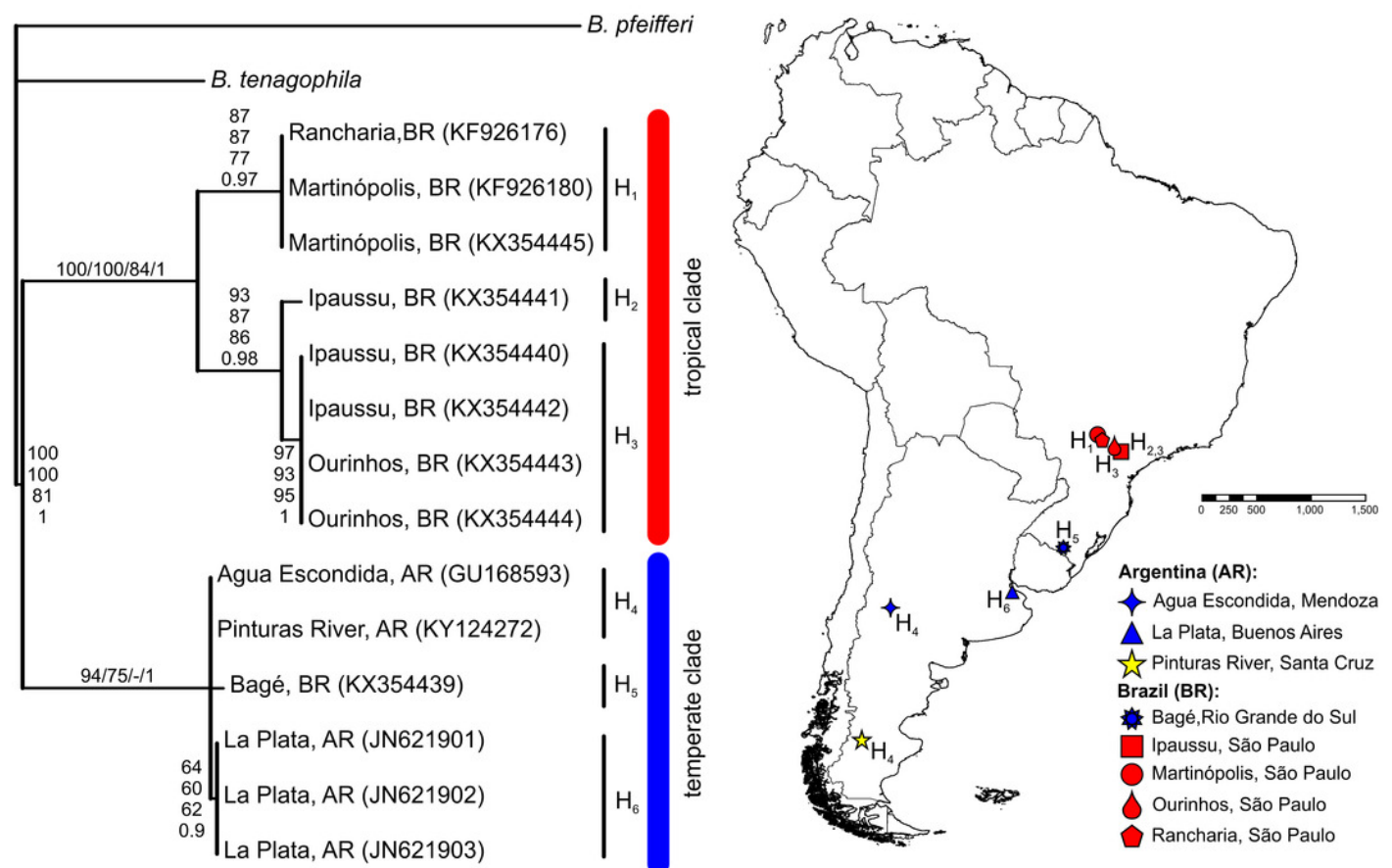



Figure 3

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.

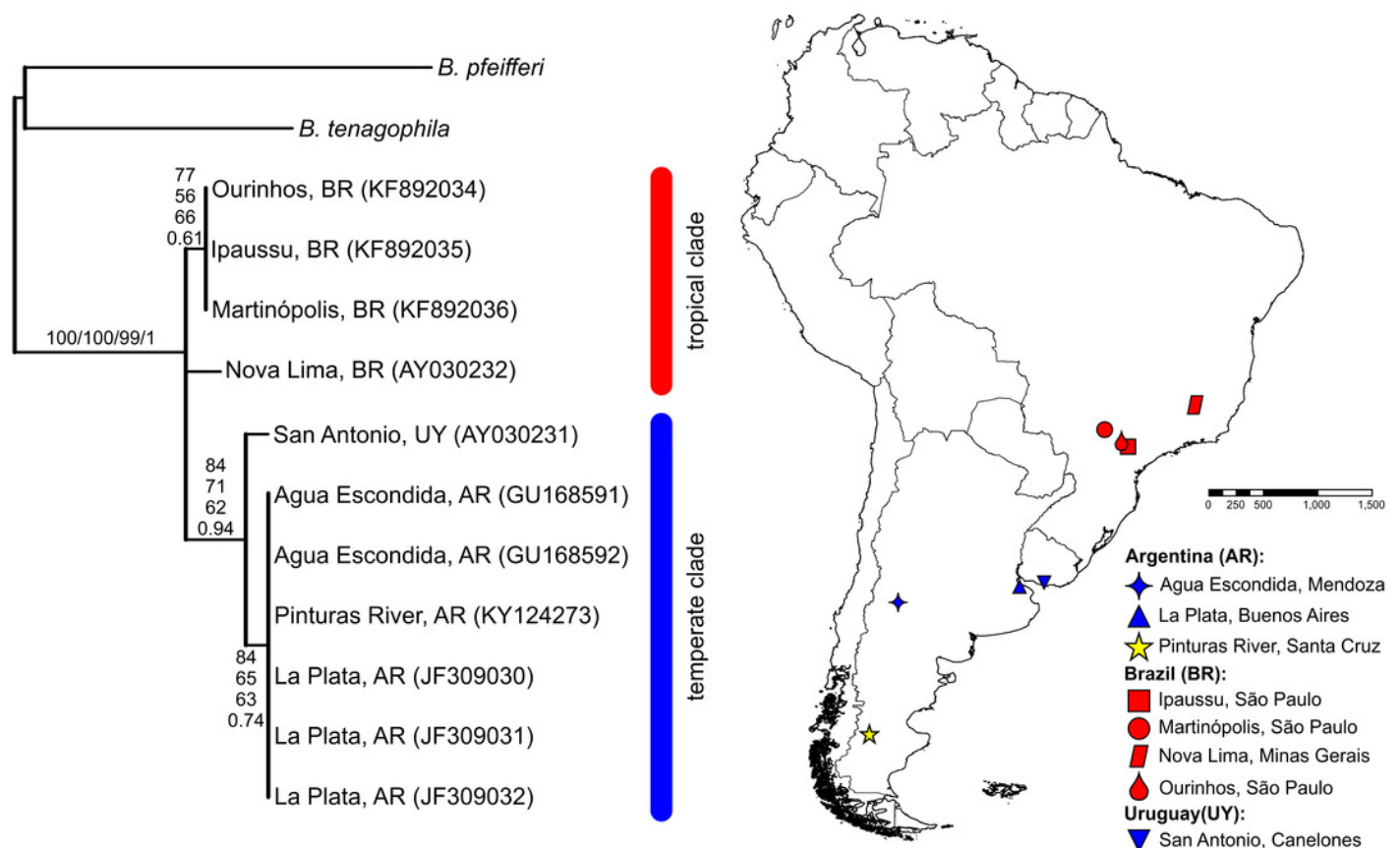



Figure 4

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.

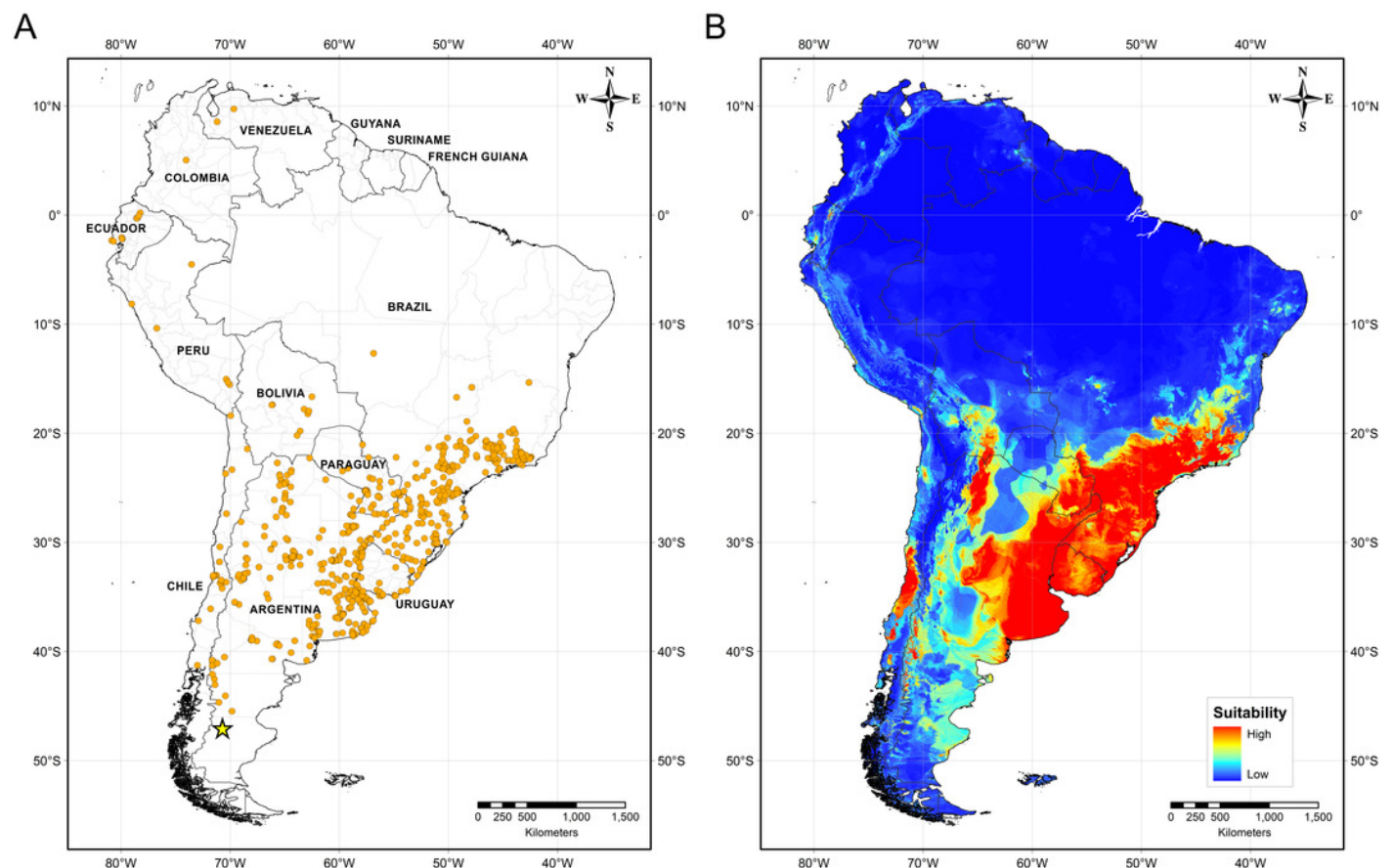
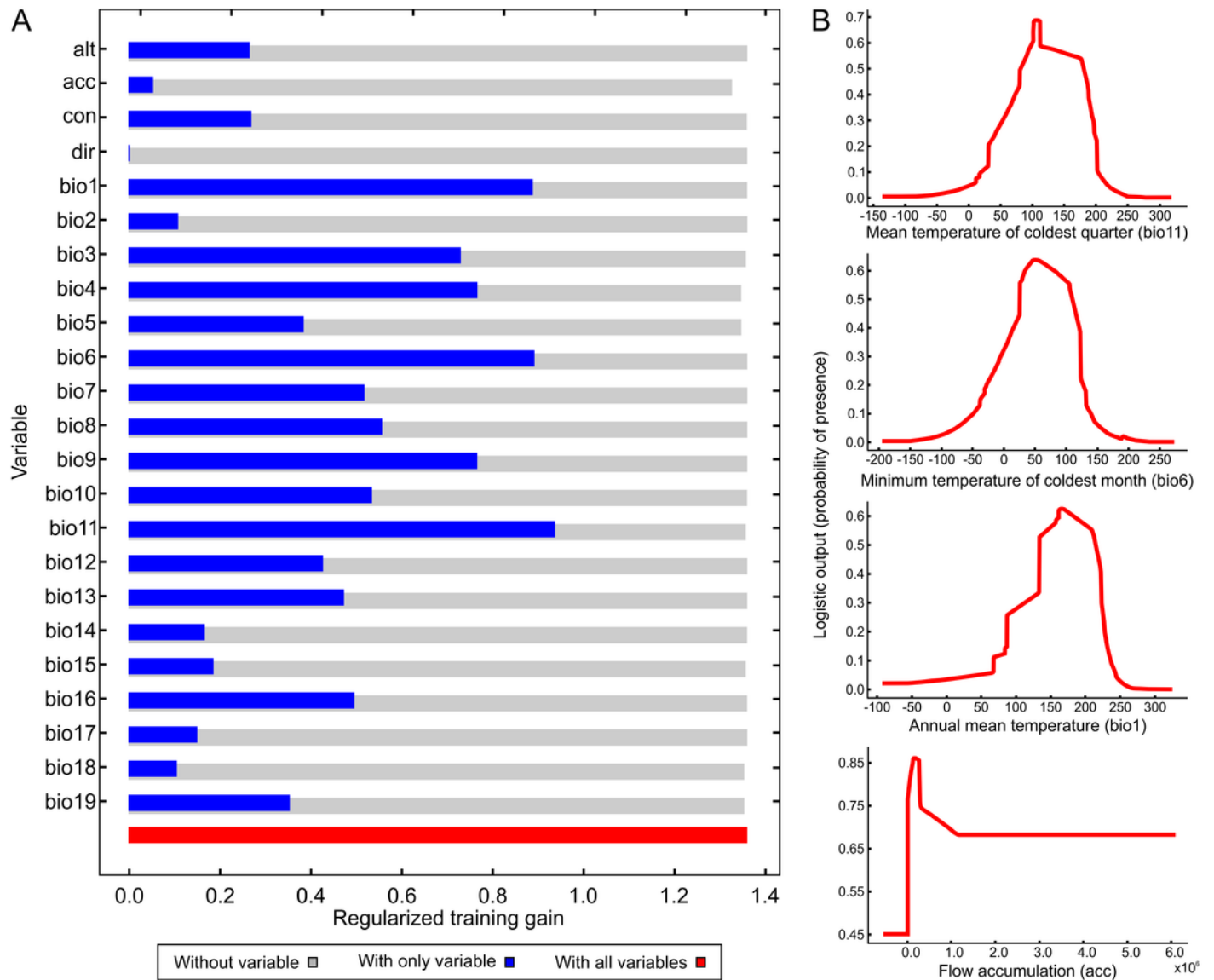



Figure 5

Relative influence of the environmental variables for the potential distribution of *Biomphalaria peregrina* in South America.

Panel A: Jackknife test determining the contribution of each environmental variable to the development of the model. In the figure, the regularized training gain is plotted on the abscissa for each of the variables indicated on the ordinate. Color code: gray, without a variable; blue, with only a single variable; red, with all variables. Panel B: Marginal-response curves for the four strongest environmental predictors. In each of the figures, the logistic output, a measure of the probability of presence, is plotted on the ordinate for—from the upper to the lower figure – the mean temperature of the coldest quarter (bio11), the minimum temperature of the coldest month (bio6), the annual mean temperature (bio1), and the flow accumulation (acc).

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	Standley et al. (2011)
				GU168592	
	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)

2 *GenBank unpublished sequences: the sequence author and submission year are indicated. **Outgroup species. LBMSU,

3 Laboratório de Bioquímica e Biologia Molecular, Superintendência de Controle de Endemias do Estado de São Paulo, Brazil;

4 MLP, Museo de La Plata, Argentina; UCH, Universidad de Chile, Chile.

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); DeJong 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); Russell (1972); Moreno González (1981) *Malacological collections*: MACN
Peru	6	Paraense (2003) *Malacological collections*: FIOCRUZ *Website*: WMSDB
Uruguay	23	Paraense (1966); DeJong et al. (2001); Scarabino (2004) *Malacological collections*: FIOCRUZ; MACN *Website*: GanBank; WMSDB
Venezuela	2	*Website*: WMSDB

2 *CECOAL, Centro de Ecología Aplicada del Litoral; FIOCRUZ, Fundação Oswaldo Cruz; IFML, Instituto Fundación Miguel

3 Lillo; MACN, Museo Argentino de Ciencias Naturales; MLP, Museo de La Plata; WMSDB, Worldwide Mollusc Species Data

4 Base

Variables used in the model development. Temperatures are expressed in ºC*10, precipitations in 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

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	–	0.012987	0.012987	0.026321	0.030198	0.028256	H_1: KF926176; KF926180; KX354445
H_2	0.012820	–	0.011116	0.032147	0.036068	0.034104	H_2: KX354441
H_3	0.012820	0.010989	–	0.032147	0.036068	0.034104	H_3: KX354440; KX354442; KX354443; KX354444
H_4	0.025641	0.031135	0.031135	–	0.003676	0.001834	H_4: KY124272; GU168593
H_5	0.029304	0.034798	0.034798	0.003663	–	0.005525	H_5: KX354439
H_6	0.027472	0.032967	0.032967	0.001831	0.005494	–	H_6: JN621901; JN621902; JN621903

2   *References to the sequences are provided in Table 1.