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Population structure and genetic diversity in the invasive freshwater snail Galba schirazensis (Lymnaeidae)

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Population structure and genetic diversity in the invasive freshwater snail *Galba schirazensis* (Lymnaeidae)

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Abstract

We studied the population genetic structure of the freshwater snail *Galba schirazensis*, a potential vector of infectious diseases such as fascioliasis. *Galba schirazensis* has now a worldwide distribution but a poorly known origin because this species has been distinguished only recently from the morphologically similar and cosmopolitan *Galba truncatula*. We developed specific microsatellite markers and sequenced a mitochondrial gene (cytochrome oxidase subunit I) to study individuals of *G. schirazensis* from the Old World and New World. We found very low genetic diversity within populations, no heterozygotes, and marked population structure—a pattern observed in other highly selfing lymnaeid species with recently enlarged distributions as a result of biological invasions. The total lack of observed heterozygosity in the few populations of *G. schirazensis* that displayed some allelic diversity suggests high selfing rates. We also found that the center of diversity, and by extension the origin area of this species, should be found in the New World, while Old World populations should rather result from a recent introduction of a genetically uniform population. The microsatellite markers developed here will help to clarify the history of expansion of *G. schirazensis* and might help understanding its role as a potential vector of infectious diseases.

Keywords: lymnaeids; *Lymnaea*; *Galba schirazensis*; *Galba truncatula*; vector; microsatellites; selfing.

1. Introduction

During the last century, human activities and climatic change have considerably increased the spread of species across their natural barriers and modified their areas of distribution (Kolar and Lodge 2001), threatening local biodiversity (Davis 2009). To better understand these threats, it is important to more fully characterize the species involved and the expansion pathways. Freshwater systems are particularly sensitive to bioinvasion risks (Beisel and Lévêque 2010). The Mollusca includes a large number of species invasive in freshwater habitats (Nunes et al. 2015). We here focus on the family Lymnaeidae in which several species have shown widespread long-distance colonization (Jabbour-Zahab et al. 1997, Meunier et al. 2001, Kopp et al. 2012). Long-distance dispersal con occur as a result of human activities, such as aquarium trade (Duggan 2010). Lymnaeids has a marked resistance to desiccation that increases their survival probability (Chapuis and Ferdy 2012). If released in conducive new environments, even one individual can found a population because lymnaeids are capable of self-fertilization, which also happens to be the main reproductive mode in some species (*e.g.*, Meunier et al. 2004*b*, Escobar et al. 2011, Lounnas et al. 2016). Such colonization events may also spread food- and water-borne trematodes carried by lymnaeids (Meunier et al. 2001, Mas-Coma et al. 2005, Correa et al. 2010), either because dispersing snails are themselves infected or because their presence in a new location facilitates subsequent transmission that originates from nearby infected vertebrates. Importantly, the worldwide expansion of the liver fluke *Fasciola hepatica*, a parasite infecting livestock and humans, has been facilitated by the dispersal of lymnaeids (Hurtrez-Boussès et al. 2001, Mas-Coma et al. 2005).

Despite their relevance to human and animal health, lymnaeids have been often misidentified (Correa et al. 2010, 2011, Lounnas et al. 2016). *Galba* species, a phylogenetically distinct group of small-shelled lymnaeids, exhibit high phenotypic plasticity in shell shape and display

93 extremely similar anatomical traits that make accurate species identification difficult in the
94 absence of molecular data (Samadi et al. 2000, Correa et al. 2011). For instance, *Galba*
95 *schirazensis* has only recently been distinguished from *Galba truncatula* in Europe and Asia and
96 from *G. truncatula, Galba cubensis*, and *Galba viator* in the Americas (Correa et al. 2010, 2011,
97 Bargues et al. 2011). However, *G. schirazensis* is now present in many regions of the world,
98 probably favored by its wide habitat range and its capacity to survive outside water for extended
99 time periods (Bargues et al. 2011). Recent molecular-based studies reported *G. schirazensis* in
100 Iran, Egypt, Reunion Island, Spain, Dominican Republic, Mexico, Colombia, Venezuela,
101 Ecuador, and Peru (Bargues et al. 2011; Correa et al. 2010, 2011, reported as *Lymnaea* sp. and
102 *Galba* sp. by the latter authors), but were of course unnoticed because the species was recently
103 described. However, only highly conserved genetic markers have been used so far in species
104 discrimination (Bargues et al. 2011; Correa et al. 2010, 2011). The population structure and
105 genetic diversity of the invasive lymnaeid *G. schirazensis* remains therefore largely unknown.
106
107 We developed microsatellite markers in *G. schirazensis* and use them to genotype 242
108 individuals from 18 localities in Peru, Ecuador, Colombia, Venezuela, USA, Spain, and Reunion
109 Island. We characterized the population structure and genetic diversity, and discuss it in light of
110 the breeding system and recent history of expansion of this species. We also assessed the
111 specificity of these markers by testing amplification in the morphologically similar and closely
112 related *Galba* species, *i.e. G. truncatula*, *G. cubensis*, and *G. viator*. We also sequenced a
113 mitochondrial gene (cytochrome oxidase subunit I; CO1) to further elucidate the
114 phylogeographic relationships among these populations and the populations studied by Bargues
115 et al. (2011) and Correa et al. (2011).
116

2. Materials and methods

Development of specific microsatellite markers

We mixed DNA from four individuals identified by Correa et al. (2011) as *Galba* sp. These individuals were collected in Río Negro, Antioquia, Colombia (N 06°07'21" W 75°26'57"). DNA was extracted from foot tissue using the DNeasy Blood and Tissue Kit (Qiagen) and individuals were identified to species using the nuclear genes 18S (GenBank accession numbers: JN614335, JN614339, JN614340, JN614342), ITS-1 (HQ283253, JN614429, JN614430, JN614432), ITS-2 (HQ283263, JN614455, JN614456, JN614458), and the mtDNA gene CO1 (JN614370, JN614371, JN614373, JN614374). All these sequences showed 99–100% of homology with the sequences of *G. schirazensis* reported by Bargues et al. (2011).

Microsatellite loci were isolated from two enriched libraries (TC10 and TG10) following protocols described in Dubois et al. (2005) and using biotin-labeled microsatellite oligoprobes and streptavidin-coated magnetic beads. The enriched molecules were cloned into the pGEMt vector used to transform XL1-Blue Supercompetent Cells. Recombinant clones were screened with TC10, TG10, and AGE1 (AAACAGCTATGACCATGATTAC) or AGE2 (TTGTAAAACGACGGCCAGTG) oligonucleotides using a modified PCR method (Waldbieser 1995). We screened 228 clones, 163 of which gave a positive signal and were sequenced using an ABI Prism 3100 sequencer (Applied Biosystems). Ninety seven sequences included a repeated motif and flanking regions allowing determination of PCR primers that were designed using Primer3 (Rozen and Skaletsky 1999). We retained the 25 loci that showed the largest uninterrupted stretches of repeated motifs and selected the 22 ones that amplified successfully (Table 1). We tested these 22 microsatellite loci in six individuals from five localities from the Americas: Finca Jocum Bucaramanga (Colombia), Huagrahuma (Ecuador), Manto de la Novia (Ecuador), Louisiana Bedico (USA; 2 individuals), and Bodoque (Venezuela; Table 2, Fig. 1).

We finally selected the 13 loci that were polymorphic in these six individuals for further population genetic analyses.

To test the specificity of microsatellite markers, we also examined individuals of *G. truncatula*, *G. cubensis*, and *G. viator* identified using the molecular markers 18S, ITS-1, ITS-2, and CO1 (GenBank accession numbers in Correa et al., 2011).

Population genetic analyses using microsatellite markers

We analyzed 238 individuals of *G. schirazensis* from 16 localities from Colombia, Ecuador, Peru, USA, and Venezuela, as well as individuals from the Old World, one from Spain and three from Reunion Island (Table 2, Fig. 1). The four latter individuals were initially identified as *G. truncatula*, but re-identified as *G. schirazensis* by analyzing the CO1 gene (GenBank accession numbers in Table 2) and/or specific microsatellite loci. The Spanish specimen was collected from within the geographic range of *G. truncatula*. The date of appearance of *G. schirazensis* in this region is currently unknown. The population from Reunion Island is not native and was first ascribed to the introduced species *G. truncatula* (Griffiths and Florens 2006). Despite the small sample sizes, we included these individuals because they constitute the first mention of *G. schirazensis* in these two areas. All samples were collected from small areas ($< 2m^2$) to prevent a Wahlund effect (Meunier et al. 2004*a*). Snails were killed in 70 °C water and immediately stored in 70% ethanol.

For each specimen, we removed the distal part of the foot, which was twice compressed between paper towels to remove excess ethanol. DNA extractions were then performed using 200 µl of 5% Chelex® (Chelex Bio Rad diluted in a Tris-EDTA buffer) solution incorporating 5 µl of proteinase K (Sigma) at a concentration of 20 mg/ml. This suspension was heated at 56 °C for 6

hours followed by gentle vortexing and a further incubation at 95 °C for 10 min. The mixture was gently vortexed and centrifuged at 10,000 g for 10 sec. The supernatant (100 µl) was collected, diluted 1: 10 in deionized water, and stored at −20 °C.

We amplified microsatellite loci in an Eppendorf Thermal Cycler, in a total volume of 10 µl containing 5 µl of Taq PCR Master Mix Kit (Qiagen), 1 µl of the primer mix, and 1 µl of DNA. PCR conditions were as follow: 15 min activation at 95 °C, 35 cycles including 30 sec of initial denaturation at 94 °C, 90 sec of annealing at 55 °C, and 60 sec of extension at 72 °C, followed by 30 min of final extension at 60 °C. For genotyping, we pooled 3 µl of diluted (1: 100) PCR products with 15 µl of Hi-Di Formamide and 0.15 µl of GeneScan-500 LIZ Size Standard and analyzed it on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). We performed multiplexed locus amplification for those PCR products characterized by different sizes and labeled with different fluorochromes. Allele sizes were estimated using GeneMapper® v.4.0 software (Applied Biosystems).

We used the individual genotypes of the 238 American individuals to estimate allelic richness, observed and expected heterozygosities, the *F*-statistic F_{IS}, and the selfing rate (s) estimated as $s = 2 F_{IS} / (1 + F_{IS})$ (Hartl and Clark 1997). The global and pairwise differentiations among populations were estimated using F_{ST} and R_{ST}. We could not use software that detect null alleles because lymnaeids are hermaphroditic. Standard Bonferroni corrections were applied in the case of multiple tests (Rice 1989). We analyzed data using Genodive (version 2.0b23; Meirmans and Van Tienderen, 2004) and FSTAT (version 2.9.3.2; Goudet, 1995) software. Individuals from Spain and Reunion Island were not included in the population genetic analysis because sample sizes were too low. However, we compared their genotypes to those of American individuals. Multilocus genotypes were clustered using a discriminant analysis of principal components

192 (DAPC) using the adegenet package of R (Jombart et al. 2010). One of the main advantages of
193 this analysis is that it does not rely on a particular population genetic model and is thus free of
194 assumptions about Hardy-Weinberg or linkage equilibrium. Six principal components were
195 retained as inferred by the α -score. The α -score gives a number of principal components
196 optimized in order to capture the best discrimination without overfitting.
197
198 **Amplification and sequencing of CO1 and phylogeographic analysis**
199 We amplified and sequenced the CO1 gene in 21 snails from 11 populations (Fig. 3). Because of
200 amplification failure, we did not sequence it in individuals from Andaracas (Ecuador), Bodoque,
201 Zea el Amparo, La Azulita, Bailadores, and San Eusebio (Venezuela) and El Rocío (Spain). The
202 PCR primers were LCO1490 (5'–GGTCAACAACTCATAAAGATATTGG–3') and HCO2198
203 (5'–TAAACTTCAGGGTGACCAAAAAATCA–3') (Folmer et al. 1994). We used 2.5 µl of
204 DNA, 12.5 µl of Taq PCR Master Mix Kit (Qiagen), 2.5 µl of each primer (2 µM), and 5 µl of
205 distilled water. PCR conditions were as follow: 15 min activation at 95 °C, 35 cycles including
206 30 sec of initial denaturation at 95 °C, 1 minute of annealing at 50 °C, and 10 min of extension at
207 72 °C, followed by 10 min of final extension at 72 °C. DNA sequencing was performed by
208 Eurofins MWG Operon (Germany). In addition, we downloaded from GenBank 13 CO1
209 sequences of *G. schirazensis* from Iran, Spain, Reunion Island, Peru, Ecuador, Colombia,
210 Venezuela, and Mexico (JF272607–JF272610, Bargues et al. 2011; JN614370–JN614378,
211 Correa et al. 2011).
212
213 The number of haplotypes, the number of polymorphic sites, and the nucleotide diversity were
214 calculated using DnaSp V5 (Librado and Rozas 2009). Divergence between haplotypes were
215 calculated using Mega7 (Kumar et al. 2016). CO1 sequences were aligned sequences using
216 Clustal-W and used to build a Maximum Likelihood tree with the best-fitting model of sequence

evolution using Mega7 (Kumar et al. 2016). Model selection was based on Bayesian Information Criterion using Mega7 (Kumar et al. 2016). Node robustness was assessed based on 500 bootstraps.

3. Results

The sequences and primers of the 22 microsatellite loci that we identified in *G. schirazensis* are available in GenBank (accession numbers in Table 1). No amplification was observed in the closely related species *G. truncatula, G. cubensis,* and *G. viator*, suggesting that these 22 loci are specific to *G. schirazensis*.

Approximate position of Table 1.

We characterized the variation at the 13 polymorphic loci in all American individuals. The number of alleles per locus was 2.846 ± 0.274 SD, the observed heterozygosity was 0 at all loci, and the mean expected heterozygosity was 0.007 ± 0.005 SD (Table 1). In most loci and populations, the lack of diversity prevented us from estimating F_{IS} (Table 2). Among the four loci and populations that showed allelic diversity at some loci (Table S1), the observed heterozygosity was always zero ($F_{IS} = 1$, $s = 1$; Table 2).

Approximate position of Table 2.

We found high genetic differentiation among populations (global $F_{ST} = 0.979$; $P < 0.001$; global $R_{ST} = 0.979$; $P < 0.001$). All population pairs coming from different countries (except for Venezuela and Colombia) showed significant F_{ST} after Bonferroni's adjustment ($P = 0.0004$; Table S2). In contrast, populations from the same country or from adjacent countries usually

242 showed no significant differentiation (Ecuador: $F_{ST} < 0.019$, NS; Venezuela and Colombia: $F_{ST} <$
243 0.235, NS). An exception is the Venezuelan population from La Trampa that differs
244 significantly from most other Venezuelan and Colombian populations with F_{ST} between 0.3 and
245 0.45 (Table S2).
246
247 Nine multilocus genotypes were found (Fig. 1). Some loci (1–8) could not be genotyped in some
248 individuals (N = 65). Thus, we did not ascribe these individuals with incomplete genotypes to
249 any multilocus genotype. However, in these individuals the loci that could be genotyped were
250 exactly the same as in the individuals were all the loci were genotyped (Table S1). To analyze
251 the genetic structure of populations using the DAPC, we used all the individuals except for 12
252 individual genotypes that had more than three loci that did not amplify (Table S1). Four clusters
253 were detected by DAPC which grouped together individuals from (1) Peru, (2) Ecuador, (3)
254 Colombia, Venezuela, Spain, and Reunion Island, and (4) USA (Fig. 2; Table S1). Each cluster
255 consisted of a single main multilocus genotype with small within-cluster variation (Table S1).
256 Differences between the main genotypes and variants did not exceed one repeat (two base pairs),
257 and was of one base pair in some cases. The exception is the specimen from Spain that differed
258 from the main type at two loci (Table S1). The main type from each cluster differed from those
259 of other clusters at five loci or more (Table S1). The largest difference was observed between
260 clusters from Ecuador and USA (all 13 loci fixed for different alleles).
261 .
262 *Approximate position of Figure 1.*
263
264 We obtained 21 CO1 sequences of *G. schirazensis* (Table 2). The analysis of mtDNA CO1
265 sequences revealed 5 haplotypes (haplotype diversity: 0.499 ± 0.083 SD) determined by 9
266 polymorphic sites without indels. The best model describing the evolution of these sequences

and sequences retrieved from GenBank (Fig. 3) was T92+G. The Maximum Likelihood tree showed that the sequences here studied were gathered in three clades: (i) one that grouped sequences from Ecuador (cluster 2 in DAPC), (ii) one with the only sequence from USA (cluster 4), (iii) and one large worldwide clade that grouped the sequences from Peru (cluster 1) and Venezuela, Colombia, and Reunion Island (cluster 3). Most CO1 sequences retrieved from GenBank—that included sequences from Iran, Spain, Venezuela, Colombia, and Reunion Island—were in the large worldwide clade (Fig. 3).

The Maximum Likelihood tree was consistent with the results from the DAPC in grouping the individuals from the same cluster in the same clade (Figs. 2 and 3). However, the tree did not reflect the same genetic distances that we did observed with the DAPC, for instance the clusters 1 and 3 were genetically distant from each other in the DAPC but they gathered in the same worldwide clade (Figs. 2 and 3). Also, the clusters 2 and 4 were genetically distant from each other in the DAPC (Fig. 2) but they were genetically close in the tree and the CO1 sequences diverged a little from each other (Table S3; Fig. 3). This inconsistency could be explained by a mitochondrial introgression among populations.

Approximate position of Figure 2.

4. Discussion

We developed 13 polymorphic genetic markers to study the population structure and genetic diversity of *G. schirazensis*, an invasive freshwater snail inhabiting Asia, Europe, and the New World. These microsatellite loci were specific to *G. schirazensis* because we did not find any amplification in the morphologically similar and most closely related species *G. truncatula, G. cubensis,* and *G. viator*. Analyzing New World populations, we observed very low genetic

diversity within populations, a total lack of heterozygotes, and marked population structure. The lack of observed heterozygosity in those few populations that showed allelic diversity suggests that *G. schirazensis* mainly reproduces by self-fertilization. This is consistent with the fact that under laboratory conditions, individuals isolated from birth are able to self fertilize (Bargues et al. 2011). Our population genetic evidence for self-fertilization in natural population only relies on five locus-by-population combinations, all involving relatively rare alleles segregated in the populations (Tables 2, S1). In the absence of self-fertilization, rare alleles are expected to be mainly in the heterozygous state, so it would be very improbable for them to appear only as homozygotes in these five cases. Therefore, a high rate of self-fertilization is the most likely explanation for the lack of heterozygosity. In addition, related lymnaeid species exhibit selfing rates in the 80–100% range, for example *G. cubensis* (Lounnas et al. 2016), *G. truncatula* (Trouvé et al. 2000, Meunier et al. 2001, 2004*a*), *Omphiscola glabra* (Hurtrez-Boussès et al. 2005), and *Pseudosuccinea columella* (Nicot et al. 2008) which also show very low or absent within-population allelic diversity, high F_{IS}, and large F_{ST}. In contrast, most populations of mainly outcrossing lymnaeids such as *Lymnaea stagnalis* (Puurtinen et al. 2007, Kopp et al. 2012, Besnard et al. 2013) and *Radix sp.* (Pfenninger et al. 2011) have high polymorphism, low F_{IS}, and moderate F_{ST}. Therefore, *G. schirazensis* shares the common characteristics of highly selfing lymnaeid species studied so far. It also shares a similar ecology with *G. cubensis*, *G. truncatula*, and *O. glabra*. All of them live mainly in temporary habitats that undergo frequent flooding and droughts that create strong bottlenecks (Meunier et al. 2004*b*). In this context, self-fertilization allows a single individual to colonize vacant habitats and to produce a new population (Meunier et al. 2004*a*). Finally, the recently analyzed transcriptomes of both *G. truncatula* and *G. schirazensis* bear molecular signatures consistent with ancient self-fertilization, dating back to their common ancestor (Burgarella et al. 2015).

317 It is very difficult to know *a priori* where *G. schirazensis* was present before 2010, hence
318 whether each particular population is native or invasive. *Galba schirazensis* has probably been
319 misidentified in previous reports of small lymnaeid species from all over the world, either as *G.*
320 *truncatula* (everywhere) or as *G. cubensis* and *G. viator* (New World; Correa et al. 2010, 2011,
321 Bargues et al. 2011) as they all have very similar shell morphology and internal anatomy. Thus,
322 it is impossible to trace invasion routes using reports from the literature, except for places known
323 from historical records to be devoid of native small-shelled lymnaeids, such as Reunion Island
324 where the *G. schirazensis* populations necessarily result from introduction(s). The geographic
325 origin of *G. schirazensis* remains unknown. Bargues et al. (2011) assumed that this species is
326 native to the Middle East as it was first described in Iran (Küster 1862). However, as is typical of
327 species descriptions of that time, only a brief description of the shell of *G. schirazensis* was
328 provided, which could in practice fit many small-shelled lymnaeids, including the very
329 widespread *G. truncatula*. Therefore, the snail lineage studied in the present paper, re-described
330 on the basis of molecular sequences as *G. schirazensis* by Bargues et al. (2011), as *Lymnaea sp.*
331 by Correa et al. (2010), and as *Galba* sp. by Correa et al. (2011), may not necessarily be (i) the
332 same snail as that named *G. schirazensis* by Küster (1862) and (ii) native from the Middle East.
333 It current occurrence in Iran (Bargues et al. 2011) could well result from an introduction.
334
335 In this confused taxonomical situation, it is important to clearly list the available evidence on the
336 possible geographical origin of *G. schirazensis*. Any mention of this species can only be
337 ascertained based on molecular evidence in order to avoid misidentification. This eliminates all
338 mentions prior to 2010, including the first description by Küster (1862). As a consequence, we
339 must rely only on genetic diversity and phylogeny to identify likely origin areas. Bargues et al.
340 (2011; Table 1) showed that all the individuals they sampled in the Old World (Iran, Egypt, and
341 Spain) have exactly the same haplotypes at the nuclear genes18S, ITS-2, ITS-1 and the

342 mitochondrial genes 16S and CO1. However, they observed two to four different haplotypes for
343 each gene in American populations (Dominican Republic, Mexico, Peru, Ecuador, and
344 Venezuela), including the Old World haplotype. Our microsatellite study corroborates this
345 pattern, as each of the four different regions sampled in the New World forms well-defined and
346 differentiated clusters and the few individuals from two very distant places in the Old World (La
347 Réunion and Spain), including one from a known recent introduction (Reunion Island), are
348 genetically related to only one of these clusters (Fig. 1). The phylogeographic tree is consistent
349 with this pattern, since American CO1 sequences are variable while the sequences from the Old
350 World showed no variation and belong all to the large worldwide clade (Fig. 3). All this suggests
351 that the center of diversity, and by extension the origin area of this species, should be found in
352 the New World, while Old World populations should rather result from a recent introduction of a
353 genetically uniform population. In other snail species with the ability to self-fertilize, genetic
354 uniformity over large distances and many populations is indeed the signature of recent
355 introductions, for example in *Lymnaea stagnalis* introduced in New Zealand (Kopp et al. 2012)
356 or *G. truncatula* introduced on the Bolivian Altiplano (Meunier et al. 2001). Finally, an
357 American origin for *G. schirazensis* is also corroborated by the phylogeny of Correa et al.
358 (2010), who concluded that the whole *Galba* clade likely has an American origin, as most of its
359 species are native there (*G. cousini, G. viator, G. neotropica, G. cubensis, G. humilis*). The
360 hypothesis posed by Bargues et al. (2011) of a post-Columbian introduction of *G. schirazensis*
361 from Europe to the New World, is not consistent with our findings: it would have been very
362 unlikely to accumulate fixed differences at 13 distinct loci (as we observed between USA and
363 Ecuador) in such a short time, while at the same time Venezuelan, Colombian, and Old World
364 populations would remain nearly identical and devoid of diversity over huge geographical
365 distances.
366

Although our data together along with the report of Bargues et al. (2011) seem sufficient to reject the hypothesis of a single recent introduction of *G. schirazensis* in the New World, more extensive sampling is needed (i) to more fully understand the genetic variation observed among South and North America isolates and (ii) to test whether all the populations from the Old World have a single recent introduced origin, an hypothesis so far consistent with the results of conservative (Bargues et al. 2011, Correa et al. 2011, this study) and polymorphic (this study) markers. The application of the genetic markers developed here to a more extensive sample that covers a worldwide range will therefore help to clarify the geographic origin and invasion routes of *G. schirazensis*.

Understanding the population structure and the genetic diversity of *G. schirazensis* can also help elucidating its role in spreading infectious diseases. However, our capacity to identify which parasites are transmitted by *G. schirazensis* has hitherto been hindered by the morphological similarity among *Galba* species (Correa et al. 2011). We need to identify by molecular means morphologically cryptic *Galba* species and to identify the role of *G. schirazensis* as an intermediate host of food- and water-borne trematodes such as *F. hepatica*.

In conclusion, our analysis provides strong support to the ideas that this species is predominantly self-fertilizing, and has an American origin with recent colonization of the Old World by a genetically uniform strain related to populations from Venezuela and Colombia—a hypothesis that awaits confirmation by more extensive sampling.

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394

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517

518

519 **Table 1.** Microsatellite loci in *Galba schirazensis*. Description of loci and genetic variation in
520 six individuals from five localities (polymorphic loci in bold characters). Annealing
521 temperature is 55 °C for all loci. Na, number of alleles detected; RAS, range of allele size
522 (in base pairs).
523

Locus	Primer sequences (5'→3')	GenBank accession number	Repeat motif	Na	RAS
Gsch_1	F: TTTTGGGTCAACATTAGGTTAGG R: GAACATTTGACACAGTAGCGTTG	KT324711	$(TC)_{13}$	3	213–249
Gsch_2	F: ACGTGCACACTTCTCCCTCT R: GCCTTGGTGCAGTTTTGTATT	KT324709	$(CT)_{23}$	1	185
Gsch_3	F: TGTAGGCAAAGGCACAAAAA R: AGGGTGTAAGGGCTGAATTG	KT324702	$(CT)_{13}$	4	152–178
Gsch_4	F: TTCATTGTCTGCTCCTGCTG R: CGCCACTGGTCGATAGACTT	KT324708	$(TC)_{20}(CT)_{13}$	2	157–160
Gsch_5	F: GGTCGTTTAGCAGCCTAGCA R: CGACCGTTCAAACGTTACTG	KT324710	$(GT)_{10}(AG)_{13}$	2	179–185
Gsch_7	F: AAACGACCGTTTCTAAGGTGA R: CCCTGAACCAACAGAGCATT	KT324707	$(AG)_{23}$	1	244
Gsch_9	F: GGCGGAAACGAAGAGAGTAA R: TACGTGCACACACTCAACCA	KT324705	$(TC)_{11}$	4	207–227
Gsch_10	F: AGGAGAGTCCCATTGAGCTG R: CGAAACTGTTGAAGGCATTG	KT324706	$(GT)_{11}$	1	218
Gsch_11	F: AACACATTTCCACCCACACA R: CTTTCTTTCGCGTGGGGTAT	KT324712	$(CT)_{18}$	2	216–235
Gsch_12	F: CTGGGGCTAACCCAAGAATC R: TTTGGGGTTGAGGGCTTTAT	KT324713	$(TG)_{10}$	1	151
Gsch_13	F: TCACGTTCTCGTGGTTCTCA R: GTCGATGGGGCTATGTGTCT	KT324714	$(CA)_{10}$	5	166–173
Gsch_14	F: CGATGGCGCCAACTTATTTA R: TCATATCGCTACGGACATTCA	KT324715	$(CA)_{10}$	2	201–222
Gsch_15	F: TGAGACGGGCAAGATTTCTC R: AGGGTTCGATTCCCATCTCT	KT324716	$(CT)_{15}$	1	154
Gsch_17	F: CTTCATCCACGCAGCAAGTA R: GGGGGCCGATATTAATTTTT	KT324717	$(AC)_{13}$	1	205
Gsch_18	F: GACGGACCGTGAATTAGAGC	KT324718	$(AG)_{11}$	1	210

	R: ATTGTTGCGGCGGATAACTA				
Gsch_19	F: CGGTATTAGGGTGTCATGTGC R: CAGGGGGAACCATAAAGTTG	KT324719	$(CA)_{12}$	1	171
Gsch_20	F: CAGTGAGACAGAGCCACGAA R: ATTGCCGACACGTTTGTTGT	KT324720	$(AG)_{31}$	1	192
Gsch_21	F: CGCTAGACCTTTCTTTCTGTCC R: TTACAAGCTCTTGGGAACGA	KT324721	$(TC)_{14}$	3	239–279
Gsch_22	F: TGTGTGTGTTTGTGGAGAGAGA R: GTGTTACGCATGGTGAACCT	KT324722	$(AG)_{11}$	3	249–322
Gsch_23	F: AATGACCCAGTGGGGAAG R: TGGGGAAGGTTCAATTGTTT	KT324723	$(AC)_{16}$	2	227–232
Gsch_24	F: GCGTGCGTGTATGTGAAAGA R: GGGGCTCTTCAAGTGTGTGT	KT324704	$(AG)_{10}$	3	167–171
Gsch_25	F: AGCCAGACAAAGGGGGATAG R: GGGCAGGTTCATTACTCTGTTC	KT324703	$(GA)_{19}$	2	188–190

Table 2. Populations of *Galba schirazensis* and their genetic variability. Genetic parameters were not calculated in the samples from Spain and La Réunion Island because of too small sample size. N, sample size; Na, mean number of alleles; He, expected heterozygosity; F_{IS}, inbreeding coefficient and *P*-value; NA, not available. Bonferroni corrections were applied ($P = 0.0125$). Observed heterozygosity was null in all populations.

Country	Location	Acronym	Coordinates	Sampling year	GenBank accession number	N	Na	He	F_{IS}	Estimated selfing rate
Peru	La Joya de Arequipa	LJ	16°28'56" S 71°49'07" W	2012	KY198250, KY198260	14	1.000	0.000	-	-
Ecuador	Huagrahuma	HU	02°47'32" S 79°16'31" W	2012	KT781302, KT781304	13	1.000	0.000	-	-
	Manto de la Novia	MN	01°24'03" S 78°17'49" W	2011	KT781305, KT781315	15	1.000	0.000	-	-
	Hacienda Cienaga	HC	00°46'18" S 78°37'10" W	2011	KT781301	12	1.000	0.000	-	-
	Andaracas	AN	00°26'10" S 78°32'22" W	2014	-	14	1.077	0.013	1 (<0.0001)	1
	Nono	NO	00°03'25" S 78°34'15" W	2014	KY198255, KY198256	16	1.000	0.000	-	-
Colombia	Finca Jocum Bucaramanga	FJ	07°06'25" N 73°04'60" W	2012	KY198253, KY198254	14	1.000	0.000	-	-
Venezuela	Bodoque	BO	08°16'15" N 71°48'51" W	2005	-	18	1.077	0.033	1 (<0.0001)	1
	Los Nevados	LN	08°27'41" N 71°04'28" W	2013	KT781320	18	1.000	0.000	-	-
	Zea el Amparo	ZA	08°21'40" N 71°46'01" W	2005	-	9	1.000	0.000	-	-
	La Azulita	LA	08°44'06" N 71°26'49" W	2005	-	11	1.000	0.000	-	-
	Bailadores	BA	08°14'05" N 71°50'26" W	2005	-	18	1.000	0.000	-	-
	San Eusebio	SE	08°38'39" N 71°23'42" W	2005	-	18	1.000	0.000	-	-
	Sabana Alto	SA	08°36'11" N 71°27'45" W	2013	KT781322, KT781323, KT781324	7	1.000	0.000	-	-

	La Trampa	LT	08°33'31" N 71°27'13" W	2013	KY198251, KY198252	20	1.154	0.048	1 (<0.0001)	1
USA	Louisiana Bedico	LB	30°26'11" N 90°15'01" W	NA	KT781332	21	1.077	0.015	1 (<0.0001)	1
Spain	El Rocío	ER	37°08'02" N 06°28'18" W	2010	-	1	-	-	-	-
La Reunion Island (France)	Ravine du Gol	RG	21°14'26" S 55°25'07" E	2009	KY198257, KY198258, KY198259	3	-	-	-	-

Figure legends

Figure 1. Geographic location of multilocus microsatellite genotypes of *Galba schirazensis*. Each multilocus genotype is figured by a color and a letter. Each pie chart represents a population. See Table 2 for population acronyms.

Figure 2. Genetic population structure of *Galba schirazensis*. Scatter plot of 230 individuals on the first two DAPC axes (first axis: 61% of total variance; second axis: 22%). Individuals group in four clusters.

Figure 3. Maximum-likelihood phylogenetic tree of *Galba schirazensis* based on mtDNA CO1 sequences. Bootstrap values are indicated at each node. Sequences are given their GenBank accession numbers. Colored sequences are those obtained in this study, the others having been retrieved from GenBank. *Galba truncatula* JN614386 sequence (Correa et al. 2011) was used as outgroup. For each sequence obtained here, the color code refers to the cluster number derived from DAPC.

Supplementary material

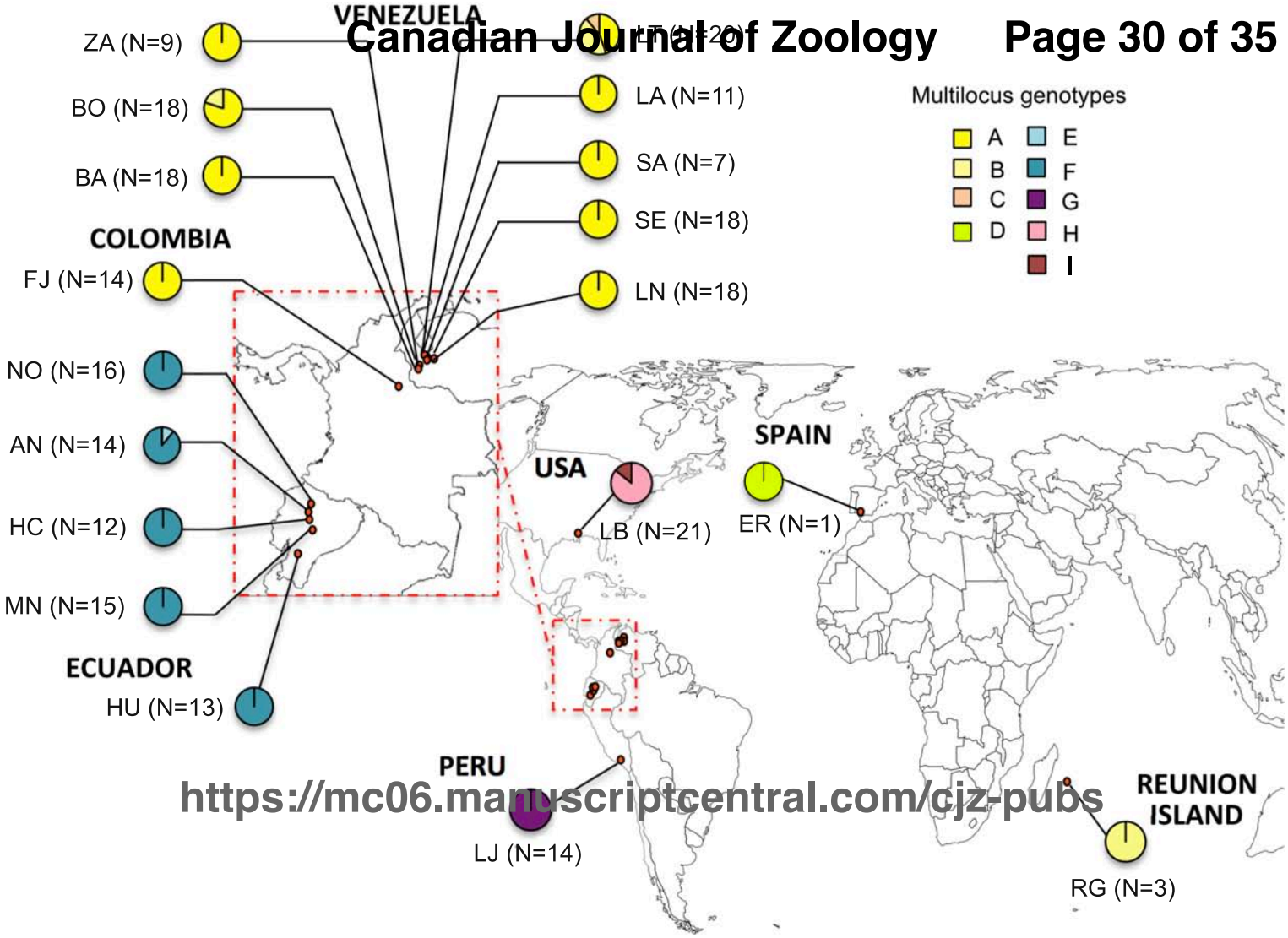
Table S1. Multilocus genotype of *Galba schirazensis*. N is the number of individuals for a given multilocus genotype. * indicates the genotypes that were discarded from DAPC due to more the three not amplified loci. Further details about loci and populations in Table 2 from main text.

Table S2. Genetic distance between populations of *Galba schirazensis*. Pairwise *FST* values on the lower diagonal, *P*-values on the upper diagonal. Bonferroni corrections were applied in all pairwise comparisons ($P = 0.0004$). FJ, Finca Jocum Bucaramanga; AN, Andaracas; HC, Hacienda Cienaga; HU, Huagrahuma; MN, Manto de la Novia; NO, Nono; LJ, La Joya de Arequipa; LB, Louisiana Bedico; LA, La Azulita; BA, Bailadores; BO, Bodoque; LN, Los Nevados; SA, Sabana Alto; SE, San Eusebio; LT, La Trampa; ZA, Zea el Amparo.

Table S3. Divergence between sequences of CO1 in *Galba schirazensis*. The number of base substitutions per site between sequences are shown. Haplotype 1, Iran, Spain, Reunion Island, Venezuela, Colombia, and Peru (the sequences here studied); Haplotype 2, Peru (the sequences studied by Bargues et al. 2011); Haplotype 3, Mexico; Haplotype 4, USA; Haplotype 5, Ecuador.

References in Supplementary Material

Bargues, M.D., Artigas, P., Khoubbane, M., Flores, R., Glöer, P., Rojas-García, R., Ashrafi, K., Falkner, G., and Mas-Coma, S. 2011. *Lymnaea schirazensis*, an overlooked snail distorting fascioliasis data: Genotype, phenotype, ecology, worldwide spread, susceptibility, applicability. PLoS ONE 6 **6**: e24567. doi:10.1371/journal.pone.0024567.



COLOMBIA
VENEZUELA
SPAIN
REUNION ISLAND

ECUADOR

PERU

USA

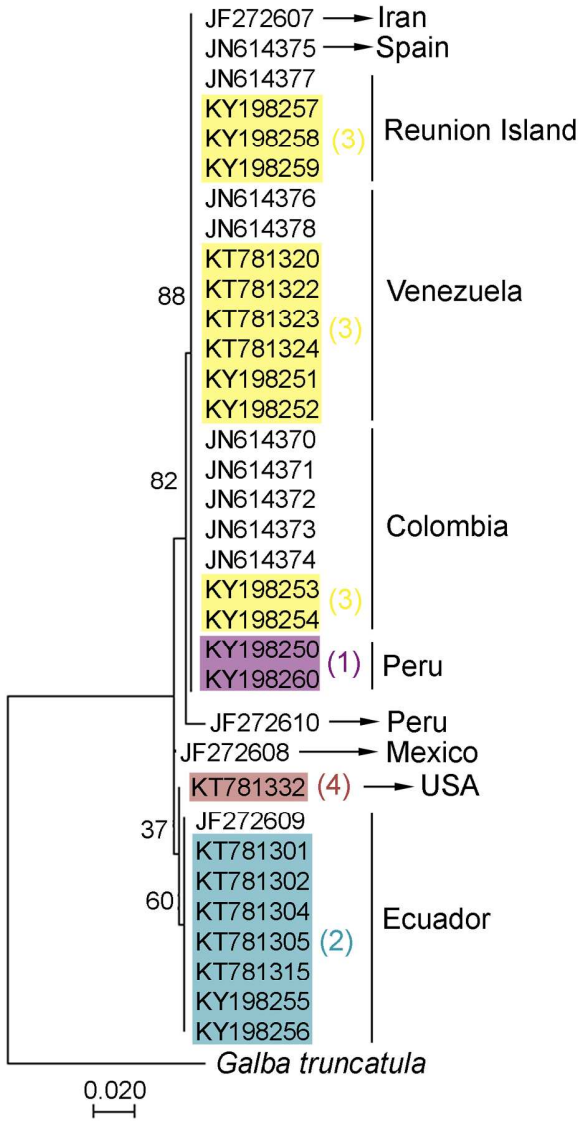


Figure 3. Maximum-likelihood phylogenetic tree of Galba schirazensis based on mtDNA CO1 sequences. Bootstrap values are indicated at each node. Sequences are given their GenBank accession numbers. Colored sequences are those obtained in this study, the others having been retrieved from GenBank. Galba truncatula JN614386 sequence (Correa et al. 2011) was used as outgroup. For each sequence obtained here, the color code refers to the cluster number derived from DAPC.

101x197mm (300 x 300 DPI)

Table S1. Multilocus genotype of *Galba schirazensis* . N is the number of individuals for a given multilocus genotype. * indicates the genotypes that were discarded from DAPC due to more the three not amplified loci. Further details about loci and populations in Table 2 from main text.

	Location	N	Multilocus genotype	Cluster	Gsch_1	Gsch_3	Gsch_4	Gsch_5	Gsch_9	Gsch_11	Gsch_13	Gsch_14	Gsch_21	Gsch_22	Gsch_23	Gsch_24	Gsch_25	
Colombia	Finca Jocum	12	A	(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
	Bucaramanga	1		(3)	213 213	-	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	157 157	185 185	207 207	-	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
Venezuela	La Azulita	8	A	(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	-	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	-	152 152	-	185 185	207 207	-	172 172	222 222	239 239	-	232 232	167 167	190 190	*
		1		(3)	213 213	152 152	-	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
	Bailadores	11	A	(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		2		(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	-	167 167	190 190	
		1		(3)	213 213	152 152	-	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	-	152 152	-	-	207 207	235 235	172 172	222 222	-	249 249	232 232	167 167	190 190	*
		1		(3)	213 213	152 152	157 157	185 185	207 207	235 235	-	222 222	-	249 249	-	167 167	190 190	
		1		(3)	-	-	-	185 185	-	235 235	172 172	222 222	-	-	-	167 167	190 190	*
		1		(3)	213 213	152 152	157 157	185 185	207 207	-	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
	Bodoque	12	A	(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		3	B	(3)	213 213	152 152	157 157	185 185	207 207	235 235	171 171	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	157 157	-	-	235 235	171 171	222 222	239 239	-	-	167 167	-	*
		1		(3)	213 213	-	-	185 185	-	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	-	185 185	207 207	-	171 171	222 222	239 239	-	232 232	167 167	190 190	
	Los Nevados	16	A	(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	-	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	-	185 185	207 207	-	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
	Sabana Alto	5	A	(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	-	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	-	185 185	207 207	-	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
	San Eusebio	15	A	(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	157 157	185 185	207 207	-	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	-	232 232	167 167	190 190	
		1		(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	-	239 239	249 249	232 232	167 167	190 190	
	La Trampa	8	A	(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		8	B	(3)	213 213	152 152	157 157	185 185	207 207	235 235	171 171	222 222	239 239	249 249	232 232	167 167	190 190	
		2	C	(3)	213 213	152 152	157 157	185 185	207 207	235 235	170 170	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	157 157	185 185	207 207	235 235	-	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	-	-	157 157	185 185	207 207	235 235	-	222 222	-	249 249	232 232	167 167	190 190	*
	Zea el Amparo	1	A	(3)	213 213	152 152	157 157	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		3		(3)	213 213	152 152	157 157	185 185	207 207	-	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	-	185 185	207 207	235 235	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	-	185 185	207 207	-	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	213 213	152 152	157 157	185 185	207 207	-	172 172	222 222	239 239	-	232 232	167 167	190 190	
		1		(3)	-	152 152	-	185 185	207 207	-	172 172	222 222	239 239	249 249	232 232	167 167	190 190	
		1		(3)	-	152 152	157 157	-	207 207	-	-	222 222	239 239	-	232 232	167 167	190 190	*
Spain	Los Andes	1	D	(3)	213 213	152 152	157 157	185 185	207 207	245 245	172 172	222 222	239 239	250 250	235 235	167 167	190 190	
La Reunion	Ravine du Gol	3	B	(3)	213 213	152 152	157 157	185 185	207 207	235 235	171 171	222 222	239 239	249 249	232 232	167 167	190 190	
Peru	La Joya de Arequipa	7	G	(1)	249 249	152 152	157 157	185 185	221 221	235 235	172 172	222 222	241 241	322 322	232 232	169 169	190 190	
		1		(1)	-	152 152	157 157	185 185	221 221	235 235	172 172	222 222	241 241	322 322	232 232	169 169	190 190	
		2		(1)	249 249	152 152	157 157	185 185	221 221	235 235	172 172	222 222	-	322 322	232 232	169 169	190 190	
		1		(1)	249 249	152 152	157 157	185 185	221 221	-	172 172	222 222	241 241	322 322	232 232	169 169	190 190	
		1		(1)	249 249	152 152	157 157	185 185	221 221	235 235	172 172	222 222	241 241	322 322	232 232	169 169	-	
		1		(1)	249 249	152 152	157 157	185 185	-	-	172 172	222 222	-	322 322	232 232	169 169	190 190	

		1		(1)	249 249	152 152	157 157	185 185	221 221	235 235	172 172	222 222	-	-	232 232	169 169	190 190	
Ecuador	Andaracas	8	E	(2)	249 249	178 178	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1	F	(2)	249 249	178 178	157 157	179 179	221 221	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	249 249	-	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	-	178 178	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	249 249	178 178	157 157	179 179	227 227	-	-	201 201	-	249 249	232 232	171 171	190 190	
		1		(2)	249 249	-	157 157	179 179	-	-	-	-	-	-	-	171 171	190 190	*
		1		(2)	-	178 178	157 157	179 179	-	216 216	166 166	-	-	249 249	-	171 171	190 190	*
	Hacienda Cienaga	9	E	(2)	249 249	178 178	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	249 249	178 178	157 157	179 179	227 227	-	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		2		(2)	-	178 178	-	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
	Huagrahuma	11	E	(2)	249 249	178 178	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	249 249	178 178	157 157	-	-	-	166 166	201 201	-	-	232 232	171 171	190 190	*
		1		(2)	249 249	-	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
	Manto de la Novia	10	E	(2)	249 249	178 178	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	-	178 178	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	249 249	178 178	157 157	-	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	249 249	178 178	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	-	190 190	
		1		(2)	-	178 178	-	179 179	227 227	-	166 166	-	-	249 249	232 232	-	190 190	*
		1		(2)	249 249	178 178	157 157	179 179	-	216 216	166 166	201 201	279 279	-	232 232	171 171	190 190	
	Nono	13	E	(2)	249 249	178 178	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	249 249	178 178	157 157	179 179	-	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	-	178 178	157 157	179 179	227 227	216 216	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
		1		(2)	249 249	178 178	157 157	179 179	227 227	-	166 166	201 201	279 279	249 249	232 232	171 171	190 190	
USA	Louisiana Bedico	12	H	(4)	233 233	162 162	160 160	185 185	209 209	235 235	170 170	222 222	239 239	284 284	227 227	169 169	188 188	
		2	I	(4)	233 233	161 161	160 160	185 185	209 209	235 235	170 170	222 222	239 239	284 284	227 227	169 169	188 188	
		1		(4)	-	162 162	-	185 185	209 209	235 235	170 170	222 222	239 239	-	227 227	169 169	188 188	
		1		(4)	233 233	162 162	-	185 185	209 209	-	170 170	222 222	239 239	-	227 227	169 169	188 188	
		1		(4)	-	162 162	160 160	185 185	209 209	235 235	-	222 222	239 239	284 284	227 227	169 169	188 188	
		1		(4)	-	162 162	160 160	185 185	209 209	-	170 170	222 222	239 239	284 284	227 227	-	188 188	
		1		(4)	233 233	162 162	160 160	185 185	209 209	235 235	-	222 222	239 239	284 284	227 227	169 169	188 188	
		1		(4)	233 233	-	-	185 185	-	235 235	-	222 222	-	284 284	-	169 169	188 188	*
		1		(4)	233 233	-	-	-	-	235 235	170 170	222 222	239 239	284 284	226 226	169 169	188 188	*
		242																

Table S2. Genetic distance between populations of *Galba schirazensis* . Pairwise F_{ST} values on the lower diagonal, P-values on the upper diagonal. Bonferroni corrections were applied in all pairwise comparisons (P = 0.00041). FJ, Finca Jocum Bucaramanga; AN, Andaracas; HC, Hacienda Cienaga; HU, Huagrahuma; MN, Manto de la Novia; NO, Nono; LJ, La Joya de Arequipa; LB, Louisiana Bedico; LA, La Azulita; BA, Bailadores; BO, Bodoque; LN, Los Nevados; SA, Sabana Alto; SE, San Eusebio;

		Colombia	Ecuador					Peru	USA	Venezuela							
		FJ	AN	HC	HU	MN	NO	LJ	LB	LA	BA	BO	LN	SA	SE	LT	ZA
Colombia	FJ	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	1,000	0.760	0.054	1,000	1,000	1,000	0.001	1,000
Ecuador	AN	0.992	-	0.721	0.752	0.323	0.311	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	HC	1,000	0.000	-	1,000	1,000	1,000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	HU	1,000	0.000	0.000	-	1,000	1,000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	MN	1,000	0.013	0.000	0.000	-	1,000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	NO	1,000	0.019	0.000	0.000	0.000	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Peru	LJ	1,000	0.991	1,000	1,000	1,000	1,000	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
USA	LB	0.987	0.986	0.991	0.990	0.991	0.992	0.987	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Venezuela	LA	0.000	0.990	1,000	1,000	1,000	1,000	1,000	0.986	-	0.741	0.129	1,000	1,000	1,000	0.004	1,000
	BA	-0.012	0.985	0.992	0.993	0.993	0.993	0.987	0.982	-0.028	-	0.044	0.519	0.777	0.501	0.001	0.924
	BO	0.204	0.964	0.970	0.972	0.974	0.975	0.955	0.964	0.177	0.150	-	0.050	0.157	0.046	0.168	0.127
	LN	0.000	0.993	1,000	1,000	1,000	1,000	1,000	0.989	0.000	0.003	0.235	-	1,000	1,000	0.001	1,000
	SA	0.000	0.988	1,000	1,000	1,000	1,000	1,000	0.984	0.000	-0.052	0.142	0.000	-	1,000	0.013	1,000
	SE	0.000	0.993	1,000	1,000	1,000	1,000	1,000	0.989	0.000	0.003	0.235	0.000	0.000	-	0.001	1,000
	LT	0.400	0.951	0.956	0.959	0.962	0.963	0.936	0.952	0.368	0.354	0.053	0.435	0.330	0.435	-	0.003
	ZA	0.000	0.989	1,000	1,000	1,000	1,000	1,000	0.985	0.000	-0.043	0.155	0.000	0.000	0.000	0.344	-

Table S3. Divergence between sequences of CO1 in *Galba schirazensis* . The number of base substitutions per site between sequences are shown. Haplotype 1, Iran, Spain, Reunion Island, Venezuela, Colombia, and Peru (the sequences here studied); Haplotype 2, Peru (the sequences studied by Bargues et al. 2011); Haplotype 3, Mexico; Haplotype 4, USA; Haplotype 5, Ecuador.

	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 4	Haplotype 5
Haplotype 1					
Haplotype 2	0.012				
Haplotype 3	0.007	0.014			
Haplotype 4	0.009	0.016	0.002		
Haplotype 5	0.012	0.019	0.005	0.002	