Systematics and geographical distribution of *Galba* species, a group of cryptic and worldwide freshwater snails

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52 biological invasions.

53

Abstract

Cryptic species are a major problem in systematics and biogeography, especially if they are invasive or transmit parasites or pathogens. Detecting cryptic species requires a pluralistic approach in which molecular markers allow to detect coherent taxonomical units that can then be analyzed using various markers (*e.g.*, internal morphology) and crosses. In asexual or selfing species, the latter criteria is of limited use. We studied a group of cryptic freshwater snail species from the family Lymnaeidae that invade almost all continents, reproduce mainly by selfing and transmit liver flukes to humans and livestock. We aim to clarify the systematics, distribution and phylogenetic relationships of *Galba* species based on a sound approach that included morphology, molecular markers, wide-scale sampling (the whole America) and data retrieved from GenBank (therefore including Old World samples). We suggest that the genus *Galba* comprises five species or species complex. *Galba cousini* is the only species that can be identified using shell morphology and internal anatomy (a derived trait) and has the most restricted distribution within the group. The other four species—*G. truncatula*, *G. cubensis*/*viator*, *G. humilis* and *G. schirazensis*—are all cryptic and constitute clades with wide variation in their genetic diversity, geographic distribution and invasiveness. Further genetic studies are required to clarify the status of the *G. cubensis/viator* (a single or two species). We highlight that no *Galba* species should be identified without molecular markers and that more sampling is required, especially in North America, Eurasia and Africa to clarify their systematics. We also discuss various hypotheses that can explain crypticity in *Galba*, such as morphological stasis or stabilizing selection.

Introduction

Distinguishing species based on morphological or other phenotypic traits may sometimes be difficult when trait variability within species trespasses that among species—such species are referred to as cryptic species (see review in Bickford et al., 2007; Fišer et al., 2018; Struck et al., 2018). They have been described in all taxonomic kingdoms, although cryptic species seems to be more often reported in animals (Struck et al. 2018). In this group, their frequency might reach a bit less than 1% of all species, an estimate that should be considered with caution given our lack of knowledge on the numbers of both cryptic species and species. They also seem to be homogeneously distributed among taxa and biogeographical regions (Pfenninger and Schwenk 2007), although it remains possible that some habitats such as caves and subterranean habitats fostered cryptic speciation (Katz et al. 2018). Several explanations have been proposed for the origin of cryptic species, partly relying on the time separating them (Fig. S1; Bickford et al., 2007; Fišer et al., 2018; Struck et al., 2018): (1) recent divergence: when morphological divergence has not accumulated yet because of lack of time, as is the case of some cave fish (Niemiller et al. 2012); (2) parallelism: when independent phenotypic trait evolves in different taxa from a similar and shared ancestral trait (Struck et al. 2018); (3) convergence: when not-too-closely related species derive from dissimilar ancestors and result in cryptic species, as observed in some sea stars (*e.g.*, Zulliger and Lessios, 2010); and (4) morphological stasis: when species remain similar over a long period of time because of limited genetic variation or stabilizing selection (*e.g.*, Gomez et al., 2004; Struck et al., 2018).

The occurrence of cryptic species is essential for understanding the speciation process (Coyne and Orr 2004; De Queiroz 2007), but also poses substantial problems with regard to at least two issues. The second issue is biological invasions. Cryptic

101 species may exhibit wide difference in invasive ability and impact on invaded
102 ecosystems and communities (Fang et al. 2014). An accurate identification at species
103 level is required in such situations to track invasions to try to prevent any harmful
104 consequences (Kolar and Lodge 2001; Dunn and Hatcher 2015; Jarić et al. 2019). The
105 third issue arises when species are involved in disease transmission and cryptic species
106 exhibit differences in this transmission. This is for example the case in the *Anopheles*
107 *gambiae* complex which includes the most important vectors of malaria in Africa
108 (Stevenson and Norris 2016). Some species indeed take blood meal from animals while
109 others also feed on humans. Accurate species identification is required for effective
110 mosquito control.

111 Snails, especially freshwater ones, are an interesting group for addressing
112 biogeography issues in cryptic species. Although taxonomists have increasingly used
113 molecular markers over the last decades to identify snail species (Dayrat et al. 2011),
114 morphological characters, especially shell shape and sculptures, remain widely used—
115 often resulting in large numbers of species names (Qian et al. 2012). However,
116 molluscan shells could show large phenotypic plasticity, for example as a reaction to
117 temperature, pollution or predation (Bourdeau et al. 2015) or could stay stable for
118 millions of years (*e.g.*, Weigand et al., 2011; Weiss et al., 2018). This resulted both in
119 the flourishing of species names and description (*e.g.*, Taylor, 2003), most of which are
120 invalid (Jarne et al. 2010; Dillon et al. 2011), and in the incorrect identification of
121 species, as well as of species invasion ability and distributional range (*e.g.*, Pfenninger
122 et al., 2006; Rama Rao et al., 2018). For example, an invasive Asian clam of the genus
123 *Sinanodonta* has been overlooked in Russia because it is morphologically
124 indistinguishable from another invasive Asian clam (Bespalaya et al. 2018). This calls
125 for an integrated approach to snail systematics and biogeography in which phenotypic

126 traits are studied together with appropriate molecular tools.

127 Here we focus on small-bodied freshwater pulmonate snails of the genus *Galba*
128 (Hygrophila, Lymnaeidae), common inhabitants of ditches, vernal ponds and muddy
129 river banks worldwide. Baker (1911) recognized 30 North American species and
130 subspecies in the subgenera *Galba* (*s.s.*) and *Simpsonia* on the basis of minor shell
131 morphological variation, which Hubendick (1951) suggested might represent as few as
132 four biological species (*humilis*, *truncatula*, *cubensis* and *bulimoides*) but among which
133 Burch (1982) continued to recognize 22. In South America, Hubendick (1951)
134 recognized only two species of small-bodied, amphibious lymnaeids (*viator* and
135 *cousini*), but workers today recognize as many as seven: *viator*, *schirazensis*, *cousini*,
136 *neotropica* and *meridensis*, as well as *truncatula* and *cubensis* (Bargues et al. 2007,
137 2011b, 2011a; Correa et al. 2010, 2011; Lounnas et al. 2017, 2018).

138 All these nominal species share a similar shell morphology and internal
139 anatomy, except *Galba cousini* (Paraense 1995), and plenty of phenotypic plasticity on
140 shell, anatomy and life-history traits (Samadi et al. 2000; Correa et al. 2011) despite
141 considerable divergence time of the order of 20 Myr based on genomic data (Burgarella
142 et al. 2015). They can therefore be considered as cryptic species, leading to
143 misidentification in many cases. For instance, *Galba schirazensis* has often been
144 confused with *Galba truncatula* or *Galba cubensis* in America (Correa et al. 2010;
145 Bargues et al. 2011a). A further difficulty is that crossing cannot be used to distinguish
146 species (Coyne and Orr 2004), as has been done in other freshwater snails (*e.g.*, *Physa*
147 species, Dillon et al., 2011), since *Galba* species mainly reproduce by self-fertilization
148 (Meunier et al. 2004; Bargues et al. 2011a; Lounnas et al. 2017, 2018). Moreover, at
149 least two species, *G. schirazensis* and *G. truncatula*, have been shown to be extremely
150 efficient invaders, aided by human activities, muddling our knowledge of species

151 distribution. The probably Eurasian *G. truncatula* has for example invaded South
152 America, especially the Bolivian Altiplano (Meunier et al. 2004). This is of special
153 concern since *Galba* snails are main vectors of the liver fluke *Fasciola hepatica* which
154 causes fasciolosis in both livestock and humans (Mas-Coma et al. 2005) and
155 transmission efficiency and invasion ability differ among species (Vázquez et al. 2018).
156 Overall, the geographic distribution of *Galba* species is poorly known. Reports from the
157 literature based on morphological identification only should be discarded or considered
158 with caution and our knowledge therefore boils down to the few available molecular
159 studies (Correa et al. 2010, 2011; Bargues et al. 2011b, 2011a, 2012; Lounnas et al.
160 2017, 2018). Our objective here is to characterize the geographic distribution of *Galba*
161 species at continental scale, based on an extensive sampling over America, and to
162 reconstruct the genus phylogeny to delimit species and to explore the origin of cripticity
163 among species. Previous studies reconstructing *Galba* phylogeny used single genes and
164 includes less than 10 sequences per species which does not account for the large
165 geographic distribution of this genus (Correa et al. 2010, 2011; Bargues et al. 2011c,
166 2011a, 2011a; Standley et al. 2013). We used morphological and molecular
167 (microsatellite loci and DNA sequences from two genes) markers to study more than
168 1,700 individuals from 161 sites. This analysis was complemented by adding all DNA
169 sequences available in GenBank to conduct a phylogenetic analysis. We used both gene
170 trees and a multispecies coalescent model to reconcile gene trees to shed light on the
171 phylogenetic relationships and on the origin of cripticity of *Galba* species.
172

173 # Material and methods

174 ## Snail sampling and species identification

175 We conducted field surveys in the New World over the last two decades (1998–2017)

searching for *Galba* species. Overall, *Galba* species were detected in 161 sites and 1,722 individuals were sampled from 9 countries: Argentina, Canada, Colombia, Cuba, Ecuador, France (French Guiana, Guadeloupe and Martinique), Peru, Venezuela and USA (Tables S1 & S2). *Galba* species have been already reported from a few of the sites from Venezuela and Ecuador in previous articles of the authors (Pointier 2015; Narváez et al. 2016).

In most cases, we sampled *Galba* species in unstable habitats that experience frequent flooding and droughts. The sampled habitats were characterized as brook, irrigation canal, ditch, oxbow lake, pond, marsh, lake, tank, rice field and river. Often individual snails were collected above the water line. Some sites were visited up to five times. Geographic coordinates were recorded in most sites.

After collection, individuals were set in water at 70 ºC during 30–45 s. This procedure allows fixation of individuals without contraction of soft parts and a proper study of snail internal anatomy. The body was carefully withdrawn from the shell using forceps and bodies and shells were stored in 70% ethanol until morphological and DNA analyses (Pointier et al. 2004).

Species were characterized using a three-step procedure involving both morphological and molecular markers (Fig. S2). Step 1 was an analysis of shell morphology and reproductive anatomy. In step 2, we used a molecular tool that enables us to distinguish *G. cubensis*, *G. schirazensis* and *G. truncatula* (Alda et al. 2018). In step 3, we sequenced mitochondrial and nuclear genes in individuals for which no PCR amplification product was obtained in step 2. DNA from individuals of those species identified in steps 1 and 2 were also sequenced in order to reconstruct the phylogeny of *Galba*. Note that the whole approach is cheaper than an approach based on simply sequencing the same genes in all individuals.

201

202 *Step 1: morphology of the shell and of internal organs*

203 We photographed the shell of three to five adult snails from each site and dissected their
204 body under a stereoscopic microscope. We drew the anatomy of the penial complex,
205 prostate and renal tube using a camera lucida attachment (Pointier et al. 2004).

206

207 *Step 2: multiplex PCR of microsatellite loci*

208 We applied the multiplex PCR designed by Alda et al. (2018) to all 1,722 individuals. It
209 is based on species-specific primers amplifying microsatellite loci targeting three
210 species and producing band sizes that are specific to these species (179–200 pb in *G.*
211 *cubensis*, 227–232 pb in *G. schirazensis* and 111–129 pb in *G. truncatula*). DNA was
212 extracted using a Chelex protocol following (Estoup and Martin 1996) adapted to 96-
213 well plates and DNA amplification and gel running followed Alda et al. (2018).

214

215 *Step 3: identification by sequencing*

216 We amplified the internal transcribed spacer 2 (ITS2) and the cytochrome oxydase
217 subunit 1 (COI) genes in 35 individuals showing no amplification products in step 2, as
218 well as in 111 individuals showing an amplification product and in one individual
219 identified as *G. cousini* in step 1 using the method described in Lounnas et al. (2017,
220 2018). The 35 individuals (1 to 5 per population) showing no amplification products
221 belong to 15 sites from Argentina, Canada and USA where all or some individuals did
222 not show a band in the gel (200 individuals, in total).

223 We used the primers NEWS2 (forward) 5' TGTGTCGATGAAGAACGCAG 3'
224 and ITS2-RIXO (reverse) 5' TTCTATGCTTAAATTCAGGGG 3' to amplify ITS2
225 (Almeyda-Artigas et al. 2000) and LCOI490 (forward) 5'

GGTCAACAAATCATAAAGATATTGG 3' and HCO2198 (reverse) 5' TAAACTTCAGGGTGACCAAAAAATCA 3' to amplify COI (Folmer et al. 1994). In both cases, PCR amplification was performed in a total volume of 25 µl containing 12.5 µl of Taq PCR Master Mix (Qiagen), 2.5 µl of each primer (10 mM) and 2 µl of DNA in an Eppendorf Thermal Cycler with an initial denaturation step at 95 ºC for 15 minutes; followed by 35 cycles of denaturation at 95 ºC for 30 seconds, annealing at 50 ºC for one minute, extension at 72 ºC for one minute; and a final elongation at 60 ºC for 30 minutes. The presence and size of amplification products were electrophoretically checked in a 1% agarose gel and stained with EZ-Vision. DNA sequencing was performed by Eurofins Genomics (Ebersberg, Germany) using PCR-amplified products as templates. All sequences were uploaded to GenBank (Table S1) and assigned to a species using the phylogenetic reconstruction.

Type localities

Because of the longstanding confusion and uncertainty regarding the systematics of the genus *Galba* worldwide, it is especially important to establish standard populations, against which unknown populations can be compared. Type localities were specified by the authors of the more recently-described species, such as *neotropica* (Bargues et al. 2007) and *meridensis* (Bargues et al. 2011b), and others have been established by subsequent use, for example *schirazensis* (Bargues et al. 2011a). But in his original description of *Limnaeus viator*, D'Orbigny (1835) simply stated "Patagonia". And Pfeiffer (1839) gave no locality data for his *Limnaeus cubensis* at all, beyond "Cuba". In such circumstances, the ICZN code provides that subsequent authors may restrict type localities to some more precise spot "within the known range of the taxon".

Type localities for all eight of the widely-recognized species in the genus *Galba*

are listed in Table 1. COI sequences from samples of all the populations inhabiting these localities have been previously uploaded to GenBank, and most have ITS1, ITS2 or 16S sequences available as well.

Retrieving data on *Galba* spp. distribution from published work

We searched the literature and GenBank for any sequence data apparently attributable to lymnaeids of the genus *Galba* for four genes: COI, ITS1, ITS2 and 16S. Coordinates were provided in most sites by the authors. When coordinates were not provided, we inferred them from the name of the sampling site using GoogleEarth. We found 132 New World sites in which *Galba* species have been molecularly characterized (Table S2), and 45 sites in the Old World (Table S3, Fig S3). The specific nomina attributed to these sequences by their depositors in GenBank were 157 *truncatula*, 152 *schirazensis*, 70 *neotropica*, 57 *cubensis*, 44 *viator*, 20 *cousini*, 9 *humilis*, 6 *meridensis* and 2 others. Together these yielded 166 COI, 163 ITS2, 118 ITS1 and 70 16S sequences.

Phylogenetic and ancestral reconstruction study

Phylogenetic analyses were conducted on the ITS2 and COI sequences obtained in this study, as well as on ITS2, COI, ITS1 and 16S sequences retrieved from GenBank. Most of these sequences have been published; a few exceptions are only in GenBank but not used in scientific articles (Tables S2–S3). We included all these 791 sequences in our analysis: 87 for 16S, 251 for COI, 120 for ITS1 and 333 for ITS2. Some GenBank accession numbers appear more than once (Tables S2–S3) because individuals with identical sequences have been registered under the same GenBank accession number.

Alignment was performed individually for each gene using MAFFT (Katoh and Standley 2013). Ambiguously aligned sites were excluded using GBLOCKS using the

default settings for a less stringent selection (Castresana 2000). The number of positions in the final sequences was 412 for 16S (83% of the original 493 positions), 609 for COI (86% of 707), 435 for ITS1 (48% of 888) and 333 for ITS2 (23% of 1,429). We examined levels of saturation for each gene and for the first and second *versus* third codon positions of COI using DAMBE (Xia 2017). We found that the third codon position of COI was highly saturated. We partitioned codon positions and unlinked substitution models in phylogenetic analyses.

We used Bayesian inference in Beast2 (Bouckaert et al. 2014) (1) to assign sequences to species; (2) to validate, and to mend if necessary, species identity for sequences retrieved from GenBank; (3) to reconstruct the phylogeny and (4) to propose the ancestral phenotypic state of *Galba* species. To address points 1 and 2, we built four independent gene trees. The best-fitting models of sequence evolution for each gene were selected using bModelTest (Bouckaert and Drummond 2017). We estimated a model for each COI partition (1st, 2nd and 3rd position). The best model describing the evolution of 16S was HKY+G+I, 123424+G+I for COI (1st codon), 121321+G+I for COI (2nd codon), TN93+G+I for COI (3rd codon), 123424+G+I for ITS1 and 121323 for ITS2. The phylogenetic analysis was done using the three unlinked COI codon partitions. The analyses were run using four gamma categories and a proportion of 0.5 invariant sites. We used a strict clock and a birth-death model as priors with lognormal birth and death rates. The MCMC ran for 200,000,000 generations with storing every 20,000 generations. The MCMC output were visualized using Tracer (Rambaut et al. 2018). The maximum credibility tree was computed TreeAnnotator (utility program distributed with the Beast package) and the trees removed as burn-in represented 10%. Gene trees were visualized and edited in FigTree (http://tree.bio.ed.ac.uk/software/figtree). These gene trees allowed us to identify or

validate species identity for all sequences, whether obtained in this study or retrieved from GenBank.

To address points 3 (species tree topology) and 4 (ancestral phenotypic state), we used StarBeast2 (Heled and Drummond 2010). We used the same models as for reconstructing the gene trees. We linked mitochondrial clocks and nuclear clocks and we used strict clock models for both partitions. We did not add an outgroup to root the tree because both *Galba* monophyly has been already ascertained (Correa et al. 2010, 2011). We used the birth-death model as tree prior with lognormal birth and death rates. We added a discrete trait partition with the phenotypic state (one for all cryptic species *vs*. another one for *G. cousini*) to infer the ancestral phenotypic state of *Galba*. The MCMC was run three times for 200,000,000 generations with storing every 20,000 generations. The MCMC output were visualized using Tracer (Rambaut et al. 2018) and sample of trees was summarized by TreeAnnotator using a 10% burn-in. The species tree was visualized and edited in FigTree, GIMP (https://www.gimp.org) and DensiTree (Bouckaert and Heled 2014).

Results

Morphology

Galba cousini was the only species that could be identified at species level based on shell morphology of adults (Fig. S4) with their larger (> 10 mm) and more globose shells with shorter spires than other species. Shell length in the other species was less than 10 mm (Fig. S4). *Galba cousini* and the other species also differed with regard to their internal anatomy (*i.e.*, ureter with two distinct flexures, ovate prostate wider than in the other species, bigger penial complex, and penis sheath about the same length than the preputium; see Fig. S4). We did not find any difference in shell morphology and

internal anatomy when comparing individuals of *G. cousini* from Ecuador and Colombia on one side and from Venezuela (referred to as *G. meridensis*, Bargues et al., 2011a) on the other side. Based on these differences in morphology, 302 individuals out of 1,722 were attributed to *G. cousini* and all were sampled in Venezuela, Colombia and Ecuador (Fig. 1). The other individuals (N = 1,420) originating from 133 sites were not distinguishable based on shell and reproductive anatomy (Fig. S4).

Multiplex PCR of microsatellite loci

DNA from these 1,420 American individuals was amplified using the multiplex PCR procedure (step 2 from Fig. S2). We identified 541 individuals of *G. cubensis*, 330 of *G. schirazensis* and 349 of *G. truncatula* (Table S1). No amplification was observed in 200 individuals sampled in one site from Argentina and 14 sites from Canada and USA (Table S1).

Identification by sequencing

Phylogenetic analysis of COI sequences returned five clusters (Figs. 2 and S6). Clusters I, II and III each contained a single type population: *truncatula*, *humilis* and *schirazensis*. Cluster IV contained the type populations of *cousini* and *meridensis*, and cluster V the type populations of *viator*, *cubensis* and *neotropica*. The posterior probabilities (PP) of clusters I, III and V were all 1.0, with PP = 0.9313 for cluster II and PP = 0.7241 for cluster IV.

Analysis of 16S, ITS1 and ITS2 sequences confirmed the COI results in almost all respects, noting that sequences were missing for at least one or two type populations in each tree (Figs. S5 and S6–S8). The *viator* type population was depicted as separate from cluster V in the ITS1 gene tree (Fig. 2 and S7). However, in the 16S, ITS2 and

351 COI gene trees, a clade containing the *viator* type population and other populations
352 from Argentina, Chile and Uruguay was included with cluster V (Fig. 2, S5–S6 and S8).
353 Genetic diversity varied among the five clusters and four genes (Fig. 2, but see
354 also Figs. S5–S8 for details). Clusters II and IV showed reduced variation, while cluster
355 V was larger and more diverse. Mitochondrial genes were more diverse than nuclear
356 genes in clusters I and V. This observation may be biased, however, because
357 mitochondrial and nuclear sequences did not always belong to the same individuals or
358 regions (some regions were better represented for some genes). Regional variation was
359 apparent in some clusters. For instance, sequences sampled from the southern USA
360 (Bosque del Apache) comprised a distinct branch within cluster V of the COI tree (Fig
361 S6), but not in the ITS2 tree (Fig. S8).
362 Most of the sequences uploaded to GenBank as belonging to one of the eight
363 species of *Galba* were accurately clustered into the five clades containing their type
364 populations. However, there were some exceptions. Eight sequences of COI uploaded to
365 GenBank as *G. truncatula* from France appeared in cluster III with *G. schirazensis* type
366 locality (Table S3; Fig. S6). We renamed these sequences as belonging to individuals of
367 *G. schirazensis*. One 16S sequence from Ethiopia was uploaded to GenBank as *G.*
368 *truncatula* but clustered at the base of *viator*/*cubensis*/*neotropica* clade V with low
369 posterior probability (Fig. S5). Another Ethiopian sequence (COI), also uploaded to
370 GenBank as *G. truncatula*, did not cluster with any of the *Galba* clades (Fig. S6). We
371 considered these sequences from Ethiopia as belonging to *G. truncatula* individual(s) in
372 spite of their ambiguous status.
373
374 *Phylogeny and state reconstruction*
375 The topology of the COI, 16S, ITS1 and ITS2 gene trees differed (Fig. 2, but see also

376 Figs. S5–S8 for details). Cluster II (*humilis*) and cluster IV (*cousini*/*meridensis*) were
377 grouped together in all gene trees. Otherwise, grouping relationships among gene trees
378 were inconsistent (Fig. 2). However, the multilocus multispecies tree returned two
379 grouping relationships: one group uniting cluster I (*truncatula*) and cluster III
380 (*schirazensis*), the other uniting clusters II (*humilis*), IV (*cousini*/*meridensis*) and V
381 (*viator*/*cubensis*/*neotropica*). Both groups showed posterior probabilities higher than
382 0.73 (Fig. 3). The multilocus multispecies tree visualized in DensiTree (Fig. S9) showed
383 that most tree topologies united the clusters in the two groups mentioned above but
384 some topologies placed clusters differently reflecting the incongruence found among the
385 gene trees.

386 The phenotypic state reconstruction showed that the most recent common
387 ancestor of *Galba* species had a cryptic phenotype. Thus, the non-cryptic phenotype of
388 *G. cousini* would be a derived state from the cryptic phenotype (Fig. 3).

389

390 ## Discussion

391 ### *Galba* comprises five species (or species complex)

392 Here we report the largest study published to date of *Galba* systematics and distribution,
393 based on extensive sampling at a very large geographical scale, an integrative approach
394 combining phenotypic approaches with molecular approaches (four genes) and using all
395 DNA sequences available in GenBank for these genes. We recovered five clades. The
396 widespread occurrence of selfing reproduction in populations of the genus *Galba* voids
397 the biological species concept, and the absence of any reliable morphological
398 distinctions obviates the typological. Thus, under evolutionary concepts, we suggest
399 that our five clades are best understood as five species (or species complex).

The oldest names corresponding to these evolutionary species are *Galba truncatula* (Muller 1774) for cluster I, *G. humilis* (Say 1822) for cluster II, *G. schirazensis* (Kuster 1862) for cluster III, *G. cousini* (Jousseaume 1887) for cluster IV and *G. viator* (d'Orbigny 1835) for cluster V. The nomen *cubensis* (Pfeiffer 1839) has also been widely applied to cluster V as well. These five genetically-defined groups have previously been recognized by other authors in other studies including less species and genes, and much lower number of individuals and populations (Correa et al. 2010, 2011; Bargues et al. 2011b, 2011a; Standley et al. 2013).

We here confirm the previous suggestions of Correa et al. (2010) and Lounnas et al. (2017) that *G. neotropica* and *G. meridensis* (Bargues et al. 2007, 2011b) should be considered synonyms of *G. cubensis*/*viator* and *G. cousini*, respectively. An unclear point is whether *G. viator* should be considered as a clade within *G. cubensis*, or a species on its own. The answer depends on the gene considered, but all *G. viator* individuals were always forming a single clade. *Galba viator* and *G. cubensis* have overlapping distributions in Argentina, Chile and Uruguay. More extensive sampling coupled with genetic population studies might be useful, but a cautious position would be to consider that they constitute a species complex or a species with wide diversity, as has been found in other freshwater snails from the Hygrophila family (*e.g.*, Ebbs et al., 2018; Mavárez et al., 2002; Pfenninger et al., 2006). We also note that if we retain a single species (clade), its name should be *viator*, and not *cubensis*, based on prior description.

In North America, Burch (1982) recognized 22 species of small, mud-dwelling lymnaeids, which he grouped into the genus *Fossaria* with two subgenera, *Fossaria* (*s.s.*) with 11 species and *Bakerilymnaea* with 11 (see Table S4 for species names). Johnson et al. (2013) transferred these species to the genus *Galba*, but otherwise

425 retained the Burch system. Included in the present analysis were topotypic samples of
426 *obrussa* from Philadelphia and *parva* from Cincinnati, both of which we here show to
427 be indistinguishable from topotypic *humilis*, collected at Owego, New York. Remigio
428 (2002) contributed to Genbank a 16S sequence from a Canadian population he
429 identified as *Fossaria obrussa*, also grouping with cluster II (*humilis*). We suggest that
430 *obrussa*, *parva* and the seven other uniquely North American specific nomina listed
431 above ascribed by Burch (1982) to the subgenus *Fossaria* are junior synonyms of *G.*
432 *humilis*, setting aside American populations of *G. truncatula* as distinct. In addition to
433 his *obrussa* sequence, Remigio (2002) contributed a 16S sequence from Oklahoma to
434 Genbank which he labelled "*Fossaria bulimoides*". This sequence grouped with cluster
435 IV (*cubensis*/*viator*) in our analysis. We suggest that all 12 specific nomina ascribed by
436 Burch (1982) to the *Fossaria* subgenus *Bakerilymnaea* (Table S4) including *bulimoides*
437 (Lea 1841), are junior synonyms of *G. cubensis*/*viator*.
438
439 **Phylogenetic relationships among *Galba* clades**
440 To elucidate the evolutionary relationships among these five species, we built separate
441 gene trees using classical approaches, as well as a species tree based on a multispecies
442 coalescent model that reconciles gene trees and provides a much better estimation
443 accuracy for species tree topology than, for instance, concatenation (Heled and
444 Drummond 2010). The inferred phylogenetic relationships among species differed when
445 considering the different genes and all species at once. Such a discordance is not
446 unusual and has been reported in many different studies (*e.g.*, Kutschera et al., 2014;
447 Stewart et al., 2014; Suh et al., 2015), including in mollusks (Krug et al. 2013; Sales et
448 al. 2013). Incomplete lineage sorting or introgressive hybridization of specific genes
449 may indeed led to such a result (Felsenstein 2004).

However, we found that *G. cubensis*/*viator*, *G. humilis* and *G. cousini* on one side and *G. schirazensis* with *G. truncatula* on the other side, are sorted as sister species based on the species tree. The result partially agrees with some gene trees published in previous work (Correa et al. 2010, 2011; Bargues et al. 2011a). However, those trees were based on a single gene and limited sampled size. Our phylogenetic analysis also revealed a few branches / sequences that stand apart. For example, Dayrat et al. (2011) reported *G. truncatula* mtDNA sequences from individual(s) from Ethiopia that create a long branch and a single clade in both mitochondrial-gene trees. Another example, here from our sampling, are the *Galba* individuals from Bosque del Apache (New Mexico, USA) which show a very long branch at the COI gene and also differ at ITS2. These examples might genuinely be long branches corresponding to up-to-now undetected species or to accelerated molecular evolution (for examples in gastropods, see Fourdrilis et al., 2016; Pinceel et al., 2005; Thomaz et al., 1996).

Although our study is based on sampling at extremely large geographic scale, especially in America, *Galba* species occur on almost all continents. Much more extensive sampling and molecular analyses are required to get a full picture of the phylogeny and distribution of the genus. Of special interest is North America, where we confirmed the occurrence of *G. humilis* and *G. cubensis*/*viator*, and reported *G. schirazensis* for the first time. We did not, however, confirm that of *G. truncatula*, despite its otherwise worldwide distribution. Populations of *Galba* also occur in Eurasia and Africa where it has generally been identified as *G. truncatula* (Vinarski 2018), but this deserves more extensive sampling based on molecular markers.

The recent arrival of *G. schirazensis* in Europe and the Middle East (Bargues et al. 2011a; Lounnas et al. 2018) and the recently described species *Galba robusta* from Yemen, based on shell and penial morphology alone, (Vinarski 2018) adds diversity to

475 the genus *Galba* and should be confirmed with molecular approaches. It would also be
476 useful to have maps of *Galba* absence, possibly associated with detection probabilities.
477 For example, *Galba* has not been found in Northern Brazil (Paraense 1982), possibly
478 because the too acid waters of the Amazon and Orinoco rivers do not allow for its
479 settling (Paraense 1982, 1983). It also does not occur in the most southern part of
480 Argentina (authors' unpublished data). Negative records in species distribution are
481 extremely important when generating and understanding accurate distribution maps and
482 species-environment associations (Brotons et al. 2004). Models based only on presence
483 data are less predictive and may become more explicative and precise if true absence
484 data and dispersal constraints are included (Václavík and Meentemeyer 2009). For
485 instance, absence data showed to be critical when addressing the spatial distribution
486 modelling of the medically relevant freshwater snail species *Bulinus globosus*,
487 *Biomphalaria pfeifferi* and *Radix natalensis* in Zimbabwe and predicting their
488 distribution range in a forecasted future climate (Pedersen et al. 2014).
489
490 **A set of cryptic species**
491 Our study confirms that *G. cousini* differs widely in size, shell shape and anatomy from
492 the other *Galba* species. However, this species is firmly entrenched in the genus and
493 derives from a *Galba* ancestor that had the cryptic morphology. Interestingly, *G. cousini*
494 is the larger species within *Galba*, occurring at high altitude in the northern Andes and
495 displays the complex reproductive anatomy of an outcrossing species (see Escobar et
496 al., 2011; Jarne et al., 2010). The other species cannot be distinguished based on shell
497 morphology or anatomy of internal organs, as already shown in previous studies
498 (Samadi et al. 2000; Correa et al. 2011). Trait variability within species is indeed larger
499 than differences among species, especially because of phenotypic plasticity (Correa et

al. 2011). This has been found in numerous groups and biogeographical regions (Bickford et al. 2007; Fišer et al. 2018; Struck et al. 2018), perhaps making up to 1% of animal species (Pfenninger and Schwenk 2007) with some habitats possibly favoring the emergence of cryptic species (like caves, Katz et al., 2018). Such a situation has been reported in other freshwater snails from the same family, such as *Ancylus* (Weiss et al. 2018) or *Radix* (Pfenninger et al. 2006), although a more common situation is a plethora of species names for individuals belonging to the same species (see above). We here considered shell shape and anatomy of reproductive organs in a qualitative way because previous studies (Samadi et al. 2000; Correa et al. 2011) showed that dimensions of internal organs depend on physiological state and phenotypic plasticity and that species cannot be distinguished by means of those measurements. A more comprehensive approach should include other anatomical traits (*e.g.*, the radula, which is widely-used in snail systematics). Reproductive and growth traits in *G. truncatula* have been shown to vary according to habitat characteristics at small geographical scale (Chapuis et al. 2007), suggesting that life-history traits would not allow to separate species.

We mentioned in Introduction several hypotheses explaining the occurrence of cryptic species (recent divergence, parallelism, convergence and stasis; Fig. S1; (Bickford et al. 2007; Fišer et al. 2018; Struck et al. 2018). We should first mention that the *Galba* genus has no closely related groups—its closest relative is probably the stagnicolids (Correa et al. 2010) which occurs in North America and Eurasia and have a very distinctive morphology. The recent divergence hypothesis might be discarded because *Galba* species are separated by several millions of years (Burgarella et al. 2015). Moreover, that the derived *G. cousini* has evolved a different morphology suggests that time is not too much of a constraint. The parallelism hypothesis also

525 seems to be unlikely given that, based on our phylogenetic reconstruction, the ancestral
526 morphology of all *Galba* species (except *G. cousini*) would be the cryptic one and that
527 the closest group (stagnicolids) has a very different morphology. The same applies to
528 the convergence hypothesis—the topology of lymnaeids (Correa et al. 2010) does not fit
529 with this idea. By default, morphological stasis would explain the occurrence of cryptic
530 *Galba* species, as has been proposed in other groups (*e.g.*, Gomez et al., 2004; Struck et
531 al., 2018) including snails. Selfing in *Galba* might have led to limited genetic variation
532 favoring stasis. Strong stabilizing selection related to particular environmental
533 conditions is another explanation. The cryptic *Galba* species indeed occupies a wide
534 variety of freshwater habitats, but can be considered much more amphibious than most
535 freshwater snails, because of their capacity to occupy humid habitats such as wet
536 prairies. Their morphology might be particularly adapted to such alternative freshwater
537 niche.

538 The occurrence of cryptic species induces several problems when studying
539 *Galba* species. The first is species misidentification, especially because they have a
540 wide and poorly-known geographical distribution that may change in time with
541 biological invasions. For example, *G. schirazensis* and *G. truncatula* have widely
542 enlarged their distribution over the last decades (Brown 1994; Bargues et al. 2001,
543 2011a; Vinarski and Kantor 2016; Lounnas et al. 2018). A second issue is ecological
544 monitoring and estimates of species diversity (Bickford et al. 2007). Cryptic species are
545 by definition hard to monitor and biodiversity might be underestimated. We showed
546 here that up to three *Galba* species were occurring in some sites (Table S1) and
547 distinguishing them would be impossible without molecular tools. *Galba* species are not
548 at extinction risk, but it is necessary to distinguish them because they are involved in the
549 transmission of fasciolosis caused by the liver fluke *F. hepatica*. Some studies showed

that lymnaeid species have different patterns of susceptibility, host-parasite compatibility and immunological resistance to *F. hepatica* (Gutiérrez et al. 2003; Vázquez et al. 2014; Dreyfuss et al. 2015). However, a systematic evaluation within *Galba* species has to be done. Moreover, transmission also depends on ecological and sociological conditions. The fact that all species can be infected under laboratory conditions (Vázquez et al. 2018) does not imply that transmission actually occurs because, for example, cattle or wildlife could not occupy the same grazing habitats than the infecting snails (Sabourin et al. 2018). Ecological studies have to be therefore performed to evaluate whether *Galba* species differ with regard to habitat type, since our current knowledge is essentially limited to *G. truncatula* (Chapuis et al. 2007).

Conclusion and future directions

Galba is a genus of interest for addressing various issues, including wide-scale biogeography, biological invasions, evolution of mating systems and host-parasite interactions. Our work is a first attempt to clarify both its phylogeny (and systematics) and its geographical distribution in the New World. Broader, worldwide sampling will be required to (i) ascertain that European, African and Asian populations referred to as *G. truncatula* actually belong to this species and to clarify its biogeographic origin (e.g., is it a *Galba* species that colonized Eurasia through the Bering strait area?); (ii) understand how the invasive species (especially *G. truncatula* and *G. schirazensis*) are spreading and what are the genetic consequences. A loss of variation has been observed in invasive populations of these species (Meunier et al. 2001; Lounnas et al. 2018), as a result of bottlenecks and founding events (Cristescu 2015). More detailed studies, based for example on long-term surveys of sites and analysis of life-history traits under laboratory conditions (*e.g.*, Chapuis et al., 2007) would help understanding species

575 interactions and the transmission of *F. hepatica* (Sabourin et al. 2018). For example, *G.*
576 *schirazensis* seems to be spreading very efficiently and genetic studies suggest that this
577 is mainly due to one genetic clone (Lounnas et al. 2018). Studying the competitive and
578 transmission ability of this clone, compared to both other *G. schirazensis* and other
579 *Galba* species would be worthwhile.
580

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591 # References

592 Albrecht C., Wolff C., Glöer P., Wilke T. 2008. Concurrent evolution of ancient sister
593 lakes and sister species: the freshwater gastropod genus Radix in lakes Ohrid and
594 Prespa. Hydrobiologia. 615:157–167.

595 Alda P., Lounnas M., Vázquez A.A., Ayaqui R., Calvopiña M., Celi-Erazo M., Dillon
596 R.T., Jarne P., Loker E.S., Muñiz Pareja F.C., Muzzio-Aroca J., Nárvaez A.O., Noya
597 O., Robles L.M., Rodríguez-Hidalgo R., Uribe N., David P., Pointier J.-P., Hurtrez-
598 Boussès S. 2018. A new multiplex PCR assay to distinguish among three cryptic *Galba*
599 species, intermediate hosts of *Fasciola hepatica*. Veterinary Parasitology. 251:101–105.

600 Almeyda-Artigas R.J., Bargues M.D., Mas-Coma S. 2000. ITS-2 rDNA sequencing of
601 Gnathostoma species (Nematoda) and elucidation of the species causing human
602 gnathostomiasis in the Americas. The Journal of parasitology. 86:537–544.

603 Baker F.C. 1911. The Lymnæidæ of North and Middle America, recent and fossil.
604 Chicago: The Academy.

605 Bargues M., Artigas P., Khoubbane M., Ortiz P., Naquira C., Mas-Coma S. 2012.
606 Molecular characterisation of *Galba truncatula*, *Lymnaea neotropica* and *L.*
607 *schirazensis* from Cajamarca, Peru and their potential role in transmission of human and
608 animal fascioliasis. Parasites & Vectors. 5:174.

609 Bargues M.D., Artigas P., Khoubbane M., Flores R., Glöer P., Rojas-García R., Ashrafi
610 K., Falkner G., Mas-Coma S. 2011a. *Lymnaea schirazensis*, an overlooked snail
611 distorting fascioliasis data: genotype, phenotype, ecology, worldwide spread,
612 susceptibility, applicability. PLoS ONE. 6:e24567.

613 Bargues M.D., Artigas P., Khoubbane M., Mas-Coma S. 2011b. DNA sequence
614 characterisation and phylogeography of *Lymnaea cousini* and related species, vectors of
615 fascioliasis in northern Andean countries, with description of *L. meridensis* n. sp.

616 (Gastropoda: Lymnaeidae). Parasites & Vectors. 4:132.

617 Bargues M.D., Artigas P., Mera y Sierra R., Pointier J.-P., Mas-Coma S. 2007.

618 Characterisation of *Lymnaea cubensis*, *L. viatrix* and *L. neotropica* n. sp., the main

619 vectors of *Fasciola hepatica* in Latin America, by analysis of their ribosomal and

620 mitochondrial DNA. Annals of Tropical Medicine & Parasitology. 101:621–641.

621 Bargues M.D., González C.L., Artigas P., Mas-Coma S. 2011c. A new baseline for

622 fascioliasis in Venezuela: lymnaeid vectors ascertained by DNA sequencing and

623 analysis of their relationships with human and animal infection. Parasites & Vectors.

624 4:1–18.

625 Bargues M.D., Vigo M., Horak P., Dvorak J., Patzner R.A., Pointier J.P., Jackiewicz

626 M., Meier-Brook C., Mas-Coma S. 2001. European Lymnaeidae (Mollusca:

627 Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA

628 ITS-2 sequences. Infection, Genetics and Evolution. 1:85–107.

629 Bespalaya Y.V., Bolotov I.N., Aksenova O.V., Gofarov M.Yu., Kondakov A.V.,

630 Vikhrev I.V., Vinarski M.V. 2018. DNA barcoding reveals invasion of two cryptic

631 *Sinanodonta* mussel species (Bivalvia: Unionidae) into the largest Siberian river.

632 Limnologica. 69:94–102.

633 Bickford D., Lohman D.J., Sodhi N.S., Ng P.K.L., Meier R., Winker K., Ingram K.K.,

634 Das I. 2007. Cryptic species as a window on diversity and conservation. Trends in

635 Ecology & Evolution. 22:148–155.

636 Bouckaert R., Heled J. 2014. DensiTree 2: Seeing trees through the forest. .

637 Bouckaert R., Heled J., Kühnert D., Vaughan T., Wu C.-H., Xie D., Suchard M.A.,

638 Rambaut A., Drummond A.J. 2014. BEAST 2: A Software Platform for Bayesian

639 Evolutionary Analysis. PLoS Computational Biology. 10:e1003537.

640 Bouckaert R.R., Drummond A.J. 2017. bModelTest: Bayesian phylogenetic site model

641 averaging and model comparison. BMC Evolutionary Biology. 17.

642 Bourdeau P.E., Butlin R.K., Brönmark C., Edgell T.C., Hoverman J.T., Hollander J.

643 2015. What can aquatic gastropods tell us about phenotypic plasticity? A review and

644 meta-analysis. Heredity. 115:312–321.

645 Brotons L., Thuiller W., Araújo M.B., Hirzel A.H. 2004. Presence-absence versus

646 presence-only modelling methods for predicting bird habitat suitability. Ecography.

647 27:437–448.

648 Brown D.S. 1994. Freshwater snails of Africa and their medical importance. London:

649 Taylor & Francis.

650 Burch J.B. 1982. North American freshwater snails. Transactions of the POETS

651 Society. 1:217–365.

652 Burgarella C., Gayral P., Ballenghien M., Bernard A., David P., Jarne P., Correa A.,

653 Hurtrez-Boussès S., Escobar J., Galtier N., Glémin S. 2015. Molecular evolution of

654 freshwater snails with contrasting mating systems. Molecular Biology and Evolution.

655 32:2403–2416.

656 Castresana J. 2000. Selection of conserved blocks from multiple alignments for their

657 use in phylogenetic analysis. Molecular Biology and Evolution. 17:540–552.

658 Chapuis E., Trouve S., Facon B., Degen L., Goudet J. 2007. High quantitative and no

659 molecular differentiation of a freshwater snail (*Galba truncatula*) between temporary

660 and permanent water habitats. Molecular Ecology. 16:3484–3496.

661 Correa A.C., Escobar J.S., Durand P., Renaud F., David P., Jarne P., Pointier J.-P.,

662 Hurtrez-Boussès S. 2010. Bridging gaps in the molecular phylogeny of the Lymnaeidae

663 (Gastropoda: Pulmonata), vectors of Fascioliasis. BMC Evolutionary Biology. 10:381.

664 Correa A.C., Escobar J.S., Noya O., Velásquez L.E., González-Ramírez C., Hurtrez-

665 Boussès S., Pointier J.-P. 2011. Morphological and molecular characterization of

666 Neotropic Lymnaeidae (Gastropoda: Lymnaeoidea), vectors of fasciolosis. Infection,
667 Genetics and Evolution. 11:1978–1988.
668 Coyne J.A., Orr A.H. 2004. Speciation. Sunderland, Massachusetts: Sinauer Associates,
669 Inc. Publ.
670 Cristescu M.E. 2015. Genetic reconstructions of invasion history. Molecular Ecology.
671 24:2212–2225.
672 Dayrat B., Conrad M., Balayan S., White T.R., Albrecht C., Golding R., Gomes S.R.,
673 Harasewych M.G., de Frias Martins A.M. 2011. Phylogenetic relationships and
674 evolution of pulmonate gastropods (Mollusca): New insights from increased taxon
675 sampling. Molecular Phylogenetics and Evolution. 59:425–437.
676 De Queiroz K. 2007. Species concepts and species delimitation. Systematic Biology.
677 56:879–886.
678 Dillon R.T., Wethington A.R., Lydeard C. 2011. The evolution of reproductive isolation
679 in a simultaneous hermaphrodite, the freshwater snail *Physa*. BMC Evolutionary
680 Biology. 11.
681 D'Orbigny A. 1835. Synopsis terrestrium et fluviatilium molluscorum, in suo per
682 Americam meridionalem itinere, ab A. D'Orbigny, collectorum. Mag de Zoologie. 5:1–
683 44.
684 Dreyfuss G., Correa A.C., Djuikwo-Teukeng F.F., Novobilský A., Höglund J., Pankrác
685 J., Kašný M., Vignoles P., Hurtrez-Boussès S., Pointier J.P., Rondelaud D. 2015.
686 Differences in the compatibility of infection between the liver flukes *Fascioloides*
687 *magna* and *Fasciola hepatica* in a Colombian population of the snail *Galba* sp. Journal
688 of Helminthology. 89:720–726.
689 Dunn A.M., Hatcher M.J. 2015. Parasites and biological invasions: parallels,
690 interactions, and control. Trends in Parasitology. 31:189–199.

691 Ebbs E.T., Loker E.S., Brant S.V. 2018. Phylogeography and genetics of the globally
692 invasive snail *Physa acuta* Draparnaud 1805, and its potential to serve as an
693 intermediate host to larval digenetic trematodes. BMC Evolutionary Biology. 18.
694 Escobar J.S., Auld J.R., Correa A.C., Alonso J.M., Bony Y.K., Coutellec M.-A., Koene
695 J.M., Pointier J.-P., Jarne P., David P. 2011. Patterns of mating-system evolution in
696 hermaphroditic animals: correlations among selfing rate, inbreeding depression, and the
697 timing of reproduction: mating-system evolution in hermaphroditic animals. Evolution.
698 65:1233–1253.
699 Estoup A., Martin O. 1996. Marqueurs microsatellites: isolement à l'aide de sondes
700 non-radioactives, caractérisation et mise au point. .
701 Fang Y.-W., Liu L.-Y., Zhang H.-L., Jiang D.-F., Chu D. 2014. Competitive ability and
702 fitness differences between two introduced populations of the invasive whitefly *Bemisia*
703 *tabaci* Q in China. PLoS ONE. 9:e100423.
704 Felsenstein J. 2004. Inferring phylogenies. Sunderland, Mass: Sinauer Associates.
705 Fišer C., Robinson C.T., Malard F. 2018. Cryptic species as a window into the
706 paradigm shift of the species concept. Molecular Ecology. 27:613–635.
707 Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for
708 amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan
709 invertebrates. Molecular marine biology and biotechnology. 3:294–299.
710 Fourdrilis S., Mardulyn P., Hardy O.J., Jordaens K., de Frias Martins A.M., Backeljau
711 T. 2016. Mitochondrial DNA hyperdiversity and its potential causes in the marine
712 periwinkle *Melarhaphe neritoides* (Mollusca: Gastropoda). PeerJ. 4:e2549.
713 Gomez S., Fleeger J.W., Rocha-Olivares A., Foltz D. 2004. Four new species of
714 *Cletocamptus* Schmankewitsch, 1875, closely related to *Cletocamptus deitersi* (Richard,
715 1897) (Copepoda: Harpacticoida). Journal of Natural History. 38:2669–2732.

716 Gutiérrez A., Pointier J.-P., Fraga J., Jobet E., Modat S., Pérez R.T., Yong M., Sanchez
717 J., Loker E.S., Théron A. 2003. *Fasciola hepatica*: identification of molecular markers
718 for resistant and susceptible *Pseudosuccinea columella* snail hosts. Experimental
719 Parasitology. 105:211–218.
720 Heled J., Drummond A.J. 2010. Bayesian inference of species trees from multilocus
721 data. Molecular Biology and Evolution. 27:570–580.
722 Hubendick B. 1951. Recent Lymnaeidae, their variation, morphology, taxonomy,
723 nomenclature and distribution. Kungl Svenska Vetenskapsakademiens Handlingar. 3:1–
724 223.
725 Jarić I., Heger T., Castro Monzon F., Jeschke J.M., Kowarik I., McConkey K.R., Pyšek
726 P., Sagouis A., Essl F. 2019. Crypticity in biological invasions. Trends in Ecology &
727 Evolution.
728 Jarne P., Pointier J.-P., David P., Koene J.M. 2010. Basommatophoran gastropods. The
729 evolution of primary sexual characters in animals. Oxford University Press. p. 173–196.
730 Johnson P.D., Bogan A.E., Brown K.M., Burkhead N.M., Cordeiro J.R., Garner J.T.,
731 Hartfield P.D., Lepitzki D.A.W., Mackie G.L., Pip E., Tarpley T.A., Tiemann J.S.,
732 Whelan N.V., Strong E.E. 2013. Conservation status of freshwater gastropods of
733 Canada and the United States. Fisheries. 38:247–282.
734 Katoh K., Standley D.M. 2013. MAFFT multiple sequence alignment software version
735 7: improvements in performance and usability. Molecular Biology and Evolution.
736 30:772–780.
737 Katz A.D., Taylor S.J., Davis M.A. 2018. At the confluence of vicariance and dispersal:
738 Phylogeography of cavernicolous springtails (Collembola: Arrhopalitidae,
739 Tomoceridae) codistributed across a geologically complex karst landscape in Illinois
740 and Missouri. Ecology and Evolution. 8:10306–10325.

741 Kolar C.S., Lodge D.M. 2001. Progress in invasion biology: predicting invaders. Trends
742 in Ecology & Evolution. 16:199–204.
743 Krug P.J., Vendetti J.E., Rodriguez A.K., Retana J.N., Hirano Y.M., Trowbridge C.D.
744 2013. Integrative species delimitation in photosynthetic sea slugs reveals twenty
745 candidate species in three nominal taxa studied for drug discovery, plastid symbiosis or
746 biological control. Molecular Phylogenetics and Evolution. 69:1101–1119.
747 Kutschera V.E., Bidon T., Hailer F., Rodi J.L., Fain S.R., Janke A. 2014. Bears in a
748 forest of gene trees: phylogenetic inference is complicated by incomplete lineage
749 sorting and sene flow. Molecular Biology and Evolution. 31:2004–2017.
750 Lounnas M., Correa A.C., Alda P., David P., Dubois M.-P., Calvopiña M., Caron Y.,
751 Celi-Erazo M., Dung B.T., Jarne P., Loker E.S., Noya O., Rodríguez-Hidalgo R., Toty
752 C., Uribe N., Pointier J.-P., Hurtrez-Boussès S. 2018. Population structure and genetic
753 diversity in the invasive freshwater snail *Galba schirazensis* (Lymnaeidae). Canadian
754 Journal of Zoology. 96:425–435.
755 Lounnas M., Vázquez A.A., Alda P., Sartori K., Pointier J.-P., David P., Hurtrez-
756 Boussès S. 2017. Isolation, characterization and population-genetic analysis of
757 microsatellite loci in the freshwater snail *Galba cubensis* (Lymnaeidae). Journal of
758 Molluscan Studies. 83:63–68.
759 Mas-Coma S., Bargues M.D., Valero M. a. 2005. Fascioliasis and other plant-borne
760 trematode zoonoses. International Journal for Parasitology. 35:1255–1278.
761 Mavárez J., Pointier J.-P., David P., Delay B., Jarne P. 2002. Genetic differentiation,
762 dispersal and mating system in the schistosome-transmitting freshwater snail
763 Biomphalaria glabrata. Heredity. 89:258–265.
764 Medeiros C., Scholte R.G.C., D'Ávila S., Caldeira R.L., Carvalho O.D.S. 2014. Spatial
765 distribution of Lymnaeidae (Mollusca, Basommatophora), intermediate host of *Fasciola*

766 *hepatica* Linnaeus, 1758 (Trematoda, Digenea) in Brazil. Revista do Instituto de
767 Medicina Tropical de São Paulo. 56:235–252.
768 Meunier C., Hurtrez-Bousses S., Durand P., Rondelaud D., Renaud F. 2004. Small
769 effective population sizes in a widespread selfing species, Lymnaea truncatula
770 (Gastropoda: Pulmonata). Molecular Ecology. 13:2535–2543.
771 Meunier C., Tirard, C., Hurtrez-Bousses S., Durand P., Bargues M.D., Mas-Coma S.,
772 Pointier J.P., Jourdane J., Renaud F. 2001. Lack of molluscan host diversity and the
773 transmission of an emerging parasitic disease in Bolivia. Molecular Ecology. 10:1333–
774 1340.
775 Narváez A.O., Aroca J.M., Alda P., Macías V., Lounnas M., Hurtrez-Boussès S., Noya
776 O., Martini Robles L., Pointier J.-P. 2016. Primer reporte de *Galba cubensis*
777 (Gastropoda: Lymnaeidae) en el Ecuador, hospedador potencial de *Fasciola hepatica* en
778 arrozales de la costa ecuatoriana. El Misionero del Agro. 13:36–47.
779 Niemiller M.L., Near T.J., Fitzpatrick B.M. 2012. Delimiting species using multilocus
780 data: diagnosing cryptic diversity in the southern cavefish, *Typhlichthys subterraneus*
781 (Teleostei: Amblyopsidae): species delimitation in cavefish. Evolution. 66:846–866.
782 Paraense W.L. 1982. Lymnaea rupestris sp. n. from Southern Brazil (Pulmonata:
783 Lymnaeidae). Memórias do Instituto Oswaldo Cruz. 77:437–443.
784 Paraense W.L. 1983. *Lymnaea columella* in northern Brazil. Memórias do Instituto
785 Oswaldo Cruz. 78:477–482.
786 Paraense W.L. 1995. *Lymnaea cousini* Jousseaume, 1887, from Ecuador (Gastropoda:
787 Lymnaeidae). Memórias do Instituto Oswaldo Cruz. 90:605–609.
788 Pedersen U.B., Stendel M., Midzi N., Mduluza T., Soko W., Stensgaard A.-S.,
789 Vennervald B.J., Mukaratirwa S., Kristensen T.K. 2014. Modelling climate change
790 impact on the spatial distribution of fresh water snails hosting trematodes in Zimbabwe.

791 Parasites & Vectors. 7:536.

792 Pfeiffer L. 1839. Bericht über die Ergebnisse maine Reise nach Kuba im Winter 1838-

793 1839. Archiv für Naturgeschichte. 5:346–358.

794 Pfenninger M., Cordellier M., Streit B. 2006. Comparing the efficacy of morphologic

795 and DNA-based taxonomy in the freshwater gastropod genus *Radix* (Basommatophora,

796 Pulmonata). BMC Evolutionary Biology. 6:100.

797 Pfenninger M., Schwenk K. 2007. Cryptic animal species are homogeneously

798 distributed among taxa and biogeographical regions. BMC Evolutionary Biology.

799 7:121.

800 Pinceel J., Jordaens K., Backeljau T. 2005. Extreme mtDNA divergences in a terrestrial

801 slug (Gastropoda, Pulmonata, Arionidae): accelerated evolution, allopatric divergence

802 and secondary contact: Molecular divergence in a terrestrial slug. Journal of

803 Evolutionary Biology. 18:1264–1280.

804 Pointier J., Noya O., Amarista M., Théron A. 2004. *Lymnaea cousini* Jousseaume, 1887

805 (Gastropoda: Lymnaeidae): first record for Venezuela. Memórias do Instituto Oswaldo

806 Cruz. 99:567–569.

807 Pointier J.-P. 2015. Freshwater molluscs of Venezuela and their medical and veterinary

808 importance. Harxheim: ConchBooks.

809 Qian Z., Yang J., Lu Y., He J. 2012. Description of three freshwater species

810 (Gastropoda) from China. Shell Discoveries. 1:30–31.

811 Rama Rao S., Liew T.-S., Yow Y.-Y., Ratnayeke S. 2018. Cryptic diversity: Two

812 morphologically similar species of invasive apple snail in Peninsular Malaysia. PLOS

813 ONE. 13:e0196582.

814 Rambaut A., Drummond A.J., Xie D., Baele G., Suchard M.A. 2018. Posterior

815 summarization in bayesian phylogenetics using Tracer 1.7. Systematic Biology.

816 Remigio E. 2002. Molecular phylogenetic relationships in the aquatic snail genus
817 *Lymnaea*, the intermediate host of the causative agent of fascioliasis: insights from
818 broader taxon sampling. Parasitology Research. 88:687–696.
819 Sabourin E., Alda P., Vázquez A., Hurtrez-Boussès S., Vittecoq M. 2018. Impact of
820 human activities on fasciolosis transmission. Trends in Parasitology. 34:891–903.
821 Sales J.B. de L., Shaw P.W., Haimovici M., Markaida U., Cunha D.B., Ready J.,
822 Figueiredo-Ready W.M.B., Schneider H., Sampaio I. 2013. New molecular phylogeny
823 of the squids of the family Loliginidae with emphasis on the genus *Doryteuthis* Naef,
824 1912: Mitochondrial and nuclear sequences indicate the presence of cryptic species in
825 the southern Atlantic Ocean. Molecular Phylogenetics and Evolution. 68:293–299.
826 Samadi S., Roumégoux A., Bargues M.D., Mas-Coma S., Yong M., Pointier J.-P. 2000.
827 Morphological studies of Lymnaeid snails from the human fasciolosis endemic zone of
828 Bolivia. Journal Molluscan Studies. 66:31–44.
829 Standley C.J., Prepelitchi L., Pietrokovsky S.M., Issia L., Stothard J., Wisnivesky-Colli
830 C. 2013. Molecular characterization of cryptic and sympatric lymnaeid species from the
831 *Galba*/*Fossaria* group in Mendoza Province, Northern Patagonia, Argentina. Parasites
832 & Vectors. 6:304.
833 Stevenson J., Norris D. 2016. Implicating cryptic and novel anophelines as malaria
834 vectors in Africa. Insects. 8:1.
835 Stewart J.E., Timmer L.W., Lawrence C.B., Pryor B.M., Peever T.L. 2014. Discord
836 between morphological and phylogenetic species boundaries: incomplete lineage
837 sorting and recombination results in fuzzy species boundaries in an asexual fungal
838 pathogen. BMC Evolutionary Biology. 14:38.
839 Struck T.H., Feder J.L., Bendiksby M., Birkeland S., Cerca J., Gusarov V.I., Kistenich
840 S., Larsson K.-H., Liow L.H., Nowak M.D., Stedje B., Bachmann L., Dimitrov D. 2018.

841 Finding evolutionary processes hidden in cryptic species. Trends in Ecology &
842 Evolution. 33:153–163.
843 Suh A., Smeds L., Ellegren H. 2015. The dynamics of incomplete lineage sorting across
844 the ancient adaptive radiation of neoavian birds. PLOS Biology. 13:e1002224.
845 Taylor D.W. 2003. Introduction to Physidae (Gastropoda: Hygrophila); biogeography,
846 classification, morphology. Revista de Biología Tropical. 51:1–287.
847 Thomaz D., Guiller A., Clarke B. 1996. Extreme divergence of mitochondrial DNA
848 within species of pulmonate land snails. Proceedings of the Royal Society of London.
849 Series B: Biological Sciences. 263:363–368.
850 Václavík T., Meentemeyer R.K. 2009. Invasive species distribution modeling (iSDM):
851 Are absence data and dispersal constraints needed to predict actual distributions?
852 Ecological Modelling. 220:3248–3258.
853 Vázquez A.A., Alda P., Lounnas M., Sabourin E., Alba A., Pointier J.-P., Hurtrez-
854 Boussès S. 2018. Lymnaeid snails hosts of *Fasciola hepatica* and *Fasciola gigantica*
855 (Trematoda: Digenea): a worldwide review. CAB Reviews. 13:1–15.
856 Vázquez A.A., Sánchez J., Pointier J.-P., Théron A., Hurtrez-Boussès S. 2014. *Fasciola*
857 *hepatica* in Cuba: compatibility of different isolates with two intermediate snail hosts,
858 *Galba cubensis* and *Pseudosuccinea columella*. Journal of Helminthology. 88:434–440.
859 Vinarski M.V. 2018. *Galba robusta* sp. nov. from Yemen (Gastropoda: Lymnaeidae).
860 Zoosystematica Rossica. 27:2–10.
861 Vinarski M.V., Kantor J.I. 2016. Analytical catalogue of fresh and brackish water
862 molluscs of Russia and adjacent countries. Moscow: A.N. Severtsov Institute of
863 Ecology and Evolution of RAS.
864 Weigand A.M., Jochum A., Pfenninger M., Steinke D., Klussmann-Kolb A. 2011. A
865 new approach to an old conundrum-DNA barcoding sheds new light on phenotypic

plasticity and morphological stasis in microsnails (Gastropoda, Pulmonata, Carychiidae): DNA BARCODING. Molecular Ecology Resources. 11:255–265.

Weiss M., Weigand H., Weigand A.M., Leese F. 2018. Genome-wide single-nucleotide polymorphism data reveal cryptic species within cryptic freshwater snail species-The case of the *Ancylus fluviatilis* species complex. Ecology and Evolution. 8:1063–1072.

Xia X. 2017. DAMBE6: new tools for microbial genomics, phylogenetics, and molecular evolution. Journal of Heredity. 108:431–437.

Zulliger D.E., Lessios H.A. 2010. Phylogenetic relationships in the genus *Astropecten* Gray (Paxillosida: Astropectinidae) on a global scale: molecular evidence for morphological convergence, species-complexes and possible cryptic speciation. Zootaxa.:1–19.

878 **Table 1.** Type localities of *Galba* species and sequences of individuals recovered in those localities. References mentioned are the ones in which
879 sequences were provided and not in which species were described, except for articles describing *Galba meridensis* and *Galba neotropica* were
880 the authors also provided sequences (Bargues et al. 2007, 2011b). The type localities for *Galba cubensis* and *Galba viator* were here restricted
881 because original description of the species did not provide a locality, authors simply stated "Cuba" and "Patagonia", respectively (D'Orbigny
882 1835; Pfeiffer 1839).

Species	Country	Locality	Coordinates	ITS1	ITS2	16S	COI	Reference
Galba cousini	Ecuador	Chanchu-Yacu	00°18'55"S 78°34'02"W	FN598157	FN598153	-	FN598161	Bargues et al. 2011b
Galba cubensis	Cuba	Vaqueria 21	23°01'N 82°32'W	AM412226	AM412223	-	AM494009	Bargues et al. 2007
Galba humilis	USA	Owego, New York	42°06'01"N 76°15'40"W	FN182193, FN182194	FN182191, FN182192	-	FN182197, FN182198	Correa et al. 2011
Galba meridensis	Venezuela	Laguna Mucubají (Mérida)	08°47'52"N 70°49'32"W	FN598159	FN598154	-	FN598164	Bargues et al. 2011b
Galba neotropica	Peru	Lima, Rio Rimac	12°02'S 76°56'–77°08'W	AM412228	AM412225	-	AM494008	Bargues et al. 2007
Galba shirazensis	Iran	Taleb Abad river, Bandar Anzali, Gilan province	37º27'46"N 49º37'07"E	JF272603	JF272601	JF272605	JF272607	Bargues et al. 2011a

Galba truncatula	Germany	Thuringia, Erfurt-Bindersleben	ND	-	-	-	EU818799	Albrecht et al. 2008
Galba viator	Argentina	Frias	40°14' S 64°10' W	JN614428	HQ283265, JN614465	-	JN614397, JN614398	Correa et al. 2011

883

884 # Figures captions

885 **Figure 1.** Geographic distribution of *Galba* species in America, based on molecular
886 identification. Pink points refer to the *viator* clade and red points refer to the *cubensis*
887 clade (see cluster V in the 16S, COI and ITS2 trees). Coordinates are given in Tables
888 S1–S2. The sites Perdriel (Argentina), Batallas and Tambillo (Bolivia), Ontario
889 (Canada), San Rafael (Mexico), Canal Salinas (Puerto Rico) are not represented since
890 coordinates are missing in the original publications.

891 **Figure 2.** Phylogenetic trees inferred in Beast2 using the mitochondrial (COI and 16S)
892 and nuclear (ITS1 and ITS2) genes. Five clades are distinguished corresponding to five
893 species / species complex. In the *G. cubensis*/*viator* clade, *G. cubensis* is represented in
894 red and *G. viator* in pink. Scale bar represents branch length expressed as number of
895 substitutions per site.

896 **Figure 3.** Most common topology of the species tree and phenotype of the most recent
897 common ancestor of *Galba* inferred in StarBeast2. Node values indicate posterior
898 probability and blue bars indicate 95% credibility intervals. The mating system is
899 indicated on the right.

Supplementary Material

Table legends

Table S1. Sampled sites from America in which *Galba* species were found. Individuals were submitted to the three-step procedures for species identification (see text and Fig. S2). For each site, we provide the country, site name, geographic coordinates, sampling date, and number of sampled individuals. Note that only a fraction of sampled individuals was sequenced. For each step (and species), we indicate the number of individuals considered. NA: not available. * indicate sites that have been resampled at different dates. Accession names in GenBank (ITS2 and COI) are indicated into parentheses. Note that in some cases a single sequence was obtained.

Table S2. Sites retrieved from literature and GenBank where *Galba* species were molecularly identified in America. *Galba cubensis* and *Galba neotropica* are considered synonymous, as are *Galba cousini* and *Galba meridensis*. Both the *Galba* and *Lymnaea* names have been used in the literature at genus level for the species considered in our study—we used *Galba* here for this monophyletic group of small lymnaeids. For each site, we report the country, site, geographical coordinates available sequences of mitochondrial (COI and 16S) and nuclear (ITS1 and ITS2) genes, species identification by specific microsatellites, bibliographic reference, and the species name used in the reference. Coordinates from Owego, New York were obtained from GoogleEarth and those from Correa et al. (2010) from Correa et al. (2011). Some coordinates were corrected in order to match the specific site: Rio Negro (Argentina) from Correa et al. (2010), Frias (Argentina) from Correa et al. (2011) and Lounnas et al. (2017a), Estanque Lagunillas (Venezuela) from Bargues et al. (2011c), Baños del Inca (Peru) from Bargues et al. (2012), Paysandú (Uruguay) from Lounnas et al. (2017a) and Geffrier (Guadeloupe) (provided by the authors). The KT461809 sequence was

925 erroneously tagged as an ITS2 sequence, but is, in fact, a COI sequence. Sequences of
926 the individuals molecularly identified by (Medeiros et al. 2014) are missing in the
927 original publication and were not uploaded to GenBank. ND, no data available.
928 **Table S3.** Sites retrieved from literature and GenBank where *Galba* species were
929 molecularly identified in Europe, Asia, and Africa. Coordinates that were not given in
930 the original articles or in GenBank were best-guess estimated. The information reported
931 for each site is as in Table S2. ND, no data available.
932 **Table S4.** Species of small, mud-dwelling lymnaeids recognized by Burch (1982) in
933 North America. The author grouped the 22 species into the genus *Fossaria* with two
934 subgenera, *Fossaria* (*s.s.*) and *Bakerilymnaea*.

Figure captions

Figure S1. Expected signatures of four evolutionary processes that can lead to cryptic species. Species with similar (identical) morphotypes denoted with 'sim.'. Panels on the left denote the phylogenetic relationships among taxa, while the panels to the right depict the evolution of morphological disparity through time for pairs of cryptic and non-cryptic species (*e.g.*, A1/ A2 *vs*. A1/A3). (A) Recent divergence: cryptic species are very closely related and only recently diverged from each other. However, the rate of morphological disparity is not necessarily substantially different from that for non-cryptic species and, as such, these taxa may not actually represent cryptic species. (B) Parallelism: the cryptic species are not very closely related to each other and the rate of morphological disparity for non-cryptic species is much greater than that for cryptic species. (C) Convergence: the cryptic species are also not closely related to each other. Initially, morphological disparity for cryptic species can change in a manner similar to that for the non-cryptic species pair. However, at some point, morphological disparity decreases for the cryptic species, while continuing to increase between non-cryptic taxa. (D) Stasis: the cryptic species are closely related to each other or are part of a species complex and diverged a long time ago. In comparison with non-cryptic species, the rate of morphological change is substantially reduced. (Figures and legends modified from Struck et al. 2018).

Figure S2. The three-step procedure followed to identify *Galba* species. The species identified at each step are indicated on the right. Fragments of the ITS2 and COI genes were sequenced in one individual of *Galba cousini* and 111 individuals of *Galba cubensis* (41), *Galba schirazensis* (41) and *Galba truncatula* (29) in order to validate steps 1 and 2.

959 **Figure S3.** Geographic distribution of *Galba cubensis*, *Galba schirazensis* and *Galba*
960 *truncatula* in the European, Asian and African samples retrieved from GenBank.
961 Coordinates are given in Table S3.
962 **Figure S4.** Shells and reproductive and urinary systems of the six *Galba* species
963 studied. *Galba cousini* is the only species that can be morphologically identified.
964 **Figure S5.** Phylogenetic tree based on Bayesian inference in Beast2 of the 16S gene.
965 All sequences were retrieved from GenBank. Sequence coloration represents species.
966 Arrows indicate sequences belonging to a type locality (see Table 1 for details).
967 Sequence data are given in Tables S1, S2, and S3.
968 **Figure S6.** Phylogenetic tree based on Bayesian inference in Beast2 of the COI gene.
969 All sequences from the current study (highlighted in yellow), as well as the ones
970 retrieved from GenBank, are included in this tree. Sequence coloration represents
971 species. Arrows indicate sequences belonging to a type locality (see Table 1 for details).
972 Sequence data are given in Tables S1, S2, and S3.
973 **Figure S7.** Phylogenetic tree based on Bayesian inference in Beast2 of the ITS1 gene.
974 All sequences were retrieved from GenBank. Sequence coloration represents species.
975 Arrows indicate sequences belonging to a type locality (see Table 1 for details).
976 Sequence data is given in Tables S1, S2, and S3.
977 **Figure S8.** Phylogenetic tree based on Bayesian inference in Beast2 of the ITS2 gene.
978 All sequences for the current study (highlighted in yellow), as well as the ones retrieved
979 from GenBank, are included in this tree. Sequence coloration represents species.
980 Arrows indicate sequences belonging to a type locality (see Table 1 for details).
981 Sequence data are given in Tables S1, S2, and S3.
982 **Figure S9.** Most probable topologies of *Galba* species based on all sequence data
983 (visualized in Densitree). Greater topological agreement is visualized by a higher

984 density of trees, whereas uncertainty in the height and distribution of nodes are
985 represented by increased transparency. The most common topologies are shown in blue,
986 the second most common topologies in red and the third in green. Solid blue lines
987 represent the consensus tree that showed the highest clade support.