This article has been published in Molecular Phylogenetics and Evolution (7, 107035; https://doi.org/10.1016/j.ympev.2020.107035)

Systematics and Geographical Distribution of *Galba* Species, a Group of Cryptic and Worldwide Freshwater Snails

Pilar Alda[1,2,3], Manon Lounnas[2], Antonio A. Vázquez[2,4], Rolando Ayaqui[5], Manuel Calvopiña[6], Maritza Celi-Erazo[7], Robert T. Dillon Jr.[8], Luisa Carolina González Ramírez[9], Eric S. Loker[10], Jenny Muzzio-Aroca[11], Alberto Orlando Nárvaez[11,12], Oscar Noya[13], Andrés Esteban Pereira[14], Luiggi Martini Robles[15], Richar Rodríguez-Hidalgo[7,16], Nelson Uribe[14], Patrice David[17], Philippe Jarne[17], Jean-Pierre Pointier[18], Sylvie Hurtrez-Boussès[2,19]

[1] *Laboratorio de Zoología de Invertebrados I, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. San Juan No. 670, B8000ICN Bahía Blanca, Buenos Aires, Argentina.*
[2] *MIVEGEC, University of Montpellier, CNRS, IRD, Montpellier, France.*
[3] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.*
[4] *Laboratory of Malacology, Institute of Tropical Medicine "Pedro Kourí", Autopista Novia del Mediodía km 6, La Habana, Cuba.*
[5] *Departamento de Microbiología y Patología, Facultad de Medicina, Universidad Nacional de San Agustín de Arequipa, Peru.*
[6] *Carrera de Medicina, Facultad de Ciencias de la Salud, Universidad de Las Américas, Quito, Ecuador.*
[7] *Instituto de Investigación en Salud Pública y Zoonosis - CIZ, Universidad Central de Ecuador, Quito, Ecuador.*
[8] *Freshwater Gastropods of North America Project, P.O. Box 31532, Charleston, SC 29417, USA.*
[9] *Grupo de Investigación "Análisis de Muestras Biológicas y Forenses" Laboratorio Clínico, Facultad de Ciencias de la Salud, Universidad Nacional de Chimborazo, Ecuador.*
[10] *Center for Evolutionary and Theoretical Immunology, Department of Biology, University of New Mexico, Albuquerque, NM87131, USA.*
[11] *Instituto Nacional de Investigación en Salud Pública INSPI, Guayaquil, Ecuador.*
[12] *Universidad Agraria del Ecuador, Facultad de Medicina Veterinaria y Zootecnia, Guayaquil, Ecuador.*
[13] *Sección de Biohelmintiasis, Instituto de Medicina Tropical, Facultad de Medicina, Universidad Central de Venezuela. Centro para Estudios Sobre Malaria, Instituto de Altos Estudios "Dr. Arnoldo Gabaldón"-Instituto Nacional de Higiene "Rafael Rangel" del Ministerio del Poder Popular para la Salud. Caracas, Venezuela.*

[14] *Grupo de Investigación en Epidemiología Molecular (GIEM), Escuela de Microbiología, Facultad de Salud, Universidad Industrial de Santander, Bucaramanga, Colombia.*
[15] *Laboratorio de Parasitologia Luiggi Martini y colaboradores, Guayaquil, Ecuador.*
[16] *Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador.*
[17] *Centre d'Ecologie Fonctionnelle et d'Evolution, UMR 5175, CNRS – Université de Montpellier – Université Paul Valéry Montpellier – EPHE - IRD, 1919 route de Mende, 34293, Montpellier Cedex 5, France.*
[18] *PSL Research University, USR 3278 CNRS–EPHE, CRIOBE Université de Perpignan, Perpignan, France.*
[19] *Département de Biologie–Ecologie, Faculté des Sciences, Université Montpellier, Montpellier, France.*

To whom correspondence should be addressed: **pilaralda@gmail.com** (P. Alda)

ABSTRACT

Cryptic species can present a significant challenge to the application of systematic and biogeographic principles, especially if they are invasive or transmit parasites or pathogens. Detecting cryptic species requires a pluralistic approach in which molecular markers facilitate the detection of coherent taxonomic units that can then be analyzed using various traits (e.g., internal morphology) and crosses. In asexual or self-fertilizing species, the latter criteria are of limited use. We studied a group of cryptic freshwater snails (genus *Galba*) from the family Lymnaeidae that have invaded almost all continents, reproducing mainly by self-fertilization and transmitting liver flukes to humans and livestock. We aim to clarify the systematics, distribution, and phylogeny of these species with an integrative approach that includes morphology, molecular markers, wide-scale sampling across America, and data retrieved from GenBank (to include Old World samples). Our phylogenetic analysis suggests that the genus *Galba* originated ca. 22 Myr ago and today comprises six species or species complexes. Four of them show an elongated-shell cryptic phenotype and exhibit wide variation in their genetic diversity, geographic distribution, and invasiveness. The remaining two species have more geographically restricted distributions and exhibit a globose-shell cryptic phenotype, most likely phylogenetically derived from the elongated one. We emphasize that no *Galba* species should be identified without molecular markers. We also discuss several hypotheses that can explain the origin of cryptic species in *Galba*, such as convergence and morphological stasis.

KEY WORDS: phylogenetics, America, Lymnaeidae, vector snails, biological invasions, self-fertilization.

1. INTRODUCTION

Cryptic species are groups of populations in which the intrapopulation phenotypic variance exceeds interspecific phenotypic variance (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 seem to be more often reported in animals (Struck et al. 2018). Their frequency might reach 10-20% of all animal species (Janzen et al. 2017), an estimate that should be considered quite approximate given our lack of knowledge on the species totals. They also seem to be homogeneously distributed among taxa and biogeographical regions (Pfenninger and Schwenk 2007). Four hypotheses have been proposed for the origin of cryptic species (Bickford et al. 2007; Fišer et al. 2018; Struck et al. 2018): (1) recent divergence, when distinguishing traits have not as yet accumulated (e.g. cave fish, Niemiller et al. 2012); (2) parallelism, where independent phenotypic traits evolve in different taxa from a similar and shared ancestral trait (Struck et al. 2018); (3) convergence, where more distantly related species evolve from dissimilar ancestors (e.g. sea stars, Zulliger and Lessios, 2010), and (4) morphological stasis, where species remain similar over long periods of time (e.g., Gomez et al. 2004; Struck et al. 2018).

Although interesting as models for study of the speciation process (Coyne and Orr 2004; De Queiroz 2007), cryptic species are problematic from at least two human perspectives: biological invasion and disease transmission. Cryptic species may exhibit wide differences in invasive ability and impact on invaded ecosystems and communities (Fang et al. 2014). An accurate identification at the species level is required in such situations to track invasions and mitigate any harmful consequences (Kolar and Lodge 2001; Dunn and Hatcher 2015; Jarić et al. 2019). Cryptic species may also exhibit

differences in disease transmission. This is the case in the *Anopheles gambiae* complex which includes the most important vectors of malaria in Africa (Stevenson and Norris 2016). Some members of the complex are broadly zoophilic, while others feed more strictly on humans. Accurate species identification is required for effective mosquito control.

Snails, especially freshwater ones, are an interesting group for addressing biogeographic issues in cryptic species. Although taxonomists have increasingly used molecular markers over the last decades to identify snail species (Dayrat et al. 2011), morphological characters, especially shell shape and sculpture, remain widely relied upon—often resulting in large numbers of synonymous species (Qian et al. 2012). The shells of some mollusk populations show significant phenotypic plasticity, however, in reaction to temperature, pollution, or predation (Bourdeau et al. 2015). Others stay stable for millions of years (e.g., Weigand et al. 2011; Weiss et al. 2018). This has resulted both in the proliferation of species names and descriptions (e.g., Taylor, 2003), most of which are invalid (Jarne et al. 2010; Dillon et al. 2011), as well as the misidentification of valid species, yielding errors in the assessment of species invasion ability and distributional range (e.g., Pfenninger et al. 2006; Rama Rao et al. 2018). For example, an invasive Asian clam of the genus *Sinanodonta* has been overlooked in Russia because it is morphologically indistinguishable from another invasive Asian clam (Bespalaya et al. 2018). Such problems call for an integrated approach to gastropod systematics and biogeography in which phenotypic traits are studied together with appropriate molecular tools (Dayrat 2005).

Here we focus on small-bodied basommatophoran pulmonate snails of the genus *Galba* (Hygrophila, Lymnaeidae), common inhabitants of unstable freshwater habitats

worldwide. Baker (1911) recognized 30 species and subspecies in the subgenera *Galba* (*s.s.*) and *Simpsonia* in North America, on the basis of minor shell morphological variation, where Hubendick (1951) suggested that as few as four biological species might be valid: *humilis*, *truncatula*, *cubensis*, and *bulimoides*. The more recent work of Burch (1982) proposed 22 North American species in the genus *Fossaria*, which we here consider a junior synonym of *Galba*. In South America, Hubendick (1951) recognized only two species of small-bodied, amphibious lymnaeids (*viator* and *cousini*), but more recent work based on single-gene approaches and on fewer than 10 sequences per species distinguishes seven species (*viator*, *schirazensis*, *cousini*, *neotropica*, *meridensis*, *truncatula*, and *cubensis*; Bargues et al. 2007, 2011b, 2011a; Correa et al. 2010, 2011; Lounnas et al. 2017, 2018). Population genetic studies suggest, however, that *neotropica* and *cubensis* could be synonymies. Uncertainty prevails in *Galba* systematics mainly because most of the nominal *Galba* species share a similar shell morphology and internal anatomy, as well as plasticity in shell, anatomy, and life-history traits (Samadi et al. 2000; Correa et al. 2011). The exceptions are *Galba cousini* (Paraense 1995) and *Galba meridensis* (Bargues et al. 2011b), which are morphologically different from other *Galba* species. However, they are both morphologically similar (shell and reproductive anatomy) and *G. meridensis* has been described from a single locality (Bargues et al. 2011b). We therefore have two groups of cryptic species, which are often misidentified within groups (Correa et al. 2010; Bargues et al. 2011a). Overall, this uncertainty calls for a wider study that sheds light on the phylogenetic relationships among *Galba* species.

A further challenge is that crossing cannot be used to distinguish species (Coyne and Orr 2004), as has been done in other freshwater snails (e.g., *Physa* species, Dillon et

al. 2011), since *Galba* populations primarily reproduce by self-fertilization (Meunier et al. 2004; Bargues et al. 2011a; Lounnas et al. 2017, 2018). Self-fertilization poses two problems here: one practical and one theoretical. On the practical side, an evaluation of whether phylogenetic groups might demonstrate reproductive isolation among each other would be prohibitively labor-intensive, many generations being required to demonstrate unsuccessful crosses at some rather-arbitrarily set confidence. The theoretical problem is that if one applies the biological species concept to a 100% self-fertilizing taxon, each individual would immediately produce a line of descent reproductively isolated from all others, and thus deserve a species name (Coyne & Orr 2004). However, strict selfing has never been reported in any biological group, including Lymnaeid snails (Cutter 2019). Population genetic studies, when variation is sufficient to test it, report low outcrossing rates in some lymnaeid populations, but generally not lower than 5-10% (Escobar et al. 2011, Lounnas et al., 2017). Rates as low as 1%, yielding recombination every 100 generations, are probably sufficient, over evolutionary time, to maintain genetic cohesion within a set of interbreeding populations, reproductively isolated from other similar sets.

Moreover, at least two species, *G. schirazensis* and *G. truncatula*, have been shown to be extremely efficient anthropogenic invaders, muddling our knowledge of species distribution. Populations of *G. truncatula*, probably from Eurasia, have invaded South America, especially the Bolivian Altiplano (Meunier et al. 2004). This is of special concern since *Galba* populations are the main vectors of the liver fluke *Fasciola hepatica* which causes fasciolosis in both livestock and humans (Mas-Coma et al. 2005) and transmission efficiency and invasion ability differ among species (Vázquez et al. 2018).

Worldwide, the geographic distribution of *Galba* species is poorly known. The dubious character of records based on morphological identification leaves us with a small sample of molecular studies (Correa et al. 2010, 2011; Bargues et al. 2011b, 2011a, 2012; Lounnas et al. 2017, 2018) upon which to base a very large-scale pattern. Our objectives here are to characterize the geographic distribution of *Galba* species at continental scale, based on an extensive sampling over America, to reconstruct the genus phylogeny to delimit species, and to explore the origin of cryptic species. We aim to delineate species—the real scientific challenge of integrative taxonomy (Dayrat 2005)—noting that in practice *Galba* species are very difficult to delineate due to the presence of cryptic species, wide geographical distribution, and mating system. Previous studies reconstructing *Galba* phylogeny have used single genes and analyzed fewer than 10 sequences per species, failing to account for the wide geographic distribution of this genus (Correa et al. 2010, 2011; Bargues et al. 2011c, 2011a; Standley et al. 2013). Here we employ morphological and molecular markers (microsatellite loci and DNA sequences from two genes) to study more than 1,700 individual *Galba* from 161 sites. Our data set was augmented with a complete sample of all the *Galba* DNA sequences available in GenBank and multiple phylogenetic analyses conducted. We used both gene trees and multispecies coalescent models to shed light on the phylogenetic relationships and on the origin of cryptic species in the genus *Galba*.

2. MATERIALS AND METHODS

2.1 Snail Sampling and Species Identification

We conducted simple searches for *Galba* populations in suitable habitats throughout the New World over a span of two decades, 1998–2017. Overall, *Galba* populations were

198 detected in 161 sites and 1,722 individuals were sampled from nine countries:
199 Argentina, Canada, Colombia, Cuba, Ecuador, France (Guadeloupe and Martinique),
200 Peru, Venezuela, and USA (Table S1). *Galba* populations have previously been
201 reported from some of these sites in Venezuela and Ecuador by the authors (Pointier
202 2015; Orlando Narváez et al. 2017). In most cases, we discovered our *Galba*
203 populations in unstable habitats subject to frequent flooding and droughts. The sampled
204 habitats were characterized as brook, irrigation canal, ditch, oxbow lake, pond, marsh,
205 lake, tank, rice field, and river. Individual snails were often collected above the water
206 line, consistent with their amphibious habit. Some sites were visited up to five times.
207 Geographic coordinates were recorded for most sites. After collection, individuals were
208 placed in water at 70 ºC for 30–45 s. This procedure allows fixation of individuals
209 without contraction of soft parts and facilitates a proper study of snail internal anatomy.
210 The body was carefully withdrawn from the shell using forceps and both body and shell
211 stored in 70% ethanol until morphological and DNA analyses (Pointier et al. 2004).
212 Species were characterized using a three-step procedure involving both
213 morphological and molecular markers (Fig. 1). Step 1 was an analysis of shell
214 morphology and reproductive anatomy. In step 2, we used a molecular tool that enables
215 us to distinguish *G. cubensis*, *G. schirazensis*, and *G. truncatula* (Alda et al. 2018). In
216 step 3, we sequenced mitochondrial and nuclear genes in individuals for which no PCR
217 amplification product was obtained in step 2. DNA from some of those individuals
218 identified in steps 1 and 2 were also sequenced in order to reconstruct the phylogeny of
219 *Galba*. Note that this three-step approach is less expensive than an approach based on
220 simply sequencing the same genes in all individuals.
221 2.1.1 Step 1: morphology of the shell and of internal organs

We photographed the shell of three to five adult snails from each site and dissected their body under a stereoscopic microscope. We drew the anatomy of the penial complex, prostate, and renal tube using a camera lucida attachment (Pointier et al. 2004). We did not record any morphological measurements or perform any quantitative tests because previous studies (Samadi et al. 2000, Correa et al. 2011) have shown that cryptic *Galba* species cannot be delimited by such means. Our observations were qualitative only.

2.1.2 Step 2: multiplex PCR of microsatellite loci

We applied the multiplex PCR test designed by Alda et al. (2018) to all the 1,420 individuals that were not distinguishable based on shell and reproductive anatomy. This method is based on species-specific primers amplifying three microsatellite loci (one each per targeted species) and producing band sizes that are specific to these species (179–200 pb in *G. cubensis*, 227–232 pb in *G. schirazensis* and 111–129 pb in *G. truncatula*). DNA was extracted using a Chelex protocol following Estoup and Martin (1996) as adapted for 96-well plates. Methods for DNA amplification and electrophoretic resolution followed Alda et al. (2018).

2.1.3 Step 3: identification by sequencing

We amplified the internal transcribed spacer 2 (ITS2) and the cytochrome oxidase subunit 1 (COI) genes in 35 individuals sampled from 15 sites in Argentina, Canada and the USA (1 to 5 per population) where at least some individuals did not demonstrate an amplification product in step 2, using the method of Lounnas et al. (2017, 2018). We also amplified ITS2 and COI in 112 individuals that did return an amplification product in step 2, including one individual identified as *G. cousini*/*meridensis*. To supplement these results, we also amplified the internal transcribed spacer 1 (ITS1) and the ARN ribosomal 16S in two individuals of *G. cubensis* from Bosque del Apache (USA) and in

one individual of *G. cousini*/*meridensis* from Ecuador (Table S1). With this approach, we obtained at least one sequence from each hypothetical species represented by the four genes and used them to delimit species. The total number of individuals amplified was 151.

We used the primers NEWS2 (forward) 5' TGTGTCGATGAAGAACGCAG 3' and ITS2-RIXO (reverse) 5' TTCTATGCTTAAATTCAGGGG 3' to amplify ITS2; Lim1657 (forward) 5' CTGCCCTTTGTACACACCG 3' and ITS1-RIXO 5' TGGCTGCGTTCTTCATCG 3' to amplify ITS1 (Almeyda-Artigas et al. 2000); LCOI490 (forward) 5' GGTCAACAAATCATAAAGATATTGG 3' and HCO2198 (reverse) 5' TAAACTTCAGGGTGACCAAAAAATCA 3' to amplify COI (Folmer et al. 1994) and forward 5' CGCCTGTTTATCAAAAACAT 3' and reverse 5' CCGGTCTGAACTCAGATCACGT 3' to amplify 16S (Remigio and Blair 1997). In all 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 confirmed in 1% agarose gels 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.

2.2 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 all 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." Pfeiffer (1839) gave no locality data for his *Limnaeus cubensis* at all, beyond "Cuba". Thus, we selected a more precise spot within these wide-ranged type localities for *viator* and *cubensis*. 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.

2.3 Retrieving Data on *Galba* spp. Distribution from Published Work

We searched the literature and GenBank for sequence data at four genes (COI, ITS1, ITS2, and 16S) apparently attributable to lymnaeids of the genus *Galba*. Coordinates were provided for most sites by the authors. When coordinates were not provided, we inferred them from the locality data 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. S1). 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. This corresponds to 166 COI, 163 ITS2, 118 ITS1, and 70 16S sequences.

294

295 2.4 Phylogenetic and Ancestral Reconstruction Study

296 Phylogenetic analyses were conducted on the ITS2 and COI sequences obtained in this
297 study, together with ITS2, COI, ITS1, and 16S sequences retrieved from GenBank. This
298 is the first phylogenetic study conducted in *Galba* species that use such a large dataset
299 including 796 sequences: 90 for 16S, 251 for COI, 122 for ITS1, and 333 for ITS2.
300 Previous studies were based on single genes and smaller sample sizes. For instance, the
301 description of *G. meridensis* is based from a single locality (Bargues et al. 2011b).
302 Some GenBank accession numbers appear more than once in Tables S2–S3 because
303 individuals with identical sequences have been registered under the same GenBank
304 accession number.

305 Alignment was performed individually for each gene using MAFFT (Katoh and
306 Standley 2013). Ambiguously aligned sites were excluded using GBLOCKS with
307 default settings for a less stringent selection (Castresana 2000). The number of positions
308 in the final sequences was 412 for 16S (83% of the original 493 positions), 609 for COI
309 (86% of 707), 435 for ITS1 (48% of 888), and 333 for ITS2 (23% of 1,429). We
310 examined levels of saturation for each gene and for the first and second *versus* third
311 codon positions of COI using DAMBE (Xia 2017). We did not find evidence of
312 saturation in the four genes analyzed, including all codon positions of COI. We
313 partitioned codon positions and unlinked substitution models in phylogenetic analyses.

314 We used Bayesian inference in Beast2 (Bouckaert et al. 2014) for five reasons:
315 (1) to assign sequences to species; (2) to validate, and to mend if necessary, species
316 identity for sequences retrieved from GenBank; (3) to delimit species; (4) to reconstruct
317 the phylogeny and estimate time divergence; and (5) to assess the ancestral phenotypic

state of *Galba* species. Because we did not have identical sets of individuals (or populations) across genes it was necessary to build four unlinked gene trees to address questions (1) and (2). 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. We linked trees for the three COI codon partitions. The analyses were run using four gamma categories and a proportion of 0.5 invariant sites. Uncorrelated relaxed-clock models were chosen for all loci. The relative clock mean priors were all lognormal (M = 0, S = 1). We used a birth-death model as priors with lognormal birth and death rates. These gene trees allowed us to evaluate species names and validate or mend species identity for all sequences, whether obtained in this study or retrieved from GenBank. All the MCMC were run for 200,000,000 generations storing every 20,000 generations. The MCMC output was visualized using Tracer (Rambaut et al. 2018) and tree samples summarized by TreeAnnotator (utility program distributed with the Beast package) using a 10% burn-in. The species tree was visualized and edited in FigTree and GIMP (https://www.gimp.org). The names of the eight widely-recognized species in the genus *Galba* were ultimately assigned by reference to their type localities. We also built haplotype networks for each gene using popART (Leigh and Bryant 2015) and compared them with gene trees.

To address our question (3), species delimitation, we built ten multispecies coalescent tree models in StarBeast2 (Ogilvie et al. 2017) differing in species assignments, some models splitting species to as many as nine while others lumping to

as few as five (Fig. S2). We assigned the species identity obtained from tasks 1 and 2 to each of the 796 sequences (Table S1–S3) and tested each scenario in turn. Species assignments were inferred from the literature and our own observations. *Galba truncatula*, *G. schirazensis*, and *G. humilis* were considered as different species in every scenario. However, *G. cousini* and *G. meridensis* were considered as a single species in half of the models and as two species in the other half since literature considers them as separate species (Bargues et al. 2011b) but we suspect they could be the same species since they both share the same distinct morphology and restricted distribution. *Galba cubensis*, *G. viator*, *G. neotropica*, and *Galba* sp. "Bosque del Apache" were considered as one, two, three, or four species depending the model since some studies considered them different species (Bargues et al. 2007) but previous genetic population studies (Lounnas et al. 2017) and the gene trees here constructed (task 1) suggest they could be synonymies. We also created an eleventh model in which we separated the populations of *G. viator* from Argentina and from Chile to test whether splitter models showed higher support than lumper models regardless of their biological meaning. In StarBeast2, not all the individuals but all the hypothetical species must be represented by at least one sequence of all genes. Thus why, we accordingly removed the sequences from Ethiopia because this hypothetical species was represented by two genes.

In all our multispecies coalescent tree models, we used the same site models as for reconstructing the gene trees. We assigned to the mitochondrial loci a gene ploidy of 0.5 and to nuclear loci a gene ploidy of 2.0 (diploid). We used constant population sizes (fixed to 1) and uncorrelated relaxed-clock models for all loci. For nuclear loci, we used the molecular clock rate reported by Coleman and Vacquier (2002) for ITS in bivalves (0.00255 per Myr). For mitochondrial loci, we retained the molecular clock rate

366 estimated by Wilke et al. (2009) for COI in invertebrates (0.0157 per Myr). We used the
367 birth-death model as tree prior with lognormal birth and death rates. We ran each model
368 using Multi-Threaded Nested Sampling analysis with 10 particles, 8 threads, a chain
369 length of 100,000,000 and sub-chain length of 5,000. Then, we compared the trees by
370 computing Bayes factor (BF), a model selection tool that is simple and well-suited for
371 comparing species-delimitation models (Leaché et al. 2014).
372 BF is the difference between the marginal likelihoods of two models: BF =
373 model 1 – model 2. If BF is larger than 1, model 1 is favored, and otherwise model 2 is
374 favored. When BF is between 20 and 150 the support is strong and when the BF is
375 above 150 the support is overwhelming (Leaché et al. 2014). We used Nested Sampling
376 implemented in the NS package to calculate the marginal likelihoods necessary to
377 obtain BF and also an estimate of the variance of the marginal likelihoods (Russel et al.
378 2019). This approach has proven to be an excellent tool for delimiting species (Leaché
379 et al. 2014; Matos-Maravi et al. 2018), assuming that tree reconstruction is of good
380 quality and that the best scenario to explain species delimitations is within the scenarios
381 tested (Leaché et al. 2014).
382 We also applied two other species-delimitation methods: Automatic Barcode
383 Gap Detection (ABGD; Puillandre et al. 2012) and Species Tree and Classification
384 Estimation in Beast2 (STACEY, Jones 2017). ABGD was run for each gene using the
385 default settings (https://bioinfo.mnhn.fr/abi/public/abgd/). ABGD does not rely on tree
386 shapes but on divergence and it requires an appropriate prior value of maximal
387 intraspecific divergence. This prior defines the threshold between intra- and
388 interspecific pairwise distances and is iterated from minimum to maximum through ten
389 steps (Puillandre et al. 2012). If this value is underestimated, the result is an oversplit

classification, and if overestimated, the classification is overlumped. Given the wide genetic diversity observed within some species (Lounnas et al. 2017), we chose the partition that showed high prior on intraspecific divergence (the penultimate partition).

The STACEY delimitation method, as the Nested Sampling analysis, also relies on a Multispecies Coalescent Model. However, STACEY does not require setting up predefined scenarios in terms of species number and sample assignment. The method distinguishes very shallow species divergences with a statistic called "collapseHeight," which we set to a small value (0.0001) following Jones (2017). Given the results obtained with ABGD and Nested Sampling analyses, we ran three multispecies coalescence models with different collapseWeight parameters: (1) a lumping model with six *Galba* species with 1/X distribution (initial value: 0.975, between 0 and 1), (2) a splitting model with nine species with 1/X distribution (initial value: 0.952 between 0 and 1), and (3) a no-prior-taxonomic model with a Beta distribution ($\alpha = 2$, $\beta = 2$). The initial value of the lumping and splitting models were calculated following Matos-Maravi et al. (2018). All other parameters were set as in the Nested Sampling analysis. We ran the three models for 250,000,000 generations with storing every 25,000 generations. The MCMC output was visualized using Tracer (Rambaut et al. 2018). For STACEY, we used only the ITS2 and COI sequences because these both genes were better represented in the dataset than ITS1 and 16S. Since the hypothetical number of species in STACEY ranges from one to the number of individuals, each of our 113 snail populations was considered as a minimal cluster.

To address our question (4), regarding species topology and divergence time, we reran the multispecies tree model that showed the highest Bayes Factor in StarBeast2. We used the same parameters as in the Nested Sampling analysis, but we ran the

MCMC for a longer time (250,000,000 generations stored every 25,000 generations). The MCMC output was visualized using Tracer (Rambaut et al. 2018) and tree samples summarized by TreeAnnotator (utility program distributed with the Beast package) using a 10% burn-in. The species tree was visualized and edited in FigTree, GIMP, and DensiTree (Bouckaert and Heled 2014). Some analyses were run in CIPRES Science Gateway (Miller et al. 2012).

Finally, to address our question (5), the ancestral phenotypic state, we applied Bayesian Binary MCMC (BBM, Ronquist and Huelsenbeck 2003), statistical dispersal-vicariance analysis (S-DIVA; Yu et al. 2010), and Statistical dispersal-extinction-cladogenesis (S-DEC; Ree and Smith 2008) in the software Reconstruct Ancestral State in Phylogenies (RASP, Yu et al. 2015). We used the splitting model (scenario A) under default settings. We added two phenotypic (shell) states: globose for *G. cousini* and *G. meridensis* and elongated for all other species.

3. RESULTS

3.1 Morphology

Most individuals (N = 1,420 from 133 sites) were not distinguishable based on shell and reproductive anatomy (Fig. S3). The exception was a single group comprising all individuals from *G. cousini* and *G. meridensis* (N = 302). These tended to demonstrate more globose shells with shorter spires, adult sizes in excess of 10 mm standard shell length. *Galba cousini* and *G. meridensis* also differed from the other species in their internal anatomy—a ureter with two distinct flexures, a wider and more ovate prostate, a larger penial complex, and a penis sheath approximately the same length as the preputium (Fig. S3). We did not find any anatomical differences between *G. cousini* and

G. meridensis, however, comparing individuals from Ecuador, Colombia, and Venezuela (Fig. 2).

3.2 Multiplex PCR of Microsatellite Loci

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

3.3 Identification by Sequencing and Phylogenetic Analysis

Phylogenetic analysis of COI sequences returned six clusters (Fig. S4). Clusters I–V each contained a single type population: *truncatula* (I), *schirazensis* (II), *humilis* (III), *cousini* (IV), and *meridensis* (V). Cluster VI contained the type populations of *cubensis*, *neotropica*, and *viator*. The posterior probabilities (PP) of all clusters were 1.0, except for cluster III (*humilis*, PP = 0.95).

Analysis of 16S, ITS1, and ITS2 sequences confirmed the COI results in almost all respects, although sequences were missing for at least one or two type populations in each tree (Figs. S5–S7). However, we detected discrepancies between the mitochondrial and nuclear gene trees. A striking example are sequences from Bosque del Apache (USA; cluster VI) that exhibited very long branches in the mitochondrial gene trees (Figs. S4–S5) but clustered tightly with the *cubensis* type population and similar populations in the nuclear gene trees (Figs. S6–S7). The mitochondrial sequences from Ethiopia (one per gene) also formed a long branch, although nuclear sequences were not

462 reported in GenBank for that population. Within cluster VI, we found that the *cubensis*
463 and *neotropica* type populations were located in separate subclades for the nuclear
464 genes but clustered together in the mitochondrial trees (Figs. S4–S7).
465 Gene trees (Figs. S4–S7) and haplotype networks (Figs. S8–S11) showed that
466 genetic diversity varied among the six clusters and four genes. Cluster II (*schirazensis*)
467 showed reduced variation, while cluster VI (*viator* / *cubensis* / *neotropica* / Bosque del
468 Apache sample) was larger and more diverse. Mitochondrial genes seemed more
469 diverse than nuclear genes. This observation may be biased, however, by a structural
470 correlation in our data between genes sequenced and regions sampled.
471 Most of the sequences uploaded to GenBank identified as one of the eight
472 species of *Galba* were accurately clustered into the six clades containing their type
473 populations. However, there were some exceptions. Eight sequences of COI uploaded
474 as *G. truncatula* from France appeared in cluster II with the *G. schirazensis* type
475 population (Table S3; Fig. S4). We reidentified these sequences as belonging to *G.*
476 *schirazensis*. The COI sequence from Ethiopia, uploaded as *G. truncatula*, clustered at
477 the base of *truncatula* clade I with low posterior probability (Fig. S4). The other
478 Ethiopian sequence (16S), also identified as *G. truncatula*, did not cluster with any of
479 the *Galba* clades (Fig. S5).
480 The topology of all gene trees showed that clusters III (*humilis*), IV (*cousini*),
481 and V (*meridensis*) grouped together. But inconsistent results among genes were
482 obtained for the remainder of the other identified clusters (Figs. S5–S8). The multilocus
483 multispecies tree returned three major groups: cluster I (*truncatula*) together with II
484 (*schirazensis*); cluster III (*humilis*) together with IV (*cousini*) and V (*meridensis*); and
485 the cluster VI group (*viator* / *cubensis* / *neotropica* / Bosque del Apache sample; Fig.

4). The multilocus multispecies tree visualized in DensiTree (Fig. 3) confirmed that most tree topologies united the clusters into the three major groups outlined above, although some topologies placed clusters differently reflecting the incongruence found among the gene trees.

3.4 Species Delimitation

Figure 3 illustrates the results obtained using the three species-delimitation methods. The Multi-Threaded Nested Sampling analysis (Fig. S12) suggested that scenario A (nine species) is the best fit to the available data, demonstrating the largest maximum likelihood estimate (Table S4). BF analysis preferred scenario A over scenario D (current taxonomy) or scenario K, separating populations of *G. viator* from Argentina and from Chile.

ABGD results varied depending on the gene analyzed (Fig. 3). Nine species (scenario A) were suggested by ITS1, while six species only were returned by our analysis of ITS2 and 16S, with *G. cubensis*, *G. viator*, *G. neotropica*, and *Galba* sp. "Bosque del Apache" lumped. ABGD analysis of the COI gene indicated that *G. viator* and *Galba* sp. "Bosque del Apache" are separate species, but that *G. cubensis* and *G. neotropica* should be lumped together. The ITS2 and COI analyses also suggested that some species (*G. cousini* and *G. truncatula*) might be represented by more than one taxon.

The species-delimitation analysis implemented in STACEY suggested that six of the nine clusters of scenario A might include more than one taxon. The exceptions were *G. viator*, *G. meridensis*, and *Galba* sp. "Bosque del Apache", the last two species

including only one population. Our STACEY results converged towards similar MCMCs regardless of which prior was used for the collapseWeight parameter (Fig. 3).

3.5 Time of Divergence and State Reconstruction

The estimated divergence time from the most recent common ancestor of the *Galba* group was 22.6 Mya [95% HPD interval: 14.6–33; Figs. 3–4]. Diversification within the species complex *G. cousini* and *G. meridensis* and the one formed by *G. cubensis*, *G. viator*, *G. neotropica*, and *Galba* sp. "Bosque del Apache" seems to have occurred 5 Mya, or less. The three analyses of phenotypic state reconstruction (S-DEC, S-DIVA, and BBM) suggested that the most recent common ancestor of all *Galba* species displayed the elongated-shell phenotype (Fig. 4). Thus, the globose-shell phenotype of *G. cousini* and *G. meridensis* should be considered as derived from the elongated one (Fig. 4).

4. DISCUSSION

4.1 *Galba* Comprises Six Species or Species Complexes

Here we report the largest study published to date of *Galba* systematics and distribution, based on extensive sampling at a very large geographical scale and integration of phenotypic and molecular approaches across all DNA sequences available in GenBank for four genes (Dayrat 2005). The widespread occurrence of self-fertilization in these populations essentially voids the biological species concept (Coyne and Orr 2004), and the absence of any reliable morphological distinction obviates the typological one. Thus, we are left with a phylogenetic approach, which suggests the existence of six clusters, perhaps corresponding to as many as nine species, or as few as six. These

findings reinforce several previously-published works that have involved fewer genes and smaller sample sizes (Correa et al. 2010, 2011; Bargues et al. 2011b, 2011a; Standley et al. 2013; Lounnas et al. 2017).

We suggest that five of our six clusters are best understood as one species each. The oldest taxonomic names for these are *Galba truncatula* (Müller 1774) for cluster I, *Galba schirazensis* (Küster 1862) for cluster II, and *Galba humilis* (Say 1822) for cluster III, *Galba cousini* (Jousseaume 1887) for cluster IV, and *Galba meridensis* (Bargues, Artigas, Khoubbane & Mas-Coma 2011) for cluster V. However, considering *Galba cousini* and *G. meridensis* as two different species is essentially based on the molecular divergence (estimated at 4.7 Mya; they always clustered together in our phylogenetic reconstructions), and should be considered with caution. The two species indeed inhabit northern regions of South America, and are not distinguishable based on shell and reproductive anatomy characters, despite the claims of Bargues et al. (2011b) given the known within-species variation for these characters. Moreover, *G. meridensis* has been sampled in a single locality, and more extensive sampling is clearly required to ascertain species status.

The sixth cluster includes the nomina *Galba viator* (d'Orbigny 1835), *Galba cubensis* (Pfeiffer 1839), *Galba neotropica* (Bargues, Artigas, Mera y Sierra, Pointier & Mas-Coma, 2007), and a population from southern USA (Bosque del Apache). However, any distinction between the sets of populations within this cluster depends on both the genes and phylogenetic methodology employed (Fig. 3). Most species delimitation methods suggest that this cluster comprises four species but others suggest two or even one species (Fig. 3). Discrepancies could be due to the genes analyzed. Note, for instance, that *G. cubensis* is not monophyletic when analyzing the COI and

ITS2 trees and haplotype networks which would be affecting the species delineation process. The distance separating *G. cubensis* and *G. neotropica* is limited (1 Mya). Moreover, microsatellite markers defined in *G. cubensis* amplified effectively in individuals of *G. neotropica* (Lounnas et al. 2017), suggesting a very short genetic distance. Additional sampling, especially in the North and South of the distribution range (Argentina and USA) and the analysis of other genes would help resolve the status of this cluster. On the whole, a cautious position would be to suggest that cluster VI corresponds to a species complex or a species with wide diversity, as has been found in other freshwater snails from the clade Hygrophila (e.g., Ebbs et al. 2018 in *Physa*; Mavárez et al. 2002 in *Biomphalaria*; Pfenninger et al. 2006 in *Radix*). We also note that if we ultimately recognize a single species; its name should be *viator*, and not *cubensis* or *neotropica*, based on prior description.

The *Galba* species tree that we constructed based on a multispecies coalescent model returned three groups: one group uniting *G. truncatula* and *G. schirazensis*, another uniting *G. humilis*, *G. cousini*, and *G. meridensis* and the last with *G. cubensis*, *G. viator*, *G. neotropica*, and *Galba* sp. "Bosque del Apache". This result partially agrees with some gene trees published in previous work (Correa et al. 2010, 2011; Bargues et al. 2011a). However, previous trees were based on single genes and smaller sample sizes. Our phylogenetic analysis also revealed some distinctive branches, including the mtDNA sequences from Ethiopia (Dayrat et al. 2011) and the samples from Bosque del Apache. These results may suggest undetected species, accelerated molecular evolution or mitochondrial introgression with non-sampled populations or species (Fourdrilis et al. 2016; Pinceel et al. 2005; Thomaz et al. 1996). More extensive

sampling and further phylogenetic analysis are required before reaching any conclusions.

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 S5 for species names). Johnson et al. (2013) transferred these species to the genus *Galba*, but otherwise retained the Burch system. Included in the present analysis were topotypic samples of *obrussa* from Philadelphia and *parva* from Cincinnati, both of which we here show to be indistinguishable from topotypic *humilis*, collected at Owego, New York. Remigio (2002) contributed to Genbank a 16S sequence from a Canadian population he identified as *Fossaria obrussa*, also grouping with cluster III (*humilis*). We suggest that *obrussa*, *parva*, and the seven other uniquely North American specific nomina listed above ascribed by Burch (1982) to the subgenus *Fossaria* are junior synonyms of *G. humilis*, setting aside American populations of *G. truncatula* as distinct. In addition to his *obrussa* sequence, Remigio (2002) contributed a 16S sequence from Oklahoma to Genbank which he labelled "*Fossaria bulimoides*". This sequence grouped with cluster VI in our analysis. We suggest that all 12 specific nomina ascribed by Burch (1982) to the *Fossaria* subgenus *Bakerilymnaea* (Table S5) including *bulimoides* (Lea 1841), are junior synonyms of the species or species complex of cluster VI (*viator* / *cubensis* / *neotropica* / Bosque del Apache sample).

Two of the three species-delimitation models here used (Nested Sampling analysis and STACEY) are based on Multispecies Coalescence, assuming gene flow within species though not between species (Sukumaran & Knowles 2017). Thus, the use of these models would be inappropriate in a fully selfing species group. As mentioned

above, the selfing rate in *Galba* species and populations is high (often ca. 0.9), but outcrossing does occur (Chapuis et al. 2007; Lounnas et al. 2017, 2018). Further research is needed to explore how selfing may affect speciation. *Galba* species would be excellent models to investigate such questions, since selfing rates vary so greatly among species and populations (Chapuis et al. 2007; Lounnas et al. 2017, 2018).

4.2 A Set of Cryptic Species

Our study has confirmed the previous reports of Samadi et al. (2000) and Correa et al. (2011) using both classical approach and geometrical morphometry that most species of *Galba* cannot be distinguished on the basis of shell morphology or internal anatomy. Trait variability within species seems to be greater than variance among species, likely attributable to phenotypic plasticity (Correa et al. 2011). Reproductive and growth traits in *G. truncatula* have been shown to vary according to habitat characteristics at small geographical scales (Chapuis et al. 2007), suggesting both that life-history traits are phenotypically plastic, and that to rely on such traits for specific identification is not advisable.

Our study also confirms that *G. cousini* and *G. meridensis* differ strikingly in adult size, shell shape, and anatomy from all other *Galba* species. Our molecular results suggest that their shell phenotype evolved from the phenotype exhibited by all other *Galba* species. Interestingly, *G. cousini* and *G. meridensis* are the largest species within the genus *Galba*, occurring in a specialized habitat and displaying a complex reproductive anatomy that resembles the anatomy of known outcrossing species rather than the simplified ones observed in selfing species (see Jarne et al. 2010; Escobar et al. 2011).

Among the freshwater pulmonates, cryptic species have previously been documented in *Ancylus* (Weiss et al. 2018) and *Radix* (Pfenninger et al. 2006). Our methods here were strictly qualitative, as was the case for *Ancylus* and *Radix*, because previous studies have shown that the dimensions of internal organs depend on physiological state and mating system and that shell shape and size depend on the environment (Samadi et al. 2000; Correa et al. 2011; see Bourdeau et al 2015 for review in gastropods). Hence species cannot be distinguished by means of such measurements. We were therefore not able to include quantitative traits to differentiate species in our reconstruction of the ancestral phenotypic state. Future, more comprehensive phylogenetic approaches should combine discrete anatomical traits, environmental variables, and mating systems to elucidate the origin and maintenance of *Galba* species. Such an approach might provide insights into how evolutionary biologists should describe and evaluate morphological and environmental diversity in cryptic species complexes.

Four hypotheses have been offered to explain the occurrence of cryptic species: recent divergence, parallelism, convergence, and stasis (Bickford et al. 2007; Fišer et al. 2018; Struck et al. 2018). The recent divergence hypothesis seems unlikely in this case. *Galba* has no closely related groups; its closest relatives are probably the stagnicoline lymnaeids of North America and Eurasia, which demonstrate a very distinctive morphology (Aksenova et al. 2018). Our analyses suggest that the several species of *Galba* are separated by more than 20 Myr (Burgarella et al. 2015). And indeed, the morphological divergence demonstrated by *G. cousini* and *G. meridensis* suggests that time has not been a significant constraint. The parallelism hypothesis also seems unlikely given that, based on our phylogenetic reconstruction, the cryptic morphology is

652 ancestral for *Galba*, and the only other morphology that has evolved in the group, as
653 demonstrated by *G. cousini* and *G. meridensis,* is derived. Nor does the topology of
654 lymnaeid phylogeny fit the convergence hypothesis (Correa et al. 2010). So, by default,
655 morphological stasis is left as the most likely hypothesis to explain the presence of
656 cryptic species in the genus *Galba*, as has been proposed in other gastropod groups
657 (e.g., Gomez et al. 2004; Struck et al. 2018).

658 The challenge of identifying cryptic *Galba* species is aggravated by their wide
659 and poorly-known geographical distributions, recently scrambled by biological
660 invasion. For example, *G. schirazensis* and *G. truncatula* have broadly expanded their
661 distribution over recent decades (Brown 1994; Bargues et al. 2001, 2011a; Vinarski and
662 Kantor 2016; Lounnas et al. 2018). We have documented up to three *Galba* species
663 occurring in some South American sites (Table S1). Their identification is not possible
664 without molecular tools.

665 The specific identity of *Galba* populations is important because they are
666 involved in the transmission of fasciolosis caused by the liver fluke *F. hepatica*. Some
667 studies have shown that lymnaeid species demonstrate different patterns of
668 susceptibility, host-parasite compatibility and immunological resistance to *F. hepatica*
669 (Gutiérrez et al. 2003; Vázquez et al. 2014; Dreyfuss et al. 2015). Although all species
670 can be infected under laboratory conditions (Vázquez et al. 2018), field transmission
671 depends on ecological and sociological conditions. Cattle or wildlife do not occupy the
672 same grazing habitats as infecting snails in many parts of the world (Sabourin et al.
673 2018). Ecological studies should be performed to evaluate whether the several cryptic
674 *Galba* species differ with regard to habitat preference, since our current knowledge is
675 essentially limited to *G. truncatula* (Chapuis et al. 2007).

4.3 Conclusions and Future Directions

Lymnaeid populations of the genus *Galba* are of interest for addressing a variety of questions, including wide-scale biogeography, biological invasions, evolution of mating systems, and host-parasite interactions. Our work is a first attempt to clarify the phylogeny, systematics, and biogeographical distribution of this interesting group in the New World. We have constructed a variety of gene trees using classical approaches, as well as a species tree based on a multispecies coalescent model that reconciles gene trees and provides a much better estimation accuracy for species tree topology than, for instance, concatenation (Heled and Drummond 2010). The inferred phylogenetic relationships among species varied, depending on the genes analyzed and techniques employed. Incomplete lineage sorting or introgressive hybridization of specific genes may indeed lead to such a result (Felsenstein 2004). Future studies could investigate which evolutionary processes (gene duplication, horizontal gene transfer, incomplete lineage sorting, hybridization) gave rise to the incongruence we have observed in gene and species trees. Although our study was conducted at an extremely large geographic scale, especially in America, *Galba* populations occur on almost all continents. Much more extensive sampling and molecular analysis will be required to get a worldwide picture of the phylogeny and distribution of the genus.

DATA AND CODE ACCESSIBILITY

Xml files for phylogenetic analyses are available from the Zenodo repository (https://zenodo.org/record/3473937#.XZiPcC0ryTd).

CRediT Author Statement

Pilar Alda: Conceptualization, Methodology, Investigation, Formal analysis, Writing - Original Draft, Reviewing and Editing **Manon Lounnas:** Investigation, Writing - Reviewing and Editing **Antonio A. Vázquez:** Investigation, Writing - Reviewing and Editing **Rolando Ayaqui:** Resources **Manuel Calvopiña:** Resources **Maritza Celi-Erazo:** Resources **Robert T. Dillon Jr.:** Resources, Writing - Reviewing and Editing **Luisa Carolina González Ramírez:** Resources **Eric S. Loker:** Resources **Jenny Muzzio-Aroca:** Resources **Alberto Orlando Nárvaez:** Resources **Oscar Noya:** Resources, Writing - Reviewing and Editing **Andrés Esteban Pereira:** Resources **Luiggi Martini Robles:** Resources **Richar Rodríguez-Hidalgo:** Resources **Nelson Uribe:** Resources **Patrice David:** Resources, Writing - Reviewing and Editing **Philippe Jarne:** Conceptualization, Resources, Writing - Reviewing and Editing **Jean-Pierre Pointier:** Resources, Writing - Reviewing and Editing **Sylvie Hurtrez-Boussès:** Conceptualization, Writing - Reviewing and Editing.

Acknowledgments

We would like to express our gratitude to Nicolás Bonel for useful comments on earlier drafts of the manuscript and Harry G. Lee for advice and assistance on the taxonomy. We thank Jimena Guerrero, Björn Stelbrink and Thomas Wilke for suggestions on phylogenetic analyses and Graham R. Jones, Patricio Maturana Russel and Remco R. Bouckaert for assistance in running STACEY and Multi-Threaded Nested Sampling. We thank the reviewers Pável Matos-Maraví and Christelle Fraïsse and the recommender from PCI in Evolutionary Biology Fabien Condamine for their thoughtful comments and suggestions. Fellowships granted by Erasmus Mundus PRECIOSA and

724 Méditerranée Infection supported research stays of PA at the Institute de Recherche pour
725 le Développement, MIVEGEC (Montpellier, France). AV was supported by a grant
726 from IRD (BEST) and ML by a doctoral fellowship from University of Montpellier and
727 a post-doctoral grant from Labex CeMeb. This study was financially supported by IRD,
728 CNRS, ECOS-SUD (A16B02) and Malacological Society of London. Version 3 of this
729 preprint has been peer-reviewed and recommended by Peer Community In Evolutionary
730 Biology (https://doi.org/10.24072/pci.evolbiol.100089).

731

732 **CONFLICT OF INTEREST DISCLOSURE**

733 The authors of this preprint declare that they have no financial conflict of interest with
734 the content of this article.

735

REFERENCES

Aksenova O.V., Bolotov I.N., Gofarov M.Y., Kondakov A.V., Vinarski M.V., Bespalaya Y.V., Kolosova Y.S., Palatov D.M., Sokolova S.E., Spitsyn V.M., Tomilova A.A., Travina O.V., Vikhrev I.V.. 2018. Species richness, molecular taxonomy and biogeography of the Radicine pond snails (Gastropoda: Lymnaeidae) in the Old World. Scientific Reports. 8: 11199.

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

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

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

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

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

Bargues M.D., Artigas P., Khoubbane M., Flores R., Glöer P., Rojas-García R., Ashrafi

760 K., Falkner G., Mas-Coma S. 2011a. *Lymnaea schirazensis*, an overlooked snail
761 distorting fascioliasis data: genotype, phenotype, ecology, worldwide spread,
762 susceptibility, applicability. PLoS ONE. 6:e24567.
763 Bargues M.D., Artigas P., Khoubbane M., Mas-Coma S. 2011b. DNA sequence
764 characterisation and phylogeography of *Lymnaea cousini* and related species, vectors of
765 fascioliasis in northern Andean countries, with description of *L. meridensis* n. sp.
766 (Gastropoda: Lymnaeidae). Parasites & Vectors. 4:132.
767 Bargues M.D., Artigas P., Mera y Sierra R., Pointier J.-P., Mas-Coma S. 2007.
768 Characterisation of *Lymnaea cubensis*, *L. viatrix* and *L. neotropica* n. sp., the main
769 vectors of *Fasciola hepatica* in Latin America, by analysis of their ribosomal and
770 mitochondrial DNA. Annals of Tropical Medicine & Parasitology. 101:621–641.
771 Bargues M.D., González C.L., Artigas P., Mas-Coma S. 2011c. A new baseline for
772 fascioliasis in Venezuela: lymnaeid vectors ascertained by DNA sequencing and
773 analysis of their relationships with human and animal infection. Parasites & Vectors.
774 4:1–18.
775 Bargues M.D., Vigo M., Horak P., Dvorak J., Patzner R.A., Pointier J.P., Jackiewicz
776 M., Meier-Brook C., Mas-Coma S. 2001. European Lymnaeidae (Mollusca:
777 Gastropoda), intermediate hosts of trematodiases, based on nuclear ribosomal DNA
778 ITS-2 sequences. Infection, Genetics and Evolution. 1:85–107.
779 Bespalaya Y.V., Bolotov I.N., Aksenova O.V., Gofarov M.Yu., Kondakov A.V.,
780 Vikhrev I.V., Vinarski M.V. 2018. DNA barcoding reveals invasion of two cryptic
781 *Sinanodonta* mussel species (Bivalvia: Unionidae) into the largest Siberian river.
782 Limnologica. 69:94–102.
783 Bickford D., Lohman D.J., Sodhi N.S., Ng P.K.L., Meier R., Winker K., Ingram K.K.,

784 Das I. 2007. Cryptic species as a window on diversity and conservation. Trends in
785 Ecology & Evolution. 22:148–155.
786 Bouckaert R., Heled J. 2014. DensiTree 2: Seeing trees through the forest.
787 https://doi.org/10.1101/012401.
788 Bouckaert R., Heled J., Kühnert D., Vaughan T., Wu C.-H., Xie D., Suchard M.A.,
789 Rambaut A., Drummond A.J. 2014. BEAST 2: A software platform for Bayesian
790 evolutionary analysis. PLoS Computational Biology. 10:e1003537.
791 Bouckaert R.R., Drummond A.J. 2017. bModelTest: Bayesian phylogenetic site model
792 averaging and model comparison. BMC Evolutionary Biology. 17.
793 Bourdeau P.E., Butlin R.K., Brönmark C., Edgell T.C., Hoverman J.T., Hollander J.
794 2015. What can aquatic gastropods tell us about phenotypic plasticity? A review and
795 meta-analysis. Heredity. 115:312–321.
796 Brown D.S. 1994. Freshwater snails of Africa and their medical importance. London:
797 Taylor & Francis.
798 Burch J.B. 1982. North American freshwater snails. Transactions of the POETS
799 Society. 1:217–365.
800 Burgarella C., Gayral P., Ballenghien M., Bernard A., David P., Jarne P., Correa A.,
801 Hurtrez-Boussès S., Escobar J., Galtier N., Glémin S. 2015. Molecular evolution of
802 freshwater snails with contrasting mating systems. Molecular Biology and Evolution.
803 32:2403–2416.
804 Castresana J. 2000. Selection of conserved blocks from multiple alignments for their
805 use in phylogenetic analysis. Molecular Biology and Evolution. 17:540–552.
806 Chapuis E., Trouve S., Facon B., Degen L., Goudet J. 2007. High quantitative and no
807 molecular differentiation of a freshwater snail (*Galba truncatula*) between temporary

808 and permanent water habitats. Molecular Ecology. 16:3484–3496.
809 Coleman A.W., Vacquier V.D. 2002. Exploring the phylogenetic utility of ITS
810 sequences for animals: a test case for *Abalone* (*Haliotis*). Journal of Molecular
811 Evolution. 54:246–257.
812 Correa A.C., Escobar J.S., Durand P., Renaud F., David P., Jarne P., Pointier J.-P.,
813 Hurtrez-Boussès S. 2010. Bridging gaps in the molecular phylogeny of the Lymnaeidae
814 (Gastropoda: Pulmonata), vectors of Fascioliasis. BMC Evolutionary Biology. 10:381.
815 Correa A.C., Escobar J.S., Noya O., Velásquez L.E., González-Ramírez C., Hurtrez-
816 Boussès S., Pointier J.-P. 2011. Morphological and molecular characterization of
817 Neotropic Lymnaeidae (Gastropoda: Lymnaeoidea), vectors of fasciolosis. Infection,
818 Genetics and Evolution. 11:1978–1988.
819 Coyne J.A., Orr A.H. 2004. Speciation. Sunderland, Massachusetts: Sinauer Associates,
820 Inc. Publ.
821 Cutter, A.D. 2019. Reproductive transitions in plants and animals: selfing syndrome,
822 sexual selection and speciation. New Phytologist. 224(3):1080–1094.
823 Dayrat B. 2005. Towards integrative taxonomy: integrative taxonomy. Biological
824 Journal of the Linnean Society. 85:407–415.
825 Dayrat B., Conrad M., Balayan S., White T.R., Albrecht C., Golding R., Gomes S.R.,
826 Harasewych M.G., de Frias Martins A.M. 2011. Phylogenetic relationships and
827 evolution of pulmonate gastropods (Mollusca): New insights from increased taxon
828 sampling. Molecular Phylogenetics and Evolution. 59:425–437.
829 De Queiroz K. 2007. Species concepts and species delimitation. Systematic Biology.
830 56:879–886.
831 Dillon R.T., Wethington A.R., Lydeard C. 2011. The evolution of reproductive isolation

in a simultaneous hermaphrodite, the freshwater snail *Physa*. BMC Evolutionary Biology. 11.

D'Orbigny A. 1835. Synopsis terrestrium et fluviatilium molluscorum, in suo per Americam meridionalem itinere, ab A. D'Orbigny, collectorum. Mag de Zoologie. 5:1–44.

Dreyfuss G., Correa A.C., Djuikwo-Teukeng F.F., Novobilský A., Höglund J., Pankrác J., Kašný M., Vignoles P., Hurtrez-Boussès S., Pointier J.P., Rondelaud D. 2015. Differences in the compatibility of infection between the liver flukes *Fascioloides magna* and *Fasciola hepatica* in a Colombian population of the snail *Galba* sp. Journal of Helminthology. 89:720–726.

Dunn A.M., Hatcher M.J. 2015. Parasites and biological invasions: parallels, interactions, and control. Trends in Parasitology. 31:189–199.

Ebbs E.T., Loker E.S., Brant S.V. 2018. Phylogeography and genetics of the globally invasive snail *Physa acuta* Draparnaud 1805, and its potential to serve as an intermediate host to larval digenetic trematodes. BMC Evolutionary Biology. 18.

Escobar J.S., Auld J.R., Correa A.C., Alonso J.M., Bony Y.K., Coutellec M.-A., Koene J.M., Pointier J.-P., Jarne P., David P. 2011. Patterns of mating-system evolution in hermaphroditic animals: correlations among selfing rate, inbreeding depression, and the timing of reproduction: mating-system evolution in hermaphroditic animals. Evolution. 65:1233–1253.

Estoup A., Martin O. 1996. Marqueurs microsatellites: isolement à l'aide de sondes non-radioactives, caractérisation et mise au point. .

Fang Y.-W., Liu L.-Y., Zhang H.-L., Jiang D.-F., Chu D. 2014. Competitive ability and fitness differences between two introduced populations of the invasive whitefly *Bemisia*

856 *tabaci* Q in China. PLoS ONE. 9:e100423.
857 Felsenstein J. 2004. Inferring phylogenies. Sunderland, Mass: Sinauer Associates.
858 Fišer C., Robinson C.T., Malard F. 2018. Cryptic species as a window into the
859 paradigm shift of the species concept. Molecular Ecology. 27:613–635.
860 Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for
861 amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan
862 invertebrates. Molecular marine biology and biotechnology. 3:294–299.
863 Fourdrilis S., Mardulyn P., Hardy O.J., Jordaens K., de Frias Martins A.M., Backeljau
864 T. 2016. Mitochondrial DNA hyperdiversity and its potential causes in the marine
865 periwinkle *Melarhaphe neritoides* (Mollusca: Gastropoda). PeerJ. 4:e2549.
866 Gomez S., Fleeger J.W., Rocha-Olivares A., Foltz D. 2004. Four new species of
867 *Cletocamptus* Schmankewitsch, 1875, closely related to *Cletocamptus deitersi* (Richard,
868 1897) (Copepoda: Harpacticoida). Journal of Natural History. 38:2669–2732.
869 Gutiérrez A., Pointier J.-P., Fraga J., Jobet E., Modat S., Pérez R.T., Yong M., Sanchez
870 J., Loker E.S., Théron A. 2003. *Fasciola hepatica*: identification of molecular markers
871 for resistant and susceptible *Pseudosuccinea columella* snail hosts. Experimental
872 Parasitology. 105:211–218.
873 Heled J., Drummond A.J. 2010. Bayesian inference of species trees from multilocus
874 data. Molecular Biology and Evolution. 27:570–580.
875 Hubendick B. 1951. Recent Lymnaeidae, their variation, morphology, taxonomy,
876 nomenclature and distribution. Kungl Svenska Vetenskapsakademiens Handlingar. 3:1–
877 223.
878 Janzen D.H., Burns J.M., Cong Q., Hallwachs W., Dapkey T., Manjunath R.,
879 Hajibabaei M., Hebert P.D.N., Grishin N.V. 2017. Nuclear genomes distinguish cryptic

880 species. Proceedings of the National Academy of Sciences. 114 (31) 8313-8318.
881 Jarić I., Heger T., Castro Monzon F., Jeschke J.M., Kowarik I., McConkey K.R., Pyšek
882 P., Sagouis A., Essl F. 2019. Crypticity in biological invasions. Trends in Ecology &
883 Evolution.
884 Jarne P., Pointier J.-P., David P., Koene J.M. 2010. Basommatophoran gastropods. The
885 evolution of primary sexual characters in animals. Oxford University Press. p. 173–196.
886 Johnson P.D., Bogan A.E., Brown K.M., Burkhead N.M., Cordeiro J.R., Garner J.T.,
887 Hartfield P.D., Lepitzki D.A.W., Mackie G.L., Pip E., Tarpley T.A., Tiemann J.S.,
888 Whelan N.V., Strong E.E. 2013. Conservation status of freshwater gastropods of
889 Canada and the United States. Fisheries. 38:247–282.
890 Jones G. 2017. Algorithmic improvements to species delimitation and phylogeny
891 estimation under the multispecies coalescent. J. Math. Biol. 74:447–467.
892 Katoh K., Standley D.M. 2013. MAFFT multiple sequence alignment software version
893 7: improvements in performance and usability. Molecular Biology and Evolution.
894 30:772–780.
895 Katz A.D., Taylor S.J., Davis M.A. 2018. At the confluence of vicariance and dispersal:
896 Phylogeography of cavernicolous springtails (Collembola: Arrhopalitidae,
897 Tomoceridae) codistributed across a geologically complex karst landscape in Illinois
898 and Missouri. Ecology and Evolution. 8:10306–10325.
899 Kolar C.S., Lodge D.M. 2001. Progress in invasion biology: predicting invaders. Trends
900 in Ecology & Evolution. 16:199–204.
901 Leaché A.D., Fujita M.K., Minin V.N., Bouckaert R.R. 2014. Species delimitation
902 using genome-wide SNP data. Systematic Biology. 63:534–542.
903 Leigh J.W., Bryant D. 2015. PopART: full-feature software for haplotype network

904 construction. Methods Ecol Evol. 6:1110–1116.
905 Lounnas M., Correa A.C., Alda P., David P., Dubois M.-P., Calvopiña M., Caron Y.,
906 Celi-Erazo M., Dung B.T., Jarne P., Loker E.S., Noya O., Rodríguez-Hidalgo R., Toty
907 C., Uribe N., Pointier J.-P., Hurtrez-Boussès S. 2018. Population structure and genetic
908 diversity in the invasive freshwater snail *Galba schirazensis* (Lymnaeidae). Canadian
909 Journal of Zoology. 96:425–435.
910 Lounnas M., Vázquez A.A., Alda P., Sartori K., Pointier J.-P., David P., Hurtrez-
911 Boussès S. 2017. Isolation, characterization and population-genetic analysis of
912 microsatellite loci in the freshwater snail *Galba cubensis* (Lymnaeidae). Journal of
913 Molluscan Studies. 83:63–68.
914 Mas-Coma S., Bargues M.D., Valero M. a. 2005. Fascioliasis and other plant-borne
915 trematode zoonoses. International Journal for Parasitology. 35:1255–1278.
916 Matos-Maravi P., Wahlberg N., Antonelli A., Penz C. 2019. Species limits in butterflies
917 (Lepidoptera: Nymphalidae): Reconciling classical taxonomy with the multispecies
918 coalescent. Systematic Entomology, 44:745–756.
919 Mavárez J., Pointier J.-P., David P., Delay B., Jarne P. 2002. Genetic differentiation,
920 dispersal and mating system in the schistosome-transmitting freshwater snail
921 Biomphalaria glabrata. Heredity. 89:258–265.
922 Meunier C., Hurtrez-Bousses S., Durand P., Rondelaud D., Renaud F. 2004. Small
923 effective population sizes in a widespread selfing species, *Lymnaea truncatula*
924 (Gastropoda: Pulmonata). Molecular Ecology. 13:2535–2543.
925 Miller M.A., Pfeiffer W., Schwartz T. 2012. The CIPRES science gateway: enabling
926 high-impact science for phylogenetics researchers with limited resources. :1.
927 Niemiller M.L., Near T.J., Fitzpatrick B.M. 2012. Delimiting species using multilocus

928 data: diagnosing cryptic diversity in the southern cavefish, *Typhlichthys subterraneus*
929 (Teleostei: Amblyopsidae): species delimitation in cavefish. Evolution. 66:846–866.
930 Ogilvie H.A., Bouckaert R.R., Drummond A.J. 2017. StarBEAST2 brings faster species
931 tree inference and accurate estimates of substitution rates. Molecular Biology and
932 Evolution. 34:2101–2114.
933 Orlando Narváez A., Aroca J.M., Alda P., Macías V., Lounnas M., Hurtrez-Boussès S.,
934 Noya O., Martini Robles L., Pointier J.-P. 2017. Primer reporte de *Galba cubensis*
935 (Gastropoda: Lymnaeidae) en el Ecuador, hospedador potencial de *Fasciola hepatica* en
936 arrozales de la costa ecuatoriana. El Misionero del Agro. 13:36–47.
937 Paraense W.L. 1995. *Lymnaea cousini* Jousseaume, 1887, from Ecuador (Gastropoda:
938 Lymnaeidae). Memórias do Instituto Oswaldo Cruz. 90:605–609.
939 Pfeiffer L. 1839. Bericht über die Ergebnisse maine Reise nach Kuba im Winter 1838-
940 1839. Archiv für Naturgeschichte. 5:346–358.
941 Pfenninger M., Cordellier M., Streit B. 2006. Comparing the efficacy of morphologic
942 and DNA-based taxonomy in the freshwater gastropod genus *Radix* (Basommatophora,
943 Pulmonata). BMC Evolutionary Biology. 14.
944 Pfenninger M., Schwenk K. 2007. Cryptic animal species are homogeneously
945 distributed among taxa and biogeographical regions. BMC Evolutionary Biology.
946 7:121.
947 Pinceel J., Jordaens K., Backeljau T. 2005. Extreme mtDNA divergences in a terrestrial
948 slug (Gastropoda, Pulmonata, Arionidae): accelerated evolution, allopatric divergence
949 and secondary contact: Molecular divergence in a terrestrial slug. Journal of
950 Evolutionary Biology. 18:1264–1280.
951 Pointier J., Noya O., Amarista M., Théron A. 2004. *Lymnaea cousini* Jousseaume, 1887

952 (Gastropoda: Lymnaeidae): first record for Venezuela. Memórias do Instituto Oswaldo
953 Cruz. 99:567–569.
954 Pointier J.-P. 2015. Freshwater molluscs of Venezuela and their medical and veterinary
955 importance. Harxheim: ConchBooks.
956 Puillandre N., Lambert A., Brouillet S., Achaz G. 2012. ABGD, Automatic Barcode
957 Gap Discovery for primary species delimitation: ABGD, automatic barcode gap
958 discovery. Molecular Ecology. 21:1864–1877.
959 Qian Z., Yang J., Lu Y., He J. 2012. Description of three freshwater species
960 (Gastropoda) from China. Shell Discoveries. 1:30–31.
961 Rama Rao S., Liew T.-S., Yow Y.-Y., Ratnayeke S. 2018. Cryptic diversity: Two
962 morphologically similar species of invasive apple snail in Peninsular Malaysia. PLOS
963 ONE. 13:e0196582.
964 Rambaut A., Drummond A.J., Xie D., Baele G., Suchard M.A. 2018. Posterior
965 summarization in bayesian phylogenetics using Tracer 1.7. Systematic Biology.
966 Ree R.H., Smith S.A. 2008. Maximum likelihood inference of geographic range
967 evolution by dispersal, local extinction, and cladogene. Systematic Biology. 57:4–14.
968 Remigio E. 2002. Molecular phylogenetic relationships in the aquatic snail genus
969 *Lymnaea*, the intermediate host of the causative agent of fascioliasis: insights from
970 broader taxon sampling. Parasitology Research. 88:687–696.
971 Remigio E.A., Blair D. 1997. Molecular systematics of the freshwater snail family
972 Lymnaeidae (Pulmonata: Basommatophora) utilising mitochondrial ribosomal DNA
973 sequences. J Mollus Stud. 63:173–185.
974 Ronquist F., Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under
975 mixed models. Bioinformatics. 19:1572–1574.

976 Russel P.M., Brewer B.J., Klaere S., Bouckaert R.R. 2019. Model selection and
977 parameter inference in phylogenetics using nested sampling. Systematic Biology.
978 68:219–233.
979 Sabourin E., Alda P., Vázquez A., Hurtrez-Boussès S., Vittecoq M. 2018. Impact of
980 human activities on fasciolosis transmission. Trends in Parasitology. 34:891–903.
981 Samadi S., Roumégoux A., Bargues M.D., Mas-Coma S., Yong M., Pointier J.-P. 2000.
982 Morphological studies of Lymnaeid snails from the human fasciolosis endemic zone of
983 Bolivia. Journal Molluscan Studies. 66:31–44.
984 Standley C.J., Prepelitchi L., Pietrokovsky S.M., Issia L., Stothard J., Wisnivesky-Colli
985 C. 2013. Molecular characterization of cryptic and sympatric lymnaeid species from the
986 *Galba*/*Fossaria* group in Mendoza Province, Northern Patagonia, Argentina. Parasites
987 & Vectors. 6:304.
988 Stevenson J., Norris D. 2016. Implicating cryptic and novel anophelines as malaria
989 vectors in Africa. Insects. 8:1.
990 Struck T.H., Feder J.L., Bendiksby M., Birkeland S., Cerca J., Gusarov V.I., Kistenich
991 S., Larsson K.-H., Liow L.H., Nowak M.D., Stedje B., Bachmann L., Dimitrov D. 2018.
992 Finding evolutionary processes hidden in cryptic species. Trends in Ecology &
993 Evolution. 33:153–163.
994 Sukumaran J. & Knowles L.L. 2017. Multispecies coalescent delimits structure, not
995 species. PNAS 114:16073–1612.
996 Taylor D.W. 2003. Introduction to Physidae (Gastropoda: Hygrophila); biogeography,
997 classification, morphology. Revista de Biología Tropical. 51:1–287.
998 Thomaz D., Guiller A., Clarke B. 1996. Extreme divergence of mitochondrial DNA
999 within species of pulmonate land snails. Proceedings of the Royal Society of London.

1000 Series B: Biological Sciences. 263:363–368.
1001 Vázquez A.A., Alda P., Lounnas M., Sabourin E., Alba A., Pointier J.-P., Hurtrez-
1002 Boussès S. 2018. Lymnaeid snails hosts of *Fasciola hepatica* and *Fasciola gigantica*
1003 (Trematoda: Digenea): a worldwide review. CAB Reviews. 13:1–15.
1004 Vázquez A.A., Sánchez J., Pointier J.-P., Théron A., Hurtrez-Boussès S. 2014. *Fasciola*
1005 *hepatica* in Cuba: compatibility of different isolates with two intermediate snail hosts,
1006 *Galba cubensis* and *Pseudosuccinea columella*. Journal of Helminthology. 88:434–440.
1007 Vinarski M.V., Kantor J.I. 2016. Analytical catalogue of fresh and brackish water
1008 molluscs of Russia and adjacent countries. Moscow: A.N. Severtsov Institute of
1009 Ecology and Evolution of RAS.
1010 Weigand A.M., Jochum A., Pfenninger M., Steinke D., Klussmann-Kolb A. 2011. A
1011 new approach to an old conundrum-DNA barcoding sheds new light on phenotypic
1012 plasticity and morphological stasis in microsnails (Gastropoda, Pulmonata,
1013 Carychiidae): DNA barcoding. Molecular Ecology Resources. 11:255–265.
1014 Weiss M., Weigand H., Weigand A.M., Leese F. 2018. Genome-wide single-nucleotide
1015 polymorphism data reveal cryptic species within cryptic freshwater snail species-The
1016 case of the *Ancylus fluviatilis* species complex. Ecology and Evolution. 8:1063–1072.
1017 Wilke T., Schultheiß R., Albrecht C. 2009. As time goes by: a simple fool's guide to
1018 molecular clock approaches in invertebrates. American Malacological Bulletin. 27:25–
1019 45.
1020 Xia X. 2017. DAMBE6: new tools for microbial genomics, phylogenetics, and
1021 molecular evolution. Journal of Heredity. 108:431–437.
1022 Yu Y., Harris A.J., Blair C., He X. 2015. RASP (Reconstruct Ancestral State in
1023 Phylogenies): A tool for historical biogeography. Molecular Phylogenetics and

1024 Evolution. 87:46–49.

1025 Yu Y., Harris A.J., He X. 2010. S-DIVA (Statistical Dispersal-Vicariance Analysis): A

1026 tool for inferring biogeographic histories. Molecular Phylogenetics and Evolution.

1027 56:848–850.

1028 Zulliger D.E., Lessios H.A. 2010. Phylogenetic relationships in the genus *Astropecten*

1029 Gray (Paxillosida: Astropectinidae) on a global scale: molecular evidence for

1030 morphological convergence, species-complexes and possible cryptic speciation.

1031 Zootaxa.:1–19.