

Figure Captions

Figure 1. The three-step procedure followed to identify the 1,722 individuals of *Galba* species. The number of individuals identified at each step is indicated in the left and the species identified are indicated on the right. In Step 1, we photographed the shell and dissected three to five adult snails from each of the 166 sites. Fragments of the ITS2 and COI genes were sequenced in 146 individuals: *Galba cousini/meridensis* (1), *Galba cubensis* (41), *Galba schirazensis* (41), *Galba truncatula* (30), *Galba humilis* (34) and *Galba viator* (1).

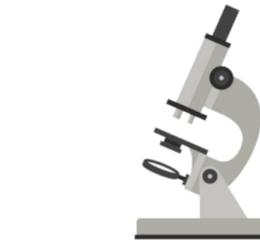
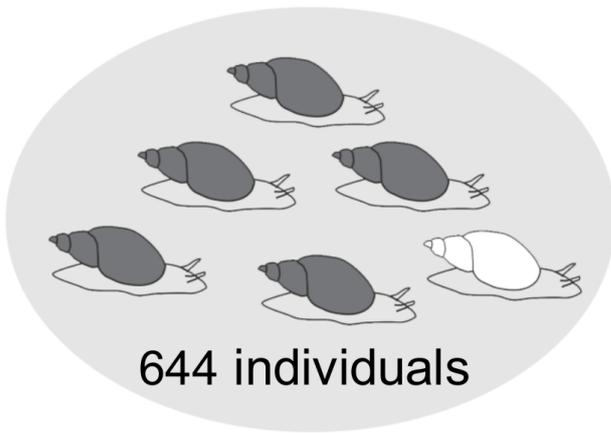
Figure 2. Geographic distribution of *Galba* species in America, based on molecular identification. Coordinates are given in Tables S1–S2. The sites Perdriel (Argentina), Batallas and Tambillo (Bolivia), Ontario (Canada), San Rafael (Mexico), Canal Salinas (Puerto Rico) are not represented since coordinates are missing in the original publications.

Figure 3. Time-calibrated phylogenetic hypotheses of the model that best approximate species status in *Galba* and species-delimitation methods. The trees showed are the most probable topologies based on the multispecies tree model that showed the highest Bayes Factor in StarBeast2 (visualized in Densitree), as a function of time (in Mya). Greater topological agreement is visualized by a higher density of trees (on the left), whereas uncertainty in the height and distribution of nodes are represented by increased transparency. The most common topologies are shown in blue, the second most common topologies in red and the third in green. The six clusters built with individual gene trees are indicated on the extreme right. The number of species recovered varied with eight approaches (ABGD with each gene, StarBeast with all genes, STACEY with a variable number of species included as prior). For each approach, the colored bars represent different species. Bars were striped when the groups included more than one species.

Clusters as appeared in text are shown in roman numbers. Scale bar represents branch length expressed as number of substitutions per site.

Figure 4. Most common topology of the species tree and phenotype of the most recent common ancestor of *Galba* inferred in StarBeast2. The probability of a cryptic phenotype in the most recent common ancestor of *Galba* species is 100% according to S -DEC and BBM and 78% according to S-DIVA. Clusters as appeared in text are shown in roman numbers. Node values indicate posterior probability and blue bars indicate 95% credibility intervals. The mating system is indicated on the right.

Step 1: Morphological study

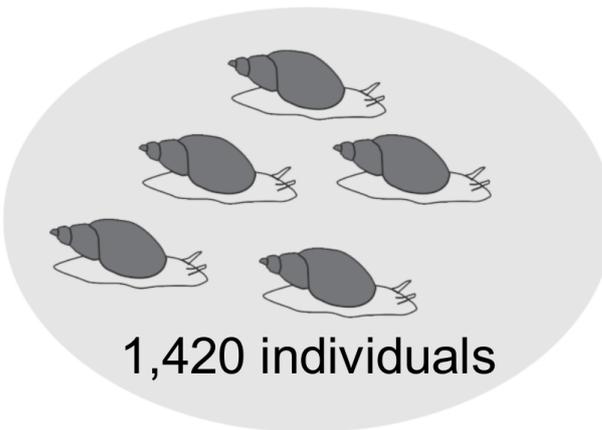


Galba cousini

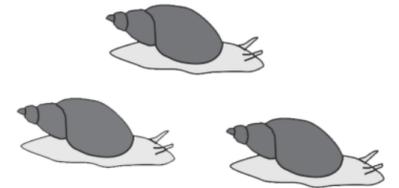


1 individual

Step 2: Multiplex PCR

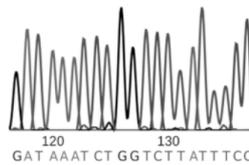
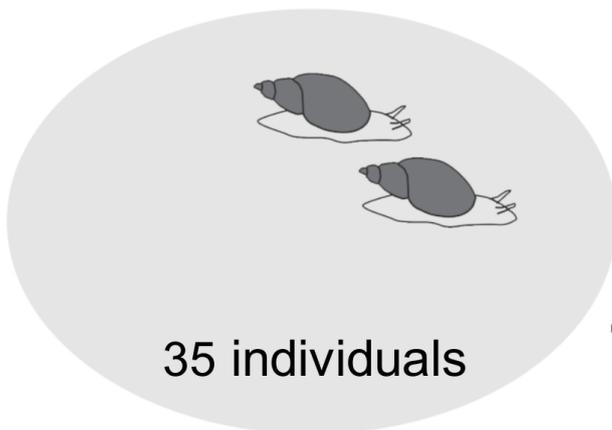


Galba cubensis
Galba schirazensis
Galba truncatula

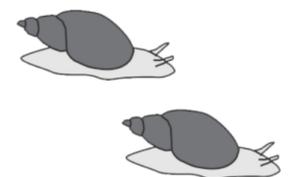


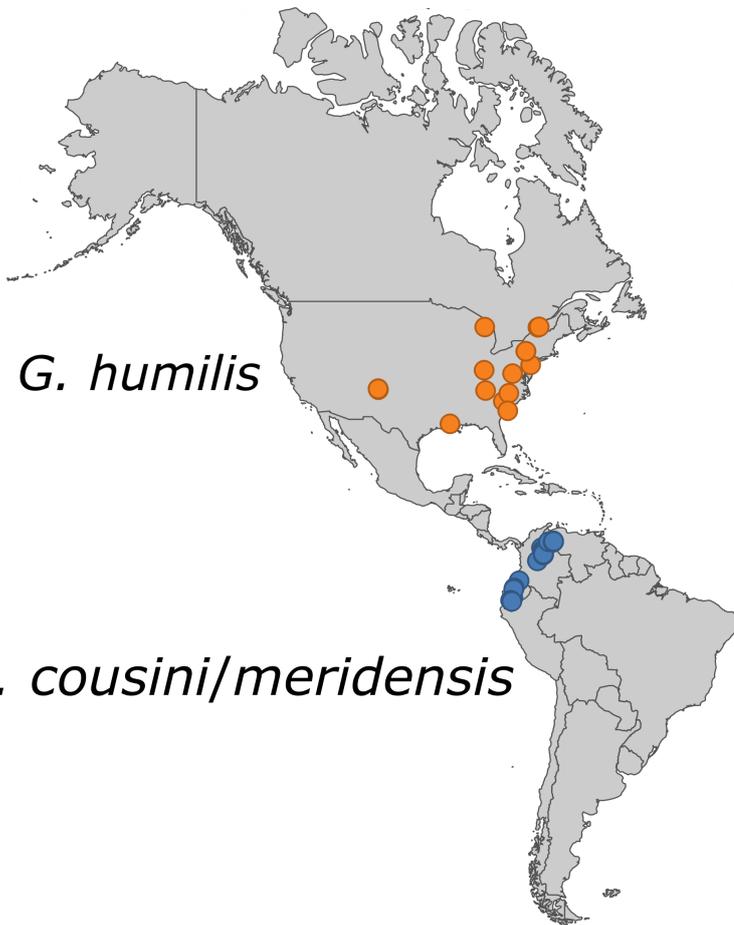
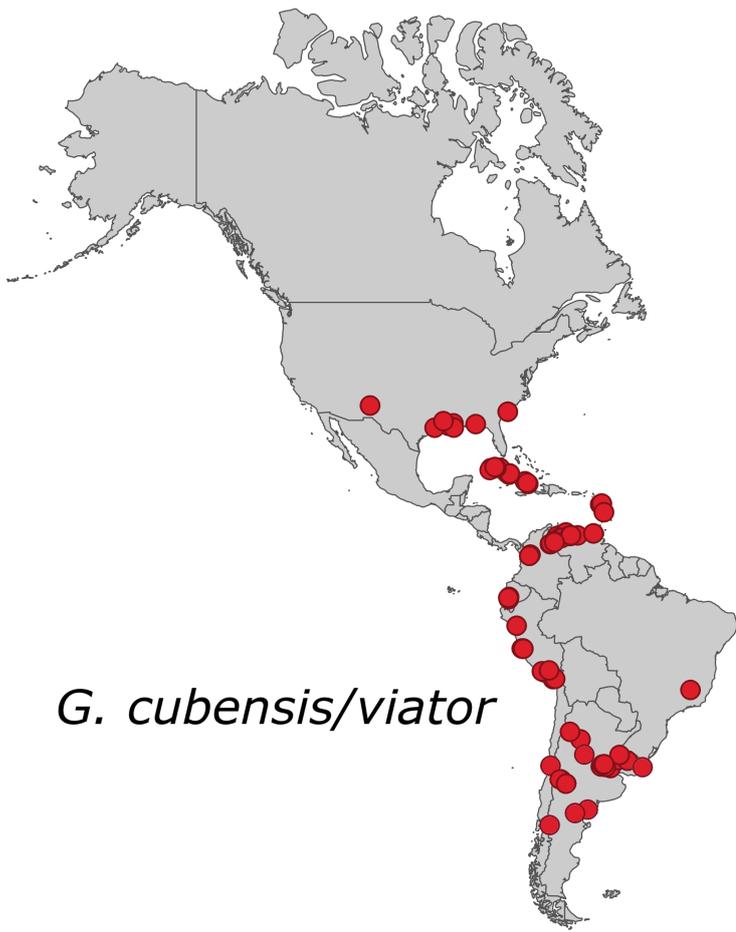
112 individuals

Step 3: ITS2 and/or COI sequencing

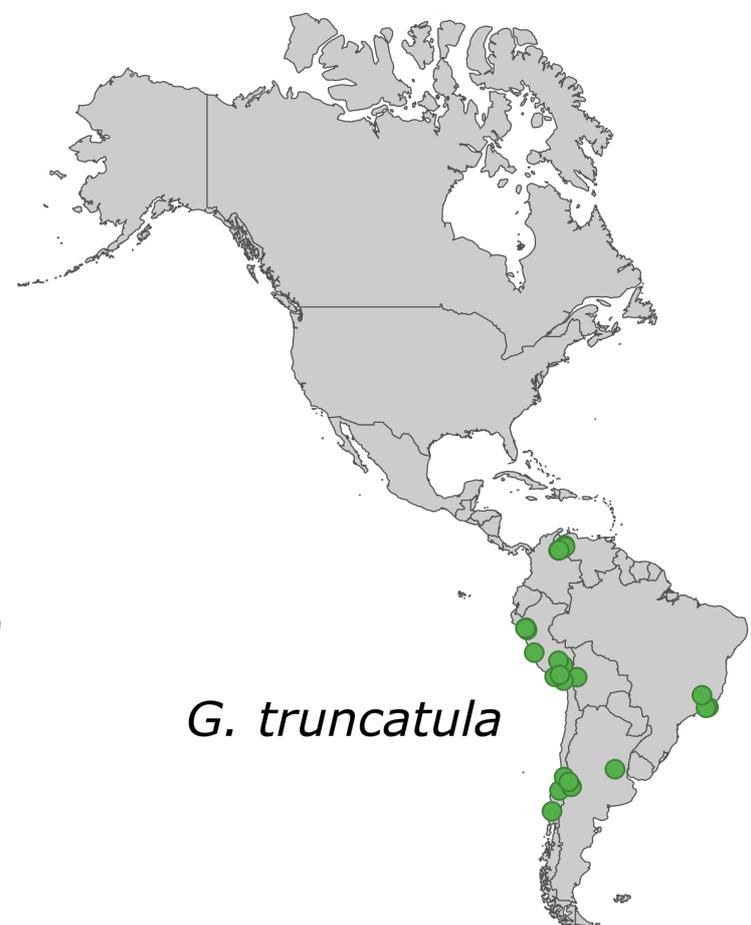


Galba humilis
Galba viator

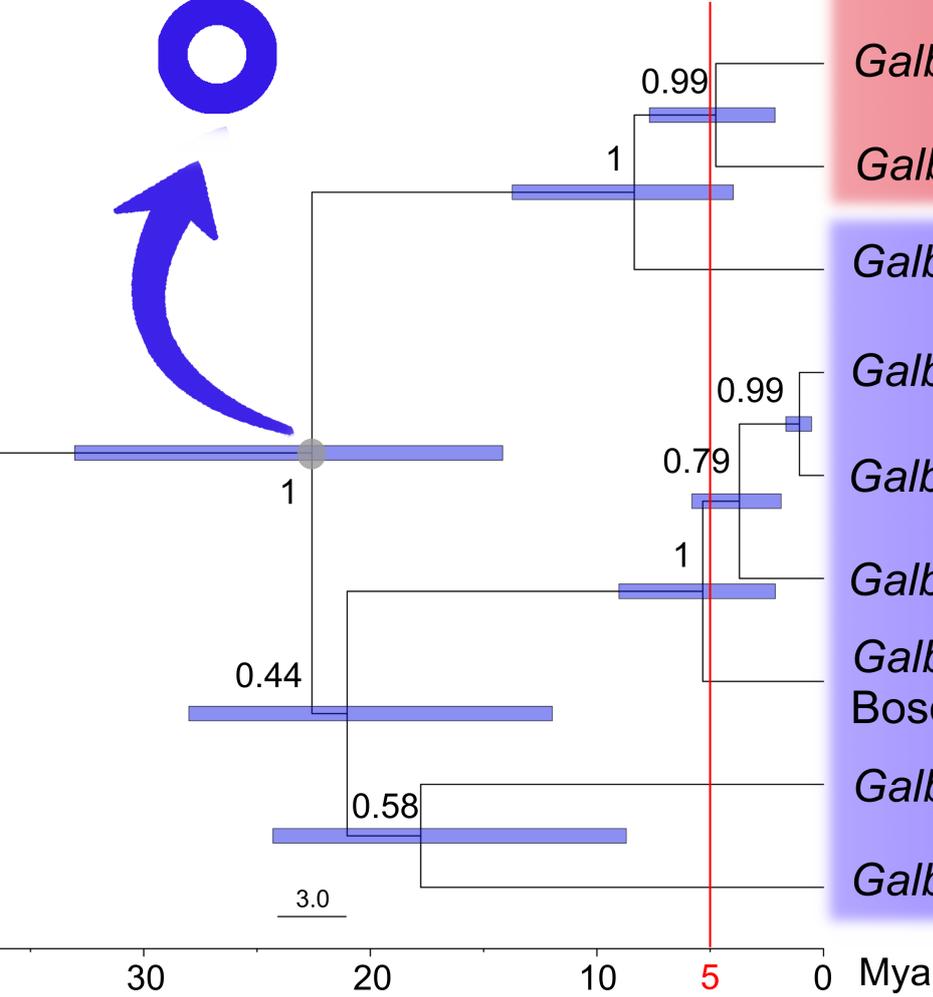
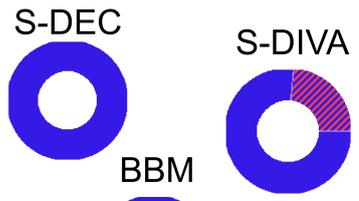




G. cousini/meridensis



Probability of cryptic phenotype in the MRCA



OUTCROSSERS

Galba cousini (IV)

Galba meridensis (V)

SELFERS

Galba humilis (III)

Galba neotropica (VI)

Galba cubensis

Galba viator

Galba sp. Bosque del Apache

Galba truncatula (I)

Galba schirazensis (II)

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

Species	Country	Locality	Coordinates	ITS1	ITS2	16S	COI	Reference
<i>Galba cousini</i>	Ecuador	Chancho-Yacu	00°18'55"S 78°34'02"W	FN598157	FN598153	-	FN598161	Bargues et al. 2011b
<i>Galba cubensis</i>	Cuba	Vaqueria 21	23°01'N 82°32'W	AM412226	AM412223	-	AM494009	Bargues et al. 2007
<i>Galba humilis</i>	USA	Owego, New York	42°06'01"N 76°15'40"W	FN182193, FN182194	FN182191, FN182192	-	FN182197, FN182198	Correa et al. 2011
<i>Galba meridensis</i>	Venezuela	Laguna Mucubají (Mérida)	08°47'52"N 70°49'32"W	FN598159	FN598154	-	FN598164	Bargues et al. 2011b
<i>Galba neotropica</i>	Peru	Lima, Rio Rimac	12°02'S 76°56'– 77°08'W	AM412228	AM412225	-	AM494008	Bargues et al. 2007
<i>Galba schirazensis</i>	Iran	Taleb Abad river, Bandar Anzali, Gilan province	37°27'46"N 49°37'07"E	JF272603	JF272601	JF272605	JF272607	Bargues et al. 2011a
<i>Galba truncatula</i>	Germany	Thuringia, Erfurt-Bindersleben	ND	-	-	-	EU818799	Albrecht et al. 2008
<i>Galba viator</i>	Argentina	Frias	40°14' S 64°10' W	JN614428	HQ283265, JN614465	-	JN614397, JN614398	Correa et al. 2011

Supplemental Information for:

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

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Figure S1. Geographic distribution of *Galba cubensis*, *Galba schirazensis* and *Galba truncatula* in the European, Asian and African samples retrieved from GenBank. Coordinates are given in Table S3.

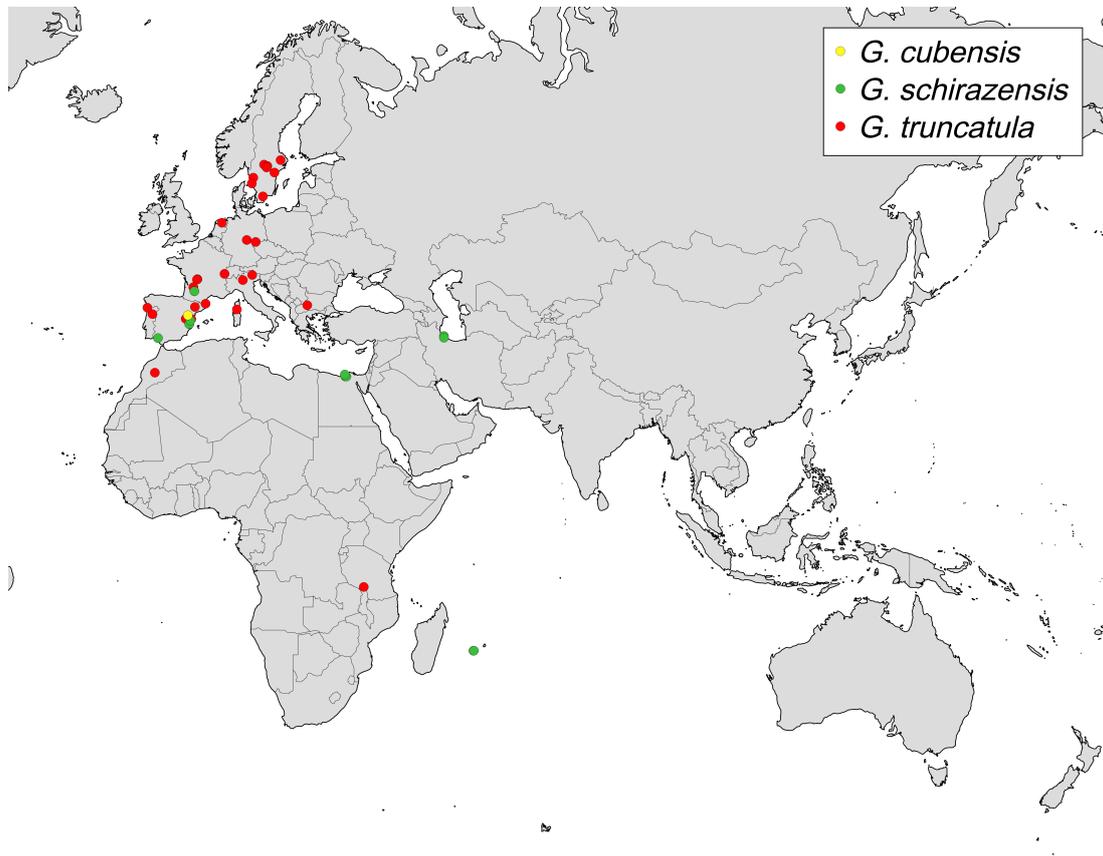


Figure S2. Scenarios for species assignments used to run the multispecies tree models using Multi-Threaded Nested Sampling in StarBeast2. The scenario K is an unreal scenario that separates the populations of *G. viator* from Argentina and Chile to test whether splitter models showed higher support than lumpers models regardless its biological sense.

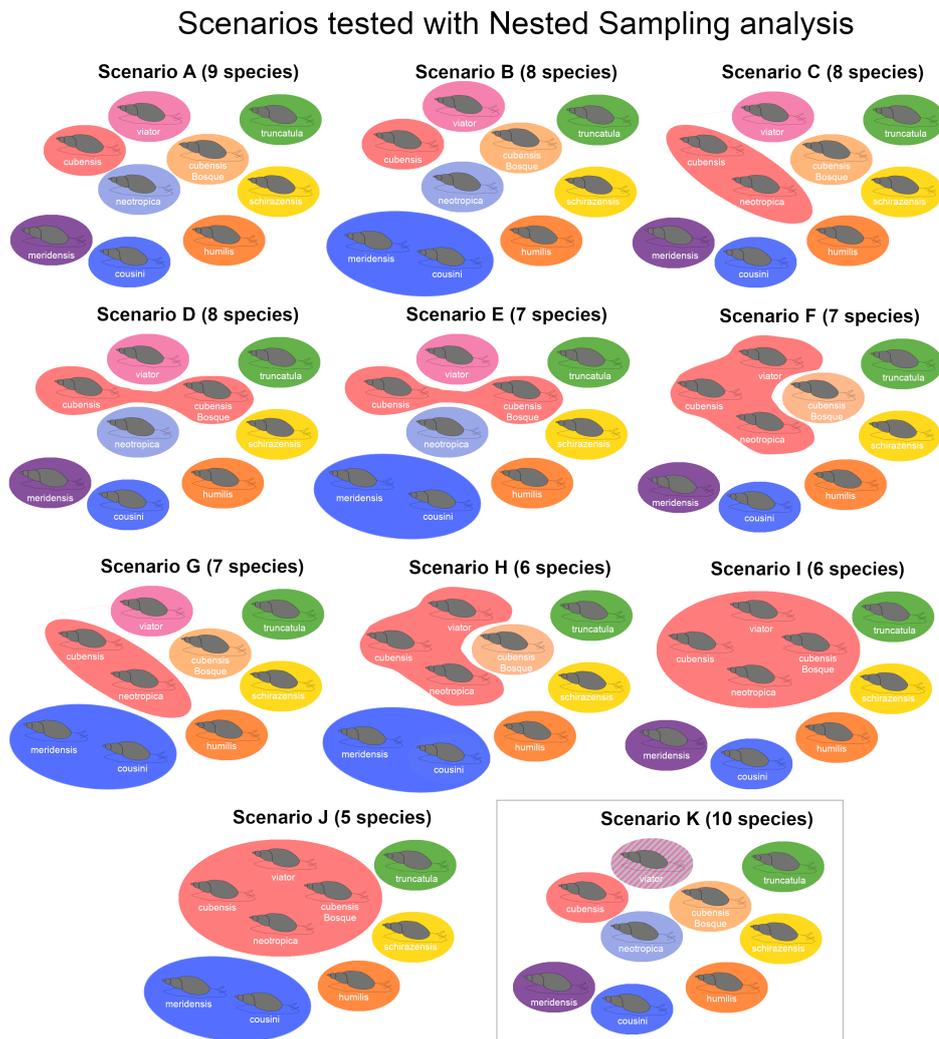


Figure S3. Shells and reproductive and urinary systems of the six *Galba* species studied. *Galba cousini/meridensis* is the only species that can be morphologically identified.

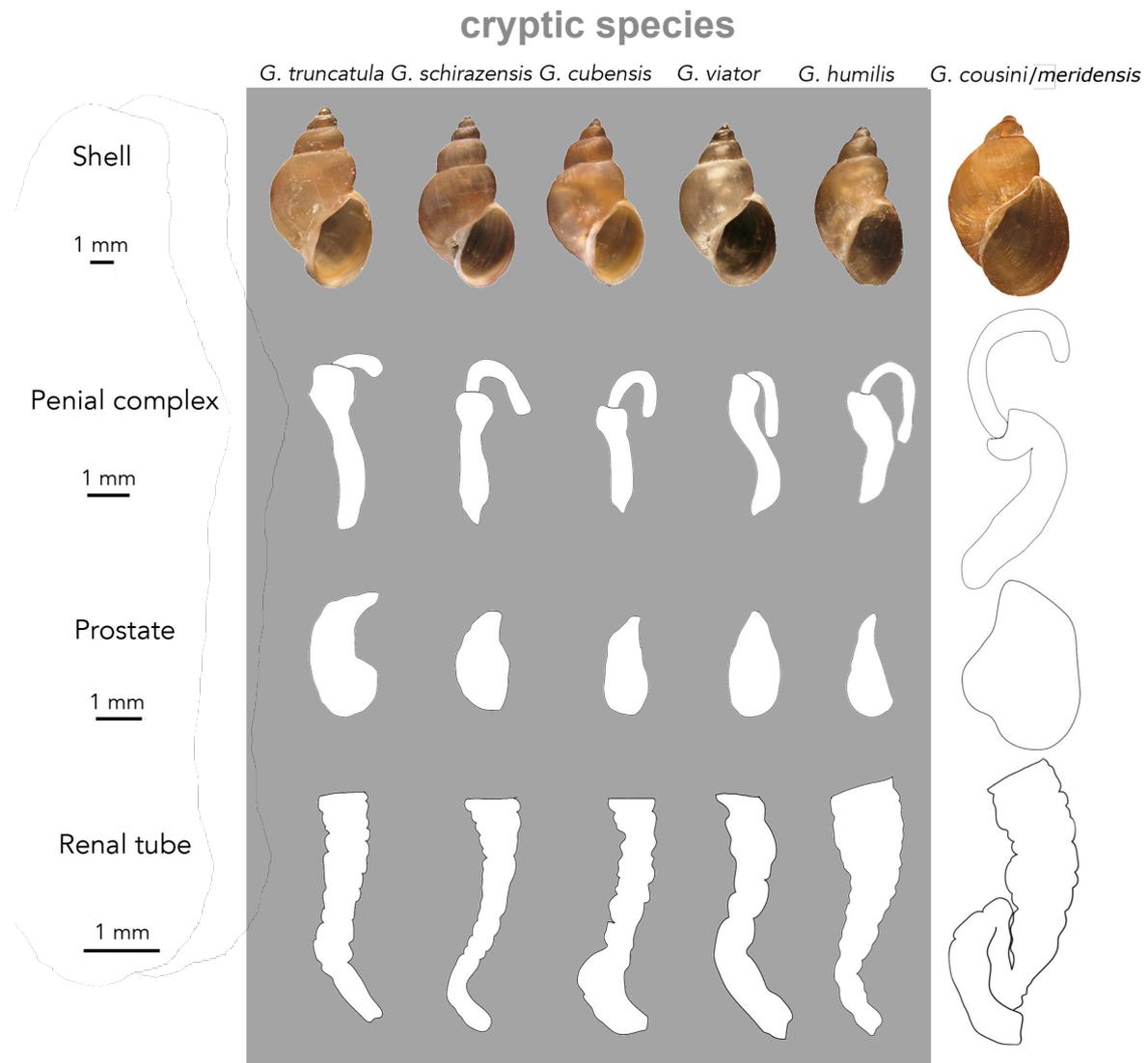


Figure S4. Phylogenetic tree of *Galba* species based on Bayesian inference in Beast2 of the COI gene. All sequences from the current study, as well as the ones retrieved from GenBank, are included in this tree. Sequence coloration represents species. Arrows indicate sequences belonging to a type locality (see Table 1 for details). Sequence data are given in Tables S1, S2, and S3.

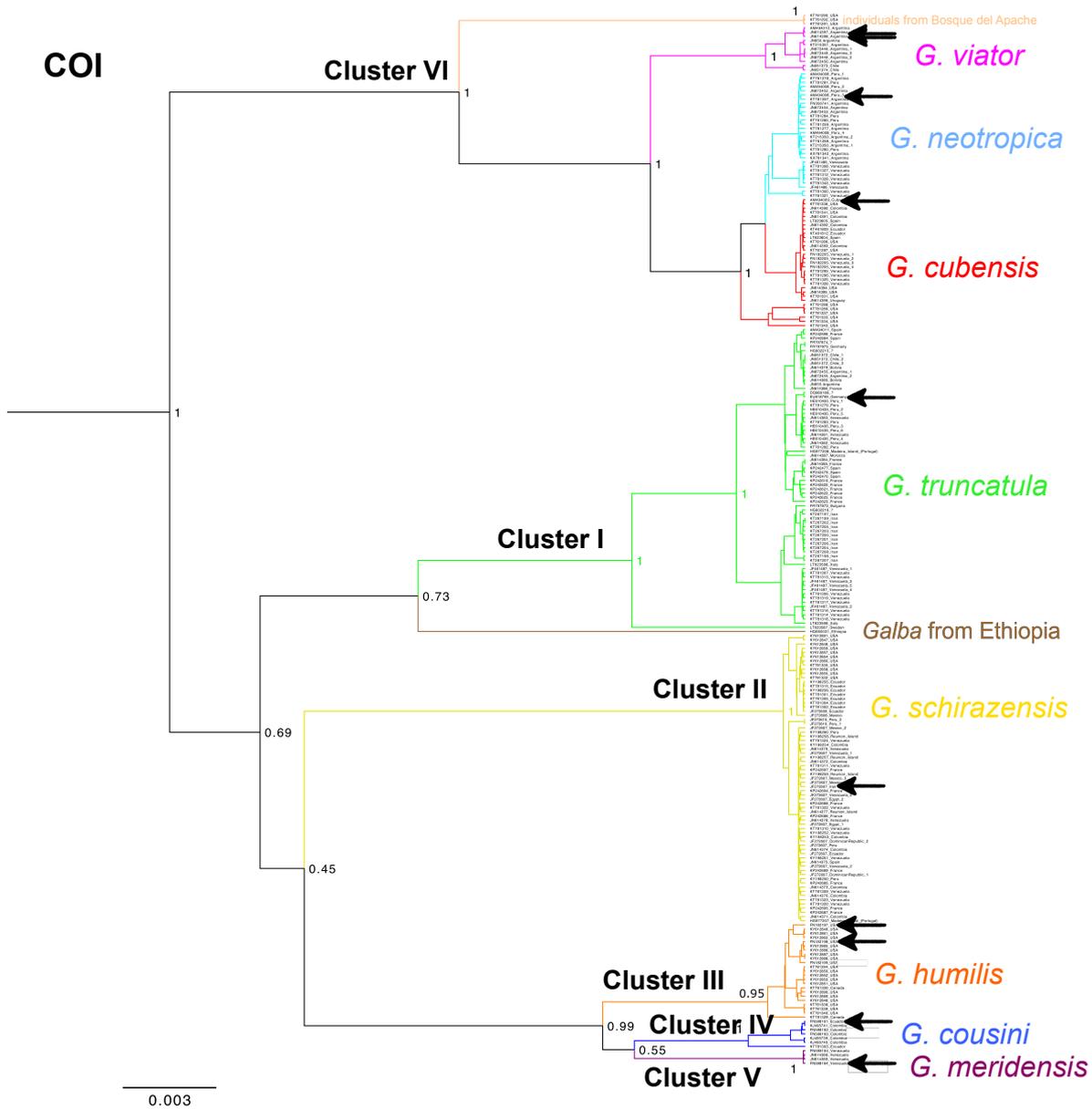


Figure S5. Phylogenetic tree of *Galba* species based on Bayesian inference in Beast2 of the 16S gene. All sequences were retrieved from GenBank except for sequences from individuals from Bosque del Apache (USA). Sequence coloration represents species. Arrows indicate sequences belonging to a type locality (see Table 1 for details). Sequence data are given in Tables S1, S2, and S3.

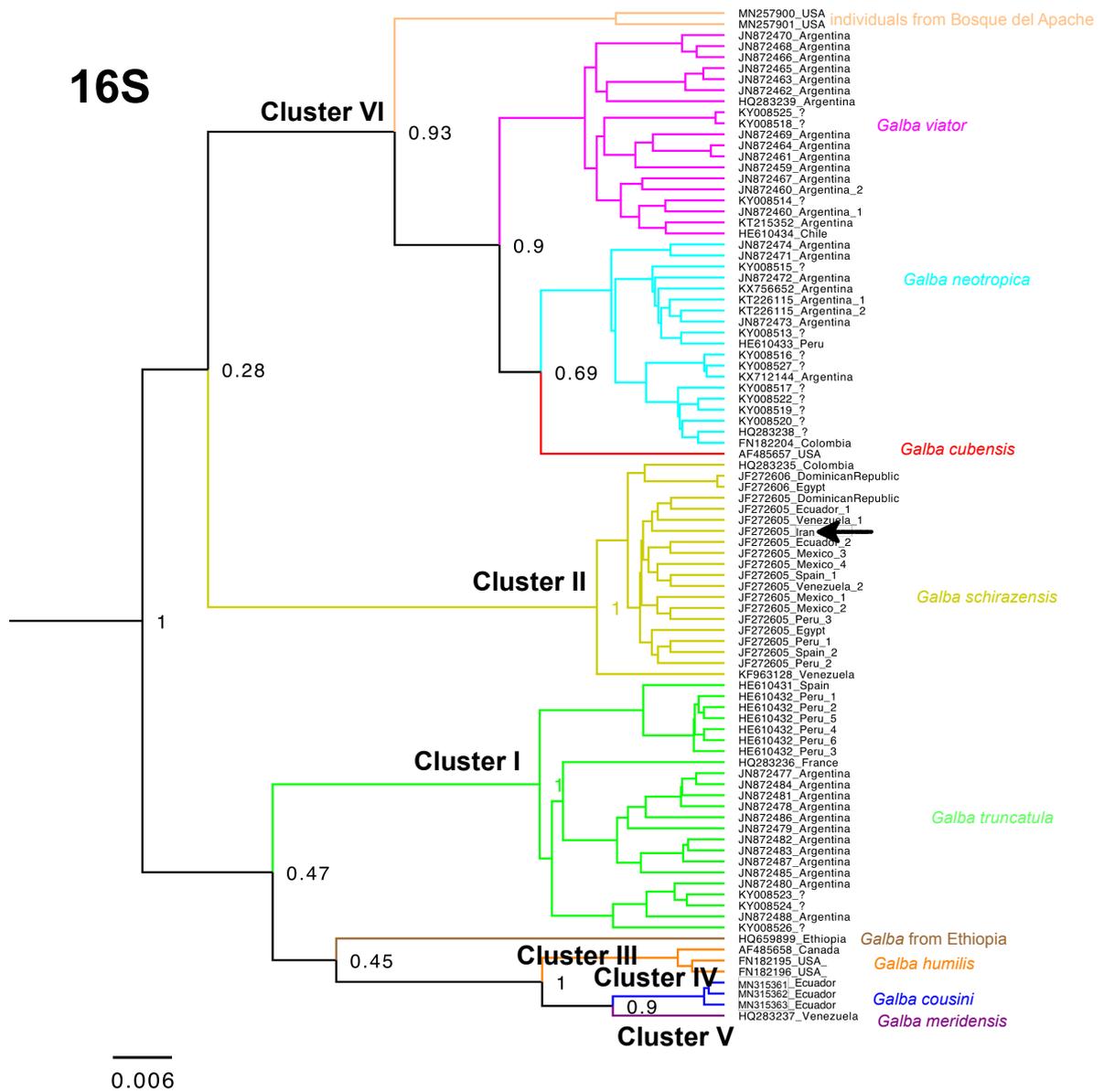


Figure S6. Phylogenetic tree of *Galba* species based on Bayesian inference in Beast2 of the ITS1 gene. All sequences were retrieved from GenBank except for sequences from individuals from Bosque del Apache (USA). Sequence coloration represents species. Arrows indicate sequences belonging to a type locality (see Table 1 for details). Sequence data is given in Tables S1, S2, and S3.

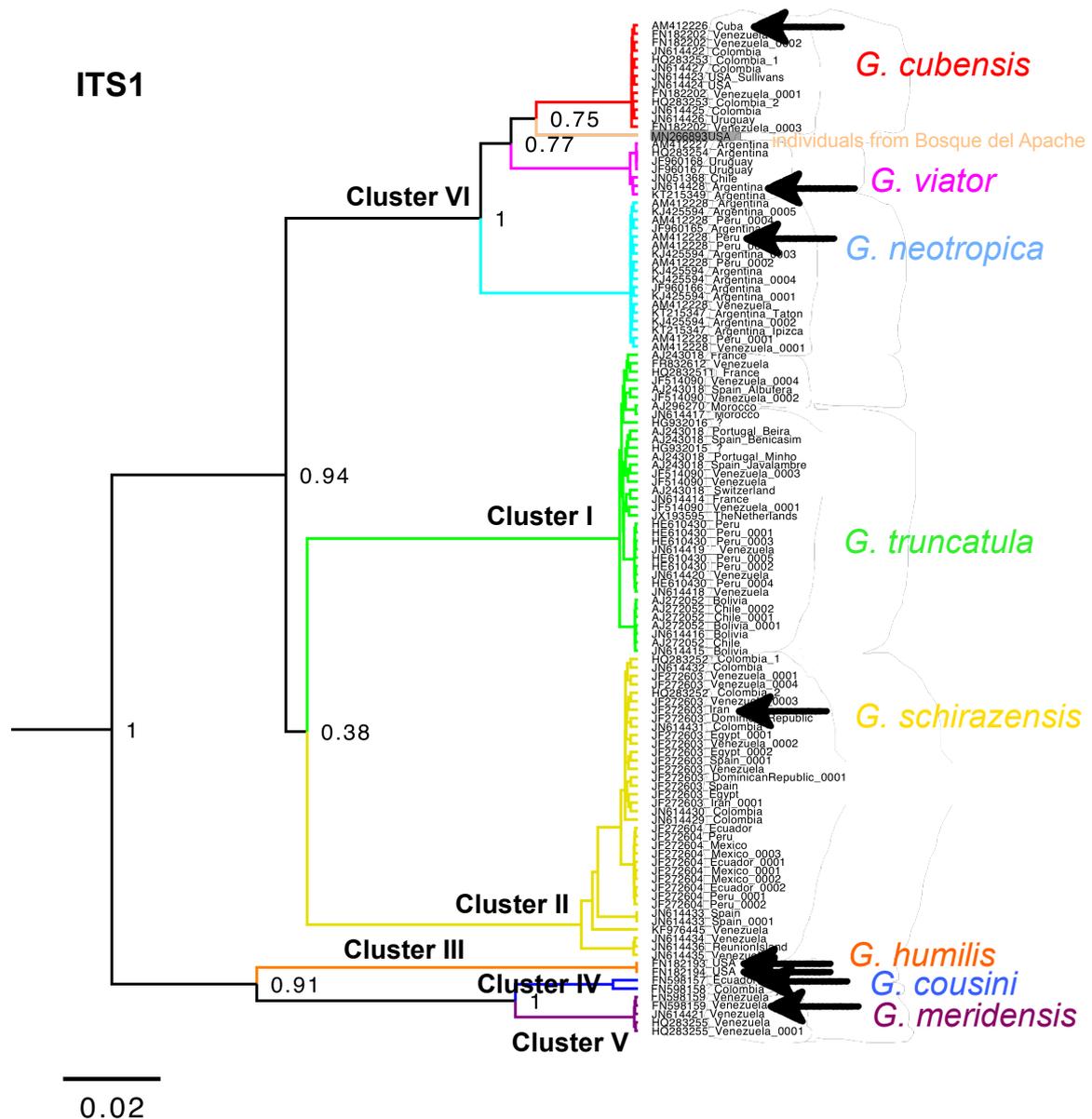


Figure S7. Phylogenetic tree of *Galba* species based on Bayesian inference in Beast2 of the ITS2 gene. All sequences for the current study, as well as the ones retrieved from GenBank, are included in this tree. Sequence coloration represents species. Arrows indicate sequences belonging to a type locality (see Table 1 for details). Sequence data are given in Tables S1, S2, and S3.

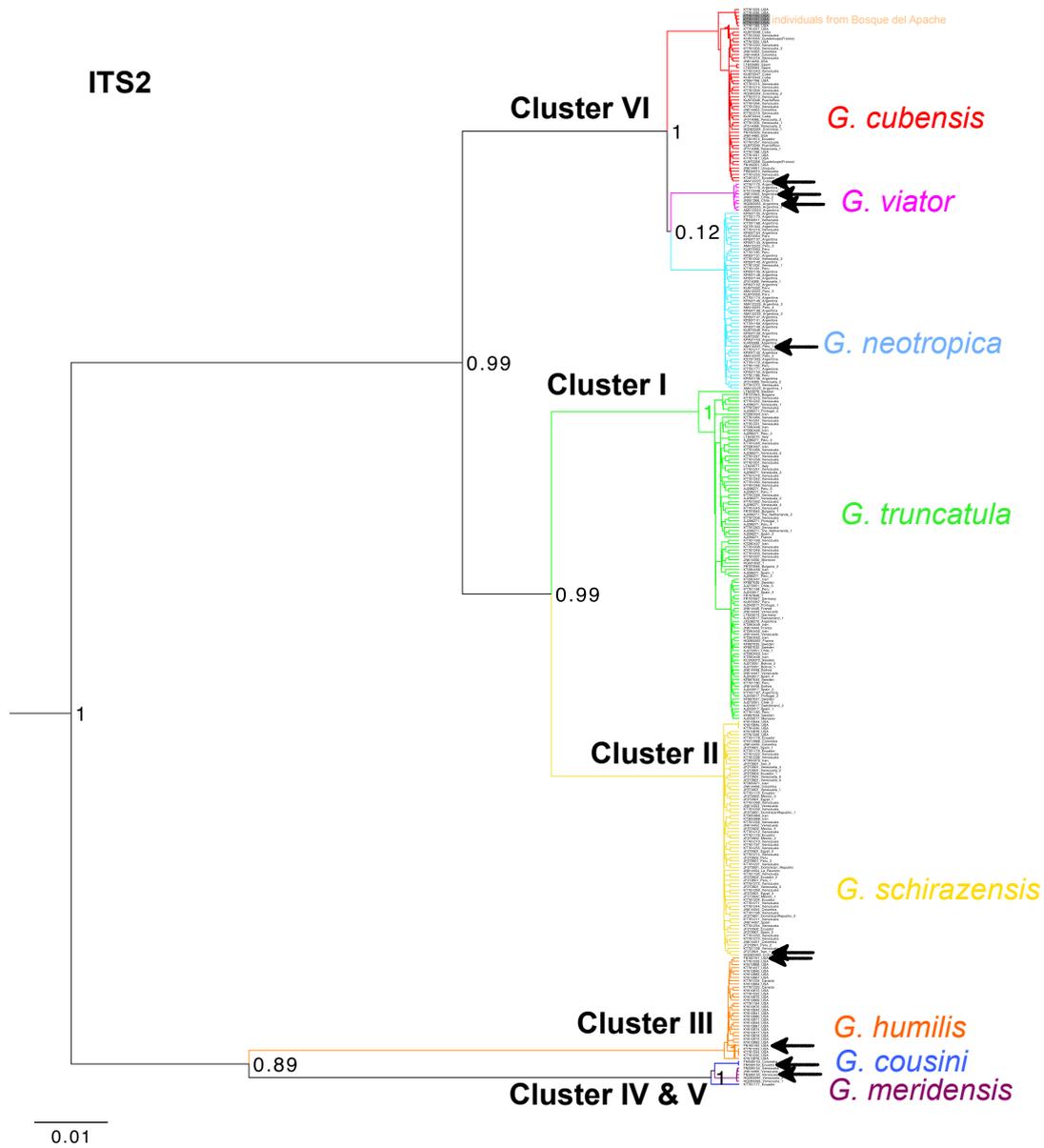


Figure S8. Haplotype network of *Galba* species based on 16S gene. Circle sizes are proportional to haplotype frequencies and colors represent species. The number of mutations separating circles are indicated by dashes. The six clusters detected in the phylogenetic analysis are represented as grey shapes. Note that a branch in Cluster I and another in Cluster VI cross other connecting branches which are themselves not connected.

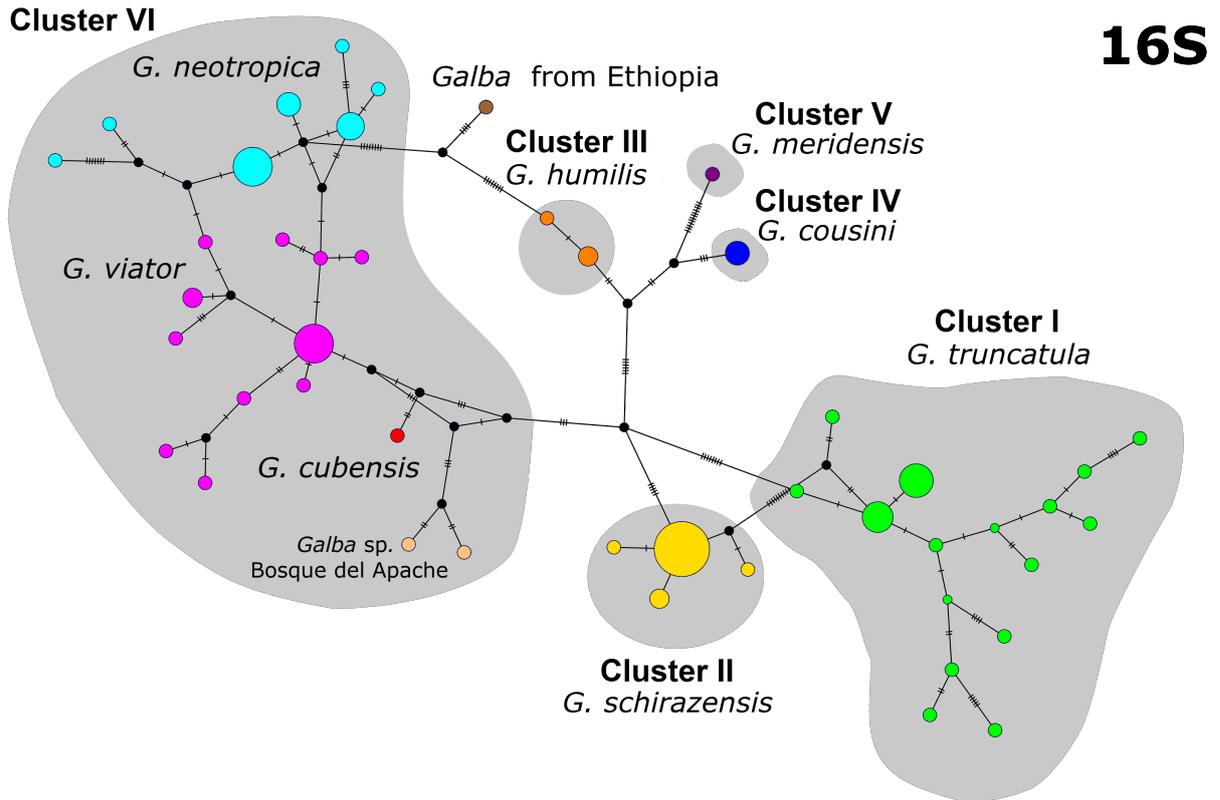


Figure S9. Haplotype network of *Galba* species based on COI gene. Circle sizes are proportional to haplotype frequencies and colors represent species. The number of mutations separating circles are indicated by dashes. The six clusters detected in the phylogenetic analysis are represented as grey shapes.

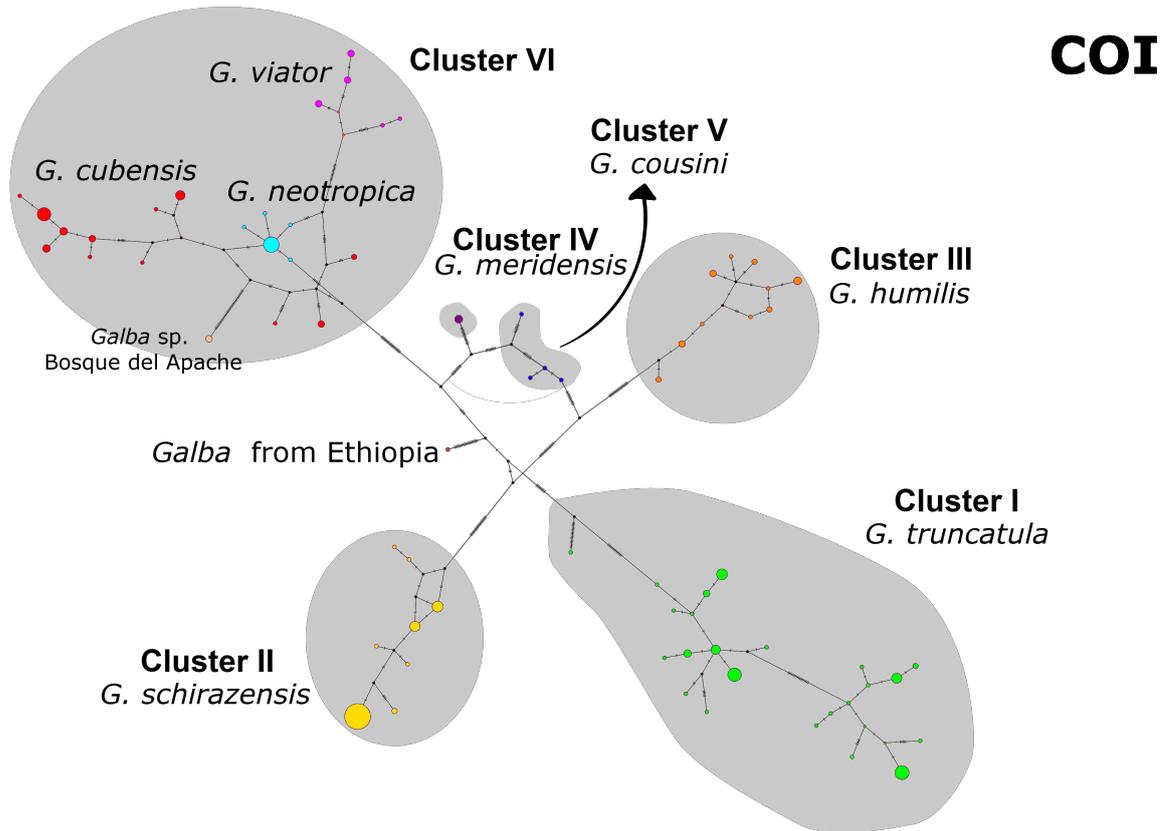


Figure S10. Haplotype network of *Galba* species based on ITS1 gene. Circle sizes are proportional to haplotype frequencies and colors represent species. The number of mutations separating circles are indicated by dashes. The six clusters detected in the phylogenetic analysis are represented as grey shapes. Note that a branch in Cluster VI crosses other connecting branches which are themselves not connected.

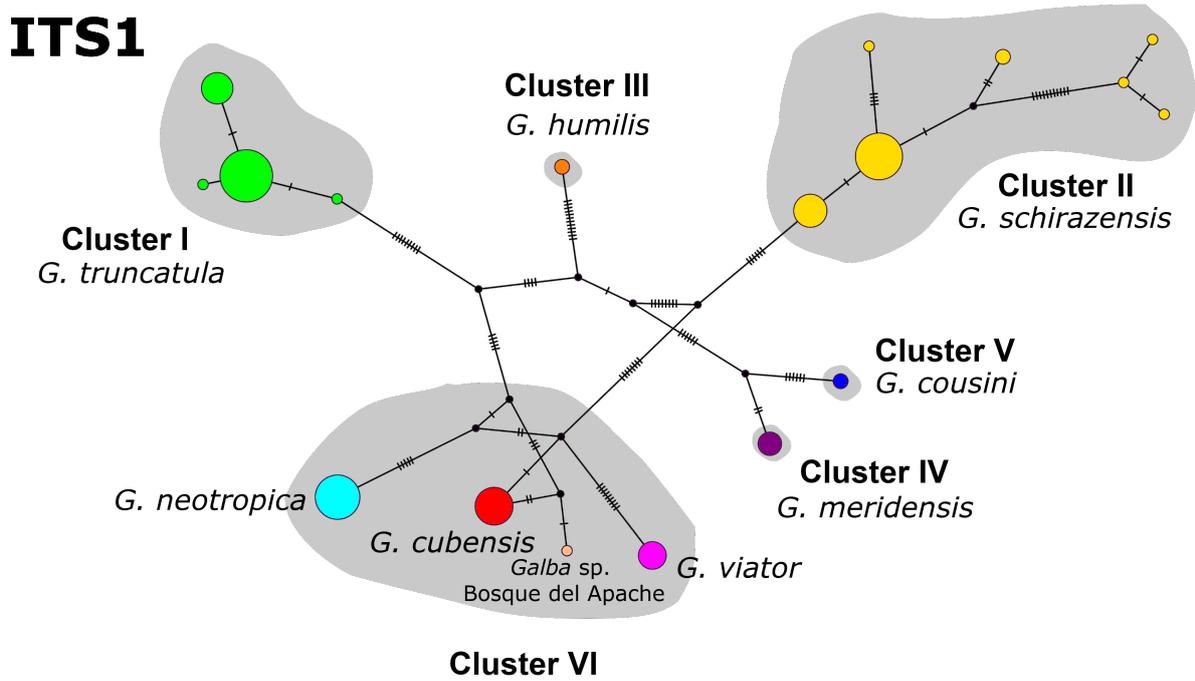


Figure S11. Haplotype network of *Galba* species based on ITS2 gene. Circle sizes are proportional to haplotype frequencies and colors represent species. The number of mutations separating circles are indicated by dashes. The six clusters detected in the phylogenetic analysis are represented as grey shapes. Note that a branch in Cluster I crosses other connecting branch which are themselves not connected.

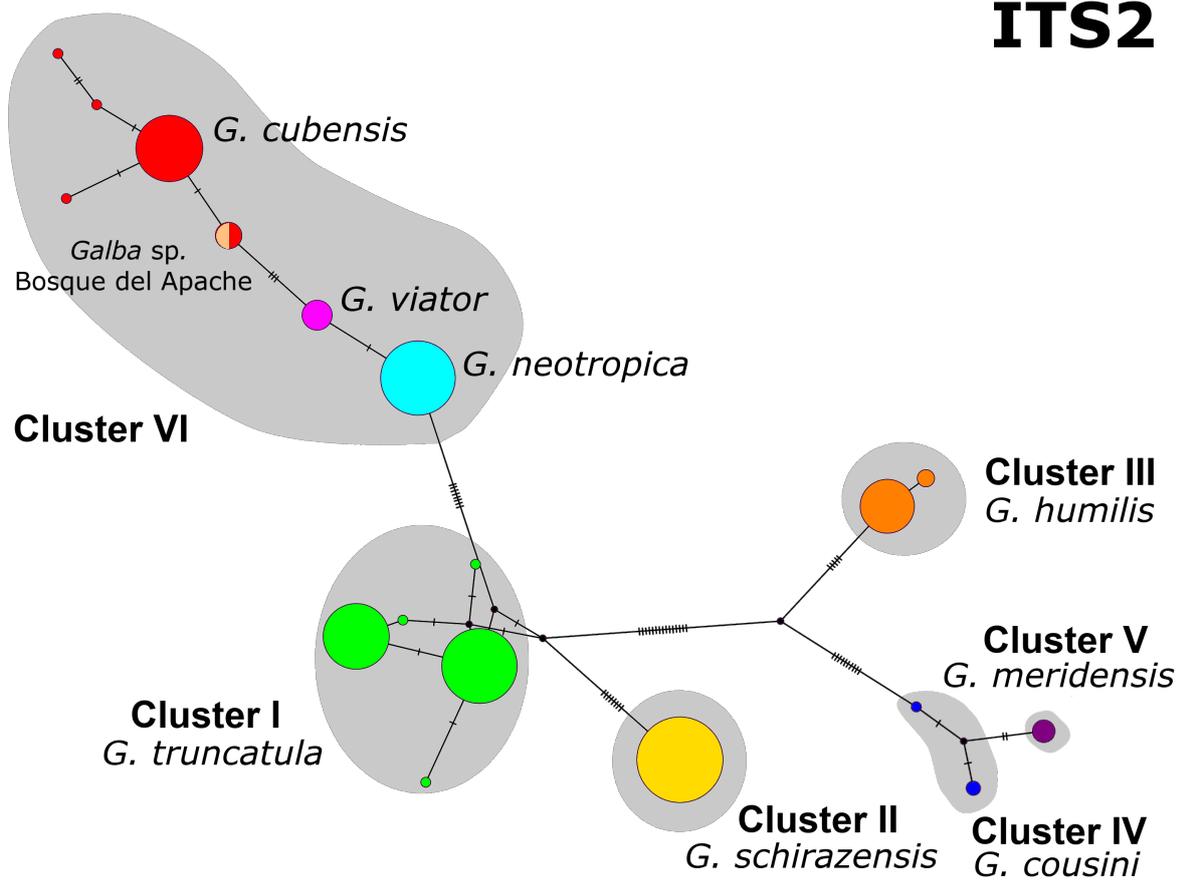


Table Legends (in Appendix 02)

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. 1). 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. The last column show the 16S and ITS1 sequences that have been incorporated to the study in order to test the species hypothesis with the multispecies coalescent models.

Table S2. Sites retrieved from literature and GenBank where *Galba* species were molecularly identified in America. 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 erroneously tagged as an ITS2 sequence, but is, in fact, a COI sequence. Sequences of the individuals molecularly identified by (Medeiros et al. 2014) are missing in the original publication and were not uploaded to GenBank. ND, no data available.

Table S3. Sites retrieved from literature and GenBank where *Galba* species were molecularly identified in Europe, Asia, and Africa. Coordinates that were not given in the original articles or in GenBank were best-guess estimated. The information reported for each site is as in Table S2. ND, no data available.

Table S4. Nested sampling results for the eleven species-delimitation models shown in Figure S11. The model with the higher Marginal Likelihood estimate is the top-ranked model. All Bayes factor (BF) calculations are made against the current taxonomy model (scenario D). Therefore, negative BF values indicate support for the current taxonomy model, and positive BF values indicate support for the alternative model.

Table S5. Species of small, mud-dwelling lymnaeids recognized by Burch (1982) in North America. The author grouped the 22 species into the genus *Fossaria* with two subgenera, *Fossaria (s.s.)* and *Bakerilymnaea*.

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