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Twenty-five samples of *Bulimulus* species are studied, partly from localities within their known distribution range, partly based on interceptions where the material originates from localities where the species seem to be recently introduced and non-native. Molecular study of cytochrome oxidase 1 (CO1) reveals the origin of some of these introductions, but is less conclusive for others. Four different methods for species delimitation were applied, which did not result in unambiguous species hypotheses. For a rapid identification of morphologically indistinct species, a more comprehensive database of sequences is needed.

Caribbean *Bulimulus* revisited: physical moves and molecular traces (Mollusca, Gastropoda, Bulimulidae)

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Key words

Orthalicoidea, distribution, alien species, phylogenetics, West Indies, Florida, Central America, Ecuador.

Abstract

Twenty-five samples of *Bulimulus* species are studied, partly from localities within their known distribution range, partly based on interceptions where the material originates from localities where the species seem to be recently introduced and non-native. Molecular study of cytochrome oxidase 1 (CO1) reveals the origin of some of these introductions, but is less conclusive for others. Four different methods for species delimitation were applied, which did not result in unambiguous species hypotheses. For a rapid identification of morphologically indistinct species, a more comprehensive database of sequences is needed.

Introduction

The genus *Bulimulus* is widespread in the Caribbean region, and on the mainland of Central America; also a number of species do occur in dispersed regions of South America (Breure, 1974; Breure & Borrero, 2008; Linares & Vera, 2012; Cuezzo et al., 2013; Ramírez et al., 2003; Simone, 2006; Thompson, 2011). Breure (1974) presented a revision of the Caribbean species, with a key to species, based on external shell morphology only. One of the most common species, *Bulimulus guadalupensis* (Bruguière, 1789), was reported then mainly from the Windward Group and supposedly imported on Jamaica, and Hispaniola. Shortly afterwards it was reported as being introduced on Curaçao (Breure, 1975). This very variable species, however, did not show infraspecific differentiation using measurements of the shells (Breure, 1974: 21). Another Caribbean species, *B. diaphanus* (Pfeiffer, 1855), was divided into two subspecies ranging from Hispaniola to St. Croix, and from St. Martin to Iles des Saintes respectively (Breure, 1974). Anatomical data of *Bulimulus* species are scarce (Breure, 1978; Miquel, 1991) and mainly concern the mainland species. Molecular data has become available during the past decades, the most extensive treatment of this genus by Breure & Romero (2012) provides seven sequences, of which five from Caribbean. In order to obtain more insight in phylogenetic relations and zoogeographical patterns, additional molecular data from *Bulimulus* species throughout the distribution range are needed. In recent years reports of introduced *Bulimulus* taxa throughout the Caribbean and in the U.S.A. have increased (D. Robinson, pers. commun.). The author frequently received photographs of *Bulimulus* species for identification. Species of this genus—for which more than 100 names are available (Breure, 1979)—typically possess few reliable external char-

acters, and are thus difficult to recognise from a photograph alone. Most of photos were from material captured by the U.S. Dept of Agriculture (USDA) at border sites, which were considered alien species, often with unclear provenance. Moreover, some Florida populations of *Bulimulus* have not been well documented as yet.

The aim of this paper is provide additional molecular and occurrence data on Caribbean (sensu lato, incl. Florida) *B. guadalupensis* and *B. diaphanus*, and to test whether recent imports of *Bulimulus* species in the U.S.A. can be traced back to a South American origin. A second aim is to test whether different species delimitation methods may be helpful to untangle morphologically very similar species (cf. Prévot et al., 2013).

Material and methods

Intercepted or collected material of *Bulimulus* species was received originating from Argentina, Bahamas, Barbados, Colombia, Dominica, Dominican Republic, Ecuador, Guadeloupe, Haiti, Honduras, Jamaica, Paraguay, St. Kitts and Nevis, and the U.S.A. Collection data, accession numbers for museum vouchers and GenBank accession numbers of all sequenced material is listed in Table 1. All tissue samples were taken from snail feet and transferred to 96% ethanol. Whole genomic DNA was extracted with DNeasy kit (Quiagen Inc.) following the manufacturer's protocol. Fragments of mitochondrial cytochrome oxidase 1 (CO1) were amplified using the Folmer primers (Folmer et al., 1994), as described in Breure & Romero (2012). New sequences obtained during the present study are indicated in Table 1. Sequences were aligned using

MAFFT as implemented in Geneious 7.1.3 (Biomatters Ltd.). The substitution model selected by jModeltest 2.1 (Posada, 2008), using the Akaike Information Criterion, was GTR+I+G.

Phylogenetic trees were inferred by application of Neighbor-Joining (NJ), Maximum Likelihood (ML), Maximum Parsimony (MP), and Bayesian Inference (BI) methods. NJ trees were constructed using MEGA6 (Tamura et al., 2013) with Kimura 2-parameter (K2P) and 500 bootstrap replicates. ML trees were inferred using PhyML 3.0 (Guindon et al., 2010), with four substitution rate categories considered, and gamma shape parameters, transition/transversation ratios, and nucleotide frequencies were estimated from the data. Nodal support of topologies was inferred by calculating aLRT statistics. MP trees were constructed using MEGA6 (Tamura et al., 2013), resulting in a most parsimous tree with length = 906; the consistency index is 0.445100 for all parsimony-informative sites. The tree was obtained using Subtree-Pruning-Regrafting and 500 bootstraps. BI trees were constructed using MrBayes 3.2.2 (Ronquist & Huelsenbeck, 2003), based on a cold chain and three incrementally heated chains (T = 0.2), running for 1,100,000 generations with a sample frequency of 200. The burn-in rate was 25% and the remaining trees were used for building a consensus tree and calculating the Bayesian posterior clade probabilities (Larget & Simon, 1999). Both ML and BI software were used as implemented in Geneious 7.1.3 (Biomatters Ltd.). All trees were rooted with an outgroup of *Drymaeus vexillum* (Broderip, 1832) and *Neopetraeus tessellatus* (Shuttleworth, 1854), for which sequences were retrieved from GenBank (Breure & Romero, 2012). Branch support was considered as well-supported if higher than 70 (bootstrapping: bs), resp. 0.9 (posterior probabilities: pp).

Evolutionary analysis of the original alignment was further explored by using SplitsTree4 v. 4.12.6 (Huson & Bryant, 2006). This program produces phylogenetic graphs with split networks; the K2P parameter was used in combination with the Neighbor-Net setting. Species delimitation was investigated using different methods, both with the original alignment and with a subset using only *B. guadalupensis*, *B. diaphanus*, and *B. sporadicus*: (1) Classical barcode gap analysis (BGA) using K2P-distances (a) 3% threshold, (b) 4% stylommatophoran threshold (both methods following Prévot et al., 2013, who also cite references debating the use of K2P); (2) Species Delimiting as implemented in Geneious 7.1.3. (Biomatters Ltd.) (SDG); (3) Genealogical Sorting Index (GSI); (4) Automated Bar code Gap Discovery (ABGD).

BGA was explored using Estimates of Evolutionary Divergence between Sequences (EDS) and estimates of Net Evolutionary Divergence between Group of Sequences (NEDGS). These were conducted in MEGA6 (Tamura et al., 2013) by calculating the number of base substitutions per site from between sequences. Standard error estimate(s) were obtained by a bootstrap procedure (500 replicates). Analyses were conducted using the Maximum Composite Likelihood model (Tamura et al., 2004). Codon positions included were 1st+2nd+3rd+Noncoding and all positions containing gaps and missing data were eliminated, resulting in 585 positions in the final dataset analysed.

The SDG plug-in in Geneious v. 7.1.3 allows evaluating the phylogenetic exclusivity of each putative species interpreted as a clade by testing the probability that this exclusivity or monophyly has occurred by chance in a coalescent process. It further assesses the probability with which a putative species can be diagnosed successfully on a phylogenetic tree by comparing intra- and

interspecific genetic distances and its ration. SDG calculates values of P ID(strict), i.e. the mean probability and its confidence interval of making a correct identification of an unknown specimen of a clade (not as sister of this clade) using placement on a tree (Masters et al., 2011). The method also calculates Rosenberg's P_{AB} (Rosenberg, 2007), a test for taxonomic distinctiveness based on the null hypothesis that monophyly is a chance outcome of random branching, and Rodrigo's P(Randomly Distinct) (Rodrigo et al., 2008), which is the probability that a clade has the observed degree of distinctiveness due to random coalescence.

The GSI method quantifies the historical relationships among groups of (putative) taxa by measuring the exclusive ancestry of a group using a rooted tree topology. A group is defined as a set of commonly labeled branch tips and exclusivity is the amount of ancestry for a group that is common to only members of the group, measured on a scale (the index), with a level of support (p-value). In the initial stages of divergence the values are at or near 0, at the final stages of genealogical sorting the values reach 1, representing exclusive ancestry (i.e. monophyly) (Cummings et al., 2008).

The ABGD method sorts the sequences into hypothetical species based on the barcode gap, which can be observed whenever the divergence among organisms belonging to the same species is smaller than divergence among organisms from different species. ABGD uses a range of prior intraspecific divergence to infer from the data a model-based one-sided confidence limit for intraspecific divergence. The method then detects the barcode gap as the first significant gap beyond this limit and uses it to partition the data. The aligned dataset was uploaded to the website (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) and run with K2P distances under

default settings. These settings are a Prior Intraspecific divergence ranging from Pmin = 0.001 to Pmax 0.1 in ten steps, a relative gap width X = 1.5, and a distance distribution Nb bins = 20 (Puillandre et al., 2012).

Results

Occurrence data

For the most common Caribbean *Bulimulus* species, *B. guadalupensis* (Bruguière, 1789), new introductions are now traced back to the following Neotropical countries: Ecuador (USDA 110210), Costa Rica (USDA 110245), and Honduras (USDA 110162). All introductions were signaled via an 'U-loop', viz. the interception at U.S.A.-borders testified that the species was present in the country of departure of the flight or shipment concerned (in all cases no stop-overs were made). However, precise locations within those countries are difficult to pinpoint. Another new record for a population of this species is in Florida, Dade County, Miami, near Coconut Grove (USDA 110205-6), and near Coral Gables (USDA 110208), where specimens have been collected by D.G. Robinson in respectively 1999 and 2010. The status of *B. guadalupensis* on Jamaica was confirmed as "introduced by humans" by Rosenberg & Muratov (2006: 140); see also under Phylogenetic analyses.

Another introduction of *Bulimulus* in Florida concerns a species that was originally reported from Jacksonville by B. Frank and H. Lee in 2009 (H. Lee, pers. commun.). Because the original spot was near a container company, it was surmised to be an alien species from an unknown source

area. Additional surveys in Jacksonville soon revealed that this, then unidentified, species was present in other spots as well and that it dispersal has taken place by rail. So far more than 20 populations have been discovered in and around Jacksonville along railroads, as far as 300 km as the crow flies SSW of Jacksonville. The species was identified by ANSP-USDA as *B. sporadicus* (d'Orbigny, 1835) (Frank & Lee, 2014), matching with specimens identified earlier as such from Houston, Texas (RMNH 114266) (D. Robinson, pers. commun.; Breure & Romero, 2012). This species is referred to in the present paper as *B.* cf. *sporadicus*, see Discussion.

Phylogenetic analyses

The NJ, ML and BI trees resulted in similar topologies (Figs 1-2; NJ: Supplementary information Fig. 1). The 17 sequences of *B. guadalupensis* fall into two sister groups that are both well-supported (bs: 100 (NJ), (ML); pp: 1.0 and 0.92 respectively). The MP tree (Supplementary information Fig. 2) also supports the division of *B. guadalupensis* into two groups (bs: 99), and suggests that the Florida population is likely from a Puerto Rico or Hispaniola source population. In the MP tree the clade with sequences from Puerto Rico (Guayabo), Honduras and Ecuador has relatively strong support (bs: 87). *B. hummelincki* and *B. diaphanus* from St. Kitts appear as paraphyletic in the same clade (bs: 71 (NJ), 78 (ML); pp: 0.99), which is sister group to all other *Bulimulus* species analysed in this study, except *B. gracilis* which is basally in all topologies. Within this second, weakly supported clade different topologies do occur, with polytomies in ML and BI. However, all methods suggest that *B. diaphanus* intercepted from the Bahamas is more closely related to the Central American *B. corneus* than to the other sequences of *B. diaphanus.* The lat-

ter are not a homogenous group, however, as the Nevis specimen is slightly differing from the Jamaican/Haitian specimens.

Detailed analysis of the alignment shows that *B. guadalupensis* sequences from the following populations can be arranged into two groups of haplotypes (h): (Group A) (h1) DR1 – Dominican Republic, La Cantera (USDA 110198); DR2 – Dominican Republic, 4 km S Cabrera (USDA 110200); DR3 – Dominican Republic, Come Pan (USDA 110197); DR5 – Dominican Republic, Santo Domingo (RMNH 106983); PR1 – Puerto Rico, Isla Vieques, Punta Arenas (USDA 110196); DO1 – Dominica, Bellevue Chopin (USDA 100706); DO2 – Dominica, Pointe Michel (USDA 100713); FL – U.S.A., Florida, Miami, Coral Gables (USDA 110208). The second haplotype (h2) differs in only one basepair at the following populations: PR2 – Puerto Rico, Isla Vieques, Florida (USDA 110199). A third haplotype (h3) links the populations at PR3 – Puerto Rico, Guayabo (USDA 110195) and those in EC – Ecuador (USDA 110210) and HO – Honduras (USDA 110162); there is a two basepair difference with h2. Group B links the following populations: (h4) GU – Guadeloupe, Saint-Anne (USDA 110209); (h5) DR4 – Dominican Republic, Las Terrenas (USDA 110201, 11 bp differences with h4); (h6) BA – Barbados, Sandy Lane (USDA 110207, 15 bp differences with h5, 13 bp with h4). The sequence from JA – Jamaica, Lucea (USDA 110194), although incomplete, is likely (nearly) identical to those from Barbados. In SplitsTrees4 group A appears to be rather homogeneous, while group B is more reticulated (Fig. 3). This was reflected in the higher values for group B in the BGA, both in EDS and NEDGS (Supplementary information, Table 4).

Fig. 4 shows the network relations of the other species. *B. diaphanus* shows up in three different branches; the specimen from Nevis (RMNH.MOL.114174) is related to *B. hummelincki* Breure, 1974 and corresponds to *B. diaphanus fraterculus* (Potiez & Michaud, 1835) (Breure, 1974). The specimens from Haiti (RMNH.MOL.114274) and Jamaica (RMNH.MOL.114173) are nearly identical, and correspond to *B. d. diaphanus* (Pfeiffer, 1855). The third sample from Bahamas (ANSP A22054) seems to be more closely related to the Central American *B. corneus* (Sowerby I, 1833); this sample may be misidentified. The three samples of *B. sporadicus* (d'Orbigny, 1835) appear to be partly unrelated; the specimens from Paraguay (RMNH.MOL.336661) and intercepted at Houston are on neighbouring branches (bootstrap 56; not shown). The sample from Florida (RMNH.MOL.336660), identified as the same species, is branching off *B. d. diaphanus* (bootstrap 86; not shown); this is corroborated in the MP tree, where the clade of *B. sporadicus* (Florida) and *B. diaphanus* (Haiti, Jamaica) has moderate support (bs: 72; not shown).

In the analyses using different species delimitation methods, the two groups within *B. guadalupensis* may be distinghuised using BGA with a 3% threshold, while at 4% threshold the difference is only marginal. Haplotype h4 shows a maximum distance of 0.041±0.008. In the SDG method using ML the B group has a relatively high intraspecific/interspecific ratio, but a relatively low P ID(strict) and non-significant values of Rosenberg's P_{AB} and Rodrigo's P(RD); however, using BI the two groups are significantly different using the latter parameter, although the other parameters hardly differ (Table 2, Supplementary information Table 3). The ABGD method resulted in one group for the recursive partition with a prior of 0.1; 13 with 0.060; 14 with 0.036; 17 with 0.022, 0.013, 0.008, 0.005, 0.003, and 0.002; 19 groups were found with prior 0.001. The initial

partition was stable on 17 groups at prior values of 0.022 and below. Of the two *B. guadalupensis* groups only group A is recognised with this method (Table 2, Supplementary information Table 4). On the contrary, in the GSI method both group A and B had a value of 1.00 (monophyly) with both ML and BI; all values were statistically significant, thus GSI suggests two species. Also within *B. diaphanus* two groups are suggested, but only one of these groups (from Jamaica and Haiti) has a significant value of 1.00. The analysed samples of *B. sporadicus* prove not to be monophyletic (Table 2, Supplementary information Table 5).

Discussion

Bulimulus guadalupensis is known to be very variable morphologically, although measurements on samples throughout its distribution area led Breure (1974) conclude that “no infraspecific differentiation occurs”. The results on this species reported in the present paper are not univocal, the two groups that are discernable depend on the species delimitation method chosen. Thus drawing nomenclatoral conclusions would – in the absence of clear external morphological differences – hinge totally on ambiguous molecular data. Since the locality data are often also not informative (e.g., in case of introduction or interception), no nomenclatorial conclusions can be drawn.

For *B. diaphanus* Breure (1974) recognised two subspecies, of which the samples studied in this paper from Haiti and Jamaica may be assigned to the nominate taxon, and the Nevis sample to

B. diaphanus fraterculus (Potiez & Michaud, 1835); more fresh tissue samples from different localities within its distribution range are needed to confirm the phylogenetic basis for the morphological differences reported earlier by Breure (1974). The polyphyly of samples of *B. diaphanus* in this study is possibly an indication of the presence of cryptic species in this group, but also here lack of data prevent me from drawing nomenclatoral conclusions.

The occurrence of alien species in Florida seems to follow a 'burst-diminishing pattern', also observed in other introduced species, from becoming very common after the introduction to diminishing to (nearly) gone after some time (B. Frank, pers. comm.). The Miami population of *B. guadalupensis* lasts currently at least ten years. The Jacksonville population of *B.* aff. *sporadicus* was only recently discovered and seems thriving, at least within the city limits, possibly resulting from the potential absence of natural enemies (such as *Euglandina* snails). Simberloff & Gibbons (2004) have suggested that population collapses of introduced species may simply be temporary lows during a more or less regular boom-and-burst cycle. The time span of such a process may vary from species to species due to specific conditions, and is as yet unknown for these *Bulimulus*. Other introductions of these species in the Caribbean are less well documented. In Curaçao (Breure, 1975) a population of *B. guadalupensis* is still present, but only in gardens in some suburban areas outside Willemstad (G. van Buurt, pers. commun.). From the network analysis (Fig. 4) it could be deduced that the introductions in Ecuador and Honduras may be traced back to the population in Puerto Rico, Guayabo, which may be linked to – in this case – export activities at the industrial park where the collection was made (Cf. Cowie et al., 2008). However, *Bulimulus*

239 species are not considered to be high-risk exotics (Cowie et al., 2009).The introduction in Ja-
240 maica of this species may be linked to a population on a different island, and in this case molec-
241 ular evidence suggests it may be traced to Barbados.

242 The phylogenetic analyses also provide some insights in the populations identified as *B. spo-*
243 *radicus* (d'Orbigny, 1835). It may be noted that only one is from a natural population, viz.
244 Paraguay, Concepción, albeit a somewhat aberrant specimen through damage of the shell at a
245 juvenile stage (Fig. 5). The two other populations sampled are alien in the U.S.A. (Houston, inter-
246 cepted by USDA; Jacksonville). This species is widespread in its area of origin and has been
247 recorded from Bolivia, Brazil (Mato Grosso, Rio Grande do Sul, Santa Catarina), Paraguay, and
248 Uruguay (Agudo-Padrón, 2014; Agudo-Padrón et al., 2014; Simone, 2006). In Uruguay and Ar-
249 gentina (Provs. Buenos Aires, Chaco, Cordoba, Corrientes, Entre Rios, Formosa, Missiones, Tu-
250 cumán) this taxon is replaced by the very similar *B. bonariensis* (Rafinesque, 1833) (Cuezzo et
251 al., 2013; Scarabino, 2003). Miquel (1991) and Cuezzo et al. (2013) consider these taxa as syn-
252 onyms, with Rafinesque's name having priority. However, the intraspecific variation within the
253 large distribution range of this species makes it likely to be a species complex. Thus it may war-
254 rant a more detailed study, including molecular research, to clarify the possible existence of
255 closely related taxa which may be difficult to distinguish on the basis of shell morphology alone.

256 This study shows that use of different species delimiting methods may produce different species
257 hypotheses, and are thus are of limited value to arrive at an unequivocal taxonomic interpretation
258 of these *Bulimulus* species, as was also observed by Prévot et al. (2013). In this case the rela-
259 tive low number of samples analysed, and the use of only one genetic marker, makes it hard to

convert the results into solid taxonomic decisions. Therefore it is suggested that the application of species delimitation methods may be of limited value as such, and should be complemented by other evidence, e.g. morphological studies.

Finally, the present study sheds some light on the usefulness of the barcoding method for rapid identification of intercepted snails of this group. Although the results strongly suggests that some of the physical moves (i.e. introduction as alien species in a distinct country) can be traced back to a source within the known distribution area, it is also clear that this holds true for the better known species only (e.g., *B. guadalupensis*). For others, especially for taxa that are morphologically very similar, the extent and reliability of the current database in GenBank is insufficient, especially with respect to areas where *Bulimulus* species are known to be native. In a wider perspective, where the effects of the global economy on non-marine gastropod introductions are becoming more and more manifest (Robinson, 1999), this is a problematic conclusion.

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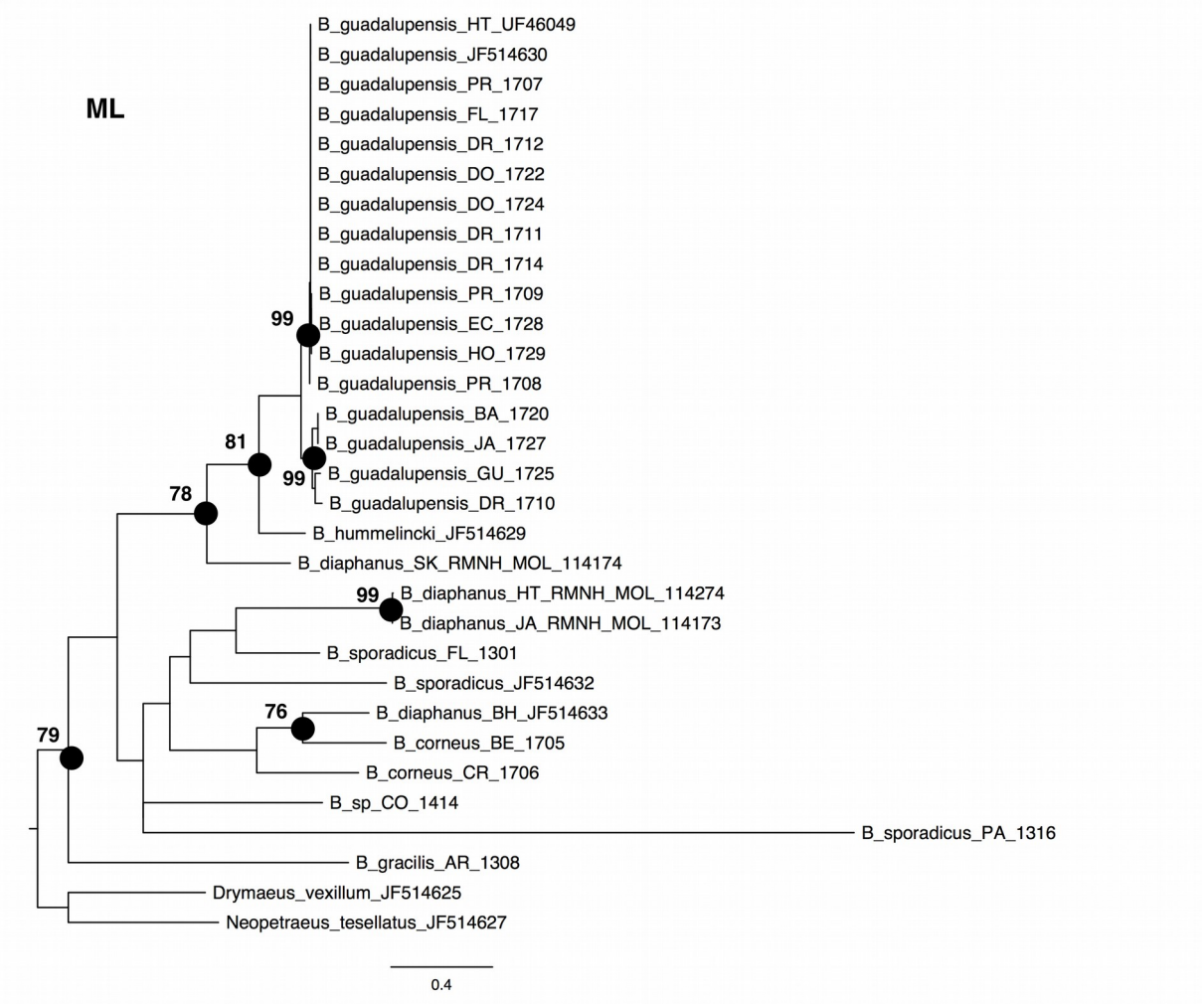
368 ——————

369 [Legends.]

370 Fig. 1. Maximum-likelyhood phylogeny for *Bulimulus* species, based on 654bp cytochrome oxi-

371 dase I mitochondrial DNA. Bootstrap values of 70 and above are presented to the left of the

372 nodes indicated by black dots. Scale bar in substitutions/site.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B_guadalupensis_PR_1707
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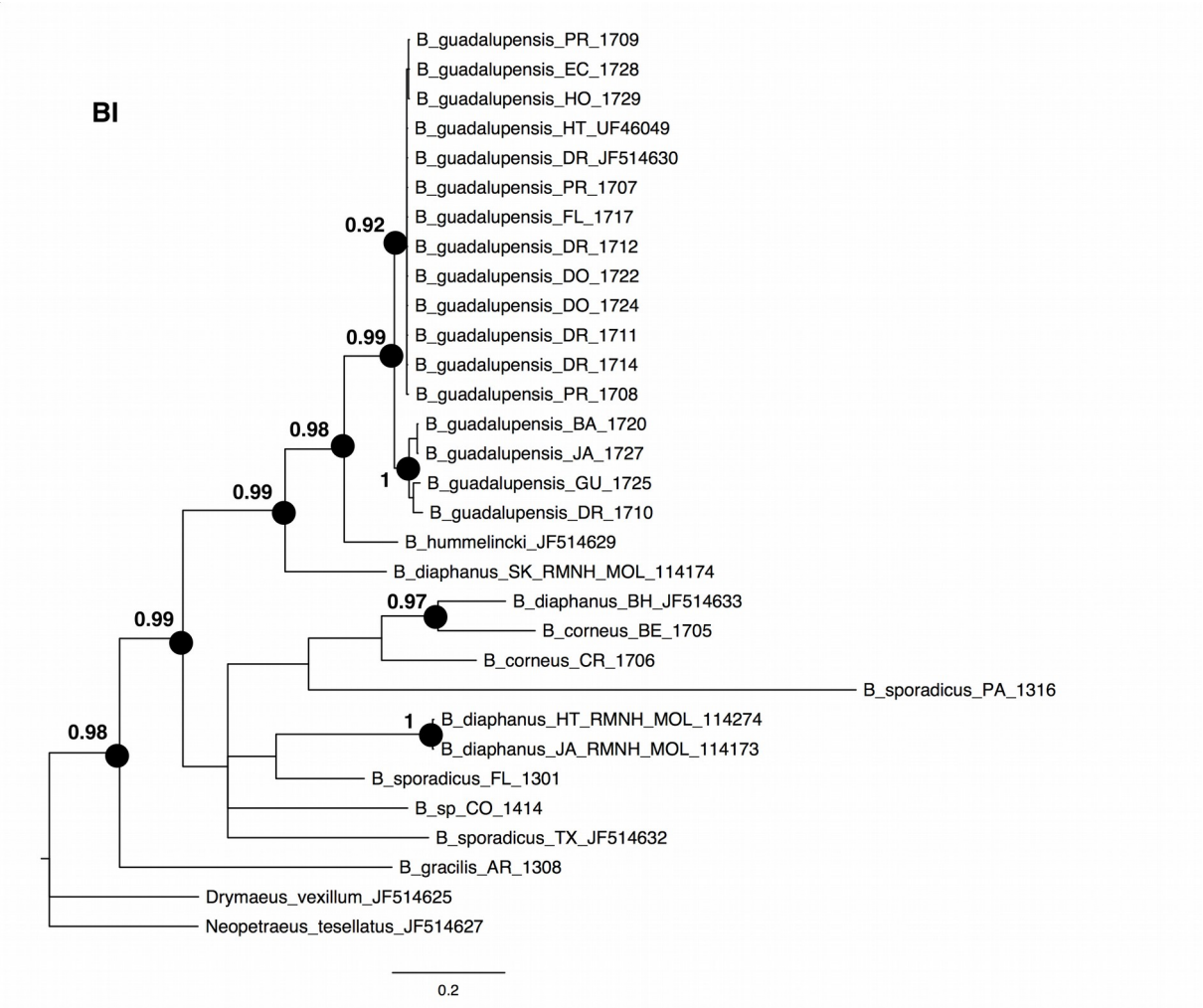
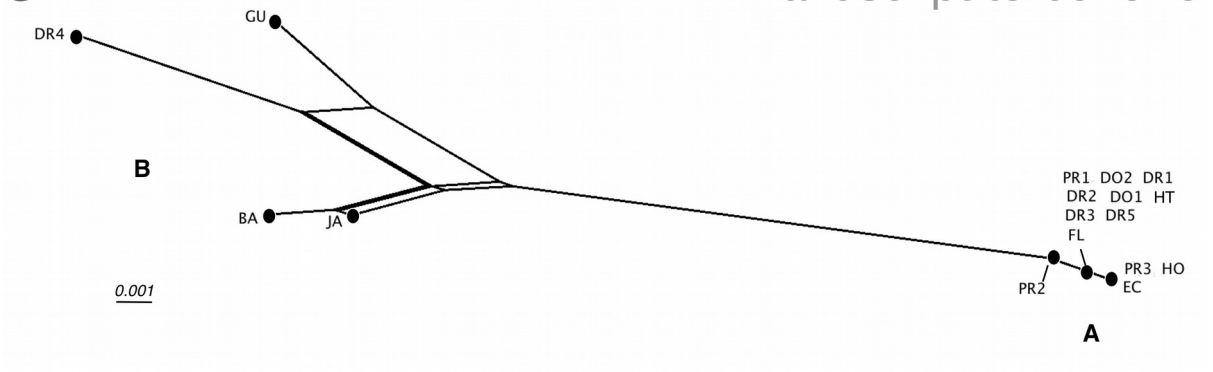
B_guadalupensis_DO_1722
B_guadalupensis_DO_1724
B_guadalupensis_DR_1711
B_guadalupensis_DR_1714
B_guadalupensis_PR_1709
99
B_guadalupensis_EC_1728
B_guadalupensis_HO_1729
B_guadalupensis_PR_1708
B_guadalupensis_BA_1720
81
B_guadalupensis_JA_1727
99
B_guadalupensis_GU_1725
78
B_guadalupensis_DR_1710
B_hummelincki_JF514629
B_diaphanus_SK_RMNH_MOL_114174
99
B_diaphanus_HT_RMNH_MOL_114274
B_diaphanus_JA_RMNH_MOL_114173
B_sporadicus_FL_1301
B_sporadicus_JF514632
76
B_diaphanus_BH_JF514633
79
B_corneus_BE_1705
B_corneus_CR_1706
B_sp_CO_1414
B_sporadicus_PA_1316
B_gracilis_AR_1308
Drymaeus_vexillum_JF514625
Neopetraeus_tesellatus_JF514627
0.4

373 Fig. 2. Bayesian phylogeny for *Bulimulus* species using MrBayes, based on the same dataset as



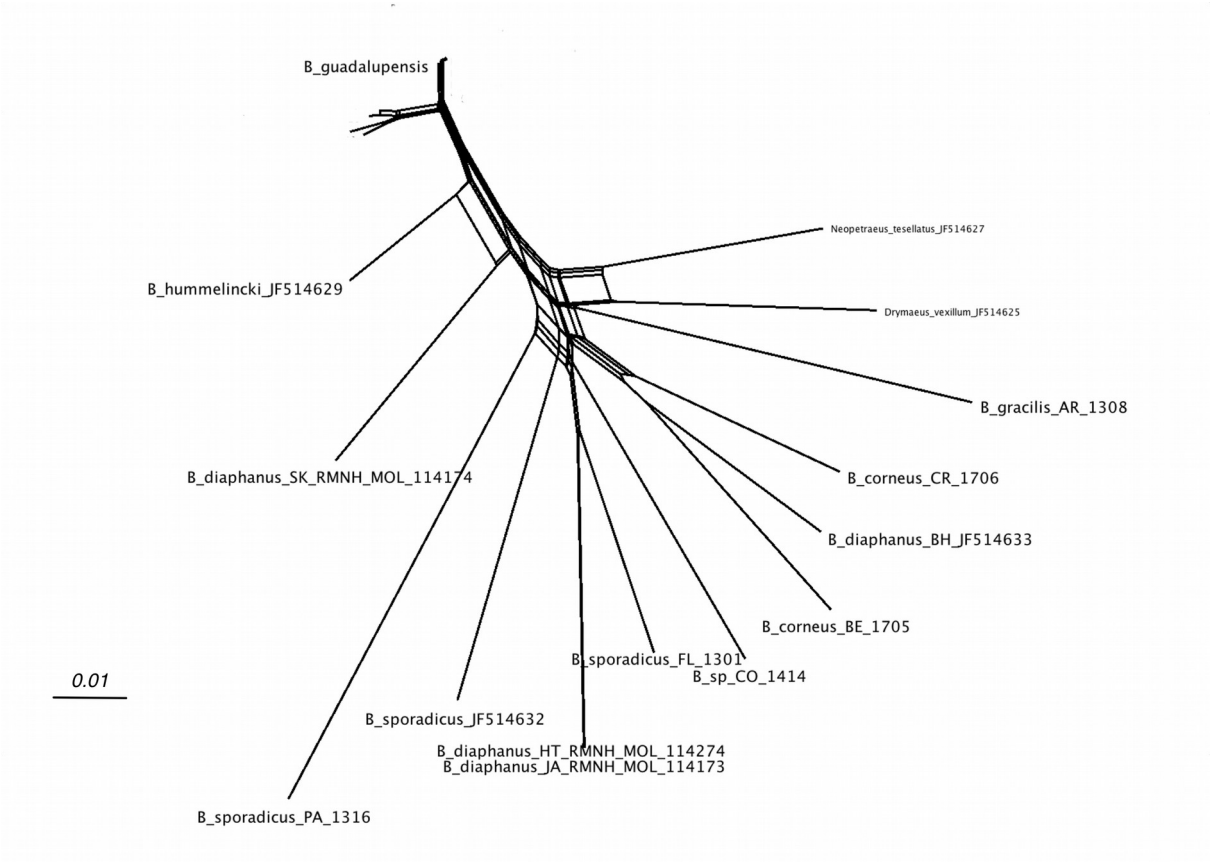
374 shown in Fig. 1. Posterior probabilities of 0.9 or above are indicated by black dots at the nodes.

375 Scale bar in substitutions/site.



376 Fig. 3. SplitsTree NeighborNet network of *Bulimulus guadalupensis* (Broderip, 1789), based on

377 K2P distances and EqualAngle representation. Label explanation given in text.



378 Fig. 4. SplitsTree NeighborNet network of all *Bulimulus* species from the analysed dataset,

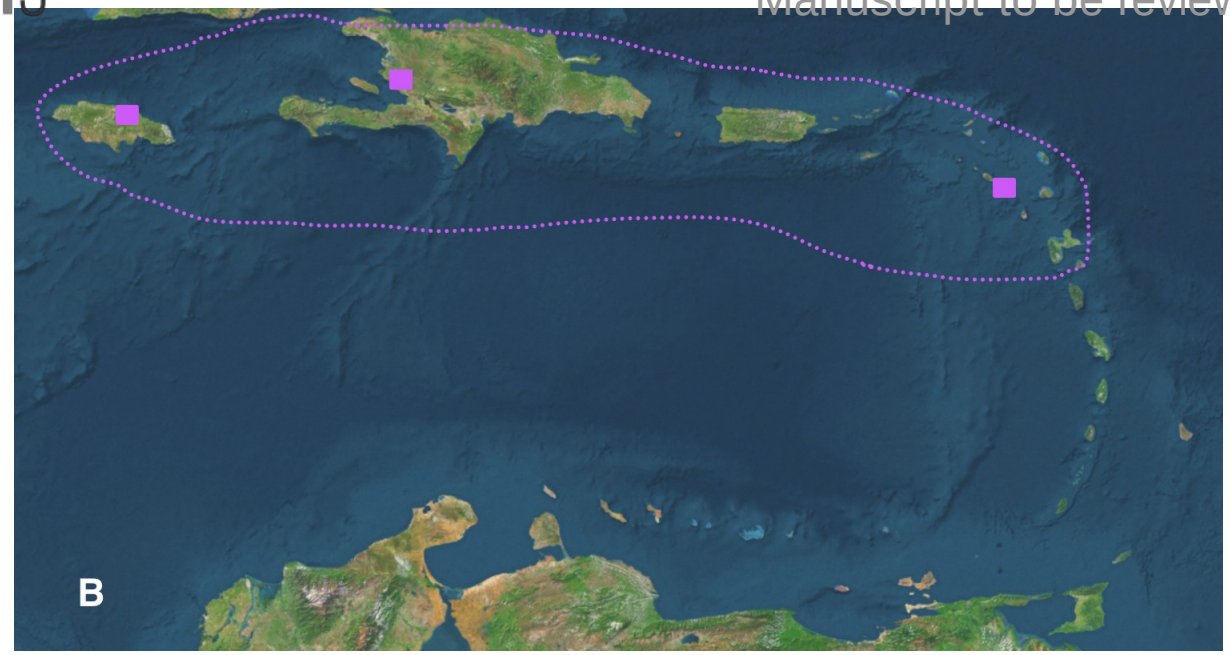
379 based on K2P distances and EqualAngle representation. Label explanation given in text.

380 Fig. 5. *Bulimulus sporadicus* (d'Orbigny, 1835), Paraguay, Concepción. U. Drechsel leg. RMNH.-

381 MOL.336661 (shell height 31.3 mm). A, living specimen (photo by courtesy U. Drechsel). B, dif-

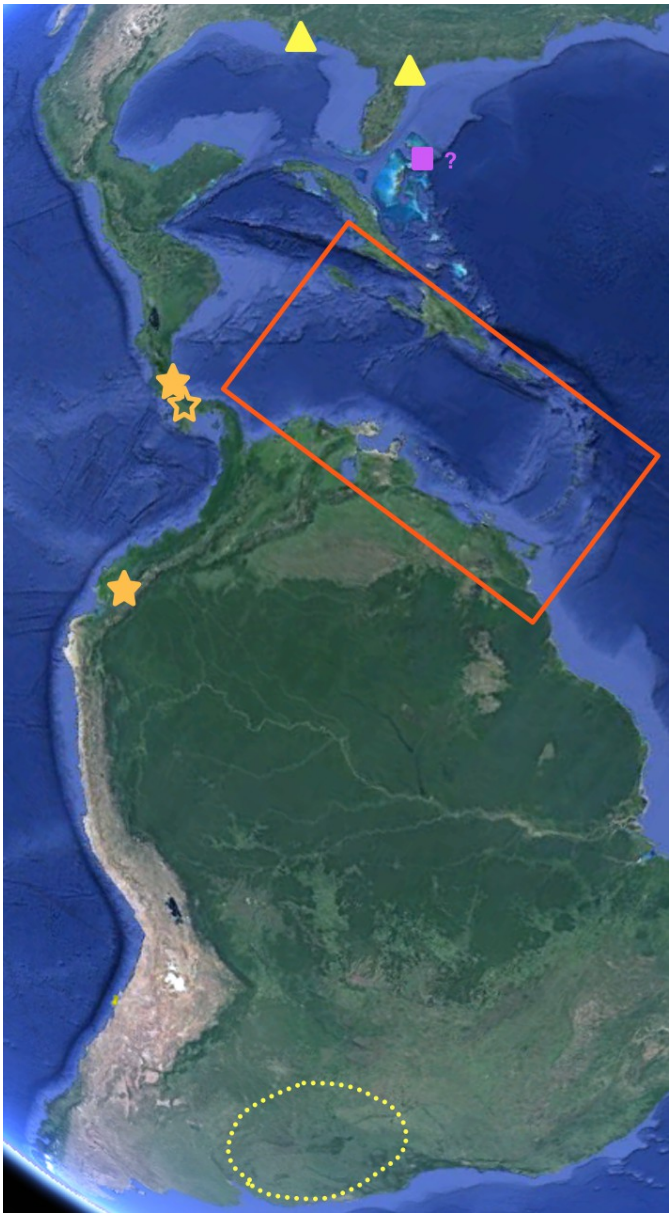
382 ferent views of the shell, arrow indicating the place where the carination starts.

383 Fig. 6. Distribution of A – *Bulimulus guadalupensis* (orange), and B – *B. diaphanus* (purple) in
384 the West Indies. Dotted lines denote generalised distribution area, solid symbols sampled locali-
385 ties. Note that the Haitian locality is only an approximation


B

386 Fig. 7. Distribution of *Bulimulus sporadicus* (yellow), *B. guadalupensis*, and *B. diaphanus* in the

387 Neotropics. Red square is area enlarged in Fig. 6. Open symbol is a locality not sampled; other



388 symbols, see legends of Fig. 6.

Table 1. Material used in this paper. Abbreviations for vouchers: ANSP, Academy of Natural Sciences, Philadelphia, U.S.A.; CMC, Cincinnati Museum, Cincinnati, U.S.A.; IML, Instituto Miguel Lillo, Tucumán, Argentina; RMNH, Naturalis Biodiversity Center (NBC), Leiden, the Netherlands; UF, Florida Museum of Natural History, Gainesville, U.S.A.; USDA, United States Department of Agriculture, Malacology Collection, ANSP, Philadelphia, U.S.A. Other abbreviations: LabID, NBC molecular laboratorium code, including a two-letter locality code; MOTU, molecular operational taxonomic unit sensu Blaxter, 2004.

MOTU	LabID	Species			Voucher	Locality				Collector(s)	CO1
s1	FL_1301	Bulimulus	sporadicus	(d'Orbigny, 1	RMNH.MOL.	USA	Florida	Nassau Coun	S Callahan	H.Lee&EW Ca	KP117235
	AR_1308	Bulimulus	gracilis	Hylton Scott,	IML-BD368	Argentina	Salta		Sierra Colora	E. Salas O.	KP117237
s2	PA_1316	Bulimulus	sporadicus	(d'Orbigny, 1	RMNH.MOL. 336661	Paraguay	Concepcion		Garay-Cue	U.Drechsel	KP117238
	CO_1414	Bulimulus	sp.		CMC-FJB78	Colombia	Magdalena		Santa Marta,	FJ Borrero	KP117236
gA	PR_1707	Bulimulus	guadalupens	(Bruguiere 17	USDA 11019	Puerto Rico		Huamaco	Isla Vieques,	DGRobinson	KP117239
gA	PR_1708	Bulimulus	guadalupens	(Bruguiere 17	USDA 11019	Puerto Rico		Huamaco	Isla Vieques,	DGRobinson	KP117249
gA	PR_1709	Bulimulus	guadalupens	(Bruguiere 17	USDA 11019	Puerto Rico		Bayamon	Guayabo, Am	DGRobinson	KP117246
gA		Bulimulus	guadalupens	(Bruguiere 17	RMNH.MOL.1	Dominican Republic			Santo Domin	J. Grego	JF514630
gB	DR_1710	Bulimulus	guadalupens	(Bruguiere 17	USDA 11020	Dominican Republic		Samana	Las Terrenas	DGRobinson	KP117253
gA	DR_1711	Bulimulus	guadalupens	(Bruguiere 17	USDA 11019	Dominican Republic		Samana	Come Pan	DGRobinson	KP117244
gA	DR_1712	Bulimulus	guadalupens	(Bruguiere 17	USDA 11019	Dominican Republic		Espaillat	La Cantera	DGRobinson	KP117241
gA	DR_1714	Bulimulus	guadalupens	(Bruguiere 17	USDA 11020	Dominican Republic			4 km S Cabre	DGRobinson	KP117245
gA	FL_1717	Bulimulus	guadalupens	(Bruguiere 17	USDA 11020	USA	Florida	Dade	Miami, Coral	DGRobinson	KP117240
gB	BA_1720	Bulimulus	guadalupens	(Bruguiere 17	USDA 11020	Barbados	St.Thomas		Sandy Lane	DGRobinson	KP117250
gA	DO_1722	Bulimulus	guadalupens	(Bruguiere 17	USDA 10070	Dominica	St.George		Bellevue Cho	DGRobinson	KP117242
gA	DO_1724	Bulimulus	guadalupens	(Bruguiere 17	USDA 10071	Dominica	St.Luke		Pointe Michel	DGRobinson	KP117243
gB	GU_1725	Bulimulus	guadalupens	(Bruguiere 17	USDA 11020	Guadeloupe	Grande-Terre		Sainte-Anne	DGRobinson	KP117252
gB	JA_1727	Bulimulus	guadalupens	(Bruguiere 17	USDA 11019	Jamaica		Hanover	Lucea, weste	DGRobinson	KP117251
gA	EC_1728	Bulimulus	guadalupens	(Bruguiere 17	USDA 11021	Ecuador			(USDA interce	A.Hansen	KP117247
gA	HO_1729	Bulimulus	guadalupens	(Bruguiere 17	USDA 11016	Honduras		ElParaiso	Yuscaran, km	H.Deschamps	KP117248
d1		Bulimulus	diaphanus	(Pfeiffer, 1855)	RMNH.MOL. 114173	Jamaica		St. Ann	Runaway Bay	DGRobinson	KP117232
d2		Bulimulus	diaphanus	(Pfeiffer, 1855)	RMNH.MOL. 114174	St. Kitts & Nevis	Nevis		Herbert Heights	DGRobinson	KP117233
d1		Bulimulus	diaphanus	(Pfeiffer, 1855)	RMNH.MOL. 114274	Haiti			(USDA interception)	D. Valleso	KP117231
d3		Bulimulus	diaphanus	(Pfeiffer, 1855)	ANSP A22054	Bahamas			(USDA interception)	G. Watkins	JF514633

396 Table 2. Number of putative species delimited by different methods applied to the dataset. BGA,

Putative species	BGA		SDG		ABGD	GSI	
	3%	4%	ML	BI		ML	BI
gA	+	-	+	+	+	+	+
gB	+	-	-	+	-	+	+
d1	+	+	-	+	+	+	+
d2	+	+	nc	nc	+	nc	nc
d3	+	+	nc	nc	+	nc	nc
s1	+	+	nc	nc	+	nc	nc
s2	+	+	nc	nc	+	nc	nc
s3	+	+	nc	nc	+	nc	nc

397 Barcode gap analysis; SDG, species delimiting in Geneious; ABGD, Automated barcode gap

398 discovery; GSI, Genealogical sorting index.

399 Supplementary information, Figure 1. Neighbour-joining phylogeny for *Bulimulus* species, based
400 on 654bp cytochrome oxidase I mitochondrila DNA. Bootstrap values of 70 and above are pre-
401 sented to the left of the nodes indicated by black dots. Scale bar in substitutions/site. (JPEG file).

402 Supplementary information, Figure 2. Maximum-parsimony phylogeny for *Bulimulus* species,
403 based on 654bp cytochrome oxidase I mitochondrila DNA. Bootstrap values of 90 and above are
404 presented to the left of the nodes indicated by black dots. (JPEG file).

405 Supplementary information, Table 1. Delimitation of MOTUs using K2P distances and standard
406 error at 3% threshold. gA and gB, *Bulimulus guadalupensis* group A respectively B. (XLSX file).

407 Supplementary information, Table 2. Delimitation of MOTUs using K2P distances and standard
408 error at 4% threshold. gA and gB, *Bulimulus guadalupensis* group A respectively B. (XLSX file).

409 Supplementary information, Table 3. Species delimiting as implemented in Geneious, using ML
410 and BI for both the total dataset and a subgroup of MOTUs. Closest species, Intraspecific dis-
411 tance, Interspecies distance, ratio of Intra/Interspecific, P ID(strict), Rosenberg's P_{AB}, and Ro-

412 drigo's P(RD) are indicated. Colours code for significance. c, d, g, gr, hu, s, and sp. correspond
413 with the respective taxon names; NA, not applicable. (XLSX file).

414 Supplementary information, Table 4. Mean p-distances between and within (diagonal) the differ-
415 ent MOTUs based on the dataset analysed. EDS, Estimates of Evolutionary Divergence between
416 Sequences; NEDGS, Net Evolutionary Divergence between Group of Sequences. c, d, g, gr, hu,
417 s, and sp. correspond with the respective taxon names; n/c, not calculated. Colours code the
418 corresponding groups. (XLSX file).

419 Supplementary information, Table 5. Results for different combinations of MOTUs, using rooted
420 trees of both ML and BI analyses, in terms of genealogical sorting index and corresponding p-
421 values based on a permutation test of 10,000 replicates. Colours code the corresponding
422 groups. (XLSX file).