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Molecular evidence supports an African affinity of the Neotropical freshwater gastropod, *Biomphalaria glabrata*, Say 1818, an intermediate host for *Schistosoma mansoni*

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Freshwater snails of the genus *Biomphalaria*, Preston 1910, are the most important and widely distributed intermediate hosts of *Schistosoma mansoni*, the blood fluke responsible for human intestinal schistosomiasis, in Africa and the Neotropics. *S. mansoni* is thought to have been imported repeatedly into the Americas during the last 500 years with the African slave trade. Surprisingly, considering that the New and Old World separated 95–106 million years (Myr) ago, the disease rapidly became established due to the presence of endemic susceptible hosts. Reconstructing the phylogenetic relationships within *Biomphalaria* may provide insights into the successful intercontinental spread of *S. mansoni*. Parsimony and distance analyses of mitochondrial and nuclear sequences show African taxa to be monophyletic and Neotropical species paraphyletic, with *Biomphalaria glabrata* forming a separate clade from other Neotropical *Biomphalaria*, and ancestral to the African taxa. A west to east trans-Atlantic dispersal of a *B. glabrata*-like taxon, possibly as recently as the Plio–Pleistocene (1.8–3.6 Myr ago) according to a general mitochondrial clock, would fit these observations. Vicariance or an African origin for *B. glabrata* followed by multiple introductions to South America over the past 500 years with the African slave trade seem unlikely explanations. Knowledge of the phylogenetic relationships among important intermediate host species may prove useful in furthering control measures which exploit genetic differences in susceptibility to parasites, and in elucidating the evolution of schistosome resistance.

Keywords: *Biomphalaria glabrata*; African and Neotropical snails; mitochondrial DNA; nuclear ribosomal DNA; phylogeny; Pleistocene

1. INTRODUCTION

Schistosomiasis infects 200 million people worldwide, with over 600 million people estimated to be at risk (WHO 1993). Freshwater snails belonging to the pulmonate genus *Biomphalaria*, Preston 1910, are the most important and widely distributed intermediate hosts of *Schistosoma mansoni*, the causative agent of human intestinal schistosomiasis, in Africa and the Neotropics. Out of these, 12 species have been identified in Africa (Brown 1994) and 19 are known in South America (Malek 1985). The presence of susceptible snails is thought to have been instrumental in the rapid establishment and spread of schistosomiasis in the New World, *S. mansoni* being repeatedly imported with African slaves within the last 500 years (Files 1951; Fletcher *et al.* 1981; Combes 1990; Després *et al.* 1993). However, considering that South America and Africa separated 95–106 million years (Myr) ago, it is puzzling why New World intermediate snail hosts should be compatible. Were African snail hosts imported with the parasite, later to become endemic to South America, or, do South American *Biomphalaria* have a closer affinity with African taxa than previously thought?

Not all *Biomphalaria* species play an equal role in schistosome transmission and even susceptible taxa vary in their compatibility to different strains of *S. mansoni* (Frandsen 1979), therefore, particular attention has focused on systematic identification and classification. However, an indication of the evolutionary relationships between snail taxa may provide insights into intermediate host–parasite coevolution and compatibility. Earlier phylogenies of *Biomphalaria* based on allozymes (Bandoni *et al.* 1995; Woodruff & Mulvey 1997) suggest that the Neotropical *Biomphalaria glabrata* has strong African affinities. However, important disagreements between these two allozyme-based phylogenies exist. Woodruff & Mulvey's (1997) phylogeny clusters African taxa with *B. glabrata*, but most importantly shows *Biomphalaria pfeifferi* as ancestral to *B. glabrata*, implying that *B. glabrata* descended from African stock. Conversely, earlier work by Bandoni *et al.* (1995) places *B. glabrata* in a sister clade to the African species, with *B. glabrata* ancestral to *B. pfeifferi* and other African taxa. However, as a phylogenetic tool, allozymes are considered to be less informative than other molecular approaches due to high levels of cryptic variation, indirect methods of comparison of allelic variants, limited resolution at the species level and disagreement about the appropriate methods of phylogenetic reconstruction (Swofford & Olsen 1990; Murphy *et al.* 1996). The conflict between allozyme data sets indicates DNA sequence analyses are required to resolve the relationships within *Biomphalaria*, which may provide insights into the intercontinental spread of *S. mansoni*.

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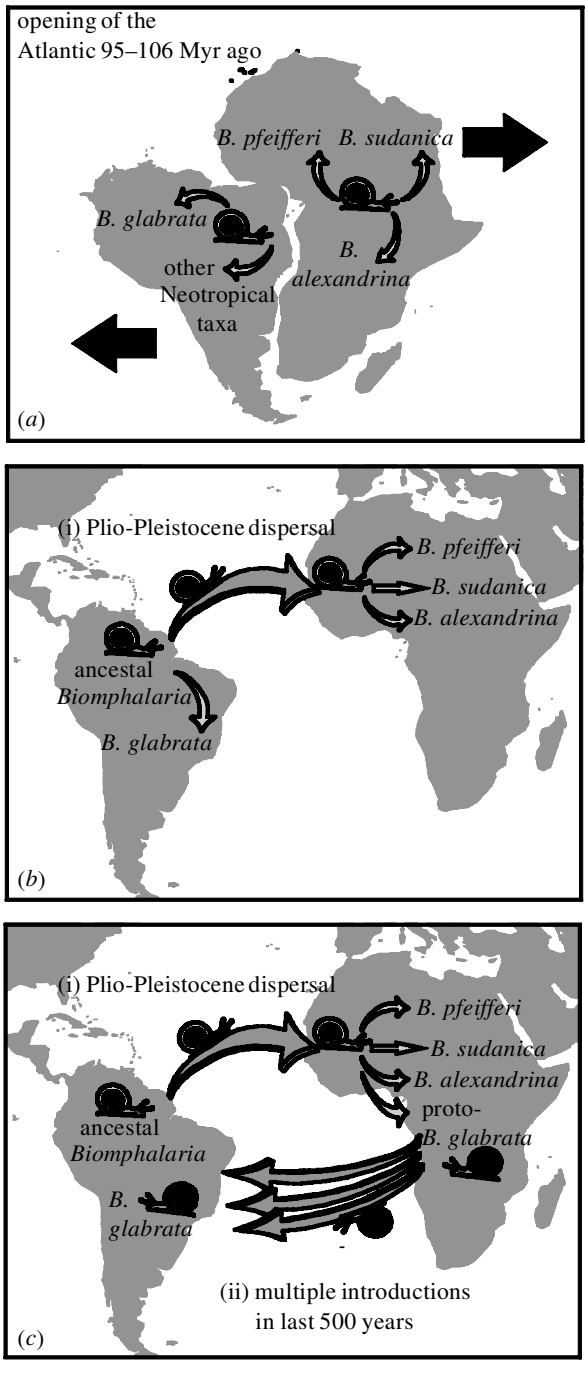

Figure 1. Colonization hypotheses of the evolutionary history of *Biomphalaria* taxa. (*a*) Simple vicariance model. Species evolved *in situ* and became separated as the continents drifted apart 95–106 Myr ago (Davis 1980). (*b*) Simple dispersal model. A west to east dispersal of South American *B. glabrata*-like taxon across the Atlantic to Africa. The African species *B. alexandrina*, *B. sudanica* and *B. pfeifferi* are recent Plio–Pleistocene (2 Myr ago) derivatives of this American form. In Africa these snails subsequently became intermediate hosts for the lateral-spined schistosome, *S. mansoni*. *B. glabrata* evolved *in situ* in the New World with other South American taxa. (*c*) Multiple dispersal model. *B. alexandrina*, *B. sudanica*, *B. pfeifferi* and proto-*B. glabrata* evolved in Africa in the Plio–Pleistocene following an earlier trans-Atlantic dispersal from the Americas, followed by multiple introductions of the taxon currently known as *B. glabrata* back to the Americas in the last 500 years in water casks of African slave traders (Woodruff & Mulvey 1997).

Here we intend to test three hypotheses regarding evolution of *Biomphalaria* (figure 1) through analysis of partial sequences of mitochondrial DNA (mtDNA) cytochrome oxidase subunit I (COI) gene and the nuclear ribosomal RNA internal transcribed spacer 1 region (ITS1). The first is a simple vicariance model, whereby species evolved *in situ* and became separated as the continents drifted apart 95–106 Myr ago (Davis 1980). Second, the hypothesis that a South American *B. glabrata*-like taxon dispersed across the Atlantic to Africa and African species *Biomphalaria alexandrina*, *Biomphalaria sudanica* and *B. pfeifferi* are Plio–Pleistocene (Van Damme 1984) derivatives of this American form. In Africa these snails subsequently became intermediate hosts for the lateral-spined schistosomes. A third view is that *B. alexandrina*, *B. sudanica*, *B. pfeifferi* and proto-*B. glabrata* evolved in Africa in the Plio–Pleistocene following an earlier trans-Atlantic dispersal from the Americas, and that the taxon currently known as *B. glabrata* returned to the Americas in the last 500 years (Woodruff & Mulvey 1997). Understanding the evolutionary relationships of these taxa in the context of their biogeography and parasite compatibility is of general relevance to studies of host–parasite coevolution and the maintenance and spread of schistosome-resistance genes.

2. MATERIAL AND METHODS

Study material (table 1) comprised seven species of *Biomphalaria*, three from the African fauna, *B. pfeifferi*, Krauss 1848, *B. sudanica*, Martens 1870, and *B. alexandrina*, Ehrenberg 1831, and four Neotropical species, *B. glabrata*, Say 1818, *B. straminea*, Dunker 1848, *B. tenagophila*, Orbigny 1835, and *B. occidentalis*, Paraense 1981. All species, with the exception of *B. occidentalis*, are responsible for the transmission of *S. mansoni*. The range of several species has recently increased through the establishment of naturalized populations due to accidental introductions. Two such taxa are incorporated into the analyses including Egyptian *B. glabrata* and *B. straminea* from Hong Kong.

Total genomic DNA was extracted from ethanol-preserved material following a modified phenol–chloroform protocol (Vernon *et al.* 1995). Primer sequences used to amplify a 579 bp fragment of mtDNA COI were obtained from Folmer *et al.* (1994). A 569 bp fragment of the ITS1 was amplified using the primers ETTS2 (Kane & Rollinson 1994) and ETTS16 (Stothard *et al.* 1996). PCR cycling conditions for COI were 94 °C for 75 s, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s. ITS1 was amplified following Stothard *et al.* (1996). PCR products were purified using Qiaquick PCR^{TM} purification columns (Qiagen, Inc., Crawley, UK) and sequenced on both strands using an Applied Biosystems, Inc. 377 automated sequencer (BigDye Terminator^{TM} Cycle Sequence Kit; ABI, Warrington, UK).

The COI ingroup comprises 20 sequences (four *B. glabrata*, six *B. pfeifferi* and ten from additional African and Neotropical taxa) and the ITS1 ingroup 14 sequences (three *B. glabrata*, six *B. pfeifferi* and five from other African and Neotropical taxa) (table 1). Between the two data sets some of the samples differ by museum accession number; being from the same country of origin but different geographical regions. Original data sets were truncated to speed up the analyses; replicates of species from the nearby localities were removed. Also included among the *B. glabrata* samples are laboratory-reared resistant (100 and 70%) and susceptible strains.

Table 1. Biomphalaria *samples used in the phylogenetic study*

(Location source and Natural History Museum (NHM) accession numbers of samples are given where applicable.)

species	NHM accession no.	location source
B. pfeifferi	1971, 1916, 1915[a]	Senegal
	1978, 1910[a]	Cameroon
	1903, 1792[a]	Mali
	1488	Ethiopia
	—	Zimbabwe
	1970	Madagascar
B. sudanica	1091	Burundi
	1908	Uganda
	1986	Kenya
	1925	Zambia
B. alexandrina	1862, 1972[a]	Egypt
B. glabrata	1141	Brazil
	1973	Egypt
	1778	Brazil (70% resistant)[b]
	1742	Brazil (susceptible)[b]
	1981	Brazil (100% resistant)[b]
	1938	Brazil
B. straminea	1107	Hong Kong
	1939	Brazil
B. tenagophila	1941	Brazil
B. occidentalis	1940	Brazil

[a] Each number refers to a different site within the country of origin.
[b] Laboratory strains of variable resistance to laboratory lines of *S. mansoni*.

Sequences were edited using SEQED^{TM} software (v. 1.03; Applied Biosystems, Inc.), and aligned in Clustal X (Thompson *et al.* 1997). Phylogenetic reconstruction was estimated using best-fit methods based on distance (Fitch–Margoliash least-squares optimization method; Fitch & Margoliash 1967), maximum-likelihood methods (quartet puzzling; Strimmer & Von Haeseler 1996), and maximum parsimony (Swofford 1996). Fitch-generated distance trees were achieved using PHYLIP 3.5 (Felsenstein 1993). The distance method applied was the Kimura two-parameter model (Kimura 1980). The heuristic search option of PAUP 4.0 v. 3.c (phylogenetic analyses using parsimony; Swofford 1996) was used with gaps set as missing data for all maximum-parsimony analyses, enabling a direct comparison of parsimony- and distance-derived phylogenies. Nodal support was assessed by bootstrap analysis (Felsenstein 1985). For each data set, 1000 bootstrap replications were performed. Computation of maximum-likelihood trees using PUZZLE 4.0.2 (Strimmer & Von Haeseler 1996) was achieved without incorporating a molecular clock, but with substitution rates following Hasegawa *et al.* (1985), and the model of rate heterogeneity set at a uniform rate. Alternative models of substitution (e.g. Tamura–Nei) and different rates of heterogeneity (e.g. gamma distribution) were implemented, but had no impact upon the main branch topology of the tree or support values.

Outgroups used in the phylogenetic analyses were chosen from other Planorbidae; *Gyraulus chinensis* for mtDNA COI and *Bulinus truncatus*, Audouin 1827, for the ITS1 data set. Different outgroups were used for the two data sets because PCR amplification of *Gyraulus chinensis* was unsuccessful with ITS1 primers.

3. RESULTS

Phylogenetic reconstruction used nucleotide sequences obtained from seven species of *Biomphalaria*, representing African and Neotropical taxa, for part of the mtDNA COI gene (579 bp) and the entire nuclear rDNA ITS1 region (563 bp). COI sequences displayed congruent topologies for the significant branches (i.e. bootstrap values > 70%) with each methodology (figure 2). Parsimony analysis gave a consistency index (CI) of 0.661, indicating relatively low levels of noise and a phylogenetically informative signal showing the Neotropical taxa as ancestral to the monophyletic African clade, with *B. tenagophila*, *B. occidentalis* and *B. straminea* ancestral to *B. glabrata*. Within the African clade *B. sudanica* and *B. alexandrina* form a sister group to *B. pfeifferi* (figure 2*a*). This is congruent for all algorithms and is consequently well supported by the data. The Neotropical species, however, are paraphyletic, with *B. glabrata* clustering with the African taxa. This apparently anomalous association is again supported by all phylogenetic methods applied. The bootstrap values (in the case of the distance and parsimony analyses) show this grouping to be supported at levels of 77 and 74%, respectively. The quartet-puzzling support values can be interpreted in a similar way (Strimmer & Von Haeseler 1996) showing a greater support for this branch at 93% (figure 2*b*). Neotropical taxa, *B. occidentalis* and *B. tenagophila*, are grouped together with high nodal support for this branching for all algorithms (98–99%). Although naturalized *B. straminea* from Hong Kong cluster with native populations from South America, the phylogenetic position of *B. straminea* remains unresolved with respect to the other Neotropical species.

Wild-caught snails from Egypt identified as *B. glabrata* (Yousif *et al.* 1996) are thought to have descended from laboratory colonies. The mtDNA COI analysis places Egyptian *B. glabrata* firmly within the *B. glabrata* group (figure 2*a*), strongly supported by a 100% bootstrap, confirming a close relationship with present day South American strains.

Phylogenetic reconstruction using the nuclear rRNA ITS1 region is congruent with the mtDNA COI results (figure 3). Parsimony analysis gives a high CI value (0.911) suggesting a robust phylogeny (figure 3*a*). For all algorithms, the Neotropical taxa are again paraphyletic, with *B. glabrata* forming a separate group with the African species, well supported by the data with bootstrap values of 89% and quartet-puzzling support values of 90%.

Mean pairwise percentage sequence divergence for all seven species, for both the mtDNA COI and ITS1, are given in table 2. Distances for mtDNA COI demonstrate a close affinity between the Neotropical *B. glabrata* and the African species (mean = 7.4%, range 5.5–9.9%), while estimates of *B. glabrata* to the other Neotropical species are more distant (mean = 9.3%, range 7.9–10%). The ITS1 distance estimates are congruent with these data (*B. glabrata* African taxa mean = 4.1%, range 0.4–5.4%; other Neotropical taxa mean = 10.6%, range 9.4–11.4%).

4. DISCUSSION

Phylogenetic reconstruction of the *Biomphalaria* species, based on homologous sequence data, is well supported by

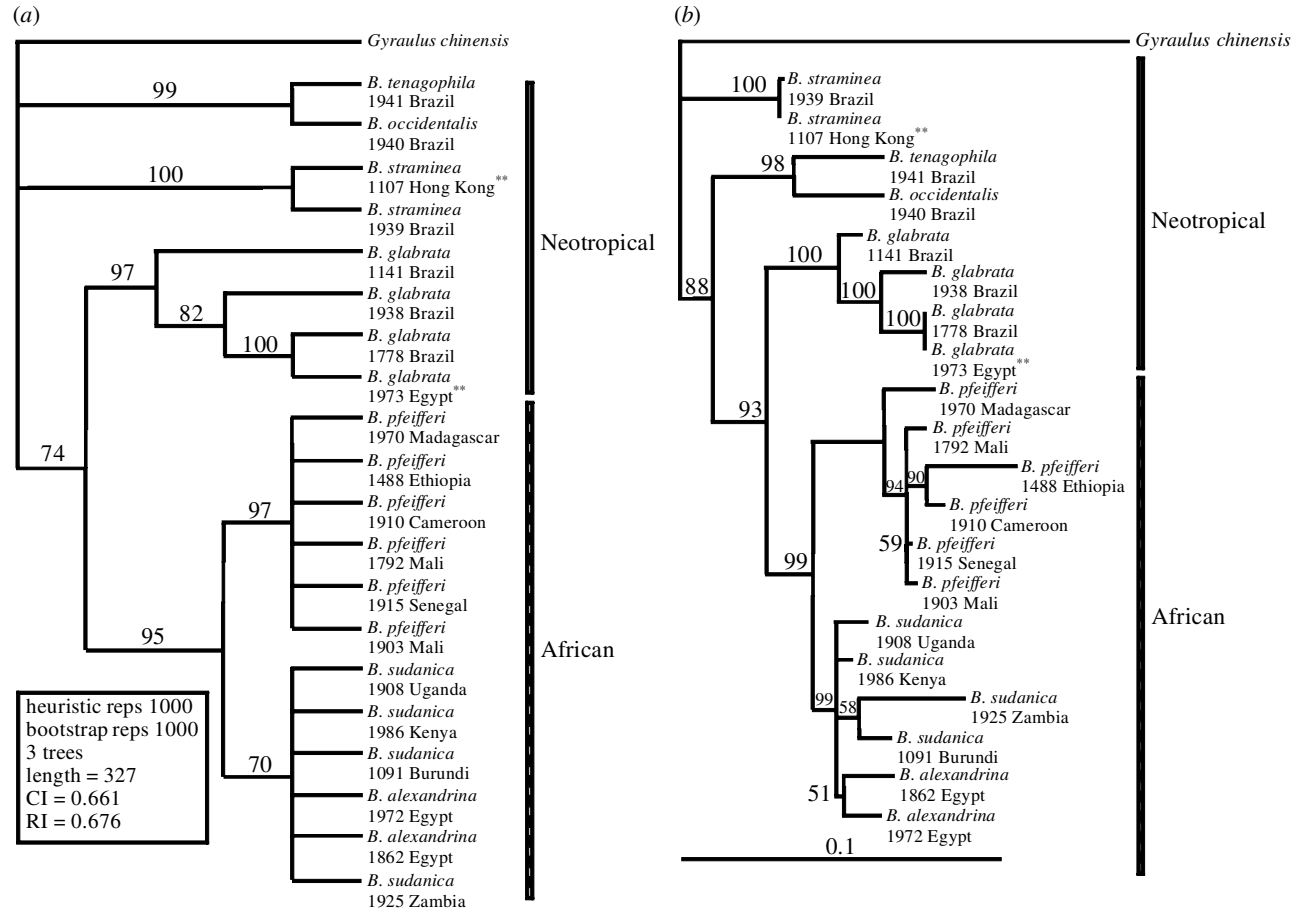

Figure 2. Phylogenetic relationships among *Biomphalaria* spp. with partial mtDNA COI sequences. (*a*) Bootstrap 50% majority rule consensus tree of the three most parsimonious. Tree search algorithm: heuristic (1000 replicates). Number at the branch nodes indicates percentage bootstrap support for 1000 replicates. CI, consistency index; RI, retention index. (*b*) Maximum-likelihood neighbour-joining tree using quartet puzzling (Strimmer & Von Haeseler 1996). Number at the branch nodes indicates percentage quartet-puzzling support values for 1000 puzzling steps. The scale denotes pairwise nucleotide substitution. African and Neotropical species are indicated by hatched and solid boxes, respectively. Taxa depicted by asterisks indicate naturalized populations which were accidentally introduced. Sequences are deposited under GenBank accession numbers AF199084–AF199111.

bootstraps and the main branch topologies of the trees were robust to all methods of analysis (parsimony and distance) and regions sequenced. Results from a partial mitochondrial gene sequence (COI) and a nuclear region (ITS1 rDNA) are congruent, indicating a stable phylogeny. Our key findings are first that Neotropical species diverged earlier than the African taxa. Second, the Neotropical species are paraphyletic, with the African taxa and the Neotropical *B. glabrata* forming a separate monophyletic clade, suggesting that *B. glabrata* is genealogically more closely related to the African species than to other Neotropical taxa. Third, proto-*B. glabrata* is ancestral to the African taxa, a finding that contradicts the perception (Woodruff & Mulvey 1997) of *B. glabrata* as a more recent African-derived taxon.

An earlier South American origin for *Biomphalaria* is consistent with fossil evidence. Early and Mid-Pleistocene freshwater gastropods are rare in Africa and the Americas but available fossil evidence shows *Biomphalaria*-like shells (nomenclature originally *Tropicus*, *Australorbis* and *Taphius*, Adams 1855) in Brazilian Palaeocene and Pleistocene deposits (Parodiz 1969). The earliest record of *Biomphalaria* (*pfeifferi*) in North Africa is in the Maghrebian lakes of the late Pliocene or Pleistocene, dating from the 'Villefranchian' Pluvial (Van Damme 1984). Furthermore, African taxa are morphologically less variable than Neotropical species (Mello 1972), substantiating the contention that African species are younger than their Neotropic congeners. Similarly, the remarkably high levels of intraspecific genetic variability of South American *Biomphalaria* species, particularly *B. glabrata* (Vidigal *et al.* 1994, 1998), has led to difficulties in generating molecular markers for species identification. Woodruff & Mulvey (1997) concluded from allozyme data that *B. glabrata* is either the most genetically variable mollusc species yet described or a syngameon of more than three semi-species. Conversely, biochemical and molecular differentiation of three African *Biomphalaria* was more readily demonstrated, owing to their more limited genetic variability (Henriksen & Jelnes 1980; Kristensen *et al.* 1998).

Although fossil, morphological and genetic evidence suggest an earlier origin for Neotropical taxa, an approximate time-frame based on sequence divergences is required to differentiate between the vicariance and dispersal hypotheses. A general time-frame can be applied to the *B. glabrata*–African species split in relation to the

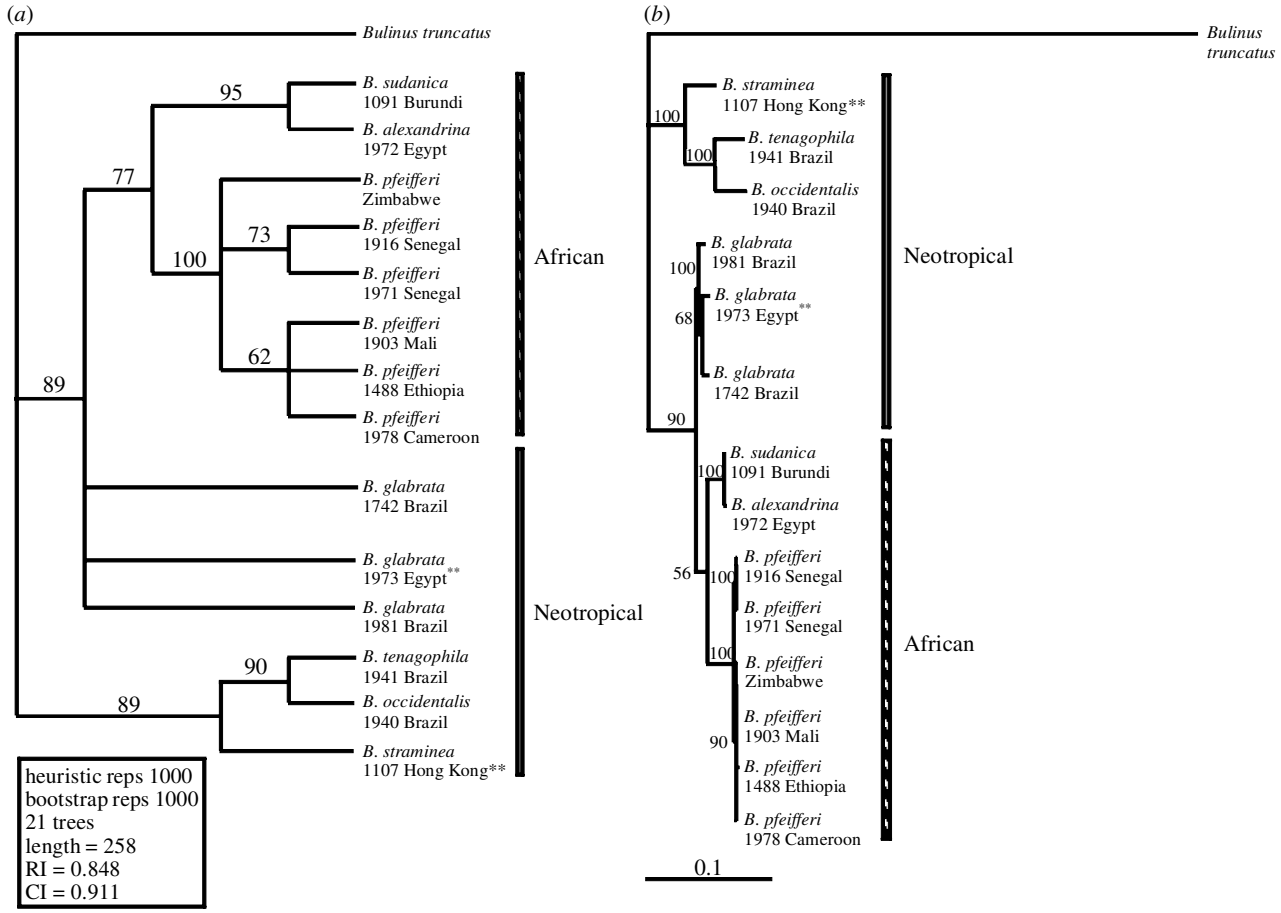

Figure 3. Phylogenetic relationships among *Biomphalaria* spp. with sequences from the entire nuclear rDNA ITS1 region. (*a*) Bootstrap 50% majority rule consensus tree of the 21 most parsimonious. Tree search algorithm: heuristic (1000 replicates). Number at the branch nodes indicates percentage bootstrap support for 1000 replicates. CI, consistency index; RI, retention index. (*b*) Maximum-likelihood neighbour-joining tree using quartet puzzling. Number at the branch nodes indicates percentage quartet-puzzling support values for 1000 puzzling steps. The scale denotes pairwise nucleotide substitution. African and Neotropical species are indicated by hatched and solid boxes, respectively. Taxa depicted by asterisks indicate naturalized populations which were accidentally introduced. Sequences are deposited under GenBank accession numbers AJ278438–41, AJ278512–3, AJ289707–8, AJ289881, AJ290976–7, AJ293580 and AJ401224–5.

Neotropical species using the average rate of mtDNA divergence across the entire molecule for most organisms of 2–4% Myr^{-1}. An average COI sequence divergence between *B. glabrata* and African species is 7.26%, indicating an approximate time-scale of 1.8–3.6 Myr. The time since divergence between *B. glabrata* and other Neotropical species is 2.3–4.6 Myr (9.3% sequence divergence). Following Després *et al.* (1992) a time-frame specific to the COI region sequenced in *Biomphalaria* can be calibrated with sequences homologous to this region from rat and mouse with a known divergence of 9–12 Myr ago (Jaeger *et al.* 1986). This was calculated at 1.7–2.2% Myr^{-1}, which encompass the lower mtDNA sequence divergence estimates assumed for the general mtDNA clock. However, these are conservative estimates as mollusc mtDNA may evolve more rapidly, as suggested by the work of Hoeh *et al.* (1996) on *Mytilus*, implying the use of the general mtDNA clock is more appropriate. These estimated dates support a dispersal model consonant with a Plio–Pleistocene arrival of snails founding the African clade, rather than a vicariance event *ca.* 100 Myr ago (figure 1*a*), which would give divergence estimates at least an order of magnitude greater.

Further support for the African affinity of *B. glabrata* comes from the observation that microsatellite loci isolated from *B. glabrata* amplified more readily in *B. pfeifferi* than in any other Neotropical taxa (Jones *et al.* 1999). The similarity of these taxa may have been paramount in allowing the rapid establishment of *S. mansoni*, hypothesized to have been repeatedly imported to the Americas via the African slave trade (Fletcher *et al.* 1981; Combes 1990; Després *et al.* 1993). Accidental introduction of a South American-derived strain of *B. glabrata* into Egypt substantiates the suitability of *B. glabrata* as an intermediate host for *S. mansoni* under natural conditions in Africa. *B. glabrata* was introduced to Egypt by 1981, when shells typical of this species were collected in Cairo (Pflüger 1982). Since this time it has successfully established itself in *B. alexandrina* habitats in the Nile Delta region, significantly increasing *S. mansoni* transmission (Yousif *et al.* 1996; Kristensen *et al.* 1999) despite the parasite's long coevolution with African snail hosts. The fact that *B. glabrata* transmits the Egyptian strain of *S. mansoni* as effectively as *B. alexandrina*, but is more tolerant of high temperatures, has a greater reproductive potential, and a longer life span, illustrates the significant impact such

Table 2. *Estimates of mtDNA COI (above diagonal) and nuclear rDNA ITS1 region (below diagonal) per cent sequence divergence with the two-parameter model of Kimura (1980)*

	African species			Neotropical species				outgroup (*Gyraulus chinesis*)
	B. pfeifferi	*B. alexandrina*	*B. sudanica*	*B. glabrata*	*B. tenagophila*	*B. occidentalis*	*B. straminea*	
B. pfeifferi	—	5.43	5.39	7.50	9.38	8.86	9.21	18.97
B. alexandrina	2.96	—	2.83	7.14	8.84	9.82	8.75	18.51
B. sudanica	2.77	2.98	—	7.15	8.91	9.76	8.77	18.20
B. glabrata	5.20	3.94	4.43	—	9.22	9.51	9.26	16.90
B. tenagophila	11.41	11.40	11.40	11.30	—	5.72	8.64	18.78
B. occidentalis	11.20	11.35	11.01	10.71	5.78	—	8.70	17.71
B. straminea	10.57	10.40	10.30	9.87	6.10	7.40	—	16.79
Bulinus truncatus (outgroup)	42.96	43.41	42.85	46.81	48.05	45.99	44.67	—

introductions can have on regional transmission dynamics (Yousif *et al.* 1996).

Our molecular phylogenies support some conclusions of previous allozyme-based studies (Bandoni *et al.* 1995; Woodruff & Mulvey 1997). Bandoni *et al.* (1995) place *B. glabrata* in a sister clade to the African species, with *B. glabrata* ancestral to *B. pfeifferi* and other African taxa. Conversely, Woodruff & Mulvey's (1997) phylogeny clusters African *B. sudanica* among *B. glabrata*, showing the two to be closely related, but most importantly *B. pfeifferi* is deemed ancestral to *B. glabrata*, implying that *B. glabrata* descended from African stock. This important disagreement between the two allozyme phylogenies indicated that further molecular analyses were required to resolve relationships within *Biomphalaria*. Molecular data from this study are concordant with Bandoni *et al.* (1995) but not Woodruff & Mulvey (1997) in showing that *B. glabrata* is ancestral to the African taxa. The greater age of *B. glabrata*, its widespread distribution in South America and presence only as a recent introduction in Egypt, points to it being an American endemic. This negates explanations of a more recently derived African *B. glabrata* dispersing to South America in the water casks of slave traders (Woodruff & Mulvey 1997) (figure 1*c*).

Our data support the hypothesis that a *B. glabrata*-like taxon, South American in origin, dispersed across the Atlantic to Africa and that the African species *B. alexandrina*, *B. sudanica* and *B. pfeifferi* are Plio–Pleistocene derivatives of this American form (figure 1*b*). In Africa these snails subsequently became intermediate hosts for the lateral-spined schistosomes. As *S. mansoni* diverged from *Schistosoma haematobium* (transmitted by the planorbid snails of the genus *Bulinus*) 10–30 Myr ago (Després *et al.* 1992) it is assumed that an alternative intermediate host was available before *Biomphalaria* reached Africa. Several modes of dispersal of *Biomphalaria* from the Americas to Africa can be envisaged. Pulmonates deposit gelatinous egg masses which may adhere to the legs, beaks and feathers of birds (Van Damme 1984). A number of wading birds have been reported to disperse from Africa to South America, but several travel in the reverse direction (Vuilleumier & Andors 1993), providing the opportunity for gastropod transmission. Snails could also have crossed the Atlantic on vegetation mats, such as those regularly washed out of the present day Amazon Basin. The return of pluvial conditions during interglacial periods may have contributed to the frequency of successful rafting.

Despite the relatively recent divergence times of Neotropical species they remain distinct and highly polymorphic. The genetic differentiation between *B. glabrata* and other Neotropical *Biomphalaria* and the speciose nature of the genus may be attributable to its ecology and the frequent and widespread climatic oscillations of the Quaternary (beginning 2.5–2.6 Myr ago) (Lowe & Walker 1997). In South America repeated fragmentation and coalescence of the rainforest may have encouraged population differentiation and speciation (Colinvaux 1987). Facultatively self-fertile freshwater snails would be especially affected, rapidly forming a series of isolated and semi-isolated demes, a population structure likely to accelerate genetic differentiation and speciation during glacials. Coalescence of divergent populations in the pluvial climate of interglacials may have left a legacy of enhanced genetic and morphological variation in extant Neotropical *Biomphalaria*, especially *B. glabrata*. Hence, the affinity of *B. glabrata* with other South American taxa might be less than anticipated, given multiple cycles of population division and coalescence; isolation followed by gene flow. However, the addition of missing representatives from each clade and further sequences might resolve the phylogeny further, removing the need to invoke scenarios promoting rapid speciation in South America.

Snail control strategies can play an important role in the reduction of schistosomiasis (Lardans & Dissous 1998), particularly since an effective vaccine remains elusive (Gryseels 2000). The fact that the two of the major intermediate hosts of *S. mansoni* are very closely related, despite being endemic to two different continents, has implications for the development of control measures which exploit genetic differences in susceptibility to parasites. A robust phylogeny of *Biomphalaria* may be useful in epidemiological risk projections and in elucidating the evolution of schistosome resistance.

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