Evolutionary Relationships and Biogeography of *Biomphalaria* (Gastropoda: Planorbidae) with Implications Regarding Its Role as Host of the Human Bloodfluke, *Schistosoma mansoni*

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The wide geographic distribution of *Schistosoma mansoni*, a digenetic trematode and parasite of humans, is determined by the occurrence of its intermediate hosts, freshwater snails of the genus *Biomphalaria* (Preston 1910). We present phylogenetic analyses of 23 species of *Biomphalaria*, 16 Neotropical and seven African, including the most important schistosome hosts, using partial mitochondrial ribosomal 16S and complete nuclear ribosomal ITS1 and ITS2 nucleotide sequences. A dramatically better resolution was obtained by combining the data sets as opposed to analyzing each separately, indicating that there is additive congruent signal in each data set. Neotropical species are basal, and all African species are derived, suggesting an American origin for the genus. We confirm that a proto-*Biomphalaria glabrata* gave rise to all African species through a trans-Atlantic colonization of Africa. In addition, genetic distances among African species are smaller compared with those among Neotropical species, indicating a more recent origin. There are two species-rich clades, one African with *B. glabrata* as its base, and the other Neotropical. Within the African clade, a wide-ranging tropical savannah species, *B. pfeifferi*, and a Nilotic species complex, have both colonized Rift Valley lakes and produced endemic lacustrine forms. Within the Neotropical clade, two newly acquired natural hosts for *S. mansoni* (*B. straminea* and *B. tenagophila*) are not the closest relatives of each other, suggesting two separate acquisition events. Basal to these two species-rich clades are several Neotropical lineages with large genetic distances between them, indicating multiple lineages within the genus. Interesting patterns occur regarding schistosome susceptibility: (1) the most susceptible hosts belong to a single clade, comprising *B. glabrata* and the African species, (2) several susceptible Neotropical species are sister groups to apparently refractory species, and (3) some basal lineages are susceptible. These patterns suggest the existence of both inherent susceptibility and resistance, but also underscore the ability of *S. mansoni* to adapt to and acquire previously unsusceptible species as hosts. *Biomphalaria schrammi* appears to be distantly related to other *Biomphalaria* as well as to *Helisoma*, and may represent a separate or intermediate lineage.

Introduction

Freshwater pulmonate snails of the genus *Biomphalaria* are best known for their role as intermediate hosts of the widely distributed parasite of humans, the digenetic trematode *Schistosoma mansoni*. This parasite, one of the causal agents of intestinal schistosomiasis, infects about 83 million people (Crompton 1999), mostly in Africa, but important foci exist in tropical South America, and infections are known from the Middle East and the Caribbean islands (Chitsulo et al. 2000).

The geographic distribution of *S. mansoni* is closely tied to that of susceptible species of *Biomphalaria* which support the development of the asexually repro-

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ducing sporocyst stages of this parasite. Although *S. mansoni* is the best studied of all platyhelminths (Snyder et al. 2001), relatively little is known about the evolution of its obligatory intermediate hosts (Morgan et al. 2001). There are 34 described species of *Biomphalaria*, of which about 18 are known to be or presumed to be (i.e., implicated in cases of natural transmission) capable of supporting the complete larval development of *S. mansoni*, nine are known to be refractory (all in the Neotropics), and seven have not been tested (Malek 1985; Brown 1994). We apply the term, susceptible, to species which have been found to be compatible with at least one strain of *S. mansoni*, but we make note of the degree of susceptibility where appropriate. Of the susceptible species, four (*Biomphalaria glabrata* in the Neotropics, and *B. pfeifferi*, *B. alexandrina*, and *B. sudanica* in Africa, Madagascar, and the Middle East) play the most significant role in transmission to humans in nature. Additionally, three African *Biomphalaria*, *B. choanomphala*, *B. camerunensis* and *B. stanleyi*, and two Neotropical species, *B. straminea* and *B. tenagophila*, play lesser but important roles in particular foci (Paraense and Corrêa 1987, 1989; Greer et al. 1990; Brown 1994).

Biomphalaria is also of interest in its own right because of its peculiar geographic distribution and the questions this has raised about its origin and diversification. The genus is confined to the tropics and subtropics, with 22 species occurring in the Americas and 12 species found in Africa, Madagascar and the Middle East. One species, *B. straminea*, has been introduced relatively recently from South America into Hong Kong (Meier-Brook 1974). Fossil forms described as *Biomphalaria* are known from the tropical Eocene and Oligocene of North America and Europe and perhaps Asia (Taylor 1988; Pierce 1993).

The origin of *Biomphalaria* was originally considered to be in Gondwanaland as much as 100 MYA (Pilsbry 1911; Davis 1980, 1992), with the present-day distribution on both sides of the Atlantic attributed to the subsequent separation of Africa and South America. If this hypothesis is correct, deep divisions should separate the South American and African representatives as the continents have been separated for more than 70 Myr (Cox 2000). It has also been suggested that *S. mansoni* was present prior to the split of the continents, thus accounting for its current presence in both South America and Africa (Davis 1980, 1992).

An alternative hypothesis based on allozyme data was forwarded, suggesting that *Biomphalaria* arose in South America after the continents split, and its presence in Africa is a consequence of a relatively recent west-to-east trans-Atlantic dispersal event in the past 2.3–4.5 Myr (Woodruff and Mulvey 1997). Transoceanic dispersal for a freshwater organism like *Biomphalaria* may have occurred in the feathers of aquatic birds or on vegetation rafted across the ocean (Woodruff and Mulvey 1997). Successful colonization would have been facilitated by the fact that *Biomphalaria* is hermaphroditic and capable of self-fertilization.

Further support for this hypothesis was recently provided by the DNA sequence data of Campbell et al. (2000). Moreover, their analyses gave strong support that the sister group of African species is the Neotropical *B. glabrata*, a relationship also noted by the allozyme-based study of Bandoni, Mulvey, and Loker (1995). This relationship is of interest because it is likely to have had profound consequences with respect to colonization of the Neotropics by *S. mansoni*.

The scenario proposed by Campbell et al. (2000) is that an ancestral Neotropical *Biomphalaria*, similar to *B. glabrata*, colonized Africa. Its descendants then spread throughout the continent and acquired a schistosome that became *S. mansoni* as we know it today. The modern presence of *S. mansoni* in the Neotropics is generally considered to be a consequence of the slave trade, on the basis of allozyme (Fletcher, LoVerde, and Woodruff 1981) and nuclear and mitochondrial ribosomal sequence data (Desprès, Imbert-Establet, and Monnerot 1993) which show very little differentiation between South American and African isolates.

Whereas the phylogenetic understanding of the genus has progressed considerably from both allozyme-based studies (Bandoni, Mulvey, and Loker 1995; Woodruff and Mulvey 1997) and DNA sequence–based studies (Campbell et al. 2000; Vidigal et al. 2000), the taxonomic coverage of the 34 species in the genus has not been extensive. The allozyme studies examined only 12 species in total, many of them from laboratory stocks. Seven species were included in the study of Campbell et al. (2000). Vidigal et al. (2000) examined 10 Neotropical species. A more complete analysis is necessary to determine if previously unsampled South American species have unexpected affinities with the African clade or if some of the rarer African species are relictual forms with close affinities to South American species other than *B. glabrata*. More complete taxon sampling can be crucial for phylogenetic accuracy (Hillis 1996), even at the species level, where the omission of taxa can lead to different phylogenies (Omland, Lanyon, and Fritz 1999).

A better understanding of the phylogenetic relationships among species of *Biomphalaria* would be helpful in several contexts. Accordingly, the purpose of this study is to use a broader representation of *Biomphalaria* species to (1) investigate relationships between the Neotropical and African species and further test the hypotheses of Woodruff and Mulvey (1997) and Campbell et al. (2000). This analysis will help to clarify where *Biomphalaria* originated and how it has diversified on two different continents; (2) ascertain relationships among the *Biomphalaria* species within continents; and (3) better understand how the phylogeography of these snails has influenced the history, the present distribution and abundance, and the possible future spread in the Neotropics of an important human parasite, *S. mansoni*. To this end, we obtained sequence data from 16 Neotropical and seven African species and, where possible, from multiple populations of each species, providing the largest sample of *Biomphalaria* species thus far investigated in any phylogenetic study. Our analysis includes all of the important hosts for *S. mansoni* and wide-rang-

ing species, as well as some of the species which are restricted in range and difficult to obtain.

Materials and Methods

Genomic DNA was extracted from 23 *Biomphalaria* species and from *Helisoma trivolvis*, primarily using a modification of a cetyl-trimethylammonium bromide–based, mollusc-specific method (Winnepenninckx, Backeljau, and De Wachter 1993). If unsuccessful, a simple alkaline-lysis method was used (Truett et al. 2000). Specimens were collected in the field, obtained from laboratory populations, or acquired through museums. Specimen information and GenBank accession numbers are given in table 1.

Three regions were amplified by PCR from each taxon: mitochondrial rDNA 16S, and nuclear rDNA ITS1 and ITS2 (only ITS2 could be amplified for *B. andecola*). Primers used to amplify a 416- to 433-nt fragment of *Biomphalaria* mtDNA 16S (451 nt for *H. trivolvis*) were 16Sar and 16Sbr (5′ CGCCTGTTTATCAAAAACAT 3′, 5′ CCGGTCTGAACTCAGATCACGT 3′; Palumbi 1996). In some cases, it was possible to amplify a fragment that included ITS1, 5.8S, and ITS2 regions, using primers which complemented conserved regions in the 3′ end of the 18S gene (ITS1-S, 5′ CCATGAACGAGGAATTCCCAG 3′) and the 5′ end of the 28S gene (BD2, 5′ TATGCTTAAATTCAGCGGGT 3′; ITS2.2, 5′ CCTGGTTAGTTTCTTTTCCTCCGC 3′). Usually, however, the ITS1 and ITS2 regions were amplified separately, using the primer ITS1-S in combination with 5.8S-AS (5′ TTAGCAAACCGACCCTCAGAC 3′), and 3SN (5′ GCGTCGATGAAGAGCGCAGC 3′) in combination with BD2 or ITS2.2. The ITS2 fragment for *B. andecola* was amplified through nested PCR, pairing the primers ITS1-S and ITS2.2, followed by amplification using 3SN and BD2. PCR products were purified using PCR Microcon columns (Millipore) and sequenced on both strands at least once using an Applied Biosystems, Inc. 377 automated sequencer (Big Dye Terminator™ cycle sequencing kit, ABI).

Sequences were aligned in CLUSTAL X (version 1.81; Thompson et al. 1997) and adjusted by eye. Unalignable regions were excluded from phylogenetic analyses (PopGen alignment, GenBank; 16S: sites 236–264; ITS1: 195–303, 415–437, 552–576; ITS2: 1153–1195, 1260–1318, 1557–1609, 1615–1648). Sequences for partial 18S and 28S, and complete 5.8S ribosomal units were identical for all taxa (except for one case, discussed later) and were consequently removed from phylogenetic analyses, leaving a total of 1,399 sites in the combined data set.

Congruence of the 16S, ITS1, and ITS2 data sets was evaluated with the incongruence length difference (ILD) test, implemented in PAUP* (Swofford 1998) as the homogeneity partition test, using 1,000 replicates and other options as for maximum parsimony (MP) previously. Invariable sites were removed before the test (Cunningham 1997).

If incongruent, some authors advocate that data sets should be analyzed separately (Miyamoto and Fitch 1995), whereas others maintain that they should be combined (Barrett, Donoghue, and Sober 1991). An alternative viewpoint is that this decision depends on the degree of incongruence (Bull et al. 1993; de Queiroz, Donoghue, and Kim 1995). If data sets are weakly incongruent, combining data should disperse noise and increase signal, thus increasing phylogenetic accuracy (de Queiroz, Donoghue, and Kim 1995), and should in the process uncover the common underlying signal of the data partitions. Although the strongest partition could swamp other partitions (Bull et al. 1993), this should be less likely in a three-partition analysis. One way to distinguish between true phylogenetic conflict and tree reconstruction artifact is that the latter is suggested by lack of statistical support such as bootstrapping for conflicting topologies (Sullivan 1996; Baldauf et al. 2000). We adopted this approach, completing phylogenetic analyses, including bootstrapping, on the three data sets individually, on each pairwise combination (16S + ITS1, 16S + ITS2, and ITS1 + ITS2), and on the three data sets combined.

All phylogenetic analyses were performed using PAUP* 4.0b4 and 4.0b8 for Windows (Swofford 1998), and trees were visualized in TreeView (Page 1996). Unweighted MP, minimum evolution (ME), and maximum likelihood (ML) analyses were performed on individual and combined data sets using the heuristic search option, random sequence addition (not allowed for ME), MULPARS on, MAXTREES set to 2,500, and tree-bisection–reconnection branch swapping. Unless otherwise noted, parsimony analyses were completed treating gaps as missing data. In order to see if including gap information had any impact on tree topology or support, two additional parsimony analyses were completed: (1) setting the gapmode option to newstate, and (2) constructing an additional data matrix by coding gap information according to the simple method of Simmons and Ochoterena (2000). Minimum evolution analyses used logdet measures of distance. ML analyses utilized the HKY85 model (Hasegawa, Kishino, and Yano 1985) with rate heterogeneity (HKY85 + I + Γ; Yang 1994), chosen by testing models of substitution through likelihood ratio tests (Huelsenbeck and Crandall 1997), with parameters estimated separately in heuristic searches (simple addition sequence) conducted under each of the models. Four rate categories, as well as the shape parameter α, were estimated in ML trees using the discrete model to approximate the gamma distribution (Yang 1994). Ten heuristic search repetitions were used for MP and ME, and one repetition for ML. Nodal support was assessed by bootstrap analysis (Felsenstein 1985), with 1,000 replicates performed for MP and ME analyses and 100 performed for ML.

Likelihood-ratio tests (Felsenstein 1988) were also used to test that the data collected were consistent with a constant rate Poisson-distributed process of substitution (molecular clock). The ML phylogeny was estimated using the HKY85 + I + Γ model, and then repeated while constraining the estimate to fit the molec-

Table 1
***Biomphalaria and H. trivolvis* specimens used in the study**

Species	Locality[a]	Country	Lab Maintained	Contributor	Accession Numbers 16S	Accession Numbers ITS
B. pfeifferi	Richard Toll	Senegal	Yes	M. Sene	AY030193	AY030361
B. stanleyi	Lake Albert*	Uganda	No	F. Kazibwe	AY030197	AY030365
B. pfeifferi	Yaoundé	Cameroon	No	R. Mimpfoundi	AY030194	AY030362
B. pfeifferi	Gezira	Sudan	No	A. Babiker	AY030195	AY030363
B. pfeifferi	Mahazoa	Madagascar	No	P. Brémond	AY030196	AY030364
B. camerunensis	Sangmelima (1999)	Cameroon	No	R. Mimpfoundi	AY030198	AY030366
B. camerunensis	Sangmelima (2000)	Cameroon	No	F. Njiokou	AY030199	AY030367
B. sudanica	Karungu	Kenya	No	E. S. Loker	AY030200	AY030368
B. sudanica	Lake Victoria, Mwanza	Tanzania	No	N. J. S. Lwambo	AY030201	AY030369
B. choanomphala	Lake Victoria, Mwanza	Tanzania	No	N. J. S. Lwambo	AY030202	AY030370
B. alexandrina	Giza (1997)	Egypt	No	E. S. Loker	AY030203	AY030371
B. alexandrina	Giza (1993)	Egypt	No	J.-P. Pointier	AY030204	AY030372
B. smithi	Lake Edward*	Uganda	No	F. Kazibwe	AY030205	AY030373
B. glabrata	Jarabacoa	Dominican Republic	No	C. Dominguez	AY030206	AY030374
B. glabrata	Rio Grande Town	Puerto Rico	No	S. File	AY030207	AY030375
B. glabrata	Salvador, Bahia	Brazil	Yes	W. L. Paraense	AY030208	AY030376
B. glabrata	Aragua	Venezuela	Yes	W. L. Paraense	AY030209	AY030377
B. kuhniana	Roseau	Dominica	No	E. S. Loker	AY030210	AY030378
B. kuhniana	Segovia	Colombia	Yes	L. E. Velásquez	AY030211	AY030379
B. kuhniana	Aragua	Venezuela	No	W. L. Paraense	AY030212	AY030380
B. straminea	Belém, Pará (PA)	Brazil	No	W. L. Paraense	AY030213	AY030381
B. straminea	São José do Rio Preto, São Paulo (SP)	Brazil	Yes	W. L. Paraense	AY030214	AY030382
B. intermedia	Itaipu Lake, Limoy	Paraguay	No	A. P. Canese	AY030215	AY030383
B. amazonica	Benjamin Constant, Amazonas (AM)	Brazil	Yes	W. L. Paraense	AY030216	AY030384
B. amazonica	Porto Velho, Rondonia (RO)	Brazil	Yes	W. L. Paraense	AY030217	AY030385
B. sp.	Santa Cruz	Bolivia	Yes	J.-P. Pointier	AY030218	AY030386
B. tenagophila	Asunción	Paraguay	Yes	W. L. Paraense	AY030219	AY030387
B. tenagophila	Formosa, Goiás	Brazil	Yes	W. L. Paraense	AY030220	AY030388
B. occidentalis	Caceres, Mato Grosso	Brazil	Yes	W. L. Paraense	AY030221	AY030389
B. prona	Lake Valencia (LkV)	Venezuela	No	R. N. Incani, M. Amarista	AY030222	AY030390
B. prona	Santa Cruz de Aragua (SaC)	Venezuela	No	R. N. Incani, M. Amarista	AY030223	AY030391
B. andecola	Near Lake Titicaca	Bolivia	No	FLMNH[b]	—	AY030392
B. sp.	Hanabanilla Lake (HbL)	Cuba	Yes	A. Gutierrez, M. Yong	AY030224	AY030393
B. sp.	Zanja Ferrer, Havana (Zf)	Cuba	Yes	A. Gutierrez, M. Yong	AY030225	AY030394
B. sp.	Salinas	Puerto Rico	No	J.-P. Pointier	AY030226	AY030395
B. temascalensis	Temascal, Oaxaca*	Mexico	Yes	J.-P. Pointier	AY030227	AY030396
B. obstructa	McKinney Falls, Texas	USA	Yes	J. T. Sullivan	AY030228	AY030397
B. obstructa	Isla del Carmen*	Mexico	Yes	W. L. Paraense	AY030229	AY030398
B. helophila	Zapata Swamp	Cuba	No	A. Gutierrez, M. Yong	AY030230	AY030399
B. peregrina	San Antonio	Uruguay	Yes	W. L. Paraense	AY030231	AY030400
B. peregrina	Nova Lima, Minas Geraís	Brazil	Yes	C. P. de Souza	AY030232	AY030401
B. schrammi	Minas Geraís	Brazil	Yes	F. Lewis	AY030233	AY030402
H. trivolvis	Pennsylvania	USA	Yes	B. Fried	AY030234	AY030403

[a] Abbreviations or year collected used to distinguish specimens from the same country are in parentheses.
[b] Florida Museum of Natural History.
* denotes type locality.

Table 2
HKY85 + I + Γ genetic distances (%) within and between selected lineages of *Biomphalaria* and *H. trivolvis* based on combined 16S, ITS1, and ITS2 sequences. Unalignable sites were removed

	African Group	*B. glabrata*	Species-rich Neotropical Group	*B. obstructa* Group	*B. peregrina*	*B. helophila*	*B. schrammi*
African group	0.08–3.78						
B. glabrata	2.34–4.29	0.31–1.55					
Species-rich Neotropical group	4.95–9.10	5.14–8.45	1.89–6.90				
B. obstructa group	6.50–8.06	6.62–7.86	6.71–9.64	0.00–0.55			
B. peregrina	11.32–14.78	12.33–13.97	11.65–15.74	12.05–13.69	—		
B. helophila	14.15–17.79	15.62–16.93	13.69–18.77	14.56–15.14	18.12	—	
B. schrammi	17.52–21.09	19.17–19.95	16.61–20.63	17.11–17.32	17.80	21.44	—
H. trivolvis	21.04–24.33	21.79–22.75	19.45–23.83	20.09–20.50	23.15	25.50	17.59

ular clock model. These estimates were used to calculate the likelihood-ratio–test statistic $\delta = 2[\ln(L_0) - \ln(L_1)]$, with $(n - 1)$ df (n is the number of taxa in the tree).

We chose as an outgroup *H. trivolvis,* which like *Biomphalaria* is a member of the family Planorbidae, and we found most parts of the *H. trivolvis* sequences to be alignable to *Biomphalaria* sequences. A close relationship between *Helisoma* and *Biomphalaria* is suggested by the histocompatibility studies of Sullivan et al. (1995). Additionally, ongoing phylogenetic studies in our laboratory of relationships within the Planorbidae indicate that *Helisoma* and *Biomphalaria* are the closest relatives of each other (unpublished data).

Results

Sequence Characterization and Pairwise Distances

Within *Biomphalaria,* there was considerable variation in the length of fragments for all three regions studied, indicating the common occurrence of indels. Sequences of mtDNA 16S had the lowest amount of length variation, with a range of 416–433 nt. ITS1 sequences had an extreme amount of length variation, with a range of 548–778 nt. The shortest ITS1 lengths were both *B. peregrina* isolates, which lack large portions of sequence, indicating probable deletion events. One of these deletions was large, approximately 170 nt. Excluding *B. peregrina,* the length range for ITS1 sequences was substantially smaller, 719–778 nt. ITS2 sequences ranged from 403 to 509 nt, but if *B. helophila* (509 nt) and *B. schrammi* (462 nt) are excluded, the range is much smaller (403–438 nt). Sequence lengths for the outgroup *H. trivolvis* were 451, 751, and 469 nt for 16S, ITS1, and ITS2, respectively. Species with the larger length differences (*B. peregrina,* ITS1; *B. helophila,* and *B. schrammi,* ITS2) also had substantial numbers of base differences compared with the rest of the *Biomphalaria* species. Pairwise distances confirm this and show these species to be not only diverged from other *Biomphalaria,* but also from each other (table 2). Additionally, as mentioned earlier, complete 5.8S sequences were identical for almost all *Biomphalaria* and *H. trivolvis,* except for *B. schrammi* which possessed 2 nt differences.

Mean base frequencies for each of the three fragments were 34.6% A, 11.9% C, 16.8% G, and 36.7% T for 16S; 21.7% A, 26.8% C, 26.7% G, and 24.8% T for ITS1; 16% A, 28.9% C, 27.5% G, and 27.6% T for ITS2. Likelihood (HKY85 + I + Γ) estimates of the transition-transversion ratio were low for all three fragments (0.845, 0.855, and 1.361 for 16S, ITS1, and ITS2, respectively), but some closely related taxa, even populations of the same species, had transition/transversion ratios of less than 1.

Phylogenetic Analyses

The data matrix used in phylogenetic analyses consisted of 1,399 total characters per taxon (except *B. andecola;* 445 were variable, 279 were parsimony informative). An MP tree based on the combined data sets is shown in figure 1. This tree is one of the 234 shortest trees produced (tree length = 889, consistency index = 0.67, retention index = 0.82, homoplasy index = 0.33), well supported at most nodes by bootstrap analyses, and identical in topology to the ML tree (−ln likelihood = 6,708.45204). The other 233 MP shortest trees differed little in topology, as indicated by the number of nodes that were present in >50% of the trees (numbered nodes indicated in boxes in fig. 1). Setting gaps as a fifth state or including the coded gap matrix did not change the tree topology significantly (*B. helophila* and *B. peregrina* switched positions). Not shown is the ME tree (score = 0.6254) which differs from the MP and ML trees in only two ways: *B. camerunensis* is the sister group of the Nilotic species complex instead of *B. pfeifferi,* and the *Biomphalaria* sp. Cuba + *B. andecola* + *B. prona* clade switches places with the *B. occidentalis* + *B. tenagophila* clade. In general, bootstrap resampling of the three combined data sets provided strong support for individual nodes (fig. 1). It should be noted that the position of *B. peregrina* was not dependent upon the ITS1 deletions. Analyses were done excluding these indel regions, with no change in the position of *B. peregrina;* also, this taxon's position was similar in 16S and ITS2 analyses for which there were not any large indels of this kind. A summary of bootstrap support values resulting from the analyses of the individual, pairwise combinations and all data sets combined is given in figure 2. Combined analyses provided more resolution than single data sets, and the three data sets combined pro-

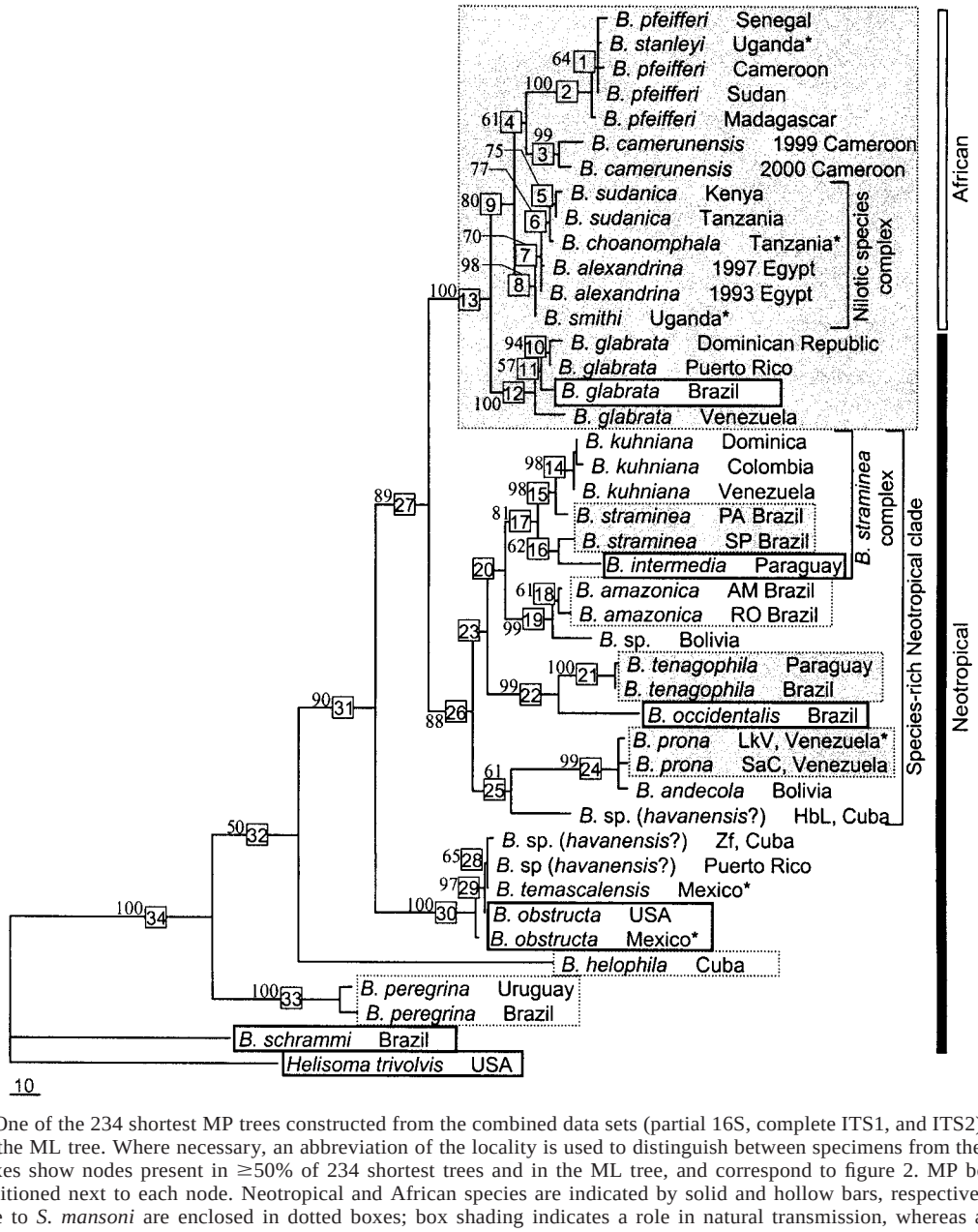

FIG. 1.—One of the 234 shortest MP trees constructed from the combined data sets (partial 16S, complete ITS1, and ITS2). The topology is identical to the ML tree. Where necessary, an abbreviation of the locality is used to distinguish between specimens from the same country. Numbered boxes show nodes present in ≥50% of 234 shortest trees and in the ML tree, and correspond to figure 2. MP bootstrap values >50% are positioned next to each node. Neotropical and African species are indicated by solid and hollow bars, respectively. Taxa which are susceptible to *S. mansoni* are enclosed in dotted boxes; box shading indicates a role in natural transmission, whereas absence of box shading indicates susceptibility known only experimentally. Solid boxes indicate resistant taxa. Taxa without boxes or shading have a susceptibility status that is unknown. Brackets highlight groups discussed in the text. Taxa depicted by asterisks indicate specimens collected from the type locality.

vided the maximum resolution and bootstrap support (fig. 2).

The results of the homogeneity partition test were 16S and ITS1, $P = 0.005$; 16S and ITS2, $P = 0.025$; ITS1 and ITS2, $P = 0.768$; 16S, ITS1, and ITS2, $P = 0.001$. These results indicated substantial congruence between ITS1 and ITS2, a result that is not unexpected given that evolutionary constraints on these sequences should be similar. Both are transcribed, but not translated, and function in the formation of rRNA subunits (Schlötterer et al. 1994). The *P* value for 16S and ITS2 allows the combining of data, according to Cunningham (1997). He noted that whenever the *P* values were greater than 0.01, combining the data improved or did not reduce phylogenetic accuracy, and this property extended to *P* values as low as 0.001. Values of *P* were quite low for 16S and ITS1 (0.005) and for all three data sets combined (0.001), and suggest caution when combining data.

The likelihood-ratio test rejected a molecular clock model for the three datasets combined, even when basal taxa with long branch lengths were removed ($P < 0.01$). However, a molecular clock model was not rejected when 16S and ITS2 data were tested individually ($P > 0.10$), regardless of whether basal taxa were removed. The ITS1 dataset rejects a molecular clock model ($P < 0.01$), regardless of whether basal taxa were removed.

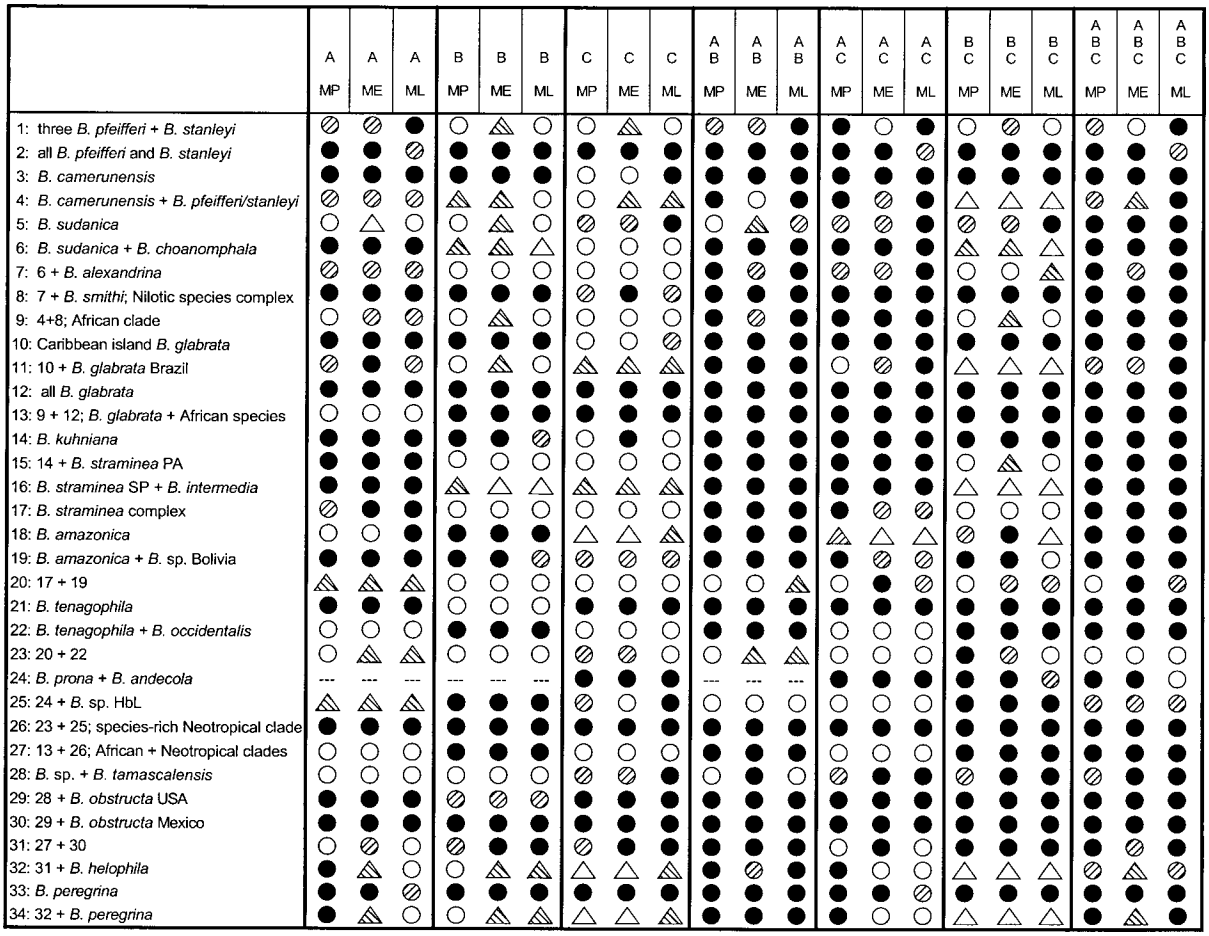

FIG. 2.—Consensus and conflict among data partitions. Phylogenetic support of individual data sets and their possible combinations (pairwise and three combined) are shown for the taxonomic groupings listed at the left (group numbering as in fig. 1). Groups correspond to nodes found in ≥50% of all the shortest MP trees and in the ML tree when all data sets were combined. Data set combinations analyzed are indicated at the top by single letters: A = partial 16S, B = ITS1, and C = ITS2. Phylogenetic criteria are also listed at the top (MP, ME, and ML). The level of bootstrap support for individual groups is indicated by empty (<50%), striped (50%–70%), and solid (>70%–100%) circles. When a particular node is not supported, and an alternative grouping is, the level of support for the alternative grouping is depicted by a striped (50%–70%) or empty (>70%–100%) triangle. Dashes indicate the absence of *B. andecola* from the data set(s).

Discussion

Data Congruence and Tree Reliability

Trees derived from the three data sets combined are probably the most reliable with respect to a number of criteria. First, a high level of similarity in topology existed between trees constructed by the three different phylogenetic methods. At most nodes, the three methods agreed, with high bootstrap support. Agreement among phylogenetic methods was lower in analyses using individual data sets or pairwise combinations (fig. 2). Agreement among methods was also evident prior to bootstrap completion, as the majority rule consensus MP and ME trees (not shown) were very similar in topology to each other and to the ML tree. Second, the phylogenetic signal appears to be additive. Analyses of our individual data sets show that there are a few strongly supported conflicts between them; but, in general, combining data improves phylogenetic resolution, as indicated by the improved bootstrap support for nodes (fig. 2). Finally, where the lack of bootstrap support by individual and pairwise combined data sets occurs, it is mostly caused by a lack of resolution rather than real phylogenetic conflict. Individual data sets in particular produced consensus bootstrap trees (not shown) with little or no resolution between species in the two species-rich clades. Rather, species and species groups from these clades formed a so-called rake topology. Thus, although the nodes supported in the combined analyses were frequently not supported by individual data sets, neither were they frequently rejected (fig. 2).

An increase in the ratio of phylogenetic signal to noise should occur when combining data, if the data sets share a common additive phylogenetic signal. The noise or random data is not likely to be additive. Despite some warning from ILD test results to avoid data combination, the greatest amount of phylogenetic resolution and consistency among phylogenetic methods was obtained when data were combined. Other studies have noted similar results (Baldauf et al. 2000; Smith 2000), even with low P values (<0.01) from ILD tests suggesting significant incongruence between data sets (Smith 2000).

Origin and Diversification of *Biomphalaria*

Neotropical species are consistently basal to African species in the trees from the three combined data sets (fig. 1) and in all other trees as well (not shown). This suggests an American origin for the genus, consistent with the oldest fossils occurring in South America (Parodiz 1969), and with the greater amount of morphological variation in Neotropical species (Mello 1972). Several additional lines of evidence support an American origin: (1) there are more recognized species in the Neotropics, (2) these species exhibit a greater range in overall size than the African *Biomphalaria*, and (3) the Neotropical species show greater diversity with respect to *S. mansoni* susceptibility (at least nine of the 22 species are refractory, whereas all the African species appear to be susceptible). In addition, Neotropical species exhibit much higher amounts of genetic variation than African species, indicating that the Neotropical species are older (table 2). The existence of North American Oligocene fossils of *Biomphalaria* raises the possibility that the genus has a North American origin (Morgan et al. 2001). Its close relative, *Helisoma*, is a predominately North American genus with few representatives in South America (Paraense 1976). Phylogenetic study of the Planorbidae, now under way in our laboratory, may help to resolve whether *Biomphalaria* originated in North or South America.

Multiple Lineages Within *Biomphalaria*

There are two species-rich groups in the genus, one Neotropical and the other consisting of *B. glabrata* plus all the African species. Basal to these are four other Neotropical lineages (*B. obstructa* + *Biomphalaria* sp. + *B. temascalensis*, *B. helophila*, *B. peregrina*, and *B. schrammi*) which are not the closest relatives of each other (fig. 1). The placement of these basal lineages does not appear to be the result of long-branch attraction. These lineages were never clustered together, and all three phylogenetic methods placed them similarly in the tree. Also, analyses were performed with these basal lineages individually removed, with no effects on the topology of the tree (not shown).

The four lineages mentioned above appear to be genetically distant from other *Biomphalaria* (table 2 and fig. 1). *Biomphalaria schrammi* is almost as distantly related from other *Biomphalaria* as *H. trivolvis*. This species is also morphologically distinct from other species in the genus (Paraense and Deslandes 1956) and is not susceptible to *S. mansoni* (Paraense, Fauran, and Courmes 1964). A similar phylogenetic position for *B. schrammi* is observed by Vidigal et al. (2000) who suggest that *B. schrammi* is intermediate between *H. trivolvis* and other *Biomphalaria*. We note that the genetic distance between *B. schrammi* and *H. trivolvis* is approximately the same as that between *B. schrammi* and other *Biomphalaria* (table 2). This suggests that *B. schrammi* is no more related to *Helisoma* than to *Biomphalaria*; it might represent a third distinct genus-level lineage. Our finding that *B. schrammi* differs by 2 nt in its 5.8S sequence from all other *Biomphalaria* and from *H. trivolvis* supports this hypothesis.

The next–most-basal branches on the tree lead to *B. helophila* and *B. peregrina*, which are also somewhat distantly related to other *Biomphalaria* (table 2 and fig. 2). Interestingly, although the amount of data is small, both these species have been experimentally infected with *S. mansoni* (Richards 1963; Paraense and Corrêa 1973). The basal position of *B. peregrina* is independent of the ITS1 deletions in this taxon, and was consistent throughout all analyses (fig. 1). There is not a discernable geographical pattern among these basal lineages as all three have wide and overlapping distributions. *Biomphalaria schrammi* is distributed throughout the Caribbean and much of South America (Paraense, Fauran, and Courmes 1964), *B. helophila* is distributed in the Caribbean, Central America, and western South America (Paraense 1996), and *B. peregrina* is widespread in South America (Paraense 1966).

Another lineage that is basal to most of the *Biomphalaria* spp. consists of *B. obstructa*, *Biomphalaria* sp. from Zanja Ferrer, Havana, Cuba, and Puerto Rico, and *B. temascalensis*, which form a group that is consistently and strongly supported in all trees and is quite closely related genetically (table 2 and branch lengths in fig. 1). The type locality, and hence the description of *B. havanensis*, is currently in dispute (Paraense and Deslandes 1958; Yong, Pointier, and Perera 1997). Our aim here is not to determine the correct type locality, but our analyses do show that there are two distinct species in Cuba that have been linked to the name *B. havanensis*. The type locality and anatomical description advocated by Yong, Pointier, and Perera (1997) is represented here by *Biomphalaria* sp. from Zanja Ferrer, Havana, Cuba. We obtained sequences from other specimens fitting this morphological description from Puerto Rico, Mexico, and the Dominican Republic, and the sequences were almost identical to *Biomphalaria* sp. Zanja Ferrer. Yong, Pointier, and Perera (1997) and Yong et al. (2001) noted that the morphology of *Biomphalaria* sp. Zanja Ferrer is very similar to *B. obstructa* (Paraense 1990) and *B. temascalensis* (Rangel-Ruiz 1987), each represented here by specimens from the type locality, so it is not surprising that they grouped together (figs. 1 and 2). There is clearly a lineage of Caribbean and Central American distribution that is basal to the Neotropical and African clades (fig. 1).

The type locality from which Paraense and Deslandes (1958) described specimens as *B. havanensis* is no longer accessible. Their description differs from that of Yong et al. (1997, 2001). Durand et al. (1998) recognized a *Biomphalaria* sp. from several locations in Cuba, that was morphologically distinct from *B. havanensis* of Yong et al. (1997) and very similar to *B. havanensis* of Paraense and Deslandes (1958). Allozymes convincingly distinguished it from *B. havanensis* of Yong et al. (1997) and from *B. helophila*. Sequences from specimens from two of the locations (Hanabanilla Lake, Guatao) reported by Durand et al. (1998) were examined and found to be identical, so only the Hanabanilla Lake specimen was used in the analyses. *Biom-*

phalaria sp. from Hanabanilla Lake is clearly distinct from *Biomphalaria* sp. Zanja Ferrer, and belongs firmly in the species-rich Neotropical clade (discussed later), although it does not have strong affinities for any group within the clade. On the basis of the disparate phylogenetic positions of specimens from Hanabanilla Lake and Zanja Ferrer, the use of the term, *B. havanensis* complex (Durand et al. 1998; Yong et al. 2001), to refer to *Biomphalaria* spp. from Cuba should be discontinued.

The susceptibility status of *B. obstructa* and *B. havanensis* should also be viewed with caution. Several investigators have attempted to infect *B. obstructa* from various geographic locations without success (Basch 1976). *Biomphalaria havanensis* has been reported to be susceptible on multiple occasions (Basch 1976), but past and continued confusion over its identity makes interpretation of these records difficult.

Species-rich Neotropical Clade

A derived Neotropical clade containing nine species was strongly supported by bootstrap values (Node 26; figs. 1 and 2), but some relationships of subgroups within the clade were not. Nodes 20 and 23 were the least supported of all the nodes in the tree and were in fact rejected in a few analyses (figs. 1 and 2). The most strongly supported group in the clade was *B. straminea, B. kuhniana,* and *B. intermedia* (Node 17; fig. 2). These species comprise a previously recognized *B. straminea* complex based on morphological similarities (Paraense 1988). *Biomphalaria straminea* is the exclusive intermediate host involved in natural transmission of *S. mansoni* in parts of northeast Brazil, despite low susceptibility (Barbosa and Figueiredo 1970; Paraense and Corrêa 1989), but *B. intermedia* is considered refractory to infection (Floch and Fauran 1954; Malek 1985). The susceptibility status of *B. kuhniana* is uncertain because although it was previously considered refractory (Floch and Fauran 1954), the naturally infected snails in Venezuela identified as *B. straminea* (de Noya et al. 1999) are perhaps *B. kuhniana.* In the analyses of the three combined data sets, the two *B. straminea* isolates did not cluster with each other. The population from northern Brazil (*B. straminea* PA) clustered with *B. kuhniana,* but the population from southern Brazil (*B. straminea* SP) clustered with *B. intermedia* (figs. 1 and 2). This result appears to be entirely derived from the 16S data set as the two *B. straminea* populations clustered together and formed the sister group of *B. kuhniana* when ITS1 and ITS2 were analyzed individually or together (Node 16 is rejected by these analyses; fig. 2). This was the most dramatic conflict among our data sets. It is possible that the close relationship among these species allows some hybridization, which could result in conflict between nuclear and maternally inherited mitochondrial sequences. Interspecific mitochondrial gene flow via hybridization has been reported in various groups of animals, including snails (Shimizu and Ueshima 2000). Hybridization is known, but not well understood, between *Biomphalaria* species (Mello-Silva et al. 1998). Vidigal et al. (2000) also noted the tendency of a northern population of *B. straminea* to cluster with *B. intermedia.* Clearly, the relationships within this group are complex and await further resolution. In addition, our report of *B. kuhniana* from Dominica, whose identification was confirmed here by sequence analysis, is the first record for this species from any Caribbean island.

The closest relative of the *B. straminea* complex appears to be *B. amazonica* (fig. 1). Vidigal et al. (2000) also found these species to cluster together. *Biomphalaria amazonica* is susceptible to *S. mansoni* (Corrêa and Paraense 1971; Paraense and Corrêa 1985). A previously unidentified specimen, designated as *Biomphalaria* sp., from Bolivia, appears to be *B. amazonica,* on the basis of sequence data, and thus extends the known range of this species (western Brazil; Paraense and Corrêa 1985) to include eastern Bolivia.

Other well-supported groups in the Neotropical clade include *B. occidentalis* with *B. tenagophila* and *B. prona* with *B. andecola* (fig. 2). *Biomphalaria tenagophila* and *B. occidentalis* have been previously recognized as very similar by morphological study (Paraense 1981), as well as by low-stringency PCR and RFLP (Pires et al. 1997; Spatz et al. 1999). The similarity between these two species is curious nonetheless because *B. tenagophila* is susceptible to some strains of *S. mansoni* (Paraense and Corrêa 1981) and is important in natural transmission in southern Brazil (Paraense and Corrêa 1963), whereas *B. occidentalis* is completely refractory to the *S. mansoni* isolates that have been tested so far (Paraense and Corrêa 1982). Although the similarity between *B. prona* and *B. andecola* has not been noted before, they are alike in the following: (1) both are found in large lakes (Lake Valencia and Lake Titicaca, respectively) and in the surrounding areas (Paraense and Deslandes 1957; Paraense et al. 1992), although *B. andecola* is also reported from streams in Chile (Malek 1985); and (2) both share a shell whorl that usually increases rapidly in width, although both species exhibit variation in this regard (Malek 1985; Paraense et al. 1992). *Biomphalaria prona* has been found naturally infected with *S. mansoni* in Venezuela, but its degree of susceptiblity or how large a role it plays in natural transmission is not known (de Noya et al. 1999).

How did the species in this diverse clade arise? Campbell et al. (2000) suggested that repeated rain forest fragmentation and coalescence caused by glacial cycles may have encouraged population differentiation and speciation, beginning in the Quaternary, 2.5–2.6 MYA. As the degree of forest fragmentation caused by aridity occurring during glacial maxima is currently debated (Colinvaux 1997), other forces, such as marine incursions during glacial minima (Lovejoy, Bermingham, and Martin 1998) and dramatic changes in drainages (Hoorn et al. 1995), should also be considered. Facultative self-fertilization was probably also an important factor in the evolution of this group.

Ancestor of *B. glabrata* Colonized Africa and Gave Rise to African Fauna

One of the best-supported nodes in all the trees (Node 13; figs. 1 and 2) confirms the African affinity of

B. glabrata, first noticed by Bandoni, Mulvey, and Loker (1995) using allozyme data and confirmed by Woodruff and Mulvey (1997) using allozymes and by Campbell et al. (2000) using DNA sequences. Our data do not support the hypothesis, however, that *B. glabrata* is derived from African stock and colonized the Neotropics via the slave trade or through an earlier event (Woodruff and Mulvey 1997). Rather, our data, including four African species not studied previously, agree with Campbell et al. (2000) that an ancestor of *B. glabrata* colonized the African continent and gave rise to the African species.

Given that the mean ITS2 sequence divergence between *B. glabrata* and the African species is 1.7% and that the substitution rates for invertebrate ITS sequences range from 0.4% to 1.2%/Myr (Schlötterer et al. 1994; Bargues et al. 2000; Wares 2001), it is estimated that the colonization of Africa occurred approximately 1.4–4.3 MYA, in the Pliocene to early Pleistocene. Substitution rate estimates for 16S sequences in invertebrates have a rather wide range (0.1%–2.2%; Cunningham et al. 1992; Romano and Palumbi 1997). Using the upper range of the divergence rates for invertebrates (1.6%–2.2%/Myr, Cunningham et al. 1992; Patarnello et al. 1996) and given the 6.4% mean divergence between *B. glabrata* and the African species, an estimate of 2.9–4.0 MYA is calculated for the colonization of Africa. These are similar to previous estimates (2.3–4.5 MYA, Woodruff and Mulvey 1997; 1.8–3.6 MYA, Campbell et al. 2000). The *B. glabrata*-African split may overestimate the timing of the colonization event, especially as *B. glabrata* shows some divergence between populations. A minimum age can be estimated using the mean sequence divergences between the African species because all African species have arisen since the colonization (1.6–2.2 and 1.1–3.2 MYA for 16S and ITS2, respectively).

These dates correspond well to the fossil record, for there are ancient *Biomphalaria* fossils in South America (Paleocene, 55–65 MYA; Parodiz 1969), as well as in other continents, but not in Africa despite a very rich and well-studied fauna of fossil molluscs (Van Damme, personal communication). In fact, the oldest fossil of *Biomphalaria* in Africa may be as recent as 0.255 MYA (Van Damme, personal communication). We calibrated the *B. glabrata*-African divergence with this date, but the rates of substitution derived are nearly an order of magnitude greater than those known for these sequences (25.2%/Myr and 6.7%/Myr for 16S and ITS2, respectively). Thus, a slightly earlier colonization is more likely in the Plio-Pleistocene. In geologic time, this is still a recent event, and both morphological data (Mello 1972) and the genetic data presented here are consistent with this idea (table 2 and branch lengths in fig. 1).

The mode of transport by which a proto-*B. glabrata* may have completed a west-to-east trans-Atlantic dispersal is presumed to have been as tiny neonate snails embedded in the feathers of aquatic birds or as eggs, neonates, or adults aboard Amazonian vegetation rafts. Woodruff and Mulvey (1997) and Wesselingh, Cadée, and Renema (1999) provide excellent discussions on this topic. Hermaphroditism and the ability of adults to aestivate may have facilitated colonization. This is not to say that colonizations were frequent, rather that they were rare. There is no indication of multiple events; no other Neotropical species exhibits any affinity with African species on the basis of sequence data.

Biogeography of African Species

Despite a relatively recent history for African *Biomphalaria*, these species exhibit interesting biogeographical patterns. The most important host for *S. mansoni*, *B. pfeifferi* has the most widespread distribution, found throughout the tropical middle of the continent and in Madagascar and Arabia. It is known from Pleistocene and Holocene fossils in the Sahara as well. There is a small amount of genetic difference between populations (0.29–0.50%, using all the data), showing that there is some genetic isolation of populations. Further investigation is needed to see what kind of genetic structure exists across populations, using a more variable marker than that employed here. Additionally, our data show that *B. stanleyi* from Lake Albert is derived from *B. pfeifferi*. One interpretation is that *B. stanleyi* is merely a lacustrine form of *B. pfeifferi;* it is no more differentiated from *B. pfeifferi* populations (genetic differences of 0.14–0.35%) than *B. pfeifferi* populations are from each other. *Biomphalaria stanleyi* has also been described from Lake Chad in sub-Saharan Africa and Lake Cohoha in Burundi (Brown 1994), and it would be interesting to obtain specimens from these locations to determine whether *B. pfeifferi* has colonized large lakes once or multiple times. Similarly, there is within the African clade a poorly differentiated Nilotic species complex (*B. alexandrina*, *B. choanomphala*, *B. smithi*, and *B. sudanica;* genetic differences of 0.14–0.58%), species which primarily inhabit the Nile basin but which have also invaded some of the great lakes in the Nile drainage. An endemic of Lake Victoria and several Rift Valley lakes, *B. choanomphala*, may be regarded as a lacustrine form of *B. sudanica*. Another lacustrine form is *B. smithi*, an endemic of Lake Edward, but its status relative to either *B. alexandrina* or *B. sudanica* is not clear as it has a basal position in the Nilotic complex (fig. 1). All three lacustrine species in the present study (*B. choanomphala*, *B. smithi*, and *B. stanleyi*), as well as other rare, African lacustrine species, share a common conchological feature: a rapid widening of the shell whorl (Brown 1994). As it has occurred in two separate African lineages, it appears to be a convergent feature induced or selected for by lacustrine environments.

A Nilotic distribution is best illustrated by *B. alexandrina*, but with the following peculiarities. This species has historically been confined to the Nile Delta in Egypt, one location in Libya and in the Sudan between Khartoum and Kosti (Brown 1994). In recent decades, however, it has colonized the Egyptian Nile from the Delta (Vrijenhoek and Graven 1992). Its close relative (fig. 1), *B. sudanica*, is also present in the Nile drainage, in the Sudan and southwards (Brown 1994).

Although the genetic distances are small and more study is needed, the branching order in our tree suggests that previous colonizations may have taken place in a southerly direction, with *B. alexandrina* giving rise to *B. sudanica.* Also, it is curious that colonization could occur in a predominantly upstream direction.

The final member of the Africa group is *B. camerunensis,* the position of which is not clear: sometimes it is the sister group of *B. pfeifferi,* and sometimes it is the sister group of the Nilotic species. Here again, there appears to be some conflict between nuclear ITS and mitochondrial 16S data (fig. 2). This species is unique in that it is primarily a rain forest species occurring in western Central Africa (Greer et al. 1990).

Biomphalaria glabrata Is a Diverse Species

Four *B. glabrata* populations formed a monophyletic group with 100% bootstrap support (fig. 1), including one population (*B. glabrata* Salvador, Brazil) that is resistant to *S. mansoni.* Populations from the Caribbean islands of Hispaniola and Puerto Rico clustered together (fig. 1), suggesting that the colonization of these two islands occurred from the same source population or that an initial colonization of one of these islands led to subsequent island-to-island colonization. There was a substantial amount of genetic variation among the four populations (0.29–1.38%), in agreement with Woodruff and Mulvey (1997) who commented that *B. glabrata* was either the most genetically diverse mollusc species yet described or a complex of more than three species. As *B. glabrata* populations form a monophyletic assemblage in all our trees, we suggest that the species is valid but that experimental studies involving *B. glabrata* should be wary of geographical variation.

Susceptibility to *S. mansoni*

All the most susceptible hosts for *S. mansoni* are found in a single clade: *B. glabrata* + African species. Pragmatically speaking, assuming that all African species belong in this clade, the three African species not yet tested for susceptibility, *B. barthi, B. rhodesiensis,* and *B. tchadiensis,* are probably also susceptible.

From the standpoint of host-parasite coevolution, there are two ways to view this susceptible clade. One is that the characteristics that rendered proto-*B. glabrata* susceptible appear to have been retained throughout the African taxa and also in modern *B. glabrata.* However, it is the parasite which is under pressure to adapt to changing snail taxa; the snails are likely to be under no pressure to retain susceptibility and, if anything, might be under pressure to evolve resistance. Thus, susceptibility in African taxa might also be the result of the continuous opportunity the parasite has had to adapt to the various African lineages. These viewpoints are not necessarily mutually exclusive, and both are probably validly used to describe what has occurred on the African continent between *Schistosoma* and *Biomphalaria*. An African schistosome, part of an already present schistosome fauna, acquired *Biomphalaria* as an additional host, eventually lost the ability to use its ancestral snail host, and diverged from other African schistosomes. It is likely that part of this process was the improvement in compatibility over time as the parasite adjusted to the snail. This can be regarded as fine-tuning by the parasite. As *Biomphalaria* differentiated into the current lineages in Africa, exploitation of these hosts was possible because of the small amount of genetic differentiation between them (table 2), and because of the ability of the parasite to fine-tune to new and evolving snail lineages.

It is also relevant to consider that *Biomphalaria* are exploited by many digenetic trematode species in addition to *S. mansoni.* Although their digenean fauna is poorly characterized (especially in South America), *Biomphalaria* are known to host at least 20 species in the Neotropics (Nasir 1980) and about 30 species in Africa (Loker, Moyo, and Gardner 1981). It seems likely that most African digeneans (a few species that use birds as definitive hosts may be global in distribution) encountered *Biomphalaria* only after it arrived in Africa. The ability to establish rapidly in *Biomphalaria* was obviously not limited to *S. mansoni.*

Our data, including six additional Neotropical and four additional African *Biomphalaria,* confirm previous studies (Woodruff and Mulvey 1997; Campbell et al. 2000) that show that the colonization of the New World by *S. mansoni* (Desprès, Imbert-Establet, and Monnerot 1993) was successful because *B. glabrata* was more closely related to the African species than to its Neotropical counterparts (table 2). The initial compatibility between them is unknown, but it was high enough so that the parasite rapidly became widely established in its new hemisphere. The high susceptibility presently exhibited by most *B. glabrata* may also be in part caused by subsequent fine-tuning by *S. mansoni.*

An interesting finding is the degree to which basal lineages in the tree include susceptible species (*B. helophila* and *B. peregrina*), especially considering the large genetic distances between them and other *Biomphalaria* as well as between each other (table 2). Susceptibility, at least weak susceptibility, does not therefore appear to be dependent upon long association with *S. mansoni,* as the parasite has only been in the New World for a few centuries. Paraense and Corrêa (1973) found *B. peregrina* from Ecuador to be susceptible, but *S. mansoni* has never occurred there. It is likely that the susceptibility of *Biomphalaria* is determined by a suite of genetic factors which combine and interact to render a species potentially susceptible, and if this suite is similar enough to previous suites the parasite has associated with, infection can occur. High susceptibility might occur only after the parasite adapts to this suite through time. This is concordant with what is known regarding the compatibility between *S. mansoni* and *Biomphalaria*: it is a complex interaction and is genetically controlled (Richards, Knight, and Lewis 1992). The weak susceptibility of basal lineages suggests that there are ancestral features retained in at least some (if not all) species of *Biomphalaria* that are keyed in on by *S. mansoni.* However, these features appear to be unique to *Biomphalaria* because the genus *Helisoma*, including

the outgroup used in this study, *H. trivolvis*, is refractory to *S. mansoni* (Brown 1994).

Features contributing to susceptibility appear to have been obscured in the clade of nine Neotropical species (fig. 1), which is the least susceptible major lineage. Yet, there are susceptible species within the clade, suggesting that such features were not totally lost (fig. 1). Of particular interest are *B. straminea* and *B. tenagophila*, which are exclusive vectors of the parasite in particular areas of Brazil, even though they are not highly susceptible (Paraense and Corrêa 1981, 1989). The acquisition of these species as hosts must have occurred after the onset of the slave trade. Paraense and Corrêa (1963, 1987) noted that A. Lutz was unable to infect *B. tenagophila* during his pioneering work on Brazilian schistosomiasis in 1916. The parasite has apparently adapted to this snail species since that time, for *B. tenagophila* is now a moderately efficient host in southeastern Brazil. Similarly, *S. mansoni* has adapted to *B. straminea* in the northeast part of the country. The acquisition of new intermediate host species has been accompanied by the differentiation of isolates of *S. mansoni* (Paraense and Corrêa 1963, 1981). Moreover, these were two separate acquisitions as *B. straminea* and *B. tenagophila* are not the closest relatives of each other, and both species are closely related to the refractory species (*B. straminea* to *B. intermedia*, and *B. tenagophila* to *B. occidentalis*; fig. 1). Continued adaptation by *S. mansoni* is probable, both in the form of increased compatibility with current hosts (*B. straminea* appears to be still especially poor) and in the adoption of new hosts in this clade. Indeed, another species within this clade, *B. amazonica*, is susceptible, though it has never been implicated in natural transmission (Corrêa and Paraense 1971; Paraense and Corrêa 1985).

Finally, the close relationships between susceptible and refractory taxa, such as those mentioned above (*B. straminea* with *B. intermedia* and *B. tenagophila* with *B. occidentalis;* fig. 1), demonstrate that the changes in the genetic suite affecting susceptibility are not necessarily accompanied by large morphological differences or by genetic isolation. The overall pattern of susceptibility appearing and disappearing in the species tree is continued within a species on a population scale as well. The most susceptible species, *B. glabrata*, includes populations that vary considerably in susceptibility, including a naturally resistant population from Salvador, Brazil.

Insights into the Future and Control of *S. mansoni*

The results of this study highlight the remarkable ability of *S. mansoni* to adapt to *Biomphalaria* snails, and this should be a point of concern as it could facilitate the spread of *S. mansoni*. The continued acquisition of new Neotropical hosts will be favored by peripatry of *Biomphalaria* species. In addition, one of the recently acquired species, *B. straminea*, shows an exceptional ability to spread, probably aided by human activity, and has established in Hong Kong (Meier-Brook 1974) and in the Caribbean (Pointier, Paraense, and Mazille 1993). Compatibility with newly acquired hosts may also increase through time.

Future efforts should be made to incorporate the rarer species of *Biomphalaria*, to gain further insight into the patterns of susceptibility and resistance and to ensure that our understanding of the intercontinental relationships within the genus are correct. Some unstudied species may be discovered to be close relatives of the already susceptible species, identifying them as potential future intermediate hosts.

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