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Distribution and identification of the genus *Biomphalaria Preston* (1910): important insights into the epidemiology of Schistosomiasis in the Amazon region

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Abstract. Schistosomiasis is a disease transmitted by flatworms of the species *Schistosoma mansoni* (Sambon, 1907). The spread of the disease is dependent on the presence of snails of the genus *Biomphalaria* (intermediate hosts). In Brazil, while 11 species and one subspecies have been identified, only three – *B. glabrata*, *B. straminea* and *B. tenagophila* – are known to eliminate cercariae into the environment. However, only *B. peregrina* and *B. amazônicaare* susceptible to infection in the laboratory. Research on schistosomiasis and its intermediate hosts in Brazil is restricted to the country's southern and southeastern regions, and little is known of the occurrence of *Biomphalaria* in the Amazon region, where the disease is probable endemic due to the ideal environmental conditions and the availability of hosts. Data on the occurrence of this genus are essential for epidemiological studies of schistosomiasis, given that the confirmation of its occurrence in a given region, and the correct identification of the species, are fundamental to the development of effective strategies for the prevention and control of schistosomiasis.

Keywords. *Biomphalaria*, identification, molecular.

Schistosomiasis: *Schistosoma mansoni* and *Biomphalaria*

Mansonic schistosomiasisis a disease that has affected human populations for thousands of years. Data from the World Health Organization (WHO) show that this is one of the most widespread parasitoses in the world and the most common of those dispersed by water (Nomura *et al.*, 2007). Schistosomiasisis a persistent disease, which has spread through anthropogenic modifications of the environment, as well as the migration of populations from endemic areas to new regions, reinforced by the lack of adequate sanitation (Silva, 2013).

In 1847, Fujii described the clinical features of the parasitoses in Japan, although the parasite itself was first described by Bilharz in Egypt in 1852. The etiological agent is a flatworm of the Class Trematoda, Genus *Schistosoma*. The trematode family Schistosomatidae is divided into two subfamilies – the Bilharzielinae, which parasitizes birds and some domestic animals, and the Schistosomatinae, composed of species that parasitize humans and wild animals. The latter includes species medically important such as *Schistosoma mansoni* (Sanbon, 1907), *S. haematobium* (Bilharz, 1852), *S. japonicum* (Katsurada, 1904), *S. mekongi* (Voge, Brickner& Bruce, 1978) and *S. intercalatum* (Fischer, 1934).

The introduction of these parasites into Brazil occurred through the importation of African and Asian slaves infected with the parasite. While a number of individuals were found to be infected by *S. haematobium* and *S. japonicum*, only *S. mansoni* has become established due to the availability of favorable conditions and compatible hosts. In Brazil, the disease is known popularly as "water belly", "bilharzia", "schistosis" or the "snail disease" (Neves, 2011). Currently, schistosomiasis occur in 54 countries, principally in Africa and the Americas. In South America, the disease occurs principally in Brazil, the Caribbean, and Venezuela. In Brazil, this parasitosis has been reported in the Federal District and 18 states – Bahia, Sergipe, Alagongas, Pernambuco, Paraíba, Rio Grande do Norte (coastal zone), Maranhão, Piauí, Ceará, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Paraná, Rio Grande do Sul, and Goiás (Brasil, 2009).

The transmission of *S. mansoni* occurs in freshwater habitats inhabited by snails of the genus *Biomphalaria*, which are involved in an important phase of the life cycle of this trematode. *S. mansoni* has a heteroxenous life cycle, which was described for the first time independently by Leiper in Egypt, in 1915, and by Lutz, in Brazil, in 1916. When adult, both male and female *S. mansoni* parasitize the mesenteric venules of their primary host (man), in which the sexual phase occurs. Approximately 300 eggs are eliminated daily in the feces of the host, which then hatch and develop in freshwater ecosystems over a period of around seven days in their ciliate, asexual larval form, the miracidia, which infect snails of the genus *Biomphalaria*, the secondary host (Neves, 2011).

In these mollusks, the miracidia are transformed into sac-shaped structures known as primary sporocysts, in which germinal cells proliferate, which produce secondary sporocysts that liberate cercaria. This asexual reproduction phase lasts four to five weeks, depending on the temperature. The cercaria eliminated by the secondary host are free-swimming and are able to penetrate the skin and blood vessels of several vertebrates, including humans whre complet the parasite's life cycle (Rey, 2011).

The Genus *Biomphalaria*

The intermediate host of *S. mansoni*are snails of the genus *Biomphalaria* belong to the Family Planorbidae, Order Basommatophora, Class Gastropoda, Phylum Mollusca. The name of the genus was proposed by Preston (1910), based on the Latin *Bis* (double) and *omphalos* or navel (Figure 1), which refers to depression found on either side of the central curve of the snail (Paraense, 1975).

The ideal temperature for the development of *Biomphalaria* ranges between 20° and 30°C, although it may tolerate temperatures as low as 18°C and as high as 32°C (Rey, 2011). This temperature range is typical of much of Brazil. Other factors also favor the development of the species, such as neutral to alkaline pH (7–8), relatively clear, shallow and slow-moving (< 30 cm/s) water, with muddy or rocky bottoms and vegetation growing near the margins. Salinity should also be lower than 3 per 1000, although individuals have been found in water with a salinity of up to 6000 mg/l (Rey, 2011; Grisoliaand Freitas, 1985).

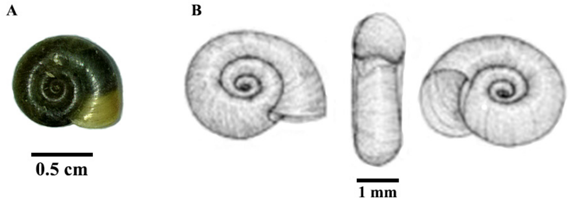

Figure 1: Snail shell from genus Biomphalaria. A: Focus on central depression present on both sides (Personal file); B: Schematic image of the shell of Biomphalaria straminea. Source: Silva (2013).

The snails of this genus inhabit aquatic habitats, either lentic or lotic, and are found on the margins of rivers, lakes, swamps and ditches. Population density tends to be higher in artificial environments, such as ditches and reservoirs, rather than natural habitats (Rey, 2011; Cunha, 1970). These snails feed primarily on algae, organic slime, bacteria, the excrementof other animals, leaves and other parts of aquatic plants (Teodoro, 2009; Neves, 2011).

These mollusks are able to tolerate major changes in the physical-chemical conditions of the environments they inhabit, due to their behavioral adaptations for survival in unfavorable conditions, such as their ability to estivate in the soil to protect themselves from changes in the environment, dehydration, predation, and washing away. Some species of *Biomphalaria* have been found buried in the soil at depths of up to 40 cm in areas treated with molluscicides (Paraense, 1955 and 1975). All the behavioral and biological characteristics of these snails favor their spread and proliferation.

Distribution patterns of *Biomphalaria* and the irrelevance to the epidemiology of schistosomiasis

The genus *Biomphalaria* has a worldwide distribution, with 26 species in the New World (Dejong *et al.,* 2001). In South America, 22 species of *Biomphalaria* have been recorded and a total of 11 species are known to occur in Brazil – *B. glabrata* (Say, 1818), *B. tenagophila* (Orbigny, 1835), *B. straminea* (Dunker, 1948), *B. peregrine* (Orbigny, 1835), *B. schrammi* (Crosse, 1864), *B. kuhniana* (Clessin, 1883), *B. intermedia* (Paraense&Deslandes, 1962), *B. amazonica* (Paraense, 1966), *B. cousin* (Paraense, 1966), *B. oligoza* (Paraense, 1975), *B. occidentalis* (Paraense, 1981), and *B. tenagophila guaibensis* (Paraense, 1984) (Tibiriçá, 2006; Neves, 2011). Of these species, four are infected naturally – *B. glabrata*, *B. straminea*, *B. tenagophila* and *B. prona* (Martens, 1873) – although experimental studies have confirmed the susceptibility of other species, such as *B. amazonica*, *B. peregrina*, *B. cousini* and *B. havannsis* (Paraense, 1966; Corrêa and Paraense, 1971; Paraense and Corrêa, 1973; Teodoro 2009 and 2010; Ohlweiler and Kawano, 2002).

B. glabrata is considered to be the most efficient vector due to its pronounced susceptibility to infection, whereas *B. straminea* is highly resistant to infection by *S. mansoni*, and is biologically less efficient in the production of cercaria in comparison with *B. glabrata* (Barbosa and Silva, 1992). Even so, *B. straminea* is important for the epidemiological transmission, due to its ample geographic distribution, and its ability to invade and colonize bodies of water. In fact, these species are present in areas with high levels of schistosomiasis (Favre *et al*., 1995).

The prevalence of the disease in most Brazilian states is due primarily to the country's long history of migrations, resulting in the transfer of the parasite to an ample range of localities (Nomura *et al*., 2007). The completion of the transmission cycle of this helminth is dependent on the availability of a series of factors associated with the interactions of the host, parasite, and the environment, such as the local occurrence of snails of the genus *Biomphalaria* (secondary host) susceptible to *S. mansoni* (Moraes *et al.*, 2014) and favorable conditions, including chemical-physical and biological factors like the composition of the soil, salinity, pH, pollution, nutrients, temperature, dissolved oxygen, conductivity (Rey, 2011). Populations of *Biomphalaria* are nevertheless affected adversely by factors such as anthropogenic impacts, predation, competition and climatic variations (Tibiriçá, 2006).

A large number of papers have focused on the distribution of the three natural host species, *B. glabrata* (Say, 1818), *B. straminea* (Dunker, 1848) and *B. tenagophila* (Orbigny, 1835). The identification of the geographic distribution of the mollusks determines the physical space within which the transmission of *S. mansoni* may occur, and the availability of these data is essential to the development of effective programs for the control of the disease (Teles *et al.*, 1991).

Paraense (1970) published the first map of the distributionof the secondary hosts of schistosomiasis in Brazil, which was subsequently updated a number of times (Carvalho *et al.*, 2008). In their book, Carvalho *et al.* (2008) present the distribution of *B. glabrata*, *B. straminea* and *B. tenagophila* by Brazilian state and municipality, based on occurrence data. These data show that at least one of the three host species of *S. mansoni* has been reported in 24 of the 26 Brazilian states, as well as the Federal District (Figure 2).

The distribution of *B. glabrata* is almost always associated with that of schistosomiasis, given its 91.6% susceptibility to the parasite, as well as its ability to eliminate a average up to 4500 cercaria per day (Tibiriçá, 2006). Just *B. glabrata* has been recorded in 16 Brazilian states (Alagoas, Bahia, Espírito Santo, Goiás, Maranhão, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Piauí, Rio Grande do Norte, Rio Grande do Sul, Rio de Janeiro, São Paulo, and Sergipe), as well as the Federal District, and in 806 municipalities. In the state of Pará, the northernmost extreme of the distribution of the species is the municipality of Quatipuru (0°53’58” S). Its occurrence has also been recorded in the municipalities of Belém, Bragança, Capanema, Irituia, Primavera, Quatipuru and Viseu (Rodrigues *et al.*, 1993; Carvalho *et al.*, 2008).

In turn, *B. straminea* has been recorded in 1325 municipalities in 24 Brazilian states (Acre, Alagoas, Amazonas, Bahia, Ceará, Espírito Santo, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Piauí, Rio Grande do Norte, Rio Grande do Sul, Rio de Janeiro, Santa Catarina, São Paulo, Sergipe, Tocantins, and Roraima), as well as the Federal District (Carvalho *et al.*, 2008).

Distribution of *Biomphalaria* in the Amazon region

The malacological fauna of the Amazon region (northern Brazil) has been the subject of a number of studies that have focused on systematics and epidemiology (Corrêa and Paraense, 1971; Rodrigues *et al.*, 1993). *Biomphalaria* is the most widespread snail Genus found in Brazilian state of Pará, where two species involved in the transmission of schistosomiasis – *B. glabrata* and *B. straminea* – are well-established in the northeast of the state (Rodrigues, 2013; Santos *et al.,* 2002).

Paraense (1986) concluded that *B. straminea* is a specie best adapted to the variety of climates and ecological conditions found in Brazil, and that the existence of localities where the species was not known to exist was due primarily to a lack of adequate research, rather than the absence of the species. In Pará, its occurrence has been reported in the municipalities of Alenquer, Altamira, Ananindeua, Belém, Belterra, Bragança, Breves, Capanema, Igarapé-Açú, Irituia, Itaituba, Monte Alegre, Nova Timboteua, Oriximiná, Primavera, Quatipuru, Santa Isabel do Pará, Santarém, and Viseu (Carvalho *et al.*, 2008). Nunes & Rodrigues (2007) recorded *Biomphalaria* in 35 of the 70 neighborhoods of Belém, capital of Pará, with cases of schistosomiasis being notified in 20.In Pará, two species, *B. glabrata* and *B. straminea*, are found in freshwater, both natural and artificial, where they are perfectly adapted to the local environmental conditions. The principal breeding grounds of these planorbids are located in the municipalities of Belém, Castanhal, Capanema, Quatipuru, Primavera, Bragança, Cachoeira do Piriá, Irituia and Km-74, where the transmission of schistosomiasis is active (Rodrigues *et al.*, 1993; Carvalho *et al.*, 2008), continuing further east to the municipality of Viseu and thelowl and plains of the neighboring state of Maranhão.

The occurrence of other *Biomphalaria* species in

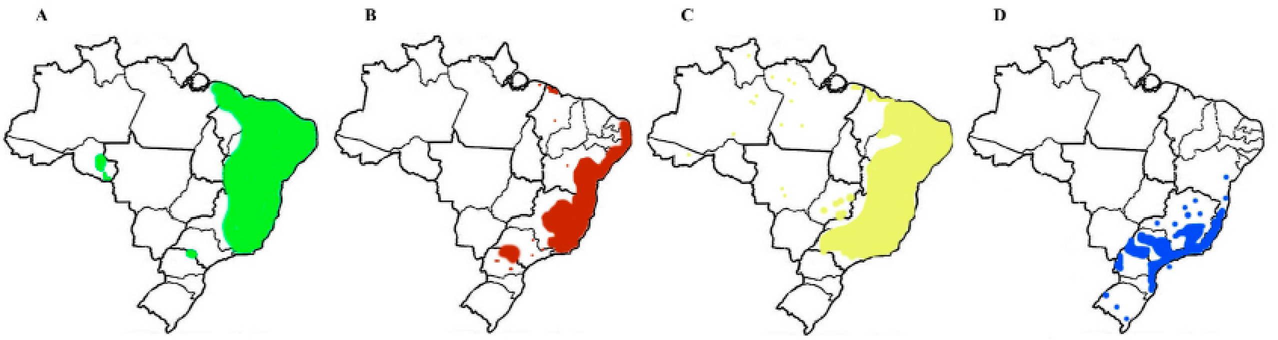

Figure 2: Map of the distribution of the genus *Biomphalaria* and its association with schistosomiasis. A: Distribution of schistosomiasis; B: Occurrence of *B. glabrata*. C: Occurrence of the specimens of *B. straminea*. D: Occurrence of the specimens of *B. tenagophila*. Source: Carvalho et al. (2008).

northern Brazil has yet to be confirmed due to the vast area of the region and the lack of malacological researchers. However, Nomura *et al.* (2007) reported 31 positive cases of *Schistosoma mansoni* in the municipality of Carajás between January 2004 and December 2005.

Machado & Martins (1951) recorded the first autochthonous cases of the disease in the municipality of Itaituba, which the result of the immigration of colonists from northeastern Brazil. In 1959, an outbreak of schistosomiasis was recorded in Quatipuru, Pará, followed by Belém, the state capital, in 1968. Paraense (1986) and Bichara *et al.* (1997) subsequently recorded autochthonous cases on Marajó Island and in Altamira.

Identification of *Biomphalaria* species

In epidemiological studies of schistosomiasis, one of the most important tasks is to identify the source of transmission. This source is detected through the identification of the areas affected by the host mollusk, and infected by *S. mansoni*, given that the occurrence of the disease is typically linked to the presence of snails (Conceição and Coura, 2012; Carvalho *et al*., 2008; Paraense, 1972; Rey, 2011). While the identification of these species is based traditionally on the comparison of the external morphology of the shell and the renal and reproductive organs, the similarities among the *Biomphalaria* species, due primarily to their small size, which is accentuated in the juvenile forms, hampers the reliable identification of taxa (Paraense, 1975; Melo, 2006).

Molecular epidemiology has been advancing in recent years due to the difficulty of identifying species reliably by traditional methods, which oftem lead to false positive or negative results. These methods involves the use of molecular techniques for the identification of species, in particular planorbids of extremely reduced size, which are difficult to determine conclusively, based on the characteristics of the shell (Traub *et al*., 2005; Caldeira *et al*., 2009). Molecular tools are also useful for the evaluation of phylogenetic patterns at the supraspecific level. In this context, an appropriate molecular marker must be selected for the problem in hand (Solé-Cava, 2001). Molecular tools thus provide an essential approach to the identification of specimens, which is especially important in Brazil, given the varying levels of susceptibility and epidemiological importance of the different species, and the extent of their geographic ranges (Melo, 2006; Paraense, 1975).

Different approaches may focus on either the mitochondrial (mtDNA) or nuclear (nDNA) genomes. For example, the Polymerase Chain Reaction analysis of Restriction Fragment Length Polymorphisms (PCR-RFLP) of the nuclear markers ITS1 and ITS2 is one of the most used approaches in studies of the systematics of *Biomphalaria* (Caldeira *et al*., 2000; Vidigal *et al*., 2001; Velásques *et al*., 2002; Spatz *et al*., 1999; Teodoro *et al*., 2010).

Using the PCR-RFLP technique to analyze an internal spacer fragment transcribed (ITS) rRNA gene, Vidigal *et al.* (2000) confirmed the efficiency of this method in the identification of 10 species and subspecies of Biomphalaria that occur in Brazil (species: *B. glabrata, B. tenagophila, B. occidentalis, B. Kuhn, B. straminea, B. intermedia, B . pilgrim, B. oligoza, B. schrammi, B. amazonica*; subspecies: *B. t. guaibensis*).The results of this study revealed low levels of intraspecific polymorphism among the different regions of the country, but distinct ITS features among species, which constitute useful markers for the reliable identification of species.

Regarding the species *B. amazonica*, Vidigal *et al.* (2000) observed patterns of variation between two sampled locations, which requires a lot of attention in the taxonomic status of *B. amazonica*. This same technique was used to confirm the presence of polymorphisms in the transcribed spacers (ITS1 and ITS2) that led to the confirmation of the existence of a new species of planorbid, *Biomphalaria edisoni*, from Colombia (Estrada *et al*., 2006).

The PCR-RFLP method has also proven effective for the definition of phylogenetic relationships and the identification of the *B. tenagophila* specimens collected in a number of different South American countries (Vidigal *et al*., 2004). It has also been important for the analysis of patterns of polymorphism, phylogenetic relationships, divergence, and the possible existence of species complexes of *Biomphalaria* in the Vale do Paranapanema region (Tuan & Santos, 2007).

The mitochondrial DNA is inherited maternally, lacks recombination and spacing sequences (introns), and is usually well conserved, but presents a high mutation rate in comparison with the nuclear DNA, given the more frequent substitution of bases, and the presence of genes that codify the ribosomal RNA, transporters, and polypeptides involved in oxidative phosphorylation, an integral process of the energetic metabolism of the mitochondria (Griffiths *et al*., 2008). This set of characteristics of the mtDNA make this genome favorable for studies of populations, intra- and inter-specific variation, and a variety of phylogenetic questions.

A number of mtDNA markers are widely-used, including the 16Sgene and subunit 1 of the cytochrome C oxidase (COI) enzyme (Palumbi *et al*., 1996). Sequences of the mitochondrial genes of two taxa of *Biomphalaria* (*B. tenagophila guaibensis* and *B. occidentalis*) from the Brazilian states of Rio Grande do Sul and São Paulo were used to estimate the levels of divergence of the *B. tenagophila* species complex and evaluate the potential of the mitochondrial COI and 16S for the identification of cryptic species (Tuan *et al*., 2012). Silva (2013) confirmed that COI can be used as a complementary tool for morphological analyses.

Preliminary studies of the distribution and biogeography of *Biomphalarias* in northern Brazil (Nobushige *et al.,* 2015; Lopes *et al.,* 2016) identified the species using mitochondrial (16S rRNA) and nuclear (ITS2) genes. The phylogenetic analyses identified *Biomphalaria kuhniana* and a monophyletic group belonging to the *B. amazonica/B. cousin* complex. The planorbids *B. amazonica* and *B. kuhniana* have already been recorded in the municipalities of Juruti and Parauapebas and in the area of the Carajás Project (Rodrigues, 1995) (Figure 3). These authors amplified the distribution of the *B. amazonica/B. cousini* complex as far as the municipalities of Bragança and Quatipuru (northeastern Pará). The third species identified in the study was *B. straminea,* which is found in the municipalities of Castanhal and São Luis.

Given the rapid expansion of the disease, and the paucity of the available data, the distribution of the host species is not adequately understood. Another limiting factor is the small number of researchers focusing on the problem (Carvalho *et al.*, 2008). Published studies on the genus are restricted to southern and southeastern Brazil, highlighting the scarcity of data from the country's northern region. As the disease is probably endemic to this region, given that the occurrence of schistosomiasis is intimately related to the local occurrence of specific hosts, given their role in the epidemiology of the disease, the reliable identification of the secondary hosts is essential to the expansions of the database and the development of effective preventive strategies for the control and combat of endemic schistosomiasis (Carvalho *et al.*, 2008).

This preliminary study presents important results, such as the amplification of the occurrence of *B. amazonica* in the northern region of Brazil, which constitute important components of the epidemiology of schistosomiasis in the region.

Comparative morphological analyses, primarily of the shell and the renal and reproductive organs, may

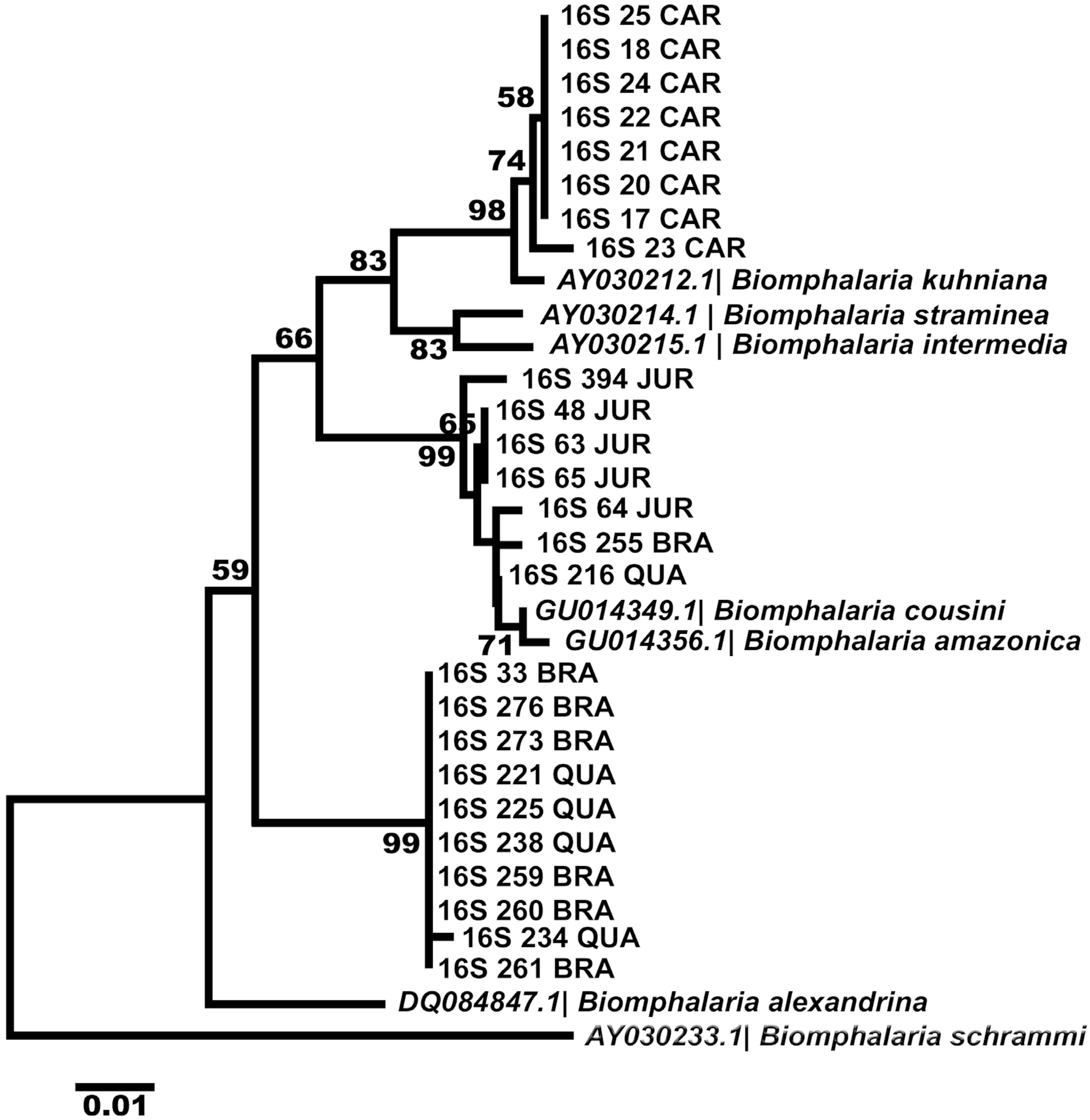

Figure 3: Phylogenetic tree of the *Biomphalaria* specimens collected in the Brazilian Amazon.

be impeded by factors such as the reduced size of some individuals, in particular immature specimens, the morphological similarities of many species, and the inadequate conservationof the specimens. Given these difficulties, molecular data provide vitally important clues for the identification of the specimens collected.

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