

***Biomphalaria* spp. (Preston, 1910) snails in the municipality of Juiz de Fora, Zona da Mata Mineira mesoregion, state of Minas Gerais, Brazil**

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This study focuses on the geographic distribution of the snail of the genus Biomphalaria and evaluates its infectivity by Schistosoma mansoni in 5264 specimens collected in the municipality of Juiz de Fora, Minas Gerais, Brazil. Of the 31 locations studied, 6 were reservoirs, 11 rudimentary holding ponds, 7 irrigation ditches, 5 lakes, 1 ornamental pond, and 1 waterfall. Intermediate hosts were found only in the rudimentary ponds and ditches, which were 100% positive. Using morphological and molecular analysis techniques, B. tenagophila, B. peregrina, and B. straminea were identified. This is the first report of B. straminea in the municipality, and evaluation of its infective potential revealed susceptibility of 25.4%. Although we did not find specimens of Biomphalaria infected by S. mansoni, the data obtained indicate the presence of intermediate hosts, especially in the irrigation ditches in Juiz de Fora, and their proximity to contaminated areas.

Key words: *Biomphalaria* - *Biomphalaria straminea* - schistosomiasis - geographic distribution - Juiz de Fora - Brazil

Schistosomiasis, an infection caused by *Schistosoma mansoni*, is one of the world's leading parasite-borne diseases, affecting 84 million people annually, in many developing countries (WHO 1998). In Brazil, where it is endemic, it affects about 6 million people a year and figures among the country's most relevant infectious diseases, principally for its high morbidity/mortality index and wide geographic distribution (Schall & Diniz 2001). It is distributed in four states of the Southeast Region. In the state of Minas Gerais, the endemic areas are located in the mesoregions of Norte de Minas, Campo das Vertentes, Oeste de Minas, Vale do Jequitinhonha, Vale do Mucuri, Vale do Rio Doce, Região Metropolitana de Belo Horizonte, Zona da Mata, and Central Mineira (Carvalho et al. 1997, Souza et al. 2001). A mesoregion is a group of microregions. A microregion is a set of contiguous municipalities in a single state, grouped according to a combination of geographic, economic and administrative characteristics.

Molecular evidence, corroborated by fossil findings, supports the hypothesis that snails of the genus *Biomphalaria*, an intermediate host of *S. mansoni*, originally

evolved in the Americas. Biogeographic studies indicate that an ancestor of *B. glabrata* from the New World dispersed in the Pliocene-Pleistocene (Campbell et al. 2000, DeJong et al. 2001), probably by rafting on vegetation or by eggs carried by migratory birds, crossing the Atlantic to Africa and giving rise to the current African species (Lockyer et al. 2004).

Among the 10 species of *Biomphalaria* found in Brazil, 7 have been identified in the state of Minas Gerais (Souza et al. 2001), including those susceptible to infection in nature: *B. glabrata* (Say 1818) *B. tenagophila* (Orbigny 1835), and *B. straminea* (Dunker 1848).

Of the state's 853 municipalities distributed in the 12 political-administrative mesoregions, 283 have been studied for the snail's prevalence. *B. glabrata* has been reported in 65.3% of the municipalities studied; *B. straminea* in 43.8%; *B. tenagophila* in 20.4%; *B. peregrina* in 20.1%; *B. schrammi* in 9.2%; *B. intermedia* in 7%, and *B. occidentalis* in 0.7% (Souza et al. 2001). The municipality in Brazil is the local political-administrative division, corresponding roughly to a county or prefecture. It is governed by a mayor and municipal council. Some municipalities are almost completely urbanized, others almost totally rural, and some, like Juiz de Fora, a mixture of the two.

The most recent scientific report on the occurrence of *Biomphalaria* in Juiz de Fora municipality, the main social and economic center in the state's Zona da Mata

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Mineira, dates from 1956 (Paraense & Deslandes) and hence contains information from 50 years ago on the location of the species found. Recently the diagnosis of a case of neuroschistosomiasis in the environs of Juiz de Fora awoke the need for updated investigations of the intermediate hosts of *S. mansoni* and on the possibility that these are infected.

So, this work focuses on the geographic distribution of *Biomphalaria* in Juiz de Fora municipality with identification of the species found, possibility of infectivity, and main types of related habitats. This updated study of the occurrence of intermediate hosts in the region confirms, for the first time, the presence of *B. straminea* in the municipality, with the study of this snail susceptibility of the Juiz de Fora strain to *S. mansoni*.

MATERIALS AND METHODS

Location under study - This study was carried out in the municipality of Juiz de Fora (21S14/42W14), in the mesoregion known as the Zona da Mata Mineira, Minas Gerais, Brazil (Fig. 1). This municipality is located 280 km from Belo Horizonte, the state capital, and 184 km from the city of Rio de Janeiro. Juiz de Fora has a land area of 1,429.875 km², with a population of 419,226 inhabitants according to the 2000 census. The municipality, with an average altitude of 800 m, has an ample hydrographic basin rich in lakes, ponds, and irrigation ditches. The spatial localization of the foci was determined by instant positioning from a GPS (Global Positioning System) apparatus.

Malacological survey - These surveys were performed in collaboration with technicians from DADS/Juiz de Fora (Directorate for Decentralized Health Actions, an organ of the State Health Secretariat). The specimens were collected from lotic and lentic ecosystems in the municipality, predominantly around the urban perimeter, where the characteristics are favorable to the occurrence and spa-

tial dispersion of snails near human populations. We captured the specimens with the aid of tongs and nets. The study was conducted in 2003 to 2004.

Snails - After collection, the snails were taken to the laboratory where the living specimens were measured. After examination under artificial light for *S. mansoni* cercariae, the snails were submitted to squeezing between glass plates to detect the presence of cercariae. Some specimens were separated for morphological identification (Paraense 1975). Another sample of specimens was packed and sent to the René-Rachou Research Center for molecular identification (Vidigal et al. 1998) and to obtain descendents (F1) for susceptibility assays.

Susceptibility of *B. straminea* - One hundred specimens F1 of *B. straminea* were exposed to 50 miracidia of *S. mansoni* (strain LE). For infection control, 50 specimens of *B. glabrata*, reared under laboratory conditions (Belo Horizonte/MG), with 8-10 mm diameter, were exposed to 10 miracidia/snail. After a 30-days exposition, the snails were individually placed under artificial light and examined by stereomicroscopy. These snails were evaluated weekly until 60 days after infection. The negative specimens were examined and submitted to squeezing between glass slides.

RESULTS

Malacological survey - The study concentrated on 31 lotic sites (ditches, waterfalls) and lentic sites (rudimentary ponds, reservoirs, ornamental ponds) distributed in the eight geographical regions of the municipality of Juiz de Fora (located in the Minas Gerais - Zona da Mata). We collected a total of 5264 specimens of *Biomphalaria* and identified them morphologically by analysis of the shell, reproductive and renal system (Paraense 1975), and by polymerase chain reaction (PCR) (Vidigal et al. 1998).



Fig. 1: map of mesoregions of the state of Minas Gerais, Brazil in 1996.

The Table shows the sites studied, their geographic position and the species found. As can be seen, of the 31 sites studied, 6 were reservoirs, 11 rudimentary holding ponds, 7 truck farms with irrigation ditches, 5 lakes, 1 ornamental pond, and 1 waterfall. We found no species of *Biomphalaria* in the reservoirs, lakes, and waterfall studied. However, we found three different species of *Biomphalaria* in 100% of the irrigation ditches studied: *B. tenagophila*, *B. Peregrina*, and *B. straminea*. Of the 11 rudimentary ponds studied, we found the species *B. tenagophila* in 27%.

The Fig. 2 shows that of the 31 sites studied, *B. tenagophila* was the species most commonly found, accounting for 60% of the positive points. *B. peregrina* was found at 20% of the points, all outside the urban perimeter. *B.*

straminea was found at 20% of the points in irrigation ditches. This is the first report of this species in the municipality.

Infection of the snails by S. mansoni - We did not find any infections by *S. mansoni* in the 5264 specimens collected, which were evaluated both by the technique of exposure to artificial light and squeezing.

Susceptibility to B. straminea - We subjected the snails collected at the coordinates 21°39'59"S, 43°25'09"W, 716 m of altitude, to the test for susceptibility to *S. mansoni* and the Juiz de Fora strain of *B. straminea*, finding 25.4% infectivity, in contrast to the Belo Horizonte strain, which showed 91.6% infectivity.

TABLE

List of sites, geographic position determined by GPS and *Biomphalaria* species studied, predominantly around the urban perimeter of Juiz de Fora, Minas Gerais, Brazil

Collection site	Type of collection site ^a	Coordinates	Region	Species of <i>Biomphalaria</i>
João Penido (Remonta)	Reservoir	21°40'47"S43°23'43"W	North	Negative
Chapéu D'Uvas	Reservoir	21°34'57"S43°33'10"W	North	Negative
João Penido (Náutico)	Reservoir	21°38'37"S43°23'41"W	North	Negative
Poço D'Anta (Cesama)	Reservoir	21°44'31"S43°24'05"W	East	Negative
Sobragi	Reservoir	21°58'44"S43°22'07"W	South	Negative
São Pedro	Reservoir	21°39'59"S43°25'09"W	West	Negative
Barreira do Triunfo	Irrigation ditch	21°39'59"S43°25'09"W	North-West	<i>B. straminea</i>
Milho Branco	Irrigation ditch	21°44'31"S43°24'25"W	North-West	<i>B. straminea</i>
Bandeirantes	Irrigation ditch	21°43'25"S43°21'20"W	North-East	<i>B. tenagophila</i>
Vivendas da Serra	Irrigation ditch	21°43'03"S43°20'56"W	North-East	<i>B. tenagophila</i>
Córrego Yung	Irrigation ditch	21°41'20"S43°18'27"W	East	<i>B. tenagophila</i>
Granjeamento Dias Tavares	Irrigation ditch	21°38'51"S43°28'39"W	North	<i>B. peregrina</i>
Grama-Ribeirão das Rosas	Irrigation ditch	21°41'23"S43°20'30"W	North-East	<i>B. tenagophila</i>
Granjeamento Dias Tavares	Rudimentary pond	21°38'46"S43°29'35"W	North	<i>B. tenagophila</i>
Army Training Camp/ Barbosa Lage	Rudimentary pond	21°42'09"S43°22'54"W	North	Negative
Vale do Oeste Paid Fishing Park/ Highway BR 040	Rudimentary pond	21°45'27"S43°26'37"W	North	Negative
Humaitá (Sítio Recanto do Sol) Humaitá	Rudimentary pond	21°44'26"S43°30'01"W	North	Negative
(Sítio Recanto do Sol Highway) Yung	Rudimentary pond	21°44'26"S43°20'01"W	North	Negative
Yung	Rudimentary pond	21°42'21"S43°18'27"W	East	<i>B. tenagophila</i>
Vivendas da Serra	Rudimentary pond	21°43'06"S43°20'51"W	North-East	Negative
Hotel Fazenda São Fidelis	Rudimentary pond	21°44'45"S43°14'48"W	South-East	Negative
Santana Farm (Sarandira Highway) Rest.Recanto das Garças/ Highway BR 040	Rudimentary pond	21°48'04"S43°10'55"W	South-East	Negative
Trevo de Torreões	Rudimentary pond	21°52'04"S43°21'01"W	South	<i>B. peregrina</i>
Mariano Procópio Museum	Rudimentary pond	21°39'59"S43° 25'03"W	North	Negative
Lake Manacás/ UFJF	Lake	21°44'78"S43°21'65"W	Center	Negative
Lake ASE	Lake	21° 46' 68"S43° 22' 22"W	West	Negative
SESC	Lake	21°42'51"S43°23'27"W	North-West	Negative
Lajinha Park	Lake	21°47'26"S43°23'57"W	West	Negative
Halfeld Park	Lake	21° 47' 56"S43° 21' 98"W	South	Negative
Vale do Ipê	Ornamental pond	21° 45' 72"S43° 21' 05"W	Center	Negative
	Waterfall	21° 45' 26" S43° 21' 96" W	Center	Negative

^a: the sites in table and map were classified using the following definition of terms: Rudimentary pond, a rustic construction to hold water; Reservoir, a reservoir behind a professionally engineered dam or dike; Lake, a large extension of water surrounded by land, normally of pluvial origin (natural or artificial); Ornamental pond, a small extension of water or small lake for ornamental purposes; Waterfall, a vertical fall of water; Irrigation ditch, small collections of water for crop irrigation.

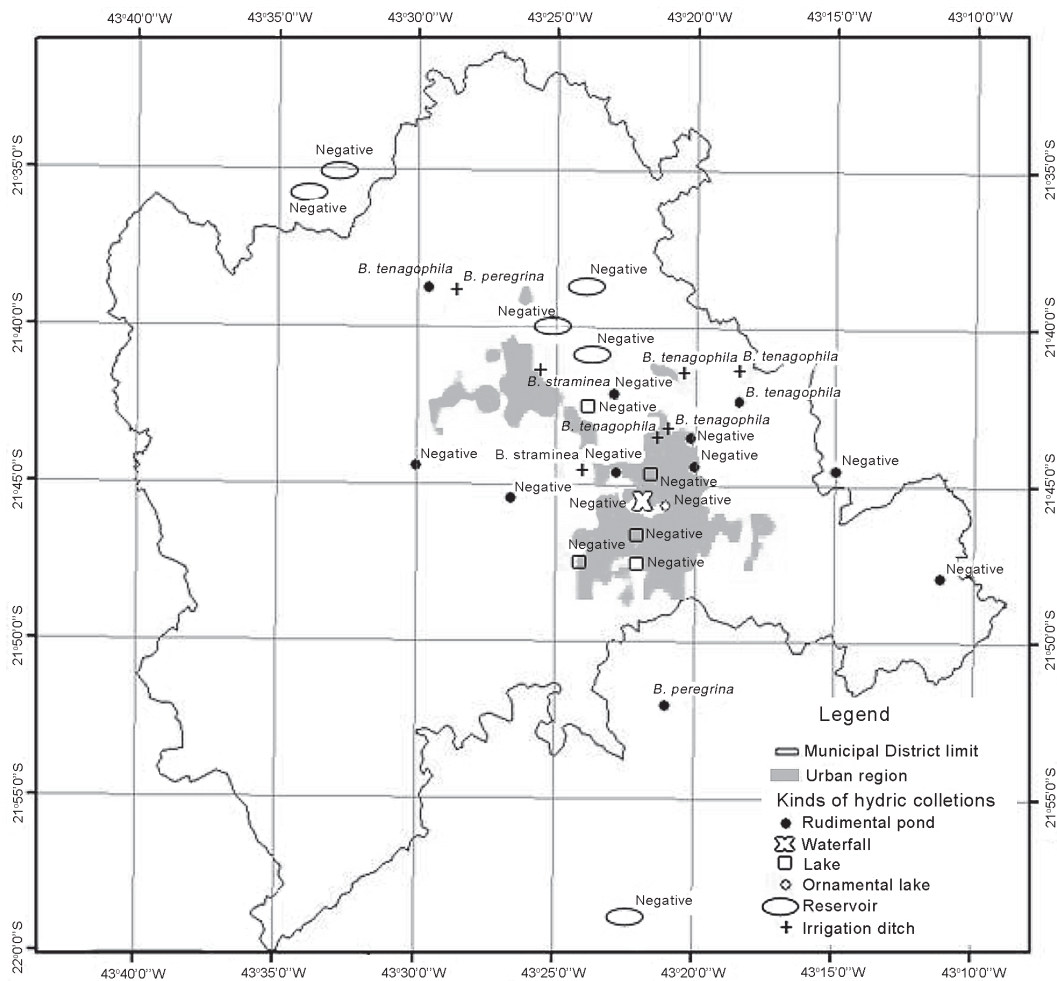


Fig. 2: map showing the distribution of the *Biomphalaria* species found in the municipality of Juiz de Fora, Minas Gerais, Brazil.

DISCUSSION

This is the first geographic mapping study, through GPS, involving snail hosts of *S. mansoni* in the municipality of Juiz de Fora, the main socioeconomic center in the Zona da Mata Mineira of the state of Minas Gerais. Up-to-date information on the occurrence of *Biomphalaria* in this region is rare. This work helps to improve knowledge of the local reality and provides support for the planorbids mapping of the state. Minas Gerais has a land area of 5,384,000 km², making it Brazil's fourth largest by geographic area. It has areas where schistosomiasis is highly endemic, a large number of water bodies and courses, and is a magnet for ecotourism. It is divided into 12 political-administrative mesoregions, among them the Zona da Mata Mineira, with 2 million inhabitants and a low municipal human development index (IDH-M). Approximately 90% of its 144 municipalities have no record in the literature on the prevalence of *Biomphalaria* sp.

Pinto and Deslandes (1953) and Paraense and Deslandes (1956) verified the occurrence of *B. glabrata*, *B. tenagophila*, and *B. peregrina* in Juiz de Fora municipality. As can be observed in this work (Table and Figure), we reaffirmed the presence of *B. tenagophila* and *B.*

peregrina, and identified *B. straminea* for the first time in the municipality, but we did not find *B. glabrata*, as reported by the previous authors. *B. glabrata* is from an epidemiological standpoint considered to be the most important host species in the Americas, due to the high biological potential of natural infection and its wide distribution. Souza et al. (2001) in a survey of planorbidae in Zona da Mata Mineira, reported finding *B. glabrata* in 100% followed by *B. tenagophila* in 40%, and *B. straminea* in 20% of the 15 municipalities studied. The fact we did not find *B. glabrata* again in the municipality of Juiz de Fora can be evaluated due to changes in a great number of biotic or abiotic factors, such as climate, water chemistry, predation, parasitism, competition, and anthropogenic perturbations. Therefore, the initial report of *B. glabrata* in Juiz de Fora, a half century ago, was due to the fact that the urban perimeters were not well defined at that time and could have been confused with the rural limits of neighboring municipalities, particularly Tabuleiro and Piau, recognized as habitats of *B. glabrata* (Souza et al. 2001). Another hypothesis involves the competitive superiority of *B. straminea*, observed in data on the population interactions of *B. glabrata* and *B. straminea*, where the first

specie has been replaced by the latter (Michelson & Dubois 1979). The lesser susceptibility of *B. straminea* to *S. mansoni* could justify action for control by substitution of one species by another. In Martinique, *B. glabrata* is mentioned as a rare species because of its possible natural substitution by *B. straminea* (Guyard & Pointer 1979). The inverse phenomenon appears to be occurring in the state of Pará, with the appearance of *B. glabrata* in places supposedly occupied only by *B. straminea* (Paraense 1986).

Such superiority might be based on some characteristics found in *B. straminea*, such as greater resistance to desiccation and to infestation by *S. mansoni*; greater vagility and capacity to disperse; or its greater fertility in relation to *B. glabrata*. One of the most characteristic traits of the snail-digenetic interaction is its specificity, and the fact that *B. glabrata* has not been found again to date explains, from the standpoint of specificity, why *S. mansoni* was not found in the municipality, even though *B. straminea* is an important host in the Brazil's Northeast Region. This *Schistosoma-B. glabrata* compatibility is a direct consequence of the complex relations between snail and host and can be considered as an amalgamation of physiological and behavioral interactions. Both the snail's susceptibility and the parasite's infectivity contribute to this highly specific relationship and are genetically defined.

The occurrence of *B. straminea* in the municipality was reported for the first time in this work, although statewide it is more amply distributed when compared to *B. tenagophila*. In Zona da Mata, *B. straminea* had already been reported in the municipalities of Ponte Nova, Ubá, and Rio Casca (Souza et al. 2001). Although *B. straminea* constitutes an intermediate host experimentally taken as less apt to transmit *S. mansoni*, due to the lower specificity of the parasite-host relationship, strains from outback and coastal zones in Brazil's Northeast Region are responsible for maintaining the high endemic prevalence in that region (Teles 1996). In Minas Gerais, the *B. straminea* specimens studied in the majority of reports have not shown infection, even though this species was pointed to as responsible for an outbreak of schistosomiasis in Paracatu municipality (Carvalho et al. 1987).

In this manner, the *B. straminea* strain found in Juiz de Fora, tested for susceptibility to *S. mansoni*, showed 25.4% infectivity in relation to the Belo Horizonte control strain. This index can be considered high when compared with the susceptibility data for the species cited in the literature (Gerken et al. 1975, Souza et al. 1981a, b, 1983, Souza 1986, Fernandez & Thiengo 2002, Massara et al. 2002).

The irrigation ditches studied here are used by truck farmers in the so-called green belt of Juiz de Fora. They are mainly smallholders who market their crops directly. Although planorbidae of the genus *Biomphalaria* can be found in various freshwater habitats, both natural and artificial, except for very deep water bodies or fast-flowing watercourses, their population density tends to be greater in artificial breeding grounds such as drainage and irrigation ditches or small collection ponds (Cunha 1970). This corroborates the fact that all the truck farms

investigated in Juiz de Fora municipality were positive for *Biomphalaria*. One of them, located on the urban perimeter (coordinates: S 21°41'23" and W 43°20'30") and colonized by *B. tenagophila*, is only 15 km from a focus of contamination within the rural limits. This evidence, associated with the large migratory movement in the microregion, the poor basic sanitation conditions and low sanitary education of the local people, form ingredients that could well lead to propagation of schistosomiasis in the urban area of Juiz de Fora municipality and its mesoregion.

Having met the objectives of systematic updating, identification and evaluation of the infectivity and susceptibility of host snails of *S. mansoni* in the municipality, the data obtained are now being used in broader efforts to help control the disease in Zona da Mata Mineira. Through a partnership between the academic community of University Federal of Juiz de Fora and the technical staff of the Directorate for Decentralized Health Actions of the State Health Secretariat (DADS/JF), the study has been expanded to the neighboring municipalities (microregion), and at the same time an Extension Project is being established to seek operational solutions with the Brazilian National Health System (SUS, Sistema Único de Saúde), to prevent the spread of this endemic disease in the region.

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