



## Article/Artigo

# Malacological survey of *Biomphalaria* snails in municipalities along the Estrada Real in the southeast of the State of Minas Gerais, Brazil

Pesquisa malacológica de *Biomphalaria* em municípios da Estrada Real, situados no sudeste do Estado de Minas Gerais

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### ABSTRACT

**Introduction:** The increasing practice of ecotourism and rural tourism in the State of Minas Gerais, Brazil, highlights the importance of studies concerning the occurrence of potential intermediate hosts of *Schistosoma mansoni*. This study aimed to identify species of *Biomphalaria* snails in municipalities along the Estrada Real, an important Brazilian tourism project. **Methods:** The specimens were collected in different water collections of 36 municipalities along the Estrada Real in the southeast of the State of Minas Gerais. *Biomphalaria* species were characterized using both morphological and molecular approaches. The research was conducted between August 2005 and September 2009 and all the sites visited were georeferenced using GPS. **Results:** Six *Biomphalaria* species were found in 30 of the 36 municipalities studied: *glabrata*, *tenagophila*, *straminea*, *peregrina*, *occidentalis* and *schrammi*. The first three species of *Biomphalaria*, recognized as intermediate hosts of *S. mansoni*, were present in 33.3%, 47.2% and 8.3% of the municipalities studied, respectively. The mollusks were found in different types of water collections and no infection by *S. mansoni* was detected. The highest occurrence of *Biomphalaria* concentration was verified in the area covered by the Caminho Novo route (Diamantina/MG to Rio de Janeiro/RJ). **Conclusions:** Considering the occurrence of schistosomiasis in the State of Minas Gerais and the socioeconomic repercussions involved in the Estrada Real Project, this work focuses on the vulnerability of water collections due to the presence of *Biomphalaria* mollusks and emphasizes the need for epidemiological surveillance and sanitary and educational measures integrated with the local community and tourism sectors.

**Keywords:** *Biomphalaria*. Schistosomiasis. Estrada Real. Tourism. Ecotourism. Spatial distribution.

### RESUMO

**Introdução:** O aumento das práticas de ecoturismo e turismo rural, em Minas Gerais, Brasil, evidencia a importância de se realizarem estudos sobre a ocorrência de hospedeiros intermediários do *Schistosoma mansoni*, no estado. O presente trabalho objetivou a busca e identificação das espécies de caramujos *Biomphalaria* encontrados em municípios mineiros pertencentes à Estrada Real, um importante projeto de turismo brasileiro. **Métodos:** Os moluscos foram coletados em 36 municípios da Estrada Real, no sudeste de Minas Gerais. A pesquisa foi realizada de agosto de 2005 a setembro de 2009 e todos os locais visitados foram georreferenciados com o uso de GPS. **Resultados:** Dos 36 municípios estudados, 30 apresentaram a ocorrência de pelo menos uma entre as seis espécies de *Biomphalaria*: *glabrata*, *tenagophila*, *straminea*, *peregrina*, *occidentalis* e *schrammi*. As três primeiras espécies citadas, reconhecidas como hospedeiras intermediárias do *S. mansoni*, estavam presentes em 33,3%, 47,2% e 8,3% dos municípios estudados, respectivamente. Os moluscos foram encontrados em diferentes tipos de coleções hídricas e em nenhum deles foi detectada infecção pelo *S. mansoni*. Houve maior ocorrência de *Biomphalaria* na área referente ao Caminho Novo (Diamantina/MG ao Rio de Janeiro/RJ). **Conclusões:** Considerando-se a ocorrência da esquistossomose, no Estado de Minas Gerais, e as repercussões socioeconômicas que envolvem o projeto Estrada Real, este trabalho aponta para a vulnerabilidade das coleções hídricas devido à presença de moluscos *Biomphalaria* e enfatiza a necessidade de vigilância epidemiológica e medidas educativas e sanitárias integradas com a comunidade local e setores de turismo.

**Palavras-chaves:** *Biomphalaria*. Esquistossomose. Estrada Real. Turismo. Ecoturismo. Distribuição espacial.

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### INTRODUCTION

Mollusks of the genus *Biomphalaria* infected with *Schistosoma mansoni* maintain the cycle of schistosomiasis in several Brazilian municipalities, despite recent improvements in the socioeconomic status of the population and education programs focusing on health, which were expected to reduce transmission of the disease. The relation between disease occurrence in human populations and the geographical presence of susceptible intermediate hosts is a well documented fact<sup>1</sup>. Poor rural communities continue to be major areas of schistosomiasis transmission<sup>1</sup>. With the phenomena of urbanization (migration to the outskirts of large cities), all aspects of the disease are reappearing, such as: neuroschistosomiasis, pulmonary hypertension, hepatosplenic schistosomiasis, acute episodes, glomerulonephritis and pyogenic liver abscesses<sup>1-13</sup>.

The State of Minas Gerais presents one of the highest prevalence rates for schistosomiasis in Brazil<sup>14,15</sup>. The presence of schistosomiasis in this state causes a number of concerns, not only for the healthcare sector, but also for economic sectors, such as tourism, which involve significant capital.

In the historical context of the economic development of State of Minas Gerais, the term *Estrada Real* refers to the routes taken by settlers from the time of the discovery of gold in Minas Gerais to the period of its exhaustion<sup>16,17</sup>. The *Estrada Real* was created by the Portuguese Crown in the 17<sup>th</sup> century with the intention of controlling the flow of wealth, in the form of gold and diamonds and goods, which passed between the State of Minas Gerais and the coastal city of Rio de Janeiro, the capital of the colony at the that time<sup>17</sup>. The association of tourist attractions, including colonial buildings, churches, museums, nature reserves, adventure sports, mineral springs and culinary attractions, led to the creation of the *Estrada Real* Project. The *Estrada Real* Project consists of 193 municipalities, 162 of which are in the State of Minas Gerais, nine in the State of Rio de Janeiro and 22 in the State of São Paulo<sup>17</sup>.

The State of Minas Gerais includes the majority of the municipalities of the *Estrada Real* Project with great potential for expanding ecotourism and rural tourism, because of the attraction represented by historical-cultural monuments, the diversity of the topography, fauna and flora and water bodies. Thus, mapping the geographical areas vulnerable to the transmission of schistosomiasis becomes a task not only of health surveillance, but also of encouraging productive sectors that drive the economy in this state.

In order to contribute to the generation of georeferenced epidemiological data and the control of schistosomiasis in the municipalities of the *Estrada Real* Project in southeastern Minas Gerais, this work reports a malacological survey designed to verify the occurrence of mollusks of the genus *Biomphalaria* and the possibility of infection by *S. mansoni*.

## METHODS

### Study area

The study municipalities are located in the southeast of the State of *Minas Gerais*, Brazil, and belong to the Regional Healthcare Administration of *Juiz de Fora* (*Gerência Regional de Saúde*, GRS/JF). The state is administratively divided into 13 macroregions. Among these, the southeastern macroregion consists of eight microregions, of which the *Juiz de Fora/Lima Duarte/Bom Jardim de Minas* microregion is under the responsibility of the GRS/JF. This microregion is composed of 37 municipalities. Of these, 36 were included in the study. The core urban area of the City of *Juiz de Fora* was not included, because the results of a local malacological survey conducted prior to this study had already been published<sup>18</sup>.

### Malacological survey

Mollusks of the *Biomphalaria* genus were collected from August 2005 to September 2009, by technicians of the GRS/JF. Catches were made in several areas of the municipalities in order to cover the largest possible area, with the aid of gloves, tongs and dip nets. In all 36 municipalities, the type and quantity of water collection sites surveyed were selected by the following criteria: reported local cases of human schistosomiasis, existence of water bodies (lotic and lentic) whose characteristics favored the occurrence of the *Biomphalaria* genus and the possibility of contact with human populations. Thus, based in these criteria, at least three water collections in each municipality were surveyed. The points were georeferenced using a Global Positioning System (GPS), Garmin Model II-12, and imported into a Geographic Information System (GIS) to permit visualization of their spatial distribution. Sites in which the presence of *Biomphalaria* was verified were photographed with a digital camera.

### Mollusk identification and examination of *Schistosoma mansoni* infection

The captured snails were counted, transported in plastic containers and maintained in the laboratory until identification. Following collection, the snails were transported to the laboratory where they were measured and examined under artificial light. Some specimens from each sample were identified at the Parasitology Laboratory in the Federal University of *Juiz de Fora* and the Entomology Laboratory of the GRS/JF, *Secretaria de Estado de Saúde de Minas Gerais* (SES/MG), according to the protocol established by Paraense<sup>19,20</sup>. Some specimens were sent to the

Laboratory of Helminthology and Malacology at the *René Rachou* Medical Research Center, Oswaldo Cruz Foundation (CPqRR/FIOCRUZ), for confirmation of species by the technique of polymerase chain reaction (PCR)<sup>21</sup>.

## RESULTS

Research on *Biomphalaria* was conducted in 36 municipalities, 19 of which form part of the *Estrada Real* Project, while the remainder are located within the coverage area (Table 1). As presented in Table 1, 30 municipalities were positive for snails of the genus *Biomphalaria*,

TABLE 1 - Presence of *Biomphalaria*, the number of catch basins surveyed and snails collected in the municipalities of the *Estrada Real* in the southeast of the State of Minas Gerais.

Municipalities	Water		Snails collected (n)
	collections studied (n)	Species of <i>Biomphalaria</i> *	
Andrelândia**	6	ausent	0
Aracitaba	6	p	2
Arantina	6	ausent	0
Belmiro Braga**	6	p	125
Bias Fortes**	6	p	64
Bicas	5	g, t	116
Bocaina de Minas	4	ausent	0
Bom Jardim de Minas	5	ausent	0
Chácara**	5	p	44
Chiador**	8	t, s	69
Coronel Pacheco**	17	g, t	567
Descoberto	5	g, t	139
Ewbank da Câmara**	8	t	55
Goianá	23	g, t	1,045
Guarará	5	g, t	20
Liberdade	6	p	8
Lima Duarte**	8	ausent	0
Mar de Espanha	6	p, o	28
Maripá de Minas	7	g, p	65
Matias Barbosa**	9	t, sc	38
Olaria**	3	p	9
Oliveira Fortes**	6	t, p	11
Passa Vinte	6	p	86
Pedro Teixeira**	6	p	35
Pequeri**	12	p	67
Piau**	14	g, t, s	394
Rio Novo	10	g, t	135
Rio Preto	3	ausent	0
Rochedo de Minas	5	g, t	160
Santa Bárbara do Monte Verde	3	p	20
Santa Rita de Jacutinga**	6	p, t	16
Santana do Deserto**	5	g, t, s	195
Santos Dumont**	6	t	15
São João Nepomuceno	5	g, t, p	38
Senador Cortes	4	p	11
Simão Pereira**	8	g, t	45

\*Species of *Biomphalaria*: t: *tenagophila*, s: *straminea*, p: *peregrina*, o: *occidentalis*, sc: *schrampi*, g: *glabrata*, \*\*Municipalities that belong to the *Estrada Real* Project. The other municipalities are part of the coverage area.

while in six, the presence of these mollusks not observed. Six *Biomphalaria* species were identified: *glabrata*, *tenagophila*, *straminea*, *peregrina*, *occidentalis* and *schrammi*. *B. tenagophila* was the most prevalent species and was found in 17 municipalities, followed by *B. peregrina*, in 16 municipalities and by *B. glabrata*, in 12. A total of 3,772 specimens of mollusks were collected, all negative for cercariae of *S. mansoni* (Table 1).

Table 2 shows the water collections visited in the municipalities studied and the number of positive sites for *Biomphalaria* snails. The sites were classified according to the following definitions intentionally left in their categories as defined in Portuguese to preserve the distinctions between site types: stream, water flowing in a channel; dam, a barrier constructed to contain the flow of water; tank, a large artificial container used to hold water; irrigation ditch, a channel for water irrigation; bog, wet spongy ground containing clay; waterfall, a cascade of water; water tank, a container for supplying water under pressure; water trough, a container providing water for animals; canal, an artificial waterway; spring, water rising from the earth; well, a hole or shaft sunk into the earth to obtain water; and reservoir, a natural or artificial pond or lake used for storage and regulation of water. Among the various water collections, streams were the most positive sites for *Biomphalaria* species, with 35 locations, followed by dams. The greatest variety of species was found in the dams. Among the 12 water collections surveyed, 10 showed the presence of *B. glabrata* and/or *B. tenagophila* (Table 2).

TABLE 2 - Type and quantity of water collection sites in which the occurrence of *Biomphalaria* was verified.

Water collection*	Positive sites (n)**	<i>Biomphalaria</i> species***
Stream	35	g, t, p, s
Dam	14	g, t, s, p, o, sc
Tank	7	t
Irrigation ditch	5	g, t
Bog	2	g, t, p
Waterfall	1	p
Water tank	1	g, t
Water trough	1	t
Canal	1	s
Spring	1	t, p
Well	1	g
Reservoir	1	g, p

\*The sites were classified according to the definitions intentionally left in their categories as defined in Portuguese to preserve the distinctions between site types (explanations in the results). \*\*Refers to the presence of *Biomphalaria*, not necessarily infected with *Schistosoma mansoni*. \*\*\*Species of *Biomphalaria*: g: *glabrata*, p: *peregrina*, t: *tenagophila*, s: *straminea*, o: *occidentalis*, sc: *schrammi*.

Figure 1 shows the coverage area of the Estrada Real Project in Brazil and highlights the positive and negative areas for *Biomphalaria* snails. A higher concentration of *Biomphalaria* occurs in the eastern region of the map, in the area covered by the Caminho Novo (Diamantina/MG to Rio de Janeiro/RJ), than in the western region

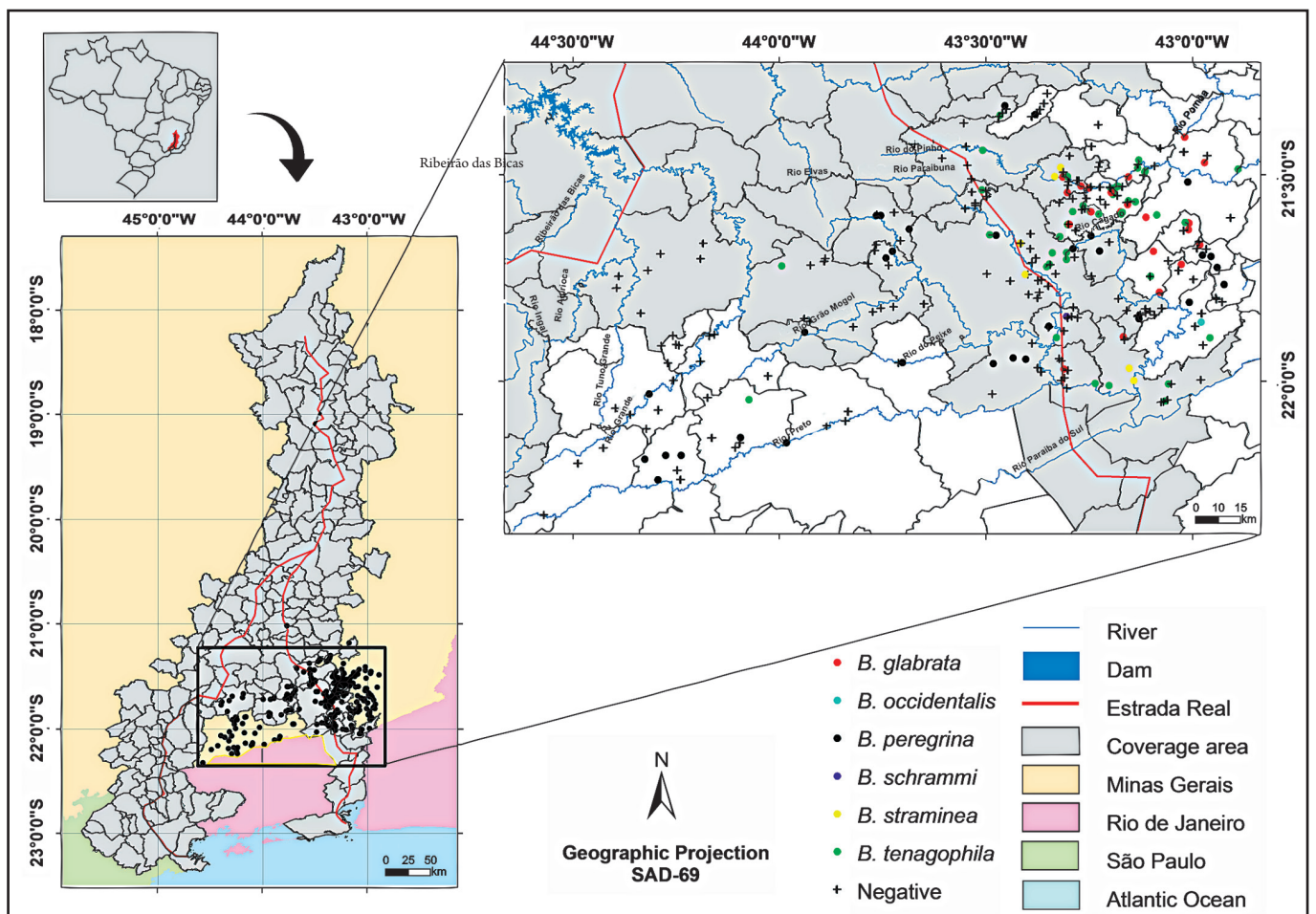


FIGURE 1 - Spatial distribution of the *Biomphalaria* genus in the municipalities of the Estrada Real, southeastern region of the State of Minas Gerais. The coverage area of the Estrada Real Project and highlighted, the *Biomphalaria* species collected in the municipalities of the Estrada Real in the southeast of the State of Minas Gerais.



of the map, which is closer to the *Caminho Velho* (*Diamantina/MG* to *Paraty/RJ*). In the eastern region, between the rivers *Pinho* and *Paraibuna*, belonging to the *Paraíba do Sul* river basin, all the species of mollusks reported in this work were found. The western region, between the rivers *Grande* and *Aiuruoca*, belonging to the *Rio Grande* water basin, is the area with just one positive collection dot for *B. peregrina*. In the southern region, between the rivers *Grande* and *Preto*, belonging to the *Rio Grande* and to the *Rio Paraíba do Sul* water basins, respectively, there are various collection dots for *B. peregrina*, but only one positive dot for *B. tenagophila* (Figure 1).

## DISCUSSION

Increased ecotourism and rural tourism in the State of *Minas Gerais* has highlighted the importance of studies regarding the occurrence of potential intermediate hosts of *S. mansoni*. This study revealed the presence of *Biomphalaria* snails in 30 municipalities, in the southeast region of the state, belonging to the *Estrada Real* Project or to its coverage area. This is a pioneering work, undertaken to investigate *Biomphalaria* snails in municipalities of the *Estrada Real* route with the use of geographic coordinates (GPS) for mollusk location.

In Brazil, there are 11 species and one subspecies of mollusks of the genus *Biomphalaria*, but only *B. glabrata*, *B. tenagophila* and *B. straminea* are recognized as intermediate hosts of *S. mansoni*. *B. peregrina* proved to be experimentally susceptible to *S. mansoni*, while *B. occidentalis* and *B. schrammi* proved to be refractory to infection<sup>22-26</sup>. *B. tenagophila* was present in 47.2% of the municipalities surveyed, *B. glabrata* in 33.3% and *B. straminea* in 8.3%. Despite the undeniable importance of *B. glabrata* in Brazil, it is interesting to observe that *B. tenagophila* is the main species transmitting schistosomiasis in the State of *São Paulo*. These data reveal the importance of surveillance and the need for a control program for schistosomiasis in such areas, since *B. glabrata* showed significant occurrence and is of great importance due to its extensive geographic distribution, high infection indices and effectiveness in the transmission of schistosomiasis<sup>27</sup>.

Among the 36 municipalities studied, the absence of *Biomphalaria* snails was verified in only six. However, it is important to highlight that the present study was conducted over four years, which means that seasonal cycles, such as temperature and rainfall, for example, may have affected the abundance of mollusk populations<sup>28,29</sup>.

This study revealed several water collections colonized by mollusks of the *Biomphalaria* genus, including streams, dams and waterfalls, which could serve for sporting practices or for the construction of pools, providing further sources of infection by schistosomiasis among tourists<sup>4,30</sup>.

The *Estrada Real* Project is based on the exploration of the touristic potential of the region, with the implementation of regional ecotourism and the preservation of the natural patrimony<sup>17</sup>. These goals also culminate, unintentionally, in maintaining the habitat of the intermediate hosts of *S. mansoni*, which may promote outbreaks and the spread of schistosomiasis in the region, since in the majority of these sites sanitation conditions are poor, representing contamination risks for water collections in cases of infected individuals.

Little information exists regarding the prevalence of schistosomiasis in the municipalities studied, because the region is generally considered to be of low endemicity<sup>18</sup>. The presence of *Biomphalaria* species was previously reported in the municipalities of *Piau*, *Rio Novo* and *Juiz de Fora*<sup>15,18,31</sup>, although considering

their geographical location, only *Juiz de Fora* has been surveyed<sup>18</sup>. Models to estimate the prevalence of schistosomiasis have been performed in the State of *Minas Gerais*<sup>14,32-34</sup> and in some *Estrada Real* municipalities<sup>16</sup>, but in both studies, due to lack of georeferenced information for *Biomphalaria*, only data concerning the prevalence of human schistosomiasis and *Biomphalaria* species in relation to the municipality were used. In this study, all the sites where the occurrence of *Biomphalaria* was verified were mapped using GPS. Knowing the exact location of the breeding/water collections of the intermediate host participant in the chain of transmission of schistosomiasis permits greater flexibility in structuring measures for disease control. Moreover, these data may favor the use of geostatistical techniques, generating predictive values for the occurrence of *Biomphalaria* at sites in the region with no information regarding the intermediate hosts<sup>34,35</sup>, which could contribute to the drafting of a map of *Biomphalaria* snails in the State of *Minas Gerais*.

Considering the occurrence of schistosomiasis in the State of *Minas Gerais* and the socioeconomic repercussions that involve the *Estrada Real* Project, this work focuses on the vulnerability of the local water collections due to the presence of *Biomphalaria* mollusks and emphasizes the need for epidemiological surveillance and sanitary and education measures integrated with the local community and tourism sectors.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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