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## *Biomphalaria* species distribution and its effect on human *Schistosoma mansoni* infection in an irrigated area used for rice cultivation in northeast Brazil

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**Abstract.** The role of irrigated areas for the spread of schistosomiasis is of worldwide concern. The aim of the present study was to investigate the spatial distribution of the intermediate snail host *Biomphalaria* in an area highly endemic for schistosomiasis due to *Schistosoma mansoni*, evaluating the relationship between irrigation and types of natural water sources on one hand, and the influence of place and time of water exposure on the intensity of human infection on the other. A geographical information system (GIS) was used to map the distribution of the intermediate snail hosts in Ilha das Flores, Sergipe, Brazil, combined with a clinical/epidemiological survey. We observed a direct correlation between the intensity of human infection with *S. mansoni* and irrigation projects. Malacological studies to identify snail species and infection rates showed that *B. glabrata* is the main species responsible for human schistosomiasis in the municipality, but that *B. straminea* also plays a role. Our results provide evidence for a competitive selection between the two snail species in rice fields with a predominance of *B. glabrata* in irrigation systems and *B. straminea* in natural water sources.

**Keywords:** *Biomphalaria glabrata*, *Biomphalaria straminea*, *Schistosoma mansoni*, geographical information system, irrigation, system, natural water sources, Brazil.

### Introduction

*Schistosoma mansoni*, a trematode worm that is endemic in 54 countries, is widespread in Africa and also occurs in Latin America. In Brazil, 42.9 million people are estimated to be at risk of contracting schistosomiasis and 6.8 million are infected (WHO, 2010; Scholte et al., 2012). This parasite occurs in 18 states including the Federal District and is most prevalent in the northeast, the southeast and the midwest (Brazil, 2009). In the northeast, *Biomphalaria straminea* and

*B. glabrata* are the snail intermediate host species responsible for transmission. The municipality of Ilha das Flores has the highest prevalence of schistosomiasis in the state of Sergipe, 46.5% (Rollemberg et al., 2011). This municipality has a furrow, irrigable perimeter of 2,600 ha used for rice production, which not only provides the local population with a multitude of water sources but also presents multiple opportunities for infection with *Schistosoma mansoni*. Many studies have described the impact of the introduction of irrigation and the onset of increased prevalence rates of schistosomiasis (Amin, 1976; Mobarak, 1982; Barbosa and Costa, 1985; Huang and Manderson, 1992; Carmo and Barreto, 1994). A large number of studies using geographical information systems (GIS) have mapped the spatial distribution and expansion of the snail intermediate hosts contributing to a better understanding of transmission dynamics, helping the schistosomiasis control programme (SCP)

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to mount necessary countermeasures (reviewed by Gazzinelli and Kloos, 2007). However, most studies used secondary data collected by the SCP and did not associate malacological studies with clinical and epidemiological data.

With the aim to better understand the factors involved in the maintenance of high prevalence of schistosomiasis, we investigated an endemic area characterized by strong transmission. GIS was used to map the spatial relationship between water sources and the breeding capability of the two main snail species involved in transmission of the disease locally. We also investigated the influence of the place and time of water exposure in the intensity of human infection.

## Materials and methods

### *Study design and endemic area*

A cross-sectional, epidemiological study was carried out by combining GIS methodology with parasitological and epidemiological surveys and a malacological investigation in Ilha das Flores municipality, located 135 km from the capital Aracaju in the state of Sergipe. This municipality, located at an altitude of 28 meters above the mean sea level (MSL), is one of the major producers of rice in the northeast, where it saddles the banks of the São Francisco River around the geographic coordinates 10° 26' 05" South and 36° 32' 21" West. In the Brazilian Geodetic System, which uses the Universal Transverse of Mercator (UTM) projection, the area is located in Meridian Zone 24.

Ilha das Flores has 8,568 inhabitants, 85.4% of whom have access to tap water (however only intermittently). Only 3.3% of the urban population has sanitation coverage (IBGE, 2007). The irrigation project takes its water from the local river with a system based on two pumping stations, one located in the city and the other in the village of Serrão. The irrigation canals are largely used by the local people for a number of activities (e.g. car washing, bicycle cleaning, playing with animals, leisure, household tasks and fishing). After irrigating the rice fields, the water is drained through a stream back into the São Francisco River. Many of these drains also receive waste from open sewers coming from houses in the vicinity. In the rainy season, the irrigation system overflows flooding rice fields, streams, fish ponds and also the streets spreading *Biomphalaria* snails around the whole municipality.

### *Creation of a georeferenced database*

Aerial photographs at the 1:25,000-scale from 2003 were obtained from the State Planning Department (SEPLAN) and a panchromatic satellite image of 2.7 m resolution from the Sino-Brazilian satellite CBERS 2B came from the National Institute for Space Research (<http://www.inpe.br>). Georeferencing was carried out based on images and photos through the acquisition of control points in environment with a hand-held global positioning system (GPS) instrument (Trimble – Juno ST) with an accuracy of 2.5 m after correction. The georeference database was created using SPRING 5.0 (National Institute for Space Research; <http://www.inpe.br>) and ArcGIS, version 9.3 (ESRI, Redlands, CA, USA). The resulting cartographic base contained maps of urban and rural areas, water sources and land use of the main thematic classes.

### *Water contact and parasitological examination*

A parasitological and epidemiological survey was carried out from 2008 to 2010. A sample of the population including 500 inhabitants was randomly selected among 100 houses in this endemic area. The sample size was calculated based on the 40% prevalence rate and a 95% confidence interval (CI) of 35.7-44.4%, determined by the SCP from the State Health Department. This project was approved by Sergipe's University Hospital ethics committee, Brazil (UFS number-CAAE 0103.0.107.107-09). All participants, including those responsible for minors, signed a consent form. Place and time of water contact was annotated. Parasitological examinations were carried out by the Kato-Katz technique (Katz et al., 1972) and Three Fecal Tests (TF) methods (Immunoassay Ind. Com. Ltda., São Paulo, Brazil).

### *Malacological study*

The malacological study was conducted from September 2009 to October 2010 and performed monthly. The presence of water sources in the city and in the villages belonging to the municipality was identified by analysis of the aerial photographs. Nine collection points for *Biomphalaria* were selected with the aim of reaching the largest possible coverage area where cases of schistosomiasis had been confirmed through the parasitological survey. The water sources were marked as collection points after their identification in the environment using GPS and digital pictures.

The snail collections were carried out by two trained

persons using a metal shell and wood tweezers to scan for snails in the radius of approximately 10 m for 45 min. The captured snails were placed in plastic boxes containing food and water, identified by date, location, type of water source and collection point. They were subsequently transported to the laboratory and the number of captured snails calculated, their species classified and the tissues tested for *S. mansoni* infection. For species identification, we used the technique of dissecting the snail genital tract (Deslandes, 1951) and morphological evaluation of the shell (Paraense, 1961, 1975; Barbosa, 1995). In order to verify *S. mansoni* infection, we used the traditional technique of exposure to light that stimulates the shedding of cercariae (Souza and Lima, 1990).

### Statistical analysis

Statistical analysis was performed using software Graphped Prisma. Differences between groups were calculated using Mann-Whitney test (Wilcoxon, 1945; Mann and Whitney, 1947). Correlation tests were carried out using the Spearman method (Spearman, 1904). A value of 5% was considered for statistical significance.

### Results

The aerial photograph used to delineate the urban and rural areas of Ilha das Flores, including the irrigation system and the collection points (water sources), is shown in Figure 1.

Table 1 presents the areas surveyed, the types of water source and the *Biomphalaria* species found, including the number of times they were found and whether or not they were infected. Out of the nine water sources surveyed, four were rice fields, two seasonal ponds, one a stream, one an irrigation drain and one an irrigation canal. Mollusks of the genus *Biomphalaria*, 16,851 specimens overall, were collected from the nine collection points within the 12-month collection period. A predominance of *B. glabrata* was found in this area. From the total number of *Biomphalaria* captured, 30.3% were of the specie *B. straminea* and 69.7% *B. glabrata*. Of this total, 230 were naturally infected by *S. mansoni*, 24.3% of *B. straminea* and 75.7% of *B. glabrata*. Additionally, infected *B. glabrata* was identified during each of the 12 collection time points in contrast to *B. straminea*.

*B. glabrata* specimens were found in three rice fields, in one canal and in one irrigation drain, whereas *B. straminea* specimens were found in two seasonal ponds, in one rice field and in one stream. A higher number of *Biomphalaria* snails was collected in the rice fields and irrigation drains. When the distribution of *B. straminea* and *B. glabrata* snails collected in nine water sources was analysed, no overlap between the two species was observed at any point of collection, demonstrating biological competition between them. *B. straminea* specimens were found in natural water sources and rice fields and *B. glabrata* snails only in water sources from the irrigation system and rice fields (Fig. 2).

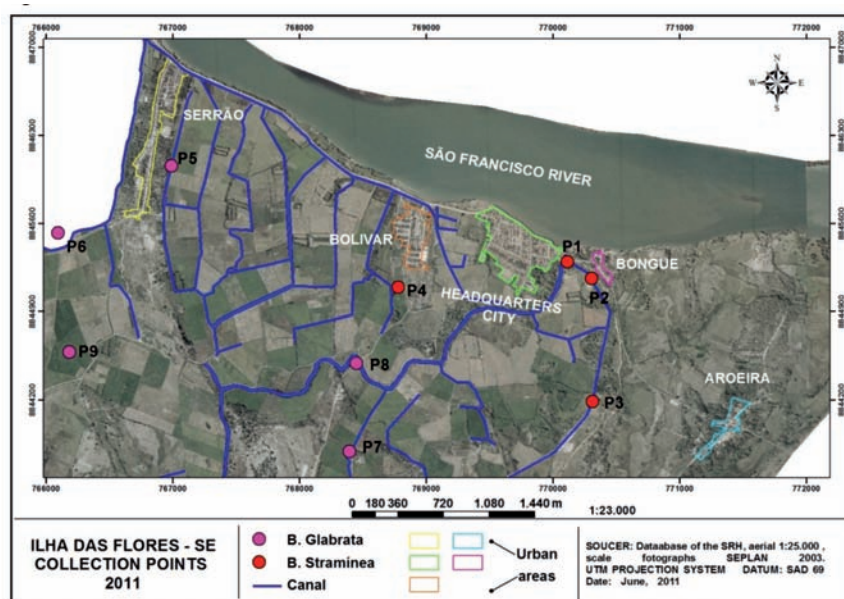


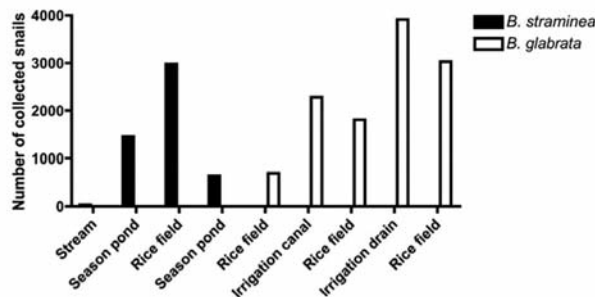
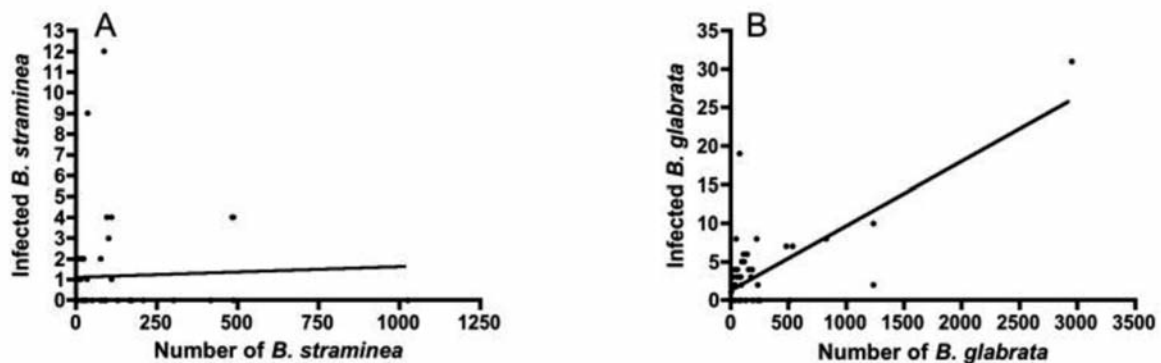
Fig. 1. Georeferenced aerial photograph of Ilha das Flores showing the urban and rural areas with the location of collection points of *Biomphalaria* and the irrigation system.

Table 1. Characterization of places where *Biomphalaria* were collected in the endemic area of Ilha das Flores from September 2009 to October 2010.

Collection points	Water sources	<i>Biomphalaria</i> species	Number of times we collected snails (/12)	Number of times we collected infected snails (/12)
P1	Stream	<i>B. straminea</i>	4	2
P2	Seasonal ponds	<i>B. straminea</i>	11	3
P3	Rice field	<i>B. straminea</i>	12	8
P4	Seasonal ponds	<i>B. straminea</i>	10	5
P5	Rice field	<i>B. glabrata</i>	12	6
P6	Irrigation canal	<i>B. glabrata</i>	12	9
P7	Rice field	<i>B. glabrata</i>	10	4
P8	Irrigation drain	<i>B. glabrata</i>	9	8
P9	Rice field	<i>B. glabrata</i>	10	4

We observed a direct correlation between the number of captured and infected *B. glabrata* (Spearman,  $r = 0.50$ ; 95% CI = 0.27-0.67;  $P < 0.001$ ), which allowed us to infer that the greater the number of snails found, the greater the number of infected ones. This was not true for *B. straminea* (Spearman,  $r = 0.24$ ; 95% CI = 0.06-0.50;  $P = 0.099$ ) (Fig. 3).

We also investigated the association between the *B. straminea* and *B. glabrata* species, their numbers and their rates of infection by *S. mansoni* with respect to harvest season and off-season. Considering all water sources together, a higher number of *B. straminea* snails was captured in the harvest season

Fig. 2. Distribution of *B. straminea* and *B. glabrata* collected in nine analysed water sources.Fig. 3. Correlation between the numbers of captured and infected *B. straminea* (A) and *B. glabrata* (B) snails. (A) Spearman  $r = 0.24$ ,  $P = 0.099$ ; (B) Spearman  $r = 0.50$ ,  $p < 0.001$ .

than in the off-season ( $P = 0.004$ , Mann-Whitney test). There was also a more significant number of infected *B. glabrata* specimens than infected *B. straminea* snails during the harvest season ( $P = 0.007$ , Mann-Whitney test) (Fig. 4). In general, not only water sources related to the irrigation system (i.e. rice fields, irrigation canals and drains) contained more captured *Biomphalaria* but also more infected ones (Fig. 5).

In an attempt to determine the influence of the irrigation system on human infection, we performed a correlation analysis between time of water contact and human infection intensity as expressed by the number of eggs/g of stool (EPG). Although there was no correlation between the EPG level and the number of hours of water contact in any of the water sources (Spearman  $r = 0.0$ ,  $P = 0.64$ ) (Fig. 6A) when only exposure to water sources related to the irrigation system was considered, a direct correlation between the EPG value and the number of hours of water contact in general was observed (Spearman  $r = 0.21$ ,  $P = 0.02$ ) (Fig. 6B).

## Discussion

The evidence for *B. glabrata* being more involved in *S. mansoni* transmission than *B. straminea* in Ilha das

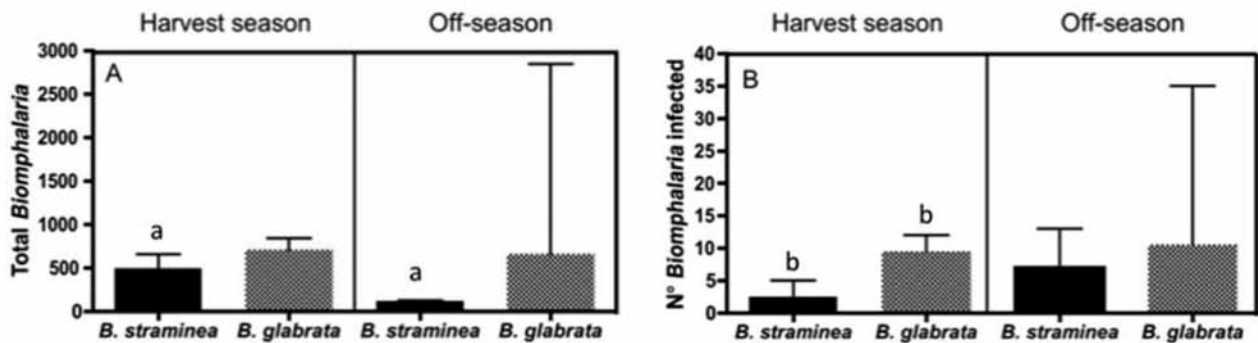


Fig. 4. The numbers of *Biomphalaria* snails collected (A) and infected (B) according to the period of rice culture, harvest season (September 2009 to February 2010 and August 2010 to October 2010) and off-season (March 2010 to July 2010). <sup>a</sup>P = 0.004; <sup>b</sup>P = 0.007; Mann-Whitney test.

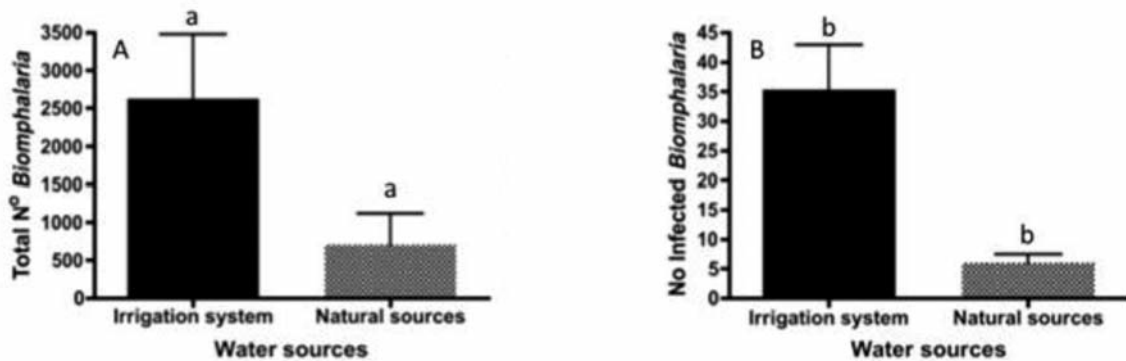


Fig. 5. The numbers of *Biomphalaria* snails captured (A) and naturally infected by *S. mansoni* (B) according to water sources surveyed at irrigation systems or natural sources. Mann-Whitney test <sup>a</sup>P = 0.047 and <sup>b</sup>P = 0.023.

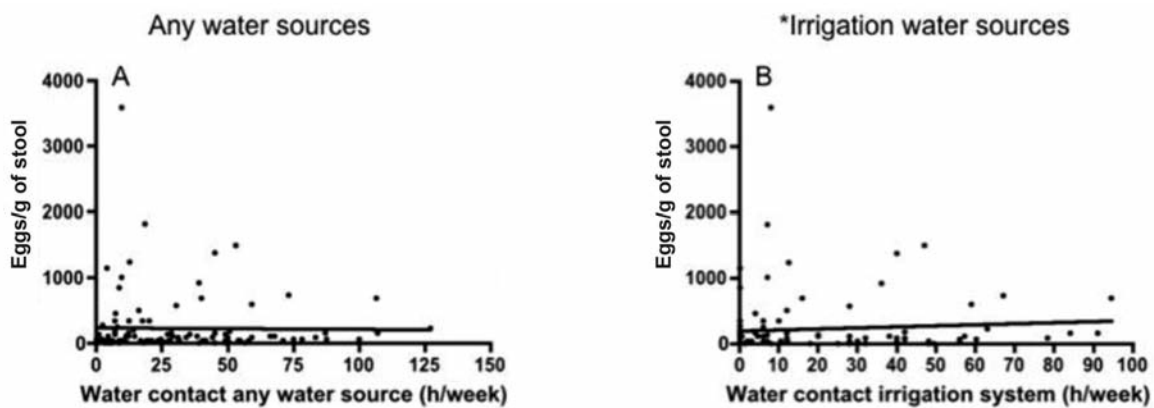


Fig. 6. Correlation between the number of hours of water contact in any water sources (A), Spearman  $r = -0.04$ ,  $P = 0.64$ ; and in the irrigation water sources (rice field, canal and drain) (B), Spearman  $r = 0.21$ ,  $P = 0.02$ .

Flores is based on the following findings:

- (i) *B. glabrata* snails were more frequently found and also more often infected than *B. straminea* snails;
- (ii) the greater the number of *B. glabrata* specimens found, the greater the number of infected ones;
- (iii) *B. glabrata* was predominately found in rice fields and the irrigation system, while *B. straminea* was

- predominately found in natural water sources; and
- (iv) the water sources related to the irrigation presented higher numbers of snails than the natural water sources.

The two species seem to be in the process of competitive displacement because in each of the collection

points only one of the species was found. There is now a predominance of *B. glabrata* over *B. straminea* in the study area. However, Mello and Barbosa (1969) and Figueiredo (1989) collected snail samples in this region of the backwoods of the São Francisco River valley and showed that *B. straminea* was the exclusive species found. Our data showing the predominance of *B. straminea* in natural water sources is indicative that this species is the natural snail found in the area, and that *B. glabrata* has overpowered *B. straminea* after the irrigation system was created in 1967. As *B. glabrata* is less resistant to dry weather conditions than *B. straminea*, the irrigation system promotes survival of the former species by changing the environment by enriching the soil with nutrients and water.

The data from the SCP from 2007 show a prevalence of 45.5%. This study shows that the irrigation and rice cultivation have an effect on the intensity of human infection by *S. mansoni*. We also show a correlation between the levels of *S. mansoni* infection and the time of activities in water sources related to the irrigation system, confirming the influence of these activities on the infection levels. This knowledge is important for understanding the process of natural selection responsible for the establishment of schistosomiasis in new areas that could potentially lead to future control designs based on natural, biological control. Snails are severely affected by *S. mansoni* infection resulting in a reduced lifespan. The particularly high susceptibility of *B. glabrata* snails to infection might be a selective disadvantage for this species that might lead to its replacement by *B. straminea*. Indeed, it has been shown that *B. straminea* replaces *B. glabrata* both in the natural environment and under laboratory conditions (Coelho, 1954; Barbosa, 1973; Michelson and Dubois, 1979; Figueiredo, 1989; Barbosa et al., 1993). However, in this area, we demonstrate that under specific environmental conditions due to irrigation, *B. glabrata* became the major species responsible for human *S. mansoni* infection.

A possible biological control of the disease could be implemented by reducing the replacement of the native snail populations in newly irrigated areas, or even repopulating areas with a predominance of *B. glabrata* with *B. straminea* or other snail species, less susceptible or resistant to *S. mansoni* infection. Indeed, this process occurred in Martinique where the substitution of *B. glabrata* for *B. straminea* interrupted transmission of schistosomiasis (Guyard and Pointier, 1979, 1982; Pointier, 1982, 1983). Indeed, it is known that *B. straminea* withstands desiccation better, has a greater ability of dispersal and fecundity,

with consequently greater aggressiveness to invade territories occupied by *B. glabrata*.

It should also be pointed out that irrigation is an important tool for the maintenance of more stable and productive agricultural schemes, and that also other factors participate in the determination of the presence or absence of the schistosomiasis, e.g.:

- (i) the type of irrigation system (intensive, mechanized, requiring little manual labour or wide-spread, non-mechanized and labour intensive);
- (ii) the relationship between humans and the environment (the cultural and behavioural practices of the population with respect to water resources); and
- (iii) socio-economic factors, which can increase environmental contamination of water sources (Barreto, 1982; Smith, 1988; Lima, 1995).

A study in Bahia, Brazil (Martins and Barreto, 2003) showed that the municipalities with the largest irrigated areas are not those with the highest rates of prevalence of schistosomiasis, and that irrigation has had little impact on the spatial profile of the disease, unlike what is commonly observed in Africa. According to this study, the extensive type of irrigation system, when compared to the intensive one, greatly influences in the spread of the disease. In the study area, the extensive, non-mechanized irrigation favours the survival of *B. glabrata* over *B. straminea* species, which results in high transmission and high prevalence of schistosomiasis.

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