

## Host-parasite relationship between *Biomphalaria amazonica* (Paraense, 1966) and *Schistosoma mansoni* (Sambon, 1907)

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(With 4 figures)

### Abstract

*Biomphalaria amazonica* is a planorbid species considered a potential host of *Schistosoma mansoni*. It is widely distributed in the Neotropical zone, particularly in the North and Centre-West of Brazil and in the North of Bolivia. The aim of the present study was to determine the host-parasite relationship between *B. amazonica* and *S. mansoni* (BH and SJ strains). Specimens of *B. amazonica* and their snail-conditioned water were examined in terms of their ability to attract miracidia. The infectivity of the mollusks was determined by exposing them to 20 miracidia of both strains. Sporocyst development and amebocyte reactions were studied after each mollusk specimen was exposed to 100 miracidia. Although no cercariae were eliminated, specimens of *B. amazonica* proved capable of attracting 77% of the miracidia they were exposed to. Viable sporocysts with no amebocyte reaction were found 96 hours after the exposure to miracidia. These results indicate the susceptibility of *B. amazonica* to the BH and SJ strains of *S. mansoni*, and therefore demonstrate the importance of this planorbid species as a potential vector of the trematode in the areas where it occurs.

**Keywords:** schistosomiasis, susceptibility, BH strain, SJ strain.

### Relação parasito-hospedeiro entre *Biomphalaria amazonica* e *Schistosoma mansoni*

#### Resumo

*Biomphalaria amazonica* é uma espécie de planorbídeo considerada vetora potencial do *Schistosoma mansoni*. É amplamente distribuída na zona neotropical, especialmente no Norte e Centro-Oeste do Brasil e Norte da Bolívia. O presente trabalho teve por objetivo estudar a relação parasito-hospedeiro entre *B. amazonica* e *S. mansoni* (linhagens BH e SJ). Espécimes de *B. amazonica* e sua água de condicionamento foram examinados em relação à sua capacidade de atração miraxonal. A infectividade dos moluscos foi testada expondo-os a 20 miracídeos de ambas as linhagens. A viabilidade dos esporocistos e o desenvolvimento de reações amebocitárias foram estudados após cada molusco ser exposto a 100 miracídeos. Apesar de não eliminarem cercárias, *B. amazonica* provou ser capaz de atrair 77% dos miracídeos a que foram expostos. Esporocistos viáveis sem reação amebocitária foram encontrados 96 horas após a exposição aos miracídeos. Esses resultados indicam a suscetibilidade de *B. amazonica* às linhagens BH e SJ de *S. mansoni* e, portanto, demonstram a importância desta espécie de planorbídeo como um vetor potencial do trematodeo na área onde ele ocorre.

**Palavras-chave:** esquistossomose, susceptibilidade, linhagem BH, linhagem SJ.

#### 1. Introduction

In Brazil, three planorbid species are regarded as natural hosts of *Schistosoma mansoni* (Sambon, 1907): *Biomphalaria glabrata* (Say, 1818); *Biomphalaria straminea* (Dunker, 1848) and *Biomphalaria tenagophila* (D'Orbigny, 1835). *Biomphalaria amazonica* (Paraense, 1966) is considered a potential vector of *S. mansoni* because it has released cercariae in experimental infections (Corrêa and

Paraense, 1971; Paraense and Corrêa, 1985; Fernandez and Thiengo, 2006). This species is widely distributed in the North and Centre-West of Brazil (Paraense and Corrêa, 1985; Fernandez and Thiengo, 2006; Coimbra Junior et al., 1984; Dorval and Silva, 1990) and has also been found in Bolivia (Pointier et al., 2002). The regions in Brazil where this species occurs have high migration rates (Coura

and Amaral, 2004), which favors the appearance of foci of *S. mansoni* schistosomiasis. This study presents the results of an assessment of the attraction of miracidia of *S. mansoni* (BH and SJ strains) (Paraense and Corrêa, 1963) to *Biomphalaria amazonica* and their ability to penetrate this species. Miracidia of *S. mansoni* are attracted to mollusks in general, particularly to their vectors (Kloetzel, 1958). Substances that are able to attract miracidia can be found in hemolymph and snail-conditioned water (SCW), where mollusks live (Chernin, 1970). For both *B. glabrata* and *B. tenagophila*, the evolution of sporocysts inside the mollusk after the penetration of miracidia has been studied in detail (Pan, 1965; Guaraldo et al., 1981). In specimens that are more susceptible to infection, sporocysts develop without facing major tissue immune reactions, whereas specimens that are more resistant to infection are encapsulated by amebocytes and killed (Guaraldo et al., 1981). The mollusks can die either because of the high number of invading miracidia during the infection or because of the development of sporocysts that cause physiological and metabolic changes during the hosts' immune responses. Tissues can also be compromised during the development of cercariae and before they are eliminated (Coelho, 1970). Since an assessment of the mollusk-parasite relationship in a laboratory is the first step in the epidemiological study of schistosomiasis in a certain area, the aim of the present study was to determine the ability of *B. amazonica* to attract miracidia, the infectivity and mortality of mollusks in relation to the miracidia of the BH and SJ strains, and the amebocyte reactions to primary sporocysts.

## 2. Material and Methods

### 2.1. Species and strains

Specimens of *B. amazonica* were obtained from mollusks collected in Barão de Melgaço, Mato Grosso, Brazil (16° 11' 40" S 55° 58' 03" W) and kindly provided by Ph.D. Wladimir Lobato Paraense. The strains of *S. mansoni* used were the sympatric species of SJ (*B. glabrata*) and BH (*B. tenagophila*) (Paraense and Corrêa, 1963).

### 2.2. Attraction of miracidia

In order to analyze the attraction of miracidia to mollusks and their snail-conditioned water (Chernin, 1972), a glass apparatus consisting of two circular, 30-mm diameter chambers, linked by a channel (40mm long and 11mm wide), was used (Brasio et al., 1985). The specimen of *B. amazonica* was introduced into one chamber of the apparatus, which was filled with dechlorinated water.

The mollusks had a diameter of between 5 and 7mm. For the SCW tests, a sample of SCW was added to one of the chambers and then 10 miracidia were placed in the center of the channel filled with dechlorinated water. After twenty minutes, the number of larvae that had moved to each compartment was counted. Ten mollusk specimens and ten different samples of SCW, for the BH and SJ strains of *S. mansoni*, were used.

### 2.3. Infectivity and mortality of mollusks

Thirty specimens of *B. amazonica* (diameter of 5 to 7-mm) were individually exposed to 20 miracidia of *S. mansoni* (BH and SJ strains) for two hours at a temperature of 28 °C under 60-W incandescent light. Susceptibility was determined from the fourth week after contact with miracidia, when cercariae were eliminated after the mollusks were exposed to light and heat. These observations were carried out until the 16<sup>th</sup> week after the infection. Every week, the water in the flasks was changed and the number of dead mollusks was registered. Feeding consisted of lettuce *ad libitum*.

### 2.4. Histopathological analysis of the cephalic region

For each strain of *S. mansoni*, twelve specimens of *B. amazonica* were individually exposed to one hundred miracidia. The mollusks were divided into groups of three specimens, according to the infection period (24 h, 48 h, 72 h and 96 h), and were fixed in Bouin's fluid for 72 h (Guaraldo et al., 1981). The cephalic region immediately above the mantle collar was separated and serial histological sections (5 µm thickness) were stained with Gomori trichrome (Guaraldo et al., 1981). Optical microscopy analysis revealed the development of primary sporocysts and an amebocyte reaction to larvae.

### 2.5. Statistical analysis

SAS® software (SAS, 2012) was used to compare the data obtained for *B. amazonica* with those obtained in a similar situation with *B. glabrata* and *B. tenagophila*, after exposure to their sympatric strains (BH and SJ, respectively).

## 3. Results

### 3.1. Attraction of miracidia

*Biomphalaria amazonica* (the mollusk and its SCW) attracted a significantly higher number of SJ miracidia than BH miracidia. When comparing the mollusk and its SCW, the latter showed a less intense attraction (Table 1). Since the strain-variable interaction was relevant ( $P > 0.0015$ ), statistical data analysis was carried out considering the

**Table 1.** Attraction of BH and SJ miracidia strains of *Schistosoma mansoni* to *Biomphalaria. amazonica* (mollusc and Snail Conditioned Water, SCW).

		N	Mean±SD	
Strain	BH	20	6.75 ± 1.45	P>0.0216
	SJ	20	7.45 ± 0.82	
Variable	SCW	20	6.55 ± 1.23	P>0.0006
	Mollusk	20	7.65 ± 0.93	

strains (BH and SJ) and the variables (SCW and mollusk). The results are shown in Tables 2 and 3. Specimens of *B. amazonica* attracted a significantly higher number of BH miracidia than their SCW. Conversely, SJ miracidia were attracted in a similar way to specimens of *B. amazonica* and their SCW (Table 2). The SCW of *B. amazonica* attracted a significantly higher number of SJ miracidia than BH miracidia, whereas specimens of *B. amazonica* showed no difference in the way they attracted miracidia (Table 3).

3.2. Infectivity and mortality

Four weeks after the mollusks had been exposed to BH and SJ miracidia, they were examined to determine the liberation of cercariae. In total, 13 specimens of *B. amazonica* that were exposed to the BH strain were alive. At the end of the experiment (16<sup>th</sup> week), only two of these mollusks were still alive and the tissue exhibited no sporocysts. Of the specimens of *B. amazonica* that were exposed to the SJ strain, 18 were alive in the 4<sup>th</sup> week and four survived until the 16<sup>th</sup> week. These mollusks did not exhibit SJ sporocysts. In the control groups, 26 specimens of *B. glabrata* were alive in the 4<sup>th</sup> week, but all of them had died by the 10<sup>th</sup> week. Conversely, 29 specimens of *B. tenagophila* were alive in the 4<sup>th</sup> week and 25 were still alive at the end of the experiment. These results can be seen in Figure 1.

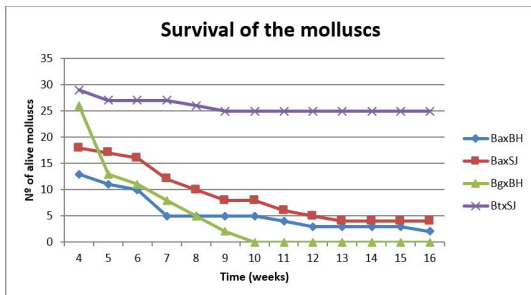


Figure 1. Survival of the molluscs exposed to 20 miracidia of *S. mansoni* (BH and SJ strains). Ba: *B. amazonica*; Bg: *B. glabrata*; Bt: *B. tenagophila*.

Table 2. Attraction of BH and SJ miracidia strains to *Biomphalaria amazonica* considering the variables Snail Conditioned Water (SCW) and mollusc.

Strain	Variable	N	Mean±SD	
BH	SCW	10	5.70 ± 1.06	P>0.0002
	Mollusk	10	7.80 ± 0.92	
SJ	SCW	10	7.40 ± 0.70	P>0.7947
	Mollusk	10	7.50 ± 0.97	

Table 3. Attraction of BH and SJ miracidia strains of *Schistosoma mansoni* to Snail Conditioned Water (SCW) or mollusc (*Biomphalaria amazonica*).

Variable	Strain	N	Mean±SD	
SCW	BH	10	5.70 ± 1.06	P>0.0005
	SJ	10	7.40 ± 0.70	
Mollusk	BH	10	7.80 ± 0.92	P>0.4872
	SJ	10	7.50 ± 0.97	

Of the 60 specimens of *B. amazonica* that were exposed to *S. mansoni*, none eliminated cercariae during the experimental period (16 weeks).

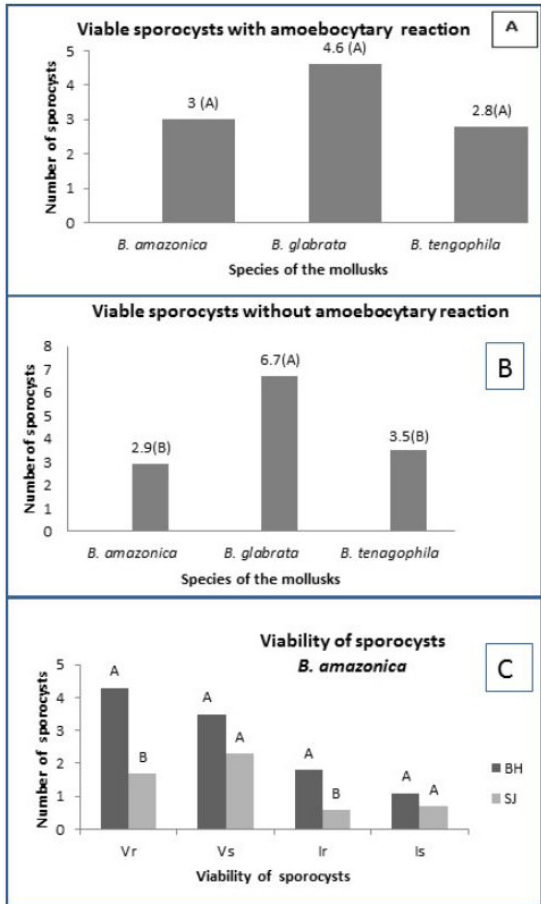
Of the specimens of *B. tenagophila* that were alive in the 4<sup>th</sup> week post-infection, two eliminated cercariae (infection rate of 6.7%). Of the 30 specimens of *B. glabrata* that were exposed to BH miracidia, nine eliminated cercariae (infection rate of 30%).

3.3. Histopathological analysis of the cephalic region

The number of sporocysts encapsulated by an amebocyte reaction was similar in all three species, although the number of viable larvae without an amebocyte reaction was significantly higher for *B. glabrata* (Figures 2A and 2B). A higher number of BH miracidia entered *B. amazonica*, and a significantly higher number of BH sporocysts were surrounded by an amebocyte reaction (Figure 2C). Ninety-six hours after the infection, viable sporocysts without an amebocyte reaction were seen in the three mollusk species (Figure 3). The largest sporocysts were found in *B. glabrata* (Figure 3C). In the case of *B. amazonica*, 53% of the sporocysts were viable. Figure 4 displays the viable sporocysts without an amebocyte reaction in *B. amazonica*. At the 96<sup>th</sup> hour, the size and number of germinative cells was similar for BH and SJ sporocysts (Figures 4A and 4B). The same aspects were observed for BH sporocysts in *B. glabrata* (Figure 4C). In *B. tenagophila*, the number of unviable sporocysts exceeded the number of viable ones (Figures 3B and 4D). Unviable sporocysts without an amebocyte reaction were observed in all three planorbid species (Figures 3 and 4E).

4. Discussion

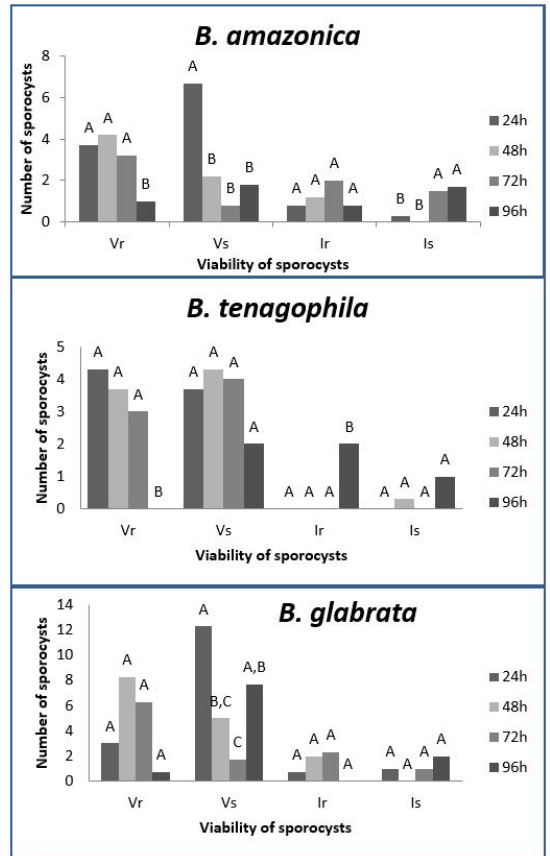
Planorbid species with a physiology that allows the parasite to develop miracidia and produce cercariae are considered a vector of *S. mansoni*. Once the cercariae are eliminated in water, they can evolve into adult worms after penetrating the definitive host. This process of infecting the mollusk is genetically controlled and depends upon



**Figure 2.** (A) Viable sporocysts with amoebocyte reaction; (B) Viable sporocysts without amoebocyte reaction in *B. amazonica*, *B. glabrata* and *B.tenagophila*; (C) Viability of BH and SJ sporocysts in *B. amazonica* (Vr: viable with reaction; Vs: viable without reaction; Ir: unviable with reaction and Is: unviable without reaction; columns with the same letter do not differ significantly amongst themselves).

the genes of the mollusks being preadapted to the genes of the parasite.

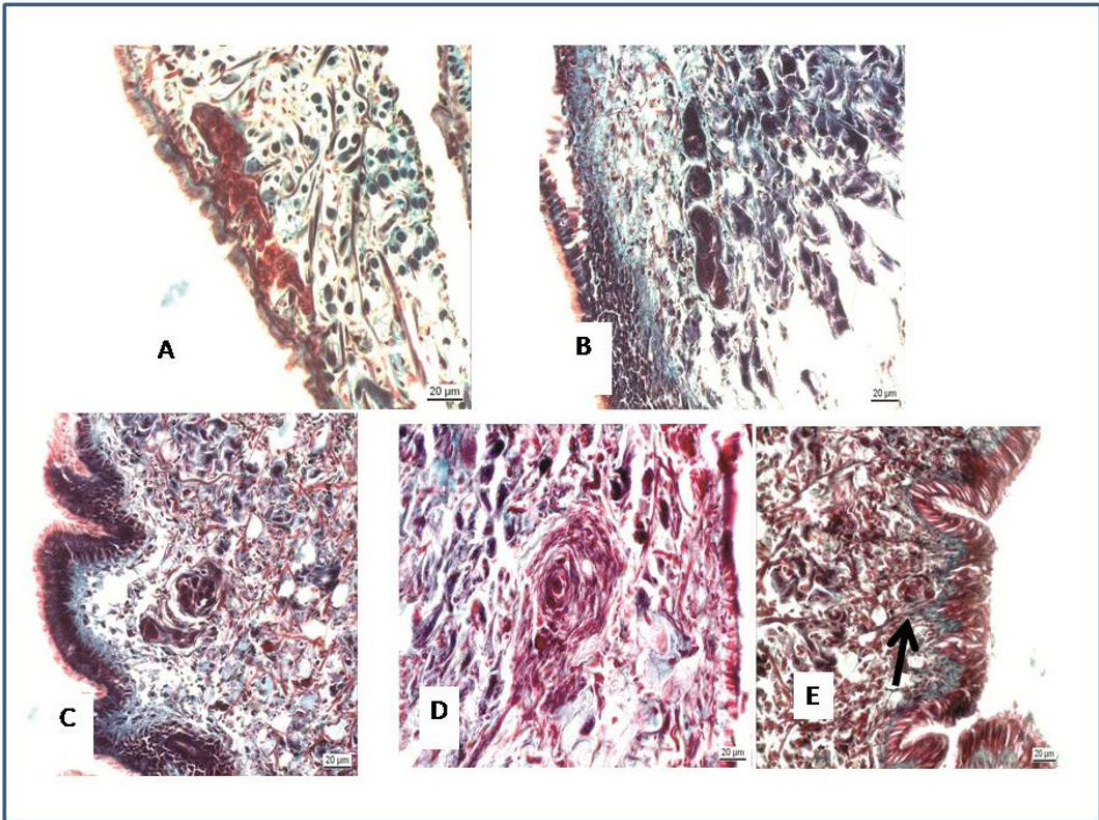
Different species of snails of the genus *Biomphalaria* exhibit different degrees of susceptibility. Some species are more susceptible and others are more (or completely) refractory to infection by *S. mansoni*. Phylogenetic studies of *Biomphalaria* have demonstrated a co-evolution in the host/parasite relationship, indicating that the parasite exerts greater pressure when mollusks adapt to taxonomic groups. High susceptibility has been confirmed after the adaptation of the parasite to the mollusk over time (DeJong et al., 2001). Although *B. amazonica* is listed among the mollusks that are susceptible to *S. mansoni*, its geographic distribution in a harmless region indicates that it is rarely exposed to parasite pressure. However, this situation can be modified through the selection of populations of trematodes that are pre-adapted to the mollusk species. Studies of *B. glabrata* and *B. tenagophila* sought to determine alterations in



**Figure 3.** Viability of sporocysts in *B. amazonica*, *B. tenagophila* and *B.glabrata*. (Vr: viable with reaction; Vs: viable without reaction; Ir: unviable with reaction; and Is: unviable without reaction; columns with the same letter do not differ significantly amongst themselves).

susceptibility and resistance to *S. mansoni*. The authors found that an increase in susceptibility was more easily obtained than an increase in resistance (Zuim et al., 2005). Even in the case of hybrid mollusks (crosses between susceptible and resistant species or populations), complete resistance to schistosome infections has never been achieved (Rosa et al., 2005; Teodoro et al., 2011). Evidence of genetic polymorphism has been reported, including comparisons of the genetic variability of mollusks that are susceptible and refractory to parasitism (Abdel-Hamid et al., 1999; Spada et al., 2002; Silva et al., 2004). This highlights the importance of the heredity of susceptibility among snails and the maintenance of foci of the disease. Other studies have reported that a similar population of snails may respond differently when exposed to different strains of *S. mansoni*, and that some of these strains exhibit pre-adapted phenotypes that can affect the degree of parasitism, thereby enabling the potential expansion of the schistosomiasis to new locations, where snail populations are found (Teodoro et al., 2011; Simões et al., 2013).

The ability to attract miracidia is the first factor to be considered in the establishment of the relationship



**Figure 4.** BH (A) and SJ (B) sporocysts of *S. mansoni* in *B. amazonica* 96 hours after the infection; (C) Viable BH sporocysts in *B. glabrata*; (D) Unviable sporocysts with amoebocyte reaction in *B. tenagophila*. Infection period of 96 hours; (E) Unviable BH sporocysts without amoebocyte reaction in *B. amazonica*. Stained with Gomori trichrome.

between the trematode and its potential host. *B. amazonica* was able to attract approximately 77% of the miracidia it was exposed to (78% for BH miracidia and 75% for SJ miracidia). The SCW of *B. amazonica* attracted less BH miracidia than the mollusk itself. Components of this SCW may have reduced the attraction of BH miracidia, although no interference was related to the SJ miracidia, given that they were attracted in the same proportion as *B. amazonica* and its SCW. Brasio et al. (1985) reported that the SCW of *B. glabrata* attracted BH and SJ miracidia in a similar manner, although the SCW of *B. tenagophila* attracted a significantly higher number of SJ miracidia. While analyzing samples of the SCW of both species, the authors noticed a distinct composition. The histological study of the infection showed more penetration of BH miracidia, as displayed in Figure 2C. The same amount of viable sporocysts without an amoebocyte reaction was found in *B. amazonica* and *B. tenagophila*. Ninety-six hours post-infection, the development patterns of the BH and SJ sporocysts in *B. amazonica* (Figures 4A and 4B) and of the BH sporocysts in *B. glabrata* were similar.

Concerning the survival of mollusks, *B. amazonica* had a shorter lifespan when exposed to the BH strain (Figure 1). Amoebocyte reactions occurred in a significantly

higher number around BH larvae (Figure 2C). The death of sporocysts was mainly followed by amoebocyte reactions, although unviable sporocysts without an amoebocyte reaction were observed in almost all specimens and infection periods (Figures 2C, 3 and 4E). Although an amoebocyte reaction is the main defense mechanism (Lie et al., 1980), the presence of degenerating larvae without an amoebocyte reaction has been reported in mollusks that were simultaneously infected with *S. mansoni* and another trematode species (Balan et al., 1993). The greater damage to parasitized tissues, either from more penetration of BH miracidia or the amoebocyte reaction of the mollusk, could explain the higher mortality of *B. amazonica* when it was exposed to the BH strain. The similar number of BH and SJ sporocysts in *B. amazonica* (Figures 2C and 3A) and the ability of this species to attract larvae allow us to conclude that it is susceptible to both strains of *S. mansoni*.

Based on these results, health surveillance and epidemiology centers located in the distribution area of *B. amazonica* should monitor the movements of people infected with *S. mansoni*, given that they could create foci of schistosomiasis in areas where the disease has not been reported as endemic.

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