From field to laboratory: isolation, genetic assessment, and parasitological behavior of Schistosoma mansoni obtained from naturally infected wild rodent Holochilus sciureus (Rodentia, Cricetidae), collected in Northeastern of Brazil

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Abstract

Wild rodent species are naturally infected by *Schistosoma mansoni*; however, the genetic characterization of the parasite, its parasitological features, and its role in human schistosomiasis are poorly understood. In this study, we isolated and characterized *Schistosoma* from naturally infected *Holochilus sciureus*, called HS strain, collected from a schistosomiasis endemic region in Maranhão State, Brazil. To isolate the parasite, miracidia obtained from the livers of *H. sciureus* were used to infect *Biomphalaria glabrata* of sympatric (called SB) and allopatric (called BH) strains, and the produced cercariae were subcutaneously inoculated into hamsters and/or BALB/c mice. Parasitological kinetics in experimentally infected hosts were evaluated, and the 16S and *cox* 1 regions of mtDNA from isolated worms were amplified and sequenced. Only miracidia obtained from infected mice, but not from hamsters, were capable of infecting *B. glabrata*, allowing maintenance of the isolated parasite. *Cox*1 and 16S mtDNA sequences showed 100% similarity with *S. mansoni*, and phylogenetic analysis showed that the HS strain of *S. mansoni* forms an assemblage with isolates from America and Kenya, confirming the conspecificity. Experimental infection of *B. glabrata* SB with *S. mansoni* HS resulted in two peaks of cercariae shedding at 45- and 70-days post-infection (dpi), and caused higher mortality than in *B. glabrata* BH. The worm recovery rate in mice was approximately 13%, and the peak of egg elimination occurred at 10th week post-infection. Therefore, *S. mansoni* obtained from *H. sciureus* was successfully isolated, genetically characterized, and maintained in mice, allowing further study of this schistosome strain.

Introduction

The blood fluke *Schistosoma mansoni* Sambon, 1907 is the etiologic agent of intestinal schistosomiasis, a fresh water-transmitted and neglected tropical disease with a great global impact on public health (Gryseels et al. 2006; McManus et al. 2018). Human schistosomiasis affects 250 million people worldwide, mainly in countries of Africa, Asia, and Latin America (Gryseels et al. 2006; Weerakoon et al. 2015). *Schistosoma mansoni* alone affects over 54 million people, especially in sub-Saharan Africa, the Caribbean islands, Puerto Rico, Suriname, Venezuela, and Brazil (WHO 2013; McManus et al. 2018; Gunda et al. 2020).

Despite the efforts of the World Health Organization (WHO) to eliminate *S. mansoni* transmission in endemic areas, human infection rates have persisted over the years (Rollinson et al. 2013; Secor 2014), indicating that the parasite would uses alternative propagation strategies. Although it is well established that humans are the most relevant definitive hosts of *S. mansoni* for parasite transmission (Gryseels et al. 2006; McManus et al. 2018; Loverde, 2019), some wild rodent species from schistosomiasis-endemic areas are frequently found to be naturally infected (Théron et al. 1992; Rey 1993; Miranda et al. 2017; Catalano et al. 2018). The involvement of wildlife hosts in *S. mansoni* transmission and maintenance in endemic areas may favor the emergence of hybrids (Leger and Webster 2017) and new genotypes or strains (Catalano et al. 2020; Miranda et al. 2017), which would negatively impact the current goal of eliminating schistosomiasis as a public health problem by 2030 (NTD Modelling Consortium Schistosomiasis Group 2019; WHO, 2022). Therefore, it is very important to implement a collective and coordinated one-health approach for the control of schistosomiasis, which considers the eco-social determinants of human health and natural infection of wild rodents with *S. mansoni*.

In Brazil, species of wild rodents in semi-aquatic habitats, mainly *Nectomys squamipes* and *Holochilus sciureus* (Rodentia, Cricetidae), are infected by *S. mansoni* and are potential sources of transmission (Rey 1993; Gentile et al. 2010; Miranda et al. 2017). These rodents are highly susceptible to *S. mansoni* infection (Souza et al. 1992;

Maldonado et al. 1994; Miranda et al. 2019), showing a high number of viable eggs in feces (Piva 1966; Dias et al. 1978; Picot 1992; Souza et al. 1992), persistent infections (Souza et al. 1992) and good pathological tolerance (Silva and Andrade 1989; Amaral et al. 2016; Miranda et al. 2019). Furthermore, previous experimental studies using *S. mansoni* from naturally infected *N squamipes* and *H. sciureus* demonstrated distinct morphological and biological characteristics between parasite strains isolated from wild rodents and humans, including differences in morphological aspects of adult worms (Machado-Silva et al. 1994), pathogenicity in mice (Bastos et al. 1984, Silva and Andrade 1989), compatibility/virulence in *Biomphalaria* snails (Bastos et al. 1982) and sensitivity to praziquantel (PZQ) (Costa-Silva et al. 2012). However, to advance the knowledge pertaining to the pathology and genetics of schistosomes from Brazilian wild rodents, it is important to isolate and maintain the parasite under laboratory conditions.

Therefore, the present work uses field and laboratory approaches to describe the isolation process of *S. mansoni* from naturally infected *H. sciureus* and assesses the genetic profile and parasitological behavior of this schistosome strain in experimentally infected vertebrate and invertebrate models.

Materials And Methods

Field collections of *Holochilus sciureus*

Holochilus sciureus was collected as part of an experimental study coordinated by the State University of Maranhão (UEMA) about the helminth fauna of this wild rodent captured in the municipalities of São Bento (02º41'45 "S 44º49'17 "O) and Peri Mirim (02º34'40" S 44º51'14"W), State of Maranhão, Brazil, between 2017 and 2018 (Fig. 1). Rodent capture and manipulation were authorized by the Biodiversity Authorization and Information System (n°67253-1) and the project was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (registration number AB9E2EC).

These municipalities have a humid tropical climate with two distinct seasons: a rainy period from February to July and a dry period from August to January. Human schistosomiasis is endemic in both municipalities, but the infection rates are low, with an estimated prevalence of 5% among residents from São Bento and 2.3% among those from Peri Mirim (Brazilian Ministry of Health 2018).

As shown in Fig. 1, capture was performed in 14 collection spots located in the naturally flooded fields of both municipalities. For each collection spot, 10 Tomahawk traps baited with a mixture of banana and peanut butter were placed 10 m apart and kept overnight, as previously described by Do Carmo-Silva et al. (2019). Each trap was checked in the morning, and rodents with the morphological characteristics of *H. sciureus*, the most common species found in this region (Brandão and Nascimento 2015; Miranda et al. 2017), were taken to the laboratory for species confirmation and necropsies. The captured animals were immediately returned to their original environments. Each collection site was inspected for two days.

Recovery of miracidia

The rodents captured in the field were euthanized with an anesthetic overdose (300 mg/kg of ketamine and 30 mg/kg of xylazine) and the livers were immediately dissected (Council of Ethics and Animal Experimentation at

UEMA; protocol nº03/2017). After dissection, specific identification of *H. sciureus* was confirmed based on skull and mandibular features (Rocha et al. 2011; Brandão and Nascimento, 2015).

The livers with macroscopic changes suggestive of schistosome infection were homogenized in cooled concentrated saline solution (2% NaCl), decanted at 4°C and the pellet containing parasite eggs was resuspended in chlorine-free water at room temperature and exposed to the artificial light for 30 min to stimulate the miracidia hatching (Standen, 1952). Miracidia were concentrated at the top of a sealed volumetric flask exposed to artificial lighting as described by Chaia (1956). The water suspension containing the miracidia was recovered, miracidia were counted under a stereomicroscope (Zeiss Stemi Dv4, Jena, Germany), and used for experimental infection of snails. The *Schistosoma* strain isolated from *H. sciureus* in the present study was designated HS.

Isolation process in snails

Biomphalaria glabrata snails descending from specimens collected in the municipality of São Bento (SB strain; sympatric strain) and from specimens collected in Belo Horizonte (BH strain; allopatric strain) were used for the experiments. The snail strains were bred and maintained separately at the molluscarium of the Laboratory of Schistosomiasis and Immunohelminthology (Instituto de Ciências Biológicas/Universidade Federal de Minas Gerais, acronym ICB/UFMG), in aquariums containing chlorine-free water, fed with lettuce (*Lectuca sativa*) previously cleaned in acetic acid solution (0.01%), and supplied with standardized powdered chow for snails (Rosa et al. 2013). Snails, 5-6 mm in diameter, were used for experimental infection with the *S. mansoni* HS strain.

For individual infection, each snail was placed in one well of a 6-well culture plate (Plate Flat Bottom, Sarstedt, Massachusetts, USA) containing approximately 6 ml of chlorine-free water and a defined number of miracidia obtained from infected *H. sciureus* liver homogenates (5–8 miracidium/well). During parasite isolation, some *H. sciureus*-infected livers yielded a small number of miracidia and did not allow for individual infections. In this situation, a mass infection was performed, in which a whole clean supernatant of liver homogenate from infected rodents was placed in a container with 8–15 *B. glabrata* of the SB strain. Plates or containers with snails and miracidia were kept for 12 h under direct light.

Snails from individual or mass infections were evaluated between 28 and 80 days post-exposure to miracidia to identify and quantify cercarial shedding. Briefly, once a week, the snails were individually placed into 6-well culture plates 6 ml of chlorine-free water and exposed to incandescent light for 4 h (Brazilian Ministry of Health, 2008). The presence of cercariae was verified, and the number of larvae was counted using a stereomicroscope (Zeiss Stemi Dv4, Oberkochen, Germany). After light exposure, snails that shed cercariae (positives) were separated from negative ones, in a dark environment at 23°C and used as a source of infective larvae for the next experimental infection.

Isolation process in mice and hamsters

Heterogenetic hamsters (*Mesocricetus auratus*) and isogenic BALB/c mice aged 6–8 weeks were acquired from an established colony at the mouse facility of UFMG and used as a vertebrate model for experimental infections using the *S. mansoni* HS strain. Experimental animals were kept at the animal facility for infected animals at the

Parasitology Department (UFMG), fed with standard chow (Presence, Primor, Brazil), and provided tap water *ad libitum*. Animal experiments were approved by the UFMG Animal Ethics and Experimentation Council (nº46/2019)

Cercariae used for hamster and mouse infections were obtained from *B. glabrata* infected with *S. mansoni* miracidia of the HS strain. Positive snails were pooled in a glass beaker containing 100 ml of chlorine-free water and exposed to light and heat for 4 h. The recovered solution containing cercariae was filtered (40 mesh/inch), concentrated in a Buchner funnel (20–30 µm porosity), and the number of cercariae was counted using a stereomicroscope (Pellegrino and Macedo 1955). When the number of cercariae was very low (less than 20 larvae/500 µL), the mass percutaneous infection was performed. Briefly, these larvae were diluted in 500 mL chlorine-free and the mice were maintained individually in contact with this solution in a bath for 1 h with artificial light exposure, as described by Brener (1956, 1959). When the infected snails shed a large number of cercariae, allowing for concentration and quantification, a defined number (from 20 to 120 cercariae/animal, as defined for each experiment) was diluted in 500µL of saline (0.9% NaCl), and the solution was subcutaneously inoculated in each experimental vertebrate model (Pellegrino and Macedo, 1956).

For qualitative confirmation of infection of vertebrates (mice or hamsters), the feces of cercarial-exposed animals were collected weekly from 35 to 62 days post-infection (dpi) and examined using the sedimentation technique (Hoffman et al. 1934). Eight weeks post-infection (wpi), cercarial-exposed animals were euthanized with an anesthetic overdose (300 mg/kg of ketamine and 30 mg/kg of xylazine) and subjected to circulatory perfusion for recovering the adult worms, which were collected individually in microtubes at -20ºC, for molecular analysis.

DNA isolation, PCR, and sequencing

Genomic DNA from individual adult male worms was isolated from mice experimentally infected with the *S. mansoni* HS strain using the QIAamp® DNA mini kit (Qiagen, California, USA), according to the manufacturer's guidelines. Polymerase chain reaction (PCR) assays and sequencing were performed for the 16S-12S region of the mtDNA (referred to as 16S herein), using the primers 16SF2 (F) 5’ GTG CTA AGG TAG CAT AAT AT 3’ and 16SR3 (R) 5’ AGA AGC AGT TTA ACC GCG AC 3, and for the cytochrome c oxidase subunit I (*cox*1) region, using the primers CO1F (F) 5-' GGC ATA TCT GTA TGA GTC TA 3’ and CO1R3 (R) 5' GCA TTT AAA TAR TCA ACA TG 3’ (Morgan et al. 2005). Both primer sets amplify a 730 bp fragment. PCR reactions were performed in a final volume of 25µL, consisting of 1.25 µL of each primer (final concentration, 0.5 µM), 12.5 µL of GoTaq® Colorless Master Mix 2x (Promega, USA), 1 µL of DNA template (about 20 ng/µL) and 9 µL of ultrapure water. Cycling conditions were as follows: 1 min at 95°C, 45 s at 50°C and 90 s at 70°C, followed by 29 cycles of 30 s at 95°C, 30 s at 50°C and 90 s at 72°C, and a final extension at 72°C for 7 min (Morgan et al. 2003). PCR products were subjected to agarose gel electrophoresis to confirm amplification. Positive PCR products were purified by enzymatic treatment with ExoSAP-IT Express (Thermo Fisher, Massachusetts, USA). Purified products were sent for sequencing at the CT-Vacinas facility (BHtec, Belo Horizonte, Brazil) using PCR primer sets (forward and reverse) according to Sanger et al. (1977).

Contiguous sequences were assembled in Geneious (Geneious Prime ver. 2022 created by Biomatters, available at http://www.geneious.com/), and their consensus was extracted and deposited in GenBank. A preliminary basic local alignment search tool (BLAST) search of the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/)

was performed to confirm the genetic proximity between the present sequences and those from representatives of Schistosomatidae.

Phylogenetic analysis

Sequences of all species of *Schistosoma* covering the same genetic region sequenced in the present study, were used for phylogenetic reconstructions (see Online Resources 1 and 2). Sequences from the same isolates (clones or others with 100% genetic similarity), those not identified to the species level, and those too short in length were not included. Sequences were aligned using M-Coffee (Notredame et al. 2000), then evaluated by the transitive consistency score to verify the reliability of aligned positions, and based on score values ambiguous aligned positions were trimmed (Chang et al. 2014). Datasets (i.e., alignments according to each genetic marker) were subjected to maximum likelihood (ML) and Bayesian inference (BI) analyses using PHYML and MrBayes, respectively (Huelsenbeck and Ronquist 2001; Guindon and Gascuel 2003). The model of evolution and its fixed parameters for each model were chosen and estimated under the Akaike information criterion using jModelTest 2 (Guindon and Gascuel 2003; Darriba et al. 2012) and are detailed in Online Resource 3. The nodal support of ML was based on 1,000 bootstrap non-parametric replications. Nodal supports for Bayesian posterior probability values were determined after running the Markov chain Monte Carlo (2 runs 4 chains) for 1×10^6 generations, with sampling frequency every 1×10^3 generations, and discarding the initial ¼ of sampled trees (1×10^6) as burn-in. The outgroup chosen was *Trichobilharzia regenti* Horak, Dvorak, and Kolarova, 1998, based on previous phylogenies of *Schistosoma* (Webster et al. 2006).

Pairwise (patristic) distance (P distances) matrixes were generated, according to each genetic marker, using the software MEGA 7.0 (Kumar et al. 2016), to evaluate intra and interspecific divergences among samples. The Kimura two-parameter (K2P) (Kimura 1980) was used as distance metric, with other parameters set to default.

Parasitological profile of *S. mansoni* HS strain under laboratory conditions

After isolating an *S. mansoni* HS strain and confirming its specific identification by genetic analysis, the next step was to describe the parasitological profile of this strain better. For this purpose, we performed standardized experimental infections of *B. glabrata* snails and BALB/c mice.

The course of infection by *S. mansoni* of HS strain in *B. glabrata* host was evaluated in two different snail strains: the BH strain (allopatric) and the SB strain (sympatric). At the time of infection, the snails, 5–6 mm in diameter, were individually exposed to three different quantities of infective larvae: 10, 15, or 20 miracidia of the *S. mansoni* HS strain. For the experiments, 20 snails of each strain were infected with each miracidia load and 20 snails of each strain were kept uninfected. Each experimental group, infected and control of both snail strains, was evaluated weekly for up to 84 days (or 12 weeks) and dead animals were counted, and the data were used to build a mortality curve. During this period, the number of cercariae shed from each infected snail was also monitored weekly from 28 to 84 dpi after exposure of the infected snails to artificial light. The number of cercaria shedding by each infected snail was estimated under an optical microscope (Nikon, New York, USA) in two 500 µL aliquots collected from a solution containing one infected snail after light exposure.

To assess the infection of mice with *S. mansoni* HS, 20 male BALB/c mice, 6-8 weeks old, were subcutaneously infected with 20 cercariae/mouse as previously described (Pellegrino and Macedo 1956), and five male BALB/c mice of the same age were kept uninfected. The experimental procedures were evaluated and approved by the Animal Ethics and Experimentation Council of UFMG (protocol nº46/2019).

Animals were monitored weekly for 12 weeks (84 days) to evaluate the mortality rate and number of eggs present in feces (infected group). The mortality rate was determined by counting the number of dead mice in each experimental and control group. Animals showing extreme cachexia, dyspnea, piloerection, and signs of apathy were euthanized and included in the mortality group.

The parasite burden was estimated by the number of adult worms recovered from portal circulation, eggs present in the feces and retained in the tissues. Fecal samples, collected from each infected mouse between the 6th and 12th weeks, were weighed and processed according to Negrão-Corrêa et al. (2004). To evaluate the number of eggs per gram of feces, two aliquots of 200 µl from each sample (final volume of 5 mL of formalin 10%) were analyzed under an optical microscope (Nikon), and the average number of eggs per aliquot of stool was regarded as the total number of eggs per gram of feces.

In addition, 8–10 infected mice were euthanized by anesthetic overdose (300 mg/kg ketamine and 30 mg/kg xylazine) and necropsied after 8 (acute schistosomiasis) and 12 weeks of infection (chronic schistosomiasis) to estimate the number of adult worms in circulation and the number of parasite eggs retained in the lungs, liver, spleen, and intestines. Adult worms of the *S. mansoni* HS strain were recovered from the portal/mesenteric circulation of each infected mouse after circulatory perfusion, as described by Pellegrino and Siqueira (1956). The recovered worms were counted separately as male and female using a stereomicroscope (Zeiss). After blood perfusion in the infected animals, the lungs, liver, spleen, and intestine (small and large) were isolated and digested in a 5% KOH solution (37ºC/4 h), as described by Cheever (1968). For egg counting, two 200 µl aliquots of each sample (5 ml final volume) were analyzed under an optical microscope, and the data were expressed as eggs/organ (Maggi et al. 2021).

Statistical analysis

The normal distribution of the measurements was evaluated using the Kolmogorov-Smirnov test. Continuous data with a normal distribution were expressed as mean and standard error, and data without a normal distribution were expressed as median and interquartile range. Categorical data frequencies were compared using Fisher's exact test. Data related to the survival curves were analyzed using log-rank tests. Statistical significance was set at $P < 0.05$. These analyses along with graphic construction were performed using GraphPad Prism software version 8 (Prism Software, Irvine, CA, USA) and STATA version 11 (Stata Corp., College Station, TX, USA).

Results

Isolation of schistosome from naturally infected *H. sciureus*

A total of 99 *H. sciureus* specimens were collected, 45 of which had eggs in feces that morphologically resembled eggs of *Schistosoma* (Table 1). During dissection, these rodents showed macroscopic pathological

changes in the liver that are typical of *Schistosoma* infection (Fig. 2a). Adult worms recovered from the hepatic portal system (Fig. 2b) and eggs observed in the liver homogenates (Fig. 2c) of *H. sciureus* also showed the characteristic morphology of *Schistosoma* species.

Fifteen livers from naturally infected *H. sciureus* collected at locations 1, 3, 7, and 11 were obtained (see Fig. 1 and Table 1). These organs were processed to isolate a *Schistosoma* strain infecting *H. sciureus* in this area. In the first attempt to isolate this schistosome strain, we used livers from *H. sciureus* captured at collection location 1, 1,894 m from the municipality of São Bento. After processing, few viable miracidia were obtained from the livers, allowing only mass infection of eight *B. glabrata* SB. The snails were examined weekly for 70 dpi for the presence of cercariae; however, all snails remained negative for schistosome infection (Table 1).

In the second isolation attempt, we used the livers of four animals, captured at collection point 3 (3,267 m away from the urban area), which also resulted in a low number of miracidia; therefore, we performed mass infection of 27 *B. glabrata* SB. Additionally, an individual infection of two *B. glabrata* SB strains with five miracidia were performed. Weekly examination of cercariae shedding showed a successful infection of only one *B. glabrata* that were individually exposed to miracidia, and the cercariae produced were used to infect three male hamsters (100 cercariae/500 µL saline/hamster). One hamster died after 8 wpi, and in the remaining two animals, the presence of *Schistosoma* eggs in the feces and adult worms in the hepatic circulatory system was confirmed. After processing the livers of these two animals, a large number of miracidia was obtained and used for individual infection of 100 *B. glabrata* BH (15 miracidia/snail). After 70 days of weekly parasitological evaluation, no cercariae were shed by snails (Table 1).

Owing to individual infection success, this technique was used in two subsequent attempts to infect *B. glabrata* snails and isolate the *Schistosoma* HS strain. In the third experiment, four livers from naturally infected *H. sciureus* collected at location 7 (2,297 m from the urban area) were used. Few miracidia were obtained from these livers, which were used to infect two *B. glabrata* SB, with six larvae each. However, after successive weekly parasitological analyses, no cercariae were shed by the exposed snails (Table 1).

In the fourth attempt, the livers of four naturally infected *H. sciureus* collected at location 11 (2,060 m from the urban area) were used. After tissue processing, a large number of miracidia were recovered and used for individual infection of 40 *B. glabrata* SB, with eight miracidia each. Fifty-six dpi, six of the 26 live snails (23%) started to shed cercariae, and the cercariae recovered from them were used for individual infections of six hamsters with approximately 90 cercariae each. These cercariae were also used for mass infection of five BALB/c mice (Table 1). Sixty-two dpi (8 weeks), only one hamster had liver lesions characteristic of *Schistosoma* infection. After processing, several miracidia were obtained from the liver, and the larvae (n=8/snail) were used for individual infection of 30 *B. glabrata* BH. However, after 80 days of weekly parasitological analyses, none of these snails had shed cercariae (Table 1). In contrast, all mass-infected mice had livers with lesions characteristic of *Schistosoma* infection. Several miracidia were obtained from these livers and used for individual infection of 18 *B. glabrata* SB, with 10–15 larvae each. Weekly parasitological examinations showed that cercariae were shed at 35 dpi in four snails (4/18; 22%). Thus, experimental infections in BALB/c mice and *B. glabrata* SB permitted successful isolation of *the Schistosoma* HS strain under laboratory conditions (Table 1).

Genetic characterization and phylogenetic analyses

Four sequences of the 16S (671–695 bp) and five of the *cox*1 (657–708 bp) mtDNA were obtained from adult *S. mansoni* HS, showing 100% genetic similarity among them and with sequences of *S. mansoni* deposited in GenBank from different geographic origins. Phylogenetic reconstructions using ML and BI showed similar patterns of nodal support and tree topology; therefore, only the BI trees are shown here (Fig. 3a, b). Trees generated from both genetic regions grouped all *S. mansoni* sequences (including the present) in a monophyletic assemblage, with high nodal support, and placed *S. rodhaini* as a sister group (Fig. 3a, b). However, although the *S. mansoni* clade showed several polytomies, mainly in the *cox*1 tree, it was possible to delimit some assemblages in the 16S tree (Fig. 3a). The present sequences clustered with strains of *S. mansoni* from the New World, that is, Puerto Rico and Brazil. Moreover, isolates of *S. mansoni* from Nigeria, Tanzania and Madagascar tended to form supported monophyletic lineages (Fig. 3a, b).

The patristic distances between the present *S. mansoni* HS 16S and *cox*1 sequences were null (P=0), indicating 100% similarity (Online Resource 4). Regarding the 16S region, sequences of S*. mansoni* from Tanzania (P=0.033), Madagascar (P=0.031), Nigeria (P=0.029), Kenya (P=0.009), Ghana (P=0.007), Senegal (P=0.004), and Guadeloupe (P=0.002) had K2P values different from 0 when compared to the newly obtained sequences. In contrast, the sequences of S*. mansoni* from Brazil, Puerto Rico, and two other samples from unidentified geographic origins (AF130787 and NC_002545) showed null distances (P=0) when compared with the sequences of *S. mansoni* HS (Online Resource 4). Regarding the *cox*1 region, sequences of *S. mansoni* from Tanzania (P=0.045), Nigeria (P=0.034), Senegal (P=0.003), and Ghana (P=0.003) had K2P values different from 0 when compared with the present sequences. However, sequences of *S. mansoni* from Puerto Rico, Guadeloupe, Brazil, Kenya, and from a sample with an unidentified origin (NC_002545), showed null values of patristic distance (P=0) when compared with the present *S. mansoni* HS sequences (Online Resource 4).

S. mansoni HS strain infection of *B. glabrata* and in BALB/c mice

Fig. 4 shows the survival curves of *B. glabrata* BH and SB during infection with crescent doses of miracidia (10, 15, or 20 miracidia/snail) of the *S. mansoni* HS strain. In general, mortality in the infected groups started between 15 and 20 dpi (period estimated to be required for migration of secondary sporocysts) and intensified after 30–40 dpi (period required for the formation and liberation of cercariae) (Fig. 4a, b). In both *B. glabrata* strains, the mortality rate induced by *S. mansoni* HS infection was miracidia dose-dependent (Fig. 4a, b). However, in *B. glabrata* BH only the experimental group infected with 20 miracidia/snail showed significantly higher mortality rates than the uninfected controls (Fig. 4a). In *B. glabrata* SB, experimental groups infected with 20 and 15 miracidia/snail showed significantly higher mortality rates than the control group, and snail mortality started earlier in this snail strain (Fig. 4b).

The susceptibility rate of both snail strains to experimental infection with *S. mansoni* HS increased in a dose-dependent manner, although the proportion of infected snails was always higher in *B. glabrata* BH (Fig. 4c, d). In this snail strain, the percentage of snails shedding cercariae increased from 15% in snails infected with 10 miracidia to 30% in snails infected with 20 miracidia (Fig. 4a). In the SB snail strain, the susceptibility rate varied from 10 to 25%, depending on the infective dose (Fig. 4b).

Cercarial shedding was first detected at 30 dpi in *B. glabrata* BH and was not dependent on the infective dose (Fig. 4c). However, the kinetics of cercarial shedding was dependent on the infective dose. BH snails infected with 20 miracidia showed a peak of cercariae shedding at 44 dpi, and no larvae were detected after 51 dpi; however,

snails infected with 10 or 15 miracidia showed maximum cercariae shedding after 51 dpi. BH snails infected with 15 miracidia were able to maintain cercarial shedding for a longer time, up to 65 dpi, which resulted in the elimination of a larger number of larvae. At 80 dpi, there was no larval shedding by *B. glabrata* BH that remained alive. Similarly, *B. glabrata* SB infected with 20 miracidia started cercarial shedding at 30 dpi, reaching a peak at 44 dpi. SB snails infected with 15 miracidia showed peak cercarial shedding at 51 dpi (Fig. 4a). Interestingly, *B. glabrata* SB infected with 10 miracidia showed two peaks of cercariae shedding: an initial peak at 44 dpi and a late peak at 72 dpi (Fig. 4d).

In BALB/c mice, *S. mansoni* HS infection was accompanied by 20% of mortality rate and the deaths occurred after 11 weeks of experimental infection in the chronic phase of the disease, resulting in a survival curve that was not significantly different from that of the control group (Fig. 5a). *S. mansoni* HS strain eggs were not observed in fecal samples of infected BALB/c mice at 6th wpi. (40-42 dpi.). Eggs in the feces were identified only after 7th wpi. (50 dpi.) and peaked at the 10th wpi. (70 dpi, beginning of the chronic phase). After this period, the number of eggs released was similar to that observed during the acute phase of infection (Fig. 5b).

Adult worms were recovered from the circulation of all mice infected with *S. mansoni* of HS strain; at 8 weeks of infection an average of 2.8 ± 1.2 worms/mouse were recovered from the portal circulation and at 12 weeks of infection 2.5 ± 0.46 worms/mouse, which represents 12.5–14% of the total cercariae inoculation dose (Table 2). The ratio of male to female worms ranged from 1:1.15 at the acute phase of infection to 1:2,33 during chronic schistosomiasis. The number of parasite eggs retained in the host tissue progressively increased during experimental infection, and the liver and intestine were the main tissues for egg retention. Conversely, parasite egg retention was low and irregular in the lung and spleen tissues. Parasite eggs were found in the lungs of only two chronically infected mice (Table 2).

Discussion

Several studies (Théron et al. 1992; Rey 1993; Miranda et al. 2017; Catalano et al. 2018) have shown that wild rodent species are susceptible to natural infections by *S. mansoni*, which may affect effective control of schistosomiasis. In Brazil, wild rodents with high rates of *S. mansoni* infection are mainly *H. sciureus* and *N. squamipes* (Rey 1993; Gentile et al. 2010; Miranda et al. 2017). Previous studies have suggested that these animals have a high tolerance to schistosomiasis (Silva and Andrade 1989; Amaral et al. 2016; Miranda et al. 2019), which results in the release of a large number of viable eggs in their feces (Piva 1966; Dias et al. 1978; Picot 1992; Souza et al. 1992). However, little is known about the biology and genetic diversity of the *S. mansoni* strains infecting these rodents. In the present study, we describe the isolation process of *S. mansoni* from naturally infected *H. sciureus* (HS strain) captured in an endemic area of schistosomiasis in Northeastern Brazil. Genetic characterization of these parasites confirmed their identity as *S. mansoni* and their close relatedness with other schistosomes isolated in Brazil, Puerto Rico, Guadeloupe, and Kenya. The present isolate *S. mansoni* HS can be maintained under laboratory conditions using a sympatric population of *B. glabrata* (SB strain) as an intermediate host and BALB/c mice, but not hamsters, as a definitive host.

Although previous studies have demonstrated the existence of different strains of *S. mansoni* isolated from humans living in different geographic regions (Warren et al., 1967; Anderson and Cheever, 1972; Martinez et al., 2003; Euzébio et al. 2012), knowledge pertaining to the parasitological, genetic, and pathological features of *S. mansoni* strains obtained from wild rodents remains unclear and may have an impact on the severity of human

schistosomiasis. Therefore, the isolation and characterization of *S. mansoni* obtained from other vertebrate hosts (e.g., wild rodents) in the laboratory is essential for a better assessment of parasite diversity and its role in human disease. In the current study, we successfully isolated the *S. mansoni* HS strain and maintained the parasite using *B. glabrata* from sympatric populations (SB strain) as intermediate hosts and BALB/c mice as definitive hosts. Indeed, there are different levels of compatibility between snails and schistosomes in different geographic regions, and the interactions between parasites and hosts are generally more suitable in sympatric than in allopatric populations (Lively 1989; Portet et al. 2019), which confirms our findings. Moreover, possible genetic variations in *S. mansoni* recovered from *H. sciureus* could also result in parasite compatibility with different snail strains and different species of vertebrate hosts. It should be highlighted that, unlike the vast majority of studies showing hamsters as good models for maintaining *S. mansoni* under laboratory conditions (Moore et al. 1949; Cheever et al. 2002; Lombardo et al. 2019), miracidia of the HS strain obtained from infected hamsters, unlike for BALB/c, did not produce cercariae in *B. glabrata*, preventing the maintenance of the parasite under laboratory conditions, in the present study. These results reinforce the hypothesis that *S. mansoni* strains naturally infecting *H. sciureus* may modify the disease transmission patterns.

In the present study, it was not possible to isolate this parasite from most of the infected *H. sciureus* (see Table 1). Although *S. mansoni* is the only currently known schistosome species that infects wild rodents in Brazil (Gentile et al. 2010; Morgan et al. 2005; Miranda et al. 2017), it is possible that *S. rodhaini* or *S. mansoni*/*S. rodhaini* hybrids also arrived in the Americas in naturally infected rodents inhabiting slave ships (Marr and Cathey, 2010; Etougbétché et al. 2020) during the 17th-19th centuries (Crellen et al. 2016). Therefore, the present unsuccessful attempts to isolate schistosomes from *H. sciureus*, captured in the State of Maranhão, Brazil, may indicate the occurrence of a great diversity of schistosomes in the region (e.g., new genotypes, hybrids, and species). Therefore, further genetic assessments of these parasites in the referred area are required.

The morphological data of schistosome eggs and worms obtained from natural infections of *H. sciureus* were confirmed by genetic analyses, in which sequences of both *cox1* and 16S showed 100% genetic similarity with those from isolates of *S. mansoni* deposited in GenBank. Although the *S. mansoni* clade tended to have a polytomic pattern (mainly in *the cox1* dataset), phylogenetic reconstruction using 16S sequences delimited some assemblages within this group, in which *S. mansoni* HS was closely related to other isolates from Brazil and Puerto Rico. These findings are similar to those of previous studies that demonstrated the close relatedness of *S. mansoni* isolates from the Americas (Morgan et al. 2005; Webster et al. 2013). In this sense, the clustering of *S. mansoni* HS with isolates from both snails and humans in Brazil and Puerto Rico suggests possible cross-infection between rodents and men.

The K2P values obtained for *cox1* sequences also demonstrated a certain proximity between *S. mansoni* HS and an isolate from Kenya, East Africa. As previously mentioned, there is strong evidence indicating that *S. mansoni* arrived in the New World between the 16th and 19th centuries through slave trade from West Africa (Lockyer et al. 2003; Morgan et al. 2005; Crellen et al. 2016). However, some studies (Morgan et al. 2013; Crellen et al. 2016) have also shown great similarity between *S. mansoni* isolates from East Africa and the Americas, and recently, Platt et al. (2022) proposed central Africa as the main source of S. mansoni lineages in Brazil. Therefore, the present results, along with those from the previously mentioned studies, suggest that New World *S. mansoni* are, in fact, originally from different geographic regions of Africa.

Experimental infections using *S. mansoni* HS demonstrated that BALB/c mice were fully susceptible to infection, since all mice inoculated with cercariae had some adult worm development and parasite eggs in feces, and the evolution of experimental schistosomiasis showed classic parasitological patterns, as previously demonstrated in mice (Anderson and Cheever 1972; Alves et al. 2016; Oliveira et al. 2022). These patterns include the beginning of egg elimination in feces around the 6th week of infection and the progressive increase in the number of eggs retained in tissues, mainly in the liver and intestine. However, only 12.5% of inoculated cercariae were recovered as adult worms, and the recovery rate was much lower when compared to mice infected by other *S. mansoni* strains (Warren et al. 1967; Anderson and Cheever 1972; Martinez et al. 2003; Freire et al. 1967; Anderson and Cheever 1972; Martinez et al. 2003; Freire et al. al. 2003), which may suggest that *S. mansoni* HS is still in the process of adaptation to laboratory vertebrate models or that BALB/c is not fully suitable for the development of this strain.

During the isolation process, viable *S. mansoni HS* cercariae were obtained only from experimental infection using *B. glabrata* SB (sympatric) as an intermediate host. However, after parasite establishment in mice, miracidia could infect both *B. glabrata* strains (SB and BH). Moreover, experimental infections of *B. glabrata* SB resulted in higher mortality rates than those of *B. glabrata* BH (allopatric), especially with higher infection doses. These findings are similar to those of Bastos et al. (1982), who demonstrated high mortality rates of *B. glabrata* from sympatric populations infected with an isolate of *S. mansoni* obtained from wild rodents, also collected in São Bento, Maranhão, Brazil. These observations suggest a more recent interaction between *S. mansoni*, wild rodents, and *B glabrata* in the study region. Moreover, although infection rates were similar between the two snail populations (SB and BH), the high mortality rate observed in *B. glabrata* SB indicates the high susceptibility of these snails to infections by *S. mansoni* HS, since they allow intense asexual multiplication of sporocysts, resulting in high tissue damage and mortality (Guaraldo et al. 1981; Portet et al. 2019). However, to unravel the intramolluscan development of *S. mansoni* HS, further experiments are necessary.

It should be highlighted that the present data also revealed an unconventional pattern of cercariae shedding by *B. glabrata* SB infected with a dose of 10 *S. mansoni* HS miracidia, that is, two peaks, one at 45 dpi and one at 70 dpi. Bastos et al. (1982) demonstrated a similar late peak of cercariae shedding in *B. glabrata* from sympatric populations infected with an isolate of *S. mansoni* from humans in São Bento, Maranhão, Brazil. This cercariae shedding pattern may be an adaptation of the parasite to local climatic conditions. The flooded fields of São Bento dry up almost completely during the dry season from August to January (Costa-Neto et al. 2011), subjecting the snails to diapause and anhydrobiosis (Dannemann and Pieri 1989), consequently affecting the propagation of *S. mansoni*. Therefore, the asynchrony observed in cercariae shedding patterns here is most likely due to the strategies of *S. mansoni* HS to overcome the seasonal drought in the region. However, such mechanisms need to be studied and confirmed.

In summary, the present study describes the successful isolation of *S. mansoni* HS obtained from naturally infected *H. sciureus*, using *B. glabrata* SB and BALB/c mice as hosts. After isolation, genetic characterization and phylogenetic analysis showed that the HS strain presented sequences clustered with strains of *S. mansoni* from America and Kenya; however, this isolated parasite strain showed differential infectivity patterns in experimental infection of vertebrate and invertebrate models.

Declarations

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Author's contribution

GSM, and DN-C: Conceptualization. GSM, JGMR, NS-S: Field collection. GSM, JGMR, MCR, SDR, GMAC, JKAOS, JCR, NS-S, and DN-C: Performed experiments. GSM, LFVF, EMLR, and FBP: Molecular analysis. GSM, JGMR, GMAC, JKAOS, LFVF, EMLR, FBP, and DN-C: Data analysis. GSM, and DN-C: Supervision and project administration. GSM, JGMR, GMAC, JKAOS, LFVF, EMLR, FBP, and DN-C: Writing and editing the original draft. All the authors reviewed and approved the final version of this manuscript.

Ethics considerations

The capture of *H. sciureus* was authorized by the Biodiversity Authorization and Information System (n°67253-1) and the project is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (registration number AB9E2EC). The experimental procedures were approved by the Ethics Committee on Animal Use of the State University of Maranhão (UEMA, Brazil; protocol nº03/2017) and of the Federal University of Minas Gerais (UFMG, Brazil; protocols nº46/2019 and nº368/2018).

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Competing interests

The authors declare no competing interests.

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Tables

Table 1

Details related to the isolation process of the *Schistosoma mansoni* HS strain, obtained from naturally infected *Holochilus sciureus* rodents captured in the municipalities of São Bento and Peri Mirim, State of Maranhão, Brazil, which are endemic areas for intestinal schistosomiasis.

Collection points	Nº of *H. sciureus* captured	Infection rate N (%) [a]	Miracidia recovery/type of infection	*B. glabrata* SB infected [b]	Hamster infected [c]	BALB/c infected [c]
P1=2°40'39.1"S 44°48'25.8"W	6	3 (50)	+ (mass infection)	-	NR	NR
P2=2°40'43.0"S 44°49'07.1"W	7	4 (57.14)	NR	NR	NR	NR
P3=2°39'56.5"S 44°48'36.9"W	7	4 (57.14)	+ (individual/mass infection)	+	+ *	NR
P4=2°40'14.2"S 44°48'51.4"W	7	3 (42.85)	NR	NR	NR	NR
P5=2°40'15.9"S 44°48'53.5"W	5	1 (20)	NR	NR	NR	NR
P6=2°40'19.2"S 44°48'56.6"W	8	4 (50)	NR	NR	NR	NR
P7=2°40'21.8"S 44°48'59.3"W	8	4 (50)	+ (individual infection)	-	NR	NR
P8=2°40'23.0"S 44°49'00.2"W	7	2 (28.57)	NR	NR	NR	NR
P9=2°40'24.1"S 44°49'00.9"W	8	5 (62.5)	NR	NR	NR	NR
P10=2°40'27.1"S 44°49'03.2"W	7	3 (42.85)	NR	NR	NR	NR
P11=2°40'28.5"S 44°49'04.1"W	8	4 (50)	+ (individual infection)	+	+ *	+
P12=2°40'28.3"S 44°49'04.4"W	6	2 (33.33)	NR	NR	NR	NR
P13=2°40'29.6"S 44°49'04.9"W	7	3 (42.85)	NR	NR	NR	NR
P14=2°41'03.7"S 44°49'17.8"W	8	2 (25)	NR	NR	NR	NR

a = based on presence of adult worms, macroscopic changes in liver and presence of eggs in this tissue (Do Carmo-Silva et al. 2019); b = based on elimination of cercariae; c = based on presence of eggs in the feces. * Not possible to finish isolation of *S. mansoni*. SB = São Bento (municipality), geographic origin of *B. glabrata* used for isolating *S. mansoni* HS strain. NR: assay not performed.

Table 2

Number of adult worms recovered from the hepatic circulatory system and number of eggs retained in different organs of BALB/c mice experimentally infected by the *Schistosoma mansoni* HS strain.

	Adult worms recovered [a]	
	Acute schistosomiasis (n=10)	**Chronic schistosomiasis (n=8)**
Males	1.5 ± 0.64	1.75 ± 0.37
Females	1.3 ± 0.58	0.75 ± 0.46
Total	2.8 ± 1.2	2.5 ± 0.46
	Eggs retained [b]	
	Acute schistosomiasis (n=10)	**Chronic schistosomiasis (n=8)**
Lung	0	0 (0-25)
Spleen	0 (0-6.25)	50 (6.25-168.8)
Liver	1,613 (956.3-1,969)	1,875 (1,035-3,556)
Intestine	3,175.5 (1,506-6,700)	4,975 (3,556-8,956)

Data presented as mean and standard error (a), and median and interquartile range (b).

Figures

Figure 1

***Holochilus sciureus* sampling areas located in an endemic region of intestinal schistosomiasis in Northeastern Brazil. A.** Brazil. **B.** State of Maranhão **C.** Municipalities of São Bento and Peri Mirim. **D.** Collection points in flooded field areas between São Bento and Peri Mirim, highlighting the points 1, 3, 7, and 11, investigated in this study. **E** and **F**. Typical ecosystem of the studied area (black rectangle in Fig. **E** indicates the presence of *H. sciureus* in natural habitat). **G.** Traps set up at night in the field collection points

Figure 2

***Schistosoma* sp. natural infection of *Holochilus sciureus* captured in the State of Maranhão, Brazil. A.** Macroscopic appearance of hepatic lobes, showing characteristic changes induced by *S. mansoni* infection. **B.** Adult worms collected from the portal hepatic circulatory system showing typical *S. mansoni* morphology. **C.** Mature egg obtained from liver homogenate showing the classic morphology of *S. mansoni*, emphasizing the presence of the lateral spike (arrow)

Figure 3

Trees generated using Bayesian inference from sequences of 16S (A) and *cox*1 (B) mtDNA genetic regions of Schistosomatidae. Full circles indicate nodal supports > 0.96 of Bayesian posterior probability (BPP) (Markov chain More Carlo, chains=4, runs=2, generations= 1×10^6, sampling frequency= 1×10^6, burn in fraction=25%) and > 90% of maximum likelihood (ML), based on 1,000 non-parametric bootstrap replications. Empty circles indicate values between 0.90-0.96 for BPP and 70-90% for ML. Values of BPP and ML below those described (low nodal supports) were not indicated and not accepted. Sequences obtained in the present study are in bold and highlighted in gray

Figure 4

Infectivity parameters of experimental infection of *Biomphalaria glabrata* of BH strain (allopatric snail strain) and SB strain (sympatric snail strain) with the *Schistosoma mansoni* of HS strain. Survival curve of uninfected *Biomphalaria glabrata* BH (**A**) and SB strain (**B**) (control group) and experimentally infected with 10, 15 or 20 miracidia of *S. mansoni* HS strain. Infectivity rate (%) of *B. glabrata* BH (**C**) or SB strain (**D**) infected with different doses (10, 15, or 20 miracidia/snail) of the *S. mansoni* HS strain. Number of cercariae shed from *B. glabrata* BH (**E**) and SB (**F**) during the experimental infection with different doses (10, 15, or 20 miracidia/snail) of the *S. mansoni* HS strain. The snails were individually infected with 10 (light gray color or circle symbol), 15 (dark grey color or square symbol) or 20 (black color or triangle symbol) miracidia of *S. mansoni* HS strain and examined weekly for 84 days of infection. Data represents values of 20 snails/group. Data of cercariae production are expressed as mean and standard error. The survival curve was analyzed by the Log-rank test and the symbol $^{\#}$ indicates value statistically different compared to the control group (not infected). mHS: miracidia of *S. mansoni* HS strain.

Figure 5

Mortality rate and parasite burden in BALB/c mice. Survival curve (**A**) and the number of eggs present in feces (**B**) of BALB/c mice uninfected and experimentally infected by the *Schistosoma mansoni* HS strain. BALB/c mice were subcutaneously infected with 20 *S. mansoni* cercariae of the HS parasite strain and monitored for 12 weeks (84 days). The Log-rank test was applied to compare mortality curves in control and infected groups with no significant differences between them. Data of egg release in feces are expressed as mean and standard error.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESM1.Genbakinformation16S.docx
- ESM2.GenbankinformationCO1.docx
- ESM3.Evolutivemodel16SeCO1.docx
- ESM4.Pairwisegeneticdistance16SeCO1.xls