

Identification of *Biomphalaria* sp. and other freshwater snails in the large-scale water transposition project in the Northeast of Brazil

Fernando Schemelzer de Moraes Bezerra^{1,2,4}, Marta Cristhiany Cunha Pinheiro³, José Damião da Silva Filho⁴, Issis Maria Nogueira de Castro¹, Roberta Lima Caldeira⁵, Mariana Silva Sousa², Albeniza Barbosa Cavalcante⁶, Alberto Novaes Ramos Júnior³

ABSTRACT

The wide eco-bio-social intervention generated by the SaoFrancisco River Integration Project (PISF) may contribute to the dispersion or introduction of schistosomiasis intermediate hosts in areas without prior recording. The objective was to characterize the limnic malacofauna and its distribution along watersheds involved in the PISF. A cross-sectional study based on the collection of mollusks from 33 water bodies, from Aurora, Brejo Santo, Jaguaratama, Jaguaribara, Jati e Mauriti municipalities in the Ceara (CE) State was developed. The conchological characteristics were used to identify snails at the genus level. The snails of the genus *Biomphalaria* were analyzed for the presence of *Schistosoma mansoni* cercariae and the molecular identification (only mollusks from Brejo Santo-CE) for differentiation between species. The following species were found: *Biomphalaria* sp.; *Drepanotrema* sp.; *Melanooides* sp.; *Physa* sp.; and *Pomacea* sp. *Pomacea* sp. (75.8%) and *Biomphalaria* sp. (72.7%) were the most prevalent species. All municipalities showed *Biomphalaria* sp. *Biomphalaria straminea* (Porcos Stream) and *Biomphalaria kuhniana* was identified in the Boi 1 and Cipo reservoirs (Brejo Santo). The evaluated municipalities under the influence of the PISF present areas with potential for schistosomiasis transmission. It is necessary to intensify control actions and health surveillance in these areas.

KEYWORDS: Mollusca. *Biomphalaria*. Molecular biology. Schistosomiasis mansoni. Epidemiology. Water resources.

INTRODUCTION

Schistosomiasis mansoni is a chronic disease caused by *Schistosoma mansoni* (Sambon, 1907), whose intermediate hosts are the species *Biomphalaria glabrata* (Say, 1818), *Biomphalaria straminea* (Dunker, 1848) and *Biomphalaria tenagophila* (Orbigny, 1835)¹. A recent parasitological survey conducted in Brazil among schoolchildren, reported a positive rate of 0.99% for *S. mansoni*². The average incidence rate of schistosomiasis in Brazil from 2001 to 2009, estimated at 22.63 cases per 100.000 inhabitants, was largely associated with transmission of *S. mansoni* in Northeast and Midwest regions and in Northern Minas Gerais State. It should be noted that the Northeast region is characterized by a sustained pattern of endemicity for schistosomiasis³.

A determinant factor for the increasing dispersion of intermediate hosts and, consequently, of schistosomiasis, is the implementation of projects that involve

¹Universidade Federal do Ceará, Faculdade de Medicina, Programa de Pós-Graduação em Patologia, Fortaleza, Ceará, Brazil

²Universidade Federal do Ceará, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas, Fortaleza, Ceará, Brazil

³Universidade Federal do Ceará, Faculdade de Medicina, Programa de Pós-Graduação em Saúde Pública, Fortaleza, Ceará, Brazil

⁴Universidade Federal do Ceará, Departamento de Análises Clínicas e Toxicológicas, Laboratório de Pesquisa em Parasitologia e Biologia de Moluscos, Fortaleza, Ceará, Brazil

⁵Fundação Oswaldo Cruz-Fiocruz, Instituto René Rachou, Grupo de Pesquisa em Helmintologia e Malacologia Médica, Belo Horizonte, Minas Gerais, Brazil

⁶Governo do Estado do Ceará, Secretaria dos Recursos Hídricos, Fortaleza, Ceará, Brazil

Correspondence to: Fernando Schemelzer de Moraes Bezerra Universidade Federal do Ceará (UFC), Departamento de Análises Clínicas e Toxicológicas, Laboratório de Pesquisa em Parasitologia e Biologia de Moluscos, Rua Capitão Francisco Pedro, 1210, Rodolfo Teófilo, CEP 60430-370, Fortaleza, CE, Brazil
Tel: +55 85 33668651

E-mail: bezerra@ufc.br

Received: 16 April 2018

Accepted: 6 July 2018

water resources in areas of greater social vulnerability^{4,5}. An important example of this is the water transposition project of the Sao Francisco River, whose objective is to guarantee water security in the Northeast of the country⁶.

The project has been carried out by the Ministry of National Integration (MI), officially known as the Sao Francisco River Integration Project (PISF). The construction began in 2007 and is divided into two axes (North and East) and comprises 477 km of channels, 27 reservoirs, 13 aqueducts, nine pumping stations, nine 230 kW substations, 270 km of high-voltage transmission lines and four tunnels, including the largest tunnel for water transportation in Latin America - Cuncas I, which is 15 km long. This project aims to benefit 12 million people in 390 municipalities in the Northeastern States, the most affected by the drought periods: Pernambuco, Ceara, Paraiba and Rio Grande do Norte⁷. However, conflicts in affected families have already been identified in all States, mainly in Ceara and Pernambuco. In fact, the Sao Francisco water transposition project exacerbates environmental conflicts involving vulnerable populations living in the affected territory, which claim access to and use of the water⁸.

These areas show intense migratory movements, generated mainly by political-economic models that do not prioritize the poorest populations that migrate in search of better living conditions. This important factor, associated with the wide distribution of schistosomiasis intermediate hosts and the precarious sanitation conditions in most municipalities, leads to the emergence of new schistosomiasis foci^{9,10}.

In addition, these projects can contribute to the dissemination of waterborne diseases in areas previously unaffected by the presence of migrant construction workers and their families. Data from the Health Secretariat of the Government of the Ceara State indicate a situation of concern, with the identification of positive test results for schistosomiasis among workers at PISF construction sites in the Penaforte municipality with 1.21% (3/247) of positivity, Mauriti with 5.5 % (23/416) and Brejo Santo with 9.1% (45/432)¹¹.

It is noteworthy that of the 184 municipalities in the Ceara State, 71 (38.5%) are endemic areas, and the Schistosomiasis Control Program has developed systematic surveillance and control actions since 1977. However, in the period from 2010 to 2015, only 22.5% (16/71) of these municipalities carried out malacological research and seven identified the presence of *B. straminea* snails carrying *S. mansoni* cercariae. The municipalities of Brejo Santo and Aurora, areas of direct influence by the Sao Francisco River, are among the ones that demonstrated active transmission of schistosomiasis¹¹.

In addition to the morbidity burden, schistosomiasis is a neglected cause of death in Brazil. A population-based study from 2000 to 2011 assessed all deaths in which schistosomiasis was mentioned as the underlying cause of death or associated with it (multiple causes of death), indicating an annual average age-adjusted mortality rate of 0.49 deaths/ 100,000 inhabitants and proportional mortality rate of 0.070%. The areas of greatest risk and impact of mortality are also located in the Northeastern region of the country, mainly in the states of Alagoas, Pernambuco and Sergipe^{12,13}.

It is essential to know and monitor the spatialization and population dynamics of snails, both at the level of diversity and density, in these areas affected by the PISF in the Ceara State, aiming to prevent or minimize possible impacts on the distribution patterns of intermediate hosts and, consequently, on the occurrence of cases of schistosomiasis.

This study sought to identify the limnic malacofauna and its distribution along the main hydrographic basins involved in the Sao Francisco River Integration Project in Ceara, as well as to investigate the presence of *S. mansoni* larvae in the snails of the *Biomphalaria* genus.

MATERIAL AND METHODS

This work was a descriptive epidemiological study of the limnic malacofauna, conducted in the Ceara State, between February 2015 and September 2016, in the municipalities of Aurora, Brejo Santo, Jaguaretama, Jaguaribara, Jati and Mauriti. These municipalities are located in an area defined by the Environmental Impact Report as directly influenced by PISF, and will undergo direct environmental transformations resulting from the enterprise. In the Ceara State, this area includes 21 municipalities⁶.

The selection of municipalities was based on the following criteria: a registry of schistosomiasis cases based on data from the Notifiable Disease Information System (SINAN) and/ or the Schistosomiasis Control Program Information System (SISPCE); being located in watersheds with eco-epidemiological characteristics indicative of transmission or potential risk for the occurrence of schistosomiasis outbreaks and having a history of the presence of intermediate hosts positive for *S. mansoni*.

A mapping of the water collections of epidemiological importance was carried out in which 33 collection points of limnic malacofauna specimens were selected within the area directly affected by the PISF in the six study municipalities (Table 1). These water collections were classified according to ecological characteristics and they were compared regarding the presence of types and number

of snails sampled. Table 2 describes the ecological and biological parameters here analyzed.

The geodetic coordinates of the capture stations of the surveyed water collections were demarcated using a Garmin Montana® 650GPS (global positioning system) receiver, using the UTM projection system.

Snail population

Mollusk sampling was carried out by scraping the submerged vegetation and the bottom of the breeding grounds, using the adequate equipment (tongs and nets). On the surface, material was carefully collected and analyzed

Table 1 - Freshwater snails (Class Gastropoda – Cuvier, 1795) distributed by municipality, in the directly affected area by the Sao Francisco River Transposition, Ceara State, Brazil, collected from February/2015 to September/2016

Municipalities	Water collections	Ampullariidae	Planorbidae		Physidae	Thiaridae
		Pomacea sp.	Biomphalaria sp.	Drepanotrema sp.	Physa sp.	Melanoides sp.
Aurora	Bleeder of the Cachoeiras Dam	X	21			
	Passagem das Pedras Dam	X	46			X
	Cachoeiras Reservoir	X	0			X
	Mofumbo Reservoir	X	56			X
	Solidade Reservoir	X	13			
	Tipi Reservoir	X	44	X	X	
	Tipi de Baixo Reservoir	X	14	X		
	Salgado River	X	450			X
Brejo Santo	Atalho Large Reservoir	X	0			X
	Cipo Reservoir		76			
	Cana Brava Stream		0			
	Pinheira Stream		4			
	Porcos Stream	X	51			X
	Torroes Stream	X	42			X
Jaguaretama	Santa Barbara Dam		0			X*
	Castanhao Large Reservoir	X	48		X	X*
	Alegre Reservoir	X	78		X	X
	Genesisio Bezerra Reservoir		0			X
Jaguaribara	Integration Channel (Piscinao)		150			X
	Irrigation Channel Curupati (Bridge)	X	14			X
	Irrigation Channel Curupati-Peixe	X	6		X	X
	Castanhao Large Reservoir	X	19		X	X*
	Castanhao Large Reservoir (Flood-gates)	X	23		X	X
	Jaguaribe River	X	6		X	
Jati	Carnaubas Reservoir	X	11			X
	Atalho Large Reservoir	X	0			X*
	Poco da Barra Stream	X	43	X	X	X
	Porcos Stream	X	70	X	X	X
Mauriti	Bom Sucesso Dam		0			
	Palestina Dam	X	10	X		X
	Sao Miguel Dam		16	X		
	Pastora Reservoir	X	0			X
	Quixabinha Reservoir	X	0			X*

Legend: X = occurrence; X* = large amounts.

Table 2 - Ecological and biological characteristics of the analyzed water collection areas, from February/2015 to September/2016, in the directly affected area by the Sao Francisco River Transposition, Ceara State, Brazil

Municipalities Water collections		Water stream		Area type		Coliforms		Shaded areas		Environment	
		Lentic	Lotic	Isolated	Peridomestician	Fecal	Total	Yes	No	Artificial	Natural
Aurora	Bleeder of the Cachoeiras Dam		X	X					X		X
	Passagem das Pedras Dam		X		X	X*	X		X	X	
	Cachoeiras Reservoir	X		X		X*	X		X	X	
	Mofumbo Reservoir	X		X		X*	X		X	X	
	Solidade Reservoir	X		X			X*		X	X	
	Tipi Reservoir	X			X	X*	X		X	X	
	Tipi de Baixo Reservoir	X			X	X*	X		X	X	
	Salgado River		X		X	X*	X		X		X
Brejo Santo	Atalho Large Reservoir	X		X		X*	X		X	X	
	Cipo Reservoir	X		X					X	X	
	Cana Brava Stream	X		X			X*		X		X
	Pinheira Stream		X	X			X*	X			X
	Porcos Stream	X		X			X*	X			X
	Torroes Stream	X		X		X*	X	X			X
Jaguaretama	Santa Barbara Dam	X			X	X	X		X	X	
	Castanhao Large Reservoir	X			X		X		X	X	
	Alegre Reservoir	X			X	X	X		X	X	
	Genesisio Bezerra Reservoir	X			X	X	X		x	X	
Jaguaribara	Integration Channel (Piscinao)		X	X			X		X	X	
	Irrigation Channel Curupati (Bridge)		X		X	X	X		X	X	
	Irrigation Channel Curupati-Peixe	X			X	X	X		X	X	
	Castanhao Large Reservoir	X		X		X	X		X	X	
	Castanhao Large Reservoir (Flood-gates)	X			X	X	X		X	X	
	Jaguaribe River		X		X	X	X		X		X
Jati	Carnaubas Reservoir	X			X		X*	X		X	
	Atalho Large Reservoir	X		X		X*	X		X	X	
	Poco da Barra Stream		X		X	X*	X	X			X
	Porcos Stream		X		X	X*	X	X			X
Mauriti	Bom Sucesso Dam	X		X		X*	X		X	X	
	Palestina Dam	X			X	X*	X	X		X	
	Sao Miguel Dam		X		X	X*	X	X		X	
	Pastora Reservoir	X			X	X*	X		X	X	
	Quixabinha Reservoir	X			X	X*	X		X	X	

Legend: X = occurrence; *presence of *Pseudomonas* sp.

for the presence of mollusks, observing the leaves and small sticks where young or small specimens were trapped. The collected mollusks were stored in a plastic container with identification of the collection point (name of the

locality, type of freshwater habitat, date of collection). The remaining material was washed and checked repeatedly until confirmation of the absence of mollusks, and then it was discarded. This examination was carried out by two

technicians, across an area of 30 m demarcated from the point where the population gets in contact with the water collection (15 m for each side), during a period of 30 min. All water collections were visited only once, between February and September 2015, with no rainy season interference, as in the last few years the Northeast of Brazil has been facing a great period of drought.

The mollusks were packed in gauze pads moistened with water. In order to minimize the loss of moisture, these pads were placed in plastic bags, which were properly identified. This material was placed in resistant thermal boxes, and transported to the Parasitology and Mollusk Biology Research Laboratory located at the Federal University of Ceara (LPBM-UFC), where the mollusks were kept in plastic containers with dechlorinated water and fed with decontaminated fresh lettuce¹⁴.

Analysis of cercarial shedding of *Biomphalaria* snails

All the collected *Biomphalaria* molluscs were exposed to artificial light (60 W lamp) at a distance of 30 cm for a period of 4 h to stimulate the elimination of *S. mansoni* cercariae or any other trematode of medical or veterinary importance. After this step, the water from the containers with snails was analyzed under stereoscopic microscope. The same experiment was repeated three times within a 30-day period.

Classification of snails at the genus level

All mollusks collected in the municipalities of Aurora, Brejo Santo, Mauriti, Jaguaratama, Jaguaribara and Jati were identified to genus level throughout conchological parameters^{15,16}.

Molecular identification of *Biomphalaria* snails

Some specimens from the municipality of Brejo Santo-CE were selected by morphological^{17,18} and molecular identification at the National Reference Laboratory in Schistosomiasis of the Rene Rachou Institute/ Fiocruz-MG.

DNA extraction was performed using the Wizard Genomic DNA Purification[®] kit from Promega (Madison, Wisconsin, EUA). DNA was submitted to the polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphism (PCR-RFLP) analysis, directed to the internal transcribed spacer region of the ribosomal RNA (ITS-rDNA), and cleaved with the enzyme *Dde*I, as previously described by Caldeira *et al.*¹⁹. For the identification of the target DNA, we run a single electrophoresis gel with the digested products obtained from

snails of the study area and the digested products of DNA extracted from *B. kuhniiana* and *B. straminea* specimens maintained in the Medical Malacology Collection (CMM-Fiocruz). This municipality was chosen because it is the first in Ceara State where the waters of the Sao Francisco River will mix with natural waters and where the largest number of new PISF reservoirs will be built.

Statistical analysis and geospatial data

A database was developed with the help of Microsoft Office Excel 2016 program (Microsoft Corp., Redmond, WA, USA) for the organization and storage of data on the surveyed water collections and collected snails.

Spatial data were stored and analyzed in ArcGis 9.3 software (Environmental Systems Research Institute, Redlands, CA, USA) for manipulation and connection of descriptive attributes to the graphical form, as well as for the elaboration of thematic maps.

Ethical aspects

The study was approved by the Research Ethics Committee of the Federal University of Ceara (N° 254/11).

RESULTS

Freshwater mollusks of five genera were found, all belonging to the Gastropoda Class (Cuvier, 1795): *Biomphalaria* (Preston, 1910); *Drepanotrema* (Crosse & P. Fischer, 1880); *Melanoides* (Olivier, 1804); *Physa* (Draparnaud, 1801) and *Pomacea* (Perry, 1810). During the malacological survey, some specimens of amphibian snails of the genus *Omalonyx* (d'Orbigny, 1837) were recovered from almost all water collections analyzed in the municipality of Jati, with the exception of Atalho Reservoir. Some species of the Bivalvia Class (Linnaeus, 1758) were found in large quantities in the Jaguaribe River and in the large reservoirs of the municipalities of Jaguaratama and Jaguaribara, mainly in the Castanhao Reservoir, but they will not be detailed in this study.

Table 1 shows the distribution of the freshwater snail species found per municipality. The *Pomacea* sp. and *Biomphalaria* sp. species were more frequently found along the studied watersheds; they were present, respectively, in 25 (75.8%) and 24 (72.7%) collection sites. The *Physa* sp. (9; 27.3%) and *Drepanotrema* sp. (6; 18.2%) were present in a smaller number of water collections surveyed. However, the exotic snails of the Thiaridae family showed an important distribution, being found in 23 (69.7%) of the water collection sites. The collection points with the

highest diversity of mollusk species were: Porcos Stream and Poço da Barra Stream, both in the municipality of Jati; followed by the Alegre Reservoir, in the municipality of Jaguaretama; the water structures supplying the Curupati irrigation and pisciculture project in the municipality of Jaguaretama; and the Castanhao Reservoir, which covers part of the municipalities of Jaguaretama and Jaguaribara. On the other hand, the water collections that did not show the presence of mollusks were: Cana Brava Stream, in the municipality of Brejo Santo and Bom Sucesso Dam, in the municipality of Mauriti.

Table 2 shows the most common ecological and biological characteristics (the percentage is representative of the sum of collection points): total coliforms (91.7%), presence of fecal coliforms (72.7%), artificial environments (72.7%), non-shaded areas (72.7%), lotic ecosystems (69.7%), and peridomiciliary areas (62.5%). The most diverse collection points were located in peridomiciliary areas (87.5%) and had total coliforms (100%) and fecal coliforms (87.5%). None of the analyzed ecological factors were found to be determinant for the presence of *Biomphalaria* sp. when evaluated alone.

The *Biomphalaria* snails did not present cercarial shedding.

Figure 1 shows the locations with the presence of specimens which belong to *Biomphalaria* genus, and the amount collected at each location. All the municipalities had some collections of water with the presence of this species. The average number of specimens of *Biomphalaria* sp. collected was from 55 snails (ranging from 4 to 450). Two collection points presented largest number of specimens collected: the Salgado River, in the municipality of Aurora (450 specimens collected), and the initial structure of the Integration Channel, known as Piscinao, in the municipality of Jaguaribara (150 specimens of *Biomphalaria* sp.).

A sample of *Biomphalaria* sp. collected in the municipality of Brejo Santo was submitted to morphological and molecular identification for differentiation. Two species were identified: *B. straminea*, a natural intermediate host species of *Schistosoma mansoni*, was identified in Porcos Stream (100% of the six specimens analyzed from this collection point); *Biomphalaria kuhniiana* (Clessin, 1883), a species not susceptible to *S. mansoni*, was found in Boi 1 and Cipo reservoirs (100% of the six specimens analyzed at each collection point) (Figure 2). In the morphological identification, only two of the three species of the *B. straminea* complex - *B. straminea* and *B. kuhniiana* presented any doubt; therefore the *B. intermedia* profile was not included in the comparison of molecular identification.

DISCUSSION

The present study broadens the knowledge on the diversity of the limnic gastropod malacofauna, as well as its distribution, and brings evidence of the integration of different components in the complex transmission dynamics of schistosomiasis in areas of PISF influence in the Ceara State. In addition, it outlines a current scenario of the distribution of *Biomphalaria* snails - intermediate hosts of schistosomiasis mansoni - in areas that will be directly affected by Brazil's largest water engineering project (the Sao Francisco River Integration Project in the Northeast River Basins). Finally, it reinforces the endemic characteristic of the disease in the studied municipalities and the potential for expansion of its transmission dynamics.

The biodiversity and the pattern of distribution of the snail species in the analyzed municipalities are irregular, even though it is a micro-geographical space. Anthropogenic habitats (water collections with total and fecal coliforms, and proximity to peridomiciliary areas) were the ones that showed the greatest diversity and population density of snails, mainly in relation to the genus *Biomphalaria*, which is known to prefer environments rich in organic matter²⁰. Climate is another preponderant factor, with the Northern part of Northeastern Brazil the region that suffers the most with intense periodic droughts, stimulating the snails to develop specific biological characteristics to adapt to these habitats subject to hydrological disturbances - flood-dry cycles²¹.

Biomphalaria straminea is the species that shows the lowest rates of natural infection by *S. mansoni*, but has a high capacity to resist desiccation, which is one of the factors that explain the wide geographical distribution of these snails in the Brazilian Northeast²². In the Ceara State, this is the only native species involved in *S. mansoni* transmission. However, the PISF will result in changes in this ecosystem balance and biodiversity: possibility of introducing new species (e.g., *B. glabrata* - the most competent vector of schistosomiasis); increase (e.g., entry of a larger number of *B. straminea*), decrease or even extinction of other species; changes in the genetic diversity of native communities, mainly by hybridization²³. Both cited species are present in several parts of the Sao Francisco River Basin²⁴. This problem has become a reality in other parts of the world⁹ and also in Brazil²⁵⁻²⁷.

The collection points that showed high amounts of *Biomphalaria* sp. are environments with large water supply, and the PISF works aim precisely at the perennization of the catchment basins, with maintenance of a high volume of water in the dams and other aquatic environments. The potential direct influence of the PISF on the population

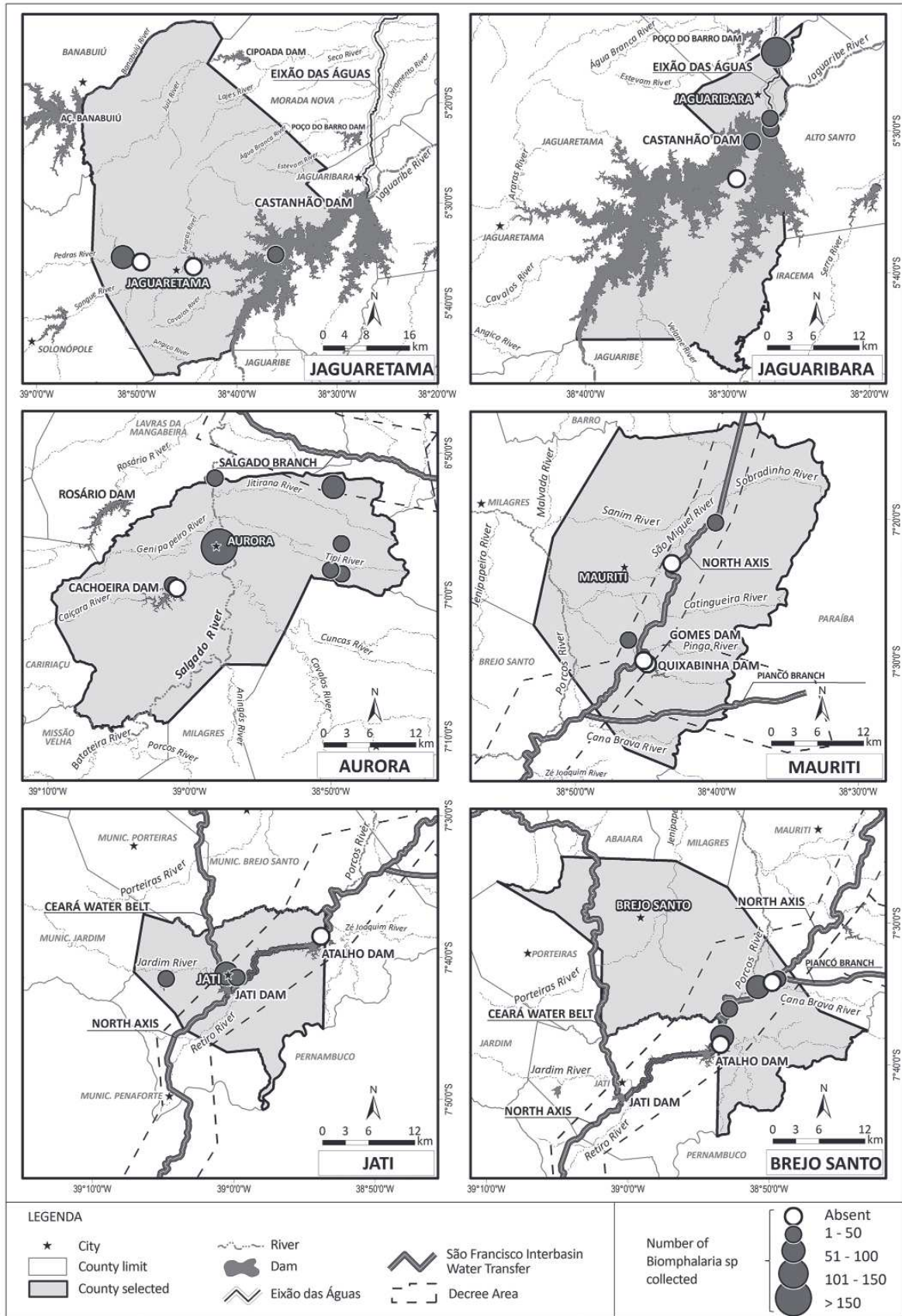


Figure 1 - Distribution of *Biomphalaria* genus in assessed River water collections obtained in the area of influence of the Sao Francisco River Integration Project in the Ceara State - Brazil

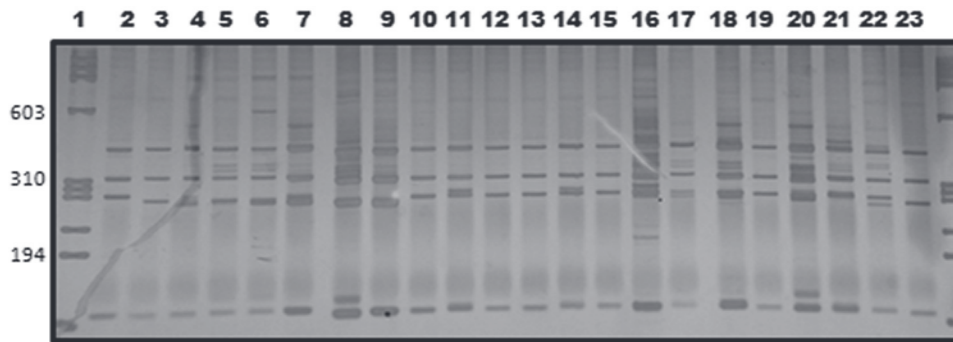


Figure 2 – Six percent silver-stained polyacrylamide gel showing the restriction profiles after digestion of the ITS region of ribosomal DNA with DdeI: band 1: Molecular-size marker phiX 174; 2 and 22: *Biomphalaria kuhniana* from Tucuruí/Para; 3 and 23: *Biomphalaria straminea* from Monte Carmelo/Minas Gerais, 4 to 9: Snails collected from Porcos Stream - Brejo Santo/Ceara; 10 to 15: Snails collected from Boi 1 Reservoirs (Brejo Santo/Ceara); 16 to 21: Snails collected from Cipo Reservoirs (Brejo Santo/Ceara). Molecular size markers are shown on the left side of the figure

density of the planorbids is related to the presence of new reservoirs, which allows greater accumulation of organic matter by reducing the velocity of the water flow²⁶ and facilitates the formation of new breeding sites²⁸. There is also the risk of snail dispersal to all municipalities through which these waters will circulate, reaching the Metropolitan Area of Fortaleza whose suburbs have favorable conditions for the disease transmission outbreaks. Moreover, migratory movements, generated by these changes, can contribute to the establishment of the transmission cycle, which was demonstrated by our research group in this region, where PISF workers already infected with *S. mansoni* were identified²⁹.

Within the scope of its operation, the PISF will enable the provision of water for various purposes, such as human supply, irrigation and animal watering, which further enhances the vulnerability of the area covered by this project⁶. The abundance of water may intensify old Northeastern customs, such as bathing and washing clothes and household utensils in natural waters³⁰, and became a tourist attraction for fishing and leisure activities, as already shown elsewhere³¹.

The areas affected by the PISF in Ceara are epidemiologically important because they have all the necessary elements for the disease transmission. Our findings demonstrated a wide distribution of *Biomphalaria* snails in all assessed municipalities, but none of the analyzed samples had larval forms of the parasite that transmit this disease. However, the PCE in Ceara State¹¹ had previously identified snails of this species, eliminating cercariae in Aurora, Brejo Santo and Mauriti, and demonstrated the existence of PISF workers with viable eggs of the parasite in their feces; 45 cases (9.1% - 45/432) and 23 cases (5.5% - 23/416) were identified at the construction site of the PISF in Mauriti and Brejo Santo, respectively. This scenario is aggravated by the social vulnerability of

these municipalities, which have low levels of education, subsistence economy, low health care coverage and insufficient basic sanitation conditions. In fact, poverty rates are factors of concern in these municipalities, where the average number of population living in poverty reaches 41.4% (average for the six surveyed municipalities), while the national average is 15.2%. The percentage of population in households with sewage collection and treatment is 28.6%, considering the average of the six surveyed municipalities, while the national average is 59.1%³². Proceedings for the treatment of these natural waters (of PISF) for domestic supply, as well as the sanitary sewage collection and treatment will be the responsibility of local municipalities, which currently do not have the financial resources to carry out these sanitation works.

Molecular identification of collected species was carried out in only three water collections (18 specimens, six at each collection point) from one of the six cities investigated, certainly constituting a limitation of the study. Our result showed that two collection points had only *B. kuhniana*, while the last point had only *B. straminea*, pointing to a competitive displacement between these two species, which has already been reported in China³³.

It is worth of note that Ceara State is the only in the Northeast region where *B. glabrata*, the main intermediate host of schistosomiasis mansoni in Brazil is not present. Both species identified in this study displaced *B. glabrata* as described elsewhere^{15,34}, perhaps warranting the non-establishment of this species in Ceara State.

Some questions persist because of the small size of the studied sample: is the population of *B. kuhniana* larger than *B. straminea* in Ceara State? If so, is this, one of the factors that could explain the low prevalence of the disease in Ceara State even with the presence of all risk factors for the maintenance of high transmission rates? Further studies conducting a comprehensive malacological

survey in all of the Hydrographic Basins of Ceara State, would require additional molecular identification for the accurate differentiation between these species. The species belonging to the *B. straminea* complex (*B. straminea*, *B. intermedia*, *B. kuhniiana* and *B. peregrina*)¹⁸ require a thorough morphological evaluation of experts with enough experience to perform this differentiation due to phenotypic similarities, size of collected specimens and inadequate fixation procedures³⁵.

The evaluated municipalities under the influence of the PISF present areas with potential for schistosomiasis transmission in different points. The construction of new reservoirs and the transposition of waters of the Sao Francisco River are factors that may lead to an increase in the diversity and population density of schistosomiasis transmitting snails. This epidemiological scenario reinforces the complexity and urgency of discussing and proposing a close association between health, environment and social factors, since this region has all the necessary conditions for schistosomiasis transmission. In this context, additional malacological studies are needed to contribute to the monitoring of schistosomiasis vector snails, and to achieve a more comprehensive understanding of the types of freshwater mollusks in this region, and how these species will be affected by this major water engineering project. It is also worth emphasizing the strengthening of public health promotion and educational actions that advocate positive changes of risk factors to prevent the disease transmission and occurrence.

ACKNOWLEDGMENTS

The authors would like to thank the Schistosomiasis Reference Laboratory of Rene Rachou Institute - FIOCRUZ-MG (in particular to Amanda Domingues de Araujo for the technical support), the Health Secretariat of Aurora, Brejo Santo, Jaguaratama, Jaguaribara, Jati and Mauriti municipalities and the Health Secretariat of the State Government of Ceara for the technical and logistic support, the Ministry of Health of Brazil for the financial support - Health Unic System Research Program (Programa Pesquisa para o Sistema Unico de Saude - PPSUS) - Call notice 03/2012 - period of validity 2012-2014.

REFERENCES

1. Brasil. Ministério da Saúde. Situação epidemiológica. [cited 2018 July 10]. Available from: <http://portalmis.saude.gov.br/saude-de-a-z/esquistossomose/situacao-epidemiologica>
2. Katz N. Inquérito nacional de prevalência da esquistossomose mansoni e geo-helminthoses. Belo Horizonte: CPqRR; 2018. [cited 2018 Jun 1]. Available from: www.cpqrr.fiocruz.br/pg/wp-content/uploads/2018/04/INPEG.pdf
3. Teixeira JC, Oliveira GS, Viali AM, Muniz SS. Estudo do impacto das deficiências de saneamento básico sobre a saúde pública no Brasil no período de 2001 a 2009. *Eng Sanit Ambient.* 2014;19:87-96.
4. Van den Broeck F, Maes GE, Larmuseau MH, Rollinson D, Sy I, Faye D, et al. Reconstructing colonization dynamics of the human parasite *Schistosoma mansoni* following anthropogenic environmental changes in Northwest Senegal. *PLoS Negl Trop Dis.* 2015;9:e0003998.
5. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis.* 2006;6:411-25.
6. Brasil. Ministério da Integração Nacional. Projeto de Integração do Rio São Francisco com Bacias Hidrográficas do Nordeste Setentrional : Relatório de Impacto Ambiental - RIMA. Brasília: Ministério da Integração Nacional; 2004. [cited 2017 Nov 25]. Available from: <http://www.integracao.gov.br/documents/10157/3678963/Rima++Relat%C3%B3rio+de+Impacto+Ambiental.pdf/4324863d-cbff-4522-9bd0-eab9d34b8fe2>
7. Brasil. Ministério da Integração Nacional. Projeto de Integração do Rio São Francisco: entenda os detalhes. [cited 2017 Oct 10]. Available from: <http://www.integracao.gov.br/web/projeto-sao-francisco/entenda-os-detalhes>
8. Silva J M, Gurgel IG, Santos MO, Gurgel AM, Augusto LG, Costa AM. Environmental conflicts and the waters of the São Francisco river. *Saude Soc.* 2015;24:1208-16.
9. Kloos H, Correa-Oliveira R, Reis DC, Rodrigues EW, Monteiro LA, Gazzinelli A. The role of population movement in the epidemiology and control of schistosomiasis in Brazil: a preliminary typology of population movement. *Mem Inst Oswaldo Cruz.* 2010;105:578-86.
10. Coura JR, Amaral RS. Epidemiological and control aspects of schistosomiasis in Brazilian endemic areas. *Mem Inst Oswaldo Cruz.* 2004;99(Suppl 1):13-9.
11. Ceará. Secretaria da Saúde. Boletim epidemiológico esquistossomose, 13 de outubro de 2016. [cited 2018 Jul 6]. Available from: <http://www.saude.ce.gov.br/index.php/boletins>
12. Martins-Melo FR, Pinheiro MC, Ramos Jr AN, Alencar CH, Bezerra FS, Heukelbach J. Spatiotemporal patterns of schistosomiasis-related Deaths, Brazil, 2000-2011. *Emerg Infect Dis.* 2015;21:1820-3.
13. Martins-Melo FR, Pinheiro MC, Ramos Jr AN, Alencar CH, Bezerra FS, Heukelbach J. Trends in schistosomiasis-related mortality in Brazil, 2000-2011. *Int J Parasitol.* 2014;44:1055-62.
14. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Vigilância

- e controle de moluscos de importância epidemiológica: diretrizes técnicas: Programa de Vigilância e Controle da Esquistossomose (PCE). 2ª ed. Brasília: Ministério da Saúde; 2008.
15. Barbosa FS, organizador. Tópicos em malacologia médica. Rio de Janeiro: Fiocruz; 1995.
 16. Sturm CF, Pearce TA, Valdés A, editors. The mollusks: a guide to their study, collection, and preservation. Los Angeles: American Malacological Society; 2006.
 17. Paraense WL. Estado atual da sistemática dos planorbídeos brasileiros. *Arq Mus Nac.* 1975;55:105-111.
 18. Paraense WL. *Biomphalaria kuhniana* (Clessin, 1883), planorbid mollusc from South America. *Mem Inst Oswaldo Cruz.* 1988;83:1-12.
 19. Caldeira RL, Teodoro TM, Jannotti-Passos LK, Lira-Moreira PM, Goveia CO, Carvalho OS. Characterization of South American snails of the genus *Biomphalaria* (Basommatophora: Planorbidae) and *Schistosoma mansoni* (Platyhelminthes: Trematoda) in molluscs by PCR-RFLP. *Biomed Res Int.* 2016;2016:1045391.
 20. Abílio FJ, Fonseca-Gessner AA, Leite RL, Ruffo TL. Gastrópodes e outros invertebrados do sedimento e associados à macrófita *Eichhornia crassipes* de um açude hipertrófico do semi-árido paraibano. *BioTerra.* 2006;1 Supl:165-78.
 21. Tuan R. Distribuição e diversidade de espécies do gênero *Biomphalaria* em microrregiões localizadas no Médio Parapanema, São Paulo, SP, Brasil. *Biota Neotrop.* 2009;9:279-83.
 22. Barbosa CS, Silva CB, Barbosa FS. Esquistossomose: reprodução e expansão da endemia no Estado de Pernambuco no Brasil. *Rev Saude Publica.* 1996;30:609-16.
 23. Teodoro TM, Jannotti-Passos LK, Carvalho OS, Grijalva MJ, Baús EG, Caldeira RL. Hybridism between *Biomphalaria cousini* and *Biomphalaria amazonica* and its susceptibility to *Schistosoma mansoni*. *Mem Inst Oswaldo Cruz.* 2011;106:851-5.
 24. Guimarães RJ, Freitas CC, Dutra LV, Felqueiras CA, Moura AC, Amaral RS, et al. Spatial distribution of *Biomphalaria* mollusks at Sao Francisco River Basin, Minas Gerais, Brazil, using geostatistical procedures. *Acta Trop.* 2009;109:181-6.
 25. Fernandez MA, Mattos AC, Silva EF, Santos SB, Thiengo SC. A malacological survey in the Manso Power Plant, State of Mato Grosso, Brazil: new records of freshwater snails, including transmitters of schistosomiasis and exotic species. *Rev Soc Bras Med Trop.* 2014;47:498-506.
 26. Rezende HR, Sessa PA, Ferreira AL, Santos CB, Leite GR, Falqueto A. Efeitos da implantação da Usina Hidrelétrica de Rosal, Rio Itabapoana, Estados do Espírito Santo e Rio de Janeiro, sobre anofelinos, planorbídeos e flebotomíneos. *Rev Soc Bras Med Trop.* 2009;42:160-4.
 27. Thiengo SC, Santos SB, Fernandez MA. Malacofauna límnic da área de influência do lago da usina hidrelétrica de Serra da Mesa, Goiás, Brasil.: I. Estudo qualitativo. *Rev Bras Zool.* 2005;22:867-74.
 28. Favre TC, Fernandez MA, Beck LC, Guimaraes RJ, Pieri OS, Thiengo SA. Assessment of schistosomiasis in the semi-arid Northeast region of Brazil: the São Francisco River large-scale water transposition project. *Rev Soc Bras Med Trop.* 2016;49:252-7.
 29. Silva Filho JD, Pinheiro MC, Sousa MS, Gomes VS, Castro IM, Ramos Jr AN, et al. Detection of schistosomiasis in an area directly affected by the São Francisco River large-scale water transposition project in the Northeast of Brazil. *Rev Soc Bras Med Trop.* 2017;50:658-65.
 30. Kloos H, Correa-Oliveira R, Quites HF, Souza MC, Gazzinelli A. Socioeconomic studies of schistosomiasis in Brazil: a review. *Acta Trop.* 2008;108:194-201.
 31. Enk MJ, Amaral GL, Costa e Silva MF, Silveira-Lemos D, Teixeira-Carvalho A, Martins-Filho OA, et al. Rural tourism: a risk factor for schistosomiasis transmission in Brazil. *Mem Inst Oswaldo Cruz.* 2010;105:537-40.
 32. Programa das Nações Unidas para o Desenvolvimento. O índice de desenvolvimento humano municipal brasileiro. 2ª ed. Brasília: PNUD, IPEA, FJP; 2013.
 33. Attwood SW, Huo GN, Qiu JW. Update on the distribution and phylogenetics of *Biomphalaria* (Gastropoda: Planorbidae) populations in Guangdong Province, China. *Acta Trop.* 2015;141:258-70.
 34. Guyard A, Pointier JP, Théron A, Gilles A. Mollusques hôtes intermédiaires de la Schistosomose intestinale dans les Petites Antilles: Hypothèses sur le rôle de *Biomphalaria glabrata* et *B. straminea* en Martinique. *Malacologia.* 1982;22:103-7.
 35. Caldeira RL, Jannotti-Passos LK, Carvalho OS. Molecular epidemiology of Brazilian *Biomphalaria*: A review of the identification of species and the detection of infected snails. *Acta Trop.* 2009;111:1-6.