

Mollusks of the genus *Biomphalaria* Preston, 1910 in Amazon Region: first report of *Biomphalaria occidentalis* Paraense, 1981 in Pará State, Brazil

Moluscos do gênero *Biomphalaria* Preston, 1910 na Região Amazônica: primeiro relato de *Biomphalaria occidentalis* Paraense, 1981 no estado do Pará, Brasil

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ABSTRACT

INTRODUCTION: In Pará State, Northern Region of Brazil, there are reports of the occurrence of *Biomphalaria glabrata*, *Biomphalaria straminea*, *Biomphalaria schrammi*, and *Biomphalaria kuhniana*. **OBJECTIVE:** To report the first existence of *Biomphalaria occidentalis* in Pará State. **MATERIALS AND METHODS:** Five mollusks were collected, examined for the presence of *Schistosoma mansoni* cercariae, and morphological and molecular taxonomies were performed. **RESULTS:** No specimens parasitized by trematode larvae were detected. *Biomphalaria occidentalis* was identified, being the first record in Pará State. **CONCLUSION:** The result obtained contributed to improve the knowledge about the dispersion and diversity of the *Biomphalaria* mollusks in Amazon Region.

Keywords: *Biomphalaria*; Mollusks; Polymerase Chain Reaction; Restriction Fragment Length Polymorphism; Schistosomiasis; Taxonomy.

RESUMO

INTRODUÇÃO: No estado do Pará, Região Norte do Brasil, há relatos da existência de *Biomphalaria glabrata*, *Biomphalaria straminea*, *Biomphalaria schrammi* e *Biomphalaria kuhniana*. **OBJETIVO:** Relatar a primeira ocorrência de *Biomphalaria occidentalis* no Pará. **MATERIAIS E MÉTODOS:** Cinco moluscos foram coletados, examinados quanto à presença de cercárias de *Schistosoma mansoni* e as taxonomias morfológica e molecular foram realizadas. **RESULTADOS:** Nenhum espécime parasitado por larva de trematódeo foi detectado. Foi identificada a espécie *Biomphalaria occidentalis*, sendo o primeiro registro no Pará. **CONCLUSÃO:** O resultado obtido contribuiu para melhorar o conhecimento sobre a dispersão e a diversidade de moluscos *Biomphalaria* na Região Amazônica.

Palavras-chave: *Biomphalaria*; Moluscos; Reação em Cadeia da Polimerase; Polimorfismo de Fragmento de Restrição; Esquistossomose; Taxonomia.

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INTRODUCTION

The genus *Biomphalaria* Preston, 1910 includes mollusks that may transmit *Schistosoma mansoni*, the etiological agent of schistosomiasis in Brazil. Among the 11 species and one subspecies of *Biomphalaria* genus that occur in the country, three are regarded as intermediate hosts of *S. mansoni*: *Biomphalaria glabrata* (Say, 1818), *Biomphalaria tenagophila* (d'Orbigny, 1835), and *Biomphalaria straminea* (Dunker, 1848). In Pará State, the species *B. glabrata*, *B. straminea*, *Biomphalaria schrammi* (Crosse, 1864), and *Biomphalaria kuhniiana* (Clessin, 1883) have been already reported¹.

The determination of species of the genus *Biomphalaria* is based on the comparison of morphological characters of shell, excretory system, and reproductive organs²; however, this can be difficult due to size of the specimens, inadequate fixation processes, and interspecific similarities³. These similarities even culminated in the grouping of some species of the genus *Biomphalaria* in two complexes: 1) *B. straminea* composed by *B. straminea*, *Biomphalaria intermedia* (Paraense & Deslandes, 1962), and *B. kuhniiana*; and 2) *B. tenagophila* containing *B. tenagophila*, *Biomphalaria tenagophila guibensis* Paraense, 1984, and *Biomphalaria occidentalis* Paraense, 1981^{4,5,6,7}. The species of *B. tenagophila* complex are indistinguishable from shell morphology and the majority of organs of the genital system. And only *B. tenagophila* is susceptible to infection with *S. mansoni*; therefore, the identification of these species is important for epidemiological studies of schistosomiasis⁷, and the knowledge of the geographical distribution of these species enable better resource distribution and adequate policies for the mollusk control⁸. The molecular taxonomy has been used as an auxiliary tool to morphology when it is not enough for the species identification.

The present work aimed to report the first occurrence of *B. occidentalis* in Pará State, Northern Region of Brazil.

MATERIALS AND METHODS

Malacological surveys were carried out in May 2017 in the municipality of Afuá, Mesorregião Marajó, in Pará State, latitude 00°09'20.1"S and longitude 50°23'02.9"W.

Five specimens were collected using tongs and nets at sewage ditches from the urban perimeter containing garbage, by the collection period of 30 min. No other species of mollusks or vegetation were observed in the sewage ditches during this time. The environment characteristics, where the specimens were collected, were favorable to the occurrence and dispersion of mollusks. A Garmin GPSMAP® 76CS Global Positioning System (GPS) receiver was used to record the geographic coordinates reading and all collection points were annotated and georeferenced.

The mollusks obtained were wrapped in gauze soaked in water, inserted in properly identified plastic bags, and stored in a rigid container in which they were transported

to Laboratory of Intestinal Parasites, Schistosomiasis, and Malacology (LPIEM) of Parasitology Section (SAPAR) of Instituto Evandro Chagas (IEC). Five specimens were measured and individually packed in glass containers with 20 mL of dechlorinated water, exposed to artificial light (60 W incandescent lamp) for 30 min, and then examined under a stereomicroscope to verify the presence of *S. mansoni* cercariae⁹. Subsequently, the mollusks were sacrificed, fixed^{10,11}, and the foot of each specimen removed for DNA extraction by modified Wizard Genomic DNA Purification Kit (Promega).

The mollusks were identified morphologically by comparison of characters of the shell and the male and female reproductive organs according to Paraense^{2,4,5}.

Molecular identification was performed by Laboratory of Helminthology and Medical Malacology, Instituto René Rachou/Fiocruz, using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) of the internal transcribed spacer (ITS) region of the RNA ribosomal genes, where the entire ITS was amplified with the primers ETTS2 (5' TAACAAGGTTTCCGTAGGTGAA 3') and ETTS1 (5' TGCTTAAGTTCAGCGGGT 3') anchored respectively in the conserved extremities of the 18S and 28S ribosomal genes¹². Subsequent was carried out cleavage of this fragment with the restriction enzymes DdeI and AluI^{7,13}.

RESULTS

All specimens that were examined for the presence of *S. mansoni* cercariae were negative for the parasite and for other trematodes larvae.

All mollusks were morphologically identified as *B. occidentalis* (Figure 1).

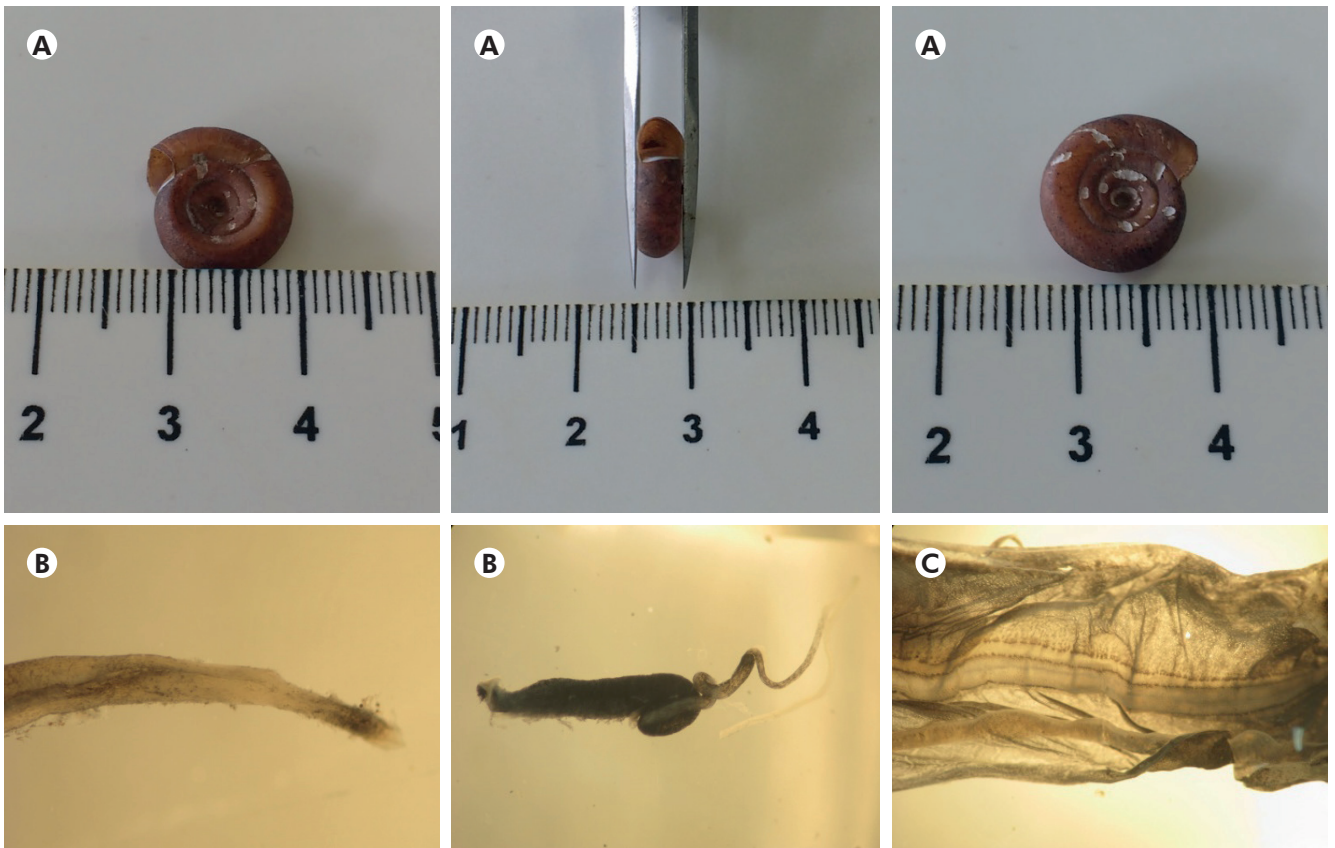
The molecular results showing restriction profiles obtained by digestion of the ITS region of DNA ribosomal with DdeI were compared to the pattern of DNA profiles obtained from the *Biomphalaria* and *Helisoma* mollusks tissue from the Medical Malacology Collection (Fiocruz/CMM) (Figure 2).

To differentiate the species, the amplicon, previously obtained of approximately 1,200 base pairs, was submitted to a new RFLP using the restriction enzyme AluI⁷, being possible to define the specie as *B. occidentalis* by molecular technique (Figure 3).

DISCUSSION

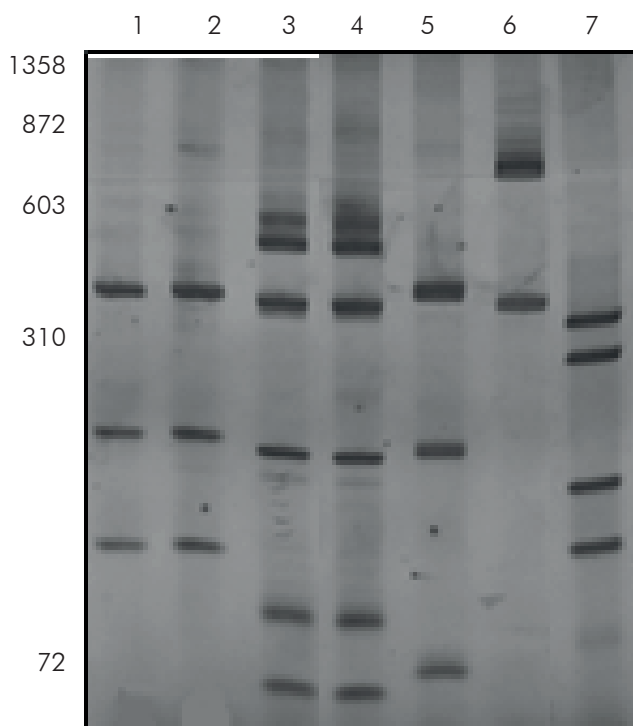
The geographic distribution of planorbid mollusks in Brazil is not well known due to the great territorial extension and the lack of human and economic resources; however, the number of studies aiming to better know the malacological fauna in some regions has increased^{14,15}.

The present study is the first report of *B. occidentalis* in Pará State. In Brazil, this species was already found in Acre, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Rio Grande do Sul, Rondônia, Santa Catarina, São Paulo States, and there are reports of shells in Amazonas too^{1,4,16}.



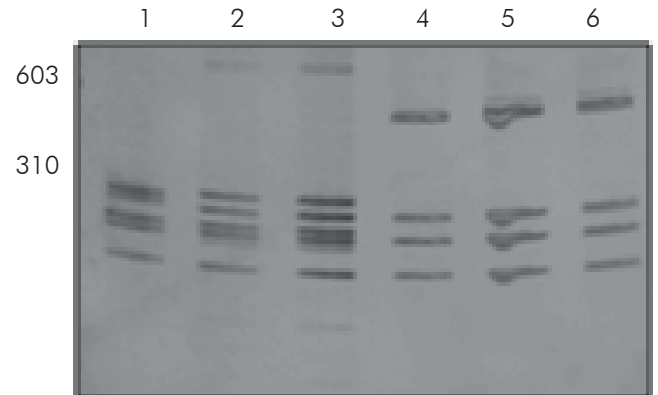
A: Shell; B: Reproductive system; C: Mantle with renal tube.

Figure 1 – *Biomphalaria* sp. from Afuá, Pará State, Brazil



Lane 1: *Biomphalaria* sp. from Afuá, Pará State; Lane 2: *B. occidentalis* from Belo Horizonte, Minas Gerais State; Lanes 3 and 4: *Helisoma* sp. from Três Lagoas, Mato Grosso do Sul State; Lane 5: *B. glabrata* from Belo Horizonte, Minas Gerais State; Lane 6: *B. tenagophila* from Sabará, Minas Gerais State; Lane 7: *Biomphalaria peregrina* from Coromandel, Minas Gerais State. The numbers on the left of the gel are the value in base pairs (bp) of the molecular size markers Phi X 174/HaeIII.

Figure 2 – Silver stained polyacrylamide gel (6%) showing restriction profiles obtained by digestion of the ITS region of DNA ribosomal with DdeI



Lane 1: *B. tenagophila* from Sabará, Minas Gerais State; Lanes 2 and 3: *B. t. guaibensis* from Santa Vitória do Palmar, Rio Grande do Sul State; Lane 4: *Biomphalaria* sp. from Afuá, Pará State; Lanes 5 and 6: *B. occidentalis* from Belo Horizonte, Minas Gerais State. The numbers on the left of the gel are the value in base pairs (bp) of the molecular size markers Phi X 174/HaeIII.

Figure 3 – Silver stained polyacrylamide gel (6%) showing restriction profiles obtained by digestion of the ITS region of DNA ribosomal with AluI

The freshwater mollusks *B. occidentalis*, *B. tenagophila*, and *B. t. guaibensis* are morphologically similar, grouped in the complex *B. tenagophila*. Despite the morphological similarity among the species of the complex, only *B. tenagophila* is susceptible to *S. mansoni*, and several studies reinforced *B. occidentalis* as refractory species^{17,18,19}; therefore the differentiation of these species is important to establish the vulnerable areas to the risk of occurrence of the schistosomiasis.

Studies of the planorbid fauna in all areas of Brazil, mainly in less investigated regions, should be motivated by the objective of knowing the geographic distribution of *Biomphalaria* mollusks aiming the better resource distribution and adequate surveillance for its control.

CONCLUSION

This study presents the first record of *B. occidentalis* in the municipality of Afuá, Pará State.

In addition, the knowledge about the dispersion and diversity of the *Biomphalaria* mollusks in Brazilian Amazon was expanded.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed to the study idealization, analysis and interpretation of data and manuscript writing, approving the published final. They declare themselves responsible for content of the article, ensuring its accuracy and integrity.

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