SHORT COMUNICATION

A Multiplex-PCR Approach to Identification of the Brazilian Intermediate Hosts of *Schistosoma mansoni*

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Due to difficulties concerning morphological identification of planorbid snails of the genus Biomphalaria, *and given a high variation of characters and in the organs with muscular tissue, we designed specific polymerase chain reaction (PCR) primers for Brazilian snail hosts of* Schistosoma mansoni *from available sequences of internal transcribed spacer 2 (ITS2) of the ribosomal RNA gene. From the previous sequencing of the ITS2 region, one primer was designed to anchor in the 5.8S conserved region and three other species-specific primers in the 28S region, flanking the ITS2 region. These four primers were simultaneously used in the same reaction (Multiplex-PCR), under high stringency conditions. Amplification of the ITS2 region of* Biomphalaria *snails produced distinct profiles (between 280 and 350 bp) for* B. glabrata, B. tenagophila and B. straminea*. The present study demonstrates that Multiplex-PCR of ITS2-DNAr showed to be a promising auxiliary tool for the morphological identification of* Biomphalaria *snails, the intermediate hosts of* S. mansoni.

Key words: *Biomphalaria* - Multiplex-PCR - internal transcribed spacer 2

Morphological identification of planorbids of the genus *Biomphalaria,* the intermediate hosts of *Schistosoma mansoni*, is based on a comparison of the characters of shell, genital and excretory systems described by Paraense (1975, 1981, 1984, 1988). However, this identification becomes difficult due to the high variation of such characters and in the organs with muscular tissue, because of its distension at the fixing moment (Paraense 1975). Paraense (1966) remarks that, due to the different environments occupied by some species, they show a high morphological intraspecific variation. Other species such as *B. straminea, B. kuhniana* and *B. intermedia* have very few morphological differences regarding the genital system, making their distinction difficult (Paraense 1988, Caldeira et al. 1998).

Relevant advancements have occurred in the field of molluskans systematics. A remarkable change was the introduction of molecular techniques. Molecular systematics may approach several problems considered insoluble by traditional methodologies. Vidigal et al. (1996), using low stringency polymerase chain reaction (LS-PCR) technique, were able to differentiate *B. glabrata* and *B. tenagophila*. Pires et al. (1997), using the same technique, differentiated *B. tenagophila* from *B. occidentalis.* Vidigal et al. (1998) used PCR and restriction fragment length polymorphism (PCR-RFLP) technique for the internal transcribed spacer (ITS) region (rDNA) analysis, which included ITS1 + 5.8S + ITS2, to differentiate these three Brazilian intermediate hosts species of *S. mansoni.* Caldeira et al. (1998) used the same methodology to differentiate *B. straminea*, *B. intermedia*, *B. kuhniana* and *B. peregrina* and Spatz et al. (1999) to distinguish *B. tenagophila*, *B. t. guaibensis* and *B. occidentalis*.

The Multiplex-PCR technique consists in using specific primers, simultaneously, under high stringency conditions. It has been used for the identification of several organisms such as fungal strains, free-living protozoon and some viruses (Fujita et al. 2001, Mosquera et al. 2002, Manguin et al. 2002, Pelandakis & Pernin 2002).

The present study was aimed at designing specific primers for Brazilian snail hosts of *S. mansoni* from available sequences of ITS2 of the ribosomal RNA gene, sequenced by Vidigal et al. (2000a), for phylogenetic studies on Brazilian *Biomphalaria*.

Studies were undertaken using snails, from different Brazilian localities, of the species *B. glabrata* (from Pará, Sergipe, Minas Gerias)*, B. tenagophila* (from Goiás, Minas Gerais, Espírito Santo) and *B. straminea* (from Piauí, Bahia, Rio Grande do Sul). The snails were killed and the foot of each specimen was removed for DNA extraction. Afterwards, they were morphologically identified, according to Paraense (1975, 1981, 1984, 1988). Total DNA was extracted from each snail's foot using the Wizard kit (Promega), as described by Vidigal et al. (2000b). From previous sequencing of the ITS2 region, one primer (ITS2f)

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was designed to anchor in the 5.8S conserved region and three other species-specific primers (BglITS2r, BtenITS2r and BstITS2r) anchored in the 28S region, flanking the ITS2 region. These four primers were simultaneously, under high stringency conditions (Multiplex-PCR). PCR amplification was undertaken in a volume of 10 µl, with 1ng template DNA, 5 pmol of each primer, 200 µM each dNTP (dNTP- PROMEGA), 0.8 U of *Taq* DNA polymerase, in a buffer solution of 10mM Tris-HCl, pH 8.5, 50 mM KCl, 1.5 mM MgCl. The reactions were covered with a drop of mineral oil and subjected to the following cycle program: initial denaturation step for 3 min at 95°C and then 24 cycles for annealing at 60°C for 1 min and extension at 72°C for 5 min. A negative control (no DNA) was included in all the experiments. PCR products were visualized in 6% silver stained polyacrylamide gels.

The amplification of the ITS2 region of *Biomphalaria* snails produced distinct fragments (between 280 and 350 bp): *B. glabrata* (280 bp), *B. tenagophila* (320 bp) and *B. straminea* (350 bp) (Figure) from the localities, mentioned above. Thus, the reproducibility of the obtained profiles was supported by use of *Biomphalaria* snails from distinct localities, in Brazil, and by increasing number of specimens (3) used (data not shown). The present study demonstrates that Multiplex-PCR of the ITS2-DNAr showed to be a promising auxiliary tool for the morphological identification of *Biomphalaria* snails, intermediate hosts of *S. mansoni*. This assay offers a rapid, simple and feasible identification method for these mollusks. It could be systematically applied as a diagnostic test in epidemiological studies, and afterwards in control measures against the expansion of schistosomiasis. We believe that this methodology may be reliably employed in the identification of these planorbids. In addition, it will be possible to assess the specificity of the designed primers for the ITS2 region, concerning other *Biomphalaria* species, especially *B. straminea* (Paraense 1988) and *B. tenagophila* (Spatz et al. 1999) complex.

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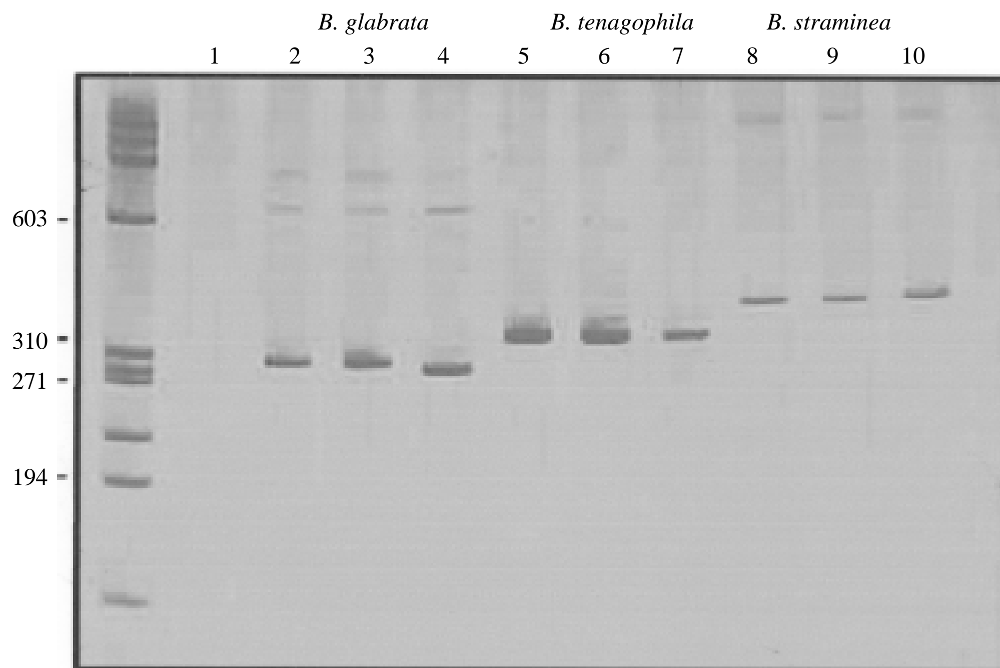

6% silver stained polyacrylamide gel showing the Multiplex-PCR amplification of the internal transcribed spacer 2 regions of the Brazilian intermediate hosts of *Schistosoma mansoni*. Lanes - 1: negative control (no DNA added); 2: *Biomphalaria glabrata* from Belém, Pará; 3: *B. glabrata* from, Aracajú, Sergipe; 4: *B. glabrata* from Sabará, Minas Gerais; 5: *B. tenagophila,* from Formosa, Goiás*;* 6: *B. tenagophila* from Vespasiano, Minas Gerais; 7: *B. tenagophila,* from, Vitória, Espírito Santo; 8: *B. straminea* from Picos, Piauí; 9: *B. straminea* from Várzea do Poço, Bahia*;* 10: *B. straminea* from Porto Alegre, Rio Grande do Sul; molecular size markers are shown on the left of the gel.

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