Biomphalaria molluscs (Gastropoda: Planorbidae) in Rio Grande do Sul, Brazil

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The present study was aimed at characterising Biomphalaria *species using both morphological and molecular (PCR-RFLP) approaches. The specimens were collected in 15 localities in 12 municipalities of the southern region of the state of Rio Grande do Sul, Brazil. The following species were found and identified:* Biomphalaria tenagophila guaibensis, Biomphalaria oligoza *and* Biomphalaria peregrina. *Specimens of the latter species were experimentally challenged with the LE* Schistosoma mansoni *strain, which showed to be refractory to infection*.

Key words: *Biomphalaria* sp - Southern Brazil - experimental infection

Freshwater snails belonging to the genus *Biomphalaria* are intermediate hosts of *Schistosoma mansoni*, the etiological agent of schistosomiasis. Among the *Biomphalaria* species that occur in Brazil, three are regarded as intermediate hosts of *S. mansoni*, namely, *Biomphalaria glabrata, Biomphalaria tenagophila* and *Biomphalaria straminea*. Investigations on experimental infection using *Biomphalaria peregrina* and *Biomphalaria amazonica* have shown that they are potential hosts for the trematode (Corrêa & Paraense 1971, Paraense & Corrêa 1973).

In Rio Grande do Sul (RS), Brazil, there have been reports on the occurrence of *B. tenagophila* (Paraense & Deslandes 1959), *B. straminea* (Cunha Neto 1972), *Biomphalaria oligoza* (Paraense 1974), *B. peregrina* (Paraense 1966), *Biomphalaria tenagophila guaibensis* (Paraense 1984) and *B. glabrata* (Carvalho et al. 1998). The presence of *S. mansoni* in the state has been associated with the occurrence of *B. glabrata* in water collections surrounding Rio dos Sinos, in the municipality of Esteio (Graeff-Teixeira et al. 1999).

Considering the fact that little is known on freshwater snail diversity in the Southern Region of Brazil, the aim of the present study was to identify the *Biomphalaria* species in this region and assess their susceptibility to *S. mansoni* infection, either as intermediate hosts and/or as potential hosts. Molluscs were collected in 15 localities of 12 municipalities of RS during 2005. The collection areas included: Arroio Grande, Bagé, Camaquã, Canguçu, Capão do Leão, Dom Pedrito, Jaguarão, Pelotas, Rio Grande, Rosário do Sul, Santa Vitória do Palmar and São Gabriel, between the 30-34° parallels and the 51-55° meridians. The molluscs collected were sent to our laboratory to obtain their F1 progeny. Morphological and molecular identification of *Biomphalaria* was undertaken according to Paraense (1975, 1981, 1984) and Vidigal et al. (2000), respectively.

Specimens of *B. peregrina* (São João Batista do Glória/MG), *B. oligoza* (Eldorado do Sul/RS), *B. t. guaibensis* (Esteio/RS) and *Biomphalaria occidentalis* [Belo Horizonte, Minas Gerais (MG)], previously identified by both morphological and molecular means, were included for comparison. The snails were identified as *B. oligoza, B. peregrina* and *B. t. guaibensis* both through their morphology and the use of molecular techniques (Fig. 1). Since the genetic profiles of *B. t. guaibensis* and *B. occidentalis* appeared to be similar when digested with the restriction enzyme *Dde*I, the amplified fragments that had been previously identified as *B. t. guaibensis* were then digested with *Alu*I since it allows differentiation between these species (Fig. 2). Table shows the field-collected snails, *B. oligoza*, *B. peregrina* and *B. t. guaibensis* in RS.

Groups of 50 F1- snails (4-6 mm) from two *B. peregrina* populations collected in the municipalities of Rio Grande and Dom Pedrito (Estrada do Meio) were individually challenged with 100 miracidia/mollusc of the LE *S. mansoni* strain. As an infection control, we included 50 *B. glabrata* specimens from Barreiro de Cima, Belo Horizonte (MG), infected with 20 miracidia /mollusc of the same strain, which had been kept in the Moluscário Lobato Paraense at Instituto de Pesquisas René-Rachou-Fiocruz (MG). The susceptibility levels in these populations were assessed by light exposure and squeezing (Souza & Lima 1997). All field-collected molluscs were determined to be *S. mansoni*-negative.

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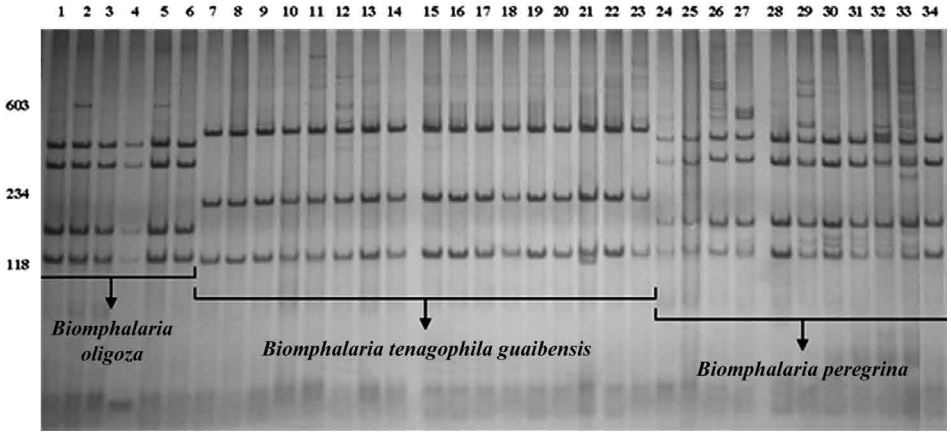

Fig. 1: 6% silver stained polyacrylamide gel showing the restriction profiles after digestion of the ITS region of ribosomal RNA with *Dde*I. Lane 1: *B. oligoza* [Eldorado do Sul, Rio Grande do Sul (RS)]; 2-3: Canguçu; 4-5: Capão do Leão (Fazenda Bela Vista); 6: São Gabriel; 7: *B. tenagophila guaibensis* [Esteio (RS)]; 8-9: Pelotas (Barragem SANEP); 10-11: Arroio Grande (Barragem Chasqueiro); 12-13: Santa Vitória do Palmar (Granja Figueira); 14-15: Dom Pedrito (Passinho do Amor); 16-17: Camaquã (Pacheca); 18-19: Santa Vitória do Palmar (Estância Ipiranga); 20-21: Jaguarão (Granja Bretanha); 22-23: Alegrete; 24: *B. peregrina* [São João Batista da Glória (MG)]; 25-26: Bagé; 27-28: Rio Grande; 29-30: Dom Pedrito (Chácara do Cedro); 31-32: Dom Pedrito (Estrada do Meio); 33-34: Rosário do Sul (Santa Ambrosina). Molecular size are on the left (X 174).

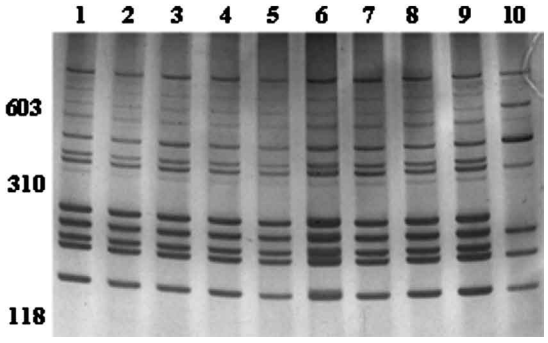

Fig. 2: 6% silver stained polyacrylamide gel showing the restriction profiles after digestion of the ITS region of ribosomal RNA with *Alu*I. Lanes 1-2: *Biomphalaria tenagophila guaibensis* (Esteio, Rio Grande do Sul); 3: *B. t. guaibensis* (Pelotas); 4: *B. t. guaibensis* (Arroio Grande); 5: *B. t. guaibensis* (Santa Vitória do Palmar/Granja Figueira); 6: *B. t. guaibensis* (Dom Pedrito/Passinho do Amor); 7: *B. t. guaibensis* (Camaquã); 8: *B. t. guaibensis* (Santa Vitória do Palmar/ Estância Ipiranga); 9: *B. t. guaibensis* (Jaguarão); 10: *Biomphalaria occidentalis* (Belo Horizonte, Minas Gerais). Molecular size are on the left (X 174).

Light exposure examinations of F1 progeny from two *B. peregrina* populations that had been challenged with *S. mansoni* miracidia showed no evidence of either cercariae or sporocysts. The mortality rate of the snails ranged from 0-16%.

The present investigation constitutes the first report on the occurrence of *B. t. guaibensis* in the municipalities of Camaquã, Dom Pedrito and Santa Vitória do Palmar. Although a survey by Teles et al. (1991) showed no evidence of such species in RS, Paraense (1984) had reported its occurrence in Arroio Grande, Jaguarão and Pelotas (RS).

B. oligoza is also being reported for the first time in Canguçu, Capão do Leão and São Gabriel and was previously reported by Paraense (1974), Fróes and Lima (1975) and Pereira et al. (2000) in the municipalities of Filipson, Gravataí, Guaíba, Lagoa Vermelha, Mariana Pimentel, Morro Reuter, Nova Petrópolis, Porto Alegre, Santo Antonio, Seival, Tramandaí, Triunfo, Tupanciretã and Viamão. In Bagé, Dom Pedrito, Rio Grande and Rosário do Sul, previous reports have described the presence of *B. peregrina* (Paraense 1966, Fróes & Lima 1975). In addition to these municipalities, the authors also reported the occurrence of *B. peregrina* in Camaquã, Jaguarão, Pelotas and São Gabriel. The results of our study, however, showed no occurrence of *B. peregrina*, which may be due to differences in the collection areas.

Early studies of shell morphology and the internal anatomy of planorbids were conducted to identify suitable traits for accurate and reliable species identification (Paraense 1972, 1975). In some *B. t. guaibensis* specimens from different populations than those analysed in our study, a dark pigmentation was observed on the renal tube, which had not been reported by Paraense (1984). Among the planorbid species described in Brazil, the only species known to have a pigmented renal tube is *B. glabrata*, the main *S. mansoni* transmitter species in Brazil (Paraense 1975). According to Paraense (1972), the specific trait found in *B. glabrata* and the main feature used to differentiate it from *B. tenagophila* is the pigmented crest that extends along the renal tube. This pigmentation found in *B. tenagophila guaibensis* might be due to either intraspecific variation or contact with staining substances present in the water collections (Paraense 1972).

Specific morphological characteristics described by Paraense (1966, 1974) were observed in the *B. peregrina* and *B. oligoza* populations analysed in our present

TABLE

Biomphalaria species identified through morphological and molecular techniques collected in municipalities in the state of Rio Grande do Sul

Municipalities	Origin of snails	species
Arroio Grande	Barragem Chasqueiro	*Biomphalaria tenagophila guaibensis*
Bagé	Bagé	*Biomphalaria peregrina*
Camaquã	Pacheca	*B. t. guaibensis*
Canguçu	Cabanha Cafundó	*Biomphalaria oligoza*
Capão do Leão	Fazenda Bela Vista	*B. oligoza*
Dom Pedrito	Passinho do Amor	*B. t. guaibensis*
Dom Pedrito	Chácara do Cedro	*B.* peregrina
Dom Pedrito	Estrada do Meio	*B. peregrina*
Jaguarão	Granja Bretanha	*B. t. guaibensis*
Pelotas	Barragem SANEP	*B. t. guaibensis*
Rio Grande	Rio Grande	*B. peregrina*
Rosário do Sul	Estância Santa Ambrosina	*B. peregrina*
Santa Vitória do Palmar	Estância Ipiranga	*B. t. guaibensis*
Santa Vitória do Palmar	Granja Figueira	*B. t. guaibensis*
São Gabriel	São Gabriel	*B. oligoza*

study. Such identification was corroborated by molecular analysis, which has been shown to be a useful tool for differentiating two morphologically similar species (Caldeira et al. 1998, Spatz et al. 2000, Vidigal et al. 2000). As both diagnostic methods are compatible, morphological traits should be used for routine identification, while molecular approaches should be employed in inconclusive cases.

The populations of *B. peregrina* used for the susceptibility tests (from Dom Pedrito and Rio Grande) showed no evidence of either *S. mansoni* cercariae or sporocysts. A similar result was obtained by Souza et al. (1988), who reported no *B. peregrina* infection in a mollusc population from Santa Rita do Sapucaí (MG), even when challenged with three different *S. mansoni* strains [From Belo Horizonte (MG), São José dos Campos (state of São Paulo) and state of Alagoas]. In addition, Paraense and Corrêa (1973) have reported similar resistance in a *B. peregrina* population collected in Pouso Alegre (MG), which is adjacent to the city of Santa Rita do Sapucaí (MG). However, *B. peregrina* populations from Lapa (state of Paraná) and from the Equator were found to be susceptible to *S. mansoni* infection, and they may be regarded as potential hosts for the trematode in these regions (Paraense & Corrêa 1973).

In the present study, by analysing snail populations susceptible to *S. mansoni* infection, we found that both *B. peregrina* populations, from Rio Grande and Dom Pedrito - Estrada do Meio, showed mortality rates ranging from 0-4.17%. These rates differ from those observed by Souza et al. (1988), who reported 0-20% mortality rates in the six populations they analysed, three of which showed a 12% mortality rate. These authors have also observed that the number of *S. mansoni* miracidia used for infection as well as the origins of the strains did not seem to affect the mortality indices.

The rates of infection and of mortality in the control group, *B. glabrata* from Barreiro de Cima, were 52% and 16%, respectively.

The susceptibility level of these different *Biomphalaria* species to infection by the same strain of *S. mansoni* varies greatly (Paraense & Corrêa 1973, 1978). Besides the difference in susceptibility observed between *Biomphalaria* species compatible with the parasite, some strain or geographical isolates of the same species of *Biomphalaria* also presented great variation in susceptibility to the parasite.

Given that *S. mansoni* is highly specific to its intermediate host, a survey of the areas where *Biomphalaria* occurs is useful, as it provides invaluable information to help design schistosomiasis control measures.

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