RESEARCH NOTE

Molecular Study of Similar *Biomphalaria* Species

Linus Spatz, Teofânia HDA Vidigal*/, Roberta L Caldeira*, Emmanuel Dias Neto***, Stella Maris González Cappa, Omar S Carvalho*/+**

Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Argentina *****Centro de Pesquisas René Rachou-Fiocruz, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte, MG, Brasil ******Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil *******Instituto Ludwig de Pesquisas sobre o Câncer, São Paulo, SP, Brasil

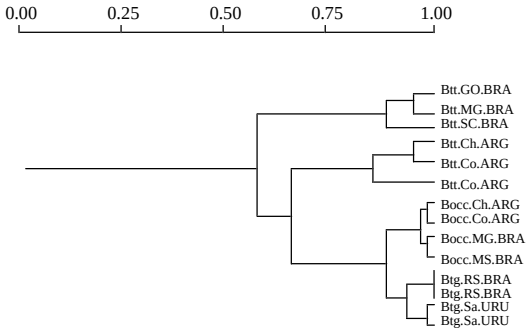
Key words: *Biomphalaria tenagophila tenagophila - Biomphalaria tenagophila guaibensis - Biomphalaria occidentalis* - snails - polymerase chain reaction - polymerase chain reaction restriction fragment length polymorphism - ribosomal RNA

Biomphalaria tenagophila tenagophila (Orbigny 1835) is a planorbid susceptible to infection with *Schistosoma mansoni* which occupies a wide range in South America (WL Paraense 1984 *Mem Inst Oswaldo Cruz 79*: 465-469) and is an important intermediate host in some areas of Brazil (WL Paraense & L Corrêa 1987 *Mem Inst Oswaldo Cruz 82*: 577). This species can not be differentiated from *B. occidentalis* or *B. tenagophila guaibensis* by shell characteristics nor morphology of most organs of the genital system (WL Paraense 1981 *Mem Inst Oswaldo Cruz 76*: 199-211, Paraense 1984 *loc.cit.*). However only *B. t. tenagophila* and *B. occidentalis* are separated by absolute reproductive isolation (Paraense 1981 *loc. cit.*). So far no reports have been published about reproductive isolation between *B. t. tenagophila* and *B. t. guaibensis* or between *B. t. guaibensis* and *B. occidentalis.*

Work partially supported by Fapemig, Capes, Fiocruz, Conicet and Fundación Roemmeres.
+Corresponding author. Fax: +55-31-295.3115. E-mail: omar@netra.cpqrr.fiocruz.br
Received 4 May 1998
Accepted 31 August 1998

The extensive intraspecific heterogeneity in the snails of the genus *Biomphalaria* at morphological (WL Paraense 1975 *Arq Mus Nac RJ 55*: 105-128) and genetic levels (THDA Vidigal et al. 1994 *Exp Parasitol 79*: 187-194), the similarity between species and the small size of some specimens difficult the correct identification of these snails. The PCR-RFLP method (polymerase chain reaction - restriction fragment length polymorphism) has been successfully employed to study genetic variation and identification of species of snails such as *Oncomelania hupensis* (M Hope & DP McManus 1994 *Acta Trop 57*: 75-82), *Bulinus* (JR Stothard et al. 1996 *Acta Trop 61*: 19-29, JR Stothard & D Rollinson 1997 *Trans R Soc Trop Med Hyg 91*: 353-357) and more recently by us in *Biomphalaria* (THDA Vidigal et al. 1998 *Exp Parasitol 89:* 180-187). Using this approach we analyze here possible sequence polymorphisms in the internal transcribed spacer region (ITS) of the rDNA by PCR amplification and restriction enzymes digestion. The entire ITS was amplified from similar species as *B. t. tenagophila*, *B. t. guaibensis* and *B. occidentalis* using the primers ETTS2 (5′-TAACAAGGTTTCCGTAGGTGAA-3′) and ETTS1 (5′-TGCTTAAGTTCAGCGGGT-3′) anchored respectively in the conserved extremities of the 18S and 28S ribosomal genes (RA Kane & D Rollinson 1994 *Mol Bioch Parasitol 63*: 153-156).

Studies were undertaken using several snail populations collected from different localities of Brazil, Argentina and Uruguay. Several enzymes were tested (*Alu*I, *Dde*I, *Hae*III, *Mnl*I, *Hpa*II, *Hfa*I, *Rsa*I). Digestion products were separated on 6 or 8% silver stained polyacrylamide gels (CJ Sanguinetti et al. 1994 *Biotech 17*: 915-918). Our results show that only the restriction profiles obtained after digestion with *Alu*I are capable of identifying the similar species *B. t. tenagophila*, *B. occidentalis* and *B. t. guaibensis*. *B. t. tenagophila* presented the most heterogeneous profile, showing some polymorphic bands when populations of Brazil and Argentina were compared. The profiles obtained with seven enzymes were used to estimate similarity between these species. A matrix of taxon/character was constructed on the basis of presence/absence of the 70 bands derived from the restriction profiles. The percentage of shared bands was then calculated using the Similarity Coefficient of Dice (LR Dice 1945 *Ecol 26*: 297-302). These data were then compared by means of UPGMA, unweighted pair group method analysis (PHA Sneath & RR Sokal 1962 *Nature 193*: 855-860) to generate a dendrogram. The analysis of this dendrogram (Figure) shows that *B. t. tenagophila* from Brazil and Argentina are clustering separately



UPGMA dendrogram of *Biomphalaria t. tenagophila* (Btt.), *B. occidentalis* (Bocc.), *B. t. guaibensis* (Btg.) constructed using the PCR-RFLPs profiles produced with seven enzymes. The numbers shown on top are indices of similarity. The letters refer to species and localities from which the snails originate. BRA (Brazil); ARG (Argentina); GO (State of Goiás, Brazil); MG (State of Minas Gerais, Brazil); SC (State of Santa Catarina, Brazil); Ch (Chaco Province, Argentina); Co (Corrientes Province, Argentina); MS (State of Mato Grosso do Sul, Brazil); RS (State of Rio Grande do Sul, Brazil); Sa (Salto, Uruguay). (Source: *J Moll studies* in press)

while the subspecies *B. t. guaibensis* seems to be more closely related with *B. occidentalis* than with *B. t. tenagophila*.

Considering geographical distribution, subspecies concepts (E Mayr et al. 1953 *Methods and Principles in Systematic Zoology*, McGraw Hill, New York, 328 pp.), similar biological aspects, morphological and molecular similarities between these species we suggest to cluster them into a *B. tenagophila* complex. However, reproductive isolation experiments should be performed in order to ilucidate this point. The present study shows that PCR-RFLP analysis of the ITS region is a promising approach to investigate the relationships between closely related species of *Biomphalaria*.

Acknowledgements: to Dr W Lobato Paraense and Dr Lygia Corrêa (Departamento de Malacologia, Instituto Oswaldo Cruz, Rio de Janeiro, RJ) who gave us the snails; to Dr David Rollinson (Natural History Museum, London, UK) for providing the oligonucleotide primers used in this study.