Larval trematodes in freshwater gastropods from Mato Grosso, Brazil: diversity and host-parasites relationships

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Abstract: A survey for freshwater gastropods carrying trematodes parasites was conducted in Manso Dam and the surrounding areas frequented by tourist, focusing particularly on the Pantanal region. Infected snails were recovered from twelve of the eighteen investigated municipalities and forty-one cercaria-snail pairings were recorded. Among these pairings were several first records of snails serving as intermediate hosts for trematodes in Brazil including *Biomphalaria amazônica* Paraense, 1966, *Biomphalaria occidentalis* Paraense, 1981, *Marisa planogyra* Pilsbry,1933, *Pomacea maculata* Perry, 1830, *Pomacea scalaris* (d'Orbigny, 1835) and *Gundlachia radiata* (Guilding, 1828). Echinostomatidae and Strigeidae were the most common trematode families (ca. 47%) and the greatest diversity of larvae were obtained from *Drepanotrema lucidum* (Pfeiffer, 1839). Paramphistomatidae, Schistosomatidae or Spirorchiidae and Notocotylidae or Pronocephalidae were recovered in *D. lucidum* for the first time extending the number of families which use this gastropod as intermediate host. Although no specimens were found harboring larval stages of *Schistosoma mansoni* Sambon, 1907 other trematode larvae were discovered, including the Schistosomatidae *Brevifurcate apharingeate cercaria* that can cause dermatitis in humans. Continued studies on the taxonomy and biology of trematodes are essential to better understand the biodiversity of these parasites as well as the epidemiological aspects for control of associated zoonosis.
Keywords: *epidemiology, cercariae, biological diversity, hydroelectric power plant, Pantanal.*

MATTOS, A.C., BOAVENTURA, M.F.F., FERNANDEZ, M.A. & THIENGO, S.C. **Larvas de trematódeos encontradas em gastrópodes límnicos provenientes de Mato Grosso, Brasil: diversidade e relações parasito hospedeiro.** Biota Neotrop. 13(4):http://www.biotaneotropica.org.br/v13n4/pt/abstract?article+bn00613042013

Resumo: Foi realizado um levantamento de trematódeos em gastrópodes límnicos provenientes da área da Usina Hidrelétrica de Manso e das proximidades com potencial turístico, como a região do Pantanal. Moluscos parasitados foram encontrados em doze dos dezoito municípios investigados e quarenta e uma interações cercária-gastrópode límnico foram observadas, entre elas *Biomphalaria amazônica* Paraense, 1966, *Biomphalaria occidentalis* Paraense, 1981, *Marisa planogyra* Pilsbry, 1933, *Pomacea maculata* Perry, 1830, *Pomacea scalaris* (d'Orbigny, 1835) and *Gundlachia radiata* (Guilding, 1828) atuando pela primeira vez no Brasil como hospedeiros intermediários de trematódeos. Echinostomatidae e Strigeidae foram as famílias de maior ocorrência (ca. 47%) e a maior variedade de tipos cercarianos foi encontrada em *Drepanotrema lucidum* (Pfeiffer, 1839). Paramphistomatidae, Schistosomatidae or Spirorchiidae and Notocotylidae or Pronocephalidae foram reportadas pela primeira vez em *D. lucidum* ampliando o número de famílias que utilizam esse gastrópode como hospedeiro intermediário. Embora nenhum espécime tenha sido encontrado com formas larvais de *Schistosoma mansoni* Sambon, 1907, outros trematódeos foram observados, como *Brevifurcate apharingeate cercaria* da família Schistosomatidae que pode causar dermatite no homem. Estudos sobre a taxonomia e a biologia de trematódeos são essenciais para melhor compreender a biodiversidade desses parasitos, assim como os aspectos epidemiológicos para o controle de parasitoses associadas.
Palavras-chave: *epidemiologia, cercárias, diversidade biológica, usina hidrelétrica, Pantanal.*

Introduction

Impoundments constitute one of the main human interventions in aquatic ecosystems, causing dramatic changes in the physical, chemical and biological structure and function of rivers (Albrecht et al. 2009). Environmental changes caused by dams interfere with water quality, through eutrophic effects and alter hydrologic patterns and ecological dynamics of the rivers and basins (Nogueira et al. 2005). According to Fundação Nacional de Saúde (the Brazilian National Health Foundation) and the Ministério da Saúde (Brazilian Ministry of Health), the construction of hydroelectric power facilities encourages human population growth and often migrants relocate to the newly dammed region from endemic schistosomiasis areas, searching for work and leisure activities (Fundação... 1995, Brasil 2007, Thiengo & Fernandez 2008). Furthermore, the modified environment generally produces favorable conditions for the reproduction of the *S. mansoni* snail hosts (Southgate 1997, Thiengo et al. 2005, Zheng et al. 2002). Several types of trematodes larvae were recovered from the snails collected in the area and identified. Flukes of the Class Trematoda have a significant medical and veterinary importance because all act as animal parasites. Additionally, invertebrates that live in other organisms may account for more than half of the species on the planet. These animals rarely are found outside of their hosts and may be underestimated (Lewinsohn et al. 2005). Studies on Trematoda in South America began in the XIX century by European helminthologists based on material collected in Brazil. In Brazil larval trematodes were first studied by Pirajá da Silva, who described the *Schistosoma mansoni* cercariae Sambon, 1907 (Cercaria blanchardi), the most important among trematode species because Schistosomiasis disease that is endemic in tropical and subtropical regions (Boaventura et al. 2002). Recently Kohn et al. (2007) reported 460 known and 75 undetermined species in South America. Dams create conditions suitable for the establishment of the mollusk vectors and also lead to increased permanent human settlement and tourism. Hence, justify further studies regarding larval helminthes in freshwater snails in those areas. To determine the distribution of freshwater snails carrying trematode larvae, particularly those acting as intermediate hosts of *Schistosoma mansoni* Sambon, 1907 vectors a qualitative study of freshwater mollusks was performed from 2002 to 2004 in the Aproveitamento Múltiplo de Manso (APM Manso) dam and also in municipalities of the Pantanal Mato-grossense, a singular wetland ecosystem near the dam, formed by the Paraguay river basin.

Material and Methods

The APM Manso is located at the Cuiabá river basin, state of Mato Grosso, and the lake is 7.30×10^9 m^3, covering an area of 427 km^2, between the municipalities of Chapada dos Guimarães and Nova Brasilândia. Freshwater snails collections were done in the reservoir, in 2002 (February, April, August, October and December), 2003 (every two months from February to December) and 2004 (February). Collections were also performed at other sites (streams, ponds, lakes, ditches, etc) in the surrounding municipalities as well as in the Pantanal Mato-grossense (Figure 1). Live snails were kept at the Laboratorio de Referencia Nacional em Malacologia Medica in aquaria containing dechlorinated tap water and at the bottom, a thin layer of a 2:1 mixture of screened soil and ground oyster shells as a source of mineral nutrients. In order to search for the larvae, snails were isolated in jars containing 4 mL filtered dechlorinated water. Jars with snails were maintained in 12:12 hr light:dark cycle for one day with exposure under an incandescent light (60 W) for 6 hours during the light cycle, with maintenance at ambient light for the remaining six, at five-day intervals to determine possible infection with trematode larvae, mainly *S. mansoni*. After, the mollusks were examined under stereoscopic microscope for larvae samples that contained cercariae were again exposed to the light:dark cycles, as described above, weekly until a sufficient number of larvae were obtained for morphological studies. Live cercarial stages recovered in this manner were studied under stereo microscope, either unstained or vitally stained with five percent neutral red. Those that were stained were fixed in 10% formalin following the protocol of Boaventura et al. (2002). Cercarial types and their possible familial affinities were identified according to Schell (1970). Snail specimens of each sample were killed following the protocol of Fernandez et al. (2008) and identified by anatomical dissection.

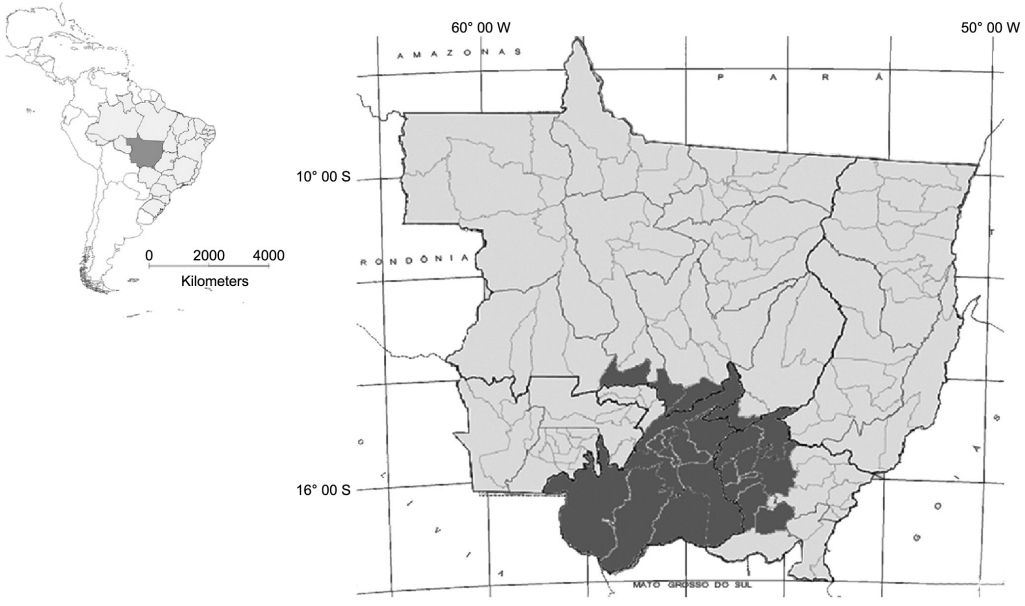

Figure 1. Map of South America, Brazil and Mato Grosso State, with investigated municipalities shaded, showing geographical location of the studied area.

Results

Ten morphologically distinct cercarial types were recovered from *Biomphalaria amazonica* Paraense, 1966, *Biomphalaria occidentalis* Paraense, 1981, *Biomphalaria schrammi* (Crosse, 1864), *Biomphalaria* sp., *Drepanotrema lucidum* (Pfeiffer, 1839), *Drepanotrema depressissimum* (Moricand, 1839), *Gundlachia radiata* (Guilding, 1828), *Physa marmorata* Guilding, 1828, *Marisa planogyra* Pilsbry, 1933, *Pomacea maculata* Perry, 1830, *Pomacea lineata* (Spix, 1827) and *Pomacea scalaris* (d'Orbigny, 1835) in twelve municipalities (Table 1). These cercarial types belong to 15 different trematode families (Table 2). Echinostomatidae and Strigeidae (Figures 2B, C) were the most common families (*ca*. 47%) and the greatest diversity of larvae was obtained from *D. lucidum* with six types. Trematode stages other than cercariae (*i.e.*cysts, rediae and metacercariae) were also observed in *B. amazonica*, *B. schrammi*, *D. lucidum*, *M. planogyra* and *P. marmorata*. Of the trematodes surveyed *Brevifurcate apharyngeate cercaria* (Figure 2A), a Schistosomatidae that can cause human dermatitis was found from *B. amazonica*, *D. lucidum* and *P. maculata*. The highest diversity of cercarial types, nine types, was found in the municipality of Poconé, in the Pantanal region.

Table 1. Trematode-freshwatwer snail interactions in Mato Grosso, Brazil.

Family	Intermediate host	Types of cercarie	Municipalities											
			Alto Paraguai	B. de Melgaço	Cáceres	C. Guimarães	Cuiabá	Jangada	Nobres	N. Brasilândia	NS. Livramento	Poconé	Pedra Preta	Rosário Oeste
Ampullariidae	*M. planogyra*	"Armatae cercaria"										x		
		"Echinostome cercaria"										x		
		"Strigea cercaria"										x		
		"Gymnocephalous cercaria"										x		
	P. figulina	"Ubiquita cercaria"					x							
	P. lineata	"Brev.-apharingeate cercaria"	x											
		"Ubiquita cercaria"					x					x		
	P. maculata	"Brev.-apharingeate cercaria"										x		
		"Ubiquita cercaria"									x			
	P. scalaris	"Echinostome cercaria"										x		
		"Strigea cercaria"										x		
		"Ubiquita cercaria"										x		
Ancylidae	*G. radiata*	"Strigea cercaria"			x	x						x		
	Died, not identified	"Brev.-pharingeate-clinostomatoide cercaria"										x		
Planorbidae	*B. amazonica*	"Brev.-apharingeate cercaria"							x					
		"Brev.-phar.-clinost. cercaria"		x					x					
		"Echinostome cercaria"		x		x								
		"Brev.-phar.-clinost. cercaria"		x										
	B. occidentalis	"Echinostome cercaria"										x		
		"Strigea cercaria"											x	
	Biomphalaria sp.	"Echinostome cercaria"				x								
	B. schrammi	"Echinostome cercaria"							x					
	D. depressissimum	"Ornatae cercaria"	x							x				

Table 2. Trematode larvae and possible definitive hosts in the Mato Grosso, Brazil.

TREMATODA		POSSIBLE DEFINITIVE HOST
Types of cercariae	Possible family(ies)	
"Amphistome cercaria"	Paramphistomatidae	Amphibious, birds and mammals
"Armatae cercaria"	Plagiorchiidae or Telorchiidae	Amphibious, birds fishes, mammals and reptiles.
"Brevifurcate-apharyngeate cercaria"	Schistosomatidae or Spirorchiidae	Birds, mammals and reptiles
"Brevifurcate-pharyngeate-clinostomatoid cercaria"	Clinostomatidae	Birds and reptiles
"Echinostome cercaria"	Echinostomatidae	Birds, mammals and reptiles
"Gymnocephalous cercaria"	Fasciolidae	Mammals
"Monostome cercaria"	Notocotylidae or Pronocephalidae	Birds, mammals and reptiles
"Ornatae cercaria"	Haplometridae or Macrodeiroididae	Amphibious and reptiles
"Strigea cercaria"	Diplostomatidae or Strigeidae	Birds and mammals
"Ubiquita cercaria"	Microphallidae	Birds, fishes and mammals

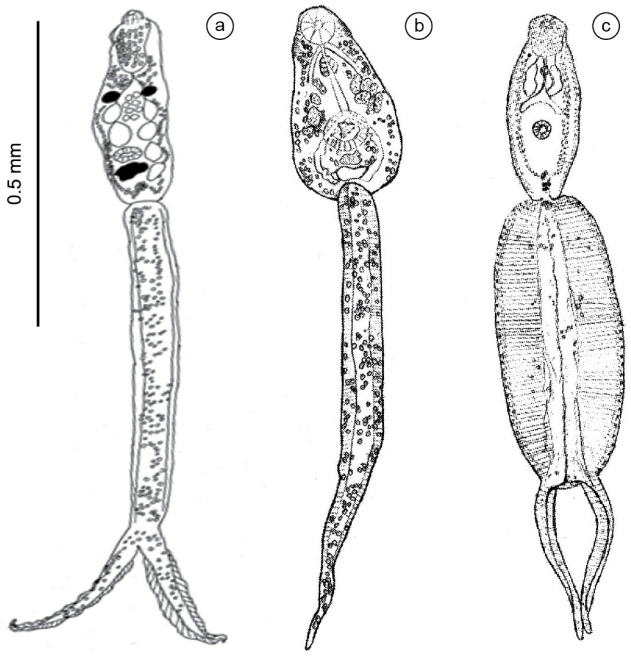

Figure 2. Schematic drawings of cercarial types of trematodes from Mato Grosso: a. Brevifurcate apharingeate cercaria; b. Echinostome cercaria; c. Strigea cercaria.

Discussion

Among the infected snails *B. amazonica, D. lucidum* and *P. maculata* should be detached because harbored *Brevifurcate apharyngeate cercaria* that can cause human dermatitis (Horák & Kolárová 2011, Bosnia et al. 1990, Hanning & Leedom 1978). Cases of human dermatitis in Brazil may be underestimated because some species of trematodes have been found in birds and intermediate hosts widely distributed throughout the country (Pinto et al. 2012). Although no specimens of freshwater snails were found harboring larval stages of *S. mansoni* other trematode larvae were recovered from twelve municipalities, mainly in the Pantanal, a wetland region often visited by tourists admiring its scenic beauty. The high incidence of Echinostomatidae and Strigeidae families, parasites of wild birds, is easily explained by the fact that approximately twenty-seven percent of Brazilian bird species occur in the Pantanal region (Vasconcelos et al. 2008). The relationship between the presence of definitive hosts and the cercarial diversity was also observed by Abdul-Salam & Al-Khedery (1992) in Kwait. Here we report, for the first time in Brazil, *B. amazonica, B. occidentalis, M. planogyra, P. maculata* and *P. scalaris* serving as intermediate hosts of trematodes. This study also extends the number of trematode families that use *D. lucidum* as intermediate host, since only Echinostomatidae, Strigeidae or Diplostomatidae, Macroderoididae or Haplometridae, and Xiphidiocercariae were previously observed in *D. lucidum*. Veitenheimer-Mendes & Almeida-Caon (1989), Veitenheimer-Mendes et al. **(**1995), Thiengo et al. (2001, 2002), Boaventura et al. (2002, 2007), Moraes et al. (2009), have reported numerous new trematode-freshwater snail relationships in Brazil, further reinforcing the important role of molluscs as intermediate hosts of trematodes, as well as their role in increasing the spread of zoonoses associated with these parasites. To accurately estimate the diversity of trematodes, it is necessary to survey their hosts. Such surveys also provides information on interactions between the species allowing a deeper understanding of the cascading effects from loss of species hosts (Lewinsohn et al. 2005). Studies on the morphology of trematode larvae are essential to better understand the taxonomy and epidemiological implications of these parasites. For effective control of their associated zoonoses and spread it is crucial that such studies be continued. Furthermore, studies with the aim of matching the adult parasite with the larval forms via molecular techniques (*i.e*. PCR, Sequencing) or those involving life cycle studies should be strongly encouraged, as they too will provide a wealth of information necessary to understanding these important parasites.

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