Rev. Inst. Med. Trop. Sao Paulo
52(6):323-327, November-December, 2010
doi: 10.1590/S0036-46652010000600007

Melanoides tuberculata AS INTERMEDIATE HOST OF *Philophthalmus gralli* IN BRAZIL

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SUMMARY

Melanoides tuberculata that naturally harbored trematode larvae were collected at the Pampulha dam, Belo Horizonte (Minas Gerais, Brazil), during malacological surveys conducted from 2006 to 2010. From 7,164 specimens of *M. tuberculata* collected, 25 (0.35%) were infected by cercariae, which have been morphologically characterized as belonging to the Megalurous group, genus *Philophthalmus*. Excysted metacercariae were used for successful experimental infection of *Gallus gallus domesticus,* and adult parasites recovered from the nictitating membranes of chickens were identified as *Philophthalmus gralli*. This is the first report of *P. gralli* in *M. tuberculata* in Brazil.

KEYWORDS: *Philophthalmus gralli*; *Melanoides tuberculata*; Eyefluke; Brazil; Snail intermediate host.

INTRODUCTION

Melanoides tuberculata (Müller, 1774), an exotic species of snail introduced in Brazil in the late 1960s[35], has been found in several Brazilian states[9]. Studies related to the interaction between *M. tuberculata* and some species of *Biomphalaria* Preston, 1910, which transmit *Schistosoma mansoni* Sambon, 1907 in the country have reported that endemic populations of planorbids coexists with these thiarids despite their displacement[12,13,32]. Other studies did not confirm the coexistence of planorbids with thiarids, but verified the significant decline or complete disappearance of *Biomphalaria* species after the introduction of thiarids[14,15]. However, the possible use of *M. tuberculata* as a biological control strategy against schistosomiasis must be considered with caution because of possible damage to the native fauna (as an alien species), and the potential of *M. tuberculata* to act as an intermediate host for parasites of medical and veterinary importance in Brazil are not fully understood. Some studies have already reported the finding of *M. tuberculata* that harbor Pleurolophocercous cercariae in Brazil[4,5,33]. Recently this cercarian type found in *M. tuberculata* from the state of Minas Gerais was identified as *Centrocestus formosanus* (Nishigori, 1924)[28].

The aim of this study is to report the natural infection of *M. tuberculata* by another type of trematode larvae in Brazil, cercariae of *Philophthalmus* Looss, 1899, which have been used for experimental life cycle studies allowing the morphological identification of the oriental eyefluke *P. gralli* Mathis and Leger, 1910.

MATERIALS AND METHODS

Mollusks were collected during 26 random malacological surveys (over minimum intervals of one month), conducted from 2006 to 2010 at Pampulha dam, an eutrophic artificial water body with an area of 260 hectares and a total water volume of 12 million m^3 located in the northern region of the city of Belo Horizonte, in the state of Minas Gerais, Brazil. The mollusks were obtained with a scoop net and long forceps, and were packed and transported to the laboratory, then placed individually in plastic receptacles containing 5 mL of tap water and left overnight at room temperature. The thiarids were examined with a stereomicroscope before and after artificial photostimulation. Emerged cercariae were studied under a light microscope with vital stains while alive (0.05% neutral red, 0.05% Nile blue, 0.05% alizarin red), or after being fixed in 10% formalin, stained with acetic carmine, cleared in beechwood creosote and mounted in Canada balsam, according to MELO (2008)[22]. To study intramolluscan parasitic stages, naturally infected snails were crushed between two glass plates and dissected under a stereoscopic microscope; the larvae found were collected and studied alive.

Young specimens of *Gallus gallus domesticus* (Linnaeus, 1758) (n = 5) were orally administered with twenty mechanically excysted metacercariae each. The chickens were sacrificed and necropsied at four weeks after infection, according to the local animal experimentation ethics committee (CETEA/UFMG). Ovigerous flukes were recovered from the nictitating membranes and conjunctival sacs of the chickens and were pressed between glass slides, fixed in cold 10% formalin and stained and mounted as described above.

Measurements of the developmental stages obtained were performed with a millimetered eyepiece. Ten larvae specimens and 13 adult parasites were measured. Drawings were made in camera lucida and photographic documentation was performed with a digital camera attached to a light microscope. The cercariae were identified and characterized according to

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previously published descriptions[7,10,30,31,34]. Adult parasites were identified with the aid of taxonomical keys and the earlier descriptions of several authors[1,6,8,11,19,21,24,25,29]. Measurements are given in micrometers (µm).

The specimens studied were deposited in the collection of the Department of Parasitology (DPIC), UFMG, under accession number 5926 a-m; e 5927.

RESULTS

In all, 7,164 specimens of *M. tuberculata* were collected and examined. A different type of cercaria emerged from 25 *M. tuberculata* specimens (0.35%) which was preliminarily characterized as belonging to the Megalurous group (Fig. 1a). Larvae tended to emerge during the morning, in small numbers, and were very active. They presented elongated body measurements of, on average, 535 (420-580) long by 128 (110-140) wide with a constriction at the level of the ventral sucker. The subterminal oral sucker is 58 (50-69) long by 55(49-65) wide and followed by a long prepharynx, a muscular pharynx and an esophagus bifurcating into two blind caeca that reach the posterior end of the body. The ventral sucker was equatorial, with a circumference averaging 68 (65-78) long by 75 (60-80) wide. The genital primordium was formed by two longitudinal cell masses dorsal to the ventral sucker. Numerous cystogenous cells were observed. The tail was simple and slender, averaging 434 (302-485) long by 54 (36-62) wide, with adhesive glands at the terminal region. These cercariae encysted rapidly at the bottom of the receptacle or on the shell of the mollusks. Metacercariae usually had a typical pyriform shape averaging 315 (300-380) long by 210 (184-236) wide (Fig. 1b).

During the dissection of naturally infected snails, mature and young rediae were found. Young rediae (Fig. 1c) were characterized by an elongated body that averaged 375 (334-457) long by 78 (68-96) wide and a muscular pharynx that averaged 53 (48-63) long by 45 (38-53) wide, with a tail-like process at the posterior end of the body. Mature rediae (Fig. 1d) were characterized by a sac-like elongated body that averaged 946 (653-1,203) long by 178 (155-206) wide. A muscular pharynx measured 73 (63-88) long by 74 (57-75) wide. Dark-colored intestinal caecum that were on average 397 (273-512) long by 50 (27-68) wide, extended up the half of the body. The birth pore was located on the anterior body, below the level of the pharynx. A pair of appendages and tail-like structures was present at the posterior region. Germ balls and cercariae were observed at different developmental stages. The morphological and biological characteristics of these larvae and of the intramolluscan stages make it possible to identify these Megalurous cercariae as belonging to the trematodes of the genus *Philophthalmus*.

In the experimental infection, 23 adult parasites were obtained from the nictitating membranes and conjunctival sacs (Fig. 2a) of all of the chickens, with a mean intensity of infection 7 (2-12) parasites. The recovered specimens (Fig. 2d) have an elongated body, that is an average of 3,610 (3,100-4,070) long by 1,020 (860-1,210) wide. The oral sucker subterminal is, on average, 330 (316-339) long by 401 (374-421) wide. The prepharynx is very small or absent. The muscular pharynx is located posterior to the oral sucker, and is on average 299 (279-316) long by 339 (326-358) wide. The ratio of the transverse diameter of the oral sucker to that of the pharynx is 1: 0.5-1.05 (1:0.86). The esophagus is small, bifurcating at the border of the ventral sucker. The ventral sucker (acetabulum) is located on the anterior third of body and is an average of

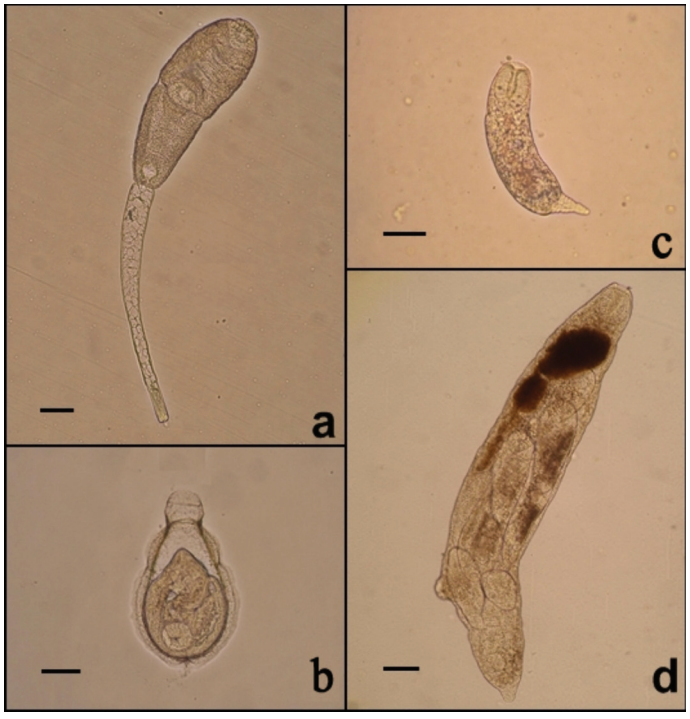

Fig. 1 - Developmental stages of *Philophthalmus gralli* obtained from naturally infected *Melanoides tuberculata* in Brazil. Cercaria (a), metacercaria (b), young redia (c) and adult redia (d). Scale bars = 100 µm.

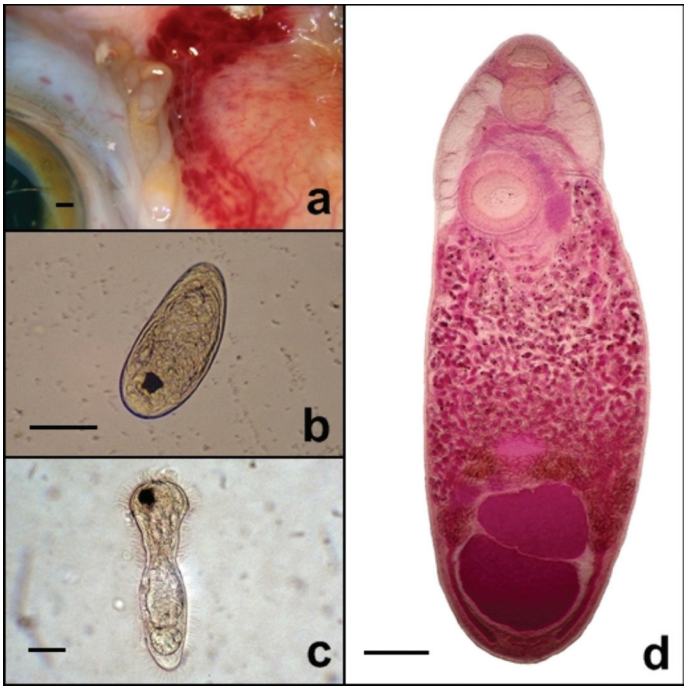

Fig. 2 - Developmental stages of *Philophthalmus gralli* obtained from chickens experimentally infected with larvae emerged from naturally infected *Melanoides tuberculata* from Brazil. These photos feature parasites in the conjunctival sac of a chicken (a), a mature egg (b), a miracidium (c), an adult parasite (d). Scale bars = 50 µm (b, c); 500 µm (a, d).

514 (479-542) long by 514 (479-542) wide. The ratio of the transverse diameter of the oral sucker to that of the acetabulum is 1: 1.22 (1:1.5). The cirrus pouch is elongated, averaging 758 (507-1,000) by 120 (86-464), with a seminal vesicle extending posteriorly to the ventral sucker. The testes are oval and are situated in tandem; the testes are located at the position post ovarian, intercecal, in the posterior part of body. The anterior testis measures, on average, 222 (179-263) long by 403 (279-453) wide. Posterior testis measures, on average, 216 (174-247) long by 351 (268-421) wide. The shape of the ovary varied from round to oval, and is situated medially in a pretesticular and post-uterine position. The ovary measures 237 (211-258) long by 266 (245-300) wide. The genital pore is medially located at the esophageal bifurcation. The location of the uterus is post-acetabular, pretesticular and intercecal (presenting mature eggs that contained miracidia with eyespots). The vitellaria are bilateral, extracecal, tubular, and extended 87% (73-97%) of the distance from the anterior testis to the ventral sucker. Mature eggs (Fig. 2b) are non-operculated and are located at the distal uterine coils. The mature eggs were an average size of 135 (120-145) long by 61 (53-65) when fresh and 73 (60-87) by 32 (27-36) after staining. The miracidium (Fig. 2c) is an average size of 131 (92-171) long by 53 (38-62) wide, and had a preformed redia stage. The main excretory ducts extended up the anterior part of the body. The excretory pore is terminal.

The morphometric data related to adult parasites obtained experimentally in the present study were compared with other neotropical records of *Philophthalmus* and the results are presented in the Table 1, represented as the amplitude followed by the mean in brackets (in micrometers) when available. The morphological and

Table 1

Measurements of *Philophthalmus gralli* obtained from chickens that were experimentally infected with trematodes larvae emerged from *Melanoides tuberculata* in Brazil, and compared with South American records of *Philophthalmus*. Morphometric data in micrometers are presented as amplitude followed by the mean in brackets.

		Philophthalmus gralli			*Philophthalmus lachrymosus*		*Philophthalmus semipalmatus*
		Present study	Muniz-Pereira and Amato, 1993	Díaz *et al.,* 2002	Freitas, 1955	Pinto *et al.*, 2005	Nasir and Díaz, 1972
Locality		Belo Horizonte, MG, Brazil	Maricá, RJ, Brazil	Aguasanta and Yaguaracal Venezuela	Manguinhos, RJ, Brazil	Foz do Iguaçu, PR, Brazil	Laguna del Peñon Venezuela
Host		*Gallus gallus domesticus*	*Anas bahamensis Amazoneta brasiliensis*	*Gallus gallus domesticus*	*Casmerodius albus*	*Hydrochaeris hydrochaeris*	*Catoptrophorus semipalmatus*
n		13	9	25	6	10	–
Body	L	3100-4070 (3610)	2120-3710 (2710)	2564-3384	4190-4620	3400-4250 (3730)	2624-4475
	W	860-1210 (1020)	604-1280 (834)	512-1205	1380-1640	850-1530 (1080)	960-1794
Oral sucker	L	316-339 (330)	204-329 (260)	237-297	300-310	220-290 (260)	216-363
	W	374-421 (401)	277-421 (332)	287-378	360-430	260-330 (300)	253-485
Ventral sucker	L	479-542 (514)	343-549 (418)	388-544	610-690	610-720 (670)	917
	W	479-542 (514)	343-494 (391)	409-505	–	630-770 (700)	958
OS/VS		1-1,22	1-1,1-1,3	1-1,3	1-2	1-2	1-2,75
Pharynx	L	279-316 (299)	183-293 (226)	227-323	310-350	190-210 (200)	225-333d
	W	326-358 (339)	183-403 (277)	227-333	380-460	90-150 (110)	–
Ovary	L	211-258 (237)	88-219 (153)	126-222	200-210	150-280 (190)	188-394d
	W	245-300 (266)	88-256 (181)	151-252	180-220	150-290 (210)	–
Anterior testis	L	179-263 (222)	161-416 (258)	272-378	360-480	140-270 (230)	188-297
	W	279-453 (403)	234-445 (329)	424-530	460-550	280-460 (370)	206-563
Posterior testis	L	174-247 (216)	161-438 (248)	272-464	380-480	150-360 (280)	188-454
	W	268-421 (351)	241-504 (319)	404-505	450-530	280-470 (360)	216-669
Eggs	L	60-87 (73)	64-90 (70)	74-80	94-97	90-110 (100)	60-69
	W	27-36 (32)	22-40 (33)	25-35	38-42	21-40 (30)	18-30
Type of vitellaria		tubular	tubular	tubular	follicular	follicular	follicular
Extension of vitellaria	%	73-97 (87)	79-89	–	–	71-90 (78.6)	–

d = diameter L = length; W = width

biological characteristics of the parasite reported here made possible the identification of the eyefluke *Philophthalmus gralli* Mathis and Leger, 1910.

DISCUSSION

Most of trematodes of the family Philophthalmidae Looss, 1899 are cosmopolitan eyeflukes of birds and mammals, and have already been reported to infect human beings[20,26]. In Brazil, two species of *Philophthalmus* have been reported only in vertebrate hosts. *Philophthalmus lachrymosus* Braun, 1902 was described in Rio de Janeiro (RJ, Brazil) in the brown-hooded gull, *Larus maculipennis* (Lichtenstein, 1823). The species has also been found in the great egret, *Casmerodius albus egretta* (Gmelin, 1789) from the same state[11], as well as the capybara, *Hydrochaeris hydrochaeris* Linnaeus, 1766 in Foz do Iguaçu (PR, Brazil)[29]. Another species, *Philophthalmus gralli* Mathis and Leger, 1910 was initially described in *Gallus* from Asia, and has also been registered in the white-cheeked pintail, *Anas bahamensis* Linnaeus, 1758 and in the Brazilian teal, *Amazonetta brasiliensis* Boetticher, 1929 in Maricá (RJ, Brazil)[24]. More recently, *P. gralli* have been found in ostriches, *Struthio camelus* Linnaeus, 1758 in Caratinga (MG, Brazil)[36]. Despite these reports, mollusks naturally infected by *Philophthalmus* have still not been reported in Brazil.

Originally described in *G. gallus domesticus* in Vietnam, the biological cycle of *P. gralli* has primarily been elucidated in North America, where the developmental stages and the participation of thiarids mollusks in its transmission have been described[1,2,3,6,37]. Since then, several studies have confirmed the participation of *M. tuberculata* as an intermediate host of *P. gralli* in different countries, such as the USA[27], Jordan[17,18], Mexico[31], the United Arab Emirates[16], Saudi Arabia[19], Venezuela[8] and Zimbabwe[23]. The biological and morphological characteristics of *P. gralli* reported here are in accordance with those described by these authors, differing from *P. lachrymosus* and *P. semipalmatus* (Nasir and Díaz, 1972) mainly by their smaller ventral sucker, the type (tubular), and the longer length of vitellaria.

In Brazil, the possible involvement of *M. tuberculata* in the transmission of *Philophthalmus* has been suggested[24,29,36], however, its occurrence has not been previously verified in nature. In the present study, the participation of *M. tuberculata* in the biological cycle of *P. gralli* in Brazil is confirmed, but the natural definitive host of *P. gralli* in Pampulha dam remains unknown. Given that *M. tuberculata* is widespread in Brazil and participates in the life cycle of *Philophthalmus* in the country, the impact of introducing and spreading these thiarids around the country must be better evaluated, with an aim toward preventing future cases of philophthalmiasis.

RESUMO

Melanoides tuberculata como hospedeiro intermediário de *Philophthalmus gralli* no Brasil

Melanoides tuberculata naturalmente infectados por larvas de trematódeos foram coletados na represa da Pampulha Belo Horizonte, Minas Gerais, Brasil durante estudos malacológicos realizados entre 2006 e 2010. De 7164 exemplares de *M. tuberculata* coletados, 25 (0,35%) apresentavam-se infectados por cercárias que foram caracterizadas morfologicamente como pertencentes ao grupo Megalura, gênero *Philophthalmus*. Metacercárias desencistadas foram utilizadas com sucesso para a infecção experimental de *Gallus gallus domesticus* e parasitos adultos recuperados da membrana nictitante das aves foram identificados como *Philophthalmus gralli*. Este é o primeiro relato de *P. gralli* em *M. tuberculata* no Brasil.

ACKNOWLEDGMENTS

To Mr Airton Lobo for technical assistance and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support and scholarship to Hudson Alves Pinto.

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Received: 26 July 2010
Accepted: 6 October 2010