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Original Article

First study on the helminth community structure of the neotropical marsupial *Metachirus myosuros* (Didelphimorphia, Didelphidae)

Primeiro estudo sobre a estrutura da comunidade de helmintos do marsupial neotropical *Metachirus myosuros* (Didelphimorphia, Didelphidae)

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Abstract

Metachirus myosuros is a marsupial species widely distributed in South America. Despite this, there is a lack of knowledge about its helminth parasites and helminth community structure. The aims of this study were to describe the species composition and determine the parasitological parameters of helminth communities of *M. myosuros* in preserved areas of the Atlantic Forest, Igrapiúna, Bahia state, northeastern Brazil. Parasites were searched from 19 specimens of this marsupial (18 were infected with at least one species), counted and identified. Ten species of helminth parasites were obtained: 7 nematodes, 2 platyhelminths and 1 acanthocephalan. The most abundant species were *Aspidodera raillieti*, *Cruzia tentaculata*, *Physaloptera mirandai* and *Viannaia conspicua* (Nematoda). These species were also the only dominant ones in the component community. Male hosts had higher prevalence of *P. mirandai* and greater abundance of *V. conspicua*. We observed a relationship between host body size and helminth abundance in both male and female hosts, and between host body size and helminth species richness in female hosts. This was the first study to analyze the helminth fauna and helminth community structure of *M. myosuros*. This was the first report of occurrences of *A. raillieti* and *Didelphonema longispiculata* in *M. myosuros*.

Keywords: Atlantic Forest, Brazil, Nematoda, parasite ecology, parasitism.

Resumo

Metachirus myosuros é uma espécie de marsupial amplamente distribuída na América do Sul. Apesar disso, existe pouca informação sobre seus helmintos parasitos e estrutura da comunidade de helmintos. Os objetivos deste estudo foram descrever a composição e determinar os parâmetros parasitológicos em comunidades de helmintos de *M. myosuros,* em áreas preservadas de Mata Atlântica, Igrapiúna, Bahia, Nordeste do Brasil. Os parasitos foram procurados em 19 espécimes desse marsupial (18 estavam infectados com pelo menos uma espécie), contados e identificados. Dez espécies de helmintos foram coletadas: 7 nematoides, 2 platelmintos e 1 acantocéfalo. As espécies mais abundantes foram *Aspidodera raillieti*, *Cruzia tentaculata*, *Physaloptera mirandai* e *Viannaia conspicua* (Nematoda). Estas espécies também foram as únicas dominantes na comunidade componente. Os hospedeiros machos tiveram maior prevalência de *P. mirandai* e maior abundância de *V. conspicua*. Foi observada uma relação significativa entre o tamanho do corpo do hospedeiro e a abundância de helmintos em hospedeiros machos e fêmeas, e entre o tamanho do corpo do hospedeiro e a riqueza de espécies de helmintos em fêmeas. Este foi o primeiro estudo a analisar a helmintofauna e a estrutura da comunidade de helmintos de *M. myosuros*. E também foi o primeiro relato da ocorrência de *A. raillieti* e *Didelphonema longispiculata* em *M. myosuros*.

Palavras-chave: Mata Atlântica, Brasil, Nematoda, ecologia de parasitos, parasitismo.

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Introduction

Parasitism plays a key role in the ecosystems because parasites can interfere in processes such as competition, migration, speciation and host reproduction, and can also affect biodiversity (Combes, 2001; Hudson, 2005). Moreover, biotic factors (such as the host gender, age, home range and species richness) and abiotic factors (such as seasonality) may alter the spatial and temporal distribution of a parasite species, thus influencing their abundance and prevalence rates (Combes, 2001).

In Brazil, 62 species of marsupials have been registered (Faria et al., 2019), but their helminth parasites are scarcely known. Most of the studies have consisted of reports on helminth species records (Gomes et al., 2003; Thatcher, 2006; Cardia et al., 2016) and taxonomic descriptions (Torres et al., 2007; Adnet et al., 2009; Araújo, 2011; Chagas-Moutinho et al., 2007; Chero et al., 2017). In addition, only a few studies have been conducted on the helminth community structure of Neotropical marsupials (Quintão e Silva & Costa, 1999; Antunes, 2005; Jiménez et al., 2011; Zabott et al., 2017; Costa-Neto et al., 2019; Ramírez-Cañas et al., 2019).

The brown four-eyed opossum *Metachirus myosuros* (Temminck, 1824) (Didelphimorphia, Didelphidae) is a strictly terrestrial marsupial species (Cunha & Vieira, 2002; Crouzeilles et al., 2010). This species has wide distribution in South America, extending from Honduras, in Central America, to northeastern Argentina (Brown, 2004). In Brazil, *M. myosuros* occurs in the Amazon, Atlantic Forest, Pantanal and Cerrado biomes (Melo & Sponchiado, 2012).

Metachirus myosuros may act as a wild reservoir for parasites and may contribute to the transmission of certain zoonoses. It has been reported to be naturally infected by *Trypanosoma cruzi* Chagas, 1909, the etiological agent of Chagas disease (Marcili et al., 2009), and by *Leishmania amazonensis* Lainson & Shaw, 1972, the etiological agent of American cutaneous leishmaniasis (Basano & Camargo, 2004) in Brazil.

In relation to helminths, all studies published on this host have been reports of species occurrence or species descriptions (Travassos, 1913; Travassos, 1920; Travassos, 1922; Freitas, 1937; Lent & Freitas, 1937; Proença, 1937; Sarmiento, 1954; Vicente, 1966; Travassos et al., 1969; Grisi & Castro, 1974; Gomes, 1979a, b; Noronha et al., 2002; Thatcher, 2006; Tantaleán et al., 2010; Barros, 2015; Lopes-Torres et al., 2019). No studies on the helminth fauna or its community structure in this marsupial have so far been published. The aims of the present study were to describe the species composition and analyze the helminth community structure of *M. myosuros* at infracommunity level (within an individual host) and component community level (set of the infracommunities of a given host population), in preserved areas of the Atlantic Forest in Igrapiúna, southern Bahia, Brazil. We also tested whether host gender and age were determinant factors for parasitism in this marsupial.

Materials and Methods

Study area

This study formed part of a comprehensive survey project on the biodiversity of the Atlantic Forest fauna and its parasites. It was conducted in preserved areas of the Pratigi Environmental Protection Area (13° 51’ S; 39° 16’ W) in the municipality of Igrapiúna, southern Bahia. The landscape of this area is formed by valleys and plains within the landholding of the Juliana Valley United Farms (Fazendas Reunidas Vale do Juliana). This comprises a set of agroforestry systems (including areas of rubber, cocoa, clove and peach palm production) and areas of dense ombrophilous forest that form patches linked by ecological corridors (OCT, 2019). The climate of this region is classified as wet equatorial (Af), according to the Köppen climate classification. The type Af is characterized by high temperatures, with high humidity and few seasonal variations, without any dry season (Alvares et al., 2013).

Sampling methods

The marsupials were collected using *Tomahawk*® live traps (16 × 5 × 5 inches), which were placed on the ground along six transects of 15 points. Captures were carried out during ten consecutive nights in August 2014 and again in March 2015. All the traps were baited with a mixture of peanut butter, banana, oatmeal and bacon.

The animals were identified by external morphology, weighed, measured, had their sex registered, and euthanised for helminth recovery. The animals were euthanised as follows. The animals were anesthetised with ketamine hydrochloride (100mg/mL) associated with xylazine hydrochloride (20mg/mL) in the proportion 1:1 with 0.1mL/100g dose. When the animal was completely anesthetised, 19.1% potassium chloride was applied intracardiac, using a 2 mL/kg body weight dose. All animals were preserved by taxidermy and deposited as voucher specimens in the Mammal Collection "Alexandre Rodrigues Ferreira" of the Santa Cruz State University (CMARF-UESC). The presence of helminths was investigated in the trachea, lungs, esophagus, heart, kidneys, liver, pancreas, spleen, small intestine, large intestine, cecum, mesenteric veins, reproductive apparatus and body cavities.

The helminths recovered were washed in physiological saline solution (0.85% NaCl). Nematodes were fixed in AFA solution (93 parts 70% ethanol, 5 parts 0.4% formalin and 2 parts 100% acetic acid) and were heated to 65 °C. The flatworms of the classes Trematoda and Cestoda were compressed in cold AFA. The specimens of acanthocephalans were kept in distilled water, fixed and compressed in cold AFA for protraction of the proboscis, as described by Amato et al. (1991). The nematodes were cleared with lactophenol and were placed between a slide and coverslip for identification under an optical microscope. The trematodes, cestodes and acanthocephalans were stained using Langeron's carmine, differentiated with 0.5% hydrochloric acid, dehydrated in an increasing alcohol series, cleared in methyl salicylate and mounted in Canada balsam as permanent preparations (Amato et al., 1991).

Specimens were counted using a stereoscopic microscope and were identified under an optical microscope (Zeiss Axio Scope A1) that was coupled to an Axio Cam MRc digital camera for photomicrography. The species were identified using morphological characteristics, as described by Vicente et al. (1997) and Anderson et al. (2009) for Nematoda; Travassos et al. (1969) for Trematoda; and Gomes (1979b) for Cestoda and Acanthocephala and other species descriptions. Voucher specimens were deposited at the Helminthological Collection of the Oswaldo Cruz Institute (Appendix 1).

The animals were captured under authorization from the Brazilian Government's Chico Mendes Institute for Biodiversity and Conservation (ICMBio; license number 17131-4) and from the Ethics Committee for Animal Use (CEUA) of the Oswaldo Cruz Foundation (license number LW-39/14) in collaboration with the Federal University of Paraíba (UFPB), Federal University of Rio de Janeiro (UFRJ) and State University of Santa Cruz (UESC). Biosecurity techniques and personal safety equipment were used during all procedures involving animal handling and biological sampling (Lemos & D'Andrea, 2014).

Data analysis

The abundance, intensity and prevalence of each helminth species were calculated according to Bush et al. (1997). The mean abundance was calculated by dividing the total number of parasites by the total number of hosts. For the mean intensity, the total number of parasites was divided by the number of infected hosts. Prevalence was calculated by dividing the number of hosts infected by the total number of hosts and multiplied by 100. The sex ratio of the most abundant helminth species was tested if it differed from 1: 1, using the χ^2 contingency test.

The mean species richness was calculated as the mean number of helminth species for each infracommunity, while the species richness was the total number of helminth species recovered. The estimated species richness was calculated using the nonparametric Jackknife 2 estimator (Magurram, 2004).

The parameters of the most prevalent and abundant species were compared in relation to host gender. The abundances were compared using the nonparametric Mann-Whitney test. The prevalences were compared using the χ^2 contingency test.

We also investigated the influence of host age, based on host body size, on the total abundance and species richness for each infracommunity, using linear regression separately for each host gender. Significance of the regression coefficient (beta) was evaluated using t test. Since body size is related to age, we hypothesized that older hosts would be more parasitized than younger ones.

The importance value, I, was calculated for each of the helminth species as described by Thul et al. (1985). From this, each species was then classified in the community as dominant ($I \geq 1.0$), co-dominant ($0.01 \leq I < 1.0$), subordinate ($I < 0.01$) or an unsuccessful pioneer ($I = 0$).

The analyses were done in accordance with Zar (1996), using the Past software, version 3.21 (Hammer et al., 2001). The data were tested for normal distribution using the Shapiro-Wilk test. In all analyses, the significant level was taken to be 5%.

Results

A total of 19 specimens of *M. myosuros* were captured (8 females and 11 males). Ninety-five percent of the hosts (18) were infected by at least one helminth species. A total of 3,299 helminths were recovered. The overall helminth species richness was 10 and the estimated species richness was 14.66. The mean helminth species richness was 4, and this ranged from zero to seven.

Seven nematode species were identified: *Aspidodera raillieti* Travassos, 1913 (Ascaridida, Aspidoderidae) and *Cruzia tentaculata* (Rudolphi, 1819) Travassos, 1917 (Ascaridida, Kathlaniidae) in the large intestine; *Physaloptera mirandai* Lent & Freitas, 1937 (Spirurida, Physalopteridae) in the stomach; and *Didelphonema longispiculata* (Hill, 1939) Wolfgang, 1953 (Spirurida, Spirocercidae), *Viannaia conspicua* Travassos, 1914 (Rhabditida, Viannaiidae), *Viannaia pusilla* Travassos, 1914 (Rhabditida, Viannaiidae) and three female specimens in the superfamily Trichostrongyloidea in the small intestine. These last specimens could not be identified at species level due to the absence of males and to the state of the material, which prevented visualization of internal structures. Two species of Platyhelminthes were identified: the digenean *Rhopalias coronatus* (Rudolphi, 1819) Stiles & Hassall, 1898 (Plagiorchiida, Rhopaliidae) and the cestode *Mathevotaenia bivittata* (Janicki, 1904) Akhumian, 1946 (Cyclophyllidea, Anoplocephalidae), both in the small intestine. One species of Acanthocephala was observed: *Oligacanthorhynchus microcephalus* (Rudolphi, 1819) Schmidt, 1972 (Archiacanthocephala, Oligacanthorhynchidae), also in the small intestine.

We recovered a total of 124 adult helminths of *P. mirandai*, 2,371 of *C. tentaculata*, 128 of *A. raillieti*, 464 of *V. conspicua* and 98 of *V. pusilla*. The nematodes *C. tentaculata* and *V. conspicua* were the most abundant species (Table 1). The prevalence was higher for the nematodes *A. raillieti*, *C. tentaculata*, *P. mirandai* and *V. conspicua* (Table 1). *Viannaia pusilla*, *R. coronatus* and the Trichostrongyloidea specimens were found only in male hosts (Table 1). The nematode *D. longispiculata* was found in a single male host in 2014 (Table 1).

The sex ratios of *A. raillieti* and *P. mirandai* did not significantly differ from 1:1 (Table 2). *Cruzia tentaculata*, *V. conspicua* and *V. pusilla* had significantly more females than males (Table 2).

The influence of host gender on the parameters found was investigated for the species *A. raillieti*, *C. tentaculata*, *P. mirandai* and *V. conspicua*. The nematodes *P. mirandai* and *V. conspicua* showed a statistically significant difference in prevalence in relation to host gender, with higher values for male hosts (Table 3). The abundance of *V. conspicua* was significantly higher in male hosts (Table 4).

A significant relationship was observed between helminth abundance and host body size for both sexes (males: regression coefficient (beta - slope) = 0.657, t = 2.617, $p = 0.028$, n = 11; females: beta = 0.707, t = 2.451, $p = 0.050$, n = 8). However, for helminth species richness, the relationship with host body size was clear only for females (males: beta = -0.189, t = -0.189, $p = 0.578$, n = 11; females: beta = 0.797, t = 3.237, $p = 0.018$, n = 8).

The nematodes were dominant in the component community of *M. myosuros* with the exception of *D. longispiculata* and *V. pusilla*, which were co-dominant and Trichostrongyloidea specimens, which were subordinate (Table 5). The cestodes were co-dominant and the trematodes were subordinate (Table 5). The acanthocephalans were co-dominant (Table 5).

Discussion

Metachirus myosuros is recorded as a new host for the nematodes *A. raillieti* and *D. longispiculata*. This study also provides the first record of the species *A. raillieti*, *C. tentaculata*, *D. longispiculata*, *V. conspicua*, *V. pusilla*, *M. bivittata*, *R. coronatus* and *O. microcephalus* in the state of Bahia, Brazil. Previous studies on helminths in *M. myosuros* were based on new taxonomic descriptions and new records of species occurrence (Table 6) without reports of abundance or prevalence indices. Twenty-four helminth species had previously been described infecting this marsupial (Table 6), including species in three phyla. Among the nine helminth species identified in the present study, seven had previously been reported infecting *M. myosuros*.

Before the present study, *A. raillieti* had been reported parasitizing *Didelphis aurita* (Wied-Neuwied, 1826), *Caluromys lanatus* (Olfers, 1818), *Didelphis marsupialis* Linnaeus, 1758, *Chironectes minimus* (Zimmermann, 1780), *Philander opossum* (Linnaeus, 1758) and *Nectomys squamipes* (Brants, 1827) in Brazil (Vicente et al., 1997). Quintão e Silva & Costa (1999) found *A. raillieti* in *Didelphis albiventris* Lund, 1840, also in Brazil. The only species of this genus previously found parasitizing *M. myosuros* was *Aspidodera subulata* (Molin, 1860) Railliet & Henry, 1912, which was

Table 1. Mean intensity and abundance (± SD), and prevalence rates (with 95% confidence interval) in relation to host gender for the helminth species found in *Metachirus myosuros* (N = 19) in Igrapiúna, state of Bahia, Brazil.

Parameters	*Aspidodera raillieti*	*Cruzia tentaculata*	*Physaloptera mirandai*	*Viannaia conspicua*	*Viannaia pusilla*
Total Mean Intensity	9.14 ± 7.89	139.47 ± 197.35	8.27 ± 12.88	29.00 ± 28.65	24.50 ± 10.15
Male	8.13 ± 8.39	181.91 ± 232.76	8.73 ± 15.03	36.27 ± 31.21	24.50 ± 10.15
Female	10.50 ± 7.71	61.67 ± 71.11	7.00 ± 4.24	13.00 ± 13.32	0
Total Mean Abundance	6.74 ± 7.88	124.79 ± 191.19	6.53 ± 11.87	24.42 ± 28.32	5.16 ± 11.07
Male	5.91 ± 7.98	181.91 ± 232.76	8.73 ± 15.03	36.27 ± 31.21	8.91 ± 13.55
Female	7.88 ± 8.13	46.25 ± 66.53	3.50 ± 4.66	8.13 ± 12.11	0
Total Prevalence	73.68 (73.57 - 73.80)	89.47 (86.72 - 92.22)	78.95 (78.78 - 79.12)	84.21 (83.80 - 84.62)	21.05 (20.89 - 21.21)
Male	72.73 (72.58 - 72.88)	100.00 (95.60 - 104.40)	100.00 (99.72 - 100.28)	100.00 (99.41 - 100.59)	36.36 (36.11 - 36.62)
Female	75.00 (74.82 - 75,18)	75.00 (73.52 - 76.48)	50.00 (49.90 - 50.10)	62.50 (62.23 - 62.77)	0
Parameters	***Didelphonema longispiculata***	**Trichostrongyloidea**	***Rhopalias coronatus***	***Mathevotaenia bivittata***	***Oligacanthorhynchus microcephalus***
Total Mean Intensity	9.00	3.00	1.00	19.20 ± 34.66	2.50 ± 2.12
Male	9.00	3.00	1.00	4.67 ± 3.21	4.00
Female	0	0	0	41.00 ± 56.57	1.00
Total Mean Abundance	0.47 ± 2.06	0.16 ± 0.69	0.05 ± 0.23	5.05 ± 18.50	0.26 ± 0.93
Male	0.82 ± 2.71	0.27 ± 0.90	0.09 ± 0.30	1.27 ± 2.61	0.36 ± 1.21
Female	0	0	0	10.30 ± 28.59	0.13 ± 0.35
Total Prevalence	5.26 (5.23 - 5.29)	5.26 (5.25 -5.27)	5.26 (5.26 -5.27)	26.32 (26.05 - 26.58)	10.53 (10.51 - 10.54)
Male	9.09 (9.04 - 9.14)	9.09 (9.07 - 9.11)	9.09 (9.09 - 9.10)	27.27 (27.22 - 27.32)	9.09 (9.07 - 9.11)
Female	0	0	0	25.00 (24.37 - 25.63)	12.50 (12.49 - 12.51)

Values without standard deviation indicates a single infected host.

Table 2. Chi-square and probability values for the sex ratio of the most abundant helminth species found in *Metachirus myosuros* in Igrapiúna, Bahia, Brazil.

Species	χ^2	*p*
Aspidodera raillieti	0.21	0.17
Cruzia tentaculata	0.0001	1.32^{-13}*
Physaloptera mirandai	0.25	0.20
Viannaia conspicua	0.0001	4.63^{-06}*
Viannaia pusilla	0.0024	0.001*

* Significant values.

Table 3. Chi-square and probability values for comparison of prevalences between host genders, for the most abundant helminth species in *Metachirus myosuros* in Igrapiúna, Bahia, Brazil.

Species	χ^2	*p*
Aspidodera raillieti	1	0.91
Cruzia tentaculata	0.17	0.08
Physaloptera mirandai	0.01	0.008*
Viannaia conspicua	0.06	0.03*

* Significant values.

Table 4. Results from the Mann-Whitney U test for comparison of helminth abundances between host genders, for *Metachirus myosuros* in Igrapiúna, Bahia, Brazil.

Species	*U*	*p*
Aspidodera raillieti	38.5	0.67
Cruzia tentaculata	24	0.11
Physaloptera mirandai	28	0.19
Viannaia conspicua	13	0.01*

* Significant values.

Table 5. Importance indices for each helminth species found in *Metachirus myosuros* in Igrapiúna, state of Bahia, Brazil.

Helminths species	Importance Indices	Classification
Aspidodera raillieti	3.428	Dominant
Cruzia tentaculata	77.101	Dominant
Didelphonema longispiculata	0.017	Co-dominant
Physaloptera mirandai	3.558	Dominant
Viannaia conspicua	14.201	Dominant
Viannaia pusilla	0.750	Co-dominant
Trichostrongyloidea	0.006	Subordinate
Mathevotaenia bivittata	0.918	Co-dominant
Rhopalias coronatus	0.002	Subordinate
Oligacanthorhynchus microcephalus	0.019	Co-dominant

Table 6. List of records of helminth species found in *Metachirus myosuros*, with locality, infection site and data sources.

Helminth	Locality	Site	References
Nematoda			
Aspidodera sp.	Peru	Large intestine	Tantaleán et al. (2010)
Aspidodera raillieti	Igrapiúna, BA, Brazil	Large intestine	Present study
Aspidodera subulata	Brazil	Large intestine	Travassos (1913)
	Brazil		Proença (1937)
	Brazil		Vicente (1966)
	Brazil		Thatcher (2006)
Capillaria sp.	Angra dos Reis, RJ, Brazil	Small intestine	Noronha et al. (2002)
Cruzia tentaculata	South America	Large intestine	Travassos (1922)
	Brazil		Thatcher (2006)
	Peru		Tantaleán et al. (2010)
	Igrapiúna, BA, Brazil		Present study
Didelphonema longispiculata	Igrapiúna, BA, Brazil	Small intestine	Present study
Dracunculus sp.	Bom Retiro, RJ, Brazil	Under the skin	Noronha et al. (2002)
Physaloptera mirandai	Angra dos Reis, RJ, Brazil	Stomach	Lent & Freitas (1937); Tantaleán et al. (2010); Barros (2015)
	Peru		Lopes-Torres et al. (2019)
	Acre and Bahia, Brazil		Present study
	Espírito Santo, Brazil		
	Igrapiúna, BA, Brazil		
Physaloptera sp.	Peru	Stomach	Tantaleán et al. (2010)
Travassostrongylus orloffi	Angra dos Reis, RJ, Brazil	Small intestine	Freitas (1937)
	Brazil		Thatcher (2006)
Travassostrongylus quatuor	Angra dos Reis, RJ, Brazil	Small intestine	Freitas (1937)
Travassostrongylus quintus	Angra dos Reis, RJ, Brazil	Small intestine	Freitas (1937)
Travassostrongylus sextus	Angra dos Reis, RJ, Brazil	Small intestine	Freitas (1937)
Travassostrongylus tertius	Angra dos Reis, RJ, Brazil	Small intestine	Freitas (1937)
	Rio de Janeiro, Brazil		Thatcher (2006)
Turgida turgida	Brazil	Stomach	Travassos (1920)
	Brazil		Travassos et al. (1969)
Viannaia conspicua	Angra dos Reis, RJ, Brazil	Small intestine	Freitas (1937)
	Igrapiúna, BA, Brazil		Present study
Viannaia pusilla	Angra dos Reis, RJ, Brazil Rio de Janeiro, Brazil	Small intestine	Freitas (1937)
	Igrapiúna, BA, Brazil		Thatcher (2006)
			Present study

Table 6. Continued...

Helminth	Locality	Site	References
Viannaia sp.	Angra dos Reis, RJ, Brazil	Small intestine	Noronha et al. (2002)
	Peru		Tantaleán et al. (2010)
Platyhelminthes			
Class Trematoda			
Duboisiella proloba	Huánuco, Peru	Caecum	Chero et al. (2017)
		intestine	
Lyperosomum silvai	Santa Maria Madalena, RJ, Brazil	Bile ducts	Grisi & Castro (1974)
			Gomes (1979a)
Plagiorchis didelphidis	Huánuco, Peru	Caecum, small intestine	Chero et al. (2017)
Podospathalium pedatum	Brazil	Intestine	Travassos et al. (1969);
	Brazil		Thatcher (2006)
Rhopalias baculifer	Huánuco, Peru	Small intestine	Chero et al. (2017)
Rhopalias coronatus	Brazil	Small intestine	Travassos et al. (1969)
	Angra dos Reis, RJ, Brazil		Gomes (1979a)
	Huánuco, Peru		Chero et al. (2017)
	Igrapiúna, BA, Brazil		Present study
Rhopalias horridus	Brazil	Intestine	Travassos et al. (1969)
Class Cestoda			
Linstowia jheringi	Brazil	Small intestine	Thatcher (2006)
Mathevotaenia bivittata	Angra dos Reis, RJ, Brazil	Small intestine	Gomes (1979b)
	Brazil		Thatcher (2006)
	Igrapiúna, BA, Brazil		Present study
Pritchardia boliviensis	Bolivia	Small intestine	Gardner et al. (2013)
Acanthocephala			
Gigantorhynchus ortizi	La Merced, Peru	Intestine	Sarmiento (1954)
	Peru, Colombia and Brazil		Thatcher (2006)
	Peru		Tantaleán et al. (2010)
Oligacanthorhynchus microcephalus	Angra dos Reis, RJ, Brazil	Small intestine, subcutaneous tissue and cysts in peritoneal cavity	Gomes (1979b)
	Igrapiúna, BA, Brazil		Present study

reported by Travassos (1913), Proença (1937), Vicente (1966) and Thatcher (2006). *Didelphonema longispiculata* was reported by Wolfgang (1953) in *Didelphis marsupialis* in North America and the Lesser Antilles.

Cruzia tentaculata was reported parasitizing *M. myosuros* by Travassos (1922) and Thatcher (2006) in Brazil and by Tantaleán et al. (2010) in Peru. *Viannaia conspicua* and *V. pusilla* were found in this marsupial by Freitas (1937) in Angra dos Reis, state of Rio de Janeiro, Brazil, and the latter was also reported by Thatcher (2006) in Brazil. Lent & Freitas (1937) reported *P. mirandai* in *M. myosuros* in Angra dos Reis, Brazil; Barros (2015) found this species in the states of Acre and Bahia, Lopes-Torres et al. (2019) in the state of Espírito Santo, Brazil, and Tantaleán et al. (2010) in Peru.

From the phylum Platyhelminthes, the trematode *R. coronatus* was reported in *M. myosuros* by Travassos et al. (1969) in Brazil, Gomes (1979a) in Angra dos Reis, Brazil, and Chero et al. (2017) in Huánuco, Peru. The cestode

M. bivittata was also reported for this marsupial by Gomes (1979b) in Angra dos Reis, Brazil. The same studies also reported *O. microcephalus* in *M. myosuros* (Gomes, 1979b).

Cruzia tentaculata, *V. conspicua* and *V. pusilla* were found in the present study to have sex ratios biased toward females, which suggests that this may be an ecological reproduction strategy in these species. A larger number of females leads to higher numbers of eggs released, thus increasing the chances of host infection. This pattern is most common in species with a polygamous mating system (Poulin, 2007), but there is no information in the literature about the mating system of these species. Nonetheless, similar results were found by Antunes (2005), Castro et al. (2017) and Costa-Neto et al. (2019) for *C. tentaculata*.

In some studies, it was observed that male marsupials of other species presented behavior of a more exploratory nature: these included *Marmosa* (*Micoureus) demerarae* (Thomas, 1905) (Morais-Júnior & Chiarello, 2005) and *Didelphis aurita* (Wied-Neuwied, 1826) (Loretto & Vieira, 2005). Such behavior may increase the chances of parasitic infection. In addition, testosterone can decrease host immunity and this can also be correlated with higher occurrence of parasites in male hosts (Combes, 2001). This may explain the greater abundance of *V. conspicua* and higher prevalence of *P. mirandai* and *V. conspicua* in male hosts, as well as the occurrence of *V. pusilla*, *R. coronatus*, *D. longispiculata* and Trichostrongyloidea specimens only in male hosts.

The helminth abundance increased with increasing host body size, suggesting that older animals were infected with more parasites than younger ones. It can be expected that larger hosts may have a larger number of parasites as well as more parasite species (Guégan et al., 1992; Morand & Poulin, 1998; Combes, 2001). For helminth species richness, this relationship could be observed only for females.

The fact that the overall estimated richness (14.66) was higher than the observed results (10) may indicate that more species could be present in the helminth fauna if the sample size were to be increased, and that rare species may also be present in the helminth community. The presence of only two subordinate species among the ten species observed corroborates this idea. The dominance of the nematodes *A. raillieti*, *C. tentaculata*, *P. mirandai* and *V. conspicua* in the component community of *M. myosuros* may indicate that these species have characteristics that make them more tolerant to the external environment and to the specificities of the host-parasite interaction, in relation to the other helminths found. Except for *P. mirandai*, the other three species have been reported to be transmitted through egg ingestion (Jiménez et al., 2011; Taylor et al., 2017), which can facilitate transmission of the parasite. Other studies have also reported that *A. raillieti* and *C. tentaculata* are dominant species in other marsupial hosts. Quintão e Silva & Costa (1999), in the state of Minas Gerais, Brazil, and Antunes (2005) in the state of Rio Grande do Sul, Brazil, found high prevalence and high mean intensity rates for *C. tentaculata* and *A. raillieti* in *D. albiventris*. Jiménez et al. (2011) found *A. raillieti* to be a dominant species in component communities in *P. opossum* and *D. marsupialis* in French Guiana. Costa-Neto et al. (2019) found *C. tentaculata* and *A. raillieti* to be the most dominant species in *D. aurita* in three areas of the Atlantic Forest in Brazil. Ramírez-Cañas et al. (2019) found a high prevalence of *C. tentaculata* in *P. opossum* in Mexico.

This was a novel study on the helminth community structure of the marsupial *M. myosuros*, albeit preliminary. This study not only contributes new records of helminth species and helminth parasitological parameters, but also enables better understanding of the parasite community and parasitism in small mammals.

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Appendix 1. Voucher specimens deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC).

Species	CHIOC numbers
Aspidodera raillieti	38911
Cruzia tentaculata	38912
Didelphonema longispiculata	38913
Mathevotaenia bivittata	38909
Oligacanthorhynchus microcephalus	38908
Physaloptera mirandai	38914
Rhopalias coronatus	38910
Viannaia conspicua	38915
Viannaia pusilla	38916