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HELMINTHS OF SMALL MAMMALS IN AN ATLANTIC FOREST BIOLOGICAL STATION IN RIO DE JANEIRO, BRAZIL

HELMINTOS DE PEQUEÑOS MAMÍFEROS EN UNA ESTACIÓN BIOLÓGICA DEL BOSQUE ATLÁNTICO EN RÍO DE JANEIRO, BRASIL

HELMINTOS DE PEQUENOS MAMÍFEROS EM UMA ESTAÇÃO BIOLÓGICA DA MATA ATLÂNTICA NO RIO DE JANEIRO, BRASIL

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ABSTRACT

Interface areas between urban and sylvatic environments increase the contact between humans and wild animals, and may favour the transmission of zoonoses. The aim of this study was to describe the helminth fauna of a small mammal community in an urban-sylvatic interface area of the Brazilian Atlantic Forest.

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Twenty helminth species were recovered in six species of small mammals. Parasite sharing was observed in two helminth species among the marsupials. This study is the first report of a helminth infection for the marsupial *Monodelphis americana* (Müller, 1776). This is the first report of the nematodes *Aspidodera raillieti* Travassos, 1913, *Viannaia hamata* Travassos, 1914 and *Trichuris* sp. parasitizing the marsupial *Marmosa paraguayana* (Tate, 1931). None of the helminth species found has been reported to infect

Keywords: Marsupials – Prevalence – Rodents – Synanthropic animals – Urbanization – Wild animals

RESUMEN

Las áreas de interfaz entre ambientes urbanos y selváticos aumentan el contacto entre humanos y animales salvajes y pueden favorecer la transmisión de zoonosis. El objetivo de este estudio fue describir la helmintofauna de una comunidad de pequeños mamíferos en un área de interfaz urbano-selvática de la Mata Atlántica brasileña. Se recuperaron veinte especies de helmintos en seis especies de pequeños mamíferos. Se observó el intercambio de parásitos en dos especies de helmintos entre los marsupiales. Este estudio es el primer reporte de una infección por helmintos para el marsupial *Monodelphis americana* (Müller, 1776). Este es el primer reporte de los nematodos *Aspidodera raillieti* Travassos, 1913, *Viannaia hamata* Travassos, 1914 y *Trichuris* sp. parasitando al marsupial *Marmosa paraguayana* (Tate, 1931). No se ha informado que ninguna de las especies de helmintos encontradas infecte a los humanos.

Palabras clave: Animales sinantrópicos – Animales salvajes – Marsupiales – Prevalencia – Roedores – Urbanización

RESUMO

Áreas de interface entre ambientes urbanos e silvestres aumentam o contato entre os seres humanos e os animais silvestres, podendo favorecer a transmissão de zoonoses. O objetivo deste estudo foi descrever a helmintofauna de uma comunidade de pequenos mamíferos em uma área de interface urbano-silvestre da Mata Atlântica brasileira. Vinte espécies de helmintos foram recuperadas em seis espécies de pequenos mamíferos. Compartilhamento de parasitos foi observado em duas espécies de helmintos entre os marsupiais. Este estudo é o primeiro relato de infecção helmíntica pelo marsupial *Monodelphis americana* (Müller, 1776). Este é o primeiro relato dos nematoides *Aspidodera raillieti* Travassos, 1913, *Viannaia hamata* Travassos, 1914 e *Trichuris* sp. parasitando o marsupial *Marmosa paraguayana* (Tate, 1931). Nenhuma das espécies de helmintos encontrada já foi relatada infectando seres humanos.

Palavras-chave: animais sinantrópicos – Animais silvestres – marsupiais – prevalência – roedores – urbanização

INTRODUCTION

The continuous process of urbanization and expansion of human activities in forested areas disrupts natural habitats and may change the patterns of species diversity and abundance. In disturbed ecosystems, generalist species, such as the common opossum *Didelphis aurita* (Wied-Neuwied, 1826), or opportunistic species, such as some sigmodontine rodents, are favoured in relation to specialist species (Gentile *et al.*, 2018). They may have their densities increased and may become abundant in rural and peridomicile areas (D'Andrea *et al*., 2007; Kajin *et al*., 2008). The occurrence of synanthropic species, such as the common rat *Rattus norvegicus* Berkenhout, 1769, and the black rat *Rattus rattus* (Linnaeus, 1758), is also observed in these areas. Such changes in the demography and distribution of natural populations, especially in the areas of the interface between urban and rural or urban and forest environments, directly influence the transmission dynamics of parasites (Kruse *et al.*, 2004). These interface areas may also harbour invasive species. The presence of invasive species and their parasites may affect the local communities and the host-parasite relationships (Lucio *et al.*, 2021).

The occurrence of human infection by helminth parasites from wild animals has been attributed to the increase of interface areas between urban or rural areas with forest fragments or reserves, where species of reservoir animals occur. Knowledge of the helminth species that parasitize wild mammals in these areas is essential for understanding their role as an etiological and/or zoonotic agent and, consequently, the potential risk to public and animal health (Bezerra-Santos *et al*., 2020), due to the continuous process of urbanization and human advance on forest areas.

There are several studies of helminth faunas of small mammals in the Brazilian Atlantic Forest (Gomes *et al*., 2003; Maldonado *et al*., 2006; Cardoso *et al*., 2016; Boullosa *et al*., 2020; Lucio *et al*., 2021). However, there is a large gap concerning helminth studies in forested reserves close to urban centres (Costa-Neto *et al*., 2019; Boullosa *et al*., 2021). The present study is complementary to Costa-Neto *et al*. (2019), which studied the structure of the helminth metacommunity of the common opossum *Didelphis aurita* in three localities, including the locality of the present study. The aim of the present study was to describe the helminth fauna of a small mammal community and the helminth population parameters in an urban-sylvatic interface area of the Atlantic Forest in Brazil.

MATERIAL AND METHODS

Study area
This study is part of a comprehensive project on biodiversity and environmental health aiming to understand the role of mammals in the transmission cycles of parasites and zoonotic agents at the FIOCRUZ Atlantic Forest Biological Station (EFMA) (Estação Biológica FIOCRUZ Mata Atlântica) and its surroundings, including areas of Pedra Branca State Park (PEPB) (Parque Estadual da Pedra Branca), in the city of Rio de Janeiro, southeast Brazil. EFMA is located on the eastern slope of the Pedra Branca massif in the western zone of the city of Rio de Janeiro, which is the second most populous city in Brazil. This area was previously occupied by sugarcane mills and coffee farms, with the construction of residences and roads. In 2003, the area was incorporated into the Fundação Oswaldo Cruz (FIOCRUZ). From then on, the master plan of the institution delimited nonedification areas and established a more effective process of environmental protection and recovery aiming to mitigate the effects of anthropic impacts. The area is formed by a mosaic of landscapes on the border of the forest reserve, including sites with vegetation in an advanced stage of ecological succession, transition sites with intermediate and initial stages of reforestation, and sites with consolidated urbanization.

Pedra Branca State Park, adjacent to EFMA, is the largest urban forest in the Americas and one of the largest remnants of the Atlantic Forest biome in the state of Rio de Janeiro. It covers an area of 12,492 ha, and the predominant phytophysiognomy is ombrophilous dense Atlantic Forest vegetation. The region's climate is humid mesothermic, with hot, rainy summers and mild winters.

The samplings were made in different types of habitat representing preserved forest areas (22°56'45"S, 43°25'00"W), transition areas between urban and forested environments, including reforestation areas (22°56'29"S, 43°24'25”W), and peridomicile areas (22°56'18”S, 43°24'11”W). See Gentile *et al*. (2018) for a more detailed description of the study area. The areas of preserved forest were located distant from human dwellings and, for the most part, above 100 m elevation. These areas have canopy heights ranging from 10 to 30 m and irregular slopes, ranging from flat to steep. The transition areas were located between the peridomicile areas and the preserved forest, with a canopy height ranging from 6 to 20 m and a flat slope. The peridomicile areas were located in the backyards of the houses, with a lower canopy ranging from 6 to 10 m, predominance of shrubs and small trees, presence of flooded areas and slope varying from flat to moderate.

Small mammal sampling
Small mammal captures were carried out from July 2012 to April 2014 every four months and in July, October and November 2017 for five consecutive nights in the three types of habitat. Mammals were collected using Tomahawk® (16 × 5 × 5 inches) and Sherman® (3 × 3.75 × 12 inches) live-traps.

Two transects of twenty points were established in each habitat. On each transect, two traps were placed on the ground at each point. All traps were baited with a mixture of peanut butter, banana, oatmeal and bacon. The total trapping effort was 9,120 trap-nights. The animals were anesthetized and submitted to euthanasia for helminth search. The mammals were identified by external morphology, cranial morphology and cytogenetic analyses, when necessary. The mammalian specimens were submitted to taxidermy and deposited in the mammalian collection of the National Museum of the Federal University of Rio de Janeiro voucher: 83156 - 83163; 83167 - 83172.

Collection and identification of helminths

Viscera, thoracic and abdominal cavities, and musculature were examined for helminths. The organs were separated in Petri dishes with saline solution (0.85% NaCl) and dissected using a stereoscopic microscope. The nematodes were fixed in AFA solution (93 parts 70% ethanol, 5 parts 0.4% formol, and 2 parts 100% acetic acid) and heated to 65°C. Some specimens were stored in 70% ethanol for further molecular analysis. Trematodes were fixed in the same solution under compression, and the cestodes and acanthocephalans were kept in distilled water for relaxation of the musculature (Amato *et al.*, 1991). Nematodes were diaphanized with lactophenol or glycerinated alcohol. Trematodes, cestodes and acanthocephalans were stained with Langeron's carmine or Delafield's hematoxylin, differentiated with 0.5% hydrochloric acid, dehydrated in a crescent alcoholic series, diaphanized in methyl salicylate and mounted in Canada balsam for permanent preparation (Amato *et al.*, 1991). The specimens were analysed under a light microscope (Axio Scope A1 light microscope – Zeiss, Göttingen, Germany) coupled to an Axio Cam MRc digital camera.

The specific morphological aspects used to identify the specimens were according to Anderson *et al.* (2009), Gibbons (2010), Gibson *et al.* (2002), Jones *et al.* (2005), Khalil *et al.* (1994), Travassos (1937), Vicente *et al.* (1997) and Yamaguti (1961). The specimens were deposited in the scientific collection of helminths at the Laboratory of Biology and Parasitology of Wild Mammal Reservoirs - IOC/FIOCRUZ-RJ using the same deposit numbers of their respective hosts.

Helminth population parameters

Population parameters were calculated for each helminth species in each host species according to Bush *et al.* (1997). The average abundance was considered as the total number of helminths divided by the number of host individuals analysed. Mean intensity was calculated as the total number of helminths divided by the number of animals infected. Prevalence was considered to be the ratio between the number of infected animals and the total number of animals analysed. These three parameters were calculated for each helminth species considering each host species. Mean helminth species richness was calculated as the average of the helminth species richness found in each infracommunity (community harboured by a single host) for each host species.

A bipartite network plot to illustrate host-parasite interactions between species (Poulin, 2010) was carried out between the small mammals and the helminth species. This plot was built based on a matrix of the presence/absence of each helminth species in each host species using the bipartite package (Dormann *et al.*, 2008).

Ethic aspects

Animals were captured under authorization of the Brazilian Government's Chico Mendes Institute for Biodiversity and Conservation (ICMBIO, licence number 13373) and the Environmental Institute of Rio de Janeiro State (INEA, licence number 020/2011). All procedures followed the guidelines for the capture, handling, and care of animals of the Ethical Committee on Animal Use of the Oswaldo Cruz Foundation (CEUA, licence numbers LW81/12, and LW-39/14). Biosafety procedures and personal safety equipment were used during all procedures involving animal handling and biological sampling.

RESULTS

Seven species of small mammals were captured, four of which were marsupials, *Didelphis aurita* (Wied Neuwied, 1826) (45), *Metachirus myosurus* (Temminck, 1924) (1), *Marmosa paraguayana* Tate, 1931 (5) and *Monodelphis americana* (Müller, 1776) (9) (Didelphimorphia, Didelphidae), and three were rodents, *Akodon*

Table 1. Mean abundance, intensity (± SD), prevalence (95% CI) and site of infection of the helminths of marsupials at FIOCRUZ Atlantic Forest Biological Reserve, Rio de Janeiro, Brazil. (-) indicates absence of the parasite species.

Hosts / Helminths (site of infection)	*Aspidodera raillieti* (large intestine)	*Brachylaima advena* (small intestine)	*Cruzia tentaculata* (large intestine)	*Duboisiela proloba* (small intestine)	*Globocephalus marsupialis* (small intestine)	*Heterostrongylus heterostrongylus* (lungs)	*Oligacanthorhynchus microcephalus* (small intestine)
Didelphis aurita							
Abundance	18.84 ± 36.68	0.46 ± 17.55	71.38 ± 133.45	4.02 ± 14.09	0.64 ± 3.49	1.78 ± 3.22	1.96 ± 6.31
Intensity	32.62 ± 43.56	17.83 ± 31.27	86.81 ± 141.62	16.45 ±25.46	9.67 ± 11.72	5.33 ± 3.50	4.40 ± 9.00
Prevalence	57.78 (42.15-72.34)	26.67 (14.60-41.94)	82.22 (67.95-92.00)	24.44 (12.88-39.54)	6.67 (01.40-18.27)	33.33 (20.00-48.95)	44.44 (29.64-60)
Marmosa paraguayana							
Abundance	0.50 ± 1.00	-	-	-	-	-	-
Intensity	2.00 ± 0.00	-	-	-	-	-	-
Prevalence	25 (0.63-80.59)	-	-	-	-	-	-
Monodelphis americana							
Abundance	-	-	-	-	-	-	-
Intensity	-	-	-	-	-	-	-
Prevalence	-	-	-	-	-	-	-

Hosts / Helminths (site of infection)	*Rhopalias coronatus* (small intestine)	*Travassostrongylus orloffi* (small intestine)	*Trichuris didelphis* (large intestine)	*Trichuris minuta* (large intestine)	*Trichuris* sp. 1 (large intestine)	*Turgida turgida* (stomach)	*Viannaia hamata* (small intestine)
Didelphis aurita							
Abundance	0.04 ± 0.30	23.53 ± 34.72	0.02 ± 0.15	2.69 ± 8.74	-	7.84 ± 7.75	104.64 ± 303.98
Intensity	2.00 ± 0.00	28.62 ± 36.39	1.00 ± 0.00	11 ± 15.36	-	9.54 ±7.44	142.70 ± 347.38
Prevalence	2.22 (0.06-11.77)	82.22 (67.95-92)	2.22 (0.06-11.77)	24.44 (12.88-39.54)	-	82.22 (67.95-92)	73.33 (58.06-85.40)
Marmosa paraguayana							
Abundance	-	-	-	-	0.25 ± 0.50	-	7.50 ± 10.85
Intensity	-	-	-	-	1.00 ± 0.00	-	15 ± 11.31
Prevalence	-	-	-	-	25 (0.63-80.59)	-	50 (06.76-93.24)
Monodelphis americana							
Abundance	-	-	-	-	-	-	4.33 ± 12.27
Intensity	-	-	-	-	-	-	19.50 ± 24.75
Prevalence	-	-	-	-	-	-	22.22 (02.81-60.01)

Table 2. Mean abundance, intensity (± SD), prevalence (95% CI) and site of infection of the helminths of rodents at FIOCRUZ Atlantic Forest Biological Reserve, Rio de Janeiro, Brazil. (-) indicates absence of the parasite species.

Hosts / Helminths (site of infection)	*Guerrerostrongylus zetta* (small intestine)	*Nippostrongylus brasiliensis* (small intestine)	*Rodentolephis akodontis* (small intestine)	*Stilestrongylus lanfrediae* (small intestine)	*Syphacia muris* (large intestine)	*Trichuris* sp.2 (large intestine)
Akodon cursor						
Abundance	-	-	2.00 ± 0.49	-	-	2.00 ± 0.76
Intensity	-	-	1.00 ± 0.00	-	-	2.00 ± 0.00
Prevalence	-	-	28.57 (3.70-71.00)	-	-	14.29 (0.40-57.90)
Oligoryzomys nigripes						
Abundance	2.67 ± 4.21	-	-	2.67 ± 6.34	-	-
Intensity	8.00 ± 2.65	-	-	12.00 ± 9.90	-	-
Prevalence	33.33 (7.50-70.10)	-	-	22.22 (02.80-60.00)	-	-
Rattus rattus						
Abundance	-	19.86 ± 52.54	-	-	103.29 ± 202.85	-
Instensity	-	139.00 ± 0.00	-	-	361.50 ± 245.37	-
Prevalence	-	14.29 (0.36-57.87)	-	-	28.57 (03.67-70.96)	-

cursor (Winge, 1887) (7), *Oligoryzomys nigripes* (Olfers, 1818) (9) (Rodentia, Sigmodontinae), and the sinantropic rodent *Rattus rattus* (Linnaeus, 1758) (7) (Rodentia, Muridae). Twenty species of helminths were collected from the small mammal community. Thirteen species were collected from *D. aurita*, three from *M. paraguayana*, one from *M. americana*, two from *A. cursor*, two from *O. nigripes* and two from *R. rattus* (Table 1). No helminth was found in the marsupial *M. myosurus*.
Forty-five individuals of *D. aurita* were analysed, and all were infected by at least one helminth species. The mean species richness was 5.4 and varied from 1 to 9 among the infracommunities. The highest abundances and intensities were observed for the nematodes *Viannaia hamata* Travassos, 1914 (Nematoda: Viannaiidae Durette-Desset & Chabaud, 1981), followed by *Cruzia tentaculata* (Rudolphi, 1819) Travassos (1917) (Nematoda: Kathlaniidae Travassos, 1918) and *Travassostrongylus orloffi* Travassos, 1935 (Nematoda: Viannaiidae Durette-Desset & Chabaud, 1981) (Table 1). The highest prevalence was recorded for *C. tentaculata*, *T. orloffi* and *Turgida turgida* (Rudolphi, 1819) Travassos, 1919 (Nematoda: Physalopteridae Leiper, 1908) (Table 1). *M. paraguayana* had two specimens infected among the five analysed. The helminth species found were *Aspidodera raillieti* Travassos, 1913 (Nematoda: Aspidoderidae (Freitas, 1956), *Trichuris* sp. 1, and *V. hamata*, the latter showing the highest prevalence and abundance in this host (Table 1). The mean species richness was 1. Only two specimens among nine *M. americana* were infected by a single helminth species, *V. hamata* (Table 1).

Considering rodents, seven individuals of *A. cursor* were analysed, and two of them were infected by helminths, the cestode *Rodentolepis akodontis* Rêgo, 1967 (Platyhelminthes, Hymenolepididae) and the nematode *Trichuris* sp. 2 (Table 2). All helminths presented low abundances and intensities. The mean species richness was 0.43. Four individuals among nine *O. nigripes* analysed were infected. The helminth species found were *Guerrerostrongylus zetta* (Travassos, 1937), Sutton & Durette-Desset, 1991 (Nematoda, Heligmonellidae) and *Stilestrongylus lanfrediae* Souza *et al.*, 2008 (Nematoda, Heligmonellidae), both with low abundance and intensity (Table 2). The mean species richness was 0.55. Seven individuals of the synanthropic rodent *R. rattus* were analysed, and two were infected. Two helminth species were found: *Nippostrongylus brasiliensis* (Travassos, 1914) (Nematoda, Heligmonellidae) in a single host and *Syphacia muris* (Yamaguti, 1935) (Nematoda, Oxyuridae) in two hosts, the latter with the largest abundance and intensity found among rodent hosts (Table 2). The mean species richness was 0.43.

The bipartite network plot between small mammals and helminths showed little parasite sharing among host species (Fig. 1). The nematode *V. hamata* was shared between the marsupials *D. aurita*, *M. paraguayana* and *M. americana* (Fig. 1). *D. aurita* and *M. paraguayana* shared the nematode *A. raillieti* (Figure 1). No other helminth species sharing was observed (Figure 1).

DISCUSSION

The marsupial *D. aurita* had the highest helminth species richness when compared to the other host species. This fact can be due to the generalist habit of this species, to its vagility (Cáceres & Monteiro-Filho, 2001), which may have contributed to a greater acquisition of parasites in different environments, to its larger body size in relation to the other mammals and to the larger number of specimens captured and analysed. The low helminth species richness found for all the other host species may be due to the high disturbance of the local environment, resulting in helminth species whose eggs may be more resistant in the soil or species less sensitive to environmental alterations. Other studies found that habitat fragmentation and disturbances could negatively affect the life cycles of some species of parasites, limiting the occurrence of some species '(Lafferty & Kuris, 1999; Cardoso *et al*., 2016). However, we cannot discard the low sample sizes of these mammals.

This study is the first report of a helminth infection for the marsupial *M. americana*, which was infected by *V. hamata.* Furthermore, this is the first report of the nematodes *A. raillieti*, *V. hamata* and *Trichuris* sp. parasitizing *M. paraguayana*,

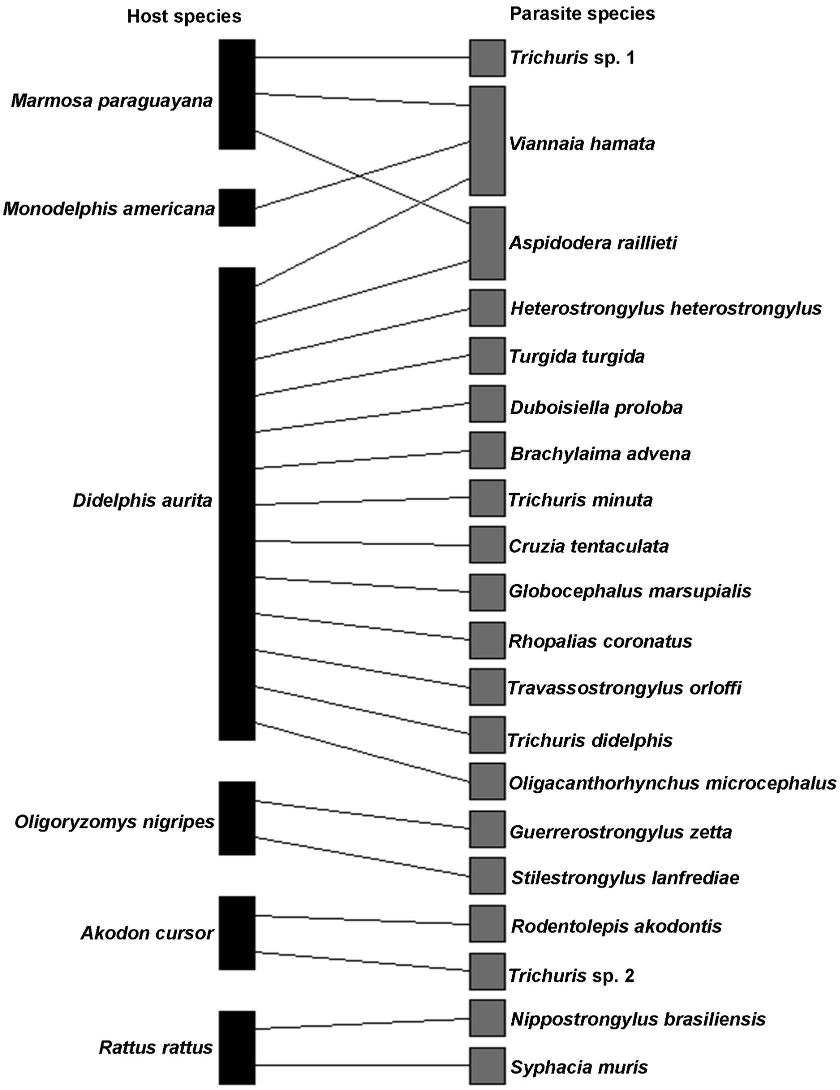

Figure 1. A bipartite network plot indicating the small mammal – helminth associations at FIOCRUZ Atlantic Forest Biological Reserve, Rio de Janeiro, Brazil.

increasing its helminth fauna to six species and adding this mammal as a new host for these helminths. Little is known about the helminth fauna of *M. paraguayana* (Santos-Rondon *et al.*, 2012). In Brazil, only two nematode species have been reported in this host, the oxyurid *Gracilioxyuris agilisis* Feijó, Torres, Maldonado Jr & Lanfredi, 2008 in the Cerrado (Neotropical savannah), state of São Paulo (Santos-Rondon *et al.*, 2012), and the filariid *Litomosoides barretti* Muller, 1980 in the state of Bahia (Muller, 1980). The cestode *Mathevotaenia bivittata* (Janicki, 1904) has also been reported in this host in Argentina (Campbell *et al.*, 2003). The helminths of *D. aurita* are discussed in detail in Costa-Neto *et al.* (2019).

Regarding the sigmodontine rodent *A. cursor*, which occurs only in the Brazilian Atlantic Forest up to 1,170 m high (Geise, 2012), the cestode *R. akodontis* had been previously reported in this host Simões *et al.* (2011), as well as a nematode of the genus *Trichuris* (*T. navonae*) by Lucio *et al.* (2021). The helminth species richness reported in the present study was very low compared to previous reports of the helminth fauna of this rodent in the

Atlantic Forest. Simões *et al*. (2011) found nine species in forest fragments, and Lucio *et al*. (2021) found eight species in open matrix areas, both in the state of Rio de Janeiro.

The two nematodes found in *O. nigripes* are commonly reported in other studies of the helminth fauna of this sigmodontine rodent (Pinto *et al.*, 1982; Gomes *et al.*, 2003; Simões *et al.*, 2011; Werk *et al.*, 2016; Panisse *et al.*, 2017; Boullosa *et al.*, 2020; Cardoso *et al.*, 2020). Likewise, the two nematodes recovered from the synanthropic rodent *R. rattus* have also been reported for this host in other studies (Gómez-Muñoz *et al.*, 2018; Panti-May *et al.*, 2020). In Argentina, Muñoz *et al.* (2018) found higher values of abundance and prevalence of *N. brasiliensis* in *R. rattus* than in this study. Panti-May *et al*. (2019) found low values of intensity, abundance and prevalence for this helminth in a region of Mexico. These authors also found high prevalence of *S. muris* positively associated with the abundance of *R. rattus.*

The helminth species were shared only within a single taxonomic group (Tribe Didelphini), with no record of parasite sharing between rodents and marsupials. The two helminths (*V. hamata* and *A. raillieti*) shared by the marsupial species can be considered core species in the present study, with high prevalence and abundances. Indeed, *A. raillieti* and *V. hamata* presented prevalence greater than 50% in *D. aurita*. This indicates that the three didelphids, *D. aurita, M. paraguayana* and *M. americana*, which share the environment, also share some helminth species. Jiménez *et al.* (2011), studying helminth component communities of didelphid marsupials from several localities, suggested that sympatric species of marsupials shared more species of parasites than parasite communities occurring in conspecific marsupials from different localities. Our results corroborate that study with regard to the local scale.

The results indicated little sharing of parasite species in these small mammal-helminth associations. Although these mammals have been reported as reservoirs of zoonotic pathogens in the study area (Berbigier *et al.*, 2021), and some of them may harbour helminths with zoonotic potential (Bezerra-Santos *et al*., 2020; Meerburg *et al.*, 2009; Araújo *et al.*, 2014), none of the identified helminth species are known to infect humans.

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