

Helminth parasites of *Galictis cuja* (Carnivora, Mustelidae), from localities in the Atlantic forest of Brazil

Helminhos parasitos de *Galictis cuja* (Carnivora, Mustelidae), em localidades de Mata Atlântica no Brasil

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Received June 9, 2016

Accepted July 27, 2016

Abstract

The current study aimed to investigate the helminth parasites of a population of *Galictis cuja* (Carnivora, Mustelidae) that occur in Atlantic Forest in the Southeastern region of Brazil. We necropsied 18 specimens of *G. cuja*, collected between January 2009 and May 2014, ran over victims on BR-040 highway, between the municipalities of Duque de Caxias, state of Rio de Janeiro and Juiz de Fora, state of Minas Gerais, localities inserted in Atlantic rainforest Biome. A total of six species of helminths were identified: *Dioctophyme renale*, *Molineus elegans*, *Physaloptera* sp., *Strongyloides* sp., *Platynosomum illiciens*, and *Pachysentis gethi*. *Molineus elegans*, *Physaloptera* sp. and *P. illiciens* were recorded for the first time in this host. Data provided in the current study when compared to the previous reports of parasitism by helminths in *G. cuja* in Brazil demonstrate that this study is the most representative with this host species.

Keywords: *Galictis cuja*, helminth, BR-040 highway, road kill, wild mammal, Brazil.

Resumo

O presente estudo teve como objetivo investigar a fauna de helmintos de uma população de *Galictis cuja* (Carnivora, Mustelidae) que ocorre em uma região de Mata Atlântica na região Sudeste do Brasil. Foram necropsiados 18 espécimes de *G. cuja*, coletados entre Janeiro de 2009 e Maio de 2014, que foram mortos acidentalmente por atropelamentos na rodovia BR-040, entre os municípios de Duque de Caxias, no Estado do Rio de Janeiro e Juiz de Fora, no Estado de Minas Gerais, localidades inseridas no Bioma Mata Atlântica. No total seis espécies de helmintos foram identificadas: *Dioctophyme renale*, *Molineus elegans*, *Physaloptera* sp, *Strongyloides* sp, *Platynosomum illiciens* e *Pachysentis gethi*. *Molineus elegans*, *Physaloptera* sp. e *P. illiciens* foram registradas pela primeira vez nesta espécie de hospedeiro. O estudo amplia a diversidade de helmintos conhecidos para o hospedeiro em questão. Os dados fornecidos no presente estudo quando comparados com os registros prévios de helmintos em *G. cuja* no Brasil demonstram que este estudo é o mais representativo com esta espécie de hospedeiro.

Palavras-chave: *Galictis cuja*, helmintos, rodovia BR-040, atropelamento, mamífero silvestre, Brasil.

Introduction

The lesser grison, *Galictis cuja* (Molina, 1872) (Carnivora, Mustelidae), is the smallest representative of the family Mustelidae. It is restricted to South America, with distribution from northeastern Brazil to southern Peru, western Bolivia, central and southern Chile, Paraguay, Uruguay, Argentina, and southeastern Brazil, at elevations from sea level to 4200 m, and habitats from Atlantic Forest, and cold steppe in Patagonia to exotic forest plantations in Chile (YENSEN & TARIFA, 2003; PREVOSTI & TRAVAINI, 2005; NABTE et al., 2009; ZÚÑIGA et al., 2009; ROCHA-MENDES et al., 2010; BORNHOLDT et al., 2013;

POO-MUÑOZ et al., 2014). According to the IUCN (2016), this species is not included in the main endangered categories, and its conservation status is of “least concern”.

To date, there have been few studies on the helminth parasite fauna of *G. cuja* in South America. The most recent compilations about helminth parasites in wild mammals in some South American countries have reported few helminth species in this host species. Lunaschi & Drago (2007) and Fugassa (2015) did not mention any occurrences of helminths in this mammal in Argentina. However, Moleón et al. (2015) provided the first report on occurrences of helminths in *G. cuja* in Argentina, consisting of the nematode *Aonchotheca putorii* (Rudolphi, 1819) (Trichocephalida, Capillariidae). In *G. cuja* from Paraguay,

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Seese et al. (in YENSEN & TARIFA, 2003) listed occurrences of unidentified nematode species of the genera *Cruzia* Travassos, 1917 (Spirurida, Kathlaniidae), *Dirofilaria* Railliet & Henry, 1910 (Spirurida, Onchocercidae), *Gnathostoma* Owen, 1836 (Spirurida, Gnathostomatidae), and *Lagochilascaris* Leiper, 1909 (Spirurida, Ascaridae), along with unidentified nematode species in the families Oxyuridae and Trichostrongylidae.

We consider that the studies about helminth parasites of *G. cuja* in Brazil have been more representative. The most recent studies on helminths in mammals in Brazil have so far recorded five species of helminths in this host, in several localities in this country (VIEIRA et al., 2008; PINTO et al., 2011). Therefore, the current study aimed to investigate the helminth parasites of a population of *G. cuja* living in a stretch of Atlantic Forest in the southeastern region of Brazil, and elaborate a checklist of helminth parasites of this host species in South America.

Material and Methods

The specimens of *Galictis cuja* examined were collected between January 2009 and May 2014, roadkilled on BR-040 highway, between the municipalities of Duque de Caxias (22°35'33"S, 43°16'48"W), state of Rio de Janeiro and Juiz de Fora (21°49'02"S, 43°23'03"W), state of Minas Gerais, localities inserted in Atlantic rainforest Biome. A total of 18 hosts were necropsied, and the skeletons and taxidermized skin of hosts parasitized are deposited at Coleção de Mastozoologia do Museu Nacional do Rio de Janeiro (MNRJ), of the Universidade Federal do Rio de Janeiro (UFRJ), under the codes: MNRJ 79162, MNRJ 79190, MNRJ 79216, MNRJ 79247, MNRJ 79261, MNRJ 79294, MNRJ 79314, MNRJ 79326, MNRJ 79358, MNRJ 79379, MNRJ 79404, MNRJ 79407, MNRJ 79421, MNRJ 79443, MNRJ 79445, MNRJ 79471, and MNRJ 79501. The hosts were identified according to Yensen & Tarifa (2003) and Bornholdt et al. (2013).

After being collected, the hosts were kept frozen in a freezer at -18 °C until necropsy. The organs were dissected in plastic trays, and

washed in saline solution 0.9%. The helminth collected were fixed in AFA (70° GL ethanol, 93 parts; 37% formalin, 5 parts; glacial acetic acid, 2 parts) for 48 hrs, and then stored in 70° GL ethanol.

For microscopic identification the nematodes were clarified in Amann's lactophenol (1: 1: 2: 1 – phenol: lactic acid: glycerin: distilled water) and mounted in temporary slides. Trematodes and acanthocephalans were stained with Mayer's carmalum (HUMASON, 1979) and mounted in Canada balsam for examination as whole mounts. The helminth species were identified in Olympus BX 41 light microscope.

The nematodes were identified at generic level according to Anderson et al. (2009). Systematic classification of nematodes is according to Hodda (2011). The specimens of the genus *Molineus* Cameron, 1923 (Strongylida, Molineoidea) were identified according to the bursal rays distribution (ANDERSON et al., 2009) and by the specific morphological descriptions made by Travassos (1921, 1937). Trematodes were identified at generic level according to Bray et al. (2008), and at specific level according to Rodrigues (1963). Acanthocephalans were identified according to Machado (1950) and Schmidt (1972). The ecological terms and quantitative parameters of infrapopulations were according to Bush et al. (1997).

Voucher specimens of helminths are deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC), Rio de Janeiro, Brazil (Table 1). The current study was performed under authorization from Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA/SISBIO (Request No 30727-4).

Results

Among the specimens of *Galictis cuja* examined in the current study, the total prevalence of parasitism was 94.4% (17 infected hosts out of the sample of 18 hosts analyzed). The helminth richness was six species, of which four were nematodes: *Diocotophyme renale*

Table 1. Species of helminths collected in *Galictis cuja* of the current study, site of infection, and ecological data of each helminth.

Helminth	Site of infection	Prevalence	Range of infections	Intensity* and mean intensity	95% Confidence interval of mean intensity	Abundance* and mean abundance	95% Confidence interval of mean abundance
Acanthocephala							
<i>Pachysentis gethi</i> (CHIOC 38100)	Small intestine	27.7%	1-9	2.6 ± 3.3	0.959; 4.241	0.7 ± 2.1	-0.344; 1.744
Trematoda							
<i>Platynosomum illiciens</i> (CHIOC 38201)	Bile duct	27.8%	1-15	5.4 ± 6.1	2.367; 8.433	1.5 ± 3.9	-0.439; 3.439
Nematoda							
<i>Diocotophyme renale</i> (CHIOC 36763)	Kidney	22.2%	1-3	1.5 ± 1.1	-0.953; 2.047	0.3 ± 0.8	-0.098; 0.698
<i>Molineus elegans</i> (CHIOC 36760)	Stomach and small intestine	61.1%	5-60	19.8 ± 24	7.865; 31.735	12.1 ± 21.7	1.309; 22.891
<i>Physaloptera</i> sp. (CHIOC 36761)	Stomach	5.5%	10	10*		0.55*	
<i>Strongyloides</i> sp. (CHIOC 36762)	Stomach and small intestine	16.7%	8-81	44 ± 36.5	25.849; 62.151	7.3 ± 21	-3.143; 17.743

* Only one specimen parasitized.

(Goeze, 1782) (Dioctophymatida, Dioctophymatidae), *Molineus elegans* (TRAVASSOS, 1921) (Rhabditida, Trichostrongylidae), *Physaloptera* sp. (Spirurida, Physalopteridae) and *Strongyloides* sp. (Panagrolaimida, Strongyloididae). There was one species of trematode, *Platynosomum illiciens* (Braun, 1901) (Trematoda, Dicrocoeliidae), and one of Acanthocephala, *Pachysentis gethi* (MACHADO, 1950) (Oligacanthorhynchida, Oligacanthorhynchidae) (Tables 1 and 2).

Until the current study, five species of helminth parasites of *G. cuja* in Brazil were known, and other six species of helminth in this host from Paraguay and Argentina was reported (Table 2).

Discussion

The genus *Molineus* (Strongylida, Molineoidea) was proposed by Cameron (1923), and its type species is *Molineus felineus* Cameron, 1923, which is a parasite of the small intestine of *Puma* (*Herpailurus yagouaroundi*) (Carnivora, Felidae) in South America. Currently, this genus has 28 nominal species, of which seven have been reported in Neotropical primates and 21 in carnivorous mammals throughout the world, except in Australia (DURETTE-DESSET et al., 2000, 2001). In carnivorous mammals in Brazil, seven species of this genus have been reported: *Molineus barbaris* Cameron, 1936, which was found to be a parasite in *Eira barbara* (Linnaeus, 1758)

Table 2. Checklist of helminth parasites of *Galictis cuja* from South America.

Helminth	Site of infection	Country	References
Phylum Acanthocephala			
Order Oligacanthorhynchida			
Family Oligacanthorhynchidae			
<i>Pachysentis gethi</i> (Machado, 1950)	Small intestine	Brazil	Machado (1950), Vieira et al. (2008), Current study
Phylum Platyhelminthes			
Class Trematoda			
Subclass Digenea			
Family Dicrocoeliidae			
<i>Platynosomum illiciens</i> (Braun, 1901)	Bile duct	Brazil	Current study
Phylum Nematoda			
Order Dioctophymatida			
Family Dioctophymatidae			
<i>Dioctophyme renale</i> (Goeze, 1782)	Kidney	Brazil	Barros et al. (1990), Vieira et al. (2008), Müller et al. (2009) Zabott et al. (2012), Pesenti et al. (2012), Current study
Order Panagrolaimida			
Family Strongyloididae			
<i>Strongyloides</i> sp.	Stomach and intestine	Brazil	Pinto et al. (2011), Current study
Order Spirurida			
Family Ascaridae			
<i>Lagochilascaris</i> sp.	Stomach	Paraguay	Seese et al. (in YENSEN & TARIFA, 2003)
Family Gnathostomatidae			
<i>Gnathostoma</i> sp.	intestine	Paraguay	Seese et al. (in YENSEN & TARIFA, 2003)
Family Kathlaniidae			
<i>Cruzia</i> sp.	Small intestine	Paraguay	Seese et al. (in YENSEN & TARIFA, 2003)
Family Onchocercidae			
<i>Dirofilaria</i> sp.	Heart	Brazil, Paraguay	Seese et al. (in YENSEN & TARIFA, 2003); Vieira et al. (2008)
Family Physalopteridae			
<i>Physaloptera</i> sp.	Stomach	Brazil	Current study
Family Oxyuridae			
Unidentified Oxyuridae	intestine	Paraguay	Seese et al. (in YENSEN & TARIFA, 2003)

Table 2. Continued...

Helminth	Site of infection	Country	References
Order Rhabdtida			
Family Trichostrongylidae			
<i>Molineus elegans</i> (Travassos, 1921)	Small intestine	Brazil	Current study
Unidentified Trichostrongylidae	Small intestine	Paraguay	Seese et al. (in YENSEN & TARIFA, 2003)
Family Metastrongylidae			
<i>Crenosoma brasiliense</i> Vieira, Muniz-Pereira, Souza Lima, Moraes Neto, Gonçalves & Luque, 2012	Lungs	Brazil	Vieira et al. (2012)
Order Trichocephalida			
Family Capillariidae			
<i>Aonchotheca putorii</i> (Rudolphi, 1819)	Small intestine	Argentina	Moleón et al. (2015)

(Mustelidae) in the state of Mato Grosso do Sul (VICENTE et al., 1997; VIEIRA et al., 2008); *M. brachiurus* Costa & Freitas, 1967, which occurs in *Chrysocyon brachyurus* (Illiger, 1815) (Canidae) in the state of Minas Gerais (VICENTE et al., 1997; VIEIRA et al., 2008); *M. felineus* Cameron, 1923, reported in *Puma (Herpailurus) yagouaroundi* (Geoffroy, 1803) (Felidae) at an unspecified locality (TRAVASSOS, 1937; VICENTE et al., 1997; VIEIRA et al., 2008); *M. major* Cameron, 1936, parasitizing *E. barbara* in the states of Mato Grosso do Sul and Rio de Janeiro (TRAVASSOS, 1937; TRAVASSOS & FREITAS, 1943; VICENTE et al., 1997; VIEIRA et al., 2008) and parasitizing *Pteronura brasiliensis* (Gmelin, 1788) (Mustelidae) in the state of Mato Grosso do Sul (VIEIRA et al., 2008); *M. nasuae* Lent & Freitas, 1938, parasitizing *Nasua nasua* (Linnaeus, 1766) (Procyonidae) in the state of Pará (LENT & FREITAS, 1938; VICENTE et al., 1997; VIEIRA et al., 2008); *M. paraensis* Travassos, 1937, reported in *Potos flavus* (Schreber, 1774) (Procyonidae) in the state of Pará; and *M. elegans* (TRAVASSOS, 1921) parasitic from *C. thous* of the state of Paraíba (TRAVASSOS, 1937; LENT & FREITAS, 1938; VICENTE et al. 1997; VIEIRA et al., 2008; LIMA et al., 2013). In Brazil, *Molineus elegans* also is reported in this type host *Saimiri sciureus* (Linnaeus, 1758) (Primates, Cebidae) of the state of Pará (CORRÊA et al., 2016). Until the current study, no reports of nematodes of the genus *Molineus* parasitizing *G. cuja* existed. Therefore, the current report of *M. elegans* in *G. cuja* in Brazil is the first report of this genus of nematodes in this host.

Currently, the genus *Physaloptera* Rudolphi, 1819 (Spirurida, Physalopteroidea), has approximately 104 nominal species, which occur in amphibians, reptiles, birds and mammals throughout the world (PEREIRA et al., 2012). According to Vieira et al. (2008) and Pinto et al. (2011), six species of this genus have been reported in wild carnivorous mammals in Brazil. *Physaloptera anomala* Molin, 1860, was found in *Panthera onca* (Linnaeus, 1758) (Felidae) at an unspecified locality (VICENTE et al., 1997; VIEIRA et al., 2008); *P. digitata* Schneider, 1866, was reported parasitizing *Leopardus wiedii* (Schinz, 1821) (Felidae) in the state of Pará (NORONHA et al., 2002; VIEIRA et al., 2008) and *Puma concolor* (Linnaeus, 1771) (Felidae) in the states of Rio de Janeiro and São Paulo (VICENTE et al., 1997; VIEIRA et al., 2008); *P. maxillaris* Molin, 1860, was reported as a parasite of *Conepatus chinga* (Molina, 1782) (Mephitidae) in the states of Pernambuco and Rio Grande do Sul (VICENTE et al., 1997; VIEIRA et al., 2008); *P. praeputialis*

Linstow, 1889, was reported in *Cerdocyon thous* (Linnaeus, 1766) (Canidae) in the state of Paraná (VIEIRA et al., 2008) and in *C. brachyurus* in the state of Minas Gerais (VICENTE et al., 1997; VIEIRA et al., 2008); *P. semilanceolata* Molin, 1860, was found parasitizing *N. nasua* in the states of Mato Grosso do Sul and Pará (VICENTE et al., 1997; VIEIRA et al., 2008); and *P. terdentata* Molin, 1860, was found in *P. concolor* in the state of Rio de Janeiro (VICENTE et al., 1997; VIEIRA et al., 2008). Unidentified species of *Physaloptera* was reported in *C. thous* in the state of Mato Grosso do Sul, in *E. barbara* in the state of Amazonas, in *L. wiedii* in the state of Pará (VIEIRA et al., 2008), in *Leopardus pardalis* (Linnaeus, 1758) (Felidae) in the states of Mato Grosso do Sul and Pará (TRAVASSOS & FREITAS, 1943; VICENTE et al., 1997; VIEIRA et al., 2008) and in *N. nasua* in the states of Mato Grosso, Mato Grosso do Sul and Rio de Janeiro (TRAVASSOS & FREITAS, 1943; VICENTE et al., 1997; VIEIRA et al., 2008). In the current study, we found only female specimens of this nematode, therefore, it was not possible to identify the species. The occurrence of an unidentified species of *Physaloptera* parasitizing *C. cuja* in the current study is the first record of this nematode in this host species.

The monotypic genus *Dioctophyme* Collet-Megret, 1802 (Enoplida, Dioctophymatoidea) is composed by *D. renale* (Goeze, 1782), which is a parasite of the kidneys or peritoneum of mammals, especially of the families Mustelidae and Canidae (VICENTE et al., 1997; ANDERSON et al., 2009). In Brazil, this species has been reported in several wild carnivorous hosts, including specimens of *G. cuja* that originated from the state of Rio de Janeiro (VIEIRA et al., 2008).

Species of *Strongyloides* Grassi, 1879 (Rhabditiida, Rhabditoidea) are parasites of several groups of domestic and wild vertebrates throughout the world (ANDERSON et al., 2009). Nevertheless, reports of this genus in wild carnivorous mammals in Brazil are rare and the occurrences have been limited to unidentified species of *Strongyloides* parasitizing *C. thous* and *Galictis vittata* (Schreber, 1776), both in the state of Rio de Janeiro (VIEIRA et al., 2008), and *G. cuja* in the state of Rio Grande do Sul (MULLER et al., 2009; PINTO et al., 2011). Species of *Strongyloides* have not been previously recorded in *G. cuja* in Atlantic forest localities in the states of Rio de Janeiro and Minas Gerais, and therefore this is the first report of this nematode in this host species in these states.

The genus *Pachysentis* Meyer, 1931 (Oligacanthorhynchida, Oligacanthorhynchidae), was proposed to accommodate *Pachysentis canicola* Meyer, 1931, a parasite of domestic dogs in South America (PETROCHENKO, 1971). Currently, this genus contains 10 nominal species that parasitize domestic and wild mammals throughout the world (PETROCHENKO, 1971; AMIN, 2013). *Pachysentis gethi* (MACHADO, 1950) was originally described by Machado (1950) and was placed in the genus *Prosthenorchis* Travassos, 1915 (Oligacanthorhynchida, Oligacanthorhynchidae), from descriptions of specimens collected from *E. barbara* in the states of Rio de Janeiro and Pará. Vieira et al. (2008) searched for information on helminth parasites in wild carnivorous mammals in Brazil that was stored in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC) and reported the same data as in the original description made by Machado (1950), along with two new records of this species, parasitizing *G. cuja* and *G. vittata*, both in the state of Rio de Janeiro.

Platynosomum illiciens (Braun, 1901) (Trematoda, Dicrocoeliidae) was described as an intestinal parasite of *Falco* sp. (Aves, Falconiformes) (TRAVASSOS, 1944). This species was reported by Travassos (1944) and Yamaguti (1958) as an exclusive parasite of the liver of some bird species in Brazil. However, Rodrigues (1963) observed large polymorphs in *P. semifuscum* Looss, 1907, which is a parasite of European birds; in *P. proxilliciens* (Canavan, 1937), which is a parasite of Asian birds; in *P. fastosum* Kossack, 1910, which is a parasite of several species of mammals in Asia and North and South America; and in *P. brauni* (Freitas & Lent, 1937), which is a parasite of Brazilian birds. This author therefore considered all these species to be junior synonyms of *P. illiciens*. Pinto et al. (2016) also considered that the morphometric differences observed in these species of *Platynosomum* were insufficient for specific differentiation, and stated that *P. illiciens* is a species with wide geographic distribution and host range, parasitizing both birds and mammals. In carnivore mammals from Brazil, *P. illiciens* has been previously reported as a parasite of *G. vittata* in the state of Rio de Janeiro (TRAVASSOS, 1944; TRAVASSOS et al., 1969; VIEIRA et al., 2008), of *P. yagouaroundi* in the state of Pernambuco (BARBOSA & PONTUAL, 1949; RODRIGUES, 1963; VIEIRA et al., 2008). Therefore, the current study is the first report of *P. illiciens* parasitizing *G. cuja*.

The quantitative data from this study could not be compared with other previous studies from helminth from *G. cuja* (Table 1), since these previous studies do not show a number of compatible hosts with the present study, or do not provide quantitative data from parasitism. Some data of mean intensity and mean abundance of certain helminth infrapopulations observed herein, showed a large standard deviation (Table 1), suggesting heterogeneity in size of these infrapopulations. When the 95% confidence intervals of the mean intensity and abundance of these data were analyzed found that the quantitative data of *P. gethi* (Confidence intervals of mean intensity 0.959, 4.241; and of mean abundance -0.344, 1.744), *P. illiciens* (Confidence interval of mean abundance -0.439, 3.439), *D. renale* (Confidence intervals of mean intensity -0.953, 2.047; and of mean abundance -0.098, 0.698), and *Strongyloides* sp. were not statistically significant (Confidence interval of mean abundance -3.143, 17.743) (Table 1), which indicate that the number of studied hosts could not estimate

the totality of the helminth infrapopulations, and that a higher sampling hosts should be analyzed for a more consistent study of the population ecology of *G. cuja* helminth at the studied area.

The most recent report of parasites in this host was in the study by Vieira et al. (2012), who proposed a new species of nematode, *Crenosoma brasiliense* Vieira, Muniz-Pereira, Souza Lima, Moraes Neto, Gonçalves & Luque, 2012 (Strongylida, Metastrongyloidea), which was collected in the lungs of one specimen of *G. cuja* in the municipality of Juiz de Fora, state of Minas Gerais.

Comparison of the data provided in the current study with the previous reports of helminth in *G. cuja* in Brazil (Table 2) demonstrates that the current study is the most representative report relating to this host species. Here, we studied a considerably sized sample of hosts (18 specimens) and recorded three new occurrences of helminths in *G. cuja* in Brazil, which is equivalent to 60% of what was previously known about this fauna.

Acknowledgements

Pilar Corrêa was supported by a student fellowship from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil). Fabiano Matos Vieira was supported by a Postdoctoral fellowship from Programa Nacional de Pós-doutorado (PNPD) CAPES/FIOCRUZ (Fundação Instituto Oswaldo Cruz) at the Programa de Pós-graduação em Biodiversidade e Saúde (PPGBS) of the Instituto Oswaldo Cruz (IOC), Rio de Janeiro, RJ.

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