Revista Brasileira de Parasitologia Veterinária
Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv

Original Article

Oncicola venezuelensis (Marteau, 1977) (Acanthocephala: Oligacanthorhynchidae) in *Puma concolor* in Rio de Janeiro, Brazil

Oncicola venezuelensis (Marteau, 1977) (Acanthocephala: Oligacanthorhynchidae) em *Puma concolor* no Rio de Janeiro, Brasil

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How to cite: Palmer JPS, Dib LV, Lobão LF, Pinheiro JL, Ramos RCF, Uchoa CMA, et al. *Oncicola venezuelensis* (Marteau, 1977) (Acanthocephala: Oligacanthorhynchidae) in *Puma concolor* in Rio de Janeiro, Brazil. *Braz J Vet Parasitol* 2020; 29(3): e009620. https://doi.org/10.1590/S1984-29612020046

Abstract

Specimens of *Oncicola venezuelensis* (Marteau, 1977) were recovered from fragments of intestinal tissue of a female *Puma concolar* (Linn, 1771) found dead in Petrópolis, Rio de Janeiro in 2017. A total of 140 helminths were recovered. Five males and 5 females of the helminths were analyzed morphologically as well as 50 parasite eggs recovered in intestinal contents. Morphologically, these helminths were compatible with the genus *Oncicola*, because of the size and shape of the proboscis, the size and disposition of the lemnisci and the morphometry of the eggs, in which the external membrane of the shell was delicate and clear. From histopathology, the helminths were deeply embeded in the mucosa reaching up to the muscle layer. One specimen was also identified molecularly with universal primers that amplified the eukaryote region ITS1-5.8S-ITS2. The helminth showed 99% identity with the gene sequence of *O. venezuelensis* deposited in GenBank. It is important to emphasize, this parasite has been very little reported in the literature, which reinforces the importance of this report.

Keywords: Acanthocephala, *Puma concolor*, morphometry, molecular biology, histopathology.

Resumo

Espécimes de *Oncicola venezuelensis* (Marteau, 1997) foram recuperados de fragmentos do tecido intestinal de uma fêmea de *Puma concolor* (Linn, 1771) encontrada morta em Petrópolis, Rio de Janeiro, em 2017. Um total de 140 helmintos foram recuperados. Cinco machos e 5 cinco fêmeas dos helmintos foram analisados morfologicamente, bem como 50 ovos dos parasitos recuperados no conteúdo intestinal. Morfologicamente, esses helmintos eram compatíveis com o gênero *Oncicola*, devido ao tamanho e formato da probóscide, o tamanho e disposição do leminisco e a morfometria dos ovos, que apresentaram membrana externa da casca delicada e clara. A partir da histopatologia, pode-se verificar que os helmintos estavam profundamente inseridos na mucosa, atingindo até a camada muscular. Um espécime também foi identificado molecularmente com *primers* universais que amplificam a região ITS-1.5.8S.ITS-2. Após as análises moleculares, foi verificado que os helmintos apresentavam 99% de identidade com sequência gênica de *O. venezuelensis* que está depositada no *Genbank*. É importante enfatizar, que esse parasito foi muito pouco relatado na literatura, demonstrando a importância deste relato.

Palavras-chave: Acanthocephala, *Puma concolor*, morfometria, biologia molecular, histopatologia.

Received April 23, 2020. Accepted May 8, 2020.
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Introduction

There are around 1298 valid species of Acanthocephala distributed in four class (Amin, 2013). In Class Archiacanthocephala, Order Oligacanthorhynchida has a single Family Oligacanthorhynchidae, which contains 12 genera, including the genus *Oncicola* (Amin, 2013). This genus has 24 species that infect carnivorous animals such as mephetids, mustelids, procyonids, felids and canids (Amin, 2013). In the case of the genus *Oncicola*, carnivores are prominent definitive hosts (Nickol et al., 2006; Núñez & Drago, 2017).

Helminths of the genus *Oncicola* inhabit the small intestine of the definitive hosts. The pathogenic condition that they cause relate mainly to the chronic inflammatory response that stems from their attachment to the host intestinal mucosa via their proboscis (Richardson, 2013; Núñez & Drago, 2017). This may produce nodules similar to granulomas and, consequently, tissue fibrosis. In homeothermic hosts, the proboscis may penetrate deeply in the various layers of the small intestine (Núñez & Drago, 2017).

Several species of *Oncicola* had already been described in Brazil, simply through morphological analyses of the adult forms. Among these species, *O. sigmoides* (Meyer, 1932) collected from *Galictis* sp. and *Conepatus* sp., and *O. luehei* (Travassos, 1917) from *Nasua nasua* (Linn., 1766), in Pará, São Paulo, Minas Gerais, Mato Grosso and Mato Grosso do Sul; *O. macrurae* (Meyer, 1931) from *Leopardus wiedii* (Schinz, 1821) in Pará; *O. magalhaesi* (Machado Filho, 1962) from *Puma concolor* (Linn., 1771) in São Paulo; *O. micracantha* (Machado Filho, 1949) from *Conepatus chinga* (Molina, 1782) in Rio Grande do Sul; *O. paracampanulata* (Machado Filho, 1963) from *Puma yagouaroundi* (Saint-Hilaire, 1803) in São Paulo, Paraná and Pará; *O. oncicola* (Ihering, 1892) from *Panthera onca* (Linn., 1758) in São Paulo and Minas Gerais, from *P. yagouaroundi* and *Leopardus pardalis* (Linn., 1758) in São Paulo and from *L. wiedii* in Pará; and *O. campanulata* (Diesing, 1851) from *L. pardalis*, *Leopardus geoffroyi*, *P. onca* and *P. concolor*, and *O. chibigouzouensis* (Machado Filho, 1963) from *L. pardalis*, in Mato Grosso (Vieira et al., 2008). The morphological description of *O. venezuelensis* form *L. pardalis* in the Serra da Capivara, Piauí was associated with molecular techniques once by Santos et al. (2017).

The aim of the present study was to report the occurrence of *Oncicola venezuelensis* infecting *Puma concolor* in the state of Rio de Janeiro, Brazil, using tools for microscopic, histopathological and molecular analysis.

Material and Methods

The result from the present analysis was inserted in a report on a larger project that was registered under SISBIO number 57635-3, authentication code 0576350320190522.

In August 2017, a female *Puma concolor* was found dead on the Brejal highway (Figure 1), in Petrópolis, state of Rio de Janeiro. The carcass was sent to the headquarters of the National Park of Serra dos Órgãos. The animal was of 1.2 m long and weighed 17 kg.

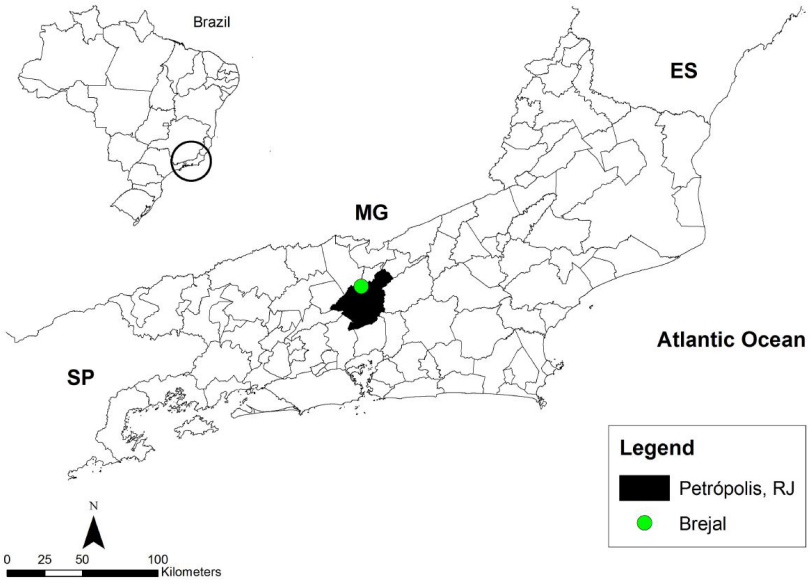

Figure 1. Location of the Brejal region in green and the city of Petrópolis in black, on a map of the state of Rio de Janeiro.

Morphology

A segment of the small intestine was sent to the Parasitology Laboratory of the Fluminense Federal University. It was washed in a sterile buffered saline solution and the parasites adhering to the mucosa were carefully removed and were viewed under a stereoscopic microscope (Diag Tech® XTL 6445, São Paulo, Brazil). The helminths were stored in receptacles containing 70% glycerinated alcohol. Subsequently, they were cleared using 10% phenol for 30 minutes and were individually laid out on microscope slides for morphological analysis.

The liquid that resulted from washing the intestinal segment was aliquoted into conical-based tubes, for subsequent recovery of eggs. These tubes were subjected to centrifugation-sedimentation for 5 minutes, at 252g. Microscope slides of the sedimented eggs were covered with a 24 × 32 mm coverslip.

Intestinal segments that contained the helminths were photographed. Parts of these tissues had been fixed in 10% formalin for 48 hours and then cut out, embedded in paraffin blocks and sectioned at 5 µm using a microtome Leika RM 2125 RT (Leica®, Germany) for mounting on microscope slides for analysis. These slides were stained with hematoxylin-eosin (hematoxylin – Confiança® São Paulo, Brazil; eosin - Reagen®, Rio de Janeiro, Brazil).

The slides with the sectioned tissues were analyzed under an optical microscope. The adult forms of the helminths and the eggs were measured and photomicrographed under an optical microscope (Olympus® BX 41, Tokyo, Japan) connected to a digital camera (Samsung® SDC415, Korea) using the software (Honestech® TVR, USA). Measurements of the helminths, their internal structures and their eggs were described using (minimum and maximum values), followed by the mean and standard deviation. So are these the specimens that were measured and included in Tables 1 and 2.

Table 1. Minimum and maximum values, means and standard deviations of the different parts of the bodies of female specimens of *Oncicola* and their eggs that were recovered in the present study and other studies.

Biometry (µm)	This study	Other reports about *Oncicola* sp.		
	Rio de Janeiro	*Oncicola venezuelensis* (Piauí. Brazil) Santos et al. 2017	*Oncicola venezuelensis* (St. John Island. U. S. Virgin Islands) Fuller, Nickol. 2011	*Oncicola venezuelensis* (Venezuela) Marteu. 1977
	Female (n=5)	Female (n=7)	Female (n=15)	Female (n=2)
Total lenght	6170-8520 7340 ± 810	6250-13250 9890	13200-18300 15500	15000-16000 NR
Total width	2010-2400 2200 ± 120	1000-1950 1470	1800-2600 2200	1900-2200 NR
Proboscis lenght	500-550 520 ± 24.49	430-600 540	432-480 458	0.50-0.55 NR
Proboscis width	550-600 602 ± 34.87	650-700 670	528-566 547	0.60-0.62 NR
Proboscis receptacle	1300-1450 1362.5 ± 64.95	970-1200 1060	1001-1390 1210	1200-1400 NR
Lemnisci	8600-13400 10100 ± 19000	NR NR	≤10000 NR	10000-12000 NR
Hooks				
I - Lenght	80-170 107 ± 23	110-140 127	115-139 127	95-145 NR
I - Base length	30-90 46.5 ± 18.51	NR NR	NR NR	NR NR

NR: Not reported.

Table 1. Continued...

Biometry (µm)	This study	Other reports about *Oncicola* sp.		
	Rio de Janeiro	*Oncicola venezuelensis* (Piauí. Brazil) Santos et al. 2017	*Oncicola venezuelensis* (St. John Island. U. S. Virgin Islands) Fuller, Nickol. 2011	*Oncicola venezuelensis* (Venezuela) Marteu. 1977
	Female (n=5)	Female (n=7)	Female (n=15)	Female (n=2)
II - Lenght	80-200 121 ± 36.73	80-120 103	120-139 131	95-135 NR
II - Base length	30-110 73.5 ± 23.51	NR NR	NR NR	NR NR
III - Lenght	70-180 106.5 ± 24.75	70-100 88	98-110 102	70-110 NR
III - Base length	20-110 53.5 ± 23.72	NR NR	NR NR	NR NR
IV - Lenght	70-190 115 ± 23.45	70-90 84	91-96 92	65-95 NR
IV - Base length	20-60 36.25 ± 10.53	NR NR	NR NR	NR NR
V - Lenght	50-130 94.5 ± 16.87	70-100 81	77-86 82	65-75 NR
V - Base length	20-60 33 ± 9.54	NR NR	NR NR	NR NR
VI - Lenght	50-110 79.45 ± 22.4	50-70 62	74-82 77	60-75 NR
VI- Base length	10-70 32.5 ± 13.74	NR NR	NR NR	NR NR
Neck lenght	250-400 314 ± 48.86	300-340 320	250-302 289	500 NR
Neck width	496-504 500 ± 2.82	400-550 476	384-422 396	620 NR
Cerebral ganglion length	130-180 152 ± 17.88	110 NR	110-149 131	NR NR
Cerebral ganglion width	110-130 112 ± 10.95	110 NR	62-86 73	NR NR
Uterine bell length	300-650 535 ± 126.19	350-500 422	499-538 520	0.65 NR
Uterine bell width	103.6-150 125.53 ± 23.52	NR NR	259-336 301	0.33 NR

NR: Not reported.

Table 1. Continued...

Biometry (µm)	This study	Other reports about *Oncicola* sp.		
	Rio de Janeiro	*Oncicola venezuelensis* (Piauí. Brazil) Santos et al. 2017	*Oncicola venezuelensis* (St. John Island. U. S. Virgin Islands) Fuller, Nickol. 2011	*Oncicola venezuelensis* (Venezuela) Marteu. 1977
	Female (n=5)	Female (n=7)	Female (n=15)	Female (n=2)
Uterus lenght	480-750 611.9 ± 117.03	400-720 610	672-816 752	1000 NR
Sphincter length	220-370 212 ± 92.17	200-400 318	264-384 330	NR NR
	eggs (n=50)	eggs (n=NR)	eggs (n=NR)	eggs (n=NR)
Length	35-74 59.8 ± 11.29	30-55 42	67-72 69	NR
Width	25-70.3 39.4 ± 10.12	22-30 26	43-50 47	NR

NR: Not reported.

Molecular study

To perform molecular analyses, five adult specimens, weighing-around 50 mg were placed in a conical-based tube containing 15 mL of sterile buffered saline solution. This tube was then subjected to three cycles of centrifugation at 447g for 10 minutes. Afterwards, the helminths were macerated on a Petri dish using a scalpel blade. The macerate was placed in a 1.5 mL microtube, 200 µL of the tissue buffer and 40 µL of the enzyme proteinase K was added. This material was then incubated at 37 °C (Nova Técnica, São Paulo, Brazil) in a bacteriological chamber for 24 hours. Subsequenthly, 20 µL of the enzyme proteinase K was added and incubate for 2 hours at 55 °C. After this incubation, no helminth tissue particles were evidenced. For DNA extraction the High Pure PCR Template Preparation (Roche®, Indianapolis, USA) commercial kit was used.

The primer for implementing the PCR was chosen after verifying the morphological characteristics. A pair of universal eukaryote primers described by Chen et al. (2010) was used. These amplified the ITS1-5.8S-ITS2 region of RNAr: forward (5'-GTCGTAACAAGGTTTCCGTA -3') and reverse (5'-TATGCTTAARTTCAGCGGGT -3'). The total volume of the reaction mix was 25 µL, consisting of 5 µL of each primer (at 10 pM), 7 µL of the DNA extracted and 8 µL of ultrapure water. For this PCR, the PuReTaq™ Ready-To-Go™ PCR (GE®, New Jersey, USA) beads were used. The reaction was performed using the following cycling: 94 °C for 2 min, followed by 40 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s and 72 °C for 7 min. Electrophoresis was performed using 1.5% agarose gel, and the bands were viewed by adding red gel. The amplified product was purified using the Promega commercial kit Wizard SV Gel and PCR Clean-Up System (Promega®, Wisconsin, USA). Following this, the purified product was subjected to genetic sequencing in an automated sequencer. The sequences were input to the BioEdit 7.2.5 (Hall, 1999) software and were compared with the reference sequences deposited in GenBank (Table 3). To compile the phylogenetic tree, the Mega® software, version 6 (Tamura et al., 2013), with the maximum likelihood (ML) algorithm in the Tamura Nei model with 5000 bootstraps, was used.

Results

Morphology

Oncicola venezuelensis (Marteau, 1977)

A total of 140 helminths and 50 eggs were recovered. Measurements were made of 10 helminths (5 males and 5 females) and on all the eggs (Tables 1 and 2). Trunk globous that was slightly wider in the anterior portion. Males 6790 µm long (5020-8210) by 2280 µm wide (2060-2550) and females 7340 µm long (6170-8520) and 2200 µm

Table 2. Minimum and maximum values, means and standard deviations of the different parts of the bodies of male specimens of *Oncicola* that were recovered in the present study and other studies.

Biometry (µm)	This study	Other reports about *Oncicola* sp.		
	Rio de Janeiro	*Oncicola venezuelensis* (Piauí. Brazil) Santos et al. 2017	*Oncicola venezuelensis* (St. John Island. U. S. Virgin Islands) Fuller, Nickol 2011	*Oncicola venezuelensis* (Venezuela) Marteu. 1977
	Male (n=5)	Male (n=3)	Male (n=10)	Male (n=2)
Total length	5020-8210 6790 ± 970	5630-12500 9170	6500-8400 8000	13500-14000 NR
Total width	2060-2550 2280 ± 180	750-1500 1170	1200-1300 1200	2000 NR
Proboscis lenght	450-550 490 ± 37.41	400-500 433	336-348 344	0.50-0.55 NR
Proboscis width	580-600 596 ± 8	370-600 507	476-480 479	0.60-0.62 NR
Proboscis receptacle	1300-1400 1334 ± 76.31	600-1180 960	1001-1390 1210	1200-1400 NR
Lemnisci	5500-13000 9700 ± 3400	NR NR	≤ 10000 NR	10000-12000 NR
Hooks				
I - Lenght	50-120 96.84 ± 23.85	120-125 NR	115-139 127	95-145 NR
I - Base length	20-70 48.95 ± 16.19	NR NR	NR NR	NR NR
II - Lenght	60-170 105.33 ± 29.18	145-150 NR	120-139 131	95-135 NR
II - Base length	30-100 60.67 ± 18.43	NR NR	NR NR	NR NR
III - Lenght	70-120 97.5 ± 14.36	125 NR	98-110 102	70-110 NR
III - Base length	20-70 38.13 ± 11.03	NR NR	NR NR	NR NR
IV - Lenght	40-120 92.78 ± 18.02	105-110 NR	91-96 92	65-95 NR
IV - Base length	20-70 37.22 ± 11.16	NR NR	NR NR	NR NR
V - Lenght	70-120 90.56 ± 16.05	95-105 NR	77-86 82	65-75 NR

NR: Not reported.

Table 2. Continued...

Biometry (µm)	This study	Other reports about *Oncicola* sp.		
	Rio de Janeiro	*Oncicola venezuelensis* (Piauí. Brazil) Santos et al. 2017	*Oncicola venezuelensis* (St. John Island. U. S. Virgin Islands) Fuller, Nickol 2011	*Oncicola venezuelensis* (Venezuela) Marteu. 1977
	Male (n=5)	Male (n=3)	Male (n=10)	Male (n=2)
V - Base length	20-60 37.78 ± 14.45	NR NR	NR NR	NR NR
VI - Lenght	50-110 92 ± 16.57	95-100 NR	74-82 77	60-75 NR
VI- Base length	20-80 36 ± 17.17	NR NR	NR NR	NR NR
Neck lenght	200-300 254 ± 40.7	220-270 245	250-302 289	500 NR
Neck width	200-550 424± 122.08	200 NR	384-422 396	620 NR
Cerebral ganglion length	140-190 157.5 ± 22.17	NR NR	110-149 131	NR NR
Cerebral ganglion width	100-140 124 ± 17.07	NR NR	62-86 73	NR NR
Anterior testis length	500-660 593.33 ± 68.23	930-1180 NR	NR NR	1800-2000 NR
Anterior testis width	370-550 460 ± 64.81	500-600 NR	NR NR	720 NR
Posterior testis length	540-730 608.33 ± 66.99	1040-1250 NR	NR NR	NR NR
Posterior testis width	300-580 430 ± 92.38	600-680 NR	NR NR	NR NR
Cement glands length	570-800 712 ± 77.04	600-700 NR	528-672 595	850 NR
Cement glands width	420-950 500 ± 244.86	550-850 NR	269-288 281	350 NR
Safftigen's pouch length	600-790 670 ± 70.59	1150-1200 NR	1056-1392 1264	NR NR
Safftigen's pouch width	310-600 466 ± 109.10	550-650 NR	288-480 384	NR NR
Copulatory bursa length	300-520 398.33 ± 73.35	1500 NR	NR NR	NR NR

NR: Not reported.

Table 2. Continued...

Biometry (µm)	This study	Other reports about *Oncicola* sp.		
	Rio de Janeiro	*Oncicola venezuelensis* (Piauí. Brazil) Santos et al. 2017	*Oncicola venezuelensis* (St. John Island. U. S. Virgin Islands) Fuller, Nickol 2011	*Oncicola venezuelensis* (Venezuela) Marteu. 1977
	Male (n=5)	Male (n=3)	Male (n=10)	Male (n=2)
Copulatory bursa width	210-620	550	NR	NR
	356.67 ± 131.23	NR	NR	NR

NR: Not reported.

Table 3. Helminth species and genera used in the phylogenetic analysis of this study.

Species	Family	Genbank acess number	Genbank Reference
Oncicola venezuelensis	Oligacanthorhynchidae	KU521566	Santos et al. (2017)
Oncicola sp.	Oligacanthorhynchidae	AF416416	Garcia-Varela et al. (2003) Unpublished
Macracanthorhynchus ingens	Oligacanthorhynchidae	AF416414.1	Garcia-Varela et al. (2003) Unpublished
Mediorhynchus sp.	Gigantorhynchydae	AF416413	Garcia-Varela et al. (2003) Unpublished
Acanthosentis cheni	Quadrigyridae	JX960752	Song et al. (2013) Unpublished
Neoechinorhynchus roseum	Neoechinorhynchidae	FJ388981	Martínez-Aquino et al. (2009)
Neoechinorhynchus emyditoides	Neoechinorhynchidae	KC004175	Pinacho-Pinacho et al. (2013) Unpublished
Neoechinorhynchus schmidti	Neoechinorhynchidae	KC004173	Pinacho-Pinacho et al. (2013) Unpublished
Pseudoacanthocephalus nguyenthileae	Echinorhynchidae	KC491890	Tkach et al. (2013)
Pseudoacanthocephalus nickoli	Echinorhynchidae	KC491884	Tkach et al. (2013)
Polymorphus minutus	Polymorphidae	AY532067	Garcia-Varela et al. (2005)
Polymorphus altmani	Polymorphidae	AY532066	Garcia-Varela et al. (2005)
Moniliformis moniliformis	Moniliformidae	AF416415	Garcia-Varela et al. (2003) Unpublished
Heteroxynema cucullatum	Heteroxynematidae	MH011309	Bell et al., 2018
Necator americanus	Ancylostomatidae	MH6658431	Monteiro et al. (2018)

wide (2010-2400). Proboscis globular, 490 µm long (450-550) by 596 µm wide (580-600) in males and 520 µm long (500-520) and 602 µm wide (550-600) in females with six rows of six hooks each. Proboscis receptacle single walled, 1334 µm long (1300-1400) in males and 1362 µm long (1300-1450) in females (Figure 2D). Cerebral ganglion, males 157.5 µm long by 124 µm wide and females 152 µm long by 112 µm wide. Neck short, males 254 µm long (200-300) by 424 µm wide (200-550) and females 314 µm long (250-400) by 500 wide (496-504). Long tubular lemnisci reaching posterior portion of trunk, occasionally rolledinged up. Leminisci in the males had 9700 µm long and in the females 10100 µm long medium (Figure 2).

Males: Reproductive tract in posterior half of trunk. Testes elliptical, in tandem. Anterior testis 593 µm long (500-600) by 460 µm wide (370-550). Eight cement glands in pairs 712 µm long (570-800) by 500 µm wide (420-950) and Safftigen pouch 670 µm long (600-790) by 466 µm wide (310-600). Copulatory bursa 398 µm long (300-520) and 356 µm wide (210-620) (Figure 2A).

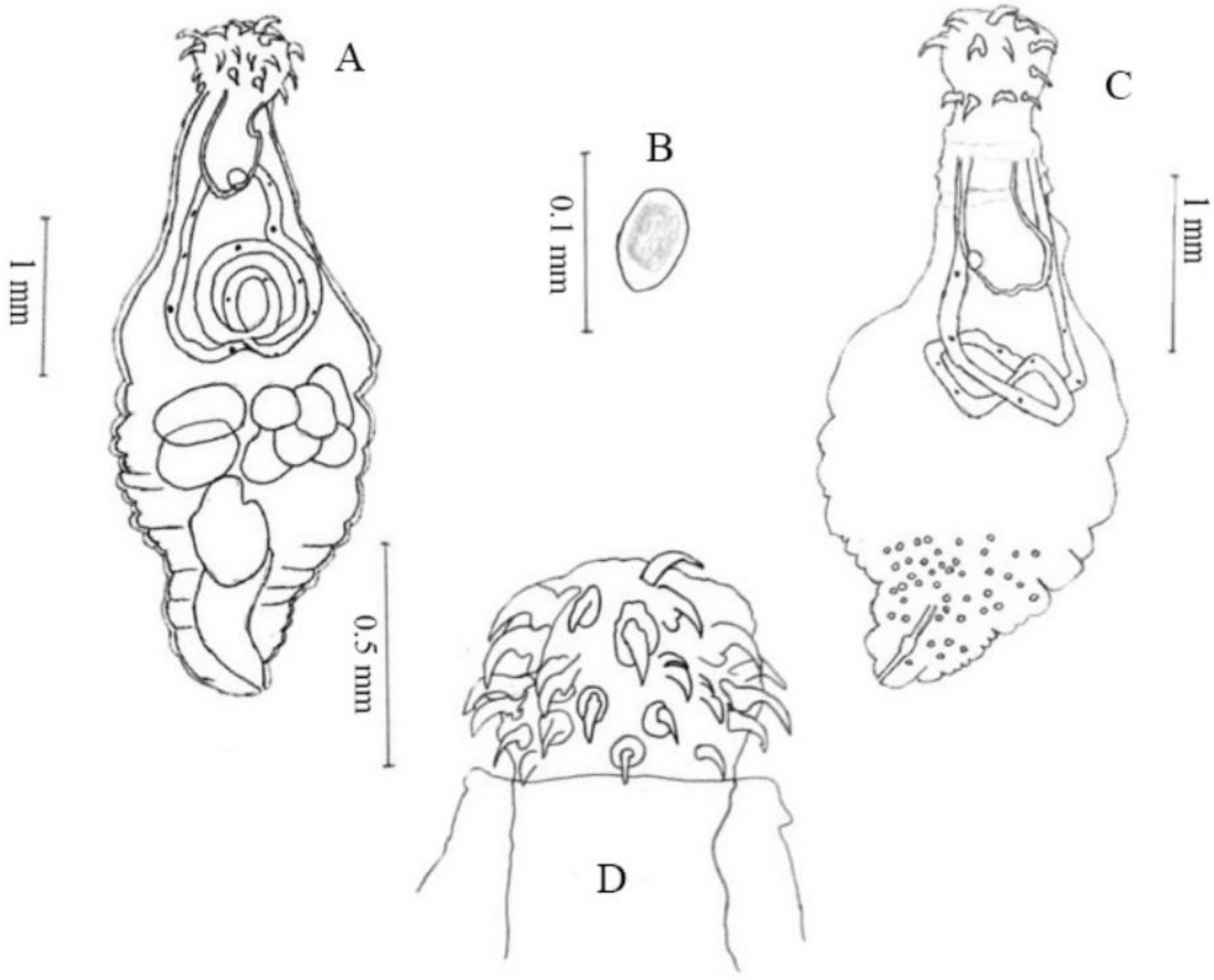

Figure 2. Line drawings of mature *Oncicola venezuelensis* from *Puma concolor*, in Rio de Janeiro. (A) Entire male; (B) Egg detected in sediment stool; (C) Outline showing shape of a gravid female; (D) Proboscis of a female.

Females: Uterine bell 535 µm long (300-650) and 125.53 µm wide (103.6-150), uterus 611 µm long (480-750), uterine bell, vagina with sphincter 212 µm long (220-370) and genital pore in the posterior region (Figure 2C). The eggs were generally very pale and slightly oval, with a very delicate external membrane with medium 59.8 µm long by 39.4 µm wide (Figure 2B). The measurements on the females, eggs and males are reported in Table 2 .

Macroscopically, adults partially attached to serous membrane intestinal wall could be seen and in mucous content (Figure 3A, B). Adults attached to the mucosa were surrounded by fibrous connective tissue (Figures 3C). Histopathological slides stained with hematoxylin-eosin showed that the parasites were attached by means of the proboscis and its hooks surrounded by collagenous tissue (Figures 3D, E). Parasite eggs are surrounded by host tissue (Figure 3F). Adult were deeply attached as far as the muscle layer (Figure 3G). The results from the morphological analysis, measurements on the parasites made it possible to identify them as members of the genus *Oncicola*.

Molecular study

After alignment of the nucleotide sequence obtained in the present study with other sequences of the phylum Acanthocephala retrieved from GenBank, it could be seen that the present sequence had 99% similarity with a sequence of *Oncicola venezuelensis* that had been recovered from a specimen of *Leopardus pardalis* in Serra da Capivara, Piauí (Figure 4).

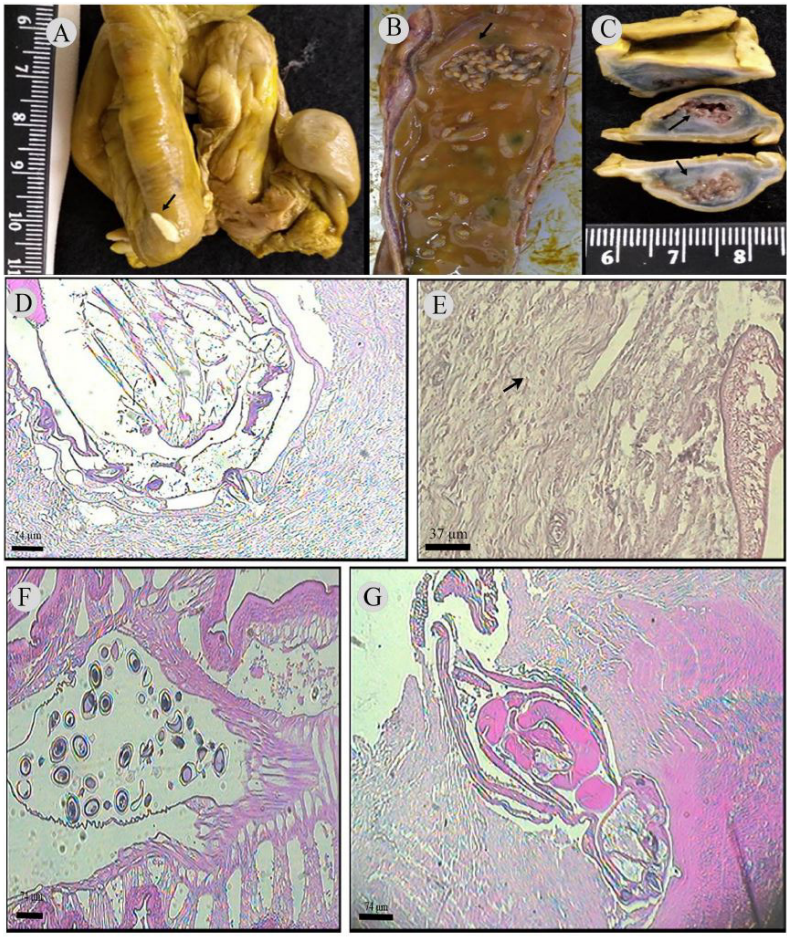

Figure 3. (A) Adult specimen attached to the serous membrane of the intestinal wall; (B) Segment jejunum with mucous content and specimens of adult attached to the mucosa; (C) Cut surface of a nodule located close to the pylorus region with parasites; (D) Histological section in which the proboscis with its hooks can be seen surrounded by collagenous tissue; (E). Collagenous tissue, in 400 x, observed in figure D; (F) Histological section through the nodule with parasite eggs surrounded by granuloma; (G) Histological section through a fragment of jejunum, in which the adult form of the parasite can be seen to be deeply attached to in muscle layer; (D-G) Hematoxylin and eosin staining.

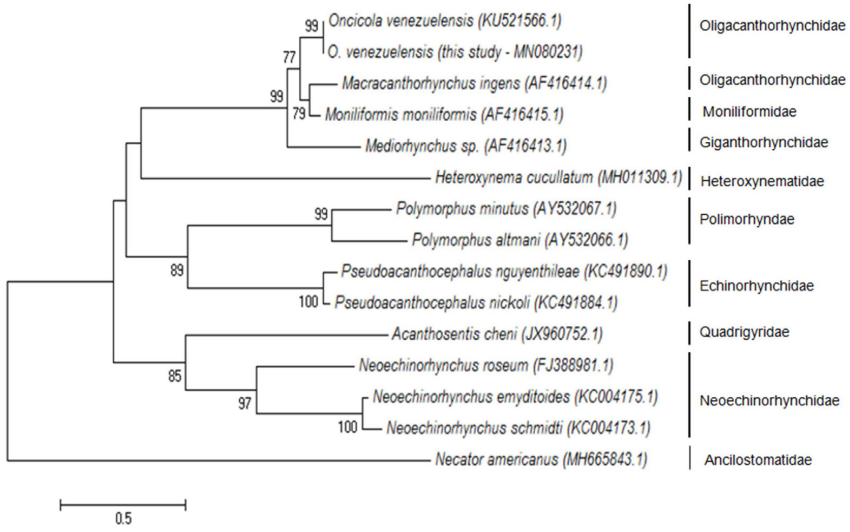

Figure 4. Maximum likelihood (ML) algorithm used with the Tamura Nei model that was based on the gene sequence obtained from the ITS1-5.8S-ITS2 region, compared with reference sequences from different species of helminths of the phylum Acanthocephala that had been detected in different animas, and from *Necator americanus*, obtained from GenBank. The numbers associated with the branches refer to the bootstrap values for 5000 replications.

Discussion

In this study, it could be seen that the helminths presented morphology compatible with the family Oligacanthorhynchidae and genus *Oncicola*, since they had a globous body in the anterior region, a spherical proboscis with six rows of hooks, a proboscis receptacle on a single wall, inserted into the base of the proboscis, and presence of a cerebral ganglion. It was also observed that the males had cement glands. However, it was difficult to quantify the number of cement glands. This fact also reported by Yamaguti (1963). *Oncicola* hooks were strongly attached to the mucosa of the small intestine of the felid. This characteristic was similar to what was described by Richardson (2013) and Núñez & Drago (2017). Through histopathology it was possible to confirm the proboscis' deep insertion in tissues, reaching the muscle layer. Unfortunately, through histopathology it was not possible to characterize the inflammatory cells in the most of the tissue, because of the autolysis process. The autolysis process occurred because the carcass was found at random in a Conservation Unit by a person, who had no experience in forensic analysis, so it is not known how long the carcass was exposed in the environment. This type of situation ends up being a reality evidenced in studies with wild animals in free living, highlighting the importance and rarity of the information that is generated with this type of material.

The eggs found in the present study had a delicate clear external shell membrane. This was morphologically similar to what was described by Yamaguti (1963) for the genus *Oncicola* and by Santos et al. (2017) and Fuller & Nickol (2011) for the species *O. venezuelensis*, which were reported infecting, an ocelot in Piauí, Brazil, and a feral cat in the U.S. Virgin Islands, respectively.

In addition, tubular lemnisci were observed extending to the posterior trunk where they tapered and rolled up. Not much is known about the importance of lemnisci, but these structures may have a function relating to transportation of fluids to the proboscis, with importance in the hydraulic system for its eversion (Núñez & Drago, 2017). In the present study, the lemnisci were long: around 9700 µm in males and 10000 µm in females. They occupied a large portion of the parasite's body. Fuller & Nickol (2011) also reported similar measurement for lemnisci in *O. venezuelensis*, of around 10000 µm.

According to Marteau (1977), few authors have reported measurements on lemnisci in species of the genus *Oncicola*. Nonetheless, this structure is fundamental in microscopy for classifying this parasite. This author provided the first description of *O. venezuelensis* infecting *L. pardalis* in Venezuela and also reported characteristics of the lemnisci that were similar to what was found in the present study, with compatible length measurements of around 10000 to 12000 µm.

Measurements of the proboscis were compatible with described by Santos et al. (2017) and Marteau (1977), who reported that the proboscis was around 500 to 550 µm in length by 600 to 620 µm in width. However, measurements the proboscis of specimens from wild felids in Brazil, both in Rio de Janeiro and in Piauí, as well as from an ocelot in Venezuela, were slightly bigger than those from worms infecting feral cats in the United States by Fuller & Nickol (2011). Changes in biometry of worms may be related to geographical locations and hosts species (Barbosa et al., 2017). Morphological examination of our specimens suggested that they might belong to the species *O. venezuelensis*. In order to ensure correct identification and study the phylogenetic interrelationships of these specimens we performed a molecular analysis using universal eukaryote primers that amplified the ITS1-5.8S-ITS2 region (Chen et al., 2010). In general, the sequence analyzed was within the Acanthocephala group next to *Macracanthorhynchus*, a genus that is also inserted into the same *Oncicola* family, Oligacanthorhynchidae. The sequence analyzed showed 99% identity with the species *O. venezuelensis*. The high branch support in the phylogenetic tree as well as the pairwise comparison evidence confirmed the identity of our specimens as *O. venezuelensis*.

The histopathological analysis on the tissues showed that the parasites were strongly attached to the deep tissue layers of the small intestine. In some places, they were surrounded by collagenous tissue, thus suggesting chronic infection. Despite the strong insertion of the parasite in the host tissue with the proboscis, in this report it was not possible to characterize the inflammatory cells, since the tissue was already very autolyzed, since the feline carcass was found in the environment for an undetermined period of the time. Although this finding is rare, Núñez & Drago (2017) emphasized that when high parasite loads are present, acanthocephalans may cross the intestinal wall, thus causing the death of the host, since they carry bacteria that can reach the peritoneal cavity.

This is the second report of *O. venezuelensis* in Brazil. However, this is the first description of this infection in *P. concolor*. It is important to emphasize that the number of nucleotide sequences in the phylum Acanthocephala deposited in GenBank remains very small. This is especially so regarding the genus *Oncicola*, which draws attention to the need to conduct molecular studies to phylogenetically validate the 24 species of *Oncicola*, which have only been reported through morphological descriptions.

Acknowledgements

We would like to thank the National Park of Serra dos Órgãos, the University Center of Serra dos Órgãos and the Primatology Center of the State of Rio de Janeiro for supplying the helminths and tissues, which were fundamental for conducting this study.

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