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HELMINTHS OF THE NUTRIA *MYOCASTOR COYPUS* (RODENTIA: MYOCASTORIDAE) IN THE FOREST OF ARAUCARIAS, BRAZIL

HELMINTOS DE NUTRIA *MYOCASTOR COYPUS* (RODENTIA: MYOCASTORIDAE) EN LA MATA DE ARAUCARIAS, BRASIL

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ABSTRACT

The nutria (*Myocastor coypus* Molina, 1782) can significantly affect ecosystems, resulting in adverse socioeconomic and health consequences for humans and animals. The present study identified the helminth fauna of *M. coypus* from a region of Mata de Araucaria in the State of Paraná, Brazil. Three free-living adult males that were found dead in the rural zone of this municipality were used. Anatomical segments from the digestive tract, respiratory tract, heart and kidneys were sieved and the material was fixed in Railliet and Henry solution. The helminths were identified and counted and the infection indicators were analyzed descriptively. A total of 341 helminths was collected belonging to the Orders and Families Trichostrongylidea: Heligmonelidae; Cestoda: Hymenolepididae; and Digenea: Notocotylidae. The prevalence, parasite intensity and abundance of the two first helminth species were observed in the animals evaluated. The region of study represents a new locality record for the three species diagnosed. *Myocastor coypus* is a new registered host for *Rondentolepis octocoronata* Linstow, 1879 in Brazil. These data contribute to the knowledge of the helminth fauna of free-living *M. coypus* in the Brazilian Araucaria Forest region.

Keywords: Brazilian Araucaria Forest – Helminth fauna – Indicators of infection – Neotropical Region – Rodents – Wild

RESUMEN

La nutria (*Myocastor coypus* Molina, 1782) puede afectar significativamente los ecosistemas, resultando en consecuencias socioeconómicas y de salud adversas para los seres humanos y los animales. El presente trabajo identificó la helmintofauna de *M. coypus* oriundos de una región de Mata de Araucária en el Estado de Paraná, Brasil. Se utilizaron tres animales de vida libre, adultos, machos, encontrados muertos en el área rural del municipio de Dos Vizinhos-PR. Los segmentos anatómicos del tracto digestivo, respiratorio, cardíaco y renal fueron tamizados y el contenido fijado en solución de Railliet y Henry. Después de la identificación y recuento de los helmintos, se elaboró un análisis descriptivo de los indicadores de infección. Se recogieron 341 helmintos, pertenecientes al Orden y Familia: Trichostrongylidea: Heligmonelidae, Cestoda: Hymenolepididae y Digenea: Notocotylidae. La prevalencia, intensidad y abundancia parasitaria media de las dos primeras especies de helmintos fueron las mayores observadas en los animales evaluados. La región de estudio representa un nuevo registro de localidad para las tres especies diagnosticadas. *Myocastor coypus* es un nuevo huésped registrado para *Rondentolepis octocoronata* Linstow, 1879, en Brasil. Estos datos contribuyen al conocimiento de la helmintofauna de *M. coypus* de vida libre en la región de la Mata de Araucária Brasileña.

Palavras clave: Helmintofauna – indicadores de infección – Mata de Araucária Brasileña – Región Neotropical – Roedores – salvaje

INTRODUCTION

Coypus (*Myocastor coypus* Molina, 1782) are rodents belonging to the family Capromyidae, natives to the South America (Woods *et al*., 1992; Carter & Leonard, 2002). They live on the banks of rivers and lakes and are herbivores with nocturnal habits (Gosling & Baker, 1981; Rodrigues & Ferigolo, 2004). They are considered opportunistic animals, known to use different food sources, depending on the vegetation present in the region, are general herbivores capable of consuming up to 25% of their body mass daily, using both aquatic and terrestrial vegetation (Gosling & Baker, 1981; Christen, 1978; Guichón *et al*., 2003). They cause adverse impacts on the ecosystem such as disruption of ecological processes, competition with native species for resources and reduction of biodiversity (Elton, 1958; Mack & D'antonio, 1998).

The Araucaria forest, a typical phytophysiognomy of the Southern Region of Brazil and also found in the highest relief points of the Southeastern Region, whose characteristic species is the Paraná Pine (*Araucaria angustifolia*), (IBGE, 2012) presents remnants dispersed in small capons, some of which are still conserved characteristics of primary forest with a flora of Pteridophytes own being part of this ecosystem. Predominates the subtropical climate, which presents/screens rigorous winters and hot summers, with rainfall indexes relatively high and well distributed during the year (Basso, 2010, IBGE, 2012).

The species of parasites and indicators of parasitic infection (prevalence, abundance, mean intensity and variation of intensity) observed in *M. coypus* vary according to the geographical region they inhabit, it is considered that cold temperatures are a limiting factor for the geographical distribution of the populations of coypus (Baroch *et al*., 2002). Microbial and endoparasite infections can cause considerable mortality, especially in times of high population density. Endoparasites rarely kill their host but may reduce the ability of coypus populations to slow population growth (Sheffels, 2013; Zanzani *et al*., 2016).

The helminth fauna of *M. coypus* has been described in studies conducted in North America, Europe and some countries of South America. Cases of natural infection with the following have been described: *Graphidioides myocastoris* (Babero, 1979), *Trichuris myocastoris* (Baruš *et al.* 1975), *Dipetalonema* sp. (Grassi, 1890), *Hippocrepis myocastoris* (Cabello & Kinoed, 1979), *Rodentolepis* sp. (Yamaguti, 1959), *Heligmosomoides* sp. (Büchner, 1889),

Strongyloides myopotami (Artigas & Pacheco, 1933), *Trichuris* sp. (Roederer, 1761), *Capillaria hepatica* (Bancroft, 1893), *Trichostrongylus colubriformis* (Leiper, 1909), *Trichostrongylus* sp. (Leiper, 1909), *Trichostrongylus duretteae* (Leiper, 1909), *Rodentolepis avetjanae* (Akhumian, 1956), *Anoplocephala* sp. (Goeze, 1782), *Hymenolepis octocoronata* (Linstow, 1879), *Dicrocoelium lanceolatum* (Braun, 1902), *Dicrocoelium* sp. (Braun, 1902) and *Fasciola hepatica* (Linnaeus, 1758) (Wenzel, 1982; Silva *et al*., 2007; El-Kouba, *et al*., 2009; Guerreiro *et al*., 2014). In Brazil, infection by *Hippocrepis fuelleborni* (Travassos & Vogelsang, 1930) and *Trichuris travassoi* (Linnaeus , 1771) has been described, along with infection by the protozoa *Giardia* sp. (Kunstler, 1882), *Cryptosporidium* sp. (Levine, 1984) and *Cystoisospora* sp. (Frenkel, 1977) (Silva *et al*., 2007; Sheffels & Sytma, 2007; Rocha *et al*., 2012).

Thus, because of the need to expand knowledge regarding helminth parasites of *Myocastor coypus* in Brazil, the present study had the objective of identifying the helminth fauna of coypus from the region of Forest in Araucaria.

MATERIALS AND METHODS

Study area
The research was conducted in the Araucarias Forest, located in the southwestern region of the state of Paraná, between the parallels 25° 44 'and 5°S and the meridians 53°3' and 31º W, in the municipality of Dois Vizinhos, Paraná, Brazil (Fig. 1).

The Subtropical Forest, also known as Forest in the Araucaria, Pine tree of Paraná among other names, has preference in areas of higher altitude (above 500m), where the temperature is milder. Although Araucaria predominates, other vegetables such as Imbuia and Mate herb are also associated with it. Currently it is reduced to approximately 3% of the initial area (IBGE, 2012).

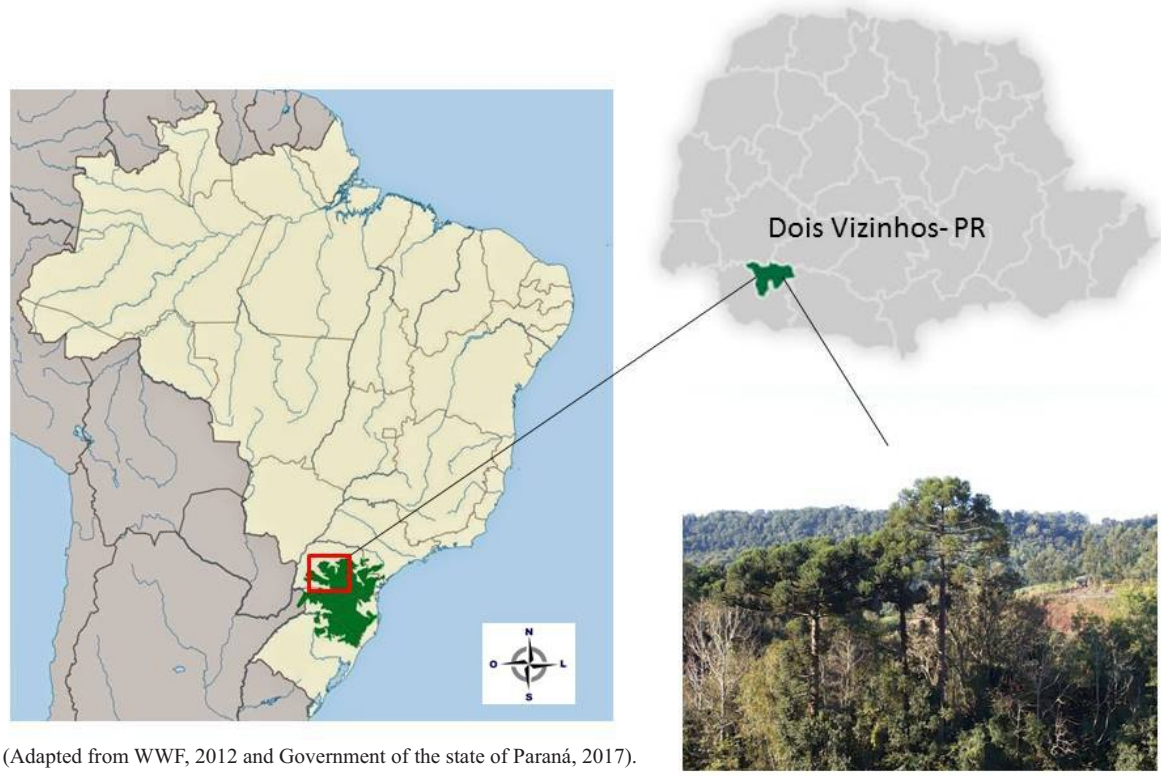
Animals
In the period from 2013 to 2014, three free-living male adults (*M. coypus*) were found dead in the rural area of the municipality of Dois Vizinhos, Southwest of the State of Paraná, and donated by rural producers to the Laboratory of Parasitology of the Federal University of the Fronteira Sul, Campus Realeza-PR.

Parasitological methods
The animals were stored in a freezer at -20 °C until the time of necropsy. Then the specimens were thawed at room temperature. Necropsies were performed at the Laboratory of Parasitology of the Federal University of the Fronteira Sul, Campus Realeza - PR. The anatomical segments of the digestive tract (esophagus, stomach, small intestine and large intestine), along with the trachea, heart, lung, liver, pancreas and kidneys, were sectioned separately and the material obtained was sieved using a Tyler 100 mesh and was fixed in Railliet & Henry solution, for subsequent identification of the helminths with the aid of a stereoscopic microscope.

The helminths were diaphanized in 80% acetic acid solution and, if necessary, diaphanized in beech creosote for studies in optical microscopy on temporary slides. For each species, at least 10 specimens of each sex were measured. In case of species with few specimens, all specimens obtained were evaluated. To obtain morphometric and morphological characteristics, we used a clear camera equipped microscope and the images of the specimens obtained with an Olympus BX-51 microscope equipped with a Q-Color 3 digital camera and the images were processed by the image analyzer software Image-Pro Plus 4.0 ,

The taxonomic identification of helminths was made following keys proposed by Linstow (1879), Travassos & Vogelsang (1930) and Artigas & Pacheco (1933). After identification and counting of the helminths, a descriptive analysis of the infection indicators according to Bush et al. (1997). Specimens of each species diagnosed were deposited in the collection of the Laboratory of Parasitology of the Federal University of the Fronteira Sul, Campus Realeza-PR.

Ethical issues
This study was approved through the Sistema de Autorização e Informação em Biodiversidade - SISBIO (Protocol N°. 45.720) and the Ethics Committee for Animal Use UFFS/Realeza (Protocol N°. 23205.005173/2014-81).



(Adapted from WWF, 2012 and Government of the state of Paraná, 2017).

Figure 1. Map representing the Araucaria Forest in Brazil, highlighting the Municipality of Dois Vizinhos, PR – Brazil.

RESULTS

A total of 341 specimens of three species representing the groups Digenea, Cestoidea and Nematoda were identified. The three species that were identified as parasitizing *M. coypus* in the study area were *Pudica maldonadoi* (Trichostrongylidea: Heligmonelidae), *Rodentolepis octocoronata (*Cestoda: Hymenolepididae) and *Hippocrepis fuelleborni* (Diginea: Notocotylidae) (Table 1).

The nematode *P. maldonadoi* was the most abundant species among the samples, with the higher prevalence, mean intensity and abundance rates. Although it presented the same prevalence as the cestode *R. octocoronata*, it was observed that the latter presented abundance and mean intensity lower than the former. The digenetic *H. fuelleborni* was the species with the lowest descriptions of infections among the animals evaluated.

The region of study represents a new record of locality for the three species of helminths diagnosed. Still, *M.coypus* is a new registered host for *R. octocoronata* in Brazil.

***Pudica maldonadoi* Artigas & Pacheco, 1933 *(*Trichostrongyloidea: Heligmonelidae)**

Thin, spiraled nematode was attenuated anterior ending; mouth small, with poorly visible papillae. Thick cuticle along the body was transversal striated and in cross-section, at midbody 12 cuticular crests with uniform striations may be observes. The male body length is 4.98 mm ± 0.40, width 0.069 mm ± 8.5. The cephalic vesicle is 0.074 mm ± 0.0095, excretory pore is 0.55 mm ± 0.17 (from the anterior ending). Long, and the esophagus is 0.43 mm ± 0.012, long and the nerve ring is 0.21 mm ± 0.022. From the anterior ending the copulatory pouch is asymmetrical, and the thin spicules are thin, is 0.46 mm ± 0.1 in length. The female is longer than the male, with total length of 10.5 mm ± 0.06, and 0.11 mm ± 0.008 wide. The vulva is at 0.13 m ± 0.023, from tail anus. Distance from the anus to the posterior extremity is 0.05 mm ± 0.026, with simple genital apparatus and acute conical tail, without a terminal spine. The caudal

Table 1. Helminth species and descriptor of infection indicators identified in three adults coypus (*Myocastor coypus*) that originated from the municipality of Dois Vizinhos, state of Paraná, Brazil.

Helminths	Site of infection	Prevalence	Abundance	Mean intensity	Range of intensity	N°
Nematoda						
Trichostrongyloidea	Small intestine	100	65.3	65.3	11 - 54	196
Pudica maldonadoi						
Cestoda						
Hymenolepididae	Small intestine	100	37	37	24 - 55	111
Rodentolepis octocoronata						
Digenea						
Notocotylidae	Large intestine	66.6	8	2	2 -12	34
Hippocrepis fuelleborni						

extremity presents an intumescent cuticle at the level of the vulva that forms dorsal a foreskin-shaped fold (Fig. 2).
Taxonomic summary:
Host: *Myocastor coypus*
Location of infection: Small intestine
Locality: Dois Vizinhos, state of Paraná, Brazil
Prevalence: 100%
Abundance: 65.3 parasites/host
Mean intensity: 65.3 parasites/host
Variation of intensity:11 to 54 parasites

***Rodentolepis octocoronata* Linstow, 1879 (Cestoidea, Hymenolepididae)**

Scolex was four oval suction cups and a face with eight prominent hooks, meander 0.062 mm in lenght. The cirrus pouch is perpendicular is the proglottids. The cirrus is long and tangled. Each proglottids has a uterus and three well-developed testicles (Fig. 3).
Taxonomic summary:
Host: *Myocastor coypus*

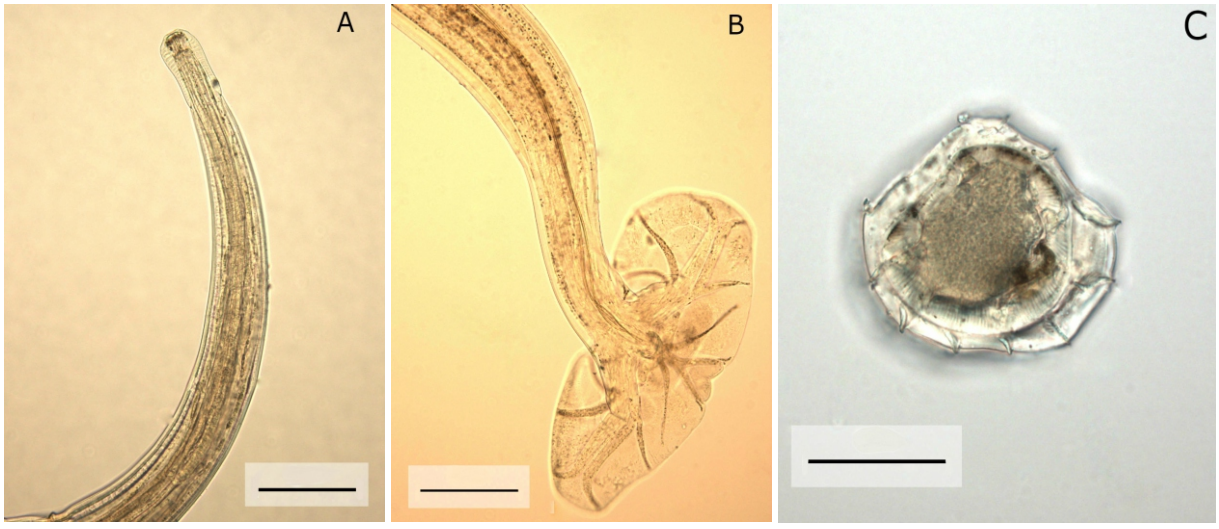


Figure 2. Morphology of *Pudica maldonadoi*: **A)** Copulatory bursa of the male; **B)** Anterior extremity; **C)** Cross-section through the synlophe. Scale bar: A and C: 50µm, B: 100µm.

Location of infection: Small intestine
Locality: Dois Vizinhos, state of Paraná, Brazil
Prevalence: 100%
Abundance: 37 parasites/host
Mean intensity: 37 parasites/host
Variation of intensity: 24 to 55 parasites

***Hippocrepis fuelleborni* Travassos & Vogelsang, 1930 (Digenea, Notocotylidae)**

Elongated body was 3.02 mm ± 1.14 in length, the anterior with is 0.33 mm ± 0.09, becoming widow at middle is 0.47 mm ± 0.19, and posterior portion of 0.55 mm ± 0.16. The oral sucker is 0.27 ± 0.08 x 0.29 ± 0.09 mm was the presence of ventral papillae. Pharynx absent and esophagus short. Male reproductive system with presence of elongated cirrus pouch, with small spines measuring 0.49 mm ± 0.16 in length. The testicules are lobates and displace diagonary. The left testicle is 0.23 ± 0.14 x 0.22 ± 0.16 mm and the right testicle is 0.21 ± 0.18 x 0.25 ± 0.15 mm. The insertion ceca join together in the end portion between the testicles. The female reproductive system is formed by a well-developed uterus and pre-testicular ovary of 0.17 ± 0.13 x 0.16 ± 0.14 mm (Fig. 4).

Taxonomic summary:
Host: *Myocastor coypus*
Location of infection: Large intestine
Locality: Dois Vizinhos, state of Paraná, Brazil
Prevalence: 66.6%
Abundance: 8 parasites/host
Mean intensity: 12 parasites/host
Variation of intensity: 2 to 22 parasites

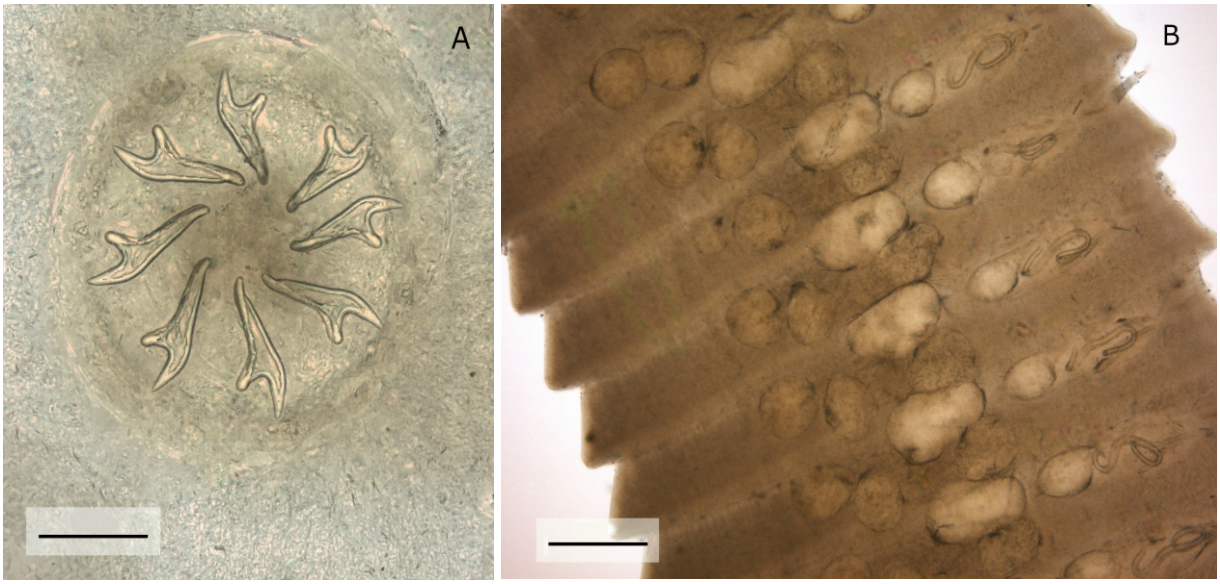


Figure 3. Morphology of *Rodentolepis octocoronata*: **A)** Presence of eight thorns; **B)** Immature proglottid. Scale bar: A: 50µm, B: 250 µm.

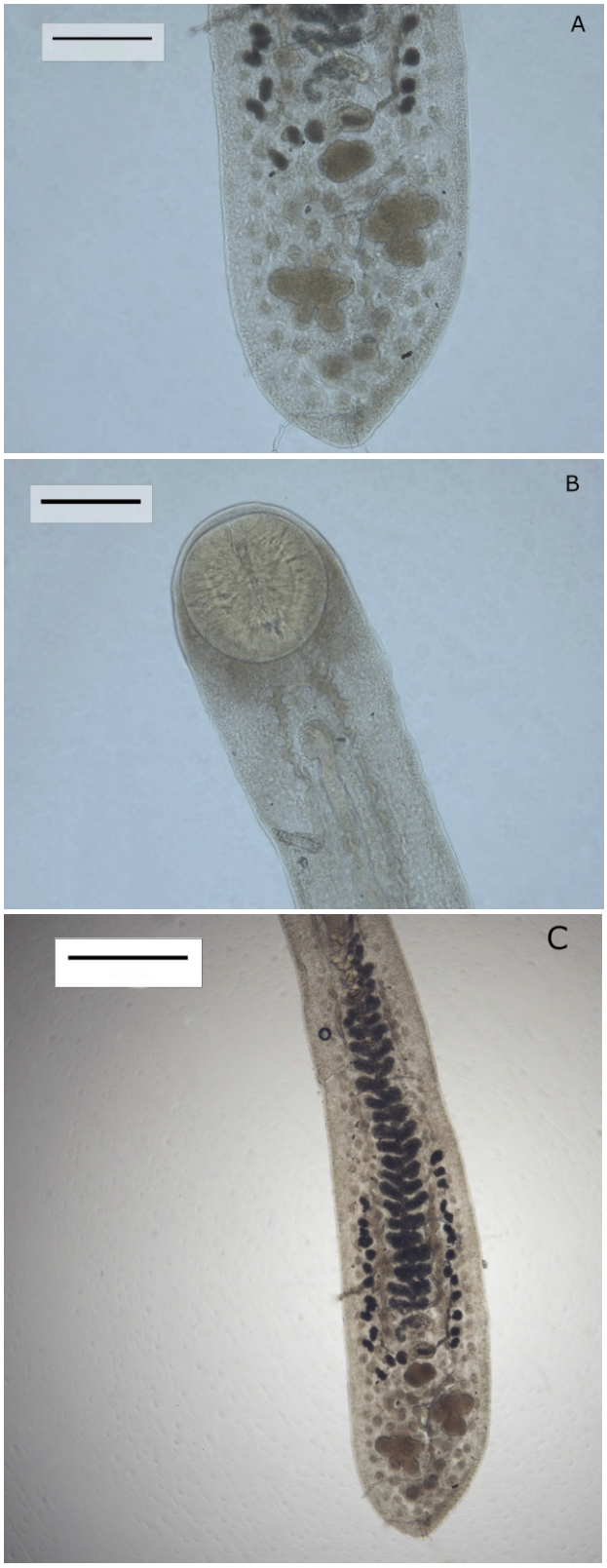


Figure 4. *Hippocrepis fuelleborni*: **A)** and **C)** Posterior extremity – reproductive system; **B)** Anterior extremity. Scale bar: A and B: 300µm, C: 500 µm.

DISCUSSION

The nematode *P. maldonadoi* was the most abundant species among the samples, with the higher prevalence, mean intensity and abundance rates. Although it presented the same prevalence as the cestode *R. octocoronata*, it was observed that the latter presented lower abundance and mean intensity. The digenean *H. fuelleborni* was the species with the lowest descriptors of infection among the animals evaluated. The region of study represents a new locality record for the three parasite species diagnosed. Nevertheless, *M.coypus* is a new host record for *R. octocoronata*.

Higher prevalence of nematodes than of other classes of helminths parasitizing coypus was also reported in a study conducted in Buenos Aires (Martino *et al*., 2012). Most cestode parasites of free-living coypus are considered to be only mildly pathogenic towards their hosts. However, because the biological cycle of trematodes and cestodes necessarily involves freshwater invertebrates, and mollusks or fish form intermediate hosts, coypus can easily become infected by these classes of helminths, consequent to their habitat and diet (Wenzel, 1982).

In Brazil, the nematode *P. maldonadoi* was only previously described in the state of Rio Grande do Sul (Artigas & Pacheco, 1933). There have been other reports of parasitism due to this trichostrongylid in Argentina (Sutton, 1974) and the United States (Babero & Lee, 1961). The species *R. octocoronata* was first described in Europe as *Hymenolepis octocoronata* (Guerreiro *et al*., 2014) and was recently reported in coypu populations in Argentina. In Brazil, the present report provides the first record of infection of *M. coypus* by *R. octocoronata*. Cases of parasitism of coypus caused by *H. fuelleborni* were recorded in Germany (Linstow, 1879) and in the state of Rio Grande do Sul, where the infection of the host was correlated with ingestion of plant material containing metacercariae of this digenean (Rocha *et al*., 2012).

The most prevalent helminths in populations of *M. coypus* in South America include the digenean *Hippocrepis myocastoris*, cestodes of the genus *Rodentolepis* and the nematodes *Dipetalonema* sp., *Graphidioides myocastoris* and *Trichuris myocastoris*. However, in Argentina, the most prevalent digenean found in coypus was *Fasciola hepatica* (Artigas & Pacheco, 1933). It is important to emphasize that the helminths identified in *M. coypus* in the municipality of Dois Vizinhos, Paraná, were specific parasites for this host. There are still no records in the literature regarding the presence of these helminths parasitizing other species of mammals.

The helminthological fauna of *M. coypus* reported in the United States comprises high diversity of parasites for this host, including 11 species of digeneans, 21 species of cestodes, one species of Acanthocephala and 31 species of nematodes (Babero & Lee, 1961). However, in the present study, only three species of helminths parasitizing *M. coypus* in the region studied were observed. This variation of diversity and prevalence of parasites for this host, obtained in the present study, may have been due to climatic factors, the presence and abundance of intermediate hosts, the social status of the population studied or even the animals' immune response (Sinkoc *et al.,* 2009).

This study describes the helminths of coypus (*Myocastor coypus*) in the municipality of Dois Vizinhos, Paraná, Brazil. These data contribute to the knowledge of the helminth fauna of wild *M. coypus* in the primary habitat of this species and register the first occurrence of *Rodentolepis octocoronata* in coypus in Brazil.

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