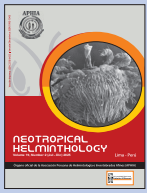
ISSN Versión Impresa 2218-6425 ISSN Versión Electrónica 1995-1403

Neotropical Helminthology, 2025, vol. 19 (2), 189-198

Neotropical Helminthology



ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

DIOCTOPHYME RENALE (GOEZE, 1782) (NEMATODA: DIOCTOPHYMATIDAE) THIRD-STAGE LARVAE IN CRAB-EATING RACCOON (*PROCYON CANCRIVORUS*) AND WHITE-EARED OPOSSUM (*DIDELPHIS ALBIVENTRIS*) IN SOUTHERN BRAZIL

LARVAS DE TERCER ESTADIO DE *DIOCTOPHYME RENALE* (GOEZE, 1782) (NEMATODA: DIOCTOPHYMATIDAE) EN MAPACHE CANGREJERO (*PROCYON CANCRIVORUS*) Y COMADREJA OVERA (*DIDELPHIS ALBIVENTRIS*) EN EL SUR DE BRASIL

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ABSTRACT

Dioctophyme renale (Goeze, 1782) is a parasitic nematode that infects the kidneys of wild and domestic carnivores, including humans. The parasite is highly prevalent in southern Brazil, affecting a wide range of wild and domestic animals. While the high rates of parasitosis in stray and semi-domesticated dogs in the region are well documented, the dynamics of the parasite's life cycle and the intermediate (freshwater invertebrates) and definitive hosts are not completely known. This study aimed to report two new wild hosts for *D. renale* third-stage larvae in southern Brazil. The study was

DOI: https://dx.doi.org/10.62429/rnh20251921971

conducted in Capão do Leão, Rio Grande do Sul, Brazil. Three road-killed mammals, including one *Procyon cancrivorus* (G. Cuvier, 1798) and two *Didelphis albiventris* Lund, 1840, were collected and sent to the Federal University of Pelotas for parasitological analysis. The parasites were removed, cleaned, preserved, and identified based on morphological and morphometric characteristics. Third-stage larvae were found encysted in the serosa and gastric submucosa of *D. albiventris* (one host with 21 larvae and the other with 34 larvae) and in the stomach submucosa, liver parenchyma, and omentum of *P. cancrivorus* (infected with 3 larvae). This study reports, for the first time, natural infections of *P. cancrivorus* and *D. albiventris* by *D. renale*. Although adult parasites were not detected, the potential of these species to act as definitive or paratenic hosts cannot be dismissed. The findings support previous studies on larval migration involving the stomach and liver before reaching the peritoneal cavity. The study also highlights the relevance of dioctophymosis in the region, where environmental factors, stray dog populations, and interactions between wild and domestic animals may facilitate transmission. Overall, these findings enhance understanding of *D. renale*'s host range and underscore the need for continued surveillance in wild and urban environments in southern Brazil.

Keywords: dioctophymosis – giant kidney worm – helminths – natural infection – wild carnivores – zoonosis

RESUMEN

Dioctophyme renale (Goeze, 1782) es un nematodo parásito que infecta los riñones de carnívoros silvestres y domésticos, incluidos los humanos. El parásito es altamente prevalente en el sur de Brasil, afectando a una amplia gama de animales silvestres y domésticos. Si bien las altas tasas de parasitosis en perros callejeros y semidomesticados en la región están bien documentadas, la dinámica del ciclo de vida del parásito y los hospederos intermediários (invertebrados de agua dulce) y definitivos no se conocen completamente. Este estudio tuvo como objetivo reportar dos nuevos hospederos silvestres para larvas de tercer estadio de *D. renale* en el sur de Brasil. El estudio fue realizado en Capão do Leão, Rio Grande do Sul, Brasil. Se recolectaron tres mamíferos atropellados, incluyendo un *Procyon cancrivorus* (G. Cuvier, 1798) y dos *Didelphis albiventris* Lund, 1840, que fueron enviados a la Universidad Federal de Pelotas para análisis parasitológicos. Los parásitos fueron removidos, limpiados, conservados e identificados con base en características morfológicas y morfométricas. Se encontraron larvas de tercer estadio enquistadas en la serosa y submucosa gástrica de *D. albiventris* (un hospedero con 21 larvas y otro con 34 larvas) y en la submucosa del estómago, el parénquima hepático y el epiplón de *P. cancrivorus* (infectado con 3 larvas). Este estudio reporta, por primera vez, infecciones naturales de *P. cancrivorus* y *D. albiventris* por *D. renale*. Aunque no se detectaron parásitos adultos, no se puede descartar el potencial de estas especies para actuar como hospederos definitivos o paraténicos. Los hallazgos respaldan estudios previos sobre la migración larval que involucra el estómago y el hígado antes de alcanzar la cavidad peritoneal. El estudio también destaca la relevancia de la dioctofimosis en la región, donde factores ambientales, poblaciones de perros callejeros y la interacción entre animales silvestres y domésticos pueden facilitar la transmisión. En general, estos hallazgos amplían la comprensión del espectro de hospederos de *D. renale* y subrayan la necesidad de una vigilancia continua en entornos silvestres y urbanos en el sur de Brasil.

Palabras clave: carnívoros silvestres – dioctofimosis – gusano gigante del riñón – helmintos – infección natural – zoonosis

INTRODUCTION

Dioctophyme renale (Goeze, 1782), also known as the giant kidney worm, is a cosmopolitan parasitic nematode that causes dioctophymosis. This parasite mainly infects the kidneys of wild and domestic carnivores, and can also affect humans (Reinhard & Fink, 2007; Norouzi *et al.*, 2017, Eiras *et al.*, 2021). Particularly, dioctophymosis has become a significant concern in the southern region of Brazil, due to a rising number of parasitosis cases in stray and semi-domesticated dogs (Rappeti *et al.*, 2017, Caye *et al.*, 2020a, Brunner *et al.*, 2022).

Records over time indicate a wide variety of wild animals affected by dioctophymosis worldwide, such as the raccoon (*Procyon lotor* [Linnaeus, 1758]) (Harkema & Miller, 1964), ranch mink (*Mustela vison* Schreber, 1777) (Dyer, 1998), harbor seal (*Phoca vitulina* Linnaeus, 1758) (Hoffman *et al.*, 2004), capuchin monkey (*Cebus apella* [Linnaeus, 1758]) (Ishizaki *et al.*, 2010), brown rats (*Ratus novergicus* [Berkenhout, 1769]) (Tokiwa *et al.*, 2011), red fox (*Vulpes vulpes* [Linnaeus, 1758]) (Hajialilo *et al.*, 2015), wolves (*Canis lupus* Linnaeus, 1758 and *Canis aureus* Linnaeus, 1758) (McNeill *et al.*, 1984, Eslahi *et al.*, 2021), ocelot

(*Leopardus pardalis* [Linnaeus, 1758]) (Goossen *et al.*, 2022) and pampa's fox (*Lycalopex gymnocercus* [Fischer, 1814]) (Fernández *et al.*, 2024). In Brazil, the known wild definitive hosts are: crab-eating fox (*Cerdocyon thous* [Linnaeus, 1766]) (Ribeiro *et al.*, 2009), ring-tailed coati (*Nasua nasua* [Linnaeus, 1766]) (Milanelo *et al.*, 2009), lesser grison (*Galictis cuja* [Molina, 1782]) (Pesenti *et a*l., 2012, Zabott *et al.*, 2012), maned wolf (*Chrysocyon brachyurus* (Illiger, 1815)) (Vulcani *et al.*, 2015, Oliveira *et al.*, 2021), river otter (*Lontra longicaudis* [Olfers, 1818]) (Echenique *et al.*, 2018) and geoffroy's cat (*Leopardus geoffroyi* [d'Orbigny & Gervais, 1844]) (Trindade *et al.*, 2018).

Dioctophyme renale preferentially infects the right kidney of definitive hosts (Mace & Anderson, 1975; Measures, 2001). The eggs of *D. renale* are released in urine and become embryonated in the environment, which are then ingested by freshwater invertebrates. After hatching, the larva migrates to the ventral blood vessels of the intermediate host and undergoes molts until it reaches the infective stage third-stage larva (L3). Infection in the definitive host occurs when ingesting the intermediate or paratenic host containing L3, which penetrates the stomach wall and changes to the fourth stage after five days of infection (Mace & Anderson, 1975).

The literature proposes three migration routes for L4 in the definitive host, with the most frequently observed route being the larvae's penetration in the duodenal wall and subsequent lodging in the right kidney of the host (Mace & Anderson, 1975). The second is that the larvae pass through the greater curvatures of the stomach wall and lodge in the left kidney (Woodhead, 1950). The third possibility is penetration of the lesser curvature gastric wall and lodging in the liver (Woodhead, 1950). The last molt occurs in the abdominal cavity. Mace & Anderson (1975) observed significant liver lesions 50 days after infecting mink (*Mustela vison* Schreber, 1777), suggesting that the organ may be in the migration route of larvae towards the right kidney.

Dioctophymosis rates in domestic animals in southern Brazil are high (Rappeti *et al.*, 2017, Caye *et al.*, 2020b, Brunner *et al.*, 2022) and the register of new definitive wild hosts become more common as research efforts increase (Pesenti *et al.*, 2012; Mascarenhas *et al.*, 2018, 2019, 2022, Echenique *et al.*, 2018, Trindade *et al.*, 2018, Scheer *et al.*, 2019). Despite the high rates of parasitosis in the region, the dynamics of the parasite's life cycle is not completely understood and the intermediate hosts are unknown. Some species of possible paratenic hosts have been recorded in the region including freshwater turtles (*Phrynops hilarii* [Duméril & Bibron,1835] and *Trachemys dorbigni* [Duméril & Bibron, 1835]), snake (*Philodryas patagoniensis* [Girard, 1858]) and fish (*Hoplosternun littorale* [Hancock, 1828]) (Mascarenhas *et al.*, 2016, 2018, 2019, 2022). In this context, this work aims to register two new wild hosts for *D. renale* in the south of Rio Grande do Sul, Brazil.

MATERIALS AND METHODS

Characterization of the study area

The area of concern is localized in the municipality of Capão do Leão, in the South of Rio Grande do Sul, Brazil (31°46'3''S - 52°26' 55''W). It is situated in the hydrographic basin of Piratini River and belongs to the Pampa biome. The municipality is crossed by important highways as the BR116, BR293 and the RS87. The annual rainfall is between 1300 and 1600 mm. The climate is humid subtropical, with a dry and temperate summer, with the mean annual temperature of 17ºC (Alvares *et al.*, 2013). The most of the territory is covered by grassland and pastures, wetlands and native forests areas (alluvial forest, riparian forest and submontane forest). It is characterized by the presence of wetlands.

Collection and identification of helminths

One adult male of *Procyon cancrivorus* (G. Cuvier, 1798) (Procyonidae) and two adults females of *Didelphis albiventris* Lund, 1840 (Didelphidae) were found ran over in the municipality of Capão do Leão. The carcasses were collected and sent to the Federal University of Pelotas, where they were frozen and later submitted to routine parasitological analysis, carried out between 2019 and 2020.

During the necropsies the organs and the abdominal cavity were individually analyzed. The parasites were carefully removed, cleaned in a physiological solution and preserved in ethanol 70%. The larvae were clarified with Amann lactophenol in semi-permanent slides (Hoffmann, 1987). The species identification followed the morphological and morphometric character description in Measures & Anderson (1985). The morphometry was taken in the microscope Olympus CX21 and the graphs were made in the Microsoft Excel 2024.

Voucher specimens were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz, Rio de Janeiro, Brazil (CHIOC-39681, CHIOC-39682 and CHIOC-39693).

Ethic aspects. The collection and analysis of biological material were carried out under the license nº 38913-5

issued by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

RESULTS

The two specimens of *D. albiventris* were parasitized by third-stage larvae, with an infection intensity of 21 and 34 larvae, which were found encysted in the serosa and gastric submucosa. *Procyon cancrivorous*, on the other hand, had three third-stage larvae encysted in the stomach submucosa, liver parenchyma and omentum (Figure 1).

The nematodes had a reddish color, two concentric rows of cephalic papillae (Figure 2a) and genital primordium in the anterior third of the body of the female larva, close to the esophagus-intestine junction (Figure 2b).

The morphometry based in a pair of females and one male larvae extracted from *P. cancrivorus* measured the body length of 7.17–8.82 mm and 8.34 mm, respectively. The two female larvae extracted from *D. albiventris* measured 16.6–20.23 mm and the pair of males 9.75-10.77 mm (Table 1).

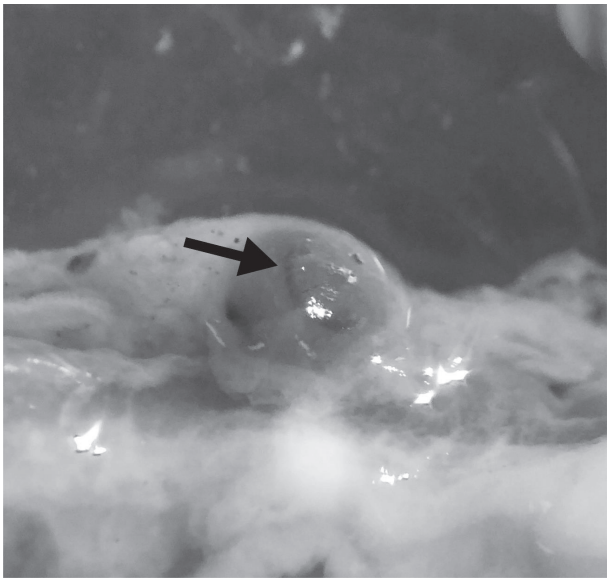

Figure 1. Liver of *Procyon cancrivorus* (Procyonidae) with an encysted *Dioctophyme renale* (Dioctophymatidae) third-stage larva (arrow).

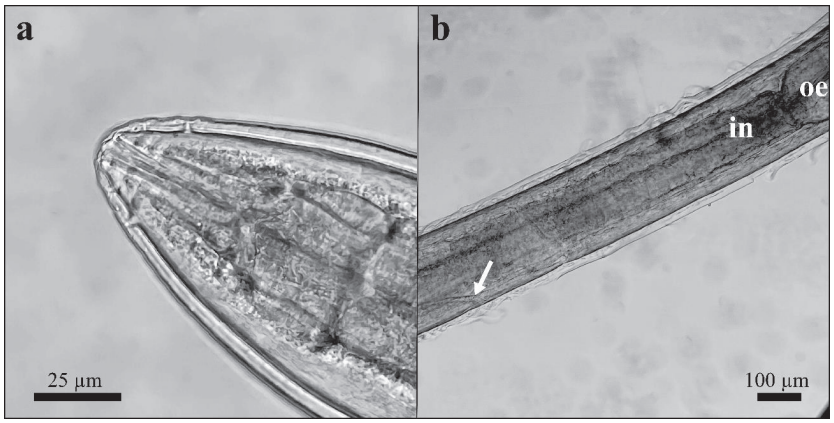

Figure 2. Microscopy of the *Dioctophyme renale* third-stage larva of a *Didelphis albiventris*. a) Anterior extremity of the larva. b) The vulvar primordium (arrow) near the intestine (in) and oesophagus (oe) junction in a female third-stage larva.

Table 1. Morphometry (mm) of third-stage larvae of *Dioctophyme renale* from *Procyon cancrivorus* and *Didelphis albiventris* in southern Rio Grande do Sul, Brazil.

	Procyon cancrivorus		*Didelphis albiventris*	
	Male (n=1)	Female (n=2)	Male (n=2)	Female (n=2)
Body length	8.34	7.17 – 8.82	9.75 – 10.77	16.6 – 20.23
Pharynx length	0.05	0.087 – 0.052	0.05 – 0.06	0.022 – 0.17
Distance from the first row of papillae to the anterior extremity	-	0.00125	0.012 – 0.015	0.03 – 0.027
Distance from the second row of papillae to the anterior extremity	-	0.0275	0.01 – 0.03	0.055 – 0.045
Distance from the nerve ring to the anterior extremity	-	0.1 – 0.087	0.07 – 0.10	0.122 – 0.102
Esophagus length	2.50	2.41 – 3.22	2.87 – 3	5.01 – 6.085
Esophagus% of the total length	29.97	33.61	27.84 – 29.49	30.18 – 30.1
Width at the esophageal-intestinal junction	0.29	0.20 – 0.202	0.14 – 0.19	0.007 – 0.122
Distance of vulvar primordium to anterior extremity	-	4.087 – 3.89	-	6.18 – 7.39
Rectum length	0.27	0.19 – 0.17	0.10 – 0.17	0.245 – 0.31

DISCUSSION

The results of this study corroborate the findings of experimental studies, which indicated the migratory route of *D. renale* larvae in definitive hosts (Woodhead, 1950; Mace & Anderson, 1975, Measures & Anderson, 1985). Mace & Anderson (1975) observed that within 24 hours after the infection in minks, *D. renale* L3 can be detected between the mucosal crypts and in the submucosa of the stomach and after five days they become coiled within the stomach wall. In the same experimental study, after a 50-day post-infection, the liver exhibited numerous lesions while the larvaer were present in the peritoneal cavity. Similarly, Measures & Anderson (1985) observed lesions within and on the surface of the liver in ferrets infected for more than 100 days, suggesting that the organ may be on the migratory route of the larvae towards the site of infection. Woodhead (1950) proposed that the larvae can penetrate the lesser or greater curvature of the stomach according to the site of infection of the adult nematode. Encysted third-stage larvae of *D. renale* was found essentially in potential paratenic hosts as *Philodryas patagoniensis* (Girard, 1858), *Hoplosternum littorale* (Hancock, 1828), *Phrynops hilarii* (Duméril & Bibron,1835), *Acanthochelys spixii* (Duméril & Bibron, 1835), *Trachemys dorbigni* (Duméril & Bibron, 1835), *Hydromedusa tectifera* Cope, 1870, *Rana pipiens* Schreber, 1782, *Ictalurus nebulosus* (Lesueur, 1819) and *Lepomis gibbosus* (Linnaeus, 1758) (Table 2). Mace & Anderson (1975), in an experimental study, found encysted larvae in *Rana pipiens* only after 25 days after the ingestion of third-stage larvae removed from the intermediate host, suggesting that the larvae do not become encysted in the first stages of the infection.

Table 2. Site of infection of the third-stage larvae of *Dioctophyme renale* in different definitive and potential paratenic host

Host species	Site of infection	Locality	Reference
Amphibians			
*Rana pipiens**	Stomach wall, peritoneal cavity, abdominal muscles (1st day); skeleticals muscles (5th day); encapsulated in the pectoral muscles, duodenal wall, legs muscles and mesentery (after 25 days of infection)	Experimental infection	Mace & Anderson (1975)
*Andrias japonicus**	Stomach wall	Japan	Hasegawa *et al.* (2002)
*Chaunus ictericus**	Colied within vesicles underneath the gastric serosa, partly in the muscular layer	Três Barras (Brazil)	Pedrassani *et al.* (2009)
Fish			
*Ictalurus nebulosus**	Encapsulated in the wall of the stomach or on the mesentery and one was in abdominal muscles.	Lake Sasajewun (Canada)	Mace & Anderson (1975)
*Lepomis gibbosus**	Encapsulated in the hypaxial musculature, epaxial musculature, stomach wall, liver, swim bladder wall and mesentery. One free larvae in the hypaxial musculature.	Algonquin Provincial Park, Ontario (Canada)	Measure & Anderson (1985)
*Hoplosternum littorale**	Encysted in the stomach serous membrane and in the celoma cavity.	Pelotas (Brazil)	Mascarenhas *et al.* (2019)
Turtles			
*Trachemys dorbigni**	Encysted in coelomic cavity; muscles; stomach (serous); mesentery; surface of various organs: heart, lung, liver, pancreas, spleen and intestines	Pelotas and Capão do Leão (Brazil)	Mascarenhas & Müller (2015)
*Phrynops hilarii**	Celomatic cavity (surface of esophagus, stomach, lung, liver, and muscles) and serous of stomach	Rio Grande do Sul Southern (Brazil)	Mascarenhas *et al.* (2022)
*Acanthochelys spixii**	Cysts in the stomach wall and large intestine	Rio Grande do Sul Southern (Brazil)	Mascarenhas *et al.* (2022)
*Hydromedusa tectifera**	Celomatic cavity (surface of stomach, and muscles) and serous of stomach	Rio Grande do Sul Southern (Brazil)	Mascarenhas *et al.* (2022)
Snakes			
*Philodryas patagoniensis**	Encysted in the coelomic cavity	Capão do Leão (Brazil)	Mascarenhas *et al.* (2018)
Birds			
*Phimosus infuscatus**	Oesophagus	Pelotas and Capão do Leão (Brazil)	Scheer *et al.* (2019)
Mammals			
*Mustela vison***	Coiled in the stomach wall and peritoneal cavity	Experimental infection	Mace & Anderson (1975)

* Potential paratenic hosts.
** Definitive hosts.

The larvae morphometry from *P. cancrivorus* and *D. albiventris* were compared with the morphometric data from *D. renale* L3 from paratenic and intermediate hosts in the Figure 3. The mean length of the larvae extracted from *P. cancrivorus* was similar to the intermediate host (*L. variegatus*) (Mace & Anderson, 1975), smaller than larvae described in freshwater turtles (Mascarenhas *et al.*, 2015), snake (Mascarenhas *et al.*, 2018) and anurans (Pedrassani *et al.*, 2009), and larger than those described in fishes (Mascarenhas *et al.*, 2019). Whereas the third-stage larvae extracted from *D. albiventris* were longer than the ones found in the *P. cancrivorus* and other hosts, but smaller than the ones extracted from *T. dorbigni* (Mascarenhas *et al.*, 2015). The third-stage *D. renale* larvae shows a significant variation in respect of the body total length. This variation may be related to the increasing in size of the larvae as the infection progress over time (Mace & Anderson, 1975), by the distinct physiological conditions of the host, the host

immunity, or to the intrinsic parasite-host relationship. Further research should be carried out to evaluate the statistical significance of the morphometric data among hosts and its implications.

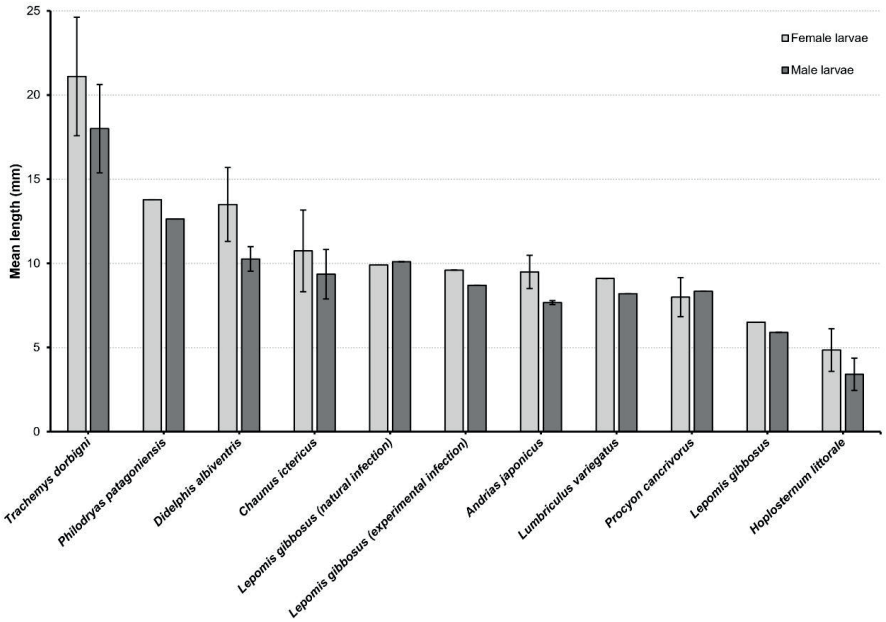

Figure 3. Mean length and standard deviation (when available) of the Dioctophyme renale third-stage larvae extracted from Trachemys dorbigni (Mascarenhas & Müller, 2015), Philodryas patagoniensis (Mascarenhas et al., 2018), Didelphis albiventris (present study), Chaunus ictericus (Pedrassani et al., 2009), Lepomis gibbosus (Measures & Anderson, 1985), Andrias japonicus (Hasegawa et al. 2002), Lumbriculus variegatus, Lepomis gibbosus (Mace & Anderson, 1975), Procyon cancrivorus (present study) and Hoplosternum littorale (Mascarenhas et al., 2019).

The role of *P. cancrivorus* and *D. albiventris* in the dioctofimatosis cycle is unknown. *Didelphis albiventris* is an abundant synanthropic species frequently impacted by road accidents in the Rio Grande do Sul state (Tres *et al.*, 2024), which has facilitated the study of its parasites. However, despite examining many specimens during our laboratory routine, this is the first recorded finding of *D. renale* in this host species. Additionally, other studies assessing the parasites diversity of *D. albiventris* in various locations (Silva & Costa, 1999; Zabott *et al.*, 2017; Cirino *et al.*, 2022) have been conducted, and none have identified an adult *D. renale* parasitizing this marsupial. Therefore, the findings in this study may reflect either an accidental infection or the early stages of infection in a definitive or paratenic host. Although the adult parasite was not found in *P. cancrivorus* and *D. albiventris* the viability of them as definitive hosts cannot be discarded.

However, the recent findings highlight the importance of dioctophymosis in the southern region of Rio Grande do Sul, particularly its presence in the wild environment (Mascarenhas *et al.*, 2018, 2019, 2022, Scheer *et al.*, 2019). The increasing prevalence of the parasite in the Pelotas and Capão do Leão areas is a cause for concern, particularly as the disease primarily affects stray and semi-domesticated dogs (Rappeti *et al.*, 2017, Caye *et al.*, 2020a, Brunner *et al.*, 2022). The maintenance of the dioctophymosis cycle in the region is favored by geographic factors of the cities of Pelotas and Capão do Leão (Mascarenhas *et al.*, 2019, 2022). The south of Rio Grande do Sul is characterized by the large presence of water bodies and wetlands, in addition to the mild climate most of the year (Sosinski, 2009; Wrege *et al.*, 2012). These municipalities also have a large population of stray and semi-domesticated dogs, the main ones affected by dioctophymosis due to the lack of selective eating habits, the hunting behavior, and frequent intake of water from open ditches (Pedrassani *et al.*, 2017, Caye *et al.*, 2020b). The wild environment associated to the high population of stray domestic animals may also favor the maintenance of the dioctophymosis cycle in the region (Pesenti *et al.*, 2012, Mascarenhas *et al.*, 2018, 2022, 2019, Trindade *et al.*, 2018, Caye *et al.*, 2020a), as a bidirectional transmission between the wild life and domestic animal has been evidenced in the Southern South America (Arce *et al.*, 2024). However, the role of wildlife to the high prevalence of dioctophymosis in urban areas remains unknown, as well as the intermediate hosts and the ecological dynamics that sustain its transmission in the region.

In this study we recorded morphometric aspects of *D. renale* third-stage larvae found in naturally infected wild mammals, *P. cancrivorus* and *D. albiventris*, which are

reported for the first time as hosts of *D. renale*. The record expands our knowledge of the biology of the nematode and the epidemiology of the parasitosis in southern Brazil.

ACKNOWLEDGMENTS

This study was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Postdoctoral fellowships were granted by the Programa Nacional de Pós-Doutorado (PNPD) at the Programa de Pós-Graduação em Microbiologia e Parasitologia, Universidade Federal de Pelotas (PPGMPar/UFPel), to CSM (2014–2019) and MRPM (2015–2020). Doctoral scholarships were also provided by CAPES to PRP and MACT (2019–2023), within the Programa de Pós-Graduação em Microbiologia e Parasitologia, Universidade Federal de Pelotas (PPGMPar/UFPel).

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Formal Analysis: PRP
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Methodology: PRP, MACT, CSM
Project administration: GM, CBO
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Software: PRP, MACT, CSM, GM, CBO
Supervision: GM, CBO
Validation: CSM
Visualization: PRP
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Received May 23, 2025.

Accepted July 19, 2025.