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Tese

**Fauna parasitária de animais silvestres no Sul do Brasil: epidemiologia e
identificação molecular**

Julia Somavilla Lignon

Pelotas, 2025

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identificação molecular**

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***“Educação não transforma o mundo. Educação muda as pessoas.
Pessoas transformam o mundo.” Paulo Freire***

Resumo

LIGNON, Julia Somavilla. **Fauna parasitária de animais silvestres no Sul do Brasil: epidemiologia e identificação molecular**. 2025. 151f. Tese (Doutorado em Ciências) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2025.

Os animais silvestres podem ser hospedeiros e/ou reservatórios de diversas doenças parasitárias, incluindo as zoonóticas, com potencial impacto significativo na saúde pública e na conservação das espécies. Nesse sentido, objetivou-se pesquisar e identificar a fauna parasitária de animais silvestres na microrregião de Pelotas, no Sul do Rio Grande do Sul, Brasil. Para tanto, carcaças de animais silvestres atropelados foram coletadas em rodovias da região e submetidas a necropsias. Fragmentos de tecidos foram amostrados e seu DNA foi extraído. Endo e ectoparasitos foram coletados e identificados morfolologicamente e molecularmente. Amostras de fezes também foram analisadas através das técnicas de Centrífugo Flutuação com Sulfato de Zinco, Sedimentação Espontânea e Esporulação de Oocistos. Percebeu-se que 87,80% dos animais coletados estavam infectados por helmintos, 51,21% infestados por ectoparasitos e 48,78% foram afetados por ambos os tipos de parasitos. Ainda, em 69,5% das amostras de fezes, encontramos ovos de helmintos e/ou cistos/oocistos de protozoários e 64,9% das amostras positivas estavam parasitadas por pelo menos um morfogrupo com agentes zoonótico. Também não foram detectadas evidências da presença do DNA de *Leishmania* spp. nos animais alvo na região estudada. Em contrapartida, novos registros da fauna parasitária foram identificados como os primeiros registros de *Hydatigera taeniaeformis* e *Ancylostoma caninum* infectando *Leopardus geoffroyi* em todo o mundo; E primeiros registros da ocorrência de *Contracaecum australe* infectando *Phalacrocorax brasilianus* e *Rhipicephalus microplus* infestando *Ozotoceros bezoarticus*, ambos no sul do Brasil. As novas informações contribuem para o conhecimento sobre a fauna parasitária de animais silvestres no Sul do Brasil e ao redor do mundo, e indicam a necessidade de estudos epidemiológicos contínuos acerca do tema nesse grupo de hospedeiros. Essa investigação é crucial para o bem-estar e conservação de animais selvagens, populações domésticas e humanos.

Palavras-chave: Epidemiologia; Ectoparasitos; Endoparasitos; Saúde única; Fauna silvestre

Abstract

LIGNON, Julia Somavilla. **Parasitic fauna of wild animals in Southern Brazil: epidemiology and molecular identification**. 2025. 151f. Thesis (Doctor degree in Sciences) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2025.

Wild animals can be hosts and/or reservoirs of several parasitic diseases, including zoonotic ones, with potentially significant impact on public health and species conservation. In this sense, the objective was to research and identify the parasitic fauna of wild animals in the microregion of Pelotas, in the south of Rio Grande do Sul, Brazil. For this purpose, carcasses of wild animals run over were collected on highways in the region and submitted to necropsies. Tissue fragments were sampled, and their DNA was extracted. Endo- and ectoparasites were collected and identified morphologically and molecularly. Fecal samples were also analyzed using the techniques of Centrifugal Flotation with Zinc Sulfate, Spontaneous Sedimentation and Oocyst Sporulation. It was observed that 87.80% of the animals collected were infected by helminths, 51.21% were infested by ectoparasites and 48.78% were affected by both types of parasites. Furthermore, in 69.5% of the fecal samples, we found helminth eggs and/or protozoan cysts/oocysts and 64.9% of the positive samples were parasitized by at least one morphogroup with zoonotic agents. Also, no evidence of the presence of *Leishmania* spp. DNA was detected in the target animals in the studied region. On the other hand, new records of parasitic fauna were identified such as the first records of *Hydatigera taeniaeformis* and *Ancylostoma caninum* infecting *Leopardus geoffroyi* worldwide; These are the first records of the occurrence of *Contracaecum australe* infecting *Phalacrocorax brasilianus* and *Rhipicephalus microplus* infesting *Ozotoceros bezoarticus*, both in southern Brazil. The new information contributes to the knowledge about the parasitic fauna of wild animals in southern Brazil and around the world and indicates the need for continued epidemiological studies on the subject in this group of hosts. Such research is crucial for the well-being and conservation of wild animals, domestic populations, and humans.

Keywords: Epidemiology; Ectoparasites; Endoparasites; One Health; Wildlife

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Lista de Abreviaturas e Siglas

BLAST	Basic Local Alignment Search Tool
BSF	Brazilian Spotted Fever
bp	Pares de base
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
DNA	Ácido desoxirribonucleico
F	Fêmea
Km ²	Quilômetro quadrado
L	Comprimento
M	Macho
mL	Mililitro
mM	Micromolar
mm	Milímetro
n	Número
NCBI	National Center for Biotechnology Information
ng	Nanograma
nm	Nanômetro
PCR	Reação em Cadeia da Polimerase
RNA	Ácido ribonucleico
RS	Rio Grande do Sul
U	Unidade
UFPeI	Universidade Federal de Pelotas
µm	Micrômetro
µL	Microlitro
µg	Micrograma
USA	Estados Unidos da América
W	Largura

Lista de Símbolos

®	Marca registarda
™	Trademark
°C	Grau Celsius
∞	Infinito
%	Por cento

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1 Introdução

O aumento populacional humano, a crescente urbanização, a expansão agropecuária, o desflorestamento excessivo, a caça, manipulação e consumo de carne animal, além do comércio ilegal de vidas silvestres, proporcionaram um maior contato entre os humanos e os seus animais domésticos com populações de animais silvestres (ELLWANGER et al., 2020). Atualmente, cerca de 75% das doenças infecciosas emergentes do homem têm origem animal (PNUMA, 2020) e 71,8% têm origem na fauna silvestre (RIBEIRO e MEDEIROS, 2017), evidenciando que a maioria das doenças infecciosas humanas tem origem zoonótica.

Os efeitos da ação antrópica no meio silvestre e sobre a saúde humana e animal podem ser observados em diferentes regiões do mundo (MÜLLER e FUX, 2023), pois muitos *habitats* foram alterados, degradados ou ocupados. Essas alterações ambientais têm desencadeado mudanças na cadeia epidemiológica de transmissão de alguns patógenos, particularmente os de caráter zoonótico, atrelado à participação de animais silvestres, sinantrópicos, domésticos e até mesmo o ser humano no ciclo epidemiológico destes agentes (LIMA, 2018). As suas consequências perturbam o equilíbrio dos ecossistemas, promovendo a propagação de doenças entre espécies — um fenômeno conhecido como transbordamento zoonótico, que pode ter um impacto significativo na saúde única (TAYLOR et al., 2001; PLOWRIGHT et al., 2017; ELLWANGER et al., 2020).

Dentre as doenças que afetam a vida silvestre, as infecções de origem parasitária são consideradas importantes bioindicadores do estado atual dos ecossistemas, e são utilizadas para avaliar a disseminação de patógenos e mudanças comportamentais (LYMBERY, 2005). Os animais silvestres podem ser reservatórios e portadores de diversas doenças parasitárias, incluindo as zoonóticas, com potencial impacto significativo na saúde pública, na conservação da vida selvagem e nos aspectos econômicos (CLEAVELAND et al., 2001).

A magnitude do problema de saúde dessas enfermidades e as suas complexas epidemiologias, apontam a necessidade da identificação de todos os elos da cadeia de transmissão. A compreensão de cada um dos focos naturais, verificando-se a

circulação destes agentes entre os animais silvestres, auxilia na implementação de estratégias efetivas de controle e é essencial para o entendimento da importância local, regional ou nacional das doenças que causam (BARBOSA et al., 2011). Esse conhecimento subsidia as ações dos serviços veterinários e de saúde pública, a fim de aplicar condutas estratégicas eficazes e sustentáveis para a vigilância das enfermidades, na tentativa de reduzir a frequência de transmissão de patógenos de animais silvestres para humanos, e assim, minimizar o risco de novos surtos, epidemias e pandemias (BARBOSA et al., 2011; ELLWANGER e CHIES, 2021).

Objetivos

Objetivo geral:

Pesquisar e identificar a fauna parasitária de animais silvestres na microrregião de Pelotas, no sul do Rio Grande do Sul, Brasil.

Objetivos específicos:

- Pesquisar o DNA de *Leishmania* spp. em amostras de tecidos, medula óssea e sangue de animais silvestres do Sul do Rio Grande do Sul, através do uso da técnica de Reação em Cadeia da Polimerase (PCR).
- Pesquisar e identificar os endoparasitos e ectoparasitos que acometem os animais silvestres no Sul do Rio Grande do Sul, através de chaves taxonômicas e técnicas moleculares como a PCR caso necessário.
- Pesquisar e identificar a ocorrência de endoparasitos por meio de técnicas coproparasitológicas em amostras de fezes de animais silvestres, no Sul do Rio Grande do Sul, através da literatura existente.
- Mapear e identificar possíveis áreas de risco para infecção humana e animal.

2 Artigos

2.1 Artigo 1

Absence of *Leishmania* spp. DNA in road-killed wild mammals in Southern Brazil

Julia Somavilla Lignon, Diego Moscarelli Pinto, Mariana Accorsi Teles, Maira Aparecida Christello Trindade, Priscila Rockenbach Portela, Silvia Gonzalez Monteiro, Kauê Rodriguez Martins, Rodrigo Casquero Cunha, Felipe Geraldo Pappen, Bianca Conrad Bohm, Fábio Raphael Pascoti Bruhn

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**Absence of *Leishmania* spp. DNA in road-killed wild mammals in Southern
Brazil**

Ausência de DNA de *Leishmania* spp. em mamíferos silvestres atropelados no Sul
do Brasil

Absence of *Leishmania* spp. in Southern Brazil

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Abstract

Leishmaniasis are neglected diseases transmitted by vectors that affect domestic and wild animals, including humans. Due to its incidence and lethality, this zoonosis is a worrying public health problem, making it essential to identify all links in the transmission chain. Infection of wild mammals by *Leishmania* spp. remains poorly understood, especially in southern Brazil. Therefore, the objective was to research, using the PCR technique, the presence of *Leishmania* spp. DNA in road-killed wild mammals in Southern Brazil. Carcasses of 96 animals were collected from highways in the Pelotas microregion, Rio Grande do Sul, southern Brazil and subjected to necropsies. Tissue fragments (spleen, skin, liver, kidney, heart, lung, lymph nodes, bone marrow and blood) were collected and genomic DNA was extracted. PCR protocols targeting the ITS1, kDNA and 18S genes were tested. We found no evidence of *Leishmania* spp. circulation in the studied population. However, epidemiological studies like this one are of great relevance, as they allow monitoring of the occurrence of pathogens and help identify possible risk areas. As these animals act as epidemiological markers for the presence of the microorganism, studies must be carried out continuously to understand whether there are sources of infection in the region.

Keywords: Leishmaniasis, Protozoa, PCR, One Health, Zoonosis.

Resumo

As leishmanioses são doenças negligenciadas transmitidas por vetores que acometem animais domésticos e silvestres, incluindo os humanos. Devido a sua incidência e letalidade, esta zoonose consiste em um problema de saúde pública preocupante sendo fundamental a identificação de todos os elos da cadeia de transmissão. A infecção de mamíferos silvestres por *Leishmania* spp. permanece pouco compreendida, especialmente no sul do Brasil. Portanto, objetivou-se pesquisar, por meio da técnica de PCR, a presença de DNA de *Leishmania* spp. em mamíferos silvestres atropelados no Sul do Brasil. Carcaças de 96 animais foram coletadas em rodovias da microrregião de Pelotas, Rio Grande do Sul, sul do Brasil e submetidas a necropsias. Fragmentos de tecidos (baço, pele, fígado, rim, coração, pulmão, linfonodos, medula óssea e sangue) foram coletados e o DNA genômico foi extraído. Protocolos de PCR visando os genes ITS1, kDNA e 18S foram testados. Não encontramos evidências de circulação de *Leishmania* spp. na população estudada. Porém, estudos epidemiológicos como este são de grande relevância, pois permitem monitorar a ocorrência de patógenos e auxiliam na identificação de possíveis áreas de risco. Como esses animais atuam como marcadores epidemiológicos da presença do microorganismo, estudos devem ser realizados continuamente para entender se existem fontes de infecção na região.

Palavras-chave: Leishmaniose, Protozoários, PCR, Saúde Única, Zoonose.

Introduction

Leishmaniasis are neglected vector-borne anthroponosis caused by infection by parasites of the genus *Leishmania* Ross, 1903 (Kinetoplastida,

Trypanosomatidae) (OPAS, 2021; Dias et al., 2022). They are heterogenic parasites whose invertebrate hosts of these protozoa are sandflies, and the vertebrate hosts include various reptiles and mammals (domestic and wild), including humans (OPAS, 2021; Lopes et al., 2023). Furthermore, there is a group of species responsible for maintaining the parasite in nature, called reservoirs. A potential reservoir differs from those that are simple hosts due to the individual persistence of the infection or infectious capacity, that is, the potential to transmit the parasite to vectors (OPAS, 2021).

Considered a complex of diseases, leishmaniasis remains one of the parasitic diseases with the greatest impact on humanity (Lopes et al., 2023). Regardless of the *Leishmania* species, infection by this parasite can be asymptomatic, as well as producing a wide spectrum of clinical manifestations, which can affect the skin, mucous membranes, and viscera. The clinical spectrum of the disease is varied and depends on the interaction of several factors related to the parasite, the vector and the host, however, the visceral form of the disease is the most serious and can lead to death in up to 90% of untreated cases (OPAS, 2021). Given its incidence and high lethality, this zoonosis is a worrying public health problem that mainly affects populations in developing countries, such as Brazil (Lopes et al., 2023), accounting for 93.5% of visceral leishmaniasis cases in the Americas (Melo et al., 2023).

Initially of wild origin and absent in the South of Brazil, leishmaniasis presented important changes in its transmission pattern and is currently found in urban centers and states in the South of the country (Santos et al., 2005; Dias et al., 2022). Some species of *Leishmania* spp. have more restricted circulation in nature. However, many of them have already adapted to urban or peri-urban cycles (e.g., *L. infantum*) (OPAS, 2021) and since 2001 the agent has been present in Rio Grande do Sul (RS), a state considered free of leishmaniasis until then, when the first autochthonous cases of cutaneous leishmaniasis in humans were reported in the cities of Santo Antônio das Missões/RS and Viamão/RS (Santos et al., 2005). In 2006, the first autochthonous case was registered in a canine in the municipality of São Borja/RS (Dias et al., 2022).

Among all new and emerging diseases, around 75% are transmitted from an intermediate animal species to humans (PNUMA, 2020) and wildlife can provide a great source of information in little-studied places. Therefore, taking into account the

magnitude of the health problem of leishmaniasis and its complex epidemiology, it is essential to identify all links in the transmission chain, in order to implement effective control strategies, which require integrated approaches such as One Health, which focuses on balancing animal, human and environmental health (PNUMA, 2020; OPAS, 2021). Understanding each source of transmission allows us to create effective and sustainable strategies for disease surveillance, for this reason, reservoir monitoring has become a focus of concern for public health bodies (OPAS, 2021), and this is necessary for health professionals to have knowledge about the epidemiology of the disease and include it in their clinical suspicions (Dias et al., 2022).

Although several studies demonstrate the infection of wild mammals by *Leishmania* spp., the transmission of these parasites in their natural cycle remains poorly understood, especially in southern Brazil. In this sense, the objective was to research, using the Polymerase Chain Reaction (PCR) technique, the presence of *Leishmania* spp. DNA in road-killed wild mammals in Southern Brazil.

Material and methods

Study area

The Pelotas microregion is situated in the south of RS, Brazil, and covers 10 municipalities: Pelotas, Capão do Leão, Pedro Osório, Cerrito, Canguçu, Morro Redondo, Turuçu, São Lourenço do Sul, Cristal and Arroio do Padre (Figure 1). The region covers an area of 10,316,601 km² for a population of 476,096 inhabitants (IBGE, 2023). The climate is subtropical, characterized by well-defined seasons with a high annual temperature range (hot summers and cold winters) in addition to well-distributed rainfall throughout the year. The region comprises the Pampa biome, with fields as the predominant landscape (Roesch et al., 2009). The region has an intense flow of people and animals.

Animal collection

Carcasses of road-killed wild animals were collected on the highways of cities belonging to the Pelotas microregion. The team traveled to three pre-established routes: Route 1 - Pelotas, Turuçu, São Lourenço do Sul and Cristal; Route 2 – Pelotas, Morro Redondo, Canguçu and Arroio do Padre; Route 3 - Pelotas, Capão do Leão,

Pedro Osório and Cerrito. Each route was carried out once a month for one year, from August 2022 to August 2023, with the starting and ending point being the city of Pelotas (31° 46' 34" S; 52° 21' 34" O), as described by Caldart et al. (2021). In addition to the driver, two veterinary medical researchers followed the routes, watching both sides of the road. Preferentially, animals with preserved and unexposed viscera were chosen, with an estimated time between one and seven hours. The collected animals were packed in plastic bags, labeled with species, sex, date, city and place where they were found and transported in isothermal boxes with ice to the laboratory of the Grupo de Estudos em Enfermidades Parasitárias at the Universidade Federal de Pelotas, where were necropsied. The species identification of the animals was confirmed according to Reis et al. (2010). Tissue fragments such as spleen, skin, liver, kidney, heart, lung, lymph nodes, bone marrow and blood from all animals were sampled and frozen at –20°C until molecular analyses were carried out, as described by Caldart et al. (2021). All study collection points were mapped using the global positioning system (GPS) with the Google Maps mobile application and inserted into the Qgis 2.14.1 software to construct the map (Figure 1).

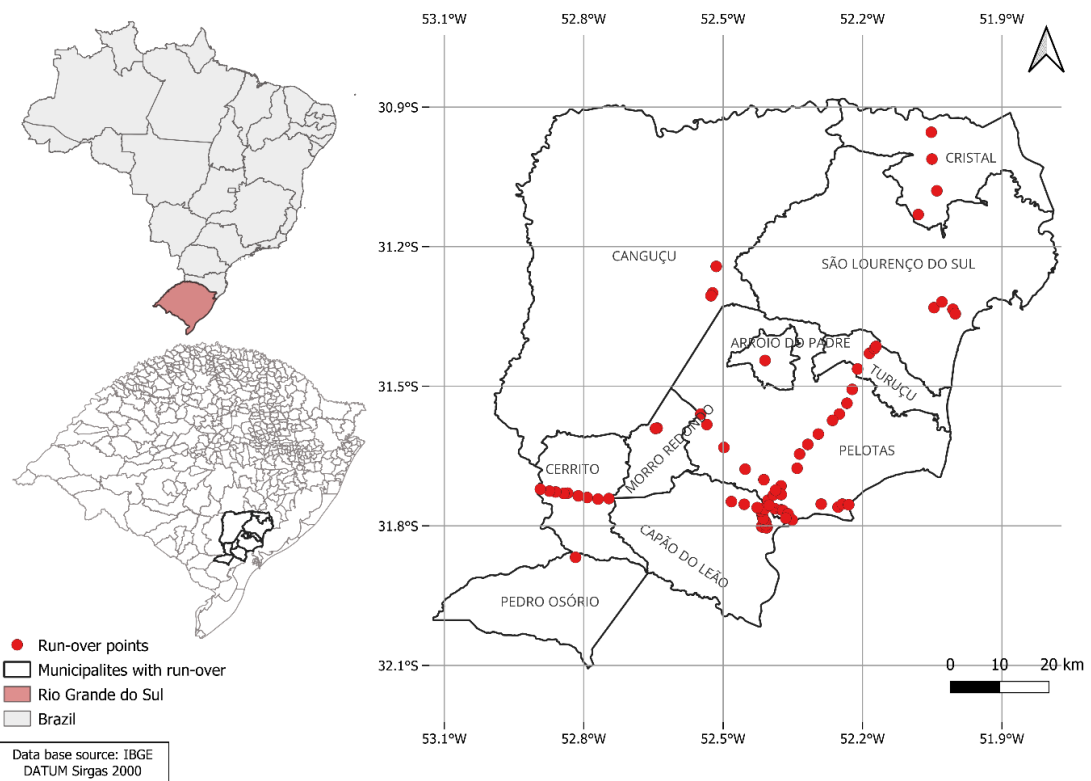


Figure 1. Run-over points where carcasses of 96 road-killed wild mammals were collected in the Pelotas microregion, southern Brazil.

DNA extraction

DNA extractions were performed with commercial kits: PetNAD™ Nucleic Acid Co-Prep Kit (GeneReach Biotechnology Corp., Taiwan, China) for blood and ID Gene™ Spin Universal Extraction Kit (ID.Vet, Grabels, France) for tissues, according to the manufacturer's instructions. The DNA samples were quantified in an ultraviolet light spectrophotometer (Thermo Scientific NanoDrop Lite Spectrophotometer, Waltham, Massachusetts, USA), to evaluate their quality by measuring their purity (260 nm/280 nm), using samples ranging from 1.8 to 2.2, and concentration in nanograms per microliter (ng/uL). Furthermore, electrophoresis was performed in 1% agarose gel to confirm the integrity of the extracted material.

Polymerase chain reaction (PCR)

For the amplification of DNA from parasites of the genus *Leishmania*, three different PCR protocols were tested according to the conditions described in Table 1. Conventional PCR was performed using the primers LITSR/L5.8SF and K13A/K13B, while nested PCR was performed using R221/R332 followed by R222/R333. In the reactions, 2.0 µL of DNA (50 ng/µL) and the mixture containing 2.0 µL of dNTP (2.5mM), 1.0 µL of each primer (10mM), 2.5µL of buffer solution (10X), 1.25 µL of MgCl₂ (50 mM), 0.25 µL of Taq DNA polymerase (5U/µL) and 15 µL of ultrapure water were used, totaling 25 µL. As a positive control, DNA from a sample known to be positive for *Leishmania braziliensis* was used, kindly provided by the Veterinary Parasitology Laboratory of the Federal University of Santa Maria. Ultrapure water was used as a negative control. The amplified products were analyzed by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide (0.5µg/mL) and visualized under ultraviolet light. A molecular weight standard of 100 bp was used (Ladder 100 bp 500 µL, Ludwig Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil).

Results and discussion

In total, carcasses of 96 animals were collected, including two *Hydrochoerus hydrochaeris*, four *Leopardus geoffroyi*, 56 *Didelphis albiventris*, one *Procyon cancrivorus*, five *Cerdocyon thous*, one *Conepatus chinga*, one *Lycalopex gymnocercus*, three *Mazama gouazoubira*, one *Euphractus sexcinctus*, two *Dasypus*

1 *novemcinctus*, 11 *Cavia aperea*, seven *Galictis cuyas*, one *Myocastor coypus* and one
2 *Coendou spinosus*. All blood and tissue samples were negative in the PCR technique.

3 *Leishmania* DNA has already been detected in wild animals in several regions
4 of Brazil (Richini-Pereira et al., 2014; Paiz et al., 2015; Caldart et al., 2021). However,
5 in the south of the country, more specifically in RS, studies are still considered scarce,
6 although cases in humans and domestic animals have increased over the years in the
7 state (Dias et al., 2022). The Pelotas microregion is considered non-endemic for
8 leishmaniasis, although human cases of visceral and cutaneous leishmaniasis have
9 been reported in recent years in the neighboring city of Rio Grande, RS (Ministério da
10 Saúde, 2023). Unofficial data indicate the occurrence of human visceral leishmaniasis
11 in the city of São Lourenço do Sul, as well as cases of canine visceral leishmaniasis in
12 Pelotas and São Lourenço do Sul (Dias et al., 2022). Cases of the disease in animals
13 often precede cases in humans, thus epidemiological surveys in non-endemic areas
14 represent an essential tool for conducting epidemiological surveillance of leishmaniasis
15 (OPAS, 2021). Early diagnosis of the disease establishment in municipalities
16 considered non-endemic allows for better planning of preventive actions aimed at
17 avoiding or minimizing problems related to this condition in areas without transmission
18 (OPAS, 2021; Dias et al., 2022). It is important to emphasize that leishmaniasis is not
19 a disease subject to eradication after its establishment and that the costs of prevention
20 are lower than those expended for disease containment, making sentinel surveillance
21 in regions considered non-endemic relevant (Dias et al., 2022), which supports the
22 importance of studying the agent in wildlife.

23 According to the literature, to date only two studies have been carried out in RS
24 with the aim of detecting the presence of *Leishmania* spp. in land mammals: one
25 involving *C. thous* (Padilha et al., 2021) and another involving *D. albiventris* (Soares,
26 2019). Both species are considered potential reservoirs of the protozoan (Azami-
27 Conesa et al., 2021; OPAS, 2021), however, marsupials stand out, as they can be
28 infected without showing clinical signs of the disease and the fact that they keep the
29 agent in their organism contributes to the maintenance of the parasite in the
30 environment (Azami-Conesa et al., 2021). Padilha et al. (2021) carried out a
31 serological survey of antibodies against zoonotic pathogens such as *L. infantum* in
32 free-ranging wild canids captured in the Pampa biome, including *C. thous*, but none of
33 the animals sampled showed the presence of antibodies. Despite the use of different

techniques, the results corroborate the present work. In contrast, Soares (2019) found a prevalence of 34% (17/50) of this protozoan in *D. albiventris* from cities such as Pelotas and Capão do Leão, in the same region as the present study, using the same diagnostic technique (PCR) and the same region target (18S gene). Likewise, the protozoan has already been detected in opossums in other Brazilian states with varying prevalence (Humberg et al., 2012; Lima et al., 2013).

However, according to Finnie (1986), animals belonging to the genus *Didelphis* are extremely adaptable to most different environments, such as forests and human civilization. Furthermore, they are nomadic animals, making it difficult to define their territory, as they travel long distances and remain in one area for relatively short periods, thus facilitating the spread of pathogens. Therefore, the authors believe that the difference in the results of the present study and the study by Soares (2019) is related to the fact that positive animals may have come from other regions that were already infected, as there are no studies that demonstrate the presence of vectors in the location studied.

Taking into account the rest of the wild animal species collected in the study, amastigote forms, antibodies or *Leishmania* DNA were detected in *D. novemcinctus* in the state of Pará, Brazil (Lainson & Shaw, 1989), in *C. chinga* in Bolivia (Telleria et al., 1999), in *C. aperea*, *P. cancrivorus* and *H. hydrochaeris* in São Paulo, Brazil (Richini-Pereira et al., 2014), in *C. cujas* in São Paulo, Brazil (Paiz et al., 2015) and in *E. sexcinctus* in Rio Grande do Norte, Brazil (Barbosa et al., 2020). We believe that the divergence between the results may be related, here too, to the absence of the vector, since most of these vertebrate species usually travel short distances. Therefore, the specimens analyzed in the study are probably native to this location and not from other regions where leishmaniasis is endemic.

Leishmania DNA was not detected in *M. gouazoubira* and *C. spinosus* in the state of Paraná, Brazil (Caldart et al., 2021) and in *M. coypus* in São Paulo (Richini-Pereira et al., 2014) and antibodies against the agent was also not found in *L. gymnocercus* in Minas Gerais (Curi et al., 2006) and Rio Grande do Sul (Padilha et al., 2021), in Brazil. These results are similar to those of the present study for these species. Still, no studies were found on the research of the protozoan in question in *L. geoffroyi* and these specimens appear to be the only ones tested to date. Considering that domestic cats are potential reservoirs of *Leishmania* (OPAS, 2021), understanding

whether free-living wild felines participate in transmission cycles is important for a better understanding of this zoonosis in the wild environment and more studies should be carried out.

We found no evidence of *Leishmania* circulation in the studied population. However, epidemiological studies like this are of great relevance as they allow monitoring of the occurrence of pathogens and help identify possible areas of risk. As these animals act as epidemiological markers for the presence of the microorganism, these studies must be continually carried out in order to understand whether there are sources of infection for humans and animals in the region.

Table 1. Main characteristics of the nucleotide sequences used in the PCR in this study.

Primer identification	Primer sequence (5'-3')	Gene	Product size (bp)	PCR conditions	References
LITSR/ L5.8S	CTGGATCATTTTCCGATG TGATACCACTTATCGCACTT	ITS1	314	Denaturation at 94°C for 2 minutes, followed by 34 cycles at 95°C for 20 seconds, 53°C for 30 seconds, 72°C for 60 seconds, final extension at 72°C for 10 minutes, and 4°C ∞.	Ranasinghe et al. (2015)
K13A/ K13B	GTGGGGGAGGGGCGTTCT ATTTTACACCAACCCCCAGTT	kDNA minicircle	120	Denaturation at 94°C for 2 minutes, followed by 40 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, final extension at 72°C for 10 minutes, and 4°C ∞.	Lachaud et al. (2002)
R221/ R332	GGTTCCTTTCCTGATTTACG GGCCGGTAAAGGCCGAATAG	18S	603	Denaturation at 94°C for 2 minutes, followed by 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, final extension at 72°C for 10 minutes, and 4°C ∞.	Caldart et al. (2021)
R222/ R333	TATTGGAGATTATGGAGCTG AAAGCGGGCGCGGTGCTG	18S	358	Denaturation at 94°C for 2 minutes, followed by 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, final extension at 72°C for 10 minutes, and 4°C ∞.	Caldart et al. (2021)

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Ethics declaration

The collection and transportation of roadkill wild animals were authorized by the Biodiversity Authorization and Information System of the Ministry of the Environment under registration 82632-3 based on Normative Instruction number 03/2014. This work was also approved by the Ethics Committee on the Use of Animals at UFPel (process number 23110.046990/2022-02).

Conflict of interest

The authors declare no conflicts of interest.

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2.2 Artigo 2

First record of *Hydatigera taeniaeformis* in Geoffroy's cat (*Leopardus geoffroyi*) in Brazil

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First record of *Hydatigera taeniaeformis* in Geoffroy's cat (*Leopardus geoffroyi*) in Brazil

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Abstract

Leopardus geoffroyi (Geoffroy's cat) is a neotropical feline considered globally threatened. In Brazil, it occurs exclusively in the Pampa biome. Its predatory habits contribute to the infection, dispersion, and continuation of the life cycle of various pathogens, including helminths, within ecosystems. However, few studies involving cestodes in wild felines are found in the literature, especially in Brazil. Therefore, we aimed to report the first case of parasitism by *Hydatigera taeniaeformis* in *L. geoffroyi*. The helminths were found in the small intestine of the necropsied feline. Specimens were analyzed morphometrically and subjected to molecular analyses for taxonomic

identification. The molecular phylogeny based on the analysis of the mitochondrial gene (COX1) allowed the identification of these parasites. Thus, this is the first description of *H. taeniaeformis* parasitizing *L. geoffroyi* in Brazil. Consequently, the number of known host species parasitized by this helminth in the country and the world is increased. Additionally, a new molecular sequence is being provided, contributing to the knowledge of *Hydatigera* in South America.

Keywords: Cestode; Wild feline; Gato-do-mato-grande; Teniasis; Rio Grande do Sul

1. Introcuccion

Leopardus geoffroyi d'Orbigny & Gervais, 1844 (Synonyms: *Felis geoffroyi*, *Oncifelis geoffroyi*) is a species of neotropical feline that lives in the subtropical and temperate regions of South America, found in countries such as Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay (Wilson and Reeder, 1993; Eisenberg and Redford, 1999). In Brazil, it is found exclusively in the south-central part of the state of Rio Grande do Sul (Oliveira and Cassaro, 2005).

Commonly known as Geoffroy's cat, the species is considered globally threatened, including within Brazil, where it is categorized as vulnerable (Almeida et al., 2013). Habitat destruction and hunting for commercial exploitation of its fur are among the main factors contributing to the species' decline (Margarido and Braga, 2004). However, retaliation for predation of domestic animals, predation by domestic dogs, and roadkill can also have an impact (Souza and Bager, 2008).

It is also known that felids are mammals adapted to predatory behavior and, as such, are susceptible to infection by various pathogens, including helminths. The consequences vary according to the parasitic species involved, host immunity, and parasite load (Monteiro, 2017), where more severe cases can cause the death of the individual. Although they may seem a minor problem for species conservation (Jorge et al., 2010), diseases can play an important role in population reduction (Funk, 2001). Conversely, carnivores help in the dispersion and continuation of the life cycle of parasites within ecosystems (Rojas et al., 2024).

Among helminths, few studies involving cestodes of the Taeniidae family Ludwig, 1886 in wild felines are found in the literature, with only the species *Taenia macrocystis* Diesing, 1850, *Taenia omissa* Luhe, 1910, *Taenia pisiformis* Bloch, 1780, and *Echinococcus oligarthrus* Diesing, 1863 being reported parasitizing *L. geoffroyi* in Brazil (Vieira et al., 2008). Therefore, we describe here the first record of parasitism by *Hydatigera taeniaeformis* Batsch, 1786 in *L. geoffroyi* in the country.

2. Material and methods

Case presentation

An adult male Geoffroy's cat (*L. geoffroyi*) that was undergoing treatment at the Wildlife Rehabilitation Center of the Federal University of Pelotas after being found run over on the highway in the municipality of Dom Pedrito, Rio Grande do Sul, died and was necropsied at the Regional Diagnostic Laboratory of the same institution. Eight specimens of cestodes with immature proglottids were found in the small intestine, which were cleaned in 0.85% saline solution and fixed in 70% ethanol solution for taxonomic identification and molecular analysis.

Morphological identification

The cestodes were stained with Carmine and mounted on permanent slides for morphological identification, as described by Amato and Amato (2010). Five specimens were analyzed morphometrically. Measurements of hooks (large and small) and suckers are given in millimeters unless otherwise indicated and are presented with minimum and maximum values (Table 1). Specimens were photographed using a Leica EZ4 HD stereomicroscope (Leica Microsystems, Wetzlar, Germany) and an optical microscope with a ZEN 2 (Blue edition) digital capture system® (Carl Zeiss Microscopy, New York, USA).

1 *DNA extraction*

2 The DNA from two cestodes was extracted using the Trizol method (Ludwig
3 Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil) according to the
4 manufacturer's instructions. DNA samples were quantified using a UV light
5 spectrophotometer (Thermo Scientific NanoDrop Lite Spectrophotometer, Waltham,
6 Massachusetts, USA), where their purity (260 nm/280 nm) was assessed - with
7 samples ranging from 1.8 to 2.2 - and their concentration in nanograms per microliter
8 (ng/μL). Additionally, to confirm the integrity of the extracted material, agarose gel
9 electrophoresis at 1% was performed.

10

11 *Polymerase Chain Reaction (PCR) and sequencing*

12 The PCR was performed using the following primers: JB3 (5'
13 TTTTGTGGGCATCCTGAGGTTTAT 3') and JB4.5 (5'
14 TAAAGAAAGAACATAATGAAAATG 3') (Bowles and McManus, 1994), amplifying a
15 fragment of approximately 420 base pairs (bp) of the mitochondrial gene subunit I of
16 cytochrome c oxidase (COX1), according to Gomez-Puerta et al. (2023). In the
17 reactions, 2.0 μL of DNA (50 ng/μL) and the mixture containing 2.0 μL of dNTP (2.5
18 mM), 1.0 μL of each primer (10 mM), 2.5 μL of buffer solution (10X), 1.25 μL of MgCl₂
19 (50 mM), 0.25 μL of Taq DNA polymerase (5U/μL), and 15 μL of ultrapure water were
20 used, totaling 25 μL. The amplifications, in a conventional thermocycler, included: initial
21 denaturation at 94°C for 2 minutes, followed by 35 cycles at 95°C for 1 minute, 50°C
22 for 1 minute, 72°C for 1 minute, final extension at 72°C for 10 minutes, and 4°C ∞. As
23 a positive control, DNA from *Taenia saginata* was used. Ultrapure water was used as
24 a negative control. The amplified products were analyzed by agarose gel
25 electrophoresis 1.5%, stained with ethidium bromide (0.5 μg/mL), and visualized under
26 ultraviolet light. A 100 bp molecular weight marker (Ladder 100 bp 500 μL, Ludwig
27 Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil) was used.

28 The amplicons were excised and purified using a Gel Purification Kit (Ludwig
29 Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil), according to the
30 manufacturer's recommendations, and submitted for sequencing using the BigDye

Terminator Cycle Sequencing v3.1 Kit (Thermo Fisher, USA) on an ABI3500 Genetic Analyzer (Applied Biosystems, USA). Consensus sequences were obtained through electropherogram analysis with Phred base calling and Phrap-assembly tool and subsequently aligned using MEGA11: Molecular Evolutionary Genetics Analysis version 11 software (Tamura et al., 2021). Multiple sequence alignment was performed using the ClustalW method. Searches for sequence similarity with sequences deposited in the National Center for Biotechnology Information (NCBI) database were conducted using the BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Evolutionary history was inferred using the Maximum Likelihood method and the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985). Evolutionary analyses were performed in MEGA11 (Tamura et al., 2021). Statistical analysis was performed by the bootstrap method with 1000 repetitions.

3. Results and discussion

In this study, the cestodes recovered from the small intestine of *L. geoffroyi* exhibited morphometric and molecular characteristics consistent with *H. taeniaeformis* (Riser, 1956; Loos-Frank, 2000; Al-Jashamy and Islam, 2007; Nakao et al., 2013; Lavikainen et al., 2016) (Table 1, Figures 1 and 2).

According to the existing literature, in *H. taeniaeformis* (Synonyms: *Taenia taeniaeformis*) adults, the scolex is composed of four large suckers found on the sides with a double crown of hooks numbering between 30 and 42 (Figure 1 - A), arranged in a circular pattern and alternating between large (Figure 1 - B) and small (Figure 1 - C) hooks. The size of the large hooks varies between 0.36-0.46 mm, while the small ones measure 0.21-0.28 mm (Loos-Frank, 2000; Al-Jashamy and Islam, 2007; Lavikainen et al., 2016) (Table 1). Behind the scolex is the neck region, which is quite small (almost nonexistent) and as wide as the scolex, recognized by the lack of a neck or as a "broad-necked" tapeworm of cats. Another observed characteristic is that immature and mature proglottids are wider than long and bell-shaped in the posterior segments. Gravid proglottids elongate (Nakao et al., 2013) but were not observed in the specimens of this study (only immature proglottids).

Based on morphological observations, the genus *Hydatigera* was treated as a junior synonym of *Taenia* Linnaeus, 1758 by Loos-Frank (2000) and Al-Jashamy and Islam (2007). According to these authors, based on all the mentioned characteristics, *H. taeniaeformis* can be clearly microscopically differentiated from all other species of *Taenia*, but mainly through the measurements and numbers of rostellar hooks (Loos-Frank, 2000). However, the use of molecular tools including the use of mitochondrial genes, such as COX1, has been used as a complement in the taxonomic identification of members of the Taeniidae family (Zhang et al., 2014; Martinez et al., 2013; Lavikainen et al., 2016; Mello et al., 2018; Gomez-Puerta et al., 2023). Evidence from DNA sequences (nuclear and mitochondrial genes) strongly supports the distinction of the genus *Hydatigera*, and thus the resurrection of the genus was proposed by Nakao et al. (2013). Currently, *Hydatigera* consists of four valid species: *H. taeniaeformis*, *H. parva* Baer, 1924, *H. krepkogorski* Schulz and Landa, 1934, and *H. kamiyai* Lavikainen et al., 2016 (Nakao et al., 2013; Lavikainen et al., 2016; Miljević et al., 2023). Furthermore, according to Lavikainen et al. (2016), *H. taeniaeformis* is a complex of three morphologically divergent entities, which can be genetically differentiated, and all three occur in the Americas (Lavikainen et al., 2016). However, these differences were not evaluated in the present study.

The sequence obtained for the COX1 gene from the cestode sample in this study was deposited in the NCBI GenBank under accession number PP860084 and showed 99-100% similarity with sequences of *H. taeniaeformis* found in GenBank under accession numbers: AB745096, OP897053, OQ786791, KT693055, MH036509, OQ569225, OQ281682, MF380375, and EF090612. Additionally, the phylogenetic study showed the positioning of the *H. taeniaeformis* isolates in the same clade (Figure 2) and distinct from all previously identified *Taenia* species parasitizing wild felids, considered for comparison purposes, supporting their distinction.

Molecular studies on *H. taeniaeformis* are considered limited in the South American continent. Martinez et al. (2013) conducted molecular analysis of *H. taeniaeformis* metacestodes collected from *Rattus norvegicus* in Argentina, analyzing the 28S ribosomal RNA and COX1 genes; however, the COX1 sequences were not deposited in GenBank. Mello et al. (2018) also molecularly analyzed larval forms collected from Ingram's squirrel (*Guerlinguetus ingrami*) in Brazil, through a different section of the COX1 gene, so the sequences from both studies could not be compared

with ours. On the other hand, sequences of *H. taeniaeformis* metacestodes recently found in small rodents from Peru (Gomez Puerta et al., 2023) and Argentina (Alonso et al., 2024) showed 99.76% and 100% identity, respectively.

Hydatigera taeniaeformis is commonly known as the feline tapeworm and has a wide geographical distribution (Al-Jashamy and Islam, 2007). The type species of its genus is the most common and widespread in domestic cats and wild felids (Lavikainen et al., 2016). Carnivores such as felids, canids, and mustelids, including domestic dogs and cats, are definitive hosts, and through their predatory behavior, ingest infected intermediate hosts such as rodents with metacestode (e.g., mice, rats, squirrels, rabbits), thereby continuing the parasite's life cycle (Nichol et al., 1981; Monteiro, 2017). This indeed explains the mode of infection in the studied animal, as this helminth has been reported in nearby locations, even in another host species (Lignon et al., 2019).

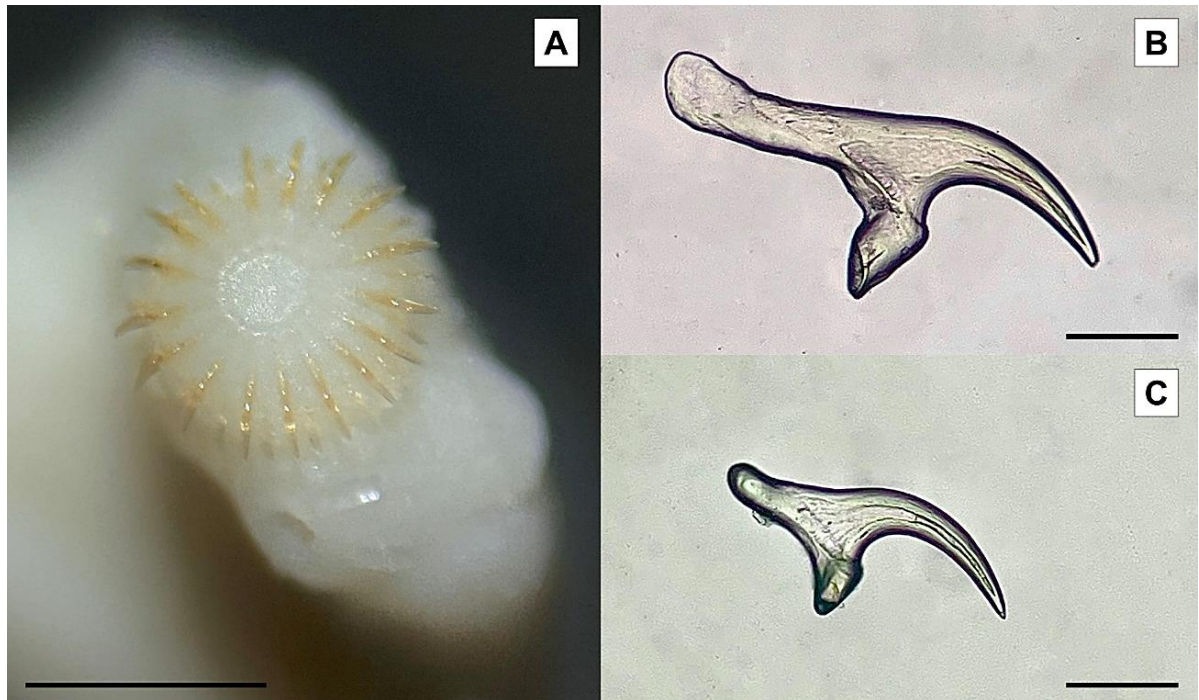
In Brazil, only four species of Taeniidae parasitizing *L. geoffroyi* have been recorded so far: *T. macrocystis*, *T. omissa*, *T. pisiformis* and *E. oligarthrus* (Vieira et al., 2008). In contrast, *H. taeniaeformis* has been identified in other felids such as domestic cats (*Felis catus*) (Wilcox et al., 2009; Lignon et al., 2019) and pumas (*Puma concolor*) (Loos-Frank, 2000; Vieira et al., 2008) in Brazil, Eurasian lynx (*Lynx lynx*) in Finland (Lavikainen et al., 2013; Lavikainen et al., 2016), and European wildcats (*Felis silvestres*) in Romania (Mederle et al., 2023). There are also sporadic reports in humans from countries such as Argentina, Sri Lanka, the Czech Republic, Denmark, and Taiwan (Stěrba and Barus, 1976; Ekanayake et al., 1999). Therefore, this is the first record of *H. taeniaeformis* parasitizing *L. geoffroyi* in Brazil, which expands the number of known host species, reinforcing the presence of the parasite and the existence of a sylvatic cycle in the region.

According to Mello et al. (2018), the discovery of *H. taeniaeformis* in wild animals may indicate the occurrence of a phenomenon known as parasite spillback, where there is transmission of infectious agents from domestic animals to wildlife (or vice versa), thus breaking down the barrier between species (Daszak et al., 2000; Ellwange and Chies, 2021). It is possible that this cestode was introduced to the South American continent through domestic cats, mediated by human intervention, since these animals did not occur in the Americas before European colonization (Lavikainen et al., 2016; Mello et al., 2018). Notably, the increasing proximity between humans and their

domestic animals with wildlife can have consequences for both populations. Generally, these adult cestodes are apathogenic, and infections are considered asymptomatic; thus, there may be undiagnosed cases (Monteiro, 2017; Taylor et al., 2017). However, studies conducted with domestic cats have reported intestinal obstruction, acute vomiting, anorexia, dyspnea, and lethargy (Wilcox et al., 2009), as well as abdominal pain, dehydration, hematochezia, and weight loss (Lignon et al., 2019), caused by this parasite. These clinical manifestations were not observed in this study, but the finding deserves attention and should serve as a warning for veterinarians at wildlife rehabilitation centers in the region, including them in their clinical suspicions.

Table 1. Characteristics of adult cestodes of *Hydatigera taeniaeformis* found in this study and in the literature.

Characteristics	Present study	Riser (1956)	Loos-Frank (2000)	Al-Jashamy and Islam (2007)	Lavikainen et al. (2016)
Sample size (n)	5	-	-	10	17
Number of hooks	30-34	-	34-36	30-40	36-42
Large hooks	0.36-0.40	0.38-0.40	0.37-0.40	0.36-0.44	0.39-0.46
Small hooks	0.24-0.27	0.25-0.26	0.21-0.26	0.25-0.27	0.24-0.28
Dimensions of sucker	0.28-0.31 x 0.23-0.26	-	0.29-0.49	-	0.28-0.32 x 0.22-0.26



1 **Figure 1.** *Hydatigera taeniaeformis* found in *Leopardus geoffroyi* in southern Brazil. A
2 - Scolex with 34 hooks arranged in two rows (Scale bar = 2 mm); B - larger Hook
3 (Scale bar = 100 μ m); C - smaller Hook (Scale bar = 100 μ m).

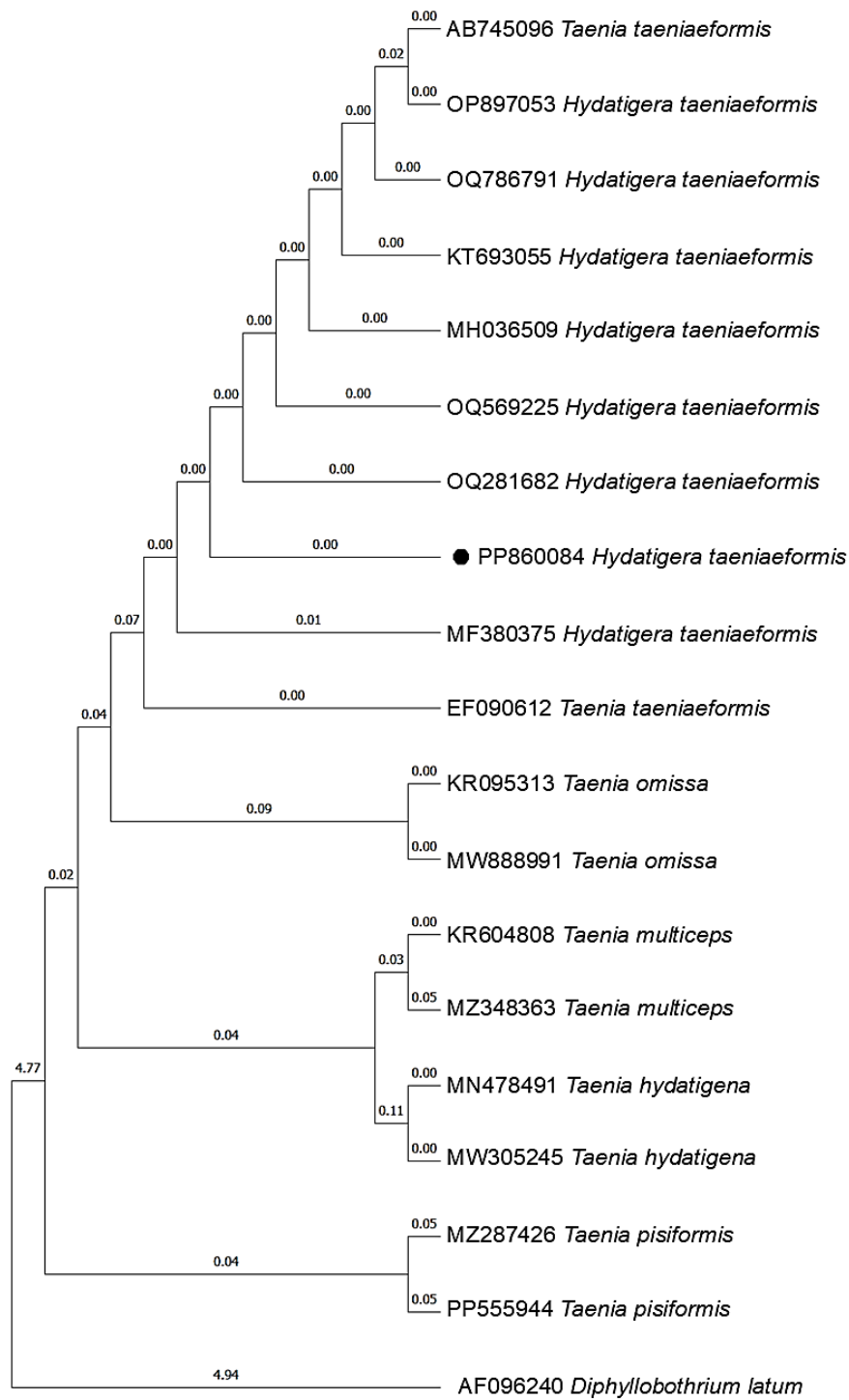


Figure 2. Phylogenetic tree inferred using the Maximum Likelihood method and the Hasegawa-Kishino-Yano model for Taeniidae species found in wild felids based on sequences of the mitochondrial gene cytochrome c oxidase subunit I (COX1). GenBank accession numbers for all sequences are provided in front of taxon names. The bootstrap consensus tree was inferred from 1000 replicates. *Diphyllbothrium latum* was used as an outgroup taxon.

4. Conclusion

We hereby document the first description of *H. taeniaeformis* parasitizing *L. geoffroyi* in Brazil. Thus, the number of known host species parasitized by this helminth in the country and worldwide is expanded. Additionally, a new molecular sequence is being provided, contributing to the understanding of *Hydatigera* in South America.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Author contributions

All authors contributed substantially to the conception and design of the study, or acquisition of data, or analysis and interpretation of data; drafting the article or revising it critically for important intellectual content; and final approval of the version to be submitted.

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2.3 Artigo 3

First record of *Contracaecum australe* (Nematoda: Anisakidae) infecting Neotropic cormorant (*Phalacrocorax brasilianus*) (Aves: Phalacrocoracidae) in southern Brazil with a phylogenetic analysis

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Abstract

Phalacrocorax brasilianus (Gmelin, 1789) (Aves: Phalacrocoracidae) is one of the few piscivorous birds inhabiting freshwater and saline environments, being considered one of the most abundant aquatic species in Rio Grande do Sul, Brazil, especially along the state's coastline. It is known that birds are hosts to a wide variety of disease-causing agents, among them, nematodes of the *Contracaecum* (Anisakidae) have a

large number of recognized species. However, little is still known about the occurrence of these parasites in the Southern region of Brazil. Herein we identified for the first time *Contracaecum australe* Garbin, Mattiucci, Paoletti, González-Acuña, and Nascetti, 2011 (Nematoda: Anisakidae) parasitizing *P. brasiliensis* in Southern Brazil. Nematodes found in the bird's proventriculus were subjected to morphometric analyses, by optical microscopy and scanning electron microscopy, and molecular analyses. Molecular phylogeny based on the analysis of the 18S, ITS-1, 5.8S and ITS-2 genes showed our sequences identical to those of *C. australe*. Therefore, this is the first record of *C. australe* in southern Brazil, expanding the geographical distribution of the parasite species in the country. Additionally, new molecular sequences are being provided, contributing to the knowledge of *Contracaecum* species parasitizing cormorants.

Keywords: Anisakidae, Neotropic cormorant, Nematoda, Rio Grande do Sul

1. Introduction

Brazil is ranked in the top three countries with the greatest known avian diversity around the world, with 1,971 of the 10,000 described species (Pacheco et al., 2021). Among them, *Phalacrocorax brasiliensis* (Gmelin, 1789) (Aves: Phalacrocoracidae) is one of the few piscivorous birds that inhabit freshwater and saltwater environments, and they are the only representatives of the genus in Brazil (Quintana et al., 2002).

Popularly known as "biguá", "mergulhão", "corvo-marinho" or "neotropic cormorant", *P. brasiliensis* have a wide geographical distribution, being found from the USA to the southern tip of South America (Sick, 1997). In southern Brazil, they are considered one of the most abundant aquatic birds, especially along the coast of Rio Grande do Sul (RS) (Accordi et al., 2001).

It is known that birds are hosts to a wide variety of disease-causing agents, including viruses, bacteria, protozoa, and helminths. Anisakidae Railliet and Henry, 1912 nematodes stand out, as they can infect several aquatic organisms at different stages of their life cycle (Anderson, 2000). With more than 100 described species (Shamsi et al., 2019) and around 40 formally accepted as valid (Bezerra et al., 2024), the *Contracaecum* Railliet and Henry, 1912 is the most numerous among anisakids

(Bezerra et al., 2024). These parasites are found in fresh, brackish and salt water, with piscivorous birds and other aquatic mammals as definitive hosts, while fish are intermediate and/or paratenic hosts (Santana et al., 2023). They typically infect the proventriculus of birds, causing lesions due to tissue attachment, which are characterized by ulcers and hemorrhages that can lead the host to death (Abollo et al., 2001). Furthermore, although the zoonotic role of these nematodes is not fully elucidated, detection in humans has been diagnosed in Australia by Shamsi and Butcher (2011), confirming a case of luminal anisakidosis due to *Contracaecum* spp. larvae.

In contrast to the great diversity of avifauna in Brazil, the helminth fauna of these animals is poorly known. Studies involving *P. brasiliensis* are considered even scarcer. The lack of information highlights the need for more studies on the distribution of helminths in different regions and host species. Herein, we identified for the first time *Contracaecum australe* Garbin, Mattiucci, Paoletti, González-Acuña, and Nascetti, 2011 parasitizing *P. brasiliensis* in southern Brazil.

2. Material and methods

Case presentation

A male specimen of *P. brasiliensis*, from Lagoa dos Patos in São Lourenço do Sul, RS, which was admitted to the veterinary hospital of the Federal University of Pelotas (UFPEL) with clinical signs of pelvic limb paresis and did not survive veterinary treatment, was necropsied at the Regional Diagnostic Laboratory of UFPEL. Nineteen nematodes were found in the proventriculus, cleaned in 0.85% saline solution and stored in 70% ethanol for morphological studies and molecular analysis. No lesions were observed in the proventriculus of the animal.

Morphological Identification

The nematodes were cleared in Amann's lactophenol (Humason, 1979) and mounted on permanent slides with Canada Balsam for morphological identification, as described by Amato and Amato (2010). They were observed under an optical microscope Primo Star 3 (Carl Zeiss Microscopy GmbH, Jena, Germany) with ZEN 2 (Blue edition) ® Carl Zeiss Microscopy digital capture system. For morphometric analyses, three males and three females, all adults, were used. Measurements are given in millimeters, unless otherwise indicated, and are presented with minimum and maximum values (Table 1).

Six nematodes (four males and two females) were subjected to dehydration using the critical point drying method (Autosamdri® 815 Critical Point Dryer, Maryland, USA). Subsequently, they were mounted on aluminum stubs with double-sided carbon tape to secure them. The samples were coated with gold (Denton Desk V, New Jersey, USA) and analyzed using a Scanning Electron Microscope (SEM) (Tescan VEGA3 LMU, Kohoutovice, Czech Republic).

The taxonomic classification follow Garbin et al. (2011), Biolé et al. (2012), Santana et al. (2023), and Garbin et al. (2024).

Specimens were deposited in the Helminthological Collection of the Oswaldo Cruz Institute under number 39675 a-b.

Molecular Identification

One specimen was used for DNA extraction using the Trizol method (Ludwig Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil) following the manufacturer's instructions. The quality and quantity of extracted DNA were measured using an ultraviolet light spectrophotometer (Thermo Scientific NanoDrop Lite Spectrophotometer, Waltham, Massachusetts, USA) and 1% agarose gel electrophoresis. The extracted DNA was stored at -20°C until PCR was performed. The 18S, ITS1, 5.8S, and ITS2 genes were amplified using primers as described in Table 2. The reactions included 2.0 µL of DNA (50 ng/µL) and a mixture containing 2.0 µL of dNTP (2.5mM), 1.0 µL of each primer (10mM), 2.5µL of buffer solution (10X),

0.75 µL of MgCl₂ (50 mM), 0.25 µL of Taq DNA polymerase (5U/µL), and 15.5 µL of ultrapure water, totaling 25 µL. DNA from adult nematodes of *Contracaecum rudolphii* Hartwich, 1964 was used as a positive control. Ultrapure water was used as a negative control. The amplified products were analyzed by 1.5% agarose gel electrophoresis, stained with ethidium bromide (0.5 µg/mL), and visualized under ultraviolet light. A 100 bp molecular weight marker (Ladder 100 bp 500 µl, Ludwig Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil) was used. The amplicons were excised and purified using a Gel Purification Kit (Ludwig Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil), according to the manufacturer's recommendations, and then subjected to sequencing using the BigDye Terminator Cycle Sequencing Kit v3.1 (Thermo Fisher, USA) on an ABI3500 genetic analyzer (Applied Biosystems, USA). Consensus sequences were obtained by electrogram analysis with Phred base calling and Phrap-assembly tool, and subsequently aligned using MEGA11: Molecular Evolutionary Genetics Analysis version 11 software (Tamura et al., 2021). Multiple sequences were aligned using the ClustalW method. Sequence similarity searches with sequences deposited in the National Center for Biotechnology Information (NCBI) database were conducted using the BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi> accessed February 20, 2024). Sequences in FASTA mode were submitted to the MultAlin platform (Corpet, 1988) for comparison. Evolutionary history for of bird-parasitic *Contracaecum* species found in GenBank was inferred using the Maximum Likelihood method and the Kimura 2-parameter model (Kimura, 1980) for the ITS1, 5.8S, and ITS2 genes and the Maximum Likelihood method and the Jukes-Cantor model (Jukes and Cantor, 1969) for the 18S gene. The MEGA11 software (Tamura et al., 2021) was also used to carry out the evolutionary analyses. Statistical analysis was performed using the bootstrap method with 1000 repetitions. *Toxocara canis* Werner, 1782 (Nematoda: Ascarididae) was used as an outgroup taxon.

3. Results and discussion

Nematodes recovered from the proventriculus of *P. brasiliensis* from Lagoa dos Patos in São Lourenço do Sul, RS, exhibited morphometric characteristics consistent with *C. australe* (Table 1, Figs. 1-3), especially noted by the well-marked constriction

in the tail (Figs. 2C, 3E-F) after the pairs of paracloacal papillae (Fig. 3E), presence of a median plate (median papilla) near the anus, long spicules (Fig. 3D) and apparently smaller and more robust body size than other *Contracaecum* species (Garbin et al., 2011; Biolé et al., 2012; Santana et al., 2023; Garbin et al., 2024). Genetic sequencing confirmed the species (Figs. 4-5).

In Brazil, two species of *Contracaecum* parasitizing *P. brasiliensis* have been recorded so far: *C. rudolphii* (see Amato et al., 2006) and *C. australe* (see Santana et al., 2023), but only *C. rudolphii* has been described parasitizing the *P. brasiliensis* in the southern region of the country (Amato et al., 2006). *Contracaecum australe* was first described by Garbin et al. (2011) parasitizing *P. brasiliensis* in Chile. Subsequently, *C. australe* was observed in this same host by Biolé et al. (2012) and Garbin et al. (2024) in Argentina. Recently, Santana et al. (2023) identified this nematode for the first time in Brazil, in the northern region of the country. Therefore, this is the first record of *C. australe* in the southern region of the country, expanding the geographical distribution of this parasite and demonstrating its wide distribution in South America. Furthermore, the finding is especially relevant considering the diversity of aquatic fauna present in the ecosystem formed in Lagoa dos Patos, which bathes the region.

Both species (*C. australe* and *C. rudolphii*) present subtle morphological differences, such as the well-marked constriction in the tail just (Figs. 2C, 3E and 3F) after the pairs of paracloacal papillae (Fig. 3E) and the presence of a median plate near the anus in *C. australe*. Although Garbin et al. (2011) suggest that these characters are not ideal for differentiating between species, as they were not described by some authors, they appear to be identified in the illustrations of *C. rudolphii* by Abollo et al. (2001), Amato et al. (2006) and Moravec and Scholz (2016). Garbin et al. (2014) even suggested that the specimens described by Amato et al. (2006) in Rio Grande do Sul could actually be *C. australe*, due to these characters. Santana et al. (2023) highlighted the importance of spicule size to distinguish between the two species. *Contracaecum australe* has larger spicules than *C. rudolphii*. *Contracaecum rudolphii* specimens described by Amato (2006) from southern Brazil have smaller spicules than *C. australe*, which presumably shows that Garbin et al. (2014) were mistaken in suggesting that those specimens were *C. australe*.

In this context, use of molecular technics combined with a good morphological description and voucher material in a museum is important. The sequences obtained for the 18S and ITS-1, 5.8S and ITS-2 genes from the adult nematode sample were deposited in NCBI GenBank under accession numbers PP739191 and PP741506, respectively. Our sequence obtained for the ITS-1, 5.8S and ITS-2 genes (GenBank acc. n. PP741506) showed 99.77% similarity with *C. australe* recently found on the northern coast of Brazil by Santana et al. (2023) (GenBank acc. n. OQ397677). For the 18S gene, no *C. australe* sequence was found in the database, but our sequence showed 100% similarity with *Contracaecum* sp. (GenBank acc. n. OQ676431). In the phylogenetic analysis of the ITS-1, 5.8S and ITS-2 genes our sequence of *C. australe* were recovered sister to that ascribed to *C. australe* (GenBank acc. n. OQ397677), both were recovered in a clade comprising all sequences of *Contraceacum* found in GenBank (Fig. 4). The study based on 18S gene recovered our sequences sister to *Contracaecum* sp. (GenBank acc. n. OQ676431), and *C. rudolphii* (GenBank acc. n. MW481243) was recovered in a clade sister to *C. australe* (Fig. 5). Our *C. australe* sequence (GenBank acc. n. PP739191) exhibited a 3bp (1%) difference from *C. rudolphii* (GenBank acc. n. MW481243). Differences and similarities between all sequences of both genes can be seen in Figures 6 and 7 (Supplementary material). Nevertheless, all genes analyzed in this study, together with morphological analyses, consistently supported the distinction between *C. australe* and *C. rudolphii*, as previously observed in piscivorous birds.

The significant role of *P. brasiliensis* in the dispersal and continuity of the parasite life cycle within ecosystems, combined with the taxonomic complexity of *Contracaecum* species, reinforces the importance of integrative taxonomy (morphology and molecular analysis) to develop our understanding of the life cycles of these parasites and the importance of some birds as hosts.

1 **Table 1.** Morphometric comparison among *Contracaecum australe* parasites from *Phalacrocorax brasilianus* in South America.

Characteristics	Present study		Santana et al. (2023)		Garbin et al. (2024)		Biolé et al. (2012)		Garbin et al. (2011)	
	Rio Grande do Sul, Brazil		Pará, Brazil		Argentina		Argentina		Chile	
	Male (n = 3)	Female (n = 3)	Male (n = 15)	Female (n = 15)	Male (n = 10)	Female (n = 10)	Male (n = 8)	Female (n = 4)	Male (n = 20)	Female (n = 10)
Body (L)	18.34-25.46	19.76-22.10	19.71-28.89	22.10-40.94	17.65-21.60	24.62-38.25	19.25-27.37	27-37	13.90-28.40	25.44-41.23
Body (W)	0.76-0.92	0.76-0.90	0.43-0.83	0.59-1.09	0.58-0.80	0.63-0.99	0.65-1.00	0.70-0.90	0.64-0.93	0.66-1.16
Lip (L)	0.08-0.10	0.09-0.10	-	-	-	-	-	-	-	-
Lip (W)	0.16-0.22	0.18-0.20	-	-	-	-	-	-	-	-
Nerve ring	0.53-0.65	0.50-0.56	0.50-0.69	0.48-0.76	0.45-0.67	0.49-0.75	0.35-0.39	0.40-0.47	0.58-0.68	0.50-0.68
Deirids	0.64	-	0.51-0.73	0.52-0.83	0.55-0.73	0.59-0.81	0.35-0.38	0.46-0.55	0.58-0.79	0.58-0.79
Oesophagus	3.14-3.52	2.30-3.50	2.36-3.64	2.89-5.09	2.51-3.45	2.75-3.68	4.12-4.40	3.62-4.50	2.62-4.60	1.52-3.95
Intestinal cecum (L)	2.23-2.38	1.45-2.70	1.50-2.66	2.06-3.46	1.65-3.04	1.79-3.15	3.57-4.00	3.70-4.25	1.56-3.24	1.30-2.86
Ventriculus (L)	0.21-0.24	0.10-0.20	0.11-0.24	0.18-0.40	0.16-0.21	0.18-0.32	0.10-0.15	0.19-0.23	0.20-0.38	0.14-0.28
Ventricular appendix (L)	1.05-1.20	0.90-1.15	0.56-1.03	0.56-1.49	0.53-0.88	0.58-0.90	0.75-0.85	0.62-0.92	0.87-1.41	0.57-0.91
Vulva	-	6.34-6.80	-	7.59-14.15	-	8.23-10.15	-	8.32-8.45	-	8.25-10.87
Head collar	0.12-0.18	0.14-0.15	-	-	-	-	-	-	-	-
Eggs (µm)	-	55	-	55-60	-	52-70	-	47-57	-	63-71
Tail	0.24-0.25	0.32-0.34	0.15-0.27	0.29-0.49	0.18-0.22	0.28-0.45	0.12-0.35	0.12-0.30	0.18-0.24	0.28-0.58
Left spicule (L)	11.55-13.50	-	-	-	9.52-11.10	-	9.20-11.00	-	-	-
Right spicule (L)	12.45-13.24	-	9.54-13.91	-	8.90-10.56	-	9.20-10.75	-	9.6-15.88	-
Precloacal papillae (pairs)	35-36	-	27-38	-	30-38	-	32-40	-	27-32	-
Postcloacal papillae (pairs)	6+1*	-	6+1*	-	-	-	6+1*	-	6+1*	-
Median papilla	1	-	1	-	-	-	1	-	1	-

2 L – Length; W - Width; n - number of specimens;

3 * – pair of phasmids.

1 **Table 2.** Main characteristics of the two nucleotide sequences used in the PCR of this study.

2

Primer identification	Primer sequence (5'-3')	Gene	Product size (bp)	PCR conditions	Authors
Nem_18S_F/ Nem_18S_R	CGCGAATRGCTCATTACAACAGC GGGCGGTATCTGATCGCC	18S	900	Initial denaturation at 94°C for 2 minutes, followed by 35 cycles at 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 1 minute, final extension at 72°C for 10 minutes, and 4°C ∞.	Floyd et al. (2005)
NC5/ NC2	GTAGGTGAACCTGCGGAAGGATCATT TTAGTTTCTTTTCCTCCGCT	ITS-1, 5.8S and ITS-2	700	Initial denaturation at 94°C for 2 minutes, followed by 40 cycles at 95°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes, final extension at 72°C for 10 minutes, and 4°C ∞.	Santana et al. (2023)

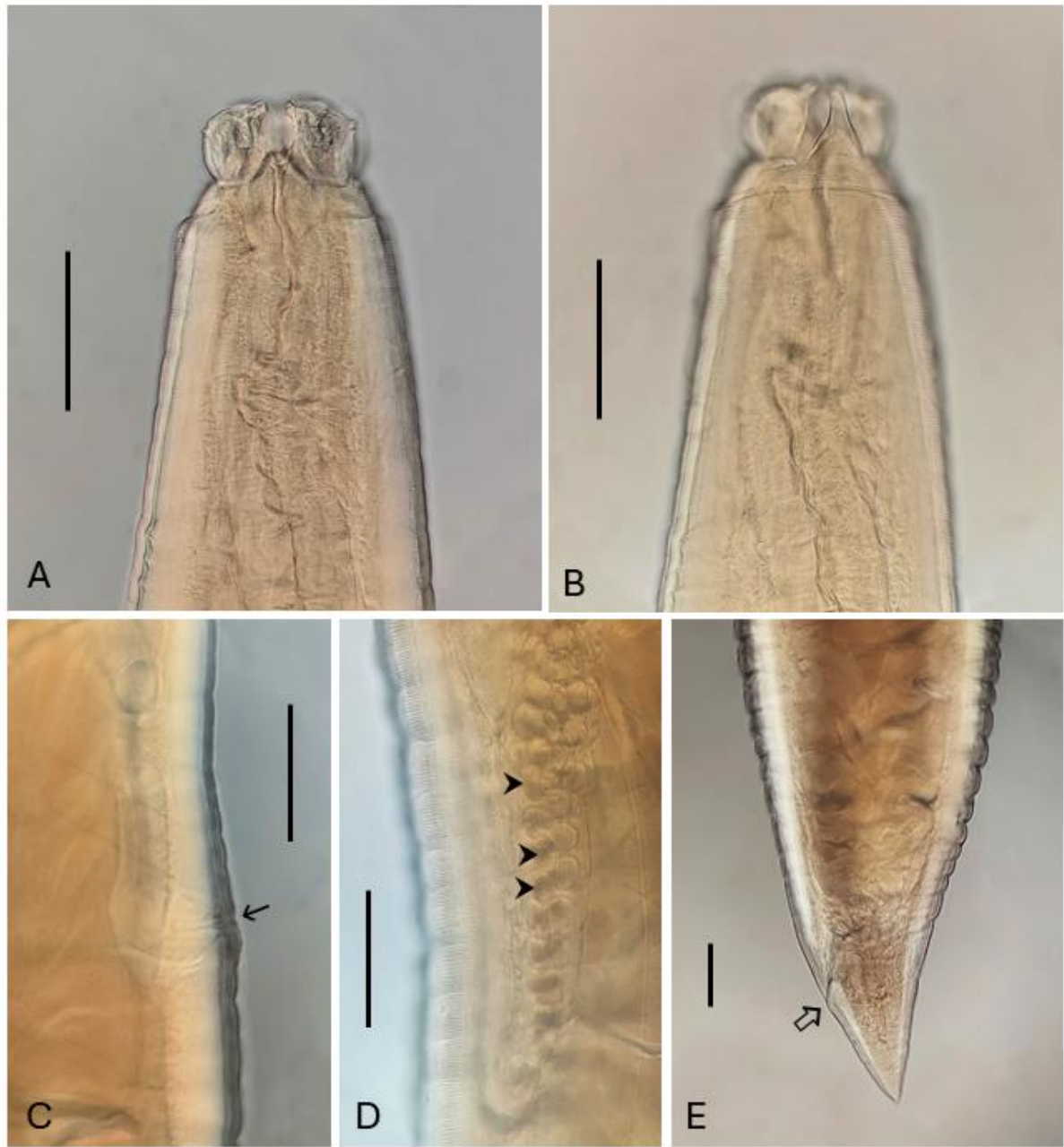


Figure 1. Female *Contracaecum australe*. A and B - Anterior region; C - Vulva (arrow); D - Uterus with eggs (arrowhead); E - Posterior region showing the anus (arrow). Scale bars 0.2 mm.

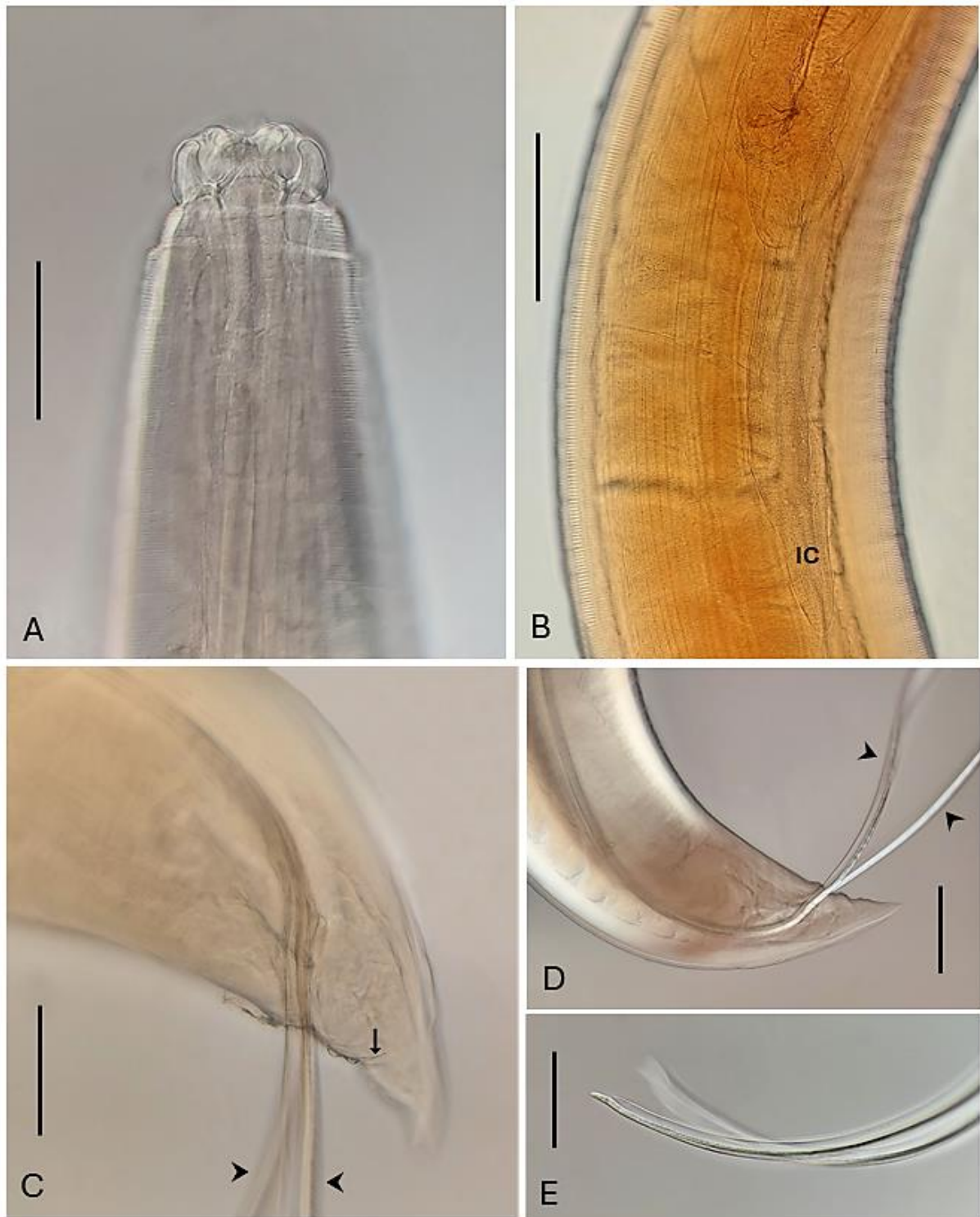


Figure 2. Male *Contracaecum australe*. A - Anterior region (scale bar 0.1 mm); B - Intestinal cecum (IC) (scale bar 0.8); C - Male tail region showing a constriction (arrow) and two spicules (arrowhead) (scale bar 0.25); D - Posterior region with spicules (arrowhead) (scale bar 0.25); E - Detail of the distal tip of the spicule (scale bars 0.25 mm).

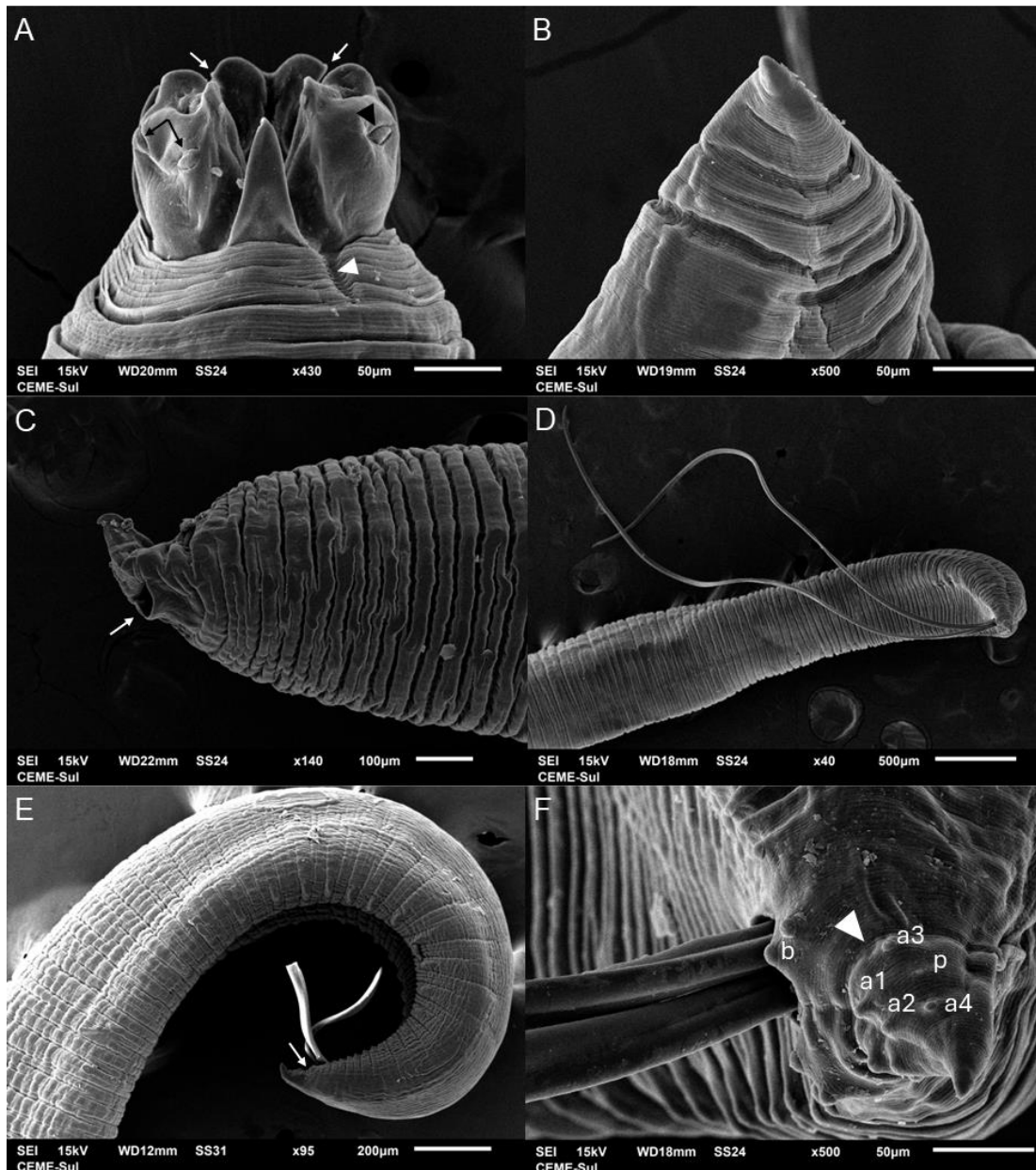


Figure 3. Scanning electron microscopy of *Contracaecum australe* (A-C female, D-F male). A - Dorsal lip with presence of two large papillae (double black arrow), ventrolateral lip with presence of voluminous papilla (black arrowhead), conspicuous auricles (white arrows), well-marked cephalic collar, with absence of striae in the lateral region, in V-shape (white arrowhead); B – Posterior end; C – Anus (white arrow); D – Posterior end with long spicules; E - Tail showing pronounced caudal constriction (white arrow) just after the pairs of paracloacal papillae; F - two pairs of subventral papillae (a1, a2), two pairs of sublateral papillae (a3, a4) and phasmids (p) located between sublateral papillae, and caudal constriction (white arrowhead) after the two pairs of paracloacal papillae (b).

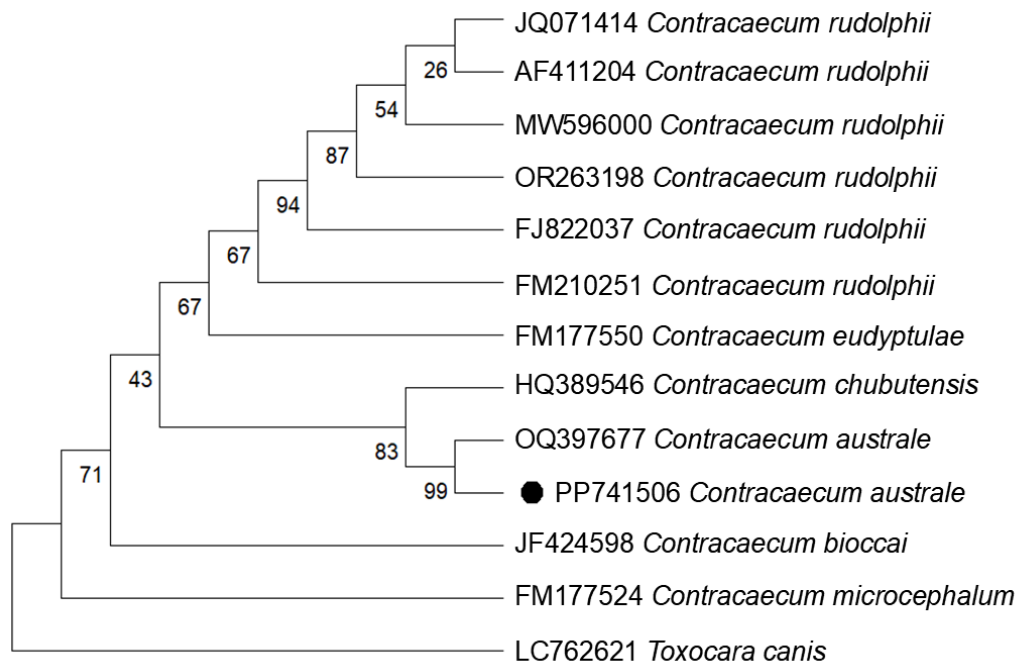


Figure 4. Phylogenetic tree inferred using the Maximum Likelihood method and the Kimura 2-parameter model for *Contracaecum* species based on sequences of the ITS-1, 5.8S, and ITS-2 genes. GenBank accession numbers for all sequences are provided in front of the taxon names. *Toxocara canis* was used as an outgroup taxon. Probability values are represented by the numbers at the nodes.

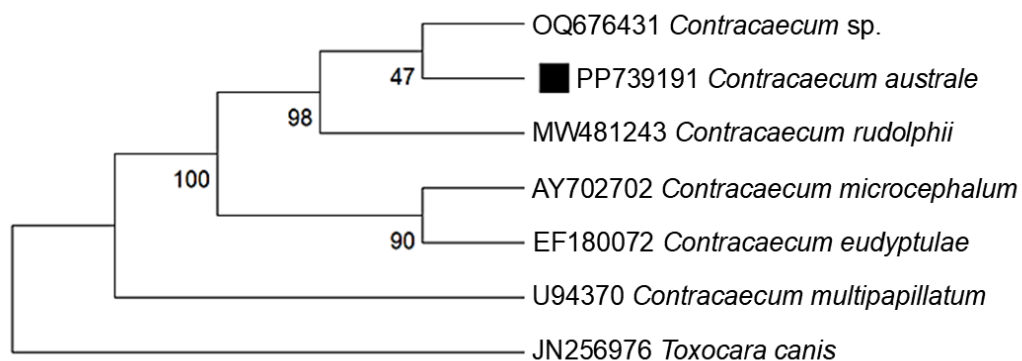


Figure 5. Phylogenetic tree inferred using the Maximum Likelihood method and the Jukes-Cantor model for *Contracaecum* species based on sequences of the 18S gene. GenBank accession numbers for all sequences are provided in front of the taxon names. *Toxocara canis* was used as an outgroup taxon. Probability values are represented by the numbers at the nodes.

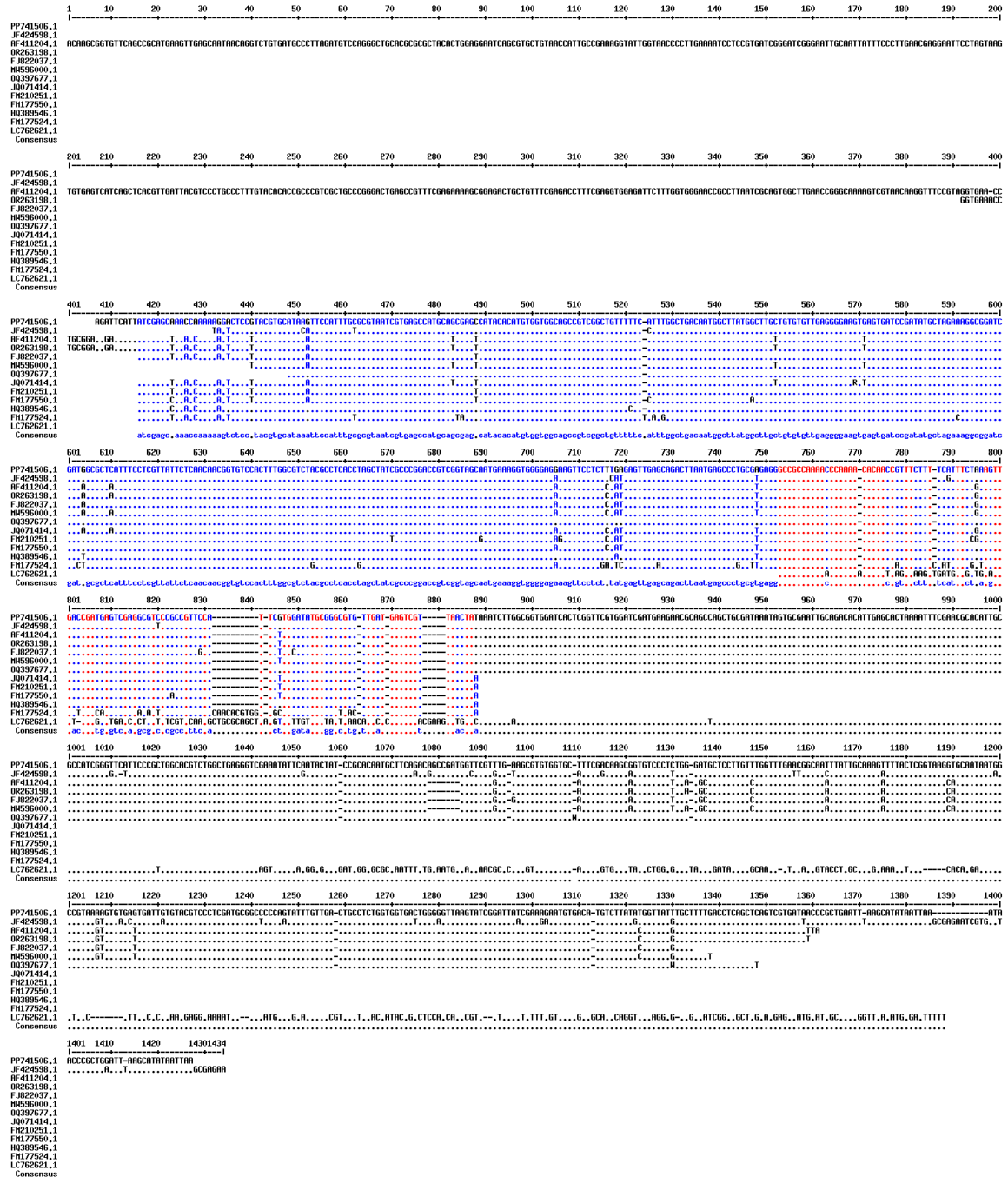


Figure 6. Alignment of the ITS-1, 5.8S and ITS-2 genes sequences using the MultAlin platform showing the differences and similarities between them. The numbers above the sequences represent nucleotide position. Letters in red represent consensus between sequences; Blue letters represent low consensus sequences.

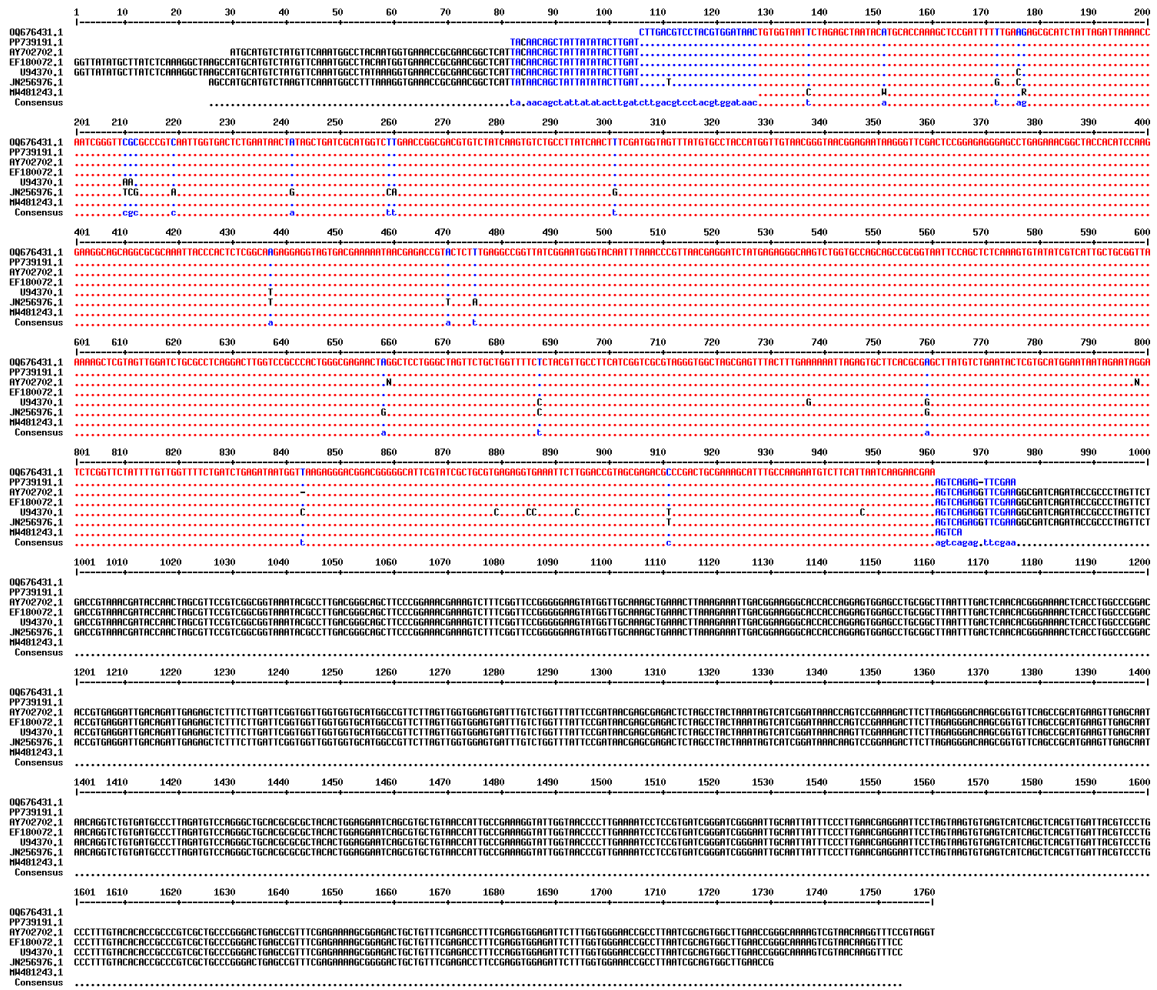


Figure 7. Alignment of 18S gene sequences using the MultAlin platform showing the differences and similarities between them. The numbers above the sequences represent nucleotide position. Letters in red represent consensus between sequences; Blue letters represent low consensus between sequences.

4. Conclusions

This is the first record of *C. australe* in southern Brazil, expanding the geographical distribution of the parasite species in the country. Additionally, new molecular sequences are provided, contributing to the knowledge of *Contracaecum* species parasitizing cormorants.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Author contributions

All authors contributed substantially to the design of the study, in addition to writing or critically reviewing the manuscript for intellectual content and approved the final version to be submitted.

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2.4 Artigo 4

New records of endoparasites and ectoparasites of free-living road-killed wild animals in the Pampa biome, Southern Brazil

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New records of endoparasites and ectoparasites of free-living road-killed wild animals in the Pampa biome, Southern Brazil

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Abstract

Wild animals host a wide variety of parasites, and the disorders caused by these parasites are among the most prevalent and significant infectious diseases affecting wildlife. The investigation of parasitic fauna is crucial for the conservation of wild animals, domestic populations, and humans. Therefore, the aim of the study was to survey endo- and ectoparasites in free-living wildlife in southern Brazil. Carcasses of road-killed wildlife were collected from highways in the Pelotas microregion, Rio Grande do Sul. All carcasses were necropsied, and endo- and ectoparasites were collected and identified. A total of 82 animals were examined; 87.80% were infected by helminths, 51.21% infested by ectoparasites, and 48.78% were affected by both types of parasites. This study presents the first records of parasitism by *Rhipicephalus microplus* in *Ozotoceros bezoarticus* in southern Brazil, and by *Ancylostoma caninum* in *Leopardus geoffroyi* worldwide. The study contributes to the knowledge about the parasitic fauna in wild animals of the Pampa biome. The presence of parasites in these

threatened species underscores the need for further research into parasitism, which is crucial for their effective management and conservation.

Keywords: Epidemiology; Parasitism; Public health; Rio Grande do Sul; Wildlife;

Introduction

Wild animals host a wide variety of parasites (Thompson et al. 2009; Rojas et al. 2024), and the disorders they cause are among the most prevalent and important infectious diseases in wildlife (Thompson 2013). Although parasites are almost always present in wild animals, they are generally in balance with the host organism, causing little harm or having minimal clinical impact (Thompson et al. 2009; Thompson 2013). Morbidity and mortality can be related to the species and parasite load, as well as the nutritional and physiological condition of the host (Catão-Dias 2003). However, when evaluating parasitism in wild animals, the effects and significance of pathogens on the hosts themselves, the transmissibility of parasites to domestic animals, and their relationship with public health must likewise be taken into account (Thompson et al. 2009).

Research on wildlife parasites is important for the conservation and welfare of these populations. Some of these parasites also have zoonotic significance and can affect humans and domesticated species (Thompson et al. 2009; Mewius et al. 2021). Increasing urbanization, industrialization, habitat fragmentation, agricultural expansion, excessive deforestation, and illegal wildlife trade have consistently facilitated interactions between people, their domestic animals, and wildlife populations to varying degrees (Otranto and Deplazes 2019). In areas with large populations of free-roaming domestic dogs and cats, as well as open herds of livestock, interactions between wildlife and domestic animal populations and the spread of parasitic infections are highly probable (Jenkins et al. 2011). Such interactions facilitate the transmission of pathogens between different species, resulting in a phenomenon known as "pathogen spillover" or "zoonotic transmission" (Plowright et al. 2017; Ellwanger and Chies 2021). This phenomenon is a key aspect of the One Health approach, which highlights the need for constant surveillance and the expanded research in new areas and among new animal species (Thompson 2013).

Knowledge about parasites affecting wildlife is still considered limited (Mathews 2009; Thompson et al. 2009), although research in this area has increased in recent years (Ramos et al. 2016; Santos et al. 2022; Benatti et al. 2023). In southern Brazil, these pathogens are studied to a relatively small extent, often in individual cases, in populations of captive animals, in main synanthropic animals, or based on fecal examinations (Ruas et al. 2008; Sprenger et al. 2018; Mewius et al. 2021; Benatti et al. 2023). Moreover, while coproparasitological examinations are practical and useful for investigating endoparasites in living animals, they have some limitations. Specifically, these examinations cannot provide complete taxonomic identification and may result in false-positive diagnoses due to pseudoparasitism, as they do not assess the adult forms of the parasites (Stuart et al. 1998).

Globally, one of the most significant challenges in conducting a parasitological survey of wildlife is the collection of biological material from the animals (Llatis et al. 2017). In this context, the use of road-killed wild animals for pathogen detection represents a viable and efficient alternative to using live animals in research. Therefore, this study aimed to survey the endo- and ectoparasites of free-living road-killed wild animals in southern Brazil.

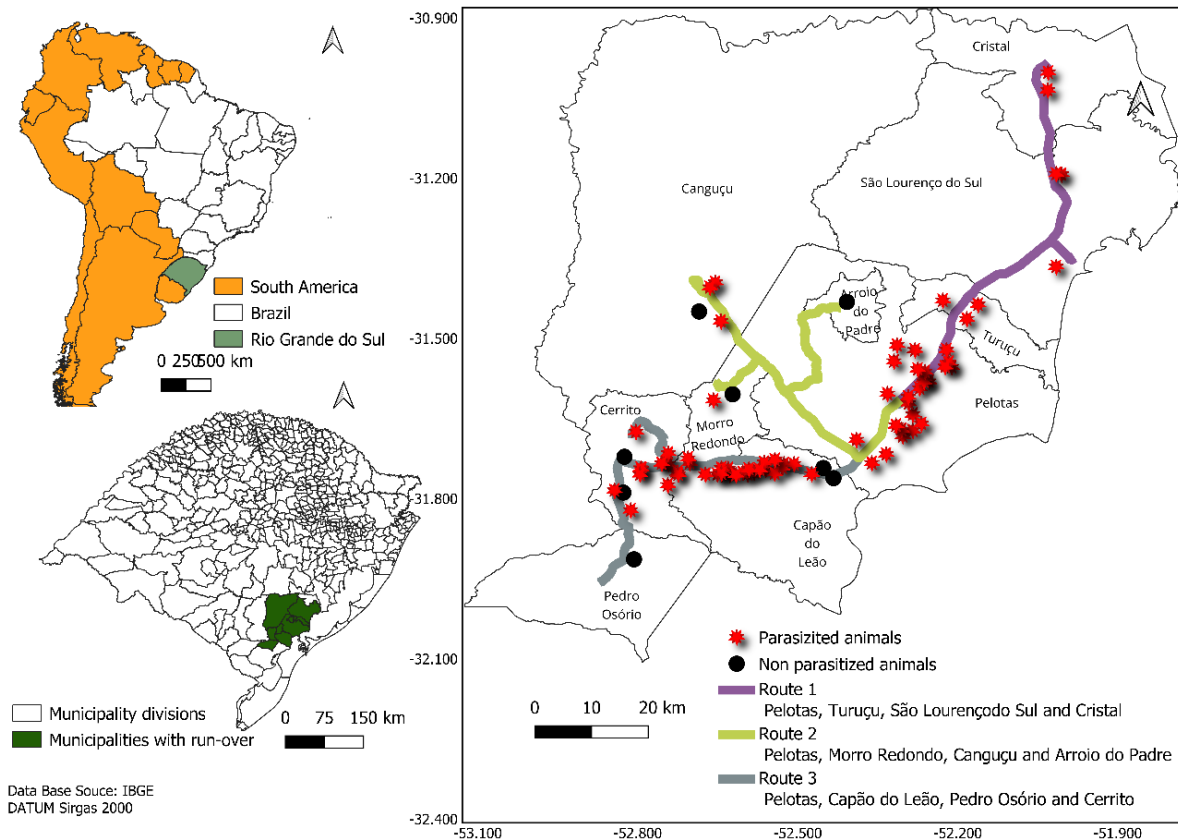
Material and Methods

Study area

The study was conducted in the Pelotas microregion, located in the southern part of the state of Rio Grande do Sul (RS), Brazil. This microregion comprises of the cities of Arroio do Padre, Canguçu, Capão do Leão, Cerrito, Cristal, Morro Redondo, Pedro Osório, Pelotas, São Lourenço do Sul, and Turuçu (Figure 1). It covers an area of 10,316.601 km² with a population of 476,096 inhabitants (IBGE 2023a). The region is part of the Pampa biome and features a subtropical climate (IBGE 2023b).

The microregion was divided into three pre-defined routes, which the study team traveled along: Route 1 – Pelotas, Turuçu, São Lourenço do Sul, and Cristal; Route 2 – Pelotas, Morro Redondo, Canguçu, and Arroio do Padre; Route 3 – Pelotas, Capão do Leão, Pedro Osório, and Cerrito. Each route was covered once a month for a year, from August 2022 to August 2023, with the city of Pelotas (31° 46' 34" S; 52° 21' 34" W) serving as the starting and ending point.

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Animal collection

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Figure 1. Study area highlighting the routes used for collecting the carcasses of road-killed wild animals on the roads in the Pelotas microregion, Rio Grande do Sul, Brazil.

Carcasses of road-killed wild animals within the cities belonging to the Pelotas microregion (Figure 1) were collected. The focus was on carcasses with preserved and unexposed viscera, free from fly larvae, and with an estimated time of death of up to 24 hours. The collected animals were placed in plastic bags, labeled with information such as species, sex, date, city, and location, and transported immediately in insulated boxes with ice to the Parasitic Diseases Study Group laboratory at the Federal University of Pelotas for necropsy. The identification of the animal species was confirmed according to described by Reis et al. (2010).

Collection and identification of parasites

The carcasses were thoroughly inspected externally for ectoparasites. When detected, the arthropods were removed with tweezers and stored in Eppendorf® microtubes with 70% ethanol. Subsequently, they were classified according to the morphological taxonomic keys with the aid of a stereoscopic microscope (Clay 1958; Price and Beer 1963; Timm and Price 1994; Bicho and Ribeiro 1998; Barros-Battesti et al. 2006; Onofrio et al. 2009).

Similarly, all the internal organs were carefully examined. Each organ was dissected individually in Petri dishes, and the contents filtered through a 100µm mesh sieve before being examined under a stereoscopic microscope to identify any endoparasites. Helminths were removed, cleaned in 0.85% saline solution, and preserved in AFA (acetic acid, formaldehyde, and ethyl alcohol) for morphological studies. Nematodes were cleared in Amann's lactophenol, while cestodes and trematodes were stained with Carmin (Amato and Amato 2010). All specimens were mounted on permanent slides for morphological identification, as described by Amato and Amato (2010). The identification of helminth species followed the existing literature (Antunes 2005; Amato et al. 2006; Ruas 2007; Vieira et al. 2008; Anderson et al. 2009; Wendt 2009; Monteiro 2017; Benatti et al. 2023).

All specimens were deposited in the parasitic collection of the Parasitic Diseases Study Group at the Federal University of Pelotas.

Molecular identification

One specimen of *Ancylostoma caninum* was used for DNA extraction using the Trizol method (Ludwig Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil) following the manufacturer's instructions. The quality and quantity of extracted DNA were measured using an ultraviolet light spectrophotometer (Thermo Scientific NanoDrop Lite Spectrophotometer, Waltham, Massachusetts, USA) and 1% agarose gel electrophoresis. The extracted DNA was stored at -20°C until PCR was performed. PCR was performed using the following primers: JB3 (5' TTTTGGGCATCCTGAGGTTTAT 3') and JB4.5 (5' TAAAGAAAGAACATAATGAAAATG 3'), amplifying a fragment of approximately 420 base pairs (bp) of the subunit I of the mitochondrial cytochrome c oxidase gene (COX1), according to Rojas et al. (2018). In the reactions, 2.0 µL of DNA (50 ng/µL)

and the mixture containing 2.0 µL of dNTP (2.5 mM), 1.0 µL of each primer (10 mM), 2.5 µL of buffer solution (10X), 1.25 µL of MgCl₂ (50 mM), 0.25 µL of Taq DNA polymerase (5U/µL), and 15 µL of ultrapure water were used, totaling 25 µL. The amplifications, in a conventional thermocycler, included: initial denaturation at 94 °C for 2 min, followed by 35 cycles at 95 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, final extension at 72 °C for 10 min, and 4 °C ∞. DNA from adult nematodes of *Toxocara cati* was used as positive control. Ultrapure water was used as a negative control. The amplified products were analyzed by 1.5% agarose gel electrophoresis, stained with ethidium bromide (0.5 µg/mL), and visualized under ultraviolet light. A 100 bp molecular weight marker (Ladder 100 bp 500 µl, Ludwig Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil) was used.

The amplicons were excised and purified using a Gel Purification Kit (Ludwig Biotechnology, Porto Alegre, Rio Grande do Sul, Brazil), according to the manufacturer's recommendations, and then subjected to sequencing using the BigDye Terminator Cycle Sequencing Kit v3.1 (Thermo Fisher, USA) on an ABI3500 genetic analyzer (Applied Biosystems, USA). Consensus sequences were obtained by electrogram analysis with Phred base calling and Phrap-assembly tool, and subsequently aligned using MEGA11: Molecular Evolutionary Genetics Analysis version 11 software (Tamura et al. 2021). Multiple sequences were aligned using the ClustalW method. Sequence similarity searches with sequences deposited in the National Center for Biotechnology Information (NCBI) database were conducted using the BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Evolutionary history for of Ancylostomatidae species found in GenBank was inferred using the Maximum Likelihood method and the Jukes-Cantor model (Jukes and Cantor 1969). The MEGA11 software (Tamura et al. 2021) was also used to carry out evolutionary analyses. Statistical analysis was performed using the bootstrap method with 1000 repetitions. *Toxocara cati* was used as an outgroup taxon.

Statistical analysis

A descriptive analysis was conducted to calculate the prevalence of wild animals affected by parasites, expressed as a percentage (%).

Georeferencing

All collection points were mapped using the global positioning system (GPS) with the Google Maps mobile application and inserted into the QGIS software, version 2.14.1, for map construction.

Results

A total of 82 wild animals were evaluated. Information about the species collected as well as the endoparasites and ectoparasites found is detailed in Table 1. The collection points of the carcasses of wild-dead wild animals can be observed in Figure 1, together with the indication that they were or not parasitized (including endoparasites and ectoparasites). Among the animals, 87.80% (72) were infected with helminths, 51.21% (42) were infested with ectoparasites, and 48.78% (40) were affected by both, indicating a high prevalence of parasitism in the region, especially in the municipalities of Pelotas/RS and Capão do Leão/RS (Figure 1). Additionally, we identified for the first time parasitism by *Rhipicephalus microplus* in *Ozotoceros bezoarticus* in southern Brazil, and also recorded for the first time parasitism by *A. caninum* in *Leopardus geoffroyi* worldwide (Figures 2-3).

Table 1. List of wild animals found and their relationship with endo and ectoparasites identified in southern Brazil.

Scientific name	Common name	Number of animals		Infected animals		Endoparasites	Infested animals		Ectoparasites
		M	F	M	F		M	F	
Birds									
<i>Phalacrocorax brasilianus</i>	Neotropic cormorant	1		1		<i>Contracaecum rudolphii</i> ^a , <i>Contracaecum australe</i> ^a , <i>Paradilepis caballeroi</i> ^b , Trematódeo ^b	0		-
		1	0	1	0		0	0	
<i>Polyborus plancus</i>	Caracara	1		0		-	1		<i>Colpocephalum</i> spp., <i>Degeeriella</i> spp.
		1	0	0	0		1	0	
<i>Athene cunicularia</i>	Burrowing owl	1		0		-	0		-
		1	0	0	0		0	0	
<i>Bubo virginianus</i>	Great Horned owl	1		0		-	0		-
		1	0	0	0		0	0	
Mammals									
<i>Didelphis albiventris</i>	White-eared opossum	37		37		<i>Aspidodera</i> spp. ^b , <i>Brachylaima</i> spp. ^b , <i>Cruzia tentaculata</i> ^b , <i>Physaloptera</i> spp. ^a	24		<i>Amblyomma aureolatum</i> , <i>Ctenocephalides felis</i> , <i>Ixodes loricatus</i>
		15	22	15	22		8	16	
<i>Cerdocyon thous</i>	Crab-eating fox	6		6		<i>Ancylostoma caninum</i> ^b , <i>Spirometra mansonoides</i> ^b , <i>Physaloptera</i> spp. ^a	6		<i>Amblyomma aureolatum</i> , <i>Amblyomma tigrinum</i>
		2	4	2	4		2	4	
<i>Ozotoceros bezoarticus</i>	Pampas deer	5		5		<i>Haemonchus</i> spp. ^b , <i>Trichostrongylus axei</i> ^b	4		<i>Rhipicephalus microplus</i>
		2	3	2	3		1	3	
<i>Cavia aperea</i>	Brazilian guinea pig	5		5		<i>Paraspidodera uncinata</i> ^b , Taeniidae ^b	0		-
		2	3	2	3		0	0	
<i>Leopardus geoffroyi</i>	Geoffroy's cat	3		3		<i>Ancylostoma caninum</i> ^b , Taeniidae ^b , <i>Toxocara cati</i> ^b	3		<i>Amblyomma aureolatum</i>
		1	2	1	2		1	2	
<i>Hydrochoerus hydrochaeris</i>	Capybara	2		2		<i>Fasciola hepatica</i> ^c , <i>Hippocrepis hippocrepis</i> ^b , <i>Protozoophaga obesa</i> ^b	2		<i>Amblyomma dubitatum</i>
		0	2	0	2		0	2	

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<i>Conepatus chinga</i>	Molina's Hog-nosed skunk	2	2			<i>Physaloptera</i> spp. ^a	0		-
		1	1	1	1		0	0	
<i>Lycalopex gymnocercus</i>	Pampas fox	1	1			<i>Spirometra mansonoides</i> ^b , <i>Ancylostoma caninum</i> ^b , <i>Toxocara canis</i> ^b	1		<i>Amblyomma aureolatum</i> , <i>Amblyomma tigrinum</i>
		0	1	0	1		0	1	
<i>Alouatta guariba</i>	Howler monkey	2	1			Taeniidae ^b	0		-
		2	0	1	0		0	0	
<i>Dasypus novemcinctus</i>	Nine-banded armadillo	1	1			<i>Physaloptera</i> spp. ^a	0		-
		1	0	1	0		0	0	
<i>Myocastor coypus</i>	Coypu	2	1			<i>Fasciola hepatica</i> ^c	0		-
		1	1	0	1		0	0	
<i>Nasua nasua</i>	Coati	1	1			<i>Cruzia</i> spp. ^b	0		-
		0	1	0	1		0	0	
<i>Euphractus sexcinctus</i>	Six-banded armadillo	1	1			<i>Ancylostoma caninum</i> ^b	0		-
		1	0	1	0		0	0	
<i>Tamandua tetradactyla</i>	Southern tamandua	1	1			<i>Physaloptera</i> spp. ^a	0		-
		1	0	1	0		0	0	
<i>Coendou spinosus</i>	Hedgehog	1	0			-	1		<i>Eutrichophilus</i> spp.
		1	0	0	0		1	0	
<i>Mazama gouazoubira</i>	Brocket deer	3	0			-	0		-
		1	2	0	0		0	0	
<i>Procyon cancrivorus</i>	Crab-eating raccoon	1	0			-	0		
		0	1	0	0		0	0	
Reptiles									
<i>Caiman latirostris</i>	Broad-snouted caiman	1	1			<i>Physaloptera</i> spp. ^a	0		-
		0	1	0	1		0	0	

<i>Salvator merianae</i>	White-and-black tegu lizard	2	2			<i>Physaloptera</i> spp. ^a	0		-
		1	1	1	1		0	0	
<i>Trachemys dorbigni</i>	D'Orbigny's slider	1		1		<i>Polystomoides</i> spp. ^d	0		-
		1	0	1	0		0	0	

Uppercase letters indicate the sex of the animals: M - male, F - female.

Lowercase letters indicate the organ where the endoparasites were found: ^a - stomach, ^b - intestine, ^c - liver, ^d - urinary bladder.

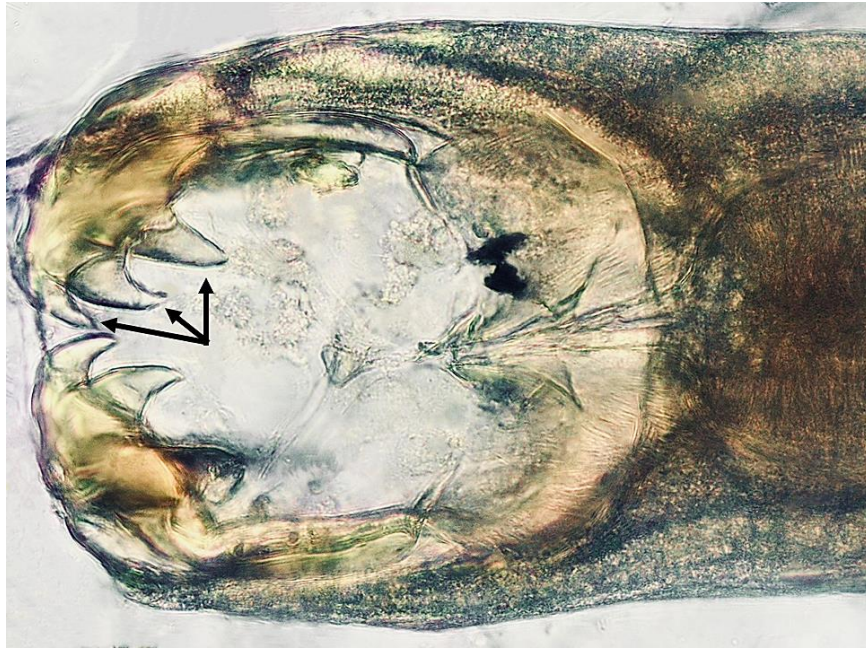


Figure 2. Buccal capsule of *Ancylostoma caninum* found in *Leopardus geoffroyi* in Southern Brazil. Arrows indicate the presence of three pairs of teeth. Magnification 400x.

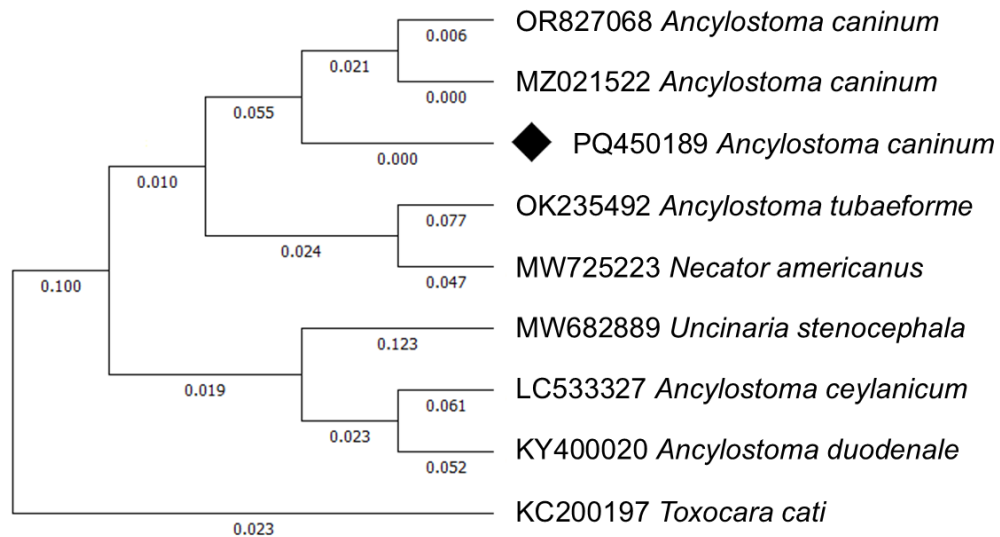


Figure 3. Phylogenetic tree inferred using the Maximum Likelihood method and the Jukes-Cantor model for Ancylostomatidae species based on COX1 gene sequences.

GenBank accession numbers for all sequences are provided in front of the taxon names. The black diamond represents the sample from this study. *Toxocara cati* was used as an outgroup taxon. Probability values are represented by the numbers at the nodes. The bootstrap consensus tree was inferred from 1000 replicates.

1 Discussion

2 Our study highlights infections by *A. caninum*, *Toxocara canis*, and *Toxocara*
 3 *cati* (syn. *Toxocara mystax*), zoonotic and cosmopolitan parasites, found in animals
 4 such as *Cerdocyon thous*, *L. geoffroyi*, *Lycalopex gymnocercus*, and *Euphractus*
 5 *sexcinctus*. These species of nematodes contributes to the syndromes of Larva
 6 Migrans, caused by the migration of these helminth larvae through the human skin,
 7 internal organs, or the eye (Monteiro 2017). These syndromes are neglected,
 8 underreported and underdiagnosed (Lee et al. 2014; Chen et al. 2018; Rodriguez-
 9 Morales et al. 2021), and have a significant socioeconomic impact, particularly in low-
 10 income communities worldwide (Chen et al. 2018; Macedo et al. 2023). Especially
 11 canids and felids may play a critical role in environmental contamination and
 12 maintenance of the life cycles of parasites transmissible to domestic animals and
 13 humans due to their higher population densities, particularly in the studied region
 14 (Morgan et al. 2013; Otranto and Deplazes 2019; Waindock et al. 2021; Holland 2023).

15 *Ancylostoma caninum* is distributed worldwide in wild and domestic canids and
 16 felines, causing chronic intestinal blood loss (Hawdon and Wise, 2021). Infections with
 17 these parasites in wild canids (e.g., *C. thous* and *L. gymnocercus*) have been observed
 18 in other studies (Ruas et al. 2008; Vieira et al. 2008), as well as in *E. sexcinctus* (Hoppe
 19 et al. 2009). These infections may result from cross-infection with infectious forms
 20 originating from sympatric carnivores. In this region, it is culturally and functionally
 21 common to use domestic dogs in the management of cattle and sheep, allowing these
 22 animals to traverse wild habitats thereby leading to niche overlap between species
 23 (Dotto et al. 2001). On the other hand, while *A. caninum* is occasionally found
 24 parasitizing felines (Coelho et al. 2011), to our knowledge, this is the first record in *L.*
 25 *geoffroyi*, which is considered endangered in Brazil and other countries, and classified
 26 as “vulnerable” in RS (Almeida et al. 2013). *Ancylostoma caninum* can be easily
 27 identified by its three pairs of teeth in the buccal capsule (Figure 2), its 12-20 mm in
 28 length, reddish-gray coloration, and claviform esophagus (Burrows 1962; Monteiro
 29 2017) and although the characteristics of *A. caninum* are well described, the use of
 30 molecular techniques combined with a good morphological description is important.
 31 Our sequence obtained for the COX1 gene (GenBank acc. n. PQ450189) showed
 32 98.47–98.72% similarity with sequences of *A. caninum* recently found in domestic dogs
 33 from Kenya (GenBank acc. n. OR827068, MZ021522). Phylogenetic analysis showed

the placement of all *A. caninum* isolates in the same clade. Therefore, the gene analyzed in this study, together with morphological analyses consistently support the identification of *A. caninum*.

Fasciola hepatica was found in capybaras (*Hydrochoerus hydrochaeris*) and coypus (*Myocastor coypus*) in our study but is not considered a natural parasite of these animals. It is believed to have been introduced to the American continent through the transport of infected European ruminants (Bargues et al. 2017). Parasitism in these wild rodents (*H. hydrochaeris* and *M. coypus*) has been previously reported in anthropized areas (Santarém et al. 2006; Labruna et al. 2018; Souza et al. 2021a) and rural areas (Silva Santos et al. 1992; Dracz et al. 2016; Bellato et al. 2009), especially where they share habitats with ruminants contributing development and maintenance of the trematode's life cycle.

In this context, nematodes of the genera *Haemonchus* spp. and *Trichostrongylus* spp., as well as ticks of the species *R. microplus*, typically have domestic ruminants as their definitive hosts (Monteiro 2017). Their presence in pampas deer (*O. bezoarticus*) may consequently suggest the occurrence of cross-infection with domestic animals. Pampas deer inhabits grassland areas in South America, resulting in close contact with livestock (cattle and sheep) (Cançado et al. 2009). While reports on the parasitism of *R. microplus* in *O. bezoarticus* exist in other states of Brazil (Pereira et al. 2000; Cançado et al. 2009), this is the first record in RS. The finding is significant because the tick species is considered the main biological vector of bovine anaplasmosis and babesiosis (Brito et al. 2006), the major causes of cattle deaths in RS (Brito et al. 2006; Dalto et al. 2018) and can be transmitted between different ruminant species (Araújo et al. 2003) such as *O. bezoarticus* (Villas-Boas et al. 2007 cited in Cançado et al. 2009). This suggests the need for additional research to clarify the susceptibility of pampas deer to these pathogens and their role in maintaining diseases in nature, especially since pampas deer is a threatened species in the study region (Chiarello et al. 2008) and have become extinct in most of their original distribution (Cançado et al. 2009).

The presence of ticks from the genus *Amblyomma* in wild animals is not uncommon (Muller et al. 2005; Ruas 2007; Perez et al. 2008; Pinto et al. 2018; Souza et al. 2021b) but deserves attention from public health services, as they are involved in the transmission cycle of rickettsiae that cause Brazilian Spotted Fever (BSF) and

other bacteria, in the state of RS (Rio Grande do Sul 2018). The occurrence of *Ixodes loricatus* and *A. aureolatum* in *D. albiventris* reinforces concerns regarding the spread of the emerging Lyme-like Brazilian disease, also known as Baggio-Yoshinari Syndrome, caused by *Borrelia burgdorferi* (Muller et al. 2005). This bacterium has been isolated from *A. aureolatum* (Barros-Battesti 1998), *I. loricatus*, and didelphimorphs (Abel et al. 2000) in Brazil. Domestic dogs, which roam the forests near homes can carry ticks from wildlife to humans, while human hunting behavior and ecotourism activities, facilitates human contact with mites, and consequently, the possibility of contracting diseases (Rio Grande do Sul 2018).

Similarly, the occurrence of *Ctenocephalides felis* in *D. albiventris*, previously documented by Antunes (2005) in Pelotas/RS and more recently in Rio Grande/RS by Lignon et al. (2023), confirms their established presence in peridomestic and domestic environments. This overlap between wild and domestic mammal species facilitates pathogen transmission. It has been suggested that *C. felis* might participate in the infection cycle for several pathogens, including *Rickettsia felis* (Peniche-Lara et al. 2016) and *Leishmania* (Ferreira et al., 2009), emerging microorganisms that infects animals (wild and domestic) and humans (Peniche-Lara et al. 2016) and were reported in various countries infecting opossums (Labruna et al. 2011; Peniche-Lara et al. 2016; Soares 2019).

Spirometra mansonoides (syn. *Spirometra mansonii*), found in *C. thous* and *L. gymnocercus*, was previously reported by Ruas (2007) in southern Brazil. The tapeworm has also been identified in domestic animals (Dall'Agnol et al. 2010; Marques et al. 2019) and is responsible for one of the main zoonoses caused by cestodes and transmitted through food. This has been estimated to infect 20 million people worldwide (Kuchta et al. 2024), including the state of RS (Fróes 1967 cited in Mentz et al. 2011). Human sparganosis cases are associated with the consumption of raw or undercooked meat from wild or domestic animals, drinking contaminated water, or contact with open wounds and intermediate hosts (Kuchta et al. 2021). Although the infective stage is not the adult parasite, its presence in wild canids in the study area predisposes the environment to contamination through the release of eggs in feces of these hosts, thereby enabling the completion of their life cycle. This finding indicates that the parasite is still present in the study region, with potential for new infections (both human and animal) through contaminated water and game meat.

Finally, we report the first record of parasitism by *R. microplus* in *O. bezoarticus* in the southern Brazil, and the first record of parasitism by *A. caninum* in *L. geoffroyi* worldwide. This discovery contributes to the knowledge of parasitic fauna in wild animals, particularly in the Pampa biome, where information on these relationships is scarce. Additionally, the presence of helminths in endangered species underscores the need to deepen and expand our understanding of parasitism in wildlife, which is crucial for the management and conservation of these species.

Ethics approval

The animals in this study are part of a scheduled research project on protozoa in wild animals from southern Brazil (Cobalto/UFPel registration 5604). The collection and transportation of carcasses of wild animals killed by vehicles were authorized by the Biodiversity Authorization and Information System of the Ministry of the Environment under registration 82632-3, based on Normative Instruction number 03/2014. This work does not require approval from the Ethics Committee on Animal Use of UFPel (process number 23110.046990/2022-02).

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Competing interests

The authors declare no competing interests.

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2.5 Artigo 5

Survey of parasitic fauna data from wild animals through coproparasitological diagnosis in Southern Brazil

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**Survey of parasitic fauna data from wild animals through coproparasitological
diagnosis in Southern Brazil**

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Abstract

Background: The proximity between people and their domestic animals with wild animal populations can result in the spread of diseases with a significant impact on public health. Infection by parasites in wildlife is considered an important bioindicator of the current state of ecosystems, and studying the epidemiology of these infections is essential for a better understanding of natural foci. However, research on parasites in southern Brazil, especially in Rio Grande do Sul (RS), is considered incipient. Therefore, in this study, we aimed to identify the parasitic fauna of wild animals in the southern region of RS through fecal parasitological diagnosis. We processed 82 fecal samples from wild animals - including birds, mammals, and reptiles - from cities within the microregion of Pelotas, using the Zinc Sulfate Centrifugal Flotation, Spontaneous Sedimentation and Oocyst Sporulation techniques.

Results: In 69.5% of the samples (93.1% of mammals, 47% of birds and 50% of reptiles), we found helminth eggs and/or protozoan cysts/oocysts, with strongylid-type eggs being the most frequent parasites (44.11%). Additionally, 64.9% of the positive samples were parasitized by at least one morphogroup with zoonotic agents (*Taeniidae*, *Capillaria*, *Strongyloides*, *Spirometra*, *Lagochilascaris*, *Sarcocystis*, *Trichuris*, *Giardia*, *Ancilostomid*, *Physaloptera*, *Toxocara*, *Fasciola*). We also recorded the first finding of *Monocystis* spp. in a Southern tamandua (*Tamandua tetradactyla*).

Conclusions: Thus, it was observed that the majority of the animals were parasitized and, consequently, susceptible to a wide range of pathogens of medical and veterinary interest, highlighting the importance of these hosts in the spread of parasites, especially those with zoonotic potential. However, the ecology of transmission and the role of these hosts in the life cycles of parasites should be further explored in other studies.

Keywords: Endoparasites; Parasitic infections; One health; Wildlife; Zoonosis;

Background

Increasing urbanization, agricultural expansion, excessive deforestation, and the illegal wildlife trade have led to greater contact between people and their domestic animals with wild animal populations (Chomel et al., 2007; Marchini et al., 2011). Currently, around 75% of infectious diseases emerging from humans have animal origins (PNUMA, 2020) and 71.8% originate from wild fauna (Ribeiro and Medeiros, 2017). Environmental changes and their consequences disrupt ecosystem balance, promoting the spread of infections between species — a phenomenon known as zoonotic spillover, which can have a significant impact on One Health (Taylor et al., 2001; Plowright et al., 2017).

Among the diseases that affect wildlife, parasitic infection is considered an important bioindicator of the current state of ecosystems, used to evaluate the spread of pathogens and behavioral changes (Lymbery, 2005). Wild animals, both in the wild and in captivity, can be reservoirs and carriers of various parasitic diseases (including zoonoses) with significant potential impact on public health, wildlife conservation, and economic aspects (Cleaveland et al., 2001).

In this context, studying the epidemiology of these infections is essential for a better understanding of natural foci to verify the circulation of these agents among wild animals and the local, regional, or national importance of the diseases they cause. This knowledge supports the actions of veterinary and public health services (Barbosa et al., 2011).

Given that environmental changes have triggered alterations in the epidemiological transmission chain of some parasites, particularly those of zoonotic

nature, involving wild, synanthropic, domestic animals, and even humans in their epidemiological cycles, and considering the scarcity of research in Southern Brazil, this study aimed to identify the parasitic fauna of wild animals in the Southern region of Rio Grande do Sul through fecal parasitological diagnosis.

Methods

A total of 82 fecal samples from wild animals, received during the years 2022 and 2023, were analyzed in the laboratory of the Grupo de Estudos em Enfermidades Parasitárias (GEEP) at the Universidade Federal de Pelotas (UFPeI). The samples were sent by the Núcleo de Reabilitação da Fauna Silvestre (NURFS) through the Laboratório Regional de Diagnóstico (LRD), both affiliated with UFPeI. All animals were free-living, although they were undergoing rehabilitation at NURFS. The rehabilitation time for each animal varied according to the type of pathology that affected it. In addition, each animal that arrives at NURFS/UFPeI undergoes screening in a specific enclosure for it, before being relocated to larger enclosures with other animals of the same species, if possible or necessary.

During animal screening, mammalian and reptile feces were collected from each animal's enclosure immediately after defecation. Bird feces were collected in pools, also from each animal's enclosure, during one shift during the day. All samples were collected using disposable gloves, identified on the day of collection and transported in isothermal containers with ice to the laboratory for analysis. They were stored at refrigerated temperature (4°C) for a maximum of 48 hours in the laboratory, during which parasitological tests were performed. For diagnosis, the following techniques were used: Centrifugal Flotation with Zinc Sulfate, modified as described by Monteiro (2017), Spontaneous Sedimentation described by Hoffmann et al. (1934) and Oocyst

Sporulation with 2% potassium dichromate described by Monteiro (2017). For identification purposes, all structures allowing the identification or differentiation of eggs/cysts/oocysts at the lowest possible taxonomic level were used, such as shell characteristics and ornaments, embryonic and larval formations, and the presence of opercula and spines. In some cases, such as strongylid-type eggs, ancilostomid eggs, anoplocephalid eggs, ascarid eggs, and some oocysts, identification remained at the morphogroup level due to the absence of diagnostic characters for species differentiation. Identification was performed by comparing the observed morphometry with that of species previously described in the literature for the host species (Papini et al., 2012; Monteiro, 2017; Taylor et al., 2017; Sprenger et al., 2018; Moraes et al., 2019; Prado et al., 2019; Teodoro et al., 2019; Bezerra-Santos et al., 2020; Uribe et al., 2021), using an Olympus CX33 series optical microscope (Olympus Corporation, Tokyo, Japan) coupled with a digital camera, with variable magnification between 40x and 100x. Micrometric eyepieces were used for morphometric analyses.

The animals in the study came from cities in the Pelotas microregion, in Rio Grande do Sul, Southern Brazil. This region includes the municipalities of Pelotas, Capão do Leão, Pedro Osório, Cerrito, Canguçu, Morro Redondo, Turuçu, São Lourenço do Sul, Cristal, and Arroio do Padre (Figure 1).

The collection of fecal samples from wild animals was authorized by the Biodiversity Authorization and Information System of the Ministry of the Environment under registration 82632-3 based on Normative Instruction number 03/2014.

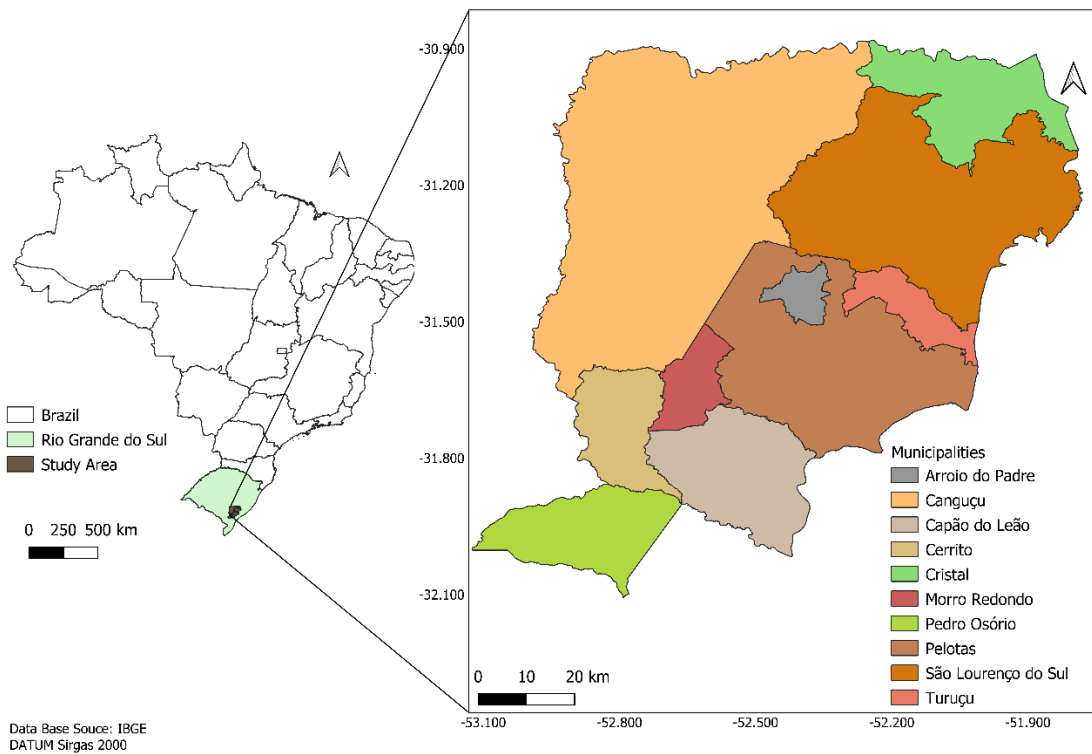


Figure 1. Study area including the cities belonging to the Microregion of Pelotas, Rio Grande do Sul, Brazil.

Results and discussion

In total, fecal samples from 34 species of wild animals were processed. Of the 82 samples analyzed - 44 mammals, 34 birds, and 4 reptiles - helminth eggs and/or protozoan cysts/oocysts were found in 69.5% (57) (Table 1). Among mammals, 93.1% were infected, as well as 47% of birds and 50% of reptiles. Photographs of the parasitic forms found can be seen in figure 2. Our results reinforce that wild animals can be infected by a wide variety of endoparasites, which typically result in subclinical infections in healthy free-living hosts but are among the main sanitary problems in captive animals (Sprenger et al., 2018; Batista et al., 2021). High population density, stress, adaptation to a new environment, or prolonged periods in a confined space can exacerbate these situations (Papini et al., 2012), highlighting the importance of

complementary examinations such as coproparasitological diagnosis, given that many of the animals in this study were undergoing rehabilitation.

Overall, strongylid-type eggs were the most frequent parasites (44.11%), followed by *Capillaria* spp. eggs (26.47%), demonstrating the diversity of parasitic species and hosts these taxa can infect. In this context, helminth infections were more common (67.64%) than protozoan infections, which were observed in 35.29% of animal species, as described by previous studies (Nath et al., 2021; Ferdous et al., 2023). However, the finding of *Giardia* spp. infections in *Cerdocyon thous* deserves attention because, besides being important causes of diarrhea in animals, they have zoonotic potential (Feng and Xiao, 2011). Furthermore, a study on the genotypes of these protozoa demonstrated that humans are likely the source of infection for these animals (Soares et al., 2011).

Among helminths, nematode infections were more prevalent compared to other classes, as reported in previous studies (Rahman et al., 2014; Shemshadi et al., 2015). This may have occurred due to the direct life cycle (at least in most species), without involvement of intermediate hosts and can be transmitted through contaminated food, water, and soil (Mir et al., 2016). On the other hand, trematodes and most cestodes require at least one intermediate host to complete their life cycle for transmission to occur. This may be the reason for the lower occurrence of infections by these helminths in this study (Atanaskova et al., 2011; Mir et al., 2016).

Although species-level identification is challenging through coproparasitological diagnosis, being a limiting factor in studies like this, many of the identified morphogroups contain species with zoonotic potential and, therefore, can infect humans. In this study, more than half of the animal species (64.9%) were parasitized by at least one morphogroup with zoonotic agents. Many of the animals evaluated

here, such as capybaras, opossums, and crab-eating foxes, are known reservoirs of various pathogens, and their proximity to other animals, including domestic (livestock and pets) and humans, in urban, peri-urban, and rural environments, can have significant public health implications. Coincidentally, these same animals (capybaras, opossums, and crab-eating foxes) were those with the highest diversity of endoparasites in this study.

Furthermore, since the diagnosis was based on fecal examination and many animals are predators, there is a possibility that some eggs, cysts, and oocysts found in the examinations belong to the preyed animal rather than the predator (spurious infection or pseudoparasitism). Thus, they act as dispersers of pathogens in the environment, representing a risk for other susceptible animals, as well as for caretakers and handlers of animals in captivity. Pseudoparasitism by *Monocystis* spp., for example, has been reported in coatis (*Nasua nasua*) (Moraes et al., 2019), nine-banded armadillos (*Dasypus novemcinctus*) (Prado et al., 2019), and more recently in opossums (*Didelphis albiventris*) (Lignon et al., 2024). Its presence is closely related to the omnivorous feeding habits of these animals since this protozoan has annelids as hosts (Velavan et al., 2010). Here, we report the finding, for the first time, in southern tamanduas (*Tamandua tetradactyla*). Although its apathogenic effect is not fully understood in vertebrates, the identification of these protozoa in the feces of individuals can lead to a misconception about the need for treatment of these animals (Prado et al., 2019), considering that the sporocyst has a similar appearance to *Trichuris* spp. eggs, albeit smaller, with approximately 10µm in length, while those of the nematode are around 55 µm (Monteiro, 2017).

In addition, the discovery of *Toxocara* spp. eggs in opossum feces and *Capillaria* spp. in *C. thous* and *Lycalopex gymnocercus* feces does not rule out the possibility of

pseudoparasitism. Although the parasitic species were not identified in our study, other authors have reported the identification of spurious infection by *Toxocara cati* eggs in *D. albiventris* (Pinto et al., 2014) and the participation of wild canids in the dispersal of *Capillaria hepatica* eggs (Ruas et al., 2002). In opossums, the possibility of interspecific coprophagy has already been suggested (Gipson et al., 2003) and their omnivorous diet also allows the ingestion of items related to their usual diet (e.g., arthropods and vegetables) contaminated by feces of other animals containing *Toxocara* spp. eggs (Cáceres, 2002). On the other hand, *C. thous* and *L. gymnocercus* can prey on hosts infected by *C. hepatica*, with the harmless passage of non-embryonated eggs through the gastrointestinal tract of these animals, eliminating them in their feces (Ruas et al., 2002). Thus, new findings in wild animals should be described, allowing the avoidance of false-positive diagnoses.

The present study represents the first survey of gastrointestinal parasite diversity, through coproparasitological diagnosis, in wild animals in Rio Grande do Sul, southern Brazil. The difficulty in species identification through fecal parasitological diagnosis, as well as the possibility of spurious infection, highlights the importance of further research including adult helminth identification, diagnostics through molecular methods, and experimental infections, which can aid in specific taxonomic identification and the epidemiology of the agents' life cycle to prove the real impact of these parasites on human and animal medicine. Furthermore, prospective epidemiological studies are suggested to be conducted over longer periods, maintaining active surveillance in the local wildlife, aiming to prevent potential future epidemics of parasitic zoonoses. Nevertheless, the findings of the present study can contribute to future diagnoses of diseases affecting these animals, as regular monitoring, coupled with appropriate therapeutic measures, can help reduce the

- 1 serious consequences of gastrointestinal parasitic infections in captive wild animals,
- 2 making preventive planning and early control more effective.

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1 **Table 1.** Data on the parasitic fauna of wild animals, through coproparasitological diagnosis, in Southern Brazil.

Scientific name	Common name	Samples	Positive samples	Endoparasites
<i>Alouatta guariba</i>	Howler monkey	2	1	Taeniidae eggs
<i>Aramides saracura</i>	Slaty-breasted wood-rail	3	2	<i>Capillaria</i> spp., <i>Heterakis</i> spp.
<i>Aramus guarauna</i>	Limpkin	2	0	-
<i>Athene cunicularia</i>	Burrowing owl	1	1	<i>Capillaria</i> spp.
<i>Bubo virginianus</i>	Great Horned owl	2	1	<i>Capillaria</i> spp.
<i>Caiman latirostris</i>	Broad-snouted caiman	1	1	<i>Strongyloides</i> spp., <i>Capillaria</i> spp., Strongylida eggs
<i>Cavia aperea</i>	Brazilian guinea pig	2	2	Strongylida eggs, Anoplocephalid eggs
<i>Cerdocyon thous</i>	Crab-eating fox	6	6	<i>Alaria</i> spp., <i>Capillaria</i> spp., <i>Spirometra</i> spp., <i>Cystoisospora</i> spp., <i>Lagochilascaris</i> spp., <i>Sarcocystis</i> spp., <i>Trichuris</i> spp., <i>Giardia</i> spp., Ancilostomid eggs, Anoplocephalid eggs
<i>Colaptes campestris</i>	Woodpecker	1	1	Anoplocephalid eggs
<i>Conepatus chinga</i>	Molina's Hog-nosed skunk	2	2	<i>Physaloptera</i> spp., <i>Spirometra</i> spp., Ancilostomid eggs, Anoplocephalid eggs
<i>Dasypus novemcinctus</i>	Nine-banded armadillo	1	1	Strongylida eggs
<i>Didelphis albiventris</i>	White-eared opossum	17	16	<i>Aspidodera</i> spp., <i>Cruzia</i> spp., <i>Physaloptera</i> spp., <i>Capillaria</i> spp., <i>Trichuris</i> spp., Strongylida eggs, <i>Alaria</i> spp., <i>Sarcocystis</i> spp., <i>Monocystis</i> spp., <i>Toxocara</i> spp., <i>Eimeria</i> spp., Anoplocephalid eggs
<i>Euphractus sexcinctus</i>	Six-banded armadillo	1	1	Strongylida eggs
<i>Furnarius rufus</i>	Rufous hornero	1	0	-

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1 **Table 1. Continuation...**

2	<i>Hydrochoerus hydrochaeris</i>	Capybara	2	2	<i>Protozoophaga obesa</i> , <i>Hippocrepis hippocrepis</i> , <i>Eimeria</i> spp., <i>Strongyloides</i> spp., Strongylida eggs, <i>Monoecocetus</i> spp., <i>Fasciola</i> spp., Ascarid eggs, Oocysts
	<i>Leopardus geoffroyi</i>	Geoffroy's cat	3	1	Ancilostomid eggs, <i>Toxocara cati</i> , Taeniidae eggs
	<i>Lycalopex gymnocercus</i>	Pampas fox	1	1	<i>Capillaria</i> spp., <i>Spirometra</i> spp., <i>Sarcocystis</i> spp., <i>Trichuris</i> spp., <i>Toxocara</i> spp., Ancilostomid eggs
	<i>Molothrus bonariensis</i>	Shiny cowbird	1	0	-
	<i>Myocastor coypus</i>	Coypu	2	2	<i>Fasciola</i> spp., <i>Paramphistomum</i> spp., Strongylida eggs
	<i>Myiopsitta monachus</i>	Monk parakeet	7	3	<i>Isospora</i> spp.
	<i>Nasua nasua</i>	Coati	1	1	<i>Sarcocystis</i> spp., <i>Cruzia</i> spp., <i>Monocystis</i> spp., Strongylida eggs, Oocysts
	<i>Ozotoceros bezoarticus</i>	Pampas deer	5	3	<i>Eimeria</i> spp., Strongylida eggs
	<i>Paroaria coronata</i>	Red-crested Cardinal	1	1	<i>Isospora</i> spp.
	<i>Passer domesticus</i>	House sparrow	1	0	-
	<i>Pitangus sulphuratus</i>	Great kiskadee	7	3	<i>Capillaria</i> spp., <i>Isospora</i> spp.
	<i>Procyon cancrivorus</i>	Crab-eating raccoon	1	0	-
	<i>Ramphastos dicolorus</i>	Green-billed toucan	2	1	<i>Capillaria</i> spp.
	<i>Saltator similis</i>	Green-winged saltator	1	1	<i>Isospora</i> spp.
	<i>Salvator merianae</i>	White-and-black tegu lizard	2	1	<i>Physaloptera</i> spp., Strongylida eggs, Oyxurid eggs
	<i>Spatula querquedula</i>	Garganey	1	0	-
	<i>Stephanophorus diadematus</i>	Diademed tanager	1	1	<i>Isospora</i> spp., <i>Ascaridia</i> spp.
	<i>Tamandua tetradactyla</i>	Southern tamandua	1	1	<i>Eimeria</i> spp., <i>Monocystis</i> spp., Strongylida eggs, Oocysts
	<i>Trachemys dorbigni</i>	D'Orbigny's slider	1	0	-
	<i>Vanellus chilensis</i>	Southern lapwing	2	1	<i>Heterakis</i> spp.
	Total		82	57	

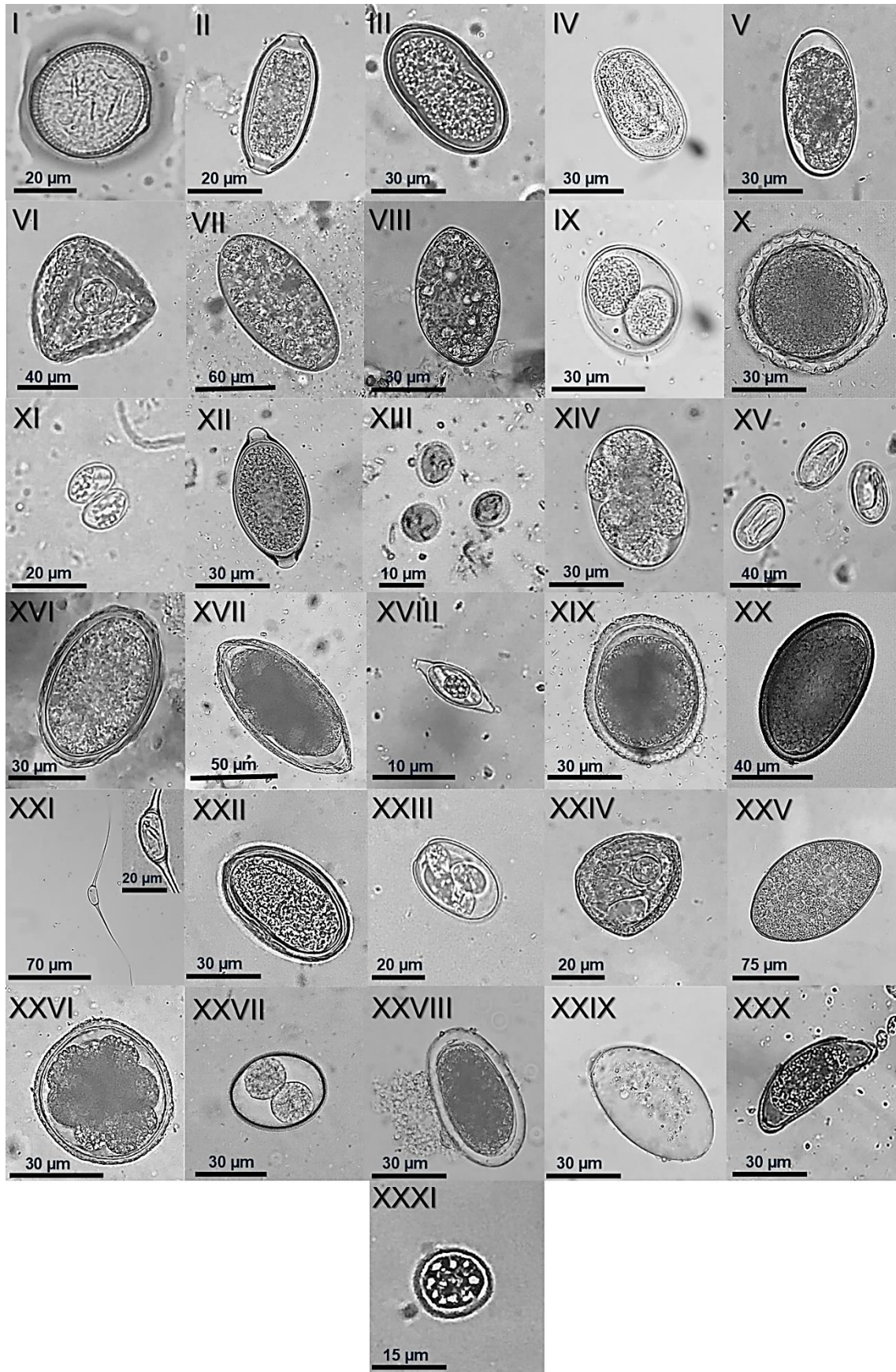


Figure 2. Illustrations of parasitic forms identified in fecal samples of wild animals evaluated in southern Brazil. I – Taeniidae egg; II – *Capillaria* spp. egg; III –

Heterakis spp. egg; IV – *Strongyloides* spp. egg; V - Strongylida egg; VI –
 Anoplocephalid egg; VII – *Alaria* spp. egg; VIII – *Spirometra* spp. egg; IX –
Cystoisospora spp. oocyst; X – *Lagochilascaris* spp. egg; XI – *Sarcocystis* spp.
 oocyst; XII – *Trichuris* spp. egg; XIII - *Giardia* spp. cysts; XIV – Ancilostomatid egg;
 XV – Physaloptera spp. eggs; XVI – *Aspidodera* spp. egg; XVII – *Cruzia* spp. egg;
 XVIII – *Monocystis* spp. sporocystis; XIX – *Toxocara* spp. egg; XX - Ascarid eggs;
 XXI - *Hippocrepis hippocrepis* egg (in detail is the miracidium capsule); XXII -
Protozoophaga obesa egg; XXIII – *Eimeria* spp. oocyst; XXIV - *Monoecocystus* spp.
 egg; XXV – *Fasciola* spp. egg; XXVI – *Toxocara cati* egg; XXVII – *Isospora* spp.
 oocyst; XXVIII – *Ascaridia* spp. egg; XXIX – *Paramphistomum* spp. egg; XXX –
 Oxyurid egg; XXXI – Unidentified oocyst.

Conclusions

It was observed that the majority of the animals were parasitized, making them
 susceptible to a wide range of pathogens of medical and veterinary interest. The
 importance of these hosts in the dispersal of parasites, especially those with zoonotic
 potential, is emphasized. However, the transmission ecology and the role of these
 hosts in the life cycles of parasites should be further explored.

Ethics approval and consent to participate

This work was approved by the Ethics Committee on the Use of Animals at UFPel
 (process number 23110.046990/2022-02).

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JSL, DMP, TSS, GRM, CGS, BCB, FGP, SGM, MPS, RTF and FRPB analyzed and interpreted the results. JSL was one of the main sources in writing the manuscript. All authors read and approved the final manuscript.

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3 Considerações Finais

Neste estudo, não foram detectadas evidências da presença do DNA de *Leishmania* spp. em animais silvestres na região estudada. Em contrapartida, novos registros da fauna parasitária foram identificados como os primeiros registros de *Hydatigera taeniaeformis* e *Ancylostoma caninum* infectando *Leopardus geoffroyi* em todo o mundo; E primeiros registros da ocorrência de *Contracaecum australe* infectando *Phalacrocorax brasilianus* e *Rhipicephalus microplus* infestando *Ozotoceros bezoarticus*, ambos no sul do Brasil.

Além disso, identificamos que 87,80% dos animais coletados estavam infectados por helmintos, 51,21% infestados por ectoparasitas e 48,78% foram afetados por ambos os tipos de parasitos. Ainda, em 69,5% das amostras de fezes, encontramos ovos de helmintos e/ou cistos/oocistos de protozoários e que 64,9% das amostras positivas estavam parasitadas por pelo menos um morfogrupo com agentes zoonóticos.

As descrições dos primeiros registros e as novas informações de ocorrência contribuem para o conhecimento sobre endo e ectoparasitos de animais silvestres no Sul do Brasil e ao redor do mundo, e indicam a potencialidade do registro de novas informações e a necessidade de estudos epidemiológicos contínuos da fauna parasitária nesse grupo de hospedeiros. Essa investigação é crucial para o bem-estar e conservação de animais silvestres, populações domésticas e humanos, especialmente no contexto da saúde única.

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Anexos

Anexo A - Documento da Comissão de Ética e Experimentação Animal (CEUA)



PARECER Nº
PROCESSO Nº

13/2023/CEUA/REITORIA
23110.046990/2022-02

ASSUNTO: Parecer CEUA processo nº 23110.046990/2022-02

"VIGILÂNCIA EPIDEMIOLÓGICA E MAPEAMENTO DE ÁREAS DE RISCO ATRAVÉS DA DETECÇÃO MOLECULAR DE *Leishmania* spp. POR MEIO DE PCR EM FAUNA SILVESTRE NA MICRORREGIÃO DE PELOTAS, RS, BRASIL"

Prezado Prof. FÁBIO RAPHAEL PASCOTI BRUHN,

Por meio deste documento, a Comissão de Ética no Uso de Animais da Universidade Federal de Pelotas (CEUA – UFPel) vem dar **ciência** da execução do projeto de pesquisa **"VIGILÂNCIA EPIDEMIOLÓGICA E MAPEAMENTO DE ÁREAS DE RISCO ATRAVÉS DA DETECÇÃO MOLECULAR DE *Leishmania* spp. POR MEIO DE PCR EM FAUNA SILVESTRE NA MICRORREGIÃO DE PELOTAS, RS, BRASIL"** sob responsabilidade do Prof. FÁBIO RAPHAEL PASCOTI BRUHN.

A proposta foi apreciada em reunião da CEUA do dia nove de fevereiro de 2023, sob processo nº 23110.046990/2022-02, e conforme detalhamento do projeto de pesquisa, salientamos que a presente não necessita da anuência da CEUA, considerando que a equipe do projeto não fará a manipulação dos animais. Tal deliberação tem como base a Resolução Normativa nº 30, de 2 de fevereiro de 2016, do Conselho Nacional de Controle de Experimentação Animal, ao qual especifica que nestes casos os pesquisadores e professores são responsáveis por todas as questões relacionadas a correta obtenção, utilização e rastreabilidade das amostras biológicas utilizados nas atividades sob sua responsabilidade, devendo agir de acordo com as exigências da Lei n. 11.794/2008, do Decreto n. 6.899/2009 e demais disposições legais pertinentes.

Atenciosamente,

Priscila Marques Moura de Leon

Coordenadora da CEUA



Documento assinado eletronicamente por **PRISCILA MARQUES MOURA DE LEON, Professor do Magistério Superior**, em 16/02/2023, às 12:45, conforme horário oficial de Brasília, com fundamento no art. 4º, § 3º, do [Decreto nº 10.543, de 13 de novembro de 2020](#).



A autenticidade deste documento pode ser conferida no site http://sei.ufpel.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **2052996** e o código CRC **F2589CF7**.

**Anexo B - Documento do Sistema de Autorização e Informação em
Biodiversidade (SISBIO)**

Autorização para atividades com finalidade científica

Número: 82632-1	Data da Emissão: 22/06/2022 17:42:38	Data da Revalidação*: 22/06/2023
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Julia Somavilla Lignon	CPF: 068.554.429-00
Título do Projeto: VIGILÂNCIA EPIDEMIOLÓGICA E MAPEAMENTO DE ÁREAS DE RISCO ATRAVÉS DA DETECÇÃO MOLECULAR DE Leishmania spp. POR MEIO DE PCR EM FAUNA SILVESTRE NA MICRORREGIÃO DE PELOTAS, RS, BRASIL	
Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Coleta de material nas rodovias	03/2022	03/2025
2	Realização de PCR	03/2022	03/2025

Equipe

#	Nome	Função	CPF	Nacionalidade
1	Natália Soares Martins	Colaboradora	030.889.700-54	Brasileira
2	Raquel Teresinha França	Colaboradora	013.807.660-01	Brasileira
3	Fábio Raphael Pascati Bruhn	Orientador	349.787.388-81	Brasileira
4	Diego Moscarelli Pinto	Colaborador	923.647.990-15	Brasileira
5	Silvia Gonzalez Monteiro	Colaboradora	745.902.190-34	Brasileira
6	FELIPE GERALDO PAPPEN	Colaborador	001.873.340-99	Brasileira
7	RODRIGO CASQUERO CUNHA	Colaborador	005.851.970-08	Brasileira

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

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Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Observações e ressalvas

1	A autorização não eximirá o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VI) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	Deve-se observar as recomendações de prevenção contra a COVID-19 das autoridades sanitárias locais e das Unidades de Conservação a serem acessadas.
3	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros).
4	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros)
5	Este documento somente poderá ser utilizado para os fins previstos na Instrução Normativa ICMBio nº 03/2014 ou na Instrução Normativa ICMBio nº 10/2010, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
6	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
7	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen .
8	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
9	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
10	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.
11	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.

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Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Outras ressalvas

1	CEMAVE Cabedelo-PB
2	CENAP Atibaia-SP
3	CPB João Pessoa-PB
4	RAN Goiânia-GO

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Rodovias	Pelotas-RS	Pampa	Não	Fora de UC Federal
2	Rodovias	São Lourenço do Sul-RS	Pampa	Não	Fora de UC Federal
3	Rodovias	Arroio do Padre-RS	Pampa	Não	Fora de UC Federal
4	Rodovias	Cristal-RS	Pampa	Não	Fora de UC Federal
5	Rodovias	Turuçu-RS	Pampa	Não	Fora de UC Federal
6	Rodovias	Morro Redondo-RS	Pampa	Não	Fora de UC Federal
7	Rodovias	Canguçu-RS	Pampa	Não	Fora de UC Federal
8	Rodovias	Pedro Osório-RS	Pampa	Não	Fora de UC Federal
9	Rodovias	Cerrito-RS	Pampa	Não	Fora de UC Federal
10	Rodovias	Capão do Leão-RS	Pampa	Não	Fora de UC Federal

Atividades

#	Atividade	Grupo de Atividade
1	Coleta/transporte de amostras biológicas in situ	Fora de UC Federal

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Coleta/transporte de amostras biológicas in situ	Crocodylia	-
2	Coleta/transporte de amostras biológicas in situ	Squamata	-
3	Coleta/transporte de amostras biológicas in situ	Chelidae	-
4	Coleta/transporte de amostras biológicas in situ	Psittaciformes	-
5	Coleta/transporte de amostras biológicas in situ	Anseriformes	-
6	Coleta/transporte de amostras biológicas in situ	Piciformes	-
7	Coleta/transporte de amostras biológicas in situ	Falconiformes	-
8	Coleta/transporte de amostras biológicas in situ	Columbiformes	-

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Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Atividades X Táxons

#	Atividade	Táxon	Qtde.
9	Coleta/transporte de amostras biológicas in situ	Passeriformes	-
10	Coleta/transporte de amostras biológicas in situ	Apodiformes	-
11	Coleta/transporte de amostras biológicas in situ	Accipitriformes	-
12	Coleta/transporte de amostras biológicas in situ	Alouatta guariba	-
13	Coleta/transporte de amostras biológicas in situ	Felidae	-
14	Coleta/transporte de amostras biológicas in situ	Procyonidae	-
15	Coleta/transporte de amostras biológicas in situ	Canidae	-
16	Coleta/transporte de amostras biológicas in situ	Mustelidae	-
17	Coleta/transporte de amostras biológicas in situ	Mephitidae	-
18	Coleta/transporte de amostras biológicas in situ	Didelphimorphia	-
19	Coleta/transporte de amostras biológicas in situ	Lagomorpha	-
20	Coleta/transporte de amostras biológicas in situ	Rodentia	-
21	Coleta/transporte de amostras biológicas in situ	Tamandua tetradactyla	-
22	Coleta/transporte de amostras biológicas in situ	Dasypus septemcinctus	-
23	Coleta/transporte de amostras biológicas in situ	Dasypus novemcinctus	-
24	Coleta/transporte de amostras biológicas in situ	Cabassous tatouay	-
25	Coleta/transporte de amostras biológicas in situ	Euphractus sexcinctus	-

A quantidade prevista só é obrigatória para atividades do tipo "Coleta/transporte de espécimes da fauna silvestre in situ". Essa quantidade abrange uma porção territorial mínima, que pode ser uma Unidade de Conservação Federal ou um Município.

A quantidade significa: por espécie X localidade X ano.

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Aves)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue
2	Amostras biológicas (Carnívoros)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue
3	Amostras biológicas (Outros mamíferos)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue

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Código de autenticação: 0826320120220622

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Autorização para atividades com finalidade científica

Número: 82632-1	Data da Emissão: 22/06/2022 17:42:38	Data da Revalidação*: 22/06/2023
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Julia Somavilla Lignon	CPF: 068.554.429-00
Título do Projeto: VIGILÂNCIA EPIDEMIOLÓGICA E MAPEAMENTO DE ÁREAS DE RISCO ATRAVÉS DA DETECÇÃO MOLECULAR DE Leishmania spp. POR MEIO DE PCR EM FAUNA SILVESTRE NA MICRORREGIÃO DE PELOTAS, RS, BRASIL	
Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
4	Amostras biológicas (Primatas)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue
5	Amostras biológicas (Répteis)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue
6	Amostras biológicas (Xenarthra)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	Universidade Federal de Pelotas	Laboratório

Autorização para atividades com finalidade científica

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Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº 03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

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Código de autenticação: 0826320120220622**Página 6/6**

Autorização para atividades com finalidade científica

Número: 82632-4	Data da Emissão: 21/06/2023 18:39:22	Data da Revalidação*: 01/05/2024
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

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Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Realização de PCR	03/2022	03/2025
2	Coleta de material nas rodovias	03/2022	03/2025

Equipe

#	Nome	Função	CPF	Nacionalidade
1	Natália Soares Martins	Colaboradora	030.889.700-54	Brasileira
2	Raquel Teresinha França	Colaboradora	013.807.660-01	Brasileira
3	Fábio Raphael Pascati Bruhn	Orientador	349.787.388-81	Brasileira
4	Diego Moscarelli Pinto	Colaborador	923.647.990-15	Brasileira
5	Silvia Gonzalez Monteiro	Colaboradora	745.902.190-34	Brasileira
6	FELIPE GERALDO PAPPEN	Colaborador	001.873.340-99	Brasileira
7	RODRIGO CASQUERO CUNHA	Colaborador	005.851.970-08	Brasileira
8	Carolina Caetano dos Santos	Colaboradora	029.070.440-52	Brasileira

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Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Observações e ressalvas

1	A autorização não eximirá o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VI) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	Todos os membros da equipe de pesquisa devem estar cientes das recomendações e boas práticas a serem seguidas neste momento de emergência zoonossanitária no Brasil devido à gripe aviária. Informe-se na página do CEMAVE na Internet: https://www.gov.br/icmbio/pt-br/assuntos/centros-de-pesquisa/cemave/destaques/gripe-aviaria/gripe-aviaria-1 .
3	Deve-se observar as as recomendações de prevenção contra a COVID-19 das autoridades sanitárias locais e das Unidades de Conservação a serem acessadas.
4	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros).
5	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros)
6	Este documento somente poderá ser utilizado para os fins previstos na Portaria ICMBio nº 748/2022, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
7	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen .
8	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
9	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
10	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.
11	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.
12	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.

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Autorização para atividades com finalidade científica

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Dados do titular

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Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Outras ressalvas

1		CEMAVE Cabedelo-PB
2		CENAP Atibaia-SP
3		RAN Goiânia-GO
4	Considerando a pandemia de COVID-19, o CPB recomenda que as atividades de pesquisa com primatas e xenartras, em vida livre ou cativeiro, dentro ou fora de UCs federais devem adotar as medidas recomendadas no comunicado disponível no link: https://www.gov.br/icmbio/pt-br/assuntos/centros-de-pesquisa/cpb/ultimas-noticias/recomendacoes-biodiversidade-e-covid-19/recomendacoes_biodiversidade_e_covid19_ucs_e_outros_ambientes_naturais.pdf	CPB João Pessoa-PB

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Rodovias	Capão do Leão-RS	Pampa	Não	Fora de UC Federal
2	Rodovias	Cerrito-RS	Pampa	Não	Fora de UC Federal
3	Rodovias	Pedro Osório-RS	Pampa	Não	Fora de UC Federal
4	Rodovias	Canguçu-RS	Pampa	Não	Fora de UC Federal
5	Rodovias	Morro Redondo-RS	Pampa	Não	Fora de UC Federal
6	Rodovias	Turuçu-RS	Pampa	Não	Fora de UC Federal
7	Rodovias	Cristal-RS	Pampa	Não	Fora de UC Federal
8	Rodovias	Arroio do Padre-RS	Pampa	Não	Fora de UC Federal
9	Rodovias	São Lourenço do Sul-RS	Pampa	Não	Fora de UC Federal
10	Rodovias	Pelotas-RS	Pampa	Não	Fora de UC Federal
11	Rodovias	Arroio Grande-RS	Pampa	Não	Fora de UC Federal
12	Rodovias	Bagé-RS	Pampa	Não	Fora de UC Federal
13	Rodovias	Santa Vitória do Palmar-RS	Pampa	Não	Fora de UC Federal
14	Rodovias	Rio Grande-RS	Pampa	Não	Fora de UC Federal

Atividades

#	Atividade	Grupo de Atividade
1	Coleta/transporte de amostras biológicas in situ	Fora de UC Federal

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Código de autenticação: 0826320420230621

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Autorização para atividades com finalidade científica

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Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Coleta/transporte de amostras biológicas in situ	Squamata	-
2	Coleta/transporte de amostras biológicas in situ	Chelidae	-
3	Coleta/transporte de amostras biológicas in situ	Crocodylia	-
4	Coleta/transporte de amostras biológicas in situ	Piciformes	-
5	Coleta/transporte de amostras biológicas in situ	Accipitriformes	-
6	Coleta/transporte de amostras biológicas in situ	Apodiformes	-
7	Coleta/transporte de amostras biológicas in situ	Passeriformes	-
8	Coleta/transporte de amostras biológicas in situ	Columbiformes	-
9	Coleta/transporte de amostras biológicas in situ	Falconiformes	-
10	Coleta/transporte de amostras biológicas in situ	Anseriformes	-
11	Coleta/transporte de amostras biológicas in situ	Psittaciformes	-
12	Coleta/transporte de amostras biológicas in situ	Alouatta guariba	-
13	Coleta/transporte de amostras biológicas in situ	Mustelidae	-
14	Coleta/transporte de amostras biológicas in situ	Canidae	-
15	Coleta/transporte de amostras biológicas in situ	Mephitidae	-
16	Coleta/transporte de amostras biológicas in situ	Felidae	-
17	Coleta/transporte de amostras biológicas in situ	Procyonidae	-
18	Coleta/transporte de amostras biológicas in situ	Rodentia	-
19	Coleta/transporte de amostras biológicas in situ	Lagomorpha	-
20	Coleta/transporte de amostras biológicas in situ	Didelphimorphia	-
21	Coleta/transporte de amostras biológicas in situ	Euphractus sexcinctus	-
22	Coleta/transporte de amostras biológicas in situ	Dasypus septemcinctus	-
23	Coleta/transporte de amostras biológicas in situ	Tamandua tetradactyla	-
24	Coleta/transporte de amostras biológicas in situ	Dasypus novemcinctus	-
25	Coleta/transporte de amostras biológicas in situ	Cabassous tatouay	-

A quantidade prevista só é obrigatória para atividades do tipo "Coleta/transporte de espécimes da fauna silvestre in situ". Essa quantidade abrange uma porção territorial mínima, que pode ser uma Unidade de Conservação Federal ou um Município.

A quantidade significa: por espécie X localidade X ano.

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Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Aves)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue
2	Amostras biológicas (Carnívoros)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue
3	Amostras biológicas (Outros mamíferos)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue
4	Amostras biológicas (Primatas)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue
5	Amostras biológicas (Répteis)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue
6	Amostras biológicas (Xenarthra)	Animal encontrado morto ou partes (carcaça)/osso/pele, Ectoparasita, Fragmento de tecido/órgão, Outras amostras biológicas(Endoparasito), Sangue

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	Universidade Federal de Pelotas	Laboratório

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Nome da Instituição: Universidade Federal de Pelotas	CNPJ: 92.242.080/0001-00

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº 03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

Este documento foi expedido com base na Instrução Normativa nº Portaria ICMBio nº 748/2022. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

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**Anexo C - Documento do Sistema Nacional de Gestão do Patrimônio Genético
e do Conhecimento Tradicional Associado (SISGEN)**



Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº AB9FEED

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro: **AB9FEED**
Usuário: **Julia Somavilla Lignon**
CPF/CNPJ: **068.554.429-00**
Objeto do Acesso: **Patrimônio Genético**
Finalidade do Acesso: **Pesquisa**

Espécie

Leishmania

Título da Atividade: **VIGILÂNCIA EPIDEMIOLÓGICA E MAPEAMENTO DE ÁREAS DE RISCO ATRAVÉS DA DETECÇÃO MOLECULAR DE Leishmania spp. POR MEIO DE PCR EM FAUNA SILVESTRE NA MICRORREGIÃO DE PELOTAS, RS, BRASIL**

Equipe

Julia Somavilla Lignon	Universidade Federal de Pelotas
Diego Moscarelli Pinto	Universidade Federal de Pelotas
Natália Soares Martins	Universidade Federal de Pelotas
Felipe Geraldo Pappen	Universidade Federal de Pelotas
Silvia Gonzalez Monteiro	UFSM
Fábio Raphael Pascoti Bruhn	UFPEL
Raquel Teresinha França	UFPEL
Rodrigo Casquero Cunha	UFPEL

Data do Cadastro: **19/04/2022 14:00:18**
Situação do Cadastro: **Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **14:19** de **19/04/2022**.



**SISTEMA NACIONAL DE GESTÃO
DO PATRIMÔNIO GENÉTICO
E DO CONHECIMENTO TRADICIONAL
ASSOCIADO - SISGEN**