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Original Article

Molecular survey of hemotropic mycoplasmas in crab-eating raccoons (*Procyon cancrivorus*) in southern Brazil

Levantamento molecular de micoplasma hemotrópico em mão-pelada (*Procyon cancrivorus*) do sul do Brasil

Renata Fagundes-Moreira[1]; Ugo Araújo Souza[1]; Viviane Kelin de Souza[1]; Nathalia de Bem Bidone[1]; Joares Adenilson May-Júnior[1,2,3,4]; Vinicius Baggio-Souza[1]; Rodrigo Ávila Mendonça[5]; Darwin Dias Fagundes[6]; Cíntia de Lorenzo[7]; Bárbara Schiller Wartchow[7]; Eloiza Teles Caldart[8]; Aline Girotto-Soares[1]; Marcelo Meller Alievi[7]; Stella de Faria Valle[7]; João Fabio Soares[1*]

[1] Laboratório de Protozoologia e Rickettsioses Vetoriais, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre, RS, Brasil
[2] Associação Onçafari, São Paulo, Brasil
[3] Panthera Corporation, New York, USA
[4] Instituto Homem Pantaneiro, Corumbá, Brasil
[5] Universidade do Sul de Santa Catarina, Tubarão, SC, Brasil
[6] Ecoeficiência Soluções Ambientais, Taquara, RS, Brasil
[7] Faculdade de Veterinária, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre, RS, Brasil
[8] Universidade Estadual de Londrina, Londrina, PR, Brasil

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Abstract

Hemoplasmas are non-cultivable bacterial parasites of erythrocytes that infect domestic and wild animals, as well as humans. Their means of transmission and pathogenesis remain contentious issues and difficult to evaluate in wild animals. *Procyon cancrivorus* is a South American carnivore and occurs in all Brazilian biomes. In this study, we aimed to investigate occurrences of hemoplasmas infecting *P. cancrivorus* and to identify their 16S rRNA gene, in southern Brazil. DNA was extracted from spleen and blood samples of *P. cancrivorus* (n = 9) from different locations. Hemoplasma DNA was detected in six samples, based on 16S rRNA gene amplification and phylogenetic analysis. Four of the six sequences belonged to the "*Mycoplasma haemofelis* group", which is closely related to genotypes detected in *Procyon lotor* from the USA; one was within the "*Mycoplasma suis* group", closely related to "*Candidatus* Mycoplasma haemominutum"; and one was within the intermediate group between these clusters. Thus, these sequences showed that the molecular identity of hemoplasmas in the population studied was very variable. In five positive animals, *Amblyomma aureolatum* ticks and a flea (*Ctenocephalides felis felis*) were collected. The present study describes the first molecular detection of mycoplasmas in *P. cancrivorus*.

Keywords: Hemoplasma, wild carnivore, procyonid, 16S rRNA gene, South America.

Resumo

Os micoplasmas hemotrópicos (hemoplasmas) são parasitas bacterianos não-cultiváveis de eritrócitos que infectam tanto animais domésticos e selvagens, como seres humanos. A transmissão e a patogênese são discutíveis e difíceis de avaliar em animais selvagens. O mão pelada (*Procyon cancrivorus*) é um carnívoro Sul-americano, que ocorre em todos os biomas brasileiros. O objetivo do presente estudo é o de investigar a ocorrência de hemoplasmas infectando *P. cancrivorus* e identificar seu gene 16S rRNA no Sul do Brasil. O DNA foi extraído do baço e amostras de sangue de *P. cancrivorus* (n= 9). O DNA de hemoplasma foi detectado em seis amostras, com base na amplificação do gene 16S rRNA e na análise filogenética. Quatro das seis sequências pertencem ao "Grupo *Mycoplasma haemofelis*", que estão intimamente relacionadas aos genótipos detectados no *Procyon lotor* dos EUA; uma dentro do "Grupo *Mycoplasma suis*", que está intimamente relacionado ao "*Candidatus* Mycoplasma haemominutum", e uma dentro do grupo intermediário entre esses *clusters*, mostrando assim que há uma diversidade genética de hemoplasmas na população estudada. Em cinco animais positivos, foram coletados carrapatos *Amblyomma aureolatum* e uma pulga *Ctenocephalides felis*. O presente estudo traz a primeira detecção molecular de micoplasmas em *P. cancrivorus*.

Palavras-chave: Hemoplasma, carnívoro selvagem, procionídeo, gene 16S rRNA, América do Sul.

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*Corresponding author: João Fabio Soares. E-mail: jfsvet@gmail.com

Introduction

Hemotropic mycoplasmas, also known as hemoplasmas and previously called *Eperythrozoon* and *Haemobartonella*, are non-cultivable bacterial parasites of erythrocytes that infect domestic and wild animals, as well as humans (Messick, 2003; Santos et al., 2008; Sykes, 2010). Some species of hemoplasma have been well described in domestic animals: they cause reduced red blood cell counts and severe anemia when associated with other pathogens (Tasker, 2010). However, in wild animals, most infections are asymptomatic (André et al., 2011) and little is known about the pathogenesis and co-infections in these occurrences (Ghazisaeedi et al., 2017). Based on studies on domestic animals, hemoplasma transmission appears to be species-host dependent, and may be transmitted by hematophagous arthropods (Senevtratna et al., 1973; Messick, 2003), contact with saliva (through fights) (Sykes, 2010), contaminated blood (Lester et al., 1995) or vertical transmission (Girotto-Soares et al., 2016). Occurrences of hemoplasmas in wild animals in southern Brazil have not commonly been reported, but they have already been found in *Didelphis albiventris* (Massini et al., 2019), *Alouatta caraya*, *Sapajus nigritus* and *Callithrix jacchus* (Cubilla et al., 2017), bats (Santos et al., 2020), capybaras (Vieira et al., 2021) and wild felids (Ribeiro et al., 2017).

The crab-eating raccoon (Procyonidae: *Procyon cancrivorus*) is a native South American wild carnivore and occurs in all six Brazilian biomes (Reis et al., 2006). This animal has a generalist and opportunistic diet that ranges from fruits to small vertebrates and fish (Pellanda et al., 2010; Quintela et al., 2014). Moreover, this species does not have any contact with the other two species of the genus: *Procyon lotor* and *Procyon pygmaeus*.

In South America, the procyonid family comprises four species: *P. cancrivorus*, *Nasua nasua*, *Bassaricyon gabbii* and *Potos flavus*. Hemoplasmas have been detected in South American coatis (*N. nasua*) in central-western and southern Brazil (Cubilla et al., 2017; Sousa et al., 2017). Recently, a new species was detected and proposed as *'Candidatus* Mycoplasma haematonasua' (Collere et al., 2021). Genetically, the closest animal species to *P. cancrivorus* is the northern raccoon (*P. lotor*), in which different species and genotypes of hemoplasmas have been detected in the USA (Volokhov et al., 2017). However, the natural habitat of *P. lotor* is limited to North and Central America (Timm et al., 2016).

In this study, we aimed to investigate occurrences of hemoplasmas infecting *P. cancrivorus* and to identify their 16S rRNA gene, in the states of Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR), southern Brazil, using molecular and bioinformatic tools.

Material and Methods

Samples

All animal samples were collected in accordance with the stipulations of the Brazilian Institute for the Environment and Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais, IBAMA), under license numbers RS 64752- 1, SC 47488-2 and PR 55384-2. Animals were identified according to municipality of origin and condition (Table 1). Blood samples (animals 5, 6 and 9) and spleen samples (animals 1, 2, 3, 4, 7 and 8) from *P. cancrivorus* were collected from different municipalities in RS, SC and PR, in southern Brazil (Figure 1). Blood smears from animal 9 were made and stained with Giemsa (Figure 2). Spleen samples were collected from necropsies on six roadkill animals. Two animals were rescued and blood was collected during anesthesia. Ectoparasites were removed manually and were placed separately in collection tubes containing 70% ethanol to preserve the material, so as to identify them subsequently according to life stage, genus and species, by means of stereomicroscopy with incident illumination, with confirmation using the dichotomous keys described in Linardi & Guimarães (2000), Barros-Battesti et al. (2006) and Martins et al. (2010).

DNA samples

DNA was extracted from 200 µL of EDTA-blood samples from the three live animals and from 10 µg of spleen samples from the six roadkill animals. For both sample types, the Invitrogen™ PureLink™ Genomic DNA extraction mini-kit (Thermo Fisher Scientific Corporation, Carlsbad, California, USA) was used following the manufacturer's instructions.

Polymerase chain reaction (PCR)

PCR for the 16S rRNA gene of mycoplasmas was performed using the forward primer HBT F 5' 97 ATACGGCCCATATTCCTACG 3' and the reverse primer HBT R 5' 98 TGCTCCACCACTTGTTCA 3', with the aim of

Table 1. BLASTn analysis on each hemoplasma 16S rRNA sequence obtained from *Procyon cancrivorus* individuals collected in the states of Rio Grande do Sul, Santa Catarina and Paraná, southern Brazil, along with their municipality of origin, condition of life and ectoparasites.

Identification	16S rRNA GenBank® Accession Number[1]	BLASTn Best Hit and Acession Number	Host and Location	Query Cover (%)	Identity (%)[2]	City of origin	Condition	Ectoparasites
1	MK751704	Genotype VI '*Candidatus* Mycoplasma sp.' [KF743733]	*Procyon lotor* (USA)	100%	100%	Caçapava do Sul/ RS#	Roadkill	1 (f)* *Ctenocephalides felis felis*; 2 (m)** *Amblyomma aureolatum*
2	-	Negative	-	-	-	Campo Novo/RS	Roadkill	Not observed
3	MK751706	Genotype V "*Candidatus* Mycoplasma sp." [KF743736]	*Procyon lotor* (USA)	100%	97.53%	Taquara/RS	Roadkill	3 (f) and 2 (m) of *A. aureolatum*
4	-	Negative	-	-	-	Venâncio Aires/RS	Roadkill	Not observed
5	MK751705	Genotype V '*Candidatus* Mycoplasma sp.' [KF743736]	*Procyon lotor* (USA)	100%	97.98%	Jaguaruna/ SC##	Captured	2 (m) of *A. aureolatum*
6	-	Negative	-	-	-	Cachoeira do Sul/RS	Captive	Not observed
7	MN276035	'*Candidatus* Mycoplasma haemominutum' [AY150974]	*Canis lupus familiaris* (Israel)	100%	99.48%	Capivarí do Sul/RS	Roadkill	2 (m) of *A. aureolatum*
8	MN276036	Uncultured *Mycoplasma* sp. [MG649987]	*Terrapene carolina carolina* (USA)	100%	94.56%	Mauá da Serra/PR###	Roadkill	1 (f) of *A.aureolatum*
9	MN543635	*Mycoplasma haemofelis* [KU645929]	*Prionailurus viverrinus* (Thailand)	100%	100%	Carlos Barbosa/RS	Rescue	1 (m) and 1 (f) of *A. relatum*

*Female (f); **Male (m). #RS = Rio Grande do Sul; ##SC = Santa Catarina; ###PR = Paraná. [1]Accession number of sequences generated in the present study. [2]Percentage similarity to sequences deposited at GenBank®.

amplifying a 595 to 620 bp fragment of the gene, as previously described (Criado-Fornelio et al., 2003). A positive sample of '*Candidatus* Mycoplasma haemobos' was set up as the positive control (Girotto et al., 2012), and the negative control was UltraPure™ DNase/RNase-free distilled water (Invitrogen™, Carlsbad, CA, USA). The PCR products were subjected to electrophoresis on 1.5% agarose gel and the results were examined using a LED transilluminator.

Sequencing and phylogenetic analysis

Amplicons of the expected size were purified using the Invitrogen™ PureLink™ Quick PCR purification kit (Thermo Fisher Scientific Corporation, Carlsbad, California, USA) and were sequenced in an automated sequencer (Sanger) in accordance with the manufacturer's protocol. The sequences thus generated were subjected to BLAST® analysis (Altschul et al., 1990) to determine the closest similarities to those in GenBank®.

Six partial sequences of the 16S rRNA gene of hemotropic mycoplasmas derived from *P. cancrivorus* were obtained and these were aligned with the corresponding 16S rRNA sequences of 48 *Mycoplasma* samples retrieved from GenBank® using Clustal/W v.1.8.1 (Thompson et al., 1994) (Figure 3). This generated a total of 397 valid positions for each sequence in the final dataset. A maximum likelihood phylogenetic tree using the T92 + G substitution model was generated using the Mega 11 software (Kumar et al., 2016) with 1000 bootstrap replicates. The substitution model was selected using the Mega 11 software (Kumar et al., 2016) according to the lowest Bayesian information criterion score. Sequence NR 113659 of *Mycoplasma pneumoniae*, a non-hemotropic mycoplasma, was used as an outgroup.

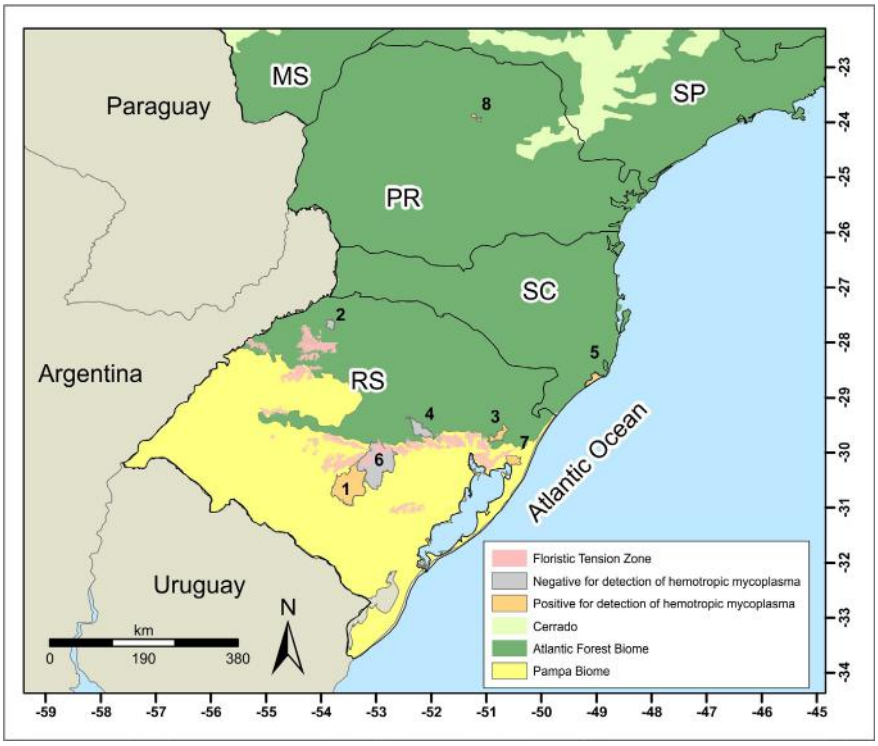

Figure 1. Localization of *Procyon cancrivorus* animals in the states of Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR), in Southern Brazil, showing the Atlantic Forest biome (in green), Pampa biome (in yellow) and the floristic tension zone between these biomes in RS (in pink). The municipalities in which positive animals were found, with their respective identification numbers, are shown in orange; municipalities with negative individuals are shown in gray.

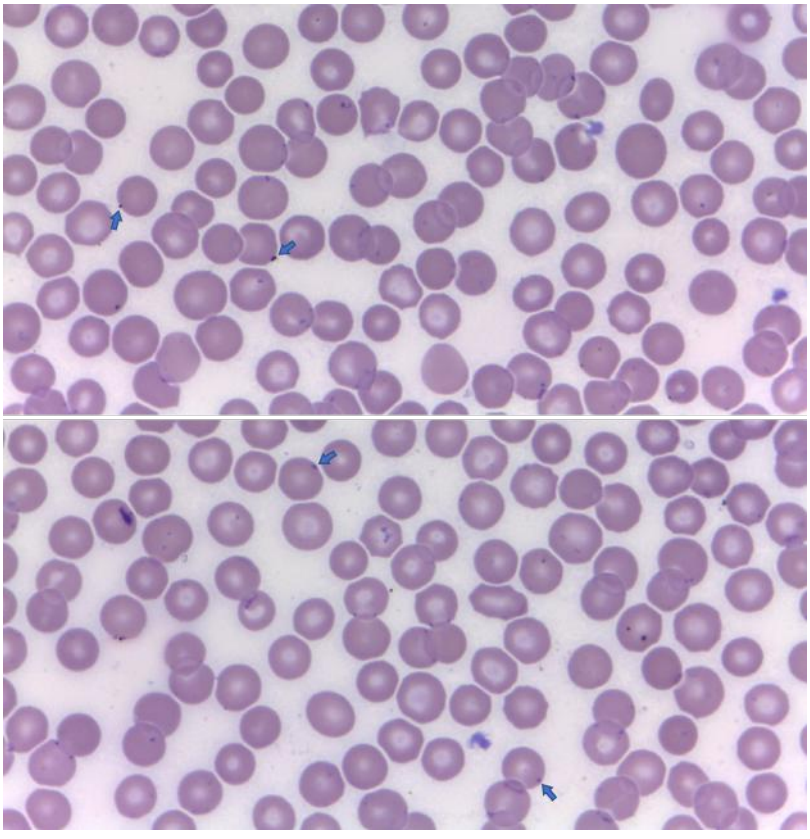

Figure 2. Light microscopy images of a quick panoptic stain blood smear from a *Procyon cancrivorus* individual (animal 9) showing small basophilic structures attached to erythrocytes (arrows) (1,000X).

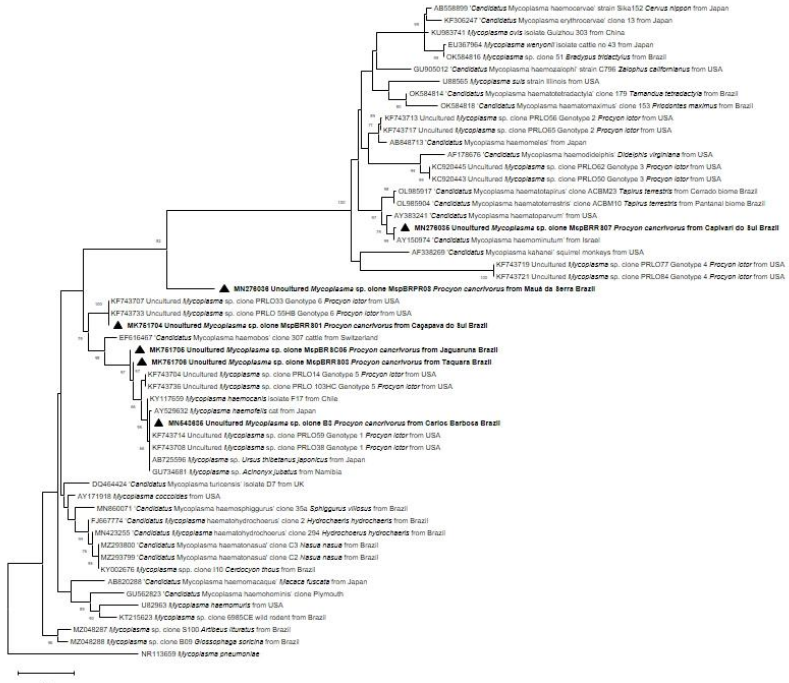

Figure 3. Maximum likelihood phylogenetic tree of 16S rRNA partial sequences of uncultured *Mycoplasma* spp. from *Procyon cancrivorus* and other hemoplasmas. Numbers on the nodes indicate bootstrap values from 1000 replicates. Only bootstrap values > 50 are shown. Numbers in brackets are GenBank® accession numbers. The *Mycoplasma* spp. from *P. cancrivorus* sequences generated in the present study are in bold and are indicated by a black arrowhead. Sequence NR 113659 of *Mycoplasma pneumoniae*, a non-hemotropic mycoplasma, was used as the outgroup.

The DNA sequences generated in the present study were deposited in GenBank® under the accession numbers MK751706 (485 bp), MK751704 (525 bp), MK751705 (594 bp), MN276035 (577 bp), MN276036 (590 bp) and MN276037 (567 bp).

The analysis to create the pairwise distance matrix from alignment of the 16S rRNA sequences detected in *P. cancrivorus* in the present study was performed using MEGA 11 (Table 2).

Results

Six out of the nine procyonids showed DNA positive for hemoplasmas. Three hemoplasma sequences (accession numbers MK751704, MK751706 and MK751705) showed 98% to 100% similarity to two distinct genotypes deposited in GenBank® that had been detected in *Procyon lotor*. The other three hemoplasma sequences (accession numbers MN276035, MN276036 and MN276037) were positioned near *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum', and near to a species deposited as "uncultured *Mycoplasma* sp." that had been detected in a turtle (*Terrapene carolina carolina*) in the USA.

Based on 16S rRNA gene amplification and phylogenetic analysis, four sequences were positioned within the "*Mycoplasma haemofelis* group", one within the "*Mycoplasma suis* group" and one within an intermediate group between these clusters. Sequences from animals 3 and 5 were found to be phylogenetically related to genotype V (similarities of 97.53% and 97.98%, respectively), and the sequence detected in animal 1 was found to be related to genotype VI (similarity of 100%) (Table 1), both proposed by Volokhov et al. (2017). Two sequences (animals 7 and 9) were found to be closely related to '*Candidatus* Mycoplasma haemominutum' and *M. haemofelis* (similarities of 99.48% and 100%, respectively). One sequence (animal 8) was found to be related to hemotropic *Mycoplasma* sp., which deserves to be highlighted, due to its low similarity to sequences from GenBank® (similarity of 94.56%). Given that 16S rRNA is a highly conserved gene, this finding strongly suggests that this sequence represented a new species of hemoplasma, but further studies would be needed to confirm this.

Table 2. Estimates of evolutionary divergence between sequences of 16S rRNA from the procyonids *Procyon cancrivorus, Procyon lotor* and *Nasua nasua.*

	MK751705 MspBRSC05 *Procyon cancrivorus* Jaguaruna Brazil	MK751706 MspBRRS03 *Procyon cancrivorus* Taquara Brazil	MN276035 MspBRRS07 *Procyon cancrivorus* Capivari do Sul Brazil	MK751704 MspBRRS01 *Procyon cancrivorus* Caçapava do Sul Brazil	MN276036 MspBRPR08 *Procyon cancrivorus* Maua da Serra Brazil	MN543635 B3 *Procyon cancrivorus* Carlos Barbosa Brazil	KF743713 PRLO56 *Procyon lotor* Genotype 2 USA	KF743717 PRLO65 *Procyon lotor* Genotype 2 USA	KC920445 PRLO62 *Procyon lotor* Genotype 3 USA	KC920443 PRLO50 *Procyon lotor* Genotype USA	KF743721 PRLO84 *Procyon lotor* Genotype USA	KF743707 PRLO33 *Procyon lotor* Genotype 6 USA	KF743704 PRLO14 *Procyon lotor* Genotype 5 USA	KF743714 PRLO59 *Procyon lotor* Genotype 1 USA	KF743719 PRLO77 *Procyon lotor* Genotype 4 USA	KF743733 PRLO55HB *Procyon lotor* Genotype 6 USA	KF743736 PRLO103HC *Procyon lotor* Genotype 5 USA	KF743708 PRLO38 *Procyon lotor* Genotype 1 USA	MZ293800 C3 *Nasua nasua* from Brazil	MZ293799 C2 *Nasua nasua* Brazil
MK751705 MspBRSC05 *Procyon cancrivorus* Jaguaruna Brazil																				
MK751706 MspBRRS03 *Procyon cancrivorus* Taquara Brazil	0.0032017062																			
MN276035 MspBRRS07 *Procyon cancrivorus* Capivari do Sul Brazil	0.1509808790	0.1408572277																		
MK751704 MspBRRS01 *Procyon cancrivorus* Caçapava do Sul Brazil	0.0467440193	0.0369844610	0.1320833990																	
MN276036 MspBRPR08 *Procyon cancrivorus* Maua da Serra Brazil	0.0890154798	0.0791085300	0.1264187385	0.0779730485																
MN543635 B3 *Procyon cancrivorus* Carlos Barbosa Brazil	0.0160232232	0.0129848165	0.1514739319	0.0592377633	0.0855999417															
KF743713 PRLO56 *Procyon lotor* Genotype 2 USA	0.1486176082	0.1406236815	0.0356742146	0.1368553188	0.1226144827	0.1407890702														
KF743717 PRLO65 *Procyon lotor* Genotype 2 USA	0.1486176082	0.1406236815	0.0356742146	0.1368553188	0.1226144827	0.1407890702	0.0014779114													
KC920445 PRLO62 *Procyon lotor* Genotype 3 USA	0.1695992135	0.1616903894	0.0644405987	0.1532282299	0.1491472578	0.1609282750	0.0554986997	0.0538429198												
KC920443 PRLO50 *Procyon lotor* Genotype USA	0.1695992135	0.1616903894	0.0644405987	0.1532282299	0.1491472578	0.1609282750	0.0554986997	0.0538429198	0.0000000000											
KF743721 PRLO84 *Procyon lotor* Genotype USA	0.1773663914	0.1652163634	0.1023540820	0.1584856408	0.1582826510	0.1730704691	0.0999468571	0.1017346139	0.1076200492	0.1076200492										
KF743707 PRLO33 *Procyon lotor* Genotype 6 USA	0.0467440193	0.0369844610	0.1320833990	0.0000000000	0.0779730485	0.0592377633	0.1368553188	0.1368553188	0.1532282299	0.1532282299	0.1584856408									
KF743704 PRLO14 *Procyon lotor* Genotype 5 USA	0.0127542586	0.0096888195	0.1491368024	0.0573776265	0.0854311779	0.0095633128	0.1384957598	0.1384957598	0.1654861793	0.1654861793	0.1733817610	0.0573776265								
KF743714 PRLO59 *Procyon lotor* Genotype 1 USA	0.0160232232	0.0129848165	0.1514739319	0.0592377633	0.0855999417	0.0000000000	0.1407890702	0.1407890702	0.1609282750	0.1609282750	0.1730704691	0.0592377633	0.0095633128							
KF743719 PRLO77 *Procyon lotor* Genotype 4 USA	0.1773663914	0.1652163634	0.1023540820	0.1584856408	0.1582826510	0.1730704691	0.0999468571	0.1017346139	0.1076200492	0.1076200492	0.0000000000	0.1584856408	0.1733817610	0.1730704691						
KF743733 PRLO55HB *Procyon lotor* Genotype 6 USA	0.0467440193	0.0369844610	0.1320833990	0.0000000000	0.0779730485	0.0592377633	0.1368553188	0.1368553188	0.1532282299	0.1532282299	0.1584856408	0.0000000000	0.0573776265	0.0592377633	0.1584856408					
KF743736 PRLO103HC *Procyon lotor* Genotype 5 USA	0.0127542586	0.0096888195	0.1491368024	0.0573776265	0.0854311779	0.0095633128	0.1384957598	0.1384957598	0.1654861793	0.1654861793	0.1733817610	0.0573776265	0.0000000000	0.0095633128	0.1733817610	0.0573776265				
KF743708 PRLO38 *Procyon lotor* Genotype 1 USA	0.0160232232	0.0129848165	0.1514739319	0.0592377633	0.0855999417	0.0000000000	0.1407890702	0.1407890702	0.1609282750	0.1609282750	0.1730704691	0.0592377633	0.0095633128	0.0000000000	0.1730704691	0.0592377633	0.0095633128			
MZ293800 C3 *Nasua nasua* from Brazil	0.0613254640	0.0585548258	0.1384663139	0.0470125325	0.0987632257	0.0724511658	0.1366601004	0.1366601004	0.1662476013	0.1662476013	0.1666247146	0.0470125325	0.0702421501	0.0724511658	0.1666247146	0.0470125325	0.0702421501	0.0724511658		
MZ293799 C2 *Nasua nasua* Brazil	0.0613254640	0.0585548258	0.1384663139	0.0470125325	0.0987632257	0.0724511658	0.1366601004	0.1366601004	0.1662476013	0.1662476013	0.1666247146	0.0470125325	0.0702421501	0.0724511658	0.1666247146	0.0470125325	0.0702421501	0.0724511658	0.0000000000	

< 0.1199 - shown in green - low divergence; > 0.1200 - shown in red - high divergence.

The following ectoparasites were observed through examining the animals of this study: one flea (*Ctenocephalides felis*) and two males of the tick *Amblyomma aureolatum* (animal 1); three females and two males of *A. aureolatum* (animal 3); two males of *A. aureolatum* (animal 5); two males of *A. aureolatum* (animal 7); one female of *A. aureolatum* (animal 8); and one male and one female of *A. aureolatum* (animal 9). No ectoparasites were found on the other individuals at the time of collection.

Discussion

The present study presents the first molecular detection of hemotropic mycoplasma in *P. cancrivorus* in South America. Through molecular investigation of blood and spleen samples, occurrences of *Mycoplasma* sp. in the genus *Procyon* in southern Brazil were determined. Some of the samples showed genotypes phylogenetically related to hemoplasmas that had been found in North American procyonids (*P. lotor*) (Volokhov et al., 2017).

Considering previous studies in Brazil, *N. nasua* specimens were found to be positive for hemoplasmas that were genetically related to *M. haemocanis*/*M. haemofelis* and possibly to a new genotype (Sousa et al., 2017), but were unrelated to the genotypes found in the present study. In another study in south Brazilian states, hemoplasmas presented similarities to the ones found in *P. lotor* and *M. haemofelis*, and to a rodent hemoplasma from the USA (Cubilla et al., 2017).

In contrast to domestic animals, the pathogenicity of hemoplasmas in wild animals remains unknown, mainly due to the impossibility of evaluating the clinical status of most of these animals. As mentioned by de Oliveira et al. (2022), hemoplasma infection in wildlife is generally chronic and does not present high levels of bacteremia, thus explaining the low pathogenicity. In the present study, hemoplasmas were detected in two live individuals through molecular techniques: one of these was rescued after being hit by a car and the other one was captured. However, neither of these individuals showed any physiological signs related to hemoplasma infection. In the blood smear evaluations on these two individuals, corpuscles suggestive of hemoplasma parasitizing erythrocytes were observed, thus suggesting the presence of ongoing bacteremia.

Occurrences of hemoplasmas in Brazilian wildlife have been recorded in the following animals: opossums (Massini et al., 2019; Pontarolo et al., 2021), non-human primates (Bonato et al., 2015; Cubilla et al., 2017), lowland tapirs (Mongruel et al., 2022), xenarthrans (Oliveira et al., 2022), bats (Santos et al., 2020; Ikeda et al., 2022), capybaras (Vieira et al., 2021) and wild felids (Ribeiro et al., 2017).

Habitat fragmentation, anthropic actions, synanthropism and other activities that can cause stress or reduce immunocompetence may be the triggering factors for these agents to cause symptomatic disease in wild animals (Neimark et al., 2001; Tasker, 2010). The states of RS, SC and PR are characterized by three biomes: Pampa (RS only), Cerrado (PR only) and Atlantic Forest (Mata Atlântica). The distribution of positive animals between the Pampa and Atlantic Forest biomes and in the zone of floristic tension between these two biomes is interesting, in that this shows the wide range of hemoplasma coverage in southern Brazil, along with its distribution in these biomes. Nonetheless, data are too scarce to be able to make inferences correlating the different hemoplasma genotypes and species with the biomes.

Conclusion

This study reports the first molecular finding of hemoplasmas in the South American procyonid, *P. cancrivorus*. Detection of different hemoplasma species demonstrates that scattered circulation of the agent occurs among individuals in southern Brazil. The species detected were similar to what has been reported in *P. lotor* in the USA, and were distant from the species detected in *N. nasua*, which is another procyonid that is much closer geographically to *P. cancrivorus* in South America. These data highlight the need for further studies on procyonids in Brazil, especially with a view to targeting other genes. The means of vectorial transmission and pathogenicity of hemotropic mycoplasmas among wild animals remain unknown. Therefore, it is suggested that further studies should be conducted in order to understand the parasite-host interactions and pathogenic potential of these hemoplasmas in procyonid populations.

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Ethics declaration

All animal samples were collected in accordance with the stipulations of the Brazilian Institute for the Environment and Natural Resources (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais, IBAMA), under license numbers RS 64752- 1, SC 47488-2 and PR 55384-2.

Conflit of interest

None.

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