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RESEARCH

Molecular survey of potentially pathogenic microorganisms in ticks collected from coatis (*Nasua nasua*) in Iguaçu National Park, Atlantic Forest biome, southern Brazil

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Abstract

Human contact with wild animals in synanthropic habits is often mediated by arthropod vectors such as ticks. This is an important method of spreading infectious agents that pose a risk to human health. Thus, this study aimed to molecularly detect *Ehrlichia* spp., *Anaplasma* spp., *Borrelia* spp., and protozoa of the order Piroplasmida in ticks collected from coatis of Iguaçu National Park (PNI), Paraná, Brazil. This study involved 553 ticks DNA, including *Amblyomma* spp. larvae, *Haemaphysalis juxtakochi* nymphs, *Amblyomma brasiliense*, *Amblyomma coelebs*, and adults of *Amblyomma ovale*. The DNA extracted from each sample was subjected to polymerase chain reaction (PCR) targeting the genes 23S rRNA for the Anaplasmataceae family, 16S rRNA for *Anaplasma* spp., *dsb* for *Ehrlichia* spp., *flaB*, 16S rRNA, *hpt*, and *glpQ* for *Borrelia* spp., and 18S rRNA for Piroplasmid protozoans. DNA from *Anaplasma* sp. was detected in ticks of the species *A. coelebs* (4/553); *Borrelia* sp. DNA was detected in *A. coelebs* (3/553), *A. ovale* (1/553), and *Amblyomma* larvae (1/553); and *Theileria* sp. was detected in *A. coelebs* (2/553). All tested samples were negative for *Ehrlichia* spp. Our study constitutes the newest report in South America of these microorganisms, which remain poorly studied.

Keywords Procyonidae · Tick-borne pathogens · *Anaplasma* · *Borrelia* · Piroplasmida · Atlantic Forest

Introduction

Ticks are a group of hematophagous ectoparasites that are of great importance to public health due to their ability to transmit numerous pathogenic agents, such as viruses, bacteria, and protozoa (Guimarães et al. 2001; Dantas-Torres et al. 2012). These ectoparasites parasitize a wide range of hosts, and may even therefore encompass humans (Barros-Battesti et al. 2006; Palomar et al. 2012).

Coatis (*Nasua nasua*) can be reservoirs of numerous zoonotic agents, such as *Leishmania* and *Trypanosoma* species (Porfírio et al. 2018). Studies have shown the importance of these procyonids in the epidemiology of these microorganisms, including those transmitted by ticks,

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such as piroplasmids and rickettsiae (Magalhães-Matos et al. 2022; Perles et al. 2023).

Iguaçu National Park (PNI) is considered a worldwide reference for nature conservation and sustainable tourism. It comprises an area of 186,000 ha of protected and rich biodiverse Atlantic Forest, housing 12 species of amphibians, 48 species of reptiles, 158 species of mammals, 175 species of fish, 390 species of birds, and more than 800 species of invertebrates (ICMBIO, 2023). Among the species of mammals in PNI, coatis stand out, as they are commonly found in flocks and often forage for food of anthropic origin (ICMBIO, 2023). These animals are highly resistant to anthropogenic pressures and can easily adapt to modified areas, mainly because of the high availability of food (Ferreira et al. 2013). Thus, the presence of people and the flow of individuals in PNI facilitates the human approach to synanthropic animals, such as coatis (Magalhães-Matos et al. 2017) and, consequently to their ectoparasites and zoonotic pathogens.

In this context, the present study aimed to conduct molecular research on *Ehrlichi*a spp., *Anaplasma* spp., *Borrelia* spp., and protozoans of the order Piroplasmida in ticks collected from coatis found in PNI, located in Foz do Iguaçu, state of Paraná, southern Brazil.

Material and methods

Samples

This study was conducted using DNA samples derived from ticks collected from coatis native to the Atlantic Forest in the environmental conservation area of PNI dependencies located in the municipality of Foz do Iguaçu, state of Paraná, southern Brazil. Samples of ticks were collected in September 2014 and March and April 2015 from 86 coatis.

Three collection points were chosen in the tourist area of the INP: points of access to two trails inside the forest (point I—25° 37′ 36″ S, 54° 27′ 39″ W and point II—25° 39′ 05″ S, 54° 26′ 16″ W) and the viewpoints of the falls (point III—25° 41′ 03″ S, 54° 26′ 24″ W, with a total length of approximately 1.2 km).

Sub-adult and adult ring-tailed coatis were attracted with banana, pineapple, or peanut butter bait and captured with a hand net (multifilament nylon, 60 × 120 cm) or Tomahawk traps (90 × 45 × 50 cm and 50 × 21.5 × 20 cm). After being contained, the ring-tailed coatis received a pre-anesthesia. Each animal was examined thoroughly to collect ticks, and the collected specimens were stored in RNAlater® and frozen at 20 °C until the moment of molecular analysis.

Taxonomic identification of the ticks was conducted based on morphology, using the specific dichotomous keys for ixodid ticks developed by Cooley (1946) and Kohls (1960) for *Haemaphysalis* nymphs, Martins et al. (2010) for *Amblyomma* nymphs, and Barros-Battesti et al. (2006) for adults ticks. Larvae from ticks were identified only at their genus level, since in Brazil there is no literature for specific identification. DNA extraction was performed individually from each tick by the phenol–chloroform method. Details of the methodology for collection and identification, conservation, transport, and DNA extraction from ticks are described in Magalhães-Matos et al. (2017) and Magalhaẽs-Matos et al. (2022).

A total of 553 ticks were used, including larvae of *Amblyomma* spp. (n=18); nymphs of *Amblyomma coelebs* (n=413), *Amblyomma brasiliense* (n=72), *Haemaphysalis juxtakochi* (n=5), and adults of *Amblyomma ovale* (n=45).

Polymerase chain reaction (PCR)

PCR assays were performed to detect the presence of DNA from potentially pathogenic bacteria and protozoa, such as *Ehrlichi*a spp., *Anaplasma* spp., *Borrelia* spp., and protozoans of the order Piroplasmida. Specific primers were used for each agent following the original protocol for each primer. Table 1 shows the primers used along with the target genes, sizes of the amplified products, and reference protocols used. DNA from *Borrelia anserina* strain AL (culture), *Babesia bigemina* (bovine positive), *Anaplasma platys* (dog positive), or *Ehrlichia canis* (dog positive) were used as positive controls, and ultrapure water was used as a negative control.

PCR products (10 µL) were applied to a 1.5% agarose gel, separated using electrophoresis (5 V/cm), stained with ethidium bromide (0.5 µg/mL), and visualized using an ultraviolet (UV) light transilluminator.

Sequencing

The material for sequencing was purified from 5 µL of the PCR product of the positive samples and treated with Exo-Sap-IT (GE Healthcare), following the manufacturer's protocol. The fragments were sequenced in both directions using an automated genetic analyzer (ABI 3730 DNA Analyzer, Thermo Fisher Scientific). The obtained sequences were aligned using the DNA Baser® program and subjected to a homology search with other sequences deposited in GenBank using the BLASTn tool.

Phylogenetic analyses

For all phylogenetic analyses, the sequences obtained in our study were aligned with those in databases using the MUSCLE tool (Edgar 2004) in the Seaview4 program (Gouy et al. 2010). Phylogenetic relationships were estimated using phylogenetic inference with the maximum likelihood (ML) method, which was implemented using

Table 1 List of primers used for the PCR analyses in the present study

Gene/*primer*	Molecular assay	Aim	Sequence (5′-3′)	Fragment	AT	Reference
Anaplasmataceae						
23S rRNA gene						
Ana23S-212f	cPCR	Screening	ATAAGCTGCGGGGAATTGTC	515 bp	55 °C	Dahmani et al. (2015)
Ana23S-723r			TGCAAAAGGTACGCTGTCAC			
Anaplasma spp.						
16S r RNA gene						
EE-1	nPCR	Characterization	TCCTGGCTCACGAACGCTGGCGGC	1433 bp	50 °C	Barlough et al. (1996)
EE-2			AGTCACTGACCCAACCTTAAATGGCTG			
EE-3			GTCGAACGGATTATTCTTTATAGCTTGC	926 bp	50 °C	
EE-4			CCCTTCCGTTAAGAAGGATCTAATCTCC			
Ehrlichia spp.						
Dsb gene						
DSB-330	snPCR	Screening	GATGATGCTTGAAGATATSAAACAAAT	349 bp	50 °C	Almeida et al. (2013)
DSB-380			ATTTTTAGRGATTTTCCAATACTTGG			
DSB-720			CTATTTTACTTCTTAAAGTTGATAWATC		52 °C	
Borrelia spp.						
flaB gene						
FlaLL	nPCR	Screening	ACATATTCAGATGCAGACAGAGGT	665 bp	55 °C	Stromdahl et al. (2003)
FlaRL			GCAATCATAGCCATTGCAGATTGT			
FlaLS			AACAGCTGAAGAGCTTGGAAT	354 bp	55 °C	
FlaRS			CTTTGATCACTTATCATTCTAATAGC			
flaB gene						
BorFlaF1	nPCR	Characterization	TACATCAGCTATTAATGCTTCAAGAA	740 bp	55 °C	Blanco et al. (2017)
BorFlaR1			GCAATCATWGCCATTGCRGATTG			
BorFlaF2			CTGATGATGCTGCTGGWATGG		55 °C	
BorFlaR2			TCATCTGTCATTRTWGCATCTT			
16S rRNA gene						
16S-Fin1 adapt	cPCR	Characterization	CCAACACCTCACAGCACGAGCTGA	733 bp	58 °C	Araújo et al. (2022)
16SLDPR2			AGCAGCTAAGAATCTTCCGCAATGG			
hpt gene						
hptf	cPCR	Screening	GCAGAYATTACAAGAGARATGG	433 bp	53 °C	Mccoy et al. (2014)
hptR			CYTCRTCACCCCATTGAGTTCC			
glpQ gene						
glpQ+1	cPCR	Screening	GGGGTTCTGTTACTGCTAGTGCCATTAC	817 bp	53 °C	Schwan et al. (2005)
glpQ-1			CAATTTTAGATATGTCTTTACCTTGTT GTTTATGCC			
Piroplasmida						
18S rRNA gene						
Bcommon-F	cPCR	Screening	GCATTTGCGATGGACCATTCAAG	200 bp	55 °C	Qurollo et al. (2017)
Bcommon-R			CCTGTATTGTTATTTCTTGTCACTACCTC			
18S rRNA gene						
BT-F3	cPCR	Characterization	TGGGGGGAGTATGGTCGCAAG	650 bp	57 °C	Seo et al. (2013)
BT-R3			CTCCTTCCTTTAAGTGATAAG			

cPCR, conventional PCR; *nPCR*, nested PCR; *snPCR*, semi-nested PCR; *AT*, annealing temperature

PhyML (Guindon and Gascuel 2003) under a sequence evolution model chosen after hierarchical testing of alternative models based on the Bayesian information criterion in MEGA version 7 (Kumar et al. 2016). Statistical support for clades was assessed using a heuristic search with 1000 bootstrap replicates. Phylogenetic relationships were visualized using FigTree v.1.4 software (Rambaut 2012).

Table 2 Tick species and frequency of positive samples for *Anaplasma* spp., *Borrelia* spp., and agents of the Order Piroplasmida, by tick species

		Anaplasma spp.		*Borrelia spp.*		Piroplasmida order	
Tick species	No.	No. pos. (%)	23S rRNA and *16S rRNA*	No. pos. (%)	*flaB and 16SrRNA*	No. pos. (%)	18S rRNA
Amblyomma spp.	18	0	-	1 (5.6)	*Borrelia* sp.	0	
Haemaphysalis juxtakochi	5	0	-	0		0	
Amblyomma brasiliense	72	0	-	0		0	
Amblyomma coelebs	413	4 (1.0)	*Anaplasma* sp.	3 (0.7)	*Borrelia* sp.	2 (0.5)	*Theileria* sp.
Amblyomma ovale	45	0	-	1 (2.2)	*Borrelia* sp.	0	
Total	553	4 (1.0)		5 (0.9)		2 (0.4)	

No, tick number; ***No. pos*.,** number of positives

Results

Of the total number of ticks analyzed using PCR (553), 1.99% (11/553) amplified DNA from at least one of the tested groups (*Anaplasma* spp., *Borrelia* spp., or Piroplasmida), and all samples were negative for *Ehrlichia* spp. The frequencies of the positive tick species are listed in Table 2.

The four samples positive for *Anaplasma* spp. in *A. coelebs* nymphs were identical and had a similarity of 94.7%

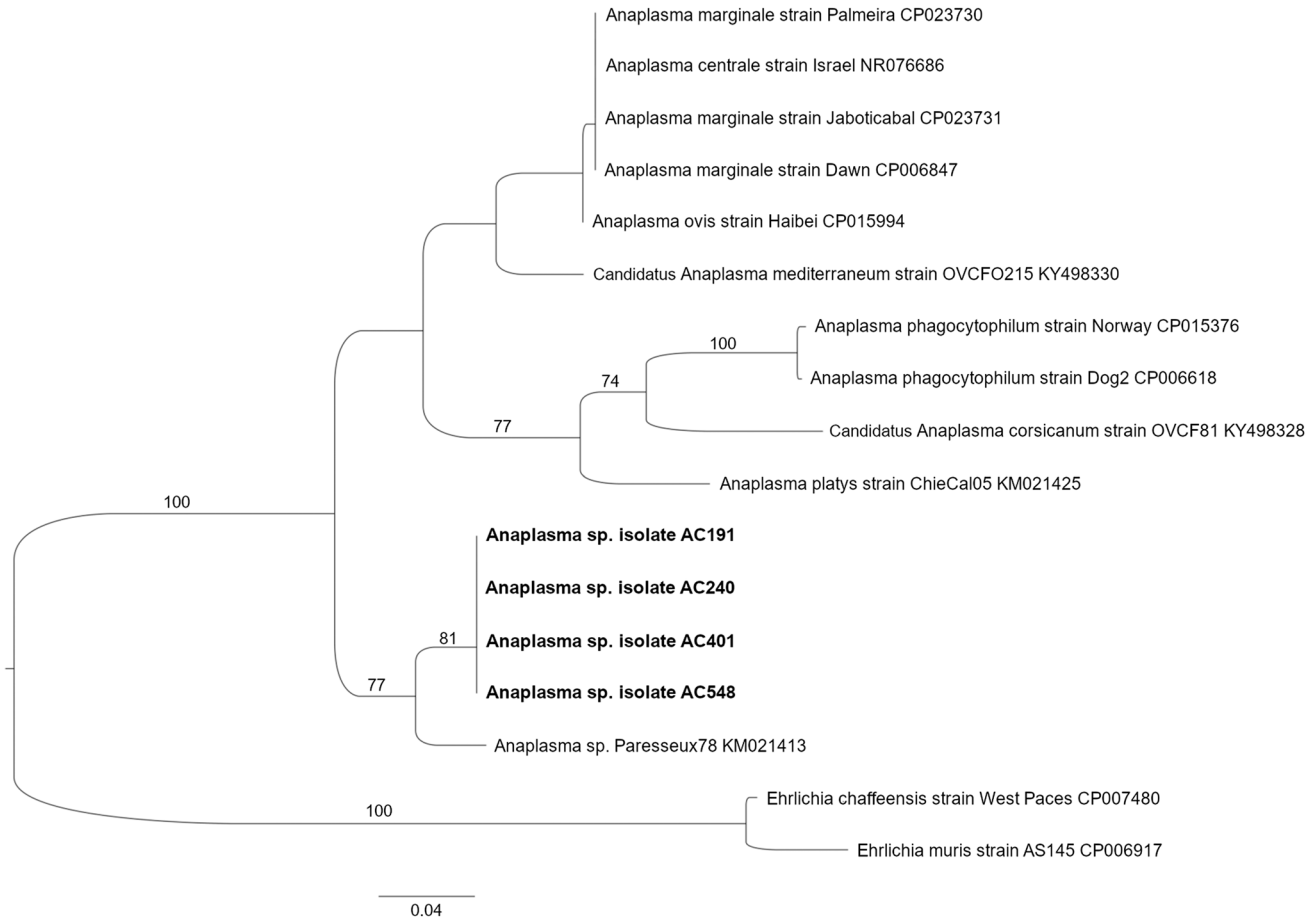

Fig. 1 Phylogenetic tree illustrating the relationships among *Anaplasma* species identified in this study (highlighted in bold). The tree is constructed based on 23S rRNA gene sequences, employing the maximum likelihood (ML) method. The numbers (>70%) presented above the branches represent bootstrap values. The scale bars correspond to an evolutionary distance of 0.03 substitutions per sequence position, and the branch labels contain GenBank accession numbers. *Ehrlichia chaffensis* (CP007480) and *Ehrlichia muris* (CP006917) were used as outgroup

(447/472) and 98.09% (820/836) with the 23S and 16S ribosomal genes of *Anaplasma marginale* cepa Florida (CP001079), respectively. The partial sequences of the *flaB* and 16S rRNA genes of *Borrelia* spp. present in ticks of the species *Amblyomma* sp. larvae, *A. coelebs* nymphs, and *A. ovale* adult female exhibited little difference (99.65 to 100% identity) and an identity 87.7% (556/634) and 99.1% (500/505), respectively, with spirochetes from the relapsing fever group (RFG) (MG944997 and KT364340, respectively). Finally, the partial sequences of the piroplasmid 18S rRNA gene in two ticks of the *A. coelebs* specie showed 97.60% (447/458) similarity with *Theileria cervi* (MW008518).

Samples positive for *Borrelia* spp. were not amplified in the PCR assays for the *hpt* and *glpQ* genes, and two of them did not amplify for the 16SrRNA gene. This was likely due to the sensitivity of the primers used and/or the fact that the samples did not have sufficient DNA concentration for amplification.

GenBank accession numbers for the partial sequences obtained in the present study are as follows: MT018000 (*Borrelia* sp. strain AC129, *flaB*), MT018001 (*Borrelia* sp. strain AC425, *flaB*), MT018002 (*Borrelia* sp. strain AO17, *flaB*), MT018003 (*Borrelia* sp. strain AC444, *flaB*), MT018004 (*Borrelia* spp. strain AC549, *flaB*), MT019342 (*Borrelia* sp. AC129, 16S rRNA), MT019525 (*Borrelia* sp. strain AC425, 16S rRNA), MT019528 (*Borrelia* sp. strain AC129, 16S rRNA), MT022490 (*Anaplasma* sp. isolate AC240, 23S rRNA), MT019664 (*Anaplasma* sp. isolate AC458, 23S rRNA), MT019625 (*Anaplasma* sp. isolate

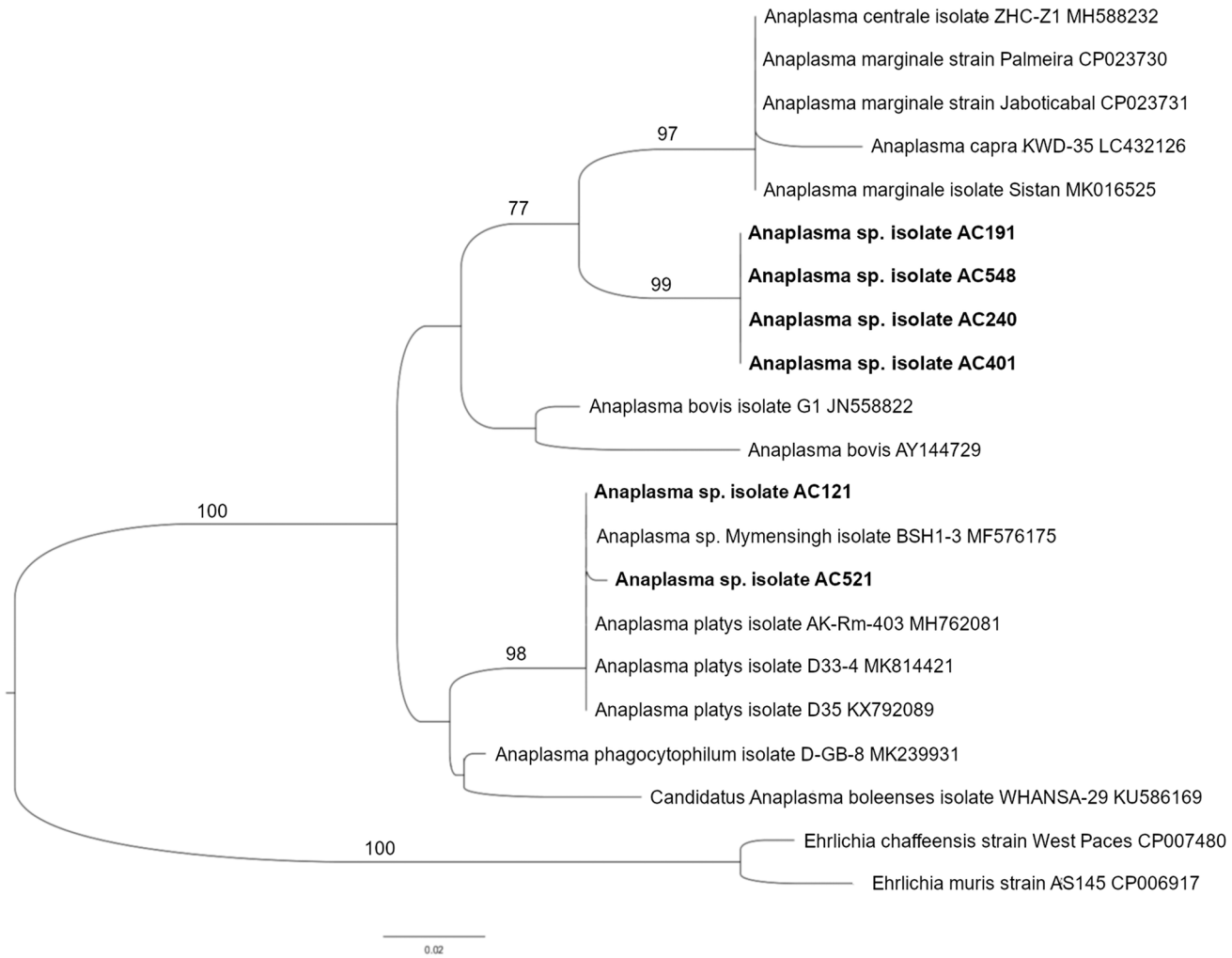

Fig. 2 Phylogenetic tree depicting the phylogenetic relationships between *Anaplasma* species detected in this investigation (highlighted in bold). The tree is constructed using 16S rRNA gene sequences and the maximum likelihood (ML) method. The numbers (>70%) displayed above the branches indicate the bootstrap values. The scale bars indicate an evolutionary distance of 0.02 substitutions per sequence position, and the branch labels include GenBank accession numbers. *Ehrlichia chaffensis* (CP007480) and *Ehrlichia muris* (CP006917) were used as outgroup

AC401, 23S rRNA), MT019564 (*Anaplasma* sp. isolate AC191, 23S rRNA), MT019536 (*Anaplasma* sp. isolated AC191, 16S rRNA), MT019537(*Anaplasma* sp. isolated AC240, 16S rRNA), MT019545 (*Anaplasma* sp. isolated AC401, 16S rRNA), MT019560 (*Anaplasma* sp. isolated AC548, 16S rRNA), MT019670 (*Theileria* sp. isolate *A. coelebs* 74), and MT019669 (*Theileria* sp. isolate *A. coelebs* 262).

Phylogenetic analyses clustered the sequences detected in *A. coelebs* with *A. marginale*, *Anaplasma centrale*, and *Anaplasma capra* (16S rRNA - Bootstrap 88%), and *Anaplasma* sp. Paresseux78 (Figs. 1 and 2) previously detected in *Bradypus tridactylus* (23S rRNA - Bootstrap 99%) (Fig. 1). For the flagellin B (Fig. 4) and 16S rRNA (Fig. 3) genes for *Borrelia* spp., the phylogenetic analyses clustered the sequences detected in *A. coelebs* and *A. ovale* with *Borrelia turcica* isolated from the hard tick *Hyalomma aegyptium*, which infests tortoises (*Testudo graeca*), and others Borreliae of Reptilian (REP) group with 92% (*fla*B) and 100% (16SrRNA) of bootstrap (Figs. 3 and 4). Regarding 18S rRNA for piroplasmids, the phylogenetic analysis indicated that the generated sequences were close to the *T. cervi* sequences available in GenBank (Fig. 5).

Discussion

The results of our study add to those of Magalhães-Matos et al. (2022), who detected DNA from *Rickettsia* spp. in ticks of coatis, reinforcing the importance of these animals as dispersers of ticks and agents transmitted by ticks in both forest and synanthropic areas of the PNI. An important finding of this study was the detection of DNA from *Anaplasma* sp. in the four positive samples of *A. coelebs*. Phylogenetic analysis revealed that they occur in the same group as *A. marginale*, *A. centrale*, and *A. capra* (Figs. 1 and 2).

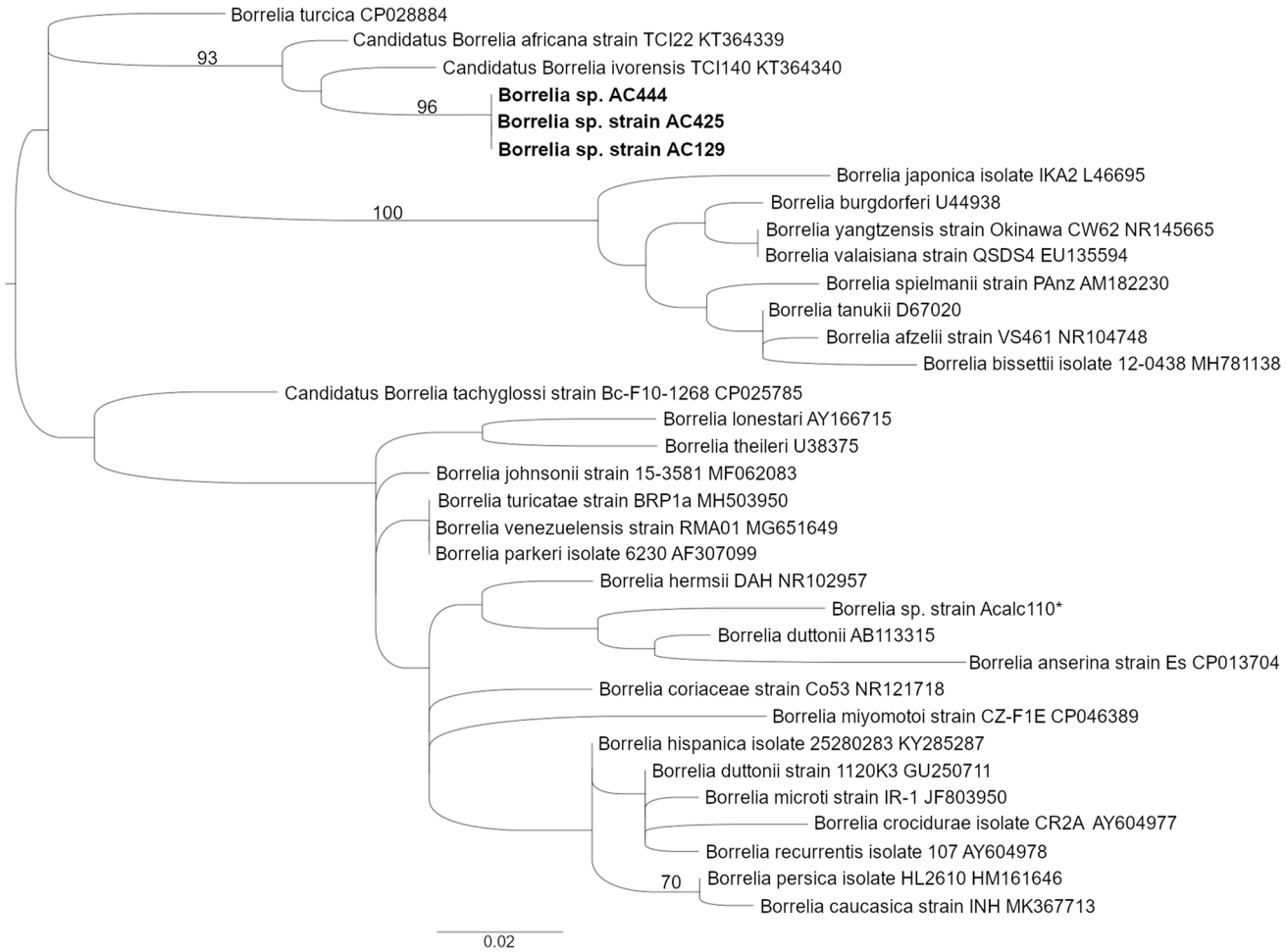

Fig. 3 Phylogenetic tree presenting the phylogenetic relationships among the *Borrelia* species identified in this study (highlighted in bold). The tree is constructed based on 16S rRNA gene sequences, utilizing the maximum likelihood (ML) method. The numbers (>70%) indicated above the branches represent the bootstrap values. The scale bars correspond to an evolutionary distance of 0.02 substitutions per sequence position, and the branch labels contain GenBank accession numbers

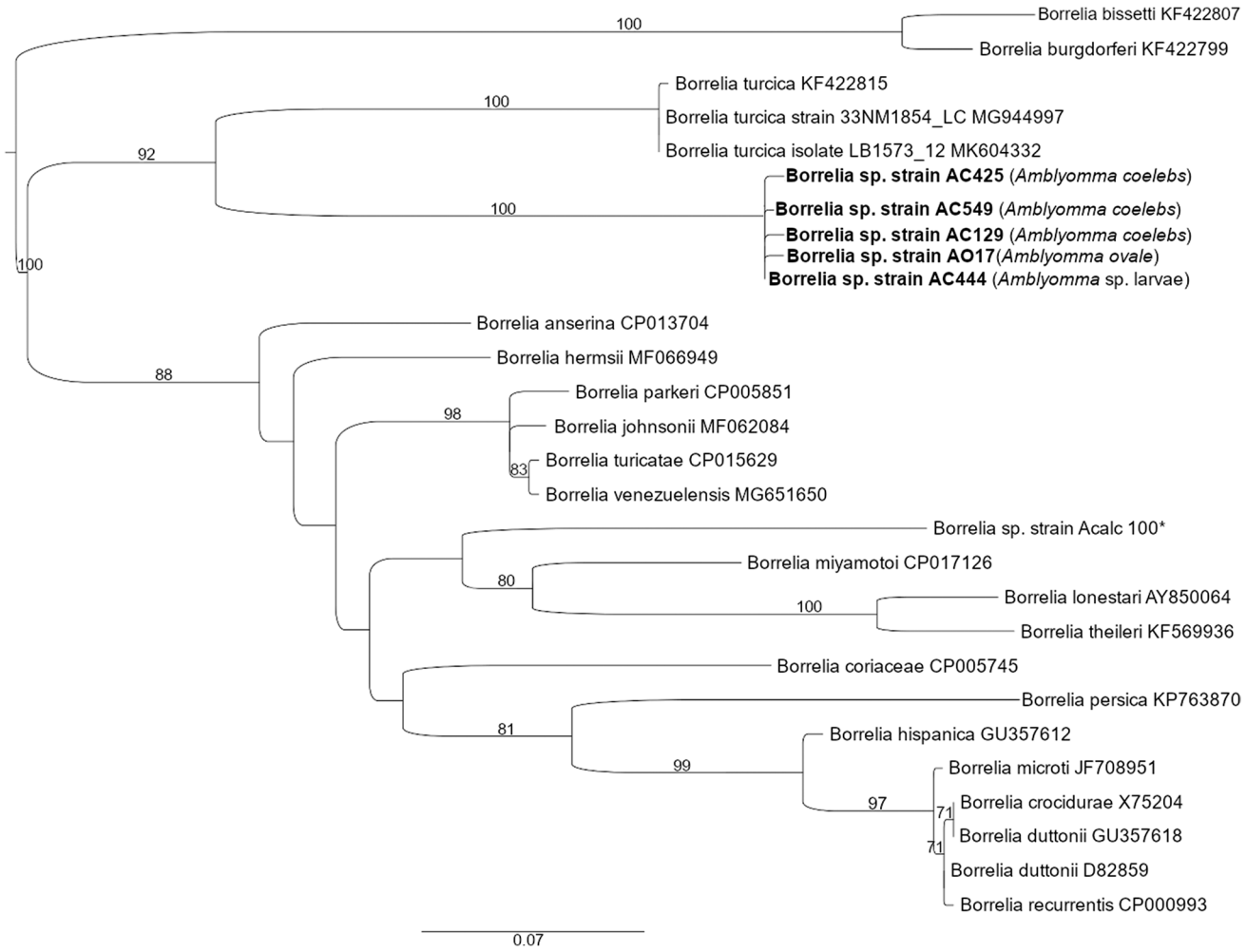

Fig. 4 Phylogenetic tree demonstrating the phylogenetic relationships between the *Borrelia* species detected in this study (highlighted in bold). The tree is constructed using *flaB* gene sequences and the maximum likelihood (ML) method. The numbers (>70%) presented above the branches indicate the bootstrap values. The scale bars indicate an evolutionary distance of 0.07 substitutions per sequence position, and the branch labels include GenBank accession numbers

Other studies seeking the amplification of the Anaplasmataceae 16SrRNA gene found high similarity with the findings of the present study. Benevenute et al. (2017) detected *Anaplasma* sp. B173 (KY391803) in a spleen fragment of a *Rattus rattus* in the state of Ceará-Brazil with 100% identity (467/467). When searching for *Anaplasma* spp. in the blood of the same group of coatis evaluated in our study, Perles et al. (2023) determined that 14.4% (7/49) were positive. The similarity of 97.78% (440/450) of the 16S rRNA gene fragment of the sequences described in coatis blood (GenBank OM811667) with those found in our study indicated that these were from two different species. However, new studies that present larger fragments and other genes are required to confirm this.

In Brazil, *Anaplasma* species are mostly detected in ticks of the genus *Rhipicephalus*, such as *A. marginale*, which infects cattle and has the tick *Rhipicephalus microplus* as its main biological vector (Vieira et al. 2019). The arthropod vectors involved in the transmission cycle of *Anaplasma* spp. among wild mammals in Brazil remain unknown (Sousa et al. 2017; Sousa et al. 2018). However, previous studies conducted in the Brazilian Pantanal detected DNA from *Anaplasma* spp. in different *Amblyomma* species collected from wild felines and carnivores, such as *Amblyomma sculptum*, *Amblyomma triste*, *Amblyomma parvum*, and *A. ovale* (Widmer et al. 2011; Sousa et al. 2017; Sousa et al. 2018). Our study extends the detection of *Anaplasma* to another tick species, *A. coelebs*. However, it must be considered that this positivity for *Anaplasma* sp. in ticks parasitizing animals may be related to the remains of the blood meal of the arthropod in the infected host (Sousa et al. 2018).

A recent study described a possible new species named "*Candidatus* Anaplasma sparouinense," suspected of an atypical case of human anaplasmosis in an Amazon

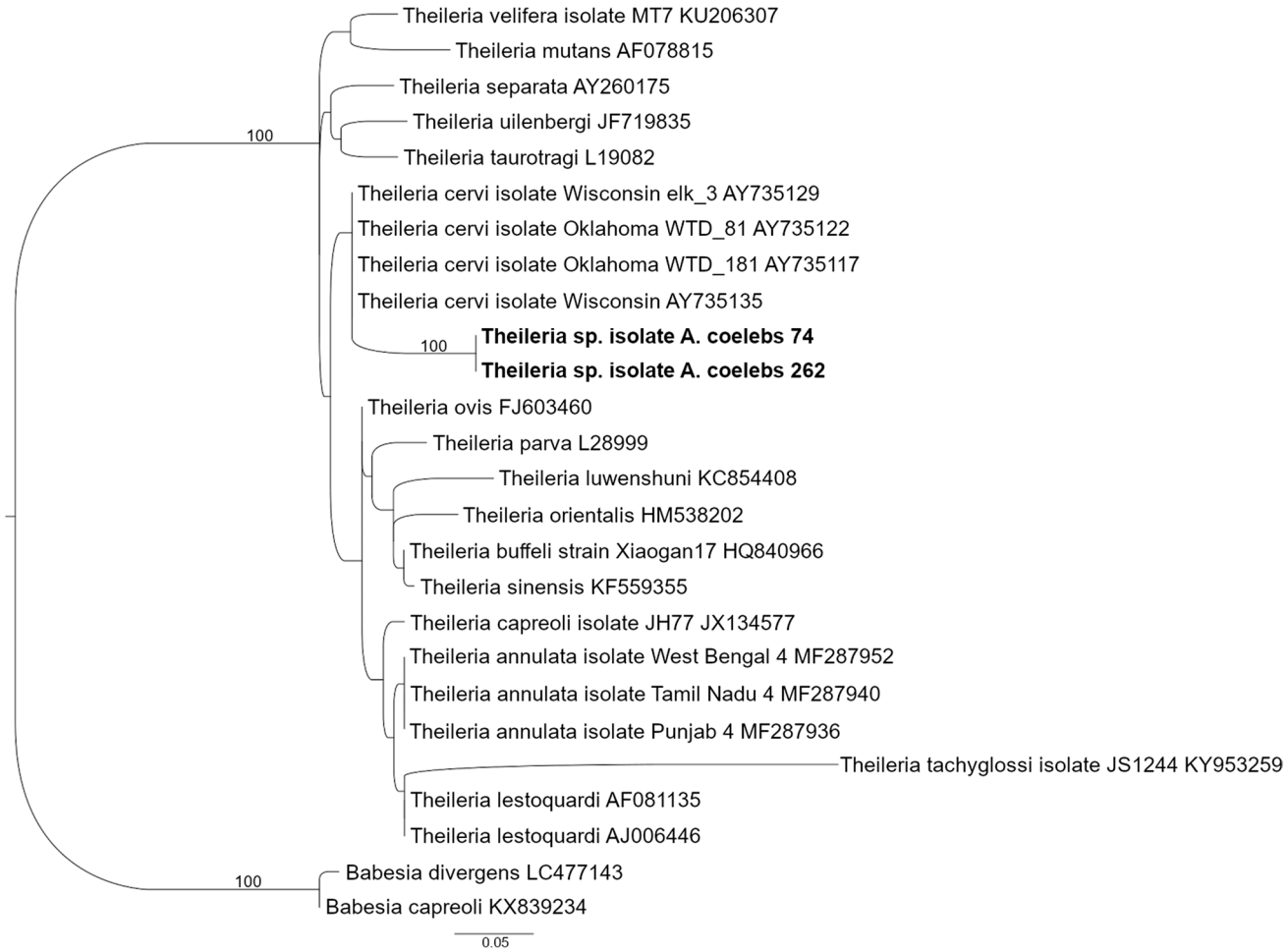

Fig. 5 Phylogenetic tree illustrating the relationships among the *Theileria* species identified in this study (highlighted in bold). The tree is constructed based on 18S rRNA gene sequences, employing the maximum likelihood (ML) method. The numbers (>70%) shown above the branches represent the bootstrap values. The scale bars correspond to an evolutionary distance of 0.05 substitutions per sequence position, and the branch labels contain GenBank accession numbers. *Babesia divergens* (LC477143) and *Babesia capreoli* (KX839234) were used as outgroup

rainforest region of French Guiana. The 16S rRNA sequence (GenBank ON513878) from "*Ca*. A. sparouinense" showed high similarity (99.8%) to the *Anaplasma* sequences detected in *A. coelebs* in our study (Duron et al. 2022). Although more data are needed, this similarity raises the possibility that our *Anaplasma* isolates pose a risk to human health.

DNA from *Borrelia* spp. was detected in ticks of the species *A. ovale*, *A. coelebs*, and *Amblyomma* sp. larvae. Phylogenetic analysis showed that the five isolates of *Borrelia* spp. detected in this study are genetically close to *B. turcica* and two strains that have been detected in *Amblyomma variegatum* collected from cattle in Côte d'Ivoire, "*Candidatus* Borrelia ivorensis" and "*Candidatus* Borrelia africana" (Ehounoud et al. 2016). They join a clade of the REP Borreliae, a new phylogenetic RGF's subgroup, which is also vectored by ticks of the genus *Amblyomma* and was initially associated with reptile hosts (Takano et al. 2010). Santos et al. (2020) described a new potential species of *Borrelia* phylogenetically close to *Borrelia* spp. from Ethiopia and Côte d'Ivoire that was detected in a nymph of *A. brasiliense* (MN650844) and, its sequence, although shorter, is 100% identical to the *Borrelia* spp. described in this study.

In South America, the first *Borrelia* spp. detected in ticks of the genus *Amblyomma* was genetically closer to the species that comprise the *Borrelia* REP group (Pacheco et al. 2019). Since then, new strains of *Borrelia* have been identified in these ixodid species. In Argentina, a species of *Borrelia* was found to infect *Amblyomma aureolatum* collected from wild birds (Cicuttin et al. 2019). In Brazil, a new strain called *Borrelia* sp. strain Acalc110 was also recently discovered in DNA of *Amblyomma calcaratum* genetically close to two species of *Borrelia* pathogenic to humans, *Borrelia miyamotoi* and *Borrelia venezuelensis* (Araújo et al. 2022). Therefore, the present study contributes to the knowledge of *Borrelia* spp. in ticks of the genus *Amblyomma*.

Adults of *A. coelebs* have a parasitic preference for tapirs (*Tapirus terrestris*) (Guglielmone et al. 2014), and in its immature stages have been found in other mammals and birds (Ogrzewalska et al. 2010; Lopes et al. 2016). In contrast, *A. ovale* is more commonly recorded in wild and domestic carnivores (Guglielmone, 2003; Magalhães-Matos et al. 2017), and small rodents are also hosts for immature stages (Guglielmone, 2003). In addition, it is important to highlight the occurrence of infestation in humans by both species of ticks (Guimarães et al. 2001; Szabó et al. 2006; Garcia et al. 2015; Ito et al. 2017). However, until now, only *A. ovale* is considered a competent transmitter of pathogens, such as *Rickettsia parkeri* strain Atlantic rainforest, which causes human rickettsiosis (Szabó et al. 2013).

None of the tick samples were amplified to *Babesia* spp. Although we did not use specific primers, *Hepatozoon procyonis* appears to be a protozoan of the Order Piroplasmida frequent in *Nasua nasua* blood, unlike *Babesia* spp. (Silva et al. 2018; Perles et al. 2023). We detected DNA from *Theileria* sp. in two ticks of the *A. coelebs* specie closed to the *T. cervi* sequences (MW008518) detected from white-tailed deer (*Odocoileus virginianus*). However, the nucleotide difference in a highly conserved gene led us to believe that it is a species that has not yet been described or that at least no molecular data have been deposited in GenBank. In Brazil, DNA from *Theileria* spp. has been detected in several wild animals such as brown deer (*Mazama gouazoubira*), pampas deer (*Ozotoceros bezoarticus*), nine-banded armadillo (*Dasypus novemcinctus*), agouti (*Dasyprocta* sp.), paca (*Cuniculus paca*), and tapir (*T. terrestris)*, including in animals with synanthropic habits such as coatis (*N. nasua*) (Silveira et al. 2013; Sousa et al. 2018; Gonçalves et al. 2020). It is notable that animals from the same groups also occur in PNI; however, additional investigations are necessary to obtain more specific information on other host species and to characterize the detected agent.

None of the samples amplified DNA from hemoparasites of the genus *Ehrlichia*. Previous studies performed in the Brazilian Pantanal have detected *Ehrlichia* spp. in several species of ticks of the genus *Amblyomma* collected from wild carnivores (Widmer et al. 2011; Melo et al. 2016; Sousa et al. 2018); however, the amplification of these samples may be related to the remains of the arthropod blood meal in the infected host (Sousa et al. 2018). Thus, there is no evidence of transmission of this pathogen in *Amblyomma* spp. and in coatis in Brazil to date (Gruhn et al. 2019).

Bioagents of the genera *Borrelia*, *Anaplasma*, and *Theileria* were found in ticks collected from coatis at the PNI in Paraná. Our study constitutes the newest report in South America of these microorganisms, which remain poorly studied. Further studies are required to clarify which species are circulating in the park and their possible hosts.

Author contributions IM Araújo and MD Cordeiro designed the project and experiments. IM Araújo, PC Magalhães-Matos, BA Baêta, CB Silva, and AH Fonseca carried out the experimental procedures. MD Cordeiro and A Guterres performed the phylogenetic analysis and interpretation of the results. IM araújo has written the first draft of the manuscript. All authors reviewed and approved the final manuscript.

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Data Availability All data and material are available in the main body of the article and supplementary material.

Declarations

Ethics approval This research was carried out after approval by the Ethics Committee for the Use of Animals of the Veterinary Institute of the Federal Rural University of Rio de Janeiro (No. 058/2014 CEUA-IV/UFRRJ). The capture of animals, field collection, and transport of biological samples were authorized by the Biodiversity Information and Authorization System (SISBio) of the Ministry of the Environment (No. 43,614–3).

Consent to participate Not applicable

Consent for publication All the authors agreed to the publication of the manuscript.

Conflict of interest The authors declare no competing interests.

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