

Article

Molecular Survey of Piroplasmids and Hemosporidians in Vampire Bats, with Evidence of Distinct Piroplasmida Lineages Parasitizing *Desmodus rotundus* from the Brazilian Amazon

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Abstract: Although bats can serve as reservoirs for several viruses and bacteria, there is limited knowledge regarding the diversity of apicomplexan protozoan belonging to the Piroplasmida and Haemosporida orders within this group of mammals. The present study aimed to investigate the occurrence and phylogenetic assessment of piroplasmids and hemosporidians in spleen samples collected from 229 vampire bats (228 *Desmodus rotundus* and 1 *Diaemus youngii*) in the states of Pará, Roraima, Amapá, and Amazonas, northern Brazil. Out of 229 bat spleen samples, 43 (18.77%) tested positive in a nested PCR for piroplasmids based on the 18S rRNA gene. Thirteen sequences (ranging from 474 to 828 base pairs) of the partial 18S rRNA gene showed 91.04–100% identity to *Theileria* sp., *Babesia* sp., and Piroplasmida previously detected in deer, tapirs, opossums, and crab-eating raccoons. The phylogenetic analysis based on the near-complete 18S rRNA gene positioned the obtained sequences from three *D. rotundus* in distinct clades (*Theileria sensu stricto*, *Tapirus terrestris*, and “South America Marsupialia”). All bat spleen DNA samples tested negative in a nested PCR assay for hemosporidians based on the *cytB* gene. The present study reported, for the first time, the presence *Babesia* sp. and *Theileria* sp. DNA in *D. rotundus*. The distinct positioning of the 18S rRNA gene sequences within different clades demonstrates the occurrence of different piroplasmid species in vampire bats.

Keywords: *Babesia*; *Theileria*; common vampire bat; Haemosporida



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1. Introduction

Bats (Chiroptera, Mammalia) comprise the second-largest group of mammals, with a great diversity of species, biological complexity, and crucial ecological importance [1,2]. Hematophagous bats, also known as vampire bats, belong to the subfamily Desmodontinae, which comprises three species: *Desmodus rotundus*, *Diphylla ecaudata*, and *Diaemus youngii* [3,4]. The high mobility, longevity, social habits, gregarious behavior, and diversity of niches, including urban environments, combined with their ability to act as hosts for various pathogens [5], highlight the importance of understanding the epidemiology of vector-borne pathogens in these animals.

Piroplasmids (Apicomplexa: Piroplasmida) are obligatory intracellular protozoa that include the genera *Theileria*, *Babesia*, *Cytauxzoon*, and *Rangelia* [6–8]. Although these tick-borne agents infect the blood cells of a wide range of mammals (wild animals [9], domestic

animals, and livestock [8,9]), as well as some bird species [10], there have been few studies on the occurrence and molecular identity of piroplasmids in chiropterans. Piroplasmids were first described in bats by Dionisi in 1899 [11], who reported the presence of structures resembling evolutionary forms of hemosporidians in blood smears from *Nyctalus noctula* and *Pipistrellus* sp. sampled in Italy. Marinkelle (1996) [12] detected *Babesia* sp. in blood smears from *Mormoops megalophylla* bats in Colombia. The first molecular detection of piroplasmids was performed in tissues from *Pipistrellus* bats in the United Kingdom, with the description of *Babesia vesperuginis* [13]. Additionally, Hornok et al. (2015) [14] detected fragments of the 18S rRNA gene of *Babesia canis* in bat fecal samples in Hungary. *Babesia vesperuginis*, *Babesia venatorum*, *Babesia crassa* (*Babesia sensu stricto* (s.s.)), and *Theileria* spp. (including *Theileria sensu stricto*) were also detected through molecular assays based on the 18S rRNA gene in bats in Romania [15]. *Babesia vesperuginis* was detected in bats and associated ectoparasites in Austria, the Czech Republic, Romania [16,17], and China [18,19]. *Babesia venatorum* was reported in bats in the United Kingdom [20]. In Madagascar, 18S rRNA gene sequences closely related to *Babesia microti* were detected in *Pteropus rufus* bats [21]. Ikeda et al. (2021) [22] first reported the occurrence of *Babesia* sp. phylogenetically associated with *Babesia vogeli* in *Artibeus lituratus* and *Artibeus planirostris* bats, as well as a putative novel Piroplasmida in *Phyllostomus discolor* in central-western Brazil. Recently, Linhart et al. (2022) [23] documented the presence of *B. vesperuginis* in *Nyctalus noctula* bats in the Czech Republic and linked the presence of the parasite to acid–base balance disturbances in the blood of infected bats during hibernation.

Hemosporidians (Apicomplexa: Haemosporida) are protozoan with reduced apical complexes that infect various species of mammals and birds [24] and are transmitted by different species of dipterans [25]. Phylogenetic studies revealed the presence of different hemosporidians in bats, some of which do not undergo schizogony in erythrocytes and therefore do not belong to the *Plasmodium* genus [26]. However, there is a certain phylogenetic duality among the hemosporidians found in bats: while *Hepatocystis* is phylogenetically related to mammalian *Plasmodium* parasites, *Nycteria* and *Polychromophilus* show closer similarity to avian *Plasmodium* species [27]. So far, nine genera of hemosporidians have been identified in bats: *Biguetiella*, *Bioccala* (possible subgenus of *Polychromophilus*), *Dionisia*, *Hepatocystis*, *Johnsprentia*, *Nycteria*, *Plasmodium*, *Polychromophilus* (*P. melanipherus* and *P. murinus* initially described in *Miniopterus schreibersii* and *Vespertilio murinus*, respectively) [11], and *Sprattiella* [28–35]. The majority of the molecular studies on hemosporidians in bats have been conducted in European and African countries [26,28,29,36]. Recently, *Polychromophilus* sp. has been detected in vespertilionid bats in Brazil [37,38].

Although the importance of vampire bats as reservoirs of virus and bacteria is recognized [5], there is a scarcity of studies that focuses on the occurrence of hemosporidians and piroplasmids in this group of animals [39]. The present study aimed to investigate the occurrence and phylogenetic positioning of piroplasmids and hemosporidians in vampire bats sampled in northern Brazil.

2. Results

All the 229 DNA samples obtained from spleen fragments of hematophagous bats tested positive in the conventional PCR (cPCR) assay targeting the endogenous mammalian gene (*gapdh*). None of the DNA samples was positive in the nested PCR for hemosporidians targeting the *cytB* gene. On the other hand, 43 (18.77%; 43/229) (95% CI (14–24%)) spleen samples from hematophagous bats tested positive in the nested PCR based on the 18S rRNA gene. Thirteen sequenced amplicons obtained from *D. rotundus* captured in the state of Pará showed identity (91.04–100%) with *Theileria* sp., *Babesia* sp., and Piroplasmida previously detected in deer, tapirs, opossums, and crab-eating raccoons (Table 1).

Table 1. BLASTn results of the partial Piroplasmida 18S rRNA gene sequences detected in *Desmodus rotundus* bats from the northern region of Brazil.

Sample (GenBank Accession Number—State)	Size (bp)	Percent Identity	Query Cover (%) / E-Value	Sequence Best Match (GenBank Accession Number, Locality)
Sample 8 OR127024 Pará	633	100%	100%/0.0	<i>Piroplasmida</i> sp. from <i>Didelphis aurita</i> —Brazil (OP751800)
Sample 12 OR127025 Pará	725	97.66%	100%/0.0	<i>Theileria cervi</i> from <i>Odocoileus virginianus</i> —USA (MW008528)
Sample 13 OR127026 Pará	769	97.27%	100%/0.0	<i>Theileria</i> sp. from <i>Tapirus terrestris</i> —Brazil (OP023835)
Sample 65 OR127027 Pará	737	99.59%	100%/0.0	<i>Piroplasmida</i> sp. from <i>Didelphis aurita</i> —Brazil (OP751799)
Sample 82 OR127028 Pará	828	99.88%	100%/0.0	<i>Piroplasmida</i> sp. from <i>Didelphis aurita</i> —Brazil (OP751800)
Sample 90 OR127030 Pará	747	96.12%	100%/0.0	<i>Theileria</i> sp. from <i>Tapirus terrestris</i> —Brazil (OP023835)
Sample 92 OR127031 Pará	670	100%	100%/0.0	<i>Theileria</i> sp. from <i>Tapirus terrestris</i> —Brazil (OP023835)
Sample 179 OR127032 Pará	497	100%	99%/0.0	<i>Piroplasmida</i> sp. from <i>Didelphis aurita</i> —Brazil (OP751800)
Sample 182 OR127033 Pará	625	100%	100%/0.0	<i>Theileria</i> sp. from <i>Tapirus terrestris</i> —Brazil (OP023835)
Sample 205 OR127034 Pará	474	91.04%	98%/0.0	<i>Theileria</i> sp. from domestic cats—Brazil (KP402164)
Sample 215 OR127035 Pará	533	99.62%	100%/0.0	<i>Piroplasmida</i> sp. from <i>Didelphis aurita</i> —Brazil (OP751800)
Sample 231 OR127036 Pará	758	99.87%	100%/0.0	<i>Babesia</i> sp. from <i>Procyon cancrivorus</i> —Uruguay (MG682489)

The positive spleen samples were obtained from 42 *D. rotundus* (97.67%) (Pará (37/206), Roraima (4/18), Amapá (1/3)) and one *D. youngii* (2.33%; 1/1) from the Pará state. Out of the 43 tested samples, 26.19% (11/42) were positive in the PCR assay targeting the near-complete 18S rRNA gene. Three amplicons obtained from *D. rotundus* bats from Pará were chosen for cloning based on their higher band intensity in agarose gel electrophoresis. All 43 samples tested negative in the PCR assays targeting the *cox1*, *cox3*, *cytB*, and *hsp70* genes and the ITS-1 intergenic region.

The phylogenetic analysis inferred by the maximum likelihood method based on a 1626 bp alignment of the 18S rRNA gene and the TIM3+I+G [40] evolutionary model positioned the sequences obtained from the clones derived from *D. rotundus* bats into three distinct clades, with each clade composed of clones obtained from the same animal. The first

group of clones derived from bat #8 was positioned within the “South America Marsupialia” clade, a recently described clade that comprises *Babesia* sp. sequences detected in opossums (*Didelphis albiventris* and *D. aurita*) and *Amblyomma dubitatum* nymphs collected from these opossums and coatis (*Nasua nasua*) in Brazil, with 100% clade support. All clones were positioned within a single subclade, closer to sequences detected in *D. aurita*. The clones obtained from bat #12 were positioned within the clade of *Theileria* sensu stricto, forming a sister clade to *Theileria cervi*, with 100% clade support. Finally, the sequences obtained from bat #91 were positioned within the *Tapirus terrestris* clade, which contains sequences of *Theileria terrestris* detected in tapirs from Brazil, with 100% clade support (Figure 1).

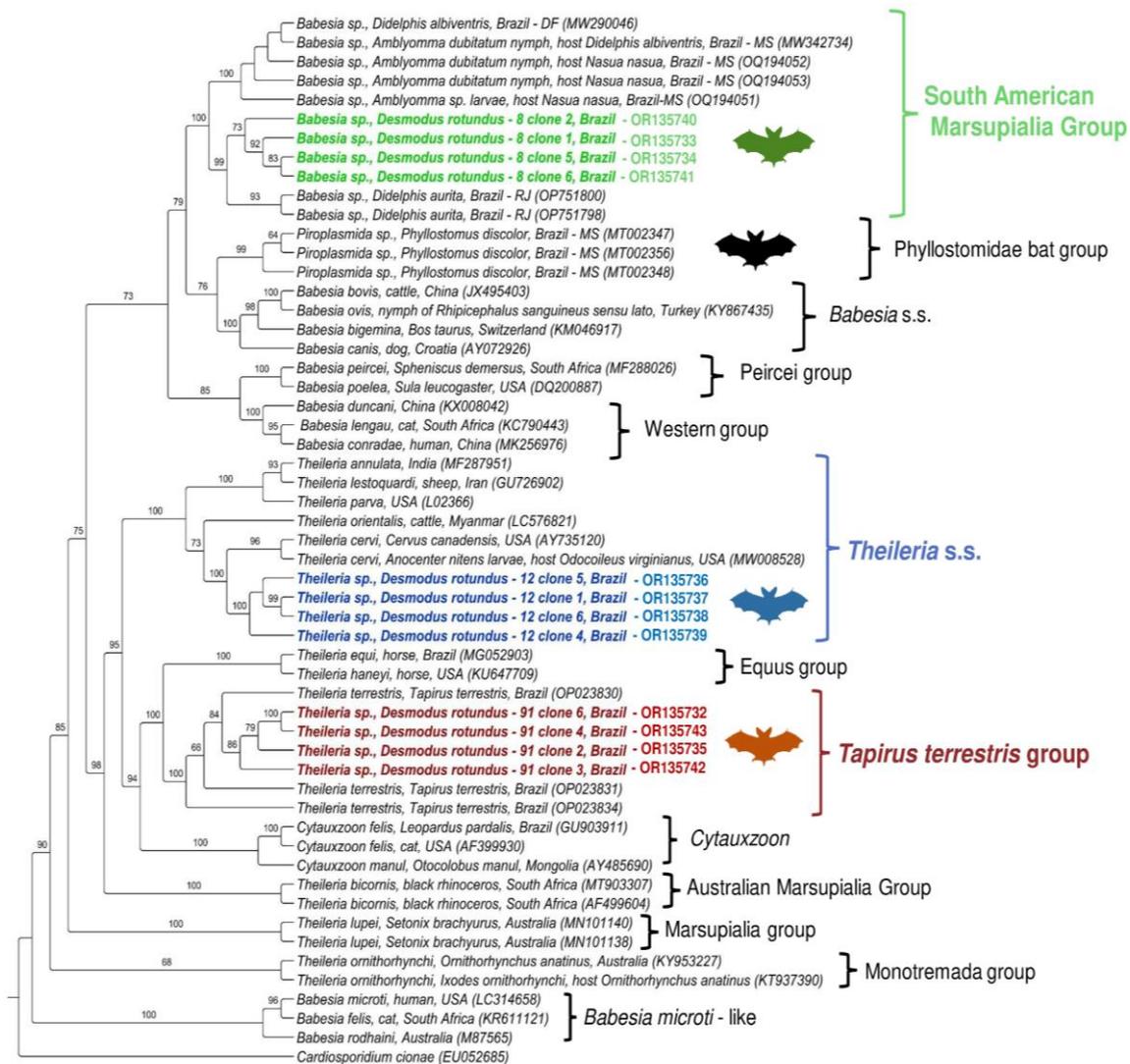


Figure 1. Phylogenetic tree based on the alignment of 1626 bp of the 18S rRNA gene, inferred using the maximum likelihood method and TIM3+I+G evolutionary model (Trifinopoulos et al., 2016) [40]. Sequences from the present study are highlighted in green, blue, and red. *Cardiosporidium cionae* was used as the outgroup. The Piroplasmida phylogenetic clades followed Jalovecka et al. (2019) [7], Ikeda et al. (2021) [22], Mongrue et al. (2022) [41], and Oliveira et al. (2023) [42].

Regarding the distance matrix analysis, the sequences obtained from bat #8 showed divergences ranging from 0.14% to 0.57% among the clones. On the other hand, divergences ranging from 0.21% to 3.85% were found among the clones obtained in this study and the other sequences within the “South American Marsupialia” clade, with the lowest divergences observed between the clones and the sequences previously detected in *D. aurita*.

The sequences from bat #12 showed divergences ranging from 0% to 0.3% among the clones. When comparing the clone sequences with those of *T. cervi*, the divergence ranged from 1.82% to 2.16%. However, the divergences were higher than 4% between the clones and the other sequences within the *Theileria* sensu stricto clade. Finally, the 18S rRNA gene cloned sequences obtained from bat #91 showed divergences ranging from 0% to 1.01% among them and from 0.25% to 0.70% with the sequences of *T. terrestris* (Supplementary Material Table S1).

3. Discussion

The present study reported, for the first time, the occurrence of *Babesia* spp. and *Theileria* spp. in vampire bats. Herein, an occurrence of 18.77% (43/229) for piroplasmids was found in hematophagous bats sampled in states of the northern region of Brazil. Previously, a similar occurrence (12.6%; 17/135) was reported in blood and spleen samples of non-hematophagous bats sampled in the midwestern region of Brazil [22]. Overall, the occurrence of piroplasmids in bats reported in other studies based on PCR assays targeting the 18S rRNA gene was lower than that observed in the present study. In Eastern Europe, Corduneanu et al. (2017) [17] reported a 4.3% positivity for *B. vesperuginis* in cardiac tissue samples of non-hematophagous bats. In Madagascar, Ranaivoson et al. (2019) [21] also detected a low positivity (4.4%) for *Babesia* sp. in blood samples from *Pteropus rufus* bats. Hornok et al. (2015) [14] reported an occurrence of 2.7% in fecal samples of non-hematophagous bats in Hungary. In China, Han et al. (2018) [18] reported a 13.3% positivity for *Babesia* spp. in blood samples from non-hematophagous bats. More recently, Linhart et al. (2022) [23] reported an 8% positivity for *B. vesperuginis* in *Nyctalus noctula* bats in the Czech Republic.

Babesia vesperuginis, a Piroplasmida species reported exclusively in bats, has been detected in bats in Europe [13,23,43] and Asia [18]. Unfortunately, due to the short size (~650 bp) of the available 18S rRNA gene sequences for this Piroplasmida species in the GenBank database, they were not included in the phylogenetic analyses of the present study.

The phylogenetic analysis of the near-complete 18S rRNA gene (1626 bp) positioned the detected piroplasmid sequences from *D. rotundus* into three distinct clades: (i.) "South American Marsupialia" clade, which contains *Babesia* spp. sequences detected in opossums and *Amblyomma* ticks; (ii.) *Theileria* s.s. clade; (iii.) *Tapirus terrestris* clade, demonstrating a high diversity of piroplasmids in these animals. Previous studies on non-hematophagous bats have also reported the molecular occurrence of a wide diversity of piroplasmid species in bats, which supports the findings of this study. These include genotypes associated with *Babesia microti* [21], *B. venatorum* [20], *B. crassa*, *B. canis* [15], *B. vogeli* [22], and *Theileria* s.s. [15]. However, caution must be taken when interpreting these findings due to the small size of the 18S rRNA gene fragment used for phylogenetic inferences and similarity analyses. For these purposes, it is desirable to use a complete or near-complete fragment of the 18S rRNA gene, along with other molecular markers (e.g., mitochondrial, *hsp-70*, etc.), in order to achieve more robust phylogenetic inferences for newly detected piroplasmid genotypes in wildlife [22,41,42,44]. For instance, Ikeda et al. (2021) [22] detected a putative novel piroplasmid species in *Phyllostomus discolor*, which was positioned in an intermediate clade between *Babesia* sensu stricto and *Theileria* s.s. In concatenated phylogenetic analyses of the 18S rRNA and *cox1* genes, it was demonstrated that sequences of *B. vesperuginis* obtained from argasid ticks (*Argas vespertilionis*) collected from *Plecotus austriacus* in Hungary do not belong to the *Babesia* s.s. clade but rather to a clade related to *Cytauxzoon felis* and *Babesia conradae* [45]. However, our attempts to amplify other molecular markers were unsuccessful, which limited our ability to draw additional phylogenetic inferences. The lack of amplification of mitochondrial and *hsp-70* genes might have been due to the non-conserved nature of such genic regions, precluding the annealing of the primers used herein. Alternatively, the low parasitemia of the infected bats might have been below the limit of detection of the PCR assays used in this study for molecular characterization.

Interestingly, the piroplasmid sequences obtained in this study were derived from the same bat species (*D. rotundus*) but clustered into different clades, indicating the presence of diverse genera (*Babesia* and *Theileria*) and piroplasmid species in common vampire bats. Previous studies have demonstrated a strong host-specific association, suggesting coevolution and co-speciation between piroplasmids and their mammalian or avian hosts [7]. However, the significant diversity of piroplasmids observed within a single vertebrate species suggests the occurrence of distinct Piroplasmida phylogenetic lineages within the same animal group [8], as observed in our study.

The pairwise analysis of the near-complete 18S rRNA gene sequences revealed low divergence among the sequences obtained from the same bat sample (0–1.01%). However, they showed higher divergence when compared to other sequences within the respective clades where they clustered in. For instance, Piroplasmida cloned sequences from bat #8 showed a divergence of 0.21% to 3.85% with other sequences within the “South American Marsupialia” clade. Interestingly, this clade split into two subclades, with the bat sequences forming a unique clade close to sequences detected in *D. aurita*. For sequences from bat #12, divergence ranged from 1.82% to 2.16% with *T. cervi* sequences and over 4% when compared to other sequences within the *Theileria* s.s. clade. The Piroplasmida sequences from bat #12 formed a unique subclade in the phylogenetic analysis. Sequences from bat #91 exhibited lower divergence, ranging from 0.25% to 0.70% with *T. terrestris* sequences, clustering with tapir-associated sequences without forming subclades. Based on the results obtained from the distance matrix and phylogenetic analyses, we can infer that at least two putative novel Piroplasmida species may occur in *D. rotundus*, as they formed distinct subclades. This hypothesis can be supported by the presence of different piroplasmid species within the same clade, forming separate subclades in the phylogeny with minimal genetic divergence between them, such as 0.14% (*T. equi* × *T. haneyi*), 0.59% (*B. poelea* × *B. piercei*), 3.35% (*B. lengau* × *B. conradae*), and 3.69% (*T. orientalis* × *T. cervi*). Therefore, future studies aiming to amplify mitochondrial gene sequences or the complete mitochondrial genomes should be conducted to explore the diversity of piroplasmids within this animal group.

It is well established that a majority of wild mammals are susceptible to piroplasmid infections [46]. Although piroplasmid species of medical and veterinary importance are primarily transmitted through the bite of ixodid ticks [8], the vectors and alternative transmission routes to bats remain largely unknown. It is plausible that social behaviors among bats, such as grooming, which serves hygienic and colony recognition purposes, could facilitate the ingestion of piroplasmid-infected ticks and potentially act as a transmission route among bats [47]. Hornok et al. (2015) [14] detected *B. canis* DNA in bat feces and suggested that this finding might be a consequence of ingesting infected vectors, such as ticks and flies. It is also possible that parasites pass from the bat’s bloodstream to the intestinal contents, as previously observed with other erythrocyte-infecting protozoa [48]. Additionally, further studies are necessary to investigate whether hematophagy, or blood feeding, could represent an additional transmission route of piroplasmids to bats.

Although hemosporidian DNA was not amplified in spleen samples from hematophagous bats sampled in northern Brazil, *Hepatocystis* sp., *Nycteria* sp., and *Polychromophilus* sp. have been previously detected in non-hematophagous bats in Central and Eastern African countries [26,35,49]. *Polychromophilus* sp. has also been detected in bats from Asia [50,51] and, more recently, Brazil [37,38]. Recently, de Mello et al. (2023) [39] reported the absence of hemosporidian DNA in liver samples from hematophagous bats sampled in several Brazilian biomes. Therefore, the diversity of hemosporidians parasitizing hematophagous bats in the Neotropics is still unknown.

4. Materials and Methods

Between 2017 and 2019, carcasses of hematophagous bats belonging to the species *Desmodus rotundus* ($n = 228$) and *Diaemus youngii* ($n = 1$) were received in the rabies diagnostic laboratory from the Instituto Evandro Chagas, collected in different states of the northern region of Brazil (Pará ($n = 206/D. rotundus$; $n = 1/D. youngii$), Roraima ($n = 18/D. rotundus$),

Amapá ($n = 3/D. rotundus$), and Amazonas ($n = 1/D. rotundus$)) (Figure 2), following approval by the “Conselho Nacional de Controle de Experimentação Animal (CONCEA) e Comitê do Uso de Animais—CEUA-FCAV/UNESP” under protocol number 015782/19.

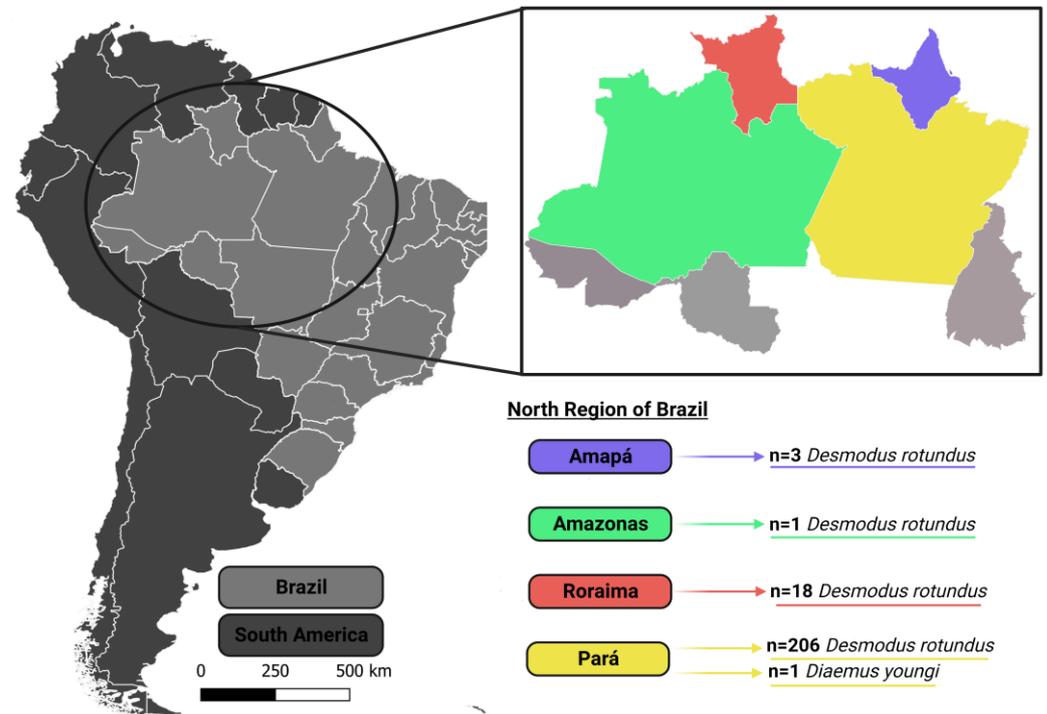


Figure 2. Geographic distribution of vampire-bat capture sites. Map created using QGIS software, version 3.28.2.

DNA extraction was performed on spleen samples from the 229 hematophagous bats using the BIOPUR Mini Spin Plus Extraction Kit (Möbius), following the manufacturer’s recommended protocol. The concentration ($\mu\text{g}/\mu\text{L}$) and purity of the DNA samples were assessed using optical spectrophotometry (Nanodrop, Thermo Scientific) based on the 260/280 ratio. The extracted DNA samples were subsequently stored at $-20\text{ }^{\circ}\text{C}$ until molecular analyses.

The DNA samples were initially subjected to a conventional PCR assay (cPCR) targeting the mammalian endogenous gene glyceraldehyde 3-phosphate dehydrogenase (*gapdh*) [52] to verify the absence of PCR inhibitors and the presence of amplifiable DNA in the samples. The samples that tested positive for the endogenous gene were subsequently subjected to PCR assays targeting molecular markers for the selected agents.

For the screening of hemosporidian DNA, a nested PCR assay based on the *cytB* gene [30] was performed. For the detection of *Babesia/Theileria* spp., a screening assay based on the 18S rRNA gene (~ 800 bp) [53] was carried out. Samples that tested positive in the screening for piroplasmids were subjected to additional PCR assays aiming to amplify six molecular markers: near-complete 18S rRNA (~ 1500 bp; [54–56]), *cox1* (~ 800 bp; [17]), *cox3* (~ 600 bp; [46]), *cytB* (~ 1 kb; [57]) genes, the intergenic region ITS-1 [58], and *hsp70* (~ 700 bp; [59]). DNA from *Babesia vogeli* (Jaboticabal strain) [60] and *Plasmodium* sp. previously detected in Orinoco goose (*Neochen jubata*) [61] were used as positive controls in the aforementioned PCR reactions. Sterile ultrapure water (Invitrogen®, Carlsbad, CA, USA) was used as a negative control in all PCR assays. The observed frequencies were expressed in percentages, and the 95% confidence interval (CI) was calculated accordingly to a previously described equation [62].

In order to obtain near-complete and high-quality sequences of a large fragment of the 18S rRNA gene, the obtained amplicons were subjected to cloning using the pGEM-T

Easy system (Promega® Madison, WI, USA) following the manufacturer's recommendations. Four clones from three positive samples were selected based on the blue/white colony screening system. The white colonies that contained the target gene fragment, previously confirmed by PCR, were subjected to plasmid DNA extraction using the commercial Wizard® Plus SV Minipreps DNA Purification Systems (Promega Madison, WI, USA), according to the manufacturer's instructions. Subsequently, plasmid DNA samples were submitted to Sanger sequencing [63] using the M13F and M13R primers [64], which flank the multiple cloning site of the pGEM-T Easy plasmid and an internal primer (5'-AGAAACGGCTACCACATCTA-3') designed in this study.

The analysis of the generated electropherograms in the sequencing and the construction of consensus sequences were performed using the Phred-Phrap software, version 23 [65,66], considering a minimum Phred quality value of 20 for the bases. The BLAST software [67] was used to compare the obtained sequences with those previously deposited in GenBank [68].

The obtained sequences were aligned with other homologous sequences from the GenBank database using the MAFFT software [69] and edited using BioEdit, v. 7.0.5.3 [70]. The W-IQ-Tree software [71] was used to perform the phylogenetic analysis using the maximum likelihood method and for selecting the evolutionary model based on the Bayesian information criterion (BIC) [40]. Clade supports for the maximum likelihood analysis were evaluated through 1000 bootstrap repetitions [72]. The editing of the phylogenetic tree, as well as the rooting (via the outgroup), was performed using the Treegraph 2.13.0 beta software [73]. Additionally, a distance matrix was calculated using the *p*-distance method in the MEGA X software [74,75]. The data were transferred to a Microsoft Excel 2016 spreadsheet to create a heatmap based on the calculated percentage values obtained in the distance matrix.

5. Conclusions

The present study reported, for the first time, the presence of *Babesia* sp. and *Theileria* sp. in *D. rotundus*. The obtained 18S rRNA gene sequences were positioned in three distinct clades ("*Theileria sensu stricto*", "*Tapirus terrestris*", and "South American Marsupialia"), demonstrating that different species of piroplasmids parasitize hematophagous bats. The detection of *Babesia* and *Theileria* species in *D. rotundus* significantly contributes to our knowledge of piroplasm diversity in vampire bats. These results suggest that these bats can harbor a wide diversity of piroplasmids, suggesting intricate host–parasite relationships and their potential role in the transmission dynamics of these pathogens. Further research is needed to fully characterize these agents, including their genetic diversity, geographic distribution, vector associations, and potential impacts on bat health. Understanding the ecology and epidemiology of piroplasmids in hematophagous bats is critical for both bat conservation and public health considerations.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/parasitologia3030026/s1>, Table S1: Pairwise analysis of the near-complete 18S rRNA sequences.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated and analyzed during the current study are available in the NCBI GenBank Nucleotide platform <https://www.ncbi.nlm.nih.gov/genbank/> (accessed on 1 August 2023) and can be accessed through accession numbers: OR135732—OR135743 and OR127024—OR127036.

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