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Tick-borne Apicomplexa in wildlife and ticks of French Guiana

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Abstract – Tick-borne Apicomplexa encompass a group of parasites responsible for significant medical and veterinary diseases, including babesiosis, theileriosis, and hepatozoonosis. In this study, we investigated the presence and diversity of tick-borne Apicomplexa in wildlife and ticks inhabiting the Amazon rainforests of French Guiana. To this end, we conducted molecular screening and typing using 18S rRNA sequences on a collection of 1161 specimens belonging to 71 species, including 44 species of wild mammals, five species of passerines, and 22 species of ticks. We characterized eight genovariants of *Babesia*, *Theileria*, *Hemolivia*, and *Hepatozoon* parasites, some matching known species, while others suggested potential novel species. These parasites were detected in wild mammals, including opossums, sloths, armadillos, porcupines, margays, greater grisons, and ticks, but not in passerines. Finally, similarities with surveys conducted in Brazil highlight the specific sylvatic transmission cycles of South American tick-borne Apicomplexa.

Key words: Ticks, Babesia, Theileria, Hemolivia, Hepatozoon, French Guiana.

Résumé – Apicomplexes transmis par les tiques chez la faune sauvage et les tiques de Guyane française. Les Apicomplexes transmis par les tiques englobent un groupe de parasites responsables de maladies médicales et vétérinaires importantes, notamment la babésiose, la theilériose et l'hépatozoonose. Dans cette étude, nous avons étudié la présence et la diversité des Apicomplexes transmis par les tiques dans la faune sauvage et les tiques habitant les forêts tropicales amazoniennes de Guyane française. À cette fin, nous avons effectué un criblage moléculaire et un typage à l'aide de séquences d'ARNr 18S sur une collection de 1 161 spécimens appartenant à 71 espèces, dont 44 espèces de mammifères sauvages, cinq espèces de passereaux et 22 espèces de tiques. Nous avons caractérisé huit géovariants des parasites *Babesia*, *Theileria*, *Hemolivia* et *Hepatozoon*, certains correspondant à des espèces connues tandis que d'autres suggéraient de nouvelles espèces potentielles. Ces parasites ont été détectés chez des mammifères sauvages, dont des opossums, des paresseux, des tatous, des porcs-épics, des margays, des grisons et des tiques, mais pas chez des passereaux. Enfin, des similitudes avec des enquêtes menées au Brésil mettent en évidence les cycles de transmission sylvatiques spécifiques des Apicomplexa transmis par les tiques d'Amérique du Sud.

Introduction

Tick-borne Apicomplexa are responsible for medically and veterinary relevant diseases, such as babesiosis, theileriosis (referred to collectively as piroplasmidosis) and hepatozoonosis [17, 22, 23, 38, 43]. Members of the phylum Apicomplexa are

unicellular eukaryotic organisms, with the majority of species being obligate endoparasites of animals, and in certain instances, of humans. Tick-borne Apicomplexa belong to two distinct orders: Piroplasmida, which includes the genera *Babesia* (M'Fadyean & Stockman, 1911), *Theileria* Bettencourt, França & Borges, 1907, and *Cytauxzoon* Neitz and Thomas, 1948 (piroplasmids), and Eucoccidiorida, which includes the genera *Hemolivia* Petit et al., 1990 and *Hepatozoon* Miller, 1908 (haemogregarines) [17, 22, 23, 28, 38, 43, 49]. Piroplasmids

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are primarily transmitted through the bite of infected ticks, but the transmission of *Hemolivia* and *Hepatozoon* rather occurs through the ingestion of infected ticks and the release of the parasites into the host's digestive system [17, 22, 23, 37, 38, 43]. Once inside the host, piroplasmids and haemogregarines mainly invade either red or white blood cells, but *Hepatozoon*, unable to replicate in these cells, further infects target organs where it causes disease [17, 22, 23, 37, 38, 43]. Despite representing a significant veterinary threat worldwide, and posing an emerging risk to humans, these blood parasites have received comparatively less attention than other vector-borne Apicomplexa, such as *Plasmodium*, their malaria-causing relative [17, 22, 23, 43].

Tick-borne Apicomplexa are the predominant group of mammalian blood parasites [17, 22, 23, 38, 43]. In domestic animals, various species elicit a spectrum of clinical signs, leading to substantial morbidity and mortality, often resulting in significant economic burdens. In cattle, representative species include *Babesia divergens*, *Babesia bovis*, *Babesia bigemina*, *Theileria annulata*, and *Theileria parva*. In dogs, *Babesia canis*, *Babesia gibsoni*, and *Babesia vogeli* are among the prominent species [17, 22, 23], while *Cytauxzoon felis* and *Hepatozoon felis* are significant in cats [23, 38, 43]. Wildlife also suffers from a wide array of tick-borne Apicomplexan species, affecting mammals, birds, reptiles, and amphibians with significant morbidity and mortality [17, 22, 23, 37, 38, 43]. Humans are not the natural hosts for tick-borne Apicomplexa, but babesiosis is increasingly recognized as an emerging zoonosis, with numerous critical clinical cases documented worldwide. Indeed, *Babesia microti*, typically found in mice and other small rodents, and *B. divergens*, mostly found in cattle, are the most common causes of human babesiosis, in North America and Europe, respectively [24, 27, 45–47].

Tick-borne Apicomplexa are classified and taxonomically identified based on their morphological, biological, and molecular characteristics [22, 23, 38, 43]. Molecular phylogenies using the 18S rRNA gene sequences have recently challenged the previous taxonomic classification of piroplasmids, revealing that *Babesia* and *Theileria* each represent polyphyletic groupings within their respective orders [23]. Hence, different *Babesia* species cluster with some *Theileria* species rather than forming cohesive monophyletic genera. However, phylogenetic approaches also indicate that each lineage of tick-borne Apicomplexa is typically associated with a restricted range of vertebrate host taxa, suggesting that these lineages have often evolved specializations to their respective vertebrate hosts [22, 23, 38, 43]. For instance, *Babesia* species of the Percei lineage exclusively infect birds, while species of the *Cytauxzoon* lineage are predominantly found in felids [23]. Members of the lineage *Babesia* sensu stricto form a notable exception, as they infect multiple and diverse mammalian and avian vertebrate hosts [23]. Nevertheless, surveys of wildlife continue to uncover new tick-borne Apicomplexa that do not fit into recognized species [4, 5, 13, 20, 21, 26, 30, 31, 34, 36, 43]. Their host range, and phenotypic and developmental characteristics remain unknown in many cases [23, 47].

In this study, we investigate the presence and diversity of tick-borne Apicomplexa in wild mammals, birds and ticks

inhabiting the Amazon rainforests of French Guiana. This territory of 84,000 km² is predominantly covered by old-growth rainforests hosting one of the highest biodiversities in the world [42]. While recent surveys have uncovered novel tick-borne microbes in French Guiana [7, 11, 12, 25, 32], including novel tick-borne pathogens infecting humans [14, 19], there have been few studies focusing on tick-borne Apicomplexa in this region. In 1988, *Babesia choloepi* was described as a novel species in Linnaeus's two-toed sloths (*Choloepus didactylus*) of French Guiana based on morphological features [18], but no molecular sequences are available to complete its description. Furthermore, a survey of blood smears revealed the presence of several undetermined piroplasmid and *Hepatozoon* species in most mammalian orders of French Guiana, except primates, and in several species of snakes [41]. However, none of these previous studies have characterized these tick-borne Apicomplexa based on molecular typing.

Materials and methods

Ethics

Mammal samples were collected and used in accordance with an international CITES permit (Convention on International Trade in Endangered Species of Wild Fauna and Flora; permit FR973A) following French legislation. Following sharing policies in French Guiana, mammal samples are registered in the JAGUARS collection (<https://kwata.net/gestion-collection-biologique>; CITES reference: FR973A) supported by Kwata NGO (accredited by the French Ministry of the Environment and the Prefecture of French Guiana, Agreement R03-2019-06-19-13), Institut Pasteur de la Guyane, Direction Générale des Territoires et de la Mer (DGTm), Collectivité Territoriale de la Guyane, and validated by the French Guianese prefectural decree No. 2012/110. The French Ministry of Higher Education and Research provides authorization for projects using wild animals for scientific purposes (reference APAFIS-37571-2022111610578451). Permits for bird sampling (French Guiana prefectural decrees Nos. 2011/003, 2013/127 and R03-2018-10-30-0092) authorized the capture, marking, sampling, holding and transport of bird samples. Bird sampling was also done with permissions from several organizations: the DGTm de Guyane, the Direction Régionale de l'Office National des Forêts (ONF) de Guyane, the Conservatoire du Littoral, the Centre National d'Études Spatiales (CNES), the Centre Spatial Guyanais (CSG), the Association pour la Découverte de la Nature en Guyane, the association Randoroura. The use of bird genetic resources is declared to the French Ministry of the Environment under reference TREL1820249A/49 in accordance with the Nagoya Protocol on Access and Benefit Sharing (ABS). The French Ministry of the Environment also validated the collection and use of tick samples under the reference TREL19028117S/156, in accordance with the ABS Nagoya Protocol. All animals were handled in strict accordance with good animal practice and ethical standards as defined by the French code of practice for the care and use of animals for scientific purposes, established by articles R214-87 to R214-137 of the French rural code.

Collection of samples

We used archived DNA templates extracted from 1161 specimens collected in French Guiana between 1994 and 2019 (Tables 1 and S1). This collection comprises samples from 71 species, including 44 species of wild mammals ($n = 626$ samples), five species of passerines ($n = 247$), and 22 species of ticks ($n = 288$). It includes blood samples (for wild mammals, and passerines), spleen samples (wild mammals), or whole body (ticks). We had primarily collected these samples as part of previous studies among wildlife, and ticks in French Guiana [7–10, 12, 14].

Molecular detection and typing of tick-borne Apicomplexa

Each DNA template underwent individual testing using semi-nested polymerase chain reaction (PCR) targeting a fragment of the 18S rRNA (SSU) gene to detect *Babesia*, *Theileria*, *Cytauxzoon*, *Hemolivia*, and *Hepatozoon* (Table S2). Semi-nested PCR amplifications were performed as follows: the first PCR run with the external primers was performed in a 10 μ L volume containing 10–50 ng of genomic DNA, 3 mM of each dNTP (Thermo Scientific, Waltham, MA, USA), 8 mM of MgCl₂ (Roche Diagnostics), 3 μ M of each primer, 1 μ L of 10 \times PCR buffer (Roche Diagnostics), and 0.5 U of Taq DNA polymerase (Roche Diagnostics). A 1 μ L aliquot of the PCR product from the first reaction was then used as a template for the second round of amplification. The second PCR was performed in a total volume of 25 μ L and contained 8 mM of each dNTP (Thermo Scientific), 10 mM of MgCl₂ (Thermo Scientific), 7.5 μ M of each of the internal primers, 2.5 μ L of 10 \times PCR buffer (Thermo Scientific), and 1.25 U of Taq DNA polymerase (Thermo Scientific). Positive (DNA templates of *Dermacentor marginatus* ticks collected in the South of France and confirmed positive for *Babesia bovis*) and negative (water) controls were included in each PCR assay. Following visualization via electrophoresis in 1.5% agarose gel, positive PCR products were sequenced by Eurofins. Sequence chromatograms were cleaned with Chromas Lite (http://www.technelysium.com.au/chromas_lite.html). New sequences obtained in this study are available in GenBank under accession numbers PP476856–PP476867.

Molecular phylogenetic analyses

Phylogenetic analyses were based on alignments of the Apicomplexa 18S rRNA gene sequences using MAFFT (<https://mafft.cbrc.jp>) for Piroplasmida and Eucoccidiorida, respectively. Sequences of Apicomplexa obtained from GenBank, including representative species of the genera *Babesia*, *Theileria*, *Cytauxzoon*, *Hemolivia*, and *Hepatozoon* were also included in the phylogenetic analyses. The Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to find 18S rRNA gene sequences available on GenBank and showing the highest nucleotide similarities with the gene sequences we characterized in this study. The Gblocks program with default parameters was used to obtain

non-ambiguous sequence alignments [16]. Phylogenetic analyses were performed using maximum-likelihood (ML) analyses on both alignments using the MEGA software package (<https://www.megasoftware.net/>). The evolutionary models that best fit the sequence data were determined using the Akaike information criterion. Clade robustness was assessed by bootstrap analysis using 1000 replicates.

Results

Detection of tick-borne Apicomplexa

Concerning the 71 species examined, molecular survey led to the identification of tick-borne Apicomplexa in 11 species (Tables 1 and S1). They were detected in seven species of mammals, including the common opossum (*Didelphis marsupialis*), gray four-eyed opossum (*Philander opossum*), Linnaeus's two-toed sloth (*Choloepus didactylus*), nine-banded armadillo (*Dasybus novemcinctus*), porcupine (*Coendou* sp.), margay (*Felis wiedii*), and greater grison (*Galictis vittata*). They were also detected in four tick species, including the two species that most commonly bite humans in South America, the Cayenne tick (*Amblyomma cajennense*) and *Amblyomma oblongoguttatum*. The other two infected species were the opossum tick (*Ixodes luciae*), and the iguana tick (*Amblyomma dissimile*). No parasites were detected in the five species of passerines surveyed.

Among the 1161 specimens examined, we detected 50 positive samples (4.31%). On the basis of 18S rDNA gene sequences (452 bp), *Babesia* spp. were found in 21 samples (1.81%), *Theileria* sp. in three samples (0.26%), *Hemolivia* spp. in 11 samples (0.95%), and *Hepatozoon* spp. in 15 samples (1.29%) (Tables 1 and S1). No *Cytauxzoon* was detected. Specifically, *Babesia* spp. were present in seven Linnaeus's two-toed sloths and 14 ticks (*A. cajennense*), *Theileria* spp. in three nine-banded armadillos, *Hemolivia* spp. in 11 ticks (*A. dissimile*), and *Hepatozoon* spp. in seven common opossums, one gray four-eyed opossum, one margay, one greater grison, and four ticks (one *I. luciae*, two *A. dissimile*, and one *A. oblongoguttatum*) (Tables 1 and S1). Infection prevalence was particularly high in *A. cajennense* (14 infected out of 15 examined, 99.33%), and *A. dissimile* (13 infected out of 21 examined, 61.90%). Infection prevalence was typically lower in mammals such as Linnaeus's two-toed sloths (seven infected out of 90, 7.78%) and common opossums (seven infected out of 51, 13.73%).

Molecular typing of tick-borne Apicomplexa

Sequencing of an 18S rDNA gene fragment from the 50 infected samples revealed the presence of eight Apicomplexa genovariants (Table S3). For piroplasmids, there were two genovariants of *Babesia* spp. (91.73% nucleotide identity between these two genovariants), and one genovariant of *Theileria* sp. (85.78%–87.97% nucleotide identities with *Babesia* genovariants). For hemogregarines, there were four genovariants of *Hepatozoon* spp. (96.31%–98.05% pairwise nucleotide identities), and one genovariant of *Hemolivia* sp. (96.10%–97.40% pairwise nucleotide identities with *Hepatozoon* genovariants).

Table 1. List of mammal, passerine and tick species examined for the presence of tick-borne Apicomplexa in French Guiana. A detailed list of specimens and sampling locations is provided in [Table S1](#).

Host species	Species local name	Order	Number of examined specimens	Number of infected specimens				
				<i>Babesia</i>	<i>Theileria</i>	<i>Cytauxzoon</i>	<i>Hepatozoon</i>	<i>Hemolivia</i>
Mammals								
<i>Caluromys philander</i>	Bare-tailed woolly opossum	Didelphimorphia	5	–	–	–	–	–
<i>Didelphis marsupialis</i>	Common opossum	Didelphimorphia	51	–	–	–	7	–
<i>Marmosa lepida</i>	Rufous mouse opossum	Didelphimorphia	1	–	–	–	–	–
<i>Marmosa murina</i>	Linnaeus’s mouse opossum	Didelphimorphia	20	–	–	–	–	–
<i>Marmosops parvidens</i>	Delicate slender opossum	Didelphimorphia	5	–	–	–	–	–
<i>Metachirus nudicaudatus</i>	Brown four-eyed opossum	Didelphimorphia	5	–	–	–	–	–
<i>Micoureus demerarae</i>	Woolly mouse opossum	Didelphimorphia	16	–	–	–	–	–
<i>Philander opossum</i>	Gray four-eyed opossum	Didelphimorphia	20	–	–	–	1	–
<i>Bradypus tridactylus</i>	Pale-throated three-toed sloth	Pilosa	108	–	–	–	–	–
<i>Choloepus didactylus</i>	Linnaeus’s wo-toed sloth	Pilosa	90	7	–	–	–	–
<i>Cyclopes didactylus</i>	Pygmy anteater	Pilosa	1	–	–	–	–	–
<i>Tamandua tetradactyla</i>	Southern tamandua	Pilosa	3	–	–	–	–	–
<i>Cabassous unicinctus</i>	Southern naked-tailed armadillo	Cingulata	2	–	–	–	–	–
<i>Dasyus novemcinctus</i>	Nine-banded armadillo	Cingulata	15	–	3	–	–	–
<i>Hydrochoerus hydrochaeris</i>	Capybara	Rodentia	2	–	–	–	–	–
<i>Holochilus sciureus</i>	Amazonian marsh rat	Rodentia	5	–	–	–	–	–
<i>Hylaeamys megacephalus</i>	Large-headed rice rat	Rodentia	15	–	–	–	–	–
<i>Hylaeamys yunganus</i>	Yungas rice rat	Rodentia	10	–	–	–	–	–
<i>Neacomys dubosti</i>	Dubost’s bristly mouse	Rodentia	1	–	–	–	–	–
<i>Neacomys paracou</i>	Paracou bristly mouse	Rodentia	8	–	–	–	–	–
<i>Nectomys rattus</i>	Small-footed bristly mouse	Rodentia	4	–	–	–	–	–
<i>Oecomys auyantepui</i>	North Amazonian arboreal rice rat	Rodentia	16	–	–	–	–	–
<i>Oecomys bicolor</i>	Bicolored arboreal rice rat	Rodentia	16	–	–	–	–	–
<i>Oligoryzomys fulvescens</i>	Fulvous pygmy rice rat	Rodentia	7	–	–	–	–	–
<i>Makalata didelphoides</i>	Brazilian spiny tree rat	Rodentia	8	–	–	–	–	–
<i>Mesomys hispidus</i>	Ferreira’s spiny tree-rat	Rodentia	13	–	–	–	–	–
<i>Proechimys cuvieri</i>	Cuvier’s spiny-rat	Rodentia	18	–	–	–	–	–
<i>Proechimys guyanensis</i>	Guyenne spiny-rat	Rodentia	20	–	–	–	–	–
<i>Coendou melanurus</i>	Black-tailed hairy dwarf porcupine	Rodentia	1	–	–	–	–	–
<i>Coendou sp.</i>	Prehensile-tailed porcupines	Rodentia	3	–	–	–	1	–
<i>Mus musculus</i>	House mouse	Rodentia	34	–	–	–	–	–
<i>Rattus rattus</i>	Black rat	Rodentia	19	–	–	–	–	–
<i>Sciurus aestuans</i>	Guianan squirrel	Rodentia	1	–	–	–	–	–
<i>Felis wiedii</i>	Margay	Carnivora	1	–	–	–	1	–
<i>Puma yagouaroundi</i>	Jaguarundi	Carnivora	5	–	–	–	–	–
<i>Eira barbara</i>	Tayra	Carnivora	4	–	–	–	–	–
<i>Galictis vittata</i>	Greater grison	Carnivora	4	–	–	–	1	–
<i>Lontra longicaudis</i>	Neotropical river otter	Carnivora	1	–	–	–	–	–
<i>Potos flavus</i>	Kinkajou	Carnivora	2	–	–	–	–	–
<i>Alouatta macconnelli</i>	Guyanese red howler	Primates	22	–	–	–	–	–

(Continued on next page)

Table 1. (Continued)

Host species	Species local name	Order	Number of examined specimens	Number of infected specimens				
				<i>Babesia</i>	<i>Theileria</i>	<i>Cytauzoon</i>	<i>Hepatozoon</i>	<i>Hemolivia</i>
<i>Saguinus midas</i>	Golden-handed tamarin	Primates	41	–	–	–	–	–
<i>Cebus apella</i>	Tufted capuchin	Primates	1	–	–	–	–	–
<i>Saimiri sciureus</i>	Guianan squirrel monkey	Primates	1	–	–	–	–	–
<i>Pithecia pithecia</i>	White-faced saki	Primates	1	–	–	–	–	–
Birds								
<i>Glyphorhynchus spirurus</i>	Wedge-billed woodcreeper	Passeriformes	96	–	–	–	–	–
<i>Pipra aureola</i>	Crimson-hooded manakin	Passeriformes	36	–	–	–	–	–
<i>Ceratopipra erythrocephala</i>	Golden-headed manakin	Passeriformes	35	–	–	–	–	–
<i>Chiroxiphia pareola</i>	Blue-backed manakin	Passeriformes	44	–	–	–	–	–
<i>Myrmotherula axillaris</i>	White-flanked antwren	Passeriformes	36	–	–	–	–	–
Ticks								
<i>Ornithodoros capensis</i>	Seabird tick	Ixodida	6	–	–	–	–	–
<i>Amblyomma cajennense</i>	Cayenne tick	Ixodida	15	14	–	–	–	–
<i>Amblyomma calcaratum</i>	–	Ixodida	1	–	–	–	–	–
<i>Amblyomma coelebs</i>	–	Ixodida	31	–	–	–	–	–
<i>Amblyomma dissimile</i>	Iguana tick	Ixodida	21	–	–	–	2	11
<i>Amblyomma geayi</i>	–	Ixodida	10	–	–	–	–	–
<i>Amblyomma goeldii</i>	–	Ixodida	5	–	–	–	–	–
<i>Amblyomma humerale</i>	–	Ixodida	10	–	–	–	–	–
<i>Amblyomma latepunctatum</i>	–	Ixodida	4	–	–	–	–	–
<i>Amblyomma longirostre</i>	–	Ixodida	23	–	–	–	–	–
<i>Amblyomma naponense</i>	–	Ixodida	5	–	–	–	–	–
<i>Amblyomma oblongoguttatum</i>	–	Ixodida	42	–	–	–	1	–
<i>Amblyomma pacae</i>	–	Ixodida	6	–	–	–	–	–
<i>Amblyomma romitii</i>	–	Ixodida	2	–	–	–	–	–
<i>Amblyomma rotundatum</i>	–	Ixodida	6	–	–	–	–	–
<i>Amblyomma scalpturatum</i>	–	Ixodida	9	–	–	–	–	–
<i>Amblyomma varium</i>	Sloth's giant tick	Ixodida	7	–	–	–	–	–
<i>Rhipicephalus microplus</i>	Asian blue tick/Tropical cattle tick	Ixodida	10	–	–	–	–	–
<i>Rhipicephalus sanguineus</i>	Brown dog tick	Ixodida	6	–	–	–	–	–
<i>Dermacentor nitens</i>	Tropical horse tick	Ixodida	55	–	–	–	–	–
<i>Haemaphysalis juxtakoichi</i>	–	Ixodida	8	–	–	–	–	–
<i>Ixodes luciae</i>	Opossum tick	Ixodida	6	–	–	–	1	–
		Total	1161	21	3	–	15	11

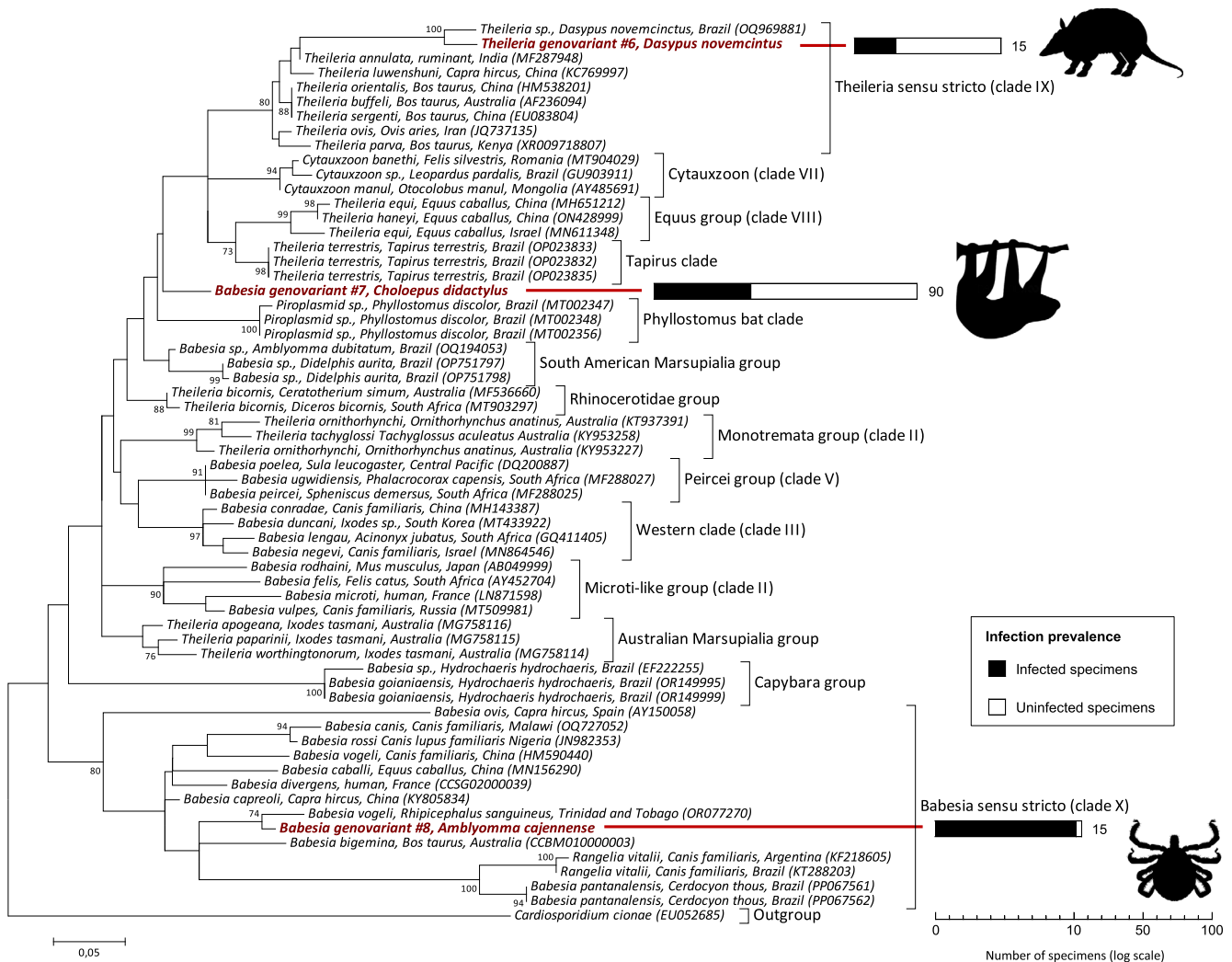


Figure 1. Phylogeny of piroplasmids (Piroplasmida order) constructed using maximum-likelihood (ML) estimations and based on 18S rDNA sequences (408 unambiguously aligned bp; best-fit approximation for the evolutionary model on the basis of Akaike information criterion: TN93+G+I). Only one 18S rDNA sequence per genovariant and per host species is shown for data produced in this study (in bold). GenBank accession numbers of sequences used in analyses are shown on the phylogenetic trees. Numbers at nodes indicate bootstrap support percentage with 1000 replicates. Only bootstrap values >70% are shown. The scale bar is in units of mean number of substitutions/site. The right part of the figure presents the prevalence of Apicomplexa infection observed for each infected species (on a logarithmic scale).

The *Hepatozoon* genovariant #2 was detected in opossums, porcupines, and the opossum tick *I. luciae*, suggesting a transmission cycle between these species in French Guiana. Similarly, the *Hepatozoon* genovariant #3 was present in margay and *A. oblongoguttatum*, a tick species feeding on a wide range of mammals. The other six genovariants were each found associated with only one species, either mammals or ticks (Table S3).

Three of the eight genovariants showed 100% nucleotide identity for their 18S rDNA sequences with known protozoan species (Table S3). *Hemolivia* genovariant #1, identified in *A. issimile*, is identical to *Hemolivia stellata*, a parasite of the cane toad *Rhinella marina* [37]. *Hepatozoon* genovariant #3, detected in margay and *A. oblongoguttatum*, matched to *Hepatozoon luiperdjie*, a recently described parasite of the African leopard [6]. *Hepatozoon* genovariant #5, found in *A. dissimile*, matched to *Hepatozoon cuorae*, a parasite of Asian reptiles

[43]. While *Hemolivia stellata* is native to South America [37], neither *Hepatozoon luiperdjie* nor *Hepatozoon cuorae* have been previously observed in the Americas [43]. The five other genovariants are distinct from taxa referenced in public databases based on their 18S rDNA sequences (Table S3).

Phylogeny of tick-borne Apicomplexa

ML phylogenetic analyses based on 18S rRNA nucleotide sequences were further conducted to examine the phylogenetic proximity of tick-borne Apicomplexa detected in this study with representative species of the genera *Babesia*, *Theileria*, *Cytauxzoon*, *Hemolivia*, and *Hepatozoon*. ML analyses confirmed that (i) the two genovariants of *Babesia* (#7 and 8) and the genovariant of *Theileria* (#6) cluster with other piroplasmids (Fig. 1), (ii) the four genovariants of *Hepatozoon* (#2, 3, 4, and 5) and the genovariant of *Hemolivia* (#1) cluster

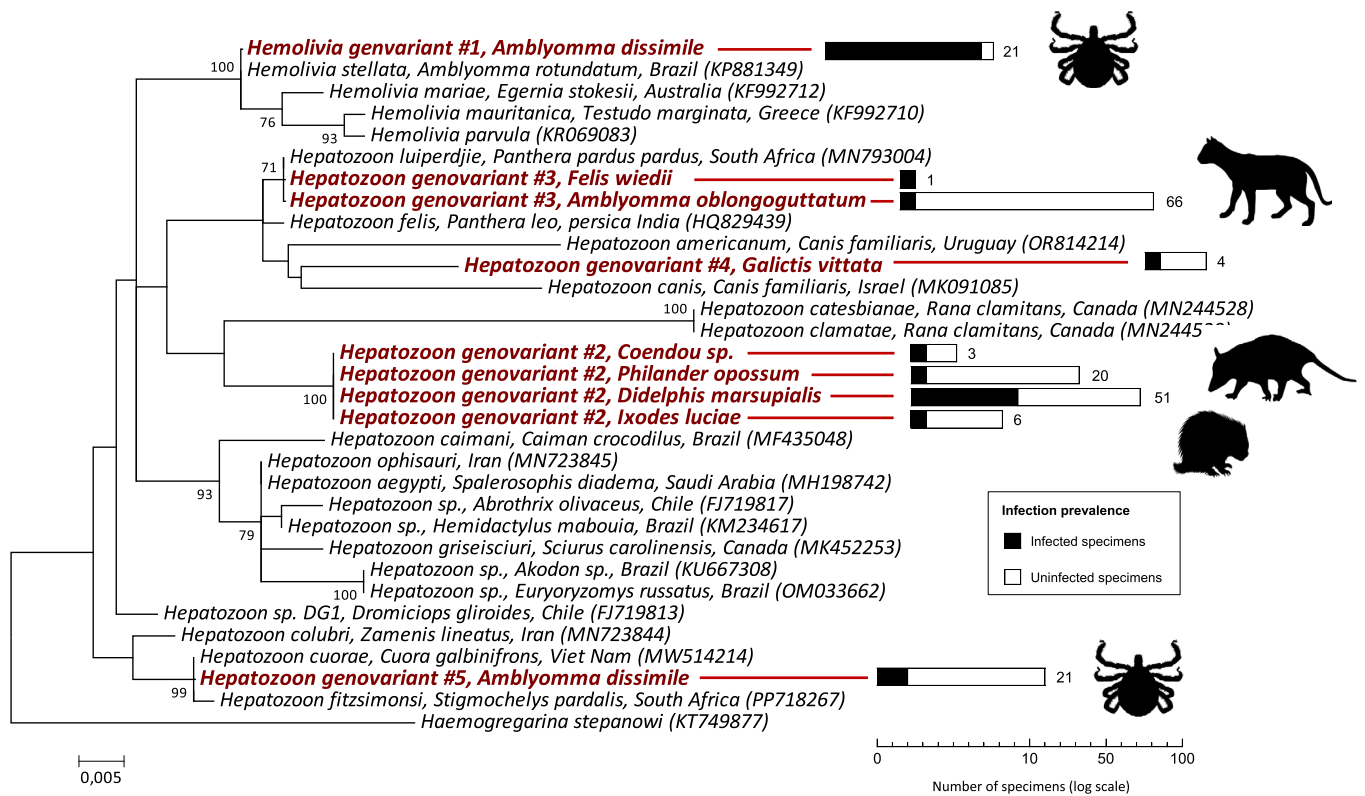


Figure 2. Phylogeny of tick-borne hemogregarines (Eucoccidiorida order) constructed using maximum-likelihood (ML) estimations based on 18S rDNA sequences (461 unambiguously aligned bp; best-fit approximation for the evolutionary model on the basis of Akaike information criterion: HKY+G). Only one 18S rDNA sequence per genovariant and per host species is shown for data produced in this study (in bold). GenBank accession numbers of sequences used in analyses are shown on the phylogenetic trees. Numbers at nodes indicate bootstrap support percentage with 1000 replicates. Only bootstrap values >70% are shown. The scale bar is in units of mean number of substitutions/site. The right part of the figure presents the prevalence of Apicomplexa infection observed for each infected species (on a logarithmic scale).

with other *Hepatozoon* and *Hemolivia* species (Fig. 2). As expected [23], Piroplasmida is formed by the genus *Cytauxzoon* and a polyphyletic assemblage of *Babesia* and *Theileria* species.

ML analyses further showed that the *Babesia* genovariants #7 and #8 and the *Theileria* genovariant #6 are distantly related within the Order Piroplasmida (Fig. 1). *Babesia* genovariant #7 from Linnaeus's two-toed sloths does not belong to a previously identified piroplasmid clade, including the recently described clades found in wildlife in Brazil, as the *Phyllostomus* bat clade [21], the *Tapirus* clade [30], the South American Marsupialia Group clade [31] and the Capybara clade [26]. *Babesia* genovariant #8 from *A. cajennense* belongs to the lineage *Babesia sensu stricto* (clade X in [23]), and is related to *Babesia vogeli*, one of the most important pathogens among *Babesia* species found in dogs [33]. *Theileria* genovariant #6 from nine-banded armadillos belongs to the *Theileria sensu stricto* lineage (clade IX in [23]), which include causative agents of tropical theileriosis, such as *Theileria orientalis* and *Theileria annulata*, two parasite species usually found in cattle [1]. In the *Theileria sensu stricto* lineage, *Theileria* genovariant #6 is closely related to another undescribed *Theileria* species recently found in nine-banded armadillos in Brazil (Fig. 1) [15, 39].

For hemogregarines, ML analyses confirmed the relatedness of *Hemolivia* genovariant #1 with *H. stellata*, and further

indicated that they are both related to *Hemolivia parvula*, *Hemolivia mauritanica*, and *Hemolivia mariae* (Fig. 2), infecting African, Palearctic, and Australian reptiles, respectively [28, 50]. *Hepatozoon* genovariant #2 found in opossums, porcupines and the opossum tick *I. luciae* forms a branch that is well divergent from all other *Hepatozoon* species, with no close relatedness to known species or isolates. *Hepatozoon* genovariant #3 clusters with *Hepatozoon luiperdjie*, and both are closely related to the common cat parasite *Hepatozoon felis*. The *Hepatozoon* genovariant #4 we detected in greater grison is more closely related to *Hepatozoon canis* and *Hepatozoon americanum*, which infect dogs in the Americas. *Hepatozoon* genovariant #5 clusters with *Hepatozoon cuorae* and *Hepatozoon colubri*, which both infect turtles and snakes (Fig. 2) [44, 48, 49].

Discussion

In our study, we have identified several genovariants of *Babesia*, *Theileria*, *Hemolivia*, and *Hepatozoon* in various wildlife species, including opossums, sloths, armadillos, and porcupines, as well as in tick species that most commonly bite humans in South America. While some genovariants match with known species, others do not, raising evidence of undescribed Apicomplexa species in French Guiana. The detection

of similar genovariants in wildlife and ticks further unveils the existence of unique sylvatic transmission cycles for these Apicomplexa. These observations in French Guiana remarkably mirror recent surveys of wildlife in Brazil, which also highlight the distinctiveness of South American tick-borne Apicomplexa and their specific sylvatic transmission cycles [4, 5, 13, 20, 21, 26, 30, 31, 34, 36, 43].

None of the three piroplasmid genovariants found in French Guiana matched with *Babesia*, *Theileria*, and *Cytauxzoon* species, strains or isolates for which 18S rRNA sequences were available in public databases. They may each represent a new putative species or an already described species for which no molecular sequences are currently available. Indeed, *Babesia choloepi*, which was morphologically characterized from erythrocytes of Linnaeus's two-toed sloths of French Guiana [18], may be the *Babesia* genovariant #7 we characterized also in Linnaeus's two-toed sloths. This *Babesia* genovariant is distinct from all already known piroplasmid clades, including those recently described in wildlife in Brazil [21, 26, 30, 31], suggesting it may form a novel piroplasmid clade. The *Theileria* genovariant #6 of the nine-banded armadillo is similar to a *Theileria* sp. infected in nine-banded armadillos in Brazil [15, 39], and both may be related to another *Theileria* sp., observed through microscopy in nine-banded armadillos in the state of Pará, Brazil, as mentioned by Lainson et al. [29], but not further described. The association of armadillos with the *Theileria* genovariant #6 is more singular since this parasite is related to species of the *Theileria sensu stricto* lineage and that are usually found in cattle [23]. The *Babesia* genovariant #8 is closely related to *Babesia vogeli*, which is commonly found in dogs and cats in Brazil [4]. Its detection in *A. cajennense*, a tick species feeding on a wide range of mammals, including humans, suggests that a diverse range of hosts is exposed to this parasite. However, *Babesia* genovariant #8 has never been detected in vertebrates, indicating that its natural hosts are currently unknown.

The diversity of *Hemolivia* and *Hepatozoon* genovariants found in French Guiana leads to quite similar observations. *Hemolivia* genovariant #1 is evidently *Hemolivia stellata*, a parasite that colonizes erythrocytes, cells of the reticuloendothelial system, and digestive cells of the cane toad [37]. However, while *A. rotundatum* is vector of this parasite in Brazil [37], we commonly observed infection in another tick species in French Guiana, the iguana tick *A. dissimile*. This tick species specializes in reptiles and amphibians, commonly feeding on cane toads, and may thus serve as an additional vector species of *Hemolivia stellata* in French Guiana. Similarly, *Hepatozoon* genovariant #5 is related to *Hepatozoon cuorae*, a species known to infect Asiatic turtles [49]. However, *Hepatozoon cuorae* has never been observed in South America, but our observation suggests that a related species may exist in French Guiana. *Amblyomma dissimile* is a potential local vector, and given the host specificity of this tick species, this parasite may infect South American reptiles or amphibians or both. *Hepatozoon* genovariant #3 is related to *Hepatozoon luiperdjie*, a parasite of the African leopard [6], previously not found outside Africa. The detection of this genovariant in margays of French Guiana, and in *A. oblongoguttatum* as a potential local vector, suggests that the *Hepatozoon luiperdjie* relatives may form a clade specific to wild felids across different

continents. *Hepatozoon* genovariant #4 found in greater grison is different to all known species but is related to *Hepatozoon canis* and *Hepatozoon americanum*, forming together a large clade associated with carnivores. On the contrary, the discovery of *Hepatozoon* genovariant #2 in two opossum species, porcupines, and the opossum tick *I. luciae* indicates that this parasite is common and widespread in the rainforests of French Guiana. However, *Hepatozoon* genovariant #2 has not been documented elsewhere, prompting inquiries into its geographic distribution.

In conclusion, the investigation into tick-borne Apicomplexa in French Guiana underscores the significance of acknowledging the diversity of these infections within this region. Moreover, the identification of potential novel species emphasizes the urgent need for ongoing research and surveillance to mitigate the health risks posed by these tick-borne parasites. A major limitation of our study is that we used a short fragment of the 18S rDNA for the typing of infection. Complete sequencing of the 18S rDNA and additional markers, such as mitochondrial markers, are now needed to confirm the existence of new species and new clades. Notably, these new typings should allow for better comparison with the new species discovered in South America, particularly for *Hepatozoon*, for which many new genovariants have been described [2, 3, 13, 35, 40].

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Conflicts of interest

The authors declare no conflict of interest.

Supplementary material

The supplementary material for this article can be found at <https://www.parasite-journal.org/10.1051/parasite/2024052/olm>.

Table S1: List and locations of samples examined for the presence of tick-borne Apicomplexa in French Guiana.

Table S2: Primers used in polymerase chain reaction (PCR) assays and Sanger sequencing for 18S rDNA (SSU) gene of tick-borne Apicomplexa.

Table S3: Genovariants of *Babesia*, *Theileria*, *Hemolivia* and *Hepatozoon* detected in French Guiana and best nucleotide identities with 18S rRNA gene sequences obtained in this study and sequences available in GenBank identified through Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) searches (Last update: 01/04/2024).

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