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Molecular screening for tick-borne bacteria and hematozoa in *Ixodes* cf. *boliviensis* and *Ixodes tapirus* (Ixodida: Ixodidae) from western highlands of Panama

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ABSTRACT

The first molecular screening for *Rickettsia*, *Anaplasma*, *Ehrlichia*, *Borrelia*, *Babesia* and *Hepatozoon* was carried out in questing *Ixodes* cf. *boliviensis* and *Ixodes tapirus* from Talamanca Mountains, Panama, using specific primers, sequencing and phylogeny. Phylogenetic analyses for the microorganisms in *Ixodes* cf. *boliviensis* confirmed the presence of *Rickettsia* sp. strain IbR/CRC endosymbiont (26/27 ticks), three genotypes of the *Borrelia burgdorferi* (*sensu lato*) complex (4/27 ticks), *Babesia odocoilei* (1/27 ticks), and *Hepatozoon* sp. (2/27 ticks), tentatively designated *Hepatozoon* sp. strain Chiriquensis. Phylogenetic analyses for the microorganisms in *I. tapirus* revealed an undescribed *Rickettsia* sp., tentatively designated *Rickettsia* sp. strain Itapirus LQ (6/6 ticks), and *Anaplasma phagocytophilum* (2/6 ticks). To the best of our knowledge, this is the first report of *B. burgdorferi* (*s.l.*) complex, *A. phagocytophilum*, *B. odocoilei*, and *Hepatozoon* sp. in *Ixodes* ticks from Central America, and also the first detection of *Rickettsia* spp. in *Ixodes* species in Panama. In light of the importance of these findings, further studies are needed focusing on the role of *I. tapirus* and *I.* cf. *boliviensis* as vectors, and the vertebrates acting as reservoirs.

1. Introduction

Ixodes (Ixodida: Ixodidae) is the most diverse genus of ticks, comprising nearly 260 species worldwide (Guglielmone et al., 2019; Saracho-Bottero et al., 2021). Of these, nearly 70 species have been reported parasitizing humans (Guglielmone & Robbins, 2018). From a public health standpoint, *Ixodes* spp. are considered among the most important arthropods, particularly in the Northern Hemisphere, because of their implications as vectors of Lyme disease (*B. burgdorferi* (*s.l.*) complex), granulocytic anaplasmosis (*A. phagocytophilum*), and to a lesser extend of babesiosis and viral diseases (CDC, 2018). In the Neotropics, where nearly 47 *Ixodes* species occur (Guglielmone et al., 2019; Saracho-Bottero et al., 2021), parasitism in humans is rare. Indeed, only *I. boliviensis*, *Ixodes pararicinus* and *Ixodes tropicalis* have been reported feeding on humans in this region (Guglielmone & Robbins, 2018; Quintero et al., 2020).

In recent decades, a diverse group of microorganisms have been detected from *Ixodes* spp. of South America. These finding include the spotted fever group rickettsia (SFGR) "*Candidatus* Rickettsia andeanae" in *I. boliviensis* (see Blair et al., 2004), *Rickettsia* spp. endosymbionts in *I. pararicinus*, *Ixodes fuscipes* and *Ixodes* cf. *affinis* (Sebastian et al., 2020), and the basal group rickettsia *Rickettsia bellii* in *Ixodes loricatus* and *I. tropicalis* (see Melis et al., 2020; Quintero et al., 2020). In addition, different genotypes of *B. burgdorferi* (*s.l.*) complex were reported from *I. fuscipes* (reported as *Ixodes aragaoi*), *Ixodes auritulus*, *Ixodes longiscutatus*, *Ixodes neuquenensis*, *Ixodes paranaensis*, *I. pararicinus*, *Ixodes sigelos* group, and *Ixodes stilesi* (see Barbieri et al., 2013; Ivanova et al., 2014; Sebastian et al., 2016; Dall'Agnol et al., 2017; Saracho-Bottero

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et al., 2017; Verdugo et al., 2017; Flores et al., 2018; Cicuttin et al., 2019; Muñoz-Leal et al., 2019, 2020; Carvalho et al., 2020). Further, "*Candidatus* Neoehrlichia chilensis" and hemoparasites of the genus *Hepatozoon* were found in *Ixodes* sp. and *I. sigelos* group, respectively (Muñoz-Leal et al., 2019). Although these *Ixodes* spp. are not of public health importance (Guglielmone & Robbins, 2018), recognizing tick species that harbor DNA of possible pathogenic microorganisms constitutes a foundational step to understand putative vector roles.

In Central America, of the 18 reported species of *Ixodes*, the only information on associated microorganisms corresponds to SFGR in *I. boliviensis*, *Ixodes minor* and *Ixodes affinis* (Troyo et al., 2014; Ogrzewalska et al., 2015; Lopes et al., 2016; Polsomboon et al., 2017; Bermúdez et al., 2021). Similar to South America, *Ixodes* spp. in Central America are not a public health concern (Guglielmone & Robbins, 2018). Nevertheless, serological evidence indicates exposure to Lyme disease in people and dogs from Costa Rica (Villalobos-Zúñiga & Somogyi, 2012; Montenegro et al., 2017), but this infection has not been confirmed.

Eleven species of *Ixodes* occur in Panama (Bermúdez et al., 2018) and there are limited data about their microorganisms (Bermúdez et al., 2021). In this study, we performed genetic screening for detection of Rickettsiales (*Rickettsia* and *Anaplasma*), *B. burgdorferi* (*s.l.*) complex, and tick-borne hematozoa (*Babesia* and *Hepatozoon*) in two species of *Ixodes* collected on vegetation in the Talamanca Mountains in Panama.

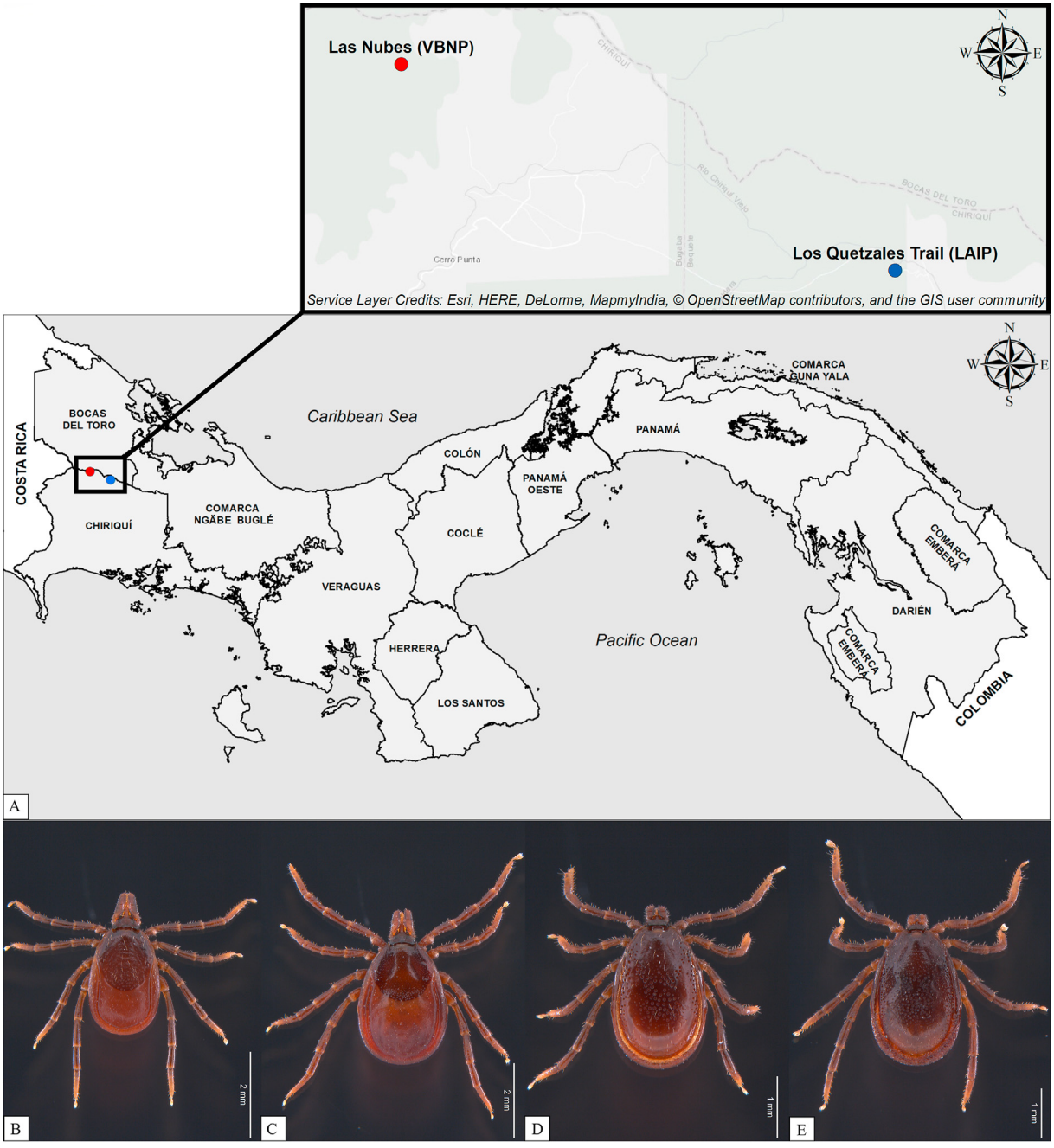

Fig. 1. A General view of Panama with Volcan Baru National Park and La Amistad International Park (black rectangle). **B** *Ixodes* cf. *boliviensis*, female. **C** *Ixodes tapirus*, female. **D** *Ixodes* cf. *boliviensis*, male. **E** *Ixodes tapirus*, male

2. Materials and methods

2.1. Sites of collection, tick collection and identification

During September 2019, prospections were performed in the Chiriqui Province, within the Las Nubes station (Volcán Baru National Park, VBNP) and in Los Quetzales trail (La Amistad International Park, LAIP), at an elevation of 2,300 and 2,500 m, respectively (Fig. 1). Both sites belong to the Talamanca mountain range, which represents the highest mountains of the country, with elevations above 3,000 m, and are among the most important hot spots of diversity in Central America, and are also close to one of the main agricultural production areas in Panama. According to the Köppen-Geiger climatic classification (Kottek et al., 2006), this region corresponds to tropical wet climate (Am), but due to the high elevation, subtropical temperatures (0–20 °C), and high humidity prevails (Anonymous, 2007, 2012).

Free-living ticks were collected using white cloth dragging and by visual search over the vegetation and preserved in 80% ethanol. The ticks were identified using a taxonomical key (Bermúdez et al., 2018).

2.2. DNA extraction and analysis

Individual ticks were bisected longitudinally using sterile scalpels and washed with distilled water to remove ethanol. DNA was extracted using the commercial kit GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania) following the manufacturer's instructions.

To compare with other Neotropical *Ixodes* spp., ticks were analyzed *via* PCR amplification of a ~460-bp fragment of the tick mitochondrial *16S* rRNA gene (Mangold et al., 1998). The identity and distances for these sequences were calculated using the Sequence Identity and Similarity calculator (Anonymous, 2021).

Extracted DNA was tested by a battery of PCR protocols targeting *Rickettsia*, family *Anaplasmataceae*, *Borrelia*, *Babesia* and *Hepatozoon*, using specific primers and published protocols for each agent (Table 1). In all PCR assays, distilled water was used as a negative control, and *Rickettsia parkeri* strain Toledo, *Ehrlichia canis* isolate P1091, *Borrelia anserina* PL and *Babesia bovis* Paysandú were included as positive controls. Five microliters of PCR amplicons were separated by electrophoresis in a 1.5% agarose gel, stained with GoodView TM Nucleic Acid Stain (Beijing SBS Genetech Co., LTd, China), and examined under UV transillumination. Amplicons were purified using GeneJET PCR purification kit (Thermo Fisher Scientific, Lithuania).

2.3. Analyses of sequences and phylogenies

Amplicons of expected size were purified and Sanger sequenced (Macrogen, Korea). Sequences were assembled and trimmed using Geneious software (Kearse et al., 2012). Consensus sequences were submitted to BLASTn analyses to compare with sequences available on GenBank. An alignment of the newly generated sequences and GenBank-retrieved homologues was built for each microorganism group with MAFFT (Katoh et al., 2002). Bayesian analyses were performed in MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001) employing four independent Markov chains, 1,000,000 metropolis-coupled MCMC generations and sampling trees every 100th generation. The first 25% of the trees were discarded as "burn-in", and the remaining trees were used to calculate the Bayesian posterior probability.

3. Results

We collected 55 adults of *Ixodes* spp., morphologically identified as *I. boliviensis* (10 males and 32 females) and *I. tapirus* (3 males and 10 females). For molecular identification of ticks, DNA was extracted from 2 females and 2 males of *I. boliviensis* and 2 females of *I. tapirus*. *Ixodes boliviensis* collected in Panama showed 92% identity with *I. boliviensis* collected in Ecuador, South America (GenBank: KM077437) (Table 2).

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

Targeted microorganism	Gene	Primer name	Sequence	Length (bp)	Reference
Tick (mitochondrial)	*16S* rRNA	16S + 1	CCGGTCTGAACTCAGATCAAG	460	Mangold et al. (1998)
		16S-1	GCTCAATGATTTTTTAAATTGCTG		
Rickettsia sp.	*gltA*	CS-78	GCAAGTATCGGTGAGGATGTAAT	401	Labruna et al. (2004)
		CS323	GCTTCCTTAAAATTCAATAAATCAGGAT		
Rickettsia sp.	*gltA*	CS-239	GCTCTTCTCATCCTATGGCTATTAT	834	Labruna et al. (2004)
		CS-1069	CAGGGTCTTCGTGCATTTCTT		
Rickettsia spotted fever group	*ompA*	Rr190.70p	ATGGCGAATATTTCTCCAAAA	532	Roux et al. (1996)
		Rr190.602n	AGTGCAGCATTCGCTCCCCCT		
		ompB-OF	GTAACCGGAAGTAATCGTTTCGTAA	511	
Rickettsia sp.	*ompB*	ompB-O	GCTTTATAACCAGCTAAACCACC		Choi et al. (2005)
		ompB SFG IF	GTTTAATACGTGCTGCTAACCAA	420	
		ompB SFG IR	GGTTTGGCCCATATACCATAAG		
Anaplasmataceae	*16S* rRNA	EHR16SD	AGAGTTTGATCCTGGCTCAG	1500	Inokuma et al. (2001)
		EHR16SR	ACGGCTACCTTGTTACGACTT		
Ehrlichia spp.	*Dsb*	Dsb-330	GATGATGTTTGAAGATATSAAACAAAT	409	Doyle et al. (2005)
		Dsb-720	CTATTTTACTTCTTAAAGTTGATAWATC		
		Dsb-380	ATTTTTAGRGATTTTCCAATACTTGG		
Ehrlichia spp.	*groEl*	HS1a	AITGGGCTGGTAITGAAAT	1297	Lotric-Furlan et al. (1998)
		HS6a	CCICCIGGIACIAIACCTTC		
		HS43	ATWGCWAARGAAGCATAGTC		
		HSVR	CTCAACAGCAGCTCTAGTAGC		
Borrelia spp.	*16S* rRNA	LoneTop	CTGGCAGTGCGTCTTAAGCA	869	Cyr et al. (2005)
		Tec1a/p	TCTTGCGAGCATACTCCCCAG		
Borrelia spp.	*flab*	Fla LL	ACATATTCAGATGCAGACAGAGGT	665	Barbour et al. (1996)
		Fla RL	GCAATCATAGCCATTGCAGATTGT		
		FlaRS	CTTTGATCACTTTCATTCTAATAGC		
		FlaLS	AACAGCTGAAGAGCTTGGAAT		
Borrelia spp.	*Flab*	Fla LS	AACAGCTGAAGAGCTTGGAATG	354	Barbour et al. (1996)
		Fla RS	CTTTGATCACTTATCATTCTAATAGC		
Piroplasmid	*18S* rRNA	BAB 143-167	CCGTGCTAATTGTAGGGCTAATACA	551	Soares et al. (2017)
		BAB 694-667	GCTTGAAACACTCTARTTTTCTCAAAG		

Table 2
Percent identity of *16S* rDNA sequences of *Ixodes* cf. *boliviensis* and other *Ixodes* spp. available on GenBank

		1	2	3	4	5	6
1	*Ixodes* cf. *boliviensis* (IbH1)	–					
2	*Ixodes* cf. *boliviensis* (IbM4)	99.02	–				
3	*Ixodes* cf. *boliviensis* (IbH25)	99.26	99.51	–			
4	*Ixodes* cf. *boliviensis* (IbM32)	99.26	99.51	100	–		
5	*Ixodes* sp. II MO-2013 (KF702351)	98.78	99.02	99.51	99.51	–	
6	*Ixodes boliviensis* (KM077437)	93.17	92.43	92.68	92.68	93.17	–

Since *I. boliviensis* corresponds to a taxon described in South America, this difference may indicate two different tick species; therefore, here we name the specimens from Panama provisionally as *Ixodes* cf. *boliviensis*. Our study also provides the first DNA sequences of *I. tapirus*. The newly generated *16S* rDNA sequences for the two tick species are available on GenBank under the accession numbers MW717930 and MW717931 (*I. tapirus* females), MW717933 and MW717934 (*I.* cf. *boliviensis* female and male from VBNP), and MW717934 and MW717935 (*I.* cf. *boliviensis* female and male from LAIP). Voucher materials (15 *I.* cf. *boliviensis* and 6 *I. tapirus*) were deposited in the Ectoparasites Collection of the "Dr. Eustorgio Méndez" Zoological Collection of the Gorgas Memorial Institute for Health Studies, under the accession numbers CoZEM-ICGES IX096-098.

A total of 33 ticks were screened for *Rickettsia*, *Anaplasma*, *Ehrlichia*, *Borrelia*, *Babesia* and *Hepatozoon*, corresponding to 27 *I.* cf. *boliviensis* (20 females and 7 males) and 6 females of *I. tapirus*. Of these, 26 *I.* cf. *boliviensis* yielded amplicons for at least one of the species of bacteria or hematozoa, and all *I. tapirus* yielded amplicons for Rickettsiales (Table 3).

DNA of *Rickettsia* spp. was detected in 96% of *I.* cf. *boliviensis* and 100% of *I. tapirus* analyzed (Table 3). Both *I.* cf. *boliviensis* (17 females and 6 males) and *I. tapirus* (6 females) yielded sequences of the *gltA* gene which showed 99.0–99.8% similarity with *Rickettsia* IRS3 (GenBank: AF140706.1). Partial *ompA* gene sequences were generated from 19 females and 6 males of *I.* cf. *boliviensis* and all *I. tapirus*; these showed 98.7–99.5% similarity to *Rickettsia* sp. IbR-CRC2 (GenBank: KJ507218). Partial *ompB* gene sequences were generated from 18 females and 6 males of *I.* cf. *boliviensis* and from all *I. tapirus*; these showed 98.6–99.2% similarity to uncultured *Rickettsia* sp. clone C23 (GenBank: MF170623). The phylogenetic analyses grouped the newly generated sequences into two clades within *Rickettsia* endosymbionts of *Ixodes* spp. of the New World (Fig. 2). While the *gltA* and *ompA* genotypes detected in *I.* cf. *boliviensis* shared a clade with *Rickettsia* sp. strain IbR/CRC albeit with low support, the genotypes detected in *I. tapirus* represented a different sister lineage (Fig. 2B). Tentatively the genotype found in *I. tapirus* is designated as *Rickettsia* strain Itapirus LQ, after the species of tick and the name of the trail (Los Quetzales).

DNA of *A. phagocytophilum* was detected in two *I. tapirus* females. The sequences generated for both *16S* rRNA and *groEl* genes were identical for each gene and showed a similarity to *A. phagocytophilum* reported on GenBank (98.6% for *16S* rDNA (AY527213, KP276588) and 100% for *groEl* (KM215261, EU246959)). Phylogenetic analyses for both genes confirmed the similarity among *A. phagocytophilum* detected in the two *I. tapirus* females, which formed a separate clade within other isolates of *A. phagocytophilum* reported from vertebrates and ticks (Fig. 3).

DNA of *Borrelia* spp. was amplified in four *I.* cf. *boliviensis* females from LAIP using the *flaB* gene primers but not with *Borrelia* genus-specific primers for the *16S* rRNA gene. BLASTn searches revealed a similarity of 97–99% of the newly generated sequences with *Borrelia carolinensis* isolate BR1972-11 (GenBank: MK604312), and three sequences showed 98% similarity with *Borrelia lanei* isolate BR1945-11 (GenBank: MK604329). The phylogenetic analysis showed that three genotypes grouped within the *B. burgdorferi* (*s.l.*) complex (Fig. 4). Tentatively these genotypes are designated as *Borrelia* sp. strain Talamanca A, B and C, after the mountainous region where they were detected (Table 3). *Borrelia* sp. Talamanca A formed a closely related monophyletic group with *B. burgdorferi* (*s.s.*).

Finally, in three *I.* cf. *boliviensis* we obtained two identical sequences of the *18S* rRNA gene, which were compatible with *Hepatozoon* sp. We tentatively designated these as *Hepatozoon* sp. strain Chiriquensis, after the province of Chiriqui. In the phylogenetic analysis, these sequences were grouped with sequences obtained from ticks or mustelids (Fig. 5); however, the support for this relationship was low. DNA of *B. odocoilei* was detected in one *I.* cf. *boliviensis*; the sequence showed 98% similarity to *B. odocoilei* (GenBank: MF357057.1). The phylogenetic analysis showed that this sequence clusters within the well-supported *B. odocoilei* clade (Fig. 6).

Table 3
Microorganisms detected in *Ixodes* cf. *boliviensis* and *Ixodes tapirus* from highlands of western Panama

Microorganism	LAIP		VBNP		
	Ixodes cf. *boliviensis*		*Ixodes* cf. *boliviensis*		*Ixodes tapirus*
	♀ ($n = 17$)	♂ ($n = 6$)	♀ ($n = 3$)	♂ ($n = 1$)	♀ ($n = 6$)
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Rickettsia sp. strain IbR/CRC	15 (88.2)	5 (83.3)	1 (33.3)	0	0
Rickettsia sp. strain Itapirus LQ	0	0	0	0	6 (100)
Anaplasma phagocytophilum	0	0	0	0	2 (33.3)
Borrelia sp. strain Talamanca A	2 (11.0)	0	0	0	0
Borrelia sp. strain Talamanca B	1 (5.8)	0	0	0	0
Borrelia sp. strain Talamanca C	1 (5.8)	0	0	0	0
Babesia odocoilei	0	1 (16.7)	1 (33.3)	0	0
Hepatozoon sp. strain Chiriquensis	2 (11.8)	0	0	0	0

Abbreviations: LAIP, La Amistad National Park, Las Nubes Station; VBNP, Volcán Barú National Park, Los Quetzales trail.

4. Discussion

Ixodes cf. *boliviensis* and *I. tapirus* have been reported in highlands of Panama since the middle of the 20th century (Fairchild et al., 1966); however, little is known about these species. Both species are common to collect in trails of VBNP and LAIP, and crawl actively in the underbrush vegetation, possibly due to the behavior of their hosts. In Panama, *I.* cf. *boliviensis* feeds mainly on wild and domestic carnivores, but also other mammals and humans are included as sporadic hosts (Fairchild et al., 1966; Bermúdez et al., 2018). Since *I.* cf. *boliviensis* could be a species different from *I. boliviensis*, this fact is relevant for public health, because the only reports of *I. boliviensis* parasitizing humans have been registered in Mexico and Central America, but not in South America (Guglielmone & Robbins, 2018). Regarding *I. tapirus*, to our knowledge, tapirs (*Tapirus pinchaque* and *Tapirus bairdii*) are the only hosts of this species (Fairchild et al., 1966; Apanaskevich et al., 2017).

To the best of our knowledge, this is the first study to molecularly detect multiple microorganisms in questing *I.* cf. *boliviensis* and *I. tapirus*. Despite the small numbers of ticks analyzed, it is interesting to note the wide variety of microorganisms found in both species. Since these findings were in adults in questing phases, the presence of these microorganisms must be a result of transstadial transmission.

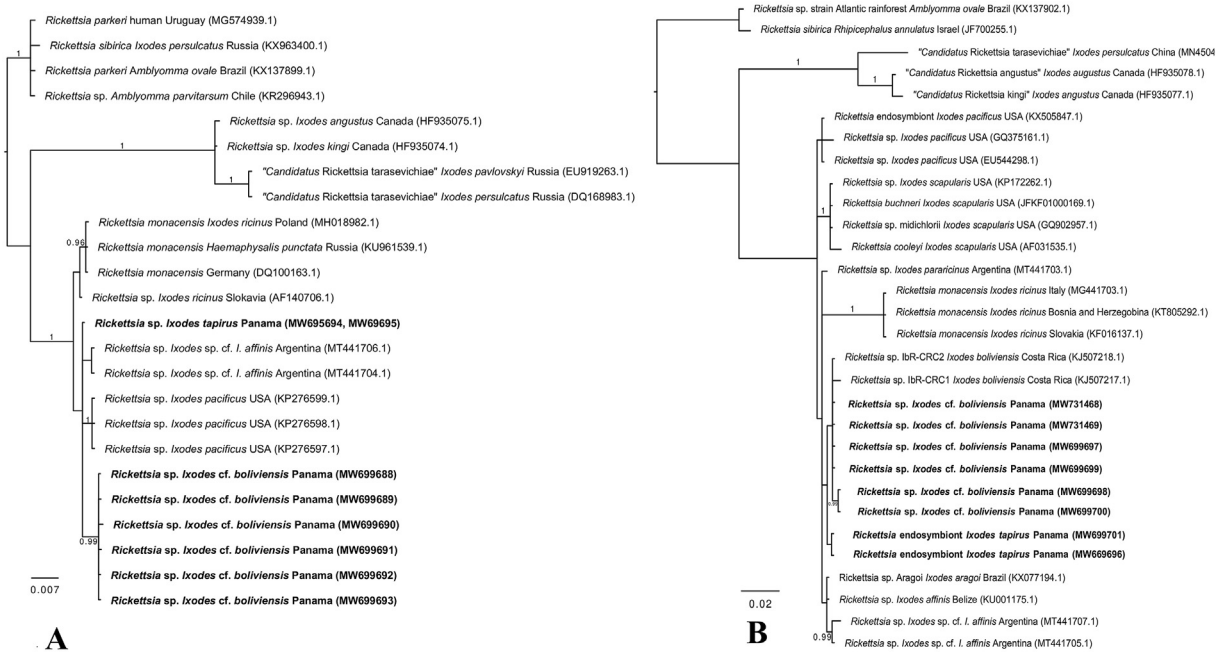

Fig. 2. Bayesian phylogenetic trees for *Rickettsia* spp. based on the *gltA* (**A**) and *ompA* (**B**) genes. Bayesian posterior probabilities are shown at the nodes (only values > 0.95 are shown). *Rickettsia sibirica* and *Rickettsia parkeri* were used as the outgroup. The newly generated sequences are indicated in bold

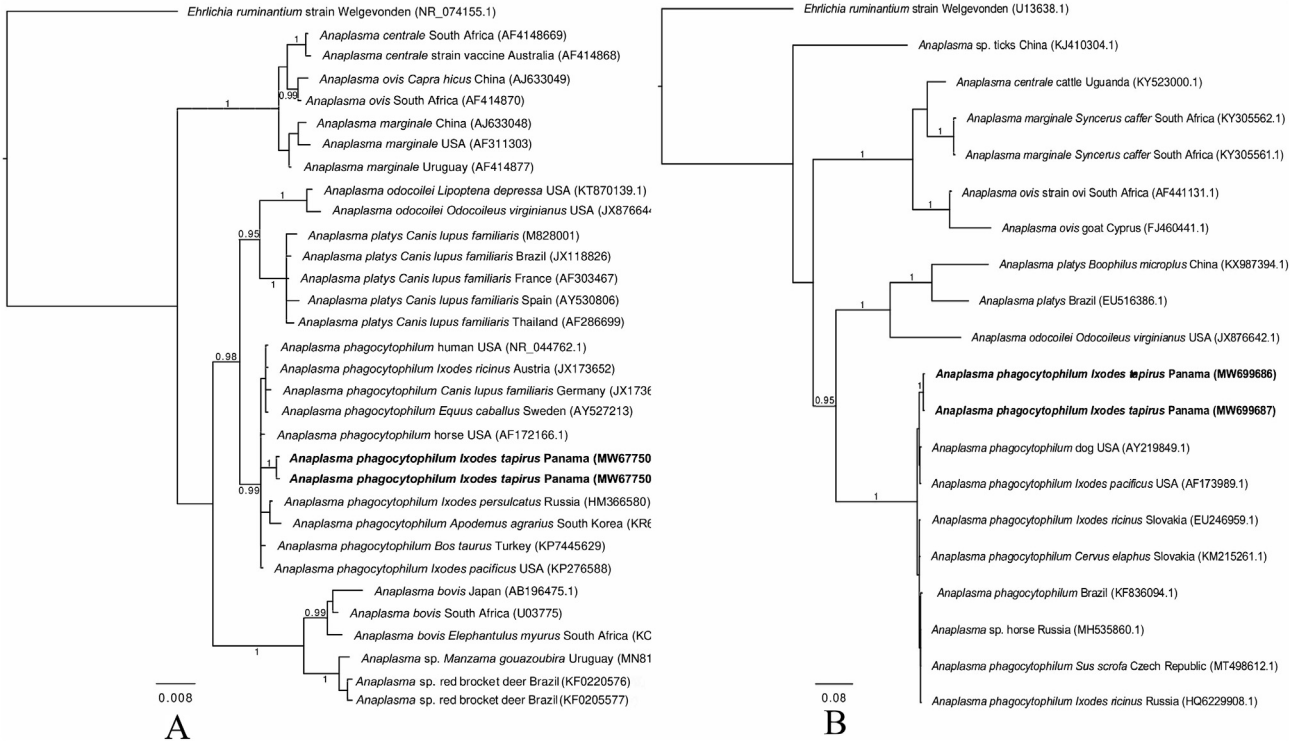

Fig. 3. Bayesian phylogenetic trees for *Anaplasma phagocytophilum* based on the *16S* rDNA (**A**) and *groEl* (**B**) genes. Bayesian posterior probabilities are shown at the nodes (only values > 0.95 are shown). *Ehrlichia ruminantium* was used as the outgroup. The newly generated sequences are indicated in bold

Rickettsia sp. strain IbR/CRC was initially found in *I. boliviensis* from Costa Rica (Troyo et al., 2014), therefore our finding was to be expected, considering that Talamanca Mountains extend to Costa Rica and represent similar environments. Our phylogenetic analysis showed that *Rickettsia* strain Itapirus LQ differs from *Rickettsia* sp. strain IbR/CRC; therefore, the degree of nucleotide dissimilarity (0.1–0.2% for *gltA* and 0.2–0.4% for *ompA*) allows these to be considered as two different endosymbionts, and not a genetic variety. Regarding the *ompB* gene, the use of a highly conserved fragment prevented the separation of these *Rickettsia* genotypes. Considering Fournier et al. (2003) and Fournier and Raoult (2009) about the differences in the homologous sequences of *gltA*, *ompA* and *ompB* genes, and the present phylogenetic analysis, both

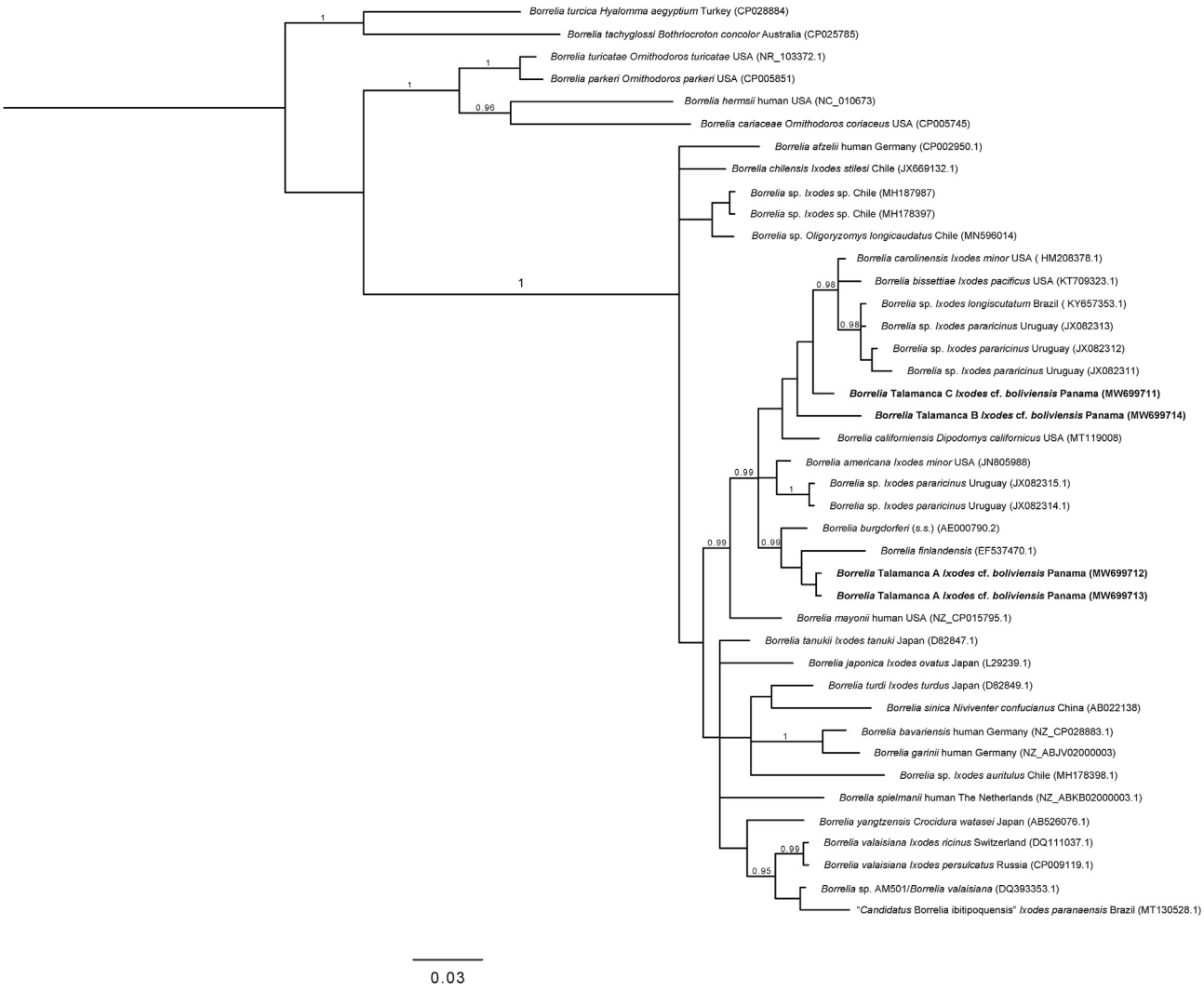

Fig. 4. Bayesian phylogenetic trees for *Borrelia* spp. based on the *flaB* gene. Bayesian posterior probabilities are shown at the nodes (only values > 0.95 are shown). *Borrelia turcica* and *Borrelia tachyglossi* were used as the outgroup. The newly generated sequences are indicated in bold

rickettsiae represent endosymbionts of the genus *Rickettsia* not yet described.

Rickettsia endosymbionts can be transmitted vertically and show a high prevalence in tick populations (Socolovschi et al., 2009; Kurtti et al., 2015), a fact that explains our findings in *I.* cf. *boliviensis* (96%) and *I. tapirus* (100%). Other *Rickettsia* endosymbionts from Central American *Ixodes* spp. include *Rickettsia* sp. strain Barva in *I. minor* from Costa Rica (Ogrzewalska et al., 2015) and *Rickettsia* spp. in *I. affinis* from Belize and Panama (Lopes et al., 2016; Polsomboon et al., 2017; Bermúdez et al., 2021). Springer et al. (2018) reported *Rickettsia monacensis* in *I. boliviensis* from Costa Rica based on multi-locus typing of seven loci; however, our phylogenetic analyses showed that *Rickettsia* sp. IbR/CRC strain from Costa Rica and Panama share, albeit with low support, the same clade with other *Rickettsia* endosymbionts reported in *Ixodes* spp. of Central and South America. This clade is separate from *R. monacensis*; therefore, because this pathogen is reported from *Ixodes ricinus* complex in Europe, its records from *Ixodes* spp. in Central America must be considered with caution.

This is the first report of *A. phagocytophilum* in *I. tapirus*. Phylogenetic results demonstrated various genotypes of *A. phagocytophilum* around the world, which have been detected in *Ixodes* spp. or from different groups of mammals (Brouqui & Matzumoto, 2007). This pathogen affects domestic mammals such as horses, ruminants and carnivores, and also causes human granulocytic anaplasmosis, a disease reported in some countries of Europe and in the USA (Grzeszczuk et al., 2007).

In the Neotropics, information of *Anaplasma* spp. includes reports in mammals and ticks from Mexico (Ojeda-Chi et al., 2019) and South America (Santos et al., 2013; Félix et al., 2020). In Ecuador, genotypes closely related to *A. phagocytophilum* were reported in *Amblyomma multipunctum* collected from *T. pinchaque* and vegetation (Pesquera et al., 2015). The finding of *Anaplasma* spp. in *A. multipunctum* and *I. tapirus* may represent a potential ticks-tapirs relationship to investigate. Since *A. phagocytophilum* is a pathogen of medical and veterinary importance, its relevance in Panama should be considered.

Our findings of three genotypes of the *B. burgdorferi* (*s.l.*) complex in *I.* cf. *boliviensis*, indicate that the Talamanca strain A is a sister group of *B. burgdorferi* (*s.s.*), while strains Talamanca B and C represent different lineages. Considering the diversity of this complex in South American *Ixodes* spp., our results indicate that *B. burgdorferi* (*s.l.*) complex may be widely distributed in Talamanca Mountains. Since *B. burgdorferi* (*s.l.*) complex includes more than 20 species of pathogens and endosymbionts (Carvalho et al., 2020), our findings do not necessarily indicate a risk to public health,

even though *Ixodes* cf. *boliviensis* is a synanthropic and anthropophilic tick in the highlands of Panama (Bermúdez et al., 2016, 2018).

Babesia odocoilei is a parasite of deer in North America and has been related to *Ixodes scapularis* (see Milnes et al., 2019). Criado-Fornelio et al. (2003) pointed out that *B. odocoilei* belongs to the "true" *Babesia* group, along with *Babesia canis*, *Babesia gibsoni* and *Babesia divergens*, and Vannier and Krausse (2009) indicated that *Babesia venatorum* is a species closely related to *B. odocoilei.*

According to Smith (1996), the genus *Hepatozoon* includes more than 300 species. Reports for *Hepatozoon* spp. from Panama include *Hepatozoon muris* and *Hepatozoon procyonis*, diagnosed by microscopy of blood samples from rats and raccoons, respectively (Schneider, 1968), and an undescribed *Hepatozoon* sp. from a blood sample form the pit viper *Bothrops asper* (see Quintero et al., 2021). Phylogenetic analyzes indicate that the *Hepatozoon* strain Chiriquensis detected here represents a putative new species.

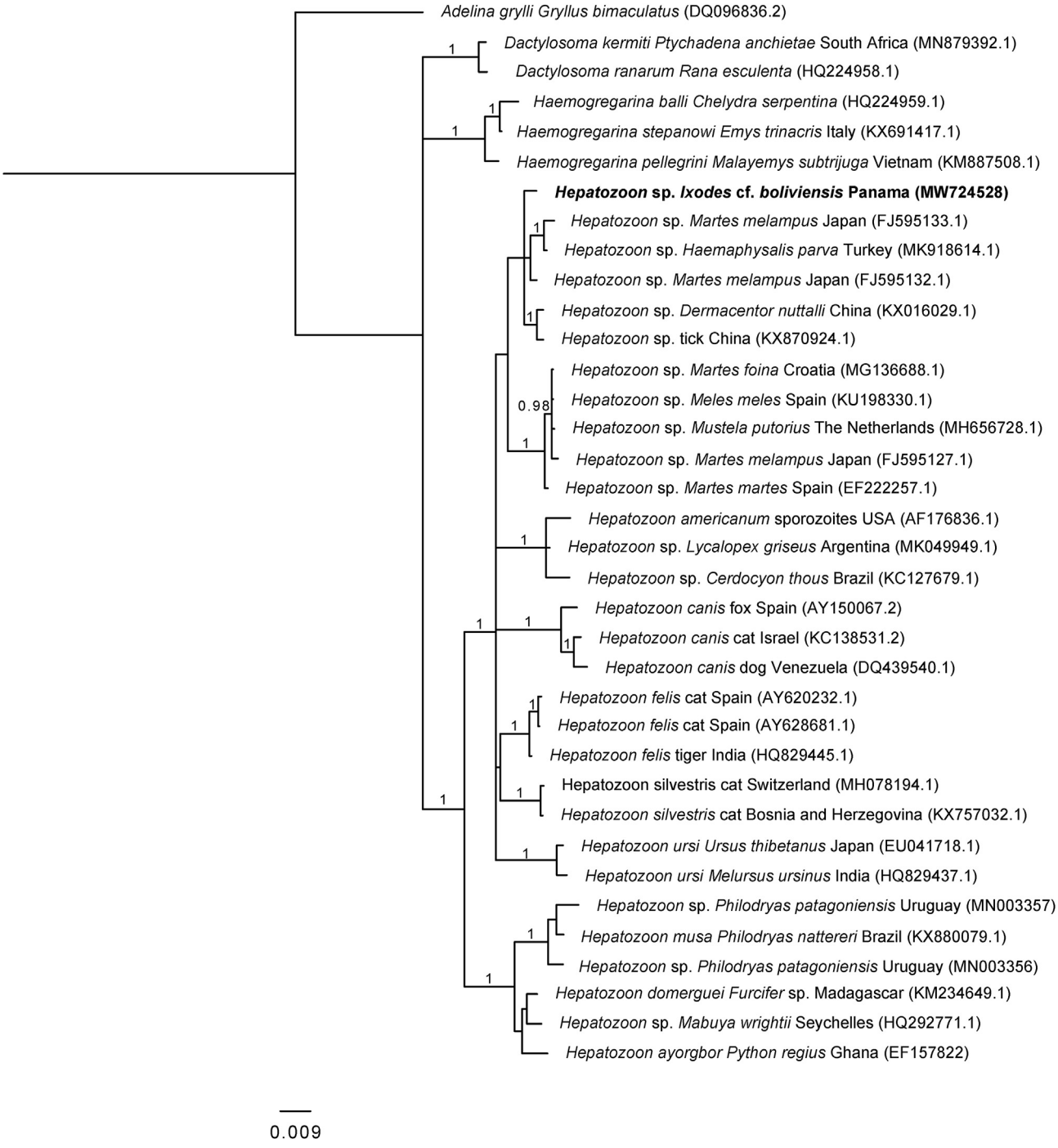

Fig. 5. Bayesian phylogenetic tree for *Hepatozoon* spp. based on the *18S* rRNA gene. Bayesian posterior probabilities are shown at the nodes (only values > 0.95 are shown). *Adelina grylli* was used as the outgroup. The newly generated sequence is indicated in bold

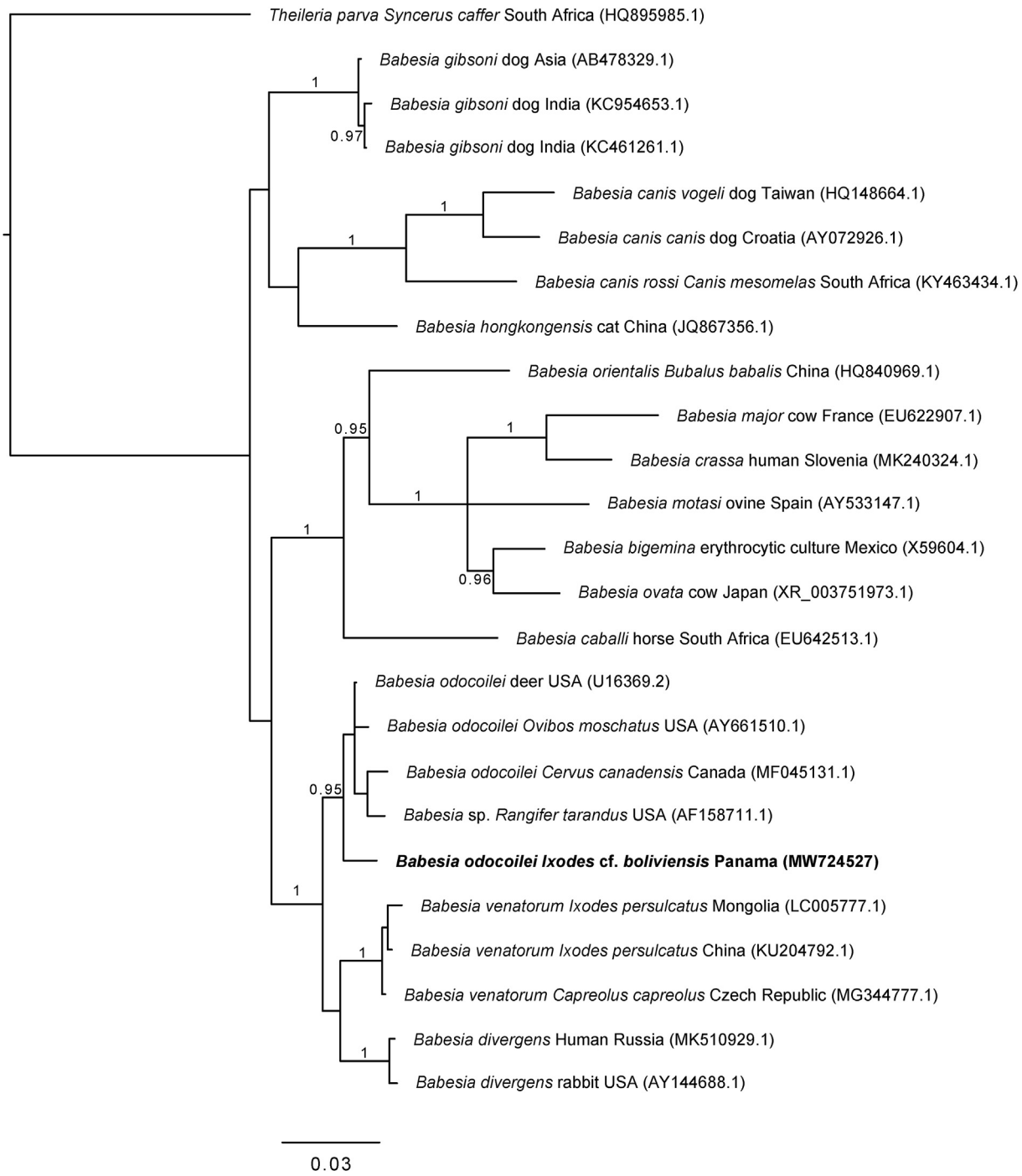

Fig. 6. Bayesian phylogenetic trees for *Babesia* based on the *18S* rRNA gene. Bayesian posterior probabilities are shown at the nodes (only values > 0.95 are shown). *Theileria parva* was used as the outgroup. The newly generated sequence is indicated in bold

5. Conclusions

In comparison to other genera of the family Ixodidae, such as *Amblyomma* or *Rhipicephalus*, there are few studies of *Ixodes* spp. from Panama and Central America (Bermúdez et al., 2016; Bermúdez & Troyo, 2018). Our results present novel data for different microorganisms associated with two species of *Ixodes*, showing the importance that these ticks may have in enzootic cycles for pathogens such as *A. phagocytophilum*, but also in the maintenance of species whose pathogenic potential remains unknown, such as *Rickettsia* spp. strains IbR/CRC and *Rickettsia* sp. strain Itapirus LQ, *Borrelia* sp. strains Talamanca, *B. odocoilei*, and *Hepatozoon* sp. strain Chiriquensis. Therefore, further studies are necessary to demonstrate the ecology of these microorganisms in the Talamanca Mountains range.

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CRediT author statement

SEBC and JMV conceived the study. SEBC, LD, NK and JMV collected ticks in the field. SEBC and LD identified ticks. MLF, JMV and SEBC performed the laboratory work and molecular analyses. SM-L and JMV performed phylogenetic analyses. SEBC and JMV drafted the manuscript. All authors contributed to reviewing the manuscript, and read and approved the final version.

Data availability

Partial gene sequences generated in this study are deposited in the GenBank database: *Rickettsia* sp. strain IbR/CRC (*gltA*: MW699688-MW699694; *ompA*: MW699697-MW699700, MW731468, MW731469; *ompB*: MW699702, MW699703, MW699706-MW699709); *Rickettsia* sp. strain Itapirus LQ (*gltA*: MW699694, MW699695; *ompA*: MW699696, MW699701; *ompB*: MW699704, MW699705); *A. phagocytophilum* (*16S* rDNA: MW677507, MW677508; *groEl*: MW699686, MW699687); *B. burgdorferi* (*s.l.*) complex Talamanca A (MW699712, MW699713); *B. burgdorferi* (*s.l.*) complex Talamanca B (MW699714); *B. burgdorferi* (*s.l.*) complex Talamanca C (MW699711); *B. odocoilei* (MW724527); and *Hepatozoon* sp. strain Chiriquensis (MW724528).

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Anonymous, 2007. Informe de la biodiversidad del Parque Internacional La Amistad Costa Rica Panamá. https://biotA.wordpress.com/2007/08/12/informe-de-la-biodiversidad-del-parque-internacional-la-amistad-costa-rica-panama/. (Accessed 24 July 2020).

Anonymous, 2012. Vegetación y flora del Parque Nacional Volcán Barú. https://biotA.wordpress.com/2012/01/11/vegetacion-y-flora-del-parque-nacional-volcan-baru/. (Accessed 24 July 2020).

Anonymous, 2021. http://imed.med.ucm.es/Tools/sias.html. (Accessed 6 March 2021).

Apanaskevich, D., Domínguez, L., Torres, S., Bernal, J., Montenegro, V., Bermúdez, S., 2017. First description of the male and redescription of the female of *Ixodes tapirus* Kohls, 1956 (Acari: Ixodidae), a parasite of tapirs (Perissodactyla: Tapiridae) from the mountains of Colombia, Costa Rica and Panama. Syst. Parasitol. 94, 413–422. https://doi.org/10.1007/s11230-017-9706-8.

Barbieri, A.M., Venzal, J., Marcili, A., Almeida, A., González, E., Labruna, M., 2013. *Borrelia burgdorferi sensu lato* infecting ticks of the *Ixodes ricinus* complex in Uruguay: first report for the Southern Hemisphere. Vector Borne Zoonotic Dis. 13, 147–153. https://doi.org/10.1089/vbz.2012.1102.

Barbour, A., Maupin, G., Teltow, G., Carter, C., Piesman, J., 1996. Identification of an uncultivable *Borrelia* species in the hard tick *Amblyomma americanum*: possible agent of a Lyme disease-like illness. J. Infect. Dis. 173, 403–409. https://doi.org/10.1093/infdis/173.2.403.

Bermudez, S., Troyo, A., 2018. A review of the genus *Rickettsia* in Central America. Res. Rep. Trop. Med. 29, 103–112.

Bermúdez, S., Castro, A., Trejos, T., García, G., Gabster, A., Miranda, R., et al., 2016. Distribution of spotted fever group rickettsiae in hard ticks (Ixodida: Ixodidae) from Panamanian urban and rural environments. EcoHealth 13, 274–284. http://link.springer.com/article/10.1007/s10393-016-1118-8.

Bermúdez, S., Apanaskevich, D., Domínguez, L., Guglielmone, A. (Eds.), 2018. Garrapatas Ixodidae de Panamá. Biblioteca Nacional, City of Panama, Panama, ISBN 978-9962-699-25-5. http://www.gorgas.gob.pa/Documentos/Garrapatas%20Ixodidae%20de%20Panama.pdf.

Bermúdez, S., Martínez-Mandiche, J., Domínguez, L., González, C., Chavarría, O., Moreno, A., et al., 2021. Diversity of spotted-fever group rickettsiae in ticks collected from wild animals in Panama. Ticks Tick Borne Dis. 12, 101723. https://doi.org/10.1016/j.ttbdis.2021.101723.

Blair, P., Jiang, J., Schoeler, G., Moron, C., Anaya, E., Cespedes, M., et al., 2004. Characterization of spotted fever group *Rickettsia*e in flea and tick specimens from northern Peru. J. Clin. Microbiol. 42, 4961–4967. https://doi.org/10.1128/JCM.42.11.4961-4967.2004.

Brouqui, P., Matzumoto, K., 2007. Bacteriology and phylogeny of *Anaplasmataceae*. Anaplasmosis in humans. In: Raoult, D., Parola, P. (Eds.), Rickettsial Diseases. Informa Healthcare, New York, pp. 179–198.

CDC, 2018. Tick-Borne Diseases of the United States. A Reference Manual for Healthcare Providers, 5th ed. https://www.cdc.gov/ticks/tickbornediseases/TickborneDiseases-P.pdf.

Carvalho, L., Maya, L., Armua-Fernandez, M., Félix, M., Bazzano, V., Barbieri, A., et al., 2020. *Borrelia burgdorferi sensu lato* infecting *Ixodes auritulus* ticks in Uruguay. Exp. Appl. Acarol. 80, 109–125. https://doi.org/10.1007/s10493-019-00435-8.

Choi, Y., Jang, W., Kim, J., Ryu, J., Lee, S., Park, K., et al., 2005. Spotted fever group and typhus group rickettsioses in humans, South Korea. Emerg. Infect. Dis. 11, 237–444.

Cicuttin, G., De Salvo, M., Venzal, J., Nava, S., 2019. *Borrelia* spp. in ticks and birds from a protected urban area in Buenos Aires city, Argentina. Ticks Tick Borne Dis. 10, 101282.

Criado-Fornelio, A., Martinez-Marcos, A., Buling-Saraña, A., Barba-Carretero, J.C., 2003. Molecular studies on *Babesia, Theileria* and *Hepatozoon* in southern Europe. Vet. Parasitol. 113, 189–201. https://doi.org/10.1016/s0304-4017(03)00078-5.

Cyr, T., Jenkins, M., Hall, R., Masters, E., McDonald, G., 2005. Improving the specificity of *16S* rDNA-based polymerase chain reaction for detecting *Borrelia burgdorferi sensu lato* - causative agents of human Lyme disease. J. Appl. Microbiol. 98, 962–970. https://doi.org/10.1111/j.1365-2672.2005.02539.x.

Dall'Agnol, B., Michel, T., Weck, B., Souza, U., Webster, A., Leal, B., et al., 2017. *Borrelia burgdorferi sensu lato* in *Ixodes longiscutatus* ticks from Brazilian Pampa. Ticks Tick Borne Dis. 8, 928–932. https://doi.org/10.1016/j.ttbdis.2017.08.003.

Doyle, C., Labruna, M., Breitschwerdt, E., Tang, Y., Corstvet, R., Hegarty, B., et al., 2005. Detection of medically important *Ehrlichia* by quantitative multicolor TaqMan real-time polymerase chain reaction of the *dsb* gene. J. Mol. Diagn. 7, 504–510.

Fairchild, G., Kohls, G., Tipton, J., 1966. The ticks of Panama (Acarina: Ixodoidea). In: Wenzel, R.L., Tipton, V.J. (Eds.), Ectoparasites of Panama. Field Museum of Natural History, Chicago, USA, pp. 167–207.

Félix, M., Armúa-Fernández, M., Parodi, P., Bazzano, V., Mangold, A., Venzal, J., 2020. Detection of a putative novel genotype of *Anaplasma* in gray-brocket deer (*Mazama gouazoubira*) from Uruguay. Exp. Appl. Acarol. 81, 575–583. https://doi.org/10.1007/s10493-020-00523-0.

Flores, F., Muñoz-Leal, S., Diaz, A., Labruna, M., 2018. Wild birds as host of *Borrelia burgdorferi sensu lato* in northwestern Argentina. Ticks Tick Borne Dis. 9, 1586–1589. https://doi.org/10.1016/j.ttbdis.2018.08.005.

Fournier, P.E., Raoult, D., 2009. Current knowledge on phylogeny and taxonomy of *Rickettsia* spp. Ann. N. Y. Acad. Sci. 1166, 1–11. https://doi.org/10.1111/j.1749-6632.2009.04528.x.

Fournier, P.E., Dumler, J.S., Greub, G., Zhang, J., Wu, Y., Raoult, D., 2003. Gene sequence-based criteria for identification of new rickettsia isolates and description of *Rickettsia heilongjiangensis* sp. nov. J. Clin. Microbiol. 41, 5456–5465. https://doi.org/10.1128/JCM.41.12.5456-5465.2003.

Grzeszczuk, A., Barat, N., Bakken, J., Dumler, S., 2007. Anaplasmosis in humans. In: Raoult, D., Parola, P. (Eds.), Rickettsial Diseases. Informa Healthcare, New York, USA, pp. 223–236.

Guglielmone, A., Robbins, R., 2018. *Hard Ticks (Acari: Ixodida: Ixodidae) Parasitizing Humans. A Global Review*. Springer Nature, New York, USA.

Guglielmone, A., Petney, T., Robbins, R., 2019. Ixodidae (Acari: Ixodoidea): descriptions and re-descriptions of all known species from 1758 to December 31, 2019. Zootaxa 4871, 1.1.

Huelsenbeck, J., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755. https://doi.org/10.1093/bioinformatics/17.8.754.

Inokuma, H., Parola, P., Raoult, D., Brouqui, P., 2001. Molecular survey of *Ehrlichia* infection in ticks from animals in Yamaguchi Prefecture, Japan. Vet. Parasitol. 99, 335–339.

Ivanova, L.B., Tomova, A., González-Acuña, D., Murúa, R., Moreno, C.X., Hernández, C., et al., 2014. *Borrelia chilensis*, a new member of the *Borrelia burgdorferi sensu lato* complex that extends the range of this genospecies in the Southern Hemisphere. Environ. Microbiol. 16, 1069–1080. https://doi.org/10.1111/1462-2920.12310.

Katoh, K., Misawa, K., Kima, K., Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30, 3059–3066. https://doi.org/10.1093/nar/gkf436.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., Rubel, F., 2006. World map of the Köppen-Geiger climate classification updated. Meteorol. Z. 15, 259–263. https://doi.org/10.1127/0941-2948/2006/0130.

Kurtti, T., Felsheim, R., Burkhardt, N., Oliver, J., Heu, C., Munderloh, U., 2015. *Rickettsia buchneri* sp. nov., a *Rickettsia*l endosymbiont of the blacklegged tick *Ixodes scapularis*. Int. J. Syst. Evol. Microbiol. 65, 965–970.

Labruna, M., Whitworth, T., Horta, M., Bouyer, D., McBride, J.W., Pinter, A., et al., 2004. *Rickettsia* species infecting *Amblyomma cooperi* ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. J. Clin. Microbiol. 42, 90–98. https://doi.org/10.1128/JCM.42.1.90-98.2004.

Lopes, M., May, J., Foster, R., Harmsen, B., Sánchez, E., Martins, T., et al., 2016. Ticks and rickettsiae from wildlife in Belize, Central America. Parasit. Vectors 9, 62.

Lotric-Furlan, S., Petrovec, M., Zupanc, T.A., Nicholson, W.L., Sumner, J.W., Childs, J.E., Strle, F., 1998. Human granulocytic ehrlichiosis in Europe: clinical and laboratory

findings for four patients from Slovenia. Clin. Infect. Dis. 27, 424–428 https://doi.org/10.1086/51468 3.

Mangold, A.J., Bargues, M.D., Mas-Coma, S., 1998. Mitochondrial *16S* rDNA sequences and phylogenetic relationships of species of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). Parasitol. Res. 84, 478–484. https://doi.org/10.1007/s004360050433.

Melis, M., Sebastian, P., Balcazar, D., Lareschi, M., Nava, S., 2020. Molecular detection of *Rickettsia bellii* in *Ixodes loricatus* (Acari: Ixodidae) ticks associated with rodents from Buenos Aires province, Argentina. Rev. Soc. Entomol. Argentina 79, 51–55.

Milnes, E.L., Thornton, G., Léveillé, A.N., Delnatte, P., Barta, J.R., Smith, D.A., Nemeth, N., 2019. *Babesia odocoilei* and zoonotic pathogens identified from *Ixodes scapularis* ticks in southern Ontario, Canada. Ticks Tick Borne Dis. 10, 670–676. https://doi.org/10.1016/j.ttbdis.2019.02.016.

Montenegro, V.M., Bonilla, M.C., Kaminsky, D., Romero-Zúñiga, J., Siebert, S., Krämer, F., 2017. Serological detection of antibodies to *Anaplasma* spp., *Borrelia burgdorferi sensu lato* and *Ehrlichia canis* and of *Dirofilaria immitis* antigen in dogs from Costa Rica. Vet. Parasitol. 236, 97–107. https://doi.org/10.1016/j.vetpar.2017.02.009.

Muñoz-Leal, S., Lopes, M.G., Marcili, A., Martins, T.F., González-Acuña, D., Labruna, M., 2019. *Anaplasmataceae, Borrelia* and *Hepatozoon* agents in ticks (Acari: Argasidae, Ixodidae) from Chile. Acta Trop. 192 https://doi.org/10.1016/j.actatropica.2019.02.002.

Muñoz-Leal, S., Ramirez, D.G., Luz, H.R., Faccini, J.L.H., Labruna, M.B., 2020. "*Candidatus* Borrelia ibitipoquensis", a *Borrelia valaisiana*-related genospecies characterized from *Ixodes paranaensis* in Brazil. Microb. Ecol. 80, 682–689. https://doi.org/10.1007/s00248-020-01512-x.

Ogrzewalska, M., Literak, I., Capek, M., Sychra, O., Calderon, V.A., Rodriguez, B.C., et al., 2015. Bacteria of the genus *Rickettsia* in ticks (Acari: Ixodidae) collected from birds in Costa Rica. Ticks Tick Borne Dis. 6, 478–482.

Ojeda-Chi, M.M., Rodriguez-Vivas, R.I., Esteve-Gasent, M.D., Pérez de León, A., Modarelli, J.J., Villegas-Perez, S., 2019. Molecular detection of rickettsial tick-borne agents in white-tailed deer (*Odocoileus virginianus yucatanensis*), mazama deer (*Mazama temama*), and the ticks they host in Yucatan, Mexico. Ticks Tick Borne Dis. 10, 365–370. https://doi.org/10.1016/j.ttbdis.2018.11.018.

Pesquera, C., Portillo, A., Palomar, A.M., Oteo, J., 2015. Investigation of tick-borne bacteria (*Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Borrelia* spp.) in ticks collected from Andean tapirs, cattle and vegetation from a protected area in Ecuador. Parasit. Vectors 24, 46. https://doi.org/10.1186/s13071-015-0662-3.

Polsomboon, S., Hoel, D.F., Murphy, J.R., Linton, Y., Motoki, M., Robbins, R., et al., 2017. Molecular detection and identification of *Rickettsia* species in ticks (Acari: Ixodidae) collected from Belize, Central America. J. Med. Entomol. 54, 1718–1726.

Quintero, J.C., Félix, M.L., Venzal, J.M., Nava, S., 2020. *Ixodes tropicalis* (Acari: Ixodidae) infestando un humano y detección molecular de *Rickettsia belli*, Colombia. Biomedica 41. https://doi.org/10.7705/biomedica.5464 (in press).

Quintero, P.I., Ríos-Carrera, N., Mack, P., Martínez, V., 2021. Endoparásitos en cuatro especies de vipéridos provenientes de la concesión minera "Cobre Panamá". Tecnociencias 23, 160–179.

Roux, V., Fournier, P., Raoult, D., 1996. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. J. Clin. Microbiol. 34, 2058–2065. https://doi.org/10.1128/jcm.34.9.2058-2065.1996.

Santos, H.A., Thomé, S.M., Baldani, C.D., Silva, C.B., Peixoto, M.P., Pires, M.S., et al., 2013. Molecular epidemiology of the emerging zoonosis agent *Anaplasma phagocytophilum* (Foggie, 1949) in dogs and ixodid ticks in Brazil. Parasit. Vectors 6, 348 https://doI.org/10.1186/1756-3305-6-348.

Saracho Bottero, M.N., Sebastian, P.S., Carvalho, L.A., Claps, L.G., Mastropaolo, M., Mangold, A.J., et al., 2017. Presence of *Borrelia* in different populations of *Ixodes pararicinus* from northwestern Argentina. Ticks Tick Borne Dis. 8, 488–493. https://doi.org/10.1016/j.ttbdis.2017.02.008.

Saracho-Bottero, M.N.S., Beati, L., Venzal, J.M., Guardia, L., Thompson, C.S., Mangold, A.J., et al., 2021. *Ixodes silvanus* n. sp. (Acari: Ixodidae), a new member of the subgenus *Trichotoixodes* Reznik, 1961 from northwestern Argentina. Ticks Tick Borne Dis. 12, 101572. https://doi.org/10.1016/j.ttbdis.2020.101572.

Schneider, C., 1968. *Hepatozoon procyonis* Richards, 1961, in a Panamanian raccoon, *Procyon cancrivorus panamensis* (Goldman). Rev. Biol. Trop. 15, 123–135.

Sebastian, P.S., Bottero, M.N.S., Carvalho, L., Mackenstedt, U., Lareschi, M., Venzal, J.M., Nava, S., 2016. *Borrelia burgdorferi sensu lato* in *Ixodes* cf. *neuquenensis* and *Ixodes sigelos* ticks from the Patagonian region of Argentina. Acta Trop. 162, 218–221. https://doi.org/10.1016/j.actatropicA.2016.06.030.

Sebastian, P.S., Flores, F.S., Saracho-Bottero, M.N., Tarragona, E.L., Venzal, J.M., Nava, S., 2020. Molecular detection of rickettsial bacteria in ticks of the genus *Ixodes* from the southern cone of America. Acta Trop. 210, 105588. https://doi.org/10.1016/j.actatropicA.2020.105588.

Smith, T., 1996. The genus *Hepatozoon* (Apicomplexa: Adeleina). J. Parasitol. 82, 565–585. https://doi.org/10.2307/3283781.

Soares, H.S., Marcili, A., Barbieri, A.R.M., Minervino, A.H.H., Moreira, T.R., Gennari, S.M., et al., 2017. Novel piroplasmid and *Hepatozoon* organisms infecting the wildlife of two regions of the Brazilian Amazon. Int. J. Parasitol. Parasites Wildl. 26, 115–121. https://doi.org/10.1016/j.ijppaw.2017.05.002.

Socolovschi, C., Medinnikov, O., Raoult, D., Parola, P., 2009. The relationship between spotted fever group rickettsiae and ixodid ticks. Vet. Res. 40, 34. https://doi.org/10.1051/vetres/2009017.

Springer, A., Montenegro, V.M., Schicht, S., Wölfel, S., Schaper, S.R., Chitimia-Dobler, L., et al., 2018. Detection of *Rickettsia monacensis* and *Rickettsia amblyommatis* in ticks collected from dogs in Costa Rica and Nicaragua. Ticks Tick Borne Dis. 9, 1565–1572. https://doi.org/10.1016/j.ttbdis.2018.08.002.

Troyo, A., Moreira-Soto, A., Carranza, M., Calderón-Arguedas, O., Hun, L., Taylor, L., 2014. Detection of an undescribed *Rickettsia* sp. in *Ixodes boliviensis* from Costa Rica. Ticks Tick Borne Dis. 5, 883–886.

Vannier, E., Krause, P.J., 2009. Update on babesiosis. Interdiscip. Perspect. Infect. Dis. 984568. https://doi.org/10.1155/2009/984568, 2009.

Verdugo, C., Jiménez, O., Hernández, C., Álvarez, P., Espinoza, A., González-Acuña, D., 2017. Infection with *Borrelia chilensis* in *Ixodes stilesi* ticks collected from *Pudu puda* deer. Ticks Tick Borne Dis. 8, 733–740. https://doi.org/10.1016/j.ttbdis.2017.05.007.

Villalobos-Zúñiga, M., Somogyi, T., 2012. Enfermedad de Lyme aguda en Costa Rica. Descripción del primer caso autóctono. Acta Med. Costarric. 54, 55–58.