

Spotted fever group *Rickettsia* and *Babesia odocoilei* in hard ticks (Acari: Ixodidae) from wild areas in Panama

Sergio BERMUDEZ CASTILLERO^{1,2,4} , María FÉLIX¹ , Lillian DOMÍNGUEZ¹ , José VENZAL³ 

¹Medical Entomology Research Department, Gorgas Memorial Institute for Health Research, Panama City, Panama

²PEDECIBA - Programa de Desarrollo de las Ciencias Básicas, Uruguay

³Laboratorio de Vectores y Enfermedades Transmitidas, Departamento de Ciencias Biológicas, CENUR Litoral Norte, Universidad de la República, Salto, Uruguay

⁴Corresponding author: sbermudez@gorgas.gob.pa

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ABSTRACT: This study evaluated the presence of *Rickettsia*, *Anaplasmataceae*, *Borrelia* and *Piroplasmida* in *Amblyomma* and *Ixodes* spp. ticks from wild areas of Panama. Molecular detection of pathogens was performed on the following species of ticks: *Amblyomma dissimile*, *Amblyomma mixtum*, *Amblyomma naponense*, *Amblyomma* cf. *oblongoguttatum*, *Amblyomma ovale*, *Amblyomma tapirellum*, *Haemaphysalis juxtakochi*, *Ixodes affinis*, and *Ixodes* sp. nymphs. Partial sequences of *Ixodes* nymphs with the 16S rRNA gene showed low similarity to other *Ixodes* species. We found evidence of DNA from *Rickettsia* spp. in *A. mixtum* (*R. amblyommatis*), *A. dissimile* ("Candidatus *R. colombianensi*"), *I. affinis* (*Rickettsia* sp.), and *Babesia odocoilei* in *I. affinis*. No evidence of DNA from the target microorganisms was detected in *A. naponense*, *A. cf. oblongoguttatum*, *A. ovale*, *A. tapirellum*, *H. juxtakochi*, or *Ixodes* sp. This is the first report of *B. odocoilei* in *I. affinis*, which represents an additional risk of zoonosis. The detection of *Rickettsia* spp. corroborates previous findings in Panama. Finally, this study was crucial to determine a putative new species of *Ixodes*, from two nymph feeding on human.

Keywords: Ixodidae, *Rickettsia* spp., *Babesia odocoilei*, wild environments, Panama

Zoobank: <http://zoobank.org/786671EA-A364-4F12-B0BE-B9AE1A152764>

INTRODUCTION

Tick-borne pathogens (TBP) include a wide range of bacteria, parasites, and viruses that can affect animals and humans and, depending to the pathogen, causing symptoms that can be mild, moderate, severe, or fatal (de la Fuente et al., 2023). Worldwide, the increase of tick-borne diseases (TBD) represents one of the main concerns in public health related to vectors, in particular in the northern hemisphere (Dixon et al., 2021; de la Fuente et al., 2023; Eisen and Eisen, 2023; Osikowicz et al., 2024). In some equatorial and Southern Hemisphere countries, TBD are still considered neglected diseases and are not included in the differential diagnosis, occurring at silent cases (Charles et al., 2021; de la Fuente et al., 2023; Foley et al., 2025). Consequently, in recent decades various investigations have been developed in several countries around the world, aimed at evaluating and monitoring potential TBPs that may be emerging agents in wild and anthropogenic environments.

In Central America, despite its limited geographical area, there is a considerable amount of information on TBP and TBD, which has been increasing in recent decades. In this sense, since the early 20th century there have been reports of cases of relapsing fevers (*Borrelia recurrentis* group, BRG), spotted fevers (mainly caused by *Rickettsia rickettsii*), and recently a suspected case of canine ehrlichiosis in humans (Daza et al., 2018; Bermúdez and Troyo, 2018). Anaplasmosis (*Anaplasma platys*, *Anaplasma marginale*), ehrlichiosis (*Ehrlichia canis*), babesiosis, and hepatozoonosis, are also known TBPs that affect domestic animals (Rojas et al., 2014; Santamaria et al., 2014). Surveillance studies have also detected pathogens in ticks, which have not

yet been associated with clinical cases in humans or animals, such as *Rickettsia parkeri*, *Ehrlichia* spp. (*E. ci. chaffeensis*, *E. ci. ewingii*, *E. minasensis*), *Anaplasma* spp. (*A. marginale*, *A. phagocytophilum*), and *Babesia* (*B. odocoilei*) (Eremeeva et al., 2009; Charles et al., 2021; Bermúdez et al., 2024a,b). Finally, other microorganisms associated with ticks have been reported but so far have not been recognized as pathogens, including more than 15 *Rickettsia* species, BRG (*Borrelia puertoricensis*), *Borrelia burgdorferi* group, and hemoparasites (*Hepatozoon* spp.) (Bermúdez and Troyo, 2018; de la Fuente et al., 2023; Bermúdez et al., 2021a,b; Romero et al., 2025). Some of these microorganisms are the product of recent research in wild areas of Central America, which have a high diversity of fauna and are widely used for ecotourism or research purposes.

Understanding the circulating TBPs is a first step towards recognizing potential new pathogens in these areas. To better understand the potential risk of TBPs in forested areas of Panama, we assessed the presence of *Borrelia*, *Rickettsia*, *Anaplasma*, *Ehrlichia* and hemoparasites in ticks collected from five sites.

MATERIALS AND METHODS

Study sites, tick collection and identification

From March 2020 to March 2022, free-living ticks were collected using a white flag in wild areas of Panama: Panama City (Summit Municipal Park), Colón (Gamboa and Soberania Nacional Park), and Darién (Natural Private Reserve Cerro Chucantí and Darién National Park) (Table 1, Fig. 1). Additionally, ticks opportunistically removed from

Table 1. Environmental characterization of the sites of collection.

SITE (Province)	DESCRIPTION
Summit Municipal Park (Panama)	Botanical garden and rescue center of wildlife, with ≈ 2.5 Km ² of secondary forest and grasslands. This area corresponds to the transition of dry and wet tropical forest, with temperatures 20-36° C.
Soberania National Park (Colon)	This park with ≈ 220 KM ² of a primary and secondary wet tropical forest and shrubs in the borders.
La Laguna-Gamboia (Colon)	This trail corresponds to a forested patch of secondary and primary forest.
Natural Private Reserve Cerro Chucanti (Darien)	This reserve has ≈ 240 Km ² among 600-1480 meters of elevation and presents primary and secondary wet forests and cloud forests.
Darien National Park (Darien)	This park with ≈ 5790 KM ² of a wet tropical forest.

humans during collections and a green iguana (*Iguana iguana*) were also included. Ticks were preserved in 95% ethanol and the adults were identified using Bermúdez et al. (2018). Nymphs of *Amblyomma* and *Ixodes* sp. ticks were identified by molecular methods (PCR and sequencing) as described below.

DNA extraction and molecular analysis

Individual ticks were bisected longitudinally using sterile scalpels and washed with distilled water to remove residual ethanol. DNA was extracted using the commercial kit GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania) following manufacturer instructions. To confirm nymph tick species, ticks were analyzed through PCR amplification of a ~ 460 base pair (bp) fragment of the tick mitochondrial 16S rRNA gene (Mangold et al., 1998).

For the detection of microorganisms, extracted DNA was tested by a battery of PCR protocols targeting *Rickettsia*, *Anaplasmatidae* family organisms, *Borrelia*, *Babesia* and *Hepatozoon* spp., using specific primers and published protocols for each agent (Table 2). Molecular grade water was used as negative control in all PCR reactions. Positive controls used included *Rickettsia parkeri* strain Toledo, *Ehrlichia canis* isolate P1091, *Borrelia anserina* PL, *Babesia bovis* Paysandú, *Hepatozoon* sp. (Z78S, P1244), and *Ixodes fuscipes* S18IpH1. Five microliters of PCR products were separated by electrophoresis in a 1.5% agarose gel, stained with GoodView TM Nucleic Acid Stain (Beijing SBS Genetech Co., Ltd), and examined under UV transillumination. Amplicons of expected size were purified using GeneJET PCR purification kit and Sanger sequenced (Macrogen, Korea).

Analyses of sequences and phylogenies

Sequences were assembled and trimmed in Geneious software (Kearse et al., 2012). Consensus sequences were submitted to BLASTn analyses to compare with sequences available in NCBI GenBank. An alignment of our sequences and GenBank-retrieved homologues was performed for each microorganism with MAFFT. The phylogeny was constructed using Maximum Likelihood algorithm provided by MEGA7 and Tamura 3 software with gamma distribution selected (Kumar et al., 2016). The support of the internal branching was assessed using 1000 bootstrap replicates.

RESULTS

One hundred fifty ticks of nine species were analyzed during this study. These species correspond to adults of *Amblyomma dissimile* (n=7), *Amblyomma mixtum* (n=21 adults), *Amblyomma naponense* (n=21), *Amblyomma* cf. *oblongoguttatum* (n=36), *Amblyomma ovale* (n=5), *Amblyomma tapirellum* (n=4), *Haemaphysalis juxtakochi* (n=48), *Ixodes affinis* (n=6), and nymphs of *Ixodes* sp. (n=2). The checklist of the ticks according to the localities and source are presented in Table 3. For nymphal ticks partial 16S rDNA sequences were submitted to BLAST and obtained 89.5% similarity to *Ixodes montoyanus* (OQ557628) and 90.4% similarity to *Ixodes tropicalis* (MT158325). This sequence is available in GenBank under access OQ883908.

In total, of 124 ticks analyzed, DNA sequences of target microorganisms were detected in 26 ticks. *Rickettsia* DNA was detected in *A. mixtum*, *A. dissimile*, and *I. affinis*. Of these, *Rickettsia amblyommatis* was sequenced from all *A. mixtum* with 100% identity to the sequences available in GenBank 99% to *ompA* gene (MN947671). "*Candidatus Rickettsia colombianensi*" was sequenced from 4/7 *A. dissimile* with 99% identity to sequences deposited to the *gltA* gene (ON159982). DNA was obtained from 5/6 *I. affinis* 100% similar to the *ompA* gene of *Rickettsia* sp. (MN947680). DNA of *Babesia odocoilei* was detected in one *I. affinis*, which showed 98% similarity to the *B. odocoilei* GenBank reference to 18S rRNA (MF357057), and 93% similarity to the *B. odocoilei* GenBank reference to COI (ON995393). Both 18S rRNA and cytochrome c oxidase subunit I (COI) phylogenetic analysis grouped these sequences within *B. odocoilei* from Panamanian *Ixodes* cf. *boliviensis* clade (Figs 2, 3).

Partial gene sequences generated in this study are deposited in the GenBank database: *R. amblyommatis* (*ompA*: PV419602), *Rickettsia* sp. endosymbiont *I. affinis* (*ompA*: submitted), "*Ca. R. colombianensi*" (*gltA*: submitted), *Rickettsia* sp. (*ompA*: PV419602), *B. odocoilei* (18S rRNA: PV059300; COI: PV387806).

No DNA of *Ehrlichia*, *Anaplasma*, *Borrelia* and *Hepatozoon* were detected in *A. mixtum*, *A. dissimile*, and *I. affinis*. No DNA of target microorganisms was evidenced in *A. naponense*, *A. cf. oblongoguttatum*, *A. ovale*, *A. tapirellum*, *H. juxtakochi*, and *Ixodes* sp.

Table 2. List of primers for PCR used in the present study.

Target	Genes	Primer name: sequence (5'–3')	Lenght (pb)	Reference
Tick (mitochondrial)	16S rRNA	16S+1: CCGGTCTGAACTCAGATCAAG 16S-1: GCTCAATGATTTTTTAAATTGCTG	460	Mangold et al. (1998)
<i>Rickettsia</i> spp.	<i>gltA</i>	CS-78: GCAAGTATCGGTGAGGATGTAAT CS323: GCTTCCTTAAAATTCAATAAATCAGGAT CS-239: GCTCTTCTCATCTATGGCTATTAT CS-1069: CAGGGTCTTCGTGCATTTCTT	401 834	Labruna et al. (2004)
<i>Rickettsia</i> SFG	<i>ompA</i>	Rr190.70p*: ATGGCGAATATTTCTCCAAAA Rr190.701n: AGTGCAGCATTCGCTCCCCCT Rr190.602n: AGTGCAGCATTCGCTCCCCCT	631 532	Regnery et al. (1991)
<i>Rickettsia</i> spp.	<i>ompB</i>	ompB-OF: GTAACCGGAAGTAATCGTTTCGTAA ompB-OR: GCTTTATAACCAGCTAAACCACC ompB SFG IF: GTTTAATACGTGCTGCTAACCAA ompB SFG IR: GGTTTGGCCCATATACCATAAG	511 420	Roux et al. (1996) Choi et al. (2005)
<i>Anaplasmataceae</i>	16S rRNA	EHR16SD**: GGTACCYACAGAAGAAGTCC EHR16SR**: TAGCACTCATCGTTTACAGC fD1: AGAGTTTGATCTGGCTCAG rP2: ACGGCTACCTTGTTACGACTT	345 ~1431	Parola et al. (2000) Weisburg et al. (1991), Inokuma et al. (2001)
<i>Ehrlichia</i> spp.	<i>dsb</i>	Dsb-330: GATGATGTTTGAAGATATSAAACAAAT Dsb-720*: CTATTTTACTTCTTAAAGTTGATAWATC Dsb-380: ATTTTATAGRATTTTCCAATACTTGG	409 349	Doyle et al. (2005) Almeida et al. (2013)
<i>Ehrlichia</i> spp.	<i>groEl</i>	HS1a: AITGGGCTGGTAITGAAAT HS6a: CCICIGGIACIAIACCTTC HS43: ATWGCWAARGAAGCATAGTC HSVR: CTCACAGCAGCTCTAGTAGC	~1400 1297	Sumner et al. (1997), Nicholson et al. (1999) Lotrić-Furlan et al. (1998)
<i>Borrelia</i> spp.	<i>flaB</i>	Fla LL: ACATATTGAGATGCAGACAGAGGT Fla RL: GCAATCATAGCCATTGCAGATTGT FlaLS: AACAGCTGAAGAGCTTGAAT Fla RS: CTTTGATCACTTATCATTCTAATAGC	665 354	Barbour et al. (1996) Barbour et al. (1996)
Piroplasmida	18S rRNA	BAB143-167: CCGTGCTAATTGTAGGGCTAATACA BAB694-667: GCTTGAAACACTCTARTTTTCTCAAAG Nbab_1F: AAGCCATGCATGTCTAAGTATAAGCTTTT 18SApiR: GGATCACTCGATCGGTAGGAG	551 1500	Soraes et al. (2017) Greay et al., (2018)
Piroplasmida	COI	COI-F: GGAAGTGGWACWGGWTGGAC COI-R: TTCGGTATTGCATGCCTTG	1080	Schreeg et al. (2016)
<i>Hepatozoon</i> spp.	18S rRNA	HEM01: TATTGGTTTAAAGAACTAATTTTATGATTG HEM02: CTTCTCCTTCTTTAAGTGATAAGGTTTAC	900	Perkins and Keller (2001)
<i>Hepatozoon</i> spp.	18S rRNA	Hep1-mod: CGCGAAATTACCCAATTCTA Hep4: TAAGGTGCTGAAGGAGTCGTTTAT	670	Spolidorio et al. (2009)

*Primer used in the first and second round, **Primers used in the initial PCR screening.

DISCUSSION

Our study showed three species of *Rickettsia* and *B. odocoilei* in ticks from wild areas in Panama, which increases knowledge of the microorganisms that circulate in ticks in these types of environments. The detection of *R. amblyommatis* infecting *A. mixtum*, "*Ca. R. colombianensi*" in *A. dissimile*, and a *Rickettsia* sp. endosymbiont in *I. affinis* were expected and consistent with previous results in Neotropical ticks (Charles et al., 2021; de la Fuente et al., 2023). *Rickettsia amblyommatis* has a wide distribution in the Americas, and it has been detected in more than 30 species across all genera of Neotropical and has a high prevalence in *A. mixtum* (Bermúdez et al., 2021a; Richardson et al., 2023; Romero et al., 2025). *Amblyomma mixtum* is considered the most anthropophilic species in Panama, found

mainly in grazing areas, secondary humid forests, and to a lesser extent in mature forests (Bermúdez et al., 2018). From a public health perspective, some studies have shown that *R. amblyommatis* can cause mild fevers in humans or animals, and can generate an immune response in animals (Moreira-Soto et al., 2016; Luz et al., 2019; Kmetiuk et al., 2019); furthermore, Rivas et al. (2015) found that *R. amblyommatis* 9-CC-3 strain from Costa Rica caused fever in guinea pigs, but also generated protection in a subsequent experimental infection of *R. rickettsii*. In Panama, *R. amblyommatis* have been reported in seroprevalence studies in humans, domestic and wild animals (Bermúdez et al. 2011, 2013, 2018), which could be related to the high prevalence found in ticks, especially *A. mixtum*.

Table 3. Ticks and microorganisms collected from wild areas in Panama during this study.

Sites	Tick Species	Host/Source	Ticks analyzed/ Total	BLAST match ($\geq 99\%$)	Prevalence (%)
SMP	<i>Amblyomma dissimile</i>	<i>Iguana iguana</i>	3/7	" <i>Ca. Rickettsia colombianensi</i> "	3/3 (100)
G	<i>Amblyomma mixtum</i>	Soil (questing)	17/17	<i>Rickettsia amblyommatis</i>	17/17 (100)
SNP	<i>Amblyomma mixtum</i>	Human	1/1	<i>Rickettsia amblyommatis</i>	1/1 (100)
SNP	<i>Amblyomma mixtum</i>	Soil (questing)	3/3	<i>Rickettsia amblyommatis</i>	3/3 (100)
G	<i>Amblyomma naponense</i>	Soil (questing)	7/21	-	0/7 (0)
G	<i>Amblyomma cf. oblongoguttatum</i>	Soil (questing)	21/21	-	0/21 (0)
SNP	<i>Amblyomma cf. oblongoguttatum</i>	Soil (questing)	15/15	-	0/15 (0)
DNP	<i>Amblyomma ovale</i>	Soil (questing)	3/3	-	0/3 (0)
PND	<i>Amblyomma ovale</i>	Soil (questing)	2/2	-	0/2 (0)
SNP	<i>Amblyomma tapirellum</i>	Soil (questing)	1/1	-	0/1 (0)
DNP	<i>Amblyomma tapirellum</i>	Soil (questing)	3/3	-	0/3 (0)
SNP	<i>Haemaphysalis juxtakochi</i>	Soil (questing)	40/48	-	0/40 (0)
SNP	<i>Ixodes affinis</i>	Soil (questing)	1/1	<i>Rickettsia endosymbiont</i>	1/1 (100)
DNP	<i>Ixodes affinis</i>	Soil (questing)	4/4	<i>Babesia odocoilei</i>	1/4 (25)
G	<i>Ixodes affinis</i>	Soil (questing)	1/1	<i>Rickettsia endosymbiont</i>	1/1 (100)
NPRCC	<i>Ixodes</i> sp.	Human	2/2	-	0/2 (0)

SMP: Summit Municipal Park; SNP: Soberanía National Park; G: Gamboa; NPRCC: Natural Private Reserve Cerro Chucantí; DNP: Darien National Park.

Regarding to "*Ca. R. colombianensi*", this species was initially reported in *A. dissimile*, *Rhipicephalus microplus*, and *Amblyomma cajennense* s.l. from Colombia (Miranda et al., 2012; Miranda and Mattar, 2014; Santodomingo et al., 2018), and was later reported in *A. dissimile* from Honduras (Novakova et al., 2015), Brazil (Luz et al., 2018), Panama (Bermúdez et al., 2021b), Costa Rica (Moreira-Soto et al., 2023). In El Salvador "*Ca. R. colombianensi*" has been reported infecting *A. dissimile* and *Amblyomma scutatum* (Romero et al., 2021). To date, this *Rickettsia* has not been shown to be pathogenic in humans or animals. *Amblyomma dissimile* parasitizes a wide variety of cold-blooded vertebrates, with sporadic reports in birds, mammals, and humans (Guglielmone and Robbins 2018). Although the presence of "*Ca. R. colombianensi*" in *A. dissimile* does not appear to pose a public health risk, its spread in forest areas is important to understand.

The novel *Rickettsia* infecting *I. affinis* may be an endosymbiont. *Rickettsia* endosymbionts can be transmitted vertically and show a high prevalence in tick populations (Socolovschi et al., 2009; Kurtti et al., 2015). Previous to this study, *Rickettsia* endosymbionts were detected in *I. affinis* from from Belize and Panama (Lopes et al., 2016; Polsomboon et al., 2017; Bermúdez et al., 2021b), but also other *Rickettsia* endosymbionts from Central American *Ixodes* spp. include *Rickettsia* sp. strain IbR/CRC in *I. cf. boliviensis* from Costa Rica and Panama (Troyo et al., 2014; Bermúdez et al., 2021a); *Rickettsia* sp. strain Barva in *Ixodes minor* from Costa Rica (Ogrzewalska et al., 2015); *Rickettsia* sp. strain Itapirus LQ in *Ixodes tapirus* (Bermúdez et al., 2021a); *Rickettsia* sp. from a close related species to *I. boliviensis* from El Salvador (Romero et al., 2025).

This study provides the first report of *B. odocoilei* in *I. affinis*, a strain is genetically close to *B. odocoilei* from *Ixodes*

cf. boliviensis of Panamanian highlands (Figs 1, 2). *Babesia odocoilei* is a parasite transmitted by *Ixodes scapularis* that affects wild ungulates, causing acute hemolytic fever in the United States and Canada (Scott et al., 2021). In addition, *B. odocoilei* is also considered a zoonotic pathogen in North America (Scott et al., 2021, 2024). Recently, a *B. odocoilei*-related strain has been found in *I. cf. boliviensis* from Panama, and *Babesia* sp. pudui, a close relative of *B. odocoilei*, has been detected in the blood of deer *Pupu puda* and in *Ixodes stilesi* ticks from Chile (Bermúdez et al., 2021a; Santodomingo et al., 2022). Since this detection was made in free-living *I. affinis* adults, the finding suggests trans-stadial perpetuation, at least during the molt nymph-adult. This fact is important, considering that the trans-stadial and transovarial transmission have been reported in the *Babesia* s.s. group (true *Babesia*) (Jalovecka et al., 2019). Although *I. affinis* is not considered a parasite of humans (Guglielmone and Robbins 2018), this finding is relevant to understanding the life cycle of Neotropical *Babesia*.

In our study, no microorganisms were detected infecting *A. naponense*, *A. cf. oblongoguttatum*, *A. ovale*, *A. tapirellum*, *H. juxtakochi*, or *Ixodes* sp. With the exception of *Ixodes* sp. nymphs, these species have been studied for the presence of microorganisms in Central America, with few results. In fact, to date no microorganisms associated with *A. naponense* and *A. tapirellum* have been detected, and there is little information on microorganisms associated with *A. cf. oblongoguttatum*, *A. ovale* and *H. juxtakochi*, including: *Rickettsia* sp. in *A. cf. oblongoguttatum* from Panama (Bermúdez et al., 2009); *R. amblyommatis* in *H. juxtakochi* from Panama (Castro et al., 2015), and *A. ovale* from Costa Rica (Troyo et al., 2016); *Rickettsia* st. Atlantic Rainforest in *A. ovale* from Belize (Lopes et al., 2016); *R. parkeri* and *Rickettsia bellii* in *A. ovale* from El Salvador (Romero et al., 2023); and *Ehrlichia chaffeensis* in *A. cf. oblongoguttatum*

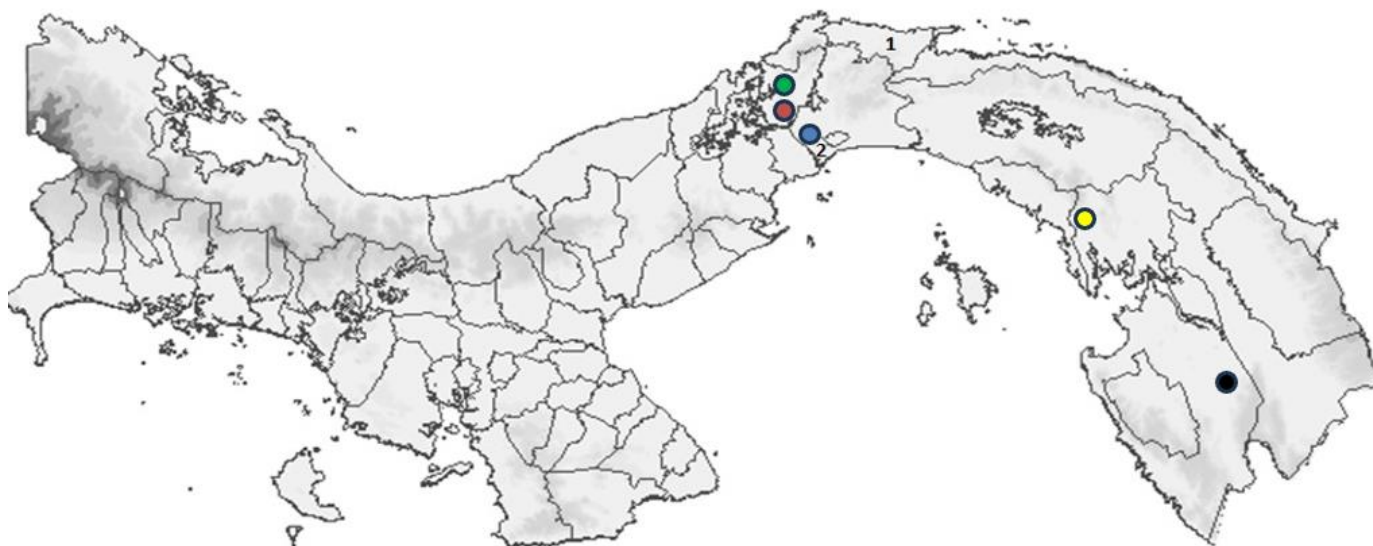


Figure 1. Samples sites in Panama. Blue spot: Summit Municipal Park, violet spot: Gamboa, green spot: Soberania Nacional Park, yellow spot: Natural Private Reserve Cerro Chucantí, black spot: Darién National Park.

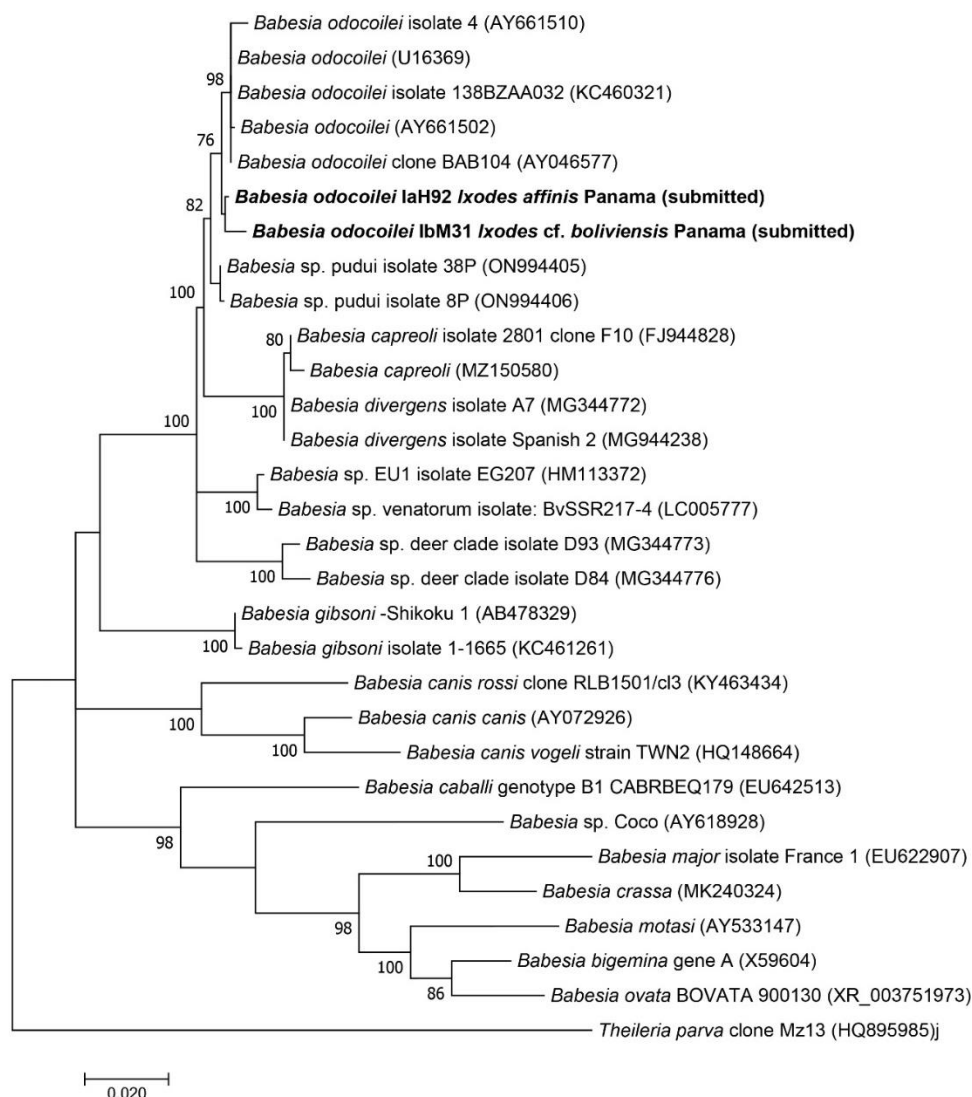


Figure 2. Maximum-likelihood phylogenetic tree generated for *Babesia* spp. based on the 18S rRNA gene. Numbers represent bootstrap support generated from 1000 replicates and branch supporting values >70% are shown. GenBank accession number is in parentheses. The newly generated sequence is indicated in bold. *Theileria parva* was used as outgroup.

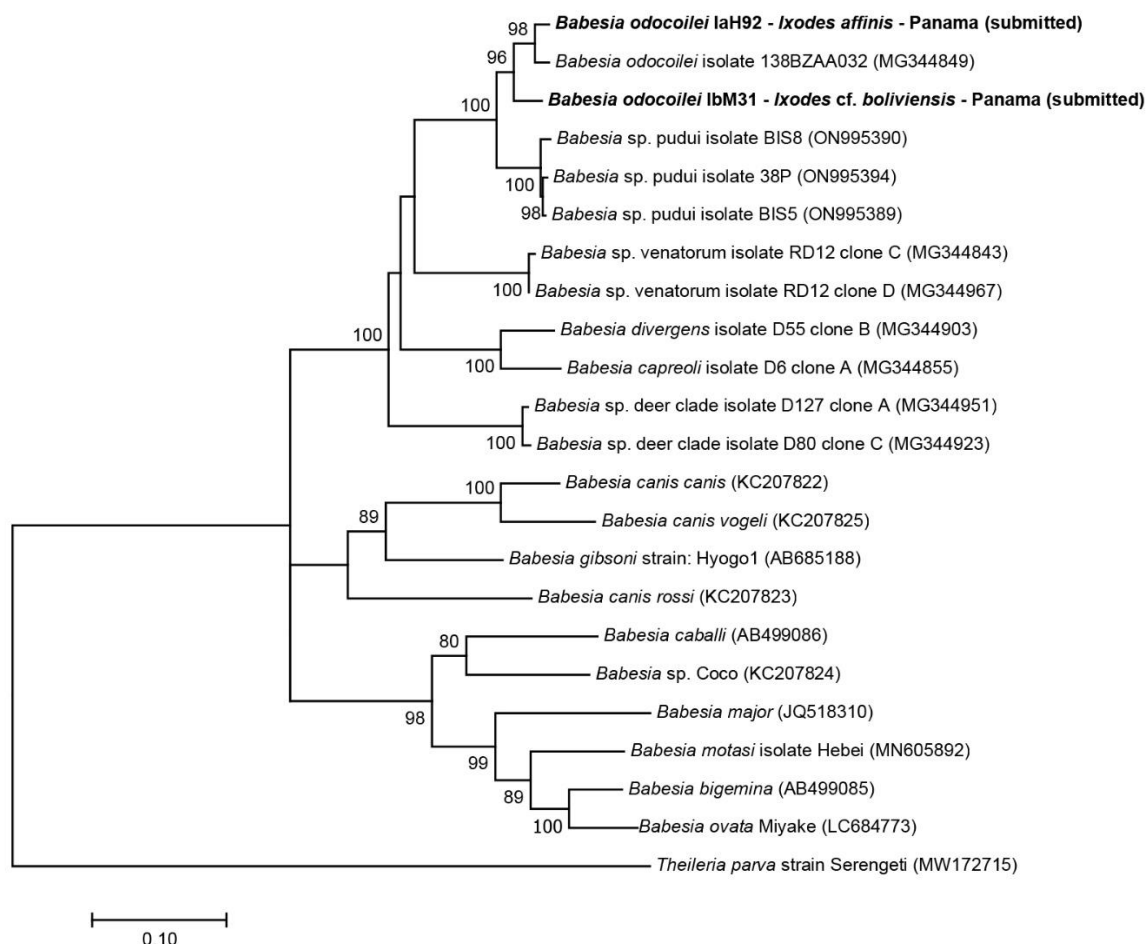


Figure 3. Maximum-likelihood phylogenetic tree generated for *Babesia* spp. based on the cytochrome c oxidase subunit I gene. Numbers represent bootstrap support generated from 1000 replicates and branch supporting values >70% are shown. GenBank accession number is in parentheses. The newly generated sequence is indicated in bold. *Theileria parva* was used as outgroup.

(Guzmán-Cornejo et al., 2024). The reason for the absence of DNA in these species in our study may be related to the low prevalence of these pathogens, so it is possible that more individuals analyzed could provide positive results.

Finally, this study was crucial to determine a putative new species of *Ixodes*, from two nymphs feeding on humans. Together with *I. cf. boliviensis* (Bermúdez et al., 2021a), this species would be another *Ixodes* reported parasitizing humans in Panama. The genus *Ixodes* is the most diverse of the family, with more than 280 described species worldwide, of which 69 feed on humans, and of these, and only *I. cf. boliviensis* (Central America), *Ixodes pararicinus* and *I. tropicalis* (South America), are from the Neotropical region (Guglielmo and Robbins 2018; Quintero et al., 2021); while the mention of *I. affinis* in a person from Costa Rica requires confirmation (Guglielmo and Robbins 2018). This finding raises the need to describe this species and determine whether it could pose a zoonotic risk within its range.

CONCLUSION

We found spotted fever group *Rickettsiae* infections in *A. mixtum*, *A. dissimile*, and *I. affinis*, which is consistent with other reports from Neotropics. This is the first report of *B. odocoilei* infecting *I. affinis*, a finding which provides new information on the ecology of this hemoparasite in tropical

wild environments. Although no pathogens were detected among the ticks, these findings highlight the need for research in wildlife environments, providing a first step toward understanding the potential risks tourists may encounter when visiting wild areas. Finally, the discovery of a potential new species of *Ixodes* biting humans indicates the need to study new environments and better characterize potential tick vectors in Neotropics.

Authors' contributions

Sergio Bermúdez Castellero: Conceived the study, performed the laboratory work and molecular analyses, drafted the manuscript. **María Félix:** Performed the laboratory work and molecular analyses, drafted the manuscript. **Lillian Domínguez:** Collected and identified ticks, drafted the manuscript. **José Venzal:** Collected ticks in the field, performed the laboratory work and phylogenetic analyses, drafted the manuscript.

Statement of ethics approval

Not applicable.

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Conflict of interest

The authors declare no conflicts of interest.

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