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New tick records in the western Brazilian Amazon, with notes on rickettsial infection and molecular evidence for *Amblyomma crassum* in Brazil

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ABSTRACT

This study evaluated the richness and abundance of ticks collected during two years in forest fragments of the state of Acre, western Brazilian Amazon. Considering all the environmental and host collections, the following 15 tick species were collected: *Amblyomma coelebs, Amblyomma crassum, Amblyomma humerale, Amblyomma latepunctatum, Amblyomma longirostre, Amblyomma naponense, Amblyomma nodosum, Amblyomma oblongoguttatum, Amblyomma ovale, Amblyomma pacae, Amblyomma rotundatum, Amblyomma scalpturatum, Haemaphysalis juxtakochi, Ixodes luciae* and *Rhipicephalus microplus.* Data from the most two abundant tick species, *A. oblongoguttatum* and *A. scalpturatum,* indicated a tendency for adults of both species to be more abundant during the dry season, and the nymphs at late rainy season of each year. The findings of *A. crassum* consisted of six nymphs collected from an amphibian (*Rhinella marina*) and a mammal (*Didelphis marsupialis*), which were morphologically unique and whose 16S rRNA partial sequences were 100 % identity to a GenBank-16S rRNA partial sequence of *A. crassum* from Colombia. This is the first confirmed record of *A. crassum* in Brazil. A total of 155 tick specimens were molecularly tested for rickettsial infection, resulting in a 6.5 % overall infection rate. *Rickettsia amblyommatis* was detected in *A. coelebs* and *A. humerale,* whereas *Rickettsia rhipicephali* was detected in *H. juxtakochi.* With the present records of *A. crassum* and *H. juxtakochi,* the tick fauna of Acre increases to 26 species, which represents 48 % of the Ixodidae fauna in Brazil. This is undoubtedly an extraordinary representation, considering that the state of Acre represents <2 % of the Brazilian territory.

1. Introduction

The Amazon Biome covers 5 % of the planet's land surface and 40 % of South America, 69 % of which is in Brazil (Ab'Saber, 1977). Due to the great diversity of terrestrial vertebrate species in the Amazon biome (WWF-Brasil, 2017), a corresponding diversity of ticks is also expected, as indicated by some studies in the Eastern Amazon (Binetruy et al., 2019; Pacheco et al., 2021a) and Western Amazon (Labruna et al., 2005a; Gianizella et al., 2018). However, given the immense size of this biome, our knowledge of its biodiversity is still somewhat modest, which is reflected in the constant description of new species of plants and animals as studies are carried out (WWF Brasil, 2017).

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The state of Acre, located in the western Brazilian Amazon, has a territorial extension of 164,173.429 km^2, corresponding to 1.93 % of the Brazilian territory and 3.27 % of the Legal Amazon (IBGE, 2024). Until 2001, only eight species of ticks had been recorded in Acre (Aragão, 1936; Guimarães et al., 2001). However, due to several field studies conducted in Acre during this century, its tick fauna currently comprises 24 species of hard ticks (Ixodidae) (Araujo et al., 2017; Fernandes et al., 2018; Lima et al., 2018; Gruhn et al., 2019; Tojal et al., 2020; 2021; Souza et al., 2016; 2023; 2025). Indeed, these data highlight Acre as a hotspot for tick diversity. This is facilitated by the fact that around 87 % of the state's area is covered by native forest, and almost 48 % of the territory is officially protected as conservation units and indigenous lands (Acre, 2017).

In Brazil, ticks are recognized vectors of two major agents of spotted fever: *Rickettsia rickettsii,* the agent of a severe illness named Brazilian spotted fever; and *Rickettsia parkeri*, the agent of a mild spotted fever rickettsiosis (Labruna, 2024). Both diseases, endemic in several regions of Brazil, have never been confirmed in the Amazon biome (Tojal et al., 2020). However, a recent study reported the infection by *R. parkeri* in its main vector (the tick *Amblyomma ovale*) in Acre, highlighting the possibilities that *R. parkeri*-caused rickettsiosis may have been neglected in the Amazon biome (Aguirre et al., 2022). In addition, several recent studies have reported different *Rickettsia* species of unknown pathogenicity infecting ticks from the wildlife of Acre (Lima et al., 2018; Gruhn et al., 2019; Mendoza-Roldan et al., 2021; Souza et al., 2023;2025).

This study aimed to gain a better understanding of the tick fauna in the state of Acre by assessing the richness and abundance of ticks in areas of the Amazon rainforest where such studies had never been carried out before. Additionally, some of the ticks were tested for infection with bacteria belonging to the genus *Rickettsia*.

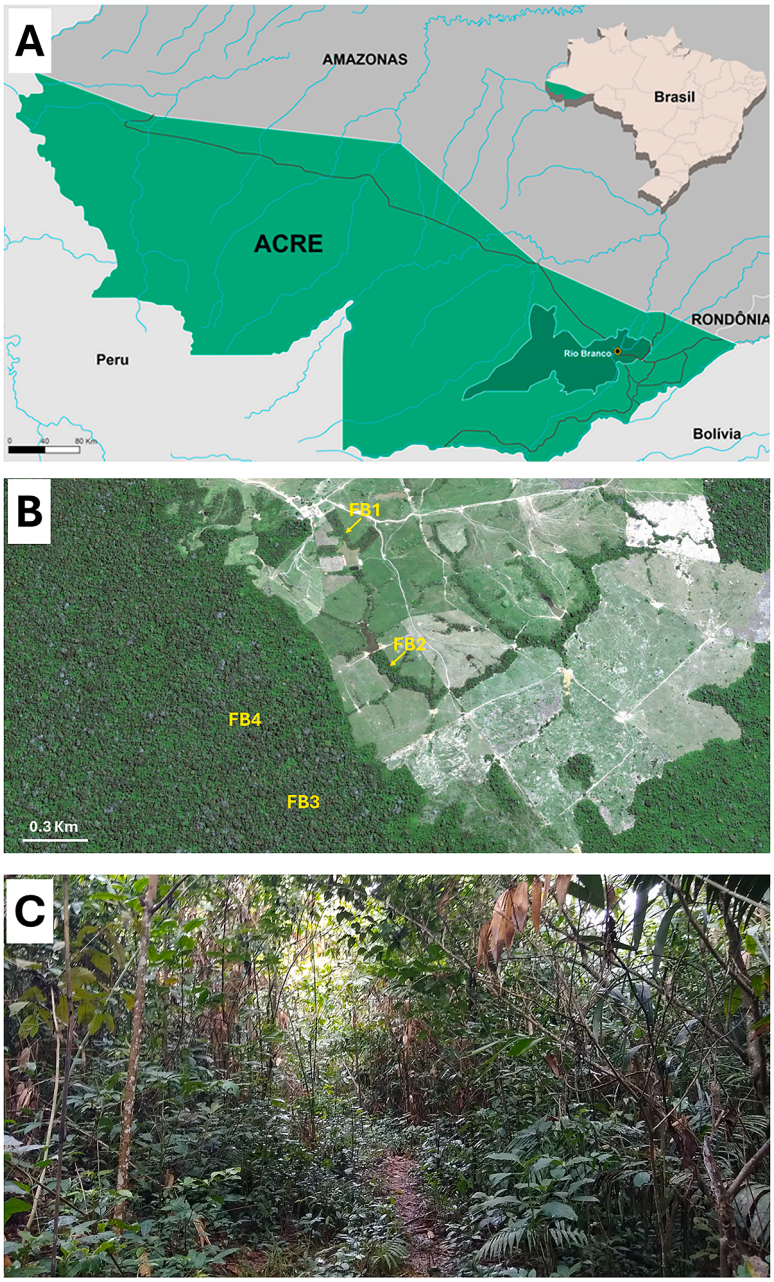

Fig. 1. (A) Map of the state of Acre, western Brazilian Amazon, indicating the area of Rio Branco Municipality. (B) Partial view of the Fazenda Batista, indicating the four sites sampled in the present study. FB1 and FB2 were small forest fragments surrounded by cattle pastures and dams; FB3 and FB4 were sites inside the main forest reserve of the farm. (C) General view of a trail where animals and ticks were sampled in the FB4 site.

2. Materials and methods

2.1. Ethics statements

The collection of ticks and the capture and handling of wild animals in this study have been authorized by the Biodiversity Authorization and Information System of the Chico Mendes Institute for Biodiversity Conservation of the Ministry of the Environment (SISBIO/ICMBio/MMA) of the Brazilian government (SISBIO license no 69.943–4), and by the UFAC Ethics Committee on the Use of Animals (protocol 36/2019 of process no 23,107.017043/2019–50). Some of the birds captured were ringed on the tarsus with a permanent metal ring engraved with a unique numerical code provided by the National Research Centre for the Conservation of Wild Birds (CEMAVE) of ICMBio/MMA, authorized by SISBIO license no 23,269–1.

2.2. Study area

This study was performed on a private farm called Fazenda Batista. This farm has a total area of 2531.77 ha and is located at the 50 Km mark of the AC-090 state highway (09°59′54''; 68°14′32″W), known as the Transacreana Road, within the municipality of Rio Branco, in the east of the state of Acre (Fig. 1A). The farm bears a large legal reserve (1231.55 ha) of Amazon primary forest, consisting of open forest with palm trees, with areas associated with patches of bamboo forest, characteristics of this part of the Amazon biome according to Silveira et al. (2008). The study region has a Tropical Climate Aw according to Köppen classification, with annual mean temperature of 25 °C and annual precipitation of around 1800 mm, more concentrated in the rainy season from October to April.

Four sites of the farm were sampled in the present study: FB1 (10°00′23''S; 68°14′20″W), FB2 (09°59′56''S; 68°14′27″W), FB3 (10°00′48''S; 68°14′31″W) and FB4 (10°00′34''; 68°14′39″W). FB1 and FB2 consisted of permanent protection areas, which were remaining forest fragments (each of around 4 ha) close to water bodies (dams); FB3 and FB4 consisted of abandoned trails inside the main forest reserve (Fig. 1B) that was contiguous to other large forest reserves, including the Chico Mendes Extractive Reserve (931,537 ha).

2.3. Collection of ticks from the environment

From August 2019 to August 2021, ticks were collected monthly from the vegetation at the four sampling points of Fazenda Batista (FB1, FB2, FB3 and FB4). In each month, ticks were collected simultaneously by the visual search method (looking for questing ticks on the tips of leaves of the vegetation bordering animal trails in the forest) and by dragging (passing a 70 cm x 120 cm white flannel over the vegetation), as previously described (Terassini et al., 2010). The dragging cloth was dispensed when the vegetation was soaked with rainwater, making it impossible to use the method for a few days during the rainy season. Collected ticks were immediately put in plastic microtubes containing absolute ethanol and transported to the laboratory.

2.4. Collection of ticks from small vertebrates

Between September 2020 to August 2021, small vertebrates (amphibians, reptiles, birds, rodents and marsupials) were trapped in sites FB3 and FB4, inside the main Forest Reserve of the Fazenda Batista, by using three methods: pitfall traps, livetraps, and mist nets. The former two were used monthly (total of 12 consecutive months), whereas mist nets were used for only seven months. We used two series of pitfall traps with a Y-shaped guide fence, according to Cechin and Martins (2000) and Bernarde et al. (2013), at each site. Each series contained four 100 L buckets intercepted by an 80 cm high plastic tarpaulin fence. The Pitfall was opened monthly for four consecutive days (96 h) and monitored daily. Livetraps consisted of 10 Tomahawk-like traps (35 cm × 16.5 cm × 16.5 cm) (Bressiani and Graipel, 2008), distributed along the trails within the forest (Fig. 1C). Livetraps were set up and baited with bacon, corn and peanut cream at sunset and inspected early the next morning for four consecutive nights, corresponding to the same monthly periods of the Pitfall traps. The combination of these two capture methods has proven to be effective in the Amazon (Teixeira et al., 2014; Botelho et al., 2024). To capture wild birds, 10 mist nets (12 m x 2.5 m; 25 mm mesh) were installed vertically along the trails, as described by Lima and Guilherme (2021). Throughout the sampling period (seven months), the mist nets were opened at sunrise (5:30 AM) and closed at sunset (5:30 PM), totaling a capture effort of 120 net/hour each day. The mist nets were monitored every 30 min and remained on the same trail for four consecutive days. The scientific nomenclature of birds is in accordance with Pacheco et al. (2021b).

Every captured vertebrate (toads, birds, small mammals) was manually restrained (using leather gloves when necessary) and had its entire body examined for the presence of ticks. When found, the ticks were collected and put in a plastic vial containing absolute ethanol (one tube per individual host). Afterwards, the animals were properly photographed to assist in taxonomic identification and subsequently released to the same place where they were captured.

2.5. Additional collections of ticks

During our study at Fazenda Batista during 2019–2021, the local community voluntarily collaborated by detecting the presence of ticks on wild animals that had been killed during subsistence hunting in the farm's forest reserve. In this case, the ticks were collected and put in plastic microtubes containing absolute ethanol previously given to the residents. When the tubes were returned to us with the collected ticks, the name of the hunted host was annotated. During the study, we also made several occasional collections of ticks from the vegetation by dragging different sites within and around the largest Forest Reserve. Most of these sites were sampled only a few times or once; thus, the results of all these occasional collections from vegetation were grouped and designated as "Others".

In June 2014, ticks were collected from a tortoise near a sustainable use conservation unit (Área de Relevante Interesse Ecológico Japiim Pentecostes: 7°40′39.3"S; 72°47′50.8″W) in the municipality of Cruzeiro do Sul, Acre (western region of the state) (Silva et al., 2019). From two other tortoises, ticks were collected on May 22 and 28, 2018, in a sustainable use conservation unit (Reserva Extrativista do Cazumbá-Iracema: 09°08′47.2"S; 069°01′15.2"W) in the municipality of Sena Madureira, Acre, as part of another study (Souza et al., 2022a).

Between July 9 and 12, 2019, ticks were collected from four bird specimens that were captured by mist nets in a forest fragment within a reserve belonging to the Federal University of Acre (Fazenda Experimental Catuaba: 10°04′38"S; 67°37′32″W), in the municipality of Senador Guiomard, Acre (Silveira et al., 2020). On February 1, 2021, a tick was collected from a bird that was captured in a full protection conservation area (Estação Ecológica Rio Acre: 10°45′00.0''; 70°31′00.0″W), in the municipality of Assis Brasil, Acre (ICMBio, 2010).

2.6. Tick identification

In the laboratory, ticks were examined in a stereoscopic microscopy, where they were identified morphologically based on current literature (Labruna et al., 2005b; Barros-Battesti et al., 2006; Onofrio et al., 2006; 2010; Martins et al., 2010). Taxonomic identification of a few immature specimens (larvae and nymphs) was expanded by molecular analysis. In this case, individual larvae (whole body) and non-engorged nymphs (extirpated legs from each nymph) were submitted to the boiling extraction method, as previously described (Horta et al., 2007). For a few engorged nymphs, a small portion of the posterior end of the idiosoma (excised with a sterile scalp) were submitted to DNA extraction using the guanidine isothiocyanate method (Sangioni et al., 2005).

Extracted DNA samples were tested by a polymerase chain reaction (PCR) protocol targeting $a \approx 460$ bp fragment of the tick mitochondrial 16S rRNA gene, as described (Mangold et al., 1998). PCR products were purified with ExoSap (USB, Cleveland, Ohio, USA) and sequenced with the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) in an automatic sequencer (model ABI 3500 Genetic Analyzer; Applied Biosystems) according to the manufacturer's protocol. The generated sequences were submitted to BLASTn analysis (www.ncbi.nlm.nih.gov/blast) to infer the closest identities to tick DNA sequences available in GenBank.

2.7. Search for rickettsia spp. in ticks

Random samples of the collected ticks were subjected to DNA extraction using the guanidine isothiocyanate method (Sangioni et al., 2005). Adults were processed individually. The whole body of non-engorged adults and a small portion of the idiosoma of engorged females (excised with a sterile scalp) were used for DNA extraction. Nymphs were separated into pools containing two to seven individuals (each pool grouping one species). Initially, the DNA samples were tested by the same above-mentioned protocol targeting the tick mitochondrial 16S rRNA gene, to validate the DNA extraction protocol. Once the extracted DNA was validated, samples were tested by a PCR assay with primers CS-78 and CS-323 targeting a 401 bp fragment of the citrate synthase gene (*gltA*), present in all species of the genus *Rickettsia* (Labruna et al., 2004). Positive samples in this assay were further tested by another PCR assay with primers Rr190.70 and Rr190.602, which amplify $a \approx 632$ bp fragment of the 190-kDa outer membrane protein gene (*ompA*), restricted to *Rickettsia* spp. of the spotted fever group (Regnery et al., 1991; Roux et al., 1996). Negative (DNA-free MiliQ water) and positive (*Rickettsia vinii* DNA) controls were used for the two assays targeting rickettsial genes. The obtained amplicons were purified, DNA-sequenced, and the sequences submitted to BLASTn analysis, as described above.

3. Results

3.1. Ticks from the vegetation

During the 25 month-period (August 2019 to August 2021) of monthly collection of ticks from the vegetation in Fazenda Batista, a total of 11 tick species were collected, totaling 1427 specimens, represented by 228 males, 266 females, 261 nymphs, and 672 larvae (Table 1). The site FB3 accounted for most of the collected ticks (73.1 %), followed by FB4 (14.4 %). These two sites were inside the main forest reserve. Sites FB1 and FB2, which consisted of small forest fragments surrounded by cattle pastures, accounted for only 1.8 % and 2.3 %, respectively, of the collected ticks. The site designated as "Others", which included occasional collections in different sites at different times within the Fazenda Batista, accounted for 8.3 % of the ticks. A total of 665 larvae (46.6 % of all ticks collected) could not be identified to species (due to lack of literature on larval specific morphology) and were retained as *Amblyomma* spp. (Table 1). Almost all these larvae were collected from site FB3. If we disregard all 665 *Amblyomma* spp. larvae from the counts, sites FB3 and FB4 accounted for 55.2 % and 26.6 %, respectively, of a total of 762 collected ticks. Among the 11 tick species, nine were collected from site FB4, eight from FB3, seven from FB1 and five from FB2. In the locality designated as "Others", nine tick species were found.

In sites FB1 to FB4, ticks were collected monthly from August 2019 to August 2021, except for January 2020 (due to heavy rain). Thus, considering the 24 sampled months, adult ticks were collected for 21 months, nymphs for 19 months, and larvae for seven months. Data from the most two abundant tick species, *Amblyomma oblongoguttatum* and *Amblyomma scalpturatum,* were plotted on a graph according to the number of nymphs and adults collected in each month (Fig. 2). There was a tendency for adults of both species to be more abundant during the dry season (between May and September) of each year. While the nymphs of *A. oblongoguttatum* showed distinct peaks during the months of the rainy season (October to April) that preceded adult peaks, the peaks of *A. scalpturatum* nymphs overlapped with the adult peak in May 2020 and preceded by one month the adult peak of 2021.

3.2. Ticks from amphibians and reptiles

During our field work in Fazenda Batista, a total of 14 cane toads (*Rhinella marina*) and 20 yellow-footed tortoises (*Chelonoidis denticulatus*) were captured and examined for ticks. Seven (50 %) of the cane toads and all 20 (100 %) yellow-footed tortoises were infested by ticks, as shown in Table 2. *Amblyomma rotundatum* was collected from both host species, whereas *Amblyomma humerale* was collected from the yellow-footed tortoises. Two larvae of *A. humerale* were molecularly identified to species based on their 16S rRNA partial sequences, which were 100 % identical to a sequence of *A. humerale* from GenBank (KY020987). In addition, four *Amblyomma* nymphs from toads, which were morphologically distinct from other *Amblyomma* species, were molecularly identified as *Amblyomma crassum* because their 16S rRNA partial sequences (413 bp) were 100 % identical (408/408 bp; 99 % query cover) to the only sequence of this species from GenBank, generated from a female specimen from Colombia (PP824641), recently reported by Alvarez-Londoño et al. (2024). After *A. crassum*, the closest identity in GenBank was 88 % with *Amblyomma cruciferum* (ON876776),

Table 1
Free-living ticks collected from vegetation in different locations of Fazenda Batista, Rio Branco municipality, state of Acre, Brazil, from August 2019 to August 2021.

Tick species	No. tick specimens according to locations in the farm[a]					TOTAL (%)	No. tick specimens per stage[b]
	FB1	FB2	FB3	FB4	Others		
Amblyomma coelebs	1	1	33	16	26	77 (5.4)	6F, 2 M, 69N
Amblyomma latepunctatum			4	7	1	12 (0.8)	4F, 6 M, 2 N
Amblyomma naponense	7	1	21	13	12	54 (3.8)	5F, 7 M, 42N
Amblyomma nodosum			2		2	4 (0.3)	1F, 1F, 2N
Amblyomma oblongoguttatum	1	1	240	65	41	348 (24.4)	100F, 164 M, 84N
Amblyomma ovale	1		9	6	3	19 (1.3)	10F, 9M
Amblyomma pacae				1		1 (0.1)	1N
Amblyomma rotundatum					1	1 (0.1)	1F
Amblyomma scalpturatum	1	1	108	88	31	229 (16.0)	99F, 85 M, 45N
Amblyomma spp.	12	28	622	3		665 (46.6)	665L
Haemaphysalis juxtakochi	1	1	4	2	2	10 (0.7)	2F, 1 M, 7N
Rhipicephalus microplus	2			5		7 (0.5)	7L
TOTAL (%)	26 (1.8)	33 (2.3)	1043 (73.1)	206 (14.4)	119 (8.3)	1427 (100)	228 M, 266F, 261 N, 672L

[a] FB1, FB2, FB3 and FB4 were fixed locations that were regularly sampled with the same sample effort during 2019–2021 in Fazenda Batista; "Others" refer to additional random locations occasionally sampled within Fazenda Batista during the course of the study.
[b] F: females; M: males; N: nymphs; L: larvae.

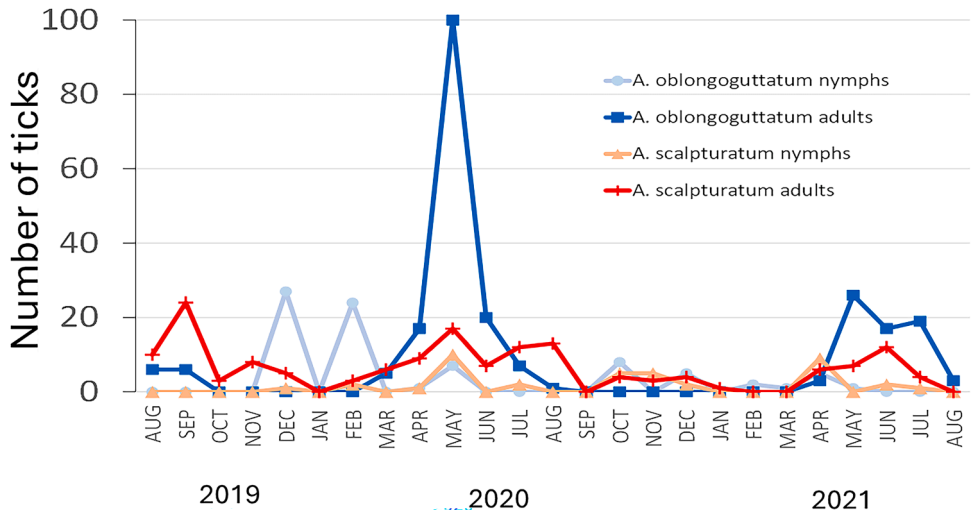

Fig. 2. Monthly numbers of nymph and adults of the two most abundant tick species (*Amblyomma oblongoguttatum* and *Amblyomma scalpturatum*) in the vegetation of Fazenda Batista, Rio Branco municipality, Acre, from August 2019 to August 2021.

Table 2
Ticks collected from amphibia, reptiles and birds (Passeriformes) in the Fazenda Batista, Rio Branco municipality, state of Acre, Brazil, during 2019 - 2021.

Host species	No. infested/ No captured (prevalence)	No. tick specimens according to species and stage[a]							Total No ticks
		A.hu	A.no	A.ob	A.ov	A.ro	A.cr	A.sp	
AMPHIBIA									
Rhinella marina	7/14 (50)					6F, 4N	4N[c]		14
REPTILA									
Chelonoidis denticulatus	20/20 (100)	198 M, 7F, 13 N, 2L[b]				4F			224
BIRDS									
Dendroplex picus	1/4 (20)		1N						1
Empidonax alnorum	1/2 (50)	1N							1
Myrmoborus leucophrys	1/2 (50)		1N					1L	2
Ramphocelus carbo	1/20 (5)	1N							1
Thamnomanes ardesiacus	1/6 (17)			1N					1
Thamnophilus aethiops	1/3 (33)	1N							1
Thamnophilus doliatus	1/7 (14)	1N							1
Turdus debilis	1/14 (7)				1N				1
Other 71 bird species[d]	0/199 (0)								0
Total birds	8/257 (3.1)	4	2	1	1			1	9

[a] A.hu: *Amblyomma humerale*; A.no: *Amblyomma nodosum*; A.ob: *Amblyomma oblongoguttatum*; A.ov: *Amblyomma ovale*; A.ro: *Amblyomma rotundatum*; A.cr.: *Amblyomma crassum*; A.sp: *Amblyomma* spp.; F: females; M: males; N: nymphs; L: larva.

[b] Taxonomic identification of these larval specimens was confirmed by generating partial DNA sequences of the tick mitochondrial 16S rRNA gene.

[c] These four nymphs were identified as *Amblyomma crassum* based on molecular analysis, as their 16S rRNA gene partial sequences were 100 % identical to *A. crassum* from GenBank (PP824641).

[d] A complete list of the bird species captured in this manuscript is shown in Table S1, with number of specimens according to bird order, family and species.

Amblyomma sabanerae (PP837379), and *Amblyomma lepidum* (OQ566204), and then 87–86 % with several sequences of *A. humerale* (e.g., MZ323405, KY020987).

Additional collections of ticks from reptiles outside the Fazenda Batista consisted of only *A. humerale,* being 16 males and 4 females from two yellow-footed tortoises from the Sena Madureira municipality, and 9 males from a yellow-footed tortoise from the Cruzeiro do Sul municipality.

3.3. Ticks from birds

Through the use of mist nets in areas FB3 and FB4 of the Fazenda Batista, a total of 257 bird specimens were collected and identified into 80 species, representing nine bird orders and 25 families. Most of these bird species (66) belonged to the order Passeriformes (Table S1). A total of nine tick specimens were collected from eight bird specimens, each one representing a different species of the order Passeriformes (Table 2). The ticks were identified as nymphs of *A. humerale* (4 specimens), *Amblyomma nodosum* (2), *Amblyomma oblongoguttatum* (1), and *Amblyomma ovale* (1), and a larva of *Amblyomma* sp.

Regarding the additional collections outside the Fazenda Batista, ticks were collected from four species of Passeriformes in the municipality of Senador Guiomard, as follows: one nymph of *Amblyomma longirostre* and a larva of *Amblyomma* sp. on Plain-brown Woodcreeper *Dendrocincla fuliginosa* (Dendrocolaptidae); four larvae of *Amblyomma* sp. on Olivaceous Woodcreeper *Sittasomus griseicapillus* (Dendrocolaptidae), Spotted Tody-Flycatcher *Todirostrum maculatum* (Rhynchocyclidae), and Tropical Kingbird *Tyrannus melancholicus* (Tyrannidae). In addition, one *Amblyomma* sp. larva was collected from Ruddy Quail-Dove *Geotrygon montana* (Columbiformes: Columbidae) in the municipality of Assis Brasil.

3.4. Ticks from wild mammals

A total of 50 specimens of wild mammals were captured in the Fazenda Batista. Most of them were small mammals (marsupials and

rodents) that were trapped in the main natural reserve (areas FB3 and FB4). Unfortunately, many of these small mammals could not be identified as species and were retained at genus level or even at family level (five Cricetidae mice) (Table 3). There were also one armadillo (*Dasypus* sp.), one collared peccary (*Dicotyles tajacu*) and one black agouti (*Dasyprocta fuliginosa*) that were hunted by local residents, who collected the ticks and gave to us.

Overall, only 8 (16 %) mammals were infested by ticks (Table 3). Among the trapped marsupials, one common opossum (*Didelphis marsupialis*) was infested by four tick species: *Amblyomma coelebs, Ixodes luciae, A. humerale,* and *A. crassum*; the latter species was represented by one larva and two nymphs that were identified by molecular methods, as their 16S rRNA partial sequences were 99.8 or 100 % (408/409 bp for larva or 413/413 bp for nymphs, respectively) identical to the only sequence of this gene for *A. crassum* in GenBank (PP824641). A short-tailed opossum (*Monodelphis* sp.) and a bristly mouse (*Neacomys* sp.) were also infested by *I. luciae.* Other tick species found on wild mammals were *Amblyomma naponense, A. oblongoguttatum, Amblyomma pacae, A. scalpturatum*, and *Haemaphysalis juxtakochi* (Table 3).

3.5. *Rickettsia spp. in ticks*

A total of 155 tick specimens were molecularly tested for rickettsial infection. *Rickettsia* spp. were found in 10 samples, comprising eight individual ticks and two nymphal pools, giving a 6.5 % overall infection rate (Table 4). All *Rickettsia*-infected ticks were from Fazenda Batista. Three males of *A. humerale* collected on yellow-footed tortoises (*C. denticulatus*), and one nymph of *A. humerale* collected on the passerine Barred Antshrike (*Thamnophilus doliatus),* yielded *gltA* (350-bp) and *ompA* (587-bp) partial sequences that were 100 % identical to corresponding sequences of *Rickettsia amblyommatis* from GenBank (CP012420 and KY053885, respectively). Two nymphs of *A. coelebs* from a common opossum (*D. marsupialis*), and one female and two nymphal pools of *A. coelebs* from vegetation, yielded *gltA* (350-bp) partial sequences that were 100 % identical to *Rickettsia* sp. (OP823398), and *ompA* (587-bp) partial sequences that were 100 % identical to *R. amblyommatis* (MW147461). In addition, one male of *H. juxtakochi* collected on vegetation yielded an *ompA* (448-bp) partial sequence that was 100 % identical to the corresponding sequence of *Rickettsia rhipicephali* (CP013133). No reliable *gltA* sequence was generated from this *H. juxtakochi* male.

3.6. *Accession numbers*

Voucher tick specimens have been deposited in the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" at the University of São Paulo, with the accession numbers CNC-5028 to CNC-5042, and in the Laboratory of Tropical Medicine of the Federal University of Acre. Tick 16S rDNA partial sequences obtained in this study have been submitted to GenBank under the following accession numbers: *A. humerale* (PV981672) and *A. crassum* (PV981673, PV981674). Partial sequences of the rickettsial genes generated in this study have been deposited in GenBank under the accession numbers PV987513, PV987515 (*R. amblyommatis gltA* and *ompA* from *A. humerale*), PV987514, PV987516 (*R. amblyommatis gltA* and *ompA* from *A. coelebs*), and PV987517 (*R. rhipicephali* from *H. juxtakochi*).

4. Discussion

Overall, 15 tick species were identified in the present study, 14 species being in the Fazenda Batista area (Rio Branco municipality). *Amblyomma longirostre* was found only in Senador Guiomard municipality. The ticks identified as *I. luciae* and *Amblyomma latepunctatum* in the present study have been previously reported by Tojal et al. (2021). To our knowledge, this is the largest study of ticks in wildlife in the state of Acre, western Brazilian Amazon. Noteworthy, we report for the first time in Acre the presence of *H. juxtakochi* and provide molecular evidence for the presence of *A. crassum*, which is the first confirmed record of this species in Brazil.

The species *A. crassum* was originally described based on a female specimen collected on a land tortoise from Colombia (Robinson, 1926). Since then, there have been few records of this species on land tortoises in Colombia, Panama, Peru and Venezuela, all based on female specimens, since the male, nymphal and larval stages have remained

Table 3
Ticks collected from wild mammals in Fazenda Batista, Rio Branco municipality, state of Acre, Brazil, from September 2020 to August 2021.

Host species	No. infested/ No captured (prevalence)	No. tick specimens according to species and stage[a]										Total No ticks
		A.co	A.hu	A. na	A.ob	A. pa	A. sc	H. ju	I.lu	A.cr	A. sp	
DIDELPHIMORPHIA												
Didelphis marsupialis	1/1 (100)	10N[b]	1 L[b], 1N						1 M	2 N[c], 1L[d]	9L	25
Marmosa sp.	0/3 (0)											0
Marmosops sp.	0/13 (0)											0
Monodelphis sp.	1/5 (20)								1N			1
CINGULATA												
Dasypus sp.	2/2 (100)	2N	1N									3
RODENTIA												
Neacomys sp.	1/1 (100)								3 N, 1L		2L	6
Oecomys sp.	0/4 (0)											0
Proechimys sp.	0/9 (0)											0
Rhipidomys sp.	0/4 (0)											0
Cricetidae	0/5 (0)											0
Myoprocta pratti	1/1 (100)			1N			1N	1N			1L	4
Dasyprocta fuliginosa	1/1 (100)					2N						2
ARTIODACTYLA												
Dicotyles tajacu	1/1 (100)			1F	2 M,1F							3
TOTAL	8/50 (16)	12	3	2	3	2	1	1	6	3	12	45

[a] A.co: *Amblyomma coelebs*; A.hu: *Amblyomma humerale*; A.na: *Amblyomma naponense*; A.ob: *Amblyomma oblongoguttatum*; A.pa: *Amblyomma pacae*; A.sc: *Amblyomma scalpturatum*; H.ju: *Haemaphysalis juxtakochi*; I.lu: *Ixodes luciae*; A.cr.: *Amblyomma crassum*; A.sp: *Amblyomma* spp.; F: females; M: males; N: nymphs; L: larvae.

[b] Taxonomic identification of these specimens was confirmed by generating partial DNA sequences of the tick mitochondrial 16S rRNA gene.

[c] These two nymphs were identified as *Amblyomma crassum* based on molecular analysis, as their 16S rRNA gene partial sequences were 100 % identical to *A. crassum* from GenBank (PP824641).

[d] This larva was identified as *A. crassum* based on molecular analysis, as its 16S rRNA gene partial sequences was 99.8 % identical to *A. crassum* from GenBank (PP824641).

Table 4
Results of molecular tests for the presence of *Rickettsia* spp. in ticks collected from wild animals and from vegetation in the state of Acre during 2019–2021.

Host or source	Tick species	No. tested[a]	No. infected (%)	*Rickettsia* species
Amphibia				
Rhinella marina	*Amblyomma rotundatum*	5F	0	
Reptilia				
Chelonoidis denticulatus	*Amblyomma humerale*	21M	3 (14)	*R. amblyommatis*
	A. rotundatum	1F	0	
Birds				
Dendrocincla fuliginosa[b]	*Amblyomma longirostre*	1N	0	
	Amblyomma sp.	1L	0	
Dendroplex picus	*Amblyomma nodosum*	1N	0	
Empidonax alnorum	*A. humerale*	1N	0	
Myrmoborus leucophrys	*A. nodosum*	1N	0	
	Amblyomma sp.	1L	0	
Ramphocelus carbo	*A. humerale*	1N	0	
Sittasomus griseicapillus[b]	*Amblyomma* sp.	1L	0	
Thamnomanes ardesiacus	*Amblyomma oblongoguttatum*	1N	0	
Thamnophilus aethiops	*A. humerale*	1N	0	
Thamnophilus doliatus	*A. humerale*	1N	1 (100)	*R. amblyommatis*
Todirostrum maculatum[b]	*Amblyomma* sp.	2L	0	
Turdus debilis	*Amblyomma ovale*	1N	0	
Tyrannus melancholicus[b]	*Amblyomma* sp.	1L	0	
Mammalia				
Didelphis marsupialis	*Amblyomma coelebs*	7N	2 (29)	*R. amblyommatis*
	Amblyomma sp.	4L	0	
Myoprocta pratti	*Amblyomma* sp.	1L	0	
Neacomys sp.	*Ixodes luciae*	2 N, 1L	0	
	Amblyomma sp.	2L	0	
Free-living				
Vegetation	*A. coelebs*	2 M, 2F	1 (25)	*R. amblyommatis*
Vegetation	*A. coelebs*	2 N[c], 6N[c]	2 (25)	*R. amblyommatis*
Vegetation	*A. ovale*	6 M, 4F	0	
Vegetation	*Amblyomma naponense*	4 M, 5F, 6N[c]	0	
Vegetation	*Amblyomma scalpturatum*	8 M, 7F, 5N[c]	0	
Vegetation	*Amblyomma latepunctatum*	4 M, 3F	0	
Vegetation	*Amblyomma oblongoguttatum*	5 M, 5F,10N[c]	0	
Vegetation	*Amblyomma nodosum*	1 M, 1F, 2N[c]	0	
Vegetation	*Haemaphysalis juxtakochi*	1 M, 2F, 5N[c]	1[d] (13)	*R. rhipicephali*
TOTAL		155	10 (6.5)	

[a] F: females; M: males; N: nymphs; L: larvae.
[b] These four birds were sampled in Senador Guiomard municipality; the remaining hosts and ticks of this table were collected in the Fazenda Batista (Rio Branco municipality).
[c] tested in a pool.
[d] refers to the male specimen.

unknown (Guglielmone et al., 2021; 2023). Because of high morphological similarities between the females of *A. crassum, A. humerale* and *A. sabanerae,* the taxonomic status of *A. crassum* has been treated with caution by some authors (Guglielmone et al., 2023). On the other hand, a recent study from Colombia reported a female of *A. crassum*, from which partial sequences of the mitochondrial 12S rRNA and 16S rRNA genes were generated (Alvarez-Londoño et al., 2024). These sequences were highly distinct from both *A. humerale* and *A. sabanerae,* suporting the classification of the female as *A. crassum* in Colombia.

In the present study, we report through molecular analysis (16S rRNA partial sequences) four nymphs of *A. crassum* on toads (*R. marina*), and two nymphs and one larva of *A. crassum* on an common opossum (*D. marsupialis*), which constitute the first records of immature stages of *A. crassum,* and also the first records of this tick species on hosts other than land tortoises. Before our molecular analysis, the six *A. crassum* nymphs were initially examined morphologically by one of us (T.F.M.), who noticed that they were distinct from all other *Amblyomma* nymphs from Brazil described so far. For this reason, we used only the legs of these nymphs for DNA extraction, and preserved the body for further morphological analyses, which are in progress. In addition, it is likely that some of the adults (197 males, seven females) here identified as *A. humerale* could be *A. crassum,* which will also be evaluated in a further study employing morphological and molecular methods.

Before this study, there were two reports of *A. crassum* in Brazil. Sauter et al. (1999) reported 22 adults (14 males, 8 females) on land tortoises from the state of Amazonas, western Brazilian Amazon. Surprisingly, the authors ignored the fact that this was the first record of this species in Brazil and did not clarify how they identified the males of *A. crassum* (since this stage remains undescribed) and how they differentiated the species from *A. humerale*, which is the most common tick on Amazonian land tortoises. The second report was made by Amorim et al. (2013), who reported two females of *A. crassum* on a green anaconda (*Eunectes murinus*) from an area of the Cerrado biome in the state of Mato Grosso do Sul. Although Amorim et al. (2013) claimed to the first report of *A. crassum* in Brazil, they also did not show how they differentiated the two specimens from *A. humerale.* In view of the great morphological similarities between *A. crassum* and *A. humerale,* these previous two records of *A. crassum* in Brazil were treated as doubtful by Guglielmone et al. (2021; 2023). Given this scenario, this study presents for the first time molecular evidence for the presence of *A. crassum* in Brazil, in sympatry with *A. humerale* in an area of the western Amazon. Indeed, new studies should review previous reports of *A. humerale* in Brazil, especially through molecular analysis, in order to determine whether some of these reports did not include any specimens of *A. crassum.*

With the addition of *A. crassum,* the Brazilian tick fauna is currently represented by 78 established tick species, being 54 Ixodidae (hard ticks) and 24 Argasidae (soft ticks) (Labruna et al., 2024). Before the present study, the tick fauna of the state of Acre was composed of 24 species, all hard ticks (Ixodidae) (Araujo et al., 2017; Fernandes et al., 2018; Lima et al., 2018; Gruhn et al., 2019; Tojal et al., 2020; 2021;

Souza et al., 2016; 2023; 2024). Paradoxically, soft ticks (Argasidae) remain to be reported in Acre, especially because the soft tick fauna in the state of Rondônia (neighboring Acre on the eastern border) is one of the richest in Brazil (Labruna et al., 2024).

As expected, during the two years of the study, higher abundance and richness of ticks were found in the main forest reserve (sites FB3 and FB4); however, it is noteworthy that as many as seven tick species were sampled in the small forest fragments (Table 1). Most of the tick species found in these small fragments (*A. coelebs, A. naponense, A. oblongoguttatum, A. ovale, A. scalpturatum*) are primarily associated with large mammals, such as tapirs (*Tapirus terrestris*) and peccaries (Labruna et al., 2005a; 2010), which were commonly observed in the area by farm residents (data not shown). It is well known that tapirs and peccaries depend on large, preserved areas of forest to become established in the Amazon biome (Norris et al., 2008; Costa et al., 2023). However, the presence of their associated tick species in small forest fragments such as FB1 and FB2 indicates that these wild mammals have also used these small fragments, besides the main forest reserve, probably facilitated by the high abundance in the forest as a whole and the proximity to fragments FB1 and FB2. The fact that only <5 % of all ticks collected from vegetation were from sites FB1 and FB2 suggest that the use of the adjacent small forest fragments by large wild mammals was minimal or/and these fragments did not provide highly suitable microclimatic conditions for the off-host stages of *A. coelebs, A. naponense, A. oblongoguttatum, A. ovale* and *A. scalpturatum.*

Interestingly, a few *Rhipicephalus microplus* ticks were collected from sites FB1 and FB4. This is an exotic tick species that was introduced with cattle in Brazil, where it has been primarily associated with cattle pastures (Labruna et al., 2005a; Guglielmone et al., 2021). Despite the presence of pastures inhabited by cattle between the sampled sites of the present study, *R. microplus* ticks represented only 0.5 % of the ticks sampled during a two-year period. Since there were no fences to prevent cattle from entering the forest fragments, and since cattle did in fact use the FB1 and FB2 fragments (as evidenced by the constant presence of bovine feces inside the fragments), our results indicate that these forested areas are not important for the *R. microplus* tick, possibly because they have very different microclimatic conditions to those of a cattle pasture in the open field.

Data from the most two abundant tick species, *A. oblongoguttatum* and *A. scalpturatum,* showed that adult ticks were more abundant in the dry season, and nymphs in the rainy season. These results are only partially concordant to those reported for several *Amblyomma* species in another area of the western Brazilian Amazon (Labruna et al., 2009) and to one study with *Amblyomma cajennense* sensu stricto in the eastern Brazilian Amazon (Araújo et al., 2023), and highly discordant for seasonal studies of *Amblyomma* spp. outside the Amazon biome in Brazil (Barbieri et al., 2019; Paula et al., 2022). In any case, further studies are needed to confirm whether the seasonal pattern of *Amblyomma* spp. ticks in the Amazon rainforest areas of Acre are really different from other regions, or whether the differences found here were simply a function of the relatively small sample numbers for more robust seasonal analyses.

Among 257 bird specimens sampled in the main forest reserve of Fazenda Batista, eight birds (3.1 %) were found infested by ticks. This infestation rate was lower than those reported from birds in other areas of the state of Acre, which varied from 6 % to 30 % (Lima et al., 2018; Souza et al., 2020; 2023; 2025). Interestingly, in the study of Souza et al. (2025), birds were sampled in five different areas, being four urban forest fragments (size varying from 4.6 to 5224 ha) and one large continuous forest area (695,303 ha). When the infestation rate was calculated separately for each of these five areas, the rates varied from 24 to 78 % among the four urban forest fragments, and was only 3.8 % for the large continuous forest area. The authors concluded that areas with a higher fragmentation index had a higher prevalence of ticks on birds, in accordance with a previous study in Brazilian Atlantic rainforest biome (Ogrzewalska et al., 2011). Based on this statement, the low infestation rate of ticks on birds found in this study (3.1 %) could be related to the large size of the main forest reserve in which birds were sampled in Fazenda Batista.

Despite several recent studies about ticks on wild birds in Acre (Lima et al., 2018; Souza et al., 2020; 2023; 2025) and in the Neotropical region (reviewed by Guglielmone et al. 2021), our records of an *A. oblongoguttatum* nymph on the dusky-throated antshrike (*Thamnomanes ardesiacus*) and of an *A. nodosum* nymph on the white-browed antbird (*Myrmoborus leucophrys*) are, to our knowledge, the first records of ticks parasitizing these two passerine species. In addition, we provide the first records of *A. humerale* on the alder flycatcher (*Empidonax alnorum*), the silver-beaked tanager (*Ramphocelus carbo*), the white-shouldered antshrike (*Thamnophilus aethiops*) and the barred antshrike (*Thamnophilus doliatus*); and of *A. ovale* on the floodplain thrush (*Turdus debilis*).

The two *Rickettsia* species, *R. amblyommatis* and *R. rhipicephali,* found in ticks of the present study belong to the spotted fever group of rickettsiae, but their pathogenicity to humans have not been properly confirmed (Parola et al., 2013; Karpathy et al., 2016). Interestingly, two genetic variants of *R. amblyommatis* were detected, each one associated with a tick species (*A. coelebs* and *A. humerale*). These tick-*Rickettsia* associations seem to be somewhat formal rather than random, because the same *ompA* haplotype of *R. amblyommatis* detected in *A. coelebs* in this study was previously detected in this same tick species from the eastern Brazilian Amazon (Souza et al., 2022b), whereas the *ompA* haplotype of *R. amblyommatis* detected in *A. humerale* in this study was previously detected in this same tick species from another area of Acre (Gruhn et al., 2019). Finally, we report the presence of *R. rhipicephali* in *H. juxtakochi* for the first time in Acre, corroborating previous studies from the Amazon and Atlantic rainforest biomes that have reported this tick-*Rickettsia* association in Brazil (Labruna et al., 2007; Soares et al., 2015; Acosta et al., 2016).

5. Conclusions

Based on sampling of amphibians, reptiles, birds, mammals and vegetation in some areas of the state of Acre, a richness of 15 tick species is reported in this study. With the present records of *A. crassum* and *H. juxtakochi*, the tick fauna of Acre increases to 26 species, which represents 48 % of the Ixodidae fauna of Brazil. This is undoubtedly an extraordinary representation, considering that the state of Acre represents <2 % of the Brazilian territory. Despite the new reports of *Rickettsia* of the spotted fever group in ticks from Acre in this study, the association of these tick-borne agents with human or animal infection in Acre needs to be proven in future studies.

CRediT authorship contribution statement

Simone Delgado Tojal: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Marcelo B. Labruna:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Data curation. **Thiago Fernandes Martins:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Dionatas Ulises de Oliveira Meneguetti:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Lina de Campos Binder:** Writing – review & editing, Validation, Software, Methodology, Investigation, Formal analysis. **Jônatas Lima:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation. **Edson Guilherme:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **André Luis Moura Botelho:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data

curation, Conceptualization. **Karoline Silva da Cruz:** Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. **Luís Marcelo Aranha Camargo:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2025.107829.

Data availability

Data will be made available on request.

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