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Ticks and tick-associated spotted fever group *Rickettsia* from birds in the Southwestern Brazilian Amazon¤

Garrapatas y su infección por Rickettsia del grupo de la fiebre manchada en aves del sudoeste de la Amazonía Brasileña

Carrapatos e sua infeção por Rickettsia do grupo da febre maculosa em aves do sudoeste da Amazonia Brasileira

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Abstract

Background: South American birds are known to play a significant role in life cycles of various hard ticks, particularly within *Amblyomma* genus. However, the tick fauna from the Amazon region has been poorly studied, being limited to very few studies. **Objective:** To report tick infestations on wild birds captured in a region of the Amazon forest, Acre state, Brazil, and to evaluate rickettsial infection in these ticks. **Methods:** Wild birds were captured by mist-nets and examined for the presence of ticks, which were all collected and identified to species level by taxonomic keys and/or molecular methods. In addition, part of these ticks was individually tested by PCR targeting portions of three rickettsial genes (*gltA, ompA, ompB*). **Results:** Among 1,322 captured birds,

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79 individuals (6.0%) were infested by one of the following ticks species: *Amblyomma nodosum* Neumann, 1899 (72 nymphs), *Amblyomma longirostre* (Koch, 1844; seven larvae, 13 nymphs), *Amblyomma humerale* Koch 1844 (four nymphs), *Amblyomma geayi* Neumann, 1899 (two larvae, two nymphs), and 421 larvae of *Amblyomma* spp *Rickettsia* sp strain NOD was detected in 3/26 *A. nodosum*, and *Rickettsia amblyommatis* in 5/8 *A. longirostre* and 1/2 *A. geayi* ticks tested. **Conclusion:** This is the first study about ticks parasitizing wild birds in Acre state, adding new host-parasite relationships, new tick species records (*A. humerale* and *A. nodosum*) and two rickettsial agents (*R amblyommatis* and *Rickettsia* sp strain NOD) for the first time in Acre.

Keywords: *Amblyomma, disease, epidemiology, rainforest, wildlife.*

Resumen

Antecedentes: En América del Sur, las aves actúan como importantes hospedadores dentro del ciclo biológico de garrapatas del género *Amblyomma,* que por su parte, se encuentran naturalmente infectadas con rickettsias patógenas. Aunque la diversidad de aves en esta región es alta, el conocimiento de la fauna de garrapatas asociadas es aún escaso. **Objetivo:** Reportar la fauna de garrapatas asociadas a aves presentes en la Amazonía, estado de Acre, Brasil, y evaluar en éstas infección por *Rickettsia.* **Métodos:** Las aves fueron capturadas con redes de niebla y examinadas en busca de garrapatas, las que a su vez fueron identificadas por medio de claves taxonómicas y métodos moleculares. Parte de estas garrapatas fueron individualmente sometidas a PCR convencional en busca de fragmentos de tres genes rickettsiales (*gltA, ompA, ompB*). **Resultados:** De un total de 1.322 aves capturadas, 79 (6,0%) se encontraron parasitadas con alguna de las siguientes especies de garrapatas: *Amblyomma nodosum* Neumann, 1899 (72 ninfas), *Amblyomma longirostre* (Koch, 1844; siete larvas, 13 ninfas), *Amblyomma humerale* Koch 1844 (cuatro ninfas), *Amblyomma geayi* Neumann, 1899 (dos larvas, dos ninfas) y 421 larvas de *Amblyomma* spp *Rickettsia* sp cepa NOD fue detectada en 3/26 *A. nodosum* y *Rickettsia amblyommatis* en 5/8 *A. longirostre*, así como también en 1/2 *A. geayi* analizadas. **Conclusión:** Este es el primer estudio sobre garrapatas parásitas de aves desarrollado en el estado de Acre. Además, se reportan nuevas relaciones parásito-hospedador, nuevos registros para dos especies de garrapatas (*A. humerale* y *A. nodosum*) y dos rickettsias (*R. amblyommatis* y *Rickettsia* sp cepa NOD) por primera vez en Acre.

Palabras clave: *Amblyomma, enfermedad, epidemiología, selva tropical, vida silvestre.*

Resumo

Antecedentes: Aves sul-americanas são conhecidas por desempenhar um papel significativo no ciclo de vida de várias espécies de ixodídeos, particularmente dentro do gênero *Amblyomma*. No entanto, a ixodofauna da região amazônica tem sido pouco estudada, sendo limitada apenas a alguns estudos. **Objetivo:** Relatar as infestações de carrapatos em aves silvestres capturadas na região de floresta amazônica, Acre, Brasil, e avaliar as infecções por riquétsias. **Métodos:** As aves silvestres foram capturadas por redes de neblina e examinadas para a presença de carrapatos, que foram coletados e identificados até espécies, utilizando chaves taxonômicas e/ou métodos moleculares. Além disso, uma parte desses carrapatos foram testados individualmente pela PCR para a pesquisa de fragmentos de três genes de riquétsias (*gltA, ompA, ompB*). **Resultados:** Entre as 1.322 aves capturadas, 79 indivíduos (6,0%) estavam infestados pelas seguintes espécies de carrapatos: *Amblyomma nodosum* Neumann, 1899 (72 ninfas), *Amblyomma longirostre* (Koch, 1844; sete larvas, 13 ninfas), *Amblyomma humerale* Koch 1844 (quatro ninfas), *Amblyomma geayi* Neumann, 1899 (duas larvas, duas ninfas) e 421 larvas de *Amblyomma* spp *Rickettsia* sp cepa NOD foi detectada em 3/26 *A. nodosum* e *Rickettsia amblyommatis* em 5/8 *A. longirostre* e 1/2 *A. geayi* dos carrapatos testados. **Conclusão:** Este é o primeiro estudo de carrapatos parasitando aves silvestres no estado do Acre, demostrando novas relações parasita-hospedeiro e registros inéditos de espécies de carrapatos (*A. humerale* e *A. nodosum*) e riquétsias (*R. amblyommatis* e *Rickettsia* sp strain NOD) para o Acre.

Palavras chave: *Amblyomma, doença, epidemiologia, floresta tropical, vida silvestre.*

Introduction

The Brazilian tick fauna is currently represented by 70 species, of which 46 are classified in the Ixodidae family (Krawczak *et al*., 2015), and 24 in the Argasidae family (Barros-Battesti *et al*., 2015; Labruna *et al*., 2016; Wolf *et al*., 2016). Among this diversity, one species of *Ornithodoros* (Ramos *et al*., 2015) and several species of *Amblyomma, Haemaphysalis* and *Ixodes* have been reported parasitizing wild birds. Nevertheless, insights derived from the latest investigations suggest that this diversity is probably much higher than currently known (Luz and Faccini, 2013). The Amazon forest, the world's largest tropical rainforest, harbors an immense biodiversity of plants and animal species (Kress *et al.,* 1998; Mittermeier *et al*., 2003). More than 1,500 bird species are found in the Amazon basin (Sick, 1997; Mittermeier *et al*., 2003; Del Hoyo *et al*., 2013), representing 40% of all bird species that occur in the Neotropical Zoogeographic Region (Stotz *et al*., 1996). Despite this high diversity, the fauna of ticks associated with avian hosts is poorly known within this ecosystem. Knowledge of ticks infesting wild birds under natural conditions in the Amazonian biome is resumed to three studies, two of them from Brazil (Amazonas and Pará states; Ogrzewalska *et al*., 2010; Martins *et al*., 2014) and one from Peru (Ogrzewalska *et al*., 2012).

A series of studies have put in evidence the occurrence of a great diversity of rickettsial agents infecting ticks hosted by different vertebrate groups (amphibians, reptiles, birds, and mammals) in the Amazon biome (Labruna *et al*., 2004a; 2007; Parola *et al*., 2007; Ogrzewalska *et al*., 2010; 2012; De Barros-Lopes *et al*., 2014; Soares *et al*., 2015). Hence, knowledge of the diversity of ticks infesting Amazonian birds is of great public health concern because many of these birds have the potential to transport *Rickettsia*-infected ticks to other New World biomes, including into the Nearctic region (Mukherjee *et al*., 2014; Cohen *et al*., 2015). The present study evaluated the diversity of ticks and their rickettsial infection in wild birds from a forest fragment at the state of Acre, located in the southwestern region of Brazilian Amazon.

Materials and methods

Ethical considerations

Bird capture was authorized by the Chico Mendes Biodiversity Conservation Institute (ICMBio) of the Brazilian Environment Ministry via SISBIO license no. 23269-1 and "Centro Nacional de Pesquisa e Conservação de Aves Silvestres" (CEMAVE/ICMBIO) of the Brazilian government through Project 1099 developed under the coordination of Edson Guilherme. This study was approved by the ethical Committee of Animal use, according to ethical principles in animal research adopted by the "Animal ethic committee" of the Federal University of Acre (protocol number 55/2015).

Study site

The Brazilian state of Acre (152,581 Km^2 in area) is situated in the extreme Southwestern portion of the Northern Region of Brazil, which is primarily composed by the Amazon biome. The Northern and Eastern borders of Acre include the Brazilian states Amazonas and Rondônia, whereas the Southern and Western borders include Bolivia and Peru. The capital of Acre, Rio Branco, with 370,550 inhabitants, is the most populous city of the state (IBGE, 2015). The city has hot and humid equatorial climate, with mean annual temperature of 26.1°C. The rainy season is long and spans from October through May, and a relatively short dry season covers the remaining four months of the year. The average annual precipitation is 1,951 mm. Acre state is located entirely within the Amazon biome and within the Inambari center of bird endemism (Cracraft, 1985; Silva *et al*., 2005) with 667 registered bird species (Guilherme, 2012).

Bird capture and tick collection

Capture of birds was performed between February 2013 and August 2014 in an Amazon forest fragment situated within the campus of the Federal University of Acre, located within the city of Rio Branco. More than 200 bird species have been previously registered in the area of the university (Guilherme, 2001). Birds were caught by mist nets (12 m long × 2 m height, 36

mm mesh) with a total capture effort of 796,464 net-hours. Birds were identified with the help of bird field-guide handbooks (Hilty and Brown, 1986; Ridgely and Tudor, 1989; 1994) and the species nomenclature followed the classification of the official Brazilian bird committee, the "Comitê Brasileiro de Registros Ornitológicos" (CBRO, 2014).

Every captured bird was rigorously examined for the presence of ticks by observing the whole body by naked eyes. When present, ticks were manually removed with forceps and stored in tubes containing 70% ethanol. Then, birds were marked with numbered rings (provided by "Centro Nacional de Pesquisa e Conservação de Aves Silvestres", Brazil) and released at the same capture site.

Tick identification

In the laboratory, ticks were morphologically identified following Martins *et al*. (2010; 2013) for species of *Amblyomma* nymphs. Tick prevalence (number of infested birds/number of captured birds × 100) was calculated following Bush *et al*. (1997).

In order to confirm taxonomic identification of nymphs, and to perform species-level identification of *Amblyomma* larvae, 27 nymphs and 12 larvae were individually submitted to DNA extraction by the Guanidine Isothiocyanate technique (Sangioni *et al*., 2005). Subsequently, attempts to amplify a ≈460-bp fragment of the tick mitochondrial rRNA gene were performed as described elsewhere (Mangold *et al*., 1998). When amplification was successful, PCR products were purified with ExoSAP-IT® and sequenced in an ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyser, Foster City, CA). Obtained sequences were assembled with Geneious R9 software (Biomatters Ltd., New Zealand) and then submitted to BLAST analyses (www.ncbi.nlm.nih.gov/blast) to determine maximal similarities with available sequences in GenBank.

Detection of Rickettsia in ticks

Tick-extracted DNA samples from larvae and nymphs were individually tested by different PCR protocols targeting rickettsial DNA. The first screening PCR was performed using primers CS-78 and CS-323, which target a 401-bp fragment of the *gltA* gene that occurs in all *Rickettsia* species (Labruna *et al*., 2004b). Positive samples with expected-size fragments were then tested with primers Rr190.70F and Rr190.701R targeting a fragment of ≈632-bp of the rickettsial *ompA* gene, present only in *Rickettsia* species belonging to the spotted fever group (SFG; Regnery *et al*., 1991), and with primers 120-M59 and 120-807, which amplify a ≈832-bp fragment of the rickettsial *ompB* gene (Roux and Raoult, 2000). PCR products were sequenced and submitted to BLAST analyses as described above.

Results

Overall, 1,322 birds belonging to 16 orders and 37 families were captured. Of these, 79 (6.0%) bird specimens corresponding to 28 species were infested by 521 specimens of the following tick species: *Amblyomma nodosum* Neumann, 1899 (72 nymphs), *Amblyomma longirostre* (Koch, 1844; seven larvae, 13 nymphs), *Amblyomma humerale* Koch, 1844 (four nymphs), *Amblyomma geayi* Neumann, 1989 (two larvae, two nymphs), and 421 larvae of *Amblyomma* spp (Table 1). PCR amplification of the mitochondrial 16S rRNA gene was successful for 31 of 39 ticks submitted to DNA extraction. However, high quality sequences were obtained for only 26 specimens, confirming the morphological diagnoses of 17 nymphs and providing the taxonomic identification of nine larvae. The remaining tick larvae were regarded as *Amblyomma* spp. Voucher tick specimens have been deposited in the "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" tick collection at the University of São Paulo, SP, Brazil (accession numbers: CNC 2799-2807).

Table 1. Ticks infesting wild birds in Acre state (Brazil) from February 2013 to August 2014.

Birds				Ticks				
Order	**Family**	**Species**	**No. infested/ No. captured**	*A. geayi*	*A. humerale*	*A. longirostre*	*A. nodosum*	*Amblyomma sp*
Columbiformes	Columbidae	*Columbina talpacoti*	1/199				1N*	
Cuculiformes	Cuculidae	*Crotophaga major*	1/1				1N*	
Coraciiformes	Momotidae	*Momotus momota*	1/18			1N*		
Piciformes	Ramphastidae	*Capito auratus insperatus*	1/2			1N*	2N*	
Passeriformes	Thamnophilidae	*Thamnomanes schistogynus*	2/16		1N*		1N*	
		Thamnophilus doliatus	2/6				2N	
		Thamnophilus schistaceus	1/2				1N*	2L
		Myrmelastes hyperythrus	4/8				9N*	
		Sciaphylax hemimelaena	1/16				1N*	
		Phlegopsis nigromaculata	2/8				5N*	3L
	Scleruridae	*Sclerurus cf. rufigularis*	1/1		1N*			
	Dendrocolaptidae	*Dendrocincla merula*	2/5			1L		3L
		Xiphorhynchus guttatus	1/10			2N	1N*	
		Dendroplex picus	3/20			1N*	3N*	
	Xenopidae	*Xenops minutus*	1/2				2N	
	Pipridae	*Pipra filicauda*	1/1	1N*				
		Pipra fasciicauda	10/69			1L	7N	8L
		Machaeropterus pyrocephalus	1/20			1N*		
	Rhynchocyclidae	*Leptopogon amaurocephalus*	1/5	1L*				
		Rhynchocyclus olivaceus	14/71	1L*	2N*	1L,1N*	17N*	4L
		Todirostrum maculatum	1/1				1N*	
	Tyrannidae	*Myiozetetes similis*	1/36				1N*	
		Empidonax alnorum	2/5			4L*	1N*	
	Troglodytidae	*Pheugopedius genibarbis*	2/32				1N*	363L
	Turdidae	*Turdus hauxwelli*	1/14			2N		
		Turdus sanchezorum	1/1			1N		
		Turdus ignobilis	5/111	1N		1N	1N	2L
	Thraupidae	*Ramphocelus carbo*	15/87			2N	14N	36L
		Total	79/1,322**	2L,2N	4N	7L,13N	72N	421L

*First record of this tick species on this specific bird species. **Total number of captured birds. Larvae: L; Nymphs: N.

Infestations in each bird specimen usually consisted of few ticks (mostly one tick per infested bird; range: 1-18). The only exception was a sole individual of *Pheugopedius genibarbis* (Swainson, 1838; Passeriformes: Troglodytidae) infested with 363 larvae of *Amblyomma* spp. No ticks were found on the following bird species (number of captured individuals in parentheses): Tinamiformes, Tinamidae: *Crypturellus undulatus* (Temminck, 1815; 1); Anseriformes, Anatidae: *Dendrocygna viduata* (Linnaeus, 1766; 1); Pelecaniformes, Ardeidae: *Tigrisoma lineatum* (Boddaert, 1783; 1), *Ixobrychus exilis* (Gmelin, 1789; 1), *Butorides striata* (Linnaeus, 1758; 4), *Pilherodius pileatus* (Boddaert, 1783; 1), Accipitriformes, Accipitridae: *Rupornis magnirostris* (Gmelin, 1788; 2); Gruiformes, Aramidae: *Aramus guarauna* (Linnaeus, 1766; 1), Rallidae: *Porphyrio martinicus* (Linnaeus, 1766; 5); Charadriiformes, Charadriidae: *Vanellus chilensis* (Molina, 1782; 3), Scolopacidae: *Tringa solitaria* (Wilson, 1813; 6), Jacanidae: *Jacana jacana* (Linnaeus, 1766; 27), Columbiformes, Columbidae: *Leptotila verreauxi* Bonaparte, 1855 (15), *Leptotila rufaxilla* (Richard and Bernard, 1792; 13), *Leptotila* sp (1), *Geotrygon montana* (Linnaeus, 1758; 9); Cuculiformes, Cuculidae: *Coccycua minuta* (Vieillot, 1817; 2), *Crotophaga ani* Linnaeus, 1758 (31); Strigiformes, Strigidae: *Megascops choliba* (Vieillot, 1817; 1); Caprimulgiformes, Caprimulgidae: *Hydropsalis albicollis* (Gmelin, 1789; 2); Apodiformes, Apodidae: *Chaetura meridionalis* Hellmayr, 1907 (1), *Chaetura brachyura* (Jardine, 1846; 1), *Tachornis squamata* (Cassin, 1853; 28); Trochilidae: *Glaucis hirsutus* (Gmelin, 1788; 18), *Phaethornis ruber* (Linnaeus, 1758; 6), *Phaethornis hispidus* (Gould, 1846; 4), *Anthracothorax nigricollis* (Vieillot, 1817; 1), *Thalurania furcata* (Gmelin, 1788; 3), *Amazilia lactea* (Lesson, 1832; 11); Coraciiformes, Alcedinidae: *Chloroceryle amazona* (Latham, 1790; 5), *Chloroceryle aenea* (Pallas, 1764; 4), *Chloroceryle americana* (Gmelin, 1788; 2), *Chloroceryle inda* (Linnaeus, 1766; 1); Galbuliformes, Bucconidae: *Bucco macrodactylus* (Spix, 1824; 4), *Monasa nigrifrons* (Spix, 1824; 9); Piciformes, Picidae: *Picumnus rufiventris* (Bonaparte, 1838; 3), *Veniliornis affinis* (Swainson, 1821; 5), *Veniliornis passerinus* (Linnaeus, 1766; 4), *Colaptes punctigula* (Boddaert, 1783; 4), *Celeus spectabilis* Sclater and Salvin, 1880 (2), *Campephilus melanoleucos* (Gmelin, 1788; 1); Psittaciformes, Psittacidae: *Aratinga weddellii* (Deville, 1851; 2), *Brotogeris sanctithomae* (Statius Muller, 1776; 3); Passeriformes, Thamnophilidae: *Myrmotherula axillaris* (Vieillot, 1817; 10), *Thamnophilus murinus* Sclater and Salvin, 1868 (3), *Taraba major* (Vieillot, 1816; 1), *Myrmelastes schistaceus* (Sclater, 1858; 4), *Hypocnemis subflava* Cabanis, 1873 (2); Dendrocolaptidae: *Dendrocincla fuliginosa* (Vieillot, 1818; 9), *Campylorhamphus trochilirostris* (Lichtenstein, 1820; 5); Furnariidae: *Furnarius leucopus* Swainson, 1838 (1); Pipridae: *Neopelma sulphureiventer* (Hellmayr, 1903; 9); Rhynchocyclidae: *Corythopis torquatus* Tschudi, 1844 (2), *Hemitriccus flammulatus* Berlepsch, 1901 (6), Tyrannidae: *Elaenia spectabilis* Pelzeln, 1868 (5), *Elaenia parvirostris* Pelzeln, 1868 (13), *Tyrannulus elatus* (Latham, 1790; 1), *Phaeomyias murina* (von Spix, 1825; 2), *Pitangus sulphuratus* (Linnaeus, 1766; 22), *Philohydor lictor* (Lichtenstein, 1823; 1), *Myiodynastes maculatus* (Statius Muller, 1776; 2), *Tyrannopsis sulphurea* (Spix, 1825; 1), *Megarynchus pitangua* (Linnaeus, 1766; 1), *Myiozetetes cayanensis* (Linnaeus, 1766; 24), *Tyrannus melancholicus* Vieillot, 1819 (39), *Pyrocephalus rubinus* (Boddaert, 1783; 4), *Cnemotriccus fuscatus* (Wied, 1831; 1); Vireonidae: *Cyclarhis gujanensis* (Gmelin, 1789) (1), *Vireo olivaceus* (Linnaeus, 1766; 2), *Vireo chivi* (Vieillot, 1817; 5), *Vireo flavoviridis* (Cassin, 1851; 1); Hirundinidae: *Stelgidopteryx ruficollis* (Vieillot, 1817; 18); Troglodytidae: *Troglodytes musculus* Naumann, 1823 (13), *Cantorchilus leucotis* Swainson, 1831 (16); Turdidae: *Turdus amaurochalinus* Cabanis, 1850 (1); Icteridae: *Cacicus cela* (Linnaeus, 1758; 4); Thraupidae: *Tangara mexicana* (Linnaeus, 1766; 1), *Tangara episcopus* (Linnaeus, 1766; 18), *Tangara palmarum* (Wied, 1821; 8), *Volatinia jacarina* (Linnaeus, 1766; 2), *Sporophila lineola* (Linnaeus, 1758; 1), *Sporophila castaneiventris* Cabanis, 1849 (11), *Sporophila angolensis* Cabanis, 1847 (1), *Sporophila* sp (1), *Saltator maximus* (Statius Muller, 1776; 4), *Saltator coerulescens* Vieillot, 1817 (4), *Saltator azarae* d'Orbigny, 1839 (2); Fringillidae: *Euphonia chlorotica* (Linnaeus, 1766; 1), Emberizidae: *Ammodramus aurifrons* (Spix, 1825; 23).

Rickettsia DNA was detected in 29.0% (9/31) of the tested ticks, from which fragments of the *gltA, ompA*, and *ompB* genes were obtained. The eight ticks with negative results for the tick

mitochondrial 16S rRNA PCR were excluded from this analysis. Sequences from three *A. nodosum* nymphs were 100% identical to *Rickettsia* sp strain NOD (GenBank accession numbers EU567177, EU567180, EU567179 for *gltA, ompA, ompB,* respectively). Sequences from four larvae and one nymph of *A. longirostre*, and one larva of *A. geayi* were 100% identical to *Rickettsia amblyommatis* (="*Candidatus* Rickettsia amblyommii"; GenBank accession numbers JX867425, KF702333, JX867427 for *gltA, ompA, ompB,* respectively). GenBank accession numbers for the partial sequences of the tick mitochondrial 16S rRNA obtained in the present study were deposited in GenBank and received the accession numbers KX999294 (*A. geayi*, larva), KX999295 (*A. longirostre*, larva), and KX999296 (*A. nodosum*, nymph). GenBank accession numbers for the partial sequences of the rickettsial genes *gltA, ompA* and *ompB* are KY008392*,* KY008394 and KY008396 for *Rickettsia* sp strain NOD, and KY008393, KY008395 and KY008397 for *R. amblyommatis*.

Discussion

Four tick species, *A. geayi, A. humerale, A. longirostre* and *A. nodosum,* were found parasitizing wild birds in Acre state, similarly to two previous studies in eastern and western Brazilian Amazon, where these four tick species were also found parasitizing passerine hosts (Ogrzewalska *et al*., 2010; Martins *et al*., 2014). While the number of studies about ticks of passerine birds is still very low for the Amazon biome, current data suggest that *A. geayi, A. humerale, A. longirostre* and *A. nodosum* are at least among the most common bird-associated ticks of the Amazon. In addition, for the first time, the present study reports 27 bird species parasitized with at least one of these four *Amblyomma* species (Table 1).

Amblyomma longirostre and *A. nodosum* were the most prevalent tick species in the present study. Adults of *A. longirostre* feed primarily on porcupines (Rodentia, Erethizontidae), whereas adults of *A. nodosum* are commonly found on anteaters, *Myrmecophaga tridactyla* Linnaeus, 1758 and *Tamandua* spp (Guglielmone *et al*., 2014). Larvae and nymphs of these two tick species seem to feed primarily on birds (reviewed by Luz and Faccini, 2013; Ogrzewalska and Pinter, 2016), an ecological association reinforced by results of the present study. These species were also found migrating to the North.

While adults of *A. humerale* feed primarily on tortoises (*Chelonoidis* spp), adults of *A. geayi* feed on sloths (*Bradypus* spp and *Choloepus* spp; Guglielmone *et al*., 2014). Based on these records of mature stages, established populations of both tick species are known to be almost restricted to the Amazon biome, yet hosts records for their immature stages are scarcely documented and currently limited to passerine birds and medium-sized mammals (Labruna *et al*., 2002; Ogrzewalska *et al*., 2010; Martins *et al*., 2013; 2014; Soares *et al*., 2015). Our results, in conjunction with these previous reports, suggest that passerine birds may also play an underestimated role as hosts for immature stages of *A. humerale* and *A. geayi*.

According to literature data (Aragão, 1936; Guimarães *et al*., 2001; Souza *et al*., 2016), the following nine tick species have been previously reported in Acre state: *Amblyomma calcaratum* Neumann, 1899, *Amblyomma coelebs* Neumann, 1899, *Amblyomma dissimile* Koch, 1844, *A. geayi*, *Amblyomma incisum* Neumann, 1906, *A. longirostre*, *Amblyomma oblongoguttatum* Koch, 1844, *Amblyomma ovale* Koch, 1844, and *Rhipicephalus microplus* (Canestrini, 1888). According to Labruna *et al*. (2005), previous reports of *A. incisum* from the Amazonian region require confirmation, as they could be misidentified with *Amblyomma scalpturatum* Neumann, 1906 or *Amblyomma latepunctatum* Tonelli-Rondelli, 1939*;* hence, we do not consider valid the report by Aragão (1936) of *A. incisum* in Acre.

Herein, we report two species, *A. humerale* and *A. nodosum,* for the first time in Acre. In addition, the CNC tick collection contains the following unpublished records of ticks in Acre: *A. scalpturatum* (CNC-1663: One male ex. vegetation, Xapuri, 17 July 2008); *Amblyomma rotundatum* (CNC-1667: One female ex. *Rhinella* sp, Assis Brasil, December 2009), and *Ixodes luciae* (CNC-1665, 1666, 2870: 10 males, eight females, ex. *Didelphis marsupialis*, Assis Brazil, December 2009). These whole data indicate that the tick fauna in Acre is currently represented by 13 tick species.

We report two SFG rickettsial agents, *Rickettsia* sp strain NOD in *A. nodosum* ticks, and *R. amblyommatis* in *A. longirostre* and *A. geayi* ticks. These *Rickettsia*-tick associations have been previously reported in other areas of Brazil (Ogrzewalska *et al*., 2009; 2010; Soares *et al*., 2015), reinforcing that these are not just occasional associations. In these previous studies, *R. amblyommatis* was reported as *R. amblyommii* or '*Candidatus* R. amblyommii'. However, a recent study reassessed the taxonomic status of this agent, which is now validated as *R. amblyommatis* (Karpathy *et al*., 2016). Despite a nearly widespread distribution of tick infection by these two rickettsial agents in South America, including the Amazon region, their role as human pathogens remains unknown. Finally, the present study provides a basic background occurrence of immature stages of *Amblyomma* spp in wild birds at the southwestern Brazilian Amazon, and for the dispersion of *Rickettsia*-infected ticks.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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