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## *Rickettsia parkeri* strain Atlantic rainforest in ticks (Acari: Ixodidae) of wild birds in Arauca, Orinoquia region of Colombia

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### ABSTRACT

Birds are important hosts for the development of the immature stages of several tick species that are vectors for disease-causing microorganisms in animals and humans. Colombia has the highest number of bird species worldwide; however, there is scarce data on the role of birds in the circulation of ticks and their associated pathogens, such as rickettsiae. The department of Arauca has a high diversity of resident and migratory (boreal and austral) birds and ticks associated with the transmission of *Rickettsia*. The objective of this research was to identify tick species parasitizing birds and to detect *Rickettsia* species in these ectoparasites. We conducted samplings in the municipalities of Arauca, Cravo Norte, and Tame between November of 2018 and August of 2019. Birds were captured using mist nets and examined for the presence of tick species. The collected ticks were morphologically and molecularly identified. Furthermore, we detected rickettsiae in ticks by amplifying fragments of the citrate synthase (*gltA*) and outer membrane protein (*ompB*) genes. We captured 606 birds belonging to 25 families and 115 species. Tick infestation rate was 3.3% (20/606) in the birds captured and eight new associations between wild birds and ticks are reported for the American continent. We identified four tick species: *Amblyomma nodosum*, *Amblyomma longirostre*, *Amblyomma mixtum*, and *Amblyomma* sp.. Moreover, we confirmed the presence of *Rickettsia parkeri* strain Atlantic rainforest in *A. nodosum*, a medically-relevant rickettsia due to cases of rickettsiosis in the American continent. This finding manifests the importance of wild birds as hosts and dispersal agents of ticks infected with pathogenic rickettsiae, as well as the need to monitor migratory birds in the Orinoquia and other regions of Colombia and America.

### 1. Introduction

Wild birds have an important role in the life cycle of several tick species since birds serve as hosts for the immature stages of larvae and

nymphs and, in some cases of ornithophilic ticks (Ogrzewalska et al., 2009a; Flores et al., 2014; Ramos et al., 2015). Ticks can act as vectors or reservoirs of pathogenic bacteria, such as rickettsiae, which can be transmitted to animals and humans (Sonenshine et al., 2002; Parola

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et al., 2013; Luz et al., 2017). Ticks have limited locomotion capacity; therefore, their dispersal largely depends on hosts (Randolph, 1998). In this sense, bird hosts can disperse ticks between habitats or even continents during migrations (Baneth, 2014; Mukherjee et al., 2014; Cohen et al., 2015; Budachetri et al., 2017). In America, there are records of several ticks species of the genera *Amblyomma*, *Ixodes*, *Haemaphysalis* (family Ixodidae), *Ornithodoros*, and *Argas* (family Argasidae) that parasitize resident and migratory birds (Barros-Battesti et al., 2006; Ogrzewalska et al., 2008, 2015; Mukherjee et al., 2014; Cohen et al., 2015). Colombia has the highest bird diversity in the world, with 1632 resident species and 139 migratory boreal and austral species (Avendaño et al., 2017). In Colombia, there are 58 tick species (43 Ixodidae and 15 Argasidae) (Rivera-Páez et al., 2018a), including eight species associated with wild birds: *Ixodes auritulus* (González-Acuña et al., 2005), *Amblyomma calcaratum*, *Amblyomma dissimile*, *Amblyomma longirostre*, *Amblyomma nodosum*, *Amblyomma ovale*, *Amblyomma varium*, and *Haemaphysalis leporispalustris* (Osorno-Mesa, 1940; Martínez-Sánchez et al., 2020). The record of *Ixodes brunneus* in *Calochaetes coccineus* should be revised (Osorno-Mesa, 1940). In addition, three *Ixodes* species, which yielded low identity DNA sequences ( $\leq 95\%$ ) to any tick species in GenBank are also known (Martínez-Sánchez et al., 2020).

Ticks can transmit bacteria that cause rickettsial diseases (Sonenshine and Clifford, 1973; Ogrzewalska et al., 2009b; Cohen et al., 2015). In particular, these diseases are attributed to infection by Gram-negative bacteria of the genus *Rickettsia*, which comprise four groups: i) the typhus group (TG) with two members, namely *Rickettsia prowazekii* and *Rickettsia typhi*; ii) the spotted fever group (SFG) that contains more than 20 species, including *Rickettsia rickettsii* and *Rickettsia parkeri*; iii) the transitional group (TRG), which comprises *Rickettsia akari* and *Rickettsia felis*; and iv) the ancestral group (AG) that includes *Rickettsia belli* and *Rickettsia canadensis* (Quintero et al., 2013). Specifically, TG and SFG generate the highest level of concern for public health (Londoño et al., 2017); for instance, *R. rickettsii* is the most pathogenic species in this genus, with reported lethality rates between 20% and 95% for the American continent (Abarca and Oteo, 2014). Particularly, in Colombia, there are lethality rates between 26.6% and 95% (Quintero et al., 2013; Miranda et al., 2017). *R. rickettsii* is the causal agent of Rocky Mountain spotted fever (RMSF), Brazilian spotted fever, or Tobia fever in Colombia (Labruna et al., 2011; Oteo et al., 2014). Moreover, there are two strains of *R. parkeri* known to cause rickettsiosis, namely *R. parkeri* sensu stricto and *R. parkeri* strain Atlantic rainforest (Londoño et al., 2019). Like in other spotted fever group (SFG) rickettsiae, *R. parkeri* is transovarially (from female to eggs) and transestadially (from one life history stage to the next) transmitted to ticks. Also, *R. parkeri* can be horizontally acquired while tick feeds on a rickettsemic host (Goddard, 2003; Walker and Ismail, 2008).

Historically, *R. rickettsii* has caused outbreaks of febrile illness in several regions of Colombia, such as the central (department of Cundinamarca) and northwestern regions (departments of Córdoba and Antioquia); therefore, these two regions of considered endemic areas for rickettsiosis (Patiño et al., 1937; Patiño, 1941; Acosta et al., 2006; Hidalgo et al., 2007a, Hidalgo et al., 2007b, 2011; Vélez et al., 2012). Additionally, the Orinoquia region has also been proposed as an endemic area for rickettsial disease associated with the SFG, due to the confirmed circulation of rickettsiae and their vector *Amblyomma mixtum* in this region (Miranda et al., 2011; Riveros-Pinilla et al., 2015; Rivera-Páez et al., 2016; Gómez-Quintero et al., 2017; Rivera-Páez et al., 2018b). In the last decade, there were reports of the pathogenic strain *Rickettsia parkeri* Atlantic rainforest associated with *Amblyomma ovale* in the departments of Córdoba and Antioquia (northwestern region).

*R. parkeri* strain Atlantic rainforest is phylogenetically closely related to *R. parkeri*, *Rickettsia africana*, and *Rickettsia sibirica* (Spolidorio et al., 2010; Londoño et al., 2014; Nieri-Bastos et al., 2018). *R. africana* and *R. sibirica* are pathogenic species distributed in the Old World, and the symptoms they cause are similar to those caused by New World species such as *R. parkeri* sensu stricto, and *R. parkeri* strain Atlantic rainforest

(Paddock et al., 2004; Parola et al., 2005; Pacheco et al., 2012). Using genetic evidence, Nieri-Bastos et al. (2018), defined that Atlantic rainforest is *R. parkeri* strain found in the southern part of South America that is transmitted by ticks of the *A. ovale* complex (i.e., *A. ovale* and *Amblyomma aureolatum*). Other tick species of the *Amblyomma maculatum* complex (*A. maculatum*, *Amblyomma triste*, and *Amblyomma tigrinum*), *A. nodosum*, *Amblyomma parvitarsum* and *Dermacentor parumapertus* have been involved in the transmission of *R. parkeri* in America (Paddock et al., 2004, 2017; Nieri-Bastos et al., 2018; Londoño et al., 2019).

*A. ovale* was found parasitizing resident and migratory birds (e.g., *Formicivora grisea* and *Parkesia noveboracensis*) in Colombia (Martínez-Sánchez et al., 2020). However, *R. parkeri* strain Atlantic rainforest has not been detected in ticks associated with birds, although it has been detected in ticks on wild and domestic mammals in the northwestern region of Colombia (Londoño et al., 2014, 2017). The only reports of genus *Rickettsia* associated with ticks in the Orinoquia region, are the reports of *R. rickettsii* on ticks of domestic animals (Rivera-Páez et al., 2018b).

Given that migratory boreal and austral birds converge in the Colombian Orinoquia region (McNish, 2007; Ocampo-Peñuela, 2010), these bird species could serve as hosts and dispersal agents of ticks infected with rickettsiae. The department of Arauca in the Orinoquia (Eastern Plains) is an ideal site to study the associations among birds, ticks, and rickettsiae. Given the above, this study aimed to identify tick species associated with wild birds and detect ticks infected with rickettsiae.

## 2. Materials and methods

### 2.1. Study area

This study was conducted in eight localities in the municipalities of Arauca, Cravo Norte, and Tame, located in the department of Arauca in the Colombian Orinoquia (Table 1, Fig. 1). The region shows a typical savanna climate with a well-defined wet season between June and July and a very dry season between December and April. This precipitation pattern leads to occasional periods of flooding and drought in the Orinoquia savanna during the corresponding seasons. This region also shows high temperatures year-round with a mean of 27 °C (McNish, 2007).

The sampled localities are divided into two sub-regions: the flooded savanna (*Savana Inundable*) and the Llanos foothills (*Piedemonte Llanero*) (Rodríguez-Durán, 2019). The flooded savanna comprises estuaries, gallery forests, and isolated forests locally known as “matas de monte” (McNish, 2007). The Llanos foothills are a sub-region between 250 m and 500 m of elevation, adjacent to the Eastern mountain range of Colombia. Both sub-regions are destined for agropecuary activities, such as rice, oil palm, corn, and plantain crops and extensive livestock farming (Vilorio de la Hoz, 2009). We conducted samplings in the eight localities to identify bird species parasitized by ticks and to detect possible infections of these ticks with rickettsiae. Localities 1–5 were sampled between November and December of 2018, locality 6 was sampled in March, and localities 7 and 8 were sampled between July and August of 2019 (Table 1, Fig. 1). The sampling dates coincided with the arrival of migratory birds from the northern (migratory boreal) and southern (austral migratory) hemispheres.

### 2.2. Bird captures and tick collection

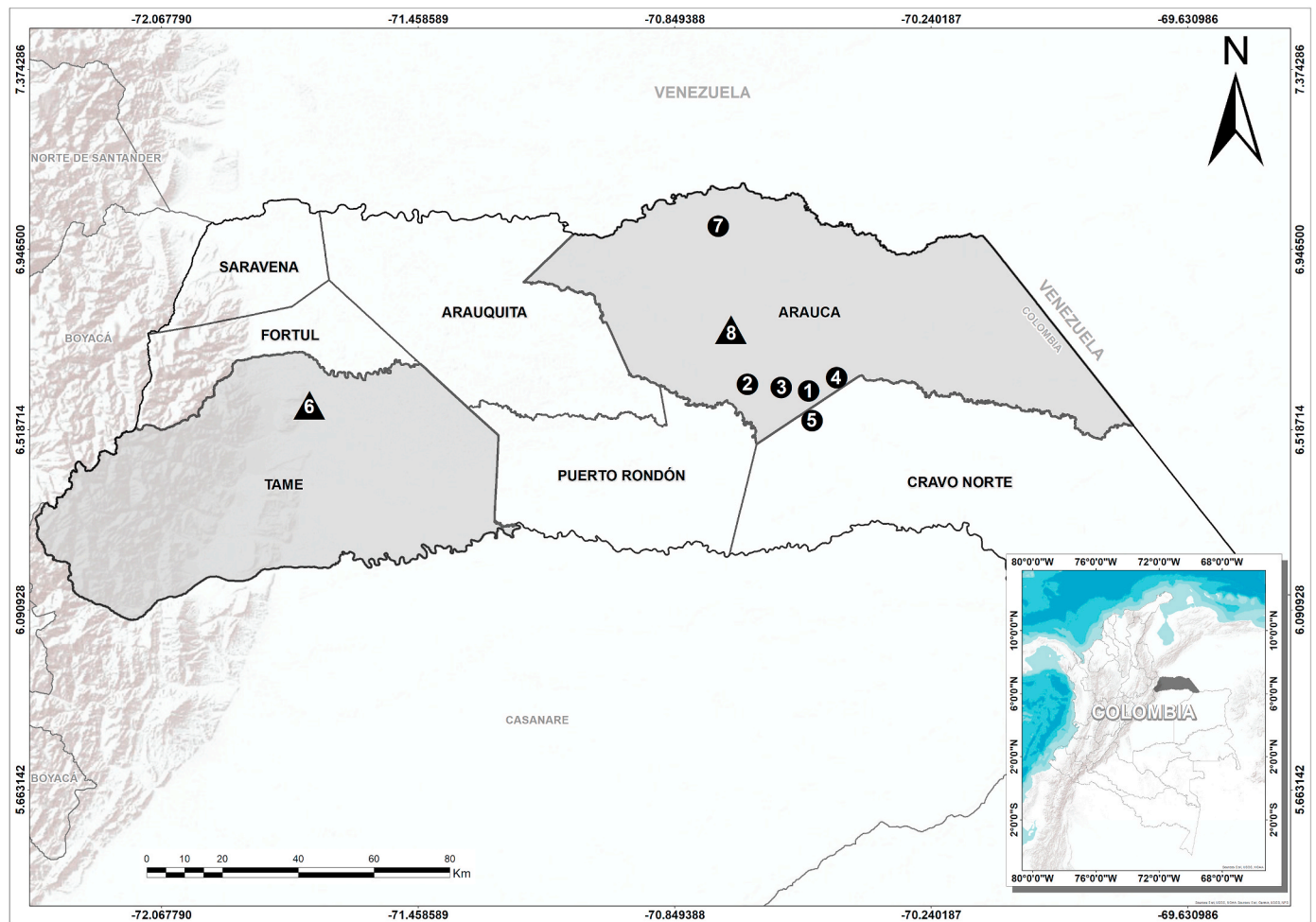
Birds were captured using eight mist nets at each locality (12 m long × 2 m wide, 36 mm mesh). The mist nets were randomly installed and opened between 6:00 and 17:00 h. The birds were taxonomically classified according to Remsen et al. (2020). The residency status of the birds (i.e., resident, migratory boreal or austral) was defined based on Avendaño et al. (2017). Each bird was completely examined for ticks for

**Table 1**

Descriptions of the localities sampled in the department of Arauca, Colombia, where birds were captured in this study.

Municipality	Locality	Locality number <sup>a</sup>	Geographical coordinates	Altitude (m above sea level)	Habitat type
Arauca	Las Plumas	1	06°36'40" N 70°31'51" W	120	Floodplain forest, Gallery forest
Arauca	Las Plumas	2	06°37'01" N 70°31'30" W	123	Floodplain forest, Medano
Arauca	Las Plumas	3	06°36'15" N 70°29'52" W	123	Floodplain forest
Arauca	Las Plumas	4	06°36'15" N 70°29'52" W	112	Floodplain forest
Cravo Norte	El Deleite	5	06°32'15" N 70°31'14" W	111	Floodplain forest
Tame	Santa Inés	6	06°24'52" N 71°32'04" W	253	Agricultural area
Arauca	Km 9, via Arauca-Arauquita	7	07°00'53" N 70°44'36" W	120	Wooded areas; High stubble
Arauca	El Socorro	8	06°46'39" N 70°42'25" W	134	Floodplain forest, Gallery forest

<sup>a</sup> Locality numbers indicated in Fig. 1.



**Fig. 1.** Localities sampled in the municipalities of Arauca, Cravo Norte, and Tame and reports of *Rickettsia* spp. in the study area (▲*Rickettsia parkeri* strain Atlantic rainforest).

5 min. The ticks were collected with entomological tweezers and conserved in Eppendorf tubes with 96% ethanol. The birds were marked with a small cut in the first rectrix of the tail to avoid re-counting the individuals and then, released at the capture site.

**2.3. Identification of ticks and rickettsiae**

The ticks collected were taxonomically identified based on their external morphology according to the literature and taxonomical keys (Kohls, 1956; Jones et al., 1972; Barros-Battesti et al., 2006; Mehlhorn, 2008; Martins et al., 2010; Nava et al., 2014, 2017). The prevalence of tick infestation in birds was calculated as (Number of infested individuals/Number of examined individuals) x 100.

The nymphs and larvae were molecularly confirmed through PCR amplification of two mitochondrial gene fragments. First, DNA extraction was performed using the DNeasy Blood and Tissue (Qiagen) and Wizard® Genomic DNA Purification (Promega) kits, according to the manufacturer’s instructions. Next, we amplified a 460 bp fragment of the 16S rDNA gene with primers 16S F 5'-CCGGTCTGAACTCAGATCAAGT-3' and 16S R 5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3' (Norris et al., 1996; Mangold et al., 1998), as well as a 700 bp fragment of the cytochrome oxidase subunit I (COI) using primers LCO1490 F 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 R 5'-TAAACTTCAGGTGACCAAAAAATCA-3' (Folmer et al., 1994). The voucher specimens of the ticks were deposited in the ectoparasite collection of the Museo de Historia Natural de la Universidad de Caldas

(MHN-UCa).

For the detection and molecular identification of *Rickettsia* species, we evaluated 30 ticks (14 larvae, 16 nymphs) and amplified a ~401 bp fragment of the citrate synthase (*gltA*) gene using primers CS-78 and CS-323 (Labruna et al., 2004). The *gltA* gene is present in all species of the genus *Rickettsia*. We performed a second PCR amplification on the samples that were positive for *gltA* using primers rompB-OF and rompB-OR, proposed by Choi et al. (2005), which amplify a fragment of 511 bp of the outer membrane protein (*ompB*) that is present in *Rickettsia* species of the spotted fever group (SFG) (Choi et al., 2005). In each set of reactions, negative (ultrapure water) and positive controls for *Rickettsia vini* DNA (kindly supplied by Dr. Marcelo Bahia Labruna) were included. The PCR products were visualized through 1.0% agarose gel electrophoresis run with TBE 1X (pH 8.0) buffer at 110 V/50 mA and stained with SYBR® Safe dye. The products were visualized on a Gel Doc-It2 310 (UVP) photodocumenter and purified using Wizard® SV Gel and PCR Clean-Up System (Promega), according to the manufacturer's instructions. The purified amplicons were Sanger sequenced at Macrogen Inc. (South Korea). The quality of the sequences was analyzed using Geneious Prime® 2019.1.3 and sequence alignments were performed with MEGAX (Kumar et al., 2018). Nucleotide divergences were estimated in MEGA X, using the Kimura 3-parameters distance model (Tamura, 1992). Species confirmation was performed using a Maximum Likelihood (ML) similarity analysis using the Kimura 3-parameters distance model and 1000 iterations in MEGA X. The alignment included 13 different sequences gathered from GenBank, where a total of 456 unequivocally nucleotide sites of the rickettsial outer membrane protein (*ompB*) gene were aligned. We identified and analyzed the tick and *Rickettsia* species based on similarity comparisons to public sequences in GenBank and BOLD (Barcode of Life Data Systems) databases. The sequences obtained in this study were deposited in GenBank.

This research was conducted under the framework permit granted to Universidad de Caldas by the Autoridad Nacional de Licencias Ambientales (ANLA) of Colombia, according to Resolution 02497 of December 31st of 2018, and the approval of the bioethics committee of the Faculty of Exact and Natural Sciences of Universidad de Caldas (June 2nd of 2017).

### 3. Results

We captured 606 birds belonging to 25 families and 115 species

**Table 2**

Wild bird species infested with ticks in the department of Arauca and results of BLAST searches for the DNA sequences of the ticks collected in this study.

Locality <sup>a</sup>	Host Bird	Tick species (number of specimens/ stage)	No. infested/No. tested (%)	Closest identity (gene: accession number)	
	<b>Family</b>				
6	Cracidae	<i>Ortalis ruficauda</i>	<i>Amblyomma mixtum</i> (2/nymphs)	1/1 (100)	<i>A. mixtum</i> [COI: MF363073.1] 100%
7,8	Thamnophilidae	<i>Formicivora grisea</i>	<i>Amblyomma nodosum</i> (4/nymphs)	2/5 (40)	<i>A. nodosum</i> [16S: MH818417.1] 99.2%
7	Furnariidae	<i>Dendroplex picus</i>	<i>A. nodosum</i> (1/larva; 1/nymphs) <sup>b</sup>	1/4 (25)	N.D <sup>c</sup>
7	Tyrannidae	<i>Camptostoma obsoletum</i>	<i>Amblyomma longirostre</i> (1/nymph) <sup>b</sup>	1/20 (5)	N.D <sup>c</sup>
6	Tytridae	<i>Pachyrhamphus polychopterus</i>	<i>A. longirostre</i> (1/larva)	1/7 (14.3)	<i>A. longirostre</i> [16S: MH818419.1] 100%
7	Troglodytidae	<i>Troglodytes aedon</i>	<i>A. nodosum</i> (440/larvae) <sup>d</sup>	2/10 (20)	<i>A. nodosum</i> [16S: FJ424402.1] 99.6%
4		<i>Campylorhynchus griseus</i>	<i>Amblyomma</i> sp. (2/larva) <sup>b</sup>	1/4 (25)	N.D <sup>c</sup>
3	Turdidae	<i>Turdus leucomelas</i>	<i>A. nodosum</i> (1/nymphs)	1/8 (12.5)	<i>A. nodosum</i> [16S: MH818417.1] 99.7%
6		<i>Turdus ignobilis</i>	<i>A. nodosum</i> (1/nymphs)	1/7 (14.3)	<i>A. nodosum</i> [16S: MH818417.1] 99.7%
6	Fringillidae	<i>Euphonia lanirostris</i>	<i>Amblyomma</i> sp. (1/larva) <sup>b</sup>	1/5 (20)	N.D <sup>c</sup>
6	Icteridae	<i>Icterus chrysater</i>	<i>A. nodosum</i> (1/nymph)	1/2 (50)	<i>A. nodosum</i> [16S: KP686064.1] 100%
7,8	Thraupidae	<i>Ramphocelus carbo</i>	<i>A. nodosum</i> (2/nymphs)	2/13 (15.4)	<i>A. nodosum</i> [16S: FJ424402.1] 100%
6		<i>Sporophila angolensis</i>	<i>A. nodosum</i> (3/nymphs)	2/5 (40)	<i>A. nodosum</i> [16S: MH818417.1] 99.5%
7		<i>Sporophila intermedia</i>	<i>A. nodosum</i> (1/nymph)	1/19 (5.3)	<i>A. nodosum</i> [16S: FJ424402.1] 99.6%
7,8		<i>Saltator coerulescens</i>	<i>A. nodosum</i> (3/nymphs)	2/9 (22.2)	<i>A. nodosum</i> [16S: FJ424402.1] 99.6%
		<b>Total: 465 Ticks (445 larvae, 20 nymphs)</b>			

<sup>a</sup> Locality numbers indicated in Table 1 and Fig. 1.

<sup>b</sup> Identification by external morphology.

<sup>c</sup> N.D: Not Done (poor DNA quality).

<sup>d</sup> Larvae were morphologically assigned to the same morphotype and a selected number of individuals were randomly selected for molecular identification.

(Table S1), including 105 resident species (576 individuals), two austral migratory species (two individuals), and eight boreal migratory species (28 individuals). The prevalence of tick infestation was 3.3% (20/606) and the infested individuals comprised 15 species (Table 2). We collected 465 ticks (445 larvae and 20 nymphs), all belonging to the genus *Amblyomma* (Acari: Ixodidae) (Table 2). The infested birds belonged mainly to the families Thraupidae (*Ramphocelus carbo*, *Sporophila angolensis*, *Sporophila intermedia*, and *Saltator coerulescens*), Turdidae (*Turdus leucomelas* and *Turdus ignobilis*), and Troglodytidae (*Troglodytes aedon* and *Campylorhynchus griseus*) (Table 2). The species *T. aedon* showed high parasite load (440 larvae), while we did not find tick infestation on migratory birds from the northern and southern hemispheres (Table S1). We molecularly confirmed the identity of *Amblyomma nodosum*, *Amblyomma longirostre*, and *Amblyomma mixtum*. It was not possible to confirm the molecular identity of six ticks (poor DNA quality); therefore, these individuals were morphologically identified (Table 2). We report 15 associations between wild birds and ticks, which involve 15 bird species and four tick species. In particular, we found eight new associations for the Americas (Table 2), *T. aedon*, *Icterus chrysater*, *S. angolensis*, *S. intermedia*, *S. coerulescens* infested with *A. nodosum*, *Ortalis ruficauda* infested with *A. mixtum*, and *Camptostoma obsoletum* with *A. longirostre*. Furthermore, *C. griseus* is documented, for the first time infested by *Amblyomma* ticks.

We detected a prevalence of 6.6% (2/30) of *Rickettsia* infection in the tick species *A. nodosum* (Table 3). The partial gene sequences of *gltA* and *ompB* for *Rickettsia parkeri* strain Atlantic rainforest showed both 100% identity to the corresponding sequences available in GenBank (Table 3). The phylogenetic analysis based on the *ompB* gene (Fig. 2) nested our sequences with *R. parkeri* strain Atlantic rainforest, *R. parkeri* strain Portsmouth, *R. parkeri* s. s and *R. africae* (bootstrap support: 82%).

The infested *A. nodosum* individuals were found associated with hosts *S. angolensis* and *Formicivora grisea* in localities 6 (Tame) and 8 (Arauca), respectively. The GenBank accession numbers of the nucleotide sequences obtained in this study are [MT471971-MT471980] for the 16S rRNA mitochondrial gene; [MT439632] for the COI mitochondrial gene; [MT501330-MT501331] for the citrate synthase *gltA* gene; [MT501328-MT501329] for the outer membrane protein *ompB* gene.

### 4. Discussion

We found eight new associations between birds and ticks, including

**Table 3**

Tick species infected with *Rickettsia* in the department of Arauca and results of BLAST searches for the DNA sequences of the rickettsiae detected in this study.

Host Bird	Tick species	No. infected/ No. tested (%)	Closest identity (%) in GenBank (accession number) according to the <i>Rickettsia</i> gene	
			gltA	ompB
<i>F. grisea</i>	<i>A. nodosum</i>	1/30 (3.33)	<i>R. parkeri</i> strain Atlantic rainforest [MN027564] 100%	<i>R. parkeri</i> strain Atlantic rainforest [CP040325] 100%
<i>S. angolensis</i>	<i>A. nodosum</i>	1/30 (3.33)	<i>R. parkeri</i> strain Atlantic rainforest [MN027564] 100%	<i>R. parkeri</i> strain Atlantic rainforest [CP040325] 100%

five between bird species *T. aedon*, *I. chrysater*, *S. angolensis*, *S. intermedia*, *S. coerulescens* and the tick species *A. nodosum*, as well as two associations between *O. ruficauda* and *C. obsoletum* and ticks *A. mixtum* and *A. longirostre*, respectively. Moreover, we provide the first report of infestation of *C. griseus* with ticks of the genus *Amblyomma*. On the other hand, seven interactions found here were previously reported in the literature (Tolesano-Pascoli et al., 2010; Ogrzewalska et al., 2011b; Luz et al., 2012; Pascoal et al., 2012; Torga et al., 2013; Ramos et al., 2015; Lugarini et al., 2015; Lima et al., 2018; Martínez- Sánchez, in 2020). These findings support the assumption of the important role of wild birds in the life cycle of several Neotropical tick species by serving as hosts for immature stages (Ogrzewalska et al., 2009a, 2009b; Budachetri et al., 2017; Nava et al., 2014; Lugarini et al., 2015). Additionally, the tick species *A. nodosum* and *A. longirostre* had not been recorded in the department of Arauca; therefore, our results expand the known distribution of these species in Colombia (Osorno-Mesa, 1940; Luque, 1948; Wells et al., 1981; Benavides-Montaña et al., 2018; Rivera-Páez et al., 2018a; Acevedo-Gutiérrez et al., 2020).

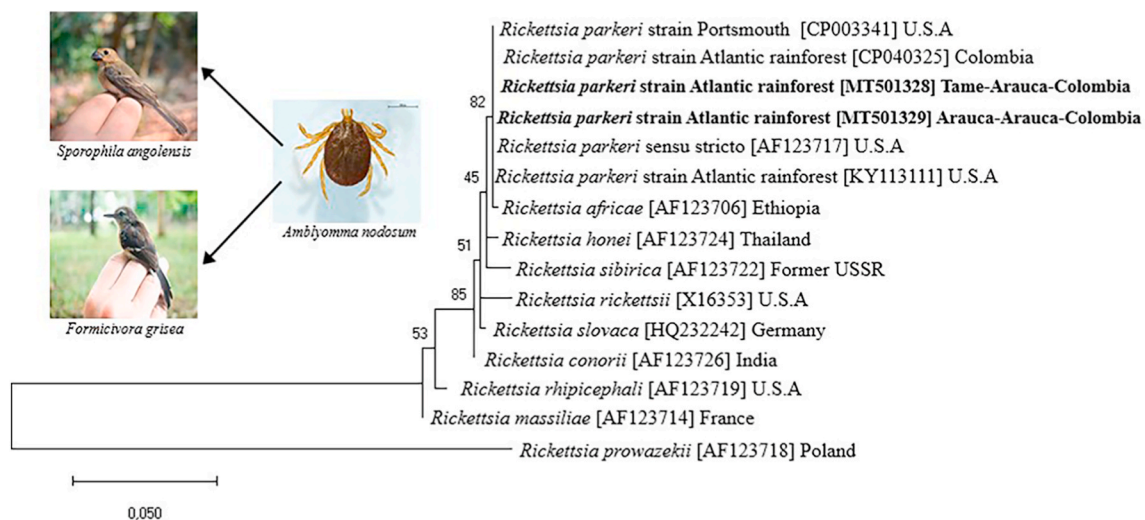
We found that 93% of the infested bird species were Passeriformes, which agrees with previous research conducted in Brazil (Labruna et al., 2007; Ogrzewalska et al., 2009a; Lugarini et al., 2015; Luz et al., 2017). Furthermore, these studies state that Passeriformes are important hosts for ticks, such as *A. longirostre* and *A. nodosum*, due to the high frequency in which immature stages of both tick species parasitize Passeriformes

birds. Therefore, Passeriformes are considered their primary hosts (Nava et al., 2017).

In this study the birds parasitized by *A. longirostre* (*C. obsoletum* and *Pachyramphus polychopterus*) seek food in canopy and subcanopy (Hilty and Brown, 1986; Restall et al., 2007; Del Hoyo et al., 1992–2011), therefore, it has been hypothesized that this tick completes its life cycle in tree canopies (Labruna et al., 2007; Nava et al., 2017; Suzin et al., 2020). Other bird species parasitized by *A. nodosum* (Table 2) inhabit in lower strata, such as in the understory and ground (Hilty and Brown, 1986; Restall et al., 2007; Del Hoyo et al., 1992–2011), which seems to be related to tick infestation as mentioned by Labruna et al. (2007).

Other bird species belonging to the orders Gruiformes, Ciconiformes, and Galliformes were found as hosts for immature stages of ticks of the *Amblyomma cajennense* complex (Labruna et al., 2007; Ogrzewalska et al., 2009a; Acevedo-Gutiérrez et al., 2020). Similarly, we found *O. ruficauda* (Galliforme) infested with the species *A. mixtum*, which belongs to the *Amblyomma cajennense* complex. The association between *O. ruficauda* and *A. mixtum* is epidemiologically relevant since this tick species is a vector of *R. rickettsii* (Rivera-Páez et al., 2016, 2018a; Bermúdez and Troyo, 2018), one of the most pathogenic rickettsiae on the Americas (Parola et al., 2013; Labruna et al., 2014). The distribution of *O. ruficauda* in northeastern and eastern Colombia (Hilty and Brown, 1986; Ayerbe-Quiñones, 2018), might play an important role as hosts and dispersers of ticks infected with rickettsia in these regions where the presence of *R. rickettsii* and its vector ticks has been reported (Rivera-Páez et al., 2016, 2018b).

*Amblyomma nodosum* represented 98.5% (458/465) of the ticks found in this study, which supports the hypothesis that this species is highly associated with Passeriformes in the Neotropics (Labruna et al., 2007; Ogrzewalska et al., 2009b; Lugarini et al., 2015; Lima et al., 2018). Particularly, two individuals of the species *T. aedon* showed high parasite loads of *A. nodosum*. In this regard, several life history traits of this bird species could favor its infestation with ticks; for example, the foraging behavior in the low strata of the vegetation and the elaboration of the nests in cavities (Kroodsmas and Brewer, 2005). These sites likely provide favorable microclimatic conditions for tick establishment and survival (Pfäffle et al., 2013). Additionally, the two *T. aedon* individuals infested with ticks were found during the incubation period so the larvae could have been acquired in the nest (Johnson and Albrecht, 2020; Pacejka et al., 1998). In the region, the high percentage of birds infested with immature stages of *A. nodosum* (75%) agrees with observations from other locations in America (Labruna et al., 2007; Ogrzewalska



**Fig. 2.** Phylogenetic tree based on partial sequences of the outer membrane protein gene *ompB* present only in SFG *Rickettsia* species. The tree was inferred through Maximum Likelihood with the Tamura 3-parameter evolution model. The sequences obtained in this study appear in bold and the GenBank accession numbers are provided within square brackets.

et al., 2009b; Lugarini et al., 2015; Lima et al., 2018). Conversely, adult ticks have been observed on mammals; for instance, in the Orinoquia, adult ticks are associated with mammal species of the order Pilosa, which are commonly found in the region (Aya-Cuero et al., 2019). These mammals are known hosts for *A. nodosum* (Luque, 1948; Witter et al., 2016; Moerbeck et al., 2018).

The detection of *R. parkeri* strain Atlantic rainforest in *A. nodosum*, which, in turn, was found infesting *F. grisea* and *S. angolensis* birds in the department of Arauca, supports the hypothesis that the Colombian Orinoquia should be considered an endemic area for rickettsial disease associated with the SFG. *Rickettsia parkeri* (strains NOD and COOPERI) were detected in *A. nodosum* collected from birds (Ogrzewalska et al., 2009b, 2011a; Lugarini et al., 2015); however, our detection of *R. parkeri* strain Atlantic rainforest in *A. nodosum* is the first association reported in the American continent for these species. *R. parkeri* strain Atlantic rainforest appears to be associated with species of the *A. ovale* complex and the NOD strain to *A. nodosum* in South America (Nieri-Bastos et al., 2018), which suggests a Neotropical origin of these strains.

Despite the distribution of *A. nodosum* includes the Neotropical region (Nava et al., 2014, 2017), there are no human reports of infestation by this ticks (Guglielmone et al., 2014; Nava et al., 2017; Moerbeck et al., 2018). In Colombia, *A. nodosum* has only been reported in the central and western areas of the country in the departments of Antioquia, Meta, Tolima, and Valle del Cauca (Osorno-Mesa, 1940; Luque, 1948; López and Parra, 1985; Benavides-Montano et al., 2018) infesting anteaters (*Tamandua tetradactyla*). Osorno-Mesa (1940), and Martínez-Sánchez et al. (2020), reported records of infested wild birds from central Colombia. Our results extend the distribution of infested birds to the Eastern planes of the country. *R. parkeri* strain Atlantic rainforest has been recorded in Colombia in *A. ovale* ticks collected from domestic (dogs) and wild mammals (*Proechimys semispinosus*) (Londoño et al., 2014, 2017). However, no cases of rickettsial infections by this strain of *R. parkeri* have yet been reported in Colombia. In contrast, in other South American countries such as Brazil, clinical cases of *R. parkeri* strain Atlantic rainforest infections mainly associated with the transmission mediated by ticks of the *A. ovale* complex (*A. ovale* and *A. aureolatum*) have been reported in the last decade (Spolidorio et al., 2010; Nieri-Bastos et al., 2016; Da Paixão-Sevá et al., 2019). Detection of *R. parkeri* strain Atlantic rainforest in ticks that parasitize wild birds, suggests the role that these vertebrates may have for the lodging and dispersal of ticks infected with rickettsia. In this context, it is necessary to carry out studies to expand the knowledge of the associations between birds, ticks, and rickettsiae.

#### 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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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijppaw.2020.09.001>.

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