



**Universidad de Caldas**

**MIGRATORY AND RESIDENT WILD BIRDS AND THEIR ROLE IN  
THE TRANSMISSION OF *Rickettsia* spp. IN TICKS (ACARI: IXODIDAE)  
IN THE DEPARTMENT OF CALDAS**

**THESIS PRESENTED ESTEFANI TATIANA MARTÍNEZ SÁNCHEZ  
TO OBTAIN THE DEGREE OF MASTER IN BIOLOGICAL SCIENCES**

Manizales, Caldas, Colombia (June, 2020)



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DIRECTED BY **GABRIEL JAIME CASTAÑO VILLA, Ph.D**  
CO-DIRECTED BY **FREDY ARVEY RIVERA PÁEZ, Ph. D**

Manizales, Caldas, Colombia (June, 2020)



ACTA DE SUSTENTACIÓN DE TESIS

Requisito para la obtención del título de Magister en Ciencias Biológicas

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A las 08:00 a.m. del día 28 de Junio de 2020 se inició el proceso de sustentación de la tesis titulada "Aves silvestres: Migratorias y residentes y su papel en la transmisión de Rickettsia spp. por garrapatas (Acari: Ixodidae) en el departamento de Caldas." desarrollada por la estudiante ESTEFANI TATIANA MARTÍNEZ SÁNCHEZ, bajo la dirección del profesor Gabriel Jaime Castaño Villa de la Universidad Caldas y la co-dirección del profesor Fredy Arvey Rivera de la Universidad Caldas. La estudiante fue informada que disponía de una (1) hora para la exposición de su tesis y que posteriormente sería evaluada por los jurados, profesores Hector Jaime Aricapa G y Giovany Guevara Cardona y directores de tesis Gabriel Jaime Castaño Villa y Fredy Arvey Rivera sobre la claridad de la exposición y corrección de escritura, originalidad, coherencia y claridad en la argumentación, actualidad y pertinencia de las fuentes.

Posterior a la sustentación de la tesis, fue conferida la siguiente calificación:

Calificación: 4.7
Recomendación de distinción: Si [x] No [ ]
Tipo de distinción: Meritoria [x] Laureada [ ]

La tesis puede ser distinguida, siempre y cuando la calificación promediada por el grupo de jurados esté entre 4,5 y 4,8 como Meritoria o si la calificación promediada está entre 4,9 y 5 como Laureada. Del otorgamiento de la distinción realizada por parte de los jurados, se remitirá desde la dirección del programa informe a la Oficina de Admisiones y Registro Académico para que se incluya la constancia en el acta de grado: Reglamento Estudiantil -Artículo 14, Acuerdo 31 de 2019 del Consejo Académico, que modifica algunos artículos de los capítulos XI y XII del Acuerdo 049 de 2007 del Consejo Académico Universidad de Caldas.

Con base en lo anterior, la estudiante fue \_Aprobada\_ para obtener el título de Magister en Ciencias Biológicas. Esta acta está firmada por el director de tesis, los dos jurados evaluadores y por el director del Programa de Maestría en Ciencias Biológicas.

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## **Dedication**

*This is dedicated to all who were a part of this process for their company, motivation, work, travel, and for being there in the good and bad times. Especially to my mother Beatriz Sánchez, who trusted me no matter what and supported me with her unconditional love and kindness.*

## DECLARATION OF ORIGINALITY

Except when it is explicitly indicated in the text, the research work contained in this thesis was carried out by Estefani Tatiana Martínez Sánchez as a student of the Master's Degree in Biological Sciences between (August) of (2017) and (June) of (2020), under the supervision and guidance of doctors Gabriel Jaime Castaño Villa and Fredy Arvey Rivera Páez.

The research reported in this thesis has not been used previously to obtain other academic degrees, nor will it be used for such purposes in the future.

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## ABSTRACT

Wild birds are associated with hard tick transport and dispersal between and within continents. Furthermore, birds play a key role in the epidemiology of tick-borne pathogens. There is documented information on the role of birds in the dispersal of Neotropical hard ticks, which, in some cases, transmit pathogenic bacteria in America (e.g., *Borrelia*, *Ehrlichia*, and *Rickettsia*). In Colombia, there are records of rickettsiae in ticks, wild and domestic mammals, and humans; however, the role of birds as hosts of ticks that carry rickettsiae is unknown. The department of Caldas is an ideal setting to study the relationships among birds, ticks, and rickettsiae, due to case reports of *Rickettsia* en humans in 2005. This research studied hard ticks from wild birds in 19 municipalities in the department of Caldas. The collected ticks were examined and morphologically identified using taxonomic keys. Molecular analyses of *16S*, *COI*, and *ITS2* gene sequences were also performed for tick identification. The detection of tick-borne *Rickettsia* was done using genes *gltA*, *ompA*, and *ompB*. A total of 2314 birds were examined, including 78 individuals (3.4%) that were infested with ticks. Ten tick species were collected and identified (e.g. *Amblyomma longirostre*, *Amblyomma varium*, *Amblyomma dissimile*, *Amblyomma ovale*, *Amblyomma nodosum*, *Amblyomma calcaratum*, *Ixodes* sp. I, *Ixodes* sp. II, *Ixodes* sp III, and *Haemaphysalis leporispalustris*). The presence of *Rickettsia amblyommatis*, *Rickettsia* sp. strain Koreansis, *Candidatus Rickettsia tarasevichiae/Rickettsia canadensis*, *Candidatus Rickettsia colombianensi/Rickettsia monacensis*, and *Rickettsia* sp. was detected in five tick species. New associations between birds and ticks are reported, mainly between migratory boreal and Neotropical ticks. Finally, the importance of birds as hosts of rickettsia-infected ticks is discussed.

Keywords: *Amblyomma*, *Ixodes*, *Haemaphysalis*, *Rickettsia*, prevalence

## RESUMEN

Las aves silvestres han sido relacionadas con el transporte y dispersión de garrapatas duras entre y dentro de los continentes. Así mismo, al parecer juegan un papel clave en la epidemiología de los patógenos asociados con las garrapatas. Principalmente se ha documentado el papel de las aves en la diseminación de garrapatas duras de origen Neotropical, en algunos casos portadoras de bacterias patógenas en América (e.g., *Borrelia*, *Ehrlichia* y *Rickettsia*). En Colombia se ha registrado la presencia de rickettsias en garrapatas, mamíferos silvestres, domésticos, y en humanos, pero se desconoce el rol de las aves como hospederos de garrapatas portadoras de bacterias de este género. El departamento de Caldas parece ser un escenario propicio para investigar las relaciones entre aves, garrapatas y rickettsias, debido a los registros de casos de *Rickettsia* en humanos en 2005. En la presente investigación, se realizó la búsqueda de garrapatas duras en aves silvestres en 19 municipios del departamento de Caldas. Las garrapatas colectadas de las aves se examinaron e identificaron con claves taxonómicas con base en su morfología y análisis moleculares con los genes *16S*, *COI* e *ITS2*. Posteriormente, para la detección de *Rickettsia* en las garrapatas se utilizaron los genes *gltA*, *ompA* y *ompB*. Se examinaron 2314 aves, de las cuales 78 individuos (3,4%) resultaron infestados por garrapatas. Diez especies de garrapatas se colectaron en aves (*Amblyomma longirostre*, *Amblyomma varium*, *Amblyomma dissimile*, *Amblyomma ovale*, *Amblyomma nodosum*, *Amblyomma calcaratum*, *Ixodes* sp. I, *Ixodes* sp. II, *Ixodes* sp III y *Haemaphysalis leporispalustris*). Se detectó la presencia de *Rickettsia amblyommatis*, *Rickettsia* sp. cepa Koreansis, *Candidatus Rickettsia tarasevichiae/Rickettsia canadensis*, *Candidatus Rickettsia colombianensi/Rickettsia monacensis* y *Rickettsia* sp. en cinco de las especies de garrapatas mencionadas. Se reportan nuevas asociaciones entre aves y garrapatas, principalmente entre aves migratorias boreales y garrapatas de origen Neotropical. Finalmente, se discute la importancia de las aves como hospederos de garrapatas infectadas por rickettsias.

**Palabras clave:** *Amblyomma*, *Ixodes*, *Haemaphysalis*, *Rickettsia*, prevalencia



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## 1. INTRODUCTORY CHAPTER

Ticks are hematophage ectoparasite arthropods that rely on several animal hosts, which serve as reservoirs and/or dispersers of associated pathogens (Jongejan and Uilenberg, 2004). This scenario poses a risk for human health since ticks are involved in the transmission of emerging and re-emerging infectious diseases worldwide (Labruna et al., 2007). Ticks are considered second to mosquitoes as vectors of medical importance and are responsible for the transmission of viruses, bacteria, and protozoa that cause diseases in domestic and wild animals and humans (Sonenshine et al., 2002). Global tick diversity comprises 956 species, which are distributed among the families Ixodidae, Argasidae, and Nuttalliellidae (Barros-Battesti et al., 2006; Guglielmone et al., 2014; Nava et al., 2017; Dantas-Torres et al., 2019). The family Ixodidae comprises 728 hard tick species distributed in Tropical and Neotropical regions around the world (Horak et al., 2002; Bowman et al., 2003; Barros-Battesti et al., 2006; Hornok et al., 2016; Labruna et al., 2016; Muñoz-Leal et al., 2016; Apanaskevich and Bermúdez, 2017; Martins et al., 2019). This tick family is involved in the transmission of diverse pathogens that cause diseases in humans worldwide (e.g., *Borrelia burgdorferi*). In Colombia, 43 hard tick species belonging to the genera *Amblyomma*, *Ixodes*, *Haemaphysalis*, *Rhipicephalus*, and *Dermacentor* (Guglielmone et al., 2003; Rivera-Paéz et al., 2018) are known. Furthermore, there are reports in Cordoba and Antioquia regarding the presence of bacteria of medical importance to humans in some of these genera (Spolidorio et al., 2010, Londoño et al., 2014, Miranda and Mattar, 2015; Nahed and McBride, 2017; Quintero et al., 2017).

In the last decades, there have been studies on wild birds infested with ticks of the genera *Amblyomma*, *Ixodes*, and *Haemaphysalis* in the American continent (Flores et al., 2014; Cohen et al., 2015; Miller et al., 2016). Wild birds play an important role in the ecology of ticks and their associated pathogens. Furthermore, birds are considered important in the long-distance dispersal of hard ticks within and between continents by crossing geographic barriers such as oceans (Olsen et al., 1995). Therefore, birds play a role in public health by dispersing vectors (arthropods) of zoonotic pathogens and contributing to re-establishing endemic disease foci across long distances (Elias et al., 2011). The transport of ticks and their

associated pathogens from one endemic area to another could affect the establishment and spread of vectors and pathogens (Hasle, 2013; Brinkerhoff et al., 2018). Although the successful establishment of a tick or the propagation of a pathogen after being transported by birds has not been proven, there is evidence that this could occur due to climate change events (Hasle et al., 2013; Cohen et al., 2015). For instance, global warming can lead to changes in the vegetation and affect the distribution of tick species. Furthermore, tick maintenance may be favored by dispersion through competent hosts, such as mammals. Moreover, bird transport is a likely mechanism that allows the establishment of ticks in sites with competent hosts across a geographic barrier (Hasle, 2013). In view of this, knowing the potential dispersers of ticks of relevance to public health provides insight into future scenarios of epidemiological interest. Several studies report tick dispersal along bird migratory routes (Klich et al., 1996; Smith et al., 1996; Rand et al., 1998; Rand et al., 2007; Ogden et al., 2008; Lommano et al., 2014). During migration, birds can cover long distances in a few days; consequently, these can transport hard ticks involved in the transmission of pathogenic agents to new geographic areas (Hornok et al., 2014). Moreover, migratory birds are reported to be involved in the migration and establishment of exotic ticks (Mukherjee et al., 2014; Ogden et al. 2008; Hamer et al. 2012; Mukherjee et al. 2014; Cohen et al. 2015).

Ticks that infest migratory birds can carry various disease-causing pathogens in humans, including bacteria such as *Rickettsia* spp., *Borrelia burgdorferi*, and several species of *Ehrlichia*. Therefore, the dispersal or migration of birds can contribute to the distribution of tick-borne pathogens (Rappole et al., 2000; Bjöersdorff et al., 2001; Parola and Raoult, 2001; Scott et al. 2012; Hornok et al. 2013; Toma et al., 2014; Lommano et al., 2015). In particular, birds serve as hosts to ticks infected with bacteria of the genus *Rickettsia* (Lugarini et al., 2015; Pacheco et al., 2012). These bacteria are obligate intracellular organisms that infect invertebrate hosts (Raoult and Roux, 1997). Several *Rickettsia* spp. cause zoonotic infectious diseases, such as rickettsiosis, which are considered globally emerging and re-emerging diseases (Parola et al., 2013). Rickettsiosis in America includes the Rocky Mountain spotted fever, Brazilian spotted fever, and Tobia fever in Colombia, caused by *Rickettsia rickettsii* (Labruna, 2009; Patiño et al., 2019). In Colombia, there are case reports of rickettsiosis in the municipality of Villeta and departments of Cordoba and Antioquia; furthermore,

rickettsiae have been detected in hard ticks collected from humans (Faccini-Martínez et al., 2015, 2016; Miranda et al., 2012; Miranda and Mattar, 2014). Specifically, outbreaks of rickettsiosis were reported in northern Caldas (associated with *R. typhi* and *R. felis*) in the municipality of Pacora in 2005 (Hidalgo et al., 2013). Although these bacterial species are dispersed by fleas that rely on domestic animals as primary hosts, it is known that wild animals and their associated ticks are also involved in the biological cycle of these bacteria (Raoult and Parola 2009).

In this context, the study of the associations between wild animals (e.g., birds) and ticks can provide a baseline to predict future interactions of hosts and vectors and, consequently, pathogen transmission (Loss et al., 2016). Colombia holds the highest bird diversity in the world; however, there are no studies to date on tick species that parasitize wild birds and infection by *Rickettsia*. Therefore, this research aimed to determine the associations between resident and migratory birds infested with ticks infected by *Rickettsia* in the department of Caldas, Colombia. Furthermore, this study contributes to the knowledge of host-tick-bacteria interactions in the American continent.

## 2. MATERIALS AND METHODS

### 2.1. *Study area*

This study was conducted in the department of Caldas, located in the Central Western region of the Colombian Andes (latitude: 6.09, longitude: -75.63) with a surface area of 8,777 km<sup>2</sup> and an elevational range between 140 and 5,350 m above sea level (a.s.l.). The mean monthly temperature varies from 13°C to 17°C and the annual precipitation ranges from 1,500 to 3,000 mm (Jaramillo-Robledo, 2006; Cardona-Salazar et al., 2020). Bird captures were conducted in 32 localities (secondary forest, crops or forest plantations) in 19 municipalities of the department of Caldas, situated in the inter-Andean valleys of the Cauca (municipality of Anserma) and Magdalena (municipalities of La Dorada, Norcasia, Samaná y Victoria) rivers, on the western (municipalities of Aranzazu, Chinchiná, Manizales, Neira, Pácora, Palestina y Supía) and eastern (municipality of Pensilvania) slopes of the central Andes mountain range, on the eastern (municipalities of Belalcázar, Marmato, Riosucio, San José y Viterbo) slope of the Western Andes mountain range, and paramo zone (municipality of Villamaría) (Cardona-Salazar et al., 2020).

### 2.2. Bird capture, collection and morphological identification of ticks

For wild bird captures, we installed eight mist nests (12 × 2.5 m; with 36 mm mesh size) in the each study localities between 2015 and 2019, for a total of 9,320 net hours. The nets were operated from 0600 h to 1800 h. Each captured bird was examined for ticks by carefully checking the head, neck, wings, and lower regions. Since the birds were released at the same site of capture. Ticks were removed and immediately preserved in 96% ethanol (Ogrzewalska et al., 2010, 2015) and were taken to the *Laboratorio de Genética* of the *Universidad de Caldas* for identification.

The morphological identification of the ticks was done following the guides of Kohls (1956), Jones et al. (1972), Estrada-Peña et al. (2005), Barros-Battesti et al. (2006), Mehlhorn (2008), Martins et al. (2010), and Nava et al. (2014, 2017).

### 2.3. Molecular identification of ticks and *Rickettsia* spp. detection

For the molecular identification of the collected ticks, DNA extraction was carried out with the DNeasy Blood and Tissue kit (Qiagen). We amplified fragments from two mitochondrial genes: a DNA barcode corresponding to the 5' region of the cytochrome oxidase subunit I (COI) (Folmer et al., 1994), and the 16S rRNA (Norris et al., 1996). Furthermore, for some samples, we amplified the ITS2 region of the nuclear rDNA gene (Zahler et al., 1995 and Mclain et al., 1995) that amplify for tick species.

For detection of *Rickettsia* spp. by PCR amplification in ticks collected on wild birds, we used primers CS-78 and CS-323, which amplify a fragment of approximately 401 bp of the citrate synthase gene (*gltA*), which is present in all *Rickettsia* species (Labruna et al. 2004). Ticks yielding PCR amplification by thus protocol were further tested by a semi-nested PCR with primers Rr 190.70 and Rr190.701 (first reaction), and Rr 190.70 and Rr 190.602 (second reaction), to amplify a final fragment of  $\approx$ 532 bp of the outer membrane protein A (*ompA*) gene of rickettsiae of the spotted fever group (Regnery et al. 1991). Additionally, these ticks were also tested by a third PCR protocol with primers *rompB* OF and *rompB* OR, which amplifies a  $\approx$ 511 bp fragment of the outer-membrane protein *rOmpB* (*ompB*) gene (Choi et al. 2005) of *Rickettsia* species of the spotted fever and typhus groups. The PCR products were loaded on 1% agarose gel through horizontal electrophoresis and visualized in a Gel Doc-It2 310 (UVP) photodocumentor. Amplicons were sequenced at Macrogen (South Korea). Generated sequences were edited by using Geneious Trial v8.14 (Drummond et al. 2009), and submitted to BLAST analyses to determine the identity to other ticks and *Rickettsia* species sequences available in GenBank and BOLD (Barcode of Life Data Systems, [www.barcodinglife.com](http://www.barcodinglife.com)) public databases. The identification criteria with the tick and *Rickettsia* sequences registered in GenBank was carried out following the suggestions of Raoult et al. (2005) and Lv et al. (2014), which present the rates for the correct identification

of the sequences of each gene used in the study. For the phylogenetic analyzes of the ticks of the genus *Ixodes* was used Maximum Likelihood (ML), with the Hasegawa-Kishino-Yano model (HKY) and 1000 bootstrap replications, using the program MEGA 7.



### 3. RESULTS

The results of this Master's thesis are presented in chapters. Each chapter consists of a scientific article, accepted, submitted or in preparation to be submitted in an international journal specialized in the area of study.

#### 3.1. CHAPTER I

**Status:** Scientific article published in a specialized international journal.

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#### 3.2. CHAPTER II

**Status:** Manuscript submitted to a specialized international journal.

**Authors:** Estefani T. Martínez-Sánchez, Marelid Cardona-Romero, Mateo Ortiz-Giraldo, William David Tobón-Escobar, Daniel Moreno López, Paula A. Ossa-López, Jorge E. Pérez-Cárdenas, Marcelo B. Labruna, Thiago F. Martins, Gabriel J. Castaño-Villa, Fredy A. Rivera-Páez.

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### **3.1. CHAPTER I**

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Associations between wild birds and hard ticks (Acari: Ixodidae) in Colombia



## Original article

## Associations between wild birds and hard ticks (Acari: Ixodidae) in Colombia

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## ABSTRACT

Ticks of the family Ixodidae are vectors of important pathogens in human and animal health. Birds are involved in long-distance transport and dispersion of hard ticks. Tick infestations on wild birds mostly involve species within the genera *Amblyomma*, *Ixodes*, and *Haemaphysalis*. In Colombia, tick research is scarce and there are no studies to date about the associations between wild birds and ticks. We aimed to contribute to the knowledge of the associations between wild birds and hard ticks based on the collection of 2314 wild birds belonging to 29 families in Caldas – Colombia between 2015 and 2019. In total, we collected 133 hard ticks that were found parasitizing 78 birds representing 45 species and 14 wild bird families. We report at least seven tick species on birds confirmed by morphological and molecular methods: *Amblyomma longirostre*, *Amblyomma varium*, *Amblyomma dissimile*, *Amblyomma ovale*, *Amblyomma nodosum*, *Amblyomma calcaratum* and *Haemaphysalis leporispalustris*. In addition, we recorded three *Ixodes* species, which yielded DNA sequences that did not have high identity ( $\leq 95\%$ ) to any species in GenBank. Ticks were found infesting resident and migratory boreal birds. This is the first study addressing the associations between wild birds and hard ticks in Colombia. We describe new associations between birds and ticks in the Americas.

## 1. Introduction

In Colombia, there are 43 hard tick species (Acari: Ixodidae) belonging to several genera reported to date, including *Amblyomma*, *Ixodes*, *Haemaphysalis*, *Rhipicephalus*, and *Dermacentor* (Guglielmine et al., 2003; Rivera-Páez et al., 2018a). Recently, ticks belonging to the genera *Amblyomma*, *Ixodes*, and *Haemaphysalis* were collected from wild birds in different countries in the Americas (Flores et al., 2014; Florin et al., 2014; Martins et al., 2014; Mukherjee et al., 2014; Lamattina et al., 2018). Wild birds are known hosts and important long-distance dispersers of a variety of hard tick species between and within

continents (Hoogstraal, 1961; 1963; Clifford et al., 1969; Olsen et al., 1995; Bjoersdorff et al., 2001). For instance, in the United States, Neotropical ticks (i.e., *Amblyomma longirostre*, *Amblyomma nodosum*, *Amblyomma calcaratum*, *Amblyomma maculatum/Amblyomma triste*, and *Amblyomma geayi*) have been collected from wild birds migrating northward from Central and South America (Scott et al., 2001; Hamer et al., 2012; Mukherjee et al., 2014; Cohen et al., 2015). Some of these ticks might contain human pathogens (Ogrzewalska et al., 2011a; Mukherjee et al., 2014; Cohen et al., 2015). Therefore, birds play an important role in the epidemiology, maintenance and geographical spread of pathogens associated with hard ticks (Hornok et al., 2014).

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Colombia has the highest bird richness in the world: 1909 bird species are reported in the country, of which 7.0 % are migratory boreal birds that use the Colombian territory as a wintering ground or transit destination on their route towards the south of the South American continent (Avendaño et al., 2017). Unfortunately, there are few records of tick infestation on wild birds in Colombia (Osorno-Mesa, 1940; Oniki and Willis, 1991). With this in mind, our study aimed to identify hard tick species associated with wild birds in the department of Caldas, Colombia, to better understand the associations between wild birds and hard ticks. Our findings are a first step to a further elucidation of the role of wild birds in the establishment and dispersal of ticks and their pathogens in Colombia.

## 2. Materials and methods

### 2.1. Study area

This study was conducted in the department of Caldas, located in the Central Western region of the Colombian Andes (latitude: 6.09, longitude: -75.63) with a surface area of 8777 km<sup>2</sup> and an elevational range between 140 and 5350 m above sea level (a.s.l.). The mean monthly temperature varies from 13 °C to 17 °C and the annual precipitation ranges from 1500 to 3000 mm (Jaramillo-Robledo, 2006; Cardona-Salazar et al., 2020). The study localities are situated in the inter-Andean valleys of the Cauca and Magdalena rivers, specifically, in the mid-elevation and high mountains of the Central and Western Andes at 148 to 3845 m a.s.l. Bird captures were conducted in 32 localities in 19 municipalities of the department of Caldas (Anserma, Aranzazu, Belalcázar, Chinchiná, La Dorada, Manizales, Marmato, Neira, Norecasia, Pácora, Palestina, Pensilvania, Riosucio, Samaná, San José, Supía, Victoria, Villamaría, and Viterbo). At the localities, we sampled secondary forests, forest plantations, monocrops, mixed crops, grazing pastures, natural pastures, and paramo zone (Neotropical high mountain biome). Detailed information on the precipitation and temperature of the study localities is described in Martínez-Sánchez et al.

(2018). The geographic location of each locality is shown in Fig. 1 and Table S1.

### 2.2. Bird capture and tick collection

For wild bird captures, we used eight mist nests (12 × 2.5 m; with 36 mm mesh size) in each of the study localities during five days on multiple occasions between months to November 2015 and March 2019, for a total of 9320 net hours. The nets were operated from 0600 h to 1800 h. Each captured bird was examined for ticks by carefully checking the head, neck, wings, and lower regions. Ticks were removed and immediately preserved in 96 % ethanol (Ogrzewalska et al., 2010, 2015). Since the birds were released at the site of capture, we marked the individuals by a small cut in the external rectrices to avoid bias in the estimation of tick infestation prevalence due to recaptures. The taxonomical identification of the birds was done following Remsen et al. (2018). The bird species were further categorized according to their residency status, such as resident or migratory boreal based on Avendaño et al. (2017). Wild bird capture and tick collection were done with the approval of the Ethics and Bioethics Committee of Universidad de Caldas and under a framework permit granted to Universidad de Caldas by the Autoridad Nacional de Licencias Ambientales (ANLA) of Colombia (resolution 1166 of October 09 of 2014 and resolution 02,497 of December 31 of 2018).

### 2.3. Morphological and molecular identifications of ticks

The morphological identification of the ticks was done following the guides of Kohls (1956); Jones et al. (1972); Estrada-Peña et al. (2005); Barros-Battesti et al. (2006); Mehlhorn (2008); Martins et al. (2010), and Nava et al. (2014, 2017). Afterward, we performed molecular identification of a subset of the individual ticks (48 larvae, 17 nymphs, and 7 adults). DNA extraction was performed using the DNeasy Blood and Tissue kit, Qiagen (Hilden, North Rhine-Westphalia, Germany). We amplified fragments from two mitochondrial genes: a DNA barcode

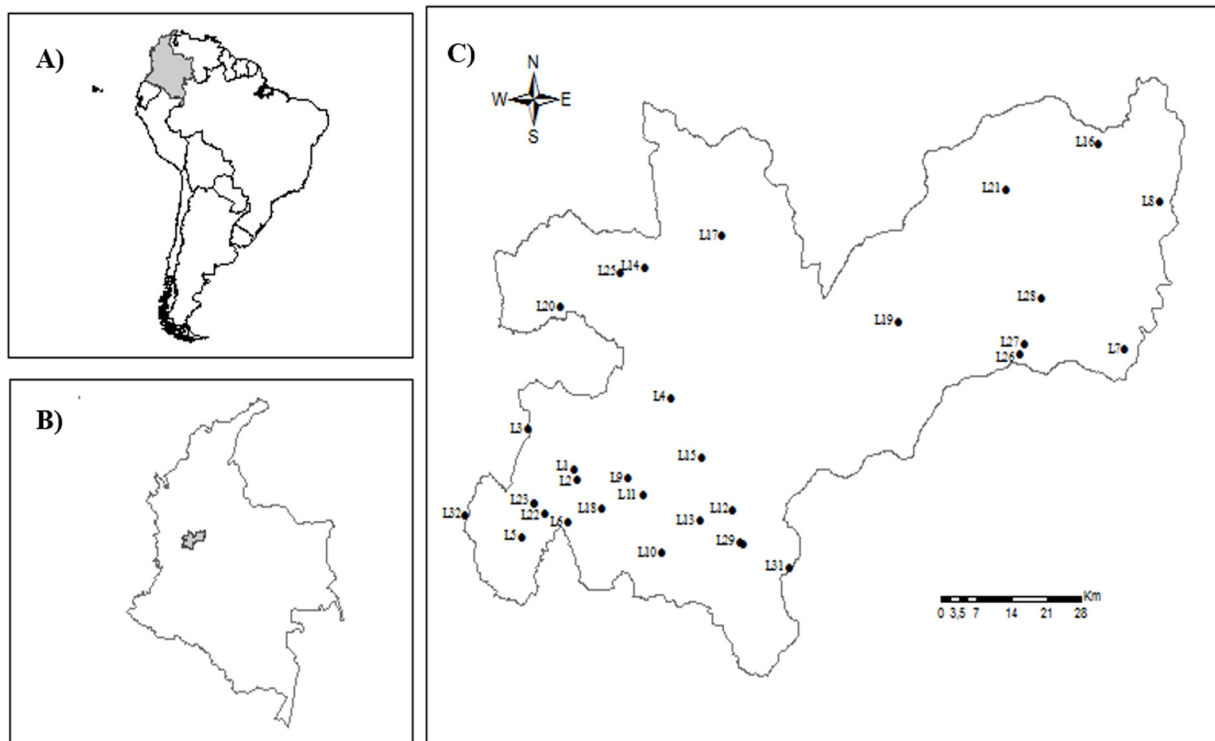


Fig. 1. Map showing the 32 sampling localities in the department of Caldas. A) South America; B) Colombia; C) Caldas. Detailed information is found in Supplementary file 1.

corresponding to the 5' region of the cytochrome oxidase subunit I (COI) gene using primers LCO1490 (F) 5'-GGTCAACAAATCATAAAGA TATTGG-3' and HCO2198 (R) 5'-TAAACTTCAGGGTGACCAAAAA TCA-3' (Folmer et al., 1994), and the 16S rRNA gene using primers 16SF 5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3' and 16SR 5'-CCGGTCTGAACCTCAGATCAAGT-3' (Norris et al., 1996). Furthermore, for some samples, we amplified the ITS2 region of the nuclear rDNA gene using primers ITS2 (F) 5'-CCATCGATGTGAAYTGCAGGACA-3' (Zahler et al., 1995) and MCLN (R) 5'-GTGAATTCTATGCTTAAATTCA GGGGGT-3' (Mclain et al., 1995).

The PCR products were assessed by horizontal electrophoresis on 1% agarose gels stained with SYBR® Safe dye and visualized on a GelDoc-It®2310 Image (UVP) photodocumenter, (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The amplicons were purified and sent to Macrogen (Geumcheon-gu, Seoul, South Korea) for Sanger sequencing. The sequences were assessed and edited using Geneious Trial v8.14 (Drummond et al., 2009). We performed BLAST searches against sequences in public databases, including GenBank and BOLD (Barcode of Life Data Systems, [www.barcodinglife.com](http://www.barcodinglife.com)). The sequences for each gene were aligned with ClustalW (Thompson et al., 1997) using MEGA version 7 (Tamura et al., 2013). Phylogenetic tree was constructed using Maximum Likelihood (ML), with the Hasegawa-Kishino-Yano model (HKY) and 1000 bootstrap replications, using the program MEGA 7.

Finally, we report the prevalence of tick infestation (number of infested birds/number of examined birds\*100) for the department of Caldas and each study locality (Ogrzewalska et al., 2011b) (Table S1).

### 3. Results

We examined 2314 birds belonging to 29 families and 232 species (207 resident and 25 migratory boreal species). In total, 3.4 % (78/2314) of the birds and 19.3 % (45/232) of the bird species were infested by ticks of the genera *Amblyomma*, *Ixodes* and *Haemaphysalis* (Table 1). The order Passeriformes showed the highest number of infested individuals, among which the families Tyrannidae (9 species) and Thraupidae (10 species) showed the highest number of tick-infested species, with a prevalence of 3.4 % and 1.6 %, respectively (Table S2). Overall, we examined 255 migratory boreal birds, of which 4.3 % (11 individuals) were infested by ticks of the genera *Amblyomma* and *Haemaphysalis*. The six migratory species with tick infestations belonged to the families Tyrannidae (*Empidonax virescens*), Turdidae (*Catharus ustulatus*), and Parulidae (*Parkesia noveboracensis*, *Oporornis agilis*, *Setophaga castanea*, and *Cardellina canadensis*). *Oporornis agilis* was caught in early November during the fall migration and *E. virescens* was caught before the spring migration began. All other migratory boreal bird species were captured during overwintering (Table S3). The tick-infested resident and migratory boreal birds were captured at an elevational range from 178 to 3845 m a.s.l. We found the highest prevalence of infested birds (42.8 % of 112 individuals examined) in tropical humid forests in the municipality of Norcasia at 204 m a.s.l. In the high-elevation paramo zone (3845 m a.s.l.), we found three bird species (*Catamenia homochroa*, *Diglossa humeralis*, and *Zonotrichia capensis*) that were infested by *Ixodes* ticks.

We collected a total of 133 ticks (7 adults, 26 nymphs, and 100 larvae) belonging to the genera *Amblyomma* (89 larvae and 26 nymphs), *Ixodes* (9 larvae and 7 adults) and *Haemaphysalis* (2 larvae). All specimens were morphologically assessed; nymphs were identified as *A. longirostre*, *Amblyomma varium*, *Amblyomma ovale*, *A. nodosum*, and *A. calcaratum*, while larvae were identified only at the genus level. Following morphological identification, we used molecular methods to confirm the identity of *A. longirostre* (36 larvae and 7 nymphs), *A. varium* (1 larva and 1 nymph), *Amblyomma dissimile* (1 larva), *A. ovale* (3 larvae and 1 nymph), *A. nodosum* (1 larva and 7 nymphs), *A. calcaratum* (1 nymph), and *Haemaphysalis leporispalustris* (2 larvae). Regarding the ticks of the genus *Ixodes*, it was not possible to assign

their identity to any valid species, since their DNA sequences always had < 95 % identity to valid species in GenBank. However we generated three different haplotypes for the 16S rRNA mitochondrial gene, suggesting that they correspond to three different species of *Ixodes* (Table 2, Fig. 3). We have classified these haplotypes as *Ixodes* sp. I (4 larvae and 2 adults), *Ixodes* sp. II (2 adults), and *Ixodes* sp. III (3 adults). It was not possible to establish the morphological identity of these adult ticks due to the poor condition of the specimens (e.g., without hypostome). With other characteristics preserved, the adult specimens of *Ixodes* sp. I were morphologically similar to *Ixodes auritulus*. The specimens *Ixodes* sp. II had some morphological features of *Ixodes spinosus* sensu lato (s.l.) Labruna et al. (2020); however, we could not confirm its taxonomic identity. The specimens of *Ixodes* sp. III were impossible to evaluate, due to their poor condition. Based on a BLAST analysis, the partial 16S rRNA sequences of *Ixodes* sp. I showed the closest match to *Ixodes* sp. I MO-2013 strain K31 [KF702352]. Furthermore, the partial sequence of *Ixodes* sp. II showed the highest 16S rDNA similarity in GenBank to *Ixodes tanuki* [AB819257], and the partial sequence of *Ixodes* sp. III was closest to *I. spinosus* s.l. [MN727313] and *Ixodes lasallei* [AF549850] (Fig. 3). The GenBank accession numbers of the nucleotide sequences obtained in this study are: [MN557237-MN557267, MT180742-MT180743] for the mitochondrial 16S rRNA gene, [MT180839-MT180854] for the COI gene, and [MN567211-MN567219] for the ITS2 region of the nuclear rDNA gene (Table 2). Voucher tick specimens were deposited in the Ectoparasite Collection in the Centro de Museos de Historia Natural de la Universidad de Caldas, Colombia.

We report 50 associations between birds and ticks, which involve 45 bird species and at least seven tick species. The dominant tick species found in this study was *A. longirostre*, which infested 71 % of the infested bird species (32 species). In particular, we highlight the association of this tick with four migratory boreal birds (i.e., *E. virescens*, *C. ustulatus*, *S. castanea*, and *C. canadensis*). In addition, *A. nodosum* infested 8.9 % of the infested birds (4 species) and was associated with a migratory boreal species (i.e., *C. ustulatus*). Finally, *A. calcaratum*, *A. dissimile*, and *H. leporispalustris* were each found associated with a single species, namely *M. marginatus*, *O. agilis*, and *E. virescens*, respectively (Fig. 2).

### 4. Discussion

We identified 50 associations between 45 bird species and at least seven tick species. Twenty of these associations are new reports for the Americas, involving 19 host species and 5 tick species (Table 1). Among the new associations, we highlight those that involve three migratory boreal species (*E. virescens*, *P. noveboracensis*, and *O. agilis*). The remaining associations have been reported previously from the Americas (Scott et al., 2001; González-Acuña et al., 2005; Labruna et al., 2007; Ogrzewalska et al., 2008, 2009a, 2009b, 2011a, 2011b, 2012, 2014; Hamer et al., 2012; Luz et al., 2012, 2016; Maturano et al., 2015; Miller et al., 2016; Budachetri et al., 2017).

Six migratory boreal bird species were infested by Neotropical ticks (*A. longirostre*, *A. nodosum*, *A. ovale*, and *A. dissimile*). These bird species breed in the United States and Canada and spend the boreal winter in South America (some reach northern Argentina). The bird *C. ustulatus* showed the highest prevalence of infestation during overwintering, specifically by *A. longirostre* and *A. nodosum*. Our infestation records complement previous reports for this species. These Neotropical ticks were previously reported during the spring migration of this bird species, along the northeast coast of the Gulf of Mexico (Cohen et al., 2015), in Louisiana (Mukherjee et al., 2014; Budachetri et al., 2017), and in northern Illinois (Hamer et al., 2012). The latter record suggests that *C. ustulatus* could play a key role in the dispersion of *A. longirostre* and *A. nodosum* throughout a large portion of the Americas. Additionally, in our study, the infestation of *E. virescens* by *A. longirostre* and *H. leporispalustris* before spring migration agrees with a report of

**Table 1**  
Wild birds infested by ticks in the department of Caldas, Colombia.

Municipality	Locality	Bird order	Bird Family	Bird species	No. infested birds/no. examined birds (% prevalence)	Ticks			
						Species	Female	Nymph	Larva
Norcasia	L16	Apodiformes	Trochilidae	<i>Phaethornis guy</i>	1/23 (4)	<i>Amblyomma longirostre</i> <sup>a</sup>			1
Riosucio	L20	Coraciiformes	Momotidae	<i>Momotus aequatorialis</i>	1/4 (25)	<i>Ixodes</i> sp. III	1		
Supía	L25	Passeriformes	Thamnophilidae	<i>Thamnophilus multistriatus</i>	1/2 (50)	<i>Amblyomma longirostre</i> <sup>a</sup>		1	
Norcasia	L16			<i>Thamnophilus atrinucha</i>	1/2 (50)	<i>Amblyomma longirostre</i>			1
Samaná	L21			<i>Formicivora grisea</i>	1/4 (25)	<i>Amblyomma ovale</i> <sup>a</sup>			11
Norcasia	L16		Furnariidae	<i>Dendrocicla fuliginosa</i>	4/4 (100)	<i>Amblyomma longirostre</i>		1	3
				<i>Glyphorhynchus spirurus</i>	2/6 (33)	<i>Amblyomma longirostre</i>			8
				<i>Xiphorhynchus</i>	4/10 (40)	<i>Amblyomma longirostre</i> <sup>a</sup>		1	5
				<i>susurrans</i>					
Anserma	L1			<i>Xiphorhynchus guttatus</i>	1/3 (33)	<i>Amblyomma varium</i> <sup>a</sup>		1	
Samaná	L21		Tyrannidae	<i>Elaenia flavogaster</i>	1/36 (2)	<i>Amblyomma longirostre</i>			2
Pácora	L17			<i>Zimmerius chrysops</i>	1/63 (1)	<i>Amblyomma varium</i> <sup>a</sup>			2
				<i>Mionectes olivaceus</i>	1/1 (100)	<i>Amblyomma longirostre</i>			1
Norcasia	L16			<i>Mionectes oleagineus</i>	5/105 (4)	<i>Amblyomma longirostre</i>		2	3
				<i>Leptopogon amaurocephalus</i>	1/7 (14)	<i>Amblyomma longirostre</i>			1
				<i>Oncostoma olivaceum</i>	2/3 (66)	<i>Amblyomma longirostre</i> <sup>a</sup>			2
Anserma	L1			<i>Poecilatriccus sylvia</i>	1/2 (50)	<i>Amblyomma longirostre</i> <sup>a</sup>			1
La Dorada	L8			<i>Todirostrum cinereum</i>	1/20 (5)	<i>Amblyomma longirostre</i> <sup>a</sup>			1
Pácora	L17			<i>Empidonax virescens</i> *	1/23 (4)	<i>Amblyomma longirostre</i> <sup>a</sup>			1
						<i>Haemaphysalis leporispalustris</i> <sup>a</sup>			2
Norcasia	L16		Pipridae	<i>Lepidothrix coronata</i>	3/6 (50)	<i>Amblyomma longirostre</i> <sup>a</sup>			5
La Dorada	L8			<i>Manacus manacus</i>	11/49 (22)	<i>Amblyomma nodosum</i>		1	
Norcasia	L16							3	
						<i>Amblyomma longirostre</i>		1	12
				<i>Machaeropterus regulus</i>	1/9 (11)	<i>Amblyomma longirostre</i> <sup>a</sup>		1	
				<i>Ceratopipra erythrocephala</i>	1/8 (12)	<i>Amblyomma longirostre</i>			1
Palestina	L18		Vireonidae	<i>Vireo olivaceus</i>	1/13 (7)	<i>Amblyomma longirostre</i>			1
Norcasia	L16		Troglodytidae	<i>Microcerculus marginatus</i>	2/4 (50)	<i>Amblyomma longirostre</i>		1	
						<i>Amblyomma calcaratum</i>		1	
Anserma	L1		Turdidae	<i>Catharus ustulatus</i> *	6/71 (8)	<i>Amblyomma nodosum</i>		4	
Manizales	L12							1	
Pácora	L17							1	
Norcasia	L16					<i>Amblyomma longirostre</i>			3
Victoria	L26								1
Norcasia	L16			<i>Turdus obsoletus</i>	1/1 (100)	<i>Amblyomma longirostre</i> <sup>a</sup>			2
Pácora	L17			<i>Turdus grayi</i>	1/19 (5)	<i>Amblyomma longirostre</i>			1
Villamaría	L31		Thraupidae	<i>Catamenia homochroa</i>	1/1 (100)	<i>Ixodes</i> sp. I	1		
				<i>Diglossa humeralis</i>	1/5 (100)	<i>Ixodes</i> sp. I			9
Norcasia	L16			<i>Iserothraupis luctuosa</i>	1/3 (33)	<i>Amblyomma longirostre</i>		1	
				<i>Eucometis penicillata</i>	3/4 (75)	<i>Amblyomma nodosum</i>		1	
						<i>Amblyomma longirostre</i>		1	1
Pácora	L17			<i>Sporophila nigricollis</i>	1/90 (1)	<i>Amblyomma longirostre</i>			1
Anserma	L3			<i>Saltator striatipectus</i>	1/22 (4)	<i>Amblyomma nodosum</i>		1	
Manizales	L12			<i>Thlyopsis superciliaris</i>	1/1 (100)	<i>Ixodes</i> sp. II	1		
Pácora	L17			<i>Tiaris olivaceus</i>	1/75 (1)	<i>Amblyomma longirostre</i> <sup>a</sup>		1	
				<i>Stilpnia heinei</i>	1/2 (50)	<i>Ixodes</i> sp. III	2		
Victoria	L26			<i>Thraupis palmarum</i>	1/47 (2)	<i>Amblyomma longirostre</i> <sup>a</sup>			1
Villamaría	L31		Emberizidae	<i>Zonotrichia capensis</i>	1/72 (1)	<i>Ixodes</i> sp. I	1		
Norcasia	L16		Cardinalidae	<i>Habia gutturalis</i>	2/3 (66)	<i>Amblyomma longirostre</i> <sup>a</sup>			11
Victoria	L26								1
Palestina	L18		Parulidae	<i>Parkesia noveboracensis</i> *	1/27 (3)	<i>Amblyomma ovale</i> <sup>a</sup>		1	
				<i>Oporornis agilis</i> *	1/14 (7)	<i>Amblyomma dissimile</i> <sup>a</sup>			1
Norcasia	L16			<i>Setophaga castanea</i> *	1/7 (14)	<i>Amblyomma longirostre</i>			1
Villamaría	L30			<i>Myiothlypis coronata</i>	1/3 (33)	<i>Ixodes</i> sp. II	1		
Norcasia	L16			<i>Cardellina canadensis</i> *	1/15 (6)	<i>Amblyomma longirostre</i>			1
			Fringillidae	<i>Euphonia lanirostris</i>	1/63 (1)	<i>Amblyomma longirostre</i> <sup>a</sup>			2

\* Migratory boreal bird species.

<sup>a</sup> New association between bird species and tick species.

infestation by *A. longirostre* in this species during spring migration in the northeast coast of the Gulf of Mexico (Cohen et al., 2015). *Oporornis agilis* was found infested by *A. dissimile* during the fall migration; similarly, Weisbrod and Johnson (1989) reported that this bird was infested by *Ixodes scapularis* (reported under the junior synonym, *Ixodes*

*dammini*) during fall migration in the northeastern United States. Therefore, the records shown here complement the information on the capacity of migratory boreal birds to transport ticks during spring and fall migrations. According to Cohen et al. (2015) nearly 4 to ~39 million ticks are transported to North America by migratory boreal birds each

**Table 2**

Results of BLAST searches of the DNA sequences generated from ticks collected from wild birds in the department of Caldas, Colombia against DNA sequences in public databases.

Tick species	Closest identity (%) in GenBank (accession number) according to tick gene examined		
	16S rDNA	COI	ITS2
<i>Amblyomma longirostre</i>	<i>A. longirostre</i> [MK605930] 100%	<i>A. longirostre</i> [KX360356] 100%	<i>A. longirostre</i> [AY887120] 97.51%
<i>Amblyomma nodosum</i>	<i>A. nodosum</i> [FJ424402] 100.00%	<i>A. nodosum</i> [KF200111] 98.20%	N.D.
<i>Amblyomma ovale</i>	<i>A. ovale</i> [MF353102] 99.75 %	<i>A. ovale</i> [KF200143] 99.47%	<i>A. ovale</i> [MT000671] 100%
<i>Amblyomma varium</i>	<i>A. varium</i> [MH818415] 99.73%	N.D.	N.D.
<i>Amblyomma calcaratum</i>	N.D.	<i>A. calcaratum</i> [KF200085] 99.69%	N.D.
<i>Amblyomma dissimile</i>	<i>A. dissimile</i> [KY389389] 100%	N.D.	N.D.
<i>Ixodes</i> sp. I*	<i>Ixodes</i> sp. [KF702352] 97.76%	N.D.	N.D.
<i>Ixodes</i> sp. II*	<i>Ixodes</i> sp. identity ≤ 95	N.D.	N.D.
<i>Ixodes</i> sp. III*	<i>Ixodes</i> sp. identity ≤ 90%	<i>Ixodes</i> sp. identity ≤ 90%	N.D.
<i>Haemaphysalis leporispalustris</i>	N.D.	N.D.	<i>H. leporispalustris</i> [JQ868582] 96.33%

N.D., not done.

\* Voucher specimen with a damaged hypostome.

year. Consequently, migratory birds could have a role in the long-distance epidemiology of vectors and pathogens and contribute to the populations of ticks and their associated pathogens in new areas.

In this study, we found tick infestations mostly on Passeriformes, corroborating other studies conducted in the Neotropical region (Gonzalez-Acuña et al., 2005; Labruna et al., 2007; Ogrzewalska et al., 2008; Luz et al., 2017). In a lesser proportion, we found infestations on individuals of the orders Apodiformes and Coraciiformes. The tick species we found parasitizing birds have been previously reported in Colombia (Osorno-Mesa, 1940; Nava et al., 2017). *Amblyomma longirostre* and *A. nodosum* were the most prevalent species in our study. These species are widely distributed in the Neotropical region and are found infesting resident and migratory birds (Guglielmono et al., 2003). Here, we found larvae and nymphs of these species, and these findings agree with Nava et al. (2010), who mention that birds are the main hosts of the immature stages. We provide the first report of *A. longirostre* on 14 bird species, including a species of the order Apodiformes (Table 1).

We morphologically examined all tick specimens collected; nymphs collected from birds were identified at the species level using morphological keys. Larvae could only be identified to the genus level. The adult tick specimens were obtained in poor condition from birds due to their strong attachment to the host at the time of capture. Particularly, these adults correspond to the genus *Ixodes*, which had to be confirmed by molecular methods.

We provide molecular evidence for three different *Ixodes* species on birds in the highlands of Colombia. A recent study also reported different *Ixodes* species on passerine birds in the highlands of Costa Rica, which similarly to the present study, had their taxonomic identifications restricted to "*Ixodes* sp." (Ogrzewalska et al., 2015). These studies highlight the unexplored diversity of ticks of the genus *Ixodes* that might be associated with passerine birds in highlands of the Neotropical region. Herein, we report the highest elevation at which a tick from the genus *Ixodes* has been found on wild birds in the Americas.

We found associations of *A. calcaratum*, *A. dissimile*, and *H. leporispalustris*, each with a single bird species. The adult stages of *A. calcaratum* are associated with mammals, mainly anteaters, while the immature stages are associated with wild birds (Guglielmono et al., 2003). We found this species infesting *M. marginatus* and this association was previously reported in Panama (Miller et al., 2016). The adult and immature stages of *A. dissimile* are mostly associated with amphibians and reptiles, and, in some cases, the immature stages are found on mammals and wild birds (Guglielmono and Nava, 2010). We provide the first report of *A. dissimile* on *O. agilis*, a migratory bird species. *Haemaphysalis leporispalustris*, a tick species with wide distribution in the Neotropical and Nearctic regions, is mainly associated with wild

rabbits and, occasionally, with wild birds (Guglielmono et al., 2003, 2014). Here, we report *H. leporispalustris* for the first time found on a migratory boreal species (*E. virescens*).

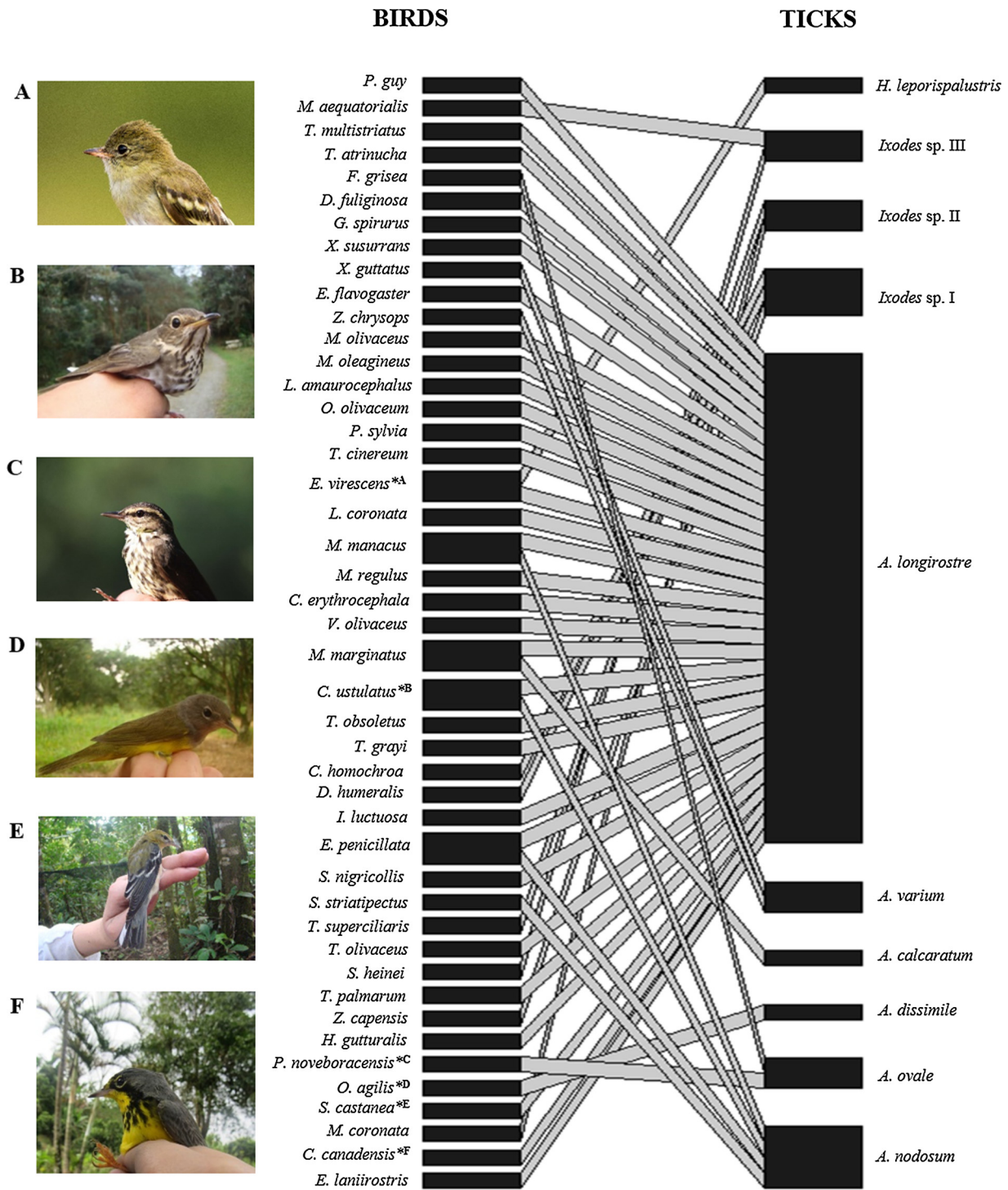
Finally, several tick species found here have been infected by pathogenic bacteria; for instance, *H. leporispalustris* collected from wild birds has been reported to be infected with *Rickettsia rickettsii* (Sonenshine and Clifford, 1973), the etiological agent of Rocky Mountain spotted fever, and *Borrelia burgdorferi* sensu lato (s.l.), causing Lyme disease (Nicholls and Callister, 1996; Scott and Durden, 2015). Other species, such as *A. nodosum* and *A. longirostre* have been found infected with *Rickettsia parkeri* strain NOD and *Rickettsia amblyommatis*, respectively, two potential human pathogens (Paddock et al., 2004; Ogrzewalska et al., 2009b, 2011a; Pacheco et al., 2012). In Colombia, ticks of these species infected with bacterial agents (i.e. *Rickettsia*) were reported on domestic and wild animals and humans in the departments of Antioquia, Casanare, Córdoba, Cundinamarca and Tolima (Miranda et al., 2012; Londoño et al., 2014; Faccini-Martínez et al., 2015, 2016; Rivera-Páez et al., 2018b; Acevedo-Gutiérrez et al., 2020). Accordingly, studying the associations between birds and ticks contributes to elucidating the current and potential scenarios of tick distributions in America that can be considered in the epidemiology of tick-borne diseases.

#### CRedit authorship contribution statement

**Estefani T. Martínez-Sánchez:** Conceptualization, Investigation, Formal analysis, Writing - original draft. **Marelid Cardona-Romero:** Conceptualization, Investigation. **Mateo Ortiz-Giraldo:** Investigation. **William David Tobón-Escobar:** Investigation. **Daniel Moreno López:** Investigation. **Paula A. Ossa-López:** Investigation, Writing - review & editing. **Jorge E. Pérez-Cárdenas:** Investigation, Writing - review & editing. **Marcelo B. Labruna:** Conceptualization, Validation, Formal analysis, Writing - review & editing. **Thiago F. Martins:** Investigation, Writing - review & editing. **Fredy A. Rivera-Páez:** Conceptualization, Validation, Formal analysis, Writing - original draft, Project administration, Funding acquisition. **Gabriel J. Castaño-Villa:** Conceptualization, Validation, Formal analysis, Writing - original draft, Project administration, Funding acquisition.

#### Declaration of Competing Interest

The authors disclose that no substantial portion of the study has been published or is under consideration for publication elsewhere and that its submission for publication has been approved by all of the authors. All authors approve the submission of this article to *Ticks and tick-borne diseases*. In case of acceptance of this manuscript, the



\*Migratory bird

Fig. 2. Associations between birds and tick species in the department of Caldas, Colombia.

copyright will be transferred to *Ticks and tick-borne diseases*. The authors have no conflicts of interest to declare.

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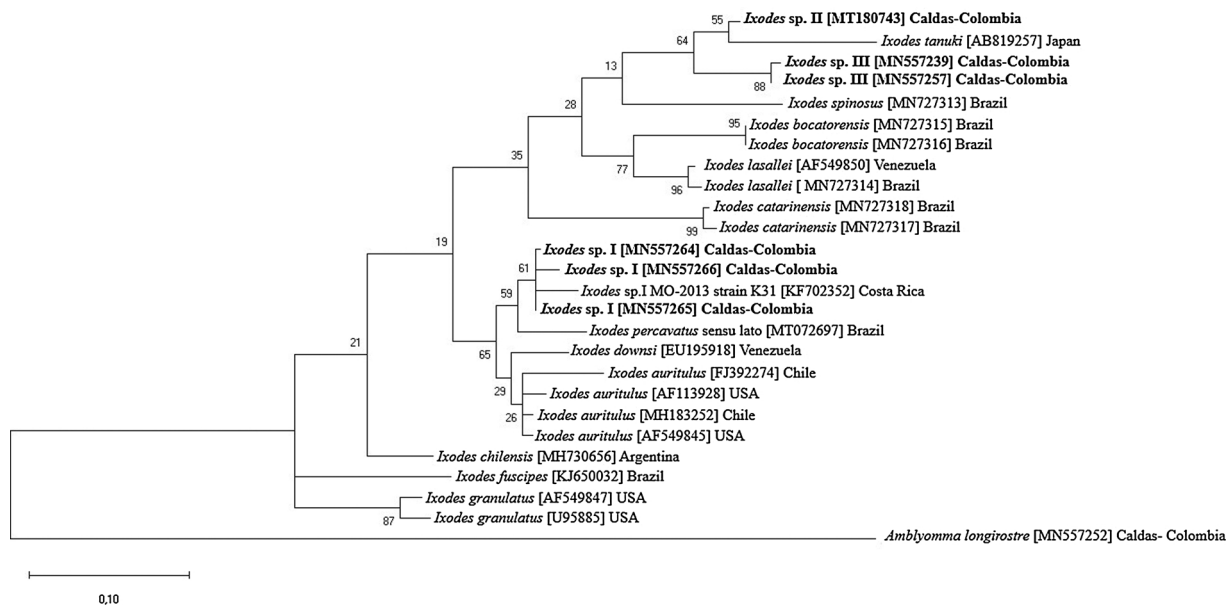


Fig. 3. Phylogenetic tree using partial mitochondrial 16S rRNA gene sequences of *Ixodes* tick specimens collected in this study (in bold) and sequences from GenBank (accession number in brackets). Numbers at nodes are bootstrap support values. The sequence of *Amblyomma longirostre* was used as the outgroup.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.tbd.2020.101534>.

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## **3.2. CHAPTER II**

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*Rickettsia* spp. in ticks (Acari: Ixodidae) from wild birds in Caldas, Colombia

## ***Rickettsia* spp. in ticks (Acari: Ixodidae) from wild birds in Caldas, Colombia**

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### **Abstract**

Bacteria belonging to the genus *Rickettsia* are the causal agents of diseases in domestic and wild animals and humans. These bacteria are considered emerging or reemerging and are transmitted by ticks, fleas, and lice vectors. In recent decades, there have been reports of rickettsias in ticks of the genus *Amblyomma*, *Ixodes*, and *Haemaphysalis* collected from wild birds. Accordingly, birds play a plausible role in the transport and spread of ticks infected by *Rickettsia* spp. In this study, we performed molecular detection of *Rickettsia* species in ticks collected from wild birds in the department of Caldas, Colombia. We detected and identified *Rickettsia amblyommatis*, *Rickettsia* sp. strain Koreansis, *Candidatus Rickettsia tarasevichiae/Rickettsia canadensis*, *Candidatus Rickettsia colombianensi/Rickettsia monacensis* and *Rickettsia* sp. This study contributes to the knowledge on infection by *Rickettsia* in ticks collected from wild birds in Colombia. We also provide the first reports of infection by *R. amblyommatis* in the genus *Ixodes* in America and the presence of *Rickettsia* at elevations above 3000 m a.s.l.

**Keywords:** *Amblyomma*, *Ixodes*, *Haemaphysalis*, *Candidatus Rickettsia tarasevichiae*.

## **Introduction**

Rickettsiae are strictly intracellular Gram-negative bacteria that have been classified into four major groups, the spotted fever group (SFG), the typhus group, the *Rickettsia bellii* group, and the *Rickettsia canadensis* group (Parola et al. 2013). In the western hemisphere, the most known tick-borne rickettsiosis is Rocky Mountain spotted fever (RMSF), a highly lethal disease affecting humans, caused by *Rickettsia rickettsii* (Parola et al. 2013). Among several other SFG *Rickettsia* species reported in ticks from South America, namely *Rickettsia parkeri*, *R. amblyommatis*, *Rickettsia rhipicephali*, and ‘*Candidatus Rickettsia colombianensi*’, only *R. parkeri* has also been shown to cause human illness, although with no fatality so far (Parola et al. 2013).

In South America, studies on rickettsiae infecting ticks from wild birds have been explored in Brazil (Ogrzewalska and Pinter, 2016), Argentina (Flores et al. 2016), Peru (Ogrzewalska et al. 2012a), and Paraguay (Ogrzewalska et al. 2014). Colombia, with the highest bird diversity of the world, harbors 1,909 wild bird species, among which 7.6% are long-distance migratory boreal or austral birds (Avendaño et al. 2017). In particular, these birds migrate across latitudes and can globally affect tick and pathogen spread. To our knowledge, few studies have addressed the possible associations of rickettsiae with bird-infesting ticks in Colombia (Cardona-Romero et al. 2020). Therefore, this study aimed to search for rickettsiae in ixodid ticks from wild birds in the department of Caldas, Colombia.

## Materials and methods

This study was conducted in the Tropical Andes of the department of Caldas, Colombia (Latitude: 6.09, Longitude: -75.63) between 2015 and 2019. Ticks were collected in 11 municipalities at elevations from 178 to 3845 m located in the inter-Andean valleys of the Cauca river (municipality of Anserma) and Magdalena river (municipalities of La Dorada, Norcasia, Samaná, and Victoria), the western slope of the Central Andes mountain range (municipalities of Manizales, Pacora, Palestina, and Supia), the eastern slope of the Western Andes range (municipality of Riosucio), and the paramo zone (municipality of Villamaria) (Cardona-Salazar et al. 2020).

The capture of birds and their ticks have been recently reported by Martínez-Sánchez et al. (2020). Therefore, details on bird capture and tick infestations, plus the taxonomic identification of ticks (morphology and molecular analyses) were provided in details in this previous study. Herein, we assessed 70 of those ticks, collected from 41 bird species, for the presence of rickettsiae. These ticks included *Amblyomma longirostre* (37 larvae, 7 nymphs), *Amblyomma nodosum* (1 larva, 6 nymphs), *Amblyomma varium* (2 larvae, 1 nymph), *Amblyomma ovale* (3 larvae, 1 nymph), *Amblyomma calcaratum* (1 nymph), *Amblyomma dissimile* (1 larva), *Ixodes* sp. I (3 larvae, 1 adult), *Ixodes* sp. II (2 adults), *Ixodes* sp. III (3 adults), and *Haemaphysalis leporispalustris* (1 larva). Wild bird capture and tick collection were done with the approval of the Ethics and Bioethics Committee of *Universidad de Caldas* and under a framework permit granted to *Universidad de Caldas* by the *Autoridad Nacional de Licencias Ambientales* (ANLA) of Colombia (resolution 1166 of October 09 of 2014 and resolution 02497 of December 31 of 2018).

For detection of *Rickettsia* spp. by PCR amplification in ticks collected on wild birds, we used primers CS-78 and CS-323, which amplify a fragment of approximately 401 bp of the

citrate synthase gene (*gltA*), which is present in all *Rickettsia* species (Labruna et al. 2004). Ticks yielding PCR amplification by this protocol were further tested by a semi-nested PCR with primers Rr 190.70 and Rr190.701 (first reaction), and Rr 190.70 and Rr 190.602 (second reaction), to amplify a final fragment of  $\approx 532$  bp of the outer membrane protein A (*ompA*) gene of rickettsiae of the spotted fever group (Regnery et al. 1991). Additionally, these ticks were also tested by a third PCR protocol with primers rompB OF and rompB OR, which amplifies a  $\approx 511$  bp fragment of the outer-membrane protein rOmpB (*ompB*) gene (Choi et al. 2005) of *Rickettsia* species of the spotted fever and typhus groups. The PCR products were loaded on 1% agarose gel through horizontal electrophoresis and visualized in a Gel Doc-It2 310 (UVP) photodocumentor. Amplicons were sequenced at Macrogen (South Korea). Generated sequences were edited by using Geneious Trial v8.14 (Drummond et al. 2009), and submitted to BLAST analyses to determine the identity to other *Rickettsia* spp. sequences available in GenBank and BOLD (Barcode of Life Data Systems, [www.barcodinglife.com](http://www.barcodinglife.com)) public databases.

## **Results**

Rickettsial DNA was detected in five out of the 10 bird-collected tick species, namely *A. longirostre*, *A. varium*, *A. dissimile*, *Ixodes* sp. I, and *Ixodes* sp. II. Accordingly, the overall prevalence of *Rickettsia* was 14% (10/70) (Table 1). Ticks with *Rickettsia* were collected from six resident bird species and one migratory boreal species (Table 1).

**Table 1.** Molecular detection of *Rickettsia* spp. in ticks collected from wild birds in the department of Caldas, Colombia.

Host birds	Tick species (number/ stages)	No. infected/No. tested (%)	Closest identity (%) in GenBank (gene: accession number)	Municipality
<i>Phaethornis guy</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Norcasia
<i>Momotus aequatorialis</i>	<i>Ixodes</i> sp. III (1/A)	0/1 (0)		Riosucio
<i>Thamnophilus multistriatus</i>	<i>Amblyomma longirostre</i> (1/N)	0/1 (0)		Supía
<i>Thamnophilus atrinucha</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Norcasia
<i>Formicivora grisea</i>	<i>Amblyomma ovale</i> (3/L)	0/3 (0)		Palestina
<i>Dendrocincla fuliginosa</i>	<i>Amblyomma longirostre</i> (2/L; 1/N)	0/3 (0)		Norcasia
<i>Glyphorhynchus spirurus</i>	<i>Amblyomma longirostre</i> (3/L)	0/3 (0)		Norcasia
<i>Xiphorhynchus susurrans</i>	<i>Amblyomma longirostre</i> (2/L)	0/2 (0)		Norcasia
<i>Xiphorhynchus guttatus</i>	<i>Amblyomma varium</i> (1/N)	0/1 (0)		Anserma
<i>Elaenia flavogaster</i>	<i>Amblyomma longirostre</i> (1/L)	1/1 (100)	<i>Rickettsia amblyommatis</i> 100% [ompA: MF034496]	Samaná
<i>Zimmerius chrysops</i>	<i>Amblyomma varium</i> (2/L)	2/2 (100)	<i>Rickettsia amblyommatis</i> 100% [ompA: KX137901]	Pácora
<i>Mionectes olivaceus</i>	<i>Amblyomma longirostre</i> (1/L)	1/1 (100)	<i>Rickettsia amblyommatis</i> 100% [ompA: MF034496]	Pácora
<i>Mionectes oleagineus</i>	<i>Amblyomma longirostre</i> (2/L)	0/2 (0)		Norcasia
<i>Leptopogon amaurocephalus</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Norcasia
<i>Oncostoma olivaceum</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Norcasia
<i>Poecilotriccus sylvia</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Anserma
<i>Todirostrum cinereum</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		La Dorada
<i>Empidonax virescens*</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Pácora
	<i>Haemaphysalis leporispalustris</i> (1/L)	0/1 (0)		Pácora
<i>Lepidothrix coronata</i>	<i>Amblyomma longirostre</i> (3/L)	0/3 (0)		Norcasia
<i>Manacus manacus</i>	<i>Amblyomma nodosum</i> (1/N)	0/1 (0)		Norcasia
	<i>Amblyomma longirostre</i> (3 L; 1/N)	0/4 (0)		Norcasia
<i>Microcerculus marginatus</i>	<i>Amblyomma longirostre</i> (1/N)	0/1 (0)		Norcasia
	<i>Amblyomma calcaratum</i> (1/N)	0/1 (0)		Norcasia
<i>Vireo olivaceus</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Palestina
<i>Catharus ustulatus*</i>	<i>Amblyomma nodosum</i> (1/L; 4/N)	0/5 (0)		Anserma, Manizales, Norcasia, Pácora
	<i>Amblyomma longirostre</i> (3/L)	0/3 (0)		Victoria
<i>Turdus grayi</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Pácora
<i>Catamenia homochroa</i>	<i>Ixodes</i> sp. I (1/A)	1/1 (100)	<i>Rickettsia amblyommatis</i> 100% [ompA: KX137901]	Villamaría
<i>Diglossa humeralis</i>	<i>Ixodes</i> sp. I (3/L)	0/3 (0)		Villamaría
<i>Islerothraupis luctuosa</i>	<i>Amblyomma longirostre</i> (1/N)	0/1 (0)		Norcasia
<i>Eucometis penicillata</i>	<i>Amblyomma nodosum</i> (1/N)	0/1 (0)		Norcasia
	<i>Amblyomma longirostre</i> (1/N)	0/1 (0)		Norcasia
<i>Sporophila nigricollis</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Pácora



Host birds	Tick species (number/ stages)	No. infected/No. tested (%)	Closest identity (%) in GenBank (gene: accession number)	Municipality
<i>Thlypopsis superciliaris</i>	<i>Ixodes</i> sp. II (1/A)	1/1 (100)	<i>Candidatus</i> <i>Rickettsia tarasevichiae</i> 99.71% [ <i>ompA</i> : MN450411]/ <i>Rickettsia canadensis</i> 98.96% [ <i>ompB</i> : MH549232]	Manizales
<i>Tiaris olivaceus</i>	<i>Amblyomma longirostre</i> (1/N)	0/1 (0)		Pácora
<i>Stilpnia heinei</i>	<i>Ixodes</i> sp. III (2/A)	0/2 (0)		Pácora
<i>Thraupis palmarum</i>	<i>Amblyomma longirostre</i> (1/L)	1/1 (100)	<i>Rickettsia amblyommatis</i> 100% [ <i>ompA</i> : MF034496]	Victoria
<i>Habia gutturalis</i>	<i>Amblyomma longirostre</i> (3/L)	0/3 (0)		Norcasia, Victoria, Palestina
<i>Parkesia noveboracensis</i> *	<i>Amblyomma ovale</i> (1/N)	0/1 (0)		Palestina
<i>Oporornis agilis</i> *	<i>Amblyomma dissimile</i> (1/L)	1/1 (100)	' <i>Candidatus</i> <i>Rickettsia colombianensi</i> ' 100% [ <i>gltA</i> : MG563768]/ <i>Rickettsia monacensis</i> 98.09% [ <i>ompB</i> : KU961543]	Palestina
<i>Setophaga castanea</i> *	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Norcasia
<i>Myiothlypis coronata</i>	<i>Ixodes</i> sp. II (1/A)	0/1 (0)		Villamaría
<i>Cardellina canadensis</i> *	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Palestina
<i>Euphonia laniirostris</i>	<i>Amblyomma longirostre</i> (1/L)	0/1 (0)		Norcasia
Total		10/70 (14)		

L: larva, N: nymph, A: adult.

\* Migratory boreal bird.

<sup>a</sup> Sequences  $\leq$  95%

Based on DNA sequences, three rickettsia agents were detected. *Rickettsia amblyommatis* was detected in *A. longirostre*, *A. varium*, and *Ixodes* sp I, which were collected from four resident bird species. The partial sequences of *gltA* and *ompA* genes showed 100% identity to sequences of *R. amblyommatis* from GenBank (Table 1). In the migratory boreal bird *Oporornis agilis*, we detected '*Candidatus* *Rickettsia colombianensi*' in *A. dissimile*, based on a partial sequence of the *gltA* gene that was 100% identical to this rickettsial agent in GenBank. The *ompB* partial sequence from this *A. dissimile* tick displayed closest identity (98.09%) to *Rickettsia monacensis* (Table 1); however, there was no available sequence of '*Candidatus* *R. colombianensi*' for the same portion of the *ompB* gene in GenBank. Finally, we detected a *Rickettsia* sp. in an *Ixodes* sp. II female that could not be assigned to any recognized species of *Rickettsia*, since its *ompA* partial sequence was 99.71% identical to '*Candidatus* *Rickettsia tarasevichiae*' and its *ompB* partial sequence was 98.96% identical to *Rickettsia canadensis* (Table 1). The GenBank accession numbers for the sequences of

*Rickettsia* obtained in this study are [MT928665-MT928670] for *gltA* gene, [MT951178-MT951180] for *ompA*, and [MT586610-MT586612] for *ompB*.

## Discussion

This research provides report of *Rickettsia* in ticks collected on wild birds in Colombia. We detected *R. amblyommatis*, ‘*Candidatus R. colombianensi*’, and a possible novel agent closely related to ‘*Candidatus R. tarasevichiae*’ and *R. canadensis*. Our detection of *R. amblyommatis* in *A. longirostre* and *A. varium* agrees with previous reports of *R. amblyommatis* in these two tick species, including some from wild birds (Ogrzewalska et al. 2010, 2011, 2012b, 2014, 2015; Pacheco et al. 2012; Mukherjee et al. 2014; Lugarini et al. 2015; Novakova et al. 2015; Ramos et al. 2015; Budachetri et al. 2017; Quintero et al. 2017; Zeringóta et al., 2017). In particular, *R. amblyommatis* was considered as the most prevalent *Rickettsia* species of the spotted fever group to infect ticks in the American continent (Karpathy et al. 2016). Therefore, we add a novel tick species for *R. amblyommatis*, *Ixodes* sp. I (closely related to *I. aurtulus*), which to our knowledge is the first record of *R. amblyommatis* in the genus *Ixodes*.

In another unidentified tick species, *Ixodes* sp. II, which is closely related to *Ixodes tanuki* (Martínez-Sánchez et al. 2020), the rickettsial gene sequences of *ompA* and *ompB* were closest to ‘*Candidatus R. tarasevichiae*’ and *R. canadensis*, which are considered to form a more basal group of rickettsiae, distinct from either the spotted fever group or the typhus group (Weinert et al. 2009). Interestingly, previous studies proposed human illness due to either ‘*Candidatus R. tarasevichiae*’ (Jia et al. 2013; Yi et al. 2014) or *R. canadensis* (Parola et al. 2013).

Our record of ‘*Candidatus R. colombianensi*’ in *A. dissimile* agrees to previous studies that reported this agent infecting *A. dissimile* collected from *Hydrochoerus hydrochaeris*, *Iguana iguana*, *Proechimys semispinosus*, and humans in Colombia (Miranda et al. 2012; Miranda and Mattar, 2014; Quintero et al. 2013; Quintero et al. 2017).

We report rickettsiae in ticks collected from a migratory boreal bird (*O. agilis*) and birds distributed in the high Andes (*C. homochroa* and *T. superciliaris*). This migratory bird species reproduces in the United States of America and Canada, and spend the winter in South America, where it reached northern Argentina and Brazil. Therefore, this bird travels long distances and passes geographical barriers that can affect the distribution of ticks and their associated pathogens. Here, we provide the first report in America of rickettsiae in ticks collected from wild birds at altitudes above 3000 m a.s.l. This finding demonstrates that rickettsiae are circulating throughout the entire altitudinal range of the department of Caldas and, likewise, this can be occurring in other regions of Colombia and America. Therefore, the department of Caldas, Colombia becomes an excellent site for researching interactions between wild birds, ticks, and pathogens to contribute information on tick-borne diseases and the possible role of wild birds in the spread of diseases.

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#### 4. FINAL CONSIDERATIONS

Several studies have demonstrated the role of wild birds in the transport of ticks and their associated pathogens. Birds are gaining greater importance in the epidemiology of various tick-borne diseases. The flight capacity of birds is one of the most important factors in the distribution and transport of potential pathogen vectors to new geographic areas. Although tick species face limiting environmental conditions for survival outside natural areas of distribution, climate change events have promoted favorable conditions for ticks to establish in other regions. In this scenario, birds play a key role to understand the spread of tick vectors since birds can cross geographic barriers. Accordingly, the study of the associations between hosts and ticks is the first step to determine future scenarios of tick-borne diseases. Several studies in the American continent show that birds participate in the biological cycle of ticks and pathogens and many of these studies have been conducted in South America (e.g., Brazil, Argentina). In Colombia, no studies have yet focused on the associations between wild birds and ticks and the infection of these vectors with *Rickettsia*. This research is the first to address the associations among wild birds, ticks, and *Rickettsia*. Furthermore, this study contributes knowledge for Colombia and broadens the existing knowledge for the American continent, involving migratory boreal birds that travel from North to South America. Finally, the presence of bacteria of the genus *Rickettsia* in Colombia has raised alarms concerning the circulation of rickettsiosis, which are unfortunately left unattended in the country due to the lack of knowledge on the cycle and origin of these diseases. Consequently, this research is a first approach to the study of *Rickettsia*-infected ticks associated with wild birds, particularly, since the pathogenicity of several reported rickettsiae is still unknown.

Knowing the ecological characteristics of bird and tick species is relevant since it provides valuable information to understand these interactions and can be included in pathogen surveillance systems. This research contributes information on the important role of migratory birds in the transport and dispersal of ticks and pathogens, in which it is necessary to comprehend the importance of migration in the epidemiological processes of tick-borne diseases. Nevertheless, other ecological characteristics of birds and ticks must be determined.



Finally, the information on the role of birds in the transmission of rickettsiae associated with hard ticks should be integrated into studies that also address the role of other tick hosts (e.g., amphibians, reptiles, and mammals), as well as other bacteria, protozoa, and viral pathogens (e.g., *Borrelia*, *Anaplasma*, *Ehrlichia*, *Babesia*, encephalitis viruses). In this regard, understanding the biological cycle of ticks and their potential hosts is crucial to elucidate the dynamics of potential pathogen transmission.

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