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Original article

Contributions to the knowledge of hard ticks (Acari: Ixodidae) in Colombia

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ABSTRACT

The known tick fauna of Colombia includes 58 species (15 Argasidae and 43 Ixodidae). To add to the knowledge of the biology of ticks in Colombia, hard ticks (Ixodidae) were collected from domestic animals or vegetation during 2014–2016 in 10 of Colombia's Departments. Ticks were identified to species through morphological examinations. Taxonomic identification was confirmed for some specimens by molecular methods, including phylogenetic analyses inferred from three tick genes (cytochrome c oxidase, 16S rDNA, second internal transcribed spacer). A total of 1745 tick specimens encompassing 8 species were collected. Overall, 5 tick species were recorded on cattle [*Amblyomma dissimile, Amblyomma mixtum, Dermacentor nitens, Rhipicephalus microplus, Rhipicephalus sanguineus* sensu lato (s.l.)], 5 on dogs (*Amblyomma maculatum, Amblyomma ovale, Amblyomma varium, R. microplus, R. sanguineus* s.l.), 3 on horses (*A. mixtum, D. nitens, R. sanguineus* s.l.), 3 on donkeys (*A. mixtum, D. nitens, R. microplus*), 1 on pig (*D. nitens*), and 2 from vegetation (*A. mixtum, A. dissimile*). This included the first records of *A. mixtum* from two Colombian Departments, indicating that the distribution of this tick in Colombia may be broader than currently known. Phylogenetic analyses confirmed that *R. sanguineus* s.l. specimens from 8 Departments belong to the "tropical species". Moreover, Colombian specimens of *A. maculatum* formed a large clade with GenBank sequences of *A. maculatum* and *A. triste,* although some Colombian specimens grouped with *A. maculatum* from the United States while others grouped with *A. triste* from Brazil. Significant polymorphisms were observed between specimens of *A. ovale* or *D. nitens*; for the former species, it is noteworthy that two distinct clades were observed. Our study provides new records for 8 tick species parasitizing domestic animals in Colombia, including species with veterinary and medical importance in the Neotropical region, such as *R. microplus, R. sanguineus, D. nitens, A. mixtum,* and *A. maculatum.* Noteworthy, we provide the first record of *A. varium* infesting a domestic mammal.

1. Introduction

The World's tick fauna currently includes 939 species of three families: Ixodidae (727 species), Argasidae (211 species) and Nuttalliellidae (1 species). Nearly one-fourth of these species are known to occur in the Neotropical region (Barros-Battesti et al., 2006; Hornok et al., 2016; Labruna et al., 2016; Muñoz-Leal et al., 2016; Apanaskevich and Bermúdez, 2017; Ash et al., 2017; Muñoz-Leal et al., 2017; Chitimia-Dobler et al., 2017; Guo et al., 2017; Nava et al., 2017). Broad-scale studies on ticks occurring in Colombia have been scarce (Osorno-Mesa, 1940; López and Parra, 1985). According to the last review of Neotropical ticks (Guglielmone et al., 2003), the following

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Table 1
Localities in Colombia where ticks were collected in the present study.

Locality number[a]	Municipality	Department
1	Leticia	Amazonas
2	Medellín	Antioquia
3	Arauca	Arauca
4	Fortul	Arauca
5	Saravena	Arauca
6	San Jacinto	Bolívar
7	Dorada	Caldas
8	Neira	Caldas
9	Norcasia	Caldas
10	Nunchía	Casanare
11	Yopal	Casanare
12	Puerto Salgar	Cundinamarca
13	San Juan de Arama	Meta
14	Ibagué	Tolima
15	Saldaña	Tolima
16	Cali	Valle del Cauca
17	Restrepo	Valle del Cauca

[a] localities indicated in.

tick species are known to occur in Colombia: Argasidae (15 species) – *Antricola mexicanus* Hoffmann, 1958, *Argas magnus* Neumann, 1896, *Argas miniatus* Koch, 1844, *Ornithodoros azteci* Matheson, 1935, *Ornithodoros brodyi* Matheson, 1935, *Ornithodoros furcosus* Neumann, 1908, *Ornithodoros hasei* (Schulzei, 1935), *Ornithodoros marinkellei* Kohls, Clifford and Jones, 1969, *Ornithodoros marmosae* Jones and Clifford, 1972, *Ornithodoros peropteryx* Kohls, Clifford and Jones 1969, *Ornithodoros puertoricensis* Fox, 1947, *Ornithodoros rossi* Kohls, Sonenshine and Clifford, 1965, *Ornithodoros rudis* Karsch, 1880, *Ornithodoros talaje* (Guérin-Méneville, 1849), and *Ornithodoros yumatensis* Cooley and Kohls, 1941; Ixodidae (38 species): *Amblyomma auricularium* (Conil, 1878), *Amblyomma cajennense* (Fabricius, 1787), *Amblyomma calcaratum* Neumann, 1899, *Amblyomma coelebs* Neumann, 1899, *Amblyomma crassum* Robinson, 1926, *Amblyomma dissimile* Koch, 1844, *Amblyomma geayi* Neumann, 1899, *Amblyomma humerale* Koch, 1844, *Amblyomma longirostre* (Koch, 1844), *Amblyomma maculatum* Koch, 1844, *Amblyomma multipunctum* Neumann, 1899, *Amblyomma naponense* (Packard, 1869), *Amblyomma neumanni* Ribaga, 1902, *Amblyomma no-*

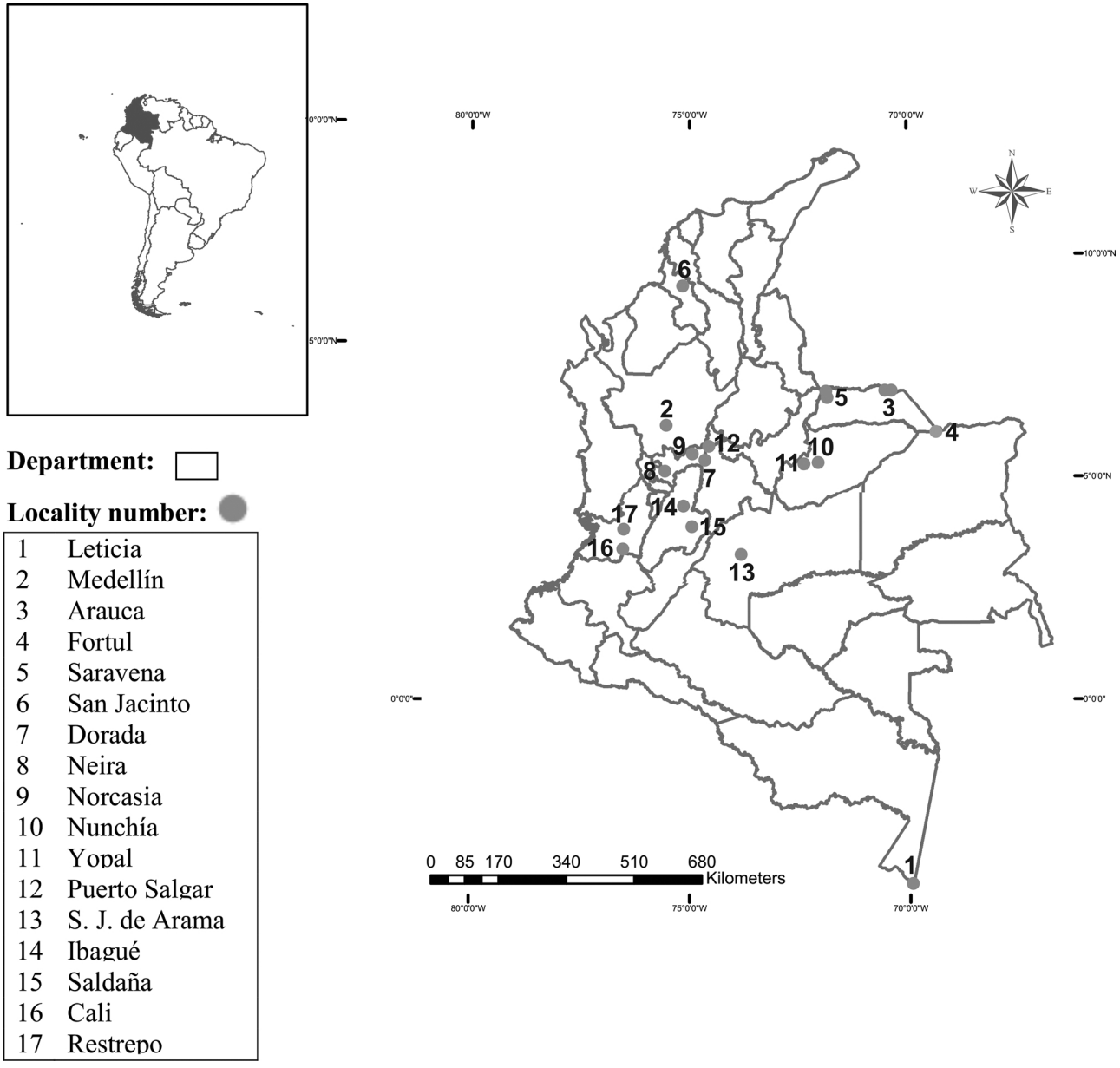

Fig. 1. Geopolitical map of Colombia showing the localities where ticks (Acari: Ixodidae) were collected during the present study.

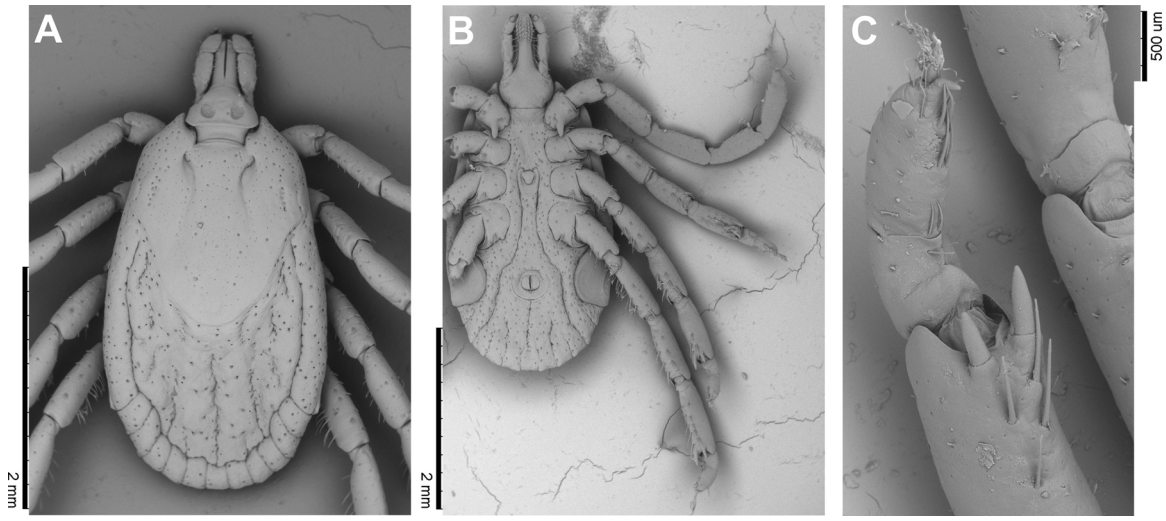

Fig. 2. A female specimen of *Amblyomma maculatum* from Ibagué-Tolima. (A) Dorsal view. (B) Ventral view. (C) Tarsus III showing two spurs.

Table 2
Ticks at different developmental stages (A: adults; N: nymphs; L: larvae) collected from different host species during 2014–2016 at different localities of Colombia.

Tick species	Stage	Host	Localities[a]
Amblyomma dissimile	A	*Bos taurus*	14
A. dissimile	N, L	vegetation	11
Amblyomma maculatum	A	*Canis lupus familiaris*	9,14,15
Amblyomma mixtum	A, N	*Bos taurus*	3,8,10,13
A. mixtum	A	*Equus asinus*	11
A. mixtum	A, N	*Equus caballus*	3,10,11
A. mixtum	A,N,L	vegetation	10,11
Amblyomma ovale	A	*C. lupus familiaris*	14,15
Amblyomma varium	A	*C. lupus familiaris*	14
Dermacentor nitens	A,N	*B. taurus*	1,3
D. nitens	A,N	*E. asinus*	6,11,14
D. nitens	A,N,L	*E. caballus*	1,3,6,10,11,14
D. nitens	A	*Sus scrofa domesticus*	3
Rhipicephalus microplus	A,N,L	*B. taurus*	1,3–5,13,14,17
R. microplus	A	*C. lupus familiaris*	5
R. microplus	A	*E. asinus*	6
Rhipicephalus sanguineus	A	*B. taurus*	1,14
R. sanguineus	A,N	*C. lupus familiaris*	1–3,5,7,9,11,12,14 – 16,17
R. sanguineus	A	*E. caballus*	3,11

[a] Locality numbers indicated in Table 1 and Fig. 1.

dosum Neumann, 1899, *Amblyomma oblongoguttatum* Koch, 1844, *Amblyomma ovale* Koch, 1844, *Amblyomma pacae* Aragão, 1911, *Amblyomma rotundatum* Koch, 184, *Amblyomma sabanerae* Stoll, 1894, *Amblyomma scalpturatum* Neumann, 1906, *Amblyomma tapirellum* Dunn, 1933, *Amblyomma triste* Koch, 1844, *Amblyomma varium* Koch, 1844, *Haemaphysalis juxtakochi* Cooley, 1946, *Haemaphysalis leporispalustris* (Packard, 1869), *Ixodes boliviensis* Neumann, 1904, *Ixodes brunneus* Kock, 1844, *Ixodes fuscipes* Kock, 1844, *Ixodes lasallei* Méndez Arocha and Ortiz, 1958, *Ixodes luciae,* Sénevet, 1940, *Ixodes montoyanus* Cooley, 1944, *Ixodes pararicinus* Keirans and Clifford, 1985, *Ixodes tapirus* Kohls, 1956, *Ixodes tropicalis* Kohls, 1956, *Ixodes venezuelensis* Kohls, 1953, *Dermacentor nitens* Neumann, 1897, *Rhipicephalus microplus* (Canestrini, 1887), and *Rhipicephalus sanguineus* (Latreille, 1806). In addition, there were reports from Colombia of *Amblyomma parvum* Aragão, 1908 (López and Parra, 1985; confirmed by Nava et al., 2017), *Ixodes affinis* Neumann, 1889 (Mattar and López-Valencia, 1998), and *Dermacentor imitans* Warburton, 1933 (Guglielmone et al., 2006). More recently, Nava et al. (2014) reevaluated the taxonomic status of *A. cajennense* and concluded that this taxon was actually represented by six valid species from which *A. cajennense* sensu stricto was not found to be present in Colombia in the examined samples, and only *Amblyomma patinoi* Labruna, Nava and Beati, 2014, and *Amblyomma mixtum* Koch, 1844 were confirmed for Colombia (Nava et al., 2014; Rivera-Páez et al., 2016). Finally, Apanaskevich and Bermúdez (2017) reported *Ixodes bocatorensis* Apanaskevich and Bermudez, 2017 from Colombia. Considering all these reports, the known tick fauna of Colombia currently includes 58 species (15 Argasidae and 43 Ixodidae), although as recently reported the presence of *I. brunneus* needs confirmation (Bermúdez et al., 2015). The present study was undertaken to expand our current knowledge of the biology of ticks in Colombia by collecting hard ticks (Ixodidae) from domestic animals and vegetation across 10 of Colombia's Departments.

2. Materials and methods

From August 2014 to May 2016, hard ticks were collected from domestic animals or vegetation in 10 Colombian Departments, encompassing 17 municipalities (Table 1, Fig. 1). Collected ticks were placed into plastic vials containing absolute ethanol and taken to the laboratory, where they were taxonomically identified based on current literature (Kohls, 1956; Jones et al., 1972; Estrada-Peña et al., 2005; Barros-Battesti et al., 2006; Martins et al., 2010; Nava et al., 2014; , 2015). The collection of specimens from animals was carried out under the Permit *"Autoridad Nacional de Licencias Ambientales ANLA Resolución 1166 de octubre 9 de 2014".*

In order to confirm morphological identifications, some specimens of each tick species were subjected to molecular taxonomic identification. DNA from individual ticks were extracted using the DNeasy Blood and Tissue kit (Qiagen, Chatsworth, California, USA) following the manufactureŕs protocol. The extracted DNA was tested by polymerase chain reaction (PCR) protocols to amplify fragments of at least one of the three following genes of the tick genome: primers 5′-GGT CAA CAA

Table 3
Results of BLAST analysis searches in public data of the DNA sequences generated from ticks collected in the present study.

Tick species (localities[a])	Closest identity (%) in GenBank (accession number) according to the tick gene		
	COI	16S rRNA	ITS2
Amblyomma dissimile (11)	*A. dissimile* [KF200114] 99%	N.A.	N.D.
Amblyomma maculatum (14)	*A. maculatum* [KU302492] 99%	*A. maculatum* [KT037651] 99%	N.D.
Amblyomma maculatum (15)	*A. triste* [KU306582] 99%	*A. triste* [KU284955] 99%	N.D.
Amblyomma maculatum (9)	N.D.	*A. maculatum* [KT037651] 100%	N.D.
Amblyomma mixtum (3)	N.D.	*A. mixtum* [KT820359] 97%	N.D.
Amblyomma mixtum (3,8,10,13)	N.D.	*A. mixtum* [KT820359] 98%	*A. cajennense* [JN866853][#] 100%
Amblyomma mixtum (8)	*A. mixtum* [KY595138] 99%	N.D.	N.D.
Amblyomma mixtum (11)	*A. mixtum* [KY595138] 99%	N.D.	N.D.
Amblyomma mixtum (13)	*A. mixtum* [KY595138] 99%	N.D.	N.D.
Amblyomma mixtum (10)	*A. mixtum* [KY595139] 100%	N.D.	N.D.
Amblyomma mixtum (11)	*A. mixtum* [KY595136] 99%	N.D.	N.D.
Amblyomma ovale (14,15)	*A. ovale* [KF200080] 99%	*A. ovale* [KU894383] 99%	N.D.
Dermacentor nitens (1)	*D. nitens* [KT906190] 96%	*D. nitens* [KY020994] 96%	N.D.
Dermacentor nitens (3,6,10,11)	*D. nitens* [KT906188] 100%	*D. nitens* [KY020994] 99%	N.D.
Dermacentor nitens (6)	*D. nitens* [KY441487] 99%	N.D.	N.D.
Rhipicephalus microplus (3–5,13,14,17)	*R. microplus* [KT906181] 100%	N.D.	N.D.
Rhipicephalus microplus (5)	*R. microplus* [KT906181] 99%	N.D.	N.D.
Rhipicephalus microplus (1)	*R. microplus* [KY678120] 100%	*R. microplus* [EU918176] 99%	N.D.
Rhipicephalus microplus (1–5,13,14,17)	N.D.	*R. microplus* [EU918176] 100%	N.D.
Rhipicephalus sanguineus (1–3,7,11,12,14-17)	N.D.	*R. sanguineus* [KY413787] 100%	N.D.
Rhipicephalus sanguineus (5)	N.D.	*R. sanguineus* [KY413787] 99%	N.D.
Rhipicephalus sanguineus (1,14,16)	N.D.	N.D.	*R. sanguineus* [KY945496] 99%
Rhipicephalus sanguineus (3,15)	N.D.	N.D.	*R. sanguineus* [JQ625707] 99%
Rhipicephalus sanguineus (11)	N.D.	N.D.	*R. sanguineus* [KF499537] 99%
Rhipicephalus sanguineus (7)	N.D.	N.D.	*R. sanguineus* [AF271283] 99%
Rhipicephalus sanguineus (14)	N.D.	N.D.	*R. sanguineus* [KF499537] 99%
Rhipicephalus sanguineus (2)	N.D.	N.D.	*R. sanguineus* [KF499537] 99%
Rhipicephalus sanguineus (3,7,11,14)	N.D.	N.D.	*R. sanguineus* [KF499537] 99%
Rhipicephalus sanguineus (11)	N.D.	N.D.	*R. turanicus* [KF958425] 99%
Rhipicephalus sanguineus (1)	N.D.	N.D.	*R. turanicus* [KF499532] 99%
Rhipicephalus sanguineus (11)	N.D.	N.D.	*R. turanicus* [KF958425] 99%

N.D.: not done.
N.A.: sequences not available in GenBank.
[#] This sequence was deposited in GenBank as *A. cajennense*; however, according to Nava et al. (2014), this sequence corresponds to *A. mixtum*.
[a] Locality numbers indicated in Table 1 and Fig. 1.

ATC ATA AAG ATA TTG G-3′ and 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′, targeting a ≈700-bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) (Folmer et al., 1994); primers 5′–CTG CTC AAT GAT TTT TTA AAT TGC TGT GG–3′ and 5′–CCG GTC TGA ACT CAG ATC AAG T-3, targeting a ≈460-bp fragment of the mitochondrial 16S rDNA gene (Norris et al., 1996); and primers 5′-CCA TCG ATG TGA AYT GCA GGA CA-3′ (Zahler et al., 1995) and 5′-GTG AAT TCT ATG CTT AAA TTC AGG GGG T-3′, which amplifies a ≈1100-bp fragment that includes the entire second internal transcribed spacer (ITS2) region of the nuclear rRNA region (Mclain et al., 1995). In addition, specimens of the following species collected in Brazil were processed by the above PCR protocols in order to obtain DNA sequences for inclusion in the phylogenetic analyses: *A. cajennense* s.s. from Governador Jorge Teixeira, Rondônia state, *A. sculptum* from Pirassununga, São Paulo state, *Amblyomma triste* from Paulicéia, São Paulo state, *R. sanguineus* s.l. from Chapada Gaúcha, Minas Gerais state, and *R. sanguineus* s.l. from São Paulo City, São Paulo state.

PCR products were purified with the QIAquick PCR purification kit (Qiagen), and sent to Macrogen Inc. (South Korea) for DNA sequencing. The sequenced fragments were evaluated and edited using Geneious Trial v8.14 (Drummond et al., 2009) and Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). In addition, the sequences were searched by MegaBlast against the public databases and deposited in GenBank and Barcode of Life Data Systems (BOLD). The sequences for each gene were aligned using ClustalW (Thompson et al., 1997), included in the program MEGA version 7 (Tamura et al., 2013). Intraspecific nucleotide divergences were estimated with the program MEGA, using the Kimura 2-Parameter distance model (K2P; Kimura, 1980). Species confirmation was carried out through a similarity analysis based on Maximum Likelihood (ML), with the K2P model and 1000 bootstrap replications, using the program MEGA. Each alignment included different sequences from GenBank, as stated by their accession numbers in the trees.

3. Results

A total of 1745 tick specimens (1543 adults, 111 nymphs, and 91 larvae) were collected from 85 individual hosts, including 28 cattle (*Bos taurus*), 26 domestic dogs (*Canis lupus familiaris*), 25 horses (*Equus caballus*), 5 donkeys (*Equus asinus*), and 1 domestic pig (*Sus scrofa domesticus*), with an additional 3 tick samples collected from vegetation (Table S1). Ticks were morphologically identified to 8 species: *A. dissimile, A. maculatum* (Fig. 2)*, A. mixtum, A. ovale, A. varium, D. nitens, R. microplus*, and *R. sanguineus* sensu lato (s.l.). Hosts and collection localities for each tick species are shown in Table 2. Overall, 5 tick species were recorded on cattle (*A. dissimile, A. mixtum, D. nitens, R. microplus, R. sanguineus* s.l.), 5 on dogs (*A. maculatum, A. ovale, A. varium, R. microplus*, *R. sanguineus* s.l.), 3 on horses (*A. mixtum, D. nitens, R.*

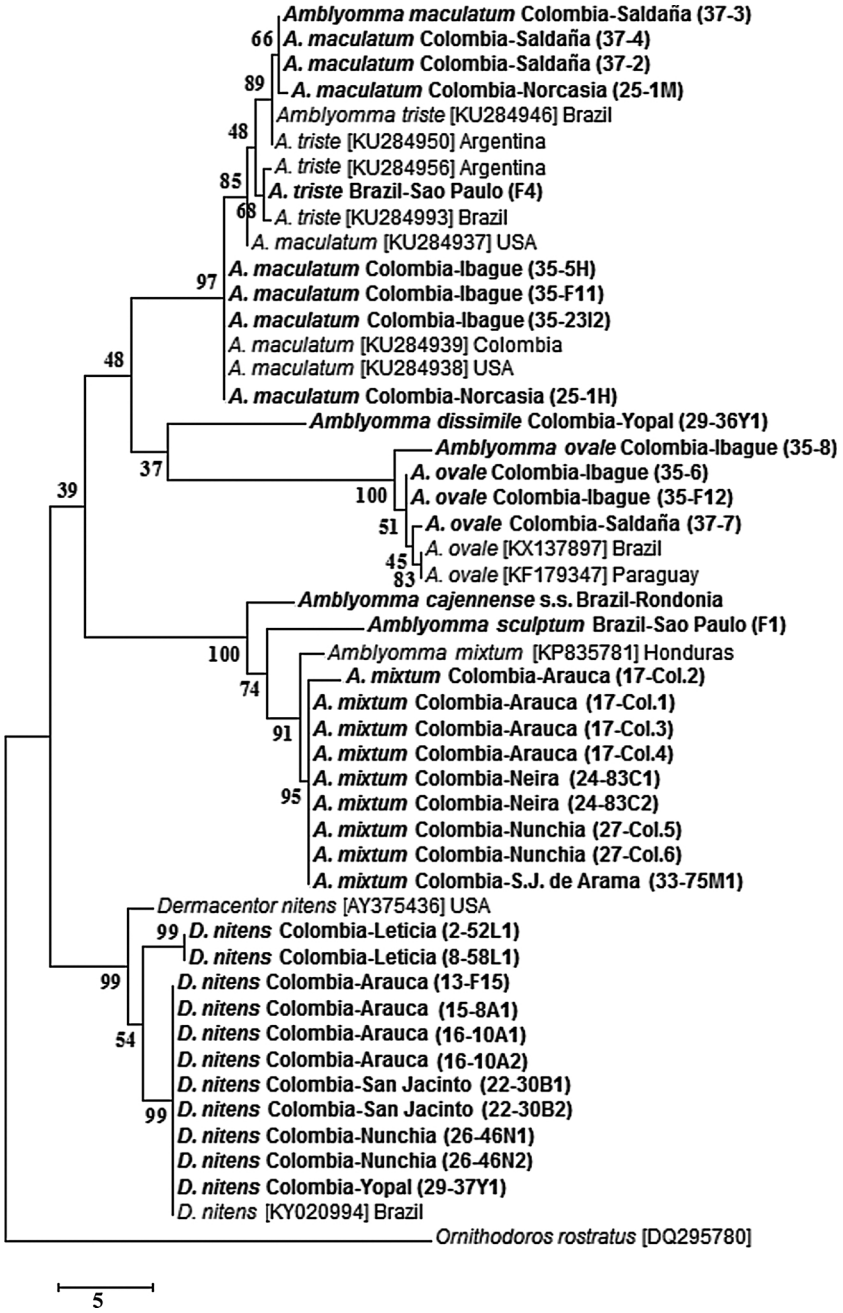

Fig. 3. Maximum Likelihood (ML) tree using sequences of the mitochondrial 16S rDNA gene partial sequences of tick specimens collected in the present study (in bold) and sequences from GenBank (accession numbers in brackets). Numbers at nodes are bootstrap support values. The sequence of *Ornithodoros rostratus* was used as outgroup.

sanguineus s.l.), 3 on donkeys (*A. mixtum, D. nitens, R. microplus*), and 1 on pig (*D. nitens*).

Partial sequences of the tick 16S rRNA and/or COI and/or ITS2 genes were generated for tick specimens of all collected tick species (Table 3), except for *A. varium*. Phylogenetical analyses based on 16S rRNA and COI partial sequences placed *A. maculatum* from Colombia mixed within a clade composed by sequences of *A. maculatum* from the United States and Colombia, and *Amblyomma triste* from Brazil and Argentina (Figs. 3 and 4). Moreover, sequences from *A. ovale* grouped with corresponding sequences of *A. ovale* from Brazil and Paraguay (Fig. 3), or Panama (Fig. 4); sequences of *D. nitens* grouped with *D. nitens* from the United States and Brazil (Fig. 3), or Colombia and Panama (Fig. 4); a sequence of *A. dissimile* grouped with *A. dissimile* from Brazil (Fig. 4); and sequences of *A. mixtum* grouped with *A. mixtum* from Honduras (Fig. 3) or Colombia, Ecuador and Panama (Fig. 4). These results for *A. mixtum* were corroborated by the ITS2 phylogenetic analysis, in which our sequences of *A. mixtum* grouped with *A. mixtum* sequences from Colombia, Costa Rica, Mexico and the United States (Fig. 5).

All 16S rRNA sequences of Colombian *R. sanguineus* s.l. generated in the present study grouped with *R. sanguineus* s.l. from Brazil, Colombia, Thailand, and South Africa (Fig. 6). In the phylogenetic analysis

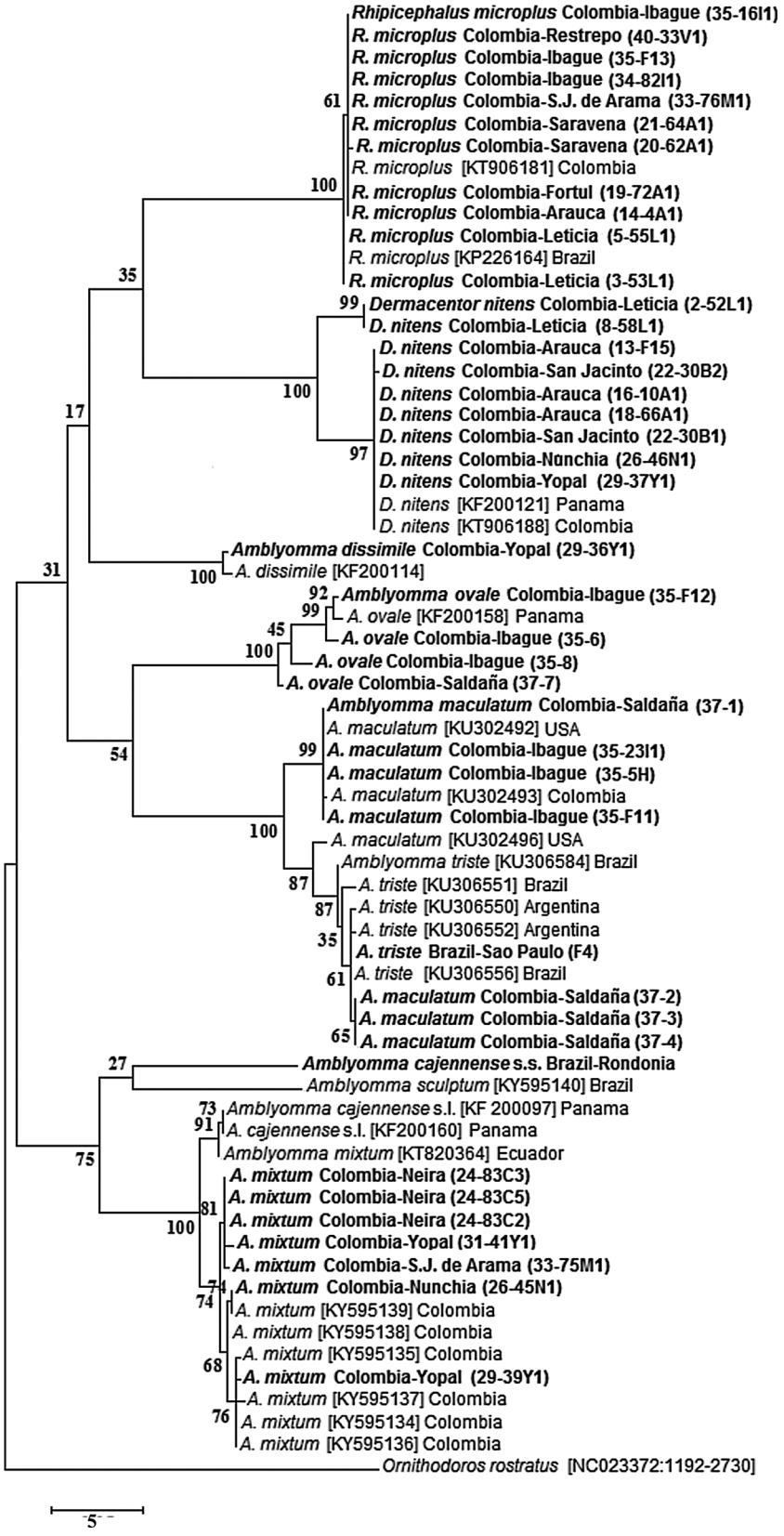

Fig. 4. Maximum Likelihood (ML) tree using sequences of the mitochondrial cytochrome c oxidase subunit I (COI) gene partial sequences of tick specimens collected in the present study (in bold) and sequences from GenBank (accession numbers in brackets). Numbers at nodes are bootstrap support values. The sequence of *Ornithodoros rostratus* was used as outgroup.

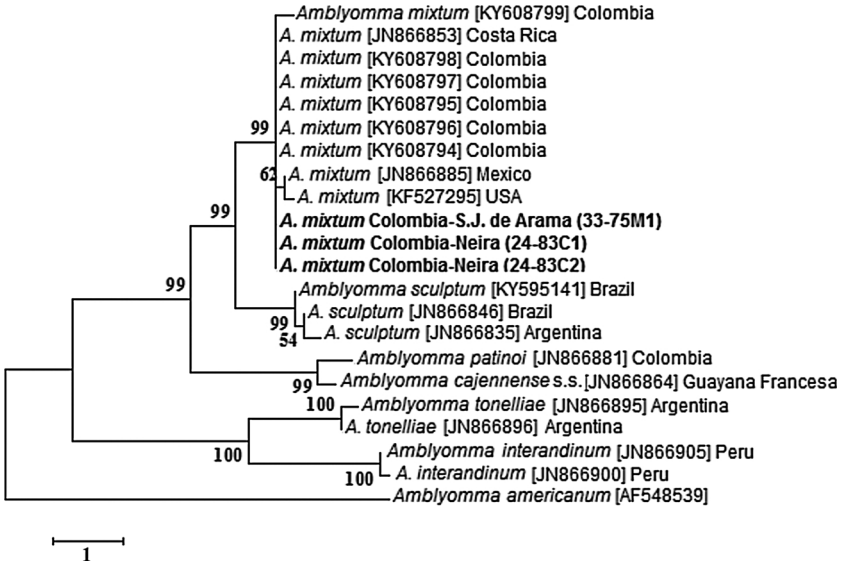

Fig. 5. Maximum Likelihood (ML) tree using sequences of the second internal transcribed space (ITS2) gene partial sequences of tick specimens collected in the present study (in bold) and sequences from GenBank (accession numbers in brackets). Numbers at nodes are bootstrap support values. The sequence of *Amblyomma americanum* was used as outgroup.

inferred by the ITS2 gene of *R. sanguineus* s.l. ticks, the Colombian specimens grouped in a large clade composed by sequences from tropical countries such as Costa Rica, Honduras, Brazil, Colombia and Thailand, but also by sequences from Australia, Egypt, and India (Fig. 7). Regarding our specimens of *R. microplus,* their 16S rRNA sequences grouped in a clade with *R. microplus* from Argentina (Fig. 6), while their COI sequences grouped with sequences of *R. microplus* from Brazil and Colombia (Fig. 4).

GenBank nucleotide sequence accession numbers for the DNA sequences obtained in the current study are: MF351562-MF351603, MF353094-MF353129 for the mitochondrial rRNA 16S gene; MF363053-MF363093 for the mitochondrial COI gene, and MF353130-MF353154 for nuclear ITS2 gene. Voucher tick specimens were deposited at the tick collection "Coleção Nacional de Carrapatos Danilo Gonçalves Saraiva" – CNC (University of São Paulo, São Paulo, Brazil).

4. Discussion

All tick species collected in the present study were previously reported from Colombia (Guglielmone et al., 2003; Rivera-Páez et al., 2016). Similarly, the tick-host associations in our study agree with previous records (López and Parra 1985; Need et al., 1991; Rivera-Páez et al., 2016; Nava et al., 2017), with the exception that we report the first confirmed collection of *A. varium* from dog.

From 7 *A. maculatum* specimens from Colombia, we generated 3 distinct haplotypes for the 16S rRNA gene, and 2 for COI. In each of the trees inferred from these two genes, the Colombian specimens formed a large clade with GenBank sequences of *A. maculatum* and *A. triste*. Noteworthy, while some Colombian specimens grouped with *A. maculatum* from the United States, other Colombian specimens grouped with *A. triste* from Brazil, for both genes. At first sight, these results could indicate that some Colombian specimens could represent the taxon *A. triste*; however, we retained these specimens as *A. maculatum* because their morphology was compatible with *A. maculatum* (two spurs on tarsi II–IV) (Fig. 2) rather than *A. triste* (one spur on tarsi II–IV), as stated in the literature (Kohls, 1956; Estrada-Peña et al., 2005). Further studies are required to elucidate the taxonomic status of *A. maculatum* and *A. triste*, since the taxonomic separation of these two species is yet to be resolved, with the possibility that they might be conspecific (Nava et al., 2017).

From up to 9 *A. mixtum* specimens from Colombia, we generated 2 distinct haplotypes for the 16S rRNA gene, 5 for COI, and 1 for ITS2. In each of the trees inferred from these three genes, the Colombian specimens grouped with GenBank sequences of *A. mixtum,* well separated from all other members of the *A. cajennense* species complex. These results corroborate a recent study that confirmed the presence of *A. mixtum* in Colombia (Rivera-Páez et al., 2016). However, whereas this previous report consisted of specimens from the Departments of Arauca and Casanare (northeastern Colombia), the present records confirm the presence of *A. mixtum* in these two Departments and expand it to two other Departments, Caldas and Meta (central Colombia). These results indicate that the distribution of *A. mixtum* in Colombia may be much broader than currently known.

From up to 30 *R. sanguineus* s.l. specimens from Colombia, we generated 2 distinct haplotypes for the 16S rRNA gene, and 10 for ITS2. In the tree inferred from the 16S rRNA gene, all Colombian specimens grouped with *R. sanguineus* specimens from Brazil and Colombia, which represent the so called "tropical species", as reported elsewhere (Burlini et al., 2010; Moraes-Filho et al., 2011; Dantas-Torres et al., 2013). These 30 *R. sanguineus* s.l. specimens were collected in 8 Departments of Colombia (Amazonas, Antioquia, Arauca, Caldas, Casanare, Cundinamarca, Tolima, Valle del Cauca), whereas the previous reports represented specimens from the Departments of Cordoba (Moraes-Filho et al., 2011), Antioquia, and Valle del Cauca (Dantas-Torres et al., 2013). These results indicate that the taxon *R. sanguineus* s.l. is possibly represented in Colombia solely by the "tropical species", which seems to be widespread in the country. In contrast to the 16S rRNA gene, the tree inferred from the ITS-2 gene did not segregate the Colombian specimens from specimens from other parts of the world. This inconclusive analysis is supported by the study of Latrofa et al. (2013), who demonstrated that the ITS-2 gene is not reliable for identification of ticks within the *Rhipicephalus* genus, especially among specimens of *R. sanguineus* s.l.

From up to 11 *R. microplus* specimens from Colombia, we generated 2 distinct haplotypes for the 16S rRNA gene, and 3 for COI. In the trees inferred from these two genes, the Colombian specimens formed a clade

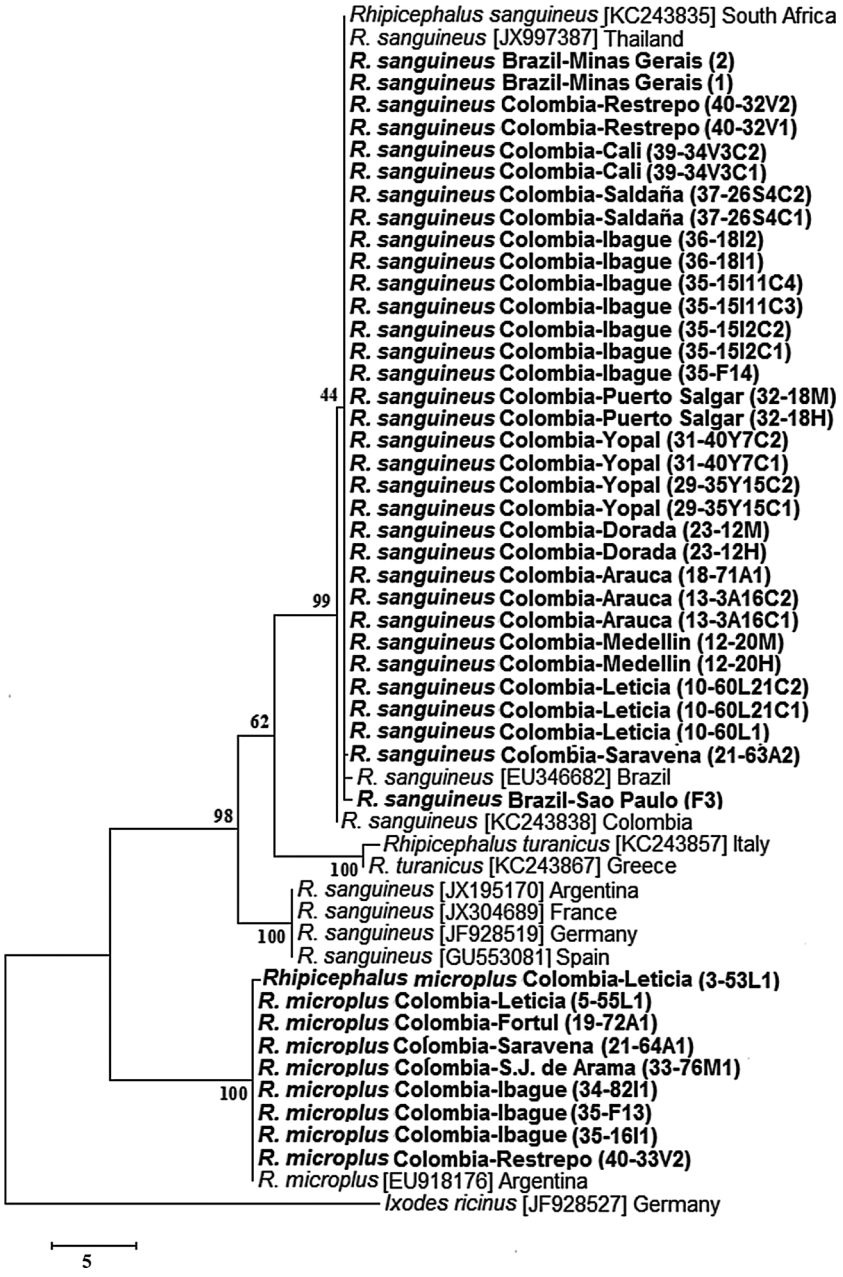

Fig. 6. Maximum Likelihood (ML) tree using sequences of the mitochondrial 16S rDNA gene partial sequences of tick specimens collected in the present study (in bold) and sequences from GenBank (accession numbers in brackets). Numbers at nodes are bootstrap support values. The sequence of *Ixodes ricinus* was used as outgroup.

with sequences from Argentina and Brazil, which correspond to bona fide records of *R. microplus* according to the studies of Labruna et al. (2009) and Estrada-Peña et al. (2012), who re-evaluated the taxonomic status of *R. microplus*. Regarding the trees that included *D. nitens* sequences, it was interesting to note some polymorphism in their 16S rRNA or COI sequences, which in both cases resulted in a clade that separated the sequences of Leticia (Amazonas Department) from the other Colombian Departments (Arauca, Bolívar, Casanare, Tolima), although we could not detect significant morphological differences between these specimens (data not shown). Finally, the relatively high polymorphism between the sequences of *A. ovale* for the two mitochondrial genes (Figs. 3 and 4) is corroborated by a study in Brazil, where four distinct haplotypes of *A. ovale,* differing up to 3.9%, were generated from a single region (Martins et al., 2016).

This study provides new records for 8 tick species parasitizing domestic animals in Colombia, including species with veterinary and medical importance in the Neotropical region, such as *R. microplus, R. sanguineus, D. nitens, A. mixtum,* and *A. maculatum* (Guglielmone et al., 2003; Nava et al., 2014, 2017). Most of these species have gone through extensive taxonomic changes or contestations over the last decade (Estrada-Peña et al., 2012; Nava et al., 2014, 2015, 2017). For this reason, phylogenetic analyses of specimens from poorly explored sites such as Colombia are crucial for our growing knowledge of tick systematics in the Neotropical region, where ticks associated with domestic animals are vectors of a number of pathogens to animals and humans (Nava et al., 2017).

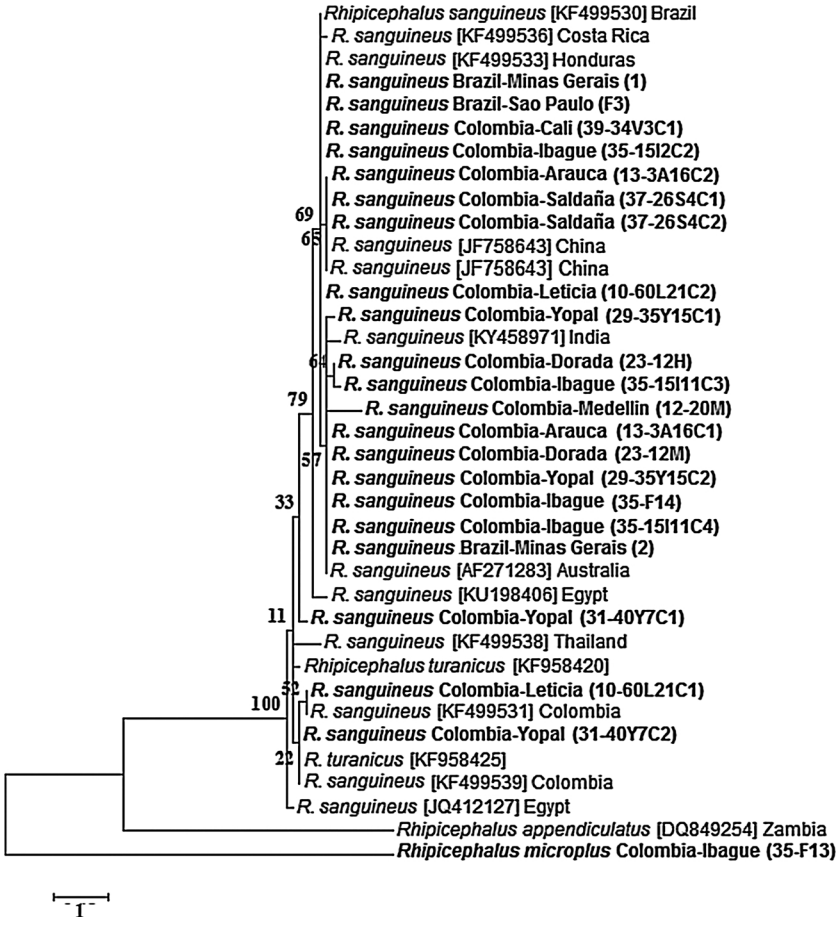

Fig. 7. Maximum Likelihood (ML) tree using sequences of the second internal transcribed space (ITS2) gene partial sequences of tick specimens collected in the present study (in bold) and sequences from GenBank (accession numbers in brackets). Numbers at nodes are bootstrap support values. The sequence of *Rhipicephalus microplus* was used as outgroup.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ttbdis.2017.10.008.

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