

ORIGINAL ARTICLE

Rickettsia spp. and Anaplasmatataceae in Ticks From Domestic Animals in Northern Colombia

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ABSTRACT

Introduction: Tick-borne diseases have a significant impact on public and animal health and represent a considerable financial burden on livestock farming. However, in many regions of Latin America, comprehensive epidemiological data, including species identification, geographical distribution and molecular profiling of ticks and their associated pathogens, remain scarce. The aims of the present study were: 1) to establish the distribution of tick species collected from domestic animals and 2) to molecularly characterise the rickettsial bacteria present in ticks from the department of Atlántico, area Caribe, Colombia.

Methods: Between January 2021 and March 2022, ticks were collected from 216 cattle and 72 sympatric domestic animals (38 dogs, 31 equids and 3 goats) on 28 farms. Specimens were identified and grouped into 297 pools. Molecular detection and characterisation of the pathogens were carried out by targeting the partial *gltA*, *ompA*, *ompB* and 16S rRNA genes of *Rickettsia* and the partial 23S rRNA and 16S rRNA genes of *Anaplasmatataceae*.

Results: A total of 1541 ticks were collected, and four species belonging to the genera *Rhipicephalus*, *Dermacentor* and *Amblyomma* were identified. A total of 137 out of 288 animals (47.6%) were infested with a mean infection rate of $9.7 \pm \text{SD } 6.8$ ticks per animal. *Rickettsia* spp. and Anaplasmatataceae DNA were detected in 2.7% (MIR: 0.5%) and 15.5% (MIR: 0.3%) of the tick pools, respectively. The obtained sequences showed high nucleotide identity (99%–100%) with sequences of *Candidatus Rickettsia colombiensis*, *Anaplasma marginale*, *Anaplasma platys*, *Ehrlichia canis* and *Ehrlichia minasensis*.

Conclusion: Our data represent the first description of *Dermacentor nitens* and *Amblyomma patinoi* in the Atlantic region of the Colombian Caribbean. Considering the risk that the tick and rickettsial species represent for public and animal health, monitoring and control programmes are necessary to prevent the spread of tick-borne pathogenic bacteria to humans.

Summary

- We report the first description of *Dermacentor nitens* and *Amblyomma patinoi* in the department of Atlántico, expanding the knowledge about the distribution of tick vectors in Colombia.
- Competent tick vectors and rickettsial bacteria of interest for public and animal health were identified.
- Vector monitoring programmes are needed to understand the interactions between animals, ticks and humans from a One Health perspective, and to reduce the risk of tick-borne diseases in the country.

1 | Introduction

Ticks are haematophagous arthropods that are considered the second and first most important vectors of pathogenic microorganisms affecting humans and animals, respectively (Dennis and Piesman 2005; De La Fuente et al. 2008; Kassiri and Nasirian 2021). Ticks and tick-borne diseases (TBD) have a serious impact on the health and socio-economic burden of the livestock sector, particularly in tropical regions (Salih et al. 2023). In this context, economic losses have been calculated at USD \$13.9–18.7 billion per year worldwide and have been associated with decreased production, treatment, and prophylaxis costs (Betancourt 2017).

To date, almost 900 species of ticks belonging to the families Ixodidae (hard ticks), Argasidae (soft ticks) and Nuttalliellidae have been identified (Guglielmone et al. 2010). Hard ticks are involved in the transmission of infectious agents (bacteria, virus, protozoa and nematodes) with a human and animal health relevance (Guglielmone et al. 2006; Rivera-Páez et al. 2018a; Madison-Antenucci et al. 2020). Among these, those belonging to the Rickettsiaceae and Anaplasmataceae families (order Rickettsiales) such as several *Rickettsia*, *Ehrlichia* and *Anaplasma* species, are of particular interest because of their zoonotic potential and their impact on animal production (André 2018; Hosseini-Chegeni et al. 2020; Salje 2021; Vouraki et al. 2022).

In Latin America, several factors such as changes in the environment, agricultural practices, unstable economies, human and animal movements, and the increase and expansion of vectors, among others, contribute to the spread of TBD (Figueredo et al. 2017; Maggi and Krämer 2019).

Despite the recent increase in studies on tick-borne pathogens in many regions of Colombia, epidemiological information regarding the species, distribution and molecular characterisation of both ticks and pathogens is lacking (Rivera-Páez et al. 2018a). Some studies have detected pathogenic Rickettsiales for humans and animals in *Rhipicephalus* spp., *Amblyomma* spp. and *Dermacentor* spp. collected from cattle, horses and dogs in this country (e.g., Miranda and Mattar 2015; Santodomingo et al. 2019; Arroyave et al. 2020; Cabrera et al. 2022; Martínez Díaz et al. 2023). However, surveillance and molecular characterisation studies are still essential to better understand the epidemiology of TBD and minimise the risk of infection from a One Health perspective.

The aims of the present study were: 1) to establish the distribution of tick species collected from domestic animals in Northern Colombia and 2) to perform a molecular characterisation of the presence of *Rickettsia*, *Ehrlichia* and *Anaplasma* species in the collected ticks.

2 | Materials and Methods

2.1 | Study Design and Sampling

A cross-sectional study was conducted to determine the frequency of species belonging to the genera *Rickettsia*, *Ehrlichia* and *Anaplasma* in ticks parasitising cattle and sympatric domestic animals in farms in the department Atlántico (Northern Colombia), a part of the Colombian Caribbean (South America). This region is divided into five geographical subregions: Northern (metropolitan), Eastern, Central, Southern and Western (coastal) (Gobernación del Atlántico 2014). The region is characterised by a tropical climate, with a mean temperature of 28°C, relative humidity >70% and an average annual rainfall exceeding 1500 mm (IDEAM 2023). Between January 2021 and March 2022, a total of 216 cattle were sampled from 28 farms (North-metropolitan, $n = 3$; East, $n = 7$; South, $n = 10$; Central, $n = 5$; Coastal, $n = 3$ farms) (Figure 1). During the same period, convenience sampling of ticks was conducted on 72 sympatric animals (including 38 dogs, 31 equids and 3 goats) at selected farms.

With the owner's consent, the entire body surface of each animal was exhaustively examined for at least 5 min. Ticks were collected in sterile tubes, labelled according to location and host, preserved at 4°C and transported for identification to the laboratory of the Centro de Investigaciones en Ciencias de la Vida, Simón Bolívar University, Barranquilla, Colombia. The epidemiological data of the sampled animals and farms were recorded whenever possible, including farm coordinates and the number and species of animals inspected and sampled.

2.2 | Morphological and Molecular Identification of Ticks

Ticks were identified to species level using morphological keys (Battesti et al. 2006; Dantas-Torres et al. 2019; Nava et al. 2017a). Tick specimens were grouped into 297 pools (mean $5.0 \pm SD 2.3$; min. 1, max. 16 ticks/pools) according to their sampling herd, host, tick species and developmental stage (larvae, nymph, male or female). The collected tick pools were macerated and homogenised in phosphate buffer solution (PBS). DNA extraction was performed using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Waltham, Massachusetts, USA) following the manufacturer's instructions. The DNA obtained was stored at -20°C until analysis.

To confirm the morphological identifications of tick species, 13 pools of ticks were randomly selected according to geographical location and were analysed by PCR using primers targeting the 16S rRNA gene that amplified an expected 460 bp fragment (Table 1), following the PCR protocol previously described by Norris et al. (1996) and Mangold et al. (1998) and subsequently

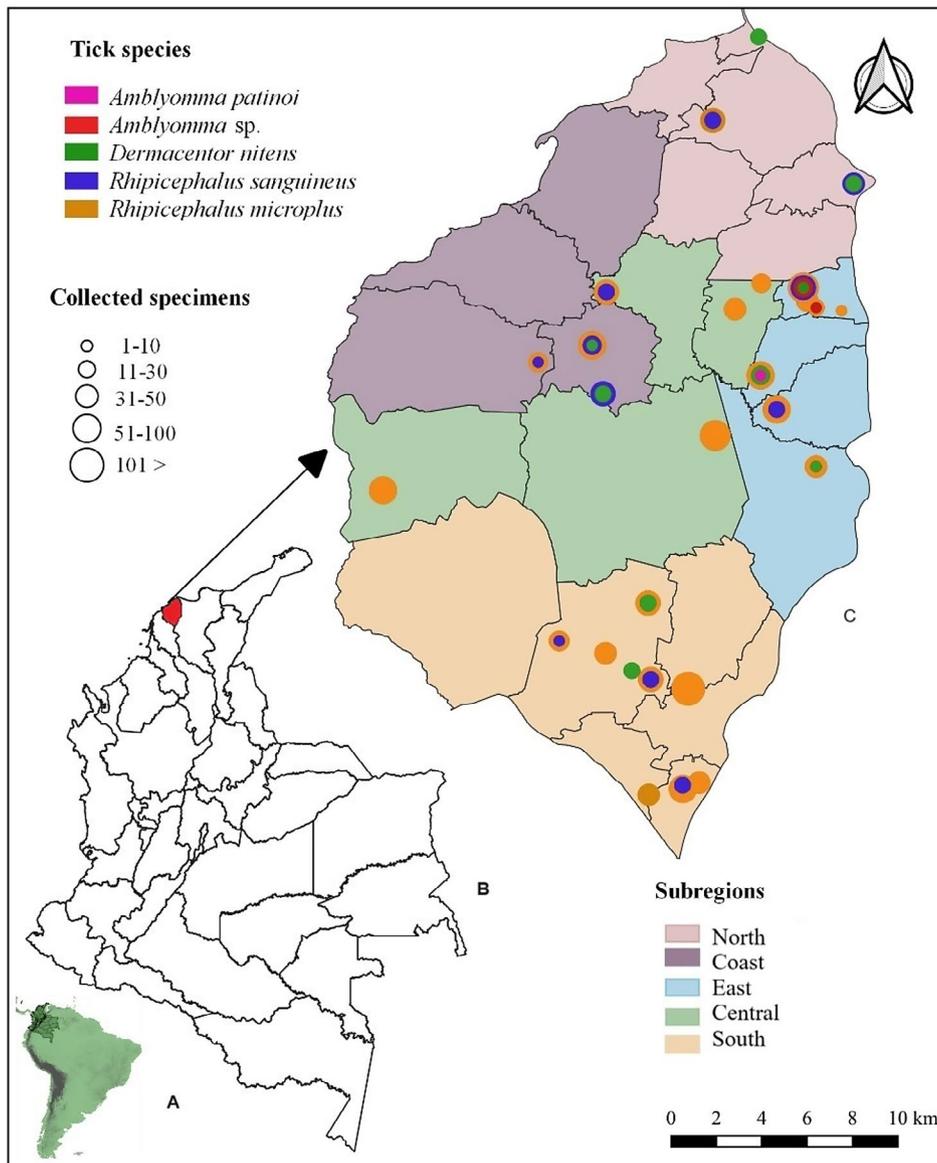


FIGURE 1 | Distribution map of tick species collected in the department of Atlántico, Colombian Caribbean.

sequenced. PCRs were performed in a CFX96 thermal cycler (Bio-Rad Laboratories, USA), using OneTaq 2X Master Mix with standard buffer (New England BioLabs Inc.). For each PCR, 5 μ L of molecular grade water and DNA from *Rhipicephalus microplus*, previously identified at the Instituto de Investigaciones Biológicas del Trópico—IIBT (Montería, Colombia), were used as negative and positive controls, respectively. The amplified products were separated and visualised under UV light in a 1.5% agarose gel.

2.3 | Molecular Analysis of the Studied Agents

A previous screening using qPCR with primers and protocols previously described for the detection of a 147bp fragment of the citrate synthase *gltA* gene using GoTaq(R) qPCR Master Mix (Promega, Biotech Ibérica, S.L) was performed for the detection of *Rickettsia* spp. (Labruna et al. 2004a) (Table 1). If qPCR analysis revealed the presence of *Rickettsia* DNA in a pool of ticks, this pool was subsequently analysed using three conventional

PCR protocols targeting a larger fragment of the *gltA* gene (401 bp), and partial the *ompA* (532 bp) and *ompB* (420 bp) genes (Choi et al. 2005; Labruna et al. 2004a; Regnery et al. 1991; Roux et al. 1996) (Table 1).

For the identification of Anaplasmataceae, PCR was performed as described above using previously described primers and protocols for the detection of a 649 bp fragment of the 23S rRNA gene (Dahmani et al. 2015). Once a pool of ticks was shown to contain Anaplasmataceae DNA, it was analysed by PCR for further identification of *Anaplasma* spp. and *Ehrlichia* spp. using previously described primers for the detection of the 16S rRNA gene (Inokuma et al. 2000) (Table 1).

For each PCR, 5 μ L of molecular grade water was used as a negative control and DNA from *Candidatus Rickettsia colombiense* and *Anaplasma phagocytophilum*, previously identified at the Instituto de Investigaciones Biológicas del Trópico—IIBT (Montería, Colombia), was used as *Rickettsia* spp. and Anaplasmataceae positive controls, respectively.

TABLE 1 | PCR primers used in the study.

	Target genes	Fragment size (bp)	Primer/sequence 5'-3'	Reference			
Tick species	16S rRNA	460	F-CCGGTCTGAACCTCAGATCAAGT R-GCTCAATGATTTTTTAAATTGCTGT	Norris et al. (1996)			
<i>Rickettsia</i> sp.	<i>gltA</i>	147	CS5- GAGAGAAAATTATATATCCAAATGTTGAT CS-6 AGGGTCTTCGTGCATTTCTT FAM-CATTGTGCCATCCAGCCTACGGT- BHQ-1	Labruna et al. (2004a)			
			<i>gltA</i>	401	CS-78 GCAAGTATCGGTGAGGATGTAAT CS-323 GCTTCCTTAAAATTCAATAAATCAGGAT	Labruna et al. (2004a)	
					<i>ompA</i> hemi-nested	631 532	Rr190.70p-ATGGCGAATATTTCTCCAAAA Rr190.701n-GTTCGGTAAATGGCAGCATCT Rr190.602n-AGTGCAGCATTCGCTCCCCCT
	<i>ompB</i> nested	511 420	rompB OF-GTAACCGGAAGTAATCGTTTCGTAA rompB OR-GCTTTATAACCAGCTAAACCACC rompB SFG IF-GTTTAATACGTGCTGCTAACCAA rompB SFG/TG IR-GGTTTTGGCCATATACCATAAG	Choi et al. (2005)			
			<i>Anaplasmataceae</i>	23S rRNA	649	Ana23S-212f-ATAAGCTGCGGGGAATTGTC Ana23S-908r-GTAACAGGTTTCGGTCTCCA	Dahmani et al. (2015)
						16S rRNA	345

2.4 | Sequencing and Phylogenetic Analysis

Fourteen PCR products, seven in which *Rickettsia* spp. and seven in which Anaplasmataceae DNA were detected, were randomly selected and sequenced. The PCR products from each of the studied subregions were analysed. All PCR products were purified with ExoSAP (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions and sequenced by the Sanger method at an external laboratory (Macrogen Inc., Seoul, Korea).

The nucleotide sequences were edited using MEGA X software (Kumar et al. 2018). In addition, the sequences were trimmed using Gblocks software to remove misaligned positions for more accurate inferences; each aligned file was trimmed to a minimum length of one block 10. Alignments were constructed using the MAFFT tool of the UGENE software using the automatic option (Okonechnikov et al. 2012), along with sequences from other available geographical areas of Ixodidae and related Rickettsiales. The consensus sequences were compared with

the sequences available in the GenBank database using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) and unique partial sequences were deposited in GenBank using BankIt v3.0 NCBI submission tool (NCBI 2015).

For phylogenetic analysis, the 16S rRNA gene for ticks, 23S rRNA and 16S rRNA for Anaplasmataceae, and *gltA*, *ompA* and *ompB* for *Rickettsia* spp. were evaluated. A maximum likelihood tree was inferred using IQ-TREE2 version 2.2.2.6; branch support values were estimated by ultrafast Bootstrap (1000 replicates). The substitution model for each partition was determined using ModelFinder (Hoang et al. 2018) according to the Bayesian information criterion BIC for each partition. Each gene was individually partitioned according to the selected best-fit model, which was as follows: tick species: 16S rRNA (*Rhipicephalus*: K3Pu+F+G4; *Dermacentor*: F81+F+G4 and *Amblyomma*: TPM2u+F+G4); *Rickettsia gltA* (K3Pu+F+G4), *ompA* (K3Pu+F+G4), *ompB* (HKY+F+I) and Anaplasmataceae: 23S rRNA (HKY+F+I+R2); 16S rRNA (HKY+F). The resulting trees were visualised using iTOL (v5) and annotated in Inkscape.

TABLE 2 | Tick infestation according to species, development stage, host species and geographical area.

Variable	Categories	<i>R. sanguineus</i> s.l. (%)	<i>R. microplus</i> (%)	<i>D. nitens</i> (%)	<i>Amblyomma</i> sp. (%)	<i>A. patinoi</i> (%)	No. of ticks (%)
Developmental stages	Male	121 (18.3)	353 (53.2)	188 (28.4)	0 (0.0)	1 (0.2)	663 (43.0)
	Female	38 (5.6)	497 (72.9)	140 (20.5)	3 (0.4)	4 (0.6)	682 (44.3)
	Nymph	7 (4.0)	93 (53.4)	74 (42.5)	0 (0.0)	0 (0.0)	174 (11.3)
	Larva	3 (13.6)	0 (0.0)	19 (86.4)	0 (0.0)	0 (0.0)	22 (1.4)
Host	Cattle	3 (0.3)	940 (98.9)	0 (0.0)	2 (0.2)	5 (0.5)	950 (61.7)
	Goat	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.2)
	Equids	0 (0.0)	0 (0.0)	421 (100)	0 (0.0)	0 (0.0)	421 (27.3)
	Horse	0 (0.0)	0 (0.0)	297 (100)	0 (0.0)	0 (0.0)	297 (19.3)
	Mule	0 (0.0)	0 (0.0)	124 (100)	0 (0.0)	0 (0.0)	124 (8.0)
	Dogs	166 (99.4)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	167 (10.8)
Location	North	40 (32.0)	32 (25.6)	53 (42.4)	0 (0.0)	0 (0.0)	125 (8.1)
	East	24 (6.2)	293 (75.1)	65 (16.7)	3 (0.8)	5 (1.3)	390 (25.3)
	South	32 (6.5)	359 (72.4)	105 (21.2)	0 (0.0)	0 (0.0)	496 (32.2)
	Centre	22 (11.6)	167 (88.4)	0 (0.0)	0 (0.0)	0 (0.0)	189 (12.3)
	Coast	51 (15.0)	92 (27.0)	198 (58.1)	0 (0.0)	0 (0.0)	341 (22.1)
No. of ticks (%)		169 (11.0)	943 (61.2)	421 (27.3)	3 (0.2)	5 (0.3)	1541 (100)
No. of pools (%)		32 (10.8)	197 (66.3)	63 (21.2)	3 (1.0)	2 (0.7)	297 (100)

Abbreviations: *A.*, *Amblyomma*; *D.*, *Dermacentor*; *R.*, *Rhipicephalus*; s.l., sensu lato.

2.5 | Statistical Analysis

The frequency of tick species, according to the developmental stage (larva, nymph, female and male adults), host and farm location, as well as the frequency of tick infestation (number of infested animals/numbers of inspected animals) was calculated. Analysis of variance (ANOVA) and post hoc tests (Tukey) were used to evaluate the association between tick infestation and tick species with host species and farm location. The frequency of infection by *Rickettsia* spp. and Anaplasmataceae was expressed as a percentage and the minimum infection rate—MIR (No. of positive tick pools/Total number of ticks), considering that only one of the ticks in each pool was positive, as previously described (Labruna et al. 2004b; Miranda et al. 2012).

Associations between positive results for *Rickettsia* spp. and Anaplasmataceae as binomial response variables, and categorical explanatory variables such as tick species and farm location were analysed using Pearson's Chi-square test or Fisher's test, ANOVA, and post hoc tests (Tukey) as corresponding. Values with *p*-values <0.05 were considered statistically significant.

All statistical analyses were performed using SPSS 25.0 software (Statistical Package for Social Sciences (SPSS), IBM Corp., Armonk, NY, USA). Quantum Geographic Information System (QGIS, Zurich, Switzerland) software was used to

represent the spatial distribution of ticks identified in the study area.

3 | Results

3.1 | Identification and Molecular Characterisation of Ticks

A total of 1541 ticks were collected in the study area. The distribution of tick species according to sex and developmental stage is shown in Table 2. Four tick species were identified. The most frequent species was *Rhipicephalus microplus* with 943 specimens (61.2%), followed by *Dermacentor nitens*, *Rhipicephalus sanguineus* sensu lato (s.l.) and *Amblyomma patinoi*. Additionally, three specimens belonging to the genus *Amblyomma* could not be identified at the species level. *Rhipicephalus sanguineus* s.l. and *D. nitens* were collected at all developmental stages, while *R. microplus* larvae were not collected, and only adult specimens of the genus *Amblyomma* were found (Table 2). The geographical distribution of the sampled farms and the tick species is shown in Figure 1.

Tick species were confirmed morphologically and by partial sequencing of the 16S RNA gene. The sequences of *R. sanguineus* s.l. (PP682357.1, PP682358.1) showed 99%–100% similarity between them and with other available *R. sanguineus* s.l. GenBank sequences. The phylogenetic analysis

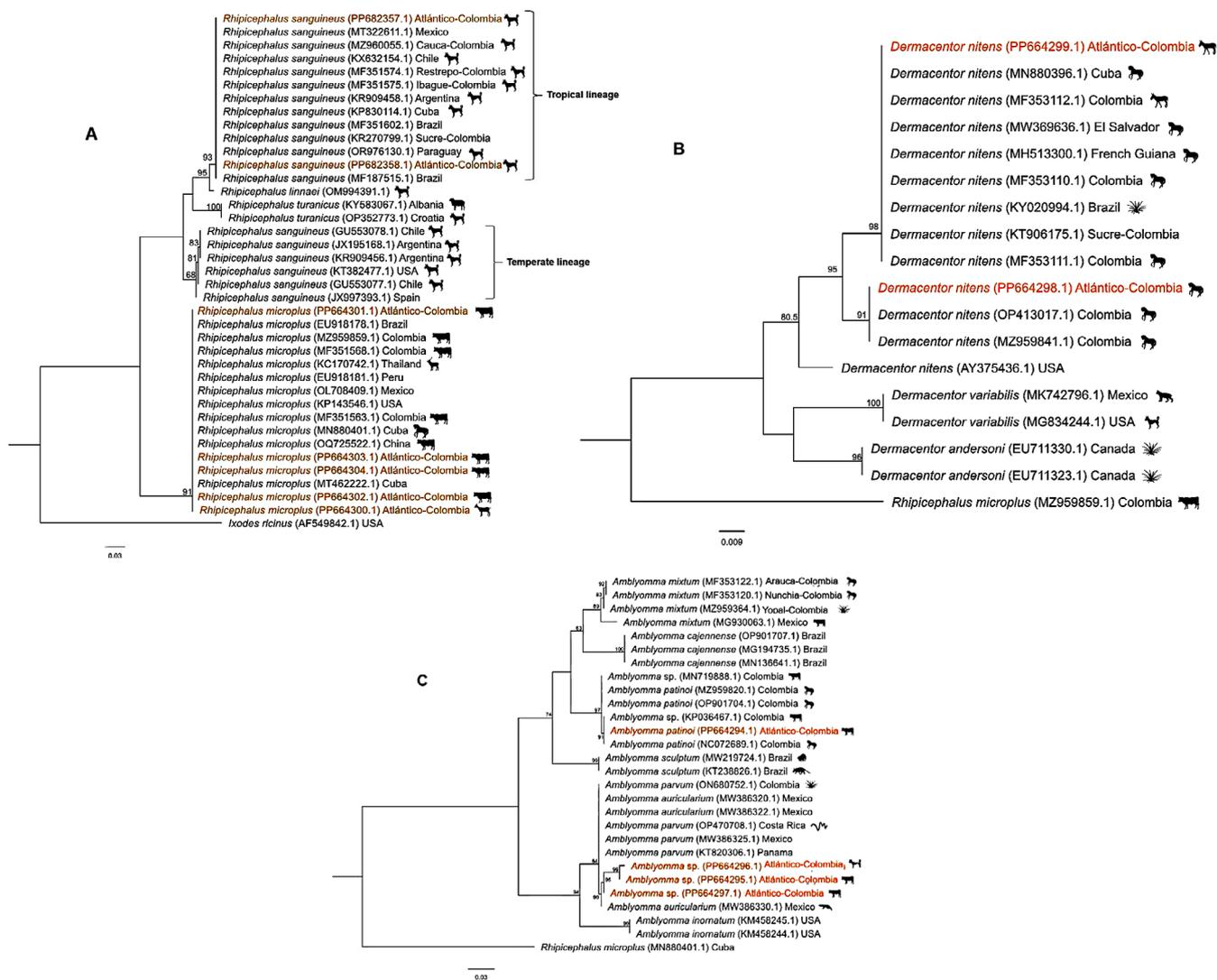


FIGURE 2 | Maximum-likelihood (ML; 1000 bootstrap replicates) phylogenetic tree clustering of the partial 16S rRNA gene of ticks.

showed that these sequences were located within the tropical lineage of *R. sanguineus* s.l. previously reported in other regions of Colombia (Valle del Cauca, MF351574.1; Tolima, MF351575.1; Sucre, KR270799.1), South and Central America (Chile, KX632154.1; Cuba, KP830114.1; Mexico, MT322611.1; Brazil, MF187515.1, MF351602.1) (Figure 2A). In addition, they showed a percentage of identity of 93%–94% with the sequences clustered in the temperate lineage of *R. sanguineus* s.l. (Chile, GU553077.1; Argentina, KR909456.1, JX195168.1, GU553078.1, GU553078.1), USA (KT382477.1) and Spain (JX997393.1) (Figure 2A).

The sequences of *R. microplus* (PP664300.1, PP664301.1, PP664304.1, PP664302.1, PP664303.1) showed 99%–100% similarity with other available *R. microplus* GenBank sequences; they were grouped with the corresponding sequences of *R. microplus* from other regions of Colombia (Arauca, MF351563.1; Cauca, MZ959859.1; Meta, MF351568.1) and from other American countries (USA, KP143546.1; Mexico, OL708409.1; Cuba, MN880401.1, MT462222.1; Peru, EU918181.1; Brazil, EU918178.1), Asia (Thailand, KC170742.1; China, OQ725522.1) and Africa (Mozambique, EU918187.1) (Figure 2A).

The two *D. nitens* sequences (PP664298.1, PP664299.1) showed 98% identity between them. One sequence was identical to sequences of *D. nitens* from other regions of Colombia (Cauca, MZ959841.1); the other sequence showed 96%–100% identity with sequences of this tick species from other regions of the country (Bolívar, MF353112.1, MF353111.1; Casanare, MF353110.1; Sucre, KT906175.1), Brazil (KY020994.1), French Guiana (MH513300.1), El Salvador (MW369636.1) and Cuba (MN880396.1) (Figure 2B). The only sequence obtained from *A. patinoi* (PP664294.1) had a percentage of identity of 99%–100% with the sequences obtained from *A. patinoi* collected from cattle and horses in other regions of Colombia (Cauca, MZ959820.1; Cundinamarca, NC072689.1, KP036467.1, KP036466.1) (Figure 2C).

The morphological characteristics of three female *Amblyomma* specimens were not sufficient to identify them at the species level. These specimens shared similar characteristics with *Amblyomma parvum* and *Amblyomma auricularium*, including a strong ventral retrograde spine on palpus I and trochanters in both sexes (Battesti et al. 2006). The 16S RNA gene sequence analyses of these specimens (PP664295.1, PP664296.1,

TABLE 3 | Prevalence of pools positive to *Rickettsia* spp. and Anaplasmataceae from ticks collected from domestic animals when considering the tick species and geographical area.

Ticks pools		No. infected/ No. tested (%) <i>Rickettsia</i> spp.	MIR ^a <i>Rickettsia</i> spp. %	No. infected/ No. tested (%) Anaplasmataceae	MIR ^a Anaplasmataceae %
Species	<i>R. sanguineus</i> s.l.	1/32 (3.1)	0.6	2/32 (6.3)	1.2
	<i>R. microplus</i>	5/197 (2.5)	0.5	44/197 (22.3)	4.7
	<i>D. nitens</i>	2/63 (3.2)	0.5	0/63 (0.0)	0.0
	<i>Amblyomma</i> sp.	0/3 (0.0)	0.0	0/3 (0.0)	0.0
	<i>A. patinoi</i>	0/2 (0.0)	0.0	0/2 (0.0)	0.0
Location	North	0/23 (0.0)	0.0	0/23 (0.0)	0.0
	East	2/76 (2.6)	0.5	15/76 (19.7)	3.8
	South	3/94 (3.2)	0.6	10/94 (10.6)	2.0
	Centre	0/48 (0.0)	0.0	11/48 (22.9)	5.8
	Coast	3/56 (5.4)	0.9	10/56 (17.9)	2.9
Total		8/297 (2.7)	0.5	46/297 (15.5)	3.0

Note: Estimated prevalence percentages at the individual level (in parentheses) were calculated based on the size of each group analysed.

Abbreviations: A., *Amblyomma*; D., *Dermacentor*; R., *Rhipicephalus*; s.l., sensu lato.

^aMIR, minimum infection rate: No. positive pools/No. ticks × 100.

PP664297.1) showed no conclusive similarity percentages between *A. parvum* and *A. auricularium* (Figure 2C). In this sense, two sequences showed 97.8% and 98.7% identity with *A. auricularium* sequences (MW386330.1) and 97.8% and 98.7% identity with *A. parvum* sequences (MW386325.1, MW386324.1, MW386323.1, MW386321.1) from Mexico; the other sequence of *Amblyomma* collected from a dog showed 94.8% identity with *A. parvum* isolate (MW386328.1, MW386327.1) and 94.7% identity with *A. auricularium* isolate (MW386322.1) from Mexico.

3.2 | Ticks' Distribution and Infestation Levels

The overall mean number of ticks collected per farm was 55.0 ± SD 43.3 (95% CI, 38.2–71.8) and the mean number of tick pools per farm was 10.6. Additionally, 137 out of 288 animals examined (47.6%) were found to be infested with ticks, with a mean of 4.9 ± SD 2.5 animals infested per farm (95% CI, 3.9–5.9; min. 1, max. 11). The highest frequency of infestation was found in cattle 49.1% (106/216), followed by dogs 44.7% (17/38), equids 41.9% (13/31) and goats 33.3% (1/3). One-way ANOVA did not reveal a significant difference between the number of ticks per farm ($F=0.36$; $df=4$; $p=0.83$), animals infested per farm ($F=1.28$; $df=4$; $p=0.31$) and farm infestation per farm location ($F=0.42$; $df=4$; $p=0.78$).

The average number of ticks collected per animal was 9.7 ± SD 6.8 (95% CI, 8.6–10.9; $F=6.18$; $df=2$; $p<0.05$). This number was significantly higher in equids (16.6 ± SD 15.8; 95% CI, 7.0–26.2) than in cattle (9.5 ± SD 6.2; 95% CI, 8.3–10.7) and dogs (9.3 ± SD 7.9; 95% CI, 5.3–13.2), with significant differences between equids and cattle ($p<0.05$) and equids compared to dogs ($p=0.01$).

Rhipicephalus and *Dermacentor* species were more widely distributed across the five subregions. The greatest diversity of

tick species was observed in cattle from the eastern subregion (Figure 1, Table 2). One-way ANOVA showed differences in tick species based on farm location ($F=2.70$; $df=4$; $p=0.03$) and host species ($F=245.60$; $df=2$; $p<0.05$), with significant differences in tick species between the Eastern and Central subregions ($p=0.02$) and between cattle and equids, as well as dogs and equids ($p<0.05$).

3.2.1 | Frequency of *Rickettsia* spp. and Anaplasmataceae

A total of 53 of the 297 tick pools (17.8%; 95% CI, 13.0–22.0) were positive for rickettsial bacteria. Table 3 shows the frequency of *Rickettsia* spp. and Anaplasmataceae expressed in percentages and MIR according to tick pools by species and geographical area. *Rickettsia* spp. DNA was detected in 8 of 297 tick pools (2.7%; 95% CI, 1.0%–5.0%) and Anaplasmataceae DNA was detected in 46 out of the 297 tick pools (15.5%; 95% CI, 11.0%–20.0%) (Table 3). *Rickettsia* spp. was detected in *R. sanguineus* s.l., *R. microplus*, and *D. nitens*, whereas Anaplasmataceae was detected in *R. sanguineus* s.l. and *R. microplus*. Tick pools of *A. patinoi* and *Amblyomma* sp. were PCR negative. Coinfections with *Rickettsia* spp. and Anaplasmataceae occurred in only one pool of *R. microplus* collected from cattle in the Southern subregion.

The frequency of *Rickettsia* spp. was higher in ticks collected in the Coastal subregion (5.4%), but there were no significant differences in the frequency of *Rickettsia* spp. among tick species ($\chi^2 0.23$; $F=0.06$; $df=4$; $p=0.99$) or the farm location ($\chi^2 3.57$; $F=0.88$; $df=4$; $p=0.47$). The frequency of Anaplasmataceae was significantly different between the tick species *R. microplus* (22.3%) and *D. nitens* (6.3%) ($\chi^2 21.60$; $F=5.72$; $df=4$; $p<0.05$), but not by farm location ($\chi^2 9.22$; $F=2.33$; $df=4$; $p=0.06$).

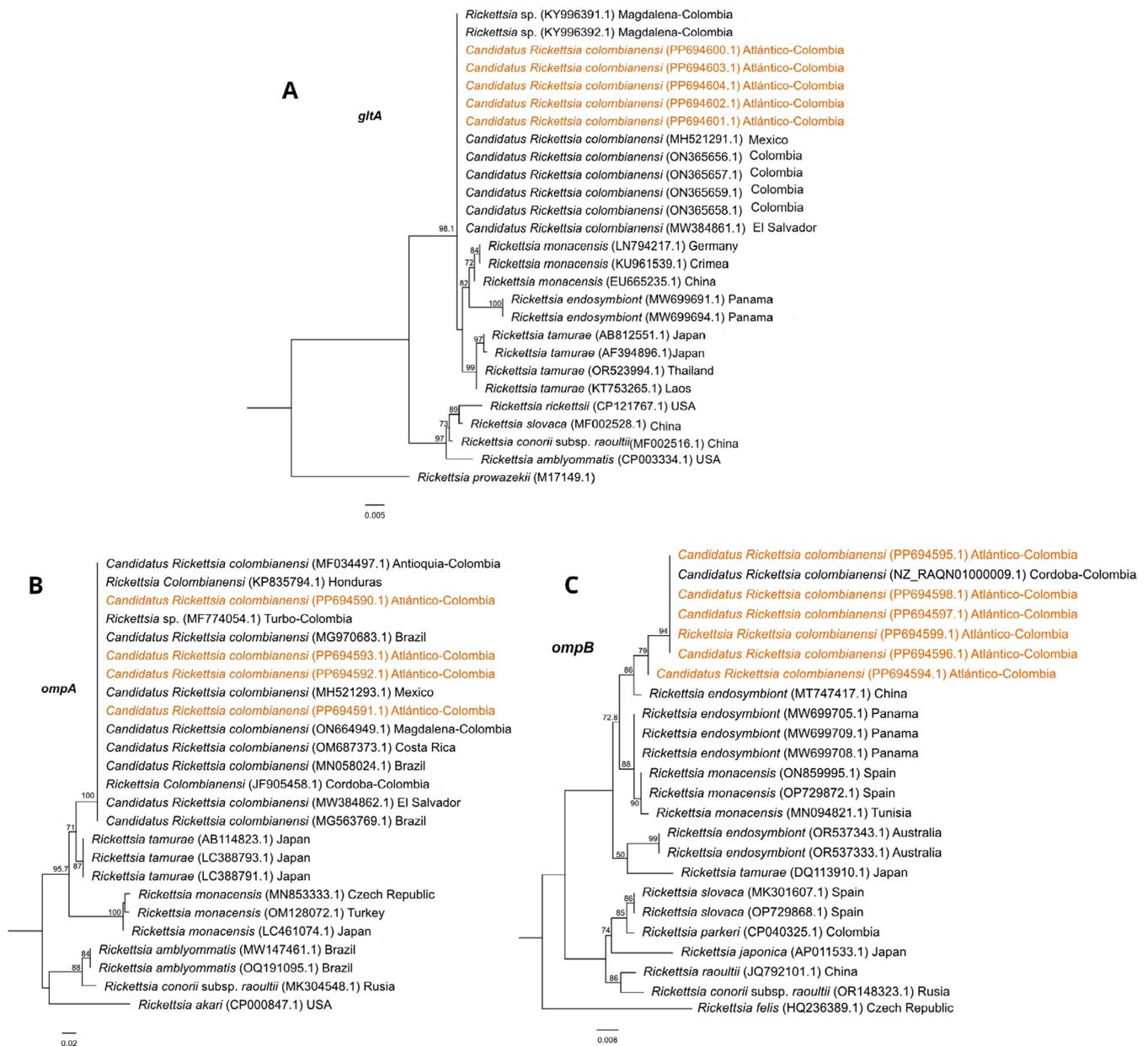


FIGURE 3 | Maximum-likelihood phylogenetic tree clustering of the partial *gltA*, *ompA* and *ompB* genes of *Rickettsia* spp.

The MIR results for *Rickettsia* spp. were similar in the three tick species identified, being higher in the specimens collected from the coastal region (0.9%) compared with those collected from other regions; whereas a high MIR of *Anaplasmataceae* was detected in *R. microplus* (4.7%) and in the specimens collected from the Central subregion (5.8%) (Table 3).

3.3 | Molecular and Phylogenetic Analysis of Rickettsial Bacteria

The *gltA* sequences obtained in the present study (PP694601.1, PP694602.1, PP694603.1, PP694604.1, PP694600.1) were identical to the sequences of *Candidatus Rickettsia colombianensis* more recently renamed as *Candidatus Rickettsia colombiense* (Oren et al. 2020), previously detected in *Amblyomma dissimile* from Colombia (ON365659.1, ON365658.1, ON365657.1, ON365656.1), El Salvador (MW384861.1), Mexico (MH521291.1)

and *Rickettsia* sp. (KY996391-KY996392) reported in Colombia (Figure 3A). The sequences of the partial *ompA* gene (PP694590.1, PP694591.2, PP694592.3, PP694593.4, PP694594.5) were identical to those of *Ca. R. colombiense* detected in *A. dissimile* from other regions of Colombia (Antioquia, MF034497.1; Córdoba, JF905458.1; Magdalena, ON664949.1), *Amblyomma* sp. from other Central-South American countries (Costa Rica, OM687373.1; Mexico, MH521293.1; El Salvador, MW384862.1; Honduras, KP835794.1) and sequences detected in cane toad (*Rhinella marina*) (MG970683.1), snake (*Bothrops atrox*) (MG563769.1) and Trombiculidae infesting *Oligoryzomys* sp. (MN058024.1) from Brazil (Figure 3B). These sequences were also identical to a *Rickettsia* sp. detected in *Amblyomma* sp. of the Cajennense complex collected from humans in Antioquia, Colombia (MF774054.1) (Figure 3B). The sequences obtained at the *ompB* partial gene (PP694594.1, PP694595.1, PP694596.1, PP694597.1, PP694598.1, PP694599.1) showed between a 99.6% and 100% identity with *Ca. R. colombiense* detected in *A.*

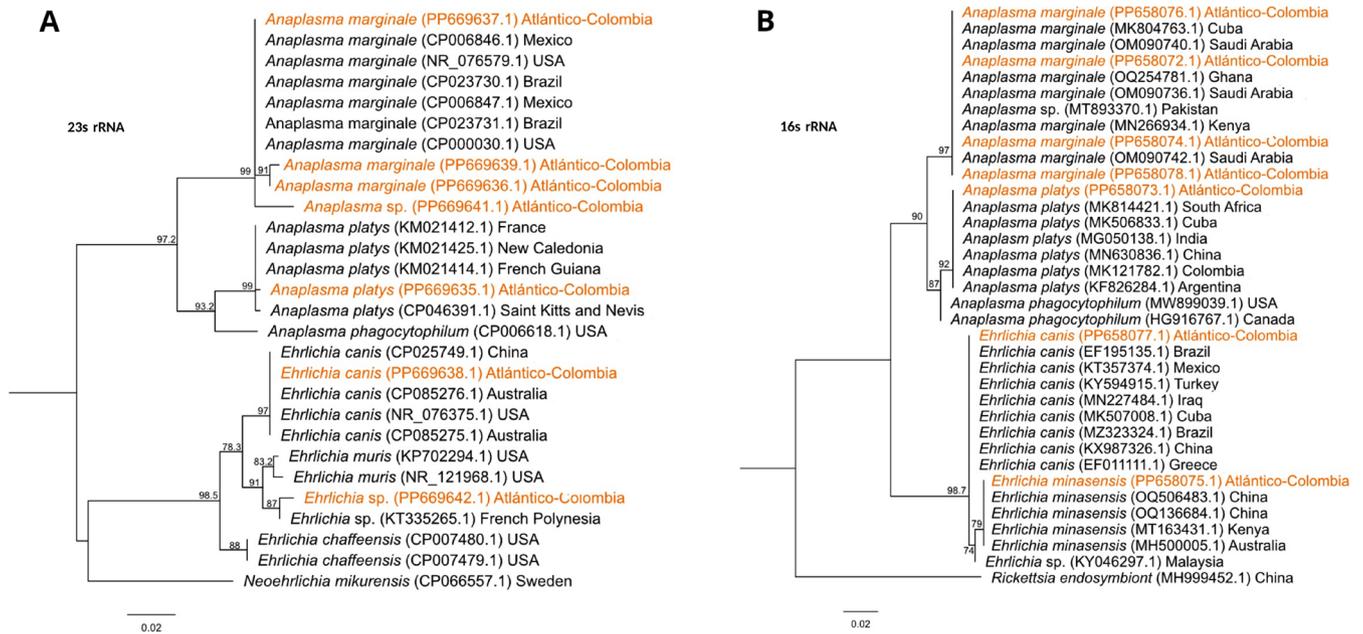


FIGURE 4 | Maximum-likelihood phylogenetic tree clustering of the partial 23S rRNA and 16S rRNA genes of Anaplasmataceae.

dissimile collected from an iguana (*Iguana iguana*) in Córdoba, Colombia (NZ_RAQN0100009.1) and 99.4% identity with a *Rickettsia endosymbiont* detected in *Haemaphysalis formosensis* from China (MT747417.1) and *Ixodes boliviensis* from Panama (MW699709.1, MW699708.1, MW699705.1) (Figure 3C).

Regarding the 23S rRNA sequences obtained for Anaplasmataceae (PP669635.1, PP669636.1, PP669637.1, PP669638.1, PP669639.1, PP669641.1, PP669642.1), three out of the five sequences obtained in *R. microplus* showed between 99.4% and 100% identity with *Anaplasma marginale* reported in cattle from countries in the Americas (USA, (NR_076579.1, CP000030.1); Mexico, (CP006847.1, CP006846.1); Brazil, (CP023731.1, CP023730.1)); one sequence named as *Anaplasma* sp. showed 98% similarity to these same sequences reported; the other sequence, obtained in *R. microplus*, showed a 97.7% identity with *Ehrlichia* sp. reported in *Rhipicephalus annulatus* from French Polynesia (KT335265.1) (Figure 4A). One of the two *Anaplasma* spp. sequences obtained from *R. sanguineus* s.l. showed more than 99.6% identity with sequences from *Anaplasma platys* from French Guiana (KM021414.1), Saint Kitts and Nevis (CP046391.1) and New Caledonia (KM021425.1); and the other sequence showed more than 99.8% identity with *Ehrlichia canis* reported in the USA (NR_076375.1), China (CP025749.1) and Australia (CP085275.1; CP085276.1) (Figure 4A).

Regarding the 16S rRNA sequences obtained for Anaplasmataceae (PP658072, PP658073, PP658074, PP658075, PP658076, PP658077, PP658078), four of the five sequences obtained from *R. microplus* were identical to a sequence of *Anaplasma marginale* obtained from cattle and camels in Africa (OQ254781.1), Saudi Arabia (OM090742.1, OM090736.1) and Cuba (MK804763.1, MT893370.1) and to a sequence of *Anaplasma* sp. obtained from cattle in Pakistan (MT893370.1); the other sequence showed more than 98% identity with *Ehrlichia minasensis* detected in Australia (MH500005.1), China (OQ506483.1, OQ136684.1) and Kenya (MT163431.1) (Figure 4B). One of the

two sequences obtained from *R. sanguineus* s.l. was identical to *A. platys* sequences obtained from dogs in Magdalena, Colombia (MK121782.1), China (MN630836.1), India (MG050138.1), Argentina (KF826284.1), South Africa (MK814421.1) and Cuba (MK506833.1). The other sequence was identical to *E. canis* detected in dogs from Cuba (MK507008.1), Mexico (KT357374.1), Turkey (KY594915.1), Iraq (MN227484.1), Brazil (EF195135.1), China (KX987326.1), Greece (EF011111.1), and in cats from Brazil (MZ323324.1) (Figure 4B).

4 | Discussion

The findings presented in this study indicate that domestic animals from the Atlantic department of the Colombian Caribbean are parasitised by a wide diversity of ticks. The identified species have been previously reported in various countries in Central and South America, including Argentina (Nava et al. 2017b), Panama (Bermúdez et al. 2018) and Brazil (Dantas-Torres et al. 2019). The presence of the tick species identified was previously reported in Colombia (Acevedo-Gutiérrez et al. 2020; Osorno-Mesa 1941; Rivera-Páez et al. 2018a). However, there are few reports on tick species and their related hosts in the department of the Atlántico, and to date only *R. sanguineus* s.l., *R. microplus* and *A. cajennense* spp. complex have been described (Acevedo-Gutiérrez et al. 2020; Osorno-Mesa 1941). To our knowledge, this is the first description of *D. nitens* and *A. patinoi* in the department of the Atlántico (northern Colombia).

Most tick species detected have domestic mammals as their main hosts (Copa et al. 2023; Guglielmone et al. 2021). The associations formed between *Rhipicephalus* and *Amblyomma* species in dogs and cattle, and *D. nitens* in horses, have been previously described (Copa et al. 2023; Cotes-Perdomo et al. 2020; Holguin-Rocha et al. 2023; Jaimes-Dueñez et al. 2023); these are important in the epidemiology of certain tick-borne pathogens with animal and human health relevance.

The high abundance of *R. microplus* in the present study is likely because cattle were the most frequently sampled host and this tick species has a feeding preference for them (Copa et al. 2023; Guglielmone et al. 2021). Notably, the presence of *R. microplus* can lead to substantial economic losses in different cattle production systems, both through direct impact (Calvano et al. 2021) and the associated borne pathogens (Scoles et al. 2007; Vecino et al. 2010; Cruz et al. 2012). In our study, the average tick load in cattle ($9.5 \pm \text{SD } 6.2$) was lower than that reported by Rocha et al. (Rocha et al. 2019) and Segura et al. (Segura et al. 2022) (ranging between 12.8 and 31.8 ticks/bovine depending on the breed), but higher than that detected by Ríos-Tobón et al. (2014) from the middle Magdalena (3.2 ticks/bovine). In any case, differences in tick infestations may be influenced by factors such as the specific epidemiological contexts or bias in the study designs and should be carefully considered when comparing studies.

Rhipicephalus sanguineus requires three hosts during its life cycle; however, it is mainly associated with dogs (Arroyave et al. 2020; Benavides-Montaña et al. 2018; Salomon et al. 2022). This is consistent with the findings of the present study, in which 99.4% of the specimens collected from dogs were identified as *R. sanguineus* s.l. In addition, the average tick load in this species ($9.3 \pm \text{SD } 7.9$) is comparable to the 12.9 ticks per dog reported in dogs from Mexico (Salomon et al. 2022).

Dermacentor nitens was the only species identified (in all development stages) feeding on the equines included in the present study. These results are not surprising since it is a one-host tick, equating to their principal hosts (Guglielmone et al. 2021; da Silva Rodrigues et al. 2017). This tick species showed the highest intensity of infection ($16.6 \pm \text{SD } 15.8$ ticks/equid) among the host species analysed, which is in accordance with those reported in Brazil (Labruna et al. 2002). These high intensities of infection can be of animal health relevance since this tick species has been associated with anaemia, ear lesions, and associated infections (Borges et al. 2000; Labruna et al. 2002).

Amblyomma species are three-host ticks, with a one-year generation pattern and a longer free-living behaviour (Labruna et al. 2002). The *Amblyomma* specimens collected in the present study were mainly found to be infesting cattle. Phylogenetic analyses confirmed that these specimens showed a high percentage of similarity to isolates of *A. patinoi* from other regions of Colombia (Faccini-Martínez et al. 2015; Segura et al. 2022; Martínez Díaz et al. 2023). This species is considered the main vector of *R. rickettsii* in animals and humans in Colombia, which is the etiologic agent of Rocky Mountain Spotted Fever (Cuéllar-Sáenz et al. 2023; Faccini-Martínez et al. 2015). This result indicates that *A. patinoi* has a wider distribution than previously reported in Colombia (Acevedo-Gutiérrez et al. 2021; Faccini-Martínez et al. 2015; Nava et al. 2014; Martínez Díaz et al. 2023), initially described in Villeta (eastern Colombia) followed by Antioquia, Bolívar, Córdoba, Magdalena, and Sucre, departments of Northwestern Colombia and the Cauca region. The high movement of infested equines and cattle between different Colombian ecoregions, along with their adaptation to climatic conditions, could facilitate the spread of *A. patinoi* to other parts of the country (Acevedo-Gutiérrez et al. 2018). In addition, three specimens of *Amblyomma* spp. that could not be characterised morphologically or molecularly shared similar

characteristics with *A. auricularium* and *A. parvum* previously reported in Colombia (López Valencia and Parra Gil 1985; Wells et al. 1981) and have been shown to be competent rickettsial vectors (Pacheco et al. 2007; Saraiva et al. 2013). Characterisation of *Amblyomma* species in the Neotropics remains a challenge; these species are included in a natural group with morphological and phylogenetic similarities (Nava et al. 2008), and analyses using cytochrome oxidase I and II genes and ITS 2 are useful for elucidating their phylogeny (Nava et al. 2017a).

Rickettsia spp. DNA was detected in 2.7% of the tick pools analysed (MIR: 0.5%) being identified in *R. sanguineus* s.l., *R. microplus* and *D. nitens*. In contrast, previous studies reported higher frequencies in other regions in Colombia (11.3%–16.1%) (Martínez Díaz et al. 2023; Rivera-Páez et al. 2018b). The occurrence of *Rickettsia* spp. in *R. sanguineus* s.l (3.1% of pools; MIR 0.6%) was similar to that reported in the Colombian Amazon region (3.8% of pools; Rivera-Páez et al. 2018b) and in Costa Rica (1.3% of pools; Troyo et al. 2016). However, another study conducted in Colombia reported higher MIR values (MIR 3.3%) (Martínez Díaz et al. 2023). Regarding *Rickettsia* spp. in *R. microplus*, the detection rate (2.5% of pools; MIR 0.5%) was similar to that reported in Costa Rica (6.6% of pools; MIR 0.4%) (Troyo et al. 2016), but lower than that reported in other studies in Colombia (MIR 9.6%) (Martínez Díaz et al. 2023; Miranda et al. 2012) and Brazil (MIR 3.0%) (Moura-Martiniiano et al. 2014). The frequency of *Rickettsia* spp. in *D. nitens* reported in the present study (3.2% of pools) was lower than that reported in Costa Rica, Cuba, Panamá, and Colombia, ranging between 10% and 20% (Bermúdez et al. 2009; Díaz-Sánchez et al. 2021; Santodomingo et al. 2019; Troyo et al. 2016); however, the MIR (0.5%) was identical to that reported in Costa Rica (Troyo et al. 2016). By contrast, other studies carried out in Colombia have not reported the presence of *Rickettsia* spp. in these tick species (Arroyave et al. 2020; Faccini-Martínez et al. 2016; Osorio et al. 2018; Zapata-Serna et al. 2022). None of the *Amblyomma* specimens were positive for rickettsial bacteria, which could be associated to the low number of specimens analysed. Other studies have also reported that *A. patinoi* may not be able to sustain *R. rickettsii* infection by transovarian transmission. Therefore, the presence of *R. rickettsii* remained low in the absence of suitable reservoirs (Martínez-Díaz et al. 2021), and to our knowledge has never been detected previously in the studied area.

The sequences of *Rickettsia* spp. obtained from the *gltA*, *ompA* and *ompB* genes in *Rhipicephalus* spp. and *Dermacentor* spp. showed high homology (>99.6%) with *Ca. R. colombiensis*. This finding is consistent with previous reports from Colombia (Miranda et al. 2012; Santodomingo et al. 2019). Other studies have associated *Rickettsia* sp. with closely related groups different to *Ca. R. colombiensis* (Holguin-Rocha et al. 2023; Martínez Díaz et al. 2023; Santodomingo et al. 2019); however, it is possible that factors such as genetic closeness or the use of a limited number of genes may not have allowed the precise identification of the species (Martínez Díaz et al. 2023). In our study, multigene analysis allowed the precise identification of this *Candidatus* species, which is widely reported in the country and most closely associated with ticks of the genus *Amblyomma*, particularly with the species *A. dissimile*, which mainly parasitises reptiles and birds (Cotes-Perdomo et al. 2022; Martínez-Sánchez et al. 2021;

Miranda et al. 2019, 2020; Rodriguez et al. 2023). It should be noted that *A. dissimile* has also been reported infesting humans in Colombia (Quintero et al. 2017). Although the pathogenicity of this *Candidatus* species is unknown, it belongs to the spotted fever group and is phylogenetically close to *Rickettsia tamurae*, a pathogenic species reported in Japan (Imaoka et al. 2011). *Candidatus* *R. colombiensis* has also been described in chigger mites (Trombidiformes: Trombiculidae) that have been shown to infest birds and small wild mammals in Brazil (Bassini-Silva et al. 2023; De Castro Jacinavicius et al. 2019), suggesting that different host–tick interactions are occurring among different host and tick species.

The detection of Anaplasmataceae species such as *A. marginale*, *A. platys*, *E. canis* and *E. minasensis* in *R. microplus* and *R. sanguineus* s.l. in the present study is consistent with findings from other regions of Colombia (Cabrera et al. 2022; Miranda and Mattar 2015). A study in Ibagué found that only 4.2% of *R. microplus* isolates were positive for Anaplasmataceae (Osorio et al. 2018). In Cauca, approximately 7.5% of pools tested positive using PCR assays targeting the *rpoB* and *groEL* genes of *Anaplasma* spp. and *Ehrlichia* spp. (Martínez Díaz et al. 2023). In addition, 30.4% and 8% of *R. sanguineus* pools (MIR 11.8% and 3.4%, respectively) in Magdalena were positive for *Ehrlichia* spp. and *A. platys*, targeting the *dsb* and *groEL* genes respectively (Arroyave et al. 2020). Additionally, in *R. microplus* and in *R. sanguineus* species, MIRs were reported for *Ehrlichia* spp. (6.2% and 2.9%, respectively) and *Anaplasma* spp. (8.4% and 0.6%, respectively), although specific *Ehrlichia* spp. and *Anaplasma* spp. were not sequenced (Martínez Díaz et al. 2023). Other studies have not reported the presence of *Ehrlichia* spp. and *Anaplasma* spp. in *R. microplus* in our study country (Arroyave et al. 2020). In the present study, no Anaplasmataceae species were detected in *D. nitens*. This finding contrasts with previous reports from other regions of Colombia, where *A. marginale* (Cotes-Perdomo et al. 2020) and *A. platys* (Santodomingo et al. 2019) were identified. However, all investigations reported low frequencies (0.4%–4.5% pools; 0.05%–1.3% MIR) (Martínez Díaz et al. 2023; Miranda and Mattar 2015). Further studies are required to determine the role of this tick species in the transmission of Anaplasmataceae in Colombia.

The tick species identified in the present study are competent vectors of pathogens relevant to animal and public health. While these tick species have been documented in other regions of the country and in Central and South America, to our knowledge, this is the first description of *D. nitens* and *A. patinoi* in the Atlantic region of the Colombian Caribbean. It is crucial to implement surveillance and control programmes for TBD, along with regular monitoring of vectors in the study area. These measures are needed to better understand animal–tick–human interactions and to mitigate the risk of TBP transmission in Colombia.

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Ethics Statement

This study was approved by the Ethics Committee of the Universidad Simón Bolívar (Barranquilla, Colombia), in accord with the ethical principles of animal research under number CIE-USB-CE-0317-00 of 28 February 2020.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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