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Infestation rates, seasonal distribution, and genetic diversity of ixodid ticks from livestock of various origins in two markets of Yaoundé, Cameroon

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Abstract

Little is known about the impact of ticks on livestock and humans in Cameroon. This study aimed to determine the prevalence, seasonal variation, and genetic diversity of hard ticks in the country. Ticks were collected during a cross-sectional survey on domestic livestock in two markets of Yaoundé in 2019 and 2020 and identified using morphological keys, 16S ribosomal DNA, (*16S rDNA*), and the cytochrome *c* oxidase subunit 1 (*Cox1*) genes. The infestation rates were 39.18%, 11.53%, and 2.74% in cattle, sheep, and goats respectively. Three genera of ticks were identified, *Rhipicephalus*, *Amblyomma*, and *Hyalomma* comprising eleven tick species. The main species were *Rhipicephalus decoloratus* (30.25%), *R. microplus* (24.43%), and *Amblyomma variegatum* (12.96%). *Rhipicephalus* spp. (81.31%) and *Amblyomma variegatum* (51.54%) were abundant during the rainy season, while *Hyalomma* spp. (83.86%) during the dry season (*p*-value <0.00001). *Cox1* and *16S rDNA* analysis showed a high level of genetic diversity among tick species with sequences close to those observed across Africa. Phylogenetic analysis revealed that our *R. microplus* belong to clade A and we identified *R. sanguineus* s.l. as *R. linnea*. This study shows a high tick infestation rate in cattle, while low in small ruminants with an extensive diversity of tick species, including several known vectors of important tick-borne diseases.

KEYWORDS
16S rDNA, cattle, Central Africa, Cox-1, ixodid ticks, small ruminant

INTRODUCTION

Ticks are obligate hematophagous Acari ectoparasites on a very wide range of vertebrate animals, including humans (Baneth, 2014). There are three main families of ticks recognized to date, the Ixodidae (hard ticks), the Argasidae (soft ticks), and the Nuttalliellidae (a primitive form of ticks) (Nava et al., 2009). Ticks are the most important vectors of viruses, bacteria, and parasites of veterinary and public health importance in the world (Jongejan & Uilenberg, 2004), particularly in temperate zones, where hard ticks are considered to be the most important vectors of pathogens (Jongejan & Uilenberg, 2004). To date, approximately 900 species of ticks have been described worldwide, with almost 700 species assigned to the Ixodidae, one species to the Nuttalliellidae, and the remainder to the Argasidae (Guglielmone et al., 2010).

An estimated 80% of the world's cattle population is at risk of tick-borne diseases (TBD) with economic losses estimated at US$22–30 billion each year (Lew-Tabor & Rodriguez Valle, 2016), which

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represent the cost of prevention and control as well as the decrease in the commercial value of infested animals (Eskezia & Desta, 2016). Tick-borne infections in humans are mostly zoonotic and pose a serious threat in Europe and North America. For example, *Babesia* infections cause disease in 255,000 persons annually, while in central Europe, the threat of tick-borne encephalitis (TBE) has led to mass vaccination programs in Switzerland and Austria. Crimean-congo hemorrhagic fever (CCHF) has become an important infection in Europe and is already widespread across Africa, and some parasitic diseases such as theileriosis are responsible for symptoms ranging from fever to haemorrhage which may lead to death in some instances (CDC, 2021).

Ticks are abundant in the Afrotropical region where they occur in climatic zones ranging from arid to wet tropical. Approximately, 200 ixodid and 40 argasid tick species have been recorded in the Afrotropical region (Madder et al., 2014). In Cameroon, little is published about the impact of ticks and tick-borne diseases on the livestock industry. Indeed, the last study, released in 1982, reported that 63% of the cattle mortality was due to TBD (Mbah, 1982).

The movement of livestock carrying infected ticks is an important contributor to the dispersal of TBDs in this region. The exchange of pathogens between neighbouring areas may have been responsible for the introduction of TBDs to previously disease-free areas in the Central African Republic due to transboundary animal movements (Motta et al., 2017). Furthermore, reported outbreaks of CCHF in many sub-Saharan African countries with a fatality rate of up to 50% have prompted the study of their Ixodid vectors (Msimang & Weyer, 2021). In addition, CCHF was found circulating in the human population in Cameroon (Sadeuh-Mba et al., 2018). Unfortunately, disease surveillance at the borders of most sub-Saharan African countries is, at best limited, but usually absent (Motta et al., 2017). The recent discovery of the cattle tick *Rhipicephalus (Boophilus) microplus* in Cote d'Ivoire in 2007, possibly introduced from Brazil was followed by a rapid spread to many other West African countries (De Clercq et al., 2013), and recently to Cameroon (Silatsa et al., 2019). This is particularly worrisome, as this tick species is an efficient vector of *Babesia bovis* which causes a virulent form of babesiosis, the most important TBD of cattle globally (Silatsa et al., 2019). In addition, resistance to safe and affordable pyrethroid insecticides is widespread in *Rhipicephalis* sp ticks, representing a serious problem for West-African countries (Adakal et al., 2013).

The identification of tick species, their abundance, and distribution, is of great importance to understanding the epidemiology of TBD and their control in every region (Taheri et al., 2014). However, traditional morphological identification requires extensive experience and can be challenging when the specimens are engorged with blood, in immature stages (larva or nymph stage), or when the ticks are physically damaged (Nava et al., 2009). Moreover, many tick species are grouped into complexes of species due to their high morphological similarities. Therefore, molecular methods based on DNA barcoding, including the *16S rDNA* gene and the cytochrome *c* oxidase subunit 1 (*Cox1*) gene, are useful in the classification of ticks.

In Cameroon, most studies on the identification of ticks are primarily based on morphological characterization and the main genus found are *Amblyomma*, *Hyalomma*, and *Rhipicephalus* (Awa et al., 2015). However, except for one study which has mainly focused on *R. microplus* (Silatsa et al., 2019), little is known about the genetic diversity of hard ticks in the country. Furthermore, only few studies have been undertaken on ticks infesting livestock in Cameroon (Stachurski et al., 1993; Awa., 1997; Ndi et al., 1998). Thus, there is scarce data on the seasonality and infestation rates of this livestock.

Therefore, this study aimed at determining the infestation rate, seasonal distribution, and species diversity of ticks in livestock in Yaoundé's markets.

MATERIALS AND METHODS

Description of the study site

This study was carried out in two of the biggest livestock markets of Cameroon, located in Yaoundé the capital city, namely Etoudi (3°55′N, 11°31′36″ E) in Yaoundé 1 district for cattle and Tsinga market (3°53′55″ N, 11°29′30″ E) in Yaoundé 2 district for goats and sheep (Figure 1), from June to September 2019 during the rainy season and from January to March 2020 within the dry season in all Cameroon regions (Fonteh & Nji, 2001; Lendzele et al., 2019). The cattle market of Etoudi is a periodic market where about 1500 to 2000 cattle originating from the Adamawa, North, Far North region of Cameroon as well as the neighbouring countries (Chad, the Central African Republic, and Sudan) are traded each market day. This market is associated with a modern slaughterhouse where the animals are usually slaughtered after the purchase to supply the city with beef. The cattle can spend two to 5 days on the way to Yaoundé and about one to 3 days in the market before being sold. The small ruminant market at Tsinga holds about 1000 goats and 1000 sheep that arrive twice a week from the Northern and Western Regions of Cameroon and are sold within 2 days.

Tick collection and morphological identification

Cattle, goats, and sheep were screened for the presence of ticks in the livestock markets of Yaoundé. Livestock was restrained on their feet, and all their body parts were examined to collect visible nymphs and adult ticks. Ticks were removed manually or with blunt steel forceps and kept in individual 15 ml falcon tubes per animal. Once in the laboratory, the ticks were washed in ethanol 70% v/v, rinsed twice with sterile water, and finally with a cell culture medium (Minimum Essential Medium). They were subsequently morphologically identified using a stereomicroscope (LEICA EZ4E, LEICA Microsystems, Wetzlar, Germany) at ×35 magnification based on published taxonomic keys (Madder et al., 2014; Walker et al., 2003). Then, the ticks were preserved in RNAlater™ Stabilization Solution (Invitrogen™, Life Technologies, Carlsbad, California, USA) and stored at −80°C until further analysis.

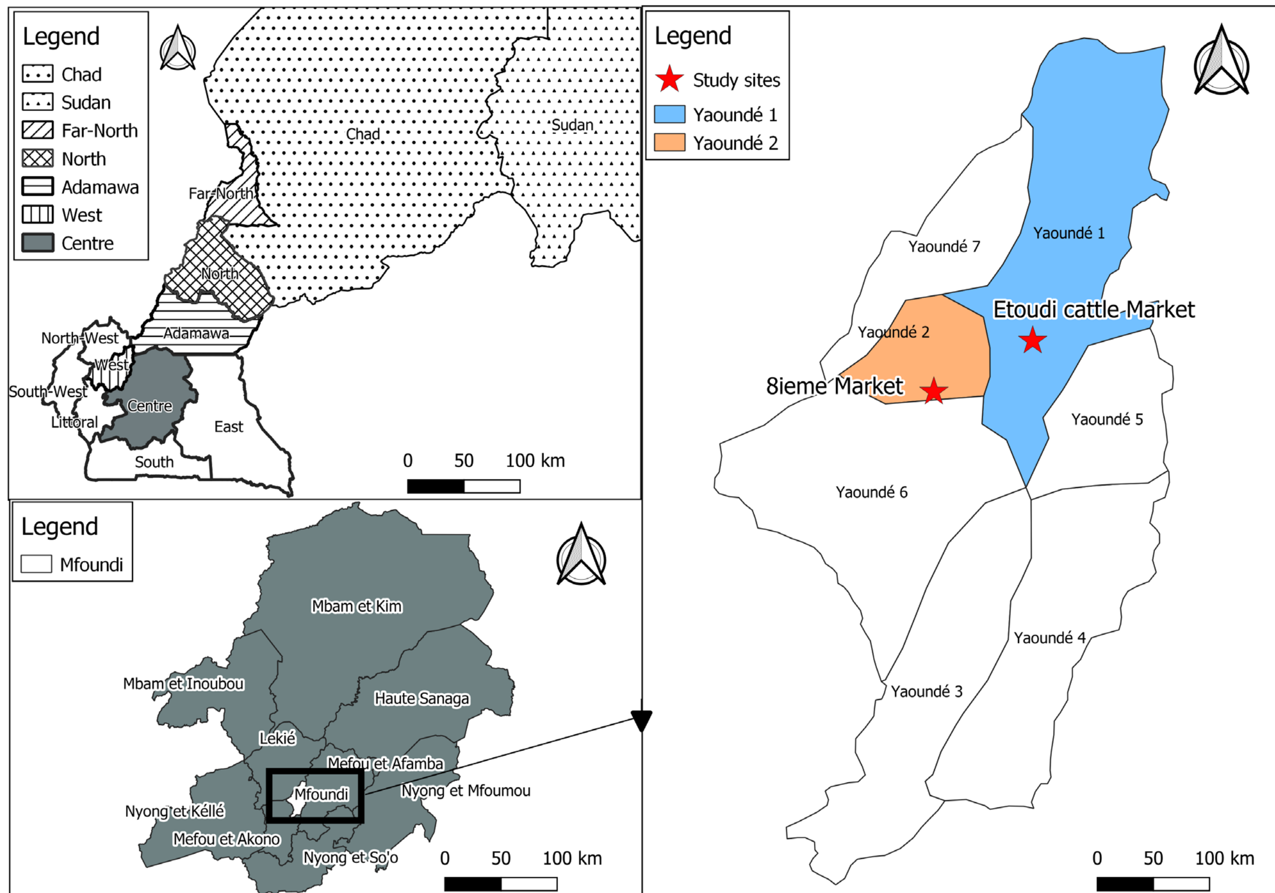

FIGURE 1 Geographic localization of the study sites. Etoudi market, where cattle are sold is located in Yaoundé I subdivision (blue), and the small ruminant market (Tsinga market) is located in the Yaoundé II subdivision (orange). The two subdivisions are found in the Mfoundi department, Centre region of Cameroon. In Cameroon, the regions of origin of animals are marked in black strips: Oblic (far north), cross strips (north), horizontal strips(Adamawa), and vertical strips (west). Chad and Sudan are shown in black dot and black triangles respectively. The map was build using shapefiles from GADM (https://gadm.org/download_country.html)

Molecular identification of different tick species

To support morphological identification, the tick species was confirmed by molecular analysis of a portion of the *Cytochrome c Oxidase subunit 1 (Cox1)* and *16S rDNA* genes as described previously (Lv et al., 2014). For each species of tick identified morphologically, ten representative individuals were randomly selected for molecular identification. For specimens only identified at the genus level, ticks were grouped according to the morphological similarities, and 10 representatives of each group were selected for molecular analysis.

DNA extraction

RNAlater preserved ticks were washed in distilled water and air-dried for 30 min then each semi- and fully engorged tick was cut into small pieces and total DNA was extracted using Livak protocol (Livak, 1984). Approximately 80 mg of ticks were individually homogenized in 200 μl of 120-mM Tris–HCl, pH 9, containing 0.5% SDS, 80 mM NaCl, 160 mM sucrose, and 60 mM EDTA. After incubation for 30 min at 65°C, 28 μl potassium acetate (8 M) was added. The mixture was then vortexed and incubated on ice for 30 min. Thereafter, the samples were centrifuged twice for 20 min at 13,500 rpm. The supernatant obtained was transferred into a new 1.5 ml tube where 400 μl ethanol 100%v/v was added. Nucleic acids were pelleted at 13,500 rpm for 15 min and the supernatant was discarded. The remaining pellet was rinsed with ethanol 70% v/v and air-dry for an hour. The nucleic acid was resuspended in 100 μl of DNAse free water and then incubate at 65°C for 10 min.

Amplification and sequencing of the *Cox1* and *16S rDNA* genes

A fragment of the *Cox1* gene (711 bp) was amplified using the following primers *Cox1*F (5′GGA ACA ATA TAT TTA ATT TTT GG3′) and *Cox1*R (5′ATC TAT CCC TAC TGT AAA TAT ATG3′). Simultaneously, a fragment of 370 bp was amplified for the *16S rDNA* gene using the following forward primer *16S*-F (5′TTA AAT TGC TGT RGT ATT3′) and reverse primer (5′CCG GTC TGA ACT CAS AWC3′) as previously described (Lv et al., 2014) using the KAPA Taq PCR Kit (Kapa Biosystems, Wilmington,

TABLE 1 Tick infestation rates per animal species and origins

Animal				Cattle (%)	Sheep (%)	Goats (%)	Total
Origin	Cameroon	West	Examined	0	0	31	31
			Infested	0	0	5 (16.13)	5 (16.13)
		Adamawa	Examined	2413	0	0	2413
			Infested	1127 (46.92)	0	0	1127 (46.71)
		North	Examined	1262	769	1774	3805
			Infested	552 (43.74)	92 (11.96)	45 (2.54)	689 (18.11)
		Far North	Examined	756	58	20	834
			Infested	249 (31.16)	8 (13.79)	/	257 (30.82)
	Chad		Examined	1873	101	38	2012
			Infested	545 (28.12)	7 (6.93)	1 (2.63)	553 (27.48)
	Sudan		Examined	28	0	0	28
			Infested	8 (30.77)	0	0	8 (28.5%)
Total (%)			Examined	6332	928	1863	9123
			Infested	2481 (39.18)	107 (11.53)	51 (2.74)	2639 (28.9%)

Massachusetts, USA). Polymerase chain reaction (PCR) was performed in a final volume of 15 μl containing 1.5 μl of Taq Buffer B (10X), 1.5 mM of $Mgcl_2$, 100 ng of DNA template and 0.4 μM of each primer using the KAPA Taq PCR Kit (Kapa Biosystems, Wilmington, Massachusetts, USA) and the amplification protocol was done as described elsewhere (Lv et al., 2014). Each PCR amplicon was separated on a 2% agarose gel using Midori-green™ (Bulldog Bio, Portsmouth, New Hampshire, USA) for visualization and the Hyper Ladder™ 100 bp (Meridian Bioscience, Bioline Cincinnati, Ohio, USA). Gels were photographed using the ENDURO™ GDS Gel documentation system (Labnet International, Edison, New Jersey, USA). The amplicons were purified using the ExoSAP-IT™ (Applied Biosystems™, Foster City, California, USA) following the manufacturer's protocol. The final concentration of purified PCR product was determined using a NanoDrop™ Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The purified amplicons were sequenced by Sanger sequencing at Genewiz (GENEWIZ, Bishop's Stortford, Hertfordshire, United Kingdom) using the same forward and reverse primers used to generate the PCR products.

Molecular analysis of the tick *16S rDNA* gene and *Cox1* gene

Sequences of both the *16S rDNA* gene and the *Cox1* were visualized and manually edited when necessary using BioEdit software version 7.1.9 (Hall, 1999). They were then compared with the available data on GenBank using BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences were then aligned to references from Africa, Asia, America, and Europe retrieved from GenBank and manually edited using BioEdit version 7.1.9 (Hall, 1999). Then, the best fitting DNA evolutionary model for the phylogenetic inference was determined using Mega X (Kumar et al., 2018). Hence the phylogenetic trees were built with 1000 bootstrap iterations using the Maximum likelihood (ML) under the Tamura-3-parameter (T92) substitution model (Tamura, 1992).

Genetic polymorphism analysis

Sequences from the multiple sequence alignment were extracted and collapsed into haplotypes using DnaSP software v6.10.01 (Rozas et al., 2017). Haplotype diversity (Hd), Nucleotide diversity (pi), and the number of haplotypes (h) values were determined. The haplotype networks were constructed to assess the genealogical relationship between haplotypes detected using TCS v1.21 (Crandall et al., 2000) and edited using tcsBU (Múrias dos Santos et al., 2016).

Statistical analysis

The infestation rate and tick species distribution per animal and season were determined using R software v4.0.3 for windows via RStudio v1.3.1093 (RStudio, T., 2020). Comparisons were made between the number of tick species observed during the rainy and the dry seasons using the Chi-square ($\chi 2$) test with significance at a *p*-value <0.05. QGIS version 3.14.16 (https://www.qgis.org), was used to generate the map presenting the study sites, and the figures presenting the seasonal variation of tick species were generated using GraphPad Prism version 8.0.2 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

RESULTS

Tick infestation rates per animal species

We examined a total of 9123 animals including 6332 cattle (477 herds), 1863 goats (52 herds), and 928 sheep (61 herds). We found 2639 (28.93%) infested animals corresponding to 2481 (94.01%) cattle, 107 (4.05%) sheep and 51 (1.94%) goats. We observed infestation rates of 39.18% (2481/6332) in cattle, 11.53% (107/928) in sheep, and

TABLE 2 Abundance of ticks per animal and origins

Origins	Cameroon							Chad			Sudan	
	West	Adamawa	North			Far north						
Species	Goats	Cattle	Cattle	Sheep	Goats	Cattle	Sheep	Cattle	Sheep	Goats	Cattle	Total (%)
Amblyomma variegatum	2	177	136	28	16	41	0	80	5	1	1	487 (12.96)
Hyalomma detritum	0	1	15	0	0	9	0	15	0	0	0	40 (1.07)
Hyalomma dromedarii	0	6	0	0	0	15	0	0	29	0	0	50 (1.33)
Hyalomma impeltatum	0	36	13	0	1	17	0	83	0	0	0	150 (3.99)
Hyalomma nitidum	0	39	3	0	0	15	0	93	0	0	0	150 (3.99)
Hyalomma rufipes	0	9	16	0	0	11	0	25	0	0	0	61 (1.63)
Hyalomma truncatum	0	18	9	0	0	77	0	46	0	0	0	150 (3.99)
Rhipicephalus annulatus	0	194	49	0	0	33	0	133	0	0	0	409 (10.88)
Rhipicephalus decoloratus	1	477	164	46	17	62	1	366	2	0	0	1136 (30.25)
Rhipicephalus microplus	0	574	255	1	0	9	0	79	0	0	0	918 (24.43)
Rhipicephalus sanguineus	0	28	0	84	31	9	10	39	5	0	0	206 (5.48)
Total (%)	3 (0.08)	1559 (41.50)	660 (17.57)	159 (4.23)	65 (1.73)	298 (7.93)	11 (0.29)	959 (25.52)	41 (1.09)	1 (0.03)	1 (0.03)	3757

2.74% (51/1863) in goats. The infestation rate was higher in cattle (39.18%) than in sheep (11.53%) and goats (2.74%), ($\chi^2 = 30.40$, *p*-value $= 0.00003$) with the most infested animals from the Adamawa, Far North and West regions for cattle (46.92%), sheep (13.79%) and goats (16.13%) respectively (Figure 1, Table 1). Ticks were collected on 50.32% (1328/2639) of examined livestock consisting of 46.23% of infested cattle (1147/2481), and all the infested small ruminants. This was due to the lack of cooperation from some shepherds in the cattle market but mostly because of the aggressive nature of some cattle. Among the 3757 ticks collected, we recorded 3058 adult females, 673 adult males, and 26 nymphs with 78.17% (2937/3757) fully engorged and 21.83% semi-engorged. The cattle examined were mostly from the Adamawa region of Cameroon representing 38.11% of all the cattle while 82.86% sheep and 63.02% goats were from the North region of the country (Figure 1, Table 1).

Morphological and molecular identification of ticks

The morphological and molecular identification of ticks revealed the presence of three genera of Ixodid ticks, *Rhipicephalus*, *Amblyomma*, and *Hyalomma* comprising respectively 71.04%, 15.99%, and 12.96% of the species observed. Most of the ticks collected were fully engorged and on many of them, the mouthpart was damaged which renders morphological identification challenging. Thus, we were able to only morphologically identify six species: *Rhipicephalus microplus*, *R. decoloratus*, *R. sanguineus* s.l, *Amblyomma variegatum*, *Hyalomma. truncatum*, *H. rufipes*, and the others were classified as *Rhipicephalus* spp. (365/3757; 9.72%), and *Hyalomma* spp. (391/3757; 10.41%). Furthermore, a 711 bp fragment of the *Cox1* gene and 370 bp fragment of the *16S rDNA* were successfully amplified and sequenced. After alignment using BLASTN (https://blast.ncbi.nlm.nih.gov/Blast.cgi), 11 species of ticks were identified. The most common were *Rhipicephalus (Boophilus) decoloratus* Koch, 1844 (30.25%), *Rhipicephalus (Boophilus) microplus* Canestrini, 1888 (24.43%), *Rhipicephalus (Boophilus) annulatus* Say, 1821 (10.88%), *Rhipicephalus sanguineus* sensu lato Latreille, 1806 (5.48%) which was further identified as *Rhipicephalus sanguineus* "tropical lineage" now known as *Rhipicephalus linnaei* Audouin, 1826, *Amblyomma variegatum* Fabricius, 1794 (12.96%), *Hyalomma truncatum* Koch, 1844 (3.99%), *Hyalomma nitidum* Schulze, 1919 (3.99%), *Hyalomma impeltatum* Schulze & Schlottke, 1930 (3.99%), *Hyalomma rufipes* Koch, 1844 (1.63%), *Hyalomma dromedarii* Koch, 1844 (1.33%), and *Hyalomma detritum* Schulze, 1919 (1.07%). We observed a 10% discrepancy between the morphological identification and the molecular markers while the two molecular makers gave a discordant result in 8% of the cases. Hence, we considered the species that was confirmed by two of the three identification methods used. The sequences obtained from this study are available on Genbank.

Tick species abundance per herds, animals, and origin

The predominant tick species was *R. decoloratus* (30.25%) followed by *R. microplus* (24.43%) (Table 2). *R. decoloratus* (30.60%) and *R. microplus*

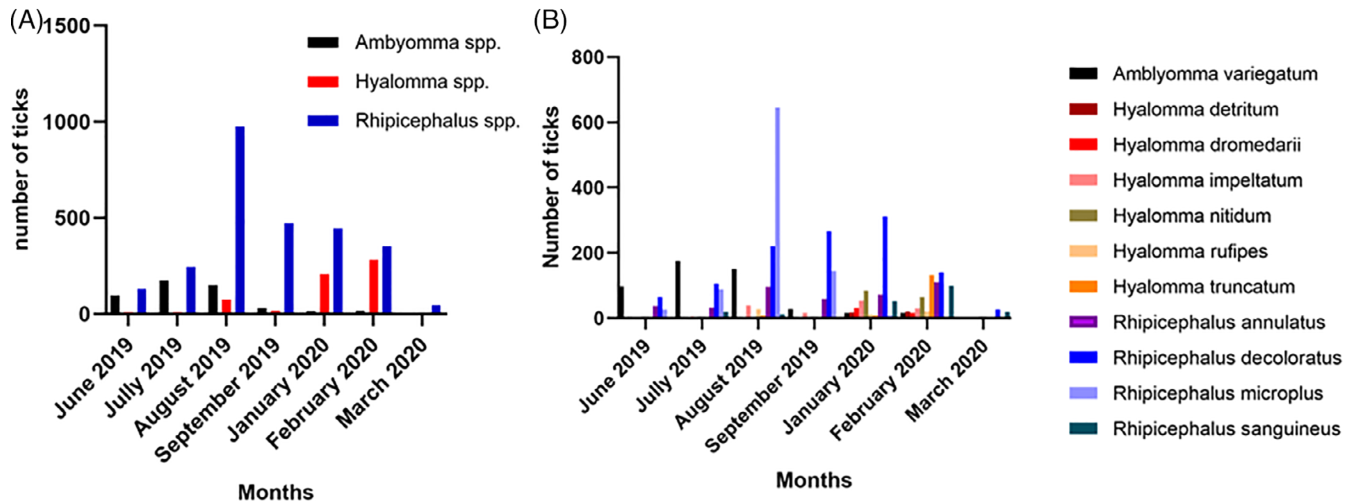

FIGURE 2 Seasonal distribution of ticks. (a) represents the seasonal distribution of ticks per genus and (b) the distribution per species of ticks. The figure was generated using GraphPad prism v8.0.2

(26.25%) were predominant in cattle while *R. sanguineus* was the main species in sheep (51.83%) and goats (42.47%) (Table 2). However, we found that the highest number of *A. variegatum* and *R. sanguineus* ticks were found on herds from the North region, *H. truncatum* from the Far North region, *R. annulatus*, *R. microplus*, *R. decoloratus* from the Adamawa region of Cameroon while *H. nitidum* and *H. dromedarii* were mostly from herds of Chad.

Infestation rates were highest in animals coming from the Adamawa region of Cameroon (41.49%) followed by animals from Chad (26.65%) (Table 2). Furthermore, the most frequently found species of ticks on animals originating from the Adamawa (36.82%) and the North (28.95%) regions were *R. microplus* ($\chi^2 = 798.34$, *p*-value: 0.225), whereas in the Far North, the predominant species was *H. truncatum* (24.93%). In addition, the predominant species of ticks on animals coming from Chad was *R. decoloratus* (36.77%) (Table 2).

Additionally, the body parts where ticks were collected on cattle vary according to the tick species. *Hyalomma* ticks were mostly found on the anus (85%) and the tail (15%), *Amblyomma* from the internal part of the thigh (74%), the scrotum and the udder for females (26%), and *Rhipicephalus* from many parts of the body (flank, ears, back, neck, shoulder, chest floor) with the main site being the fore under part (60%). However, on small ruminants, ticks were mostly collected on the ears (88%) and the remaining on the scrotum and udder part of females (12%).

Seasonal distribution of ticks from the livestock markets of Yaoundé

Our analyses revealed that ticks were most predominant in August (32%) which corresponds to the rainy season in the country, and we found a smaller number of ticks during March (1.25%) which is within the dry season in Cameroon. Furthermore, *A. variegatum* (35.93%) and *Rhipicephalus* ticks (36.56%), were most abundant during the rainy season, in July and August respectively and *Hyalomma* ticks (47.1%) were most predominant in February, during the dry season (Figures 2 and 3). The difference obtained between the rainy and the dry seasons was statistically significant ($\chi^2 = 13.52$, *p*-value <0.0002).

Polymorphism statistics and phylogenetic trees

Genetic diversity analysis was performed with four tick species (*R. decoloratus*, *R. microplus*, *R. sanguineus*, *A. variegatum*) on a 371 bp fragment of *16S rDNA* (Table 3) and in three species (*R. microplus*, *R. sanguineus*, and *R. decoloratus*) on a fragment of *Cox1* gene spanning 711 bp (Table 4). In addition, phylogenetic analysis were performed on *H. truncatum*, *H. nitidum*, and *H. dromedarii* sequences.

Genetic diversity of *R. decoloratus* ticks sampled from livestock and sequence analysis of the *16S rDNA* and *Cox1* genes

Analysis of fifteen sequences of *16S rDNA* gene of *R. decoloratus* revealed a low polymorphism defining four haplotypes from four substitution sites, with an overall haplotype diversity of 0.543 and nucleotide diversity of 0.0028. The haplotype H1 was dominant (10/15, 66.66%) and present in all the four studied populations (Adamawa, North, Far North, and Chad) (Figure 3a, Table 3). However, haplotypes H2 (1/15, 6.67%) and H4 (3/15, 20.00%) were detected only in animals from the Adamawa region, and haplotype H3 (1/15, 6.67%) was exclusively found in animals from the North region (Figure 3a). There was one mutational step between H1 and H3, H4 (Figure 3a). H2 differs from H1 by a single nucleotide polymorphism (SNP) at position 110 (A/G), H3 and H4 by two SNP at positions 211 (T/C) and 213 (G/A) in H3 then at position 184 (A/G), 211 (T/C) for H4 (Figure 3b). The negative values of the neutrality tests (Tajima's D and

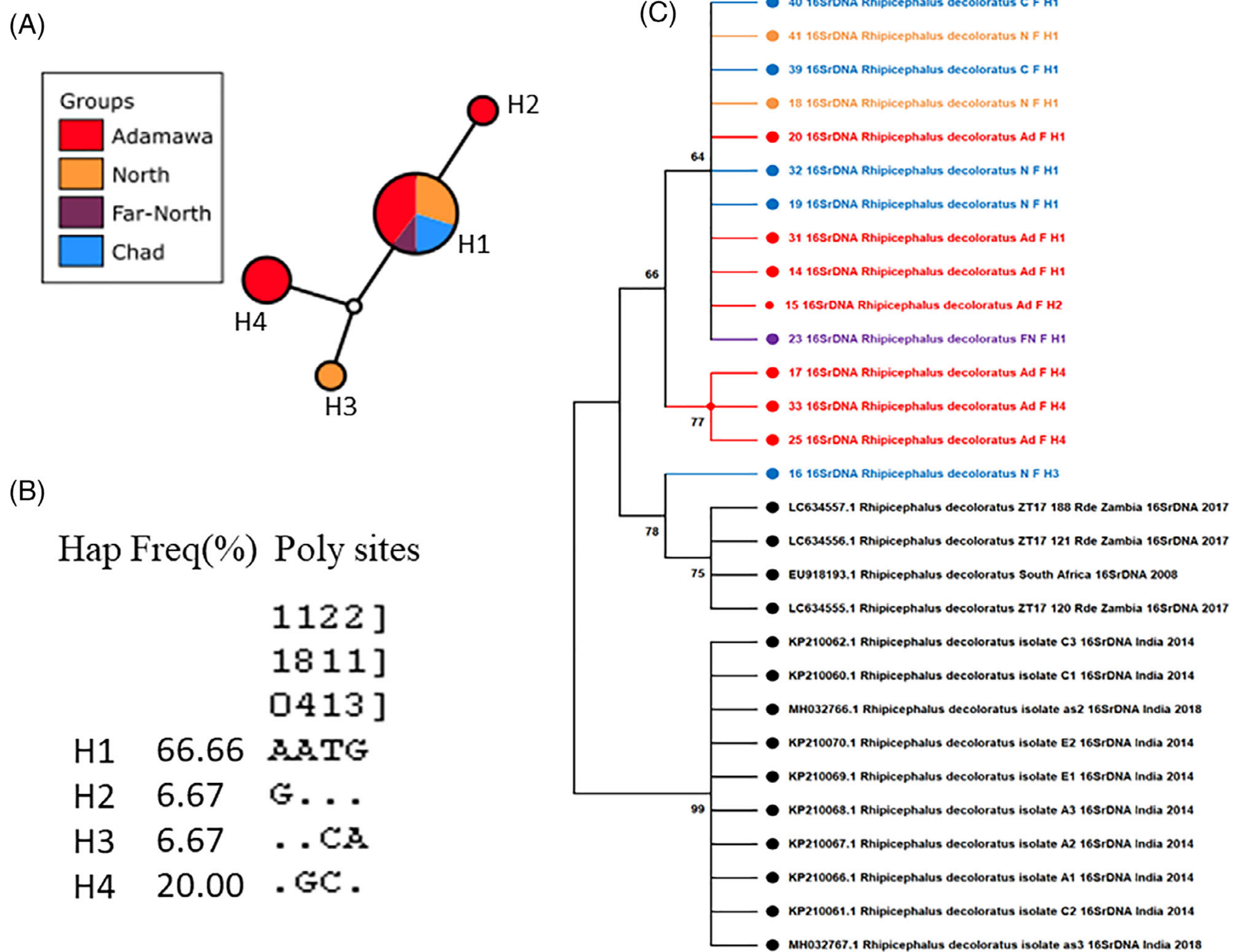

FIGURE 3 Genetic analysis of *16S rDNA* gene of *R. decoloratus*. (a) and (b) represent the haplotype network and haplotype frequencies respectively. Haplotypes H1–H4 represent the different nucleotide sequence variants, each colour represents the locality where ticks were collected. Mutational steps are symbolized by a small white dot between haplotypes, and the diameter of the circles is proportional to the number of individuals that belong to each haplotype. The nucleotide alignment shows the polymorphic sites and the frequencies of mutations per haplotype. These analysis were done using TCS v1.21 software. Panel C represent the phylogenetic tree built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 50% are shown. The sequences generated in the present study are highlighted in purple, blue, red, and orange. Hap: Haplotype, Freq: Frequencies, poly: Polymorphic

TABLE 3 Summary of polymorphism statistics of the *16S rDNA* gene

Species	*n*	Sites	S	Hap	Hd		D	D*
Rhipicephalus decoloratus	15	371	4	4	0.540	0.0028	−0.5254	−0.5961
Rhipicephalus sanguineus	12	370	16	4	0.636	0.0163	0.6213	0.9932
Rhipicephalus microplus	8	399	7	3	0.714	0.0079	0.5293	1.4401
Amblyomma variegatum	9	368	9	4	0.806	0.0072	−0.9733	−1.2794

Abbreviations: D and D*, Tajima's and Fu and Li's statistics; h, number of haplotypes; Hd, haplotype diversity; *N*, number of sequences (*n*); S, number of polymorphic sites; π, nucleotide diversity.

Fu & Li's D) suggest a recent population expansion or decrease in genetic variation due to positive selection (Table 3). The ML tree of *R. decoloratus* based on the *16S rDNA* (Figure 3c) showed that sequences of haplotypes H1 and H2 clustered together and formed a clade, haplotype H3 sequences comprised another clade, and sequences of H4 formed the third clade. Almost all our *R. decoloratus 16S rDNA* sequences formed a separate clade. Although, H3 formed a clade that is closed to sequences from Zambia.

Genetic polymorphism analysis using 14 sequences of the *R. decoloratus Cox1* gene generated a total of 9 substitution sites

TABLE 4 Summary of the polymorphism statistics of the *Cox1* gene

Species	n	Sites	S	Hap	Hd		D	D*
Rhipicephalus decoloratus	14	766	9	7	0.846	0.0033	−0.6189	−1.3518
Rhipicephalus sanguineus	13	716	56	8	0.897	0.03801	2.1666	1.3936
Rhipicephalus microplus	7	736	56	5	0.905	0.02362	−1.4659	−1.5903

Abbreviations: D and D*, Tajima's and Fu and Li's statistics; h, number of haplotypes; Hd, haplotype diversity; *N*, number of sequences (*n*); S, number of polymorphic sites; π, nucleotide diversity.

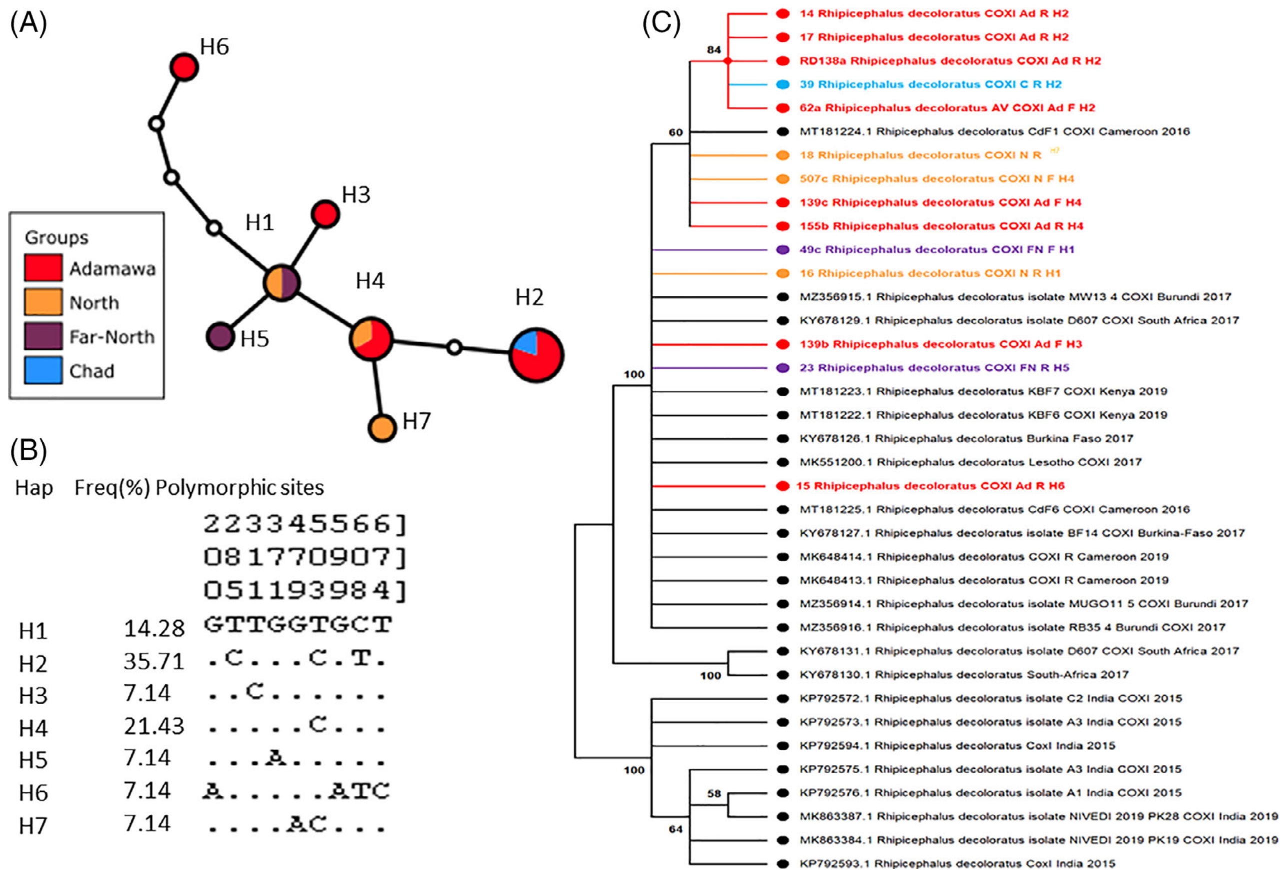

FIGURE 4 Genetic analysis of *Cox-1* gene of *R. decoloratus*. (a) and (b) represent the haplotype network and haplotype frequencies respectively. Haplotypes H1–H7 represent the different nucleotide sequence variants, each colour represents the locality where ticks were collected. Mutational steps are symbolized by a small white dot between haplotypes, and the diameter of the circles is proportional to the number of individuals that belong to each haplotype. The nucleotide alignment shows the polymorphic sites and the frequencies of mutations per haplotype. These analysis were done using TCS v1.21 software. Panel (c) represent the phylogenetic tree built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 50% are shown. The sequences generated in the present study are highlighted in purple, blue, red, and orange. Hap: Haplotype, Freq: Frequencies, poly: Polymorphic

7 haplotypes, and with a haplotype diversity of 0.846 and nucleotide diversity of 0.0033 (Figure 4a, Table 4). A high genetic diversity of the *R. decoloratus Cox1* was observed in the Adamawa and North regions where we detected the presence of four haplotypes H2 (5/14, 35.71%), H3 (1/14, 7.14%), H4 (3/14, 21.43%), and H6 (1/14, 7.14%), and three haplotypes H1 (2/14, 14.28%), H4 and H7 (1/14, 7.14%) respectively. Though haplotype H1 was present in both the North and Far North, haplotype 5 (1/14, 7.14%) was found only in the Far North (Figure 4a). There were three mutational steps between H1 and H6 and one between H2 and H4 (Figure 4b). H2 differs from H1 by three SNPs at positions 285 (T/C), 503 (T/C), 608 (C/T), H3 differs by one SNP at position 311 (T/C), H4 by one SNP at position 503 (T/C), H5 by one SNP at position 371 (G/A), H6 by four SNPs at positions 200 (G/A), 599 (G/A), 608 (C/T), 674 (T/C) then H7 differ by two SNPs at positions 479 (G/A) and 503 (T/C) (Figure 4b). The negative values of Tajima's D and Fu & Li's D* tests obtained suggest a recent population expansion or decrease in genetic variation due to positive selection (Table 4).

The ML tree of *R. decoloratus* based on the *Cox1* gene (Figure 4c) produced three main clades. Clade one was made up of five

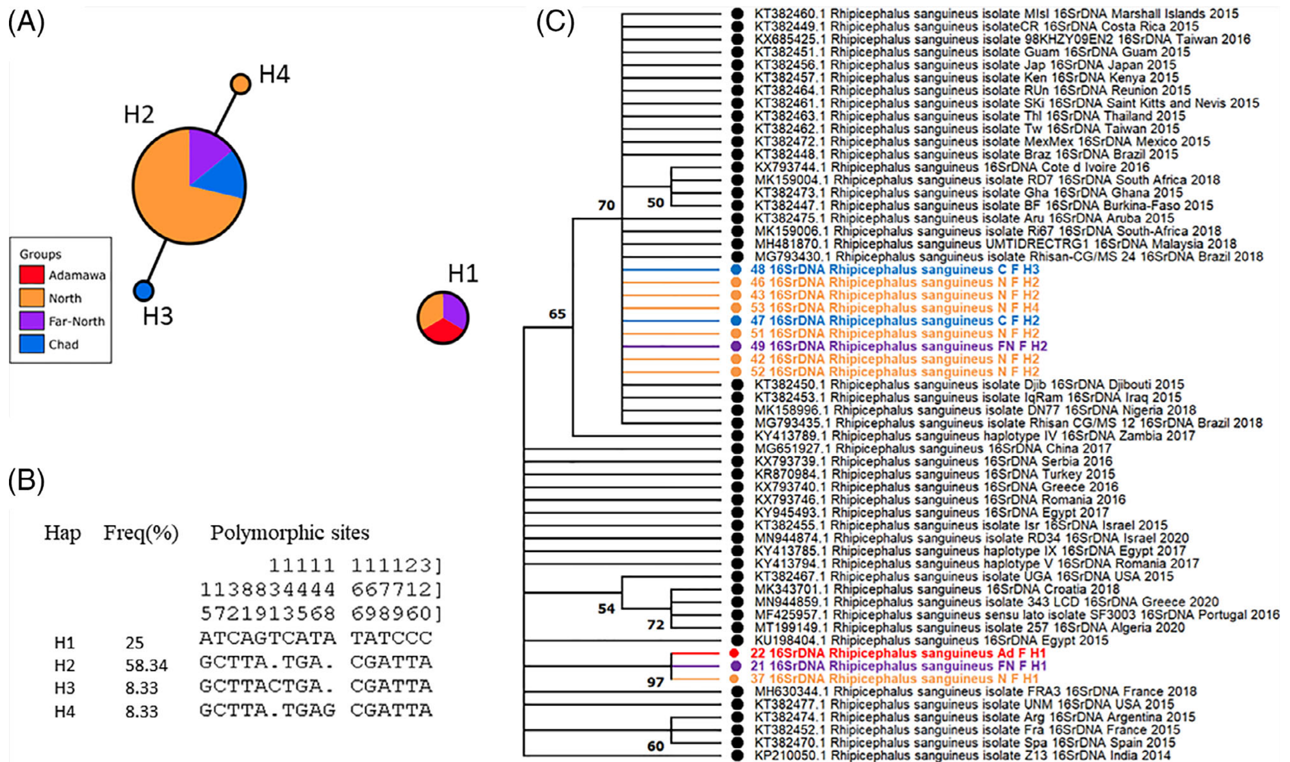

FIGURE 5 Genetic analysis of *16S rDNA* gene of *R. sanguineus* s. l. (a) and (b) represent the haplotype network and haplotype frequencies respectively. Haplotypes H1–H4 represent the different nucleotide sequence variants, each colour represents the locality where ticks were collected. The diameter of the circles is proportional to the number of individuals that belong to each haplotype. The nucleotide alignment shows the polymorphic sites and the frequencies of mutations per haplotype. These analysis were done using TCS v1.21 software. Panel (c) represent the phylogenetic tree built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 50% are shown. The sequences generated in the present study are highlighted in purple, blue, red, and orange. Hap: Haplotype, Freq: Frequencies, poly: Polymorphic

sequences, all from haplotype H2. Sequences forming haplotypes H4 and H7 clustered with other Cameroonian sequences to produce a second clade and sequences of haplotypes H1, H3, H5, and H6 comprised the third clade. The last four haplotypes clustered with sequences from East, South, and Central Africa downloaded from GenBank (Figure 4c). It should however be noted that genetic polymorphism analysis of *R. decoloratus* revealed higher haplotype diversity (Hd) and nucleotide diversity (Π) with sequences obtained from *Cox1* gene compared to those obtained from *16S rDNA*.

Genetic diversity of *R. sanguineus* ticks sampled from livestock and sequence analysis of the *16S rDNA* and *Cox1* genes

Twelve sequences of *R. sanguineus 16S rDNA* gene used for the genetic analysis generated four haplotypes and 16 substitution sites with a haplotype diversity of 0.636 and nucleotide diversity of 0.0163 (Figure 5a Table 3). Haplotypes H1 (3/12, 25%) and H2 (7/12, 58.34%) were both presents in at least three of the four studied populations, while H3 (1/12, 8.33%) was only present on animals from Chad and H4 (1/12, 8.33%) was specific to those from North region of Cameroon (Figure 5a). The haplotype H1 comprises sequences of *Rhipicephalus linnaei*, a subspecies of the complex *R. sanguineus* s.l. revealed a high level of genetic diversity with many single nucleotide polymorphisms when compared with the other three haplotypes and was distantly isolated from the other haplotypes in the network showing the existence of more than 20 mutational steps between H1 and the other haplotypes (Figure 5a). Haplotype H2 differs from H3 and H4 by a single nucleotide polymorphism at position 148 (T/C) for H2 and H3 then at 131 (A/T) for H2 and H4; then H1 differs from H2 by 14 SNPs (Figure 5b).

The ML tree of *R. sanguineus* based on *16S rDNA* (Figure 5c) showed that all the sequences from different populations forming the haplotype H1 clustered together to form a clade and were very close to the reference sequences from Argentina, the USA, and Portugal while the reference sequences from Aruba, La Reunion, and Thailand were closer to the sequences of haplotypes H2, H3, and H4.

Genetic polymorphism analysis of the 13 *Cox1* sequences of *R. sanguineus* originating from the North, Far North, and Adamawa regions revealed a total of eight haplotypes and 56 substitution sites with overall haplotype diversity and nucleotide diversity of 0.897 and 0.03801 respectively (Figures 6a and 7b, Table 4). This analysis revealed a high level of diversity of this gene in the North region, where

(B)

```
Hap  Freq(%)                    Polymorphic sites

                    1111 1111122222 2222233333 3333444555 5555555556 666666]
              1277991123 6799900234 5588900124 6778147112 4444558993 377899]
              8189365772 2723912273 5858119878 9387471092 0369688470 959709]
H1   15.39    CTTACAGTCA CGTCCTCCTG CCCAATCGAC TGTCATCCAA AATCCATGGC TGCACG
H2   7.69     ..C...A... .....A.... .......... .......... G......... ......
H3   7.69     ......A... .....A.... .......... .......... G......... ......
H4   7.69     .......... .....A.... .......... .......... .....G.... ......
H5   15.39    ......A... .......... .......... .......... .......... ......
H6   7.69     ......A... .....A.... .......... .A........ .G...G.... ......
H7   30.77    TCA.TG.CTG TAATTATTCA TTTTCCTTTT C.CTTCTTCG ..GTT.CAAT CATGTA
H8   7.69     TCAGTG.CTG TAATTATTCA TTTTCCTTTT C.CTTCTTCG ..GTT.CAAT CATGTA
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FIGURE 6 Genetic analysis of *Cox-1* gene of *R. sanguineus* s. l. (a) and (b) represent the haplotype network and haplotype frequencies respectively. Haplotypes H1–H8 represent the different nucleotide sequence variants, each colour represents the locality where ticks were collected. Mutational steps are symbolized by a small white dot between haplotypes, and the diameter of the circles is proportional to the number of individuals that belong to each haplotype. The nucleotide alignment shows the polymorphic sites and the frequencies of mutations per haplotype. These analysis were done using TCS v1.21 software. Panel (c) represent the phylogenetic tree built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 50% are shown. The sequences generated in the present study are highlighted in purple, red, and orange. Hap: Haplotype, Freq: Frequencies, poly: Polymorphic

the eight haplotypes were found circulating. Indeed, H2 (1/13, 7.69%), H3 (1/13, 7.69%), H4 (1/13, 7.69%), H5 (2/13, 15.39%), H6 (1/13, 7.69%), H8 (1/13, 7.69%) were exclusively from the North. However, the haplotypes H1 (2/13, 15.39%) and H7 (4/13, 30.77%) were also present in the Far North and Adamawa regions respectively. There was one mutational step between H1 and H4, and between H5 and H6 while two mutational steps were observed between H4 and H6. Furthermore, H7 and H8 were distantly isolated from the other haplotypes with more than 20 mutational steps as shown by the lack of represented steps (Figure 6a). *R. sanguineus* polymorphism analysis revealed a high haplotype diversity (Hd) with sequences obtained from *Cox1* gene compare to those obtained from *16S rDNA* (Figure 6a). Haplotype H1 differs from H2 by 4 SNPs at positions 78 (T/C), 115 (G/A), 201 (T/A), and 540 (A/G). Haplotype H3 differs from H1 by three SNPs at positions 115 (G/A), 201 (T/A), and 540 (A/G) while it differs from H4 by two SNPs at positions 201 (T/A) and 558 (A/G). Haplotype H3 differs respectively fromH5 by a single nucleotide polymorphism at position 115 (G/A), and H6 by five SNPs at positions 115 (G/A), 201 (T/A), 373 (G/A), 543 (A/G), and 558 (A/G). Haplotype H7 differs respectively from H1 by 50 SNPs and from H8 by a single nucleotide polymorphism at position 79 (A/G) (Figure 6b). Tajima's D and Fu & Li's D values of *16S rDNA* and *Cox1* genes of this tick species were positive, indicating that these genes have undergone balancing selection (Tables 3 and 4). The ML tree of *R. sanguineus* based on the *Cox1* gene (Figure 6a) showed that the sequences of haplotype H1 comprised one clade, sequences of haplotype H5 formed another clade, then H4 and H6 sequences were in the same clade with sequences from Brazil. Sequences of haplotype H2 and H3 clustered to form the fourth clade and sequences of H7 and H8 form a fifth clade that clustered with reference sequences from Portugal and China.

Genetic diversity of *R. microplus* complex per origin of livestock for the *16S rDNA* and *Cox1* genes

Eight sequences of *16S rDNA* gene of *R. microplus* complex were used for analysis and results revealed the presence of three haplotypes and seven substitution sites with a haplotype diversity and nucleotide diversity of 0.714 and 0.0079 respectively (Figure 7a, Table 3). Both haplotypes H1 (2/8, 25%) and H2 (4/8, 50%) were found in animals from the Adamawa and North regions. However, the haplotype H3 (2/8, 25%) was found in the Adamawa region of Cameroon and Chad (Figure 7a,b). Haplotype H1 was distant from the other haplotypes

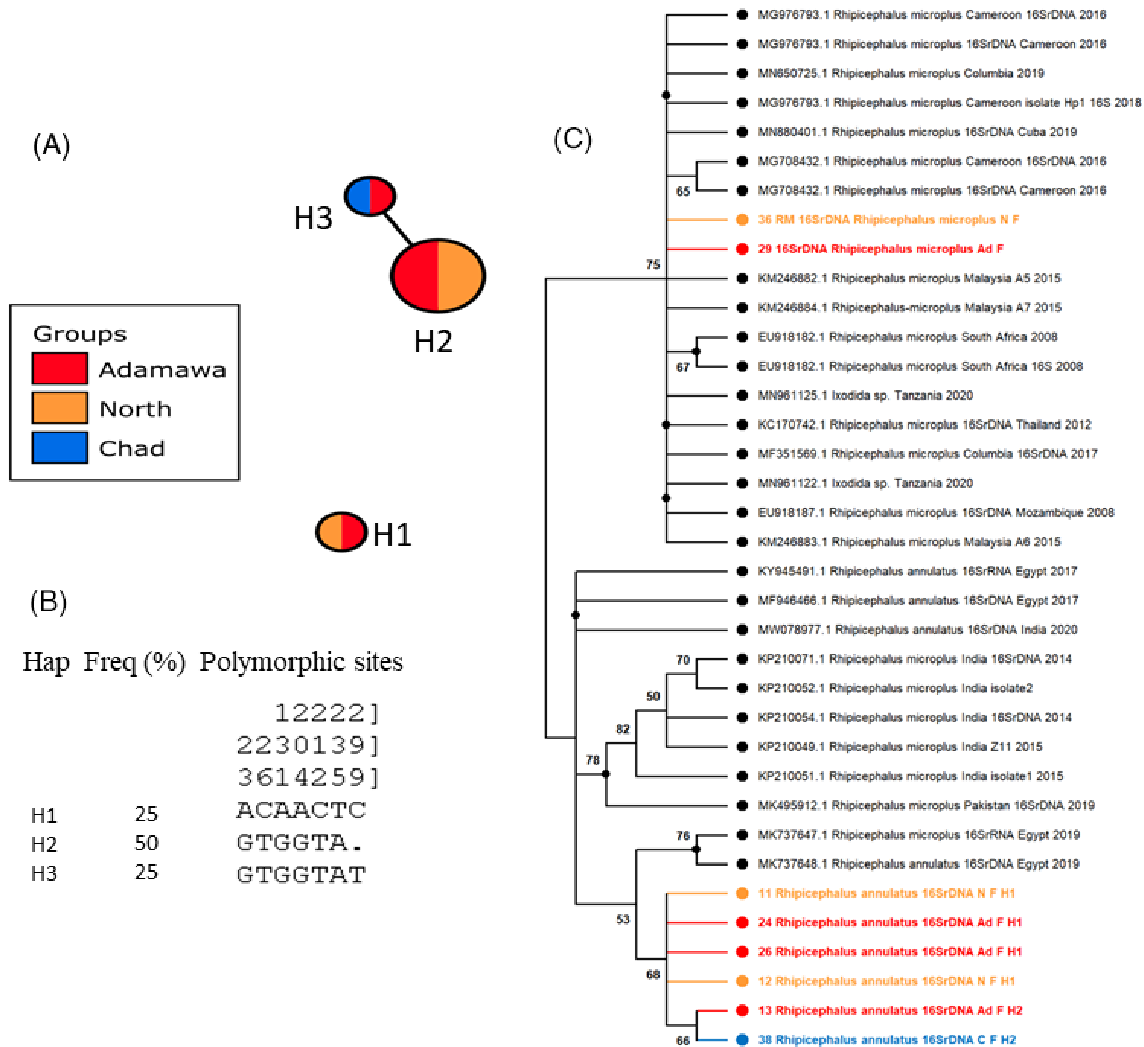

FIGURE 7 Genetic analysis of *16S rDNA* gene of *R. microplus* complex. (a) and (b) represent the haplotype network and haplotype frequencies respectively. Haplotypes H1–H3 represent the different nucleotide sequence variants, each colour represents the locality where ticks were collected. The diameter of the circles is proportional to the number of individuals that belong to each haplotype. The nucleotide alignment shows the polymorphic sites and the frequencies of mutations per haplotype. These analysis were done using TCS v1.21 software. Panel (c) represent the phylogenetic tree built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 50% are shown. The sequences generated in the present study are highlighted in blue, red, and orange. Hap: Haplotype, Freq: Frequencies, poly: Polymorphic

with more than 20 mutational steps (Figure 7a). Indeed, H1 differs from H2 by six SNPs at positions 23 (A/G), 26 (C/T), 131 (A/G), 204 (A/G), 212 (C/T), 235 (T/A) then H2 differs from H3 by a single nucleotide polymorphism at position 299 (C/T) (Figure 7b). Tajima's D and Fu & Li's D positive values suggest a balancing selection that needs to be confirmed (Table 3).

The ML tree of *R. microplus* complex based on the *16S rDNA* gene (Figure 7c) showed that our sequences formed two different clades one made of *R. microplus* species that clustered with those already described in Cameroon and Malaysia, another made of *R. annulatus* that was closed to sequences from Egypt and Pakistan.

Seven sequences of *R. microplus* complex used for analysis based on the *Cox1* gene revealed the existence of five haplotypes and 56 substitution sites with a haplotype diversity and nucleotide diversity of 0.905 and 0.02362 respectively (Figure 8a, Table 3). The haplotype H1 (2/7, 28.58%) was found in the Adamawa and North regions, H3 (1/7, 14.28%) and H5 (1/7, 14.28%) were exclusive from the Adamawa, haplotype H2 (2/7, 28.58%) in the North and H4 (1/7, 14.28%) in the Far North region of Cameroon (Figure 8a). Furthermore, H2 differs from H1 by four SNPs at positions 99 (C/T), 183 (A/T), 318 (G/A), and 484 (C/T), H3 differs from H2 by a SNP at position 708 (C/T). Then H4 differs from H1 by two SNPs at positions 484 (C/T) and 648 (C/T) whereas, H5 differs from H1 by 53 SNPs. This is confirmed by the haplotype network, where H5 is distant from the other haplotypes (Figure 8a,b). It should be noted that from H2 to H1 and H4, we observed three mutational steps. The negative values of the Tajima's D and Fu & Li's D suggest a recent population expansion or decrease in genetic variation due to positive selection (Table 4). The ML tree of *R. microplus* complex based on *Cox1*

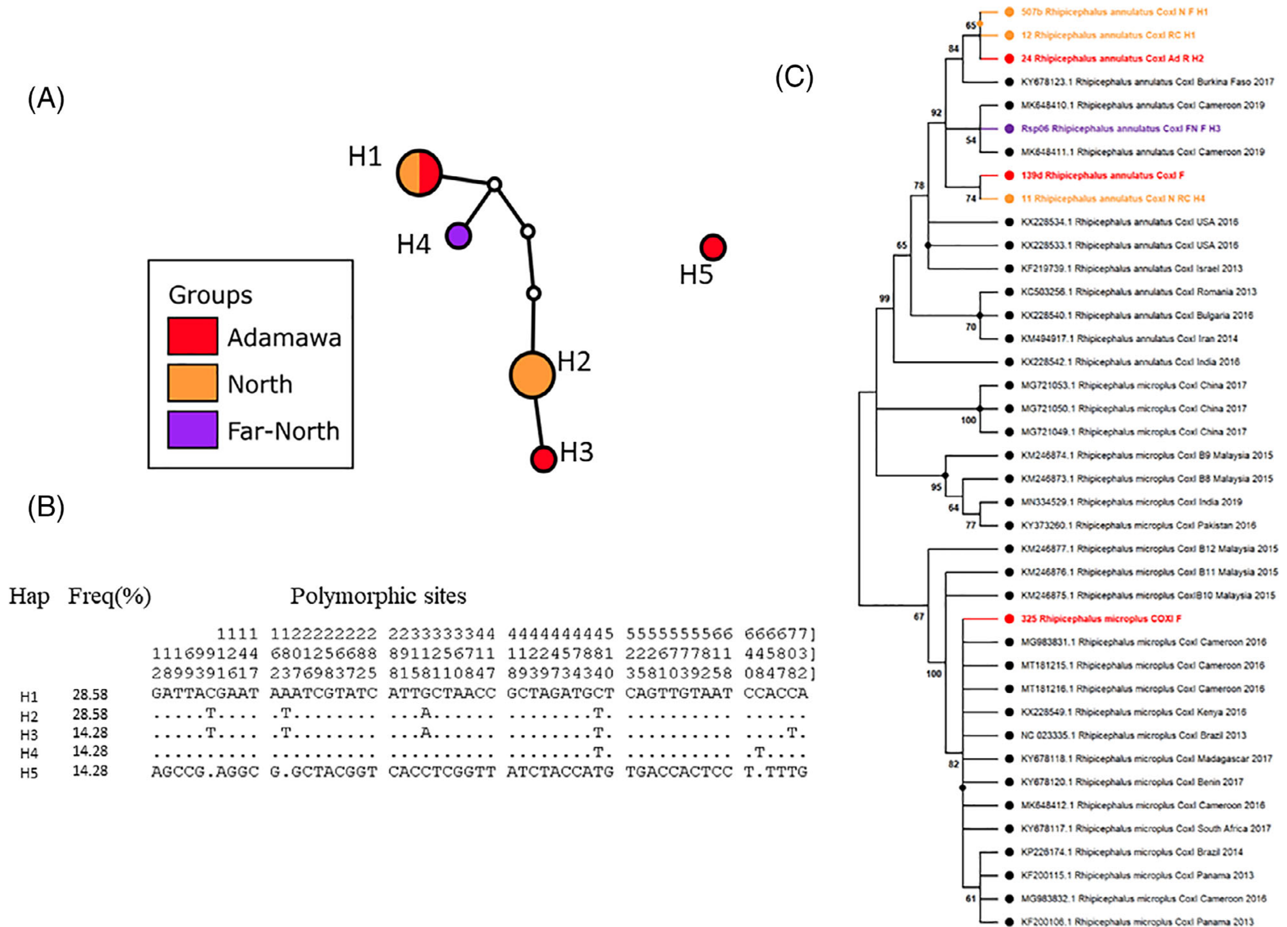

FIGURE 8 Genetic analysis of *Cox1* gene of *R. microplus* complex. (a) and (b) represent the haplotype network and haplotype frequencies respectively. Haplotypes H1–H5 represent the different nucleotide sequence variants, each colour represents the locality where ticks were collected. Mutational steps are symbolized by a small white dot between haplotypes, and the diameter of the circles is proportional to the number of individuals that belong to each haplotype. The nucleotide alignment shows the polymorphic sites and the frequencies of mutations per haplotype. These analysis were done using TCS v1.21 software. Panel C represent the phylogenetic tree built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 50% are shown. The sequences generated in the present study are highlighted in purple, red, and orange. Hap: Haplotype, Freq: Frequencies, poly: Polymorphic

gene (Figure 8c) showed the formation of four clades, the three first clades were made of sequences from *R. annulatus* (H1, H2, H3, and H4) and clustered with sequences from Cameroon and Burkina-Faso. Sequences from H5 (*R. microplus*) form the last clade and clusters with sequences previously described in Cameroon.

Rhipicephalus microplus complex polymorphism analysis produced higher haplotype diversity (Hd) and nucleotide diversity (Π) for sequences of *Cox1* gene compare to those for *16S rDNA* gene.

Genetic diversity analysis of *a. variegatum* per origin of livestock using the *16S rDNA* and *Cox1* genes

Nine sequences of *A. variegatum* were used for the analysis based on the *16S rDNA* gene, we found four haplotypes and 9 substitution sites with a haplotype diversity and nucleotide diversity of 0.806 and 0.0072 respectively (Figure 9a, Table 3). The haplotype H2 (3/8, 33.33%) and H4 (3/8, 33.33%) were the major ones with H2 that was made essentially of sequences from the North, and H4 was from the North, Far North, and Chad. Then haplotype H3 (2/8, 22.23%) was composed of sequences from the Adamawa and the North and H1 (1/8, 11.11%) from Far North (Figure 9b). Haplotype H1 was distant from other haplotypes and differs from H2 by seven SNPs at positions 12 (T/A), 53 (T/C), 104 (A/T), 120 (A/G), 157 (T/C), 178 (C/T), and 206 (C/T), then haplotype H2 differs from H3 by a SNP at position 113 (A/T) and haplotype H3 differs from H4 by a SNP at position 349 (A/G) (Figure 9b). The negative values of Tajima's D and Fu & Li's D obtained suggest a recent population expansion or decrease in genetic variation due to positive selection (Table 3).

The ML tree of *A. variegatum* based on *16S rDNA* analysis showed that there are three principal clusters (Figure 9c). The haplotypes H4 and H3 clustered with sequences from West African countries, H2 clustered with sequences from West and East Africa (Nigeria, Senegal, and Ethiopia) then H1 clustered with sequences from East Africa.

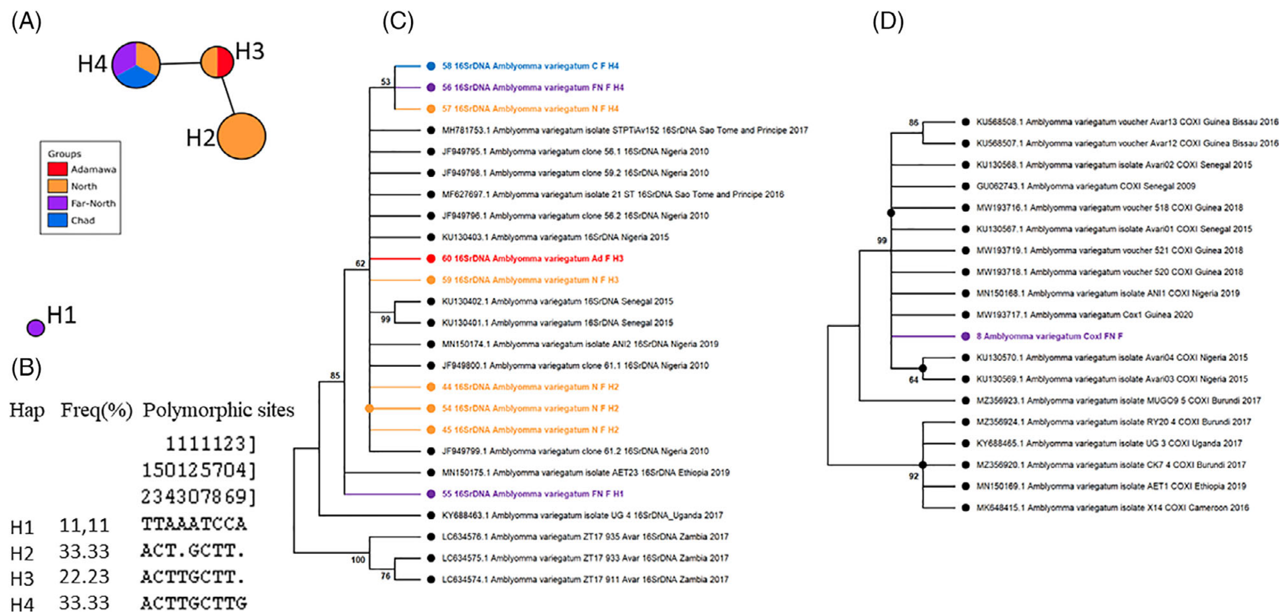

FIGURE 9 Genetic analysis of *16S rDNA* gene of *a. variegatum*. (a) and (b) represent the haplotype network and haplotype frequencies respectively. Haplotypes H1–H4 represent the different nucleotide sequence variants, each colour represents the locality where ticks were collected. The diameter of the circles is proportional to the number of individuals that belong to each haplotype. The nucleotide alignment shows the polymorphic sites and the frequencies of mutations per haplotype. These analysis were done using TCS v1.21 software. Panel (c) and panel (d) represent the phylogenetic tree of *16S rDNA* and *Cox-1* respectively built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 50% are shown. The sequences generated in the present study are highlighted in purple, blue, red, and orange. Hap: Haplotype, Freq: Frequencies, poly: Polymorphic

Although we did not have enough sequences for *Cox1* gene for the polymorphism analysis, the ML tree shows that the sequence generated from this study clustered with those from West African countries (Nigeria, Senegal, and Guinea) (Figure 9d).

Phylogenetic analyses of *H. truncatum* and *H. nitidum 16S rDNA* and *Cox1* genes

The ML tree of three *H. nitidum* based on *16S rDNA* analysis showed that the sequences obtained from this study clustered with those from East and West Africa (Figure S1A).

The ML tree of four sequences of *H. truncatum* based on the *Cox1* gene showed that the sequences formed two different clusters that are closed to those already described earlier in Cameroon (Figure S1B).

Phylogenetic analyses of *H. dromedarii 16S rDNA* and *Cox1* genes

The ML tree of one sequence of *H. dromedarii* based on *16S rDNA* analysis showed that the sequence obtained from this study clusters with those from North Africa (Tunisia, Egypt) (Figure S2A). For the *Cox1* gene, this sequence clusters with those from Asia (Saudi Arabia) and North Africa (Tunisia, Egypt) (Figure S2B). Some sequences of *H. rufipes* and *H. impeltatum* were obtained (Figure S3 and S4).

DISCUSSION

We surveyed hard ticks in the livestock markets of Yaoundé to gain more insight into the extent of tick and tick-borne disease burden in Cameroon. We found a high infestation rate (2639/9123; 28.93%) of all animal species. Therefore, we collected ticks on 49.56% (1308/2639) of the infested animals. Indeed, although we were able to screen more animals, we were not granted permission for tick collection on many occasions by the handlers and shepherds. Then we had to cope with extracting ticks by hand from often aggressive cattle, though this was not a problem with the small ruminants.

We have morphologically identified 6 species out of 11 found. Many factors can explain this challenge. First, most of the ticks found in this study were fully engorged (78.17%), which can create confounding factors when using identification keys. In addition, during the sampling of the ticks from the animals, the mouthparts were damaged on some occasions which therefore made the identification more problematic. These inconsistencies have already been described elsewhere (Nava et al., 2009). Finally, some species of ticks can only be classified as belonging to a complex including *R. (Boophilus) microplus* complex that currently consists of five taxa, namely *R. australis*, *R. annulatus*, *R. (B.) microplus* clade A, *R. microplus* clade B, and *R. (B.) microplus* clade C. Hence, the morphological classification obtained may not always reflect the molecular results (Nava et al., 2009). In this study where we have used both *Cox1* and *16S rDNA* as well as morphologic identification keys for the taxonomic assignation of ticks, the species was determined by an identical outcome with at least two of

the three tools used as shown elsewhere (Filipe et al., 2021; Paternina et al., 2016).

The infestation rates were higher in cattle (39.18%) compared to sheep (11.53%) and goats (2.74%). This difference can be explained by the mode of farming used for cattle and small ruminants. Indeed, in regions from where most of our animals arrive, cattle are reared as free-range on open land; they cover an impressive distance for pasture in the savanna and forest which therefore increases the likelihood of contact with questing ticks. However, small ruminants are usually reared around the houses and hence are less exposed to ticks. The larger skin surface of cattle could also explain the difference in tick infestation rate. A comparable situation has been observed elsewhere, between large ruminants including buffaloes and cattle, and small ruminants (Rehman et al., 2017).

The result of this study also confirmed that different tick species prefer to attach and feed on specific body parts of animals. *Hyalomma* ticks were mostly found on the anus (80%) and the tail (20%), *Amblyomma* on the internal part of the thigh (70%), the scrotum and the udder part of the females (30%), *Rhipicephalus* on the whole body (flank, ears, back, neck, shoulder, fore under) with the main site being the fore under part (60%). Indeed, ticks were mostly found on well-vascularised body parts where there are little or no hairs which facilitate the blood meal intake (Opara et al., 2005).

The 11 species of ticks found show a very diverse tick population in Cameroon as compared to other studies. Although all these tick species have previously been described in Cameroon mainly morphologically, they have never been found together in a single study (D. N. Awa et al., 2015). The case of *H. nitidum* that was first described in Cameroon in 1919 and since then is still circulating (Tomassone et al., 2005) combined with the high diversity in this tick genus could be a concern as the range of pathogens transmitted collectively by this group is very broad. These include tick-parasites and bacteria as previously shown (Ndip et al., 2004; Silatsa et al., 2020), and also viruses like Crimean-Congo Hemorrhagic fever (CCHFV) (González Gordon & Bessell, 2022; Sadeuh-Mba et al., 2018). In addition, the infestation by *A. variegatum* ticks has been implicated in the transmission of heartwater disease and the exacerbation of dermatophilosis in Cameroon and also leads to wounds and lesions of dermatophilosis (Awa., 1997; Stachurski et al., 1993).

The predominant tick species of small ruminants in this study was *R. sanguineus*, known as the most widespread tick in the world. *R. sangineus*, is a tick usually found in dogs. Indeed, dogs are recurrently found around shepherds as they are used to control and protect the farmed animals. Hence, dog-specific *R. sangineus* can have an opportunistic behaviour and feed on other animals, including humans especially when the dog population is oversized by other vertebrates (Chaligiannis et al., 2016). Interestingly, *R. sangineus* is a vector of many zoonotic pathogens, such as *Coxiella burnetii*, *Ehrlichia canis*, *Rickettsia conorii*, and *R. rickettsia* (Dantas-Torres, 2008). Hence, its opportunistic behaviour can favour the interspecies transmission of pathogens.

In general, ticks were highly abundant during the rainy season in August with mostly *Rhipicephalus* ticks. However, *Hyalomma* ticks were mostly found within the dry season in February (Fonteh & Nji, 2001; Lendzele et al., 2019). This observation is intrinsically associated with tick biology and environmental conditions. It has been demonstrated that *Hyalomma* spp. preferentially parasitize livestock in warm and semi-arid habitats, which resemble the conditions during the dry season in Cameroon, while *Rhipicephalus* spp. is associated with warm and humid conditions (Ayalew et al., 2014). This fluctuation in the relative abundance of hard ticks species throughout the year was also recorded in a previous study in South Africa (Nyangiwe et al., 2013). Thus, it is necessary to identify and monitor ticks throughout the year as it can have a direct implication on tick-borne disease periodic transmission as exemplified by Lumpy skin disease (LSD) of which outbreaks are common during the summer in endemic countries (Lubinga, 2013).

Rhipicephalus decoloratus and *R microplus*, which were early described in Cameroon (Silatsa et al., 2019) were the most abundant tick found in our study. This not only confirms the circulation of *R. microplus* in Cameroon but also shows the progressive establishment of this species locally. It was hypothesized that the cross-border animal market and transhumance could contribute to the introduction and spread of *R. microplus* from Nigeria to Cameroon and its dissemination throughout tropical and subtropical regions. *Rhipicephalus microplus* is considered the most important tick species infesting livestock in the world (Rodríguez-Vivas et al., 2014). Furthermore, these two species were found to be implicated in the transmission of viral, bacterial, and parasitic diseases in various regions of the world (Yawa et al., 2021). Indeed, it was demonstrated that *R. decoloratus* plays a key role in the transmission of LSD virus, a poxvirus in the genus *Capripoxvirus* that causes the LSD, an economically important and debilitating disease of cattle which is endemic to Africa (Lubinga, 2013).

Phylogenetic and polymorphism analysis of the collected ticks showed that there were two to three different clusters for the same species and two to six haplotypes showing the occurence of an intraspecies variation within *R. decoloratus*, *R. annulatus*, *R. sanguineus*, and *A. variegatum*. Indeed, this observation was greater with *Cox1* gene than with *16S rDNA*. Thus we observed a more diverse and variable tick population while analysing the *Cox1* than the *16S rDNA* as previously described in *R. microplus* showing that *16S rDNA* gene is more conserved. It has been shown elsewhere that *16S rDNA* gene seems to have better features for interspecific phylogenetic analyses while *Cox1* gene appears to be more useful for intraspecific genetic variability studies (Filipe et al., 2021; Paternina et al., 2016). This highlights the difficulty of using these two genes and the need for more genetic characterization using whole-genome sequencing (Burger et al., 2014; Low et al., 2015).

The *Cox1* gene has previously been shown to have a greater intraspecies resolution within the *R. microplus* complex compared to *12S*, *16S* genes, or internal transcribed spacer 2 (ITS2) regions (Burger et al., 2014; Low et al., 2015). Based on *Cox1* phylogenetics, five distinct generic clusters were shown to occur within the *R. microplus* species complex such as Clade A (ticks from Africa, Asia, and South America), Clade B (southern China and northern India), Clade C (ticks from Malaysia and India), *Rhipicephalus australis* and *R. annulatus*

(Burger et al., 2014; Low et al., 2015). *Rhipicephalus australis* and *R. annulatus* are closely related to ticks in Clade B. The Cameroonian *R. microplus* sequences analysed in this study clustered with the African *R. microplus* reference sequences into Clade A (Burger et al., 2014) with *16S rDNA* and *Cox1* genes. This result confirms the circulation of *R. microplus* Clade A in Cameroon as observed in ticks collected in 2016 (Silatsa et al., 2019).

Hyalomma tick sequences were mostly closed to those already described in Cameroon. Although, *H. dromedarii* was closely related to countries where its preferential hosts (camels) are prevalent such as Egypt, Tunisia, and Saudi Arabia, it has also been demonstrated that this tick species can also infest other ruminants including sheep, goats, cattle, and horses (Walker et al., 2003). *Hyalomma* is a vector of many viral (CCHF virus, Kadam virus, Dera–Ghazi–Khan Virus, and Dhori virus), bacterial (e.g., *Coxiella burnetti*), and protozoan pathogens (e.g., *Theleria Annulata*, *T. Camelensis*) (Walker et al., 2003).

The results concerning the *R. sanguineus* complex suggested the presence of two lineages, *R. sanguineus* "temperate lineage" and *R. sanguineus* s.l. "tropical lineage" (*R. linnaei*). Indeed, 14 species within the *R. sanguineus* complex have been already recognized although, the morphological differentiation of these species is difficult (Bakkes et al., 2020). The recognition of multiple species within the *R. sanguineus* species complex by the use of molecular markers (in particular mitochondrial DNA) has raised a significant taxonomic issue because at least four potential species have been previously classified as "lineage" (*R. sanguineus* s.l. "temperate lineage", "Southeastern Europe lineage", "tropical lineage" and "Afrotropical lineage" (Šlapeta et al., 2021). Three of these "lineages" have now been formally recognized as species with the "temperate lineage" as *R. sanguineus* s.s., the "Afrotropical lineage" as *R. afranicus*, and the "tropical lineage" as *R. linnaei*. The classification of the remaining "lineages" is yet to be resolved (Šlapeta et al., 2021). However, morphological differentiation of *R. linnaei* from *R. sanguineus* s.l. remains complex in the absence of robust morphological distinctive traits (Nava et al., 2009). Therefore, molecular data is still the only tool that provides a clear identification of the specimens of the *R. sanguineus*, Latreille, (1806) complex.

The negative values of the neutrality tests (Tajima's D and Fu & Li's D) obtained in some tick species (*R. decoloratus*, and *A. variegatum*) suggest a recent population expansion or decrease in genetic variation due to positive selection. Furthermore, the positive values obtained in other species (*R. microplus* and *R. sanguineus*) suggest a balanced selection that needs to be confirmed. Therefore, further investigations are required to obtain a definite conclusion regarding the population history of these species of ticks.

Globally, the mean number of ticks per cattle was not obtained because as shepherds were selling their animals, we could not keep these animals for a long time. Furthermore, as we were using a questionnaire to determine the origin of animals, we noted that some animals from different regions were transported together which constitutes a limitation of our study. However, there are three main life cycles for the development of hard ticks from larvae to adults: one-host tick, two-host ticks, and three-host ticks. The development of a tick from larvae to adult can take from 1 to 3 years in natural conditions and up to approximately 88 to 134 days in the laboratory (Apanaskevich et al., 2013). It has been shown that it is on rare occasions that a tick may move from one host to another if it is not to moult from one stage to another (Apanaskevich et al., 2013; Baron et al., 2018). However, in the present study, the main genus of ticks collected, *Rhipicephalus* is known as one host tick (*R. microplus*, *R. decoloratus*, *R. annulatus*) (Apanaskevich et al., 2013; Baron et al., 2018), while *Hyalomma* (*H. rufipes*, *H. dromedarii*, *H. truncatum* and *H. impeltatum*) and *A. variegatum* are two or three-host ticks. Hence, although, ticks may have moved from one animal to another during the transportation to the market, it is highly probable that the animals have travelled with ticks from their place of origin. Nevertheless, more investigations need to be done on the cattle and small ruminant breeding sites to have the real situation in these regions. In addition, the amplification of the tick *Cox1* gene was problematic for some species such as *A. variegatum* from which only one sequence was obtained with our primer set (Lv. Lv et al., 2014). These primers could be species-specific and suggest that future research should focus on designing species-specific primers for amplification and sequencing of each species (Baron et al., 2018) or multiplex protocol that will directly detect tick species based on the amplicon sizes.

CONCLUSION

Our results provide contemporary data on the range of hard ticks on livestock in Cameroon. The study revealed the highest tick infestation rates in cattle (39%), far higher than in sheep (11.53%) or goats 2.74%) with a high diversity of tick species. The most common tick species found *was R. decoloratus* followed by the most common tick pest globally, *R. microplus*. *Hyalomma* spp., the main vectors of CCHF were predominant during the dry season which means that the efforts to control this virus may be focused on this specific period. We observed high genetic diversity of ticks from Cameroon, particularly from ticks collected in the Adamawa and North regions where most haplotypes were detected. The results of this study indicate that there are probably higher tick infestation rates in the livestock production regions of Cameroon. Thus, further investigations are warranted to map tick distribution in different locations of the main cattle production sites of the country. Continuous nationwide surveillance of tick-borne diseases would enable early detection and response to any disease outbreaks.

AUTHOR CONTRIBUTIONS

Conception and design: Huguette Simo Tchetgna and Charles S. Wondji. Data collection on the field: Huguette Simo Tchetgna, Francine Sado Yousseu and Doumani Djonabaye.Laboratory experiments: Huguette Simo Tchetgna and Francine Sado Yousseu. Data analysis: Huguette Simo Tchetgna and Francine Sado Yousseu.

Manuscript writing: Francine Sado Yousseu and Huguette Simo Tchetgna. Manuscript review: Huguette Simo Tchetgna, Basile Kamgang, Roland Ndip Ndip, Philip J. McCall and Charles S. Wondji. All authors read and approved the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in GenBank under the accession numbers OK576059 to OK576100 for *Cox-1* and OK353695 to OK353745 for *16S rDNA*.

ETHICS DECLARATION

The study protocol was implemented with approval from the regional delegation of the Ministry of Livestock, Fisheries, and Animal Industries (MINEPIA), Centre. Consent for tick sampling on livestock was obtained from owners.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Figure S1. Phylogenetic trees of *16S rDNA* of *H. truncatum* (A) and *H. nitidum* (B). These phylogenetic trees were built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 50% are shown. The sequences generated in the present study are highlighted in red.

Figure S2. Phylogenetic trees of *16S rDNA* (A) and *Cox1* (B) genes of *H. dromedarii*. These phylogenetic trees were built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 50% are shown. The sequences generated in the present study are highlighted in purple.

Figure S3. Phylogenetic trees of *16S rDNA* (A) and *Cox1* (B) genes of *H. rufipes*. These phylogenetic trees were built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap

replicates. Only bootstrap values over 40% are shown. The sequences generated in the present study are highlighted in red, orange, and purple.

Figure S4. Phylogenetic trees of *16S rDNA* of *H. impeltatum* (A) and *H. detritum* (B) genes. These phylogenetic trees were built using the ML under the Tamura-3-parameter (T92) substitution model and 1000 bootstrap replicates. Only bootstrap values over 40% are shown. The sequences generated in the present study are highlighted in red.

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