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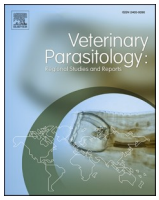

Original Article

Analysis of the tick communities associated to domestic mammals in rural areas of the Yungas montane forest from Argentina

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ABSTRACT

The aim of this work was to describe the tick community associated to domestic mammals in rural areas from the Yungas lower montane forest of Argentina. The circulation of tick-borne pathogens was also analyzed. Samples of ticks parasitizing cattle, horses, sheep and dogs were carried out in different seasons, and questing ticks were collected from vegetation and analyzed to detect the presence of *Rickettsia, Ehrlichia, Borrelia* and *Babesia* by a battery of different PCRs. The structure of the tick communities was analyzed through the Chao1 species richness estimator, the Shannon–Wiener index and the Horn index of community similarity. Eight tick species were collected in the study area: *Amblyomma sculptum, Rhipicephalus microplus, Amblyomma hadanii, Dermacentor nitens, Amblyomma ovale, Haemaphysalis juxtakochi, Ixodes pararicinus* and *Rhipicephalus sanguineus* sensu stricto. However, *A. sculptum* was by far the dominant species in the tick assemblages analyzed, and this was reflected in the low diversity values obtained. *Dermacentor nitens, A. sculptum* and *R. microplus* were the three species associated to horses. The predominance of *A. sculptum* was also observed in the tick samples obtained from dogs, even on two tick species, namely *A. ovale* and *R. sanguineus* s.s., which have dogs as the principal domestic host. *Rhipicephalus microplus* and *A. sculptum* were the most abundant ticks on cattle, while few specimens of *I. pararicinus, A. hadanii* and *D. nitens* were found on bovines. *Dermacentor nitens* ticks were found to be infected with *B. caballi,* which indicate the circulation of this pathogen of horses in the Yungas area. The detection of a strain of *Borrelia* sp. belonging to the *B. burgdorferi* s.l. complex in *I. pararicinus* is consistent with previous findings made in Argentina, but the public health relevance of this vector-microorganism association is far from being similar to that occurs in the northern hemisphere because there are practically no records of these tick species parasitizing humans in South America. The tick community of rural areas of the Yungas lower montane forest is composed by species which are potential vectors of pathogenic microorganism with veterinary and public health importance, circulating in a human-wildlife-livestock interface.

1. Introduction

Hard ticks (Acari, Ixodida: Ixodidae) are blood-feeding ectoparasites of amphibian, reptile, avian and mammal hosts, including humans. Considering the relevance on animal and public health issues, ticks are one of the most important group of ectoparasites of the world because of their role as vectors of pathogenic microorganism and also due to the deleterious physical effects that tick parasitism provokes in hosts (e.g. reduced weight gain and milk production, hidden damage, increased mortality and morbidity, secondary myiasis) (Späth et al., 1994;

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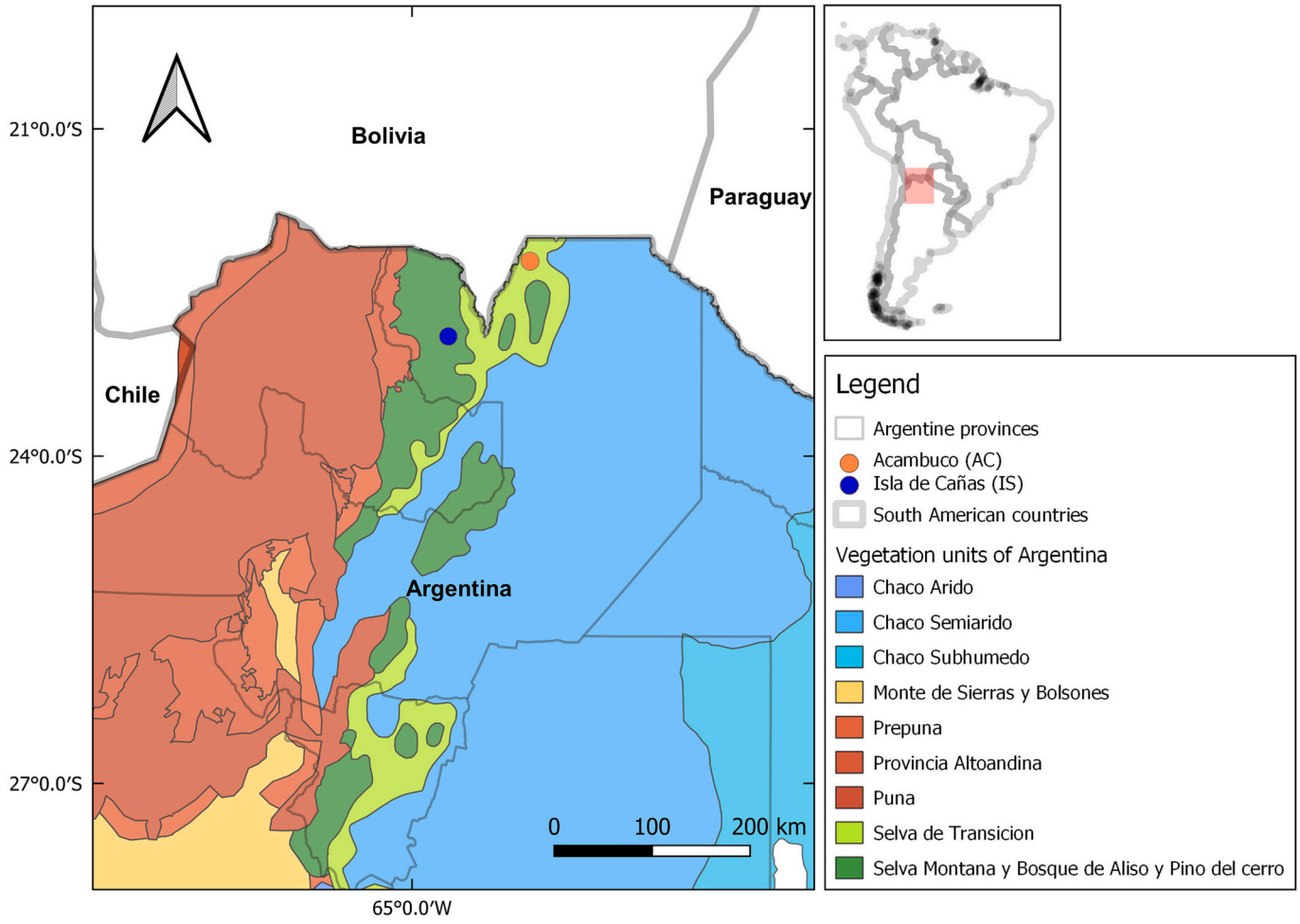

Fig. 1. Study area in Isla de Cañas and Acambuco localities in northwestern of Salta Province, Argentina, in relation to the combination of the eco-regions of Argentina as defined in Oyarzabal et al., 2018.

Jongejan and Uilenberg, 2004; Sonenshine et al., 2002; Reck et al., 2014; Guglielmone and Robbins, 2018). Different species of hard ticks are common parasites of domestic mammals in South America, and some of them have a sympatric and parapatric distribution (Nava et al., 2017; Guglielmone et al., 2021). In this sense, understanding dynamics and structure of parasite communities on host animals in a rapidly changing global environment is instrumental to control and manage parasite infestations (Kim, 2006).

The subtropical mountain forest belonging to the Yungas Biogeographic Province (Yungas forest) extend from northern Peru to northwestern Argentina and is located on the western slopes of the Andes, at an altitude ranging from 300 to 3500 m.a.s.l. (Morrone, 2006). In northwestern Argentina, the Yungas forest extends along discontinuous mountain ranges across the provinces of Jujuy, Salta, Tucumán and Catamarca, covering an area of four million ha (Brown et al., 2001), where anthropogenic activities such as extensive cattle-grazing, agriculture and tourism are developed. A total of 13 species of hard ticks of the genera *Amblyomma, Dermacentor, Haemaphysalis, Ixodes* and *Rhipicephalus* were found in the Yungas forest of Argentina (Nava et al., 2017; Saracho-Bottero et al., 2018; Saracho Bottero et al., 2021). Saracho-Bottero et al. (2018) studied the tick species that infest cattle and humans throughout an altitudinal gradient in the Yungas forest of Argentina, but there are no further studies characterizing the ticks and tick-borne microorganisms with veterinary and public health importance as a part of an analysis of the community structure for this ecological area. In view of the above mentioned, the objective of this work was to study the tick community composed by species that infest domestic mammals (i.e. cattle, horses, sheep and dogs) in rural areas from a north-south corridor of the Yungas lower montane forest of Argentina. The natural infection by tick-borne microorganisms in ticks was also analyzed in order to identify the risk of pathogen transmission and to increase and deepen the knowledge about the epidemiology of the diseases that these pathogens produce, which can be instrumental to the subsequent planning preventive measures.

2. Materials and methods

The study was performed in two sites belonging to the Yungas forest from northwestern Argentina, in the north of Salta Province. One site was located in “Isla de Cañas” (IC) (Iruya department, 22°54′11.44″S, 64°39′57.22″; 803 m.a.s.l), and the second sampling site was located at the “Acambuco” (AC) (General José de San Martín department, 22°12′48.20″S, 63°55′0.42″W; 798 m.a.s.l). Both sites occur in the “Provincia Fitogeográfica de las Yungas” (PFY) according to the definition given by Oyarzabal et al. (2018). Within PFY, IC corresponds to the vegetation unit named as “Selva montana y Bosque de Aliso ”, and AC corresponds to the vegetation unit named as “Selva de transiciòn o pluvial semicaducifolia pedemontana” (Oyarzabal et al., 2018) (Fig. 1). IC represents the middle floor (between 700 and 1500 m. a.s.l.) of the Yungas, whereas AC belong to the lower level of the Yungas (between 400 and 700 m. a.s.l), in ecotonal areas with the “Chaco semiárido” of the “Provincia Fitogeografica Chaqueña” sensu Oyarzabal et al. (2018). The lower level is the stratum of the Yungas that has historically been most modified by anthropogenic activities (Oyarzabal et al., 2018), and

Table 1
Ticks collected from vegetation in Isla de Cañas and Acambuco. LL: larvae; NN: nymphs, FF: females; MM: males.

		Amblyomma hadanii				*Amblyomma ovale*				*Amblyomma sculptum*				*Haemaphysalis juxtakochi*				*Ixodes paranicinus*				*Rhipicephalus microplus*			
Locality	Season	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM
Isla de Cañas	Spring		85	1						11,249	1722	16	78		1							24			
	Summer		30	4	2			1		434	600	152	200								2				
	Autumn		1		1					18,309	3		9			1									
	Winter									11,686					2					1					
Acambuco	Spring									97	42														
	Summer										92	40	53		2						1				
	Autumn									1579				1	1		1								

this fact applies to AC.

Three samplings were carried out in the two localities: September 2019 (spring), December 2019 (summer), and April 2021 (autumn). A winter sample (July 2019) was also taken in IC. Ticks were manually collected on cattle, dogs, horses and sheep. In AC, 22 bovines, nine dogs, 10 horses and seven sheep were examined, while in IC 46 bovines and 14 dogs were examined for ticks. Additionally, free-living ticks were collected from vegetation by flagging along different transects in each locality. All ticks collected were stored in 96% ethanol until posterior taxonomic determination and for testing microorganism infection in the laboratory. In some cases, engorged larvae and nymphs collected on host were allowed to molt to the subsequent stage under laboratory conditions to improve the accuracy of morphological determination. Ticks (adults and nymphs) were determined following Nava et al. (2017) and Saracho-Bottero et al. (2020), while larvae were determined by comparison with known laboratory-reared material deposited in the Tick Collection of Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Argentina. In the case of the ticks belonging to the *Rhipicephalus sanguineus* group, the taxonomic determination was made following the descriptions and criteria of Nava et al. (2018), by complementing the morphological comparison with the analysis of sequences of the mitochondrial 16S rRNA gene according to the protocol of Mangold et al. (1998).

Chao1 species richness estimator as defined in Chao and Chiu (2016) for each tick community was calculated with the SpadeR software (Chao et al., 2015) by using the following formula:

$$Chao1 = S + \left(\frac{n-1}{n}\right)\left(\frac{f1(f1-1)}{2(f2+1)}\right)$$

where, S is the total number of species observed, n is the total number of tick specimens collected, $f1$ is the number of singletons (number of species of which only one specimen was collected) and $f2$ is the number of doubletons (number of species of which only two specimens were collected).

The Shannon–Wiener index was also obtained with the formula:

$H_e' = -\sum p_{ie} * \ln p_{ie}$, where H_e' is the Shannon entropy index of each tick community e and p_{ie} is the relative abundance of the species i in the community e. This index is a measure of the amount of uncertainty to predict to which species an individual chosen at random from a sample of S species and n individuals will belong, i.e. the larger the value of H_e', the more equitable the species abundances distribution will be (Krebs, 1999). Finally, The Spade R software was used to calculate community similarity measures with the Horn index as defined in Krebs (1999). Comparisons between data from each community were performed (considering absolute abundances) by selecting q = 1 to incorporate species abundances in the analysis, without focusing on dominant species, with a bootstrap of 1000 replications.

Unfed ticks collected from vegetation were molecularly analyzed to detect the infection with *Rickettsia, Ehrlichia* (Rickettsiales: Rickettsiaceae and Anaplasmataceae), *Borrelia* (Spirochaetales: Spirochaetaceae) and *Babesia* (Piroplasmida: Babesiidae). Specimens of *Dermacentor nitens* were the exception, because they were only collected on hosts (see results). Ticks genomic DNA was extracted by using a phenol/chloroform assay described by Mangold et al. (1998). All DNA samples were processed individually by real-time PCR (rt-PCRs) assay amplifying a 177 bp fragment of the 16S rRNA gene from bacteria of the Anaplasmataceae family (Monje et al., 2019) and 147 bp fragment of the citrate synthase gene (*gltA*) from Rickettsiaceae family following the protocols and primers described by Monje et al. (2019) and Labruna et al. (2004) and Guedes et al. (2005), respectively. The rt-PCR-positive samples were further tested by a battery of conventional PCRs to amplify a 345 bp fragment of the 16S rRNA gene, a 409 bp fragment of the specific *dsb* gene of bacteria of the genus *Ehrlichia* and a 830 and 532 bp fragments of two rickettsial genes citrate synthase gene (*glt*A) and 190-kDa outer membrane protein (*ompA*) following the protocols and

Table 2
Ticks collected on domestic mammals in Isla de Cañas. LL: larvae; NN: nymphs, FF: females; MM: males.

		Amblyomma sculptum				*Rhipicephalus microplus*				*Amblyomma ovale*				*Rhipicephalus sanguineus*				*Ixodes paranicinus*			
Season	Host	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM
Spring	Dog	5	45	2	1																
	Cattle	3	100	27	36	3		7													
Summer	Dog		5	9	19						1	2	6		1	1	2				
	Cattle		8	115	146		13	45	35												
Autumn	Dog	3					0					1									
	Cattle	51	3	11	47		7	99	21											1	
Winter	Cattle		2				5	7	14												

Table 3
Ticks collected on domestic mammals in Acambuco. LL: larvae; NN: nymphs, FF: females; MM: males.

		Amblyomma sculptum				*Rhipicephalus microplus*				*Amblyomma ovale*				*Dermacentor nitens*				*Amblyomma hadanii*			
Season	Host	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM	LL	NN	FF	MM
Spring	Dogs		1																		
	Cattle	1	21	2	3		25	118	66									1			
	Horse		2	2	6																
	Sheep		1																		
Summer	Dogs			2	5								1								
	Cattle		2	5	40		58	32	26						2						
	Horse				6											2	3				
	Sheep		1	8	3																
Autumn	Dogs		1	1	2							1									
	Cattle							1	1												
	Horse							5	1						8	23	75				
	Sheep																				

primers described by Parola et al. (2000), Doyle et al. (2005) and Labruna et al. (2004), respectively. In addition, tick DNAs were screened by PCR for detection of *Borrelia* DNA following the methods detailed in Barbieri et al. (2013). Nested PCR was performed targeting the flagellin gene (*fla*) of *Borrelia* spp. with the primers presented in Barbour et al. (1996). Finally, the DNA samples were tested for the presence of *Babesia* by applying a PCR assay targeting a fragment of the 18S rDNA gene following the protocol and primers described by Soares et al. (2011). As the epidemiology of *Babesia bovis, Babesia bigemina* and *Anaplasma marginale* has already been extensively studied in northern Argentina, these pathogens were not included in the current study.

The sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) and aligned with the program Clustal W (Larkin et al., 2007). Phylogenetic analyses were performed by the Maximum-likelihood (ML) method with gaps considered to be missing characters. The best-fitting substitution model was selected with the Akaike Information Criterion (AIC) using the ML model test implemented in MEGA 5 (Tamura et al., 2011). Support for the topologies was tested by bootstrapping over 1000 replicates.

3. Results

Eight tick species were collected in the study area: *Amblyomma sculptum* (n: 47114[1]; 98.2% of the total ticks), *Rhipicephalus microplus* (n: 613; 1.3% of the total ticks), *Amblyomma hadanii* (n: 125; 0.26% of the total ticks), *D. nitens* (n: 113; 0.23% of the total ticks), *Amblyomma ovale* (n: 13; 0.03% of the total ticks), *Haemaphysalis juxtakochi* (n: 9; 0.02% of the total ticks), *Ixodes paranicinus* (n: 5; 0.01% of the total ticks), and *Rhipicephalus sanguineus* sensu stricto (n: 4; 0.01% of the total ticks). The 16S sequences obtained from the four specimens determined a priori as belonging to the *R. sanguineus* group were identical among each other (Genbank accession numbers: OQ408536) and they matched with the sequences (99% of similarity) of *R. sanguineus* s.s. from France (Genbank accession numbers: MH630342- MH6303444). Seven of these eight tick species were collected in Isla de Cañas (IC from now on): *A. sculptum* (n: 45096[2]; 99% of the total ticks), *R. microplus* (n: 280; 0.62% of the total ticks), *A. hadanii* (n: 124; 0.27% of the total ticks), *A. ovale* (n: 11; 0.02% of the total ticks), *R. sanguineus* (n: 4; 0.01% of the total ticks), *I. paranicinus* (n: 4; 0.01% of the total ticks) and *H. juxtakochi* (n: 4; 0.01% of the total ticks). Seven tick species were also collected in Acambuco (AC from now on): *A. sculptum* (n: 2018; 84.67% of the total ticks), *R. microplus* (n: 333; 11.19% of the total ticks), *D. nitens* (n: 113; 3.8% of the total ticks), *H. juxtakochi* (n: 5; 0.2% of the total ticks), *A. ovale* (n: 2; 0.07% of the total ticks), *A. hadanii* (n: 1; 0.03% of the total ticks) and *I. paranicinus* (n: 1; 0.03% of the total ticks). Ticks collected on vegetation were *A. sculptum* (all stages), *A. hadanni* (adults and nymphs), *A. ovale* (adults), *H. juxtakochi* (nymphs and adults), *I. paranicinus* (adults) and *R. microplus* (larvae). All parasitic stages of *A. sculptum* and *R. microplus,* adults of *I. paranicinus,* larvae of *A. hadanii* and nymphs of *D. nitens* were collected on cattle. Tick species collected on dogs were *A. sculptum* (all stages), *A. ovale* (nymphs and adults) and *R. sanguineus* (nymphs and adults). Adults and nymphs of *A. sculptum,* adults of *R. microplus* and nymphs and adults of *D. nitens* were collected parasitizing horses in AC, where nymphs and adults of *A. sculptum* were also found on sheep. Details on tick species, abundance of each parasitic stage and season of ticks collected from vegetation in IC and AC are showed in Table 1, while data from ticks collected on hosts in IC and AC are presented in Tables 2 and 3, respectively. Proportions of the number of each tick species collected on cattle, dogs and horses are depicted in Fig. 2.

The overall values of species richness of the tick assemblies estimated with Chao1 was 7 in both localities, while this index taking into account only species collected from vegetation was 6 in IC and 3 in AC. Shannon–Wiener indices (H`) were separately obtained for the tick assemblages of ticks collected from vegetation in each locality, and calculated including and excluding *A. sculptum,* which is the dominant tick species

[1] This value is notably incremented by the collection of larvae, which are prone to be collected by flagging from vegetation

[2] Idem footnote 1

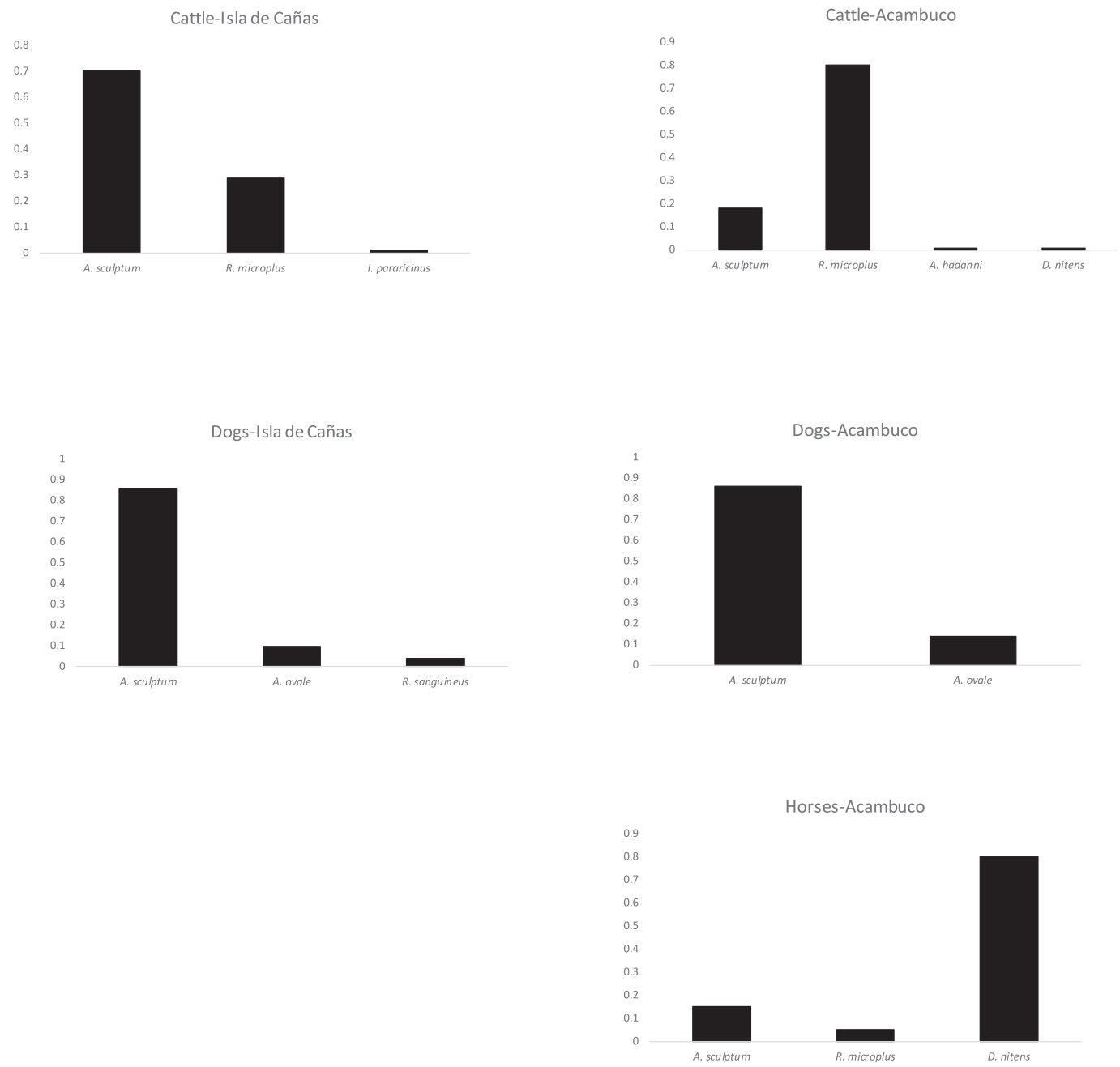

Fig. 2. Proportions of the number of each tick species collected in Isla de Cañas and Acambuco.

in both localities (see Table 2). The values of H` were as follow: H'_{IC} with *A. sculptum* = 0.032; H'_{IC} without *A. sculptum* = 0.607; H'_{AC} with *A. sculptum* = 0.021; H'_{AC} without *A. sculptum* = 0.410. The values (mean ± SE) of the Horn similarity index between IC and AC was 0.3424 ± 0.0006 (95% CI = 0.3411–0.3436).

One-hundred and three specimens of *D. nitens* were processed for tick-borne pathogens detection in 25 pools of three, four or five ticks each. Two pools of 5 males from AC were positive to *Babesia caballi.* The two 18S sequences of these positive samples (GenBank accession numbers OQ418456 and OQ418457) were identical. They form a strongly supported monophyletic clade (93% bootstrap support) with other sequences of *B. caballi* form different continents (Fig. 3). The 18S sequences of *B. caballi* from Argentina are closely related to 18S sequences *B. caballi* reported from Spain, USA, China and Saint Kitts and Nevis, which form a different clade (91% bootstrap support) from the South African strains of *B. caballii* (Fig. 3).

All specimens of *I. pararicinus* collected in this work were processed for tick-borne pathogens detection, and one male from Acambuco was positive for *Borrelia* (haplotype NC67; GenBank accession number: OQ418084). The ML phylogenetic tree generated with *fla*B sequences shows that the *Borrelia* haplotype found in this study belong to the *Borrelia burgdorferi* sensu lato complex (Fig. 4), and it clustered (83% bootstrap support) with a group formed by haplotypes A, B and C of *Ixodes fuscipes* from Uruguay (GenBank accession numbers: JX082311, JX082312 and JX082313), haplotype Pampa of *Ixodes longiscutatus* from Brazil (GenBank accession number: KY657353), haplotypes FSF1 and FSF8 of *Ixodes* sp. cf. *I. affinis* from Argentina (GenBank accession numbers: MT596703, MT596705 and MT596706) and also with a *Borrelia* haplotype (MP30) previously detected in *I. pararicinus* from northwestern Argentina (GenBank accession number: KY595468).

All samples of *A. sculptum* (n: 105), *A. hadanii* (n: 56), *A. ovale* (n: 1), *H. juxtakochi* (n: 8) and *R. sanguineus* s.l. (n: 4) processed for *Rickettsia*

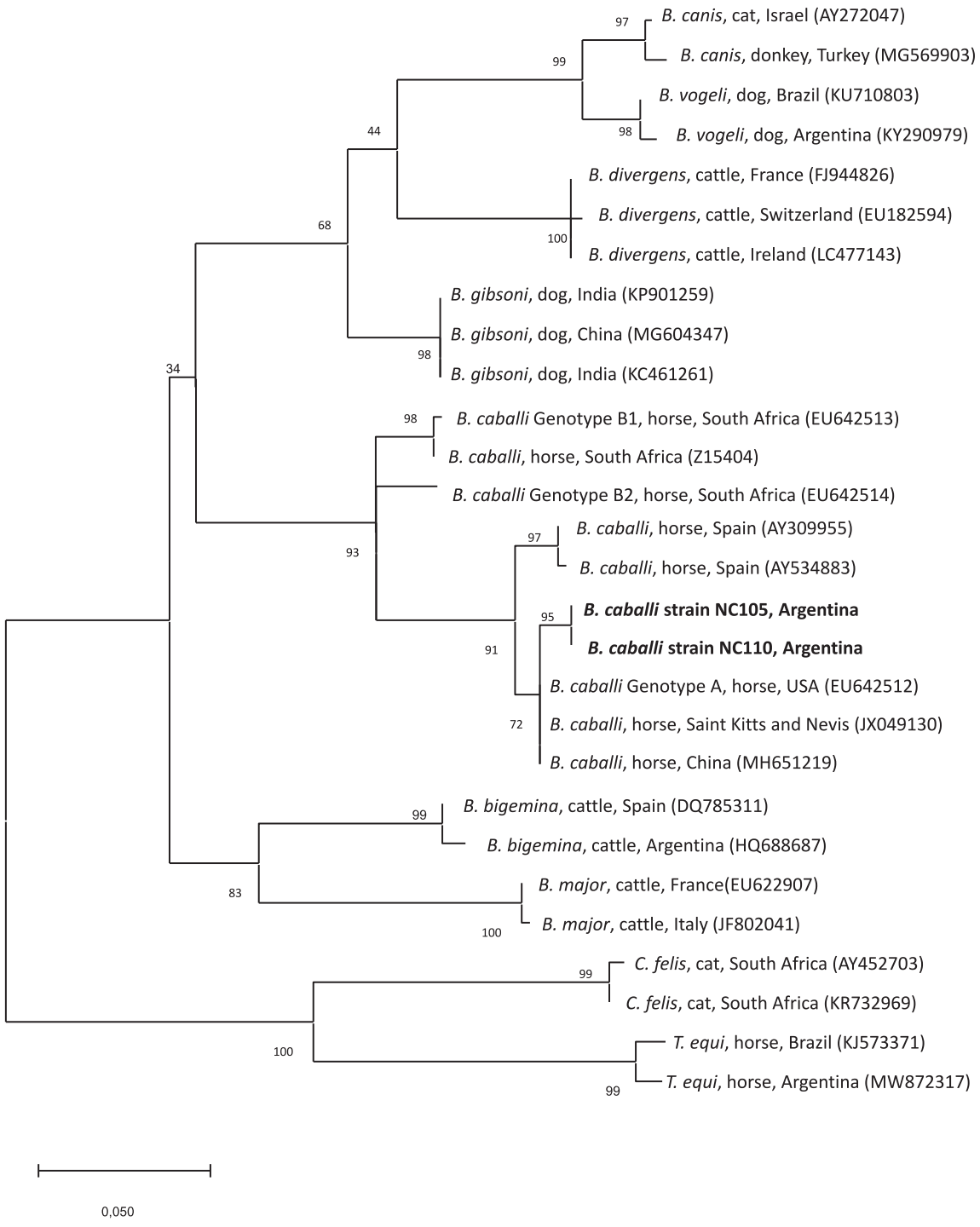

Fig. 3. Maximum-likelihood tree constructed from 18S rDNA sequences for different *Babesia* species. Numbers represents bootstrap support generated from 1000 replications. GenBank accession numbers are given in brackets. Sequences generated in this study are in bold. The substitution model was GTR (G + I).

spp. and *Ehrlichia* spp. detection were negative.

4. Discussion

Although seven/eight species belonging to five different genera were found in the two representative localities of the study area, the diversity of AC is lower than in IC, which could be related no only to the micro-climatic and landscape differences between both sites, but also to the greater anthropogenic disturbance of AC. *Amblyomma sculptum* is by far the dominant species in both tick assemblages. This phenomenon was observed both in the samples of free-living ticks from vegetation and from those collected on hosts. The dominance of *A. sculptum* beforementioned is reflected in the low values of Shannon-Wiener index in both localities. In fact, when the Shannon-Wiener index was calculated excluding *A. sculptum,* the values of this index increased notably in the two sites analyzed, regardless the difference among them in terms of specific richness and diversity. The large number of *A. sculptum* larvae found questing on vegetation is a remarkable fact that contribute to this quantitative difference with respect to the remaining tick species collected in the study area, as mentioned in footnotes 1 and 2. *Amblyomma sculptum* is a member of the *Amblyomma cajennense* group (Nava et al., 2014b) which has a one-year life cycle where larvae are

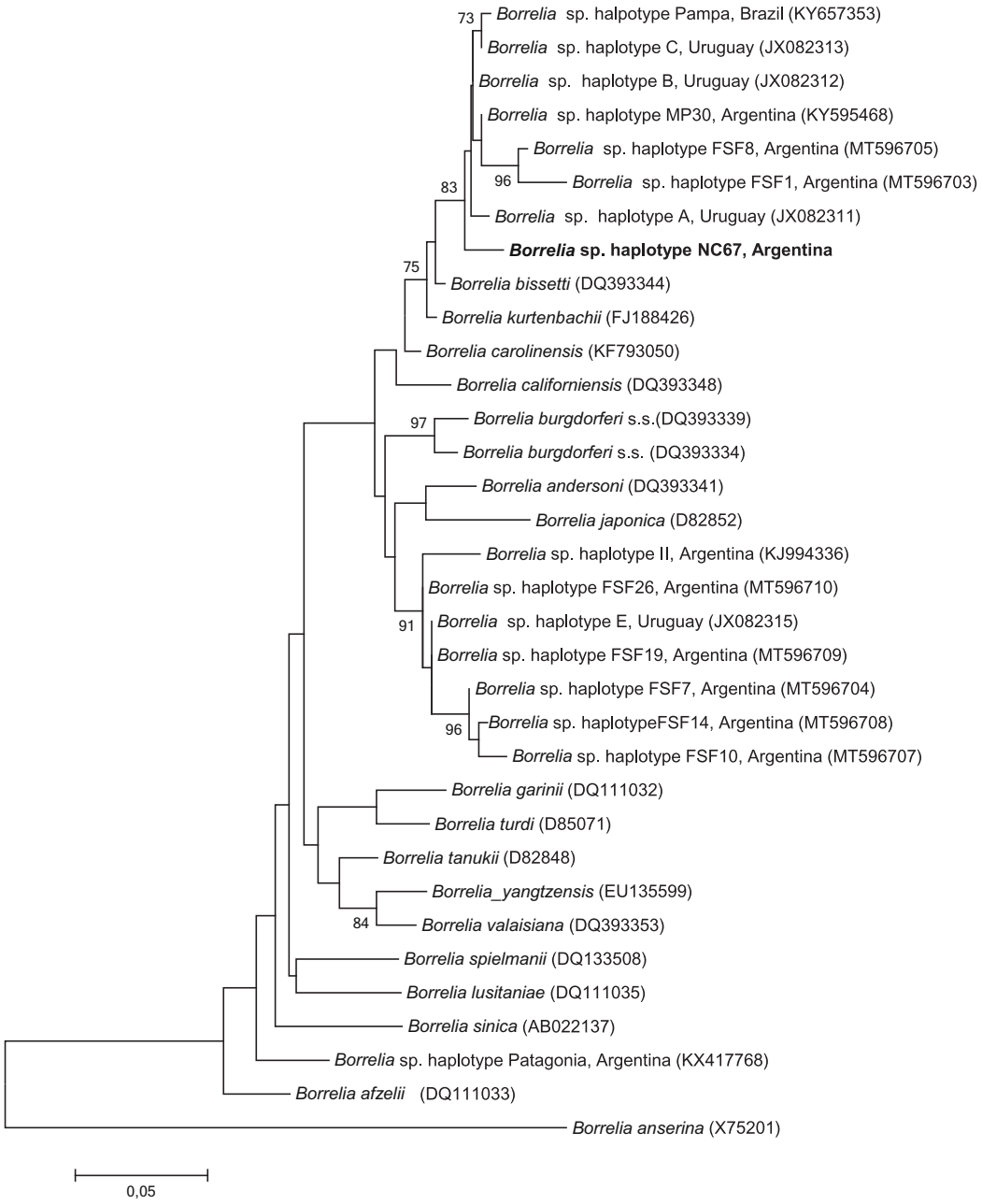

Fig. 4. Maximum-likelihood tree constructed from *fla* partial sequences of *Borrelia* spp. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are given in brackets. Sequences generated in this study are in bold. The substitution model was GTR (G + I).

active from late summer to late winter, nymphs from early winter to early summer, and adults are active throughout all year but with the peak of abundance in early and mid-summer (Tarragona et al., 2018). Therefore *A. sculptum* ticks can be found throughout the entire year. The finding of a large number of larvae, nymphs and adults of *A. sculptum* questing on vegetation in all seasons is consequence of its phenology, and explains the ubiquitous presence of numerous immature and adult specimens of this tick on domestic mammals, but also highlight its public health importance since this remarkable abundance of free-living specimens increases the risk of tick-bites in humans. In fact, *A. sculptum* was the principal tick recorded on cattle and biting humans in a previous work performed in the Yungas from Argentina (Saracho-Bottero et al., 2018). *Amblyomma sculptum* is also one of the most recorded tick species parasitizing humans in South America (Nava et al., 2017; Guglielmone and Robbins, 2018; Nogueira et al., 2022), where it is the principal vector of *Rickettsia rickettsii,* the etiologic agent of a lethal spotted fever in humans (Labruna et al., 2014, 2017). The evidence obtained in this work, added to those presented in Saracho-Bottero et al. (2018), clearly indicate that *A. sculptum* is the most relevant tick species as parasite of domestic mammals and humans in rural areas of the Yungas lower montane forest from northern Argentina.

Dermacentor nitens, A. sculptum and *R. microplus* were the three species associated to horses. This result is not unexpected since records of these tick species parasitizing horses are usual, and *D. nitens* has horses as its principal host (Nava et al., 2017; Guglielmone et al., 2021). The associations formed between these three ticks with horses are relevant from a veterinary perspective because of the direct effect of tick infestation but also of the role of ticks as vectors of pathogenic microorganisms. *Dermacentor nitens* is a proven biological vector of *Babesia caballi*, the etiologic agents of equine babesiosis (Friedhoff and Soulé, 1996; Scoles and Ueti, 2015). Clinical cases of equine babesiosis were diagnosed in Argentina, including cases in Salta Province (Ibáñez et al.,

1974; Aguirre et al., 2004). A sample of *D. nitens* males collected on horses during this work was found to be infected with *B. caballi,* which indicate the circulation of this pathogen of horses in the Yungas area. The *B. caballi* strain detected is genetically similar to *B. caballi* strains that circulate in other regions of the world (i.e. USA, Spain, China, Caribbean) (Fig. 3). Bhoora et al. (2009) showed that sequences of 18S rRNA gene of *B. caballi* split in two clades, one formed by strains from USA and Spain (group A sensu Bhoora et al., 2009), and another one containing strains from Africa (group B sensu Bhoora et al., 2009). Sequences from Argentina, China and the Caribbean belong to the group A sensu Bhoora et al. (2009). The impact of this sequence heterogeneity for epidemiology and diagnostic of equine babesiosis by *B. caballi* is unknown. Additionally to the role of *D. nitens* in the epidemiology of equine piroplasmosis, *R. microplus* is a competent vector of *Theileria equi*, and ticks from the *A. cajennense* complex were involved as vectors of *T. equi* and Venezuelan equine encephalomyelitis virus (Linthicum et al., 1991, 1992; Kerber et al., 2009; Scoles and Ueti, 2013, 2015; Scoles et al., 2015). All these lines of evidence analyzed as a whole highlight the epidemiological relevance of the tick-horse associations recorded for the Yungas from Argentina.

The predominance of *A. sculptum* was also observed in the tick samples obtained from dogs, even on two tick species, namely *A. ovale* and *R. sanguineus* s.s., which have dogs as the principal domestic host (Guglielmone et al., 2003; Nava et al., 2017). The low representation of *R. sanguineus* s.s. in the samples is due to the fact that this species is a frequent parasite of dogs in urban areas but not in rural or wild areas. Regarding cattle, the dominance of *R. microplus* and *A. sculptum* is largely predictable based on the known affinity of these species for cattle (Nava et al., 2017; Guglielmone et al., 2021). *Ixodes pararicinus, A. hadanii* and *D. nitens* were poorly represented in cattle samples, with only a few specimens of each species collected. Although infestations with *I. pararicinus* in cattle are usual in the Yungas of northwestern Argentina, this tick is commonly found in the Upper Montane Forest of the Yungas, above 1500 m.a.s.l., and not in the lower montane forest (Saracho-Bottero et al., 2018). This strong ecological preference of *I. pararicinus* for certain environments within the altitude gradient of the Yungas forest clearly determine the incidence of *I. pararicinus* parasitism on cattle in this area. Cattle appear to be occasional hosts for *A. hadanii,* in coincidence with the conclusions presented by Saracho-Bottero et al. (2018), whereas that it is widely known that *D. nitens* has horses as its principal host and no other domestic hosts as cattle (Nava et al., 2017; Guglielmone et al., 2021).

The detection of a strain of *Borrelia* sp. belonging to the *B. burgdorferi* s.l. complex in *I. pararicinus* is consistent with previous findings made in Argentina. Ticks from the *Ixodes ricinus* complex, namely *I. pararicinus* and *Ixodes* sp. cf. *I. affinis*, were found to be infected with different strains of *Borrelia* sp. from the *B. burgdorferi* s.l. complex in different provinces from northern Argentina, including areas belonging to the Yungas Biogeographic Province[3] (Nava et al., 2014a; Saracho-Bottero et al., 2017, 2018; Flores et al., 2020). The haplotype detected in this work, which represent a genospecies with unknown pathogenicity to humans, is phylogenetically related (see Fig. 4) to haplotypes previously detected in *I. pararicinus* from Salta Province (haplotype MP30; Saracho-Bottero et al., 2017) and *Ixodes* sp. cf. *I. affinis* from Chaco Province also in Argentina (haplotypes FSF1 and 8, Flores et al., 2020), and in *I. fuscipes* from Uruguay (haplotypes A, B, C; Barbieri et al., 2013; named as *I. pararicinus* (see Labruna et al., 2020)) and *I. longiscutatus* from Brazil (haplotype Pampa; Dall'Agnol et al., 2017). In spite of the ubiquitous presence of *Borrelia* sp. in ticks from the *I. ricinus* complex in Argentina as *I. pararicinus* and *Ixodes* sp. cf. *I. affinis*, the public health relevance of this vector-microorganism association is far from being similar to that occurs in the northern hemisphere because there are practically no records of these tick species parasitizing humans in South America (see Guglielmone and Robbins, 2018; Guglielmone et al., 2021).

Most of the tick species that form the communities analyzed in this study, with the exception of *R. microplus, D. nitens* and *R. sanguineus* s.s., have wild mammals as primeval hosts (Nava and Guglielmone, 2013; Nava et al., 2017; Guglielmone et al., 2021), but *A. sculptum, A. ovale, H. juxtakochi* and *I. pararicinus* also have the capacity to develop their parasitic phase (completely or in the adult stages) on domestic mammals as cattle, horses or dogs. Moreover, *A. sculptum* and *A. ovale* are frequent parasites of humans (Guglielmone and Robbins, 2018). The tick community characterized in this work for rural areas of the Yungas lower montane forest, is composed by species which are potential vectors of pathogenic microorganism with veterinary and public health importance, circulating in a human-wildlife-livestock interface. This pattern clearly reveals the need of an exhaustive epidemiological vigilance in the Yungas area regarding tick-borne diseases affecting humans and domestic mammals.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Santiago Nava reports financial support was provided by Instituto Nacional de Tecnología Agroecuaria (INTA PEI109). Santiago Nava reports a relationship with National Institute of Agricultural Technology that includes: employment. no.

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