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Ticks infesting cattle and humans in the Yungas Biogeographic Province of Argentina, with notes on the presence of tick-borne bacteria

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Abstract This study was performed to determine the tick species that infest cattle and humans throughout an altitudinal gradient in the Yungas Biogeographic Province of Argentina. The presence of tick-borne bacteria of the genera *Rickettsia, Ehrlichia* and *Borrelia* in the collected ticks was also evaluated. Samples of ticks parasitizing cattle and humans were carried out in different seasons. Questing ticks (adults and nymphs) were collected from vegetation and analyzed to detect the presence of *Rickettsia, Ehrlichia* and *Borrelia* by a battery of different PCRs. Five species of hard ticks were found parasitizing cattle: *Amblyomma sculptum, Amblyomma tonelliae, Amblyomma hadanii, Haemaphysalis juxtakochi* and *Ixodes pararicinus*. *Amblyomma sculptum* (immature and adults), *A. tonelliae* (immature and adults), *A. hadanii* (larvae) and one nymph of *I. pararicinus* were found attached to humans. *Rickettsia amblyommatis* was detected in one nymph of *A. hadanii.* DNA of a *Borrelia* genospecies belonging to the *B. burgdorferi* s.l. complex (phylogenetically related to haplotypes previously reported in *Ixodes aragaoi* from Uruguay and *I. pararicinus* from Argentina) was detected in adults of *I. pararicinus. Amblyomma sculptum* and *I. pararicinus* appear to be the tick species more frequent on cattle in the YBP from Argentina, and *A. sculptum* and *A. tonelliae,* were the main ticks found attached to humans. The medical importance of the bacteria of the genus *Rickettsia* and *Borrelia* detected in this work remains unknown.

Keywords Ixodidae · Cattle · Humans · Bacteria · Yungas · Argentina

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Introduction

Ticks (Acari: Ixodida) are hematophagous ectoparasites of terrestrial vertebrates with impact on public health and animal production because they have capacity to transmit disease agents to domestic animals and humans and contribute to the development of toxicosis, myiasis and secondary infections (Sonenshine and Mather 1994; Jongejan and Uilenberg 2004). Fifty one species of ticks have been recorded in Argentina, and several of them are usual parasites of domestic animals and human and associated with tick-borne microorganisms of medical and veterinary relevance (Nava et al. 2017).

The Yungas Biogeographic Province (YBP) is extended from northern Peru to northwestern Argentina and consists of the western slopes of the Andes, at an altitude ranging from 300 to 3500 m a.s.l. (Morrone 2006). In northwestern Argentina, the subtropical mountain forests or Yungas extend along discontinuous mountain ranges across the provinces of Jujuy, Salta, Tucumán and Catamarca, covering an area of four million ha (Brown et al. 2001). Anthropogenic activities such as extensive cattle-grazing, agriculture and tourism are developed in areas included within the YBP of Argentina. Although 13 species of hard ticks (Acari, Ixodida: Ixodidae) belonging to the genera *Amblyomma, Dermacentor, Haemaphysalis, Ixodes* and *Rhipicephalus* were recorded in the YBP of Argentina (Nava et al. 2017), there is a lack of systematic studies evaluating which species are frequently associated to cattle and humans and their potential role as vector of tick-borne pathogens. This basic information is important for epidemiological inferences on the risk of tick-borne pathogen transmission in a given area, as well as for designing eco-epidemiological studies on this subject. Therefore, a study was implemented to determine the tick species that infest cattle and humans throughout an altitudinal gradient in the YBP of Argentina, including the diagnosis of tick-borne bacteria of the genera *Rickettsia, Ehrlichia* and *Borrelia* with potential sanitary relevance.

Materials and methods

Tick collection was performed in and around Villa Monte (24°18′S, 64°31′W), Jujuy Province, northwestern Argentina. The three main forest types characteristic of the YBP are represented in this area, namely Piedmont Forest, Lower Montane Forest and Upper Montane Forests (Brown et al. 2001). These forest types, which are described in Brown et al. 2001, are differentiated along an altitudinal gradient: Piedmont Forest from 400 to 700 m a.s.l.; Lower Montane Forest from 700 to 1500 m a.s.l.; and Upper Montane Forest from 1500 to 3000 m a.s.l. Climate in the YBP is defined as subtropical with the annual rainfall concentrated from late spring to late summer and occasional frost during the coldest part of the year. Annual rainfall averages of 800 mm (500–1400 mm), 1800 mm (1100–2300 mm) and 1100 mm (800–1400 mm) occur in Piedmont Forest, Lower Montane Forest and Upper Montane Forest, respectively (Bianchi and Yañez 1992). Water input due to fog interception constitutes a complementary water source in the montane forest (Hunzinger 1997).

Seasonal cattle tick-sampling sessions were carried out on 10 cattle grazing in representative areas of the three types of forests of the YBP mentioned above. Details about type forest, date and season of each sample are shown in Table 1. Additionally, free-living ticks were collected from vegetation along different transects that cross the three main types of forests of the YBP. After each sampling the operators controlled their bodies in search of fixed ticks, which were recovered using dissection forceps. All ticks collected were stored

in 96% ethanol until posterior taxonomic determination in the laboratory. In some cases engorged larvae and nymphs collected on cattle were allowed to molt to the subsequent stage under laboratory conditions to improve the accuracy of morphological determination. Ticks were determined following Nava et al. (2014b, c, 2017) and by comparison with known laboratory-reared material deposited in the Tick Collection of Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela, Argentina.

Questing ticks (adults and nymphs) were collected from vegetation and analyzed to detect the presence of *Rickettsia, Ehrlichia* and *Borrelia*. Genomic DNA was extracted by using a phenol/chloroform assay described by Mangold et al. (1998). For the detection of rickettsial DNA, ticks were screened by a real time-PCR (rt-PCR) targeting a 147 bp fragment of the citrate synthase gene (*glt*A) following the protocols described by Labruna et al. (2004) and Guedes et al. (2005) with the primers CS5 5′-GAGAGAAAATTATAT CCAAATGTTGAT-′3 and CS6 5′-AGGGTCTTCGTGCATTTCTT-′3. The rt-PCRs were performed in a Rotor-Gene-Q6000 (Qiagen). The rt-PCR-positive samples were further tested by a battery of PCRs to amplify fragments of two rickettsial genes, those for citrate synthase gene (*glt*A) with the primers CS239 5′-GCTCTTCTCATCCTATGGCTA TTAT-′3 and CS1069 5′-CAGGGTCTTCGTGCATTTCTT-3′ (Labruna et al. 2004), and 190-kDa outer membrane protein (*omp*A) with the primers Rr 190.70p 5′-ATGGCGAAT ATTTCTCCAAAA-′3 and Rr 190.602n 5′-AGTGCAGCATTCGCTCCCCCT-′3 (Regnery et al. 1991). All PCR runs were managed with two negative controls (water and none-template control) and a positive control (*Rickettsia massiliae*). The detection of ehrlichial DNA in ticks was performed by the usage of a PCR assay described by Doyle et al. (2005) which amplifies a fragment of the gene *dsb* with the primers *dsb* 330 5-GATGATGTCTGA AGATATGAAACAAAT-03′ and dsb 728 5′ CTGCTCGTCTATTTTACTTCTTAAAG-′3. *Ehrlichia canis* was used as positive control. Additionally, ticks were screened by PCR for detection of *Borrelia* DNA following the methods detailed in Barbieri et al. (2013). Twenty-one adults and six nymphs of the genus *Ixodes* were processed in pools as follow: three pools of two nymphs each, two pools of two males each, one pool of three males, three pools of three females each, and one female processed individually. Nested PCR was performed targeting the flagellin gene (*fla*) of *Borrelia* spp with the primers presented in Barbour et al. (1996). The primers Fla LL (5′-ACA TAT TCAGAT GCA GAC AGA GGT-3′) and Fla RL (5′-GCA ATC ATA GCC ATTGCA GAT TGT-3′) were used in the first reaction, and the primers Fla LS (5′-AAC AGC TGA AGA GCT TGG AAT G-3′) and Fla RS (5′-CTT TGATCA CTT TC ATT CTA ATA GC-3′) were employed in the second reaction. Nuclease free water was used as negative control and DNA of *Borrelia anserina* served as positive control in the PCR reactions. Positive PCR-amplicons were purified (Wizard SV Gel and PCR Clean-Up System, Promega) and sequenced. Phylogenetic analyses were performed with Maximum-likelihood (ML) methods by using the program Mega 5 (Tamura et al. 2011), and best fitting substitution models were determined with the Akaike Information Criterion using the ML model test also implemented in MEGA 5. Support for the topologies was tested by bootstrapping over 1000 replications and gaps were excluded from the comparisons.

Results

Five species of hard ticks were found parasitizing cattle during this study, namely *Amblyomma sculptum, Amblyomma tonelliae, Amblyomma hadanii, Haemaphysalis juxtakochi* and *Ixodes pararicinus* (Table 1). Data on season and the forest types of the YBP in which these tick species were detected on cattle are shown in Table 1. Both immature and adults stages of *A. sculptum, A. tonelliae* and *A. hadanii* were found associated to cattle, while *I. pararicinus* and *H. juxtakochi* were represented only by adults (Table 1). Four of these five species were recorded infesting humans in the study area: larvae (n: 12), nymphs (n: 18) and adults (n: 7) of *A. sculptum,* nymph (n: 1) and adults (n: 3) of *A. tonelliae,* larvae (n: 5) of *A. hadanii,* and one nymph of *I. pararicinus*. No specimen of *H. juxtakochi* was detected biting humans.

Fifty-one adults and 323 nymphs of *A. sculptum,* 60 nymphs of *A. hadanii* and 17 nymphs of *H. juxtakochi* were tested for infection with *Rickettsia* and *Ehrlichia*. All samples of *A. sculptum* and *H. juxtakochi* were negative. One nymph of *A. hadanii* was positive to *Rickettsia*. The phylogenetic analysis performed with *omp*A sequences has shown that the positive nymph of *A. hadanii* was infected with *Rickettsia amblyommatis* (Fig. 1). The similarity between the *omp*A sequence of *R. amblyommatis* obtained in this work and

Table 1 Ticks collected on cattle in the Yungas Biogeographic Province of Argentina

Type of forest[a]	Date and season	Tick species	n and tick stage
Piedmont forest	January 2012; early-summer	*Amblyomma tonelliae*	22NN
	January 2012; early-summer	*Amblyomma tonelliae*	23MM; 18FF
	January 2012; early-summer	*Amblyomma sculptum*	1M; 1F
Lower montane forest	September 2015; early-spring	*Amblyomma sculptum*	28MM; 40FF; 10NN
	October 2015; mid-spring	*Amblyomma sculptum*	15MM; 34FF; 50NN
	December 2015; early-summer	*Amblyomma sculptum*	75MM; 114FF; 6NN
	December 2015; early-summer	*Amblyomma hadanii*	1N
	December 2015; early-summer	*Haemaphysalis juxtakochi*	1F
	June 2017; late-autumn	*Amblyomma sculptum*	13MM; 2FF; 1N; 170LL
	June 2017; late-autumn	*Haemaphysalis juxtakochi*	2MM; 1H
Upper montane forest	October 2013; mid-spring	*Ixodes pararicinus*	11M; 65F
	October 2013; mid-spring	*Amblyomma sculptum*	1M
	February 2014; mid-summer	*Amblyomma hadanii*	1M; 1F; 1N
	July 2014; mid-winter	*Ixodes pararicinus*	8MM; 165FF
	July 2014; mid-winter	*Haemaphysalis juxtakochi*	1M; 3HH
	January 2015; early-summer	*Ixodes pararicinus*	9FF
	January 2015; early-summer	*Amblyomma sculptum*	4FF
	May 2015; mid-autumn	*Ixodes pararicinus*	16MM; 69FF
	October 2015; mid-spring	*Ixodes pararicinus*	4MM; 31FF
	October 2015; mid-spring	*Amblyomma sculptum*	1M

M male, *F* female, *N* nymph, *L* larva

[a]See description of each type of forest in the "Materials and methods" section

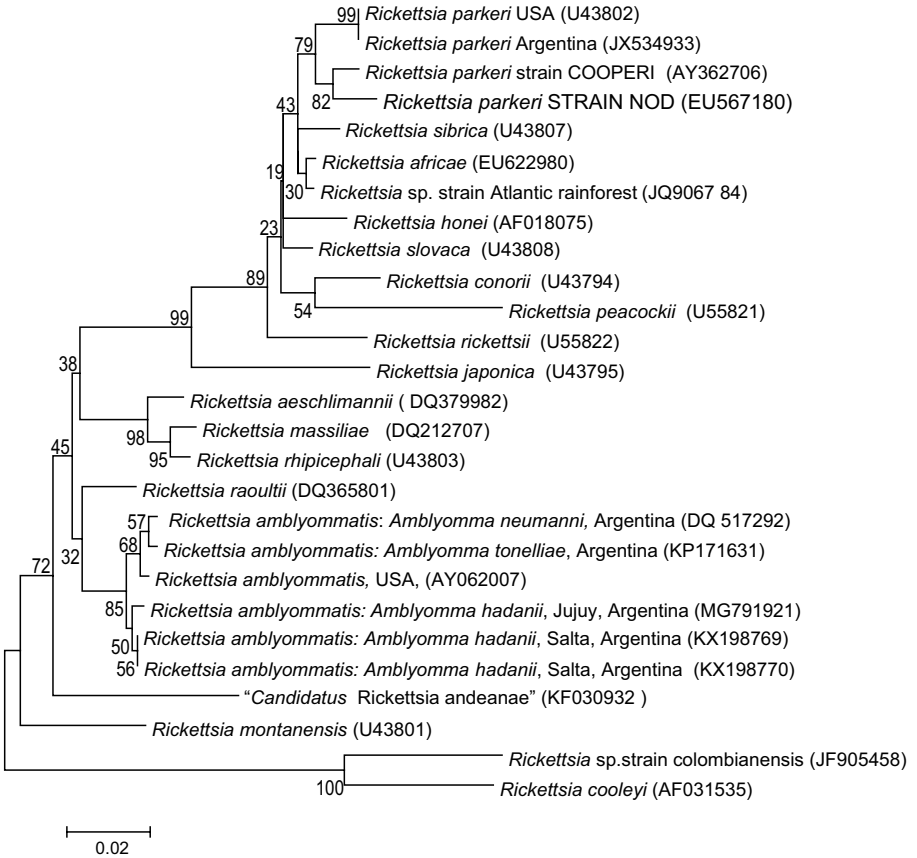

Fig. 1 Maximum-likelihood tree constructed from *omp*A partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets. The substitution model was GTR (G + I)

those detected in other *Amblyomma* species from South America and USA was never lower than 99%. The *omp*A sequence obtained from this nymph (GenBank accession number: MG791921) was phylogenetically more closely related to the sequences of *R. amblyommatis* (named as '*Candidatus* Rickettsia amblyommii') which were previously reported in *A. hadanii* ticks (GenBank accession numbers: KX198769 and KX198770) from Salta Province in Argentina (Fig. 1). The comparison using BLAST (www.ncbi.nlm.nih.gov/blast) of the *glt*A sequence (GenBank accession number: MG791922) obtained from the positive nymph of *A. hadanii* with those sequences *Rickettsia* deposited in GenBank confirmed the determination of *R. amblyommatis* reached with the *omp*A sequence.

In order to detect molecularly the presence of bacteria of the genus *Borrelia*, 21 adults and six nymphs of *I. pararicinus* were processed in pools. Three pools of two nymphs each were negative, two pools of two males each were positive, one pool of three males was positive, three pools of three females each were positive, and one female processed individually was also positive. Two unique haplotypes (A and B) differing in four bases were obtained after sequencing the positive samples (Genbank accession numbers: MG791919

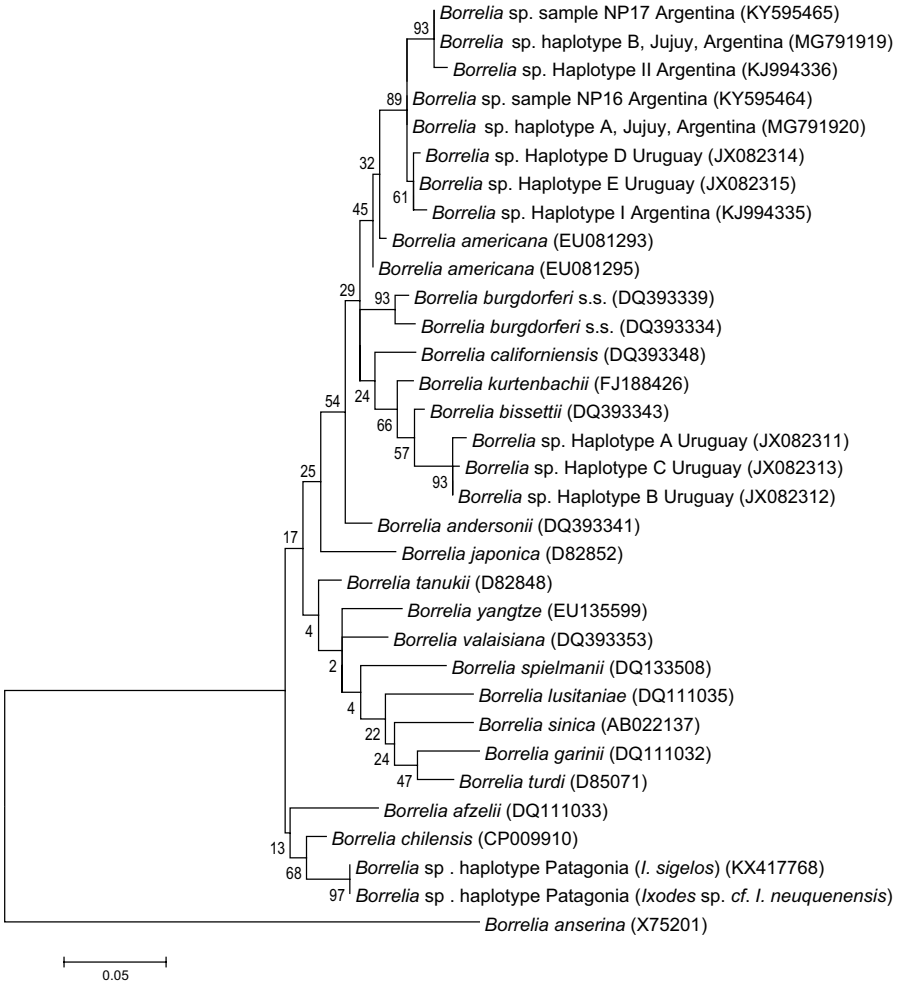

Fig. 2 Maximum-likelihood tree constructed from *Borrelia fla* partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets. The substitution model was GTR (G + I)

and MG791920). The phylogenetic tree generated with the *fla* sequences shows that the *Borrelia* genospecies found in this work belong to the *B. burgdorferi* s.l. complex (Fig. 2). The *fla* sequences were phylogenetically related to the haplotypes D and E of the *Borrelia* genospecies reported in *Ixodes aragaoi* (named as *I. pararicinus*) from Uruguay (Genbank accession numbers: JX082315 and JX082315) and, as expected, to the haplotypes of the same *Borrelia* genospecies previously detected in *I. pararicinus* ticks from the Jujuy and Tucumán Provinces, Argentina (Genbank accession numbers: KJ 994335, KJ 994336, KY595464-KY595467) (Fig. 2).

Discussion

Five species of hard ticks were found parasitizing cattle in the YBP of Argentina. *Amblyomma tonelliae, A. sculptum* and *I. pararicinus* were the principal tick species affecting cattle in the Piedmont Forest, Lower Montane Forest and Upper Montane Forest, respectively (see Table 1). Although the *A. sculptum* infestation in cattle predominated in Lower Montane Forest, few specimens of this tick were also collected on cattle in Piedmont Forest and Upper Montane Forest. Conversely, *A. tonelliae* and *I. pararicinus* were restricted to only one type of forest, the first one to the Piedmont Forest and the second one to the Upper Montane Forest. Few specimens of *A. hadanii* and *H. juxtakochi* were detected on cattle in Lower Montane Forest and Upper Montane Forest, but not in Piedmont Forest. The results herein presented are not unexpected because all these cattle-tick associations have previously been recorded in Argentina (Guglielmone et al. 1992; Guglielmone and Nava 2013; Tarragona et al. 2015; Nava et al. 2017).

The results of this work suggest that cattle can sustain the complete cycle of *A. sculptum* and *A. tonelliae* in the YBP from Argentina because the immature and adult stages of these tick species were found feeding on the bovines examined. The capacity of the species belonging to the *A. cajennense* complex (represented in the YBP by *A. sculptum* and *A. tonelliae*) to develop their complete parasitic phase on a recently introduced host such as cattle was already underscored (Guglielmone et al. 1990; Guglielmone and Nava 2013; Nava and Guglielmone 2013; Tarragona et al. 2015), and has epidemiological relevance. Non-indigenous species may act as suitable hosts for native parasites, and this interrelationship can have a negative effect on native fauna due to acquisition and amplification of the native parasite by an introduced host (Kelly et al. 2009; Mastitsky and Veres 2010; Tompkins et al. 2011), which in turn could also increase the risk of bites in humans. Thus, the epidemiological importance of the *A. sculptum* and *A. tonelliae* infestation in cattle is not only related to the deleterious effect of the tick parasitism on cattle per se, but also to the role that this host could has as amplifier of a potential vector of pathogenic microorganisms.

The infestation with adults of *I. pararicinus* in cattle was noticeable (see Table 1), but it was completely restricted to the Upper Montane Forest. The role of cattle as host for the adults of *I. pararicinus* in the YBP is known (Nava et al. 2017). But unlike what happens with *A. sculptum* and *A. tonelliae*, who are able to develop a surrogate cycle independent of native hosts, *I. pararicinus* still depends on primeval and native hosts such as small mammals and passerine birds (see Nava et al. 2017) to sustain their larvae and nymphs. It is unknown whether the *I. pararicinus* parasitism has a deleterious effect on cattle, but the data obtained in this work strongly suggest that this host can amplify the *I. pararicinus* populations due to its role as host for adult stages.

Nymphs and adults of *A.hadanii* and adults of *H. juxtakochi* were recorded on cattle in both Lower Montane Forest and Upper Montane Forest, but they were found in low number in relation to the values observed for *A. tonelliae, A. sculptum* and *I. pararicinus*. *Haemaphysalis juxtakochi* has been previously found parasitizing cattle in different ecological regions from Argentina (Guglielmone et al. 1992; Guglielmone and Nava 2013), and there is a record of a nymph of *A. hadanii* on cattle in a locality belonging to the YBP (Nava et al. 2014c). But according to the data obtained in this work, the role of cattle as host for *H. juxtakochi* and *A. hadanii* in the study area appears to be less relevant than for *A. sculptum, A. tonelliae* and *I. pararicinus.*

The presence of *R. amblyommatis* in *A. hadanii* ticks was detected in two different localities of the YBP in Salta and Jujuy Provinces, Argentina (Mastropaolo et al. 2016; this study). Phylogenetically, the *R. amblyommatis ompA* sequences obtained from *A. hadanii* are more closely related to each other than to the *ompA* sequences of *R. amblyommatis* obtained from other *Amblyomma* species (see Mastropaolo et al. 2016 and the Fig. 1 of this work), but the differences among haplotypes are too small (1%) to consider them a priori different species. *Amblyomma hadanii* could be a potential vector of this rickettsia because its larvae and nymphs were found parasitizing humans (Nava et al. 2014c; this work). But although Apperson et al. (2008) have suggested that some cases of rickettsiosis in USA may have been caused by *R. amblyommatis* (named as "*Candidatus* R. amblyommii"), this *Rickettsia* species is currently regarded as being of unknown pathogenicity (Parola et al. 2013).

The species of the *A. cajennense* complex, namely *A. sculptum* and *A. tonelliae,* were the main ticks found attached to humans in the samples performed in the three forest types of the YBP. Immature and adult stages of these two species are known to be aggressive to humans (Nava et al. 2017). Although tick-borne pathogens were not found in these two tick species, they are known to have vectorial competence to transmit *Rickettsia rickettsii* under experimental conditions (Soares et al. 2012; Labruna et al. 2014; Tarragona et al. 2016). In this sense, ticks from the *A. cajennense* complex were incriminated as the vector in the fatal cases of spotted fever in humans that were reported in areas located within the YBP in Argentina (Paddock et al. 2008).

All pools of *I. pararicinus* adults were positive to a *Borrelia* genospecies belonging to the *B. burgdorferi* sensu lato complex (Fig. 2). Two haplotypes were identified, which are identical to the haplotypes of previously detected in *I. pararicinus* ticks from Jujuy and Tucumán Provinces in Argentina (Nava et al. 2014a; Saracho-Bottero et al. 2017). These haplotypes are phylogenetically related to the haplotypes D and E detected in *I. aragaoi* from Uruguay (Barbieri et al. 2013). Taking into account the results of the current study and those presented by Nava et al. (2014a) and Saracho-Bottero et al. (2017), it can be stated that the presence of *Borrelia* genospecies belonging to the *B. burgdorferi* s.l complex in *I. pararicinus* ticks from the YBP is widespread. But the epidemiological relevance of this fact should not be overrated by two main reasons: first, the *Borrelia* genospecies detected in *I. pararicinus* are of unknown pathogenicity, because they have not been associated with human disease so far; second, in contrast to that occurring with some *Ixodes* species in the northern hemisphere, *I. pararicinus* is not prone to bite humans, which is demonstrated by the lack of previous records of this tick infesting humans along its distribution in South America (Guglielmone et al. 2006; Nava et al. 2017). In this work, only one nymph was found attached to human in an area of the mountains with an altitude above 1500 m.a.s.l. (upper montane forest) where the presence of people is sporadic. In conclusion, the presence of *Borrelia* in *I. pararicinus* ticks should not be directly related to a great risk of human disease.

Amblyomma sculptum and *I. pararicinus* appear to be the tick species more frequent on cattle in the YBP from Argentina. There are records of *Rhipicephalus* (*Boophilus*) *microplus* and *Amblyomma neumanni*, two common parasites of cattle in Argentina, in the YBP of Argentina (Nava et al. 2017), but they were not found on cattle during the current study. Infestations with *R. microplus* and *A. neumanni* in cattle were observed in localities near the study area in the ecotone between Yungas and Chaco Biogeographic Provinces (S. Nava and AJ Mangold, unpublished). The main findings of this study suggest that, although *R. microplus* and *A. neumanni* can be occasionally observed on cattle in the YBP,

the microclimatic conditions of the YBP preclude the establishment of large populations of these two tick species within this ecological area.

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