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Borrelia burgdorferi sensu lato in *Ixodes cf. neuquenensis* and *Ixodes sigelos* ticks from the Patagonian region of Argentina

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ABSTRACT

This study was conducted to detect *Borrelia burgdorferi* sensu lato infection in ixodid ticks from the Patagonia region in the south of Argentina. Therefore, ticks were collected on rodents in the provinces of Chubut, Río Negro and Santa Cruz. These ticks were identified as nymphs of *Ixodes cf. neuquenensis* and *Ixodes sigelos*. The *B. burgdorferi* s.l. infection was tested by a battery of PCR methods targeting the gene flagellin (*fla*) and the *rrfA-rrlB* intergenic spacer region (IGS). Three pools of *I. sigelos* nymphs from Chubut and Santa Cruz provinces as well as one pool of *I. cf. neuquenensis* nymphs from Río Negro province were tested positive in the *fla*-PCR. The samples of *I. sigelos* were also positive for the IGS-PCR. Phylogenetically, the haplotypes found in the positive ticks belong to the *B. burgdorferi* s.l. complex, and they were closely related to *Borrelia chilensis*, a genospecies isolated from *Ixodes stilesi* in Chile. The pathogenic relevance of the *Borrelia* genospecies detected in both *I. neuquenensis* and *I. sigelos* is unknown.

1. Introduction

Borrelia burgdorferi sensu lato is a group which includes at least 20 genospecies (Casjens et al., 2011; Margos et al., 2011, 2014; Stanek and Reiter, 2011; Ivanova et al., 2014). They are principally transmitted by tick species belonging to the *Ixodes ricinus* complex (Steere et al., 2005). Most of the genospecies of *B. burgdorferi* s.l. were reported in the Holarctic region (Margos et al., 2011; Clark et al., 2014; Vollmer et al., 2013). To date, the only reliable reports of *B. burgdorferi* s.l. in South America were made in Argentina (Nava et al., 2014), Chile (Ivanova et al., 2014), and Uruguay (Barbieri et al., 2013), where the ticks *Ixodes pararicinus, Ixodes stilesi* and *Ixodes aragaoi* were found to be positive to *B. burgdorferi* s.l., respectively. There is a report of *B. burgdorferi* s.l. infecting the tick *Dermacentor nitens* in Brazil (Goncalves et al., 2013), but this finding is subject to confirmation because it was only based on sequences of a short fragment (*circa* 184 bp) of the *rrfA-rrlB* intergenic spacer region (IGS), which exhibits low levels of polymorphism within the genus *Borrelia*.

Ticks of the genus *Ixodes* are distributed in practically all biogeographic regions of the world. In total, over 240 species were described so far in the world, from them 10 were found in Argentina (Guglielmone and Nava, 2005, 2014; Lamattina et al., 2016). In Argentina, *Ixodes sigelos* and *Ixodes neuquenensis* are principally found in the Patagonian region (Guglielmone et al., 2004; Guglielmone and Nava 2005; Sanchez et al., 2010). *Ixodes sigelos*, *I. neuquenensis*, *I. stilesi* and *I. abrocomae* conform a group of species phylogenetically closely related (Guglielmone and Nava 2011). With the exception of *I. stilesi* (see Ivanova et al., 2014), no studies on *Borrelia* infection in this group of species have been performed. In this work we report the detection of a new genotype of *B. burgdorferi* s.l. in two South American species of the genus *Ixodes* phylogenetically not related to the *I. ricinus* complex.

2. Materials and methods

Ticks were collected on rodents (Cricetidae: Sigmodontinae) at different localities belonging to the Patagonian region of Argentina. The ticks collected on the rodents were stored in 96% ethanol and

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Table 1
Used primer pairs for the detection of *Borrelia burgdorferi* sensu lato.

target	primer sequence	reference
flagellin (*fla*)[a]	Fla LL; 5′-ACA TAT TCA GAT GCA GAC AGA GGT-3′ Fla RL; 5′-GCA ATC ATA GCC ATT GCA GAT TGT-3′ Fla LS; 5′-AAC AGC TGA AGA GCT TGG AAT G-3′ Fla RS; 5′-CTT TGA TCA CTT ATC ATT CTA ATA GC-3′	Barbour et al. (1996)
intergenic spacer region (*rrfA-rrlB*)[b]	IGSb; 5′-GTT AAG CTC TTA TTC GCT GAT GGT A-3′) IGSa; 5′-CGA CCT TCT TCG CCT TAA AGC-3′)	Derdáková et al. (2003)

[a] Nested PCR; Fla LL and Fla RL first reaction – Fla LS and Fla RS second reaction.
[b] 5S (*rrfA*)–23S (*rrlB*) intergenic spacer region.

transferred to the laboratory by their taxonomic determination. The identification of the ticks was carried out following Keirans et al. (1976) and Guglielmone et al. (2004, 2005). Rodents were determined by U.F.J. Pardiñas (IDEAUS – CENPAT, Instituto de Diversidad y Evolución Austral, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Puerto Madryn, Argentina). DNA extraction of ticks was managed using a DNA extraction Kit (AxyPrep Multisource Genomic DNA Miniprep Kit; Axygen Biosciences, Union City, CA, USA) following the manufactures instructions. The detection of *Borrelia* spp. DNA was carried out by a battery of PCR assays reported by Barbieri et al. (2013). Briefly, nested PCR was performed targeting the flagellin gene (*fla*) of *Borrelia* spp. Positive samples were further used to amplify a 225- to 255-bp fragment of the *rrfA-rrlB* intergenic spacer region (IGS). The primers used are detailed in Table 1. For all PCR reactions, nuclease free water was used as negative control. DNA of the *B. burgdorferi* s.l. haplotype D isolated from *I. aragaoi* ticks from Uruguay served as positive control. All positive samples were purified and afterwards sequenced using the appropriate primers. The received sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999) with manual edition whenever it was necessary and aligned with the program Clustal W (Thompson et al., 1994). Phylogenetic analysis was performed with the maximum-likelihood (ML) method. The best-fitting substitution model was determined with the Bayesian Information Criterion using the ML model test implemented in MEGA 5 (Tamura et al., 2011). A tree based on *Borrelia fla* partial sequences was generated with the Tamura 3-parameter model by using a discrete gamma-distribution (+G). Support for the topologies was tested by bootstrapping over 1000 replications. The number of variable nucleotide positions between sequences of *fla* and IGS were used to calculate pairwise estimates of percent sequence divergence among sequences of *Borrelia* spp. found in different areas of the world. Gaps were excluded in the pairwise distance estimation.

3. Results

In total, 65 nymphs (26 *I. cf. neuquenensis* and 39 *I. sigelos*) were tested for *Borrelia* spp. infection (Table 2). Hereby, two pools of *I. sigelos* were positive in the *fla*L-PCR: one pool of four nymphs collected on *Phyllotis xanthopygus* at Lago Cardiel (Santa Cruz Province), and one pool of 5 nymphs also collected on *P. xanthopygus* captured in Lago Blanco (Chubut Province). Furthermore, one nymphal pool of *I. cf. neuquenensis* and *I. sigelos* showed a positive PCR result in the *fla*S-PCR. The *I. sigelos* pool (7 nymphs) was collected from *P. xanthopygus* also at Lago Cardiel. The pool of *I. cf. neuquenensis* (7 nymphs) was collected at Meseta Somuncurá in the province of Río Negro (undetermined host species). All positive samples were sequenced. Fig. 1 shows the result of the phyloge-

Table 2
Distribution of the collected ticks and results of the pathogen detection. All ticks analysed were nymphs.

ID	Tick species	No. ticks	Host	Locality/Province	Latitude/Longitude		*fla*[a]	*fla*[b]	*rrf*A-*rrl*B[c]
NP 30	*I. sigelos*	2	*Phyllotis xanthopygus*	Lago Cardiel/Santa Cruz	S 48° 55′ 11”	W 71° 12′ 18”	0	0	n.d.
NP 31	*I. sigelos*	7	*Phyllotis xanthopygus*	Lago Cardiel/Santa Cruz	S 48° 55′ 11”	W 71° 12′ 18”	0	1	1
NP 32	*I. sigelos*	4	*Phyllotis xanthopygus*	Lago Cardiel/Santa Cruz	S 48° 55′ 11”	W 71° 12′ 18”	1	1	1
NP 33	*I. sigelos*	1	*Phyllotis xanthopygus*	Lago Cardiel/Santa Cruz	S 48° 55′ 11”	W 71° 12′ 18”	0	0	n.d.
NP 34	*I. sigelos*	1	*Phyllotis xanthopygus*	Lago Cardiel/Santa Cruz	S 48° 55′ 11”	W 71° 12′ 18”	0	0	n.d.
NP 35	*I. sigelos*	1	*Abrothrix olivacea*	Punta Quilla/Santa Cruz	S 50° 6′ 58”	W 68° 24′ 44”	0	0	n.d.
NP 36	*I. sigelos*	3	*Abrothrix hirta*	Lago Blanco/Chubut	S 45° 53′ 1”	W 71° 12′ 43”	0	0	n.d.
NP 37	*I. sigelos*	1	*Abrothrix hirta*	Lago Blanco/Chubut	S 45° 53′ 1”	W 71° 12′ 43”	0	0	n.d.
NP 38	*I. sigelos*	2	*Abrothrix olivacea*	Pali Aike/Santa Cruz	S 51° 54′ 54”	W 69° 54′ 36”	0	0	n.d.
NP 39	*I. sigelos*	2	*Abrothrix hirta*	Lago Blanco/Chubut	S 45° 53′ 1”	W 71° 12′ 43”	0	0	n.d.
NP 40	*I. sigelos*	5	*Phyllotis xanthopygus*	Lago Blanco/Chubut	S 45° 53′ 1”	W 71° 12′ 43”	1	n.d.	1
NP 41	*I. sigelos*	3	*Loxodontomys micropus*	El Maitén/Chubut	S 42° 2′ 57”	W 71° 10′ 0”	0	0	n.d.
NP 42	*I. sigelos*	1	*Reithrodon auritus*	Pico Salamanca/Chubut	S 45° 34′ 26”	W 67° 20′ 13”	0	0	n.d.
NP 43	*I. sigelos*	2	*Phyllotis xanthopygus*	Lago Blanco/Chubut	S 45° 53′ 1”	W 71° 12′ 43”	0	0	n.d.
NP 44	*I. sigelos*	2	*Euneomys sp*	Leleque/Chubut	S 42° 24′ 44”	W 71° 4′ 4”	0	0	n.d.
NP 45	*I. sigelos*	2	*Abrothrix hirta*	El Maitén/Chubut	S 42° 2′ 57”	W 71° 10′ 0”	0	0	n.d.
NP 46	*I. cf. neuquenensis*	1	*Phyllotis xanthopygus*	Estancia Talagapa/Chubut	S 42° 11′ 41”	W 68° 20′ 29”	0	0	n.d.
NP 47	*I. cf. neuquenensis*	5	underdetermined host	Meseta Somuncurá/Río Negro	S 41° 30′ 0”	W 67° 15′ 0”	0	0	n.d.
NP 48	*I. cf. neuquenensis*	7	underdetermined host	Meseta Somuncurá/Río Negro	S 41° 30′ 0”	W 67° 15′ 0”	0	1	0
NP 49	*I. cf. neuquenensis*	5	underdetermined host	Meseta Somuncurá/Río Negro	S 41° 30′ 0”	W 67° 15′ 0”	0	0	n.d.
NP 50	*I. cf. neuquenensis*	5	underdetermined host	Meseta Somuncurá/Río Negro	S 41° 30′ 0”	W 67° 15′ 0”	0	0	n.d.
NP 51	*I. cf. neuquenensis*	1	*Phyllotis xanthopygus*	Laguna Blanca/Río Negro	S 41° 25′ 36”	W 66° 57′ 20”	0	0	n.d.
NP 52	*I. cf. neuquenensis*	2	*Phyllotis xanthopygus*	Estancia Talagapa/Chubut	S 42° 11′ 41”	W 68° 20′ 29”	0	0	n.d.
total	***I. sigelos***	**39**					**2**	**2**	**3**
total	***I. cf. neuquenensis***	**26**					**0**	**1**	**0**

0: negative PCR result; 1: positive PCR result; n.d.: not done.
[a] PCR method described by Barbour et al. (1996) detecting the Flagellin gene; used primers FlaLL and FlaRL, see Table 1.
[b] PCR method described by Barbour et al. (1996) detecting the Flagellin gene; used primers FlaLS and FlaRS, see Table 1.
[c] PCR method described by Derdáková et al. (2003) detecting the intergenic spacer region; see Table 1.

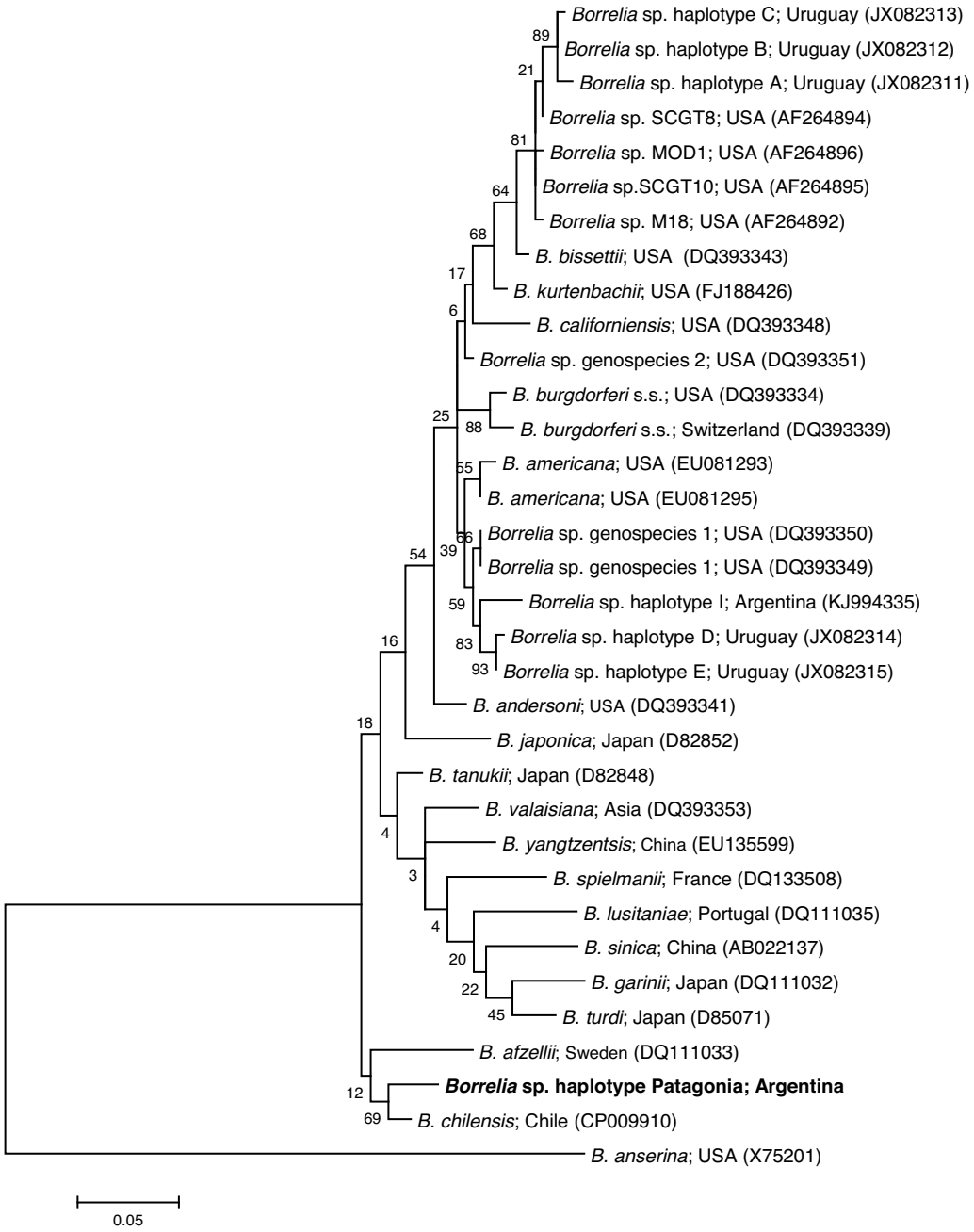

Fig. 1. Maximum-likelihood tree constructed from *fla* partial sequences. Numbers represent bootstrap support generated from 1000 replications. GenBank accession numbers are in brackets (*B.*: *Borrelia*).

netic analyses of the obtained with *flaS* DNA partial sequences. The two *flaS* sequences of *B. burgdorferi* s.l. obtained from the DNA of *I. cf. neuquenensis* and *I. sigelos* were identical among each other and conform a haplotype, namely *Borrelia* sp. haplotye Patagonia (see Fig. 1) (GenBank accession number: KX417768). This haplotype is phylogenetically related to the corresponding sequence of *B. chilensis*, a member of the *B. burgdorferi* s.l. complex (GenBank accession number CP009910) isolated from *I. stilesi* in Chile (see Fig. 1). The similarity between the haplotype Patagonia with *B. chilensis* was 97.5%. In the cases of the three *I. sigelos* samples, the PCR targeting the IGS region also was positive whereas this gene could not be amplified in the *flaS* positive sample of *I. cf. neuquenensis*. The IGS sequences belonging to the *Borrelia* detected in *I. sigelos* also showed the highest similarities with the corresponding sequence of *B. chilensis*. The similarities ranged from 89.7% (samples ID NP 31 and NP 40, see Table 2; GenBank accession number: KX417769) to 90.4% (sample ID NP 32, see Table 2; GenBank accession number: KX417770).

4. Discussion

The findings presented in this work were based on the analyses of two different loci – *fla* and *rrfA-rrlB* (IGS region). *Borrelia* DNA was identified in the two tested tick species, *I. cf. neuquenensis* and *I. sigelos*. In all the cases the *Borrelia* haplotypes detected in both tick species in the four positive samples are phylogenetically closely related to *B. chilensis*, a member of the *B. burgdorferi* s.l. complex detected in *I. stilesi* from Chile by Ivanova et al. (2014). So far for South America, genospecies of *B. burgdorferi* s.l. in *Ixodes* ticks were described for Argentina, Chile and Uruguay (Nava et al.,

2014; Ivanova et al., 2014; Barbieri et al., 2013). In Chile, *B. chilensis* was found in *I. stilesi* whereas in Uruguay and Argentina two new haplotypes of *B. burgdorferi* s.l. were detected in *I. aragaoi* and *I. pararicinus*, respectively (Barbieri et al., 2013; Nava et al., 2014). Both *I. aragaoi* and *I. pararicinus* belong to the *Ixodes ricinus* complex, but not *I. stilesi* (Venzal et al., 2005; Guglielmone and Nava 2011; Onofrio et al., 2014). The two ixodid species tested in this study, *I. cf. neuquenensis* and *I. sigelos*, are not members of the *I. ricinus* complex, but they conform a phylogenetic group with *I. stilesi* (Guglielmone and Nava 2011). Therefore, it is not unexpected that the *Borrelia* haplotypes found in this work are closely related to the *Borrelia* genospecies detected in *I. stilesi* from Chile.

The results of this work confirm that in the Southern Cone of America other *Ixodes* species besides them of the *I. ricinus* complex could play a role in the epidemiology of *B. burgdorferi* s.l., as proposed by Nava et al. (2014). Additionally, it is probably that the rodent species which are hosts for the immature stages of *I. sigelos* and *I. cf. neuquenensis* (see Guglielmone and Nava (2011) and Table 2 of this work) can be competent reservoirs for the *Borrelia* sp. detected in this two tick species. No cases of human parasitism by *I. sigelos*, *I. neuquenensis* and *I. stilesi* were reported to date (Guglielmone et al., 2006, 2014). Therefore, the epidemiological risk that implies the infection with the *Borrelia* genospecies associated to this group of tick species seems to be low. The isolation and culture of the new genotypes of *B. burgdorferi* s.l. described in this work are needed to characterize them in a definitive way.

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