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Original Article

Rodents as potential reservoirs for *Borrelia* spp. in northern Chile

Roedores como potenciais reservatórios de *Borrelia* spp. no norte do Chile

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Abstract

Small mammals play an essential role in the transmission and maintenance cycles of *Borrelia* spirochetes. In Chile, recent studies have characterized novel *Borrelia* genotypes in ticks collected from small mammals, a fact that suggests these vertebrates are hosts for spirochetes from this genus. Considering this evidence, the goal of this study was to determine the presence of *Borrelia* DNA in small mammals inhabiting northern Chile. In winter of 2018, 58 small mammals were captured in five localities. Blood samples were collected from rodents and DNA was extracted to determine the presence of *Borrelia* DNA by PCR targeting the *flaB* gene and *rrs–rrlA* intergenic spacer (IGS). From three individuals (5%), belonging to two rodent species of Cricetidae family (*Phyllotis xanthopygus* and *Oligoryzomys longicaudatus*), we retrieved three *flaB* and two IGS *Borrelia* genotypes. Phylogenetic analyses performed with both Maximum Likelihood and Bayesian inferences showed that our sequences grouped with homologous genotypes from the relapsing fever and Lyme borreliosis groups. Our findings suggest that *P. xanthopygus* and *O. longicaudatus* rodents may play a role as reservoirs for borrelial spirochetes in Chile.

Keywords: *Borrelia*, infectious diseases, small mammals, reservoirs, rodent, Chile.

Resumo

Pequenos mamíferos possuem um papel essencial na transmissão e manutenção de espiroquetas do gênero *Borrelia*. No Chile, estudos recentes têm descrito novos genótipos de *Borrelia* em carrapatos, parasitando pequenos mamíferos. Isso sugere que esses vertebrados podem atuar como possíveis reservatórios dessas espiroquetas. Considerando-se essa evidência, o objetivo deste estudo foi determinar a presença de DNA de *Borrelia* em pequenos mamíferos da região norte do Chile. Durante o inverno de 2018, 58 pequenos mamíferos foram capturados em cinco localidades. Amostras de sangue obtidas a partir dos indivíduos capturados foram submetidas à extração de DNA e ensaios de PCR, para a detecção de *Borrelia* spp. baseados no gene *flaB* e espaçador intergênico *rrs–rrlA* (IGS). A partir de três espécimes (5%) pertencentes a duas espécies de roedores da família Cricetidae (*Phyllotis xanthopygus* e *Oligoryzomys longicaudatus*) obtiveram-se três genótipos de *Borrelia* para o gene *flaB* e dois para IGS. Análises filogenéticas inferidas, usando-se os métodos Bayesiano e de Máxima Verossimilhança, indicaram que as sequências geradas neste estudo agrupam-se com borrelias do grupo da Febre Recorrente e Borreliose de Lyme. Os achados deste estudo sugerem que roedores *P. xanthopygus* e *O. longicaudatus* poderiam atuar como possíveis reservatórios para *Borrelia* spp. no Chile.

Palavras-chave: *Borrelia*, doenças infecciosas, pequenos mamíferos, reservatórios, roedor, Chile.

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Introduction

In Chile, studies on bacterial infections in small mammals have been performed mostly in the central and southern regions of the country (Müller et al., 2018; Llanos-Soto & González-Acuña, 2019). In the northern region of Chile, research regarding bacterial infection on mammals consists in three surveys focusing on *Escherichia* and *Salmonella* of marine vertebrates (*Otaria flavescens*) (Salinas et al., 2010; Sturm et al., 2011; Toro et al., 2015). Data on vector-borne bacterial pathogens transmitted by mites or ticks are absent for this region of the country.

Rodentia is among the most diverse mammal order in Chile, with 69 species distributed along the country (MMA, 2018). This group of vertebrates plays an important role in the maintenance and propagation of tick-borne pathogens (bacterial, protozoan and viral) in urban and natural environments (Llanos-Soto & González-Acuña, 2019). Globally, rodents act as hosts for tick populations and serve as reservoirs for zoonotic pathogenic agents, such as *Borrelia* species (Cutler, 2015; Cutler et al., 2017).

Spirochetes in the genus *Borrelia* merge their transmission cycles with vertebrates and their associated ticks in wild ecosystems (Kurtenbach et al., 1995; Talagrand-Reboul et al., 2018). For instance, in the Northern Hemisphere, human-pathogenic *Borrelia burgdorferi* sensu lato (s.l.) spirochetes use rodents and hard ticks (Acari: Ixodidae) of the *Ixodes ricinus* species complex as reservoirs and vectors, respectively, and are the causative agents of Lyme borreliosis (LB) in humans (Donahue et al., 1987; Kurtenbach et al., 1995; Hazler & Ostfeld, 1995; Rauter & Hartung, 2005). Moreover, small mammals and some species of soft ticks (Acari: Argasidae) of the *Ornithodoros* genus maintain natural foci of relapsing fever (RF) borreliae in tropical and subtropical ecosystems in both hemispheres (Talagrand-Reboul et al., 2018).

Despite their importance for the maintenance of *Borrelia* infections elsewhere, the role of rodents as sylvatic reservoirs for this genus of spirochetes has not been properly addressed in Chile, since most studies have focused on vectors rather than potential vertebrate reservoirs. For instance, only one valid genospecies, *Borrelia chilensis,* has been identified in *Ixodes stilesi* ticks collected from the environment, and from long-tailed pygmy rice rats (*Oligoryzomys longicaudatus*) (Ivanova et al., 2014). In this case, although ticks were infected with *B. chilensis*, this does not necessarily mean that *O. longicaudatus* were carrying the spirochetes, since positive nymphs could have acquired the bacterium through a previous blood meal (Guttman et al., 1996). A similar scenario encompasses the recent findings of novel *Borrelia* genotypes in *Ixodes sigelos* s.l. group, and an *Ornithodoros* sp. closely related with *Ornithodoros atacamensis* in northern Chile (Muñoz-Leal et al., 2019a, b), for which their associated hosts, i. e. rodents of genus *Phyllotis*, were not assessed for *Borrelia* infection (Muñoz-Leal et al., 2019a). In this context, to elucidate the identity of vertebrate reservoirs for *Borrelia* is still a crucial step to understand transmission cycles of these bacteria in Chilean ecosystems. In this study, we aimed to assess the role of rodents and marsupials from northern Chile as potential reservoirs for *Borrelia* spp. through molecular analyses performed in blood obtained from these mammals.

Material and Methods

Study area

This study surveyed rodents in five localities belonging to hyper-arid hydrographic regions from northern Chile (Figure 1) during July (Austral winter) of 2018. Hyper-arid hydrographic region in Chile is characterized by having an annual precipitation and potential evapotranspiration ratio <0.05; the annual precipitation does not exceeds 100 mm, presenting an annual water deficit higher than 1200 mm; and dryness prevails throughout the year with a short peak of humidity that lasts one month (MMA, 2018).

Sample collection

Rodents and marsupials were captured using Sherman-like traps. Eighty traps remained active during two consecutive nights (10 hours per night) in each locality, and were placed along four parallel lines distanced approximately 100 m from each other, with 20 traps per line (spaced 10 m between each other). Animal handling was performed according to protocols used in field and laboratory studies on rodents (Herbreteau et al., 2011). Fifty microliters of blood were collected from each captured rodent through puncture of the caudal ventral vein and stored in sterile tubes with 96% ethanol (Sigma-Aldrich®). All rodents were identified to the species level using a taxonomic guide (Iriarte, 2008), and after blood collection, were released in the same place of capture.

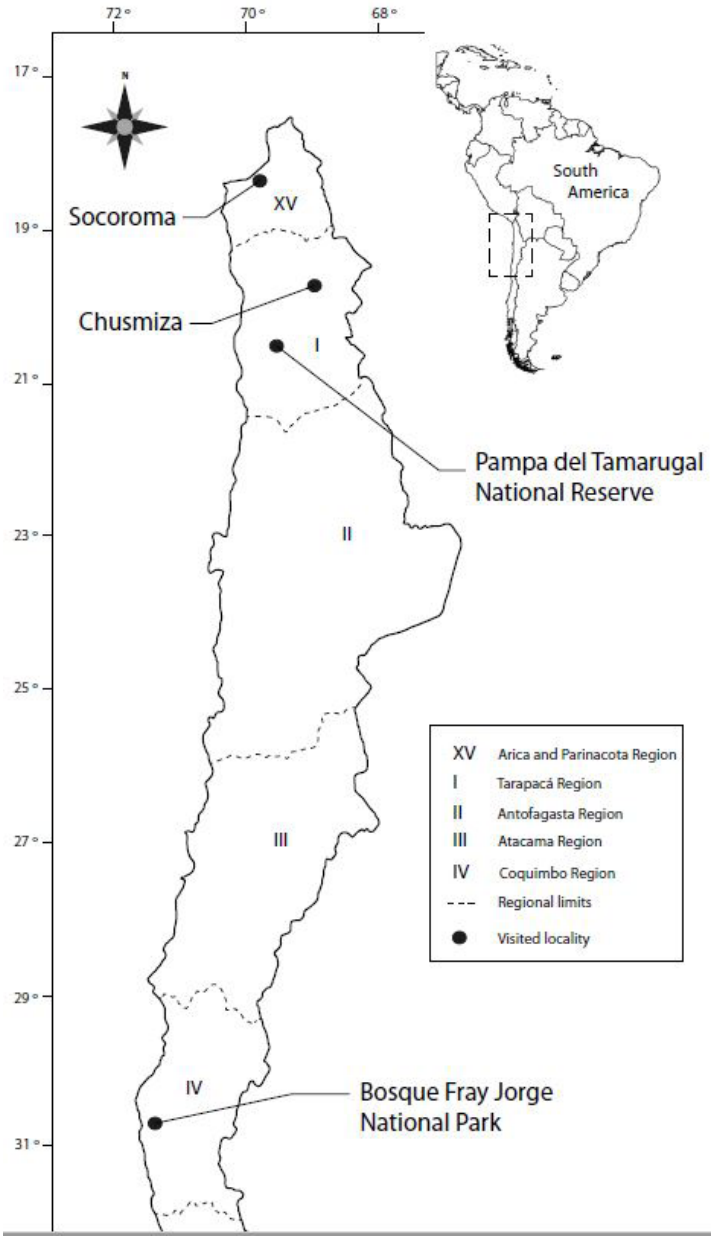

Figure 1. Map of northern Chile with the locations (black dots) were small mammals were captured.

Authorization for small mammal captures was granted by the Servicio Agrícola y Ganadero (SAG; Resolution N° 1532/2019 and 9071/2018). Field work in national parks and reserves was authorized by the Corporación Nacional Forestal (CONAF; Permits 39/2018; 67/2019; 05/2018; 76/2018; 66/2018). All procedures were approved and carried out according to the Bioethics Committee of the School of Veterinary Sciences, Universidad de Concepción (Form CBE-19-2017).

DNA extraction and gene amplification

DNA was extracted from blood using the DNeasy Blood & Tissue Kit (QUIAGEN, GERMANY). Forty microliters of Buffer AE (10 mM Tris-Cl; 0.5 mM EDTA, pH 9.0) were used to suspend the final DNA yield. To test successful extractions, and rule out the presence of PCR inhibitors, DNA quantity (concentration) and quality (purity and integrity) of DNA was assessed by A_{260} / A_{280} absorbance (A) in each sample using an Epoch™ Microplate Spectrophotometer. Samples with an A_{260} / A_{280} DNA ratio ranging between 1.6–2.0 were considered pure, and suitable for PCR amplification (Khare et al., 2014). Additionally, samples were tested through conventional PCR targeting endogenous *gapdh* (glyceraldehyde-3-phosphate dehydrogenase) gene as an internal control following Birkenheuer et al. (2003).

Successfully extracted samples were screened for *Borrelia* flagellin gene (*flaB)*, and *rrs–rrlA* intergenic spacer (IGS) through nested PCR protocols, using primers listed in Table 1. Reactions were performed into a final volume of 25 µL containing 12.5 µL of Dream Taq Green PCR Master Mix (Thermo Scientific, USA), 1 µL of each primer (10 pmol), 2 µL of DNA for conventional PCR, 1 µL of the product for nested rounds, and ultra-pure water to complete the final volume of the mix. Amplicons were verified in 1.5% agarose gels stained with SYBR Safe (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA), and visualized through UV light. Positive samples were purified and sequenced in both directions at AUSTRAL-omics (Valdivia, Chile).

Table 1. List of primers used for the detection and characterization of *Borrelia* DNA in this study.

Target	Primer	Sequence	Expected length (bp)	Reference
Flagelin gene fragments (*flaB*)	FlaLL	5´-ACATATTCAGATGCAGACAGAGGT-3´	658	(Stromdahl et al., 2003)
	FlaRL	5´-GCAATCATAGCCATTGCAGATTGT-3´		
	FlaLS	5´-AACAGCTGAAGAGCTTGGAATG-3´	354	
	FlaRS	5´-CTTTGATCACTTATCATTCTAATAGC-3´		
rrs–rrlA intergenic spacer (IGS)	IGS-F	5´-GTATGTTTAGTGAGGGGGGTG-3´	987	(Bunikis et al., 2004)
	IGS-R	5´-GGATCATAGCTCAGGTGGTTAG-3´		
	IGS-Fn	5´-AGGGGGGTGAAGTCGTAACAAG-3´	945	
	IGS-Rn	5´-GTCTGATAAACCTGAGGTCGGA-3´		

Phylogenetic analyses

The obtained sequences were edited with ProSeq Version (V) 3 (Filatov, 2009), compared using BLASTn (https://blast.ncbi.nlm.nih.gov), and aligned with records from the NCBI database using the ClustalW algorithm (Thompson et al., 1994) implemented in MEGA 7.0 (Kumar et al., 2016). Alignments for *flaB* gene and IGS were used to construct both Bayesian Inference (BI) and Maximun Likelihood (ML) trees with MrBayes 3.2.2. (Ronquist et al., 2012), and IQ-TREE v1.6.12 (Nguyen et al., 2015), respectively. We chose these methods because they are based on models of molecular evolution (Huelsenbeck et al., 2001; Felsenstein, 2004). Evolutionary models for BI were selected using MrBayes 3.2.2, employing the option "lset nst=mixed rates=gamma", and the Bayesian Information Criterion (BIC) (Schwarz, 1978). ModelFinder (Kalyaanamoorthy et al., 2017) with the option "-m MFP+MERGE", and the BIC were employed to select best evolutionary models for the ML analysis (Schwarz, 1978).

BI was performed with two independent tests of 10^7 generations, running four MCMC chains, sampling trees every 1000 generations, and discarding the first 25% as burn-in. MCMC chain correlation was confirmed with Tracer v1.7.1 (Rambaut et al., 2018). Statistical support of internal nodes was evaluated employing Bayesian posterior probabilities (BPPs) and considering values ≥0.70 as strong support (Huelsenbeck & Ronquist, 2001). The ML analysis was carried using rapid hill-climbing and stochastic disturbance methods, evaluating the robustness of the inferred tree with 1000 pseudo-replicates of ultrafast bootstrapping. We used the criteria of Minh et al. (2013) to evaluate the ultrafast bootstrap: values <70% were considered non-significant statistical support; values between 70-94% as moderately significant; and values ≥ 95% as highly significant.

Results

Positive animals and PCR

A total of 58 small mammals belonging to 12 species in the families Cricetidae, Muridae and Didelphidae were captured (Table 2). Although DNA purity obtained after measurements of A_{260} / A_{280} absorbance ratio was optimal in 56/58 of the samples (97%), five samples (including the two with low A_{260} / A_{280} absorbance ratio) were negative after GAPDH gene PCR and excluded from further analyses. Three out of 53 rodents (5%) were positive for *Borrelia flaB* screening, and two of these samples were positive for IGS (4%) (Table 2).

Table 2. Number and identity of small mammals captured in Northern Chile. Specimens positive for *Borrelia* by PCR assays are highlighted in bold. Abbreviations: BFJNP, Bosque Fray Jorge National Park; Ch, Chusmiza; Par, Parinacota; PTNR, Pampa del Tamarugal National Reserve; Soc, Socoroma.

Order	Family	Species	Locality	Geographic coordinates	No. Positive/ No. Capture
Didelphimorphia	Didelphidae	*Thylamys elegans*	BFJNP	30°39'07.07"S, 71°41'09.44"W	0/2
Didelphimorphia	Didelphidae	*Thylamys pallidor*	Soc	18°16'44.30"S, 69°35'28.40"W	0/1
Rodentia	Cricetidae	*Abrothrix andinus*	Soc	18°12'00.00"S, 69°16'00.12"W	0/1
Rodentia	Cricetidae	*Abrothrix andinus*	Par	18°12'00.00"S, 69°16'00.12"W	0/4
Rodentia	Cricetidae	*Abrothrix berlepschii*	Par	18°12'00.00"S, 69°16'00.12"W	0/1
Rodentia	Cricetidae	*Abrothrix berlepschii*	Soc	18°16'44.30"S, 69°35'28.40"W	0/4
Rodentia	Cricetidae	*Abrothrix jelskii*	Par	18°12'00.00"S, 69°16'00.12"W	0/1
Rodentia	Cricetidae	*Abrothrix longipilis*	BFJNP	30°39'07.07"S, 71°41'09.44"W	0/1
Rodentia	Cricetidae	*Abrothrix olivacea*	BFJNP	30°39'07.07"S, 71°41'09.44"W	0/3
Rodentia	**Cricetidae**	***Oligoryzomys longicaudatus***	**BFJNP**	**30°39'07.07"S, 71°41'09.44"W**	**1/1**
Rodentia	Cricetidae	*Phyllotis limatus*	Soc	18°16'44.30"S, 69°35'28.40"W	0/1
Rodentia	Cricetidae	*Phyllotis magister*	Soc	18°16'44.30"S, 69°35'28.40"W	0/1
Rodentia	Cricetidae	*Phyllotis xanthopygus*	Ch	19°41'01.27"S, 69°11'53.05"W	0/4
Rodentia	Cricetidae	*Phyllotis xanthopygus*	PTNR	20°28'14.03"S, 69°40'25.16"W	0/4
Rodentia	Cricetidae	*Phyllotis xanthopygus*	Par	18°12'00.00"S, 69°16'00.12"W	0/5
Rodentia	**Cricetidae**	***Phyllotis xanthopygus***	**Soc**	**18°16'44.30"S, 69°35'28.40"W**	**2/16**
Rodentia	Muridae	*Mus musculus*	Soc	18°16'44.30"S, 69°35'28.40"W	0/2
Rodentia	Muridae	*Rattus rattus*	PTNR	20°28'14.03"S, 69°40'25.16"W	0/1
Total					**3/53**

Three different genotypes were obtained for *flaB* gene. Two *flaB* sequences of 304 bp (99.67% of identity between them) were retrieved from blood of two *Phyllotis xanthopygus* collected in Socoroma (named as *Borrelia* sp. A10 and *Borrelia* sp. A44). BLASTn comparisons revealed that *flaB* sequences for *Borrelia* sp. A10 and *Borrelia* sp. A44 were 97.48% (271/278 bp, 91% query cover, 0 gap, 5e-130 E-value) and 97.12% (270/278 bp, 91% query cover, 0 gap, 2e-128 E-value) identical with *Borrelia* sp. 95325 (HM583797) characterized from undetermined *Ornithodoros* sp. from Bolivia (Parola et al., 2011), respectively. On the other hand, a different genotype of *flaB* gene (307 bp) was obtained from one *O. longicaudatus* captured in Bosque Fray Jorge National Park. After BLASTn comparisons, this sequence (named as *Borrelia* sp. A53) was 98.70% (303/307 bp, 94% query cover, 0 gap, 3e-151 E-value) identical to *Borrelia* spp. characterized from ticks belonging to *I. sigelos* group in Chile (MH187987 and MH178397; Muñoz-Leal et al., 2019a). The sequences of *flaB* from *Borrelia* sp. A10, A44, and A53 were deposited in GenBank under accession numbers MN596012, MN596013, and MN596014, respectively.

Two different IGS sequences were obtained from the same samples of *P. xantophygus* positive for *flaB* gene. Sequences were 96.49% identical between them and 94.36% identical (202/214 bp, 41% query cover, 3 gaps, 8e-85 E-value) to *Borrelia* sp. TM (DQ000283; referred as "cf. *Borrelia crocidurae*" amplified from ticks). IGS sequences from *Borrelia* sp. A10 and A44 were deposited in Genbank under accession numbers MN598782 and MN598783, respectively.

Phylogenetic analyses

Overall, BI and ML phylogenetic trees for *flaB* and IGS depicted similar and well-supported logic topologies, grouping *Borrelia* spp. into LB and RF groups. In particular, phylogenetic analyses for *flaB* gene positioned our sequences within a clade with *Borrelia* sp. 95325 (HM583797) having statistically significant support (BPP=1

in Figure 2A, and Bootstrap=100 in Figure 2B). For the BI analysis, the clade composed by our sequences and *Borrelia* sp. 95325 formed a clade with high statistical support (BPP=0.98 in Figure 2A) with *Borrelia latyschewii* (JF708952), *Borrelia microti* (JF708951), *Borrelia duttonii* (NC011229), *Borrelia recurrentis* (CP000993), *Borrelia crocidurae* (CP004267), *Borrelia hispanica* (MF432465), and *Borrelia persica* (NZAYOT01000225). However, the internal relations of this clade were undefined. On the other hand, with high support (Bootstrap=97 in Figure 2B), the ML analysis supported *Borrelia* sp. A10 and A44 as independent branches into a monophyletic group with *Borrelia* sp. 953225 (HM583797). On the other hand, and with high statistical support (BPP= 0.84 in Figure 2A, and Bootstrap= 90 in Figure 2B), *Borrelia* sp. A53 clustered with *Borrelia* genotypes characterized from ticks belonging to the *I. sigelos* group (MH187987, MH178397), with *B. chilensis* VA1 (CP009910), and *Borrelia* sp. ISIG1 (KX417768) obtained from *I.* cf. *neuquenensis,* and *I. sigelos* from Argentina as sister groups (Figure 2).

With high statistical support (BBP= 1 in Figure 3A, and Bootstrap= 96 in Figure 3B), phylogenetic analyses for IGS sequences showed that *Borrelia* sp. A10 and *Borrelia* sp. A44 form an independent clade related to *Borrelia* spp. belonging to the relapsing fever group (Figure 3).

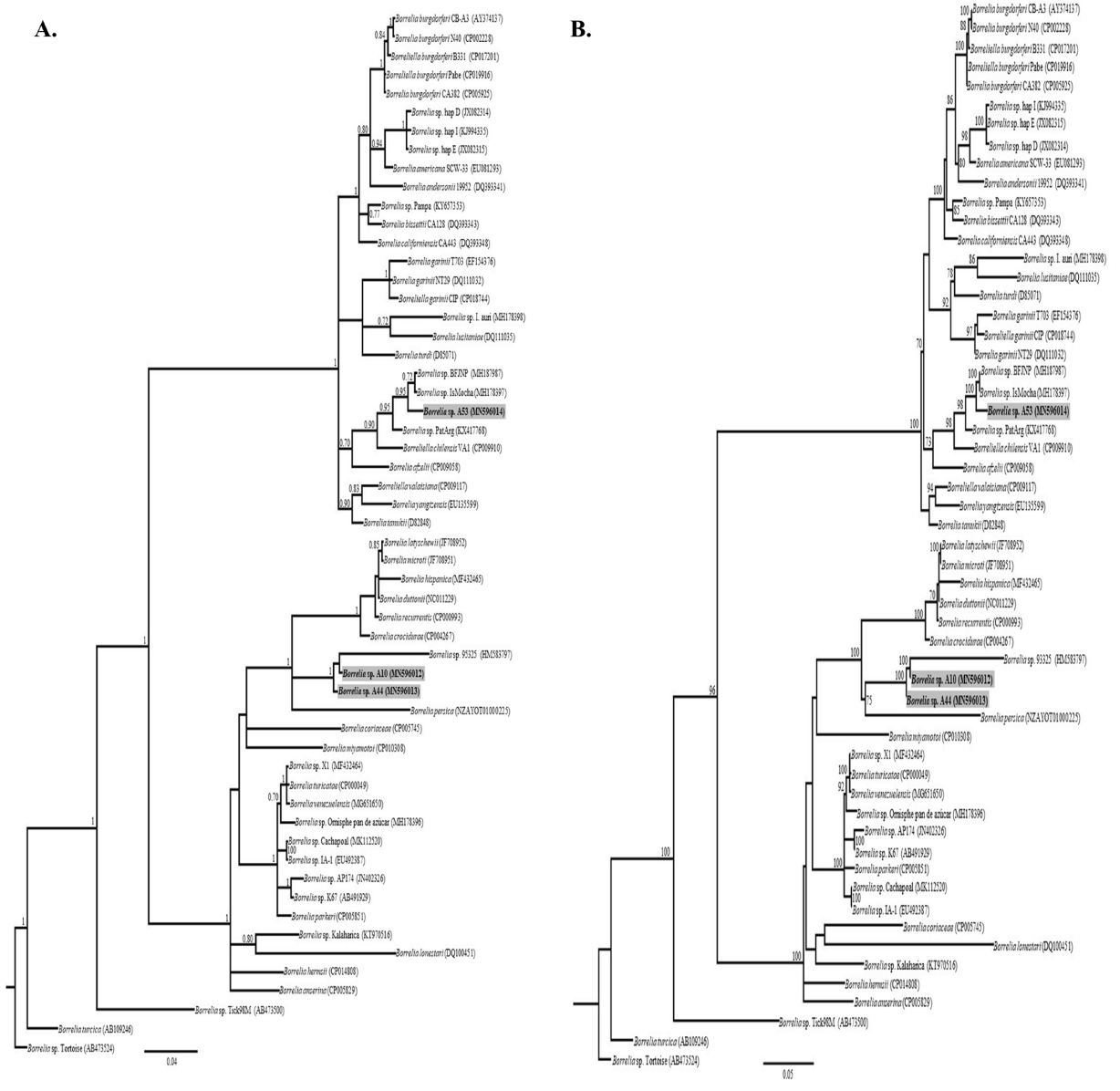

Figure 2. Bayesian (A) and Maximum Likelihood (B) phylogenetic trees for *Borrelia flaB* gene (alignment length, 657 bp). Otherwise omitted, numbers above branches represent BPP and bootstrap values ≥ 0.70 and ≥ 70%, respectively. GenBank accession numbers for the sequences included in the analyses are embedded in each tree. The position of *Borrelia* sp. A10, A44, and A53 are highlighted in bold with a gray background.

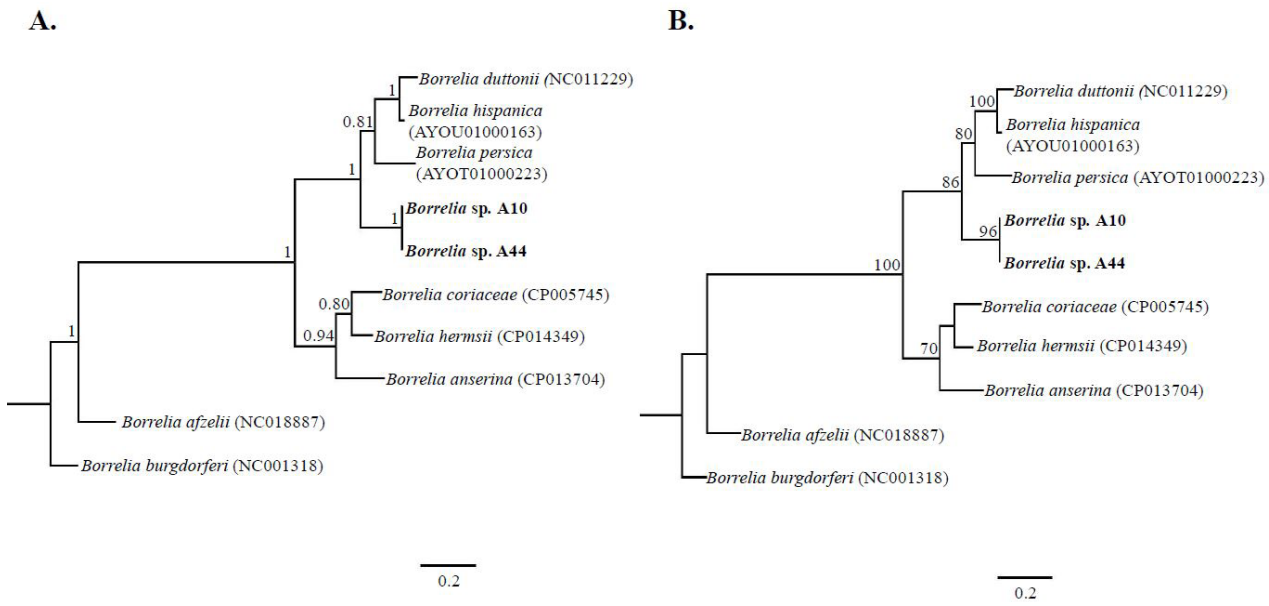

Figure 3. Bayesian (A) and Maximum Likelihood (B) phylogenetic trees for *Borrelia* IGS sequences gene (alignment length, 713 bp). Otherwise omitted, numbers above branches represent BPP and bootstrap values ≥ 0.70 and ≥ 70%, respectively. GenBank accession numbers for the sequences included in the analyses are embedded in each tree. The position of *Borrelia* sp. A10, and A44 are highlighted in bold with a gray background.

Discussion

The identification of wild vertebrate reservoirs implicated in the maintenance of pathogenic agents should be considered a permanent task in scientific research (Karesh et al., 2012). Rodents are important sylvatic reservoirs, as at least 217 out of 2777 known species harbor 66 zoonotic agents (Han et al., 2015). Considering this scenario, and the recent detection of *Borrelia* spp. in rodent-associated ticks in Chile, we aimed to assess the presence of *Borrelia* DNA in 12 species of small mammals (ten rodents and two marsupials) from this country. We detected DNA of *Borrelia* spp. belonging to the LB and RF groups in two cricetid rodents, namely *P. xanthopygus* and *O. longicaudatus*, respectively. While cricetid rodents (i. e. *Peromyscus leucopus*) have been previously reported as competent reservoirs for *Borrelia burgdorferi* sensu stricto (s.s.) in North America (Levine et al., 1985; Hofmeister et al., 1999; Bunikis et al., 2004), high prevalence for *Borrelia burgdorferi* s.l. have been reported in synanthropic murid rodents (*Mus musculus* and *Rattus rattus*) too (Solís-Hernández et al., 2016). In our study, synanthropic *M. musculus* were negative to *Borrelia* detection, a fact that could be attributed to the small sample of this species that was analyzed.

Apart from rodents, we assessed blood from marsupials, but with negative results. Nevertheless, *Borrelia*-like spirochetes have already been isolated from opossums (Marsupialia) in the United States (Hanson, 1970), so the role of Chilean marsupials as hosts for *Borrelia* spp. should not be discarded. Studies focusing on small mammals and enzootic cycles of borrelial spirochetes have been performed only for Northern (Levine et al., 1985; Hofmeister et al., 1999; Bunikis et al., 2004) and Central American species (Solís-Hernández et al., 2016). Whether rodents may act as reservoirs for *Borrelia* in South America remained unknown until the current study.

Parola et al. (2011) detected a RF *Borrelia* sp. (*Borrelia* sp. 95325, HM583797) in an undetermined *Ornithodoros* sp. collected in Bolivia. Remarkably, the *flaB* gene sequence retrieved by Parola et al. (2011) formed an independent clade with the sequences obtained in our study (*Borrelia* sp. A10 and *Borrelia* sp. A44), suggesting a close phylogenetic relationship. The phylogenetic resolution of the *flaB* gene has been useful to define lineages in the genus *Borrelia* (Fukunaga et al., 1996). In agreement with this fact, our BI and ML analyses indicated with high support that the detected genotypes constitute putatively new species (Figure 2). According to these results, the BI and ML phylogenies for IGS also point that the sequences of *Borrelia* sp. A10 and *Borrelia* sp. A44 constitute novel taxa, related to RF borreliae (Figure 3). To date, only one rodent parasitized by an *Ornithodoros* sp. has been proposed as putative reservoir for a RF *Borrelia* sp. in Chile (Muñoz-Leal et al., 2019b). However, the sequences detected in this study differ from *Borrelia* genotypes previously detected in soft ticks from this country (Muñoz-Leal et al., 2019a).

Different genotypes of *flaB* gene belonging to the LB group have been reported in Chile, namely *Borrelia* sp. Navarino (MH178398), characterized from *Ixodes auritulus* collected in the bird a *Troglodytes musculus*, and

several genotypes related to *B. chilensis* from ticks of the *I. sigelos* group (MH178397, MH187987, CP009910; Ivanova et al.2014; Muñoz-Leal et al., 2019a). Even though those studies have made valuable contributions to the understanding of the diversity of *Borrelia* in Chile, evidence of mammal hosts acting as reservoir for these agents was previously non-existent. In this study, sequences of *Borrelia flaB* gene retrieved from blood of *O. longicaudatus* (*Borrelia* sp. A53) branched as independent genotypes within the LB group of borreliae (Figure 2). Remarkably, *Borrelia* sp. A53 was also related to *B. chilensis* (CP009910), a genospecies previously reported in *I. stilesi* parasitizing the same rodent species in Southern Chile (Ivanova et al., 2014). Moreover, genotypes of *Borrelia* detected in larvae and nymphs of *I. sigelos* s.l. collected on *P. darwini* and *Octodon degus* (Muñoz-Leal et al., 2019a) clustered with *Borrelia* sp. A53 as well. This fact suggests that LB genotypes of *Borrelia* associated with rodents could constitute a monophyletic group related to *B. chilensis*. A similar hypothesis pointing a natural group of borreliae infecting rodents has been proposed through phylogenetic analyses using *Borrelia* genotypes detected in rodent-associated ticks in Southern Argentina (Sebastian et al., 2016).

To our knowledge, this is the first report of infection by *Borrelia* spp. in small mammals from Chile and South America, and the first isolation of DNA from these spirochetes in *P. xanthopygus* and *O. longicaudatus*. Our results suggest that these rodents may act as potential reservoirs for novel *Borrelia* genotypes in natural ecosystems of northern Chile. However, future studies are needed to further determine the competence of these rodents in maintaining *Borrelia* infections, and to investigate if other species of small mammals participate in the enzootic cycles of these spirochetes as well.

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