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Borrelia burgdorferi sensu lato infecting *Ixodes auritulus* ticks in Uruguay

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Abstract
In the southern cone of South America different haplotypes of *Borrelia burgdorferi* sensu lato (Bbsl) have been detected in *Ixodes* spp. from Argentina, southern Brazil, Chile, and Uruguay. So far, Lyme borreliosis has not been diagnosed in Uruguay and the medical relevance of the genus *Ixodes* in South America is uncertain. However, the growing number of new genospecies of Bbsl in the southern cone region and the scarce information about its pathogenicity, reservoirs and vectors, highlights the importance of further studies about spirochetes present in Uruguay and the region. The aim of this study was to determine the presence of Bbsl in *Ixodes auritulus* ticks collected from birds and vegetation in two localities of southeastern Uruguay. In total 306 *I. auritulus* were collected from 392 passerine birds sampled and 1110 ticks were collected by flagging in vegetation. Nymphs and females were analyzed for *Borrelia* spp. by PCR targeting the flagellin (*fla*) gene and the *rrfA-rrlB* intergenic spacer region (IGS). The phylogenetic analysis of *Borrelia* spp. positive samples from passerine birds and vegetation revealed the presence of four *fla* haplotypes that form a clade within the Bbsl complex. They were closely related to isolates of *Borrelia* sp. detected in *I. auritulus* from Argentina and Canada.

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Introduction

Borrelia burgdorferi sensu lato (Bbsl) is a complex of spirochaetal species, which includes at least 23 genospecies, mostly associated to hard ticks of the genus *Ixodes* (Casjens et al. 2011; Ivanova et al. 2014; Margos et al. 2011; Scott et al. 2017; Stanek and Reiter 2011). Within this complex, *Borrelia burgdorferi* sensu stricto, *Borrelia afzelii*, and *Borrelia garinii* are the major etiological agents of Lyme borreliosis (Baranton et al. 1992; Burgdorfer et al. 1982), a tick-borne infectious disease of humans. Additionally, other species as *B. spielmanii, B. mayonii B. bavariensis, B. bissettiae, B. kurtenbachii, B. lusitaniae* and *B. valaisiana* have also been associated to Lyme borreliosis in humans (Le Fleche et al. 1997; Margos et al. 2010, 2013, 2016; Pritt et al. 2016; Richter et al. 2006; Wang et al. 1999). Four tick species belonging to the *Ixodes ricinus* complex are major vectors for the transmission of Bbsl to humans: *I. scapularis* and *I. pacificus* in North America, and *I. ricinus* and *I. persulcatus* in Europe and Asia (Steere et al. 2016). In Uruguay, there are four *Ixodes* species currently identified: *I. auritulus*, *I. longiscutatus, I. loricatus* and *I. aragaoi* (Nava et al. 2017; Onofrio et al. 2014). The only member of the *I. ricinus* complex in Uruguay is *I. aragaoi* (Nava et al. 2017), and to the present there are no reports of it parasitizing humans (Guglielmone et al. 2014). In the southern cone of America, different haplotypes of Bbsl have been detected in *Ixodes* spp. from Argentina, southern Brazil, Chile, and Uruguay (Barbieri et al. 2013; Cicuttin et al. 2019; Dall'Agnol et al. 2017; Ivanova et al. 2014; Nava et al. 2014; Saracho-Bottero et al. 2017; Sebastian et al. 2016). Uruguayan *Borrelia* genotypes isolated from *I. aragaoi* belong to five different haplotypes of the flagellin (*fla*) gene, called A, B, C, D and E. From those five haplotypes, haplotypes A to C are similar to *Borrelia bissettiae*, whereas haplotypes D and E are associated with *Borrelia americana* (Barbieri et al. 2013). In Argentina, two genospecies belonging to the Bbsl complex were detected and isolated from *I. pararicinus*: one highly similar to haplotypes A to C from Uruguay, and another, similar to haplotypes D and E (Nava et al. 2014; Saracho-Bottero et al. 2017). The similarities between the Argentinian and Uruguayan *Borrelia* haplotypes detected in the ticks may be due to the fact that *I. aragaoi* and *I. pararicinus* are closely related to one another (Nava et al. 2017; Onofrio et al. 2014; Saracho-Bottero et al. 2017; Venzal et al. 2005a). Furthermore, in Chile, Argentina and Brazil, Bbsl have been detected infecting *Ixodes* species that are not members of the *I. ricinus* complex. In Chile, *Borrelia chilensis* was described in association with *I. stilesi* and *Borrelia* sp. Navarino in *I. auritulus* (Ivanova et al. 2014; Muñoz-Leal et al. 2019). In the Argentinean Patagonia, *I. neuquenensis* and *I. sigelos,* both conforming a phylogenetic group with *I. stilesi*, were found to be infected with a new genospecies of Bbsl, named *Borrelia* sp. haplotype Patagonia, phylogenetically related to the corresponding sequence of *B. chilensis* (Sebastian et al. 2016). In Buenos Aires city, Argentina, *I. auritulus* was found infected with a *Borrelia* sp. related to a *Borrelia* sp. detected in *I. auritulus* from Canada (Cicuttin et al. 2019). In Rio Grande do Sul, the southernmost state of Brazil, *Borrelia* sp. haplotype Pampa has been detected in *I. longiscutatus* (Dall'Agnol et al. 2017). It is worth noting that these reports do not necessarily involve a risk for human health, since the pathogenicity of these new genospecies is unknown. With the exception of a single record of a *I. pararicinus* nymph parasitizing human in the Yungas forests of Argentina

(Saracho-Bottero et al. 2018), none of the *Ixodes* species, from which the *Borrelia* genospecies were detected, has been found parasitizing humans.

Ixodes auritulus is a tick species with a worldwide distribution that parasitizes several orders of birds, with passerine birds probably the main hosts (González-Acuña et al. 2005). Rodents are considered exceptional hosts for this tick, there are no records of *I. auritulus* on other mammals (including humans) (Guglielmone et al. 2014; Nava et al. 2017). It has been reported transstadial transmission of *B. burgdorferi* in the larva-nymph and nymph-adult molts of *I. auritulus* suggesting vector competence for Bbsl (Scott et al. 2015, 2018a). Furthermore, Scott et al. (2015, 2018a) showed that *I. auritulus* is involved in the enzootic maintenance cycle of Bbsl in British Columbia, Canada.

The aim of this study was to determine the presence of Bbsl in *I. auritulus* collected from birds and vegetation in southeastern Uruguay.

Materials and methods

Tick collection and identification

Ticks were retrieved from birds and collected on vegetation from April 2013 to December 2014. Eight tick samplings were made, two per season, in two localities of southeastern Uruguay: Reserva Natural Salus (Lavalleja Department; 34° 25′ S, 55° 18′ W) and Laguna Negra (Rocha Department; 34° 03′ S, 53° 40′ W). Both localities belong to the ecoregion Sierras del Este sensu Brazeiro et al. (2012). Free-living ticks were collected by flagging vegetation along animal trails and footpaths. Birds were captured using mist nets, which remained active from dawn to dusk. Birds were caught with permission of Uruguayan authorities from the Departamento de Fauna, Ministerio de Ganadería, Agricultura y Pesca (Resolution 368/14). Bird species were determined in the field following Narosky and Yzurieta (2003) and Olmos (2011) taxonomic keys. Nomenclature follows the convention of Clements et al. (2016). Each bird was examined for ticks using entomological forceps, and then released. The ticks obtained were immediately stored in 95% ethanol. At the laboratory, ticks were morphologically identified using a stereoscopic microscope and keys for larval, nymph and adult stages (Durden and Keirans 1996; Keirans and Clifford 1978; Kleinjan and Lane 2008; Nava et al. 2017; Onofrio et al. 2006).

DNA extraction and PCR amplification

For molecular analysis, adult ticks and nymphs were pooled by stage (1–20 adult or nymphs per pool) according to source (bird/vegetation) and date of collection. Larvae were not included in this study. Ticks were bisected longitudinally using sterile scalpel blades and forceps, rinsed with distilled water to remove ethanol, and crushed with a homogenization pestle. DNA was extracted using Pure Link™ Genomic DNA Kit (Invitrogen™ USA) following the manufacturer's instructions. Molecular screening of *Borrelia* spp. was done as previously described by Barbieri et al. (2013). Briefly, nested-PCR targeting the *flagellin* gene (*fla*) of *Borrelia* spp. was performed using primers FlaRL (5′-GCA ATC ATA GCC ATT GCA GAT TGT-3′) and FlaLL (5′-ACA TAT TCA GAT GCA GAC AGA GGT-3′) that amplify a fragment of 665 bp, and for nested amplification primers FlaRS (5′-CTT TGA TCA CTT ATC ATT CTA ATA GC-3′) and FlaLS (5′-AAC AGC TGA AGA GCT TGG AAT G-3′) that targets a fragment of 354 bp of *fla* gene (Barbour et al. 1996). Some

positive samples to *fla* gene were further analyzed by PCR for the presence of a 225 to 255 bp fragment of the *rrfA-rrlB* intergenic spacer region (IGS) using primers IGSb (5′-AGC TCT TAT TCG CTG ATG GTA-3′) and IGSa (5′-CGA CCT TCT TCG CCT TAA AGC-3′) (Derdáková et al. 2003). All PCR reactions were performed including water and *B. anserina* DNA as negative and positive control, respectively. PCR products were analyzed in a 1.5% agarose gel by electrophoresis. Amplicons were purified and sent to the Institut Pasteur de Montevideo (Uruguay) for sequencing.

Sequence comparison and phylogenetic analysis

The sequences were assembled and compared using Lasergene software (DNAStar, Madison, WI). The alignments and phylogenetic analysis were performed using MEGA 6.06 (Tamura et al. 2013). The *fla* and IGS partial sequences (354 bp and 252 bp, respectively) of *Borrelia* spp. obtained in this study were aligned with the respective sequences of Bbsl genotypes retrieved from the GenBank. The best fitted nucleotide substitution model (GTR + gamma) for ours datasets was selected using jModelTest (Posada 2008). Maximum likelihood trees were conducted with 1000 bootstrap replicates. Sequences of *Borrelia hermsii* and *B. anserina* were included in *fla* phylogenetic inference as out-groups.

Results

Ticks

During the study, 392 birds corresponding to 43 species belonging to five orders and 18 families were captured (Table 1). Of those, 108 (27.5%) were captured in autumn, 90 (23.0%) in winter, 133 (33.9%) in spring, and 61 (15.6%) in summer. Of the 392 birds sampled, 355 (90.6%) corresponded to the order Passeriformes, split in 14 families. During this study, we found bird-feeding ticks parasitizing only specimens of the order Passeriformes. Seventy-eight of the total birds examined (19.9%), were found parasitized with *I. auritulus* (Table 1). The prevalence of *I. auritulus* infestation was 21.3% in autumn, 23.3% in winter, 12.8% in spring and 27.9% in summer (Table 1). Three hundred and six *I. auritulus* ticks (167 larvae, 115 nymphs and 24 females) were retrieved from bird specimens belonging to eight families of passerines (Table 2a), and a total of 1110 *I. auritulus* free-living ticks were collected from vegetation: 847 larvae, 186 nymphs and 77 females (Table 2b).

Among the bird families parasitized, the Turdidae family, represented by three species, accumulated 79.7% (244/306) of the total *I. auritulus* collected on birds in the study. The prevalence of *I. auritulus* infestation in *Turdus* spp. was 41.6% in autumn, 71.4% in winter, 46.7% in spring and 94% in summer.

We described only the results about *I. auritulus* ticks, although during the study *I. aragaoi*, *Haemaphysalis juxtakochi* and *Amblyomma* spp. were also retrieved from birds and collected on vegetation, which information will be used in future analyzes.

Table 1 Birds collected and infested with *Ixodes auritulus* in southeastern Uruguay (2013–2014)

Order	Family	Species	No birds	Birds/birds infested with *I. auritulus*			
				Autumn	Winter	Spring	Summer
Gruiformes	Rallidae	*Aramides cajaneus*	1	1/0			
Columbiformes	Columbidae	*Leptotila verreauxi*	4		1/0	1/0	2/0
Apodiformes	Trochilidae	*Chlorostilbon lucidus*	3			2/0	1/0
		Hylocharis chrysura	4			4/0	
		Leucochloris albicollis	17	3/0		8/0	6/0
Piciformes	Picidae	*Colaptes melanochloros*	3	1/0		1/0	1/0
		Picumnus nebulosus	2	1/0			1/0
		Veniliornis spilogaster	3	1/0	1/0	1/0	
Passeriformes	Thamnophilidae	*Thamnophilus caerulescens*	1				1/1
		Thamnophilus ruficapillus	2		1/1	1/1	
	Furnariidae	*Cranioleuca pyrrhophia*	5	1/0	3/0		1/0
		Furnarius rufus	5	4/0	1/0		
		Lochmias nematura	2			2/1	
		Synallaxis spixi	5	2/0	1/1	1/1	1/0
		Syndactyla rufosuperciliata	1	1/0			
	Tyrannidae	*Elaenia parvirostris*	28	2/0		25/0	1/0
		Knipolegus cyanirostris	3			3/0	
		Lathrotriccus euleri	1			1/0	
		Myiarchus swainsoni	2			2/0	
		Phylloscartes ventralis	10	5/0	3/1		2/0
		Pitangus sulphuratus	7	3/0	2/0	2/0	
		Serpophaga subcristata	7		5/0	2/0	
		Tyrannus savana	2			2/0	
	Tityridae	*Pachyramphus polychopterus*	2			2/0	
	Vireonidae	*Cyclarhis gujanensis*	5	3/0	2/0		
		Vireo olivaceus	8			7/0	1/0
	Troglodytidae	*Troglodytes aedon*	12	4/0	3/1	5/0	
	Turdidae	*Turdus albicollis*	38	16/10	7/7	11/8	4/3
		Turdus amaurochalinus	13	5/1	5/2	2/2	1/1
		Turdus rufiventris	53	15/4	9/6	17/4	12/12
	Mimidae	*Mimus triurus*	1		1/0		
	Parulidae	*Basileuterus culicivorus*	25	8/2	7/1	3/0	7/0
		Myiothlypis leucoblephara	12	4/0	1/0	3/0	4/0
		Setophaga pitiayumi	8		6/0		2/0
	Thraupidae	*Poospiza cabanisi*	13	5/3	1/0	4/0	3/0
		Saltator similis	2	2/0			
		Sicalis flaveola	3			3/0	
		Stephanophorus diadematus	6	3/2		2/0	1/0
		Tangara preciosa	23	3/0	18/0	2/0	
	Emberizidae	*Zonotrichia capensis*	46	15/1	12/1	10/0	9/0
	Cardinalidae	*Cyanoloxia glaucocaerulea*	1			1/0	

Table 1 (continued)

Order	Family	Species	No birds	Birds/birds infested with *I. auritulus*			
				Autumn	Winter	Spring	Summer
	Icteridae	*Molothrus bonariensis*	2			2/0	
	Fringillidae	*Spinus magellanicus*	1			1/0	
		Total	392	108/23	90/21	133/17	61/17
		Birds per season (%)		27.5	23	33.9	15.6
		Birds infested with ticks (%)		21.3	23.3	12.8	27.9

Molecular detection of *Borrelia* spp.

Seventy-three pools (55 pools of nymphs and 18 pools of females), corresponding to 139 *I. auritulus* (115 nymphs and 24 females) retrieved from 60 birds, were tested by *fla* PCR for detection of *Borrelia* spp. Fifteen pools of nymphs (minimum infection rate: 13%) and two pools of females (minimum infection rate: 8.3%) resulted in Bbsl positives. They corresponded to ticks obtained from 17 birds (Table 3a). Furthermore, *fla* PCR analysis of 263 *I. auritulus* (186 nymphs and 77 females) collected from vegetation revealed the presence of *Borrelia* spp. in 12 pools of nymphs (minimum infection rate: 6.4%) and two pools of females (minimum infection rate: 2.6%) of 35 pools tested (19 and 16 of nymphs and females, respectively) (Table 3b). There were *Borrelia* spp. positive samples through all seasons. The *Borrelia* spp. *fla* gene fragments obtained from all positive tick pools were sequenced. They revealed the presence of four different *fla* haplotypes; named as *Borrelia* sp. *I. auritulus* UY1, UY2, UY3, and UY4 (registered in GenBank accession numbers MK160129, MK160130, MK160131, and MK160132, respectively). We did not observe a correlation between haplotype and bird species or collection season. UY4 was the most common haplotype, found in 11 pools of ticks collected from birds and in all pools from vegetation (Table 3). As the screening was done using tick pools we don't discard the possible presence of more haplotypes. In the phylogenetic reconstruction of the *fla* gene, those four Uruguayan haplotypes formed a well-supported monophyletic clade within the Bbsl complex. The four haplotypes are closely related to sequences of *Borrelia* spp. detected in *I. auritulus* from Argentina (haplotypes G1138 and G1000; GenBank accession number MK984829 and MK984824) and Canada (isolate Cn186-BbPCR2; GenBank accession number KT827332) (Fig. 1). The *fla* haplotypes UY3 and UY4 were subjected to an additional PCR targeting an IGS fragment. Both of them resulted positive and were successfully sequenced (GenBank accession numbers MK160133, MK160134). The IGS phylogenetic tree showed the Uruguayan haplotypes clustering together with Bbsl British Columbia genotype 1 sequences obtained by Scott et al. (2010) from *I. auritulus* of Canada (GenBank accession numbers EU019112.1, EU019121.1 and EU019120.1) (Fig. 2).

Table 2 Larvae (L), nymphs (N) and adults (A) of *Ixodes auritulus* collected from (a) passerine birds and (b) vegetation

(a) Birds species	No birds	No infested birds	*I. auritulus*													Mean intensity	Mean abundance
			Autumn			Winter			Spring			Summer					
			L	N	A	L	N	A	L	N	A	L	N	A	Total		
Thamnophilidae																	
T. caerulescens	1	1										0	1	0	1	1.00	1.00
T. ruficapillus	2	2				5	0	0	0	1	0				6	3.00	3.00
Furnariidae															0		
L. nematura	2	1							0	0	1				1	1.00	0.50
S. spixi	5	2				4	0	0	0	1	0				5	2.50	1.00
Tyrannidae																	
P. ventralis	10	1				0	1	0							1	1.00	0.10
Troglodytidae																	
T. aedon	12	1				1	0	0							1	1.00	0.08
Turdidae																	
T. albicollis	38	28	45	13	0	47	14	6	4	9	4	4	9	1	156	5.57	4.10
T. amaurochalinus	13	6	1	0	0	5	6	2	0	2	0	0	1	0	17	2.83	1.31
T. rufiventris	53	26	14	4	1	6	9	6	0	4	2	4	20	1	71	2.73	1.34
Parulidae																	
B. culicivorus	25	3	9	1	0	2	0	0							12	4.00	0.48
Thraupidae																	
P. cabanisi	13	3	11	16	0										27	9.00	2.08
S. diadematus	6	2	0	3	0										3	1.50	0.50
Emberizidae																	
Z. capensis	46	2	4	0	0	1	0	0							5	2.50	0.11
Total:	226	78	84	37	1	71	30	14	4	17	7	8	31	2	306		
Ticks per season (%)			122 (39.9)			115 (37.6)			28 (9.1)			41 (13.4)					

Table 2 (continued)

(b) Vegetation	Autumn			Winter			Spring			Summer			No ticks
	L	N	A	L	N	A	L	N	A	L	N	A	
Ticks	147	39	1	665	101	14	31	28	58	4	18	4	1110
Ticks per season (%)	187 (16.9)			780 (70.3)			117 (10.5)			26 (2.3)			

Mean intensity: number of ticks per number of infested birds. Mean abundance: number of ticks per number of birds

Table 3 Detection of *Borrelia* spp. by nested PCR targeting the *fla* gene in *Ixodes auritulus* pools of nymphs (N) and adults (A) collected from (a) birds and (b) vegetation

(a) Autumn			Winter			Spring			Summer		
Parasitized bird	Pools	Positive PCR	Parasitized bird	Pools	Positive PCR	Parasitized bird	Pools	Positive PCR	Parasitized bird	Pools	Positive PCR
T. albicollis[a]	1 (1 N)		*P. ventralis*[b]	1 (1 N)		*T. ruficapillus*[b]	1 (1 N)		*T. caerulescens*[a]	1 (1 N)	1 (1 N)[2]
T. albicollis[a]	1 (3 N)										
T. albicollis[a]	1 (1 N)		*T. albicollis*[b]	1 (3 N)		*L. nematura*[a]	1 (1A)	1 (1A)[4]	*T. albicollis*[b]	1 (6 N)	1 (6 N)[4]
T. albicollis[b]	1 (3 N)	1 (3 N)[4]	*T. albicollis*[b]	1 (1 N)					*T. albicollis*[b]	2 (3 N, 1A)	
T. albicollis[b]	1 (1 N)		*T. albicollis*[b]	1 (1A)		*S. spixi*[b]	1 (1 N)				
T. albicollis[b]	1 (1 N)		*T. albicollis*[b]	1 (5 N)	1 (5 N)[1]				*T. amaurochalinus*[b]	1 (1 N)	1 (1 N)[2]
T. albicollis[b]	1 (1 N)		*T. albicollis*[b]	1 (1 N)		*T. albicollis*[a]	2 (1 N, 1A)	1 (1A)[4]			
T. albicollis[b]	1 (2 N)		*T. albicollis*[b]	3 (4 N, 2A, 3A)	1 (4 N)[4]	*T. albicollis*[a]	1 (3 N)		*T. rufiventris*[a]	1 (3 N)	
						T. albicollis[a]	2 (1 N, 1A)		*T. rufiventris*[b]	1 (1 N)	
T. rufiventris[a]	2 (2 N, 1A)		*T. amaurochalinus*[b]	2 (6 N, 2A)		*T. albicollis*[a]	1 (1 N)		*T. rufiventris*[b]	1 (5 N)	1 (5 N)[4]
T. rufiventris[a]	1 (1 N)					*T. albicollis*[b]	1 (1A)		*T. rufiventris*[b]	1 (2 N)	
T. rufiventris[a]	1 (1 N)		*T. rufiventris*[b]	2 (1 N, 2A)	1 (1 N)[4]	*T. albicollis*[b]	1 (2 N)		*T. rufiventris*[b]	1 (3 N)	1 (3 N)[1]
			T. rufiventris[b]	1 (1 N)		*T. albicollis*[b]	2 (1 N, 1A)		*T. rufiventris*[b]	1 (1 N)	
B.culicivorus[a]	1 (1 N)		*T. rufiventris*[b]	2 (2 N, 1A)					*T. rufiventris*[b]	1 (3 N)	
			T. rufiventris[b]	1 (1 N)		*T. amaurochalinus*[b]	1 (1 N)		*T. rufiventris*[b]	1 (1 N)	1 (1 N)[4]

Table 3 (continued)

(a) Autumn			Winter			Spring			Summer		
Parasitized bird	Pools	Positive PCR	Parasitized bird	Pools	Positive PCR	Parasitized bird	Pools	Positive PCR	Parasitized bird	Pools	Positive PCR
P. cabanisi[a]	1 (3 N)	1 (3 N)[4]	*T. rufiventris*[b]	1 (2A)		*T. amauro-chalinus*[b]	1 (1 N)		*T. rufiven-tris*[b]	2 (1 N, 1A)	
P. cabanisi[a]	1 (10 N)	1 (10 N)[3]	*T. rufiventris*[b]	2 (4 N, 1A)							
P. cabanisi[a]	1 (3 N)	1 (3 N)[4]				*T. rufiventris*[a]	2 (1 N, 1A)				
						T. rufiventris[a]	1 (1 N)				
S. diadema-tus[a]	1 (1 N)	1 (1 N)[2]				*T. rufiventris*[a]	1 (1A)				
S. diadema-tus[a]	1 (2 N)	1 (2 N)[4]				*T. rufiventris*[a]	1 (2 N)				

(b) Vegetation	Pools	PCR positive pools
Autumn	6: (5 N, 4 N)[a] (10 N, 10 N, 10 N, 1A)[b]	2: (10 N, 10 N)[b,4]
Winter	10: (10 N, 10 N, 10 N, 10 N, 7 N, 14 N, 20 N, 20 N, 6A, 8A)[b]	6: (10 N, 10 N, 10 N, 10 N, 7 N, 6A)[b,4]
Spring	16: (4 N, 3A)[a] (8 N, 8 N, 8 N, 5A, 5A, 5A, 5A, 5A, 5A, 5A, 5A, 5A, 5A, 5A)[b]	5: (4 N)[a,4] (8 N, 8 N, 8 N, 5A)[b,4]
Summer	3: (9 N)[a] (9 N, 4A)[b]	1: (9 N)[b,4]

[a]Birds captured in Reserva Natural Salus, Lavalleja Department

[b]Birds captured in Laguna Negra, Rocha Department

[1,2,3,4]Haplotypes UY1, UY2, UY3 and UY4

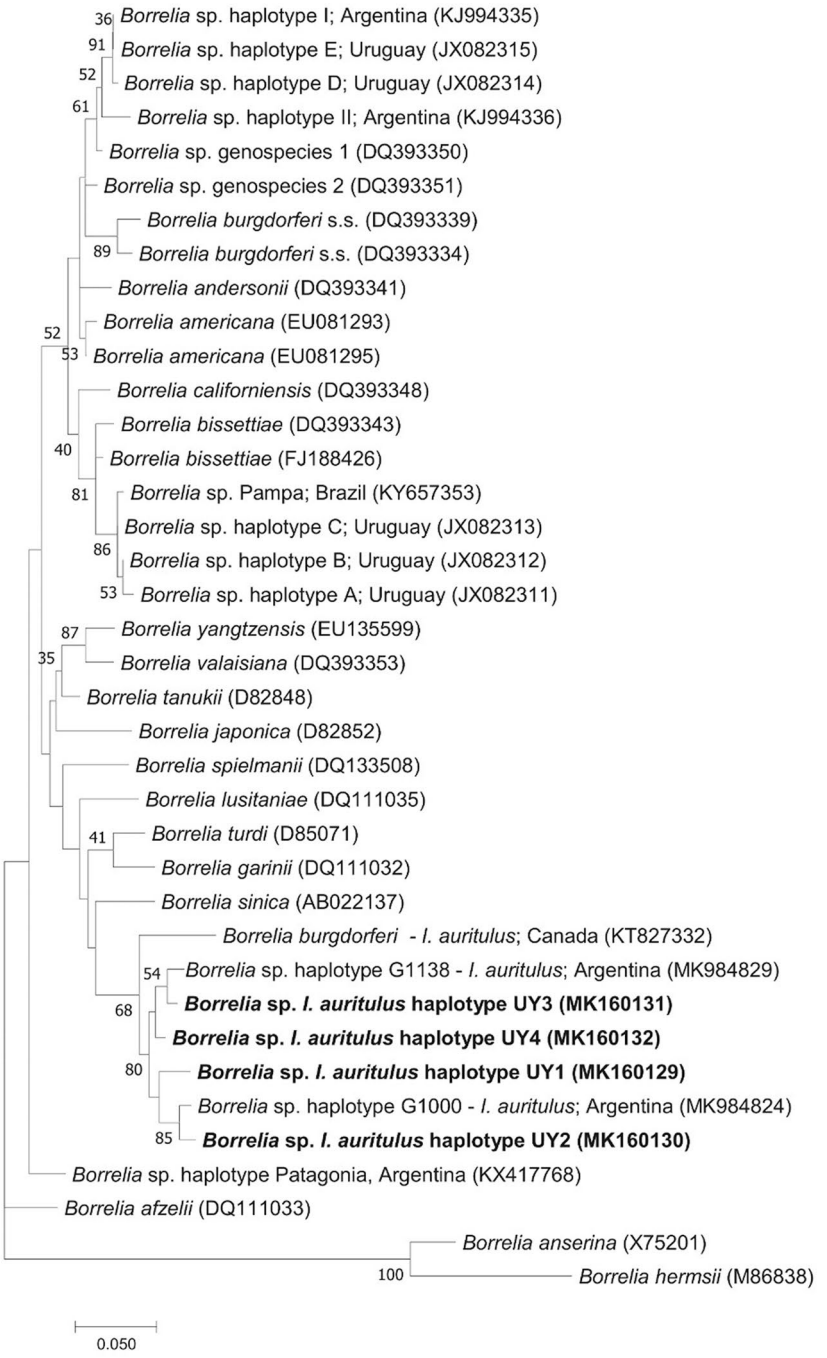

Fig. 1 Maximum likelihood tree constructed of *Borrelia* spp. *fla* partial sequences. Numbers represent bootstrap support generated from 1000 replications. The sequences obtained in this study were highlighted bold and GenBank accession numbers are in brackets. *B. anserina* and *B. hermsii* were included as outgroup

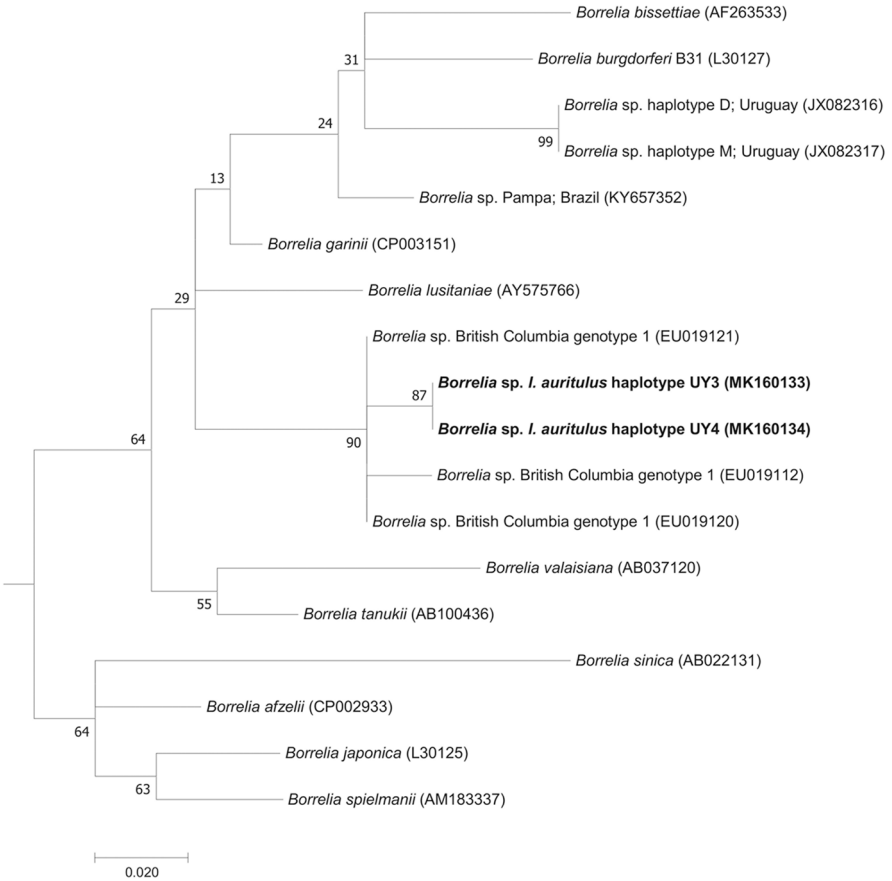

Fig. 2 Maximum likelihood tree constructed of *Borrelia* spp. IGS partial sequences. Numbers represent bootstrap support generated from 1000 replications. The sequences obtained in this study were highlighted bold and GenBank accession numbers are in brackets

Discussion

Ixodes auritulus, the avian coastal tick, is found in the Australian, Ethiopian, Nearctic and Neotropical zoogeographical regions (Guglielmone et al. 2014). During this survey, we captured 78 passerine birds infested with *I. auritulus*. Among those, members of the family Turdidae represented 77% (n = 60) of the parasitized birds. Moreover, 79.7% of the total ticks collected were found in specimens from this family (Table 2a). In a previous study about ticks on wild birds of Uruguay, birds from the genus *Turdus* were the most infested with larvae and nymphs of ixodids (Venzal et al. 2005b). Wild birds are frequently infested by ticks, and some bird species act as reservoirs of zoonotic pathogens (Richter et al. 2000). There are several reports discussing the role of members of the family Turdidae as hosts of *I. auritulus* (da Cunha Amaral et al. 2013; Arzua and Barros-Battesti 1999; González-Acuña et al. 2005; Scott et al. 2012, 2015). These authors and other researchers highlighted the role of birds belonging to the genus *Turdus*, as reservoirs of *Borrelia* spp. and their participation in the dissemination of

infected ticks (Rudenko et al. 2014; Saracho-Bottero et al. 2017; Scott et al. 2012; Scott and Foley 2016). In our study, another bird species found highly infested with *I. auritulus* was *Poospiza cabanisi*. Previous studies about bird-tick relationships have shown that tick infestation is more common among birds that forage primarily on the ground and in the shrub layer (Morshed et al. 2005). In this context, the genera *Turdus* and *Poospiza* include birds characterized by living in low forest stratum and are frequently found on the ground (Narosky and Yzurieta 2003), which may explain the high infestation levels reported in this study.

The previous findings of Bbsl genotypes in *I. auritulus* were made in ticks collected on Canadian birds (Morshed et al. 2005; Scott et al. 2010, 2012, 2015, 2018a; Scott and Foley 2016). *Borrelia* haplotypes found in these birds corresponded to *B. burgdorferi* s.s., British Columbia genotype 1, British Columbia genotype 2, British Columbia genotype 3 (Morshed et al. 2005; Scott et al. 2010) and, similarly, *B. americana* (Scott and Foley 2016). Recently, Muñoz-Leal et al. (2019) found a *Borrelia* genospecies belonging to the Bbsl complex (*Borrelia* sp. Navarino) retrieved from *I. auritulus* collected on a bird, *Troglodytes musculus*, in Chile.

In the present study, the results of the PCR using *fla* gene revealed the presence of *Borrelia* in 17 pools, (minimum infection rate: 12.2%), of nymphs and females of *I. auritulus*, collected from birds (Table 3a). Nine positive pools of *I. auritulus* were obtained from passerine birds of Laguna Negra and eight from Reserva Natural Salus. At the same time that our study was carried out, Cicuttin et al. (2019) studied the presence of *Borrelia* spp. in a protected urban area of Buenos Aires city, Argentina. They found a prevalence of Bbsl in *I. auritulus* collected on birds of 27.3%. The phylogenetic tree generated with the *fla* sequences in our study suggests that the *Borrelia* haplotypes UY1-4 detected in *I. auritulus* belongs to the Bbsl complex and are closely related to the haplotypes G1000 and G1138 detected by Cicuttin et al. (2019) in *I. auritulus,* and are related to an isolate of *Borrelia* sp. obtained from *I. auritulus* in Canada (Isolate Cn186-BbPCR2) (Fig. 1). Cicuttin et al. (2019) showed that the same haplotypes from Argentina, Uruguay and Canada conform a monophyletic group. The IGS phylogenetic reconstruction showed that Bbsl sequences of *I. auritulus* from Uruguay form a well-supported clade with British Columbia genotype 1 of *I. auritulus* from Canada (Fig. 2). The difference between the IGS fragment sequences obtained from *I. auritulus* of Canada and Uruguay is a single nucleotide. However, the fragment of IGS used is very short, so further analyses using other genes (or longer fragments of the genes) are required to properly determine the genetic variation between haplotypes from the two geographic locations. Migratory birds could be involved in the probable relation between the Canadian haplotypes and the haplotypes found in Argentina and Uruguay. Migratory birds are increasingly considered important in the global dispersal of zoonotic pathogens; they can transport the tick vectors as well as the pathogens (Ogden et al. 2008). Some species of migratory birds have been described as efficient reservoirs of some genotypes of Bbsl (Ogden et al. 2008; Scott et al. 2018b). Neotropical passerines migrate across national and intercontinental borders, and become long-range vectors for any zoonotic pathogen that they harbor. Overall, dispersal of Bbsl-infected ticks along migration routes is an important mechanism in the establishment of new endemic foci of tick-borne diseases (Scott et al. 2014, 2018b). *Ixodes auritulus* parasitizes several orders of birds, with passerine birds probably the main hosts, sustaining tick populations throughout the Neotropic and the Nearctic (González-Acuña et al. 2005; Scott et al. 2015).

The spirochete detected in *I. auritulus* during this study represents the third genospecies of Bbsl reported for Uruguay. The two previous Uruguayan genospecies were associated with *I. aragaoi* ticks obtained in the same locations of this study (Barbieri et al. 2013). Until now, Lyme borreliosis does not represent a problem for public health in the southern cone of South America. In Brazil, it has been described a disease named as Lyme disease-like syndrome, or Baggio-Yoshinari syndrome, with some clinical manifestations similar to those observed for Lyme disease (Mantovani et al. 2007; Miziara et al. 2018; Yoshinari et al. 2010). However, a more recent study performed a critical evaluation of the diagnostic methods that were used for this Baggio-Yoshinari syndrome, and concluded that they might not represent a *Borrelia*-caused disease (de Oliveira et al. 2018). In Uruguay, although one case described in 1996 and many suspected clinical cases with Lyme disease-like symptoms, tick-borne borreliosis has not been diagnosed (Conti-Díaz 2001; Nava et al. 2014 Protasio et al. 1996). Furthermore, even though in the Holarctic region ticks bites in humans by *Ixodes* spp. are very common, the situation in South America is different, with only a few reports of human bites by ticks of the genus *Ixodes* (Guglielmone et al. 2006, 2014; Nava et al. 2014; Saracho-Bottero et al. 2017). However, Scott et al. (2018a) described that if there are two or more tick species feeding concurrently on a host, they can transmit Bbsl, via the reservoir host, from one cofeeding tick species to another cofeeding tick species. Alternatively, one tick species can infect a reservoir-competent host and, after the blood meal, another tick species (that could be one that bite humans) can subsequently acquire Bbsl from this spirochetemic host. Also, although *I. auritulus* only parasitizes avifauna, both birds and mammals eat these ixodid ectoparasites, and may become systematically infected by oral inoculation (Scott et al. 2018a). These findings mean that medical implications due to the presence of Bbsl in *I. auritulus* cannot be dismissed. The isolation and culture of these *Borrelia* genospecies will be essential to study their pathogenicity.

The growing number of publications describing the presence of new genospecies of Bbsl in the southern cone region of America (Barbieri et al. 2013; Ivanova et al. 2014; Nava et al. 2014; Saracho-Bottero et al. 2017; Sebastian et al. 2016), and the scarce information about its pathogenicity, reservoirs and vectors, highlights the importance of further studies about spirochetes presence in Uruguay and the region.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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