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Original article

Geographical distribution and phylogenetic analysis of *Rhipicephalus sanguineus* sensu lato in northern and central Chile

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ABSTRACT

The presented study analyzed the presence and geographical distribution of the tropical and temperate lineages of *Rhipicephalus sanguineus* sensu lato in Chile. *R. sanguineus* s.l. ticks were collected from dogs at 14 sites in northern and central Chile for morphological and genetic analysis based on the 16S rDNA gene. Phylogenetic studies proved the existence of both, the tropical and the temperate lineages. The former was represented by a single haplotype and occurred in the far north; the latter included four haplotypes and was observed from the Tarapacá Region southwards. In four sites at latitudes from 20°S to 22°S, both lineages were found to coexist. Our study discovered for the first time the existence of the tropical lineage in Chile and demonstrated that distributions of the tropical and temperate lineages overlap, forming a transitional zone of approximately 200 km in northern coastal Chile.

1. Introduction

Rhipicephalus sanguineus sensu lato (s.l.) is a cluster of species containing some of the most widespread tick species in the world, with remarkable economical, medical, and veterinary importance (Nava et al., 2015; Hekimoğlu et al., 2016). Despite its known relevance as a vector of important parasitic and bacterial pathogens such as *Ehrlichia canis, Anaplasma platys, Hepatozoon canis*, *Babesia vogeli,* and *Rickettsia* spp. (Dantas-Torres, 2008; Dantas-Torres and Otranto, 2015), the exact taxonomic differences and relationships among *R. sanguineus* s.l. remain uncertain. The main reasons for these controversies are the unreliable original description of *R. sanguineus* (Latreille, 1806), the loss of the type specimen, and the high morphological similarity among the members of the species complex (Dantas-Torres and Otranto, 2015; Nava et al., 2015).

To address these taxonomic uncertainties, various molecular methods, mainly analyzing mitochondrial 16S and 12S rDNA, have been developed. These studies have supported the existence of various, genetically divergent *R. sanguineus* s.l. lineages (Burlini et al., 2010; Dantas-Torres et al., 2013; Liu et al., 2013; Moraes-Filho et al., 2011; Nava et al., 2012; Oliveira et al., 2005; Sanches et al., 2016; Szabó et al., 2005; Chitimia-Dobler et al., 2017). In South America, two lineages have been identified: a tropical lineage in tropical areas in Brazil, Paraguay, Colombia, Peru, and Argentina, and a temperate lineage in subtropical and temperate regions in Brazil, Uruguay, Argentina, and Chile (Moraes-Filho et al., 2011; Nava et al., 2012). Comparative studies of specimens from a wider geographical range suggest the existence of further lineages in Europe, Africa, and Asia (Dantas-Torres et al., 2013; Hekimoğlu et al., 2016; Chitimia-Dobler et al., 2017). Beside its taxonomic relevance, the recognition of different lineages might also have important clinical implications, since, as recently demonstrated, both tropical and temperate lineages have different vectorial competence to transmit the canine pathogen *E. canis* (Moraes-Filho et al., 2015; Cicuttin et al., 2017).

In Chile, ticks of the *R. sanguineus* complex were first described in the central Metropolitan Region by the mid-1970s (Tagle, 1976).

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Table 1
Sampling localities in northern and central Chile and *Rhipicephalus sanguineus* s.l. haplotypes obtained by phylogenetic analysis of 16S rDNA partial sequences.

Study Sites						Samples (n)		Haplotypes
N°	Region	Locality	Coordinates	AAT (°C)[a]	Altitude (m.a.s.l.)	Collected	Analyzed	
1	XV	Arica	18°29′01“S 70°18′24“W	18.7	2	13	7	Trop2
2	XV	Cuya	19°09′40“S 70°10′38“W	18.2	102	5	4	Trop2
3	I	Huara	19°59′50“S 69°46′18“W	16.0	1103	10	3	Trop2
4	I	Iquique	20°13′56“S 70°08′46“W	18.1	1	10	4 1	Trop2 Temp2
5	I	Alto Hospicio	20°15′57“S 70°06′05“W	17.4	951	10	2	Temp2
6	I	Chanavayita	20°42′02“S 70°11′16“W	18.4	10	10	2 2 1	Trop2 Temp3 Temp4
7	I	San Marcos	21°06′52“S 70°07′22“W	19.3	10	10	2 1	Trop2 Temp3
8	II	Tocopilla	22°05′20“S 70°11′46“W	20.5	39	11	3 1	Trop2 Temp2
9	II	María Elena	22°20′34“S 69°39′44“W	16.1	1155	10	7	Temp2
10	II	Baquedano	23°20′00“S 69°50′29“W	16.6	1029	10	1	Temp2
11	II	Mejillones	23°05′54“S 70°27′04“W	18.3	12	7	4	Temp2
12	II	Antofagasta	23°39′22“S 70°24′04“W	17.9	40	10	7 1	Temp2 Temp1
13	IV	Coquimbo	29°58′03”S 71°20′14”W	15.1	15	10	5 3	Temp2 Temp3
14	RM	Santiago	33°36′33”S 70°34′31”W	14.6	700	10	9	Temp2

AAT, average annual temperature; m.a.s.l., meters above sea level.
[a] Source: https://es.climate-data.org.

Currently, this species complex is known to be endemic from Arica in the far north (18°29′01“S) to Valdivia in southern Chile (39°49′11“S) (Abarca et al., 2016; González-Acuña and Guglielmone, 2005; López et al., 2015), but the presence and distribution of different lineages of *R. sanguineus* s.l. have not been studied systematically yet. The presented study aimed to analyze the spatial distribution and limits of *R. sanguineus* s.l. lineages and haplotypes in northern and central Chile.

2. Material & methods

2.1. Tick sampling

Ticks from five administrative regions in northern and central Chile, ranging from Arica (18°29′01“S) to Santiago (33°36′33”S), were included. Specimens (maximum three per dog) were collected from stray dogs in 12 study sites from Arica to Antofagasta during January to February 2016 and from household dogs in Coquimbo and Santiago in 2014 and 2015 (Table 1). Dogs were restrained and examined by a veterinarian, which carefully removed ticks with tweezers and placed them in ethanol 70%. Samples were kept at room temperature and sent to the Laboratorio de Infectología y Virología Molecular, Pontificia Universidad Católica de Chile in Santiago, Chile, for taxonomical identification according to Walker et al. (2000) and subsequent molecular analysis.

The study protocol was reviewed and approved by the Comité Ético Científico (Approval N°12-170), and by the Comité de Bienestar Animal (Approval N°12-033) of the Faculty of Medicine, Pontificia Universidad Católica de Chile in Santiago, Chile.

2.2. Molecular analysis and sequencing

Subgroups of 5–10 male ticks per site were used for further molecular analysis by partial amplification of 16sDNA gene. Dried ticks were mechanically triturated and re-suspended in PBS1X DNAase free buffer. DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the manufactureŕs instructions. Subsequently, a partial fragment of 405 base pairs (bp) of the constitutive mitochondrial gene 16S rDNA was amplified using 5′-CCG-GTC-TGA-ACT-CAG-ATC-AAG-T-3′ and 5′-GCT-CAA-TGA-TTT-TTT-AAA-TTG-CTG-T-3′ as forward and reverse primers, respectively (Mangold et al., 1998). The PCR protocol included the following steps: 2 min at 94 °C, followed by 7 cycles of 30 s at 94 °C – 30 s at 45 °C – 45 s at 72 °C continued by 28 cycles of 30 s at 94 °C – 30 s at 47 °C – 45 s at 72 °C, with increasing annealing temperatures (0.3 °C per cycle), and a final step of 10 min at 72 °C. For amplification, Platinum® PCR SuperMix High Fidelity (cat: 12532016) (Thermo Fisher Scientific, Waltham, MA, USA) was used. All PCRs were performed in a ProFlex 3 × 32 well PCR system (Thermo Fisher Scientific). PCR products were separated in 2% agarose gel stained with SYBR®Safe DNA gel stain (Thermo Fisher Scientific) and visualized in a trans-illuminator. Sequencing was carried out by Macrogen Corp. (Bethesda, MD, USA).

2.3. Phylogenetic analysis

The obtained sequences were manually edited and aligned in BioEdit 7.2.5 version (Hall, 1999). The phylogenetic analysis was inferred by Neighbor-Join (NJ), Maximum Likelihood (ML), and Maximum Parsimony (MP) methods using the software MEGA6 (Tamura et al., 2013). The search of the most appropriate model of nucleotide substitution for phylogenetic analysis was performed in MEGA6, according to the Bayesian information criterion (BIC), which was applied to the methods of NJ and ML. For the ML method, initial trees for the heuristic search were obtained automatically by applying NJ and Bio NJ algorithms to a matrix of pairwise distances estimated using the

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