Ticks and Tick-borne Diseases 9 (2018) 792–797

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

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

Fabián E. Díaz[a,b,1], Constanza Martínez-Valdebenito[b,c,1], Javier López[d], Thomas Weitzel[e,**,1], Katia Abarca[b,c,f,*,1]

[a] Escuela de Postgrado y Postítulo, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile
[b] Departamento de Enfermedades Infecciosas e Inmunología Pediátricas, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[c] Laboratorio de Infectología y Virología Molecular, Red Salud UC, Santiago, Chile
[d] Veterinary Hospital Puente Alto, Santiago, Chile
[e] Laboratorio Clínico, Clínica Alemana de Santiago, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, Chile
[f] Millennium Institute on Immunology and Immunotherapy, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

ARTICLE INFO

Keywords:
Rhipicephalus sanguineus sensu lato
Epidemiology
16 rDNA
Phylogenetic analysis
Chile

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).

* Corresponding author at: Departamento de Enfermedades Infecciosas e Inmunología Pediátricas, Pontificia Universidad Católica de Chile, Diagonal Paraguay 362, Santiago, Chile.
** Corresponding author at: Laboratorio Clínico, Clínica Alemana, Universidad del Desarrollo, Av Vitacura 5951, Santiago, Chile.
E-mail addresses: modular@veterinaria.uchile.cl (F.E. Díaz), cmartinezv@med.puc.cl (C. Martínez-Valdebenito), javievet@gmail.com (J. López), tweitzel@alemana.cl (T. Weitzel), katia@med.puc.cl (K. Abarca).
[1] Equal contributors.

https://doi.org/10.1016/j.ttbdis.2018.03.004
Received 1 November 2017; Received in revised form 24 January 2018; Accepted 1 March 2018
Available online 05 March 2018
1877-959X/

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	Trop2
							1	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	Trop2
							2	Temp3
							1	Temp4
7	I	San Marcos	21°06′52“S 70°07′22“W	19.3	10	10	2	Trop2
							1	Temp3
8	II	Tocopilla	22°05′20“S 70°11′46“W	20.5	39	11	3	Trop2
							1	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	Temp2
							1	Temp1
13	IV	Coquimbo	29°58′03”S 71°20′14”W	15.1	15	10	5	Temp2
							3	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

Table 2
16S rDNA reference sequences of the included *Rhipicephalus* isolates.

Species	Origin	Genbank accession number	Lineage	Reference
R. sanguineus	Chile (Metropolitan Region)	GU553077.1	Temperate	Moraes-Filho et al. (2011)
R. sanguineus	Argentina (Chaco Province)	KR909456.1	Temperate	Cicuttin et al. (2015)
R. sanguineus	Argentina (Santa Fé Province)	JX195168.1	Temperate	Nava et al. (2012)
R. sanguineus	Argentina (Santa Fé Province)	JX195167.1	Temperate	Nava et al. (2012)
R. sanguineus	Argentina (Formosa Province)	JX206980.1	Tropical	Nava et al. (2012)
R. sanguineus	Brazil (Northeast)	GU553074.1	Tropical	Moraes-Filho et al. (2011)
R. sanguineus	Brazil (Northeast)	GU553075.1	Tropical	Moraes-Filho et al. (2011)
R. camicasi	Kenya	KU746973.1	NA	Unpublished
R. turanicus	Greece	KC243867.1	NA	Dantas-Torres et al. (2013)
R. turanicus	Turkmenistan	KF145150.1	NA	Dantas-Torres et al. (2013)
R. pussilus	Spain	KU513962.1	NA	Almeida et al. (2017)
R. rossicus	Romania	KY111472.1	NA	Unpublished
R. guilhoni	Nigeria	KC243854.1	NA	Dantas-Torres et al. (2013)
R. appendiculatus	Unknown	L34301.1	NA	Black and Piesman (1994)

Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. In addition, MP method was used to corroborate the topology of the obtained phylogenetic tree. The MP tree was obtained using the Subtree-Pruning-Regrafting algorithm with level 1 search in which the initial trees were obtained by random addition of sequences (10 replicates). For all the methods, the support of the topology was based in a bootstrapping of 1000 replicates, and the positions equivalent to gaps or missing data were deleted. Additionally, we included sequences available in GenBank of *R. sanguineus* s.l. from other regions in South America as well as other species belonging to the *R. sanguines* group such as *R. camicasi, R. guilhoni*, *R. turanicus*, *R. pusillus*, and *R. rossicus* (Table 2). A sequence of *R. appendiculatus* was used as an outgroup.

3. Results

A total of 136 ticks were collected, which were all morphologically identified as *R. sanguineus* s.l.; after amplification, sufficient genetic material was available from 70 of those specimens (Table 1). The phylogenetic analysis included a total of 84 partial sequences of the mitochondrial gene 16S rDNA, 70 from our samples and 14 from sequences published in GenBank. The T92 model (Tamura, 1992) was chosen (using MEGA 6), as the most accurate nucleotide substitution model for our dataset. Based on this model, we were able to determine six haplotypes, which grouped in two phylogenetic clades with marked divergence. In accordance with the reference sequences, these clades were identified as the tropical and temperate lineages (Fig. 1). The dichotomy was supported by a high bootstrap value (99%) and similar topologies of phylogenetic trees obtained by NJ and MP analyses (data not shown).

Specimens of the tropical lineage belonged to a single haplotype (Trop2) and occurred in seven of eight northern study sites, ranging from Arica to Tocopilla over a distance of approximately 400 km (Fig. 2). Four haplotypes of the temperate lineage were detected from Iquique to Santiago. One dominating haplotype (Temp2) was found all over this range, whereas the other three (Temp1, Temp3, Temp4) were less frequent and had a narrower geographical distribution (Table 1). The pairwise distance between the six haplotypes from study isolates and reference strains in GenBank revealed a genetic heterogeneity of 0.3% between the tropical haplotypes Trop1 and Trop2 and of 0.3–1.0% among temperate haplotypes. The pairwise distance between both lineages, on the other hand, ranged from 5.8% to 6.9% (Table 3). Noteworthy, four study sites from Iquique to Tocopilla were endemic for both lineages, indicating an area of coexistence of the Trop2 and different temperate lineage haplotypes over a distance of approximately 200 km (Fig. 2). Sequences obtained in this study were deposited in GenBank and are available under the access numbers KX632153 (Temp1), KX632154 (Trop2), KX632155 (Temp3), and KX632156 (Temp4).

4. Discussion

The genetic diversity of the morphotype *R. sanguineus* has raised controversies on its taxonomic position and systematic relationships, leading to the idea of a complex of various morphologically indistinguishable but genetically distinct lineages and/or species (Dantas-Torres and Otranto, 2015; Nava et al., 2015; Sanches et al., 2016). In South America, a tropical and a temperate lineage have been reported (Moraes-Filho et al., 2011; Nava et al., 2012). Ancestral biogeographic reconstructions of *R. sanguineus* s.l. suggest that the American continent was independently colonized by European and African tick populations (Hekimoğlu et al., 2016). European ancestors of the temperate lineage could have been introduced during the “Colombian exchange”, while the tropical lineage derived from African populations, which colonized the New World during the period of Atlantic slave trade (Burlini et al., 2010). As suggested by in vivo experiments of Labruna et al. (2017), the further spatial establishment of the lineage in South America might be related to their ecological preferences. The pronounced behavioral diapause of the temperate lineage might lead to a better adaptation and higher survival in colder regions, while the lack of diapause of the tropical lineage could be beneficial in tropical climates with less seasonal temperature variations.

In Chile, only the temperate lineage has so far been reported, but the studies included only few samples from the central region (Santiago, Viña del Mar) as well as from Easter Island (Moraes-Filho et al., 2011; Nava et al., 2012). Our study, providing the first comprehensive molecular data over a wide geographical range, proved that both lineages are endemic in Chile and that the genetic differences among and between lineages and haplotypes are similar to those observed in other South American regions (Dantas-Torres et al., 2013; Moraes-Filho et al., 2011; Nava et al., 2012). The tropical lineage was detected in the far north; it was defined by a single haplotype (Trop2), which clustered with a haplotype with a wide distribution in Latin America (Argentina, Brazil, Venezuela, Panama, Costa Rica, and Mexico) (Cicuttin et al., 2015; Dolz et al., 2015; Moraes-Filho et al., 2011). Interestingly, the included sequence of *Rhipicephalus camicasi* from Africa (Kenya) also grouped within this cluster, which is in accordance with a recent analysis from northern Africa (Chitimia-Dobler et al., 2017). The temperate lineage inhabited a wider geographical range and was represented by four haplotypes, indicating a wider genetic diversity. Temp2 showed the widest geographical distribution extending from far north to central Chile; this haplotype was phylogenetically closely related to the haplotype II described by Nava et al. in Argentina (Nava et al., 2012). Temp 3 haplotype was much less prevalent and Temp4 was represented by a single tick specimen, collected from a locality where both lineages coexisted. This haplotype showed the highest divergence from the tropical

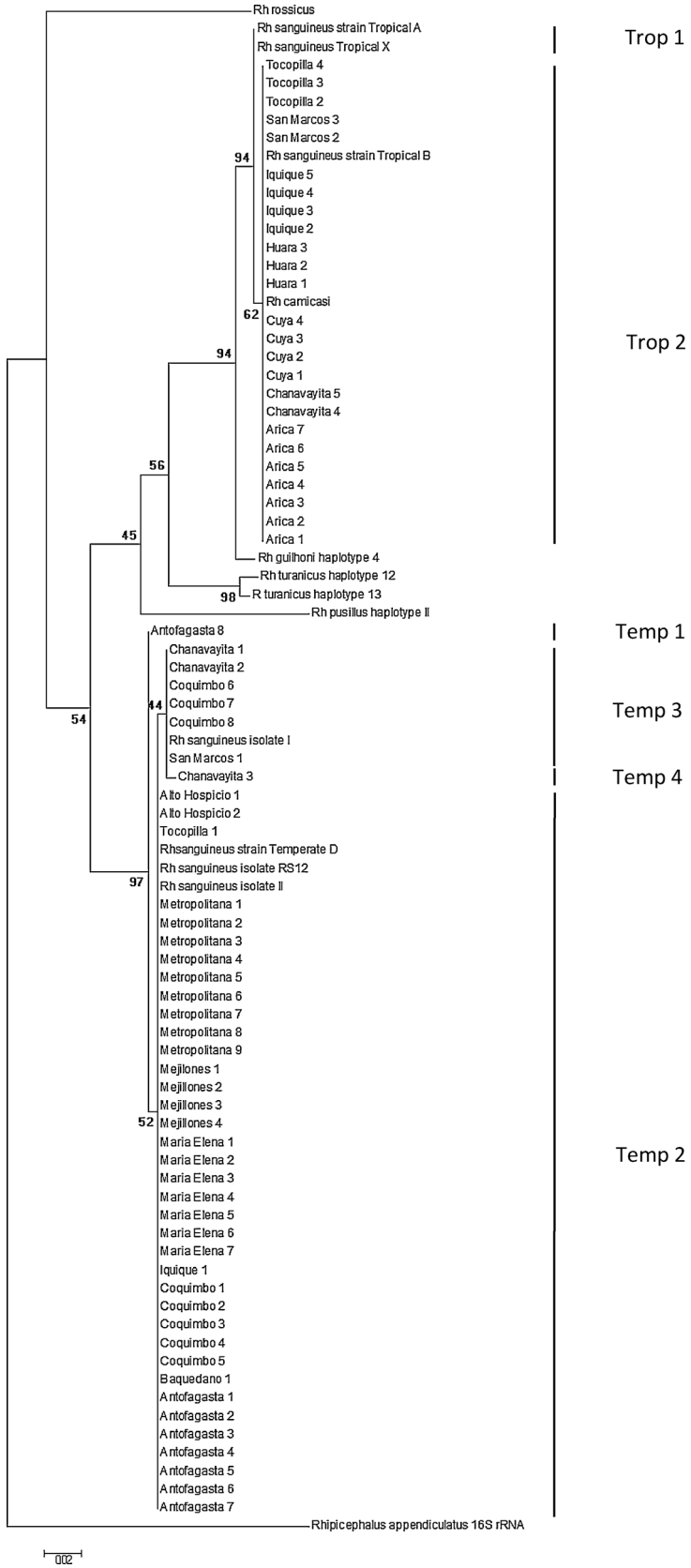

Fig. 1. Maximum likelihood tree based on partial sequences of *R. sanguineus* s.l. 16S rDNA gene included in this study. Numbers at internal nodes represent support values from bootstrap based on 1000 replications.

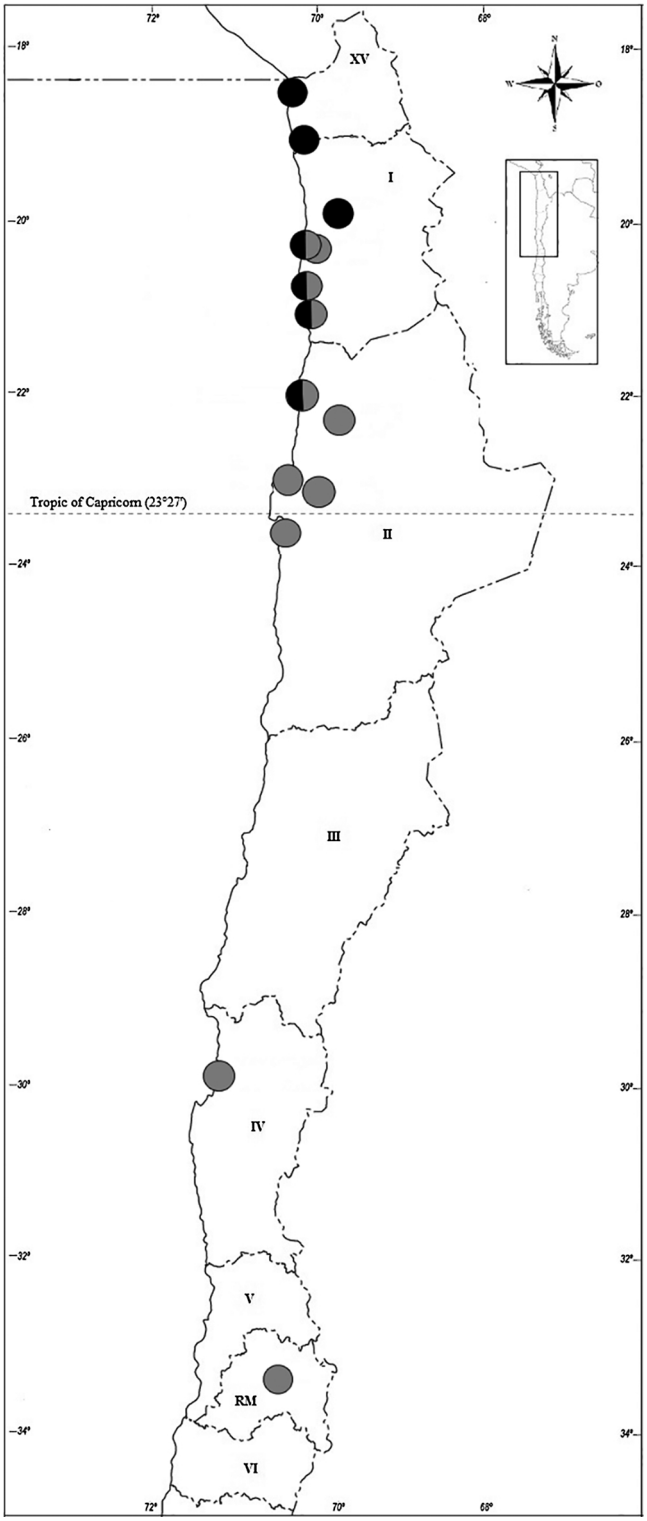

Fig. 2. Study regions in northern and central Chile. Dots mark the geographical locations of the 14 study sites (named from North to South in Table 1). Black color indicates the presence of tropical and grey color of the temperate lineage. Roman numerals stand for the different Chilean Regions, RM indicates Metropolitan Region.

lineage; it might originate from this area, since BLAST analysis did not show homology to other published 16S rDNA gene sequences. Deeper molecular studies of low frequency haplotypes are necessary to understand the co-evolution of the lineages and haplotypes in this unique transitional zone. Our study confirmed the latitudinal pattern of the distribution of *R. sanguineus* s.l. lineages in the Southern Cone of South America, but proved a wider range of the tropical lineage, occurring up to a southern latitude of 22°S in Chile (Argentina, 23°S; Paraguay, 25°S; Brazil, 28°S). We also established the current northern limit of the temperate lineage in Chile, which was endemic to latitude 20°S. The latitudinal differences on both sides of the Andes might be related to differences in the environmental and climatic conditions, but also to ecological factors related to the endophilous and peridomestic nature of the species complex (Dantas-Torres, 2010). Climatic factors are known to influence the endemicity of *R. sanguineus* s.l. lineages; the exact mechanisms, however, which determine the evolution of different lineages/species are uncertain (Zemtsova et al., 2016; Labruna et al., 2017). Zemtsova et al. (2016) suggested that temperature was a crucial factor and that the tropical lineage occupied regions with average annual temperatures (AAT) above 20 °C and the temperate lineage below this temperature. This hypothesis was recently supported by data from North America (Jones et al., 2017). For the Chilean haplotype of the tropical lineage, this threshold was not confirmed, since most sites where it was detected had AAT below 20 °C, the lowest was 16.0 °C in Huara (Table 1). This adaptation could partly be explained by relatively mild pacific climate with low daily and seasonal temperature fluctuations in the low altitude coastal sites, but not in further inland at higher altitudes, where temperature changes are more pronounced. Our data suggest that beside the AAT other factors related to on-host and off-host ecological conditions, including temperature fluctuations, might be of importance for the establishment of tick populations in certain geographical locations.

Table 3
Interspecific pairwise distances (%) calculated among partial 16S rDNA haplotypes of *Rhipicephalus sanguineus* s.l. identified in this study.

Haplotype	Temp1	Temp2	Temp3	Temp4	Trop1	Trop2
Temp1	—					
Temp2	0.3	—				
Temp3	0.7	0.3	—			
Temp4	1.0	0.7	0.3	—		
Trop1	5.8	6.1	6.5	6.1	—	
Trop2	6.1	6.5	6.9	6.5	0.3	—

An important factor for the epidemiology and evolution of *R. sanguineus* s.l. in the Americas is the sympatry of lineages in geographic areas of transition (Burlini et al., 2010; Nava et al., 2012). In 2012, Nava et al. reported the presence of both lineages in the Formosa Province in Argentina, suggesting an ecotonal zone between latitudes 24° and 25°S. Still, in this study, the two lineages were never found in the same locality. Recently, both lineages were also described in Texas, but again, no mixed populations occurred within the same study site (Jones et al., 2017). Our study demonstrated a transition area, which extended from latitudes 20°S to 22°S (approximately 200 km). In contrast to the studies cited above, we demonstrated for the first time, that both lineages were not only inhabiting similar geoclimatic habitats, but were also coexisting in close vicinity within the same localities. These sites were all at low altitude and close to the coastline (INE, 2016). One higher altitude site within the transition zone (Alto Hospicio, 950 m a.s.l.) was only inhabited by the temperate lineage. Nevertheless, further north, the tropical lineage was found in altitudes up to 1100 m a.s.l.; further studies are necessary to address the possible influence of altitude on the coexistence of the lineages. The geographical overlap observed in our study has important implications, since it raises the possibility of crossbreeding and the existence of hybrids. As shown by in vivo data, cross-breeding between Argentinean and Brazilian *R. sanguineus* ticks was possible, but produced non-fertile progeny (Szabó et al., 2005); similar results were reported between North American and African strains (Levin et al., 2012). Further in vivo and in vitro studies using ticks from the Chilean transition area should address this important question.

The epidemiology of the different lineages has also important

implications for veterinary medicine. As suggested by experimental studies of Moraes-Filho et al. (2015), only the tropical lineage is capable to transmit *E. canis*. This concept is in concordance with epidemiological findings from Uruguay, Brazil, and northern Argentina (Nava et al., 2012, Cicuttin et al., 2015), but was recently questioned by observations from Buenos Aires, Argentina, where the template lineage is endemic (Cicuttin et al., 2016; Cicuttin et al., 2017). These studies molecularly identified *E. canis* in 6.7% of dog samples from this region and also from 11 of 32 tick specimens, all belonging to the temperate lineage. The authors propose three possible explanations for these findings, I) the introduction of *E. canis* by chronically infected dogs, which acquired the pathogen in other areas; II) the transmission of *E. canis* within the region by transient populations of the tropical lineage, which yet not have been detected; and III) the fact that the template lineage of this region has a low vector capacity to transmit *E. canis*.

In Chile, canine infections by *E. canis* have only been reported in Arica in the far north (López et al., 2012), where only the tropical lineage is endemic. *Anaplasma platys*, on the other hand, has been diagnosed in central Chile (Abarca et al., 2007), indicating that the temperate lineage haplotype in this region (Temp2) is an effective vector of this pathogen.

In conclusion, our study added important epidemiological data on the geographical distribution of *R. sanguineus* s.l. lineages and haplotypes in northern and central Chile, proving the endemicity of both, the tropical and temperate lineages, with a zone of coexistence and potential hybridization at latitudes between 20°S and 22°S. Further studies are necessary to better understand the geoclimatic factors including temperature and altitude that determine the endemicity and coexistence of the different lineages of this important ectoparasite in South America.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

Parts of this work have been presented at the V Latin American Congress of Rickettsial Diseases in Mérida, Mexico, in September 2015. The study was funded by the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT N° 1130817 and N° 1170810). We are grateful to Paulina Lepe for her valuable help with tick identification and Santiago Nava for his advice regarding the molecular markers.

References

Abarca, K., López, J., Perret, C., Guerrero, J., Godoy, P., Veloz, A., Valiente-Echeverria, F., León, U., Gutjahr, C., Azócar, T., 2007. *Anaplasma platys* in dogs, Chile. Emerg. Infect. Dis. 9, 1392–1395.

Abarca, K., López, J., Gárate, D., Acosta-Jamett, G., 2016. Flea and ticks species from dogs in urban and rural areas in four districts in Chile. Arch. Med. Vet. 48, 109–115.

Almeida, C., Simões, R., Coimbra-Dores, M.J., Rosa, F., Dias, D., 2017. Mitochondrial DNA analysis of *Rhipicephalus sanguineus* s.l. from the western Iberian peninsula. Med. Vet. Entomol. 31, 167–177.

Black IV, W.C., Piesman, J., 1994. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. Proc. Natl. Acad. Sci. U. S. A. 91, 10034–10038.

Burlini, L., Teixeira, K.R., Szabó, M.P., Famadas, K.M., 2010. Molecular dissimilarities of *Rhipicephalus sanguineus* (Acari: Ixodidae) in Brazil and its relation with samples throughout the world: is there a geographical pattern? Exp. Appl. Acarol. 50, 361–374.

Chitimia-Dobler, L., Langguth, J., Pfeffer, M., Kattner, S., Küpper, T., Friese, D., Dobler, G., Guglielmone, A.A., Nava, S., 2017. Genetic analysis of *Rhipicephalus sanguineus* sensu lato ticks parasites of dogs in Africa north of the Sahara based on mitochondrial DNA sequences. Vet. Parasitol. 239, 1–6.

Cicuttin, G.L., Taragona, E.L., De Salvo, M.N., Mangold, A.J., Nava, S., 2015. Infection with *Ehrlichia canis* and *Anaplasma platys* (Rickettsiales: Anaplasmataceae) in two lineages of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) from Argentina. Ticks Tick Borne Dis. 6, 724–729.

Cicuttin, G.L., De Salvo, M.N., Gury Dohmen, F.E., 2016. Molecular characterization of *Ehrlichia canis* infecting dogs, Buenos Aires. Ticks Tick Borne Dis. 7, 954–957.

Cicuttin, G.L., De Salvo, M.N., Silva, D.A., Brito, M., Nava, S., 2017. *Ehrlichia canis* (Rickettsiales: Anaplasmataceae) en garrapatas *Rhipicephalus sanguineus* sensu lato del linaje templado (Acari: Ixodidae) provincia de Buenos Aires, Argentina. Rev. Fave-Secc. Cienc. Vet. 16, 93–96.

Dantas-Torres, F., Otranto, D., 2015. Further thoughts on the taxonomy and vector role of *Rhipicephalus sanguineus* group ticks. Vet. Parasitol. 208, 9–13.

Dantas-Torres, F., Latrofa, M.S., Annoscia, G., Giannelli, A., Parisi, A., Otranto, D., 2013. Morphological and genetic diversity of *Rhipicephalus sanguineus* sensu lato from the new and old worlds. Parasit.Vectors 6, 213.

Dantas-Torres, F., 2008. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. Vet. Parasitol. 152, 173–185.

Dantas-Torres, F., 2010. Biology and ecology of the brown dog tick, *Rhipicephalus sanguineus*. Parasit.Vectors 3, 26.

Dolz, G., Solórzano-Morales, A., Ábrego, L., Barrantes, A., Jiménez-Rocha, A.E., 2015. Haplotipos de *Rhipicephalus sanguineus* s.l. positivos a *Ehrlichia canis* presentes en Costa Rica – resultados preliminares. Rev. Bioméd. 26, 43.

González-Acuña, D., Guglielmone, A.A., 2005. Ticks (Acari Ixodoidea: Argasidae, Ixodidae) of Chile. Exp. Appl. Acarol. 35, 147–163.

Hall, T.A., 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Hekimoğlu, O., Sağlam, İ.K., Özer, N., Estrada-Peña, A., 2016. New molecular data shed light on the global phylogeny and species limits of the *Rhipicephalus sanguineus* complex. Ticks Tick Borne Dis. 7, 798–807.

INE (Instituto Nacional De Estadísticas), 2016. Medio Ambiente –Informe Anual 2015. http://historico.ine.cl/medioambiente/descargas/2016/medio_ambiente_2016.pdf (Accessed 20 October 2017).

Jones, E.O., Gruntmeir, J.M., Hamer, S.A., Little, S.E., 2017. Temperate and tropical lineages of brown dog ticks in North America. Vet. Parasitol. Reg. Stud. Rep 7, 58–61.

López, J., Abarca, K., Mundaca, M.I., Caballero, C., Valiente-Echeverría, F., 2012. Identificación molecular de *Ehrlichia canis* en un canino de la ciudad de Arica, Chile. Rev. Chil. Infectol. 29, 527–530.

López, J., Pinto, V., Rojas, J., Weitzel, T., Abarca, K., 2015. Detección y posterior diseminación de *Rhipicephalus sanguineus* en Chile. Rev. Bioméd. 26, 151–152.

Labruna, M.B., Gerardi, M., Krawczak, F.S., Moraes-Filho, J., 2017. Comparative biology of the tropical and temperate species of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) under different laboratory conditions. Ticks Tick Borne Dis. 8, 146–156.

Latreille, P.A., 1806. Genera crustaceorum et insectorum: secundum ordinem naturalem in familia disposita, iconibus exemplisque plurimis explicata. Paris et Argentorati 1, 302.

Levin, M.L., Studer, E., Killmaster, L., Zemtsova, G., Mumcuoglu, K.Y., 2012. Crossbreeding between different geographical populations of the brown dog tick, *Rhipicephalus sanguineus* (Acari: Ixodidae). Exp. Appl. Acarol. 58, 51–68.

Liu, G.H., Chen, F., Chen, Y.Z., Song, H.Q., Lin, R.Q., Zhou, D.H., Zhu, X.Q., 2013. Complete mitochondrial genome sequence data provides genetic evidence that the brown dog tick *Rhipicephalus sanguineus* (Acari: Ixodidae) represents a species complex. Int. J. Biol. Sci. 9, 361–369.

Mangold, A.J., Bargues, M.D., Mas-Coma, S., 1998. Mitochondrial 16S rRNA sequences and phylogenetic relationships of *Rhipicephalus* and other tick genera among Metastriata (Acari: Ixodidae). Parasitol. Res. 84, 478–484.

Moraes-Filho, J., Marcili, A., Nieri-Bastos, F.A., Richtzenhain, L.J., Labruna, M.B., 2011. Genetic analysis of ticks belonging to the *Rhipicephalus sanguineus* group in Latin America. Acta Trop. 117, 51–55.

Moraes-Filho, J., Krawczak, F., Costa, F., Soares, J.B., Labruna, M., 2015. 2015. comparative evaluation of the vector competence of four south american populations of the *Rhipicephalus sanguineus* group for the bacterium *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis. PLoS One 10, e0139386.

Nava, S., Mastropaolo, M., Venzal, J.M., Mangold, A.J., Guglielmone, A.A., 2012. Mitochondrial DNA analysis of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) in the southern cone of south america. Vet. Parasitol. 190, 547–555.

Nava, S., Estrada-Peña, A., Petney, T., Beati, L., Labruna, M., Szabó, M., Venzal, J.M., Mastropaolo, M., Mangold, A., Guglielmone, A.A., 2015. The taxonomic status of *Rhipicephalus sanguineus* (Latreille, 1806). Vet. Parasitol. 208, 2–8.

Oliveira, P.R., Bechara, G.H., Denardi, S.E., Saito, K.C., Nunes, E.T., Szabó, M.P., Mathias, M.I., 2005. Comparison of the external morphology of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) ticks from Brazil and Argentina. Vet. Parasitol. 129, 139–147.

Sanches, G.S., Évora, P.M., Mangold, A.J., Jittapalapong, S., Rodriguez-Mallon, A., Guzmán, P.E.E., Bechara, G.H., Camargo-Mathias, M.I., 2016. Molecular, biological, and morphometric comparisons between different geographical populations of *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae). Vet. Parasitol. 215, 78–87.

Szabó, M.P., Mangold, A.J., Joao, C.F., Bechara, G.H., Guglielmone, A.A., 2005. Biological and DNA evidence of two dissimilar populations of the *Rhipicephalus sanguineus* tick group (Acari: Ixodidae) in South America. Vet. Parasitol. 130, 131–140.

Tagle, I., 1976. Presencia accidental de *Rhipicephalus sanguineus* en un perro de Santiago de Chile. http://www2.inia.cl/medios/biblioteca/agritec/NR23393.pdf (Accessed 13 september 2016).

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Tamura, K., 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C content biases. Mol. Biol. Evol. 9, 678–687.

Walker, J.B., Keirans, J.E., Horak, I.G., 2000. The Genus *Rhipicephalus* (Acari: Ixodidae): A Guide to the Brown Ticks of the World. Cambridge University Press, Cambridge, pp. pp643.

Zemtsova, G.E., Apanaskevich, D.A., Reeves, W.K., Hahn, M., Snellgrove, A., Levin, M., 2016. Phylogeography of *Rhipicephalus sanguineus* sensu lato and its relationships with climatic factors. Exp. Appl. Acarol. 69, 191–203.