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Expansion in the latitudinal distribution of *Rhipicephalus sanguineus* sensu stricto (Acari: Ixodidae) to southern Chile

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Abstract

Rhipicephalus sanguineus is a species complex with a challenging diagnosis. There are two main lineages: a temperate lineage—now recognized as *R. sanguineus* sensu stricto (s.s.)—and a tropical lineage. In the Americas, several studies examined the distribution of both lineages. Meanwhile, in Chile, the distribution of the tropical lineage was reported from 18° to 22° S and *R. sanguineus* s.s. from 20° to 33° S; the morphological diagnosis located *R. sanguineus* in south-central Chile. In this study, we assessed the morphological and genetic identity of ticks collected from 47 dogs in southern Chile. Six dogs (12.8%) were parasitized by ticks that were morphologically and genetically assigned to *R. sanguineus* s.s. This finding would represent the southernmost record of this species in the country, located at over 308 km further south than the previous record, suggesting a latitudinal expansion of its distribution. Experimental studies are further required to understand how this tick survives extreme weather conditions when compared to other *R. sanguineus* s.s. from lesser latitudes.

Keywords Ticks · *Rhipicephalus sanguineus* sensu stricto · Southern Chile · Temperate lineage · Dog

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Introduction

Ticks are a group of parasites that is of increasing interest to researchers given the importance of these parasites from a veterinary and public health point of view (Moraes-Filho et al. 2011; Nava et al. 2012). Ticks act as vectors of several parasites such as helminths, protozoans, bacteria, and viruses, and some can pose zoonotic concerns, such as *Coxiella burnetti*, *Ehrlichia canis*, *Rickettsia conorii*, *Rickettsia felis*, *Rickettsia massiliae*, and *Rickettsia rickettsii* (Dantas-Torres 2010; Dantas-Torres et al. 2010, 2012; Abarca et al. 2013). Ticks hold importance as there may be changes in their distribution due to climate change and loss of habitat; consequently, this may affect the pathogens they could transmit (Dantas-Torres 2010; Carlson et al. 2017).

The brown dog tick *Rhipicephalus sanguineus* sensu lato (s.l.) (Latreille) (Acarina: Ixodidae) is a cosmopolitan tick, associated with urban and periurban areas where domestic dogs act as its main host. However, this species also parasitizes other domestic and wild mammals, even humans (Dantas-Torres 2010; Nava et al. 2017). The endophilic behavior of this tick represents a potential threat to public health. Although poorly reported in people from South America (Dantas-Torres 2010), this close interaction could result in the transmission of zoonotic pathogens (Dantas-Torres et al. 2010, 2012; Abarca et al. 2013).

The original description of *R. sanguineus* s.l. by Latreille was very brief and non-informative and its type locality was not clearly stated (Nava et al. 2015, 2018). As a result, several conflicting identifications have been made and have since been revealed through molecular tools; thus, *R. sanguineus* s.l. has been presented alongside 10 other species form a species complex (Nava et al. 2012). This species complex has been the subject of several molecular studies, which have highlighted the presence of two genetic lineages: a temperate lineage—now recognized as *R. sanguineus* sensu stricto (s.s.)—and a tropical lineage. Both lineages would be distributed as two different populations around the globe; *R. sanguineus* s.s. would be distributed between latitudes 22° South and 25° North, and the tropical lineage would be found under latitudes 30° South and above 29° North (Burlini et al. 2010).

It is suggested that there may have been different origins at the moment this species was introduced into the Americas (Szabó et al. 2005; Burlini et al. 2010; Moraes-Filho et al. 2011; Nava et al. 2012, 2018). *Rhipicephalus sanguineus* s.s. would come from European countries during the colonization of the Americas, whereas the tropical lineage would come from Africa during the slavery trade in Atlantic countries (Szabó et al. 2005; Burlini et al. 2010). In the Americas, the tropical lineage would be distributed from northern Argentina to Mexico, and *R. sanguineus* s.s. would be associated with cold and temperate areas, such as Argentina, Brazil, Chile, Uruguay, and the USA (Nava et al. 2017).

Meanwhile in the Southern Cone, the tropical lineage and *R. sanguineus* s.s. have been geographically separated between latitudes 24° and 25° S (Nava et al. 2012). Currently, both have been found in Chile, with the tropical lineage restricted to northern Chile, whereas *R. sanguineus* s.s. is found in north to south-central Chile. The two lineages coexist between latitudes 20° and 22° S (Díaz et al. 2018).

In continental Chile, reports of ticks parasitizing domestic dogs have been published since the 1970s, and additional reports have also been made for islands such as San Juan Bautista Island and Easter Island (González-Acuña et al. 2008; Moraes-Filho et al. 2011). Currently, the geographic distribution of *R. sanguineus* s.l. in the country is as follows: the northernmost record is from Arica (Arica and Parinacota region, latitude 18° S), and the southernmost record is from Angol (La Araucanía region, latitude 37° S) (Abarca et al.

2013; Moreno and González-Acuña 2015; Díaz et al. 2018). The aim of this study was to determine the molecular identification and phylogenetic position of *R. sanguineus* s.l. specimens collected from southern regions and to expand its distribution in Chile.

Materials and methods

Collection of ticks

The ticks (n = 119) were collected during November 2016, August–December 2017, January–March 2018, and October 2019 from both stray and owned dogs of different ages and sexes in southern Chile: Valdivia (39°48′50.854″ S 73°14′45.549″ W; Los Ríos region) and Osorno (40°34′23.96″ S 73°8′9.243″ W; Los Lagos region) (Fig. 1). In terms of the seasons during which ticks were collected, 15, 14, and 10 dogs were sampled during winter, autumn, and spring, respectively, plus eight other dogs during summer. The following areas were inspected at greater detail: neck, legs (axillary, inguinal, and interdigital areas), and ears. All collected ticks were preserved immediately in 70% ethanol following collection.

Identification of ticks

All ticks (n = 119) were identified morphologically at the species level according to the approaches of Walker et al. (2000) and Nava et al. (2017, 2018). For the molecular analysis, we selected one tick per parasitized dog. Each tick (n = 6) was washed individually with PBS1X solution and its idiosome was cut in half with a sterile scalpel. The material
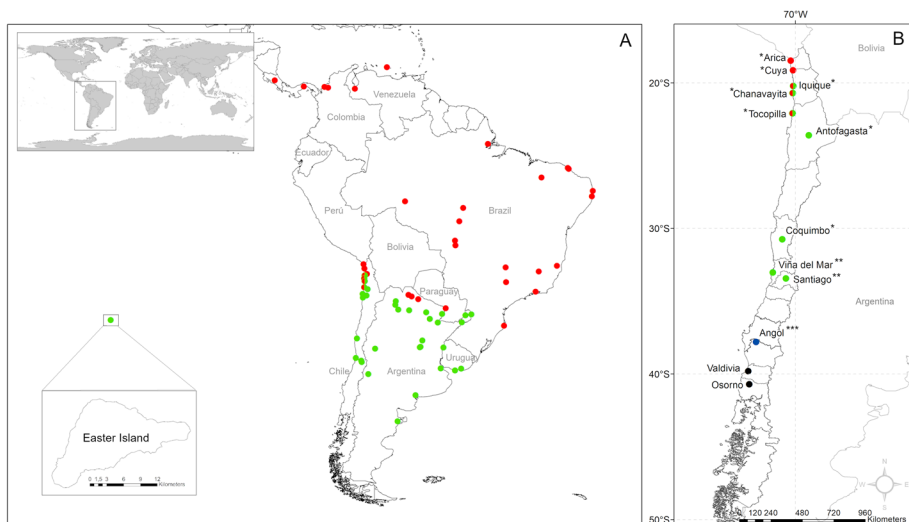

Fig. 1 Distribution of *Rhipicephalus sanguineus* lineages identified with molecular analysis in Latin America and Chile (**a**). Detail of localities where ticks were molecular identified as belonging to the tropical and temperate linages in Chile, and new reports of the present study (**b**). Lineages are indicated as follows: red, tropical lineage; green, *R. sanguineus* sensu stricto; red/green, both lineages are present; blue, *R. sanguineu*s identified only morphologically by Abarca et al. (2013); ***, the more austral register in Chile; and black, present study. Previous records from Chile: *, Díaz et al. (2018); **, Moraes-Filho et al. (2011)

used to handle the ticks was sterilized between every sample by flaming and washing with ethanol. DNA was extracted from the posterior end of the idiosome using a commercial kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. A 405-bp fragment from the 16S rDNA gene was amplified using 5′-CCGGTCTGAACTCAGATCAAGT-3′ and 5′-GCTCAATGATTTTTTAAATTGCTGT-3′ as forward and reverse primers, respectively (Mangold et al. 1998). Reactions were performed in a final mixture volume of 26 μL with 12.5 μL of GoTaq Green Master Mix 2X (Promega, Madison, WI, USA), plus 5.5 μL of ultrapure water free of nucleases, 2 μL of forward primer (10 μM), 2 μL of reverse primer (10 μM), and 4 μL of DNA sample. Tubes with the PCR mixture with the addition of 4 μL of distilled water replacing DNA acted as the negative control. The conditions for the PCR protocol included the following steps: 2 min to 94 °C, followed by seven cycles of 30 s to 94 °C, 30 s to 45 °C, and 45 s to 72 °C. Following this, there were 28 cycles of 30 s to 94 °C, 30 s to 47 °C, and 45 s to 72 °C, with increased annealing temperatures (0.3 °C per cycle), and a final step of 10 min to 72 °C (Díaz et al. 2018). The PCR products were visualized in 1.5% agarose gel stained with SYBR Safe, and sequenced in both directions at Macrogen (Seoul, South Korea).

The obtained DNA sequences (n = 5) were edited and aligned using the Codon Code Aligner software (Codon Code Corporation, Centerville, MA, USA). To test the phylogenetic relationships of ticks, a phylogenetic tree with Bayesian probabilities was constructed, including sequences from ticks deposited in GenBank and using *R. appendiculatus* as an outgroup (for more details, see Supplementary file, Table S1). The model used was GTR + G, the analysis was made with 2.5 million generations, with samples taken every 1,000 trees. In all, 25% of samples were deleted to construct the final tree, using the MrBayes v.3.2 software (Ronquist et al. 2012). The final tree was displayed and annotated in FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). To estimate evolutionary divergences, the sequences were grouped by locality and lineage. The number of base differences per site are shown as p-distances; the standard error was calculated with 10,000 bootstraps, using MEGA X (Kumar et al. 2018).

All sequences obtained in this study are deposited in GenBank under accession numbers MT551042 to MT551046.

Results

Forty-five stray dogs (31 from Valdivia and 14 from Osorno), and two owned dogs (one from Valdivia and one from Osorno) were sampled for ticks. Of these samples, only six dogs (12.8%) were parasitized by ticks: five from Valdivia (four stray dogs and one owned dog), and one from Osorno (owned dog). Four dogs were found to be infested with ticks during the spring from all two sampled locations (three from Valdivia, and one from Osorno), and the other two were sampled from Valdivia during the summer. In total 119 ticks were collected, with 39 adult males and 80 engorged and non-engorged adult females collected.

All ticks collected were morphologically identified as *R. sanguineus* s.s. following descriptions by Nava et al. (2018). With regards to molecular analysis, a total of five partial 16S rDNA gene sequences were obtained; amplification was unsuccessful for one tick. The phylogenetic tree shows two well differentiated clades that belong to the tropical lineage and *R. sanguineus* s.s. The first one includes sequences from North, Central (Mexico) and South America (Colombia, Brazil, Argentina, Chile). The second,

R. sanguineus s.s., was related with sequences from Europe, USA, Uruguay, Argentina and Chile (Fig. 2). The sequences of ticks analyzed in this study were phylogenetically related to *R. sanguineus* s.s., with a strong support for this clade (98%), except one obtained from Osorno that showed a polytomy (Fig. 2). The sequences of ticks collected from Valdivia showed intraspecific divergences (ranging from 0.4 to 1.7%) and were 0.6% different from Osorno, and 0.4–1% divergent from other sequences of *R. sanguineus* s.s. from Chile. These values are into the divergence range of all the other sequences from *R. sanguineus* s.s. (0–1.7%), that includes sequences from Argentina, Uruguay, USA, Spain, Italy and France. On the other hand, the tropical lineage has greater intraspecific divergences than *R. sanguineus* s.s. (5.4–6.8%). In Chile,
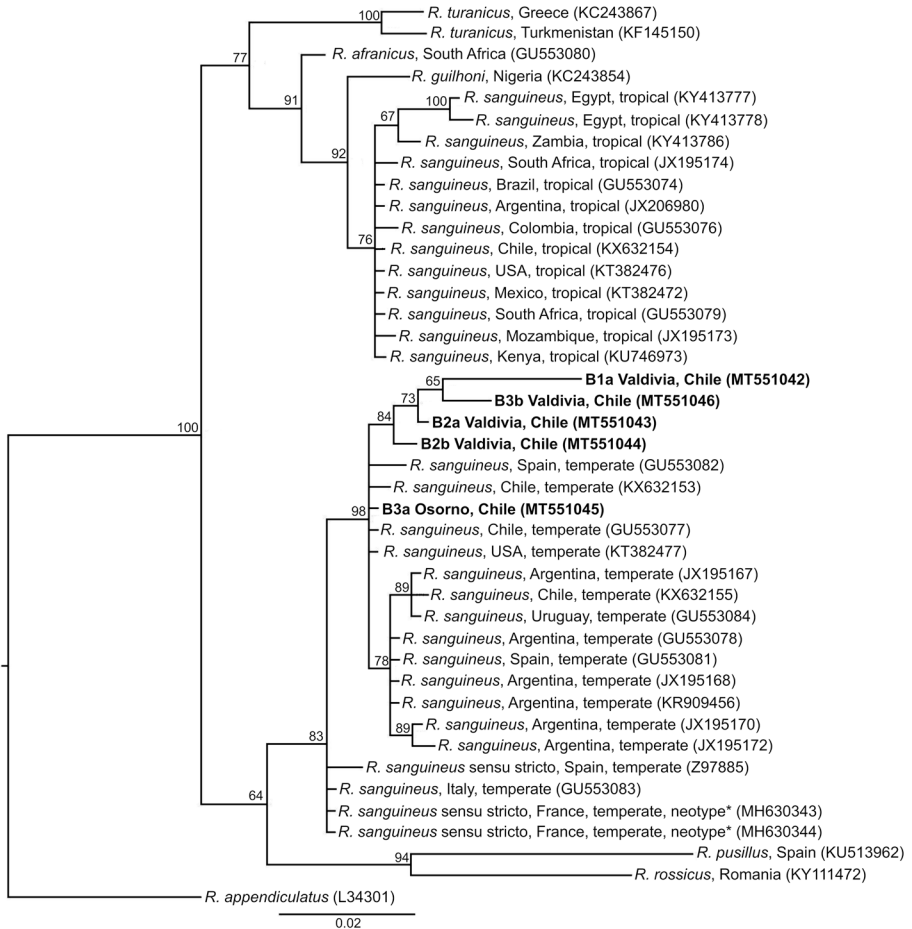

Fig. 2 Phylogenetic tree of *Rhipicephalus sanguineus* based on the 16S gene. This tree was constructed using a Bayesian method, with the substitution model GTR+R. The Bayesian probability values for the nodes are shown over the tree branches; the scale bar shows the number of substitutions per site. The GenBank accession numbers are shown in parentheses. The area or country of origin is specified for all *R. sanguineus* lineages. Sequences obtained in this study are shown in bold, neotype of *R. sanguineus* are indicated with asterisk

differences between sequences from tropical and *R. sanguineus* s.s. were between 5.8 and 6.2% (for more details, see Table S2).

Discussion

Phylogenetic analyses, genetic divergences, and morphological traits support the notion that the samples of *R. sanguineus* from Valdivia and Osorno corresponded to *R. sanguineus* s.s. Of the analyzed sequences, four were aligned in one clade with strong nodal support (84%), whereas the sequence from Osorno did not form part of this small clade, being a polytomy. These small differences could mean that there are different haplotypes (e.g., Moraes-Filho et al. 2011; Díaz et al. 2018). However, additional molecular analyses of a larger sample size are required to clarify these relationships.

In terms of geographical criterion, the present results place the distribution of *R. sanguineus* s.s. about 308 km south to the previously established record in the country (see Abarca et al. 2013). Using an online survey to veterinarians, López et al. (2015) reported the presence of 'ticks' in Valdivia; however, the actual specimens were not collected. Thus, the results presented in the current study confirm the preliminary results of the aforementioned study, while also reporting a new location in the Los Lagos region, in a southern direction. Given that there are previous records of *R. sanguineus* s.s. in Argentina at nearby latitudes (south of the 43th parallel; Nava et al. 2012; Zemtsova et al. 2016), these results seem to be expected. Currently, according to the present results and those from Moraes-Filho et al. (2011) and Díaz et al. (2018), *R. sanguineus* s.s. in Chile would be distributed from 18° to 40° S (southern Chile), at least. Consequently, in Chile, there would be a tropical lineage, restricted to northern Chile, and *R. sanguineus* s.s., with a large distribution. The tropical lineage still needs further analysis to establish its identity within this complex species (Moraes-Filho et al. 2011), even if there are hybrids present in areas of sympatry of both lineages (Burlini et al. 2010; Nava et al. 2012), as can occur in Iquique, Chanavayita (Tarapacá region) and Tocopilla (Antofagasta region; Díaz et al. 2018). Based on the aforementioned data, *R. sanguineus* s.s. would be the dominant species in the country (Fig. 1).

The latitudinal expansion of this tick from Central Chile to southern Chile would be explained because *R. sanguineus* s.s. is adapted to low temperatures and can survive winter conditions through behavioral diapause (Labruna et al. 2017). It is interesting to note that the authors of that study found that extreme winter conditions (10 °C) were deleterious for *R. sanguineus* s.s., with no molting nor hatching of eggs. This was in stark contrast with the environmental conditions experienced during winter for the present locations, with temperatures falling below 7 °C in Valdivia and Osorno (Center for Climate and Resilience Research, CR2; http://explorador.CR2.cl). Thus, according to Koch and Tuck (1986), larvae and nymphs of *R. sanguineus* would suffer diapause under temperatures below 10 °C, avoiding questing behavior during winter (Venzal et al. 2007). Furthermore, the mean temperature in the southern latitudes of Chile—11.3 °C for Valdivia and 14.7 °C for Osorno (Center for Climate and Resilience Research, CR2; http://explorator.CR2.cl)—seems a critical abiotic factor for the development of ticks during the rest of the year. If these findings are compared with the results of Labruna et al. (2017), where temperatures of 13 °C were established as 'winter conditions', then this would negatively affect the development of *R. sanguineus* s.s. Zemtsova et al. (2016) suggested that *R. sanguineus* s.s. would be distributed in regions with temperatures below 20 °C; however, the current temperatures at these latitudes in Chile seem extreme when compared with the temperatures at other

latitudes. Although a previous study examined the effects of seasonality on *R. sanguineus* in Santiago, Central Chile (Alcaíno et al. 1990), where the environmental conditions currently correspond to *R. sanguineus* s.s. (Moraes-Filho et al. 2011; Díaz et al. 2018), it is important to note that the mean and minimal temperatures at this latitude are higher when compared with the sampled locations (CR2; http://explorator.CR2.cl). Hereafter, we suggest that additional studies be conducted to examine the dynamics of these most southern populations to understand how these ticks could survive and develop at these latitudes with low mean temperatures and lower temperatures during the winter (see Labruna et al. 2017).

Lineage identification is of critical importance as vector-borne pathogens are transmitted differently among the different lineages or species (Dantas-Torres 2010; Moraes-Filho et al. 2015). Thus, the potential implications on human health and veterinary medicine may be different between countries that feature different lineages distributed at various geographical locations, as occurs in Chile (Díaz et al. 2018). For example, ticks of a tropical lineage are the only competent vector for *E. canis* (Venzal et al. 2007; Cicuttin et al. 2015; Moraes-Filho et al. 2015). Conversely, *Anaplasma platys* has been found in tropical lineage and *R. sanguineus* s.s. in Argentina (Cicuttin et al. 2015), although in Chile, it has only been recorded in dogs (Abarca et al. 2007). These, and other pathogens and parasites, need to be tested in *R. sanguineus* s.s. to establish their transmission competence (Dantas-Torres et al. 2013). Furthermore, ongoing climate change would cause modifications in the geographical distribution of vectors, such as ticks, with new records found in areas that were previously considered to be free of these parasites (Dantas-Torres 2010; Carlson et al. 2017). Thus, future analyses would need to establish whether pathogens may be transmitted to dogs and other mammals, even humans, at these latitudes of the country.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interests.

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