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Genetic variability of Ehrlichia canis TRP36 in ticks, dogs, and red foxes from the Eurasia continent

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Corresponding Author:	Domenico Otranto University of Bari Valenzano (Bari), Italy
First Author:	Marcos Antônio Bezerra-Santos
Order of Authors:	Marcos Antônio Bezerra-Santos
	Viet-Linh Nguyen
	Roberta Iatta
	Ranju Ravindran Santhakumari Manoj
	Maria Stefania Latrofa
	Adnan Hodžić
	Filipe Dantas-Torres
	Jairo Alfonso Mendoza-Roldan
	Domenico Otranto
Abstract:	Ehrlichia canis is among the most prevalent tick-borne pathogens infecting dogs worldwide, being primarily vectored by the brown dog tick, Rhipicephalus sanguineus sensu lato (s.l.). Genetic variability within E. canis isolates has been assessed by analysis of different genes (e.g., disulfide bond formation protein gene, glycoprotein 19, Tandem Repeat Protein 36 -TRP36) in the Americas, Africa, Asia, and in a single dog sample from Europe (i.e., Spain). Therefore, this study aims to assess the variations in the TRP36 gene of E. canis detected in naturally infected canids and R. sanguineus s.l. ticks from different countries in Asia and Europe. For this, DNA samples of dogs (n = 644), foxes (n = 146), and R. sanguineus s.l. ticks (n = 658) from Austria, Italy, Iran, Pakistan, India, Indonesia, Malaysia, the Philippines, Singapore, Thailand, Vietnam, and Taiwan were included in this study. Ehrlichia canis 16S rRNA positive samples (n = 115 from the previous studies; n = 14 from Austria in this study) were selected for molecular examination by analyses of TRP36 gene. Out of 129 E. canis 16S rRNA positive samples from dogs (n = 88), foxes (n = 7), and R. sanguineus s.l. ticks (n = 34), in 52 the TRP36 gene was amplified. The phylogenetic analysis of the TRP36 pre-repeat, tandem repeat, and post repeat regions showed that most samples were genetically close to the United States E. canis genogroup, whereas two samples from Austria and one from Pakistan clustered within the Taiwan genogroup. TRP36 sequences from all samples presented a high conserved nucleotide sequence in the tandem repeat region (from 6 to 20 copies), encoding for nine amino acids (i.e., TEDSVSAPA). Data herein obtained confirms the US genogroup as the most frequent group in dogs and ticks, whilst the Taiwan genogroup was present in a lower frequency. Besides, this study described for the first time the US genogroup in red foxes, thus revealing that these canids share identical strains with domestic dogs and R. sanguineus s.l. vectors.
Suggested Reviewers:	Martin Pfeffer pfeffer@vetmed.uni-leipzig.de
	Gad Baneth gad.baneth@mail.huji.ac.il

	Rafael Antonio Nascimento Ramos rafaelanramos10@yahoo.com.br
	David Modrý modryd@vfu.cz
Opposed Reviewers:	

To Editor-in-Chief of
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Dear Editor,
Please find herewith attached the manuscript entitled "Genetic variability of *Ehrlichia canis* TRP36 in ticks, dogs, and red foxes from the Eurasia continent" by Marcos Antônio Bezerra-Santos, Viet-Linh Nguyen, Roberta Iatta, Ranju Ravindran Santhakumari Manoj, Maria Stefania Latrofa, Adnan Hodžić, Filipe Dantas-Torres, Jairo Alfonso Mendonza-Roldan and myself, to be considered for publication in *Veterinary Microbiology*.

As you know, *Ehrlichia canis* is among the most prevalent tick-borne pathogens infecting dogs worldwide. However, studies on the genetic variability of this bacterium from different geographical areas are scant. This study aims to assess the variations in the TRP36 gene of *E. canis* strains detected in naturally infected canids and *R. sanguineus* s.l. ticks from different countries in Asia and Europe. For this, DNA samples from the blood of dogs (n = 589), the spleen of foxes (n = 146), and from *R. sanguineus* s.l. (n = 658) obtained from previous studies, and dog blood DNA samples (n = 55) from Austria were included in this study. *Ehrlichia canis* 16S rRNA positive samples were selected for molecular examination by analyses of TRP36 gene. Out of 129 *E. canis* 16S rRNA positive samples from dogs (n = 88), foxes (n = 7), and *R. sanguineus* s.l. ticks (n = 34), 52 scored positive to TRP36 gene. The phylogenetic analysis of the TRP36 gene showed that most samples were genetically closed to the US *E. canis* genogroup, whereas three samples from Austria (n = 2) and Pakistan (n = 1) clustered within the Taiwanese genogroup. TRP36 sequences from all samples presented a high conserved amino acid sequence (i.e., TEDSVSAPA). Data herein obtained confirms the US genogroup as the most frequent group in dogs and ticks. Besides, this study described for the first time the US genogroup in red foxes, thus revealing that these canids share identical strains with domestic dogs and *R. sanguineus* s.l.

I hope you will find the manuscript of interest for the readers of your journal.
Thank you for handling our manuscript and for your insightful suggestions on it,

Regards,
Domenico Otranto

Domenico Otranto
DVM, PhD, DipEVPC, EBVS® European Veterinary Specialist in Parasitology, FRES
Professor of Parasitic Diseases
Head of Department of Veterinary Medicine, University of Bari
President – World Association for the Advancement of Veterinary Parasitology
Visiting Professor of Parasitology and Parasitic Diseases - Faculty of Veterinary Sciences, Bu-Ali Sina University, Hamedan, IRAN
Tel +39 080 4679944/9839
e.mail: domenico.otranto@uniba.it

Highlights

The US *Ehrlichia canis* genogroup is the most frequent among canids and tick vectors

New geographical ranges have been identified for the US *Ehrlichia canis* genogroup

The Taiwan *Ehrlichia canis* genogroup is reported for the first time in Europe

Detection of the US *Ehrlichia canis* genogroup in red foxes is first to science

Red foxes share identical *Ehrlichia canis* genogroup with dogs and ticks

Genetic variability of *Ehrlichia canis* TRP36 in ticks, dogs, and red foxes from the Eurasia continent

Marcos Antônio Bezerra-Santos[a*], Viet-Linh Nguyen[a*], Roberta Iatta[a], Ranju Ravindran Santhakumari Manoj[a], Maria Stefania Latrofa[a], Adnan Hodžić[b], Filipe Dantas-Torres[c], Jairo Alfonso Mendoza-Roldan[a], Domenico Otranto[a, d**]

[a] *Department of Veterinary Medicine, University of Bari, Valenzano, Italy*

[b] *Department of Pathobiology, Institute of Parasitology, University of Veterinary Medicine Vienna, Vienna, Austria.*

[c] *Aggeu Magalhães Institute, Fundação Oswaldo Cruz (Fiocruz), Recife, Brazil*

[d] *Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran*

*These authors have equally contributed to the study

**Corresponding author: Department of Veterinary Medicine, University of Bari, Valenzano 70010, Bari, Italy. Phone: +39-0804679944/9839. Email: domenico.otranto@uniba.it

Abstract

Ehrlichia canis is among the most prevalent tick-borne pathogens infecting dogs worldwide, being primarily vectored by the brown dog tick, *Rhipicephalus sanguineus* sensu lato (s.l.). Genetic variability within *E. canis* isolates has been assessed by analysis of different genes (e.g., disulfide

bond formation protein gene, glycoprotein 19, Tandem Repeat Protein 36 -TRP36) in the Americas, Africa, Asia, and in a single dog sample from Europe (i.e., Spain). Therefore, this study aims to assess the variations in the TRP36 gene of *E. canis* detected in naturally infected canids and *R. sanguineus* s.l. ticks from different countries in Asia and Europe. For this, DNA samples of dogs (*n* = 644), foxes (*n* = 146), and *R. sanguineus* s.l. ticks (*n* = 658) from Austria, Italy, Iran, Pakistan, India, Indonesia, Malaysia, the Philippines, Singapore, Thailand, Vietnam, and Taiwan were included in this study. *Ehrlichia canis* 16S rRNA positive samples (*n* = 115 from the previous studies; *n* = 14 from Austria in this study) were selected for molecular examination by analyses of TRP36 gene. Out of 129 *E. canis* 16S rRNA positive samples from dogs (*n* = 88), foxes (*n* = 7), and *R. sanguineus* s.l. ticks (*n* = 34), in 52 the TRP36 gene was amplified. The phylogenetic analysis of the TRP36 pre-repeat, tandem repeat, and post repeat regions showed that most samples were genetically close to the United States *E. canis* genogroup, whereas two samples from Austria and one from Pakistan clustered within the Taiwan genogroup. TRP36 sequences from all samples presented a high conserved nucleotide sequence in the tandem repeat region (from 6 to 20 copies), encoding for nine amino acids (i.e., TEDSVSAPA). Data herein obtained confirms the US genogroup as the most frequent group in dogs and ticks, whilst the Taiwan genogroup was present in a lower frequency. Besides, this study described for the first time the US genogroup in red foxes, thus revealing that these canids share identical strains with domestic dogs and *R. sanguineus* s.l. vectors.

Keywords: *Ehrlichia canis*, Genetic diversity, TRP36 gene, Dogs, Ticks, Red foxes

1. Introduction

Canine monocytic ehrlichiosis caused by *Ehrlichia canis* is among the most common tick-borne diseases affecting dogs across the globe (**Harrus and Waner, 2011**). This Gram-negative obligatory intracellular bacterium is vectored by the brown dog tick, *Rhipicephalus sanguineus* sensu lato (s.l.) (**Groves et al., 1975)**, which is the most common tick species infesting dogs worldwide (**Dantas-Torres, 2010**). Indeed, the wide distribution of *E. canis* is associated with the ubiquitous distribution of its tick vector, which is highly adapted to urban environments of tropical and temperate regions (**Dantas-Torres, 2010; Dantas-Torres and Otranto, 2015**). The infection caused by *E. canis* in dogs may occur with an acute, subclinical, and chronic presentation as observed in experimental studies (**Stich et al. 2008; Mylonakis et al. 2019**). The acute phase starts after an incubation period of about 8–20 days, usually involving a variety of clinical signs (e.g., anemia, anorexia, ataxia, conjunctivitis, depression, fever, leukopenia, ocular discharge, thrombocytopenia, and vomiting) (**Stich et al. 2008; Mylonakis et al. 2019**). The acute presentation may be followed by a subclinical phase that lasts months or years, with no clinical signs. Later on, dogs may develop chronic phase presenting ulcerative stomatitis, hind limb and scrotal edema, neurological signs (e.g., seizures, ataxia, vestibular dysfunction, and cervical pain), cutaneous and mucosal petechiae and ecchymoses, epistaxis, hematuria, melena, and prolonged bleeding from venipuncture sites (**Stich et al. 2008; Sainz et al., 2015; Mylonakis et al. 2019**).

Despite its wide distribution and veterinary importance, information regarding the genetic variability of *E. canis* from different geographical areas is scant (**Hsieh et al., 2010**), being most of the epidemiological studies based on the analysis of 16S rRNA gene, which is highly conserved among samples isolated worldwide (**Aguiar et al., 2013**). However, genetic variations for this bacterium have been assessed based on the Tandem Repeat Protein 36 (TRP36) gene,

demonstrating different amino acid sequences among *E. canis* strains from different geographical areas (**Zhang et al., 2008; Hsieh et al., 2010; Aguiar et al., 2013; Nambooppha et al., 2018; Aktas and Özübek, 2019; Arroyave et al., 2020**). The TRP36 has a major antibody epitope, and along with other tandem repeat (TR) proteins (e.g., TRP19, TRP140) plays an important role in the pathogen mechanisms within the host (e.g., adhesion, internalization, actin nucleation, and immune evasion) (**Doyle et al., 2006; McBride and Walker, 2011**). Variations in TR sequences and/or number may alter the biological function of the TRP36 protein, possibly resulting in different forms of disease presentation (**Zhang et al., 2008; Aguiar et al., 2013; Ferreira et al., 2014**). To date, four *E. canis* genogroups from United States, Taiwan, Brazil, and Costa Rica have been detected in dogs based on the TRP36 analysis (**Arroyave et al., 2020**), with the Costa Rica genogroup being firstly identified in humans from the same country (**Bouza-Mora et al., 2016**), and, more recently, in dogs from Peru (**Geiger et al., 2018**). Information about the genetic diversity of *E. canis* in Europe and in several Asian regions is lacking. Therefore, the aim of this study is to fill this gap by analyzing the variations in the TRP36 gene sequences of *E. canis* in naturally infected canids and *R. sanguineus* s.l. ticks from different countries in Asia and Europe.

2. Material and methods

2.1 Study area and sampling

The 16S rRNA gene was used to select positive samples to be analyzed for the TRP36 gene. *Ehrlichia canis* 16S rRNA positive samples (n = 115), were selected out of 1393 DNA samples from dog blood (n = 589), foxes' spleen (n = 146), and *R. sanguineus* s.l. ticks (n = 658) obtained from previous studies (**Manoj et al. 2020; Iatta et al., 2020; Nguyen et al., 2020; Sgroi et al.,**

2020) in Asia countries (i.e., India, Indonesia, Iran, Malaysia, Pakistan, the Philippines, Singapore, Taiwan, Thailand, Vietnam) and Italy (**Figure 1)**. In addition, blood DNA samples of dogs (*n* = 55) from Austria (including imported dogs from unknown European countries) were tested for 16S rRNA and TRP36 genes (**Table 1**).

2.2 Polymerase chain reaction for 16S rRNA and TRP36 gene

Samples from Austria (*n* = 55) were PCR screened using the primers EHR16SD: (5′-GGTACCYACAGAAGAAGTCC-3′) and EHR16SR: (5′-TAGCACTCATCGTTTACAGC-3′) targeting a portion of 345 bp of the 16S rRNA for *Ehrlichia* spp./ *Anaplasma* spp. (**Parola et al., 2000**). Thereafter, the 16S rRNA positive samples from this (*n* = 14) and the previous studies (*n* = 115), were tested by conventional PCR using the forward EC36-F (5′-GTATGTTTCTTTTATATCATGGC-3′) and reverse EC36-R primers (5′-GGTTATATTTCAGTTATCAGAAG-3′) targeting a portion of ~1000 bp of *E. canis* TRP36 gene (**Hsieh et al., 2010**). The PCR reactions to amplify the TRP36 gene were performed using the following parameters: 95°C for 10 minutes followed by 35 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1.5 minutes, with a final extension of 72°C for 10 min. Amplified PCR amplicons were examined in 2% agarose gel stained with GelRed (VWR International PBI, Milan, Italy) and visualized on a GelLogic 100 gel documentation system (Kodak, New York, USA).

2.3 Sequencing and phylogenetic analysis of TRP36 gene

Positive samples were purified and sequenced in both directions in an automated sequencer ABI-PRISM 377 (ThermoFisher Scientific, https://www.thermofisher.com) by the Sanger's method using the same pair of primes of PCR. Sequences obtained were analyzed using Geneious Prime software (https://www.geneious.com) and compared each other and with other sequences available

in the GenBank database through BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). For phylogenetic analysis, the representative sequences of *E. canis* TRP36 obtained were included along with those available in the GenBank database. Phylogenetic relationships were inferred using the Maximum Likelihood (ML) method based on Le_Gascuel_2008 model (**Le and Gascuel, 2008**) with discrete Gamma distribution (+G) to model evolutionary rate differences among sites for TRP36 selected by best-fitting model. Evolutionary analyses were conducted with 1000 bootstrap replications using MEGA7 software (**Kumar et al., 2016**). Homologous sequence from *Ehrlichia chaffeensis* (DQ085430) was used as an outgroup.

Bayesian analyses was also performed with the program MrBayes v3.1.2 (**Huelsenbeck and Ronquist, 2001**). The first 25% of these trees represented the "Burn in" and the rest of the trees were used to calculate Bayesian analyses, performed with the General Time Reversible model, using a discrete Gamma distribution to model evolutionary rate differences among sites (+G). The rate variation model allowed for some sites to be evolutionarily invariable ([+I]), with 2,000,000 generations. The first 25% of these trees represented the "Burn in" and the rest of the trees were used to calculate Bayesian analyses.

3. Results

The TRP36 gene was amplified in 52 out of 129 *E. canis* 16S rRNA positive samples (**Table 1**). Amplicons ranged in size from around 600 to 1,000 bp. Most samples (*n* = 49; 94.23% - ST1–ST4 and ST7–ST8) presented sequences of 429 bp in length, encoding for 143 amino acids (aa) in the pre-repeat regions, whilst two sequences from Austria (ST6) and one from Pakistan (ST5) were shorter (i.e., 426 bp encoding for 142 aa).

In the ST1–ST4 and ST7–ST8 aa sequences, molecular signatures in the pre-repeat region were represented by a glycine (G) at position 117 and a putative glycosylation sequon (i.e., NPS) with asparagine (N) at position 125, whilst in three isolates (i.e., two from Austria - ST6, and one from Pakistan - ST5) a gap was present at position 117, and "NSS" at position 125 (**Figure 2**).

All samples presented a highly conserved TR region, encoding for nine aa "TEDSVSAPA", with the number of repeats ranging from 6 to 20 copies (**Figure 2**). The length of aa sequences at the post-repeat region diverged among samples, with the number of translated aa varying from 1 to 36, with the most frequent aa length being 22 (n = 8), followed by 17 (n = 6), 9 (n = 5), and 15 (n = 4).

BLAST analysis showed a nucleotide identity ranging from 99.44% to 100% with sequences belonging to the US and Taiwan genogroups available from the GenBank database (**Table 2**). The molecular identification of *E. canis* genogroups was supported by the distinct separation of clades inferred by the phylogenetic analyses. In particular, the ML tree grouped ST5 and ST6 in the Taiwan genogroup as a monophyletic clade of the US genogroup, which includes the other STs supported by strong bootstrap value (99%) with the exclusion of Costa Rica and Brazil genogroups. Further analysis using the Bayesian inference also confirmed the same cladding with a high bootstrap value (**Figure 3**).

Sequences obtained in this study were submitted to GenBank and are available under the following accession numbers: xxxxxxxx to xxxxxxxxxx.

4. Discussion

This study reports the genetic diversity of *E. canis* TRP36 gene detected in canids and ticks from Eurasian countries, with two main genogroups being identified based on the analysis of the pre-tandem, and TR regions. Most samples herein analyzed belonged to the US genogroup (94.23%), presenting a highly conserved amino acid sequence in the TR region (TEDSVSAPA) varying only in the number of copies. Conversely, variations in molecular signatures of the pre-repeat region of samples from Austria (n = 2) and Pakistan (n = 1) placed these strains in the Taiwan genogroup, presenting a high genetic diversity. Up to date, four *E. canis* genogroups have been identified worldwide based on the analysis of the TRP36 gene: the US genogroup, characterized by the "TEDSVSAPA" amino acid sequences (**Doyle et al., 2005)**; the Taiwan genogroup, also characterized by the TR "TEDSVSAPA" sequence, but with important variation in the pre-repeat region (**Hsieh et al., 2010**); the Brazil genogroup, with eight TR amino acids sequences "ASVVPEAE" (**Aguiar et al., 2013**); and the Costa Rica genogroup, which has been recently detected in human blood, presenting 28 "EASVVPAAEAPQPAQQTEDEFFSDGIEA" and 29 "EASVVPAAEAPQPAQQTEDEFFSDGIE" amino acids at the TR region (**Bouza-Mora et al. 2017**).

Results herein reported include, for the first time, Austria, and Pakistan in the list of countries for the occurrence of the Taiwan genogroup. However, most dogs from Austria were imported from unknown European countries, and since *R. sanguineus* s.l. vector is not endemic in Austria (**Duscher et al., 2015**), dogs probably acquired the infection abroad. Austria, Italy, India, Indonesia, Malaysia, Singapore, the Philippines, and Vietnam were herein recorded as new geographical ranges for the US *E. canis* genogroup. Accordingly, the *E. canis* US genogroup is the most represented in dogs and *R. sanguineus* ticks worldwide, being previously reported in North America, Colombia, Brazil, Cameroon, Nigeria, Spain, China, Turkey, Israel, Pakistan, and

Thailand (**Doyle et al., 2005; Aguiar et al., 2013; Kamani et al., 2013; Zweygarth et al., 2014; Nambooppha et al., 2018; Aktas and Özübek, 2019; Cabezas-Cruz et al., 2019; Arroyave et al., 2020; Mengfan et al., 2020**). On the other hand, the Taiwan genogroup has been only reported in dog samples from China, Taiwan, Thailand, and Turkey, and in tick cell culture in South Africa (**Hsieh et al., 2010; Zweygarth et al., 2014; Nambooppha et al., 2018; Aktas and Özübek, 2019; Mengfan et al., 2020**). Again, the classification of *E. canis* genogroups has been controversial. For example, a recent study considered the Taiwan genogroup as US II (**Aktas and Özübek, 2019**) due to the identical amino acid sequences "TEDSVSAPA" present in the TR region described in North America (**Doyle et al., 2005**). However, other studies have confirmed that this genogroup has a high genetic diversity in the pre-repeat region when compared to the strains belonging to the US group, placing it into a different clade (**Nambooppha et al., 2018; Cabezas-Cruz et al., 2019**).

The number of TR copies from most of the isolates were previously recorded in samples from Spain (7 TRs), Brazil (7, 8, 11, 13 TRs), Nigeria (8, 12 TRs), South Africa (8 TRs), Thailand (8, 9, 13 TRs), Turkey (8, 14 TRs), Pakistan (9 TRs), Israel (10 TRs), Taiwan (10, 12, 13, 14 TRs), Colombia (10, 11, 12, 13, 14, 17 TRs) and USA (12 TRs) (**Zhang et al., 2008; Hsieh et al., 2010; Aguiar et al., 2013; Kamani et al., 2013; Ferreira et al., 2014; Zweygarth et al., 2014; Nambooppha et al., 2018; Aktas and Özübek, 2019; Cabezas-Cruz et al., 2019; da Costa et al., 2019; Arroyave et al., 2020**). The exception for this pattern was the TR numbers detected in Pakistan (6, 15, 20 TRs) and Austria (15 TRs), which are herein recorded for the first time. Whether these variations in TR overlap differences in pathogenicity of these strains warrants further investigations.

The *E. canis* genogroups, including those herein reported, have been recorded only in domestic dogs and *R. sanguineus* s.l. ticks (**Hsieh et al., 2010; Aguiar et al., 2013; Kamani et al., 2013; Zweygarth et al., 2014; Nambooppha et al., 2018; Aktas and Özübek, 2019; Cabezas-Cruz et al., 2019; Arroyave et al., 2020; Mengfan et al., 2020**), with the exception of Costa Rica genogroup, which displayed a zoonotic potential, as it was detected in human blood bank donors in Costa Rica (**Bouza-Mora et al. 2017)**. Later on, the *E. canis* genogroup above was characterized in dogs (**Geiger et al., 2018**) showing the overlap of the same genogroup among people and domestic canids. The above suggests the possibility for the transmission of potentially zoonotic *E. canis* strains and supports the importance of further studies on the genetic variability of this bacterium to detect variations in its pathogenicity, its occurrence in competent vectors, and susceptible hosts, especially in high endemic regions.

The detection of *E. canis* TRP36 genogroups in red foxes is first to science and it confirms that this animal species harbor *E. canis* strains (i.e., US genogroup) identical to those reported in domestic dogs (**Aguiar et al., 2013; Kamani et al., 2013; Ferreira et al., 2014; Nambooppha et al., 2018; Aktas and Özübek, 2019; Arroyave et al., 2020**). The above suggests that wild and domestic canids share the same ecological niches (**André, 2018; Otranto et al., 2015**), which ultimately may affect the transmission dynamics of *E. canis* among these hosts. Since it is the first isolation of the TRP36 *E. canis* gene in Italy, further studies including domestic dogs, other wild canid species, and ticks are advocated to confirm this overlapping of *E. canis* genogroups among these vertebrate hosts and vectors.

This study brings important information on the genetic diversity of *E. canis* in countries from Asia and Europe, reporting for the first time the characterization of the TRP36 gene of this pathogen in most countries herein evaluated. These results suggest that the US genogroup is the most frequent

group in dogs and ticks in the studied areas, and the Taiwan genogroup occurs with a lower frequency. Finally, this is the first detection of the US genogroup in red foxes confirming that these canids share identical strains with domestic dogs.

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Figure legends

Figure 1. Distribution map of *Ehrlichia canis* TRP36 genogroups detected in this and previous studies worldwide in dogs, foxes, and/or ticks. Orange represents countries where only the US genogroup was detected; green represents countries where only the Taiwan genogroup was detected; and yellow represents countries where both genogroups were reported.

Figure 2. Differences in the *Ehrlichia canis* TRP36 pre-repeat region and Tandem repeat variation number of samples evaluated in this study. Notice that ST5 and ST6, which belongs to the Taiwan genogroup, present a high amino acid variability in the pre-repeat region when compared to US genogroup samples.

Figure 3. Molecular phylogenetic analysis by Maximum Likelihood (ML) method inferred from the TRP36 gene of 37 sequences of *Ehrlichia canis* with *Ehrlichia chaffeensis* (DQ085430) as outgroup. Nodal support is also indicated by Bayesian inference (BI) analysis. Numbers at nodes are the support values ordered as ML/BI. Sequence types (STs) obtained in this study are indicated in bold.

Figure 1

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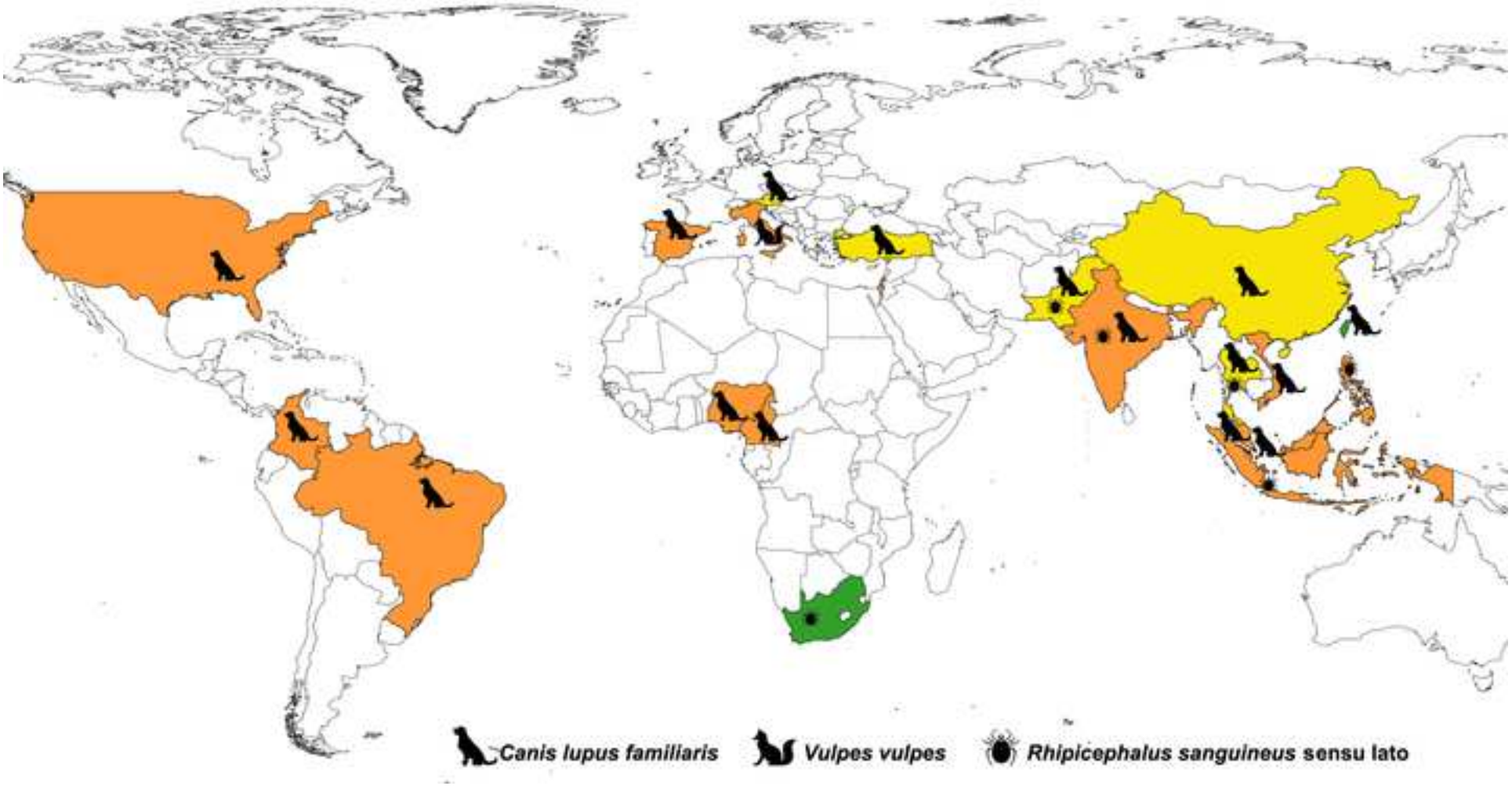

Figure 2

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Amino acid position of variable regions (*Molecular signatures)

Sample	19	23	24	26	30	33	35	37	41	44	45	46	48	49	50	51	58	60	62	66	74
MN159542	N	A	H	F	Q	I	G	S	L	D	I	E	Y	P	E	H	N	N	H	Y	Y
ST1	.	.	.	L	.	R	.	.	.	.	.	.	H	.	G	.	K	.	R	D	D
ST2	.	.	.	.	.	R	.	.	.	.	V	.	H	.	G	.	K	.	R	D	D
ST3	.	.	.	.	.	R	.	.	.	.	.	.	H	.	G	Y	K	.	R	D	D
ST4	.	.	.	.	.	R	.	.	.	.	.	.	H	.	G	.	K	.	R	D	D
ST5	G	S	N	.	S	R	V	V	I	E	V	G	S	G	.	.	K	G	.	D	.
ST6	G	S	N	.	S	R	I	V	I	E	V	G	S	G	.	.	K	G	.	D	.
ST7	.	S	.	.	.	R	.	.	.	.	.	.	H	.	G	.	K	.	.	D	D
ST8	.	S	.	.	.	R	.	.	.	.	.	.	H	.	G	.	K	.	R	D	D

Sample	78	79	80	82	84	102	108	116	117*	123	124	125*	128	129	130	133	Pre-repeat length	Tandem Repeats[a]
MN159542	N	M	K	H	L	N	F	D	G	Y	G	NPS	R	P	A	N	143	TEDSVSAPA[23]
ST1	.	.	.	.	.	.	.	.	.	H	E	---	.	.	.	.	143	TEDSVSAPA[8, 10, 11, 13]
ST2	.	.	.	.	.	.	.	.	.	H	E	---	.	.	.	.	143	TEDSVSAPA[12, 13, 17, 20]
ST3	.	.	.	.	.	.	.	.	.	H	E	---	.	.	.	.	143	TEDSVSAPA[10, 11, 12, 14]
ST4	.	.	.	.	.	.	.	.	.	H	.	---	.	.	.	.	143	TEDSVSAPA[7 - 14]
ST5	D	.	.	R	.	K	.	G	*	H	.	NSS	L	L	P	S	142	TEDSVSAPA[15]
ST6	D	.	.	R	.	K	S	G	*	H	.	NSS	L	L	P	S	142	TEDSVSAPA[15]
ST7	.	V	M	N	.	.	.	.	.	H	.	---	P	.	.	.	143	TEDSVSAPA[10]
ST8	.	.	.	.	F	.	.	.	.	H	E	---	P	.	.	.	143	TEDSVSAPA[8]

Figure 3

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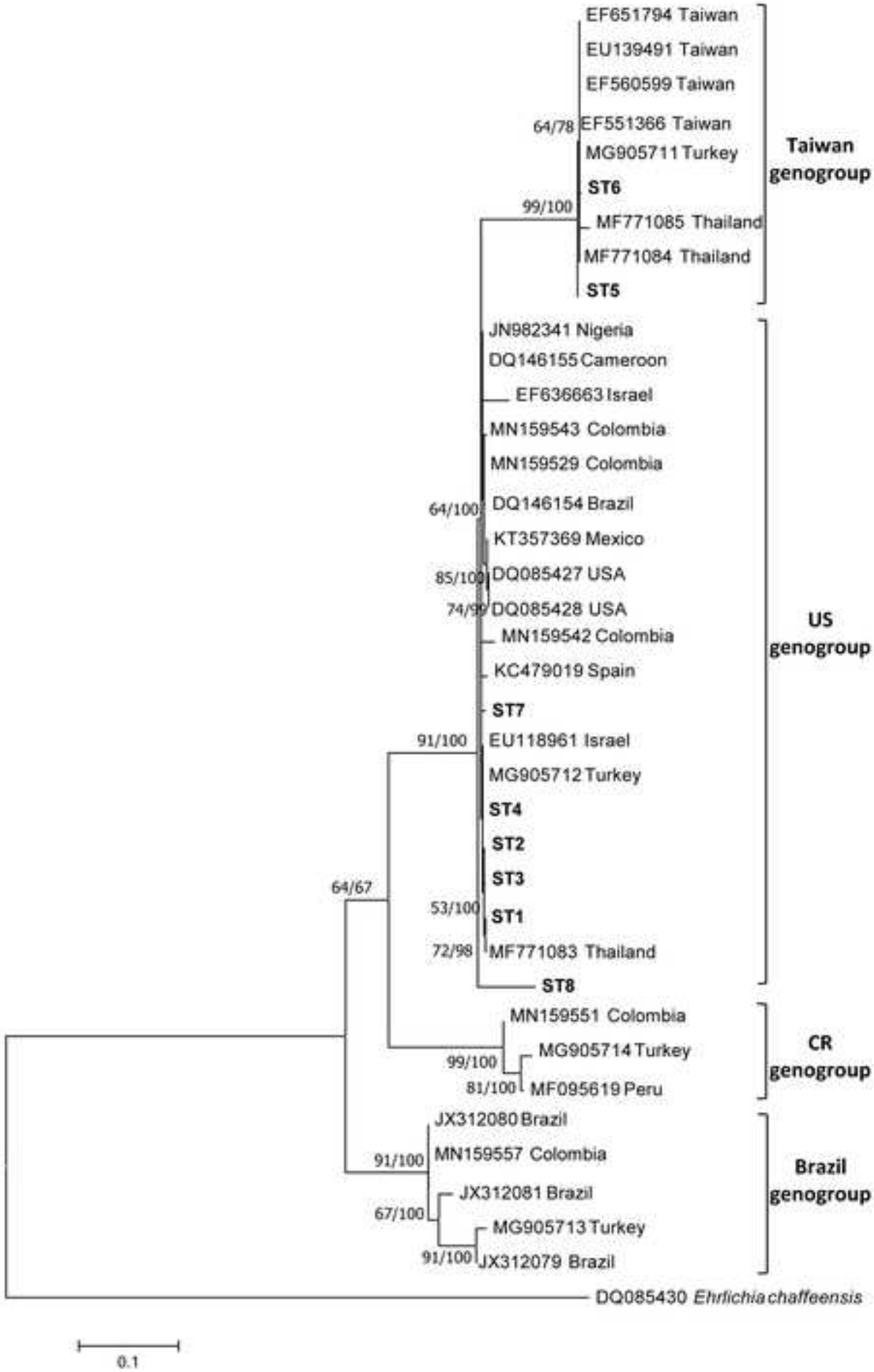

Table 1. Dog, fox, and tick samples from Asia and Europe evaluated for the TRP36 gene.

Country	Host (*n*)			16S rRNA positive	TRP36 positive
	Dog	Fox	Tick		
Austria	55	-	-	14 dogs	4 dogs
India	230	-	294	37 dogs; 27 ticks	25 dogs; 2 ticks
Indonesia	8	-	79	2 dogs; 2 ticks	1 tick
Iran	248	-	-	17 dogs	0
Italy	-	146	-	7 foxes	5 foxes
Malaysia	5	-	3	2 dogs	1 dog
Pakistan	50	-	-	10 dogs	9 dogs
Philippines	12	-	90	2 dogs; 2 ticks	2 ticks
Singapore	6	-	4	1 dog	1 dog
Taiwan	4	-	25	1 dog	0
Thailand	16	-	46	1 dog; 3 ticks	1 tick
Vietnam	10	-	117	1 dog	1 dog
Total	644	146	658	129	52

Table 2. Sequence type detected through the phylogenetic analysis of samples positive for the TRP36 gene in the Eurasia continent.

Sequence type (ST)	Host			Total n (%)	Genogroup	Accession number and nucleotide identity percentage
	Dog (country)	Fox (country)	Tick (country)			
ST1	4 (Vietnam, Austria, Singapore, Malaysia)	-	4 (Indonesia, Thailand, Philippines)	8/52 (15.4%)	US	MF771083 (100%)
ST2	7 (Pakistan)	-	2 (India)	9/52 (17.3%)	US	MH549195 (99.72%)
ST3	7 (India)	-	-	7/52 (13.5%)	US	MH549195 (99.72%)
ST4	18 (India)	5 (Italy)	-	23/52 (44.2%)	US	EU118961 (100%)
ST5	1 (Pakistan)	-	-	1/52 (1.9%)	Taiwanese	EU139491 (99.87%)
ST6	2 (Austria)	-	-	2/52 (3.8%)	Taiwanese	EU139491 (99.87%)
ST7	1 (Austria)	-	-	1/52 (1.9%)	US	MN159539 (99.44%)
ST8	1 (Pakistan)	-	-	1/52 (1.9%)	US	MH549195 (99.66%)

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: