

Molecular Characteristics of Rickettsia in Ticks Collected Along the Southern Border of Mongolia

Michael E. von Fricken (✉ mvonfric@gmu.edu)

George Mason University College of Health and Human Services <https://orcid.org/0000-0003-2938-4549>

Matthew A. Voorhees

US Army Medical Research Institute of Infectious Diseases

Jeffrey W. Koehler

US Army Medical Research Institute of Infectious Diseases

Carmen Asbun

George Mason University College of Health and Human Services

Brandon Lam

Johns Hopkins University School of Medicine

Barbara Qurollo

North Carolina State University

Kathryn M. Hogan

George Mason University College of Health and Human Services

Uyanga Baasandagwa

National Center for Zoonotic Disease

Battsetseg Jigjav

National Center for Zoonotic Disease

Randal J. Schoepp

US Army Medical Research Institute of Infectious Diseases

Research

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Abstract

Introduction: Tick-borne infectious *Dermacentor* and diseases represent a significant threat to public health, particularly in regions where individuals frequently enter tick habitats. This is especially true in Mongolia, where 26% of the population are pastoral herders whose lifestyle results in high risk of tick-borne diseases, which include Rickettsial diseases. In this study, ticks collected along Mongolia's southern border were tested for the presence of *Rickettsia* spp. DNA to better understand the risk of this bacterial infection in the region.

Methods: *Dermacentor* and *Hyalomma* ticks (n = 4,022) collected across southern Mongolia (aimags Bayankhongor, Dornogovi, Govi-Altai, Khovd, and Omnogovi) were pooled and tested for *Rickettsia* spp. by real-time PCR. Subsequent melt-curve analyses and Sanger sequencing were used to identify specific *Rickettsia* species.

Results: Approximately 64% of the 786 tick pools tested positive for *Rickettsia* bacteria. Melt curve analyses identified between four and six different *Rickettsia* species circulating in these tick pools. Amplicon sequencing of the *ompA* gene from selected positive samples identified *Rickettsia* spp. that closely resembled *R. raoultii* and *R. sibirica*. Detection rates varied greatly by sampling region and tick genus. *Dermacentor* ticks from the Govi-Altai region had the highest maximum likelihood estimation (MLE) infection rate of 48.4% (95% CI: 41.7-56.5%) while *Hyalomma* ticks collected in Omnogovi had an MLE rate of 7.6% (95% CI: 6.2-9.2%).

Conclusions: Multiple *Rickettsia* species were found to circulate at high rates within native tick species in southern Mongolia. Further studies are required to investigate the clinical burden of disease associated with these *Rickettsia* spp. in exposed Mongolian populations.

Background

Rickettsial infections are on the rise globally and pose an emerging threat to human health [1–3]. These pathogenic bacteria are transmitted by arthropods such as ticks and fleas causing mild to fatal illnesses (ex. spotted fevers and typhus) characterized by non-specific fever, myalgia nausea, and rash that can progress to lymphadenopathy and encephalitis [4]. Treatment needs to be started promptly after suspected Rickettsial infection to improve prognosis. Spotted Fever Group (SFG) rickettsial organisms are gram-negative obligate intracellular coccoid-shaped bacteria that can infect a variety of mammalian species including livestock and humans. Tick-borne SFG *Rickettsia* are distinguished from the *Rickettsia* typhus group (TG) by vector, clinical presentation, and the presence of the outer membrane protein *ompA*, which is absent in the TG *Rickettsia* [5]. The epizootiology of *Rickettsia* spp. is complicated by transovarial and transstadial transmission within their vector tick species [6].

Mongolia, a vast landlocked country, has up to 26% of the three million residents engaging in traditional pastoral herding. This subset of the population is at an increased risk of exposure to zoonotic and vector-borne infectious diseases [7–9]. Tick-borne rickettsioses can have a significant impact on health and

livelihood within this at-risk population, with peak tick bite counts occurring during productive months paired with low healthcare seeking rates despite presence of symptoms [10]. Mongolian lives are also indirectly affected by tick-borne diseases through economic losses incurred from illness in livestock [7].

Multiple *Rickettsia* species including *R. raoultii*, *R. sibirica sibirica*, *R. sibirica mongolitimonae*, *R. heilongjiangensis* and “*Candidatus R. tarasevichiae*” have been identified in *Dermacentor* spp., *Ixodes persulcatus*, and *Haemaphysalis concinna* ticks collected in and around Mongolia [2, 8, 11–14]. Of these, only *R. raoultii* (scalp eschar and neck lymphadenopathy after tick bite, or SENLAT) and *R. sibirica sibirica* (Siberian tick typhus) are known to cause human disease [15].

Characterization of *Rickettsia* species is especially important, since they are implicated in serious clinical diseases in neighboring countries [16–21]. Previous studies by our group collected and tested *Dermacentor nuttalli* and *Hyalomma asiaticum* tick pools for the presence of Crimean-Congo hemorrhagic fever virus by real-time RT-PCR [9]. Here, we utilized this same sample set to assess the presence of different *Rickettsia* spp.

Methods

Sample collection, study location, and processing.

Questing environmental ticks and ticks removed from livestock were collected in 2013 and 2014 by the National Center for Zoonotic Diseases (Ulaanbaatar, Mongolia) from five aimags in southern Mongolia (Khovd, Govi-Altai, Bayankhongor, Omnogovi, and Dornogovi; Fig. 1). *Dermacentor nuttalli* (n = 2,396) and *Hyalomma asiaticum* (n = 1,626) ticks were pooled into 2,011 pools based on identity, sex, geographic location, and engorgement. Tick pools were homogenized [SPEX SamplePrep MiniG® 1600 tissue homogenizer (Metuchen, New Jersey, USA)] and total nucleic acid extracted [TRIzol LS® reagent, KingFisher Flex Purification System, MagMax 96 for MicroArrays Total RNA Isolation Kit (ThermoFisher Scientific)]. These homogenates were further pooled for testing (786 pools of 2–6 ticks each). All extracted nucleic acid and homogenized tick pools were stored at -70 °C until testing.

Rickettsia spp. testing.

Five µl nucleic acid pools were tested in duplicate for *Rickettsia* spp. utilizing a real-time PCR assay with melt curve analysis targeting the 23 s-5 s ITS region with 0.4 µM (final concentration) primers Rick23-5 F (5'- AGCTCGATTGATTTACTTTGCTG - 3') and Rick23-5 R (5'- CCACCAAGCTAGCAATACAAA-3') and SsoAdvanced SYBR Green Supermix (Bio-Rad) in a 25 µL reaction [22]. Cycling conditions were: 98 °C for 3 min; 40 cycles of (98 °C for 15 sec, 62 °C 15 sec, and 72 °C for 15 sec) followed by a melt curve analysis of the 75–90 °C range with measurements in 0.5 °C increments. Samples were run on the LightCycler 480 (Roche). Melt curve analysis was used as a rationale to identify candidates for sequencing, based on amplicon melt temperatures potentially indicating different *Rickettsia* spp.: *R. amblyomma* (78 °C), *R. bellii* (76.5 °C), *R. canadensis* (76.5 °C), *R. conori* (77.5 °C), *R. montanensis* (77 °C), *R.*

parkeri (78 °C), *R. typii* (75.5 °C), *R. rickettsia* (77 or 78 °C), *R. rhipicephi* (78 °C), *R. felis* (78 °C), *Candidatus R. amblyommii* (78.5 °C), *R. honei* (78 °C), and *R. raoultii* (78 °C) [22].

A 172 base pair amplicon of *ompA* was amplified and sequenced using the Big Dye Direct Sanger Sequencing Kit (ThermoFisher Scientific) for samples selected based on the melt curve analysis. Amplification used the primers Rick-ompA-F (5'-TGTAACGACGGCCAGTGCTTTATTACCCACCTCAAC) and

Rick-ompA-R (5'-CAGGAAACAGCTATGACCTRATCACCACCGTAAGTAAAT) modified for the Big Dye Direct Sanger Sequencing kit (underlined sequence). Sequences from the forward and reverse primers were assembled and analyzed using CLC Genomics Workbench v10.1.2.

Statistical analysis.

Maximum likelihood estimates (MLE) and minimum infection rates (MIR) were calculated to estimate the likelihood of pathogen detection from pooled samples based on laboratory findings, both of which are common measurements used when examining pooled samples.

Results

Rickettsia spp. detection.

A total of 4,022 ticks [*Dermacentor nuttalli* (n = 2,306) and *Hyalomma asiaticum* (n = 1,626)] were collected across southern Mongolia. A pooling strategy was implemented, resulting in 467 *D. nuttalli* and 319 *H. asiaticum* tick pools. Initial testing by real-time PCR found 64% of tick pools tested positive (505/786) for *Rickettsia* spp. with *D. nuttalli* and *H. asiaticum* detection rates of 86% and 33%, respectively. The highest *Rickettsia* spp. pool detection rate was observed in Govi-Altai at 95% (195/204). Table 1 depicts a summary of the tick collection locations, species identification, and testing results.

Table 1

Maximum Likelihood estimates and Minimum Infection Rate by region based on qPCR results, including 95% confidence intervals.

Province	Genus	Positive pools (%)	Total # of Ticks	MLE			MIR		
				Point	Low	High	Point	Low	High
Bayankhongor	<i>Dermacentor</i>	55/67 (82%)	334	30.1	22.9	37.3	16.5	12.5	20.4
Dornogovi	<i>Dermacentor</i>	10/12 (83%)	58	39.6	17.4	61.3	17.2	7.5	27.0
Govi-Altai	<i>Dermacentor</i>	195/204 (96%)	1058	48.9	41.2	55.8	18.4	16.1	20.8
Khovd	<i>Dermacentor</i>	34/46 (74%)	238	23.2	16.4	30.4	14.3	9.8	18.7
Omnogovi	<i>Dermacentor</i>	107/138 (78%)	708	26.9	21.6	30.8	15.1	12.5	17.8
Dornogovi	<i>Hyalomma</i>	6/27 (22%)	144	4.6	2.1	9.2	4.2	1.0	7.4
Khovd	<i>Hyalomma</i>	2/3 (66%)	10	34.6	7.3	69.7	20.0	0.0	44.8
Omnogovi	<i>Hyalomma</i>	96/289 (33%)	1478	7.6	6.2	9.2	6.5	5.2	7.8
Total	<i>Dermacentor</i>	401/467 (86%)	2396	33.2	30.1	36.2	16.7	15.2	18.2
Total	<i>Hyalomma</i>	104/319 (33%)	1626	7.4	6.1	8.9	6.4	5.2	7.6

Overall, maximum likelihood estimates (MLE) showed *Rickettsia* spp. present at an average prevalence of 33.2% (95% CI: 30.1–36.2%) in *Dermacentor* ticks, and 7.4% (95% CI: 6.1–8.9%) *Hyalomma* ticks collected along the southern border. Bayankhongor had an 82% prevalence (55/67 positive tick pools) with an MLE of 30.1% (95% CI: 22.9–37.3%) and a MIR of 16.5% (95% CI: 12.5–20.4%). In contrast, Omnogovi aimag had the highest percentage of *Rickettsia* positive *Hyalomma* ticks, with a pool positive rate of 33% (96/289) and an MLE of 7.59% (95% CI: 6.24–9.16) and MIR of 6.5% (95% CI: 5.2–7.6%).

Rickettsia spp. in circulation.

Melt curve analysis of the amplicon generated at the end of the real-time PCR reaction can differentiate some species of *Rickettsia* based on the impact nucleic acid compositional differences have on strand binding kinetics. Analysis of these melt curves identified at least eight distinct curves, suggesting a wide diversity of *Rickettsia* spp. in circulation in the Mongolian tick population.

Thirty samples were selected for Sanger sequencing based on the melt curve analysis, tick species, and geographic distribution. An approximately 212 base pair segment of *ompA* was amplified, sequenced, and BLAST identified. *Rickettsia raoultii* (n = 14), *R. sibirica mongolitimoniae* (n = 6), and *R. sibirica* (n = 1) were detected, and one unique isolate closely related to *R. slovaca* was identified (Fig. 2).

Discussion

Rickettsia spp. are circulating at a high rate within native tick species in Mongolia. The highest MLE rate of 48.4% (95% CI: 41.7–56.5%) was observed in *Dermacentor* ticks from the Govi-Altai region. Additionally, an MLE rate of 7.6% (95% CI: 6.2–9.2%) was observed in *Hyalomma* ticks collected in Omnogovi, warranting further testing. Overall, a large percentage of *D. nuttalli* pools (86%) tested positive for *Rickettsia* spp. by real-time PCR, and nearly all the ticks tested from the Govi-Altai region tested positive. Melt curve analysis found a high amount of *Rickettsia* spp. diversity; *ompA* sequencing identified three species of *Rickettsia* (*R. raoultii*, *R. sibirica mongolitimoniae*, and *R. sibirica*) known to cause human disease. Speck and colleagues (2012) found prevalence rates of *R. raoultii* (82%) and *R. sibirica* (4%) in *Dermacentor nuttalli* ticks in northern Mongolia, with 5% of the identified *Rickettsia* spp. not able to be assigned to a specific tick species [11]. Most infected ticks were found in the Selenge and Khentii aimags with *R. sibirica* being found exclusively in ticks from Arkhangai aimag [11]. PCR analysis of ticks collected at Sino-Russian and Sino-Mongolian borders found a 53.4% prevalence of *Rickettsia* phylogenetically belonging to *R. raoultii* [16]. Boldbaatar and colleagues (2017) detected a 15.1% prevalence of *R. raoultii* in *D. nuttalli* in Dornogovi (a southern aimag), while higher maximum likelihood estimates (MLEs) found in the northern aimags of Selenge and Tov [8]. This study found an MLE of 39.6 (95% CI: 17.4–61.3%) in Dornogovi among *Dermacentor* ticks, but our sample size was only 12 pools. *Hyalomma* ticks collected from the same area had a much lower MLE, 4.6 (95% CI: 2.1–9.2), with only 6/27 pools testing positive. In the same study, *Candidatus R. tarasevichiae* was found to have a 46.6% MLE in *I. persulcatus* ticks collected from the Selenge aimag exclusively [8].

With regard to human risk of disease exposure, those living in more northern aimags (Selenge and Tov) were 2.8 times more likely to be seropositive for *Rickettsia* in comparison to those living in southern aimags (Dornogovi) [7].

Given the high positive detection rates, especially in the Govi-Altai region, continued vector surveillance is warranted. The high positive seroprevalence rates of *Dermacentor* ticks contrasts with a previous study of *D. nuttalli* collected from Dornogovi, which detected *R. raoultii* in 15.1% of ticks included in the study [8]. A previous study that focused primarily on *Rickettsia* prevalence rates in ticks collected from northern aimags found *Rickettsia* spp. rates in *D. nuttalli* as high as 84% and averaging 64% [11]. The *Rickettsia* detection rates determined in this study show similarly high rates in southern aimags of Mongolia. Enhanced serological and syndromic surveillance are needed to determine the clinical burden of SFG *Rickettsia* in Mongolia, paying particular attention to pastoral herding populations. Such studies will help characterize the relationship between the high detection rates of *Rickettsia* found in Mongolian ticks and their impact on both human and animal health. Considering the severity of the clinical symptoms of

Rickettsia isolates reported in neighboring China and Russia, it is possible that these same pathogens are circulating in Mongolia.

Rickettsial pathogens have a complex disease ecology, with a wide distribution of hosts that includes mammals, humans, and ectoparasites. This study highlights the need for continued disease surveillance of both humans and local tick vectors in order to further characterize the epidemiology of SFG *Rickettsia* in Mongolia. Additionally, efforts to increase awareness can be used to inform populations within these regions of their disease risks, especially the Mongolian pastoralists who have regular contact with livestock. Further studies should focus on contributing to a comprehensive epidemiological profile of *Rickettsia* in humans, livestock, vectors, and reservoir hosts within Mongolia.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors have given consent for publication.

Availability of data and materials

The datasets used and analyzed are available upon request from the corresponding author. Sequence data FASTA files for included in supplementary file S1.

Competing interests

No competing interests to declare

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Authors' contributions

MEV designed study, conducted biological analysis, sequencing, drafted manuscript, and performed statistical analysis. MAV and JWK conducted DNA extraction, biological analysis, and sequencing. CA and BL drafted manuscript and conducted biological analysis. BQ drafted manuscript, provided controls and protocols, and assisted with optimization of lab assays. KMH performed statistical analysis and data visualization, UB and BJ collected samples and organized original dataset. RJS designed study, coordinated efforts, and drafted manuscript. All authors read and approved the final manuscript.

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Author Details

¹*Department of Global and Community Health, George Mason University, Fairfax, VA, United States.*

²*United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Frederick, MD, United States.* ³*Johns Hopkins School of Medicine, Baltimore, MD, United States.* ⁴*Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States.*

⁵*National Center for Zoonotic Diseases, Ulaanbaatar, Mongolia*

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Figures

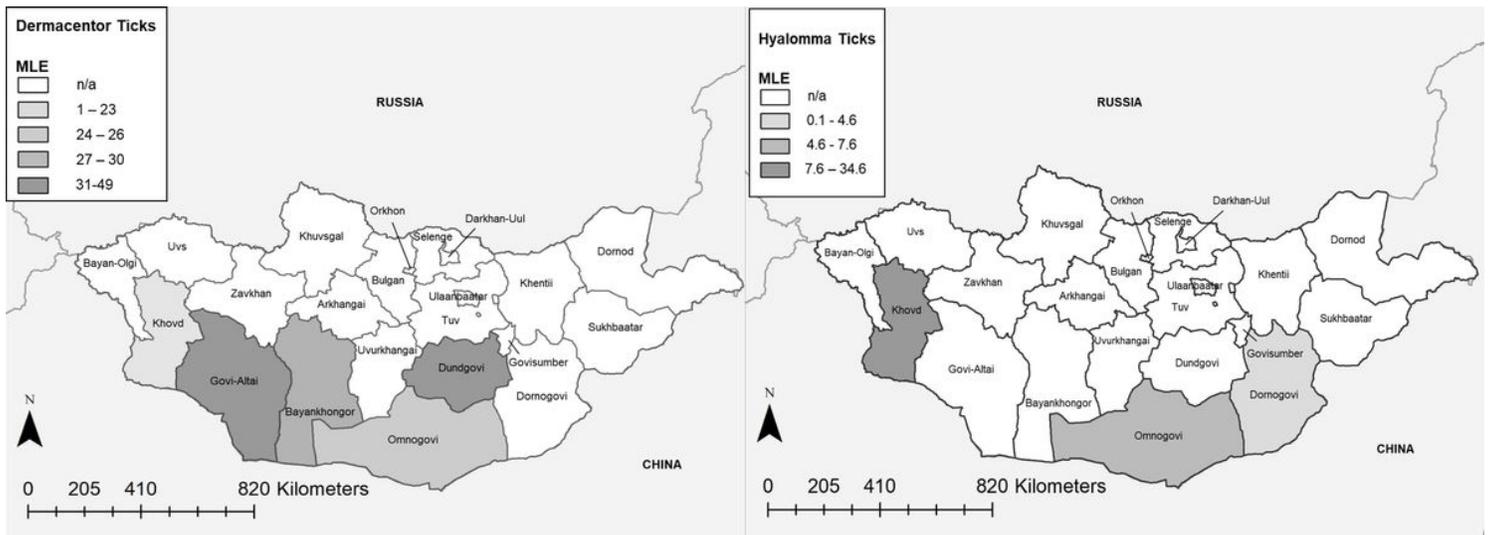


Figure 1

MLE by Aimag for Dermacentor and Hyalomma ticks. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.'

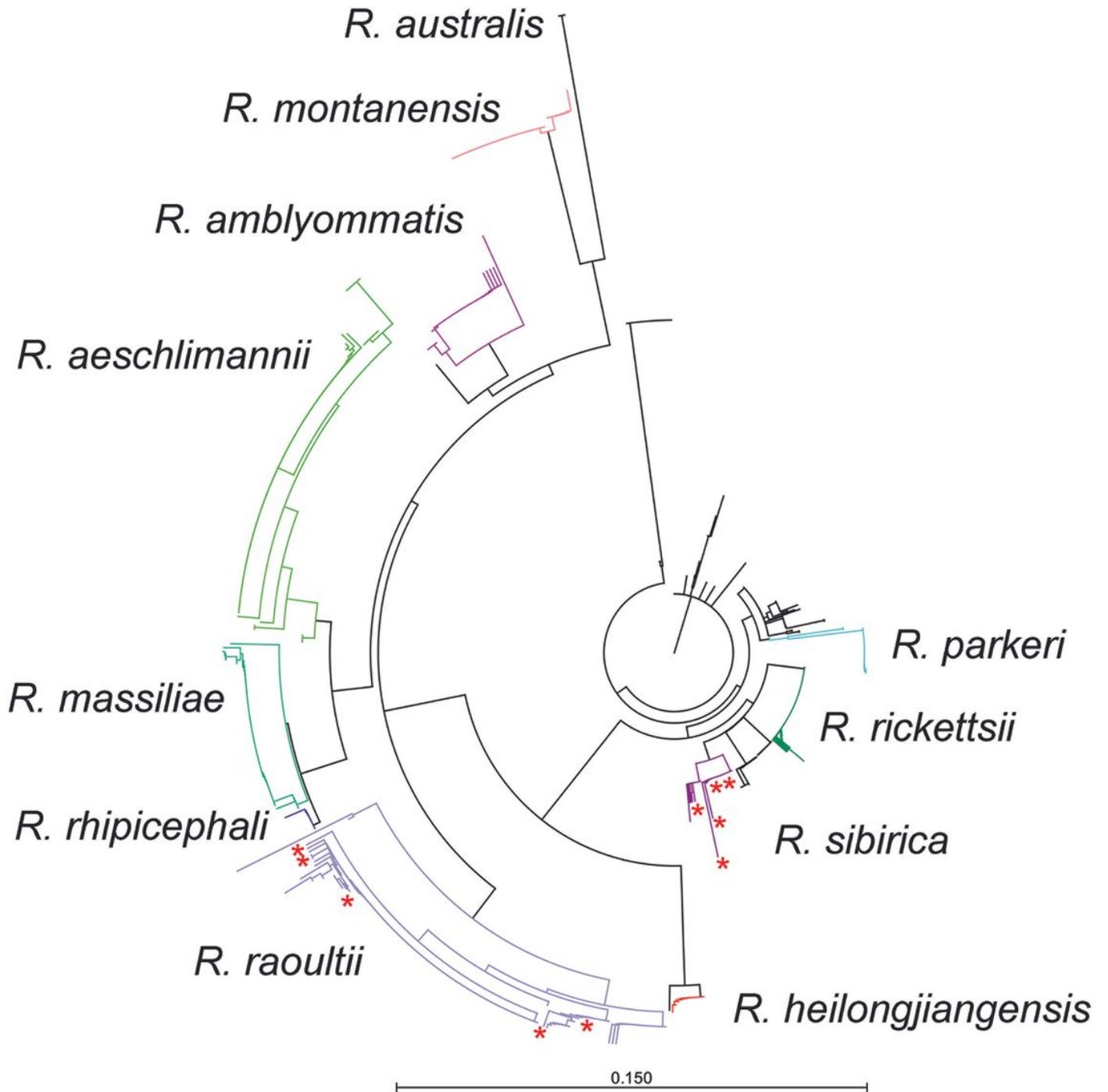


Figure 2

. Sequence analysis of *ompA* gene fragment. *Rickettsia ompA* sequences from the Mongolian tick samples (stared, in red) were aligned with *ompA* sequences from multiple *Rickettsia* species found in GenBank. A phylogenetic tree (Neighbor Joining, Jukes-Cantor) highlights the genetic diversity of the detected *Rickettsia* species.

Supplementary Files

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