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A new spotted fever group *Rickettsia* genotype in *Haemaphysalis leporispalustris* from Maine, USA

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Abstract

Spotted fever group (SFG) rickettsioses are increasingly recognized worldwide as threats to public health. *Rickettsia rickettsii*, *Rickettsia parkeri*, and *Rickettsia rickettsii* subspecies *californica* cause spotted fever rickettsioses, including Rocky Mountain spotted fever. These disease agents are transmitted to humans by various tick vectors in the United States. There is growing concern that other tick species, such as *Haemaphysalis leporispalustris*, may also transmit new and potentially unrecognized SFG rickettsial pathogens. In this study, we found that 6.1% of 296 questing *H. leporispalustris* ticks (21 larvae, 260 nymphs, 9 males, and 6 females) collected from 38 towns across nine counties in Maine, USA, were positive for *Rickettsia* spp. Further multilocus sequence typing and phylogenetic analysis revealed that this is a new *Rickettsia* genotype (*Rickettsia* sp. ME2023) belonging to the SFG group and close to *Candidatus Rickettsia lanei*. Tick vectors and rickettsial species associated with (SFG) rickettsioses in New England warrant further investigation. Additionally, the role of *H. leporispalustris* in pathogen enzootic cycles and transmission requires further study.

Keywords

Spotted fever group; *Rickettsia*; Genotype; *Haemaphysalis leporispalustris*; *Rickettsia rickettsii*; Maine

Introduction

Rickettsia are obligate intracellular bacteria responsible for various vector-borne diseases in humans and animals. A pangenomic meta-analysis showed that *Rickettsia* species diverged into five phylogenetically relevant categories including two spotted fever groups (SFGI and

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SFGII), the Typhus group (TG), the Canadensis group (CG), and the Bellii group (BG) (El Karkouri et al., 2022). Tick-borne rickettsioses are mainly caused by *Rickettsia* species within the SFG groups. The SFG rickettsioses incidence in the United States has generally increased over time with a distinct increase since the mid-1990s, rising from 1,985 cases in 2010 to 6,248 cases in 2017. However, reported cases slightly decreased to 5,207 in 2019 (Bishop et al., 2022). Cases from 2020 to 2022 were much lower, ranging from 1,100 to 1,300. This sharp decline was likely due in large part to a 2020 change in the case definition (Centers for Disease Control and Prevention, 2025).

In the United States, the most serious and commonly reported spotted fever is Rocky Mountain spotted fever (RMSF), caused by *Rickettsia rickettsii*. The surveillance case definition for RMSF was modified to encompass the broader category of spotted fever rickettsiosis in 2010. This change was made because some cases reported as RMSF might actually be caused by other SFG rickettsiae, rather than the RMSF pathogen specifically (Parola et al., 2013). Rocky Mountain spotted fever cases associated with *Dermacentor variabilis* are primarily found in the eastern United States, while *Dermacentor andersoni* is more commonly associated with RMSF in the Rocky Mountain region and farther west. Additionally, RMSF cases linked to *Rhipicephalus sanguineus* sensu lato are concentrated in the Southwest (Parola et al., 2013). Although *D. variabilis* has been historically treated as a major vector of the RMSF agent in the eastern United States, the infection rate of that tick species has been found to be extremely low (Stromdahl et al., 2011). *Rhipicephalus sanguineus* sensu lato may be a more important vector of the RMSF agent than previously recognized, especially in the southwestern United States (Demma et al., 2005). While RMSF remains the most significant spotted fever disease, several other rickettsial pathogens and their tick vectors have been identified as causing human disease in the United States. *Rickettsia parkeri* rickettsiosis, caused by *R. parkeri* transmitted by the Gulf Coast tick, *Amblyomma maculatum*, is distributed primarily in the southeastern United States (Paddock et al., 2004). Pacific Coast tick fever is caused by the bacterium *Rickettsia rickettsii* subspecies *californica*, which is transmitted by the Pacific Coast tick, *Dermacentor occidentalis*, in the western United States (Padgett et al., 2016). Genomic, phenotypic, ecological, and clinical data recently identified *Rickettsia* 364D ("*R. philipii*") as a distinct subspecies of *R. rickettsii* (Paddock et al., 2024). Interestingly, a recently recognized SFG pathogen, determined to be the cause of a severe RMSF-like illness in California, was likely transmitted by *Haemaphysalis leporispalustris* (Eremeeva et al., 2018; Probert et al., 2024).

The rabbit tick (*H. leporispalustris*) is a three-host tick and the only tick species that is widely distributed throughout North and Central America to Argentina (Kohls, 1960). The adult and immature stages of *H. leporispalustris* primarily feed on wild rabbits (*Sylvilagus* spp.) and hares (*Lepus* spp.), while larvae and nymphs may also infest other small animals and several species of birds (Campbell and Glines, 1979; Kollars and Oliver, 2003). The tick can go through two life cycles in one year in the southern United States but has a 2-year life cycle in southwestern Nova Scotia in Canada (Campbell and Glines, 1979). *Haemaphysalis leporispalustris* does bite humans, but not frequently (Eisen, 2022; Harrison et al., 1997).

In North America, *H. leporispalustris* can transmit or carry several *Rickettsia* species, including human pathogenic *R. rickettsii* (Parker et al., 1951), *R. parkeri* (Henning et al.,

2014), *Rickettsia felis* (Roth et al., 2017), non-pathogenic *Rickettsia bellii* (Backus et al., 2023) and *Rickettsia canadensis* (McKiel et al., 1967). This tick species is usually not considered the primary vector for human rickettsioses agents in the United States, but it plays an important role in the enzootic maintenance of *R. rickettsii* in nature (Parker et al., 1951). However, Fuentes et al. isolated two highly virulent isolates of *R. rickettsii* from *H. leporispalustris* in Costa Rica (Fuentes et al., 1985). These two isolates were physiologically and serologically indistinguishable from human strains. Moreover, Probert et al. found severe RMSF-like illness in two patients in northern California over a span of 20 years, and these two case patients might have encountered questing *H. leporispalustris* infected with a new *Rickettsia* species: *Candidatus Rickettsia lanei* (also known as CA6269/CA23RLA) or may have encountered other tick species infected with this pathogen (Eremeeva et al., 2018; Probert et al., 2024). The potential role of *H. leporispalustris* in the maintenance and transmission of SFG *Rickettsia* is underexplored.

In this study, we found that 18 of 296 questing *H. leporispalustris* ticks collected from Maine were *Rickettsia* spp. positive using *RRI6* Taqman real-time PCR assays, which were originally designed to be *R. rickettsii*-specific (Kato et al., 2013). Further multilocus sequence typing (MLST) and phylogenetic analysis revealed that this is a new *Rickettsia* genotype closely related to *Candidatus Rickettsia lanei*.

2. Materials and Methods

2.1 Tick Collection and Morphological Identification

The questing *H. leporispalustris* were collected around Maine residential and non-residential forested areas using 0.5-m² white flannel tick flags during June to August, 2020–2024. The collection sites comprised 38 towns across nine counties in Maine (Table 1). These collections were part of the New England Center of Excellence in Vector-Borne Diseases (NEWVEC) Project ITCH (Is Tick Control Helping). Ticks were identified to species level using standard morphological keys (Egizi et al., 2019) and confirmed through a Taqman real-time PCR method after DNA extraction.

2.2 DNA Extraction and Molecular Identification

DNA was extracted from each tick using Epicenter Master Complete DNA and RNA Purification Kits (Epicenter Technologies, Madison, WI, USA) following the manufacturer's protocols. The real-time PCR assay was performed in 16-μL reaction volumes using the Brilliant III qPCR Master Mix (Agilent, La Jolla, CA, USA) in a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The reaction contained 8-μL 2X Master Mix, 200 nM forward primer, 200 nM reverse primer, 200 nM dual-labeled probe, 1-μL template DNA, and water up to 16 μL. Cycling conditions included an initial activation of the Taq DNA polymerase at 95 °C for 10 min, followed by 40 cycles: 95 °C for 15 s and 60 °C for 1 min. All assays were performed under the same set up and cycling conditions.

Morphologically identified *H. leporispalustris* ticks were confirmed using our newly developed real-time PCR assay targeting the internal transcribed

spacer 2 (*ITS2*) gene, with forward primer (CGGTGGAACGGACGCATTC), reverse primer (TCCACACCCTTCGGGAGAAG) and dual-labeled probe (Cy5 - TCGCAGCCGATTCACAGACATCCAAGAT -Iowa Black). TaqMan probe and primer sequences were designed to be specific for *H. leporispalustris ITS2* gene (GenBank accession number [JQ868568](#)). Assay specificity was first checked for primer and probe sequences using the BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and then was tested with genomic DNA from humans, mice (Promega, Madison, WI), soil, and 18 common tick species in the United States (Xu et al., 2022). The sensitivity of the assay was determined using a serial dilution of gBlock DNA (Integrated DNA Technologies Inc., Coralville, IA) fragment of *H. leporispalustris ITS2* gene. The minimum detection level was 32.9 copies of *ITS2* gene; the maximum detection level was 3.292×10^7 copies. The real-time PCR efficiency was 84.5% with standard curve $Y = -3.758 \cdot \log(X) + 20.31$, $RSq = 0.995$.

Rickettsia spp. was detected and confirmed by two assays, both targeting the *RRi6* gene. The *RRi6*-based assay was originally developed for the specific detection of *R. rickettsii* (Denison et al., 2014), and although *R. rickettsii* is quite rare, it can cause deadly disease in New England. Therefore, we employed both *RRi6* assays to cross-check and confirm the findings. Ticks were first screened using an assay with forward primer (TAAGTTCAGTAAGTAAGATGCCAACC), reverse primer (AGATTGATTAACCCCTCCACTACC) and dual-labeled probe (HEX - AGTGAGTCCTCTCCAATCAGCGATTGAGGC -BHQ1). *Rickettsia* spp. positive samples were confirmed by the Centers for Disease Control and Prevention (CDC) *RRi6* assay (Kato et al., 2013), using forward primer (AAATCAACGGAAGAGCAAAAC), reverse primer (CCCTCCACTACCTGCATCAT) and dual-labeled probe (HEX - TCCTCTCCAATCAGCGATTG -BHQ1). A gBlock containing the *RRi6* gene served as a positive control, with water as the negative control.

2.3 Multilocus Sequence Typing (MLST) and Phylogenetic Analysis

Fragments of four *Rickettsia* genes commonly used for MLST were amplified as previously described: *gltA* (382bp), *ompA* (629bp), *ompB* (856bp) and *sca4* (924bp) (Eremeeva et al., 2018). Positive PCR products were treated using Exosap-IT kit (Applied Biosystems, Foster City, CA, USA) then sequenced with an ABI 3730 sequencer (Applied Biosystems) following the manufacturer protocols. Obtained sequences were aligned with reference sequences from GenBank using ClustalW (Thompson et al., 1994). Phylogenetic analysis was conducted using maximum likelihood methods implemented in MEGA X software (Kumar et al., 2018).

3. Results

Project ITCH collected 2,536 questing ticks from Maine, including six species: *Ixodes scapularis*, *Ixodes cookei*, *Ixodes dentatus*, *Ixodes marxi*, *D. variabilis* and *H. leporispalustris*. Among them, 296 (11.7%) *H. leporispalustris* ticks were collected from 38 towns across nine Maine counties (Table 1). All ticks were collected from June to August, 2020–2024. The species was identified as *H. leporispalustris* using both morphological

characteristics and the molecular assay described in this paper. The collection comprised six adult females, nine adult males, 260 nymphs, and 21 larvae (Table 1).

Rickettsia spp. were detected by both *RRi6* TaqMan real-time assays. The results from both assays were congruent. The overall prevalence of *Rickettsia* spp. in *H. leporispalustris* ticks was 6.1% (18 of 296 ticks, 95% confidence interval 3.6% to 9.4%). The *Rickettsia* spp. positive ticks were one adult male, two larvae, and 15 nymphs. The cycle threshold (Ct) values of these *Rickettsia* spp. positive samples averaged 23.90 (range 20.84 to 26.75).

Multilocus Sequence Typing (MLST) was further applied to characterize these *Rickettsia* spp. positive samples. Four gene fragments (*gltA*, *ompA*, *ompB*, and *sca4*) were successfully amplified and sequenced from 16 *Rickettsia* spp. positive ticks. The *gltA*, *ompB*, and *sca4* sequences from 16 ticks were identical (GenBank accession no: PQ633344 - PQ633346). The *ompA* sequences from 15 of the 16 ticks were identical, whereas the last tick had a single nucleotide difference (C/T) (GenBank accession no: PQ633343 and PQ633347). These *Rickettsia* spp. positive samples appear to belong to the same *Rickettsia* genotype, which we describe herein as *Rickettsia* sp. ME2023.

Phylogenetic analysis showed that the 4-gene sequence concatenated fragment of *Rickettsia* sp. ME2023 not only belonged to the SFG group, but also clustered with three pathogenic *Rickettsia* species: *R. rickettsii* and two California species (*R. rickettsii* subspecies *californica* and *Candidatus Rickettsia lanei*) (Fig. 1). It formed a sister clade with *Candidatus Rickettsia lanei*. When comparing *Rickettsia* sp. ME2023 with *Candidatus Rickettsia lanei*, the nucleotide sequence identities were 99.7%, 98.8%, 99.2%, and 96.4% for the *gltA*, *ompA*, *ompB*, and *sca4* genes, respectively (Table 2). The sequence identity of *Rickettsia* sp. ME2023 was more distant from *R. rickettsii* subspecies *californica* and *R. rickettsii*.

4. Discussion

In this paper, we found a new spotted fever group *Rickettsia* genotype (*Rickettsia* sp. ME2023) in *H. leporispalustris* from Maine, USA. According to the sequence identity cutoff value guideline (Eremeeva et al., 2018; Fournier et al., 2003), *Rickettsia* sp. ME2023 may be classified as a new *Rickettsia* species or subspecies with support of additional genetic or phenotypic characteristics (Parola et al., 2005; Zhu et al., 2005). Recently, Paddock et al. combined phenotypic, ecological, and clinical data with whole-genome sequencing of 11 contemporary isolates and identified *Rickettsia* 364D as *R. rickettsii* subspecies *californica* (Paddock et al., 2024). The taxonomic designation of *Rickettsia* sp. ME2023 may also need an integrative taxonomic approach.

It has been demonstrated that larvae, nymphs, and adults of *H. leporispalustris* are able to acquire and maintain *R. rickettsii* infection by transstadial and transovarial transmissions within the tick population, with active transmission of the bacterium to rabbits by all parasitic stages (Freitas et al., 2009). *Rickettsia* sp. ME2023 may also be transmitted both transovarially and transstadially since it was found in questing larvae, nymphs and adults of *H. leporispalustris*. We did not find *Rickettsia* sp. ME2023 in *I. scapularis*, *I. cookei*, *I. dentatus*, *I. marxi* or *D. variabilis* ticks. However, it is worth looking for this *Rickettsia* in

other tick species that feed on the same hosts as *H. leporispalustris*. Finding this *Rickettsia* genotype in five Maine counties indicated that this genotype is widely distributed in Maine and may also be distributed in the greater New England region where *H. leporispalustris* is endemic.

The newly discovered *Rickettsia* genotype is likely pathogenic. It is grouped together with three known pathogens: widely distributed *R. rickettsii* and two California species (*R. rickettsii* subspecies *californica* and *Candidatus Rickettsia lanei*). Further research is needed to clarify the possible human disease incidence, clinical manifestations, and epidemiology of this putative pathogen. *Rickettsia rickettsii* subspecies *californica* infection is associated with milder disease than RMSF and presents with a localized eschar (necrotic wound) but without a petechial rash (Padgett et al., 2016). *Candidatus Rickettsia lanei* is a rarely encountered pathogen in California and no typical rash was observed in the first human case in 2023 (Probert et al., 2024). Interestingly, a 65-year-old Maine resident tested positive for SFG-reactive IgG antibodies in 2020 but lacked the typical rash progression from wrists/ankles to trunk (Ray, 2020). Ray (2020) suspected that other less-severe SFG diseases with cross-reactive antibodies are possible causative agents. Whether this genotype is linked to probable or less-severe SFG diseases in Maine and New England warrants further investigation.

The identification of *Rickettsia* sp. ME2023 in the rabbit tick raises attention to the potential role for *H. leporispalustris* to serve as a vector for transmission or maintenance of rickettsial agents. The CG group rickettsiae: *R. canadensis* was first detected in *H. leporispalustris* from Canada (McKiel et al., 1967) and later was proven to be an established, ecologically stable *Rickettsia* species in North America (Philip et al., 1982). Burgdorfer noted the potential importance of this three-host species with broad distribution from Alaska to Argentina, remarking that “it is the only species of tick that occurs in all parts of North America where spotted fever has been recognized in man.” (Burgdorfer, 1969). The *R. rickettsii* isolates from *H. leporispalustris* in the United States are of low virulence for guinea pigs and serologically differ from isolates obtained from human patients (Parker et al., 1952; Parker et al., 1951). However, Fuentes et al. suggested that *H. leporispalustris* plays a role as an effective reservoir for maintenance of *R. rickettsii* in Costa Rica because they obtained two high virulent isolates from *H. leporispalustris* collected from the common tapeti *Sylvilagus brasiliensis*. These isolates were physiologically and serologically indistinguishable from human strains (Fuentes et al., 1985). *Haemaphysalis leporispalustris* has not been frequently reported to bite humans (Eisen, 2022). It tends to drop off hares and rabbits during the day when these hosts spend most of their time in their resting places (Burgdorfer, 1969). For these two reasons, this tick is usually not considered the primary vector for human rickettsioses agents in the United States. However, the recent finding of *Candidatus Rickettsia lanei* as the cause of severe RMSF-like illnesses in two patients suggests that these patients might have encountered *Rickettsia* infected *H. leporispalustris* (Eremeeva et al., 2018; Probert et al., 2024).

In New England, Cape Cod and the offshore islands were identified as a hotspot for RMSF as early as 1937. Hazard et al. studied 13 RMSF cases reported in this area, highlighting the region as an important endemic area (Hazard et al., 1969). Rocky Mountain spotted

fever incidence now has significantly decreased in New England compared to historical trends (Chapman et al., 2006; Treadwell et al., 2000). During 2008–2012, 63% of reported SFG rickettsiosis cases originated from five states: Arkansas, Missouri, North Carolina, Oklahoma, and Tennessee (Biggs et al., 2016). Vector competition may play a role in the RMSF decrease in New England. For example, the increase in *I. scapularis* populations might have affected the distribution of *D. variabilis*, the primary vector for the RMSF agent. These two tick species show distinct host preferences: immature stages of *I. scapularis* primarily feed on white-footed mice (*Peromyscus leucopus*), with adults preferring white-tailed deer (*Odocoileus virginianus*), while *D. variabilis* immature stages feed almost exclusively on rodents, with adults targeting dogs and medium-sized mammals (Sonenshine, 2018). How *H. leporispalustris* will compete with these ticks is not clear, although it is a common tick species in Cape Cod (Snow et al., 2023). It is also worth noting that while *D. variabilis* is historically identified as the major vector for the RMSF agent in New England, none of the rickettsial strains isolated from Massachusetts populations of *D. variabilis* in past half century have been identified as *R. rickettsii* (Keirans, 1977; St John et al., 2016). The tick vectors and rickettsial species associated with SFG rickettsioses in New England and the effect of *H. leporispalustris* in the pathogen enzootic cycles and transmission also warrant further investigation.

The CDC *RRi6* assay was designed specifically to detect *R. rickettsii* by targeting the *RRi6* gene that is absent in other rickettsial genomes including *R. rickettsii* subspecies *californica* (Denison et al., 2014; Kato et al., 2013). The *RRi6* gene was initially believed to be unique to *R. rickettsii*, leading to the development of an *RRi6*-based assay for *R. rickettsii*-specific detection (Denison et al., 2014). Homologues of this gene may, however, exist in other *Rickettsia* species. This has resulted in the potential misidentification of *Candidatus Rickettsia lanei* as *R. rickettsii* when using the *RRi6* assay (Probert et al., 2024). *Rickettsia* sp. ME2023 and *Candidatus Rickettsia lanei* may have homologues of the *RRi6* gene. The reverse primer and probe sequences of the newly developed *Candidatus Rickettsia lanei*-specific Taqman real-time PCR assay by Probert 2024 are identical to the sequence found in *Rickettsia* sp. ME2023. As a result, the assay may not be able to distinguish between *Candidatus Rickettsia lanei* and *Rickettsia* sp. ME2023, despite a two-nucleotide difference within the forward primer. DNA sequences of the *ompA* and *ompB* genes can provide unique distinguishing characteristics for *Rickettsia* sp. ME2023 and closely related *Rickettsia* species.

5. Conclusion

We report a new spotted fever group *Rickettsia* genotype (*Rickettsia* sp. ME2023) found in questing *H. leporispalustris* from Maine, USA. Multilocus phylogenetic evidence strongly supports the monophyly of this genotype with the pathogenic spotted fever group rickettsiae, including *Candidatus Rickettsia lanei*. This discovery expands our understanding of the diversity and distribution of rickettsial species in the northeastern United States. Future work is needed to understand the role of *H. leporispalustris* as a vector for enzootic maintenance and transmission of this putative pathogen. While its genetic relatedness to known pathogenic species points to its potential clinical relevance, further epidemiological investigation is warranted to evaluate its public health risk.

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References:

- Backus LH, Lopez-Perez AM, Marcek J, Shultz L, Zazueta OE, Shooter S, Foley J, 2023. Rickettsial antibodies and *Rickettsia bellii* detection in lagomorphs and their ectoparasites in Northern Baja California, Mexico. J. Med. Entomol. 60, 1073–1080. 10.1093/jme/tjad085. [PubMed: 37410023]
- Biggs HM, Behravesh CB, Bradley KK, Dahlgren FS, Drexler NA, Dumler JS, Folk SM, Kato CY, Lash RR, Levin ML, Massung RF, Nadelman RB, Nicholson WL, Paddock CD, Pritt BS, Traeger MS, 2016. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever and other spotted fever group rickettsioses, Ehrlichioses, and Anaplasmosis - United States. MMWR Recomm. Rep. 65, 1–44. 10.15585/mmwr.rr6502a1.
- Bishop A, Borski J, Wang HH, Donaldson TG, Michalk A, Montgomery A, Heldman S, Mogg M, Derouen Z, Grant WE, Teel PD, 2022. Increasing incidence of spotted fever group rickettsioses in the United States, 2010–2018. Vector Borne Zoonotic Dis. 22, 491–497. 10.1089/vbz.2022.0021. [PubMed: 36037000]
- Burgdorfer W, 1969. Ecology of tick vectors of American spotted fever. Bull. World Health Organ. 40, 375–381. [PubMed: 5306621]
- Campbell A, Glines MV, 1979. Development, survival, and oviposition of the rabbit tick, *Haemaphysalis leporispalustris* (Packard) (Acari: Ixodidae), at constant temperatures. J. Parasitol. 65, 777–781. [PubMed: 512768]
- Centers for Disease Control and Prevention, 2025. Data and statistics on spotted fever rickettsiosis. <https://www.cdc.gov/rocky-mountain-spotted-fever/data-research/facts-stats/index.html> (accessed 1 February 2025).
- Chapman AS, Murphy SM, Demma LJ, Holman RC, Curns AT, McQuiston JH, Krebs JW, Swardlow DL, 2006. Rocky Mountain spotted fever in the United States, 1997–2002. Vector Borne Zoonotic Dis. 6, 170–178. 10.1089/vbz.2006.6.170. [PubMed: 16796514]
- Demma LJ, Traeger MS, Nicholson WL, Paddock CD, Blau DM, Ereemeeva ME, Dasch GA, Levin ML, Singleton J Jr., Zaki SR, Cheek JE, Swardlow DL, McQuiston JH, 2005. Rocky Mountain spotted fever from an unexpected tick vector in Arizona. N. Engl. J. Med. 353, 587–594. 10.1056/NEJMoa050043. [PubMed: 16093467]
- Denison AM, Amin BD, Nicholson WL, Paddock CD, 2014. Detection of *Rickettsia rickettsii*, *Rickettsia parkeri*, and *Rickettsia akari* in skin biopsy specimens using a multiplex real-time polymerase chain reaction assay. Clin. Infect. Dis. 59, 635–642. 10.1093/cid/ciu358. [PubMed: 24829214]
- Egizi AM, Robbins RG, Beati L, Nava S, Vans CR, Occi JL, Fonseca DM, 2019. A pictorial key to differentiate the recently detected exotic *Haemaphysalis longicornis* Neumann, 1901 (Acari, Ixodidae) from native congeners in North America. Zookeys 818, 117–128. 10.3897/zookeys.818.30448.
- Eisen L, 2022. Tick species infesting humans in the United States. Ticks Tick Borne Dis. 13, 102025. 10.1016/j.ttbdis.2022.102025. [PubMed: 35973261]
- El Karkouri K, Ghigo E, Raoult D, Fournier PE, 2022. Genomic evolution and adaptation of arthropod-associated *Rickettsia*. Sci. Rep. 12, 3807. 10.1038/s41598-022-07725-z. [PubMed: 35264613]
- Ereemeeva ME, Weiner LM, Zambrano ML, Dasch GA, Hu R, Vilcins I, Castro MB, Bonilla DL, Padgett KA, 2018. Detection and characterization of a novel spotted fever group *Rickettsia*

- genotype in *Haemaphysalis leporispalustris* from California, USA. *Ticks Tick Borne Dis.* 9, 814–818. 10.1016/j.tbd.2018.02.023. [PubMed: 29545107]
- Fournier PE, Dumler JS, Greub G, Zhang J, Wu Y, Raoult D, 2003. Gene sequence-based criteria for identification of new *Rickettsia* isolates and description of *Rickettsia heilongjiangensis* sp. nov. *J. Clin. Microbiol.* 41, 5456–5465. 10.1128/JCM.41.12.5456-5465.2003. [PubMed: 14662925]
- Freitas LH, Faccini JL, Labruna MB, 2009. Experimental infection of the rabbit tick, *Haemaphysalis leporispalustris*, with the bacterium *Rickettsia rickettsii*, and comparative biology of infected and uninfected tick lineages. *Exp. Appl. Acarol.* 47, 321–345. 10.1007/s10493-008-9220-4. [PubMed: 19067185]
- Fuentes L, Calderon A, Hun L, 1985. Isolation and identification of *Rickettsia rickettsii* from the rabbit tick (*Haemaphysalis leporispalustris*) in the Atlantic zone of Costa Rica. *Am. J. Trop. Med. Hyg.* 34, 564–567. 10.4269/ajtmh.1985.34.564. [PubMed: 3923853]
- Harrison BA, Engber BR, Apperson CS, 1997. Ticks (Acari: Ixodida) uncommonly found biting humans in North Carolina. *J. Vector Ecol.* 22, 6–12. [PubMed: 9221733]
- Hazard GW, Ganz RN, Nevin RW, Nauss AH, Curtis E, Bell DW, Murray ES, 1969. Rocky Mountain spotted fever in the eastern United States. *N. Engl. J. Med.* 280, 57–62. 10.1056/NEJM196901092800201. [PubMed: 4881723]
- Henning TC, Orr JM, Smith JD, Arias JR, Norris DE, 2014. Spotted fever group rickettsiae in multiple hard tick species from Fairfax County, Virginia. *Vector Borne Zoonotic Dis.* 14, 482–485. 10.1089/vbz.2013.1534. [PubMed: 24978651]
- Kato CY, Chung IH, Robinson LK, Austin AL, Dasch GA, Massung RF, 2013. Assessment of real-time PCR assay for detection of *Rickettsia* spp. and *Rickettsia rickettsii* in banked clinical samples. *J. Clin. Microbiol.* 51, 314–317. 10.1128/JCM.01723-12. [PubMed: 23135935]
- Keirans JE, 1977. Rocky Mountain spotted fever: occurrence in Massachusetts. *Science* 195, 1284. 10.1126/science.195.4284.1284-a. [PubMed: 402694]
- Kohls GM, 1960. Records and new synonymy of new world *Haemaphysalis* ticks, with descriptions of the nymph and larva of *H. juxtakochi* Cooley. *J. Parasitol.* 46, 355–361. [PubMed: 14410554]
- Kollars TM Jr., Oliver JH Jr., 2003. Host associations and seasonal occurrence of *Haemaphysalis leporispalustris*, *Ixodes brunneus*, *I. cookei*, *I. dentatus*, and *I. texanus* (Acari: Ixodidae) in southeastern Missouri. *J. Med. Entomol.* 40, 103–107. 10.1603/0022-2585-40.1.103. [PubMed: 12597662]
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. 10.1093/molbev/msy096. [PubMed: 29722887]
- McKiel JA, Bell EJ, Lackman DB, 1967. *Rickettsia canada*: a new member of the typhus group of rickettsiae isolated from *Haemaphysalis leporispalustris* ticks in Canada. *Can. J. Microbiol.* 13, 503–510. 10.1139/m67-065. [PubMed: 6039198]
- Paddock CD, Sumner JW, Comer JA, Zaki SR, Goldsmith CS, Goddard J, McLellan SL, Tamminga CL, Ohl CA, 2004. *Rickettsia parkeri*: a newly recognized cause of spotted fever rickettsiosis in the United States. *Clin. Infect. Dis.* 38, 805–811. 10.1086/381894. [PubMed: 14999622]
- Paddock CD, Karpathy SE, Henry A, Ryle L, Hecht JA, Hacker JK, Padgett KA, Kjemtrup AM, Bullock H, Lane RS, Ladner JT, 2024. *Rickettsia rickettsii* subsp. *californica* subsp. nov., the etiologic agent of Pacific Coast tick fever. *J. Infect. Dis.* 10.1093/infdis/jiae512.
- Padgett KA, Bonilla D, Eremeeva ME, Glaser C, Lane RS, Porse CC, Castro MB, Messenger S, Espinosa A, Hacker J, Kjemtrup A, Ryan B, Scott JJ, Hu R, Yoshimizu MH, Dasch GA, Kramer V, 2016. The eco-epidemiology of Pacific Coast tick fever in California. *PLoS Negl. Trop. Dis.* 10, e0005020. 10.1371/journal.pntd.0005020. [PubMed: 27706171]
- Parker RR, Bell JF, Chalgren WS, Thraikill FB, McKee MT, 1952. The recovery of strains of Rocky Mountain spotted fever and tularemia from ticks of the eastern United States. *J. Infect. Dis.* 91, 231–237. 10.1093/infdis/91.3.231. [PubMed: 13011395]
- Parker RR, Pickens EG, Lackman DB, Belle EJ, Thraikill FB, 1951. Isolation and characterization of Rocky Mountain spotted fever rickettsiae from the rabbit tick *Haemaphysalis leporispalustris* Packard. *Public Health Rep.* 66, 455–463. [PubMed: 14816519]

- Parola P, Paddock CD, Raoult D, 2005. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin. Microbiol. Rev. 18, 719–756. 10.1128/CMR.18.4.719-756.2005. [PubMed: 16223955]
- Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, Abdad MY, Stenos J, Bitam I, Fournier PE, Raoult D, 2013. Update on tick-borne rickettsioses around the world: a geographic approach. Clin. Microbiol. Rev. 26, 657–702. 10.1128/CMR.00032-13. [PubMed: 24092850]
- Philip RN, Casper EA, Anacker RL, Peacock MG, Hayes SF, Lane RS, 1982. Identification of an isolate of *Rickettsia canada* from California. Am. J. Trop. Med. Hyg. 31, 1216–1221. 10.4269/ajtmh.1982.31.1216. [PubMed: 6756179]
- Probert WS, Haw MP, Nichol AC, Glaser CA, Park SY, Campbell LE, Trivedi KK, Romo H, Saunders MEM, Kjemtrup AM, Padgett KA, Hacker JK, 2024. Newly recognized spotted fever group *Rickettsia* as cause of severe Rocky Mountain spotted fever-like illness, Northern California, USA. Emerg. Infect. Dis. 30, 1344–1351. 10.3201/eid3007.231771. [PubMed: 38816345]
- Ray EJ, 2020. Is Rocky Mountain spotted fever transmitted in Maine? J Maine Med. Assoc. 2, 10. 10.46804/2641-2225.1045.
- Roth T, Lane RS, Foley J, 2017. A molecular survey for Francisella tularensis and Rickettsia spp. in Haemaphysalis leporispalustris (Acari: Ixodidae) in northern California. J. Med. Entomol. 54, 492–495. 10.1093/jme/tjw202. [PubMed: 28031350]
- St John HK, Adams ML, Masuoka PM, Flyer-Adams JG, Jiang J, Rozmajzl PJ, Stromdahl EY, Richards AL, 2016. Prevalence, distribution, and development of an ecological niche model of *Dermacentor variabilis* ticks positive for *Rickettsia montanensis*. Vector Borne Zoonotic Dis. 16, 253–263. 10.1089/vbz.2015.1856. [PubMed: 26900673]
- Snow AA, Pearson P, Xu G, Allen DN, Santamaria R, Rich SM, 2023. Tick densities and infection prevalence on coastal islands in Massachusetts, USA: establishing a baseline. Insects 14, 628. 10.3390/insects14070628. [PubMed: 37504634]
- Sonenshine DE, 2018. Range expansion of tick disease vectors in North America: implications for spread of tick-borne disease. Int. J. Environ. Res. Public Health 15, 478. 10.3390/ijerph15030478 [PubMed: 29522469]
- Stromdahl EY, Jiang J, Vince M, Richards AL, 2011. Infrequency of *Rickettsia rickettsii* in *Dermacentor variabilis* removed from humans, with comments on the role of other human-biting ticks associated with spotted fever group Rickettsiae in the United States. Vector Borne Zoonotic Dis. 11, 969–977. 10.1089/vbz.2010.0099. [PubMed: 21142953]
- Thompson JD, Higgins DG, Gibson TJ, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680. 10.1093/nar/22.22.4673. [PubMed: 7984417]
- Treadwell TA, Holman RC, Clarke MJ, Krebs JW, Paddock CD, Childs JE, 2000. Rocky Mountain spotted fever in the United States, 1993–1996. Am. J. Trop. Med. Hyg. 63, 21–26. 10.4269/ajtmh.2000.63.21. [PubMed: 11357990]
- Xu G, Ribbe F, McCaffery J, Luo CY, Li AY, Rich SM, 2022. Development of a Taqman real-time PCR for the identification of Haemaphysalis longicornis (Acari: Ixodidae). J. Med. Entomol. 59, 1484–1487. 10.1093/jme/tjac074. [PubMed: 35640022]
- Zhu Y, Fournier PE, Ereemeeva M, Raoult D, 2005. Proposal to create subspecies of *Rickettsia conorii* based on multi-locus sequence typing and an emended description of *Rickettsia conorii*. BMC Microbiol. 5, 11. 10.1186/1471-2180-5-11. [PubMed: 15766388]

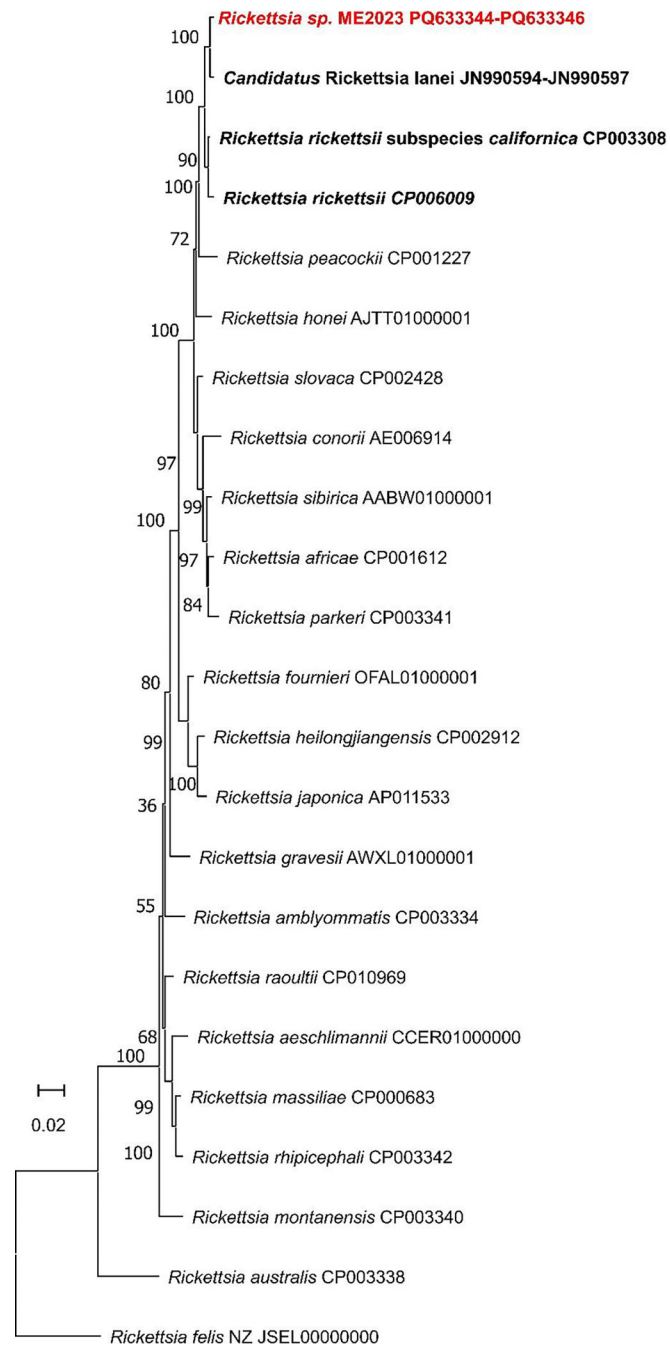


Fig. 1: Phylogenetic tree of concatenated *Rickettsia* genes: *gltA*, *ompA*, *ompB* and *sca4* (length: 2,671bp), constructed by the minimum evolution method of MEGA X software with GTR+G as the best model. The numbers on the branches represent bootstrap support with 500 bootstrap replicates.

Table 1.

County of collection, number of towns sampled, total number of *Haemaphysalis leporispalustris* ticks collected by life stage (L: Larva; N: Nymph; M: Male adult; F: Female adult) and total number of the spotted fever group *Rickettsia* genotype (*Rickettsia* sp. ME2023) with infection rate and 95% confidence intervals (CI) in Maine, USA.

Maine County	Town #	Tick #	<i>Rickettsia</i> sp. ME2023 positive #, (infection rate, 95% CI)
Androscoggin	1	1 (1N)	0, (0%, 0–97.5)
Hancock	4	12 (7N, 5M)	1 (1M), (8.3%, 0–38.5)
Kennebec	6	11 (11N)	1 (1N), (9.1%, 0–41.3)
Knox	4	10 (10N)	0, (0%, 0–30.1)
Lincoln	2	2 (1M, 1F)	0, (0%, 0–84.2)
Penobscot	13	195 (6L, 184N, 2M, 3F)	12 (12N), (6.2%, 3.2–10.5)
Sagadahoc	1	20 (14L, 5N, 1M)	2 (2L), (10.0%, 1.2–31.7)
Waldo	5	42 (41N, 1F)	2 (2N), (4.8%, 0.6–16.2)
York	2	3 (1L, 2N)	0, (0%, 0–70.8)
Total	38	296 (21L, 260N, 9M, 6F)	18(1M, 15N, 2L), (6.1%, 3.6–9.4)

Table 2.

Comparison of sequence identity of the spotted fever group *Rickettsia* genotype (*Rickettsia* sp. ME2023, GenBank accession no: [PQ633344](#) - [PQ633346](#)) with *Candidatus Rickettsia lanei* (GenBank accession no: [JN990594](#) - [JN990597](#)), *Rickettsia rickettsii* subspecies *californica* (GenBank accession no: [CP003308](#)) and *Rickettsia rickettsii* (GenBank accession no: [CP006009](#)).

<i>Rickettsia</i> sp. ME2023 target sequence	<i>Candidatus Rickettsia lanei</i> CA6269 sequence identity	<i>R. rickettsii</i> subspecies <i>californica</i> sequence identity	<i>R. rickettsii</i> sequence identity
<i>gltA</i> (382bp)	99.7%	99.2%	98.1%
<i>ompA</i> (629bp)	98.8%	97.3%	96.8%
<i>ompB</i> (856bp)	99.2%	98.7%	98.4%
<i>sca4</i> (924bp)	96.4%	98.9%	98.8%