

Rickettsia japonica and Novel *Rickettsia* Species in Ticks, China

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PCR amplification indicated the minimum infection rate of *Rickettsia* spp. was 0.66% in *Haemaphysalis longicornis* ticks collected from Shandong Province, China. Phylogenetic analysis based on the *rrs*, *gltA*, *ompA*, and *ompB* genes indicated that the ticks carried *R. japonica*, *Candidatus Rickettsia longicornii*, and a novel *Rickettsia* species related to *R. canadensis*.

Rickettsia species are gram-negative obligate intracellular bacteria that infect humans and a variety of vertebrates through the bite of arthropod vectors. Hard-body ticks are the primary vector of spotted fever group (SFG) rickettsiae; recently, several emerging and reemerging SFG rickettsiae were found to infect humans (1). *Rickettsia japonica* is the pathogenic agent of Japanese spotted fever that has been reported in Japan, South Korea, and Thailand since 1984 (2–4). Japanese spotted fever is a severe zoonosis and develops abruptly with headache, fever, shaking chills, skin eruptions, tick bite eschars, and malaise (2). *R. canadensis* was initially isolated from ticks in Canada; a serologic study indicated the presence of *R. canadensis* antibodies in febrile patients (5). The presence of *Rickettsia* species and their distributions in China are not very clear. In this study, we analyzed *Rickettsia* species in *Haemaphysalis longicornis* ticks collected from Shandong Province, China, and found *R. japonica*, *Candidatus Rickettsia longicornii*, and a novel *Rickettsia* species closely related to *R. canadensis* in the ticks.

The Study

We collected questing ticks by flagging during April–July 2013–2015. We collected them in Jiaonan County (35°35′–

36°8′ N and 119°30′–120°11′E), Shandong Province, China. Jiaonan County is located on the Pacific coast of China and has a maritime monsoon-type climate. We identified tick species individually by morphology and confirmed by PCR amplification and DNA sequencing of the 16S rRNA gene of 2 nymphs and 2 adult ticks of each species as described previously (6,7).

For detection of *Rickettsia* DNA, we pooled ticks according to their developmental stages, with each pool consisting of 20 nymphs or 10 adult ticks. We homogenized them with Tissue Lyser II (QIAGEN, <http://www.qiagen.com>). We extracted total nucleic acids from the tick suspension using the AllPrep DNA/RNA Mini Kit (QIAGEN).

Initially, in all the tick pools, we amplified nucleic acid preparations with rickettsial universal primers targeting *rrs*, *gltA*, and *ompB* (B1–B4). We further amplified *Rickettsia* clones in the tick pools closely related to *R. japonica* with primers of *ompA*, an SFG rickettsia unique gene. The clones positive with *rrs* and *gltA* gene primers but negative with *ompB* primers (B1–B4) we further amplified with primers Cand-1 to Cand-4, which were designed from the *R. canadensis ompB* gene because the *Rickettsia* clones from these tick pools were closely related to *R. canadensis* on the basis of the *rrs* and *gltA* gene sequences (Table). We used distilled water as a negative control in each run.

We performed electrophoresis on the PCR products in 1.2% agarose gels, stained them with ethidium bromide, and visualized them under UV light. DNA bands with the expected size were excised and extracted by Gel Extraction Kit (Omega Bio-tek, <https://www.omegabiotek.com>). We cloned the purified PCR products into pMD19-T vector (Takara, <https://www.takara-bio.com>) and engaged Sangon Biotech (Shanghai, China) (<https://www.life-biotech.com>) to conduct sequencing on both strands. We compared nucleotide sequences with BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and constructed a phylogenetic tree using the maximum-likelihood method with MEGA version 6.0 (<https://www.megasoftware.net>). We deposited the *Rickettsia* genes obtained in this study in GenBank under accession nos. MF496152–MF496168 (*rrs*), MF496169–MF496185 (*gltA*), MF496186–MF496199 (*ompB*), and MK102707–MK102720 (*ompA*).

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Table. Primer sequences and PCR conditions used in study of *Rickettsia* species, China

Target gene	Primer name	Sequence, 5' → 3'	Amplicon size, bp	Annealing temp, °C	Reference
<i>rrs</i>	S1	TGATCCTGGCTCAGAACGAAC	1,486	55	(8)
	S2	TAAGGAGGTAATCCAGCCGC			
	S3	AACACATGCAAGTCGRACGG	1,371		
	S4	GGCTGCCTCTTGCCTTAGCT			
<i>gltA</i>	gltA1	GATTGCTTTACTTACGACCC	1,087	52	(9)
	gltA2	TGCATTTCTTCCATTGTGC			
	gltA3	TATAGACGGTGATAAAGGAATC	667		
	gltA4	CAGAACTACCGATTCTTTAAGC			
<i>ompB</i>	B1	ATATGCAGGTATCGGTACT	1,355	56	(9)
	B2	CCATATACCGTAAGCTACAT			
	B3	GCAGGTATCGGTACTATAAAC	843		
	B4	AATTTACGAAACGATTACTTCCGG			
<i>ompB</i>	Cand-1	CCGGACTTTGCGGTGTAGAT	1,136	52	This study
	Cand-2	AAAGCCAGAAGGTGAGGCTG			
	Cand-3	ACCGCACTTGTATCGGTAGT	874		
	Cand-4	AAGCAGGTGGTGTAGTCGGA			
<i>ompA</i>	Rr190.70p	ATGGCGAATATTTCTCCAAAA	631	50	(10)
	Rr190.701n	GTTCCGTTAATGGCAGCATCT			
Tick mitochondrial 16S RNA	Forward	AGTATTTTACTATACAAAGGTATTG	408	55	(7)
	Reverse	GTAGGATTTTAAAGTTGAACAAACTT			

We collected a total of 2,560 *H. longicornis* ticks, 2,080 nymphs and 480 adults. PCR amplification indicated that 14 tick pools were positive with *rrs*, *gltA*, and *ompB* (B1–B4) primers and further positively amplified by PCR with *ompA* primers. In addition, 3 clones were positive with *rrs*, *gltA*, and *ompB* (Cand-1 to Cand-4) primers. The minimum infection rate of *Rickettsia* in the ticks was 0.66% (17/2,560), assuming 1 tick was positive in each positive pool of ticks.

Sequence analysis indicated that 3 clones (J84, J85, and J217) detected from the tick pools were closely related to *R. canadensis*, showing sequence homology of 98.7%–99.1% for *rrs*, 97.8%–98.4% for *gltA* and 94.8%–95.1% for *ompB*. One clone (J244) was highly homologous to *Candidatus Rickettsia longicornii*, showing sequence homology of 99.2% for *rrs*, 100% for *gltA*, and 99.7% for *ompA*. The remaining 13 clones were homologous to each other and to *R. japonica*, showing sequence homology of 99.2%–100% for *rrs*, 99.1%–100% for *gltA*, 99.3%–99.4% for *ompB*, and 97%–97.3% for *ompA* of a variety strains of *R. japonica* (Appendix Tables 1–4, <https://wwwnc.cdc.gov/EID/article/25/5/17-1745-App1.xlsx>).

Phylogenetic analysis based on the concatenated sequences of *rrs*, *gltA*, *ompB*, and *ompA* showed that *Rickettsia* clones (J84, J85, and J217) were clustered in the same clade with, but distinct from, *R. canadensis*; clone J244 was in the same clade as *Candidatus Rickettsia longicornii*; the remaining 13 clones were in the same clade as *R. japonica*. These results indicated that clones J84, J85, and J217 were a novel *Rickettsia* species; clone 244 was *Candidatus Rickettsia longicornii*; and other clones were *R. japonica* (Figure).

Conclusions

In this study, we demonstrated that *H. longicornis* ticks from China were infected with multiple *Rickettsia* species, including *R. japonica*, *Candidatus Rickettsia longicornii*, and a novel *Rickettsia* species. We named the novel species *Candidatus Rickettsia jiaonani* after the sampling site. The exact classification of *Candidatus Rickettsia jiaonani* needs to be further studied by sequencing the whole genomes of the organisms.

R. japonica infection in humans has been reported recently in Anhui Province in central China (11), suggesting that *R. japonica* is widely distributed in China and its epidemiology needs to be further investigated. *Candidatus Rickettsia longicornii* was previously detected in *H. longicornis* ticks collected from South Korea (12). *Candidatus Rickettsia jiaonani* is closely related to *R. canadensis*, which was first isolated from *H. leporispalustris* ticks removed from rabbits in Ontario, Canada, in 1963 and then from a *H. leporispalustris* tick removed from a black-tailed jackrabbit in California in 1980 (13).

H. longicornis ticks are native to East Asia, including China, Korea, and Japan, and they were introduced into Oceania, including Australia, New Zealand, Fiji, and Hawaii, through cattle importation (6). Recently, this tick species was found in 8 states in the eastern United States (14). This study and previous studies demonstrated that *H. longicornis* ticks carry *R. japonica*, *Candidatus Rickettsia longicornii*, *Candidatus Rickettsia jiaonani*, *Anaplasma phagocytophilum*, *Ehrlichia*, and severe fever with thrombocytopenia syndrome virus (12,15). These pathogens need to be monitored in countries in East Asia in which the *H. longicornis* tick is native and in the countries that this tick species has invaded.

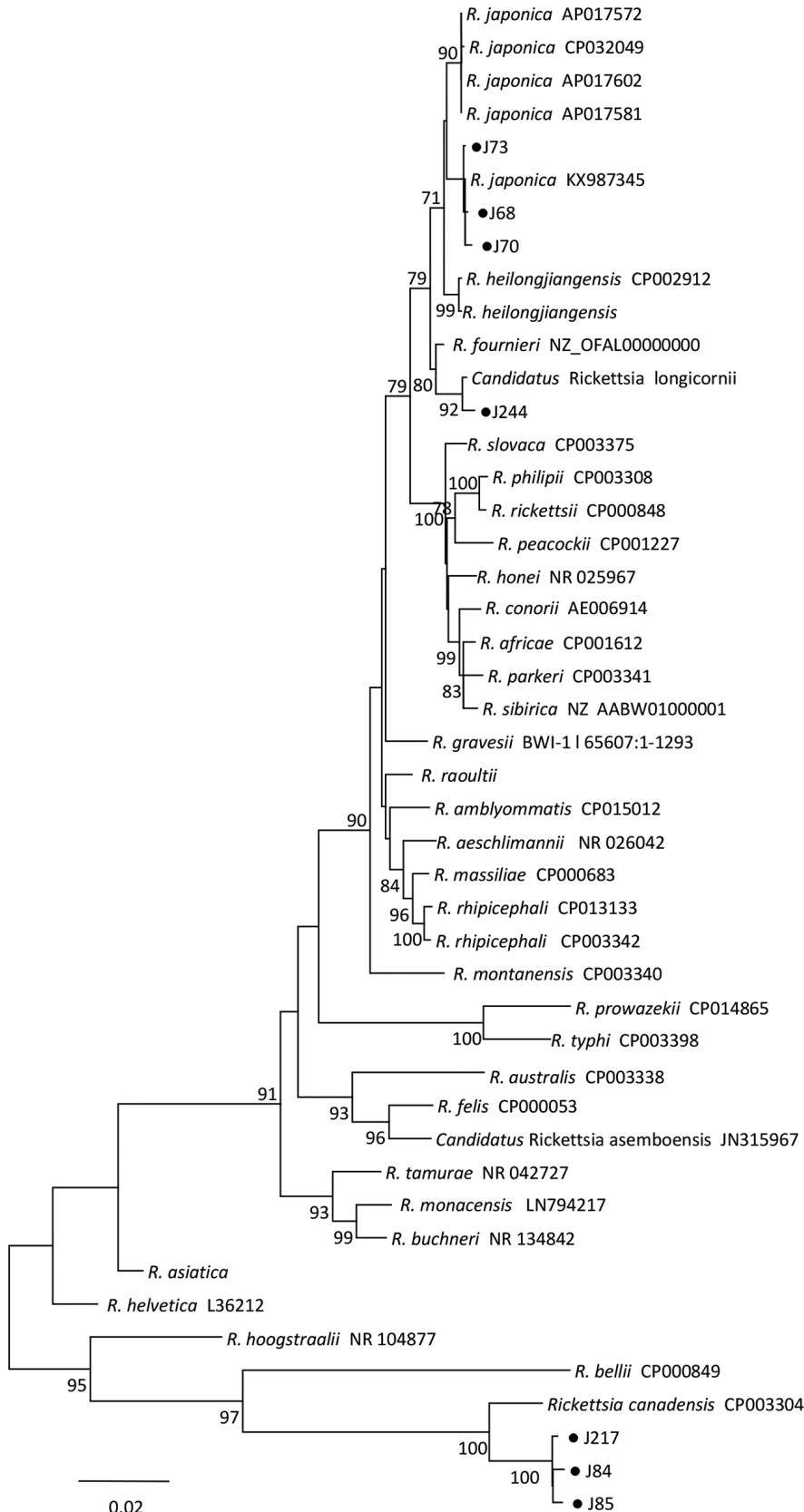


Figure 1. Phylogenetic tree of isolates from study of *Rickettsia* species in China (black dots) and comparison isolates. The tree was generated using the concatenated sequences of *rrs*, *gltA*, *ompB*, and *ompA* of *Rickettsia* species by the maximum-likelihood method in MEGA6 software (<http://www.megasoftware.net>) with 1,000 replicates for bootstrap testing. Numbers (>70) above or below branches are posterior node probabilities. Dots indicate rickettsial sequences obtained in this study. *Rickettsia* clones J69, J70, and J73 represent 13 similar clones in the phylogenetic analysis. Scale bar indicates nucleotide substitutions per site. The *Rickettsia* species name and complete genome GenBank accession no. appear on each line. For the *Rickettsia* species without complete genome sequences, the GenBank accession nos. in the order of *rrs*, *gltA*, *ompB* and *ompA* are NR_074469, KT899087, and AY280712, AF179362 for *R. heilongjiangensis*; KY474575, KX963389, KU310593, and KX506738 for *R. raoultii*; MG906672, MG906678, and MG906676,0020 for *Candidatus Rickettsia longicornii*; and AF394906, AF394901 and DQ110870 for *R. asiatica*.

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References

1. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev*. 2013;26:657–702. <http://dx.doi.org/10.1128/CMR.00032-13>
2. Mahara F. Japanese spotted fever: report of 31 cases and review of the literature. *Emerg Infect Dis*. 1997;3:105–11. <http://dx.doi.org/10.3201/eid0302.970203>
3. Gaywee J, Sunyakumthorn P, Rodkvamtook W, Ruang-areerate T, Mason CJ, Sirisopana N. Human infection with *Rickettsia* sp. related to *R. japonica*, Thailand. *Emerg Infect Dis*. 2007;13:657–9. <http://dx.doi.org/10.3201/eid1304.060585>
4. Chung MH, Lee SH, Kim MJ, Lee JH, Kim ES, Lee JS, et al. Japanese spotted fever, South Korea. *Emerg Infect Dis*. 2006;12:1122–4. <http://dx.doi.org/10.3201/eid1207.051372>
5. Bozeman FM, Elisberg BL, Humphries JW, Runcik K, Palmer DB Jr. Serologic evidence of *Rickettsia canada* infection of man. *J Infect Dis*. 1970;121:367–71. <http://dx.doi.org/10.1093/infdis/121.4.367>
6. Teng K, Jiang Z. Economic insect fauna of China Fasc 39 Acari: *Ixodidae*. Fauna Sinica Beijing: Science Press. 1991.
7. Luo LM, Zhao L, Wen HL, Zhang ZT, Liu JW, Fang LZ, et al. *Haemaphysalis longicornis* ticks as reservoir and vector of severe fever with thrombocytopenia syndrome virus in China. *Emerg Infect Dis*. 2015;21:1770–6. <http://dx.doi.org/10.3201/eid2110.150126>
8. Huang Y, Zhao L, Zhang Z, Liu M, Xue Z, Ma D, et al. Detection of a novel *Rickettsia* from *Leptotrombidium scutellare* mites (Acari: *Trombiculidae*) from Shandong of China. *J Med Entomol*. 2017;54:544–9. <http://dx.doi.org/10.1093/jme/tjw234>
9. Igolkina YP, Rar VA, Yakimenko VV, Malkova MG, Tancev AK, Tikunov AY, et al. Genetic variability of *Rickettsia* spp. in *Ixodes persulcatus*/*Ixodes trianguliceps* sympatric areas from Western Siberia, Russia: identification of a new *Candidatus Rickettsia* species. *Infect Genet Evol*. 2015;34:88–93. <http://dx.doi.org/10.1016/j.meegid.2015.07.015>
10. Roux V, Fournier PE, Raoult D. Differentiation of spotted fever group rickettsiae by sequencing and analysis of restriction fragment length polymorphism of PCR-amplified DNA of the gene encoding the protein rOmpA. *J Clin Microbiol*. 1996;34:2058–65.
11. Li J, Hu W, Wu T, Li HB, Hu W, Sun Y, et al. Japanese spotted fever in eastern China, 2013. *Emerg Infect Dis*. 2018;24:2107–9. <http://dx.doi.org/10.3201/eid2411.170264>
12. Jiang J, An H, Lee JS, O'Guinn ML, Kim HC, Chong ST, et al. Molecular characterization of *Haemaphysalis longicornis*–borne rickettsiae, Republic of Korea and China. *Ticks Tick Borne Dis*. 2018;9:1606–13. <http://dx.doi.org/10.1016/j.ttbdis.2018.07.013>
13. Philip RN, Casper EA, Anacker RL, Peacock MG, Hayes SF, Lane RS. Identification of an isolate of *Rickettsia canada* from California. *Am J Trop Med Hyg*. 1982;31:1216–21. <http://dx.doi.org/10.4269/ajtmh.1982.31.1216>
14. Haddow AD. The consequences of medically important invasive arthropods: the longhorned tick, *Haemaphysalis longicornis*. *Clin Infect Dis*. 2019;68:530–1.
15. Qin XR, Han FJ, Luo LM, Zhao FM, Han HJ, Zhang ZT, et al. *Anaplasma* species detected in *Haemaphysalis longicornis* tick from China. *Ticks Tick Borne Dis*. 2018;9:840–3. <http://dx.doi.org/10.1016/j.ttbdis.2018.03.014>

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