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Characterization of Spotted Fever Group *Rickettsia* in Ticks Collected from South Korea

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Recently, numbers of newly emerging spotted fever group *Rickettsia spp.* were observed in ticks. Here, we explored to determine the prevalence of rickettsia species found in ticks from 2 northeastern provinces (Gyeonggi, and Gangwon) of South Korea. A total of 1,798 *Ixodid* ticks were collected by tick drag methods from August to September 2009 in two northeastern provinces. 204 samples were screened for the rickettsial 17-kDa antigen gene using nested PCR (nPCR), and 25 samples showed positivity. Of the 25 samples, 16 (64%), 11 (44%), and 9 (36%) were positive for rickettsial outer membrane protein A (*ompA*) gene, outer membrane protein B (*ompB*) gene, and surface cell antigen 4 (*sca4*) gene, respectively. BLAST results of *ompB, ompA* and *sca4* sequences were obtained from the two identified genotypes designated as *Rickettsia sp. str. koreansis*, formerly named as HlR/D91, (from *Haemaphysalis longicornis*) and InR/D372 (from *Ixodes nipponensis*). The *ompA* small part (645bp) of *Rickettsia sp. str. koreansis* shared 99.8% similarity with *Rickettsia* sp. FuJ98 (firstly identified in China) while *ompA* large part (1651bp) of *Rickettsia sp. str. koreansis* showed 98.8% similarity with *R. heilongjiangensis*. Further, *ompB* (1848bp) and *sca4* (1705bp) of *Rickettsia sp. str. koreansis* showed 97.9% and 97.6% sequence similarities with *R. hulinensis* and *R. japonica,* respectively, while *ompA* (small & large parts), *ompB*, and *sca4* derived from *Rickettsia* sp. InR/D372 clustered with *R. monacensis* (99.8%) and *R. tamurae* (99.6%), respectively. These data suggest that the potentially new *Rickettsia sp. str. koreansis* was closely related to *R. japonica*, *R. heilongjiangensis* in northeastern Korea.

Key Words: *ompA*, *ompB*, *sca4*, Spotted fever group *Rickettsia*

INTRODUCTION

Rickettsia spp., an obligate intracellular bacteria, is widely known to cause rickettsiosis on diverse vertebrate hosts including, but not limited to, human, through arthropod vectors such as tick (1). The rickettsiosis infect human and infected patient shows clinical manifestation of fever, headache, myalgia, and rash after 2 to 14 days of incubation. The rickettsiosis is also considered as hard-to-diagnose

diseases due to intracellular features of *Rickettsia spp.* As climate change gets severe, the prevalence of pathogenic Rickettsia spp. was reported globally, and their emergence and re-emergence became more frequent (2). This study involves rickettsial samples obtained previously and preserved for future diagnostics because diagnostic accuracy was not sufficiently to differentiate the strains of *Rickettsia spp*.

In South Korea, the spotted fever group *Rickettsia* (SFGR)-related nucleotide sequence was first identified in 2003 in *Haemaphysalis longicornis* (3). The detection of DNA sequences and antibodies of several SFGRs including *R. japonica*, *R. conorii*, *R. akari*, *R. australis*, and *R. monacensis* has been reported in South Korea (3-10). *R. japonica* was first detected from *Haemaphysalis* spp. ticks and human sera in 2003 and 2004, respectively, while *R. monacensis* was first detected from *Haemaphysalis* spp. ticks in 2009 (3, 6, 9).

R. japonica and *R. heilongjiangensis* were identified in Japan and Far East (11). The first clinical case caused by *R. japonica* was identified in Japan in 1984 and was reported as Japanese spotted fever (JSF) (12, 13). Since then, *R. japonica* has been detected in Japan, Philippines, South Korea, and Thailand (13-16). *R. heilongjiangensis* was first isolated from *Dermacentor silvarum* ticks in the Heilongjiang province of China in 1983. It was classified under the *R. japonica* subgroups in SFGR (17). Rickettsioses caused by *R. heilongjiangensis* have been reported from China, Russia, Kazakhstan, and Japan (18-21).

Ixodid ticks, especially *H. longicornis* and *H. flava*, are commonly found in South Korea (22, 23). Other tick species such as *Ixodes nipponensis*, *Amblyomma testudinarium, Haemaphysalis phasiana*, and *I. turdus* have also been reported in South Korea (24, 25).

H. longicornis ticks are major vectors of severe fever with thrombocytopenia syndrome (SFTS). They are widely distributed in the Asia Pacific region, China, Japan, and South Korea (26). Yun et al. reported that 12 (70.5%) out of 17 nucleotide sequences detected from *H. longicornis* in Korea were closely related to SFTS reported in China and Japan (27). In China, which is geographically close to South Korea, *H. longicornis* ticks are the main vectors for the livestock and act as carriers of *Rickettsia* as well as *Theileria*, *Babesia*, *Ehrlichia,* and *Anaplasma* (28).

Recently, along with climate change, novel pathogenic *Rickettsia spp.* have been discovered in diverse hosts and vectors from different regions of the world (29). South Korea is not an exception. In order to make rapid response against the changing landscape on emergence of novel *Rickettsia spp.*, it is imperative to determine their transmission via molecular, genomic analyses. The purpose of this study was to investigate and characterize the new *Rickettsia*-related sequences in ticks inhabiting the two northeastern provinces of South Korea.

MATERIALS AND METHODS

Collection and identification of ticks

Ticks were collected using tick drag methods in two northeastern provinces (Gyeonggi and Gangwon) of South Korea from April 2009 to September 2009 (Supplementary Table 1 & Supplementary Fig. 1). A total of 1798 ticks were pooled (n = 204 pools, 1-35 ticks/pools), according to genus, sex, developmental stage, and collection site. Tick pools were prepared and each tick was transferred into 2 mL microcentrifuge tubes, screw-capped, and stored at -70°C.

DNA extraction

Tick samples were washed with 70% ethanol and rinsed with distilled water. Total DNA was extracted from each sample using the G spin total DNA extraction kit (iNtRON, Gyeonggi, South Korea). This DNA extraction kit was exclusively used for the DNA extraction from the whole blood, cells, tissues, and bacteria. Total genomic DNA samples were stored at -20°C before use.

nPCR for detection of rickettsial agents

Screening of tick pools for rickettsial DNA was conducted by nested PCR (nPCR) using genus-specific primers for the 17-kDa antigen gene as described previously (30, 31). *Rickettsia*-positive samples were subjected to the amplification of genes in the outer membrane protein A (*ompA*), outer membrane protein B (*ompB*), and surface cell antigen (*sca4*) by nPCR to generate amplicons for sequencing. To amplify the *ompA* gene, the *ompA* gene sequences coding the N- and C-terminal conserved regions (position 1-645 and 2829-4479 and amplicon size 645 bp and 1651 bp, respectively) were analyzed (30, 31). Details of *ompA* amplification (including primer sequences) are presented in Table 1. In addition, to amplify the citrate synthase gene (*gltA*), the primer pair (RpCS.62p, RpCS.1258n) was used as previously described (30, 31), and the primer pairs fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and Rc16S.452n (5'-AACGTCATTATCTTCCTTGC-3') were used to amplify the 16S rRNA gene (32).

Table 1. Oligonucleotide primers for detection rickettsial target genes

Target gene[a]	Primers	Nucleotide sequences (5' → 3')	Product size (bp)	PCR condition (°C / sec)		
				Denaturation	Annealing	Extension
17 kDa	Rr17k.1p	TTTACAAAATTCTAAAAACCAT	539	95	47	72
	Rr17k.539n	TCAATTCACAACTTGCCATT				
ompA	190-3588F	AACAGTGAATGTAGGAGCAG	1,651	94	50	72
	RompARm4433R	GAATTTAAGGTTACTATACCTTC				
ompB	RompB11F[c]	ACCATAGTAGCMAGTTTTGCAG	1,892	94	50	72
	RompB1902R[bc]	CCGTCATTTCCAATAACTAACTC		30	30	120
	RompB11F[c]	ACCATAGTAGCMAGTTTTGCAG	1,442	94	46	72
	RompB1452R[bc]	SGTTAACTTKACCGYTTATAACTGT		30	30	90
	120-607F[c]	AATATCGCTGACGGTCAAGGT	1,296	94	47	72
	RompB1902R[bc]	CCGTCATTTCCAATAACTAACTC		30	30	80
	RompBRm11F[e]	RCCATAGTRGCCAGTTKTGCAG	1,846	94	50	72
	RompBRm1902R[be]	CCGTMATTTCCAATAACTAACTC		30	30	110
	RompBRm155F[ce]	CGAGTTACCTTAGATTCTGT	1,612	94	40	72
	RompBRm1766R[bce]	CTCCAATAGTACCGATACC		30	30	100
	120-807R[bc]	CCTTTTAGATTACCGCCTAA				
	RompB1009F[c]	ACATKGTTATACARAGTGYTAATGC				
	RompBRm961R[bce]	AAATTTGGTTTGTAATTGTA				
	RompBRm845F[ce]	GTTGGTACATTTGGTACTACT				
sca4	RrD749F	TGGTAGCATTAAAAGCTGATGG	1,937	94	56	72
	RrD2685R[bc]	TTCAGTAGAAGATTTAGTACCAAAT		30	30	120
	RrD928F[c]	ATTTATACACTTGCGGTAACAC	1,758	94	45	72
	RrD2685R[bc]	TTCAGTAGAAGATTTAGTACCAAAT		30	30	110
	RrD1713F[c]	CTCTGAATTAAGCAATGCGGAAA				
	Sca4_1500R[bc]	CGCATAGCTACTGTAGCTTCAAGC				
	RrDRh.Rj1826R[bc]	TCTAAATTCTGCTGCATCAAT				
	RrDRm1826R[bce]	TCTAAATTCTGTTGCATCAAT				
	Sca4_ 2230F[c]	TGAAGGCAAAGGAGGTCCTG				

[a]omp: outer membrane protein B gene, sca4: surface cell antigen gene; [b]reverse orientation; [c]primers for sequencing; [e]specially designed primers for *R. monacensis*.

The reaction mixture was prepared by transferring 2 μL DNA extract and 8 pmol of each primer to a tube of AccuPower® PCR premix (Bioneer Corp., Daejeon, Korea) containing 1U of Taq DNA polymerase, 250 μM each of dNTP, 50 mM of Tris-HCl (pH 8.3), 40 mM of KCl, and 1.5 mM $MgCl_2$. The final volume was adjusted to 20 μL with distilled water. The nPCR reactions were run on Veriti™ 96-well Thermal Cycler (Applied Biosystems, Carlsbad, USA). Subsequently, the nPCR products were cleaned and prepared for sequencing using the QIAquick spin PCR purification kit (Qiagen) as described by the manufacturer.

Sequencing analysis

Primers used to sequence *Rickettsia* species are listed in Table 1. Sequencing was performed by Genotech Co. Ltd. (Daejeon, Korea). To obtain accurate nucleotide sequences for the 17-kDa antigen gene, *ompA, ompB,* and *sca4,* all the amplicons were sequenced bidirectionally. Sequence analyses were performed with MegAlign software (DNASTAR, London, UK). A concatenated alignment tree was constructed using the maximum likelihood (ML) method, the neighbor-joining (NJ) method, and the Tamura-Nei model (33). Phylogenetic analyses were performed using the Molecular Evolutionary Genetics Analysis 6.0 software and bootstraps were executed with 1,000 replications.

GenBank accession numbers

Sequences obtained in this study have been deposited in GenBank with accession numbers of KC888947-KC888948, KC888949-KC888950, KC888951-KC888952, KC888953-KC888954, KC888955-KC888956, and MK224716-MK224719 for 17-kDa, *ompA*-large part, *ompA*-small part, *ompB*, *sca4*, *gltA* and 16S rRNA, respectively.

RESULTS

Tick collection

A total of 1798 ticks were collected from two northeastern provinces of South Korea from August 2009 to September 2009. *H. flava* was the predominant (65.0%) tick collected followed by *H. longicornis* (20.9%) (Table 2). The total tick numbers by developmental stage were as follows: larvae 1,343 (74.7%), nymphs 417 (23.2%), and adults 38 (2.1%). The tick species were morphologically classified into *H. flava* (n = 1,169), *H. longicornis* (n = 376), and *I. nipponensis* (n = 253).

nPCR for screening of rickettsial agents

Twenty-five out of 204 (12.3%) tick pool samples tested positive based on PCR screening using primers specific for the rickettsial 17-kDa antigen gene. These positive samples were further assessed by nPCR to amplify *ompA*, *ompB,* and *sca4* genes using gene-specific primers. Of these 25 samples, 16 (64%), 9 (36%), and 11 (44%) were positive for rickettsia species using primers of *ompA*, *ompB*, and *sca4*, respectively (Table 2, Supplementary Table 2).

Sequencing of rickettsial *ompA, ompB, and sca4* genes

To identify *Rickettsia* spp. in the 17-kDa antigen-positive pooled tick samples, the *ompA* (small & large parts), *ompB*, and *sca4* genes were sequenced partially. Out of 16 positive samples of *ompA* gene, 9 samples (results based on both small and large part) were highly similar to *R. heilongjiangensis*-*R. japonica* (98.6%-99.0%), five samples were highly similar to *R. monacensis* (99.1%-99.6%), and two samples showed that *ompA* small and large part were similar to *R. rickettsii* (97.4%) and *Candidatus R. andeanae* (97.8%), respectively (Supplementary Table 2).

Table 2. Numbers of ticks collected from northeastern province in South Korea

Province	Species	Stage	No. of ticks (No. of tested pools)	No. of 17-kDa PCR positive (%)#	No. of *ompA* PCR positive (%)#	No. of *ompB* PCR positive (%)#	No. of *sca4* PCR positive (%)#
Gyeonggi	*H. longicornis*	Larva[a]	140 (9)	1 (0.71)	0	-	1
		Nymph[b]	214 (49)	15 (7.0)	10	5	7
		Adult male[c]	1 (1)	0	-	-	-
		Adult female[c]	15 (15)	1 (6.6)	1	1	0
	H. flava	Larva	254 (12)	0	-	-	-
		Nymph	164 (39)	2 (1.22)	2	1	0
		Adult male	12 (12)	1 (8.3)	1	1	0
		Adult female	5 (5)	0	-	-	-
	I. nipponensis	Larva	93 (7)	0	-	-	-
		Nymph	7 (5)	3 (42.8)	1	0	2
		Adult male	1 (1)	0	-	-	-
		Adult female	1 (1)	0	-	-	-
Gangwon	*H. longicornis*	Larva	1 (1)	0	-	-	-
		Nymph	4 (3)	0	-	-	-
		Adult male	0	0	-	-	-
		Adult female	1 (1)	1 (100.0)	0	0	0
	H. flava	Larva	708 (14)	0	-	-	-
		Nymph	24 (7)	0	-	-	-
		Adult male	2	0	-	-	-
		Adult female	0	0	-	-	-
	I. nipponensis	Larva	147 (5)	0	-	-	-
		Nymph	4 (3)	1 (33.3)	1	1	1
		Adult male	0	0	-	-	-
		Adult female	0	0	-	-	-
		Total	1,798 (204)	25 (1.39)	16 (64.0)	9 (36.0)	11 (44.0)

[a]1-35 larvae/pool, [b]1-6 nymphs/pool, [c]1 per adults
#MFIR (Minimum field infection rate/100 ticks)= no. of positive pools/no. of examined ticks

The *ompB* gene showed high sequence similarity to *R. heilongjiangensis* (97.1%-98.6%) in seven samples. The sequence similarity between *R. monacensis* (99.8%) and *R. raoultii* (94.5%) was found in one sample each. In the case of *sca4* gene, five samples showed high sequence similarity to *R. tamurae* (98.1%-99.6%), another five samples contained sequences similar to *R. africae* (98.4%-98.5%), and one sample carried sequences similar to *R. japonica* (97.6%).

Based on BLAST analysis, the detected sequences were classified into two types of *Rickettsia* isolates: *Rickettsia sp. str. koreansis*, formerly named as HlR/D91, obtained from *H. longicornis* and InR/D372 obtained from *I. nipponensis*. The

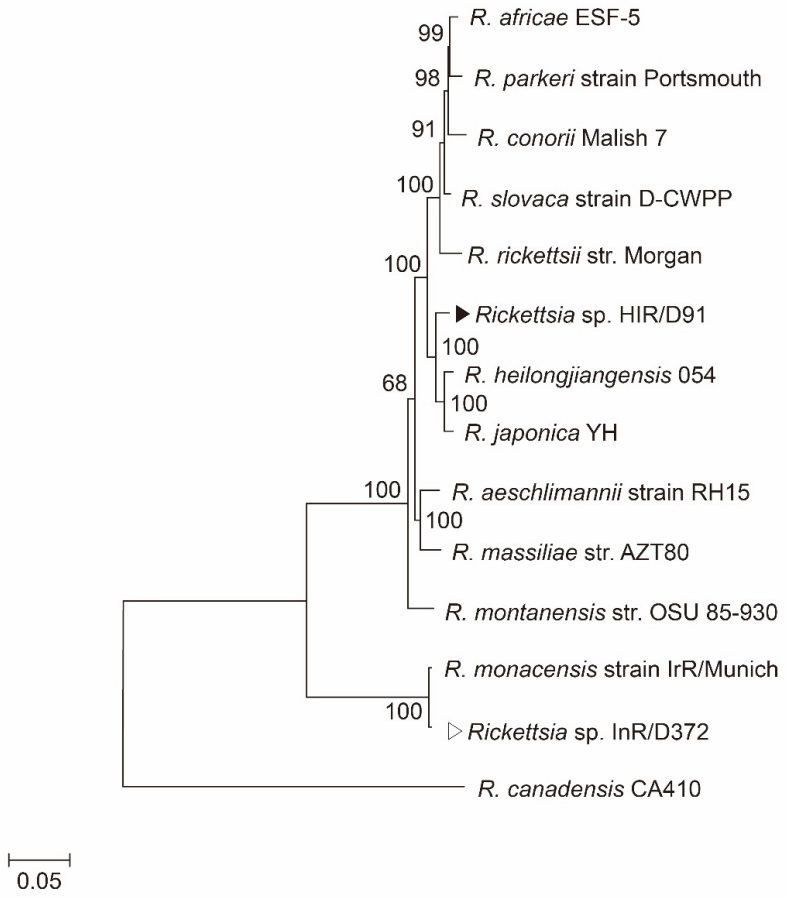

Fig. 1. Phylogenic tree consisting of concatenated fragments of *ompA* (small & large, 2296 bp), *ompB* (1848 bp), and *sca4* (1705 bp) genes from *Rickettsia sp. str. koreansis* (formerly named as HIR/D91) (▶) and InR/D372 (▷) between various *Rickettsia* species with multi-phylogenetic relationships. The evolutionary history was inferred by using ML method.

sequence of *ompA* small part containing the 645 bp fragment from *Rickettsia sp. str. koreansis* showed 94.9 to 99.8% similarity with *Rickettsia* sp. FuJ98, *R. japonica*, and *R. heilongjiangensis* (Fig. 1) (Supplementary Table 2). The amplicon sequence of the large part of *ompA* of 1651 bp, derived from *Rickettsia sp. str. koreansis* showed 98.5 to 98.8% similarity with sequences of rickettsiae previously reported as *R. heilongjiangensis* (Fig. 1). The 1848 bp sequence of *ompB* from *Rickettsia sp. str. koreansis* showed 97.0 to 97.9% similarity with *R. heilongjiangensis, R. japonica* (Fig. 1) while the 1705 bp fragment of *sca4* showed 96.1 to 97.6% similarity with *R. japonica* and *R. heilongjiangensis* (Fig. 1). The sequences of *ompA* small and large parts derived from InR/D372 showed 99.8 to 99.9% similarity with *R. monacensis* (Fig. 1). Similarly, *ompB* sequences of InR/D372 shared 99.8% similarity with *R. monacensis*. On the other hand, the sequence of *sca4* from InR/D372 shared 99.6% similarity with *R. tamurae* (Fig. 1). In addition, the nucleotide sequences of the *gltA* gene and the 16S rRNA gene were analyzed and compared with representative sequences. The nucleotide sequences of the *gltA* genes, *Rickettsia sp. str. koreansis* and InR/D372 showed 100% sequence similarity with *Candidatus R. jingxinensis* (GenBank accession no. MH500217) and *R. monacensis* Cesa (GenBank accession no. MH589997.1), respectively. The nucleotide sequence of *16S rRNA* genes, *Rickettsia sp. str. koreansis* and InR/D372 showed 100% sequence similarity with uncultured *Rickettsia* YN03 (GenBank accession no. KY433580.1) and *R. monacensis* 1187 ISE6 (GenBank accession no. LC388771.1), respectively. In order to confirm of phylogenetic tree, the neighbor-joining tree were generated using the Kimura's two-parameter model (Supplementary Fig. 2).

DISCUSSION

Rickettsiae with a high degree of similarity to *R. japonica* YH (GenBank accession number AP011533) in the ticks of Korea were identified in 2003 (3). *R. japonica* was detected in Korean human sera in 2004 and 2005 (4, 6, 9). *R. heilongjiangensis* is considered genetically similar to *R. japonica* but distinct enough to be classified as a new species (34). In this study, we found that the nucleic acids of rickettsial agents were closely related to *R. heilongjiangensis*-*R. japonica* genogroup in *H. longicornis* ticks and *R. monacensis* in *I. nipponensis* ticks, in two northeastern provinces of South Korea. To identify new *Rickettsia* molecular isolates, we used partial sequencing of the gene coding for 17-kDa protein and outer membrane protein genes (*ompA*, *ompB*, and *sca4*). In a previous study, we analyzed a set of PCR primers of rickettsial *ompA* (small and large parts) gene targeting *ompA* upstream and downstream of the repeat regions (30). The domain of *ompA* gene contains 6 to10 tandem repeat units with mostly identical sequences. The number and sequences of *ompA* repeat units vary with the rickettsial species except for the highly conserved region (30). The *ompA, ompB*, and *sca4* encoding genes exhibit higher sequence variability among SFGR than the other genes such as the 17-kDa, *gltA*, and 16S rRNA. Of course, in this study, the partial 16S rRNA and *gltA* sequences of the *Rickettsia sp. str. koreansis* showed 100% sequence similarity with *Rickettsia* sp. YN03, and *Candidatus R. jingxinensis* and *Rickettsia* sp. InR/D372 showed 100% sequence similarity with *R. monacensis* ISE6 and *R. monacensis* Cesa, respectively.

Rickettsia sp. str. koreansis, the *ompA* partial sequence, showed 99.8 to 99.9% nucleotide similarity with the corresponding sequence of *R. japonica* and/or *R. heilongjiangensis* (Supplementary Table 2). The partial *ompB* gene showed 97.9% similarity with *R. hulinensis* while the nucleotide sequence of *sca4* showed 97.6% similarity with *R. japonica* and 97.0% similarity with *R. heilongjiangensis*. Recently, the partial 17-kDa and *ompA* sequence of *Rickettsia sp. str. koreansis* showed close similarities with rickettsial isolates detected from *H. longicornis* in 2013 in southwestern province of South Korea (23). Furthermore, *Rickettsia* sp. InR/D372 showed similar nucleotide sequences with *R. monacensis* derived from *I. nipponensis* which was collected from south Jeolla province suggesting that *Rickettsia sp. str. koreansis* and InR/D372 exist without geographical or regional variation in South Korea (30).

Our results revealed that the small part of *ompA* derived from *Rickettsia sp. str. koreansis* was found in *Rickettsia* sp. FuJ98 (GenBank accession no. AF169629.1). Although the small part of *ompA* is no larger than 645 bp and the large part of *ompA* measured 1651 bp, the *ompA* of *Rickettsia* sp. FuJ98 has yet to be annotated. Moreover, *Rickettsia vini*, a new species detected in *Ixodes arboricola* has been isolated and identified in the Czech Republic, sharing 99.3% similarity with the nucleotide sequence of *ompB* gene from *Rickettsia sp. str. koreansis* (35).

New SFG rickettsiae species exhibit sequence similarity of < 98.8% for *ompA*, < 99.2% for *ompB*, and < 99.3% for *sca4* (17). Our study showed that the phylogenetic tree based on *ompA*-large part sequence, *ompB*, and *sca4* sequences showed an independent clade (Fig. 1). Therefore, this study suggests that *Rickettsia sp. str. koreansis* is a new *Rickettsia* species, but there are some limitations. First, only a partial sequence of several genes was analyzed. Secondly, it was confirmed only in *H. longicornis* ticks. Therefore, in order to make a decision as a new species of *Rickettsia*, isolation and typing of multiple genes are required.

This study was conducted to identify various ticks in two northeastern provinces (Gangwon, Gyeonggi) of South Korea and analyze their dominant species, developmental stages, and *Rickettsia* genus-specific genes (*ompA*, *ompB*, and *sca4*). Rickettsial molecular detection closely related to various rickettsiae including *R. heilongjiangensis*, *R. japonica*, and *R. monacensis* were identified. Our results also suggested a potentially new species of *Rickettsia sp. str. koreansis* in two northeastern provinces of South Korea.

CONFLICT OF INTEREST

The authors have no conflicts.

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J.Y, Kim analyzed the data and wrote the manuscript. T.U, Kang wrote and revised the manuscript. H.C, Kim and S.T, Chong conceived the trapping sampling. H.J, Park, D.Y, Song, and S.H, Shin performed the experiments. All authors were involved in manuscript review and discussion. Funding for portions of this work was provided by the Armed Forces Health Surveillance Branch-Global Emerging Infections Surveillance and Response System (AFHSB-GEIS), Silver Spring, Maryland, USA. The views expressed in this article are those of the authors do not reflect the official policy or position of the Department of the Army, the Department of the Navy, the Department of Defense, nor the US Government.

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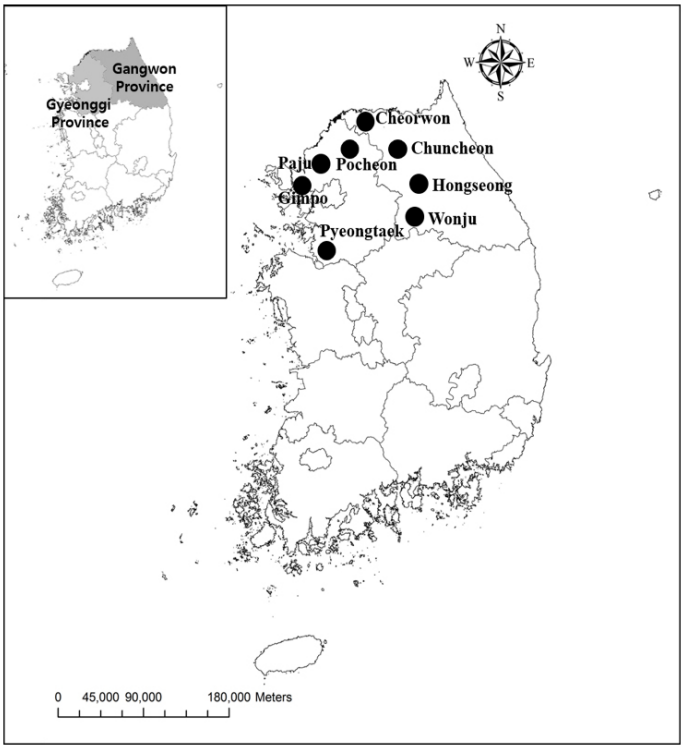
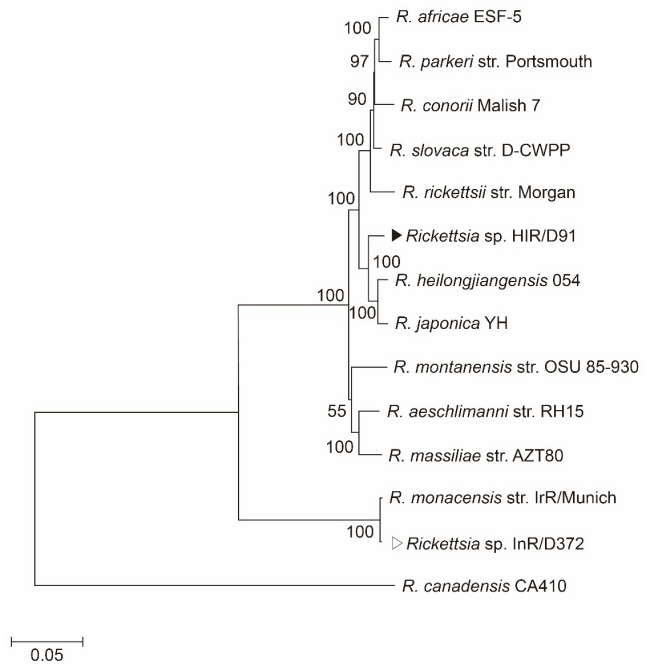
SUPPLEMENTARY DATA



Supplementary Fig. 1. Map of the northeastern region (Gyeonggi & Gangwon) in South Korea.



Supplementary Fig. 2. Phylogenic tree representing multi-phylogenetic relationships between various *Rickettsia* species and *ompA* (small & large), *ompB*, and *sca4* PCR product amplified from *Rickettsia sp. str. koreansis* (formerly named as HlR/D91) (▶) and InR/D372 (▷). The evolutionary history was inferred by using NJ method.

Supplementary Table 1. Sampling sites for ticks

	Province	Sites	Grid Coordinates
Northeastern	Gyeonggi	Paju-si	34°35'43.7"N, 126°38'23.4"E
		Pyeongtaek-si	36°89'81.8"N, 126°99'20.1"E
			36°05'64.2"N, 127°35'26.8"E
			36°95'48.1"N, 127°62'58.9"E
		Gimpo-si	37°53'51.4"N, 126°43'43.0"E
			37°44'25.3"N, 126°32'03.1"E
		Pocheon-si	38°06'75.3"N, 127°35'69.4"E
			38°07'11.0"N, 127°35'16.1"E
			38°06'26.4"N, 127°36'18.4"E
			38°10'28.2"N, 127°44'60.6"E
	Gangwon	Wonju-si	37°25'44.3"N, 127°58'02.6"E
		Hongseong-gun	37°48'47.3"N, 127°45'59.9"E
		Chuncheon-si	37°48'50.2"N, 127°45'59.1"E
		Cheorwon-gun	38°05'18.1"N, 127°15'57.3"E
			38°09'12.5"N, 127°17'58.9"E

Supplementary Table 2. Result of sequencing analysis of the PCR products

Sample ID	Tick species	R17k PCR results	Sequencing results		
			ompA (small / large*)	*ompB*	*sca4*
91	*H. longicornis*	+	*R. heilongjiangensis /R. japonica**	*R. heilongjiangensis*	*R. japoncia*
187	*I. nipponensis*	+	*R. heilongjiangensis /R. japonica**	N.D	*R. tamurae*
195	*H. longicornis*	+	N.D	*R. heilongjiangensis*	*R. africae*
201	*H. longicornis*	+	N.D	N.D	N.D
208	*H. flava*	+	*R. heilongjiangensis#*	N.D	N.D
213	*H. flava*	+	*R. heilongjiangensis#*	*R. raoultii*	N.D
228	*H. longicornis*	+	*R. japonica#*	N.D	N.D
230	*H. longicornis*	+	*R. monacensis#*	N.D	N.D
231	*H. longicornis*	+	*R. rickettsii /Can. R. andeanae**	N.D	N.D
233	*H. longicornis*	+	*R. monacensis#*	N.D	*R. africae*
234	*H. longicornis*	+	N.D / *R. japonica**	*R. heilongjiangensis*	N.D
236	*H. longicornis*	+	N.D	N.D	N.D
237	*H. longicornis*	+	N.D	*R. heilongjiangensis*	N.D
238	*H. longicornis*	+	*R. rickettsii /Can. R. andeanae**	N.D	N.D
239	*H. longicornis*	+	N.D	N.D	N.D
240	*H. longicornis*	+	*R. heilongjiangensis /R. japonica**	*R. heilongjiangensis*	*R. tamurae*
242	*H. longicornis*	+	*R. heilongjiangensis /R. japonica**	N.D	N.D
243	*H. longicornis*	+	*R. heilongjiangensis /R. japonica**	N.D	N.D
252	*H. longicornis*	+	*R. heilongjiangensis /R. japonica**	*R. heilongjiangensis*	*R. africae*
292	*H. longicornis*	+	N.D	N.D	*R. africae*
317	*I. nipponensis*	+	N.D	N.D	N.D
356	*H. longicornis*	+	N.D	*R. heilongjiangensis*	*R. africae*
372	*I. nipponensis*	+	*R. monacensis#*	*R. monacensis*	*R. tamurae*
404	*I. nipponensis*	+	*R. monacensis#*	N.D	*R. tamurae*
409	*I. nipponensis*	+	*R. monacensis#*	N.D	*R. tamurae*
Total		25	16	9	11

*: indicates the result of *ompA* large part, #: indicates the same results of *ompA* both small& large, N.D: Not determined