

# Large-Scale Survey for Tickborne Bacteria, Khammouan Province, Laos

## Technical Appendix

**Technical Appendix Table 1.** Primers and probes used for screening quantitative PCR in large-scale survey for tickborne bacteria, Khammouan Province, Laos\*

Bacteria	Gene	Primers and probes used to screen samples		Reference†
		Primer or probe	Sequence, 5'→3'	
<i>Rickettsia</i> spp.	17 kDa	R17K128F2	F-GGGCGGTATGAAYAAACAAG	(5)
Spotted fever group	17 kDa	R17K238R	R-CCTACACCTACTCCVACAAG	(5)
Spotted fever group	17 kDa	R17K202TAQP	P-CCGAATTGAGAACCAAGTAATGC	(5)
<i>Borrelia</i> spp.	23S rRNA	Bb23Sf	F-CGAGTCTTAAAAGGGCGATTTAGT	(6)
<i>Borrelia</i> spp.	23S rRNA	Bb23Sr	R-TATTTATGGCCAGGCTGAAGC	(6)
<i>Borrelia</i> spp.	23S rRNA	Bb23Sp	P-AGATGTGGTAGACCCGAAGCCGAGTG	(6)
<i>Coxiella</i> spp.	IS1111	IS1111f	F-CAAGAAACGTATCGCTGTGGC	(7)
<i>Coxiella</i> spp.	IS1111	IS1111R	R-CACAGAGCCACCGTATGAATC	(7)
<i>Coxiella</i> spp.	IS1111	IS1111 probe	P-CCGAGTTCGAAACAATGAGGGCTG	(7)
<i>Ehrlichia</i> spp.	16S rRNA	EHR16S-17	F-GCGGCAAGCCTAACACAT	(8)
<i>Ehrlichia</i> spp.	16S rRNA	EHR16S-97	R-CCCGTCTGCCACTAACAATTATT	(8)
<i>Ehrlichia</i> spp.	16S rRNA	EHR16S-38	P-AGTCGAACGGACAATTGCTTATAACCTTTTGGT	(8)
<i>Anaplasma</i> spp.	msp2	ApMSP2f	F-ATGGAAGGTAGTGTGGTTATGGTATT	(6)
<i>Anaplasma</i> spp.	msp2	APMSP2r	R-TTGGTCTTGAAGCGCTCGTA	(6)
<i>Anaplasma</i> spp.	msp2	ApMSP2p	P-TGGTGCCAGGGTTGAGCTTGAGATTG	(6)

\*F, forward; IS, insertion sequence; msp2, major surface protein 2; P, probe; R, reverse.

†References are in the text of the article.

**Technical Appendix Table 2.** Primers used for sequencing in large-scale survey for tickborne bacteria, Khammouan Province, Laos\*

Bacteria	Gene	Primers used to sequence bacteria-positive samples			Reference
		Primer	Sequence, 5'→3'	Size, bp	
<i>Rickettsia</i> spp.	17 kDa	R17kM61F†	F-ACTTTACAAAATTCTAAAAACCATATACT	524	(1)
Spotted fever group	17 kDa	R17K31F‡	F-CATTGTCCGTCAGGTTGGCG	524	(1)
Spotted fever group	17 kDa	Rr2608Rnew†‡	R-CATTGTCCGTCAGGTTGGCG	434	(1)
Spotted fever group	<i>gltA</i>	Cs1dF†‡	F-ATGACTAATGGCAATAATAA	1,237	(2)
Spotted fever group	<i>gltA</i>	RpE CS877p‡	F-CATAACCAAGTGTAAAGCTG	1,237	(2)
Spotted fever group	<i>gltA</i>	CS1273R†	R-GGGGGCCTGCTCACGGCGG	382	(2)
Spotted fever group	<i>gltA</i>	RpE CS1258n‡	R-ATTGCAAAAAGTACAGTGAACA	382	(2)
Spotted fever group	<i>sca4</i>	RrD749F†‡	F-TGGTAGCATTAAAAGCTGATGG	1,078	(3)
Spotted fever group	<i>sca4</i>	RrD928F‡	F-ATTTATACACTTGCGGTAACAC	1,078	(3)
Spotted fever group	<i>sca4</i>	RrD1826R†‡	R-TCTAAATKCTGCTGMATCAAT	899	(3)
Spotted fever group	<i>ompA</i>	RompAM50F†	R-TTGCGTTATAACACTTTTTAAGTGA	692	(4)
Spotted fever group	<i>ompA</i>	190–70F‡	F-ATGGCGAATATTTCTCCAAAA	692	(4)
Spotted fever group	<i>ompA</i>	RompA642R†	R-ATTACCTATTGTTCCGTTAATGGCA	632	(4)
Spotted fever group	<i>ompA</i>	190–701R‡	R-GTTCCGTTAATGGCAGCATCT	632	(4)
Spotted fever group	<i>ompB</i>	RompB11F†‡	F-ACCATAGTAGCMAGTTTTGCAG	1,902	(5)
Spotted fever group	<i>ompB</i>	120–607F‡	F-AATATCGCTGACGGTCAAGGT	1,902	(5)
Spotted fever group	<i>ompB</i>	RompB1902R†‡	R-CCGTCATTTCCAATAACTACTC	1,265	(5)
Spotted fever group	<i>ompB</i>	RAK1452R‡	R-SGTTAACTTKACCGYTTATAACTGT	1,452	(5)
<i>Borrelia</i> spp.	<i>fla B</i>	280F†	F-GCAGTTCARTCAGGTAACGG	1,452	(6)
<i>Borrelia</i> spp.	<i>fla B</i>	754R†	R-TAGCAAGTGATGTATTRGCATCAAC	475	(6)
<i>Borrelia</i> spp.	<i>fla B</i>	301F‡	F-ACATATTCAGATGCAGACAGAGG	475	(6)
<i>Borrelia</i> spp.	<i>fla B</i>	737R‡	R-GCATCAACTGRGTTGTAAACATTAACAGG	437	(6)
<i>Ehrlichia</i> spp.	16 S rRNA	Ehr16SF	F-GTACCYACAGAAGAAGTCC	437	(7)
<i>Ehrlichia</i> spp.	16 S rRNA	Ehr16SR	R-GCTGTAAACGATGAGTGCTAA	345	(7)

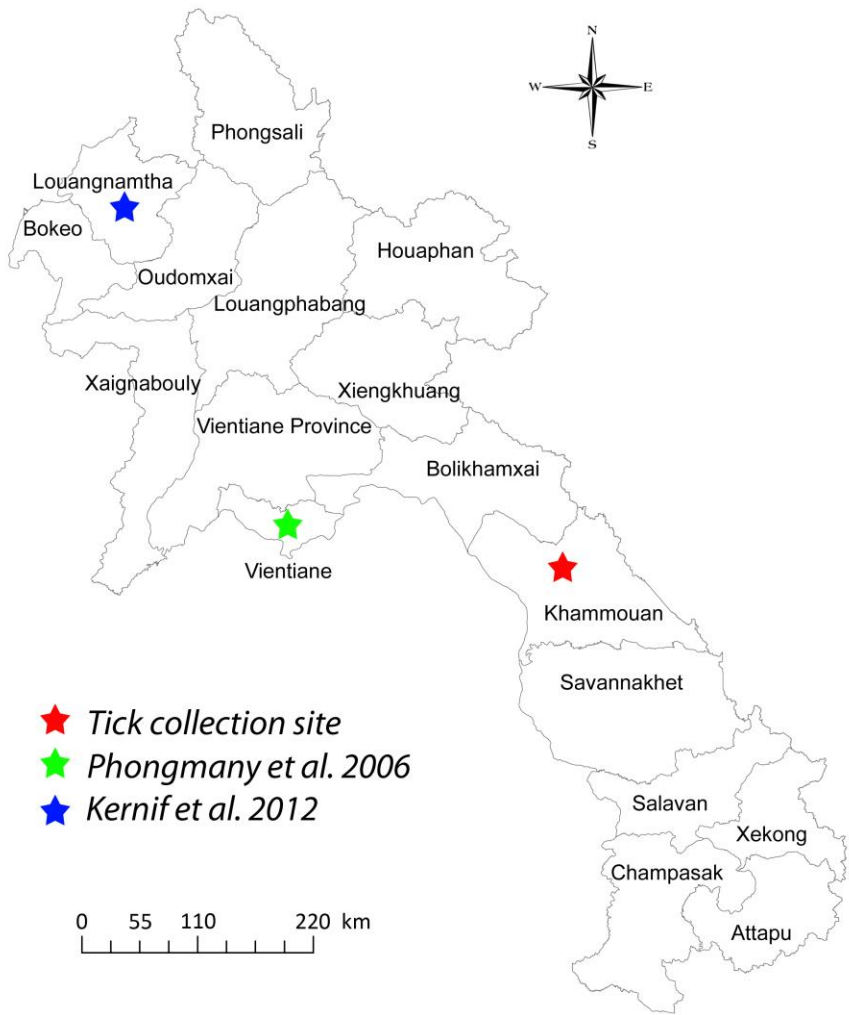
\*F, forward; R, reverse.

†First reaction.

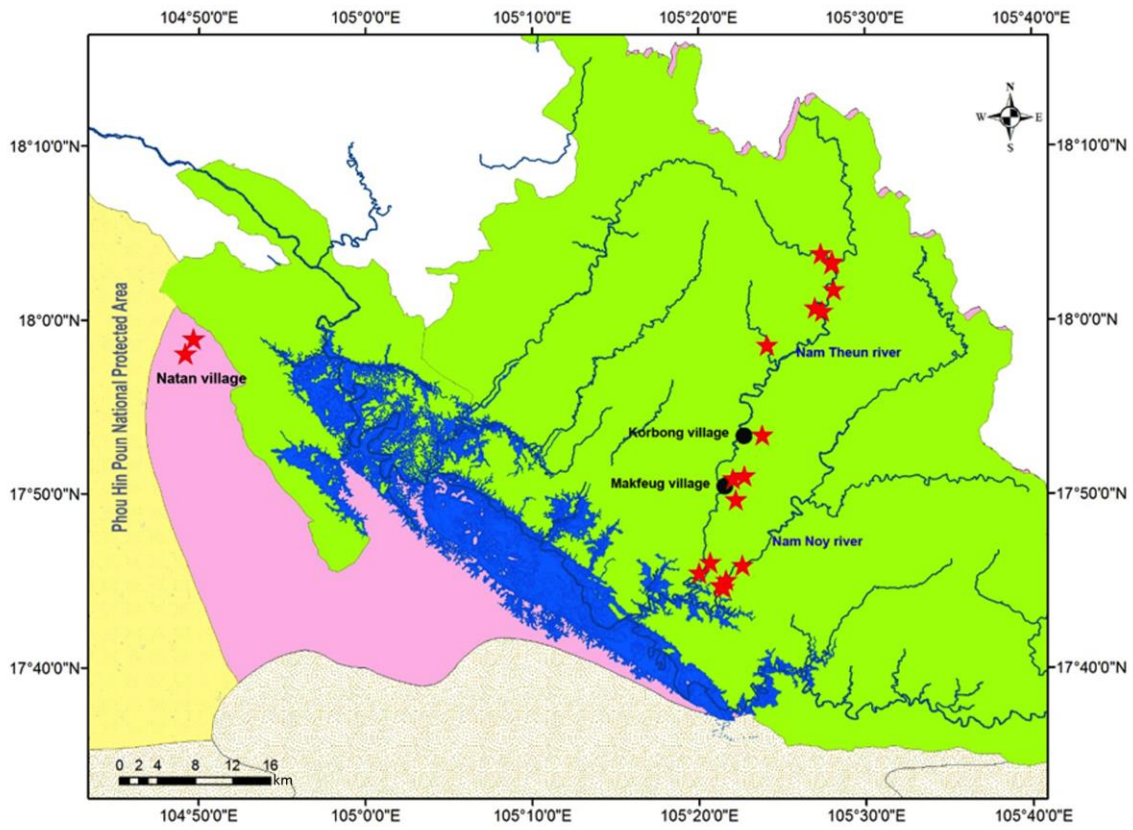
‡Second reaction.

## References

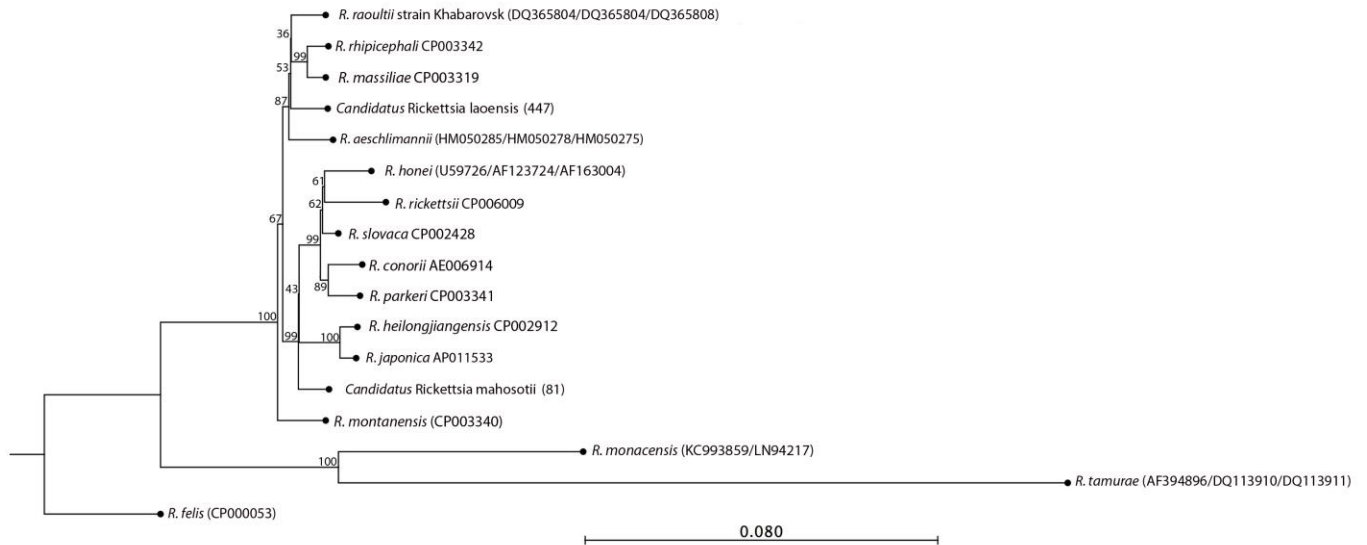
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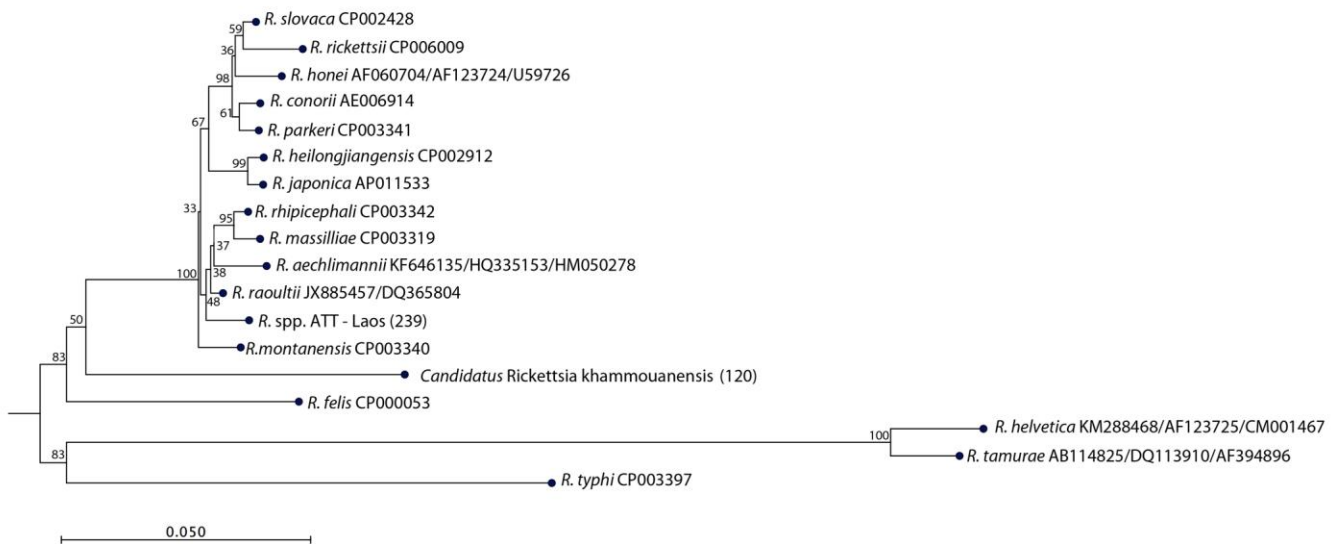
**Technical Appendix Figure 1.** Khammouan Province (red star), Laos, where ticks were collected in this study (see Technical Appendix Figure 2). Locations of previous studies investigating *Rickettsia* spp. in Laos are shown by the green star (8) and blue star (9).



**Technical Appendix Figure 2.** Location of tick collections sites (red stars) in Khammouan Province, Laos.



**Technical Appendix Figure 3.** Phylogenetic analysis of *gltA*, *sca4*, and *ompB* genes of candidate novel *Rickettsia* spp., Kammouan Province, Laos. The tree was constructed by using concatenated partial nucleotide sequences (2,845–2,920 bp) of *gltA*, *sca4*, and *ompB* genes; the Kimura-80 model; and the neighbor-joining method. Analyses were supported by using bootstrap analysis with 1,000 replications. Numbers along branches are bootstrap values. Sample numbers identifying each tick pool from this study are shown in parentheses after the sequence name. Scale bar indicates nucleotide substitutions per site.



**Technical Appendix Figure 4.** Phylogenetic analysis of *gltA*, 17 kDa, and *ompB* genes of *Rickettsia* spp., Kammouan Province, Laos. The tree was constructed by using concatenated partial nucleotide sequences (1,114–1,117 bp) of concatenated sequences of *gltA*, 17-kDa, and *ompB* genes; the Kimura-80 model; and the neighbor-joining method. Analyses were supported by using bootstrap analysis with 1,000 replications. Numbers along branches are bootstrap values. Sample numbers identifying each tick pool from this study are shown in parentheses after the sequence name. Scale bar indicates nucleotide substitutions per site.