



RESEARCH ARTICLE

A new record of *Rickettsia japonica* in ticks infesting a Burmese ferret-badger in Thailand

Hirunkanokpun, S.¹, Ahantarig, A.², Baimai, V.², Pramual, P.³, Trinachartvanit, W.^{2*}

¹Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok, 10240, Thailand

²Biodiversity Research Cluster, Department of Biology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok, 10400, Thailand

³Department of Biology, Faculty of science, Mahasarakham University, Maha Sarakham, 44150, Thailand

*Corresponding author: wachareeporn.tri@mahidol.ac.th

ARTICLE HISTORY

Received: 14 December 2021

Revised: 20 January 2022

Accepted: 21 January 2022

Published: 31 March 2022

ABSTRACT

Ticks are important vectors of arthropod-borne diseases and they can transmit a wide variety of zoonotic pathogens to humans, domestic and wild animals. *Rickettsia japonica* is a member of SFG rickettsiae causing Japanese spotted fever (JSF) and can transmit to humans via infected ticks. In this study, we report the first case of *Rickettsia japonica* in *Haemaphysalis hystricis* tick collected from a roadkill Burmese ferret-badger (*Melogale personata*) in Loei province, northeastern Thailand. According to the DNA sequences and phylogenetic analyses of the outer membrane protein A and B genes (*ompA* and *ompB*), the detected *R. japonica* was identical to those found in JSF patients in Korea, Japan, and China, and closely related to *Rickettsia* detected by *ompA* in a tick from Thailand. Further study on the prevalence of *R. japonica* and diversity of mammalian reservoir hosts will be useful to gain a better understanding of JSF epidemiology.

Keywords: *Rickettsia japonica*; spotted fever group; tick; Thailand.

INTRODUCTION

Hard ticks (Acari: Ixodidae) have been considered as important vectors of various infectious agents in Southeast Asia, including the spotted fever group (SFG) rickettsia member, *Rickettsia japonica* causing Japanese spotted fever (JSF) in humans (Low *et al.*, 2020). Although ticks are known as the potential vectors of SFG rickettsiae (Parola *et al.*, 2013), there is very little knowledge about the interaction between JSF vector and mammalian reservoir hosts in Thailand. Few investigations of *R. japonica* and JSF have been documented. Previously, *R. japonica* was detected in wild rats by serological method (Okabayashi *et al.*, 1996). In addition, a male patient infected with *Rickettsia* sp. was also reported that the case was related to *R. japonica* (Gaywee *et al.*, 2007). Later, the strain TCM1 of *Rickettsia* sp. isolated from a male *Haemaphysalis hystricis* tick collected from Mt. Doi Suthep in the northern region was reported as closely related to *R. japonica* while the identity of its host was unknown (Takada *et al.*, 2009).

Burmese ferret-badger is a native terrestrial mammal species of Southeast Asia including Thailand. Local people of some remote areas consume Burmese ferret-badger as food and/or medicinal recipe (Duckworth *et al.*, 2016). They are known to be parasitized by ticks such as *Amblyomma testudinarium*, *Haemaphysalis langrangei*, *Haemaphysalis heinrichi* and *H. hystricis* (Hoogstraal *et al.*, 1968; Petney *et al.*, 2019). Additionally, Sukmak *et al.* (2015) reported the detection of three species of *Babesia* and an unknown *Cytauxzoon* sp. in

the blood of a single male Burmese ferret-badger. Little information is known about the association between Burmese ferret-badger and tick-borne bacterial pathogens in Thailand.

This study aims to investigate the presence of *Rickettsia* spp. in ticks infesting a Burmese ferret-badger and characterize the detected *Rickettsia* sp. using partial DNA sequencing of 17-kDa, *gltA*, *ompA*, and *ompB* genes. Awareness of *R. japonica* related to its host and vector is also provided and we suggested that this is a new record of *R. japonica* in tick infested mammal in Thailand. For a global perspective, we update information that might expand knowledge related JSF epidemiology in Southeast Asia.

MATERIALS AND METHODS

Tick collection and identification

To investigate the association of mammalian host parasitized by ticks infected with SFG rickettsiae, a total of 16 ticks were removed from a fresh roadkill Burmese ferret-badger (*Melogale personata*) in Loei province, northeastern Thailand in October 2019. All tick samples were morphologically and molecularly identified based on protocols as previously described (Wassef & Hoogstraal, 1984; Tanskul & Inlao, 1989; Black & Piesman, 1994). The ticks in this study were identified as unfed nymphs of *H. hystricis* (n = 8) and *Dermacentor auratus* (n = 8).

DNA extraction and PCR amplification

Each individual nymph was washed in 10% bleach, 70% ethanol, and sterile distilled water three times (1 min each). DNA was extracted from each individual nymph ($n = 8$) of *D. auratus* and two pools of *H. hystricis* nymphs (4 nymphs/pool) using the QIAamp DNA Extraction Kit for Tissue (QIAGEN) according to the manufacturer's protocol. The presence of *Rickettsia* spp. was examined by Polymerase Chain Reaction (PCR) targeting a portion of the rickettsial 17-kDa antigen gene (Webb *et al.*, 1990), the citrate synthase gene (*gltA*) (Regnery *et al.*, 1991), outer membrane protein A (*ompA*) (Regnery *et al.*, 1991), and B (*ompB*) genes. PCR amplification was performed under conditions optimized for each primer pair as follows: 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min for the 17-kDa protein fragment; 35 cycles of denaturation at 95°C for 20 s, annealing at 48°C for 30 s, and extension at 60°C for 2 min for the *gltA* and *ompA* gene fragments. For amplification of the rickettsial *ompB* region (~800 bp product size), the forward primer was RicF: CAG CAA GGT AAT AAG TTT AAT AC and the reverse primer was RicR: GCT ATA CCG CCT GTA GTA ACA G; Cycling conditions in PCR were 95°C for 5 min, 30 cycles of 95°C for 1 min, 56°C for 1 min, 72°C for 1 min, and a final cycle of 72°C for 10 mins (Table 1).

DNA sequencing and phylogenetic analysis

PCR products were purified using Wizard® SV Gel and PCR Clean-Up System kit (Promega) according to the manufacturer's protocol and sequenced in both directions on an ABI 3730xl automated DNA Analyzer (Applied Biosystems). All DNA sequences were edited using BioEdit (Alzohairy, 2011). Edited sequences were assembled into a contig using SeqMan software (DNASTAR, Lasergene) and subjected to BLASTn analysis (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) for similarity to known sequences. The partial sequences of *Rickettsia* sp. in a pool of *H. hystricis* nymphs (HHT) were assigned GenBank under numbers: MW415892 (17-kDa antigen), MW415894 (*gltA*), MW415896 (*ompA*), and MW415898 (*ompB*). A partial sequence of *H. hystricis* tick was also deposited to GenBank under number OM232105 (16S rRNA). Four phylogenetic methods, i.e., neighbor-joining (NJ), maximum parsimony (MP) in MEGA (Kumar *et al.*, 2018), maximum likelihood (ML) in RaxML (Kozlov *et al.*, 2019), and Bayesian (BA) in MrBayes (Ronquist *et al.*, 2012) were used to infer genetic relationships between *Rickettsia* sp. obtained in present study and those reported in GenBank (Table 2).

RESULTS AND DISCUSSION

Rickettsia sp. was detected in one pool of *H. hystricis* nymphs infesting a Burmese ferret-badger using the 17-kDa specific primers, and it was subsequently sequenced with primers specific to 17-kDa, *gltA*, *ompA*, and *ompB* genes. No rickettsial DNA was detected in any of *D. auratus* nymphs. BLAST analyses revealed that the sequences of these four genes were closely related or identical to those *R. japonica* sequences deposited in GenBank, i.e., 17-kDa (100%; 434/434 bp with KY484162), *gltA* (100%; 382/382 bp with DQ909073), *ompA* (99.25%; 529/533 bp with DQ019319), and *ompB* (100%; 742/742 bp with CP047359). The variable *ompA* and *ompB* were employed for phylogenetic analysis in this study. BLAST results revealed that the *ompA* sequence obtained from *H. hystricis* nymphs removed from the Burmese ferret-badger was nearly identical (99.06-99.25%) to *R. japonica* infecting humans in China (access. no. CP047359, KY347792-3), Japan (access. no. AP017581, LC101443, U43795), Korea (access. no. DQ019319), and 98.37% identical to the strain TCM1 isolated from *H. hystricis* in Thailand (access. no. AB359459). However, the sequence was further distantly related to *Rickettsia* sp. infecting humans in Thailand (access. no. DQ909072) (Figure 1A). The sequence of the *ompB* gene of the same sample was identical to *R. japonica* infecting humans in China (access. no. CP047359, CP032049) and *H. hystricis* (access. no. AP017586-8, AP017579) in Japan. Unfortunately, sequences of the *ompB* gene from *H. hystricis* and from humans in Thailand were not available and could not be included in this study (Figure 1B). Based on nucleotide BLAST results, the unknown *Rickettsia* sp. detected in the *H. hystricis* ectoparasites of Burmese ferret-badger is *R. japonica* (Fournier *et al.*, 2003; Raoult *et al.*, 2005). This was supported by phylogenetic analyses based on partial *ompA* and *ompB* gene sequences. All phylogenetic methods revealed similar tree topologies, thus for convenience, only the neighbor-joining trees are shown (Figure 1A and B). It is obvious that our *Rickettsia* sp. was clustered with *R. japonica* with strong support (>90%).

The results thus clearly demonstrated that one pool of *H. hystricis* nymphs was infected with *R. japonica*. In Thailand, *H. hystricis* was reported as ectoparasite of human (*Homo sapiens*), hog-badger (*Arctonyx collaris*), and sambar deer (*Rusa unicolor*) (Sumrandee *et al.*, 2016; Tanskul *et al.*, 1983). It was also found on vegetation (Arthan *et al.*, 2015). This study is the first reported of *H. hystricis* collected from Burmese ferret-badger (*M. personata*) encountered in Loei province. The

Table 1. Primers used for PCR detections of rickettsial and tick DNA

Organism	Target gene	Primer name	Primer sequences (5'-3')	Amplification fragment size (bp)	References
<i>Rickettsia</i> spp.	17-kDa antigen	Rr17.61p Rr17.492n	GCTCTTGCAACTTCTATGTT CATTGTTCGTGAGTTGGCG	434	Williams <i>et al.</i> (1992)
	Citrate synthase (<i>gltA</i>)	RpCS.877p RpCS.1258n	GGGGGCTGCTCACGGCGG ATTGCAAAAAGTACAGTGAACA	381	Regnery <i>et al.</i> (1991)
	190-kDa protein antigen (<i>ompA</i>)	Rr190.70p Rr190.602n	ATGGCGAATATTTCTCCAAAA AGTGCAGCATTGCTCCCCCT	532	Regnery <i>et al.</i> (1991)
	120-kDa protein antigen (<i>ompB</i>)	RicF RicR	CAGCAAGGTAATAAGTTTAATAC GCTATACCGCTGTAGTAACAG	~800	Newly designed in this study
Tick	16S mitochondrial DNA	16S+1 16S-1	CTGCTCAATGATTTTTTAAATTGCTGTGG CCGGTCTGAACCTCAGATCAAGT	460	Black and Piesman (1994)

Table 2. GenBank accession numbers of rickettsial *ompA* and *ompB* genes used to infer the phylogenetic relationship with *Rickettsia japonica* obtained in this study

Gene	Species	GenBank accession No.
<i>ompA</i>	<i>Rickettsia japonica</i>	DQ909072, MH385342, AP017581, CP047359, KY347792, KY347793, LC101443, DQ019319, U43795, D28766, MG906665*, MK102707*, MK102708*, MK102709*, MK102710*, MK102711*, MK102712*, MK102713*, MK102714*, MK102715*, MK102716*, MK102717*, MK102718*, MK102719*
	<i>Rickettsia heilongjiangensis</i>	AB473813, KT899082, KT899083, AH012829, AF179362
	<i>Rickettsia raoultii</i>	MT321626
	<i>Rickettsia africae</i>	MK905241*, MK90542*
	<i>Rickettsia parkeri</i>	MK801772*, MK962698*, MH194358*, MF536975*, MH247927*, MF034495*, KY113110*, KJ158741*, KJ174528*, MK962699*, MN027565
	<i>Rickettsia vini</i>	KJ626329*, KF192804*, MT062907*, JF758828*
	<i>Rickettsia</i> sp.	AB359459, DQ402517
<i>ompB</i>	<i>Rickettsia japonica</i>	CP047359, CP032049, AP017579, AP017586, AP017587, AP017588, KY364904, AP017572*, AP017573*, AP017574*, AP017575*, AP017576*, AP017577*, AP017578*, AP017580*, AP017581*, AP017582*, AP017583*, AP017584*, AP017585*, AP017589*, AP017590*, AP017591*, AP017592*, AP017593*, AP017594*, AP017595*, AP017596*, AP017597*, AP017598*, AP017599*, AP017600*, AP017601*, AP017602*, AP011533*
	<i>Rickettsia heilongjiangensis</i>	AP019862*, AP019863*, AP019864*, AP019865*, CP002912*
	<i>Rickettsia rickettsii</i>	CP018913*, CP018914*, CP006010*, CP000766*, CP003311*, CP003318*, CP003306*, CP003307*, CP003309*
	<i>Rickettsia conorii</i>	AE006914
	<i>Rickettsia parkeri</i>	CP040325, CP003341
	<i>Rickettsia peacockii</i>	CP001227

* GenBank accession No. omitted in phylogenetic tree (triangularly collapsed for visualization).

results of current study expand knowledge on the distribution of *R. japonica* and its association with tick vector and host.

Haemaphysalis hystricis is a three-host tick that has been reported in India, Sri Lanka, China, Taiwan, Japan, Southeast Asia including Thailand (Hoogstraal *et al.*, 1965). In Japan, *H. hystricis* tick has been reported as the important vector of *R. japonica* (Mahara, 1997). The adult stage of this tick species parasitizes a wide range of medium to large carnivores, including deer, domestic dogs, wild boars, tiger, and sometimes attacks humans (Hoogstraal *et al.*, 1965). In addition to the adult stage, cases of human tick bite by a nymphal stage of *H. hystricis* have been reported in Japan (Yamauchi *et al.*, 2009). Several SFG rickettsiae are transmitted transovarially and transstadially in their tick vectors. *Haemaphysalis hystricis* was documented as having transovarial transmission of *R. japonica* because it was isolated from their unfed larvae (Akter *et al.*, 2017). The identification of *R. japonica* in *H. hystricis* nymph infesting the second host, Burmese ferret-badger, in this study poses a question regarding the origin of infection, whether this infected nymph arises from a previously transovarially infected larva or transstadially from a larva acquiring *R. japonica* by feeding on infected primary host. Therefore, further research and study on this aspect are strongly encouraged.

We have demonstrated for the first time an association of *H. hystricis* ticks infected with *R. japonica* and their host (*M. personata*) in Thailand. Further investigations on the abundance and distribution of *H. hystricis* ticks parasitizing wild mammals and the prevalence of rickettsial infection in the vectors and hosts are necessary to gain a better understanding of the epidemiology of SFG rickettsiae and other tick-borne diseases in Thailand and Southeast Asia.

ACKNOWLEDGEMENTS

We thank Dr. Adrian Plant for invaluable comments. This research was partially supported by Mahidol University, the Thailand Research Fund-Chinese Academy of Science Grant (DBG6180027), and the Center of Excellence on Biodiversity, Office of Higher Education Commission (BDC-PG3-163005 and BDC-PG3-161006).

Conflicts of interest

The authors declare that they have no conflict of interest.

REFERENCES

- Akter, A., Ooka, T., Gotoh, Y., Yamamoto, S., Fujita, H., Terasoma, F., Kida, K., Taira, M., Nakadouzo, F., Gokuden, M. *et al.* (2017). Extremely low genomic diversity of *Rickettsia japonica* distributed in Japan. *Genome Biology and Evolution* 9: 124-133. <https://doi.org/10.1093/gbe/evw304>
- Alzohairy, A. (2011). BioEdit: an important software for molecular biology. *GERF Bulletin of Bioscience* 2: 60-61.
- Arthan, W., Sumrandee, C., Hirunkanokpun, S., Kitthawee, S., Baimai, V., Trinachartvanit, W. & Ahantari, A. (2015). Detection of *Coxiella*-like endosymbiont in *Haemaphysalis* tick in Thailand. *Ticks and Tick-borne Diseases* 6: 63-68. <https://doi.org/10.1016/j.ttbdis.2014.09.005>
- Black, W.C. & Piesman, J. (1994). Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proceedings of the National Academy of Sciences of the United States of America* 91: 10034-10038. <https://doi.org/10.1073/pnas.91.21.10034>

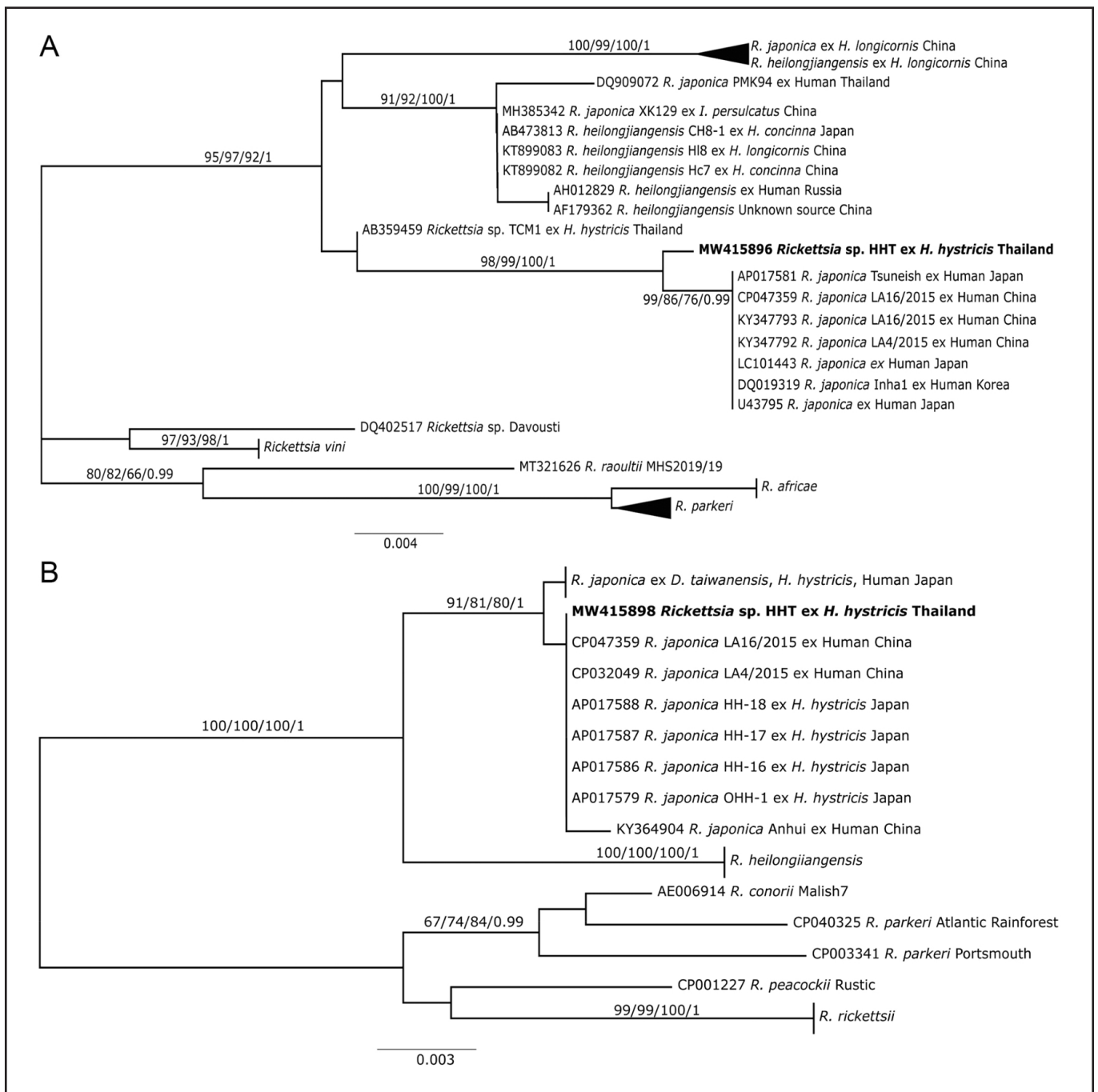


Figure 1. Neighbor-joining trees of *Rickettsia japonica* amplified from pooled nymphs of *Haemaphysalis hystricis* in Thailand (HHT: indicated in bold characters) based on partial sequences of the *ompA* (A) and *ompB* (B) genes. Variability within some clades were collapsed into triangles for visualization. The bootstrap values for neighbor-joining, maximum parsimony, maximum likelihood and probability for Bayesian analysis were shown above branch. Scale bar indicates nucleotide substitutions per site. The name of each sequence containing the GenBank accession number is followed by the name of the *Rickettsia* species, host species and country of origin.

Duckworth, J.W., Long, B., Willcox, D.H.A., Coudrat, C.N.Z., Timmins, R.J., Abramov, A.V., Chan, B. & Chutipong, W. (2016). *Melogale personata*. *The IUCN Red List of Threatened Species* 2016: e.T41627A45209826. <https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T41627A45209826.en>

Fournier, P.E., Dumler, J.S., 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. *Journal of Clinical Microbiology* 41: 5456-5465. <https://doi.org/10.1128/JCM.41.12.5456-5465.2003>

Gaywee, J., Sunyakumthorn, P., Rodkvamtook, W., Ruangareerate, T., Mason, C.J. & Sirisopana, N. (2007). Human infection with *Rickettsia* sp. related to *R. japonica*, Thailand. *Emerging Infectious Diseases* 13: 657-659. <https://doi.org/10.3201/eid1304.060585>

Hoogstraal, H., Trapido, H. & Kohls, G.M. (1965). Studies on Southeast Asian *Haemaphysalis* ticks (Ixodoidea, Ixodidae). The identity, distribution, and hosts of *H. (Kaiseriana) hystricis* Supino. *The Journal of Parasitology* 51: 467-480. <https://doi.org/10.2307/3275974>

- Hoogstraal, H., Santana, F.J. & van Peenen, P.F.D. (1968). Ticks (Ixodoidea) of Mt. Sontra, Danang, Republic of Vietnam. *Annals of the Entomological Society of America* **61**: 722-729. <https://doi.org/10.1093/aesa/61.3.722>
- Kozlov, A.M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. (2019). RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* **35**: 4453-4455. <https://doi.org/10.1093/bioinformatics/btz305>
- Kumar, S., Stecher, G., Li, M., Nnyaz, C. & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**: 1547-1549. <https://doi.org/10.1093/molbev/msy096>
- Low, V.L., Tan, T.K., Khoo, J.J., Lim, F.S. & AbuBakar, S. (2020). An overview of rickettsiae in Southeast Asia: vector-animal-human interface. *Acta Tropica* **202**: 105282. <https://doi.org/10.1016/j.actatropica.2019.105282>
- Mahara, F. (1997). Japanese spotted fever: report of 31 cases and review of the literature. *Emerging Infectious Diseases* **3**: 105-111. <https://doi.org/10.3201/eid0302.970203>
- Okabayashi, T., Tsutiya, K., Muramatsu, Y., Ueno, H. & Morita, C. (1996). Serological survey of spotted fever group rickettsia in wild rats in Thailand in the 1970s. *Microbiology and Immunology* **40**: 895-898. <https://doi.org/10.1111/j.1348-0421.1996.tb01157.x>
- Parola, P., Paddock, C.D., Socolovschi, C., Labruna, M.B., Mediannikov, O., Kernif, T., Abdad, M.Y., Stenos, J., Bitam, I., Fournier, P.E. et al. (2013). Update on tick-borne rickettsioses around the world: a geographic approach. *Clinical Microbiology Reviews* **26**: 657-702. <https://doi.org/10.1128/CMR.00032-13>
- Petney, T.N., Sajjuntha, W., Boulanger, N., Chitimia-Dobler, L., Pfefer, M., Eamudomkarn, C., Andrews, R.H., Ahamad, M., Putthasorn, N., Muders, S.V. et al. (2019). Ticks (Argasidae, Ixodidae) and tick-borne diseases of continental Southeast Asia. *Zootaxa* **4558**: 1-89. <https://doi.org/10.11646/zootaxa.4558.1.1>
- Raoult, D., Fournier, P.E., Eremeeva, M., Graves, S., Kelly, P.J., Oteo, J.A., Sekeyova, Z., Tamura, A., Tarasevich, I. & Zhang, L. (2005). Naming of rickettsiae and rickettsial diseases. *Annals of the New York Academy of Sciences* **1063**: 1-12. <https://doi.org/10.1196/annals.1355.002>
- Regnery, R.L., Spruill, C.L. & Plikaytis, B.D. (1991). Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *Journal of Bacteriology* **173**: 1576-1589. <https://doi.org/10.1128/jb.173.5.1576-1589.1991>
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539-542. <https://doi.org/10.1093/sysbio/sys029>
- Sukmak, M., Wajjwalku, W., Lorsunyaluck, B., Kaolim, N. & Buddhakosai, W. (2015). Molecular detection of Piroplasmida's multiple infection in Burmese ferret badger (*Malogale personata*). Proceedings of 53rd Kasetsart University Annual Conference: Plants, Animals, Veterinary Medicine, Fisheries, Agricultural Extension and Home Economics. Bangkok, Thailand.
- Sumrandee, C., Baimai, V., Trinachartvanit, W. & Ahantarig, A. (2016). Molecular detection of *Rickettsia*, *Anaplasma*, *Coxiella* and *Francisella* bacteria in ticks collected from Artiodactyla in Thailand. *Ticks and Tick-borne Diseases* **7**: 678-689. <https://doi.org/10.1016/j.ttbdis.2016.02.015>
- Takada, N., Fujita, H., Kawabata, H., Ando, S., Sakata, A., Takano, A. & Chaithong, U. (2009). Spotted fever group *Rickettsia* sp. closely related to *Rickettsia japonica*, Thailand. *Emerging Infectious Diseases* **15**: 610-611. <https://doi.org/10.3201/eid1504.071271>
- Tanskul, P. & Inlao, I. (1989). Keys to the adult ticks of *Haemaphysalis* Koch, 1844, in Thailand with notes on changes in taxonomy (Acari: Ixodoidea: Ixodidae). *Journal of Medical Entomology* **26**: 573-601. <https://doi.org/10.1093/jmedent/26.6.573>
- Tanskul, P., Stark, H.E. & Inlao, I. (1983). A checklist of ticks of Thailand (Acari: Metastigmata: Ixodoidea). *Journal of Medical Entomology* **20**: 330-341. <https://doi.org/10.1093/jmedent/20.3.330>
- Wassef, H.Y. & Hoogstraal, H. (1984). *Dermacentor* (Indocentor) *auratus* (Acari: Ixodoidea: Ixodidae): identity of male and female. *Journal of Medical Entomology* **21**: 169-173. <https://doi.org/10.1093/jmedent/21.2.169>
- Webb, L., Carl, M., Malloy, D.C., Dasch, G.A. & Azad, A.F. (1990). Detection of murine typhus infection in fleas by using the polymerase chain reaction. *Journal of Clinical Microbiology* **28**: 530-534. <https://doi.org/10.1128/jcm.28.3.530-534.1990>
- Williams, S.G., Sacci, J.B., Jr, Schriefer, M.E., Andersen, E.M., Fujioka, K.K., Sorvillo, F.J., Barr, A.R. & Azad, A.F. (1992). Typhus and typhuslike rickettsiae associated with opossums and their fleas in Los Angeles County, California. *Journal of Clinical Microbiology* **30**: 1758-1762. <https://doi.org/10.1128/jcm.30.7.1758-1762.1992>
- Yamauchi, T., Shimazu, Y. & Mizuta, H. (2009). A case of human tick bite by a nymphal tick, *Haemaphysalis hystricis* (Acari: Ixodidae), in Japan. *Medical Entomology and Zoology* **60**: 135-137. <https://doi.org/10.7601/mez.60.135>