BIOTROPIA ISSN: 0215-6334 | e-ISSN: 1907-770X
The Southeast Asian Journal of Tropical Biology
Vol. 32 No. 1, 2025: 111 - 117
DOI: 10.11598/btb.2025.32.1.2107

Short Communication

TICK-BORNE PATHOGENS DETECTION FROM TICKS INFESTING *Malayopython reticulatus* (REPTILIA: PYTHONIDAE) SNAKES IN INDONESIA

Supriyono, Hana Faizah Sophia, Upik Kesumawati Hadi, and Susi Soviana*

School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor 16680, Indonesia

ARTICLE HIGLIGHTS

- A total of 38 ticks were collected from *M. reticulatus*, comprising 13 *A. helvolum* and 25 *A. varanense*.
- Spotted fever group *Rickettsia* spp. (7.89%) and reptile-associated *Borrelia* sp. (2.63%) were detected in male *Amblyomma helvolum* ticks collected from *Malayopython reticulatus* snakes in Indonesia.
- Snake-associated ticks may harbor emerging pathogens, underscoring the importance of tick surveillance in reptiles for early disease detection and zoonotic prevention.

Article Information

Received : 19 October 2023
Revised : 8 August 2024
Accepted : 2 September 2024

***Corresponding author, e-mail:**
susiso@apps.ipb.ac.id

ABSTRACT

Ticks are important arthropod vectors of numerous diseases in humans and animals. Furthermore, ticks are also established vectors and reservoirs of pathogens important to wildlife and human health. *Rickettsia* and *Borrelia* are two genera of bacteria that may be transmitted by ticks, and some pathogenic species are zoonosis. This research investigated the prevalence of *Rickettsia* spp. and *Borrelia* sp. in *Amblyomma helvolum* and *Amblyomma varanense* ticks fed on *Malayopython reticulatus* and *Python bivittatus* snakes in Indonesia. A total of 38 ticks were collected from three *M. reticulatus* snakes, while no ticks were found on the *P. bivittatus* snake. The 38 ticks consisted of 13 individuals *A. helvolum* and 25 individuals *A. varanense*. PCR analysis revealed that three (3/38; 7.89%) male *A. helvolum* ticks were positive for spotted fever group *Rickettsia* spp. and one (1/38; 2.63%) male *A. helvolum* tick was positive for a reptile-associated group *Borrelia* sp. Although the overall prevalence of tick-borne pathogens was low, this study underscores the importance of monitoring the prevalence and prevention of tick-borne diseases. Surveillance of ticks infesting reptiles can facilitate the early detection of disease transmission to both animals and humans. These findings also suggested that snake-associated ticks may harbor emerging tick-borne pathogens.

Keywords: *Amblyomma helvolum, Borrelia,* Indonesia, *Malayopython reticulatus, Python bivittattus, Rickettsia*

INTRODUCTION

Malayopython reticulatus (Reptilia: Pythonidae) is a non-venomous snake commonly found in Indonesia. This snake is also one of the wild animals susceptible to infestations by various ectoparasites, including ticks. Five tick species have been separately reported to infest *M. reticulatus* snakes, which are *Amblyomma helvolum* (Anastos 1950; Anderson & Tzianabos 1989; Andoh *et al.* 2015), *A. varanense* (Anastos 1950; Andoh *et al.* 2015), *A. cordiferum* (Auffenberg 1988), and *Rhipicephalus sanguineus* (Chao *et al.* 2013). *Amblyomma latum* was also reported to infest *Python regius* snakes in Ghana and Togo (Pandit *et al.* 2011; Mariana *et al.* 2011; Sumrandee *et al.* 2014; Andoh *et al.* 2015).

Ticks known to be associated with pathogens include *Ixodes* spp., *Rhipicephalus* spp., *Haemaphysalis* spp., *Amblyomma* spp., and *Dermacentor* spp. (Estrada-Peña & Jongejan 1999; Raoult *et al.* 2002; Takano *et al.* 2014). Several pathogens are associated with ticks, a few of which are *Borrelia* sp. and *Rickettsia* sp. The major groups of *Borrelia* impacting animal and human health are relapsing fever *Borrelia*, Lyme disease *Borrelia*, and reptile-associated (REP) *Borrelia* (Bunikis & Barbour 2005; Takano *et al.* 2010; Franke *et al.* 2013). *Borrelia* spp. was previously detected in *A. varanense* infesting *P. reticulatus* in Thailand (Trinachartvanit *et al.* 2016). Meanwhile, *Rickettsia* sp. belonging to the spotted fever group (SFG) was also detected from *A. transversale* and

A. trimaculatum ticks infesting *P. regius* and *Boiga forsteni* snakes, respectively (Andoh *et al.* 2015). This research aimed to determine the presence of *Rickettsia* spp. and *Borrelia* sp. in ticks that infest wild snakes in Indonesia. In addition, phylogenetic analyses of detected pathogens were also presented.

MATERIALS AND METHODS

Ticks were collected within the period of 2021-2022 from three wild-caught *M. reticulatus* snakes in Bogor (6°35'42.1368" S and 106°48'59.8860" E) and Jakarta (20°58'50.736" N and 89°40'45.876" W) and one *P. bivittatus* snake found in Jakarta (20°58'50.736" N and 89°40'45.876" W). The snakes were handled in accordance with good animal welfare practices and released to their original habitats upon examination.

From the skin beneath their scales, ticks were collected using forceps and stored in 70% ethanol. Tick species, stage, and sex were identified based on morphologic features following taxonomic keys and molecular analysis (Anastos 1950; Kohls 1957).

The collected ticks were washed individually and homogenized in 200 µL 10x PBS solution. Tick DNA was individually extracted using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Oligonucleotide primer pairs used in this study were 16SrDNA (mt-rrs 1 5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3'; mt-rrs 2 5'-CCGGTCTGAACTCAGATCAAGTA-3') for tick identification, 17-kDa antigen (R1 5'-TCAATTCACAACTTGCCATT-3',R2 5'-TTTACAAAATTCTAAAAACC-3') for *Rickettsia* detection, and *flaB* (PAD 5'-GATCARGCWCAAYATAACCAWATGCA-3', PDU 5'-AGATTCA AGTCTGTTTTGGAAAGC-3') for *Borrelia* detection (Anderson & Tzianabos 1989; Takano *et al.* 2010).

Amplifications were performed with the following conditions: 95 °C for 5 minutes, 94 °C for 45 seconds, 50-52 °C for 30 seconds, 72 °C for 45 seconds, and 72 °C for 10 minutes. PCR products were visualized using electrophoresis on 1.2% agarose gel stained with ethidium bromide in 1X TAE buffer. Electrophoresis was carried out at 100 V for 25 minutes. DNA from positive samples then amplified again as much as 50 µL for sequencing and the sample was sent to PT. Genetika Science Indonesia, Tangerang.

The sequences of positive samples were compared with sequences in the NCBI GenBank database by nucleotide BLAST. Phylogenetic analyses were performed using the MEGA7 software (www.megasoftware.net) (Tamura *et al.* 2007). The phylogenetic trees were constructed by the neighbor-joining method. Bootstrap analyses (1,000 replicates) were carried out according to the Kimura 2-parameter model. All sequences were deposited in GenBank (Accession numbers: *Rickettsia* sp. ST9 (OQ164644), *Rickettsia* sp. ST10 (OQ164645), *Rickettsia* sp. ST13 (OQ054254), and *Borrelia* sp. ST10 (OQ187772)).

RESULTS AND DISCUSSION

From the three *M. reticulatus* snakes, a total of 38 ticks were collected which consisted of 13 individuals of *A. helvolum* (11 males, 2 females) and 25 individuals of *A. varanense* (20 males, 5 females) which were confirmed by morphological examination. There were no ticks found infesting the *P. bivittatus*.

Amblyomma helvolum had 3/3 dentition, long and narrow palps, and oval porose areas on the rectangular basis capituli. The male ticks had an ovoid scutum with metallic and yellowish patches of ornamentation. The female ticks, on the other hand, had no scutum ornamentations. The eyes were flat and located at the lateral margins of the scutum. Coxa I bore a pair of triangular spurs, with the external spur about twice as long as internal one. Coxae II-IV each bore a single, triangular spur.

Amblyomma varanense had 3/3 dentition, long and narrow palps, and oval porose areas on the rectangular basis capituli. The male ticks had a reddish-brown round scutum, nearly as broad as long, with five metallic-green spots of variable thickness, shape, and intensity. The female ticks had a reddish-brown cordiform scutum with three greenish metallic spots. Coxa I bore two short, distinct, and separated spurs; the external spur was slightly longer than the internal. Coxae II-IV each bore a single, blunt spur about as wide as long. PCR was used to confirm the identification of ticks.

Amblyomma helvolum has a natural distribution that extends from the Nicobar Islands of India eastward through parts of Thailand, Laos, Malaysia, Singapore, Vietnam, Indonesia, the Philippines, and Taiwan (Auffenberg 1988; Kolonin 1995; Petney & Keirans 1995; Chao *et al.* 2013). *Amblyomma helvolum* has been reported to infest

various reptiles, such as *Python* spp., *Ptyas korros* (Zamensis), and *Naja naja* (Kohls) (Imaoka *et al.* 2011) in Malaysia (Kohls 1957). Quite similarly, *A. varanense* is distributed in India, Thailand, Myanmar, Singapore, and Indonesia (Anastos 1950; Supriyono *et al.* 2019). *Amblyomma varanense* commonly infests reptiles, but is also known to feed on various mammals (Burridge 2001).

All collected ticks were individually examined by PCR for *Rickettsia* spp. and *Borrelia* spp. The results showed that three male *A. helvolum* ticks infested on *M. reticulatus* were positive for *Rickettsia* spp. and one male *A. helvolum* tick infested on *M. reticulatus* was positive for *Borrelia* sp. The detected *Rickettsia* spp. and *Borrelia* sp. were compared with sequences in the NCBI GenBank database by nucleotide BLAST. There were no pathogens detected in *A. varanense* in this study.

The 17-kDa gene sequences of two detected *Rickettsia*, *Rickettsia* sp. ST10 and *Rickettsia* sp. ST13, showed 100% (500/500) and 99.4% (497/500) similarity, respectively, to *Rickettsia* sp. RT2 (GenBank: LC428381) detected from *A. varanense* ticks infesting an Asian water monitor (*Varanus salvator*) in Indonesia. However, *Rickettsia* sp. ST9 was 99.2% (499/503) in similarity to *Rickettsia* sp. (GenBank: AB795164) detected from *A. sparsum* ticks infesting tortoises (*Geochelone pardalis*) from Zambia (Fig. 1). The detected *Rickettsia* spp. belonged to the spotted fever group (SFG), which are potential human pathogens.

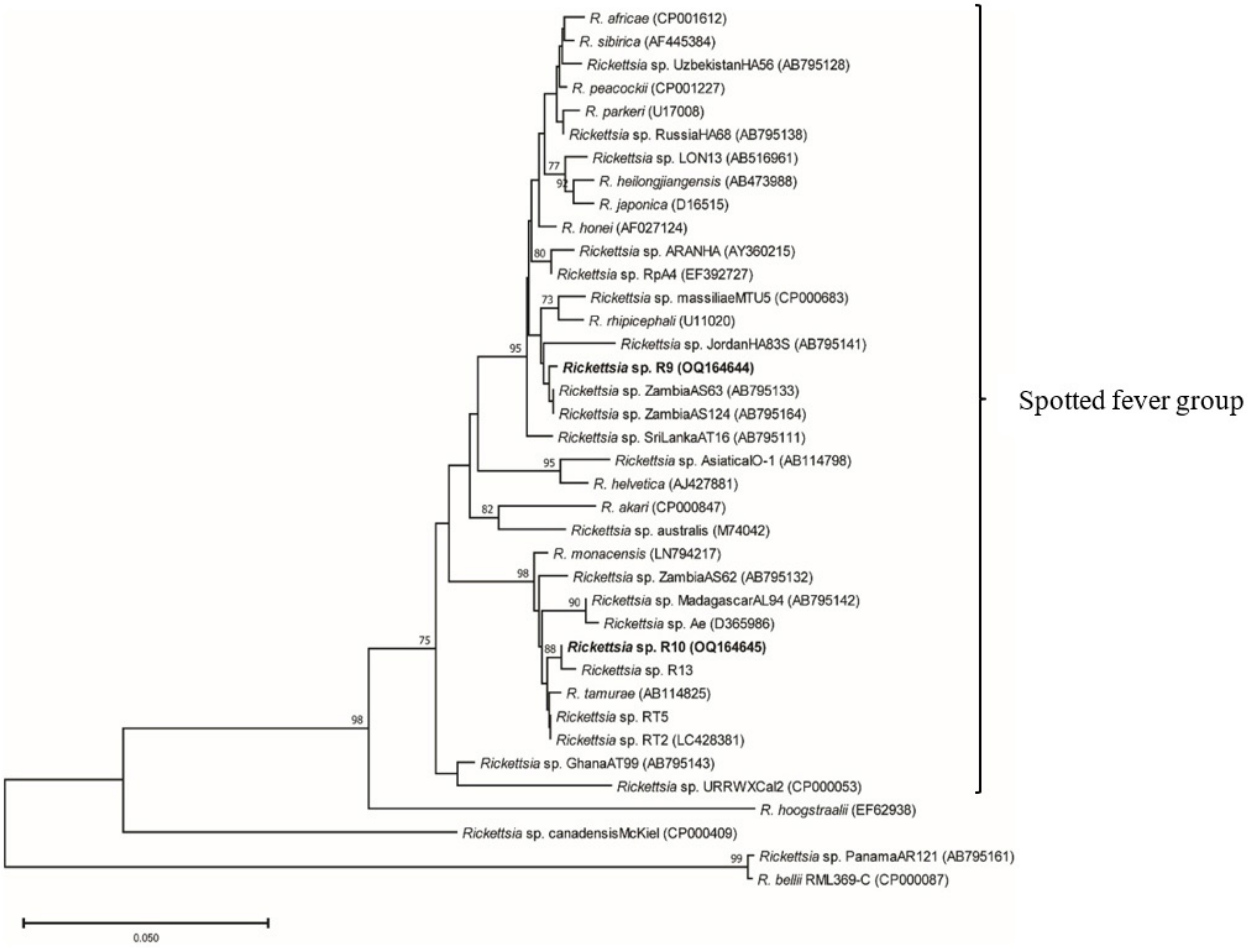

Figure 1 Phylogenetic analysis of 17-kDa of *Rickettsia* spp.

Notes: The phylogenetic branches showed support of > 70% by tree, which was constructed using the neighbor-joining method and bootstrap tests (1,000 replicates) carried out according to the Kimura 2-parameter model. The bar indicates the percentage of sequence divergence. Bold letters indicate the samples analyzed in the present study.

Several studies have reported the detection of SFG in ticks from various countries in Southeast Asia, including Japan, Thailand, Malaysia, and Indonesia (Imaoka *et al.* 2011; Doornbos *et al.* 2013; Kho *et al.* 2015; Supriyono *et al.* 2019). *Rickettsia* sp. RT2, closely related to *R. tamurae*, was detected in *A. varanense* ticks infesting an Asian water monitor (*V. salvator*) in Indonesia (Supriyono *et al.* 2019). Although there have been no reports of SFG infection in humans due to tick bites in Indonesia thus far, tick infestations pose a potential risk to human health. Infection caused by *R. tamurae*, a member of the SFG *Rickettsia*, has been reported in Japan and Laos (Phongmany *et al.* 2006; Imaoka *et al.* 2011). Additionally, antibodies against SFG infection in humans have been reported in rural areas in Malaysia (Tay *et al.* 2000). The results of this study indicated that there was a natural circulation of SFG in ticks infesting snakes.

Analysis of the *flaB* gene showed that the detected *Borrelia* sp. was 99.3% (296/298) in similarity to *Borrelia* sp. BF16 (GenBank: AB473488) detected from *A. trimaculatum* ticks infesting *B. forsteni* snakes. The detected *Borrelia* sp. was also 99.2% (370/373) in similarity to uncultured *Borrelia* sp. (GenBank: KT758064) detected from *A. varanense* ticks infesting *P. reticulatus* in Thailand (Trinachartvanit *et al.* 2016).

The constructed phylogenetic tree details that the detected *Borrelia* sp. in this study belongs to the REP *Borrelia* group and differs from the Lyme disease-associated *Borrelia* and the relapsing fever-associated *Borrelia* (Fig. 2).

REP *Borrelia* has been detected in ticks infesting various reptiles, including snakes, turtles, and monitor lizards in Thailand, Indonesia, and Japan (Takano *et al.* 2010; Trinachartvanit *et al.* 2016; Supriyono *et al.* 2019). REP *Borrelia* has also been detected in ticks infesting imported captive-bred *Geochelone sulcata* tortoises and wild-caught *Chelonoidis carbonarius* and *C. denticulatus* tortoises in Indonesia (Sophia *et al.* 2023).

Malayopython reticulatus has a habitat distribution in rainforests, woodlands, and nearby grasslands. It is also associated with rivers and is found in areas with nearby streams and lakes (Murray-Dickson *et al.* 2017). The snakes in this study were wild-caught in rural areas and thus, have potential to transmit diseases via hematophagous arthropods which include ticks.

This data is essential for vector surveillance to predict the risk of emerging diseases and zoonoses, primarily caused by tick-borne bacteria. Although there are no reports of *A. helvolum* and *A. varanense* infestations in humans in Indonesia, the detected tick pathogens can pose risks to wildlife and human health. It is not uncommon that many reptiles, including snakes, are kept as pets that live close to humans or other animals.

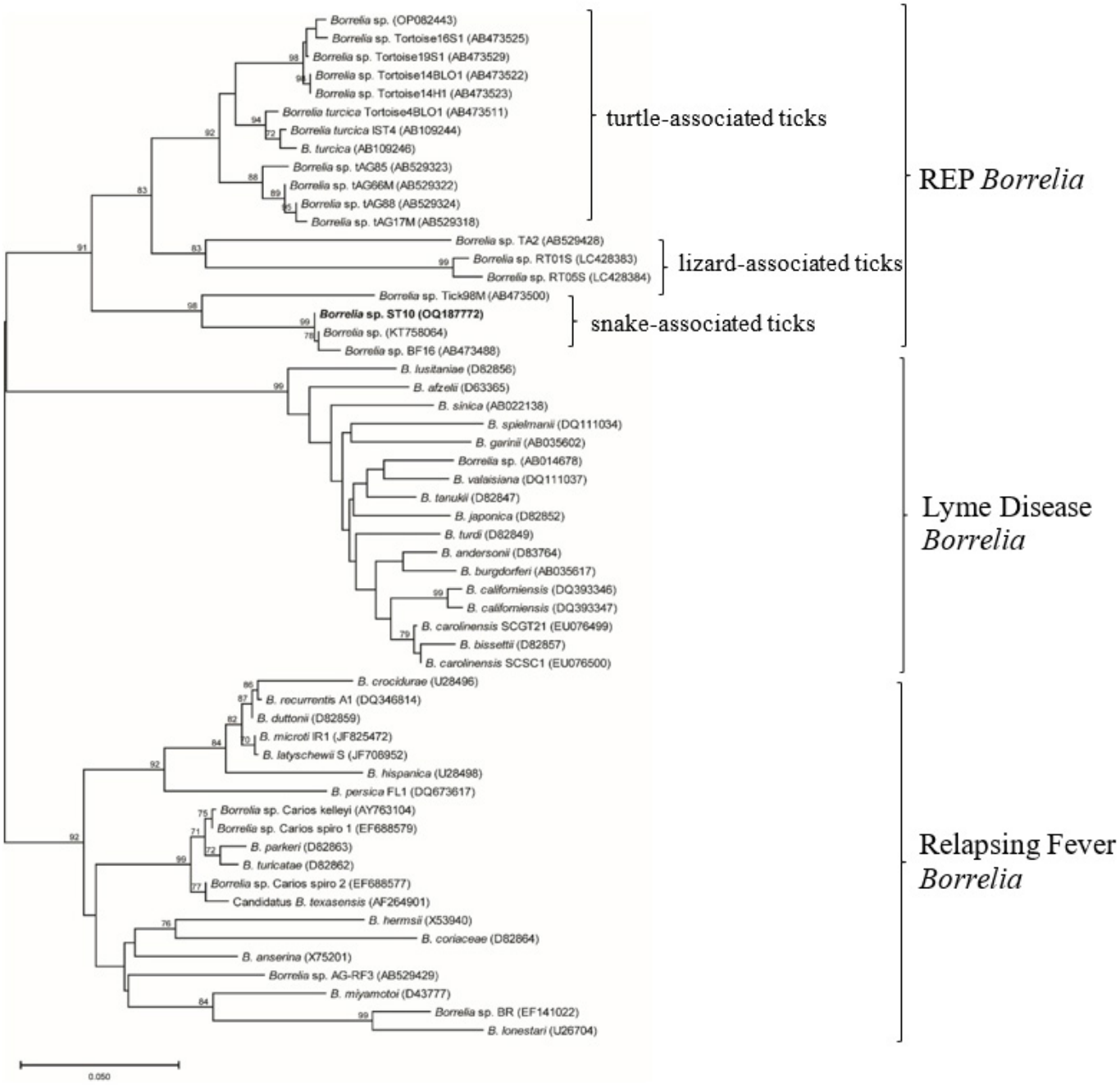

Figure 2 Phylogenetic analysis of *flaB* of detected *Borrelia* sp.

Notes: The phylogenetic branches showed support of > 70% by tree, which was constructed using the neighbor-joining method and bootstrap tests (1,000 replicates) carried out according to the Kimura 2-parameter model. The bar indicates the percentage of sequence divergence. Bold letters indicate the samples analyzed in the present study.

CONCLUSION

Rickettsia spp. and *Borrelia* sp. were detected in *A. helvolum* which belong to spotted fever group and reptile-associated borrelia, respectively. The prevalence of spotted fever group *Rickettsia* spp. in these ticks was 7.89%, and the prevalence of reptile-associated *Borrelia* sp. was 2.63%. These findings provide new insights into the parasite-host dynamics within the tick and tick-borne pathogen system in wild snakes in urban areas of Indonesia, enhancing our understanding of the ecology and interactions among wildlife, ticks, and tick-borne pathogens in the region. Monitoring the circulation patterns of tick-borne bacteria is crucial for assessing infection risks to wildlife and humans, as well as for developing strategies for disease control, mitigation, and early warning systems in case of outbreaks. Further studies are required to assess the prevalence of tick-borne bacteria in other animal species across Indonesia.

ACKNOWLEDGEMENTS

We thank the Laboratory of Medical Entomology and the Animal Teaching Hospital, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia.

REFERENCES

Anastos G. 1950. The Scutate ticks, or Ixodidae, of Indonesia. Entomol Am 1-4:1-144.

Anderson BE, Tzianabos T. 1989. Comparative sequence analysis of a genus-common rickettsial antigen gene. J Bacteriol 171(9):5199-201. DOI: 10.1128/jb.171.9.5199-5201.1989

Andoh M, Sakata A, Takano A, Kawabata H, Fujita H, Une Y … Ando S. 2015. Detection of *Rickettsia* and *Ehrlichia* spp. in ticks associated with exotic reptiles and amphibians imported into Japan. PLoS One 10(7):e0133700. DOI: 10.1371/journal.pone.0133700

Auffenberg T. 1988. *Amblyomma helvolum* (Acarina: Ixodidae) as a parasite of varanid and scincid reptiles in the Philippines. Int J Parasitol 18(7):937-45. DOI: 10.1016/0020-7519(88)90176-2

Bunikis J, Barbour AG. 2005. Third *Borrelia* species in white-footed mice. Emerg Infect Dis 11(7):1150-1. DOI: 10.3201/eid1107.041355

Burridge MJ. 2001. Ticks (Acari: Ixodidae) spread by the international trade in reptiles and their potential roles in dissemination of diseases. Bull Entomol Res 91(1):3-23.

Chao LL, Hsieh CK, Shih CM. 2013. First report of *Amblyomma helvolum* (Acari: Ixodidae) from the Taiwan stink snake, *Elaphe carinata* (Reptilia: Colubridae), collected in southern Taiwan. Ticks Tick Borne Dis 4(3):246-50. DOI: 10.1016/j.ttbdis.2012.11.002

Doornbos K, Sumrandee C, Ruang-Areerate T, Baimai V, Trinachartvanit W, Ahantarig A. 2013. *Rickettsia* sp. closely related to *Rickettsia raoultii* (Rickettsiales: Rickettsiaceae) in an *Amblyomma helvolum* (Acarina: Ixodidae) tick from a *Varanus salvator* (Squamata: Varanidae) in Thailand. J Med Entomol 50(1):217-20. DOI: 10.1603/me12010

Estrada-Peña A, Jongejan F. 1999. Ticks feeding on humans: A review of records on human-biting Ixodoidea with special reference to pathogen transmission. Exp Appl Acarol 23(9):685-715. DOI: 10.1023/a:1006241108739

Franke J, Hildebrandt A, Dorn W. 2013. Exploring gaps in our knowledge on Lyme borreliosis spirochaetes: Updates on complex heterogeneity, ecology, and pathogenicity. Ticks Tick Borne Dis 4(1-2):11-25. DOI: 10.1016/j.ttbdis.2012.06.007

Imaoka K, Kaneko S, Tabara K, Kusatake K, Morita E. 2011. The first human case of *Rickettsia tamurae* infection in Japan. Case Rep Dermatol 3(1):68-73. DOI: 10.1159/000326941

Kho KL, Koh FX, Tay ST. 2015. Molecular evidence of potential novel spotted fever group rickettsiae, *Anaplasma* and *Ehrlichia* species in *Amblyomma* ticks parasitizing wild snakes. Parasites Vectors 8:112. DOI: 10.1186/s13071-015-0719-3

Kohls GM. 1957. Malaysian parasites XVII ticks (Ixodoidea) of Borneo and Malaya. Stud Inst Med Res Malaya 28:65-94.

Kolonin GV. 1995. Review of the ixodid tick fauna (Acari: Ixodidae) of Vietnam. J Med Entomol 32:276-82. DOI: 10.1093/jmedent/32.3.276

Mariana A, Vellayan S, Halimaton I, Ho TM. 2011. Acariasis on pet Burmese python, *Python molurus bivittatus* in Malaysia. Asian Pac J Trop Med 4(3):227-8. DOI: 10.1016/S1995-7645(11)60075-8

Murray-Dickson G, Ghazali M, Ogden R, Brown R, Auliya M. 2017. Phylogeography of the reticulated python (*Malayopython reticulatus* ssp.): Conservation implications for the worlds' most traded snake species. PLoS One. 12(8):e0182049. DOI: 10.1371/journal.pone.0182049

Pandit P, Bandivdekar R, Geevarghese G, Pande S, Mandke O. 2011. Tick infestation on wild snakes in northern part of western Ghats of India. J Med Entomol 48(3):504-7. DOI: 10.1603/me10164

Petney TN, Keirans JE. 1995. Ticks of the genera *Amblyomma* and *Hyalomma* from South-East Asia. Trop Biomed 12:45-56.

Phongmany S, Rolain JM, Phetsouvanh R, Blacksell SD, Soukkhaseum V, Rasachack B … Newton PN. 2006. Rickettsial infections and fever, Vientiane, Laos. Emerg Infect Dis 12(2):256-62. DOI: 10.3201/eid1202.050900

Raoult D, Lakos A, Fenollar F, Beytout J, Brouqui P, Fournier PE. 2002. Spotless rickettsiosis caused by *Rickettsia slovaca* and associated with *Dermacentor* ticks. Clin Infect Dis 34(10):1331-6. DOI: 10.1086/340100

Sophia HF, Supriyono, Soviana S, Novianto D, Hadi UK. 2023. Molecular detection of *Borrelia* spp. (Spirochaetales: Borreliaceae) in ticks (Acari: Ixodidae) collected from tortoises in Java, Indonesia. Biodiversitas 24(12): 6852-7. DOI: 10.13057/biodiv/d241246

Sumrandee C, Hirunkanokpun S, Doornbos K, Kitthawee S, Baimai V, Grubhoffer L … Ahantarig A. 2014. Molecular detection of *Rickettsia* species in *Amblyomma* ticks collected from snakes in Thailand. Ticks Tick Borne Dis 5(6):632-40. DOI: 10.1016/j.ttbdis.2014.04.013

Supriyono, Takano A, Kuwata R, Shimoda H, Hadi UK, Setiyono A … Maeda K. 2019. Detection and isolation of tick-borne bacteria (*Anaplasma* spp., *Rickettsia* spp., and *Borrelia* spp.) in *Amblyomma varanense* ticks on lizard (*Varanus salvator*). Microbiol Immunol 63(8):328-33. DOI: 10.1111/1348-0421.12721

Takano A, Fujita H, Kadosaka T, Takahashi M, Yamauchi T, Ishiguro F … Kawabata H, 2014. Construction of a DNA database for ticks collected in Japan: application of molecular identification based on the mitochondrial 16S rDNA gene. Med Entomol Zool 65(1):13-21. DOI: 10.7601/mez.65.13

Takano A, Goka K, Une Y, Shimada Y, Fujita H, Shiino T, Watanabe H, Kawabata H. 2010. Isolation and characterization of a novel *Borrelia* group of tick-borne borreliae from imported reptiles and their associated ticks. Environ Microbiol 12(1):134-46. DOI: 10.1111/j.1462-2920.2009.02054.x

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24(8):1596-9. DOI: 10.1093/molbev/msm092

Tay ST, Ho TM, Rohani MY, Shamala D. 2000. Antibody prevalence of *Orientia tsutsugamushi*, *Rickettsia typhi* and TT118 spotted fever group rickettsiae among febrile patients in rural areas of Malaysia. Trans R Soc Trop Med Hyg 94:280-4

Trinachartvanit W, Hirunkanokpun S, Sudsangiem R, Lijuan W, Boonkusol D, Baimai V, Ahantarig A. 2016. *Borrelia* sp. phylogenetically different from Lyme disease- and relapsing fever-related *Borrelia* spp. in *Amblyomma varanense* from *Python reticulatus*. Parasites Vectors 9:359. DOI: 10.1186/s13071-016-1629-8