Current Research in Parasitology & Vector-Borne Diseases 7 (2025) 100250

Contents lists available at ScienceDirect

Current Research in Parasitology & Vector-Borne Diseases

journal homepage: www.sciencedirect.com/journal/current-research-in-parasitology-and-vector-borne-diseases

Unveiling hidden threats: Molecular surveillance of bacterial and protozoan infections in Asian water monitors (*Varanus salvator*) at Thailand's Khao-zon Wildlife Breeding Station

Witchuta Junsiri [a], Jitkamol Thanasak [b], Tawewan Issarankura Na Ayudhaya [c], Somjit Chaiwattanarungruengpaisan [d], Piyanan Taweethavonsawat [a,e,*]

[a] Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand
[b] Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, 73170, Thailand
[c] Department of Preclinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, 73170, Thailand
[d] The Monitoring Surveillance Center for Zoonotic Diseases in Wildlife and Exotic Animals, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, 73170, Thailand
[e] Biomarkers in Animals Parasitology Research Unit, Chulalongkorn University, Bangkok, 10330, Thailand

ARTICLE INFO

Keywords:
Asian water monitor
Blood parasites
Phylogenetic analysis
Thailand
Varanus salvator

ABSTRACT

Asian water monitors (*Varanus salvator*) are often exposed to harmful microorganisms such as species of *Hepatozoon*, *Anaplasma*, *Rickettsia*, and *Borrelia*. These pathogens pose significant risks to wildlife and public health. In this study, we aimed to investigate the presence and genetic diversity of pathogenic microorganisms in *V. salvator* from the Khao-zon Wildlife Breeding Station, Ratchaburi, Thailand. Sixteen *V. salvator* were collected, and thin blood smears and polymerase chain reaction (PCR) assays of the DNA isolated from blood were used to identify infections. Blood smear analysis revealed two positive samples (2/16; 12.5%) for *Hepatozoon* sp. PCR results confirmed the presence of *Hepatozoon* sp. (6/16; 37.5%), *Anaplasma* sp. (3/16; 18.75%), *Rickettsia* sp. (2/16; 12.5%), *Borrelia* sp. (4/16; 25.0%) and *Burkholderia* sp. (1/16; 6.25%). Phylogenetic analysis revealed 99.86% similarity of *Hepatozoon* sp. sequences with *Hepatozoon ophisauri* (GenBank: MN723845) in *Pseudopus apodus* from Iran, whereas the *gltA* gene of *Rickettsia* sp. was closely related to *Rickettsia conorii raoultii* (GenBank: MF002515) in *Dermacentor nuttalli* from China. This study represents the first detection of the five pathogens in *V. salvator* from Thailand and provides valuable insights into the genetic diversity of these microorganisms. Our findings suggest that *V. salvator* may serve as reservoir for multiple pathogens, posing potential threats to both wildlife and humans. The presence of zoonotic agents such as *Rickettsia* spp., *Anaplasma* spp., and *Borrelia* spp. underscores the importance of continued surveillance in wildlife populations to mitigate the risk of emerging infectious diseases.

1. Introduction

The Asian water monitor (*Varanus salvator*) is under wildlife protection according to the list of Thailand's protected species (Wild Animal Reservation and Protection Act, B.E. 2535 of 1992) (Lauprasert and Thirakhupt, 2001), since its population has decreased due to the loss of habitat or hunting for consumption or leather products (Shine et al., 1996; Mahaprom et al., 2015). *Varanus salvator* is one of the most common monitor lizards throughout much of southern Asia, from Sri Lanka through Southeast Asia, including Thailand (Böhme, 2003; Koch et al., 2013), where the species is most threatened due to its proximity to human habitats and agriculture (Cota, 2009). In Thailand, during the past few years, the living zones of water monitors have extended into human communities such as Samut Prakan, Samut Songkhram, Samut Sakhon and Ratchaburi provinces, due to the increase in the number of water monitors (Cota, 2009).

Water monitors are often exposed to harmful microorganisms such as *Rickettsia* spp., *Anaplasma* spp., *Ehrlichia* spp., *Borrelia* spp. and/or *Hepatozoon* spp. (Thanasak et al., 2022; Yean et al., 2024). However, only a limited number of studies have reported the presence of bacteria

* Corresponding author. Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, 10330, Thailand.
E-mail address: Piyanan.T@chula.ac.th (P. Taweethavonsawat).

https://doi.org/10.1016/j.crpvbd.2025.100250
Received 24 October 2024; Received in revised form 20 February 2025; Accepted 21 February 2025
Available online 21 February 2025

in ticks associated with *Varanus* spp. in Asian countries (Doornbos et al., 2013; Koh et al., 2018; Supriyono et al., 2019; Khan et al., 2022). Thicks of the genus *Amblyomma* are potential vectors and *Amblyomma*-infested reptiles have been reported in several studies (Doornbos et al., 2013; Sumrandee et al., 2015; Trinachartvanit et al., 2016; Kaenkan et al., 2020). In Thailand, reports of bacteria in ticks infesting varanid lizards, including *Rickettsia* spp. and *Borrelia* spp. (Doornbos et al., 2013; Kaenkan et al., 2020), and protozoans of the genus *Hepatozoon* are rare (Junsiri et al., 2024) and the information on bacterial and parasitic infections in *V. salvator* is still limited. Identification of bacterial and parasitic pathogens from *V. salvator* is crucial for comprehending the health dynamics of wild animals in Thailand. The aim of this study was to molecularly characterize bacteria and blood parasites carried by *V. salvator* in Ratchaburi Province of Thailand.

2. Materials and methods

2.1. Sample collection

Asian water monitors (*V. salvator*) were captured by hand from November 2023 to May 2024 at the Khao-zon Wildlife Breeding Station, Ratchaburi Province of Thailand (13°35′07.4"N, 99°29′17.0"E). The adult males and females, which had more than 3 kg of body weight and more than 100 cm in length from the snout to the anus, were randomly chosen for blood collection and subsequently released. The clinical signs, including injuries, wounds, emaciation, overall body condition, and tick infestation, were checked. Ten ml of peripheral blood samples were drawn from the caudal tail vein using an 18-gauge needle into an EDTA blood collection tube. All samples were maintained at 4 °C while they were being handled in the laboratory. Blood smears were obtained, air-dried, fixed in methanol, and stained with Giemsa (Rosenblatt, 2009). Parasites were observed under an Olympus CX31 biological microscope (Olympus, Tokyo, Japan). The remaining EDTA blood sample was preserved at −20 °C for subsequent molecular analysis.

2.2. DNA extraction, amplification, and sequencing

DNA was extracted from individual blood samples using the NucleoSpin® Blood, (Macherey-Nagel, Düren, Germany) kit following the manufacturer's instructions. Amplification of *Hepatozoon* and bacterial DNA was performed using a set of primers provided in Table 1. DNA was amplified in a 20-μl reaction system, each including 2 μl of DNA template, 1× GoTaq® Green Master Mix (Promega, Madison, USA), 0.2 mM each of reverse and forward primer, and filled to volume with nuclease-free water. The reaction was performed in a thermal cycler (BIOER Technology, Hangzhou, China). The PCR primers, amplification sizes (base pairs), and annealing temperatures are listed in Table 1. Nuclease-free water was used as the negative control for each PCR. DNA-positive samples for each pathogen were used as a positive control. Prior to sequencing, the quality of the PCR products was checked with 1.5% agarose gel electrophoresis, and the products were stained with RedSafe™ Nucleic Acid Staining Solution (INtRON Biotechnology, Gyeonggi, Korea). If the PCR product quality was suboptimal, the product was fully replicated. If the PCR product was of good quality, the product was purified using the NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel). The purified DNA fragments were then sequenced using Sanger sequencing (U2Bio Co., Ltd, Seoul, South Korea) and subsequently analyzed for phylogenetic and haplotype diversity.

2.3. Data analysis

The sequences were edited using BioEdit v.7.0.5.3 (Hall, 1999) and identified through comparative analysis with sequences deposited in GenBank, using the National Center for Biotechnology Information (NCBI) BLAST search engine. Multiple sequence alignment was performed using MEGA software version 11.0.13 (Kumar et al., 2018). Phylogenetic trees were reconstructed with the maximum likelihood (ML) method with 1000 bootstrap replicates to assess tree stability (Felsenstein, 1985; Nei and Kumar, 2000) and the Bayesian inference (BI) algorithm in MrBayes, version 3.1.2 (Huelsenbeck and Ronquist, 2001) with 5,000,000 generations of trees sampled every 1000 generations. The sequences were analyzed in DnaSP software, v.5.10.01 to

Table 1
Primers used for confirmatory PCR testing.

Gene	Description	Target organisms	Primer sequence (5′-3′)	T (°C)	Reference
18S rRNA	18S ribosomal RNA	*Hepatozoon* spp.	TATTGGTTTTAAGAACTAATTTTATGATTG CTTCTCCTTCCTTTAAGTGATAAGGTTCAC	48	Perkins and Keller (2001)
16S rRNA	16S ribosomal RNA	Eubacteria	AGAGTTTGATCCTGGCTCAG ACGGCTACCTTGTTAGGACTT	52	Weisburg et al. (1991)
		Ehrlichia/Anaplasma spp.	AGAACGAACGCTGGCGGCAAGCC CGTATTACCGCGGCTGCTGGCA	60	Dawson et al. (1994)
		Borrelia spp.	ATAACGAAGAGTTTGATCCTGGC CAGCCGCACTTTCCAGTACG	64	Masuzawa et al. (1999)
gltA	Citrate synthase protein	*Rickettsia* spp.	GACCATGAGCAGAATGCTTCT[a] ATTGCAAAAAGTACAGTGAAC[a]	44	Ishikura et al. (2003)
			GGGGGCCTGCTCACGGCGG[b] ATTGCAAAAAGTACAGTGAAC[b]	48	
17 kDa	17 kDa outer membrane antigen	*Rickettsia* spp.	TTTACAAAATTCTAAAAACCAT[a] TCAATTCACAACTTGCCATT[a] GCTCTTGCAACTTCTATGTT[b] TCAATTCACAACTTGCCATT[b]	57	
groEL	60 kDa heat-shock protein	*Ehrlichia/Anaplasma* spp.	GAAGATGCWGTWGGWTGTACKGC[a] AGMGCTTCWCCTTCWACRTCYTC[a] ATTACTCAGAGTGCTTCTCARTG[b] TGCATACCRTCAGTYTTTTCAAC[b]	57	Tabara et al. (2007)
flaB	Flagellin protein	*Borrelia* spp.	ACATATTCAGATGCAGACAGAGGT[a] GCAATCATAGCCATTGCAGATTGT[a] AACAGCTGAAGAGCTTGGAATG[b] CTTTGATCACTTATCATTCTAATAGC[b]	55	Stromdahl et al. (2003)

Note: W = A, T; K = G, T; M = A, C; R = A, G; Y = C, T.
Abbreviation: T (°C), annealing temperature.
[a] First round PCR.
[b] Nested PCR.

calculate DNA polymorphisms and haplotype information (Librado and Rozas, 2009). The haplotype networks were estimated by means of the Templeton, Crandall, and Sing (TCS) network in Population Analysis with Reticulate Trees (PopART) software (Clement et al., 2002; Leigh and Bryant, 2015).

3. Results

3.1. Morphology of the gamonts

Examination of the blood smears obtained from 16 *V. salvator* samples revealed gamonts resembling *Hepatozoon* in two samples (Fig. 1). The mature gamonts were identified within the cytoplasm of infected erythrocytes and showed an elongated shape with broad, rounded ends. The gamonts were approximately 4.45 μm in width and 19.0 μm in length with pale basophilic cytoplasm and central oval basophilic nucleus.

3.2. PCR amplification and sequencing

Blood samples collected from *V. salvator* tested positive for *Hepatozoon* sp. (6/16; 37.5%) and four bacterial species: *Anaplasma* sp. (3/16; 18.75%), *Rickettsia* sp. (2/16; 12.5%), *Borrelia* sp. (4/16; 25.0%), and *Burkholderia* sp. (1/16; 6.25%). All sequences generated in this study have been deposited in the GenBank database (Table 2). Sequencing of the positive PCR products confirmed the presence of several pathogenic microorganisms and parasites. BLAST analysis revealed that the 18S rDNA sequences of *Hepatozoon* sp. showed 99.86% similarity with *H. ophisauri* (GenBank: MN723845) from *Pseudopus apodus* in Iran. The 16S rDNA sequences of *Anaplasma* sp. showed 98.3% similarity to sequences identified as uncultured *Anaplasma* sp. (GenBank: OR494603) detected in *Amblyomma gervaisi* ticks from monitor lizards in Pakistan, whereas the *groEL* gene sequences showed 98.71% similarity to *Anaplasma* sp. (GenBank: LC428379) isolated from *Amblyomma varanense* ticks that had fed on water monitors (*V. salvator*) in Indonesia. Furthermore, the 16S rRNA gene sequences of *Rickettsia* sp. showed a similarity of 98.9% with sequences from *Amblyomma helvolum* ticks collected from Asiatic water snakes (*Xenochrophis piscator*) in Thailand, and the 17 kDa gene sequences of *Rickettsia* sp. showed 98.9% similarity with *Rickettsia* sp. (GenBank: LC428381) isolated from *Amblyomma varanense* ticks fed on a water monitor (*V. salvator*) in Thailand. Interestingly, the *gltA* gene sequences of *Rickettsia* sp. showed 98.59% similarity with the sequence of *R. conorii raoultii* (GenBank: MF002515) isolated from *Dermacentor nuttalli* in China. The flagellin gene sequences of *Borrelia* sp., presented 93% similarity with a sequence identified as *Borrelia* sp. (isolate STN2; GenBank: MK462207), which was found in *Amblyomma varanense* ticks in Bengal monitors in Thailand. This finding suggests a very high degree of similarity between the two sequences, but it does not necessarily mean that they are identical. Additionally, the sequence of *Burkholderia* sp. was 99.88% similar to that of uncultured *Burkholderia* sp. from China (GenBank: OQ678120) as well as *Burkholderia cepacian* isolated from soil and *Rubus idaeus* in the USA and Mexico (GenBank: CP022083, OR426199).

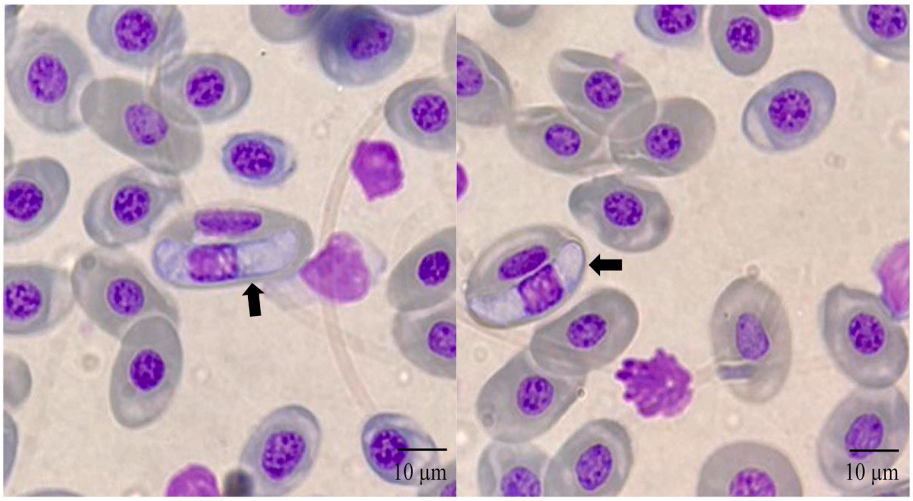

Fig. 1. Light photomicrographs of hemogragarine gamonts from *Varanus salvator* (Giemsa stained). Arrows indicate gamonts of *Hepatozoon* sp.

Table 2
DNA sequences generated in this study.

Isolate	Pathogen species/Gene							
	Hepatozoon sp.	*Rickettsia* sp.			*Borrelia* sp.	*Anaplasma* sp.		*Burkholderia* sp.
	18S rRNA	16S rRNA	*gltA*	17 kDa	*flaB*	16S rRNA	*groEL*	16S rRNA
CM1	PP767327	PP767754	PQ272554	PQ272548				
CM2	PP767328					PP767320	PQ272556	
CM4						PP767321	PQ272557	
CM5	PP767329						PQ272558	
CM6	PP767330				PQ272550			
CM9								PP767324
CM10					PQ272551			
CM11	PP767331	PP767755	PQ272555	PQ272549				
CM12					PQ272552			
CM14	PP767332							
CM16					PQ272553			

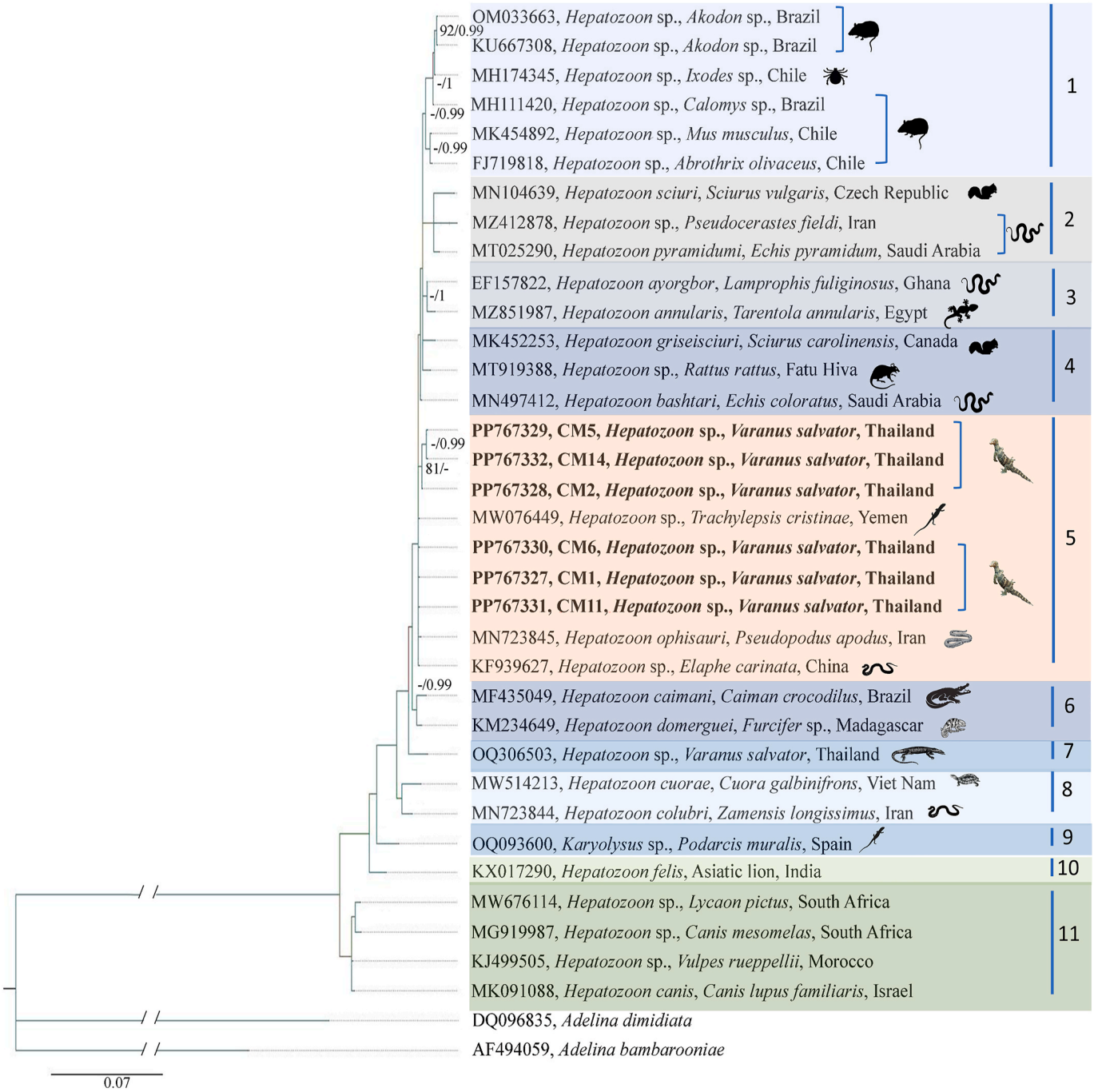

Fig. 2. Phylogenetic tree reconstructed by maximum likelihood (ML) and Bayesian inference (BI) analyses using 18S rDNA sequences for *Hepatozoon* spp. The ML bootstrap and BI posterior probability values are given at the nodes as ML/BI; only values > 70% (ML) and 0.95 (BI) are shown. The scale-bar indicates the number of substitutions per site. The newly generated sequences are indicated in bold. *Adelina dimidiata* and *Adelina bambarooniae* were used as outgroups.

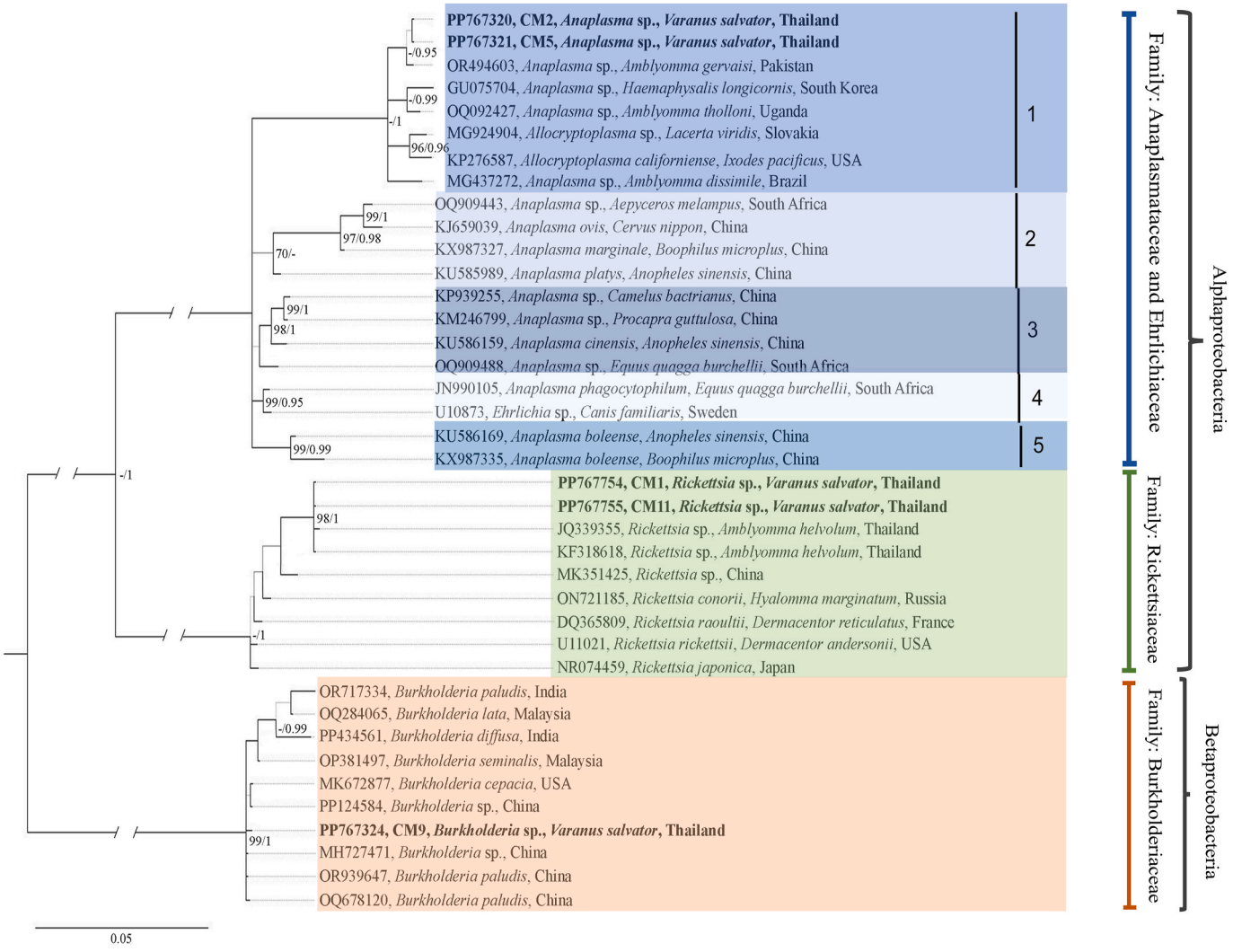

Fig. 3. Phylogenetic tree reconstructed by maximum likelihood (ML) and Bayesian inference (BI) analyses using 16S rDNA sequences for *Anaplasma* spp., *Rickettsia* spp. and *Burkholderia* spp. The ML bootstrap and BI posterior probability values are given at the nodes as ML/BI; only values > 70% (ML) and 0.95 (BI) are shown. The scale-bar indicates the number of substitutions per site. The newly generated sequences are indicated in bold. Note that the tree is unrooted.

3.3. Phylogenetic analyses

Phylogenetic analysis was conducted by combining the six *Hepatozoon* sp. 18S rDNA sequences detected in this study with a previously recorded *Hepatozoon* sp. sequence (GenBank: OQ306503). The phylogenetic tree also included sequences obtained from the GenBank database that were either closely matched to sequences from this study or represented *Hepatozoon* spp. described in ticks, reptiles, amphibians, and small mammals. The phylogeny showed several different clades (Fig. 2). The newly generated *Hepatozoon* sp. sequences grouped in Clade 5, along with sequences from *Trachylepsis cristinae* found in Yemen (GenBank: MW076449), from *Pseudopus apodus* in Iran (GenBank: MN723845), and from *Elaphe carinata* in China (GenBank: KF939627).

The phylogenetic tree based on the 16S rDNA gene (Fig. 3) showed that the sequence of *Burkholderia* sp. from this study was resolved as a basal lineage compared with other *Burkholderia* sp. sequences. The newly generated sequences for *Rickettsia* sp. clustered with *Rickettsia* sp. sequences (GenBank: JQ339355 and KF318618) obtained from *Amblyomma helvolum* ticks in Thailand (Fig. 3).

The phylogeny based on 17 kDa gene of *Rickettsia* spp. also revealed a monophyletic group comprising a clade with *Rickettsia* sp. resolved as sister group to *Rickettsia* sp. sequences obtained from ticks (GenBank: OR537322, OR537323, and MN150181) (Fig. 4). Interestingly, the phylogenetic tree for *Rickettsia gltA* gene showed the closest clustering of the new *Rickettsia* sp. sequence with that of *R. conorii raoultii* (GenBank: OL687227) from ticks in South Korea (Fig. 5).

The phylogenetic tree for 16S rRNA gene of *Anaplasma* spp. formed a monophyletic group with five main clades, one of which included the newly generated *Anaplasma* sp. sequence along with *Anaplasma* sp. sequence recently detected in *Amblyomma gervaisi* ticks in Pakistan (Fig. 3). Based on the *groEL* gene, the new *Anaplasma* sp. sequence showed a similar grouping to the 16S rDNA tree. The combined tree

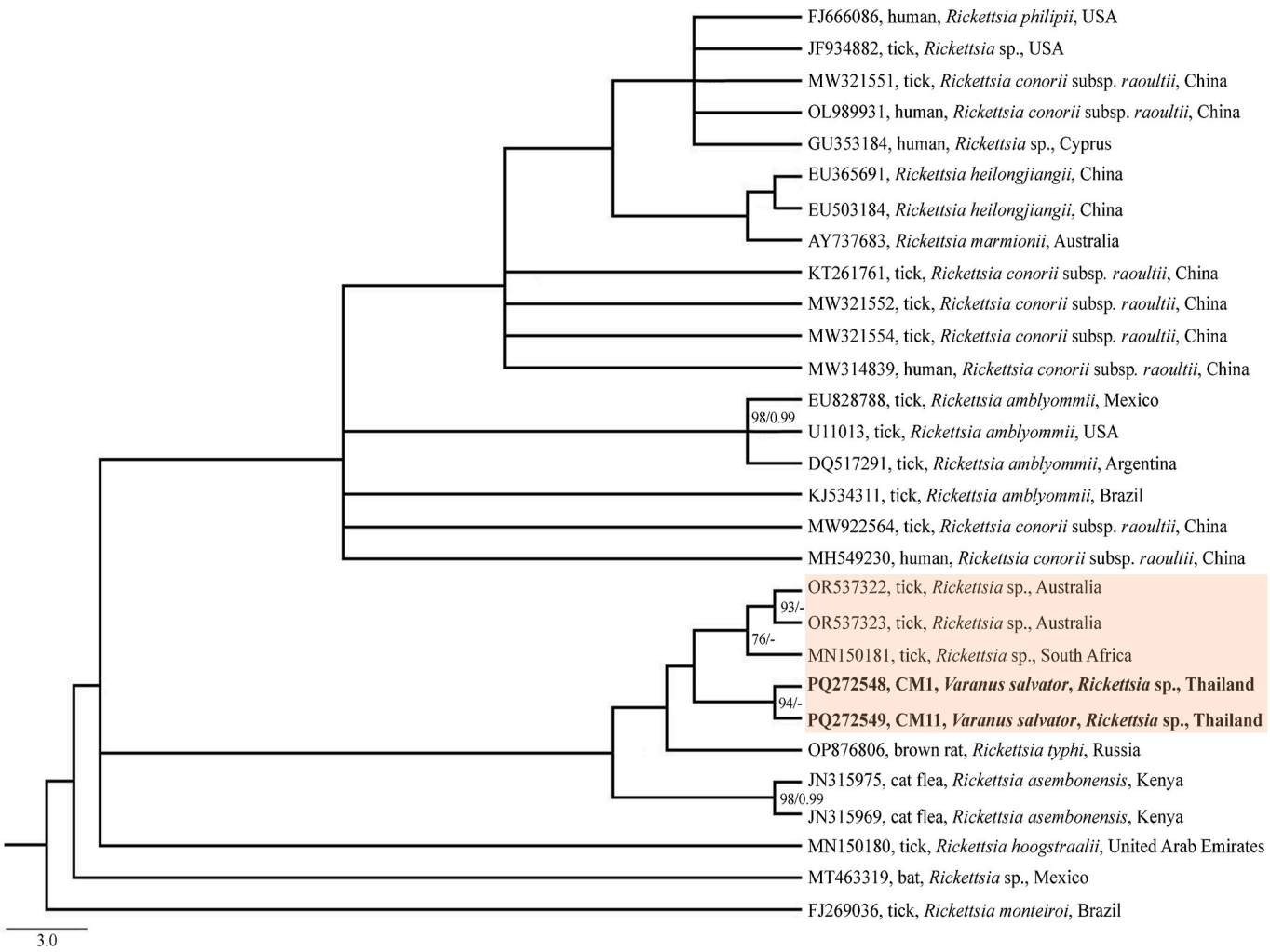

Fig. 4. Phylogenetic tree reconstructed by maximum likelihood (ML) and Bayesian inference (BI) analyses using 17 kDa sequences for *Rickettsia* spp. The ML bootstrap and BI posterior probability values are given at the nodes as ML/BI; only values > 70% (ML) and 0.95 (BI) are shown. The scale-bar indicates the number of substitutions per site. The newly generated sequences are indicated in bold. Note that the tree is unrooted.

revealed that the three new *Anaplasma* sp. sequences clustered together with a sequence of *Anaplasma* sp. (GenBank: LC428379) obtained from *Amblyomma varanense* ticks collected from *V. salvator* in Indonesia (Fig. 6).

The phylogeny based on *flaB* gene of *Borrelia* spp. showed that *Borrelia* sp. from *V. salvator* formed a monophyletic group, clustering with several *Borrelia* sp. haplotypes within the reptile-associated (REP) clade of *Borrelia* (Fig. 7).

3.4. Haplotype diversity

Nucleotide diversity (π), haplotype diversity (h), number of haplotypes, and the mean number of nucleotide differences (k) of the sequences obtained in the present study and GenBank sequences were analyzed (Table 3). The haplotype network based on 18S rDNA sequences of *Hepatozoon* spp. comprised 26 different haplotypes (Fig. 8). *Hepatozoon* sp. isolated from *V. salvator* in Thailand represented Haplotype 1 shared with *H. ophisauri* isolated from *Pseudopus apodus* in Iran and *Hepatozoon* sp. isolated from *Elaphe carinata* in China. In addition, the rest of haplotypes has also been detected in other hosts and other countries worldwide including *Trachylepis cristinae* in Yemen, *Canis lupus familiaris* in Israel, *Vulpes rueppellii* in Morocco, *Canis mesomelas* and *Lycaon pictus* in South Africa, *Echis coloratus* in Saudi Arabia, *Lamprophis fuliginosus* in Ghana, *Abrothix olivaceus* in Chile, *Caiman crocodilus* in Brazil, *Rattus rattus* in Fatu Hiva, *Sciurus carolinensis* in Canada, *Tarentola annularis* in Egypt, *Sciurus vulgaris* in the Czech Republic, *Cuora galbinifrons* in Vietnam, *Podarcis muralis* in Spain, Asiatic lion in India, *Furcifer* sp. in Madagascar. Moreover, *Hepatozoon* spp. from canine and rodent hosts formed two groups, Haplotypes 8–11 and Haplotypes 14–17 and 19, respectively, as illustrated in Fig. 8.

The haplotype network for *Rickettsia* spp. *gltA* gene shown in Fig. 9 comprised 24 haplotypes from 16 countries including Thailand, Costa Rica, Pakistan, Brazil, Colombia, North Macedonia, Australia, Turkey, South Korea, Portugal, China, Germany, Spain, Russia, Slovakia and Vietnam. The sequences of *Rickettsia* sp. isolated from *V. salvator* in Thailand represented Haplotype 1 and closely related to uncultured *Rickettsia* sp. isolated from *Homo sapiens* in Colombia and *R. sibirica* isolated from *Homo sapiens* in North Macedonia (Haplotype 5), *R. conorii*

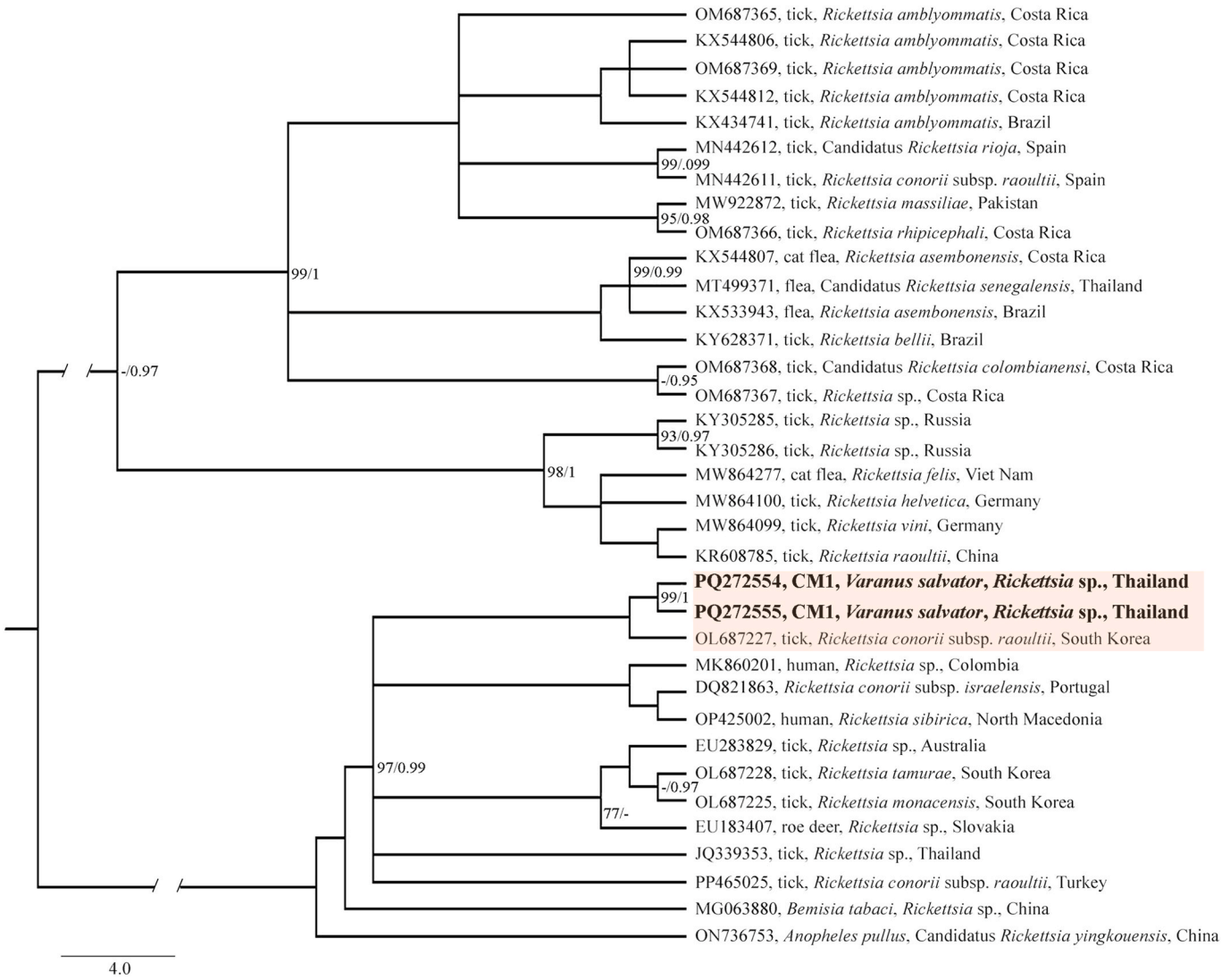

Fig. 5. Phylogenetic tree reconstructed by maximum likelihood (ML) and Bayesian inference (BI) analyses using *gltA* sequences for *Rickettsia* spp. The ML bootstrap and BI posterior probability values are given at the nodes as ML/BI; only values > 70% (ML) and 0.95 (BI) are shown. The scale-bar indicates the number of substitutions per site. The newly generated sequences are indicated in bold. Note that the tree is unrooted.

raoultii isolated from *Amblyomma helvolum* fed on *V. salvator* from Thailand and *Dermacentor marginatus* from Turkey (Haplotype 7) and *Haemaphysalis flava* from South Korea (Haplotype 10), and *R. conorii israelensis* from Portugal (unknown host) (Haplotype 11) (Fig. 9).

The haplotype network for *Anaplasma* spp. *groEL* gene comprising 22 haplotypes from 12 countries including Thailand, Indonesia, USA, Somalia, Italy, Tunisia, China, Portugal, Germany, Canada, Uganda and Cyprus (Fig. 10) demonstrated that the new sequences from *V. salvator* represented two haplotypes: Haplotype 1 shared with *Anaplasma* sp. from *Amblyomma varanense* ticks that fed on *V. salvator* from Indonesia and Haplotype 2 (Fig. 10).

The haplotype network for *Borrelia* spp. *flaB* gene comprised 22 haplotypes from 12 countries including Thailand, Spain, USA, Panama, Colombia, Iran, Brazil, Mali, Japan, Indonesia, Turkey and Poland (Fig. 11). Interestingly, the result revealed two groups of *Borrelia* spp., reptile-associated *Borrelia* spp. (REP) and relapsing fever *Borrelia* spp. (RF). The network revealed a grouping of the four *Borrelia* sp. haplotypes (Haplotypes 1–4) in *V. salvator* studied here, associated with a group of *Borrelia* sp. haplotypes in ticks from water monitors previously reported from Thailand (Haplotypes 5–7) and Indonesia (Haplotypes 21 and 22) within the REP group comprising isolates from other countries including Indonesia, Brazil, USA, Japan, Turkey and Poland (Fig. 11).

4. Discussion

To our knowledge, this is the first study investigating bacterial and parasitic pathogens affecting *V. salvator* at the Khao-zon Wildlife Breeding Station, Ratchaburi Province, Thailand. Our findings offer valuable insights into the distribution and genetic diversity of *Hepatozoon* sp., *Anaplasma* sp., *Rickettsia* sp., and *Borrelia* sp. in Thailand. Additionally, molecular analysis based on 16S rDNA confirmed the presence of *Burkholderia* sp. The identification of bacterial and parasitic

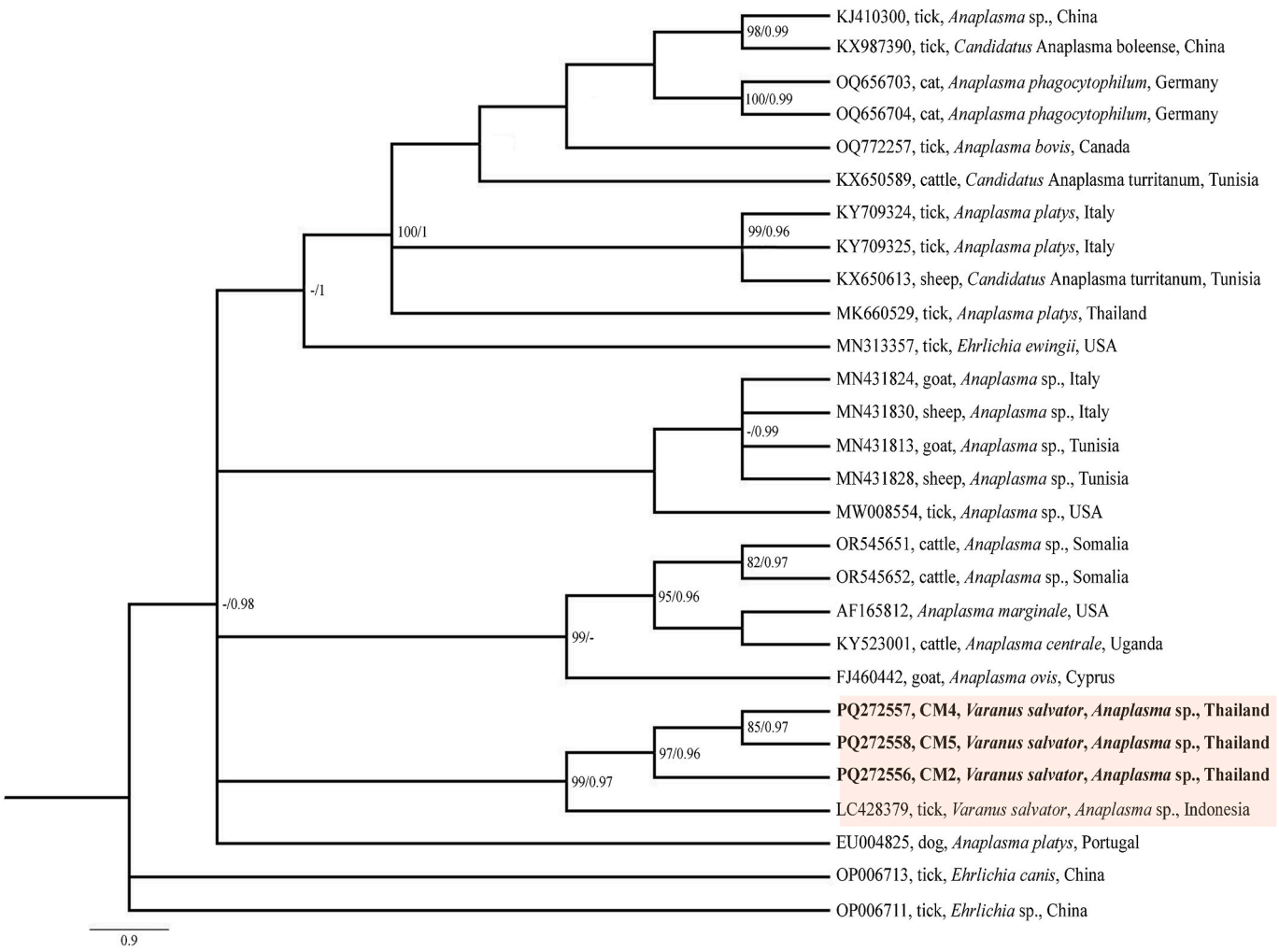

Fig. 6. Phylogenetic tree reconstructed by maximum likelihood (ML) and Bayesian inference (BI) analyses using *groEL* sequences for *Anaplasma* spp. The ML bootstrap and BI posterior probability values are given at the nodes as ML/BI; only values > 70% (ML) and 0.95 (BI) are shown. The scale-bar indicates the number of substitutions per site. The newly generated sequences are indicated in bold. *Ehrlichia canis* and *Ehrlichia* sp. were used as outgroups.

pathogens in water monitors from this region holds ecological importance, as these pathogens can significantly impact the health of water monitors, other wildlife species, and the overall ecosystem.

In this study, we identified potential pathogenic microorganisms within the collected blood samples. In particular, our analysis confirmed the presence of *Hepatozoon* sp. Within the haemogregarine genera, *Hepatozoon* is commonly reported to infect reptiles and other vertebrates, including amphibians and mammals (Telford, 2009). *Hepatozoon* spp. may be transmitted through a wide range of arthropod vectors such as flies, mosquitoes, ticks, fleas, lice, and mites (Smith, 1996; Thomas et al., 2024). Based on microscopic examination of cells parasitized by *Hepatozoon* sp. gamonts, the results were verified in two samples. The presence of *Hepatozoon* sp. has already been reported in *V. salvator* in Thailand (Salakij et al., 2014; Thanasak et al., 2022; Junsiri et al., 2024) and in several species of reptiles sampled in Asia (Han et al., 2015; Sumrandee et al., 2015). The average size of *Hepatozoon* sp. gametocytes (19.0 × 4.45 μm) was similar to that of the previously described *Hepatozoon* sp. in *V. salvator* (Thanasak et al., 2022). However, the morphology of the vast majority of haemogregarines is rather similar, preventing easy differential diagnosis. Unfortunately, the morphological and biological features of *Hepatozoon* sp. detected in *V. salvator* are still lacking for diagnosis. Despite morphological comparisons in the former publications of various authors using their own approaches, methods, and optic systems, a degree of uncertainty is always introduced. Over the past decade several phylogenetic studies using 18S rDNA sequences, have provided useful insight into the evolutionary relationships of *Hepatozoon* parasites, as well as their ability to distinguish between species (Perkins and Keller, 2001). However, because the 18S rDNA gene is a relatively conserved marker, certain nodes remain unresolved (Cook et al., 2016). Furthermore, molecular confirmation based on *Hepatozoon* 18S rDNA revealed high similarity with *H. ophisauri* (GenBank: MN723845) detected in *Pseudopus apodus* from Iran. According to the phylogenetic and haplotype analyses, the *Hepatozoon* sp. sequences generated here were related to *Hepatozoon* sp. described from reptiles (*Elaphe carinata*, the king rat snake) and *H. ophisauri* from *Pseudopus apodus*, Iran. Therefore, our results revealed that a novel *Hepatozoon* sp. closely related to *H. ophisauri* was discovered in *V. salvator* in Thailand. This finding differed from our previous report that *Hepatozoon* sp. in *V. salvator* shared 99% identity with *H. caimani* (Junsiri et al., 2024). This finding suggests that the habitats of freshwater wetlands and urban

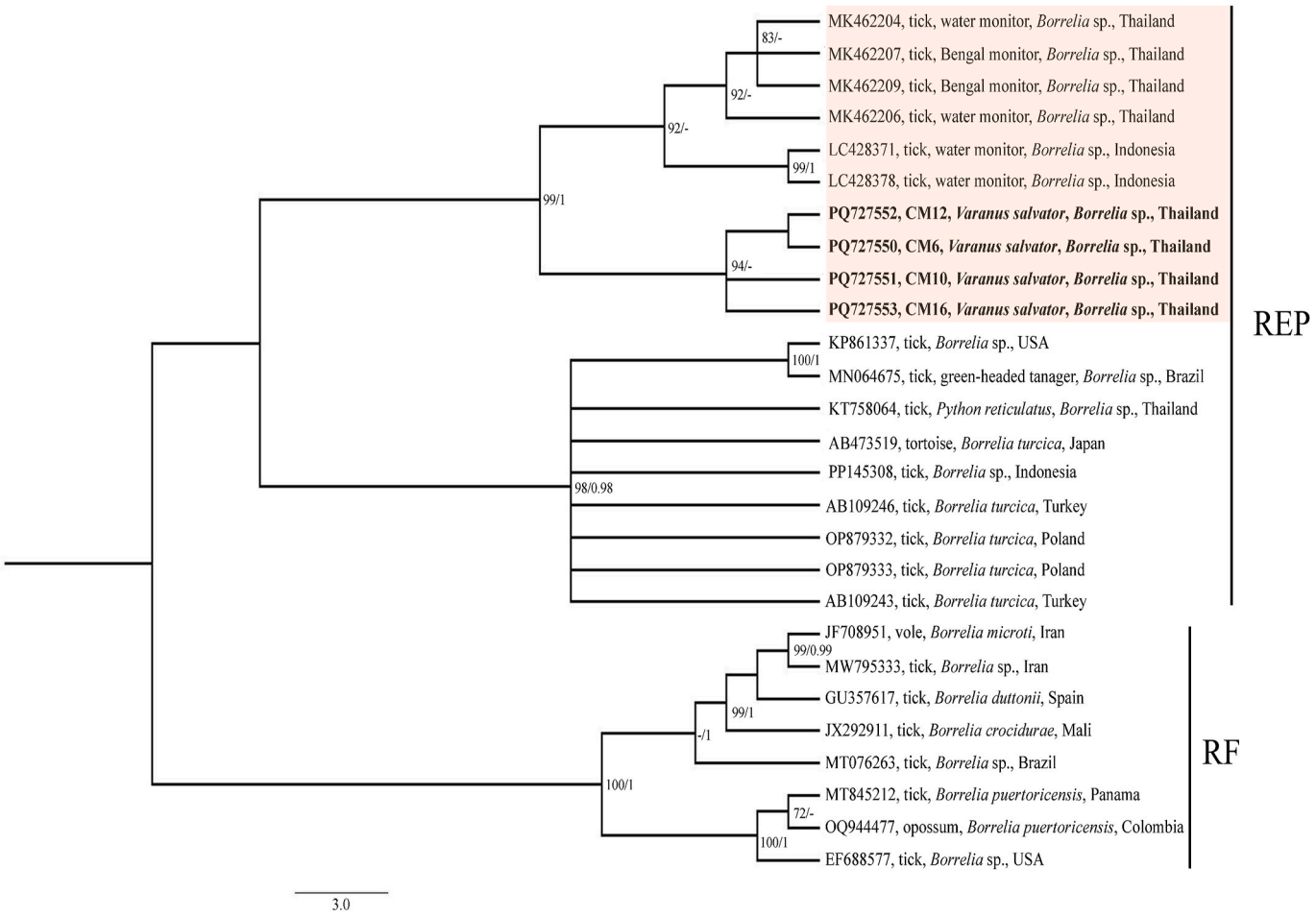

Fig. 7. Phylogenetic tree reconstructed by maximum likelihood (ML) and Bayesian inference (BI) analyses using the *flaB* sequences for *Borrelia* spp. The ML bootstrap and BI posterior probability values are given at the nodes as ML/BI; only values > 70% (ML) and 0.95 (BI) are shown. The scale-bar indicates the number of substitutions per site. The newly generated sequences are indicated in bold. *Abbreviations*: REP, reptile-associated *Borrelia* spp.; RF, relapsing fever *Borrelia* spp. The RF group was used as the outgroup.

Table 3
Polymorphism and genetic diversity of *Hepatozoon* sp., *Anaplasma* sp., *Rickettsia* sp. and *Borrelia* sp. detected in *Varanus salvator*.

Gene	Size (bp)	*n*	VS	GC%	N_{hap}	*h* (mean ± SD)	π (mean ± SD)	*k*
18S rRNA *Hepatozoon* sp.	1826	34	100	40.1	26	0.948 ± 0.031	0.0148 ± 0.0024	18.045
groEL Anaplasma sp.	528	28	118	47.2	22	0.981 ± 0.015	0.4271 ± 0.0260	54.664
gltA Rickettsia sp.	569	35	114	34.6	24	0.978 ± 0.011	0.4220 ± 0.0204	51.916
flaB Borrelia sp.	882	27	96	45.1	22	0.983 ± 0.015	0.1297 ± 0.0058	36.841

Abbreviations: *n*, number of analyzed sequences; VS, number of variable sites; GC, G×C content; N_{hap}, number of haplotypes; *h*, haplotype diversity; π, nucleotide diversity (per site); *k*, mean no. of nucleotide differences; SD, standard deviation.

waterways of *V. salvator* may have contributed to parasite divergence (Cota et al., 2008, 2009). *Hepatozoon ophisauri* is known to infect wild animals, including rodents, birds, reptiles, and amphibians (Perles et al., 2019; Perison et al., 2022). For example, a mixed infection of two *Hepatozoon* sp. was recorded in African leopards (van As et al., 2020). In this case, confirmation of the presence of *Hepatozoon* sp. in *V. salvator* is still needed, as information about this parasite in these reptiles is limited.

Rickettsiosis is a vector-borne zoonotic disease, and many rickettsiae belong to the spotted fever group (SFG) of *Rickettsia* spp. (Parola et al., 2005). The presence of *Rickettsia* sp. in *V. salvator* warrants attention. To date, phylogenetic analysis based on 16S rRNA gene sequences has frequently been used (Raoult and Roux, 1997). A multigenic approach, combining several genes, including those encoding *gltA* and 17 kDa, was suggested to be potentially helpful and appropriate for the reconstruction of the evolutionary relationships of diverse but closely related species of *Rickettsia* (Fournier and Raoult, 2009; Aung et al., 2014). Moreover, *gltA* has frequently been used as a target for genetic diagnostics based on PCR because this approach can easily identify a number of *Rickettsia* spp.; this gene is also highly conserved

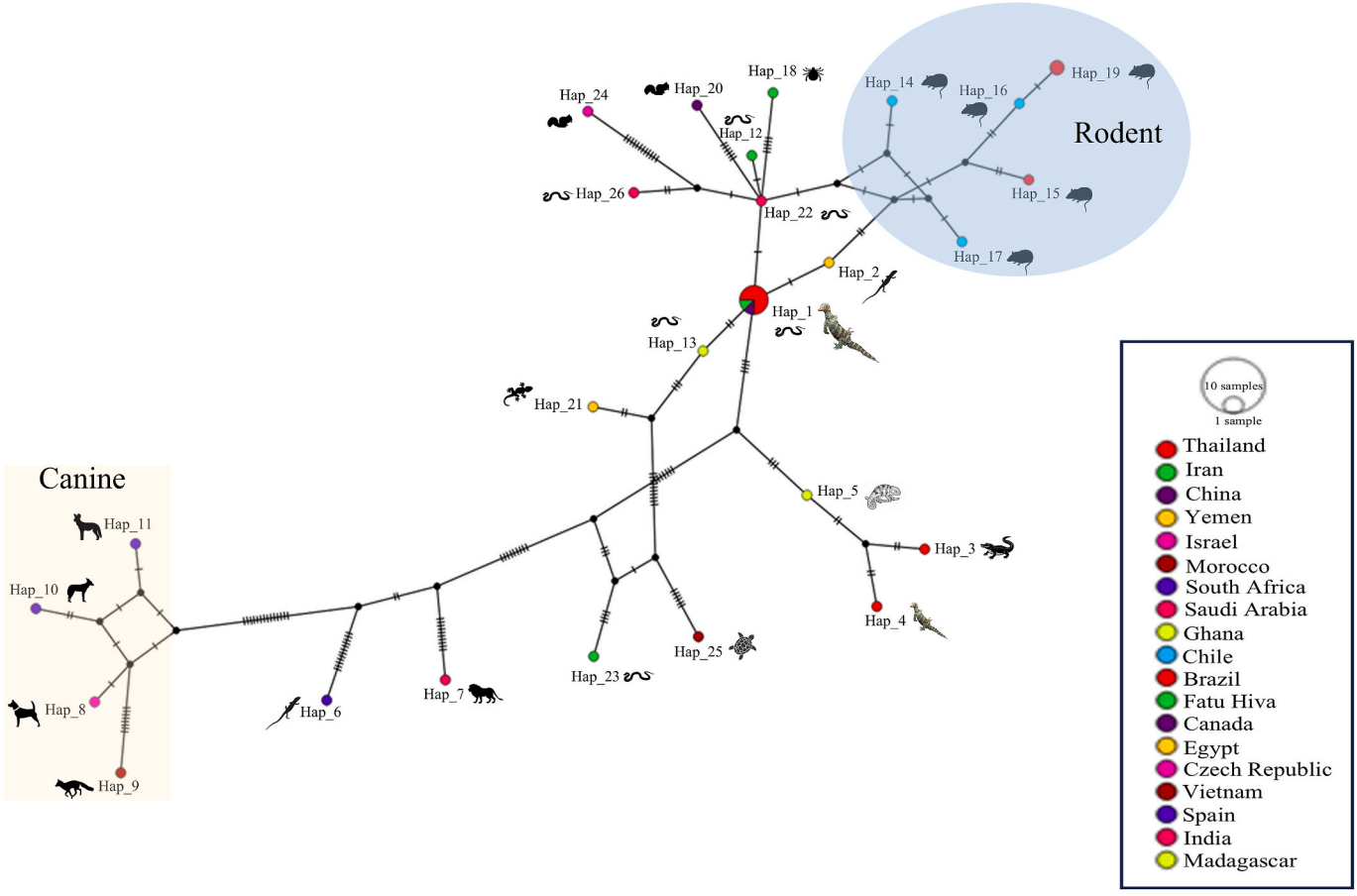

Fig. 8. TCS haplotype network for *Hepatozoon* spp. 18S rRNA gene sequences examined in Thailand and worldwide. Each circle represents a haplotype, and the area of the circle is proportional to the haplotype frequency; black circles represent unsampled haplotypes; hatch marks represent mutational steps; colors correspond to different areas of origin of the sequence.

(Chaloemthanetphong et al., 2023). Similarly, based on our results, the phylogenetic trees based on 16S rDNA, 17kDA, and *gltA* of *Rickettsia* sp. were aligned with reference to published *Rickettsia* spp. sequences. The phylogenetic relationships based on the 16S rRNA and 17 kDa genes in this study were similar, while the tree of *gltA* gene indicated that the *Rickettsia* sp. infecting *V. salvator* is closely evolutionarily related to *R. conorii raoultii* (GenBank: OL687227). These results agree with the results of previous studies (Doornbos et al., 2013). Therefore, *Rickettsia* sp., closely related to *R. conorii raoultii*, was discovered in *V. salvator* in Thailand. *Rickettsia conorii raoultii* has been detected in different tick species collected from reptiles in different countries including Thailand, India and Malaysia (Doornbos et al., 2013; Kho et al., 2015; Nallan et al., 2023), but the pathogenicity of this species is still unclear. However, *R. raoultii* is pathogenic to humans, with cases of spotted fever and tick-borne lymphadenopathy described in previous reports (Switaj et al., 2012). Furthermore, the study of the transmission and role of vectors, including ticks, is highly warranted. In addition, the potential geographical and host distribution, characterization and culture of *Rickettsia* sp. found in *V. salvator* should be investigated to determine whether this bacterium is pathogenic or symbiotic.

Species of *Anaplasma* can cause anaplasmosis, another tick-borne disease impacting both animal and human populations (Pascoe et al., 2019). Through molecular analysis, we ascertained the presence of *Anaplasma* sp. However, the bacteria revealed in our study did not align with classified species. Additionally, the geographical diversity of our samples may have contributed to the uniqueness of the identified sequences. To date, the *groEL* gene has become more commonly used for inferring eubacterial phylogenies and can be useful as a marker to clarify evolutionary relationships among *Anaplasma* spp. (Chisu et al., 2018; Karlin and Brocchieri, 2000) where 16S rDNA sequences are highly conserved (Portillo et al., 2011). In this study, the phylogenetic relationships based on the 16S rRNA and *groEL* genes were similar, with both trees indicating that *Anaplasma* sp. infecting *V. salvator* is closely related to *Anaplasma* sp. obtained from ticks, which is similar to that previously reported by Supriyono et al. (2019). These *Anaplasma* spp. are detected worldwide because of the high similarity of 16S rDNA sequences (Portillo et al., 2011). Because ticks harbor the same bacterial endosymbiont regardless of their geographical origin, these bacteria may be endosymbionts that are distributed worldwide (Niebylski et al., 1997). Therefore, the bacteria may infect lizards and be transmitted by ticks. Recently, the presence of *Anaplasma bovis* and *Anaplasma phagocytophilum* in monitor lizards (*V. salvator* and *V. bengalensis*) has been reported in Malaysia (Koh et al., 2018).

Species of *Borrelia* are known as pathogens of arthropod-borne diseases of public health concern. The newly generated *Borrelia* sp. sequences belong to the group referred to as reptile-associated (REP)

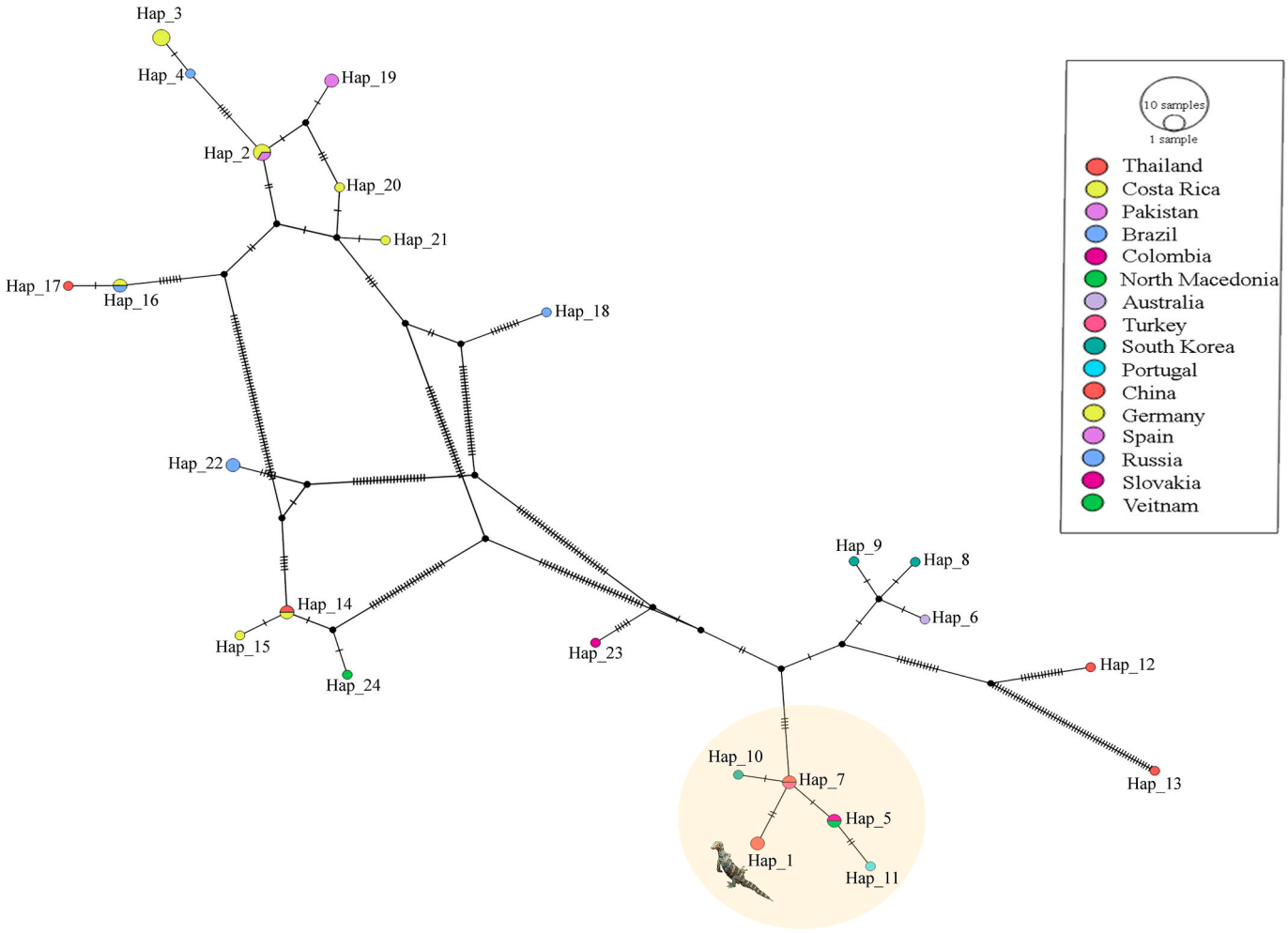

Fig. 9. TCS haplotype network for *Rickettsia* spp. *gltA* gene sequences examined in Thailand and worldwide. Each circle represents a haplotype, and the area of the circle is proportional to the haplotype frequency; black circles represent unsampled haplotypes; hatch marks represent mutational steps; colors correspond to different areas of origin of the sequence.

borreliae (Takano et al., 2011). Reports on REP *Borrelia* spp. associated with *Varanus* spp. (or varanid) hosts have already been documented in Tanzania, Australia, Indonesia, Pakistan, and Thailand (Takano et al., 2011; Panetta et al., 2017; Supriyono et al., 2019; Kaenkan et al., 2020; Khan et al., 2022; Gofton et al., 2023). To date, the *flaB* gene of *Borrelia* spp. is a useful marker for phylogenetic studies and has adequate diversity within closely related species of *Borrelia* (Zakham et al., 2021). The *flaB* gene has also been widely used for the detection of *Borrelia* spp. in tick and reptile samples (Trinachartvanit et al., 2016; Kaenkan et al., 2020). The phylogenetic analysis of *flaB* sequences indicated that varanid-associated *Borrelia* formed a monophyletic clade within the REP *Borrelia* spp. lineage. This finding is similar to that previously reported on the presence of *Borrelia* DNA from *Amblyomma varanense* ticks on *V. salvator* in Indonesia (Supriyono et al., 2019). However, their existence in these areas could be considered a risk factor in the future. Finally, the presence of *Burkholderia* sp. based on 16S rDNA was determined. The sequence of *Burkholderia* sp. was 99.88% similar to that of uncultured *Burkholderia* sp. from China, the USA, and Mexico. In reptiles, isolation of *B. pseudomallei* has been anecdotally documented in crocodiles (Sprague and Neubauer, 2004), and pet green iguanas in Belgium (Hellebuyck et al., 2018). *Burkholderia* sp., especially *B. pseudomallei*, is endemic throughout Southeast Asia and increased awareness and diagnostic capabilities in recent years have greatly expanded the known distribution of this bacterium (Dance, 2015).

5. Conclusions

This study provides valuable data on bacterial and parasitic infections in *V. salvator* at the Khao-zon Wildlife Breeding Station, Ratchaburi Province, Thailand. The results suggest that *V. salvator* may serve as a reservoir host and carrier for many disease agents such as *Hepatozoon* spp., *Rickettsia* spp., *Anaplasma* spp. and *Borrelia* spp. in this region. Identification of these pathogenic microorganisms in *V. salvator* has multiple implications. First, the presence of these pathogens highlights the potential risks to both animal and human health. In addition, *Anaplasma* spp., *Rickettsia* spp. and *Borrelia* spp. are linked to diseases that can range in severity in humans. Therefore, knowledge of the diversity of these pathogens in *V. salvator* populations is a pivotal component of public health preparedness. Identification of pathogenic microorganisms underscores the importance of water monitor

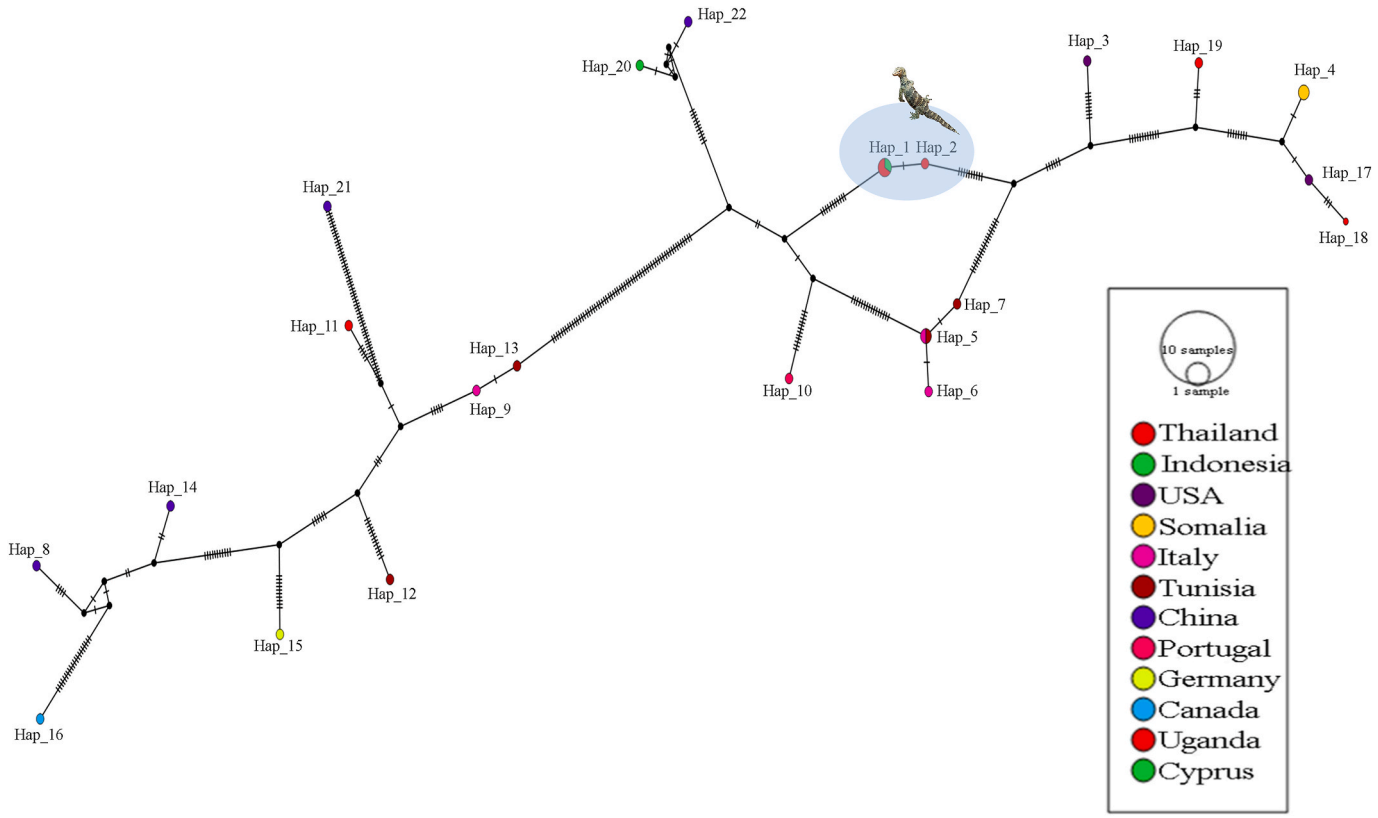

Fig. 10. TCS haplotype network for *Anaplasma* spp. *groEL* gene sequences examined in Thailand and worldwide. Each circle represents a haplotype, and the area of the circle is proportional to the haplotype frequency; black circles represent unsampled haplotypes; hatch marks represent mutational steps; colors correspond to different areas of origin of the sequence.

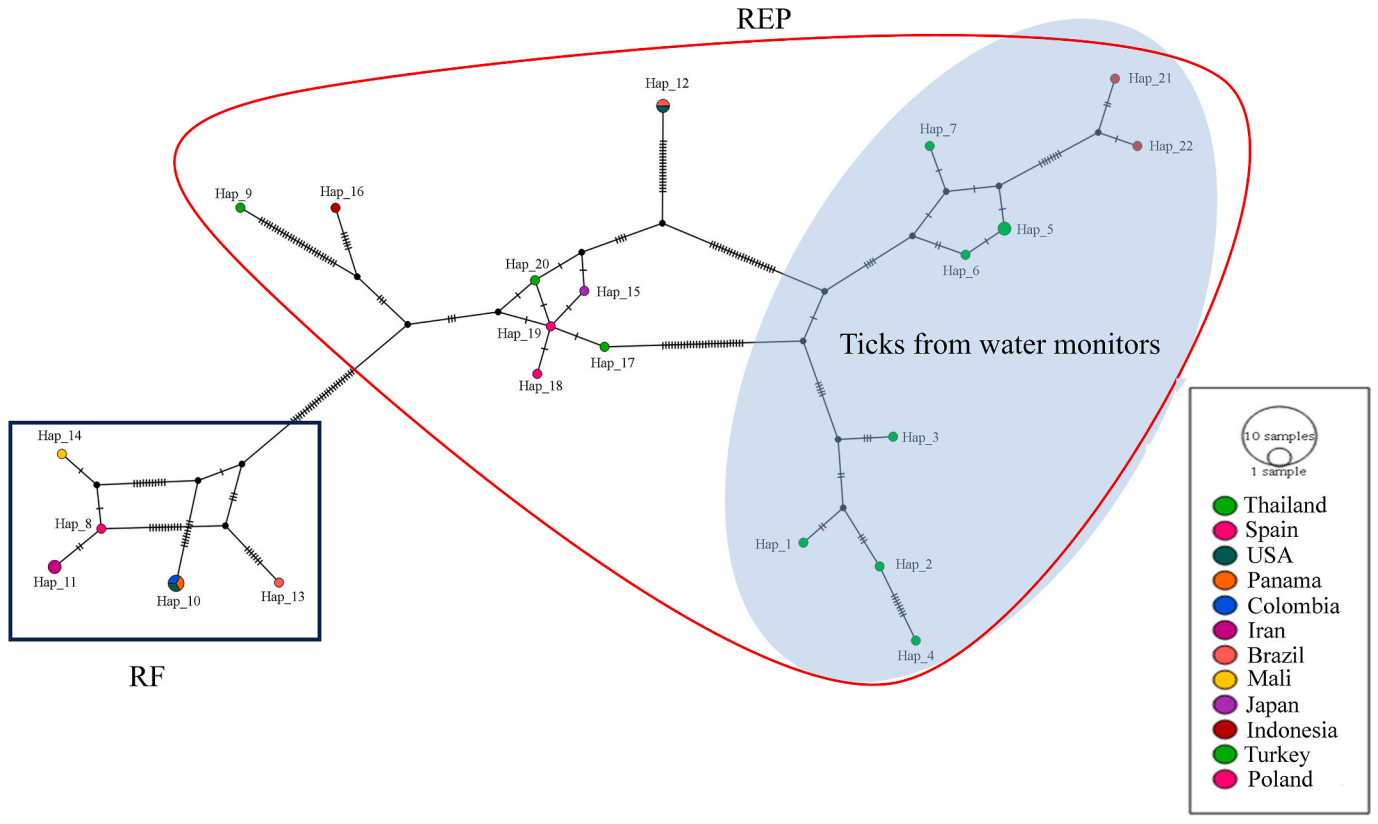

Fig. 11. TCS haplotype network for *Borrelia* spp. *flaB* gene sequences examined in Thailand and worldwide. Each circle represents a haplotype, and the area of the circle is proportional to the haplotype frequency; black circles represent unsampled haplotypes; hatch marks represent mutational steps; colors correspond to different areas of origin of the sequence. *Abbreviations*: REP, reptile-associated *Borrelia* spp.; RF, relapsing fever *Borrelia* spp.

surveillance for disease assessment.

CRediT authorship contribution statement

Witchuta Junsiri: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. **Jitkamol Thanasak:** Investigation, Resources, Writing - review & editing. **Tawewan Issarankura Na Ayudhaya:** Investigation, Resources, Writing - review & editing. **Somjit Chaiwattanarungruengpaisan:** Investigation, Resources, Writing - review & editing. **Piyanan Taweethavonsawat:** Supervision, Visualization, Conceptualization, Writing - review & editing.

Ethical approval

The Asian water monitors were handled in accordance with good animal practices required by the Animal Ethics Procedures of the Faculty of Veterinary Science-Animal Care and Use Committee (FVS-MU-IACUC), Mahidol University, Thailand (Protocol No. MUVS-2024-06-39) and the Biosafety Committee of Chulalongkorn University, Faculty of Veterinary Science (Protocol No. IBC 2231037).

Funding

This research project is funded by the Thailand Science Research and Innovation Fund, Chulalongkorn University (FOODF68310009).

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the staff of Parasitology Unit, Faculty of Veterinary Science and the Second Century Fund (C2F), Chulalongkorn University and the staff at Khao-zon Wildlife Breeding Station, the Department of National Park Wildlife and Plant Conservation.

Data availability

All data generated or analyzed during this study are included in this published article. The newly generated sequences were submitted to the GenBank database under the accession numbers PP767327-PP767332 (18S rRNA gene of *Hepatozoon* sp.), PP767754-PP767755 (16S rRNA gene of *Rickettsia* sp.), PQ272554-PQ272555 (*gltA* gene of *Rickettsia* sp.), PQ272548-PQ272549 (17 kDa gene of *Rickettsia* sp.), PQ272550-PQ272553 (*flaB* gene of *Borrelia* sp.), PP767320-PP767321 (16S rRNA gene of *Anaplasma* sp.), PQ272556-PQ272558 (*groEL* gene of *Anaplasma* sp.), PP767324 (16S rRNA gene of *Burkholderia* sp.).

References

Aung, A.K., Spelman, D.W., Murray, R.J., Graves, S., 2014. Rickettsial infections in Southeast Asia: Implications for local populace and febrile returned travelers. Am. J. Trop. Med. Hyg. 91, 451.

Böhme, W., 2003. Checklist of the living monitor lizards of the world (family Varanidae). Zool. Verhandel. 34, 3–43.

Chaloemthanetphong, A., Ahantarig, A., Apanaskevich, D.A., Hirunkanokpun, S., Baimai, V., Trinachartvanit, W., 2023. A novel *Rickettsia*, Candidatus Rickettsia takensis, and the first record of Candidatus *R*ickettsia laoensis in *Dermacentor* from northwestern Thailand. Sci. Rep. 13, 10044.

Chisu, V., Zobba, R., Lecis, R., Sotgiu, F., Masala, G., Foxi, C., et al., 2018. *GroEL* typing and phylogeny of *Anaplasma* species in ticks from domestic and wild vertebrates. Ticks Tick Borne Dis. 9, 31–36.

Clement, M., Snell, Q., Walker, P., Posada, D., Crandall, K., 2002. TCS: Estimating gene genealogies. In: Proceedings 16th Parallel and Distributed Processing Symposium. https://doi.org/10.1109/IPDPS.2002.1016585.

Cook, C.A., Netherlands, E.C., Smit, N.J., 2016. Redescription, molecular characterisation and taxonomic re-evaluation of a unique African monitor lizard haemogregarine *Karyolysus paradoxa* (Dias, 1954) n. comb. (Karyolysidae). Parasites Vectors 9, 347.

Cota, M., 2009. Study and conservation of varanids of Thailand: Past achievements and future challenges. J. Wildl. Thailand 16, 11–12.

Cota, M., Chan-Ard, T., Mekchai, S., Laoteaw, S., 2008. Geographical distribution, instinctive feeding behavior and report of nocturnal activity of *Varanus dumerilii* in Thailand. Biawak 2, 152–158. https://varanidae.org/Vol2_No4_Cota_et_al.pdf.

Dance, D.A.B., 2015. Melioidosis in Puerto Rico: The iceberg slowly emerges. Clin. Infect. Dis. 60, 251–253.

Dawson, J.E., Stallknecht, D.E., Howerth, E.W., Warner, C., Biggie, K., Davidson, W.R., et al., 1994. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to infection with *Ehrlichia chaffeensis*, the etiologic agent of human ehrlichiosis. J. Clin. Microbiol. 32, 2725–2728.

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, 217–220.

Felsenstein, J., 1985. Phylogenies and the comparative method. Am. Nat. 125, 1–15.

Fournier, P.E., Raoult, D., 2009. Current knowledge on phylogeny and taxonomy of *Rickettsia* spp. Ann. N. Y. Acad. Sci. 1166, 1–11.

Gofton, A.W., Popa-Baez, A., Takano, A., Soennichsen, K., Michie, M., Short, M., et al., 2023. Characterisation and comparative genomics of three new *Varanus*-associated *Borrelia* spp. from Indonesia and Australia. Parasites Vectors 16, 317.

Hall, T.A., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Han, H., Wu, Y., Dong, H., Zhu, S., Li, L., Zhao, Q., et al., 2015. First report of *Hepatozoon* (Apicomplexa: Adeleorina) from king ratsnakes (*Elaphe carinata*) in Shanghai, with description of a new species. Acta Parasitol. 60, 266–274.

Hellebuyck, T., Wattiau, P., Boyen, F., Moeremans, I., Roosens, N.H., Vanneste, K., et al., 2018. Isolation of *Burkholderia pseudomallei* from a pet green iguana, Belgium. Emerg. Infect. Dis. 24, 2331–2333.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Ishikura, M., Ando, S., Shinagawa, Y., Matsuura, K., Hasegawa, S., Nakayama, T., et al., 2003. Phylogenetic analysis of spotted fever group rickettsiae based on gltA, 17-kDa, and rOmpA genes amplified by nested PCR from ticks in Japan. Microbiol. Immunol. 47, 823–832.

Junsiri, W., Kamkong, P., Taweethavonsawat, P., 2024. First molecular detection and genetic diversity of *Hepatozoon* sp. (Apicomplexa) and *Brugia* sp. (Nematoda) in a crocodile monitor in Nakhon Pathom, Thailand. Sci. Rep. 14, 3526.

Kaenkan, W., Nooma, W., Chelong, I.A., Baimai, V., Trinachartvanit, W., Ahantarig, A., 2020. Reptile-associated *Borrelia* spp. in *Amblyomma* ticks, Thailand. Ticks Tick Borne Dis. 11, 101315.

Karlin, S., Brocchieri, L., 2000. Heat shock protein 60 sequence comparisons: Duplications, lateral transfer, and mitochondrial evolution. Proc. Natl. Acad. Sci. USA 97, 11348–11353.

Khan, M., Islam, N., Khan, A., Islam, Z.U., Muñoz-Leal, S., Labruna, M.B., Ali, A., 2022. New records of *Amblyomma gervaisi* from Pakistan, with detection of a reptile-associated *Borrelia* sp. Ticks Tick Borne Dis. 13, 102047.

Kho, K.L., Koh, F.X., Tay, S.T., 2015. Molecular evidence of potential novel spotted fever group rickettsiae, *Anaplasma* and *Ehrlichia* species in *Amblyomma* ticks parasitizing wild snakes. Parasites Vectors 8, 112.

Koch, A., Ziegler, T., Böhme, W., Arida, E., Auliya, M., 2013. Pressing problems: Distribution, threats, and conservation status of the monitor lizards (Varanidae: *Varanus* spp.) of Southeast Asia and the Indo-Australian archipelago. Herpetol. Conserv. Biol. 8, 1–62.

Koh, F.X., Panchadcharam, C., Sitam, F.T., Tay, S.T., 2018. Molecular investigation of *Anaplasma* spp. in domestic and wildlife animals in Peninsular Malaysia. Vet. Parasitol. Reg. Stud. Reports 13, 141–147.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. Mega X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.

Lauprasert, K., Thirakhupt, K., 2001. Species diversity, distribution and proposed status of monitor lizards (family Varanidae) in southern Thailand. Nat. Hist. J. Chulalongkorn Univ. 1, 39–46. https://li01.tci-thaijo.org/index.php/tnh/article/view/102876/82420.pdf.

Leigh, J.W., Bryant, D., 2015. PopART: Full-feature software for haplotype network construction. Methods Ecol. Evol. 6, 1110–1116.

Librado, P., Rozas, J., 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism. Bioinformatics 25, 1451–1452.

Mahaprom, R., Duengkae, P., Chuaynkern, Y., 2015. Population density and morphometry analysis for sex determination in *Varanus salvator* from Bangkachao, Samutprakran province. Thai J. Forestry 34, 109–123.

Masuzawa, T., Fukui, T., Miyake, M., Oh, H.-B., Cho, M.-K., Chang, W.-H., et al., 1999. Determination of members of a *Borrelia afzelii*-related group isolated from *Ixodes nipponensis* in Korea as *Borrelia valaisiana*. Int. J. Syst. Bacteriol. 49, 1409–1415.

Nallan, K., Ayyavu, V., Ayyanar, E., Thirupathi, B., Gupta, B., Devaraju, P., et al., 2023. Molecular evidence of *Rickettsia conorii* subsp. *raoultii* and *Rickettsia felis* in *Haemaphysalis intermedia* ticks in Sirumalai, Eastern Ghats, Tamil Nadu, South India. Microorganisms 11, 1713.

Nei, M., Kumar, S., 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York.

Niebylski, M.L., Peacock, M.G., Fischer, E.R., Porcella, S.F., Schwan, T.G., 1997. Characterization of an endosymbiont infecting wood ticks, *Dermacentor andersoni*, as a member of the genus *Francisella*. Appl. Environ. Microbiol. 63, 3933–3940.

Panetta, J.L., Šíma, R., Calvani, N.E.D., Hajdušek, O., Chandra, S., Panuccio, J., Šlapeta, J., 2017. Reptile-associated *Borrelia* species in the goanna tick (*Bothriocroton undatum*) from Sydney, Australia. Parasites Vectors 10, 616.

Parola, P., Paddock, C.D., Raoult, D., 2005. Tick-borne rickettsioses around the world: Emerging diseases challenging old concepts. Clin. Microbiol. Rev. 18, 719–756.

Pascoe, E.L., Stephenson, N., Abigana, A., Clifford, D., Gabriel, M., Wengert, G., et al., 2019. Human seroprevalence of tick-borne *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, and *Rickettsia* species in northern California. Vector Borne Zoonotic Dis. 19, 871–878.

Perison, P.W.D., Amran, N.S., Adrus, M., Khan, F.A.A., 2022. Detection and molecular identification of blood parasites in rodents captured from urban areas of southern Sarawak, Malaysian Borneo. Vet. Med. Sci. 8, 2059–2066.

Perkins, S.L., Keller, A.K., 2001. Phylogeny of nuclear small subunit rRNA genes of hemogregarines amplified with specific primers. J. Parasitol. 87, 870–876.

Perles, L., Roque, A.L.R., D'Andrea, P.S., Lemos, E.R.S., Santos, A.F., Morales, A.C., et al., 2019. Genetic diversity of *Hepatozoon* spp. in rodents from Brazil. Sci. Rep. 9, 10122.

Portillo, A., Pérez-Martínez, L., Santibáñez, S., Santibáñez, P., Palomar, A.M., Oteo, J.A., 2011. *Anaplasma* spp. in wild mammals and *Ixodes ricinus* from the north of Spain. Vector Borne Zoonotic Dis. 11, 3–8.

Raoult, D., Roux, V., 1997. Rickettsioses as paradigms of new or emerging infectious diseases. Clin. Microbiol. Rev. 10, 694–719.

Rosenblatt, J.E., 2009. Laboratory diagnosis of infections due to blood and tissue parasites. Clin. Infect. Dis. 49, 1103–1108.

Salakij, C., Salakij, J., Prihirunkit, K., Narkkong, N., Sanyathitiseree, P., Kranjanapitukkul, K., 2014. Quantitative and qualitative morphologic, cytochemical, and ultrastructural characteristics of blood cells in captive Asian water monitors. Vet. Clin. Pathol. 43, 538–546.

Shine, R., Harlow, P.R., Boeadi, J.S.K., 1996. Commercial harvesting of giant lizards: The biology of water monitors *Varanus salvator* in southern Sumatra. Biol. Conserv. 77, 125–134.

Smith, T.G., 1996. The genus *Hepatozoon* (Apicomplexa: Adeleina). J. Parasitol. 82, 565–585.

Sprague, L.D., Neubauer, H., 2004. Melioidosis in animals: A review on epizootiology, diagnosis and clinical presentation. J. Vet. Med. B Infect. Dis. Vet. Public Health 51, 305–320.

Stromdahl, E.Y., Williamson, P.C., Kollars Jr., T.M., Evans, S.R., Barry, R.K., Vince, M.A., Dobbs, N.A., 2003. Evidence of *Borrelia lonestari* DNA in *Amblyomma americanum* (Acari: Ixodidae) removed from humans. J. Clin. Microbiol. 41, 5557–5562.

Sumrandee, C., Baimai, V., Trinachartvanit, W., Ahantarig, A., 2015. *Hepatozoon* and *Theileria* species detected in ticks collected from mammals and snakes in Thailand. Ticks Tick Borne Dis. 6, 309–315.

Supriyono, Takano, A., Kuwata, R., Shimoda, H., Hadi, U.A., Setiyono, A., et al., 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, 328–333.

Switaj, K., Chmielewski, T., Borkowski, P., Tylewska-Wierzbanowska, S., Olszynska-Krowicka, M., 2012. Spotted fever rickettsiosis caused by *Rickettsia raoultii* - case report. Przegl. Epidemiol. 66, 347–350.

Tabara, K., Arai, S., Kawabuchi, T., Itagaki, A., Ishihara, C., Satoh, H., et al., 2007. Molecular survey of *Babesia microti*, *Ehrlichia* species and *Candidatus* Neoehrlichia mikurensis in wild rodents from Shimane Prefecture, Japan. Microbiol. Immunol. 51, 359–367.

Takano, A., Fujita, H., Kadosaka, T., Konnai, S., Tajima, T., Watanabe, H., et al., 2011. Characterization of reptile-associated *Borrelia* sp. in the vector tick, *Amblyomma geoemydae*, and its association with Lyme disease and relapsing fever *Borrelia* spp. Environ. Microbiol. Rep. 3, 632–637.

Telford Jr., S.R., 2009. Haemoparasites of the Reptilia. Color Atlas and Text. CRC Press, Boca Raton, FL, USA.

Thanasak, J., Tansatit, T., Taowan, J., Hirunwiroj, N., Chitthichanonte, S., Wongmack, T., 2022. The peripheral blood mononuclear cells preparation and the hematology of *Varanus salvator*. PLoS One 17, e0269108.

Thomas, R., Santodomingo, A., Saboya-Acosta, L., Quintero-Galvis, J.F., Moreno, L., Uribe, J.E., Muñoz-Leal, S., 2024. *Hepatozoon* (Eucoccidiorida: Hepatozoidae) in wild mammals of the Americas: A systematic review. Parasites Vectors 17, 108.

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.

van As, M., Netherlands, E.C., Smit, N.J., 2020. Molecular characterization and morphological description of two new species of *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina: Hepatozoidae) infecting leukocytes of African leopards *Panthera pardus pardus* (L.). Parasites Vectors 13, 222.

Weisburg, W.G., Barns, S.M., Pelletier, D.A., Lane, D.J., 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173, 697–703.

Yean, S., Prasetyo, D.B., Marcombe, S., Hadi, U.K., Kazim, A.R., Tiawsirisup, S., et al., 2024. Challenges for ticks and tick-borne diseases research in Southeast Asia: Insight from the first international symposium in Cambodia. PLoS Negl. Trop. Dis. 18, e0012269.

Zakham, F., Jääskeläinen, A.J., Castrén, J., Sormunen, J.J., Uusitalo, R., Smura, T., et al., 2021. Molecular detection and phylogenetic analysis of *Borrelia miyamotoi* strains from ticks collected in the capital region of Finland. Ticks Tick Borne Dis. 12, 101608.