scientific reports

Check for updates

OPEN

Molecular prevalence of *Coxiella* like endosymbionts and the first record of *Coxiella burnetii* in hard ticks from Southern Thailand

Wanwipa Nooma[1], Warissara Kaenkan[1], Wachareeporn Trinachartvanit[1], Visut Baimai[1] & Arunee Ahantarig[1,2]✉

Eight hard tick species were identified among a total of 466 samples collected from vegetation in southern Thailand: *Dermacentor compactus* (*n* = 150), *D. steini* (*n* = 100), *D. auratus* (*n* = 85), *D. tricuspis* (*n* = 41), *Haemaphysalis hystricis* (*n* = 69), *H. semermis* (*n* = 3), *H. shimoga* (*n* = 2) and *Amblyomma testudinarium* (*n* = 16). In 93 ticks from these 8 species, *Coxiella* bacteria were detected via 16 S rRNA, *groEL* (60-kDa chaperone heat shock protein B) and *rpoB* (β subunit of bacterial RNA polymerase) genes. Interestingly, *Coxiella burnetii* was detected for the first time in *H. hystricis* and *D. steini* in Songkhla Province. *Coxiella*-like endosymbionts (CLEs) were also found in 84 ticks from 7 species, namely, *D. compactus*, *D. auratus*, *D. tricuspis*, *H. hystricis*, *H. semermis*, *H. shimoga* and *A. testudinarium*. Among these, CLEs associated with *D. compactus* and *H. semermis* were reported for the first time in Thailand. Phylogenetic analysis and generation of a haplotype network clearly revealed 2 distinct groups of *Coxiella* bacteria, namely, *C. burnetii* and CLEs. The nucleotide alignment of *Coxiella* 16 S rRNA revealed differences in bases at 3 positions between *C. burnetii* and CLEs. Thus, these differences could be used as liable molecular markers for discriminating these 2 groups in hard ticks.

Keywords Tick species diversity, *Coxiella* bacteria, *Coxiella burnetii*, Tick-borne diseases

The hard ticks of the family Ixodidae currently includes approximately 762 species worldwide[1]. In Southeast Asia, the tropical climate, with high rainfall and humidity, contributes to the region's rich biodiversity and makes it a unique environment for various species of ticks. More than 90 species of hard ticks have been found in this region, including 58 species in Thailand[2]. In addition to the diversity of tick species, numerous tick-borne diseases, such as babesiosis, ehrlichiosis, hepatozoonosis, Lyme borreliosis, rickettsiosis and query fever (Q fever), affecting both animals and humans have been recorded in Southeast Asia[2,3]. Ticks are a potential source of *Coxiella* spp. For example, ticks can be responsible for transmission of *Coxiella burnetii* (the causative agent of Q fever) in animals and humans[4]. *Dermacentor*, *Haemaphysalis* and *Amblyomma* ticks, which have been reported to be associated with various microorganisms, including *Coxiella* bacteria, are of particular interest[5–7].

Coxiella is a genus of obligate intracellular gram-negative bacteria, including pathogenic agents, e.g. *C. burnetii*, that cause zoonotic Q fever in humans and coxiellosis in animals, and some nonpathogenic agents known as *Coxiella*-like endosymbionts (CLEs)[7]. *Coxiella burnetii* infection has been reported in numerous genera of ticks, including *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes* and *Rhipicephalus*[8–10]. Among mammals, ruminants such as cattle, goats and sheep are the main reservoirs of *C. burnetii*, serving as primary sources for human infection[11]. In animals, *C. burnetii* infection is often asymptomatic but can cause significant reproductive problems, such as infertility, abortion and stillbirth[4,12,13]. In Thailand, *C. burnetii* infections in animals and humans have been reported since 1966[14]. Several studies have reported the seroprevalence of antibodies against *C. burnetii* in animals (including cattle, goats and sheep) and in humans who have been exposed to these animals (including farmers and others who work with livestock, zoo animals and wildlife)[15,16]. Only one report described the presence of *C. burnetii* in 2 *Rhipicephalus microplus* ticks collected from dairy cattle in northern Thailand[17].

Coxiella-like endosymbionts have been reported in several species of soft and hard ticks[18]. In addition, CLEs are known to be essential for tick survival because they provide nutrients lacking from blood meals and reproductive benefits[19,20]. Some reports of CLE-harbouring ticks collected from vegetation or animals have

[1]Biodiversity Research Cluster, Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand. [2]Center of Excellence for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Nakhon Pathom 73170, Thailand. ✉email: arunee.aha@mahidol.ac.th

documented CLEs in several tick genera worldwide, including *Amblyomma*, *Dermacentor*, *Haemaphysalis* and *Rhipicephalus* in Thailand[5–7,21–27].

Phylogenetically, some CLEs are closely related to *C. burnetii*, but they have different roles and functions[18]. In this context, *C. burnetii* may be misidentified as a CLE via PCR[18]. Therefore, gene sequencing targeting highly conserved genes such as 16 S rRNA is typically used to separate these 2 groups of *Coxiella* bacteria[18,28]. Fewer investigations of the prevalence of *C. burnetii* and CLEs in ticks have been conducted in Thailand than in many other countries. In particular, data related to biodiversity-rich areas, such as southern Thailand, are lacking. Most methods used to detect *Coxiella* involve gene sequencing, which targets highly conserved genes such as 16 S rRNA, *groEL* (60 kDa chaperone heat shock protein B) and *rpoB* (β subunit of bacterial RNA polymerase)[18]. The objectives of this study were to investigate the presence of *Coxiella* bacteria in diverse species of hard ticks collected from vegetation in Songkhla Province, southern Thailand based on the 16 S rRNA, *groEL* and *rpoB* genes. Additionally, phylogenetic analysis and the haplotype network of *Coxiella* bacteria (*C. burnetii* and CLE groups) are discussed.

Materials and methods

Tick collection and identification

Adult tick samples were collected (by using forceps) from the dorsal and ventral sides of leaves along trails in Hat Yai District, Songkhla Province, southern Thailand (Fig. 1), covering an area of approximately 2 square kilometres (6.94206°N, 100.24916°E), every 2 months from July 2021 to May 2022. All tick samples were kept in tubes containing 70% ethanol and stored at -20 °C for further study at Mahidol University. Each tick sample was morphologically identified according to its external characteristics listed in standard taxonomic descriptions[29–34]. Ticks were subsequently confirmed via molecular identification with PCR targeting the mitochondrial 16 S rRNA[35], cytochrome c oxidase I (*COI*)[36] and internal transcribed spacer 2 (*ITS2*)[37] genes, followed by DNA sequencing. The primers used in this study are shown in Table 1.

DNA extraction

All samples were surface sterilized by rinsing with 70% ethanol, followed by 10% sodium hypochlorite, and then washed with sterile distilled water three times for 1–2 min each to remove external contamination. Next, DNA was extracted from individual samples with the QIAamp DNA Tissue Kit (Qiagen, Germany) according to the manufacturer's protocol. The extracted DNA was assessed for quality by amplifying the tick 16 S rRNA gene and then stored at -20 °C until further use.

PCR amplification of *Coxiella* bacteria and DNA sequencing

The primer sets targeting the 16 S rRNA[38], *groEL*[39] and *rpoB*[39] genes were used for PCR amplification to detect *Coxiella* bacteria, as described in Table 1. PCR was performed with a SimpliAmp™ Thermal Cycler (Applied Biosystems, USA) under the following conditions: initial denaturation at 93 °C for 3 min; 35 cycles of denaturation at 93 °C for 30 s, annealing at 58 °C for 30 s for the 16 S rRNA gene, at 56 °C for 30 s for the

Fig. 1. Geographical location of Songkhla Province, southern Thailand, where adult tick samples were collected from vegetation. The red star represents the Hat Yai District. The map was created with QGIS 3.36.2 (https://www.qgis.org).

Organism	Target gene	PCR assay	Primer name	Primer sequence (5′–3′)	Product size (bp)	Annealing temperature (°C)	Reference
Tick spp.	16 S rRNA	Conventional PCR	16 S + 1	CTGCTCAATGATTTTTTAAATTGCTGTGG	460	55	[35]
			16 S-1	CCGGTCTGAACTCAGATCAAGT			
	COI	Conventional PCR	RON	GGAGCYCCWGATATAGCTTTCCC	463	55	[36]
			TCOIR	WGGRTGRCCAAARAATCAAAATA			
	ITS2	Conventional PCR	ITS2-F	ACATTGCGGCCTTGGGTCTT	1,200-1,600	55	[37]
			ITS2-R	TCGCCTGATCTGAGGTCGAC			
Coxiella spp.	16 S rRNA	Conventional PCR	COX-16 S rRNA (F)	GGGGAAGAAAGTCTCAAGGGTAA	532	58	[38]
			COX-16 S rRNA (R)	TGCATCGAATTAAACCACATGCT			
	groEL	Nested PCR	CoxGrF1	TTTGAAAAYATGGGCGCKCAAATGGT	655	56	[39]
			CoxGrR2	CGRTCRCCAAARCCAGGTGC			
			CoxGrF2	GAAGTGGCTTCGCRTACWTCAGACG	619		
			CoxGrFR1	CCAAARCCAGGTGCTTTYAC			
	rpoB	Nested PCR	CoxrpoBF2	GGGCGNCAYGGWAAYAAAGGSGT	607–610	56	[39]
			CoxrpoBR1	CACCRAAHCGTTGACCRCCAAATTG			
			CoxrpoBF3	TCGAAGAYATGCCYTATTTAGAAG	539–542		
			CoxrpoBR3	AGCTTTMCCACCSARGGGTTGCTG			

Table 1. Primer sets for PCR amplification.

first and second rounds for the *groEL* and *rpoB* genes and extension at 72 °C for 1 min; and a final extension at 72 °C for 5 min. The PCR products were subjected to electrophoresis on a 1% agarose gel stained with ethidium bromide and visualized under a UV transilluminator to identify the presence of target genes. The positive PCR products were subsequently excised from the agarose gel and purified with a NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, Germany) following the manufacturer's instructions. The purified PCR products were confirmed via DNA sequencing in both directions according to the Sanger method (Macrogen, South Korea).

Phylogenetic tree analysis

The nucleotide sequences were trimmed and assembled in the MEGA X program version 10.2.6[40]. The consensus sequences were subsequently compared with other DNA sequences deposited in the GenBank database from NCBI using BLASTn (The Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/BLAST) to confirm the *Coxiella* species. Multiple sequence alignment of *Coxiella* 16 S rRNA was performed with the ClustalW algorithm, as implemented in the MEGA X program, based on representative *Coxiella* sequences (we chose all *C. burnetii* and selected positive samples of CLEs from all tick species) and related *Coxiella* species. The aligned DNA sequences were then used to construct a phylogenetic tree according to the neighbour-joining (NJ) or maximum likelihood (ML) method with 1,000 bootstrap replicates in the MEGA X program version 10.2.6.

Haplotype analysis

The alignment of the 16 S rRNA sequences obtained in this work and the previously detected *Coxiella* sequences downloaded from GenBank was analysed using DnaSP v5 software[41] to determine haplotypes. A haplotype network was subsequently constructed via the TCS network method[42] implemented in PopART software[43].

Results

Tick identification

A total of 466 adult ticks were morphologically identified as belonging to 8 species in 3 genera: *Dermacentor*, *Haemaphysalis* and *Amblyomma*. These findings reflect the species diversity of hard ticks in this specific location of Songkhla Province, southern Thailand (Table 2). *Dermacentor* was the predominant genus, comprising 376 ticks, including 150 *D. compactus* (77 females, 73 males), 100 *D. steini* (51 females, 49 males), 85 *D. auratus* (46 females, 39 males) and 41 *D. tricuspis* (23 females, 18 males) individuals. The remaining 90 tick samples were composed of 69 *H. hystricis* (31 females, 38 males), 3 *H. semermis* (3 females), 2 *H. shimoga* (2 females) and 16 *A. testudinarium* (14 females, 2 males) individuals. *Dermacentor compactus* was the most common species (150/466), whereas *H. shimoga* was the least common (2/466).

To confirm the morphological species, representatives of each tick species were randomly selected for molecular identification and sequenced for the 16 S rRNA, *COI* and *ITS2* genes. These nucleotide sequences were deposited in GenBank under accession numbers MZ330742-MZ330748 and PQ213158 for 16 S rRNA, PQ811578-PQ811584 for *COI* and PQ819929-PQ819936 for *ITS2* (Table S1). BLASTn analysis of 16 S rRNA sequences (ranging from 375 to 433 bp) from all tick species compared with those available in GenBank revealed percent identities ranging from 92.64 to 100% (Table 3): *A. testudinarium* (99.77% identity with MG874022 and MZ490781 from Thailand), *D. compactus* (identical to isolates MZ005661, MZ005666 and MZ005651 from Malaysia) and *D. steini* (99.49% identity with MK296403 from Malaysia). The *D. auratus* sequence exhibited 100% identity with those from Malaysia (PP107965 and MZ005647) and Thailand (KC170746). Additionally, the *D. tricuspis* sequence shared 100% identity with *D. atrosignatus* sequences from Malaysia (MZ005642

Tick species (no.)	No. of adult tick		No. of PCR positive for *Coxiella* bacteria	
	F	M	CB	CLE
Dermacentor compactus (150)	77	73	0	7 (4 F, 3 M)
Dermacentor steini (100)	51	49	7 (2 F, 5 M)	0
Dermacentor auratus (85)	46	39	0	1 (1 F)
Dermacentor tricuspis (41)	23	18	0	22 (15 F, 7 M)
Haemaphysalis hystricis (69)	31	38	2 (1 F, 1 M)	38 (15 F, 23 M)
Haemaphysalis semermis (3)	3	-	0	2 (2 F)
Haemaphysalis shimoga (2)	2	-	0	2 (2 F)
Amblyomma testudinarium (16)	14	2	0	12 (10 F, 2 M)
Total (466)	247	219	9 (3 F, 6 M)	84 (49 F, 35 M)

Table 2. Tick species and molecular detection of *Coxiella* bacteria. *F* female, *M* male, *CB Coxiella burnetii*, *CLE Coxiella*-like endosymbiont.

Tick species	Code	16 S rRNA		
		Closely related sequence	GenBank accession no.	Percent identity
Amblyomma testudinarium	L3_25	*A. testudinarium*	MG874022, MZ490781	99.77%
Dermacentor compactus	S1_4	*D. compactus*	MZ005661, MZ005666, MZ005651	100.00%
Dermacentor steini	t7_6	*D. steini*	MK296403	99.49%
Dermacentor auratus	t1_1	*D. auratus*	PP107965, MZ005647, KC170746	100.00%
Dermacentor tricuspis	L3_28	*D. tricuspis* (*D. atrosignatus*)	MZ005642, MZ005643	100.00%
Haemaphysalis hystricis	L3_32	*H. hystricis*	LT593118, OL741743, MZ848135	99.72–100.00%
Haemaphysalis semermis	L1_28	*H. lagrangei*	MZ490789	92.64%
Haemaphysalis shimoga	S1_2	*H. shimoga*	KC170730	100.00%

Table 3. BLASTn analysis of ticks for 16 S rRNA gene.

and MZ005643). However, recent taxonomic revisions of this group of *Dermacentor* clearly revealed that *D. atrosignatus* was synonymous with *D. tricuspis*[34]. Moreover, the *H. hystricis* sequence shared 100% identity with that from Malaysia (LT593118) and 99.72% identity with those from Japan (OL741743) and China (MZ848135). The *H. shimoga* sequence was 100% identical to that of the Thai isolate KC170730. Notably, no molecular data for the 16 S rRNA and *ITS2* sequences of *H. semermis* are available in GenBank. However, the *COI* gene of *H. semermis* exhibited 100% identity with that from Malaysia (PQ288529). In addition, *H. semermis* in this study presented an identity of 92.64% (16 S rRNA gene) with *H. lagrangei* (MZ490789) from Thailand. BLASTn analysis of the sequences of the *COI* and *ITS2* genes compared with the GenBank sequences for all tick species is shown in Table S2.

Molecular detection of *Coxiella* bacteria

Using PCR targeting the 16 S rRNA gene, *Coxiella* bacteria were detected in 93 out of 466 tick samples. Interestingly, all species of ticks were positive for *Coxiella* bacteria, and the results are as follows: *D. compactus* (7), *D. steini* (7), *D. auratus* (1), *D. tricuspis* (22), *H. hystricis* (40), *H. semermis* (2), *H. shimoga* (2) and *A. testudinarium* (12) (Table 2). All tick samples positive for *Coxiella burnetii* were collected in January 2022.

DNA sequence and phylogenetic analyses

A phylogenetic tree constructed from the partial sequences of the 16 S rRNA gene clearly revealed 2 groups of *Coxiella* bacteria, namely, *C. burnetii* (9 sequences) and CLEs (21 sequences) (Fig. 2). In the first group, the 7 sequences of *C. burnetii* found in *D. steini* (L1_29, L1_32.4, L1_34, L1_35, L1_36, L1_40 and L1_47) and the 2 sequences found in *H. hystricis* (L1_7 and L11_18.2) were clustered with *C. burnetii* detected in ticks, goats and humans (Fig. 2) (DNA sequence identities = 99.60–100%). In the second group, the remaining 21 sequences exhibited variation within this cluster, with close similarity to the CLE group, with identities ranging from 96.57 to 99.80% (Table 4). The CLEs were phylogenetically clustered into various clades, comprising CLEs in *A. testudinarium*; CLEs in *D. tricuspis*, *D. compactus* and *D. auratus*; and CLEs in *H. shimoga*, *H. semermis* and *H. hystricis* (Fig. 2). *Coxiella*-like endosymbionts in *A. testudinarium* were clustered with those found in *A. testudinarium* from Thailand (KY462724 and OK161265) and *Amblyomma* sp. from Malaysia (LT009437), and they exhibited close relationships with CLEs in *A. testudinarium* from Thailand (MZ182314 and MZ182315). Interestingly, CLEs in *D. tricuspis* formed an independent clade with high bootstrap value support according to 16 S rRNA gene analysis. In contrast, CLE sequences identified in *D. compactus* and *D. auratus* were clustered with CLEs from *Rhipicephalus* ticks, including CLEs isolated from *R. sanguineus* s.l. in France (KP994843), *Rhipicephalus* sp. in Cote d'Ivoire (KP994849), *R. pusillus* in France (KP994841) and *Candidatus* Coxiella

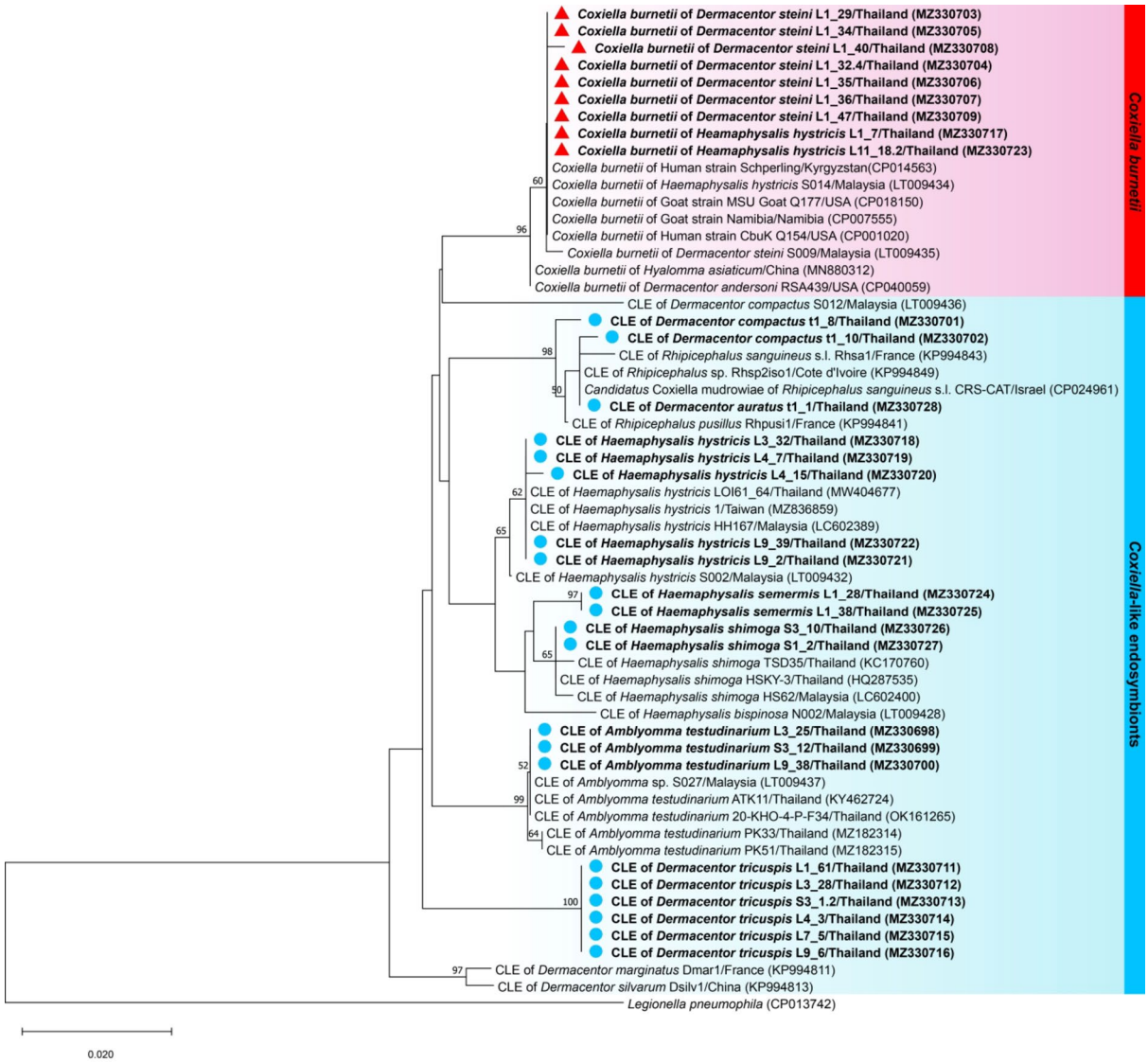

Fig. 2. Phylogenetic analysis of *Coxiella* bacteria was performed via the neighbour-joining method with the Kimura 2-parameter model based on a 472 bp alignment of the 16 S rRNA gene. The tree was generated using MEGA X software, with bootstrap support calculated from 1000 replicates. Only bootstrap values greater than 50% are shown above the branches. *Coxiella* sequences identified in ticks from this study are in bold type; red triangles denote *C. burnetii*, and blue circles denote CLEs. *Legionella pneumophila* was used as an outgroup. *CLE Coxiella*-like endosymbiont.

mudrowiae in Israel (CP024961). Additionally, the results of the 16 S rRNA gene analysis of CLEs detected in *H. shimoga* clustered with those of *H. shimoga* reported in Thailand (KC170760 and HQ287535) and Malaysia (LC602400). These CLEs were closely related to an independent clade of CLEs in *H. semermis* identified in this study. Furthermore, CLEs in *H. hystricis* based on 16 S rRNA were related to those occurring in *H. hystricis* from Thailand (MW404677), Taiwan (MZ836859) and Malaysia (LC602389). There are clearly revealed 2 groups of *Coxiella* bacteria (*C. burnetii* and CLEs) for *groEL* and *rpoB* genes compared to 16 S rRNA (Figs. S1 and S2). The accession numbers of the *Coxiella* genes (16 S rRNA, *groEL* and *rpoB*) are shown in Table S3, and BLASTn analyses of the sequences of the *groEL* and *rpoB* genes are presented in Table S4.

Sequence alignment of *C. burnetii* and CLEs

A detailed comparison of the 16 S rRNA sequences detected in this study with those in the NCBI database revealed significant differences in DNA sequences between *C. burnetii* and CLEs. The alignment included 61 selected DNA sequences: 37 from *C. burnetii* (2 from this study and 35 from the references) and 24 from CLEs (8 from this study and 16 from the references). The results revealed that *C. burnetii* and CLEs are closely related, making them very difficult to distinguish. However, 3 distinct sites differed between *C. burnetii* and CLEs, i.e., positions 235, 349, and 350 of the DNA sequence (Fig. 3). *Coxiella burnetii* had A, C and T bases at positions 235, 349, and 350, respectively, whereas CLEs had G, T and C bases, respectively (Fig. 3). These findings suggest that

Group	Tick species	Code	Sex	GenBank accession no.	Closely related sequence for 16 S rRNA gene (GenBank accession no.)	Percent identity
CB_1	*Dermacentor steini*	L1_29	M	MZ330703	*Coxiella burnetii* (CP014563, LT009434, CP018150, CP007555, CP001020)	99.60–100.00%
		L1_32.4	M	MZ330704		
		L1_34	F	MZ330705		
		L1_35	F	MZ330706		
		L1_36	M	MZ330707		
		L1_40	M	MZ330708		
		L1_47	M	MZ330709		
	Haemaphysalis hystricis	L1_7	F	MZ330717		
		L11_18.2	M	MZ330723		
CLE_1	*Amblyomma testudinarium*	L3_25	M	MZ330698	CLE of *Amblyomma* sp. isolate S027 (LT009437)	99.60–99.80%
		L9_38	F	MZ330700		
		S3_12	F	MZ330699		
CLE_2	*Dermacentor auratus*	t1_1	F	MZ330728	CLE of *R. pusillus* isolate Rhpusi1 (KP994841)	98.99%
CLE_3	*Dermacentor compactus*	t1_8	M	MZ330701	CLE of *Rhipicephalus* sp. isolate Rhsp2iso1 (KP994849)	99.40–99.60%
		t1_10	F	MZ330702		
CLE_4	*Dermacentor tricuspis*	L1_61	F	MZ330711	CLE of *O. sonrai* isolate Oson1 (KP994797)	96.57%
		L3_28	F	MZ330712		
		S3_1.2	F	MZ330713		
		L4_3	F	MZ330714		
		L7_5	F	MZ330715		
		L9_6	F	MZ330716		
CLE_5	*Haemaphysalis hystricis*	L3_32	M	MZ330718	CLE of *H. hystricis* isolate HH167 (LC602389)	99.60–99.80%
		L4_7	M	MZ330719		
		L4_15	F	MZ330720		
		L9_2	F	MZ330721		
		L9_39	M	MZ330722		
CLE_6	*Haemaphysalis semermis*	L1_28	F	MZ330724	CLE of *H. shimoga* isolate HS62 (LC602400)	98.99%
		L1_38	F	MZ330725		
CLE_7	*Haemaphysalis shimoga*	S3_10	F	MZ330726	CLE of *H. shimoga* isolate HS62 (LC602400), HSKY-3 (HQ287535)	99.77–99.80%

Table 4. BLASTn analysis of sequences of 16 S rRNA gene. *F* female, *M* male, *CB Coxiella burnetii*, *CLE Coxiella*-like endosymbiont.

nucleotide polymorphisms within the 16 S rRNA gene, specifically at positions 235, 349, and 350, could be used as reliable evidence to distinguish *C. burnetii* from CLEs associated with hard tick species in Thailand.

Haplotype analysis

A haplotype network of *Coxiella* based on partial 16 S rRNA sequences (434 bp) revealed that the haplotypes were divided into 2 main groups (*C. burnetii* and CLEs), comprising a total of 23 haplotypes. *Coxiella burnetii* was found in 4 haplotypes (Hap_1, Hap_2, Hap_12 and Hap_13), while the remaining 19 haplotypes were CLEs (Fig. 4). The network revealed CLE haplotypes in a variety of tick species, including *A. testudinarium*, *Amblyomma* sp., *D. auratus*, *D. compactus*, *D. tricuspis*, *D. marginatus*, *D. silvarum*, *H. hystricis*, *H. shimoga*, *H. semermis*, *H. bispinosa*, *R. sanguineus* s.l., *R. pusillus* and *Rhipicephalus* species.

The *C. burnetii* sequences detected in this study were grouped into 2 haplotypes (Hap_1 and Hap_2). Hap_1 included the sequences of *C. burnetii* in *D. steini* (excluding L1_40) and *H. hystricis* obtained from this study, along with other *C. burnetii* strains isolated from tick (*H. hystricis*), goats and humans. Hap_2, Hap_12 and Hap_13 were separated from Hap_1 by a single mutational event. Hap_2 and Hap_13 included one *C. burnetii* sequence in *D. steini* (L1_40) from this study and from Malaysia, respectively. Two sequences of *C. burnetii* from *D. andersoni* and *Hyalomma asiaticum* belonged to Hap_12. *Coxiella burnetii*, Hap_1 was the most common haplotype, consisting of sequences from Thailand, Malaysia, Kyrgyzstan, the USA and Namibia.

Coxiella-like endosymbionts in this study were identified in 9 haplotypes (Hap_3, Hap_4, Hap_5, Hap_6, Hap_7, Hap_8, Hap_9, Hap_10, and Hap_11). CLEs of *A. testudinarium* (4 sequences) from this study were grouped within Hap_3, along with other sequences from Thailand and Malaysia. CLEs from *Dermacentor* in this study were found in 4 haplotypes: Hap_4 and Hap_5 (both from *D. compactus*), Hap_6 (*D. auratus*) and Hap_7 (*D. tricuspis*). Haplotype 6 contained the *D. auratus* CLE sequence from Thailand, which clustered together with other CLEs of *Rhipicephalus* from other countries (Israel and Cote d'Ivoire). Notably, 3 unique haplotypes, Hap_4, Hap_5 and Hap_7, were identified that were unique to Thailand. Additionally, CLEs from *Haemaphysalis* in this study were divided into 4 haplotypes: Hap_8 and Hap_9 (both from *H. hystricis*), Hap_10 (*H. semermis*) and Hap_11 (*H. shimoga*). In addition, Hap_8 clustered with CLEs of *H. hystricis* from Malaysia and Taiwan,

Fig. 3. Multiple sequence alignment of *Coxiella* bacteria, including *C. burnetii* and CLEs, using partial sequences of the 16 S rRNA gene (450 bp). The alignment divides sequences into 2 groups, shown in pink for *C. burnetii* and blue for CLEs. Nucleotide differences between *C. burnetii* and CLEs at positions 235, 349, and 350 are indicated by different colours: green for adenine (A), pink for guanine (G), red for thymine (T), and cyan for cytosine (C). The sequences obtained from this study are shown in bold type. *CB Coxiella burnetii*, *CLE Coxiella*-like endosymbiont, *Am Amblyomma* sp., *At Amblyomma testudinarium*, *Da Dermacentor auratus*, *Dc Dermacentor compactus*, *Dm Dermacentor marginatus*, *Ds Dermacentor steini*, *Dsi Dermacentor silvarum*, *Dt Dermacentor tricuspis*, *Ha Hyalomma asiaticum*, *Hb Haemaphysalis bispinosa*, *Hh Haemaphysalis hystricis*, *Ho Haemaphysalis obesa*, *Hs Haemaphysalis semermis*, *Hsh Haemaphysalis shimoga*, *Rh Rhipicephalus* sp., *Rs Rhipicephalus sanguineus* s.l.

Figure 3. (continued)

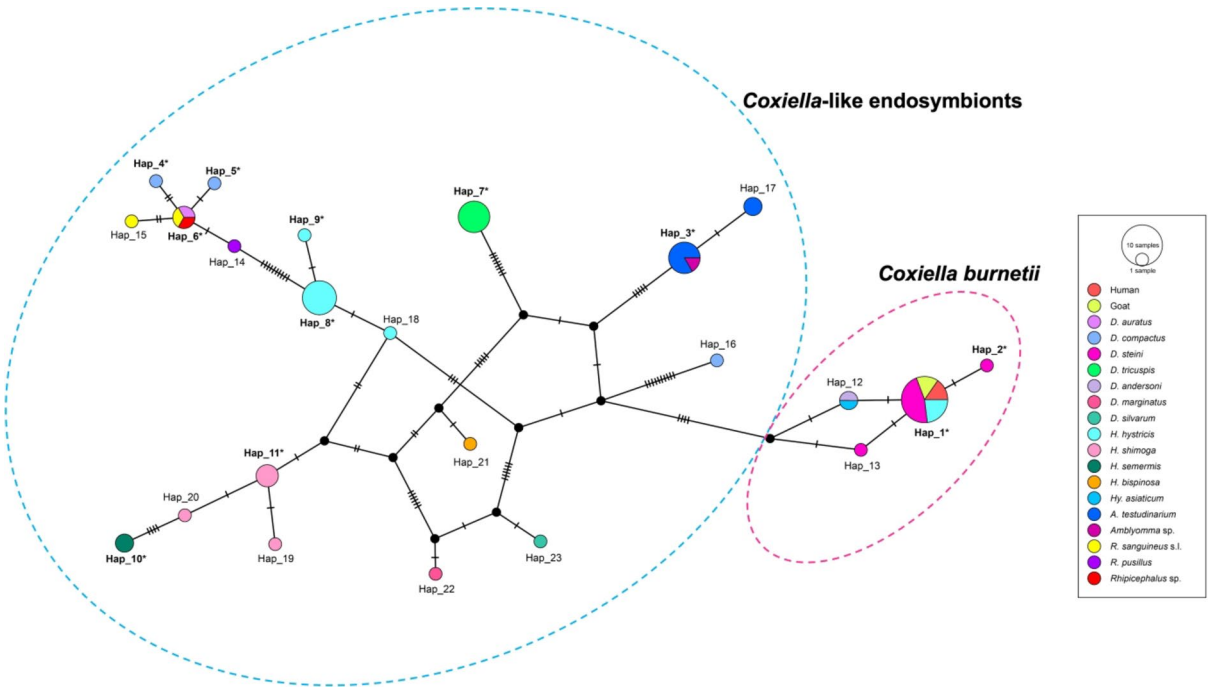

Fig. 4. TCS network of *Coxiella* bacterial haplotypes based on the partial 16 S rRNA gene sequences (434 bp), including *Coxiella* sequences from 17 *C. burnetii* and 41 CLEs. The circle size is proportional to the number of sequences in each haplotype. Each dash on the branch between haplotypes indicates the number of mutation steps. The colours represent *Coxiella* hosts. *Coxiella* haplotypes identified in ticks from this study are bolded and marked with an asterisk.

whereas the remaining *Haemaphysalis* haplotypes (Hap_9, Hap_10, and Hap_11) identified in this study were unique to Thailand.

Discussion

A total of 8 tick species found in a relatively small area of vegetation in Songkhla Province, southern Thailand, reflect the diverse species of ticks in this particular region. Previous studies have documented that these tick species (with the exceptions of *D. compactus* and *H. shimoga*) occur in various areas of Thailand, including the southern region, such as Nakhon Si Thammarat and Satun Provinces[29,44,45].

Sequence analyses of the 16 S rRNA gene revealed that *C. burnetii* (a pathogenic bacteria) was present in 9 out of the 30 selected positive tick samples, in individuals exclusively of the species *D. steini* and *H. hystricis*. In contrast, CLEs were identified in the remaining 21 positive tick samples of 7 species. These results are in accordance with those previously reported by Khoo et al. (2016) and Chisu et al. (2021), suggesting that CLEs are more likely than *C. burnetii* to be associated with various species of ticks[28,46]. Moreover, these results present the first report of CLEs associated with *H. semermis* and *D. compactus* in Thailand.

Our findings indicated that 16 S rRNA gene sequencing accurately discriminated *C. burnetii* from CLEs. DNA sequence alignments between *C. burnetii* and CLEs clearly differed at 3 base positions. Therefore, the *Coxiella* 16 S rRNA gene could be used as a molecular marker for discriminating *C. burnetii* from CLEs in hard tick species. Additionally, our phylogenetic results of CLEs showing diverse tick genera clustering within a single clade seem to be consistent with those of several other authors[47–49]. These observations appear to demonstrate that CLEs horizontally transmit among tick genera through host–vector interactions.

There have been reports of *C. burnetii* infections in several tick species from Southeast Asia, e.g. *R. sanguineus* s.l. from dogs in the state of Selangor, Malaysia[50], and *R. microplus* from ruminants in Luzon, Philippines[51]. Furthermore, Khoo et al. (2016) detected *C. burnetii* in *D. steini* and *H. hystricis* from wild boars in the state of Selangor, Malaysia, through PCR targeting the 16 S rRNA and *rpoB* genes[46]. However, Muramatsu et al. (2014) reported the identification of *C. burnetii* in 2 *R. microplus* ticks from dairy cattle in northern Thailand via RFLP nested PCR targeting the *com1* gene[17]. To our knowledge, there are no reports of *C. burnetii* in ticks collected from vegetation in Thailand. Our results constitute the first record of *C. burnetii* associated with *D. steini* and *H. hystricis* from the vegetation area of Songkhla Province, southern Thailand. Thus, *C. burnetii* appears to be distributed from northern to southern Thailand and down to peninsular Malaysia. These findings seem to suggest a wider range of distributions of *C. burnetii* in association with some species of ticks in Southeast Asia than previously recognized. To date, certain species of *Dermacentor* and *Haemaphysalis* ticks seem to favourably serve as vectors for *C. burnetii*. Nevertheless, the potential pathogenicity of *C. burnetii* in *D. steini* and *H. hystricis* ticks remains unknown.

Coxiella burnetii has been documented in more than 40 tick species[52]. Some of these ticks can transmit *C. burnetii* through vertical or horizontal transmission[53,54]. *Dermacentor steini* primarily parasitizes wild pigs, whereas *H. hystricis* favourably infests a broader range of mammals and birds[55–57]. Notably, these 2 tick species have also been found in humans[55,57], indicating the potential risk of *C. burnetii* transmission through tick bites and circulation in the environment via animal hosts. However, the genetic relationships between the *C. burnetii* strains detected in ticks in this study and those found in human isolates are unclear. Further research is necessary to determine the prevalence of *C. burnetii* across various tick species, especially those belonging to *Dermacentor* and *Haemaphysalis*, which commonly occur in different environments, particularly dairy farms, forests and ecotourism areas. It is important to examine the role of ticks in transmitting these pathogenic bacteria to humans and animals. Additionally, investigations should emphasize the pathogenic potential of *C. burnetii* in different geographic areas of Thailand and neighbouring countries. Therefore, further research on this topic is warranted to elucidate the potential pathogenic role of *C. burnetii* and its route of transmission among different tick vectors and related hosts. Importantly, ongoing surveillance and monitoring of tick-borne diseases should continue to effectively minimize potential threats to the health of humans as well as domestic and wild animals in Thailand and neighbouring countries.

Data availability

The DNA sequences of tick species and *Coxiella* bacteria from this study are available in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/).

Received: 14 October 2024; Accepted: 14 March 2025
Published online: 24 March 2025

References

1. Guglielmone, A. A., Nava, S. & Robbins, R. G. Geographic distribution of the hard ticks (Acari: Ixodida: Ixodidae) of the world by countries and territories. *Zootaxa* **5251**, 1–274. https://doi.org/10.11646/zootaxa.5251.1.1 (2023).
2. Yean, S. et al. 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. https://doi.org/10.1371/journal.pntd.0012269 (2024).
3. Ahantarig, A., Trinachartvanit, W. & Milne, J. Tick-borne pathogens and diseases of animals and humans in Thailand. *Southeast. Asian J. Trop. Med. Public. Health*. **39**, 1015–1032 (2008).
4. Celina, S. S. & Cerny, J. *Coxiella burnetii* in ticks, livestock, pets and wildlife: A mini-review. *Front. Vet. Sci.* **9**, 1068129. https://doi.org/10.3389/fvets.2022.1068129 (2022).
5. Arthan, W. et al. Detection of *Coxiella*-like endosymbiont in *Haemaphysalis* tick in Thailand. *Ticks Tick. Borne Dis.* **6**, 63–68. https://doi.org/10.1016/j.ttbdis.2014.09.005 (2015).
6. Sumrandee, C., Baimai, V., Trinachartvanit, W. & Ahantarig, A. Molecular detection of *Rickettsia*, *Anaplasma*, *Coxiella* and *Francisella* bacteria in ticks collected from artiodactyla in Thailand. *Ticks Tick. Borne Dis.* **7**, 678–689. https://doi.org/10.1016/j.ttbdis.2016.02.015 (2016).

7. Nooroong, P., Trinachartvanit, W., Baimai, V. & Ahantarig, A. Phylogenetic studies of bacteria (*Rickettsia*, *Coxiella*, and *Anaplasma*) in *Amblyomma* and *Dermacentor* ticks in Thailand and their co-infection. *Ticks Tick. Borne Dis.* **9**, 963–971. https://doi.org/10.1016/j.ttbdis.2018.03.027 (2018).
8. Psaroulaki, A. et al. Ticks, tick-borne rickettsiae, and *Coxiella burnetii* in the Greek Island of cephalonia. *Ann. N Y Acad. Sci.* **1078**, 389–399. https://doi.org/10.1196/annals.1374.077 (2006).
9. Knap, N., Zele, D., Glinsek Biskup, U., Avsic-Zupanc, T. & Vengust, G. The prevalence of *Coxiella burnetii* in ticks and animals in Slovenia. *BMC Vet. Res.* **15**, 368. https://doi.org/10.1186/s12917-019-2130-3 (2019).
10. Ni, J. et al. *Coxiella burnetii* is widespread in ticks (Ixodidae) in the Xinjiang areas of China. *BMC Vet. Res.* **16**, 317. https://doi.org/10.1186/s12917-020-02538-6 (2020).
11. Pexara, A., Solomakos, N. & Govaris, A. Q fever and seroprevalence of *Coxiella burnetii* in domestic ruminants. *Vet. Ital.* **54**, 265–279. https://doi.org/10.12834/VetIt.1113.6046.3 (2018).
12. Arricau-Bouvery, N. & Rodolakis, A. Is Q fever an emerging or re-emerging zoonosis? *Vet. Res.* **36**, 327–349. https://doi.org/10.1051/vetres:2005010 (2005).
13. Espana, P. P., Uranga, A., Cilloniz, C. & Torres, A. Q Fever (*Coxiella burnetii*). *Semin Respir Crit. Care Med.* **41**, 509–521. https://doi.org/10.1055/s-0040-1710594 (2020).
14. Sangkasuwan, V. & Pongpradit, P. *SEATO medical research study on rickettsial diseases in Thailand* (1967).
15. Suputtamongkol, Y. et al. Q fever in Thailand. *Emerg. Infect. Dis.* **9**, 1186–1187. https://doi.org/10.3201/eid0909.030086 (2003).
16. Doung-Ngern, P. et al. Seroprevalence of *Coxiella burnetii* antibodies among ruminants and occupationally exposed people in Thailand, 2012–2013. *Am. J. Trop. Med. Hyg.* **96**, 786–790. https://doi.org/10.4269/ajtmh.16-0336 (2017).
17. Muramatsu, Y. et al. Seroepidemiologic survey in Thailand of *Coxiella burnetii* infection in cattle and chickens and presence in ticks attached to dairy cattle. *Southeast. Asian J. Trop. Med. Public. Health*. **45**, 1167–1172 (2014).
18. Duron, O., Sidi-Boumedine, K., Rousset, E., Moutailler, S. & Jourdain, E. The importance of ticks in Q fever transmission: what has (and has not) been demonstrated? *Trends Parasitol.* **31**, 536–552. https://doi.org/10.1016/j.pt.2015.06.014 (2015).
19. Smith, T. A., Driscoll, T., Gillespie, J. J. & Raghavan, R. A *Coxiella*-like endosymbiont is a potential vitamin source for the lone star tick. *Genome Biol. Evol.* **7**, 831–838. https://doi.org/10.1093/gbe/evv016 (2015).
20. Ben-Yosef, M. et al. *Coxiella*-like endosymbiont of *Rhipicephalus sanguineus* is required for physiological processes during ontogeny. *Front. Microbiol.* **11**, 493. https://doi.org/10.3389/fmicb.2020.00493 (2020).
21. Trinachartvanit, W. et al. *Coxiella*-like bacteria in fowl ticks from Thailand. *Parasit. Vectors*. **11**, 670. https://doi.org/10.1186/s13071-018-3259-9 (2018).
22. Trinachartvanit, W. et al. Co-infection with *Coxiella*-like bacteria and *Babesia* in goat ticks from Southern Thailand. *Southeast. Asian J. Trop. Med. Public. Health*. **50**, 643–650 (2019).
23. Takhampunya, R. et al. Metagenomic approach to characterizing disease epidemiology in a disease-endemic environment in Northern Thailand. *Front. Microbiol.* **10**, 319. https://doi.org/10.3389/fmicb.2019.00319 (2019).
24. Takhampunya, R. et al. The bacterial community in questing ticks from Khao Yai National park in Thailand. *Front. Vet. Sci.* **8**, 764763. https://doi.org/10.3389/fvets.2021.764763 (2021).
25. Usananan, P. et al. Phylogenetic studies of *Coxiella*-like bacteria and spotted fever group rickettsiae in ticks collected from vegetation in Chaiyaphum Province, Thailand. *Front. Vet. Sci.* **9**, 849893. https://doi.org/10.3389/fvets.2022.849893 (2022).
26. Usananan, P., Kaenkan, W., Trinachartvanit, W., Baimai, V. & Ahantarig, A. *Coxiella*-like bacteria in *Haemaphysalis wellingtoni* ticks associated with great Hornbill, *Buceros bicornis*. *Trop. Biomed.* **39**, 191–196. https://doi.org/10.47665/tb.39.2.009 (2022).
27. Hirunkanokpun, S. et al. Spotted fever group *Rickettsia*, *Anaplasma* and *Coxiella*-like endosymbiont in *Haemaphysalis* ticks from mammals in Thailand. *Vet. Res. Commun.* **46**, 1209–1219. https://doi.org/10.1007/s11259-022-09980-x (2022).
28. Chisu, V., Mura, L., Foxi, C. & Masala, G. Coxiellaceae in ticks from human, domestic and wild hosts from Sardinia, Italy: high diversity of *Coxiella*-like endosymbionts. *Acta Parasitol.* **66**, 654–663. https://doi.org/10.1007/s11686-020-00324-w (2021).
29. Tanskul, P. & Inlao, I. Keys to the adult ticks of *Haemaphysalis* Koch, 1844, in Thailand with notes on changes in taxonomy (Acari: Ixodoidea: Ixodidae). *J. Med. Entomol.* **26**, 573–600. https://doi.org/10.1093/jmedent/26.6.573 (1989).
30. Voltzit, O. V. & Keirans, J. E. A review of Asian *Amblyomma* species (Acari, Ixodida, Ixodidae). *Acarina* **10**, 95–136 (2002).
31. Wassef, H. Y. & Hoogstraal, H. *Dermacentor* (*Indocentor*) *compactus* (Acari: Ixodoidea: Ixodidae): identity of male and female. *J. Med. Entomol.* **20**, 648–652. https://doi.org/10.1093/jmedent/20.6.648 (1983).
32. Wassef, H. Y. & Hoogstraal, H. *Dermacentor* (*Indocentor*) *auratus* (Acari: Ixodoidea: Ixodidae): identity of male and female. *J. Med. Entomol.* **21**, 169–173. https://doi.org/10.1093/jmedent/21.2.169 (1984).
33. Wassef, H. Y. & Hoogstraal, H. *Dermacentor* (*Indocentor*) *steini* (Acari: Ixodoidea: Ixodidae): identity of male and female. *J. Med. Entomol.* **23**, 532–537. https://doi.org/10.1093/jmedent/23.5.532 (1986).
34. Apanaskevich, D. A., Apanaskevich, M. A., Nooma, W., Ahantarig, A. & Trinachartvanit, W. Reinstatement of *Dermacentor tricuspis* (Schulze, 1933) n. comb., n. stat. (Acari: Ixodidae) as a valid species, synonymization of *D. atrosignatus* Neumann, 1906 and description of a new species from Indonesia, Malaysia and Thailand. *Syst. Parasitol.* **98**, 207–230. https://doi.org/10.1007/s11230-021-09972-6 (2021).
35. Black, W. C. & Piesman, J. Phylogeny of hard- and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proc. Natl. Acad. Sci. USA*. 91, 10034–10038. https://doi.org/10.1073/pnas.91.21.10034 (1994).
36. Simon, C. et al. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* **87**, 651–701. https://doi.org/10.1093/aesa/87.6.651 (1994).
37. Lv, J. et al. Assessment of four DNA fragments (COI, 16S rDNA, ITS2, 12S rDNA) for species identification of the Ixodida (Acari: Ixodida). *Parasit. Vectors*. **7**, 93. https://doi.org/10.1186/1756-3305-7-93 (2014).
38. Almeida, A. P. et al. *Coxiella* symbiont in the tick *Ornithodoros rostratus* (Acari: Argasidae). *Ticks Tick. Borne Dis.* **3**, 203–206. https://doi.org/10.1016/j.ttbdis.2012.02.003 (2012).
39. Duron, O. et al. The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*. *PLoS Pathog*. **11**, e1004892. https://doi.org/10.1371/journal.ppat.1004892 (2015).
40. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549. https://doi.org/10.1093/molbev/msy096 (2018).
41. Librado, P. & Rozas, J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452. https://doi.org/10.1093/bioinformatics/btp187 (2009).
42. Clement, M., Posada, D. & Crandall, K. A. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**, 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x (2000).
43. Leigh, J. W. & Bryant, D. Popart: full-feature software for haplotype network construction. *Methods Ecol. Evol.* **6**, 1110–1116. https://doi.org/10.1111/2041-210x.12410 (2015).
44. Tanskul, P., Stark, H. E. & Inlao, I. A checklist of ticks of Thailand (Acari: metastigmata: Ixodoidea). *J. Med. Entomol.* **20**, 330–341. https://doi.org/10.1093/jmedent/20.3.330 (1983).
45. Cornet, J. P., Demoraes, F., Souris, M., Kittayapong, P. & Gonzalez, J. P. Spatial distribution of ticks in Thailand: a discussion basis for tick-borne virus spread assessment. *Int. J. Geoinformatics*. **5**, 57–62 (2009).
46. Khoo, J. J. et al. *Coxiella* detection in ticks from wildlife and livestock in Malaysia. *Vector Borne Zoonotic Dis.* **16**, 744–751. https://doi.org/10.1089/vbz.2016.1959 (2016).
47. Machado-Ferreira, E. et al. *Coxiella* symbionts are widespread into hard ticks. *Parasitol. Res.* **115**, 4691–4699. https://doi.org/10.1007/s00436-016-5230-z (2016).

48. Papa, A., Tsioka, K., Kontana, A., Papadopoulos, C. & Giadinis, N. Bacterial pathogens and endosymbionts in ticks. *Ticks Tick. Borne Dis.* **8**, 31–35. https://doi.org/10.1016/j.ttbdis.2016.09.011 (2017).
49. Rahal, M. et al. Molecular identification and evaluation of *Coxiella*-like endosymbionts genetic diversity carried by cattle ticks in Algeria. *Ticks Tick. Borne Dis.* **11**, 101493. https://doi.org/10.1016/j.ttbdis.2020.101493 (2020).
50. Watanabe, M. et al. Molecular screening for *Rickettsia*, *Anaplasmataceae* and *Coxiella burnetii* in *Rhipicephalus sanguineus* ticks from Malaysia. *Trop. Biomed.* **32**, 390–398 (2015).
51. Galay, R. L. et al. Molecular detection of *Rickettsia* spp. and *Coxiella burnetii* in cattle, water Buffalo, and *Rhipicephalus* (*Boophilus*) *microplus* ticks in Luzon Island of the Philippines. *Trop. Med. Infect. Dis.* **5**, 54. https://doi.org/10.3390/tropicalmed5020054 (2020).
52. Sprong, H. et al. Prevalence of *Coxiella burnetii* in ticks after a large outbreak of Q fever. *Zoonoses Public. Health*. **59**, 69–75. https://doi.org/10.1111/j.1863-2378.2011.01421.x (2012).
53. Siroky, P. et al. Tortoise tick *Hyalomma aegyptium* as long term carrier of Q fever agent *Coxiella burnetii*–evidence from experimental infection. *Parasitol. Res.* **107**, 1515–1520. https://doi.org/10.1007/s00436-010-2037-1 (2010).
54. Korner, S. et al. Uptake and fecal excretion of *Coxiella burnetii* by *Ixodes ricinus* and *Dermacentor marginatus* ticks. *Parasit. Vectors.* **13**, 75. https://doi.org/10.1186/s13071-020-3956-z (2020).
55. Wassef, H. Y. & Hoogstraal, H. *Dermacentor* (*Indocentor*) *steini* (Acari: Ixodoidea: Ixodidae): hosts, distribution in the Malay Peninsula, Indonesia, Borneo, Thailand, the Philippines, and New Guinea. *J. Med. Entomol.* **25**, 315–320. https://doi.org/10.1093/jmedent/25.5.315 (1988).
56. Lim, F. S. et al. Bacterial communities in *Haemaphysalis*, *Dermacentor* and *Amblyomma* ticks collected from wild boar of an Orang Asli community in Malaysia. *Ticks Tick. Borne Dis.* **11**, 101352. https://doi.org/10.1016/j.ttbdis.2019.101352 (2020).
57. Guglielmone, A. et al. *The Hard Ticks of the World (Acari: Ixodida: Ixodidae)* (Springer Netherland, 2014).

Acknowledgements

We would like to thank Professor Dmitry A. Apanaskevich for supervising tick taxonomy. The authors (W.N. and A.A.) would like to thank the Thailand Research Fund for providing W.N. PhD scholarship. This research project was partially supported by Royal Golden Jubilee Ph.D. (RGJ-PHD) Scholarship (Grant No. PHD 0096/2558), Center of Excellence on Biodiversity, Office of Higher Education Commission (BDC-PG3-163005), Mahidol University, and additional grant from the Faculty of Science, Mahidol University in 2024.

Author contributions

W.T., V.B. and A.A. conceptualized and designed the experiments. W.N. carried out the field collection of samples, performed the experiments, analyzed data and wrote original draft of the manuscript. W.K. analyzed the data and helped preparing the manuscript. W.T., V.B. and A.A. contributed to manuscript editing, provided critical feedback and supervised the study. All authors discussed the results, revised the manuscript, read and approved the final version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

The study was approved by the SCMU-ACUC committee, protocol No. MUSC64-012-561, and conducted in accordance with relevant guidelines and regulations.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-94605-x.

Correspondence and requests for materials should be addressed to A.A.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.