Phylogenetic Studies of Coxiella-Like Bacteria and Spotted Fever Group Rickettsiae in Ticks Collected From Vegetation in Chaiyaphum Province, Thailand

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Ticks can transmit a wide variety of pathogens, including bacteria. Here, we report the detection of tick-associated bacteria in Chaiyaphum Province, northeastern Thailand. There have been few reports of tick-borne bacterial pathogens in the study areas, which are evergreen forests dominated by plateaus at elevations of approximately 1,000 m. In total, 94 ticks were collected from vegetation. They were screened for the presence of Coxiella, Francisella, Rickettsia, and Borrelia bacteria using PCR assays. In this study, we found ticks from two genera, Haemaphysalis and Amblyomma, that were positive for Coxiella-like bacteria (CLB) and Rickettsia. Francisella and Borrelia spp. were not detected in these two tick genera. The results revealed the evolutionary relationships of CLB in Amblyomma testudinarium, Haemaphysalis lagrangei, and Haemaphysalis obesa ticks using the 16S rRNA and rpoB markers, which clustered together with known isolates of ticks from the same genera. In contrast, the groEL marker showed different results. On the basis of the groEL phylogenetic analysis and BLAST results, three groups of CLB were found: (1) CLB from A. testudinarium grouped as a sister clade to CLB from Ixodes ricinus; (2) CLB from Haemaphysalis lagrangei was distantly related to CLB from Haemaphysalis wellingtoni; and (3) CLB from A. testudinarium grouped as sister clade to CLB from Amblyomma from French Guiana and Brazil. For Rickettsia studies, phylogenetic trees of the gltA, ompB, and sca4 genes revealed two groups of Spotted Fever Group (SFG) Rickettsiae: (1) SFG Rickettsiae that formed a sister clade with Rickettsia tamurae AT-1 (belong to the Rickettsia helvetica subgroup) in A. testudinarium and (2) SFG Rickettsiae that formed a distantly related group to Rickettsia rhipicephali 3-7-female6-CWPP (belong to the Rickettsia massiliae subgroup) in A. testudinarium. This study expanded our knowledge of the diversity of tick-borne Coxiella and Rickettsia bacteria. The pathogenic roles of these bacteria also need to be investigated further.

Keywords: Coxiella-like bacteria, Rickettsia, Haemaphysalis, Amblyomma, tick
INTRODUCTION

Ticks are important hematophagous ectoparasites of both humans and animals. They can transmit a wide range of pathogens, such as Coxiella, Francisella, Borrelia, and Rickettsia bacteria (1–4). Hard ticks, such as the genera Amblyomma, Dermacentor, and Rhipicephalus, have been identified as the main vectors of Spotted Fever Group (SFG) Rickettsiae (5). Several tick species have been reported in Chaiyaphum Province, northeastern Thailand. These include Amblyomma testudinarium, Haemaphysalis asiatica, Haemaphysalis hystricis, Haemaphysalis semermis, Rhipicephalus haemaphysaloides, and Ixodes granulatus (6). Rickettsia spp. are gram-negative bacteria that cause SFG diseases. SFG rickettsioses have been reported (7) that is closely related to Rickettsia tamurae (8). Haemaphysalis semermis (9), which is closely related to Rickettsia tamurae, has been reported in many regions of the world, including Japan, northern China, Korea (7–9), and Thailand (10). SFG rickettsioses are characterized by fever, headache, muscle pain, maculopapular rash, and developing eschar at the site of tick bites (11). In Thailand, Rickettsia spp. have been reported near the Thai-Myanmar border, such as Rickettsia sp. strain RDla420 identified in Dermacentor auratus ticks obtained from a bear and Rickettsia sp. strain RDla440 detected in Dermacentor larval ticks from a wild pig nest (12). In addition, Sumrandee et al. (13) reported the first evidence of a Rickettsia sp. that is closely related to Rickettsia tamurae in Rhipicephalus (Boophilus) microplus ticks from Thailand. In Chaiyaphum Province, Malaisiri et al. (14) reported a phylogenetic analysis of new Rickettsia genotypes that were closely related to Rickettsia tamurae and Rickettsia monacensis and might be pathogenic to humans.

Q fever is a zoonotic disease caused by Coxiella burnetii; infection mainly arises through the inhalation of airborne particles contaminated with bacteria. The clinical features of Q fever include flu-like symptoms to pneumonia and granulomatous hepatitis in serious cases (15). Coxiella burnetii infections and Coxiella-like bacteria (CLB) have been found in humans and other animals in Thailand (16–19). For example, C. burnetii was found in Thai patients in Khon Kaen Province, northeastern Thailand (20). CLB can promote the reproductive fitness and development of Haemaphysalis longicornis ticks (21). In addition, CLB can interfere with the colonization and transmission of pathogens. For example, CLB can impact pathogen susceptibility in ticks, e.g., CLB can defend their Rhipicephalus haemaphysaloides tick hosts against the pathogenic microbe Babesia microti (22). Interestingly, CLB was determined to be the cause of death in a female eclectus parrot (Eclectus roratus) (23). In Thailand, CLB was also detected in Haemaphysalis ticks, such as Haemaphysalis shimoga and Haemaphysalis lagrangei (24). Moreover, Trinachartvanit et al. (25) reported CLB in Haemaphysalis wellingtoni tick-infested fowl from various parts of Thailand.

The objectives of this study were to identify the presence of bacteria in ticks collected from vegetation and to reveal the overall diversity of bacterial infections and species in ticks using PCR and phylogenetic analysis.

MATERIALS AND METHODS

Tick Collection and Identification

In 2014–2015, ticks (larvae, nymphs, and adults) were collected from vegetation from forests in Chaiyaphum Province, Thailand (16°16′25.2″N 101°29′02″E; 16°22′23.0″N 101°46′38.7″E; and 16°12′18.9″N 101°52′22.9″E). The areas being studied are evergreen forests dominated by plateaus at elevations of approximately 1,000 m. We placed the ticks into tubes that were held in a container with liquid nitrogen and stored them in a freezer after returning to the laboratory. Morphological identification of all tick stages was performed under a stereomicroscope (26, 27). Tick identification was performed using molecular methods on the ticks that were positive for either Coxiella or Rickettsia bacteria; previously published primers (16S+1/16S-1) for the amplification of mt 16S rDNA were used (28). There were seven pools of Haemaphysalis ticks at the immature stage, including nymphs (four pools: three pools of five and one pool of four) and larvae (three pools: one pool of six, one pool of seven, and one pool of 15). For the Amblyomma immature stage (nymphs), there were five pools of three nymphs.

DNA Extraction

Before DNA extraction, ticks were cleaned three times with 70% ethanol, 10% sodium hypochlorite, and sterile distilled water. DNA extraction was conducted using a DNeasy Blood and Tissues Kit (Qiagen) according to the manufacturer’s protocol. The DNA products were stored at −20°C until use as templates for the PCR assay.

Molecular Analysis

All extracted DNA samples were used as templates for PCR assays with specific bacterial primers for detecting the presence of Coxiella, Rickettsia, Francisella, and Borrelia. Coxiella-positive bacteria were identified through the 16S rRNA, groEL (60-kDa chaperone heat shock protein B), and rpoB genes (β subunit of bacterial RNA polymerase). Rickettsia species were screened by PCR, targeting the 17-kDa antigen, citrate synthase (gltA), outer membrane protein A (ompA), outer membrane protein B (ompB), and cell surface antigen (sca4) genes. PCR primer pairs for the detection of bacterial species, primer name, target genes, and size of the amplicons (bp) are shown in Table 1. The PCR product of the expected size from each corresponding primer pair (gene) was cloned and sequenced as a positive control. A sterile distilled water negative control was also included.

DNA Purification, Sequencing, and Phylogenetic Analysis

The positive PCR products were purified with a Nucleospin Gel and PCR Clean-up Kit (Düren, Germany) and sequenced. The DNA sequence alignment of representative positive samples generated by this study was created using the CLUSTALW program. The nucleotide sequences were analyzed and blasted with the National Center for Biotechnology Information BLASTn database. Afterward, phylogenetic analyses were carried out using the maximum likelihood (ML) (38) and neighbor-joining
TABLE 1 | Primers for PCR amplification used in this study.

| Organism | Target gene | Primer name | Sequence (5′-3′) | References |
|----------|-------------|-------------|------------------|------------|
| Tick (Acari) | mt 16S rDNA | 16S+1 | CTGCTCAAGATTTTTAAATTGTGTGGG | (28) |
|           |             | 16S-1 | CCGGGTCTGAACTCAGATCAAGT | |
| Rickettsia | 17-kDa antigen | RR17.61p | CATTGTTCGTCAGGTTGGCG | (29) |
|           |             | RR17.42p | GCTCTTGCAAATCTCATATTGG | |
|           |             | RpCS.877p | GGCCGCTGCTCAAGGCGG | (30) |
|           |             | RpCS.1258n | ATTCGAAAGATACAGTGAACA | |
|          | ompA | RR190.70p | ATGGCGAATATTCTCCAAA | (31) |
|           |             | RR190.602n | ATGGCGAATATTCTCCAAA | |
|           | ompB | RIC-F | CAACCCAGCAAGGTAATGTTTA | (32) |
|           |             | RIC-R | GCTATACCAGCGCTGTAACAG | |
|           | sca4 | RdD749F | TGCTAGCATTAAGAAGCTGATGG | (33) |
|           |             | RdD1826R | TCTAAATKCTGCTGATGTCAT | |
|          | groEL | CoxGrF1 | TTTGGAAAYATGGGCGKCAAATGGT | (34) |
|           |             | CoxGrR2 | TTTGGAAAYATGGGCGKCAAATGGT | |
|           |             | CoxGrF2 | CAGCTGATGCTGTCGATGACG | |
|           |             | CoxGrR1 | CCAAROCAGAGTGGCTTTTYAC | |
|           | rpoB | CoxrpoBF2 | GGCGGNCAAYGGWAAAAAGGGGT | (35) |
|           |             | CoxrpoBR1 | CAGCRAAHCGTGGACCRCAAAATGGT | |
|           |             | CoxrpoBF3 | TGAAAGAVAGGCTGTTGGGAAAG | |
|           |             | CoxrpoBR3 | AGCTTTMCCACGSAAROGGTGGCTGGT | |
|          | 16S rDNA | Borrelia | ATACCAAGAGATTTGATGCG | (36) |
|           |             | Franciscella | TACGGTCAGAAACAGACTGT | (37) |
|           | 16SR | Franciscella | TACGGTCAGAAACAGACTGT | |

(NJ) (39) methods (evaluated by bootstrap analysis with 1,000 replicates) for Coxiella and Rickettsia spp., respectively.

RESULTS

Tick Collection

A total of 94 ticks were collected and identified. The species, number, and life stage of the ticks are shown in Table 2. The ticks belonged to two genera, Haemaphysalis and Amblyomma. Of the adult ticks, 13 males and 12 females belonged to H. lagrangei. The remaining ticks belonged to A. testudinarium (two female ticks). In the immature stage, Haemaphysalis sp. (22 nymphs and 28 larvae) was the most commonly collected, followed by Amblyomma sp. (17 nymphs). The species of all ticks included in the phylogenetic trees were confirmed by molecular methods, and their sequences were submitted to GenBank with the accession numbers shown in Tables 3, 4.

Detection of Bacteria

Coxiella and Rickettsia bacteria were detected in two genera of ticks (Haemaphysalis and Amblyomma), whereas Franciscella sp. and Borrelia sp. were not identified in this study (Table 2). Single infection with Coxiella was detected in 16 of 25 H. lagrangei adult ticks (six of 13 males and 10 of 12 females), and single infection with Rickettsia was detected in one of 25 H. lagrangei females. Adult A. testudinarium ticks were infected with Coxiella (two of two females) and Rickettsia (one of two females). Single infection with Coxiella was detected in three of three nymphs and four of seven pools of Haemaphysalis ticks (one pool of four nymphs, two pools of five nymphs, and one pool of 15 larvae). In addition, two of two nymphs and five of five pools of Amblyomma ticks were positive for Coxiella and two of two nymphs and four of five pools were positive for Rickettsia. However, infection with Rickettsia was not detected in the immature stage of Haemaphysalis. Moreover, coinfection with these two bacteria was detected in infected adults of H. lagrangei. In addition, adults of A. testudinarium were coinfected with Coxiella and Rickettsia. Coinfection with these two bacteria was also present in Amblyomma nymphal ticks.

DNA Sequencing and Phylogenetic Analysis

A phylogenetic tree based on the partial sequences of 16S rRNA revealed that all Coxiella sequences detected in this study belonged to two endosymbiotic groups (Figure 1A).
TABLE 2 | Species, number, life stage of ticks, and results of bacterial infection in ticks analyzed by PCR (positive result of each bacterium) collected from vegetation in Chaiyaphum Province, Thailand.

| Tick species               | Number of collected ticks | No. of PCR positive/No. of ticks |
|---------------------------|---------------------------|---------------------------------|
|                           | Male | Female | Nymph | Larva | Coxiella | Rickettsia |
| H. lagrangei              | 13   | 12     | 0     | 0     | 16/25    | 1/25      |
| Haemaphysalis sp.         | 0    | 0      | 22    | 28    | 3/3 individual, 4/7 pool | 0/3 individual, 0/7 pool |
| A. testudinarum           | 0    | 2      | 0     | 0     | 2/2      | 1/2       |
| Amblyomma sp.             | 0    | 0      | 17    | 0     | 2/2 individual, 5/5 pool | 2/2 individual, 4/5 pool |
| Total                     | 13   | 14     | 39    | 28    | 32/44    | 8/44      |

TABLE 3 | Details of GenBank accession numbers of the Coxiella gene sequences and BLAST analysis of these sequences from tick samples collected from vegetation in Chaiyaphum Province, Thailand.

| Tick species and stage | Code (accession number of tick 16S rRNA) | Percent identity (matching nucleotides/total) with closest Coxiella spp. sequences for each gene |
|-----------------------|------------------------------------------|--------------------------------------------------------------------------------------------------|
|                       |                                          | 16S rRNA | groEL | rpoB |
| Amblyomma testudinarum| PK33 (MZ490780)                          | 99.6% (484/486) Coxiella sp. S027 (LT009437)  | 91.5% (529/578) Coxiella endosymbiont of ixodes hexagonus hexa1 (KP985500) | 99% (486/491) Coxiella sp. S027 (LT174617) |
| Amblyomma testudinarum| PK48 (MZ490781)                          | 99.8% (470/471) Coxiella sp. S027 (LT009437)  | 91.7% (530/578) Coxiella endosymbiont of ixodes hexagonus hexa1 (KP985500) | 99% (486/491) Coxiella sp. S027 (LT174617) |
| Amblyomma testudinarum| PK138-140 (MZ490788)                     | 99.9% (470/471) Coxiella sp. S027 (LT009437)  | 91.7% (530/578) Coxiella endosymbiont of ixodes hexagonus hexa1 (KP985500) | 99% (486/491) Coxiella sp. S027 (LT174617) |
| Amblyomma testudinarum| PK51 (MZ490782)                          | 99.6% (484/486) Coxiella sp. S027 (LT009437)  | 91.7% (530/578) Coxiella endosymbiont of ixodes hexagonus hexa1 (KP985500) | 99% (486/491) Coxiella sp. S027 (LT174617) |
| Amblyomma testudinarum| PK67-69 (MZ490783)                        | 99.8% (483/484) Coxiella sp. S027 (LT009437)  | 91.7% (529/577) Coxiella endosymbiont of ixodes hexagonus hexa1 (KP985500) | 99% (486/491) Coxiella sp. S027 (LT174617) |
| Amblyomma testudinarum| PK81-83 (MZ490784), PK100-102 (MZ490785) | 99.8% (485/486) Coxiella sp. S027 (LT009437)  | 91.7% (530/578) Coxiella endosymbiont of ixodes hexagonus hexa1 (KP985500) | 99% (486/491) Coxiella sp. S027 (LT174617) |
| Amblyomma testudinarum| PK121-123 (MZ490787)                      | 99.6% (484/486) Coxiella sp. S027 (LT009437)  | 91.7% (530/578) Coxiella endosymbiont of ixodes hexagonus hexa1 (KP985500) | 99% (486/491) Coxiella sp. S027 (LT174617) |
| Amblyomma testudinarum| PK168 (MZ490790)                          | 99.8% (485/486) Coxiella sp. S027 (LT009437)  | 91.2% (527/578) Coxiella endosymbiont of ixodes hexagonus hexa1 (KP985500) | 99% (486/491) Coxiella sp. S027 (LT174617) |
| Haemaphysalis lagrangei  | PK16 (MZ490778), PK17 (MZ490779)          | 99.8% (485/486) Coxiella sp. HLS03 (JQ764262) | 88.2% (516/585) Coxiella sp. TR332 (MG874471) | 94.3% 482/490 Coxiella sp. S027 (LT174612) |
| Haemaphysalis lagrangei  | PK146-150 (MZ490789)                      | 99.8% (485/486) Coxiella sp. HLS03 (JQ764262) | 88.2% (516/585) Coxiella sp. TR332 (MG874471) | 94.3% 482/490 Coxiella sp. S027 (LT174612) |
| Haemaphysalis obesa larva| PK104-118 (MZ490786)                      | 100% (486/486) Coxiella sp. TPS08 (KC170759) | 87.7% (514/585) Coxiella sp. TR332 (MG874471) | 92.2% 452/490 Coxiella sp. S027 (LT174612) |

Coxiella sequences detected in H. lagrangei and Haemaphysalis obesa were in the first group. PK16, PK17, and PK146-150 clustered with CLB of HLS03 found in H. lagrangei, whereas PK104-118 grouped with CLB of TPS08 detected in H. obesa. These sequences were closely related to those of CLB in H. hystricis S002 (LT009432), H. longicornis 47 (AY342035), and H. longicornis A (AB001519). Coxiella sequences in A. testudinarium and Amblyomma sp. formed a monophyletic clade and clustered together with CLB found in Amblyomma sp. S027 and A. testudinarium AMTKK2.1 from Malaysia and Thailand, respectively (Figure 1A). BLAST analysis of the Coxiella 16S rRNA and groEL and rpoB gene sequences from Haemaphysalis and Amblyomma ticks is shown in Table 3.

Interestingly, the BLAST results of the groEL gene sequences from this work showed DNA sequence identities of < 92% compared to the existing sequences. Phylogenetic trees based on the partial sequences of groEL revealed that CLB in this study were clustered into three groups (Figure 1B). All sequences of Coxiella spp. in Amblyomma (except PK48 and PK138-140)
grouped together as sister clades to the CLB of *I. ricinus*. CLB in *Haemaphysalis* grouped as sister clades with CLB detected in *H. wellingtoni* TRG32 (MG874471). Interestingly, the groEL sequences of PK48 and PK138-140 clearly formed separate clades from the other *Coxiella* spp. in *A. testudinarium* obtained in the first group, although they still formed a sister clade with the *Amblyomma* genus from other geographical regions. CLB in *Haemaphysalis* (PK16, PK17, PK104-118, and PK146-150) from this study (Figure 1B) formed a distinct clade from those of *Amblyomma* in *Coxiella* spp. Importantly, the BLAST results of the rpoB gene sequences from this work exhibited DNA sequence identities < 95% compared to the existing sequences for *Haemaphysalis* (PK16, PK17, PK146-150, and PK104-118). Phylogenetic trees based on the partial sequences of *rpoB* revealed that all CLB sequences detected in this study were also divided into two groups. The first group included *Coxiella* spp. detected in *Amblyomma* ticks that formed a monophyletic clade and grouped together with CLB in *Amblyomma* sp. S027 (LT174617) from Malaysia. The second group included *Coxiella* sequences found in *Haemaphysalis* that formed an independent clade and grouped with CLB in *H. hystricis* S002 (LT174612) from Malaysia (Figure 1C).

BLAST analysis of the *Rickettsia* 17-kDa antigen, *gltA*, *ompA*, *ompB*, and *sca4* gene sequences from *Amblyomma* and *Haemaphysalis* ticks is shown in Table 4. The bands for *ompA* and *ompB* from PK16 were faint and could not be sequenced. Interestingly, the BLAST search results of all *ompB* genes studied herein showed < 97% DNA sequence similarity compared to the existing *ompB* genes in *Rickettsia* spp. In addition, the BLAST search results of PK100-102 showed that the *Rickettsia* *ompB* gene sequences from *A. testudinarium* nymphal ticks had 96% sequence similarity to *Rickettsia rhipicephali* 3-7-female 6-CWPP (CP003342). Moreover, the BLAST search results of the *sca4* gene of PK100-102 showed 97.3% identity to *Candidatus Rickettsia thierseensis* (MT424980), 97.3% (873/897) *R. founieri* AUS118 (KF666472). Phylogenetic trees based on the partial sequences of the 17-kDa, *gltA*, *ompB*, and *sca4* genes from *Rickettsia* spp. are shown in Figures 2A–D.
Usananan et al.  
Coxiella and Rickettsia Bacteria in Tick  

**FIGURE 1** | Phylogenetic tree of *Coxiella* species gene sequences using the maximum likelihood method with 1,000 bootstrap replicates (bootstrap values < 50% are not shown). (A) 16S rRNA gene: *Legionella longbeachae* was used as the outgroup. (B) groEL gene: *Legionella longbeachae* was used as the outgroup. DNA from *Coxiella* spp. amplified from ticks identified in this study is indicated by black dots and bold font. The scale bar indicates nucleotide substitutions per site.
FIGURE 2 | Phylogenetic tree of *Rickettsia* species using the neighbor-joining method with 1,000 bootstrap replicates (bootstrap values < 50% are not shown). (A) 17-kDa antigen gene: *Rickettsia bellii* was used as the outgroup. (B) *gltA* gene: *Rickettsia bellii* was used as the outgroup. (C) *ompB* gene: *Rickettsia typhi* and *Rickettsia prowazekii* were used as outgroups. (D) *sca4* gene: *Rickettsia bellii* was used as the outgroup. DNA from *Rickettsia* spp. amplified from ticks identified in this study is indicated by black dots and bold font. The scale bar indicates nucleotide substitutions per site.

based on the partial sequences of the 17-kDa (Figure 2A) and *gltA* (Figure 2B) genes indicated that the *Rickettsia* spp. detected in this study formed three groups. The first group (PK16) was clustered with *Rickettsia* sp. HOT2 and *Rickettsia* sp. 315 (17-kDa gene), and this group was within the *Rickettsia massiliae* subgroup of *Rickettsia*. Phylogenetically, the *gltA* PK16 sequence grouped with the *Rickettsia raoultii* clade (*R. massiliae* subgroup) and was closely related to the clades containing *Rickettsia japonica* and *Rickettsia heilongjiangensis*. The second group included PK100-102, which clustered together with *Rickettsia* sp. in *A. testudinarium* from Laos. The third group (including PK48, PK51, PK67-69, PK81-83, PK138-140, and PK168) formed a sister clade with *R. tamurae* based on their 17-kDa and *gltA* gene sequences.

However, phylogenetic analysis based on the partial sequence of the *ompA* gene was not included in this study because the *ompA* amplicons might be the products of contaminated reactions. In addition, phylogenetic trees based on the partial sequences of the *ompB* gene revealed that *Rickettsia* spp. formed two groups: the first group (including PK100-102) formed a sister clade to *R. raoultii*, *R. rhipicephali*, and *R. massiliae* (*R. massiliae* subgroup), and the second group (including PK48, PK51, PK67-69, PK81-83, PK138-140, and PK168) grouped within the clade containing *R. tamurae* (*Rickettsia helvetica* subgroup) (Figure 2C). A phylogenetic tree of the *sca4* gene (not including sequence PK138-140) showed the presence of two groups within SFG *Rickettsia* (Figure 2D), similar to the results for the *ompB* gene.

**DISCUSSION**

CLB have been identified in several tick genera, including *Haemaphysalis* and *Amblyomma*, and in at least two-thirds of tick species (15, 40, 41). Moreover, the tissue distribution of this symbiont within ticks showed that CLB specifically colonized the ovaries of female *Amblyomma cajennense* (42) and *H. longicornis* ticks (43), which also indicated that CLB is associated with the regulation of tick reproductive fitness (42, 44, 45). By using...
specific fluorescent foci, CLB were also observed in several tick tissues, including Malpighian tubules, salivary glands, and the midgut (46). In Thailand, the presence of CLB in the Haemaphysalis genus has also been documented (13, 24, 25). Our results added information on CLB in H. lagrangei, H. obesa, and A. testudinarium ticks from Chaiyaphum Province.

The phylogenetic tree based on ML analysis using the 16S rRNA and rpoB genes showed that the detected CLB from both Haemaphysalis and Amblyomma ticks obtained in this study were clustered in the same clade as CLB gene sequences from similar genera. However, some groEL gene sequences of CLB in A. testudinarium ticks (accession numbers MZ173557 and MZ173558) detected in this study formed two separate clades. The groEL gene sequence of CLB in A. testudinarium (accession numbers MZ173557 and MZ173558) clustered with Amblyomma from other countries. Another group clustered with the Ixodes genus. This clade of groEL genes was also close to CLB associated with H. wellingtoni collected from domestic fowl from Trang Province, Thailand (25). The reason that the infection pattern shown by the groEL gene of CLB found in A. testudinarium was grouped with other previously published Coxiella sequences from the other tick genera may be horizontal gene transfer. A few examples have shown that accidental horizontal transmission occurs among host individuals, including during cofeeding. For example, the highly efficient exchange of the rompA gene of Rickettsia conorii israelensis was demonstrated between infected and uninfected Rhipicephalus sanguineus ticks feeding nearby each other on a dog that was not formerly infected with these bacteria (47). Interestingly, the results for the 16S rRNA and rpoB markers in this study revealed that the CLB in A. testudinarium, H. lagrangei, and H. obesa ticks clustered together with known isolates, in contrast to the results found with the groEL marker. On the basis of the groEL phylogenetic analysis and BLAST results in this work, we found three groups of CLB: (1) CLB from A. testudinarium grouped as a sister clade with CLB from I. ricinus; (2) CLB from H. lagrangei was distantly related to CLB from H. wellingtoni; and (3) CLB from A. testudinarium grouped as a sister clade with CLB from Amblyomma ticks from French Guiana and Brazil. Rickettsia spp. detected in ticks from this study grouped with SFG Rickettsiae, which are pathogenic bacteria. On the basis of the phylogenetic analysis, we showed that Rickettsia sp. detected from H. lagrangei tick (PK16) obtained in this study were clustered in different clades containing the rickettsial genes of Amblyomma ticks. Moreover, Rickettsia sp. detected from the H. lagrangei tick (PK16-MZ490778) grouped with Rickettsia sp. HOT2, which clustered with the R. raoultii clade based on the 17-kDa gene. It has been reported that the presence of HOT2 Rickettsia has been detected in Haemaphysalis ornithophila ticks from Khao Yai National Park, Thailand (48). On the basis of the gltA gene sequence, PK16 grouped within the R. raoultii clade (R. massiliae subgroup). However, the ompA and ompB genes of PK16 could not be sequenced due to the presence of faint bands.

The BLAST analysis showed that the ompA genes of Rickettsia spp. collected from Amblyomma ticks were identical (100% DNA sequence identity) to those from R. tamurae AT-1 in all Rickettsia samples detected in this study. In this scenario, it is possible that the detection of the ompA sequences may have arisen due to PCR bias, resulting in this very surprising finding. An alternative explanation could be that the ompA amplicons were the product of a contaminated reaction. We did not include the phylogenetic analysis of the ompA gene in this study.

On the basis of the gltA, ompB, and sca4 phylogenetic analyses and BLAST results from this work, we found two groups of SFG Rickettsiae: (1) SFG Rickettsiae that grouped as a sister clade with R. tamurae AT-1 (belonging to the R. helvetica subgroup) in A. testudinarium and (2) SFG Rickettsiae that was distantly related to R. rhipicephali 3-7-female 6-CWPP (belonging to the R. massiliae subgroup) in A. testudinarium. The pathogenic roles of these bacteria need to be studied further.

CONCLUSIONS

From the results of the groEL phylogenetic analysis, CLB clades were found to group as a sister clades to CLB from I. ricinus, CLB from H. wellingtoni, and CLB from Amblyomma ticks from French Guiana and Brazil. In addition, on the basis of the gltA, ompB, and sca4 phylogenetic analyses, SFG Rickettsiae formed two groups: a sister clade to R. tamurae AT-1 (belonging to the R. helvetica subgroup) and a clade distantly related to R. rhipicephali 3-7-female 6-CWPP (belonging to the R. massiliae subgroup). This study demonstrates the diversity of CLB and Rickettsia bacteria with their host ticks, which may act as potential vectors.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

AA wrote and edited manuscript and planned and designed the research with advice from VB and WT. PU wrote the original draft. VB and WT edited the manuscript. WK analyzed the data of the phylogenetic tree and writing. RS took care of the experiments. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by grants from Mahidol University, the Center of Excellence on Biodiversity (BDC) and the Office of Higher Education Commission (BDC-PG3-163005). This research was also supported by the Thailand Research Fund through the Royal Golden Jubilee PhD Program (Grant No. PHD 0175/2560) to PU.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Surachai Chansri for ticks from his field collections.
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