Spotted fever group *Rickettsia*, *Anaplasma* and *Coxiella*-like endosymbiont in *Haemaphysalis* ticks from mammals in Thailand

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Abstract
Ticks are ectoparasites of vertebrates and vectors of various pathogenic microorganisms. In this study, the presence of bacteria and protozoa was evaluated by PCR and DNA sequencing in 233 mammal ticks collected from 8 provinces in Thailand. Sequence and phylogenetic analyses of partial rickettsial *ompA*, *ompB*, *sca4* and partial *Coxiella* 16S rRNA, *GroEL*, *rpoB* genes clearly revealed, for the first time, a co-infection of SFG *Rickettsia* belonging to *R. massiliae* subgroup and *Coxiella*-like endosymbiont (CLE), Cox-hein, in a male of *Haemaphysalis heinrichi* tick infesting Burmese ferret-badger in Loei province. Moreover, a male of *H. hystricis* tick infesting the same host was infected with another CLE, Cox-hys. Based on the 16S rRNA gene sequence, *Anaplasma* sp., closely related to *Anaplasma bovis* was also detected in a male of *H. heinrichi* infesting the same Burmese ferret-badger. In addition, the third CLE, Cox-asia, found in *H. asiatica* collected from Asian palm civet in Chiang Rai province, was different from both Cox-hein and Cox-hys. This study provided important data and broadened our knowledge on tick-borne pathogens and endosymbionts in Thailand and Southeast Asia.

Keywords Tick · SFG *Rickettsia* · *Coxiella* · *Anaplasma* · Burmese ferret-badger · Asian palm civet

Introduction
Ticks are important vectors of infectious pathogens including bacteria, protozoa, and viruses. The obligate intracellular bacteria, *Rickettsia*, is notorious for causing infection and mild to severe diseases in humans and other mammals (Raoult and Roux 1997). Based on phylogenomic analyses, members of the genus *Rickettsia* are classified into four groups, namely, spotted fever group (SFG), typhus group (TG), transitional group (TRG), and ancestral group (AG) (Gillespie et al. 2007).

*Coxiella burnetii* is the etiological agent of a worldwide zoonotic disease called Q fever. In Thailand, infective endocarditis caused by *C. burnetii* has been reported (Pachirat et al. 2012). Both animals and humans can be infected by this bacterium, usually through inhalation of contaminated aerosols. Its reservoir hosts comprise mammals, birds, and arthropods, particularly ticks (Raoult and Marrie 1995). However, the role of ticks as the vector of *C. burnetii* remains controversial. Many Ixodidae and Argasidae ticks, including *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes*, *Rhipicephalus*, Argas, and *Ornithodoros* also harbor *Coxiella*-like endosymbionts (CLE) (Duron et al. 2015). Thus far, there is no evidence of the transmission of CLE by ticks to vertebrates. In addition to CLE, another endosymbiont commonly found in many arthropods and filarial nematodes is *Wolbachia* bacteria. In arthropods, *Wolbachia* has been reported to manipulate host reproduction including cytoplasmic incompatibility, parthenogenesis, feminization of genetic males, and male killing. Therefore, current research focuses on *Wolbachia* application to protect humans from vector-borne diseases (Werren et al. 2008).

*Anaplasma* and *Ehrlichia* are tick-transmitted bacteria in the family Anaplasmataceae, order Rickettsiales. *Anaplasma*
is an obligate intracellular bacterium living in mammal blood cells. Members of this genus include tick-borne pathogens causing fatal infectious diseases in humans and catastrophic diseases in animals (Dumler et al. 2001). Anaplasmosis in humans is principally caused by Anaplasma phagocytophilum. Anaplasma has also been found in ticks of the family Ixodidae, including the genera Amblyomma, Dermacentor, Ixodes, and Rhipicephalus (Dumler et al. 2001). Ehrlichia spp. are obligatory intracellular bacteria that cause ehrlichiosis in animals and humans. In Thailand, several Ehrlichia spp. that are the etiologic agents of ehrlichiosis including E. canis, E. chaffeensis, and E. platys, have been reported (Parola et al. 2003; Pinyoowong et al. 2008). Lyme disease is a tick-borne disease caused by Borrelia burgdorferi, whose main vectors are Ixodes ticks, i.e., Ixodes ricinus and Ixodes scapularis. This bacterial pathogen has also been reported to be carried by other tick genera, namely Amblyomma, Dermacentor, Haemaphysalis, Hyalomma, and Rhipicephalus. Moreover, other members of the genus Borrelia are known to be the cause of human diseases, for example, B. afzelii and B. garinii (Margos et al. 2009).

Babesia spp. and Hepatozoon canis are tick-transmitted apicomplexan parasites that are causative agents found in dogs, cattle, and wild animal species (Baneth 2011; Gray et al. 2019). Babesiosis is caused by several Babesia spp. which are transmitted by hard ticks such as Rhipicephalus sanguineus, H. longicornis, and H. elliptica (Gray et al. 2019). Hepatozoon canis is the causative agent of hepatoponosisis widely distributed in many countries (Baneth 2011). Although the brown dog tick, R. sanguineus, is the main vector of H. canis, this pathogen also infects several other tick species, e.g., Amblyomma ovale, H. flava, H. longicornis, R. microplus, and R. turanicus (Baneth 2011; Demoner et al. 2013; Giannelli et al. 2017).

The study of pathogens that are transmitted by ticks will be useful for understanding the role of ticks as vectors of human and other animal disease agents. This information is crucial for effective monitoring and control of tick-borne diseases. Thus, the aim of this study was to investigate the presence of bacteria and protozoa in mammal ticks collected from several regions in Thailand using molecular approaches.

Materials and methods

Tick collection and identification

A total of 233 ticks were collected from domestic and road-killed mammals (23 hosts belonging to 7 species) in 8 provinces of Thailand during 2015–2019 (Table 2). All tick samples were kept in labeled collection vials individualized per host, containing 70% ethanol. They were identified morphologically by using a stereomicroscope following previously published taxonomic keys (Cooley 1946; Wassef and Hoogstraal 1984; Tanskul and Inlao 1989). Species of ticks were molecularly identified by partial sequencing of 16S rRNA gene (Black and Piesman 1994) to confirm their morphological identifications.

DNA extraction and PCR amplification

Each individual tick was rinsed in 10% sodium hypochlorite, 70% ethanol, and sterile distilled water three times (1 min each). Genomic DNA was extracted from individual adults, nymphs, pooled nymphs, and pooled larvae using the QIAamp DNA Extraction Kit for Tissue (QIAGEN) according to the manufacturer’s protocol. The presence of bacteria and protozoa, including Anaplasma, Borrelia, Coxiella, Ehrlichia, Rickettsia, Wolbachia, Babesia, and Hepatozoon in tick samples was initially screened by Polymerase Chain Reaction (PCR) using primers as shown in Table 1. For Rickettsia characterization, the primers Rr17.61p/Rr17.492n were initially used to amplify a 434 bp fragment of the 17-kDa antigen gene. Subsequently, the 17-kDa-positive samples were amplified and sequenced with primers specific to gltA, ompA, ompB, and sca4 genes (Webb et al. 1990; Regnery et al. 1991; Roux and Raoult 2000; Jiang et al. 2005). For Coxiella, the 16S rRNA positive samples were further amplified with primers targeting rpoB (DNA directed RNA polymerase beta) and GroEL (60 kDa chaperone heat shock protein B) genes (Duron et al. 2014, 2015). Additionally, the positive sample tested with EHR16SD/EHR16SR primers was amplified and sequenced with Anaplasma 16S rRNA primers as previously described by Zobba et al. (2014) (Table 1).

DNA sequencing and phylogenetic analysis

All positive amplicons were purified using the GF-1 Ambi Clean kit (Vivantis) according to the manufacturer’s instructions and sequenced in both directions on an ABI 3730xl DNA analyzer (Applied Biosystems). All obtained DNA sequences were assembled and edited using BioEdit (Alzhairy 2011). Edited sequences were assembled into a contig using SeqMan software (DNASTAR, Lasergene), and thus were subjected to BLASTn analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find sequence similarity to known sequences. Phylogenetic analyses were performed using the maximum parsimony method (PAUP v. 4.0b1) and bootstrap analysis was calculated with 1,000 replicates.

Results

Identification of ticks

A total of 23 mammals from 8 provinces of Thailand were examined for tick infestation. These mammals belonged...
| Target organism  | Target gene                        | Primer name   | Primer sequences (5'-3')                                      | Amplification fragment size (bp) | References                  |
|------------------|------------------------------------|---------------|--------------------------------------------------------------|---------------------------------|-----------------------------|
| *Rickettsia* spp. | 17-kDa antigen                     | Rr17.61p      | GCTCTTGGCAACTTCT ATGTT                                       | 434                             | Webb et al. 1990            |
|                  |                                    | Rr17.492n     | CATTTGTCGTCAAGTGG GGGCG                                      |                                 |                             |
| Citrate synthase (gliA) |                         | RpCS.877p     | GGGGGGCTGCTCAGG GC GG                                        | 381                             | Regnery et al. 1991         |
|                  |                                    | RpCS.1258n    | ATTGAAAAAAGTACA GTGAACA                                      |                                 |                             |
| 190-kDa protein antigen (ompA) |                              | Rr190.70p     | ATGCCGAATATTTTCCA AAA                                         | 532                             | Regnery et al. 1991         |
|                  |                                    | Rr190.602n    | AGTGACCTCCGCTCCCCCCT                                         |                                 |                             |
| 120-kDa protein antigen (ompB) |                              | RicF          | CAGCAAGTAATAAGTTT AATAC                                       | ~800                            | Hirunkanokpun et al. 2022   |
|                  |                                    | RicR          | GCTATAACGCGCTGTAACAG                                         |                                 |                             |
| 120-kDa cytoplasmic protein (sca4) |                              | RrD749F       | TGGTAGACCCTAAAAAG CTGTTG                                     | ~1,090                          | Jiang et al. 2005           |
|                  |                                    | RrD1826R      | TCTAAATKCTGCTG MATCAAT                                        |                                 |                             |
| *Coxiella* spp.  | 16S rRNA                           | 16S rRNA F    | GGGGAAGAAAAATGCTTC AAGGTAATATCCTT                            | 532                             | Almeida et al. 2012         |
|                  |                                    | 16S rRNA R    | TGCATCGAATAAACACG AGTCTCCACCGC                               |                                 |                             |
| rpoB             | CoxrpoBF2                          |               | GGGCGNCAYGGWAAY AAAGGSGT                                     | 607–610                         | Duron et al. 2015           |
|                  | CoxrpoBR1                          |               | CACCRAAHCGTGACGCCR CAAATIG                                    |                                 |                             |
|                  | CoxrpoBF3                          |               | TCGAAGAYATGCCYTATT TAGAAG                                     | 539–542                         |                             |
|                  | CoxrpoBR3                          |               | AGCTTTMCCACCCSARG GGTGCG                                      |                                 |                             |
| *GroEL*          | CoxGrF1                            |               | TTTGAAAAAYATG GGGCGKCAAATGTT                                  | 655                             | Duron et al. 2014           |
|                  | CoxGrR2                            |               | CGRTCRCRAAARCCA GGTGC                                        | 619                             |                             |
|                  | CoxGrF2                            |               | GAAGTGGGCTTCGCT ACWTCAGAG                                    |                                 |                             |
|                  | CoxGrFR1                          |               | CCAARCCAGGTGCT TTYYAC                                        |                                 |                             |
| Anaplasma- ceae  | 16S rRNA                           | EHR16SD       | GTTACCCYACAGAAGA AGTCC                                       | 345                             | Parola et al. 2000          |
|                  |                                    | EHR16SDR      | TAGCACTCAGTTTACA GC                                          |                                 |                             |
| *Anaplasma* spp. | 16S rRNA                           | AnaplsppF     | AGAAGAAGTCCCCGGC AAACCT                                       | ~800                            | Zobba et al. 2014           |
|                  |                                    | AnaplR3       | GAGACGACTTTTACGGGAT TAGCTC                                   |                                 |                             |
| *Babesia* spp.   | 18S rRNA                           | F             | GTTCTCAGTCGCCAGGC TTGAC                                      | 422–440                         | Hilpertshauer et al. 2006   |
|                  |                                    | R             | CAAGACAAAGTCTGCTG TTTGAC                                     |                                 |                             |
**Table 1** (continued)

| Target organism | Target gene | Primer name | Primer sequences (5'-3') | Amplification fragment size (bp) | References |
|-----------------|-------------|-------------|--------------------------|--------------------------------|------------|
| *Borrelia* spp. | 16S rRNA    | 16SF1       | ATAACGAAGAGTTTG ATCCTGGC | 1,350                          | Masuzawa et al. 1999 |
|                 |             | 16SR        | CAGCCGCACCTTTCCA GTACG  |                                |            |

**Table 2** PCR results of *Rickettsia*, *Coxiella*, and *Anaplasma* in mammal ticks collected from 8 provinces in Thailand

| Province         | Mammal host (No.) | Tick species (No.) | No. of samples tested | PCR results: No. of positive ticks |
|------------------|--------------------|--------------------|-----------------------|-----------------------------------|
|                  |                    |                    |                       | Rickettsia | Coxiella | Anaplasma |
| 1. Chiang Rai    | Dog (2)            | *Rhipicephalus sanguineus* (22) | 12M, 10F | - | - | - |
|                  | Cow (2)            | *Rhipicephalus microplus* (9) | 4M, 5F | - | - | - |
|                  | Asian palm civet (1) | *Haemaphysalis asiatica* (15) | 9M, 6F | - | 6M | - |
| 2. Loei          | Burmese ferret-badger (1) | *Haemaphysalis heinrichi* (6) | 1M | - | 1M | - |
|                  |                    |                     | 1M | 1M* | 1M* | - |
|                  |                    |                     | 2M, 2F | - | - | - |
|                  |                    | *Haemaphysalis hystricis* (1) | 1M | - | 1M | - |
|                  |                    | *Dermacentor auratus* (8) | 8N | - | - | - |
| 3. Roi Et        | Cow (1)            | *Haemaphysalis bispinosa* (11) | 5M, 6F | - | - | - |
|                  | Cow (1)            | *Rhipicephalus microplus* (15) | 4M, 2F | - | - | - |
|                  |                    |                     | 1PN (*n*=5) | - | - | - |
|                  |                    |                     | 1PN (*n*=4) | - | - | - |
| 4. Chumphon      | Dog (2)            | *Rhipicephalus sanguineus* (18) | 9M, 9F | - | - | - |
| 5. Surat Thani   | Dog (3)            | *Rhipicephalus sanguineus* (21) | 7M, 9F | - | - | - |
|                  |                    |                     | 1PN (*n*=5) | - | - | - |
| 6. Ranong        | Dog (2)            | *Rhipicephalus sanguineus* (10) | 6M, 4F | - | - | - |
|                  | Cow (1)            | *Rhipicephalus microplus* (15) | 4M, 11F | - | - | - |
| 7. Phang Nga     | Cow (2)            | *Rhipicephalus microplus* (32) | 14M, 18F | - | - | - |
|                  | Sheep (1)          | *Haemaphysalis bispinosa* (11) | 2M, 9F | - | - | - |
|                  | Goat (1)           | *Haemaphysalis bispinosa* (20) | 3M, 17F | - | - | - |
| 8. Satun         | Cat (1)            | *Haemaphysalis bispinosa* (15) | 1PN (*n*=3) | - | - | - |
|                  |                    |                     | 1PN (*n*=3) | - | - | - |
|                  |                    |                     | 1PL (*n*=5) | - | - | - |
|                  |                    |                     | 1PL (*n*=4) | - | - | - |
|                  | Sheep (1)          | *Haemaphysalis bispinosa* (2) | 2M | - | - | - |
|                  | Goat (1)           | *Haemaphysalis bispinosa* (2) | 1PN (*n*=2) | - | - | - |
| Total            | 23                 | 233                | 210                    | 1 | 8 | 1 |

*F* female; *M* male; *PN* pooled nymphs; *PL* pooled larvae; - PCR negative; * co-infection
to seven species: dogs (Canis lupus familiaris), cat (Felis catus), sheep (Ovis aries), goats (Capra aegagrus hircus), cattle (Bos primigenius taurus), Asian palm civet (Paradoxurus hermaphroditus), and Burmese ferret-badger (Melogale personata). In total, 233 tick specimens comprising seven species belonging to three genera were identified as follows: R. sanguineus (71/233; 30.5%), R. microplus (71/233; 30.5%), H. bispinosa (61/233; 26.2%), H. asiatica (15/233; 6.4%), H. heinrichi (6/233; 2.6%), H. hystricis (1/233; 0.4%), and Dermacentor auratus (8/233; 3.4%) (Table 2).

The results of tick molecular identifications were consistent with morphological identifications using taxonomic key. The partial mitochondrial 16S rRNA gene sequences of ticks were submitted to GenBank under accession numbers as follows: ON055731 (H. asiatica), ON062951 (H. hystricis), and ON074588 (H. heinrichi).

**Detection of tick-borne bacteria and protozoa in tick samples**

A total of 233 tick specimens were molecularly detected for bacterial and protozoal microorganisms by PCR technique. All tick samples collected from domestic mammals showed PCR negative results while some of the ticks collected from Burmese ferret-badger in Loei province and from Asian palm civet in Chiang Rai province were positive for Rickettsia (0.4%; 1/233) and Coxiella (3.4%; 8/233) (Table 2). Rickettsial DNA was detected in a male of H. heinrichi. Coxiella-like endosymbionts were detected in a male of H. hystricis (Cox-hys), six males of H. asiatica (Cox-asia), and a male of H. heinrichi (Cox-hein) which was co-infected with SFG Rickettsia sp. Interestingly, Anaplasma DNA was detected in another male of H. heinrichi infesting Burmese ferret-badger from Loei province (Table 2). None of the 233 mammal ticks gave specific PCR products of Borrelia, Ehrlichia, Wolbachia, Babesia, and Hepatozoon.

**DNA sequencing and phylogenetic analysis of tick-borne bacteria**

The partial sequences of Rickettsia sp. in a male of H. heinrichi were submitted to GenBank under accession numbers MW415893 (17-kDa antigen), MW415895 (gltA), MW415897 (ompA), OK031073 (ompB), and OK031072 (sca4). The DNA sequences of CLE detected in H. heinrichi (Cox-hein), H. hystricis (Cox-hys), and H. asiatica (Cox-asia) were submitted to GenBank under accession numbers: MW404679, MW404677, and MW404676 for partial 16S rRNA; OK031069, OK031070, and OK031071 for partial GroEL; OK031066, OK031067, and OK031068 for partial rpoB. The GenBank accession number of partial 16S rRNA of Anaplasma sp. detected from a male of H. heinrichi was MW405449.

The BLAST result of partial sequences of 17-kDa amplified from a male of H. heinrichi indicated a high nucleotide sequence similarity (99.08–99.54%) to SFG rickettsiae members, namely R. rhipicephali (MN477896), R. massiliae (KY069262), and R. raoultii (MW321554). Likewise, the BLAST result of partial gltA derived from the same tick showed high similarity (98.17–98.69%) to R. rhipicephali (KX018048), R. massiliae (KY640405), and other members of SFG rickettsiae, including R. raoultii, R. japonica, and R. heilongjiangensis. However, a more variable gene, ompA partial sequence obtained from this tick, was highly similar (98.12–98.31%) only to members of R. massiliae subgroup: R. rhipicephali (CP003342) and R. massiliae (MT309019, MW779485, etc.). Correspondingly, the results for partial ompB and sca4 sequences demonstrated the highest similarity to members of R. massiliae subgroup; 96.65% with R. rhipicephali (AF123719, CP003342) and 96.97% with R. massiliae (CP003319, DQ503429). Phylogenetic trees inferred from partial sequences of these genes revealed three groups of rickettsiae, comprising SFG, TRG, and TG. The SFG rickettsiae sequences utilized in phylogenetic analysis contained two subgroups: R. rickettsii and R. massiliae. Rickettsia sp. detected in this study was grouped in the SFG clade, clustered with members of the R. massiliae subgroup, including R. rhipicephali, R. massiliae, R. aeschimannii, Rickettsia sp. TwKM01, and Rickettsia sp. Bar29 (Fig. 1).

Based on three partial sequences of 16S rRNA, GroEL, and rpoB, all Coxiella spp. amplified from three Haemaphysalis tick species were CLE. In contrast, C. burnetii was not detected in this study. Nucleotide sequences of the three CLE (Cox-hein, Cox-hys, and Cox-asia) were different from each other. The BLAST results of these Coxiella partial 16S rRNA sequences indicated that their nucleotide sequences were highly similar to other CLE of several Haemaphysalis tick species represented in GenBank (98.52–100%). However, BLAST results of partial GroEL and rpoB sequences showed lower level of nucleotide sequence similarity to other CLE available in GenBank than that found with 16S rRNA. The nucleotide sequence similarity of CLE detected in this study was 89.42–93.64% for GroEL and 91.04–100% for rpoB. Phylogenetic analysis of partial 16S rRNA revealed that Cox-hein and Cox-hys in ticks removed from Burmese ferret-badger were grouped in the same subclade of clade D, while Cox-asia in ticks removed from Asian palm civet was placed in another subclade (Fig. 2). In contrast, the phylogenetic trees generated from the partial sequences of GroEL and rpoB, Cox-hein were more closely related to Cox-asia than to Cox-hys (Fig. 2). However, all three CLE detected in this study were grouped in clade D with other CLE of Haemaphysalis ticks from previous reports.
Anaplasma sp. was found only in a male of *H. heinrichi* using PCR with 16S rRNA primers. BLAST results of the partial sequences of 16S rRNA illustrated a remarkably high similarity (99.73%; 730/732 bp) to several strains of *A. bovis*, for example, Zhengxiaocon-goat-48 (MH255939), ZJ69 (KP062958), sika35 (LC060988), NR07 (AB196475), and many other strains. Based on phylogenetic analysis of partial 16S rRNA gene sequences, *Anaplasma* sp. detected in this study manifestly belonged to the same clade with *A. bovis* with strong bootstrap support (100%) (Fig. 3). The results of BLAST and phylogenetic analysis thus explicitly demonstrated that a male of *H. heinrichi* was infected with *Anaplasma* sp. closely related to *A. bovis*.

**Discussion**

Ticks serve as vectors and/or reservoirs of several pathogens such as *Anaplasma*, *Coxiella*, *Ehrlichia*, and *Rickettsia* (Walker and Yu 2012). To better understand tick-associated microorganisms among various mammal ticks in Thailand, we conducted specific molecular screening for the presence of bacterial and protozoal microorganisms. Previous studies showed that *Rickettsia* spp. were widely distributed throughout Thailand in various locations, hosts, and in tick species, including *H. ornithophila*, *Amblyomma testudinarium*, *H. shimoga*, *H. lagrangei*, *A. helvolum*, and *A. varanense* (Hirunkanokpun et al. 2003; Ahantarig et al. 2011; Sumrander et al. 2014; Malaisri et al. 2015; Noorooong et al. 2018). In this study, a SFG *Rickettsia* sp. was detected in a male of *H. heinrichi* infesting Burmese ferret-badger in Loei province, northeastern Thailand. The members of SFG rickettsiae have been categorized into four subgroups: *R. rickettsii*, *R. massiliae*, *R. helvetica*, and *R. akari* (Merhej and Raoult 2011). Our results revealed five partial rickettsial sequences of 17-kDa, *gltA*, *ompB*, and *sca4* genes that were amplified and sequenced from a *H. heinrichi* tick. The analysis of partial 17-kDa and *gltA* sequences indicated that the *Rickettsia* sp. was a member of SFG rickettsiae. However, these two genes could not differentiate whether it belonged to the *R. massiliae* or *R. rickettsii* subgroups. Since 17-kDa and *gltA* genes are highly conserved among SFG rickettsiae, previous studies have suggested that less conservative rickettsial genes such as *ompA*, *ompB*, and *sca4* should be more suitable for the comparisons of closely related SFG species (Robinson et al. 2019). Therefore, these gene sequences were used for characterization of *Rickettsia* sp. in this study. BLAST and phylogenetic analyses of the partial *ompA*, *ompB*, and *sca4* genes showed that the detected SFG *Rickettsia* sp. found in this study was a member of *R. massiliae*.
Fig. 2 Maximum parsimony tree of *Coxiella* spp. based on the partial sequences of 16S rRNA (a), *GroEL* (b), and *rpoB* (c) genes. The bootstrap values were shown above or near the branch. *Coxiella* endosymbionts detected in this study are indicated in bold. Three clades of *Coxiella* are indicated as A, B, and D which were originally defined by Duron et al. 2015.

Fig. 3 Maximum parsimony tree of *Anaplasma* spp. based on the partial 16S rRNA gene sequences. The bootstrap values are shown above the branch. The *Anaplasma* sp. sequence obtained in present study is indicated in bold.
subgroup and was closely related to both *R. rhipicephali* and *R. massiliae*. However, it remains uncertain whether this *Rickettsia* sp. is pathogenic *R. massiliae* or non-pathogenic *R. rhipicephali*. Further characterizations by amplification and sequencing of additional genes, such as *sco2* and *GroEL*, are required to clarify this point. In Thailand, a *Rickettsia* sp. closely related to members of the *R. massiliae* subgroup has been reported in *H. lagrangei* collected from sambar deer (Sumrandee et al. 2016). We reported herein for the first time SFG *Rickettsia* sp. belonging to the *R. massiliae* subgroup in *H. heinrichi* infesting a wild Burmese ferret-badger.

The distribution and diversity of tick species may be crucial to the investigation of tick-borne diseases. Cornet et al. (2009) indicated that *Haemaphysalis* was one of the most common genera widely distributed throughout Thailand and was capable of transmitting tick-borne diseases to humans. Humans bitten by infected *Haemaphysalis* ticks are consequently exposed to a high risk of bacterial infection. *Haemaphysalis heinrichi* was previously found infesting four species of mammal hosts: *Arctonyx collaris*, *Bos domesticus*, *Canis familiaris*, and *Melogale personata* in Chiang Mai, Chiang Rai, Khon Kaen, Nak hon Ratchasima, Prachinburi, and Ubon Ratchathani (Tanskul et al. 1983). Our findings have provided additional evidence of *H. heinrichi* infesting Burmese ferret-badger in Loei province, northeastern Thailand. Further study of the abundance and distribution of *H. heinrichi* and related tick species and the prevalence of bacterial infection will provide a better understanding of the epidemiology of rickettsioses and other tick-borne diseases in Thailand.

*Coxiella*-like endosymbionts have frequently been found in ticks (Ahantarig et al. 2011; Almeida et al. 2012; Arthan et al. 2015; Duron et al. 2015). Multilocus sequence analysis of five *Coxiella* housekeeping genes indicated that *Coxiella* endosymbiont in ticks served as the common ancestor of *C. burnetii* (Duron et al. 2015). The endosymbiont may play a vital role in providing vitamin and cofactor biosynthesis pathways and in defining the reproductive fitness of tick hosts (Smith et al. 2015). Consequently, understanding its role in sustaining growth and survival of ticks may provide new methods for control and management of tick populations. Q fever and seroprevalence of *C. burnetii* have been reported in rural areas of Malaysia and Thailand (Suputtamongkol et al. 2003; Bina Rai et al. 2011). In Malaysia, both *C. burnetii* and *Coxiella* endosymbiont were reported in *H. hystricis* ticks collected from the same wild boar. In this study, *C. burnetii* was not detected in all tick samples and *CLE* were detected solely in one (*Haemaphysalis*) of three analyzed tick genera. We identified three different types of CLE, namely Cox-hein in *H. heinrichi*, Cox-hys in *H. hystricis*, and Cox-asia in *H. asiatica*. Phylogenetic analysis with five concatenated gene sequences by Duron et al. (2015) showed that the genus *Coxiella* was divided into four clades (A-to-D) corresponding to the host genera. In this study, the phylogenetic trees generated from partial 16S rRNA, *GroEL*, and *rpoB* gene sequences demonstrated that the three CLE were clustered with those of *Haemaphysalis* ticks and were placed in clade D. The result of CLE partial 16S rRNA sequences obtained in this study was in accordance with previous authors who suggested that CLE detected from the same host genera were clustered within the same clade (Khoo et al. 2016; Trinachartvanit et al. 2018). However, *GroEL* and *rpoB* gene sequences did not cluster together with those previously reported in the same host genera (Khoo et al. 2016; Trinachartvanit et al. 2018). In Thailand, co-infections of *Rickettsia* and *Coxiella* have been reported in *H. lagrangei* and *A. testudinarium* (Nooroong et al. 2018; Sumrandee et al. 2016). Our results have also demonstrated the first case of co-infection of CLE and SFG *Rickettsia* sp. closely related to *R. massiliae* subgroup in *H. heinrichi* infesting Burmese ferret-badger. Interestingly, many wild animals have been reported as reservoirs of pathogenic bacterial agents of humans, including *A. phagocytophilum*, *C. burnetii*, and *Rickettsia* spp. (Meerburg and Reusken 2011; Silaghi et al. 2014). Further investigation in this field of research will provide more information on the prevalence of tick-borne pathogens in various tick species infesting wild mammals in Thailand before any conclusion can be made.

Anaplasmosis is an infectious hemotropic disease of cattle, sheep, goats, and other ruminants worldwide. Several *Anaplasma* species are causative agents of anaplasmosis in ruminants, e.g., *A. phagocytophilum*, *A. marginale*, and *A. bovis* (Inokuma 2007). In Thailand, *Anaplasma* spp. have been reported in both domestic and wild mammals and their associated ticks, including *A. platys* which have been detected from blood of mammal hosts i.e., cats, dogs, rodents, sambar deer, and wild boar, or from their parasitic ticks (Parola et al. 2003; Pinyoowong et al. 2008; Foongladda et al. 2011; Salakij et al. 2012; Sumrandee et al. 2016). *Anaplasma marginale* and *A. phagocytophilum* were found in water buffalo and ticks collected from vegetation, respectively (Nguyen et al. 2020; Nooroong et al. 2018); *A. bovis* has been reported in ticks infesting bear, sambar deer, rodents, and ticks collected from vegetation (Parola et al. 2003; Malaisri et al. 2015; Sumrandee et al. 2016; Takhampunya et al. 2019). In this study, an *Anaplasma* sp. closely related to *A. bovis* was detected in *H. heinrichi* removed from Burmese ferret-badger. Thus, the results of the present and previous studies in Thailand demonstrated that *A. bovis* were commonly found in ticks parasitizing domestic and wild mammals.

Ixodid ticks of the genera *Ixodes*, *Dermacentor*, *Rhipicephalus*, and *Amblyomma* are the main vectors of *Anaplasma* bacteria (Dumler et al. 2001). However, this
bacterium has also been found in *Haemaphysalis* ticks in Asia and North America (Goethert and Telford 2003; Kim et al. 2003; Qin et al. 2018; Fukui and Inokuma 2019). In Thailand, *Anaplasma* spp. have been detected in ticks such as *A. platys* in *D. auratus* (Parola et al. 2003) and *R. sanguineus* (Foongladda et al. 2011); *A. phagocytophilum* in *D. auratus* (Nooroong et al. 2018); *A. bovis* in *H. lagrangei* (Parola et al. 2003; Sumrandee et al. 2016), *H. shimoga* (Malaisri et al. 2015), *H. obesa* (Sumrandee et al. 2016), and *H. bandicota* (Takhampunya et al. 2019). In this study, the presence of *Anaplasma* sp. closely related to *A. bovis* in *H. heinrichi* has been demonstrated by sequence and phylogenetic analysis of partial *Anaplasma* 16S rRNA. Our findings support previous studies in Thailand and other countries in Asia in which *A. bovis* is mostly found in *Haemaphysalis*, and this tick genus also associates with the transmission of *Anaplasma* (Goethert and Telford 2003; Parola et al. 2003; Lee and Chae 2010; Yoshimoto et al. 2010; Malaisri et al. 2015; Sumrandee et al. 2016). To our knowledge, this is the first report of *Anaplasma* sp. closely related to *A. bovis* in *H. heinrichi* tick-infested Burmese ferret-badger.

In summary, we identified tick-borne bacteria among various mammal ticks collected from 8 provinces of Thailand. Based on DNA sequencing and phylogenetic analyses, we found SFG *Rickettsia* sp. in the *R. massiliae* subgroup in *H. heinrichi* and three CLE, namely Cox-hein, Cox-hys in *H. heinrichi* and *H. hystricis* ticks infesting a Burmese ferret-badger, and Cox-asia in *H. asiatica*. Co-infection of SFG *Rickettsia* sp. and CLE (Cox-hein) was detected in *H. heinrichi*. This study also provided the first evidence for the presence of an *Anaplasma* sp. closely related to *A. bovis* in a *H. heinrichi* tick. Our results extended the knowledge of geographic distribution of ticks parasitizing different species of mammals and vector-borne bacteria in Southeast Asia.

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**Authors’ contributions** All authors contributed to the study conception and design. AA, SH, PR and PP carried out the field collection of samples. The majority of the laboratory works were carried out by SH, AA, and WT. SH wrote the manuscript with guidance from VB. All authors discussed the results and commented on the previous version as well as approved the final versions of this manuscript.

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**Data availability** All data included in this study are available on request to the corresponding author.

### Declarations

**Competing interests** The authors declare no competing interests.

**Ethical approval** All applicable institutional, national and international guidelines for the care and use of animals were followed.

**Consent to participate** Not applicable.

**Consent for publication** All authors read and approved the final manuscript.

**Conflict of interest** The authors declare no conflict of interest.

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