Diversity and phylogenetic relationships among *Bartonella* strains from Thai bats

Clifton D. McKee1,2*, Michael Y. Kosoy1, Ying Bai1, Lynn M. Osikowicz1, Richard Franka3, Amy T. Gilbert3,4, Sumalee Boonmar5, Charles E. Rupprecht6, Leonard F. Peruski7

1 Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, United States of America, 2 Department of Biology, Colorado State University, Fort Collins, CO, United States of America, 3 Division of High-Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA, United States of America, 4 National Wildlife Research Center, USDA/APHIS/Wildlife Services, Fort Collins, CO, United States of America, 5 Faculty Sciences and Public Health, Rajapruk University, Nonthaburi, Thailand, 6 LYSSA LLC, Lawrenceville, GA, United States of America, 7 Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

* xmm9@cdc.gov

Abstract

Bartonellae are phylogenetically diverse, intracellular bacteria commonly found in mammals. Previous studies have demonstrated that bats have a high prevalence and diversity of *Bartonella* infections globally. Isolates (*n* = 42) were obtained from five bat species in four provinces of Thailand and analyzed using sequences of the citrate synthase gene (*gltA*). Sequences clustered into seven distinct genogroups; four of these genogroups displayed similarity with *Bartonella* spp. sequences from other bats in Southeast Asia, Africa, and Eastern Europe. Thirty of the isolates representing these seven genogroups were further characterized by sequencing four additional loci (*ftsZ*, *nuoG*, *rpoB*, and ITS) to clarify their evolutionary relationships with other *Bartonella* species and to assess patterns of diversity among strains. Among the seven genogroups, there were differences in the number of sequence variants, ranging from 1–5, and the amount of nucleotide divergence, ranging from 0.035–3.9%. Overall, these seven genogroups meet the criteria for distinction as novel *Bartonella* species, with sequence divergence among genogroups ranging from 6.4–15.8%. Evidence of intra- and intercontinental phylogenetic relationships and instances of homologous recombination among *Bartonella* genogroups in related bat species were found in Thai bats.

Introduction

Of the emerging infectious diseases in humans, most are zoonoses originating in wildlife [1,2]. Thus, surveillance and characterization of zoonotic infections is fundamental to protecting public health and understanding infectious disease ecology. Following the discovery that numerous severe viral infections are linked to bats [3], efforts to detect and understand the dynamics of viral, bacterial, and fungal zoonotic infections of bats have increased in recent years.
One genus of bacteria, *Bartonella*, is frequently found in wildlife. Bartonellae (Rhizobiales, Alphaproteobacteria) are fastidious, facultative, hemotropic bacteria that infect a variety of mammalian groups, including ungulates, rodents, carnivores, and primates [4]. Hematophagous arthropods (e.g., flies, ticks, fleas, and mites) are believed to be the primary vectors of *Bartonella* spp. infections [5,6] and studies have demonstrated the competence of a small number of vector species [7–11]. Over 30 *Bartonella* species have been named and characterized, and candidate species are being discovered as new mammal species and their ectoparasites are sampled. Among the named *Bartonella* species, approximately half have now been identified as zoonotic and can cause a wide spectrum of illnesses in people ranging from self-limiting fever to endocarditis [12–14]. Given the potential health impacts of bartonellae and increasing risk of zoonoses due to human interaction with wildlife and their vectors, surveillance for new *Bartonella* species, especially in new mammalian groups and geographic regions, is important to capture the substantial diversity of this genus and to study the natural cycles of transmission in the hosts.

*Bartonella* spp. infections have now been found in over 60 bat species representing over 40 genera, 11 families, and both suborders from Central and South America, Africa, Europe, and Southeast Asia. Diversification of *Bartonella* species in bats appears to have followed the diversification of bats, with clades of *Bartonella* spp. confined to particular bat families, superfamilies, and suborders [15,16]. Very recent studies of *Bartonella* spp. in bats from Algeria [17], Madagascar and the Union of Comoros [18], French Guiana [19], Saint Kitts [20], South Africa [21], the Republic of Georgia [22], China [23], France and Spain [24], the United States [25], Argentina [26], and Brazil [27] have only added to this substantial diversity. Despite apparent phylogenetic patterns linking bats and bat-associated bartonellae, there is evidence that spillover of bartonellae from bats into other mammals is possible, particularly to humans and dogs [22,28–32].

In the present study, we characterize the diversity of *Bartonella* spp. found in bats sampled in Thailand. Thailand possesses a high diversity of mammal species, particularly bats, many of which may carry zoonotic infections [33]. Bats and their ectoparasites within the region (e.g., China, Laos, Vietnam, Taiwan, and Malaysia) have been laboratory-confirmed to harbor bartonellae [23,32,34,35]. We hypothesized that *Bartonella* species identified in Thailand may likely have phylogenetic relationships with *Bartonella* species previously identified in related bat hosts. We utilized gene sequencing of the citrate synthase gene (*gltA*) and multi-locus sequence typing (MLST) of four additional loci to characterize *Bartonella* spp. isolates from five bat species from four regions in Thailand. MLST is an approach frequently applied to distinguish among *Bartonella* species and epidemiologically relevant strains [36–38]. Utilizing multiple loci can elucidate distant evolutionary relationships, clonal stability, and recombination events among *Bartonella* species [38,39]. This study expands our knowledge of the host range and diversity of *Bartonella* spp. in bats from Southeast Asia and enriches our understanding of the evolutionary history of this diverse genus globally.

### Materials and methods

#### Bartonella spp. cultures

The current study is based on comparative characterization of 42 cultures of *Bartonella* species (Table 1) selected from isolates obtained from whole blood of bats collected in four Thai provinces: Chiang Rai (north), Kamphaeng Phet (west), Khon Kaen (northeast), and Sa Kaeo (east), as described [40]. Captured bats were anesthetized by intramuscular injection of ketamine hydrochloride (0.05–0.1 mg/g body weight) and euthanized under sedation in accordance with the field protocol approved by the CDC Institutional Animal Care and Use Committee; the
CDC IACUC also specifically approved this study. The Supplementary Material (S1 Text) contains additional details regarding sampling locations (Fig A in S1 Text), bat capture, species distributions (Fig B in S1 Text), culturing procedures, and infection prevalence among species and locations (Table A in S1 Text). Bartonella spp. bacteria were cultured from blood of 34 (36.6%) of 93 bats distributed among all four provinces and representing five bat species: the wrinkle-lipped free-tailed bat (*Chaerephon plicatus*, Molossidae), the great roundleaf bat (*Hipposideros armiger*, Hipposideridae), the fulvus roundleaf bat (*H. fulvus*), the intermediate roundleaf bat (*H. larvatus*), and the black-bearded tomb bat (*Taphozous melanopogon*, Emballonuridae).

**DNA purification and multi-locus sequence typing (MLST)**
A suspension of pure cultured isolate was heated to 95°C for 10 min followed by 1 min centrifugation at 8000 rpm for the lysed cells to precipitate. The supernatant was then moved to a clean microcentrifuge tube for storage until examination. Isolates were initially verified as *Bartonella* spp. and genotyped by PCR amplification of a fragment of the citrate synthase gene.

| Host         | Province       | Isolate | ftsZ | gltA | ITS | nuoG | rpoB | ST | Genogroup |
|--------------|----------------|---------|------|------|-----|------|------|----|-----------|
| *C. plicatus*| Sa Kaeo        | SK128   | 1    | 1    | 1   | 1    | 1    | ST1| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK130   | 1    | 1    | 1   | 1    | 1    | ST1| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK144   | 1    | 1    | 1   | 1    | 1    | ST1| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK157   | 1    | 1    | 1   | 1    | 1    | ST1| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK166   | 1    | 1    | 1   | 1    | 1    | ST1| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK168   | 1    | 1    | 1   | 1    | 1    | ST1| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK189   | 1    | 1    | 1   | 1    | 1    | ST1| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK191   | 1    | 1    | 1   | 1    | 1    | ST1| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK202   | 1    | 1    | 1   | 1    | 1    | ST1| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK170   | 1    | 2    | 1   | 1    | 1    | ST2| Cp1       |
| *C. plicatus*| Sa Kaeo        | SK194   | 2    | 3    | 2   | 2    | 2    | ST3| Cp2       |
| *C. plicatus*| Sa Kaeo        | SK197   | 2    | 3    | 3   | 3    | 2    | ST4| Cp2       |
| *C. plicatus*| Sa Kaeo        | SK163   | 3    | 4    | 4   | 3    | 3    | ST5| Cp3       |
| *C. plicatus*| Sa Kaeo        | SK165   | 3    | 4    | 5   | 3    | 3    | ST6| Cp3       |
| *C. plicatus*| Sa Kaeo        | SK180   | 3    | 4    | 4   | 3    | 3    | ST5| Cp3       |
| *C. plicatus*| Sa Kaeo        | SK198a  | 3    | 4    | 4   | 3    | 3    | ST5| Cp3       |
| *H. armiger* | Khon Kaen      | KK182   | 4    | 5    | 6   | 5    | 4    | ST7| H3        |
| *H. larvatus*| Kamphaeng Phet | KP270   | 4    | 6    | 6   | 4    | 4    | ST8| H3        |
| *H. larvatus*| Kamphaeng Phet | KP215   | 5    | 5    | 7   | 4    | 5    | ST9| H3        |
| *H. fulvus*  | Chiang Rai     | CR224   | 6    | 7    | 8   | 6    | 6    | ST10| H3        |
| *H. larvatus*| Kamphaeng Phet | KP277   | 7    | 8    | 9   | 7    | 7    | ST11| H2        |
| *Hipposideros sp.* | Sa Kaeo | KP174 | 8    | 9    | 10  | 4    | 8    | ST12| H1        |
| *H. larvatus*| Kamphaeng Phet | KP287a  | 9    | 9    | 10  | 8    | 7    | ST13| H1        |
| *H. larvatus*| Kamphaeng Phet | KP216a  | 8    | 9    | 10  | 8    | 8    | ST14| H1        |
| *H. larvatus*| Kamphaeng Phet | KP268a  | 8    | 9    | 10  | 8    | 8    | ST14| H1        |
| *H. larvatus*| Kamphaeng Phet | KP292   | 8    | 9    | 10  | 8    | 8    | ST14| H1        |
| *H. armiger* | Khon Kaen      | KK200a  | 8    | 9    | 10  | 8    | 9    | ST15| H1        |
| *H. larvatus*| Kamphaeng Phet | KK290   | 8    | 9    | 10  | 8    | 9    | ST15| H1        |
| *H. larvatus*| Kamphaeng Phet | KP293a  | 9    | 9    | 10  | 8    | 8    | ST16| H1        |
| *T. melanopogon* | Kamphaeng Phet | KP283b  | 10   | 10   | 11  | 9    | 10   | ST17| Tm        |

Genogroups were determined using a combination of sequence typing, phylogenetic analysis, and population clustering.

[https://doi.org/10.1371/journal.pone.0181696.t001](https://doi.org/10.1371/journal.pone.0181696.t001)
(gltA) following previous studies [41,42]. PCR products were separated by 1.5% gel electrophoresis and visualized by ethidium bromide staining. Positive PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Valencia, CA) according to manufacturer protocols and sequenced in both directions with the same primers on an Applied Biosystems Model 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were first verified as _Bartonella_ spp. DNA using BLAST (National Center for Biotechnology Information, Bethesda, MD) and closely matching sequences (>95% sequence identity) were downloaded as references.

Further characterization of 30 isolates from _Hipposideros_ spp., _C. plicatus_, and _T. melanopogon_ was performed using MLST of four additional loci (ftsZ, nuoG, rpoB, and ITS) previously used for characterization of _Bartonella_ strains [38,39,41,43–46]. Primers and associated references for protocols are provided in Table 2. PCR purification, sequencing, and alignment used the same methods as with gltA. All sequences were aligned with MAFFT v7.187 using the local, accurate L-INS-I algorithm [47], trimmed to equal lengths with Gblocks v0.91b [48], and compared with other _Bartonella_ strains from bats, bat ectoparasites, and known _Bartonella_ species (Table B in S1 Text).

Each unique variant was assigned to a sequence type (ST) based on the allelic profile (Table 1). Nucleotide polymorphisms and diversity of the five MLST loci were examined in MEGA v7.0.21 [49] among all 17 STs. The correct open reading frame for protein coding loci (ftsZ, gltA, nuoG, and rpoB) was determined by checking all starting positions and choosing the frame that contained no premature stop codons. Ratios of non-synonymous to synonymous substitutions (dN/dS) were calculated using the Nei-Gojobori method for each of the loci to check for evidence of selection. Nucleotide diversity (π) and mean pairwise sequence distance were estimated for the five loci separately and for concatenated sequences; sequence distances were calculated as the number of substitutions per site. ITS sequences contained gap regions, so the total lengths of sequences for this locus and concatenated sequences include gaps.

### Phylogenetic analysis

The optimal substitution model was selected with jModelTest v2.1.6 [50] using Akaike’s information criterion corrected for finite sample sizes (AICc) [51]. The generalized time-reversible model with four gamma-distributed rate categories and a proportion of invariant sites (GTR method).
+Γ+I) was the best available model for all loci. Separate maximum likelihood (ML) phylogenetic trees for each locus (gltA, ftsZ, nuoG, rpoB, and ITS) were created for the 30 isolates characterized by multiple loci to visualize clustering of strains and to identify potential recombination events among loci. Trees were generated without reference sequences with RAxML v8.2.10 [52] using 1000 bootstrap replicates to estimate node support.

A Bayesian phylogeny of gltA sequences from Thai bats, known Bartonella species, and select Bartonella strains from bats and their ectoparasites from the Americas [19,25,34,53], Europe [22,31,54,55], Africa [18,38,42], and Southeast Asia [23,32,35]. This selection of bat-associated Bartonella strains is representative of phylogenetic clades found in previous phylogenies of gltA sequences from bats [16,22]. Closely matching sequences (>95% sequence identity) based on the initial BLAST search from free-ranging dogs from Thailand [28] and other bats and ectoparasites from Vietnam, Laos, and Malaysia [34,35] were included in the phylogenetic analysis. The gltA phylogeny was inferred by Markov chain Monte Carlo (MCMC) sampling in BEAST v1.8.4 [56,57] using the GTR+Γ+I model. Well-supported clades (posterior probability > 0.9) of bat-associated Bartonella strains were collapsed within the gltA phylogeny and labeled with the host families and countries represented in the clade to reduce complexity.

A second Bayesian tree was generated from concatenated sequences of five loci (ftsZ, gltA, nuoG, rpoB, and ITS) from Thai bats (30 isolates), known Bartonella species, and other Bartonella strains from bats [31,32,38,42]. The selection of bat strains for the multi-locus phylogeny was taken from the same set of strains used in the gltA analysis, with the added restriction that the strains had been characterized by at least two of the loci we had sequenced from Thai bats. This restricted set contained strains from the Americas [19,25], Europe [22,31,55], Africa [38,42], and Southeast Asia [32] and is representative of clades found in previous multi-locus phylogenies of bat-associated Bartonella species [22,38]. For the multi-locus phylogeny, we used separate partitions for each of the five loci; each locus was analyzed under the GTR+Γ+I substitution model, but parameters were allowed to vary for each partitioned locus. All loci were linked with the same clock model and speciation model. GenBank accession numbers for all sequences used in the gltA and multi-locus phylogenetic analyses are listed in the Supplementary Material (Table B in S1 Text).

For both BEAST phylogenies, we set the number of MCMC iterations to 2×10⁸, sampling every 2×10⁴th iteration. No codon partitions were used for either the gltA or multi-locus analyses due to the short sequence length of all loci which could substantially reduce the effective sample size of estimated parameters for each codon position. A strict molecular clock was chosen for both phylogenies because we did not seek to accurately estimate branch times. Additionally, all of the isolates from Thai bats were cultured around the same date and therefore could not be used to calibrate another clock model. We chose to use the birth-death model with incomplete sampling to represent patterns of speciation in the phylogeny [58]. All priors were kept at the default, diffuse settings for both the gltA and multi-locus analyses (see S1 Text for details). Three separate chains were run and effective sample sizes (ESS) and mixing of parameters during MCMC sampling were assessed using Tracer v1.6 [56]. Chains were then combined and the maximum clade credibility tree was found using TreeAnnotator [56,57].

Recombination and admixture analysis

To assess the level of recombination among sequence types, a phylogenetic network was inferred using the Neighbor-Net algorithm in SplitsTree v4.13.1 [59] from concatenated sequences of all five loci (ftsZ, gltA, nuoG, rpoB, and ITS) from the 30 Bartonella isolates from Thai bats evaluated by MLST. The pairwise homoplasy index [60] was calculated in SplitsTree to test for significant recombination among the sequence types. Bayesian population clustering
was performed with STRUCTURE v2.3.4 [61] using concatenated sequences of all five loci from the 30 isolates evaluated by MLST. The program was run five times for each value of K (the number of population clusters) ranging from 3–10 for \(5 \times 10^4\) iterations and \(5 \times 10^4\) burn-in iterations using the admixture model with correlated allele frequencies. Convergence of MCMC chains for each run was assessed by visual analysis of trace diagrams for all measured parameters. The optimal value of K was estimated according to the \(\Delta K\) method [62] with STRUCTURE HARVESTER v0.6.94 [63]. We did not evaluate K = 2 due to our prior observation of at least three distinct clades in the multi-locus phylogeny based on host genus (Hipposideros spp., Chaerephon sp., and Taphozous sp.) and a recently observed bias towards the selection of K = 2 as the optimal number of populations in studies that use the \(\Delta K\) method [64].

**Nucleotide sequence accession numbers**

Unique alleles from this study were submitted to GenBank with the following accession numbers: KY232154 to KY232182 and MF288092 (ftsZ), KY232183 to KY232224 (gltA), KY232254 to KY232282 and MF288099 (nuoG), KY232283 to KY232311 and MF288103 (rpoB), and KY232225 to KY232253 and MF288133 (ITS).

**Results**

**Analysis of gltA genotypes**

Initial phylogenetic analysis based on gltA sequences (Fig 1) demonstrated the presence of three genogroups found in Hipposideros spp. bats (H1-3), three other genogroups found in Chaerephon plicatus (Cp1-3), and a distinct genogroup in Taphozous melanopogon (Tm). Posteriors distributions for the gltA tree likelihood and all estimated parameters of the substitution model and the birth-death speciation model converged and had sufficient effective sample sizes (ESS > 200) for each of the three chains separately and combined. Sequences from one genogroup (H1) from Thai H. armiger and H. larvatus were nearly identical (>99% sequence identity) to sequences found in H. armiger and H. larvatus from Vietnam [35]. This genogroup formed a well-supported clade (posterior probability = 1) with other sequences from hipposiderid and rhinolophid bats and their bat flies from Vietnam [35], Malaysia [34], Kenya [42], and Georgia [22]. Another group of sequences from H. larvatus (H2) formed a clade (posterior probability = 0.93) with Bartonella genotypes from Megaderma lyra in Vietnam [35], Hipposideros vittatus (previously identified as H. commersoni) from Kenya [42,65], and community dogs from Thailand [28], with sequence identities ranging from 88–90% in this clade. The third genogroup (H3) from H. armiger, H. fulvus, and H. larvatus clustered (88–90% sequence identity) with Bartonella species found in pteropodid bats: Bartonella species found in Eidolon helvum from Africa [38,42], sequences from bat flies collected from Pteropus hypomelanus in Malaysia [34], and bat flies from Ptenochirus jagori and Harpyionycteris whiteheadi in the Philippines [34]. However, the posterior support for this clade was only 0.45 based on data from gltA sequences alone.

Two genogroups found in C. plicatus (Cp1 and Cp2) are closely related (94% sequence identity) to each other and formed a well-supported clade (posterior probability = 1). These two genogroups were more distantly related (87% sequence identity) to the third cluster (Cp3). Finally, the single genogroup from T. melanopogon (Tm) is very closely related (95% sequence identity) to the Bartonella strain found in Coleura afra, another emballonurid bat, in Kenya [42]. These two groups form a well-supported clade (posterior probability = 1) with Bartonella species from African pteropodid bats (Eidolon helvum and Rousettus aegyptiacus) [42]. Sequence divergence was ≤3.1% within a genogroup and 6.2–16.2% among genogroups.
Fig 1. Phylogenetic relationships among citrate synthase (*gltA*) sequences of *Bartonella* strains from Thai bats. The phylogeny was inferred by Bayesian analysis in BEAST using the GTR+Γ+I substitution model and a birth-death speciation.
All of these separate clusters are sufficiently distinct from one another based on gltA sequences (<96% DNA similarity) to be considered putative new *Bartonella* species [66]. However, as we acknowledged above, genogroup H1 appears to have been discovered previously in *H. armiger* and *H. larvatus* from Vietnam [35], but was not cultured or characterized by additional genetic loci.

### Allelic profiles and sequence types

Based on allelic profiling, the MLST analysis distinguished 17 sequence types (ST) among the 30 isolates (Table 1). All five sequenced loci distinguished either eight or nine alleles among the isolates. Genogroups Cp1–3 contained isolates from only *C. plicatus*. Genogroup Cp1 was almost entirely clonal with nine isolates characterized as ST1 and a single isolate of ST2; the distance among STs based on concatenated sequences of the five loci was 0.036%. Genogroup Cp2 had two distinct isolates, characterized as ST3 and ST4, with a distance of 0.035% among STs. Genogroup Cp3 was also nearly clonal, with three isolates characterized as ST5 and one isolate as ST6; the distance among STs was 0.14%.

Genogroup H1 was comprised of isolates from *H. armiger*, *H. larvatus*, and another *Hippposideros* sp. bat. This group was the most variable, with five distinct STs (ST12–16) with a maximum sequence distance of 3.9%. Genogroup H2 from *H. larvatus* was a single, distinct type (ST11). Genogroup H3 had isolates from *H. armiger*, *H. fulvus*, and *H. larvatus* with four distinct sequence types (ST7-10) with a maximum sequence distance of 2.0%.

Some of the unique sequence types arose from apparent homologous recombination events among genogroups, highlighted in the individual gene trees (Figs C-G in S1 Text). One strain from *H. larvatus* (isolate KP287a, ST13) clustered with genogroup H1 for all loci except for *rpoB* where it clustered with genogroup H2. Another strain from the *Hippposideros* sp. bat (isolate KP174, ST12) clustered with genogroup H1 for all loci except *nuoG* where it clustered with genogroup H3. Even with these recombinant strains, genogroups remained distinct, with ≤3.9% sequence distance within a genogroup and 6.4–15.8% distance among genogroups.

### Patterns of selection and diversity in nucleotide sequences

The five analyzed loci revealed different levels of variation over the length of sequenced fragments (Table 3), ranging from 21.6% variable sites for *ftsZ* to 45.3% for ITS. Mean pairwise sequence distances ranged from 8.3% for *ftsZ* to 22.8% for ITS. Nucleotide diversity showed a similar pattern, with values ranging from 8.0% for *ftsZ* to 12.7% for ITS. Based on concatenated sequences from all five loci, there were 895 (28.7%) variable sites among the 30 STs over the length of the alignment with 9.5% nucleotide diversity and a mean pairwise distance of 11.0%. Calculated dN/dS ratios from protein coding loci were generally low, ranging from 0.03 for *ftsZ* to 0.09 for *gltA*, indicating that purifying selection is dominant for these genes.

### Phylogenetic analysis of multiple loci

The Bayesian tree assembled by partitioned analysis of *ftsZ*, *gltA*, *nuoG*, and *rpoB* sequences (Fig 2) clarified the phylogenetic position of the seven genogroups identified by gltA sequences.
relative to other *Bartonella* strains associated with bats. As with the gltA analysis, the posterior distributions for all relevant model parameters (for each partitioned locus) and the combined tree likelihood converged and had large effective sample sizes (ESS > 200). Posterior support for nodes was higher across the tree as compared to the gltA due to the added sequence information. The multi-locus phylogeny shows that genogroups Cp1-3 form a unified clade (posterior probability = 0.99) that is part of a larger clade (posterior probability = 1) with a *Bartonella* species isolated from *Eidolon helvum* in Africa [38,42] and multiple species isolated from *Myotis blythii* and *Rhinolophus ferrumequinum* in Georgia [22].

Genogroup H1 formed a clade (posterior probability = 1) with *Bartonella* species from *Triaenops persicus* in Kenya [42] and *Rhinolophus ferrumequinum* in Georgia [22] while genogroup H2 was linked (posterior probability = 1) to the *Bartonella* species from *Hipposideros vitatus* from Kenya [42]. All three genogroups from *Hipposideros* spp. bats (H1-3) were closely linked (posterior probability = 0.91) and more distantly related to *Bartonella* species from *Eidolon helvum* in Africa and *Rhinolophus euryale* in Georgia [22,38,42]. Strains KP287a from *H. larvatus* and KP174 from a *Hipposideros* sp. bat diverge slightly from the rest of genogroup H1 due to recombination events with genogroups H2 and H3, respectively. Similar to the gltA phylogeny, genogroup Tm was very similar to the *Bartonella* species from *Coleura afra* in Kenya and more distantly related to *Bartonella* species from other African fruit bats, *Eidolon helvum* and *Roussettus aegyptiacus* [42]. Genogroups H1-3 and Tm are all members of a large and well-supported clade (posterior probability = 1) composed entirely of bat-associated *Bartonella* species from Africa and Eurasia recognized in previous multi-locus phylogenetic analyses [22,38].

Recombination and admixture analyses

The network phylogeny from SplitsTree (Fig 3) generated from concatenated sequences of five loci (*ftsZ*, *gltA*, *nuoG*, *rpoB*, and ITS) supported the distinction between the seven genogroups (Cp1-3, H1-3, and Tm). However, the pairwise homoplasy index [60] test found significant recombination among the isolates (mean = 0.19, variance = 6.6×10⁻⁶, p-value < 0.0001). These recombination events can been seen in the web-like linkage between genogroups H1 and H2 for strain KP287a and the linkage between genogroups H1 and H3 for strain KP174. The optimal number of populations within the isolates was seven according to the AK method [62,63] after Bayesian clustering analysis using STRUCTURE [61]. All seven of these populations

Table 3. Nucleotide polymorphism and diversity among *Bartonella* strains from Thai bats.

| Genes   | Size (bp) | # alleles | V | N | V (%) | N (%) | dN | dS | dN/dS | π (%) | Mean pairwise distance (%) |
|---------|-----------|-----------|---|---|-------|-------|----|----|-------|------|---------------------------|
| *ftsZ*  | 886       | 10        | 191| 17| 21.6  | 5.8   | 0.01| 0.31| 0.03   | 8.0  | 8.3                       |
| *gltA*  | 356       | 10        | 101| 19| 28.4  | 16.1  | 0.03| 0.32| 0.09   | 9.3  | 9.3                       |
| *nuoG*  | 353       | 9         | 86 | 10| 24.4  | 8.5   | 0.02| 0.31| 0.06   | 8.6  | 8.9                       |
| *rpoB*  | 833       | 10        | 205| 20| 24.6  | 7.2   | 0.02| 0.36| 0.04   | 9.0  | 9.9                       |
| ITS     | 689       | 11        | 312| NA| 45.3  | NA    | NA | NA | NA     | 12.7 | 22.8                      |
| Concatenate, no ITS | 2428       | 15        | 583| 66| 24.0  | 8.2   | 0.02| 0.33| 0.05   | 8.6  | 9.1                       |
| Concatenate | 3117       | 17        | 895| NA| 28.7  | NA    | NA | NA | NA     | 9.5  | 11.0                      |

Values are calculated from all individuals (n = 30). ITS is not a protein coding locus and contains large insertions and deletions, thus only nucleotide diversity is calculated. The length of ITS sequences is based on aligned sequences, which includes gaps. π, average number of nucleotide differences per site; V, number of variable sites; N, number of non-synonymous sites; S, number of synonymous sites; dS, number of synonymous changes per synonymous site; dN, number of non-synonymous changes per non-synonymous site; NA, not applicable. Mean pairwise distance was calculated using the number of substitutions per site and dN and dS were calculated using the Nei-Gojobori method.

https://doi.org/10.1371/journal.pone.0181696.t003
Fig 2. Phylogenetic relationships among Bartonella genogroups from Thai bats, other strains from bats, and named Bartonella species assessed by multiple loci. The Bayesian tree was inferred in BEAST by partitioned analysis of
matched with the genogroups distinguished by the MLST profiles and phylogenetic analysis. The clustering analysis showed that strain KP287a was mostly composed of genogroup H1 with some genetic material from genogroup H2 and strain KP174 was almost entirely composed of genogroup H1 with some admixture with genogroup H3. This admixture is confirmed by the maximum likelihood analysis of the five sequenced loci (Figs C-G in S1 Text), showing that the \textit{nuoG} sequence of strain KP174 clustered with genogroup H3 and the \textit{rpoB} sequence of strain

Fig 3. Network phylogeny of \textit{Bartonella} strains from Thai bats. The network was inferred using the NeighborNet algorithm in SplitsTree based on concatenated sequences of five loci (\textit{ftsZ}, \textit{gltA}, \textit{nuoG}, \textit{rpoB}, and ITS) from 30 \textit{Bartonella} isolates analyzed by MLST. Distinct genogroups are named next to clusters of isolates. Recombinant isolates are labeled individually.

https://doi.org/10.1371/journal.pone.0181696.g002
KP287a clustered with genogroup H2. The relative amount of admixture (Fig 4) in these recombinant strains was also proportional to the size of \textit{nuoG} (353 bp) and \textit{rpoB} (833 bp) sequences. STRUCTURE analysis was also able to discern some admixture between strain CR224 from \textit{H. fulvus} in Chiang Mai (genogroup H3) with genogroup Tm. This admixture was not as obvious as with strains KP287a and KP174, but is observable from the distinction of strain CR224 from all other members of genogroup H3 at the five sequenced loci (Figs C-G in S1 Text) and some web-like connections between genogroups H3 and Tm in the network phylogeny (Fig 3).

Discussion

\textit{Bartonella} is a highly diverse genus of bacteria and bats have been distinguished as particularly reliable sources of novel \textit{Bartonella} species. The present study focused on characterization of \textit{Bartonella} isolates from bat species in Thailand. We identified seven novel \textit{Bartonella} genogroups in five species of bats using sequences of the gltA gene. Genogroups H1 through H3 were found in roundleaf bats (\textit{H. armiger}, \textit{H. larvatus}, and \textit{H. fulvus}). Genogroups Cp1 through Cp3 were found in free-tailed bats (\textit{C. plicatus}) and genogroup Tm was found in sheath-tailed bats (\textit{T. melanopogon}).

Comparison with previous gltA sequences on GenBank showed that genogroup H1 had been previously detected in \textit{H. armiger} and \textit{H. larvatus} in Vietnam [35] and is closely related to sequences found in a bat fly (\textit{Phthiridium fraterna}) removed from a \textit{Hipposideros} sp. bat from Malaysia [34] and sequences found in \textit{Rhinolophus} spp. from Vietnam and Georgia [22,35]. Genogroup H2 was found to be related to sequences found in \textit{Hipposideros vittatus} (previously reported as \textit{H. commersoni}) from Kenya [42,65], \textit{Megaderma lyra} from Vietnam [35], and community dogs from Thailand [28]. Genogroup H3 clustered with \textit{Bartonella} species identified in \textit{Eidolon helvum} in Africa [38,42] and bat flies from \textit{Pteropus hypomelanus}, \textit{Ptenochirus jagori}, and \textit{Harpyionycteris whiteheadi} in Malaysia and the Philippines [34]. Genogroup Tm was found to be very closely related to a \textit{Bartonella} species from \textit{Coleura afra} in Kenya [42].

Phylogenetic analysis of multiple loci confirmed that genogroups H1-3, Cp1-3, and Tm are divergent enough to be considered separate \textit{Bartonella} species according to previously established criteria based on individual loci, with genogroups differing by 6.5–15.6% sequence identity [66]. Most genogroups displayed clonal behavior with very little variation at multiple loci, however genogroups H1 and H3 showed measurable genetic variation at several loci. Additionally, these groups showed some evidence of homologous recombination. These heterogeneous patterns of genetic variation and homologous recombination have been observed in other \textit{Bartonella} species found in bats [38].

Host specificity of \textit{Bartonella} species in bats has been a subject of some discussion [42,67,68]. As more studies have been performed, it is clear that \textit{Bartonella} species are typically shared among bats in the same families, superfamilies, and suborders [16]. Transmission may be facilitated by shared ectoparasites when species roost in sympatry [22]. All five of the focal bat species in this study inhabit caves and manmade structures and host a variety of ectoparasite families. \textit{Chaerephon plicatus} has been found infested with bat flies (Diptera: Nycteribiidae), fleas (Siphonaptera: Ischnopsyllidae), ticks (Ixodida: Argasidae, Ixodidae), mites (Trombidiiformes: Myobiidae; Sarcoptiformes: Sarcoptidae, Listrophoridae), and bat bugs (Hemiptera: Cimicidae) in Malaysia, the Philippines, and Thailand [69–72]. \textit{Taphozous melanopogon} hosts bat flies (Diptera: Nycteribiidae, Streblidae), ticks (Ixodida: Argasidae), mites (Trombidiiformes: Myobiidae, Trombiculidae; Sarcoptiformes: Listrophoridae; Mesostigmata: Macronyssidae, Spinturnicidae), and bat bugs (Hemiptera: Polycenidae) in Thailand, Malaysia, Burma, Sri Lanka, Indonesia, and India [69,73–77]. Bat flies (Diptera: Nycteribiidae, Streblidae), ticks (Ixodida: Argasidae, Ixodidae), and mites (Trombidiiformes: Myobiidae, Trombiculidae;
Sarcoptiformes: Listrophoridae; Mesostigmata: Macronyssidae, Spinturnicidae) are known to parasitize Hipposideros spp., including *H. armiger* and *H. larvatus* in Vietnam, Indonesia, China, Thailand, Malaysia, and Burma [69,73,78–87]. Of these ectoparasite groups, nycteribiid and streblid bat flies, cimicid bugs, ischnopsyllid fleas, argasid and ixodid ticks, and macronyssid and spinturnicid mites are suspected vectors of *Bartonella* spp. in bats [17,34,53,88–93]. Trombiculid mites parasitizing rodents have been found harboring *Bartonella* spp. and may also be vectors of *Bartonella* spp. in bats [94,95].

Based on our observations of separate *Bartonella* species infecting *Chaerophon plicatus*, *Taphozous melanopogon*, and *Hipposideros* spp., we may surmise that transmission among these genera is uncommon, due in part to the specificity of *Bartonella* spp. to related host species and perhaps reinforced by the specificity of ectoparasites to their bat hosts. Although our focal species share a range of ectoparasite families, there are likely specific associations of ectoparasites to one or a few related bats. There are few data available concerning the host range of ectoparasites in Southeast Asia, so more study is warranted to fully understand the ecology and transmission dynamics of *Bartonella* spp. in bats and their ectoparasites in this region.

**Supporting information**

**S1 Text. Supplementary material.** This file includes additional information on field and laboratory methods and supplementary tables and figures.

(HTML)
Acknowledgments
Thanks to Boonlert Lumlertdacha, Brett Petersen, Felix Jackson, and Kis Robertson for their participation in the fieldwork, and to Pongpun Sawatwong and Possawat Jorakate for their participation in culturing bacteria from bat blood. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of CDC.

Author Contributions
Conceptualization: Michael Y. Kosoy, Sumalee Boonmar, Charles E. Rupprecht, Leonard F. Peruski.

Data curation: Clifton D. McKee, Michael Y. Kosoy, Ying Bai, Lynn M. Osikowicz, Richard Franka, Amy T. Gilbert.

Formal analysis: Clifton D. McKee.

Funding acquisition: Michael Y. Kosoy, Charles E. Rupprecht.

Investigation: Clifton D. McKee, Michael Y. Kosoy, Ying Bai, Lynn M. Osikowicz, Richard Franka, Amy T. Gilbert, Sumalee Boonmar, Charles E. Rupprecht, Leonard F. Peruski.

Resources: Clifton D. McKee, Michael Y. Kosoy, Ying Bai, Lynn M. Osikowicz, Richard Franka, Amy T. Gilbert, Sumalee Boonmar, Charles E. Rupprecht, Leonard F. Peruski.

Software: Clifton D. McKee.

Supervision: Michael Y. Kosoy, Sumalee Boonmar, Charles E. Rupprecht, Leonard F. Peruski.

Visualization: Clifton D. McKee.

Writing – original draft: Clifton D. McKee, Michael Y. Kosoy, Leonard F. Peruski.

Writing – review & editing: Clifton D. McKee, Michael Y. Kosoy, Ying Bai, Lynn M. Osikowicz, Richard Franka, Amy T. Gilbert, Sumalee Boonmar, Charles E. Rupprecht, Leonard F. Peruski.

References
1. Woolhouse MEJ, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. Emerg Infect Dis. Center Disease Control; 2005; 11: 1842–1847. https://doi.org/10.3201/eid1112.050997 PMID: 16485468
2. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008; 451: 990–993. https://doi.org/10.1038/nature06536 PMID: 18288193
3. Calisher CH, Childs JE, Field HE, Holmes K V, Schountz T. Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev. 2006; 19: 531–545. https://doi.org/10.1128/CMR.00017-06 PMID: 16847084
4. Kosoy MY. Ecological associations between bacteria of the genus Bartonella and mammals. Biol Bull. 2010; 37: 716–724. https://doi.org/10.1134/S1062359010070071
5. Billeter SA, Levy MG, Chomel BB, Breitschwerdt EB. Vector transmission of Bartonella species with emphasis on the potential for tick transmission. Med Vet Entomol. 2008; 22: 1–15. https://doi.org/10.1111/j.1365-2915.2008.00713.x PMID: 18380649
6. Tsai Y-L, Chang C-C, Chuan S-T, Chomel BB. Bartonella species and their ectoparasites: selective host adaptation or strain selection between the vector and the mammalian host? Comp Immunol Microbiol Infect Dis. Elsevier Ltd; 2011; 34: 299–314. https://doi.org/10.1016/j.cimid.2011.04.005 PMID: 21616536
7. Chomel BB, Kasten RW, Floyd-Hawkins K, Chi B, Yamamoto K, Roberts-Wilson J, et al. Experimental transmission of Bartonella henselae by the cat flea. J Clin Microbiol. 1996; 34: 1952–1956. PMID: 8818889
8. Bouhsira E, Fernandez Y, Liu M, Franc M, Boulouis H, Biville F. *Ctenocephalides felis* an in vitro potential vector for five *Bartonella* species. Comp Immunol Microbiol Infect Dis. Elsevier Ltd; 2013; 36: 105–111. https://doi.org/10.1016/j.cimid.2012.10.004 PMID: 23200028

9. Morick D, Krasnov BR, Khokhlova IS, Gottlieb Y, Harrus S. Transmission dynamics of *Bartonella* sp. strain OE 1–1 in Sundevall’s jirds (*Meriones crassus*). Appl Environ Microbiol. 2013; 79: 1258–1264. https://doi.org/10.1128/AEM.03011-12 PMID: 23241972

10. Kernil T, Leulmi H, Socolovschi C, Berenger J-M, Lepidi H, Bitam I, et al. Competence of *Cimex lectularius* bed bugs for the transmission of *Bartonella quintana*, the agent of trench fever. PLoS Negl Trop Dis. 2015; 9: e0003789. https://doi.org/10.1371/journal.pntd.0003789 PMID: 26009794

11. Leulmi H, Bitam I, Berenger J-M, Lepidi H, Rolain J-M, Almeras L, et al. Competence of *Cimex lectularius* and *Ctenocephalides felis* for the transmission of *Bartonella quintana* by the cat flea, *Ctenocephalides felis felis*. Mol Ecol. 2014; 23: 1204–1212. https://doi.org/10.1111/mec.12663 PMID: 24400877

12. Breitschwerdt EB, Maggi RG, Chomel BB, Lappin MR. *Bartonella* species. Comp Immunol Microbiol Infect Dis. Elsevier B.V.; 2017; 44: 382–394. https://doi.org/10.1016/j.cimid.2017.04.004 PMID: 28221109

13. Leulmi H, Aouadi A, Bitam I, Bessas A, Benakhla A, Raoult D, et al. Detection of *Bartonella tammiae*, *Coxiella burnetii* and *Rickettsia* spp. in bats from France and Spain. Emerg Infect Dis. 2016; 22: 457–462. https://doi.org/10.3201/eid2203.150269 PMID: 26885624

14. Vayssier-Taussat M, Moutailler S, Fémenia F, Raymond P, Croce O, La Scola B, et al. Identification of novel zoonotic activity of *Bartonella* spp., France. Emerg Infect Dis. 2016; 22: 457–462. https://doi.org/10.3201/eid2203.150269 PMID: 26885624

15. Lei BR, Olival KJ. Contrasting patterns in mammal-bacteria coevolution: *Bartonella* and *Leptospira* in bats and rodents. PLoS Negl Trop Dis. 2014; 8: e2738. https://doi.org/10.1371/journal.pntd.0002738 PMID: 24651646

16. McKee CD, Hayman DTS, Kosoy MY, Webb CT. Phylogenetic and geographic patterns of *bartonella* host shifts among bat species. Infect Genet Evol. Elsevier B.V.; 2016; 44: 382–394. https://doi.org/10.1016/j.meegid.2016.07.033 PMID: 27473781

17. Leulmi H, Aouadi A, Bitam I, Bessas A, Benakhla A, Raoult D, et al. Detection of *Bartonella tammiae*, *Coxiella burnetii* and *ricketsiae* in arthropods and tissues from wild and domestic animals in northeastern Algeria. Parasit Vectors. Parasites & Vectors; 2016; 9: 27. https://doi.org/10.1186/s13071-016-1316-9 PMID: 26791781

18. Wilkinson DA, Duron O, Cordonin C, Gornard Y, Ramasindrazana B, Mavingui P, et al. The bacteriome of bat flies (*Nycteribiidae*) from the Malagasy region: a community shaped by host ecology, bacterial transmission mode, and host-vector specificity. Appl Environ Microbiol. 2016; 82: 1778–1788. https://doi.org/10.1128/AEM.03011-12 PMID: 23241972

19. Davoust B, Marie J-L, Dahmani M, Berenger J-M, Bompar J-M, Blanchet D, et al. Evidence of *Bartonella* spp. in blood and ticks (*Omithodorus hasel*) of bats, in French Guiana. Vector-Borne Zoonotic Dis. 2016; 16: 516–519. https://doi.org/10.1089/vbz.2015.1918 PMID: 27305604

20. Reeves WK, Beck J, Orlova M V, Daly JL, Pippin K, Revan F, et al. Ecology of bats, their ectoparasites, and associated pathogens on Saint Kitts Island. J Med Entomol. 2016; 53: 1218–1225. https://doi.org/10.1093/jme/tjw078 PMID: 27282816

21. Dietrich M, Tjalle MA, Weyer J, Kearney TE, Seaman ECJ, Nel LH, et al. Diversity of *Bartonella* and *Ricketsia* spp. in bats and their blood-feeding ectoparasites from South Africa and Swaziland. Fenton B, editor. PLoS One. 2016; 11: e0152077. https://doi.org/10.1371/journal.pone.0152077 PMID: 26999518

22. Urushadze L, Bai Y, Oskowicz LM, McBee CD, Kandaurov A, Kuzmin I V, et al. Prevalence, diversity, and host associations of *Bartonella* strains in bats from Georgia (Caucasus). PLoS Negl Trop Dis. 2011; 11: e0005428. https://doi.org/10.1371/journal.pntd.0005428 PMID: 22999125

23. Han H-J, Wen H, Zhao L, Liu J-W, Luo L-M, Zhou C-M, et al. Novel *Bartonella* species in insectivorous bats, northern China. Munderloh UG, editor. PLoS One. 2017; 12: e0167915. https://doi.org/10.1371/journal.pone.0167915 PMID: 28081122

24. Stuckey MJ, Boulouis H-J, Cliquet F, Picard-Meyer E, Servat A, Aréchiga-Ceballos N, et al. Potentially zoonotic *Bartonella* in bats from France and Spain. Emerg Infect Dis. 2017; 23: 539–541. https://doi.org/10.3201/eid2303.160934 PMID: 28221109

25. Lilley TM, Wilson CA, Bernard RF, Willcox EV, Vesterinen EJ, Webber QMR, et al. Molecular detection of *Candidatus* Bartonella mayotimonensis in North American bats. Vector-Borne Zoonotic Dis. 2017; XX: vzb.2016.2080. https://doi.org/10.1089/vbz.2016.2080

26. Cicuttin GL, De Salvo MN, La Rosa I, Dohmen FEG. *Neorickettsia risticii*, *Rickettsia* sp. and *Bartonella* sp. in *Tadarida brasiliensis* bats from Buenos Aires, Argentina. Comp Immunol Microbiol Infect Dis. Elsevier; 2017; 52: 1–5. https://doi.org/10.1016/j.cimid.2017.04.004 PMID: 28673455
27. Ikeda P, Seki MC, Carrasco AOT, Rudiak LV, Miranda JMD, Goncalves SMM, et al. Evidence and molecular characterization of *Bartonella* spp. and hemoplasmas in neotropical bats in Brazil. Epidemiol Infect. 2017; 1–15. https://doi.org/10.1017/S0950268817000966 PMID: 28502279

28. Bai Y, Kosoy MY, Boonmar S, Sawatwong P, Sangmaneeted S, Peruski LF. Enrichment culture and molecular identification of diverse *Bartonella* species in stray dogs. Vet Microbiol. Elsevier B.V.; 2010; 146: 314–319. https://doi.org/10.1016/j.vetmic.2010.05.017 PMID: 20570065

29. Lin EY, Tsigeleis C, Baddour LM, Lepidi H, Rolain J-M, Patel R, et al. *Candidatus* Bartonella mayotimonensis and endocarditis. Emerg Infect Dis. 2010; 16: 500–503. https://doi.org/10.3201/eid1603.081673 PMID: 20202430

30. Podsiadly E, Chmielewski T, Karbowiak G, Jedrzejewski B, Wierzbanowska S. The occurrence of spotted fever rickettsioses and other tick-borne infections in forest workers in Poland. Vector-Borne Zoonotic Dis. 2010; 11: 985–989. https://doi.org/10.1089/vbz.2010.0080 PMID: 21083370

31. Veikkolainen V, Vesterinen EJ, Lilley TM, Pulliainen AT. Bats as reservoir hosts of human bacterial pathogen, *Bartonella mayotimonensis*. Emerg Infect Dis. 2014; 20: 960–967. https://doi.org/10.3201/eid2006.130995 PMID: 24856523

32. Lin J-W, Hsu Y-M, Chomel BB, Lin L-K, Pei J-C, Wu S-H, et al. Identification of novel *Bartonella* spp. in bats and evidence of Asian gray shrew as a new potential reservoir of *Bartonella*. Vet Microbiol. Elsevier B.V.; 2012; 156: 119–126. https://doi.org/10.1016/j.vetmic.2011.09.031 PMID: 22005177

33. Han BA, Kramer AM, Drake JM. Global patterns of zoonotic disease in mammals. Trends Parasitol. Elsevier Ltd; 2016; 32: 565–577. https://doi.org/10.1016/j.pt.2016.04.007 PMID: 27316904

34. Morse SF, Olival KJ, Kosoy MY, Biller SA, Patterson BD, Dick CW, et al. Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Streblidae, Nycteribiidae). Infect Genet Evol. Elsevier B.V.; 2012; 12: 1717–23. https://doi.org/10.1016/j.meegid.2012.06.009 PMID: 22771358

35. Anh PH, Van Cuong N, Son NT, Tue NT, Kosoy MY, Woolhouse MEJ, et al. Diversity of *Bartonella* spp. in bats, southern Vietnam. Emerg Infect Dis. 2015; 21: 1266–1267. https://doi.org/10.3201/eid2107.141760 PMID: 26079810

36. Arvand M, Feil EJ, Gilardi M, Boulouis H-J, Viezens J. Multi-locus sequence typing of *Bartonella henselae* isolates from three continents reveals hypervirulent and feline-associated clones. Redfield R, editor. PLoS One. 2007; 2: e1346. https://doi.org/10.1371/journal.pone.0001346 PMID: 18094753

37. Chaloner GL, Ventosilla Palma, Birtles RJ. Multi-locus sequence analysis reveals profound genetic diversity among isolates of the human pathogen *Bartonella bacilliformis*. Picardeau M, editor. PLoS Negl Trop Dis. 2011; 5: e1248. https://doi.org/10.1371/journal.pntd.0001248 PMID: 21811647

38. Bai Y, Hayman DTS, McKee CD, Kosoy MY. Classification of *Bartonella* strains associated with straw-colored fruit bats (*Eidolon helvum*) across Africa using a multi-locus sequence typing platform. PLoS Negl Trop Dis. 2015; 9: e0003478. https://doi.org/10.1371/journal.pntd.0003478 PMID: 25635826

39. Buffet J-P, Pisanu B, Brisse S, Roussel S, Félix B, Halos L, et al. Deciphering *Bartonella* diversity, recombination, and host specificity in a rodent community. Skurnik M, editor. PLoS One. 2013; 8: e68956. https://doi.org/10.1371/journal.pone.0068956 PMID: 23894381

40. Kosoy MY, Bai Y, Boonmar S, Sawatwong P, Jorakate P, Peruski LF, et al. *Bartonella* species in bats from Thailand. International Conference and Emerging Infectious Diseases Program and Abstract Book. 2012. p. 129.

41. Norman AF, Regnery R, Jameson P, Greene C, Krause DC. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J Clin Microbiol. 1995; 33: 1797–1803. PMID: 7545181

42. Kosoy MY, Bai Y, Lynch T, Kuzmin I V, Niezgoda M, Franka R, et al. *Bartonella* spp. in bats, Kenya. Emerg Infect Dis. 2010; 16: 1875–1881. https://doi.org/10.3201/eid1612.100601 PMID: 21122216

43. Zealter Z, Liang Z, Raoult D. Genetic classification and differentiation of *Bartonella* species based on comparison of partial ftsZ gene sequences. J Clin Microbiol. 2002; 40: 3641–3647. https://doi.org/10.1128/JCM.40.10.3641-3647.2002 PMID: 12354859

44. Colborn JM, Kosoy MY, Motin VL, Telepnev M V, Valbuena G, Myint KSA, et al. Improved detection of *Bartonella* DNA in mammalian hosts and arthropod vectors by real-time PCR using the NADH dehydrogenase gamma subunit (*nuoG*). J Clin Microbiol. 2010; 48: 4630–4633. https://doi.org/10.1128/JCM.00470-10 PMID: 20926707

45. Renesto P, Gouvernet J. Use of rpoB gene analysis for detection and identification of *Bartonella* species. J Clin Microbiol. 2001; 39: 430–437. https://doi.org/10.1128/JCM.39.2.430-437.2001 PMID: 11158086

46. Diniz PP, Maggli RG, Schwartz DS, Cadenas MB, Bradley JM, Hegarty BC, et al. Canine bartonellosis: serological and molecular prevalence in Brazil and evidence of co-infection with *Bartonella henselae*
and *Bartonella vinsonii* subsp. *berkhoffii*. Vet Res. 2007; 38: 697–710. https://doi.org/10.1051/vetres:2007023 PMID: 17583666

47. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013; 30: 772–780. https://doi.org/10.1093/molbev/mst010 PMID: 23329690

48. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol. 2000; 17: 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334 PMID: 10742046

49. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013; 30: 2725–2729. https://doi.org/10.1093/molbev/mst197 PMID: 24132122

50. Darrida B, Taboada GL, Doallo R, Posada D. JModelTest 2: more models, new heuristics and parallel computing. Nat Methods. Nature Publishing Group; 2012; 9: 772–777. https://doi.org/10.1038/nmeth. 2109 PMID: 22847109

51. Burnham KP, Anderson DR. Multimodel inference: understanding AIC and BIC in model selection. Sociol Methods Res. 2004; 33: 261–304.

52. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014; 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033 PMID: 24451623

53. Judson SD, Frank HK, Hadly EA. Bartonellae are prevalent and diverse in Costa Rican bats and bat flies. Zoonoses Public Health. 2015; 62: 609–617. https://doi.org/10.1111/zph.12188 PMID: 25810119

54. Concannon R, Wynne-Owen K, Simpson VR, Birtles RJ. Molecular characterization of haemoparasites infecting bats (Microchiroptera) in Cornwall, UK. Parasitology. 2005; 131: 489–496. https://doi.org/10.1017/S0031182005008097 PMID: 16174413

55. Stadler T. On incomplete sampling under birth–death models and connections to the sampling-based coalescent. J Theor Biol. Elsevier; 2009; 261: 58–66. https://doi.org/10.1016/j.jtbi.2009.07.018 PMID: 19631666

56. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012; 29: 1969–1973. https://doi.org/10.1093/molbev/mss075 PMID: 22367748

57. Pritchard J, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155: 945–59. PMID: 10835412

58. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 2005; 14: 2665–2681. https://doi.org/10.1083/0966-842 X(05)00597-6 PMID: 16489234

59. Lachner MH, Steinbiss M, Kiel PC, Tullous K, Pfeifer R, Hecht H, et al. Identification of Bartonella species in German bats by real-time PCR and 16S rRNA gene sequencing. Parasitology. 2011; 138: 1655–1663. https://doi.org/10.1017/S0031182011001958 PMID: 21856059

60. Kading RC, Gilbert AT, Mossel EC, Crabtree MB, Kuzmin IV, Niezgoda M, et al. Isolation and molecular characterization of Fikirini rhabdovirus, a novel virus from a Kenyan bat. J Gen Virol. 2013; 94: 2393–2398. https://doi.org/10.1099/vir.0.053983-0 PMID: 23939976

61. Bai Y, Kosoy MY, Recuenco S, Alvarez Castilla D, Moran D, Turmelle AS, et al. Bartonella spp. in bats, Guatemala. Emerg Infect Dis. 2011; 17: 1269–1272. https://doi.org/10.3201/eid1707.101867 PMID: 21762584
68. Bai Y, Recuenco S, Gilbert AT, Osikowicz LM, Gomez J, Rupprecht CE, et al. Prevalence and diversity of Bartonella spp. in bats in Peru. Am J Trop Med Hyg. 2012; 87: 518–523. https://doi.org/10.4269/ajtmh.2012.12-0097 PMID: 22826480

69. Beck AJ. A survey of bat ectoparasites in West Malaysia. J Med Entomol. 1971; 8: 147–152. PMID: 5157835

70. Corpuz-Raros LA, Lit IL Jr. List of mites (Acari) inhabiting Philippine caves and cave-dwelling vertebrates. Museum Publ Nat Hist. 2015; 4: 26–50.

71. Williams JE, Imlarp S, Top FH Jr., Cavanaugh DC, Russell PK. Kaeng Khoi virus from naturally infected bedbugs (Cimicidae) and immature free-tailed bats. Bull World Health Organ. 1976; 53: 365–369. PMID: 1086729

72. Alvarez JD V, Lit IL Jr, Alviola PA. Bat flies (Diptera: Nycteribiidae) from Mount Makiling, Luzon Island: New host and distribution records, with a checklist of species found in the Philippines. Check List. 2015; 11: 1509. https://doi.org/10.15560/15.1.1509

73. Jobling B. A revision of the genus Nycteribosca Speiser (Diptera Pupipara, Streblidae). Parasitology. 1934; https://doi.org/10.1017/S0031182000023349

74. Maa TC. Records and descriptions of Nycteribiidae and Streblidae (Diptera). Pacific Insects. 1962; 4: 417–436.

75. Maa TC. On new Diptera, Pupipara from the Oriental region. Pacific Insects. 1974; 16: 465–486.

76. Zade V, Thakare V, Malik LA, Kali A, Dandge P. Diversity of ectoparasites present on some species of bats from Navegaon National Park, Maharashtra, India. Biol Forum. 2012; 4: 35–41.

77. Traub R, Starcke H. The function of combs in ectoparasitic insects. Proceedings of the International Conference on Fleas. 1977. pp. 79–87.

78. Aroon S, Hill III JG, Archawakom T, Kupittayanant S, Thanee N. Ectoparasites associated with bats in tropical forest of northeastern Thailand. J Agric Technol. 2015; 11: 1781–1792.

79. Azhar I, Anwarali Khan FA, Ismail N, Abdullah MT. Checklist of bat flies (Diptera: Nycteribiidae and Streblidae) and their associated bat hosts in Malaysia. Check List. 2015; 11: 1777. https://doi.org/10.15560/11.5.1777

80. Bush SE, Robbins RG. New host and locality records for Ixodes simplex Neumann and Ixodes vespertilionis Koch (Acari: Ixodidae) from bats (Chiroptera: Hipposideridae, Rhinolophidae and Vespertilionidae) in southern China. Int J Acarol. 2012; 38: 1–5. https://doi.org/10.1080/01647954.2011.569509

81. Wilson N. New distributional records of ticks from Southeast Asia and the Pacific (Metastigmata: Argasidae, Ixodidae). Orient Insects. 1970; 4: 37–46. https://doi.org/10.1080/00305316.1970.10433939

82. Ahmed M, Ibrahim H, Bujang MK, Mohd Sah S-A, Mohamad N, Nor SM, et al. A survey of acarine ectoparasites of bats (Chiroptera) in Malaysia. J Med Entomol. 2013; 50: 140–146. https://doi.org/10.1603/ME11240 PMID: 23427663

83. Domrow R. The Asian species of Whartonia (Acarina, Trombiculidae). Treubia. 1962; 26: 1–9.

84. Mariana A, Zuraidawati Z, Ho T, Mohd Kulaimi B, Saleh I, Shukor M, et al. A survey of ectoparasites in Gunung Stong Forest Reserve, Kelantan, Malaysia. Southeast Asian J Trop Med Public Health. 2005; 36: 1125–1131. PMID: 16438136

85. Nadchatram M. Two new species of Old World Whartonia (Acar: Prostigmata: Trombiculidae). J Med Entomol. 1980; 17: 324–327.

86. Theodor O. New species and new records of Diptera Pupipara II. Species from Asia and Africa. J Med Entomol. 1973; 10: 556–569. PMID: 4779919

87. Iuchikawa K, Kobayashi T. A contribution to the ectoparasite fauna of bats in Thailand II. Blood-sucking Acari (Argasidae, Spinturnicidae and Macronyssidae). Contrib from Biol Kyoto Univ. 1978; 25: 249–254.

88. Billeter SA, Hayman DTS, Peel AJ, Baker KS, Wood JLN, Cunningham AA, et al. Bartonella species in bat flies (Diptera: Nycteribiidae) from western Africa. Parasitology. 2012; 139: 324–329. https://doi.org/10.1017/S0031182011002113 PMID: 22309510

89. Reeves WK, Loftis AD, Gore JA, Dasch GA. Molecular evidence for novel bartonella species in Trichobius major (Diptera: Streblidae) and Cimex adjunctus (Hemiptera: Cimicidae) from two southeastern bat caves, U.S.A. J Vector Ecol. 2005; 30: 339–341. PMID: 16599175

90. Brook CE, Bai Y, Dobson AP, Osikowicz LM, Ranaivoson HC, Zhu Q, et al. Bartonella spp. in fruit bats and blood-feeding ectoparasites in Madagascar. Vinetz JM, editor. PLoS Negl Trop Dis. 2015; 9: e0003532. https://doi.org/10.1371/journal.pntd.0003532 PMID: 25706653

91. Reeves WK, Rogers TE, Durden LA, Dasch GA. Association of Bartonella with the fleas (Siphonaptera) of rodents and bats using molecular techniques. J Vector Ecol. 2007; 32: 118–122. https://doi.org/10.3376/1081-1710(2007)32[118:AOBWTF]2.0.CO;2 PMID: 1763432
92. Loftis AD, Gill JS, Schriefer ME, Levin ML, Eremeeva ME, Gilchrist MJR, et al. Detection of *Rickettsia*, *Borrelia*, and *Bartonella* in *Carios kelleyi* (Acari: Argasidae). J Med Entomol. 2005; 42: 473–480. https://doi.org/10.1603/0022-2585(2005)042[0473:DORBAB]2.0.CO;2 PMID: 15962801

93. Hornok S, Kovács R, Meli ML, Gónczi E, Hofmann-Lehmann R, Kontschán J, et al. First detection of bartonellae in a broad range of bat ectoparasites. Vet Microbiol. 2012; 159: 541–543. https://doi.org/10.1016/j.vetmic.2012.04.003 PMID: 22551590

94. Klangthong K, Promsthaporn S, Leepitakrat S, Schuster AL, McCardle PW, Kosoy M, et al. The distribution and diversity of *Bartonella* species in rodents and their ectoparasites across Thailand. Shao R, editor. PLoS One. 2015; 10: e0140856. https://doi.org/10.1371/journal.pone.0140856 PMID: 26484537

95. Loan HK, Cuong N Van, Takhampunya R, Klangthong K, Osikowicz L, Kiet BT, et al. *Bartonella* species and trombiculid mites of rats from the Mekong Delta of Vietnam. Vector-Borne Zoonotic Dis. 2015; 15: 40–47. https://doi.org/10.1089/vbz.2014.1604 PMID: 25629779