

Prevalence And Genetic Diversity of *Bartonella* Spp. In Northern Bats (*Eptesicus Nilssonii*) And Their Blood-Sucking Ectoparasites In Hokkaido, Japan

Kei Nabeshima

Nihon University

Shingo Sato

Nihon University

R. Jory Brinkerhoff

University of Richmond

Murasaki Amano

Nihon University

Hidenori Kabeya

Nihon University

Takuya Ito

Nihon University

Soichi Maruyama (✉ maruyama.soichi@nihon-u.ac.jp)

Nihon University College of Bioresource Sciences Graduate School of Bioresource Sciences: Nihon Daigaku Seibutsu Shigen Kagakubu Seibutsu Shigen Kagaku Kenkyuka <https://orcid.org/0000-0003-3207-1092>

Research Article

Keywords: Bartonella, bat flea, Japan, Northern bats

Posted Date: July 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-670515/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

We investigated the prevalence of *Bartonella* in northern bats (*Eptesicus nilssonii*) and their ectoparasites from Hokkaido, Japan. Two batbugs (*Cimex japonicus*) and 174 bat fleas (*Ischnopsyllus needhami*) were collected from the bats. *Bartonella* bacteria were isolated from 32 (26.0%) of 123 bats examined. Though *Bartonella* DNA was detected in 79 (45.5%) of bat fleas, the bacterium was isolated from only one bat flea (0.6%). The *gltA* sequences of the isolates were categorized into genotypes I, II, and III, which were found in common between the bats and fleas. The *gltA* sequences of genotypes I and II showed 97.6% similarity with the gene from *E. nilssonii* in Finland and a bat flea from *E. serotinus* in the Netherlands. The *rpoB* sequences of the genotypes showed 98.9% similarity with strain 44722 from *E. serotinus* in Georgia. The *gltA* and *rpoB* sequences of genotype III showed 95.9% and 96.7% similarity with strains from two different species of shrews in Kenya and France, respectively. Phylogenetic analysis revealed that *Bartonella* isolates of genotypes I and II clustered with strains from *Eptesicus* bats in Georgia and Finland, *Myotis* bats in Romania and the UK, and a bat flea from an *Eptesicus* bat in Finland. In contrast, genotype III formed a clade with *B. florencae*, *B. acomydis*, and *B. birtlesii*. These data suggest that northern bats in Japan harbor two *Bartonella* species and *I. needhami* likely serves as a potential vector of *Bartonella* transmission in the bats.

Introduction

A total of 1,314 bat species are currently recognized in the world [1]. The largest bat family Vespertilionidae, known as Vesper bats, consists of 426 bat species and *Pipistrellus*, *Myotis*, *Vespertilio*, *Plecotus*, *Murina*, and *Eptesicus* are the common genera in Japan [2]. Recently, it has been reported that Vesper bats carry pathogenic bacteria, including *Campylobacter*, *Clostridium*, *Listeria*, *Salmonella*, *Shigella*, *Vibrio*, *Yersinia*, *Borrelia*, *Grahamella*, *Neorickettsia*, *Leptospira*, and *Bartonella* [3].

Bartonella are small, fastidious Gram-negative bacteria that parasitize erythrocytes and endothelial cells of various mammals. *Bartonella* infections have been found in various bat species belonging to Vesper bats, genera *Myotis* and *Eptesicus* in Europe, Asia, and the Americas [4]. *Bartonella mayotimonensis* was first detected from an aortic valve of a patient with endocarditis [5], and then a *B. mayotimonensis*-like organism was also found in *Eptesicus* bats in Finland and *Myotis* bats in Finland, France, and Spain [6, 7]. Additionally, it has been reported that the *gltA* sequences of *Bartonella* bacteria isolated from *Eptesicus* and *Myotis* bats in Georgia are closely related to those from forest workers in Poland [8]. These data suggest that *Bartonella* bacteria in *Eptesicus* and *Myotis* bat species are transmissible between the bats and humans and are pathogenic for humans.

Ectoparasites such as cat fleas [9], body lice [10], sand flies [11], and the deer ked [12] are known to be competent vectors of *B. henselae*, *B. quintana*, *B. bacilliformis*, and deer associated strains, respectively. Previous studies have shown that some ectoparasites, such as bat flies may be involved in *Bartonella* transmission among bats. *Bartonella* DNA has been frequently detected in nycteribiid and streblid bat flies collected in Japan [13] and other 19 countries [4, 14–20]. In particular, *Bartonella* bacteria were

isolated from a *Cyclopodia* bat fly on *Eidolon helvum* in Ghana [19] and from three *Nycteribia* species that infested *Miniopterus fuliginosus* in Japan [13]. These findings suggest that bat flies may serve as the major vector for the transmission of *Bartonella* among bats regardless of country.

We previously reported that *Bartonella* bacteria are prevalent in 24% (12/50) of *Miniopterus fuliginosus* in Japan [21]. Furthermore, detection of *Bartonella* have been also reported in various Asian bat species at high prevalence, such as *Taphozous melanopogon* (100%; 1/1), *Hipposideros* sp. (37.2%; 16/43), and *Chaerephon plicatus* (39.5%; 17/43) in Thailand [22], *Hipposideros* sp. (45.5%; 5/11), *Rhinolophus* spp. (43.3%; 13/30), *Megaderma* spp. (33.3%; 1/3), and *Megaerops niphanae* (50%; 1/2) in Vietnam [23], *Miniopterus schreibersii* (42.9%; 6/14) in Taiwan [24], and *Rhinolophus* spp. (23%; 3/13) and *Myotis* spp. (30.4%; 24/79) in China [25]. Despite *Myotis* and *Eptesicus* bats are suspected of having pathogenic *Bartonella* species for humans [6], no epidemiological studies of the bacteria and the vectors have not been conducted in these bat species in Japan so far.

The aims of the present study were to investigate the prevalence and genetic properties of *Bartonella* bacteria in northern bats (*Eptesicus nilssonii*) and to identify a potential vector for the organism in the bat species in Japan.

Materials And Methods

2.1 Collection of blood and ectoparasites from bats

A total of 123 northern bats (*Eptesicus nilssonii*) were captured in Yakumo City (42°29'N, 140° 18'E), Hokkaido Prefecture, located in the northern part of Japan. Bat samples were separately collected in August 2017 (N = 49), June 2018 (N = 38), and August 2018 (N = 36). The bats were living within the exterior walls of an abandoned building in dense colonies as shown in the supplementary video. Before performing the present study, we obtained individual permissions to capture bats from the Oshima general sub-prefectural bureau, Hokkaido government (license #: Oshima 27, 28, 29, 122, 123, and 124).

Blood samples were aseptically collected from the bats via heart puncture using the same procedures as previously reported [21]. The blood samples and the carcasses were immediately sent to the Laboratory of Veterinary Public Health at the Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University under frozen conditions with dry ice. All of the samples were stored at -70°C until they were examined and tested.

A total of 174 bat fleas and two batbugs were obtained from the surfaces of the carcasses in the laboratory. The ectoparasites were morphologically identified at the species level under a stereo microscope SZX16 (Olympus, Tokyo, Japan) with taxonomic keys [26, 27]. Rates of parasitism by species were compared using chi-square tests with *P* values < 0.01 and considered to be statistically significant.

2.2 Isolation of *Bartonella* bacteria from bats

Isolation of *Bartonella* bacteria from the bat blood samples exactly followed a previously described method [21]. *Bartonella* bacteria were tentatively identified by colony morphology (small, white, round shape) and three colonies per sample were sub-cultured on a fresh blood agar plate using the same conditions as the primary culture.

2.3 Detection of *Bartonella* DNA from ectoparasites

To avoid bacterial and fungal contamination on the surface of the ectoparasites, each sample was sterilized by immersing in 500 μ l of 70% ethanol containing 0.35% povidone-iodine (Shionogi & Co., Ltd, Osaka, Japan) for 10 minutes. Then, the samples were once washed with 1 ml of 0.01 M PBS with 0.5% FBS and transferred into shatter-resistant 2.0 ml tubes (SSIbio, Lodi CA, USA). After adding 400 μ l of sucrose phosphate glutamate (SPG; 10 mM sodium phosphate, 220 mM sucrose, and 0.5 mM L-glutamic acid), each sample was homogenized using a bead crusher μ T-12 (TAITEC corp., Saitama, Japan) at 3,000 rpm for 1 min. Genomic DNA was extracted from the homogenate aliquot (100 μ l) by InstaGene Matrix (Bio-Rad Laboratories, Inc. Hercules, CA, USA).

Real-time PCR targeting the transfer-mRNA (*ssrA*) gene of the genus *Bartonella* was used for screening *Bartonella* DNA [28]. Real-time PCR assays were performed in 20 μ l reaction mixtures containing 10 μ l of TB Green Premix Ex Taq II (Takara Bio Inc., Shiga, Japan), 2 μ l of DNA sample (approximately 10 to 20 ng/ μ l), 1 μ l of each primer (10 nM), and 6 μ l of nuclease-free water. The PCR conditions were as follows: 95°C for 30 seconds, followed by 40 cycles at 95°C for 5 seconds and 60°C for 30 seconds. The genomic DNA from the northern bat isolate (EN2-1) was used as a positive control and nuclease-free water as a negative control in this study. The targeted DNA was amplified with a Thermal Cycler Dice Real Time System II (Takara Bio Inc). Melting curve analysis was applied to the results with a Ct value lower than 35 cycles. When a sample showed $80 \pm 1^\circ\text{C}$ melting temperature, the sample was defined as positive by the assay. Each positive sample was additionally analyzed by conventional PCR targeting the citrate synthase gene (*gltA*) [29]. The conventional PCR products were separated on 3% agarose gels by electrophoresis and were visualized by staining with ethidium bromide under UV light. When samples tested positive for both PCRs, the samples were defined as being infected with *Bartonella* bacteria. A band showing the expected size for *gltA* (approximately 380 bp) was purified by using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) following the manufacturer's instructions.

The purified PCR products of the *gltA* gene were directly sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit and a Genetic Analyzer model 3130 (Applied Biosystems). When double peaks were detected in the DNA sequencing chromatograms, the PCR products were cloned using the plasmid pGEM-T Easy vector system (Promega) and resequenced. The plasmids were purified from the transformed cell lysates with a plasmid purification kit (PureYield Plasmid Miniprep System; Promega) and then were sequenced using the primers SP6 (5'-CAAGCTATTTAGGTGACACTATAG-3') and T7 (5'-TAATACGACTCACTATAGGG-3').

2.4 Isolation of *Bartonella* bacteria from ectoparasites

The homogenates of *Bartonella* DNA-positive ectoparasites were subsequently used for the isolation of *Bartonella* bacteria following the previously described method [13].

2.5 PCR amplification of the *gltA* and *rpoB* genes from *Bartonella* isolates

Genomic DNA was extracted from each sub-cultured colony by using InstaGene Matrix (Bio-Rad Laboratories) and subjected to genus-specific PCR targeting the *gltA* [29] and RNA polymerase beta-subunit-encoding (*rpoB*) genes [30]. The PCR products were separated on 2% agarose gels by electrophoresis and visualized by staining with ethidium bromide under UV light. Any samples showing the expected band sizes for *gltA* and *rpoB* (approximately 850 bp) were considered as members of the genus *Bartonella*. The PCR products were then purified using the Wizard SV Gel and PCR Clean-Up System (Promega) and directly sequenced as previously described [21].

2.6 Genotyping.

Genotypes of the *gltA* genes were determined when unique sequence variants with ≥ 1 nucleotide difference were found in each isolate by comparing the *gltA* sequences using Genetyx software Ver 12 (Genetyx Corporation, Tokyo, Japan). Representative isolates from each genotype were further analyzed by sequencing their *rpoB* genes.

2.7 Sequence homology analysis

The *gltA* and *rpoB* sequences of representative strains were compared with genomic sequences of prokaryotes registered in the GenBank/EMBL/DDBJ database using the BLAST program.

2.8 Phylogenetic analysis

Based on evolutionary model selection using JModelTest2 [31] with Akaike's information criterion corrected for finite sample sizes (AICc) [32], the generalized time-reversible substitution model with four gamma-distributed categories and a proportion of invariant sites (GTR + G + I) model was the best available model for the phylogenetic analyses based on the *gltA* sequences.

A phylogenetic tree of the *gltA* sequences was constructed using the maximum-likelihood method based on the GTR + G + I model in MEGA 7 [33]. Known *Bartonella* species (N = 40), bat-associated *Bartonella* strains (N = 420) derived from 36 countries, and *Brucella melitensis* 16M as an outgroup were included in this analysis. Strain names, host types, host scientific names, countries where the bats and ectoparasites were collected are summarized together with references and the *gltA* accession numbers in supplementary Table 1.

Results

3.1 Ectoparasite fauna in the northern bats

All bat fleas (N = 174) were morphologically identified as *Ischnopsyllus needhami* and the two batbugs as *Cimex japonicus*. Parasite prevalence of *I. needhami* and *C. japonicus* in bats were 59.3% (71/123) and 2.4% (2/123), respectively and the prevalence of *I. needhami* was significantly higher than that of *C. japonicus* ($p < 0.01$). Parasite prevalence of bat fleas varied by sampling period: 63.2% (31/49) in August 2017, 71.1% (27/38) in June 2018, and 36.1% (13/36) in August 2018 (Table 1).

Table 1 Prevalence of *Bartonella* and *I. needhami* in Japanese northern bats by sample collection date

Sample collection date	Number of bats examined	Number of <i>Bartonella</i> positive bats (%)	Number of <i>I. needhami</i> positive bats (%)
2017-Aug	49	7 (14.2)	31 (63.2)
2018-June	38	11 (28.9)	27 (71.1)
2018-Aug	36	14 (38.9)	13 (36.1)
Total	123	32 (26.0)	71 (59.3)

3.2 Isolation of *Bartonella* bacteria from northern bats and their ectoparasites, and *gltA* genotypes of the isolates

Bartonella bacteria were isolated from 26% (32/123) of the northern bats and 0.6% (1/174) of *I. needhami*, but not from *C. japonicus*. The prevalences of *Bartonella* in the bats by sampling period were 14.2% (7/49) in August 2017, 28.9% (11/38) in June 2018, and 38.9% (14/36) in August 2018 (Table 1). A total of 99 isolates were obtained from 32 bat and one flea samples (3 isolates per one positive sample). In 71 northern bats with *I. needhami*, 28.2% (20/71) were positive for *Bartonella* bacteria, whereas 23.1% (12/52) of the bats without the bat fleas were positive for *Bartonella* bacteria. There was no significant difference in the rates between *I. needhami* positive and negative bats.

The bat isolates were conveniently classified into three genotypes as genotypes I (33 isolates), II (33 isolates), and III (30 isolates) based on the *gltA* sequences. None of the bats was co-infected with multiple genotypes. Three isolates from one *I. needhami* were classified into the genotype I. The *gltA* and *rpoB* sequences of representative bat strains (EN2-1, EN19-2, and EN36-1) were registered in the GenBank/EMBL/DDBJ database (Table 2).

Table 2 Genotypes and accession numbers of *gltA* and *rpoB* genes of representative strains from Japanese northern bats and bat fleas

Genotype	No. of isolates	Representative strain	<i>gltA</i> accession #	<i>rpoB</i> accession #
I	36*	EN2-1	LC596942	LC596945
II	33	EN19-2	LC596943	LC596946
III	30	EN36-1	LC596944	LC596947

* Three of the isolates were derived from bat fleas.

3.3 Detection of Bartonella DNA from bat ectoparasites and *gltA* genotypes of Bartonella DNA

Bartonella DNA was detected from 79 *I. needhami* (45.5%; 79/174), but not from *C. japonicus*. The *gltA* sequences from the bat fleas were also classified into genotypes I, II, and III, which are common with those of the bat isolates. The numbers of *I. needhami* harboring only one genotype were 43 for genotype I, 21 for genotype II, and 10 for genotype III, respectively (Table 3). Fleas harboring two genotypes were found in five individuals: genotypes I and II in two fleas, genotypes II and III in one flea, and genotypes I and III in two fleas.

Table 3 The *gltA* genotypes of *Bartonella* DNA detected in bat fleas (*I. needhami*)

<i>gltA</i> genotypes	No. of bat fleas
I	43
II	21
III	10
I+II	2
II+III	1
I+III	2
Total	79

3.4 Sequence similarities of the *gltA* and *rpoB* genes between the three genotypes and other Bartonella strains

The sequence similarities between the three genotypes and the closest *Bartonella* strains based on BLAST searches are shown in Table 4.

Table 4 Sequence similarities in the *gltA* and *rpoB* genes between the representative northern bat strains and closest strains.

Strain (genotype)	Closest strain (Host) in <i>gltA</i> sequences	% similarity	Closest strains (Host) in <i>rpoB</i> sequences	% similarity
EN2-1 (I)	<i>Bartonella</i> sp. 1157/3 (<i>Eptesicus nilssonii</i>)	97.9%	<i>Bartonella</i> sp. 44722 (<i>Eptesicus serotinus</i>)	98.8%
EN19-2 (II)	Uncultured <i>Bartonella</i> sp. clone 1F40 (<i>Ischnopsyllus variabilis</i>)			
EN36-1 (III)	<i>Bartonella</i> sp. B28303 (<i>Crocidura olivieri</i>)	95.9%	<i>B. florencae</i> R4 ^T (<i>Crocidura russula</i>)	96.7%

The *gltA* sequences of strains EN2-1 (genotype I) and EN19-2 (genotype II) showed 97.6% similarity with those of strain 1157/3 from a northern bat in Finland and strain 1F40 from a bat flea (*Ischnopsyllus variabilis*) in the Netherlands. Only one nucleotide base in the *gltA* was different between strains EN2-1 and EN19-2. The *gltA* sequence of strain EN36-1 (genotype III) showed 95.9% similarity with that of strain B28303 from an African giant shrew (*Clocidura olivieri*) in Kenya.

The *rpoB* sequences of strains EN2-1 and EN19-2 were identical each other and showed 98.9% similarity with that of strain 44722 from a serotine bat (*E. serotinus*) in Georgia. The *rpoB* sequence of EN36-1 showed 96.7% similarity with *B. florencae* R4^T from a greater white-toothed shrew (*Clocidura russula*) in France.

3.5 Phylogenetic analysis of bat isolates based on the *gltA* sequences

Three representative strains were divided into two different clades. Strain EN2-1 (genotype I) and strain EN19-2 (genotype II) formed clade A with strains from *Myotis* bats in the UK and Romania, *Eptesicus* bats in Georgia and Finland, and a flea (*I. variabilis*) infesting *E. serotinus* in the Netherlands. On the other hand, strain EN36-1 (genotype III) clustered in clade B with *B. acomydis* KS2-1^T from a golden spiny mouse (*Acomys russatus*), *B. florencae* R4^T, and *B. birtlesii* IBS325^T from a wood mouse (*Apodemus speciosus*) (Fig. 1).

Discussion

In the present study, 26% (32/123) of Japanese northern bats, *E. nilssonii* were found to be infected with *Bartonella* bacteria. According to previous studies, *Bartonella* bacteria were also isolated from *Eptesicus* bats: *E. nilssonii* (33.3%; 1/3) in Finland [6] and *E. serotinus* (25%; 4/20) in Georgia [8]. The prevalence rate of *Bartonella* bacteria in Japanese northern bats seems to be quite similar to those of previous two countries. In contrast, no *Bartonella* was detected in *E. serotinus* in China [25] or France [7]. Since these results suggest that *Bartonella* prevalence in *Eptesicus* bats may vary depending on the locations of the bat populations and/or bat species, more investigations will be necessary to clarify the causes of the differences in *Bartonella* prevalence in different regions and bat species.

Our study showed that Japanese northern bats were infested with two species of ectoparasites: bat fleas (*Ischnopsyllus needhami*) and batbugs (*Cimex japonicus*). The parasitic rate of *I. needhami* was significantly higher than that of *C. japonicus* ($p < 0.01$), indicating that a dominant ectoparasite in Japanese northern bats is *I. needhami*. Previous studies have shown that various ectoparasites such as *Ischnopsyllus* spp. and *Myodopsylla borealis* (bat fleas), *Cimex* spp. (batbug), *Carios kelleyi* (soft tick), and *Basilisa forcipata* (bat fly) parasitize *Eptesicus* bats in Russia and the USA [34, 35]. However, we did not find any other ectoparasites other than *I. needhami* and *C. japonicus* in this study. Hence, the fauna of ectoparasites on *Eptesicus* bats may depend on the season, habitat, or bat species.

Bartonella DNA was frequently detected from bat fleas (45.4%; 79/174) and *Bartonella* bacteria were isolated from one bat flea. In the present study, the isolation rate is much lower than the detection rate by

PCR. The isolation of *Bartonella* from fleas was performed after finishing identification of all ectoparasites and detection of *Bartonella* DNA, and it took about 1 to 2 months. In the previous studies, the isolation rate of *Bartonella* from fresh deer ked samples was reported to be considerably high as 51.5% [12] and 73.3% [36]. Therefore it is necessary to use samples as fresh as possible for understanding the actual prevalence of the viable bacteria in ectoparasites. Since the northern bats that we sampled were living in dense conditions within the exterior walls of a building (Supplementary video), it seems that the fleas can easily move between bat bodies in the colony. When comparing the *gltA* genotypes of *Bartonella* between the bats and the fleas, multiple genotypes were detected in the fleas, but the bats were infected with only one genotype. The evidence supports the possibility that the bat fleas actively move and suck blood of northern bats in the colony, and the bat fleas are likely to transmit *Bartonella* bacteria to individual bats.

In the BLAST searches, the *gltA* and *rpoB* sequences of strains EN2-1 (genotype I) and EN19-2 (genotype II) showed the highest similarities (97.9% in *gltA* and 98.8% in *rpoB*) with strains 1157/3 from a northern bat in Finland, 1F40 from *Ischnopsyllus variabilis* in the Netherlands, and 44722 from *Eptesicus serotinus* in Georgia. On the other hand, EN36-1 (genotypes III) showed the highest similarities (95.9% in *gltA* and 96.7% in *rpoB*) with a shrew (*Clocidura* spp.) strain B28303 in Kenya and *B. florencae* R4^T in France.

In the phylogenetic analysis based on the *gltA* sequences, strains EN2-1 (genotype I) and EN19-2 (genotype II) formed clade A with strains from bat fleas collected from an *Eptesicus* bat in the Netherlands, *Eptesicus* bats in Georgia and Finland, and *Myotis* bats in the UK and Romania. Strain EN36-1 (genotypes III) formed clade B with *B. florencae*, *B. acomydis*, and *B. birtlesii* from shrews, wood mouse, and golden spiny mouse, respectively.

These results suggest that genotypes I and II are Vesper bat-specific *Bartonella* species and genotype III is closely related to shrew and rodent-derived *Bartonella* species. Interestingly, strains in clade B were distinct from other known bat-associated *Bartonella* strains. A previous study [37] suggested that host switching of *Bartonella* is a common event as has occurred at least five times between bats and other small mammals. From the results of BLAST search and phylogenetic analysis based on the *gltA* sequences, such an event might have occurred in genotype III between Japanese northern bats and shrews. However, the identity of a “bridge” vector capable of transmitting *Bartonella* bacteria between bats and other small mammal species is currently unknown. Further investigation will be necessary to clarify the bridge vectors of northern bats.

Our study showed that northern bats in Japan harbor two species of *Bartonella*: Vesper bat-specific *Bartonella* species and a *B. florencae*-like bacterium. Additionally, bat fleas (*Ischnopsyllus needhami*) were found to be dominant ectoparasites in the bats and serve as a potential vector of *Bartonella* transmission among northern bats in Japan.

Declarations

Acknowledgements

This work was supported by Grant-in Aid for Scientific Research from Japan Society for the Promotion of Science [Grant number 18K06003]. We would like to thank Dr. Yuki Kobayashi, Nihon University for her support of sample collections.

Funding

This work was supported by Grant-in Aid for Scientific Research (No. 18K06003) from the Japan Society for the Promotion of Science.

Conflicts of interest/Competing interests

The authors have no conflicts of interest directly relevant to the content of this article.

Availability of data and material, Code availability

Not applicable.

Authors' contributions

Kei Nabeshima: Writing - Original Draft, Methodology, Investigation, Shingo Sato: Methodology, Investigation, Writing - Review & Editing, Murasaki Amano: Investigation, Jory R. Brinkerhoff: Methodology, Investigation, Writing - Review & Editing, Hidenori Kabeya: Writing - Review & Editing, Itou Takuya: Investigation, Soichi Maruyama: Resources, Conceptualization, Supervision, Writing - Review & Editing

References

1. IUCN (2020) The IUCN Red List of Threatened Species. Version 2020-3. <https://www.iucnredlist.org>. Accessed 1 March 2021
2. Matsue M, Fujiwara N, Oshio T et al (2006) A draft of the guideline for ecological surveys on bat species (in Japanese). <http://www.nilim.go.jp/lab/bcg/siryuu/tnn/tnn0354.htm>. Accessed 1 March 2021
3. Mühldorfer K (2013) Bats and bacterial pathogens: A review. *Zoonoses Public Health* 60:93–103. <https://doi.org/10.1111/j.1863-2378.2012.01536.x>
4. Stuckey MJ, Chomel BB, de Fleurieu EC et al (2017) *Bartonella*, bats and bugs: A review. *Comp Immunol Microbiol Infect Dis* 55:20–29. <https://doi.org/10.1016/j.cimid.2017.09.001>
5. Lin EY, Tsigrelis C, Baddour LM et al (2010) *Candidatus Bartonella mayotimonensis* and endocarditis. *Emerg Infect Dis* 16:500–503. <https://doi.org/10.3201/eid1603.081673>
6. Veikkolainen V, Vesterinen EJ, Lilley TM, Pulliainen AT (2014) Bats as reservoir hosts of human bacterial pathogen, *Bartonella mayotimonensis*. *Emerg Infect Dis* 20:960–967.

<https://doi.org/10.3201/eid2006.130956>

7. Stuckey MJ, Boulouis HJ, Cliquet F et al (2017) Potentially zoonotic bartonella in bats from France and Spain. *Emerg Infect Dis* 23:539–541. <https://doi.org/10.3201/eid2303.160934>
8. Urushadze L, Bai Y, Osikowicz L et al (2017) Prevalence, diversity, and host associations of *Bartonella* strains in bats from Georgia (Caucasus). *PLoS Negl Trop Dis* 11:1–19. <https://doi.org/10.1371/journal.pntd.0005428>
9. Chomel BB, Kasten RW, Floyd-Hawkins K et al (1996) Experimental transmission of *Bartonella henselae* by the cat flea. *J Clin Microbiol* 34:1952–1956. <https://doi.org/10.1128/JCM.34.8.1952-1956.1996>
10. Bonilla DL, Kabeya H, Henn J et al (2009) *Bartonella quintana* in body lice and head lice from homeless persons, San Francisco, California, USA. *Emerg Infect Dis* 15:912–915. <https://doi.org/10.3201/eid1506.090054>
11. Garcia-Quintanilla M, Dichter AA, Guerra H, Kempf VAJ (2019) Carrion's disease: more than a neglected disease. *Parasit Vectors* 12:141. <https://doi.org/10.1186/s13071-019-3390-2>
12. Sato S, Kabeya H, Ishiguro S et al (2021) *Lipoptena fortisetosa* as a vector of *Bartonella* bacteria in Japanese sika deer (*Cervus nippon*). *Parasites Vectors* 14:73. <https://doi.org/10.1186/s13071-021-04585-w>
13. Nabeshima K, Sato S, Kabeya H et al (2020) Detection and phylogenetic analysis of *Bartonella* species from bat flies on eastern bent-wing bats (*Miniopterus fuliginosus*) in Japan. *Comp Immunol Microbiol Infect Dis* 73:101570. <https://doi.org/10.1016/j.cimid.2020.101570>
14. Reeves WK, Loftis AD, Gore JA, Dasch GA (2005) 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* 30:339–41
15. Judson SD, Frank HK, Hadly EA (2013) Bartonellae are prevalent and diverse in Costa Rican bats and bat flies. *Zoonoses Public Health* 62:609–617. <https://doi.org/10.1111/zph.12188>
16. Brook CE, Bai Y, Dobson AP et al (2015) *Bartonella* spp. in fruit bats and blood-feeding ectoparasites in Madagascar. *PLoS Negl Trop Dis* 9:1–9. <https://doi.org/10.1371/journal.pntd.0003532>
17. Dietrich M, Tjale MA, Weyer J et al (2016) Diversity of *Bartonella* and *Rickettsia* spp. in bats and their blood-feeding ectoparasites from South Africa and Swaziland. *PLoS One* 11:1–9. <https://doi.org/10.1371/journal.pone.0152077>
18. Kamani J, Baneth G, Mitchell M et al (2014) *Bartonella* species in bats (Chiroptera) and bat flies (Nycteribiidae) from Nigeria, West Africa. *Vector-Borne Zoonotic Dis* 14:625–632. <https://doi.org/10.1089/vbz.2013.1541>
19. Billeter SA, Hayman DTSS, Peel AJ et al (2012) *Bartonella* species in bat flies (Diptera: Nycteribiidae) from western Africa. *Parasitology* 139:324–329. <https://doi.org/10.1017/S0031182011002113>
20. Morse SF, Olival KJ, Kosoy M et al (2012) Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Streblidae, Nycteribiidae). *Infect Genet Evol* 12:1717–1723. <https://doi.org/10.1016/j.meegid.2012.06.009>

21. Nabeshima K, Sato S, Kabeya H et al (2020) Isolation and genetic properties of *Bartonella* in eastern bent-wing bats (*Miniopterus fuliginosus*) in Japan. *Infect Genet Evol* 83:104354. <https://doi.org/10.1016/j.meegid.2020.104354>
22. McKee CD, Kosoy MY, Bai Y et al (2017) Diversity and phylogenetic relationships among *Bartonella* strains from Thai bats. *PLoS One* 12:1–19. <https://doi.org/10.1371/journal.pone.0181696>
23. Anh PH, Van Cuong N, Son NT et al (2015) Diversity of *Bartonella* spp. in bats, southern Vietnam. *Emerg Infect Dis*. <https://dx.doi.org/10.3201/eid2107.141760>
24. Lin J-WW, Hsu Y-MM, Chomel BB et al (2012) Identification of novel *Bartonella* spp. in bats and evidence of Asian gray shrew as a new potential reservoir of *Bartonella*. *Vet Microbiol* 156:119–126. <https://doi.org/10.1016/j.vetmic.2011.09.031>
25. Han H-JJ, Wen HL, Zhao L et al (2017) Novel *Bartonella* species in insectivorous bats, northern China. *PLoS One* 12:e0167915. <https://doi.org/10.1371/journal.pone.0167915>
26. Sakaguti K (1962) *Pacific Insects Monograph*. Bernice P. Bishop Mus, Honolulu
27. Usinger R (1966) *Monograph of Cimicidae (Hemiptera - Heteroptera)*. Entomological Society of America, College Park
28. Diaz MH, Bai Y, Malaria L et al (2012) Development of a novel genus-specific real-time PCR assay for detection and differentiation of *Bartonella* species and genotypes. *J Clin Microbiol* 50:1645–1649. <https://doi.org/10.1128/JCM.06621-11>
29. Norman AF, Regnery R, Jameson P et al (1995) Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. *J Clin Microbiol* 33:1797–1803. <https://doi.org/10.1128/JCM.33.7.1797-1803.1995>
30. Renesto P, Gouvernet J, Drancourt M et al (2001) Use of *rpoB* gene analysis for detection and identification of *Bartonella* species. *J Clin Microbiol* 39:430–437. <https://doi.org/10.1128/JCM.39.2.430-437.2001>
31. Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772. <https://doi.org/10.1038/nmeth.2109>
32. Burnham KP, Anderson DR (2004) Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociol Methods Res* 33:261–304. <https://doi.org/10.1177/0049124104268644>
33. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol* 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>
34. Orlova MV (2011) Ectoparasite associations of bats from Urals (Russia). *Hystrix It. J. Mamm.* 2011;22(1). <https://doi.org/10.4404/hystrix-22.1-4470>
35. Pearce RD, O'Shea TJ (2007) Ectoparasites in an urban population of big brown bats (*Eptesicus fuscus*) in Colorado. *J Parasitol* 93:518–530. <https://doi.org/10.1645/GE-973R.1>
36. Dehio C, Sauder U, Hiestand R (2004) Isolation of *Bartonella schoenbuchensis* from *Lipoptena cervi*, a Blood-Sucking Arthropod Causing Deer Ked Dermatitis. *J Clin Microbiol* 42:5320–5323. <https://doi.org/10.1128/JCM.42.11.5320-5323.2004>

Compressed maximum likelihood phylogenetic tree based on *gltA* sequences. Evolutionary distances were calculated by the maximum likelihood method with GTR+G+I model in MEGA 7. The triangles represent compressed branches containing various sequences from bat-associated *Bartonella*. The uncompressed tree is available as a Supplementary Figure. Representative isolates from *E. nilssonii* examined in this study, the 420 bats and their ectoparasite-associated *Bartonella* strains, and 40 type strains were included in the analysis. *Bartonella* isolates from *E. nilssonii* in Japan are indicated by red circles. The clades that contain the isolates in this study are enlarged on the right side of the figure.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFig.tif](#)
- [Supplementaryvideo201207.mp4](#)
- [Table.S1200702.xlsx](#)