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## Isolation and genetic properties of *Bartonella* in eastern bent-wing bats (*Miniopterus fuliginosus*) in Japan

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### ABSTRACT

The prevalence and genetic characteristics of *Bartonella* species in eastern bent-wing bats (*Miniopterus fuliginosus*) from Japan were investigated. *Bartonella* bacteria were isolated from 12/50 (24%) of bats examined. Analyses of sequence similarities of the citrate synthase gene (*gltA*) and RNA polymerase beta-subunit-encoding (*rpoB*) gene indicated that the isolates from *M. fuliginosus* were distinct from those present in known *Bartonella* species as the levels of similarity for both of the genes were lower than the cut-off values for species identification in *Bartonella*. A phylogenetic analysis of the *gltA* sequences revealed that the *Miniopterus* bat-associated strains fell into five genotypes (I to V). Though genotypes I to IV formed a clade with *Bartonella* from *Miniopterus* bats from Taiwan, genotype V made a monophyletic clade separate from other bat isolates. In a phylogenetic analysis with the concatenated sequences of the 16S rRNA, *gltA*, *rpoB*, cell division protein (*ftsZ*) gene, and riboflavin synthase gene (*ribC*), isolates belonging to genotypes I to IV clustered with *Bartonella* strains from Taiwanese *Miniopterus* bats, similar to the outcome of the phylogenetic analysis with *gltA*, whereas genotype V also made a monophyletic clade separate from other bat-associated *Bartonella* strains. The present study showed that *M. fuliginosus* in Japan harbor both genus *Miniopterus*-specific *Bartonella* suggesting to be specific to the bats in Japan.

### 1. Introduction

Bats (Order Chiroptera) are widely distributed and are found on all continents except the polar regions. Thirty-five species of bats are now recognized to be present in Japan (Matsue et al., 2006); the genera *Pipistrellus*, *Myotis*, *Rhinolophus*, *Eptesicus*, and *Miniopterus* inhabit throughout the country. Bats can serve as reservoirs for various viral zoonotic pathogens such as rabies virus in *Desmodus* bats in South America, Nipah virus and Hendra virus in *Pteropus* bats in India and Australia, and SARS coronavirus in *Rhinolophus* bats in China (Calisher et al., 2006). Other zoonotic bacteria, such as *Campylobacter jejuni* and *Leptospira* spp., have also been detected in *Myotis* bats in the Netherlands and in *Artibeus*, *Phyllostomus*, and *Sturnira* bats in Peru (Muhldorfer, 2013).

*Bartonella* bacteria are small, fastidious, and Gram-negative bacilli that parasitize erythrocytes and endothelial cells in many species of mammals. Several *Bartonella* spp. are known to cause human diseases

including cat-scratch disease (*B. henselae*), trench fever (*B. quintana*), and Carrión's disease (*B. bacilliformis*). Blood-sucking ectoparasites have been shown to play an important role in the transmission of *Bartonella* species among their hosts: cat fleas transmit *B. henselae* between cats (Chomel et al., 1996) and lice transmit *B. quintana* between humans (Bonilla et al., 2009). There are evidences that bats also serve as a reservoir for pathogenic *Bartonella* species. *B. mayotimonensis* was first detected in the aortic valve of a patient with endocarditis in the USA (Lin et al., 2010); subsequently, it was detected in *Myotis* bats in Finland (Veikkolainen et al., 2014), the USA (Lilley et al., 2017), France, and Spain (Stuckey et al., 2017b). Interestingly, the citrate synthase gene (*gltA*) sequence of *Bartonella* strains from *Myotis* bats in Georgia is closely related to that of *Bartonella* DNA identified in forest workers in Poland (Urushadze et al., 2017).

*Bartonella* species have been detected in many bat genera, such as *Pipistrellus*, *Myotis*, *Nyctalus*, *Rhinolophus*, *Eptesicus*, *Miniopterus*, *Hipposideros*, *Megaderma*, *Cynopterus*, *Coleura*, *Triaenops*, *Epomophorus*,

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**Table 1**  
Genotyping of bat isolates based on the *gltA* sequences and GenBank accession numbers for five housekeeping genes.

Genotype	Representative strain	Accession numbers in:				
		<i>gltA</i>	<i>rpoB</i>	16S rRNA	<i>ftsZ</i>	<i>ribC</i>
I	bat2-1	LC483820	LC483825	LC483830	LC483835	LC483841
II	bat23-1	LC483822	LC483827	LC483832	LC483837	LC483844
III	bat8-3	LC483821	LC483826	LC483831	LC483836	LC483843
IV	bat43-1	LC483824	LC483829	LC483833	LC483838	LC483842
V	bat24-1	LC483823	LC483828	LC483834	LC483839	LC483840

**Table 2**  
Sequence similarities of the *gltA* and *rpoB* genes among strains from *M. fuliginosus*.

Genotype (strain)	Sequence similarities (%) by strain:				
	I (bat2-1)	II (bat23-1)	III (bat8-3)	IV (bat43-1)	V (bat24-1)
I (bat2-1)	–	99.4 <sup>a</sup>	99.4 <sup>a</sup>	99.0 <sup>a</sup>	84.4 <sup>a</sup>
II (bat23-1)	97.7 <sup>b</sup>	–	99.4 <sup>a</sup>	99.7 <sup>a</sup>	85.1 <sup>a</sup>
III (bat8-3)	97.2 <sup>b</sup>	99.3 <sup>b</sup>	–	99.7 <sup>a</sup>	85.1 <sup>a</sup>
IV (bat43-1)	97.7 <sup>b</sup>	100 <sup>b</sup>	99.3 <sup>b</sup>	–	85.4 <sup>a</sup>
V (bat24-1)	87.9 <sup>b</sup>	87.7 <sup>b</sup>	87.6 <sup>b</sup>	87.7 <sup>b</sup>	–

<sup>a</sup> Sequence similarities of *gltA*.

<sup>b</sup> Sequence similarities of *rpoB*.

*Rousettus*, *Eidolon*, and *Megaerops* (Stuckey et al., 2017a). *Miniopterus* bats have been found to show up to 56.3% (49/87) *Bartonella* prevalence in Kenya (Kosoy et al., 2013), 88.9% (24/27) in Georgia (Urushadze et al., 2017), and 42.9% (6/14) in Taiwan (Lin et al., 2012). To date, however, no information on the prevalence and genetic properties of *Bartonella* species is available in any bat species in Japan. The present study was conducted to determine the prevalence and genetic characteristics of *Bartonella* species in *Miniopterus fuliginosus* bats in Japan.

## 2. Materials and methods

### 2.1. Collection of blood and ectoparasites from bats

In March 2013, fifty *M. fuliginosus* were captured at a headrace tunnel in Wakayama Prefecture located in the western part of Japan (33°40'N, 135°23'E) after gaining the permission (license # Nishi 4 and 5) of sample collection from the local government. Blood samples were aseptically collected via cardiac puncture from bats euthanized following the guidelines for euthanasia of the Japan Veterinary Medical Association. The blood samples were transferred to a blood collection tube containing EDTA, the samples immediately frozen in dry ice, and transported to the Laboratory of Veterinary Public Health, the Department of Veterinary Medicine, College of Bioresource Sciences, Nihon University. The blood samples were stored at –70 °C until examined. Ectoparasites were also collected from the bat carcasses during autopsy in the laboratory.

**Table 3**  
Sequence similarities of the *gltA* and *rpoB* genes between representative isolates from *M. fuliginosus* and their closest strains.

Geno type	Strain	<i>gltA</i>			<i>rpoB</i>		
		Closest strain	Host (country)	% similarity	Closest strain	Host (country)	% similarity
I	bat2-1	No. 5	<i>Miniopterus schreibersii</i> (Taiwan)	100%	No. 15	<i>Miniopterus schreibersii</i> (Taiwan)	99.4%
II	bat23-1	No. 7		100%	No. 5		100%
III	bat8-3	No. 7		99.7%	No. 5		99.3%
IV	bat43-1	No. 7		99.7%	No. 5		100.0%
V	bat24-1	44,544	<i>Myotis blythii</i> (Georgia)	89.0%	<i>B. quintana</i> Fuller <sup>T</sup>	Human (Yugoslavia)	89.1%

### 2.2. Isolation of *Bartonella* bacteria from bats and identification of ectoparasites

Frozen blood samples were thawed at room temperature and 100 µl of each blood sample was separately transferred to sterile 1.5 ml conical tubes. The blood was mixed with 100 µl of medium 199 supplemented with 1 mM sodium pyruvate solution and 20% fetal bovine serum (Life Technologies, Carlsbad, CA, USA). Aliquots (100 µl) of the mixture were placed on two heart infusion agar plates (Difco, Sparks Glencoe, MI, USA) containing 5% rabbit blood. The inoculated plates were incubated at 35 °C in a moist atmosphere under 5% CO<sub>2</sub> for up to 4 weeks. *Bartonella* bacteria were tentatively identified by colony morphology (small, gray or cream-yellow, round shape); three colonies were picked from each positive sample and sub-cultured on a fresh blood agar plate using the same conditions as the primary culture.

The identification of ectoparasites on the bats was performed by morphological characteristics under a stereomicroscope SZX16 (Olympus, Tokyo, Japan) by the reference of bat flies in Japan (Sato and Mogi, 2008).

### 2.3. PCR amplification of the *gltA* and *rpoB*

Genomic DNA was extracted from each sub-culture colony using InstaGene Matrix (Bio-Rad Laboratories, Inc. Hercules, CA, USA). Genus-specific PCR targeting the *gltA* and the RNA polymerase beta-subunit-encoding gene (*rpoB*) was then performed to confirm *Bartonella* identification (Norman et al., 1995; Renesto et al., 2001). The genomic DNA of *B. alsatica* IBS382<sup>T</sup> and nuclease-free distilled water were used as positive and negative controls, respectively. The PCR amplicons were separated by electrophoresis on 3% agarose gels and visualized by staining with ethidium bromide. Samples showing product sizes of approximately 390 bp for the *gltA* and approximately 900 bp for the *rpoB* were considered to be positive for the genus *Bartonella*.

### 2.4. DNA sequencing and genotyping

PCR amplicons were purified using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Subsequently, the nucleotide sequences of the amplicons were determined using a Genetic Analyzer model 3130 (Applied Biosystems, Foster City, CA, USA). The *gltA* genotype was determined when unique sequence variants with ≥ 1 nucleotide difference were found in each isolate by comparing the gene

**Table 4**Sequence similarities of the *gltA* and *rpoB* genes in representative isolates from *M. fuliginosus* in Japan and closest type strains of *Bartonella* species.

Genotype	Strain	<i>gltA</i>		<i>rpoB</i>	
		Closest species	% similarity	Closest species	% similarity
I	bat2-1	<i>B. quintana</i> Fuller <sup>T</sup>	91.0%	<i>B. callosciuri</i> BR11-1 <sup>T</sup>	88.7%
II	bat23-1	<i>B. capreoli</i> IBS193 <sup>T</sup>	91.0%	<i>B. melophagi</i> K-2C <sup>R</sup>	88.4%
III	bat8-3	<i>B. quintana</i> Fuller <sup>T</sup>	91.0%	<i>B. melophagi</i> K-2C <sup>R</sup>	88.5%
IV	bat43-1	<i>B. capreoli</i> IBS193 <sup>T</sup>	91.3%	<i>B. melophagi</i> K-2C <sup>R</sup>	88.4%
V	bat24-1	<i>B. rochalimae</i> BMGH <sup>T</sup>	87.2%	<i>B. quintana</i> Fuller <sup>T</sup>	89.1%

sequences by using Genetyx software Ver 12 (Genetyx Corporation, Tokyo, Japan). Representative isolates from each genotype were further analyzed by sequencing of the *rpoB*, the 16S rRNA, the cell division protein gene (*ftsZ*), and the riboflavin synthase gene (*ribC*). The PCR protocol used for amplification of the 16S rRNA (Heller et al., 1997), *ftsZ* (Zeaiter et al., 2002), and *ribC* (Johnson et al., 2003) was based on the conditions described in previous reports on *Bartonella*.

### 2.5. Sequence homology analysis

Nucleotide sequences of the *gltA*, *rpoB* and 16S rRNA were compared between the representative bat isolates and the strains of prokaryotes registered in the GenBank/EMBL/DDBJ database using the BLAST program. Sequence similarities in the *gltA* and *rpoB* genes were additionally compared with the type strains of *Bartonella* species.

### 2.6. Phylogenetic analysis

Based on evolutionary model selection using JModelTest2 (Darriba et al., 2012) with Akaike's information criterion corrected for finite sample sizes (AICc; Burnham and Anderson, 2004), 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 in the phylogenetic analyses based on the *gltA* sequences and the concatenated sequences with the *gltA*, *rpoB*, *ribC*, *ftsZ*, and 16S rRNA.

A phylogenetic tree based on the *gltA* sequences of the *Bartonella* isolates was constructed using the Maximum Likelihood method based on the GTR + G + I model in MEGA 7 (Kumar et al., 2016). Known *Bartonella* species ( $N = 41$ ) and bat-associated *Bartonella* strains ( $N = 249$ ) derived from Taiwan (Lin et al., 2012), China (Han et al., 2017), Vietnam (Anh et al., 2015), Thailand (Mckee et al., 2017), France (Stuckey et al., 2017b), UK (Concannon et al., 2005), USA (Lilley et al., 2017), Guatemala (Bai et al., 2011), Mexico (Stuckey et al., 2017c), Peru (Bai et al., 2012; Becker et al., 2018), Belize (Becker et al., 2018) Madagascar (Brook et al., 2015), Kenya (Kosoy et al., 2013), and Georgia (Urushadze et al., 2017) were included in this analysis. Strain names, host species, countries where the bats were collected, and the *gltA* accession numbers are summarized in Supplementary Table 1.

A phylogenetic tree based on the concatenated sequences of the 16S rRNA, *gltA*, *rpoB*, *ftsZ*, and *ribC* was constructed using the Maximum Likelihood based on the GTR + G + I model in MEGA 7 (Kumar et al., 2016). The representative isolates in the present study were also compared with six Taiwanese *Bartonella* strains (No. 5, No. 6, No. 7, No. 8, No. 15, and No. 16) from *M. schreibersii* (Lin et al., 2012) in this phylogenetic analysis. Support for nodes in both trees was assessed by bootstrapping with 1000 replicates.

## 3. Results

### 3.1. Isolation of *Bartonella* bacteria from bats, genotyping the bat isolates based on the *gltA* sequences, and identification of ectoparasites on bats

*Bartonella* bacteria were isolated from 12/50 (24%) bats examined and totally 36 bacterial isolates were obtained. The *gltA* sequences of the isolates were classified into five genotypes (I to V, Table 1). Eleven of the bats were infected by a single *Bartonella* genotype, but the other bat was co-infected with two genotypes (I and III). The *gltA*, *rpoB*, 16S rRNA, and *ribC* sequences of all genotypes were deposited in the GenBank/EMBL/DDBJ database (Table 1).

In total, 52 ectoparasites were collected from the bats and identified as the bat flies, genus *Nycteribia* ( $N = 34$ ; species was not identified) and as *Penicillidia jenymsii* ( $N = 18$ ).

### 3.2. Sequence homology of the *gltA*, *rpoB* and 16S rRNA

Sequence similarities among representative genotypes I (Strain bat2-1), II (Strain bat23-1), III (Strain bat8-3), and IV (Strain bat43-1) ranged from 99.0 to 99.7% for the *gltA* and 97.2 to 100% for the *rpoB*. Sequence similarities between genotype V and genotypes I to IV ranged from 84.4 to 85.4% for the *gltA* and 87.6 to 87.9% for the *rpoB* (Table 2).

Sequence similarities between representative genotypes I (Strain bat2-1), II (Strain bat23-1), III (Strain bat8-3), and IV (Strain bat43-1) and strains No. 5, No. 7, and No. 15 from *Miniopterus* bats in Taiwan ranged from 99.7 to 100% for the *gltA* and 99.2 to 100% for the *rpoB*. In contrast, representative genotype V (Strain bat24-1) showed 89.0% similarity for the *gltA* with strain 44,544 from Georgian mouse-eared bats (*Myotis blythii*) and 89.1% similarity for the *rpoB* with *Bartonella quintana* strain Fuller<sup>T</sup> (Table 3).

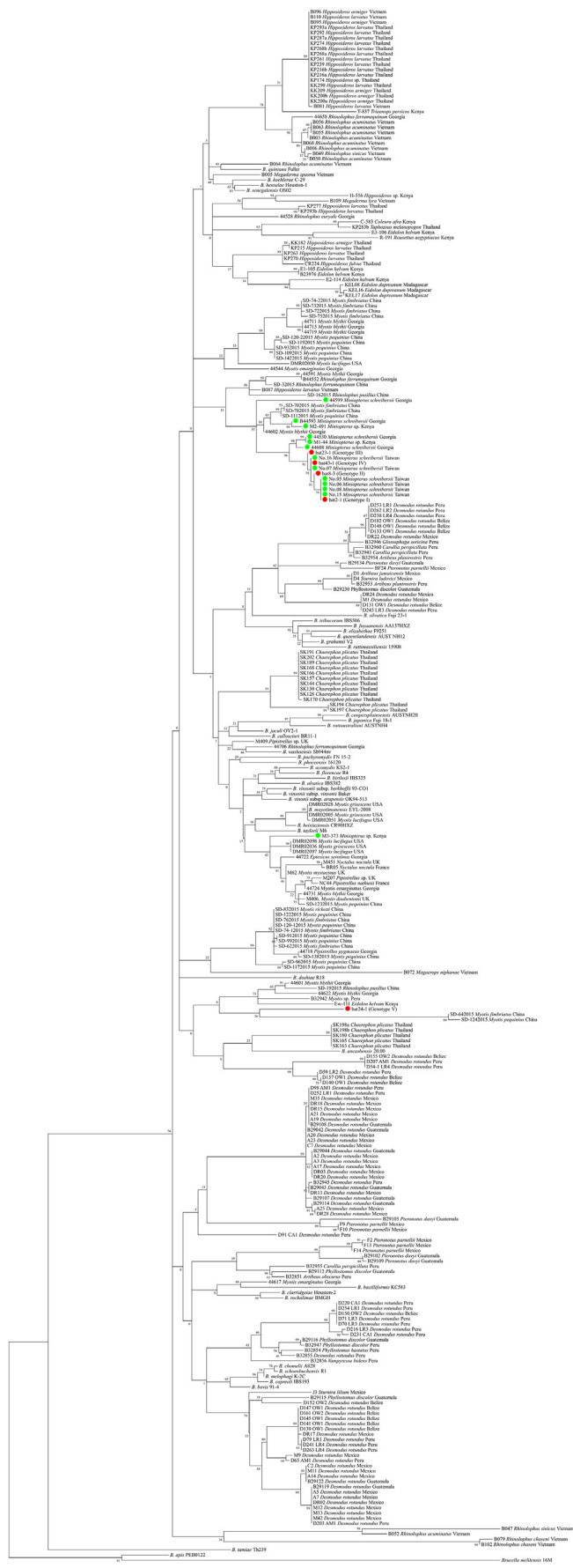
In addition, 16S rRNA sequences of genotypes I to IV were identical to that of *Bartonella* sp. strain No.5 from *Miniopterus* bats in Taiwan. On the other hand, genotype V showed 97.9% similarity with *Bartonella* sp. strain F2 isolated from *Pteronotus parnellii* in Mexico (data not shown).

Sequence similarities for the *gltA* and *rpoB* between representative isolates and type strains of existing *Bartonella* species ranged from 87.2 to 91.3% and 88.2 to 89.1%, respectively (Table 4).

### 3.3. Phylogenetic analysis based on the *gltA* sequences and concatenated sequences of five genes

In the phylogenetic analysis based on the *gltA* sequences, four representative isolates derived from genotypes I (Strain bat2-1), II (Strain bat23-1), III (Strain bat8-3), and IV (Strain bat43-1) clustered with the isolates from *Miniopterus* bats in Taiwan (No. 5, No. 6, No. 7, No. 8, No. 15, and No. 16). Genotype V (Strain bat24-1) made a monophyletic clade distinct from the other known *Bartonella* strains (Fig. 1).

In the phylogenetic analysis with the concatenated sequences of five genes (16S rRNA, *gltA*, *rpoB*, *ftsZ*, and *ribC*), four representative isolates from genotypes I–IV grouped in a lineage with strains No. 5, No. 6, No. 8, and No. 15 from *Miniopterus* bats in Taiwan; no known *Bartonella* species was present in the lineage. Strain bat24-1 of genotype V made a



**Fig. 1.** Phylogenetic relationship of bat-associated *Bartonella* strains based on the *gltA* sequences. Evolutionary distances were calculated by Maximum Likelihood method with GTR + G + I model in MEGA 7. The analysis included five representative isolates from *M. fuliginosus*, 249 bat-associated *Bartonella* strains, and 41 known type strains of *Bartonella* species. *Bartonella* isolates from *M. fuliginosus* in Japan are indicated by red circles and isolates from *Miniopterus* bats in Taiwan, Kenya and Georgia are shown by green circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

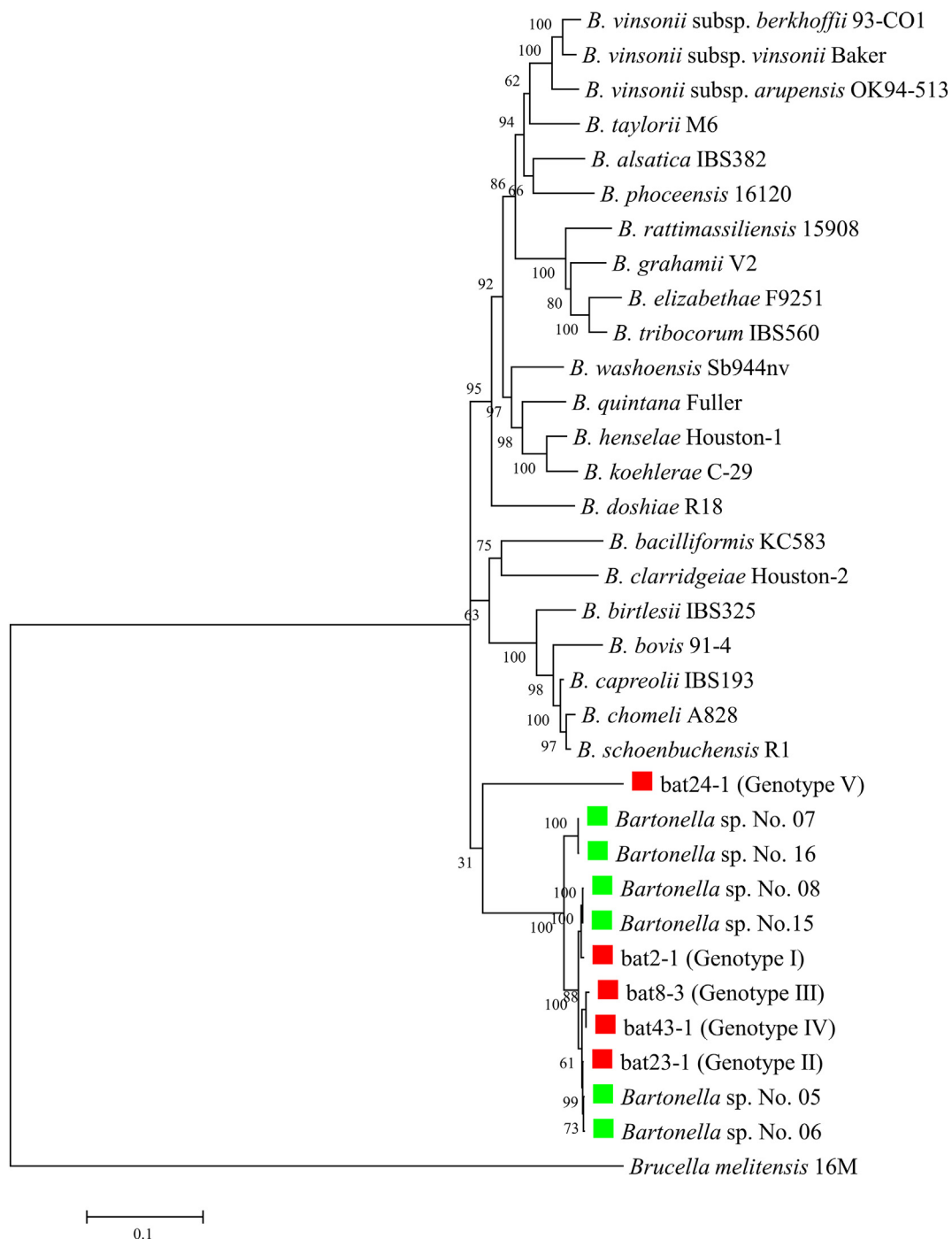
monophyletic clade, consistent with the result of the phylogenetic analysis using the *gltA* sequences and was clearly different from known *Bartonella* species (Fig. 2).

**4. Discussion**

The present study showed that 24% (12/50) of *M. fuliginosus* in Japan harbored *Bartonella* bacteria in their blood. The genus *Miniopterus* is insectivorous and is distributed worldwide, with the exception of polar regions (IUCN, 2019). In previous studies, *Bartonella* bacteria have been isolated from *Miniopterus* bats: 49/87 (56.3%; Kosoy et al., 2013) in Kenya; 24/27 (88.9%; Urushadze et al., 2017) in Georgia; and 6/14 (42.9%; Lin et al., 2012) in Taiwan. Although we used almost similar procedure for the isolation of *Bartonella* as reported in the previous studies, the prevalence in *Miniopterus* bats in Japan found to be lower than those in Kenya, Georgia, and Taiwan. It is suggested that the prevalence of *Bartonella* in Japanese *Miniopterus* bats examined may be basically lower than other countries. With regard to other bat genera, *Bartonella* has been reported in 140/445 (31.5%) of *Myotis* bats, 10/172 (5.8%) of *Pipistrellus* bats, 5/53 (9.4%) of *Eptesicus* bats, and 47/120 (39.2%) of *Rhinolophus* bats (Stuckey et al., 2017a). Thus, the rate of *Miniopterus* individuals carrying *Bartonella* bacteria is relatively high compared with other insectivorous bat genera. *Miniopterus* form large colonies, especially in winter, and show considerable inter-individual contact (Serra-Cobo and López-Roig, 2017). In the present study, we found many colonies consisted of several dozens to hundreds of individuals in the sampled tunnel. Furthermore, ectoparasitic bat flies (*Nycteribia* sp. and *P. jenyensis*) were recovered from the bats. The high colony density of *Miniopterus* and the presence of ectoparasitic bat flies may have contributed to the higher prevalence and horizontal transmission of *Bartonella* bacteria within the population.

Only one bat was co-infected with two genotypes (I and III) of *Bartonella* bacteria. Co-infection with different species and genotypes of *Bartonella* has been reported in various wild mammals such as bats (Urushadze et al., 2017; Han et al., 2017), rodents (Gutiérrez et al., 2015), carnivores (Henn et al., 2009), and ruminants (Sato et al., 2012). Urushadze et al. (2017) reported that the co-infection rate of *Bartonella* in *Miniopterus* bats was 29.2% (7/24) of the positive samples by analyzing all morphologically unique colonies. Since we examined three colonies per positive samples in the present study, more colonies are needed to detect detailed co-infection status in the bats.

La Scola et al. (2003) suggested that newly encountered *Bartonella* isolates should be considered as new species if the *gltA* fragment (327 bp) and *rpoB* fragment (825 bp) showed < 96.0% and < 95.4% sequence similarities with those of validated *Bartonella* type strains, respectively. Here, the comparisons of the *gltA* and *rpoB* sequences between bat isolates and type strains of *Bartonella* species yielded similarities for both genes that were lower than the suggested cut-off values for species identification in *Bartonella*. In the homology analysis among representative strains, genotypes I to IV showed high sequence similarities for the *gltA* and *rpoB*, whereas genotype V showed low similarities with other genotypes. From the BLAST search, the *gltA* and *rpoB* sequences of genotypes I to IV showed the highest similarity (> 99.7% for *gltA* and > 99.2% for *rpoB*) with three strains from *Miniopterus* bats in Taiwan. However, genotype V showed low similarity (89.0%) with strain 44,544 from *Myotis* for the *gltA* and 89.1% with *B.*



**Fig. 2.** Phylogenetic relationship based on concatenated sequences of bat-associated *Bartonella* strains. Evolutionary distances were calculated by Maximum Likelihood method with the GTR + G + I model and are shown as number of base substitutions per site. The analysis includes five representative isolates from *M. fuliginosus*, six *Bartonella* strains (No. 5, No. 6, No. 7, No. 8, No. 15, and No. 16) from *Miniopterus* bats in Taiwan, and 22 known type strains of *Bartonella* species. Evolutionary analyses were conducted in MEGA7. *Bartonella* isolates from *M. fuliginosus* in Japan are indicated by the red squares on the left and isolates from *Miniopterus* bats in Taiwan are shown by green squares. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*quintana* Fuller<sup>T</sup> for the *rpoB* sequence.

In the phylogenetic analysis based on the *gltA* sequences, the representative isolates from *M. fuliginosus* fell into two lineages. Urushadze et al. (2017) reported that bat-associated *Bartonella* strains tended to be grouped by genus and/or family of the bat in a phylogenetic analysis of the *gltA*. Corduneanu et al. (2018) also showed that *Miniopterus* bat-associated *Bartonella* clustered in the same clade in a phylogenetic analysis of *gltA*. Genotypes I to IV found in the present

study formed a lineage with strains from *Miniopterus* in Taiwan, suggesting that these strains are specific to *Miniopterus* living in Japan and Taiwan. However, genotype V formed a monophyletic clade clearly separate from any bat-associated *Bartonella* strains. Thus, Japanese *M. fuliginosus* may harbor a unique *Bartonella* lineage which has not been found in any other bat species.

As reported by the *ad hoc* committee for the re-evaluation of species definition in bacteriology, the description of a new species should be

based on the sequence analysis of housekeeping genes using at least five genes (Stackebrandt et al., 2002). The phylogenetic tree based on the concatenated sequences of five genes showed that genotypes I to IV were closely related to the isolates from *Miniopterus* in Taiwan, as in the phylogenetic tree for the *gltA*. These data suggest that *Miniopterus* bats in both Japan and Taiwan harbor *Bartonella* bacteria with high genetic similarities. However, strain bat 24-1 of genotype V formed a clearly independent clade that was separate from other bat isolates and known *Bartonella* species. These results suggest that *M. fuliginosus* in Japan harbor two novel *Bartonella* species: one is similar to species found in Taiwanese *Miniopterus* and another is specific to Japanese bats.

Previous studies have shown that some of the blood-sucking arthropods that infest bats may be involved in *Bartonella* transmission among bats (Brook et al., 2015; Muhldorfer, 2013). Bat flies have been suggested to be a potential vector for *Bartonella* transmission in bat population in Africa, South America, and Asia as *Bartonella* DNAs are often detected in these ectoparasites (Stuckey et al., 2017a). In the present study, we identified bat flies belonging to *Nycteribia* sp. and *P. jenkinsii* on *Miniopterus* bats; these bat fly species are known as blood-sucking obligate ectoparasites. Further studies are necessary to clarify the role of bat flies as transmission vectors of *Bartonella* bacteria among *M. fuliginosus*.

## 5. Conclusion

In the present study, we showed that 24% (12/50) of eastern bent-wing bats (*Miniopterus fuliginosus*) harbored *Bartonella* bacteria in their blood for the first time in Japan. Phylogenetic analyses based on the *gltA* and the concatenated sequences with the *gltA*, *rpoB*, *ribC*, *ftsZ*, and 16S rRNA of the isolates clarified that two novel *Bartonella* species are present in the bats; one is close to the isolates from Taiwanese *Miniopterus* bats, and another is distinct from other known bat-associated *Bartonella* suggesting to be specific to the bats in Japan.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meegid.2020.104354>.

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## Declaration of Competing Interest

The authors have no conflict of interest.

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