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Identification of novel *Bartonella* spp. in bats and evidence of Asian gray shrew as a new potential reservoir of *Bartonella*

Jen-Wei Lin [a], Yuan-Man Hsu [b], Bruno B. Chomel [c], Liang-Kong Lin [d], Jai-Chyi Pei [e], Sheng-Hai Wu [f], Chao-Chin Chang [g,*]

[a] *Department of Veterinary Medicine, College of Veterinary Medicine, National Chung Hsing University, Taichung 402, Taiwan*
[b] *Department of Biological Science and Technology, College of Life Sciences, China Medical University, Taichung, Taiwan*
[c] *Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, USA*
[d] *Department of Life Science, Tunghai University, Taichung 407, Taiwan*
[e] *Institute of Wildlife Conservation, National Pingtung University, Pingtung 912, Taiwan*
[f] *Department of Life Sciences, National Chung Hsing University, Taichung 402, Taiwan*
[g] *Graduate Institute of Microbiology and Public Health, National Chung Hsing University, Taichung 402, Taiwan*

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ABSTRACT

Many studies indicated that small mammals are important reservoirs for *Bartonella* species. Using molecular methods, several studies have documented that bats could harbor *Bartonella*. This study was conducted to investigate the relationship of *Bartonella* spp. identified in bats and small mammals living in the same ecological environment. During May 2009 and March 2010, a total of 102 blood specimens were collected. By whole blood culture and molecular identification, a total of 6 bats, 1 rodent and 9 shrews were shown to be infected by *Bartonella* species. After sequencing and phylogenetic analyses of the sequences of *glt*A, *fts*Z, *rpo*B and *rib*C genes, these specific isolates from bats were not similar to the known *Bartonella* species (the similarity values were less than 91.2%, 90.5%, 88.8%, and 82.2%, respectively); these isolates formed an independent clade away from other known *Bartonella* type strains. The *Bartonella* spp. isolated from small mammals, which were closely related to *Bartonella tribocorum*, *Bartonella elizabethae*, *Bartonella grahamii*, *Bartonella rattimassiliensis* and *Bartonella queenslandensis*, were similar to the findings in previous studies worldwide. Therefore, the results implied that the species of *Bartonella* strains isolated from small mammals were different from those identified in bats. Our results strongly suggested that the bat isolate could be a new *Bartonella* species. This study is also the first one to isolate *Bartonella* organisms from Asian gray shrews, *Crocidura attenuata tanakae*.

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1. Introduction

Many studies have shown that small mammals are important reservoirs for most of the *Bartonella* species. Most of these species can be harbored by rodents, such as *Bartonella elizabethae* (Ying et al., 2002), *Bartonella grahamii* (Ellis et al., 1999), *Bartonella vinsonii* subsp. *arupensis* (Welch et al., 1999), *Bartonella doshiae* (Birtles et al., 1995), *Bartonella taylorii* (Birtles et al., 1995), *Bartonella vinsonii* subsp. *vinsonii* (Ellis et al., 1999), *Bartonella tribocorum* (Heller et al., 1998), *Bartonella washoensis* (Kosoy et al., 2003), *Bartonella rattimassiliensis* (Gundi et al., 2004), *Bartonella phoceensis* (Gundi et al., 2004), *Bartonella birtlesii* (Bermond et al., 2000), *Bartonella rochalimae* (Lin et al., 2008), *Bartonella japonica* (Inoue

* Corresponding author. Tel.: +886 4 2284 0894x706; fax: +886 4 2285 2186.
E-mail address: changcc@dragon.nchu.edu.tw (C.-C. Chang).

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et al., 2010), and *Bartonella silvatica* (Inoue et al., 2010). The order Rodentia has been reported as the reservoir for several zoonotic *Bartonella* species, such as *B. elizabethae* (Daly et al., 1993), and *B. washoensis* (Kosoy et al., 2003) and *B. grahamii* (Kerkhoff et al., 1999).

Up to date, more than 4600 species of mammals have been identified in Class Mammalia. The top two orders within mammalian consist of Rodentia and Chiroptera, which are with over 2000 and 930 species, respectively (Wong et al., 2007). Since the Middle Ages (A.D. 500–1400), many historical records and recent studies have shown that rodents play major roles for transmission of important zoonotic diseases, such as plague and hemorrhagic fever (Meerburg et al., 2009). Since the 1990s, some studies have also shown that bats were associated with several emerging zoonotic agents, including Hendra, Nipah, rabies, Ebola, and coronavirus-like viruses (Calisher et al., 2006; Maeda et al., 2008; Wong et al., 2007). Concannon et al. (2005) first demonstrated that bats could be infected with *Bartonella* in the United Kingdom. In the study using molecular identification of the *glt*A gene, 5 (two *Pipistrellus* spp., one *Nyctalus noctula*, one *Myopterus daubentonii*, and one *Myotis mystacinus*) of 60 blood samples collected from bats were detected to harbor *Bartonella* DNAs (Concannon et al., 2005). Reeves et al. (2005, 2007) further detected *Bartonella* DNA in ectoparasites of bats, such as *Trichobius major*, *Cimex adjunctus*, and *Sternopsylla texanus*, using PCR of the 16S–23S rDNA intergenic spacer (ITS) region. Recently, using whole blood culture, *Bartonella* organisms were successfully isolated from bats in Kenya, and 106 of the 331 bats tested by whole blood culture were shown *Bartonella* bacteremic (Kosoy et al., 2010).

Therefore, this study was conducted to investigate if bats could be reservoirs of *Bartonella* spp. in Taiwan, and to further understand if novel *Bartonella* spp. could be identified in bats and small mammals trapped from the same ecological environment. Moreover, this study was the first time to investigate if *Bartonella* organisms could be isolated from Asian gray shrews, *Crocidura attenuata tanakae*.

2. Materials and methods

2.1. Specimen collection

During the period of May 2009 and March 2010, a total of 54 bats, 23 rodents and 25 shrews were collected in Taiwan. The animals were trapped from several sites, including Taipei, Taichung, Nantou, Pingtung, Hualian counties, and Dongsha island. The trapping sites for rodents and bats included caves, air-aid shelters, abandoned farm houses, chicken/pig/cattle farms and deep trenches. The specimens were initially collected for the purposes of understanding the ecology of these small mammals, and investigating zoonotic pathogens (i.e., rabies virus, influenza virus, Japanese encephalitis virus and hantavirus) in these animals. The captured animals were humanely anesthetized with zoletil 50 (Virbac Laboratories, 06516 Carros, France) and blood was collected via cardiac puncture using 3 ml syringes fitted with 22 gauge, 1.5 in. needles. The blood specimen was collected in an EDTA tube (10 μl, 1.5 mg/ml) and stored at −80 °C for further isolation of *Bartonella* species.

2.2. Cultivation of Bartonella organisms

A total of 102 whole blood samples collected from bats, rodents, and shrews lived in the same ecological environment were used for *Bartonella* isolation. For bacterial isolation, 50 μl of thawed whole blood sample was plated onto chocolate agar. The plates were kept at 35 °C in 5% CO_2 incubator for at least 1 month (Boulouis et al., 2005). The plates were examined weekly for the growth of putative colonies of *Bartonella*. At least two colonies were randomly selected from the suspected plates and each colony was separately sub-cultured onto two different chocolate agar plates. After harvesting pure bacteria culture, the DNA was extracted and used for further molecular confirmation of *Bartonella* species by polymerase chain reaction (PCR) and sequence analysis.

2.3. DNA extraction and polymerase chain reaction (PCR) for confirmation of Bartonella species

The DNA of each isolate was extracted using Viogene DNA/RNA Extraction Kit (Viogene Biotek Corp., Taipei, Taiwan) following the manufacturer's instructions. The primers BhCS.781p and BhCS.1137n were used for amplifying the partial fragment (approximately 390 bp) of the *glt*A gene of *Bartonella* species (Norman et al., 1995). The PCR-positive samples for the *gltA* gene were further analyzed by PCR of the 16S/23S rRNA intergenic spacer region (ITS) (Jensen et al., 2000). For further phylogenetic analysis, confirmed *Bartonella* isolates were analyzed by partial sequences of the *fts*Z, *rpo*B and *rib*C genes. For PCR amplification of the *fts*Z gene, a set of primers Bfp1 and Bfp2 was used to amplify an expected 900-bp product (Zeaiter et al., 2002). Primers 1400F and 2300R were used to amplify a 900-bp fragment of the *rpo*B gene (Renesto et al., 2001). For PCR amplification of the *rib*C gene, a set of primers ribC-F and ribC-R was used to amplify an expected 800-bp DNA fragment (Inoue et al., 2009). As the 16S rRNA gene has been well-accepted for species identification of bacteria, the primers P8 and Pc1544 were used for amplifying approximately 1300–1400 bp of the 16S rRNA gene (Heller et al., 1997).

2.4. Sequencing and phylogenetic analyses for Bartonella species

The confirmed PCR products were sent for automated sequencing (Mission Biotech, Taipei, Taiwan). By phylogenetic analysis, the sequences obtained from the isolates were compared to the sequences of *Bartonella* type/reference strains. The accession numbers of the *Bartonella* type/reference strains used for comparison were listed in Table 2. The sequences of the 16S ribosomal DNA, *fts*Z, *glt*A, *rib*C and *rpo*B genes, and the 16S/23S rRNA ITS of the *Bartonella* strain isolated in this study have been submitted to the GenBank. The accession numbers of the partial sequences of the *glt*A, *rib*C, *rpo*B, *fts*Z and 16S rRNA genes for the isolates in this study were serial numbers of

Table 1
Prevalence of *Bartonella* bacteraemic among bats and small mammals.

Small mammals	Animal species	Prevalence (%) of *Bartonella* bacteremia	Total
Bats	*Rhinolophus monoceros*	0.0 (0/4)	11.1 (6/54)
	Hipposideros terasensis	0.0 (0/1)	
	Pipistrellus abramus	0.0 (0/35)	
	Miniopterus schreibersii	42.9 (6/14)	
Rodents	*Rattus culturatus*	0 (0/16)	4.4 (1/23)
	Rattus coxinga niviventer	100 (1/1)	
	Unidentified	0 (0/6)	
Shrews	*Crocidura attenuata tanakae*	77.8 (7/9)	36 (9/25)
	Crocidura horsfieldi kurodai	0 (0/3)	
	Unidentified	15.4 (2/13)	

JF500507–JF500522, JF500523–JF500538, JF500539–JF500554, JF500491–JF500506 and JF500555–JF500560, respectively. The sequences of the 16S/23S rRNA ITS region and *glt*A gene of the isolates in this study were analyzed first by using the blastn program of the NCBI website to search for the closest *Bartonella* species in the GenBank database. Then, the sequences of 16S ribosomal DNA, *fts*Z, *glt*A, *rib*C and *rpo*B genes were aligned by Clustal W method of BioEdit program version 7.0.8 (Tom Hall, Ibis Biosciences, Isis Pharmaceuticals, Carlsbad, CA, USA).

Using sequences of the *glt*A gene and the merged set of sequences of five genes (16S ribosomal DNA, *fts*Z, *glt*A, *rib*C and *rpo*B), further phylogenetic analysis was performed to show the relationship of the *Bartonella* type/reference strains and the bat isolates in this study. Phylogenetic analysis was performed on the aligned DNA sequences using maximum-parsimony as implemented in PHYLIP version 3.6 (Joseph Felsenstein, Department of Genome Sciences and Department of Biology, University of Washington, Seattle, WA, USA). Bootstrap support was calculated by using 1000 bootstrap data replicates as implemented by SEQBOOT of the PHYLIP program.

2.5. Sequence analysis of the ftsY gene for the inference of the genomic DNA G+C content

As reported by Fournier et al. (2006), the prokaryote genomic DNA G+C content can be inferred from the sequences of *fts*Y genes. Therefore, the primers BartftsyF and BartftsyR were used to amplify the fragment of the *fts*Y gene of *Bartonella* species by the procedures developed by Fournier et al. (2007).

3. Results

3.1. Isolation of Bartonella species

Among the 102 small mammals tested, *Bartonella* organisms were isolated from 16 animals (15.7%) using whole blood culture (Table 1). The isolates were from six bats (*Miniopterus schreibersii*), one rodent (*Rattus coxinga niviventer*) and nine shrews (including seven *Crocidura attenuata tanakae* and two without species information). All isolates were confirmed to be *Bartonella* after sequence analysis of the 16S/23S rRNA ITS region and *glt*A gene. *Bartonella* isolates Nos. 5, 6, 7, 8, 15, and 16 were from *M. schreibersii* and No. 9 was from *Rattus coxinga niviventer*. The isolates from *Crocidura attenuata tanakae* were numbered as Nos. 1, 2, 3, 4, 12, 13, and 14. The two isolates from the unknown species of shrews were numbered as Nos. 10 and 11 (Table 1).

3.2. Gene sequence comparison and phylogenetic analyses

The *glt*A sequences obtained from the bat isolates were very similar to each other ranging from 99.0% to 100.0%. Further compared to the sequences of the *Bartonella* type/reference strains listed in Table 2, the results indicated that the isolates from bats were not close to any known *Bartonella* species, with DNA similarity values less than 91.2%. However, the isolate from *Crocidura attenuata tanakae* and the isolate from *Rattus coxinga niviventer* were closely related to *Bartonella queenslandensis* with DNA similarity values of 99.0% and 99.7%, respectively. In comparison with the sequences of the *glt*A gene of the *Bartonella* type/reference strains, except for the isolate No. 1 from *Crocidura attenuata tanakae*, other isolates from shrews were shown DNA similarity values of 95.1–95.5%, closest to *B. tribocorum*, followed by *B. grahamii* (DNA similarity value: 94.2–94.5%), and *B. elizabethae* (DNA similarity value: 93.5–93.8%).

With regard to the sequences of the *fts*Z gene, the bat isolates were shown DNA similarity values of 97.1–100% between each other and were distant from all known *Bartonella* type/reference strains (DNA similarity values <90.5%). One isolate from *Crocidura attenuata tanakae* and one isolate from *Rattus coxinga niviventer* were close to *B. queenslandensis* with DNA similarity values from 99.5% to 99.9%. Except for one isolate (No. 1) from *Crocidura attenuata tanakae*, other isolates from shrews were shown closest to *B. tribocorum* (96.7%), followed by *B. elizabethae* (96.4%), and *B. queenslandensis* (96.2%).

More divergence of the sequences of the *rpo*B and *rib*C genes were observed for the bat isolates, as the DNA similarity values were shown to be 95.1–99.9% and 94.9–100%, respectively. The sequences of these bat isolates were still distinct from the sequences of all known *Bartonella* type/reference strains obtained in this study; the DNA similarity value for the *rpo*B gene was less than 88.8% and that for the *rib*C gene was less than 82.2%). The *rpo*B gene sequences obtained from one isolate from *Crocidura attenuata tanakae* and one isolate from *Rattus*

Table 2
Accession numbers of the (a) sequences of the type/reference *Bartonella* species and (b) reference *Bartonella* species sequences used for phylogenetic analysis in this study.

	gltA gene	*ftsZ* gene	*rpoB* gene	*ribC* gene	16S rDNA
(a) *Bartonella* type/reference strain					
Bartonella alsatica IBS 382^T	AF204273	AF467763	AF165987	AY116630	AJ002139
B. bacilliformis KC583^T	AB292601	AB292602	AF165988	AJ236918	Z11683
B. birtlesii IBS 325^T	AF204272	AF467762	AB196425	AY116632	AF204274
B. bovis 91-4^T	AF293394	AF467761	AY166581	AY116637	AF293391
B. capreoli IBS 193^T	AF293392	AB290192	AB290188	AB290194	AF293389
B. chomelii A828^T	AY254308	AB290193	AB290189	AB290195	AY254309
B. clarridgeiae Houston-2 cat^T	U84386	AF141018	AF165990	AB292604	AB292603
B. doshiae R18^T	Z70017	AF467754	AF165991	AY116627	Z31351
B. elizabethae F9251^T	Z70009	AF467760	AF165992	AY116633	L01260
B. grahamii V2^T	Z70016	AF467753	AF165993	AY166583	Z31349
B. henselae Houston-1^T	BX897699	AF061746	AF171070	AJ132928	BX897699
B. japonica sp. nov. Fuji 18-1^T	AB242289	AB440633	AB242288	AB440635	AB440632
B. koehlerae C-29^T	AF176091	AF467755	AY166580	AY116634	AF076237
B. phoceensis strain 16120	AY515126	AY515135	AY515132	–	–
B. queenslandensis	EU111801	EU111779	EU111790	–	–
B. quintana Fuller^T	Z70014	AB292605	AF165994	AJ236917	M11927
B. rattimassiliensis strain 15908	AY515124	AY515133	AY515130	–	–
B. schoenbuchensis R1^T	AJ278183	AF467765	AY167409	AY116628	AJ278187
B. silvatica sp. nov. Fuji 23-1^T	AB242287	AB440637	AB242292	AB440639	AB440636
B. taylorii M6^T	Z70013	AF467756	AF165995	AY116635	Z31350
B. tribocorum IBS 506^T	AJ005494	AF467759	AF165996	AB292600	AJ003070
B. vinsonii subsp. *arupensis* OK 94-513^T	AF214557	AF467758	AY166582	AY116631	AF214558
B. vinsonii subsp. *berkhoffii* 93-CO1^T	U28075	AF467764	AF165989	AY116629	L35052
B. vinsonii subsp. *vinsonii* Baker^T	Z70015	AF467757	AF165997	AY116636	M73230
B. washoensis	AF470616	–	–	–	–
B. washoensis subsp. *cynomysii* strain CL8606co	–	DQ825692	DQ825688	–	–
(b) *Bartonella* strain					
KK49	FJ946851	–	–	–	–
KK61	FJ946852	–	–	–	–
M62	AJ871612	–	–	–	–
M207	AJ871614	–	–	–	–
M406	AJ871613	–	–	–	–
M409	AJ871611	–	–	–	–
M451	AJ871615	–	–	–	–
Bartonella isolates in bats in Kenya	HM363764–HM363768	–	–	–	–
Bartonella isolates in bats in Kenya	HM545136–HM545141	–	–	–	–
Brucella melitensis 16M^T	NC_003317	NZ_GG703780	NC_003317	NC_003318	NC_003317

coxinga niviventer were similar to *B. queenslandensis* (DNA similarity values: 98.9–99.5%). However, the sequences of the *rib*C gene for these two isolates were closest to *B. tribocorum* (DNA similarity values: 96.1–96.8%). Except for one isolate (No. 1) from *Crocidura attenuata tanakae*, the sequences of the *rpo*B gene for the other shrew isolates were 96.2–96.4% most similar to that of *B. queenslandensis*, followed by *B. tribocorum* (95.4–95.5%), *B. elizabethae* (94.3–94.4%), and *B. grahamii* (94.2–94.3%). Using the sequences of the *rib*C gene, except for one shrew isolate (No. 1), the other shrew isolates were closest to *B. tribocorum* (94.8–95.0%), followed by *B. elizabethae* (92.1–92.3%).

The phylogenetic tree constructed by the *glt*A gene (Fig. 1) showed that the bat isolates were grouped into an independent clade, separated from all *Bartonella* type/reference strains. The rodent and shrew isolates belonged to the same clade of *B. tribocorum*, *B. elizabethae*, *B. grahamii*, *B. rattimassiliensis* and *B. queenslandensis* (Fig. 1). In this specific clade, one isolate from *Crocidura attenuata tanakae* and one isolate from *Rattus coxinga niviventer* were formed a specific group with *B. queenslandensis* (bootstrap value >90%). Using the merged set of concatenated sequences of five genes (16S ribosomal DNA, *fts*Z, *glt*A, *rib*C, and *rpo*B) to construct the phylogenetic relationship, the results also shown that the isolates from bats formed a significantly distinct clade comparing to the other *Bartonella* type/reference strains (Fig. 2).

After sequence analysis of the *fts*Y gene, the genomic DNA G+C content of the *Bartonella* isolates in bats in Taiwan were estimated to be from 30.29% to 31.15%.

4. Discussion

In this study, we investigated the epidemiology of *Bartonella* infections in bats and other small mammals from the same ecological environment. This is the first report that *Bartonella* organisms were isolated from bats, *M. schreibersii*, in Taiwan. Through sequence and phylogenetic analyses of various genes, the results indicated that the isolates from *M. schreibersii* were not similar to the known *Bartonella* species as previously described. However, *Bartonella* spp. isolated from small mammals, were closely related to *B. tribocorum*, *B. elizabethae*, *B. grahamii*, *B. rattimassiliensis*, and *B. queenslandensis*, similar to the

Fig. 1. Phylogenetic analysis of different *Bartonella* species on the basis of partial DNA sequences of the *gltA* gene. The phylogenetic relationship was constructed by using the maximum-parsimony method of the PHYLIP version 3.6 program, and bootstrap analysis was performed with 1000 trials of bootstrap data (bootstrap values not shown if lower than 70%).

findings in previous studies worldwide (Bai et al., 2009; Gundi et al., 2009; Hsieh et al., 2010; Liu et al., 2010).

In this study, it was also found that the *Bartonella* infections could occur among Asian gray shrew (*Crocidura attenuata tanakae*). To the best of our knowledge, this is the first study to demonstrate the *Bartonella* infection in *Crocidura attenuata tanakae*. This shrew is a subspecies belonged to the species of *Crocidura attenuate*. The species is generally found in the tropical and subtropical zone of Asia, such as India and China.

According to the study by Concannon et al. (2005), two blood specimens of bats from *Pipistrellus* spp. have been detected by molecular identification. In our study, a total of 35 blood specimens from the bats, *Pipistrellus abramus*, belonging to the same genus, were collected. However, no *Bartonella* isolates have been obtained from these specimens by whole blood culture. It might be related to the limited samples tested in our study, or related to the sensitivity of different detection methods. Using the sequences of the partial *glt*A gene, when the sequences found in *Pipistrellus* spp. by Concannon et al. (2005) were compared to the sequences of the bat isolates in our study, the DNA similarity values of 86.7–90.9% indicated that *Bartonella* species in bats in the United Kingdom were not closely related to the isolates in Taiwan. Our phylogenetic analysis further supported that bat isolates in Taiwan formed a unique clade, which was separated from the sequences of *Bartonella* in bats in the United Kingdom (Fig. 1). Recently, Kosoy et al. (2010) reported *Bartonella* isolates from bats in Kenya. Among the 331 bats tested, *Bartonella* were isolated from 106 animals using whole blood culture. The *Bartonella*-bacteremic bat species were *Eidolon helvum* (23/88), *Rousettus aegyptiacus* (22/105), *Coleura afra* (4/9), *Triaenops persicus* (7/8), *Hipposideros commersoni* (1/4), and *Miniopterus* spp. (49/87) (Kosoy et al., 2010). In our study, we also tested the bats from the same genus of *Miniopterus* (*M. schreibersii*) and *Hipposideros* (*Hipposideros terasensis*). Due to only one sample of *H.*

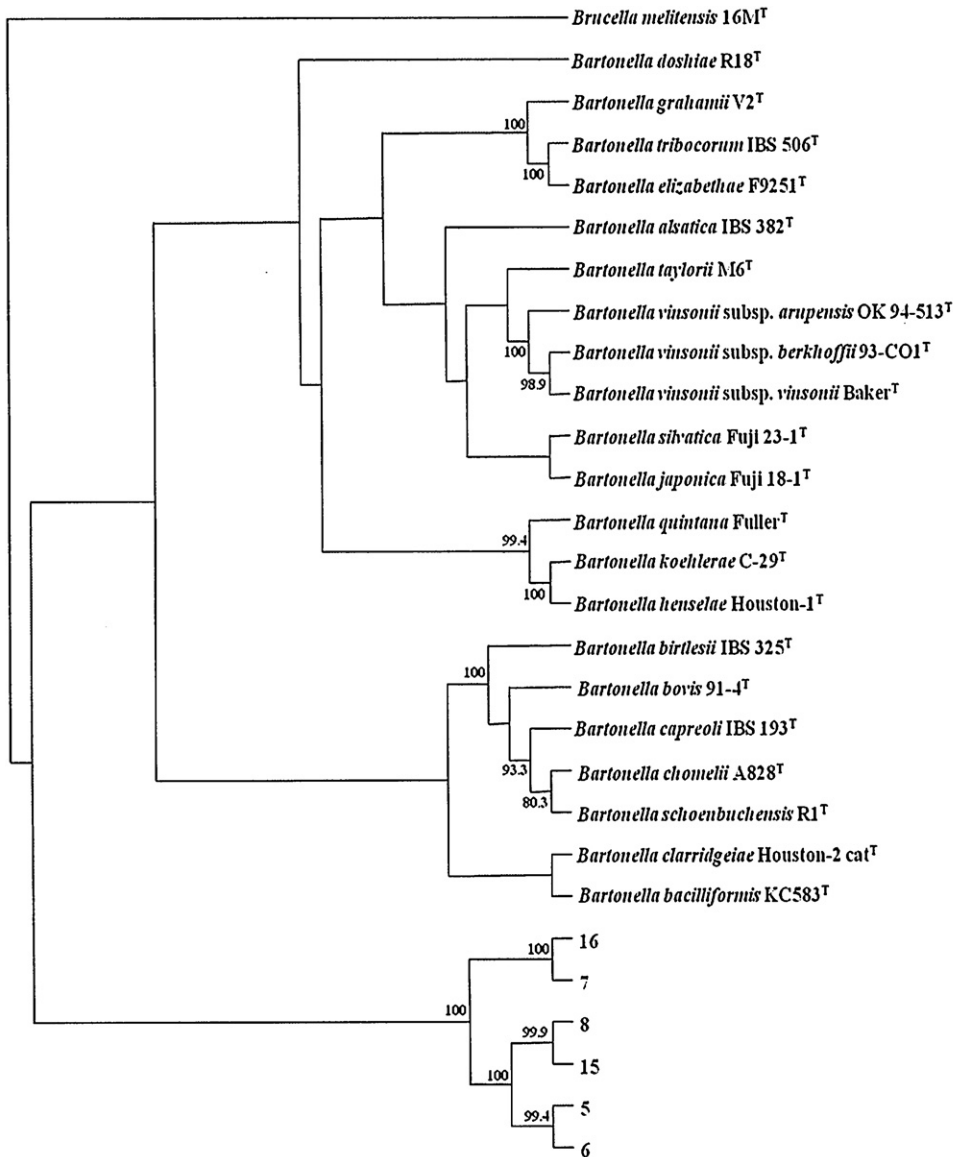

Fig. 2. Phylogenetic analysis of different *Bartonella* species based on the sequences of a merged set of concatenated sequences of five genes (16S rRNA, *ftsZ*, *gltA*, *ribC* and *rpoB* genes). The phylogenetic relationship was constructed by using the maximum-parsimony method of the PHYLIP version 3.6 program, and bootstrap analysis was performed with 1000 trials of bootstrap data (bootstrap values not shown if lower than 70%).

terasensis tested, no *Bartonella* isolate was obtained in our study. However, we cultured *Bartonella* isolates from the blood of 42.9% (6/14) of the *M. schreibersii*. After sequence analysis, the results indicated that the DNA identity of these isolates from *M. schreibersii* in our study was not closely related to the strains isolated in *Miniopterus* spp. in Kenya (95.8–96.1% to the strain HM545139, and 92.5–92.9% to the strain HM545140).

Of major interests, after comparing the sequences of the partial *glt*A gene of the bat isolates in Taiwan to the available sequence data in the GenBank, it was found that the bat isolates were most similar to the uncultured *Bartonella* spp. isolates KK49 and KK61, with DNA similarity value of 95.5–96.8%. The KK49 and KK61 sequences were amplified from stray dogs in Thailand in 2010 (Bai et al., 2010). Therefore, further investigation should be conducted to determine whether these *Bartonella* organisms could be possibly transmitted between bats and dogs.

Arthropods play an essential role in the transmission cycle of *Bartonella* organisms between hosts. The studies by Reeves et al. (2005, 2007) collected ectoparasites from bats and successfully detected *Bartonella* DNA from the *T. major*, *C. adjunctus*, and *S. texanus* using PCR of the 16S–23S rRNA intergenic spacer (ITS) region. Further research should be conducted to investigate whether the *Bartonella* isolates found in bats in Taiwan could be transmitted in bats or between bats and dogs through common vectors.

In this study, it was further identified at least two genotypes of the bat isolates in Taiwan. Based on the

sequence comparison, the sequences of the *glt*A genes and 16S ribosomal DNA were highly similar among the *Bartonella* isolates in bats in Taiwan (DNA similarity values: 99–100%, and 99.9–100%, respectively). However, the sequences of the *fts*Z, *rpo*B and *rib*C genes were slightly divergent (DNA similarity values: 97.1–100%, 95.1–99.9% and 94.9–100%, respectively). On the basis of sequencing and phylogenetic analyses of *glt*A, *fts*Z, *rpo*B, *rib*C genes and 16S ribosomal DNA, the bat isolates No. 7 and No. 16 could belong to one genotype, as their *rpo*B and *rib*C sequences were not similar to the other *Bartonella* isolates in bats in Taiwan (divergence values: 4.3–4.8% and 4.7–5.1%, respectively).

It is of major interests to understand if the bat isolates in Taiwan could be a new *Bartonella* species. In our study, to study the bat isolates, except for the *glt*A gene, sequences of the 16S rRNA, *fts*Z, *rib*C, and *rpo*B gene were also analyzed. As reported by the ad hoc committee for the re-evaluation of the species definition in bacteriology, descriptions of a new species could be based on housekeeping gene (encoded metabolic functions) sequence analysis with a comparison of at least five genes (Stackebrandt et al., 2002). The study by La Scola et al. (2003) also documented that newly encountered *Bartonella* isolates should be considered as a new species if the 327-bp *glt*A fragment and 825-bp *rpo*B fragment shared $<$96.0% and $<$95.4% sequence similarity with those of validated species, respectively. In comparison with the sequences of the *glt*A, *fts*Z, *rpo*B and *rib*C genes of the *Bartonella* type/reference strains, the DNA similarity values of the bat isolates in Taiwan were less than 91.2%, 90.5%, 88.8%, and 82.2% to all known *Bartonella* species, respectively. After phylogenetic analyses using the merged set of concatenated sequences of the 16S ribosomal DNA, *glt*A, *fts*Z, *rpo*B, *rib*C genes, all the results strongly suggested that the isolates from *M. schreibersii* in Taiwan could be considered as a new *Bartonella* species.

The results of the estimated genomic DNA G+C content of the *Bartonella* isolates in bats in Taiwan using the sequences of the *fts*Y gene were from 30.29% to 31.15%, which were much less than the *Bartonella* type/reference strains (ranging from 37% to 41%) (Bermond et al., 2000, 2002). It was also found that the segment of the *fts*Y genes in the bat isolates in Taiwan was shorter than *Bartonella* species tested, including *Bartonella australis*, *Bartonella henselae*, *Bartonella quintana*, *Bartonella grahamii*, *Bartonella bacilliformis*, and *B. tribocorum* (Fournier et al., 2006, 2007). Although it is still unclear why the *fts*Y gene of the bat isolates in Taiwan differs from the *Bartonella* type/reference strains, further studies will be conducted to examine the possible relationship between the divergence of the *fts*Y gene and host specificity.

At present, no bat-associated *Bartonella* infection was documented in humans. In Taiwan, bats and small mammals live very closely to the human living environment. As bats play important roles for the transmission of certain emerging zoonoses, physicians and veterinarians should be aware of accidental *Bartonella* infections from bats.

5. Conclusion

Our results implied that the species of *Bartonella* strains isolated from small mammals were different from those identified in bats. Furthermore, the bat isolate could be a new *Bartonella* species. This study is also the first one to isolate *Bartonella* organisms from Asian gray shrews, *Crocidura attenuata tanakae*.

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