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Population genetic structure based on mitochondrial DNA analysis of Ikonnikov's whiskered bat (*Myotis ikonnikovi*—Chiroptera: Vespertilionidae) from Korea

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Abstract

Background: Ikonnikov's whiskered bat (*Myotis ikonnikovi*) is found throughout the Korean Peninsula, as well as in Kazakhstan, Russia, Mongolia, China, and Japan. It is small-sized and primarily inhabits old-growth forests. The decrease and fragmentation of habitats due to increased human activity may influence the genetic structure of bat populations. This study was designed to elucidate the population genetic structure of *M. ikonnikovi* using mitochondrial genes (*cytochrome oxidase I* and *cytochrome b*).

Results: The results showed that *M. ikonnikovi* populations from Korea have high genetic diversity. Although genetic differentiation was not detected for the *COI* gene, strong genetic differentiation of the *Cytb* gene between Mt. Jeombong and Mt. Jiri populations was observed. Moreover, the results indicated that the gene flow of the maternal lineage may be limited.

Conclusions: This study is the first to identify the genetic population structure of *M. ikonnikovi*. We suggest that conservation of local populations is important for sustaining the genetic diversity of the bat, and comprehensive studies on factors causing habitat fragmentation are required.

Keywords: *Myotis ikonnikovi*, Population structure, Genetic diversity, *Cytochrome oxidase I*, *Cytochrome b*

Background

Bats are small mammals found globally, except in polar regions, and have a high worldwide biodiversity of 1200 species (Schipper et al. 2008). Bats play important ecological roles, including in insect population control, seed dispersion, and pollination. Their voracious feeding significantly contributes to pest control as they consume over 30% of their body weight in insects per night. Their roles in pest control and seed dispersion have been recognized for their economic value, and the importance of ecological services provided by bats is being emphasized (Fenton 2003; Boyles et al. 2011; Kunz et al. 2011).

The distribution of *Myotis ikonnikovi*, a typical forest bat, spans from the Altai Mountains of Kazakhstan to the Siberian and Ussuri regions of Russia, southern Mongolia, northeastern China, Korea, and Honshu and Hokkaido of Japan (Simmons 2005). This species has been found in multiple areas of Korea, including Mt. Jeombong (JB), Mt. Odae (OD), Mt. Sobaek (SB), and Mt. Jiri (JR), where they roost in hollow trees or under the bark of living or dead trees in forests (Kim et al. 2014).

The home range of forest bats is associated with their weight (body size) (Fenton 1997). Species in the genus *Myotis* are mostly small and travel only a short distance from their roost site to foraging ground, and they use multiple roost sites within a relatively narrow home

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range (Kurta et al. 1996; Ormsbee 1996; Fenton 1997; Broders et al. 2006). *Myotis ikonnikovi* prefers mature forests that can satisfy its ecological needs regarding both food and shelter, and inhabits small areas within the habitat (Kim et al. 2014). Similarly to other *Myotis* species, this species changes its roost sites within a radius of 500 m or reoccupies previously abandoned roost sites (Lewis 1995; Brigham et al. 1997; Menzel et al. 2002; Kunz and Lumsden 2003; Kim et al. 2014).

The decrease and fragmentation of habitats due to increased human activity may influence the genetic differentiation of bat populations (Rossiter et al. 2000; Wright et al. 2018). We thus hypothesized that the Korean populations of *M. ikonnikovi* would be largely influenced by these factors, as the habitats of individual bats are usually within 0.5 km from their roost sites during their active periods (Kim et al. 2014). In this study, mitochondrial genes were analyzed to investigate the genetic diversity and differentiation among populations of *M. ikonnikovi*. Moreover, the study examined phylogenetic relationships among Russian, Chinese, and Japanese populations. The findings of the present study could be helpful for establishing efficient methods that consider genetic characteristics for the conservation management of *M. ikonnikovi* populations in Korea.

Materials and methods

Collection of samples

Forty-two *M. ikonnikovi* individuals were captured from six foraging sites in four regions (Mt. Jeombong, Mt. Odae, Mt. Sobaek, and Mt. Jiri) using mist nets from mid-May to early August in 2014 to 2016 (Table 1, Fig. 1). Tissue samples were collected from the chiropatagium of one wing of each bat using a 3-mm-diameter biopsy punch, and a ring band carved with a serial number was attached to a leg of each individual (Faure et al. 2009). After sampling, all bats were released at the capture site, and the tissue samples were stored in 100% ethanol at − 20 °C.

DNA extraction and amplification

Genomic DNA was extracted from each tissue sample stored in 100% ethanol using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and stored at − 20 °C. To amplify the two target mitochondrial regions *COI* and *Cytb*, universal primers (LCO1490-HCO2198 for *COI*, L14724-MVZ16 and L15162-H15915 for *Cytb*) and an *M. ikonnikovi*-specific primer that was designed for the present study were used (Additional file 1: Table S1). The specific primers (MICOI-F, -R and MICytbL-F, -R) were designed for more effective PCR amplification based on the sequences of the PCR products that were amplified using the universal primer sets. The *Cytb* gene sequence was divided into two parts to facilitate the PCR amplification and sequencing. The fore part was amplified using the L14724-MVZ16 or MICytbF-MICytbR primer sets, and the rear part was amplified using the L15162-H15915 primer set. PCR amplifications were performed using premixed ready strips (FastMix Frenche™ PCR i-Taq, iNtRon Biotechnology, Seongnam, Republic of Korea) with 16 μl distilled water, 1 μl (10 pmol) of each primer, and 2 μl template DNA. All PCR amplifications were conducted in a PeqSTAR Universal Gradient thermocycler (Peqlab GmbH, Erlangen, Germany). The temperature profile included an initial denaturation step of 3 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at each annealing temperature (Additional file 1: Table S1), 1~2 min at 72 °C, and a final extension step of 5 min at 72 °C.

The PCR amplicons were separated and visualized using 1% agarose gel electrophoresis with TopGreen Nucleic Acid Gel Stain (Genomic Base, Seoul, Republic of Korea) and then purified using a Fragment DNA purification kit (iNtRon Biotechnology) for sequencing. The purified PCR products were read in both directions.

Population genetic and phylogenetic analyses

The resultant sequences were assembled and edited with the ClustalW algorithm embedded in MEGA7 software

Table 1 Sampling sites of *Myotis ikonnikovi* in Korea

Population	Sample size	Date	Site	Latitude	Longitude
Mt. Jeombong (JB)	5	16 Jun 2016	Jindong-ri, Girin-myeon, Inje-gun, Gangwon-do, Korea	38.043960 N	128.474633 E
	1	17 Aug 2016			
Mt. Odae (OD)	15	31 Jul 2014	Dongsan-ri, Jinbu-myeon, Pyeongchang-gun, Gangwon-do, Korea	37.765082 N	128.577128 E
	12	1 Aug 2014			
Mt. Sobaek (SB)	3	24 May 2014	Jwaseok-ri, Dansan-myeon, Yeongju-si, Gyeongsangbuk-do, Korea	37.008669 N	128.585180 E
	1	14 Aug 2015			
Mt. Jiri (JR)	4	12 Jul 2014	Jungsan-ri, Sicheon-myeon, Sancheong-gun, Gyeongsangnam-do, Korea	35.320792 N	127.755091 E
	1	13 Jul 2014	Naedong-ri, Toji-myeon, Gurye-gun, Jeollanam-do, Korea	35.268310 N	127.576770 E

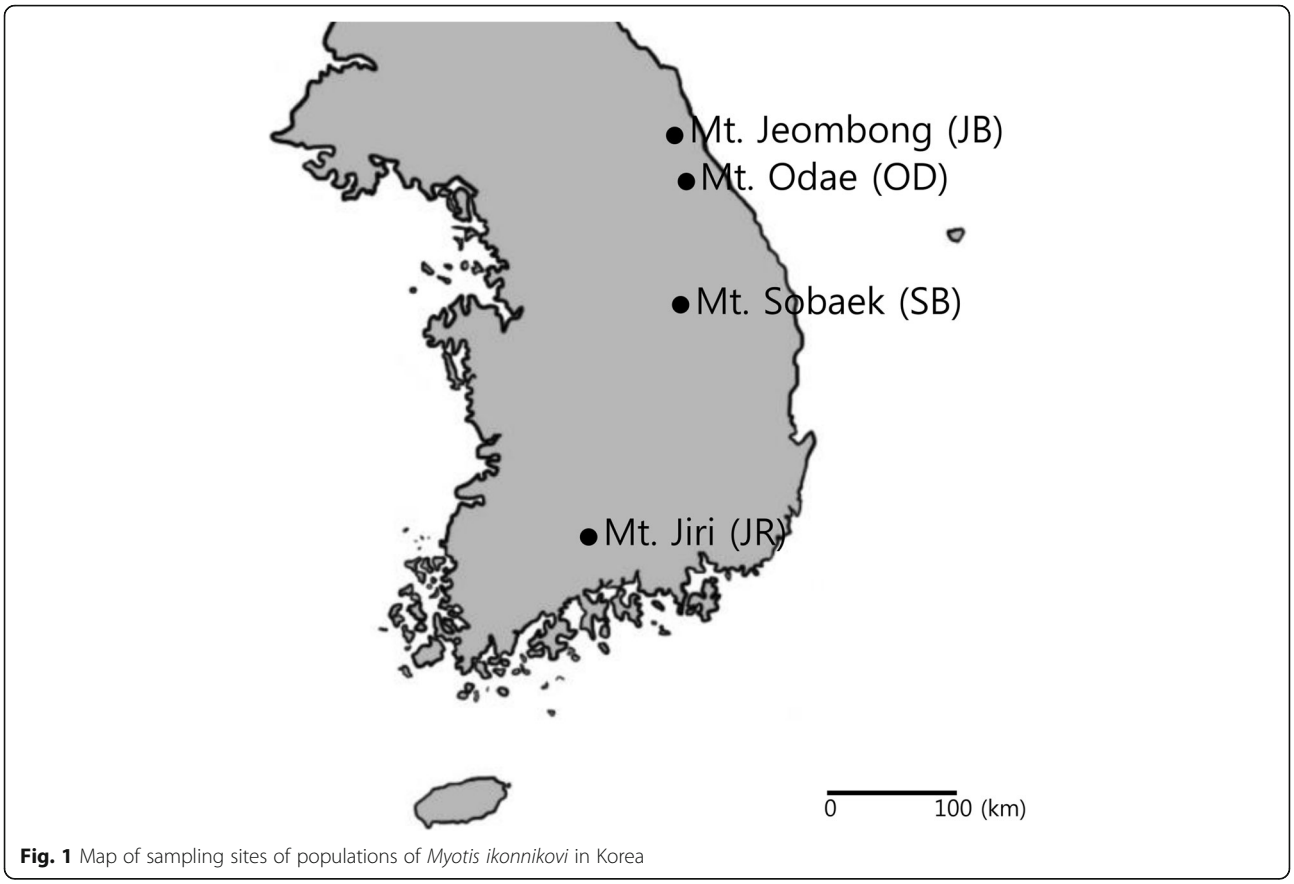

Fig. 1 Map of sampling sites of populations of *Myotis ikonnikovi* in Korea

(Kumar et al. 2016). The haplotypes of each gene were determined using DnaSP v5 (Librado and Rozas 2009). For population genetic analyses, samples were assorted into four mountain populations according to collection sites (Table 1). The network relationships among the haplotypes were inferred with the median-joining algorithm and were visualized in Network 5.0 (Bandelt et al. 1999). The haplotype network was reconstructed with the regional data in PowerPoint 2013 (Microsoft, Redmond, WA, USA).

Molecular diversity indices were generated for four populations and each gene using ARLEQUIN (Excoffier and Lischer 2010). For comparing populations with different sample sizes, haplotype richness (H_R) was calculated after rarefaction based on the smallest sample size (ADZE 1.0) (Szpiech et al. 2008). The population genetic structure and neutrality of populations were tested by analysis of molecular variance (AMOVA), including the overall fixation index statistics (F_{ST}) and pairwise F_{ST} with 1000 permutations, in ARLEQUIN. The Mantel test was used to test isolation by distance (IBD) between geographical distance and pairwise F_{ST} in ARLEQUIN.

Maximum-likelihood (ML) trees for the haplotypes of two genes (*COI*, 597 bp and *Cytb*, 1006 bp) were generated with 1000 bootstrap replicates after selecting the best-fit substitution model in MEGA7. The Tamura 3-parameter+gamma (T92 + G) and Hasegawa–Kishino–Yano+gamma (HKY + G) models were selected for ML analysis of the *COI* and *Cytb* genes, respectively. For both phylogenetic trees, sequences of *Myotis bombinus* from the GenBank database were used as the outgroup. Because of limitations of genetic data availability, only the phylogenetic relationships among Russian, Chinese, and Korean populations for the *COI* gene and between Japanese and Korean populations for the *Cytb* gene were inferred.

Results

Genetic diversity and haplotype network

A total of 11 and 21 haplotypes were identified from the *COI* and *Cytb* genes, respectively (GenBank accession numbers MN528478–528561). The lowest haplotype diversity value for the *COI* gene was observed in the Sobaek (SB) population (0.5), and the highest was observed in the Jeombong (JB) population (0.8667). The lowest nucleotide diversity value was observed in the Jiri (JR) population (0.00163), and the highest was observed in the JB population (0.00878). For the *Cytb* gene, the SB haplotype diversity value was between 0.8 and 0.96, and the nucleotide diversity value was between 0.00437

Table 2 Molecular diversity indices of mitochondrial DNA across four populations of *Myotis ikonnikovi* in Korea

Gene		JB (6)	OD (27)	SB (4)	JR (5)
COI	*h*	4	7	2	3
	k	10	10	6	2
	H_d	0.8667	0.8148	0.5	0.8
	H_R	3.2	3.02615	2	2.8
	π	0.00878	0.00540	0.00488	0.00163
Cytb	*h*	3	17	3	3
	k	10	32	11	8
	H_d	0.8	0.9601	0.8333	0.8
	H_R	2.8	3.76746	3	2.8
	π	0.00530	0.00765	0.00580	0.00437

h number of haplotypes, *k* number of polymorphic sites, H_d haplotype diversity, H_R haplotype richness, and π nucleotide diversity

and 0.00765, indicating similar haplotype and nucleotide diversity among the four populations (Table 2). Accounting for sample size, the highest haplotype richness of the *COI* and *Cytb* genes was observed in the JB population (3.2) and Odae (OD) population (3.76746), respectively (Table 2).

According to the haplotype network, the number of different nucleotides between haplotypes of *COI* was smaller than that between haplotypes of *Cytb* (Fig. 2). The JB and OD populations shared haplotypes in both genes (Fig. 2). In addition, specific haplotypes for each of the four populations appeared in both genes. In the *COI* gene, the OD population shared haplotypes with the remaining three populations. In the *Cytb* gene, the JB and OD populations shared haplotypes with each other, while the SB and JR populations shared only a single haplotype. Conversely, the JR population did not share any haplotype with the JB or OD populations.

AMOVA, IBD, and phylogenetic analysis

The *COI* gene exhibited non-significant genetic differentiation among the populations ($F_{ST} = 0.01467$, $p > 0.1$), whereas the *Cytb* gene exhibited high and significant differentiation among populations ($F_{ST} = 0.19769$, $p < 0.01$). However, in both genes, > 80% of the genetic variation was explained by differences among individuals within a population, while a small amount of the variation was attributable to differences among populations (Table 3). In the analysis of pairwise differences by population, the *COI* gene showed F_{ST} values ranging between − 0.05719 and 0.17917, whereas the genetic differentiation among

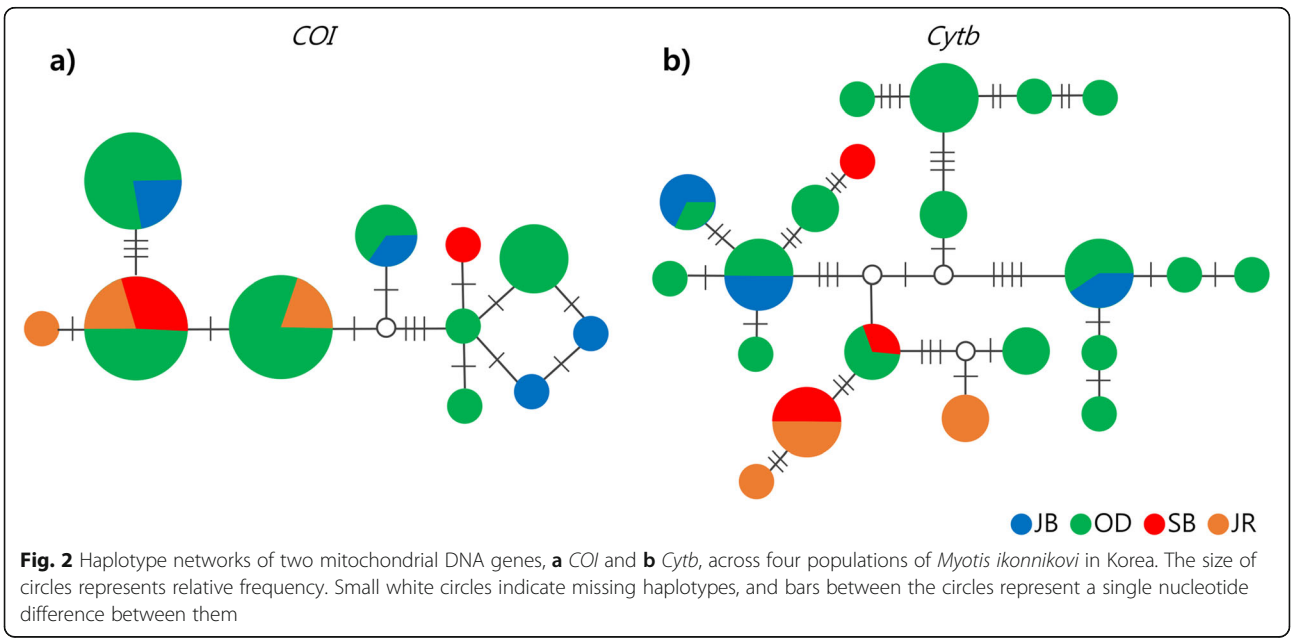

Fig. 2 Haplotype networks of two mitochondrial DNA genes, **a** *COI* and **b** *Cytb*, across four populations of *Myotis ikonnikovi* in Korea. The size of circles represents relative frequency. Small white circles indicate missing haplotypes, and bars between the circles represent a single nucleotide difference between them

Table 3 AMOVA results for the *COI* and *Cytb* sequences of *Myotis ikonnikovi* in Korea

Gene	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
COI	Among populations	3	5.553	0.02475	1.47
	Within populations	38	63.185	1.66277	98.53
	Total	41			
	F_{ST} and p value	$F_{ST} = 0.01467$, p value > 0.05			
Cytb	Among populations	3	29.699	0.84889	19.77
	Within populations	38	130.920	3.44527	80.23
	Total	41	160.619	4.29417	
	F_{ST} and p value	$F_{ST} = 0.19769$, p value < 0.01			

all of the regions was not significant. Conversely, the *Cytb* gene was significantly different between the JB and JR, OD and JR, and OD and SB populations (Table 4). The F_{ST} between JB and JR populations was the highest ($F_{ST} = 0.48594$). In addition, Slatkin's linearized F_{ST} between JB and JR populations was the highest value (Additional file 1: Table S2). For the neutrality test of each population, the results were not significant, which indicated that the genetic diversity was achieved not by selection but by drift (Table 5).

The results of the Mantel test for both the *COI* and *Cytb* genes, performed to investigate IBD, indicated that, even at far geographical distances, large genetic differentiation was not observed (*COI*, $p > 0.1$, $r = -0.365$; *Cytb*, $p > 0.1$, $r = 0.506$). While the genetic differentiation between JB and JR populations, which were farthest apart from each other, was highest, the correlations between geographical and genetic distances were not significant among other regions (Table 4).

According to the ML phylogenetic tree of the *COI* gene (597 bp), haplotypes appearing in the OD and JB populations clustered in the same clades as those from Russia (Fig. 3). This revealed that Korean populations might undergo genetic exchange with Russian populations. In phylogenetic trees of the *Cytb* gene, the haplotypes were divided into two clades (Fig. 3). One clade included only Korean haplotypes, while the other clade included Japanese haplotypes (Fig. 3). This could be interpreted as an effect of geographical isolation between Korea and Japan. Our findings are similar to those reported for various bat species in Europe, including *Myotis*, which were genetically differentiated between populations from Britain and continental Europe (Atterby et al. 2010; Razgour et al. 2013; Moussy et al. 2015; Wright et al. 2018).

Table 4 Pairwise differentiation (F_{ST}) for *COI* (above the diagonal) and for *Cytb* (below the diagonal) across four populations of *Myotis ikonnikovi* in Korea

	JB	OD	SB	JR
JB		0.01504	− 0.01408	0.17917
OD	0.05810		− 0.05719	0.03300
SB	0.26821	*0.14581*		− 0.02362
JR	*0.48594*	*0.29715*	0.03359	

Italic values represent statistical significance at p value < 0.05

Discussion

We analyzed mitochondrial DNA markers to investigate the genetic diversity and differentiation among populations of *M. ikonnikovi* from Korea. The mitochondrial genes of *M. ikonnikovi* exhibited high genetic diversity. Although genetic differentiation was not detected for the *COI* gene, strong genetic differentiation between JB and JR populations was observed for the *Cytb* gene. Moreover, the results of the present study indicated that the gene flow of the maternal lineage may be limited.

The genetic diversity of the Korean *M. ikonnikovi* populations based on *Cytb* sequences was higher than that calculated using *COI* sequences; this result is similar to that of Russian populations of *Myotis dasycneme* (Andersen et al. 2019). Other cases of high genetic diversity that are similar to that of the *M. ikonnikovi* populations herein include *Myotis lucifugus* and *Myotis septentrionalis* inhabiting North America; these species, however, were examined using the mitochondrial control region (Johnson et al. 2015). The average haplotype diversity (0.491) of European *M. myotis* using the mitochondrial control region was lower than that of *M. ikonnikovi* (Ruedi and Castella 2003). Therefore, the mitochondrial genetic diversity of Korean *M. ikonnikovi* is very high when compared with other *Myotis* species.

The closest populations (i.e., OD and JB) did not show any genetic differentiation, and the most distant populations (i.e., JB and JR) had the highest genetic differentiation. This result is consistent with the calculated IBD, which showed that genetic differentiation increases with greater distance. Interestingly, however, the SB

Table 5 Tests of the model of neutrality across four populations of *Myotis ikonnikovi* in Korea

Gene		JB (6)	OD (27)	SB (4)	JR (5)	Total (42)
COI	Tajima's *D*	1.39940	0.90959	− 0.80861	0.24314	0.33967
		p value > 0.1				
	Fu's FS	1.32594	0.99739	2.94444	− 0.47542	− 0.91940
		p value > 0.1				
Cytb	Tajima's *D*	1.30798	−0.27061	− 0.27914	1.02753	− 0.39228
		p value > 0.1				
	Fu's FS	3.11818	− 3.54325	1.74722	2.05468	− 3.65575
		p value > 0.1				

population showed significant genetic differentiation over distance despite being closest to the OD population. Such genetic differentiation was also observed between the Mt. Odae and Mt. Sobaek populations of *Rhinolophus ferrumequinum* in Korea (Byeon et al. 2018).

The SB population appears to have participated in genetic exchanges with the JB population to the north and with the JR population to the south; this suggests that the SB population acts as a bridge between the northern and southern populations of *M. ikonnikovi* in Korea. Thus, the conservation of SB populations is particularly important for effective management of *M. ikonnikovi* in Korea. However, little is known regarding the SB population; therefore, further investigation of *M. ikonnikovi* in this area and in the area between the SB and JR populations is needed.

Conclusions

Myotis ikonnikovi is a small, nocturnal bat that forages within forests. These traits hamper the collection of the bats for population-level studies. Therefore, few population genetic studies at foraging sites have been performed, and little is known regarding the genetics of bat species from Northeast Asia (Kawai et al. 2003; Kruskop et al. 2012). This study is the first to identify the genetic

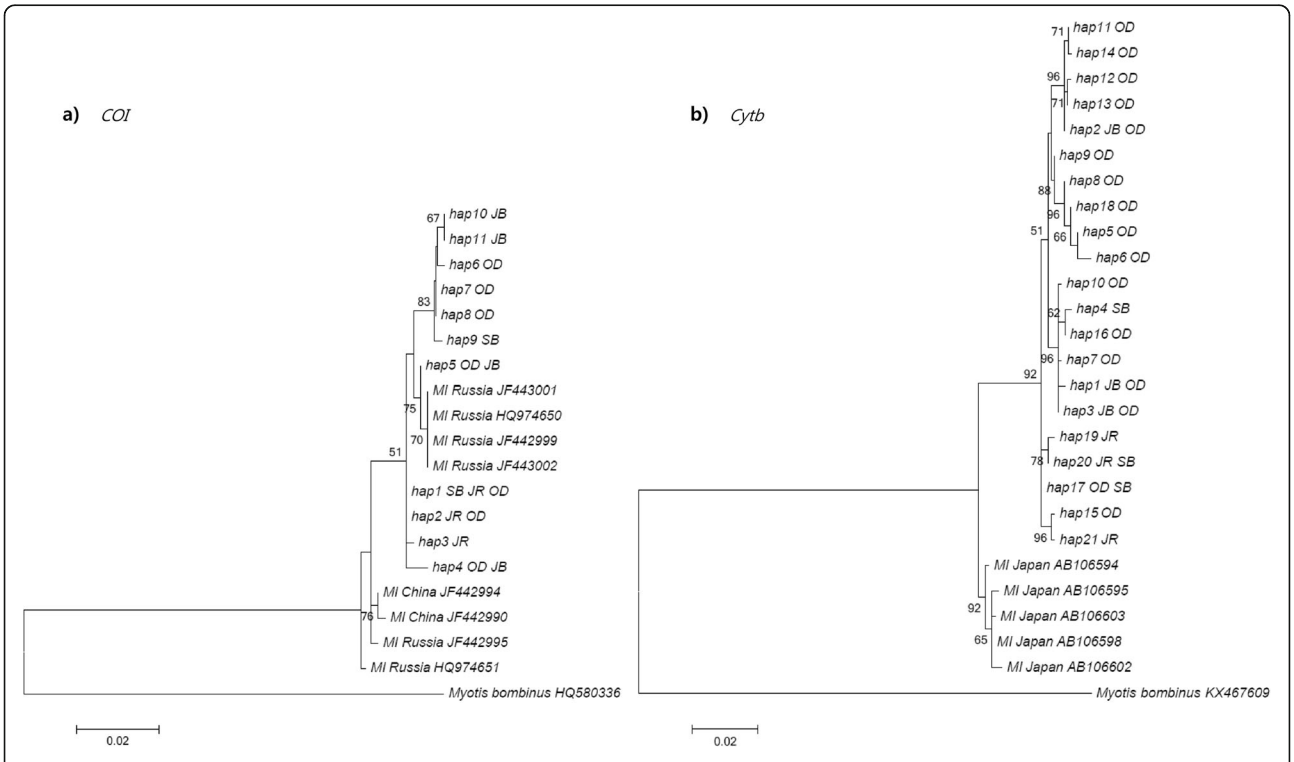

Fig. 3 Maximum-likelihood phylogenetic tree for **a** *COI* and **b** *Cytb* of *Myotis ikonnikovi* in Korea, Russia, China, and Japan. Sequences from Russia, China, Japan, and the outgroup (*Myotis bombinus*) were obtained from the GenBank database. GenBank accession numbers are shown after the location of samples. Numbers at branch nodes refer to the bootstrap support (bootstrap value = 1000)

population structure of *M. ikonnikovi*, which is vulnerable to habitat fragmentation and loss as it inhabits old-growth forests of over 40 years of age (Kim et al. 2014). According to the results of the present study, the differentiation between local populations is evident; thus, we believe that all local populations should be individually preserved. Extensive research on factors causing habitat fragmentation will contribute to effective conservation and management of *M. ikonnikovi* populations in the future.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s41610-019-0140-5.

Additional file 1: Table S1. Primers used in the present study [Akmali et al. 2015; Irwin et al. 1991; and Vrijenhoek 1994]. **Table S2.** Slatkin's linearized F_{ST} for *COI* (above the diagonal) and for *Cytb* (below the diagonal) across four populations of *Myotis ikonnikovi* in Korea.

Abbreviations

AMOVA: Analysis of molecular variance; *COI*: *Cytochrome oxidase I*; *Cytb*: *Cytochrome b*; F_{ST}: Fixation index statistics; IBD: Isolation by distance; JB: Jeombong; JR: Jiri; ML: Maximum-likelihood; OD: Odae; SB: Sobaek

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Authors' contributions

SP and PN contributed equally to this work. SSK designed the study and conducted the field study. YSC and SBJ collected the samples. SP and PN analyzed the data and wrote the manuscript. GJ reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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