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Review article

Bat study in the Kharaa region, Mongolia

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ABSTRACT

Our study objectives were to determine bat species composition and to study the genetic variations and sound characteristics in bats of the Kharaa, Shatan, and Ulgii areas of Mongolia. This study is the first bat survey in this area. Nineteen species were from Mongolia. Six bat species belonged to three genera. We performed mitochondrial DNA sequencing of *Myotis bombinus*, *Myotis gracilis,* and *Myotis petax* to confirm the morphological identification of these species. We also determined the sound frequencies of the six bat species, based on their echolocation calls. The conservation status was determined using World Conservation Union red list categories and criteria. Sixteen bats from three species were ringed during this study and three artificial boxes were placed on trees in the Kharaa River Valley. Other than the northern bat, all species were eastern Palearctic. The northern bat (*Eptesicus nilssonii*) species is widespread in the northern Palearctic region.

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Introduction

Most species of mammals distributed in the Kharaa region are usually animals of the Siberian taiga and temperate zone. The Khentii biogeographic zone is included in the Siberian taiga region (Bannikov 1954). The unique mammalian diversity of the region is composed central Asian steppe species and temprate zone taiga mountain species. Our study area was in the Khentii Mountain biogeographic region. There are seven orders of mammals distributed in this area such as hedgehogs, insectivoras, bats, rabbits, rodents, carnivores, and ungulates (Punsalpaamuu et al 2012). Diverse habitat heterogeneity of the region supports diverse biodiversity, which includes rare species. Bats have not been studied in the study area. Therefore, our study objectives were to determine bat species composition in the Kharaa, Shatan, and Ulgii areas of Mongolia and to study their genetic variations (to confirm morphological identification) and their sound characteristics.

Materials and methods

Geographical characteristics of the study area

The study area was in Batsumber Soum, the Tov province (N48.52117, E107.83190), and 120 km away from the north of Ulaanbaatar. The Kharaa region is a subwatershed of the Tuul River and the Orkhon-Selenge watershed (Figure 1). The maximum altitude is 1,300–1,700 m and the mean altitude is 500–800 m. The surface soil of the Kharaa region is composed of Imperial granite and mafic rock minerals of the Paleozoic era (Punsalpaamuu et al 2012). The highest mountain, Tsogt Hairhan, is 1,628 m and on the west side of our camp site; the lowest altitude was at the confluence of the Shatan and Kharaa rivers.

Study period and method

This study was conducted during June 2–15, 2005 and 2010–2015. We used mist nets with two 3-m poles for capturing the bats. Every day during this study, our capture work began 30 minutes before sunset and ended at midnight (approximately 1 AM). We placed mist nets at five different sites in Kharaa, Ulgii, Shatan Railway station, and the confluence of the rivers of Kharaa and Shatan (Figures 1 and 2). During the day, tree and rock holes were searched for bats by boreoscope. We collected 41 individual bats.

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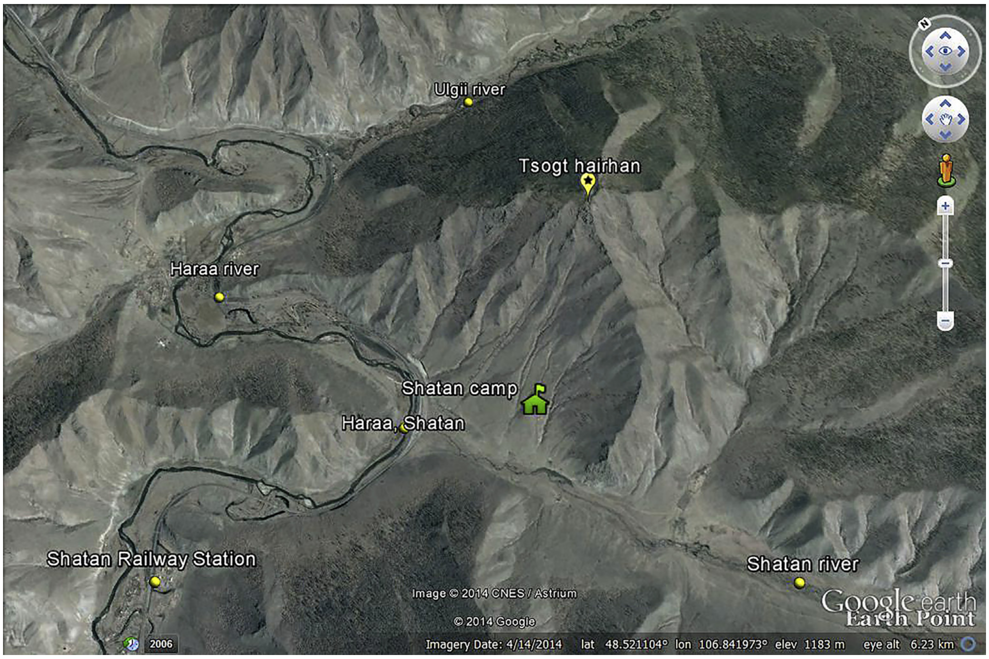

Figure 1. Study sites in the Kharaa, Ulgii, and Shatan areas.

We measured the body size, weight, and sex. Ectoparasites were preserved in 70% ethanol. Genetic samples were preserved in 96% ethanol. Before releasing the bats, we recorded their call using the Anabat detector (Titley Scientific Co., Columbia, Missouri, USA), and analyzed the data on Analook (Titley Scientific Co.) and Microsoft Excel (Microsoft, Redmond, WA, USA). We computed the descriptive statistical parameters: maximum, minimum, average, and standard deviation of the body measurements. All photos presented in this paper were taken during the field research. We used the mitochondrial cytochrome c oxidase subunit I (COI) DNA sequencing of *Myotis bombinus*, *Myotis gracilis* and *Myotis petax* to confirm the morphological identification.

Figure 2. Main study sites. A, Kharaa River (N48°31′57.4″ E106°49′29.5″; altitude, 1048 m); B, Ulgii River (N48°32′02.8″ E106°51′14.0″; altitude, 1052 m); C, The confluence of the Kharaa and Shatan rivers (N48°31′11.3″ E106°49′51.4″; altitude, 1096 m); D, Shatan valley (N48°30′02.4″ E106°50′53.7″; altitude, 1099 m).

Principle components analysis (PCA) is a variable-reduction technique and reorganizes a larger set of variables into a smaller set of components that account for most variances in the original variables (Manly 2004). The PCA was performed using a correlation matrix of morphological variables to discriminate the parameters and components between species. For the analysis of variance (ANOVA), I used JMP software (SAS institute 2010).

The equipment used for the study included the Anabat detector (Titley Scientific Co.), GPS Garmin map 62 (Garmin Co., Kansas city, USA), micro CA-300 inspection camera (Ridge Tool Company, Ohio, US), a camera with an 18–55 mm lens (Nikon D3100; Nikon Co., Tokyo, Japan), custom-made poles 3–5 m in height, mist nets (9 m × 3 m, 16-mm mesh; Ecotone Co., Gdynia, Poland), custom-made cotton bags, electronic scales (Pesola Co., Schindellegi, Switzerland), Digimatic calipers (Mitutoyo Co., Illinois, USA), boxes for samples, ethanol, tweezers, and headlights.

Results

In this study, we identified six bat species belonging to three genera: Amur bat (*Myotis bombinus* Thomas, 1906), Ussuri whiskered bat (*Myotis gracilis* Ognevi, 1927), Ikonnikov's bat *(Myotis ikonnikovi* Ognev, 1912), eastern water bat (*Myotis petax* Hollister, 1912), Ognev's long-eared bat (*Plecotus ognevi* Kishida, 1927), and the northern bat [*Eptesicus nilssonii* (Keyserling et Blasius, 1839)].

Amur bat (Myotis bombinus Thomas, 1906)

Amur bats are mostly distributed in deciduous forests and mixed forests in south Siberia, Mongolia, Korea and Japan. We recorded this species from the Balj River as a new record for Mongolia (Ariunbold et al 2008). A second capture was conducted in the Ulgii River region. The Amur bat can be identified by the strong hairs of the tail, which are on the membrane of tail (Appendix 1, Figure S1). The call frequency of the Amur bat ($n = 70$) was 26.03–62.42 kHz (MinFreq–MaxFreq; Fc, 33.94 kHz; Appendix 2, Figure S7).

Ussuri whiskered bat (Myotis gracilis Ognevi, 1927)

The Ussuri whiskered bat species is a common species in the Mongolian forest and forest steppe regions. We captured this species from the Shatan River Valley (Appendix 1, Figure S2). The Ussuri whiskered bat is widely distributed in the northern part of the east Palearctic region, which includes northern Mongolia. The call frequency of the Ussuri whiskered bat ($n = 16$) had a MinFreq–MaxFreq of 38.41–73.77 kHz (Fc 41.02 kHz; Appendix 2, Figure S8).

Ikonnikov's bat (Myotis ikonnikovi Ognev, 1912)

The Ikonnikov's bat species is distributed in eastern Asia and very rare in northern Mongolia. We have observed this species in the study area (Appendix 1, Figure S3). Ikonnikov's bat distribution in Mongolia is in the Khentii, Khuvsgul, Khangai, and western Khyangan mountains, and forest along the Selenge river basin. The call frequency of the Ikonnikov's bat **(**$n = 11$) was 38.87–79.46 kHz (Fc 43.93 kHz; Appendix 2, Figure S9).

Eastern water bat (Myotis petax Hollister, 1912)

Eastern water bat is distributed from western Siberia to the Japanese forests and steppes, and mostly in wetlands (Appendix 1, Figure S4). The call frequency of the eastern water bat ($n = 23$) had a MinFreq–MaxFreq of 37.73–78.14 kHz (Fc, 44.58 kHz; Appendix 2, Figure S10).

Northern bat (Eptesicus nilssonii Keyserling and Blasius, 1839)

The northern bat is distributed from the Arctic region to the forests and steppes of the Palearctic region (Wilson and Reeder 2005). We captured several individual northern bats from under

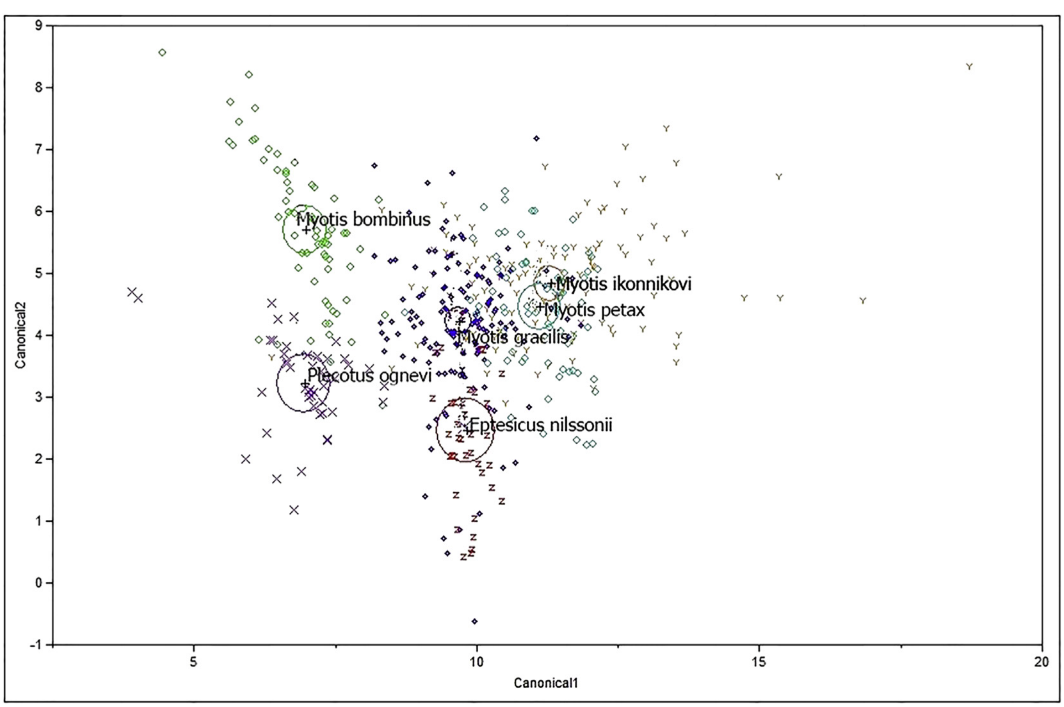

Figure 3. Principle component analysis ordination of the six species by call frequency.

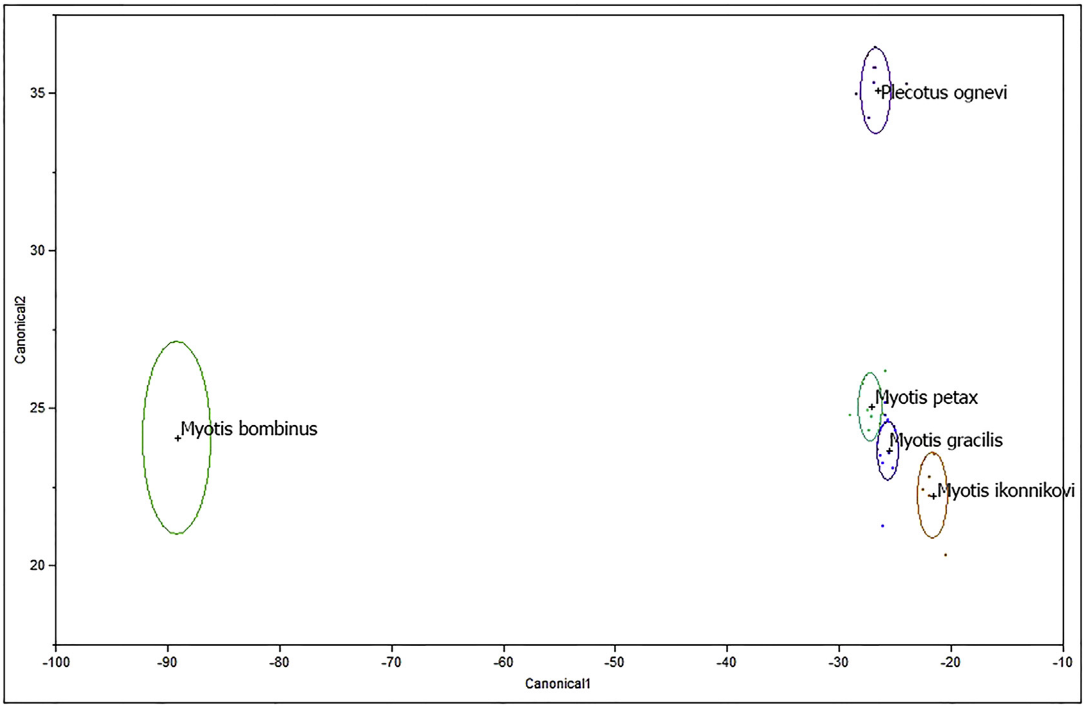

Figure 4. Principle component analysis ordination of the five species by morphological metrics.

train station's light when they were hunting for moths (Appendix 1, Figure S5). They feed on insects such as Noctuidae, Hymenoptera and Coleoptera. The call frequency of the northern bat ($n = 22$) had a MinFreq–MaxFreq of 28.82–53.70 kHz (Fc, 31.42 kHz; Appendix 2, Figure S11).

Ognev's long-eared bat (Plecotus ognevi Kishida, 1927)

Ognev's long-eared bat is distributed in the Siberian forest, Amur basin, central Siberia, Sahalin, and islands of Kuril. In Mongolia, this species is distributed in Mongol Altai, the wetlands of major lakes, Khuvsgul Lake, Khangai Mountain, and Khentii Mountain. Ognev's long-eared bats mostly live in mixed forests and the taiga; they are uncommon in western Mongolia (Appendix 1, Figure S6). Their major components of diet are Lepidoptera and blood-sucking insects. They also feed on the caterpillars of butterflies and spiders. The call frequency of Ognev's long-eared bat ($n = 11$) has a MinFreq–MaxFreq of 27.51–44.87 kHz (Fc, 30.2 kHz; Appendix 2, Figure S12).

The species clustered into six areas of the PCA (Figure 3), which corresponded with the call frequency of six species. The call frequency was generally segregated by each species with its specialty. Four of the six species are more discriminated by their higher call frequency.

The species were clustered into five areas in the PCA (Figure 4), which corresponded to the morphological metrics of five species. The morphological characteristics were generally differentiated by each species specialty; however, three of five species were more similar to each other than to the remaining species.

Morphological metric comparison was made between the bat species captured in the study areas. These six species were differentiated by the following characteristics: the largest bat is the northern bat; the Amur bat's third finger is the longest; Ognev's long-eared bat has the longest fifth finger, tail, ear, tibia and hind foot; Ussuri whiskered bat and Ikonnikov's bat are similar but Ikonnikovi's bat has the smaller body size, and the eastern water bat has a medium body size (Appendix 3, Table S1).

Analysis of variance (ANOVA) was conducted by comparing the morphological metrics of the species using Tukey–Kramer honest significant difference. The six species were significantly different from each other, based on their morphological characteristics ($p < 0.005$). *Myotis bombinus* is substantially differentiated greatly from the other species, and is only similar with two species in total length (Table 1).

Table 1. Morphological metrics comparisons of six species using Tukey–Kramer honest significant difference.

Species name	Mass	L. total	L. forearm	L. 3rd finger	L. 5th finger	L. thumb	L. claw	L. ear	L. tragus	W. tragus	L. tibia	L. hind foot	Wing span
Myotis bombinus	A	BC	A	B	C	D	A	B	C	C	C	A	.
Eptesicus nilssonii	B	A	B	A	AB	.	.	B	B	.	.	.	AB
Myotis petax	B	B	C	A	AB	AB	BC	B	B	B	B	BC	AB
Plecotus ognevi	B	BC	BC	A	A	A	B	A	A	A	A	B	A
Myotis ikonnikovi	B	BC	E	B	B	CD	C	B	B	B	B	C	AB
Myotis gracilis	B	C	D	B	B	BC	C	B	B	B	B	C	B
Prob > *F*	<0.0001	00.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0054

(A; B; C; D) denoted significant differences of the morphological metrics among the six species; L - length; W - width denoted significant differences of the morphological metrics among the six species; L-length; W- width.

Discussion

We recorded six species of bats belonging to three genera of the family Vespertilionidae from the study areas. All species, other than the northern bat, were from the eastern Palearctic region. The northern bat (*Eptesicus nilssonii*) species is widespread in the northern Palearctic region. The percentages of the six species were 32% for Ognev's long-eared bat, 24% for the Ussuri whiskered bat, 22% for the eastern water bat, 12% for Ikonnikov's bat, 5% for the Amur bat, and 5% for the northern bat.

The morphological identification matched by molecular determination using the mitochondrial DNA sequencing results of the species *Myotis bombinus*, *Myotis gracilis*, and *Myotis petax*. Our DNA results matched the NCBI gene bank results by Kruskop et al (2012): *Myotis bombinus* (100%), *Myotis petax* (98%), and *Myotis gracilis* (99%).

The eastern Palearctic Ussuri whiskered bat *Myotis gracilis* should be synonymous with *Myotis sibirica* Kastschenko, 1905 by Kruskop et al (2012), and differences between these two species should be considered subspecies; thus, specific population study is necessary in our country. The western Palearctic species *Myotis brandtii* and the eastern Paleacrtic species *M. gracilis* of high mountain species nearly resemble each other and are closely related by origination (Ruedi et al 2013). *Myotis petax* and *Myotis daubentonii* were initially synonymous; they are now separate species and are not closely related (Datzmann et al 2012; Kruskop et al 2012). However, our statistical analyses showed that *M. gracilis* and *M. petax* are significantly different from each other and only share some morphological characteristics (Table 2).

We placed three artificial nest boxes on trees (Figure 5). However, bats have not hosted in the boxes. We captured 41 individual bats, measured their morphological characteristics, and ringed some of them. This may give some data about their movement, home range, age, growth, and habitat selection.

We ringed 24 bats from five species. We used 2.9-mm numbered rings. The eastern water bats were marked by blue plastic and split metal bat bands with numbers 145, 146, 149, UC0981, UC0982, UC0983; Ussuri whiskered bats were marked by plastic and split metal bat bands with numbers 141, 143, 144 (blue), 41 (black) UC0978, UC0979, UC0980; Ikonnikov's bats were marked by blue plastic rings with number 150; northern bats were marked by split metal bat bands with the number UC0984; Ognev's long-eared bats were marked by split metal bat bands with numbers UC0969, UC0970, UC0971, UC0972, UC0973, UC0974, UC0975, and UC0976, UC0977.

Ognev's long-eared bats were ringed in spring 2014. This species was recaptured in 2015, which showed that it stays permanently in one place.

Table 2. World Conservation Union (International Union for Conservation of Nature) evaluation of bats recorded from study areas.

	Species name: Mongolian	Latin	International Redlist	Mongolian Redlist
1	Eastern water bat	*Myotis petax*	NE	LC
2	Ussuri whiskered bat	*Myotis gracilis*	NE	DD
3	Ikonnikov's bat	*Myotis ikonnikovi*	LC	DD
4	Amur bat	*Myotis bombinus*	NT	NE
5	Northern bat	*Eptesicus nilssonii*	LC	LC
6	Ognev's long-eared bat	*Plecotus ognevi*	LC	LC

DD, data deficient; LC, least concern; NE, not evaluated; NT, near-threatened.

Figure 5. Artificial nest box for bats.

In addition, conservation status was stated by using World Conservation Union [International Union for Conservation of Nature (IUCN)] red list categories and criteria. Three species are categorized as "least concern", one species is categorized by "near-threatened," and two species have not been evaluated worldwide; three species are "least concern", two species are "data deficient," and one species has not been evaluated in regional status (Clark et al 2006; Table 2). The six species are not listed in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Convention on Migratory Species (CMS), and Mongolian Red Book.

The following species may be recorded with additional collection in the region: greater tube-nosed bat (*Murina hilgendorfi*), steppe whiskered bat (*Myotis aurascens*), fraternal Myotis (*Myotis frater*), and particoloured bat (*Vespertilio murinus*). Their morphological identification issues need to be resolved using molecular analyses.

Acknowledgments

We are grateful to members of the Mongolian and Korean Biological Expedition, especially Dr Peak Woon Kee, Jin Seon-Deok, and Paik In-Hwan.

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Appendix 1. Photos of six species captured in the study areas.

Figure S1. Amur bat (*Myotis bombinus* Thomas, 1906).

Figure S2. Ussuri whiskered bat (*Myotis gracilis* Ognevi, 1927).

Figure S3. Ikonnikov's bat (*Myotis ikonnikovi* Ognev, 1912).

Figure S4. Eastern water bat (*Myotis petax* Hollister, 1912).

Figure S5. Northern bat [*Eptesicus nilssonii* (Keyserling and Blasius, 1839)].

Figure S6. Ognev's long-eared bat (*Plecotus ognevi* Kishida, 1927).

Appendix 2. Call frequency of the six species captured in our study.

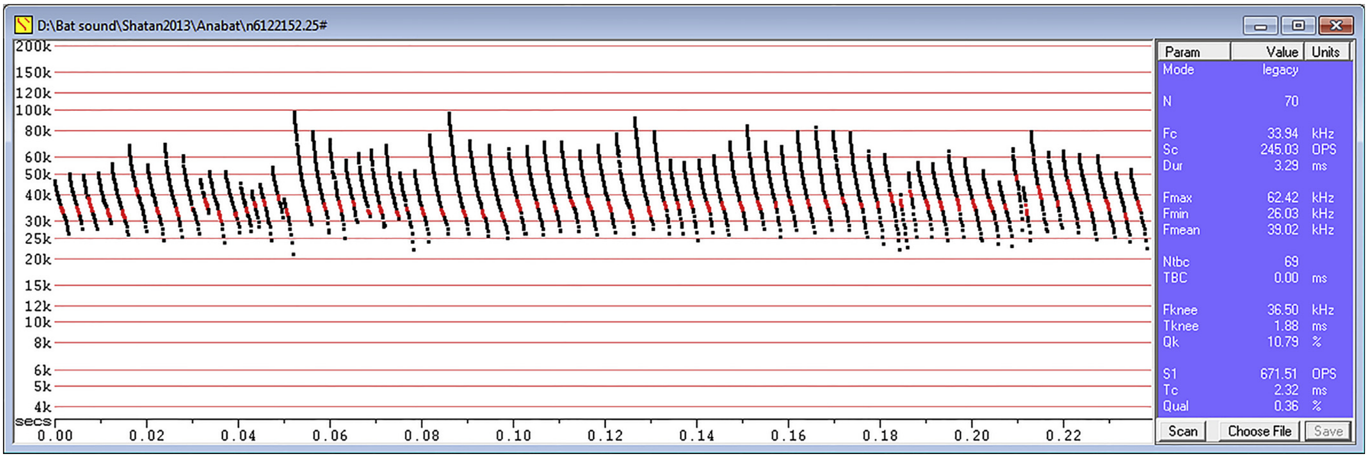

Figure S7. Amur bat's voice frequency measured by Anabat detector (Titley Scientific Co., Columbia, Missouri, USA).

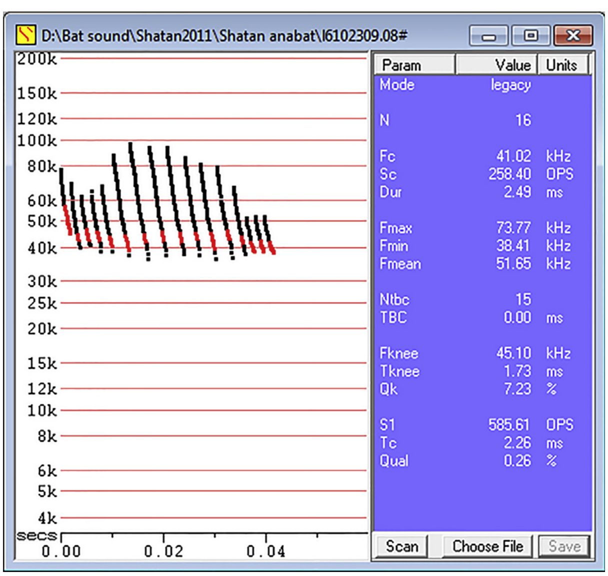

Figure S8. Ussuri whiskered bat's call frequency measured by Anabat detector (Titley Scientific Co., Columbia, Missouri, USA).

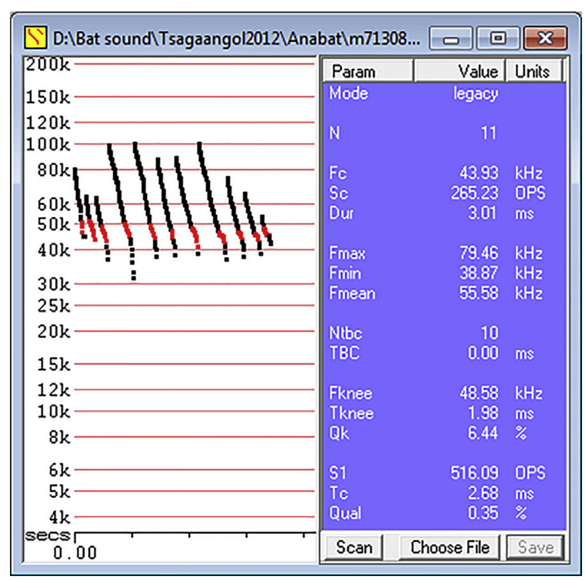

Figure S9. Ikonnikov's bat sound frequency measured by Anabat detector (Titley Scientific Co., Columbia, Missouri, USA).

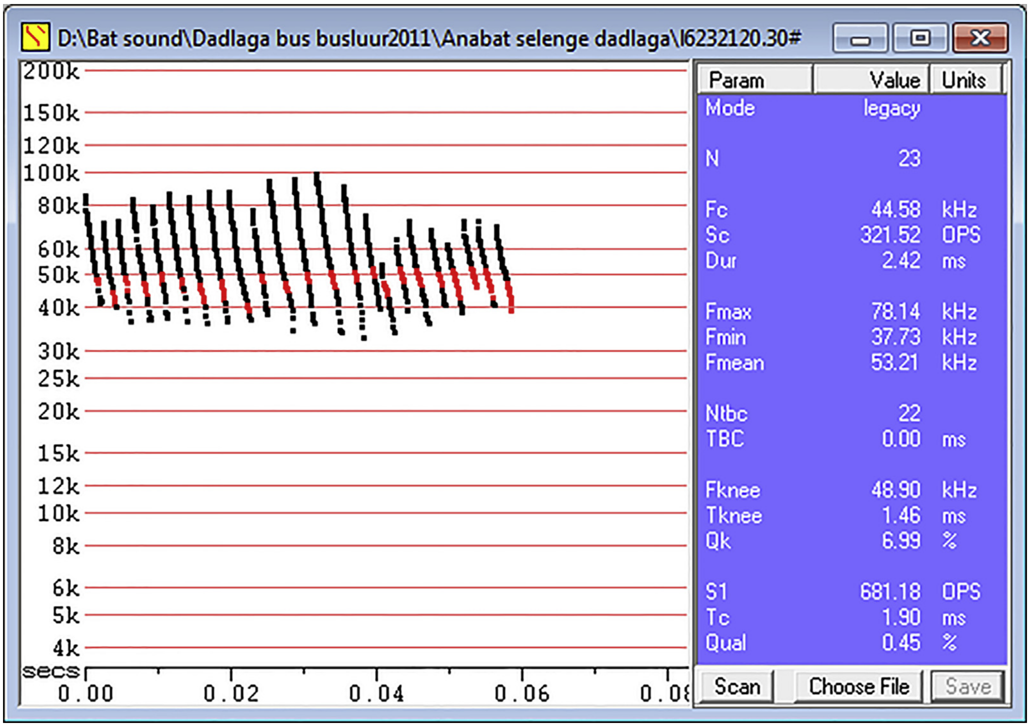

Figure S10. Eastern water bat call frequency measured by Anabat detector (Titley Scientific Co., Columbia, Missouri, USA).

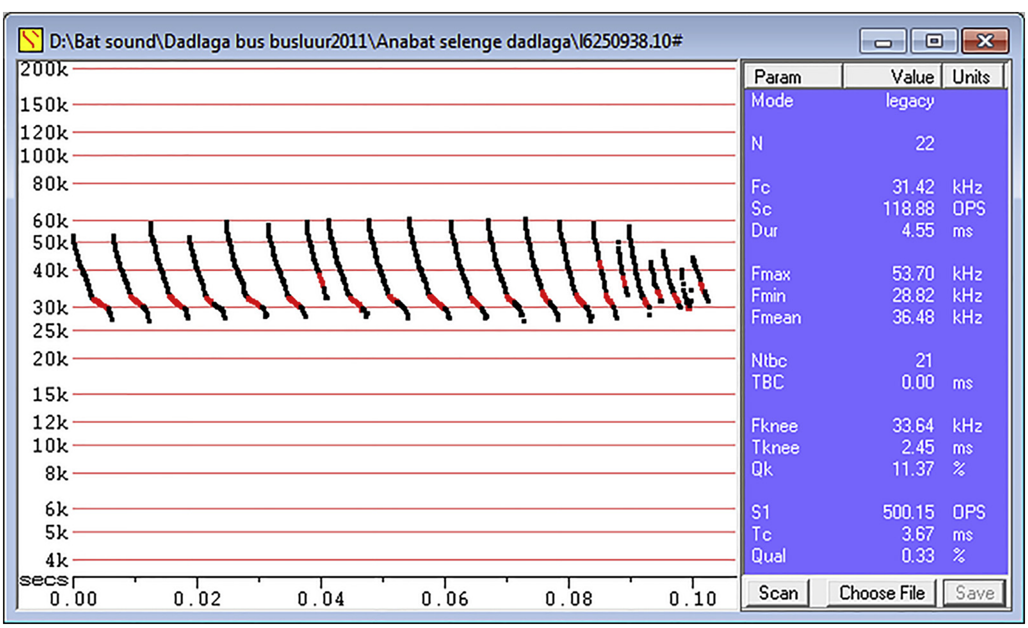

Figure S11. Northern bat call frequency measured by Anabat detector (Titley Scientific Co., Columbia, Missouri, USA, USA).

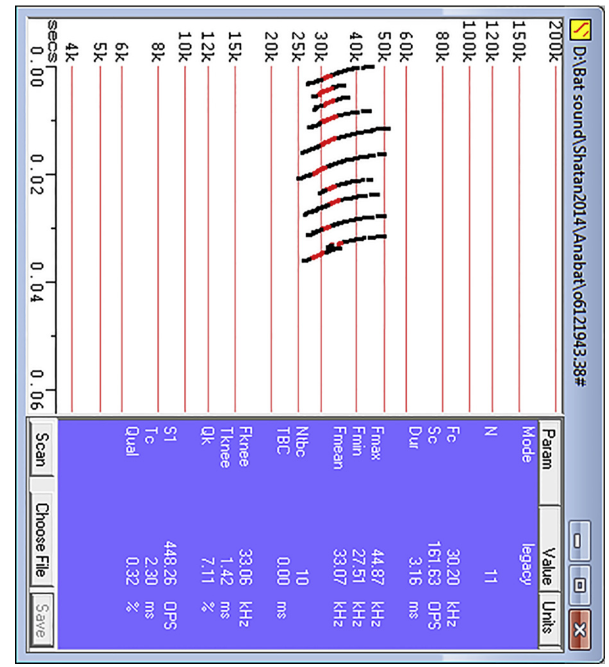

Figure S12. Ognev's long-eared bat call frequency measured by Anabat detector (Titley Scientific Co., Columbia, Missouri, USA).

Appendix 3. Morphological characteristics metrics of the six species captured in our study.

Table S1. Morphometric parameters of six species collected from study sites.

	Amur bat ($n = 2$)	Ussuri whiskered bat ($n = 10$)	Ikonnikov's bat ($n = 5$)	Eastern water bat ($n = 9$)	Northern bat's ($n = 2$)	Ognev's long-eared bat ($n = 13$)
Mass (g)	4.4–7.5 (5.95 ± 1.55)	3.9–6.2 (5.4 ± 0.69)	5.0–6.5 (5.5 ± 0.63)	3.5–8.8 (7.0 ± 1.59)	7.0–7.8 (7.4 ± 0.57)	4.2–9.5 (6.8 ± 1.8)
Head-body length (mm)	46.8–47 (46.9 ± 0.1)	39.1–46.7 (43.2 ± 2.65)	42.5–48.5 (45.2 ± 2.42)	45.6–54.5 (49.2 ± 3.17)	58.5–60.0 (59.3 ± 1.06)	36.5–53.0 (44.4 ± 7.12)
Tail length (mm)	42.7–45 (43.85 ± 1.1)	33.8–43.0 (37.4 ± 2.47)	33.8–37.8 (36.2 ± 1.73)	34.6–43.0 (40.9 ± 2.77)	40.0–44.8 (42.4 ± 3.39)	32.4–48.0 (40.9 ± 5.27)
Forearm length (mm)	39.9–41.4 (40.65 ± 0.7)	34.5–37.1 (35.9 ± 0.73)	32.1–33.6 (32.9 ± 0.68)	36.2–39.5 (38.2 ± 0.99)	40.8–41.0 (40.9 ± 0.14)	37.0–42.6 (39.2 ± 1.65)
3^{th} finger length (mm)	67.7–68 (67.85 ± 0.1)	48.0–55.1 (51.9 ± 2.32)	42.5–54.0 (50.3 ± 4.47)	56.3–61.6 (58.9 ± 1.66)	64.0–64.5 (64.3 ± 0.35)	46.5–68.8 (62.5 ± 5.73)
5^{th} finger length (mm)	47.9–50.2 (49.05 ± 1.1)	40.0–56.4 (43.6 ± 4.80)	41.2–48.5 (43.3 ± 1.35)	42.0–49.4 (46.3 ± 2.70)	46.0–46.3 (46.2 ± 0.21)	45.5–54.2 (49.4 ± 2.56)
Thumb length (mm)	6.3–6.7 (6.5 ± 0.2)	4.0–6.1 (5.5 ± 0.58)	3.1–5.0 (4.5 ± 0.78)	4.3–7.4 (6.3 ± 0.92)	–	4.2–8.0 (6.7 ± 1.11)
Claw length of thumb (mm)	2.3–2.8 (2.55 ± 0.25)	1.2–2.1 (1.6 ± 0.27)	1.0–2.0 (1.6 ± 0.42)	1.3–2.6 (2.1 ± 0.40)	–	2.0–3.5 (2.7 ± 0.39)
Ear length (mm)	12.8–17.3 (15.05 ± 2.25)	10.3–18.7 (12.9 ± 2.34)	8.5–12.0 (10.8 ± 1.35)	11.2–13.4 (12.7 ± 0.76)	12.0–12.1 (12.1 ± 0.07)	25.3–36.3 (31.1 ± 3.50)
Tragus length (mm)	7.5–10.7 (9.1 ± 1.6)	3.5–7.6 (6.1 ± 1.15)	4.5–6.9 (5.7 ± 0.87)	4.6–6.8 (5.6 ± 0.74)	4.5–6.0 (5.3 ± 1.06)	11.4–15.2 (14.0 ± 1.16)
Tibia length (mm)	17.8–17.8 (1.87 ± 0.37)	13.8–17.7 (15.8 ± 1.35)	14.3–16.0 (15.0 ± 0.71)	15.4–18.0 (16.6 ± 0.96)	–	16.8–21.4 (19.2 ± 1.36)
Hind foot length (mm)	8–8.2 (8.1 ± 0.1)	5.6–7.8 (6.6 ± 0.63)	5.9–6.3 (6.1 ± 0.16)	4.5–7.9 (7.1 ± 1.08)	–	6.2–9.0 (7.7 ± 0.99)
MinFreq–MaxFreq (kHz)	26.03–62.42 ($n = 70$)	38.41–73.77 ($n = 16$)	38.87–79.46 ($n = 11$)	37.73–78.14 ($n = 23$)	28.82–53.70 ($n = 22$)	27.51–44.87 ($n = 11$)
Characteristic frequency (kHz)	33.94 ($n = 70$)	41.02 ($n = 16$)	43.93 ($n = 11$)	44.58 ($n = 23$)	31.42 ($n = 22$)	30.2 ($n = 11$)

MaxFreq, maximum frequency; MinFreq, minimum frequency.