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New records and an updated checklist of the genus *Myotis* (Chiroptera, Vespertilionidae) in Xizang, China

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ABSTRACT

Xizang, located on the southwestern Qinghai-Xizang Plateau of China, hosts a mammalian fauna whose true diversity remains incompletely understood. This knowledge gap is particularly pronounced in bat clades with high cryptic diversity such as *Myotis* (Chiroptera: Vespertilionidae). To clarify species boundaries and diversity patterns of *Myotis* in this region, we conducted a series of field surveys in border areas of Xizang from 2023 to 2025 and employed an integrative taxonomic approach combining mitochondrial cytochrome b (cyt *b*) phylogenetic analyses, detailed morphological comparisons, and echolocation calls assessments. Our results confirm the distribution of 9 *Myotis* species in Xizang, significantly updating its species diversity. Notably, we document *Myotis himalaicus* and *Myotis sicarius* from China for the first time, substantially extending their known distributions. In addition, we critically reassess the occurrence of *Myotis siligorensis* and *Myotis longipes*, demonstrating that valid records of both species in China are restricted to Xizang, near their type localities in India and Nepal. Consequently, previous records of these taxa from China likely represent misidentifications and necessitate a thorough re-evaluation. Overall, this study establishes a robust taxonomic baseline for future systematic updates and conservation planning in the biogeographically complex region of Xizang.

Keywords: Xizang (China); New records; *Myotis*; Species diversity; Taxonomic revision

INTRODUCTION

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Xizang (China) represents a globally critical region for biodiversity, conservation, and biogeographic research (Gautam et al., 2023; Jiao et al., 2024; Lei et al., 2015; Liu et al., 2024; Wang et al., 2020, 2022; Yang et al., 2025). In recent years, substantial progress has been made in documenting the species diversity, distribution, and adaptive evolution of various taxonomic groups within this region, particularly among large mammals (Li et al., 2024) and herpetofauna (Ma et al., 2024; Wang et al., 2024). Nevertheless, research effort and sampling intensity remain highly uneven across taxa. Bats (Chiroptera), despite their fundamental roles in ecosystem functioning and services (Liu et al., 2025; Ramírez-Francel et al., 2022; Tuneu-Corral et al., 2023), represent one of the most pronounced knowledge gaps in Xizang. Their nocturnal and cryptic habits, combined with the severe logistical constraints of high-altitude fieldwork (Aylen et al., 2022; Gong et al., 2023), have resulted in chronically under-sampled assemblages and a scarcity of targeted surveys. This deficiency is particularly acute in remote and access-restricted border regions.

The genus *Myotis* Kaup, 1829 represents a particularly challenging taxonomic group. As the most species-rich bat genus, comprising over 140 extant species (Simmons & Cirranello, 2025), *Myotis* exemplifies a rapid mammalian evolutionary radiation (Burgin et al., 2018; Yu et al., 2014). However, its exceptional diversity is accompanied by pervasive morphological conservatism and convergence, a high prevalence of cryptic species/complexes, and a historical scarcity of vouchered material from biogeographically critical regions. In Xizang, these challenges are further intensified by long-standing under-sampling, especially in remote international border zones. Consequently, published accounts of *Myotis* diversity in the region have been notably unstable (Table 1): different monographs (e.g., Feng et al., 1986; Smith

Received: 17 April 2026; Accepted: 25 May 2026; Online: 17 June 2026

Foundation items: This work was supported by the National Natural Science Foundation of China (32370469, 32300363, 31970394, 32192421), Survey of Wildlife Resources in Key Areas of Xizang (ZL202203601), Special Foundation for National Science and Technology Basic Research Program of China (2021FY100303), and College Students' Innovative Training Program of Guangzhou University (202411078045, S202511078165).

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Table 1 Revisions and updates of the *Myotis* species checklist in Xizang

Species	Feng et al. (1986)	Wang (2003)	Simmons (2005)	Smith & Xie (2009)	Jiang et al. (2015)	Wei et al. (2022)	Liu et al. (2022)	This study
<i>M. formosus</i>	–	–	+	–	–	+	+	○
<i>M. himalaicus</i>	–	–	–	–	–	–	–	●
<i>M. laniger</i>	–	–	+	+	+	–	+	○
<i>M. longipes</i>	–	–	–	–	–	–	–	●
<i>M. muricola</i>	+	+	–	+	+	+	+	○
<i>M. nipalensis</i>	–	–	+	–	+	+	+	○
<i>M. sibiricus</i>	–	–	–	–	–	–	–	○
<i>M. sicarius</i>	–	–	–	–	–	–	–	●
<i>M. siligorensis</i>	–	–	–	–	–	–	–	●

“+” indicates that the corresponding literature recognizes the distribution record of the species in Xizang, while “–” indicates the opposite. “○” represents species considered distributed in Xizang in this study, with “●” denoting new national records or first confirmed occurrences in China. Note that according to the conclusions of Kruskop et al.(2012), previous Chinese records of *M. brandtii* are assigned to *M. sibiricus*.

& Xie, 2009; Wei et al., 2022) report varying numbers of species. Meanwhile, Kruskop et al.(2012) suggested that records of *Myotis brandtii* (Eversmann, 1845) from China should be reassigned to *Myotis sibiricus* Kastschenko, 1905, a taxonomic perspective that has not yet been adopted by current Chinese taxonomic frameworks (Wei et al., 2022, 2025). This case exemplifies the growing taxonomic instability within the genus.

More importantly, Ruedi et al.(2021) provided a comprehensive reassessment of *Myotis* diversity along the southern slopes of the Himalayas, revealing substantial discrepancies with the taxonomic framework traditionally applied in China. These inconsistencies largely reflect the lack of well-documented specimens from key regions, particularly Xizang’s border areas along the northern and eastern Himalayan sectors. As core components of the Himalayan biogeographic zone, these regions are likely to host taxa bridging South and East Asian faunas (Rana et al., 2020; Regmi et al., 2023). Consistent with this expectation, neighboring regions across the Himalayas exhibit markedly higher documented *Myotis* diversity (Acharya & Adhikari, 2010; GBIF, 2025; Saikia et al., 2025). Together, unstable historical records, regional sampling gaps, and recent taxonomic revisions underscore a fundamental unresolved issue: the true diversity, distributional limits, and biogeographic relationships of *Myotis* in Xizang remain poorly understood.

To address these deficiencies, we conducted a series of bat surveys in the border zones of Xizang, with particular emphasis on the northern slopes and eastern Himalayan sector (e.g., Mêdog, Nyalam, Zayü, etc.; see Figure 1 for details). We generated a comprehensive, vouchered dataset integrating morphological examination and molecular evidence. Specifically, our objectives were to: 1) provide an evidence-based reassessment of *Myotis* species diversity in these under-surveyed areas; 2) incorporate Xizang populations into recent Himalayan-wide taxonomic frameworks for comparison and systematization; and 3) clarify taxonomic ambiguities and phylogenetic relationships among key taxa. By doing so, this study establishes a faunal baseline for future evolutionary, ecological, and conservation research in one of the Asian most biodiverse yet least explored regions.

MATERIALS AND METHODS

Sampling

From 2023 to 2025, we conducted a series of bat surveys in

the Xizang Autonomous Region of China (Medog, Nyalam, Zayü, and other areas), focusing on border areas, and collected a number of *Myotis* specimens using harp traps and mist nets. All sampling sites are shown in Figure 1, and the corresponding Global Positioning System (GPS) coordinates are provided in Supplementary Table S2. To enable accurate species identification within a more rigorous and comprehensive comparative framework, we incorporated corresponding *Myotis* specimens from other regions of China as comparative material for subsequent morphological analysis, based on historical distribution records and species checklists. All voucher specimens and sequence data used in this study are provided in Supplementary Table S1. Voucher specimens were confirmed as adults based on the degree of epiphyseal-diaphyseal fusion (Brunet-Rossini & Wilkinson, 2009), fixed by immersion in 75% ethanol solution and deposited at the Biodiversity Research Center of South China, Guangzhou University.

Morphological data collection and analyses

Following the Chiroptera Morphometric Standard (Yang et al., 2007), 5 external and 13 craniodental characteristics (Table 2; Supplementary Table S2) were taken using MNT-150 digital calipers (Meinert Industrial Co., Ltd., China; accuracy 0.01 mm). External measurements included forearm length (FA); tibia length (TIB); hindfoot length including claw (HF); ear length (EL); and tail length (TL). Craniodental measurements comprised: greatest length of skull (GTL); condylocanine length (CCL); zygomatic breadth (ZB); mastoid width (MAW); braincase width (BCW); height of braincase (BCH); palatal bridge length (PBL); maxillary toothrow length (C^1M^3L); upper canine width (C^1C^1W); width across the upper molars (M^3M^3W); mandibular toothrow length (C_1M_3L); mandible length (ML); and mandibular height (MH). Missing values were absent in our morphometric dataset.

To preliminarily assess size and shape differentiation among species, we generated bivariate scatter plots using two pairs of key measurements: FA vs. HF for external morphology, and GTL vs. C^1M^3L for craniodental morphology. These plots provide a basic visualization of the raw data without dimensionality reduction. For a more comprehensive multivariate assessment, we further performed principal component analyses (PCAs) separately on the external and craniodental datasets, and extracted the first two principal components to generate scatter plots. These analyses were conducted using the R packages: factoextra (Kassambara & Mundt, 2020) and FactoMineR (Lê et al., 2008) in R v.4.3.2.

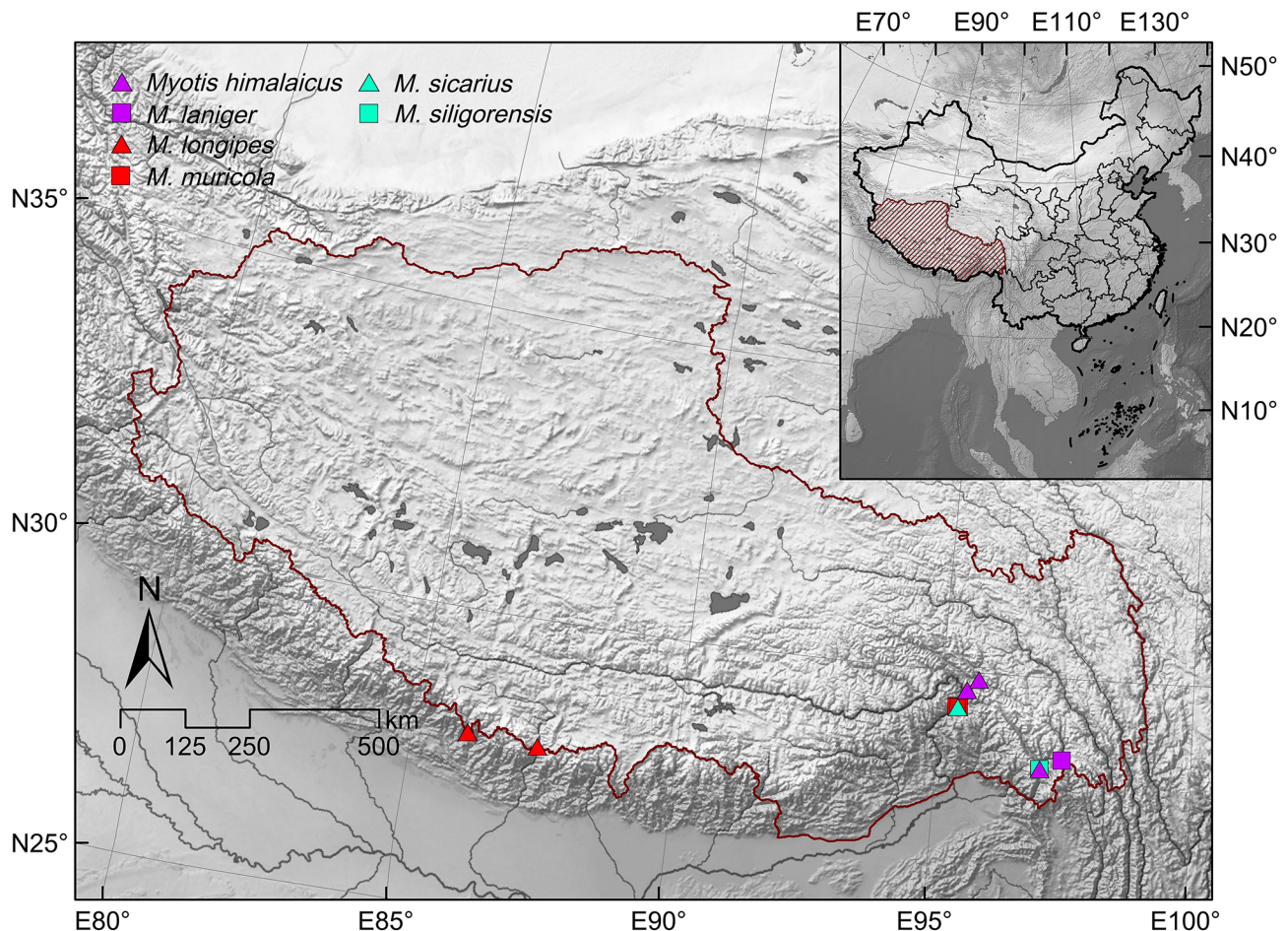


Figure 1 Map showing sampling localities in the Xizang Autonomous Region, China

Detailed Global Positioning System (GPS) coordinates of each sampling site are provided in Supplementary Table S2.

By integrating these results with detailed morphological comparisons, we compiled an updated identification key to the *Myotis* species of Xizang, providing a scientific reference for subsequent research and conservation efforts.

Genetic data (cyt *b*) acquisition and phylogenetic analysis

Total genomic DNA was extracted from approximately 20 mg of muscle tissue using a commercial kit (DNU333-03, Maibao Biotechnology Co., Ltd., China). The mitochondrial cytochrome *b* (cyt *b*) gene was amplified by PCR using primers from Irwin et al. (1991) and Ruedi et al. (2013). Each 30 μ L reaction contained: 13 μ L of 2 $\times$ Accurate Taq Premix (AG11009, Accurate Biotechnology Co., Ltd., China), 0.5 μ L of each forward and reverse primer (10 μ mol/L), 14 μ L of RNase-free water, and 2 μ L of DNA template (~20 ng/ μ L). Thermal cycling profile was: initial denaturation at 94 $^{\circ}$ C for 5 min; 37 cycles of 94 $^{\circ}$ C for 45 s, 46 $^{\circ}$ C for 30 s, and 72 $^{\circ}$ C for 60 s; and a final extension at 72 $^{\circ}$ C for 5 min. Amplification products were verified by agarose gel electrophoresis and submitted to Sangon Biotech (Shanghai) for bidirectional Sanger sequencing.

The 18 newly generated cyt *b* sequences from this study were combined with homologous *Myotis* sequences and *Kerivoula furva* sequences (as outgroup) that were downloaded from GenBank (Supplementary Table S1). All sequences were assembled, aligned, and edited manually using Geneious v.8 (Kearse et al., 2012). Subsequently, phylogenetic analyses were performed using both Bayesian inference (BI) and maximum likelihood (ML) methods.

Bayesian inference was performed under the GTR+I+ Γ model, while maximum likelihood analysis used the best-fit model (TPM2u+F+I+R2) selected by ModelFinder (Kalyaanamoorthy et al., 2017). BI analyses were conducted using MrBayes v.3.2.7 (Ronquist et al., 2012). Four Markov chains were run for 10 million generations, with sampling every 1 000 generations, and the first 25% of trees were discarded. ML analyses were performed using IQ-TREE v.2 (Minh et al., 2020), and branch support was evaluated with 1 000 ultrafast bootstrap replicates.

Given the inherent limitations of a single mitochondrial marker (e.g., insufficient phylogenetic signal, maternal inheritance bias), relying solely on its phylogenetic topology may lead to overestimation of species diversity or misleading inferences. To delimit species boundaries more robustly, we employed two species delimitation methods that do not require a priori assignment of specimens to putative species — assemble species by automatic partitioning (ASAP, Puillandre et al., 2021) and Poisson tree process (PTP, Zhang et al., 2013)—for cross-validation. ASAP analyses were conducted using the online platform (<https://bioinfo.mnhn.fr/abi/public/asap>), and PTP analyses were performed using the PTP webserver (<https://species.h-its.org/ptp/>). Additionally, pairwise genetic distances (interspecific and intraspecific) were calculated under the Kimura 2-parameter (K2P) model (Kimura, 1980) using MEGA v.11 (Tamura et al., 2021).

Echolocation call recording and analysis

Echolocation calls of *Myotis* bats were recorded in flight state

Table 2 External and craniodental characteristics measurements (in millimeters) and PCA factor loadings of 9 *Myotis* species

Traits	PC1 loading	PC2 loading	<i>M. alticraniatus</i> <i>n</i> =6	<i>M. annatessae</i> <i>n</i> =5	<i>M. formosus</i> <i>n</i> =2	<i>M. himalaicus</i> <i>n</i> =4	<i>M. laniger</i> <i>n</i> =8	<i>M. longipes</i> <i>n</i> =6	<i>M. muricola</i> <i>n</i> =5	<i>M. sicarius</i> GZHU 24198	<i>M. siligorensis</i> —
FA	0.545	0.118	34.22±0.65 32.99–34.85	32.77±1.62 31.28–35.52	51.06 50.72	41.30±0.91 40.42–42.40	36.48±0.85 35.08–37.52	35.27±0.38 34.65–35.74	36.55±1.22 35.65–38.65	53.55	35.18±1.34 (9) 32.89–36.76
TIB	0.505	0.296	15.24±0.53 14.29–15.67	16.19±0.44 15.70–16.75	24.76 24.01	21.36±0.54 20.96–22.15	16.21±0.66 15.26–17.29	15.16±1.15 13.02–15.99	16.95±1.04 15.66–18.55	23.58	15.03±1.12 (9) 13.21–17.03
HF	0.240	−0.753	9.22±0.53 8.74–9.98	6.80±0.25 6.57–7.13	13.14 11.12	7.97±0.95 7.13–9.08	10.26±0.46 9.77–10.98	8.37±0.79 7.49–9.40	6.73±1.10 5.39–7.87	6.98	7.63±0.66 (9) 6.80–8.89
EL	0.348	−0.506	11.99±0.80 11.12–12.95	12.18±0.91 10.94–13.23	17.31 16.2	11.46±1.25 9.97–12.66	15.02±1.11 13.59–17.17	14.64±0.51 13.97–15.20	13.38±1.05 11.94–14.22	19.23	12.41±0.96 (9) 11.25–13.90
TL	0.519	0.275	34.84±1.08 33.60–36.35	34.53±1.95 31.09–35.84	57.85 57.19	45.50±2.92 41.15–47.28	35.97±2.27 33.07–39.25	38.23±0.79 37.20–39.12	40.07±3.59 35.11–43.60	53.59	35.74±2.12 (9) 32.40–38.84
GTL	0.292	0.178	12.72±0.23 12.35–12.97	12.96±0.17 12.81–13.21	18.34 17.75	14.21±0.17 13.98–14.35	14.42±0.30 13.83–14.72	12.98±0.58 12.03–13.62	14.01±0.43 13.49–14.66	18.56	12.53±0.17 (5) 12.33–12.78
CCL	0.286	0.215	11.22±0.32 10.85–11.68	11.47±0.14 11.32–11.62	16.77 14.66	12.96±0.19 12.69–13.13	12.76±0.22 12.53–13.10	11.37±0.27 10.95–11.66	12.25±0.59 11.24–12.72	16.73	10.89±0.34 (5) 10.51–11.39
ZB	0.287	−0.050	7.69±0.27 7.37–8.05	7.94±0.11 7.80–8.09	11.78 11.69	9.12±0.19 8.96–9.39	8.37±0.39 7.92–9.24	7.94±0.25 7.54–8.20	8.59±0.50 7.83–9.12	11.96	7.66±0.22 (5) 7.45–8.00
BCW	0.284	−0.106	6.53±0.16 6.30–6.74	6.44±0.10 6.32–6.59	8.57 9.19	7.49±0.23 7.31–7.82	7.13±0.31 6.57–7.68	6.53±0.14 6.34–6.68	6.70±0.40 6.42–7.36	8.88	6.46±0.07 (5) 6.33–6.51
BCH	0.204	−0.839	5.41±0.61 4.75–6.13	6.13±0.31 5.74–6.52	7.09 8.35	6.85±0.77 5.72–7.44	5.93±0.65 5.12–6.85	5.99±0.39 5.51–6.65	5.35±0.43 4.86–5.90	8.11	6.24±0.23 (5) 6.04–6.56
PBL	0.238	0.309	5.11±0.27 4.90–5.61	4.95±0.32 4.51–5.39	7.77 7.74	6.32±0.54 5.86–7.07	6.64±0.80 5.53–7.72	5.33±0.27 4.91–5.70	6.34±1.16 5.15–7.95	9.96	5.60±0.49 (5) 5.04–6.11
MAW	0.280	−0.017	7.01±0.42 6.71–7.84	6.81±0.13 6.65–6.98	8.95 9.42	7.66±0.18 7.42–7.84	7.33±0.24 7.10–7.86	6.72±0.23 6.31–6.91	7.26±0.32 6.81–7.59	9.21	6.66±0.12 (5) 6.52–6.79
C ¹ M ³ L	0.288	0.225	4.56±0.17 4.42–4.89	4.49±0.23 4.11–4.73	7.43 7.26	5.35±0.05 5.30–5.41	5.55±0.14 5.40–5.84	4.80±0.12 4.61–4.94	5.19±0.27 4.75–5.42	7.34	4.59±0.15 (5) 4.45–4.77
C ¹ C ¹ W	0.285	−0.133	3.18±0.13 3.04–3.33	3.15±0.15 3.00–3.38	5.21 5.42	4.05±0.19 3.81–4.25	3.50±0.21 3.25–3.97	3.23±0.15 3.00–3.39	3.52±0.16 3.28–3.71	5.17	3.18±0.03 (5) 3.14–3.21
M ³ M ³ W	0.285	0.057	5.04±0.23 4.75–5.40	5.08±0.12 4.98–5.24	8.05 7.89	6.15±0.22 5.86–6.34	5.92±0.57 4.87–6.66	5.36±0.18 5.14–5.56	5.85±0.19 5.60–6.02	8.13	5.05±0.06 (5) 4.99–5.12
C ₁ M ₃ L	0.288	0.069	4.99±0.17 4.78–5.19	4.97±0.17 4.76–5.21	8.05 7.7	5.76±0.31 5.34–6.08	5.88±0.36 5.48–6.48	5.12±0.15 4.93–5.29	5.55±0.22 5.32–5.89	8.06	5.12±0.17 (5) 4.93–5.34
ML	0.295	0.059	9.17±0.17 8.87–9.38	9.31±0.16 9.19–9.54	14.55 14.46	10.93±0.18 10.66–11.06	10.67±0.35 10.28–11.20	9.60±0.25 9.35–9.95	10.37±0.18 10.11–10.52	14.38	9.09±0.18 (5) 8.94–9.34
MH	0.277	−0.170	2.41±0.14 2.22–2.56	2.64±0.40 2.17–3.26	5.25 4.87	3.22±0.26 2.88–3.48	2.76±0.26 2.48–3.18	2.59±0.15 2.39–2.77	3.09±0.18 2.89–3.35	5.01	2.68±0.20 (5) 2.38–2.90

Values are given as mean±standard deviation, range (min–max), and sample size (*n*). Factor loadings for PC1 and PC2 are provided for each trait based on separate PCAs of external (5 traits) and craniodental (13 traits) datasets. *M. alticraniatus* was collected from non-Xizang regions and is primarily used for comparison with *M. siligorensis*, whereas *M. annatessae* also from non-Xizang areas is presented for future comparisons with *M. nipalensis*.

using an ultrasound detector (M500-384, Pettersson Elektronik AB, Sweden). Recordings were conducted in a quiet room (approximately 3 m×3 m×3 m). Acoustic data were analyzed using BatSound v.4.0 (sampling frequency: 300 kHz, Fast Fourier Transform (FFT) size: 1024 points). For each species, more than 10 high-quality pulses were selected to measure three key parameters: peak frequency, pulse duration, and inter-pulse interval. Given that the *Myotis* calls typically consist of broadband frequency-modulated (FM) signals with multiple harmonics, where the first harmonic is the strongest and other harmonics (2nd–4th) decrease successively, only the first harmonic was analyzed in this study.

RESULTS

Morphological descriptions

Through morphological comparison with existing voucher specimens, consultation of original descriptions, and cross-referencing with historical distribution records and known species checklists (Table 1), we assessed the taxonomic placement of *Myotis* specimens collected from Xizang. These taxa were classified as *Myotis himalaicus* Saikia et al., 2025; *Myotis laniger* (Peters, 1871); *Myotis longipes* (Dobson, 1873); *Myotis muricola* (Gray, 1846); *Myotis sicarius* Thomas, 1915; and *Myotis siligorensis* (Horsfield, 1855). Among these, *M. himalaicus* and *M. sicarius* represent the first confirmed

records for China. Meanwhile, *M. longipes* and *M. siligorensis*, although previously listed in Chinese checklists, were based on misidentifications outside Xizang. Our study confirms their genuine distribution in China through specimens collected from Xizang, thereby re-establishing their valid distributional status. To facilitate future taxonomic research and conservation practice, we provide detailed morphological descriptions based on voucher specimens, accompanied by illustrations to aid accurate identification.

Myotis himalaicus is a medium-sized *Myotis* (Figures 2–3), with a forearm length of 40.42–42.40 mm (Table 2). The dorsal fur is dark brown with slightly golden-brown tips; ventral hairs have dark bases and lighter tips (Figure 2). The ear is relatively short, with a deep notch at mid-height of the outer margin. The tragus is slender and extends to the ear notch. The wing membrane attaches to the base of the toe. The hind foot (incl. claws) length is less than half the tibia length (Supplementary Table S2). The skull is globose in profile (Figure 3), with a greatest length of skull (GTL) of 13.98–14.35 mm (Table 2). The rostrum is short, and the frontal part of the braincase rises abruptly. The sagittal crest is very weak, while the lambdoid crest is moderate. Dentition is relatively weak (Figure 3); the second upper premolar (P^3) is small and displaced lingually. The height of the upper and lower canines (C^1/C_1) exceeds that of the corresponding third premolars (P^4/P_4).

Myotis longipes is a small-sized species (Figures 2–3), with an FA of 34.65–35.74 mm (Table 2). The ventral fur is nearly black at the base with grayish-white tips, while the dorsal fur is

uniformly grayish-brown. The conch is narrow and elongated, broadest medially and tapering rapidly toward the top, and the tragus is short, less than half of ear length. The wing membrane attaches at the midpoint of the metatarsus. The hind foot is small, significantly less than half the tibia length (Supplementary Table S2). The skull is domed in profile, with GTL of 12.03–13.62 mm (Table 2). The rostrum is short, rising abruptly and inflating sharply toward the braincase. The braincase height and width differ by less than 1 mm (Supplementary Table S2). C^1 is small, not exceeding P^4 in crown height. P^3 is relatively small, its height is approximately one-fourth that of C^1 . C_1 is distinctly larger than P_2 . P_4 is much less than half the size of P_2 (Figure 3).

Myotis sicarius is noticeably larger than other *Myotis* species recorded in Xizang (Figures 2–3), with an FA of 53.55 mm (Table 2). Its most prominent character is a distinct golden-yellow patch on the mid-venter, and the dorsal fur is uniformly dark brown to brown (Figure 2). The ear is relatively long, with the outer margin distinctly flared near the basal one-third. The hind foot is very small, significantly less than half the tibia length (Supplementary Table S2). The wing membrane attaches at the base of the toes. The skull is robust overall, with a relatively long rostrum (Figure 3). The braincase is relatively flat, rising gently from the interorbital region. The zygomatic arches are robust, and the sagittal and lambdoid crests are prominent. C^1 is strong, noticeably larger than P^4 (Figure 3). P^2 is relatively weak, its height less than half of C^1 . P^3 is displaced inwards, not visible in lateral view. C_1 is about equal in size to P_2 , while P_3 is displaced lingually and is



Figure 2 External morphology of 6 *Myotis* species collected from Xizang region in this study

For each species, the panel shows external morphology: lateral head view, dorsal fur, ventral fur, and the location of the wing membrane attachment. Photos by Wen-Hua Yu and Yi Wu.

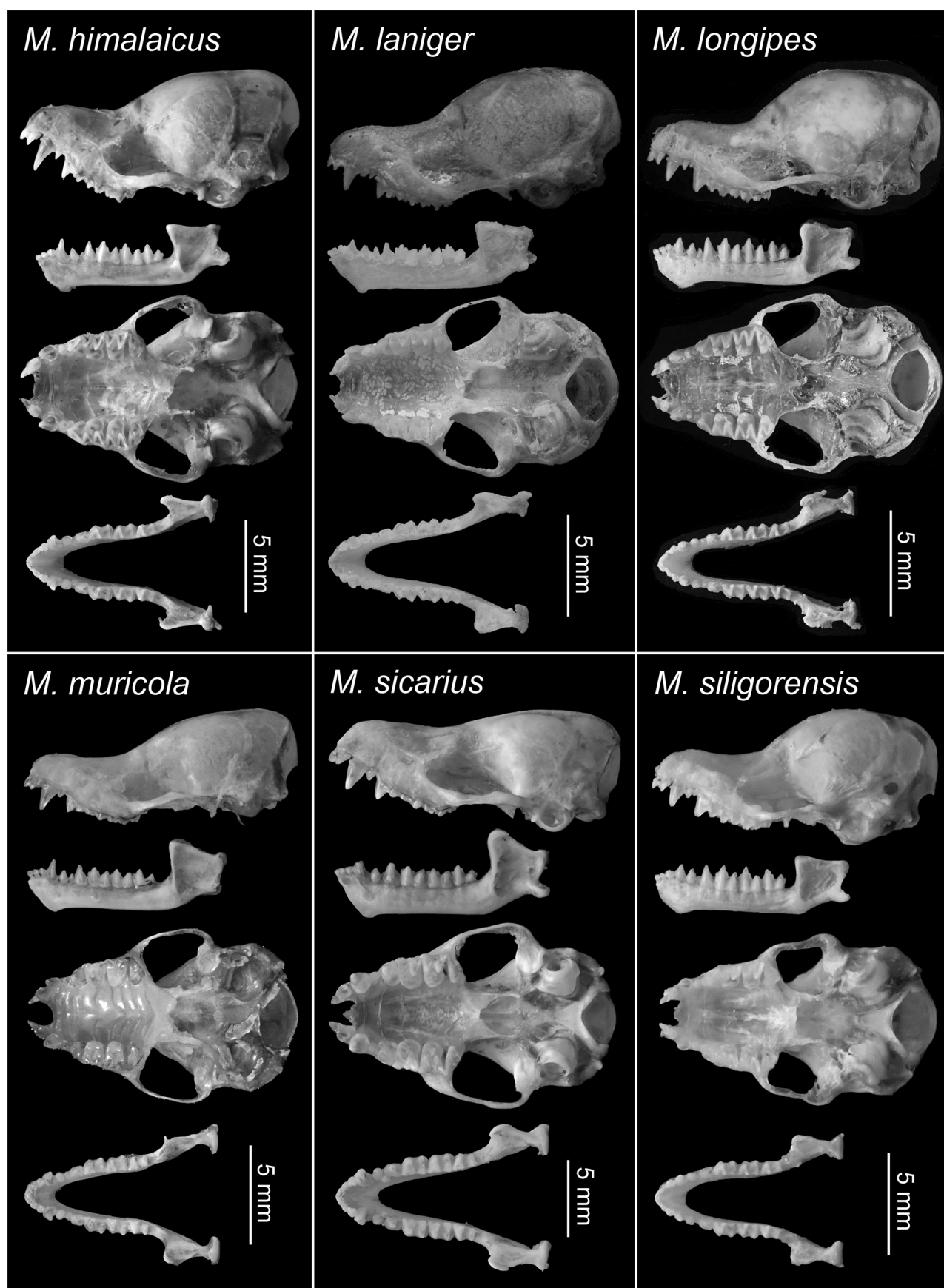


Figure 3 Craniodental characteristics of 6 *Myotis* species collected from the Xizang region

For each species, the panel shows craniodental characters: ventral and lateral views of the maxilla, and lateral and frontal views of the mandible. Scale bar=5 mm. Photos by Kun-Hao Chen.

scarcely visible in lateral view.

Myotis siligorensis is a small-sized species (FA 33–37 mm; Table 2). Its dorsal fur is dark brownish-black, while the ventral fur varies from dark brown at the base to pale yellow at the tip (Figure 2). The conch is relatively long, extending slightly beyond the tip of the muzzle when folded forward. The height of tragus is less than half the ear length. The wing membrane attaches at the midpoint of the metatarsus. The hind-foot length is about half the tibia length (Supplementary Table S2). The braincase is inflated and rounded (Figure 3), rising abruptly from the rostrum, and its height is nearly equal to its width (ratio>0.9). The zygomatic arches are well-developed and robust. The sagittal crest is absent, while the lambdoid crest is weak and only faintly visible. C¹ is weak, nearly equal in size to P⁴. P³ is vestigial and almost invisible in lateral view. C₁ is connected to P₂ and is shorter than P₄. P₃ is small and displaced lingually (Figure 3).

Bivariate and multivariate morphometric patterns

Bivariate analyses of raw morphometric data showed that large-bodied species were clearly separated from medium- and small-bodied species, whereas small-bodied species overlapped extensively with each other (Figure 4). In the external measurement plot (FA vs. HF; Figure 4A), the large-sized *Myotis formosus* (Hodgson, 1835) and *M. sicarius* formed a distinct cluster in the upper-right region, while the medium-sized *M. himalaicus* occupied an intermediate position. The remaining six small-sized species — *Myotis alticraniatus* Osgood, 1932, *M. laniger*, *M. longipes*, *M. muricola*, *Myotis annatessae* Kruskop & Borisenko, 2013, and

M. siligorensis—showed significant overlap. In the craniodental plot (GTL vs. C¹M³L; Figure 4B), *M. sicarius* and *M. formosus* again stood out as the largest species, while the remaining species were mainly arranged along the GTL-axis in the lower-left corner of the morphospace. Notably, *M. alticraniatus* and *M. siligorensis* were separated from each other along the HF axis in the external measurement plot, but overlapped markedly in the craniodental plot.

Principal component analyses (PCAs) were performed separately on the external and craniodental datasets (Figure 4C, D). For the external measurements, the first two principal components explained 63.2% and 21.2% of the total variance, respectively (Figure 4C). PC1 was strongly positively correlated with overall body size indicators (e.g., FA, TIB, TL; Table 2) and was therefore interpreted as a general size axis. PC2 was mainly negatively correlated with EL and HF (Table 2), reflecting morphological variation independent of body size. Along the PC1 axis, the large-sized *M. formosus* and *M. sicarius*, together with the medium-sized *M. himalaicus*, were clearly separated from a cluster formed by the six smaller species (*M. alticraniatus*, *M. longipes*, *M. laniger*, *M. muricola*, *M. annatessae*, and *M. siligorensis*). These six small-sized taxa overlapped extensively on PC1, while on PC2, *M. laniger* and *M. annatessae* were positioned above and below a central cluster containing the remaining four species.

For the craniodental measurements, the first two principal components explained 86.3% and 5.0% of the variance, respectively (Figure 4D). PC1 was strongly correlated with

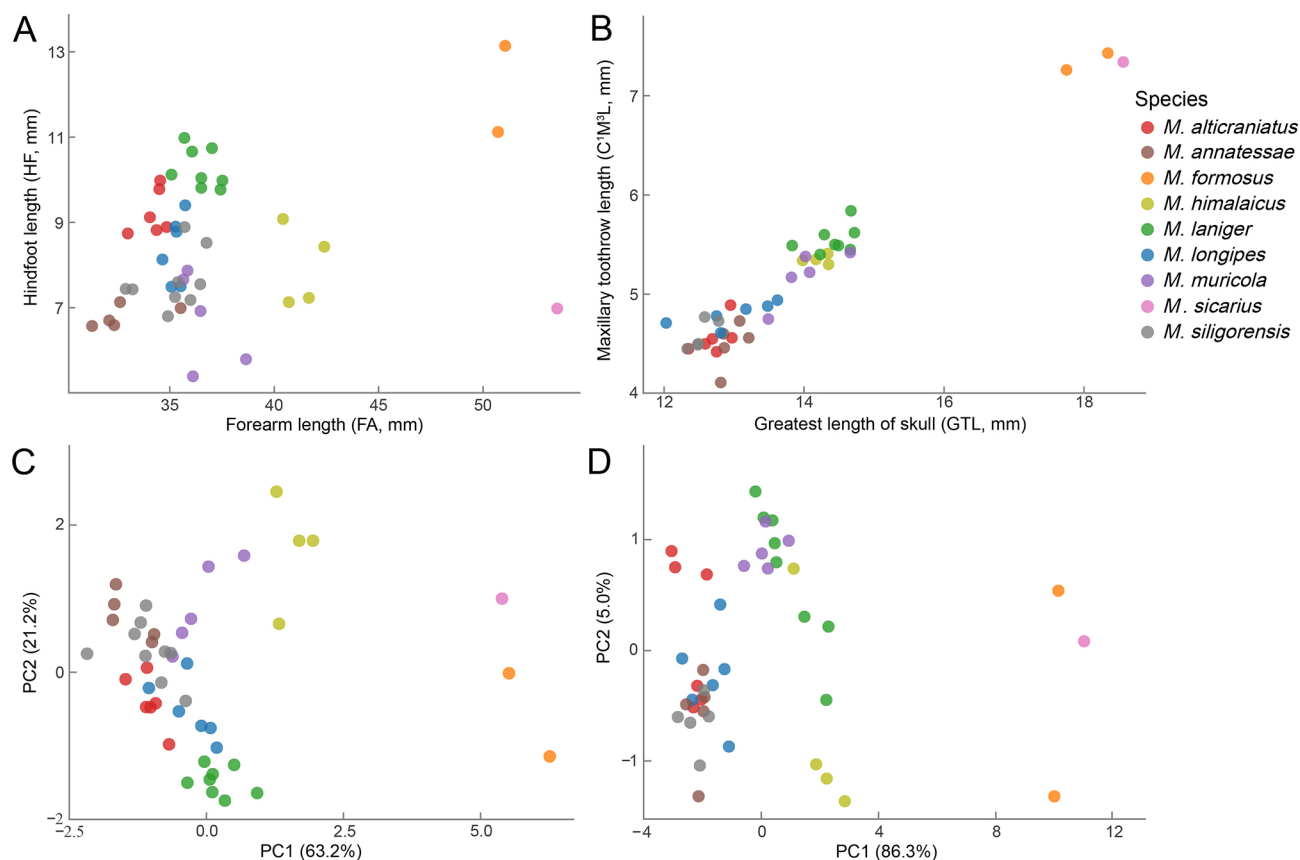


Figure 4 Morphometric analyses of 9 *Myotis* species

A: Bivariate plot of forearm length (FA) vs. hindfoot length (HF); B: Bivariate plot of greatest skull length (GTL) vs. maxillary tooththrow length (C¹M³L); C: Principal component analysis (PCA) based on 5 external measurements; D: PCA based on 13 craniodental metric dataset. See Table 2 for factor loadings of traits.

most cranial dimensions (e.g., GTL, CCL, ML; Table 2). PC2 was strongly negatively correlated with BCH and positively correlated with PBL and all toothrow length indices (C^1M^3L and C_1M_3L ; Table 2), indicating that it coincides with morphological variation. In this PCA, *M. laniger* and *M. muricola* overlapped in morphospace and were not separated along the PC2 axis, whereas *M. alticraniatus*, *M. longipes*, *M. annatessae*, and *M. siligorensis* clustered tightly on the left side of the morphospace. Overall, small-sized species were further differentiated along the PC2 axis, a differentiation primarily associated with craniodental traits such as BCH, PBL, and C^1M^3L and C_1M_3L , revealing craniodental distinctions that were not evident from external traits alone.

Molecular phylogeny and species delimitation

Phylogenetic reconstruction using the *cyt b* gene yielded robust support (PP=1, BS=100%) for nearly all species-level nodes (Figure 5). At the top of the phylogenetic tree, the *M. alticraniatus* clade comprised a mixed clade including *Myotis badius* Tiunov, Kruskop & Feng, 2011, *Myotis kalkoae* Tiunov et al., 2025, *Myotis sowerbyi* Howell, 1926, and the misidentified “*M. siligorensis*” (FJ215679), with its sister group being the *M. laniger* clade containing two specimens from Xizang (GZHU 24145/46). ASAP and PTP species delimitation analyses indicated that these two species clades could be divided into multiple inconsistent groups, suggesting the possible presence of cryptic diversity within *M. alticraniatus* and *M. laniger*, while also reflecting the limitations of single-gene markers in resolving recently diverged lineages (Figure 5). In the sister clade to the above-mentioned species, the Xizang specimens clustered with topotypic *M. longipes* (MW054878/79) and true *M. siligorensis* (MW054882/83) sequences from India, respectively. Notably, paratype sequences of *Myotis csorbai* Topál, 1997 (MW054873/74) were robustly nested within this *M. longipes* cluster (PP=1, BS=100%; Figure 5). Moreover, both ASAP and PTP methods consistently supported *M. longipes* and *M. siligorensis* as distinct species.

Meanwhile, samples identified as *M. himalaicus* from Xizang and the topotypic *Myotis soror* Ruedi et al., 2015 sequence (KP187901) from Taiwan formed a sister group (Figure 5). Specimen from Xizang (GZHU 24198) also formed a highly supported clade with sequences of *M. sicarius* from Nepal and Vietnam (PP=1, BS=100%; Figure 5). These phylogenetic results confirm the first records of *M. himalaicus* and *M. sicarius* in China. In addition, consistent with results from Kruskop et al. (2012), our phylogeny recovered a sister relationship between *M. sibiricus* and *M. brandtii*. Moreover, specimens identified as “*M. nipalensis*” from other regions of China (outside Xizang) were all found to cluster with *M. annatessae* (PP=1, BS=96%), rather than with true *Myotis nipalensis* Dobson, 1871 sequences from India (MW054881/94), indicating that previous Chinese records of “*M. nipalensis*” likely represent misidentifications of *M. annatessae*.

In light of recent taxonomic revisions (Liu et al., 2023; Ruedi et al., 2021), this study further re-examined all *cyt b* sequences in public databases (NCBI-nt; see Supplementary Table S4). This review revealed extensive mislabeling, particularly for Asian *Myotis* sequences. Specifically, most sequences labeled as “*M. davidii*” from China and adjacent regions actually represent *M. alticraniatus* or *M. laniger* (Supplementary Figure S1; Supplementary Table S4).

Concurrently, all sequences labeled “*M. siligorensis*” (FJ215679; Zhang et al., 2009) and “*M. s. alticraniatus*” from outside India, including those identified as *M. badius* from Yunnan (MF630872, MW054890/91), correspond to *M. alticraniatus*. Notably, all previous records of “*M. longipes*” outside of India and Nepal were actually *M. laniger*, including those from China (Huang et al., 2018; Zhang et al., 2009). A revised compilation of these sequences is provided (Supplementary Table S4) to aid future verification and research.

Pairwise genetic distances

K2P genetic distance analysis quantified the genetic differentiation both among and within the 12 examined *Myotis* species (Tables 3; Supplementary Table S4). Interspecific distances ranged from 9.1% to 20.5% (Table 3). Specifically, the genetic distance between *M. alticraniatus* and true *M. siligorensis* was 13.7%, as was that between *M. laniger* and *M. longipes*. Meanwhile, our analysis supports the conclusion of Kruskop et al. (2012), with the genetic distance between *M. brandtii* and *M. sibiricus* being approximately 11.5% (Table 3). Furthermore, specimens previously collected from other regions of China (outside Xizang) and misidentified as “*M. nipalensis*” were found to be more closely related to *M. annatessae*, while showing a distance of 7.9% from true *M. nipalensis* (Table 3), indicating that previous Chinese records of “*M. nipalensis*” likely represent misidentifications of *M. annatessae* (Baker & Bradley, 2006).

At the intraspecific level, most sequences within the *M. alticraniatus* complex showed pairwise distances below 2% (Supplementary Table S3), supporting their conspecific status (Baker & Bradley, 2006). However, sequences MF630872 and FJ215679 exhibited divergences of 4.5–8.9% from others in the complex; combined with the results of phylogenetic and species delimitation analyses, their internal relationships require further verification. Within the *M. longipes* group (including *M. csorbai*), all pairwise genetic distances were below 2% (Supplementary Table S3). For *M. siligorensis*, the distance between Xizang and Indian specimens ranged from 3.3% to 3.8%. In addition, we found that the distance between *M. sicarius* from Vietnam and Nepal was greater than 8% (consistent with Györrösy et al., 2024), whereas the *M. sicarius* specimen collected from Xizang was more closely related to Nepalese individuals, with a divergence of less than 2% (Supplementary Table S3).

Echolocation call characteristics

Analysis of ultrasonic characteristics recorded in flight across 8 *Myotis* species revealed pronounced interspecific divergence (Figure 6). Based on peak frequency, these species can be broadly categorized into two distinct acoustic groups (Table 4). The high-frequency group comprised *M. siligorensis* (88.49 ± 19.59 kHz), *M. alticraniatus* (73.01 ± 1.38 kHz), *M. annatessae* (72.53 ± 3.20 kHz), *M. longipes* (70.57 ± 4.81 kHz), and *M. muricola* (69.37 ± 3.70 kHz). A low-frequency group included *M. himalaicus* (64.54 ± 3.99 kHz), *M. laniger* (47.13 ± 1.64 kHz) and *M. sicarius* (43.33 ± 1.15 kHz), with *M. sicarius* exhibiting the lowest peak frequency (Figure 6; Table 4).

DISCUSSION

The taxonomy of *Myotis* in Xizang has long been hindered by inadequate sampling and an overreliance on labile

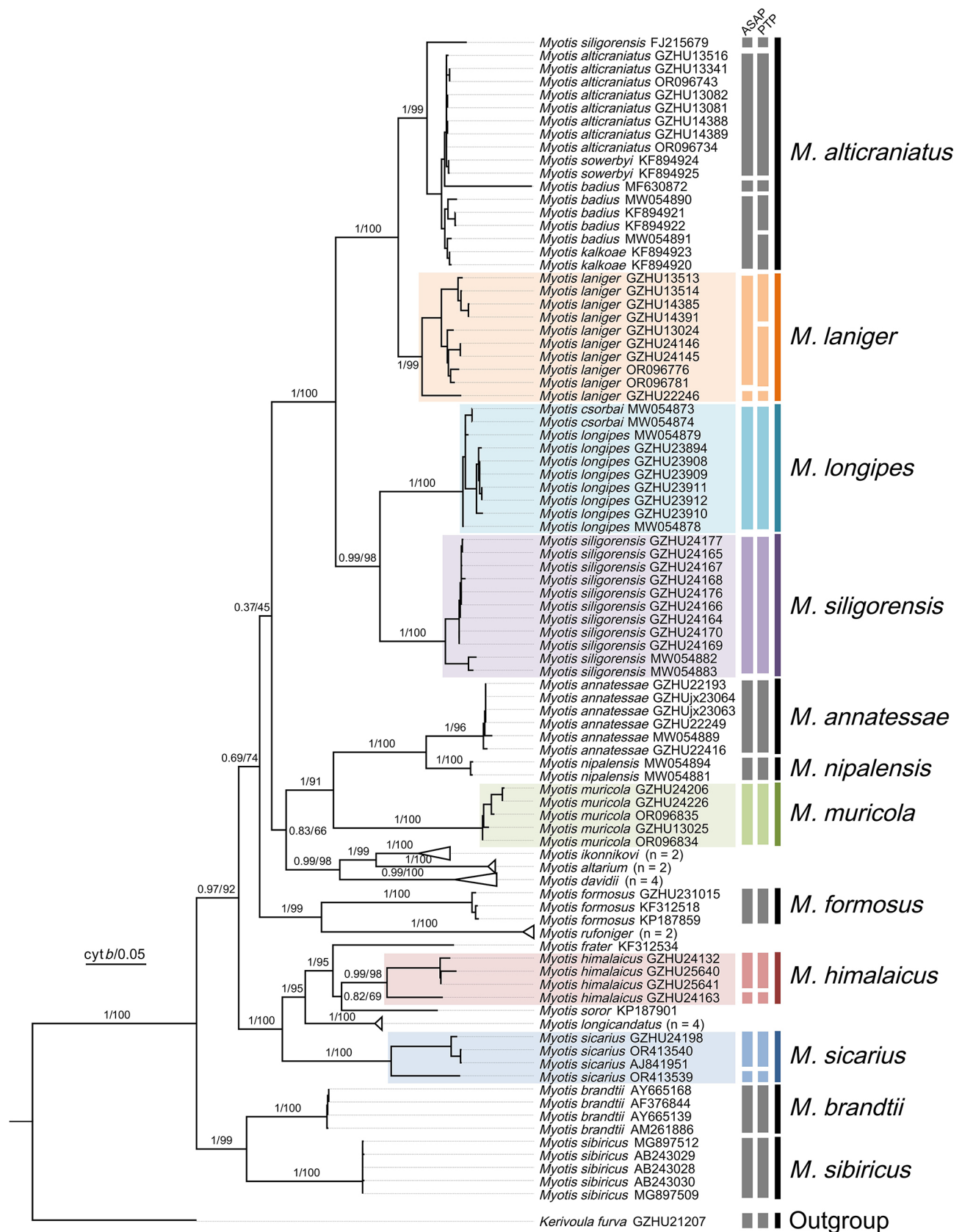


Figure 5 Bayesian inference and maximum likelihood phylogenetic tree of *Myotis* based on the mitochondrial cytochrome b (cyt b) gene sequence

The values on the branches represent the posterior probability (PP) and bootstrap support (BS), respectively. The image only shows the branching patterns of the focal species. The 6 colored blocks represent the species collected in Xizang for this study (see Supplementary Table S1), while the bars on the right indicate the results of different species delimitation methods.

Table 3 K2P genetic distances for 12 *Myotis* species, including those distributed in Xizang and corresponding comparative species

Species	1	2	3	4	5	6	7	8	9	10	11
1 <i>M. alticraniatus</i>											
2 <i>M. annatessae</i>	0.173										
3 <i>M. brandtii</i>	0.167	0.190									
4 <i>M. formosus</i>	0.190	0.195	0.184								
5 <i>M. himalaicus</i>	0.191	0.209	0.184	0.204							
6 <i>M. laniger</i>	0.083	0.178	0.169	0.185	0.187						
7 <i>M. longipes</i>	0.136	0.184	0.155	0.190	0.181	0.137					
8 <i>M. muricola</i>	0.196	0.168	0.205	0.195	0.205	0.184	0.190				
9 <i>M. nipalensis</i>	0.162	0.079	0.194	0.191	0.194	0.163	0.174	0.170			
10 <i>M. sibiricus</i>	0.185	0.178	0.115	0.189	0.195	0.179	0.155	0.182	0.178		
11 <i>M. sicarius</i>	0.189	0.194	0.174	0.205	0.170	0.198	0.175	0.204	0.187	0.179	
12 <i>M. siligorensis</i>	0.137	0.167	0.180	0.196	0.190	0.137	0.114	0.168	0.173	0.168	0.191

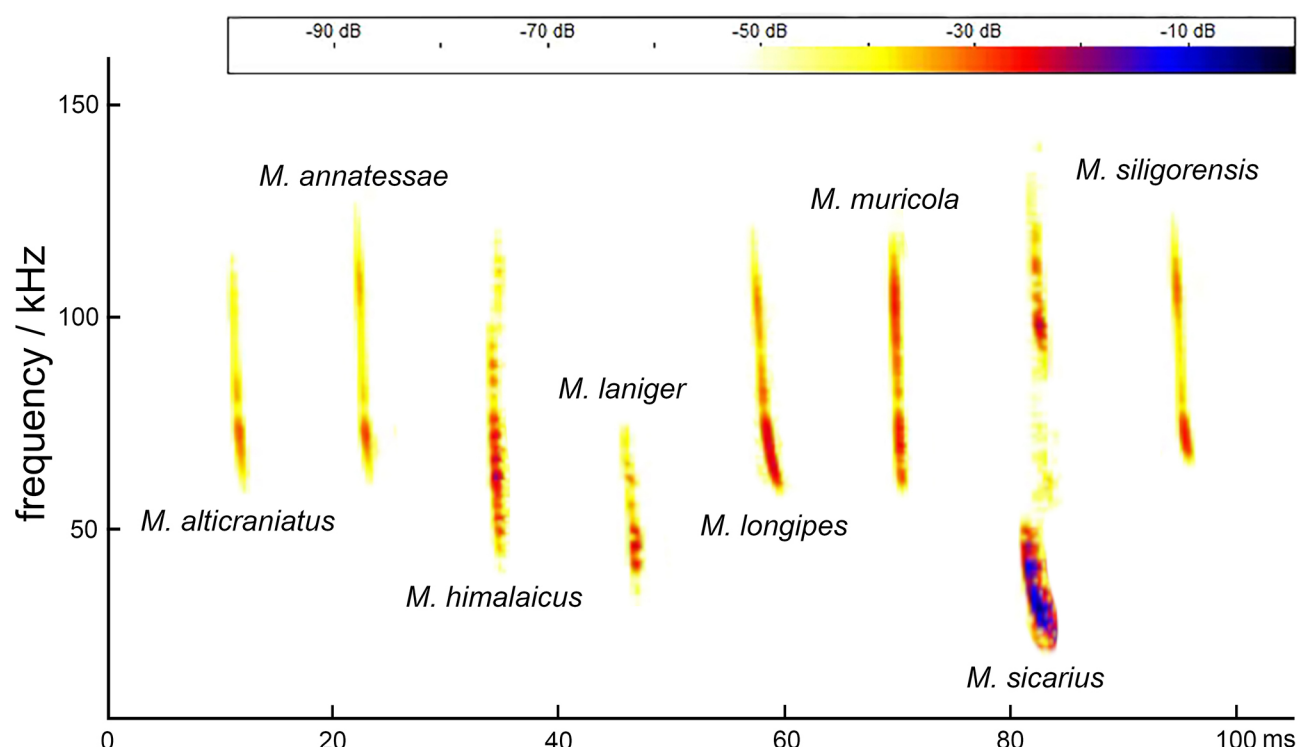


Figure 6 Spectrogram of echolocation calls of 8 *Myotis* species in flight state

Table 4 Echolocation call data from 8 *Myotis* species

Species	Duration/ms		Peak frequency/kHz		Interval/ms	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
<i>M. alticraniatus</i>	3.02±0.37	2.60–3.60	73.01±1.38	71.00–75.40	41.11±19.48	17.80–72.40
<i>M. annatessae</i>	3.48±0.66	2.30–4.40	72.53±3.20	65.70–77.00	30.50±9.66	19.10–44.60
<i>M. himalaicus</i>	3.26±0.30	2.80–3.60	64.54±3.99	58.60–71.30	24.90±11.60	17.00–56.20
<i>M. laniger</i>	2.72±0.28	2.10–3.00	47.13±1.64	42.70–48.60	37.36±19.67	18.80–69.40
<i>M. longipes</i>	4.20±0.71	3.20–5.70	70.57±4.81	65.70–84.30	40.57±23.52	23.00–105.50
<i>M. muricola</i>	3.23±0.27	2.70–3.60	69.37±3.70	60.80–73.30	34.04±23.27	12.60–92.00
<i>M. sicarius</i>	3.74±0.55	3.00–5.00	43.33±1.15	42.10–46.20	168.30±32.35	142.00–257.00
<i>M. siligorensis</i>	4.03±0.59	3.30–4.70	88.49±19.59	67.00–112.60	51.88±16.66	22.80–73.40

morphological traits, underscoring the broader challenges in species delimitation across the Qinghai-Xizang Plateau (Table 1). The situation is further complicated by the fact that many historical records from China were based on misidentified specimens, particularly for species whose true distributions lie in the Himalayan region. Through newly collected specimens from border areas and a combination of

morphometric, phylogenetic, and acoustic analyses, we provide an evidence-based reassessment that corrects several long-standing misidentifications, clarifies species distributional limits, and establishes a solid foundation for future biogeographic inference and conservation planning.

The long-standing conflation of *M. alticraniatus* and *M. siligorensis* exemplifies how uncritical reliance on superficial

morphological similarity has obscured taxonomic resolution within *Myotis*. Since its description from Vietnam (Osgood, 1932), *M. alticraniatus* was subsequently treated as a subspecies of *M. siligorensis* due to apparent similarities in cranial morphology (Corbet & Hill, 1992). However, accumulating phylogenetic evidence has consistently demonstrated that these similarities reflect convergent adaptations rather than common ancestry (Morales et al., 2019; Ruedi et al., 2021; Stadelmann et al., 2007). By integrating morphological and phylogenetic evidences, Ruedi et al. (2021) reinstated *M. alticraniatus* to species rank and restricted *M. siligorensis* sensu stricto to the central and eastern Himalayas (Bates et al., 2005), corresponding closely to our sampling area in Xizang (Zayü County, Nyingchi). Their revision indicated that populations historically identified as “*M. siligorensis*” in China and Indochina represent *M. alticraniatus*. Nevertheless, due to scarce voucher specimens and limited critical reappraisal, Chinese taxonomic accounts did not adopt these changes (e.g., Wei et al., 2022, 2025), leading to numerous persistent misidentifications (e.g., Chen et al., 2025; Ding et al., 2025; Xiao et al., 2017).

Our integrated analysis further confirms the distinctness of *M. alticraniatus* and *M. siligorensis*. They are consistently separable by pelage coloration, and craniodental traits (Figures 2–3). Phylogenetically, they form reciprocally monophyletic clades with genetic distances far exceeding conventional species thresholds (Baker & Bradley, 2006), and their echolocation peak frequencies are also clearly divergent (Table 4). Re-examination of all Chinese sequences formerly labeled as “*M. siligorensis*” reveals that they actually fall within the *M. alticraniatus* clade (Supplementary Figure S1). However, this clade is phylogenetically complex, containing not only sequences misidentified as “*M. siligorensis*” but also those of *M. badius*, *M. kalkoae*, and *M. sowerbyi* (Figure 5). Species delimitation analyses (ASAP and PTP) further split this assemblage into multiple, partially inconsistent putative species, suggesting unresolved cryptic diversity. Consequently, although we have verified the conjecture of Chen et al. (2026) and confirmed that the true *M. siligorensis* in China is restricted to Xizang, the taxonomic boundaries within the *M. alticraniatus* complex remain unclear and require further clarification.

Chinese records of *M. longipes* have similarly been obscured by taxonomic confusion, stemming from incomplete original descriptions (Peters, 1871) and subsequent misinterpretations based on misidentified specimens (Allen, 1938; Topál, 1997). Although molecular tools promised clarity, early misidentified sequences (Ruedi et al., 2013; Zhang et al., 2009) propagated phylogenetic error. Recent reappraisals diverge sharply: Liu et al. (2023) re-assigned historical Chinese *M. longipes* records to *M. laniger*, whereas Wei et al. (2025) tentatively classified them as *M. csorbai* following Peacock (2019). These conflicting interpretations reflect both morphological complexity and insufficient consultation of type material.

Our integrative analysis provides evidence to resolve this controversy. 6 specimens from Nyalam, Xizang—adjacent to Nepal and India—align morphologically with true *M. longipes* (Figures 2–3) and form a highly supported monophyletic group with sequences (MW054878/79) from India (PP=1, BS=100%; Figure 5). This represents the first confirmed record of *M. longipes* in China. Re-evaluation of all previous Chinese records supports the conclusion of Liu et al. (2023) that these

populations belong to *M. laniger* (Supplementary Figure S1; Supplementary Table S4), thereby clarifying species boundaries. Notably, *M. csorbai* has been synonymized with *M. longipes* (Simmons & Cirranello, 2025), and its paratype sequences are nested within the *M. longipes* clade (Figure 5). Accordingly, we propose the following taxonomic recommendations: previous Chinese records of “*M. longipes*” from outside Xizang should be revised to *M. laniger*; true *M. longipes* in China is confined to Xizang; and *M. csorbai* should no longer be recognized as a separate species in the Chinese checklist.

Historically, “*M. brandtii*” was reported from Xizang and northern China (Feng et al., 1986; Wei et al., 2022). Kruskop et al. (2012) re-examined specimens from the Russian Altai and proposed that Chinese records should be assigned to a distinct species, *M. sibiricus*. Our phylogenetic tree (Figure 5) recovers a well-supported sister relationship between *M. sibiricus* and *M. brandtii*, with a K2P distance of approximately 11.5% (Table 3), strongly supporting their status as separate species and corroborating the conclusion of Kruskop et al. (2012). Given that our sampling did not include Xizang specimens of either species, we tentatively revise the Chinese records of “*M. brandtii*” to *M. sibiricus* following Kruskop et al. (2012). Future surveys in northern and western Xizang are needed to further clarify the distributions of these two closely related taxa.

Furthermore, our phylogenetic tree reveals that all specimens previously identified as “*M. nipalensis*” from outside Xizang (e.g., Fujian, Hunan, Jiangxi) do not cluster with the true *M. nipalensis* specimens from Nepal (MW054881/94). Instead, they form a highly supported clade with *M. annatessae* (MW054889; PP=1, BS=100%; Figure 5), with a K2P genetic distance of less than 2% between them (Supplementary Table S3). Therefore, the majority of historical Chinese records of “*M. nipalensis*” (Jiang et al., 2015; Liu et al., 2022; Wei et al., 2022) are likely misidentifications of *M. annatessae*, while true *M. nipalensis* appears to be restricted to the southern Himalayan slopes (India, Nepal). Although our study did not collect *M. nipalensis* from Xizang, considering that the border areas of Xizang are adjacent to Nepal, we suggest retaining the record of *M. nipalensis* for Xizang, while revising the corresponding records from the rest of China to *M. annatessae*.

Based on the integrative evidences presented above, we herein revise the *Myotis* fauna of Xizang to comprise 9 validated species. Among these, *M. himalaicus* and *M. sicarius* are newly recorded species for China. Previous Chinese checklists (e.g., Smith & Xie, 2009; Wei et al., 2022) listed *M. longipes* and *M. siligorensis*, but these records were based on misidentifications; we therefore confirm their authentic occurrence in China for the first time. Additionally, the presence of *M. laniger* and *M. muricola* in Xizang is corroborated by the present study. Furthermore, through a critical re-evaluation of historical literature (Table 1), we preliminarily infer the occurrence of three other species in Xizang, namely *M. formosus*, *M. nipalensis*, and *M. sibiricus*. It should be noted that the latter two have undergone taxonomic revisions: following the conclusion of Kruskop et al. (2012), previous Chinese records of “*M. brandtii*” are assigned to *M. sibiricus*; meanwhile, historical records of “*M. nipalensis*” from outside Xizang are re-identified as *M. annatessae*, while true *M. nipalensis* is tentatively retained for Xizang due to its adjacency to Nepal. In summary, this framework establishes

an updated baseline for assessing *Myotis* diversity across the Qinghai-Xizang Plateau. To facilitate future research and identification, a diagnostic key to the recognized species is provided.

Several limitations merit consideration. Geographic coverage remains incomplete, specimen representation for certain taxa is limited, and some phylogenetic relationships remain unresolved amid ongoing taxonomic revisions. Addressing these gaps will require targeted field surveys in currently unsampled areas of Xizang and adjacent regions to obtain additional vouchered specimens, particularly along international borders and ecotonal zones. Methodologically, moving beyond single-locus mitochondrial data to incorporate multi-nuclear markers and genomic-scale datasets (Leaché et al., 2014) is essential to resolve early-diverging nodes (Gaughran et al., 2025; Yao et al., 2024). While techniques such as environmental DNA (eDNA) (Deiner et al., 2017; Ruppert et al., 2019) and acoustic monitoring (Bergmann et al., 2022; Thomas & Davison, 2022) are increasingly used in biodiversity assessment, their effective application relies heavily on a robust taxonomic foundation for calibration. Consequently, sustained investment in integrative, voucher-based taxonomy remains essential for advancing *Myotis* systematics and for underpinning effective conservation strategies across the Himalayan region.

Identification key of *Myotis* species in Xizang

- 1 Pelage brightly coloured, golden-yellow *M. formosus*
– Pelage dull-coloured, dull, dark brown or grayish-white 2
- 2 Large size, forearm length more than 45 mm *M. sicarius*
– Smaller size, forearm length less than 45 mm 3
- 3 Relatively large size, forearm 40 mm or more
..... *M. himalaicus*
– Small size, forearm length less than 40 mm 4
- 4 Hind foot length distinctly less than half of tibia length 5
– Hind foot length approximately 1/2 to 2/3 of tibia length 8
- 5 Canines weak, smaller than third premolar; margin of conch without notch *M. siligorensis*
– Canines robust, distinctly larger than third premolar; concha with distinct notch 6
- 6 Second upper premolar displaced inwards (lingually), not visible laterally *M. muricola*
– Second upper premolar situated within toothrow, clearly visible in lateral view 7
- 7 Ventral fur pale white; dorsal fur brown, tips with metallic sheen *M. sibiricus*
– Ventral fur brown; dorsal fur darker, lacking metallic sheen *M. nipalensis*
- 8 Ear narrow and elongated, extending more than 5 mm beyond tip of nose *M. longipes*
– Ear short, not reaching or just reaching tip of nose
..... *M. laniger*

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

All fieldwork and sampling complied with relevant laws and regulations and followed the guidelines for the use of wild mammals (Sikes & The Animal Care and Use Committee of the American Society of Mammalogists, 2016). The research was approved by the People's Government of Xizang Autonomous Region (permit number: ZL202203601).

DATA AVAILABILITY

All newly generated DNA sequences have been deposited in the GenBank under accession numbers PZ316293–PZ316310. Additionally, all datasets generated in this study are available on the Science Data Bank

(<http://www.scidb.cn/>) under DOI: 10.57760/sciencedb.38857.

SUPPLEMENTARY DATA

The article's supplementary data are available online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

W.H.Y., Y.W., X.Y.W., and K.H.C. conceived the research; staff from the bat lab of W.H.Y. and Y.W. were responsible for field surveys and sample collection; L.B.Z. contributed some valuable specimen materials; K.H.C., Y.G., Y.X.M., and L.Y.X. performed the data analyses; K.H.C. wrote the original draft of the manuscript; W.H.Y., X.Y.W., K.H.C., Y.Y., Y.W., and L.B.Z. reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We thank our lab members Hao-Hui Su, Xia-Qin Zhan, Xin-Dan Fan, and Zhuo-Yu Li for their assistance in fieldwork, and Yu-Nuo Ding for help with data analysis. We also thank Ting-Lei Jiang of the Jilin Provincial Key Laboratory of Animal Resource Conservation and Utilization, Northeast Normal University, for providing specimen data and identification guidance. We are deeply grateful for the solid, constructive and detailed insights provided by the four anonymous reviewers.

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