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New Insights into the Taxonomy of *Myotis* Bats in China Based on Morphology and Multilocus Phylogeny

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Abstract: The genus *Myotis* is one of the most diverse and widely distributed mammals, providing a good model for studies of speciation and diversification across large geographic scales. However, the classification within this genus has long been chaotic. Taxonomic revisions based on multiple data sources are essential and urgent. In this study, morphometrics and genetic markers with different modes of inheritance were used to clarify the taxonomy of *Myotis* distributed in China. Based on 173 mitochondrial *Cytb* sequences and five morphological characteristics, 114 specimens collected nationwide over the past 20 years were assigned to 11 *Myotis* species. All Chinese samples classified into *M. davidii* and *M. longipes* were revised to *M. alticraniatus* and *M. laniger*. Then, two nuclear fragments (*Rag2* and *Chd1*) and *Cytb* sequences from representative individuals of Chinese *Myotis* were used for multilocus phylogeny reconstruction and genetic divergence evaluation. The phylogenetic relationships were clearly demonstrated in the species tree: *M. alticraniatus* and *M. laniger*; *M. fimbriatus*, *M. pilosus*, *M. macrodactylus*, and *M. petax*; and *M. pequinius*, *M. chinensis*, and *M. blythii* formed three strongly supported monophyletic clades. Mitochondrial divergence was almost 10 times that of nuclear divergence, with interspecific K2P distances ranging from 8% to 20% for *Cytb* and 0.3% to 2.3% for concatenated nuclear genes. Low levels of genetic divergence were observed between *M. alticraniatus* and *M. laniger*, as well as *M. fimbriatus* and *M. pilosus*. These results provide new insights into the taxonomy and phylogeny of *Myotis* bats in China and are important for the future research and conservation of Chinese *Myotis*.

Keywords: *Myotis*; taxonomy; multilocus phylogeny; morphometrics; conservation

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1. Introduction

Bats (Order Chiroptera) have been of increased concern in recent years. Chiroptera is one of the most widely distributed and successfully evolved mammalian orders [1,2]. Innovations, such as powered flight and laryngeal echolocation, allow bats to successfully occupy diverse nocturnal niches and form rich species diversity with rapid radiations. Until now, more than 1400 existing bat species have been identified, accounting for a quarter of all mammalian species [3–5].

Myotis is the most diverse chiropteran genus, with more than 126 known extant species [6,7]. It is the only mammalian genus naturally distributed on every continent except Antarctica [8]. The genus of *Myotis* provides an excellent model system for the investigation of speciation and diversification at a large geographic scale [8,9]. Early morphology-based studies divided the genus *Myotis* into three [10], four [11,12], or even seven subgenera [13]. However, subsequent research found the subgenera mentioned

above were not genetically monophyletic [14–16]. The similarity in morphologies is more likely to reflect convergent adaptation or likeness in predation behavior rather than close phylogenetic relationships [7,16–18].

Accurate identification of evolutionary relationships among species is difficult but essential for the study of speciation and diversification. The systematics of genus *Myotis* have been studied based on mitochondrial markers, such as *Cytb*, *ND1*, and *COI* genes [14–16]. With the widespread use of nuclear markers, *Rag2* gene, self-developed anonymous nuclear loci, and UCE loci were used in *Myotis* phylogenetic inference [7–9,19,20]. Although much work has been carried out on the genus *Myotis*, inaccurate specimen identification and wrongly labelled GenBank sequences made the phylogenetic relationships more complicated [21]. Meanwhile, the published research on *Myotis* is primarily concentrated in North America and Europe. There is poor knowledge of the species diversity, phylogenetic relationship, and the degree of differentiation of *Myotis* distributed in East Asia, which could be a critical origin center of all *Myotis* lineages [8].

The taxonomy of *Myotis* species in China remains largely confused and needs to be revised based on multiple source data. Only one research studied the phylogenetic status of *Myotis* in China, including six species (*M. fimbriatus*, *M. longipes*, *M. siligorensis*, *M. altarium*, and two unknown species) [22]. However, according to a recent study that critically evaluated the small Myotinae's systematic position in the Himalayas [21], the species delimitation in Zhang et al. [22] was problematic and outdated. In addition, due to rapid urbanization and environmental changes, the populations of some *Myotis* species are declining. Three *Myotis* species, *M. pilosus*, *M. blythii*, and *M. chinensis*, which are widely distributed in China, are listed as vulnerable species on the IUCN Red List. Thus, it is urgently necessary to study the Chinese *Myotis* to clarify their taxonomic status, determine their phylogenetic relationships, and help with conservation.

In this study, we collected hundreds of *Myotis* samples across China, revised their taxonomy with morphological and genetic data, and reconstructed the phylogenetic tree based on an updated species classification. We aimed to (a) clarify the taxonomic status of collected specimens, (b) determine the phylogenetic relationships among *Myotis* species based on multilocus datasets, and (c) evaluate the genetic differentiation of Chinese *Myotis*. The results will help elucidate the taxonomic status and phylogenetic relationships among Chinese *Myotis* and provide a good basis for *Myotis* conservation in China.

2. Materials and Methods

2.1. Samples Collection

Morphological and genetic data of 195 bats were used in this study, including 114 samples of Chinese *Myotis* collected nationwide over the past 20 years and 81 specimens recorded in literature (Table S1). All the genetic data generated in this study were based on wing membrane biopsies. All the studies were reviewed and approved by the Laboratory Animal Welfare and Ethics Committee of Jilin Agricultural University.

2.2. Genetic and Morphological Data Acquisition

Genomic DNA was extracted using UNIQ-10 column animal genomic DNA extraction kit (Sangon Biotech, Shanghai, China), and then the DNA quality was detected by 1% agarose gel electrophoresis. Mitochondrial cytochrome b gene (*Cytb*) and two nuclear genes (*Rag2* and *Chd1*) were amplified with primers L14724 and H15915 [23], 179F and 1458R [8], and EX26F and EX27R [24], respectively. The PCR amplification products were qualified by electrophoresis and sent to Shanghai Sangon Biotech for Sanger sequencing. In addition, we downloaded a set of published and unpublished sequences from GenBank (Table S1). SeqMan v.7 [25], Bioedit v.7 [26], and Geneious v.8 [27] were used to edit and align DNA sequences. DnaSP v.6 [28] was implemented to identify haplotypes. The Iss index in DAMBE v.6 [29] was evaluated to measure substitution saturation.

Morphological characteristics, including forearm length (FA), tibial length (TIB), hindfoot length (FL), ear length (EH), and tail length (TAIL), were collected from 97 specimens

by measuring in the field or retrieving literature (Table S2). Because we focused on the overall morphological difference among species, no age, sex, or geographic variations were controlled. Principal component analysis (PCA) was performed on the standardized morphological data, and the first two principal components were extracted to draw a scatterplot to visualize morphological variation among *Myotis* species. All analyses above were implemented in R v.4.2.1 [30].

2.3. Phylogenetic Analysis

The Bayesian inference (BI) and maximum likelihood (ML) approaches were employed for phylogenetic reconstruction from the mitochondrial *Cytb* gene and concatenated nuclear genes (*Rag2* and *Chd1*). The BI tree was reconstructed with MrBayes v.3.2 [31]. Five million generations were run with a sampling frequency of 100 generations and burn in of the first 25% iterations. IQ-TREE2 [32] was used to infer ML tree with 1000 ultrafast bootstrap replicates. Most specimens were successfully amplified in *Cytb* gene, and some downloaded *Cytb* sequences were recently revised by Ruedi et al. [21], so the BI and ML trees based on *Cytb* were used for taxonomic revision.

Mitochondrial (*Cytb*) and nuclear (*Rag2* and *Chd1*) sequences of representative specimens with revised taxonomy were used for species tree estimation. The species tree was reconstructed with the multispecies coalescent model of *BEAST in BEAST v.1.8 [33]. The site models, clock models, and gene trees were set to unlinked across loci. An uncorrelated relaxed clock and Yule prior were used. Ten million generations were run in BEAST with a sampling frequency of 1000 generations. Convergence was tested in Tracer [34] to ensure all ESS values exceeded 200. The same method was applied to two nuclear genes to compare with the BI and ML trees constructed from the concatenated nuclear dataset.

Bayesian and maximum likelihood methods are sensitive to nucleotide substitution models, so we selected optimal nucleotide substitution models using BIC criteria in ModelFinder [35]. For the mitochondrial *Cytb* gene, the optimal model for each of the codon positions is TIM2e + G4, TPM2u + F + I + G4, and TN + F + I + G4, respectively. The optimal models for nuclear *Rag2* and *Chd1* are HKY + F + G4 and HKY + F, respectively. The sequences of *Eptesicus fuscus* were downloaded as outgroups. The final phylogenetic tree was visualized and edited in FigTree v.1.4.4 [36].

2.4. Genetic Divergence Evaluation

Level of genetic divergence among *Myotis* species was evaluated using the Kimura two-parameter (K2P) model with 1000 bootstrap replications in MEGA v.10.0.5 [37]. Both mitochondrial and nuclear divergence were estimated. The concatenated nuclear dataset was used for nuclear divergence estimation.

3. Results

3.1. Taxonomic Revision

Mitochondrial phylogeny and morphological data were used for taxonomic revision. One hundred and four *Cytb* sequences were successfully amplified in this study. Combined with the sequences downloaded from GenBank, 174 sequences (including two outgroups) were used for mitochondrial phylogenetic reconstruction. A consistent topology was obtained from different methods (BI and ML, Figure S1). Individuals of *M. siligorensis* ("C4"), *M. frater* ("C5"), *M. fimbriatus* ("C7"), *M. macrodactylus* ("C8"), *M. petax* ("C9"), *M. pequinius* ("C10"), *M. chinensis* ("C11"), *M. blythii* ("C12"), and *M. muricola* ("C14") formed strongly supported monophyletic clades (PP/BP = 1.00/100, Figure 1).

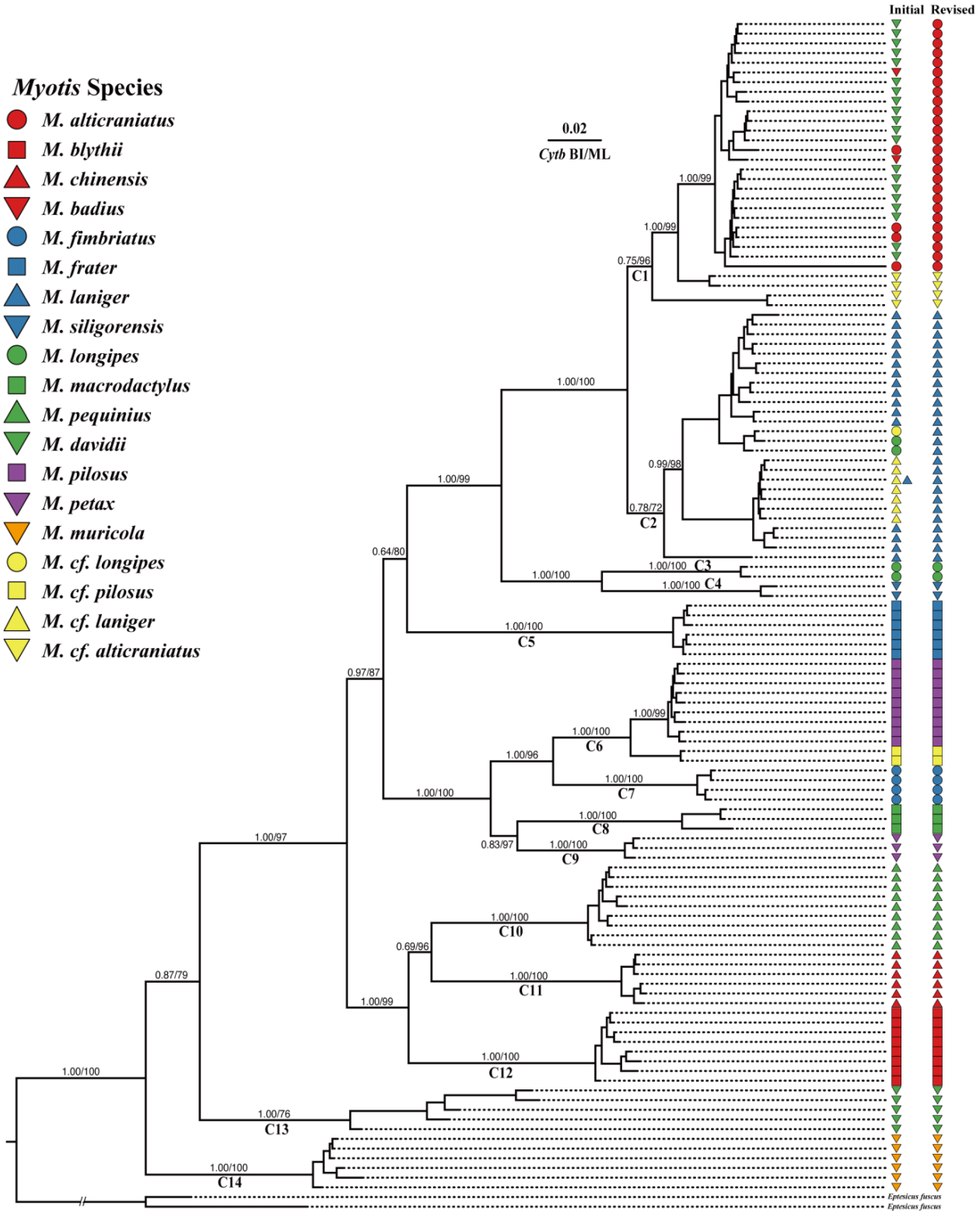

Figure 1. Mitochondrial phylogenetic tree reconstructed based on 123 *Cytb* haplotypes. Values on the branches represent posterior probability (PP) and bootstrap percentage (BP). Geometries with different colors and shapes represent *Myotis* species. "Initial" represents the initially filed identification or the species information labelled in GenBank. "Revised" means the revised species names. The information on mitochondrial haplotypes was described in Table S3.

The individuals of *M. davidii* were placed in two very distinct clades (Figure 1). One clade ("C13") includes all the genuine *M. davidii* sequences mentioned in Ruedi et al. [21] and near the base of the tree. The other clade consists of two sister clades, "C1" and "C2". Clade "C1" includes sequences initially identified as *M. davidii*, *M. badius*, and *M. alticraniatus*. According to Ruedi et al. [21], all Chinese sequences available in the GenBank labelled as "*M. davidii*" were actually "*M. alticraniatus*", which is the same species as "*M. badius*". For the sequences in clade "C1", those grouped with *M. badius* or *M. alticraniatus* were assigned to "*M. alticraniatus*", and the others were labelled as "*M.* cf. *alticraniatus*".

Clade "C2" comprised the samples of *M. davidii*, *M. laniger*, and *M. longipes*, and formed two well-supported subclades (Figure 1). In the first subclade, all individuals and sequences were classified as *M. laniger*, except two individuals of unknown origin were grouped with two sequences downloaded from GenBank labelled "*M. longipes*". The genuine *M. longipes* sequences revised by Ruedi et al. [21] were monophyletic (clade "C3") and close to the clade "C2". Therefore, the two unknown individuals were labelled "*M.* cf. *longipes*". The second subclade includes individuals and GenBank sequences identified as *M. laniger* and *M. davidii*. Considering most sequences in clade "C2" were from *M. laniger*, and the genuine *M. davidii* was placed in a distant clade, we labelled those "*M. davidii*" sequences as "*M.* cf. laniger". In addition, in the highly supported clade "C6", another two specimens of unknown origin were sister to *M. pilosus* and labelled as "*M.* cf. *pilosus*".

According to the morphological analysis, *M. blythii*, *M. chinensis*, *M. frater*, *M. pequinius*, and *M. pilosus* were different from each other, whereas the *M. alticraniatus*, *M. laniger*, *M. macrodactylus*, and *M. fimbriatus* were relatively similar (Figure 2). Individuals of *M.* cf. *alticraniatus*, *M.* cf. *laniger*, and *M.* cf. *longipes* overlapped with those *M. laniger* and *M. alticraniatus*. Two specimens of *M.* cf. *pilosus* were much smaller than *M. pilosus* (Table S2) and distinct from the individuals of *M. pilosus* but close to its closely related species, *M. fimbriatus*. However, the four individuals of *M. fimbriatus* were pretty scattered.

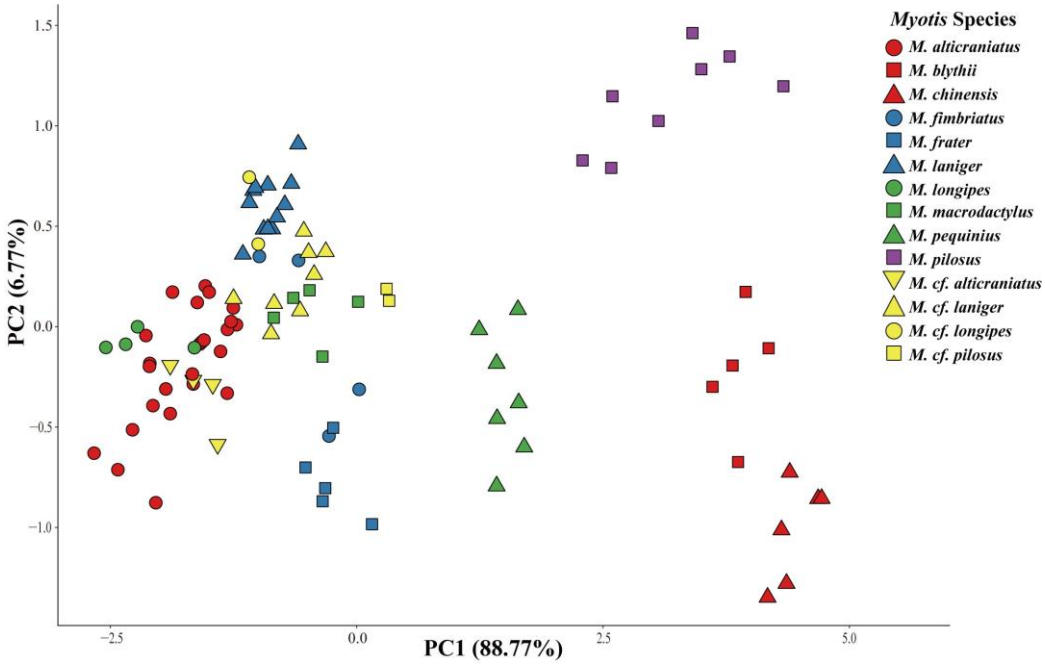

Figure 2. Principal component analysis based on five morphological characteristics. The first two principal components explained 88.77% and 6.77% of the total variance, respectively. Geometries with different colors and shapes represent *Myotis* species.

3.2. Phylogeny of Chinese Myotis

According to the revised taxonomy presented above, representative samples of 11 Chinese *Myotis* species were chosen for phylogenetic relationship inference based on the nuclear genes. *Rag2* and *Chd1* sequences were successfully amplified from 30 and 26 samples, respectively (Table S1). The BI and ML trees constructed based on concatenated nuclear genes were consistent in topology (Figure S2). In the BI/ML tree, sequences of each species formed highly supported monophyletic clades (PP/BP $\geq$ 0.98/79, Figure 3A), and the phylogenetic relationships among those species were mostly congruent with the tree inferred by *BEAST based on the same datasets, only the phylogenetic location of *M. petax* and *M. muricola* shows difference (Figure 3B).

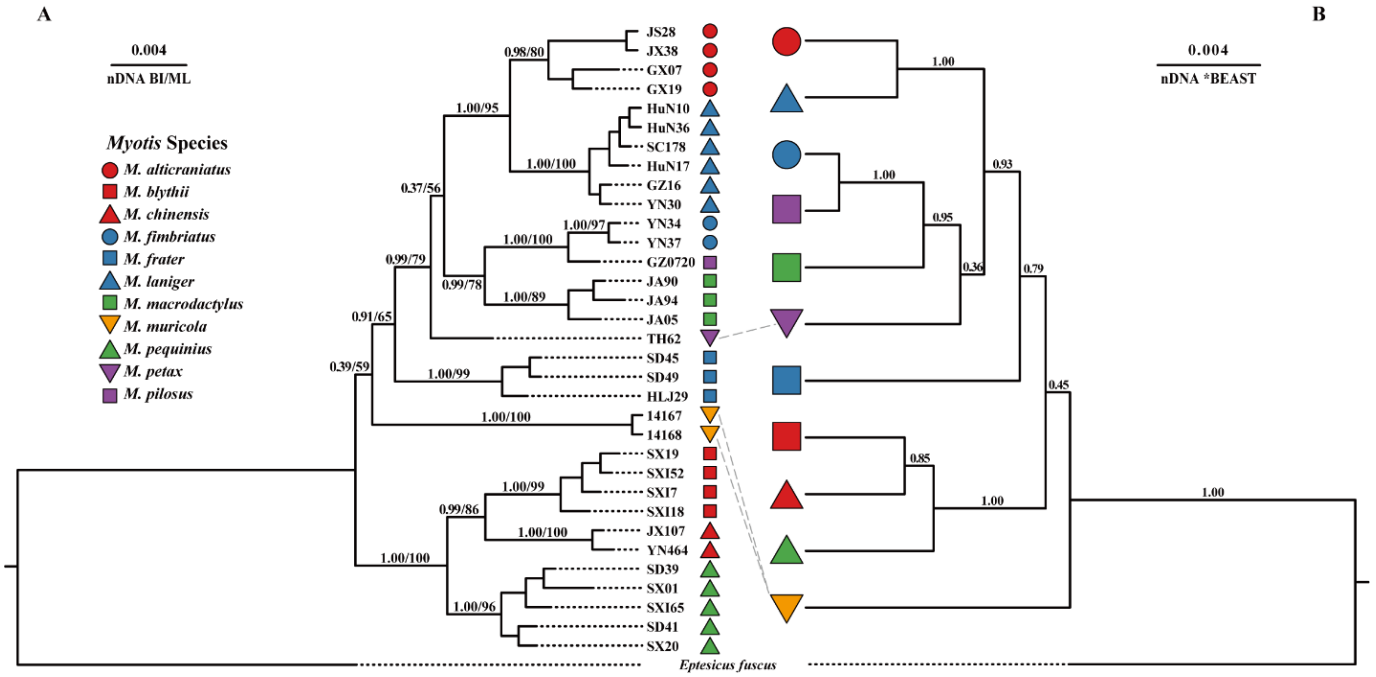

Figure 3. (**A**) Phylogenetic tree based on concatenated nuclear genes. Values on the branches represent posterior probability (PP) and bootstrap percentage (BP). (**B**) Species tree constructed in *BEAST based on nuclear *Rag2* and *Chd1* genes. Values on the branch represent posterior probability. Geometries with different colors and shapes represent *Myotis* species.

In the BI/ML tree, *M. petax* was sister to a group of closely related species, including *M. alticraniatus*, *M. laniger*, *M. fimbriatus*, *M. pilosus*, and *M. macrodactylus* (Figure 3A). While in the results of *BEAST, *M. petax* clustered with a well-supported clade that included *M. fimbriatus*, *M. pilosus*, and *M. macrodactylus*, and formed a sister group relationship with another highly supported *alticraniatus–laniger* clade (Figure 3B). The BI/ML tree was divided into two clades, *M. muricola* was at the basal position of one clade, but in the *BEAST tree, *M. muricola* was distantly related to all other assayed *Myotis* species and at the base of the tree (Figure 3).

Thirty-three *Cytb* sequences were chosen from the same individuals as *Rag2* and *Chd1* sequences to reconstruct the multilocus species tree (Figure 4A). Most clades were highly supported (PP $\geq$ 0.98) and suggested a close relationship between *M. alticraniatus* and *M. laniger*; *M. fimbriatus*, *M. pilosus*, *M. macrodactylus*, and *M. petax*; and *M. pequinius*, *M. chinensis*, and *M. blythii* (Figure 4A). *Myotis frater* was close to the strongly supported *alticraniatus–laniger* clade and its sister clade. The latter includes four species, as *M. fimbriatus* and *M. pilosus* formed a strongly monophyletic clade that was sister to the well-supported *macrodactylus–petax* clade. All these four species were grouped together with high support value (PP = 1.00). *Myotis muricola* was at the basal position of the species tree and supported with a posterior probability of 1.00.

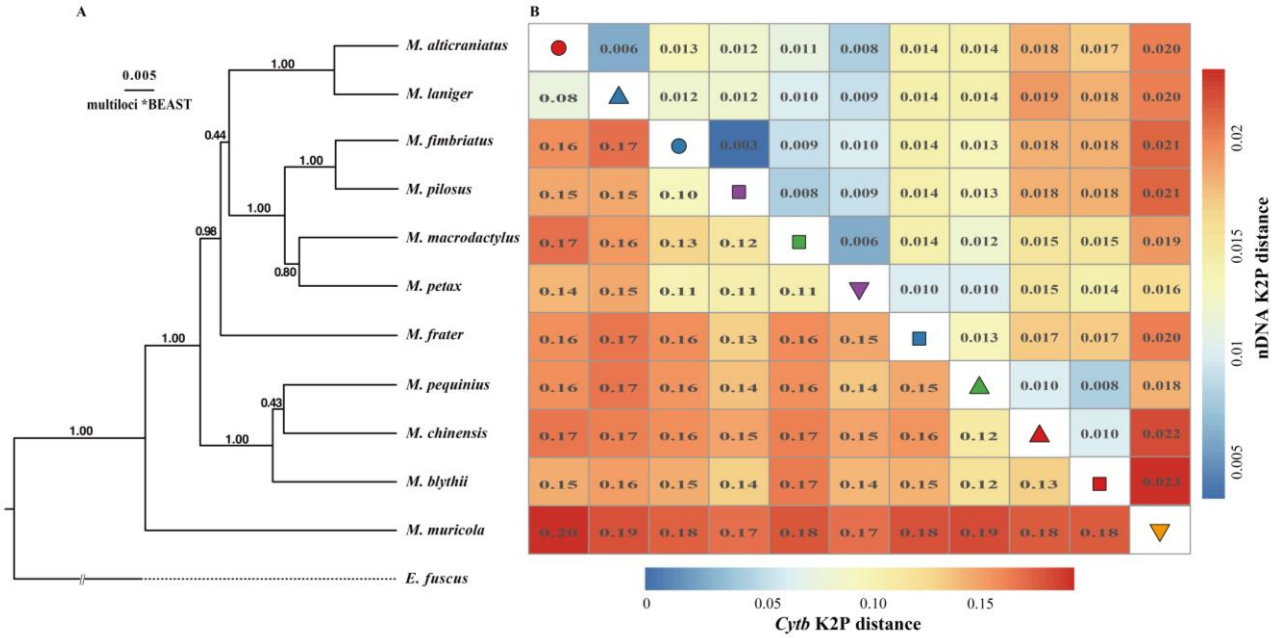

Figure 4. (**A**) Species tree constructed in *BEAST based on *Cytb*, *Rag2*, and *Chd1* genes. Values on the branch represent posterior probability. (**B**) Heatmap of K2P genetic distance calculated based on mitochondrial *Cytb* gene (lower triangular) and concatenated nuclear genes (upper triangular). Geometries with different colors and shapes represent *Myotis* species and corresponds to the species on the left side.

3.3. The Level of Genetic Divergence

Mitochondrial divergence was almost ten times of nuclear divergence (Figure 4B). For mitochondrial *Cytb*, the interspecific K2P distances among species ranged from 8% to 20%. Most mitochondrial distances were higher than 10%, except for *alticraniatus–laniger* and *fimbriatus–pilosus*. The highest values were observed between *M. muricola* and other species. For concatenated nuclear genes, the interspecific K2P distances among species ranged from 0.3% to 2.3%. The lowest value was found between *M. fimbriatus* and *M. pilosus*.

4. Discussion

This study presents the first phylogenetic analysis focusing on the *Myotis* in China based on comprehensive sampling and integrated morphological and genetic data. Importantly, we reconstructed the phylogeny of Chinese *Myotis* with a revised and updated taxonomy according to Ruedi et al. [21]. The results of this study clarified the species status and relationships among *Myotis* bats in China and provided a reasonable basis for the research and conservation of the genus *Myotis*.

Accurate species delimitation is an essential premise for evolutionary research and plays a fundamental role in understanding biodiversity [38]. Traditional taxonomic identification is mainly based on morphological and other phenotypic characteristics as indicators of reproductive isolation. With the development of sequencing technologies, genetic markers are widely used in species identification and classification. However, many studies have found that inconsistency may be between phenotypic and genetic data and genetic markers of different inheritance patterns [39]. Genus *Myotis* has undergone rapid species diversification and is one of the most successful species radiations among extant mammals [20]. Discordances between genetic and morphological characters have been identified in this genus [8,9,20] and caused years of taxonomic controversy.

Morphological characters are easy to measure, while in many cases, they do not reflect actual relatedness. Even in the same species, geographic variation would lead to discordance between morphology and genetics, which has been reported in many organisms,

such as the *Blandfordia grandiflora* [40], *Drosophila* [41], snakes [42], and primates [43]. In this study, two individuals of unknown origin were labelled as "*M.* cf. *pilosus*" because of their genetic similarity with *M. pilosus*, but they were clearly distinguished in morphology (Figures 1 and 2, Table S2). However, in this case, the discordance seems unrelated to the geographic variation as the other individuals of *M. pilosus* were collected from different localities and well-grouped in PCA analysis (Figure 2). Errors in the measurement and genetic introgression could also lead to inconsistency. To determine the taxonomy of those two specimens, morphological and genetic data from more *M.* cf. *pilosus* samples of the same locality are required.

In another case, distantly related species showed some morphological similarity due to convergent adaptation, such as the evolution of sensitive hearing to high-frequency sounds between echolocating bats and cetaceans [44]. Convergent adaptations have been reported in many taxa, including carnivorous plants [45], marine tetrapods [46], frogs [47], etc. In *Myotis*, multiple studies found that the phenotype is more likely to be a convergent adaptation in a specific situation, which may be related to predation behavior and could not fully reflect the proximity of kinship [16–18]. In this study, we found that *M. pilosus* and *M. fimbriatus* were similar in genetics but different in morphology (Figures 1 and 2). *Myotis pilosus* is the only known piscivorous bat in East Asia, while *M. fimbriatus* is a trawling insectivorous bat. The differences in morphology are likely related to predation behavior. According to Chang et al. [48], the morphological advantages of *M. pilosus*, such as the giant forearm, hind foot, and body size, help it forage on fish.

Discordance between morphological and genetic data was also detected in *M. longipes*. The genuine *M. longipes* collected from India formed a monophyletic mtDNA clade ("C3"). In contrast, in the morphology, the four voucher specimens of *M. longipes* collected from the Guangdong and Hunan Provinces of China [49,50] were mixed with *M. alticraniatus* and *M. laniger* in Figure 2. Four voucher specimens were smaller than the topotypes collected from India [51]. They were classified as *M. longipes* based on their close phylogenetic relationships to the *M. longipes* from Laos reported by Ruedi et al. [8]. However, Ruedi et al. [21] revised this sample to *M. laniger* in the recently published paper. Thus, the *M. longipes* and *M.* cf. *longipes* collected from China, which were nested in the clade "C2", belong to *M. laniger*. According to Topál [52], *M. longipes* is likely endemic to Afghanistan and India. The records of *M. longipes* in China (such as Guangxi, Hunan, Guangdong, and Guizhou [53]) were most likely to be *M. laniger* and require further validation.

Myotis laniger and *M. alticraniatus* represent two different feeding-foraging modalities [7]. *Myotis laniger* is a trawling bat with large feet, while *M. alticraniatus* is an aerial hawking bat with tiny feet [7,21]. All the individuals of *M.* cf. *laniger* have relatively long feet (FL: 9.77–11.36 mm) and high foot/tibia ratios (range from 60–68%), while the individuals of *M.* cf. *alticraniatus* have relatively small-sized feet (FL: 7.70–8.95 mm) and low foot/tibia ratios (range from 54–59%). However, the five external morphological characteristics used in this study were insufficient to distinguish those two species (Figure 2). Craniodental characteristics could provide critical information in species delimitation, especially for the morphology conserved *Myotis*, while only biopsy samples were obtained in this study, and no wet specimens nor skulls were included. In future studies, we should better consider more external and craniodental characteristics of the small-sized *Myotis* for a more accurate and reliable species delimitation, and more importantly, the voucher specimens, topotypes, and/or holotypes should be incorporated.

According to Baker and Bradley [54], the genetic variations from mitochondrial *Cytb* among bats were 1.4–1.9% for intraspecific divergence, 3.3–14.7% for interspecific differentiation of sister taxa, and 8.4–15.7% for interspecific differentiation of non-sister species. For the clade "C1", although the morphological characteristics of *M. alticraniatus* and *M.* cf. *alticraniatus* were similar, their mitochondrial K2P genetic distance was 5.85%, suggesting an interspecific differentiation. Further taxonomic scrutiny was required for the individuals of *M.* cf. *alticraniatus*. The individuals of *M. alticraniatus* were diverged from *M. laniger* with a mitochondrial K2P genetic distance of 8%, indicating an interspecific

differentiation of sister taxa. Based on the morphological and genetic evidence, we updated the distribution information of these two closely related *Myotis* species and confirmed that *M. laniger* and *M. alticraniatus* are widely distributed in southern China and overlap to a large extent (Table S1).

In addition, *M. alticraniatus* and *M. davidii* were often confused in many studies, such as Kawai et al. [15], You et al. [55], and You et al. [56]. Ruedi et al. [21] found that *M. alticraniatus* and *M. davidii* are distinct in craniodental and mandibular morphologies by reexamining the museum specimens of these two species, including the holotype of *M. davidii* from Beijing (MNHN 1987–296). According to our genetic results, all Chinese sequences initially identified as *M. davidii* were *M. alticraniatus* and *M. laniger*, and distantly placed from the sequences of genuine *M. davidii,* which were in the clade "C13" near the base of the mitochondrial tree (Figure 1). This finding is consistent with the results of Ruedi et al. [21], who found all Chinese "*M. davidii*" sequences available in the GenBank exceed 13% genetic divergence compared to the genuine *M. davidii* but within 5% divergence to *M. alticraniatus*. However, the genetic sequences of genuine *M. davidii* used in Ruedi et al. [21] and this study were generated from *M. davidii* as redefined by Benda et al. [57]. Genetic data from the holotype or topotypes of *M. davidii* from Beijing were required for further confirmation.

The phylogenetic relationships of the genus *Myotis* have been explored in several studies, such as Kawai et al. [15], Zhang et al. [22], Ruedi et al. [8], Morales et al. [7], and Ruedi et al. [21]. All the studies using *Cytb* or *Rag2* as genetic markers supported a closer relationship between *M. laniger* and *M. alticraniatus*, while in Morales et al. [7], who used 1610 UCEs, a closer relationship was observed among *M. laniger*, *M. fimbriatus*, and *M. petax*; *M. alticraniatus* and *M. pilosus* had relatively distant relationships with the above three species. None of these studies yield consistent phylogenetic relationships, especially for the basal relationships. Compared with previous studies, we focused on the *Myotis* distributed in China and generated a highly supported species tree with one mitochondrial and two nuclear markers. Our results provided some useful phylogenetic information for the Chinese *Myotis*, such as the lowest genetic distance between *M. laniger* and *M. alticraniatus*, and close relationships within four trawling bats (*M. fimbriatus*, *M. petax*, *M. pilosus*, and *M. macrodactylus*) or three gleaning bats (*M. pequinius*, *M. chinensis*, and *M. blythii*). However, there are still two weakly supported clades that are likely to be incorrect topologies. More genetic/genomic and phenotypic data, especially the craniodental characteristics, should be considered to reconstruct a more convincing species tree.

Large-scale wildlife diversity monitoring and investigation are being carried out worldwide. Still, the taxonomic research on bats is lagging due to their particularity of flying and nighttime activity [58]. Until now, studies on Chinese *Myotis* have been primarily focusing on single species, such as the phylogeographic studies of *M. pequinius* [59] and *M. pilosus* [60]. The interspecific phylogenetic relationships among Chinese *Myotis* remain chaotic. The results of this study provide new insights into the taxonomy and phylogeny of *Myotis* bats in China and are essential for the future research and conservation of Chinese *Myotis*. However, there are still many unknown species and taxonomic controversies within the *Myotis* genus. Multiple sources of data, such as genetic markers, genomic SNPs, external morphological and craniodental characteristics, evolutionary history, and geographical and ecological information, should be incorporated in species delimitation to obtain more accurate and reliable taxonomy.

5. Conclusions

In conclusion, this study combined morphological and genetic data to investigate the taxonomy and phylogeny of Chinese *Myotis*. With the broad geographic scale sampling and data collection, we revised the taxonomic status of 114 Chinese *Myotis* specimens. All individuals initially identified as *M. davidii* and *M. longipes* were reassigned to *M. alticraniatus* and *M. laniger*. The phylogenetic relationships of Chinese *Myotis* were reconstructed with the updated taxonomy and multiple genetic markers and showed three highly sup-

ported monophyletic clades. One includes *M. laniger* and *M. alticraniatus*, which have low genetic distance, one contains four trawling bats (*M. fimbriatus*, *M. petax*, *M. pilosus*, and *M. macrodactylus*), and the other one includes three gleaning bats (*M. pequinius*, *M. chinensis*, and *M. blythii*). This study emphasizes the importance of using an updated taxonomy in species classification and phylogeny reconstruction and provides essential background information for the conservation of *Myotis* in China.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15070805/s1, Table S1: Sampling information of specimens and sequences used in this study; Table S2: Morphological data used in this study; Table S3: Haplotypes of mitochondrial *Cytb* sequences; Table S4: Haplotypes of nuclear sequences; Figure S1: Phylogenetic trees reconstructed based on 123 mitochondrial *Cytb* haplotypes. Values on the branches represent posterior probability obtained with MrBayes (A) and bootstrap percentage obtained with IQ-TREE (B). Geometries of different colors and shapes represent *Myotis* species. The information on mitochondrial haplotypes was described in Table S3; Figure S2: Phylogenetic trees reconstructed based on 20 nuclear Rag2 haplotypes (A,B), 13 nuclear *Chd1* haplotypes (C,D), and concatenated nuclear sequences (E,F). Values on the branches represent posterior probability obtained with MrBayes (BI) and bootstrap percentage obtained with IQ-TREE (ML). Geometries of different colors and shapes represent *Myotis* species. The information on nuclear haplotypes was described in Table S4.

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