Molecular and morphological revision of small Myotinae from the Himalayas shed new light on the poorly known genus *Submyotodon* (Chiroptera: Vespertilionidae)

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Abstract

The systematics status of the constituent species of the *M. mystacinus* morphogroup in the Himalayan region has long been marred by uncertainty. Lack of integrative studies combining morphological and genetic data from specimens recently collected in this region has hampered our understanding of cryptic variations in this complex taxonomic group. To address this issue, new material from the Himalayan region of India and Nepal was obtained and vouchered specimens in the holdings of various museums were also re-examined. As comparative material, a large series of relevant specimens from South and Southeast Asia were also included in this revision. Using a combination of multivariate analysis of craniodental characters and molecular reconstructions, we critically evaluated the systematic position of the small Myotinae in the Himalayas. We establish that *M. nipalensis* forms a very distinct lineage (which also includes the recently described *M. annatessae*) and refute previous taxonomic suggestions that it is related to *M. davidii*. Our study also conclusively proved the common occurrence of the poorly known genus *Submyotodon* in the Himalayan region (Afghanistan, Pakistan, India, Nepal and China) and evidenced species-level divergences within that genus. *Submyotodon* species share nyctalodont or semi-nyctalodont lower molar configuration with few other small and unrelated Myotinae from Asia suggesting that these unusual dental characters are homoplasious in this subfamily. We also noticed a very confused taxonomic situation associated with many DNA sequences of Asian *Myotis* deposited in public repositories and call for possibilities of better data curation.

Keywords *Myotis* · *muricola* · Systematics · Barcode

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Introduction

The Himalayan foothills are a megadiverse ecoregion supporting a high number of mammalian taxa (Olson et al. 2001; Orme et al. 2005). Its bat fauna has been studied in some details during the last centuries, especially during the

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British Raj, when naturalists like Blyth, Hodgson, and Hut-ton collected many specimens from these hilly regions, some of them representing new species (e.g. Hodgson 1835; Dobson 1878). During the twentieth century, global revisions of the speciose genus *Myotis* based on extensive museum material of Eurasian origin (Tate 1941; Hill 1983) suggested that many of those named forms from the Himalayas were only colour or minor size variants of more widespread taxa, which resulted in a considerable reduction in the numbers of accepted species (Ellerman and Morrison-Scott 1951; Corbet and Hill 1992). However, owing to the limited resolving power of classical morphological taxonomy in groups such as *Myotis* characterized by remarkably conservative and convergent phenotypic characters (Ruedi and Mayer 2001; Stadelmann et al. 2007; Morales et al. 2019), part of these taxonomic inferences based solely on morphology likely do not reflect natural groupings. One of these debated species complexes consists of a group of small-sized *Myotis* collectively known as the *mystacinus*-morphogroup and thought to be widely distributed over the Palearctic and Indomalayan regions (Thomas 1926; Benda and Tsytsulina 2000; Benda et al. 2016). These small “whiskered” *Myotis* (forearm 30–35 mm) are all characterized by small feet, rather short ears and wing membranes inserted to the base of toes and indeed resemble to the European *M. mystacinus* (Kuhl 1817) (type locality: Germany).

Building upon observations of the early collectors, and also his own morphological comparisons, Tate (1941) revised the systematics of species belonging to the *mystacinus*-morphogroup and recognized three distinct taxa within Asian representatives of this group: (1) a relatively large and dark form originally described as *Vespertilio* [= *Myotis*] *muricola* (Horsfield 1855) (type loc. Nepal), with *V. moupinensis* (Tomes 1859) (type loc. Szechwan, China) considered as a junior synonym; (2) a smaller, also relatively dark form originally described as *V.* [= *Myotis*] *caliginosus* (Tomes 1859) (type loc. India), with *V. blanfordi* (Dobson 1871) (type loc. “Sikkim, Simla, Dalhousie”, India) considered as a synonym; and (3) a larger and ventrally much paler (whitish) form originally known as *V.* [= *Myotis*] *nipalensis* (Dobson 1871) (type loc. Kathmandu, Nepal) to which he associated with some reservation *M. meinertzhageni* (Thomas 1926) (type loc. Ladakh, Kashmir) and *M. kukunorensis* (Bobrinskoy 1929) (type loc. S of Qinghai Lake, Tibet). He also considered a further, more distinct species characterized by much smaller canines and particularly doomed braincase above the orbits and originally named as *V.* [= *Myotis*] *siligorensis* (Horsfield 1855) (type loc. Siligori, Nepal [correctly Siligori, Darjeeling district, West Bengal, India]) and to which he associated the Indochinese *alticraniatus* (Osgood 1932) (type loc. Tonkin, Vietnam) and *sowerbyi* (Howell 1926) (type loc. Fukien, China). Subsequent revisions of the group merged the first two dark-bellied forms as a single

species under the name *M. muricola*, while the ventrally whitish form (*nipalensis*) was relegated to the synonymy of *M. mystacinus*. The taxon *thaianus* (Shamel 1942) (type loc. Chiangmai, Thailand) was added as a subspecies to *M. siligorensis* (Ellerman and Morrison-Scott 1966; Hill 1983; Koopman 1994; Bates and Harrison 1997).

A new revision of members of the whole *mystacinus* morphogroup concentrating on morphometric, bacular and dental characteristics of Central Asian and Western Palearctic specimens (Benda and Tsytsulina 2000), however, again challenged the prevailing taxonomic arrangement of Asian whiskered bats and demonstrated that *M. mystacinus* sensu stricto (s. str.) is a species confined to the Western Palearctic region and the Caucasus with northern Iran representing its easternmost limit of distribution (Benda et al. 2012). In that region, it occurs in sympatry with another distinct morphotype initially called *M. aurascens* (Kuzjakin 1935) (type loc. Caucasus). Aided by more comprehensive series of samples from Central and Eastern Asia, Tsytsulina et al. (2012) again surveyed the variation of these bats with both genetic and morphometric analyses and corroborated most of Benda and Tsytsulina (2000) hypotheses, including the distinctness of European *M. mystacinus* s. str. and the species status and very widespread occurrence of *M. aurascens* across Eurasia. However, because they lacked any material from south of the Himalayas, they could not definitively evaluate the status of the Himalayan taxon *M. nipalensis* but only assumed that the steppe form *przewalskii* could be a subspecies of that species. The same taxonomic conclusions (including doubts about the systematic position of *nipalensis* sensu Benda and Tsytsulina) emerged from an independent survey of genetic variation of East Palearctic bats (Kruskop et al. 2012).

In a last twist given to the confusing taxonomic arrangement of Asian members of the *mystacinus*-morphogroup, Benda and colleagues (Benda and Gaisler 2015; Benda 2016) re-examined the scant material from this region and considered that most Asian forms, including *aurascens*, *przewalskii*, *sogdianus*, *meinertzhageni*, *nipalensis*, etc. were all junior synonyms of *V.* [= *Myotis*] *davidii* (Peters 1869) (type loc. Beijing, China). According to this new view, *M. davidii* would be distributed from the Bosphorus eastward across Central Asia to South Korea and would represent the “most widespread, morphologically plastic and ecologically universal [...]” species in this group (Benda and Gaisler 2015:327). Nevertheless, no genetic material from the Himalayas was evaluated to substantiate this new hypothesis of synonymy.

Although *M. muricola* was widely accepted to be a common bat occurring throughout most of the Indomalayan region, with numerous taxa listed under its synonymy, genetic evidence from both mitochondrial and nuclear genes (Lack et al. 2010; Ruedi et al. 2013) showed that at least one of the synonymized taxon from Taiwan, “*Myotis*”

muricola latirostris (Kishida 1932) was phylogenetically not only distant from other forms of *M. muricola*, but also very divergent from all assayed species of *Myotis*. These authors concluded that this taxon should therefore be classified in a distinct genus. Owing to its peculiar skull and molar shapes, Ruedi et al. (2015) placed it in the morphologically similar fossil genus *Submyotodon* described by Ziegler (2003) from Miocene deposits of Europe. These authors further suggested that some small forms formerly considered synonyms of *M. muricola* (i.e. *caliginosus*, *blanfordi* and *moupinensis*) also corresponded to *Submyotodon*, a view substantiated in a morphological revision by Benda and Gaisler (2015). Species in this genus all share a nyctalodont (see Fig. S3 in Ruedi et al. 2013) or semi-nyctalodont configuration of the first two pairs of lower molars (Ziegler 2003; Ruedi et al. 2015), unlike in most other *Myotis* that have a myotodont configuration (Menu and Sigé 1971). The only exceptions among the Myotinae in this respect are taxa included in the *M. siligorensis* species complex (Borisenko et al. 2008; Tiu-nov et al. 2011).

Despite many decades of efforts, the paucity of museum specimens and lack of reference genetic data from the Himalayas has hampered progress in our understanding of species diversity and taxonomy in this difficult group of bats. We thus setup a series of field surveys in various parts of Northern India and Nepal with the aim to get primary access to new specimens from this area that includes several type localities for these taxa. We present here results of these surveys of Himalayan Myotinae and, with help of a combined morphological and molecular approach, we shed new light on the taxonomy of members of the *mystacinus* morphogroup and also on the little-known genus *Submyotodon*.

Materials and methods

The current revision is based on a series of field studies conducted in the Himalayan region of Nepal (between 1994 and 2016) and India (between 2009 and 2019) and also re-examination of museum specimens lodged in various institutions of Asia and Europe.

In Himachal Pradesh, India, the surveyed areas included the Outer Himalayas (Happy Valley, Salogra, Mt. Karol and Devthal, between 960 and 1865 m a. s. l.) in Solan district; the Middle Himalayas (Narkanda, c. 2700 m a. s. l.) in Shimla district; and the Trans-Himalayas (Sangla, c. 2720 m a. s. l.) in Kinnaur district. Localities in Northeastern India were also surveyed in the East Jaintia Hills District (c. 670 m a. s. l.), Meghalaya, and Phalang III (c. 730 m a. s. l.) in Tamenglong District, Manipur.

In Nepal the collecting localities included Langtang Himal, Bagmati Pradesh (between 1800 and 2800 m a. s. l.), Tanahun, Kaski and Syangja Districts, Gandaki Pradesh

(between 670 and 2900 m a. s. l.), Taplejung and Tehratum Districts, Province No. 1. (between 1600 and 3000 m a. s. l.), and the Kathmandu Valley, Bagmati Pradesh (between 1400 and 1500 m a. s. l.).

Morphological methods

Bats were captured with mist nets, hand nets, and a harp trap set across bodies of water or across presumed flyways in forests. Animals were preliminarily identified in the field with keys in Bates and Harrison (1997), measured, photographed and biopsied (Worthington-Wilmer and Barratt 1996) before being released within an hour of capture. A small number of voucher specimens were taken for more thorough identification involving cranial or dental characters. Animals were handled according to the recommendations of the American Society of Mammalogists (Sikes and Animal Care and Use Committee of the American Society of Mammalogists 2016). Except for hindfoot length (HF) which included claws, standard external measurements were taken as defined by Bates and Harrison (1997) and taken to the nearest 0.1 mm with a dial calliper. These measurements were forearm length (FA), tail length (TL), head and body length (HB), tibia length (TIB), ear length (EAR), tragus length (TRA) and body mass (W, expressed in grams). For vouchered specimens, the following craniodental measurements were also taken with a digital calliper (to the nearest 0.01 mm) and also corresponded to those proposed by Bates and Harrison (1997): greatest length of skull including incisors (GTLi); condylo-basal length (CBL); condylo-canine length (CCL); zygomatic breadth (ZB); breadth of braincase (BB); interorbital breadth (IOB); width across the upper canines (CCW); width across the upper molars (M3M3W); maxillary tooththrow length (CM3L); mandible length, including incisors (MLi); mandibular tooththrow length (cm3L); in addition, we also took the height of the coronoid process measured from its tip to the closest point of the base of the ramus (coh) (Yu et al. 2020). The prepared specimens were compared to original descriptions of the types, or directly with the type material available in museums. To visualise shape and size variation, measurements of intact skulls were also subjected to multivariate analyses via principal components extracted from the correlation matrix in SPSS-X (Norusis 1993); few missing data from slightly damaged skulls were replaced by mean values of the species.

Material for comparisons

Besides the newly captured bats, we also examined and re-identified small Myotinae specimens from Northern India and Nepal and some extra-limital material held in various museum collections, the acronyms of which are BMNH: the Natural History Museum (formerly British Museum (Natural

History)), London, UK; CDZTU: Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal; HNHM: Hungarian Natural History Museum, Budapest, Hungary; HZM: Harrison Institute (formerly Harrison Zoological Museum), Sevenoaks, UK; MHNG: Muséum d'histoire naturelle de Genève, Geneva, Switzerland; MNHN, Muséum national d'histoire naturelle, Paris, France; NMP: National Museum Prague, Czech Republic; TESRI: Taiwan Endemic Species Research Institute, Taiwan; THUMB: the Department of Biology, Tunghai University, Taiwan; ZMMU: Zoological Museum of Moscow University, Russia; ZSI: Zoological Survey of India, Regional Centres at Shillong and Solan, India. The list of Asian specimens examined for the morphological comparisons is as follows (an * indicates that the specimen was also analysed genetically):

***Myotis alticraniatus*:** Laos PDR (Xiengkhouang)—MHNG 1956.089*, 1956.090*, 1956.091, 1956.092, 1956.093; China (Guizhou)—HNHM 2011.13.7.*, 2011.13.8*, 23,765*; Vietnam (Ninh Binh) – HNHM 88.51.1.

***Myotis annatessae*:** Laos PDR (Phongsaly)—MHNG 1926.044*; Vietnam (Ha Tinh) – ZMMU S-164986, S-164989 (both paratypes).

***Myotis cf. annatessae*:** China (Jiangxi)—HNHM 25,348*.

***Myotis badius*:** China (Fagudian, Yunnan)—HNHM 25,839*, 25,347* (topotypes).

***Myotis blythii*:** India (Himachal Pradesh)—ZSI CW-34–35, M-34, VMERS 405*.

***Myotis davidii*:** China (Beijing)—MNHN 1987–296 (holotype), 1911–739; Mongolia—MHNG 1933.092*, 1933.094, 1933.095, 1933.096 (ssp. *mongolicus*).

***Myotis csorbai*:** Nepal (Syangja)—HNHM 97.2.1*, 97.2.3* (paratypes).

***Myotis fimbriatus*:** China (Fagudian, Yunnan)—HNHM 25,872*, 25,898*.

***Myotis laniger*:** India (Meghalaya)—ZSI VMERS 263, 370–371, HNHM 92.108.1–3, MHNG 1981.074*, 1991.081* (plus 17 M and 10 F released, including M1607, M1934*, M1636*, M1937* and M2047); China (Banfangzi)—NMP 90,546; Taiwan (Taichung)—THUMB T-3474*.

***Myotis longipes*:** India (Kashmir)—HNHM 92.104.35–38, 92.104.40* (topotypes); India (Himachal Pradesh)—ZSI VMERS 445, CW36–38, M25, M35, 307, VMERS 438*, 439, 440, 441, 442* (plus 2 F released); Pakistan—HNHM 99.15.1.

***Myotis muricola*:** Nepal—BM(NH) 45.1.8.143 (holotype), HNHM 98.5.24. (topotype); India (Darjeeling)—HNHM 92.106.1.; India (Manipur)—ZSI VMERS 450; Cambodia (Mondolkiri)—HNHM 2005.82.20–21.; China (Hong Kong)—HNHM 2005.83.1.; Vietnam (Dak Lak)—HNHM 2014.26.11; Vietnam (Lo Go Xa Mat)—ZMMU

S-172626; Vietnam—ZMMU S-172615; Laos PDR (Phongsaly)—MHNG 1926.036*; Malaysia (Selangor)—MHNG 1970.064–69, MNHN 1975–544.1, 1975–544.3, 1975–544.6, 1975–544.7, 1983–1892, 1983–1893, 1983–1895, 1983–1910, 1983–1897, 1983–1902, 1983–1898; Malaysia (Terengganu)—MHNG 1970.061, 1970.062*, 1970.065*; Indonesia (Java)—MHNG 1972.084*.

***Myotis nipalensis*:** Nepal—CDZTU B9* (topotype); India (Himachal Pradesh)—ZSI M-38, VMERS 445* (plus 1 F released).

***Myotis phanluongi*:** Vietnam (Phu Khanh)—ZMMU S-175155* (holotype).

***Myotis siligorensis*:** India (West Bengal)—BM(NH) 79.11.21.125 (holotype); India (Darjeeling, topotype of *darjelingensis*)—HNHM 92.106.2., 92.107.1.; India (Meghalaya)—VMERS599, 600*; MHNG 1991.083 (plus 1 M* and 1 F released); India (Assam)—VMERS601*.

***Submyotodon caliginosus*:** “Himalayas”—BM(NH) 75.10.27.1–2 (cotypes of *blanfordi*); India (Himachal Pradesh)—BM(NH) 23.9.1.13, ZSI M-50, VMERS417*, 421* (plus 2 F and 1 M released; topotypes); India (Sikkim)—BM(NH) 91.10.7.57; India (Kashmir)—BM(NH) 8.7.6.10; Nepal—HNHM 98.8.7.; Pakistan—BM(NH) 71.1574–1577.

***Submyotodon latirostris*:** Taiwan—HNHM 2003.36.7., 2003.36.15–16., 2003.36.17–18., 2004.19.9., 2004.19.18., 2004.19.21.; MHNG 1998.052*, 1998.057; TESRI B0276–80, THUMB 7973, t-4549–52, t-4548, t-4550, t-4604, B000105, B03010*, B030011–12, B030036–37, B030039–40 (topotypes).

***Submyotodon moupinensis*:** China (Szechwan)—MNHN 1870–588 (holotype), 1870–589a–b (paratypes), 1962–2660; China (Yunnan)—BM(NH) 23.4.1.2; HNHM 26,564*.

***Submyotodon cf. moupinensis*:** China (Shaanxi)—NMP 90,544*.

DNA analyses

Wing biopsies or tissue samples preserved in alcohol were extracted for genomic DNA using the Qiagen DNeasy blood and tissue kit (Bangalore, India) according to manufacturer's manual and eluted in a final volume of 200 µl TE buffer. Total genomic DNA from extra-limital samples was obtained with the same methods. Two mitochondrial genes commonly used in bat systematics were amplified: the cytochrome *b* (abbreviated hereafter CYTB) and the traditional barcode gene, part of the cytochrome C oxidase subunit 1 (COI). They were amplified with Molcit-F/Cytb-H (Ibáñez et al. 2006; Weyeneth et al. 2008) and UTyr/C1L705 (Hassanin et al. 2012) or VF1d/VR1d (Ivanova et al. 2007), respectively. PCR were carried out in 25 µl reactions containing 50–500 ng of genomic DNA. Each PCR was run with

negative controls to monitor possible contaminants. PCR products were cleaned using ExoSAP Kit (Thermo Fisher Scientific, India) and sequenced in both directions with the PCR primers. Chromatographs were checked, assembled, and edited using Sequencher 4.1 (Gene Codes Corp., USA). In addition, we downloaded a set of published and unpublished sequences of various representatives of Eurasian *Myotis* taken from both the GenBank and BOLD (see Online Resource 1). This was a complicated task as several hundred CYTB and COI sequences of potential interest were available, but many are associated with problematic species labels. The spotted problematic species identifications included obvious labelling errors, old names that were not updated following recent taxonomic changes, and inaccurate or wrong specimen identification. To illustrate this problem, we cite here the whole mitogenome from a *M. muricola* specimen from Vietnam (Platt et al. 2018), which is wrongly labelled in the Genbank as “*Myotis atacamen-sis*”, an endangered species endemic to Chile. In the Online Resource 1, we list the downloaded sequences used in the phylogenetic reconstruction and give the original species name as it appears in the databank along with our revised species assignment.

Phylogenetic analyses

Because most publicly available sequences came from disparate specimens for both markers or lacked data for either gene, aligned sequences were analysed separately for the COI and CYTB genes. Phylogenetic relationships were estimated with maximum likelihood (ML) and Bayesian inferences (BI). All analyses were done with a fully partitioned model, the best-fitting model for these partitions being evaluated with Modelfinder (Kalyanamoorthy et al. 2017) and the Akaike Information Criterion. We inferred the ML tree using the edge-linked partition model in iQ-TREEv 2.0 (Nguyen et al. 2015; Chernomor et al. 2016) and obtained branch supports with 1000 non-parametric bootstrap resampling. For the BI, we run twice independently four parallel MCMC chains for 10 million generations obtained in MrBayes v3.2.0 (Ronquist and Huelsenbeck 2003). The chains were checked for convergence and appropriate effective sample size (ESS > 200) with TRACER v1.5 (Rambaut and Drummond 2009). The initial 10% of sampled trees were discarded as burn-in. Posterior probabilities (PP) were subsequently computed from the consensus of the remaining trees. For all molecular reconstructions we used three species of Indian *Miniopterus* (Table 1) as a composite outgroup to Myotinae.

Level of genetic divergence between sequences were evaluated with the Kimura two parameter model implemented in MEGA X (Kumar et al. 2018) for comparison with values

given for bats (Bradley and Baker 2001; Kruskop et al. 2012; Benda et al. 2016).

Results

Based on re-examination of the chiropteran holdings at ZSI Solan, we revised taxonomic identification of a few *Myotis* species from Himachal Pradesh (Saikia et al. 2011). The first revision pertains to a female specimen (registration number M-50) which was collected near Chamba, at 2480 m a.s.l. and was identified as “*M. muricola*” by Saikia et al. (2011). We captured six new individuals of the same taxon in various places of Himachal Pradesh, including in the forested areas of Mt. Karol, Sangla, and Narkanda. Externally, this small whiskered *Myotis* had a dense, shaggy dark brown fur. Individual hairs on the dorsum had light brown tips and the roots were blackish. The lighter tips gave a glossy sheen to the dorsal pelage. The ventral hairs also had dark roots and greyish brown tips but lacked the overall glossy appearance. The animals had a dark, hairy face with prominent bristle-like hairs and small feet with a wing insertion to the base of toe (Fig. 1a). However, contrary to the usual notched rear edge of ears found for instance in *M. mystacinus* or *M. muricola* (Fig. 1b), the deep emargination in the ears of this unusual taxon was even more pronounced and projected forwards as an arched lobe near the base of the ear (Fig. 1a). This peculiar emargination of the ear was noticed by Tomes as a typical attribute of *V. caliginosus* (Tomes 1859) and resembles the antitragus of *Rhinolophus*, as also mentioned by Dobson in the original description of *V. blanfordi* (Dobson 1871: 214). This ear lobe is also clearly visible in *Submyotodon latirostris* from Taiwan (Lin et al. 2004; Cheng et al. 2015) and on specimens of *V. moupinensis* from China (see Online Resource 4). The shape of the tragus also differs in being curved forwards and only tapering near the tip (Fig. 1a), reminiscent of many pipistrelles but unlike that of most *Myotis* species, the tragus of which being usually straight and tapering regularly towards the tip. In lateral view, the braincase of our specimens appears particularly flattened when compared to other species in the region (Fig. 2a); the skull also has a raised occipital part and pointed rostrum. This typical skull shape closely resembles that of the type of *V. moupinensis* (Fig. 1e) or *S. latirostris* (e.g. Figure 4a in Ruedi et al. 2015). The above three taxa also share a nyctalodont or semi-nyctalodont configuration of lower molars characterising the genus *Submyotodon* (Ziegler 2003), and all differ considerably from the skull of few other small nyctalodont Myotinae such as *M. alticraniatus* or *M. siligorensis* (Fig. 2d) (Kruskop and Borisenko 2013). The distinctive specimens from Himachal Pradesh, thus, correspond in all respects to *Vespertilio* (= *Submyotodon*) *blanfordi* described from the same area (Tomes 1859;

Table 1 Sequences of the CYTB (1140 bp) and COI (652 bp) mitochondrial genes from diverse Myotinae and outgroups generated in this study and with corresponding GenBank numbers

Species name	Sequence name	CYTB	COI	Voucher	Locality/Province	Country/Region
<i>Myotis alcaethoe</i>	Myalcaethoe M941	AJ841955	MW054896	MHNG 1828.073	Le Chenit, Vaud	SWITZERLAND
<i>M. alticraniatus</i>	Myalticraniatus HNHM2011 13 7	MW054869 ^S	MW054897	HNHM 2011.13.7	Zhejiang	S CHINA
<i>M. alticraniatus</i>	Myalticraniatus HNHM23772	MW054871 ^S		HNHM 2011.13.8	Jiangxi	S CHINA
<i>M. alticraniatus</i>	Myalticraniatus HNHM23765	MW054870 ^S	MW054898	HNHM 23.765	Guizhou	S CHINA
<i>M. annatessae</i>	Myannatessae M1200	KF312514	MW054899	MHNG 1926.044	Hat Hin, Phongsaly	N LAOS
<i>M. cf. annatessae</i>	Mycf_annatessae HNHM25348	MW054889	MW054927	HNHM 25.348	Jiangxi	S CHINA
<i>Myotis badius</i>	Mybadius HNHM25839	MW054890	MW054928	HNHM 25.839	Fagudian, Yunnan	S CHINA
<i>Myotis badius</i>	Mybadius HNHM25847	MW054891	MW054929	HNHM 25.847	Fagudian, Yunnan	S CHINA
<i>M. blythii</i>	Myblythii M2206	MW054872	W054901	ZSI VMERS 405	Barog, Himachal Pradesh	NW INDIA
<i>M. csorbai</i>	Mycsorbai HNHM97 2 1	MW054873 ^S		HNHM 97.2.1	Syangja	NEPAL
<i>M. csorbai</i>	Mycsorbai HNHM97 2 3	MW054874 ^S		HNHM 97.2.3	Syangja	NEPAL
<i>M. daubentonii</i>	Mydaubentonii M1861		MW054904	MHNG 1989.005	Vich, Vaud	SWITZERLAND
<i>M. davidii mongolicus</i>	Mydavidii mongolicus M1260		MW054905	MHNG 1933.092	Boon Tsagaan Nuur, Bayan-hongor	MONGOLIA
<i>M. fimbriatus</i>	Myfimbriatus HNHM25872	MW054892	MW054930	HNHM 25.872	Fagudian, Yunnan	S CHINA
<i>M. fimbriatus</i>	Myfimbriatus HNHM25898	MW054893	MW054931	HNHM 25.898	Dali, Yunnan	S CHINA
<i>M. laniger</i>	Mylongipes M1636		MW054909	M1636 no voucher	Lobang, Assam	NE INDIA
<i>M. laniger</i>	Mylongipes M1937		W054910	M1937 no voucher	Shella, Meghalaya	NE INDIA
<i>M. laniger</i>	Mylongipes M1934			M1934 no voucher	Shella, Meghalaya	NE INDIA
<i>M. laniger</i>	Mylaniger M1606	MW054875	MW054906	MHNG 1981.074	Kseh, Meghalaya	NE INDIA
<i>M. laniger</i>	Mylaniger Myl Yunnan	MW054877		My1 no voucher	Mile, Yunnan	S CHINA
<i>M. laniger</i>	Mycflanager M629		MW054903	THUMB T-3474	Heping, Taichung	TAIWAN
<i>M. longipes</i>	Mylongipes M2210	MW054878	MW054907	ZSI VMERS 438	Mt. Karol, Himachal Pradesh	NW INDIA
<i>M. longipes</i>	Mylongipes M2219	MW054879	MW054908	ZSI VMERS 442	Mt. Karol, Himachal Pradesh	NW INDIA
<i>M. longipes</i>	Mylongipes HNHM92 104 40	MW054876 ^S		HNHM 92.104.40	Kashmir	NW INDIA
<i>M. muricola</i>	Mymuricola M1567		MW054911	MHNG 1970.062	Jertih, Terengganu	MALAYSIA
<i>M. muricola</i>	Mymuricola M1570		MW054912	MHNG 1970.065	Ulu Gombak, Selangor	MALAYSIA
<i>M. muricola</i>	Mymuricola M1600		MW054913	MHNG 1972.084	Perlabuhan Ratu, Cimaja	JAVA
<i>M. muricola</i>	MycfaterMYOT4		W054902	SJR MOYT4 (no voucher)	Longmen, Guangdong	S CHINA
<i>M. muricola</i>	Myaterssp M1165	KF312509	MW054900	MHNG 1926.036	Long Nai Mai, Phongsaly	LAOS
<i>M. mystacinus</i>	Mymystacinus M701		MW054915	MHNG 1807.033	Pili, Macedonia	GREECE
<i>M. mystacinus</i>	Mymystacinus M803		MW054916	MHNG 1807.035	Rendina, Macedonia	GREECE
<i>M. mystacinus</i>	Mymystacinus M2275		MW054914	MHNG 3002.087	Morzier, Haute-Savoie	FRANCE
<i>M. mystacinus</i>	MymystacinusM2291	MW054880		MHNG 3003.057	Bischofszell, Thurgovie	SWITZERLAND
<i>M. nipalensis</i>	Mynipalensis CDZTU B9	MW054894	MW054917	CDZTU B9	Lalitpur	NEPAL
<i>M. nipalensis</i>	Mynipalensis M2199	MW054881	MW054918	ZSI VMERS 445	Solan, Himachal Pradesh	NW INDIA
<i>M. siligorensis</i>	Mysiligorensis 5 Myo	MW054882	MW054919	5 Myo sili (no voucher)	Shillong, Meghalaya	NE INDIA
<i>M. siligorensis</i>	Mysiligorensis M1908		MW054920	VMERS 600	Lobang, Assam	NE INDIA
<i>M. siligorensis</i>	Mysiligorensis M1637	W054883	MW054920	VMERS 601	Lobang, Assam	NE INDIA
<i>Submyotodon caliginosus</i>	Sucaliginosus M2221	MW054885	MW054921	ZSI VMERS 417	Sangla, Himachal Pradesh	NW INDIA
<i>S. caliginosus</i>	Sucaliginosus M2217	MW054884	MW054922	ZSI VMERS 421	Mt Karol, Himachal Pradesh	NW INDIA
<i>S. latirostris</i>	Sulatirostris M2138		MW054923	MHNG 1998.052	Syuejian, Miaoli	TAIWAN
<i>S. moupinensis</i>	Sumoupinensis HNHM26564	MW054895	MW054932	HNHM 26.564	Xishuangbanna, Yunnan	S CHINA
<i>Miniopterus fuliginosus</i>	Mifuliginosus M2262	MW054886	MW054924	ZSI M2262	Solan, Himachal Pradesh	NW INDIA
<i>Mi. magnater</i>	Mimagnater M1614	MW054887		MHNG 1981.071	Kseh, Meghalaya	NE INDIA
<i>Mi. magnater</i>	Mimagnater M1928		MW054925	MHNG 1991.079	Lakadong, Meghalaya	NE INDIA
<i>Mi. pusillus</i>	Mipusillus M2036	MW054888	MW054926	ZSI VMERS 570	Umlyngsha, Meghalaya	NE INDIA

An ^S indicates CYTB sequences for which only the initial 410–413 bp could be sequenced due to degraded conditions of the tissue samples

Dobson 1871) or to its senior synonym *V.* (= *Submyotodon*) *caliginosus*, also described from this part of India.

Specimens of genuine *M. muricola* from Central Nepal and North-eastern India (Table 1) could be easily distinguished externally by their larger dimensions, by a combination of relatively uniform dark-brown fur (Fig. 1b), small ears and feet, and strong canines (significantly exceeding the height of third upper premolars, Fig. 2b), as noted by Benda (2010). The lower molars had a myotodont configuration, as observed in the examined type specimen.

Another misidentified specimen included in the ZSI Solan collections is a female individual (No. M-38) collected in the city of Solan (at 1500 m a.s.l.) and reported as “*M. siligorensis*” by Saikia et al. (2011). This specimen had a whitish ventral pelage (albeit barely discernible due to prolonged exposure to excessive UV-light), a globose braincase without sharply raising frontal parts and a relatively strong dentition, which are all characters excluding genuine *siligorensis* (Bates and Harrison 1997) but instead are typical for another poorly known Himalayan species, *M. nipalensis*. We captured two adults of this same taxon with mist nets set over the Narag River, 12 km SE of Solan. One was a pregnant female, which was quickly released after basic measurements and the other, a male, kept as a voucher for detailed morphological examination (Fig. 1c). The pelage of these animals was characterized by light brown dorsum and extensive whitish ventrum (usually only slightly lighter in other Himalayan Myotinae), strong but relatively small feet with wing membranes attaching to the base of the outer toe, and unnotched ears (Fig. 1c). Thumbs were thin and relatively long. The skull had a globose braincase, a slight concavity in the frontal parts when viewed in profile and a much-elevated coronoid process compared to the condyloid process (Fig. 2c). The dentition was relatively weak, however, not to the extent as seen in *M. siligorensis* where the canine hardly exceeds the length of the third upper premolar (Fig. 2d). The lower molars were all myotodont, like in most other *Myotis*. These morphological features concur very well with the original descriptions of *M. nipalensis* (e.g. Dobson 1871, 1878) and are in contradiction to the key characters (Bates and Harrison 1997) given for *M. siligorensis*.

Indeed, specimens of genuine *M. siligorensis* captured further east in North-eastern India (c. 400 km away from type-locality of that species) and housed in the ZSI Shillong collection (Table 1) were different. Although they superficially resembled *M. muricola* or *M. nipalensis*, they were darker and had only a slightly lighter venter without the prominent white fur typical of the latter (Fig. 1d) and smaller body dimensions than the former. They differed from the similar-sized *S. caliginosus* by their relatively larger feet and thumbs (compare Fig. 1a and d) and by the ears lacking deep emargination to the outer edge. The wing insertion, as in the other “whiskered” *Myotis*, was to the base of the outer

toe. Craniodental characteristics of *M. siligorensis* were very distinctive, with a weak dentition (particularly the tiny, conical lower canines, see Benda 2010), sharply raised frontals and a globose braincase (Fig. 2d). The first two lower molars (m1 and m2) were nyctalodont but the last one (m3) was myotodont, in conformity with the type of *M. siligorensis* examined.

Finally, we also re-examined the series of specimens housed in the ZSI Solan collection identified initially as “*M. mystacinus*” by Saikia et al. (2011). They were all captured around Solan and apparently were relatively common. The relatively long feet and wing membrane attaching to the ankle immediately indicated that this taxon does not belong to a species from the *mystacinus*-morphogroup, but to a typical trawling *Myotis* in the *longipes-laniger* group (Topál 1997). Compared to the series of Kashmiri *M. longipes* used by Topál in his revision of the group (Topál 1997), these Himachal Pradesh specimens proved to be morphologically identical (albeit somewhat darker). As these bats could be easily distinguished by external characters from true members of “whiskered” *Myotis*, they were not included in the following morphometric analyses.

Multivariate morphological comparisons

Global PCA analyses of craniodental variation of Indomalayan whiskered bats suggest that size was correlated with the main component, which accounted for 70% of the total variance (Fig. 3). Indeed, all variables were highly and positively correlated with this first component. Specimens were thus ordered accordingly along this component, the skulls of the smaller species (*alticraniatus*) appearing on the negative side, and the larger ones (*muricola*) on the positive side of the axis (Fig. 3). The second component accounted for 11.3% of the total variance and separated essentially the *Myotis* from the *Submyotodon* species with little overlap between both taxa in this morphospace. The skull of nyctalodont *M. alticraniatus* were thus strongly differentiated from all *Submyotodon* individuals possessing similar cusp structure on lower molars.

To focus on the more difficult groups, we repeated this PCA on the *Myotis* species only. For this second analysis, the first axis (83.3% of variance) was also essentially a size component, which ordered the measured skulls according to overall size (Fig. 4). The second axis (6.8% of variance) was more strongly correlated with two measurements expressing the broadness of skull (BB and POC). According to this analysis, all *M. muricola* specimens were set in a distinct group, while those identified as *M. davidii* and *M. nipalensis* stood out owing to their relatively broader skulls. This morphospace separated most *M. alticraniatus* from the skulls of other small species (*M. siligorensis* and *M. annatessae*), while the single *M. phanluongi* was even more distinct. It is

Fig. 1 Dorsal, ventral and portrait views of different Myotinae from northern India and Southeast Asia. **a** *Submyotodon caliginosus* (voucher M2217, ZSI VMERS421), Mt Karol, Himachal Pradesh; **b** *Myotis muricola* (M1570, MHNG1970.065), Peninsular Malaysia; **c** *Myotis nipalensis* (voucher M2199, ZSI VMERS445), Solan, Himachal Pradesh; **d** *Myotis siligorensis* (voucher M1908, MHNG1991.083), Jaintia Hills, Meghalaya. Animals are not shown to scale

interesting to note that the five intact skulls of *M. siligorensis* from the Himalayas and the three skulls of *M. annatesae* analysed here were very similar in this morphospace, although both possess distinct lower molar configurations (nyctalodont versus myotodont, respectively) (Kruskop and Borisenko 2013).

Molecular analyses

We successfully generated 26 new sequences of CYTB and 40 of COI from a total of 48 specimens (Table 1). Except for the older specimens from the HNHM collections for which we could only amplify a shorter segment (about

Fig. 2 Skull and mandible of a selection of Myotinae from the Indomalayan Region. **a** *Submyotodon caliginosus* (voucher M2217, ZSI VMERS421), Mt Karol, Himachal Pradesh; **b** *Myotis muricola* (ZSI VMERS450), Tamenglong, Manipur; **c** *Myotis nipalensis* (voucher M2199, ZSI VMERS445), River Narag, Solan, Himachal Pradesh; **d** *Myotis siligorensis* (voucher M1637, MHNG1981.073), Jaintia Hills, Meghalaya; **e** *Submyotodon moupinensis* (paratype, voucher MNHN 1870-589A), Tibet, China; **f** *Myotis alticraniatus* (voucher N196, MHNG 1956.091), Xieng Khouang, Laos PDR. Some images were rotated to match orientations in all specimens, but all are shown to scale

Fig. 3 Principal component analysis based on eleven craniodental and mandibular measurements taken in selected Asian taxa of *Myotis* and *Submyotodon*. Due to entirely missing data for some taxa, the lower tooththrow length (cm3L) was excluded from this analysis. Besides species of *Submyotodon*, taxa possessing nyctalodont configuration of lower molars include *M. alticraniatus*, *M. phanluongi*, and *M. siligorensis*

400 bp) of the CYTB gene, these sequences were 1140 bp and 652 bp long for the CYTB and COI, respectively. All new sequences were deposited in the GenBank under numbers MW054869-MW054932 (Table 1).

For both markers, mean intraspecific comparisons within the Himalaya were within 1% of sequence divergence (K2P model; Online Resources 2 and 3). Species sampled over wider geographic areas were more distinct, with mean K2P genetic distances ranging up to 5% within *M. muricola* sensu lato (s.l.). Comparisons involving distinct species were usually higher than 9%. Exceptions for the CYTB dataset included the two partial sequences of paratypes of *M. csorbai* from Nepal that were almost identical to the western Himalayan *M. longipes* (K2P 0.2–0.5%), topotypes of *M. badius* and *M. alticraniatus* from China (<1%), or *M. annamiticus* and some *M. laniger* (down to 2.2%). These pairwise comparisons were much below what is expected for inter-specific comparisons (Baker and Bradley 2006). Within the genus *Submyotodon* inter-specific distances ranged from 9 to 14% for both mitochondrial markers, whereas they were higher when compared to sequences of *Myotis* (16 to 23% K2P distance).

Phylogenetic relationships of the Myotinae inferred with the different methods (BI and ML) and for each gene were mostly congruent (Figs. 5 and 6). Discordances were essentially due to the poorly resolved, deeper nodes (with less than 75% support), indicating either that the datasets were

Fig. 4 Principal component analysis of twelve craniodental and mandibular measurements taken in seven representative species of small Asian *Myotis*

insufficient to resolve these ancient nodes, or that basal relationships in these Myotinae reflect unresolved polytomies (Whitfield and Lockhart 2007). One consistent relationship was the basal position of the strongly monophyletic *Submyotodon* clade, which branched off early in the Myotinae radiation. Crown groups were much better resolved and concordant between both genes. The focal taxa from the Himalayas were all placed with strong support in the following clades. The diverse clade comprising the samples of *M. muricola* s.l. was monophyletic and formed a sister group relationship with another group of closely related taxa including *M. nipalensis*, *M. annatessae* (Fig. 5) and *M. ancricola* (Fig. 6). Indian samples of *M. siligorensis* were firmly placed as a sister species of *M. longipes* with strong support in both genes. The latter species included the two partial CYTB sequences of *M. csorbai* as well, supporting their close relationship (Topál 1997). The other taxa often associated phenetically with *M. siligorensis* (i.e. *alticraniatus*, *phanluongi* or *badius*), however, were placed in a very distinct and strongly supported clade that included *M. laniger* and *M. annamiticus*. The geographically widespread samples of *M. davidii* formed a well-supported monophyletic clade that was related to *M. mystacinus* and to *M. ikonnikovii* (Figs. 5 and 6). This *M. davidii* clade included notably the two partial sequences of CYTB (AY699844) and COI (JX008074) considered as “*M. nipalensis*” by Tsytsulina et al. (2012) and Kruskop et al. (2012), respectively.

Discussion

Concordant with previous molecular reconstructions based on multiple mitochondrial and nuclear markers (Lack et al. 2010; Ruedi et al. 2015; Morales et al. 2019), nyctalodont or semi-nyctalodont species of Myotinae do not form a monophyletic unit as the three *Submyotodon* species and the few Asian *Myotis* taxa showing this dental character (*siligorensis*, *alticraniatus*, *phanluongi*, *badius*) (Tiunov et al. 2011) were phylogenetically unrelated (Figs. 5 and 6). Clearly, the special molar configuration in these small Myotinae evolved several times independently and may not serve as a good indicator of phylogenetic relationship in this subfamily. Re-examination of the external morphology of the three known species of recent *Submyotodon* also indicates that they can easily be distinguished from true *Myotis* by their unique shape of the ears: they all possess a prominent, arched lobe to the rear edge projecting forwards at a right angle (Fig. 1a and Online Resource 4). This arched lobe was not observed in any *Myotis* and thus provides an external diagnostic character to identify members of *Submyotodon* in the field. Genetically, all three species of *Submyotodon* examined here differ conspicuously from one another (9.4–11.9%; Online Resources 2 and 3), which supports their specific distinction.

A further sample referred here as *S. cf. moupinensis* sampled north of the Qinling mountains in central China (Online Resource 1) differs genetically from these three species, suggesting additional taxonomic diversity in this poorly known genus.

Submyotodon caliginosus appeared to be relatively common in parts of Himachal Pradesh (Dobson 1871; present study), Uttarakhand (Chakravarty et al. 2020) and Sikkim (India), and also in Langtang Himal (Nepal; material in HNHM). This species also occurs in Punjab and Khyber Pakhtunkhwa, Pakistan (material in BMNH and HZM, respectively) and according to Benda and Gaisler (2015) occurs as far west as the Hindu Kush mountains in eastern Afghanistan, suggesting that the species is relatively widespread in the southern slopes of the Himalayas. On the other hand, *S. moupinensis* is probably much rarer and known from a few specimens recorded in southern China (Szechwan, Yunnan and Fujian) (Milne-Edwards 1872; Allen 1938), whereas *S. latirostris* is quite common but endemic to the mountains of Taiwan (Cheng et al. 2015; Ruedi et al. 2015). Museum collections holding potential representatives of these poorly known species need to be re-examined, to provide realistic distribution ranges for all of them.

Besides the distinctive *Submyotodon* species, our phylogenetic reconstructions showed that a number of small Asian *Myotis* characterized by a raised frontal part of the skull, doomed braincase, very small canines (Fig. 2) and nyctalodont or semi-nyctalodont lower molars, and formerly collectively known as *M. siligorensis* s.l. (e.g. Kruskop and Borisenko 2013) did not form a natural grouping. Indeed, genuine (North-east Indian) *M. siligorensis* analysed here for the first time shared no genetic similarities with other Asian species usually included in this group (i.e. *M. alticraniatus*, *M. phanluongi*, *M. badius* and by extension *M. thaianus*). This again suggests that similar craniodental traits shared by *M. siligorensis* s. str. and the latter species result from convergent morphological evolution.

Taxonomically, these new morphological and phylogenetic results imply that *M. siligorensis* s. str. (including the junior synonym *darjelingensis* Horsfield, 1855) must be considered as a species on its own. Its distribution range is thus probably restricted to Central and Eastern Himalaya, including parts of India, Nepal and Myanmar (Bates et al. 2005). The other taxa found further east into China and Indochina should be referred to as *M. alticraniatus* or to the allied taxa *M. thaianus*, *M. phanluongi* and *M. badius*. Sequences from topotypes of *M. badius* are intermingled among various geographic lineages of *M. alticraniatus* (Figs. 5 and 6) suggesting that they represent variants of a single species. Notice that all Chinese sequences available in the GenBank and labelled as “*M. davidii*”, including a complete mitochondrial genome (KM233172; Wang et al. 2016), certainly represent *M. alticraniatus* as well (see Online Resource 2) as they are

Fig. 5 Maximum Likelihood tree recovered with iQTree from the alignment of 1140 bp of the CYTB gene. Strongly supported nodes for both ML and BA reconstructions (i.e. $\geq 95\%$ bootstrap and posterior probability support, respectively) are indicated with a black dot, while nodes with moderate support (comprised between 75 and 94%) are symbolized with a greyed circle; other nodes with no statistical support in either analysis (i.e. $< 75\%$) are left unmarked

all within 0–5% of sequence divergence compared to our reference data in this group. Genuine *M. davidii*, as redefined by Benda and Gaisler (2015) and as understood here, are part of another, much more divergent (13–18%) clade related to *M. mystacinus* (Morales et al. 2019; Coraman et al. 2020). The main phylogenetic relationships presented here (Figs. 5 and 6) are largely corroborated by more global molecular

analyses of over 100 *Myotis* species and based on a much denser sampling of up to 1610 nuclear loci (Morales et al. 2019), and hence unlikely to reflect only particular relationships of the mitochondrial genome.

The widespread and polytypic species *M. davidii* itself has also been the source of much taxonomic confusion. This Chinese taxon, originally described from Beijing, is quite

Fig. 6 Maximum Likelihood tree recovered with iQTree from the alignment of 652 bp of the COI gene. Node support illustrated as in Fig. 5

distinctive morphologically (Dobson 1878; Allen 1938; Benda and Gaisler 2015; Benda et al. 2016), but fell into oblivion when it was included in the long list of purported synonyms of “*M. mystacinus*” (e.g. Findley 1972) or ignored in global revision of Asian *Myotis* (e.g. Corbet and Hill 1992). The status of this taxon was revised by Benda and colleagues with a combination of morphologic and genetic

characters, first under “*M. nipalensis*” (Benda and Tsytsulina 2000), then under “*M. aurascens*” (Tsytsulina et al. 2012) and finally as *M. davidii* (Benda et al. 2016). Although we concur with these revisions that *M. davidii* is a well-defined species that is morphologically and genetically distinct from any other species in the *mystacinus* morphogroup (Zhigalin 2019), we demonstrate here that the overlooked

Indian congener *M. nipalensis* is different from *M. davidii*, as opposed to the suggestion of Benda et al. (2016). Indeed, the genetic material analysed here represent for the first time genuine *M. nipalensis* and shows that it is phylogenetically very distinct from any other *Myotis*, including *M. davidii* (Figs. 5 and 6). Its closest relatives appear to be members of the Indochinese *M. annatessae* s. l. (at about 8% sequence divergence; Online Resources 2 and 3). Morphologically, the examined specimens from India and Nepal perfectly fit to the very detailed description given by Dobson for the type of *M. nipalensis* (Dobson 1878). Likewise, records of “*M. mystacinus*” from the Indian Subcontinent (Bates and Harrison 1997) and Myanmar (Bates et al. 2005) also fit to the characters of genuine *M. nipalensis* and should all be reassigned to that species. According to this new taxonomic view, *M. nipalensis* is a typical species of the southern slopes of the Himalayan region but its precise geographic range needs to be evaluated with more museum material.

The novel systematics arrangement of *M. nipalensis* proposed here also calls into question the possible occurrence of *M. “annatessae”* s. l. into the Himalayas and the specific distinction of the closely related *M. anricola* (Kruskop and Borisenko 2013; Kruskop et al. 2018). Indeed, none of these new taxa were adequately compared with genuine *M. nipalensis* as the later was wrongly assumed to be related to *M. davidii*. Our phylogenetic results rather suggest that *M. nipalensis*, *M. annatessae*, *M. cf. annatessae* and *M. anricola* are genetically remarkably similar (Online Resources 2 and 3), despite considerable geographic distances separating the samples. Compared to genetic distances observed in other *Myotis* bats, these taxa should be considered conspecific. Morphologically they also appeared similar in our PCA analyses (Fig. 5), but specific comparisons involving the type material should be done to support this taxonomic interpretation.

Given the accumulation of confused or wrong species identifications associated with sequences deposited in the GenBank (Bidartondo et al. 2008; Leray et al. 2019), a more general consequence of the taxonomic changes proposed here for several Myotinae of the Himalayas is that it becomes urgent to curate such useful genetic databases with more taxonomic attention. It is indeed more and more popular to identify species from difficult groups such as *Myotis* bats with barcode approaches (Francis et al. 2010; Clare et al. 2011), including in epidemiological studies of zoonotic diseases (Nadin-Davis et al. 2012; Mohammadi et al. 2019), but such approaches depend entirely on the reliability of taxonomic identification of reference sequences. Unfortunately, with an exponentially increasing number of submitted sequences with questionable species tagging, it becomes even harder now to evaluate the accuracy of these reference databases. The BOLD system partly solved this problem by building a reference system

based on barcode index numbers—BIN (Ratnasingham and Hebert 2013) rather than on binomial names, which group similar sequences together in a MOTU, regardless of their actual species identification. Still, when we submitted to the BOLD identification machine (as of 18/07/2020) our COI sequence of *Myotis badius* topotypes, no less than four different species (*daubentonii*, *mystacinus*, *siligorensis* and *davidii*) received a 100% probability of correct placement, none of which corresponding to the correct identification of that sequence. This system is furthermore largely restricted to sequences of the COI gene, impeding critical comparisons with other genes. GenBank has a far greater scope of genetic markers (Porter and Hajibabaei 2018), but because there is currently no possibility to correct or even flag problematic species assignation of sequences, it may actually become increasingly misleading to rely on it for automatized barcoding approaches (Shen et al. 2013). For instance, in a recent review evaluating species-level genetic diversity for mammalian species based on CYTB, Schrago and Mello (2020) provided practical guidelines to improve the performance of unsupervised algorithms for species delimitation. To highlight species deserving attention for possible cryptic lineages, they give the example of the endangered *M. atacamensis* showing “unusually high genetic differences” in that Neotropical species, overlooking the fact that one of the two reference sequences taken from the Genbank (MF143493; Platt et al. 2018) is actually a mislabelled *M. muricola* (see Online Resource 1), hence inflating their “intra-specific” statistics.

As shown in this study, a key to the success in resolving long-standing taxonomic riddles among Indomalayan Myotinae is the inclusion of new genetic material from near the type localities of these taxa. We think this approach should be applied to solve other taxonomic problems in the Indomalayan fauna or flora. Given the rapidly accelerating habitat degradation (Betts et al. 2017) and the possible loss of these unique elements of biodiversity (Ceballos and Ehrlich 2009), this should be considered as a priority area of research.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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References

- Allen GM (1938) The mammals of China and Mongolia vol 11, part. 1. Natural history of Central Asia. The American Museum of Natural History, New York
- Baker RJ, Bradley RD (2006) Speciation in mammals and the genetic species concept. *J Mammal* 87:643–662
- Bates PJJ, Harrison DL (1997) Bats of the Indian Subcontinent, vol 43. Harrison Zoological Museum, Sevenoaks
- Bates PJJ et al (2005) A review of the genera *Myotis*, *Ia*, *Pipistrellus*, *Hypsugo*, and *Arielulus* (Chiroptera: Vespertilionidae) from Myanmar (Burma), including three species new to the country. *Acta Chiropterol* 7:205–236
- Benda P (2010) On a small collection of bats (Chiroptera) from western Sabah (North Borneo, East Malaysia). *Vespertilio* 13–14:45–76
- Benda P, Gaisler J (2015) Bats (Mammalia: Chiroptera) of the Eastern Mediterranean and Middle East. Part 12. Bat fauna of Afghanistan: revision of distribution and taxonomy*. *Acta Soc Zool Bohem* 79:267–458
- Benda P, Tsytsulina KA (2000) Taxonomic revision of *Myotis mystacinus* group (Mammalia: Chiroptera) in the western Palearctic. *Acta Soc Zool Bohem* 64:331–398
- Benda P et al (2012) Bats (Mammalia: Chiroptera) of the Eastern Mediterranean and Middle East. Part 10. Bat fauna of Iran. *Acta Soc Zool Bohem* 76:163–582
- Benda P, Gazaryan S, Vallo P (2016) On the distribution and taxonomy of bats of the *Myotis mystacinus* morphogroup from the Caucasus region (Chiroptera: Vespertilionidae). *Turk J Zool* 40:842–863. <https://doi.org/10.3906/zoo-1505-47>
- Betts MG et al (2017) Global forest loss disproportionately erodes biodiversity in intact landscapes. *Nature* 547:441–444. <https://doi.org/10.1038/nature23285>
- Bidartondo MI et al (2008) Preserving accuracy in GenBank. *Science* 319:1616a. <https://doi.org/10.1126/science.319.5870.1616a>
- Bobrinskoy N (1929) Bats of Central Asia. *Ežegod Zool Muz Akad* 1929:217–244
- Borisenko AV, Kruskop SV, Ivanova NV (2008) A new mouse-eared bat (Mammalia: Chiroptera: Vespertilionidae) from Vietnam. *Russian J Theriol* 7:57–69
- Bradley R, Baker R (2001) A test of the genetic species concept: cytochrome b sequences and mammals. *J Mammal* 82:960–973
- Ceballos G, Ehrlich PR (2009) Discoveries of new mammal species and their implications for conservation and ecosystem services. *Proc Natl Acad Sci USA* 106:3841–3846. <https://doi.org/10.1073/pnas.0812419106>
- Chakravarty R, Ruedi M, Ishtiaq F (2020) A recent survey of bats with descriptions of echolocation calls and new records from the western Himalayan region of Uttarakhand, India. *Acta Chiropterol* 22:197–224
- Cheng HC, Fang YP, Chou CH (2015) A photographic guide to the bats of Taiwan, 2nd edn. The Agriculture Committee Taiwan Endemic Species Research Institute, Taipei (**[in Chinese]**)
- Chernomor O, von Haeseler A, Minh BQ (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Syst Biol* 65:997–1008. <https://doi.org/10.1093/sysbio/syw037>
- Clare EL, Lim BK, Fenton MB, Hebert PDN (2011) Neotropical bats: estimating species diversity with DNA barcodes. *PLoS ONE* 6:e22648
- Coraman E, Dundarova H, Dietz C, Mayer F (2020) Patterns of mtDNA introgression suggest population replacement in Palaearctic whiskered bat species. *R Soc open sci* 7:191805. <https://doi.org/10.1098/rsos.191805>
- Corbet GB, Hill JE (1992) The mammals of the Indomalayan region: a systematic review. Oxford University Press, Oxford
- Dobson GE (1871) Notes on nine new species of Indian and Indo-Chinese Vespertilionidae, with remarks on the synonymy and classification of some other species of the same family. *Proc Asiat Soc Bengal* 1871:210–215
- Dobson GE (1878) Catalogue of the Chiroptera in the collection of the British Museum. Order of the Trustees. Taylor & Francis, London
- Ellerman JR, Morrison-Scott TCS (1951) Checklist of Palaearctic and Indian mammals, 1758 to 1946, 1st edn. Tonbridge Printers LTD., Tonbridge
- Ellerman JR, Morrison-Scott TCS (1966) Checklist of Palaearctic and Indian mammals, 1758 to 1946, 2nd edn. Alden Press, Oxford
- Findley JS (1972) Phenetic relationships among bats of the genus *Myotis*. *Syst Zool* 21:31–52
- Francis C et al (2010) The role of DNA barcodes in understanding and conservation of mammal diversity in Southeast Asia. *PLoS ONE* 5:e12575. <https://doi.org/10.1371/journal.pone.0012575>
- Hassanin A et al (2012) Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. *CR Biol* 335:32–50. <https://doi.org/10.1016/j.crv.2011.11.002>
- Hill JE (1983) Bats (Mammalia: Chiroptera) from Indo-Australia. *Bull Br Museum (Natural History) Zool* 45:103–208
- Hodgson BH (1835) Synopsis of the Vespertilionidae of Nepal. *J Asiat Soc Bengal* 4:699–701
- Horsfield T (1855) Brief notices of several new or little-known species of mammalia, lately discovered and collected in Nepal, by Brian Houghton Hodgson, Esq. *Annals Mag Nat Hist* 16:101–114
- Howell AB (1926) Three new mammals from China. *Proc Biol Soc Wash* 39:137–140
- Ibáñez C, García-Mudarra JL, Ruedi M, Stadelmann B, Juste J (2006) The Iberian contribution to cryptic diversity in European bats. *Acta Chiropterol* 8:277–297. [https://doi.org/10.3161/1733-5329\(2006\)8\[277:TICTCD\]2.0](https://doi.org/10.3161/1733-5329(2006)8[277:TICTCD]2.0)
- Ivanova N, Zemlak T, Hanner R, Hebert P (2007) Universal primer cocktails for fish DNA barcoding. *Mol Ecol Notes* 7:544–548

- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 14:587–589. <https://doi.org/10.1038/nmeth.4285>
- Kishida K (1932) Notes on a Formosan whiskered bat. *Lansania* 4:153–160
- Koopman KF (1994) Chiroptera: Systematics. In: Niethammer J, Schliemann H, Starck D (eds) *Handbuch der Zoologie*, vol 8. de Gruyter, Germany, pp 100–109
- Kruskop SV, Borisenko AV (2013) A new species of South-East Asian *Myotis* (Chiroptera: Vespertilionidae), with comments on Vietnamese ‘Whiskered bats.’ *Acta Chiropterol* 15:293–305. <https://doi.org/10.3161/150811013x678937>
- Kruskop SV, Borisenko AV, Ivanova NV, Lim BK, Eger JL (2012) Genetic diversity of northeastern Palaearctic bats as revealed by DNA barcodes. *Acta Chiropterol* 14:1–14. <https://doi.org/10.3161/150811012x654222>
- Kruskop SV, Borisenko AV, Dudorova AV, Artyushin IV (2018) Description of a new Indochinese *Myotis* (Mammalia: Chiroptera: Vespertilionidae), with additional data on the “*M. annatessae*” species complex. *Russian J Theriol* 17:17–31
- Kuhl H (1817) *Die deutschen Fledermäuse*. Hanau, Deutschland
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35:1547–1549
- Kuzjakín AP (1935) Novye dannye po sistematike i geografičeskomu rasprostraneniú letučih myšej (Chiroptera) v SSSR (in Russian with a summary in German). *Bull Mosk Obš Ispyt Prir Otd Biol* 44:428–438
- Lack JB, Roehrs ZP, Stanley CE Jr, Ruedi M, Van Den Bussche RA (2010) Molecular phylogenetics of *Myotis* indicate familial-level divergence for the genus *Cistugo* (Chiroptera). *J Mammal* 91:976–992. <https://doi.org/10.1644/09-Mamm-a-192.1>
- Leray M, Knowlton N, Ho SL, Nguyen BN, Machida RJ (2019) GenBank is a reliable resource for 21st century biodiversity research. *Proc Natl Acad Sci USA* 116:22651–22656. <https://doi.org/10.1073/pnas.1911714116>
- Lin LK, Lee LL, Cheng HC (2004) *Bats of Taiwan* [in Chinese], 2nd edn. National Museum of Natural Sciences, Taipei
- Menu H, Sigé B (1971) Nyctalodontie et myotodontie, importants caractères de grades évolutifs chez les chiroptères entomophages. *CR Acad Sci* 272:1735–1738
- Milne-Edwards A (1872) *Mémoire de la faune mammalogique du Tibet Oriental et principalement de la principauté de Moupin*. In: Milne-Edwards H (ed) *Recherches pour servir à l'histoire naturelle des mammifères comprenant des considérations sur la classification de ces animaux*, vol 1. G. Masson, Paris, pp 231–304
- Mohammadi Z, Shahabi S, Ghorbani F, Khajeh A (2019) Efficiency of the mitochondrial DNA markers (COI, cyt b) and a nuclear DNA marker (RAG1) in molecular identification of zoonotic diseases’ hosts. *J Health Sci Surv Syst* 7:86–93. <https://doi.org/10.30476/jhss.2020.84774.1042>
- Morales AE, Ruedi M, Field K, Carstens BC (2019) Diversification rates have no effect on the convergent evolution of foraging strategies in the most speciose genus of bats, *Myotis*. *Evolution* 73:2263–2280. <https://doi.org/10.1111/evo.13849>
- Nadin-Davis SA, Guerrero E, Kimberley Knowles M, Feng Y (2012) DNA barcoding facilitates bat species identification for improved surveillance of bat-associated rabies across Canada. *Open Zool J* 5:27–37. <https://doi.org/10.2174/1874336601205010027>
- Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 32:268–274. <https://doi.org/10.1093/molbev/msu300>
- Norusis MJ (1993) *SPSS for Windows: base system user’s guide*, release 6.0. Chicago
- Olson DM et al (2001) *Terrestrial Ecoregions of the World: a new map of life on earth: a new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity*. *Bioscience* 51:933–938
- Orme CDL et al (2005) Global hotspots of species richness are not congruent with endemism or threat. *Nature* 436:1016–1019
- Osgood WH (1932) *Mammals of the Kelley-Roosevelt and Delacour Asiatic expedition*. *Field Mus Nat Hist* 18:191–339
- Peters W (1869) *Bemerkungen über neue oder weniger bekannte Fledertiere, besonders des Pariser Museums*. *Ber Akad Wiss Berlin* 1869:392–406
- Platt RN et al (2018) Conflicting evolutionary histories of the mitochondrial and nuclear genomes in New World *Myotis* bats. *Syst Biol* 67:236–249. <https://doi.org/10.1093/sysbio/syx070>
- Porter TM, Hajibabaei M (2018) Over 2.5 million COI sequences in GenBank and growing. *PLoS ONE* 13:e0200177. <https://doi.org/10.1371/journal.pone.0200177>
- Rambaut A, Drummond AJ (2009) *Tracer v1.5*. Available from <https://beast.bio.ed.ac.uk/Tracer>
- Ratnasingham S, Hebert PDN (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0066213>
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574
- Ruedi M, Mayer F (2001) Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. *Mol Phylogenet Evol* 21:436–448
- Ruedi M et al (2013) Molecular phylogenetic reconstructions identify East Asia as the cradle for the evolution of the cosmopolitan genus *Myotis* (Mammalia, Chiroptera). *Mol Phylogenet Evol* 69:437–449. <https://doi.org/10.1016/j.ympev.2013.08.011>
- Ruedi M, Csorba G, Lin LK, Chou CH (2015) Molecular phylogeny and morphological revision of *Myotis* bats (Chiroptera: Vespertilionidae) from Taiwan and adjacent China. *Zootaxa* 3920:301–342. <https://doi.org/10.11646/zootaxa.3920.2.6>
- Saikia U, Thakur ML, Bawri M, Bhattacharjee PC (2011) An inventory of the chiropteran fauna of Himachal Pradesh, northwestern India with some ecological observations. *J Threat Taxa* 3:1637–1655
- Schrage CG, Mello B (2020) Employing statistical learning to derive species-level genetic diversity for mammalian species. *Mammal Rev* 50:240–251. <https://doi.org/10.1111/mam.12192>
- Shamel HH (1942) A collection of bats from Thailand (Siam). *J Mammal* 23:317–328
- Shen Y-Y, Chen X, Murphy RW (2013) Assessing DNA barcoding as a tool for species identification and data quality control. *PLoS ONE* 8:e57125. <https://doi.org/10.1371/journal.pone.0057125>
- Sikes RS, Animal Care and Use Committee of the American Society of Mammalogists (2016) 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *J Mammal* 97:663–688. <https://doi.org/10.1093/jmammal/gyw078>
- Stadelmann B, Kunz TH, Lin LK, Ruedi M (2007) Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. *Mol Phylogenet Evol* 43:32–48
- Tate GH (1941) A review of the genus *Myotis* (Chiroptera) of Eurasia, with special reference to species occurring in the East Indies. *B Am Mus Nat Hist* 78:537–565
- Thomas O (1926) A new *Myotis* from Ladak. *Annals Mag Nat Hist* 17:609–610
- Tiunov MP, Kruskop SV, Feng J (2011) A new mouse-eared bat (Mammalia: Chiroptera, Vespertilionidae) from South China. *Acta Chiropterol* 13:271–278. <https://doi.org/10.3161/150811011x624758>
- Tomes RF (1859) Descriptions of six hitherto undescribed species of bats. *Proc Zool Soc Lond* 1859:68–79

- Topál G (1997) A new mouse-eared bat species, from Nepal, with statistical analyses of some other species of subgenus *Leuconoe* (Chiroptera, Vespertilionidae). *Acta Zool Acad Scient Hung* 43:375–402
- Tsytsulina K, Dick MH, Maeda K, Masuda R (2012) Systematics and phylogeography of the steppe whiskered bat *Myotis aurascens* Kuzyakin, 1935 (Chiroptera, Vespertilionidae). *Russian J Theriol* 11:1–20
- Wang S-Q, Li Y-J, Yin A-G, Zhang W, Jiang J-J, Wang W-L, Hu M (2016) The complete mitochondrial genome of David's myotis, *Myotis davidii* (*Myotis*, Vespertilionidae). *Mitochondrial DNA Part A* 27:1587–1588. <https://doi.org/10.3109/19401736.2014.958681>
- Weyeneth N, Goodman SM, Stanley WT, Ruedi M (2008) The biogeography of *Miniopterus* bats (Chiroptera: Miniopteridae) from the Comoro Archipelago inferred from mitochondrial DNA. *Mol Ecol* 17:5205–5219
- Whitfield JB, Lockhart PJ (2007) Deciphering ancient rapid radiations. *Trends Ecol Evol* 22:258–265
- Worthington-Wilmer J, Barratt E (1996) A non-lethal method of tissue sampling for genetic studies of Chiropterans. *Bat Res News* 37:1–3
- Yu W-H, Csorba G, Wu Y (2020) Tube-nosed variations—a new species of the genus *Murina* (Chiroptera: Vespertilionidae) from China. *Zool Res* 41:70–77. <https://doi.org/10.24272/j.issn.2095-8137.2020.009>
- Zhigalin A (2019) New data on David's myotis, *Myotis davidii* (Peters, 1869) (Mammalia, Chiroptera, Vespertilionidae), in Siberia and the Urals. *Biodivers Data J*. <https://doi.org/10.3897/BDJ.7.e34211>
- Ziegler R (2003) Bats (Chiroptera, Mammalia) from Middle Miocene karstic fissure fillings of Petersbuch near Eichstätt, Southern Franconian Alb (Bavaria). *Geobios* 36:447–490

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