

Echolocation calls, wing shape, diet and phylogenetic diagnosis of the endemic Chinese bat *Myotis pequinius*

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We describe the echolocation calls, flight morphology and diet of the endemic Chinese bat *Myotis pequinius* Thomas, 1908. Orientation calls are broadband, and reach low terminal frequencies. Diet comprised 80% beetles by volume. Wing shape and call design suggest that the bats fly in cluttered habitats, and the possession of moderately long ears and the dietary composition imply they forage at least sometimes by gleaning. *Myotis pequinius* resembles a larger Oriental version of the western Palaearctic species *M. nattereri*. Phylogenetic analysis based on sequences of the cytochrome *b* gene of mitochondrial DNA (1,140 base pairs) from a range of Palaearctic *Myotis* species confirmed that *M. pequinius* is close to the *nattereri* group, and is a sister-species to the eastern Palaearctic *M. bombinus*. One bat sequenced from China could not be identified from available species descriptions. It was smaller than *M. pequinius*, and also differed from it in sequence divergence by 6.7%, suggesting the existence of additional, cryptic taxonomic diversity in this group. Our phylogenetic analysis also supports the recognition of *M. schaubi* as a species distinct from *M. nattereri* in Transcaucasia and south-western Asia. *Myotis nattereri tschuliensis* is more closely related to *M. schaubi* than to *M. nattereri*, and is best considered either as a subspecies of *M. schaubi*, or possibly as a distinct species.

Key words: cytochrome *b*, echolocation calls, diet, wing shape, *Myotis*

INTRODUCTION

The Peking myotis, *Myotis pequinius* Thomas, 1908 is endemic to China, and has been recorded from the provinces of Hebei, Beijing, Shandong, Jiangsu, Henan and Sichuan (Wang, 2003). The species was first described by Thomas (1908). Allen (1938:

212) stated “except for the two original specimens taken in a cave thirty miles west of Peiping, nothing further seems to be known of this bat”. Despite this poor knowledge, this endemic species is classified as Lower Risk, Near Threatened in the IUCN Red List for microchiropteran bats (Hutson *et al.*, 2001). To our knowledge, nothing has

been published on the ecology of *M. pequinius* except for some geographic range records (Allen, 1938; Wang, 2003).

In his revision of Eurasian *Myotis*, Tate (1941) proposed to separate this genus into seven subgenera, one of which (*Isotus* Kolenati, 1856) was typified by *Myotis nattereri* (Kuhl, 1817). Tate (1941) considered that the subgenus *Isotus* was limited to the north temperate zone of Eurasia with the single exception of *M. thysanodes* Miller, 1897, a North American species. Tate (1941) included the Asian taxa *bombinus*, *tshuliensis* and *amurensis* as subspecies of the European *M. nattereri*. Tate (1941) followed Thomas (1908) and regarded *M. pequinius* as belonging to a distinct subgenus, *Leuconoe* Boie, 1830, typified by *M. daubentonii* (Kuhl, 1817), because the feet of *M. pequinius* largely exceed half the length of the tibiae (Thomas, 1908; Allen, 1938). In his comprehensive phenetic analyses of representatives of most species of *Myotis*, Findley (1972) reduced Tate's (1941) categories to only three subgenera (*Myotis*, *Selysius* and *Leuconoe*). Findley (1972) also established the close phenetic resemblance of *pequinius* with *nattereri* and *thysanodes*, and proposed to include all three species in the same *nattereri* group of the subgenus *Myotis*. Findley's (1972) view prevailed in most subsequent classifications (e.g., Koopman, 1993, 1994). In their revision of the *nattereri* group, Horáček and Hanák (1984) included also several fossil taxa in an attempt to reconstruct the phylogenetic history of this group. Most remarkably, they established that the extant *M. nattereri araxenus* Dahl, 1947 from Iran and Transcaucasia was very similar to the Plio-Pleistocene form *M. schaubi* Kormos, 1934 found in Hungary. As a consequence, Horáček and Hanák (1984) proposed to include *araxenus* as a subspecies of *M. schaubi*. These authors also considered that the important geographic gap and morphological differences

separating the West Palaearctic *nattereri* from the East Palaearctic *bombinus* warranted full species rank for *bombinus*. They retained *tshuliensis* Kuszajkin, 1935 from south-west Asia as a subspecies of *M. nattereri*, but considered the Russian Far East *M. amurensis* Ognev, 1927 as a subspecies of the Japanese *M. bombinus* Thomas, 1905. Horáček and Hanák (1984) confirmed that *M. pequinius* was another independent species belonging to the *nattereri* group that is not related to any *Leuconoe* species.

This systematic arrangement has been adopted by most modern taxonomists (e.g., Koopman, 1993, 1994; Simmons, 2005). Evidence from multiple mitochondrial DNA (mtDNA) data sets demonstrate that none of the Tate's (1941) or Findley's (1972) proposed subgenera are natural groupings, i.e., they do not form monophyletic groups (Ruedi and Mayer, 2001; Hofer and Van Den Bussche, 2003; Kawai *et al.*, 2003; Bickham *et al.*, 2004; Stadelmann *et al.*, 2004a, 2004b). Rather, each subgenus as defined by morphological traits comprises independent lineages that evolved convergently in distinct continents, and are better described as ecomorphs (e.g., *M. nattereri* in Europe and *M. thysanodes* in North America). In particular, Kawai *et al.* (2003) showed that the Japanese '*nattereri*' (= *M. bombinus*) did not form a monophyletic group with European *M. nattereri*, raising the possibility that even within this group, convergent evolution might exist. Sharing of primitive morphological characters (symplesiomorphic traits) among distantly related taxa may also result from the retention these traits by several lineages at the time of divergence: of course this must be carefully excluded in order to show convergent evolution (Lee *et al.*, 2002).

In this paper we describe the echolocation calls, wing shape, and diet of *M. pequinius*. To test whether the *nattereri* group as defined by Horáček and Hanák (1984)

forms a natural clade within the *Myotis* radiation, we performed a phylogenetic analysis using sequences of the cytochrome *b* gene (1,140 bp). *Myotis pequinius* bears morphological similarities with *M. nattereri*, and so we aimed to determine whether these resemblances are the outcome of a shared evolutionary history, or, as with the structural similarities among trawling *Myotis* species (Gaisler and Zúkal, 2004; Stadelmann *et al.*, 2004a) are the consequence of convergent evolution. In addition, we present findings that suggest additional taxonomic diversity within the *M. nattereri* group in China.

MATERIALS AND METHODS

Study Sites

We captured bats at Foguang Temple, Shanxi Province (39°20'N, 113°23'E). The temple is reputed to be the oldest standing wooden building in the world (857 AD), and is of enormous heritage value. The roof space above the temple is occupied by several thousand *M. pequinius*, and the bats cause problems at the temple, mainly through the large quantities of droppings produced by such a large colony. We visited the site on 16 August 2001 to collect droppings, capture bats, and to record echolocation calls. PB mist-netted above a water pit in Daguping, Foping District, Shaanxi Province (33°35'N, 107°46'E; ca. 1,190 m a.s.l.) on 17 September 2004 and captured a bat that is referred to here as *M. cf. pequinius*.

Morphology

We captured three male bats at Foguang Temple, traced their wing outlines, digitized the outlines on a Summagraphics SummaSketch III digitizing tablet and calculated wing parameters following Norberg and Rayner (1987). Forearm lengths were measured to the nearest 0.1 mm with dial calipers. We did not measure body mass, and so used measurements reported by Liang (1990) in 1981–1984. One of these measurements was collected before hibernation (November), the other set (Series 1) at the end of hibernation (April: 12 ♀♀, 2 ♂♂), and so measurements probably span much of the normal range of body mass found in this species. Size-

independent descriptors of wing loading and aspect ratio were calculated from equation (9) in Norberg and Rayner (1987). Skull measurements (see Table 2) were taken to the nearest 0.05 mm by using dial callipers.

Echolocation Calls

Echolocation calls were recorded as bats emerged late in the evening from the temple and flew across relatively open terrain about 20 m from the roost. We captured one bat and recorded it on release to confirm identification. Recordings were made with a Pettersson D-980 bat detector, which time-expanded calls 10× and downloaded them to a Sony TCD-D8 DAT recorder. Measurements were made from a Kay digital Sona-Graph model 5500 using a 512-point FFT size and a Hanning Window. Duration measurements were made from waveforms, frequency of most energy was measured from power spectra. Upper and lower frequencies were measured by inspecting power spectra and spectrograms simultaneously, and reading frequency values from spectrograms at points where calls rose clearly above background noise.

Diet

We collected 120 g of droppings from the roost site, and analysed 50 selected individually at random from this accumulation. The droppings had accumulated over many years, and we were unaware of any other species roosting in the building that may have contaminated the sample. Insect fragments were identified by observation under a low power binocular microscope (10× magnification) and identified to order using keys available in the literature (e.g., McAney *et al.*, 1991). Prey composition was estimated according to percentage volume.

Sequencing and Phylogenetic Analysis

For the phylogenetic analyses, we used ethanol-preserved tissue samples from one *M. pequinius* sampled at Foguang Temple, and from the adult male referred to as *M. cf. pequinius* captured by PB in Shaanxi Province. The final taxon sampling retained here (see Table 1) comprises all species of *Myotis* usually included in the *nattereri* group, and an array of pertinent taxa available in GenBank. The latter include notably the American *M. thysanodes*, the European *M. emarginatus* and several species of large *Myotis*, which appeared to be related to *M. nattereri*

TABLE 1. Current taxonomy (Simmons, 2005) of the *Myotis* bats sequenced in this paper, with collecting localities, GenBank accession numbers, and location of voucher specimens. Those taken directly from the GenBank are referred to the authors who produced them. Acronyms of institutions are as follows: National Museum Prague (NMP), Zoological Museum of Moscow State University (ZMMU)

Taxon	Locality	GenBank #	Voucher/Reference
<i>Myotis bombinus</i>	Aomori, Japan	AB106606	Kawai <i>et al.</i> , 2003
<i>M. b. amurensis</i>	Primorskiy, E Russia	AM284169	ZMMU
<i>M. bechsteini</i>	Jura, Switzerland	AF376843	Ruedi and Mayer, 2001
<i>M. b. blythii</i>	Os, Kirghizstan	AF376840	Ruedi and Mayer, 2001
<i>M. b. ancilla</i>	Fangshan Co, S China	AM284170	no voucher
<i>M. brandtii</i>	Neuhaus, Germany	AF376844	Ruedi and Mayer, 2001
<i>M. daubentonii</i>	Bavaria, Germany	AF376847	Ruedi and Mayer, 2001
<i>M. emarginatus</i>	Thessaloniki, Greece	AF376849	Ruedi and Mayer, 2001
<i>M. myotis</i>	Bavaria, Germany	AF376860	Ruedi and Mayer, 2001
<i>M. mystacinus</i>	Württemberg, Germany	AF376861	Ruedi and Mayer, 2001
<i>M. nattereri</i>	Peloponnese, Greece	AF376863	Ruedi and Mayer, 2001
<i>M. n. tschuliensis</i>	Sarikamis, Kars, Turkey	AM284171	NMP 90568
<i>M. pequinius</i>	Foguang Temple, Shanxi, China	AM284172	no voucher
<i>M. cf. pequinius</i>	Daguping, Shaanxi, China	AM284173	NMP 90556
<i>M. punicus</i>	Meknes, Morocco	AF376842	Ruedi and Mayer, 2001
<i>M. schaubi</i>	Choplu, Azerbaijan, Iran	AF376868	Ruedi and Mayer, 2001
<i>M. thysanodes</i>	USA	AF376869	Ruedi and Mayer, 2001

in previous molecular analyses (Ruedi and Mayer, 2001; Stadelmann *et al.*, 2004a, 2004b). We also included *M. brandtii* as a functional outgroup.

Prior to DNA extraction, about 10–30 mg of tissue was soaked for 1–2 hours in sterile water. Total genomic DNA was extracted following a salting out protocol developed by Miller *et al.* (1988), as detailed in Castella *et al.* (2001). The final extraction product was diluted into 50 μ l of low TE buffer and stored at -20°C until further analyses. The complete cytochrome *b* gene (*Cyt b*) was amplified and sequenced with the primer pairs L14724 (Irwin *et al.*, 1991) and BSVES268H (Stadelmann *et al.*, 2004b). In short, PCR cocktails included 2–10 μ l of DNA extract, 0.2 μM of each primer, 2.5–4 mM of MgCl_2 , 0.2 mM each of 4 dNTPs, 1 unit of Taq DNA polymerase (Qiagen, Inc., Switzerland) with appropriate buffer and ddH_2O . Thermal profiles of amplifications started with three minutes of denaturation at 94°C , followed by 39 cycles at 94°C (45 sec), $45\text{--}53^{\circ}\text{C}$ (45 sec) and 72°C (1.5 min), with a final extension at 72°C (5 min). Final PCR products were purified and sequenced directly (ABI Prism 377 automated DNA sequencer) in both directions using the same two primers. Sequences were assembled manually, edited, aligned and translated into amino acids to check for reading frame with the software BioEdit (Hall, 2001).

Probabilistic methods were used to reconstruct phylogenetic trees with Maximum Likelihood (ML) implemented in PAUP* 4.0b10 (Swofford, 2002).

The best-fitting model of nucleotide substitution (General Time Reversible model with rate variation among sites and a proportion of invariable sites) for the *Cyt b* data set was identified with Modeltest 3.04 (Posada and Crandall, 1998). To find the ML topology, heuristic searches were started from a neighbour-joining tree, followed by complete branch-swappings. Reliability of nodes was assessed with 1,000 non-parametric bootstrap replicates. Bayesian posterior probabilities were also calculated using a Metropolis-coupled, Markov chain, Monte Carlo (MCMCMC) sampling approach, as implemented in MRBAYES 3.0b4 (Ronquist and Huelsenbeck, 2003) and using the same model of DNA substitutions. Four simultaneous Markov chains were run for 1,000,000 generations with trees sampled every 1,000 generations. After about 20,000 generations the log-likelihoods of trees reached an asymptote. These initial trees were thus discarded as 'burn-in'. Posterior probabilities were computed from the consensus of the remaining 980 sampled trees.

Cladistic analyses were also performed to reconstruct phylogenetic trees, using the principle of Maximum Parsimony (MP) implemented in PAUP*. All substitutions were weighted equally. The most parsimonious tree was estimated through a heuristic search with 25 random additions of taxa, and complete tree-bisection-reconnection branch swapping for each iteration. Nodal support under the MP framework was assessed by bootstrap analyses with 1,000 replicates.

RESULTS

Myotis pequinius resembles a larger version of *M. nattereri* of the western Palearctic in appearance (Fig. 1) and possesses a fringe of stiff hairs along the interfemoral membrane. Both bats possess relatively long ears, but the tragus is notably shorter in *pequinius*, barely reaching the ear notch (it is much longer in *nattereri*). We also confirm that the feet of *M. pequinius* are relatively enlarged (Fig. 1). Other external differences with *M. nattereri* include larger forearms and longer thumbs. Measurements of skull morphology taken from museum specimens (Table 2) show that *M. pequinius* has a larger skull than *M. bombinus*, *M. nattereri*, *M. schaubi* and *M. n. tschuliensis*. The specimen of *M. cf. pequinius* was intermediate in skull measurements between *M. pequinius* and *M. bombinus amurensis*, both of which occur in China. The forearm length of *M. cf. pequinius* was 43.8 mm, notably smaller than all other *M. pequinius* measured (Tables 3 and 4). *Myotis cf. pequinius* had longer ears than *M. pequinius*, and a tragus of similar length despite it being a smaller bat (Table 3). The former also had a smaller foot and tibia than *M. pequinius*. Overall, its longer ears made it more like *M. nattereri* in appearance than *M. pequinius*.

Wing Shape

Myotis pequinius has a relatively low wing loading and aspect ratio for its body size (Table 4). Since no females were captured, it is unknown whether wing shape is sexually dimorphic, as is usual of other species of *Myotis* where females are typically slightly larger. Size-independent descriptors of wing loading and aspect ratio were between -2.31 and -1.44 and -0.06 and 0.36 respectively [based on the mean body mass values of April Series 1 and the heavier

TABLE 2. Skull measurements (means, in mm) from museum specimens of bats in the *M. nattereri* clade in Fig. 3. Measurements were taken from Benda and Horáček (1995), supplemented by material listed in the footnote. Sample sizes are in parentheses. *Myotis pequinius* Series 1: 1 ♂ (BMNH 8.8.7.2., type), 30 km W of Beijing, China. – 2 ♂♂ (BMNH without numbers), China; *M. pequinius* Series 2: (♀♀ SZC00061-1 and 00061-2), Lingqiu, Shanxi Province; *M. bombinus bombinus*: 5 ♀♀ (BMNH 6.1.4.13-17, incl. 6.1.4.14, type specimen), Kin-Shin, Japan. – 2 ♂♂ (KMC 2758, 2759), Omogo, Ehime, Japan; *M. bombinus amurensis*: 1 ♂ (ZMMU 1317, type specimen), Amur river, Russia. – 1 indeterminate (ZMMU S104379), Far East of Russia. NMP = National Museum Prague; BMNH = Natural History Museum London, U.K.; ZMMU = Zoological Museum, Moscow State University, Russia; KMC = Kishio Maeda collection, Japan; SZC = Shuyi Zhang collection, Beijing

Character	<i>M. pequinius</i>		<i>M. cf. pequinius</i>		<i>M. bombinus</i>		<i>M. nattereri</i>		<i>M. schaubi</i>		<i>M. nattereri</i> <i>tschuliensis</i>	
	Series 1 (3)	Series 2 (2)	NMP 90556	<i>bombinus</i> (7)	<i>amurensis</i> (2)	(117)	(9)	(15)				
Greatest skull length	18.20	19.98	16.40	14.91	15.25	15.76	17.18	16.12				
Condylobasal length	17.70	17.75	15.13	14.03	14.17	14.72	16.12	15.13				
Zygomatic width	12.00	11.79	10.59	9.25	9.40	9.93	10.69	10.15				
Interorbital width	4.75	5.38	3.75	3.69	3.80	3.83	4.08	3.69				
Upper canines width	5.25	5.11	4.10	3.75	3.91	4.05	4.49	4.10				
Upper tooth-row length	7.02	7.39	6.33	5.73	5.81	6.13	6.74	6.40				
Mandible length	14.37	14.68	12.02	10.81	10.90	11.48	12.55	11.81				
Lower tooth-row length	7.43	7.76	6.62	6.14	6.05	6.51	7.18	6.78				



FIG. 1. Photographs of *M. pequinius* — individual captured at Foguang Temple, Shanxi

individual captured in November by Liang (1990)]. Therefore both wing loading and aspect ratio were lower than predicted for a bat of its body size.

Echolocation Calls

When flying, *M. pequinius* emitted broadband echolocation calls with peak energy around 33 kHz and which reached relatively low terminal frequencies (Fig. 2 and Table 5). Most calls were generally almost linearly modulated in frequency (Fig 2B,

right), though some longer pulses were more curved in their lower frequency portion (Fig 2B, left).

Diet

Beetle remains dominated in droppings examined from *M. pequinius* (80% by volume — Table 6). Small amounts of Hemiptera, Lepidoptera and Diptera were recorded, together with single mites (presumably an ingested ectoparasite), a lepidopteran larva, and a wing fragment from a dragonfly.

Sequencing and Phylogenetic Analysis

Except for *M. n. tschuliensis* [for which we could not obtain clean sequences for the initial third (about 400 bp) of *Cyt b*], all other bats sequenced easily and aligned readily with other known sequences of *Cyt b*. The five new *Cyt b* sequences obtained in this study have been deposited in GenBank under accession numbers AM284169–AM284173 (Table 1). The different methods used for phylogenetic reconstructions gave results similar to the ML tree of Fig. 3, but bootstrap support or

Table 3. External measurements (in mm) from *M. pequinius* (Lingqiu, Shanxi Province, specimens SZC00061-1 and 00061-2 from collection of Shuyi Zhang, Beijing) in comparison with the smaller individual collected by PB from Daguping, Foping District, Shaanxi Province (*M. cf. pequinius*)

Character	<i>M. pequinius</i>	<i>M. cf. pequinius</i>
Forearm length	52.70, 51.78	43.8
Ear length	14.94, 15.98	19.9
Tragus length	8.92, 10.72	9.7
Thumb length (incl. claw)	9.34, 9.96	7.9
Hindfoot length (incl. claw)	11.80, 10.9	8.4
Tibia length	21.80, 21.4	16.6

TABLE 4. Wing morphology of *M. pequinius*. Data are from three adult males captured at Foguang Temple, Shanxi, except for data on body mass (also used to calculate mean wing loading), taken from Liang (1990). The body mass data are from two sets of measurements (April and November — see text), and wing loading is calculated for the mean of the first series, and for the heavier individual captured on a second occasion

Parameter	\bar{x}	Range
Body mass (g)	10.89	9.80–11.50 ¹
	–	14.05 ²
Forearm length (mm)	50.27	49.60–50.70
Hand wing area (cm ²)	67.01	66.70–67.54
Arm wing area (cm ²)	91.45	87.34–94.40
Wing area (cm ²)	190.25	188.56–191.10
Wing span (cm)	34.23	34.00–34.60
Hand wing length (cm)	8.33	8.00–8.50
Arm wing length (cm)	7.07	6.70–7.30
Tip Length Ratio	1.18	1.10–1.27
Tip Area Ratio	0.73	0.71–0.77
Tip Shape Index	1.66	1.49–1.93
Aspect Ratio	6.16	6.05–6.35
Wing loading (Nm ⁻²)	–	5.62–7.24

¹ — April ($n = 14$)

² — November ($n = 1$)

posterior probabilities of several nodes were low. This lack of support indicates that some lineages do not have a firm position within this tree, and longer sequences might be necessary to gain more resolution. For example, *M. nattereri* from Greece is either placed at the basis of the *nattereri* clade (ML and MB reconstructions — Fig. 3), or sister to the *myotis-blythii-punicus* group (MP reconstructions); none of these reconstructions reached 50% support for the phylogenetic placement of *M. nattereri*. All reconstructions, however, were concordant in placing with strong support (95–100%)

the two representatives of the *pequinius* lineage as sister to *M. bombinus*. The *pequinius* sample differed from that of *M. cf. pequinius* by 6.7% in sequence divergence (Table 7), although the two were sister taxa (Fig. 3). Likewise, the two forms from the Near East, *M. n. tschuliensis* and *M. schaubi* differ from each other by 3.7% and are clearly distinct from the remaining taxa within the *nattereri* clade (Table 7). As a comparison, *M. blythii ancilla*, a taxon endemic to central and eastern China is closely related to the nominal form, *M. b. blythii* from Central Asia (1.7% sequence divergence, results not shown).

DISCUSSION

Myotis pequinius is a relatively large bat (forearm length ca. 50 mm) within its own genus. The relatively large ears, hairs along the interfemoral membrane and the wing shape all bring to mind a scaled-up version of *M. nattereri*. It differs externally, however, from the west Palaearctic *M. nattereri* in many ways, including possession of a relatively shorter tragus, longer forearms and thumbs, and longer feet. Its flight morphology fits in the morphological space described for hover-gleaners and ground-gleaners in Norberg and Rayner (1987: 406). The wing shape is similar to that of *M. nattereri*, as are the echolocation calls (broadband, and reaching low terminal frequencies, though not as broadband as those of *M. nattereri*). Calls became slightly more curved in their frequency/time course when

TABLE 5. Echolocation call characteristics from *M. pequinius* emitting orientation calls while flying away from the roost. Measurements from 13 individuals (1 call/individual) are reported

Parameter	\bar{x}	SD	Minimum	Maximum
Pulse interval (ms)	93.38	30.26	56.56	144.40
Pulse duration (ms)	5.72	1.04	3.91	7.50
Upper frequency (kHz)	84.18	10.06	62.40	99.20
Lowest frequency (kHz)	16.86	1.71	13.60	19.20
Frequency of most energy (kHz)	32.80	5.82	23.20	40.00

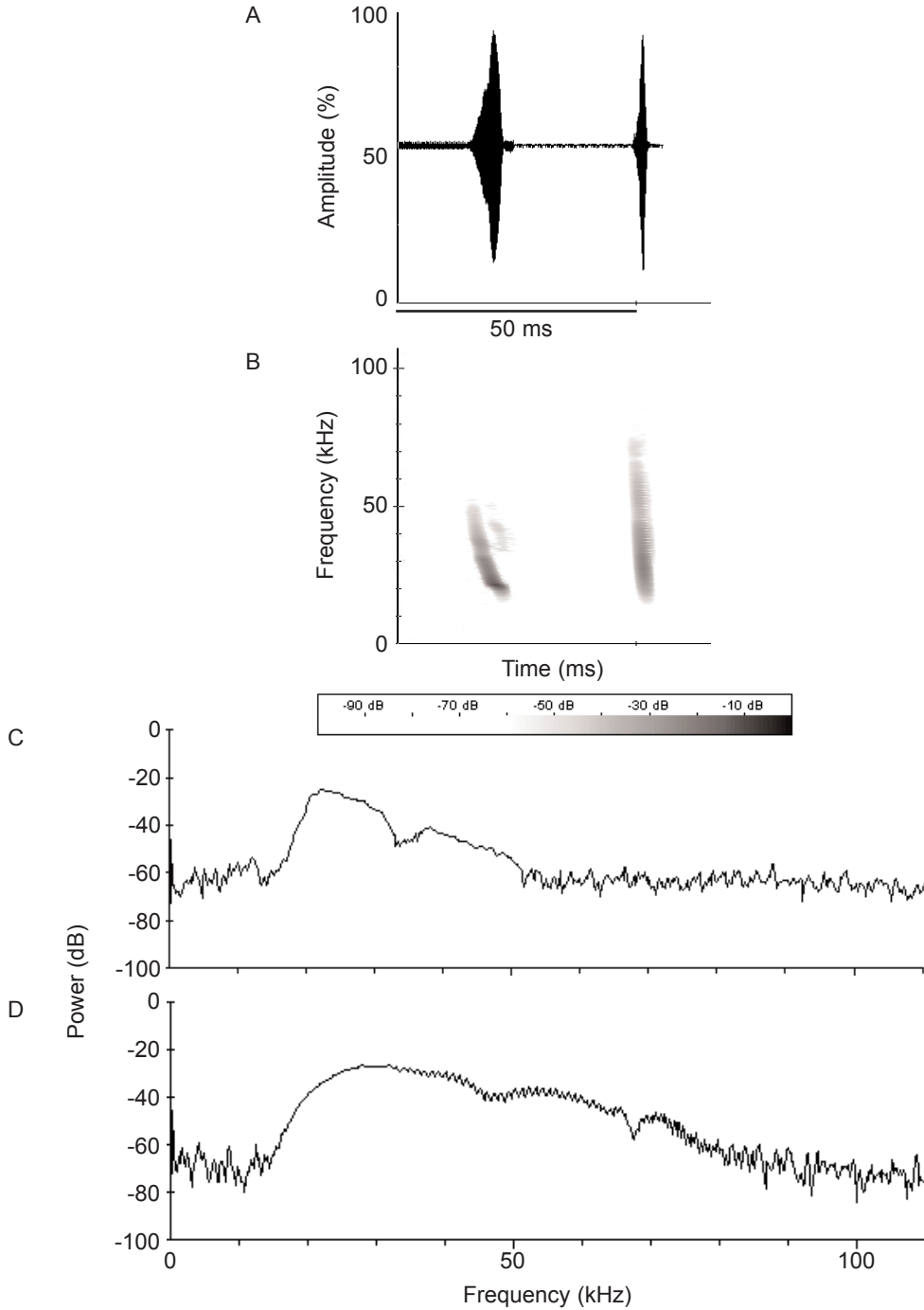


FIG. 2. Echolocation calls of *M. pequinius*. (A) and (B) are waveforms and spectrograms respectively of two calls, the left-hand one recorded from a bat flying in open space, the right-hand one from a bat flying closer to the roost. The spectrogram was performed using a 512-point FFT size and a Hanning Window, and the time axis applies to both waveform and spectrogram. (C) is a power spectrum on the left-hand call, (D) is a power spectrum of the right-hand call, again using a 512-point FFT

TABLE 6. Diet of *M. pequinius* as determined from analysis of 50 droppings collected from a long-term accumulation at Foguang Temple, Shanxi Province on 18 August 2001. Means and SDs of percent contribution by volume are presented

Order	Percentage volume	
	\bar{x}	SD
Coleoptera	80.00	25.46
Hemiptera	10.00	22.50
Lepidoptera	5.20	13.44
Diptera	3.50	9.38
Lepidopteran larvae	0.60	4.24
Odonata	0.60	4.24
Acari	0.10	0.70

longer in duration. In many bat species longer pulses are emitted in more open habitats because echoes return later and pulse-echo overlap is avoided (Kalko and Schnitzler, 1993). More curved calls would also be predicted from bats flying at faster speed in open habitats, as curvature increases Doppler tolerance for long duration pulses, an effect that is important for accurate ranging performance at higher flight speeds (Boonman *et al.*, 2003). The relatively low frequency of most energy, low terminal frequency, and relatively long pulse duration and pulse interval for a *Myotis* species are as predicted from the scaling of these parameters with body mass (Jones, 1999).

The broadband echolocation calls, wing shape and relatively long ears of *M. pequinius* leads us to predict that the species hunts in a way similar to *M. nattereri*, by

taking prey from close to vegetation (Siemers and Schnitzler, 2000) and by gleaning (Swift and Racey, 2002). Unlike *M. nattereri*, which eats mainly spiders and many non-volant Diptera (Vaughan, 1997; Geisler and Dietz, 1999), *M. pequinius* ate mainly beetles. The presence of a lepidopteran larva in droppings suggests gleaning in this species (although the larva could have been hawked while suspended from a thread), and the presence of a fringe of hairs along the tail membrane is also suggestive of this behaviour. The dietary composition resembles another gleaning bat, *M. myotis*, which also eats many beetles and some lepidopteran larvae (Arlettaz, 1996a). *Myotis myotis* reduces the intensity of echolocation calls immediately prior capturing prey on the ground (Arlettaz *et al.*, 2001), and it would be illuminating to determine if *M. pequinius* shows similar behaviour. More intriguing are the unusually elongated feet, an attribute also found in trawling foragers such as *M. daubentonii*, *M. capaccinii* or *M. dasycneme*, all of which feed by capturing prey on or close to the water surface (Siemers *et al.*, 2001). The diet of *M. pequinius* is composed essentially of beetles, and hence it is unlikely that it hunts over the water. It is however possible that it hunts prey by trawling in grass, as has been reported in *M. nattereri* (Arlettaz, 1996b). Radiotelemetric studies could bring some clues to the hunting behaviour of this bat in its natural habitat.

TABLE 7. Kimura 2-parameter distance matrix among seven Eurasian taxa of the *nattereri* group measured at the cytochrome *b* gene. For sake of comparison, the North American *M. thysanodes* was also included in this table

Taxon	1	2	3	4	5	6	7	8
1. <i>M. nattereri</i>	–							
2. <i>M. pequinius</i>	0.139	–						
3. <i>M. n. tschuliensis</i>	0.155	0.143	–					
4. <i>M. schaubi</i>	0.172	0.144	0.037	–				
5. <i>M. cf. pequinius</i>	0.139	0.067	0.151	0.149	–			
6. <i>M. bombinus</i>	0.138	0.088	0.146	0.140	0.089	–		
7. <i>M. b. amurensis</i>	0.154	0.086	0.164	0.156	0.098	0.045	–	
8. <i>M. thysanodes</i>	0.157	0.182	0.203	0.189	0.185	0.199	0.210	–

Although the different phylogenetic reconstructions based on the *Cyt b* gene did not reach statistical support, these molecular data suggest a monophyletic evolution of the Old World species traditionally allied to the *nattereri* group (Fig. 3). As already shown in Ruedi and Mayer (2001), the American *M. thysanodes* is not part of this group [contra Findley (1972) or Koopman (1994)], which contradicts the validity of the subgenus *Isotus* proposed by Tate (1941). Rather, evidence from a broad range of taxa shows that *M. thysanodes* falls within the New World clade of *Myotis* (Ruedi and Mayer, 2001; Hoofer and Van Den Bussche, 2003). Although *M. thysanodes* shares with *M. nattereri* external features such as fringing, stiff hairs along the rear margin of

the uropatagium, and long and narrow ears, these features evolved independently, probably as adaptations to similar ecological niches.

Our molecular reconstructions strongly support (100% support in all reconstructions) the placement of *M. pequinius* within the *nattereri* group as a sister-species to the eastern Palaearctic *M. bombinus*. An unexpected result was the relatively large genetic distance found between the two specimens (*M. pequinius* and *M. cf. pequinius*) sampled in China. They differ by 6.7% sequence divergence at the *Cyt b* gene (Table 7). Intra-specific divergences in *Myotis* (Ruedi and Mayer, 2001) or in species of bats in general (Bradley and Baker, 2001) are usually in the range of

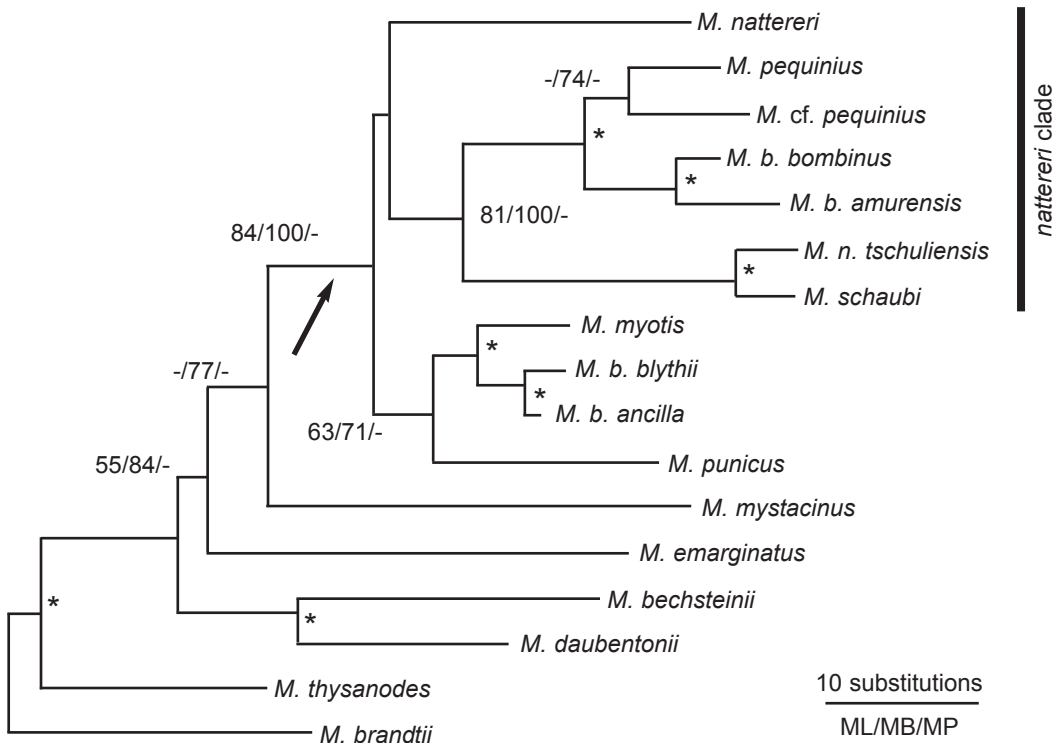


FIG. 3. Maximum likelihood (ML) tree of taxa in the *nattereri* group and a selection of other species of the genus *Myotis*. Bayesian reconstructions (MB) based on the same dataset resulted in the same topology, but phylogenetic reconstructions using maximum parsimony (MP) placed the pair *M. n. tschuliensis* — *schaubi* in a more basal position (indicated by an arrow). Bootstrap supports or posterior probabilities of nodes higher than 95% in all three methods are indicated by a star (*); support values are indicated in order for ML, MB and MP reconstructions. The symbol ‘-’ is used where bootstrap support is less than 50%

0–2%. The specimen from Daguping was much smaller (forearm length 43.8 mm and skull length 16.4 mm) than bats captured at the temple, and was also smaller than the measurements given by Allen (1938) for the type specimens of *pequinus*. This clearly points to the existence of additional cryptic taxonomic variation in this group, and will require more thorough morphological and genetic investigations for clarification.

Our molecular reconstructions are also relatively concordant with the systematic arrangement proposed by Horáček and Hanák (1984), except for the position of *M. n. tschuliensis*. These authors viewed this form as belonging undoubtedly to a larger subspecies of *nattereri*, and thus distinct from *M. schaubi araxenus*. All molecular reconstructions show *M. schaubi* and *M. n. tschuliensis* as closely related sister taxa (3.7% sequence divergence; 100% support in all analyses), while the European lineage of *M. nattereri* diverged earlier from these two taxa and is basal to the whole clade. *Myotis n. tschuliensis* is currently known from Turkey, Transcaucasia and Turkmenistan, and possibly also from north western Iran. Our results suggest that *M. n. tschuliensis* is best considered either as a subspecies of *M. schaubi*, or, given the extensive sequence divergence, as a species in its own right. Further research in Transcaucasia is needed to establish whether *M. n. tschuliensis* and *M. schaubi* are syntopic, and whether they interbreed.

In conclusion, the morphological similarities between *M. pequinus* and *M. nattereri* appear to be the consequence of a shared evolutionary history, although several other related species evolved in the same group. Both species have wing shapes adapted for slow flight in relatively cluttered habitats, and emit broadband echolocation calls that are short in duration. Such calls are adapted for capturing prey close to vegetation, although the larger *M. pequinus*

forages more on beetles than does *M. nattereri*, which eats mainly Diptera.

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