Cryptic diversity in Mongolian vespertilionid bats (Vespertilionidae, Chiroptera, Mammalia). Results of the Mongolian-German biological expeditions since 1962, No. 299

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In contrast to the Eastern Palaearctic region a high degree of cryptic diversity was discovered among temperate bats of the Western Palaearctic region in the last ten years. Climatic oscillations caused severe changes in the distribution of species throughout the Palaearctic region during the Pleistocene. Exploring multiple taxa can help to understand general evolutionary differentiation processes. In the present study genetic variation within and among 94 Mongolian vespertilionid bats of six genera (*Hypsugo*, *Eptesicus*, *Vespertilio*, *Myotis*, *Plecotus*, and *Nyctalus*) was screened by sequencing a 798 bp fragment of the mitochondrial ND1 gene and then subsequently compared with those of Western Palaearctic taxa. This allowed first insights in the differentiation among a wide range of bats across the Palaearctic region. A total of 16 distinct mitochondrial lineages were found in Mongolia. Thirteen lineages differed by at least five percent sequence divergence from Western Palaearctic species. Only three lineages (*Eptesicus nilssonii*, *Vespertilio murinus*, and *Nyctalus noctula*) showed lower divergence values. Our data demonstrate a substantial differentiation between most Western and Eastern Palaearctic vespertilionid bats. Estimations of divergence times showed that most divergence appeared prior to the Pleistocene, but current distributions of bats were most likely shaped by the usage of multiple refugia during glaciations.

Key words: biogeography, Chiroptera, Palaearctic region, cryptic species, mtDNA sequence divergence, phylogeny

Introduction

Since the Middle Miocene strong climatic oscillations have led to warm and ice ages. They have strongly affected and changed the floral and faunal distribution patterns and have led to many geographic separations and speciation events. Especially in the Western Palaearctic region, glacial refugia of many taxa are well known, including those of vespertilionid bats (Ibáñez *et al.*, 2006). The fossil record and particularly the genetic investigations revealed the Iberian, Italian and Balkan Peninsulas in the Mediterranean as the major glacial refugia in Europe. Colonization from more northern or from more eastern refugia could not be excluded in many taxa. The main reason for this is a lack of comprehensive biogeographic investigations about most taxa from Eastern Europe and the Eastern Palaearctic region (Taberlet *et al.*, 1998; Hewitt, 2000, 2004*a*, 2004*b*; Schmitt, 2007).

This also applies to the well-studied group of bats, which represents the second largest mammalian order. Most bat species in the Palaearctic region belong to the family Vespertilionidae and the distribution ranges of some species extend far towards the north. Simmons (2005) listed a total of 44 vespertilionid bats for the Western Palaearctic region that were mainly discovered while studying

morphological characters. Today, molecular approaches combined with morphological analyses suggest the existence of at least 54 vespertilionid bat species in the Western Palaearctic region (Mayer *et al.*, 2007). The newly discovered species resemble morphologically well-known ones, but genetically they differ from each other. Due to the lack of obvious morphological differentiation they are called cryptic species. Little is known about the distribution of many presumed cryptic species. For example, *Myotis alcathoe* had initially only been found in Greece and Hungary and therefore a small distribution range had been assumed (von Helversen *et al.*, 2001). Later, *M. alcathoe* was recorded in most parts of Europe (Niermann *et al.*, 2007), even Northern Europe (Ahlén, 2010; Jan *et al.*, 2010).

Until recently, it has been assumed that 40 vespertilionid bat species occur in the Eastern Palaearctic region (Simmons, 2005; see Bannikov, 1954 and Stubbe and Chotolchu, 1968 for Mongolian bats). Many of these species seem to have a wide distribution range comprising large proportions of the Western and Eastern Palaearctic region. So far, DNA sequencing has not widely been applied in order to study species diversity and distribution patterns of bats from the Eastern Palaearctic region. One exception is the work of Kawai *et al.* (2003) about Japanese and East Asian *Myotis*, another is the recent revision of the genus *Plecotus* (Spitzenberger *et al.*, 2006), which incorporates morphological and genetic data. A high degree of cryptic diversity was found in specimens of the genus *Plecotus* from the Eastern Palaearctic region. At least 11 Eastern Palaearctic mtDNA lineages were found and Western and Eastern Palaearctic taxa were characterized by high mitochondrial divergence as well as differing morphological characters (Spitzenberger *et al.*, 2006). The authors postulated many glacial refugia within the Palaearctic region, especially in the East, resulting in numerous isolation and expansion events. Also, Kawai *et al.* (2003) found many putative cryptic species in the genus *Myotis*. Some of the morphologically similar species among Japan and the Western Palaearctic region belong to different phylogroups according to their phylogenetic reconstructions. They possibly represent cryptic species.

In this study we surveyed genetic diversity of the mitochondrial ND1 gene (subunit one of NADH dehydrogenase) among all vespertilionid genera from Mongolia. This is the first comprehensive genetic analysis of a whole bat family from the Eastern Palaearctic region.

This study had a twofold purpose: first, we aimed at identifying the number of distinct mtDNA lineages within a large sample of 94 bats from Mongolia comprising the six genera *Hypsugo*, *Eptesicus*, *Vespertilio*, *Myotis*, *Plecotus*, and *Nyctalus* as a measure of diversity and an indication of species diversity in this region. The 'Generalized Mixed Yule-Coalescent' (GMYC) approach was used among others for delimiting genetic clusters (Pons *et al.*, 2006; Fontaneto *et al.*, 2007; Monaghan *et al.*, 2009); secondly, a comparison of the DNA sequences from Mongolian specimens with published sequences from other Asian regions and from Europe allowed a first intended insight in the divergence between the Western and Eastern Palaearctic bat fauna and the biogeographic patterns underlying the evolution of the observed species diversity.

Materials and Methods

Sample Collection

Mitochondrial ND1 sequences of 94 vespertilionid bats from different Mongolian localities (Fig. 1) were collected, analyzed, and compared to Western and other Eastern Palaearctic specimens (157 sequences). *Miniopterus schreibersii* and *Tadarida teniotis* were used as outgroup taxa. Altogether, we incorporated 253 sequences (see Appendix).

Sequence and Genetic Analyses

DNA isolation and amplification of the mitochondrial protein-coding gene NADH dehydrogenase subunit one (ND1) were performed as described in Mayer and von Helversen (2001). We sequenced the product using the primers ER70 and ER89 (Mayer and von Helversen, 2001). Multiple sequence alignments were built using the ClustalW algorithm implemented in BioEdit v7.0.5.3 (Thompson *et al.*, 1994; Hall, 1999). The alignment was truncated on the 5'-end at the start codon ATG, which represents the beginning of the "open reading frame" (ORF). Furthermore, the alignment was cut at nucleotide 798 (3'-end).

Neither did we find any insertions or deletions, nor a second start codon or any stop codon within the sequences. Therefore, the reading frame was not altered. A low value for the overall transition/transversion ratio R of 2.6 was found (Tamura *et al.*, 2004). Basically, we would expect that transversions are equally distributed among the codon positions in older pseudogenes. However, transversional changes occurred almost always at third codon positions in the whole data set. All these findings provide no indications for nuclear mitochondrial DNA segments ('numts' — Sorenson and Quinn, 1998) in our data set.

Species assignment of the Mongolian specimens were based on ND1 sequence comparisons with previously published sequences of identified European and Asian species. Mongolian specimens with less than five percent sequence divergence to an established species were assigned to one species following the approach of Mayer *et al.* (2007). Only few, but interesting cases, showed that morphologically distinct species can differ
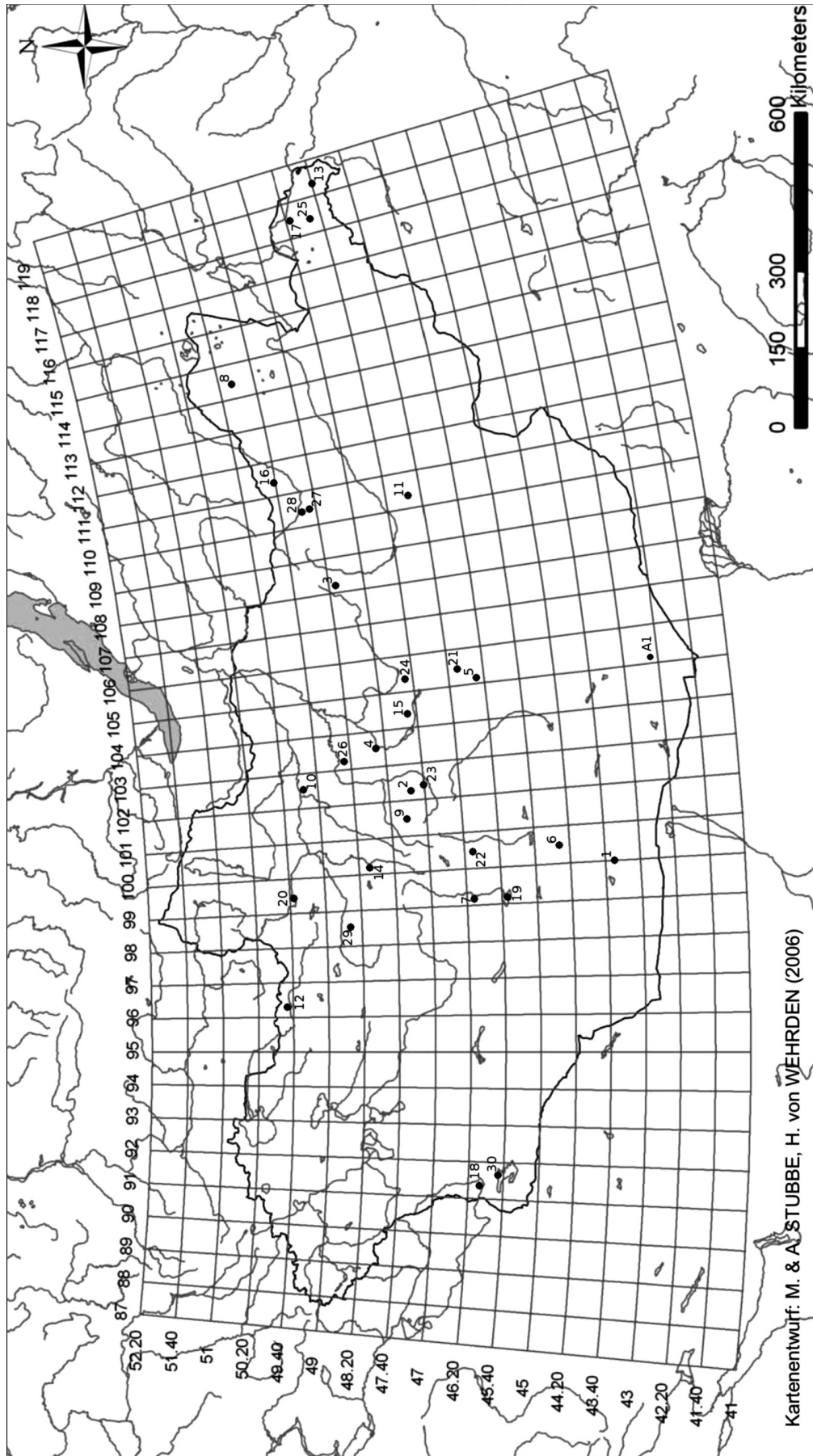
FIG. 1. Sample localities of Mongolia (see hereto locality code in the Appendix). Longitude and latitude are given along the edge of the map and the scale is in km

by much less than 5%, or intraspecific variability can exceed 5% (e.g., Francis *et al.*, 2010; Clare *et al.*, 2011). Therefore, we discuss the actual level of differentiation for each taxon separately in these cases.

Species assignment within the genus *Plecotus* was conducted by sequencing a short DNA fragment and by building subsequent DNA sequence comparisons with published sequences from Spitzenberger *et al.* (2006). Therefore, we amplified a part of the 16S rRNA gene according to the protocol of Palumbi *et al.* (1991), which was modified by Kiefer (2007).

Phylogenetic Analyses

Pairwise sequence comparisons were calculated with Mega 4 (Kumar *et al.*, 2004; Tamura *et al.*, 2007). All values in percentage are uncorrected genetic distances (*p*-distance). We applied the neighbor-joining algorithm with 1,000 bootstrap replications and Kimura-2-parameter (K2P) to visualize the genetic distances between the haplotypes. Phylogenetic trees were built separately for each genus.

Genetic clusters were delimited by the 'Generalized Mixed Yule-Coalescent' (GMYC) package (Pons *et al.*, 2006; Fontaneto *et al.*, 2007; Monaghan *et al.*, 2009) and the statistical software R (R Development Core Team, 2008). We used the multiple threshold version of GMYC, which starts with a single threshold, but uses an iterative algorithm to test whether relaxation of the single threshold leads to a significant improvement in model fit. The single threshold specifies the transition from between to within species branching. We used three percent sequence divergence as lower limit and ten percent as upper limit for the starting presets of the threshold in the multiple version. We were aiming at finding the optimal threshold for species delimitation by the algorithm. Ultrametric input trees were computed with the software BEAST v1.5.3 (Drummond and Rambaut, 2007). Bayesian sampling of the trees were performed with 10 million steps and a sample frequency of 1,000 in two independent runs for each genus. We used a burnin of 10% for each run and afterwards combined both runs with the software LogCombiner. We used the software TreeAnnotator to obtain the ultrametric maximum clade credibility tree out of 18002 sampled trees. TreeAnnotator and LogCombiner are provided as part of the BEAST package. Decay indices (DI) were obtained with TreeRot v3 (Sorenson and Franzosa, 2007) in combination with the phylogenetic software Paup 4.0b (Swofford, 2002).

Estimation of Divergence Times

We combined ND1 sequences of the three genera *Hypsugo*, *Myotis*, and *Plecotus* and performed analyses of divergence times with the software BEAST v1.6.1 (Drummond and Rambaut, 2007). *Miniopterus schreibersi* and *Tadarida teniotis* were used as outgroup. The searches were conducted under Hasegawa-Kishino-Yano HKY+Γ (Hasegawa *et al.*, 1985) with four rate categories as model of evolution. We did not use a more complex model, because there was a trade-off between computational power and model complexity. It was not possible to get a consistent phylogenetic reconstruction between different runs under the GTR+Γ model in reasonable time. We partitioned our mitochondrial data set into codon positions and calculated separate evolutionary model parameters for the first, second and third codon position to account for the rate heterogeneity among these positions. A Bayes factor comparison between unpartitioned and partitioned analyses, revealed

a better performance of the partitioned data set and high rate differences among the codon positions. Further, we analyzed different time calibration settings within strict or uncorrelated lognormal relaxed clock (UCLN) models. Bayes factor calculations were performed with the software Tracer v1.4 (Drummond *et al.*, 2006). The goal of these analyses was not to correctly infer all divergence times within the genera *Hypsugo*, *Myotis*, and *Plecotus*, especially not at basal splits, because our taxon sampling is far away from complete. Instead, we only aimed to clarify if the divergences between Western and Eastern Palaearctic lineages were in the Pleistocene or previously.

Calibration of the Molecular Clock

We incorporated two different calibration points including: (1) the divergence between Molossidae and Vespertilionidae set at 37 million years ago (MYA) in the middle Eocene (McKenna and Bell, 1997). We used the proposed age of the fossils and a lognormal distribution to model the minimum age constraint for this specific node ($\bar{x}$ = 3, SD = 0.95, offset = 37 in real space). The maximum age constraint was arbitrarily set to 50 MYA; (2) divergence between *Myotis nattereri* and *M. schaubi* set at 6 MYA in the upper Miocene (Horáček and Hanák, 1983–84). We used a normal distribution centered around six MYA with a standard deviation of ± 1 million year to model the divergence time of these two *Myotis* lineages.

RESULTS

Hypsugo

Mongolian bats of the genus *Hypsugo* formed one haplogroup, which had a ND1 sequence divergence up to 0.2%. They built a well-supported clade representing *H. alaschanicus* with a bootstrap support (BS) value of 92 and a decay index (DI) of 13 (Fig. 2 and Table 1). The GMYC method also revealed this cluster. The most closely related congeneric Western Palaearctic species differed by at least 9.0 (*H. savii*) or 9.2 (*H. cf. darwinii*) percent ND1 sequence divergence.

Eptesicus

We found three distinct haplogroups in our sample. Two haplogroups were also supported by the GMYC method. Firstly, two Mongolian specimens grouped together with one Russian (from Nischni Nowgorod) and one Japanese *E. nilssonii* sample forming an Eastern Palaearctic clade at up to 0.5% ND1 sequence divergence (Fig. 3 and Table 2). Further, we found up to 2.0% sequence divergence to Western Palaearctic *E. nilssonii* and *E. serotinus* samples, which formed a monophyletic clade for themselves with a maximum intraclade sequence divergence of only 1.3%. These low distance values between western and eastern haplogroups were not
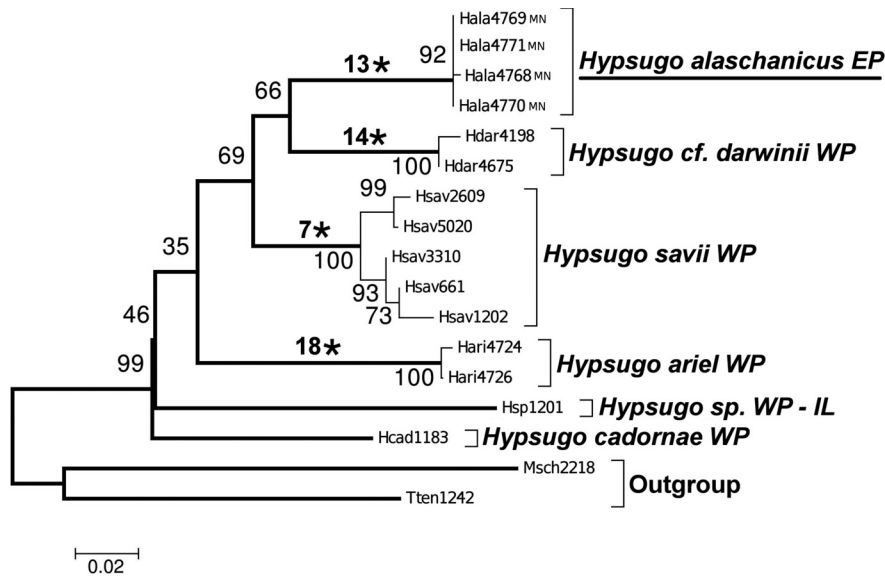
FIG. 2. Neighbor-joining (NJ) tree of the genus *Hypsugo*. NJ tree is built with K2P distances. Numbers at branching points are bootstrap support values (BS). Meaningful decay indices (DI) are given above the branches and asterisks (*) indicate delimited clusters according to the GMYC approach. EP — Eastern Palaearctic, WP — Western Palaearctic, IL — Israel, MN — Mongolian specimen, sp. — unidentified species, cf. — confer

high enough to be detected as discrete by the GMYC method.

Secondly, the sister clade of all *E. nilssonii/ E. serotinus* samples was the *E. gobiensis* lineage (Fig. 3), which has a strict Eastern Palaearctic distribution. Haplotypes between both clades differed by 5.2 and 7.3%, as intraspecific sequence divergence among the six Mongolian *E. gobiensis* samples reached 2.4% (Table 2).

Thirdly, two Mongolian bats, which resembled in their morphology *E. serotinus*, represent a highly distinct lineage. Surprisingly, their sequences differed by 7.1 to 8.0% from sequences of Western Palaearctic *E. serotinus* samples (Table 2). However, the Mongolian haplotypes grouped with those of the subspecies *E. s. turcomanus* (Simmons, 2005) and differed by only 0.3% (Table 2). The Western Palaearctic *E. bottae* lineage appeared as sister clade (Fig. 3) and differed by 6.5 to 7.3% sequence divergence.

Vespertilio

Western and Eastern Palaearctic *V. murinus* samples had rather similar haplotypes that did not differ by more than 1.7% sequence divergence. A Mongolian and a German specimen showed only 0.3% sequence divergence. *Vespertilio murinus* could be distinguished from its Eastern Palaearctic congener *V. sinensis* by 9.0 to 14.1% (Fig. 3 and Table 2). The haplotypes of the Mongolian and Japanese specimens (Kawai *et al.*, 2002) of *V. sinensis* showed a maximum intraspecific sequence divergence of 1.0% (see also Kawai *et al.*, 2010).

Myotis

We sequenced 39 Mongolian specimens of the *M. mystacinus* morphogroup. Sequences of 35 specimens showed high similarities to those of

TABLE 1. ND1 inter- and intraspecific genetic distances of the genus *Hypsugo*. Number of analyzed individuals (*n*) and sample locality (R) are given: EP — Eastern Palaearctic, WP — Western Palaearctic, IL — Israel, sp. — unidentified species, cf. — confer. All distances are uncorrected pairwise *p*-distances. Intraspecific distances are given at the diagonal in bold. One unrealistic high genetic distance is tagged with an exclamation mark. This high value arose probably from a short alignment overlap

No.	Lineage	*n*	R	1	2	3	4	5	6
1	*H. alaschanicus*	4	EP	**0.0–0.2**					
2	*H.* cf. *darwinii*	2	WP	9.2–10.1	**0.6**				
3	*H. savii*	5	WP	9.0–13.0	8.6–11.4	**0.3–3.9**			
4	*H. ariel*	2	WP	13.9–15.5	13.2–13.7	11.8–13.1	**0.4**		
5	*H. cadomae*	1	EP	14.9–17.0	12.9–12.9	12.0–12.6	14.8–15.0	–	
6	*Hypsugo* sp.	1	IL	11.0–27.3!	17.5–17.6	14.0–16.5	15.1–15.5	16.4	–

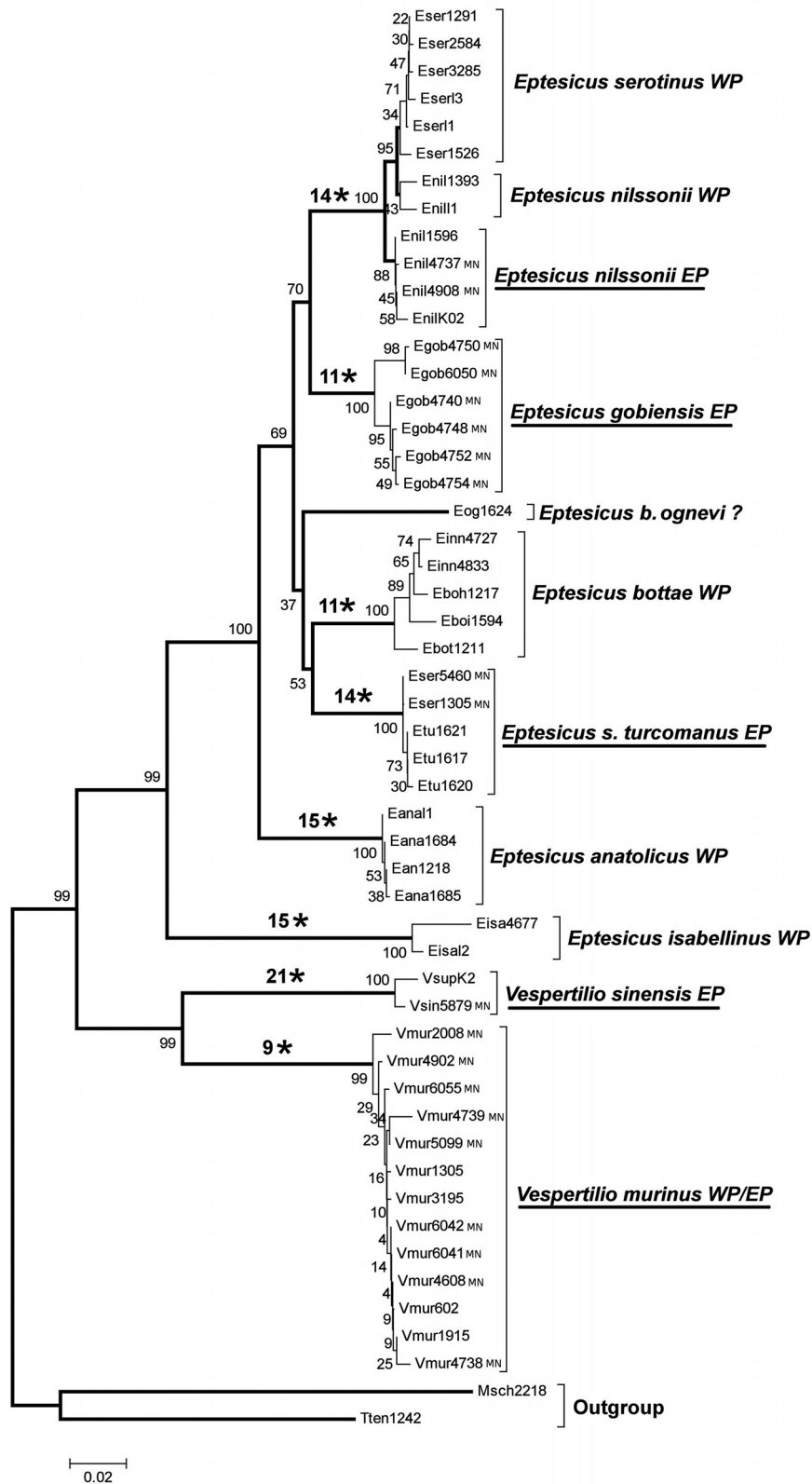FIG. 3. Neighbor-joining (NJ) tree of the genera *Eptesicus* and *Vespertilio*. NJ tree is built with K2P distances. Numbers at branching points are bootstrap support values (BS). Meaningful decay indices (DI) are given above the branches and asterisks (*) indicate delimited clusters according to the GMYC approach. EP — Eastern Palaearctic, WP — Western Palaearctic, ? — unknown origin, MN — Mongolian specimen

TABLE 2. ND1 inter- and intraspecific genetic distances of the genera *Eptesicus* and *Vespertilio*. Number of analyzed specimens (*n*) and sample locality (R) are given: EP — Eastern Palaearctic, WP — Western Palaearctic, ? — unknown origin, b — Western and Eastern Palaearctic. All distances are uncorrected pairwise *p*-distances. Intraspecific distances are given at the diagonal in bold

No.	Lineage	n	R	1	2	3	4	5	6	7	8	9	10	11
1	*E. serotinus*	6	WP	**0.1–0.9**										
2	*E. s. turcomanus*	5	EP	7.1–8.0	**0.0–0.3**									
3	*E. nilssonii*	2	WP	0.8–1.3	7.3–0.8	**1.1**								
4	*E. nilssonii*	4	EP	1.0–1.9	6.4–7.4	1.5–2.0	**0.0–0.5**							
5	*E. gobiensis*	6	EP	5.8–7.3	7.3–8.7	5.8–7.1	5.2–6.6	**0.0–2.4**						
6	*E. bottae*	5	WP	7.6–8.6	6.5–7.3	8.3–8.8	7.5–8.2	6.5–8.4	**0.5–2.4**					
7	*E. anatolicus*	4	WP	8.7–9.5	9.5–10.4	8.5–9.2	7.9–8.9	7.8–9.0	8.5–10.1	**0.0–0.1**				
8	*E. isabellinus*	2	WP	14.6–16.6	16.8–17.9	14.5–16.5	14.0–16.5	14.5–16.2	15.0–17.7	14.3–15.1	**2.4**			
9	*E. b. ognevi*	1	?	8.4–9.2	7.8–8.0	8.9–9.4	8.2–8.4	7.8–8.7	8.3–8.8	10.4–11.0	13.6–17.3	–		
10	*V. sinensis*	2	EP	19.0–20.9	19.5–20.3	20.3–20.9	20.1–20.7	18.9–20.8	18.9–20.1	19.2–19.8	20.2–21.5	19.4–19.5	**1.0**	
11	*V. murinus*	13	b.	18.0–20.5	18.6–20.5	18.8–20.8	18.3–20.1	18.6–20.7	18.3–21.7	18.1–20.4	19.4–25.6	18.3–20.0	9.0–14.1	**0.0–1.6**

M. aurascens from Bulgaria and Asia. Four specimens clustered with those of *M. ikonnikovi* from Japan and Russia (BS 99, DI 25, GMYC support; Fig. 4). Intraspecific sequence divergence among all *M. ikonnikovi* sequences did not exceed 3.5%. The clade of *M. aurascens* was well supported (BS 99, DI 15, GMYC support; Fig. 4) and split in a western and an eastern subclade. Due to incomplete sequences, we restricted the calculation of intraspecific variation within the eastern clade to 26 specimens from Mongolia and the first 443 base pairs of the ND1 gene. Intraclade divergences were up to 0.9% for the eastern and up to 0.5% for the western clade. Interclade divergences ranged from 3.4 to 4.3%. Differences to the lineages *M. mystacinus* and *M. ikonnikovi* were in the range of 6.0 to 13.5% (Fig. 4 and Table 3).

We were not able to confirm the appearance of *M. brandtii* in Mongolia. Instead, morphologically *M. brandtii*-like specimens represent *M. gracilis*, since they have grouped together with published sequences of *M. gracilis* from Japan (Fig. 4). This clade was well supported (BS 99, DI 10, GMYC support) and showed little intraspecific sequence divergence (up to 0.9%; Table 3). Phylogenetic reconstructions supported a sister species relationship between *M. brandtii* and *M. gracilis* (Fig. 4). Both species differed by 9.7 to 11.9% sequence divergence (see also Kawai *et al.*, 2006).

Sequences of six bats, which resembled in their morphology *M. daubentonii*, formed a well-supported clade (BS 99, DI 25, GMYC support; see Fig. 4) and clustered with three published sequences of *Myotis petax* (Kawai *et al.*, 2002, 2003; Matveev *et al.*, 2005; K. Tsytsulina, M. H. Dick, K. Maeda, and R. Masuda, unpublished data). Intraspecific sequence divergence reached 0.7%, but differences to Western Palaearctic *M. daubentonii* samples were at least 12.7% (Table 3).

One specimen from the north-east of Mongolia, which resembled in its morphology *M. nattereri*, clustered with a Japanese specimen of *M. bombinus* (Horáček *et al.*, 2000) on a well-supported branch (BS 99, DI 26, GMYC support; see Fig. 4). We found 3.5% sequence divergence between both haplotypes. Surprisingly, these specimens differed by at least 11.4% from Western Palaearctic *M. nattereri* sensu stricto and sequence differences were even higher to other representatives of the *M. nattereri* species group including *M. escalerai*, *M. schaubi*, and '*M. nattereri*' from northern Iberia and Austria or Morocco (Fig. 4 and Table 3).

FIG. 4. Neighbor-joining (NJ) tree of the genus *Myotis*. NJ tree was built with Kimura-2-Parameter distances. Numbers at branching points are bootstrap support values (BS). Meaningful decay indices (DI) are given above the branches and asterisks (*) indicate delimited clusters according to the GMYC approach. EP — Eastern Palaearctic, WP — Western Palaearctic, SI — South Iberia, NI — North Iberia, AT — Austria, MA — Morocco, ? — unknown origin, MN — Mongolian specimen, s.s. — sensu stricto, cf. — confer

Plecotus

We found four divergent lineages among Mongolian long-eared bats (Fig. 5). Sequencing a fragment of the 16S rRNA gene and comparisons with published sequences (Spitzenberger *et al.*, 2006) allowed the assignment of these four lineages to the four species *P. ognevi*, *P. turkmenicus*, *P. strelkovi*, and *P. kozlovi*. These four species showed ND1 sequence divergences in the range of 4.2 to 16.6% to each other (Table 4). Intraspecific sequence divergence among twelve specimens of *P. ognevi* reached 4.0%. A high bootstrap support value (100), a decay index of 8, and the GMYC approach supported this taxon. Calculations of intraspecific sequence divergences for the other three Mongolian lineages were not possible, because we had only one sequence of each taxon.

Phylogenetic analyses of the genus *Plecotus* suggested a monophyletic assemblage of all Eastern Palaearctic sequences (Fig. 5), although statistical support was low. The Eastern Palaearctic clade included a fifth lineage that was represented by one specimen from Japan (*P.* cf. *auritus* from Kawai *et al.*, 2002). Sequence differences of at least 9.1 and up to 28.7% were found between Western and Eastern Palaearctic species (Table 4). For example, *P. ognevi* differed by 14.6 to 17.0% sequence divergence from *P. auritus*, its putative Western

Palaearctic conspecies. In addition, *P. auritus* differed from Japanese *P.* cf. *auritus* by at least 14.1% sequence divergence. The divergence between *P. strelkovi* and *P. austriacus* was 17.5% at the minimum (see Spitzenberger *et al.*, 2006).

Nyctalus

We found one specimen from the south-west of Mongolia, which resembled in its morphology *Nyctalus noctula*. It clustered with German samples of *N. noctula* (Mayer and von Helversen, 2001; Mayer *et al.*, 2007) on a well-supported branch (BS 100, DI 9, GMYC support; Fig. 6). ND1 divergences were in the range of 0.3 to 1.3%. *Nyctalus noctula* differed from *N. aviator* and *N. lasiopterus* roughly by 5.5 to 7.5% and from *N. leisleri* and *N. plancyi* by at least 12% (Fig. 6 and Table 5).

Divergence Times of the Genera Hypsugo, Myotis, *and* Plecotus

Bayes factor comparisons revealed that the uncorrelated lognormal relaxed clock model outperformed the strict clock model. A coefficient of variation of 1.205 indicated high rate variation among the analyzed taxa (Drummond *et al.*, 2007). In the final analysis, we performed two independent runs under the uncorrelated lognormal relaxed clock
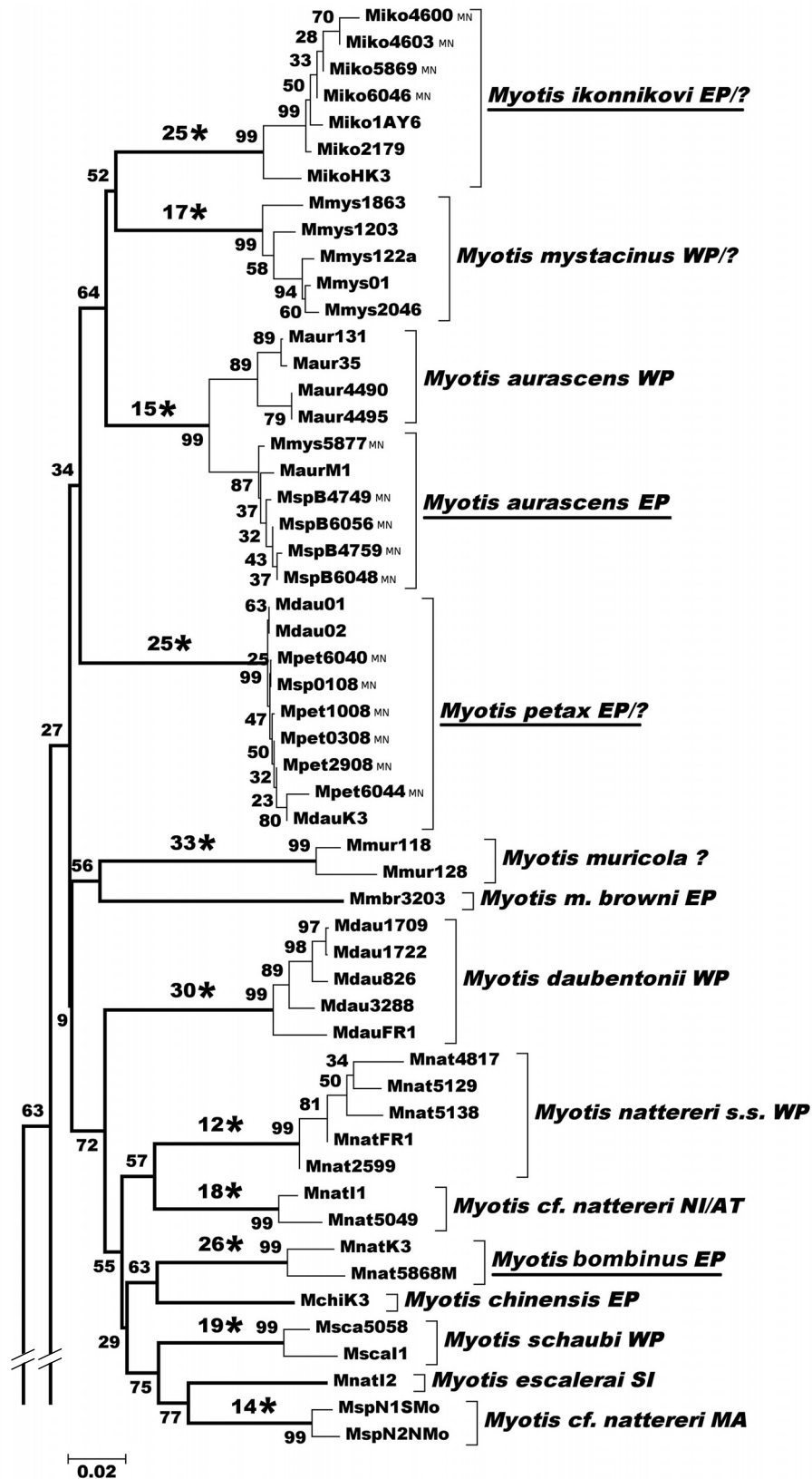
FIG. 5. Neighbor-joining (NJ) tree of the genus *Plecotus*. NJ tree was built with K2P distances. Numbers at branching points are bootstrap support values (BS). Meaningful decay indices (DI) are given above the branches and asterisks (*) indicate delimited clusters according to the GMYC approach. EP — Eastern Palaearctic, WP — Western Palaearctic, MN — Mongolian specimen, cf. — confer

model (UCLN) with 50 million steps each. We used a burnin of 20% and combined both runs (Bayes factor < 0.5) with the software LogCombiner. The maximum clade credibility tree was computed with the software TreeAnnotator. The analysis revealed that the divergence (point estimates) of species within the genera *Hypsugo*, *Myotis*, and *Plecotus* happened prior to the Pleistocene (Fig. 7).

DISCUSSION

Species with a Whole Palaearctic Distribution

Genetic screening of a 798 bp fragment of the mitochondrial ND1 gene revealed a wide Western and Eastern Palaearctic distribution range in only three (*E. nilssonii*, *V. murinus*, and *N. noctula*) out of 16 Mongolian bat species of the family Vespertilionidae. In comparison to all other investigated species, the distribution of *E. nilssonii*, *V. murinus*, and *N. noctula* extends further to the north and even breeding above the Arctic Circle was described for *E. nilssonii* (Rydell, 1993; Rydell and Baagøe, 1994; Rydell *et al.*, 1994; Simmons, 2005). Thus their populations were likely not as strongly repelled to the south during glaciations as those of other species, which have a more southern distribution today. Perhaps *E. nilssonii*, *V. murinus*, and *N. noctula* had

a much wider distribution range during Pleistocene glaciations as seen today. Probably they were repelled to higher latitudes and altitudes after the last glacial maximum (LGM), because temperatures started to rise drastically. Similar minor intraspecific divergences were found in several other cold adapted or at least cold tolerant species, as in the reindeer *Rangifer tarandus*, the arctic fox *Alopex lagopus*, or the snowy owl *Bubo scandiacus* (Dalen *et al.*, 1994; Flagstad and Røed, 2003; Marthinsen *et al.*, 2009). Almost no geographic structuring and little morphological variation across the Palaearctic populations were found. The authors came to the conclusion that each species has formed one large panmictic population in the past and maybe still today. A recent range expansion was detected in all data sets. The spread of populations created a pattern of low genetic structure, but relatively high genetic diversities could be observed as in the highly mobile bat species *E. nilssonii*, *V. murinus*, and *N. noctula*.

Distinct Lineages in The West and East

The other 13 Mongolian lineages showed distinct mtDNA haplotypes that differed by at least 5% from Western Palaearctic lineages. Nine of these 13 taxa are well known species from the Eastern Palaearctic region (Simmons, 2005). Mongolian *H. alaschanicus* (Bobrinskoj, 1926)
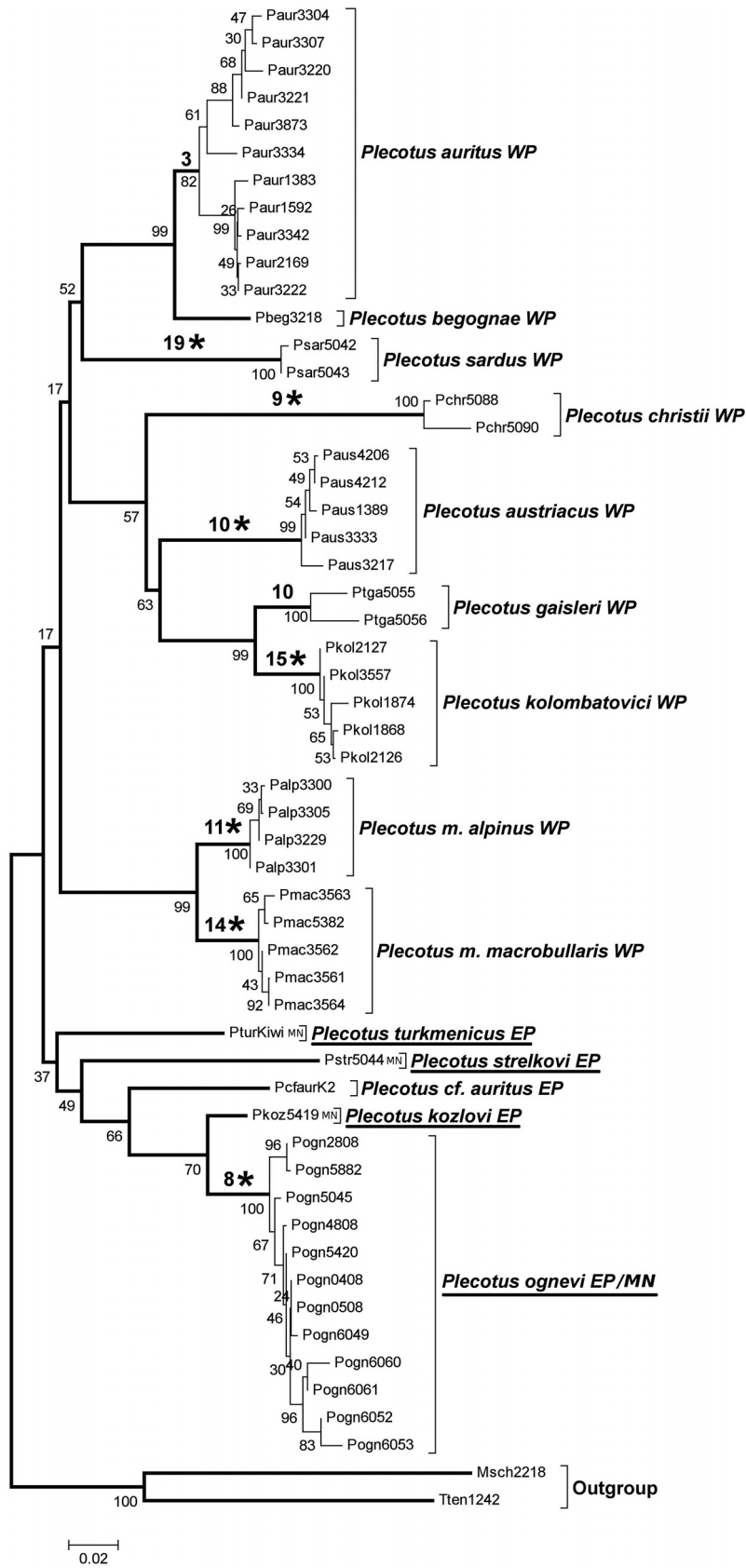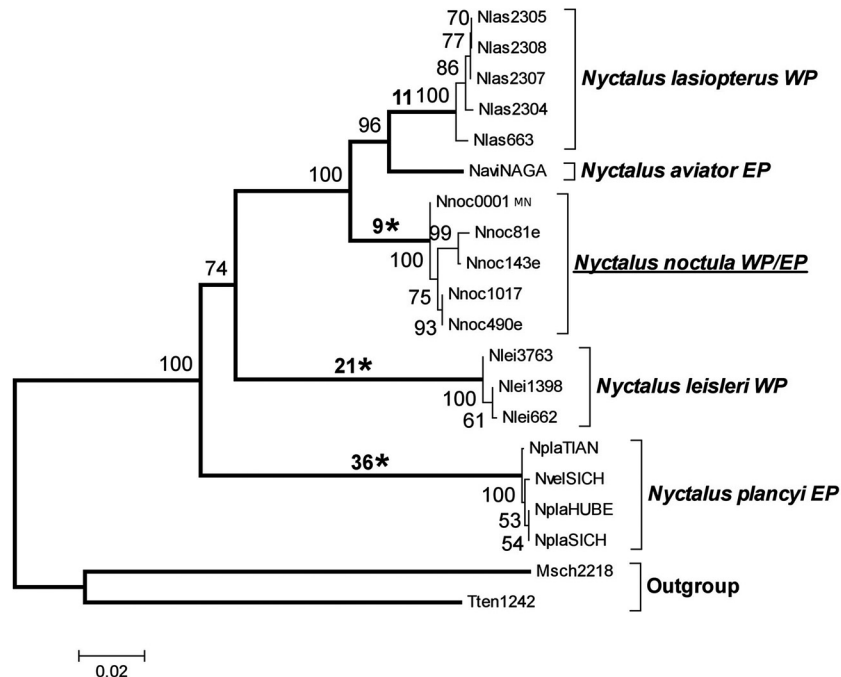

FIG. 6. Neighbor-joining (NJ) tree of the genus *Nyctalus*. NJ tree was built with K2P distances. Numbers at branching points are bootstrap support values (BS). Meaningful decay indices (DI) are given above the branches and asterisks (*) indicate delimited clusters according to the GMYC approach. EP — Eastern Palaearctic, WP — Western Palaearctic, MN — Mongolian specimen

TABLE 3. ND1 inter- and intraspecific genetic distances of the genus *Myotis*. Number of analyzed specimens (*n*) and sample locality (R) are given: EP — Eastern Palaearctic, WP — Western Palaearctic, SI — South Iberia, NI — North Iberia, AT — Austria, MA — Morocco, ? — unknown origin, sp. — unidentified species, s.s. — sensu stricto, cf. — confer. All distances are uncorrected pairwise *p*-distances. Intraspecific distances are given at the diagonal in bold. Nine specimens of *M. aurascens* were excluded in the calculation of intra- and interclade divergences of Western and Eastern Palaearctic samples, because too much sequence information was missing for them (values are marked with asterisks)

No.	Lineage	n	R	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*M.* sp. *auras*	26*/35	EP	**0.0–0.9***																	
2	*M. aurascens*	4	WP/?	3.4–4.3*	**0.0–0.5***																
3	*M. mystacinus*	5	WP/?	7.7–13.2	6.0–13.1	**0.6–3.0**															
4	*M. ikonnikovi*	7	EP/?	9.6–13.5	10.0–13.4	11.0–14.2	**0.0–3.5**														
5	*M. petax*	9	EP/?	10.8–12.9	11.3–11.8	10.7–14	11.2–15.6	**0.0–0.7**													
6	*M. daubentonii*	5	WP	11.3–16.6	13.3–14.8	14.1–15.0	14.8–18.8	12.7–14.9	**0.1–3.8**												
7	*M. muricola*	2	?	14.0–17.4	14.7–16.8	14.05–16.7	15.2–19.3	14.3–18.8	15.0–17.7	**2.9**											
8	*M. m. browni*	1	EP	14.4–17.4	14.7–16.1	14.2–15.0	15.6–17.9	14.4–16.7	14.6–16.2	14.6–16.0	**—**										
9	*M. schaubi*	1	WP	11.9–15.5	13.8–15.1	14.5–17.0	13.0–18.3	14.0–16.6	11.6–15.4	14.0–17.1	14.4–16.3	**2.7**									
10	*M. escalerai*	1	SI	13.7–16.3	14.8–16.2	15.6–17.1	14.2–16.2	14.2–16.6	13.4–14.4	16.2–17.9	16.1	11.2–11.3	**—**								
11	*M. bombinus*	2	EP	12.3–16.3	13.7–15.4	14.1–16.7	13.5–18.8	14.9–17.6	12.2–14.0	15.3–17.3	16.0–16.8	12.0–13.2	11.7–12.9	**3.5**							
12	*M. chinensis*	1	EP	12.2–13.9	12.8–13.0	13.2–14.6	12.3–15.1	13.8–15.4	12.2–13.3	14.5–16.5	14.4	10.8–11.5	12.6	9.8–10.4	**—**						
13	*M.* cf. *nattererii*	2	NI/AT	9.8–14.6	12.6–13.7	11.9–15.9	12.3–15.4	13.6–14.9	11.9–14.8	15.7–18.2	14.4–15.9	11.3–12.1	12.2–13.3	12.1–15.9	10.4–11.4	**2.1**					
14	*M. nattererii* s.s	5	WP	12.6–16.6	14.2–17.3	14.0–16.5	12.8–17.8	12.9–15.9	12.5–16.5	15.1–20.5	15.8–19.8	10.7–14.1	12.6–16.4	11.4–17.1	9.9–11.4	8.6–13.5	**0.0–3.4**				
15	*M.* cf. *nattererii*	2	MA	13.0–18.0	13.2–15.2	14.8–16.1	13.0–17.9	15.3–17.4	13.1–14.4	14.7–17.0	15.3–15.5	9.3–10.7	8.9–9.3	12.0–12.4	11.5–11.9	11.4–12.3	10.7–17.9	**1.6**			
16	*M. alcathoe*	5	WP	11.4–14.6	13.4–14.2	13.5–14.8	13.7–16.8	12.4–15.8	15.3–16.2	14.8–17.2	15.9–16.2	14.7–16.5	12.7–13.2	15.1–16.3	12.9–13.4	12.1–14.2	12.1–16.9	14.7–15.9	**0.0–1.4**		
17	*M. brandtii*	5	WP	14.2–19.3	15.3–17.2	14.8–16.5	14.4–17.1	14.9–16.5	16.1–17.5	15.9–17.8	15.1–15.5	16.1–17.3	15.5–16.5	15.7–16.2	15.4–15.8	12.9–13.4	15.4–15.8	13.7–19.9	14.5–15.2	**0.6–1.0**	
18	*M. gracilis*	10	EP	11.9–17.9	15.5–16.4	15.0–18.0	14.5–18.5	14.9–18.5	15.8–19.8	15.3–18.7	14.9–16.7	15.3–18.7	15.0–18.3	15.0–18.3	15.5–18.3	15.4–16.7	16.4–21.5	15.4–17.9	15.5–17.9	9.7–11.9	**0.0–0.9**

samples formed a highly differentiated mtDNA lineage with little intraspecific variation. Our study is in accordance with the current view that *E. gobiensis* and *V. sinensis* have only an Eastern Palaearctic distribution (Simmons, 2005). Their ND1 sequences were highly distinct from other Mongolian and Western Palaearctic congeneric taxa (this paper and Kawai *et al.*, 2010). Our data confirm the eastern distribution of the four taxa *P. turkmenicus*, *P. ognevi*, *P. strelkovi*, and *P. kozlovi*, as it was shown by Spitzenberger *et al.* (2006) and show their appearance in Mongolia. *Plecotus turkmenicus*, *P. ognevi*, and *P. strelkovi* have a predominantly Eastern Palaearctic distribution, although they were sporadically notified in eastern parts of the Western Palaearctic region. As far as we know, *P. kozlovi* is reported only in the Far East including Mongolia and China (Spitzenberger *et al.*, 2006). None of the Western Palaearctic species of the genus *Plecotus* were found in Mongolia. Thirty-five samples of the *M. mystacinus* species group could be genetically assigned to the species *M. aurascens* and only four samples to the species *M. ikonnikovi* (Benda and Tsytsulina, 2000). *Myotis ikonnikovi* has an Eastern Palaearctic distribution (Simmons, 2005; Kawai *et al.*, 2006). The appearance of *M. aurascens* in Mongolia shows that the distribution range of this species extends further to the East than previously thought (Benda *et al.*, 2011).

A wide distribution allover the Palaearctic region has been assumed in the remaining four species: *M. brandtii*, *M. daubentonii*, *M. nattereri*, and *E. serotinus* (Simmons, 2005). In contrast to this view, we found highly distinct mitochondrial haplotypes between bats of the Western Palaearctic region and Mongolia. All Mongolian specimens, which resembled in their morphology *M. brandtii*, belonged to a distinct mitochondrial lineage, which substantially differed from European *M. brandtii*, but clustered with *Myotis gracilis* haplotypes from Japan (Kawai *et al.*, 2003, 2006). Slight morphological differences between both taxa support the view that both lineages represent two independent species (Horáček *et al.*, 2000; Kawai *et al.*, 2006; Stadelmann *et al.*, 2007, but see also Sokolov and Orlov, 1980; Tinnin *et al.*, 2002; Simmons, 2005; Dolch *et al.*, 2007). Therefore, *M. brandtii* is not present in Mongolia or is at least rather rare.

Six specimens of '*M. daubentonii*' from Central and East Mongolia clustered with samples of Asian '*M. daubentonii*' from Japan and Russia (Kawai *et al.*, 2002, 2003) and differed by at least 12.7% from *M. daubentonii* haplotypes from

Europe. Therefore, our data support the conclusions of Matveev *et al.* (2005). They have raised Eastern Palaearctic forms of '*M. daubentonii*' to a new species, which they designated to *Myotis petax* Hollister, 1912.

One Mongolian specimen, which resembled in its morphology *M. nattereri*, differed in its ND1 sequence from those of Western Palaearctic *M. nattereri* by at least 11.4%, but clustered with a *M. bombinus* sample from Japan (Horáček *et al.*, 2000;

Kawai *et al.*, 2003). Interestingly, Eastern Palaearctic samples of *M. bombinus* did not form the sister group to Western Palaearctic samples of *M. nattereri*. Instead, they clustered more closely with *Myotis chinensis* Tomes, 1857.

The diversity of the mitochondrial genome of *E. serotinus* remains unclear. We obtained ND1 sequences of two Mongolian specimens, which resembled in their morphology *E. serotinus*, that differed by more than 7% from Western European samples of
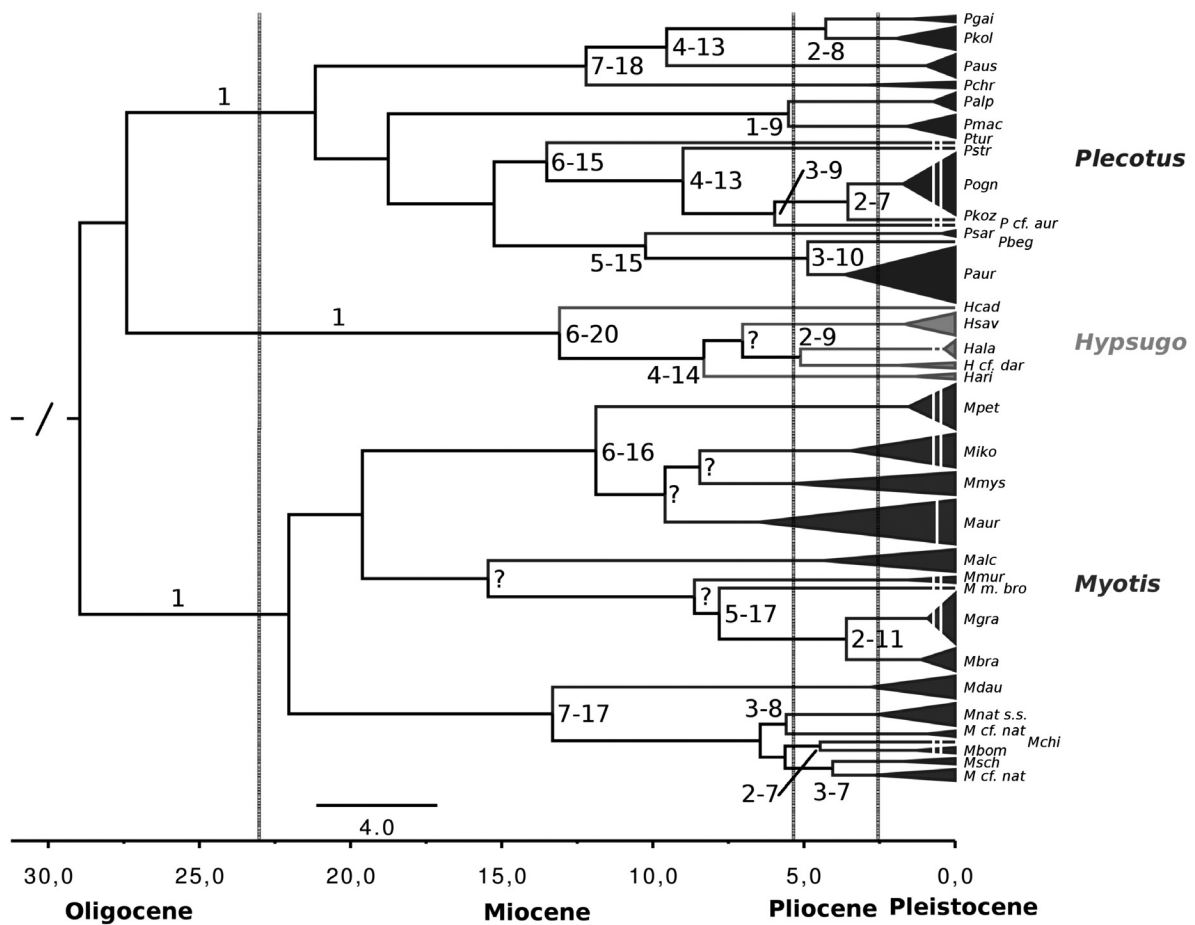

FIG. 7. Bayesian dating. Bayesian inference of divergence times among the genera *Plecotus*, *Hypsugo*, and *Myotis* under the UCLN model in BEAST resulted in the featured maximum clade credibility tree, which was based on 16,002 sampled trees. The Geological Time Scale (2004) of The International Commission on Stratigraphy (ICS) was used as a timetable. The time axis was scaled in million years ago (MYA). *Plecotus* was represented by 53 specimens in 14 differentiated lineages, *Hypsugo* by 14 specimens in five lineages, and *Myotis* by 73 specimens in 16 lineages. Broken lines at the tips mark Eastern- and solid lines Western Palaearctic lineages. Only one analyzed taxon (*M. aurascens*) seems present in both parts of the Palaearctic region (marked with only one dash). The monophyly of the genera *Plecotus*, *Hypsugo*, and *Myotis* was supported by a posterior probability of 1 in each case. Numbers at the nodes represent confidence intervals (95% HPD) in million years ago rounded to nearest integer. Only nodes that are relevant to the divergence of lineages were tagged. Nodes with question marks had a posterior probability lower than 0.5 and therefore calculation of confidence intervals were not possible for them. Most species divergences happened in the late Miocene and following Pliocene (point estimates). Species names were abbreviated: *Plecotus*: Pgai — *gaisleri*, Pkol — *kolombatovici*, Paus — *austriacus*, Pchr — *christii*, Palp — *alpinus*, Pmac — *macrobullaris*, Ptur — *turkmenicus*, Pstr — *strelkovi*, Pogn — *ognevi*, Pkoz — *kozlovi*, P cf. *aur* — cf. *auritus*, Psar — *sardus*, Pbeg — *begognae*, Paur — *auritus*; *Hypsugo*: Hcad — *cadornae*, Hsav — *savii*, Hala — *alaschanicus*, H cf. *dar* — cf. *darwinii*, Hari — *ariel*; *Myotis*: Mpet — *petax*, Miko — *ikonnikovi*, Mmys — *mystacinus*, Maur — *aurascens*, Malc — *alcathoe*, Mmur — *muricola*, M m. *bro* — *muricola browni*, Mgra — *gracilis*, Mbra — *brandtii*, Mdau — *daubentonii*, Mnat s.s. — *nattereri* sensu stricto, M cf. *nat* — cf. *nattereri*, Mchi — *chinensis*, Mbom — *bombinus*, Msch — *schaubi*

TABLE 4. ND1 inter- and intraspecific genetic distances of the genus *Plecotus*. Number of analyzed specimens (*n*) and sample locality (R) are given: EP — Eastern Palaearctic, WP — Western Palaearctic, cf. — confer. All distances are uncorrected pairwise *p*-distances. Intraspecific distances are given at the diagonal in bold

No.	Lineage	n	R	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	P. auritus	11	WP	**0.0–4.4**													
2	P. begognae	1	WP	4.9–7.1	–												
3	P. sardus	2	WP	11.3–14.2	13.1–13.5	**0.3**											
4	P. christi	2	WP	15.5–19.6	16.5–17.5	18.0–18.1	**2.1**										
5	P. austriacus	5	WP	14.7–18.4	16.3–16.8	15.8–16.9	15.0–16.2	**0.0–1.8**									
6	P. gaisleri	2	WP	15.5–18.5	16.8–18.4	19.7–20.0	17.6–18.8	11.6–13.5	**3.3**								
7	P. kolombatovici	5	WP	14.5–17.7	16.7–17.8	17.9–19.5	15.2–20.0	10.4–12.4	5.9–7.1	**0.0–0.9**							
8	P. m. alpinus	4	WP	12.1–14.6	12.8–14.2	14.6–16.0	17.8–19.6	15.2–19.1	17.2–19.1	16.0–18.5	**0.0–0.4**						
9	P. m. macrobullaris	5	WP	12.8–14.7	13.7–14.2	15.0–15.9	18.0–21.4	16.5–18.0	17.5–18.9	18.1–18.6	4.6–5.3	**0.0–0.9**					
10	P. turkmenicus	1	EP	13.0–14.4	13.2	14.2–14.5	18.6–21.0	16.3–17.0	18.1–18.6	16.3–18.4	14.1–14.6	14.0–14.6	–				
11	P. strelkovi	1	EP	15.6–18.2	15.4	17.0–17.0	20.8–23.4	17.5–18.3	18.1–18.1	15.4–16.5	14.1–14.6	16.0–16.9	14.1	–			
12	P. cf. auritus	1	EP	14.1–16.7	15.6	16.1–16.3	15.8–18.7	17.5–18.0	18.2–18.9	18.7–19.7	15.2–16.3	16.0–16.9	14.9	15.2	–		
13	P. kozlovi	1	EP	13.6–18.1	15.4	14.2–14.7	9.1–12.0	15.6–16.6	17.3–17.4	17.5–18.8	16.3–16.7	16.0–16.7	12.5	13.4	9.1	–	
14	P. ognevi	12	EP	14.6–17.0	15.9–17.8	15.2–17.9	24.7–28.7	17.1–20.0	18.0–21.7	17.9–21.3	14.1–17.3	13.0–13.7	13.6–15.7	13.7–16.6	10.9–13.3	4.2–7.8	**0.0–4.0**

E. serotinus and *E. nilssonii*. ND1 divergences of Western European *E. serotinus* samples fall within the range of intraspecific variation of *E. nilssonii*, which is most likely the result of mitochondrial introgression of the *E. nilssonii* mitochondrial genome into populations of the Western European *E. serotinus* lineage (Ruedi and Mayer, 2001; Artyushin *et al.*, 2009). Therefore, the distinct *Eptesicus* haplotype from Mongolia might represent the 'original' lineage of Western European *E. serotinus*, which was presumably found in Eastern European samples of *E. serotinus* (Artyushin *et al.*, 2009). Alternatively, it could be a separate divergent lineage, which only occurs in the Eastern Palaearctic region. Currently we cannot distinguish between these options, because no ND1 sequences are available from Eastern European populations of *E. serotinus*.

Applying a molecular clock in a phylogenetic analysis allowed the estimation of divergence times among Western and Eastern Palaearctic lineages. We used fossils of the genus *Myotis* to calibrate the clock. The reconstruction resulted in deep divergences between Western and Eastern Palaearctic bats that likely occurred prior to the Pleistocene (Fig. 7). Such substantial divergences of mitochondrial lineages among the Western and Eastern Palaearctic region are in accordance with studies of other temperate animals and arctic plants (Hewitt, 2000).

The distribution of glacial refugia and physical barriers (e.g. Ural mountains) during the range expansions have likely shaped the current distribution of taxa. The Western Palaearctic region was mainly populated from species of southern refugia during interglacials: the Iberian, Italian, and Balkan Peninsulas in the Mediterranean region and the Caucasus in the East (Taberlet *et al.*, 1998). The refugia of the Eastern Palaearctic region are less clear. Many eastern refugia were postulated, but comprehensive data are still lacking for most species. Especially mountainous regions, such as the Altai Heights, the Beringian upland tundra, the Central Siberian Plateau, the Putoran Mountains, or the Southern Ural were notified as possible refugia of many taxa (Hewitt, 2004a, 2004b). To which degree particular regions were also refugia of the different bat lineages cannot be answered with the existing data. A wide geographic sampling of selected bat taxa, especially from the Eastern Palaearctic region, is required to answer this question.

A comparable high degree of differentiation was also found among hipposiderid bats from South-

TABLE 5. ND1 inter- and intraspecific genetic distances of the genus *Nyctalus*. Number of analyzed specimens (*n*) and sample locality (R) are given: EP — Eastern Palaearctic, WP — Western Palaearctic. All distances are uncorrected pairwise *p*-distances. Intraspecific distances are given at the diagonal in bold

No.	Lineage	*n*	R	1	2	3	4	5
1	*N. lasiopterus*	5	WP	**0.0–0.9**				
2	*N. leisleri*	3	WP	12.2–13.0	**0.0–0.2**			
3	*N. noctula*	5	WP/EP	5.6–6.6	12.6–13.4	**0.0–1.3**		
4	*N. plancyi*	4	EP	15.9–16.2	15.4–16.3	14.1–15.2	**0.0–0.4**	
5	*N. aviator*	1	EP	4.4–4.5	11.7–12.3	5.7–7.4	15.3–15.4	–

east Asia (Murray *et al.*, 2012). Deep genetic divergences within several species suggest a high number of cryptic species in this region. Another study revealed a high degree of differentiation in neotropical bats (Clare *et al.*, 2011). Intraspecific variability ranged from 0 to almost 12% among traditional species. Such high values are a strong indicator for the presence of cryptic species. Kruskop *et al.* (2012) also found deep phylogeographic splits in pairs of vicariant species of Palaearctic bats.

CONCLUSIONS

Screening a fragment of the mitochondrial ND1 gene revealed a strong divergence between Western and Eastern Palaearctic vespertilionid bats. Most comparisons among western and eastern bats resulted in highly differentiated lineages with more than five percent ND1 sequence divergence. Surprisingly, bats that closely resemble each other in morphology were genetically not necessarily closely related. Vespertilionid bats can have a conserved and indecisive morphotype making it difficult to distinguish them at the species level. On the other hand, independent adaptations in Western and Eastern Palaearctic lineages may have happened and resulted in strikingly similar morphological phenotypes, as it was shown for example in bats of the genus *Myotis* (Ruedi and Mayer, 2001). Deep mitochondrial divergences between Western and Eastern Palaearctic lineages seem to result from relatively old species splittings prior to the Pleistocene. Several Western and Eastern Palaearctic refugia during Pleistocene glaciations and restricted gene flow between these parts may have influenced the current distribution of bats. Unfortunately, we have no evidence for the existence and strength of reproductive barriers. This would require the analysis of populations in regions of secondary contact. Therefore, the question whether all of the detected lineages represent biological species remains unanswered at this time.

LITERATURE CITED

AHLÉN, I. 2010. Nymffladdermus *Myotis alcathoe* — en nyupptäckt art i Sverige. [Alcathoe's bat *Myotis alcathoe*, a new member of Sweden's bat fauna.] Fauna och Flora, 105(4): 8–15.

ARTYUSHIN, I. V., A. A. BANNIKOVA, V. S. LEBEDEV, and S. V. KRUSKOP. 2009. Mitochondrial DNA relationships among North Palaearctic *Eptesicus* (Vespertilionidae, Chiroptera) and past hybridization between common serotine and northern bat. Zootaxa, 2262: 40–52.

BANNIKOV, A. G. 1954. Mammals of the Mongolian Peoples Republic. Academy of Sciences of the USSR, Moscow, 669 pp.

BENDA, P., and K. A. TSYTSULINA. 2000. Taxonomic revision of *Myotis mystacinus* group (Mammalia: Chiroptera) in the western Palearctic. Acta Societatis Zoologicae Bohemicae, 64: 331–398.

BENDA, P., S. AULAGNIER, A. M. HUTSON, K. TSYTSULINA, A. KARATAŞ, J. PALMEIRIM, and M. PAUNOVIĆ. 2011. *Myotis aurascens*. IUCN red list of threatened species. Available from http://www. iucnredlist.org/details/136553/0.

BOBRINSKOJ, N. A. 1926. Note préliminaire sur les Chiroptères de l'Asie Centrale. Comptes Rendus de l'Académie des Sciences de l'URSS, A: 95–98.

CLARE, E. L., B. K. LIM, M. B. FENTON, and P. D. N. HEBERT. 2011. Neotropical bats: estimating species diversity with DNA barcodes. PloS ONE, 6(7): e22648.

DALEN, L., E. FUGLEI, and P. HERSTEINSSON. 1994. Population history and genetic structure of a circumpolar species: the arctic fox. Biological Journal of the Linnean Society, 84: 79–89.

DOLCH, D., N. BATSAIKHAN, K. THIELE, F. BURGER, I. SCHEFFLER, A. KIEFER, F. MAYER, R. SAMJAA, M. STUBBE, L. KRALL, and D. STEINHAUSER. 2007. Contributions to the

Chiroptera of Mongolia with first evidences on species communities and ecological niches. Erforschung biologischer Ressourcen der Mongolei (Halle/Saale), 10: 407–458.

DRUMMOND, A. J., and A. RAMBAUT. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology, 7: 214.

DRUMMOND, A. J., S. Y. W. HO, M. J. PHILLIPS, and A. RAMBAUT. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biology, 4(5): e88.

DRUMMOND, A. J., S. Y. W. HO, N. RAWLENCE, and A. RAMBAUT. 2007. A rough guide to BEAST 1.4. Available from https://code.google.com/p/beast-mcmc/.

FLAGSTAD, O., and K. H. RØED. 2003. Refugial origins of reindeer (*Rangifer tarandus*) inferred from mitochondrial DNA sequences. Evolution, 57: 658–670.

FONTANETO, D. E., C. HERNIOU, M. BOSCHETTI, G. CAPRIOLI, C. R. MELONE, and T. G. BARRACLOUGH. 2007. Independently evolving species in asexual bdelloid rotifers. PLoS Biology, 5(4): e87.

FRANCIS, C. M., A. V. BORISENKO, N. V. IVANOVA, J. L. EGER, B. K. LIM, A. GUILLÉN-SERVENT, S. V. KRUSKOP, I. MACKIE, and P. D. N. HEBERT. 2010. The role of DNA barcodes in understanding and conservation of mammal diversity in Southeast Asia. PLoS ONE, 5(9): e12575.

HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95–98.

HASEGAWA, M., H. KISHINO, and T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution, 22: 160–174.

HEWITT, G. M. 2000. The genetic legacy of the Quaternary ice ages. Nature, 405: 907–913.

HEWITT, G. M. 2004a. Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London, 359B: 183–95.

HEWITT, G. M. 2004b. The structure of biodiversity — insights from molecular phylogeography. Frontiers in Zoology, 1: 4.

HORÁČEK, I., and V. HANÁK. 1983–84. Comments on the systematics and phylogeny of *Myotis nattereri* (Kuhl, 1818). Myotis, 21–22: 20–29.

HORÁČEK, I., V. HANÁK, and J. GAISLER. 2000. Bats of the Palaearctic region: a taxonomic and biogeographic review. Pp. 11–157, *in* Proceedings of the VIIIth European Bat Research Symposium (B. W. WOŁOSZYN, ed.). Institute of Systematics and Evolution of Animals PAS, Kraków, 280 pp.

IBÁÑEZ, C., J. L. GARCIA-MUDARRA, M. RUEDI, B. STADELMANN, and J. JUSTE. 2006. The Iberian contribution to cryptic diversity in European bats. Acta Chiropterologica, 8: 277–297.

JAN, C. M. I., K. FRITH, A. M. GLOVER, R. K. BUTLIN, C. D. SCOTT, F. GREENAWAY, M. RUEDI, A. C. FRANTZ, D. A. DAWSON, and J. D. ALTRINGHAM. 2010. *Myotis alcathoe* confirmed in the UK from mitochondrial and microsatellite DNA. Acta Chiropterologica, 12: 471–483.

KAWAI, K., M. NIKAIDO, M. HARADA, S. MATSUMURA, L. K. LIN, Y. WU, M. HASEGAWA, and N. OKADA. 2002. Intra- and interfamily relationships of Vespertilionidae inferred by various molecular markers including SINE insertion data. Journal of Molecular Evolution, 55: 284–301.

KAWAI, K., M. NIKAIDO, M. HARADA, S. MATSUMURA, L. K. LIN, Y. WU, M. HASEGAWA, and N. OKADA. 2003. The status of the Japanese and East Asian bats of the genus *Myotis* (Vespertilionidae) based on mitochondrial sequences. Molecular Phylogenetics and Evolution, 28: 297–307.

KAWAI, K., N. KONDO, N. SASAKI, D. FUKUI, H, DEWA, M. SATO, and Y. YAMAGA. 2006. Distinguishing between cryptic species *Myotis ikonnikovi* and *M. brandtii gracilis* in Hokkaido, Japan: evaluation of a novel diagnostic morphological feature using molecular methods. Acta Chiropterologica, 8: 95–102.

KAWAI, K., D. FUKUI, M, SATO, M. HARADA, and K. MAEDA. 2010. *Vespertilio murinus* Linnaeus, 1758 confirmed in Japan from morphology and mitochondrial DNA. Acta Chiropterologica, 12: 463–470.

KIEFER, A. 2007. Phylogeny of Western Palaearctic long-eared bats (Mammalia, Chiroptera, *Plecotus*) — a molecular perspective. PhD Thesis, Gutenberg University, Mainz, 132 pp.

KRUSKOP, S. V., A. V. BORISENKO, N. V. IVANOVA, K. L. BURTON, and J. L. EGER. 2012. Genetic diversity of northeastern Palaearctic bats as revealed by DNA barcodes. Acta Chiropterologica, 14: 1–14.

KUMAR, S., K. TAMURA, and M. NEI. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics, 5: 150–163.

MARTHINSEN, G., L. WENNERBERG, R. SOLHEIM, and J. T. LIFJELD. 2009. No phylogeographic structure in the circumpolar snowy owl (*Bubo scandiacus*). Conservation Genetics, 10: 923–933.

MATVEEV, V. A., S. V. KRUSKOP, and D. A. KRAMEROV. 2005. Revalidation of *Myotis petax* Hollister, 1912 and its new status in connection with *M. daubentonii* Kuhl, 1817 (Vespertilionidae, Chiroptera). Acta Chiropterologica, 7: 23–37.

MAYER, F., and O. VON HELVERSEN. 2001. Cryptic diversity in European bats. Proceedings of the Royal Society of London, 268B: 1825–1832.

MAYER, F., C. DIETZ, and A. KIEFER. 2007. Molecular species identification boosts bat diversity. Frontiers in Zoology, 4: 4.

MCKENNA, M. C., and S. K. BELL. 1997. Classification of mammals above the species level. Columbia University Press, New York, 631 pp.

MONAGHAN, M. T., R. WILD, M. ELLIOT, T. FUJISAWA, M. BALKE, D. J. G. INWARD, D. C. LEES, R. RANAIVOSOLO, P. EGGLETON, T. G. BARRACLOUGH, and A. P. VOGLER. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. Systematic Biology, 58: 298–311.

MURRAY, S. W., P. CAMPBELL, T. KINGSTON, A. ZUBAID, C. M. FRANCIS, and T. H. KUNZ. 2012. Molecular phylogeny of hipposiderid bats from Southeast Asia and evidence of cryptic diversity. Molecular Phylogenetics and Evolution, 62: 597–611.

NIERMANN, I., M. BIEDERMANN, W. BOGDANOWICZ, R. BRINKMANN, Y. LE BRIS, M. CIECHANOWSKI, C. DIETZ, I. DIETZ, P. ESTÓK, O. VON HELVERSEN, *et al.* 2007. Biogeography of the recently described *Myotis alcathoe* von Helversen and Heller, 2001. Acta Chiropterologica, 9: 361–378.

PALUMBI, S., A. MARTIN, S. ROMANO, W. O. MCMILLAN, L. STICE, and G. GRABOWSKI. 1991. The simple fool's guide to PCR version 2.0. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu, 44 pp.

PONS, J., T. G. BARRACLOUGH, J. GOMEZ-ZURITA, A. CARDOSO, D. P. DURAN, S. HAZELL, S. KAMOUN, W. D. SUMLIN, and A. P. VOGLER. 2006. Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic Biology, 55: 595–609.

R DEVELOPMENT CORE TEAM. 2008. R: a language and environment for statistical computing. R Foundation for

Statistical Computing, Vienna, Austria. http://www.R-project.org.

RUEDI, M., and F. MAYER. 2001. Molecular systematics of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. Molecular Phylogenetics and Evolution, 21: 436–448.

RYDELL, J. 1993. Eptesicus nilssonii. Mammalian Species, 430: 1–7.

RYDELL, J., and H. J. BAAGØE. 1994. Vespertilio murinus. Mammalian Species, 467: 1–6.

RYDELL, J., K.-B. STRANN, and J. R. SPEAKMAN. 1994. First record of breeding bats above the Arctic Circle: northern bats at 68–70°N in Norway. Journal of Zoology (London), 233: 335–339.

SCHMITT, T. 2007. Molecular biogeography of Europe: Pleistocene cycles and postglacial trends. Frontiers in Zoology, 4: 11.

SIMMONS, N. B. 2005. Order Chiroptera. Pp. 312–529, *in* Mammal species of the World: a taxonomic and geographic reference, 3rd edition (D. E. WILSON and D. M. REEDER, eds.). The Johns Hopkins University Press, Baltimore, 2142 pp.

SOKOLOV, V. E., and V. N. ORLOV. 1980. Identification guide to the mammals of Mongolia. Nauka Press, Moscow, 351 pp.

SORENSON, M. D., and E. A. FRANZOSA. 2007. TreeRot, version 3. Boston University, Boston, MA. http://people.bu.edu/msoren/TreeRot.html.

SORENSON, M. D., and T. W. QUINN. 1998. Numts: a challenge for avian systematics and population biology. Journal of the American Ornithologists' Union, 115: 214–221.

SPITZENBERGER, F., P. P. STRELKOV, H. WINKLER, and E. HARING. 2006. A preliminary revision of the genus *Plecotus* (Chiroptera, Vespertilionidae) based on genetic and morphological results. Zoologica Scripta, 35: 187–230.

STADELMANN, B., L. K. LIN, T. H. KUNZ, and M. RUEDI. 2007.

Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. Molecular Phylogenetics and Evolution, 43: 32–48.

STUBBE, M., and N. CHOTOLCHU. 1968. Zur Säugetierfauna der Mongolei. Mitteilungen aus dem Zoologischen Museum Berlin, 44: 5–121.

SWOFFORD, D. L. 2002. PAUP* phylogenetic analysis using parsimony. Sinauer Associates, Sunderland, MA. Available from http:// paup.csit.fsu.edu/.

TABERLET, P., L. FUMAGALLI, A. WUST-SAUCY, and J. COSSON. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology, 8: 1923–1934.

TAMURA, K., M. NEI, and S. KUMAR. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the USA, 101: 11030–11035.

TAMURA, K., J. DUDLEY, M. NEI, and S. KUMAR. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24: 1596–1599.

THOMPSON, J. D., D. G. HIGGINS, and T. J. GIBSON. 1994. CLUSTAL-W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22: 4673–4680.

TINNIN, D. S., J. L. DUNNUM, J. SALAZAR-BRAVO, N. BATSAI-KHAN, M. S. BURT, S. L. GARDNER, and T. L. YATES. 2002. Contributions to the mammalogy of Mongolia, with a checklist of species for the country. Special Publication of the Museum of Southwestern Biology, 6: 1–38.

VON HELVERSEN, O., K.-G. HELLER, F. MAYER, A. NEMETH, M. VOLLETH, and P. GOMBKÖTÖ. 2001. Cryptic mammalian species: a new species of whiskered bat (*Myotis alcathoe* n. sp.) in Europe. Naturwissenschaften, 88: 217–223.

APPENDIX

List of specimens. Specimens with GenBank accession numbers, specimen and country code, as well as locality code and full name of the locality with corresponding geographic coordinates (Geo-Ref.) are given. Voucher specimens are marked with crosses. Abbreviations (country code) are used to tag the following sample countries: AT — Austria, BG — Bulgaria, BY — White Russia, CH — Swiss, CN — China, DE — Germany, EG — Egypt, ES — Spain, GR — Greece, HR — Croatia, HU — Hungary, IE — Ireland, IL — Israel, IR — Iran, IT — Italy, JO — Jordan, JP — Japan, LA — Laos, LY — Libya, MA — Morocco, MN — Mongolia, PH — Philippines, RU — Russia, TR — Turkey, ? — unknown origin. All Mongolian taxa are in bold. Nine specimens of *M. aurascens* were excluded in the calculation of intra- and interclade divergences of Western and Eastern Palaearctic samples, because too much sequence information was missing for them (included specimens are marked with asterisks)

Specimen	Individual code	Country code	GenBank number	Mongolian locality	Locality code	Geo-Ref.	Voucher
Miniopterus schreibersii	Msch2218	HU	AF401463				
Tadarida teniotis	Tten1242	GR	DQ915087				
Eptesicus s. turcomanus	Eser5460	**MN**	FR848495	Bordzongijn-gobi	A1	042°29'00.00"N 105°15'00.00"E	
	Eser1305	**MN**	FR848494	Oasis Zulganai	1	043°35'02.50"N 100°04'05.70"E	x
	Etu1617	?	EU786995				
	Etu1620	?	EU786998				
	Etu1621	?	EU786999				

APPENDIX. Continued

Specimen	Individual code	Country code	GenBank number	Mongolian locality	Locality code	Geo-Ref.	Voucher
Eptesicus serotinus	Eser1291	DE	AF401472				
	Eser3285	DE	DQ915024				
	Eser2584	HU	DQ915023				
	EserI3	GR	AY033950				
	EserI1	ES	DQ120803				
	Eser1526	GR	AF401471				
Eptesicus nilssonii	Enil1393	DE	AF401474				
	EnilI1	DE	AY033987				
	Enil1596	RU	AF401473				
	Enil4737	**MN**	DQ915025	Orkhon river (near waterfall)	2	047°19'51.80"N 102°22'47.90"E	x
	Enil4908	**MN**	FR848493	Baruun Church river	3	048°14'10.50"N 108°20'36.50"E	
	EnilK02	JP	AB079822				
Eptesicus gobiensis	Egob4740	**MN**	FR848487	Har Buhyn Balgas (ruin)	4	047°53'13.50"N 103°53'36.80"E	x
	Egob4748	**MN**	FR848488	Unsted Hiid, (ruined monastery)	5	045°46'09.70"N 105°14'05.50"E	
	Egob4750	**MN**	FR848489	Orog lake	6	044°49'09.90"N 100°48'29.90"E	
	Egob4752	**MN**	FR848486	Orog lake	6		
	Egob4754	**MN**	FR848490	Orog lake	6		
	Egob6050	**MN**	FR848491	08-LR3	7	046°07'31.10"N 099°15'22.80"E	
Eptesicus bottae	Einn4833	IL	DQ915027				
	Einn4727	EG	DQ915026				
	Eboh1217	?	EU786945				
	Eboi1594	?	EU786939				
	Ebot1211	?	EU786938				
Eptesicus b. ognevi	Eog1624	?	EU787001				
Eptesicus anatolicus	EanaI1	TR	DQ120805				
	Eana1684	GR	AF401477				
	Eana1685	GR	AF401476				
	Ean1218	?	EU786936				
Eptesicus isabellinus	EisaI2	ES	DQ120804				
	Eisa4677	MA	DQ915028				
Vespertilio sinensis	VsupK2	JP	AB079823				
	Vsin5879	**MN**	FR848582	Chuch lake	8	049°31'44.50"N 114°39'06.70"E	x
Vespertilio murinus	Vmur602	DE	AF401468				
	Vmur1915	RU	AF401469				
	Vmur1305	DE	AF401470				
	Vmur3195	CH	DQ915021				
	Vmur4738	**MN**	FR848575	Orkhon river (near waterfall)	2		x
	Vmur4739	**MN**	FR848576	Orkhon river (near waterfall)	2		x
	Vmur5099	**MN**	FR848578	Orog lake	6		
	Vmur6041	**MN**	FR848579	Zencheriyn river (08-L02)	9	047°27'42.40"N 101°46'31.90"E	
	Vmur6042	**MN**	FR848580	Zencheriyn river (08-L02)	9		
	Vmur6055	**MN**	FR848581	08-LR3	7		
	Vmur2008	**MN**	FR848573	Chuch lake	8		
	Vmur4902	**MN**	FR848577	Selenge river (near Hutag-Ondor)	10	049°20'45.80"N 102°50'07.90"E	x
	Vmur4608	**MN**	FR848574	Ondörkhan	11	047°14'44.90"N 110°34'30.00"E	
Hypsugo savii	Hsav661	TR	AF401417				
	Hsav1202	IL	DQ915020				
	Hsav3310	TR	AF401418				
	Hsav5020	HR	DQ915019				

APPENDIX. Continued

Specimen	Individual code	Country code	GenBank number	Mongolian locality	Locality code	Geo-Ref.	Voucher
Hypsugo savii	Hsav2609	GR	AF401419				
Hypsugo sp.	Hsp1201	IL	DQ915016				
Hypsugo cf. *darwinii*	Hdar4675	MA	DQ915018				
	Hdar4198	MA	DQ915017				
Hypsugo alaschanicus	Hala4768	**MN**	FR848496	Orog lake	6		x
	Hala4769	**MN**	FR848497	Orog lake	6		x
	Hala4770	**MN**	FR848498	Orog lake	6		
	Hala4771	**MN**	FR848499	Orog lake	6		
Hypsugo cadornae	Hcad1183	LA	DQ120797				
Hypsugo ariel	Hari4726	EG	DQ915015				
	Hari4724	EG	DQ915014				
Plecotus begognae	Pbeg3218	ES	AF516273				
Plecotus auritus	Paur3222	HU	DQ915077				
	Paur2169	HR	AF401369				
	Paur3342	AT	DQ915076				
	Paur1592	RU	AF401372				
	Paur1383	DE	AF401373				
	Paur3334	IT	DQ915079				
	Paur3873	IE	DQ915075				
	Paur3221	CH	AF516277				
	Paur3220	HU	DQ915073				
	Paur3307	IT	DQ915074				
	Paur3304	IT	DQ915072				
Plecotus sardus	Psar5042	IT	DQ915061				
	Psar5043	IT	DQ915062				
Plecotus christii	Pchr5088	JO	DQ915081				
	Pchr5090	EG	DQ915080				
Plecotus austriacus	Paus4206	GR	DQ915066				
	Paus4212	GR	DQ915067				
	Paus3333	IT	DQ915065				
Plecotus austriacus	Paus3217	ES	AF516270				
	Paus1389	DE	AF401366				
Plecotus gaisleri	Ptga5055	LY	DQ915064				
	Ptga5056	LY	DQ915060				
Plecotus kolombatovici	Pkol2127	HR	AF401362				
	Pkol3557	GR	DQ915063				
	Pkol1874	GR	AF401364				
	Pkol1868	GR	AF401365				
	Pkol2126	HR	AF401363				
Plecotus m. alpinus	Palp3300	AT	AF516274				
	Palp3305	CH	AF516272				
	Palp3301	AT	AF516275				
	Palp3229	CH	AF516269				
Plecotus m. macrobullaris	Pmac3561	GR	DQ915068				
	Pmac3562	GR	DQ915069				
	Pmac3563	GR	DQ915070				
	Pmac3564	GR	DQ915071				
	Pmac5382	GR	FR848558				
Plecotus turkmenicus	PturKiwi	**MN**	FR848572	Oasis Zulganai	1		x
Plecotus ognevi	Pogn5045	**MN**	FR848563	Tes river	12	049°45'32.00"N 096°34'05.70"E	
	Pogn5420	**MN**	FR848569	Mongolia		?	
	Pogn5882	**MN**	FR848564	Numrog river	13	047°00'07.00"N 119°22'20.50"E	x
	Pogn6049	**MN**	FR848565	Tschulutyn river (08-L03)	14	048°09'55.80"N 100°17'47.90"E	
	Pogn6052	**MN**	FR848570	08-LR3	7		
	Pogn6053	**MN**	FR848566	08-LR3	7		

APPENDIX. Continued

Specimen	Individual code	Country code	GenBank number	Mongolian locality	Locality code	Geo-Ref.	Voucher
Plecotus ognevi	Pogn6061	**MN**	FR848568	Ulmental (08-LR5)	15	047°16'07.10"N 104°30'29.20"E	
	Pogn6060	**MN**	FR848567	08-LR3	7		
	Pogn0408	**MN**	FR848559	Baldsh river	16	049°03'41.70"N 111°32'01.40"E	
	Pogn0508	**MN**	FR848560	Baldsh river	16		
	Pogn2808	**MN**	FR848561	Sumber Sum	17	047°38'04.30"N 118°38'55.80"E	
	Pogn4808	**MN**	FR848562	Baruun Church river	3		
Plecotus strelkovi	Pstr5044	**MN**	FR848571	Bulgan river	18	046°08'40.10"N 091°29'55.30"E	
Plecotus cf. *auritus*	PcfaurK2	JP	AB079817				
Plecotus kozlovi	Pkoz5419	**MN**	FR848557	Böön Tzagaan lake (salt lake)	19	045°37'19.20"N 099°14'58.20"E	
Myotis aurascens	MspB4598*	**MN**	FR848517	Har Buhyn balgas	4		x
	MspB4599*	**MN**	FR848518	Har Buhyn balgas	4		x
	MspB4602*	**MN**	FR848519	Delger Mörön river (near Mörön)	20	049°37'31.30"N 099°42'00.40"E	x
	MspB4604	**MN**	FR848520	Orkhon river (near waterfall)	2		x
	MspB4741	**MN**	FR848521	Sum Hoh Burd	21	046°09'41.60"N 105°45'02.80"E	
	MspB4742*	**MN**	FR848522	Sum Hoh Burd	21		
	MspB4743*	**MN**	FR848523	Sum Hoh Burd	21		
	MspB4744	**MN**	FR848524	Sum Hoh Burd	21		
	MspB4745	**MN**	FR848525	Sum Hoh Burd	21		
	MspB4746*	**MN**	FR848526	Sum Hoh Burd	21		
	MspB4747*	**MN**	FR848527	Sum Hoh Burd	21		
	MspB4749*	**MN**	FR848528	Oasis Zulganai	1		
	MspB4755*	**MN**	FR848529	Orog lake	6		
	MspB4756	**MN**	FR848530	Orog lake	6		
	MspB4757	**MN**	FR848531	Böön Tzagaan lake	19		
	MspB4758	**MN**	FR848532	Böön Tzagaan lake	19		
	MspB4759	**MN**	FR848533	Bayanhongor (Tuy bridge)	22	046°10'42.30"N 100°43'44.80"E	
	MspB4760	**MN**	FR848534	Orkhon river (base near Hujirt)	23	047°01'37.20"N 102°39'51.30"E	
	MspB4761*	**MN**	FR848535	Orkhon river (near Hujirt)	23		
	MspB4763*	**MN**	FR848536	Orkhon river (near Hujirt)	23		
	MspB4764*	**MN**	FR848537	Tuul river, near Öndörshireet	24	047°15'37.40"N	
Myotis aurascens	MspB4765*	**MN**	FR848538	Tuul river, near Öndörshireet	24	105°28'03.30"E	x
	MspB4766*	**MN**	FR848539	Tuul river, near Öndörshireet	24		
	MspB6048*	**MN**	FR848540	Tschulutyn river (08-L03)	14		
	MspB6051*	**MN**	FR848541	08-LR3	7		
	MspB6054*	**MN**	FR848542	08-LR3	7		
	MspB6056*	**MN**	FR848543	08-LR3	7		
	MspB6057*	**MN**	FR848544	08-LR3	7		
	MspB6058*	**MN**	FR848545	08-LR3	7		
	MspB6059*	**MN**	FR848546	08-LR3	7		
	Mmys1508*	**MN**	FR848512	Chuch lake	8		x
	Mmys1608*	**MN**	FR848513	Chuch lake	8		x
	Mmys3808*	**MN**	FR848514	South of Tsagaa lake	25	047°14'37.00"N 118°33'16.80"E	x
	Mmys5877*	**MN**	FR848515	Chuch lake	8		x
	Mmys5878*	**MN**	FR848516	Chuch lake	8		x
	MaurM1	?	AY699860				
	Maur4495*	BG	DQ915037				
	Maur4490*	BG	DQ915036				
	Maur35*	?	AY699856				
	Maur131*	?	AY699858				

APPENDIX. Continued

Specimen	Individual code	Country code	GenBank number	Mongolian locality	Locality code	Geo-Ref.	Voucher
Myotis mystacinus	Mmys1863	GR	AY027846				
	Mmys1203	IL	DQ915034				
	Mmys01	?	AY699865				
	Mmys122a	DE	AY027848				
	Mmys2064	BG	DQ915033				
Myotis ikonnikovi	MikoHK3	JP	AB106579				
	Miko2179	RU	AY027850				
	Miko1AY6	?	AY699866				
	Miko4600	**MN**	FR848508	Orkhon river (crater near Orkhon)	26	048°32'33.60"N 103°17'03.10"E	x
	Miko4603	**MN**	FR848509	Orkhon river (crater)	26		
	Miko5869	**MN**	FR848510	Baldsh river	16		x
	Miko6046	**MN**	FR848511	Tschulutyn river (08-L03)	14		
Myotis daubentonii	Mdau1709	BY	AF401450				
	Mdau826	GR	AF401447				
	Mdau1722	BY	AF401449				
	Mdau3288	ES	DQ915046				
	MdauFR1	DE	AY033985				
Myotis petax	Mdau01	?	AY699872				
	Mdau02	RU	AB106568				
	MdauK3	JP	AB079824				
	Mpet6040	**MN**	FR848554	Zencheriyn river (08-L02)	9		
	Mpet6044	**MN**	FR848555	Zencheriyn river (08-L02)	9		
	Msp0108	**MN**	FR848556	Orchon tuul		?	
	Mpet0308	**MN**	FR848551	Onon river I	27	048°35'48.20"N 110°42'04.50"E	
	Mpet1008	**MN**	FR848552	Onon river II	28	048°50'24.70"N 110°38'32.80"E	x
	Mpet2908	**MN**	FR848553	Sumber Sum	17		
Myotis muricola	Mmur128	?	AY699871				
	Mmur118	?	AY699870				
Myotis browni	Mmbr3203	PH	AY033958				
Myotis brandtii	Mbra1590	RU	AY027858				
	Mbraspb4	?	AY699869				
	Mbra4621	GR	DQ915035				
	Mbra2044	BG	AY027856				
	Mbra2312	HU	AY027853				
Myotis gracilis	Mgra4605	**MN**	FR848500	Orkhon river (crater)	26		x
	Mgra4606	**MN**	FR848501	Ideriyn river	29	048°40'44.30"N 098°53'59.50"E	x
	Mgra4607	**MN**	FR848502	Orkhon river (waterfall)	2		x
	Mgra4608	**MN**	FR848503	Orkhon river (waterfall)	2		x
	Mgra6043	**MN**	FR848506	Zencheriyn river (08-L02)	9		
	Mgra6047	**MN**	FR848507	Tschulutyn river (08-L03)	14		
	MmysK3	JP	AB106583				
	MspK3	JP	AB079826				
	Mgra5870	**MN**	FR848504	Baldsh river	16		x
	Mgra5871	**MN**	FR848505	Baldsh river	16		
Myotis nattereri	Mnat5868	**MN**	FR848547	Baldsh river	16		x
	MnatK3	JP	AB106584				
	MnatI1	ES	DQ120801				
	Mnat5049	AT	DQ915049				
	Mnat2599	HU	AF401439				
	MnatFR1	GR	AY033984				
	Mnat4817	TR	FR848548				
	Mnat5129	GR	FR848549				
	Mnat5138	TR	FR848550				
	MspN1SMo	MA	EU360612				

APPENDIX. Continued

Specimen	Individual code	Country code	GenBank number	Mongolian locality	Locality code	Geo-Ref.	Voucher
Myotis nattereri	MspN2NMo	MA	EU360613				
Myotis escalerai	MnatI2	ES	DQ120802				
Myotis alcathoe	Malc2199	GR	AY027837				
	Malc2313	HU	AY027836				
	Malc2315	HU	AY027835				
	Malc65a	GR	AY027834				
	Malc82a	GR	AY027832				
Myotis chinensis	MchiK3	CN	AB106567				
Myotis schaubi	Msca5058	TR	DQ915050				
	MscaI1	IR	AY033955				
Nyctalus noctula	Nnoc81e	DE	DQ915009				
	Nnoc1017	DE	AF401430				
	Nnoc143e	DE	DQ915008				
	Nnoc490e	DE	AF401422				
	Nnoc0001	**MN**	FR851291	Menc river	30	045°59'00.00"N 091°58'00.00"E	x
Nyctalus lasiopterus	Nlas2304	HU	AF401436				
	Nlas2305	HU	AF401435				
	Nlas2307	HU	AF401434				
	Nlas2308	HU	AF401433				
	Nlas663	GR	AF401432				
Nyctalus leisleri	Nlei1398	TR	DQ915011				
	Nlei3763	IE	DQ915012				
	Nlei662	GR	DQ915010				
Nyctalus plancyi	NplaSICH	CN	EU005650				
	NplaTIAN	CN	EU005651				
	NvelSICH	CN	AB079820				
Nyctalus aviator	NaviNAGA	JP	AB079819				