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A NEW SPECIES OF *BARBASTELLA* (CHIROPTERA: VESPERTILIONIDAE) FROM NORTH CHINA

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A new species of *Barbastella* is described, originally discovered in 2001 in Beijing, northern China. The description of the new species is based on both morphological and molecular data. The morphology of the skull and ears of the new bat is more similar to that of the Egyptian barbastelle (*B. leucomelas*) and *B. barbastellus* distributed in Europe than to *B. leucomelas* found in southern China and Taiwan. Projections and notches occur along the posterior margin of each ear, and a small lobe (vaulted process) protrudes from the middle outer edge of each pinna. The skull and body size of the new species are larger than in *B. leucomelas*. Echolocation calls were of 2 types, a brief frequency-modulated call that was alternated with longer calls with a convex frequency–time course. The calls were very similar to those of *B. barbastellus* recorded in Europe, although they may be slightly lower in frequency. Molecular phylogenies were reconstructed from cytochrome-*b* (*Cytb*) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*ND1*) gene sequences. Cladograms of *ND1* indicated that barbastelles from the Beijing area form a monophyletic group, which is the sister to *B. leucomelas* from Egypt. The clade including the new species and Egyptian barbastelle clusters with *B. barbastellus*, but not with *B. leucomelas* from Sichuan, Taiwan, and Japan. The genetic distances (corrected Kimura 2-parameter) between *Barbastella* sp. nov. and most bats from other localities (including all *B. barbastellus*) were 14.31–17.69% at the *ND1* gene and 15.01–17.36% at the *Cytb* gene. However, *ND1* divergence is 12.79% between *Barbastella* sp. nov. and *B. leucomelas* from Egypt. All these results support the hypothesis that the barbastelle from Beijing is a new species. Additionally, because Egypt is the type locality of *B. leucomelas*, the paraphyletic nature of *B. leucomelas* suggests that barbastelles from Sichuan, Taiwan, and Japan—which are currently classified as *B. leucomelas darjelingensis*—should not be considered conspecific with *B. leucomelas*.

Key words: *Barbastella*, China, molecular phylogeny, morphology, new species

Barbastelle bats, genus *Barbastella* (Vespertilionidae, Chiroptera), currently comprise 2 species: the western barbastelle (*B. barbastellus* (Schreber, 1774)) and the eastern barbastelle (*B. leucomelas* (Cretzschmar, 1826)—Nowak 1999; Simmons 2005). *B. barbastellus* is found in the western Palearctic, from the United Kingdom and western Europe through to the Caucasus Mountains, Bulgaria, Turkey, Crimea (Ukraine), Morocco, Mediterranean islands, and Canary Islands (where it exists as an endemic subspecies *B. barbastellus guanchae*—Juste et al. 2003; Trujillo et al. 2002). *B. leucomelas* is widely distributed from the Sinai (Egypt), Eritrea, northern Iran, and the Caucasus to Afghanistan, the Pamirs, India, Nepal, western China, Japan, and possibly Indo-China (Corbet 1978; Nowak 1999; Simmons 2005). *B. leucomelas* is considered to comprise 2 subspecies—*B. l. leucomelas* (Sinai, Egypt, northern Iran, and possibly Eritrea) and *B. l. darjelingensis* (rest of range—Corbet 1978). *B. barbastellus* and *B. leucomelas* are reported as being sympatric in the Caucasus (Corbet 1978).

The eastern and western barbastelle species are often distinguished by morphological differences: *B. barbastellus* has a prominent projecting lobe of the posterior margin of the ear

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(Corbet 1978), but *B. leucomelas* lacks the lobe. However, Hackethal et al. (1988) suggested that because of morphological and geographical variation in *B. barbastellus*, the projecting lobe may not be accurately used to distinguish barbastelle species. *B. leucomelas* is often described as a larger species, having a greater forearm length (41–45 mm in *B. leucomelas* and 31–43 mm in *B. barbastellus*) and condylobasal length (14.2–15.0 mm in *B. leucomelas* and 12.4–14.1 mm in *B. barbastellus*) than *B. barbastellus*. *B. leucomelas* also has a wider baculum (0.40–0.57 mm) than has *B. barbastellus* (0.27–0.30 mm—Rydell and Bogdanowicz 1997). However the measurements of *B. l. leucomelas* from the Middle East are smaller than those of Asian *B. leucomelas darjelingensis* (forearm length 37.3–39.2 mm in Egypt—Dietz 2005), and the taxonomic identity of bats from the Middle East remains unclear (Rydell and Bogdanowicz 1997). Indeed, bats now considered as *B. leucomelas* were 1st described as *Vespertilio leucomelas* Cretzschmar, 1826, from Sinai and *Plecotus darjelingensis* Hodgson, in Horsfield, 1855, from northern India (synonyms = *Vespertilio leucomelas* Cretzschmar, 1830–1831; *Barbastella blandfordi* Bianchi, 1917; and *Barbastella darjelingensis* Dobson, 1875), and were only later combined into a single species by Tate (1942) and Ellerman and Morrison-Scott (1951).

Uchida and Ando (1972) analyzed the karyotypes of Taiwanese barbastelles and found that they were identical to those of the Japanese barbastelles. Lin et al. (1997) 1st used the name *Barbastella formosanus* for barbastelles from Taiwan, but without providing an adequate description of these bats as a new species. Lin et al. (2002) designated the *Barbastella* of Taiwan as a new record of *B. leucomelas*, which was classified as *B. l. darjelingensis* through morphological comparison. Subsequently, the name *Barbastella leucomelas* instead of *B. formosanus* was presented in the 2nd edition of *Bats of Taiwan* (Lin et al. 2004). Therefore, the Latin name *Barbastella formosanus* is nomen nudum (also see Simmons 2005). Yoshiyuki (1989) determined that the average ear length of the Japanese *B. leucomelas* (15.3 mm) was shorter than that of the Indian *B. leucomelas* (19.0 mm) and suggested that they may be distinct species because of rather invariant dimensions of the ear. Horáček et al. (2000) also suggested that the western subspecies of *B. leucomelas* (i.e., *B. l. leucomelas*) may be conspecific with *B. barbastellus*, and that Japanese populations may be distinct at the species level.

Mitochondrial DNA genes, such as cytochrome *b* (*Cytb*) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*ND1*) are inherited maternally and evolve more rapidly than nuclear genes. These genes are used frequently to study the evolutionary histories of closely related species and investigate the phylogenetic relationships among different animals at species and higher levels (Baker et al. 1994; Li et al. 2006; Ruedi and Mayer 2001). Employing molecular methods permits the detection of cryptic species that have become reproductively isolated but resemble each other morphologically. Morphological distinctions, nevertheless, are still the most convenient means for the identification of new species.

In this paper, we describe a new species of barbastelle through morphological characters and echolocation calls, and perform a molecular phylogenetic analysis of this genus based on *Cytb* and *ND1* gene sequences.

Materials and Methods

Bat sampling and morphological comparisons.—On 14 August 2001, a female barbastelle was captured with a mist net from the San-Qing Cave (39°45′N, 115°45′E) at Wang-Lao-Pu Village, Fangshan District, southwestern Beijing, and the echolocation calls of this bat were recorded. The bat was photographed and later released. On 4 November 2002, we captured 1 hibernating male barbastelle bat at the same cave, and the specimen was kept (specimen IOZ-BRG00065; Bat Research Group, Institute of Zoology, Chinese Academy of Sciences). Another male specimen (IOZ-BRG00054) of *Barbastella* was caught with a mist net in an abandoned tunnel around the Darwin Bat Research and Conservation Center at San-Liu-Shui Village (39°43′N, 115°45′E), Fangshan District, on 25 September 2003. Both specimens were preserved in 75% ethanol (2 with extracted skulls). On 21 August 2006, 4 individuals of *Barbastella* were found in the same tunnel. They were set free after measurements were taken and wing-membrane sampling was performed (collection nos. 20060821A0197–20060821A0200). One specimen (IOZ-BRG-FLW007) of *B. leucomelas* from Sichuan (= Szechwan) Province (preserved in 75% ethanol) and 2 specimens (THU12920(7184) and THU6977(12454)) of *B. leucomelas* from Tunghai University of Taiwan (preserved as dry skins with skulls) also were examined.

Captured bats were weighed to the nearest 0.1 g, and a set of 14 external measurements was taken for each specimen. The external measurements were taken to the nearest 0.1 mm with dial calipers. A set of 15 cranial and dental measurements was taken in the laboratory to the nearest 0.01 mm with dial calipers.

The following external measurements were taken: body mass, total length (head and body), tail length, forearm length, ear length, ear width, tragus length, tragus width, foot length (including claws, measured to the distal part of claw), tibia length, calcar length, and length of the metacarpals of the 3rd, 4th, and 5th digits.

The following cranial and dental measurements, followed by abbreviations in parentheses, were taken: greatest length of skull (GLS), condylobasal length (CBL), condylocanine length (CCL), braincase breadth (BB), braincase height (BH; height of braincase posterior to the auditory bullae), zygomatic width (ZW), least interorbital breadth (IOB), rostrum length (RL; rostral length from preorbital foramina to the alveolus of the inner incisor), rostrum width (RW; rostral width at the level of the preorbital foramina), auditory bullae length (ABL; length of the skull at the level of the auditory bullae), mandible length (ML), C-M3 length (C-M^3), c-m3 length (C-M_3), C-C width (C^1-C^1), and M3-M3 width (M^3-M^3).

We compared morphological characters of the specimens we examined with published values for both *B. barbastella* and *B. leucomelas* (Harrison and Bates 1991; Rydell and Bogdanowicz 1997; Trujillo et al. 2002).

Table 1.—The collection localities of the bats analyzed, with the corresponding GenBank accession numbers. The sequences obtained from GenBank are AF401365 and AF401376 (Mayer and von Helversen 2001); AB079816 (Kawai et al. 2002); AF513745, AF513749, AF513752, and AF513753 (Juste et al. 2003); AF513771 (Juste et al. 2004); DQ915030–DQ915032 (Mayer et al. 2007); and AY665169 and AY699874 (Tsytsulina et al. 2004). A dash indicates that the corresponding sequences were unsuccessfully amplified or not yet unavailable in GenBank. *ND1* = nicotinamide adenine dinucleotide dehydrogenase subunit 1; *Cytb* = cytochrome *b*.

Specimen	Species	Locality	GenBank accession no.		Voucher
			ND1	*Cytb*	
Bbei1.Beijing	*Barbastella* from Beijing	San-Liu-Shui Village, Fangshan District, Beijing, China	EF534767	EF534760	Biopsy
Bbei2.Beijing	*Barbastella* from Beijing	San-Liu-Shui Village, Fangshan District, Beijing, China	EF534768	EF534761	Biopsy
Bbei3.Beijing	*Barbastella* from Beijing	Wang-Lao-Pu Village, Fangshan District, Beijing, China	EF534769	EF534762	IOZ-BRG00065
Bbei4.Beijing	*Barbastella* from Beijing	San-Liu-Shui Village, Fangshan District, Beijing, China	EF534770	—	IOZ-BRG00054
Bleu.Sichuan	*B. leucomelas*	Sichuan, China	EF534774	EF534766	IOZ-BRG-FLW007
Bleu1.Taiwan	*B. leucomelas*	Taiwan, China	EF534772	EF534763	Biopsy
Bleu2.Taiwan	*B. leucomelas*	Taiwan, China	EF534771	EF534764	Biopsy
Bleu3.Taiwan	*B. leucomelas*.	Taiwan, China	EF534773	EF534765	Biopsy
Bleu.Japan	*B. leucomelas*	Japan	AB079816	—	
Bleu.Egypt	*B. leucomelas*	Egypt	DQ915030	—	
Bbar.Greece	*B. barbastellus*	Greece	DQ915031	—	
Bbar.Hungary	*B. barbastellus*	Hungary	DQ915032	—	
Bbar.Germany	*B. barbastellus*	Germany	AF401376	—	
Bbar.Turkey	*B. barbastellus*	Thrace, Turkey	—	AF513753	
Bbar.Spain	*B. barbastellus*	Canary Islands, Spain	—	AF513745	
Bbar.Switzerland	*B. barbastellus*	Valais, Switzerland	—	AF513749	
Bbar.Morocco	*B. barbastellus*	Azrou, Morocco	—	AF513752	
Plecotus1	*Plecotus auritus*		AY699874	AY665169	
Plecotus2	*Plecotus austriacus*		AF401365	AF513771	

Recording and analysis of echolocation calls.—We recorded the calls from 1 bat that we captured from the San-Qing Cave on 14 August 2001, released into open space. The calls were recorded using a Pettersson D-980 bat detector (Pettersson Elektronik, Uppsala, Sweden) at 10× expansion onto a Sony TC-3 DAT recorder (Sony, Tokyo, Japan). The calls were analyzed using BatSound version 3.31 (Pettersson Elektronik). We measured call durations and pulse intervals from waveforms, and frequency measurements from spectrograms except for the frequency of most energy, which was measured from the peak of the power spectrum. Spectral analysis was performed with a 1,024-point fast Fourier transform, using a Hanning window.

Molecular data collection.—We took 3-mm biopsy punches from wing membranes of 4 live individuals (collection nos. 20060821A0197–20060821A0200, but only 2 were used for analysis in view of their absolutely identical nucleotides) and 2 specimens (IOZ-BRG00065 and IOZ-BRG00054) from Beijing, 3 from Taiwan (not the 2 dry specimens mentioned in the previous context), and 1 voucher specimen (IOZ-BRG-FLW007) from Sichuan. The punches were preserved in 99% ethanol and stored at 4°C. The handling of all bats conformed to guidelines for animal care and use established by the American Society of Mammalogists (Gannon et al. 2007) and all voucher specimens are deposited at the Institute of Zoology, Beijing, at present. Some published mitochondrial sequences also were obtained from the GenBank database, including *B. leucomelas* from Japan and Egypt, and *B. barbastellus* from much of its range—Canary Islands (Spain), Turkey, Switzerland, Morocco, Germany, Hungary, and Greece (Juste et al. 2003; Kawai et al. 2002; Mayer et al. 2007; Mayer and von Helversen 2001). *Plecotus auritus* and *Plecotus austriacus* were used as outgroups because they represent the larger clade of plecotine bats to which *Barbastella* is the sister (Bogdanowicz et al. 1998). See sampling localities and GenBank accession numbers in Table 1 for details.

We used DNeasy Blood & Tissue Kits (QIAgen, Inc., Basel, Switzerland) to extract mitochondrial genomic DNA. *ND1* and *Cytb* genes were amplified separately using 2 pairs of primers as follows: L16S (5′-CCT CGA TGT TGG ATC AGG-3′) and HtMet (5′-GTA TGG GCC CGA TAG CTT-3′—Cao et al. 1998) for the *ND1* gene, and Bat_*Cytb*_1 (5′-TAG AAT ATC AGC TTT GGG TG-3′—Li et al. 2006) and Bat_*Cytb*_2 (5′-AAA TCA CCG TTG TAC TTC AAC-3′—G. Li, pers. comm.) for the *Cytb* gene.

Polymerase chain reaction profiles for *ND1* and *Cytb* sequences were the same: 1st denaturation at 95°C for 5 min, followed by 35 cycles at 95°C (30 s for denaturing), 55°C (30 s for annealing), and 72°C (80 s for extending), with the final extension at 72°C for 10 min. Each 50-μl polymerase chain reaction cocktail included 25 μl of 2 × ExTaq polymerase (TAKARA, Inc., Shiga, Japan), 1 μl of each primer (10 μM), and 2 μl of DNA templates (50 μg/μl). The polymerase chain reaction products were purified using the Agarose Gel DNA Purification Kit version 2.0 (TAKARA, Inc.) and sequenced in both directions with polymerase chain reaction primers using Big-Dye Terminator version 3.1 and an ABI3730 automated DNA sequencer (Applied Biosystems, Inc., Foster City, California) by the Sanger method. Base calling and quality trimming of the sequences were carried out using Phred (Ewing

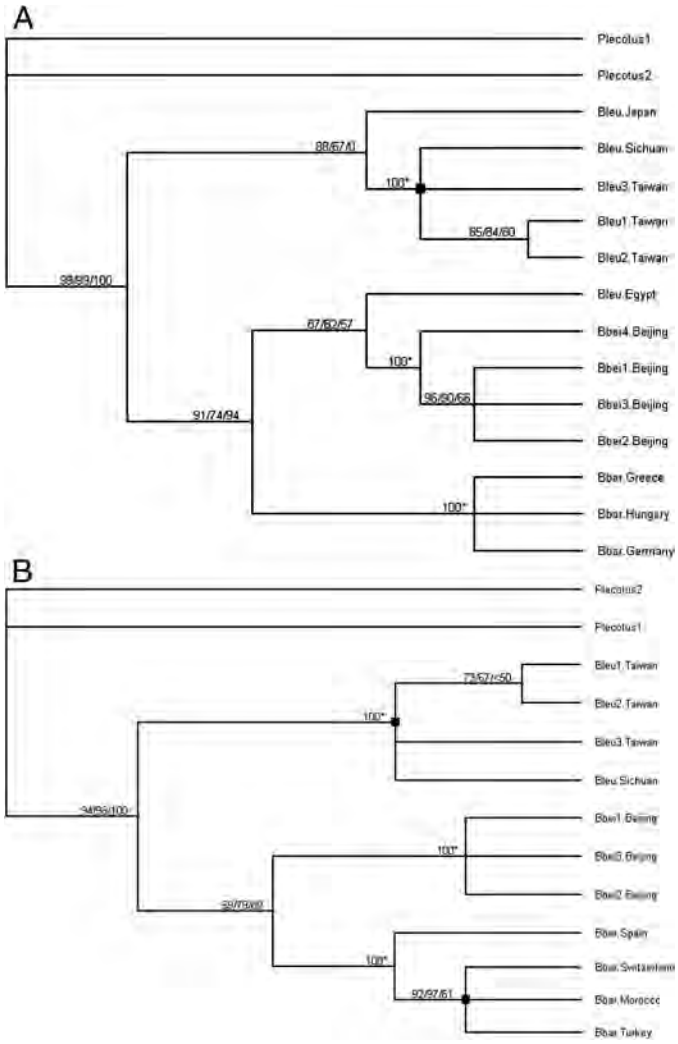

Fig. 1.—A) Cladogram without branch lengths by integrating neighbor-joining, maximum-parsimony, and maximum-likelihood trees based on nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*ND1*) gene sequences. B) Cladogram without branch lengths by integrating neighbor-joining, maximum-parsimony, and maximum-likelihood trees based on cytochrome *b* (*Cytb*) gene sequences. Three numbers at the nodes separated by "/" from left to right are bootstrap values following neighbor-joining (2,000 replicates), maximum-parsimony (2,000 replicates), and maximum-likelihood (100 replicates) approaches, respectively. An asterisk (*) indicates 3 approaches obtained the same bootstrap values. Black squares indicate that the interior topologies of the 3 clades are disparate by 3 methods despite high bootstrap values. The number "0" in the *ND1* cladogram tree indicates that the Japanese clade in maximum-likelihood tree locates at other position but with a poor bootstrap support (see text for details). Species abbreviations are defined in Table 1.

et al. 1998). The 2 overlapping fragments of the *ND1* or *Cytb* gene for each individual were compiled using AssemblyLIGN 1.0.9 software (Oxford Molecular Group PLC 1998).

Phylogenetic analyses.—After acquiring the sequences of *ND1* and *Cytb* genes, we aligned them respectively using ClustalX1.81 (Thompson et al. 1997), and edited them manually after a final alignment adjusted by eye. Sequence variation and divergence were calculated by the program MEGA3.1 (Kumar et al. 2004)—in order to be comparable with the database in Baker and Bradley (2006), we chose the Kimura 2-parameter model (Kimura 1980) to calculate sequence divergence for both genes.

The program PAUP 4.0*b10 (Swofford 2002) was used to construct a maximum-likelihood tree, with the best-fit model identified by MODELTEST 3.6 (Posada and Crandall 1998) and the following executive parameters: heuristic search = 10 replicates, random addition of taxa, and tree-bisection-reconnection branch-swapping. Robustness of the topology was assessed with 100 bootstrap replicates. In contrast, 2,000 bootstrap replicates were carried out to assess robustness of the neighbor-joining (with best-fit model distances) and the maximum-parsimony trees obtained with PAUP 4.0*b10 (Swofford 2002). We constructed separate trees for *Cytb* and *ND1* sequences because some bats could not have their DNA sequenced for both genes because of tissue degradation, and because *Cytb* and *ND1* sequences on GenBank did not always come from the same individual.

Results

Molecular phylogenetic analysis.—We did not obtain a *Cytb* sequence from 1 Beijing specimen (Bbei4: the holotype, IOZ-BRG00054) because no genomic DNA could be extracted, probably because of tissue degradation. Fortunately, a complete *ND1* sequence was obtained soon after the bat was captured. We obtained 7 sequences (4 *ND1* sequences and 3 *Cytb* sequences) from 4 individuals (Bbei1–Bbei4; Table 1). The mitochondrial *Cytb* and *ND1* gene sequences of barbastelle bats are available in GenBank (accession nos. EF534760–EF534774; Table 1).

After sequence alignment and manual editing, we finally obtained *ND1* sequences of 795 base pairs (bp) and *Cytb* sequences of 680 bp, where our data overlapped with published sequences. The program MODELTEST3.6 (Posada and Crandall 1998) selected HKY85+G (base frequencies: A = 0.3349, C = 0.2903, G = 0.1280, T = 0.2468; transition : transversion [Ts/Tv] = 7.5088; gamma shape parameter = 0.2654) and K81uf+G (base frequencies: A = 0.3009, C = 0.2991, G = 0.1270, T = 0.2731; gamma shape parameter = 0.2085) as the best-fit models by hierarchical Likelihood Ratio Tests for the *ND1* gene and *Cytb* gene, respectively.

All phylogenetic trees produced display similar topologies (Figs. 1A and 1B). The genus *Barbastella* is divided into 2 major groups. One major group includes barbastelles from Sichuan and Taiwan, with intraspecific genetic distances at the *ND1* gene of 0.38–1.27% and at the *Cytb* gene of 0.29–0.59%, indicating their monotypic status. This clade presumably represents bats currently described as *B. leucomelas darjelingensis*. *Barbastella* from Beijing and *B. barbastellus* form the 2nd major clade. Individuals from the same species cluster together, forming separate clades, with genetic distances at the *ND1* gene of 14.31–15.31% and at the *Cytb* gene of 16.90–17.36% (Table 2), separating *Barbastella* from Beijing

from *B. barbastella*. The intraspecific *ND1* sequence divergence of *B. barbastellus* from Germany, Hungary, and Greece varies between 0.38% and 1.14%, whereas the intraspecific *Cytb* sequence divergence varies between 0.74% and 2.40% from Morocco, Switzerland, and Turkey. The divergence of the subspecies *B. barbastellus guanchae* from Canary Islands (Spain) with the mainland barbastelles is larger (3.80–5.69%) than divergence values seen among the mainland barbastelles at the *Cytb* gene (Juste et al. 2003). As for intraspecific variation in *Barbastella* from Beijing, the pairwise genetic distances of the 3 individuals (Bbei1–Bbei3) are 0% at partial *ND1* and *Cytb* sequences studied, so we consider them as 1 haplotype. Obviously, Bbei4 is another haplotype, with an *ND1* sequence divergence of 1.40% between the 2 haplotypes.

Although we obtained only 1 *ND1* sequence for Egyptian and Japanese *B. leucomelas*, their genetic divergence to the other barbastelles and positions in the phylogenetic trees are still remarkable. The Egyptian haplotype has a smaller divergence with *B. barbastellus* (14.44–15.07%) than with *B. l. darjelingensis* (17.88–18.72%), and divergence of 12.79% exists between the Egyptian sample and *Barbastella* from Beijing. These relationships contradict the current classification and are also reflected in the phylogenetic trees—the Egyptian haplotype is nested in the group that includes *B. barbastellus* and *Barbastella* from Beijing with firm support from all 3 phylogenetic methods. Moreover, it appears as sister to *Barbastella* from Beijing with high bootstrap support in the neighbor-joining and maximum-parsimony trees, although the maximum-likelihood tree has a bootstrap value of only 57.

The Japanese haplotype also has a large *ND1* divergence from other barbastelles: 16.90% with *Barbastella* from Beijing, 18.72% with *B. leucomelas* from Egypt, 18.17–19.01% with *B. barbastellus*, and 14.35–14.74% with *B. leucomelas* from Sichuan and Taiwan. It is sister to the Sichuan–Taiwan *B. leucomelas* with bootstrap support of 88 on the neighbor-joining tree and 67 on the maximum-parsimony tree (Fig. 1A). On the maximum-likelihood tree, however, the Japanese haplotype forms 1 branch of a basal trichotomy within *Barbastella*, the other branches corresponding to *B. l. darjelingensis* from Sichuan–Taiwan and the barbastelles from Europe, Egypt, and Beijing. The phylogenetic analysis thus supports the hypothesis that the barbastelle from Beijing is an undescribed species, which is named and compared with other congeners below.

TABLE 2.—Corrected genetic divergences of group means (Kimura 2-parameter model) based on partial mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 1 sequences (823 bp, below the diagonal) and partial cytochrome-*b* sequences (680 bp, above the diagonal), calculated by MEGA version 3.1 (Kumar et al. 2004).

	Barbastella from Beijing	*B. leucomelas*, Sichuan	*B. leucomelas*, Taiwan	*B. leucomelas*, Japan	*B. leucomelas*, Egypt	*B. barbastellus*, Greece	*B. barbastellus*, Hungary	*B. barbastellus*, Germany	*B. barbastellus*, Switzerland	*B. barbastellus*, Morocco	*B. barbastellus*, Turkey	*B. barbastellus*, Canary Islands, Spain
Barbastella from Beijing		0.1551	0.1501	—	—	—	—	—	0.1736	0.1695	0.1728	0.169
B. leucomelas, Sichuan	0.1729		0.0059	—	—	—	—	—	0.1592	0.1533	0.1586	0.1627
B. leucomelas, Taiwan	0.1769	0.0059		—	—	—	—	—	0.1581	0.1522	0.1575	0.1616
B. leucomelas, Japan	0.169	0.1435	0.1474		—	—	—	—	—	—	—	—
B. leucomelas, Egypt	0.1279	0.1788	0.1794	0.1872		—	—	—	—	—	—	—
B. barbastellus, Greece	0.1448	0.1597	0.1648	0.1834	0.1461		—	—	—	—	—	—
B. barbastellus, Hungary	0.1431	0.158	0.1631	0.1817	0.1444	0.0038		—	—	—	—	—
B. barbastellus, Germany	0.1531	0.1678	0.173	0.1901	0.1507	0.0114	0.0101		—	—	—	—
B. barbastellus, Switzerland	—	—	—	—	—	—	—	—		0.0074	0.0224	0.0396
B. barbastellus, Morocco	—	—	—	—	—	—	—	—	—		0.024	0.038
B. barbastellus, Turkey	—	—	—	—	—	—	—	—	—	—		0.0569
B. barbastellus, Canary Islands, Spain	—	—	—	—	—	—	—	—	—	—	—	

Barbastella beijingensis, new species

Beijing Barbastelle

Holotype.—Institute of Zoology, Chinese Academy of Sciences, IOZ-BRG00054, adult male in alcohol with skull extracted, collected by Drs. Jie Ma and Li-Biao Zhang on 25 September 2003. GenBank accession number for *ND1* is EF534770.

Type locality.—Darwin Bat Research and Conservation Center at San-Liu-Shui Village, Fangshan District, Southwestern Beijing, China (39°43′N, 115°45′E), 407.8 m above sea level. We captured the specimen in an abandoned tunnel (approximately 1 km long, 3.5–4 m high) in this village.

Paratype.—Institute of Zoology, Chinese Academy of Sciences, IOZ-BRG00065, adult male in alcohol with skull

FIG. 2.—*Barbastella beijingensis* (IOZ-BRG00054), male. Photo by Shu-Yi Zhang.

extracted, collected by Dr. Hui-Hua Zhao and Jin-Shuo Zhang on 4 November 2002.

Referred material.—One specimen (IOZ-BRG-FLW007, male) of *B. leucomelas* from Sichuan (= Szechwan) Province, was deposited at the Bat Research Group, Institute of Zoology, Chinese Academy of Sciences. Two specimens (THU12920(7184), male and THU6977(12454), female) of *B. leucomelas* from Taiwan were preserved at the Department of Life Science, Tunghai University.

Distribution and habitat.—So far known only from the type locality and a nearby cave. We found the new barbastelle species 4 times in Fangshan District, about 100 km southwest of Beijing. San-Liu-Shui Village is in a mountainous region with riparian woodland, and the roost site was an abandoned tunnel, more than 1 km in length. Other caves also were important diurnal roost sites. The surrounding vegetation is classified as warm temperate zone forest. Most of the native forest consists of Chinese pine (*Pinus tabulaeformis*), arborvitae (*Sabina chinensis*), and oak (*Quercus mongolica* and *Q. liaotungensis*). In this region at least 4 bat species are sympatric with the Beijing barbastelle: *Rhinolophus ferrumequinum*, *Myotis ricketti*, *Myotis blythii*, and an unidentified *Murina* in light of our field surveys. In 2001 and 2002, we also captured 2 barbastelles at Wang-Lao-Pu Village, Fangshan District (39°45′N, 115°45′E).

Etymology.—We nominate this species using the name of the type locality, Beijing (formerly Peking), capital city of the People's Republic of China.

Diagnosis.—This is a relatively small bat among species of the order Chiroptera in China, but relatively large in the genus of *Barbastella* (forearm length 41.1 mm), with a large skull (greatest length of skull [GLS] 15.7 mm) and a long condylobasal length (CBL 14.5 mm). Interorbital breadth is relatively wider than in *B. leucomelas* (IOB 3.9 mm). Therefore, its skull measurements fall within the range reported for *B. leucomelas* rather than those of *B. barbastellus*. The posterior margin of the ear has projections and notches. There is a small lobe (vaulted process) protruding from the outer edge of each ear, which is less conspicuous than that in many *B. barbastellus*. This species can be distinguished from the other species of *Barbastella* based on the *ND1* and *Cytb* sequences.

Description.—The dorsal fur is dark black with brown-gray tips, the ventral fur is lighter than the dorsal pelage. The flat and wide muzzle has pronounced glandular swellings. A few long whiskers and dense hairs line the margin of the upper and lower lips. The relatively large nasal aperture is broad. Ear length is 15.5 mm; the ears are brownish black and have transverse ridges. The outline of the ears is nearly square; as in many *B. barbastellus* there is a slender and delicate projecting lobe or vaulted process on the outer edge of the pinna. The ears are forward facing and join across the forehead. The tragus is triangular and large, and is more than half the height of the pinna (see Fig. 2). In contrast to *B. leucomelas*, the skull is relatively large with a condylocanine length of 14.3 mm. The upper surface of the rostrum is smooth, slightly concave, and the rostrum is relatively wider than in *B. leucomelas*. The postdental extension is poorly developed, with a blunt median spine. The coronoid process of each half mandible is short. The condyle of each mandible is narrow. The angular process projects for a relatively large distance (see Fig. 3).

Upper toothrow length (C-M^3) is 4.7 mm. The 1st upper incisor (I2) is bicuspidate and the 2nd upper incisor (I3) is very small. The canine is slender with a well-developed cingulum but lacks secondary cusps. The 1st upper premolar (P2) is very small between the canine and the 2nd upper premolar (P4) and is displaced inward. P4 is large and attains two-thirds the height of the canine. P4 is wider than the 1st upper molar (M1). There are no hypocones on M1 and M2 and the mesostyle is weaker than the parastyle and metastyle. M3 consists of 3 commissures and a metacone. In the lower dentition, the 3 incisors are overlapping. The well-developed anterior cingular cusp of the weak lower canine is higher than the 3rd incisor (i3). The lower canine (c) is higher than p4. p2 is very small, about one-third the height of p4. p4 is similar in height to m1 (see Fig. 3).

Echolocation calls.—The bat alternated 2 signal types. One call type followed a convex frequency–time course ($n = 10$ all data: start frequency 42.7 ± 1.6 kHz, end frequency 25.1 ± 1.4 kHz, frequency of most energy 39.4 ± 0.7 kHz, duration 8.2 ± 1.7 ms, pulse interval 99.0 ± 28.0 ms). This call type was interspersed with brief, frequency-modulated signals with a lower frequency of most energy ($n = 6$ all data: start frequency 39.2 ± 1.6 kHz, end frequency 26.8 ± 0.5 kHz, frequency of most energy 32.1 ± 1.9 kHz, duration 5.1 ± 0.8 ms, pulse interval 72.2 ± 6.2 ms). Representative calls are illustrated in Fig. 4.

Comparisons.—*Barbastella beijingensis* can be distinguished from *B. leucomelas* of South China and Taiwan by its larger body size (Table 3) and by its projecting ear lobes (vaulted processes). The ear lobes of this new species are delicate and slender, and do not look as buttonlike as in many western barbastelles. Ear lobes were not present in specimens of *B. leucomelas* from Sichuan and Taiwan that were examined. Interestingly, although *B. leucomelas* is larger than *B. barbastella* (Rydell and Bogdanowicz 1997), *B. beijingensis* is larger than *B. leucomelas*. The size of the skull of *B.*

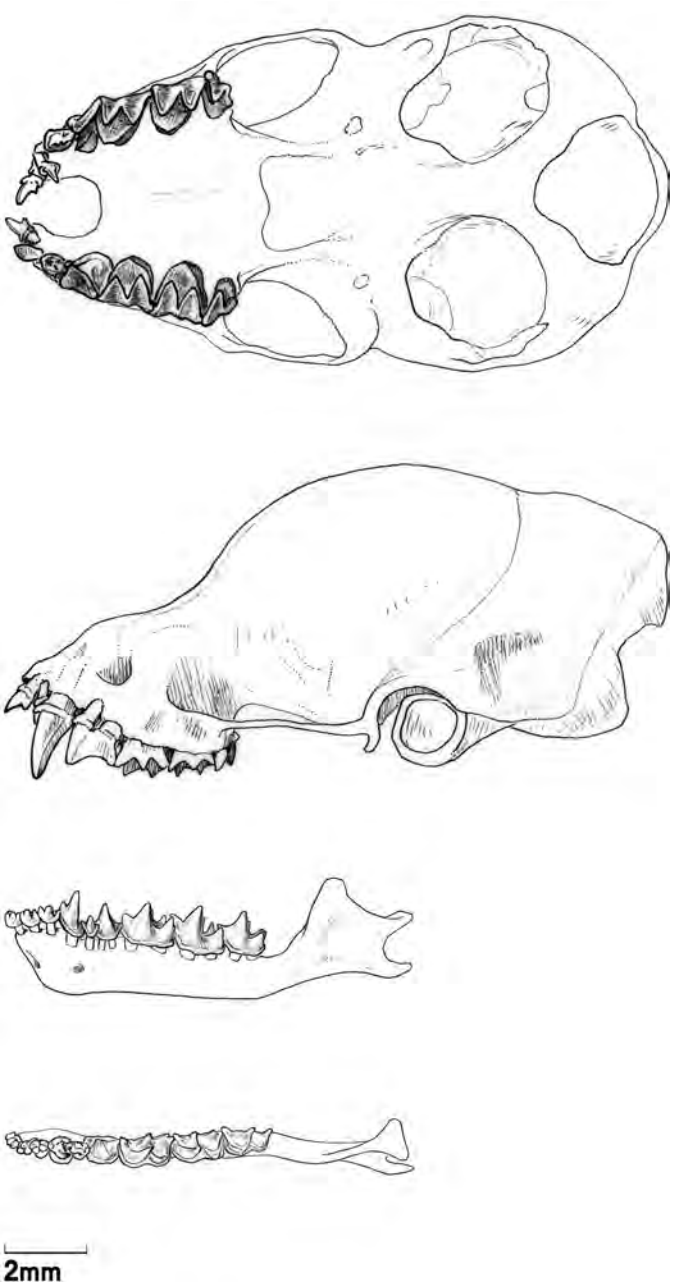

Fig. 3.—Ventral and lateral views of the skull of *Barbastella beijingensis* (IOZ-BRG00054), male, with lateral and dorsal views of the lower jaw.

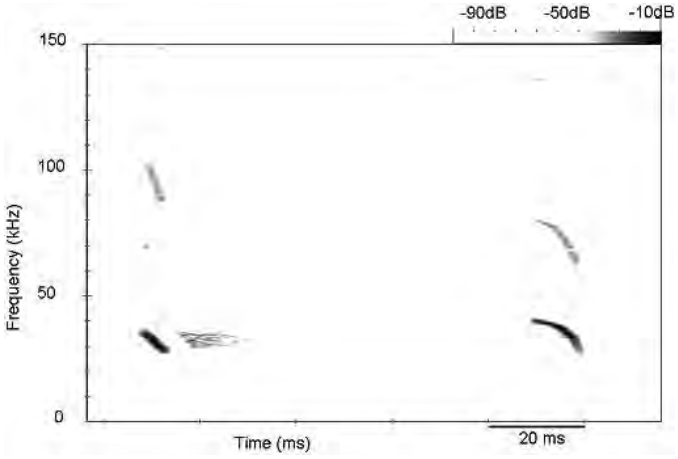

Fig. 4.—Spectrogram of consecutive echolocation calls produced by *Barbastella beijingensis*. The spectrogram was made using a 1,024-point fast Fourier transform and a Hanning window. A brief frequency-modulated signal is followed by a longer call with a convex frequency–time course.

beijingensis is relatively larger than that of *B. leucomelas* from Taiwan (Table 4). Measurements of p4 from the holotype and paratype are higher than that of the Taiwan specimen—the reason might be that the Taiwan specimen may be an old individual with worn premolars. In contrast to *B. leucomelas* from Taiwan and Iran (see Bates and Harrison 1997), the canine and P4 of this new species are stronger and better developed. We propose that size of P4 might prove to be a diagnostic dental character in a larger sample of bats. For bat identification, the fur color is not a consistent character to identify different species because of variation related to age and geography. However, the pelage color of Beijing's barbastelle is quite similar to that described for the Egyptian barbastelle (both of them are browner than that of *B. barbastellus*, see Harrison and Bates 1991). Bats captured in Beijing in 2006 appeared much darker than those captured previously, although all bats captured were adults. Although we did not have access to voucher specimens or sequences from India, Nowak (1999) and Simmons (2005) stated that *B. l. darjelingensis* is distributed in southwestern China, suggesting that barbastelle bats from Sichuan are the same subspecies as *B. leucomelas* from India, and this conclusion is strongly supported through comparisons of their morphological data (Tables 3 and 4; Lin et al. 2002). Meanwhile, although the barbastelle from Japan was suggested as a distinct species from *B. l. darjelingensis* (Horáček et al. 2000; Yoshiyuki 1989), we still considered it as *B. l. darjelingensis* in our analysis. We note that cranial characters of the new species are quite similar to the eastern and western barbastelles, but body size and projecting ear lobes are keys to distinguishing and identifying *B. beijingensis*.

Discussion

Integrated analysis is important for recognizing cryptic species by using phenotypic, phylogenetic, and other biological data (e.g., echolocation calls and karyology) when there is significant genetic divergence among taxa but relatively little morphological divergence or vice versa. For example, integrated techniques have been used on morphologically similar pipistrelles in Britain (Barratt et al. 1997; Jones and Van Parijs 1993), long-eared bats (*Plecotus*) in Europe (Kiefer et al. 2002; Spitzenberger et al. 2006), and house bats (*Scotophilus*) in South Africa (Jacobs et al. 2006). Barbastelle bat populations at different sites have morphological similarities, but large genetic differences of mitochondrial DNA. In this paper we use molecular data to support assignment of species status to *B. beijingensis*. The echolocation calls of *B. beijingensis* are very

TABLE 3.—External measurements, to nearest 0.1 mm, of the holotype and paratype as well as other specimens of *Barbastella beijingensis* sp. nov. (length in mm, mass in g). Additionally, data from *B. leucomelas* from Sichuan and Taiwan are presented, together with information collated by Rydell and Bogdanowicz (1997) for *B. barbastellus*, *B. barbastellus guanchae* from the Canary Islands (Trujillo et al. 2002), and *B. leucomelas* from Israel and Sinai (Harrison and Bates 1991).

	B. beijingensis sp. nov.						*B. leucomelas* from Sichuan and Taiwan			*B. l. leucomelas*	*B. barbastellus*	*B. b. barbastellus*	*B. b. guanchae*
Parameter	Holotype IOZ-BRG00054	Paratype IOZ-BRG00065	20060821A0197	20060821A0198	20060821A0199	20060821A0200	IOZ-BRG-FLW007	THU12920 (7184)	THU6977 (12454)	Cited by Harrison and Bates 1991	Cited by Rydell & Bogdanowicz 1997	Cited by Trujillo et al. 2002	Cited by Trujillo et al. 2002
Sex	Male	Male	Female	Female	Female	Male	Male	Male	Female		Males and females		
Body mass			11.9	13.9	13.0	10.5					5.6–13.7		
Total length	52.6	49.7	53.4	53.7	54.1	54.3	41.1	51.5	47.9		45–60		
Tail length	47.0	32.7					40.0	27.0	41.7	33.2–51.0	36–52		
Forearm length	41.1	41.9	45.1	44.9	46.4	43.3	37.8	40.0	42.1	37.3–39.5	31–43	37.2–41.8	37.2–42.0
Ear length	15.5	14.6	15.0	15.9	13.1	15.4	10.5	12.8	11.9	18.0			
Ear width	12.8	9.8						11.9	8.4				
Tragus length	6.9	7.4						6.9	5.3				
Tragus width	3.5	3.1						3.4	2.6				
Foot length	7.9	6.2	8.4	8.1	9.6	9.2	5.3	6.5	6.5	6.5–6.8			
Tibia length	19.1	23.1					18.4	18.1	19.2				
Calcar length	5.5	5.6						5.6	5.6				
3rd digit metacarpal	39.9	40.9						35.1	39.6				
4th digit metacarpal	38.4	39.2						40.0	36.0				
5th digit metacarpal	36.7	37.8						38.1	37.9				

TABLE 4.—Cranial and dental measurements, to nearest 0.1 mm, of the holotype and paratype of *Barbastella beijingensis* sp. nov. and *B. leucomelas* and *B. barbastellus* (length in mm).

Parameter	*B. beijingensis* sp. nov.		*B. leucomelas* from Taiwan		*B. l. leucomelas*	*B. barbastellus*	*B. b. barbastellus*	*B. b. guanchae*
	Holotype	Paratype	THU12920 (7184)	THU6977 (12454)	Cited by Harrison and Bates 1991	Cited by Rydell and Bogdanowicz 1997	Cited by Trujillo et al. 2002	Cited by Trujillo et al. 2002
Greatest length of skull (GLS)	15.7	15.2	14.6	14.9	14.6		13.4–14.4	13.9–14.2
Condylobasal length (CBL)	14.5	14.2	13.7	14.0	13.4	12.4–14.1	12.8–13.5	12.8–13.6
Condylocanine length (CCL)	14.3	13.9	13.2	13.4				
Braincase breadth (BB)	8.6	8.5	8.0	8.0	7.0–7.2			
Braincase height (BH)	6.0	7.9	6.9	6.8				
Zygomatic width (ZW)	8.8	8.0	7.5	7.4	7.4–7.5	7.0–8.2	7.2–7.7	7.2–7.7
Interorbital breadth (IOB)	3.9	4.1	3.8	3.7		3.4–3.9		
Rostrum length (RL)	3.5	3.3	4.2	4.0				
Rostrum width (RW)	3.2	3.1	3.7	3.4				
Auditory bullae length (ABL)	2.9	3.0	3.2	3.0				
Mandible length (ML)	9.9	9.5	9.4	9.6		8.4–9.5		
C-M^3	4.7	4.9	5.0	4.9	4.1–4.5	4.3–5.0	4.4–4.8	4.3–4.7
C-M_3	5.3	5.1	5.5	5.4	4.6–4.9			
C^1-C^1	4.3	4.0	3.7	3.5				
M^3-M^3	5.7	6.5	5.7	5.4		5.1–5.7	5.4–5.6	

similar to those of *B. barbastellus*, which also alternated a brief frequency-modulated signal with a longer call with a convex frequency–time course (Denzinger et al. 2001). The frequency values we recorded are similar to those described for *B. barbastellus* in Europe (Denzinger et al. 2001; Parsons and Jones 2000; Russo and Jones 2002). The echolocation calls of *B. leucomelas* from Egypt start at 40 kHz and end at 28 kHz for the short (3- to 4-ms) frequency-modulated type, and span 44–27 kHz for the longer call type (8–12 ms—Dietz 2005), values very similar to those we recorded for *B. beijingensis*. Although barbastelle bats can be readily identified to genus by their distinctive calls, we doubt whether echolocation call parameters will offer diagnostic features for individual species.

Baker and Bradley (2006) argued that species are genetically isolated and should show consistent levels of sequence divergence in *Cytb* comparisons. For bats, they documented levels of variation at *Cytb* between 0.2% and 3.8% within populations, 0% and 5.9% within species, 4.8% and 18.7% among species within genera (nonsister species), and 2.3% and 14.7% between sister taxa. In our study, in addition to the *Cytb* gene, we also chose the *ND1* gene as a robust supplementary gene, in consideration of less published molecular data for *Cytb* gene than *ND1* gene, and of their approximately identical rate of evolution in vespertilionid bats (Ruedi and Mayer 2001). The congruence between slight morphological differences and substantial sequence divergence of at least 5% indicates that distinct species may be present.

In our research, the phylogenetic trees constructed by 2 protein coding genes reveal that *B. beijingensis* clusters with *B. barbastellus* (also with *B. l. leucomelas* in view of *ND1* gene) rather than with *B. l. darjelingensis* (Figs. 1A and 1B). The sequence divergences between *B. beijingensis* and European *B. barbastellus* (not including *B. b. guanchae*) are almost 3–4 times those between *B. b. guanchae* and *B. b. barbastellus* in southern Europe and Morocco (Juste et al. 2003).

The original proposal that *B. leucomelas* from Sinai (Egypt) and *B. darjelingensis* from northern India represent separate species (Horáček et al. 2000) may be correct after all, and therefore the appropriate names for the taxa would be *B. leucomelas*, type locality in Sinai, Egypt, and *B. darjelingensis*, type locality in Darjeeling, India. The latter, we also speculate, may contain cryptic species as indicated by the Japanese *Barbastella* in our study, having an *ND1* genetic distance of 14.35–14.74% with the bats from Sichuan and Taiwan, which appears compatible with the findings of Yoshiyuki (1989) and Horáček et al. (2000). However, we withhold firm conclusions because of inadequate data and no voucher specimens of Indian and Japanese barbastelles.

In the late summer of 2006, we caught 4 individuals of *B. beijingensis* from the same colony in a fissure in the tunnel at San-Liu-Shui Village. We estimated there were 15 or more bats in the fissure because of audible sounds of bats and, upon our approach, 2 bats flew out from the fissure. We had previously captured 1 in 2001, 1 in 2002, and another in 2003 in the same tunnel and in a cave in Fangshan District. DeBlasé (1980) suggested *B. leucomelas* is generally a nonsocial species roosting and hunting singly or, in the former United Soviet Socialist Republic, roosting in groups of no more than 3 individuals. We hypothesize that the ecology of *B. beijingensis* might be different from that of *B. leucomelas* or *B. barbastellus*, and therefore further study is required. In Europe *B. barbastella* typically roosts in trees during the summer (Russo et al. 2004, 2005).

Allen (1938) suggested that *B. leucomelas* is not a common bat species throughout much of its range. Bates and Harrison (1997) noted that there have been no recent population assessments, but outlined the potential threat that deforestation could pose. *B. leucomelas* was assessed as Lower Risk/Least Concern in 1996 and 2003 listings of the *Red List* of IUCN and Lower Risk/Least Concern in the IUCN/SSC *Action Plan*

(Hutson et al. 2001). In the *China Species Red List,* Wang and Xie (2004) listed *B. leucomelas* as Vulnerable. We conducted many field surveys in Fangshan, Beijing, from 1997 to 2006 and found *B. beijingensis* only 4 times, suggesting that it might be rare.

In conclusion, barbastelles show morphological similarities but considerable genetic divergence over their range. Barbastelles around Beijing are more similar genetically to European *B. barbastellus* than they are to Asian *B. leucomelas*, but are sufficiently different genetically to warrant specific status. Further sampling of populations between Beijing and western Europe will be needed to determine whether the Beijing bats are isolated, or part of a cline of increasing genetic divergence extending eastward, and whether any natural divisions occur between *B. leucomelas* and *B. barbastellus* over the species' ranges.

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