# Molecular studies on the classification of *Miniopterus schreibersii* (Chiroptera: Vespertilionidae) inferred from mitochondrial cytochrome *b* sequences

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A b s t r a c t . Mitochondrial cytochrome *b* sequences have been identified in *Miniopterus schreibersii* (Kuhl, 1817) from the Oriental-Australasian areas. All phylogenetic analyses indicate that the Oriental-Australasian *M. schreibersii* diverged from the Spanish *M. schreibersii*, with the mean percentage sequence differences ranging from 15.81% to 18.92% between them. A large and significant percentage sequence difference (10.91%) also separated the Chinese/Japanese specimens from the Australian specimens. Our molecular results corroborate a previous report based on morphological characters by M a e d a (1982), which suggested that *Miniopterus schreibersii* in Europe, Asia and Australia should be regarded as three distinct species, named *Miniopterus schreibersii*, *M. fuliginosus* and *M. oceanensis*. However, the specimen from Hainan should be grouped together with the other Chinese specimens in one species. The results also confirmed Appleton's recent molecular study on Oriental-Australasian *Miniopterus schreibersii*.

**Key words**: phylogeny, species status, mitochondrial DNA

## Introduction

Schreibers' long fingered bat, *Miniopterus schreibersii*, is a vespertilionid species with a very wide distribution covering southern Europe, Asia, Northern Africa, the Solomon Islands, Philippines and Northern and Eastern Australia (C o r b e t & H i l l 1980, N o w a k 1991, T a n 1992). Due to the extensive overlap of morphological variations within the cosmopolitan *M. schreibersii* complex, the detailed classification of *M. schreibersii* is highly debated. While some authors treat the entire complex as a single species throughout the Old World, with several subspecies (T a t e 1941, W i l s o n & R e e d e r 1993), others divide it into several species (M a e d a 1982). Many authors have followed Tate's classification (E l l e r m a n & M o r r i s o n - S c o t t 1951, C o r b e t 1978, C o r b e t & H i l l 1980), in spite of serious reservations by others (M a e d a 1982). Based on the exact measurements for external and skull characters, M a e d a (1982) regarded *M. schreibersii* in Europe, Asia (excluding Hainan Island) and Australia as three distinct species which he named *M. schreibersii*, *M. fuliginosus* and *M. oceanensis*. The species from Hainan Island was given

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a separate species status as *M. macrodens* (Maeda 1982). However, Koopman (1993) and Corbet & Hill (1992) disagreed with Maeda's classification. Molecular studies, which focused on the taxonomy of *M. schreibersii* (Cardinal & Chirstidis 2000, Miller-Butterworth et al. 2002, Barragan et al. 2002), did not address the detailed classification scheme. Most recently, Appleton et al. (2004) reported on the molecular systematic and biogeography of *M. schreibersii* using mitochondrial ND2 gene analysis. They studied *M. schreibersii* from the Palearctic-Ethiopian regions and the Oriental-Australasian regions and suggested that *M. schreibersii* was a paraphyletic assemblage comprising several species. Two major lineages were identified, with one restricted to the Palearctic-Ethiopian and the other to the Oriental-Australasian regions. However, their data used for the Oriental-Australasian classification was based upon only a few specimens. In this paper, we re-examine the classification of the Oriental-Australasian *M. schreibersii* group based on a much larger data pool using mitochondrial cytochrome-*b* gene sequences. In addition, we included a Hainan specimen to test Maeda's hypothesis.

Mitochondrial DNA has many merits, such as its simple framework, its maternal inheritance, the limited number of combinations, and the fact that it evolves rapidly and thus provides ample information on evolutionary changes (Brown 1981, Brown 1983). It is therefore considered to be an appropriate molecular marker and is widely used to investigate the population genetics and molecular phylogenetic relationships of bats (Cardinal & Chirstidis 2000, Baker et al. 1994, Hoofer & Van den Bussche 2001, Kawai et al. 2002). In contrast, since the evolving rate of the mitochondrial cytochrome-*b* gene is moderate, a short fragment of the cytochrome-*b* gene reveals phylogenetic information ranging from the intraspecies level to interspecies and even interfamily levels (Meyer et al. 1990).

## Material and Methods

### Material

Samples for genetic analysis were collected from Guangxi (China), Hainan (China) and Nara (Japan). The identification of specimens was limited to the level of species. Muscle tissues from two individuals of each area were obtained in April 2001 and preserved in 75% ethanol at -20 °C.

The Miniopterinae are recognized as the sister group to all other Vespertilionids (Jones et al. 2002, Kawai et al. 2002). Consequently, we included another species of Vespertilionidae, *Murina leucogaster*, as the outgroup. In order to determine the variation between species of *Miniopterus*, we also analyzed the Cyt-*b* sequence of *Miniopterus australis*. The sequences of our specimens have been deposited in GenBank under accession numbers (AY208138-208140) (Table 1).

### Methods

#### DNA extraction

Genomic DNA was extracted from muscle tissue by SDS/proteinase K treatment followed by phenol/chloroform extraction and ethanol precipitation (Maniatis et al. 1982). The products were inspected on 1.2% agarose gel.

**Table 1.** Localities sampled for genetic study and accession number.

| specimens | collection location | Accession number |
|---|---|---|
| *Miniopterus schreibersii* | Hainan (China) | AY208138 |
| *Miniopterus schreibersii* | Guangxi (China) | AY208139 |
| *Miniopterus schreibersii* | Nara (Japan) | AY208140 |
| *Miniopterus schreibersii* | Kumamoto (Japan) | AB085735* |
| *Miniopterus schreibersii* | Spain | AF376830* |
| *Miniopterus schreibersii* | Australia | AF217442* |
| *Miniopterus schreibersii* | Australia | AF217443* |
| *Miniopterus schreibersii* | Australia | AF217444* |
| *Miniopterus schreibersii* | Australia | AF217441* |
| *Miniopterus australis* | Australia | AF217440* |
| *Murina leucogaster* | Japan | AB085733* |

*indicated: the sequences of Cyt-*b* obtained from GenBank.

## PCR reactions and sequencing

A primer pair of L14724 and H15915R (Irwin et al. 1991) was used to amplify a complete Cyt-*b* gene. The PCR amplification was performed in a 50μl reaction volume containing 10×buffer, 1.5mM $Mg^{2+}$, 0.2μM of each primer, 1.25U of Takara TaqDNA polymerase and $ddH_2O$ for 35 cycles, with denaturation for 1 min at 94 °C, annealing for 1 min at 50 °C, and extension for 30 second at 72 °C, and finally by a 10-min elongation at 72 °C. The PCR products were inspected on 1.2% agarose gel and visualized with an ethidium bromide stain. The size of the PCR product was identical to the expected size. Subsequently, the products were sequenced by Bioasia Technology (Shanghai). The sequencing primers were L14724 + H15915R (Irwin et al. 1991) and L360 (5'-ATTAGCTGTGATAGCAACGGCATTC-3'). L360 was designed in the study.

## DNA analysis

Two sequences from China and one from Japan were determined in this study, the other sequences are from the GenBank (details see Table 1). The results from our sequencing analysis were tied together by using the Bioedit version 5.0.9 software (Hall 2001) and aligned by blast in GenBank in order to test whether the sequencing results were correct. Subsequently, DNA sequences were aligned with Clustal W (Thompson et al. 1994), and genetic distances were calculated according to Kimura's two-parameter method using MEGA program, Version 2 (Kumar et al. 2001). The phylogenetic analysis was conducted with the maximum-parsimony method and the neighbor-joining method using PAUP4.0a (Swofford 1998). Robustness of branching patterns was assessed using 1000 bootstrap replicates for the maximum parsimony and neighbor-joining trees. Meanwhile in order to compare with the sequences of Australian *M. schreibersii*, we used the same analysis methods to compare a part of our data with 354 base-pair (bp) sequences from the Australian *M. schreibersii*. Other sequences used in this study were obtained from GenBank whose accession numbers are shown in Table 1. In the analysis of 354bp, *M. australis* (Vespertilionidae, Miniopterinae) and *Murina leucogaster* (Vespertilionidae, Murininae) were selected as outgroups.

## Results

### Cyt-*b* sequences variation

The size of the complete mitochondrial Cyt-*b* was 1140bp and the sequences from 2 individuals within the same population were identical.

The comparison included all *M. schreibersii* in five sites examined and the outgroup *Murina leucogaster*. A total of 346 variable sites were observed in the 1140bp length of the entire Cyt-*b*. However, only 48 amino acid residue variations were observed in the entire 1140bp length of Cyt-*b*. The mean base composition was: A (28.1%), C (30.0%), T (27.4%), G (14.5%).

Genetic distances between *M. schreibersii* and the outgroup are listed in Table 2. The genetic distances among *M. schreibersii* from the five sites ranged between 0.89% and 16.40%. The genetic distance between two Japanese populations was very low (0.8%), and was slightly higher (1.43%) between any two Chinese populations. While the mean genetic distance between Chinese *M. schreibersii* and Japanese *M. schreibersii* was 6.46%. In contrast, the Spanish *M. schreibersii* differed greatly from the others analyzed here (15.04% – 16.40%).

The 354 bp fragment of the Cyt-*b* sequence differences among *M. schreibersii* and the outgroup are listed in Table 2. A total of 122 variable sites were observed in the 354 bp Cyt-*b* fragment. Only 20 amino acid residue variations were found in the 354 bp length, with an averaged base composition of: A (25.6%), C (30.9%), T (28.1%), G (15.4%).

The genetic distances among *M. schreibersii* from all sites generally varied over a wide range from 0.57% to 18.92% (Table 2). Specifically, the mean distance between Chinese *M. schreibersii* and Japanese *M. schreibersii* was 5.06%, and that between Japanese *M. schreibersii* and Australian *M. schreibersii* was 8.13%, while the mean distances between Chinese and Australian *M. schreibersii* differed by 10.91%.

As expected, the distances between populations within each geographic area were very low: 0.86% between the two Chinese populations, 0.57% between the two Japanese populations, and ranging between 1.73% to 6.03% between four Australian populations. Interestingly, however, the distances between the Spanish *M. schreibersii* and the others were very high (15.81% – 18.92%), even exceeding the difference between *M. schreibersii* and *M. australis* (12.70% – 16.50%).

### Phylogenetic tree

A phylogenetic analysis of the 1140bp data sets resulted in highly concordant branching topologies using neighbor-joining and maximum parsimony methods. The data set consisted of 1140 characters, of which 118 characters were parsimony informative. The unweighted parsimony analysis of this Cyt-*b* data set produced a single most parsimonious tree of 163 steps, with a consistency index of 0.8282 and retention index of 0.7926 (Fig. 1). Both neighbor-joining tree (Fig. 1) and maximum parsimony tree indicated that the two populations of *M. schreibersii* from China and Japan, respectively, formed sister groups: The two populations from China (Hainan and Guangxi) clustered together in a clade as did the populations from Japan (Nara and Kumamoto). Bootstrap support for each of these clades was generally high (100%). In contrast, the populations of *M. schreibersii* from Spain failed to cluster with the Chinese – Japanese clade. The outgroup (*Murina leucogaster*) was located at the base of the trees.

**Table 2.** DNA sequence divergences of Cyt-*b* (354bp & 1140bp) among *Miniopterus schreibersii* and two outgroups *Miniopterus australis* and *Murina leucogaster*).

| Taxon names | *M.schreibersii* (Hainan) | *M.schreibersii* ( Guangxi ) | *M.schreibersii* (Nara) | *M.schreibersii* (Kumamoto) | *M.schreibersii* (Spain) | *M.schreibersii* (Australia) 1 | *M.schreibersii* (Australia) 2 | *M.schreibersii* (Australia) 3 | *M.schreibersii* (Australia) 4 | *M.australis* |
|---|---|---|---|---|---|---|---|---|---|---|
| *M.schreibersii* (Guangxi) | 0.0086/ 0.0143 | | | | | | | | | |
| *M.schreibersii* (Nara) | 0.0568/ 0.0646 | 0.0474/ 0.0665 | | | | | | | | |
| *M.schreibersii* (Kumamoto) | 0.0506/ 0.0626 | 0.0475/ 0.0646 | 0.0057/ 0.0089 | | | | | | | |
| *M.schreibersii* (Spain) | 0.1581/ 0.1504 | 0.1619/ 0.1512 | 0.1883/ 0.1640 | 0.1808/ 0.1605 | | | | | | |
| *M.schreibersii* 1 (Australia) | 0.1073 | 0.1038 | 0.0795 | 0.0731 | 0.1813 | | | | | |
| *M.schreibersii* 2 (Australia) | 0.1216 | 0.1180 | 0.0963 | 0.0898 | 0.1813 | 0.0233 | | | | |
| *M.schreibersii* 3 (Australia) | 0.1073 | 0.1038 | 0.0861 | 0.0797 | 0.1657 | 0.0477 | 0.0539 | | | |
| *M.schreibersii* 4 (Australia) | 0.1073 | 0.1038 | 0.0762 | 0.0698 | 0.1892 | 0.0173 | 0.0292 | 0.0603 | | |
| *M.australis* | 0.1310 | 0.1310 | 0.1303 | 0.1270 | 0.1463 | 0.1650 | 0.1572 | 0.1421 | 0.1572 | |
| *Murina leucogaster* | 0.3229/ 0.2920 | 0.3278/ 0.2975 | 0.3264/ 0.2970 | 0.3174/ 0.2916 | 0.2837/ 0.2716 | 0.3229 | 0.3132 | 0.3037 | 0.3328 | 0.2717 |

Note: The values below the line indicated the DNA sequence divergences of Cyt-b 1140 bp. The others indicated the DNA sequence divergences of Cyt-*b* 354 bp.

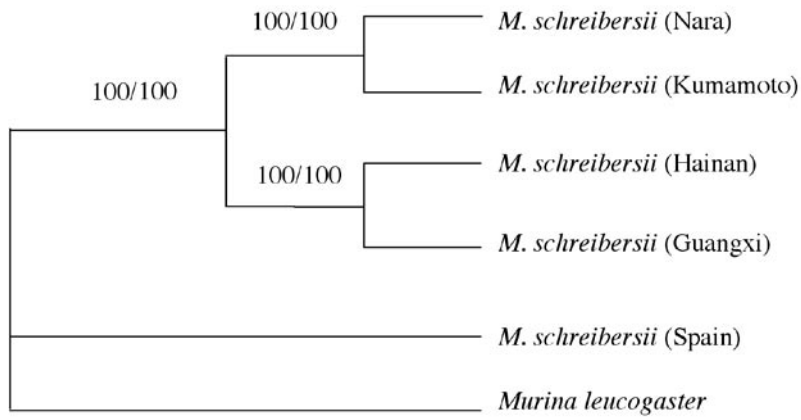


**Fig. 1.** Neighbor-joining tree using bootstrap method with heuristic search based on 1140bp of Cyt-*b*. Maximum parsimony topology was identical. The bootstrap support, based on 1000 replicates, is indicated above each node with the 50% majority-rule tree values below each node (The values above the line indicated the bootstrap values of neighbor-joining tree; The values below the line indicated the bootstrap values of maximum parsimony tree).

Similarly, our phylogenetic analysis of the 354bp data sets also resulted in highly concordant branching topologies using neighbor-joining and maximum parsimony methods. This Cyt-*b* data set consisted of 354 characters, of which 67 characters were parsimony informative. The unweighted parsimony analysis of the Cyt-*b* data set produced a single most parsimonious tree of 137 steps using heuristic search, with a consistency index of 0.6194 and retention index of 0.6710 (Fig. 2). With the exception of the Spanish *M. schreibersii*, the others clustered together and formed a monophyletic group which

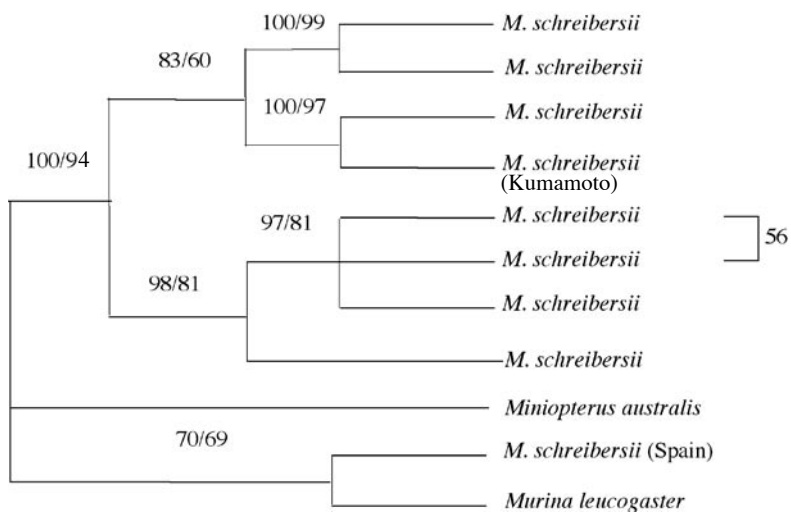


**Fig. 2.** Neighbor-joining tree using bootstrap method with heuristic search based on 354bp of Cyt-*b*. Maximum parsimony topology was identical. The bootstrap support, based on 1000 replicates, is indicated above each node with the 50% majority-rule tree values below each node (The values above the line indicated the bootstrap values of neighbor-joining tree; The values below the line indicated the bootstrap values of maximum parsimony tree).

was supported by 94% bootstrap in the MP tree (Fig. 2) and by 100% bootstrap in the NJ tree (Fig. 2). The analysis revealed that, all populations from China, Japan, and Australia, respectively, clustered together. Bootstrap support ranged from 81% to 99% in the MP tree and from 98% to 100% in the NJ tree. The populations from China, together with those from Japan comprised a clade supported by 60% bootstrap in the MP tree and by 83% in the NJ tree. The Australian clade and the Chinese – Japanese clade assembled together and formed a monophyletic relationship. The bootstrap values were 94% in the MP tree and 100% in the NJ tree. Within the Australian clade, three populations (1, 4, 2) clustered together as one group, which then with the remaining population3 constructed the Australian clade. Finally, one of the most striking outcomes of our analysis was that the Spanish *M. schreibersii* separated from the others and even clustered together with the more distant outgroup, *Murina leucogaster*, which together with the other outgroup (*M. australis*) was located at the base of the trees.

## Discussion

Synonymous mutations presumably occupied large portions on variable sites of the Cyt-*b* analyzed in our study: based on a set of 1140 bp and 354 bp, we found only 48 and 20 amino acid substitutions, respectively. The number of codon nucleotide substitutions differed from that of amino acid substitutions, which was probably linked to the function of the Cyt-*b* protein: Cyt-*b* is one of the main components of the mitochondrial electron transfer chain (Wang et al. 1998). It is located on the inner-membrane of mitochondria and, in association with other proteins, builds succinic dehydrogenase, which catalyzes the electron transfer to coenzyme Q. Because of this central role in the respiratory chain common to all mammals, the gene codon for Cyt-*b* of survivable species underwent only few mutations and therefore the Cyt-*b* amino acid sequence is highly conserved among mammals.

In general, we found a large variation in the genetic distances among the *M. schreibersii* in distinct areas included in our study. The smallest difference of less than 1% occurred between the two Japanese populations (Nara and Kumamoto). This might suggest that despite a geographic distance of more than 600 km, which is far more than their known migration distances of 100–350 km (Schober & Grimmberger 1997), gene flow has occurred between these two populations, consequently reducing their genetic diversity.

In contrast, the genetic distances between the Spanish *M. schreibersii* and other *M. schreibersii* from China, Japan and Australia were much greater (15.04% – 16.40% based on 1140 bp, and 15.81% – 18.92% based on 354 bp). These distances were also higher than species-level divergences for Cyt-*b* among bats of the family Rhinolophidae (9,91%) or Hipposideridae (11,66%) (Wang et al. 2003), the genera *Chiroderma* (4.1–15.3 %) (Brown 1983), *Phyllostomus* (7.0–13.4%) (Van den Bussche & Baker 1993), *Pipistrellus* (11%) (Barratt et al. 1997) and *Myotis* (11.8%) (Cooper et al. 2001). This suggests that the Asian and Australian *M. schreibersii* already diverged from the Spanish *M. schreibersii*. Based on previous morphologic classification, *M. schreibersii* is distributed all over Southern Europe, and the specimen from Spain should remain known as *M. schreibersii*. In addition, the results of our phylogenetic analysis also clearly showed that the Spanish *M. schreibersii* did not cluster with the *M. schreibersii* from other locations and, instead, constructed a monophyly. In the NJ tree and based on 354bp, the Spanish *M. schreibersii* and one of the outgroups, *Murina leucogaster*, even formed a clade supported by a 70% bootstrap value. Our results therefore suggest that the Oriental-Australasian

*M. schreibersii* should be considered to represent separate species, distinct from the Spanish *M. schreibersii*.

Moreover the genetic distances between the Asian *M. schreibersii* (in China and Japan) and the Australian *M. schreibersii* were 6.98% – 12.16%, identical to the results of Appleton et al. (2004) that divergences separating the Australia-New Guinea-Solomon Islands assemblage from the Philippines-Japan-China assemblage ranged from 8.7% to 12%. These genetic distances exceed 10%, which was the typical intraspecies difference among *M. schreibersii* (Cardinal & Chirstidis 2000). This result indicates that the Asian and Australian *M. schreibersii* are probably distinct species. The relationships of four populations from Australia are identical with the results obtained by Cardinal & Chirstidis (2000). In view of the genetic distance (1.73% – 6.03%) among the Australian populations, we cautiously regard them as reflecting intraspecies level differences.

From the phylogenetic relationship based on analyzing 1140 bp and 345 bp, the specimens from China and Japan formed sister taxa, which is consistent with the previous study (Appleton et al. 2004). The topology of the Australian clade was identical to the previous results (Cardinal & Christidis 2000).

Due to the phylogenetic relationship and the genetic distances between this group and the true Spanish *schreibersii*, the Oriental/Australasian specimens are clearly not *M. schreibersii*. This result corroborates the previous report by Maeda (1982) that *M. schreibersii* in Europe, Asia (excluding Hainan Island) and Australia should be regarded as three distinct species, which he named *M. schreibersii*, *M. fuliginosus* and *M. oceanensis*. In addition, our analysis suggests that the Hainan specimen should belong to the same species as the Guangxi specimen, which is most likely to be *M. fuliginosus*.

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## LITERATURE

APPLETON B.R., MCKENZIE J.A. & CHIRSTIDIS L. 2004: Molecular Systematics and Biogeography of the Bentwing Bat Complex *Miniopterus schreibersii* (Kuhl, 1817) (Chiroptera: Vespertilionidae). *Mol. Phylogen. Evol. 31: 431–439.*

BAKER R.J., TADDEI V.A., HUDGEONS J.L. & VAN DEN BUSSCHE R.A. 1994: Systematic relationships within *Chiroderma* (Chiroptera: Phyllostomidae) based on cytochrome *b* sequence variation. *J. Mammal. 75(2): 321–327.*

BARRAGAN M.J.L., MARTINEZ S., MARCHAL J.A., BULLEJOS M., DIAZ DE LA GUARDIA R. & SANCHEZ A. 2002: Repeated DNA sequences in the microbat species *Miniopterus schreibersii* (Chiroptera: Vespertilionidae). *Hereditas 137: 65–71.*

BARRATT E.M., DEAVILLE R., BURLAND T.M., BRUFORD M.W., JONES G., RACEY, P.A. & WAYNE R.K. 1997: DNA answers the call of pipistrelle bat species. *Nature 387: 138–139.*

BROWN W. M. 1981: Mechanism of evolution in animal mitochondrial DNA. *Ann. N. Y. Acad. Sci. 61:119–134.*

BROWN W. M. 1983. Evolution of animal mitochondrial DNA. In: Nei M. & Koehn R.K. (eds), Evolution of genes and proteins. *Sinauer Associates, Sunderland, MA: 62–85.*

CARDINAL B.R. & CHRISTIDIS L. 2000: Mitochondrial DNA and morphology reveal three geographically distinct lineages of the large bentwing bat (*Miniopterus schreibersii*) in Australia. *Austr. J. Zool. 48: 1–19.*

COOPER S.J.B., DAY P.R., REARDON T.B. & SCHULZ M. 2001: Assessment on species boundaries in Australian Myotis (Chiroptera: Vespertilionidae) using mitochondrial DNA. *J. Mammal. 82 (2): 328–338.*

CORBET G.B. 1978: The Mammals of the Palearctic Region: A Taxonomic Review. *B.M. (Nat. His.) London: p. 62.*

CORBET G.B. & HILL J.E. 1980: A world list of mammalian species. *Brit. Mus. (Nat. Hist.), London, p. 83, 314.*

CORBET G.B. & HILL J.E. 1992: The mammals of the Indomalayan region: a systematic review. *Oxford University Press, Oxford.*

ELLERMAN J.R. & MORRISON-SCOTT T.C.S. 1951: Checklist of Palearctic and India Mammals 1758–1946. *B.M. (Nat. His.) London: 182–184.*

HALL T. 2001: Bioedit: Biological sequence alignment editor. Ver. 5.0.9. *Department of Microbiology, North Carolina State University.*

HOOFER S.R. & VAN DEN BUSSCHE R.A. 2001: Phylogenetic relationships of Plecotine bats and allies based on mitochondrial sequences. *J. Mammal. 82:131–137.*

IRWIN D.M., KOCHER T. D. & WILSON A.C. 1991: Evolution of the cytochrome-*b* gene in mammals. *J. Mol. Evol. 32:128–144.*

JONES K.E., PURVIS A., MACLARNON A., BININDA-EMONDS O.R.P. & SIMMONS N.B. 2002: A phylogenetic supertree of the bats (Mammalia: Chiroptera). *Biol. Rev. 77: 223–259.*

KAWAI K., NIKAIDO M., HARADA M., MATSUMURA S., LIN L.K., WU Y., HASEGAWA M. & OKADA N. 2002: Intra-and interfamily relationships of Vespertilionidae inferred by various molecular makers including SINE insertion data. *J. Mol. Evol. 55:284–301.*

KOOPMAN K.F. 1993: Order Chiroptera. In: Wilson D.E. & Reeder D.M. (eds), Mammal species of the world, a taxonomic and geographic reference. 2nd ed. *Smithsonian Institution Press, Washington DC: p. 231.*

KUMAR S., TAMURA K., JAKOBSEN I.B. & NEI M. 2001: MEGA2: Molecular Evolutionary Genetics Analysis software. *Bioinformatics 17 (12): 1244–1245.*

MAEDA K. 1982: Studies on the classification of *Miniopterus* in Eurasia, Australia and Melanesia. Honyurui kagaku (Mammalian Science) Suppl. 1. *Mammal Research Association, Japan, p. 1–176.*

MANIATIS T., FRITSCH E.F. & SAMBROOK J. 1982: Molecular cloning (A laboratory manual). *Cold Spring Harbor Laboratory Press, New York: p. 545.*

MEYER A., KOCHER T.D., BASASIBWAKI P. & WILSON A.C. 1990: Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequence. *Nature 347: 550–553.*

MILLER-BUTTERWORTH C.M., JACOBS D.S. & HARLEY E.H. 2002: Isolation and characterization of highly polymorphic microsatellite loci in Schreibers' long-fingered bat, *Miniopterus schreibersii* (Chiroptera: Vespertilionidae). *Molecular Ecology Notes 2: 139–141.*

NOWAK R.M. 1991: Walker's mammals of the World Fifth Edition Volume I. *The Johns Hopkins University Press, Baltimore and London: 366–369.*

SCHOBER W. & GRIMMBERGER E. 1997: The bats of Europe and North America. *T.F.H. Publications, Neptune City, NJ, USA: 1–160.*

SWOFFORD D.L.P. 1998: Phylogenetic Analysis Using Parsimony (PAUP), Version4.0. *Sinauer Associates, Sunderland, Massachusetts.*

TAN B.J. 1992: A Systematic List of the Mammals. *China Science and Technology Press, Beijing: p. 118.*

TATE G.H.H. 1941: Results of the Archbold Expeditions. No. 40. Notes on vespertilionid bats of the subfamilies Miniopterinae, Murininae, Kerivoulinae and Nyctophilinae. *Bulletin of the American Museum of Natural History 78:567–597.*

THOMPSON J.D., HIGGINS D.G. & GIBSON T.J. 1994: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, osition specific gap penalties and weight matrix choice. *Nucl. Acids. Res. 22 (22): 4673–4680.*

VAN DEN BUSSCHE R.A. & BAKER R.J. 1993: Molecular phylogenetics of the New World bat genus *Phyllostomus* based on cytochrome-*b* DNA sequence variation. *J. Mammal. 74: 793–802.*

WANG H., LIANG B., FENG J., SHENG L. & ZHANG S. 2003: Molecular phylogenetic of Hipposiderids (Chiroptera: Hipposideridae) and Rhinolophids (Chiroptera: Rhinolophidae) in China based on mitochodrial cytochrome *b* sequences. *Folia Zool. 52(3): 259–268.*

WANG K. R., XUE S. B. & LIU H. T. 1998: Cell Biology. *Beijing Normal University Publishing House, Beijing.*

WILSON D.E. & REEDER D.M. 1993: Mammals of the World: a Taxonomic and Geographic Reference. *Smithsonian institution: Washington, DC.*