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Molecular Phylogeny of Whiskered Bats (*Myotis*, Vespertilionidae, Chiroptera) in Palearctic Region

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ABSTRACT

Morphological features and mitochondrial DNA (mtDNA) sequences were analysed to study the phylogenetic relationships among eight species of whiskered bats (genus *Myotis*, subgenus *Selysius*) in the Palearctic region. The mtDNA phylogeny did not support the current division of the subgenus into species groups based on morphological data and species distributions, but showed instead that several species in the subgenus *Selysius* originated from distant lineages. The morphological similarity in some species could be due to convergence as a result of adaptation to similar habitats.

Keywords: Chiroptera, *Myotis*, Taxonomy, Morphology, Mitochondrial DNA, Cytochrome *b*, ND1

INTRODUCTION

The problem of sibling, or cryptic, species is one of the most topical and at the same time the most difficult questions in animal taxonomy. Recently, many revisions of the bat family Vespertilionidae based on molecular phylogenetic analysis showed that several common bat species actually consist of two or more sibling species [1, 2]. However, taxonomical studies of whiskered bats (genus *Myotis*, subgenus *Selysius*) have been made based only on morphology [e.g. 3, 4]. Phylogenetic studies of the genus *Myotis* [5] have mostly concentrated on the relationships among subgenera, and have not included a detailed analysis of the subgenus *Selysius*, because the taxonomy within the subgenus is still poorly resolved. Although recently several subspecies of *Myotis mystacinus* (the type species of the subgenus *Selysius*) were recognized as separate species based on morphological analysis [4], their phylogenetic relationships are still unclear because of lack of data

on their genetics. To date, among whiskered bats, only *Myotis alcathoe* has been described as a new species based on both molecular and morphological data [6].

The purpose of the present study was to compare morphological data and molecular phylogenetic relationships obtained from mitochondrial DNA sequences of species from the subgenus *Selysius*, including recently recognized species such as *M. auraszens* and *M. hajastanicus*, and to revise the taxonomical composition and phylogeny of this subgenus. The subgenus *Selysius* includes nine Palearctic and eight Oriental species that are arranged into four species groups: *frater*, *mystacinus*, *muri-cola* and *siligorensis*. The *siligorensis* group includes a single species, *M. siligorensis*, distributed in Central and South-Eastern Indo-Malayan regions. This species was not included into the present study because no specimens were available.

MATERIALS AND METHODS

Morphological Analysis

Two hundred seventeen specimens of both sexes of ten species from the subgenus *Selysius* were included in the analysis. For the morphometric analysis, 16 basic skull measurements were made: condylobasal length, width of the skull on the level of auditory bullae, width of braincase, height of braincase posterior to the auditory bullae, interorbital constriction, rostral width on the level of preorbital foramina, C-M³ length, length and width of the upper canine cingulum base, length of interval between cingulums of upper canine and large premolar («pseudodiasteme»), molariform row length, width of M³, length of M³, width between outer margins of upper canines, width between outer margins of M³, lower jaw length from alveole of i₁ to the articulated process, and length of maxillary tooth row. Only adult specimens identified without any doubt were used for the analysis. All the measurements were first analysed by factor analysis, and then the ten most substantial characters were used for multidimensional scaling. The scaling was based on Mahalanobis distances between centroids of the groups.

Phylogenetic Analysis

Entire sequences (1140 base-pairs: bp) of the mitochondrial cytochrome *b* gene from 23 samples of eight species and partial sequences (350 bp) of the mitochondrial ND1 gene from 20 samples of seven species of the subgenus *Selysius* were determined for the phylogenetic analysis. Phylogenetic relationships among nucleotide sequences were constructed by the neighbor-joining (NJ; [7]) method using

MEGA software [8] and the maximum parsimony method (MP) using PAUP* v 4.0b10 [9].

RESULTS AND DISCUSSION

Morphological Differences

The crown shapes of upper canines significantly differed from species to species in the subgenus *Selysius* (Fig. 1, [10], [11]). The upper canines were relatively round (*M. frater*, *M. bucharensis*), triangular with different orientations (*M. mystacinus*, *M. ikonnikovi*, *M. brandtii*), polyhedral (*M. muricola*) or rhomboid (*M. auraszens*, *M. hajastanicus*). The Palearctic species (*M. mystacinus*, *M. brandtii*, *M. ikonnikovi*, *M. auraszens* and *M. hajastanicus*) have triangular or rhomboid canine crowns, while Oriental species (*M. muricola*, *M. hosonoi*, *M. frater* and *M. bucharensis*) have round or polyhedral crowns. Analysis of skull morphometry showed that *Selysius* bats from the Palearctic and Oriental regions belong to separate species groups (Fig. 2). This result is in congruence with the current taxonomical structure of this subgenus [e.g. 12].

Molecular Phylogeny

Phylogenetic relationships among the species included based on cytochrome *b* (Fig. 3) and ND1 (Fig. 4) genes were not in agreement with the current division of the subgenus into species groups. The division into species groups within this subgenus has been based mostly on distribution patterns - all the Palearctic species are placed into the *mystacinus* species group, whereas Oriental species are placed into the *muricola* and *frater* species groups. The results of the present study showed that despite

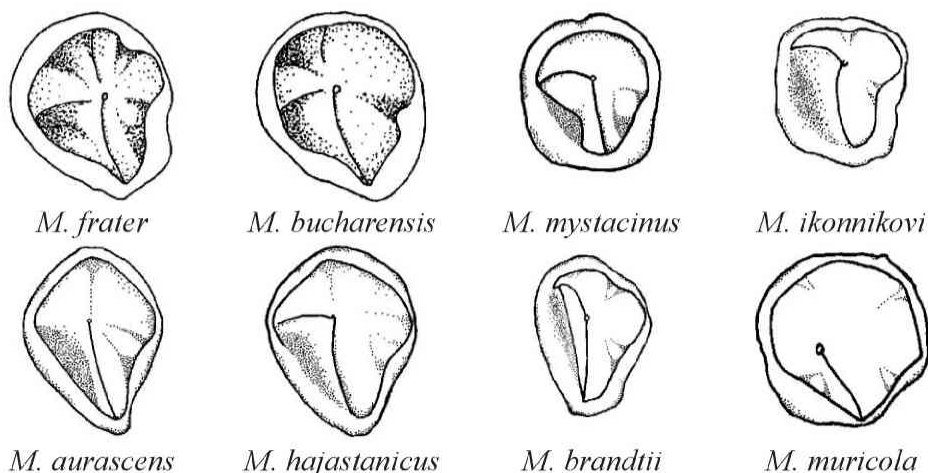


Fig. 1 Upper canines of eight species included in the study (views from below).

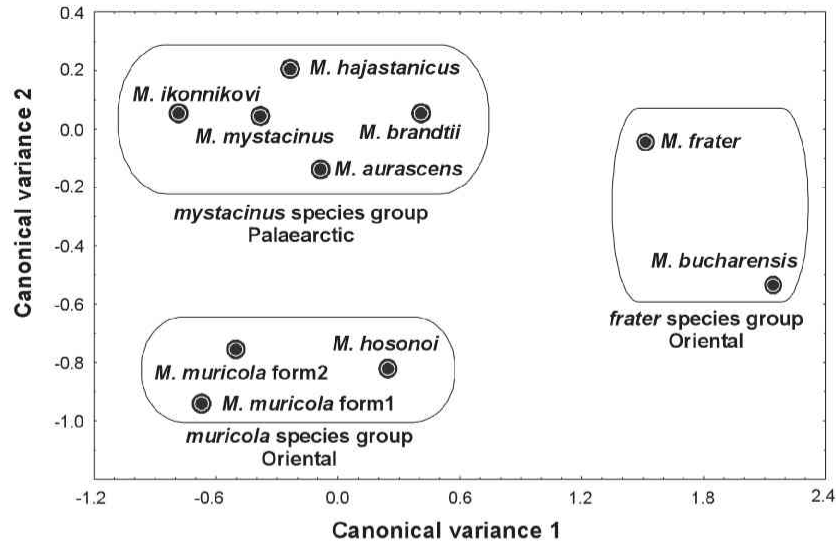


Fig. 2 Multi-dimensional scaling of 16 skull measurements in the space of two canonical variances.

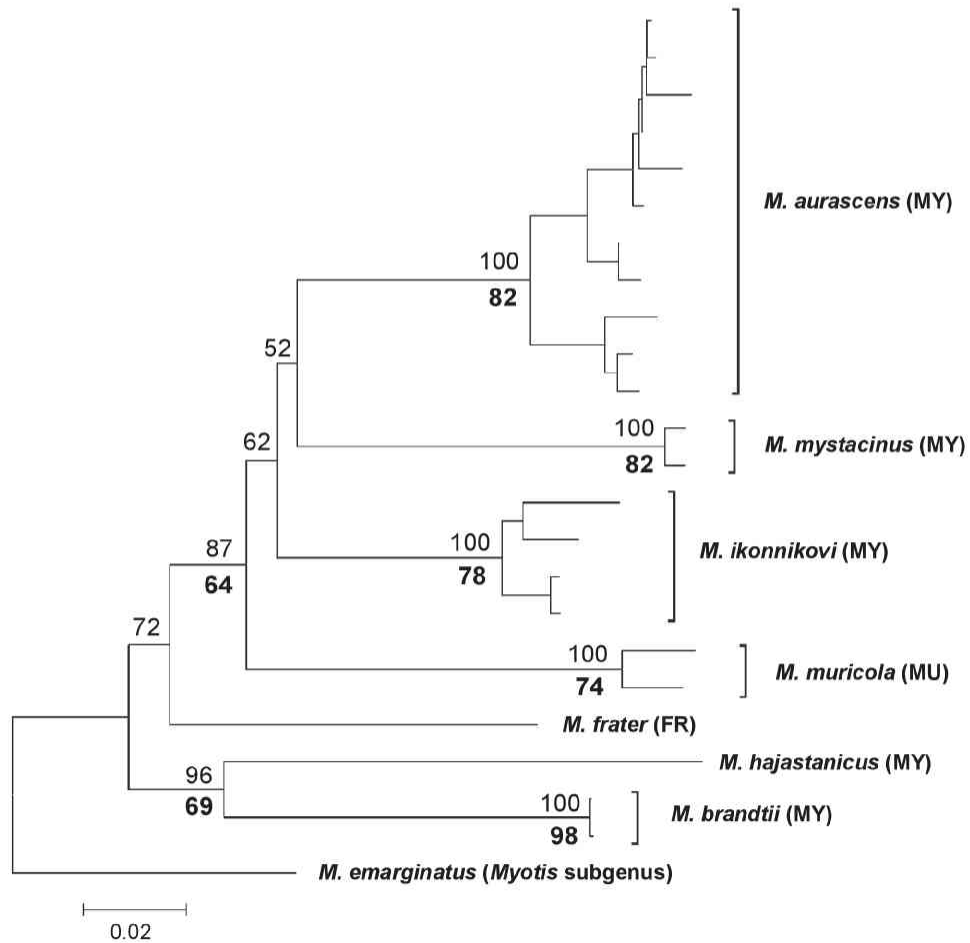


Fig. 3 Neighbor-joining relationships among *Selysius* species obtained from complete sequences of the cytochrome *b* gene (1140 bp). The scale shows genetic distances calculated by Kimura's two parameter model. Bootstrap values (derived from 1000 replications) are indicated above the internal branches and parsimony bootstrap scores > 50% are under the branches. MY, *mystacinus* species group; MU, *muricola* species group; FR, *frater* species group.

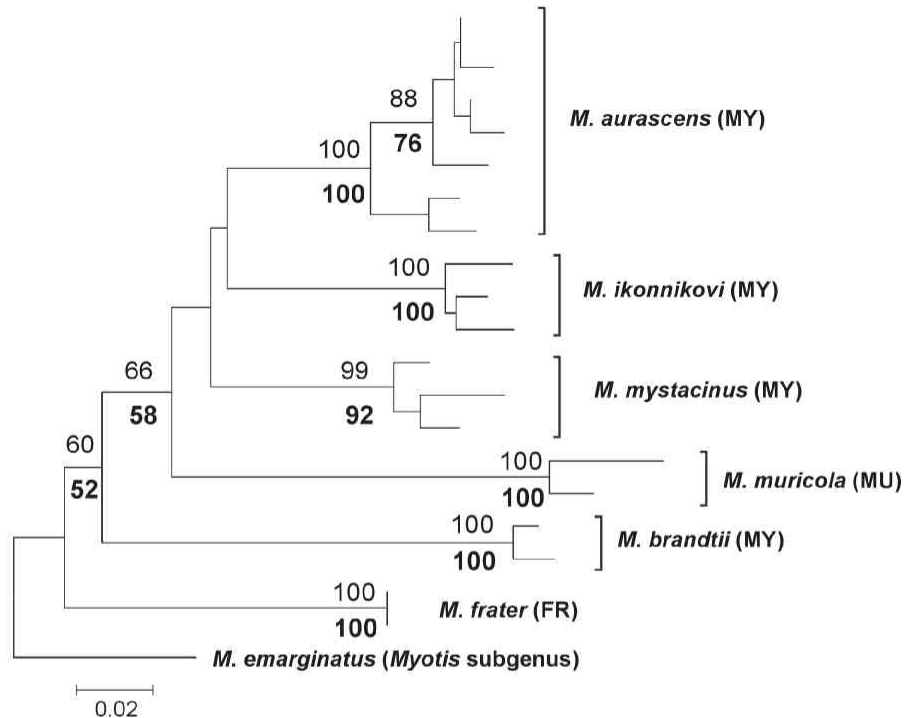


Fig. 4 Neighbor-joining relationships among *Selysius* species obtained from partial sequences of the ND1 gene (350 bp). The scale shows genetic distances calculated by Kimura's two parameter model. Bootstrap values (derived from 1000 replications) are indicated above the internal branches and parsimony bootstrap scores > 50% are under the branches. MY, *mystacinus* species group; MU, *muricola* species group; FR, *frater* species group.

all the samples of each species clustering separately with high bootstrap support, Palearctic species formed two different clades separated by Oriental *M. muricola* and *M. frater* (Fig. 3) or by Oriental *M. muricola* with *M. frater* basal to other species included (Fig. 4). The phylogenetic positions of *M. aurascens* and *M. hajastanicus* were distinct from the other species in the cytochrome *b* gene tree (Fig. 3, [13]). *Myotis hajastanicus* is close in appearance to *M. aurascens* and well distinguished from *M. brandtii* (Figs. 1 and 2). By contrast, molecular phylogenetic trees showed that *M. hajastanicus* is distant from *M. aurascens* and clustered with *M. brandtii* with high bootstrap support (96% in NJ and 69% in MP), separately from other species. As shown in a previous study [5], *M. brandtii* is associated with American species of *Myotis* and distant from other Eurasian species. Thus, in spite of the morphological similarity, *M. hajastanicus* could have originated from a lineage different from *M. aurascens*. The relationships between *M. aurascens* and *M. hajastanicus* were similar to those between *M. mystacinus* and *M. brandtii* shown in the previous study [5].

The distribution range of *M. hajastanicus*, which

is endemic to the Caucasus, lies within that of *M. aurascens*. Throughout Eurasia, *M. aurascens* inhabits the forest-steppe and steppe parts of the temperate arid zone, including mountains. In both gene trees *M. aurascens* has two clades (Figs. 3 and 4). One clade includes samples from the western part of the distributional area, whereas another clade includes samples from the eastern part. However, both clades include samples from Kazakhstan, i.e. the distributional areas are overlapping. This suggests that during the last glacial period there were several refuges: in the Caucasus and Middle East areas, and in Asia. After the last glacial period, the species expanded their ranges northward, and populations from western and eastern areas joined to form the current range of *M. aurascens*. At present, the distributional history of *M. hajastanicus* is unclear, but our results indicate that the morphological similarity between *M. aurascens* and *M. hajastanicus* as well as between *M. mystacinus* and *M. brandtii* could be due to convergence as a result of adaptation to similar habitats of those species respectively.

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