



Short communication

## *Myotis hajastanicus* is a local vicariant of a widespread species rather than a critically endangered endemic of the Sevan lake basin (Armenia)

Christian Dietz<sup>a</sup>, Astghik Gazaryan<sup>b</sup>, George Papov<sup>b</sup>, Heliana Dundarova<sup>c</sup>, Frieder Mayer<sup>d,e,\*</sup>

<sup>a</sup> Biologische Gutachten Dietz, Balingen Straße 15, 72401 Haigerloch, Germany

<sup>b</sup> Yerevan State University, Department of Zoology, Aleq Manooguan 1, Yerevan 0025, Armenia

<sup>c</sup> Bulgarian Academy of Sciences, Institute of Biodiversity and Ecosystem Research, 1 Tsar Osvoboditel, 1000 Sofia, Bulgaria

<sup>d</sup> Museum für Naturkunde—Leibniz Institute for Evolution and Biodiversity Science, Invalidenstrasse 43, 10115 Berlin, Germany

<sup>e</sup> Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Altensteinstraße 6, 14195 Berlin, Germany

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## ABSTRACT

The Armenian whiskered bat, *Myotis hajastanicus*, is assumed to represent an independent species with an exceptionally small distribution range that is restricted to the basin of Lake Sevan in the Southern Caucasus. The species has not been recorded during the last 25 years and thus was classified as critically endangered by the International Union for Conservation of Nature (IUCN). In 2013, we found an extant population of the Armenian whiskered bat and caught one male and eleven pregnant females next to Lake Sevan. Morphological analysis revealed close similarity and largely overlapping phenotypic variation between *M. hajastanicus* and *Myotis auraszensis*. This morphological similarity and the lack of genetic differentiation in a fast-evolving mitochondrial gene between both taxa strongly argue for a single species (*M. auraszensis*) and the rejection of a local endemism.

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The Armenian highlands and the Sevan lake basin harbor a unique flora and fauna as a result of a variety of climatic conditions and habitats. About 1.600 plant species with at least 23 endemics are known (Babayan et al., 2005). Among vertebrates, two endemic fish species and one bird species are known within the basin and nearby mountain ridges (Babayan et al., 2005). In addition, an endemic mammal species has been described from the area as well: the Armenian whiskered bat, *Myotis hajastanicus*. It was described as a subspecies of the widely distributed *Myotis mystacinus* by Argyropulo (1939). A detailed review of the Western Palearctic whiskered bats pointed out the unique morphological characters of the Lake Sevan specimens and raised them to species rank: *M. hajastanicus* (Benda and Tsytsulina, 2000). Referring to that publication, *M. hajastanicus* was also given species rank by Simmons (2005) in the reference list of mammal species of the world. Genetic analysis of a museum specimen placed the species within the *Myotis brandtii* clade, surprisingly distant to the morpho-

logically more similar *M. mystacinus* clade (Tsytsulina and Masuda, 2004).

IUCN classified the endemic species as critically endangered (IUCN, 2008) with the following justification: “The species is only known from a very restricted area. There are no records since 1980s despite two surveys. The species might be extinct. Assessed as Critically Endangered (CR); further surveys are urgently required to determine whether the species persists.” and “population size unknown, the species is known only from five sites, probably of records from nursery colonies (no male individuals have yet been recorded)”. This ranks the Armenian whiskered bat among the 30 rarest bat species in the world; 25 of them are classified as critically endangered and five mostly insular species are extinct (IUCN, 2008). Within the western Palearctic region and the range of the EUROBATS agreement *M. hajastanicus* is the most endangered and one of the least known species (Eurobats, 2015a,b).

The aim of our study was to find an extant population of the Armenian whiskered bat to assess its morphological and genetic characters and their variability in order to assess its taxonomic rank and distribution.

In June 2013, we conducted a field expedition to search for the enigmatic species in northern Armenia, focused on the Sevan Lake

\* Corresponding author at: Museum für Naturkunde—Leibniz Institute for Evolution and Biodiversity Science, Invalidenstrasse 43, 10115 Berlin, Germany.

E-mail address: [frieder.mayer@mf-n-berlin.de](mailto:frieder.mayer@mf-n-berlin.de) (F. Mayer).

**Table 1**Selected characters and comparison of *Myotis hajastanicus* with morphological similar species. Measurements are given in the form MW ± SD (n) (min–max).

	Lake Sevan bats 2013 <sup>c</sup>	<i>M. hajastanicus</i> <sup>a</sup>	<i>M. aurascens</i> <sup>a</sup>	<i>M. nipalensis</i> <sup>a</sup>	<i>M. mystacinus</i> <sup>b</sup>	<i>M. alcaethoe</i> <sup>c</sup>	<i>M. brandtii</i> <sup>a</sup>
forearm (mm)	35.78 ± 0.8 (12) (34.7–37.5)	35.59 ± 0.8 (11) (34.5–37.3)	34.68 ± 1.1 (49) (32.9–36.4)	34.80 ± 1.6 (6) (32.2–37.3)	33.29 ± 1.5 (66) (30.4–35.6)	32.03 ± 1.3 (63) (30.1–34.8)	34.96 ± 1.1 (81) (32.2–37.1)
metacarp of fifth finger (mm)	30.72 ± 0.9 (12) (28.9–31.8)	32.76 ± 1.1 (11) (31.2–35.2)	30.71 ± 1.1 (48) (28.2–33.1)	30.90 ± 1.8 (5) (27.8–32.3)	29.53 ± 1.5 (60) (26.5–32.0)	25.92 ± 0.9 (31) (24.0–27.1)	30.11 ± 1.1 (81) (27.7–32.2)
proximal phalange of fifth finger (mm)	8.78 ± 0.5 (12) (7.6–9.4)	8.50 ± 0.5 (11) (7.8–9.3)	8.03 ± 0.5 (48) (7.2–9.1)	7.60 ± 1.0 (5) (6.0–8.6)	7.41 ± 0.6 (60) (6.1–8.4)	6.84 ± 0.3 (31) (6.5–7.4)	7.65 ± 0.4 (81) (6.7–8.7)
canine shape type	hajastanicus (n = 8) & aurascens (n = 4)	hajastanicus	aurascens	nipalensis/transcaspicus	mystacinus	mystacinus	brandtii
penis	thin (n = 1)	unknown	thin	thin	thin	thin	club shaped

<sup>a</sup> Benda and Tsytsulina (2000).<sup>b</sup> Benda and Tsytsulina (2000), including *M. alcaethoe*.<sup>c</sup> Own data.

basin and the surrounding mountain areas, including the type locality of *M. hajastanicus*. During daytime we checked a wide variety of possible roosting sites, mainly bridges of roads and railways, rock crevices, stone walls, historical buildings, tree crevices and small caves. At night we conducted acoustic surveys and set mistnets to catch foraging bats. Nets were placed across small streams, at possible foraging sites or at entrances of possible roosts. Captured bats were immediately taken from the nets and kept individually in cotton bags until they were processed. Bats were identified following Dietz and von Helversen (2004) and Dietz et al. (2009) and whiskered bats by Benda and Tsytsulina (2000), Tsytsulina (2000) and Tsytsulina and Masuda (2004). All bats were measured (lengths of forearm, fifth finger, third finger, thumb, tibia and hindfeet, in whiskered bats also lengths of metacarpals and phalanges of the fifth finger) with a mechanical Hommel calliper to the nearest of 0.01 mm. Close-up photographs of all external characters were taken with a Nikon D300 camera, Nikon 105 mm macro-lens and a Nikon close-up multiflash-system in hand and in a flight tent. Dental characters were inspected with an optical otoscope combined with a magnifying lens and LED-lights.

The first whiskered bat was found on June 4th, 2013 in a fissure at the entrance of a small cave close to the eastern shore of Lake Sevan, 2.5 km northwest of the village of Tsapatagh (40°25'N, 45°26'E, 1929 m asl.). The locality is only 17 km from the original collection locality of the paratype specimen in Shorzha and most other records come from a 50 km radius around our capture site. In the night we set mistnets at the entrance of the cave and a second one nearby. A total of 39 bats were captured, eleven of which were pregnant female whiskered bats. The other individuals represented three other species; *Myotis oxygnathus*, *Myotis cf. nattereri*, and *Plecotus macrobullaris*. A fifth species, *Hypsugo cf. savii* could be observed and was recorded, but not captured. The twelve whiskered bats were very similar to each other. Their general appearance and external characters closely resembled European *Myotis aurascens* (Table 1, Dietz and von Helversen, 2004; Dietz et al., 2009), yet the following combination of characteristics and measurements clearly assigned the bats to *M. hajastanicus* as defined by Benda and Tsytsulina (2000). In general *M. hajastanicus* is a poorly differentiated species (Benda et al., 2016). All metric data of the forearm, skull and teeth fall within the variation of closely related species (Figs. 3–12 in Benda and Tsytsulina, 2000). However *M. hajastanicus* is described to have narrow canines with a rhombic shape of cingulum and basis of the canine crown, a rather slender point and four more or less well developed mesial, distal, palatal and labial edges. While this type of canines has been found also typically in *M. aurascens*, canines of *M. hajastanicus* are described to be

shorter than in related species (text p. 344 and Figs. 42 & 58 in Benda and Tsytsulina, 2000). In our sample eight individuals showed this *hajastanicus* type with very short and narrow canines, while four females had longer canines resembling much the *aurascens* type.

In metric data only the lengths of the metacarpals of the fifth finger and the first phalange of the fifth finger differ from other species and populations (Table 2 and Figs. 13 and 14 in Benda and Tsytsulina, 2000). Our bats clearly had much longer proximal phalanges of the fifth finger than any other westpaleartic whiskered bat species (Table 1), clearly assigning our specimen to *M. hajastanicus*. So far no data on penis or baculum morphology have been available, since all specimen had been females. In the first male ever caught the penis shape was clearly different of *Myotis brandtii* and *Myotis gracilis*, and had the typical thin shape of all species of the *M. mystacinus*-clade (Table 1).

In the field, biopsy punches with a diameter of 3 mm were taken from the wing membrane and stored in 80% ethanol for genetic analyses. DNA was isolated according to a salt-chloroform procedure (Müllenbach et al., 1989). The complete mitochondrial gene *nd1* (NADH dehydrogenase subunit 1) gene was amplified with the primers ER65 (5'-CCTCGATGTTGGATCAGG-3') and ER66 (5'-GTATGGCCCGATAGCTT-3'), which are located in the 16s rRNA and the *tRNAMet* genes, respectively (Petit et al., 1999). The amplifications were carried out in a volume of 25 µl containing 0.4 µM of each primer, 200 µM of each dNTP, 0.5 units Taq Polymerase (5000 U/ml; NEB) and 1x of its reaction buffer (10× ThermoPol Reaction Buffer). After an initial denaturation of 94 °C for 4 min, a temperature cycle of 94 °C for 30 s, 55 °C for 40 s and 72 °C for 90 s was repeated 40 times, followed by a final extension of 72 °C for 8 min. Sanger cycle sequencing were performed with primer ER70 (5'-CAGACCGAGTAATCCAGGTCGGTT-3'; Petit et al. 1999) and the Big Dye Version 2 kit by following the manufacturer's protocol (Applied Biosystems). Electrophoresis was performed on a ABI 3130xl Sequencer (Applied Biosystems). We sequenced the first 450 bp of the *nd1* gene, which represents the first 150 amino acid codons of that gene. Sequences were obtained for ten of the twelve whiskered bats caught at Lake Sevan. Sequences were aligned in CodonCode Aligner (CodonCode Corporation, MA, USA) together with the *nd1* sequences retrieved from GenBank (Table 2). Sequence divergence among haplotypes (uncorrected p-distances) were calculated in MEGA6 (Tamura et al., 2013) and visualized as a minimum spanning network by using HapStar (Teacher and Griffiths, 2011).

All specimens from Lake Sevan shared one mitochondrial haplotype, which was also reported for an individual of *M. aurascens* from Turkey (GenBank reference KF218453, Çoraman et al., 2013).

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