



Short communication

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

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ARTICLE INFO

Article history:

Received 21 March 2016

Accepted 20 June 2016

Handled by Danilo Russo

Available online 23 June 2016

Keywords:

Phylogeography

Endemism

Taxonomy

Conservation

Chiroptera

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

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Table 1Selected 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 ^c	<i>M. hajastanicus</i> ^a	<i>M. aurascens</i> ^a	<i>M. nipalensis</i> ^a	<i>M. mystacinus</i> ^b	<i>M. alcaethoe</i> ^c	<i>M. brandtii</i> ^a
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

^a Benda and Tsytsulina (2000).^b Benda and Tsytsulina (2000), including *M. alcaethoe*.^c 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).

Table 2
Individuals included in the genetic analysis of the mitochondrial *nd1* gene.

Taxon	Gender	Country	Locality	Haplotype	ID#	Genbank Accession#	References
<i>Myotis cf. hajastanus</i>			<i>icus</i>				
	male	Armenia	Tsapatagh, Lake Sevan	H9	8300	KX530149	This study
	female	Armenia	Tsapatagh, Lake Sevan	H9	8312	KX530149	This study
	female	Armenia	Tsapatagh, Lake Sevan	H9	8321	KX530149	This study
	female	Armenia	Tsapatagh, Lake Sevan	H9	8322	KX530149	This study
	female	Armenia	Tsapatagh, Lake Sevan	H9	8323	KX530149	This study
	female	Armenia	Tsapatagh, Lake Sevan	H9	8325	KX530149	This study
	female	Armenia	Tsapatagh, Lake Sevan	H9	8329	KX530149	This study
	female	Armenia	Tsapatagh, Lake Sevan	H9	8331	KX530149	This study
	female	Armenia	Tsapatagh, Lake Sevan	H9	8333	KX530149	This study
	female	Armenia	Tsapatagh, Lake Sevan	H9	8335	KX530149	This study
<i>Myotis aurascens</i>		Iran	Western, Azerbaijan	H8		AY699861	Tsytulina et al. (2012)
		Iran	Western, Azerbaijan	H8		AY699862	Tsytulina et al. (2012)
		Iran		H10		KF218452	Çoraman et al. (2013)
		Turkey		H9		KF218453	Çoraman et al. (2013)
		Russia	Stavropol Region	H14		AY699856	Tsytulina et al. (2012)
		Russia	Volgograd region	H12		AY699857	Tsytulina et al. (2012)
		Russia	North Caucasus, Tuapse	H13		AY699858	Tsytulina et al. (2012)
		Russia	Tuva	H11		AY699859	Tsytulina et al. (2012)
		Mongolia	Chuch lake	H2	1508	FR848512	Datzmann et al. (2012)
		Mongolia	Chuch lake	H2	1608	FR848513	Datzmann et al. (2012)
		Mongolia	South of Tsagaa lake	H2	3808	FR848514	Datzmann et al. (2012)
	male	Mongolia	Chuch lake	H2	5877	FR848515	Datzmann et al. (2012)
	female	Mongolia	Chuch lake	H2	5878	FR848516	Datzmann et al. (2012)
	female	Mongolia	Har Buhyn balgas	H3	4599	FR848518	Datzmann et al. (2012)
		Mongolia	Sum Hoh Burd	H4	4742	FR848522	Datzmann et al. (2012)
		Mongolia	Sum Hoh Burd	H4	4743	FR848523	Datzmann et al. (2012)
		Mongolia	Sum Hoh Burd	H4	4746	FR848526	Datzmann et al. (2012)
		Mongolia	Sum Hoh Burd	H4	4747	FR848527	Datzmann et al. (2012)
		Mongolia	Oasis Zulganai	H5	4749	FR848528	Datzmann et al. (2012)
		Mongolia	Orog lake	H4	4755	FR848529	Datzmann et al. (2012)
		Mongolia	Orkhon river (near Hujirt)	H4	4761	FR848535	Datzmann et al. (2012)
		Mongolia	Orkhon river (near Hujirt)	H4	4763	FR848536	Datzmann et al. (2012)
		Mongolia	Tuul river, near Öndörshireet	H6	4764	FR848537	Datzmann et al. (2012)
		Mongolia	Tuul river, near Öndörshireet	H4	4766	FR848539	Datzmann et al. (2012)
	male	Mongolia	Tschulutyn river	H7	6048	FR848540	Datzmann et al. (2012)
	female	Mongolia		H4	6051	FR848541	Datzmann et al. (2012)
	female	Mongolia		H4	6054	FR848542	Datzmann et al. (2012)
	female	Mongolia		H4	6056	FR848543	Datzmann et al. (2012)
	male	Mongolia		H4	6057	FR848544	Datzmann et al. (2012)
	male	Mongolia		H4	6058	FR848545	Datzmann et al. (2012)
	female	Mongolia		H4	6059	FR848546	Datzmann et al. (2012)
		South Korea	Gangwon Do, Yongwol Gun	H1		AY699860S	Tsytulina et al. (2012)

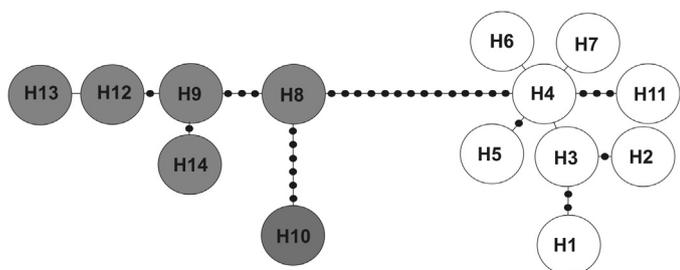


Fig. 1. Minimum spanning network of mitochondrial haplotypes based on a 450 bp fragment of the *nd1* gene. Open circles represent sampled haplotypes from the eastern Palearctic region, grey circles are haplotypes found in the Caucasus region and small black circles denote hypothetical haplotypes. Each line codes for one nucleotide substitution.

Sequence divergence to the other whiskered bats, *M. mystacinus*, *M. brandtii* and *Myotis alcaethoe*, always exceeded 9.7%. A detailed analysis of genetic variation within *M. aurascens* and the animals from Lake Sevan revealed a total of 38 polymorphic sites. Sequences differed by up to 25 nucleotides (5.3%). The sequences grouped into two groups separated by at least 15 substitutions (3.3%; Fig. 1). One group comprised individuals from the eastern Palearctic region (Mongolia, Eastern Siberia and South Korea) and the other one is represented by animals from the western Palearctic region (Cauca-



Fig. 2. Portrait of a female *Myotis hajastanicus* from the Sevan lake basin, Armenia.

sus, Russia, Turkey and Iran), including the individuals from Lake Sevan. Sequence divergence within this group reached a maximum of 2.0%.

Our first finding of an extant population of the critically endangered Armenian whiskered bat, *M. hajastanicus*, since the 1980s proves that it is not extinct as it has been believed (IUCN, 2008, Figs. 2 and 3). The assignment of our specimen to *M. hajastanicus* is



Fig. 3. A male *Myotis hajastanicus* from the Sevan lake basin, Armenia.

well justified: the capture location is close to the type locality (in the same basin 17 km apart), habitats are identical in both areas, lying at about 2000 m asl. at the foothills of the high mountain ridge along the east bank of Lake Sevan. Although *M. hajastanicus* is a poorly differentiated species, all morphological criteria of our specimen fit well to the species description by Benda and Tsytsulina (2000), especially the unusual long proximal phalanges of the fifth finger are clearly diagnostic. However we detected some overlap in dental characters with *M. aurascens* and *M. nipalensis* and most other metric data were similar to related species (Benda et al., 2016).

When bringing the morphological and genetic criteria assessed in our twelve specimen from Lake Sevan together with all the data and descriptions published by Benda and Tsytsulina (2000), Benda and Karataş (2005), Benda et al. (2016), Tsytsulina (2000), Tsytsulina et al. (2012) and Tsytsulina and Masuda (2004) in a synoptical way, the three species, *M. aurascens*, *Myotis nipalensis* and *M. hajastanicus*, rather seem to belong to one quite variable morphotype than to separated groups (see also Benda et al., 2016). We were able to detect a significant overlap in all mentioned dental characters and size. Coloration was also very variable and showed extensive overlap. Remaining subtle size differences might rather reflect local adaptations to foraging habitats or climate or reflect the population history than being indicative for a different species status. This monotypic scenario is also supported by the genetic analyses of the mitochondrial *nd1* gene. A maximum sequence divergence of 2.0% typically falls within the range of intraspecific variation (Mayer et al., 2007).

Tsytsulina et al. (2012) found only one genetic lineage in Iran and assigned both morphotypes of *aurascens* and *nipalensis* sensu Benda and Tsytsulina (2000) to be conspecific and named it *M. aurascens*. Similarly, Benda et al. (2012, 2016) assembled several taxa

previously believed to be different from each other together into the species *M. davidii* Peters 1869, originally described from China. Basing on morphological and genetical comparisons, Benda et al. (2012, 2016) now regard *aurascens* and *nipalensis* as junior synonyms of *M. davidii*. These changes in taxonomic opinions clearly indicate a previous over splitting due to an overvaluation of subtle and quite variable dental characters and measurements. The changes also show that a correct assignment of whiskered bats is complicated because they are often morphologically similar and hence lack synapomorphic characters but exhibit strong phenotypic similarities due to similar ecological adaptations. Although not further evaluated, this can be regarded as first indication to doubt the morphological discreteness of *M. hajastanicus* as well.

According to the genetic results of Tsytsulina and Masuda (2004) we expected the first ever caught male to show a club shaped penis similar to the closely related *M. brandtii* and *M. gracilis*. However the penis shape was similar to the species of the *M. mystacinus* clade, indicating the first discrepancies between published genetic results and morphology. The disagreement could be solved when it became clear that the published sequence AY665138 of *M. hajastanicus* in Tsytsulina and Masuda (2004) was a chimera of cytochrome b sequences of *M. gracilis* with parts of *Bos indicus* (see Ruedi et al., 2013).

So far, there is no bat species known that is endemic to a small geographic area that is not geographically separated from other ecologically suitable areas for example by water, high mountains or deserts. Bats are highly mobile, migrate between summer and winter roosts and individuals of different colonies gather at swarming sites in autumn where mating occurs. Such behaviour allows gene flow across large distances and prevents divergence of populations and thus also speciation. Therefore, the long-term persistence of species in a small confined area would be unexpected. Our finding is in line with these biogeographic hypotheses since *M. hajastanicus* and *M. aurascens* were expected to occur in close proximity (Benda and Tsytsulina, 2000).

All together we were able to show that the whiskered bat population of the Lake Sevan basin still persists and at least two nursery colonies exist in the *terra typica* of the taxon *hajastanicus*. However, neither morphological nor genetic criteria support the distinctness of the taxon from closely related other species of the *M. mystacinus* clade. Morphological and genetical characters place *hajastanicus* clearly together with *aurascens*, a taxon lately regarded to be a synonym of *Myotis davidii* (Benda et al., 2012, 2016). Therefore, the validity of the species *M. hajastanicus* can be rejected. Instead, *M. aurascens/davidii* has a wide distribution in arid steppe like habitats from Eastern Europe into Central Asia and northern China, being not rare in some places and thus reflecting a rather low red list category of Least Concern rather than a category of more endangered species. Interestingly a persistence of *hajastanicus* has been suggested when inferring extinction risk of mammals by cox regression models by Fisher and Blomberg (2011), a prediction confirmed by our findings.

Acknowledgements

We are grateful to Isabelle Waurick, she extracted the DNA and performed DNA sequencing. Emrah Çoraman, Radek Lučan and an anonymous referee helped much to improve the manuscript.

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