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First record of *Myotis alcathoe* (Chiroptera: Vespertilionidae) in Slovakia

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A b s t r a c t . *Myotis alcathoe* has been recorded in the territory of Slovakia for the first time. On 2 August 2001, two individuals of the species were netted at the entrance to the Stĺpová jaskyňa cave in the basaltic plateau of the Cerová vrchovina Mts (48° 12' 23" N, 19° 54' 37" E; 525 m a. s. l; ca. 2 km south of the village of Šurice). The species identification was confirmed by sequencing part of the mitochondrial gene ND1 in two specimens. Measurements and description of external and cranial characters, as well as description of the baculum of these two reference specimens are given for further comparisons of this poorly known species.

Key words: *Myotis alcathoe*, Slovakia, first record, identification characters, mtDNA

Introduction

The Alcathoe's bat, *Myotis alcathoe* Helversen et Heller, 2001, has been distinguished primarily on the basis of genetic, karyological and echolocation characters (V o l l e t h 1987, H e l v e r s e n 1989, H e l v e r s e n et al. 2001) from other, very similar species belonging to *M. mystacinus* group. Although these characters separate very clearly *M. alcathoe* from either *M. mystacinus* (Kuhl, 1817), *M. aurascens* Kusjakin, 1935, *M. brandtii* (Eversmann, 1845), or from *M. ikonnikovi* Ognev, 1911 (H e l v e r s e n et al. 2001, M a y e r & H e l v e r s e n 2001), its external morphology and cranial characters are rather similar to these species. H e l v e r s e n et al. (2001), and later R u e d i et al. (2002), gave a series of morphological characters which should help to discriminate this new species from other European *Myotis*. These characters include a reddish brown dorsal pelage (without golden tips commonly found in *M. mystacinus*, *M. brandtii* or *M. aurascens*), brownish colour of face and ears (not blackish), and a shorter tragus (hardly projecting beyond the ear notch). External dimensions of thumb, claws, feet and forearms are also smaller than those of other European *Myotis*. In short, the general appearance of *M. alcathoe* is a combination of a very small *M. mystacinus*, but with the brownish coloration typical of *M. daubentonii* (Kuhl, 1817), but the limited number of Alcathoe's bats measured so far is still too small to estimate the full variation range of these morphological characters.

Originally *M. alcathoe* has been recorded at two sites in the southern part of the Pindus Mts (Thessaly, Central Greece), at two sites in the Greek Rhodopes Mts, and at two sites in northern Hungary (the Bükk and Mátra Mts; H e l v e r s e n et al. 2001) (Fig. 1). The

species was therefore supposed to be an eastern element inhabiting the Balkan Peninsula and reaching only marginally into central Europe. However, *M. alcathoe* has been found recently in western France as well (R u e d i et al. 2002), suggesting that its geographic range is much wider than indicated by H e l v e r s e n et al. (2001). The existence of this new species in western Europe further indicates that the name *M. alcathoe* Helversen et Heller, 2001 might be a junior synonym of an older name included so far in the synonymy list of *M. mystacinus* s. l. (for details see B e n d a & T s y t s u l i n a 2000 and R u e d i et al. 2002).

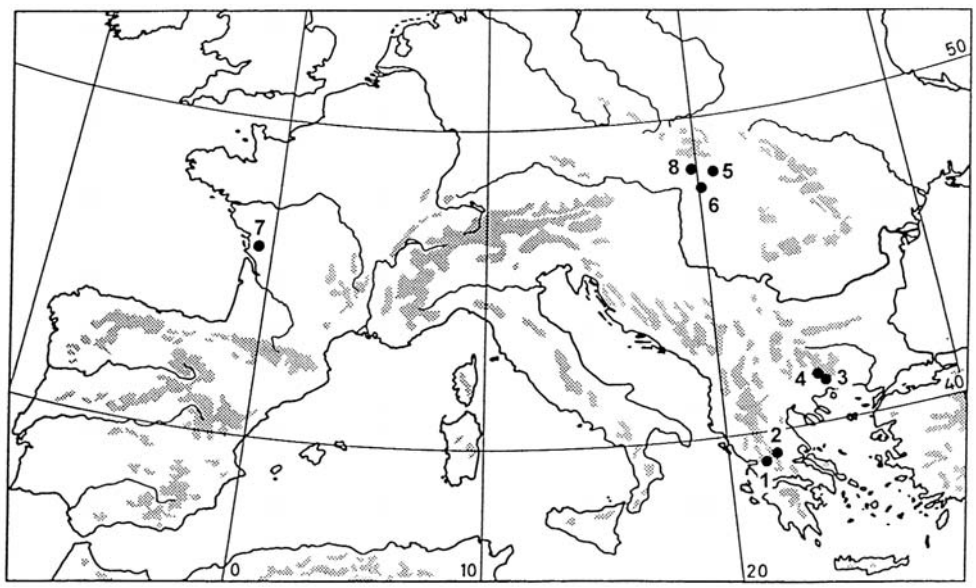

Fig. **1**. Records of *M. alcathoe*, confirmed by genetic analyses. Explanation: Greece (H e l v e r s e n et al. 2001): **1** – Kleistos, Fournikos river (type loc.), **2** – Loutropigi, **3** – Loutra Thermia, Nestos river, **4** – Skaloti; Hungary (H e l v e r s e n et al. 2001): **5** – Kacs (Bükk Mts), **6** – Parad (Matra Mts); France (R u e d i et al. 2002): **7** – Port-d'Euvaux; Slovakia (own data): **8** – Pohanský hrad.

This paper describes the first record of *M. alcathoe* in Slovakia, which extends its known distribution range slightly north into central Europe (Fig. 1). Thus, the Slovak bat fauna counts two more species in recent times – together with records of *Pipistrellus pygmaeus* in eastern Slovakia (D a n k o & P j e n č á k 2002, F u l í n 2002). Although species identification of *M. alcathoe* is still based here on DNA characters, we also give a more detailed description of the Slovak individuals in order to contribute new data useful to a morphological identification of *M. alcathoe*.

Material and Methods

C a p t u r e s i t e

On 8 August 2001, five bats preliminarily identified as *M. mystacinus* (3 males, 2 females: forearm$^+$ length 30.2–32.9 mm, mean 31.7 mm; weight 3.9–4.3 g, mean 4.1 g) were mist-netted in a boulder field at the Stĺpová jaskyňa cave, ca. 2 km south of the village of Šurice

(district of Lučenec, Cerová vrchovina Mts, southern-central Slovakia). One male and one female were collected for further analysis and are deposited in the collection of the Department of Zoology, National Museum (Natural History), Praha, under numbers NMP 50446 and 50447, respectively.

The Stĺpová jaskyňa cave (48° 12' 23" N, 19° 54' 37" E; 525 m a. s. l.; mapping square no. 7885) is situated at the western slope of the Pohanský hrad hill (and the National Nature Reserve of the same name). The cave was created by the gravitation (slope movements) of an extensive Pliocene basaltic plateau, in a complex including 30 similar pseudokarstic caves in the area (G a á l & G a á l 1997). The hill is covered by 80 to 100-year-old stands of deciduous woods (mostly *Acerion pseudoplatani*) and bush assemblages of *Prunion spinosae* with solitary old trees (*Quercus patraea* agg., *Q. cerris*, *Carpinus betulus*, *Pyrus communis*, etc.). Vegetation around the rocky fields is a typical mixture of *Lolio-Cynosurenion* and *Alopecurion* (G a á l o v á 1996). Other bats recorded in the same area include *Rhinolophus hipposideros*, *Myotis myotis*, *M. daubentonii*, *Eptesicus serotinus*, *Nyctalus noctula*, *N. leisleri*, *Barbastella barbastellus*, *Plecotus auritus*, and *P. austriacus* (U h r i n & B e n d a 1995, unpubl. data).

G e n e t i c a n a l y s i s

Total genomic DNA was extracted from ethanol-preserved tissues with the DNeasy Tissue kit of QIagen, Inc. as described in R u e d i et al. (2002). The complete nicotinamide-dehydrogenase subunit 1 gene (*ND1*) was obtained by PCR with specific primer pairs ER65 and ER66 designed by P e t i t et al. (1999) and M a y e r & H e l v e r s e n (2001). The 25 µl reaction volume PCR cocktail contained: 3 µl of DNA extract, 0.5 µl of each primers (10 µM), 2 µl of $MgCl_2$ (25 mM), 0.5 µl dNTP (40 mM), 0.15 µl of Taq polymerase, 2.5 µl of buffer and completed with ddH_2O. Amplifications included an initial step of denaturation at 94 °C, followed by 37 cycles at 94 °C (45''), 50 °C (45'') and 72 °C (1'), with a final extension at 72 °C (1'30''). The 500 initial bases of ND1 were sequenced directly from the purified PCR product, using ER70 primer (P e t i t et al. 1999, M a y e r & H e l v e r s e n 2001), and followed by BigDye sequencing protocols (Applied Biosystems).

The partial *ND1* sequences were aligned with the program Sequencher 4.1 (Gene Codes Corp.), and compared to other homologous sequences of all European *Myotis* (H e l v e r s e n et al. 2001, M a y e r & H e l v e r s e n 2001, R u e d i & M a y e r 2001).

Results and Discussion

Both Slovak specimens could be sequenced and aligned without ambiguity with the *M. alcathoe* available from Greece, Hungary and France. The initial 500 bp of ND1 of these two Slovak individuals differ by 5 mutations from each other (1% sequence divergence). All mutations are synonymous third codon positions, as is usually observed among closely related sequences (see e.g. R u e d i & M a y e r 2001). These variable nucleotides are found at position 27 (G(A), at position 96 (A→G), at position 120 (G→A), at position 153 (C→T) and at position 438 (G→A). In fact the sequence of the Slovak female (NMP 50447) corresponds exactly to the haplotype found in all Greek specimens of *M. alcathoe* (including the three paratypes), while the male (NMP 50446) has exactly the same sequence as the two Hungarian specimens (see H e l v e r s e n et al. 2001) and the two French ones (R u e d i et al. 2002). Thus the two different haplotypes of *M. alcathoe* found so far in western and south-

eastern Europe, coexist in the same locality in Slovakia and still provide the best way to identify this new species in Europe.

The external, cranial and dental measurements of these two Slovak specimens of *M. alcathoe* are given in Table 1. Their external measurements agree with those given in the type series (H e l v e r s e n et al. 2001), and also with values given for French specimens (R u e d i et al. 2002). Two exceptions include tibia and thumb claw lengths, but these differences probably may result from different measuring methods used. Tragus is relatively short in both Slovak *M. alcathoe* with tips slightly exceeding beyond the notch in lateral margin of the auricle. Again, this corresponds to previous descriptions of the species (H e l v e r s e n 1989, R u e d i et al. 2002). No epiblema was noted and the tail projects 1 mm from the uropatagium, as in Greek *M. alcathoe*. The general colour of dorsal hairs of both Slovak specimens is 6–7 mm long, with proximal parts (ca. $^{2}/_{3}$) dark brown (or black-brown) and distal ends light brown. Ventral hairs are up to 6 mm long with proximal parts (ca. $^{3}/_{4}$) black-brown and distal ends light ochre. Hairs on throat are darker than on belly. The limits between the dark back and lighter belly is not sharp on shoulders, like in *M. mystacinus* (B e n d a & T s y t s u l i n a 2000). The facial skin and ears are brown with bases of the ears less pigmented. Wing membranes are somewhat darker brown than the facial parts, but are still lighter that typical *M. mystacinus* or *M. brandtii*. Pelage and bare skin colours are therefore not unlike those of adult *M. daubentonii* or *M. emarginatus*. As noted before (H e l v e r s e n et al. 2001, R u e d i et al. 2002), both Slovak *M. alcathoe* are therefore browner and lighter in general appearance than species of the *M. mystacinus* group and, along with the shorter tragus and smaller dimensions, provide good external characters to identify *M. alcathoe*.

Cranial measurements of Slovak individuals of *M. alcathoe* correspond to or are slightly larger than those of the type series, but do not reach mean values found in *M. mystacinus* s. str. The skull shape in Slovak *M. alcathoe* resembles that of *M. mystacinus*, however, it has lower rostrum and the fronto-maxillary region is markedly more concave (the same as in the type specimen, see H e l v e r s e n et al. 2001); the frontal region is markedly convex with parietal regions being rather flat (Fig. 2a). The upper canines are small, short in mesiodistal direction (RCn values in both Slovak specimens are smaller than mean values in *M. mystacinus*); their cingula are oval to triangular with a rounded mesial edge; crown of canines have a pseudotriangular basis, with larger palatodistal concavity, a smaller labiodistal concavity and a convex mesial surface. These characters can be found in *M. mystacinus* as well (Fig. 2b). The upper and lower row of premolars (P^2P^3 and P_2P_3) are relatively long, especially P^3 and P_3 which are relatively larger compared to those of *M. mystacinus* (see Tab. 1). The crown of P^3 in both individuals reaches $^{1}/_{2}$ of P^2 height; these premolars are located in the axis of the tooth-row (Fig. 2b, c). In both Slovak specimens, the large upper premolar (P^4) has relatively high cusps on the mesiopalatal edges of the cingulum, these cusps are higher than mean values in *M. mystacinus*, but lower than in *M. brandtii* (see B e n d a & T s y t s u l i n a 2000). Paraconuli and paralophi are present on all upper molars in both specimens; metalophi are absent, while the female has poorly developed metaconuli. In both Slovak specimens, the second upper incisive has two protuberances on palatal and labial sides of the main cusp (like in *M. mystacinus*).

In the male Slovak *M. alcathoe* (NMP 50446), the unpigmented penis is narrow and cylindrical, the distal straight part being 3 mm long and 0.9 mm wide. The baculum is oval, 0.48 mm long and 0.27 mm wide; diaphysis is flat, concave from ventral side, with broad

Table 1. Measurements of Slovak specimens of *M. alcathoe* in comparison with published data on the type series of *M. alcathoe* (Helversen et al. 2001) and of French specimens (Ruedi et al. 2002). Measurements of a central European population of traditionally understood *M. mystacinus* s. str. (Benda & Tsytsulina 2000) is also given for comparison (but this sample might include some undetected *M. alcathoe*, see text). Explanation: LC – head and body length, LCd – tail length, LAt^{+} – forearm length (wrist incl.), LAt^{+} – forearm length (without wrist), LPol – thumb length (without claw), LUp – thumb claw length, LdIII – third finger length, LdV – fifth finger length, LTp – hind foot length (without claws), LTib – tibia length, LA – ear length, LT – tragus length, G – weight, LCr – greatest length of skull, LCb – condylobasal length, LaZ – zygomatic width, LaI – interorbital width, LaN – braincase width, AN – braincase height, CC – rostral width over upper canines, M^3M^3 – rostral width over third upper molars, CM^3 – length of upper teeth-row, CP^4 – length of upper teeth-row betw. C and P^4 (incl.), P^2P^3 – length of upper tooth-row betw. P^2 and P^3 (incl.), LMd – length of mandible, LCo – height of coronoid process, CM_3 – length of lower tooth-row, LCn – length of upper canine, LaCn – width of upper canine, RCn – ratio LCn/LaCn, P^3 – length of second upper premolary P^3, ACin – height of cingulum cusp on third upper premolary P^4.

	M. alcathoe, Slovakia		*M. alcathoe*, type series			*M. alcathoe*, France		*M. mystacinus*, Central Europe			
	male	female	type (male)	paratypes				males		females	
	NMP 50446	NMP 50447	SMF 90249	mean±SD	n	mean	n	mean±SD	n	mean±SD	n
LC	44	39	–	–		–		–		–	
LCd	37	36	–	–		–		–		–	
LAt^{+}	32.5	32.9	–	–		–		33.08±1.51	19	33.94±1.17	26
LAt^{-}	31.3	31.6	30.5	31.4±0.5	13	31.67	20	–		–	
LPol	4.2	4.4	4.0	4.6±0.3	9	3.95	2	4.47±0.28	19	4.59±0.34	26
LUp	1.6	1.6	1.0	–		1.70	2	–		–	
LdIII	52.4	52.6	50	51.4±1.0	10	51.26	20	–		–	
LdV	40.2	41.0	40	40.3±1.3	12	41.58	20	–		–	
LTp	5.3	5.6	5.5	6.1±0.3	12	–		–		–	
LTib	14.0	14.3	–	13.4±0.6	8	–		14.97±0.85	18	14.69±0.80	25
LA	14.3	14.0	–	–		–		–		–	
LT	6.3	6.5	–	–		–		–		–	
G	4.2	4.3	–	4.2±0.4		4.78	20	–		–	
LCr	12.66	13.18	–	–		–		13.42±0.27	45	13.49±0.26	55
LCb	12.18	12.56	11.70	12.30±0.27	7	–		12.75±0.26	44	12.84±0.27	54
LaZ	8.22	8.16	7.87	8.13±0.16	7	–		8.17±0.22	30	8.22±0.21	33
LaI	3.28	3.28	3.27	3.34±0.11	7	–		3.41±0.11	45	3.40±0.11	57
LaN	6.41	6.35	–	–		–		6.62±0.15	44	6.64±0.15	56
AN	4.73	4.71	–	–		–		4.67±0.16	45	4.69±0.13	55
CC	3.37	3.39	3.17	3.26±0.08	7	–		3.31±0.11	44	3.32±0.15	55
M^3M^3	5.26	5.23	5.15	5.16±0.10	7	–		5.17±0.16	44	5.23±0.16	56
CM^3	4.90	4.98	4.80	4.94±0.09	7	–		5.03±0.15	45	5.04±0.11	56
CP^4	2.32	2.37	–	–		–		2.43±0.10	45	2.41±0.09	57
P^2P^3	0.79	0.81	–	–		–		0.74±0.05	44	0.74±0.05	55
LMd	9.31	9.43	9.01	9.50±0.26	7	–		9.55±0.24	45	9.61±0.25	57
LCo	2.73	2.76	2.77	2.75±0.10	7	–		2.65±0.10	43	2.65±0.12	56
CM_3	5.20	5.31	5.05	5.27±0.16	7	–		5.41±0.12	45	5.42±0.14	57
LCn	0.80	0.80	–	–		–		0.83±0.03	22	0.84±0.04	30
LaCn	0.68	0.66	–	–		–		0.66±0.03	22	0.67±0.03	30
RCn	1.17	1.21	–	–		–		1.26±0.05	22	1.25±0.05	30
P^3	0.39	0.38	–	–		–		0.34±0.03	44	0.35±0.03	55
ACin	0.16	0.15	–	–		–		0.11±0.03	42	0.11±0.03	56

"wings" while both epiphyses have thickened parts (Fig. 2d). Thus both penis and baccular characteristics are very similar to those of *M. mystacinus*, but distinct from both *M. brandtii* and *M. aurascens* (Benda & Tsytsulina 2000, Tupinier & Aellen 2001).

In general, cranial and dental characters given above are still based on a too limited number of specimens to understand their full range of variation in *M. alcathoe*. Most of

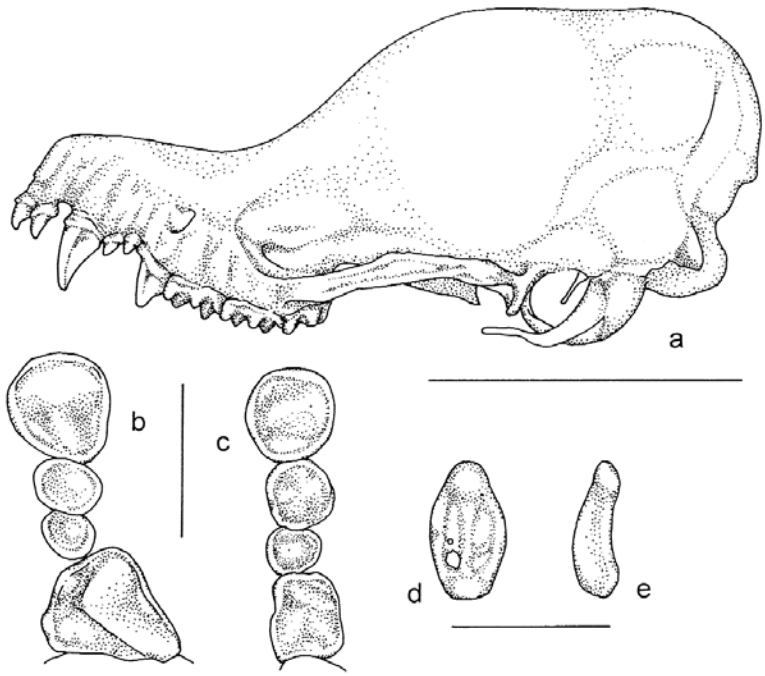

Fig. **2**. Skull (a), anterior maxillary (b) and mandibular (c) tooth-rows and baculum (dorsal – d, and lateral view – e) of *M. alcathoe* from Slovakia (NMP 50446). Scale bar 5 mm (a), 1 mm (b, c), 0.5 mm (d, e).

these characters show apparently a slight overlap with values given for a sample of central European *M. mystacinus* (B e n d a & T s y t s u l i n a 2000 and Table 1). However, this "reference" population of *M. mystacinus* s. str. might include unnoticed *M. alcathoe*, as both species were not yet distinguished, nor where they distinguished in the values given in the recent volume of Handbuch der Säugetiere Europas (T u p i n i e r & A e l l e n 2001). Thus, before such a reference population of *M. mystacinus* (i. e., specimens clearly identified as such by molecular characters) has been clearly established, it is premature to draw any definitive picture of the degree of morphological overlap between cranial measurements of *M. alcathoe* and species of the *mystacinus* group. As mentioned above, external characters relating to general colour, tragus length, colour of bare parts, and size can be good indicators of *M. alcathoe*, but in this case too, we still need to assess variation due to age or season, and measure them on a larger sample to be able to identify reliably living individuals.

In any case, the new finding of *M. alcathoe* in Slovakia suggests that the distribution range and ecological requirements of the species are wider than expected. These include not only the eastern parts of sub-Mediterranean Europe but also more mesic habitats of central and western Europe (H e l v e r s e n 1989, H e l v e r s e n et al. 2001, J o u r d e 2000, B a r d e t & H u e t 2001, R u e d i et al. 2002). Furthermore, whether any of the older names considered so far as junior synonyms of *M. mystacinus* might be applied to *M. alcathoe* is still an open question (R u e d i et al. 2002). It will require a critical re-examination of the type material with, preferably, a molecular diagnosis of those material still conserved in Museums.

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