

## New records of the Alcatheo bat, *Myotis alcatheo* in Moravia (Czech Republic)

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**Abstract.** A male *Myotis alcatheo* was identified morphologically among 119 road traffic casualties sampled in 2007 in southern Moravia (Czech Republic). Two additional male *M. alcatheo* were found among six carcasses collected in 1999 on roads in northern Moravia. Identification of all three specimens was confirmed by sequencing of mtDNA. The first photo of a *M. alcatheo* mandible is presented. The species, described in 2001, has actually been known from 12 localities within the Czech Republic.

**Key words:** *Myotis alcatheo*, morphometry, genetic identification, mtDNA

### Introduction

A new species of whiskered bat, *Myotis alcatheo*, was originally described from Greece and Hungary (Hellersen et al. 2001). Soon it was evident that its range was in fact much greater including France (Ruedi et al. 2002), Slovakia (Benda et al. 2003), Bulgaria (Shunger et al. 2004), Switzerland (Stadelmann et al. 2004), Iberian Peninsula (Agirre-Mendi et al. 2004, Ibáñez et al. 2006), Germany, Poland, Albania, European part of Turkey (Niermann et al. 2007). Using the morphological data and measurements in both the original description and the handbook of European and NW African bats (Dietz et al. 2007), we identified *M. alcatheo* among road casualties collected systematically in 2007 (Gaisler et al., in press). This became a stimulus to investigate earlier material of bat carcasses accidentally found on roads and preliminary determined as *M. mystacinus*. In the meantime a paper was published that evidenced a wide European distribution of *M. alcatheo* in moist deciduous forests with old trees and water streams (Niermann et al. 2007). The species has also been recorded at 9 sites on the territory of the Czech Republic (Lučan et al., in prep.). In spite of this, *M. alcatheo* still belongs to bats little known in Europe and the aim of this note is to contribute to define its status.

### Material and results of standard processing

Systematic sampling of traffic casualties the results of which are published elsewhere (Gaisler et al., in press) revealed 119 bat carcasses of 11–12 species picked up from emergency stopping lanes of a road between Pohořelice and Perná in southern Moravia (southeastern part of the Czech Republic). Within the sample, a male *M. alcatheo*, labelled M1, was found on 6 August 2007 at Pasohlávky, district Břeclav (N48°54'05", E16°35'05", elevation

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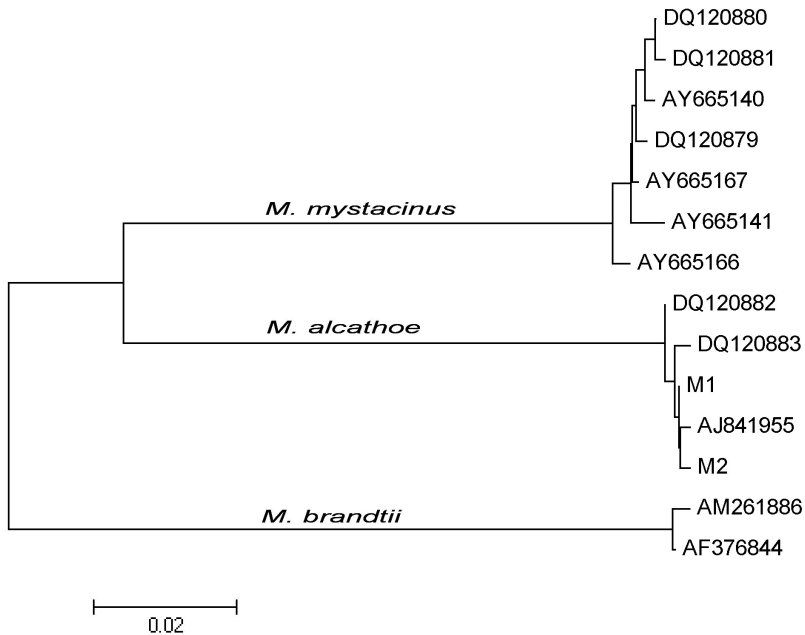
**Fig. 1.** Right mandible of *M. alcaethoe*, a male No. M1 – buccal view (top), lingual view (bottom); see text for details.

170 m). Measurements taken on the fresh carcass are as follows: forearm 31.2, thumb 4.5, tibia 14.0 mm. The small size, ear shape and ear colour of the specimen correspond well to the species' characters given by Dietz et al. (2007). The carcass was then transferred to a dermestarium to obtain remains of its skeleton. Following measurements of postcranial bones could be taken: (metacarpals) mt<sub>2</sub> 25.1, mt<sub>3</sub> 26.9, mt<sub>4</sub> 26.6, mt<sub>5</sub> 26.0, scapula 8.8 × 4.7, femur 12.2, tibia 13.0, tarsalia + phalanges (combined) 6.0, claws 1.7 mm. The skull is missing but the right mandible is well preserved measuring 9.3 × 3.0, with C-M<sub>3</sub> 5.3 mm. The attached document (Fig. 1) seems to be the first photo of a *M. alcaethoe* mandible published.

Further two male *M. alcaethoe*, M2 and M3, were identified among road traffic casualties accidentally found in northern Moravia. M2 was recorded on 25 September 1999 near the Volenský fishpond at Šenov (49°46'59"N, 18°22'06"E, ele 230 m) and M3 on 5 September 1999 at Hodoňovice in the Ostravice river valley (49°37'49"N, 18°21'34"E, ele 330 m), district Frýdek-Místek (both cases). Only two measurements were taken on each of the carcasses: forearm M2 32.5, M3 33.7; tibia M2 14.5, M3 14.8 mm. Ear shape, fur colour and its distribution on head as well as the shape of penis of M2 corresponded to the description of *M. alcaethoe* while external characters (as far as preserved) of M3 were rather questionable and the species was identified only genetically.

### Results of mitochondrial DNA sequencing

After storing each carcass in a refrigerator, a wing punch was taken from it and preserved in 96% alcohol for molecular identification. DNA was extracted from the membrane by quick HotShot method (Trett et al. 2000) and partial *cyt b* fragments were amplified by using the primers Molcit-F (Ibáñez et al. 2006) and MVZ-16 (Smith & Patton 1993) using the protocol described in Ibáñez et al. (2006). Sequencing reactions were performed with BigDye Terminators v. 3.1 (Applied Biosystems) and analysed on ABI3130



**Fig. 2.** Unrooted Neighbour Joining phylogenetic tree of analysed sequences. Two newly described haplotypes from the Czech Republic (M1 and M2) apparently belong to *M. alcahloe*.

Genetic Analyser (Applied Biosystems). Sequences of two different haplotypes of *Myotis brandtii* (GenBank accession numbers AM261886, AF376844), seven haplotypes of *Myotis mystacinus* (AY665140, AY665141, AY665166, AY665167, DQ120879, DQ120880, DQ120881), and three haplotypes of *Myotis alcahloe* (DQ120882, DQ120883, AJ841955) were used for genetic identification of carcasses. Three new sequences obtained during this study were aligned with the sequences from GenBank by using Clustal W (Thompson et al. 1994). Alignment was checked by eye and unrooted phylogenetic tree was constructed by the Neighbour Joining method implemented in Molecular Evolutionary Genetic Analysis (MEGA) version 4.0 (Tamura et al. 2007).

Sequencing of three samples of putative *M. alcahloe* revealed two different partial *cyt b* sequences (737 bp). We call them here M1 (from the individual M1) and M2 (identical in individuals M2 and M3) and they are deposited in GenBank under accession numbers EU795690 and EU795691, respectively. Both haplotypes are very similar and differ only in 1–2 substitutions with respect to those described from Northern Iberia (DQ120882; Ibáñez et al. 2006) and Switzerland (AJ841955; Stadelmann et al. 2004). All specimens examined obviously belong to the *M. alcahloe* haplogroup as evidenced from the Neighbour Joining phylogenetic analysis (Fig. 2).

## Discussion

At Pasohlávky, *M. alcahloe* was a part of a lowland bat community inhabiting patches of old broadleaved forest with ponds and artificial lakes. In addition to carcass collecting, this community was also studied by ultrasound detecting and *M. alcahloe* was likely among the detected species. However, its high frequency echolocation signals are very similar to that of

*M. emarginatus* which is common in the territory. Therefore the two could be identified as a couple of species only, *M. emarginatus/alcaethoe* (G a i s l e r et al., in press). The second carcass was found at Šenov situated in an industrial lowland landscape of the Ostrava basin near the Ostrava agglomeration. Fishponds having originated as a result of former or present coal mining and broadleaved groves are common there. The third specimen was found at Hodoňovice in an upland along the Ostravice river close to the Beskydy Mts piedmont. It is an agricultural area with broadleaved woods and human settlements. In general our findings correspond to that from neighbouring countries Germany, Poland, Slovakia and Hungary that *M. alcaethoe* is a species preferring moist alluvial or mesic broadleaved forests (H e l v e r s e n et al. 2001, B e n d a et al. 2003, N i e r m a n n et al. 2007). The present distribution of the species in the Czech Republic shows a patchiness resulting from its restriction to well preserved oak-hornbeam forests in lower to mid-elevations (L u č a n et al., in prep.). Lučan and co-authors netted the species in nine localities which represent three mutually distant territories. None of our records was made in or close to any of these territories. Thus the new records broaden the knowledge of *M. alcaethoe* distribution in the country and in central Europe.

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