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Alcathoe bat (*Myotis alcathoe*) in the Czech Republic: distributional status, roosting and feeding ecology

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Between 2001 and 2008, we recorded *Myotis alcathoe* at nine sites within three distant areas in the Czech Republic. The species identification was confirmed with cyt *b* sequences and four distinct haplotypes were identified. All the localities exhibit surprisingly uniform habitat characteristics: (1) old full-grown oak-hornbeam forests, with (2) numerous large trees in advanced stages of decay are present, and (3) a very small to large water bodies and/or patches of riparian vegetation surrounded by the forest. Using radiotracking techniques, we discovered 27 day roosts of *M. alcathoe*, located mostly in big oak, birch and lime trees inside extensive forest stands. All roosts were fissures or small cavities in a tree trunk and in branches in the canopies, some 16 m above the ground. Bats preferred trees that were higher, had higher canopy and canopy basement and had larger diameter at breast height than other available trees. Roost trees were surrounded by lower trees with lower canopy basements than available trees. Roost trees were in a poorer condition than other available trees. Roosts were occupied by up to 83 individuals in July but usually single individuals were found in the roosts in September. In contrast to syntopic *M. mystacinus* and *M. brandtii*, *M. alcathoe* has never been found in an anthropogenic roost (except for a fissure in concrete electricity pole). Preliminary analysis of the diet showed that nematoceran flies were the most important prey item along with spiders, caddis flies, small moths and neuropterans. In the observed ecological characteristics, *M. alcathoe* markedly differs from other European species of the genus *Myotis*. Its restricted habitat requirements are perhaps responsible for an islet-like pattern of its distribution and suggest an essential conservation value of the habitats of its occurrence.

Key words: radio tracking, diet analysis, tree roosts, forest bats, *Myotis alcathoe*

Introduction

The influx of the techniques of molecular taxonomy into domain of bat research by the end of the 20th Century revealed unexpected amount of cryptic variation. In Europe, at least seven new species not recognized before were distinguished (Ibáñez *et al*., 2006; Dietz *et al*., 2007; Mayer *et al*., 2007). The questions which are the biogeographical, ecological and behavioural specificities of particular species and which are the background factors responsible for sympatry of sibling species present a new challenge to current study of European bats. The results obtained subsequently for the common pipistrelle group, i.e. *Pipistrellus pipistrellus* (Schreber, 1774) and *Pipistrellus pygmaeus* (Leach, 1825), demonstrate significance of such studies and even enable to test various models of speciation dynamics (e.g., Barratt *et al*., 1997; Barlow and Jones, 1999; Hulva *et al*., 2004; Davidson-Watts *et al*., 2006). Unfortunately, the detailed information available on the common pipistrelle group is rather exceptional. In most instances the current information on new cryptic species is limited to data on their molecular differences and enumeration of to-date available records, which is often quite scarce.

The Alcathoe bat (*Myotis alcathoe* von Helversen and Heller, 2001) is an extreme example of such a situation. The species formerly included in *M. mystacinus* was described by von Helversen *et al*. (2001), based mostly on differences in mitochondrial DNA sequences (ND1 and 12S rRNA), karyology and echolocation calls. Despite close morphological similarity to the whiskered bat, *M. mystacinus* (Kuhl, 1817), and Brandt's bat, *M. brandtii* (Eversmann, 1845), the Alcathoe bat does not represent a sister clade to any of these two species. Rather, it is more closely related to Geoffroy's bat, *M. emarginatus* among the European congeners (Mayer and von Helversen, 2001).

The emerging picture of distribution currently summarized by Niermann *et al*. (2007) suggests that the geographical range of *M. alcathoe* covers a large part of Europe, from northern Spain and France to central Germany, southern Poland, and Greece, though it apparently represents a rare bat in all the regions from which it was reported. The listed records suggest that the species may be restricted to mountain regions in the south and throughout its range it prefers natural, moist and deciduous forests with old trees and water streams. Unfortunately, still almost no further data on biology of this enigmatic bat species are available. So far, only one day-roost has been discovered, a fissure in a plane tree trunk in Greece inhabited by three females and two juveniles (von Helversen *et al*., 2001).

No information is available on the diet of *M. alcathoe*. Although trophic ecology of its sibling congeners *M. brandtii* and *M. mystacinus* has not been studied into particular details, the essential characteristics of their trophic niche are robustly supported (Taake, 1992; Beck, 1995; Rindle and Zahn, 1997; Andreas, 2002; Pithartová, 2007). *Myotis mystacinus* and *M. brandtii* feed especially on small nematoceran Diptera (Nematocera), spiders (Araneae) and small moths (Lepidoptera) or alternatively Trichoptera (*M. mystacinus* — Pithartová, 2007). All the above mentioned members of the phenotypic group show a wing morphology of slow hawkers (Norberg and Rayner, 1987). A high proportion of spiders and even other non-flying prey (Beck, 1995) in the diet suggests possible foliage gleaning though other studies contradict it (Siemers and Schnitzler, 2004). Whether the Alcathoe bat fits that pattern is not known.

The records of *M. alcathoe* in the Czech Republic, first reported in the present paper, considerably enlarge the information on roosting habits, habitat requirements and feeding ecology of this species and enable even some preliminary comparisons with two cryptic species living in sympatry, *M. mystacinus* and *M. brandtii*.

Materials and Methods

An extensive search for *M. alcathoe* in the Czech Republic begun in 2001 when the species was described. The list of sites where the Alcathoe bat was recorded since then (including number of captured *M. alcathoe* individuals and number of all bats) is shown in Table 1. Numerous sites where we searched for the species are omitted in this paper but all are available elsewhere (Hanák and Anděra, 2006).

Capture of Bats

In most instances, the *M. alcathoe* was captured using mist nets in the vicinity of water bodies in forests. Usually, 2–6 nets (6–18 m of total length) were opened from dusk until 1.00 am (Central European Summer Time). Out of a large number of underground swarming sites that were investigated with the aid of mist-netting, in only two sites was the species actually recorded — one of them is the mass swarming site Ledové sluje near Vranov nad Dyjí in the Podyjí National Park, continously studied since 1991 (Reiter *et al*., 1997). All captured bats were measured, sexed and their reproductive status was recorded.

Identification of the Species

The preliminary field identification was based on the characters summarized by Dietz and von Helversen (2004) and Dietz *et al*. (2007). At the same time, nearly all individuals of the phenotypic group were sampled for DNA using biopsy wing punches, which were stored in alcohol for molecular identification. Total genomic DNA was extracted using DNeasy blood and tissue kit (Qiagen). Partial sequence (402 bp) of the mitochondrial gene for cytochrome *b* was amplified using a protocol published by Hulva *et al*. (2004). Sequences were aligned by eye and the dataset was collapsed into haplotypes. The species identification was verified by comparison with *M. alcathoe* cytochrome *b* sequences published in GenBank (Stadelmann *et al*., 2004; Ibáñez *et al*., 2006). In all cases (samples from sites 2, 4, 6, 7, and 8) the molecular identification completely confirmed the field identification by morphological characters.

Radio Tracking and Location of Roosts

In July and August 2007, we radio-tagged two individuals (adult female and juvenile male) of *M. alcathoe* in the Křivoklátsko Biosphere Reserve (Central Bohemia, Czech Republic, site 4 — see Table 1). Further, in 2008, we radio-tagged 14 individuals at site 8 (Kostelecký Forest): nine in the second

TABLE 1. Characteristics of sites of occurrence of *M. alcathoe* in the Czech Republic. Dates of nettings for each particular site are also given. Only data from nights when Alcathoe bats were captured are provided. Swarming sites are marked in bold. The numbers following dates refer to number of Alcathoe bats, proportion (%) of Alcathoe bats to all captured bats and number (n) of all bats captured

Site No.	Locality, coordinates, altitude	Habitat	Dates and samples
1	Havraníky – Devět mlýnů 48°48.5'N, 15°59'E; 240 m a.s.l.	Artificial channel at a former water mill, alluvial forest in canyon of the Dyje river, thermophilous forests present at slopes of the canyon	29.6.2001: 1 (7.1%), n = 14
2	Klíčava Dam 50°04'N, 13°54'E; 340 m a.s.l.	Old parkland surrounded by oak-hornbeam forests, dike of a big water reservoir	21.7.2005; 16.8.2006: 11 (15.9%), n = 69
3	Bílavsko, Ochozy Forest 49°23'N, 17°37'E; 285 m a.s.l.	Oak-hornbeam forest, three small ponds at the forest edge	27.7.2006: 1 (5.9%), n = 17
4	Vůznice Natural Reserve 50°01'N, 13°59'E; 320 m a.s.l.	Old oak-hornbeam forest, bank of water reservoir	15.8.2006; 31.7.2007; 2.8.2007; 1.10.2007; 3.9.2008: 19 (17.9%), n = 106
5	**Zbečno 50°03'N, 13°55'E; 300 m a.s.l.**	**Oak-hornbeam forest, two small galleries (< 30 m long)**	**17.8.2006: 1 (4.8%), n = 21**
6	Tvořihráz, Purkrábka 48°54'N, 16°07'E; 250 m a.s.l.	Oak-hornbeam to thermophilous oak forest, small stream inside forest	2.8.2006: 3 (30.0%), n = 10
7	**Čížov, Ledové sluje 48°51'N, 15°52'E; 290 m a.s.l.**	**Oak-hornbeam to thermophilous oak forest, bolder sea, pseudokarstic caves, slope of the canyon of Dyje river**	**1–2.9.2006; 17–18.4.2007; 17–18.7.2007; 1–2.9.2008: 16 (1.1%), n = 1502**
8	Líšná, Kostelecký Forest 49°24'N, 17°32'E; 262 m a.s.l.	Oak-hornbeam forest, small pond in the forest	6.7.2007; 13.–21.7.2008; 31.8.–9.9.2008: 29 (15.8%), n = 184
9	Šumná, Vlkov pond 48°56'N, 16°54'E; 390 m a.s.l.	Remnants of oak-hornbeam forest changed to extensive conifer plantation, riparian vegetation, pond dam	1.8.2007: 2 (22.2%), n = 9

half of July and five in the first half of September 2008. We used LB-2N transmitters (Holohil System, Carp, Ontario, Canada), each of 0.37 g, i.e. 8.4 ± 0.9% ($\bar{x}$ ± SD) of body mass (4.5 ± 0.5 g, n = 16) of radiotracked individuals. Although the mass of transmitters exceeded the 5% rule recommended by some authors (e.g., Aldridge and Brigham, 1988), we did not observe any signs of detrimental effect of radio transmitter mass on bat behaviour. Moreover, we recaptured an adult radio tracked female at site 4 (see Table 1) three months after the transmitter fell off. This individual was obviously in good condition and its body mass was equal to three other captured Alcathoe bats. Upon initial capture, the bats were measured, their sex, body mass and reproductive stage were recorded and then we attached transmitters to the interscapular part of their back using cyanoacrylate glue (Encarnação *et al*., 2004). From the day following the night of capture and radiotagging, the bats were tracked for 91 bat-days in total (6.1 ± 2.3 days) to their day-roosts using AR 8000 (AOR Ltd., Japan) or LA 12-Q (AVM Instruments, Colfax, California, USA) receivers and three-element Yagi antenae. A detailed summary of radiotracking data will be published elsewhere.

Description of Roost Trees and Plots

All detailed data on roosts were gathered at Kostelecký forest (site 8, see Table 1), where most of the radiotracking study was conducted. We recorded seven features of each roost tree: tree species, tree height, height of the roost, height of canopy basement, height of canopy, diameter at breast height (DBH), and tree condition. In many cases, we were not able to locate the precise roost position on a tree. Therefore, we report data on the heights of roosts only for those roosts of which precise position was located by observation of the emerging bats. We used binoculars (10 × 50) to inspect the type of roosting cavity in cases where we located the precise position of a roost in tree. We defined the height of canopy basement as the height of the first branch longer > 2 m that had another branches above it. Tree height and the height of canopy basement were measured using clinometer. Canopy height was calculated by subtracting the height of canopy basement from the tree height. DBH was measured using a tree caliper. We classified tree condition into five classes based on the percentage of dead branches on tree trunk as follows: class 1 — up to 25% of dead branches; class 2 — 25.1–50%; class 3 — 50.1–75%; class 4 — more than 75%; class 5 — dead tree. We recorded the same features (except for tree condition) for microplot in each roost-tree. The microplot was defined as five nearest trees with DBH ≥ 10 cm surrounding the roost tree. We recorded tree species composition, density and DBH within a radius of 17.8 m (0.1 ha plot) of each roost tree.

Available Trees and Plots

To assess roosting preferences of Alcathoe bats, we also gathered data on available (random) trees and plots. We defined an available tree as a tree with DBH ≥ 25 cm (cf. Ruczyński and Bogdanowicz, 2008) located 100 paces in a random direction from roost tree. Random directions were selected by a repeated lot for 36 numbers, each referring to one tenth of 360° compass azimuth. We recorded one available tree and plot for each roost tree in Kostelecký forest. We selected available trees and plots for 22 of 23 roost trees. For each available tree and surrounding 0.1 ha plot, we recorded the same parameters as in the roost trees.

Survey of Synanthropic Roosts

During summer months (July, August) of 2005, 2006 and 2007, we checked more than 150 potential roosts of forest-dwelling bats in man-made structures (forest houses, cottages. huts, haylofts, etc.) in the Křivoklátsko Biosphere Reserve (ca. 400 km^2, incl. sites 2, 4, 5), where all three species from the *M. mystacinus* morphogroup (sensu Benda and Karataş, 2005; i.e. *M. mystacinus*, *M. brandtii* and *M. alcathoe*) live in sympatry and often even in syntopy. Special attention has been paid on window shutters of cottages located in forests or along forest edges, i.e. the roosts frequently used by several bat species in the Czech Republic, including *M. mystacinus* and *M. brandtii* (Hanák and Anděra, 2006; authors' unpublished data).

Analysis of Faecal Pellets

Bats were kept individually in cloth sacks and their faecal pellets were collected for later analysis under binocular microscope. Particular prey categories were identified using comparative slides, methodological works (McAney *et al.*, 1991) and entomological keys. In total, we collected data on prey composition from three *M. alcathoe* individuals in Vůznice (site 4 — see Table 1; 33 pellets), 13 individuals in Kostelecký forest (site 8; 66 pellets) and two individuals in Ledové sluje (site 7; 10 pellets). All samples were collected in the first decade of September 2008.

Data Analysis

Characteristsics of roost and available trees, microplots and plots were compared using paired *t*-test. All tested variables had normal distribution. Differences in tree species occurrence and tree condition were tested using Chi-square test. We tested a proportion of *M. brandtii*, *M. mystacinus* (pooled together prior analysis) and *M. alcathoe* to all captured bats at foraging versus swarming sites using Chi-square test with Yates' correction. All tests were performed using Statistica 8.0 (Statsoft Inc.). To express results of diet analyses we modified formulas of McAney *et al.* (1991). Percentage volume (% vol) is expressed as the sum of all relative volumes of particular prey category in individual pellets in the studied set of faeces samples divided by the number of faecal pellets in the analysed set of samples. Percentage volume shows what proportion of volume represents particular prey in the set of faeces samples analysed. All values are given as mean ± SD.

RESULTS

Since 2001 when intensive search for *M. alcathoe* in the Czech Republic begun it was recorded at nine sites within three regions (Křivoklátsko Biosphere Reserve — sites 2, 4, 5; Podyjí National Parkand and surroundings — sites 1, 6, 7, 9; and Central Moravia, sites 3, 8 — see Table 1 and Fig. 1). In total, we captured 83 individuals (21 adult ♂♂, 21 juvenile ♂♂, 22 adult ♀♀, 17 juvenile ♀♀, and two individuals of unknown sex) of

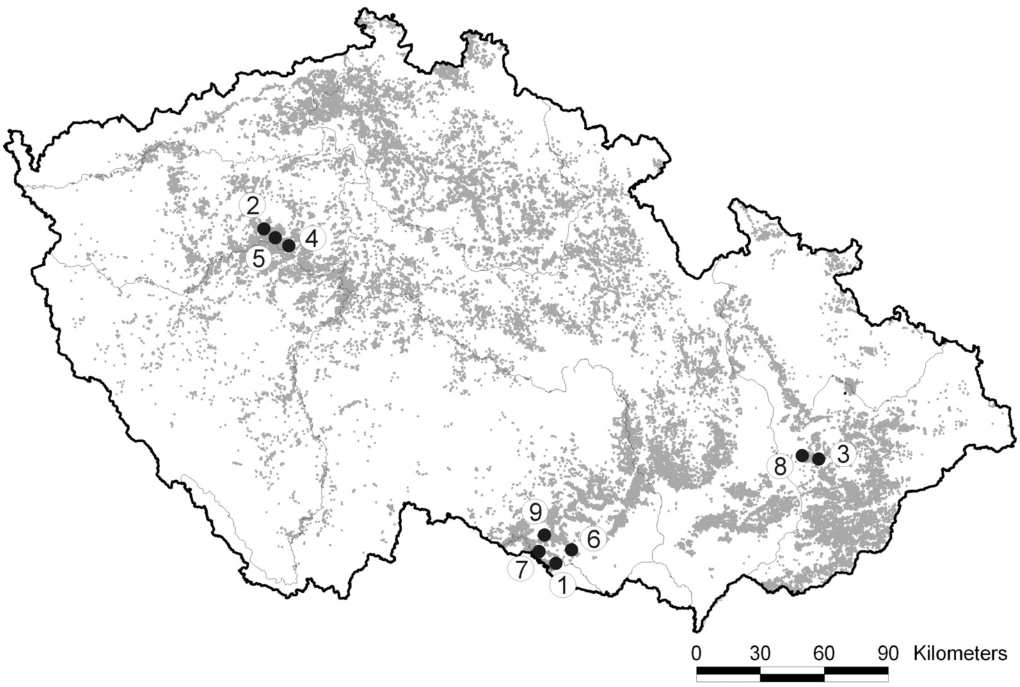

FIG. 1. Distribution of Alcathoe bat in the Czech Republic: sites of confirmed occurrence (numbers refer to the site numbers in Table 1) and current distribution of oak-hornbeam forests in the territory of the Czech Republic (after Chytrý *et al.*, 2001), i.e. the areas of potential occurrence of *M. alcathoe* (grey)

M. alcathoe in the Czech Republic (Table 1). Regarding the relatively large number of individuals of the *M. mystacinus* morphogroup that were handled throughout the study period in the Czech Republic the records of Alcathoe bat are apparently rather rare and limited in geographical respect.

Until now DNA samples of 16 individuals of *M. alcathoe* (sites 2, 4, 6, 7, and 8) have been examined and four cytochrome *b* haplotypes suggesting a shallow genetic substructure were identified. One lineage compounds from haplotype 1, which is widespread (eight individuals at sites 4, 6, and 7) and retains basal position, and haplotypes 2 (two individuals at site 7) and 3 (two individuals at site 8) which differs from each other by less than 1% (1–2 mutational steps). Haplotype 4 (four individuals at sites 2 and 6) represent separate lineage with p-distance of 2% (8–10 mutational steps) from the cluster mentioned above [The accession numbers of the respective sequences are: EU541661 (haplotype 1), EU541662 (haplotype 2), EU541663 (haplotype 3), and EU541664 (haplotype 4)].

Habitats

All localities where *M. alcathoe* was recorded possessed a surprisingly uniform habitat characteristics which could be summarized as follows: (1) they are located in old full-grown oak-hornbeam forests (*Melampyro nemorosi-Carpinetum* and *Carici pilosae-Carpinetum* associations) with transitions to thermophilous types of the oak forests (*Quercion petraeae* association) in some cases, in which (2) numerous large trees (> 60 cm in DBH) in advanced stages of decay are present, and (3) a very small to large water bodies and/or patches of riparian vegetation are available within the forest. In one case, much of the former oak-hornbeam forest stand had been changed to coniferous monoculture, yet still some patches of natural habitats are present. We recorded up to 16 more bat species at a single site and representation of Alcathoe bats varying from 5.9 to 30.0% (Table 1). Typically, the sites of capture of *M. alcathoe* were located in the vicinity of water bodies or small streams inside forests. Beside captures of the Alcathoe bats at summer foraging sites, we also recorded this species at two swarming sites close to entrances to the underground spaces in April, July and September where its proportion was 1.1–4.8% (of 1,502 and 21 bats, respectively). Significant differences ($\chi^2 = 4.5$, $d.f. = 1$, $P < 0.05$) between Alcathoe bats and other members of the morphogroup (i.e. Brandt's and whiskered bats) were that the proportion of the latter to all captured bats was nearly the same both at foraging (11.0%) and swarming (8.4%) sites.

Roosts and Roosting Preference

We found three day roosts at site 4 in 2007 and 24 day roosts at site 8 in 2008. All but one roost were in trees. Twelve roosts (44.4%) were in oaks (*Quercus robur*), five (18.5%) in limes (*Tilia cordata*), five (18.5%) in birches (*Betula pendula*), one of each in alder (*Alnus glutinosa*), maple (*Acer pseudoplatanus*), aspen (*Populus tremula*) and poplar (*Populus × canadensis*). One roost was discovered in a hollow concrete pole of power line at the edge of the forest. This roost had the entrance height of 7 m and was occupied by a colony of 23 Alcathoe bats in the second half of July 2008. All tree roosts that we were able to inspect using binocular ($n = 10$) were long and narrow fissures in a tree trunk or a large side branch. They were located 16.5 ± 1.5 m above the ground ($n = 10$). In nine of 10 cases, the roosts were located above the canopy basement of roosting trees. We counted bats during evening emergence at 14 roost trees. We observed 1–83 (16.5 ± 23.2, median = 8, $n = 10$) and 1–2 (1.3 ± 0.4, median = 1, $n = 4$) bats leaving these roost trees in the second half of July and first half of September, respectively.

The incidence of tree species on used versus available trees was statistically different ($\chi^2 = 71.3$, $d.f. = 4$, $P < 0.001$). Oaks were more abundant in roost trees compared to available trees whereas birches and limes where rather underrepresented (Fig. 2). In general, roost trees had larger DBH ($t = 4.3$, $d.f. = 21$, $P < 0.001$), were higher ($t = 2.6$, $d.f. = 21$, $P < 0.05$), and were surrounded by lower trees ($t = -4.5$, $d.f. = 21$, $P < 0.001$) than available trees. Trees in microplots had lower canopy basement ($t = -2.7$, $d.f. = 21$, $P < 0.01$) and had lower canopies ($t = -2.6$, $d.f. = 21$, $P < 0.05$) in roost trees than in available trees (Table 2). Roost trees had significantly different condition than available trees ($\chi^2 = 47.2$, $d.f. = 4$, $P < 0.001$). Whereas most available trees were assigned to class 1, most roost trees were in classes 2 or 3 (Fig. 3). However, only one roost was located in a completely dead tree.

We recorded 17 tree species in plots surrounding roost trees ($n = 1,265$ trees) and 12 tree species in plots surrounding available trees ($n = 1,106$ trees). The most abundant were lime and hornbeam (both ≈35% of proportion) followed by birch (≈16%) and oak (5%). All other species represented < 10% of all stems. Tree density was slightly higher in 0.1 ha

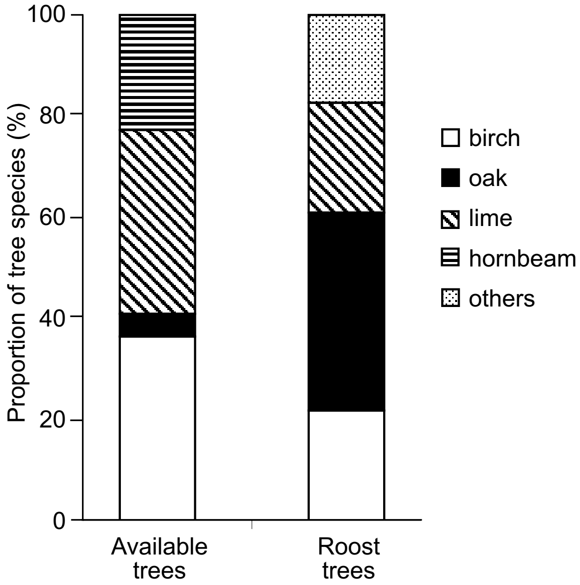

FIG. 2. Proportion of tree species used as roosts by *M. alcathoe* (n = 22) relative to available trees (n = 22)

plots with roosts than in available plots, however, the values were slightly below the significance level (t = 1.7, $d.f.$ = 21, P = 0.09 — see Table 2). We did not find any other differences between roost and available trees.

Survey of Synanthropic Roosts

During survey of more than 150 potential roosts in man-made structures (mostly houses and cottages) in summer of 2005, 2006, and 2007 in the Křivoklátsko Biosphere Reserve, we found 14 and three roosts of *M. mystacinus* and *M. brandtii*, respectively (mostly behind opened shutters). Individuals or small groups of four more bat species were also recorded: *Eptesicus serotinus*, *Pipistrellus pipistrellus* sensu stricto, *P. nathusii*, and *Plecotus austriacus*. However, no *M. alcathoe* was discovered during this survey. All roosts of *M. mystacinus* and *M. brandtii* were located behind open window shutters of houses and cottages, 1.5–6 m ($\bar{x}$ = 3 m, n = 17) from the ground level, and were occupied either by single bats or small nursery groups of up to five bats (in total 19 *M. mystacinus* and 10 *M. brandtii*). Usually, a group of bats was scattered in more than one such roost within one building.

Diet Analysis

In total 15 taxonomic categories were identified in diet, those with volume percentage larger than 5% are surveyed in Fig. 4. Nematocera (mostly Chironomidae and Tipulidae in a lesser extent) was the most important prey item along with Araneae, Trichoptera, small Lepidoptera, and Neuroptera.

DISCUSSION

Based on records of *M. alcathoe* in three different areas within the Czech Republic, including reproductive females and newly fledged juveniles, we provide the first evidence of reproducing populations of the species in this country. These findings are not too surprising given the reports of *M. alcathoe* in neighbouring countries, e.g., Germany, Poland, Slovakia, Austria and Hungary (von Helversen *et al.*, 2001; Benda *et al.*, 2003; Niermann *et al.*, 2007; Ohlendorf and Funkel, 2008; Spitzenberger *et al.*, 2008). Nevertheless, similarly as in other

TABLE 2. Characterics of roost trees and available trees (n = 22, except for 'nearest tree' variable, where n = 21). Probability level: ns — not significant, * P < 0.05), ** P < 0.01, *** P < 0.001

Parameter	Roost trees		Other available trees		t-test
	x ± SD	Range	x ± SD	Range	
		Plot (0.1 ha)			
DBH (cm)	54.5 ± 16.9	32–90	34.9 ± 10.0	25–60	4.3***
Height of tree (m)	29.7 ± 4.0	23–38	26.2 ± 4.0	20–38	2.6*
Height of canopy basement (m)	13.1 ± 3.1	8–21	12.3 ± 3.6	3–20	ns
Height of canopy (m)	16.5 ± 5.0	7–26	13.9 ± 4.1	7–22	ns
Distance to the nearest tree (m)	2.5 ± 1.4	0.5–5.5	2.6 ± 1.6	0.5–5	ns
Number of trees in 0.1 ha plot	58.2 ± 19.5	27–98	50.7 ± 12.7	29–89	ns
Mean DBH in 0.1 ha plot (cm)	26.2 ± 4.3	19.9–36.1	28.1 ± 4.1	22.2–40.5	ns
Number of tree species in 0.1 ha plot	4.7 ± 1.2	3–8	4.5 ± 1.1	3–7	ns
		Microplot (five surrounding trees)			
DBH (cm)	24.6 ± 7.2	16.4–43.2	27.5 ± 6.7	16.6–40.2	ns
Height of trees (m)	19.5 ± 4.1	11.5–27.3	22.9 ± 2.8	18.5–28.4	-4.6***
Height of canopy basement (m)	9.5 ± 2.1	5.9–14.9	11.1 ± 2.4	7.9–16.0	-2.7*
Height of canopy (m)	10.0 ± 3.0	5.6–17.6	11.8 ± 2.1	8.6–17.0	-2.6*

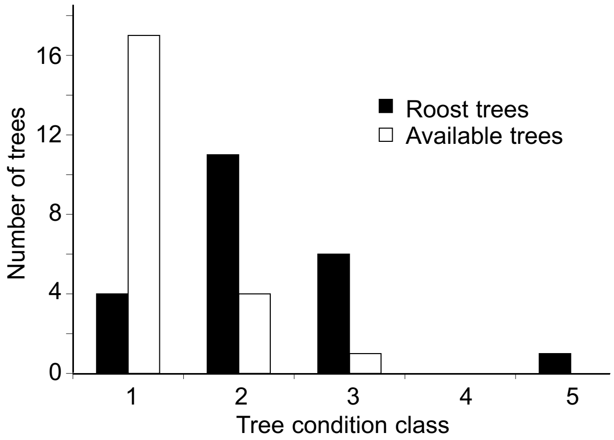

FIG. 3. Differences in tree condition in roost trees (n = 22) and available trees (n = 22)

countries (cf. Niermann *et al.*, 2007; Spitzenberger *et al.*, 2008) also in the Czech Republic *M. alcathoe* apparently ranks among rare species with islet-like distribution.

The population of *M. alcathoe* in the Czech Republic is relatively homogenous in the studied mitochondrial marker. Nevertheless, the shallow genetic substructuring demonstrated by the present study is worth of a comment. The pattern within the group of haplotypes 1, 2, and 3 suggests a star phylogeny accompanying recent expansions from relatively small founder population. Their sympatry with a more distinct haplotype 4 suggests a more intricate story: either colonization by two allopatrically evolved lineages or a high level of gene flow connected with relatively small effective size of population. This pattern could be a signature of very fast increase of population numbers, as usually accompanies recent expansion of the range. In any case, these preliminary results show that phylogeography of *M. alcathoe* presents quite attractive but still largely unknown topic.

In contrast to other regions of Central Europe where records of *M. alcathoe* are mostly limited to single or few individuals at a particular locality, we discovered at least two areas (Křivoklátsko Biosphere Reserve, Central Bohemia and Kostelecký forest, Central Moravia), where the Alcathoe bats reach quite high abundance. All the records of *M. alcathoe* were restricted to one and the same habitat: old, well preserved oak-hornbeam forests in lower to mid-elevations. This fact [(which is in good accord to the habitat characteristics suggested for Austrian and Hungarian records by Spitzenberger *et al.* (2008)] is particularly surprising because that habitat shows quite a fragmentary distribution in the Czech Republic (Chytrý *et al.*, 2001) and even locally its patches are much rarer than alluvial or mesic broadleaved forests suggested as preferred habitat of the species by Niermann *et al.* (2007). In that respect the local pattern of distribution contrasts to that observed in two other Central European sibling congeners, *M. mystacinus* and *M. brandtii*, which apparently inhabit a wide spectrum of woodland types including forest edges and semi-opened forest stands (Tupinier, 2001; Tupinier and Aellen, 2001; Hanák and Anděra, 2006). Consequently, our results sugest that *M. alcathoe* is the most specialized species of the morphogroup concerning its habitat requirements, and that the scarce islet-like pattern of its records, obviously contrasting to an extensive distribution range, may actually to be a result of its dependency upon the habitat. This distribution shows such an islet-like pattern throughout most of Europe.

The day roosts we recorded were located high in the canopies of large trees that were in poorer condition than available trees. These conditions are in good accord with the only day-roost reported for the species until now by von Helversen *et al.* (2001) and von Helversen (2004) (a fissure in a plane tree trunk situated 8 m above the ground in a dense stand of *Platanus* forest in Greece). These records not only support a view of *M. alcathoe* as a dendrophilous species but suggest that, in comparison to other

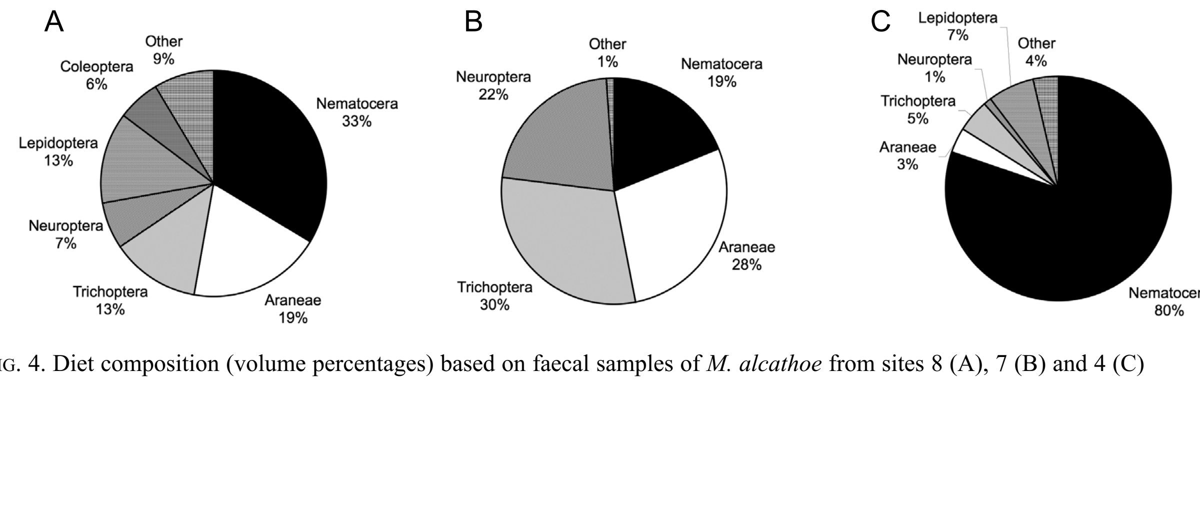

FIG. 4. Diet composition (volume percentages) based on faecal samples of *M. alcathoe* from sites 8 (A), 7 (B) and 4 (C)

European tree-dwelling *Myotis* bats (e.g., *M. daubentonii*, *M. nattereri*, and *M. bechsteinii*) which roosts are located usually in the sub-canopy level (Červený and Bürger, 1989; Boonman, 2000; Kaňuch, 2005), *M. alcathoe* seems to be a strict canopy-dwelling species.

The fact that intensive surveys of possible roosts of bats in man-made structures throughout the Czech Republic provided numerous records of *M. mystacinus* and *M. brandtii* (Hanák and Anděra, 2006) but no *M. alcathoe* (and corresponding results of the systematic survey in two of the three regions where *M. alcathoe* was found abundant) suggests that *M. alcathoe* most probably does not use roosts in buildings. However, the finding of the summer colony in a concrete pole of power line may indicate that at least some man-made structures could be used and that Alcathoe bat can be relatively flexible in its roosting requirements.

We proved that summer colonies may count more than 80 individuals, however they were much smaller in most instances. In contrast to summer, the autumn occupancy of the roosts was limited to single individuals or very small groups only. This reminds the pattern of roost occupancy in other dendrophilous species, including Brandt's and whiskered bats, which form large maternity colonies during a short period in June and first half of July and than disperse onto high number of roosts occupied usually by single or very few bats (Hanák and Anděra, 2006). While *M. mystacinus* and *M. brandtii* have been encountered in underground sites on regular basis during swarming and hibernation, records of *M. alcathoe* in these sites were rather exceptional. No winter record is available from the Czech Republic, and obviously as yet almost nothing is known on hibernacula of that species.

The first data on foraging ecology of Alcathoe bat presented in this paper can be summarized as follows: (1) Nematocera (mostly chironomids) is the essential component of diet; (2) The high proportions of other taxa, particularly Araneae and Trichoptera are worth of mentioning, the former one indicates even foliage gleaning; (3) A high degree of between sample variation in diet composition suggests considerable plasticity of trophic niche and considerable seasonal and/or local differences in diet composition can be expected; (4) It seems clear that *M. alcathoe* is not a foraging specialist (such as *Barbastella barbastellus* or *Myotis myotis*, for example); (5) The radiotracked individuals foraged mostly in high layers of canopy and alternatively in uncluttered spaces above water bodies. Also diet composition indicates that the foraging strategy of this species combines a slow manoeuvrable flight close to foliage complemented perhaps with a gleaning and aerial hawking of swarming nematocerans in uncluttered places.

In conclusion, observed combination of ecological characteristics suggest that *M. alcathoe* markedly differs from other European species of the genus *Myotis*. It seems to be strictly dendrophilous canopy-dwelling species, the patchy distribution of which is most probably related to its strict preference of old, well-preserved and structured broadleaved forests, i.e. the habitat which underwent considerable decline throughout Europe. Consequently, *M. alcathoe* seems to be the species worth of a considerable conservation interest. Its conservation priorities should be focused particularly on management and preservation of species' habitats. Rich bat assemblages counting up to 16 more species were recorded at the same sites as *M. alcathoe*, stressing the essential conservation value of such habitats.

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