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NEW GENETICALLY CONFIRMED RECORD OF *MYOTIS ALCATHOE* (CHIROPTERA, MAMMALIA) FROM UKRAINE

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New Genetically Confirmed Record of *Myotis alcathoe* (Chiroptera, Mammalia) from Ukraine. Vlaschenko, A., Yerofieiva, M., Tovstukha, I., Holovko, O., Kornieikov, O., Ruedi, M., Muzyka, D. — *Myotis alcathoe* is one of the iconic species among cryptic European bats, described only 20 years ago. Despite research efforts, there is still limited knowledge about its distribution and ecology, leading to its Data Deficient status on the IUCN Red List. Ukraine lies on the eastern border of the species' range, with only a few known records. Here, we document a new genetically confirmed record of *M. alcathoe* from the Central-Western part of Ukraine (Rivne Region, in "Dermansko-Ostrozkyi" National Nature Park). Eight individuals were mist-netted in late April 2023 near a mine entrance, alongside a few *Myotis nattereri* and *Myotis bechsteinii*. This

record extends the known range of *M. alcathoe* 30–40 km to the northeast, marking the second genetically confirmed record of this species in Ukraine. The woodlands of the National Park area, characterized by wet old-growth oak and hornbeam forests, seem to be suitable summer habitats for this species. Continued bat surveys during the breeding season are necessary in the area to better understand its habitat preferences and distribution.

Key words: bats, Eastern Europe, species distribution.

Among European mammals, bats are one of the groups that have continued to provide taxonomists with new species in recent decades. Several European bat species are difficult to recognize and identify by external morphology, but the recent advances of molecular methods facilitated the detection of new taxa (e. g., Mayer and von Helversen, 2001; Spitzenberger et al., 2006; Barratt et al., 1997; Jones and Froidevaux, 2020). One of the iconic bat species described in the 21st century is the Alcathoe Bat *Myotis alcathoe* von Helversen and Heller, 2001 that had been so far confused with the widespread and commoner Whiskered Bat *Myotis mystacinus* (von Helversen et al., 2001).

The discovery of a new cryptic species of bats in Europe after more than two centuries of intensive research has sparked enormous interest in all aspects of the biology and ecology of *M. alcathoe*. Its ecology, roosting habits and habitat requirement, as well as its current distribution has been extensively evaluated (e. g., Budinski and López-Baucells, 2023). Despite these research efforts, data on the natural history and population trends of *M. alcathoe* remain insufficient, resulting in its Data Deficient status on the Global IUCN Red List (Hutson and Paunović, 2016). However, on the European level, this species is evaluated as Near Threatened (Russo and Cistrone, 2023).

Identifying *M. alcathoe* in the field is still challenging and often relies on genetic evidence to be ascertained (e. g., Bashta et al., 2011; Kmiecik et al., 2020). Consequently, the current distribution of *M. alcathoe* in Europe appears patchy, either due to a real patchiness of populations or to a lack of reliable data (Hutson and Paunović, 2016; Budinski and López-Baucells, 2023).

The known range of *M. alcathoe* covers most of Europe, patchily presented in the Anatolia peninsula (Turkey), and at the East extends to the western and south-western regions of Ukraine. However, only a few records (locations) have been documented in this country since the species was described (e. g.: Bashta et al., 2011; Bashta, 2014; Bashta et al., 2018; Zagorodniuk, 2018; Godlevska et al., 2022; Godlevska and Savchenko, 2022). Determining the eastern limit of the species' distribution, which lies somewhere in Ukraine, is of particular interest.

In this note, we present new, genetically confirmed records of *M. alcathoe* in central-western Ukraine, which represent some of the most north-eastern records for the species in Ukraine and in the Eastern-Europe (Budinski and López-Baucells, 2023).

Bat mist-netting was conducted near the entrance of an abandoned limestone mine in the "Peklo" tract of "Dermansko-Ostrozkyi" National Nature Park (50°27'05" N, 26°20'48" E) for three consecutive nights in April 2023. The research was authorized by the administration of the "Dermansko-Ostrozkyi" National Nature Park, and was conducted under special permission for the project P781 of the Science and Technology Center in Ukraine. For each captured bat, forearm length measurements were taken using calipers (accuracy 0.1 mm), and body mass was measured using an electronic scale (accuracy 0.1 g). Standard wing punches (3 mm in diameter) were taken from the left wing of bats showing atypical morphology

Fig. 1. Overall and lateral view of one of the individuals of *M. alcathoe* from the "Dermansko-Ostrozkyi" National Nature Park (Rivne Region, Ukraine). Notice the flesh-coloured face and ears, which differ from much darker colouration found in Whiskered bats (*M. mystacinus*)

Table 1. **Bat-ring number, sex, age, body mass and forearm length of *M. alcathoe* captured in "Dermansko-Ostrozkyi" National Nature Park in 26–29 April 2023**

Ring number/ID	Sex/Age	Body mass, g	Forearm length, mm]	GenBank number
BT09201	F ad	4.1	32.5	—
BT09203	M ad	3.8	32.7	PQ615934
BT09204	F ad	4.1	32.5	—
BT09205	M ad	3.9	32.4	PQ615935
BT09206	M ad	4.1	33.1	—
BT09207	F ad	3.5	30.5	PQ615936
BT09208	M ad	4.0	32.4	—
BT09209	M ad	3.5	32.8	—

Note. For the three individuals identified by barcode DNA, the corresponding GenBank number is also mentioned.

and preserved in 96% ethanol for subsequent genetic analysis. All bats were ringed following the protocol described by Vlaschenko et al. (2020) and then released back inside the mine.

Fourteen bats were captured, including *Myotis bechsteinii* (2 F and 3 M), *M. nattereri* (1 M), and eight smaller *Myotis* bats exhibiting traits that differed from typical *M. mystacinus* (Kuhl, 1817); these small *Myotis* were preliminarily identified as *M.* cf. *alcathoe* (Fig. 1 and Table 1). The individuals preliminarily identified as *M.* cf. *alcathoe* had very small overall size, distinct color of fur and ears, and distinctive shape and size of ears and tragus (Fig. 1). These differences correspond to some of the discriminant characters of *M. alcathoe* described in more details elsewhere (e. g. Dietz and Kiefer, 2016; Budinski and López-Baucells, 2023).

Further molecular identification of three of these *M.* cf. *alcathoe* was based on comparisons of the standard barcode DNA (COI, Hebert et al., 2003) with homologous fragments from all other species of European *Myotis*, following protocols and

recommendations of Ruedi et al. (2023). Sequences generated here were also submitted to the BOLD identification (Ratnasingham and Hebert, 2007) and deposited in the GenBank (PQ615934, PQ615935, PQ615936).

A map of the current distribution of *M. alcathoe* in Ukraine was generated based on data and generalization of Zagorodniuk (2018) updated with information from Kusnezh (2014), Kusnezh & Chaika (2013), Pokynchereda and Kusnezh (2014), Chaika & Kusnezh (2018), Godlevska et al. (2022), Godlevska and Savchenko (2022).

Bat surveys on the territory of "Dermansko-Ostrozkyi" National Nature Park (NPP) have been conducted in previous years, as summarized by Godlevska et al. (2016). In particular, bat mist-netting was done during the autumn swarming period (September 2016) near the entrance of the same limestone mine in the "Peklo" track where we caught *M. alcathoe*. Tens of individuals of seven species were mist-netted in 2016: *Myotis myotis* (Borkhausen, 1797), *M. bechsteinii*, *Myotis daubentonii* (Kuhl, 1817), *M. nattereri*, *Myotis mystacinus*, *Plecotus auritus* (Linnaeus, 1758), and *Barbastella barbastellus* (Schreber, 1774). One particular individual (this-year-born female) from the *M. mystacinus* s. l. group was identified as *M. mystacinus* s.str. (Godlevska et al., 2016). This previous capture might suggest that both *M. mystacinus* s. str. and *M. alcathoe* coexist in this place or misidentification of *M. alcathoe*.

Results from the genetic comparisons of three barcode DNA sequences of the small *Myotis* confirmed that they were 99–100% identical to a range of COI sequences of *M. alcathoe* sampled in western Europe or the Balkans. We therefore document here new, genetically confirmed occurrence of *M. alcathoe* in the central-western part of Ukraine (Fig. 2). This record was made during the spring departure from wintering roosts, indicating that the limestone mine in the "Peklo" tract serves as a wintering site for this species (individuals of both sexes were captured). This mine is

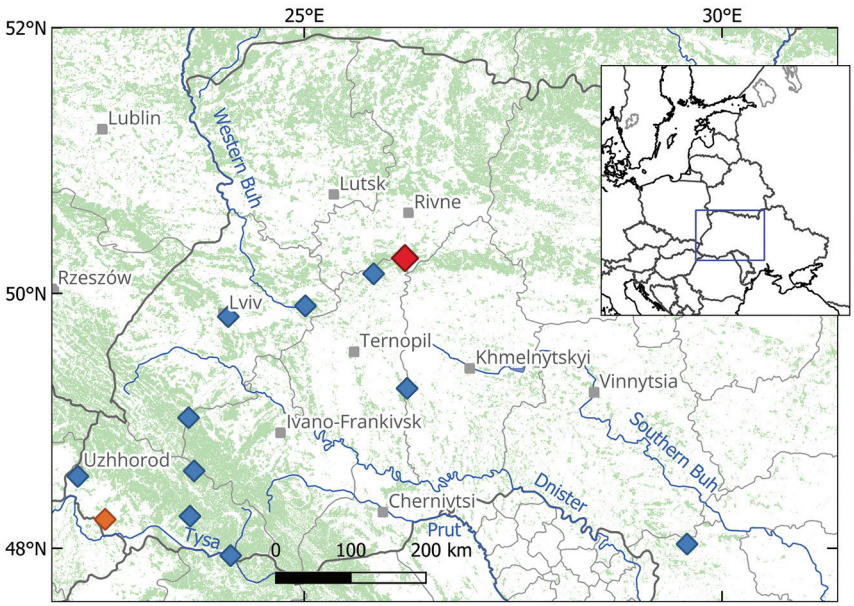

Fig. 2. Map of the western part of Ukraine with known records of *M. alcathoe*: identified without genetic methods (blue diamonds), genetically confirmed previously (orange diamond) and our new record (red diamond) in the country

located in the oak-hornbeam forest belt, which spans 200 km from west to east and up to 20 km from north to south in the Forest-steppe Nature zone within the Temperate-broadleaf Forest biome (Fig. 2).

The current record of *M. alcathoe* is located just 30–40 km from a previous record in "Kremenetski Hory" National Nature Park (Godlevska and Savchenko, 2022), which is essentially the same woodland area situated on limestone hills. The woodlands of the "Dermansko-Ostrozkyi" NPP are characterized by wet oak and hornbeam forests with patches of old-growth forest. Our new record of *M. alcathoe* was made in a relatively typical habitat for this species, as described previously (Budinski and López-Baucells, 2023). Continued bat surveys during the breeding season are necessary to better understand the habitat preferences and distribution of this species in Ukraine.

Ukraine ranks among the top European countries in terms of preserving biological species and habitat diversity. However, the country ranks among the lowest in biodiversity monitoring and inventory programs. Furthermore, more than two years of full-scale war have severely hindered field research and conservation initiatives, setting back progress in many areas. Researchers and conservationists have to keep monitoring programs as far as is it possible in Ukraine in the current circumstances.

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Conflict of interest. The authors declare no conflict of interests.

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