New records of the Alcathoe bat, *Myotis alcathoe* (Vespertilionidae) for Italy

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DOI: http://dx.doi.org/10.14709/BarbJ.7.1.2014.01

Spanish title: Nuevas citas de murciélago ratonero bigotudo pequeño, *Myotis alcathoe* (Vespertilionidae) en Italia

Abstract: The Alcathoe bat (*Myotis alcathoe*) is a recently described cryptic species; in Europe its distribution range is poorly known. In Italy this species has been recorded in a small number of locations in Abruzzo (central Italy) and Campania (southern Italy). Our report refers to three bats captured in a mountainous area dominated by forest habitats in the Appennino Lucano Val d’Agri Lagonegrese National Park (Basilicata region). The identification of bats captured was confirmed by molecular analysis using the technique of DNA barcoding. In this paper we present new recordings that highlight the presence of the species in other regions of southern Italy and that help define its distributional status in Europe.

Key words: *Myotis alcathoe*, cryptic species, DNA barcoding, coxl.

accepted: October 4, 2013
illuminated to distinguish juveniles from adults (Antony 1988).

Bats captured were subjected to a skin biopsy using a sterile punch of a 3 mm diameter from the tail membrane (uropatagium) (Worthington Wilmer and Barratt 1996).

Samples were stored in sterile tubes containing 95% ethanol for subsequent molecular analysis.

Total genomic DNA was extracted from tissue samples using 5 PRIME, ArchivePure DNA Purification Kit. A fragment of ca. 650 bp of the mitochondrial subunit 1 of cytochrome c oxidase, suitable for echolocating bats identification (Galimberti et al. 2012), was amplified for the three sampled bats using the primers VF1d 5’- TTCTCAACCAACCACAARGAYATYGG-3’ and VR1d 5’-TAGACTTCTGGGTGGCCRAARAAYCA-3’ from Ivanova et al. (2007).

PCR reactions were performed in 20μl reactions using ca. 1 ng of genomic DNA, 0.2μl of VF1d (0.2 mM), 0.2μl of VR1d (0.2 mM), 2μl of total dNTPs (0.2 mM), 0.1μl of (0.5U) of MasterTaq Eppendorf®, 2μl 1x Buffer including MgCl2 at 1.5 mM and 14.5μl of water. PCR conditions were: 1 min at 94°C, followed by 5 cycles of 30 s at 94°C, 40 s at 50 °C, and 1 min at 72°C, followed by 35 cycles of 30 s at 94°C, 40 s at 55°C, and 1 min at 72°C, and ending with 10 min at 72°C. The light strands were sequenced using an ABI3730XL by Macrogen Inc. Chromatographs were checked by eye and sequences were edited, when necessary, using the BioEdit sequence alignment editor (version 7.0.5.3; Hall 1999).

To assess species attribution each sequence was compared using the BLAST algorithm in GenBank, where sequences belonging to almost all the Italian echolocating bats species are available thanks to a previous study (Galimberti et al. 2012). All sequences have been deposited in GenBank (HG325822-23-24).

We caught three lactating females (FAL = 33.6, 32.9 and 33.0 mm respectively; weight = 4.7, 4.9 and 4.0) from the “M. mystacinus group”, two in Marsico Nuovo (altitude 1167 m a.s.l) and one in Calvello (altitude 1304 m a.s.l.). All there caught in beech forest sites.

Molecular analysis of these samples allowed the taxonomic identification of the three bats as belonging to the species *M. alcathoe*. The high similarity matches with reference deposited sequences excluded the misidentification with other congenerics.

In central and eastern Europe, *M. alcathoe* seems to prefer deciduous forests with old trees and streams (Niermann et al. 2007; Řehák et al. 2008; Lučan et al. 2009; Bashta et al. 2011). Our data adds further records for the presence of *M. alcathoe* in beech forests of southern Italy (Tereba et al. 2008).

**ACKNOWLEDGEMENTS**

Fieldwork was conducted under licence from the Italian Environment Ministry (prot. PNM-2012-0000644). We are indebted with FEM2 Ambiente srl of the Università of Milano-Bicocca for performing the molecular taxonomic identification. The authors are grateful also to Danilo Russo (University of Naples) for his valuable advice and Antonio Conte for logistic support during fieldwork.

This work was funded by the Appennino Lucano National Park Authority.

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DOI: http://dx.doi.org/10.1371/journal.pone.0040122


