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THE ALPINE LONG-EARED BAT (*PLECOTUS ALPINUS)* KIEFER AND VEITH, 2001) IS PRESENT ALSO IN PIEDMONT REGION: FIRST RECORD REVEALED BY DNA ANALYSIS

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RIASSUNTO - **L'orecchione alpino (*Plecotus alpinus* Kiefer e Veith, 2001) è presente anche in Piemonte: prima segnalazione accertata mediante analisi del DNA.** Viene riportata la prima segnalazione per il Piemonte della specie *Plecotus alpinus* (Kiefer e Veith, 2001) recentemente descritta. Per l'esatta determinazione specifica si è fatto ricorso a tecniche genetiche, in quanto non sono state ancora messe a punto tecniche discriminanti basate su parametri biometrici. La presenza di questa nuova specie anche in Piemonte dovrebbe indurre ad un monitoraggio su larga scala, per definirne in dettaglio la distribuzione e le preferenze di habitat, finalizzate anche alla determinazione dello *status* delle popolazioni presenti.

Parole chiave: Chiroptera, *Plecotus alpinus*, distribuzione, conservazione, Piemonte.

European bat species belonging to the genus *Plecotus* can be considered sibling species, a phenomenon well known among other European bat genera: e.g. *Myotis mystacinus* and *M. brandtii*, *M. myotis* and *M. blythii*. (e.g. Arlettaz, 1996; Arlettaz *et al.,* 1997). In general, sibling species are difficult to discriminate based on traditional body measurements or analysis of echolocation calls, and this also holds for the genus *Plecotus* (Kiefer and Veith, 2001; Spitzenberger *et al*., 2002). Thanks to the recent developments in molecular biology, in particular using mitochondrial DNA sequencing, some new species have been described or proposed in the genus *Plecotus*: *Plecotus kolombatovici*, *P. alpinus* and *P. sardus* (Kiefer and Veith, 2001; Mucedda *et al.,* 2002). In Italy *P. alpinus* is presently recorded only in Trentino Alto Adige (Kiefer and Veith, 2001; Chirichella *et al.*, present issue) and Lombardy region (Trizio *et al.*, in press). Here, we present the first record of the occurrence of alpine long-eared bat (*Plecotus alpinus*) in Piedmont region, NW Italy.

In September 2001, a field survey of bats, promoted by Ente Parchi e Riserve Naturali Lago Maggiore as part of an Italian - Swiss Interreg Project, was carried out, using mist-nets placed at intensively used foraging sites (Agnelli *et al*. in press). Bats were captured while in nocturnal flight at Premeno

(Verbano-Cusio-Ossola province), a village located at 800 m a.s.l. and surrounded by meadows and hardwoods, about 2,5 km away from Maggiore Lake.

Trapped individuals were removed immediately and held in cotton sacks until the nets were closed. Animals were measured and patagium tissue samples were taken, releasing each animal at the point of capture. A calliper (±0.1 mm) was used to measure: (1) forearm length, from wrist joint to elbow joint; (2) thumb (1st finger) length, from nail insertion to posterior thumb articulation; (3) thumb claw length, from nail insertion to nail tip. Each bat was classified as juvenile, sub-adult or adult by observing the closure of epiphyseal growth plates in the metacarpal-phalangeal joint of the fourth finger (Kunz, 1988) against a bright light source; body size and development of genitals were also taken into account. Preliminary species determination in the field was carried out according to the existing literature (Lanza, 1959; Schober and Grimmberger, 1997) and specific identification keys (Roesli and Moretti, 2000).

Mitochondrial DNA for genetic analysis was extracted from patagium tissue samples as described elsewhere (Trizio *et al*, in press). Mt-DNA was amplified and analysed following methods proposed by Kiefer and Veith (2001). The obtained sequences were aligned to previously published sequences of all European *Plecotus* species (GenBank Accession Nos. AY134012-134026, AF529229-529230 – Kiefer and Veith, 2001; Kiefer *et al.*, 2002) using Clustal X software (Thomson *et al.*, 1997). The phylogenetic relationships were inferred both by distance methods, applying the neighbor-joining algorithm (Saitou and Nei, 1987) and by maximum parsimony (MP). Other methods did not produce significant different tree topologies. All calculations were carried using software package (Felsenstein, 1989).

Three individuals of the genus *Plecotus*, two adult females and an aduld male, were captured. According to the discriminant function suggested by Maddalena and Moretti (1994) and based on the three body measurements taken, the two females were determined as *P. auritus* and the male as *P. austriacus*. However, the penis shape of the latter did not allow to identify it as belonging to any of these two species, looking intermediate between the morphological types described (Maddalena e Moretti, 1994; Roesli and Moretti, 2000). In effect, the sequence of the 550 bp mt-DNA that was analysed (Kiefer and Veith, 2001), showed that all three individuals belonged to the recently described species *Plecotus alpinus*. We therefore suggest that the discriminant function proposed by Maddalena and Moretti (1994) needs to be revised, since considered the presence of only two species in the Alps.

The classification on genetic basis of *Plecotus* species is presently essential, in order to undertake studies on distribution, ecological preferences and population status of the various sibling species.

We hope that these aspects will be expanded in a near future, in order to increase the knowledge needed to implement effective conservation action plans.

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