

NATURAL HISTORY NOTE

Confirmation of the presence of *Plecotus macrobullaris* Kuzyakin, 1965 in Liguria (North-West Italy) (Chiroptera: Vespertilionidae)Mara Calvini^{1,*}, Roberto Toffoli¹

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ABSTRACT

We present the second confirmed record of the Alpine Long-eared Bat, *Plecotus macrobullaris* in Liguria, province of Imperia (NW Italy) on the basis of a male *Plecotus* found dead in Sanremo. The specific attribution of the specimen was based on the morphological characters and subsequently confirmed through genetic analysis of the mitochondrial COI DNA barcoding sequence. This record suggests the tendency of the species to occupy areas at lower altitudes in coastal areas close to the mountains, as well as confirms the presence of the species in this part of the region after 45 years. This new report extends the distribution range of *P. macrobullaris* further eastward than it was previously known. In order to know the real distribution of this species and acquire enough data to assess its conservation status, it would be essential to conduct more research targeting this species in future projects.

Plecotus macrobullaris Kuzyakin 1965 was considered as a subspecies of *Plecotus auritus* (Kuzyakin 1965) until species rank was confirmed by Kiefer & Veith (2001) and Spitzenberger et al. (2002). *Plecotus* species are overall very similar to each other, which makes their field identification extremely complex and sometimes impossible. In particular, their biometric characteristics greatly overlap between species, and the lack of easily recognizable morphological characters (Ashrafi et al. 2010, Tvrtkovic et al. 2005), strongly suggests that genetic analyses need to be performed in order to correctly identify the various species, especially outside their common distribution range (Alberdi et al. 2016). The identification uncertainty is the basis for the scarcity of circumstantiated records of *Plecotus macrobullaris*, and as a result, its conservation status is Data Deficient (DD) in the Italian Red List of Mammals (Rondinini et al. 2013).

Plecotus macrobullaris is characterized by a mountainous distribution range with stable populations in the Pyrenees, Alps, Dinaric Alps, Greece, Crete, Corsica, Turkey, Middle East, and Caucasus (Dietz & Kiefer 2014). In Italy, *P. macrobullaris* is rarely reported in the mountain areas of the Northern Regions, in particular the species is reported in Piemonte (Toffoli et al. 2016, Trizio et al. 2003), Valle d'Aosta (Debernardi & Patriarca 2008), Lombardia (Trizio et al. 2005), Veneto (Ferretto & Pereswiet-Soltan 2016, Kiefer & Veith 2001), Trentino (Chirichella et al. 2003), and in Friuli Venezia-Giulia (Lapini et al. 2013, Spitzenberger et al. 2006). In Liguria, the species is reported on the basis of a single specimen of unknown sex preserved in the Natural History Museum of Wien (NMW 150005), collected at Sanremo (Imperia) on 7th July 1972 (Spitzenberger et al. 2002).

Here we present the second confirmed recording of *Plecotus macrobullaris* in Liguria after 45 years and some elements to better define the species' distribution range in the Italian Alps.

We analyzed an adult male *Plecotus*, in accordance with epiphysis ossification of the phalanges (Dietz & von Helversen 2004), found dead near Sanremo (Imperia, NW Italy; 43°50'12"N 7°45'22"E) on 28th August 2010 at a very low altitude (about 30 m asl) (Fig. 1). The locality is characterized by a vegetation mosaic with Mediterranean scrub forest alternating with Olive groves, small forest patches of *Quercus pubescens*, and rocky outcrops near isolated houses.

The specimen was measured with a digital caliper (accuracy 0.01 mm, see Table 1) and subsequently frozen. A tissue sample preserved in Ethanol 95% was later examined (September 2016) by the Dept. of Mammalogy and Ornithology at the Natural History Museum of Geneva (Switzerland), where the standardized COI barcode sequence was amplified using the primers UTyr and C1L705, following the method suggested by Hassanin et al. (2009). The generated sequence was compared to other *Plecotus* DNA barcoding reference sequences retrieved in GenBank using the BLAST algorithm.

The specimen is now preserved in the Mammalogy collection of the Museo Civico di Storia Naturale of Carmagnola (TO, Piedmont, Italy) with the acronym and catalogue number: MCCI 1043.

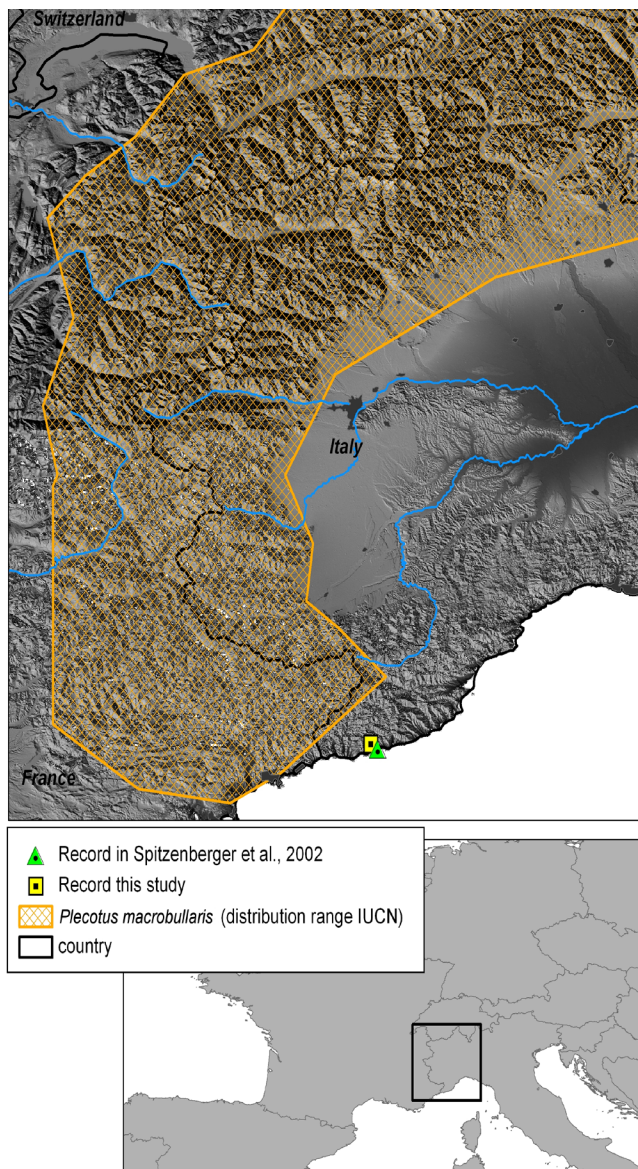


Fig. 1 - Record of *Plecotus macrobullaris* in Liguria and the distribution area in the Western Alps according to IUCN (2017).

The morphology and measurements of the specimen from Sanremo greatly recall those of the *Plecotus macrobullaris* described in the literature. In particular, the dorsal fur is constituted by tricolor hairs with a black base, light grey in the mid-part, and dark grey at the top, while the ventral side is light grey. The fur color agrees with that described by Kiefer & Veith (2001) for *Plecotus macrobullaris*, even if other authors have reported different color patterns for the species, including individuals with greyish or yellowish underparts (Spitzberger et al. 2002, Garin et al. 2003, Tvrtkovi et al. 2005, Spitzberger et al. 2006).

A hard triangular pad extends from the lower lip towards the chin. The triangular chin pad is considered a good diagnostic characteristic of *Plecotus macrobullaris* by different authors (Dietz & Kiefer 2014, Spitzberger et al. 2006, Tvrtkovi et al. 2005, Spitzberger et al. 2002), although they do not ensure a 100% correct identification like DNA analysis. Unfortunately, the shape of the penis was not clearly detected, due to the degeneration of the soft tissues, which occurs almost immediately after the death, particularly in the genital region. Genetic analyses conducted on the tissue sample returned the 730 bp long nucleotide sequence deposited on GenBank with the number KY364276. This sequence was 100% similar to other reference COI sequences of *P. macrobullaris* of Western lineages (Alberdi et al. 2015b), from Spain, France, and Italy, as previously suggested by the morphological and biometrical observations. As previously reported by Galimberti et al. (2012), *P. macrobullaris* can be univocally distinguished from other European congeners by using the mitochondrial COI.

This new specimen, found in the same locality of the only other *P. macrobullaris* from Liguria, confirms the presence of the species in this part of the region after 45 years (Spitzberger et al. 2002), even sporadically. This species is probably more common than is thought based on the known records. Additionally, our results seem to indicate that the distribution range extends further eastward than was previously known, almost in continuity with the populations of the Roja valley, Alpes Maritimes (France), and extend very close to the Italian border (Drousie et al. 2016). The Sanremo records also confirm the tendency of the species to occupy areas at lower altitudes, as has been previously recorded in Mediterranean France and in other parts of the Alps (Drousie et al. 2016, Alberdi et al. 2013). In particular, *Plecotus macrobullaris* tends to occupy the mountain reliefs with steep slopes and rocky outcrops, such as those observed in Western Liguria, not only in the Sanremo area (Alberdi et al. 2014). The place where the specimen has been found, even if it is characterized by habitats not perfectly suitable for the species, is not that far from the ecological optimum areas of the Ligurian and Maritimes Alps (Alberdi et al. 2014). This fact combined with the documented daily movements of 5-6 km with an additional altitudinal shift of several hundred meters (Alberdi et al. 2014), suggests the possibility that some individuals regularly occur in the coastal areas close to the mountains. Finally, the date of the specimen recovery could also indicate post-breeding dispersal movements, even if they have never been documented for the species.

On the other hand, our analysis confirms the validity of the morphological characteristics suggested for the species, particularly the triangular pad extending towards the chin at the lower lip. To know the real distribution range of this

Table 1 - Biometric measures (mm) detected on the specimen of *Plecotus macrobullaris* and typical ranges of the species according to Dietz & Kiefer (2014) in parentheses.

Data	Locality	Province	Sex	Forearm	Digit 3	Digit 4	Foot	Tragus
28th August 2010	Sanremo	Imperia	♂	40,38	67,10	49,50	8,44	16,52
				(37,3-46,0)	(63,0-72,0)	(49,0-58,0)		(15,5-19,0)

elusive species and acquire the needed data to assess its conservation status, we strongly suggest targeted research in Western Liguria and in other areas suitable for *Plecotus macrobullaris*.

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