Journal of
Bat Research & Conservation

OPEN ACCESS
www.secemu.org
DOI: 10.14709/BarbJ.15.1.2022.08

NATURAL HISTORY NOTE

The tale of an ice-preserved Alpine Long-Eared Bat in the Pyrenees

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ABSTRACT

The Alpine Long-eared Bat *Plecotus macrobullaris* (Kuzjakin, 1965) is a relatively recently defined species and one of the few bats able to occupy the high-mountain environment. The phylogenetic studies of this species suggest that it originated before the Middle Pleistocene. However, it has never been reported in ancient chronologies, no fossils are known, and the older recognized specimens are those from museum collections. One of the main reasons for this lack of ancient and fossil record is that the skeletal anatomy of this species has not been described in detail yet, making difficult the correct assignation of skeletal remains. We present a Long-eared Bat individual that was ice-preserved within the perennial ice in a high-mountain ice cave, SO-01, in the Pyrenees. The bat skin was radiocarbon dated, pointing that the specimen died more likely in a timeframe between 1760 and 1800 AD. Following previously published criteria based on cranial diagnostic features the recovered specimen was assigned to *P. macrobullaris*, which represents the most ancient record of this species in the Pyrenees and probably worldwide. In addition, some new observations have been described on both cranial and postcranial anatomical traits.

DOI: https://doi.org/10.14709/BarbJ.15.1.2022.08

Keywords: Chiroptera, *Plecotus macrobullaris*, high-mountain, ice cave, Pyrenees, Spain

received: June, 24th 2022
accepted: December, 15th 2022

The Alpine Long-eared Bat *Plecotus macrobullaris* (Kuzjakin, 1965) is a mid-XX century defined species whose biogeographic distribution extends along the main mountainous regions of the southwestern and central Palearctic, including the Pyrenees, Alps, Dinaric Alps, Pindos Mountains, several mountain ranges in Turkey (Kaçkar, Taurus, Guneydogu Toroslar), Greater Caucasus, Lower Caucasus, Anti-Lebanon and the Iranian Alborz and Zagros Mountains (Alberdi & Aizpurua 2018, and references therein). This is one of the few bats able to occupy high-mountain environments (Alberdi et al. 2014). Its elevational range spans from the sea level to about 2800 m (Alberdi & Aizpurua 2018), and in the Pyrenees it has been observed hibernating in ice caves higher than 2100 m. It is adapted to rocky and open landscapes and tolerates a wide temperature range (mean summer temperatures ranging from 6 to 22°C, Alberdi et al. 2014). The molecular data known to date suggest that the first appearance of this species might have occurred before the Middle Pleistocene (Alberdi et al. 2015). However, this taxon has never been reported in ancient chronologies, no fossils are known, and the older recognized specimens are those from museum collections. In this work, we present a Long-eared Bat individual that was ice-preserved (Fig. 1A) within the perennial ice in the SO-01 high-mountain ice cave, in the Pyrenees.

The SO-01 (30T 0709030 4731005, 2547 m a.s.l, UTM coordinates) is a small ice cave which opens in the south face of the Collarada massif (Central Western Pyrenees), with an entrance located at the bottom of a cliff and a ramp that connects with the perennial ice body. This ice body (Fig. 1B) comprised ca. 10 m of layered congelation ice, where some unconformities suggest different ablation phases in the past (Fig. 1C). The cave shows a statodynamic behaviour (Luetscher & Jeannin 2004): the mean air annual temperature within is ~0.5 °C (2019-2020), while the mean annual air temperature outside the cave is ca. 5.8 °C (2019-2020), with a maximum and minimum of ca. 9.6 °C and ca. 3.5 °C.

The studied bat specimen was almost fully preserved (Fig. 1A) and was recovered in June 2015. The bat skin was ^{14}C dated by means of Accelerator Mass Spectroscopy (AMS) and analysed in the Direct AMS laboratories (Seattle, Washington, USA). Radiocarbon dates were calibrated using CALIB Rev 7.0.4 (Stuiver & Braziunas 1993) and the INTCAL 20 calibration curve (Reimer et al. 2020) they invariably require revision as new data become available and our understanding of the Earth system improves. In this volume the international ^{14}C calibration curves for both the Northern and Southern Hemispheres, as well as for the ocean surface layer, have been updated to include a wealth of new

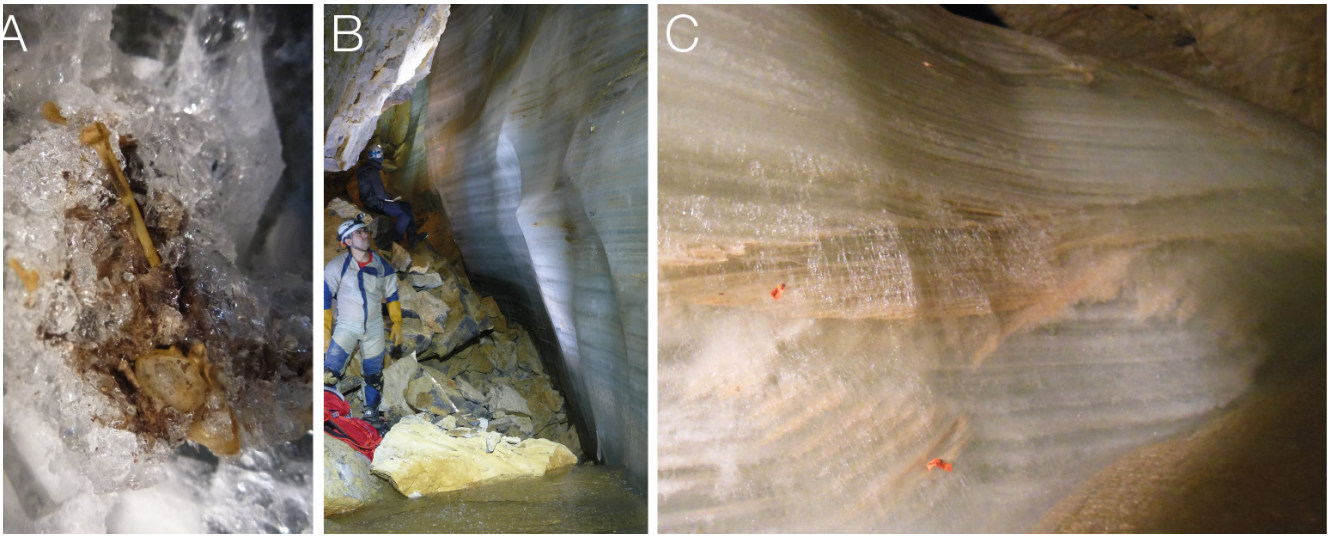

Fig. 1 - Sample state of preservation and origin. A: ice-preserved bat before picking it up. B: perennial deposit of layered congelation ice within the cave. C: detailed image of the ice deposits showing layers and unconformities.

data and extended to 55,000 cal BP. Based on tree rings, IntCal20 now extends as a fully atmospheric record to ca. 13,900 cal BP. For the older part of the timescale, IntCal20 comprises statistically integrated evidence from floating tree-ring chronologies, lacustrine and marine sediments, speleothems, and corals. We utilized improved evaluation of the timescales and location variable ^{14}C offsets from the atmosphere (reservoir age, dead carbon fraction. The skin provided an age range of between 0 to 300-year cal BP (2σ), with a maximum probability between 147-191-year cal BP. Thus, the specimen died, then get trapped within the ice, more likely in a timeframe between 1760 and 1800 AD.

The taxonomic assignation was based on the skeletal anatomy, as the external characters were mostly destroyed once the cold chain was interrupted. The collections consulted for anatomical morphology comparison with the other two *Plecotus* species present in the Iberian Peninsula, i.e. *Plecotus auritus* and *Plecotus austriacus*, belong to the Smithsonian Institution (USA; *P. auritus* n=8; *P. austriacus* n=3), the Hungarian Natural History Museum (Hungary; *P. auritus* n=10; *P. austriacus* n=7) and the National Museum of Natural History of Paris (France; *P. austriacus* n=2). The species assignation follows Dupuis (1986), Menu & Popelard (1987), Sevilla García (1988), Spitzenberger et al. (2002) and (2006) for cranial elements; Felten et al. (1973) and Dodelin (2002) were consulted for humerus description. The cranial parameters measured on our specimen (Fig. 2A, B) were the condylobasal-length (CBL), the upper canine to upper third molar distance (CM3), the total length of the mandible (mdL) and the lower canine to lower third molar distance (cm3), chosen because of both the better discrimination power of antero-posterior lengths (Galán et al. 2019) and the availability of *Plecotus macrobullaris* biometric data in the literature (Spitzenberger et al. 2002, 2006).

The bat skeleton recovered presents diagnostic characters of the genus *Plecotus*, such as the dental formula (2.1.2.3/3.1.3.3), the mandibular ramus morphology (trapezoidal with a high coronoid process and a long, well-developed angular process, Fig. 2B), the P4 occlusal outline (subtriangular, with a slightly anteriorly displaced heel),

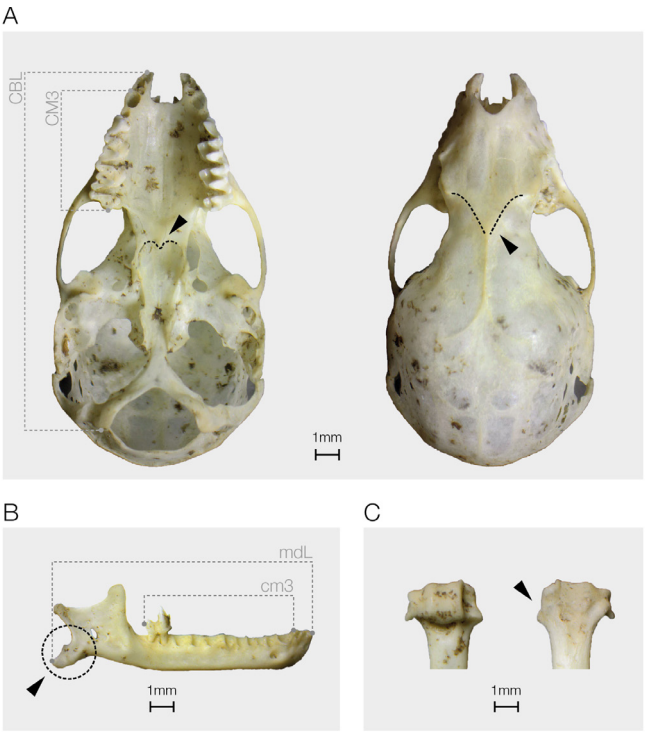

Fig. 2 - Skeletal anatomical features analysed on the recovered specimen. A: skull (ventral view at the left, arrow indicating the nasal spine; dorsal view at the right, arrow indicating the two ridges at the anterior part of the skull). B: mandible (lingual view, arrow indicating the angular process). C: humeral distal epiphysis (external view at the left; internal view at the right, arrow indicating the angle between the union of the epitrochlea and the styloid process).

the M1 and M2 occlusal outline at the protocone base (rounded, with a very regular cingulum) and the humerus distal epiphysis (in external view, it presents a curvature of the proximal edge between the trochlea and the condyle, and the epitrochlea is completely visible; Fig. 2C). However, the skeletal anatomy and biometry of the different species conforming the genus *Plecotus* has only been extensively described in two of the three taxa present in the Iberian Peninsula: *P. auritus* and *P. austriacus.* According to the available data, the CBL and cm3 size of our specimen

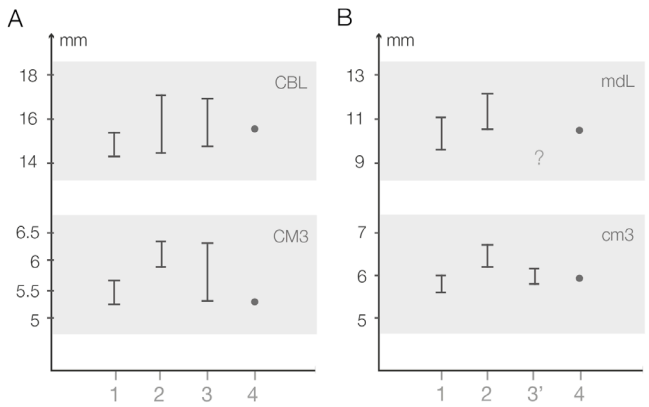

Fig. 3 - Cranial biometry comparison between *Plecotus auritus* (1, from Menu & Popelard 1987), *Plecotus austriacus* (2, from Menu & Popelard 1987), *Plecotus macrobullaris* (3, from Spitzenberger et al. 2006; 3', Spitzenberger et al. 2002) and the specimen from SO-01 (4). A: skull biometry. B: mandible biometry.

falls within the *P. macrobullaris* variation and is almost intermediate between *P. austriacus* and *P. auritus* (Fig. 3A, B). On the other hand, the CM3 size mostly falls within the *P. auritus* variability (Fig. 3A). The mdL size is intermediate between *P. austriacus* and *P. auritus* (Fig. 3B). Other skeletal features that are coincident with *P. macrobullaris* (according to Spitzenberger et al. 2002; Fig. 2A) are a light expression of the two ridges separated by a groove on the anterior part of the skull (similar to *P. auritus*, more strongly marked on *P. austriacus*) and a relatively short and light angular process if compared with *P. austriacus* (although in our specimen it is stronger than in most *P. auritus* consulted specimens (Fig. 2B). Finally, some morphological features on the cranial and postcranial anatomy of our specimen (not previously referred to in the available anatomical descriptions of *P. macrobullaris*) have been compared with the consulted specimens of the other two *Plecotus* species present in the Iberian Peninsula. It presents a nasal spine (Fig. 2A) more developed than in the considered specimens of both *P. austriacus* and *P. auritus.* On the other hand, the P4 lacks an anterior cingulum cone, and the distal epiphysis of the recovered humeri shows a straight union between the epitrochlea and the styloid process in internal view (Fig. 2C); both morphologies are considered to be distinctive to *P. austriacus* versus *P. auritus* (Felten et al. 1973, Dupuis 1986, Sevilla García 1988, Spitzenberger et al. 2006), thus they might be features shared by *P. austriacus* and *P. macrobullaris*. A larger sample would be needed in order to stablish if these characters are distinctive of the alpine species, or simply reflect intraspecific variations.

ACKNOWLEDGMENTS

We thanks to the ECZ (Espeleo Club de Zaragoza) their help during the sampling and the cave topography. J.G. is the recipient of a UPV/EHU contract for PhD research staff specialization 2020 (ESPDOC20/83). C.N.-L. is the recipient of a Juan de la Cierva-Formación fellowship (FJC2020-044561-I). Miguel Bartolomé was supported by OCHESTRA-ref 2552/2020. This work has been supported by the IEA (Ayudas a la investigación del IEA, 2014), the Gobierno Vasco-Eusko Jaurlaritza project (IT1418-19) and OCHESTRA project (ref 2552/2020).

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