

## A new species of long-eared bat (Chiroptera, Vespertilionidae) from Sardinia (Italy)

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We describe a new species of long-eared bat, genus *Plecotus*, from the island of Sardinia (Italy). The new species is clearly distinguishable from other European *Plecotus* species by its mitochondrial 16S rRNA gene (4.1–9.6% sequence divergence) as well as by a unique combination of morphological characters such as brownish colour of dorsal pelage, a relatively large thumb and thumb claw, an almost cylindrical form of the penis and the characteristic shape of the baculum. The most important morphological diagnostic character is a relatively long ( $\geq 18$  mm) and wide ( $\geq 6$  mm) tragus. The new species is currently known from three localities on Sardinia. In addition to the new species we discovered a lineage of *P. auritus*, which is substantially differentiated from continental *P. auritus* at subspecific level (1.2–2.7% of sequence divergence of the 16S rRNA gene). The existence of these two endemic bat taxa on Sardinia highlights the island's importance in the conservation of the European bat community.

*Key words:* *Plecotus* sp. nov., *Plecotus auritus*, *Plecotus austriacus*, long-eared bat, Sardinia, DNA sequence analysis, 16S rRNA, morphology, conservation

### INTRODUCTION

Molecular techniques have greatly enhanced our understanding of the evolutionary relationships of organisms and application to European bats has recently enabled the detection of cryptic species. Two distinct phonic types (Weid and von Helversen, 1987) of the widespread pipistrelle *Pipistrellus pipistrellus* (Schreber, 1774), corresponded to genetically well-defined lineages that are differentiated at the species level (Jones and Parijs, 1993; Barratt *et al.*, 1997; Mayer and von Helversen, 2001). Within European *Myotis mystacinus* group, the morphologically cryptic *Myotis alcathoe* (von Helversen *et al.*, 2001) was discovered

when applying molecular techniques to bats from Greece.

Recently, two new species of long-eared bats (genus *Plecotus*) were discovered in Europe. Species rank was assigned to *P. kolombatovici* Dulic, 1980, formerly considered a subspecies of *P. austriacus* Fischer, 1829 (Mayer and von Helversen, 2001), and a formerly unknown cryptic species *P. alpinus* Kiefer and Veith, 2002 (= *P. microdontus* Spitzenberger, 2002 — see Kock, 2002) was discovered in the Alps and adjacent mountains of Southern Europe.

Knowledge of the Sardinian bat fauna is currently expanding. The Centre for the Study and Protection of Sardinian Bats (Centro per lo Studio e la Protezione dei

Pipistrelli in Sardegna) recently mentioned 18 species: four rhinolophids, 13 vespertilionids and one molossid (Mucedda *et al.*, 1999). All of these species also occur on the European mainland.

Until 1959, the only species of long-eared bats (genus *Plecotus*) known to occur on Sardinia was the brown long-eared bat, *P. auritus* (Linnaeus, 1758) (Lanza, 1959). Mucedda *et al.* (2002) reconfirmed its presence and also recorded the grey long-eared bat, *P. austriacus*. To better define priorities for conservation it was desirable to genetically compare Sardinian *Plecotus* to conspecific populations from the European mainland (Mitchell-Jones *et al.*, 1999). More specifically, we were interested in determining if Sardinian *Plecotus* are genetically similar to mainland populations because they only recently colonised the island, or do they constitute derived, genetically distinct lineages that deserve special attention for national and international wildlife conservation? To clarify the genetic identity of Sardinian brown and grey long-eared bats we compared partial mitochondrial DNA sequences of Sardinian specimens to published sequences of all currently known European species of *Plecotus* (Kiefer *et al.*, 2002). Here we report the discovery of two genetically distinct lineages of Sardinian *Plecotus*, one of which deserves recognition at the species level.

## MATERIALS AND METHODS

### *Specimens Studied*

We sampled, under license, 22 specimens of long-eared bats from three localities in central Sardinia (Appendix). Samples for DNA extraction were obtained from wing tissue, using sterile biopsy punches (Worthington Wilmer and Barrett, 1996). We recorded the following morphometric (hand-held caliper measurements to the nearest 0.05 mm) and other morphological characters, according to Stebbings (1967) and Häussler and Braun (1991): HB = head-and-body length; Tail = tail length; FA = forearm

length (including carpals); F2 = length of the 2nd finger (incl. carpals); F3 = length of 3rd finger (incl. carpals); F4 = length of 4th finger (incl. carpals); F5 = length of 5th finger (incl. carpals); HF = hind foot length (excl. claws); CaL = calcar length; Ear = ear length; TL = tragus length (TL<sub>Blatt</sub> in Häussler and Braun 1991); TW = tragus width; TH = thumb length excl. claw; CL = claw length; SL = skull length; CBL = condylobasal length; SH = skull height (incl. bullae); IOW = interorbital constriction width; M<sup>3</sup>-M<sup>3</sup> = width across upper molars; C-M<sup>3</sup> = length of maxillary toothrow; C-M<sub>3</sub> = length of mandibular toothrow; M<sub>3</sub>-M<sub>3</sub> = width across lower molars; ML = mandible length; MW = mastoid width; CsupL = length of upper canines; MBD = maximal bulla diameter; ZW = zygomatic width; MDB = minimal distance between bullae; BL = length of baculum; BW = basal width of baculum.

The baculum of the holotype was extracted following the procedure of Anderson (1960). It was photographed with a Leitz photomicroscope DMRB to obtain the drawing and then measured with the same device to the nearest of 0.01 mm.

### *DNA Extraction and Sequencing*

DNA was extracted using QiAmp tissue extraction kits (Qiagen). Double-stranded PCR was used to amplify mitochondrial DNA fragments. Primers and cycling procedures were as follows: 16SA (light chain; 5' - CGC CTG TTT ATC AAA AAC AT - 3') and 16SB (heavy chain; 5' - CCG GTC TGA ACT CAG ATC ACG T - 3') of Palumbi *et al.* (1991) amplified to a ca. 555 bp section of the mitochondrial 16S ribosomal RNA gene. PCR cycling procedure was as follows: initial denaturation step: 90 s at 94°C, 33 cycles: denaturation for 45 s at 94°C, primer annealing for 45 s at 55°C, extension for 90 s at 72°C. PCR products were purified using the Qiaquick purification kit (Qiagen). We sequenced single-stranded fragments on an ABI 377 automatic sequencer using standard protocols. We sequenced 555 bp of the 16S rRNA gene that are homologous to the base pair positions 2215–2490 of the *Pipistrellus abramus* complete mitochondrial genome (Nikaido *et al.*, 2001). These sequences were aligned to previously published sequences of all European *Plecotus* species (GenBank Accession Nos. AY134012–134026, AF529229–529230 — Kiefer *et al.*, 2002) using the Clustal X software (Thompson *et al.*, 1997). Only different haplotypes were included in the analysis. For hierarchical outgroup comparison we included *Barbastella barbastellus* (Schreber, 1774) and *Myotis bechsteinii* (Kuhl, 1817) (GenBank AF529231 and AY134027, respectively — Kiefer *et al.*, 2002).

## Molecular Data Analysis

We determined the number and distribution of base substitutions. The amount of phylogenetic signal was assessed by generating  $10^6$  random trees and calculating the skewness ( $g_1$ ) and kurtosis ( $g_2$ ) of the resulting tree length distribution (with PAUP\*, version 4.08b — Swofford, 2001). Prior to model assessment we performed a  $\chi^2$ -test for base distribution across sequences to rule out non-homogeneous base compositions that require the use of the paralinear LogDet distance instead of specific substitution models (Lockhart *et al.*, 1994). Using a hierarchical likelihood ratio test (LRT), we tested the goodness-of-fit of nested substitution models for homogeneous data partitions (for ingroup taxa only). We used MODELTEST version 3.06 (Posada and Crandall, 1998) to determine a specific substitution model to be used for further analyses. For our 16S rRNA gene a Tamura-Nei (TrN) substitution model (Tamura and Nei, 1993) with no invariable sites ( $I = 0$ ), and among site substitution rate variation with a  $\gamma$ -shape parameter = 0.4882 was selected.

We used the neighbor-joining algorithm (NJ — Saitou and Nei, 1987), applying the selected substitution model, for phylogenetic tree reconstruction. We calculated maximum parsimony tree (MP), treating gaps as missing characters and giving equal weight to transitions and transversions (heuristic search with the TBR branch swapping algorithm). We used PAUP\* (Swofford, 2001) for tree reconstruction. Robustness of NJ and MP tree topologies was tested by bootstrap analyses (Felsenstein, 1985), with 2,000 replicates each (Hedges, 1992).

## RESULTS

### Phylogenetic Hypothesis

Of the 555 bp of the sequence, 125 were variable and 84 bp were parsimony informative. Skewness ( $g_1$ ) and kurtosis ( $g_2$ ) were estimated to -0.4958 and 0.1821, respectively. Bases were distributed homogeneously among sequences, and we applied the specific substitution model and gamma shape parameter.

The neighbor-joining (Fig. 1) and maximum parsimony (not shown) trees consistently show the same topology. Both

analyses reveal two major clades. The first major clade contains *P. kolombatovici* and *P. austriacus* (incl. the Sardinian samples 3, 6, 9, 10, 11 and 12); the second one comprises *P. auritus* [incl. the Sardinian samples 4, 5, 14, 16 and 17 (haplotypes 4, 5, 14, 17 — see Appendix)], *P. alpinus*, and a Sardinian clade consisting of samples 1, 2, 13, 15, 20, 21, and 22 (haplotypes 1, 2, and 13). All clades are supported by bootstrap values > 90%. Mean substitution rates and TrN distances among lineages of each of the two major clades are in the same range (0.43–0.54 and 0.057–0.067, respectively — Table 1). Substitution rates for the 16S rRNA gene of ca. 5% correspond to substitution rates of 11–12% for protein coding mitochondrial genes like ND1, ND2 or cytochrome *b* (authors' own data). The latter indicate differentiation at the species level (see Smith and Patton, 1993, and Bradley and Baker, 2001 for mammals in general, and Cooper *et al.*, 2001, and Mayer and von Helvesen, 2001 for bats). Consequently, and in accordance with morphological data (see below), we describe the specimens characterized by the geographically restricted Sardinian clade (samples Sar1, Sar2, Sar13, Sar15, Sar20, Sar21, and Sar22 — see Appendix) as a new species.

The Sardinian subclade within *P. auritus* shows substitution rates and molecular TrN distances to other *P. auritus* subclades that range from 0.12–0.27 and 0.019–0.022, respectively, i.e., within the same limits as for the Iberian sample Paur7 (Fig. 1). Sardinian *P. austriacus* haplotypes are nested within other European *P. austriacus* haplotypes with no apparent sub-structuring.

### *Plecotus sardus* sp. nov.

#### Derivatio nominis

The specific name *sardus* refers to the island of Sardinia (Italy, Mediterranean Sea) where the taxon is found.

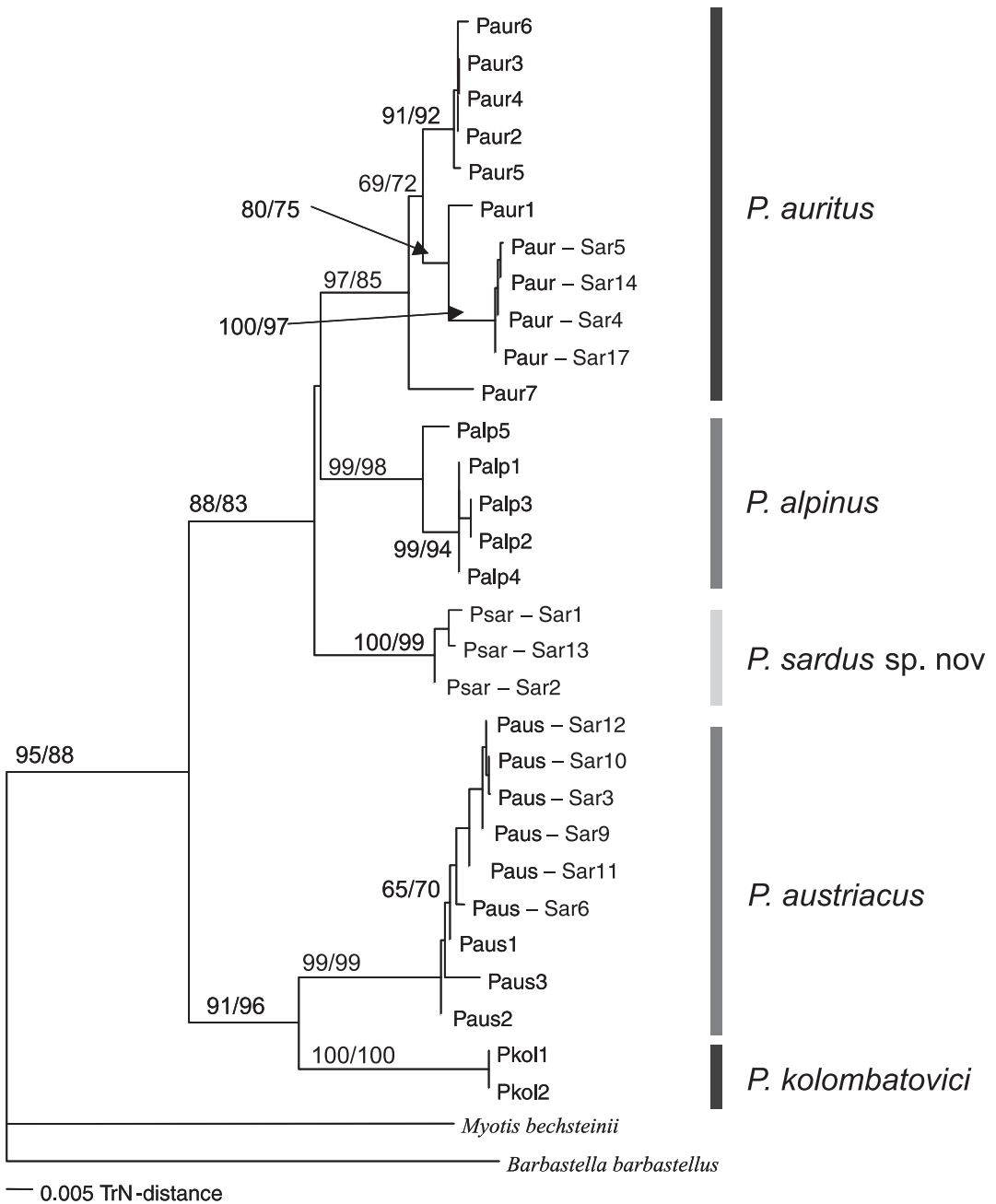


FIG. 1. Neighbor-joining tree of European long-eared bats, based on 555 bp of mitochondrial 16S gene fragment (TrN substitution model with  $I = 0$  and  $\gamma$ -shape parameter  $\alpha = 0.4882$ ); bootstrap support values are indicated for neighbor-joining (left) and maximum parsimony (right) trees; 2,000 replicates were analysed. Abbreviations of haplotypes are the same as in Kiefer *et al.* (2002) except for the samples from Sardinia Sar1–Sar22 (see Appendix)

TABLE 1. Corrected molecular distances (TrN + G, above diagonal) and mean substitution rates (below diagonal) among major *Plecotus* lineages (ranges are given in parentheses)

Lineage	(1)	(2)	(3)	(4)	(5)
(1) <i>P. auritus</i>	–	0.057 (0.049–0.064)	0.060 (0.058–0.063)	0.112 (0.106–0.114)	0.118 (0.108–0.123)
(2) <i>P. alpinus</i>	0.049 (0.043–0.054)	–	0.053 (0.052–0.054)	0.113 (0.111–0.115)	0.106 (0.102–0.113)
(3) <i>P. sardus</i> sp. nov.	0.049 (0.045–0.053)	0.043 (0.041–0.045)	–	0.119 (0.112–0.125)	0.113 (0.110–0.120)
(4) <i>P. austriacus</i>	0.082 (0.069–0.094)	0.088 (0.083–0.092)	0.090 (0.085–0.096)	–	0.062 (0.057–0.067)
(5) <i>P. kolombatovici</i>	0.086 (0.082–0.091)	0.081 (0.079–0.085)	0.082 (0.080–0.084)	0.054 (0.049–0.058)	–

### Specimens Examined

#### Holotype

Adult male, skin, skull and baculum, from the collection of the Department of Zoology and Biological Anthropology of the University of Sassari (Dipartimento di Zoologia e Antropologia Biologica — DZAB 0023); found dead by M. Mucedda and E. Pidinchredda on September 22, 2001 in the interior of a cave at Lanaitto's Valley, Oliena District, Nuoro Province, middle-east Sardinia, Italy (40°15'29"N, 9°29'13"E, 150 m a.s.l.). Measurements (in mm): HB, 45; Tail, 51; Ear, 37.5; TL, 18.5; TW, 6.5; FA, 41.2; TH, 6.0; CL, 3.1; HF, 7.7; F2, 35.8; F3, 71.8; F4, 57.0; F5, 56.0; CaL, 18; SL, 17.10; CBL, 15.90; SH, 7.80; IOW, 3.65; M<sup>3</sup>–M<sup>3</sup>, 6.25; M<sub>3</sub>–M<sub>3</sub>, 4.00; C–M<sup>3</sup>, 5.75; C–M<sub>3</sub>, 6.20; ML, 11.30; MW, 9.30; CsupL, 1.50; MBD, 4.75; ZW, 9.20; MDB, 1.20; BL, 0.80; BW, 0.71.

#### Other specimens

One juvenile; found dead by M. Mucedda and E. Pidinchredda in the interior of a cave at Baccu Addas valley, Baunei district, province of Nuoro. Five individuals, 1 ♂ and 4 ♀♀ — mist-netted by M. Mucedda, E. Pidinchredda and M. L. Bertelli near the Omodeo Lake (Ula Tirso District, Oristano Province), and subjected to morphometric measurements (see Table 2), drawing of wing patterns and photography (Fig. 2), and then released. We took tissue samples for genetical analysis from all these individuals.

#### Diagnosis

*Plecotus sardus* sp. nov. is unambiguously identifiable through DNA sequence analysis. The partial 16S rRNA sequence of the holotype (GenBank Accession No. AY175822) reads: tattagaggcactgcctgccagc

TABLE 2. Body measurements of *Plecotus sardus* sp. nov.

Character	Sar 13 ♂–holotype	Sar 22 ♂	Sar 2 ♀	Sar 15 ♀	Sar 20 ♀	Sar 21 ♀	̄	SD	n
Forearm length	41.2	41.1	42.3	42.2	42.2	40.9	41.65	0.65	6
Thumb length	6.0	6.0	6.0	6.0	6.4	6.0	6.07	0.16	6
Claw length	3.1	2.0	2.4	2.5	2.5	2.6	2.52	0.35	6
Ear length	37.5	38.0	38.6	39.0	–	–	38.28	0.66	4
Tragus length	18.5	18.0	18.0	19.8	18.9	19.2	18.73	0.71	6
Tragus width	6.5	6.2	6.0	6.4	6.5	6.4	6.33	0.20	6
Hind foot length	7.7	7.5	7.0	7.6	6.8	6.7	7.22	0.44	6





FIG. 2. *Plecotus sardus* sp. nov. [note the shape and length of tragus (an individual released after capture)]

gactccagttaaacggccggtatcctgaccgtcaaagg  
tagcataatcatttgttctctaaatagggacttgatgaatgg  
ccccacgaggggttaactgtctcttacttttaatcagtg  
aaattgacactcccgtgaagagggcgggaattaaaaaata  
agacgaWaagaccctatggagctttaattaactcac  
aaattataactaatactacaagagacaagctaaacttgatt  
gagttaacaatttNNgttggggcgaccttggataaagatc  
aacctccgagatagatctactaagacctacaagcaaggtt  
atatactatacattgatccgccaatagcgaacgaaca  
agttaccctagggataacagcgcaatcctattaagagtc  
atatcgacaattagggttacgacctcgatgttgatcagga  
catccaatggtgcagcagctattaatgtgtcgtttgttcaa  
cgattaagtctactacgtgatctgagt.

It differs in 24 substitutions (21 transitions, tis, and 3 transversions, tvs) from *P. auritus* (GenBank AY134013), 21 substitutions (19 tis and 2 tvs) from *P. alpinus* (AY134017), 44 substitutions (37 tis and 7 tvs) from *P. austriacus* (AY134022), and 40 substitutions (35 tis and 5 tvs) from *P. kolombatovici* (AY134025), respectively.

Like *P. alpinus*, *P. sardus* sp. nov. combines typical morphological features of both

*P. auritus* and *P. austriacus* (Table 3). It is similar to *P. auritus* in its brownish colour of dorsal pelage, length of thumb and length of thumb-claw; similar to *P. austriacus* in its whitish colour of ventral pelage, broadest width of tragus and length of forearm (Table 3); and similar to *P. alpinus* in the shape of the penis (Fig. 3). However, *P. sardus* sp. nov. differs from all other European *Plecotus* spp. in the length of the tragus and the shape of the baculum (Fig. 4). Additionally, it differs from *P. kolombatovici* in the forearm and ear lengths (Table 3).

#### Description

*Plecotus sardus* sp. nov. is larger than both *P. auritus* and *P. kolombatovici*, reaching the size of specimens of *P. austriacus* and *P. alpinus*. The dorsal fur is brown rather than reddish as in some *P. auritus*. The hair is very fine and woolly, ca. 10 mm long and tri-coloured: to the first 6 mm are

TABLE 3. Morphological characteristics of European *Plecotus* spp. (data from Häussler and Braun, 1991<sup>1</sup>; Spitzenberger *et al.*, 2002<sup>2</sup>; Kiefer and Veith, 2002<sup>3</sup>; A. Kiefer and O. von Helversen, unpubl. data<sup>4</sup>; authors' own data<sup>5</sup>)

Character	<i>auritus</i>	<i>austriacus</i>	<i>kolombatovici</i>	<i>alpinus</i>	<i>sardus</i> sp. nov.
Colour of dorsal fur	brown to reddish <sup>4</sup>	grey <sup>4</sup>	brownish <sup>4</sup>	greyish-brown <sup>2</sup>	brown <sup>5</sup>
Colour of ventral fur	yellowish-brown to creamy <sup>3</sup>	grey <sup>3</sup>	whitish <sup>4</sup>	pale grey <sup>3</sup>	whitish to pale brown <sup>5</sup>
Forearm length	35.1–43.5 <sup>2</sup> 37.5–39.7 <sup>1</sup> 36.0–43.5 <sup>4</sup>	33.9–42.1 <sup>2</sup> 38.4–42.0 <sup>1</sup>	36.2–39.3 <sup>2</sup>	white <sup>3</sup> , white-grey <sup>2</sup> 39.6–43.5 <sup>2</sup> 39.7–42.2 <sup>3</sup> 40–45 <sup>4</sup>	40.9–42.3 <sup>5</sup>
Tragus width	4.5–5.5 <sup>1</sup> , <5.5 <sup>4</sup>	5.7–6.3 <sup>1</sup> , >5.5 <sup>4</sup>	4.5–5.0 <sup>4</sup>	5.5–6.0 <sup>3,4</sup>	6.0–6.5 <sup>5</sup>
Tragus length	12.0–13.7 <sup>1</sup> , <15.5 <sup>4</sup>	13.5–16.1 <sup>1</sup> , 14–16 <sup>4</sup>	12–14 <sup>4</sup>	16–19 <sup>4</sup>	18.0–19.8 <sup>5</sup>
Ear length	35.0–38.0 <sup>1</sup> 26.2–40.4 <sup>2</sup>	35.0–39.0 <sup>1</sup> 28.6–41 <sup>2</sup>	29.7–34.1 <sup>2</sup>	34–38.3 <sup>2</sup>	37.5–39.0 <sup>5</sup>
Thumb length	>6.5 <sup>4</sup>	<6.5 <sup>4</sup>	<6.5 <sup>4</sup>	>6.5 <sup>3</sup> , 6.5–7.0 <sup>3</sup>	6.0–6.4 <sup>5</sup>
Claw length	>2 <sup>4</sup>	<2 <sup>4</sup>	<2 <sup>4</sup>	>2 <sup>3</sup> , 2.0–2.8 <sup>3</sup>	2.0–3.1 <sup>5</sup>
Hind foot length	8.2–8.9 <sup>1</sup> , >9 <sup>4</sup>	6.8–7.9 <sup>1</sup> , 7–8 <sup>4</sup>	<8 <sup>4</sup>	>8.5–9.0 <sup>3</sup> , 8 <sup>4</sup>	6.7–7.7 <sup>5</sup>
C–M <sup>3</sup>	5.3–5.5 <sup>1</sup>	5.8–6.3 <sup>1</sup>	5.16–5.42 <sup>2</sup>	5.36–5.74 <sup>2</sup>	5.75 <sup>5</sup>
C–M <sub>3</sub>	4.85–5.61 <sup>2</sup>	5.40–6.29 <sup>2</sup>	5.53–5.83 <sup>2</sup>	5.82–6.16 <sup>2</sup>	6.20 <sup>5</sup>
Upper canine length	5.42–6.00 <sup>2</sup>	6.14–6.83 <sup>2</sup>	1.61–1.75 <sup>2</sup>	1.77–1.99 <sup>2</sup>	1.50 <sup>5</sup>
Size of the protuberances over the eyes	1.43–1.85 <sup>2</sup> large (> 2 mm) <sup>5</sup>	1.93–2.18 <sup>2</sup> small (< 1 mm) <sup>5</sup>	small (< 1 mm) <sup>5</sup>	medium (ca. 1–2 mm) <sup>5</sup>	medium (ca. 1–2 mm) <sup>5</sup> (smaller than in <i>alpinus</i> )
Penis shape	narrowing towards the end <sup>4</sup>	club-shaped <sup>4</sup>	club-shaped <sup>4</sup>	almost cylindrical, pointed only at the tip <sup>4</sup>	almost cylindrical, pointed only at the tip <sup>5</sup>
Triangular pad at the chin	no <sup>2,4</sup>	no <sup>2,4</sup>	no <sup>2,4</sup>	yes <sup>2,4</sup>	no <sup>5</sup>

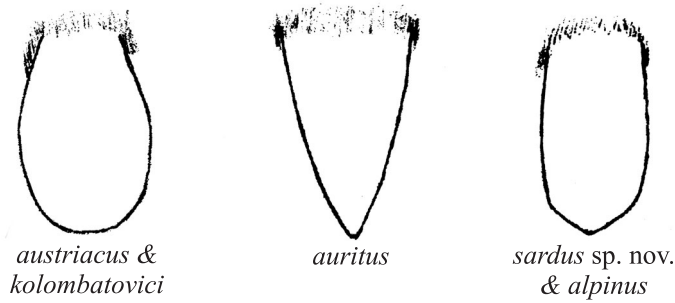


FIG. 3. Shape of the penis (dorsal view) from the five European *Plecotus* spp.

very dark brown-grey, the next 2.5 mm are whitish-light brown, and the terminal portion (1.5 mm) brown. The ventral pelage is whitish, tending to pale brown. The hair is ca. 7 mm long and bi-coloured: the basal 2/3 is dark brown, the terminal 1/3 is whitish. The brown colour of dorsal fur spreads slightly towards the neck and the change in colour between dorsal and ventral fur is abrupt and evident.

The wing membranes are brown, tending slightly towards reddish. The plagiopatagium inserts at the base of the 5th toe. The tail is 51 mm long, with about 2.5 mm of the last caudal vertebra extending beyond the uropatagium. The calcar is 18 mm long and slightly bent, with a small lobe at the tip; it reaches approximately half the length of the edge of the uropatagium. The hind foot is similar in size to that of *P. alpinus*,

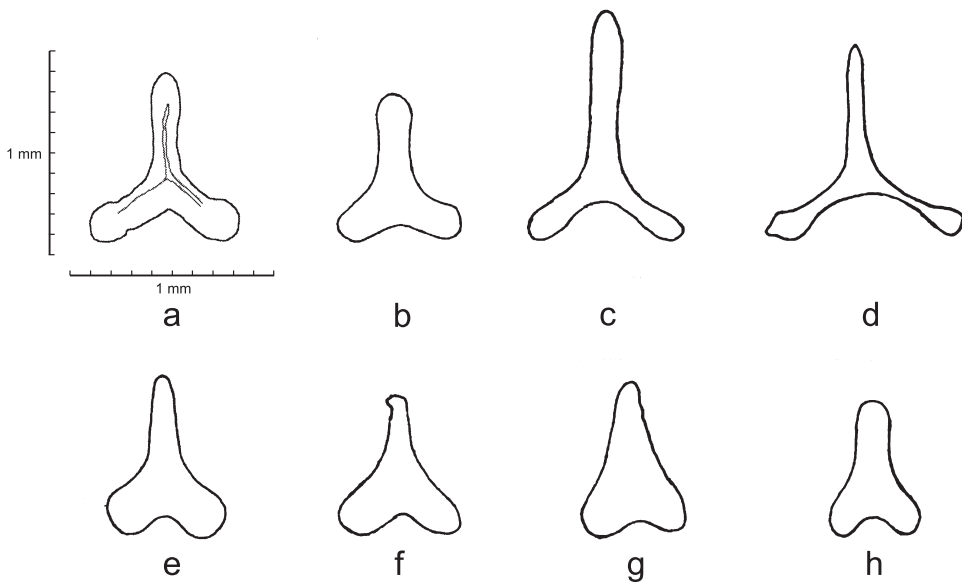


FIG. 4. Dorsal view comparing the bacular shape of: (a) *P. sardus* sp. nov., (b) *P. alpinus* (Kiefer and Veith, 2002); (c) *P. auritus* (Topál, 1958); (d) *P. teneriffae* (Ibáñez and Fernandez, 1985); (e) *P. kolombatovici* (Đulić, 1980); (f) *P. austriacus christii* (Qumsiyeh, 1985); (g) *P. austriacus wardi* (Strelkov, 1988); and (h) *P. austriacus* (Topál, 1958). All bacula are redrawn in the same scale



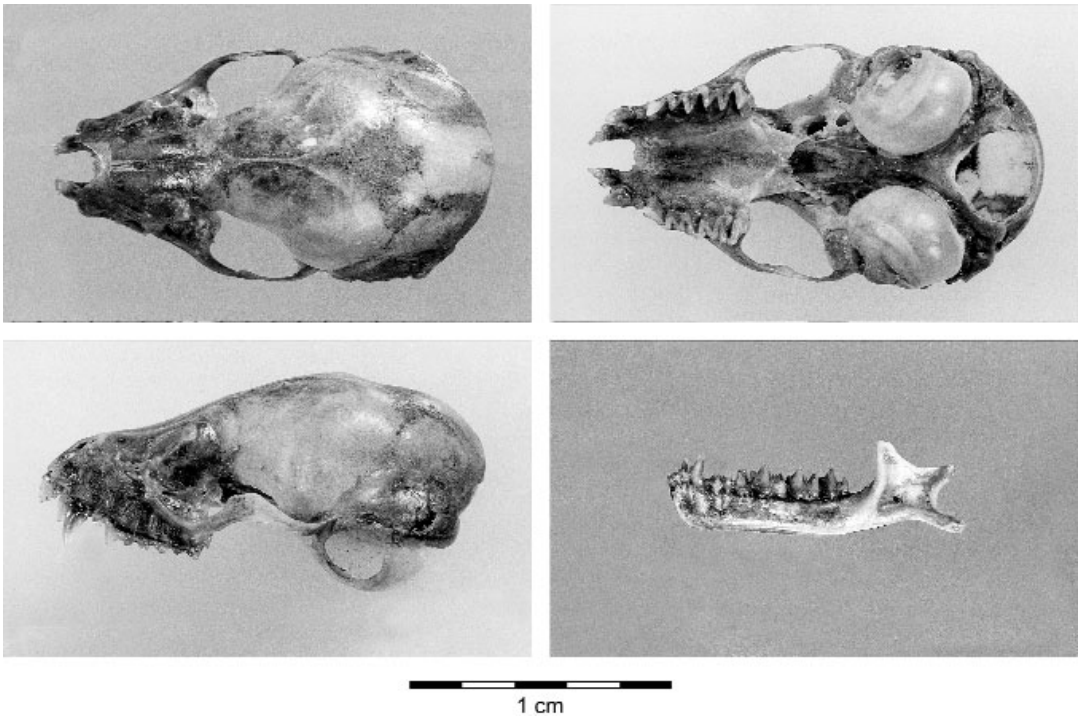


FIG. 5. Cranium (dorsal, ventral, and lateral views) and mandible of the holotype of *P. sardus* sp. nov.

and almost as large as in *P. auritus*, but the hairs on the toes are shorter than in *P. auritus*.

The ears are large, ca. 37.5 mm long, pale-brown with a reddish hue. The tragus is very large, 18.5 mm long, pale brown tending towards yellowish-white, and it is more or less straight (see Fig. 2). It is the longest tragus among the European long-eared bats and is one of the most important characters for distinguishing this species from other European *Plecotus* (Table 3). The maximum tragus width is 6.5 mm, which is similar to *P. austriacus*.

The muzzle is narrower and less swollen than in *P. auritus*. Its colour is pale rosy-brown, without the dark mask typical for *P. austriacus*. The protuberances over the eyes are 1 mm wide (Table 3), with a few long and straight hairs. Evident under the chin is a glandular wart that lacks hairs.

The penis is almost cylindrical, only slightly rounded, and pointed only at the tip

(Fig. 3). Although the shape of the penis resembles that of *P. alpinus*, the shape of the baculum is clearly different (Fig. 4). The shape of the baculum resembles that of *P. auritus*, but is smaller and proportionally wider at the base, 0.80 mm long and 0.71 mm wide. The proximal part is ventrally concave.

According to the skull of the holotype (Fig. 5), *P. sardus* sp. nov. is different in its C–M<sup>3</sup> and C–M<sub>3</sub> lengths from other European *Plecotus* species, except *P. austriacus*. The upper canine from *P. sardus* sp. nov. is as small as in *P. auritus* (Table 3). Compared to the upper canine and the 2nd upper premolar, the 1st upper premolar is very small.

#### Distribution

The species is currently known only from the type locality and two additional locations on Sardinia. These three localities

are separated by a distance of about 60 km and occur within the most wooded regions of the island. Two localities, including the type locality, are situated in limestone mountain regions of middle-east Sardinia. There are numerous natural caves, included in the 'National Park of Gennargentu and Orosei Gulf', which is relatively close to the sea coast. The third locality is situated at a low elevation above sea level in the central part of the island, where the Tirso River is fed from an artificial lake.

## DISCUSSION

### *Comparisons*

Despite of several morphological similarities (e.g., Kiefer and Veith, 2002), *P. sardus* sp. nov. differs from all other European *Plecotus* species in the length of the tragus and the shape of the baculum (Figs. 2 and 4; see also Topál, 1958; Đulić, 1980; Kiefer and Veith, 2002; Spitzenberger *et al.*, 2002). Additionally, it differs from *P. kolombatovici* in the forearm and ear lengths (Table 3). The ears are longer than in *P. kolombatovici* and reach the maximum size of those of *P. auritus*, *P. alpinus* and *P. austriacus* (e.g., Đulić, 1980; Häussler and Braun, 1991; Spitzenberger *et al.*, 2002). *Plecotus sardus* sp. nov. has the longest tragus among the European long-eared bats and its length is one of the most important characters for distinguishing this species from other European *Plecotus* (Table 3).

The protuberances over the eyes are 1 mm wide, intermediate in size between those of *P. auritus* and *P. austriacus* (e.g., Strelkov, 1988, 1989a; von Helversen, 1989) and slightly smaller than in *P. alpinus* (Kiefer and Veith, 2002). The hard triangular pad reported by Spitzenberger *et al.* (2002) for *P. alpinus* is lacking. The penis differs in shape from that of *P. auritus*, *P. austriacus* and *P. kolombatovici* (Schober

and Grimmberger, 1989; von Helversen, 1989; A. Kiefer and O. von Helversen, unpubl. data) in being almost cylindrical, only slightly rounded, and pointed only at the tip (see Fig. 3). The shape of the baculum resembles that of *P. auritus*, but is smaller and proportionally wider at the base (Lanza, 1960; Strelkov, 1989a; De Paz, 1994); it is also thinner distally than that of *P. alpinus* (Kiefer and Veith, 2002; Spitzenberger *et al.*, 2002) and is different in shape from that of *P. kolombatovici* (Đulić, 1980), *P. austriacus* (Topál, 1958), *P. teneriffae* (Ibáñez and Fernandez, 1985), *P. austriacus wardi* (Strelkov, 1988), *P. christii* (Qumsiyeh, 1985) and *P. balensis* (Kruskop and Lavrenchenko, 2000).

### *Taxonomy*

During the last 200 years, several names have been suggested for *Plecotus* populations from Europe, Africa and Asia. Kiefer and Veith (2002) pointed out that some of these are *nomina nuda* and that other names require further discussion (see also Yoshiyuki, 1991). Nevertheless, we note that none of the suggested taxa were described from Sardinia. This is the only area where we identified haplotypes of *P. sardus* sp. nov. among more than 300 specimens of long-eared bats we analysed from all over Europe and Asia (authors' unpubl. data). As a consequence, we conclude that none of the available names is applicable to Sardinian material described in this paper.

Among the Asian *Plecotus*, Strelkov (1988, 1989a, 1989b) recognised *P. auritus sacrimontis* Allen, 1908, *P. austriacus wardi* Thomas, 1911, *P. austriacus turkmenicus* Strelkov, 1985, and *P. austriacus kozlovi* Bobrinskoy, 1926. Noteworthy, none of them morphologically fits *P. sardus* sp. nov. (e.g., fur colour, bacular morphology).

In the genus *Myotis*, Castella *et al.* (2000) have shown a close relationship

between North African and Sardinian populations. Therefore, we reviewed names for North African *Plecotus*. According to Qumsiyeh (1985), two distinct taxa occur in North Africa. One is a non-desert form, referable to the wide-spread *P. austriacus*. For the other form, known from arid areas only, Qumsiyeh (1985) used the name *P. austriacus christii* Gray, 1838. Comparing Qumsiyeh's (1985) information with our data reveals that the bacula of *P. a. christii* and *P. sardus* sp. nov. are quite different. In addition, the Pleistocene *P. abeli* Wettstein, 1931, known only from fossil material from Austria, is not believed to be conspecific with any of the extant *Plecotus* spp. (A. Kiefer and R. Hutterer, unpubl. data).

#### *Status of Sardinian P. auritus*

The level of substitution rates and TrN molecular distances of the Sardinian subclade within *P. auritus* is in the same range as for the Iberian sample Paur7, which represents the subspecies *P. auritus begognae* De Paz, 1994 (Kiefer *et al.*, 2002; J. Juste, C. Ibáñez, D. Trujillo, J. Muñoz, P. Benda, and M. Ruedi, unpubl. data), indicating differentiation of these Sardinian brown long-eared bats may be at the subspecific level. However, since haplotype Paur1 from continental Europe (Switzerland) and the Sardinian *P. auritus* samples form a monophyletic unit with respect to all other *P. auritus* haplotypes, we await information at a broader geographic scale before describing the Sardinian sample as representing a new subspecies.

#### *Implications for Conservation*

Based on our molecular analyses we confirm the presence of *P. auritus* and *P. austriacus* in Sardinia and add a third, *P. sardus* sp. nov. to Sardinia's bat fauna. The Sardinian *P. auritus* lineage is differentiated at the subspecific level from mainland

*P. auritus* samples. Consequently, two endemic lineages of long-eared bats may inhabit the island. Sardinia is therefore of extraordinary importance for European bat diversity. We suggest that bat conservation in Sardinia should be strengthened in general, since this will be beneficial for all species, including *P. sardus*. Furthermore we advocate the immediate establishment of a specific action plan for the conservation of its single potentially endemic bat species.

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## APPENDIX

Specimens used in the genetic analyses. Abbreviations: SMF = Forschungsinstitut Senckenberg, Frankfurt am Main, Germany; ZMFK = Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany; DZAB = Dipartimento di Zoologia e Antropologia Biologica, Sassari, Italy. Names of Sardinian samples are in parentheses in the voucher column

Haplotype	Locality	GenBank Accession No.	Voucher
<i>Plecotus austriacus</i> Paus-1	Bavaria, Germany ( $n = 2$ )	AY134022	O. von Helversen, Univ. Erlangen, Germany
<i>P. austriacus</i> Paus-2	Villavelayo, Spain	AY134023	PAT98082501, private collection of J. Juste, Sevilla, Spain
<i>P. austriacus</i> Paus-3	La Junguera, Spain	AY134024	SMF 97.207
<i>P. austriacus</i> Paus-Sar3	Monte Albo, Sardinia	AY175816	voucher not preserved (Sar 3)
<i>P. austriacus</i> Paus-Sar6	Supramonte, Sardinia	AY175817	voucher not preserved (Sar 6)
<i>P. austriacus</i> Paus-Sar9	Monte Albo, Sardinia	AY175823	voucher not preserved (Sar 9)
<i>P. austriacus</i> Paus-Sar10	Monte Albo, Sardinia	AY175815	voucher not preserved (Sar 10)
<i>P. austriacus</i> Paus-Sar11	Supramonte, Sardinia	AY175820	voucher not preserved (Sar 11)
<i>P. austriacus</i> Paus-Sar12	Supramonte, Sardinia	AY175814	voucher not preserved (Sar 12)
<i>Plecotus kolombatovici</i> Pkol-1	Orebic, Croatia ( $n = 2$ )	AY134025	D. Kovacic, Univ. Zagreb, Croatia
<i>P. kolombatovici</i> Pkol-2	Proastio, Greece	AY134026	voucher not preserved
<i>P. kolombatovici</i> Pkol-2	Dirrachi, Greece	AY134026	voucher not preserved
<i>Plecotus alpinus</i> Palp-1	Duvin, Switzerland,	AY134017	ZFMK 2001.328, coll. M. Lutz
<i>P. alpinus</i> Palp-1	Ristolas, France	AY134017	ZFMK 2001.325, coll. P. Favre & C. Joulot
<i>P. alpinus</i> Palp-2	Spaizzo, Italy	AY134018	voucher not preserved
<i>P. alpinus</i> Palp-3	Fischertratten, Austria	AY134019	ZFMK 2001.327, coll. G. Reiter
<i>P. alpinus</i> Palp-4	Waisach, Austria	AY134020	ZFMK 2001.326, coll. G. Reiter
<i>P. alpinus</i> Palp-5	Tymphristos, Greece	AY134021	O. von Helversen, Univ. Erlangen, Germany
<i>Plecotus sardus</i> Psar-1	Baunei, Sardinia	AY175819	private collection of M. Mucedda, Sassari, Italy (Sar 1)
<i>P. sardus</i> Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 2)
<i>P. sardus</i> Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 15)
<i>P. sardus</i> Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 20)
<i>P. sardus</i> Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 21)
<i>P. sardus</i> Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 22)
<i>P. sardus</i> Psar-13	Oliena, Sardinia	AY175822	holotype, DZAB 0023 (Sar 13)
<i>Plecotus auritus</i> Paur-1	Curaglia, Switzerland	AF529229	coll. No. 2513, Univ. Zürich, Switzerland
<i>P. auritus</i> Paur-1	Guarda, Switzerland	AF529229	ZFMK 2001.344, coll. M. Lutz
<i>P. auritus</i> Paur-1	Masein, Switzerland	AF529229	ZMFK 2001.343, coll. M. Lutz
<i>P. auritus</i> Paur-1	Winterthur, Switzerland	AF529229	coll. No. 2387, Univ. Zürich, Switzerland



## APPENDIX. Continued

Haplotype	Locality	GenBank Accession No.	Voucher
<i>P. auritus</i> Paur-2	Zagreb, Croatia ( $n = 2$ )	AF529230	D. Kovacic, Univ. Zagreb, Croatia
<i>P. auritus</i> Paur-3	Styria, Austria ( $n = 8$ )	AY134012	private collection of B. Freitag, Graz, Austria
<i>P. auritus</i> Paur-4	Bavaria, Germany ( $n = 2$ )	AY134013	O. von Helversen, Univ. Erlangen, Germany
<i>P. auritus</i> Paur-5	Moscow, Russia ( $n = 2$ )	AY134014	P. P. Strelkov, Inst. Zool., St. Petersburg, Russia
<i>P. auritus</i> Paur-6	Hall, Admont, Austria	AY134015	private collection of B. Freitag, Graz, Austria
<i>P. auritus</i> Paur-7	Villoslada, Spain	AY134016	PAR9808071, private collection of J. Juste, Sevilla, Spain
<i>P. auritus</i> Paur-Sar4	Bolotana, Sardinia	AY175824	voucher not preserved (Sar 4)
<i>P. auritus</i> Paur-Sar5	Supramonte, Sardinia	AY175818	voucher not preserved (Sar 5)
<i>P. auritus</i> Paur-Sar17	Supramonte, Sardinia	AY175821	voucher not preserved (Sar 17)
<i>P. auritus</i> Paur-Sar14	Bolotana, Sardinia	AY175825	voucher not preserved (Sar 14)
<i>P. auritus</i> Paur-Sar14	Bolotana, Sardinia	AY175825	voucher not preserved (Sar 16)
<i>Barbastella barbastellus</i>	Darmstadt, Germany	AF529231	SMF 84.732, coll. G. Herzig
<i>Myotis bechsteini</i>	Boos, Germany	AY134027	voucher not preserved