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

MAURO MUCEDDA¹, ANDREAS KIEFER², ERMANNO PIDINCHEDDA¹, and MICHAEL VEITH²

¹Centro per lo Studio e la Protezione dei Pipistrelli in Sardegna – Gruppo Speleologico Sassarese, Via dei Navigatori 7, 07100 Sassari, Italy; E-mail of MM: mucedda@ssmain.uniss.it
²Institute of Zoology, Department of Ecology, Mainz University, Saarstraße 21, D-55099 Mainz, Germany

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 (≥ 18 mm) and wide (≥ 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 alcatheo (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 genet-
cially 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 geneti-
cally similar to mainland populations because they only recently colonised the island, or do they constitute derived, genet-
cally 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 Pleco-
tus (Kiefer et al., 2002). Here we report the discovery of two genetically distinct lineag-
es of Sardinian Plecotus, one of which de-
serves 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 Sar-
dinia (Appendix). Samples for DNA extraction were obtained from wing tissue, using sterile biopsy punch-
es (Worthington Wilmer and Barrett, 1996). We recorded the following morphometric (hand-held cal-
ipher measurements to the nearest 0.05 mm) and oth-
er morphological characters, according to Stebbins (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_{Blatt} in Häussler and
Braun 1991); TW = tragus width; TH = thumb length
excl. claw; CL = claw length; SL = skull length; CBL = condylar length; SH = skull height (incl. bul-
lae); IOW = interorbital constriction width; M3-M2^3 = width across upper molars; C-M^3 = length of maxil-
lar toothrow; C-M^3 = length of mandibular tooth-
row; M1-M3 = width across lower molars; ML = mandible length; MW = mastoid width; CsPL = length of upper canines; MBD = maximal bulla diameter; ZW = zygomatic width; MDB = minimal dis-
tance between bullae; BL = length of baculum; BW = basal width of baculum.

The baculum of the holotype was extracted fol-
lowing the procedure of Anderson (1960). It was pho-
tographed 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 extrac-
tion kits (Qiagen). Double-stranded PCR was used to ampli-
fy 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) am-
plified 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 an-
nealing for 45 s at 55°C, extension for 90 s at 72°C.
PCR products were purified using the Qiaquick pu-
rification kit (Qiagen). We sequenced single-stranded
fragments on an ABI 377 automatic sequencer us-
ing standard protocols. We sequenced 555 bp of the 16S
rRNA gene that are homologous to the base pair po-
sitions 2215–2490 of the Pipistrellus abramus
complete mitochondrial genome (Nikaido et al., 2001).
These sequences were aligned to previously pub-
lished 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 dif-
f erent haplotypes were included in the analysis. For hierarchi-
 cal outgroup comparison we included Bar-
bastella 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 χ^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 γ-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 Helversen, 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 γ-shape parameter α = 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)

<table>
<thead>
<tr>
<th>Lineage</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) <em>P. auritus</em></td>
<td>0.057</td>
<td>0.060</td>
<td>0.112</td>
<td>0.118</td>
<td>0.118</td>
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<tr>
<td></td>
<td>(0.049–0.064)</td>
<td>(0.058–0.063)</td>
<td>(0.106–0.114)</td>
<td>(0.108–0.123)</td>
<td></td>
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<tr>
<td>(2) <em>P. alpinus</em></td>
<td>0.049</td>
<td>0.053</td>
<td>0.113</td>
<td>0.106</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.043–0.054)</td>
<td>(0.052–0.054)</td>
<td>(0.111–0.115)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(3) <em>P. sardus</em> sp. nov.</td>
<td>0.049</td>
<td>0.043</td>
<td>0.119</td>
<td>0.113</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.045–0.053)</td>
<td>(0.041–0.045)</td>
<td>(0.112–0.125)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4) <em>P. austriacus</em></td>
<td>0.082</td>
<td>0.090</td>
<td>0.119</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.069–0.094)</td>
<td>(0.083–0.092)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5) <em>P. kolombatovici</em></td>
<td>0.086</td>
<td>0.082</td>
<td>0.054</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>(0.082–0.091)</td>
<td>(0.079–0.085)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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. Pidinchedda 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; M3–M3, 6.25; M3–M3, 4.00; C–M3, 5.75; C–M3, 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. Pidinchedda 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. Pidinchedda 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: tattaggageactgctgccaggt

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

<table>
<thead>
<tr>
<th>Character</th>
<th>Sar 13 ♂–holotype</th>
<th>Sar 15 ♂</th>
<th>Sar 22 ♂</th>
<th>Sar 2 ♀</th>
<th>Sar 15 ♀</th>
<th>Sar 20 ♀</th>
<th>Sar 21 ♀</th>
<th>p</th>
<th>SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>forearm length</td>
<td>41.2</td>
<td>41.1</td>
<td>42.3</td>
<td>42.2</td>
<td>42.2</td>
<td>40.9</td>
<td>41.65</td>
<td>0.65</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>thumb length</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.07</td>
<td>0.16</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>claw length</td>
<td>3.1</td>
<td>2.0</td>
<td>2.4</td>
<td>2.5</td>
<td>2.5</td>
<td>2.6</td>
<td>2.52</td>
<td>0.35</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>ear length</td>
<td>37.5</td>
<td>38.0</td>
<td>38.6</td>
<td>39.0</td>
<td>–</td>
<td>–</td>
<td>38.28</td>
<td>0.66</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>tragus length</td>
<td>18.5</td>
<td>18.0</td>
<td>18.0</td>
<td>19.8</td>
<td>18.9</td>
<td>19.2</td>
<td>18.73</td>
<td>0.71</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>tragus width</td>
<td>6.5</td>
<td>6.2</td>
<td>6.0</td>
<td>6.4</td>
<td>6.5</td>
<td>6.4</td>
<td>6.33</td>
<td>0.20</td>
<td>6</td>
<td></td>
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<tr>
<td>hind foot length</td>
<td>7.7</td>
<td>7.5</td>
<td>7.0</td>
<td>7.6</td>
<td>6.8</td>
<td>6.7</td>
<td>7.22</td>
<td>0.44</td>
<td>6</td>
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<thead>
<tr>
<th>Character</th>
<th>auritus</th>
<th>austriacus</th>
<th>kolombatovici</th>
<th>alpinus</th>
<th>sardus sp. nov.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of dorsal fur</td>
<td>brown to reddish&lt;sup&gt;4&lt;/sup&gt;</td>
<td>grey&lt;sup&gt;3&lt;/sup&gt;</td>
<td>brownish&lt;sup&gt;4&lt;/sup&gt;</td>
<td>greyish-brown&lt;sup&gt;2&lt;/sup&gt;</td>
<td>brown&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colour of ventral fur</td>
<td>yellowish-brown to creamy&lt;sup&gt;3&lt;/sup&gt;</td>
<td>grey&lt;sup&gt;3&lt;/sup&gt;</td>
<td>whitish&lt;sup&gt;4&lt;/sup&gt;</td>
<td>white&lt;sup&gt;3&lt;/sup&gt;, white-grey&lt;sup&gt;2&lt;/sup&gt;</td>
<td>whitish to pale brown&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Forearm length</td>
<td>35.1–43.5&lt;sup&gt;2&lt;/sup&gt;</td>
<td>33.9–42.1&lt;sup&gt;2&lt;/sup&gt;</td>
<td>36.2–39.3&lt;sup&gt;2&lt;/sup&gt;</td>
<td>39.6–43.5&lt;sup&gt;2&lt;/sup&gt;</td>
<td>40.9–42.3&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Forearm length</td>
<td>37.5–39.7&lt;sup&gt;1&lt;/sup&gt;</td>
<td>38.4–42.0&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>40–45&lt;sup&gt;4&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td>Tragus width</td>
<td>4.5–5.5&lt;sup&gt;1&lt;/sup&gt;, &lt;5.5&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5.7–6.3&lt;sup&gt;1&lt;/sup&gt;, &gt;5.5&lt;sup&gt;4&lt;/sup&gt;</td>
<td>4.5–5.0&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5.5–6.0&lt;sup&gt;3&lt;/sup&gt;, 4</td>
<td>6.0–6.5&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>13.5–16.1&lt;sup&gt;1&lt;/sup&gt;, 14–16&lt;sup&gt;4&lt;/sup&gt;</td>
<td>12–14&lt;sup&gt;4&lt;/sup&gt;</td>
<td>16–19&lt;sup&gt;4&lt;/sup&gt;</td>
<td>18.0–19.8&lt;sup&gt;5&lt;/sup&gt;</td>
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<tr>
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<td>35.0–39.0&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>Ear length</td>
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<td>28.6–41&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>&lt;6.5&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&lt;6.5&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&gt;6.5&lt;sup&gt;1&lt;/sup&gt;, 6.5–7.0&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>Claw length</td>
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<td>&lt;2&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&lt;2&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&gt;2&lt;sup&gt;3&lt;/sup&gt;, 2.0–2.8&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.0–3.1&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>6.8–7.9&lt;sup&gt;1&lt;/sup&gt;, 7–8&lt;sup&gt;4&lt;/sup&gt;</td>
<td>8&lt;sup&gt;4&lt;/sup&gt;</td>
<td>&gt;8.5–9.0&lt;sup&gt;3&lt;/sup&gt;, 8&lt;sup&gt;5&lt;/sup&gt;</td>
<td>6.7–7.7&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>C–M&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>5.16–5.42&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5.36–5.74&lt;sup&gt;2&lt;/sup&gt;</td>
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<tr>
<td>C–M&lt;sub&gt;3&lt;/sub&gt;</td>
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<td></td>
<td>5.53–5.83&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5.82–6.16&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>1.93–2.18&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.61–1.75&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.77–1.99&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.50&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>over the eyes</td>
<td>large (&gt; 2 mm)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>small (&lt; 1 mm)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>small (&lt; 1 mm)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>medium (ca. 1–2 mm)&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>small (&lt; 1 mm)&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>medium (ca. 1–2 mm)&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>Penis shape</td>
<td>narrowing towards the end&lt;sup&gt;4&lt;/sup&gt;</td>
<td>club-shaped&lt;sup&gt;4&lt;/sup&gt;</td>
<td>club-shaped&lt;sup&gt;4&lt;/sup&gt;</td>
<td>almost cylindrical, pointed only at the tip&lt;sup&gt;4&lt;/sup&gt;</td>
<td>almost cylindrical, pointed only at the tip&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>Triangular pad at the chin</td>
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<td>no&lt;sup&gt;2&lt;/sup&gt;, 4</td>
<td>no&lt;sup&gt;2&lt;/sup&gt;, 4</td>
<td>yes&lt;sup&gt;2&lt;/sup&gt;, 4</td>
<td>no&lt;sup&gt;5&lt;/sup&gt;</td>
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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 plagio- patagium 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 calcare 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*,

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

**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. tenerifae* (Ibáñez and Fernandez, 1985); (e) *P. kolombatovici* (Dulić, 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.
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$^3$ and C–M$_3$ 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. tenerifae* (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 subsclade 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 differentiat-
ed 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.

**ACKNOWLEDGEMENTS**

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**LITERATURE CITED**


A new species of long-eared bat from Sardinia


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## APPENDIX. Continued

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*Note:* The table continues with information about additional specimens and their details.