Phylogeny of Western Palaearctic long-eared bats

(Mammalia, Chiroptera, *Plecotus*)

– a molecular perspective

Dissertation

zur Erlangung des Grades

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Mainz, im Dezember 2007

P. auritus (links) und *P. macrobullaris* (rechts)

Wir können alles schaffen
genau wie die tollen
dressierten Affen es schaffen
wir müssen nur wollen…
(Wir sind Helden)

Die Kapitel 3- 6 wurden in leicht abgewandelter Form veröffentlicht:

Kapitel 3:

KIEFER, A., MAYER, F., KOSUCH, J., VON HELVERSEN, O., VEITH, M. (2002): Conflicting molecular phylogenies of European long-eared bats (*Plecotus*) can be explained by cryptic diversity. – *Mol. Phyl. Evol*. **25**: 557-566.

Die ND1-Sequenzen wurden von Frieder Mayer, Erlangen, analysiert. Joachim Kosuch hat einen Teil der Laborarbeiten durchgeführt.

Kapitel 4:

KIEFER, A., VEITH, M. (2002): A new species of long-eared bat from Europe (Mammalia, Chiroptera, Vespertilionidae). – *Myotis*, **39**: 5-16.

Kapitel 5:

MUCEDDA, M., KIEFER, A., PIDINCHEDDA, E., VEITH, M. (2002): A new species of long-eared bat (Chiroptera, Vespertilionidae) from Sardinia (Italy). – *Acta Chiropterologica* **4**: 121-135.

Mauro Mucedda hat umfangreiche Aufsammlungen in Sardinien getätigt. Ermanno Pidinchedda hat bei diesen Aufsammlungen mitgeholfen.

Kapitel 6:

BENDA, P., KIEFER, A., HANÁK, V., VEITH, M. (2004): Systematic status of African populations of long-eared bats, genus *Plecotus* (Mammalia: Chiroptera). – *Folia Zoologica, Monograph 1*, **53**: 1-47.

Die umfangreichen morphologischen Analysen und Interpretationen wurden von Petr Benda, Museum Prag, durchgeführt, der auch zahlreiche afrikanische Fledermausbelege beigesteuert hat.

Die Publikationen der Kapitel 3 – 5 wurden von mir geschrieben. Die Daten hierfür wurden zum größten Teil von mir erhoben und analysiert. Die oben nicht erwähnten Autoren waren Betreuer (Veith) oder haben neben Sammlungsmaterial auch wichtige Beiträge zur Diskussion geliefert. (Hanák, von Helversen). Die Publikation des Kapitels 6 wurde gemeinsam von Petr Benda und mir geschrieben, wobei er den morphologischen Teil und ich den genetisch-phylogenetischen Teil formulierte. Der Rest dieser Arbeit war eine echte Teamarbeit, bei der Petr Benda und ich gleiche Anteile an der Arbeit hatten. Ich war an der Planung, Auswertung und am Schreiben des Artikels beteiligt.

Contents

1. Abstract

Phylogeny of Western Palearctic long-eared bats (Mammalia, Chiroptera, *Plecotus*) – a molecular perspective

Long-eared bats are an enigmatic group of bats that inhabit most parts of Europe up to the polar circle. Numerous taxa have been described in the past, but for a long time only two species were regarded valid. Further species were known from Northern Africa, the Canary Islands and Asia.

In the present thesis I used molecular data, partial sequences of the mitochondrial genes for 16S rRNA, ND1 and of the mitochondrial control region to analyse the phylogenetic relationship within and among lineages of Western Palaearctic long-eared bats. I estimated the best fitting substitution models and constructed phylogenetic trees using four different approaches: neighbor joining (NJ), maximum likelihood (ML), maximum parsimony (MP) and a Bayesian approach.

Seven lineages of long-eared bats are well differentiated at species level: *Plecotus auritus*, *P. austriacus*, *P. balensis*, *P. christii*, *P. sardus*, *P. teneriffae* and *P. macrobullaris*. I described three new taxa in this thesis: *Plecotus sardus*, *P. kolombatovici gaisleri* (= *Plecotus teneriffae gaisleri, Benda et al. 2004*) and *P. macrobullaris alpinus* [=*Plecotus alpinus*, Kiefer & Veith 2002). Morphological characteristics for field determination are described for the new taxa. Three of the species are polytypic: *P. auritus* (a western and eastern European lineage, and a most recently discovered Caucasian lineage), *Plecotus kolombatovici* (*P. k. kolombatovici* and *P. k. gaisleri and P. k. ssp*) and *P. macrobullaris* (*P. m. macrobullaris* and *P. m. alpinus*). A formerly fourth *P. auritus* subspecies, the Iberian *P. begognae* is now regarded as a distinct species (see chapter 7 and Ibanez *et al.* 2006, Mayer *et al.* 2007). The distribution areas of most species are refined based on genetically identified specimens.

The detection of a considerable amount of cryptic diversity among Western Palaearctic long-eared bats will have impact on species conservation. First steps towards better protection of the endemic Sardinian long-eared bats have been initiated, but until now it did not enter national and international legislation, such as the EU-habitat directive.

2. General introduction

In his “Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species cum characteribus, differentiis, synonymis, locis. Editio decima, reformata” Carl von Linné described in 1758 *Vespertilio auritus*, today known as the brown long-eared bat, *Plecotus auritus*. A second variant was described by Fischer in 1829 as *Vespertilio auritus var b austriacus* (=*Plecotus austriacus*), the grey long-eared bat. However, *P. austriacus* was considered conspecific with *P. auritus* by most coeval scientists and therefore suffered the same fate as numerous other old forms of *Plecotus* that were published in the 19th century and which never achieved scientific appreciation (Hanak 1966). Therefore, for most of the 20th century *P. auritus* was considered the only valid European species of long-eared bats.

Around 1960, several scientists discovered that two forms of *Plecotus* occurred in syntopy throughout Europe (Lanza 1959, Bauer 1960). However, it was Bauer (1960) who affiliated one of them to *P. austriacus*, the form previously described by Fischer (1829). Consequently, he resurrected the grey long-eared bat back into species rank. His enumeration of diagnostic morphological characters allowed future field discrimination of both forms. However, there still remained regional uncertainties in the determination of European long-eared bats, especially in the Alps and the Balkans. Hybrid status of such “dubious” specimens was assumed (Bauer, in Aellen 1961), although introgression has never been proven (Moretti et al. 1993).

Within *Plecotus auritus*, two geographically restricted subspecies were added to the nominotypical form: *P. a. macrobullaris* Kuzjakin 1965[1] in the Caucasus Mountains and *P. a. begognae* de Paz 1994 in the Iberian Peninsula. A single additional subspecies of the grey-long-eared bat, *P. austriacus kolombatovici* Dulic 1980, was described from the Mediterranean coast of former Yugoslavia.

A first African species of long-eared bat was already described in 1838 by Gray (*P. christii* from Egypt). Offshore the African continent, Barrett-Hamilton (1907) discovered a comparatively large species which was regarded endemic to the Canary Islands. He classified it as *Plecotus auritus teneriffae*. After showing that it was morphologically clearly differentiated from both *P. auritus* and *P. austriacus,* Ibanez & Fernandez (1985a) considered it a full species.

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[1] The description of *P.a. macrobullaris* is poor and published in Russian language only. It is not clear why, but all western European scientists ignored this species description, as did all Russian bat specialists (except Kuzjakin himself). Even P.P. Strelkow in his outstanding review of the genus *Plecotus* in the former U.S.S.R ignored Kuzjakin’s work.

Since the mid 1980s, the development and application of molecular techniques to phylogenetic questions accelerated the recognition of new species. Based on sequence analyses of mainly mitochondrial DNA, scientists started to re-examine seemingly well established phylogenies. Often they not only ended up in surprisingly new hypotheses on the phylogenetic relationships of species, they also discovered morphologically cryptic lineages that formerly had been regarded as populations of well-known species. A first amazing example was the discovery that an already known call variant of the Pipistrelle bat in fact resembled a species of its own, *Pipistrellus pygmaeus* Leach, 1825 (Barrett et al. 1997).

Indication for more cryptic speciation among European bats came from a broad survey on mitochondrial DNA variation of European bats (Mayer and von Helversen 2001). Among others, they showed that within the genus *Plecotus* three lineages were differentiated at species level: *P. auritus*, *P. austriacus* and *P. kolombatovici*, with the latter two being sister taxa. Interestingly, in the same year, Spitzenberger *et al.* (2001) published an alternative mitochondrial DNA phylogeny of European *Plecotus*, with *P. kolombatovici* being sister species of *P. auritus*. The results of Mayer and von Helversen (2001) and Spitzenberger *et al.* (2001) are mutually exclusive. They simply may be due to differential geographical sampling. This, however, inevitably leads to the assumption that Europe harbours at least four taxa of *Plecotus*.

A broad geographical sampling of all known species and subspecies of *Plecotus* from Europe and adjacent regions will provide new insight in the amount of cryptic speciation in European long-eared bats. In the present thesis I therefore

(1) test the hypothesis that the discordant results of Mayer and von Helversen (2001) and Spitzenberger *et al.* (2001) are due to the existence of a forth European *Plecotus* species;
(2) test for coexistence of *Plecotus* species on Sardinia, a well known western Mediterranean centre of endemism;
(3) study the phylogenetic relationship of European long-eared bats to North-African and Caucasian representatives of the genus;
(4) make, where necessary, taxonomical changes.

Finally, and in synopsis with the most recent (preliminarily) revision of the genus *Plecotus* published by Spitzenberger *et al.* in 2006, I will show that the story of finding more cryptic *Plecotus* species is still ongoing.

3. Conflicting molecular phylogenies of European long-eared bats (*Plecotus*) can be explained by cryptic diversity

3.1 Introduction

The application of molecular methods has added new insights into organismic evolution. Recently, some spectacular cases drastically changed long-held beliefs of taxa affiliations like the paraphyly of crustaceans with respect to insects (Burmester 2001; Garcıιa-Machado *et al.*1999) or the phylogenetic position of turtles as a sister group of the Archosauria (crocodiles and birds; Zardoya & Meyer 1998). Conflicts between molecular data and classical taxonomy often result from convergent morphological evolution during a species' radiation into vacant ecological niches. This produces similar phenotypes among non-related lineages (Schluter 2000; Wägele *et al.* 1999). Beak morphology of Darwin finches is a classic example (Grant 1986). In bats, Ruedi & Mayer (2001) showed that similar ecomorphs evolved convergently among unrelated Palearctic and Nearctic species of the genus *Myotis*.

Morphological similarity among species that occupy similar ecological niches complicates the recognition of species on the basis of morphological characters. Since differences accumulate with time in neutrally evolving genomic regions, DNA sequence analysis is a powerful tool to discover morphologically cryptic species diversity. Within European bats, several morphologically near-indistinguishable pairs of species are known, although genetically they are very distinct. In some cases they even do not group as sister taxa in phylogenetic analyses and thus have to be considered as similar ecomorphs that occupy similar ecological niches (Mayer & von Helversen 2001).

In 2001, two studies on mitochondrial gene sequences revealed inconsistent phylogenetic relationships among European *Plecotus* lineages. Mayer & von Helversen (2001) inferred a sister relationship of *P. kolombatovici* to its former conspecific, *P. austriacus* (Fig. 3-1a), while Spitzenberger *et al.* (2001) found evidence for a sister relationship of *P. kolombatovici* and *P. auritus* (Fig 3-1b). In both studies, bootstrap support for the respective sister relationship was high (98 versus 70 %). In both cases, the studied *P. kolombatovici* specimens met the original morphological description of Dulic (1980).

How can different genes of the same molecule (the bat mitochondrial genome is a single ring-molecule of about 16.650 bp length; Pumo *et al.* 1998) produce significant but conflicting results? Due to non-recombination, mitochondrial genes share the same genealogy, forming the rationale to derive phylogenies from combined mitochondrial data sets.

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Two hypotheses may account for the conflicting results of Mayer & von Helversen (2001) and Spitzenberger *et al.* (2001). (i) The ND1 and D-loop partitions of the mitochondrial *Plecotus* genome evolved differently. Due to different functional constraints, the processes of molecular evolution may differ among genes, and even among single base positions (Steward & Wilson 1987; Luo *et al.* 1989; Wolfe *et al.* 1989; Bull *et al.* 1993; Huelsenbeck *et al.* 1996). (ii) The *P. kolombatovici* specimens used in both studies may represent two different lineages with different affiliations to the known *Plecotus* species.

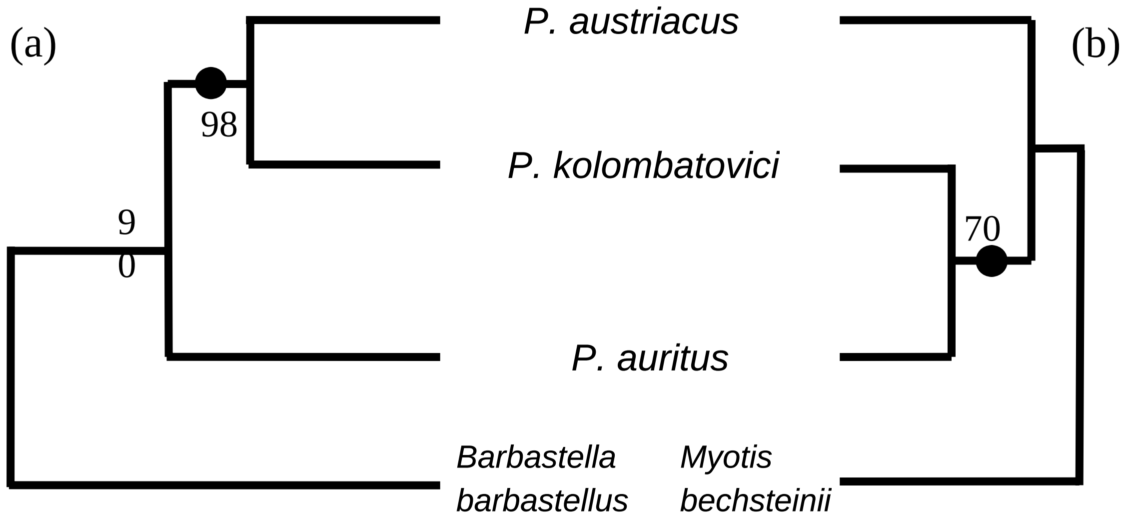

Figure 3-1. Two conflicting hypothesis about the phylogenetic relationships among European long-eared bats: (a) 800 bp of ND1 (Mayer & von Helversen 2001), (b) ca. 250 bp of the D-loop (Spitzenberger *et al.* 2001); black circles indicate significant relationships (bootstrap support) of *P. kolombatovici* to alternative sister groups.

To decide between these two hypotheses and to clarify species diversity we sequenced parts of the mitochondrial ND1 and 16S genes and of the D-loop of different *Plecotus* lineages. First, we analysed *Plecotus* specimens from a wide range of localities in Europe, including the samples studied by Mayer & von Helversen (2001). Second, we cross-linked our study with the data of Spitzenberger *et al.* (2001) by analysing the homologous fragment of the D-loop.

3.2 Material and Methods

3.2.1 Samples

Particularly long ears (>25mm) are characteristic for European species of the genus *Plecotus* (Geoffroy, 1818). In 1758, Linnaeus described only one species, *Plecotus (Vespertilio) auritus*. Several other European *Plecotus* species have been described since Linnaeus. However, only *P. austriacus* (Fischer, 1829) is currently regarded as valid (Bauer, 1960). It is larger than *P. auritus* and can also be distinguished by some fur characteristics, the thumb length and tragus shape. Only two subspecies, *Plecotus austriacus kolombatovici* (Dulic, 1980) and *P. auritus begognae* (de Paz, 1994) have been described for Europe. In 2001, species rank was suggested for the taxon *P. kolombatovici*, based on substantial genetic differences in mitochondrial DNA sequences (ND1 gene: Mayer & von Helversen 2001; control region: Spitzenberger *et al.* 2001).

We genetically analysed 35 long-eared bats from 17 localities in Europe (appendix 3-1). They covered all currently acknowledged taxa of European long-eared bats. All specimens were identified using external morphology in accordance with standard references (Dulic 1980; Strelkov 1988; 1989; von Helversen 1989). Some specimens could not be identified unambiguously as *P. auritus*, *P. austriacus*, or *P. kolombatovici*. Using the same set of characters (e.g., forearm length, thumb length, claw length, hind foot length, fur colour) they appeared to be between *P. auritus* and *P. austriacus*. For the time being we named them *Plecotus* indet.. Tissues were obtained from either fresh wing tissue or from tissue samples of ethanol-preserved or mummified specimens. For hierarchical outgroup comparison we consistently included *Barbastella barbastellus* and *Myotis bechsteinii*, the outgroups used by Spitzenberger *et al.* (2001) and Mayer & von Helversen (2001), respectively.

3.2.2 DNA sequencing

DNA was extracted using the QiAmp tissue extraction kits (Qiagen). Double-stranded PCR was used to amplify mitochondrial DNA fragments. The primers and cycling procedures were:

16S: 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 45 s at 94°C, primer annealing for 45 s at 55°C, extension for 90 s at 72°C.

ND1: DNA amplification and sequencing protocols are described in Mayer & von Helversen (2001).

D-loop: A partial sequence of the mitochondrial D-loop was amplified using the primers Phe (Haring *et al.* 2000) and Ple2+ (Spitzenberger *et al.* 2001). For PCR conditions see Spitzenberger *et al.* (2001).

PCR products were purified using the Qiaquick purification kit (Qiagen). We sequenced single-stranded fragments on an ABI 377 automatic sequencer using standard protocols.

Sequences obtained (lengths refer to the aligned sequences, including gaps) were comprised of 555 bp (16S), 901 bp (ND1) and 258 bp (D-loop) homologous to base pair positions 2215-2490, 2783-3446, and 16776-16926 of the *Pipistrellus abramus* mitochondrial genome (Nikaido *et al.* 2001). Sequences were aligned using the Clustal X software (Thompson *et al.* 1997).

3.2.3 Phylogenetic analyses

We tested for congruence among data partitions (Huelsenbeck *et al.* 1996; Whelan *et al.* 2001) using the parsimony method of Farris *et al.* (1994) as implemented in PAUP* (100 replicates, heuristic search using the TBR branch swapping algorithm).

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 40b8; Swofford 2001).

Prior to model assessment we performed a χ^2-Test for base distribution across sequences in order to rule out non-homogeneous base compositions that require the use of the paralinear LogDet distance measure 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). modeltest version 2.0 (Posada & Crandall 1998) was used to calculate the test statistic δ = 2 log Λ with Λ being the ratio of the likelihood of the null model divided by the likelihood of the alternative model (for details see Huelsenbeck & Crandall 1997). Due to the performance of multiple tests, we adjusted the significance levels of rejection of the null hypothesis via the sequential Bonferroni correction to α=0.01 (Rice 1989). We used the best fitting substitution model for further analyses.

Data were subjected to three different methods of phylogenetic reconstruction: (i) neighbor-joining (NJ) (Saitou & Nei 1987) using the selected substitution model; (ii) maximum parsimony (MP) with gaps treated as a fifth character state; transitions and transversions given equal weight; heuristic search with the TBR branch swapping algorithm; and

(iii) maximum likelihood (ML) analysis based on the selected substitution model. All analyses were run with PAUP* (Swofford 2001). Robustness of NJ and MP tree topologies was tested by bootstrap analyses (Felsenstein 1985), with 2000 replicates each (Hedges 1992). Only bootstrap values ≥70% indicate sufficiently resolved topologies (Huelsenbeck & Hillis 1993), those between 50 and 70 % were regarded as tendencies. Despite some reasonable criticism (Cao *et al.* 1998) but due to computational constraints, we used Quartet Puzzling (Strimmer & von Haeseler 1996) with 2000 permutations to infer reliability values (which are usually slightly higher than bootstrap values; Cao *et al.* 1998) for ML tree topologies. To increase confidence in ML topologies derived from Quartet Puzzling we also calculated ML trees based on 100 bootstrap replicates.

3.2.4 Cross-comparison with Spitzenberger's *et al.* (2001) lineages

To assign our samples and those of Spitzenberger's *et al.* (2001) to the same taxonomic groups we aligned our D-loop sequences with their sequences (GenBank accession numbers AY030054-AY030078). We added one museum specimen from Ogulin/Lika (Senckenberg Museum, Frankfurt SMF 44898) that had been described by Dulic (1980) as an intergrade between *P. austriacus* and *P. kolombatovici*. Only 180 bp could be sequenced for this specimen, which restricted the alignment to the respective number of bp. For optimal comparison we used the same substitution model (HKY85; Hasegawa *et al.* 1985) as Spitzenberger *et al.* (2001). We calculated a neighbor-joining tree with 2000 bootstrap replicates.

3.2.5 Molecular clock calibration

For molecular clock calibration we only used the ND1 fragment since it showed almost no signs of transition saturation (see results section).

To test for rate constancy among *Plecotus* haplotypes we conducted a likelihood ratio test using TREE-PUZZLE (Schmidt *et al.* 2000) with *Barbastella barbastellus* as the outgroup. We used the Tamura-Nei substitution model (Tamura & Nei 1993) with base frequencies and a gamma distribution shape parameter (α=0.4) estimated from the data.

A standard substitution rate of 2% per million years is usually applied to mammalian cytochrome *b* sequences (Jones & Avise 1998). Since Ruedi & Mayer (2001) showed that in the bat genus *Myotis* the cytochrome *b* and ND1 genes evolve at exactly the same rate (for overall mutation rate constancy among mammalian genes see Kumar & Subramanian 2002), this seems to be a standard substitution rate applicable to mitochondrial protein coding genes. We applied this substitution rate to date splits among major *Plecotus* lineages; 95% confidence intervals were calculated via mean time of divergence ±1.96 standard deviation of pairwise species comparisons.

3.3 Results

3.3.1 DNA sequence polymorphism

Standard sequence statistics from all three mitochondrial genome regions and their combinations are given in table 3-1. With the exception of the highly variable D-loop, transitions (ti's) by far outnumber transversions (tv's) up to a rate of 8.7 : 1 in ND1. This indicates that transitions are only weakly saturated in 16S and at best slightly saturated in ND1. A test for partition homogeneity revealed no conflicting phylogenetic signals among the three gene fragments (p = 1.00). Consequently, we combined all three mitochondrial genome regions (1714 base pairs) for further analyses. Nucleotides were homogeneously distributed across all 17 *Plecotus* haplotypes (χ^2 = 31.3, df = 54, p = 0.994). The likelihood ratio test selected the Tamura-Nei model (Tamura and Nei, 1993) with no invariable sites (I=0) and a gamma shape parameter of α=0.4826 as the most likely substitution model (-lnL = 7218.5483).

Table 3-1. Number of base pairs (bp), number of variable (VS) and parsimony informative (PI) sites, empirical base frequencies (π_A, π_G, π_C, π_T), skewness (g_1) and kurtosis (g_2) of four alignments of mitochondrial gene fragments; ti/tv ratios were calculated for ingroups only.

alignment	# bp	# VS	# PI	π_A	π_G	π_C	π_T	g_1	g_2	ti/tv ratio
16S/ND1/ D-loop	1714	607	439	0.325	0.241	0.154	0.281	0.603	0.657	6.03
16S	555	120	75	0.323	0.206	0.199	0.270	0.748	0.587	5.16
ND1	901	366	270	0.317	0.129	0.258	0.297	0.689	0.529	8.71
D-loop	258	121	94	0.360	0.113	0.278	0.251	0.948	0.842	2.58

3.3.2 Phylogenetic relationships

All long-eared bats form a monophylum with respect to the chosen outgroups. Within *Plecotus*, two major clades with two sub-clades each consistently emerge from all analyses (NJ, MP and both types of ML trees; only the NJ tree is shown in Fig. 3-2). Monophyly of *Plecotus*, sister clade relationships and monophyly of sub-clades are all supported by bootstrap values above 89% in all tree-evaluating approaches.

Specimens of *P. auritus* from Croatia, Germany, Switzerland, Hungary, Austria, Spain and Russia form a sister sub-clade to the specimens of *P.* indet. from Italy, Switzerland, Austria, France and Greece. The second sister relationship emerges between *P.*

austriacus from Spain, Austria, Switzerland and Germany and *P. kolombatovici* from Croatia and Greece.

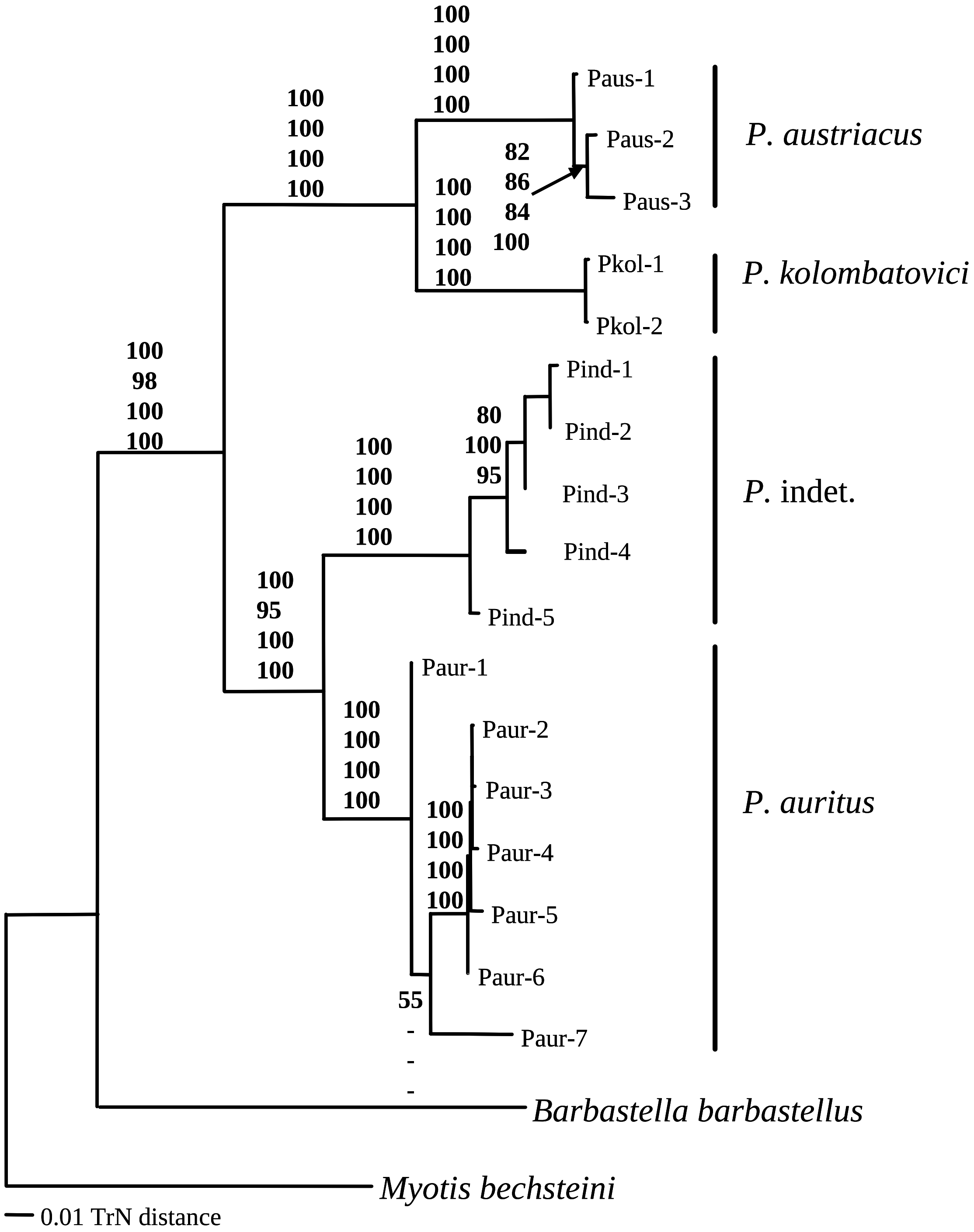

Figure 3-2. Neighbor-joining tree of 35 European Plecotus samples, based on 1714 bp of partial 16S, ND1 and D-loop sequences (Tamura–Nei substitution model with *I* = 0 and α = 0:4826); bootstrap supports are indicated for neighbor-joining, maximum parsimony, and maximum likelihood trees (upper, NJ; upper middle, MP; lower middle, ML; lower, Bayesian inference); 2000 replicates or 2000 puzzle steps were analysed;), this clade was not represented in the respective bootstrap tree.

Levels of genetic differentiation range from 0.33 to 0.43 TrN distance between haplotypes of the two major clades (table 3-2). Within major clades the level of genetic differentiation is lower, but within a similar range: 0.166 between *P. auritus* and *P.* indet., and 0.173 between *P. kolombatovici* and *P. austriacus*.

Within *P. auritus*, three more recently diverged lineages can be distinguished: (i) a lineage represented by a Spanish sample from the vicinity of the type locality of *Plecotus auritus begognae*, (ii) a German-Swiss lineage, and (iii) a wide-spread lineage that contains specimens from all over Europe.

The average TrN genetic distance among these lineages is 0.0227±0.0209. Within *P.* indet. the single Greek specimen belongs to a different lineage than animals from the Alps. Both lineages are differentiated by 0.0192 ± 0.0405 TrN distances. The two clades in *P. austriacus* reflect the classification of *P. a. austriacus* in central Europe and *P. a. hispanicus* on the Iberian Peninsula (Bauer 1956, 1960). No apparent pattern is visible within *P. kolombatovici*.

Table 3-2. Mean, minimum and maximum Tamura-Nei genetic distances within and among major *Plecotus* lineages.

lineage	(1)	(2)	(3)	(4)
(1) *P. auritus*	0.023	-	-	-
	(0.002-0.056)			
(2) P. indet.	0.166	0.009	-	-
	(0.104-0.220)	(0-0.026)		
(3) *P. austriacus*	0.3316	0.436	0.014	-
	(0.268-0.3638)	(0.3046-0.504)	(0.097-0.0019)	
(4) *P. kolombatovici*	0.3409	0.397	0.173	0.002
	(0.293-0.380)	(0.352-0.447)	(0.165-0.181)	

A comparison with the published sequences of Spitzenberger *et al.* (2001) was only possible for the D-loop region. As in the combined 16S/ND1/D-loop data set, we found two major lineages, with two sub-clades each (Fig. 3-3). Our samples clustered with the clade 3 sequences of Spitzenberger *et al.* (2001, their Pleaur-1, -2, -4, -8, -9, -10, -11, -12, -13, -14; here Paur-7, -8, -9, -10, -11, -12, -13, -14, -15, -16), while our *P. austriacus* samples clustered with their *P. austriacus* samples (their Pleaus-1, -2, -5, -7, -8, -11, -12; here Paus-4, -5, -6, -7, -8, -9, -10). Surprisingly, our *P. kolombatovici* samples and those of Mayer & von Helversen (2001) clustered with their unknown *Plecotus* taxon from Turkey (their Plesp-TR; here Pkol-3), while their *P. kolombatovici* haplotypes (their Pleaus-3, -9, -

10, -13, Pleaur-3, Plespec-K; here Pind-7, -8, -9, -10, -11, -12) clustered with our *P.* indet. Bootstrap supports for all four mixed clades were 90% and higher. The specimen from Ogulin/ Croatia (Pind-6) that was described by Dulic (1980) as an intergrade between *P. austriacus* and *P. kolombatovici* clustered together with our *P*. indet. samples.

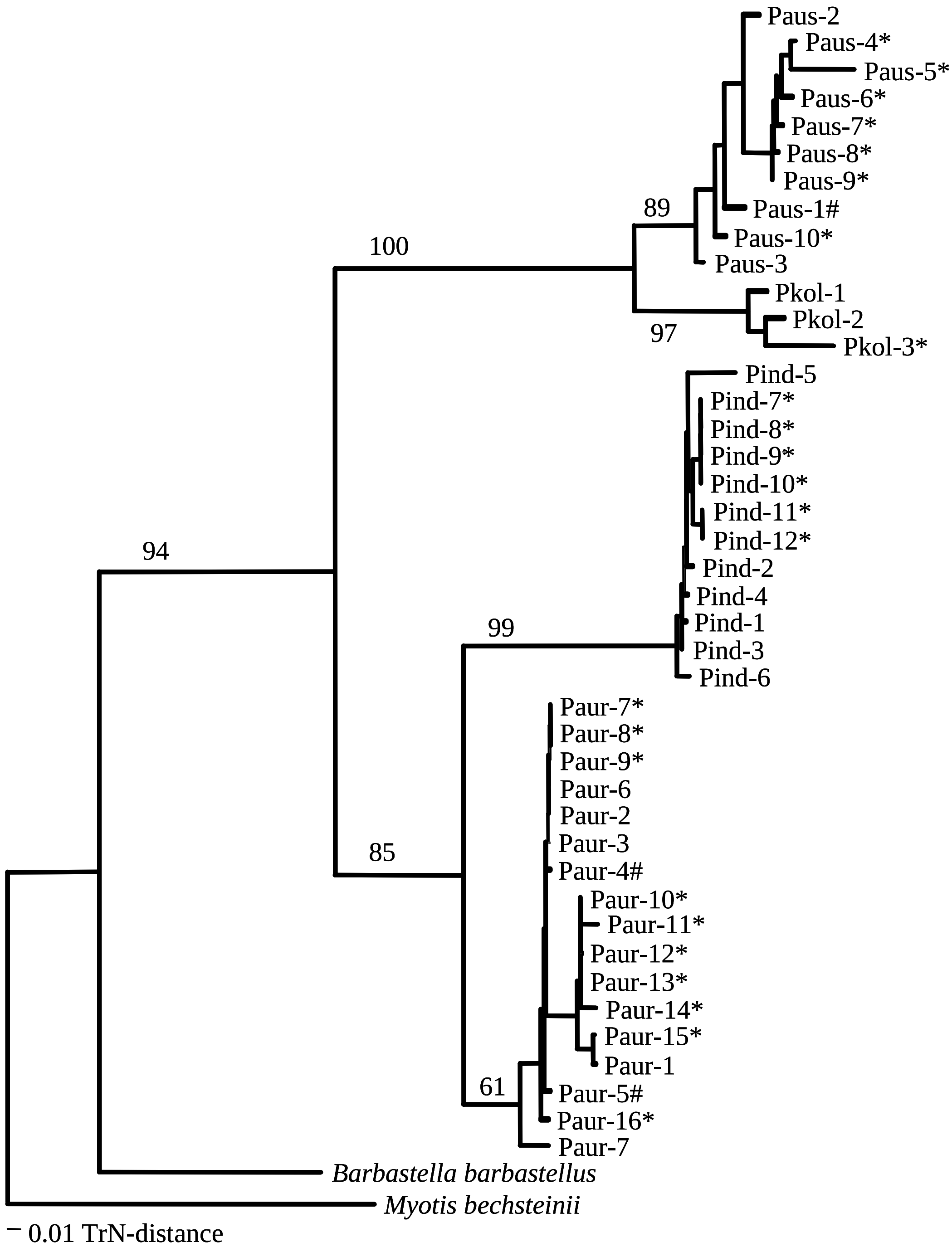

Figure 3-3. Neighbor-joining tree of 43 *Plecotus* D-loop haplotypes, based on the HKY85 substitution model; * = haplotypes from Spitzenberger *et al.* (2001), # = samples from Mayer & von Helversen (2001); bootstrap values for 2000 replicates are indicated.

3.3.3 Test for substitution rate constancy and molecular clock calibration

The molecular clock test revealed rate constancy among *Plecotus* lineages. The more complex tree (no clock; logL= -3340.16) was not significantly better than the simpler tree (molecular clock enforced; logL = -3352.00) on the 5% level.

The split between the two major clades can be dated to 4.22 million years ago (mya). Both clades almost simultaneously separated into sub-clades at 3.01 and 3.21 mya, respectively (Tab. 3-3).

Table 3-3. Estimated time of divergence between *Plecotus* sub-clades when applying a standard vertebrate substitution rate of 2% per million years (my) for the ND1 gene; n = number of pair-wise comparisons; the lower and upper 95% confidence limits (CI) were calculated from ±1.96 standard deviation of all pair-wise comparisons.

		estimated time of divergence [in my]		
split	n	lower 95% CI	mean	upper 95% CI
P. austriacus / *P. kolombatovici*	6	2.81	3.01	3.22
P. auritus / *P.* indet.	45	2.68	3.21	3.74
among major clades	70	3.73	4.22	4.71

3.4 Discussion

The partition homogeneity test unambiguously shows that the three gene regions do not produce conflicting phylogenetic results. Consequently, we can rule out the hypothesis that the use of different sections of the mitochondrial genome was responsible for the topological incongruity between Spitzenberger *et al.* (2001) and Mayer & von Helversen (2001). In contrast, a combined analysis of the sequences of Spitzenberger *et al.* (2001) and our samples (including the *P. kolombatovici* samples of Mayer & von Helversen, 2001) showed that two different evolutionary lineages (species) had been named *Plecotus kolombatovici* in previous phylogenetic analyses. These two lineages (species) are clearly distinct from *P. austriacus* and *P. auritus* and indicate the existence of at least four European species of long-eared bats.

3.4.1 Which lineage represents the true *Plecotus kolombatovici* Dulic 1980?

P. kolombatovici was described by Dulic (1980) on the basis of cranial and body morphology. She characterised *P. kolombatovici* as a small subspecies of *Plecotus austriacus* with a brownish dorsum and a whitish venter, which in some measurements, such as forearm, was even smaller than *P. auritus*. Spitzenberger *et al.* (2001) used cranial morphology to affiliate Dulic's long-eared bats to either species. Their *P. kolombatovici* specimens show a skull size intermediate to *P. auritus* and *P. austriacus*, which fit Dulic's (1980) description. In contrast, Mayer & von Helversen (2001) used external morphology to identify *P. kolombatovici*. They affiliated specimens from the Adriatic coast and Greece that were smaller than *P. austriacus* and *P. auritus* to *P. kolombatovici*. Consequently, none of the character sets used by either Spitzenberger *et al.* (2001) or Mayer & von Helversen (2001) covered the whole range of diagnostic characters and thus allowed for unambiguous affiliation of specimens to *P. kolombatovici*. Unfortunately, neither of the two studies analysed specimens from the type locality.

Ecological characteristics may therefore be used to separate *P. kolombatovici* from the fourth, currently undescribed species. From Dulic (1980) it becomes evident that *P. kolombatovici* is a coastal lowland form (type locality: Korcula, an Adriatic island of Croatia, 276 m a.s.l.). This also holds true for the *P. kolombatovici* specimens analysed by Mayer & von Helversen (2001) and for our specimens from the same clade. It is presently known from a narrow zone along the Adriatic coast of former Yugoslavia and to coastal habitats of Greece and Turkey.

In contrast and based on their own samples, Spitzenberger *et al.* (2001) regard *P. kolombatovici* as a faunal element of the mountainous regions of the Balkan peninsula and the Southern Alps. Most of their specimens originated from mountainous habitats and clustered in the D-loop tree (Fig. 3-3) with our *P.* indet. samples that came almost exclusively from high elevation localities above 800 m a.s.l. in the Swiss, Austrian, Italian and Croatian Alps and the Pindos mountains in Greece. This sharply contradicts Dulic's (1980) description of *P. kolombatovici* as an Adriatic lowland form. We may therefore conclude that the whole clade represents an as yet undescribed species of mountainous long-eared bats, erroneously described by Spitzenberger *et al.* (2001) as *P. kolombatovici*.

Evidence for the existence of a fourth *Plecotus* species comes from Dulic (1980) and Spitzenberger *et al.* (2001) themselves. In her original description of *P. kolombatovici* Dulic (1980) mentioned four morphologically distinct groups of long-eared bats: *Plecotus auritus*, *P. austriacus*, *P. kolombatovici*, and intergrades between *P. auritus* and *P. austriacus*. The latter came from Lika and Bosna (former Yugoslavia). We sequenced one of Dulic's (1980) intergrades from Ogulin/Lika. It unambiguously clustered into our *P.* indet. clade.

Four morphological groups of *Plecotus* also emerge from figure 3 of Spitzenberger *et al.* (2001). Their long-eared bats from Greece (not including Thrace) and Asia Minor are morphologically intermediate between clusters 3 (*P. auritus*) and 2 (their *P. kolombatovici*). They originate from areas where we found *P. kolombatovici*. In contrast, their cluster 2 comprises bats from areas inhabited by our *P.* indet. Consequently, our *P.* indet. and Spitzenberger's *et al.* (2001) cluster 2 (their *P. kolombatovici*) represent a fourth *Plecotus* lineage rather than *P. kolombatovici* (sensu Dulic, 1980). Meanwhile Kiefer & Veith (2002 = chapter 4) described this new taxon as a distinct species named *Plecotus alpinus*.

3.4.2 Altitudinal niche separation of European *Plecotus* species

In many areas of Europe *Plecotus auritus* and *P. austriacus* are regarded as an altitudinally vicariant pair of species. While *P. austriacus* usually forms nursery colonies in lowland roosts up to 400 m a.s.l. with a modal value of 300 m a.s.l., colonies of brown long-eared bats are usually found up to 1100 m a.s.l. with a modal value of 600 m a.s.l. (von Helversen *et al.* 1987; Stutz 1989; Müller 1993).

Within the Alps the situation becomes more complicated. Low elevation habitats are virtually lacking, and consequently *P. austriacus* would not be expected to occur in this region. Nevertheless, in most alpine regions a bimodal altitudinal distribution of long-eared bats is still evident (Spitzenberger 1993; Arlettaz *et al.* 1997). However, modal values are shifted towards higher altitudes (e.g., 600 m and 1100 m a.s.l. in Carinthia/Austria; Spitzenberger 1993). Nursery colonies of the lowland species *P. austriacus* are recorded at 1100 m a.s.l. in the Val Bregaglia in Grisons/Switzerland (Zingg & Maurizio 1991) and up to 1500 m a.s.l. in Carinthia (Spitzenberger 1993). Deuchler (1964) even mentioned a mixed colony of *P. austriacus* and *P. auritus* at 1640 m a.s.l. from Grisons. Interestingly, Arlettaz *et al.* (1997) described high altitude specimens of *P. auritus* as exceptionally large, sometimes even larger than *P. austriacus*.

It is likely that such bimodal altitudinal distributions represent *P. auritus* and *P*. indet.. Our data show that *P*. indet. occurs almost exclusively at high altitudes (appendix 3-1). Occasional high altitude records of *P. austriacus* (e.g., Aellen 1971, Deuchler 1964) can therefore most probably be attributed to *P*. indet..

Plecotus austriacus, *P. auritus* and *P*. indet. occupy different altitudinal niches. Whereas *P. austriacus* dominates open lowland habitats, *P. auritus* is typical for montane forest habitats, mostly below 1000 m a.s.l.. Consequently, both species widely co-occur throughout Central and Eastern European highlands. In the Alps, *P. auritus* is sympatric with *P*. indet., which usually prefers open habitats above 800 m a.s.l.. *Plecotus kolombatovici* replaces *P. austriacus* in eastern Mediterranean coastal areas.

3.4.3 A paleobiogeographic scenario of *Plecotus* evolution in Europe

Mayer & von Helversen (2001) concluded from their analysis of ND1 that *P. kolombatovici* is differentiated from its sister taxon *P. austriacus* at a level above that of hybridising European bat species (Mayer & von Helversen 2001). Sympatric occurrence in Thrace, Greece (von Helversen, unpublished) supports the species status of *P. kolombatovici*. Genetic divergence of *Plecotus auritus* and *P*. indet. is in the same range as for *P. austriacus* and *P. kolombatovici*. Again, sympatry of both species in Delphi, Greece (Spitzenberger *et al.* 2001), Northern Italy and Grisons, Switzerland (Kiefer, unpublished) adds support for assigning species status to *P*. indet..

Based on our molecular clock calibration, all four *Plecotus* species are of mid- or late Pliocene origin. This corresponds to the assumed origin of many Palearctic and Nearctic bat species of the genus *Myotis* (Castella *et al.* 2000; Ruedi & Mayer 2001). Although having diverged several million years ago all four species remained morphologically very similar. Until 1960 only a single species of *Plecotus* was recognised in Europe.

3.5 Conclusions

Molecular phylogenetic analyses are without doubt extremely valuable for deriving hypotheses on the evolution of species. We could demonstrate that two significant but conflicting hypotheses, both derived from the analysis of a single mitochondrial gene fragment, simply arose due to the misidentification of lineages. Since current taxonomy is based and will be based on designation of type specimens, we have to keep in mind that molecular phylogenetic analyses do not free systematists from a thorough inclusion of morphological and ecological data.

Abstract

Conflicting phylogenetic signals of two data sets that analyse different portions of the same molecule are unexpected and require an explanation. In the present paper we test whether (i) differential evolution of two mitochondrial genes or (ii) cryptic diversity can better explain conflicting results of two recently published molecular phylogenies on the same set of species of long-eared bats (genus *Plecotus*). We sequenced 1714 bp of three mitochondrial regions (16S, ND1, and D-loop) of 35 *Plecotus* populations from 10 European countries. A likelihood ratio test revealed congruent phylogenetic signals of the three data partitions. Our phylogenetic analyses demonstrated that the existence of a previously undetected *Plecotus* lineage caused the incongruities of previous studies. This lineage is differentiated on the species level and lives in sympatry with its sister lineage, *Plecotus auritus*, in Switzerland and Northern Italy. A molecular clock indicates that all European *Plecotus* species are of mid or late Pliocene origin. *Plecotus* indet. was previously described as an intergrade between *P. auritus* and *Plecotus austriacus* since it shares morphological characters with both. It is currently known from elevations above 800 m a.s.l. in the Alps, the Dinarian Alps and the Pindos mountains in Greece. Since we could demonstrate that incongruities of two molecular analyses simply arose from the misidentification of one lineage, we conclude that molecular phylogenetic analyses do not free systematists from a thorough inclusion of morphological and ecological data.

4. A new species of long-eared bat (*Plecotus*; Vespertilionidae, Mammalia) in Europe

4.1 Introduction

Particular long ears (>25mm) are characteristic for all long-eared bats. They are widely distributed throughout the Northern Hemisphere and comprise the Palearctic genus *Plecotus* (Geoffroy, 1818) and the Nearctic genera *Corynorhinus*, *Idionycteris* and *Euderma*. The Nearctic taxa were included as subgenera in the genus *Plecotus* by Handley (1959). However, this view was rejected, based on cytogenetic (chromosome banding, e.g. Fedyk & Ruprecht 1983, Stock 1983, Qumsiyeh & Bickham 1993, Volleth & Heller 1994) and/or morphological data (Frost & Timm 1992, Bogdanowicz *et al.* 1998). Recently, Hoofer & van den Bussche (2001) used mitochondrial DNA sequences to re-evaluate vespertilionid phylogeny. According to their analysis, long-eared bats represent a tribus of their own, the Plecotini. It includes the Nearctic *Corynorhinus*, *Idionycteris* and *Euderma*, and the Palearctic genera *Plecotus* and *Barbastella*.

In the past, species designation within the genus *Plecotus* has frequently changed. Linnaeus (1758) only recognised the brown long-eared bat, *Plecotus auritus*. Subsequently, several new species were described (e.g. Fischer 1829, Jenyns 1828, Koch 1860, Barrett-Hamilton 1907, Thomas 1911b, see also Bree & Dulic 1963), but none of these was accepted until 1960. Bauer (1960) re-validated *Plecotus austriacus* (Fischer, 1829), formerly a variety of *P. auritus*. Since then, only two further subspecies, *Plecotus auritus begognae* (de Paz, 1994) and *P. austriacus kolombatovici* (Dulic, 1980) have been described from Europe.

Plecotus austriacus is widely distributed from the Cape Verde Islands, northern Africa, Central Europe, and the Arabian Peninsula to the Himalayas (Strelkov 1988, 1989, Swift 1998). *Plecotus auritus,* a more montane species, is widespread from Ireland through Central and northern Europe, the Ural and Caucasus Mountains, Mongolia to northeast China and Japan (Strelkov 1988, 1989, Corbet & Hill 1991, Swift 1998). However, the systematics and taxonomy of eastern Palearctic populations of both species still remain unclear (e.g. Strelkov 1988, 1989a, Yoshiyuki 1991).

Recently, several *Plecotus* species have been recognized. *Plecotus teneriffae* (Barret-Hamilton, 1907), formerly a subspecies of *P. auritus*, is now treated as a species endemic to the Canary Islands (Ibáñez & Fernandez 1985a). *Plecotus balensis* (Kruskop & Lavrenchenko, 2000) was newly discovered in the Bale Mountains, Ethiopia.

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Using mitochondrial DNA, Mayer & von Helversen (2001) and Spitzenberger *et al.* (2001) recognized three *Plecotus* lineages in Europe, namely *P. auritus*, *P. austriacus* and *P. kolombatovici*. Both studies argued that *P. kolombatovici* is clearly differentiated at the species level. Surprisingly, Mayer & von Helversen (2001) demonstrated a sister relationship of *P. kolombatovici* and *P. austriacus*, while Spitzenberger *et al.* (2001) found *P. auritus* to be the sister species of *P. kolombatovici*. As shown by in chapter 3, Spitzenberger *et al.* (2001) incorrectly assigned the name *P. kolombatovici* to a clade that obviously represented a fourth, currently unknown species (Fig. 4-1, Tab. 4-1). We here describe this new species and present preliminary data on its morphological variation.

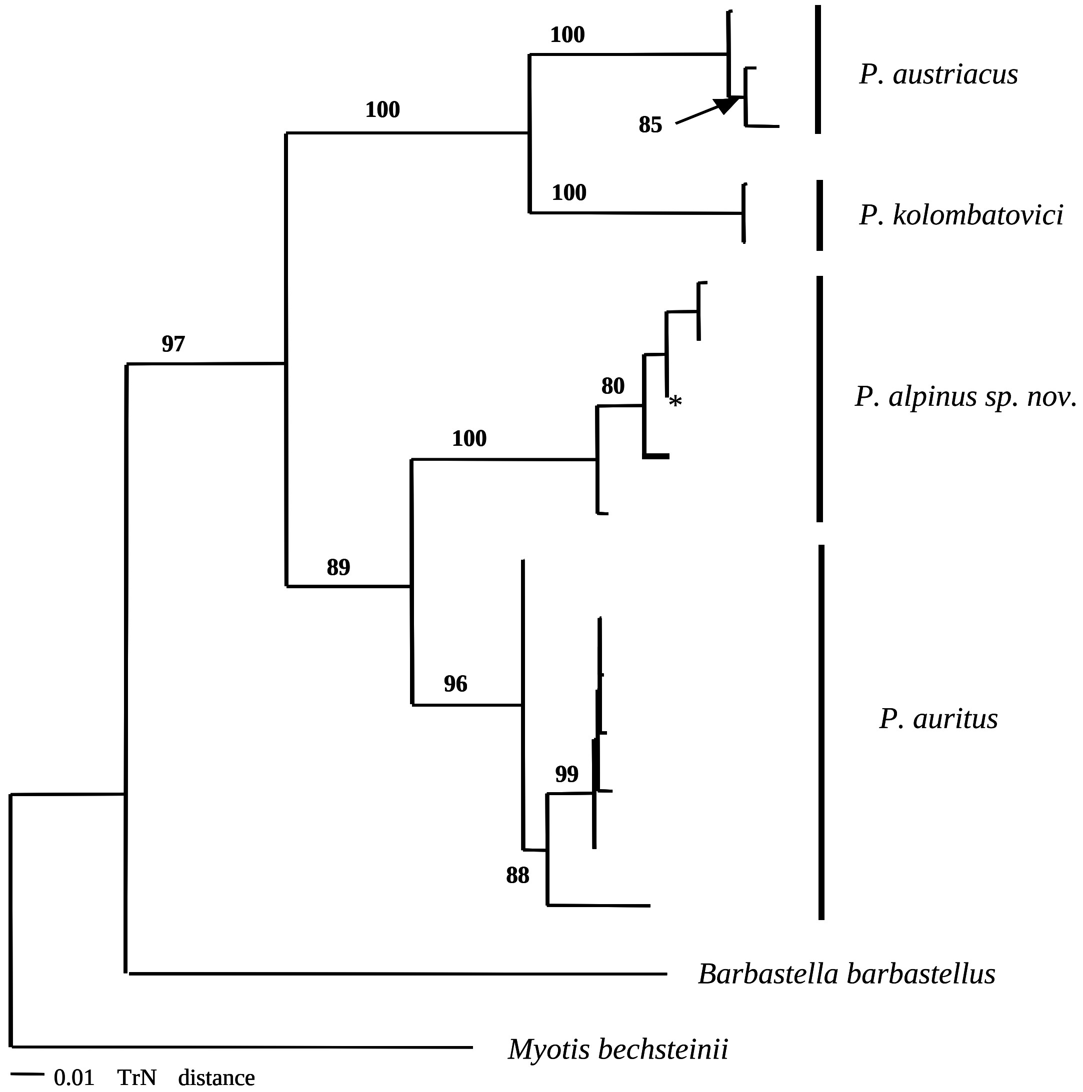

Figure 4-1. Neighbor-joining tree of European *Plecotus* samples (modified after chapter 3), based on 1714 bp of mitochondrial 16S, ND1 and D-loop gene fragments (Tamura-Nei substitution model with I=0 and G=0.4826; for details see chapter 3). An asterisk indicates the position of a specimen with a D-loop sequence identical to the holotype.

4.2 Material and methods

All specimens of the new species were identified using parts of the mitochondrial 16S or D-loop genes (for details see chapter 3). A total of six specimens (four males, two females) were investigated. Five specimens were dry skins, one specimen is preserved in alcohol. We used five extracted skulls and two bacula for cranial and bacular morphology. Three voucher specimens are stored in the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn (ZFMK), one specimen in the Senckenberg Institute, Frankfurt (SMF) and one specimen is deposited in the private collection of O. von Helversen, Erlangen, Germany. For comparison we analysed specimens of all European *Plecotus* species which previously have been identified using DNA-sequencing.

We took the following measurements: FA = forearm length (with wrist), HF = hind foot length (without claws), TL = tragus length, TW = tragus width, TH = thumb length without claw, CL = claw length, TA = tail length, SL = skull length, CBL = condylobasal length, SH = skull height (with bullae), IOW = interorbital constriction width, M^3-M^3 = width across upper molars, C-M^3 = length of maxillary toothrow, C-M_3 = length of mandibular toothrow, ML = mandible length, MW = mastoid width, CsupL = Length of upper canines, MBD = maximum bulla diameter, ZW = zygomatic width, MDB = minimal distance between bullae, 3MT = length of 3rd finger, 5MT = length of 5th finger, BL = length of baculum, BW = basal width of baculum, VHL = total ventral hair length, %WT = percentage of white tip in ventral hair length.

The bacula were obtained following the maceration procedure of Anderson (1960). They were photographed with a digital imaging unit (Leica DC 300) on a Leica photomicroscope and compared to published drawings of bacula of other *Plecotus* species. Two size parameters were scored for the Y-shaped bacula: LB – length of baculum and BW – basal width of baculum. We also analysed qualitative features of external and cranial morphology.

Table. 4-1. Mean Tamura-Nei genetic distances within and among major European *Plecotus* lineages (modified after chapter 3).

lineage	*(1)*	*(2)*	*(3)*	*(4)*
(1) *P. auritus*	0.023	-	-	-
(2) *P. alpinus* sp. nov.	0.166	0.009	-	-
(3) *P. austriacus*	0.331	0.436	0.014	-
(4) *P. kolombatovici*	0.340	0.397	0.173	0.002

4.3 Results and description

Molecular data unambiguously separate the new *Plecotus* species from *P. auritus*, *P. austriacus* and *P. kolombatovici* and therefore constitute excellent characters for species determination, useful in species descriptions (e.g. Veith *et al.* 2001). The molecular differences are corroborated by the external, cranial and bacular morphology.

Plecotus alpinus Kiefer & Veith, 2002.

Holotype.

Adult male, ZFMK 2001.325; Ristolas, Haute-Alpes, France, 06°57' N, 44°46'E, 1600 m a.s.l., August 24, 2001; collected dead on road by Philippe Favre (Groupe Chiroptères de Provence); dry skin, skull and baculum, with carcass in alcohol (Figs 4-2, 4-3, 4-4).

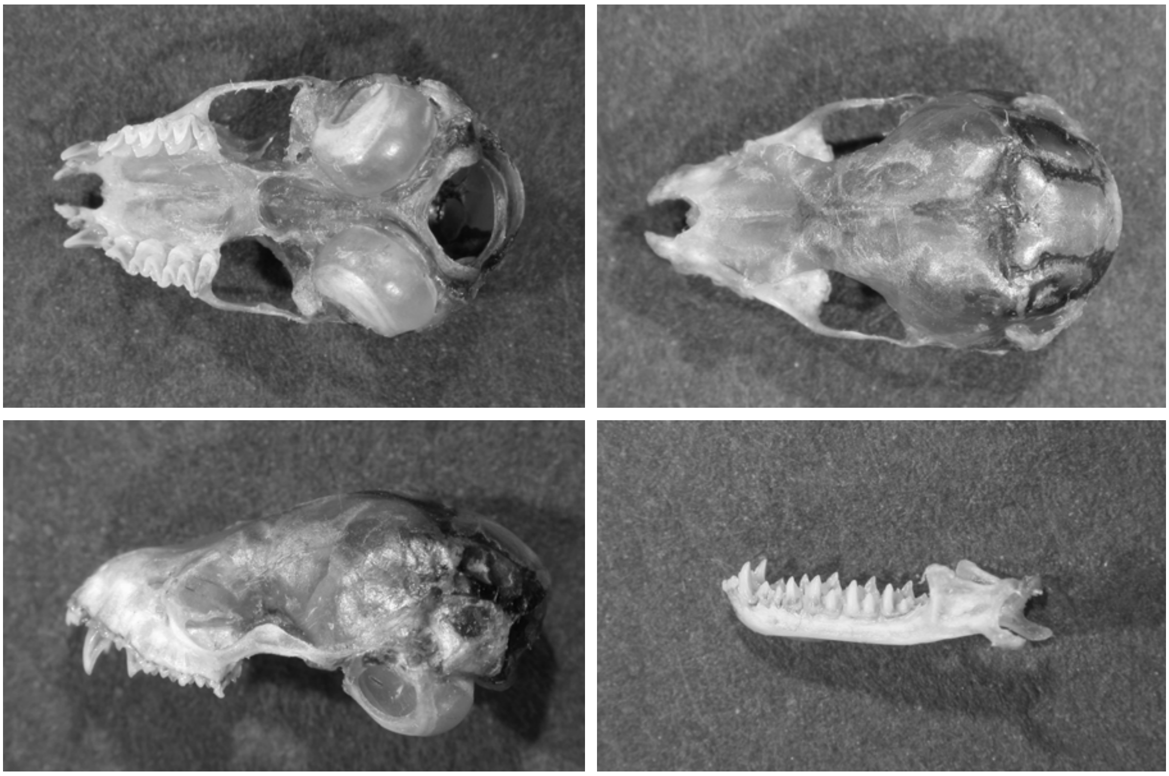

Figure 4-2. Skull (lateral, ventral and dorsal view) and mandible of *Plecotus alpinus* sp. nov. (holotype, ZFMK 2001.325).

Other specimens studied.

Adult male, private collection of O. von Helversen, Tymphristos, Karpenissi, Greece, 38°55' N, 21°55’E, 1800 m a.s.l., June 12, 2001; collected by O. von Helversen; dry skin, skull and baculum, with carcass in alcohol.

Adult female, ZFMK 61.451, Schaan, Liechtenstein, 51°06' N, 06°28' E, August 23, 1961; collected by E. von Lehmann; skin and skull.

Adult female, ZFMK 2001.327, Fischertratten, Austria, 46°56' N, 13°31' E, 768 m a.s.l., May 15, 2000; collected by G. Reiter; skin and skull.

Adult male, SMF 44898, Ogulin, Lika, Croatia, 1972; collected by J. Gelencir; skin in alcohol with skull.

Juvenile male, ZFMK 2001.328, Duvin, Switzerland, 46°43' N, 09°12' E, 1080 m a.s.l., August 29, 2000; collected by Miriam Lutz; dry skin with skull.

Diagnosis.

Long-eared bat of medium to large size with a condylobasal length of 15.5 – 15.8 mm. The overall impression of the ventral fur is dense and more whitish than in all other European *Plecotus* species. Thumb, claw and forearm are comparative large and the tragus is remarkably long (>16 mm). It differs from all European *Plecotus* species in its DNA sequences of the 16S and D-loop mitochondrial genes.

16S; GenBank accession number AY081062 (homologous to positions 2215-2490 of the *Pipistrellus abramus* mitochondrial genome, GenBank accession number AB061528):

gtattagaggcattgcctgcccagtgactctagttaaacggccgcggtatcctgaccgtgcaaaggtagcataatcatt
tgttctctaaatagggacttgtatgaatggcctcacgagggtttaactgtctcttacttttaatcagtgaaattgacactcccgtgaa
gaggcgggaattaaaaaataagacgagaagaccctatggagcttcaattaactaacttataagttataatactaatctaaaa
gagacaaatcaaacttgactaagttaacaatttgggttggggcgacctcggaataaaaatcaacttccgagatagatctacta
agacctacaagtcaaagttatcttaccacacattgatccgccaatgacgatcaacgaaacaagttaccctagggataacagc
gcaatcctatttaagagcccatatcgacaattagggtttacgacctcgatgttggatcaggacatcccaatggtgcagcagcta
ttaatgtgttcgtttgttcaacgattaaagtcctacgtgatctgagt.

D-Loop; GenBank accession number AY081061 (homologous to positions 16776-16926 of the *Pipistrellus abramus* mitochondrial genome, GenBank accession number AB061528):

tcttgccaaaccccaaaaacaagaagaataatattacgacacttatagacttaactcatactctgcaccaaatctata
actttctcccaccacaaagtcacaccccctctactttaaagatacaatttccttagacagacatgtcctcagatctgcaaacgggc
cttcaaacacaacacgc.

Description and comparison.

Plecotus alpinus Kiefer & Veith 2002 is a medium to large-sized representative of the genus with a dense fur. The tip of the ventral hair is white, not grey as in *P. austriacus* or *P. kolombatovici* or yellowish-brown to creamy as in *P. auritus*. Its ventral fur appears more whitish than in all other European *Plecotus* since the white tips of the ventral fur cover more than 50% of total hair length (*P. auritus*: 33%, *P. kolombatovici*: 37%, *P. austriacus*: 51%) The total length of the ventral hair is with more than 9.5 mm much longer than in any other European *Plecotus* species. The dorsal fur is pale-grey with a black base, a whitish central part and darker tips and resembles that of *P. austriacus*. In the juvenile specimen the ventral fur is completely white, the darker base is absent.

The protuberances in front of the eyes are larger than in *P. austriacus* but smaller than in *P. auritus*. Forearm length is medium to large; thumb and claw are as big as in *P. auritus* (Dulic 1980, Häussler & Braun 1991, von Helversen 1989). The hind foot is medium to large sized and covered with long patulous hairs like in *P. auritus* (von Helversen 1989). The tragus is longer than in *P. auritus*, *P. austriacus* and *P. kolombatovici* (Dulic 1980, von Helversen 1989, Häussler & Braun 1991). The proximal part of the ear and the tragus is flesh-coloured, whereas the distal part of the tragus is dark as in *P. austriacus*.

The skull (Fig. 4-2) is medium-sized with a comparatively long rostrum and medium-sized tympanic bulla (Hának 1966, Dulic 1980, Häussler & Braun 1991). The processus angularis mandibulae has no well-marked horn like in *P. austriacus* and its end has no club-shaped widening like in *P. auritus* (Fig. 4-2, see Ruprecht 1965). Shape and size of the baculum (Fig. 4-3, Tab. 4-2) are intermediate between *P. auritus* and *P. austriacus* (Topál 1958, Lanza, 1960, Strelkov, 1988, 1989a). In addition, Dulic (1980) herself pointed out that the baculum of a specimen from Lika is atypical for both *P. austriacus* and *P. auritus*.

Specimens examined for comparison.

Plecotus auritus: ZFMK 2.001.329 (m); ZFMK 2.001.330; ZFMK 2.001.331 (f), ZFMK 2.001.332; ZFMK 2.001.333 (f); ZFMK 2.001.334 (f); ZFMK 2.001.335; ZFMK 2.001.336; ZFMK 2.001.337; ZFMK 2.001.338; *Plecotus austriacus*: ZFMK 77.49; ZFMK 77.50; ZFMK 2.001.339 (f); ZFMK 2.001.340; ZFMK (f); ZFMK 2.001.341 (f); ZFMK 2.001.342 (f). *Plecotus kolombatovici*: ZFMK 79.214 (m).

Etymology.

Named after the alpine region, where the new species is regularly found. We suggest the following vernacular names: Alpine long-eared bat (English), Alpenlangohr (German), Oreillard des Alpes (French).

Distribution and life history.

Plecotus alpinus Kiefer & Veith 2002 is currently known from the Alps (France, Austria, Liechtenstein, Switzerland, Italy, Slovenia), the Dinarian Mountains (Croatia), and the Pindos Mountains in Greece (chapter 3). It occurs at altitudes above 800 m a.s.l., with only one known exception from Italy (Pesina, province of Verona, 480 m a.s.l., see Fig. 4-4). However, these specimens were caught directly at the foot of the Monte Baldo massif.

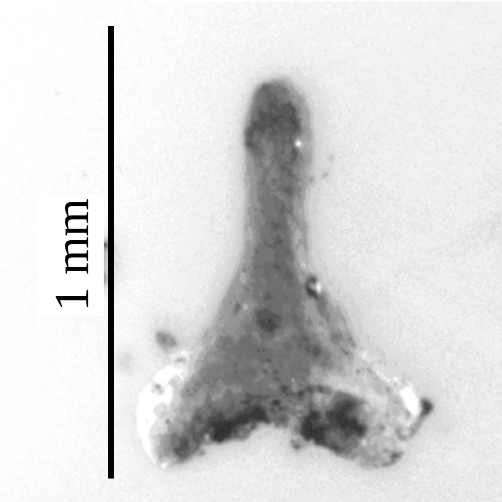

Figure 4-3. Baculum of *Plecotus alpinus* Kiefer & Veith 2002 (dorsal view; holytype, ZFMK 2001.325).

Table 4-2. Individual measurements (mean and standard deviation, SD) of male (m) and female (f) adult specimens of *Plecotus alpinus* Kiefer & Veith 2002, including the holotype (no. 1).

measurement	*1 (m)*	*2 (m)*	*3 (f)*	*4 (f)*	*5 (m)*	*mean ± SD*
FA	40.5	39.7	39.7	39.8	42.2	40.38 ± 1.07
HF	8.5	9.0	8.5	8.5	8.5	8.60 ± 0.22
TL	16.0	18.0	17.0	17.0	-	17.00 ± 0.82
TW	5.5	6.0	5.5	5.5	-	5.63 ± 0.25
TH	7.0	7.0	7.0	6.5	6.8	6.86 ± 0.22
CL	2.3	2.0	2.8	2.3	-	2.35 ± 0.33
TA	-	-	-	-	53.5	
SL	16.8	16.8	16.8	16.9	16.8	16.80 ± 0.07
CBL	15.8	15.5	15.7	15.8	15.5	15.67 ± 0.17
SH	7.5	7.7	8.0	7.7	-	7.73 ± 0.19
IOW	3.7	3.5	3.8	3.5	3.6	3.64 ± 0.13
M^{3}-M^{3}	6.5	6.6	6.6	6.5	6.1	6.45 ± 0.21
C-M^{3}	5.7	5.7	6.3	5.5	5.6	5.76 ± 0.31
C-M_{3}	6.2	6.0	6.2	6.5	6.0	6.18 ± 0.20
ML	10.9	10.9	10.9	10.8	10.7	10.85 ± 0.10
MW	9.1	9.1	8.9	9.1	9.0	9.03 ± 0.07
CsupL	1.3	1.2	1.2	1.8	-	1.38 ± 0.27
MBD	4.6	4.6	4.6	4.6	4.5	4.59 ± 0.05
ZW	8.9	9.1	8.8	8.8	8.5	8.81 ± 0.21
MDB	0.8	0.9	0.9	0.8	-	0.85 ± 0.09
3 MT	64.0	67.0	62.0	64.0	-	64.25 ± 2.06
5 MT	51.0	52.5	51.0	51.0	-	51.38 ± 0.75
BL	0.61	0.51	-	-	-	0.56 ± 0.07
BW	0.85	0.85	-	-	-	0.85 ± 0.00
VHL	10.0	10.0	9.7	-	8.3	9.90 ± 0.17
% WT	53	55	56	-	50	53.67 ± 1.15

1 = ZFMK 2001.325 (holotype); 2 = Tymphristos [= *P. macrobullaris macrobullaris*]; 3 = ZFMK 61.451 (Schaan); 4 = ZFMK 2001.327 (Fischertratten); 5 = SMF 44898 (Ogulin, data from Kock 1974).

4.4 Discussion

Plecotus alpinus Kiefer & Veith 2002 is an alpine sister species of the brown long-eared bat, *P. auritus.* The new species can unambiguously be distinguished from other European *Plecotus* species based on molecular data (Fig. 4-1). It shares morphological similarities with *P. auritus* and *P. austriacus*, although being well distinct from either species in several traits. However, its combination of characteristic traits makes the species to look like an intermediate between *P. auritus* and *P. austriacus*. This is probably the reason why it has not been discovered until recently. In fact, "intermediates" between known *Plecotus* species have been described from the distribution range of the Alpine long-eared bat (e.g., Dulic 1980), one of which proved to represent a specimen of *P. alpinus* Kiefer & Veith 2002 (from Ogulin, Lika, Croatia; see Kiefer *et al.* 2002, chapter 3).

Aellen (1961) caught bats at the Col de Bretolet at the French-Swiss border at ca. 2000 m a.s.l. One of these, a very large female *Plecotus,* was examined by Bauer (in Aellen 1961) who suggested that it was a hybrid between *P. auritus* and *P. austriacus*. From its external characters, it represented a large *P. auritus* whereas its skull was more typical for *P. austriacus*. However, an introgression between *P. auritus* and *P. austriacus* could not be proved (Moretti *et al.* 1993). High altitude populations of long-eared bats were described from the Alps which were difficult to assign either to *P. auritus* or to *P. austriacus* based on morphological characters (e.g. Spitzenberger & Mayer 1988). These populations probably represent *P. alpinus* Kiefer & Veith 2002. as well.

Figure 4-4. *Plecotus alpinus* Kiefer & Veith 2002 (Pesina, Italy). Note the nearly white ventral fur and the colour of the tragus (photo A. Kiefer; specimen released after capture).

Available names

Several Central Asian and African *Plecotus* species have been described (e.g., *Plecotus wardi* Thomas, 1911b, *Plecotus cristiei* Gray, 1838*, Plecotus balensis* Kruskop & Lavrenchenko, 2000) during the last two centuries. All of them live far out of the range of *Plecotus alpinus* Kiefer & Veith 2002 and are morphologically well distinct. We therefore can exclude them as potentially conspecific with the Alpine long-eared bat.

Several names are available from Europe (see Lanza 1959, 1960 and Bree & Dulic 1963 for a comprehensive list). Most of them are not available for the Alpine long-eared bats since they represent either nomina nuda (e.g. *Macrotus europaeus* Leach, 1816, *Plecotus bonapartii* Gray, 1838) or were described from areas were *Plecotus alpinus* Kiefer & Veith 2002 does not occur (e.g. *Vespertilio otus* Boie, 1825, *P. brevimanus* Jenyns, 1828, *Plecotus cornutus* Faber, 1826, *Plecotus homochrous* Hodgson, 1847). Only the following names must be discussed in more detail.

Plecotus communis Lesson, 1827 and *Plecotus vulgaris* Desmarest, 1829: The holotypes of these two species are lost, probably during the 19th century (M. Tranier, pers. comm.), and the brief descriptions of these two taxa do not diagnose a specific species of long-eared bat.

Schinz (1840) mentioned *Plecotus mogalatos* Brehm as a synonym of *P. auritus* (later in this paper spelled *P. megalotos*), which had been described by “Herrn Brehm”. This most likely was Christian Ludwig Brehm, an ornithologist from Saxony, Germany. To the best of our knowledge, neither a formal description nor a holotype exists for *Plecotus mogalatos/megalotos*. In the checklist of Ellerman & Morrison-Scott (1966) this synonym is mentioned as "*Plecotus megalotos* Schinz, 1840"). We therefore regard *Plecotus megalotos* a nomen nudum, too.

Plecotus auritus meridionalis was described by Martino & Martino (1940) from Slovenia. Based on their description, this species is clearly distinguishable from *Plecotus alpinus* Kiefer & Veith 2002 in the condylobasal-length and the length of the bulla. Their data on cranial morphology even indicate that *P. auritus meridionalis* may be identical with *P. austriacus* (Bauer 1960). In addition, they mentioned “… specimens from Switzerland and N. Italy [which] are probably intermediate forms” of *Plecotus auritus meridionalis* and *P. auritus auritus.* This perfectly corresponds to the "intermediate" individuals mentioned by Dulic (1980), which now turned out to be *Plecotus alpinus* Kiefer & Veith 2002 (chapter 3).

Plecotus auritus hispanicus (Bauer 1956) was thought to be an endemic of the Iberian Pensinsula. Bauer (1960) himself re-defined it as a local race of *P. austriacus*.

Koch (1862/63) mentioned three variations of *Plecotus*. His *Plecotus brevipes* (= *Plecotus kirschbaumii* in Koch 1860) clearly is *Plecotus austriacus.* His *Plecotus* var. *typus* is medium-sized, grey-brown and found in the lowlands. His *Plecotus* var. *montanus* is smaller and reddish-brown. We agree with Kock (1994) that the two latter forms are within the variation of *Plecotus auritus*.

Ongoing research on morphological and echolocation characteristics of *Plecotus alpinus,* Kiefer & Veith 2002 will show whether it will be possible to identify the four European *Plecotus* species in the field.

Abstract

A new species of bat of the genus *Plecotus* from the Alps and other mountainous regions of Europe is described. *Plecotus alpinus* sp. nov. can be distinguished from other *Plecotus* species by its sequence of parts of the 16S and D-loop region of the mitochondrial DNA. *Plecotus alpinus* sp. nov. is genetically a close relative to *Plecotus auritus*, although in some morphological characters it shows a closer relation to *Plecotus austriacus*. *Plecotus alpinus* sp. nov. shares morphological similarities with *P. auritus* and *P. austriacus*, so in former studies it appeared as intermediate between *P. auritus* and *P. austriacus*. However, a combination of characteristic traits distinguishes *P. alpinus* sp. nov. clearly from its closely related taxa.

Taxonomic remark in addition to Kiefer & Veith, 2002

Spitzenberger *et al.* (2003) unambiguously showed that *Plecotus alpinus* (Kiefer & Veith 2002) and *Plecotus* (*auritus*) *macrobullaris* (Kuzjakin, 1960) are synonyms. Here I use *Plecotus alpinus* because I assume that both forms represent different taxa. *Plecotus alpinus* represents the western lineage of this clade, whereas *P. macrobullaris* the eastern lineage. Both forms differ in ca. 2 % (genetic divergence) of the 16S rRNA gene and in ca. 4.6 % (range 4.3–4.8 %) of the ND1 gene (Mayer *et al.* 2007). In addition, both forms differ in some morphological characters such as fur colour and some measurements (see fig. 6 in Spitzenberger *et al.* 2003). To avoid further confusion I will use the names *P. macrobullaris alpinus* and *P. macrobullaris macrobullaris* in the following chapters of this work. At present it remains open if the two represent species or subspecies.

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

5.1 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 & 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 & 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 & von Helversen 2001), and a formerly unknown cryptic species *P. macrobullaris* Kuzjakin 1960 (= *P. alpinus* Kiefer & Veith 2002 and *P. microdontus* Spitzenberger 2002 - see Kock 2002 and Spitzenberger *et al.* 2003) 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 1 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.* (2002b) 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* (chapter 3). Here we

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report the discovery of two genetically distinct lineages of Sardinian *Plecotus*, one of which is differentiated at the species level.

5.2 Materials and Methods

5.2.1 Specimens studied

We sampled, under license, 22 specimens of long-eared bats from three localities in central Sardinia (Appendix 5-1). Samples for DNA extraction were obtained from wing tissue, using sterile biopsy punches (Worthington-Wilmer & Barrett 1996). We recorded the following morphometric (hand-held calliper measurements to the nearest 0.05 mm) and other morphological characters, according to Stebbings (1967) and Häussler & Braun (1991):

FA = forearm length (including carpals), HF = hind foot length (excl. claws), TL = tragus length (TLBlatt in Häussler & Braun 1991), TW = tragus width, TH = thumb length without claw, CL = claw length, EAR = ear length, SL = skull length, CBL = condylobasal length, SH = skull height (with bullae), IOW = interorbital constriction width, M^3–M^3 = width across upper molars, C–M^3 = length of maxillary toothrow, C–M_3 = length of mandibular toothrow, M_3–M_3 = 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, F3 = length of 3rd finger with wrist, F5 = length of 5th finger with wrist, 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.

5.2.2 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 Numbers AY134012-134026, AF529229-529230; chapter 3) 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 Accession Numbers AF529231 and AY134027, respectively; chapter 3).

5.2.3 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 (g1) and kurtosis (g2) of the resulting tree length distribution (with PAUP*, version 40b10; 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 & 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 & Nei 1993) with no invariable sites (I=0), and among site substitution rate variation with a gamma shape parameter α=0.4882 was selected.

We used the neighbor-joining algorithm (NJ; Saitou & 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).

5.3 Results and Discussion

5.3.1 Phylogeny

Of the 555 bp of the sequence, 125 were variable and 84 bp were parsimony informative. Skewness (g1) and kurtosis (g2) 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. 5-1) and maximum parsimony (not shown) trees consistently show the same topology. Both analyses show two major clades. One contains *P. kolombatovici* and *P. austriacus* (including the Sardinian samples 3, 6, 9, 10, 11 and 12). The second clade comprises *P. auritus* (including the Sardinian samples 4, 5, 14, 16 and 17), *P. alpinus*, and a Sardinian clade consisting of samples 1, 2, 13, 15, 20, 21 and 22 (haplotypes 1, 2, 13, see appendix 5-1). 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 5-1). Substitution rates for the 16S rRNA gene of ca. 5% correspond to substitution rates of 11-12% for protein coding mitochondrial genes like ND 1, ND 2 or Cyt *b* (own data). The latter indicate differentiation at the species level (see Smith & Patton 1993, Bradley & Baker 2001 for mammals in general and Cooper *et al.* 2001, Mayer & 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, Sar22) 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. This is in the same range as for the Iberian samples Paur7 which represents the subspecies *P. auritus begognae*[1] De Paz 1994 (chapter 3; Juste *et al.* 2004), 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 monophylum 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. Sardinian *P. austriacus* haplotypes are nested within other European *P. austriacus* haplotypes with no apparent sub-structuring.

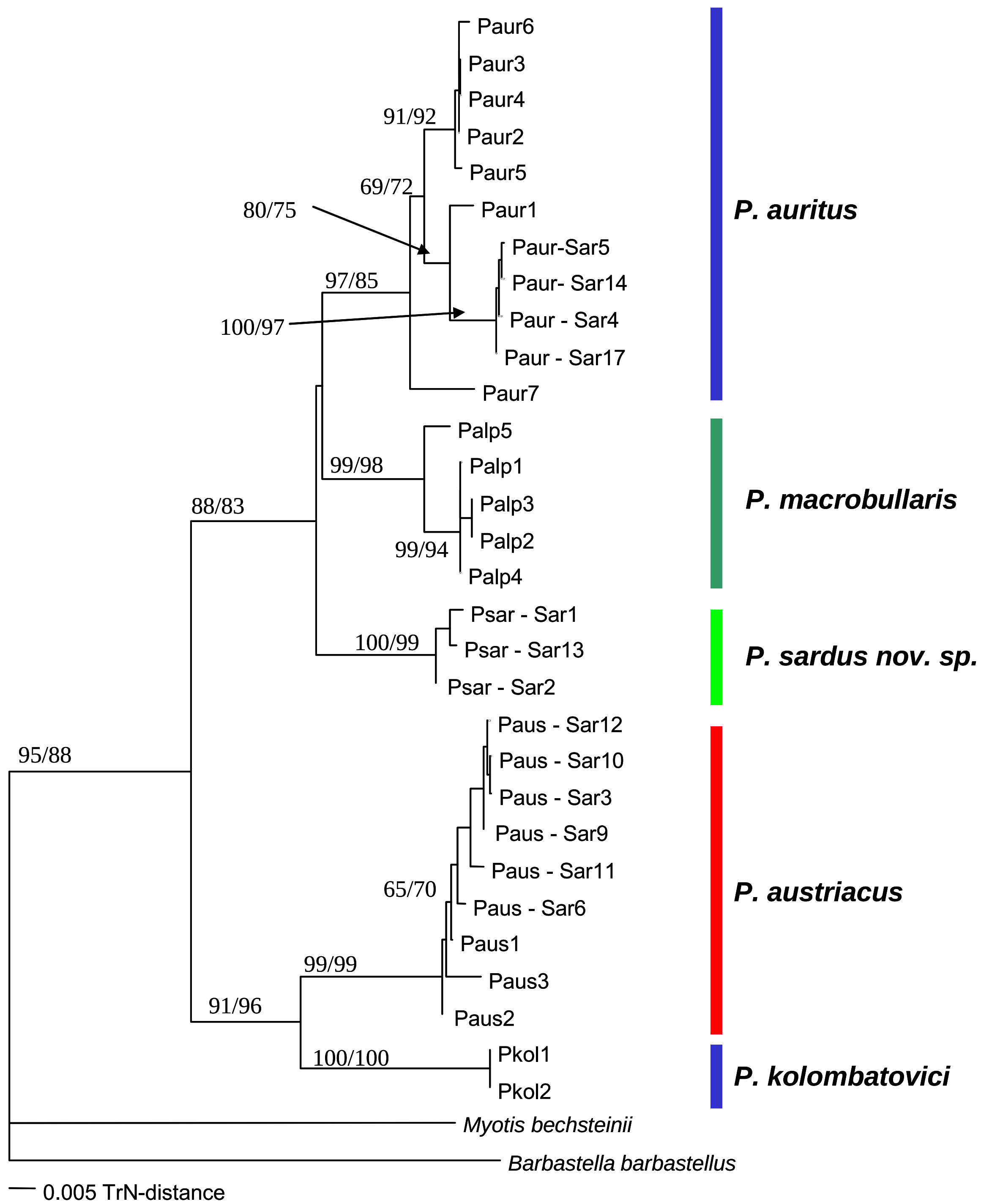

Figure 5-1. Neighbor-Joining tree of European long-eared bats, based on 555 bp of mitochondrial 16S gene fragment (TrN substitution model with I=0 and Γ-shape parameter α=0.4882); bootstrap supports are indicated for neighbor-joining and maximum parsimony trees (left=NJ, right=MP); 2000 replicates were analysed; abbreviations of haplotypes are the same as in chapter 3, except the samples from Sardinia Sar1-Sar22 (see appendix 5-1).

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[1] New analysis shows that *P. auritus begognae* is genetically more differentiated then the three other *P. auritus* sublineages. Therefore some scientists think that *P. begognae* represents a species of its own (Mayer *et al.* 2007 and Ibanez *et al.* 2006).

Table 5-1. Corrected molecular distances (TrN+I+G, above diagonal) and mean substitution rates (below diagonal) among major *Plecotus* lineages (ranges are given in brackets).

lineage	(1)	(2)	(3)	(4)	(5)
(1) *P. auritus*	-	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) *P. macrobullaris*	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) *P. sardus*	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) *P. austriacus*	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) *P. kolombatovici*	0.086 (0.082-0.091)	0.081 (0.079-0.085)	0.082 (0.080-0.084)	0.054 (0.049-0.058)	-

***Plecotus sardus* sp. nov.**

Derivatio nominis

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

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 Mauro Mucedda and Ermanno Pidinchedda on September 22, 2001 in the interior of a cave at Lanaitto's Valley, Oliena district, province of Nuoro, middle-east Sardinia, Italy (40°15'29'' N, 9°29'13'' E, 150 m a.s.l.).

Other specimens examined

One juvenile; found dead by Mauro Mucedda and Ermanno Pidinchedda in the interior of a cave at Baccu Addas valley, Baunei district, province of Nuoro. Five specimens, 1 male and 4 females; mist-netted by Mauro Mucedda, Ermanno Pidinchedda and Maria Luisa Bertelli near the Omodeo Lake (Ula Tirso district, province of Oristano), and subjected to morphometric measurements, drawing of wing patterns and photography, and then released. We took tissue samples for genetic analysis from all these specimens.

Figure 5-2. *Plecotus sardus*; note the length of tragus.

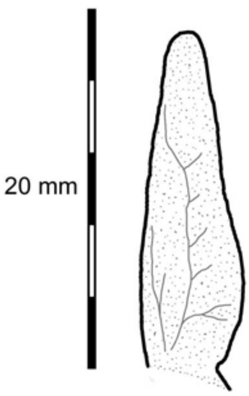

Figure 5-3. Shape and length of tragus.

Figure 5-4. Shape and colour of the penis.

Diagnosis

Plecotus sardus sp. nov. is unambiguously identifiable through DNA sequence analysis. The partial 16S rRNA sequence of the holotype, homologous to bp 2215 and

2490 of the *Pipistrellus abramus* complete mitochondrial genome (Nikaido *et al.* 2001), reads:

tattagaggcactgcctgcccagtgactccagttaaacggccgcggtatcctgaccgtgcaaaggtagcataatcatt tgttctctaaatagggacttgtatgaatggccccacgagggtttaactgtctcttacttttaatcagtgaaattgacactcccgtgaa gaggcgggaattaaaaaataagacgaWaagaccctatggagctttaattaattaactcacaaattataatactaatctacaa gagacaagctaaacttgattgagttaacaatttNNgttggggcgaccttggaataaagatcaacctccgagatagatctacta agacctacaagtcaaggttatatactatacattgatccgccaatagcgatcaacgaaacaagttaccctagggataacagcg caatcctatttaagagtccatatcgacaattagggtttacgacctcgatgttggatcaggacatcccaatggtgcagcagctatt aatgtgttcgtttgttcaacgattaaagtcctacgtgatctgagt (GenBank accession number AY175822).

It differs in 24 substitutions (21 ti's and 3 tv's) from *P. auritus* (GenBank accession number AY134013), 21 substitutions (19 ti's and 2 tv's) from *P. macrobullaris alpinus* (GenBank accession number AY134017), 44 substitutions (37 ti's and 7 tv's) from *P. austriacus* (GenBank accession number AY134022), and 40 substitutions (35 ti's and 5 tv's) from *P. kolombatovici* (GenBank accession number AY134025), respectively.

Like *P. macrobullaris*, *P. sardus* sp. nov. combines typical morphological characters of both *P. auritus* and *P. austriacus* (chapter 4). It is similar to *P. auritus* in its brownish colour of dorsal pelage, length of thumb and length of thumb-claws (see Table 5-1; von Helversen 1989; Schober and Grimmberger 1989). It is similar to *P. austriacus* in its whitish colour of ventral pelage, broadest width of tragus and length of forearm (Table 5-1). It is similar to *P. macrobullaris* in the shape of the penis (Fig. 5-4).

However, *P. sardus* sp. nov. differs from all other European *Plecotus* species in the length of the tragus and the shape of the baculum (Fig. 5-3–5-5; Topál 1958; Dulic 1980; chapter 4, Spitzenberger *et al.* 2002). Additionally it differs from *P. kolombatovici* in forearm length and ear length (Table 5-3; Spitzenberger *et al.* 2002; Dulic 1980).

Description

Plecotus sardus sp. nov. is larger than both *P. auritus* and *P. kolombatovici*, reaching the size of specimens of *P. austriacus* and *P. macrobullaris*. Its head and body length is 45 mm, length of forearm is 41.2 mm, length of thumb 6 mm, length of thumb-claw 3.1 mm, length of hind-foot 7.7 mm. In the wing, the length of the 2nd finger (including carpals) is 35.8 mm, the 3rd finger is 71.8 mm, the 4th finger is 57 mm, and the 5th finger is 56 mm.

The dorsal fur is brown rather than reddish as in some *P. auritus*. The hairs are very fine and woolly, ca. 10 mm long and tri-coloured: to the first 6 mm are 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 hairs are 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.

Table 5-2. Body and skull measurements of *Plecotus sardus*.

	Male Holotype	Male	Female	Female	Female	Female	Mean	Stand. dev.	n
sample name	Sar 13	Sar 22	Sar 2	Sar 15	Sar 20	Sar 21			
Locality	Lanaitto	Omodeo	Omodeo	Omodeo	Omodeo	Omodeo			
FA	41.2	41.1	42.3	42.2	42.2	40.9	41.65	0.65	6
TH	6.0	6.0	6.0	6.0	6.4	6.0	6.07	0.16	6
CL	3.1	2.0	2.4	2.5	2.5	2.6	2.52	0.35	6
TL	18.5	18.0	18.0	19.8	18.9	19.2	18.73	0.71	6
TW	6.5	6.2	6.0	6.4	6.5	6.4	6.33	0.20	6
HF	7.7	7.5	7.0	7.6	6.8	6.7	7.22	0.44	6
Ear	37.5	38.0	38.6	39.0	-	-	38.28	0.66	4
SL	17.10	-	-	-	-	-	-	-	-
CBL	15.90	-	-	-	-	-	-	-	-
SH	7.80	-	-	-	-	-	-	-	-
IOW	3.65	-	-	-	-	-	-	-	-
M^3M^3	6.25	-	-	-	-	-	-	-	-
M_3M_3	4.00	-	-	-	-	-	-	-	-
CM^3	5.75	-	-	-	-	-	-	-	-
CM_3	6.20	-	-	-	-	-	-	-	-
ML	11.30	-	-	-	-	-	-	-	-
MW	9.30	-	-	-	-	-	-	-	-
CsupL	1.50	-	-	-	-	-	-	-	-
MBD	4.75	-	-	-	-	-	-	-	-
ZW	9.20	-	-	-	-	-	-	-	-
MDB	1.20	-	-	-	-	-	-	-	-
F3	71.80	-	-	-	-	-	-	-	-
F5	56.00	-	-	-	-	-	-	-	-
BL	0.80	-	-	-	-	-	-	-	-
BW	0.71	-	-	-	-	-	-	-	-

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 in the living animal 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. macrobullaris*, and almost as large as in *P. auritus* (Table 5-3), 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 ears are longer than in *P. kolombatovici* and reach the maximum size of those of *P. auritus*, *P. macrobullaris* and *P. austriacus* (Table 5-3; Dulic 1980; Häussler & Braun 1991, Spitzenberger *et al.* 2002). The tragus is very large, 18.5 mm long, pale brown tending towards yellowish-white, and it is more or less straight (Fig. 5-3). 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. 5-3). The maximum tragus width is 6.5 mm, which is similar to *P. austriacus* (Table. 5-3).

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, intermediate in size between those of *P. auritus* and *P. austriacus* (Table. 5-3; Strelkov 1988, 1989a; von Helversen 1989) and slightly smaller than in *P. macrobullaris* (chapter 4), with a few long and straight hairs. Evident under the chin is a glandular wart that lacks hairs. The hard triangular pad reported by Spitzenberger *et al.* (2002) for *P. macrobullaris* is lacking.

The penis differs in shape from that of *P. auritus, P. austriacus* and *P. kolombatovici* (Dietz & von Helversen 2004, von Helversen 1989, Kiefer & von Helversen 2004, Schober & Grimmberger 1989), in being almost cylindrical, only slightly rounded, and pointed only at the tip (Fig. 5-4). The shape of the penis resembles that of *P. macrobullaris* whereas the shape of the baculum is clearly different (Fig. 5-5).

The shape of the baculum (Fig. 5-5) resembles that of *P. auritus*, but is smaller and proportionally wider at the base, 0.80 mm long and 0.71 mm wide (Lanza 1960; Strelkov 1989a; De Paz 1994). It is also thinner in the distal part than that of *P. macrobullaris* (chapter 4; Spitzenberger *et al.* 2002) and it is different in shape from that of *P. kolombatovici* (Dulic 1980), *P. austriacus* (Topal 1958), *P. teneriffae* (Ibanez and Fernandez 1985a), *P. austriacus wardi* (Strelkow 1988), *P. christii* (Qumsijeh 1985) and *P. balensis* (Kruskop & Lavrenchenko 2000, not shown in Fig. 5-5). The proximal part is ventrally concave.

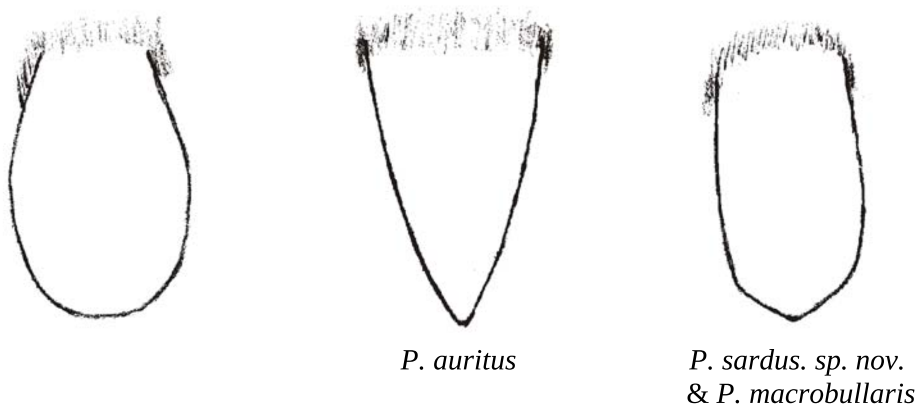

Figure 5-4. Shape of the penis form from the 5 European *Plecotus* species.

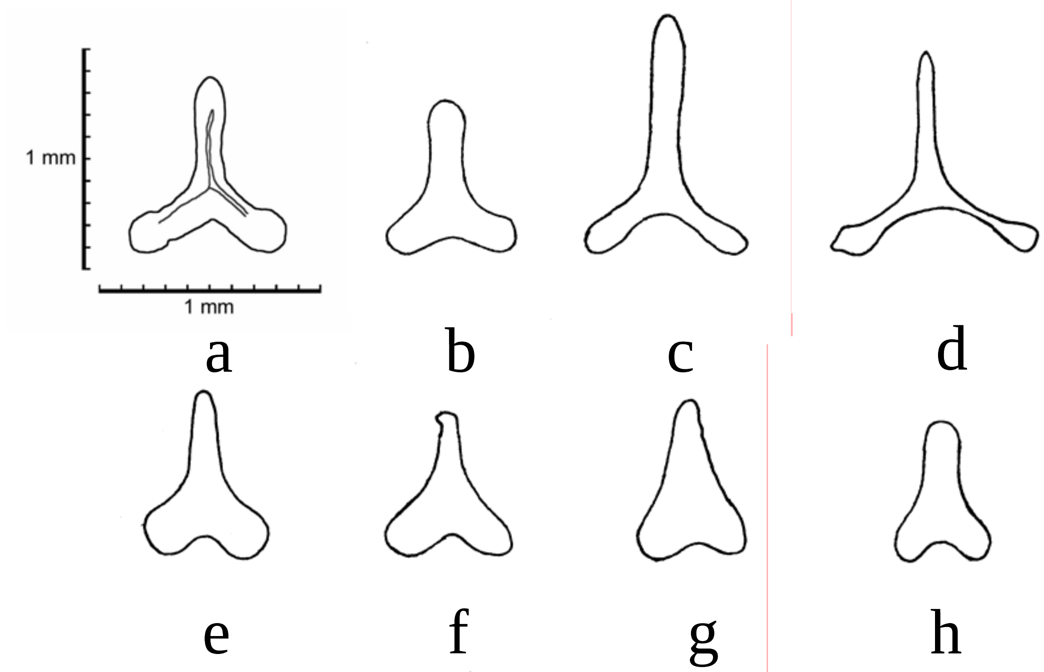

Figure 5-5. Comparison of the shape of *P. sardus* sp. nov (a) to that from other *Plecotus* taxa (b: *P. macrobullaris alpinus* (chapter 4); c: *P. auritus* (Topal 1958); d: *P. teneriffae* (Ibanez & Fernandez 1985b); e: *P. kolombatovici* (Dulic 1980); f: *P. austriacus christii* (Qumsiyeh 1985); g: *P. austriacus wardi* (Strelkow 1988); h: *P. austriacus* (Topal 1958)). All bacula were redrawn in equal scale and dorsal view.

Cranial measurements are given in Tab. 5-2. According to the skull of the holotype, *P. sardus* sp. nov. is different in its CM3 and CM3 length from other European *Plecotus* species, except *P. austriacus*. The upper canine from *P. sardus* sp. nov. is as small as in *P. auritus* (Table. 5-3). Compared to the canine and the second premolar, the first upper premolar is very small and is similar to that of *P. austriacus* (Swift 1998).

Table 5-3. Morphological characteristics of European *Plecotus* (data from Häussler & Braun 1991[1]; Spitzenberger *et al.* 2002[2]; chapter 4[3]; Kiefer & von Helversen 2004[4], own data[5]).

	P. auritus	*P. austriacus*	*P. kolombatovici*	*P. macrobullaris*	*P. sardus*
Colour of dorsal fur	brown to reddish[4]	grey[4]	brownish[4]	greyish brown[2] pale grey[3]	brown[5]
Colour of ventral fur	yellowish-brown to creamy[3]	grey[3]	Whitish[4]	white[3], white-grey[2]	whitish to pale brown[5]
FA - Forearm length [mm]	35.1-43.5[2] 37.5-39.7[1] 36.0-43.5[4]	33.9-42.1[2] 38.4-42.0[1]	36.2-39.3[2]	39.6-43.5[2] 39.7-42.2[3] 40-45[4]	40.9-42.3[5]
TW - Tragus width [mm]	4.5-5.5[1], <5.5[4]	5.7-6.3[1], >5.5[4]	4.5-5.0[4]	5.5-6.0[3,4]	6.0-6.5[5]
TL – Tragus length [mm]*	12.0-13.7[1], <15.5[4]	13.5-16.1[1], 14-16[4]	12-14[4]	16-19[4]	18.0-19.8[5]
Ear - Ear length [mm]	35.0-38.0[1], 26.2-40.4[2]	35.0-39.0[1], 28.6-41[2]	29.7-34.1[2]	34-38.3[2]	37.5-39[5]
TH – Thumb length [mm]	>6.5[4]	<6.5[4]	<6.5[4]	>6.5 [3], 6.5-7.0[3]	6.0-6.4[5]
CL - Claw length [mm]	>2[4]	<2[4]	<2[4]	>2 [3], 2.0-2.8[3]	2.0-3.1[5]
HF - Hind-foot [mm]	8.2-8.9[1], >9[4]	6.8-7.9[1], 7-8[4]	<8[4]	>8.5-9.0 [3], 8[4]	6.7-7.7[5]
CM^3	5.3-5.5[1], 4.85-5.61[2]	5.8-6.3[1], 5.40-6.29[2]	5.16-5.42[2]	5.36-5.74[2]	5.75[5]
CM_3	5.8-6.0[1], 5.42-6.00[2]	6.4-6.7[1], 6.14-6.83[2]	5.53-5.83[2]	5.82-6.16[2]	6.20[5]
CsupL - upper canine length [mm]	1.43-1.85[2]	1.93-2.18[2]	1.61-175[2]	1.77-1.99[2]	1.50[5]
Size of the protuberances over the eyes	large (> 2mm)[5]	small (<1mm)[5]	small (<1mm)[5]	medium (ca. 1-2mm)[5]	medium (ca. 1-2mm)[5] (smaller than in alpinus)
Penis shape	narrowing towards the end[4]	club-shaped[4]	club-shaped[4]	almost cylindrical, (pointed only at the tip)[4]	almost cylindrical (pointed only at the tip)[5]
Triangular pad at the chin	no[2,4]	no[2,4]	no[2,4]	yes[2,4]	no[5]

* =TL_{Blatt} in Häussler & Braun (1991)

Distribution

The species is currently known only from the type locality (cave at Lanaitto's Valley, Oliena district, province of Nuoro, middle-east Sardinia) and two additional locations on Sardinia. These three localities are situated within approximately 60 kilometres of each other, located in 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 one 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.

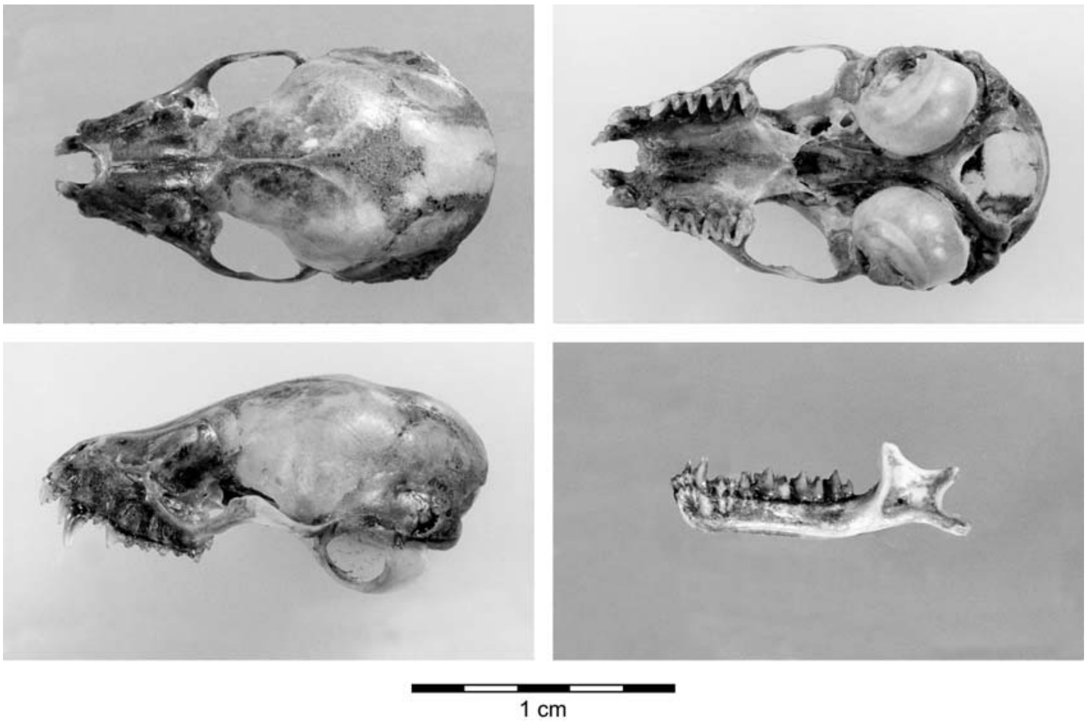

Figure 5-6. Skull (dorsal, ventral, lateral view) and mandible of the holotype of *Plecotus sardus* sp. nov.

5.4 Discussion

5.4.1 Taxonomy

During the last 200 years, several names have been suggested for *Plecotus* populations from Europe, Africa and Asia. N chapter 3 we 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 (own unpublished 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*, Strelkow (1988, 1989a, 1989b) recognised *P. auritus sacrimontis* Allen 1908, *P. austriacus wardi* Thomas 1911, *P. austriacus turkmenicus* Strelkow, 1985, *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 *Plecotus 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).

5.4.2 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.

Abstract

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 from other European *Plecotus* spp.) 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. Morphological diagnostic characters are a relatively long (≥18mm) and wide (≥6mm) 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.

6. Systematic status of African populations of long-eared bats, genus *Plecotus* (Mammalia: Chiroptera)

6.1 Introduction

Long-eared bats, genus *Plecotus* Geoffroy Saint-Hilaire 1818, are primarily distributed in the temperate zone of Eurasia (Hanák 1966, Corbet 1978, Strelkov 1988b); at least six species of this genus live in the western part of the Palaearctic realm (chapter 3, Juste *et al.* 2004). The long-eared bats only reach the margins of the African continent (Hayman & Hill 1971, Kingdon 1997). Records in Africa were summarised by Kock (1969). He and later Kingdon (1997) and Kruskop & Lavrenchenko (2000) interpreted the sparse records of long-eared bats in Africa as being fragments of their continuous distributional range, which occupies the whole coastal belt of the northern part of Africa from Senegal over the Mediterranean shore up to Eritrea, the Nile Valley and the Ethiopian Highlands. The present knowledge of the distribution of *Plecotus* in Africa, however, shows a refuge distribution in several regions, isolated by sea or desert and situated around the Sahara desert (Fig. 6-1).

Long-eared bats are known to occur in the whole belt of the Mediterranean climate and vegetation of north-western Africa (= Maghreb) from the south-western Anti-Atlas Mts. over almost the whole region of Morocco (Aulagnier & Thevenot 1986, Juste *et al.* 2004), northern Algeria (Kowalski & Rzebik-Kowalska 1991, Zagorodniuk 2001), Tunisia (Aellen & Strinati 1969, Kock 1969) and up to north-western Tripolitania (Qumsiyeh 1985, Qumsiyeh & Schlitter 1982, own data). This population is probably isolated by a desert along the Great Syrtis from the distribution range recorded in a relatively small territory of Mediterranean Cyrenaica in north-eastern Libya (Qumsiyeh & Schlitter 1982, Hanák & Elgadi 1984, Qumsiyeh 1985, Juste *et al.* 2004). Another separate population of long-eared bats inhabits the Nile valley from Cairo, Lower Egypt, up to the Fifth Cataract of the Nile in northern Sudan (Anderson 1902, Flower 1932, Kock 1969, Qumsiyeh 1985). This population also extends to the area of the Siwa-Al Jaghbub oases in the Libyan Desert in the west (De Beaux 1928, Hayman 1948, Lanza 1960) and to the Red Sea Mts. in the east (Frauenfeld 1856, Osborn 1988).

Two *Plecotus* populations were reported from the Afro-tropical region: in eastern Africa there are known to be at least seven records from the Ethiopian Highlands of Ethiopia and Eritrea (Rüppell 1842, Sordelli 1902, Largen *et al.* 1974, Yalden *et al.* 1996, Kruskop & Lavrenchenko 2000, Juste *et al.* 2004) and in western Africa, there are five records along the Senegal river (Rochebrune 1883). Nevertheless, the latter range is considered unlikely (see Grubb & Ansell 1996). Long-eared bats also live in all three main archipelagos of Macarone-

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sia: on Madeira (Mathias 1988), on three western islands of the Canary Islands (Trujillo 1991) and on the Cape Verde Islands (Dorst & Naurois 1966). Bats of the genus *Plecotus* are also known from some Mediterranean islands which are close to the African coast, e.g. Pantelleria (Felten & Storch 1970), Malta (Borg *et al.* 1997) and Crete (Hanák *et al.* 2001).

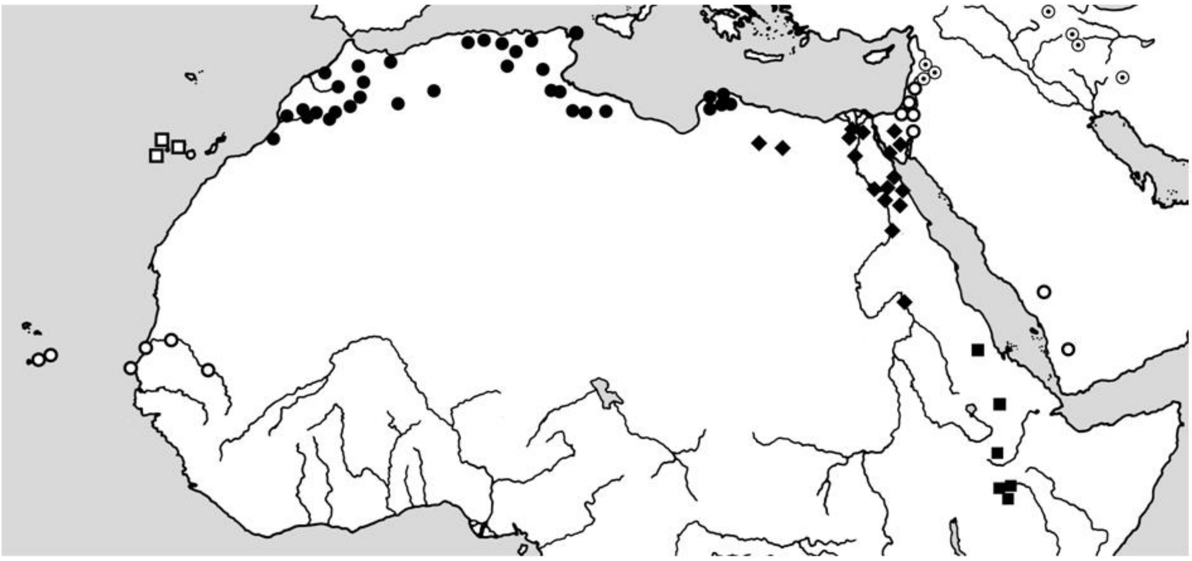

Fig 6-1: Distribution of *Plecotus* in Africa and the Middle East. Modified after Kock (1969), Nader & Kock (1990), other sources (see text) and own data. The symbols denote individual taxa (see text), open circles denote unknown taxonomic status of an individual or population.

Systematic position of the African populations of long-eared bats was primarily constrained by the opinion that all populations belonged to only one taxon (Hanák 1966, Hayman & Hill 1971, Strelkov 1998). Therefore, all the African populations were primarily assigned to the only species recognised in the genus, *Plecotus auritus* (Linnaeus, 1758) (Fischer 1829, Rüppell 1842, Peters 1866, Loche 1867, Dobson 1878, Lataste 1885, Anderson 1902, De Beaux 1928, Flower 1932, Zavattari 1934, 1937, Laurent 1939, Rode 1947, Panouse 1953, Brosset 1955, 1960, 1963, Deleuil & Labbé 1955, etc.), or to the subspecies *P. auritus christii* Gray, 1838[1] (Ellerman & Morrison-Scott 1951, Toschi 1954, Bauer 1956, Setzer 1957) described from the Nile Valley in Egypt (Qumsiyeh 1985). On only a few occasions has the theory been put forward that the African (Egyptian) population represents a separate species, *P. christii*. (Thomas 1911b, Hayman 1948).

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[1] * Gray (1838) described the species *Plecotus christii* in honour of Dr. Turnbell Christie, who delivered the type specimen (Flower 1932). Therefore the misspelling name *christiei* was used for a long time.

Later on, another species was differentiated from the species rank of *P. auritus* on the basis of morphological characters, *Plecotus austriacus* (Fischer, 1829) (Bauer 1960, Lanza 1960). All known African populations of long-eared bats were subsequently assigned to this new form (Hanák 1962, Hayman & Hill 1971, Hufnagl 1972, Largen *et al.* 1974, Madkour 1977, Corbet 1978, Aulagnier & Thevenot 1986, Gaisler & Kowalski 1986, Kowalski & Rzebik-Kowalska 1991, Yalden *et al.* 1996, etc.), usually under the subspecific name *P. austriacus christii* (Harrison 1964, Hill 1964, Hanák 1966, Kock 1969, Gaisler *et al.* 1972, Atallah 1977, Strelkov 1988, Horáček *et al.* 2000), although occasionally under the name *P. austriacus aegyptius* (Fischer, 1829) (Aellen & Strinati 1969, Anciaux de Faveaux 1976, Gaisler 1983). However, the latter is nomen preoccupatum by the name *Vespertilio pipistrellus* var. *aegyptius* Fischer, 1829 (Ellerman & Morrison-Scott 1951, Kock 1969, 1999).

Nevertheless, the concept of a single African form, covered by the species *P. auritus* and later by *P. austriacus* was not completely accepted: Flower (1932) and later on Kock (1969) discussed the possible existence of two forms in Africa; the desert form *christii*, and another one inhabiting the Mediterranean and the mountains of Ethiopia. They also mentioned the possible occurrence of true *P. auritus*. Koopman (1975) preferred assigning the form *christii* to *P. auritus*, which fits more with the morphological status of that species according to his analyses.

Qumsiyeh & Schlitter (1982) first showed morphological differences between populations of long-eared bats occurring in the Mediterranean parts of Libya (northernmost parts of Tripolitania and Cyrenaica), and desert populations of the Nile valley and the Siwa-Al Jaghbub oases in the Libyan Desert. The same conclusion was also given by Hanák & Elgadi (1984) and Qumsiyeh (1985). They named the Mediterranean form *P. austriacus austriacus*, and the desert form *P. austriacus christii*. The same opinion was also presented by Nader & Kock (1990).

Two morphological revisions of the south-western Palaearctic populations (Ibáñez & Fernández 1985a, de Paz 1994) provided a new view of the systematic position of some African populations. Ibáñez & Fernández (1985a) assigned species status to the Canary Islands population, *P. teneriffae* Barret-Hamilton, 1907. de Paz (1994) confirmed the specific status of *P. teneriffae*, assigned the population of *P. auritus* from southern Iberia to an independent subspecies, *P. a. begognae de* Paz, 1994, and for the first time showed the morphological similarity of the north-African population (under the name *P. austriacus christii*) to the subspecies *P. austriacus kolombatovici* Đulić, 1980 – a form described from the Dalmatine Islands of Croatia (Đulić 1980). Another morphological analysis, carried out by Kruskop & Lavrenchenko (2000), defined the population inhabiting the Ethiopian Highlands as a new species, *P. balensis* Kruskop et Lavrenchenko, 2000.

Several recent molecular studies have revised the systematic status of European populations of long-eared bats (chapter 4, chapter 3, Mayer & von Helversen 2001, chapter 5, Spitzenberger *et al.* 2001, 2002). The existence of five species of the genus *Plecotus* in Europe has been described: in addition to *P. auritus* and *P. austriacus*, there are also *P. kolombatovici*, *P. alpinus* Kiefer et Veith, 2002 (= *P. microdontus* Spitzenberger, 2002) and *P. sardus* Mucedda, Kiefer, Pidinchedda et Veith, 2002. The genetic study by Pestano *et al.* (2003) confirmed an exclusive position of the Canary Islands population of *P. teneriffae*. All these species belong to two main lineages (chapters 3, 4 & 5, Pestano *et al.* 2003): the *auritus* group, including the species *P. auritus*, *P. alpinus*, and *P. sardus*, and the *austriacus* group, including the species *P. austriacus*, *P. kolombatovici*, and *P. teneriffae*. Most recently, Spitzenberger *et al.* (2003) showed that a Middle Eastern and Caucasian form, previously assigned to the subspecies *P. austriacus wardi* Thomas, 1911 (Hanák 1966, Strelkov 1988, Koopman 1994, Benda & Horáček 1998) is actually a separate species, named *P. macrobullaris* Kuzjakin, 1965 (described from the northern slope of the Greater Caucasus Mts., Bobrinskij *et al.* 1965). In addition, *P. macrobullaris* was shown by its genetic and some morphological traits to be very close to the Euro-alpine *P. alpinus*, suggesting conspecificity of both forms under the former name.

The comprehensive genetic study by Juste *et al.* (2004) on *Plecotus* populations of the circum-Mediterranean origin (including southern Europe, Middle East, Canary Islands and parts of northern Africa) confirmed most of the results of the previous analyses (chapters 3, 5, Spitzenberger *et al.* 2002, 2003):

- the separation of two main lineages of long-eared bats in the western Palaearctic;
- the genetic similarity of pairs of European species: *P. auritus* and *P. macrobullaris* s. l., and *P. austriacus* and *P. kolombatovici*, respectively; and
- a close relationship between the European *P. alpinus* and the Middle Eastern *P. macrobullaris*.

However, the analysis by Juste *et al.* (2004) has also shown the position of other Mediterranean and African populations in the tree of these two lineages:

- the form *P. auritus begognae* from southern Iberia deserves subspecific status as given by de Paz (1994);
- the species *P. austriacus* is monotypic, it occurs in southern Europe only (incl. Madeira and some Mediterranean islands) and does not cross the Gibraltar Strait;

- the Ethiopian long-eared bat, *P. balensis* is a species of its own, related to *P. austriacus* (contra Kruskop & Lavrenchenko 2000); and
- *P. teneriffae* from the Canary Islands and long-eared bats from the Mediterranean part of northern Africa (= Afro-Mediterranean populations) are forms very close to *P. kolombatovici* from the Balkans and Asia Minor.

However, the systematic position of the north African populations was not sufficiently resolved in this analysis, and the taxonomy of these populations remains open.

The aim of the present study is to describe the systematic status of north African populations of long-eared bats using morphological and genetic analyses to unravel the problems suggested by Juste *et al.* (2004). More specifically, we focus on three major questions:

- What is the systematic position of the north-east African desert population of long-eared bats, currently assigned to *P. austriacus christii*?
- What is the position of the population of long-eared bats inhabiting the region of Mediterranean climate and vegetation of Maghreb and Cyrenaica (= Afro-Mediterranean)?
- Is the isolated population of long-eared bats on Cyrenaica morphologically and/or genetically distinct from the Maghreb population?

6.2 Material and methods

6.2.1 Morphological analysis

In the morphological analysis, museum materials of long-eared bats from northern Africa, the Middle East and central and southern Europe were used. Examined specimens of African long-eared bats (56 specimens from Ethiopia, Egypt, Libya, Tunisia, Algeria, Morocco, and Canary Islands) are listed in Appendix 6-1. The following non-African taxa were included for comparison: *P. auritus auritus* (30 specimens from central Europe and 13 specimens from the Balkans), *P. auritus begognae* (15 spec. from Spain), *P. austriacus* (29 spec. from central Europe, 29 spec. from the Balkans, and 9 spec. from Spain, incl. the type series of *P. auritus hispanicus* Bauer, 1956), *P. balensis* (4 spec. from Ethiopia), *P. m. alpinus* (9 spec. from the Alps, the Pyrenees and Greece, incl. the holotype of *P. alpinus* Kiefer et Veith, 2002), *P. kolombatovici* (22 spec. from the Balkans and Asia Minor), and *P. m. macrobullaris* (31 spec. from Turkey, Syria, Georgia, Armenia and Iran, incl. the paratype of *P. auritus macrobullaris* Kuzjakin, 1965 from the Russian Caucasus); see Benda & Ivanova (2003) for the list of examined comparative specimens of European species (*P. auritus* and *P. austriacus* from central Europe and the Balkans), and see Appendix 6-2 for the list of specimens of *P. a. begognae*, *P. austriacus* (Spain), *P. kolombatovici*, *P. m. alpinus*, and *P. m. macrobullaris*.

We primarily used skulls for morphological analyses. The specimens were measured in a standard way using mechanical or optical callipers. The measurements were taken according to Stebbings (1967), Häussler & Braun (1991) and Rabeder (1972, 1974), and the dental measurements were taken including cingula of the respective teeth (Menu 1983). Bacula were extracted in 6% solution of KOH and coloured with alizarin red.

The statistical analyses of morphometric data were performed with use of the software Statistica 6.0. One-way analysis of variance (ANOVA) between pairs of samples (Tab. 6-3) and a canonical discriminant analysis (Tab. 6-2, Figs 6–3, 6-4) of skull and bacular data were used to describe the most variable characters among lineages. Principal components were extracted for the description of differences between bacular measurements (Fig. 6-6).

6.2.2 Genetic analysis

We analysed a fragment of the mitochondrial 16S rRNA gene of 32 specimens of long-eared bats from Africa (Libya, Morocco and Ethiopia) and the Middle East (Turkey, Syria, Iran). Most of the tissue samples are associated with voucher specimens at the National Museum Prague (Appendix 6-2). DNA extraction, PCR amplification and sequencing protocols were the same as described in chapter 4. 16S rDNA sequences of *P. teneriffae* were obtained from Pestano *et al.* (2003) (GenBank accession numbers: AJ431657=Pten1, AJ431656=Pten2, AJ431654=Pten3). All sequences were aligned to previously published sequences of all European *Plecotus* species (GenBank Accession Numbers AY134015–AY134017, AY134021, AY134022, AY134025, AY134026, AY175816, AY175821, and AF529229 (chapter 3, chapter 5) 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 Accession Numbers AF529231 and AY134027, respectively; chapter 3).

6.2.4 Phylogenetic analysis

We computed a neighbor-joining (NJ; TrN model, α=0.73) tree using the MEGA software (version 2.1, Kumar *et al.* 2001). Maximum parsimony (MP) and maximum likelihood (ML) analyses and Quartet Puzzling (QP) were run using PAUP* 4.0b10 (Swofford 2002). Substitution model parameters for ML searches were obtained with MODELTEST 3.06 (Posada & Crandall 1998). Quartett Puzzling was run with 50,000 puzzling steps to infer reliability values for the ML tree topologies. The statistical robustness of groupings in NJ and MP was evaluated by the bootstrap analysis (Felsenstein 1985), with 2000 replicates each. The MP analysis was performed with the heuristic search algorithm (1000 replicates of random taxon additions, TBR branch swapping), treating gaps as fifth character and giving transitions and transversions equal weight. Finally we applied the Bayesian method using the general time reversible model (GTR; Rodríguez *et al.* 1990) with a gamma shape parameter α estimated for eight rate categories of equal weight from the data (software: MRBAYES of Huelsenbeck & Ronquist 2001). We ran four simultaneous Metropolis-coupled Monte Carlo Markov chains for 500,000 generations. We sampled a tree every 100 generations and calculated a consensus topology for 4000 trees by omitting the first 1000 trees (burn-in).

Abbreviations

Measurements: LCr = greatest length of skull; LCb = condylobasal length of skull; LCc = condylocanine length of skull; LaZ = zygomatic width; LaI = width of interorbital constriction; LaInf = rostral width between foramina infraorbitalia; LaN = neurocranium width; ANc = neurocranium height; ACr = skull height (incl. tympanic bullae); LBT = length (largest diameter) of tympanic bulla; CC = rostral width between canines (incl.); P^4P^4 = rostral width between third upper premolars (incl.); M^3M^3 = rostral width between third upper molars (incl.); I^1M^3 = length of upper teeth-row between I^1M^3 (incl.); CM^3 = length of upper teeth-row between CM^3 (incl.); M^1M^3 = length of upper teeth-row between M^1M^3 (incl.); CP^4 = length of upper teeth-row between CP^4 (incl.); LI^1 = mesiodistal length of first upper incisor; LaI^1 = palatolabial width of first upper incisor; AI^1 = height of first upper incisor crown (from cingulum to the tip of the higher cusp); LCn = mesiodistal length of upper canine; LaCn = palatolabial width of upper canine; ACn = height of upper canine crown (from cingulum to the tip); LP^3 = mesiodistal length of first upper premolar; LaP^3 = palatolabial width of first upper premolar; AP^3 = height of first upper premolar crown (from cingulum to the tip); LM^1 = mesiodistal length of first upper molar (over parastyle and metastyle edges); LaM^1 = largest palatolabial width of first upper molar from parastyle; LM^3 = mesiodistal length of third upper molar over metaconus; LaM^3 = palatolabial width of third upper molar from parastyle; ACin = height of mesiopalatal cingular cusp of P^4; LMd = mandible length; ACo = height of coronoid process; I_1M_3 = length of lower teeth-row between I_1M_3 (incl.); CM_3 = length of lower teeth-row between CM_3 (incl.); M_1M_3 = length of lower teeth-row between M_1M_3 (incl.); CP_4 = length of lower teeth-row between CP_4 (incl.); LAt = forearm length (incl. wrist); LPol = thumb length (excl. claw); LBc = largest length of baculum; LaBc = largest width of baculum; LCBc = length of baculum body (over medial axis); LaCBc = width of baculum body (in the point of bifurcation); LArBc = length of larger baculum arm (from medial axis); AnBc = angle of baculum arms (to medial axis).

Collections: CUP – Department of Zoology, Charles University, Prague, Czech Republic; EBD – Estacion Biologica Doñana, Seville, Spain; IVB – Institute of Vertebrate Zoology, Brno, Czech Republic; JGUM – Department of Ecology, Johannes Gutenberg-University, Mainz, Germany; MUB – Department of Zoology and Ecology, Masaryk University, Brno, Czech Republic; NMP – National Museum, Prague, Czech Republic; SMF – Senckenberg Museum, Frankfurt am Main, Germany; VMO – Regional Museum Olomouc, Czech Republic; ZFMK – Zoological Institute Alexander Koenig, Bonn, Germany; ZIN – Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; ZMMU – Zoological Museum, Moscow State University, Russia.

6.3 Results

6.3.1 Morphological analyses

Basic statistical parameters of wing, cranial, and dental characters of individual samples are given in Appendix 6-1. The comparison of two of the most distinguishable cranial measurements (Fig. 6-2), the length of the upper teeth-row (CM^3) and the largest horizontal diameter of the tympanic bulla (LBT) (Bauer 1960, Hanák 1966, Strelkov 1988, Nader & Kock 1990, Spitzenberger *et al.* 2001, Benda & Ivanova 2003, etc.), shows the basic size relations between the compared samples of long-eared bats from the western Palaearctic, including the African samples. All three samples of *P. auritus* (incl. *P. a. begognae*) clearly differed by a smaller tympanic bulla from the remaining material. Due to this one dimension, the western Palaearctic *P. auritus* were very well distinguishable from the other samples compared, formerly included in the broadly understood *P. austriacus* s. l., i.e., samples of *P. austriacus* s. str., *P. kolombatovici*, *P. m. alpinus*, *P. m. macrobullaris* and all of the African specimens. The African samples grouped into two major clusters with only a slight overlap: one was composed of smaller specimens (CM^3 < 5.4 mm) from the north-east African deserts (i.e., the Upper Egypt along the Nile Valley and Al Jaghbub oasis, eastern Libya) and of larger specimens (CM^3 5.4–5.7 mm) from the Ethiopian Highlands; this group fell within the variation of European *P. kolombatovici* and *P. m. alpinus* (CM^3 5.1–5.7 mm). Larger bats (CM^3 > 5.55 mm) came from Mediterranean Africa (i. e., Cyrenaica and Maghreb, incl. Tripolitania) and form a cluster of specimens of almost the same size as *P. m. macrobullaris* from the Middle East, but generally with a smaller tympanic bulla (mean 4.53 mm in the African, and 4.66 mm in the Middle Eastern sample, respectively). The largest African sample was formed by the Canarian *P. teneriffae*, and fell within the size of *P. austriacus* s. str. The sample of five long-eared bats from Pantelleria Island grouped together with both main clusters of African specimens. Because of the clear morphological difference of *P. auritus* from the African and Middle Eastern specimens and from the other compared European taxa (Fig. 6-2), the *P. auritus* samples were excluded from the following statistical evaluation of skull and dental characters.

The results of a discriminant analysis of the first two canonical variables of nine skull measurements in which the *F*-values were most significant (LCr, LaI, LaN, ANc, LBT, CC, CM^3, CP^4, ACo, and CP_4; Tab. 6-1; 1st CV = 62.18%, 2nd CV = 17.62% of variance) showed differences between all the samples, with the exception of those within *P. austriacus* (Fig. 6-3).

The north-east African desert specimens grouped into one cluster of the smallest samples. The Ethiopian *P. balensis* grouped into a cluster between *P. m. macrobullaris* and *P. m. alpinus*. The Afro-Mediterranean specimens grouped partly with *P. austriacus*, but almost clearly differed from *P. m. macrobullaris* from the Middle East (see also Tab. 6-3) and reasonably differed from the smaller specimens of middle-sized *Plecotus* populations (*P. kolombatovici*, *P. m. alpinus*, and north-east-African desert population). Canarian *P. teneriffae* grouped into a cluster separate from that of *P. austriacus* and the Afro-Mediterranean population. The results of the discriminant analysis of the first two canonical variables of seven dental measurements (LI^1,AI^1, LCn^1, $LaCn^1$, LP^3, LM^1, LM^3, and LaM^3; Tab. 6-1; 1st CV = 70.28%, 2nd CV 13.75% of variance) clearly separated three main groups (Fig. 6-4): (1) *P. austriacus* and *P. teneriffae*, (2) the Afro-Mediterranean population, and (3) a group of all other samples (*P. kolombatovici*, *P. m. alpinus*, *P. m. macrobullaris*, *P. balensis*, and north-east African desert bats).

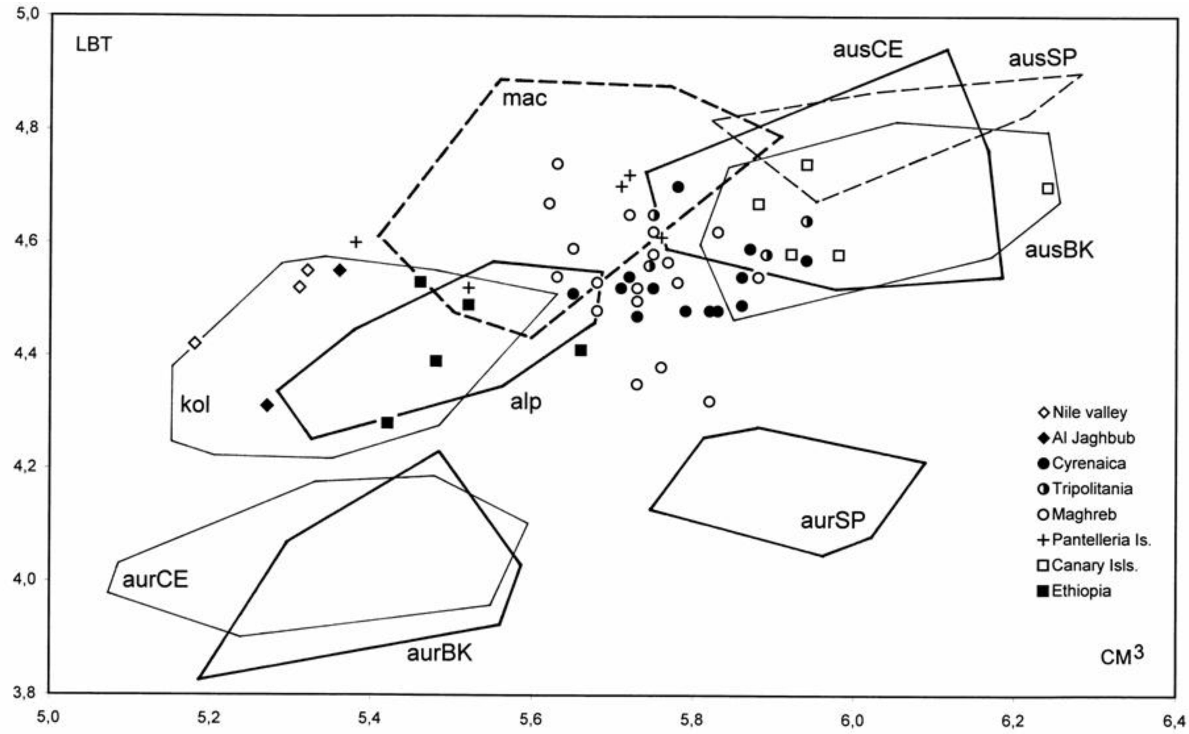


Fig. 6-2. Bivariate plot of west-Palaearctic forms of *Plecotus*: CM^3 against LBT. Symbols denote African specimens, polygons denote the comparative non-African samples (alp – *P. m. alpinus*, aur – *P. auritus*, aus – *P. austriacus*, kol – *P. kolombatovici*, mac – *P. m. macrobullaris*, BK – the Balkans, CE – Central Europe, SP – Spain).

This analysis confirmed the results of the discriminant analysis of cranial measurements, i.e. the significant separation of African samples from the Euro-Asian ones (excluding sample of *P. teneriffae*). The Afro-Mediterranean *Plecotus* clearly differed from *P. austriacus* in dental characters; the most significant difference was in the width of M^3, but there were also differences in almost all other cranial and dental measurements (mainly LBT, I^1M^3, CM^3, CP^4, I_1M_3, and CM_3, see Tab. 6-2).

Tab. 6-1. *F*-values and significance levels of the discriminant analysis of inter-population comparison of individual samples (Figs. 6-3, 6-4, 6-6).

	F	*p*	*F*	*p*		*F*	*p*	*F*	*p*
LCr	2.889	0.002	4.241	0.000	LP^3	6.189	0.000	8.206	0.000
LCb	1.875	0.051	–	–	LaP^3	2.941	0.002	–	–
LaI	3.726	0.000	5.687	0.000	AP^3	3.677	0.000	–	–
LaInf	1.816	0.061	–	–	LM^1	4.848	0.000	7.229	0.000
LaN	3.988	0.000	4.241	0.000	LaM^1	3.503	0.000	–	–
ANc	6.188	0.000	5.116	0.000	LM^3	3.671	0.000	6.188	0.000
LBT	4.539	0.000	5.123	0.000	LaM^3	4.359	0.000	4.638	0.000
CC	8.751	0.000	5.749	0.000	ACin	2.114	0.026	–	–
P^4P^4	2.112	0.026	–	–	LMd	1.451	0.162	–	–
M^3M^3	3.343	0.001	–	–	ACo	4.017	0.000	4.377	0.000
I^1M^3	4.305	0.000	–	–	I_1M_3	2.520	0.007	–	–
CM^3	11.903	0.000	19.950	0.000	CM_3	0.721	0.704	–	–
M^1M^3	3.559	0.000	–	–	M_1M_3	1.725	0.078	–	–
CP^4	6.979	0.000	11.206	0.000	CP_4	5.472	0.000	4.606	0.000
LI^1	4.241	0.000	5.141	0.000	LBc	3.760	0.008	39.224	0.000
LAI^1	3.726	0.000	–	–	LaBc	1.296	0.287	–	–
AI^1	5.767	0.000	5.278	0.000	LCBc	0.845	0.526	–	–
LCn	6.582	0.000	6.723	0.000	LaCBc	11.966	0.000	27.589	0.000
LaCn	12.388	0.000	12.260	0.000	LArBc	0.377	0.860	–	–
ACn	4.333	0.000	–	–	AnBc	9.817	0.000	11.744	0.000

The north-east African desert bats had the most slender teeth of all the samples; they showed a tendency to group slightly outside the bats with relatively small teeth, *P. kolombatovici*, *P*. *m*. *alpinus*, and *P. m. macrobullaris*. *Plecotus balensis* clustered close to this group. Unlike other African bat samples, the Canarian bats showed similar dental characters to *P*. *austriacus*. Our analysis also showed a close similarity between the samples of *P*. *m*. *alpinus* and *P*. *m*. *macrobullaris*: although these populations differed in cranial measurements (*P*. *m*. *macrobullaris* was significantly larger, see Fig. 6-2), they were almost identical in significant dental characters. Statistical differences in individual measurements between African samples are given in Tab. 6-2.

Next page: Tab. 6-2. F-values of an ANOVA of cranial and dental characters between (1) north-east African desert population and Afro-Mediterranean population; (2) *P. austriacus* (all European samples) and Afro-Mediterranean population; (3) *P. t. kolombatovici* and north-east African desert population; (4) *P. m .macrobullaris* and Afro-Mediterranean population; (5) Maghrebian population and Cyrenaica population; (6) *P. teneriffae* and Afro-Mediterranean population; (7) north-east African desert population and *P. balensis*; (8) *P. austriacus* and *P. auritus* (samples from Central Europe and the Balkans); (9) *P. t. kolombatovici* and Afro-Mediterranean population. The significant values are underlined (p<0.050) or printed by bold (p<0.005); df – degrees of freedom.

	(1)			*(2)*			*(3)*			*(4)*		
	df	*F*	p	df	*F*	p	df	*F*	p	df	*F*	p
LCr	47	**25.587**	0.000	108	**62.000**	0.000	26	0.011	0.917	72	**12.997**	0.001
LCb	46	**32.520**	0.000	106	**75.161**	0.000	26	0.609	0.442	71	2.530	0.116
LCc	44	**38.553**	0.000	104	**73.176**	0.000	26	0.531	0.473	64	0.447	0.506
LaI	47	**18.400**	0.000	108	2.101	0.150	26	0.604	0.444	72	1.704	0.196
LaInf	47	**22.945**	0.000	108	**59.391**	0.000	26	3.438	0.075	72	2.009	0.161
LaN	46	**19.386**	0.000	107	**47.833**	0.000	26	3.635	0.068	71	**12.481**	0.001
ANc	46	<u>5.298</u>	0.026	106	0.096	0.757	26	<u>5.680</u>	0.025	71	<u>7.652</u>	0.007
ACr	45	<u>6.083</u>	0.018	107	**16.397**	0.000	25	<u>5.553</u>	0.027	71	**28.440**	0.000
LBT	47	2.969	0.091	108	**88.596**	0.000	26	3.054	0.092	72	**21.591**	0.000
CC	46	**132.249**	0.000	106	**53.819**	0.000	26	**35.662**	0.000	71	**26.702**	0.000
P^4P^4	47	**56.841**	0.000	107	**58.941**	0.000	26	**13.459**	0.001	71	**10.739**	0.002
M^3M^3	47	**50.863**	0.000	106	**70.324**	0.000	26	2.860	0.103	72	1.455	0.232
I^1M^3	46	**124.782**	0.000	106	**102.398**	0.000	26	**8.163**	0.008	71	0.019	0.891
CM^3	46	**145.813**	0.000	107	**123.335**	0.000	26	1.076	0.309	71	**44.557**	0.000
M^1M^3	46	**28.559**	0.000	107	**31.999**	0.000	26	1.961	0.173	71	**11.569**	0.001
CP^4	46	**38.673**	0.000	107	**89.415**	0.000	26	1.159	0.292	71	**12.305**	0.001
LI^1	40	**21.610**	0.000	100	<u>6.032</u>	0.016	26	<u>5.940</u>	0.022	61	**47.526**	0.000
LaI^1	40	**29.653**	0.000	100	**14.964**	0.000	26	3.218	0.084	61	**30.314**	0.000
AI^1	34	3.036	0.090	95	**25.368**	0.000	25	<u>4.494</u>	0.044	54	0.097	0.756
LCn	40	**75.006**	0.000	101	1.782	0.185	26	3.613	0.068	61	**93.125**	0.000
LaCn	40	**78.411**	0.000	101	**11.598**	0.001	26	**22.242**	0.000	61	**138.522**	0.000
ACn	34	**15.237**	0.000	96	**41.581**	0.000	25	<u>6.482</u>	0.017	54	0.264	0.610
LP^3	41	**23.625**	0.000	102	**16.101**	0.000	26	0.136	0.715	62	<u>5.048</u>	0.028
LaP^3	40	**33.638**	0.000	102	0.463	0.498	25	<u>5.200</u>	0.031	62	<u>4.049</u>	0.049
AP^3	36	0.974	0.330	98	**33.758**	0.000	25	0.004	0.948	56	**23.935**	0.000
LM^1	39	**11.170**	0.002	100	0.460	0.499	26	0.003	0.955	58	**14.611**	0.000
LaM^1	39	**19.680**	0.000	100	**18.908**	0.000	26	**23.282**	0.000	58	0.864	0.357
LM^3	38	0.055	0.816	99	**53.569**	0.000	26	0.013	0.909	57	<u>4.427</u>	0.040
LaM^3	38	2.281	0.139	99	**68.629**	0.000	26	2.356	0.137	57	3.707	0.059
ACin	37	1.660	0.206	99	<u>7.348</u>	0.008	24	3.470	0.075	57	<u>4.246</u>	0.044
LMd	47	**58.180**	0.000	108	**65.688**	0.000	25	0.288	0.597	72	0.060	0.808
ACo	47	**25.858**	0.000	108	**42.046**	0.000	25	<u>7.478</u>	0.011	72	<u>6.888</u>	0.011
I_1M_3	47	**95.413**	0.000	108	**109.043**	0.000	25	<u>8.630</u>	0.007	72	2.615	0.110
CM_3	47	**75.367**	0.000	108	**123.345**	0.000	25	1.198	0.284	72	0.590	0.445
M_1M_3	47	**31.228**	0.000	108	**39.429**	0.000	25	<u>8.120</u>	0.009	72	0.986	0.324
CP_4	47	**47.867**	0.000	108	**19.723**	0.000	25	<u>5.702</u>	0.025	72	0.604	0.440

(5)			(6)			(7)			(8)			(9)		
df	*F*	p	df	*F*	p	df	*F*	p	df	*F*	p	df	*F*	p
37	1.081	0.305	47	**21.504**	0.000	8	7.690	0.024	99	**359.733**	0.000	63	**71.643**	0.000
36	2.447	0.126	46	**30.324**	0.000	9	4.777	0.057	98	**498.063**	0.000	62	**119.248**	0.000
34	0.440	0.512	44	**29.239**	0.000	9	4.556	0.062	98	**588.699**	0.000	60	**134.575**	0.000
37	**12.168**	0.001	47	0.002	0.963	9	**47.499**	0.000	99	**11.301**	0.001	63	**29.558**	0.000
37	4.032	0.052	47	**15.042**	0.000	7	3.522	0.103	99	**129.349**	0.000	63	**18.613**	0.000
36	0.328	0.570	46	**28.732**	0.000	8	**16.838**	0.003	97	**56.327**	0.000	62	**10.913**	0.002
36	**14.897**	0.000	46	6.053	0.018	8	7.116	0.028	97	**12.999**	0.000	62	0.013	0.909
36	**18.280**	0.000	46	**12.905**	0.001	7	11.865	0.011	98	**132.245**	0.000	62	0.018	0.894
37	0.016	0.900	47	**13.168**	0.001	9	0.560	0.473	99	**1273.543**	0.000	63	**35.880**	0.000
36	2.970	0.093	46	0.128	0.723	8	**25.224**	0.001	99	**173.407**	0.000	62	**140.650**	0.000
37	6.084	0.018	47	1.678	0.202	7	16.386	0.005	96	**43.856**	0.000	63	**65.160**	0.000
37	1.952	0.171	47	**10.606**	0.002	8	3.248	0.109	98	**119.613**	0.000	63	**88.609**	0.000
36	6.455	0.016	46	**32.410**	0.000	8	**21.300**	0.002	98	**349.012**	0.000	62	**166.693**	0.000
36	3.323	0.077	46	**36.229**	0.000	9	**15.740**	0.003	99	**631.469**	0.000	62	**214.280**	0.000
36	1.443	0.237	46	**29.440**	0.000	5	3.731	0.111	99	**333.436**	0.000	62	**50.108**	0.000
36	1.137	0.293	46	1.101	0.300	5	0.712	0.437	99	**424.961**	0.000	62	**86.233**	0.000
30	0.073	0.789	40	2.206	0.145	8	11.200	0.010	98	0.034	0.854	56	**14.469**	0.000
30	2.689	0.112	40	3.530	0.068	8	0.992	0.348	98	**23.116**	0.000	56	**50.727**	0.000
25	3.277	0.082	35	0.126	0.724	7	0.568	0.476	97	**69.785**	0.000	51	0.122	0.728
30	0.328	0.571	40	6.131	0.018	8	**10.356**	0.012	99	**336.915**	0.000	56	**165.555**	0.000
30	**9.691**	0.004	40	0.000	0.991	8	**11.009**	0.011	99	**323.094**	0.000	56	**132.279**	0.000
25	1.148	0.294	35	0.747	0.393	7	0.042	0.844	99	**330.432**	0.000	51	**10.421**	0.002
31	1.128	0.296	41	0.575	0.453	8	**15.284**	0.004	99	4.781	0.031	57	**61.084**	0.000
31	**15.714**	0.000	41	0.111	0.740	7	15.416	0.006	99	**74.466**	0.000	57	**59.785**	0.000
27	1.333	0.258	37	**18.556**	0.000	7	0.074	0.793	99	2.916	0.091	53	4.296	0.043
29	0.253	0.619	39	4.493	0.040	8	6.500	0.034	99	**236.400**	0.000	55	**36.805**	0.000
29	0.008	0.927	39	**15.449**	0.000	8	8.875	0.018	99	**89.232**	0.000	55	1.948	0.168
28	2.757	0.108	38	**27.298**	0.000	8	0.733	0.417	99	3.344	0.070	54	0.072	0.790
28	**11.037**	0.002	38	**11.624**	0.002	8	0.000	1.000	99	**31.162**	0.000	54	0.318	0.575
28	**21.245**	0.000	38	4.420	0.042	7	**28.655**	0.001	99	**48.325**	0.000	53	7.535	0.008
37	0.000	0.986	47	**19.176**	0.000	8	6.554	0.034	99	**497.810**	0.000	62	**155.578**	0.000
37	0.167	0.685	46	**11.093**	0.002	8	1.288	0.289	98	**363.965**	0.000	62	**24.924**	0.000
37	1.272	0.267	47	**22.047**	0.000	7	**17.474**	0.004	98	**573.273**	0.000	62	**143.951**	0.000
37	0.124	0.727	47	**22.020**	0.000	8	7.597	0.025	97	**728.601**	0.000	62	**119.323**	0.000
37	0.918	0.344	47	**11.592**	0.001	6	0.037	0.853	99	**241.237**	0.000	62	**33.218**	0.000
37	0.219	0.642	47	1.599	0.212	7	7.994	0.026	97	**574.773**	0.000	62	**86.975**	0.000

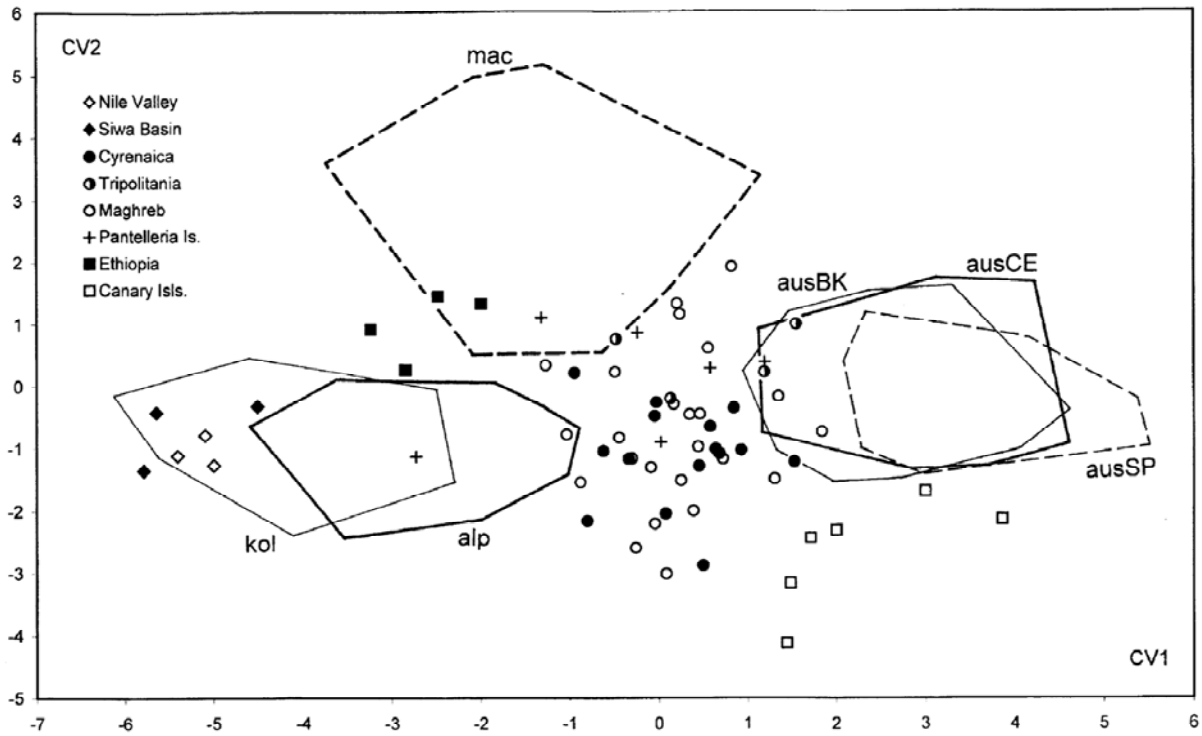


Fig. 6-3. Bivariate plot of the first two canonical axes of the nine cranial of samples of *Plecotus* (for details see text). For abbreviations see fig. 6-2.

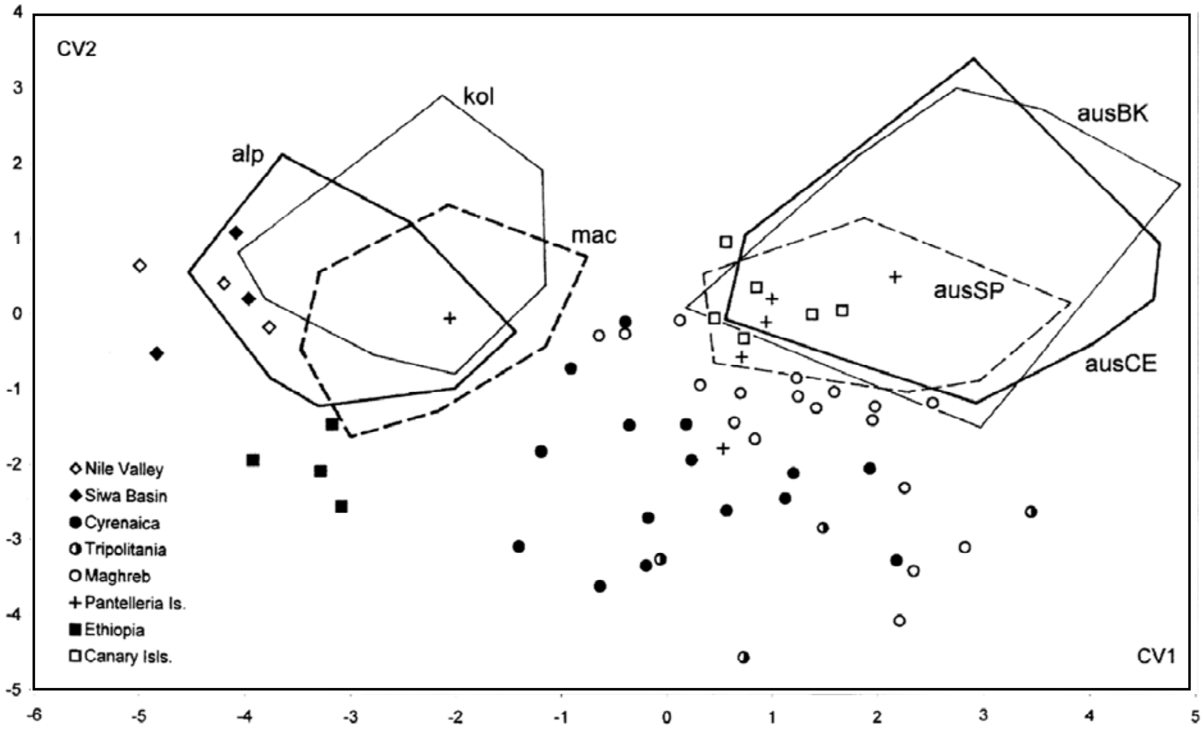


Fig. 6-4. Bivariate plot of the first two canonical axes of seven dental measurements of samples of *Plecotus* (for details see text). For abbreviations see fig 6-2.

The sample of bats from Pantelleria Island showed a similar divergence into two clusters in discriminant analyses (Figs 6-3, 6-4) and in the bivariate comparison (Fig. 6-2): one specimen was consistently included in a cluster of smaller bats (*P. kolombatovici* and *P. m. alpinus*), while the remaining specimens grouped with *P. austriacus* or Afro-Mediterranean bats.

The comparison of bacula of the African bats with additional samples showed four major shape types (Fig. 6-5):

(1) larger or middle-sized bones with a broad bulky body, broad proximal arms and an obtuse angle of arms; this type was found in the Afro-Mediterranean bats and in *P. m. macrobullaris*;

(2) a smaller baculum with broad arms of an obtuse angle and a rather narrow body was present only in bats of the north-east African deserts;

(3) larger or middle-sized bones with a rather narrow body and longer narrow arms of an obtuse angle; this type was found in *P. auritus*, *P. balensis* (after Kruskop & Lavrenchenko 2000), and *P. teneriffae* (after Ibáñez & Fernández 1985a); and

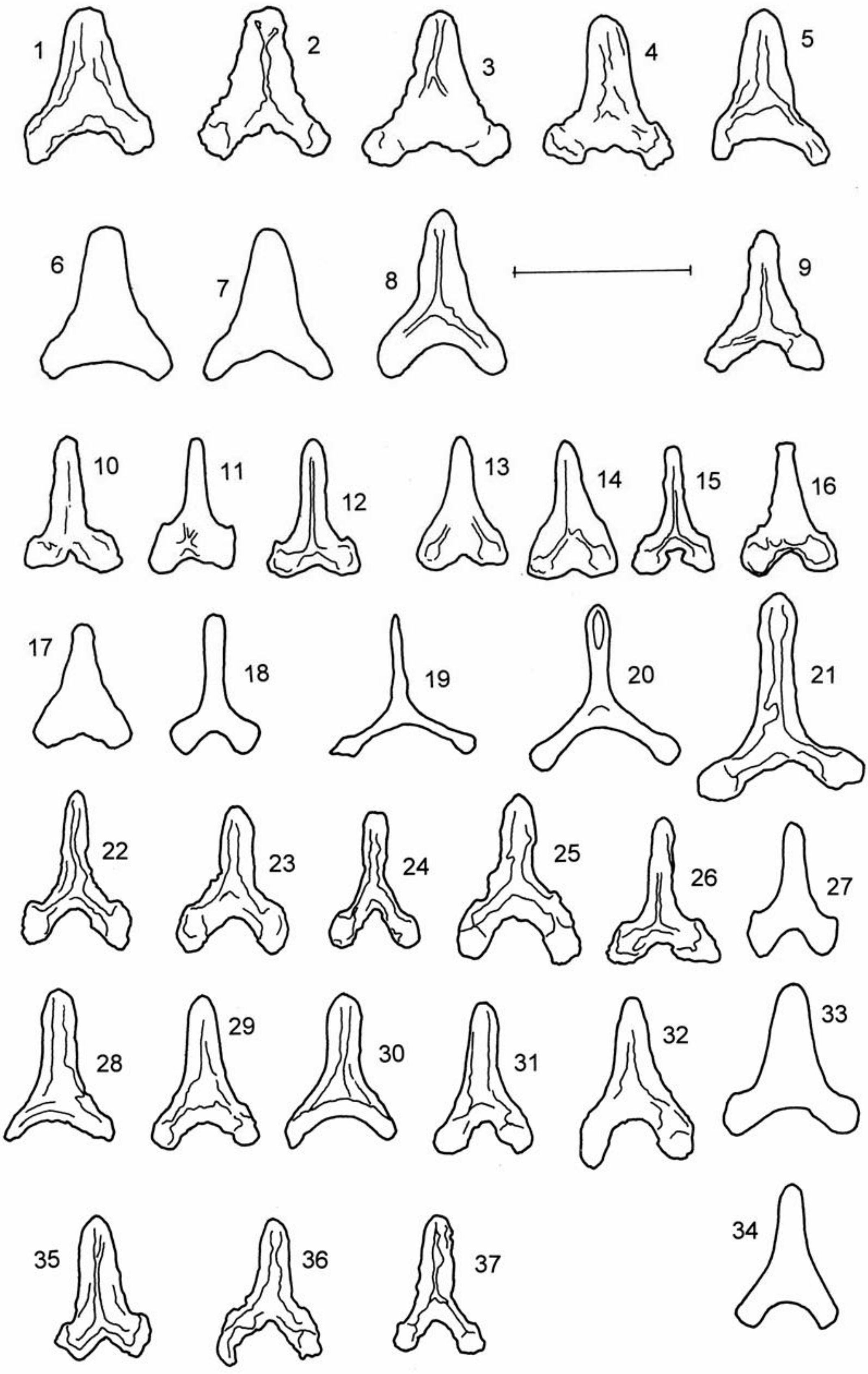
(4) smaller or middle-sized bones with a narrow body and arms and a acute angle of arms; this type was found in *P. kolombatovici* and *P. austriacus*.

The differences between these bacular types were also shown by the principal component analysis of three bacula characters with the most significant *F*-value (LBc, LaCBc, AnBc; Tab. 6-1; 1st PC = 48.12%, 2nd PC = 34.57% of variance): the type (3) was clearly separated from all the others. Among the remaining samples, which cluster together, the African samples were the most differentiated. The north-east African desert bats had the smallest baculum of type (2), while Afro-Mediterranean bats had the largest of type (1).

In conclusion, the morphological analyses showed that four more or less distinct populations of long-eared bats live in Africa. One population of small bats with a characteristic baculum, slightly built teeth, and a slight reduction of M^3 inhabits the desert habitats of north-eastern Africa; another population of similar, but slightly larger bats lives in the Ethiopian Highlands. Middle-sized bats with a large and very broad baculum and more heavily built teeth (but more reduced M^3) form the third group of populations; these bats occur in the Mediterranean regions of northern Africa, from Morocco to Tripolitania (Maghreb) and Cyrenaica. The fourth African group is the Canarian sample, with the largest skulls, slightly built unicuspidal teeth, heavily built molars and a characteristic asterisk-like baculum.

The most pronounced differences were found between the western and eastern north African populations, i.e. the north-eastern desert and the Afro-Mediterranean bats. They differed significantly in almost all characters (Tab. 6-2), with the only exceptions being the cranial ones (LBT) and some dental characters (heights of I^1, of P^3, and of cingular cusp on P^4, and measurements of M^3). No essential differences were found between the bats of Maghreb and Cyrenaica; among cranial characters the only difference was found in the width of the interorbital constriction (LaI), measurements of the braincase (ANc, ACr), and among dental measurements in the palatolabial width of upper teeth (LaCn, LaP^3, LaM^3). Nevertheless, the differences between these subpopulations were the smallest among all samples (Tab. 6-2). The small north-east African desert long-eared bats were close to *P. balensis* and *P. kolombatovici* in several characters, however, these three populations differed significantly in the height of the braincase (ANc), the rostral widths (CC, P^4P^4), the lengths of teeth-rows (I^1M^3, I_1M_3, CP_4), and in some dental measurements (LaCn, LaP^3, and LaM^1). Although the Afro-Mediterranean sample had the position of middle-sized or larger specimens among the compared bats, it differed significantly in most characters from *P. austriacus*, *P. m. macrobullaris*, and *P. teneriffae* (Tab. 6-2). The Canarian sample was very close in most characters to the European *P. austriacus*, but it differed significantly in unicuspide teeth-rows (CP^4, CP_4), skull widths (LaN, CC, P^4P^4) and in the measurements of the upper canine and first upper molar. The sample from Pantelleria Island was composed of two morphotypes of long-eared bats.

Fig. 6-5 (opposite page). Bacula of *Plecotus* from African populations and of comparative taxa from the Balkans and the Middle East. Drawings are based on the original preparates (1–5, 9, 10, 21–26, 28–32, 35–37) and on published data (see below). All drawings are adjusted to the same magnification. 1–9 – *P. t. gaisleri* subsp. n. (1–3 – NMP 49905–49907, Wadi al Kuf, Libya, 4 – NMP 49916, Qasr ash Shahdayn, Libya, 5 – NMP 49920, Wadi al Kuf, Libya, 6 – Shahat, Libya [Hanák & Elgadi 1984], 7 – Quariat al Faioah, Libya [Hanák & Elgadi 1984], 8 – Wadi al Kuf, Libya [Qumsiyeh 1985], 9 – NMP 49965, Nanatalah, Libya); 10–18 – *P. christii* (10 – NMP 49862, Al Jaghbub, Libya, 11, 12 – al Jaghbub, Libya [Lanza 1960], 17 – Al Jaghbub, Libya [Hanák & Elgadi 1984], 13 – Cairo, Egypt [Lanza 1960], 14 – Luxor, Egypt [Lanza 1960], 15 – Egypt, undefined [Lanza 1960], 16 – Dandara Temple, Qena Prov., Egypt [Qumsiyeh 1985], 18 – Egypt, undefined [Wassif & Madkour 1972a]); 19 – *P. t. teneriffae*, Altos de Arafo, Tenerife I., Canary Islands (Ibáñez & Fernández 1985a); 20 – *P. balensis*, Harrena Forest, Bale Mts., Ethiopia (Kruskop & Lavrenchnko 2000); 21 – *P. auritus auritus*, NMP 50441, Rilski manastir, Bulgaria; 22–27 – *P. t. kolombatovici* (22 – NMP 49092, Hvar Is., Croatia, 23, 24 – NMP 48726, 48728, Kombotades, Greece, 25 – NMP 48087, Çevlik, Turkey; 26 – CUP T93/64, Narlikuyu, Turkey, 27 – Hvar Is., Dalmatia [Đulić 1980]); 28–34 – *P. m. macrobullaris* (28–30 – NMP 48139–48141, Takht-e-Suleyman, Iran, 31 – NMP 48849, Ras al Ain, Syria, 32 – NMP 48994, Maalula, Syria, 33, 34 – Armenia, undefined [Strelkov 1989]); 35–37 – *P. austriacus* (35 – NMP 49131, Ploski, Bulgaria, 36 – NMP 50438, Lakatnik, Bulgaria, 37 – NMP 49134, General Todorov, Bulgaria). Scale line – 1 mm.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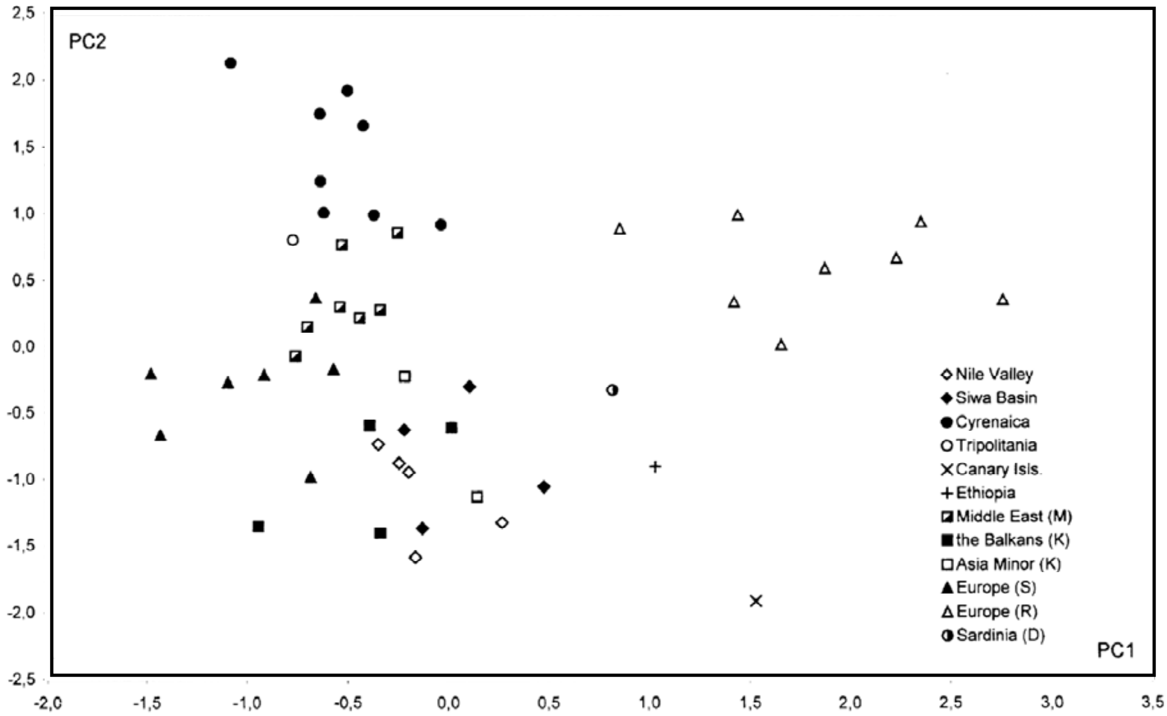

Fig. 6-6. Bivariate plot of the first two principle components of three bacular measurements. For details see text. Abbreviations: D – *P. sardus*, K – *P. t. kolombatovici*, M – *P. macrobullaris*, R – *P. auritus*, S – *P. austriacus*.

6.3.2 Genetic analyses

We consistently achieved 554 bp of the 16S rRNA gene for all samples, with 128 variable sites, 84 of which were parsimony informative. Empirical base frequencies of ingroups (all *Plecotus*) were as follows: π_A=0.3271, π_C=0.1992, π_G=0.2028, π_T=0.2709. The likelihood ratio test (LRT test) implemented in MODELTEST selected the TrN model (Tamura & Nei 1993) with among site substitution rate variation (gamma shape parameter α=0.73) and a proportion of invariable sites of I=0.65.

Two major clades of *Plecotus* were consistently found in all analyses (Fig. 6-7) except the position of *P. sardus*, which was outside the *P. auritus* clade sensu Spitzenberger *et al.* (2003) in the Bayesian approach. Independent from this we name the clade that comprised all *P. auritus*, *P. m. macrobullaris*, *P. m. alpinus* and *P. sardus* haplotypes (clade support values are 89, 92 and 82 for NJ, ML and MP, respectively) the *P. auritus* clade. All remaining haplotypes, including the African ones, formed a second well-defined clade, which we name the *austriacus*-clade (92/89/100/94 for NJ/ML/BAYES/MP). The mean pairwise genetic difference (uncorrected *p*) between haplotypes of the *auritus* and the *austriacus* clade were between 0.066 and 0.088 (Tab. 6-3).

Within the *auritus* clade, the *P*. *m*. *macrobullaris* and *P*. *m*. *alpinus* haplotypes formed a well-supported subclade (most support values >98%), with the Alpine haplotype Palp1 (= *P*. *m*. *alpinus*) being the sister lineage of an eastern *P*. *m*. *macrobullaris* clade. The mean genetic differentiation between *P*. *m*. *macrobullaris* and *P*. *m*. *alpinus* was 0.012 ± 0.004.

Within the well supported *austriacus* clade (92/89/100/87 for NJ/ML/BAYES/MP), at least four distinct lineages emerged that were differentiated from each other at a level of 3.9–6.8% (Tab. 6-3): European *P*. *austriacus*, Ethiopian *P*. *balensis*, the east Libyan sample (belonging to the form traditionally named *P*. *austriacus christii*, i.e. north-east African bats) and a mixed clade comprising the *P*. *teneriffae* from the Canaries, the south-east European *P*. *kolombatovici* and all remaining African haplotypes. According to Juste *et al.* (2004) we name this mixed clade the *P*. *teneriffae/kolombatovici* clade. Within the latter, the position of our Cyrenaican (north-eastern Libya), Tripolitanian (north-western Libya) and Maghrebidian (Moroccan) samples, which are morphologically similar among each other but well distinguishable from all other African samples (see above), is poorly resolved. Genetic distances among sublineages of the *P*. *teneriffae/kolombatovici* clade range between 1.8 and 2.2% (Tab. 6-3).

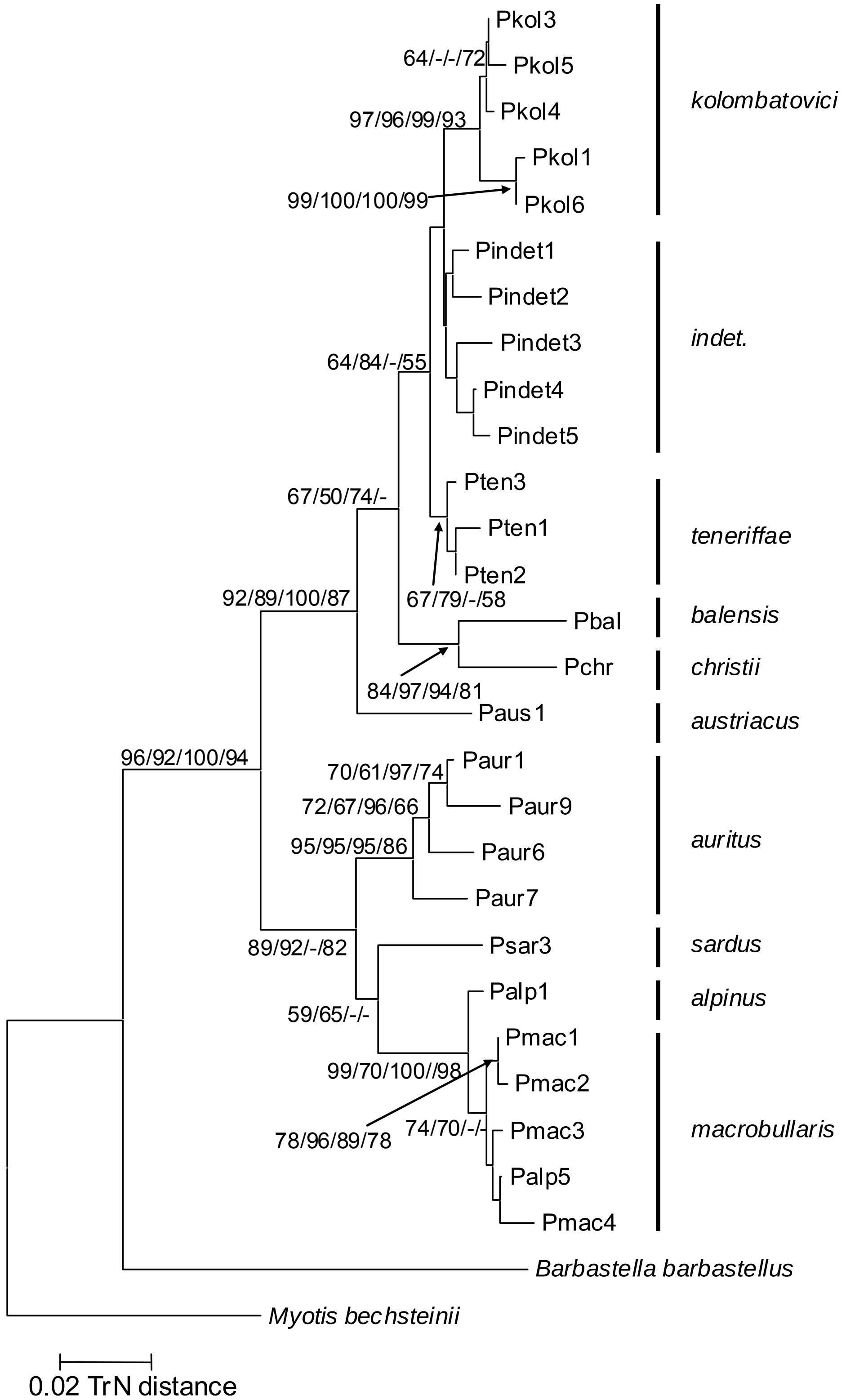

Fig.6-7. Neighbor-joining tree based on 554 bp of partial 16S rDNA sequences (Tamura-Nei model with α=0.65) with *Myotis bechsteinii* defined as the outgroup. Support values are indicated for neighbor-joining (NJ; left), maximum likelihood (QP=Quartet Puzzling; left middle), Bayesian inference (right middle) and maximum parsimony (MP; right); – support values less than 50 % are not shown.

Tab. 6-3. Uncorrected *p*-distances within and among major *Plecotus* lineages; mean (below diagonal) and standard deviation (above diagonal) are given. Abbreviations of lineages: Pbal – *P. balensis*, Pchr – *P. christii*, Psar – *P. sardus*, Paur-w – *P. auritus* (W-European samples), Paur-e – *P. auritus* (E-European samples), Paur-sp – *P. auritus* (Iberian samples), Paur-sa – *P. auritus* (Sardinian samples), Pten – *P. t. teneriffae*, Pkol – *P. t. kolombatovici*, Pindet – *P. t. gaisleri* subsp. n., Palp – *P. m. alpinus*, Pmac – *P. m. macrobullaris*, Paus – *P. austriacus*. Framed are the values under 0.040.

	Pbal	Pchr	Psar	Paur-w	Paur-e	Paur-sp	Paur-sa	Pten	Pkol	Pindet	Palp	Pmac	Paus
Pbal		0.008	0.010	0.011	0.011	0.011	0.011	0.008	0.009	0.009	0.011	0.011	0.011
Pchr	0.041		0.012	0.011	0.012	0.012	0.012	0.008	0.009	0.008	0.011	0.011	0.011
Psar	0.066	0.087		0.009	0.009	0.009	0.009	0.011	0.011	0.010	0.008	0.008	0.012
Paur-w	0.066	0.077	0.048		0.005	0.006	0.005	0.011	0.011	0.010	0.008	0.008	0.011
Paur-e	0.070	0.083	0.048	0.017		0.006	0.006	0.011	0.011	0.011	0.009	0.008	0.011
Paur-sp	0.074	0.088	0.048	0.026	0.022		0.007	0.011	0.011	0.011	0.009	0.009	0.012
Paur-sa	0.074	0.088	0.048	0.013	0.022	0.026		0.011	0.011	0.011	0.009	0.009	0.012
Pten	0.039	0.041	0.077	0.074	0.078	0.073	0.082		0.005	0.004	0.011	0.011	0.008
Pkol	0.055	0.054	0.080	0.078	0.083	0.078	0.085	0.022		0.004	0.011	0.011	0.008
Pindet	0.050	0.045	0.076	0.072	0.077	0.076	0.082	0.018	0.020		0.010	0.011	0.008
Palp	0.075	0.083	0.042	0.044	0.048	0.050	0.050	0.081	0.080	0.074		0.004	0.012
Pmac	0.078	0.086	0.043	0.044	0.047	0.047	0.050	0.085	0.086	0.081	0.011		0.012
Paus	0.068	0.064	0.085	0.077	0.070	0.077	0.085	0.048	0.048	0.043	0.083	0.084	

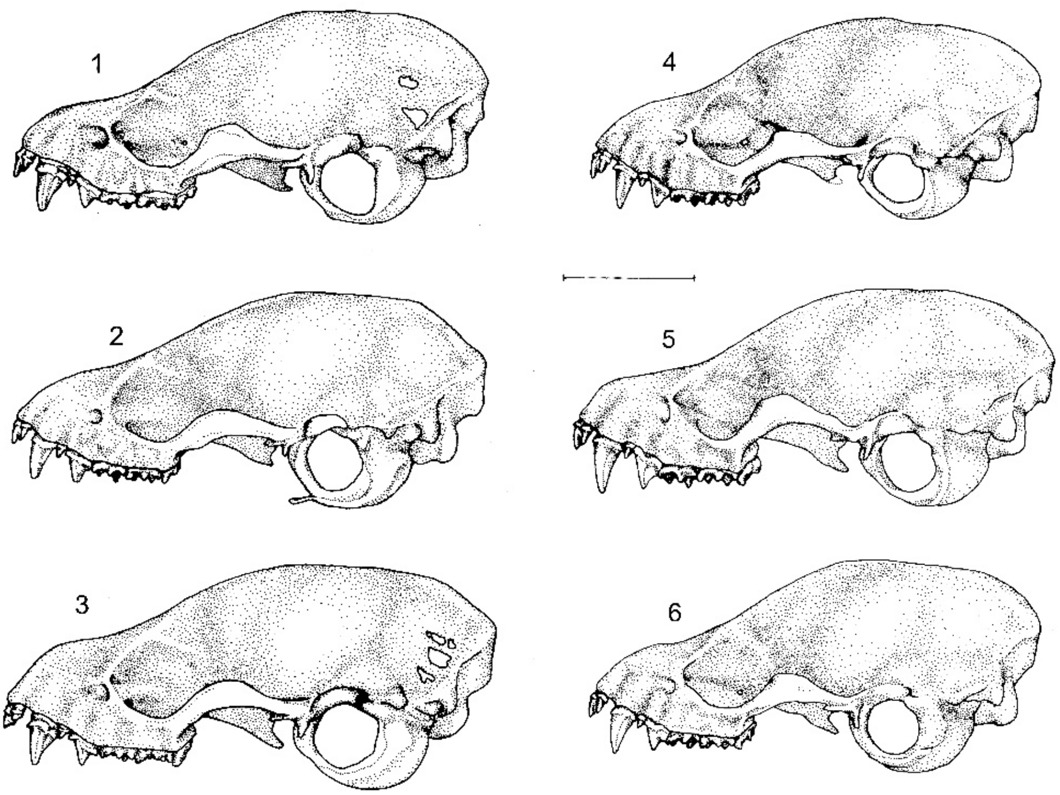

Fig. 6-8. Skulls of *Plecotus* from African populations and of comparative taxa from the Balkans and the Middle East. 1 – *P. t. gaisleri* subsp. n., holotype (NMP 49911, female, Wadi Al Kuf, Libya); 2 – *P. christii* (NMP 49863, female, Al Jaghbub, Libya); 3 – *P. m. macrobullaris* (NMP 48139, male, Takht-e Suleyman, Iran); 4 – *P. auritus* (NMP 48567, male, Paraliá Skotínas, Greece); 5 – *P. austriacus* (NMP 49045, female, Papagianni, Greece); 6 – *P. t. kolombatovici* (NMP 48726, male, Kombotades, Greece). Scale line – 5 mm.

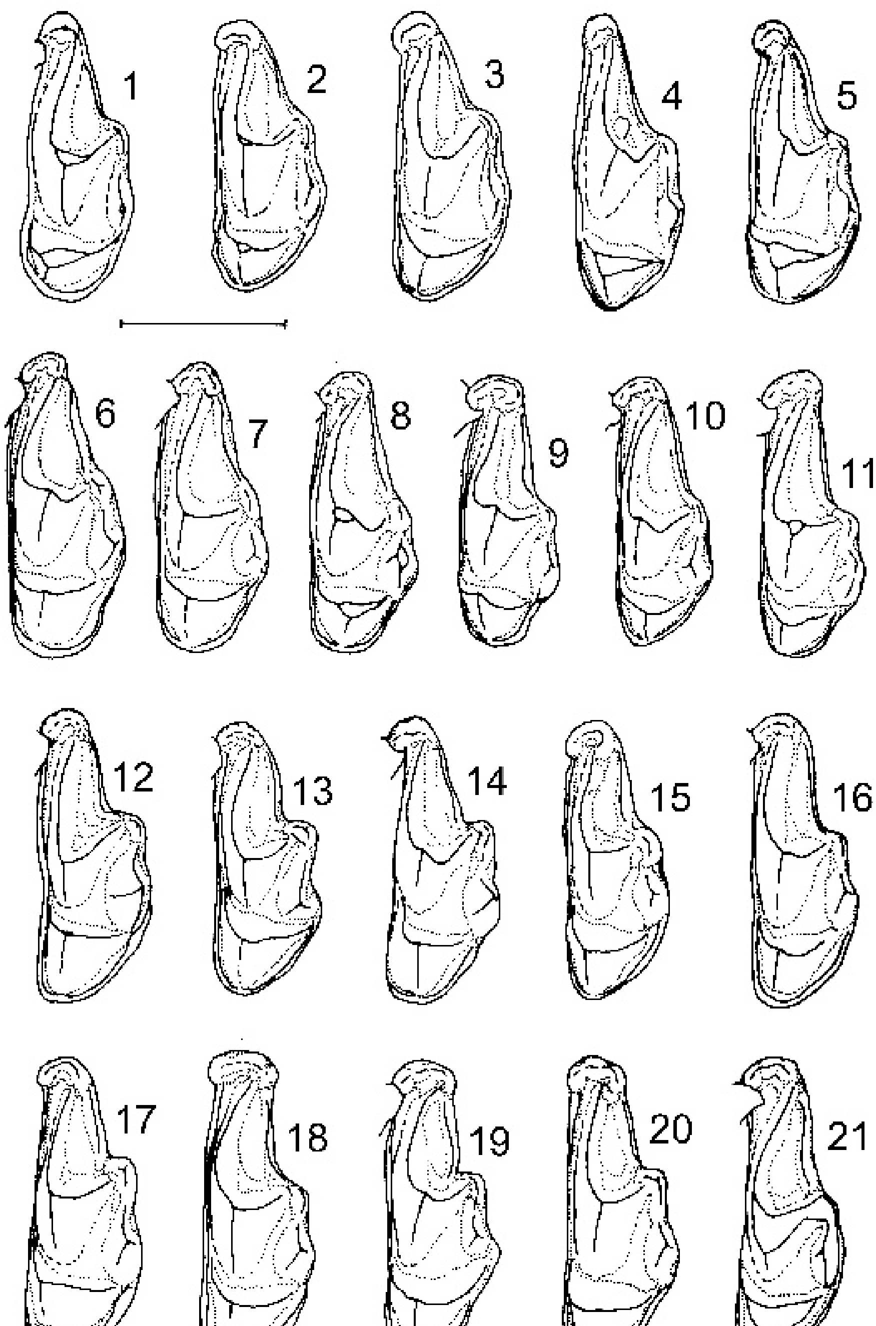

Fig. 6-9. Variation of the shape of the left upper third molar (M^3) in several species of *Plecotus*. 1–5 – *P. christii* (1 – IVB 100, Valley of the Kings, Egypt, 2 – NMP E-71, Bir Nagat, Egypt, 3 – NMP E-72, Bir Kohila, Egypt, 4, 5 – NMP 49863, 49862, Al Jaghbub, Libya); 6–8 – *P. t. gaisleri* subsp. n. (NMP 49920–49922, Wadi Al Kuf, Libya), 9–11 – *P. t.* cf. *gaisleri* subsp. n. (9–10 – NMP 49857, 49856, Ain Az Zarqa, Libya, 11 – NMP 49966, Nanatalah, Libya); 12–16 – *P. t. kolombatovici* (12 – NMP 49091, Zavala, Croatia, 13 – ZFMK 97.214, Korfu, Greece, 14 – NMP 48727, Kombotades, Greece, 15 – JGUM, Letoon, Turkey, 16 – 48087, Çevlik, Turkey); 17–21 – *P. m. macrobullaris* (17 – NMP 48993, Maalula, Syria, 18 – NMP 48053, Yabroud, Syria, 19 – NMP 47911, Van, Turkey, 20 – NMP 48126, Choplu, Iran, 21 – NMP 48139, Takht-e-Suleyman, Iran). Scale line – 1 mm.

Fig. 6-10. Portraits of *Plecotus christii* (Photos: P. Benda).

6.4 Discussion

In the morphological and genetic analyses, we examined samples of most west Palaearctic populations of long-eared bats that can also be considered to occur on the African continent. The analyses have clearly separated two populations of long-eared bats in Africa. The first population of smaller bats with slightly built teeth and a narrow baculum with broad arms inhabits the Nile Valley and oases of the Libyan and Egyptian deserts. This population was traditionally referred to as *P. austriacus christii* (Hanák & Elgadi 1984, Qumsiyeh 1985, Nader & Kock 1990). However, morphological and molecular evidence shows its position as a sister group to *P. balensis.* Since the north-east African desert form differs from *P. balensis* by 4.1%, it indicates that this lineage is a true species, which should be named *P. christii* Gray, 1838. Kruskop & Lavrenchenko (2000) previously described morphological differences between *P. balensis* and *P. christii*, and our analyses confirm their conclusions. Differences in colouration between both forms were mentioned several times (Kock 1969, Nader & Kock 1990, Kruskop & Lavrenchenko 2000). Both species originated from one centre, possibly east-African or east-Mediterranean. The *P. balensis* group (*P. christii and P. balensis*) is evidently a parallel lineage to the *P. austriacus* and *P. teneriffae/kolombatovici* lineages, inhabiting southern Europe, north-western Africa and Macaronesia (Juste *et al.* 2004). The *auritus* lineage, composed at least of three species – *P. auritus*, *P. macrobullaris/alpinus* and *P. sardus* (chapter 5, Spitzenberger *et al.* 2003, Juste *et al.* 2004, our results) – does not reach the African continent. According to our analyses, this lineage occurs in the northern Mediterranean only, from Iberia in the west to Crete and the Levantine Mountains in the east (see also Juste *et al.* 2004).

Both our morphological and molecular analyses support the close relationship between the Middle Eastern populations, recently considered as the independent species *P. macrobullaris* (Spitzenberger *et al.* 2003), and the recently described *P. alpinus*. Spitzenberger *et al.* (2003) and Juste *et al.* (2004) suggested including both forms into one species on the basis of their genetic similarity. Although several morphological features, mostly cranial, are in accordance with the molecular evidence, *P. macrobullaris* and *P. alpinus* differ markedly in body size, colouration and some other cranial characters such as breadth of braincase without bullae (Spitzenberger 2003; figure 6-4) and largest diameter of bullae (LBT; figure 6-2). This supports the opinion of Spitzenberger *et al.* (2003) about a subspecific division of the species *P. macrobullaris* into the Middle Eastern *P. m. macrobullaris* Kuzjakin, 1965 and the Euro-alpine *P. m. alpinus* Kiefer et Veith, 2002.

A third African population that is well differentiated by our morphological analyses inhabits the Mediterranean part of northern Africa in Maghreb and Libya. It was formerly assigned to *P. austriacus* (Gaisler 1983, Hanák & Elgadi 1984, Qumsiyeh 1985, Nader & Kock

1990, Kowalski & Rzebik-Kowalska 1991, Koopman 1994) and later included in the *P. teneriffae/kolombatovici* group (Juste *et al.* 2004). These bats are medium to large-sized and have a very typically broad and large baculum, clearly different from other species of the genus *Plecotus*, mainly from *P. auritus*, *P. balensis*, *P. teneriffae*, *P. kolombatovici*, and *P. austriacus*. Molecular evidence affiliates this population to another African taxon, *P. teneriffae* from the Canary Islands. However, *P. teneriffae* differs from all Afro-Mediterranean populations in coloration (it is darker and more greyish), in body and skull size (it is significantly larger), and in the distinct shape of the baculum, which in general is similar to that of *P. auritus* (Ibáñez & Fernández 1985a, own data). Although the Afro-Mediterranean population is very young from the point of view of molecular differentiation (1.8–2.2% divergence from the two other sublineages of the *P. teneriffae/kolombatovici* clade, and thus below the level usually found between *Plecotus* species), it is a morphologically well-defined form. Therefore we propose that the Afro-Mediterranean population be assigned to a new taxon at the subspecific level within a species that also includes the *P. teneriffae* and *P. kolombatovici* lineages. As in *P. m. macrobullaris* and *P. m. alpinus*, these three sublineages clearly show that morphology may differ substantially among genetically similar populations of long-eared bats. Consequently, the whole group is composed of three lineages, which, in concordance with degrees of genetic differentiation in other *Plecotus* lineages, are differentiated at the subspecific level: *P. teneriffae teneriffae*, *P. teneriffae kolombatovici*, and a clade formed by Afro-Mediterranean long-eared bats, *P. teneriffae* subsp. They form a monophylum, with the Afro-Mediterranean haplotypes potentially being paraphyletic with respect to *kolombatovici* and *teneriffae* (low bootstrap support). More molecular data are needed to unambiguously resolve the splits within this group. Due to the occurrence of two morphologically distinct forms in the island of Pantelleria (see below) we cannot exclude that all three lineages may in fact represent true biological species.

Specimens from the African offshore island of Pantelleria seem to be a key group in resolving the taxonomic status of the *P. teneriffae/kolombatovici* sublineages. They morphologically resemble specimens of the Afro-Mediterranean population. However, one specimen morphologically groups with *P. kolombatovici*. This may indicate sympatric occurrence of both forms, which in fact would confirm the specific status of both the populations. Nevertheless, these statements must be confirmed by a broader analysis of the Pantellerian long-eared bats and other geographically proximal populations, i.e. from Tunisia, Sicily, Italy and Malta.

In conclusion, our analyses clearly show that at least four different forms of *Plecotus* are allopatrically distributed in Africa: *Plecotus teneriffae teneriffae* (Canary Islands), *Plecotus teneriffae* subsp. (northern Cyrenaica and very probably all the Mediterranean Africa), *Plecotus christii* (north-eastern deserts of Africa), and *Plecotus balensis* (Ethiopian Highlands).

Another African population of Plecotine bats, which inhabits western Africa (Senegal and Cape Verde Islands) remains of uncertain systematic position. The collection of the Natural History Museum in Paris has a male specimen (No. 1983-1467 in alcohol, without skull) from the Cape Verde Islands but the measurements of forearm and thumb (LAt 40.4 mm, LPol 5.5 mm) only confirm that this specimen does not belong to *P*. *teneriffae* or to the Afro-Mediterranean population. This population was mentioned only once (Rochebrune 1883, Dorst & Naurois 1966), and we therefore consider its status as uncertain because of the possibility of misidentification of collected specimens and/or misinterpretation of their origin (see also Grubb & Ansell 1996).

6.5 Taxonomy of the African populations of *Plecotus*

6.5.1 *Plecotus christii* Gray, 1838

Vespertilio auritus aegyptius Fischer, 1829: 117 [nec *Vespertilio pipistrellus aegyptius* Fischer, 1829].

Plecotus christii Gray, 1838: 495.

Plecotus aegyptiacus Fitzinger, 1866: 545 [partim].

Plecotus auritus: Dobson 1878: 179 [partim]; Anderson 1902: 114; De Beaux 1928: 42; Flower 1932: 380; Zavattari 1934: 887; Zavattari 1937: 547; Ryberg 1947: plate 42 [partim]; Koopman 1975: 416; etc.

Plecotus christiei [sic]: Thomas 1911b: 160; Hayman 1948: 39.

Plecotus auritus aegyptius: Allen 1939: 96.

Plecotus auritus christiei [sic]: Ellerman & Morrison-Scott 1951: 181; Toschi 1954: 246, Bauer 1956: 314.

Plecotus auritus cf. *christii*: Setzer 1957: 49.

Plecotus wardi: Lanza 1960: 10 [partim].

Plecotus austriacus: Hanák 1962: 91 [partim]; Hufnagl 1972: 33; Madkour 1977: 182; Wassif *et al.* 1984: 8; Koopman 1993: 224 [partim]; etc.

Plecotus austriacus christiei [sic]: Harrison 1964: 181 [partim]; Hanák 1966: 64; Kock 1969: 179 [partim]; Hayman & Hill 1971: 35 [partim]; Gaisler *et al.* 1972: 28–33; Atallah 1977: 309 [partim]; Corbet 1978: 61 [partim]; Qumsiyeh 1985: 65; Osborn 1988: 597; Nader & Kock 1990: 319; Harrison & Bates 1991: 101; Koopman 1994: 110 [partim]; Kruskop & Lavrenchenko 2000: 8; etc.

Type locality. Nile Valley between Qena and Aswan, southern Egypt (restricted by Qumsiyeh 1985: 65).

Description. Small long-eared bat. Forearm is short (LAt 36.7–40.2 mm), thumb short (LPol 5.5–5.6 mm). Skull is small (LCb 14.9–15.6 mm), with medium-sized tympanic bullae (LBT 4.3–4.6 mm). Rostral part of skull is relatively short (I^1M^3/LCr 0.37–0.38), low and very narrow (CC 3.3–3.5 mm; absolute and relative narrowest from the compared samples, see App.1; CC/CM^3 0.63–0.65). Concavity of the frontal region is relatively flat with low and short sagittal crest (Fig. 6-8). The braincase is relatively medium in height, but long and very narrow (LaN/LCr 0.45–0.50, most narrow of the compared samples). Zygomatic arches bear very low orbital process. Mandible is proportionally short, coronoid process low (ACo 2.7–2.9 mm; ACo/LMd 0.26–0.29). First upper incisors are mesiodistally short (0.52–0.58 mm). Cingula of upper canines are mesiodistally short (LCn 0.86–1.02 mm) and relatively narrow (LCn/LaCn 1.25–1.41). Crowns of upper canines are low, first upper premolars (P^3) are minute, their cingula are mesiodistally short (LP^3 0.37–0.44 mm) and palatolabially narrow (LaP^3 0.41–0.50 mm). Second upper premolars (P^4) bear a very low cusp on the mesiopalatal edge of cingulum (ACin 0.02–0.06 mm). Third upper molars (M^3) are robust (Fig. 9) and mesiodistally relatively long (LM^3/LaM^3 0.36–0.41), with broad and long protofossae, small and simple parastyles, short preparacristal parts and long premetacristal parts. Muzzle is blunt (Fig. 6-10), with large oval preorbital glands, rounded supraorbital glands are small (ca. 0.9 mm in diameter), the glands behind the mouth corner are very tiny (close to the state of *P. kolombatovici* after Spitzenberger *et al.* 2002: Fig. 6). The distal part of the glans penis is broad (ca. 2.5 mm in the broadest part) and rounded (of the *austriacus* & *kolombatovici* type after chapter 5: Fig. 3). The baculum is small and narrow, its proximal arms are short and broad, in an obtuse or (more often) an acute angle, proximal extremity is narrow and pointed (Fig. 6-5, Lanza 1960, Wassif & Madkour 1972a, Hanák & Elgadi 1984, Qumsiyeh 1985). The coloration of pelage and naked parts is generally very pale; the distal parts of the dorsal hairs are very light brownish-grey or umber, the proximal parts of the dorsal hairs are chestnut brown. Distal parts of ventral hairs are creamy to white, proximal parts chestnut brown. Wing membranes are light grey, distal parts are lighter up to the creamy margins of the plagiopatagium. Ears and tragi are pale umber, unpigmented (only tips and inner distal margins of tragi are slightly brownish), face is light greyish brown. The end of the tail projects ca. 1.5 mm from the uropatagium. For cranial, dental, and wing measurements incl. basic statistical evaluation see App. 6-1. Some other morphological notes are given by Wassif & Madkour (1969, 1970, 1971, 1972b, c, 1973, 1974) and by Madkour (1987).

Distribution. Confirmed distribution of *P. christii* is restricted only to the north-eastern African desert region, which probably also includes the Sinai (Kock 1969, Koopman 1975, Qumsiyeh 1985). Most records come from the Nile Valley, from the river delta in northern Egypt up to the Fifth Cataract of the Nile in northern Sudan (Fitzinger 1866, Anderson 1902, Flower 1932, Lanza 1960, Hoogstraal 1962, Kock 1969, Gaisler *et al.* 1972, Madkour 1977, Wassif *et al.* 1984, Qumsiyeh 1985). Easternmost records are available from the Qatar Range, Red Sea Mts. (Frauenfeld 1856, Osborn 1988), or from southern Sinai, respectively (Anderson 1902, Flower 1932, Madkour 1977, Qumsiyeh 1985). The species also inhabits oases in the Siwa Basin, incl. the oasis of Al Jaghbub, in the Western (= Libyan) Desert on the Libyan-Egyptian border (De Beaux 1928, Zavattari 1934, 1937, Hayman 1948, Lanza 1960, Hanák 1966, Hanák & Elgadi 1984, own data). The occurrence of *P. christii* in the Arabian Peninsula remains open (Harrison & Bates 1991).

6.5.2 *Plecotus balensis* Kruskop et Lavrenchenko, 2000

Plecotus auritus: Rüppell 1842: 156; Peters 1866: 18; Ryberg 1947: plate 42 [partim].
Plecotus aegyptiacus Fitzinger, 1866: 545 [partim].
Plecotus austriacus: Hayman & Hill 1971: 35 [partim]; Largen *et al.* 1974: 246; Nader & Kock 1990: 319 [partim]; Yalden *et al.* 1996: 89.
Plecotus austriacus christiei [sic]: Kock 1969: 179 [partim]; Atallah 1977: 309 [partim]; Koopman 1994: 110 [partim].
Plecotus balensis Kruskop & Lavrenchenko, 2000: 6; Juste *et al.* 2004: in press.

Type locality. Harenna Forest, Bale Mountains National Park, southern Ethiopia (Kruskop & Lavrenchenko 2000).

Description. Small to middle-sized long-eared bat. Forearm and thumb are rather short (LAt 36.3–41.2 mm, LPol 5.4–6.2 mm). Skull is rather medium-sized (LCb 15.2–15.9 mm), with medium-sized tympanic bullae (LBT 4.3–4.5 mm). Rostral part of skull is relatively short (I^1M^3/LCr 0.37–0.39) and rather narrow (CC 3.5–3.8 mm; CC/CM^3 0.65–0.68). Concavity of the frontal region is relatively flat (Kruskop & Lavrenchenko 2000: Fig. 1). Braincase is of relatively medium height and very broad (LaN/LCr 0.50–0.53, most broad from the compared samples). Coronoid process of mandible is very low (ACo 2.7–2.8 mm; ACo/LMd 0.26–0.27). First upper incisors are mesiodistally rather long (0.60–0.64 mm). Cingula of upper canines are mesiodistally medium in length (LCn 1.01–1.07 mm) and relatively narrow (LCn/LaCn 1.26–1.36). Crowns of upper canines are low, first upper premolars (P^3) are medium-sized, the cingula are mesiodistally short (LP^3 0.46–0.49 mm) and palatolabially broad (LaP^3 0.50–0.55 mm). Second upper premolars (P^4) bear a medium height cusp on the mesiopalatal edge of cingulum (ACin 0.08–0.13 mm). Third upper molars (M^3) are mesiodistally relative long (LM^3/LaM^3 0.37–0.44). Muzzle is blunt, with smaller preorbital glands and tiny rounded supraorbital glands (Kruskop & Lavrenchenko 2000: Fig. 5). Information on the external shape of the glans penis is not available. The baculum is asterisk-shaped, overall very broad with long and narrow proximal arms in an obtuse angle, distal part is narrow, all three epiphyses are expanded and the distal epiphysis is perforated terminally (Fig. 6-5, Kruskop & Lavrenchenko 2000: Fig. 7). The coloration of the dorsal pelage is dark chestnut brown, ventral pelage pale greyish, and naked parts are dark brown or blackish brown (see also Kruskop & Lavrenchenko 2000). For cranial, dental, and wing measurements including basic statistics see App. 6-1. For further details of description see Kruskop & Lavrenchenko (2000).

Distribution. The known distribution of the species is restricted to the Ethiopian Highlands, both to the south and to the north of the Rift Valley. All records come from mountainous areas above 2000 m a. s. l. In Ethiopia *P. balensis* was found in Shoa (= Shewa) or the vicinity of Addis Ababa, respectively (Rüppell 1842, Peters 1866, Kruskop & Lavrenchenko 2000), in the Bale Mts. (Yalden *et al.* 1996, Kruskop & Lavrenchenko 2000), and near Abune Yusef (Juste *et al.* 2004). The record from Asmara, Eritrea (Sordelli 1902) also probably belongs to this species, originating from a high elevation (ca. 2350 m a. s. l.).

6.5.3 *Plecotus teneriffae teneriffae* Barret-Hamilton, 1907

Plecotus teneriffae Barret-Hamilton, 1907: 520; Ibáñez & Fernández 1985a: 147; Ibáñez & Fernández 1985b: 310; Trujillo 1991: 77; Trujillo & Barone 1991: 106; Koopman 1993: 225; Horáček *et al.* 2000: 135; Pestano *et al.* 2003: 302.
Plecotus austriacus: Koopman 1993: 224 [partim].
Plecotus austriacus teneriffae: Koopman 1994: 110.
For older synonymy see Ibáñez & Fernández (1985a: 147).

Type locality. Orotava, Tenerife Island (Barret-Hamilton 1907: 520)

Description. Large long-eared bat. Forearm is long – LAt 41.1–42.6 mm (own data), 42.0–45.4 mm (Ibáñez & Fernández 1985a, b), 40.1–46 mm (Trujillo 1991), 44.0 mm in holotype specimen (Barret-Hamilton 1907). Thumb is moderately long – LPol 6.3–6.7 mm (own data), 6.0–6.5 (Ibáñez & Fernández 1985a). Skull is large – LCb 16.2–16.6 mm (own data), 16.4–17.2 mm (Ibáñez & Fernández 1985a), with large tympanic bullae – LBT 4.58–4.74 mm (own data), 4.6–4.9 mm (Ibáñez & Fernández 1985a). Rostral part of skull is relatively long (I^1M^3/LCr 0.39–0.41) and rather narrow (CC 3.8–4.0 mm; CC/CM^3 0.64–0.66). Braincase is relatively low, short and broad (LaN/LCr 0.49–0.51). Coronoid process of mandible is very high (ACo 3.3–3.4 mm; ACo/LMd 0.29–0.30). First upper incisors are mesiodistally long (0.62–0.66 mm). Cingula of upper canines are mesiodistally medium in length (LCn 1.07–1.12 mm), but relatively very broad (LCn/LaCn 1.12–1.19). Crowns of upper canines are relatively low, first upper premolars (P^3) are medium-sized, the cingula are mesiodistally short (LP^3 0.40–0.49 mm) and palatolabially broad (LaP^3 0.51–0.58 mm). Second upper premolars (P^4) bear a medium high cusp on the mesiopalatal edge of cingulum (ACin 0.06–0.10 mm). Third upper molars (M^3) are mesiodistally relative long (LM^3/LaM^3 0.37–0.42). Muzzle is relatively narrow and "pointed", with long oval preorbital glands and larger rounded supraorbital glands (Trujillo 1991: Photos No. 33, 34, 39, 40), but generally resembling the *P. austriacus* type after Strelkov (1988: Ris. 1). Information on the external shape of the glans penis is not available. Baculum is asterisk-shaped, overall very broad with long and thin proximal arms in an obtuse angle with extended epiphyses, the distal part is thin and pointed distally (Fig. 5, Ibáñez & Fernández 1985a: Fig. 2). The colouration of distal parts of hairs of the dorsal pelage is dark ashy grey or greyish brown, the ventral pelage light grey, the basal parts of hairs on both sides are blackish; the naked parts are dark grey or brownish grey (see also Trujillo 1991: 77–78, and above mentioned photos). The end of the tail projects from the uropatagium (Trujillo 1991). For cranial, dental, and wing measurements including basic statistics see App. 6-1, for other particulars of description see Ibáñez & Fernández (1985a, b) and Trujillo (1991).

Distribution. Trujillo (1991) mentioned confirmed records on three western islands of the Canarian archipelago (i.e., the islands of Tenerife, La Palma and El Hierro), and suggested a possible occurrence on the fourth island, La Gomera. Records from the three mentioned islands only are also reported by Pestano *et al.* (2003).

6.5.4 *Plecotus teneriffae gaisleri* subsp. n.

Plecotus auritus: Loche 1867: 78; Lataste 1885: 66; Rode 1947: 138; Ryberg 1947: plate 42 [partim]; Panouse 1951: 96; Panouse 1953: 97; Brosset 1955: 304; Deleuil & Labbé 1955: 48; Brosset 1960: 249; Brosset 1963: 442.
Plecotus auritus auritus: Laurent 1939: 279.
Plecotus auritus christiei [sic]: Ellerman & Morrison-Scott 1951: 181 [partim].
Plecotus austriacus: Hanák 1962: 91 [partim]; Baker *et al.* 1971: 701; Qumsiyeh & Schlitter 1982: 387; Aulagnier & Destre 1985: 333; Kowalski & Rzebik-Kowalska 1991: 111; Koopman 1993: 224; Bogdanowicz *et al.* 1998: 86; Zagorodniuk 2001: 102 [partim]; etc.
Plecotus austriacus christiei [sic]: Hill 1964: 86; Hanák 1966: 64 [partim]; Kock 1969: 179 [partim]; Hayman & Hill 1971: 35 [partim]; Atallah 1977: 309 [partim]; Corbet 1978: 61 [partim]; Aulagnier & Thevenot 1986: 53.
Plecotus austriacus aegyptius: Aellen & Strinati 1969: 427; Anciaux de Faveaux 1976: 71; Gaisler 1983: 365.
Plecotus austriacus austriacus: Hanák & Elgadi 1984: 180–181; Qumsiyeh 1985: 70; Nader & Kock 1990: 319 [partim]; Koopman 1994: 110 [partim].
Plecotus cf. *kolombatovici*: Juste et al. 2004

Type material. Holotype: Adult female, NMP 49911, specimen in alcohol, skull extracted; 20 May 2002, Wadi al Kuf, SW Massah, Cyrenaica, Libya, leg. P. Benda, V. Hanák, M. Andreas, A. Reiter & M. Uhrin. Paratypes: Four males and two females, NMP 49905–

49907, specimens in alcohol, skulls extracted, and NMP 49908–49910, alcohol specimens; the same date, locality and collectors as for the holotype specimen.

Type locality. Central part of the Wadi Al Kuf (Jabal Akhdar Mts.), ca. 8 km south-west from Massah, Al Jabal Al Akhdar Dist., Cyrenaica, north-eastern Libya, 32° 42' N, 21° 35' E; ca. 330 m a. s. l.

Etymology. Patronymic; named in honour of Professor Jiří Gaisler (Brno, the Czech Republic) who significantly contributed to the knowledge of the North African bat fauna.

Dimensions of the holotype specimen. External measurements: body length 55 mm, tail length 48 mm, LAt 40.4 mm, LPol 6.2 mm, ear length 36.8 mm, tragus length 17.2 mm, weight 8.3 g. Cranial measurements: LCr 17.08 mm, LCb 16.07 mm, LCc 15.56 mm, LaZ 8.93 mm, LaI 3.47 mm, LaInf 4.32 mm, LaN 8.07 mm, ANc 5.48 mm, ACr 7.57 mm, LBT 4.48 mm, CC 4.02 mm, P^4P^4 5.14 mm, M^3M^3 6.34 mm, I^1M^3 6.83 mm, CM^3 5.83 mm, M^1M^3 3.39 mm, CP^4 2.71 mm, LMd 11.07 mm, ACo 3.17 mm, I_1M_3 7.15 mm, CM_3 6.25 mm, M_1M_3 3.82 mm, CP_4 2.46 mm. Dental dimensions: LI^1 0.64 mm, LaI^1 0.49 mm, AI^1 0.80 mm, LCn 1.23 mm, LaCn 0.92 mm, ACn 1.46 mm, LP^3 0.49 mm, LaP^3 0.60 mm, AP^3 0.46 mm, LM^1 1.40 mm, LaM^1 1.61 mm, LM^3 0.61 mm, LaM^3 1.58 mm, ACin 0.02 mm.

Description. Medium-sized long-eared bat. Forearm is medium in length (LAt 37.2–40.9 mm), thumb medium-sized (LPol 6.1–6.8 mm). Skull is medium-sized to large (LCb 15.6–16.3 mm), with medium-sized to large tympanic bullae (LBT 4.5–4.7 mm). Rostral part of skull is relatively long (I^1M^3/LCr 0.39–0.41), medium in height and quite broad (CC 3.9–4.2 mm; CC/CM^3 0.68–0.71). Concavity of the frontal region is relatively deep with a low sagittal crest (Fig. 6-8). Braincase is relatively high, short and broad (LaN/LCr 0.47–0.51). Zygomatic arches bear distinct orbital process (Fig. 6-8). Coronoid process of mandible is high (ACo 2.8–3.4 mm; ACo/LMd 0.26–0.31). First upper incisors are mesiodistally long (0.59–0.68 mm). Cingula of upper canines are mesiodistally long (LCn 1.09–1.24 mm), relatively rather broad (LCn/LaCn 1.19–1.36). Crowns of upper canines are high, first upper premolars (P^3) are large, the cingula are mesiodistally long (LP^3 0.44–0.54 mm) and palatolabially broad (LaP^3 0.50–0.62 mm). Second upper premolars (P^4) often bear a very low cusp on the mesiopalatal edge of cingulum (ACin 0.00–0.07 mm). Third upper molars (M^3) are rather fine (Fig. 6-9) and mesiodistally relatively long (LM^3/LaM^3 0.36–0.40), with short and narrow protofossae, long parastyles with a horseshoe-like convolute crest (the state similar to *P. macrobullaris* s. l.), long preparacristal parts and distinctly short premetacristal parts. Muzzle is narrow (Fig. 6-11), in shape generally resembling that of European *P. austriacus* (sensu Strelkov 1988: Ris. 1) but broader, with large oval preorbital glands, rounded supraorbital glands are moderately small (ca. 1.2 mm in diameter), the glands behind the mouth corner are very tiny (close to the state of *P. kolombatovici* after Spitzenberger *et al.* 2002: Fig. 6-6). Distal part of the glans penis is broad (the most broad part is ca. 3 mm) and rounded (of the *austriacus* & *kolombatovici* type after Mucedda *et al.* 2002 (chapter 5): Fig. 5-3). The baculum is large and broad, its proximal arms are long and broad, in a relatively acute angle, the proximal extremity is broad and blunt (Fig. 6-5, Hanák & Elgadi 1984, Qumsiyeh 1985); it is a large bone, in shape slightly resembling that of *P. m. macrobullaris* but more massive (Fig. 6-5). The coloration of the pelage is generally dark. Dorsal hairs are 7–8 mm long, their distal quarter is hazelnut brown or dark umber, the proximal 4–5 mm segment is dark chestnut brown. The ventral side is overall light brown, the hairs are 7–8 mm long. Distal parts of ventral hairs are ochre, proximal parts dark greyish-brown. Face, wing membranes, ears and tragi are dark brown to dark greyish-brown. Ears and tragi are pigmented equally in the whole length, but are lighter in the most proximal parts. The end of the tail projects ca. 1 mm from the uropatagium. For cranial, dental, and wing measurements including basic statistical evaluation see App. 6-1.

Differential diagnosis. *Plecotus t. gaisleri* subsp. n. differs significantly from other west-Palaearctic and African species of *Plecotus*. It markedly differs in sequences of part of the mitochondrial 16S gene from the long-eared bats of the *auritus* group (*P. a. auritus*, *P. a. begognae*, *P. m. alpinus*, *P. m. macrobullaris* and *P. sardus*), from the *balensis* lineage (*P. christii* and *P. balensis*), and from *P. austriacus* (see Fig. 6-7 and Results chapter for more details). The morphological differences are as follow.

Plecotus t. gaisleri subsp. n. differs from *P. t. teneriffae* Barret-Hamilton, 1907 in coloration and some morphological features; the coloration of pelage and naked parts of *P. t. gaisleri* subsp. n. is lighter, that of dorsal hairs is more brownish. The forearm length, longitudinal dimensions of skull and rostrum (LCr, LCb, LCc, I^1M^3, CM^3, M^1M^3, CP^4) and all the mandibular dimensions (excl. of CP_4) are significantly larger in *P. t. teneriffae* than in *P. t. gaisleri* subsp. n. (see App. 6-1 and Tab. 6-2). *P. t. teneriffae* has a significantly broader upper part of rostrum and the braincase, a relatively higher coronoid process of mandible, and has mesiodistally longer and relatively narrower upper canines, and significantly larger molars, M^3 is relatively and absolutely longer. The most important and distinct character is the shape of baculum; in *P. t. teneriffae* it is a very gracile thin asterisk-shape bone with thin proximal arms in an obtuse angle, while in *P. t. gaisleri* subsp. n. the baculum is broad and triangle-shape with broad proximal arms in a more acute angle (Fig. 6-5). *Plecotus t. gaisleri* subsp. n. differs from *P. t. kolombatovici* Đulić, 1980, in coloration and some morphological features; the coloration of pelage and naked parts is more dark and greyish in *P. t. gaisleri* subsp. n., while in *P. t. kolombatovici* it is brownish with light brown naked parts. *Plecotus t. gaisleri* subsp. n. has a significantly longer forearm and thumb; *P. t. kolombatovici* has a smaller skull in almost all dimensions (see App. 6-1, most significantly different in LCr, LCb, LCc, LaZ, CC, P^4P^4, M^3M^3, I^1M^3, CM^3, M^1M^3, CP^4, LMd, I_1M_3, CM_3, CP_4), with a relatively shorter rostrum (I^1M^3/LCr 0.38–0.40), relatively broader and higher braincase (LaN/LCr 0.47–0.51), significantly shorter and relatively broader first upper molar (M^1), and overall smaller and gracile teeth. The shape of M^3 is very distinct (Fig. 6-3). The baculum of *P. t. kolombatovici* differs from that of *P. t. gaisleri* subsp. n. in size (smaller) and in the width of the distal extremity and proximal arms (broader, see Fig. 6-5).

Plecotus t. gaisleri subsp. n. differs from *P. christii* Gray, 1838 in coloration and some morphological characters; the colouration of pelage of *P. christii* is very pale greyish brown, wing membranes and ears in *P. christii* are almost unpigmented pale greyish. In *P. t. gaisleri* subsp. n. colouration of all parts is very significantly darker. Like *P. t. kolombatovici*, *P. christii* has a smaller forearm, thumb, and skull than *P. t. gaisleri* subsp. n. (see App. 6-1 and Tab. 6-2). *Plecotus christii* has a relatively shorter, lower and narrower rostrum, smaller tympanic bullae, distinctly shorter but relatively broader upper canines, a relatively lower coronoid process and narrower braincase. The shape of M^3 is very distinct (Fig. 6-9). The baculum of *P. t. gaisleri* subsp. n. is larger and broader than that of *P. christii*, with the proximal extremity broad and blunt (see Fig. 6-5). *Plecotus t. gaisleri* subsp. n. differs from *P. balensis* Kruskop et Lavrenchenko, 2000 in colouration and some morphological characters; the colouration of pelage and naked parts of *P. balensis* is more dark, the pelage is dark chestnut brown, without greyish tinges, and naked parts are blackish brown, while in *P. t. gaisleri* subsp. n. they are dark brown. *P. balensis* has a smaller thumb and skull than *P. t. gaisleri* subsp. n. (see App. 6-1), it is significantly different in all longitudinal skull dimensions. *Plecotus balensis* has a relatively shorter and narrower rostrum, smaller tympanic bullae, shorter but relatively distinctly broader upper canines, a relatively and absolutely lower coronoid process and broader braincase. The baculum in *P. balensis* is a very gracile thin asterisk-shape bone with thin proximal arms in obtuse angle and distal perforated thickness; while in *P. t. gaisleri* subsp. n. the baculum is broad and triangle-shaped with broad proximal arms in a more acute angle (Fig. 6-5).

Plecotus t. gaisleri subp. n. differs from *P. austriacus* (Fischer, 1829) in colouration and some morphological characters; the colouration of the pelage and naked parts of *P. t. gaisleri* subsp. n. is more brownish than in *P. austriacus*, which is dominated by greyish shades. *Plecotus austriacus* is significantly larger in most cranial and dental dimensions (see App. 6-1 and Tab. 6-2, Figs. 6-2 – 6-4, 6-8). *Plecotus austriacus* has a relatively lower braincase, higher rostrum (but equally broad), and some higher coronoid processes than *P. t. gaisleri* subsp. n. Like in *P. t. kolombatovici*, the baculum of *P. austriacus* is smaller and more gracile than that of *P. t. gaisleri* subsp. n. *Plecotus. t. gaisleri* subsp. n. differs from *P. auritus* (Linnaeus, 1758) in colouration and some morphological features; the colouration of *P. auritus* is lighter and more brownish than that of *P. t. gaisleri* subsp. n. *Plecotus auritus* has a distinctly longer thumb and very small tympanic bullae, *P. a. auritus* has a smaller skull and more gracile teeth than *P. t. gaisleri* subsp. n. (Tab. 6-1, Figs. 6-2, 6-8). The baculum of *P. auritus* is a very large bone with relatively thin proximal arms in an obtuse angle and a relatively thin distal part and completely differs from that of *P. t. gaisleri* subsp. n. (Fig. 6-5). *Plecotus auritus* also has a different shape of glans penis (see chapter 5).

Plecotus m. macrobullaris Kuzjakin, 1965 differs from *P. t. gaisleri* subsp. n. in several morphological characters; due to its similar ecological requirements, *P. m. macrobullaris* barely differs in coloration, it is slightly more brownish. Like *P. auritus*, *P. m. macrobullaris* markedly differs from *P. t. gaisleri* subsp. n. in the length of thumb. *Plecotus m. macrobullaris* has larger tympanic bullae and a higher rostrum, but generally a smaller skull than *P. t. gaisleri* subsp. n.; *P. m. macrobullaris* further differs very distinctly in absolute and relative dimensions of upper molars and canines (App. 6-1 and Tab. 6-2). The baculum of *P. m. macrobullaris* is very similar to that of *P. t. gaisleri* subsp. n. in shape but is smaller and more gracile (Fig. 6-5). *Plecotus m. alpinus* Kiefer et Veith, 2002 differs from *P. t. gaisleri* subsp. n. in colouration and some morphological characters; the colouration of *P. m. alpinus* is dark greyish in the dorsal pelage and white in ventral hair (chapter 4), *P. t. gaisleri* subsp. n. is more brownish on both sides and lighter in the dorsal hairs. Like *P. kolombatovici*, *P. m. alpinus* generally has a smaller skull with smaller tympanic bullae and more slender teeth, smaller but relatively broader upper canines and a relatively lower coronoid process (Tab. 6-1). *Plecotus m. alpinus* also has a different shape of glans penis and baculum from *P. t. gaisleri* subsp. n. (Fig. 6-5, chaper 4, chapter 5).

<u>Comment</u>. Compared to other *Plecotus* taxa, *P. t. gaisleri* subsp. n. is probably very young (according to its genetic similarity with other forms of the *teneriffae* lineage, *P. t. teneriffae* and *P. t. kolombatovici*). Within the *teneriffae* lineage, significant morphological differences have evolved among populations, which correspond to or are even more significant than between other *Plecotus* species (see chapter 3). This strong morphological differentiation is not mirrored by genetic traits. Thus, with insufficient details for the description of a species, we evaluated the population of Cyrenaica, Libya as *P. t. gaisleri* subsp. n. Since we did not find profound morphological differences between this population and those inhabiting the Maghreb part of Mediterranean Africa (see also Tab. 6-3) we expect that the latter also belongs to the new subspecies. However, a more detailed genetic and morphological analysis of the Maghreb population is needed. Hence, the synonymy mentioned above covers the whole presumed range of the new subspecies, see “Distribution” below, while the subspecific description defines only the Cyrenaica population.

In the Maghreb population, a tendency towards light coloration was observed in the specimens from more arid desert and semi-desert habitats (i.e., Anti-Atlas Mts., Saharan Atlas Mts., or Jebel Nafusa Mts.). In contrast, populations from coastal and montane regions are as dark as described above. Because the same phenomenon was also observed in different populations of *P. m. macrobullaris* in the Middle East, we regard this colour polymorphism as irrelevant for taxonomy. However, in other *Plecotus* species a tendency for colour changes along a humidity gradient is not observed.

Fig. 6-11. Portrait of *P. teneriffae gaisleri* subsp. n. Left: Cyrenica population, right: Maghreb population (photos: P. Benda).

Distribution. *Plecotus t. gaisleri* subsp. n. is distributed in the belt of Mediterranean climate in northern Cyrenaica, Libya. The known distribution range covers a restricted area of ca. 10,000 sq. km of mountainous and coastal vegetation between Al Mari in the West and Darnah in the East (ca. 20° 30' – 22° 30' N). This bat was recorded on at least nine sites in the northern Cyrenaica: Wadi al Kuf and 6 km SE Qasr Maqdam (Qumsiyeh & Schlitter 1982, Qumsiyeh 1985), Quariat el Faioah (Hanák & Elgadi 1984), Shahat (Cyréné) (Hanák & Elgadi 1984, Juste *et al.* 2004), Wadi al Minshiyah, Sidi Muhammad al Mabkhut and three places in Wadi al Kuf (own records, see App. 6-2).

The distributional range of the north-west African (Maghreb) population which probably also belongs to *P. t. gaisleri* subsp. n. covers the entire belt of Mediterranean climate from Morocco over to Algeria and Tunisia and up to north-western Tripolitania, Libya. Most records come from Morocco (Panouse 1951, 1953, Brosset 1955, 1960, 1963, Hill 1964, Aulagnier & Destre 1985, Aulagnier & Thevenot 1986, Juste *et al.* 2004, etc.), and the range covers all the mountain ridges of the country from the Rif Mts. in the north up to the Anti-Atlas Mts in the south-west and Er Rachidia and Figuig in the south-east. From Algeria we know of several records, from the northern part of the country, extending from the sea coast southwards up to the southern slope of the Saharan Atlas Mts. (Loche 1858, 1867, Gaisler 1983, Gaisler & Kowalski 1986, Kowalski *et al.* 1986, Kowalski & Rzebik-Kowalska 1991, Zagorodniuk 2001). Tunisian records were mentioned from all parts of the country (Anderson 1892, Laurent 1939, Deleuil & Labbé 1955, Kock 1969, Aellen & Strinati 1969). In Tripolitania this bat was found at three sites in the Jebel Nafusa Mts. (Qumsiyeh 1985, Qumsiyeh & Schlitter 1982, own records, see App. 6-2).

Abstract

Long-eared bats of the genus *Plecotus* are widespread over most of temperate Eurasia, marginally reaching the African continent and Macaronesia. Previously, all African populations were assigned to one species, *P. auritus*, and later to *P. austriacus*. We analysed museum specimens of African long-eared bat populations using both morphologic and genetic techniques. Based on morphological evidence we recognise four well-defined allopatric populations in northern Africa. They differ in fur colouration, skull morphology and bacular traits. The molecular data support a division of the African populations into at least three well-separated evolutionary lineages. With a combination these data we define three species of *Plecotus* occurring in Africa (incl. the Canary Islands) and describe a new subspecies. Small, very pale greyish-brown Egyptian long-eared bats (*P. christii* Gray, 1838) inhabit desert and semi-deserts habitats of eastern Sahara (Libyan Desert, Nile Valley of Egypt and northern Sudan). Smaller to medium-sized, dark brown Ethiopian long-eared bats (*P. balensis* Kruskop et Lavrenchenko, 2000) inhabit the Ethiopian Highlands above 2000 metres a. s. l. This form represents the only Afro-tropical species of *Plecotus*. Large, dark greyish Canarian long-eared bats (*P. teneriffae teneriffae* Barret-Hamilton, 1907) occur on the three western islands of the Canarian Archipelago. A medium-sized greyish-brown Gaisler's long-eared bat, *P. teneriffae gaisleri* subsp. n., is described from the Mediterranean region of Cyrenaica, north-eastern Libya. Due to the lack of substantial morphological differences we preliminarily consider the Maghrebian population of long-eared bats to be consubspecific with *P. teneriffae gaisleri* subsp. n. The systematic position of the population of Cape Verde Islands remains uncertain.

7. General conclusions

To face the current biodiversity crisis, profound knowledge of species and their distribution is crucial. Tropical habitats with their extraordinary species richness are therefore in the focus of conservation biologists. In contrast, temperate biomes were for a long time regarded as sufficiently studied, at least with respect to vertebrates. It was therefore surprisingly that even among mammals, apart from birds the best studied group of vertebrates, several new European species were described during the last few years.

Many morphologically uniform but widespread species harbour extensive genetic variation (Avise 2000, Omland *et al.* 2000). This interspecific variation is sometimes so distinct that delimitation of new, morphologically cryptic species is needed. The discoveries of the Soprano Pipistrelle (*Pipistrellus pygmaeus* Leach 1825) by Barrett *et al.* (1997) and the Alcathoe's bat (*Myotis alcathoe* Helversen *et al.* 2001) are examples that accentuate the value of molecular tools in systematics. Most recently, two papers (Ibanez et al. 2006 and Mayer et al. 2007), show an unexpected high number of undiscovered species even in a seemingly well known group (bats) in an intensively sampled geographic region as the Western Palaeartic.

The genus *Plecotus* is probably the most outstanding example of how molecular markers have changed our view of lineage diversity and their phylogenetic relationships in a seemingly well-studied European vertebrate group. During the first half of the 20th century, only a single European species, *P. auritus*, was considered valid by competent taxonomists. Only in 1960, Bauer recognized *P. austriacus* as being a valid second species, a view that did not change for another three decades. Today, many more evolutionary *Plecotus* lineages can be discriminated.

Systematics of Western Palaearctic long-eared bats

In my first study I could show that contradictory phylogenies of *Plecotus* species inferred from two different contemporary studies simply arose from cryptic diversity. Differential geographical sampling within seemingly homogeneous taxa resulted in insufficient recognitions of the true diversity. My broader taxon sampling clearly showed that continental Europe is inhabited by four clear-cut evolutionary lineages of long-eared bats, each of which is differentiated at species level: *P. auritus, P. austriacus, P. kolombatovici* and *P. spec.* (now *P. macrobullaris*). This illustrates that taxonomic conclusions drawn from a geographically restricted sampling in general have to be interpreted carefully.

One of the new species constitutes a mixture of morphological characters usually diagnostic for either *P. auritus* or *P. austriacus*. It occurs in syntopy with both of them, with no signs of introgression. I therefore described it in the second part of this thesis as a new species, *Plecotus alpinus* (now *P. macrobullaris*), the Alpine long-eared bat. Genetically, *P. macrobullaris* is the sister species of *P. auritus.* Preliminary data on its distribution and ecology highlight a pronounced altitudinal niche separation among all three species.

My third approach was to illuminate the status of Sardinian long-eared bats. Three species occur on Sardinia: the grey long-eared bat, the brown long-eared bat and a previously unknown species. Based on its molecular and morphological differentiation I described it as *Plecotus sardus*, a species endemic to the island.

Finally I included samples from the Canary Islands, Northern Africa and the Caucasian Mountains. This synopsis of all western Palearctic Plecotine bat taxa covered molecular and morphological characters and looked for combined evidence of taxon delimitation. Seven well defined lineages are differentiated at species level. In northern Africa, including the Canary Islands, five lineages can be distinguished, two of which belong to *P. kolombatovici*.

Spitzenberger *et al.* (2006) treat *P. teneriffae* from the Canary Islands as a valid species because of its morphological distinctness (especially in the baculum). This is supported by molecular data from different mitochondrial genes (Juste *et al.* 2004, Spitzenberger *et al.* 2006 and chapter 6) as well as from nuclear genes (Schütte *et al*. unpublished). Therefore I now (contra chap. 6) follow Spitzenberger *et al.* (2006) in treating *P. teneriffae* as a valid species.

The situation within the "*kolombatovici-gaisleri*"-group is complex. Depending on the samples, two (chap. 6) or three (Juste *et al.* 2004, Spitzenberger *et al.* 2006) groups can be distinguished. This discord arises due to differential sampling in the Moroccan/Atlas Mountains area. At least two lineages inhabit NW Africa, with their phylogenetic position within the *P. kolombatovici* clade being unresolved (Figs. 6-7, 7-1; Juste *et al.* 2004). Due to the comparatively low genetic variation among lineages of the *P. kolombatovici* complex and their allopatric distribution, I now preliminarily regard them as different subspecies (following Spitzenberger *et al.* 2006): *P. k. kolombatovici* (from the Balkans and Asia Minor), *P. k. gaisleri* (from the Cyrenaica) and *P. k.* ssp. (from the Maghrebian). In the morphological analysis of skull and dental characters (chap. 6), the Maghrebian population is only slightly differentiated from the *P. k. gaisleri* from the Cyrenaica (Fig. 6-4). However the morphological analysis of Spitzenberger *et al.* (2006) using qualitative and quantitative characters separated three groups within *P. kolombatovici*: the north-eastern Mediterranean (Balkans and Asia Minor), the Cyrenaican (Lybian) and a Maghrebian population (Fig. 7 in Spitzenberger *et al.* 2006).

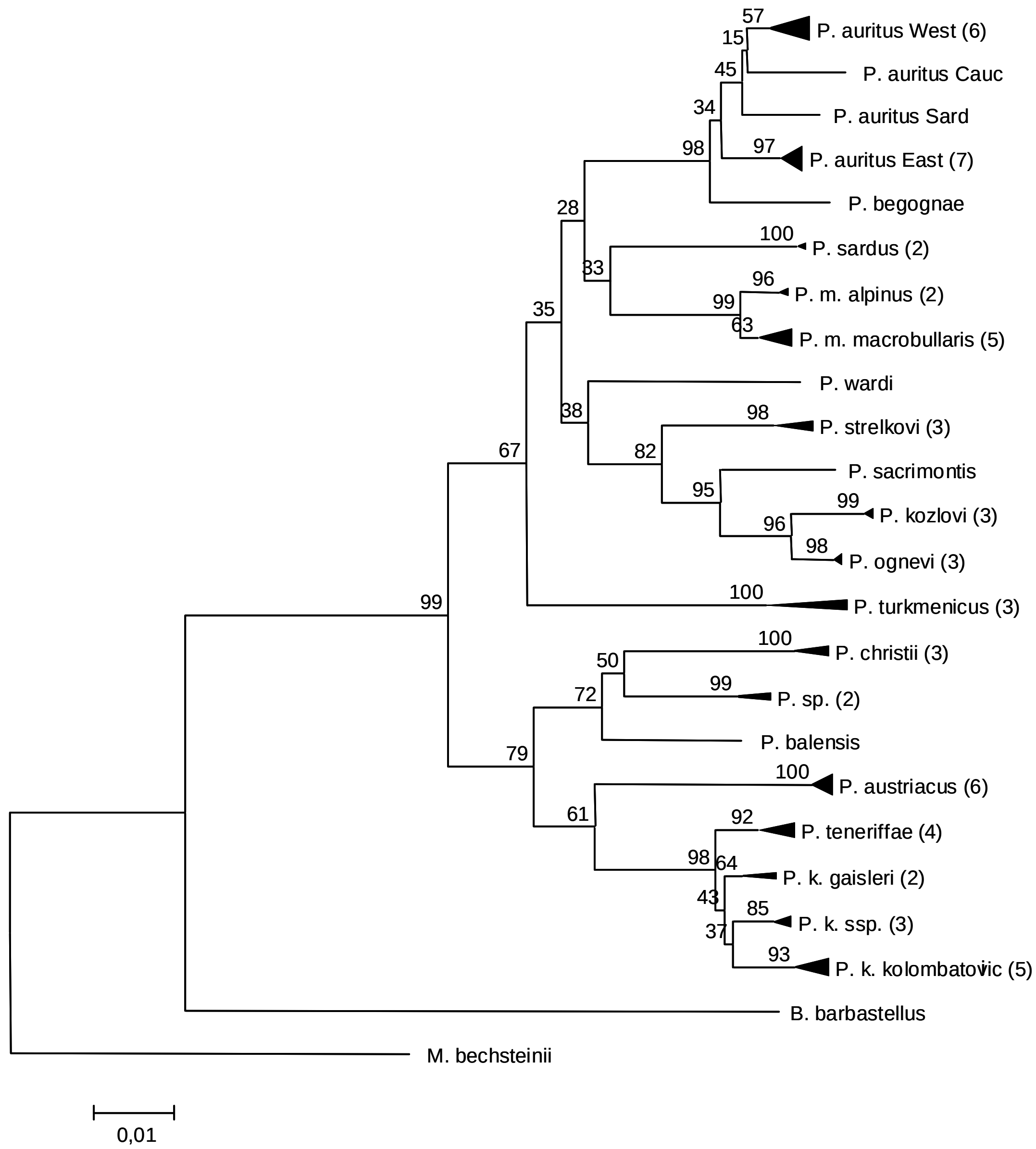

Fig. 7-1. Neighbor-joining tree based on 516 bp of partial 16S rDNA sequences (LogDet; 2000 bootstrap replicates; 68 haplotypes of 16 Palaeartic *Plecotus* species, all haplotypes within a lineage are compressed, with the number of included haplotypes shown in brackets; Kiefer unpublished).

Recently, Spitzenberger *et al.* (2006) showed in their preliminary review of the taxonomy of the Palaeartic genus *Plecotus* that it consists of 13-16 described and 3 undescribed species (see also Fig. 7-1). Only two of the west-Palaeartic members of the genus (*P. auritus* and *P. macrobullaris*) reach the Ural in the North and the Caucasus Mountains in the South. All eastern Palaeartic lineages which where formerly included in *P. auritus* (e.g. *P. sacrimontis*, *P. ognevi*) or *P. austriacus* (e.g. *P. kozlovi*, *P. turkmenicus*) represent species of their own. (Fig. 7-1).

In the CR-tree presented by Spitzenberger *et al.* (2006) there are four sub-clades within *P. auritus*. These four lineages are not equally differentiated from each other. Two of them, the western and the eastern sub-clades, have a mean genetic difference clearly below 5 %. This haplotypes from Caucasus and Iberia are divergent above 5 % (here: 5.5.-8.0 %).

One of them, "*begognae*" from the Iberian Peninsula, is morphologically and genetically clearly distinct and lives in sympatry with the western lineage of *P. auritus* (shown by Ibanez *et al.* 2006). Schütte (2005) shows in her total genetic evidence tree (3 mt DNA and 3 nuclear DNA genes) that *P. begognae* is a group of its own compared to other *P. auritus* sublineages. I therefore follow Juste *et al.* (2004), Ibanez *et al.* (2006) and Mayer *et al.* (2007) in treating *P. begognae* as a species. The Caucasian subclade might represent a taxon of its own. Also, its rank as species or subspecies is not known (Spitzenberger *et al.* 2006). Their morphological data also support the separate position of the Caucasian clade. Whether the western and eastern sub-clades of *P. auritus* constitute subspecies remains open; although genetically different (chapter 4), they are morphologically very similar (Spitzenberger *et al.* 2006) and gene flow is known between these lineages (Veith et al. 2004). On the other hand, there seems to be a small morphometric difference which fits to two biogeographically distinct areas (the Dinarids and the Pannonian) in Croatia (Tvrtkovic et al. 2005). This difference should be investigated in other areas especially in Central Europe in the future. It also remains open if the Sardinian *P. auritus*-subclade represents a lineage or subspecies of its own (chapter 5 and fig. 7-1), or if it falls within the variation of the western *P. auritus*-subclade (fig. 5-1). Until now a morphological analysis of the Sardinian clade is lacking because the taxon is very rare and it has so far been impossible to sample individuals for a reliable examination.

The taxonomic treatment of the sublineages of the *P. macrobullaris* lineage is still a matter of debate. Spitzenberger *et al.* (2006, p. 197) write in their description of CR-subtrees and clades: "Within *P. macrobullaris* two groups (subclades 2a and 2b) can be distinguished corresponding to the eastern (2a) and the western (2b) part of their distribution range which may suggest two glacial refugia. Compared to the subclades of *P. auritus* the average genetic distance between subclades 2a and 2b is rather low. The geographic border between the two groups may be located in the South Alpine region, since the westernmost individual of the eastern clade (Plesp18) originated from Scrutto, Italy." Later they write (p. 206): "In the light of other, partly contradictory, genetic and morphological results, this subspecific division of *P. macrobullaris* seems questionable. Juste *et al.* (2004) found in their CR and cyt *b* trees that *P. macrobullaris* from Syria, Iran, Iberia, Switzerland and Crete form a single cluster without geographic subdivision." The latter interpretation of Juste *et al.* (2004) needs some critical discussion: In their CR tree a clear East-West pattern emerges, with the Mediterranean island population from Crete standing intermediate in the phylogenetic tree. The cytochrome *b* tree of Juste *et al.* (2004) in fact shows no clear geographic pattern, as bootstrap support values for nodes are extremely low, making any geographical interpretation dispensable. The syntopic occurrence of eastern and western haplotypes within a NE Italian (Friuli) population was also taken as evidence by Spitzenberger *et al.* (2006) for discarding the existence of an eastern and western *P. macrobullaris* lineage. However, the presence of an eastern haplotype within one of the most eastern populations of the western haplolineage could be interpreted as a sign of secondary contact following postglacial range expansions of both haplolineages from their respective refugia. If these lineages should finally be treated as con-specific or con-subspecific is a matter of taste and is solely based on a phenetic interpretation of molecular distances. However, for the sake of taxonomic stability I herein prefer sustaining their treatment as two distinct subspecies.

Distribution of Western Palearctic long-eared bats

Sufficient data are now available to draw a first picture of the distribution of long-eared bats throughout Europe and the Circum-Mediterranean realm (Fig. 7-2 and Fig. 7-3).

Plecotus auritus is the by far the most widespread species. Its eastern lineage reaches far into Scandinavia to the polar circle. In the South it reaches some Mediterranean Peninsulas (Sardinian lineage). The western lineage is currently known from northern Iberia and western and central Europe. Following Spitzenberger *et al.* (2006), the Caucasian lineage reaches Asia towards the Ural in the North-East and the Caucasus Mountains in the South-East (see. Fig 1 from Spitzenberger *et al.* 2006). A distinct species, *P. begognae*, inhabits the Iberian Peninsula.

Plecotus austriacus is distributed all over central and southern Europe, with a few populations known from southern England and South Sweden. It occurs also on Madeira in the Atlantic Ocean and Sardinia and Corsica in the Mediterranean Sea. Former records from North Africa turned out to be *P. kolombatovici* or *P. christii*.

Two lineages of *P. macrobullaris* inhabit Europe: a western lineage is distributed in the Pyrenees, the entire Alpine ridge including the Dinarids in former Croatia and on Corsica; an eastern lineage is known from the northern part of the Dinarian Alps throughout Greece (including Crete) until Anatolia, the Caucasus and the Near East.

Plecotus kolombatovici occurs in several disjunctive areas. The subspecies *P. k. kolombatovici* occupies coastal habitats from Croatia in the West to southern Turkey in the East. *P. k. gaisleri* lives along the N African coast, from the Cyrenaica in the East to the Maghreb in the West. A third lineage inhabits NW Africa, namely the Moroccan and Algerian Atlas Mountains.

Plecotus teneriffae is endemic to the Canary Islands. *Plecotus sardus* is currently known only from Sardinia. *Plecotus christii* is restricted to the deserts of Libya, Egypt and the Sinai Peninsular, with the westernmost populations reaching the Cyrenaica. *Plecotus balensis* is endemic to the Bale Mountains, Ethiopia, while a currently undescribed species inhabits large parts of the Arabian Peninsula.

Implications for conservation

Within a few years molecular analyses have allowed the detection of four new taxa of long-eared bats in Europe and Mediterranean Africa. Conservation efforts were immediately started for the endemic Sardinian long-eared bat (*P. sardus*), and *P. macrobullaris* will soon be included in national Red Data Books of several countries. Long-eared bats are an excellent example of how phylogenetic analyses may have strong impact on nature conservation and how priorities in species conservation can be outlined as soon as formerly cryptic lineages are discerned.

Western Palaearctic long-eared bats – the story goes on

In my thesis I could draw a rather precise picture of the diversity within the genus *Plecotus* in the Western Palaearctic realm. However, molecular techniques will be continuously used to delimit further evolutionary lineages of long-eared bats (see the most recently published studies on cryptic diversity of European bats by Ibanez *et al.* 2006 and

Mayer *et al.* 2007). The existence of a distinct lineage of *P. auritus* on Sardinia, the unclear phylogenetic affiliation of NW African long-eared bats to the *P. kolombatovici* lineages, and the discovery of a currently unnamed distinct species on the Saudi-Arabian Peninsula (*P. sp*. in Fig 7-1) show that the number of taxa will certainly increase.

Distribution areas of all lineages have to be further specified applying DNA barcoding. Based on thorough geographical sampling, phylogeographic approaches such as Nested Clade Phylogeographic Analysis (Templeton, 2004) or coalescence simulation (Knowles 2004, Knowles & Maddison 2002) will substantially enhance our understanding of the spatial and temporal evolutionary scenarios of the western Palaeactic long-eared bat.

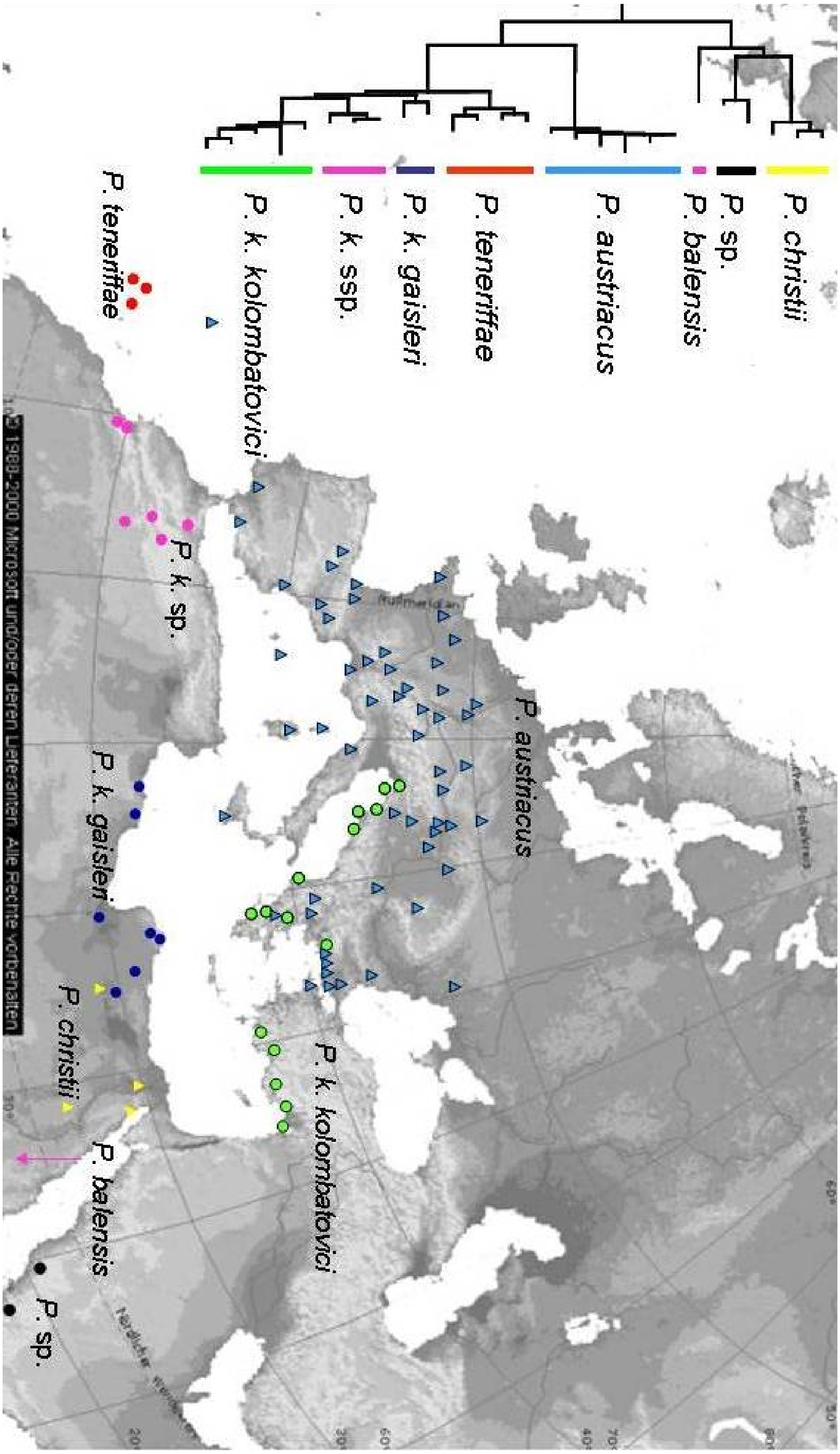

Fig. 7-2. Distribution of the *Plecotus austriacus* group based on genetically validated specimens; compiled from published records and own data (Kiefer unpublished).

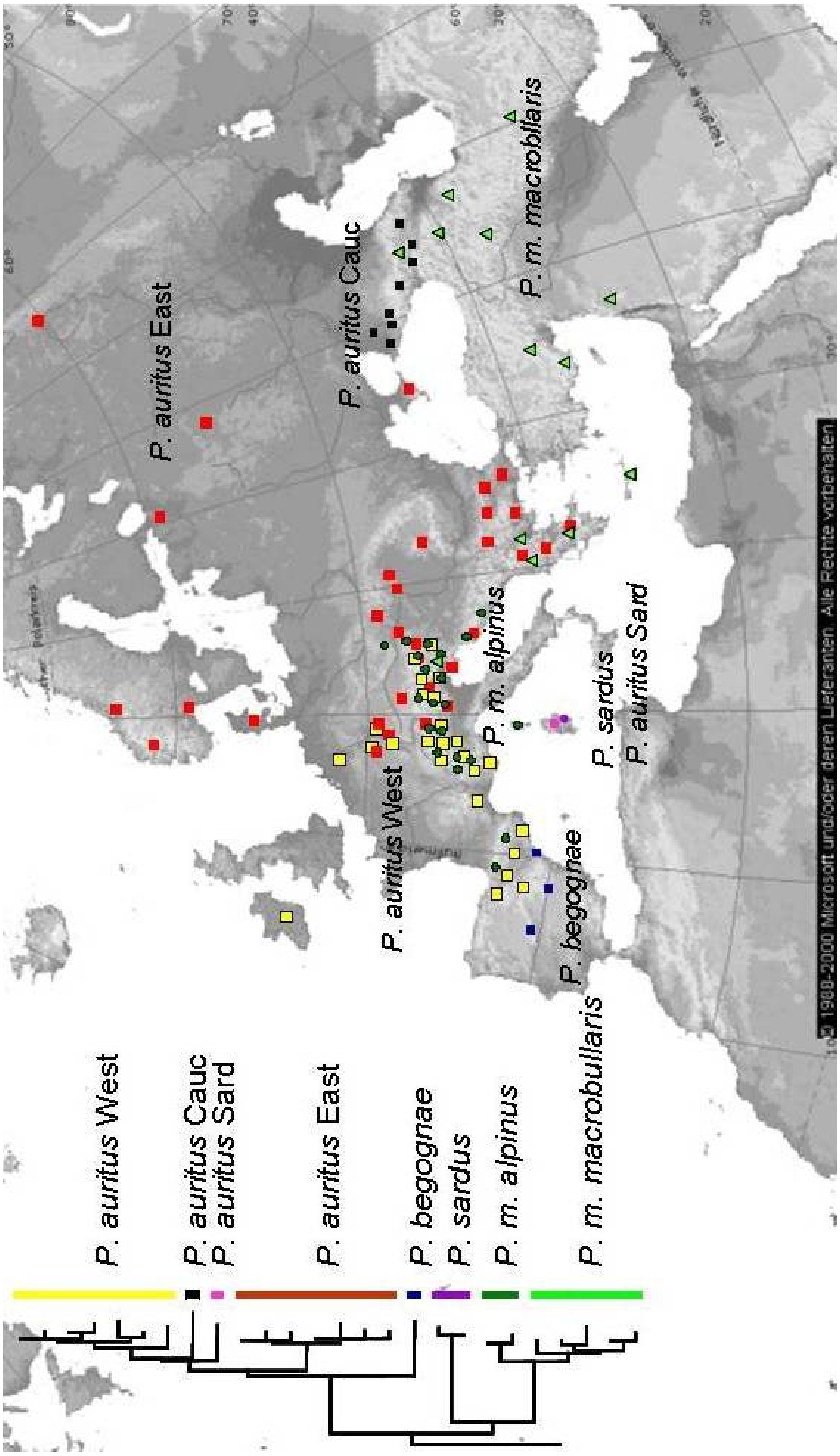

Fig. 7-3. Distribution of the *Plecotus auritus* group based on genetically validated specimens; compiled from published records and own data (Kiefer unpublished).

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Appendix 3-1: Specimens used in the genetic analyses in chapter 3.

Haplotye	Locality	Genbank Accession Number 16S	ND1	D-Loop	Voucher
Plecotus austriacus Paus-1	Bavaria, Germany (n=2)	AY134022	AF401367	AY1344006	O. von Helversen, Univ. Erlangen
Plecotus austriacus Paus-2	Villavelayo, Spain	AY134023	AF516270	AY134007	J. Juste, PAT98082501
Plecotus austriacus Paus-3	La Junguera, Spain	AY134024	AF516271	AY134008	SMF 97.207
Plecotus kolombatovici Pkol-1	Orebic, Croatia (n=2)	AY134025	AF401363	AY134009	D. Kovacic, Univ. Zagreb
Plecotus kolombatovici Pkol-2	Proastio, Greece	AY134026	AF401365	AY134010	voucher not preserved
Plecotus kolombatovici Pkol-2	Dirrachi, Greece	AY134026	AF401365	AY134010	voucher not preserved
Plecotus indet. Pind-1	Duvin, Switzerland,	AY134017	AF516269	AY134000	ZFMK 2001.328, collected by M. Lutz
Plecotus indet. Pind-1	Ristolas, France	AY134017	AF516269	AY134000	ZFMK 2001.325, collected by P. Favre, C. Joulot
Plecotus indet. Pind-2	Spaizzo, Italy	AY134018	AF516269	AY134001	voucher not preserved
Plecotus indet. Pind-3	Fischertratten, Austria	AY134019	AF516274	AY134002	ZFMK 2001.327, collected by G. Reiter
Plecotus indet. Pind-4	Waisach, Austria	AY134020	AF516275	AY134003	ZFMK 2001.326, collected by G. Reiter
Plecotus indet. Pind-5	Tymphristos, Greece	AY134021	AY131290	AY134004	O. von Helversen, Univ. Erlangen
Plecotus indet. Pind-6	Ogulin, Croatia	—	—	AY134005	SMF 44898
Plecotus auritus Paur-1	Curaglia, Switzerland	AF529229	AF516277	AY133993	Univ. Zürich 2513
Plecotus auritus Paur-1	Guarda, Switzerland	AF529229	AF516277	AY133993	ZFMK 2001.344, collected by M. Lutz
Plecotus auritus Paur-1	Masein, Switzerland	AF529229	AF516277	AY133993	ZMFK 2001.343, collected by M. Lutz
Plecotus auritus Paur-1	Winterthur, Switzerland	AF529229	AF516277	AY133993	Univ. Zürich 2387
Plecotus auritus Paur-2	Zagreb, Croatia (n=2)	AF629230	AF401369	AY133994	D. Kovacic, Univ. Zagreb
Plecotus auritus Paur-3	Styria, Austria (n=8)	AY134012	AY131291	AY133995	private collection B. Freitag, Graz
Plecotus auritus Paur-4	Bavaria, Germany (n=2)	AY134013	AF401374	AY133996	O. von Helversen, Univ. Erlangen
Plecotus auritus Paur-5	Moscow, Russia (n=2)Þ	AY134014	AF401371	AY133997	P.P. Strelkov. Univ. Moscow
Plecotus auritus Paur-6	Hall, Admont, Austria	AY134015	AF516276	AY133998	private collection, B. Freitag, Graz
Plecotus auritus Paur-7	Villoslada, Spain	AY134016	AF516273	AY133999	J. Juste, PAR9808071
Barbastella barbastellus	Germany	AF529231	AF401376	AF529232	SMF 84.732
Myotis bechsteinii	Germany	AY134027	AY033978	AY134011	voucher not preserved

Appendix 5-1. Specimens used in the genetic analyses in chapter 5. Abbrevations: SMF = Forschungsinstitut Senckenerg, Frankfurt am Main, Germany; ZFMK = Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany, DZAB = Dipartimento di Zoologia e Anthropologia Biologica, Sassari, Italy. Names of Sardinian samples are in parentheses in the voucher column.

Haplotype	Locality	GenBank accession #	Voucher (sample name for Sardinian samples)
Plecotus austriacus Paus-1	Bavaria, Germany (n=2)	AY134022	O. von Helversen, Univ. Erlangen
Plecotus austriacus Paus-2	Villavelayo, Spain	AY134023	PAT98082501, private collection of J. Juste, Sevilla, Spain
Plecotus austriacus Paus-3	La Junguera, Spain	AY134024	SMF 97.207
Plecotus austriacus Paus-Sar3	Monte Albo, Sardinia	AY175816	voucher not preserved (Sar 3)
Plecotus austriacus Paus-Sar6	Supramonte, Sardinia	AY175817	voucher not preserved (Sar 6)
Plecotus austriacus Paus-Sar9	Monte Albo, Sardinia	AY175823	voucher not preserved (Sar 9)
Plecotus austriacus Paus-Sar10	Monte Albo, Sardinia	AY175815	voucher not preserved (Sar 10)
Plecotus austriacus Paus-Sar11	Supramonte, Sardinia	AY175820	voucher not preserved (Sar 11)
Plecotus austriacus Paus-Sar12	Supramonte, Sardinia	AY175814	voucher not preserved (Sar 12)
Plecotus kolombatovici Pkol-1	Orebic, Croatia (n=2)	AY134025	D. Kovacic, Univ. Zagreb
Plecotus kolombatovici Pkol-2	Proastio, Greece	AY134026	voucher not preserved
Plecotus kolombatovici Pkol-2	Dirrachi, Greece	AY134026	voucher not preserved
Plecotus alpinus Palp-1	Duvin, Switzerland,	AY134017	ZFMK 2001.328, coll. M. Lutz
Plecotus alpinus Palp-1	Ristolas, France	AY134017	ZFMK 2001.325, coll. P. Favre, C. Joulot
Plecotus alpinus Palp-2	Spaizzo, Italy	AY134018	voucher not preserved
Plecotus alpinus Palp-3.	Fischertratten, Austria	AY134019	ZFMK 2001.327, coll. G. Reiter
Plecotus alpinus Palp-4	Waisach, Austria	AY134020	ZFMK 2001.326, coll. G. Reiter
Plecotus alpinus Palp-5	Tymphristos, Greece	AY134021	O. von Helversen, Univ. Erlangen
Plecotus sardus Psar-1	Baunei, Sardinia	AY175819	private collection M. Mucedda, Sassari: Italy (Sar 1)
Plecotus sardus Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 2)
Plecotus sardus Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 15)
Plecotus sardus Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 20)
Plecotus sardus Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 21)
Plecotus sardus Psar-2	Ula Tirso, Sardinia	AY175826	voucher not preserved (Sar 22)
Plecotus sardus Psar-13	Oliena, Sardinia	AY175822	holotype, DZAB 0023 (Sar 13)
Plecotus auritus Paur-1	Curaglia, Switzerland	AF529229	Univ. Zürich 2513
Plecotus auritus Paur-1	Guarda, Switzerland	AF529229	ZFMK 2001.344, coll. M. Lutz
Plecotus *auritus* Paur-1	Masein, Switzerland	AF529229	ZMFK 2001.343, coll. M. Lutz

Appendix 5-1.continued

Haplotype	Locality	GenBank accession #	Voucher (sample name for Sardinian samples)
Plecotus auritus Paur-1	Winterthur, Switzerland	AF529229	Univ. Zürich 2387
Plecotus auritus Paur-2	Zagreb, Croatia (n=2)	AF529230	D. Kovacic, Univ. Zagreb
Plecotus auritus Paur-3	Styria, Austria (n=8)	AY134012	private collection B. Freitag, Graz
Plecotus auritus Paur-4	Bavaria, Germany (n=2)	AY134013	O. von Helversen, Univ. Erlangen
Plecotus auritus Paur-5	Moscow, Russia (n=2)	AY134014	P. P. Strelkov. Univ. Moscow
Plecotus auritus Paur-Sar17	Supramonte, Sardinia	AY175821	voucher not preserved (Sar 17)
Plecotus auritus Paur-Sar14	Bolotana, Sardinia	AY175825	voucher not preserved (Sar 14)
Plecotus auritus Paur-Sar14	Bolotana, Sardinia	AY175825	voucher not preserved (Sar 16)
Barbastella barbastellus	Darmstadt, Germany	AF529231	SMF 84.732, coll. G. Herzig
Myotis bechsteinii	Boos, Germany	AY134027	voucher not preserved

Appendix 6-1

Basic statistics of morphological measurements of the examined samples. For abbreviations see Material and Methods in chapter 6.

	P. christii					*P. balensis*					*P. t. teneriffae*				
	n	M	min	max	SD	n	M	min	max	SD	n	M	min	max	SD
LAt	4	38.17	36.70	40.20	1.638	5	39.42	36.30	41.20	1.886	5	42.04	41.10	42.60	0.627
LPol	2	5.55	5.50	5.60	0.071	5	5.74	5.40	6.20	0.297	4	6.50	6.30	6.70	0.183
LCr	5	16.34	16.02	16.62	0.257	4	17.05	16.56	17.90	0.601	5	17.49	17.33	17.73	0.158
LCb	5	15.24	14.93	15.57	0.282	5	15.60	15.21	15.91	0.269	5	16.44	16.18	16.64	0.193
LCc	5	14.69	14.35	15.07	0.268	5	15.08	14.64	15.38	0.333	5	15.83	15.71	16.16	0.187
LaZ	5	8.36	8.07	8.62	0.219	1	8.67				5	9.23	9.13	9.42	0.122
LaI	5	3.15	2.98	3.27	0.118	5	3.60	3.46	3.71	0.101	5	3.39	3.33	3.47	0.054
LaInf	5	3.90	3.75	4.07	0.127	3	4.08	3.85	4.21	0.200	5	4.36	4.23	4.51	0.103
LaN	5	7.83	7.49	8.08	0.234	4	8.69	8.30	9.07	0.349	5	8.75	8.49	8.98	0.184
ANc	5	5.23	5.02	5.52	0.227	4	5.57	5.43	5.81	0.166	5	5.53	5.47	5.63	0.062
ACr	4	7.39	7.20	7.57	0.180	4	7.68	7.63	7.78	0.071	5	7.74	7.48	7.87	0.155
LBT	5	4.47	4.31	4.55	0.104	5	4.42	4.28	4.53	0.097	5	4.65	4.58	4.74	0.072
CC	5	3.40	3.33	3.47	0.054	4	3.66	3.54	3.77	0.112	5	3.89	3.81	4.02	0.090
P^4P^4	5	4.50	4.33	4.66	0.135	3	4.80	4.75	4.83	0.046	5	5.00	4.88	5.14	0.107
M^3M^3	5	5.72	5.42	5.94	0.223	4	5.98	5.76	6.11	0.153	5	6.36	6.28	6.41	0.049
I^1M^3	5	6.15	6.02	6.29	0.125	4	6.51	6.42	6.63	0.104	5	7.01	6.80	7.32	0.192
CM^3	5	5.29	5.18	5.36	0.068	5	5.51	5.42	5.66	0.092	5	5.99	5.88	6.24	0.143
M^1M^3	5	3.17	3.11	3.24	0.055	4	3.22	3.13	3.32	0.079	5	3.58	3.47	3.68	0.084
CP^4	5	2.43	2.37	2.55	0.073	4	2.55	2.51	2.60	0.040	5	2.61	2.52	2.80	0.115
LI^1	5	0.55	0.52	0.58	0.025	4	0.61	0.60	0.64	0.021	5	0.65	0.62	0.66	0.019
LaI^1	5	0.37	0.32	0.40	0.029	4	0.40	0.38	0.46	0.042	5	0.43	0.39	0.46	0.030
AI^1	5	0.82	0.72	0.94	0.083	4	0.77	0.67	0.86	0.082	5	0.85	0.77	0.95	0.074
LCn	5	0.94	0.86	1.02	0.066	4	1.04	1.01	1.07	0.026	5	1.07	1.05	1.12	0.029
LaCn	5	0.71	0.66	0.76	0.043	4	0.78	0.76	0.80	0.016	5	0.93	0.89	0.95	0.025
ACn	5	1.39	1.30	1.52	0.079	4	1.37	1.15	1.59	0.242	5	1.54	1.33	1.66	0.141
LP^3	5	0.40	0.37	0.44	0.035	4	0.48	0.46	0.49	0.014	5	0.45	0.40	0.49	0.045
LaP^3	5	0.45	0.41	0.50	0.040	4	0.53	0.50	0.55	0.023	5	0.54	0.51	0.58	0.025
AP^3	5	0.43	0.34	0.48	0.063	4	0.42	0.40	0.46	0.031	5	0.52	0.46	0.57	0.039
LM^1	5	1.26	1.23	1.29	0.024	4	1.33	1.30	1.39	0.042	5	1.47	1.41	1.52	0.046
LaM^1	5	1.47	1.42	1.53	0.045	4	1.56	1.51	1.60	0.046	5	1.72	1.60	1.79	0.084
LM^3	5	0.58	0.55	0.62	0.027	4	0.61	0.58	0.66	0.038	5	0.67	0.65	0.69	0.016
LaM^3	5	1.54	1.50	1.57	0.028	4	1.55	1.50	1.58	0.039	5	1.68	1.61	1.72	0.040
ACin	5	0.04	0.02	0.06	0.018	4	0.10	0.08	0.13	0.018	5	0.08	0.06	0.10	0.016
LMd	5	10.15	9.98	10.41	0.160	4	10.53	10.15	10.69	0.257	5	11.30	11.22	11.54	0.137
ACo	5	2.82	2.69	2.92	0.105	4	2.78	2.73	2.82	0.044	5	3.36	3.32	3.38	0.027
I_1M_3	5	6.42	6.31	6.66	0.153	3	6.81	6.70	6.91	0.105	5	7.25	7.16	7.51	0.147
CM_3	5	5.70	5.60	5.87	0.111	4	5.90	5.76	6.02	0.109	5	6.44	6.35	6.66	0.127
M_1M_3	5	3.59	3.47	3.76	0.116	4	3.63	3.53	3.71	0.077	5	3.96	3.82	4.08	0.098
CP_4	5	2.07	1.95	2.23	0.124	3	2.29	2.26	2.32	0.031	5	2.31	2.24	2.41	0.062

Appendix 6-1. continued

P. t. gaisleri subsp. n. and *P. t.* cf. *gaisleri* subsp. n.

	Cyrenaica					Tripolitania					Maghreb				
	n	M	min	max	SD	n	M	min	max	SD	n	M	min	max	SD
LAt	19	39.11	37.20	40.90	1.002	4	40.70	39.50	41.50	0.849	19	39.69	36.90	41.60	1.260
LPol	19	6.38	6.10	6.80	0.174	4	6.22	6.00	6.40	0.171	19	5.65	5.20	6.10	0.265
LCr	15	17.02	16.54	17.43	0.255	4	17.13	16.76	17.63	0.392	19	16.91	16.17	17.37	0.325
LCb	15	15.94	15.63	16.33	0.213	4	16.11	15.81	16.42	0.276	19	15.79	15.43	16.21	0.249
LCc	15	15.35	15.05	15.60	0.185	4	15.51	15.10	15.84	0.323	17	15.29	14.88	15.73	0.223
LaZ	14	8.98	8.78	9.14	0.115	4	9.06	8.94	9.22	0.126	18	8.98	8.58	9.23	0.174
LaI	15	3.50	3.44	3.62	0.049	4	3.42	3.37	3.48	0.052	19	3.34	2.88	3.59	0.164
LaInf	15	4.20	4.02	4.32	0.104	4	4.15	4.05	4.32	0.117	19	4.09	3.84	4.37	0.139
LaN	15	8.29	8.04	8.76	0.177	4	8.28	8.08	8.47	0.160	19	8.34	7.75	8.68	0.237
ANc	15	5.50	5.36	5.69	0.100	4	5.37	5.28	5.44	0.072	19	5.33	5.07	5.58	0.142
ACr	15	7.64	7.53	7.81	0.093	4	7.54	7.51	7.57	0.028	19	7.47	7.30	7.64	0.117
LBT	15	4.53	4.47	4.70	0.060	4	4.61	4.56	4.65	0.044	19	4.53	4.30	4.74	0.120
CC	15	3.99	3.88	4.15	0.081	4	3.89	3.56	4.05	0.223	18	3.94	3.71	4.12	0.114
P^4P^4	15	5.04	4.92	5.18	0.087	4	5.03	4.88	5.17	0.128	19	4.91	4.48	5.14	0.174
M^3M^3	15	6.23	6.12	6.34	0.077	4	6.29	6.14	6.54	0.177	19	6.15	5.88	6.45	0.151
I^1M^3	15	6.77	6.62	6.95	0.097	4	6.86	6.76	6.99	0.111	18	6.67	6.53	6.83	0.092
CM^3	15	5.79	5.65	5.94	0.078	4	5.83	5.75	5.94	0.099	18	5.73	5.62	5.88	0.073
M^1M^3	15	3.38	3.26	3.50	0.064	4	3.34	3.29	3.39	0.045	18	3.40	3.16	3.50	0.088
CP^4	15	2.66	2.55	2.76	0.058	4	2.79	2.74	2.85	0.054	18	2.68	2.49	2.83	0.080
LI^1	15	0.64	0.59	0.68	0.031	4	0.60	0.58	0.64	0.026	12	0.63	0.54	0.70	0.046
LaI^1	15	0.46	0.37	0.54	0.049	4	0.47	0.40	0.50	0.048	12	0.47	0.42	0.53	0.030
AI^1	15	0.85	0.67	0.99	0.090	4	0.93	0.88	0.97	0.046	7	0.90	0.82	0.95	0.048
LCn	15	1.16	1.09	1.24	0.054	4	1.15	1.09	1.25	0.068	12	1.14	1.03	1.26	0.061
LaCn	15	0.91	0.81	0.99	0.057	4	0.99	0.94	1.04	0.039	12	0.96	0.87	1.07	0.059
ACn	15	1.60	1.42	1.75	0.105	4	1.73	1.64	1.84	0.091	7	1.66	1.55	1.76	0.090
LP^3	15	0.49	0.44	0.54	0.033	4	0.49	0.46	0.53	0.035	13	0.47	0.39	0.53	0.040
LaP^3	15	0.57	0.50	0.62	0.036	4	0.53	0.49	0.55	0.030	13	0.53	0.49	0.59	0.028
AP^3	15	0.46	0.41	0.53	0.040	4	0.46	0.41	0.52	0.049	9	0.43	0.38	0.51	0.041
LM^1	15	1.41	1.36	1.47	0.030	4	1.38	1.32	1.42	0.043	11	1.36	1.26	1.43	0.060
LaM^1	15	1.61	1.56	1.70	0.039	4	1.61	1.53	1.66	0.064	11	1.62	1.54	1.72	0.069
LM^3	15	0.61	0.57	0.64	0.023	4	0.53	0.51	0.54	0.016	10	0.57	0.52	0.65	0.037
LaM^3	15	1.58	1.54	1.63	0.032	4	1.51	1.46	1.57	0.049	10	1.63	1.55	1.71	0.056
ACin	15	0.04	0.00	0.07	0.020	4	0.01	0.00	0.03	0.016	10	0.08	0.04	0.12	0.026
LMd	15	10.90	10.72	11.17	0.167	4	11.16	10.85	11.48	0.309	19	10.88	10.32	11.23	0.229
ACo	15	3.16	2.83	3.37	0.144	4	3.19	3.02	3.34	0.150	19	3.15	2.82	3.37	0.141
I_1M_3	15	7.02	6.74	7.20	0.123	4	7.14	7.07	7.21	0.058	19	6.96	6.54	7.18	0.149
CM_3	15	6.18	5.93	6.32	0.101	4	6.34	6.27	6.42	0.079	19	6.19	5.65	6.38	0.158
M_1M_3	15	3.84	3.71	4.16	0.108	4	3.80	3.76	3.84	0.039	19	3.86	3.63	4.03	0.087
CP_4	15	2.38	2.26	2.48	0.072	4	2.57	2.48	2.63	0.066	19	2.36	2.21	2.52	0.073

Appendix 6-1. continued

	P. t. kolombatovici					*P. m. alpinus*					*P. m. macrobullaris*				
	n	M	min	max	SD	n	M	min	max	SD	n	M	min	max	SD
LAt	21	37.64	36.10	39.00	0.859	5	40.12	39.30	42.10	1.158	30	42.28	39.80	45.60	1.495
LPol	16	5.81	5.40	6.40	0.317	5	6.50	6.10	7.00	0.339	30	7.16	6.50	8.00	0.369
LCr	22	16.32	15.87	16.97	0.262	9	16.56	15.87	17.18	0.395	31	17.25	16.68	18.02	0.350
LCb	22	15.16	14.68	15.73	0.245	9	15.44	14.68	16.07	0.449	31	16.00	15.38	16.85	0.338
LCc	22	14.63	14.15	15.18	0.230	9	14.86	14.13	15.41	0.407	26	15.38	14.85	16.07	0.273
LaZ	21	8.57	8.36	8.97	0.137	8	8.64	8.42	8.78	0.122	29	9.04	8.65	9.93	0.279
LaI	22	3.21	2.93	3.48	0.146	9	3.37	3.08	3.62	0.153	31	3.45	3.22	3.67	0.126
LaInf	22	3.99	3.70	4.23	0.150	9	4.08	3.62	4.32	0.253	31	4.20	3.88	4.57	0.177
LaN	22	8.12	7.62	8.67	0.249	8	8.21	8.02	8.37	0.122	31	8.50	7.71	8.83	0.229
ANc	22	5.39	5.13	5.58	0.114	9	5.18	4.82	5.37	0.198	31	5.48	5.17	5.66	0.121
ACr	22	7.55	7.24	7.88	0.134	8	7.38	7.08	7.62	0.196	31	7.74	7.27	8.07	0.182
LBT	22	4.37	4.22	4.57	0.114	9	4.43	4.25	4.58	0.109	31	4.66	4.43	4.89	0.132
CC	22	3.62	3.50	3.88	0.086	8	3.68	3.44	3.84	0.117	31	3.82	3.58	3.98	0.097
P^4P^4	22	4.69	4.50	4.88	0.108	9	4.93	4.71	5.27	0.196	30	5.09	4.77	5.42	0.146
M^3M^3	22	5.87	5.66	6.15	0.135	9	6.19	6.02	6.48	0.138	31	6.24	6.02	6.44	0.119
I^1M^3	22	6.33	6.12	6.64	0.125	9	6.52	6.27	6.75	0.186	31	6.74	6.43	7.21	0.160
CM^3	22	5.36	5.15	5.64	0.130	9	5.51	5.28	5.69	0.151	31	5.62	5.41	5.92	0.102
M^1M^3	22	3.24	3.13	3.42	0.076	9	3.17	3.03	3.29	0.085	31	3.33	3.18	3.53	0.072
CP^4	22	2.49	2.37	2.62	0.070	9	2.55	2.41	2.71	0.095	31	2.76	2.62	2.96	0.077
LI^1	22	0.59	0.53	0.64	0.034	9	0.60	0.57	0.66	0.032	27	0.57	0.51	0.62	0.028
LaI^1	22	0.40	0.35	0.44	0.030	9	0.41	0.38	0.48	0.035	27	0.42	0.36	0.47	0.029
AI^1	22	0.89	0.72	1.03	0.067	9	0.74	0.58	0.83	0.084	25	0.88	0.71	1.01	0.091
LCn	22	0.98	0.91	1.07	0.040	9	0.99	0.92	1.10	0.050	27	1.03	0.93	1.15	0.043
LaCn	22	0.78	0.74	0.83	0.030	9	0.79	0.73	0.86	0.040	27	0.79	0.73	0.84	0.029
ACn	22	1.53	1.24	1.67	0.109	9	1.57	1.33	1.72	0.114	25	1.62	1.33	1.78	0.111
LP^3	22	0.41	0.38	0.47	0.029	9	0.45	0.42	0.48	0.020	27	0.46	0.41	0.53	0.028
LaP^3	22	0.48	0.42	0.53	0.026	9	0.49	0.46	0.54	0.026	27	0.53	0.47	0.59	0.032
AP^3	22	0.43	0.37	0.48	0.035	9	0.46	0.36	0.52	0.049	25	0.52	0.38	0.62	0.055
LM^1	22	1.28	1.20	1.36	0.045	9	1.32	1.23	1.40	0.052	25	1.33	1.23	1.42	0.045
LaM^1	22	1.59	1.48	1.66	0.053	9	1.56	1.45	1.64	0.071	25	1.60	1.53	1.71	0.049
LM^3	22	0.59	0.55	0.67	0.026	9	0.62	0.61	0.65	0.016	25	0.61	0.57	0.65	0.021
LaM^3	22	1.59	1.50	1.72	0.050	9	1.64	1.55	1.69	0.053	25	1.63	1.54	1.72	0.050
ACin	21	0.09	0.00	0.23	0.061	9	0.07	0.03	0.10	0.026	25	0.07	0.02	0.19	0.042
LMd	21	10.23	9.88	10.52	0.166	9	10.54	10.24	10.92	0.275	31	10.91	10.30	11.46	0.262
ACo	21	2.97	2.73	3.20	0.105	9	2.94	2.75	3.10	0.121	31	3.06	2.67	3.28	0.161
I_1M_3	21	6.59	6.43	6.87	0.117	9	6.80	6.40	7.08	0.217	31	7.06	6.77	7.32	0.153
CM_3	21	5.79	5.37	6.13	0.160	9	5.86	5.07	6.21	0.342	31	6.17	5.93	6.75	0.165
M_1M_3	21	3.71	3.58	3.87	0.075	9	3.62	3.50	3.74	0.092	31	3.83	3.63	4.32	0.133
CP_4	21	2.17	2.06	2.31	0.063	9	2.23	2.11	2.32	0.078	31	2.38	2.23	2.54	0.070

Appendix 6-1. continued

	P. auritus auritus CE					*P. auritus auritus* BK					*P. auritus begognae* SP				
	n	M	min	max	SD	n	M	min	max	SD	n	M	min	max	SD
LAt	27	38.84	35.60	41.20	1.278	12	39.72	38.40	41.20	0.957	1	40.40	40.40	40.40	
LPol	15	7.03	6.60	7.40	0.294	12	7.38	6.90	7.80	0.286	1	6.10	6.10	6.10	
LCr	30	16.19	15.41	16.90	0.379	13	16.27	15.69	16.53	0.240	15	16.92	16.57	17.25	0.201
LCb	29	14.94	14.08	15.58	0.378	13	15.09	14.62	15.40	0.240	15	15.83	15.46	16.18	0.215
LCc	29	14.40	13.53	14.95	0.337	13	14.52	14.13	14.78	0.209	15	15.29	14.95	15.58	0.197
LaZ	25	8.73	8.05	9.05	0.218	12	8.71	8.54	8.85	0.090	7	9.08	8.83	9.38	0.180
LaI	30	3.45	3.20	3.72	0.106	13	3.38	3.14	3.64	0.121	15	3.42	3.14	3.63	0.120
LaInf	30	4.04	3.63	4.23	0.115	13	4.09	3.96	4.27	0.114	15	4.23	3.88	4.47	0.139
LaN	28	8.31	7.66	8.63	0.189	13	8.19	7.78	8.39	0.192	15	8.34	8.08	9.16	0.247
ANc	28	5.31	5.03	5.52	0.140	13	5.25	5.08	5.44	0.106	15	5.30	5.07	5.52	0.107
ACr	29	7.31	6.82	7.58	0.164	13	7.28	7.12	7.42	0.107	15	7.09	3.47	7.59	1.008
LBT	30	4.03	3.85	4.18	0.082	13	4.03	3.82	4.22	0.104	15	4.16	4.04	4.26	0.076
CC	30	3.81	3.54	4.14	0.127	13	3.80	3.68	3.92	0.076	15	4.16	3.97	4.32	0.095
P^4P^4	28	5.01	4.73	5.27	0.143	13	5.01	4.91	5.18	0.078	15	5.41	5.33	5.56	0.070
M^3M^3	30	6.13	5.72	6.33	0.147	13	6.14	5.99	6.27	0.088	14	6.78	6.61	6.93	0.100
I^1M^3	30	6.31	5.86	6.69	0.172	13	6.37	5.48	6.68	0.306	15	6.96	6.76	7.14	0.112
CM^3	30	5.31	4.88	5.60	0.147	13	5.42	5.18	5.58	0.112	15	5.91	5.75	6.10	0.094
M^1M^3	30	3.19	3.08	3.42	0.069	13	3.21	3.11	3.37	0.078	15	3.50	3.37	3.58	0.066
CP^4	30	2.44	2.18	2.59	0.106	13	2.55	2.45	2.66	0.060	15	2.63	2.55	2.79	0.073
LI^1	30	0.62	0.53	0.70	0.040	13	0.59	0.54	0.63	0.025	15	0.63	0.60	0.67	0.021
LaI^1	30	0.40	0.34	0.48	0.038	13	0.41	0.37	0.47	0.032	15	0.46	0.42	0.48	0.022
AI^1	29	0.84	0.69	0.94	0.073	13	0.86	0.77	1.01	0.076	15	0.95	0.88	1.05	0.057
LCn	30	0.96	0.83	1.08	0.052	13	0.94	0.89	1.06	0.052	15	1.08	1.01	1.18	0.050
LaCn	30	0.81	0.71	0.90	0.047	13	0.77	0.71	0.93	0.055	15	0.94	0.90	0.98	0.026
ACn	30	1.41	1.11	1.58	0.122	13	1.44	1.32	1.59	0.095	15	1.69	1.49	1.89	0.114
LP^3	30	0.44	0.39	0.54	0.044	13	0.43	0.40	0.48	0.022	15	0.45	0.40	0.53	0.038
LaP^3	30	0.50	0.44	0.57	0.034	13	0.48	0.44	0.51	0.021	15	0.54	0.48	0.59	0.031
AP^3	30	0.50	0.36	0.58	0.053	13	0.51	0.46	0.63	0.052	15	0.53	0.48	0.60	0.039
LM^1	30	1.27	1.18	1.37	0.040	13	1.30	1.24	1.33	0.028	15	1.47	1.39	1.56	0.045
LaM^1	30	1.56	1.42	1.65	0.058	13	1.60	1.52	1.70	0.051	15	1.81	1.73	1.91	0.053
LM^3	30	0.64	0.57	0.71	0.034	13	0.64	0.54	0.68	0.040	15	0.66	0.63	0.69	0.018
LaM^3	30	1.65	1.49	1.74	0.057	13	1.65	1.53	1.71	0.053	15	1.80	1.74	1.86	0.037
ACin	30	0.11	0.00	0.20	0.043	13	0.13	0.08	0.19	0.033	15	0.10	0.06	0.15	0.024
LMd	30	10.30	9.62	10.66	0.231	13	10.32	9.88	10.64	0.199	15	11.20	10.83	11.57	0.191
ACo	29	2.81	2.54	3.08	0.131	13	2.87	2.65	3.03	0.103	15	3.24	2.97	3.60	0.157
I_1M_3	29	6.60	6.19	6.93	0.149	13	6.71	6.52	6.88	0.113	15	7.28	7.12	7.51	0.105
CM_3	28	5.72	5.41	6.07	0.131	13	5.84	5.61	6.24	0.163	15	6.39	6.27	6.61	0.107
M_1M_3	30	3.60	3.47	3.84	0.087	13	3.72	3.55	4.16	0.166	15	3.94	3.79	4.11	0.089
CP_4	28	2.14	2.01	2.24	0.068	13	2.19	2.06	2.31	0.075	15	2.33	2.21	2.49	0.080

Appendix 6-1. continued

	P. austriacus CE					*P. austriacus* BK					*P. austriacus* SP				
	n	M	min	max	SD	n	M	min	max	SD	n	M	min	max	SD
LAt	29	40.07	38.80	43.50	1.102	29	39.80	37.00	42.30	1.189	8	40.10	39.20	41.00	0.682
LPol	21	5.61	5.00	6.10	0.292	19	5.68	5.30	6.10	0.227	8	5.85	5.60	6.20	0.233
LCr	29	17.39	17.02	18.06	0.227	29	17.39	16.72	17.92	0.327	9	17.53	17.34	17.88	0.162
LCb	29	16.27	15.96	16.88	0.190	29	16.30	15.62	16.76	0.275	8	16.48	16.17	16.88	0.202
LCc	29	15.73	15.39	16.27	0.192	29	15.69	15.02	16.02	0.245	8	15.91	15.62	16.28	0.199
LaZ	26	9.24	9.02	9.68	0.149	29	9.20	8.75	9.50	0.182	9	9.35	9.06	9.66	0.186
LaI	29	3.33	3.07	3.47	0.098	29	3.38	3.09	3.55	0.107	9	3.50	3.31	3.74	0.130
LaInf	29	4.33	4.03	4.48	0.142	29	4.33	4.20	4.58	0.103	9	4.42	4.35	4.63	0.087
LaN	29	8.55	8.25	8.83	0.148	29	8.51	8.12	8.83	0.145	9	8.77	8.64	8.88	0.066
ANc	29	5.38	5.02	5.62	0.128	29	5.41	5.05	5.81	0.164	8	5.49	5.32	5.75	0.158
ACr	29	7.66	7.42	7.95	0.130	29	7.64	7.23	7.95	0.177	9	7.70	7.59	7.90	0.114
LBT	29	4.73	4.52	4.96	0.100	29	4.67	4.47	4.81	0.086	9	4.80	4.68	4.91	0.073
CC	29	4.11	3.90	4.35	0.112	29	4.11	3.82	4.37	0.123	8	4.19	4.06	4.38	0.109
P^4P^4	29	5.17	4.88	5.41	0.130	29	5.18	4.97	5.51	0.122	9	5.17	5.01	5.45	0.155
M^3M^3	28	6.46	6.13	6.80	0.160	29	6.47	6.08	6.82	0.176	8	6.40	6.18	6.65	0.153
I^1M^3	28	6.96	6.75	7.20	0.119	29	7.01	6.78	7.27	0.134	9	6.99	6.74	7.28	0.156
CM^3	29	5.99	5.74	6.18	0.116	29	6.01	5.82	6.26	0.119	9	6.04	5.83	6.28	0.144
M^1M^3	29	3.50	3.26	3.63	0.101	29	3.53	3.32	3.71	0.090	9	3.44	3.34	3.53	0.062
CP^4	29	2.84	2.65	2.96	0.072	29	2.86	2.72	3.00	0.082	9	2.81	2.62	2.91	0.094
LI^1	28	0.62	0.50	0.70	0.045	29	0.60	0.44	0.68	0.046	9	0.61	0.59	0.65	0.022
LaI^1	28	0.44	0.34	0.49	0.042	29	0.44	0.39	0.49	0.028	9	0.46	0.41	0.51	0.036
AI^1	28	0.97	0.80	1.11	0.067	29	0.95	0.77	1.07	0.055	9	0.94	0.77	1.03	0.078
LCn	29	1.19	1.06	1.30	0.059	29	1.14	1.06	1.30	0.056	9	1.18	1.10	1.23	0.050
LaCn	29	1.00	0.91	1.10	0.051	29	0.97	0.88	1.07	0.049	9	0.96	0.89	1.04	0.042
ACn	29	1.83	1.50	1.98	0.113	29	1.81	1.47	1.93	0.094	9	1.75	1.46	1.95	0.150
LP^3	29	0.46	0.39	0.52	0.032	29	0.44	0.39	0.51	0.032	9	0.45	0.42	0.49	0.020
LaP^3	29	0.57	0.47	0.62	0.037	29	0.54	0.47	0.64	0.035	9	0.55	0.51	0.59	0.023
AP^3	29	0.53	0.40	0.62	0.051	29	0.51	0.40	0.59	0.047	9	0.49	0.38	0.55	0.064
LM^1	29	1.39	1.32	1.45	0.033	29	1.40	1.32	1.49	0.041	9	1.42	1.38	1.48	0.035
LaM^1	29	1.66	1.58	1.74	0.045	29	1.67	1.56	1.77	0.043	9	1.66	1.61	1.72	0.036
LM^3	29	0.66	0.59	0.76	0.041	29	0.65	0.61	0.71	0.032	9	0.61	0.55	0.65	0.030
LaM^3	29	1.71	1.60	1.82	0.059	29	1.72	1.59	1.80	0.057	9	1.68	1.60	1.76	0.060
ACin	29	0.07	0.02	0.14	0.028	29	0.07	0.02	0.15	0.027	9	0.06	0.00	0.12	0.032
LMd	29	11.25	10.94	11.70	0.173	29	11.21	10.86	11.62	0.214	9	11.36	11.18	11.64	0.127
ACo	29	3.28	3.07	3.50	0.115	29	3.33	3.08	3.54	0.124	9	3.40	3.09	3.57	0.149
I_1M_3	29	7.24	7.05	7.47	0.102	29	7.27	7.05	7.53	0.123	9	7.28	7.14	7.54	0.122
CM_3	29	6.44	6.23	6.65	0.098	29	6.45	6.23	6.70	0.109	9	6.48	6.28	6.63	0.115
M_1M_3	29	3.97	3.74	4.11	0.109	29	4.02	3.79	4.24	0.096	9	3.91	3.82	4.03	0.062
CP_4	29	2.47	2.37	2.60	0.056	29	2.47	2.35	2.59	0.057	9	2.44	2.31	2.60	0.102

Appendix 6-2

List of material examined in the morphological analyses.
Abbreviations: S – skull, A – alcohol specimen, B – dry skin (balg); m – male, f – female, ind. – sex undetermined, for other abbreviations see Material and Methods.

Plecotus christii Gray, 1838
Egypt: 1 m (IVB 100 [S+B]), Luxor, Valley of the Kings, 30 April 1969, leg. J. Gaisler; – 1 m (NMP E-71 [S+B]), Bir Nagat, Qattar Mts., 4 June 1984, leg. D. J. Osborn; – 1 m (NMP E-72 [S+B]), Bir Kohila, Qattar Mts., 4 June 1984, leg. D. J. Osborn.
Libya: 1 m, 1 f (NMP 49862, 49863 [S+A]), Al Jaghbub, 13 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter & M. Uhrin.

Plecotus balensis Kruskop et Lavrenchenko, 2000
Ethiopia: 1 m (SMF 28225 [S+B]), Schoa, 1842, ded. E. Rüppell; – 1 m, 2 f (EBD 25842–25844 [S+B]), Abune Yusef, 26 January 2000, leg. J. Juste.

Plecotus t. teneriffae Barret-Hamilton, 1907
Canary Islands, Spain: 1 ind. (EBD 16887 [S+Sk]), La Palma Is., Cueva de los Tilos, Los Sauces, 24 September 1987, leg. C. Ibáñez; – 1 m (EBD 16014 [S+B]), La Palma Is., Barranco de S. Juan, 5 km S Los Sauces, 25 September 1987, leg. C. Ibáñez; – 3 m (EBD 16011, 16012, 16043 [S+B]), Teneriffae Is., Fte del Riachnelo, PN Los Cañadas del Teide, 12 September 1987, leg. C. Ibáñez.

Plecotus t. gaisleri subsp. n.
Cyrenaica, Libya: 1 m ad (NMP Li-6 [S]), Shahhat (= Cyréné), ruins, 14 April 1979, leg. V. Hanák, 2 m (NMP 48330, 48331 [S+A]), the same site, 11 October 1999, leg. P. Benda & P. Nová; – 1 m (NMP 49916 [S+A]), Qasr ash Shahdayn, ruins, 21 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter & M. Uhrin; – 1 m (NMP Li-76 [S]), Quariat al Faioah, 6 km S, 30 April 1980, leg. V. Hanák; – 1 m (NMP 49920 [S+A]), Wadi al Kuf, estuary, 21 may 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter & M. Uhrin; – 4 m ad, 3 f ad (NMP 49905–49907 [A+S], 49908–49910 [A]), Wadi al Kuf, ca. 8 km SW Massah, 20 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter & M. Uhrin; – 4 m (NMP 49899, 49900 [S+A], 49898, 49901 [A]), Wadi al Kuf, 5 km SW Al Bayda, 19 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter & M. Uhrin; – 1 f (NMP 49883 [S+A]), Wadi al Minshiyah, estuary, 17 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter & M. Uhrin.

Plecotus teneriffae cf. *gaisleri* subsp. n.
Tripolitania, Libya: 2 f (NMP 49856, 49857 [S+A]), Ain Az Zarqa, 10 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter & M. Uhrin; – 1m, 1 f (NMP 49965, 49966 [S+A]), Nanatalah, 28 May 2002, leg. M. Andreas, P. Benda, V. Hanák, A. Reiter & M. Uhrin.

Tunisia: 1 f (SMF 22352 [S+B]), El Houaria, Cap Bon, 12 March 1963, leg. J. Kiepenhauer & K. Linsenmair; – 1 f (MNHN 1962-2631 [S+A]), Tatahouine, May 1961, leg. M. Blanc.
Algeria: 1 m (MUB A-57 [S+B]), Aokas, 30 May 1981, leg. J. Gaisler; – 2 f (MUB A-509, VMO ZO-5138 [S+B]) Brezina, 22 July 1983, leg. J. Gaisler; – 1 m (VMO ZO-4670 [S+B]), Setif, 1 November 1981, leg. J. Gaisler, 2 m (MUB A-122, VMO ZO-4671 [S+B]), 17 November 1981, the same site and collector, 1 m (MUB A-334 [S+B]), 8 October 1982, the same site and collector; – 1 m, 4 f (MUB A-459, A-460, VMO ZO-5123–5125 [S+B]), Tikdja, Djurdjura, 2 July 1983, leg. J. Gaisler.
Morocco: 1 m (MNHN 1983-511 [S+B]), Bou Izakarne, 4 February 1953; – 2 f (EBD 15355, 15357 [S+B]), Jbel Tissouke, 5 km E Chafchaouen, 11 May 1987, leg. C. Ibáñez; – 1 f (EBD 15509 [S+B]), 5 km W Moulay Abdeslan, 16 May 1987, leg. C. Ibáñez.
Pantelleria, Italy: 2 m, 4 f (SMF 37048, 37050, 37052–37055 [S+A], 37049 [S+B]), 17–28 September 1969, leg. H. Felten & G. Storch.

Plecotus t. kolombatovici Đulić, 1980
Croatia: 1 m (NMP 49092 [S]), Stari Grad, Hvar Is., 1 Sept 1977, leg. J. Červený & B. Kryštufek; – 1 m (NMP 49091 [S]), Zavala, Hvar Is., Belušica pećina cave, 29 Aug 1977, leg. J. Červený & B. Kryštufek.
Greece: 1 m (SMF 44918 [S+B]), Agia Fotia, Híos Is., 23 May 1972, leg. D. Kock; – 1 m (NMP 48569 [S]), Delfí (Fokída Dist), 23 Sept 1988, leg. V. Hanák & V. Vohralík; – 1 m (NMP 48585 [S+B]), Kleidoniá (Ioánnina Dist.), above the Voidomatis river, 27 Sept 1988, leg. V. Hanák & V. Vohralík; – 1 f (NMP 48725 [S+A]), Kombotades (Fthiótida Dist.), above the Sperhiás river, 9 Sept 1996, leg. M. Andreas, P. Benda & M. Uhrin; – 3 m (NMP 48726–48728 [S+A]), Kombotades (Fthiótida Dist.), a cave, 10 Sept 1996, leg. M. Andreas, P. Benda & M. Uhrin; – 1 m (ZFMK 97.214 [S+B]), Lefkimmi, Kerkira Is., 27 March 1961, leg. J. Niethammer; – 1 f (NMP 48572 [S+B]), Micro Pápigo (Iónnina Dist.), above a pool, 25 Sept 1988, leg. V. Hanák & V. Vohralík; – 4 m (NMP 48573–48576 [S+B]), Pápigo (Iónnina Dist.), cave, 26 Sept 1988, leg. V. Hanák & V. Vohralík; – 1 m (NMP 48609 [S+B]), Petralóna (Halkidikí Dist.), a cave above the village, 28 Sept 1988, leg. V. Hanák & V. Vohralík.
Turkey: 1 m (NMP 48087 [S+A]), Çevlik (Hatay Dist.), ancient ruins, 1 July 1997, leg. P. Benda; – 1 f (JGUM [S]), Karadere (Mugla Dist.), Letoon, ruins, Feb 1999, leg. A. Kiefer; – 1 m (CUP T93/64 [S+A]), Narlikuyu (Icel Dist.), cave, 29 Oct. 1993, leg. P. Benda & I. Horáček; – 2 m, 1 f (CUP T93/73–75 [S]), Bozagaç (Icel Dist.), Yalan Dünya magara cave, 30 Oct. 1993, leg. P. Benda & I. Horáček.

Plecotus austriacus (Fischer, 1829)
Spain: 1 ind. (ZFMK 97.259 [S]), National Park Ordessa, 25 May 1972; – 1 ind. (ZFMK 61.42 [S+B]), Rubials, 25 Oct. 1959; – 1 m, 2 f, 1 ind. (ZFMK 34.19, 46.289, 46.292, 46.293 [S+B], holoptype and three paratypes of *P. auritus hispanicus* Bauer, 1956), Langunilla (Salamanca), 3 Febr. 1934, 6–8 July 1940, leg. H. Wolf; – 3 m (ZFMK 35.73, 53.36, 61.201 [S+B]), Linares de Riofrio (Salamanca), 2 May 1935, 3 Febr. 1953, 6 March 1961, leg. H. Wolf.

Plecotus auritus begognae de Paz, 1994
Spain: 1 f (ZFMK 46.288 [S+B], paratype of *P. auritus hispanicus* Bauer, 1956), Langunilla (Salamanca), 10 July 1940, leg. H. Wolf; – 1 m, 14 f (SMF 18137–18140, 18142–18145, 20690–20695 [S+B]), Linares de Riofrio (Salamanca), 8–10 June 1958, 27 August 1958, 6 June 1962, leg. H. Wolf.

Plecotus m. macrobullaris Kuzjakin, 1965
Turkey: 1 f (NMP 47911 [S+A]), Van, 27 July 1992, leg. P. Benda.
Syria: 1 f (MNHN 1983-1996 [S+A]), Djeroud, 1908, leg. H. Gadeau de Kerville; – 1 m, 5 f (NMP 48989–48994 [S+A]), Maalula, 30 April 2001, leg. P. Munclinger & P. Nová; – 2 m, 3 f (NMP 48849–48853 [S+A]), Eas Al Ain, 22 may 2001, leg. M. Andreas, P. Benda, A. Reiter & D. Weinfurtová; – 2 f (NMP 48052, 48053 [S+A]), Yabroud, 27 June 1998, leg. M. Andreas, P. Benda & M. Uhrin.
Russia: 1 ind. (ZMMU S-96545 [S], paratype of *P. auritus macrobullaris* Kuzjakin, 1965), surroundings of Vladikavkaz, summer 1914, leg. L. Bëma.
Georgia: 1 m (ZIN 8699 [S+A]), Ahalkalaki, 1894, leg. Jablokov.
Armenia: 1 f (ZIN 72366 [S+B]), Mormarik, Ankovan, 8 August 1971, leg. E. Javrujan & L. Saharjan; – 1 m (ZIN 21442 [S+B]), northern bank of the Sevan lake, Tel', 20 km Elenovki, 15 November 1931, leg. A. I. Argyropulo.
Azerbaijan: 1 m (MUB 1.2.103 [S+B]), Kjal'vaz, Lerik Dist., 9 July 1968, leg. I. Rahmatulina.
Iran: 2 m, 5 f (NMP 48123–48129 [S+A]), 20 km E Choplu, 2 October 1998, leg. M. Andreas, P. Benda, A. Reiter & M. Uhrin; – 4 m (NMP 48138–48141 [S+A]), Takht-e Suelyman, 3 Ocober 1998, leg. M. Andreas, P. Benda, A. Reiter & M. Uhrin.

Plecotus m. alpinus Kiefer et Veith, 2002
Andorra: 1 f (JGUM [S+B]), Ordino, 20 Nov 2001, leg. M. J. Dubourq.
Austria: 1 f (ZFMK 2001.327 [S+B]), Fischertratten, 15 May 2000, leg. G. Reiter; – 1 f (63.225 [A]), Gailtal, Kärnten, 1 July 1960, leg. M. Eisentraut; – 1 ind. (JGUM [S]), Tirol, leg. A. Voraner.
Croatia: 1 m (SMF 44898 [S]), Ogulin, 1972, leg. J. Galencir; – 1 f (SMF 32962 [S]), Rovinj, 17 June 1967, leg. V. Brendov.
France: 1 ind. (JGUM [S]), Sardière, Savoie, July 1999, leg. S. Vincent; – 1 m (ZFMK 2001.325 [S+B], holotype of *P. alpinus* Kiefer et Veith, 2002), Ristolas, Haute Alpes, 24 Aug 2001, leg. P. Favre.
Greece: 1 m (JGUM [S+B]), Timfristos (Fthiótida Dist.), 12 June 2001, leg. O. von Helversen.
Liechtenstein: 1 m (ZFMK 61.451 [S+B]), Schaan, 23 Aug 1961, leg. E. von Lehmann.

Appendix 6-3. List of material used in the genetical analyses in chapter 6.

Haplotype	Locality	GenBank accession #	Voucher (sample name for Sardinian samples)
Plecotus austriacus Paus1	Bavaria, Germany (n=2)	AY134022	O. von Helversen, Univ. Erlangen
Plecotus kolombatovici Pkol1	Orebic, Croatia (n=2)	AY134025	D. Kovacic, Univ. Zagreb
Plecotus kolombatovici Pkol3	Letoon, Turkey	AY531617	A. Kiefer, Univ. Mainz
Plecotus kolombatovici Pkol4	Letoon, Turkey	AY531616	A. Kiefer, Univ. Mainz, voucher not preserved
Plecotus kolombatovici Pkol5	Narlikuyu (Icel), Turkey	AY531618	CUP T64/93
Plecotus kolombatovici Pkol5	Cevlik (Hatay)), Turkey	AY531618	NMP 48087
Plecotus kolombatovici Pkol6	Kombatades (n=2) (Lamia), Greece	AY531619	NMP 48726, 48727
Plecotus sardus Psar3	Oliena, Sardinia	AY175822	holotype, DZAB 0023
Plecotus m. alpinus Palp1	Ristolas, France	AY134017	ZFMK 2001.325, collected by P. Favre, C. Joulot
Plecotus m. macrobullaris Pmac1	Yabroud (n=2)(Dimashq), Syria	AY531625	NMP 48052, 48053
Plecotus m. macrobullaris Pmac1	Ras al Ain (n=2) (Dimashq), Syria	AY531625	NMP 48852, 48853
Plecotus m. macrobullaris Pmac2	Maalula (n=2) (Dimashq), Syria	AY531628	NMP 48993, 48994
Plecotus m. macrobullaris Pmac3	Choplu (n=2), Iran	AY531626	NMP 48126, 48127
Plecotus m. macrobullaris Pmac4	Tahkt-e-Suleyman (n=2), Iran	AY531627	NMP 48139, 48141
Plecotus m. macrobullaris Palp5	Tymphristos, Greece	AY134021	O. von Helversen, Univ. Erlangen
Plecotus t. gaisleri subsp. n. Pindet1	Wadi al Minshiyah, Lybia	AY531621	NMP 49883
Plecotus t. gaisleri subsp. n. Pindet1	Wadi al Kuf (n=5), Lybia	AY531621	NMP 49900, 49905, 49907, 49911, 49920
Plecotus t. gaisleri subsp. n. Pindet1	Qasr, ash Shahdayn, Lybia	AY531621	NMP 49916
Plecotus t. gaisleri subsp. n. Pindet1	Sidi Muhammamd Al Mablehut (n=2), Lybia	AY531621	NMP 49926, 49927
Plecotus t. gaisleri subsp. n. Pindet2	Nanatalah (n=2), Lybai	AY531624	NMP 49965, 49966
Plecotus t. gaisleri subsp. n. Pindet2	Ain as-Zarqa (n=2), Lybia	AY531624	NMP 49857, 49858
Plecotus t. gaisleri subsp. n. Pindet3	Agadir, Morocco	AY531623	J. Juste, Pat1
Plecotus t. gaisleri subsp. n. Pindet4	Tetouan, Morocco	AY531625	J. Juste, Pat51
Plecotus t. gaisleri subsp. n. Pindet5	Errachidia, Morocco	AY531622	J. Juste, Pat2
Plecotus christii Pchr	Al Jaghbub (n=2), Lybia	AY531615	NMP 49862, 49863
Plecotus auritus Paur1	Curaglia, Switzerland	AF529229	Univ. Zürich 2513
Plecotus auritus Paur6	Hall, Admont, Austria	AY134015	private coll. B. Freitag, Graz
Plecotus auritus Paur7	Villoslada, Spain	AY134016	J. Juste, PAR9808071
Plecotus auritus Paur9	Supramonte, Sardinia	AY175821	voucher not preserved
Plecotus balenssi Pbal	Abune Yusef, Ethiopia (n=2)	AY531614	J. Juste, EBD25842, 25844
Plecotus teneriffae teneriffae Pten1	El Hierro, Canary Islands, Spain	AJ431657	Pestano et al. 2003
Plecotus teneriffae teneriffae Pten2	Tenerife, Canary Islands, Spain	AJ431656	Pestano et al. 2003
Plecotus teneriffae teneriffae Pten3	La Palma, Canary Islands, Spain	AJ431654	Pestano et al. 2003
Barbastella barbastellus	Germany	AF529231	SMF 84.732
Myotis bechsteinii	Germany	AY134027	voucher not preserved

10. Danksagung

Hinweis: Aus datenschutzrechtlichen Gründen ist es mir an dieser Stelle nicht erlaubt, all denen dank zu sagen, die es verdient hätten.

@ Alle: Seit euch meines Dankes bewusst.

11. Zusammenfassung

Die Phylogenie der Westpaläarktischen Langohren (Mammalia, Chiroptera, *Plecotus*) – eine molekulare Analyse

Die Langohren stellen eine Fledermausgattung dar, die fast alle westpaläarktischen Habitate bist zum Polarkreis hin besiedeln und in vielerlei Hinsicht rätselhaft sind. In der Vergangenheit wurden zahlreiche Formen und Varietäten beschrieben. Trotzdem galt für lange Zeit, dass nur zwei Arten in Europa anerkannt wurden. Weitere Arten waren aus Nordafrika, den Kanaren und Asien bekannt, aber auch deren Artstatus wurde vielfach in Frage gestellt.

In der vorliegenden Dissertation habe ich mittels molekularer Daten,der partiellen Sequenzierung mitochondrialer Gene (16S rRNA und ND1), sowie der mitochondrialen Kontrollregion, eine molekular Analyse der phylogenetischen Verwandtschaftsverhältnisse innerhalb und zwischen den Linien der westpaläarktischen Langohren durchgeführt. Die besten Substitutionsmodelle wurden berechnet und phylogenetische Bäume mit Hilfe vier verschiedener Methoden konstruiert: dem neighbor joining Verfahren (NJ), dem maximum likelihood Verfahren (ML), dem maximum parsimony Verfahren (MP) und dem Bayesian Verfahren.

Sechs Linien der Langohren sind genetisch auf einem Artniveau differenziert: *Plecotus auritus, P. austriacus, P. balensis, P. christii, P. sardus,* und *P. macrobullaris*. Im Falle der Arten *P. teneriffae, P. kolombatovici* und *P. begognae* ist die alleinige Interpretation der genetischen Daten einzelner mitochondrialer Gene für eine Festlegung des taxonomischen Ranges nicht ausreichend. Ich beschreibe in dieser Dissertation drei neue Taxa: *Plecotus sardus*, *P. kolombatovici gaisleri* (=*Plecotus teneriffae gaisleri*, Benda et al. 2004) and *P. macrobullaris alpinus* [=*Plecotus alpinus*, Kiefer & Veith 2002). Morphologische Kennzeichen, insbesondere für die Erkennung im Feld, werden hier dargestellt.

Drei der sieben Arten sind polytypisch*: P. auritus* (eine west- und ein osteuropäische Linie, eine sardische Linie und eine aktuell entdeckte kaukasische Linie, *Plecotus kolombatovici* (*P. k. kolombatovici, P. k. gaisleri und P. k.* ssp.) und *P. macrobullaris* (*P. m. macrobullaris* und *P. m. alpinus*). Die Verbreitungsgebiete der meisten Arten werden in dieser Arbeit erstmals ausschließlich anhand genetisch zugeordneter Tiere dargestellt.Die Untersuchung der ökologischen Einnischung der nun anerkannten Formen, insbesondere in Gebieten sympatrischer Verbreitung, bietet ein spannendes und lohnendes Feld für zukünftige Forschungen. Nicht zuletzt muss sich die Entdeckung eines beachtlichen Anteils kryptischer Diversität innerhalb der westpaläarktischen Langohren auch bei der Entwicklung spezieller Artenschutzkonzepte widerspiegeln.

Ich erkläre, dass ich die vorgelegte Thesis selbstständig, ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt habe, ich in der Thesis angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der Satzung der Johannes Gutenberg-Universität Mainz zur Sicherung guter wissenschaftlicher Praxis niedergelegt sind, eingehalten.