



MORPHOLOGY AND MITOCHONDRIAL
DNA SEQUENCES SHOW THAT
PLECOTUS ALPINUS KIEFER & VEITH, 2002
AND *PLECOTUS MICRODONTUS* SPITZENBERGER,
2002 ARE SYNONYMS OF *PLECOTUS*
MACROBULLARIS KUZJAKIN, 1965

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Genetic and morphological analyses of long-eared bats from Northern Ossetia and other Caucasian localities revealed that the sister clade of *Plecotus auritus* discovered in the Eastern Alps (SPITZENBERGER *et al.*, 2001) and described as a new species by Spitzenberger (SPITZENBERGER *et al.*, 2002) (*Plecotus microdontus*) and by KIEFER & VEITH (2002) (*Plecotus alpinus*) is conspecific with *Plecotus macrobullaris* Kuzyakin, 1965, described as a subspecies of *P. auritus*, from the vicinity of Vladikavkaz. The valid name for this species therefore is *Plecotus macrobullaris*. *P. macrobullaris* also occurs in central and eastern Turkey. Morphological as well as genetic analyses differentiate between an eastern and a western group within *P. macrobullaris*. From a morphological comparison of specimens of *P. macrobullaris* with the type specimens of *Plecotus wardi* from Kashmir we conclude that *macrobullaris* is not conspecific with *wardi*.

Key words: Vespertilionidae, *Plecotus*, mitochondrial control region, morphological characters, Caucasus, Asia Minor, Alps, Croatia, Northern Italy, taxonomy, nomenclature

Spitzenberger, F., Strelkov, P. & Haring, E.: Morfologija i sekvence mitohondrijske DNK pokazuju da su *Plecotus alpinus* Kiefer & Veith, 2002 i *Plecotus microdontus* Spitzenberger, 2002 sinonimi *Plecotus macrobullaris* Kuzjakin, 1965. *Nat. Croat.*, Vol. 12, No. 2, 39–53, 2003, Zagreb.

Genetske i morfološke analize dugouhkih šišmiša iz Sjeverne Osetije i drugih kavkaskih lokaliteta otkrile su da je sestrinska grupa vrste *Plecotus auritus*, otkrivena u Istočnim Alpama (SPITZENBERGER *et al.*, 2001) i opisana kao nova vrsta od Spitzenberger (SPITZENBERGER *et al.*, 2002) (*Plecotus microdontus*) i KIEFER & VEITH (2002) (*Plecotus alpinus*), konspicijna s vrstom *Plecotus macrobullaris* Kuzyakin,

1965, opisanom kao podvrsta od *P. auritus* iz blizine Vladikavkaza. Zato je validno ime ove vrste *Plecotus macrobullaris*. *P. macrobullaris* se također javlja u središnjoj i istočnoj Turskoj. Morfološke i genetičke analize pokazuju razliku istočne i zapadne grupe unutar *P. macrobullaris*. Iz morfološke usporedbe primjeraka *P. macrobullaris* s tipskim primjercima *Plecotus wardi* iz Kašmira zaključujemo da *macrobullaris* nije konspicijozan s *wardi*.

Ključne riječi: Vespertilionidae, *Plecotus*, mitohondrijska kontrolna regija, morfološke značajke, Kavkaz, Mala Azija, Hrvatska, Alpe, sjeverna Italija, taksonomija, nomenklatura

INTRODUCTION

In the year 2001 SPITZENBERGER *et al.* reported the existence of a distinct clade of *Plecotus* exists in the Austrian Alps, different from the species *P. auritus* and *P. austriacus*. In that paper the newly discovered clade was erroneously assigned to *Plecotus kolombatovici*. In the following year the clade was described as a new species by KIEFER & VEITH (»*Plecotus alpinus*«) and SPITZENBERGER (»*Plecotus microdontus*«) almost simultaneously (KOCK, 2002).

In this paper we report about investigations on long-eared bats from Asia Minor and the Caucasus, showing that *Plecotus auritus macrobullaris* Kuzyakin, 1965, described from the vicinity of Vladikavkaz, Northern Ossetia on the northern slope of the Great Caucasus, is conspecific with the species that had been named *P. alpinus* and *P. microdontus* respectively.

MATERIAL AND METHODS

Genetic analysis

DNA extraction, PCR amplification, cloning and sequencing protocols were the same as described previously (SPITZENBERGER *et al.*, 2002). For the phylogenetic analysis a section of approximately 245 bp comprising the 3'-end of the mitochondrial control region (CR) and a partial sequence of the *tRNA-Phe* gene (SPITZENBERGER *et al.*, 2001) was amplified using the PCR primers Ple2+ (5'-TCTTGCCAAACCCCAAAAAC-3') and Phe- (5'-AAGCATTTTCAGTGCTTTGCTT-3'). From the 31 sequences included in the phylogenetic analysis nine have been published previously (SPITZENBERGER *et al.*, 2001) and 22 have been determined in the course of the present study (designation of specimens corresponds to those in Fig. 1; locality and voucher numbers of museum collections are given in parentheses): *P. auritus*: Italy: Plesp10 (Belluno); Ukraine: Ple2 (Alushta, S Crimea, ZIANP 82991); Russian Federation: PleKB173 (Ladoga, Leningradskaya oblast, NMW 63227); Greece: Ple27793 (Parnassós mountains, NMW 27793); *P. macrobullaris*: Italy: Plesp8 (Cimolais, Pordenone), Plesp12 (Pordenone), Plesp13 (Porcia, Pordenone), Plesp16 (Gorizia, MFSN 1321), Plesp18 (Scrutto, Udine); Croatia: Ple49 (northern Velebit mountains, CNHM 6133), Ple5990 (southern Velebit mountains, CNHM 5994), Ple6112 (northern Velebit mountains, CNHM 6112), Ple6098 (northern Velebit mountains, CNHM 6098); Turkey: Ple34421 (Vil. Bitlis, NMW 34421), Ple74 (Cilician Toros mountains, ZMB 92995), Ple20651 (Nevşehir, NMW 20651); Armenia: Ple28 (ZIANP 51415), Ple91 (ZIANP 21442); Russian Federation, Severo-Osetinskaya Respublica: Ple88, Ple89 (Zaramak Alagyrskii

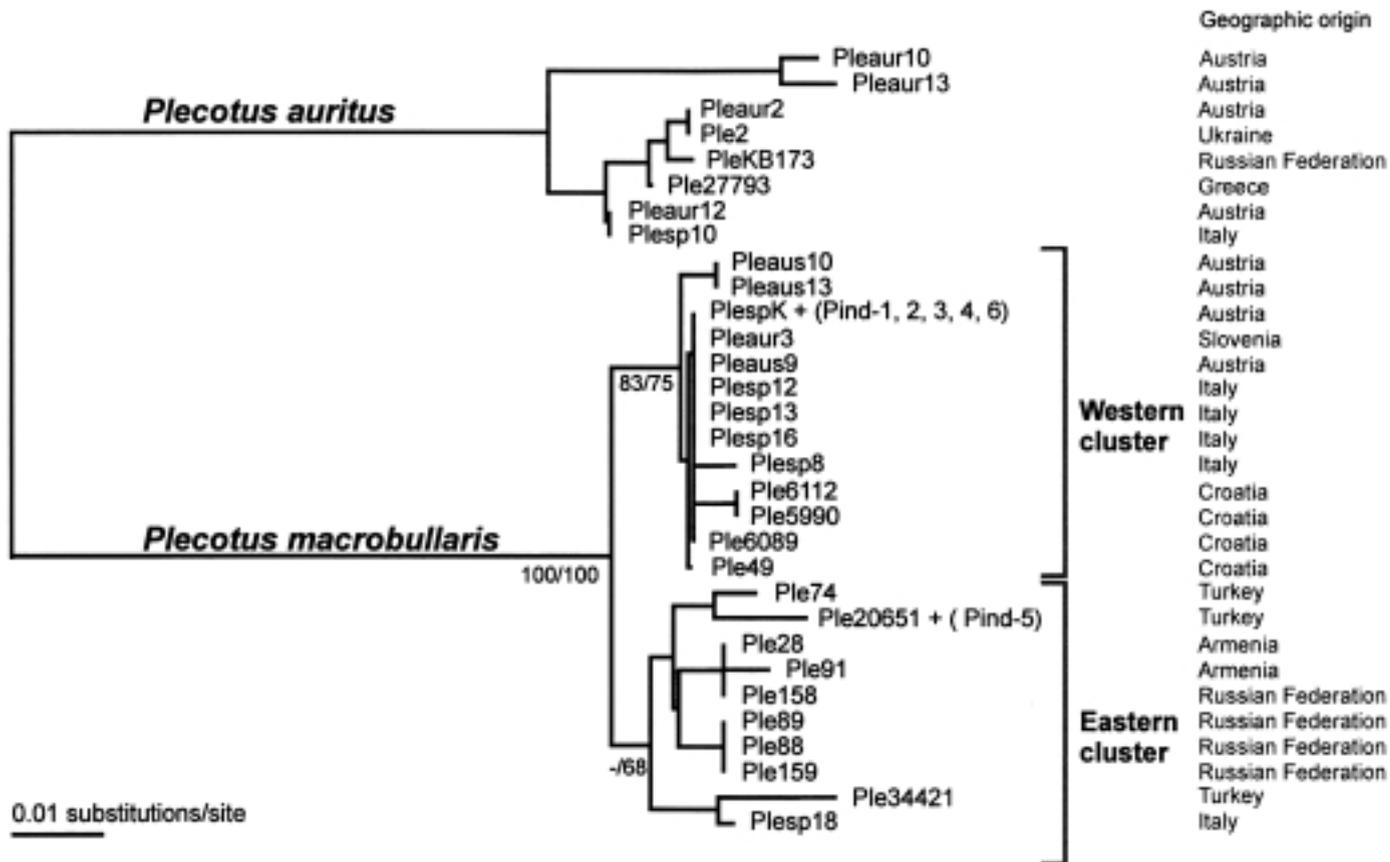


Fig. 1. NJ tree of partial CR sequences (midpoint rooting). Geographic origins of the 31 samples are indicated on the right. Bootstrap values (1000 replicates) of the three major nodes (defining *P. macrobullaris* and its two clusters) are depicted: left: NJ, right: MP. Positions of the sequences published by KIEFER *et al.* (2002) are indicated: Pind-1 to Pind-6 (see text).

district, ZIANP 72326, ZIANP 72327) Ple158, (Tzargasta, N Caucasus, ZIANP 85701), Checheno-Ingushkaya Respublica: Ple159 (N Caucasus, ZIANP 85702). Editing and alignment of sequences was performed using the BioEdit software package version 5.0.9 (HALL, 1999). Neighbour-joining (NJ; SAITOU & NEI, 1987) and maximum parsimony (MP) dendrograms were calculated with the software package PAUP (version 4.0b10; SWOFFORD, 2002). MP trees were calculated with heuristic search using the TBR (tree bisection reconnection) algorithm and a random taxon addition sequence; gaps were treated as fifth character state. For the NJ tree uncorrected distances (p-distances) were used. The sequences determined in the course of the present study are registered under the following GenBank accession numbers: AY324402–AY324423.

Morphological analysis

In addition to the specimens used in the description of *P. microdontus* (SPITZENBERGER *et al.*, 2002) from Austria, Italy, and Slovenia, 14 specimens from the Caucasus and 12 specimens from Asia Minor were examined:

P. macrobullaris: Russian Federation, Severo-Osetinskaya Respublica: near Vladikavkaz (until 1990 Ordzhonikidze) (1 skull) 1914 (ZIANP 1435=holotype of *P. macrobullaris*); Zaramak, Alagyrskiï district 2 ♂, 1 ♀ (3 skins + 3 skulls) 1957 (ZIANP 72326, 72327, 72328); Tzargasta: 1 ♀ (1 alc) 1978 (ZIANP 85701). – Checheno-Ingushkaya Respublica: 4 ♂, 4 ♀ (measurements of the forearm). – Armenia: 1 specimen (1 alc.) 1963 (ZIANP 51415). – Turkey: Vil. Nevşehir 1 ♂, 9 ♀ (5 skins + 5 alc. + 10 skulls) 4.–16.7.1975 (NMW 20651–20656, 20658–20660, 20663); Cilician Toros mountains 1 ♂ (1 alc. + 1 skull) 30.4.1904 (ZMB 92995); Vil. Bitlis (1 skin + 1 skull) 16.7.1984 (NMW 34417).

Comparative material:

Plecotus wardi: India, Kashmir: near Leh, Ladakh (1 ♂, 2 ♀) (3 skins + 2 skulls) 10.6.1906 (type series BMNH 6.10.3.1, 6.10.3.2=holotype, 8.7.6.1).

Abbreviations

The abbreviations of the measurements are the same as in SPITZENBERGER *et al.* (2002).

BMNH British Museum of Natural History London

CNHM Croatian Natural History Museum Zagreb

MFSN Museo Friulano di Storia Naturale Udine

NMW Naturhistorisches Museum Wien

ZIANP Zoological Institute Academy of Sciences St. Petersburg

ZMB Zoologisches Museum Berlin

RESULTS

Genetic analysis

The alignment of CR sequences has a length of 203 positions and includes 31 sequences, among them eight sequences of *P. auritus*. Of the 51 variable characters 44 are parsimony-informative. Fig. 1 shows a NJ dendrogram calculated from this

alignment. The tree is composed of two major clades. One contains the *P. auritus* sequences, whereas the main clade comprises sequences of individuals representing the new species as defined in SPITZENBERGER *et al.* (2002) (*P. microdontus*) and KIEFER & VEITH, 2002 (*P. alpinus*) as well as individuals from Italy, Croatia, Turkey, Armenia, and the Russian Federation. Among them are four individuals from the vicinity of Vladikavkaz representing *P. a. macrobullaris* Kuzyakin, 1965. Essentially the same topology is obtained in an MP analysis (not shown; two equally parsimonious trees, TL=59, CI =0.881, RI=0.973, RC=0.857; bootstrap values are included in Fig. 1). Minor differences between MP trees and the NJ tree are found within subclades only. The average distance between the two clades is 15.5 % (range: 14.4 % to 18.4 %). In contrast, the average distance within the main clade measures only 1.7 % (0–4.0 %) which is similar to that found within the *P. auritus* clade (1.9 %; range: 0–4.5 %). Thus, the members of the main clade obviously represent a single species. The main clade is further divided into two clusters that can be assigned to two geographic regions: a western cluster composed of sequences representing Austria, Italy, Slovenia, and Croatia, and an eastern cluster representing individuals mainly from Turkey, Armenia, the Russian Federation (Caucasus) as well as one individual from Italy. Average distance between the two clusters is 2.5 % (range 2.0–4.0%), within clusters 0.3 % (range: 0–1.0 %) for the western and 1.7 % (range 0–3.5 %) for the eastern cluster.

Several CR sequences isolated from individuals of *P. alpinus* (then designated *Plecotus indet.*) were published by KIEFER *et al.* (2002): Pind-1 (Ristolas, France), Pind-2 (Spaizzo, Italy), Pind-3 (Fischertratten, Austria), Pind-4 (Waisach, Austria), Pind-5 (Tymphristos, Greece), Pind-6 (Ogulin, Croatia). Including them into our data set results in a shorter alignment which has a length of 180 positions since the section sequenced by KIEFER *et al.* (2002) is somewhat shorter than that used in our study. On the basis of this alignment, calculation of an MP tree (not shown) indicated that, with the exception of Pind-5 from Greece, these sequences belong to the western cluster. Four of these sequences are identical to the main haplotype represented by the specimen PlespK and one (Pind-6) differs at one position. The Greek sample is found in the eastern group and clusters with Ple20651 from Turkey. The assignment of these sequences (depicted in Fig. 1) illustrates that the individuals investigated by KIEFER *et al.* (2002) also belong to *P. macrobullaris*. Their affiliation to the western or eastern cluster respectively follows the same geographic pattern also found in the other individuals.

Altogether, the phylogenetic analysis proves that *Plecotus macrobullaris* Kuzyakin, 1965 described from the northern slope of the Great Caucasus is conspecific with *P. alpinus* Kiefer & Veith, 2002 described from the Western Alps (Hautes-Alpes in France) and *P. microdontus* Spitzenberger, 2002 described from the Eastern Alps (Eastern Tyrol, Austria). The oldest available and therefore valid name for this species is *Plecotus macrobullaris*.

The presence of two distinct clusters within *P. macrobullaris* reflecting two geographic groups of individuals indicates that the western and eastern group might represent two different subspecies. The specimens from Greece, central and eastern Turkey, Armenia, and Northern Ossetia belong to the eastern group whereas the in-

dividuals from the Alpine regions, northern Italy, and Croatia are found in the western group. The only exception is an individual from northern Italy found in the eastern cluster (Plesp18).

Morphological analysis of geographical variation within *Plecotus macrobullaris*

KUZYAKIN in BOBRINSKIJ *et al.* (1965) described *macrobullaris* as subspecies of *Plecotus auritus* from the vicinity of Ordzhonikidze (now Vladikavkaz) in Northern Ossetia. As distribution area of this taxon he adduced regions of Northern Ossetia and Kabardino-Balkaria in the high mountains of the Great Caucasus and Lake Sevan in Armenia. As distinguishing characters he recorded very large bullae (4.5–4.9 mm), dark dorsal fur (»as in European *auritus*«, without mentioning which *Plecotus* population he had in mind), tips of ventral fur white, bases of all hairs black or dark brown.

Within the vast area from the Caucasus to the western Alps, the colouration and measurements of *P. macrobullaris* are subject to geographical variation. In this paper the analysis of this variation is restricted to a comparison of two eastern populations (Caucasus and Turkey) and one western population (Eastern Alps). The Western Alpine population could not be included in this comparison, because measurements of only one individual (the holotype of *P. alpinus*) are available (KIEFER & VEITH, 2002) and the colouration of the pelage of the western Alpine population is known only from two photographs.

Variation of colouration

In Turkish *P. macrobullaris* the dorsal fur is a shade lighter and paler brown and the ventral fur is somewhat whiter than in specimens of the Eastern Alps as described in SPITZENBERGER *et al.* (2002). All hairs are bicoloured, the dorsal hair length is 10.5 mm, the ventral hair length only a little shorter (10.1 mm). Ears and patagia are light brown.

From two photographs it may be assumed that the western and southern Alpine *P. macrobullaris* differ from the Eastern Alpine by a much darker face and strikingly white ventral fur: a live animal depicted in KIEFER & VEITH (2002) was caught in Pesina, prov. Verona, Italy. A photograph of a bat labelled as *Plecotus austriacus* probably taken in Switzerland (HAUSSER, 1995) is very similar in colouration to the one from Pesina.

Morphometric variation (Tab. 1)

Because of insufficient material of males, comparison of measurements is restricted to females.

The length of the forearm decreases from East to West.

Skull and teeth of Caucasian, Turkish and Eastern Alpine specimens are similar in general structure (Fig. 2 and 3). Length of C sup. increases from East to West, length of I¹ does not vary between the populations. For this reason the ratio between the lengths of I¹ and upper canine in the eastern populations is different from the ratio in western populations, where it can be used to distinguish *P. macro-*

Tab. 1. Body- and skull measurements of *Plecotus macrobullaris*

| | | male | | | | | female | | | | | |
|--------------------------------|------------|-------|------|-------|-------|---|--------|------|-------|-------|-------|---|
| | | mean | s | min | max | n | mean | s | min | max | n | |
| FA+ | Caucasus | 42.77 | 0.48 | 42.10 | 43.20 | 6 | 43.10 | 1.45 | 40.70 | 44.20 | 5 | |
| | Asia Minor | | | | 40.90 | 1 | 42.67 | 1.23 | 40.50 | 44.20 | 9 | |
| | East. Alps | 40.59 | 0.59 | 39.60 | 41.50 | 7 | 41.91 | 0.86 | 40.50 | 43.50 | 11 | |
| Gsl | Caucasus | | | 16.60 | 17.50 | 3 | | | | | 17.32 | 1 |
| | Asia Minor | | | 17.40 | 17.50 | 2 | 17.68 | 0.44 | 16.88 | 18.30 | 10 | |
| | East. Alps | 17.07 | 0.21 | 16.70 | 17.37 | 7 | 17.46 | 0.22 | 17.08 | 17.86 | 11 | |
| Ccl | Caucasus | | | 14.80 | 15.60 | 3 | | | | | 14.98 | 1 |
| | Asia Minor | | | 15.15 | 15.23 | 2 | 15.45 | 0.38 | 14.72 | 16.00 | 10 | |
| | East. Alps | 14.79 | 0.08 | 14.68 | 14.91 | 7 | 15.19 | 0.19 | 14.90 | 15.63 | 11 | |
| Cbl | Caucasus | | | 15.50 | 16.30 | 3 | | | | | 15.72 | 1 |
| | Asia Minor | | | 15.81 | 15.86 | 2 | 16.10 | 0.39 | 15.31 | 16.75 | 10 | |
| | East. Alps | 15.47 | 0.13 | 15.24 | 15.61 | 7 | 15.94 | 0.19 | 15.70 | 16.41 | 11 | |
| Bh ⁺ | Caucasus | | | | 7.70 | 1 | | | | | 7.28 | 1 |
| | Asia Minor | | | 7.75 | 7.95 | 2 | 7.78 | 0.17 | 7.51 | 8.06 | 10 | |
| | East. Alps | 7.61 | 0.10 | 7.43 | 7.77 | 7 | 7.72 | 0.11 | 7.51 | 7.94 | 10 | |
| Bb | Caucasus | | | 8.92 | 9.30 | 3 | | | | | 8.79 | 1 |
| | Asia Minor | | | 8.90 | 9.20 | 2 | 9.28 | 0.27 | 8.66 | 9.50 | 10 | |
| | East. Alps | 8.33 | 0.16 | 8.08 | 8.51 | 7 | 8.46 | 0.16 | 8.25 | 8.76 | 11 | |
| Mb | Caucasus | | | | 9.15 | 1 | | | | | 8.92 | 1 |
| | Asia Minor | | | 9.30 | 9.66 | 2 | 9.41 | 0.23 | 8.95 | 9.70 | 10 | |
| | East. Alps | 9.05 | 0.15 | 8.80 | 9.24 | 7 | 9.25 | 0.09 | 9.10 | 9.40 | 11 | |
| Zb | Caucasus | | | | 8.70 | 1 | | | | | | |
| | Asia Minor | | | 8.87 | 9.39 | 2 | 9.09 | 0.18 | 8.87 | 9.40 | 10 | |
| | East. Alps | 8.66 | 0.15 | 8.37 | 8.84 | 7 | 8.90 | 0.12 | 8.75 | 9.14 | 10 | |
| Iob | Caucasus | | | 3.30 | 3.68 | 3 | | | | | 3.48 | 1 |
| | Asia Minor | | | 3.70 | 3.88 | 2 | 3.60 | 0.09 | 3.41 | 3.72 | 10 | |
| | East. Alps | 3.68 | 0.11 | 3.50 | 3.85 | 7 | 3.63 | 0.07 | 3.54 | 3.76 | 11 | |
| C-C | Caucasus | | | 3.60 | 3.99 | 3 | | | | | 3.62 | 1 |
| | Asia Minor | | | 3.85 | 4.04 | 2 | 3.87 | 0.16 | 3.66 | 4.23 | 10 | |
| | East. Alps | 3.68 | 0.10 | 3.50 | 3.85 | 7 | 3.82 | 0.10 | 3.66 | 3.99 | 11 | |
| M ³ -M ³ | Caucasus | | | 6.20 | 6.68 | 3 | | | | | 6.17 | 1 |
| | Asia Minor | | | 6.22 | 6.58 | 2 | 6.40 | 0.17 | 6.17 | 6.63 | 10 | |
| | East. Alps | 6.17 | 0.16 | 5.92 | 6.42 | 7 | 6.29 | 0.13 | 6.00 | 6.49 | 11 | |
| C-M ³ | Caucasus | | | 5.50 | 5.80 | 3 | | | | | 5.34 | 1 |
| | Asia Minor | | | 5.78 | 5.82 | 2 | 5.76 | 0.11 | 5.56 | 5.97 | 10 | |
| | East. Alps | 5.47 | 0.07 | 5.36 | 5.55 | 7 | 5.62 | 0.09 | 5.49 | 5.74 | 11 | |
| DBt | Caucasus | | | 4.57 | 4.80 | 2 | | | | | 4.62 | 1 |
| | Asia Minor | | | 4.57 | 4.70 | 2 | 4.66 | 0.11 | 4.52 | 4.85 | 10 | |
| | East. Alps | 4.47 | 0.06 | 4.40 | 4.58 | 7 | 4.56 | 0.10 | 4.43 | 4.73 | 11 | |
| Cl | Caucasus | | | | 1.77 | 1 | | | | | 1.84 | 1 |
| | Asia Minor | | | 1.53 | 1.86 | 2 | 1.79 | 0.11 | 1.66 | 2.00 | 10 | |
| | East. Alps | 1.87 | 0.05 | 1.77 | 1.95 | 7 | 1.93 | 0.04 | 1.85 | 1.99 | 11 | |
| I ^h | Caucasus | | | | 0.87 | 1 | | | | | 0.88 | 1 |
| | Asia Minor | | | 0.66 | 0.94 | 2 | 0.90 | 0.08 | 0.75 | 1.02 | 10 | |
| | East. Alps | 0.89 | 0.05 | 0.81 | 0.97 | 7 | 0.89 | 0.04 | 0.84 | 0.97 | 9 | |
| M | Caucasus | | | 11.20 | 11.25 | 2 | | | | | 10.86 | 1 |
| | Asia Minor | | | 10.88 | 10.95 | 2 | 11.35 | 0.24 | 10.91 | 11.73 | 10 | |
| | East. Alps | 10.71 | 0.18 | 10.44 | 10.87 | 7 | 10.97 | 0.15 | 10.74 | 11.23 | 11 | |
| C-M ₃ | Caucasus | | | 6.00 | 6.24 | 2 | | | | | 6.07 | 1 |
| | Asia Minor | | | 6.20 | 6.21 | 2 | 6.16 | 0.15 | 5.89 | 6.41 | 10 | |
| | East. Alps | 5.93 | 0.07 | 5.82 | 6.00 | 7 | 6.04 | 0.11 | 5.83 | 6.16 | 11 | |
| Corh | Caucasus | | | 2.60 | 3.09 | 2 | | | | | 2.96 | 1 |
| | Asia Minor | | | 3.12 | 3.16 | 2 | 3.12 | 0.13 | 2.91 | 3.29 | 10 | |
| | East. Alps | 2.92 | 0.09 | 2.83 | 3.08 | 7 | 3.00 | 0.08 | 2.90 | 3.16 | 11 | |



Fig. 2. Skull of *Plecotus macrobullaris* from the vicinity of Alagyr, Severo-Osetinskaya Respublika (ZIANP 72328)



Fig. 3. Skull of *Plecotus macrobullaris* from Lienz, Austria (holotype of *Plecotus microdontus* (NMW 34857)

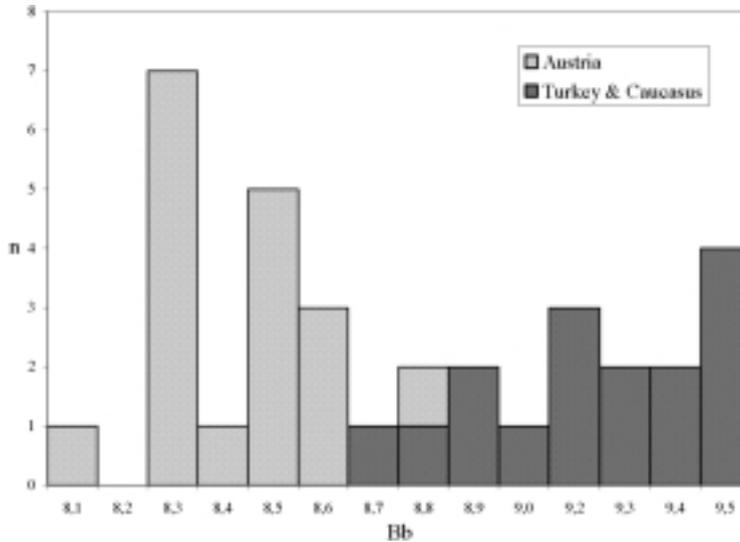


Fig. 4. Variation of breadth of braincase without bullae (Bb) in *Plecotus macrobullaris* in the western (Eastern Alps) and eastern part (Caucasus and Asia Minor) of its range

bullaris from the three other Western Palearctic *Plecotus* species (SPITZENBERGER *et al.*, 2002). Skull size decreases from East to West. While in most measurements the difference between the eastern and western population is small, some measurements of the skull breadth, especially Bb (Fig. 4), Mb, Zb, and the length of the facial part (C-M³ – Fig. 5 and C-M₃) as well as the auditory bullae are considerably smaller in the Eastern Alps. The correlation of skull length (Ccl) and skull breadth (Bb) (Fig. 6) separates the eastern from the western populations well.

Baculum

The baculum of the holotype of *Plecotus microdontus* (SPITZENBERGER *et al.*, 2002) is similar in shape and size to two bacula of *P. macrobullaris* from Armenia depicted in STRELKOV (1988) and STRELKOV (1989). Number »R« in Fig. 2 in STRELKOV (1988) and number »19« in Fig. 3 in STRELKOV (1989) represent the baculum of *P. macrobullaris* ZIANP 21442 designated Ple91 in the genetic analysis. Its measurements are: total length 0.82 mm, maximum width 0.72 mm. A baculum of a second individual from Armenia (ZIANP 61746) that was not analysed genetically is characterised by the following measurements: total length 0.80 mm, maximum width 0.57 mm.

Morphological comparison of *Plecotus macrobullaris* with other *Plecotus* taxa described from the Palearctic

The discrimination of *P. macrobullaris* from the western Palearctic *Plecotus* species *auritus*, *austriacus*, and *kolombatovici* has been described in SPITZENBERGER *et al.*, 2002. Among taxa recorded from the central Palearctic, *P. homochrous* Hodgson, 1847 and

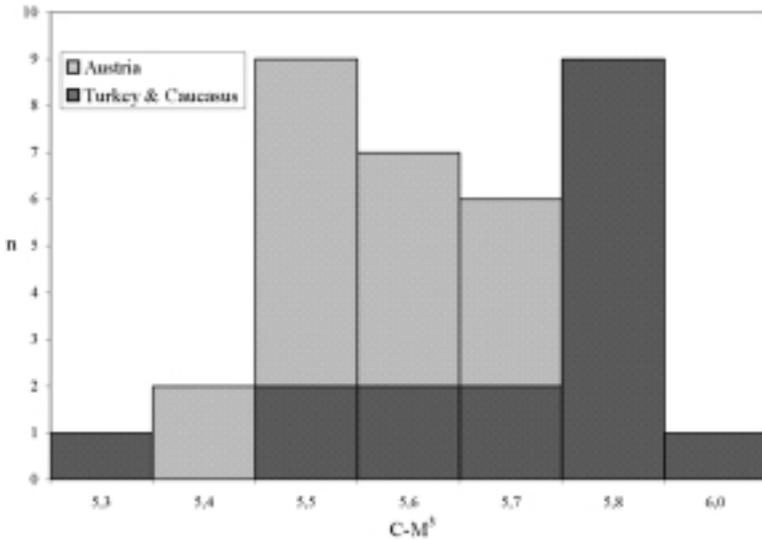


Fig. 5. Variation of the distance C-M³ in *Plecotus macrobullaris* in the western (Eastern Alps) and eastern part (Caucasus and Asia Minor) of its range

P. puck Barret-Hamilton, 1907 differ from *P. macrobullaris* by their smaller size, *P. austriacus turkmenicus* Strelkov, 1985 and *P. auritus kozlovi* Bobrinskij, 1926 by their larger size, *P. mordax* Thomas, 1926 by larger teeth, *P. christiei* Gray, 1838 by smaller

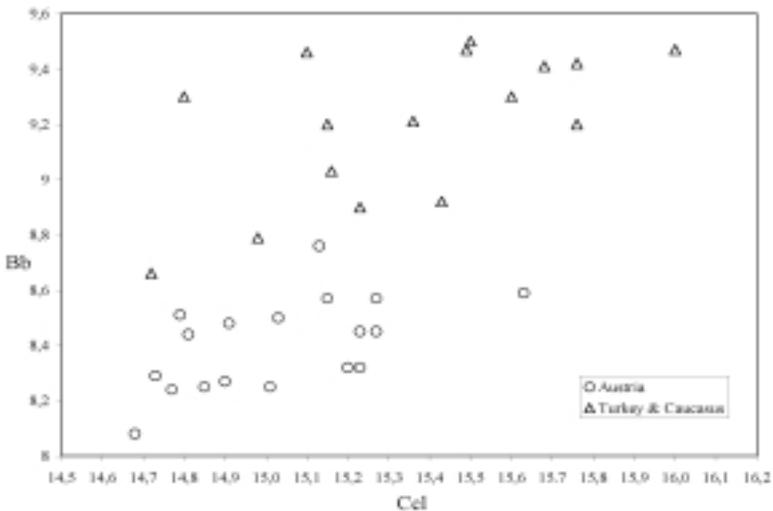


Fig. 6. Bivariate scatter plot of skull length (Ccl) against skull breadth (Bb) for *Plecotus macrobullaris* from the western (Eastern Alps) and eastern part (Caucasus and Asia Minor) of its range

forearms, and *P. ariel* Thomas, 1911 by dark brown colour. *Plecotus wardi* Thomas, 1911 described from the Ladakh range in Kashmir, however, resembles *P. macrobullaris* in many characters. The name *wardi* has been widely used for Asian long-eared bats not related to *auritus*, and many authors assigned Caucasian *austriacus*-like bats to *wardi* (e.g. GROMOV *et al.*, 1963; PAVLINOV & ROSSOLIMO, 1987; STRELKOV, 1988; KOOPMAN in WILSON & REEDER, 1993; HORAČEK *et al.*, 2000).

To exclude the possibility that *P. macrobullaris* is conspecific with *P. wardi* Thomas, 1911 described from Ladakh, Kashmir, we examined the type specimens of *P. wardi* preserved in the Natural History Museum in London.

From Turkish *P. macrobullaris*, which are lighter than those from the Alps, the three specimens forming the type series of *P. wardi* differ in colour (pale buff) and texture (very dense) of the dorsal, and shortness (6.3 mm) and sparseness of the ventral hair. The dorsal hair of *P. wardi* is tricoloured, the bases of all hairs are dark brown.

From Tables 1 and 2 it is visible that forearm and skull measurements of *wardi* fall within the variation of *macrobullaris*.

From *P. macrobullaris* the type specimens of *P. wardi* (Fig. 7) differs in markedly larger posterior upper incisivus and canine, shorter but plumper P², the lack of a distance between the upper canine and P² (small distance present in *P. macrobullaris*), shorter and broader anterior part of the zygomatic arch, shallower anterior palatal emargination. There is a well developed median palatal processus in *P. wardi*, which is lacking or much less well developed in *P. macrobullaris*. The part of the maxilla containing the alveoli is thicker and more sturdy in *P. wardi* than in *P. macrobullaris* in which it is thinner and more delicate (Fig. 2, 7).

Shape and size of baculum of topotypic *wardi* are unknown.

Tab. 2. Body and skull measurements of *Plecotus wardi* from Kashmir.

| | BMNH ♀ 6.10.3.2. holotype | BMNH ♀ 6.10.3.1. paratype |
|--------------------------------|---------------------------------|---------------------------------|
| FA ⁺ | 45.40 | 42.54 |
| Gsl | 17.61 | 17.50 |
| Ccl | 15.47 | 15.40 |
| Cbl | 16.30 | 16.04 |
| Bh ⁺ | 7.50 | 7.42 |
| Bb | 9.08 | 8.92 |
| Mb | 9.21 | 9.14 |
| Zb | – | 8.76 |
| Iob | 3.54 | 3.80 |
| C–C | 3.84 | 3.87 |
| M ³ –M ³ | 6.31 | 6.51 |
| C–M ³ | 5.85 | 5.86 |
| DBt | 4.46 | 4.56 |
| Cl | 1.55 | 1.52 |
| I ¹ | 0.89 | 0.80 |
| M | 11.15 | 11.24 |
| C–M ₃ | 6.23 | 6.33 |
| Corh | 2.83 | 2.96 |



Fig. 7. Skull of the holotype of *Plecotus wardi* (BMNH 6.10.3.2)

DISCUSSION

Both the genetic and the morphological results presented here show that the new *Plecotus* species described by Spitzenberger in SPITZENBERGER *et al.* (2002) and in KIEFER & VEITH (2002) have to be synonymized with *Plecotus macrobullaris* Kuz'yakin, 1965. Conspecificity with other Palearctic species, especially with *P. wardi*, can be excluded according to morphological characters.

The section of the mitochondrial control region selected for the genetic analysis is quite short, because only small DNA fragments can be amplified from old tissues of museum specimens. This may cause comparatively low bootstrap values at some nodes. Nevertheless, this section provides sufficient information to serve as a diagnostic character for clarifying intraspecific relationships and for taxon assignment. The allocation of the holotype of *P. alpinus* and the Eastern Alpine populations described as *P. microdontus* to *Plecotus macrobullaris* was only possible with the aid of molecular techniques, although KUZ'YAKIN (1965) had reported already the chief characters typical of Caucasian as well as Alpine long-eared bats (intermediate position between *P. auritus* and *P. austriacus*, white ventral fur and large size of the bullae in comparison to Caucasian *P. auritus*).

The present investigation shows that *P. macrobullaris* has a more or less continuous distribution extending from the Caucasus through Turkey and the Balkan peninsula to the Alps. Genetic as well as morphological characters indicate a sub-specific division between a western and an eastern group.

Only one specimen, Plesp 18, is not in accordance with this geographic separation. Plesp 18 originates from Italy but belongs genetically to the eastern lineage. It suggests either incomplete lineage sorting or recent gene flow between the separated populations. The genetic divergence seems to correspond to pronounced differences in skull proportions of the marginal populations. Future investigations of additional populations will show whether geographic patterns revealed by genetic and morphological methods are identical.

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References

- GROMOV, I., GUREYEV, A., NOVIVKOV, G., SOKOLOV, I., STRELKOV, P. & CHAPSKII, K., 1963: Mljekopitayushthshye fauny SSSR. Moskva, Leningrad.
- HALL, T. A., 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. **41**, 95–98.
- HAUSSER, J. (ed.), 1995: Säugetiere der Schweiz: Verbreitung, Biologie, Ökologie. Birkhäuser, Basel.
- HORÁČEK, I., HANÁK, V. & GAISLER, J., 2000: Bats of the Palearctic Region: A taxonomic and biogeographic review. Proc. VIIIth EBRS 1, 11–57.
- 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.
- KIEFER, A. & VEITH, M., 2002: A new species of long-eared bat from Europe (Chiroptera: Vespertilionidae). Myotis **39**, 5–16.
- KOCK, D., 2002: The publication dates of *Plecotus alpinus* Kiefer and Veith, 2002 and of *Plecotus microdontus* Spitzenberger, 2002. Acta Chir. **4**, 219–220.
- KOOPMAN, K., 1993: Order Chiroptera. 137–241. In: WILSON, E. & REEDER, D.: Mammal species of the world. Smithsonian Inst. Press, Washington and London.
- KUZYAKIN, A., 1965: Otrjad Rukokrylyje. Ordo Chiroptera. 79–116. In: BOBRINSKIY, N., KUZNETSOV, B. & KUZJAKIN, A.: Opredelitel mljekopitayushtshikh SSSR. Isd. Prosveshtshenije. Moskva.
- PAVLINOV, I. & ROSSOLIMO, O., 1987: Sistematika mljekopitayushtshikh SSSR-Isd-Mosk. univer-siteta.
- SAITOU, N. & NEI, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution **4**, 406–425.
- SPITZENBERGER, F., PIÁLEK, J. & HARING, E., 2001: Systematics of the genus *Plecotus* (Mammalia, Vespertilionidae) in Austria based on morphometric and molecular investigations. Folia zool. **50**, 161–172.
- SPITZENBERGER, F., HARING, E. & TVRTKOVIĆ, N., 2002: *Plecotus microdontus* (Mammalia, Vespertilionidae), a new bat species from Austria. Nat. Croat. **11**, 1–18.
- STRELKOV, P. P., 1988: Brown (*Plecotus auritus*) and Grey (*P. austriacus*) bats (Chiroptera, Vespertilionidae) in the USSR. Zool. Zh. **67**, 90–101.
- STRELKOV, P. P., 1989: New Data on the Structure of Baculum in Palearctic Bats. I. The Genera *Myotis*, *Plecotus*, and *Barbastella*. European Bat Research 1987 Praha, 87–94.
- SWOFFORD, D. L., 2002: PAUP* – Phylogenetic Analysis Using Parsimony (*and other methods). Ver. 4.0b6–10. Sinauer, Sunderland, Mass.