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## Does *Microtus majori* occur in Europe?

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### Abstract

Voles from Mt. Pelister, Macedonia, which are known from the literature as *Microtus majori*, were subjected to morphometric, karyotypic and electrophoretic analyses, and compared with *Microtus subterraneus* from Slovenia and Montenegro, and *Microtus majori* from Asia Minor. The diploid chromosome number of the Pelister voles ( $2n = 52$ ) is the same as in *M. subterraneus* from the Balkans. Genetic distances, as revealed by electrophoretic analysis of 27 gene loci between voles from Mt. Pelister and *M. subterraneus* from Slovenia and Montenegro correspond to those generally observed among subspecies of Arvicolidae. Discriminant analysis of 12 raw skull measurements separated successfully *M. majori* from *M. subterraneus*. According to this, the Pelister population should be allocated to the latter, which means that there is no reason to include *M. majori* in the list of the European fauna.

### Introduction

*Microtus majori* Thomas, 1906 is considered to inhabit the Caucasus, northern Turkey and north-western Iran (probably incorrectly given as north-eastern Iran by GROMOV and BARANOVA 1981), but it was recently also reported from the Balkans (MUSSEY and CARLETON 1993). Namely, MALEC and STORCH (1963) and later FELTEN and STORCH (1965) point out the existence of two size classes amongst voles belonging to the *Microtus subterraneus* group from Macedonia. The larger morphotype, from Mt. Pelister, was first ascribed to *Pitymys multiplex* (FATIO, 1905) but later identified as *Pitymys majori* (FELTEN et al. 1971) or *Microtus majori* (STORCH 1982). Subsequent authors reported additional localities for *Microtus majori* in the Balkans: KIVANÇ (1986) in European Turkey and NIETHAMMER (1986) in northern Greece. However, other recent studies of the rodent fauna of the Balkans have not confirmed the presence of this species in Greek Macedonia (VOHRALIK and SOFIANIDOU 1987) or failed to mention it within the territory of the former Yugoslavia (PETROV 1992).

The aim of the present study is to reevaluate the identity of the voles from Mt. Pelister which form the basis of the inclusion of *M. majori* in the list of European mammals. This population was compared with *M. majori* from Asia Minor, and with two populations of *M. subterraneus* (de Selys Longchamps, 1936) from south-eastern Europe by biometric, karyotypic and electrophoretic analyses.

### Material and methods

We examined 107 voles. Material included voles from museum collections, as well as freshly collected animals. Standard museum specimens are housed in the following collections (acronyms in brackets): British Museum (Natural History), London (BMNH); Forschungsinstitut und Natur-Museum Senckenberg, Frankfurt am Main (SMF); Naturhistorisches Museum Wien, Vienna (NMW); and Slovene Museum of Natural History, Ljubljana (PMS). Material was pooled into four samples: sample

1: *Microtus majori*, Asia Minor (vicinity of Trabzon, including the type of *majori*; BMNH); sample 2: *M. subterraneus*, Slovenia (PMS); sample 3: Mt. Pelister, Macedonia (NMW, PMS, SMF); sample 4: *M. subterraneus*, Mt. Lovćen, Montenegro (PMS).

All our specimens from sample 2 were collected at Kopanki, Mt. Pelister, or from its vicinity, i.e. in the same area from which voles ascribed to *M. majori* originated.

Chromosomes: Nine individuals were examined karyologically (Pelister 7; Lovćen 2). Standard flame-dried preparations were made directly from the bone marrow of colchicined animal (FORD and HAMERTON 1956). In most animals, metaphase spreads were differentially stained by following the slightly modified G-banding and C-banding methods of SEABRIGHT (1971) and SUMNER (1972). Nucleolar organiser regions (NORs) were revealed by the silver staining technique of HOWELL and BLACK (1980).

Allozyme analysis: Tissue samples from 14 fresh specimens (Pelister 6; Lovćen 2; Slovenia 6) were collected and transported in liquid nitrogen to the laboratory, where they were preserved at  $-80^{\circ}\text{C}$  until processed. Homogenates for electrophoresis were obtained from portions of muscle tissue crushed in distilled water. Electrophoretic analysis was carried out on 27 loci, encoding 21 enzymes:  $\alpha$ -Glycerophosphatase dehydrogenase (E.C. 1.1.1.8;  $\alpha$ Gpdh), Sorbitol dehydrogenase (E.C. 1.1.1.4; Sdh), Lactate dehydrogenase (E.C. 1.1.1.27 Ldh-1 and Ldh-2), Malate dehydrogenase (E.C. 1.1.1.37; Mdh-1 and Mdh-2), Malic enzyme (E.C. 1.1.1.40; Me-1 and Me-2), Isocitrate dehydrogenase (E.C. 1.1.1.42; Idh-1), 6-Phosphogluconate dehydrogenase (E.C. 1.1.1.44; 6Pgdh), Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49; G6pdh), Indophenol oxidase (E.C. 1.15.1.1; Ipo), Nucleoside phosphorylase (E.C. 2.4.2.1; Np), Glutamateoxaloacetate transaminase (E.C. 2.6.2.1; Got-1 and Got-2), Hexokinase (E.C. 2.7.1.1; Hk), Creatine kinase (E.C. 2.7.3.2; Ck), Adenylate kinase (E.C. 2.7.4.3; Adk), Phosphoglucomutase (E.C. 2.7.5.1; Pgm-1 and Pgm-2), Esterases (E.C. 3.1.1.1; Est-1 and Est-3), Acid phosphatase (E.C. 3.1.3.2; Acph), Adenosine deaminase (E.C. 3.5.4.4; Ada), Aldolase (E.C. 4.1.2.13; Aldo), Mannose phosphate isomerase (E.C. 5.3.1.8; Mpi), Glucose phosphate isomerase (E.C. 5.3.1.9, Gpi).

Electrophoretic procedures follow those described in FILIPPUCCI et al. (1988). Isozymes were numbered in order of decreasing mobility from the most anodal. Allozymes were designated numerically according to their mobility relative to the most common allele in the reference population from Mt. Pelister. Allozymic data were analysed as genotype frequencies with the BIOSYS-1 program of Swofford and Selander (1981). The amount of genetic divergence between populations was estimated using the indices of standard genetic identity (I) and distance (D) proposed by Nei (1978). A dendrogram of the genetic relationships between populations was obtained using unweighted pair-group analysis, UPGMA (Sokal and Sneath 1963).

Morphometric analysis: Specimens were preserved as skulls with skins or in alcohol (skulls extracted). Only adult, undamaged skulls (total 53: sample 1: 11; sample 2: 16; sample 3: 24; sample 4: 2) were used for multivariate analysis. Twelve skull measurements were taken from each skull (Fig. 1) using a vernier calliper, accurate to the nearest 0.1 mm. The abbreviations used were: CbL – condylobasal length; RoL – rostrum length; NcL – neurocranial length; DiL – diastema length; MxT – maxillary toothrow length; ZgB – zygomatic breadth; BcB – braincase breadth; IoC – interorbital constriction; BcH – braincase height per bullae; Bc – braincase height without bullae; RoH1 – height of rostrum at the anterior alveoli of the first upper molar; RoH – height of rostrum across the second upper molar.

Variations in metrical characters among samples were analysed by discriminant analyses of raw data using the Statgraphics statistical program (version 5).

## Results and discussion

### Karyotype

The diploid chromosome number of voles from Pelister (sample 2) is  $2n = 52$ . The karyotype comprises one pair of large subtelocentric and one pair of large submetacentric autosomes. The remaining autosomes are acrocentrics of decreasing size, except for the smallest pair which is metacentric. Distinct short arms can be seen in most of the acrocentric chromosomes. The X chromosome is a large metacentric and the Y is a large acrocentric (Fig. 2).

There are at least four pairs of acrocentric chromosomes possessing NORs. These are located in the telomeric region of one pair of medium-sized chromosomes, and in the pericentromeric area of one small pair. In two pairs of autosomes they are displayed on the apparent short arms (Fig. 3a). The G-banded sex chromosomes are shown in figure 3b. Whereas the X chromosome seems to be of a standard type as far as both size and banding

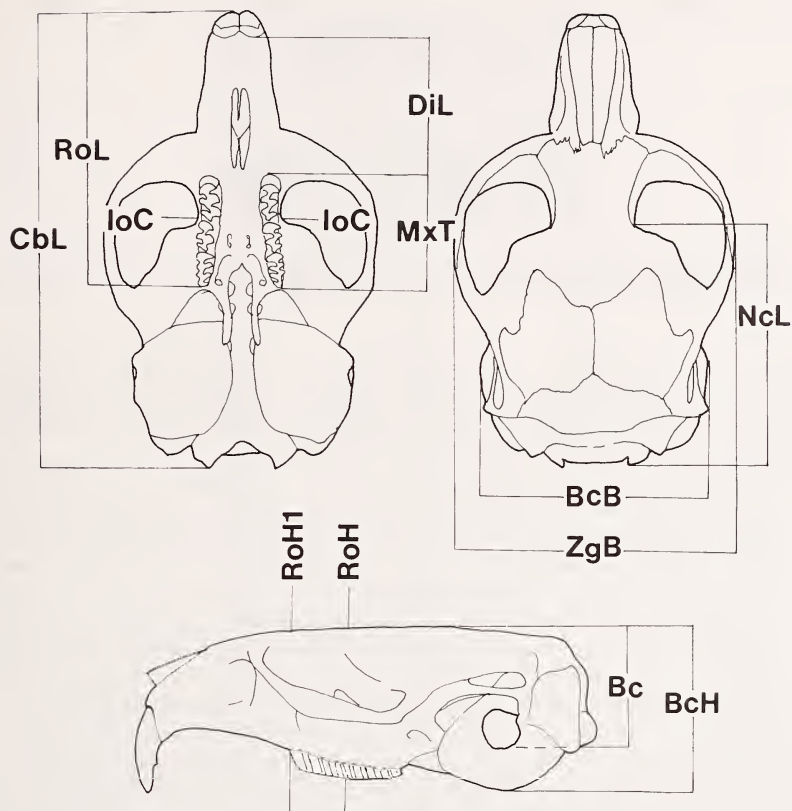


Fig. 1. Cranial measurements of voles used in this study. See text for abbreviations

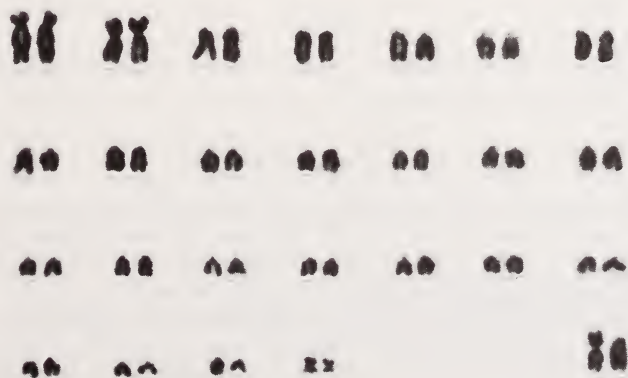


Fig. 2. Conventionally stained karyotype of *Microtus subterraneus* from Mt. Pelister

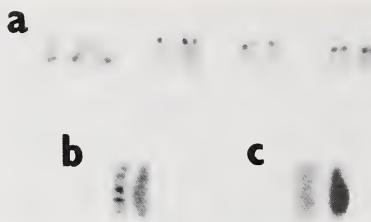


Fig. 3. Chromosomes of *Microtus subterraneus* from Mt. Pelister. a: Four chromosome pairs carrying NORs; b: G-banded; c: C-banded sex chromosomes (the Y on the right)

pattern are concerned, the Y is conspicuously large (nearly as large as the X) and without any obvious G-banding pattern. Only weak C-bands were revealed in some acrocentric autosomes in C-banded metaphases. The X chromosome displays no C-bands. In contrast, the Y chromosome is entirely heterochromatic (Fig. 3c).

The karyotype of the Lovćen specimens appears to be similar to that of the Pelister voles:  $2n = 52$ , with one pair of large subtelocentrics, one pair of large submetacentrics, and one pair of small metacentric chromosomes. Since C-banding was not successful in the only male available from Lovćen, the question of Y chromosome size remains open.

The same diploid number of chromosomes (i.e.  $2n = 52$ ) has already been reported for *Microtus subterraneus* from the former Yugoslavia, including populations from Slovenia (ŽIVKOVIĆ et al. 1975), and from Mt. Pelister (PETROV and ŽIVKOVIĆ 1979). *Microtus majori* from the Caucasus displays a different diploid number,  $2n = 54$  (ZIMA and KRÁL 1984), while the karyotype of *M. majori* from its type locality (in the vicinity of Trabzon, northern coast of Asia Minor) has not been studied yet. A karyotype of  $2n = 52$  chromosomes was also found in *M. subterraneus* from various parts of Europe (see NIETHAMMER 1982; SABLINA et al. 1989; ZIMA and KRÁL 1984, for review). A large Y chromosome was reported from the Austrian Alps (GAMPERL et al. 1982) whereas, in certain other regions of Europe, only the standard, smaller Y has been found (ZIMA 1984; SABLINA et al. 1989). It should be noted, however, that the Y chromosome of the Alpine population is only slightly different from the standard Y chromosome, while the element found in the Pelister population is about twice as large as the standard Y. Thus, this phenomenon cannot be interpreted as being a consequence of a varying degree of chromosome spiralisation in individual preparations, but should be considered to be a specific feature of the study population.

### Electrophoretic analysis

Seventeen of the twenty-seven loci analysed were monomorphic and fixed for the same allele in all the populations studied:  $\alpha$ Gpdh, Sdh, Ldh-1, Ldh-2, Mdh-1, Mdh-2, Me-2, Idh-1, Ipo-1, Np, Got-2, Hk, Ck, Adk, Pgm-2, Aldo, Pgi. The allele frequencies of the polymorphic loci in the populations analysed are given in table 1.

Two loci (Got-1 and Pgm-1) partially discriminated the Mt. Pelister population from *M. subterraneus* from Mt. Lovćen and Slovenia.

From the allele frequencies at the 27 loci tested, Nei's values of genetic identity and distance were calculated amongst populations using all pairwise comparisons (Tab. 2). An UPGMA dendrogram summarizing the genetic relationships between the samples is given in figure 4.

The lowest genetic distance value found was between populations from Slovenia and Mt. Lovćen ( $D = 0.011$ ). The population from Mt. Pelister displayed higher values of



Table 1. Allelic frequencies observed at the polymorphic loci analysed in Balkan populations of *M. subterraneus*.

See text for explanation

Locus	Allele	Slovenia	Lovćen	Pelister
Me-1	100	0.83	1.00	1.00
	104	0.17	—	—
6Pgdh	100	0.80	1.00	0.92
	104	0.20	—	0.08
G6pdh	95	—	—	0.08
	100	1.00	1.00	0.92
Got-1	100	0.08	—	1.00
	105	0.92	1.00	—
Pgm-1	100	0.08	—	0.75
	105	0.92	1.00	0.25
Ada	95	—	—	0.33
	100	0.75	0.75	0.67
	105	0.25	0.25	—
Mpi	95	—	—	0.17
	100	0.92	1.00	0.83
	105	0.08	—	—
Acph	100	0.42	1.00	1.00
	105	0.58	—	—
Est-1	95	0.08	—	0.08
	100	0.92	1.00	0.92
Est-3	95	—	—	0.08
	100	0.83	1.00	0.92
	104	0.17	—	—

genetic distance with those from Slovenia ( $D = 0.067$ ) and Montenegro ( $D = 0.062$ ). These values correspond to those generally observed among subspecies of Arvicolidae ( $D = 0.064$ ; GRAF 1982) and more generally in other rodents (FILIPPUCCI et al. 1991).

### Phenetics

*Microtus majori* possesses three pairs of teats, two inguinal and one pectoral, while the pectoral teats are absent in *M. subterraneus* (NIETHAMMER 1972). All of our seven lactating females from Mt. Pelister had only the two inguinal pairs. In contrast, one standard museum skin from Mt. Pelister (SMF 23,585) clearly shows an additional pectoral pair of teats; this is the only female in the SMF collection which was obviously lactating. The possibility that the number of teats may be polymorphic in the marginal population of *M. subterraneus* is further supported by a personal communication from B. PETROV. Amongst four females that he collected on Mt. Orjen, Montenegro, from which only the *M. subterraneus* karyotype has been reported (ŽIVKOVIĆ et al. 1975), the two lactating females had an additional pair of pectoral teats. A polymorphism in this character has also been reported in *Microtus savii* (de Selys Longchamps, 1838), which has either 2 or 3 pairs of teats (KRAPP 1982).

Table 2. Values of genetic identity (Nei's I, above the diagonal) and distance (Nei's D, below the diagonal), between Balkan samples of *M. subterraneus*, based on 27 loci

	Slovenia	Lovćen	Pelister
Slovenia	—	0.989	0.935
Lovćen	0.011	—	0.940
Pelister	0.067	0.062	—

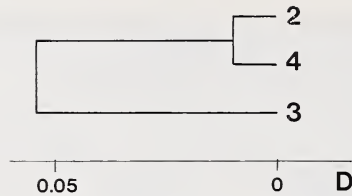


Fig. 4. UPGMA dendrogram summarizing genetic relationships between three populations of *Microtus subterraneus*. D = Nei's (1978) unbiased genetic distance, based on 27 enzyme loci. The cophenetic correlation coefficient is 0.997

The tail appears to be relatively longer in *M. majori* from Asia Minor than in *M. subterraneus* from Slovenia. In the voles from Mt. Pelister the tail is as short as in Slovenian *M. subterraneus* (Fig. 5). However, since external measurements were taken by different collectors, they are likely to have been affected by differences in measuring techniques.

The first two functions resulting from the discriminant analysis of 12 raw skull measurements of four samples (which were responsible for 91.8 % of the variance) clearly distinguished European *M. subterraneus* from Asian *M. majori* (Fig. 6). According to their skull morphology, the Pelister population should thus be allocated to *M. subterraneus*. All specimens of *M. majori* were classified correctly, whilst there was some overlap between samples 2 to 4 (Tab. 3). A total of 9 specimens (= 17 %) was misclassified.

In the next step, samples 2 to 4 (*M. subterraneus*) were pooled and discriminant analysis was repeated. Specimens of both taxa were allocated into their actual groups. Removing five cranial variables (NcL, Dil, NcL, BcH, Mxt) from the discriminant analysis did not affect the classification results. The discriminant function, based on 7 raw skull measurements, could be useful in distinguishing the two species in museum material (Fig. 7):

$$DF = -0.60888 \times \text{CbL} - 1.19402 \times \text{ZgB} + 3.08761 \times \text{BcB} - 3.00499 \times \text{IoC} \\ + 3.18741 \times \text{Bc} + 2.79813 \times \text{RoH1} - 3.81314 \times \text{RoH} - 25.8104$$

The discriminant function has values lower than 1.1 in *M. subterraneus* and higher than 1.5 in *M. majori*.

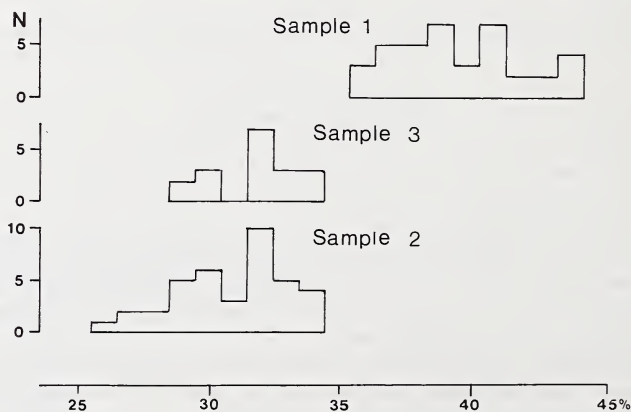


Fig. 5. Frequency histogram of the relative tail length ( $100 \times \text{tail} / \text{head and body length}$ ) in *Microtus subterraneus* (sample 2), *M. majori* (sample 1) and voles from Mt. Pelister (sample 3)

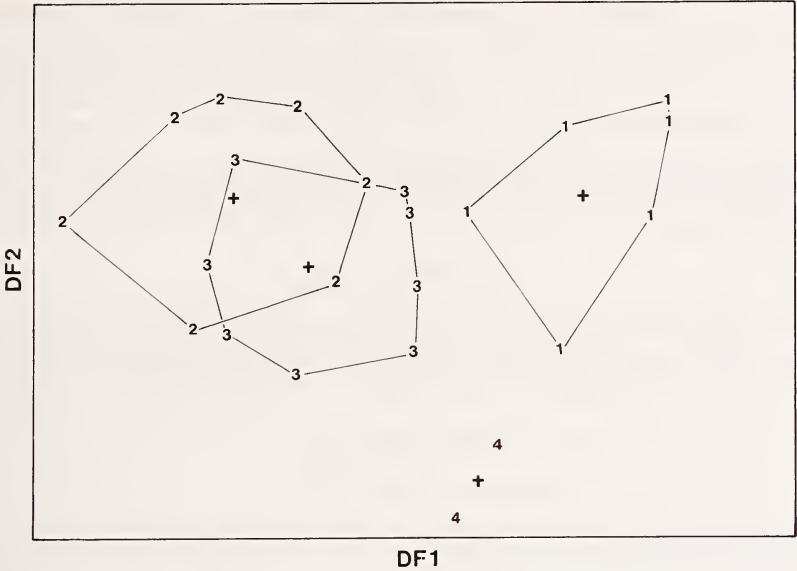


Fig. 6. Projection of four samples of 53 voles on the first two discriminant variates. Polygons enclose scores for all individuals within a locality group, and crosses are placed on group centroids. See text for identifying numbers

Table 3. Classification table for analysis based on four groups of voles  
Rows are actual and columns are predicted groups (in %)

Actual group	Predicted group				Sample size
	1	2	3	4	
1 <i>M. majori</i>	100.0	0.0	0.0	0.0	11
2 Slovenia	0.0	75.0	25.0	0.0	16
3 Pelister	0.0	16.6	79.2	4.2	24
4 Lovćen	0.0	0.0	0.0	100.0	2

Taxonomic conclusions

The present evidence does not suggest the inclusion of the Pelister voles into Asia Minor's *M. majori*. For the time being, it seems much more appropriate to include them in *M. subterraneus*. This also means that there is no reason to include *M. majori* in the European fauna. Anyhow electrophoretic, as well as karyological data, indicate that the Pelister

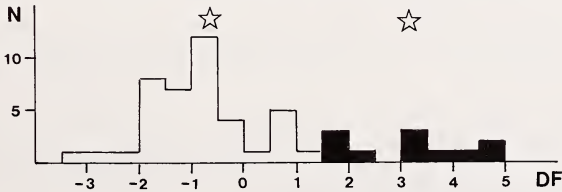


Fig. 7. Distribution of the specimens of *Microtus majori* (black) and *Microtus subterraneus* (white) in the discriminant function axis. Stars represent placement of group centroids

population may be distinct from *M. subterraneus* from the rest of Europe. Its taxonomic relations to other large-sized Balkan voles, usually ascribed to *M. subterraneus* (*hercegovinensis*, *brauneri*), remain open. According to the electrophoretic analysis, very large voles from Mt. Lovćen, which are even bigger than *M. majori*, are genetically closer to small *M. subterraneus* from Slovenia than to the Pelister voles. The fact that *M. s. hercegovinensis* (Martino, 1940) and other populations of similarly large *M. subterraneus* from Bosnia and Herzegovina have recently been placed into *Microtus multiplex* (PETROV 1992) suggests that the taxonomy of large "*M. subterraneus*" in the Balkans continues to remain a source of debate.

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### Zusammenfassung

#### *Kommt Microtus majori in Europa vor?*

Wühlmäuse aus dem Pelister-Gebirge in Makedonien, die in der Literatur als *Microtus majori* geführt werden, wurden morphologischen, karyologischen und elektrophoretischen Untersuchungen unterworfen und mit *M. subterraneus* aus Slowenien und Montenegro sowie *M. majori* aus Kleinasien verglichen. Die diploide Anzahl der Chromosomen ( $2n = 52$ ) der Wühlmäuse vom Pelister stimmt mit *M. subterraneus* vom Balkan überein. Die genetischen Distanzen, ermittelt durch elektrophoretische Analyse von 27 Genloci, zwischen Wühlmäusen vom Pelister und *M. subterraneus* aus Slowenien und Montenegro entsprechen denen, die gewöhnlich zwischen Subspezies von Arvicolidae festgestellt werden. Mittels einer Diskriminanzanalyse von 12 Schädelmaßen ließ sich *M. majori* und *M. subterraneus* erfolgreich trennen. Danach muß die fragliche Population aus dem Pelister-Gebirge letztgenannter Art zugeordnet werden, was bedeutet, daß *M. majori* aus der Liste der europäischen Fauna zu streichen ist.

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