

Evolutionary divergence of *Microtus liechtensteini* (Rodentia, Arvicolidae) based on the first lower molar

Patrick BRUNET-LECOMTE and Boris KRYŠTUFEK

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Morphometrical analysis of the first lower molar (M_1) confirmed *Microtus liechtensteini* (Wettstein, 1927) to be closer to *M. multiplex* than to *M. subterraneus*. In the terms of the inclination of the pitomyan rhombus, *M. liechtensteini* appeared to be more evolved than *M. multiplex*. Significant interpopulation variability was recorded as regards morphometrical analysis of M_1 in *M. liechtensteini*: *M. l. petrovi* was larger than the nominate race *M. l. liechtensteini*, while populations from the sub-Mediterranean zone differed by more open anterior loop.

Laboratoire de Paléontologie analytique et Géologie sédimentaire (URA CNRS 157) et Laboratoire de Préhistoire et Paléoécologie du Quaternaire de l'EPHE, Centre des Sciences de la Terre, 6 Bd Gabriel, 21000 Dijon, France (PB-L); Slovene Museum of Natural History, Prešernova 20, P.O. Box 290, 61001 Ljubljana, Slovenia (BK)

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Introduction

Microtus liechtensteini (Wettstein, 1927) was described on the base of a single specimen collected from the peak Mali Rajinac, 1699 m a.s.l. (Velebit Mts., north-western part of the Dinaric Alps). Ellerman and Morrison-Scott (1951) considered this vole as a subspecies of *Microtus subterraneus*, while Djulić and Mirić (1967) treated it as a form of *Microtus multiplex*. Petrov and Živković (1971) found voles from the type locality of *M. liechtensteini* to have diploid number $2n = 46$ and the fundamental number of arms $NF = 52$. They were thus different from *M. multiplex* with the chromosomal set $2n = 48$ and $NF = 54$ (Meylan 1970). Consequently, *M. liechtensteini* was regarded as an independent species. Its distribution area extends from southeastern Alps in Austria and Slovenia to the northwestern Dinaric Alps. Presumably isolated populations were reported from the southern margin of Pannonian plain in Croatia, from Bosnia, and from Mt Tara in western Serbia (Král *et al.* 1978, Petrov and Živković 1979, Tvrtković *et al.* 1979, Kryštufek 1984, Purger and Karanović 1991). Ground voles with the *liechtensteini* karyotype were reported also from the eastern part of the Italian Alps (Storch and Winking 1977).

Near Calliano (North Italy) two hybrids with intermediate karyotype were found on the contact zone of *M. multiplex* and *M. liechtensteini* (Storch and Winking 1977). This gave reason to regard *multiplex* and *liechtensteini* as parapatric karyological forms of one species (Niethammer and Krapp 1982). Graf and Meylan (1980) who produced interfertile hybrids in captivity assumed that only one polytypic species occurs with distribution area extending from France to the Balkans. Such an opinion of the taxonomic position of *M. liechtensteini* is still not generally accepted. While it is by some authorities as a chromosomal race of *M. multiplex* (Graf and Meylan 1980, Niethammer and Krapp 1982, Petrov 1992), others concern it as an independent species (Corbet 1978, Nowak 1991). According to available zoogeographical and karyological evidence it is clear that *M. liechtensteini* and *M. multiplex* are two parapatric biospecies of a superspecies. Therefore we cannot agree with Petrov (1992) who synonymized *M. liechtensteini* and *M. multiplex* on the base of the "broad species concept".

Intraspecific variation of *M. liechtensteini* was demonstrated among populations from Slovenia and Croatia. Živković *et al.* (1975) described geographic variation in the position of centromere on both heterochromosomes. Populations from the sub-Mediterranean part of this area are larger than the continental ones and were described as a new subspecies *M. liechtensteini petrovi* (Kryštufek 1983). The subspecies populates dry and warm sub-Mediterranean regions with scarce forests, while the nominate race lives mainly in continental beech and spruce forests, frequently in higher altitudes. *M. l. petrovi* is very little known form, and only few specimen were collected by snap traps. The majority of material was obtained from owl pellets, where its dominance may attain values up to 15% (Lipej 1988). The karyotype of *M. l. petrovi* has not been studied as yet.

Enamel pattern of the first lower molar (M₁) in *M. liechtensteini* is predominantly of the medio-European *subterraneus-multiplex* type characterized by a long anterior part and a closed anterior loop. In some populations the Mediterranean type of M₁, with an open anterior loop and a short anterior part, has been also observed. However, such a rough approach towards the study of M₁ enamel pattern cannot tell whether *M. liechtensteini* is closer to *M. multiplex* than to *M. subterraneus* in terms of M₁ morphology, nor to analyse divergences among different populations. Morphometrical analysis of *M. liechtensteini* populations from different biogeographical regions, and comparison with M₁ patterns of *M. multiplex* and *M. subterraneus*, was undertaken to answer these two questions.

Material and method

We examined 163 teeth of *M. liechtensteini* from 28 samples from Slovenia and Croatia (1 – Planica Valley, 2 teeth; 2 – Kobarid, 3 teeth; 3 – Mt Pokljuka, 1 tooth; 4 – Mt Jelovica, 4 teeth; 5 – Jezersko, 1 tooth; 6 – Kamniška Bistrica, 1 tooth; 7 – Vojsko, 2 teeth; 8 – Ajdovščina, 2 teeth; 9 – Nova Gorica, Vogrsko, 4 teeth; 10 – Ljubljana, Ig, 3 teeth; 11 – Mt Travnica gora, 4 teeth; 12 – Sevnica, 1 tooth; 13 – Mokro polje, 1 tooth; 14 – Mt Snežnik, 8 teeth; 15 – Klivnik, 2 teeth; 16 – Mt Risnjak, 12 teeth;

17 – Kubed, 20 teeth; 18 – Sočerga, 1 tooth; 19 – Stena, 2 teeth; 20 – Mirna 1, 30 teeth; 21 – Mirna 2, 30 teeth; 22 – Mirna 3, 11 teeth; 23 – Mt Učka, 11 teeth; 24 – Štirovača, Alan, 1 tooth; 25 – Zavižan, 2 teeth; 26 – Baške Oštarije, 1 tooth; 27 – Plitvice, 2 teeth; 28 – Mt Makljen, 1 tooth), and compared them with *M. multiplex* (166 teeth from: Col du Lautaret, Hautes-Alpes, France, 30 teeth; Col du Montgenèvre, Hautes-Alpes, France, 17 Teeth; Les Vigneaux, Hautes-Alpes, France, 10 teeth; La-Chapelle-en-Vercors, Drôme, France, 12 teeth; Saint-Martin-de-la-Cluze, Isère, France, 10 teeth; Zermatt, Wallis, Switzerland, 12 teeth; Gudo, Ticino, Switzerland, 12 teeth; Meride, Ticino, Switzerland, 10 teeth; Varenzo, Ticino, Switzerland, 11 teeth; Mazzolla, Toscana, Italy, 30 teeth; Fivizzano, Toscana, Italy, 12 teeth) and *M. subterraneus* (226 teeth from: Fougères, Ile-et-Vilaine, France, 30 teeth; Quetteville, Calvados, France, 29 teeth; Hostiaz, Ain, France, 30 teeth; La Roquille, Gironde, France, 24 teeth; Localities from Tarn, France, 11 teeth; Bussy, Loire, France, 30 teeth; Champéry, Valais, Switzerland, 22 teeth; Chavradura, Grisons, Switzerland, 10 teeth; Montricher, Vaud, Switzerland, 10 teeth; Niepołomice, Poland, 30 teeth).

The specimens of *M. liechtensteini* were assigned to 10 geographical samples (sample A = samples 17 and 18; sample B = samples 14, 15, and 16; sample C = samples 1, 2, 3, and 4; sample D = sample 23; sample E = samples 7, 8, and 9; sample F = samples 24, 25, 26, and 27; sample G = samples 10 and 11; sample H = sample 20; sample I = sample 21; sample J = sample 22) for the analysis of morphometric variations within and between species (Fig. 1). Sample areas were kept as small as possible and selected so as to represent an integral geographic area. Samples A, H, I, and J were from the sub-Mediterranean zone as defined by Jovanović *et al.* (1986), and samples B to G from the continental zone. According to Šugar (1984), samples A and I were from the boundary between the two zones, but still in the sub-Mediterranean region. The samples 5, 6, 12, 13, 19, and 28 were removed from the analysis because of the low number.

Affinities among the three taxa and among geographic samples of *M. liechtensteini* were assessed by the canonical discriminant analysis of 21 parameters measured on the M_1 (Fig. 2): measures 1 to 6, measures 7 to 13 and measures 15 to 22. The measures 14 and 23 were removed from the analysis because of the difficulty to take the measures in the worn teeth. Variations of total length of M_1 (TL = measure 6), anterior part of M_1 [AP = (measure 6 – measure 3) / measure 6], pitymyan rhombus (PR = measure 4 – measure 3) and anterior loop (AL = measure 20 – measure 18) among the three species and among *M. liechtensteini* geographic groups as defined by previous discriminant analysis were analysed by one-way analysis of variance completed by the Scheffé contrast method.

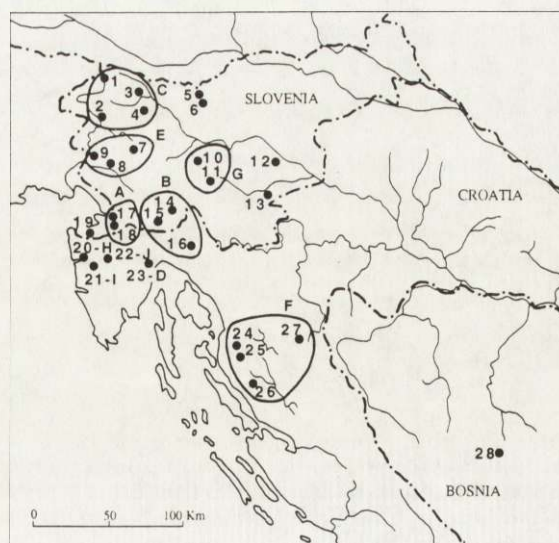


Fig. 1. Geographic distribution of samples of *Microtus liechtensteini* analysed in this study.

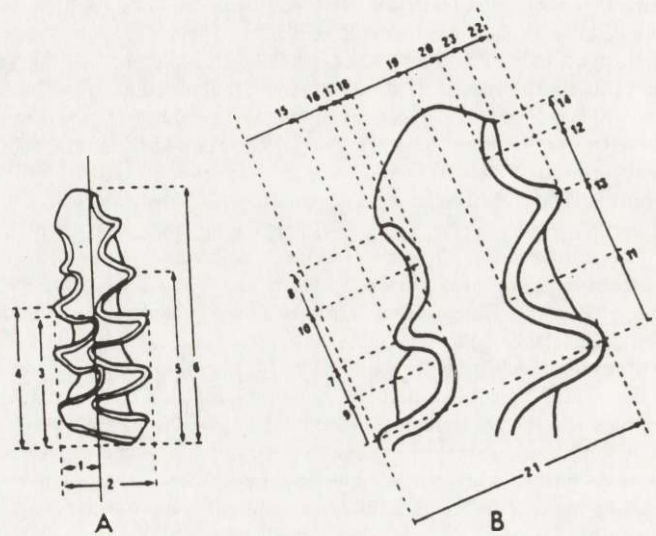


Fig. 2. Morphometry of the first lower molar. A – reference axis of the tooth and general measures. B – measures of the anterior complex.

Results

At first step the three species were subjected to the canonical discriminant analysis. The first two canonical axes explained 40% ($p < 0.001$) and 14% ($p < 0.001$) of the variance between populations. The first canonical axis was significantly correlated ($p < 0.05$) with measure 2 (0.84), measure 21 (0.82), measure 22 (0.79), measure 5 (0.75), and measure 3 (0.75). The second canonical axis had

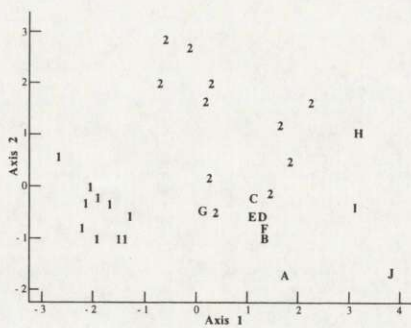


Fig. 3. Projection of centroids of the samples of *Microtus liechtensteini* (labels A to J), *M. subterraneus* (labels 1) and *M. multiplex* (labels 2) in the plan 1 – 2 of interspecific canonical discriminant analysis.

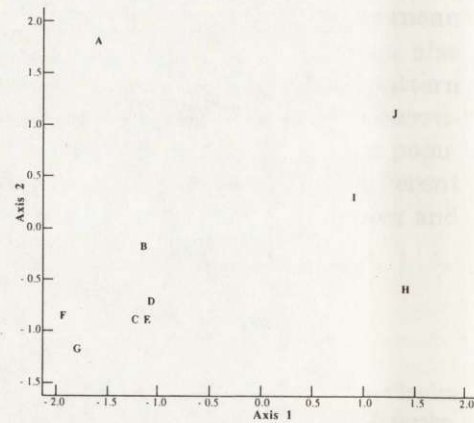


Fig. 4. Projection of centroids of the samples of *Microtus liechtensteini* (labels A to J) in the plan 1 – 2 of intraspecific canonical discriminant analysis.

significant negative correlations ($p < 0.05$) with measure 18 (-0.48), measure 17 (-0.35), and measure 22 (-0.28). Populations belonging to *M. subterraneus* were clearly discriminated from other *M. multiplex* and *M. liechtensteini* populations on the first axis (Fig. 3), showing thereby that *M. liechtensteini* is morphologically closer to *M. multiplex* than to *M. subterraneus*. As the first axis discriminated samples according to the size, *M. subterraneus* appeared to be the smallest of the species. The combination of the first two axes provided reasonably good discrimination between *M. liechtensteini* and *M. multiplex* samples. Second canonical axis was explained as shape vector. It is evident that size alone was not sufficient to discriminate *M. liechtensteini* from *M. multiplex* since they were of the same general size. Samples A, H, I, and J, all belonging to *M. liechtensteini*, were obviously quite apart from the remaining *M. liechtensteini* samples.

Morphometric characteristics of the M_1 in three species are given in Table 1. The total length of the M_1 is significantly different between the three species, *M. liechtensteini* having the greatest total length of the M_1 and *M. subterraneus* the smallest. The anterior part is significantly shorter in *M. multiplex* than in *M. subterraneus*, *M. liechtensteini* having an anterior part between that of *M. multiplex* and *M. subterraneus*. The pitmyan rhombus is significantly less inclined in *M. multiplex* than in *M. subterraneus* and *M. liechtensteini*. The

Table 1. Description, analysis of variance and Scheffé's test of the total length (TL), the anterior part (AP), the inclination of the pitmyan rhombus (PR) and the close of the anterior loop (AL) of the first lower molar in *Microtus subterraneus* (*M. s.*), *M. multiplex* (*M. m.*) and *M. liechtensteini* (*M. l.*). Units for TL, PR and AL are 10^{-2} mm, AP is without unity. SD – standard deviation. * Means with the same letter are not significantly different.

Variable	Species	<i>n</i>	Mean	SD	Scheffé's test ($p < 0.05$)*
TL	<i>M. s.</i>	226	249.0	9.9	A
	<i>M. m.</i>	166	259.9	21.3	B
	<i>M. l.</i>	156	270.1	14.8	C
Analysis of variance: $p < 0.001$					
AP	<i>M. s.</i>	226	0.524	0.013	A
	<i>M. m.</i>	166	0.518	0.017	B
	<i>M. l.</i>	156	0.521	0.017	AB
Analysis of variance: $p = 0.002$					
PR	<i>M. s.</i>	226	-0.7	3.5	A
	<i>M. m.</i>	166	3.5	4.6	B
	<i>M. l.</i>	156	-0.5	3.8	A
Analysis of variance: $p < 0.001$					
AL	<i>M. s.</i>	226	15.5	6.1	A
	<i>M. m.</i>	166	22.9	8.6	B
	<i>M. l.</i>	156	24.1	9.7	B
Analysis of variance: $p < 0.001$					

Table 2. Description, analysis of variance and Scheffé's test of the total length (TL), the anterior part (AP), the inclination of the pitomyan rhombus (PR) and the close of the anterior loop (AL) of the first lower molar in the three groups of *Microtus liechtensteini*. Units for TL, PR and AL are 10^{-2} mm, AP is without unity. SD – standard deviation. * Means with the same letter are not significantly different.

Variable	Group	n	Mean	SD	Scheffé's test ($p < 0.05$)*
TL	1	64	265.2	13.8	A
	2	21	275.3	13.3	B
	3	71	273.0	15.1	A B
Analysis of variance: $p = 0.002$					
AP	1	64	0.526	0.017	A
	2	21	0.530	0.014	A
	3	71	0.514	0.015	B
Analysis of variance: $p < 0.001$					
PR	1	64	0.6	3.7	A
	2	21	-1.2	3.1	A
	3	71	-1.3	3.9	A
Analysis of variance: $p = 0.012$					
AL	1	64	23.3	9.7	A
	2	21	13.8	6.8	B
	3	71	27.9	7.9	C
Analysis of variance: $p < 0.001$					

anterior loop is evidently more closed in *M. subterraneus* than in *M. multiplex* and *M. liechtensteini*.

Projection of centroids of ten *M. liechtensteini* samples on to the first two canonical axes (51% and 19% respectively of variance between classes) revealed the existence of three sample groups (Fig. 4): group 1: samples B to G, group 2: sample A, group 3: samples H, I, and J. Group 3 was discriminated from groups 1 and 2 by the first canonical axis, while axis 2 separated groups 1 and 2. The first canonical axis was significantly positively correlated ($p < 0.05$) with variables measure 16 (0.74), measure 5 (0.61), measure 20 (0.58) and measure 22 (0.57). The second axis had significant positive correlations ($p < 0.05$) with variables measure 18 (0.71), measure 1 (0.50), measure 8 (0.48) and measure 17 (0.47).

Morphometric characteristics are given in Table 2. The total length of the M_1 is significantly greater in group 2 than in group 1, while group 3 was intermediate between groups 1 and 2. The anterior part is significantly shorter in group 3 than in the other two groups. None of the three groups differs significantly from the others with regard to the tilt of the inclination of the pitomyan rhombus. However, combined groups 2 and 3 are significantly different ($p < 0.05$) from group 1 with a less inclined pitomyan rhombus. The anterior loop is significantly different

between the 3 groups, group 2 having the most closed anterior loop and group 3 the least closed.

Discussion and conclusion

General morphological analysis of 31 samples of *M. subterraneus*, *M. liechtensteini* and *M. multiplex* confirmed the close kinship between *M. liechtensteini* and *M. multiplex* compared with *M. subterraneus*. This is in accordance with taxonomic arrangement of these species (Niethammer and Krapp 1982). *M. liechtensteini* is characterized by a long M₁. Its pitomyan rhombus, which is more tilted than in *M. multiplex* suggesting to be more evolved than *M. multiplex* (Brunet-Lecomte 1988, 1990).

Among *M. liechtensteini*, samples B to G were morphologically closer to *M. multiplex*, than samples A, H, I, and J. The independent position of samples A, H, I, and J was confirmed also by the discriminant analysis of ten *M. liechtensteini* samples. The distribution pattern of the three groups recognized (Fig. 4) corresponds well with main plant communities. Group 1 with the medio-European morphotype of M₁ is limited to the continental zone. Group 2, which is also characterized by a medio-European morphotype, populates the sub-Mediterranean zone but close to the continental zone. Group 3, which is of medio-European type with marked Mediterranean morphotype trends, originate from the sub-Mediterranean zone.

Group 1 includes also topotypes of *M. l. liechtensteini*, so it should be ascribed to the nominate subspecies. Group 2, which originates from the type locality of *M. l. petrovi*, is ascribed to that subspecies. Taxonomic position of group 3 is left open, also because of lack of other (e.g. karyological) data.

The following hypothetical evolution of *M. liechtensteini* and the three above recognized groups is suggested: *M. liechtensteini* derived directly from *M. multiplex* or from a common ancestor with *M. multiplex*, by cladogenesis during the upper Pleistocene in a region that favoured the isolation of populations (in glacial refuge). Next, group 2 (*petrovi*) evolved from group 1 (*liechtensteini*) in a region on the boundary of continental and the sub-Mediterranean zones. Either group 1 or 2 evolved into group 3 in the sub-Mediterranean zone. But it should be stressed that there are great differences in shape between groups 2 and 3 although they are both located in the same biogeographic region and are close in terms of distance. Therefore we cannot exclude the possibility that the group 3 does not belong to *M. liechtensteini*, and had evolved from a different lineage than *M. liechtensteini* / *M. multiplex*. If we accept that chromosome divergence precedes morphological divergence, a considerable chromosome differences should be also expected between groups 2 and 3.

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