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Variation in the baculum of the European souslik, *Spermophilus citellus*

By B. KRYŠTUFEK and V. HRABĚ

Slovene Museum of Natural History, Ljubljana, Slovenia and Institute of Landscape Ecology, Brno, Czech Republic

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Abstract

We analysed 35 bacula from six geographical subsamples of the European souslik, *Spermophilus citellus* (Linnaeus, 1766) from the Pannonian Plain and the Balkan Peninsula. Highly significant between-sample differences were found in all four scored parameters: baculum length, basal breadth, breadth of the spoon-shaped distal spatula, and the number of tooth-like projections (denticles) along the spatula's border. The greatest interpopulation differences found were in the spatula breadth and in the number of denticles. Interpopulation differentiation shown by the bacular characters follows the pattern of variation previously observed in non-metric cranial traits, and is not affected by simple geographic distance. In *S. citellus* the baculum contains more phyletic information than do conventional taxonomic characters such as the size and proportions of the skull.

Introduction

The baculum or os penis is a heterotopic bone in most mammals. Its morphology varyies considerably between species but it generally remains constant within species. Its function is to support the penis during copulation and it may also play a role in "internal courtship" (sensu EBERHARD 1985). Since the work of THOMAS (1915) it has been known that the analysis of bacular characters is often superior to the use of cranial traits for phylogenetic reconstructions in groups such as squirrels (Sciuridae). Accordingly, bacular morphology has often been used to elucidate taxonomic problems in Nearctic sciurids (e.g. WHITE 1953; BURT 1960), although only rarely applied to the study of Palaearctic squirrels (e.g. RESHETNIK and BALAKHNIN 1967).

The European souslik, *Spermophilus citellus* (Linnaeus, 1766), is the most western Eurasian ground squirrel, and is characterised by a high degree of interpopulation variation. Untill now, no less than 9 subspecies have been described, usually on the basis of conventional taxonomic characters such as colour, size and skull proportions (RUŽIĆ 1978; KRYŠTUFEK 1995). Although these characters do not allow the recognition of clearly defined races (KRYŠTUFEK 1995), variation in nonmetric cranial traits demonstrates significant interpopulation differences, at least in one segment of the species' range (KRYŠTUFEK 1990). This is particularly evident in the Balkans, where differences between samples have been ascribed to allopatric divergence, whilst the relative homogeneity seen in Pannonian sousliks presumably reflects their comparatively recent colonisation of the Pannonian Plain (see KRYŠTUFEK 1990, 1995).

The baculum of the European souslik was first described and drawn by DIDIER (1952), although he did not indicate the origin of the single specimen available to him. RESCHET-

NIK and BALAKHNIN (1967) drew the baculum of *S. citellus*, and compared it with the same bone in *S. suslicus* and *S. pygmaeus*. MECZYNSKI (1971) studied the male genitalia including the bacula of five European sousliks from Opole voivodship in Poland, and RUŽIĆ (1978) drew a specimen from southern Banat, Serbia. KAYA and ŞIMŞEK (1986) studied bacular variability in a representative sample of *S. citellus thracius* and compared it with *S. xanthoprymnus*; unfortunately their figures are too small to show finer details of bacular structure.

Despite several attempts to describe the baculum of the European souslik, the nature and range of variation of this structure remains obscure. The aim of the present study is to describe baculum interpopulation variability in six geographic samples of the European souslik, and to evaluate the phyletic weight of bacular morphology in the study of evolutionary divergence within this species.

Material and methods

We examined 35 bacula, originating from six geographically distinct regions (Fig. 1). Samples, their designation numbers, and sample sizes (in brackets) are as follows: Sample 1 – Czech Republic: Třebíč, Studenec (n = 2). Sample 2 – Serbia, Srem: Indjija; Fruška gora Mt. (n = 9). Sample 3 – Serbia, Deliblatska

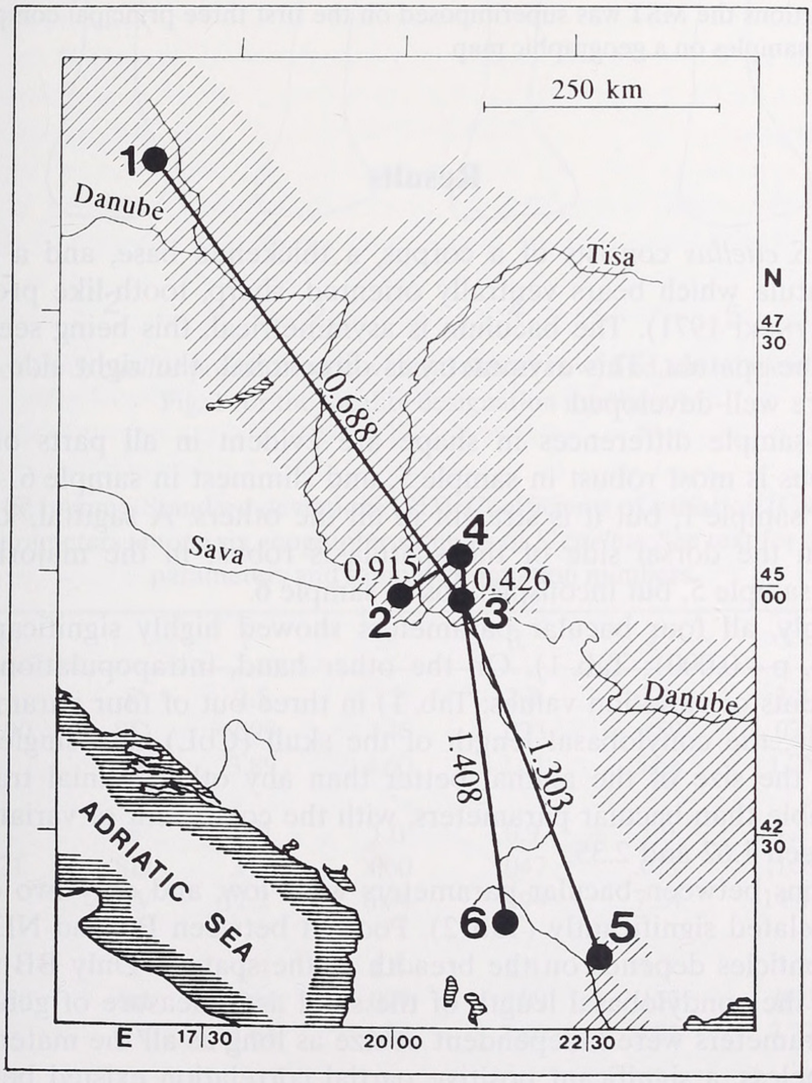

Fig. 1. Site localities for the six samples of *S. citellus*, connected by the Minimum Spanning Tree with the length of the edges. See text for designation numbers of samples. Approximate distribution (shaded) is modified from RUŽIĆ (1978).

peščara (south): Banatska Palanka; Samoš (n = 5). Sample 4 – Serbia, Deliblatska peščara (north): Orlovat (n = 4). Sample 5 – Macedonia: lowlands along the Vardar River: Lake Dojran; Gevgelija (n = 6). Sample 6 – Macedonia: Jakupica Mt.: Gorno Begovo; Solunsko pole (n = 9). Sample 3 is topotypical with *S. c. laskarevi*, sample 5 with *S. c. gradojevici*, and sample 6 with *S. c. karamani*. Only adult males (those having attained at least their second calendar year) were selected for analysis. Sousliks were aged by tooth wear and date of collection (Ružić 1966). One year old specimens strongly predominated in our material with 77%. The rest (i. e. 17%) were two years old animals, while older sousliks (2 in all) were exceptional. Consequently we presume that age did not influence our results.

Glans penis and adjoining part of the corpus were removed from freshly killed animals and preserved in 70% ethanol until processed. We adopted the method described by Anderson (1960), i. e. maceration of the glans penis for 2 to 4 days in 2–3% potassium hydroxide with a few drops of a saturated alcoholic solution of Alizarin Red S. The stained baculum was placed in glycerine, with a small quantity of thymol added as a disinfectant. The material is stored, together with standard museum specimens, in the Natural History Museum of Slovenia at Ljubljana.

Four linear parameters were scored for each baculum using a dissecting microscope fitted with an eyepiece graticule: LB – length of baculum, BB – basal breadth, BS - breadth of spatula, ND – number of denticles.

Differences between and within groups were analysed by One way Analysis of Variance (ANOVA), Principle Components Analysis (PCA), and Clustering (UPGMA) performed on the Average Taxonomic Distance (ATD) matrix obtained from z-standardized data. Procedures were performed using the NTSYS-pc routine (Rohlf 1989) and the Statgraphics (version 5) statistical programme. A Minimum Spanning Tree (MST) based on sample means was calculated from the ATD matrix. In an attempt to detect local distortions the MST was superimposed on the first three principal components axes and on the projection of samples on a geographic map.

Results

The baculum of *S. citellus* consists of a corpus, a thickened base, and a spoon-like expanded distal spatula which bears ventrally oriented, sharp, tooth-like projections along its margin (Meczynski 1971). The baculum is asymmetrical, this being seen most clearly in the shape of the spatula. This asymmetry is directional, the right side of the spatula usually being more well-developed.

Marked inter-sample differences in shape are evident in all parts of the baculum (Fig. 2). The corpus is most robust in sample 5, and slimmest in sample 6. The basal border is concave in sample 1, but it is straight in all the others. A sagittal, knob-like, anterior projection on the dorsal side of the spatula is robust in the majority of samples, particularly so in sample 5, but inconspicuous in sample 6.

Not surprisingly, all four bacular parameters showed highly significant interlocality variation ($F > 8.0$, $p < 0.0001$; Tab. 1). On the other hand, intrapopulation variation was high (see coefficients of variation values; Tab. 1) in three out of four parameters (BB, BS, ND). For example, the conylobasal length of the skull (CbL) as a single measurement which represents the size of the animal better than any other cranial trait (Kryštufek 1995) is less variable than bacular parameters, with the coefficient of variation in samples 2 to 6 being between 1.62 and 2.35.

Intercorrelations between bacular parameters were low, and only two of six pairwise comparisons correlated significantly (Tab. 2). Poor fit between BS and ND suggests that the number of denticles depends on the breadth of the spatula. Only BB was correlated significantly with the condylobasal length of the skull as a measure of general body size. Other bacular parameters were independent of size as long as all the material was pooled together. Nevertheless, a significant positive partial correlation existed between LB and CbL when sample 5 was removed from the analysis ($r = 0.66$, $p < 0.0005$). Thus, the length of the baculum cannot be predicted from the general size of the animal. The largest European sousliks (sample 5) have relatively shortest bacula, measuring between 5.9 and

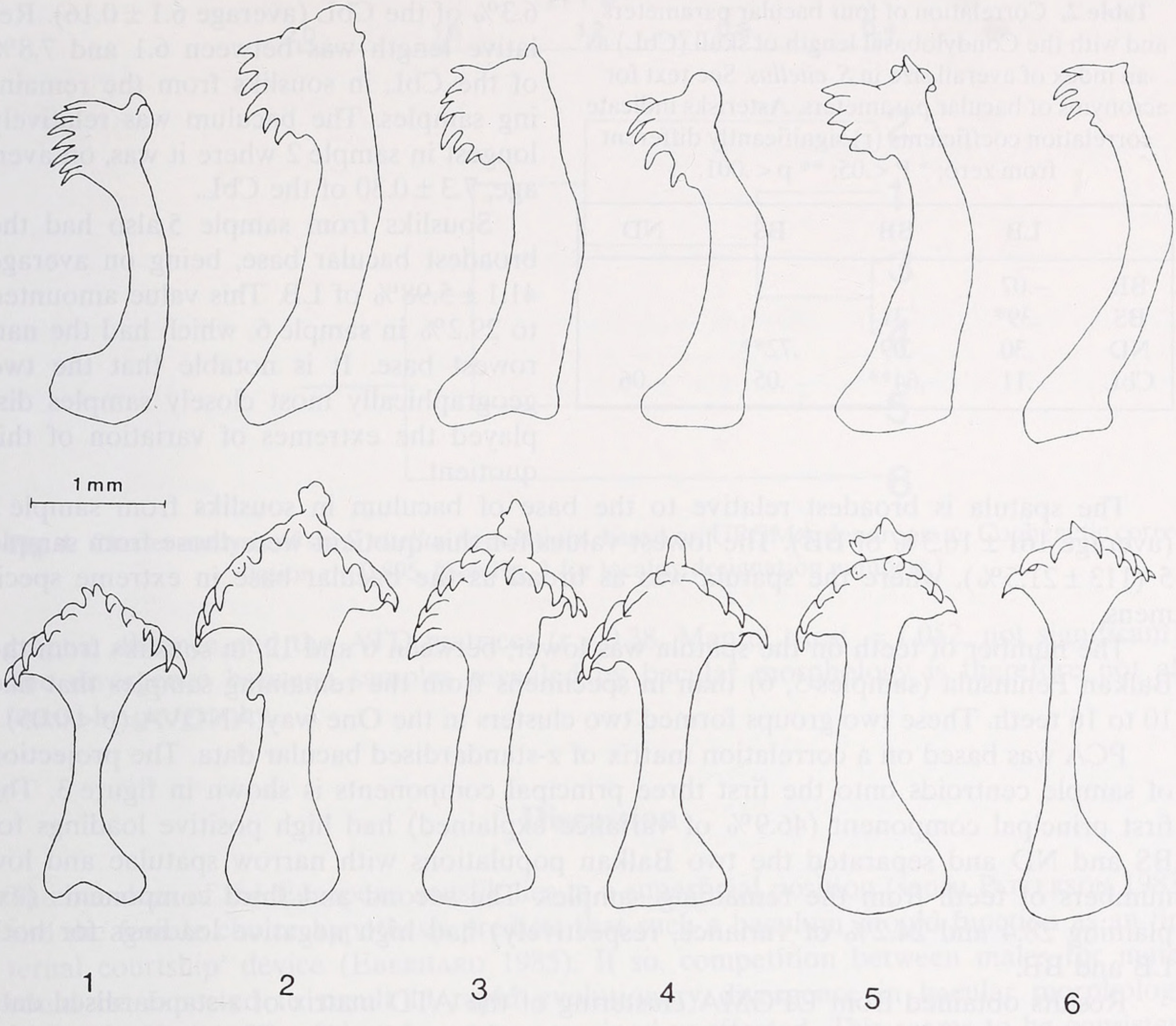

Fig. 2. Bacula of six *S. citellus* populations in ventral (lower row) and lateral view (upper row). (See Fig. 1 for the locality designation numbers.)

Table 1. Means (x̄; in mm), Standard deviations (SD), Coefficients of variation (CV) and F-test values of four bacular parameters among six geographic samples of *S. citellus*. See text for acronyms of bacular parameters and sample designation numbers.

Sample		1	2	3	4	5	6
LB	x̄	2.5	3.2	2.8	2.8	2.8	2.9
F = 16.90	SD	.099	.146	.073	.159	.035	.118
	CV	3.89	4.60	2.61	5.66	1.27	4.02
BB	x̄	0.9	1.0	0.9	1.0	1.2	0.9
F = 8.21	SD	.106	.060	.047	.071	.168	.063
	CV	11.2	6.04	4.94	7.24	14.4	7.30
BS	x̄	1.4	1.6	1.4	1.5	1.3	1.1
F = 27.30	Sd	.064	.077	.109	.152	.092	.060
	CV	4.64	4.84	7.80	9.92	7.22	5.32
ND	x̄	13.0	12.7	12.4	12.8	9.8	8.7
F = 8.21	SD	1.41	0.87	2.61	2.22	1.60	1.41
	CV	10.9	6.85	21.0	17.4	16.3	16.3

Table 2. Correlation of four bacular parameters and with the Condylobasal length of skull (CbL) as an index of averall size in *S. citellus*. See text for acronyms of bacular parameters. Asterisks indicate correlation coefficients (r) significantly different from zero; * P <.05; ** p < .001.

	LB	BB	BS	ND
BB	–.07			
BS	.39*	.31		
ND	.30	.09	.72**	
CbL	.11	.64**	.05	–.06

6.3% of the CbL (average 6.1 ± 0.16). Relative length was between 6.1 and 7.8% of the CbL in sousliks from the remaining samples. The baculum was relatively longest in sample 2 where it was, on average, 7.3 ± 0.30 of the CbL.

Sousliks from sample 5 also had the broadest bacular base, being on average 41.1 ± 5.98% of LB. This value amounted to 29.2% in sample 6, which had the narrowest base. It is notable that the two geographically most closely samples displayed the extremes of variation of this quotient.

The spatula is broadest relative to the base of baculum in sousliks from sample 2 (average 161 ± 16.3% of BB). The lowest values for this quotient were those from sample 5 (113 ± 21.5%), where the spatula was as broad as the bacular base in extreme specimens.

The number of teeth on the spatula was lower, between 6 and 12, in sousliks from the Balkan Peninsula (samples 5, 6) than in specimens from the remaining samples that had 10 to 16 teeth. These two groups formed two clusters in the One way ANOVA ($p < 0.05$).

PCA was based on a correlation matrix of z-standardised bacular data. The projection of sample centroids onto the first three principal components is shown in figure 3. The first principal component (46.9% of variance explained) had high positive loadings for BS and ND and separated the two Balkan populations with narrow spatulae and low numbers of teeth from the remaining samples. The second and third components (explaining 28.4 and 24.2% of variance, respectively) had high negative loadings for both LB and BB.

Results obtained from UPGMA clustering of the ATD matrix of z-standardised data showed the same intergroup relations as suggested by ordination (Fig. 4). However, the projection of the MST onto a geographic map of the localities (Fig. 1) suggests that differences exist between geographic distance and phenetic distance, expressed as ATD. Two samples from closely-placed sites in the Balkans (5 and 6) showed no mutual relationships, but both were linked to sample 3. There was no significant correlation between geo-

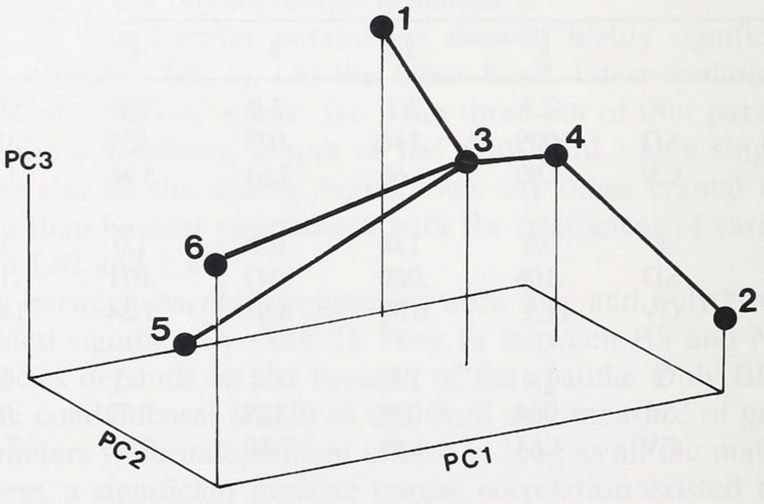

Fig. 3. Projection of group centroids of six *S. citellus* populations onto the first three Principal components axes. Centroids are connected by the Minimum Spanning Tree. (See Fig. 1 for locality designation numbers.)

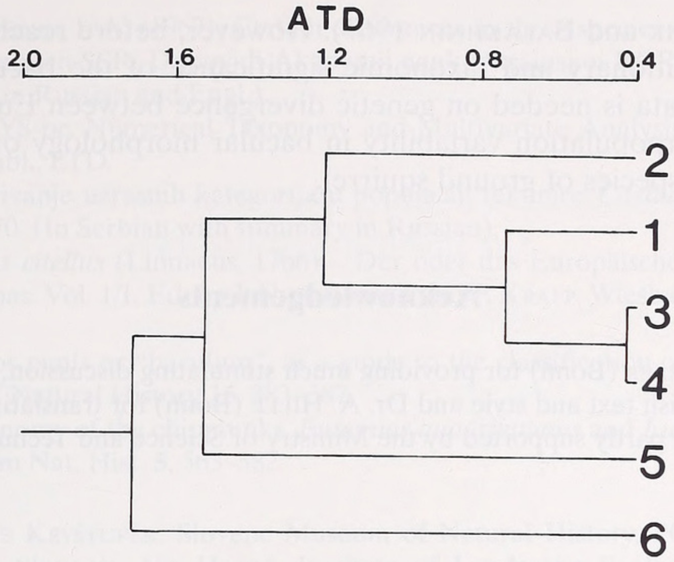

Fig. 4. Cluster analysis of six *S. citellus* populations, based on UPGMA dendrogram. Cophenetic correlation r = 0.895. (See Fig. 1 for locality designation numbers.)

graphic distance and the ATD matrices (r = 0.38, Mantel t-test = 1.052, not significant). The divergence between samples revealed by bacular morphology is therefore not affected by geography.

Discussion

The baculum of the European souslik lies in a superficial position (sensu PATTERSON 1983) and the female choice hypothesis predicts that such a baculum should function as an 'internal courtship' device (EBERHARD 1985). If so, competition between males for mates could be expected to result in rapid evolutionary divergence in bacular morphology, whilst the remainder of the phenotype remained unaffected. This seems to be consistent with the observations on the European souslik. The characters which are under direct environmental selection (size, colour) diverged between *S. citellus* populations to a lesser degree (KRYŠTUFEK 1995) than bacular characters.

Interpopulation relations between five souslik samples from Serbia and Macedonia suggested by the clustering of bacular parameters are the same as those obtained from nonmetric cranial traits (KRYŠTUFEK 1992). Accepting the assumption that nonmetric skeletal traits reflect genetic differentiation between populations (e. g. HARTMAN 1980), the structure of the baculum is likely to contain a considerable ammount of phyletic information, making it an appropriate structure for the study of evolutionary divergence in *S. citellus*. For example, bacular morphology suggests *S. c. karamani* (sample 6) to be the most distinct, which is consistent with analyses of cranial traits, both nonmetric (KRYŠTUFEK 1992) and metric ones (KRYŠTUFEK 1995). The basic difference between the two sets of data is categorical interpopulation variation of baculum, while cranial data overlap to a large extent between samples. It is thus possible to ascribe each baculum of *S. citellus karamani* to the actual group, which is not the case with the skull. Anyhow, we should underline that we have data on bacular variation from only one part of the species distribution range. Any increase in samples studied could diminish the diagnostic value of the baculum shape as is the case with cranial morphology (compare KRYŠTUFEK 1993, 1995).

Differences in bacular morphology in some Nearctic ground squirrels (BURT 1960) are of approximately the same magnitude as those demonstrated in this study between geographic samples of *S. citellus*, and a similar impression is obtained from studying drawings of the bacula of three closely-related western Palaearctic taxa: *S. citellus*, *S. pygmaeus* and

S. suslicus (Reshetnik and Balakhnin 1967). However, before reaching any definite conclusions about evolutionary and taxonomic significance of the bacular variability in the European souslik, data is needed on genetic divergence between European souslik populations, data on interpopulation variability in bacular morphology on a larger geographic scale, and for other species of ground squirrel.

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Zusammenfassung

Variabilition des Baculum zwischen Populationen des Europäischen Ziesels Spermophilus citellus

Untersucht wurden 35 Bacula des Europäischen Ziesels *Spermophilus citellus* (Linnaeus, 1766) in 6 geographischen Stichproben von der Pannonischen Ebene und der Balkanhalbinsel. Alle 4 untersuchten Merkmalsstrukturen – Länge des Baculum, Breite an der Basis, Breite der löffelförmigen distalen Spatula und Zahl der Zähnchen (Dentikel) am Außenrand der Spatula – waren hochsignifikant zwischen den Stichproben verschieden. Die größten Unterschiede entfielen auf die Spatulabreite und die Zahl der Dentikel. Die Populationsunterschiede in den Baculumausprägungen assoziieren mit bereits festgestellter Merkmalsvariation in qualitativen Schädelmerkmalen und zeigen sich stabil gegenüber der geographischen Interdependenz der Stichproben. Verschiedene Baculumstrukturen von *S. citellus* enthalten daher mehr phyletische Information als konventionelle taxonomische Merkmale wie etwa Größe oder Schädelproportionen.

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Authors' addresses: BORIS KRYŠTUFEK, Slovene Museum of Natural History, PO Box 290, 1001 Ljubljana, Slovenia; VIT HRABĚ, Institute of Landscape Ecology, AS CR, Května 8, 603 65 Brno, Czech Republic.