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DISTINGUISHING *MUS SPICILEGUS* FROM *MUS MUSCULUS* (RODENTIA, MURIDAE) BY USING CRANIAL MEASUREMENTS

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The skulls of the two members of the genus *Mus*, the outdoor, aboriginal *Mus spicilegus* and the commensal *M. musculus* can be differentiated using the zygomatic coefficient (CZ). However, the CZ-method does not differentiate all *Mus* specimens because the cranial measurements needed to calculate CZ are subjective due the unfixed endpoints. Measurement error can contribute significantly to erroneous species classification of specimens if only a single set of measurement is used. The primary aim of our study was to define and test the measurable characters on a large number of skulls in order to definite separate of the two species. Based on the *F*-values derived from Discriminant Function Analysis, we found the following three variables contributed considerable to the total discrimination power: width of the zygomatic arch (B), width of first upper molar (MW) and width of the upper ramus of the zygomatic process of maxilla (A). By the classification functions the most discriminating character is MW. For discriminating the above-mentioned two species Fisher's linear discriminant functions were calculated using the two most powerful discriminating variables. This lead us to formulate the following identification function key for *M. musculus* and *M. spicilegus*: 2.1MW–B = 1.46 mm. We infer that with the use of this key the correct identification approaches 100%. The separation of the two species based on the width of M^1 (first upper molar) is useful from a paleontological viewpoint because teeth are generally the best preserved element among vertebrate fossil remains.

Key words: morphometry, identification function key, house mouse, mound-building mouse, age-group

INTRODUCTION

There are two species of the genus *Mus* in Hungary, commensal house mouse, *Mus musculus* LINNAEUS, 1758, and outdoor mound-building mouse, *M. spicilegus* PETÉNYI, 1882. *M. spicilegus* is unique and easily identifiable by its genetically determined (ORSINI *et al.* 1983) mound-building behaviour. However, although this species is rather distinct in some cranial and dental traits from other Western Palaearctic mouse taxa (MACHOLÁN 1996*b*), field identification is difficult. Several studies (BIHARI 2003, DEMETER *et al.* 1996) have attempted to delimit the range of *M. spicilegus* in Hungary but this task is hampered by morphological similarity of the species with the sympatric house mouse. ORSINI *et al.* (1983) attempted to distinguish the two species using the zygomatic coefficient (CZ), i.e., the ratio of dimension A to B (Fig. 1). However, DEMETER *et al.* (1996) found that this ratio is not reliable to distinguish samples collected in Hungary. This is due perhaps, to equivocal positions of endpoints of both dimensions. Another character distinguishing *M. spicilegus* from other European mouse species is the lingual outline of M^2 (second upper molar) described by MACHOLÁN (1996*a*) (see also KRYŠTUFEK & MACHOLÁN 1998). Finally, DEMETER *et al.* (1996) examined the mandibles of these species. Their analyses of both linear measurements and variable shapes pointed out differences between the two mouse species in the shape of the coronoid process.

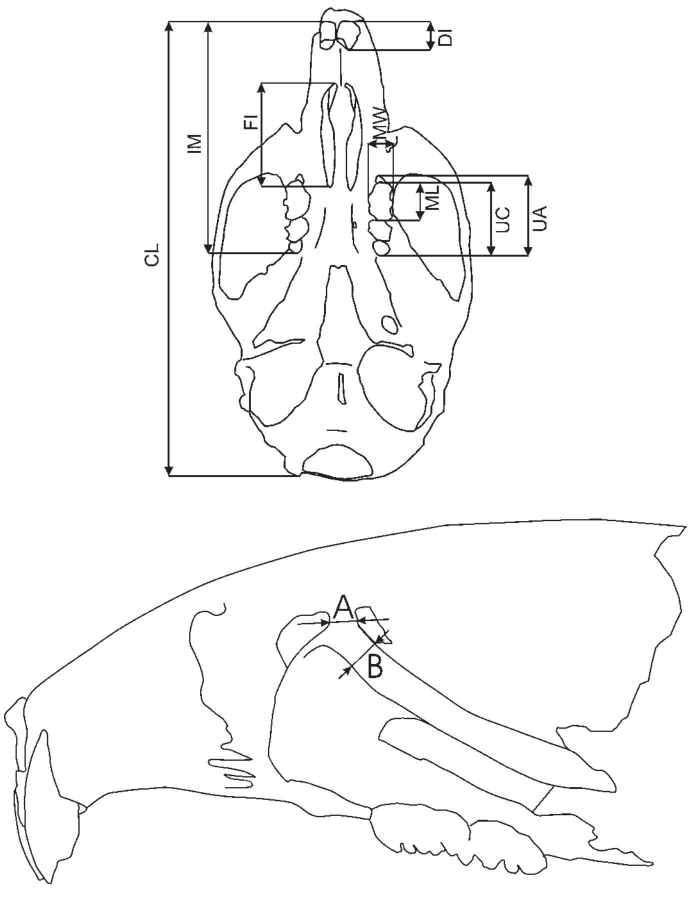

Fig. 1. Endpoints of distance measures taken from the cranium. Abbreviations are explained in text.

Table 1. Number of specimens examined from different populations.

No.	Geographic region	*M. spicilegus*	*M. musculus*
1.	Borsodi Mezőség	204	34
2.	Hevesi Füves Puszták	19	4
3.	Hortobágy	30	12
4.	Kiskunság		33
5.	Sárrét	20	28
	Further sporadic locality	16	2
		289	113

The primary aim of our study was to quantitatively test what set of measurable characters could be used to separate the two species. We hope this will lead to a reliable key that will allow mouse skulls recovered from owl-pellets to be accurately identified as to species.

MATERIAL AND METHODS

Of the 514 specimens measured, 402 consisted fragmented skulls from owl-pellets which were collected at 34 localities from five distinct geographic regions: Borsodi Mezőség, Kiskunság, Sárrét, Hevesi Füves Puszták, and Hortobágy (Fig. 2 and Table 1). Variation among populations within each region is assumed to be negligible because the localities within a region were situated in close proximity. Individual localities are given in CSERKÉSZ (2005). All the skulls were deposited in the collection of the Bükk Mammalogical Society, Eger.

In addition to the 402 studied specimens from owl-pellets, sexual dimorphism was studied on 112 museum specimens (Mammal Collection of the Hungarian Natural History Museum). These specimens consisted of 40 females and 40 males of *M. musculus*, and 15 females and 17 males of *M. spicilegus*, collected in the Carpathian Basin.

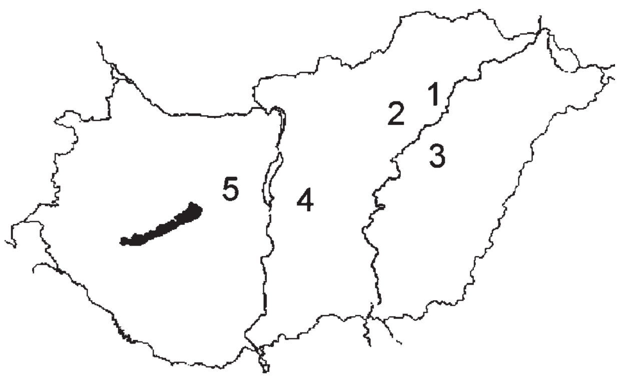

Fig. 2. Map of Hungary showing the collection regions. 1–5: geographic regions (see Table 1)

Following DEMETER and LÁZÁR (1984) and ORSINI *et al.* (1983), six cranial and three dental characters were measured (Fig. 2): A – width of the upper ramus of the zygomatic process of maxilla, B – width of the zygomatic arch, UA – length of the upper tooth row at alveoli, IM – distance between incisor and M^3 (third upper molar), UC – length of the upper tooth row at crowns, FI – length of foramen incisivum, DI – cross-sectional depth of incisor, ML and MW – length and width of first upper molar (M^1). A derived index, the zygomatic coefficient (CZ=A/B) was studied in detail. MW, UC, A, B, FI and CL (condylobasal length) were recorded on museum specimens. Usually the neurocranium is broken on skulls from owl pellet, so CL can not be measured on these specimens. Measurements were made by the first author with a digital calliper connected to a computer and recorded to the nearest hundredth of a millimeter. A stereo microscope was used to assess tooth wear. The skulls were graded into six age groups based on tooth wear following CHOU *et al.* (1998). To avoid undesirable variation due to potential asymmetry, only the left side was measured in paired characters.

Morphometric variation was studied using Principal Component Analysis and the differentiation was estimated using Discriminant Function Analysis. All previously mentioned measurements except condylobasal length (CL), were included in the analyses. CL was left out because it could only be measured on museum specimens. Mahalanobis generalized distances (D^2) and Fisher's linear discriminant functions were calculated from Discriminant Function Analysis (DFA). Normality was tested by Shapiro-Wilk W-test. Hotelling T^2 – and Student t-tests were applied to test the sexual dimorphism. Correlation between the measurements and age groups was analyzed using the Pearson correlation coefficient (r_p). Univariate and multivariate analyses were conducted using the statistical package StatSoft Inc. (2006). Only the Fisher's linear discriminant functions were performed using SPSS (SPSS Inc. 2005). Basic statistical parameters: mean (x), standard deviation (SD), and coefficient of variation ($CV=SD/x$) as an estimation of variability were calculated for each skulls separately for both species.

RESULTS

Significant sexual dimorphisms were not found on museum specimens in either of the species (*M. musculus*: $T^2 = 0.09$, $p = 0.49$; *M. spicilegus*: $T^2 = 0.09$, $p = 0.83$). Cranial measurements were greater for females in *M. musculus* (Table 2). In the case of *M. spicilegus* more skulls are needed to address this question however difference were not found in the actual samples. Thus we pooled the sexes in all subsequent analyses.

Using the keys of MACHOLÁN (1996*a*) and ORSINI *et al.* (1983) 113 specimens of *M. musculus* and 289 specimens of *M. spicilegus* were determined a priori. The measurements of complete skulls (143 specimens) with full dataset were loaded into a Discriminant Function Analysis (DFA) in two species group. Both groups were split into adult and juvenile subgroups. The clouds of points (Fig. 3) are very clearly distinguished and non-overlapping (the tiny overlap of ellipses is probably results from the small sample size in adult specimens of *M. musculus*). The separation between the two species is highly significant ($D^2 = 37.11$; F (9, 143) = 111.06; $p < 0.00005$). The D^2-values showing the distance between groups are in the Table 3.

Table 2. Cranial measurement means for male and female *M. musculus*.

	x_{male} (mm)	x_{female} (mm)	$t_{student}$	p
MW	1.03	1.04	–1.04	0.300
UC	3.19	3.23	–1.80	0.075
A	0.55	0.55	–0.31	0.755
B	0.96	0.96	–0.06	0.952
FI	4.50	4.60	–1.45	0.152

Table 3. Morphometric divergence between age-groups examined as indicated by Mahalanobis distances (D^2).

	Juv. *M. spicilegus*	Ad. *M. spicilegus*	Juv. *M. musculus*
Ad. *M. spicilegus*	2.57		
Juv. *M. musculus*	33.41	40.97	
Ad. *M. musculus*	37.57	41.04	3.43

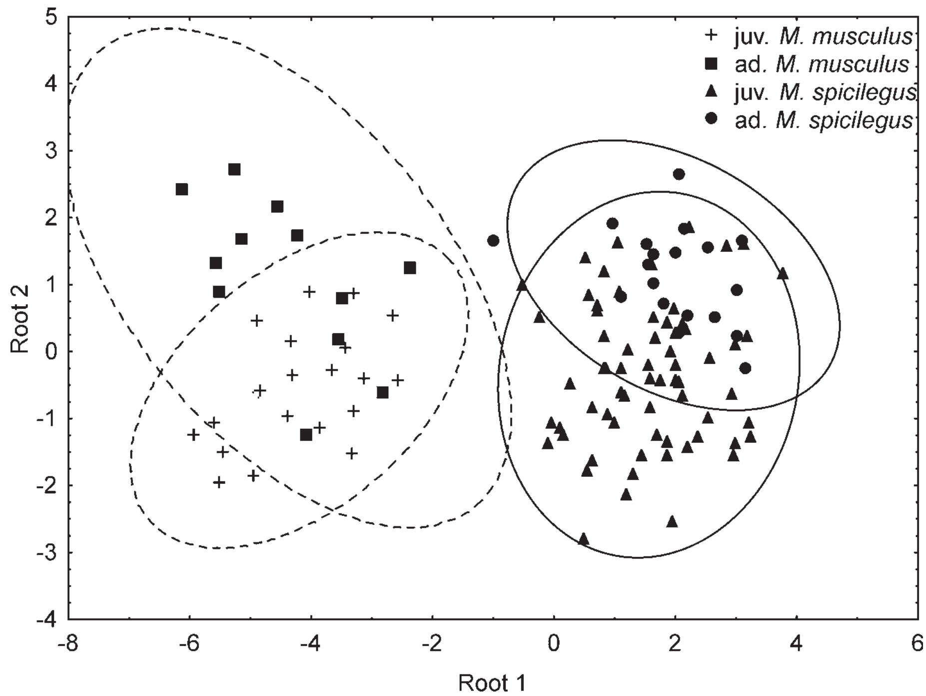

Fig. 3. Two-dimensional plot of the two canonical variates based on 10-character DFA scores.

Table 4. Results of discriminant function analysis: *F*-values for the variables and classification functions for the species and the variables.

			Classification functions	
	F	*p*	*M. musculus*	*M. spicilegus*
B	40.92	0.000	95.15	22.12
MW	34.62	0.000	544.30	641.47
A	17.29	0.000	134.42	98.81
ML	1.11	0.29	78.61	64.55
IM	7.17	0.008	26.77	34.80
FI	5.43	0.02	38.13	32.13
UA	1.81	0.18	15.63	10.90
DI	0.32	0.56	0.71	–14.02
Constant			–598.97	–658.14

Interestingly, when only interspecific differences are considered, the distance between adults are the highest ($D^2 = 41.04$) but high and significant distance ($D^2 = 37.57$ and 40.97) could be also find between the juvenile and adult groups. The distance is relative smaller between the juveniles (Table 3). Variables with the highest *F*-values contribute most in determining the separation among species. They contribute to total discrimination in the following order: B, MW, A, IM, FI, UA, ML, DI by *F*-values and by the classification functions the most discriminating character is MW (Table 4).

It was two a priori *M. spicilegus* which were included into the musculus group according to the squared Mahalanobis distance (D^2) measured from the group centroids. However, classification with the wrong group was not strongly supported (44%).

Correlation between measurements of *M. spicilegus* and the age groups was analyzed using the Pearson correlation coefficient (r_p). The highest value was for IM while A and B, the two components of CZ, were intermediate (Table 5). The characters connected with the tooth-row varied the least as a function of age; therefore, they must have a fundamental function in the determination.

Table 5. Correlation coefficients (r_p) and p-values (p) for measurements of *M. spicilegus* and age-groups.

	A	B	UC	UA	DI	FI	ML	MW	IM
r_p	0.46	0.35	0.11	0.32	0.41	0.49	–0.17	0.12	0.61
p-values	0.000	0.000	0.247	0.000	0.000	0.000	0.028	0.116	0.000
N	158	158	113	144	149	150	155	159	100

Table 6. Statistical characters of PCA.

Component	R^2X	R^2X(Cumul.)	Eigenvalues	Q^2	Limit
1	0.37	0.37	4.12	0.34	0.09
2	0.32	0.69	3.49	0.68	0.11
3	0.11	0.80	1.21	0.78	0.12
4	0.06	0.86	0.64	0.85	0.13

The two species groups separated by DFA were loaded into Principal Component Analysis (PCA: correlation matrix, unrotated) for summarizing the morphometric variation. For each component an R^2X value was calculated. This value represents the percentage of observable variation in the data (Table 6). The first principal component (PC1) explains 37.48% of the observable variations, while PC2 explains 31.80%.

As seen in Figure 4 the two groups are not absolutely separated by the PC1 axis. In the majority of PCAs the PC1 describes the size; therefore, it is treated as the 'size vector'. All other components are assumed to describe shape. The question of whether PC1 represents a size vector was tested by inspecting coefficients

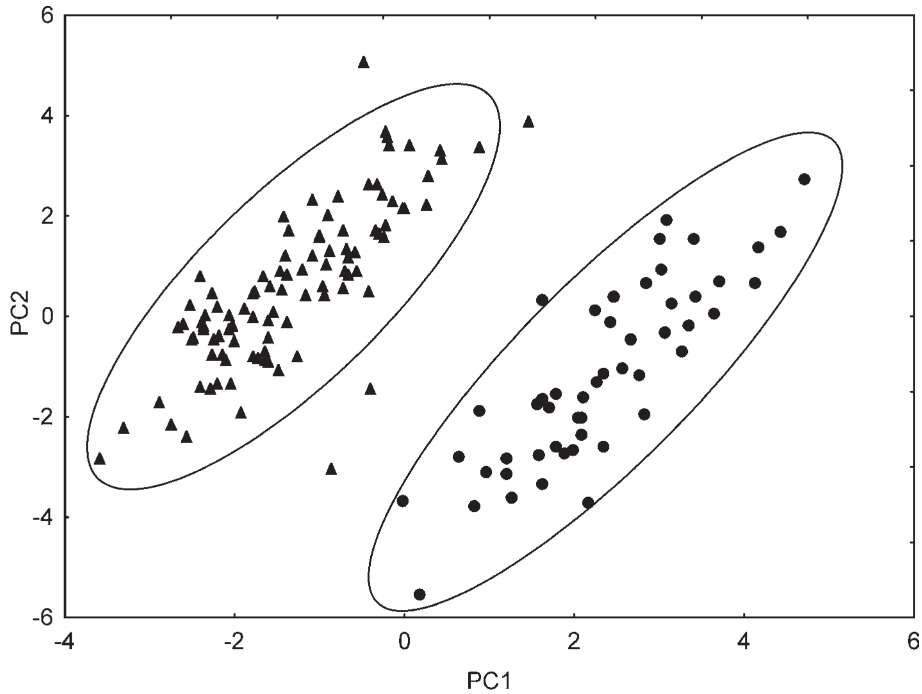

Fig. 4. Bivariate plot of individual scores on PC1 and PC2.

Table 7. Eigenvectors and loadings of PC1.

	Eigenvectors of	Loadings of	
	PC1	PC1	PC2
A	0.12	0.24	0.59
B	0.44	0.89	–0.16
UC	–0.03	–0.06	0.76
UA	0.23	0.46	0.61
DI	0.31	0.63	0.54
FI	0.29	0.59	0.59
ML	–0.15	–0.31	0.42
MW	–0.37	–0.76	0.52
IM	0.19	0.38	0.82

of the first eigenvector and of the loadings. If size variation is present in the data and the coefficients of PC1 are either all positive or all negative, then this PC can be said to summarize the within-sample size variation (BOOKSTEIN 1989). The tooth traits showed negative loadings on PC1 as compared with positive loadings of skull loadings (Table 7). The first eigenvectors do not have the same sign nor similar magnitude; therefore, PC1 may also include a part of shape information as well.

Correlation analysis between the loadings of PC1 and correlation coefficients of variables with age resulted in a high correlation coefficient ($r = 0.68$; $p < 0.05$). The most age-dependent variable has the highest loadings on PC1 so it could be treated as the 'growth factor'. PC1 had high positive loading for B and high negative loading for MW (Table 7). Thus these characters appear to contribute most to morphometric separation (cf. also results of DFA) and it indicates that species with wide MW also have narrow B.

There is some evidence (DAYAN *et al.* 2002, PANKAKOSKI *et al.* 1987, SOULÉ 1982) that the relative error of a measurement, expressed as the coefficient of variation (CV), begins to grow rapidly when the mean exceeds a threshold value. No or negative correlation between CV and the mean value indicates high reliability of measurement. Therefore, we estimated the measurement reliability in a set of characters which appeared to be contributing the most discrimination. The dependency of CV on the mean is clearly non-linear, most of hyperbolic (Fig. 5). The character A and MW have similar means but different coefficients of variation. Due to the high correlation of A and B with the age, the standard deviation will be larger and it emerges in the CV with the relative error. Because MW has a small mean value we

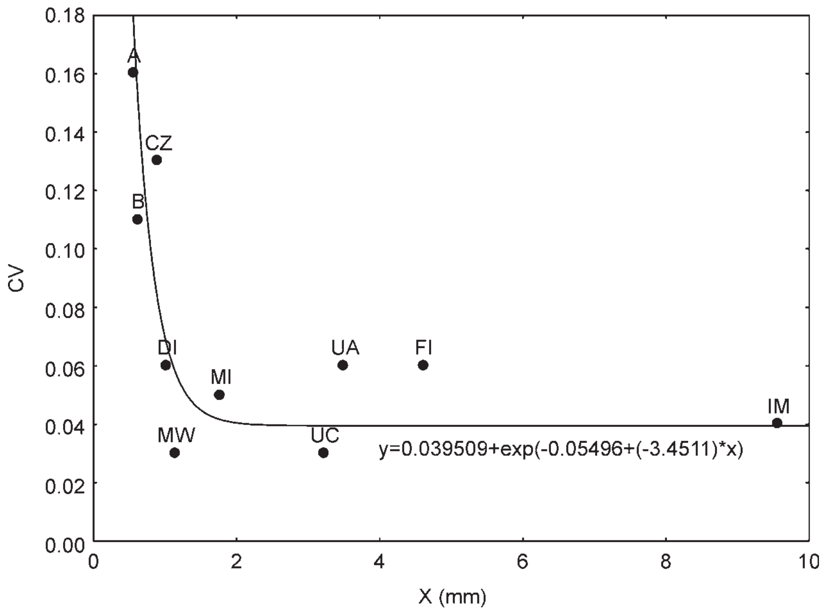

Fig. 5. The hyperbolic regression of coefficient of variation (CV) on mean (X). The regression of the joint exponential grow curve: $r = 0.86$ (proportion of variance accounted for: 0.73).

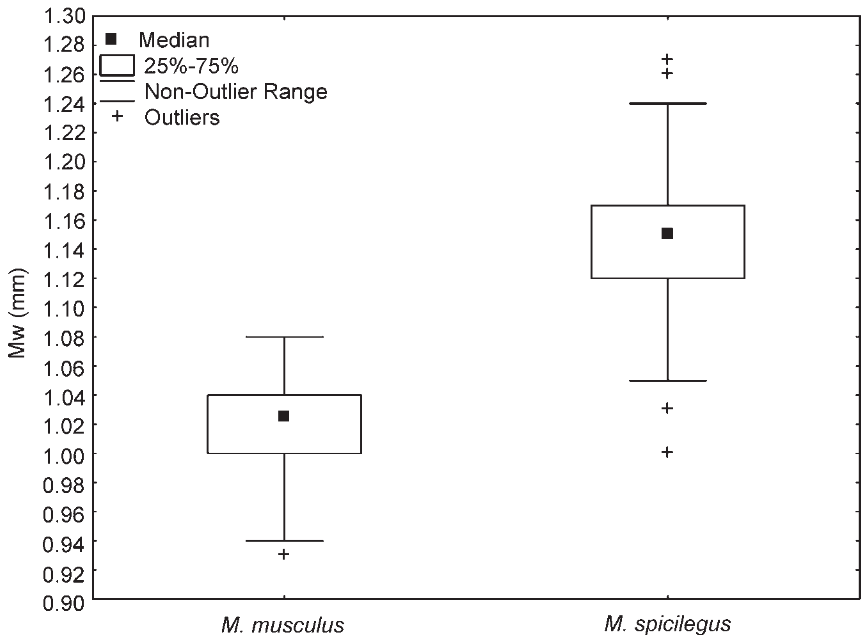

Fig. 6. Box-plot of width of M1 (MW) for the species.

would expect a high CV. But the CV value of MW is low indicating that the standard deviation is small and that it can be measured with a small amount of error.

The overlap of MW between species is 0.025 mm if we ignore outliers (Fig. 6). Seven *M. spicilegus* were in the total sample. The MW's of these seven samples are below the maximum value (1.08 mm) of *M. musculus*; at least 97.5% of the skull can be identified with the MW-method. For remaining skulls an equation is presented. As a part of DFA, Fisher's linear discriminant functions were calculated using the two most discriminating variables, B and MW, to obtain discrimination equation 2.1MW–B=1.46 mm. With this equation the identification becomes more accurate (Fig. 7), the probability of correct classification approaches 100%.

The species of the skulls, which were left out from multivariate statistics because of breakage, were identified with the help of the MW-method and use of the discrimination equation (2.1MW–B = 1.46 mm), and the number of skulls misidentified by the CZ-method was calculated. The lingual outlines of M^2 (MACHOLÁN 1996*a*, KRYŠTUFEK & MACHOLÁN 1998) of these skulls were also checked. Finally 6.6% of *M. spicilegus*, and only 3.5% of *M. musculus* were misidentified using CZ measurement.

The descriptive statistics pertaining to cranial measurements and derived CZ ratios with the results of t-tests are presented in the Appendix to this paper.

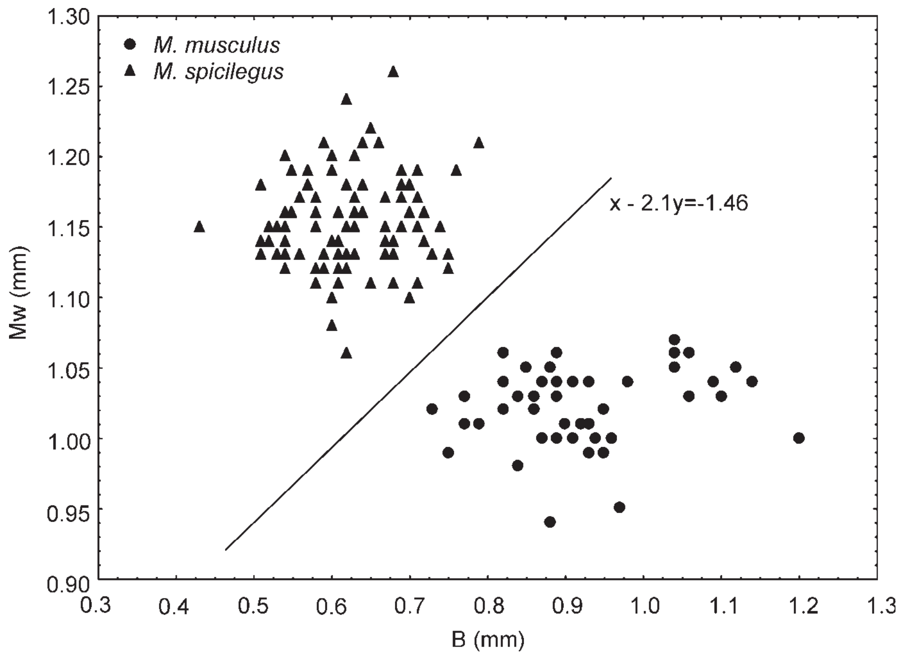

Fig. 7. Bivariate plot of MW and B with the discrimination equation and line.

DISCUSSION

The aim of our research was to find measurable morphologic characters of skull fragments, found in owl pellets, that distinguish *M. spicilegus* from *M. musculus*. We examined some of the known distinguishing criteria and tried to find new ones. Studies of owl-pellets bring about several problems. For example, the time- and habitat-related variations are highly pronounced because the exact habitat of prey populations and the time of predation are not known. Part of the variation in habitat, season, sex and age must remain unknown. Further bias can be expected because of the relatively high occurrence of juvenile individuals in owl-pellets (CSERKÉSZ 2005, FREUDENTHAL *et al.* 2002, LONGLAND & JENKISS 1987) owing to less efficient antipredator behaviour (LAY 1974) and to the seasonally high frequency of juveniles in the field.

Differences between most of the measured characters were highly significant, yet ranges of all the measurements overlapped. We focused primarily on the zygomatic coefficient (CZ) which other authors have suggested is a major criterion for distinguishing aboriginal species of mice (*M. spretus*, *M. macedonicus*, *M. spicilegus*) from commensal house mice (LYALYUKHINA *et al.* 1991, MACHOLÁN 1996*b*, ORSINI *et al.* 1983). Our results are in agreement with those of MACHOLÁN (1996*a*) who found a small overlap in CZ values between *M. musculus* and *M. spicilegus*. Therefore, using this variable alone we would expect incorrect identification in a small number of cases. Moreover, mean values of CZ as well as the values suggested as a rule of thumb for discrimination differ among morphometric studies (cf. LYALYUKHINA *et al.* 1991, MACHOLÁN 1996*c*, ORSINI *et al.* 1983, and this paper).

Another problem, often encountered in morphometric studies is that characters are more pronounced with age. We can avoid this problem by excluding juveniles from our analysis. In our study we tested the correlation between several measurements and the ages estimated from the amount of tooth-wear. Our results confirm the interpretation of CSERKÉSZ (2005) and ROOD (1965). That is that the measures of the tooth rows change to a small extent after the development of M^3. Among the examined characters we found a significant increase at the IM, FI, DI and the components of CZ. On the other hand, the estimation of age based on tooth wear is somewhat unreliable because tooth wear shows individual variation and depends on habitat and food. Moreover, it follows from Table 5 that all but two (UC and MW) measurements are significantly correlated with age. For this reason we focused on the width of the first upper molar (MW) the measurement of which was significantly different for the two species and less age-dependent than other variables. Though slightly overlapping, this measure differentiated the species quite well, with *M. spicilegus* molars considerably wider than those of *M. musculus*. With some reservation, we consider specimens with MW < 1.08 mm as *M. musculus* and those with MW > 1.08 mm as *M. spicilegus*. ENGELS (1980) described a mean value of MW of 1.08 mm in *M. spicilegus* from Austria, however, we are not sure if the author's samples did not consist of *M. musculus* or, possibly, a mixture of both species owing to unclear taxonomy and/or a lack of reliable determination criteria before the advent of biochemical and molecular methods. Besides ENGEL's paper we could not find additional discussions of MW in the literature; therefore its discriminating power in *Mus* taxonomy is new information. It would be interesting to test this criteria on samples collected outside of the Carpathian Basin as well. The separation of these two species based on the width of the M^1 would be useful from a paleontological viewpoint, considering that teeth are generally the best-preserved element among vertebrate fossil remains.

However, because the MW of some *M. spicilegus* specimens (< 2.5%) is less than 1.08 mm, the MW-method is not reliable in all cases. For greater reliability we present a discrimination equation based on MW and B, the two most discriminating variables, which we suggest can be used for species identification: if 2.1MW–B < 1.46 mm the skull should be considered as *M. musculus*, and if higher than 1.46 it should be considered *M. spicilegus*. As stated earlier the probability of correct identification approaches 100% using this equation.

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APPENDIX

Descriptive statistics and results of t-tests comparing cranial measurements (mm) and the derived ratio CZ for *M. musculus* and *M. spicilegus*. Statistics are given as mean ±SD, sample size, range (minimum–maximum), and coefficient of variation (CV=SD/X). Significance is indicated as follows: n.s. = $p \geq 0.05$; * = $0.01 \leq p \leq 0.05$; ** = $0.001 \leq p \leq 0.01$; ***= $0.0001 \leq p \leq 0.001$; ****= $p < 0.0001$

	M. musculus	*M. spicilegus*
A ****	0.51±0.008, 102	0.56±0.09, 234
	(0.28–0.74) 0.17	(0.34–0.86) 0.16
B ****	0.90±0.12, 102	0.62±0.07, 234
	(0.57–1.2) 0.13	(0.3–0.88) 0.11
CZ (A/B) ****	0.57±0.07, 102	0.90±0.12, 233
	(0.34–0.76) 0.12	(0.59–1.24) 0.13
UC ****	3.14±0.13, 75	3.22±0.09, 156
	(2.87–3.44) 0.04	(2.98–3.58) 0.03
UA (n.s.)	3.54±0.17, 67	3.50±0.20, 171
	(3.2–4.1) 0.05	(3.06±4.17) 0.06
DI (n.s.)	1.02± 0.07, 55	1.02±0.06, 176
	(0.88–1.18) 0.07	(0.91–1.18) 0.06
FI (n.s.)	4.61±0.28, 69	4.61±0.26, 186
	(3.97–5.14) 0.06	(4.00–5.17) 0.06
ML ***	1.73±0.07, 63	1.78±0.06, 190
	(1.54–1.91) 0.04	(1.56–1.96) 0.05
MW ****	1.02±0.03, 76	1.14±0.03, 230
	(0.93–1.08) 0.03	(1.01–1.24) 0.03
IM *	9.40±0.46, 66	9.56±0.43, 129
	(8.38–10.41) 0.05	(8.49–10.57) 0.04
CL#	19.27±0.77, 68	19.42±0.99, 24
	(17.55–20.8) 0.03	(17.95–21.31) 0.05

#: it was measured on museum specimens