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Craniometric comparison and discrimination of two sibling species of the genus *Mus* (Mammalia, Rodentia) from Slovakia

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Abstract. Craniometric characteristics of two sibling species of the genus *Mus*, the indoor house mouse (*Mus musculus*) and the outdoor mound-building mouse (*M. spicilegus*), from Slovakia were compared and evaluated in order to discriminate them. Twenty-one skull and dental variables were evaluated on 107 skulls of adult house mice, *M. musculus* and 80 skulls of mound-building mice, *M. spicilegus*. The parametric unpaired t-test and discriminant function analysis showed variations in the cranial variables between the two species. We identified four dental variables (LaM^1, LaM_1, LM_1 and LOID) suitable for the differentiation.

Key words: *Mus musculus*, *Mus spicilegus*, skull and dental parameters, PCA, DFA

Introduction

The house mouse (*Mus musculus*, Linnaeus, 1758) and mound-building mouse (*Mus spicilegus*, Petényi, 1882) are two sibling species of the genus *Mus* distributed in Slovakia (Krištofík & Danko 2003a, b, Krištofík 2012, Krištofík & Stollmann 2012). Moreover, the mound-building mouse has the northern border of its geographical distribution in south Slovakia (Krištofík & Stollmann 2012). The great similarity and overlap of some morphological patterns between species of the genus *Mus* has caused a great deal of confusion in systematic identification (Macholán 1996a). Several authors have shown that besides the difference in DNA sequences (Gerasimov et al. 1990, She et al. 1990, Auffray & Britton-Davidian 1992, Macholán 1996b), skull and/or teeth traits are also reliable for distinguishing species (Orsini et al. 1983, Sokolov et al. 1990, Lyalyukhina et al. 1991, Demeter et al. 1996, Macholán 1996a, b, 2001, 2006, 2008, Zagorodniuk 1996, Unterholzner & Willenig 2000, Balčiauskienė et al. 2004, Cserkész et al. 2008).

More recent morphological analyses of both species of the genus *Mus* from Slovakia were noted in our previous papers (Čanády et al. 2008, 2014, Csanády & Mošanský 2018). However, we have not yet published any discrimination analysis of cranial measurements, and no detailed analyses were carried out. Previous studies (Orsini et al. 1983, Sokolov et al. 1990, Lyalyukhina et al. 1991, Macholán 1996a, Unterholzner & Willenig 2000) have attempted to discriminate the species using the zygomatic coefficient, i.e. the ratio between two dimensions: A (breadth of the upper ramus of the zygomatic process of the maxilla) to B (breadth of the zygomatic arch). Unfortunately, data from the literature on the mean values of the zygomatic index (A/B) for populations of *M. spicilegus* as well *M. musculus* are highly variable (see Cserkész et al. 2008, Čanády et al. 2014, Csanády & Mošanský 2018). The mentioned authors assume that using this variable alone, one would expect incorrect identification in a small number of cases. This is why several authors (Macholán 1996a, b, 2001, 2006, 2008, Balčiauskienė et al. 2004, Cserkész et al. 2008) have recommended using dental characteristics, whose specific dimensions, especially the shape of molars, are specific to each species. In Hungary, which borders Slovakia, greater attention

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has been paid to distinguishing these sibling species using skull characteristics (Cserkész et al. 2008).
In the present study, we provide discriminant analysis to reveal which set of cranial traits could be used to discriminate the two species. The obtained results should facilitate identification of museum collections and bones recovered from owl pellets and help to distinguish *Mus* skulls obtained by trapping.

Material and Methods

The analysed samples included 107 skulls (57 males, 50 females) of the adult house mouse (*M. musculus*) and 80 skulls (43 males, 37 females) of the mound-building mouse. The 21 skull measurements used were described in previous studies (Čanády et al. 2014, Csanády & Mošanský 2018). Their abbreviations and definitions are as follows: LD – length of upper diastema, LOSD – length of upper molar row (alveoli), LM^1 – length of first upper molar, LaM^1 – width of first upper molar, LM^{2+3} – length of second and third upper molar, LOID – length of lower molar row (alveoli), LM_1 – length of first lower molar, LaM_1 – width of first lower molar, LB – basal length, LCb – condylobasal length, LN – length of *os nasale*, LFI – length of *foramen incisivum*, LMd – length of mandible, LaZ – zygomatic breadth, LaI – interorbital width, ACr – brain-case height with the choanae, FL – facial length, i.e. distance from the gnathion after the aboral edge of the hard palate, CI – thickness of the incisor, IBW – width of the braincase, A – breadth of the upper ramus of the zygomatic process of the maxilla, and B – breadth of the zygomatic arch. Skull measurements were taken with a digital calliper with accuracy of 0.01 mm. Dental traits were measured with an Olympus SZ 400 stereomicroscope. The values of all measures were presented in previous studies (Čanády et al. 2014, Csanády & Mošanský 2018). In this study we used dataset obtained from our previous study. Herein we present only the mean values shown in Table 1. We also evaluated our previously obtained raw data in relation to the discrimination of both species.
The obtained dataset (untransformed data) was evaluated using the statistical parameters of means and standard deviations (M ± SD). Normal distribution was tested using two normality tests (the D'Agostino-Pearson omnibus K^2 test and the Shapiro-Wilk W-test). Before other analyses, measurements were $\log_{10}$-transformed to reduce intra-sample variation and to improve normality. We then re-checked the normality and confirmed that the measurements had normal distribution. The parametric unpaired t-test was used to detect and validate the statistical significance of the variability among traits between species. Morphometric variation was also examined by means of principal component analysis (PCA). Correlations between the measurements were analysed using the Pearson correlation coefficient (r_p). We examined PCA, including both species, in the case of 14 variables which give clear size differences between the species (according to results of the t-test: LN, LCb, LD, LaZ, LaI, ACr, LM^1, LaM^1, LM_1, LaM_1, CI, LOID, A, B). Species differentiation was estimated using discriminant function analysis (DFA). Mahalanobis generalized distances (D^2) and Fisher's linear discriminant functions were calculated from the DFA.
We also used published material on craniometric data from adult *M. musculus* and *M. spicilegus* across the species range. We evaluated six traits: LCb, FI, LM^1, LOID, A and B (Table 5). Populations in the central part of the range came from the European part of Russia, central and south-eastern Ukraine, Moldova (Sokolov et al. 1990, Lyalyukhina et al. 1991),

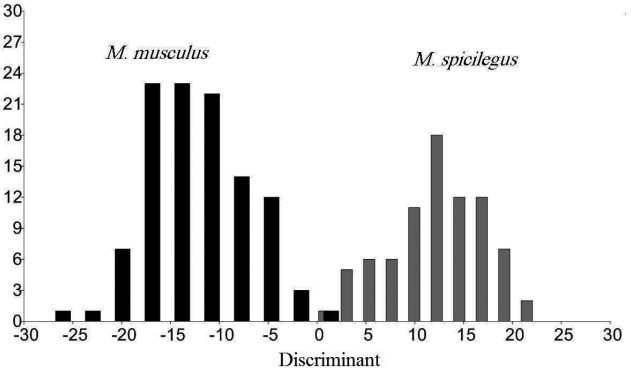

Fig. 1. Frequency histogram of the canonical scores for the standard DFA between the species *M. musculus* (black bars) and *M. spicilegus* (grey bars) from Slovakia.

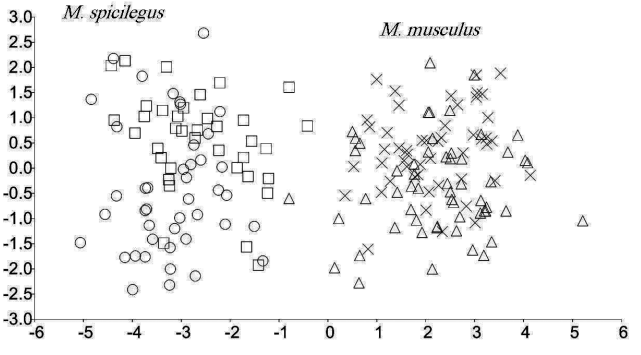

Fig. 2. Results of discriminant analysis calculated with all the variables of two sibling species of genus *Mus* from Slovakia, the house mouse (*Mus musculus*) and the mound-building mouse (*Mus spicilegus*). Symbols: open square – female *M. spicilegus*, open circle – male *M. spicilegus*, open triangle – male *M. musculus*, and cross – female *M. musculus*.

Table 1. Standard statistics for cranial and dental measurements (in mm) of two sibling species of the genus *Mus*, the house mouse (*Mus musculus*) and the mound-building mouse (*Mus spicilegus*) from Slovakia. Statistics given as sample size (N); mean (M); standard deviation (SD); degree of freedom (df). Significant differences are marked as: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

	Mus spicilegus (Čanády et al. 2014)		*Mus musculus* (Csanády & Mošanský 2018)				
Trait	N	M ± SD	N	M ± SD	t-test	df	*p*
LB	78	19.52 ± 0.80	98	19.21 ± 0.82	2.54	174	0.0119*
LCb	76	18.32 ± 1.11	95	19.03 ± 1.44	3.44	169	0.0007***
LN	76	7.05 ± 0.50	87	6.64 ± 0.36	5.98	161	< 0.0001***
LFI	79	4.43 ± 0.19	105	4.39 ± 0.23	1.17	181	0.2433
FL	79	10.39 ± 0.48	100	10.22 ± 0.55	2.14	177	0.0337*
LD	80	4.89 ± 0.26	106	4.67 ± 0.29	5.37	184	< 0.0001***
LMd	76	10.22 ± 0.59	104	10.37 ± 0.58	1.73	178	0.0849
LOSD	80	3.33 ± 0.16	107	3.27 ± 0.15	2.75	185	0.0065**
LOID	77	2.93 ± 0.13	104	2.72 ± 0.13	10.31	179	< 0.0001***
LaZ	80	11.18 ± 0.47	101	10.79 ± 0.37	6.07	179	< 0.0001***
IBW	76	9.16 ± 0.32	90	9.14 ± 0.25	0.59	164	0.558
LaI	80	3.43 ± 0.22	105	3.16 ± 0.23	8.02	183	< 0.0001***
ACr	76	6.51 ± 0.34	95	6.39 ± 0.21	2.85	169	0.005**
LM^1	80	1.69 ± 0.07	107	1.61 ± 0.08	6.62	185	< 0.0001***
LM_1	77	1.46 ± 0.04	101	1.34 ± 0.06	16.90	176	< 0.0001***
LM^{2+3}	79	1.51 ± 0.10	107	1.37 ± 0.09	10.06	184	< 0.0001***
LaM^1	80	1.11 ± 0.04	107	0.98 ± 0.04	21.79	185	< 0.0001***
LaM_1	77	0.85 ± 0.03	101	0.78 ± 0.03	18.70	176	< 0.0001***
CI	80	0.99 ± 0.09	100	0.96 ± 0.08	2.74	178	0.0067**
A	78	0.61 ± 0.07	97	0.57 ± 0.08	3.99	173	< 0.0001***
B	78	0.69 ± 0.07	95	0.91 ± 0.12	15.01	171	< 0.0001***

southern Hungary (Cserkész et al. 2008) and western Austria (Unterholzner & Willenig 2000). Data on the eastern part of the range came from northern and southern Bulgaria (Gerasimov et al. 1990), and data on the northern part came from Poland (Sokolov et al. 1990, Lyalyukhina et al. 1991).

All descriptive analyses were performed using MS Excel 2003 for Windows XP and the statistical analysis system GraphPad Prism version 5.01 (GraphPad Software, Inc., San Diego, California, U.S.A.). PCA and Fisher's linear discriminant functions were done using Statistical Software OriginPro8.6 (Microral Software Inc., Northamptom, U.S.A.). DFA was evaluated using the program PAST version 3.14 (Hammer et al. 2001). Published data do not allow for an unpaired t-test, PCA and DFA, as the raw data are not available. Nevertheless, the F and t-test from the parameters were used to compare our craniological measurements for each species with data from different parts of the range, using the program PAST version 3.14 (Hammer et al. 2001).

Results

Results of the unpaired t-test revealed statistically significant differences among the mean values of measured cranial parameters in both species. Most of the traits in the mound-building mouse were significantly greater than those in the house mouse (Table 1).

PCA on the size-free variables show separation of the species (Table 1, 2). The first two principal components (PC1-PC2) explained 52 % of the variation. The first (PC1) explained 38.5 % of the total variance. This was associated mainly with the length of the lower molar row (alveoli) (LOID) and the width of the first lower and upper molar (LaM_1 and LaM^1). PC2 accounted for 13.5 % and was correlated with the inner length of the *os nasale* (LN) and the breadth of the zygomatic arch (B). PC3 accounted for 9.6 % of the overall variation and was highly associated with the condylobasal length (LCb) and breadth of the upper ramus of the zygomatic process of the maxilla (A).

Table 2. Loading values of Principal Component Analysis (PCA) for the three main components (PC1-PC3) in selected cranial and dental variables for pooled data of two sibling species of genus *Mus* from Slovakia, the house mouse (*Mus musculus*) and the mound-building mouse (*Mus spicilegus*); their eigenvalues; percentage (variability %) and cumulative percentage (cumulative %) expressions. All abbreviations and measures are explained in Material and Methods.

	PC1	PC2	PC3
LN	0.30	0.35	0.06
LCb	–0.21	–0.27	0.51
LD	0.24	0.16	0.38
LaZ	0.12	0.13	0.22
LaI	0.32	0.30	–0.18
ACr	0.17	0.33	0.00
LM^1	0.24	–0.25	–0.38
LaM^1	0.34	–0.32	0.01
CI	0.22	0.31	0.24
LOID	0.35	0.08	–0.11
LM_1	0.33	–0.28	0.02
LaM_1	0.34	–0.27	0.09
A	0.12	–0.14	0.50
B	–0.28	0.34	0.03
Eigenvalue	5.39	1.89	1.34
Percentage (%)	38.5	13.5	9.6
Cumulative (%)	38.5	52	61.6

analyses resulted in two functions. The first, with an Eigenvalue of 6.63, explains 96.3 % of variance, and the second, with Eigenvalue of 0.18, explains 2.7 %, respectively.

In particular, the *Mus musculus* was separated from the samples of mound-building mice along function 1. This was mainly influenced by LaZ, LaM^1 and B. Similarly, the samples were separated along function 2, mainly based on LN and LaM^1.

Analysis of the t-test revealed significant differences in traits between the compared populations for both species, respectively (Table 5).

Discussion

Ambiguity in the determination of species of the genus *Mus* by somatic characteristics (Macholán 1996a, b, Čanády et al. 2008) has led to their discrimination through cranial and dental characteristics. Based on differences in interorbital width, the shape of molars and the zygomatic index (A/B), two aboriginal outdoor species, *Mus spretus* and *M. spicilegus*, were distinguished from the indoor *M. musculus* (Darviche & Orsini 1982, Orsini et al. 1983). In this study, we used the parametric unpaired t-test and discriminant function analysis (DFA) to show variations and distinguishing criteria in skull variables between *M. musculus* and *M. spicilegus*. First, by comparing *Mus* species using the t-test we found significant differences in 18 characteristics. We then used DFA for 14 selected traits to distinguish them. Our study showed that out of 14 investigated cranial traits, seven showed clear separation of the groups of both species (LaM_1, LM_1, LOID, B, LaI and LM^1). We primarily identified four dental variables (LaM^1, LaM_1, LM_1 and LOID) suitable for the differentiation. We also compared published data on *M. musculus* and *M. spicilegus* skull measurements across the species range. The high variability in both species, expressed in skull characteristics, was confirmed by different samples from different parts of range. Our results showed that the data on six selected characteristics was found to be very unequal. The analysis is thus restricted by the availability of data. Cserkész et al. (2008) showed the

Table 3. Morphometric divergence between sex-groups of two sibling species of the genus *Mus* from Slovakia, the house mouse (*Mus musculus*) and the mound-building mouse (*Mus spicilegus*), examined as indicated by Mahalanobis distances (D^2).

	male *M. spicilegus*	female *M. spicilegus*	male *M. musculus*
female *M. spicilegus*	1.60		
male *M. musculus*	29.95	24.45	
female *M. musculus*	29.31	23.06	0.83

Discriminant analysis on 14 selected variables (LN, LCb, LD, LaZ, LaI, ACr, LM^1, LaM^1, LM_1, LaM_1, CI, LOID, A, B) showed clear separation of the groups ($\lambda_{Wilks'\ 1} = 0.103$, $F_{42,\ 505} = 13.83$, $p < 0.0001^{***}$, Fig. 1, 2), and 99.5 % of cases were grouped correctly. Separation among these species was highly significant ($D^2 = 25.74$, $p = 0.0005^{***}$). The D^2-values showing the distance between genders are in Table 3. Variables with the highest F-values that contribute the most to determining the separation among the different parts of Slovakia studied are shown in Table 4. They contribute to the total discrimination in the following order: LaM^1, LaM_1, LM_1, LOID, B, LaI and LM^1. The F-values and classification functions revealed that the most discriminating characteristics were the width of first upper and lower molar (LaM^1 and LaM_1) and the length of the first lower molar (LM_1). Discriminant

Table 4. Results of discriminant function analysis, F-values for the variables and classification functions for the cranial and dental variables of two sibling species of genus *Mus* from Slovakia, the house mouse (*M. musculus*) and the mound-building mouse (*M. spicilegus*). Significant variables are shown with the significance levels: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

	F	*p*	Classification function
LCb	11.86	$p < 0.0001^{***}$	16.22
LN	35.72	$p < 0.0001^{***}$	36.52
LD	28.86	$p < 0.0001^{***}$	–4.10
LOID	106.35	0.0^{***}	39.34
LaZ	36.89	$p < 0.0001^{***}$	–0.47
LaI	64.29	$p < 0.0001^{***}$	61.96
ACr	8.09	0.005^{**}	32.49
LM^1	44.31	$p < 0.0001^{***}$	–63.10
LM_1	285.54	0.0^{***}	98.62
LaM^1	478.12	0.0^{***}	216.34
LaM_1	349.56	0.0^{***}	113.82
CI	7.49	0.007^{**}	–23.66
A	15.93	$p < 0.0001^{***}$	11.41
B	68.72	$p < 0.0001^{***}$	–36.18
Constant			128.72

mm) stated by Cserkész et al. (2008) is a good tool for distinguishing the two species.

Several studies have also dealt with the problem of differences between the two sympatric species, *M. spicilegus* and *M. musculus*. There is valuable data from Austria, which is the north-western edge of the species *M. spicilegus* (Unterholzner & Willenig 2000), and also from central Ukraine (Sokolov et al. 1990, Lyalyuchina et al. 1991). By comparing *M. spicilegus* and *M. musculus* from the Austrian populations, Unterholzner & Willenig (2000) found statistically significant differences in all cranial and dental characteristics measured. In the Slovak population of the studied adult *M. spicilegus*, a higher average value was reached in most of the characteristics compared to *M. musculus*, and they were often statistically significant. These results agree with those of (Macholán 1996b, Unterholzner & Willenig 2000): that *M. spicilegus* individuals reach higher dimensions in the measured characteristics compared to *M. musculus*. In our material, the first upper molar (LM^1) was broader in relation to its length for *M. spicilegus*, whereas in *M. musculus* it was narrower. The same results were obtained for the first lower molar

Table 5. Comparison of quantitative parameters with *p*-values of cranial and dental variables of two sibling species of genus *Mus*. Populations compared are: for *M. musculus*: A – Slovakia, B – Hungary, C – Central Ukraine, D – Poland, E – European part of Russia, F – northern Bulgaria, G – southern Bulgaria, H – Austria (male), I – Austria (female); for *M. spicilegus*: A – Slovakia, B – Hungary, C – central Ukraine, D – Moldova, E – south-eastern Ukraine, F – northern Bulgaria, G – southern Bulgaria, H – Austria (male), I – Austria (female). Legend: LCb – condylobasal length; LFI - length of *foramen incisivum*; LM^1 – length of first upper molar; LOID – length of lower molar row (alveoli); A – breadth of the upper ramus of the zygomatic process of the maxilla, and B – breadth of the zygomatic arch.

	Traits	A-B	A-C	A-D	A-E	A-F	A-G	A-H	A-I
M. musculus	LCb	0.0607	0.0001	0.4379	0.0119	< 0.0001	< 0.0001	1.0	0.2117
	LFI	< 0.0001	-	-	-	< 0.0001	< 0.0001	< 0.0001	0.0434
	LM^1	< 0.0001	-	-	-	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	LOID	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	A	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0051	0.5939	-	-
	B	0.557	< 0.0001	< 0.0001	< 0.0001	0.4767	0.1367	-	-
M. spicilegus	LCb	< 0.0001	0.3504	< 0.0001	0.0737	< 0.0001	-	< 0.0001	< 0.0001
	LFI	< 0.0001	-	-	-	< 0.0001	-	< 0.0001	0.0009
	LM^1	< 0.0001	-	-	-	< 0.0001	-	1.000	0.0002
	LOID	< 0.0001	< 0.0001	0.021	< 0.0001	< 0.0001	-	< 0.0001	< 0.0001
	A	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	-	-	-
	B	< 0.0001	0.43	0.626	< 0.0001	< 0.0001	-	-	-

following three variables contributed considerably to the total discrimination power: the width of first upper molar (LaM^1), breadth of the upper ramus of the zygomatic process of the maxilla (A) and breadth of the zygomatic arch (B). These results are consistent with our findings, which confirmed that the identification function key ($2.1LaM^1 – B = 1.46$ (LM^1). Our results indicate that the limit values of the width of the first upper and lower molars (LaM^1 and LaM_1) do not overlap (Čanády et al. 2014, Csanády & Mošanský 2018), except for LaM^1 adult females in 0.04 % of cases.

The usability of cranial and dental features to determine *Mus* species was mentioned earlier by

Gerasimov et al. (1990). Kryštufek & Macholán (1998) used 31 cranial variables to examine the population of the Adriatic coast (Ulcinj, Montenegro), originally described as *Mus hortulanus* ssp. Metric and non-metric morphological features, coupled with typical mound-building activity, allowed the studied population to be associated with *M. spicilegus*. Similarly, Macholán & Vohralík (1997) confirmed the species status of mice skulls of the genus *Mus* from Albania and northwestern Greece deposited in the collections of Charles University and the National Museum in Prague. The analysis revealed similarities to *M. spicilegus* specimens from the Adriatic coast described at the same time as a different subspecies of *M. s. adriaticus* (Kryštufek & Macholán 1998).

At the same time, some non-metric features are important for distinguishing the two species. For example, Macholán (1996a) stated that the second upper molar (M^2) in *M. spicilegus* is characterized by a concave contour on the lingual side of the tooth, while that of *M. musculus* is rather convex in shape. Another potentially suitable sign, according to Unterholzner & Willenig (2000), is the shape of the proximal end of the *foramen incisivum*, which is mostly rounded in *M. spicilegus* and with a tip in *M. musculus*. Individuals belonging to the species *M. spicilegus* from eastern Slovakian were recognized using these non-metric determinants. At the same time, the incidence of *M. spicilegus* in older museum collections from eastern Slovakia was not confirmed. This is consistent with the data found in 2002 by L. Mošanský (cited by Krištofík & Danko 2003a).

Another problem often encountered in previous morphometric studies was the usability of the zygomatic index (Darviche & Orsini 1982, Orsini et al. 1983, Macholán 1995). The review of metric and non-metric morphological features unambiguously demonstrated the mistakenness of the initial assignment to *M. spicilegus*, but also attributed the individual to *M. musculus*. Later, the same author (Macholán 1996b, 2006), using a detailed morphometric analysis of the genus *Mus*, confirmed that the zygomatic index, despite the distinction between the species, is not necessarily credible for overlapping values. He also offered other determinants, such as the size and shape of the molars. Similarly, different zygomatic index values (A/B) were found within the European populations of *M. spicilegus* and *M. musculus*. Unterholzner & Willenig (2000) reported values for *M. spicilegus* (0.95) and *M. musculus* (0.43) for the Austrian population. Sokolov et al. (1990) and Lyalyuchina et al. (1991) reported a significantly lower index value for *M. spicilegus* (0.5) and *M. musculus* (0.4), but the difference between the two species was confirmed. In Slovak populations, *M. spicilegus* had a zygomatic index of value of 0.90, while *M. musculus* had 0.63. Thus, a highly significant difference was confirmed (Čanády et al. 2014, Csanády & Mošanský 2018). For individuals of *M. musculus*, values not less than 0.70 were found to be consistent with those of Orsini et al. (1983) and Macholán (1996a). Similarly, Cserkész et al. (2008) reported mean values of 0.90 for the zygomatic index of the Hungarian population of *M. spicilegus* and a smaller value of 0.57 for *M. musculus*. These results, obtained for adult individuals in their study, were in accordance with the above-mentioned data. All the mentioned discrepancies in the zygomatic index values are why several authors (Demeter et al. 1996, Macholán 1996b, 2006, Cserkész et al. 2008, Čanády et al. 2014, Csanády & Mošanský 2018) recommend using for determination dental characteristics, whose dimensions, but especially their shape, are specific to the two compared species.

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