Morphometric analysis of European house mice

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Skulls of 892 house mice of five species (*Mus* Linnaeus, 1758), collected from 136 localities across Europe and Morocco, were studied. The analysis revealed that variations in size affected most of the characters considered, indicating a need to size-adjust the data. *M. domesticus* was morphologically the most variable of all the European mice yet this variability was not consistent with the distribution of subspecies *domesticus* and *brevirostris*. The population from Albania was distinct within the *M. domesticus* samples, resembling *M. musculus* in overall size. In *M. musculus*, a W-E gradient of size was found in some variables, especially in females, and a sex dimorphism appeared also in populations from western parts of its range. Among 619 mice from 66 samples across the Czech and Slovak Republics and western Ukraine, but not from populations from western Bohemia, only *M. musculus* was substantiated. In spite of the fact that *M. spicilegus* and *M. macedonicus* are genetically and morphologically very close, as many as 9 variables (both untransformed and size-adjusted) proved to be different between the two species while *M. spretus* was found to be morphologically intermediate. The NW distribution limit of *M. spicilegus* is discussed.

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Introduction

House mice are a group of closely related species of Palaearctic origin representing the last offshoot of the genus *Mus* (Boursot *et al.* 1993). They are characterized by rather inconspicuous interspecific morphological differences which tend to be obscured by intrinsically non-categorical geographical and ecological variation in pelage colour and other characters. The systematics of house mice remained confused for a long time with as many as 133 specific or subspecific taxa described (Berry 1981), the descriptions being highly author-dependent and frequently based on one or few individuals only. In the 1940s, Schwarz and Schwarz (1943) attempted to simplify mouse taxonomy by recognizing only a single species, *Mus musculus*, and their approach was followed by other authors (Ellerman and Morrison-Scott 1951, Serafiński 1965, Corbet 1978, Reichstein 1978). However, this concept oversimplified hierarchical relationships among

house mouse taxa ignoring such phenomena as absence of interbreeding between some of them etc (Sage 1978, Bonhomme *et al.* 1983).

The introduction of biochemical and molecular genetics into population and systematic biology has clarified the evolutionary history and relationships within the house mice complex (for the most recent reviews on evolution of mice, see Boursot *et al.* 1993 and Sage *et al.* 1993). It has been shown that there are five taxa of house mice in Europe (Marshall and Sage 1981, Thaler *et al.* 1981, Bonhomme *et al.* 1984) representing two separate major lineages. One lineage consists of three allopatric "aboriginal" (Sage 1981) outdoor species: *Mus spretus* Lataste, 1883, *M. spicilegus* Petényi, 1882, and *M. macedonicus* Petrov *et* Ružić, 1983. Two commensal, indoor taxa *M. musculus* Linnaeus, 1758, and *M. domesticus*, Schwarz *et* Schwarz, 1943 (after Corbet 1988), belong to the second major lineage. The two last forms meet along a narrow, 1200 km long, hybrid zone in Europe extending from Jutland to the Black Sea. Whereas there is a wide agreement about the specific status of the former three taxa (although see Marshall 1986 and Mezhzherin 1994 for different viewpoints), there is no consensus on the classification of the latter two forms. One approach is to regard all commensal mice as members of a single species, *Mus musculus* (Bonhomme and Guénet 1989, Auffray *et al.* 1990a), whereas an alternative approach is to recognize both the major evolutionary lineages within the group of commensal mice as distinct species (Ferris *et al.* 1983, Sage *et al.* 1986, 1993, Prager *et al.* 1993). Following Marshall (1981, p. 20) and Sage *et al.* (1993, p. 525), all the European house mouse taxa are regarded as separate species throughout this paper.

Advances in biochemical and molecular methodology with an unequivocal genetical characterisation of investigated animals has enabled methods such as morphometrics to make substantial progress in mouse systematics. Various papers in the last two decades have dealt with the uni- and/or multivariate morphometrics of house mice although they mostly focused on only one or a few species (Sans-Coma *et al.* 1979, Thorpe *et al.* 1982, Engels 1980, 1983, Darviche and Orsini 1982, Davis 1983, Palomo *et al.* 1983, Lyalyukhina *et al.* 1991, Scriven and Bauchau 1992, Lavrenchenko 1994). Gerasimov *et al.* (1990), using electrophoretically determined individuals, established diagnostic keys for all the European mouse species. Unfortunately, because of the complexity of the discriminant equations and equivocal description of individual variables they seem to be of little practical use. Moreover, it is not certain if these keys can also be used for populations from other parts of Europe.

The aims of this study are (1) to determine the basic statistics for and (2) reveal morphometric and morphological relationships between populations of the five European house mouse species, (3) to evaluate the specific status of central European populations of mice, and (4) to describe the occurrence/absence of *M. spicilegus* in the territory of the former Czechoslovakia and specify the NW border of its range.

Material and methods

Mouse skulls used in this study are deposited in the collections of the Institute of Landscape Ecology in Brno, National Museum and Charles University in Prague, Museum of Natural History in Vienna, University of Lausanne, and University of Montpellier.

A total of 892 skulls were measured, originating from 136 localities throughout Europe and northern Africa. Since only one or few skulls were available from some sites, samples were pooled into geographically natural groups so long as interpopulation differences within such groupings were negligible (Table 1). *M. musculus* populations were thus pooled into Bohemian (MC), Moravian (MM), Slovakian (MS), and Hungarian (MH), samples; mice from two Ukrainian localities (MU), previously regarded as *M. spicilegus* (Štěpánek 1934), have been recently reassigned as *M. musculus* (Macholán 1995). *M. domesticus* samples were grouped into three groups: DCH from south and south-western Switzerland (presumably *M. d. domesticus*), DA from Albania (*M. d. brevirostris*), and finally, a somewhat heterogenous group DWM from western Mediterranean islands, representing also *M. d. brevirostris* (Ellerman and Morrison-Scott 1951, Keller 1976, Reichstein 1978, Marshall 1981). A group of populations from the *domesticus/musculus* hybrid zone in western Bohemia (Macholán and Zima 1994) was included as a separate sample (MSP).

Table 1. List of population groups. A code and name of a respective group, specific appurtenance, and number of animals examined within each group are given.

Code	Species	Origin	*n* of animals
MC	*M. musculus*	Bohemia (Czech)	83
MM	"	Moravia (Czech)	322
MS	"	Slovakia	181
MH	"	Hungary	44
MU	"	Ukraine	33
MSP	*Mus* sp.	W Bohemia	29
DA	*M. domesticus*	Albania	10
DCH	"	Switzerland	32
DWM	"	W Mediterranean	59
GR	*M. macedonicus*	Greece	45
AUT	*M. spicilegus*	Austria	20
SPR	*M. spretus*	France, Spain, Morocco	34
Total			892

Aboriginal mice samples were pooled into specific groups: AUT – *M. spicilegus* (the vicinity of Lake Neusiedl, Austria), GR – *M. macedonicus* (Greece), and SPR – *M. spretus* from France, Spain and Morocco. Although this paper concentrates on European house mice populations, mice from Morocco are included in the *spretus* sample in order to increase the number of animals of the species given that there were no significant morphometric differences between European and African populations (M. Macholán, unpubl. Ph D thesis). All the localities and numbers of animals examined are listed in Appendix 1.

The study is based on 19 cranial and dental variables: A – breadth of the upper ramus of the zygomatic process of maxilla, B – breadth of the zygomatic process of maxilla, LCb – condylobasal length, LB – basal length, LN – length of os nasale, LaR – rostral breadth, LaC – breadth of the skull per bullae, LaZ – zygomatic breadth, hC – height of the braincase, LD – length of the diastema, LM1i – length of the first lower molar, LaM1i – M_1 breadth, LM2i – M_2 length, LM3i – M_3 length, LaM3i
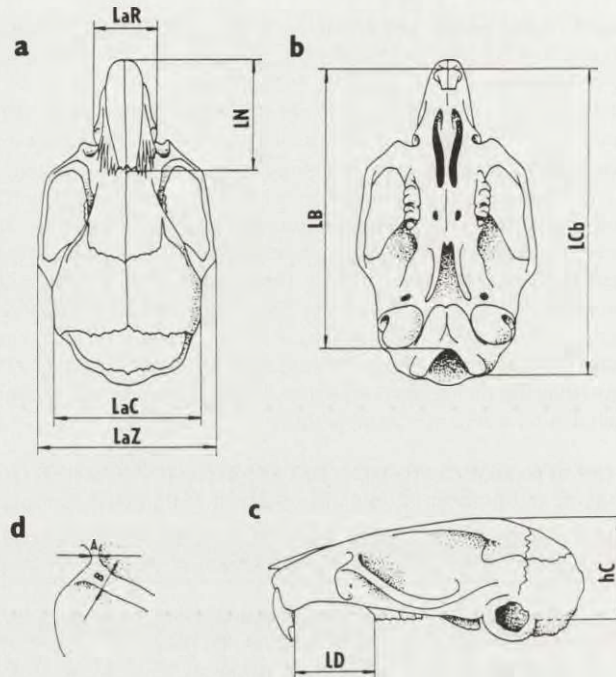
Fig. 1. Cranial measures used in the study. Dorsal (a), ventral (b), and lateral (c) views of the mouse skull; (d) a detail of the upper part of the zygomatic plate with the zygomatic process of maxilla. Consult the Material and methods section for expalantions of symbols.

– M_3 breadth, LM13i – length of the lower molars row, LM1s – length of the first upper molar, LM2s – M^2 length, LM13s – length of the upper molars row (see also Fig. 1). All the measurements were taken with a calliper. To avoid undesirable variation due to potential asymmetry, the right side was always measured in paired characters, with the exception of the variables A and B which were measured on both sides and averaged. In addition to the skull variables, two body dimensions were taken from museum records: the head-and-body length (LC) and the length of the tail (LCd).

Adult individuals only were measured. Determination of adulthood involved taking into account the condition of the reproductive organs; all mice weighing less than 10 g were considered juvenile (Laurie 1946, Pelikán 1981); age was also assessed from tooth wear (Lidicker 1966) – animals older than two months (age classes 3–8) only were taken. When possible, all the three approaches were combined since the latter two are influenced to some extent by nutritional conditions and possibly other factors. All individuals, in which there were any doubts as to age, were excluded from the subsequent investigation.

One-way analysis of variance (ANOVA) was used to detect variation among groups for each variable. As there were very high between-group differences in numbers of animals analysed, all those specimens with missing variables were excluded from the large groups and 50 individuals were then randomly chosen from these samples using a random number generator. Before computing ANOVA, the Kolmogorov-Smirnov test for the normal distribution of variables and Bartlett's test for homogeneity of variances were applied.

To improve normality, the Box-Cox transformation was used on those variables appearing to have a non-normal distribution (Sokal and Rohlf 1981). Since the deviation from the normal distribution of a particular variable was not of the same character across the populations (for example, one sample might be right-skewed while another skewed to the left and yet another platykurtic), a "compromise" approach had to be used seeking for an approximate value of which would both improve normality and reduce heteroscedasticity of *individual subsamples*. In variables still displaying considerable deviations from normality and/or homogeneity of variances after the transformation, the non--parametric Kruskal-Wallis test was used instead of ANOVA. In order to establish which of the populations are distinct, the Tukey HSD multiple pairwise comparisons were performed. As this procedure requires counts per population to be equal, the Tukey-Kramer adjustment by a harmonic mean was applied. In non-parametric variables, pairwise comparisons using the Mann-Whitney U-test with Bonferroni-adjusted probabilities were used instead of the Tukey HSD (Bonferroni critical values guarantee that the Type I error rate will not be greater than a chosen critical value divided by the total number of comparisons). The SYSTAT package (Wilkinson 1990) was used for all statistical procedures.

Results

Due to limited space, the basic descriptive statistics (means, standard deviations, minimal and maximal values) for raw data are not shown here (they are available from the author upon request). Even though the analysis is focused on skull variables both body dimensions, LC and LCd, were included because the relative tail length (LCd/LC) is frequently used for the discrimination of *M. domesticus* (tail longer than body), *M. musculus* (tail of medium length, slightly shorter than body) and the three aboriginal species (short tail).

As shown in the box plot of the relative length of the tail (Fig. 2), mice separated into three groups, one consisting of animals with the tail on average slightly more than 100% of the body (*domesticus* and the W Bohemian hybrid population); the second mainly *musculus* populations with the relative tail length about 90%; and finally, *spicilegus*, *spretus* and *macedonicus* mice with tails of 70–85% of the body length. Nevertheless, there were large overlaps between *M. musculus* and *M. domesticus* and between *M. musculus* and *M. spicilegus* + *M. macedonicus* which

Fig. 2. A notched box-and-whiskers plot of the relative tail length. The median is marked by the centre horizontal line. Edges of the central box are the lower and upper hinges, ie the box represents the interquartile range (Hspread). The whiskers show the range of values which fall within 1.5 Hspreads of the hinges. They do not necessarily extend to the inner fences which represent exactly hinges ± 1.5 Hspread. Values outside the inner fences are marked with asterisks; values outside the outer fences (hinges ± 3 Hspreads) are indicated by circles. The boxes are notched at the median and the width of the notched area displays the 95% confidence interval of the median. See Table 1 for explanations of abbreviations.
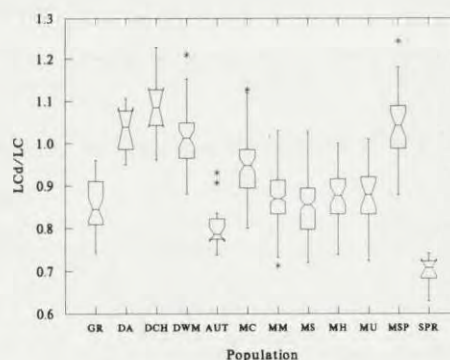
could muddle identification. More strikingly, a slight overlap was found even between *M. domesticus* and both eastern aboriginal species, *M. spicilegus* and *M. macedonicus*.

However, as the body dimensions were almost exclusively taken from various collection records (with the likelihood of different or nonstandard ways of measuring) their reliability could not be guaranteed and neither variable was taken into account in further analyses. In addition, LN was also rejected as the nasals were damaged in many of the skulls examined.

No significant sex dimorphism was detected within the *macedonicus*, *spicilegus*, *spretus*, and *domesticus* populations. On the other hand, females from some *M. musculus* populations, especially from western parts of the range, tended to have significantly higher values of "size" variables (LCb, LB, LaZ), and an apparent west-to-east gradient of this dimorphism was revealed. In Fig. 3, the LCb values are plotted against the longitude: while the condylobasal length significantly decreased from west to east in females ($p \leq 0.001$), for males the slope of the regression line was not significantly different from zero ($p \geq 0.05$).

For all variables, the results of either ANOVA or the Kruskal-Wallis test were highly significant. The results of Tukey HSD multiple pairwise comparisons are summarized in Appendix 2. The following conclusions can be drawn from it: (1) All the *musculus* populations, including that from Ukraine, are homogenous. (2) MC females resemble *domesticus* more than do MC males and other *musculus* mice. The MSP hybrid population is very close to *M. domesticus*. (3) As many as 7 variables were significantly different between DA and DWM. Mice from Albania (DA) are strikingly similar to the *M. musculus* populations, probably due to their small size. (4) *M. spicilegus* from Austria resemble *M. domesticus* (1–4 different variables $\alpha = 0.001$) and *M. spretus* (2 variables) more than *M. macedonicus* (9 variables).
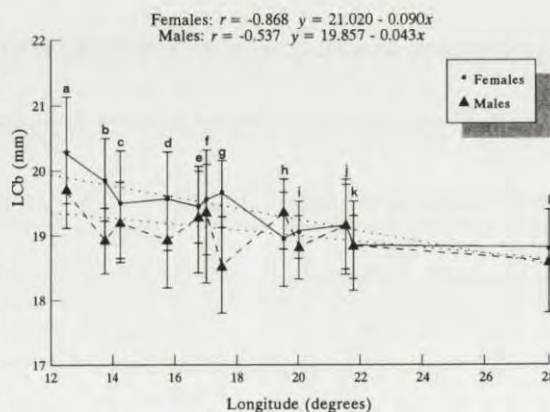

Fig. 3. A plot of LCb against the longitude. Localities: (a) Kostelní Bříza, (b) Vimperk (4 localities close to each other), (c) Milevsko, (d) Brtnice, (e) Brno, (f) Břeclav, (g) Dunajská Streda, (h) Kiskunhalaza, (i) Rimavská Sobota (2 localities), (j) Hajdu-Bihar (2 localities), (k) Ruská Poruba, (l) Ukraine (2 localities). Note: four animals from Ukraine (l) were only available with determined sex, one female and three males.

Table 2. List of cranial and dental variables showing significant differences between pairs of species. DA population from Albania was excluded from the *M. domesticus* sample.

Pairs of taxa	Levels of significance	
	$0.001 < p \leq 0.01$	$p \leq 0.001$
musculus/domesticus	LM13s	LCb, LB, LaC, LaZ, hC, LD, LM1i, LaM1i, LM2i, LM13i, LM1s
musculus/macedonicus		A, B, LCb, LB, LaC, LaZ, hC, LD, LM1i, LaM1i, LM2i, LM3i, LaM3i, LM13i, LM1s, LM2s, LM13s
musculus/spicilegus	LM2i	A, B, LaR, LaC, LaZ, hC, LM1i, LM3i, LM13i, LM2s, LM13s
musculus/spretus		A, B, LD, LM1i, LaM1i, LM2i, LM3i, LaM3i, LM13i, LM1s, LM2s, LM13s
domesticus/macedonicus	hC, LM1s	A, B, LaR, LaZ, LaM1i, LM2i, LM3i, LaM3i, LM13i, LM2s, LM13s
domesticus/spicilegus	LaM1i	A, B, LM1s, LM2s
domesticus/spretus	hC, LM1i, LaM1i, LM3i	A, B, LM13i, LM13s
macedonicus/spicilegus	hC	B, LaR, LaM1i, LM2i, LM3i, LaM3i, LM13i, LM1s, LM13s
macedonicus/spretus	LM2i, LM2s, LM13s	LCb, LB, LaR, LaC, LaZ, hC, LM3i, LaM3i, LM13i
spicilegus/spretus	B, hC, LM1i	LaM1i, LM2i, LM1s

The results presented in Appendix 2 and Table 2 suggest that variations in size (whether due to the different age structure of populations, ecological variables or other factors) affected most of the analyzed characters, thus making difficult the assessment of interrelationships. Removal of this influence is thus necessary. Two approaches were used: the first method was based on removing the size information from the data using a kind of bivariate transformation; the second approach took relations between selected variables (eg the length-to-width of the skull or molars) as a rough indicator of shape.

Numerous univariate and multivariate transformations are available for negating size information from data. Among the most popular techniques are the logarithmic transformation, creation of a ratio between particular variable and some standard size measure, and taking the logarithm of that ratio. Because of some undesirable statistical properties of these transformations (Atchley *et al.* 1976, Atchley 1978, Thorpe 1983b, Reist 1985) several techniques have been suggested derived from allometric relations between body or skull parts (Thorpe 1975, Corruccini 1977, Kuhry and Marcus 1977; see also Reist 1985 for a review). Because each original specimen should be represented by a set of transformed variates in this study rather than by some multivariate component scores, a matrix was created of allometrically adjusted variates, ie measures adjusted to those

expected for a mean skull size by an allometric formula of Thorpe (1975). Such adjusted variates are predictions of what an individual's value of a particular variable would be if the individual was of overall mean size. Two possible types of slopes may be extracted from the data. To use the total pooled sample for computing the slope of the regression line, equality of within-group regression slopes is required. This assumption was tested and when violated (as in the case of LaZ) the within-group slopes were computed.

No differences were found between males and females of *M. musculus*; similarly, the whole *musculus* sample appeared to be homogenous when as few as two pairwise differences were ascertained in total (LaZ between MM and MS; and LM2s between MH and MU, $\alpha = 0.001$). Within the *M. domesticus* group, the most distinct populations appeared to be DA and DWM (LaR, LaC*, LM1i, LaM1i*, LM1s*, LM13s*, $\alpha = 0.001$ and $\alpha = 0.01$*, respectively), whereas the DCH vs DWM comparison revealed 3 variables (LM1i*, LaM1i, LM13s*), and DA vs DCH one measure (LaR) only.

Table 3. List of cranial and dental variables showing significant differences between pairs of species/populations. DA population from Albania is included.

Pairs of species/populations	Levels of significance	
	$0.001 < p \leq 0.01$	$p \leq 0.001$
musculus / domesticus	LaM1i	LaR, LM13i, LM1i, LM1s
musculus / DA		LaR
musculus / macedonicus	LaC	A, B, LaZ, hC, LM1i, LaM1i, LM2i, LM3i, LaM3i, LM13i, LM1s, LM2s, LM13s
musculus / spicilegus	LaC, hC	A, B, LaR, LaZ, LM1i, LM3i, LM13i, LM2s, LM13s
musculus / spretus	LaR, LaM3i, LM2s	A, LD, LM1i, LM2i, LM3i, LM13i, LM1s, LM13s
domesticus / DA	LM1i	LaR
domesticus / macedonicus	LaC	A, B, LaR, LaZ, LaM1i, LM2i, LM3i, LaM3i, LM13i, LM2s, LM13s
domesticus / spicilegus	LM1s	A, B, LaZ, LM3i, LM2s
domesticus / spretus	hC, LM2i	A, B, LM1i, LaM1i, LM3i, LM13i, LM2s, LM13s
macedonicus / DA	LM2s	A, B, LaR, LaC, LaZ, LM1i, LaM1i, LM2i, LM3i, LaM3I, LM13i, LM1s, LM13s
macedonicus / spicilegus		B, LaR, LaM1i, LM2i, LM3i, LaM3i, LM13i, LM1s, LM13s
macedonicus / spretus	B, LaZ	LaC, hC, LM3i, LaM3i
spicilegus / DA	LaZ, LM2s, LM13s	A, B, LaR, LaC, LM13i
spicilegus / spretus	B, LaZ, LM1i	LaC, hC, LaM1i, LM1s
spretus / DA	LaZ	A, B, LaR, LM1i, LaM1i, LM2i, LM13i, LM1s, LM13s

Table 3 shows significant differences between pairs of species. The Albanian sample was kept separate in order to check its relation to the *musculus* group. Rather surprisingly, as many as 9 variables were found to differ between *M. macedonicus* and *M. spicilegus* at α = 0.001 whereas only 4 variables (6 at α = 0.01) and 5 variables (8 at α = 0.01) were significantly different between *macedonicus* and *spretus*, and between *spicilegus* and *spretus*, respectively. Apparently, dental measures, both untransformed (Table 2) and size-adjusted (Table 3), were those which discriminated best between these species and also between the aboriginal and commensal lineages.

The lengths of the lower and upper tooth-rows, adjusted for the skull size, displayed the highest values in the outdoor species and all the species (+ the DA
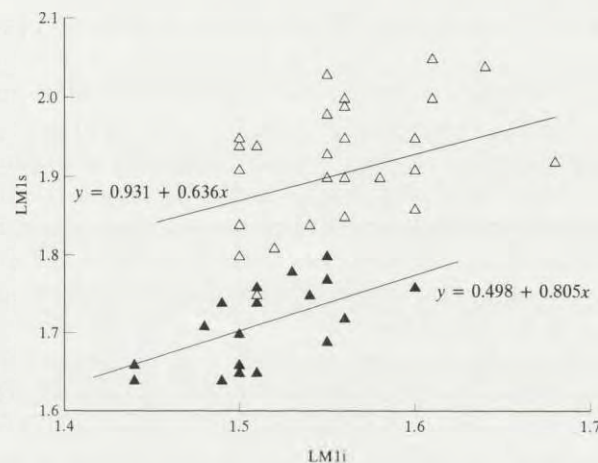

Fig. 4. A scatter plot of LM1s against LM1i with regression lines for *M. macedonicus* (open triangles) and *M. spicilegus* (closed triangles).

population) were ranked in the following increasing order: DA < MUS < DOM < SPI < SPR < MAC. On the other hand, when the upper and lower first molars were compared, there was a clear separation of *M. spicilegus* from *M. macedonicus*, regardless of whether the raw or transformed data were taken (Fig. 4).

If the variables are summed whose means differ between the groups at α = 0.01, the samples appear to be separated into two main clusters, namely the group of the aboriginal species and the commensal taxa, closely related to each other. *M. macedonicus* groups with *M. spretus* while *M. spicilegus* is the most distinct member of the aboriginal group. As there is just single significant difference between the DA population and the *musculus* sample whereas two differences are found between the former and the *domesticus* group, the Albanian mice are strikingly clustered with *M. musculus* rather than with *M. domesticus*.
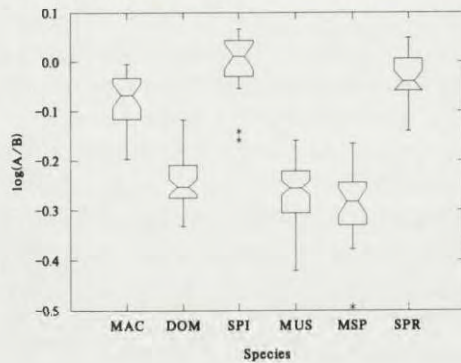
Fig. 5. A notched box plot of the zygomatic index, expressed as log(A/B). Abbreviations: MAC – *M. macedonicus*, DOM – *M. domesticus*, SPI – *M. spicilegus*, MUS – *M. musculus*, MSP – the *musculus/domesticus* hybrid population from western Bohemia, SPR – *M. spretus* (see Fig. 2 for more explanations).

As indicated above, a comparison of two measurements of an object (length to width, for example) can serve as a rough guide of its shape. Even though creation of ratios can have undesirable consequences for some statistical analyses (Atchley *et al.* 1976, Thorpe 1983b), using simple ratios has an advantage over multivariate analyses of shape in that they are easily understandable and clearly attributable to particular characters. The condition required is the linear relationship between compared variables. To render linearity logarithms were taken from the ratios. The requirement that the relationship between the numerator and denominator of a ratio should pass through the origin (Thorpe 1983b) could be neglected because we are not attempting to adjust for size.

The first ratio, the so called zygomatic index (ie breadth of the upper ramus of the zygomatic process of maxilla / breadth of the zygomatic process of maxilla = A/B ratio, see Fig. 1d) is not, strictly speaking, "a shape ratio" yet it is included here as it has been claimed as the diagnostic factor distinguishing aboriginal and commensal mice (Darviche and Orsini 1982, Kratochvíl 1986a, b, Lyalyukhina *et al.* 1991). However, even though this ratio clearly distinguished both groups there was an overlap of values between them (Fig. 5) and this key cannot be considered absolute.

When the length-to-width relation of the third and first lower molars were assessed, two pictures emerged. Whereas the former ratio indicated that M_3 of the commensal species were, on average, wider than longer (while the opposite was true for the aboriginal taxa), the relation LM1i/LaM1i clearly separated *M. spicilegus* (M_1 relatively very narrow) from *M. macedonicus* (broad M_1) with the third group of *domesticus, musculus*, and *spretus* intermediate. When LaM1i is plotted against LM1i in *spicilegus* and *macedonicus* mice their mutual distinctness is illuminative (Fig. 6). Although differences among taxa were rather inconspicuous in both the relative width of the skull and the zygomatic breadth it could be seen that the skull of *M. spicilegus* and *M. musculus* were relatively broad compared to *M. domesticus*; the same picture was displayed by the LaZ/LCb ratio though zygomatic arches of *macedonicus* were more vaulted than those of *musculus*.
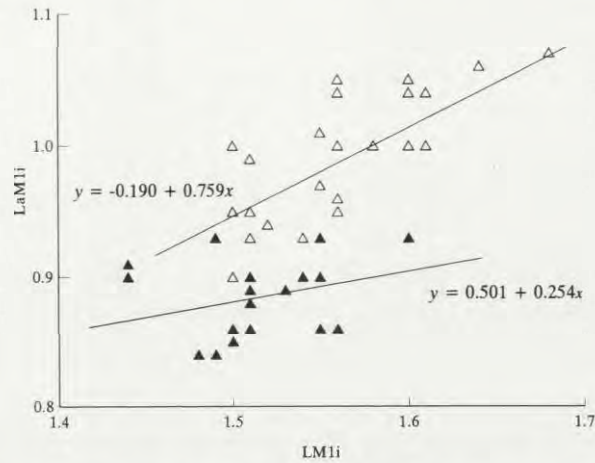
Fig. 6. A scatter plot of the width (LaM1i) against the length (LM1i) of the first lower molar with regression lines for *M. macedonicus* (open triangles) and *M. spicilegus* (closed triangles).

Relative rostral width appears to be another helpful guide of the shape differences between mouse taxa, whether based on condylobasal length (not shown here) or to skull breadth (Fig. 7). In the former case, individual populations show a west-to-east gradient of the LaR/LCb ratio across *musculus* populations whereas in the latter, the pooled *M. musculus* group is shown as there were no differences among individual *musculus* populations. *M. musculus* has the broadest rostrum among the house mouse species, followed by *M. macedonicus* and *M. spretus*; *M. spicilegus* was the species with the relatively narrowest rostrum while there was a great variation across the *M. domesticus* sample, the Albanian population showing far the lowest values of both the LaR/LCb and LaR/LaC ratios of all the groups studied.

In Fig. 8a, rostral breadth is compared with the length of the diastema in relation to condylobasal length. In this trivariate graph, the highest relative values approximate the tops of the triangle whereas the lowest values are close to the
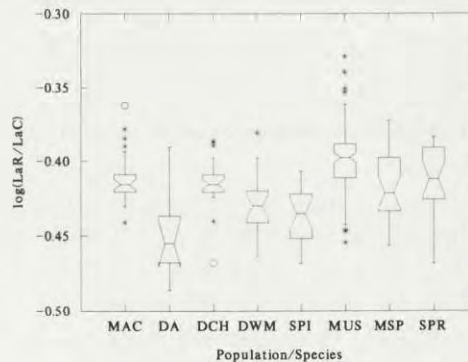

Fig. 7. Relative rostral width expressed as the logarithms of the LaR/LaC ratios. All the *musculus* populations were pooled in the plot. Populations and species labelled as in previous figures.
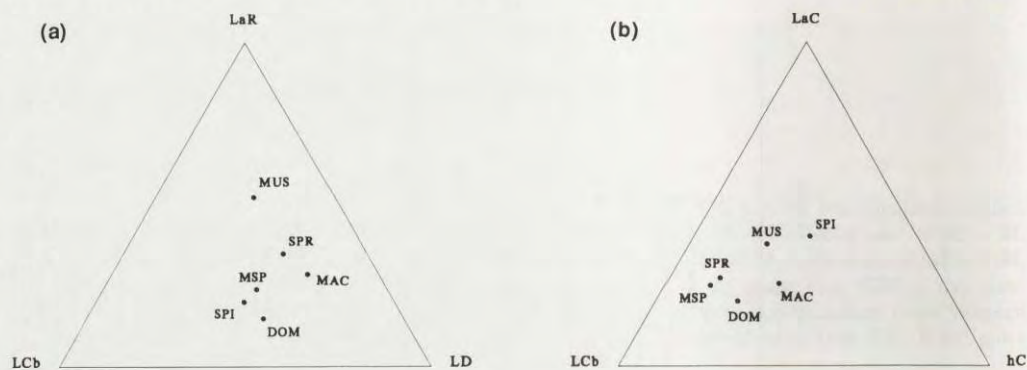
Fig. 8. Trivariate plots of the LaR-LD-LCb (a) and LaC-hC-LCb (b) relations among group centroids. Mean values for each of the variables for each species are transferred to relative values to give the sum of 100% (see Wilkinson 1990 for details).

opposite base (see Wilkinson 1990 for details). *M. musculus* has, on average, a relatively very short and broad rostrum; in the contrast, the rostrum of *M. spicilegus* and *M. domesticus* is long and narrow with *spretus* and *macedonicus* falling in between. Similarly, when cranial length and width, and height of braincase are compared (Fig. 8b) *musculus* and *spicilegus* have a relatively short and broad skull in contrast to narrow and long skull of *domesticus* and *spretus*; in hC, only minor differences were revealed, with the braincase of *macedonicus* and *spicilegus* being slightly higher than *musculus*, *domesticus*, and *spretus* (the last species has relatively the flattest braincase of all the species studied except for the MSP hybrid population).

Discussion and conclusions

Although absolute "size" measures can be highly heritable (Thorpe 1981, 1983a, Atchley 1983, Thorpe and Leamy 1983) and provide valuable information on the similarity or dissimilarity of taxa, the size variation in organisms with inde-terminate growth can cause a substantial bias which is likely to perturb an assessment of between-groups relationships. In this study, the bias in the growth stage was minimized by considering adult individuals only. Nevertheless, absolute morphometric variables are usually dependent on size so that a high within-group correlation between them merely indicates that each character is repeatedly measuring a similar facet of the phenotype (Thorpe 1983a). Moreover, nutritional, sexual, ecological, seasonal and other factors (Leamy 1981, Leirs *et al.* 1993) are also likely to affect morphological characters, obscuring the major patterns of interracial and interspecific variation. Data transformations to adjust for size are thus essential; comparisons in terms of size variation are only discussed in the following text when it is relevant to the study.

M. domesticus appeared to be morphologically the most variable species of all the European house mouse taxa examined, which is consistent with the level of genetic variability of this species (Sage 1981, Berry 1981). Nevertheless, even though fairly large differences were found between *domesticus* samples these were not congruent with traditional classification into subspecies *domesticus* and *brevirostris*. This suggests that these taxa, distinguished on the basis of coat colour differences, are not natural units and using the subspecific categories is inadvisable (Thorpe 1987). This conclusion is supported by other morphological and genetical studies (Engels 1980, Darviche and Orsini 1982, Ferris *et al.* 1983, Wilson *et al.* 1985).

Numerous size and shape differences of the Albanian mice from other *M. domesticus* populations and their close similarity to *M. musculus* implies either the introgression of genes across the *domesticus/musculus* hybrid zone or a distinct systematic position of this population within commensal house mice. However, given the geographical distance of the sampling sites from the zone and highly significant differences in such morphometrical characters as the rostral width the former possibility does not seem likely. Moreover, diagnostic nonmetrical traits (see Macholán 1996) classified these mice as *M. domesticus* (M. Macholán, unpubl. Ph D thesis). Nevertheless, the question of their systematic relationships to other commensal populations remains open untill larger sample or more variables are evaluated.

In comparison with *M. domesticus*, *M. musculus* appeared to be more homogenous though an apparent decline of certain "size" variables (LC, LCb, LB, LaZ) from the west to the east was revealed. This tendency was more marked in females and significant differences exist between sexes in populations from the western parts of the species range. On the other hand, differences between *M. musculus* populations in size-adjusted variates were uncommon, supporting tentative attribution of mouse populations from the former Czechoslovakia and western Ukraine to the Linné's house mouse, *M. musculus* (Macholán 1995). The only exception was in the westernmost part of Bohemia where morphologically and genetically *domesticus* mice and hybrid individuals with *musculus* occur (Macholán and Zima 1994).

In spite of data from 619 mice from 64 localities across the Czech and Slovak Republics and 2 sites in western Ukraine, the occurrence of the mound-building (hillock) mouse, *M. spicilegus*, was not substantiated (Fig. 9). This conflicts with other reports of this species or its "transitional forms" from Slovakia (Jeitteles 1862, Babor 1943, Hanzák and Rosický 1949, 1950, Ferianc 1956, Feriancová-Masárová and Hanák 1965, Štollmann 1985). From published ecological, morphological and biological data, however, it is obvious that these resulted from a misidentification of *M. musculus* when the discrimination was mostly based on the coat colour and/or the relative tail length (Ferianc 1949, Mošanský 1957, Serafiński 1965). Although there were reports on the occurrence of typical mounds of *M. spicilegus* from southern parts of Slovakia in the last and the beginning of this century (Petényi 1841 and M. Uher cited in Ferianc 1949, p. 31 and p. 33, respectively) their

Fig. 9. Collecting sites in NE Austria and S Slovakia. Open triangles – *M. spicilegus*, open circles – *M. musculus*. The occurrence of the mound-building mouse outside the Lake Neusiedl Plateau has not been substantiated in the material under study. The sites from which *spicilegus* and *musculus* mice were studied electrophoretically are indicated by closed symbols.

contemporary presence even in adjacent areas south to the Danube was not sustained (M. Macholán, unpubl.). So it seems that this species has recently retreated southwards and the Parndorf Plateau may be considered the NW border of its range (though rare migrations to the north cannot be completely excluded).

Although *M. spicilegus* and *M. macedonicus* are genetically and morphologically very similar (Bonhomme *et al.* 1983, 1984, Gerasimov *et al.* 1990) as many as 9 variables were found to be significantly different ($p < 0.001$) between these species (as opposed to only one variable reported by Gerasimov *et al.* 1990) regardless if the raw or transformed data were used. On the other hand, *M. spretus* appeares to be morphologically intermediate. When untransformed variables were assessed, *M. spretus* displayed more variables in common with *M. spicilegus* whereas evaluation of size-adjusted data revealed greater similarity with *M. macedonicus*. This agrees with the conclusions of Marshall and Sage (1981) who described the skull of *macedonicus* (therein referred as *M. abbotti*) to be similar to that of *spretus* but larger. Whether this similarity mirrors the ecological vicariance of these species (Auffray *et al.* 1990a, b) is not yet clear.

Using ratios is very common in systematic studies and many taxonomic keys are based on them. Here, the two most frequently used ratios, relative tail length and zygomatic index (Darviche and Orsini 1982, Orsini *et al.* 1983, Kratochvíl 1986a, b, Auffray *et al.* 1990b, Macholán 1996) were evaluated. Whereas the former was only a very rough guide for discriminating between three groups, *domesticus* × *musculus* × *spicilegus, spretus,* and *macedonicus,* the latter distinguished between commensal and aboriginal mice rather efficiently even though it cannot

be regarded as absolute because of the overlap of values (see also Lyalyukhina *et al.* 1991).

Other bivariate and trivariate comparisons were employed to examine the species interrelationships in the shape of the skull and molars. *M. spicilegus* and *M. musculus* have, on average, relatively short and broad skulls while *M. domesticus* and *M. spretus* are characterized by long and narrow skulls; the skull of *M. macedonicus* appeared to be of an intermediate width but with more vaulted zygomatic arches. *M. musculus* had by far the broadest and shortest rostrum as opposed to *M. domesticus* and especially *M. spicilegus* with their long and narrow rostrum. These results agree well with descriptions given in Marshall and Sage (1981), and Marshall (1981, 1986).

Dental traits show a tendency in the commensal species for the reduction of M3 (Engels 1980) which is regarded as a derived character in murids (Jacobs 1985). This reduction is also apparent in the maxillary and mandibular tooth-row lengths, adjusted for the skull size, which were found to be significantly lower in the commensal mice group. On the other hand, the shape of M_1 separated the two eastern outdoor species, *M. macedonicus* with a relatively broad molar, from *M. spicilegus*, in which M_1 was the narrowest of all the species studied. It should be noted that the molar measures, when size-adjusted, are more informative than the skull variables and yielded the most discriminating criteria.

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APPENDIX 1. List of localities and numbers of animals investigated (in parentheses).

Mus musculus Linnaeus, 1758
    BOHEMIA (MC): *Sokolov district*: Kostelní Bříza (18), Rudolec (1); *Karlovy Vary distr.*: Stráž n.
O. (4); *Prachatice distr.*: Jaroškov (1), Masákova Lhota (4), Vimperk (7), Zdíkovec (12); *Most distr.*:
Horní Jiřetín (6); *Písek distr.*: Milevsko (21); *Louny* (5); *Teplice* (4).
    MORAVIA (MM): *Břeclav distr.*: Břeclav (118), Lednice (20), Sedlec (2), Týnec (2); *Brno* (28);
Jihlava distr.: Brtnice (45); *Kroměříž distr.*: Chropyně (10); *Žár n. S. distr.*: Dolní Rožinka (1);
Prostějov distr.: Doloplazy (2); *Přerov distr.*: Prusy (1); *Hodonín distr.*: Čejkovice (5), Dubňany (1),
Dolní Bojanovice (3), Hodonín (7), Jindřichov (5), Josefov (7), Lužice (2), Mutěnice (9), Prušánky (1);
Vyškov (13); *Blansko distr.*: Kulířov (2), Lažánky (3), Vilémovice (2); *Šumperk distr.*: Jeseník (7),
Šumperk (1); *Znojmo distr.*: Šatov (1); *Opava* (9); *Senica distr.*: Holíč (3), Popudinské Močidlany (8),
Radimov (3), Vrádište (1).
NOTE: although Senica district belongs politically to Slovakia as it is geographically closer to southern
Moravian localities (from the geological point of view it is a part of the Czech Basin in fact) the latter
four localities were included into the MM sample.
    SLOVAKIA (MS): *Trnava distr.*: Boleráz (10); *Zvolen distr.*: Dobrá Niva (4); *Poprad distr.*: Dolný
Smokovec (7); *Dolný Kubín* (2); *Dunajská Streda distr.*: Dunajská Streda (2), Gabčíkovo (9), Kl'účovec
(5); *Lučenec* (1); *Nové Zámky* (3); *Levice distr.*: Plášovce (1), Tupá (3); *Bratislava distr.*: Ivanka pri
Dunaji (1); *Trebišov distr.*: Král'ovský Chlmec (4), Leles (7), Vel'ké Kapušany (9), Viničky (9);
Rimavská Sobota (14); *Rožňava distr.*: Rožňava (18), Silica (2); *Košice distr.*: Šaca (3); *Komárno distr.*:
Zemianská Ol'ča (3); *Vranov n. T. distr.*: Ruská Poruba (64).
    HUNGARY (MH): Budapest (3); Kiskunhalaza (11); Kétpo (17); Hajdu-Bihar pusztas (4); Szeged
(8); Szolnok (1).
    UKRAINE (MU): Mukačevo (11); Velké Komňaty (22).

Mus sp. Linnaeus, 1758:
    WESTERN BOHEMIA (MSP): *Cheb distr.*: Dolnice (7), Horní Ves (6), Hrozňatov (1), Klest (2),
Polná (4), Střížov (7), Trojmezí (3).

Mus domesticus Schwarz *et* Schwarz, 1943:
    ALBANIA (DA): Queparo (6); Sarandë (4).
    SWITZERLAND (DCH): *Ticino*: Chiasso (1), Claro (10), Grüsch (1), Lumino (1), Olivone (1),
Quartino (2), San Vittore (2); *Vaud*: Froideville (1), Noville (1); *Valais*: Ardon (1), Leytron (1), Réchy
(2), Riddes (1), Sembracher (1); *Graubünden*: Leggia (1), Müstair (1), Poschiavo (2); *France*:
Brenthomme (1).
    WESTERN MEDITERRANEAN ISLANDS (DWM): *Corse*: Ajaccio (7), l'Ospedale (5), Monaccia
(19), Palveroso (1), Porto Vecchio (5), Sagone (4), Talone (4); *Sicily* (8); *Malta*: Gozo (1); *Imera* (3);
Lampedusa (2).

Mus macedonicus Petrov *et* Ružić, 1983:
    GREECE (GR): *Alexandroupoli* (3); *Komotini distr.*: Porto Lagos (2); *Thessaloniki distr.*: Gefyra
(4), Thessaloniki (2); *Serres distr.*: Strymonikon (2); *Ioannina distr.*: Konitsa (1), Perama (1);
Samothraki: Kamariotissa (18); Makrilos (2); *Arta distr.*: Vlaherna (2); *Dráma distr.*: Koudounia (3);
Lesbos (2); *Samos* (3).

Mus spicilegus Petényi, 1882:
    AUSTRIA (AUT): Zurndorf vicinity (20).

Mus spretus Lataste, 1883 (SPR):
    FRANCE: Grenade (5), La Gardiole (11), La Clape (1), La Capelle Masmolène (2), Petit Travers (2).
    SPAIN: Albarracin (1), Garsala Lerida (1), San Quintin de Mediona (2), Sierra Nevada (2).
    MOROCCO: Agadir (5), Casablanca (2).

APPENDIX 2. List of cranial and dental variables (numbers 1–18) showing significant differences between pairs of taxa (* − $p \leq 0.001$). Note: In all the *M. musculus* populations, males and females are pooled together. Where a difference was only found for one sex, then it is marked by m (males) or f (females). Populations of the same species are bracketed in bold boxes.

	GR	DA	DCH	DWM	AUT
DA	1, 4*, 5, 6, 7, 8, 10, 11, 12*, 13, 14, 15, 16, 17, 18				
DCH	1, 2, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18	7			
DWM	1, 5*, 7, 12*, 13, 14, 15, 17, 18	6, 8, 10, 11, 15, 16, 18	18		
AUT	2, 5, 11, 12, 13, 14, 15, 16, 18	1, 7, 10, 15 18*	1*, 13*, 16*, 17	1, 2, 11, 16	
MC	1, 2f, 3m, 4m, 6, 7m, 8, 9m, 10, 11, 12, 13, 14, 15, 16, 17, 18	2f, 5, 7f	3m, 4m, 7m, 8m, 9m, 10*, 11*, 15, 16	3m, 4m, 8, 9m, 10, 11, 12*, 13*, 15, 16, 18	1, 2, 7m, 8m, 10, 13, 15, 17, 18
MM	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	5	3, 4, 8*, 9, 10*, 15	3*, 4*, 8, 9, 10, 11, 13*, 15, 16, 18	1, 2, 5*, 7, 8, 10, 13, 15, 17, 18*
MS	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	5	3, 4, 7, 9, 10, 12, 15, 16	3, 4, 6, 8, 9, 10, 11, 12, 13, 15, 16, 18	1, 2, 5*, 6, 7, 8, 10, 13, 15, 17, 18
MH	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18	5	1*, 3, 4, 7, 9, 10, 12*, 15*	3, 4, 6, 8, 9, 10, 11, 12, 15, 16	1, 2, 5, 7, 8*, 10, 15, 17
MU	1, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17*, 18	5	1*, 3, 4, 7, 9, 10, 12, 15, 16	3, 4, 6, 8, 9, 10, 11, 12, 13*, 15, 16, 18	1, 2, 5, 6*, 7, 9*, 10, 13, 15, 17, 18
MSP	1, 2, 6*, 8, 10, 11, 12, 13, 14, 15, 16, 17*, 18	2, 7*, 16*		8*	1, 2
SPR	3*, 4*, 5*, 6, 7, 8, 12, 13, 14, 15, 18	1, 10, 11, 15, 16, 18	1, 2, 10, 11, 13, 15, 18	1, 2, 8	8*, 10*, 11, 12*, 16

APPENDIX 2 – continued. Note: MCm and MCf are subpopulations of males and females from Bohemia, respectively.

	MCm
MCf	7

Number codes:

1 – A		10 – LM1i
2 – B		11 – LaM1i
3 – LCb		12 – LM2i
4 – LB		13 – LM3i
5 – LaR		14 – LaM3i
6 – LaC		15 – LM13i
7 – LaZ		16 – LM1s
8 – hC		17 – LM2s
9 – LD		18 – LM13s

	MC	MM	MS	MH	MU	MSP
MM						
MS	2f*, 3f, 7f, 8f*	7				
MH	2f*, 3f, 7f, 18					
MU	2f*, 3f, 4f*, 7f, 9f	7*		17		
MSP	3m*, 7m, 10, 11, 12*, 15, 16, 17*, 18	3*, 4*, 9*, 10*, 11*, 12*, 15, 16*, 18*	3, 4, 6*, 7, 9, 10, 11, 12, 14, 15, 16, 18	3, 4*, 7, 9, 10, 11, 12, 15	3, 4, 7, 9, 10, 11, 12, 15, 16, 17, 18	
SPR	1, 2, 10, 11, 12, 13, 15, 16, 17m, 18	1, 2, 9, 10, 11, 12, 13, 15, 16, 17, 18	1, 2, 3*, 7, 9, 10, 11, 12, 13, 14, 15, 16, 18	1, 2, 9, 10, 11, 12, 13, 15, 16, 18	1, 2, 3*, 7, 9, 10, 11, 12, 13, 15, 16, 17, 18	1, 2, 10, 11*, 13*, 15, 18*