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Comparison of craniological parameters in *Mus musculus musculus* Linnaeus, 1758 and *Mus hortulanus* Nordmann, 1840

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To analyse craniological parameters of *M. m. musculus* and *M. hortulanus*, 14 measurements were taken, two indices were calculated, and three qualitative traits were considered. The material comprised 169 skulls of *M. m. musculus* captured in the European part of the U.S.S.R., in central Ukraine, and in Poland, and 225 skulls of *M. hortulanus* captured in central and south-eastern Ukraine and in Moldova. It has been found that these two species can be differentiated using the following parameters: breadth of zygomatic process of maxilla, index K, zygomatic index, shape of masseter plate, and frequency of zygomatic foramen. In order to identify an individual to species, it is necessary to consider the total set of these characteristics, and first of all quantitative traits.

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Introduction

Most researches argued that the house mouse, *Mus musculus* Linnaeus, 1758 has many forms, and they considered *M. hortulanus* Nordmann, 1840 as one of the subspecies of *M. musculus* (Argiropulo 1940, Schwarz and Schwarz 1943, Bobrinskij *et al.* 1965, Gromov and Baranova 1981, and others). According to other authors, *M. m. musculus* and *M. hortulanus* are separate species (Val'ch 1927, Pisareva 1948, Ladygina 1964, Thaler *et al.* 1981a, b; Marshall 1986 and other). Based on the study of the polymorphisms of proteins, Thaler *et al.* (1981a, b) have concluded that *M. m. musculus* and *M. hortulanus* are two sympatric species, genetically isolated under natural conditions. These species also differ in ecological parameters (Naumov 1940, Lyalyukhina 1984), eco-physiological characteristics (Lyalyukhina 1964) and ethological characteristics (Sokolov *et al.* 1983, 1990; Meshkova *et al.* 1984, 1986). They also show specific differences in olfactory signals (Sokolov *et al.* 1984, Kotenkova *et al.* 1989).

Poland and a large part of the USSR are occupied by *M. m. musculus* (Bonhomme *et al.* 1984, Thaler 1986). Transcaucasia is inhabited by hybrids of *M. m. domesticus* and *M. m. bactrianus* (Mezherin and Kotenkova 1989). *M. m. musculus* and *M. hortulanus* are sympatric

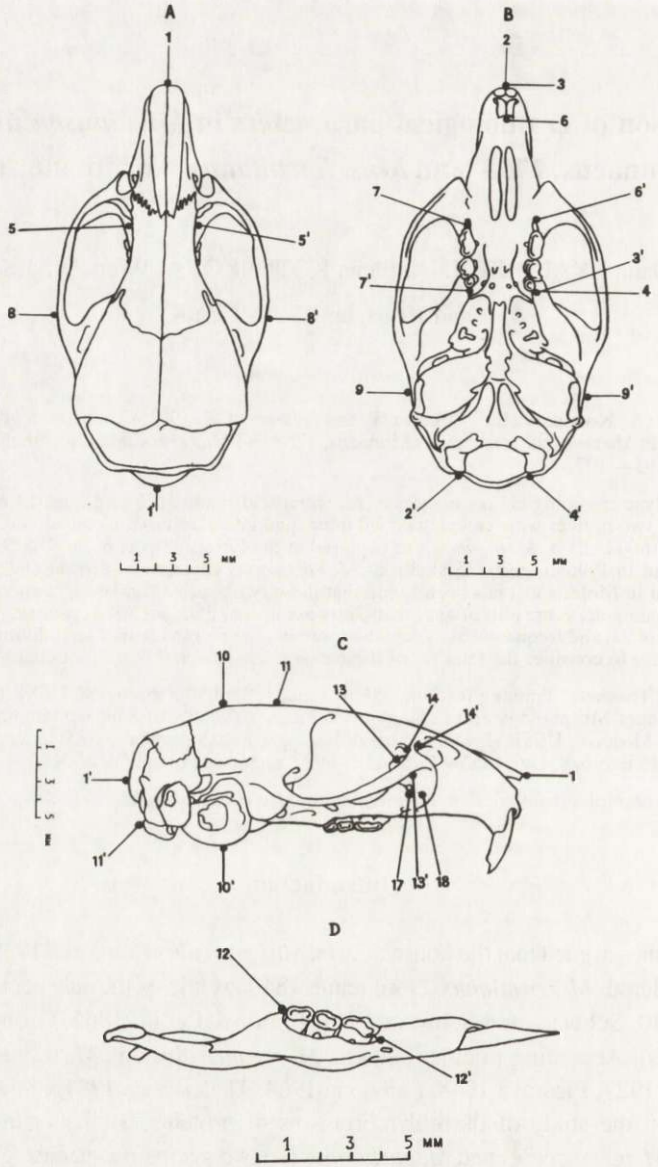

Fig 1. Skull characters used for the study (drafted by V. A. Lapshov). 1 – 1' – Profile length, 2 – 2' – Condylobasal length, 3 – 3' – Rostrum length, distance between the most distal point of pallatine process of premaxilla to the posterior edge of molar row, 4 – 4' – Brain-case length, distance from the posterior edge of the last molar to the posterior edge of occipital condyle, 5 – 5' – Interorbital constriction, 6 – 6' – Length of diastema, 7 – 7' – Length of maxillary tooth-row, 8 – 8' – Zygomatic breadth, 9 – 9' – Maximum brain-case breadth, 10 – 10' – Hight of brain-case through tympanic bullas, 11 – 11' – Distance between occipital condyle and crossing of frontal and coronal sutures, 12 – 12' – Length of mandibular tooth-row, 13 – 13' – Breadth of zygomatic process of maxilla, 14 – 14' – Minimum breadth of upper ramus of zygomatic process of maxilla, 15 – Index K = 11 – 11'/13 – 13', 16 – Zygomatic index = 14 – 14'/13 – 13', 17 – Zygomatic foramen, 18 – Masseter plate.

in Ukraine and Moldova (Lyalyukhina 1984), and they do not cross under natural conditions (Mezherin 1987, Mezherin and Zagorodnjuk 1989).

The purpose of this paper is to describe the geographical variation, to check the validity of the formerly used and of other potential parameters distinguishing *M. m. musculus* and *M. hortulanus*, in particular when variations due to age are taken into account.

Material and methods

M. m. musculus used for craniological measurements were caught in dwelling places located in central-European parts of the USSR (Moscow), central Ukraine (Kirovograd), and Poland (near Warsaw). *M. hortulanus* specimens were captured in agrocoenoses of central Ukraine (Zaporozhe region) and of Moldova (near Kishinev). The total material consisted of 169 skulls of *M. m. musculus* and 225 skulls of *M. hortulanus* of different ages

Table 1. Material used for the estimation of craniological parameters in *M. m. musculus* and *M. hortulanus*.

Species	Region	Number of individuals		
		adult	subadult	juvenile
M. m. musculus	European USSR (Moscow)	32	–	–
	Central Ukraine (Kirovograd)	39	22	19
	Poland (near Warsaw)	57	–	–
M. hortulanus	Central Ukraine (Kirovograd region)	132	24	25
	South-eastern Ukraine (Zaporozhe region)	20	–	–
	Moldova (Kishiniev region)	24	–	–

(Table 1). The age of animals was estimated from the tooth wear (Varshavskij 1950, Keller 1974) rather than from the lens weight, the latter being less reliable (Berry and Treslove 1968). Three age groups were distinguished: adults, subadults, and juveniles.

Skulls were measured by means of a slide calliper and MBS-1 microscope with a micrometer in the ocular. A total of 14 skull measurements were taken. These measurements are detailed in Fig. 1. In addition to the variables indicated in the figure we calculated index K as the ratio of distance between occipital condyle and crossing of frontal and coronal sutures (11)* to the breadth of zygomatic process of maxilla (13), and after Orsini *et al.* (1983), a zygomatic index was calculated as the ratio of the minimum breadth of upper ramus of zygomatic process of maxilla (14) to the breadth of zygomatic process of maxilla (13).

Besides, qualitative characteristics of skulls were recorded, i.e. (1) shape of the lower edge of enlarged part of zygomatic process of maxilla (masseter plate), (2) presence or absence of zygomatic foramen on masseter plate, (3) shape of coronal suture. These traits were proposed by Orsini *et al.* (1983) based on the analysis of skulls of the different forms of the house mouse, and used by Kratochvíl (1986a, b) for diagnosis of different forms of mice of the genus *Mus*. The frequency of the occurrence of each of the qualitative parameters for *M. m. musculus* and *M. hortulanus* was calculated.

The significance level of at least 0.05 was used for all differences.

Table 2. Mean values of quantitative parameters ($\bar{x}$) for *M. m. musculus* (n – number of individuals, *p* – significance level, NS – nonsignificant difference).

Measurement, index	Central Ukraine			Poland			Central Europe			Significance
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	level, *p*
1. Profile length	39	20.6	1.14	57	20.2	1.12	32	20.9	1.00	0.05
2. Condylobasal length	39	18.5	1.14	57	18.9	1.24	32	19.5	1.11	0.01
3. Rostrum length	39	8.8	0.65	57	9.5	0.54	32	9.9	0.62	0.001
4. Brain-case length	39	9.5	0.65	57	9.5	0.64	32	9.6	0.53	NS
5. Interorbital constriction	39	3.5	0.11	57	3.6	0.10	31	3.6	0.14	0.001
6. Length of diastema	39	5.2	0.39	57	5.2	0.46	32	5.4	0.44	NS
7. Length of maxillary tooth-row	39	3.4	0.19	57	3.5	0.15	32	3.5	0.18	0.05
8. Zygomatic breadth	39	11.1	0.44	57	10.8	0.52	31	10.9	0.49	0.05
9. Maximum breadth of brain-case	39	9.8	0.24	57	9.7	0.35	32	9.6	0.28	0.05
10. High of brain-case through tympanic bullas	39	7.3	0.28	57	7.4	0.22	32	7.1	0.23	0.001
11. Distance between occipital condyle and crosing of frontal and coronal sutures	39	6.0	0.43	53	6.8	0.27	32	6.4	0.29	0.001
12. Lenght of mandibular tooth-row	39	3.1	0.24	54	3.0	0.17	32	3.0	0.12	0.05
13. Breadth of zygomatic process of maxilla	39	1.1	0.15	57	1.0	0.14	32	1.1	0.14	NS
14. Minimum breadth of upper ramus of zygomatic process of maxilla	39	0.4	0.09	57	0.4	0.12	32	0.4	0.09	NS
15. Index K	39	5.7	0.92	53	6.2	0.87	32	6.1	0.82	0.01
16. Zygomatic index	39	0.4	0.08	57	0.4	0.11	32	0.4	0.08	NS

Table 3. Mean values of quantitative parameters ($\bar{x}$) for *M. hortulanus* (n – number of individuals, p – significance level, NS – nonsignificant difference).

Measurement, index	Central Ukraine			Moldova			South-eastern Ukraine			Significance
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	level, p
1. Profile length	132	20.3	1.26	22	20.7	1.07	20	20.2	0.80	NS
2. Condylobasal length	132	18.5	1.45	22	19.7	1.09	20	18.8	0.81	0.01
3. Rostrum length	132	9.3	0.91	24	10.0	0.60	20	9.5	0.35	0.001
4. Brain-case length	132	9.4	0.74	22	9.6	0.62	20	9.4	0.51	NS
5. Interorbital constriction	132	3.5	0.14	24	3.5	0.13	19	3.5	0.10	NS
6. Length of diastema	132	5.3	0.56	24	5.6	0.41	20	5.1	0.32	0.05
7. Length of maxillary tooth-row	132	3.5	0.24	24	3.6	0.17	20	3.4	0.12	0.05
8. Zygomatic breadth	105	10.9	0.61	24	10.9	0.44	17	10.8	0.41	NS
9. Maximum breadth of brain-case	130	9.7	0.28	22	9.6	0.32	19	9.7	0.24	NS
10. High of brain-case trough tympanic bullas	130	7.5	0.31	21	7.4	0.28	18	7.3	0.26	NS
11. Distance between occipital condyle and crossing of frontal and coronal sutures	130	6.5	0.51	21	6.8	0.23	19	6.8	0.28	0.01
12. Length of mandibular tooth-row	132	3.1	0.28	23	3.0	0.19	20	3.1	0.14	NS
13. Breadth of zygomatic process of maxilla	132	0.7	0.10	24	0.7	0.13	20	0.8	0.10	0.001
14. Minimum breadth of upper ramus of zygomatic process of maxilla	96	0.4	0.01	24	0.5	0.01	20	0.4	0.08	0.001
15. Index K	129	9.0	1.52	21	10.5	1.90	19	8.7	1.37	0.001
16. Zygomatic index	93	0.5	0.12	24	0.7	0.20	20	0.5	0.11	0.001

Table 4. Comparison of quantitative parameters of *M. m. musculus* and *M. hortulanus* by Duncan multiple test (values of probability are given, NS – nonsignificant). Populations compared are: for *M. hortulanus*: A – central Ukraine, B – Moldova, C – south-eastern Ukraine, for *M. m. musculus*: D – central Ukraine, E – Poland, F – central European USSR.

Parameters of skull	A – D	A – E	A – F	B – D	B – E	B – F	C – D	C – E	C – F
1. Profile length	NS	NS	0.05	NS	NS	NS	NS	NS	NS
2. Condylobasal length	NS	NS	0.01	0.01	0.05	NS	NS	NS	NS
3. Rostrum length	0.01	NS	0.01	0.01	0.05	NS	0.05	NS	NS
4. Brain-case length	NS	NS	NS	NS	NS	NS	NS	NS	NS
5. Interorbital constriction	NS	0.01	0.01	NS	0.05	NS	NS	NS	NS
6. Length of diastema	NS	NS	NS	0.01	0.01	NS	NS	NS	NS
7. Length of maxillary tooth-row	0.05	NS	NS	0.01	0.05	0.05	NS	NS	NS
8. Zygomatic breadth	NS	NS	NS	NS	NS	NS	NS	NS	NS
9. Maximum breadth of brain-case	0.05	NS	NS	NS	NS	NS	NS	NS	NS
10. Hight of brain-case through tympatic bullas	0.01	NS	0.01	NS	NS	NS	NS	NS	0.05
11. Distance between occipital condyle and crossing of frontal and coronal sutures	0.01	0.01	NS	0.01	NS	0.01	0.01	NS	0.01
12. Length of mandibular tooth-row	NS	NS	NS	NS	NS	NS	NS	NS	NS
13. Breadth of zygomatic process of maxilla	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
14. Minimum breadth of upper ramus of zygomatic process of maxilla	NS	0.01	0.01	NS	NS	NS	NS	NS	0.05
15. Index K	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
16. Zygomatic index	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Table 5. Mean values of quantitative taxonomic parameters for *M. m. musculus* and *M. hortulanus* at different ages. For each age class all differences in the parameters between the two species are significant at $p < 0.01$ (Student *t*-test, n – number of individuals).

Parameter	Age	*M. m. musculus*					*M. hortulanus*				
		n	$\bar{x}$	SD	ANOVA		n	$\bar{x}$	SD	ANOVA	
					F	*p*				*F*	*p*
Breadth of zygomatic process of maxilla	juv.	19	1.01	0.21			25	0.68	0.13		
	subad.	22	1.05	0.19	1.69	NS	24	0.71	0.11	2.43	NS
	ad.	39	1.10	0.15			132	0.73	0.10		
Index K	juv.	19	6.24	1.12			21	10.04	2.01		
	subad.	22	5.45	0.93	3.34	0.05	24	8.75	1.01	4.64	0.02
	ad.	39	5.71	0.92			129	9.03	1.52		
Zygomatic index	juv.	19	0.36	0.11			25	0.49	0.09		
	subad.	22	0.36	0.13	0.81	NS	24	0.47	0.09	2.23	NS
	ad.	39	0.39	0.08			93	0.52	0.12		

Table 6. Frequency of occurence (in per cent) of qualitative traits in *M. m. musculus* and *M. hortulanus* (n – number of individuals).

Traits			*M. m. musculus*			*M. hortulanus*		
			Central Ukraine	Poland	Central European USSR	Central Ukraine	South-eastern Ukraine	Moldova
Shape of coronal suture	brace	%	31.8	57.9	28.1	69.0	65.0	16.7
		n	14	33	9	91	13	4
	bow	%	40.9	14.0	12.5	19.0	30.0	37.59
		n	18	8	4	25	6	9
	obtuse angle	%	25.0	28.1	56.3	9.0	0.0	45.9
		n	11	16	18	12	0	11
	other shapes	%	2.3	0.0	3.1	3.0	5.0	0.0
		n	1	0	1	4	1	0
Shape of the lower edge of masseter plate	not protruding	%	28.9	36.8	18.7	81.0	70.0	87.4
		n	13	21	6	107	14	21
	protruding	%	66.7	40.2	81.3	16.0	30.0	12.6
		n	30	23	26	21	6	3
	other shapes	%	4.4	22.8	0.0	3.0	0.0	0.0
		n	2	13	0	4	0	0
Zygomatic foramen	present	%	65.9	45.6	50.0	8.0	35	4.2
		n	29	26	16	10	7	1

Results

Quantitative craniological parameters of *M. m. musculus* and *M. hortulanus* in different parts of their ranges

Analysis of variance was used to compare craniological measurements and indices of each species in different parts of the range. This analysis has shown that *M. m. musculus* living in different parts of the range show similarity in five parameters (4, 6, 13, 14 and 16) and significant differences in 11 parameters (1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 15) (Table 2). *M. hortulanus* from various parts of Ukraine and Moldova were similar in seven parameters (1, 4, 5, 8, 9, 10, 12) (Table 3) and differed in nine parameters (2, 3, 6, 7, 11, 13, 14, 15, 16).

The Duncan multiple test was used to compare quantitative parameters of skull between the two species. Using this test, it is possible to show similarities and differences in individual parameters between the two species from different regions. Thus, each parameter of *M. m. musculus* from the central European USSR was compared with respective parameters of *M. hortulanus* from central and south-eastern Ukraine and from Moldova. A similar comparison was made for *M. m. musculus* from central Ukraine and Poland. It has been found that *M. hortulanus* from each of the study areas significantly differs from *M. m. musculus* in central European USSR, central Ukraine, and Poland with respect to three parameters: breadth of zygomatic process of maxilla, index K, and zygomatic index (13, 15, 16) (Table 4). For this reason these parameters are proposed as taxonomic characters.

Comparison of quantitative craniological parameters of *M. m. musculus* and *M. hortulanus* at different ages

Analysis of variance (ANOVA) was used to compare taxonomic parameters in three age classes (juvenile, subadult, adult) for *M. m. musculus* and *M. hortulanus* separately, and Student *t*-test for testing differences between these species. Within species the taxonomic parameters were similar, except for the index K (Table 5). Differences between *M. m. musculus* and *M. hortulanus* were statistically significant for each age class ($p < 0.001$) if the compared individuals were from the same or different age class. So individuals at different ages can be identified to species.

Qualitative craniological parameters of *M. m. musculus* and *M. hortulanus* in different parts of their ranges

In *M. m. musculus* various shapes of coronal suture occur with different frequency within the range of the species (Table 6). In central-European USSR, the posterior edge of frontal bones forms an obtuse angle for more than half of individuals. In mice from Poland, this shape is less frequent by half, and the most frequent shape of suture resembles a brace. The coronal suture of mice from central Ukraine is most frequently bow-like. A different picture emerged from the analysis of the shape of masseter plate. Typically, in *M. m. musculus* it tended to be round with a protruding lower egde. Only in Poland the proportions of individuals with protruding and not protruding edge of masseter plate were similar. The proportion of skulls with zygomatic foramen present was high and different in various parts of the range.

The coronal suture of *M. hortulanus* was typically of the shape of a brace, except for the individuals from Moldova where most frequently it formed an obtuse angle (Table 6). Most

individuals of this species had masseter plate with not protruding lower edge. Zygomatic foramen was rare in individuals from Moldova and central Ukraine, but relatively frequent in those from south-eastern Ukraine.

The comparison of qualitative traits of *M. m. musculus* and *M. hortulanus* has shown that none of the proposed qualitative traits taken separately can be used for identification the species. However, because of differences in the frequency of the shape of the lower edge of masseter plate and zygomatic foramen, they can be helpful in mouse identification (Table 7).

Table 7. Craniological parameters differentiating between *M. m. musculus* and *M. hortulanus*.

Parameter	*M. m. musculus*	*M. hortulanus*
Breadth of zygomatic process of maxilla	> 1.0	< 0.8
Index K	4.5 – 6.6	7.3 – 14.1
Zygomatic index	< 0.42	> 0.50
Shape of lower edge of masseter plate	more often with protruding lower edge	more often with nonprotruding lower edge
Zygomatic foramen	often present	often absent

Discussion

Long ago, zoologists noticed that house mice from various parts of their range differ in craniological traits. Agriropulo (1940), who considered the house mouse as a species comprising five subspecies, described the configuration, proportions, and structure of the skull of *M. m. musculus*, including the range of variation for nine parameters of the skull. His characteristics of skulls were however, limited to only superficial comparisons (e. g. relatively bigger, massive, or relatively smaller) and to some measurements. Numerical values of most of these measurements overlapped for different subspecies. As a result, the diagnostic value of such comparison was small. Ognev (1916) took measurements of *M. hortulanus* skulls. He suspected that this species differed at the level of subspecies from the synanthropic house mouse with respect to the length and proportions of some parts of the skull and in the shape of coronal suture. Gulij (1930) analysed geographical and age-related variations in craniometry of mice, he considered as *M. hortulanus*, presented an opposite view on the taxonomic value of these indices. He has concluded that most of the craniological traits cannot be used for the diagnosis of *M. hortulanus*. It should be noted, however, that the materials of Ognev (1916) and Gulij (1930) were likely to contain individuals of both *M. hortulanus* and *M. m. musculus*. Migulin (1937) was the first to note differences in the breadth of zygomatic process of maxilla (or, using his terminology, "zygoma hight in the anterior one third of its length") and proposed this trait for identification of mouse species. A similar view was presented by Sharleman (1937). According to Pisareva (1948), the value of this trait in *M. m. musculus* is twice that in *M. hortulanus*. In recent years, the taxonomy of the house mouse was revised by biochemists, and this gave rise to the reanimation of craniological parameters as less labour consuming and

generally more accessible than biochemical methods (Marshall and Sage 1981, Kratochvíl 1986a, b, 1987).

Orsini *et al.* (1983) proposed a zygomatic index for diagnostic of *M. musculus* and *M. hortulanus*. For six populations from Greece, Bulgaria, and Austria, they found the values of this index ranging from 0.45 to 0.48 for *M. musculus* and from 0.74 to 0.80 for *M. spicilegus* (= *M. hortulanus*). For *M. musculus* these values are comparable to our data (Table 2) but not for *M. hortulanus* (Table 3). Presumably the value of this index varies in different parts of the range of *M. hortulanus*, or depends on measuring technique. This does not change, however, the fact that the values of the zygomatic index show significant differences between *M. musculus* and *M. hortulanus* in all the study areas, thus is a good diagnostic trait. Auffrey *et al.* (1990) have found that using this index it is possible to differentiate between *Mus "spretoides"* and *M. m. domesticus* occurring in Israel.

In this paper we use the new index K to differentiate between *M. m. musculus* and *M. hortulanus* which yielded a parameter clearly different between these species. Differences in the values of this index between the species compared are greater than differences in the zygomatic index, facilitating their identification. We also calculated index K for *M. hortulanus* collected from eastern Ukraine (Dnepropetrovsk region) by Pisareva (1948). Its value ($K = 9.8 \pm 0.6$) totally agrees with our result (Table 3). Thus, using zygomatic index and index K, it is possible to indentify the species of individual mouse independently on the age of the animal.

Unlike Kratochvíl (1986a, b) and Orsini *et al.* (1983), we have found that none of the analysed qualitative traits separately can be used for differentiating between *M. m. musculus* and *M. hortulanus*. According to Orsini *et al.* (1983), each species has a distinct set of traits. For example, the lower edge of masseter plate in *M. m. musculus* is rounded and protruding forwards, and the zygomatic foramen is always present. Kratochvíl (1986a, b), shows a drawing of coronal suture which is similar to a triangle in this species. According to these authors, distinct qualitative traits are characteristic of *M. hortulanus*. Our data do not confirm those reported by Orsini *et al.* (1983), who show that the shape of masseter plate and the presence or absence of zygomatic foramen are important taxonomic traits. Our results show that each qualitative trait taken separately can specify individuals only with some probability. A high degree of the polymorphism of coronal suture reduces the diagnostic value of this trait. Gulij (1930) concluded that this trait was of a diagnostic value only when used with other traits, and we totally agree with him.

More complicated is the identification to species of individuals showing traits of both *M. m. musculus* and *M. hortulanus*. We analysed such individuals, and have found that most of them share only one trait of the other species. Only one individual of *M. hortulanus* from central Ukraine shared two traits with *M. m. musculus*, and another individual shared three traits (shape of coronal suture and the shape of the edge of masseter plate, and also the presence of zygomatic foramen). But the values of index K and of zygomatic index indicated that these individuals belonged to *M. hortulanus*. Similar results were obtained for one individual of *M. m. musculus*, in which the shape of the edge of masseter plate was typical of *M. hortulanus*, zygomatic foramen was absent, but the values of index K and zygomatic index showed that this was *M. m. musculus*. Thus, our study provides evidence that craniological parameters can

reliably be used for diagnosis of *M. m. musculus* and *M. hortulanus* but only when a set of quantitative and qualitative traits is examined, i.e. breadth of zygomatic process of maxilla, index K, zygomatic index, shape of lower edge of masseter plate, and the frequency of occurence of zygomatic foramen, and first of all, quantitative traits should be used (Table 7).

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