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UDC 591.4:599.323.5(234)(477.8) DIAGNOSTIC CRITERIA FOR IDENTIFICATION OF MICROTUS S. L. SPECIES (RODENTIA, ARVICOLIDAE) OF THE UKRAINIAN CARPATHIANS

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Diagnostic Criteria for Identification of *Microtus* **s. l. Species (Rodentia, Arvicolidae) of the Ukrainian Carpathians. Barkaszi, Z.** — The present paper deals with the issues of diagnostics of morphologically similar vole species (*Microtus* **s.** l.) common in the fauna of the Ukrainian Carpathians. Three groups of characters have been analysed: external traits (linear body dimensions, coloration features, etc.), morphological and anatomical features of the skull (size of its structures, form of certain bones and sutures, etc.), and morphology of molar teeth (number and form of enamel lobes and triangles). The analysis of museum specimens of voles collected in the region of the Ukrainian Carpathians has shown that external and cranial non-metric characters allow reliable identification to genus level, while for species diagnostics it is necessary to use a complex of characters including structural features of molars and dimensions of skull structures. The height and width of the braincase have the largest diagnostic value among cranial characters for the sibling species *Microtus agrestis–Microtus arvalis* such value have the upper molars length, condylobasal length, and braincase height. The most significant, revised and newly described, diagnostic characters have been generalized into an identification key, especially convenient for use during work with collection materials.

Key words: Carpathians, morphological variation, species identification, voles.

Introduction

Nowadays, when due to intense anthropogenic pressure on natural ecosystems significant habitat disturbances and disappearances occur, investigation of biodiversity dynamics and of fauna changes within certain regions becomes especially relevant. Habitat disturbance and economic activity often lead to the appearance of alien species, changes in the distributional range of indigenous species and in the structure of autochthonous communities. Scientific collections amassed and deposited at natural history museums may provide considerable help in tracing the history of biodiversity changes. The fauna composition of a certain

region during different periods, changes in species diversity and in the structure of communities can be determined by analysis of collection samples. Specimens obtained from raptor pellets, digestive tract of reptiles and mammals allow investigating the feeding habits of animals and trophic relationships within ecosystems (Litvaitis, 2000; Rozhenko, 2006).

Collection samples usually originate from several sources. In addition to specimens collected by specialists, a notable part of collections may consist of specimens gathered by enthusiastic naturalists, students (especially in case of zoological museums at universities), or experts in neighbouring branches who are not involved into the issues of systematics of certain animal groups. Nevertheless, materials collected and passed by them to museums have a high value, especially in studies of taxonomy, variability, evolutional morphology, geographic distribution, and ecology. Besides, stock collections deposited at museums are unique basis for long-term monitoring of biodiversity (Klymyshyn, 2015).

The first and probably the most important stage in working with collection materials is the correct identification of specimens. At the same time, the set of diagnostic characters used for identification always corresponds to the currently accepted views on the systematics of the group. Such views constantly develop from the most obvious morphological differences (e. g., 'Linnaean' species) to tiny details in the skull structure, tooth system, and to use of molecular diagnostic methods (e. g., modern classification schemes recognizing sibling species).

Identification of museum specimens of small mammals is mainly based on principles of comparative morphology involving a complex of metric and non-metric characters of the skull and its tiny structures. At the same time, the most difficult task is to find characters allowing identification of morphologically similar species, and even more so sibling ones.

Voles are a quite homogenous group according to their morphology; therefore, the species identification is often problematic. Because of this, a large number of descriptions and available collection specimens represent a small set of the most common species, although under such descriptions or labels could be 'hidden' other species that slightly differ by their morphology. Hence, development of diagnostic keys and searching for characters convenient to use are of key importance not only for field zoology, but also for museum work. Regarding the fauna of Ukraine, similar criteria were developed and have been successfully applied in practice for such groups of mammals as hamsters (Zagorodniuk & Atamas', 2005), mole rats (Korobchenko, 2012), whitetoothed shrews (Tovpinets, 2012 b), sibling species complex *M. arvalis* s. str.–*M. levis* (Tovpinets, 2012 a), and other groups of animals as well.

Traditional large-scale identification keys and diagnostic descriptions (e. g., Korneyev, 1952; Gromov et al., 1963; Pucek, 1984; Rekovets, 1994; Zagorodniuk, 2002) usually cover the fauna of not natural but administrative regions (e. g., Ukraine or the entire former USSR). Therefore, they include a considerable number of taxa the absence of which in the fauna of a certain natural region, particularly of the Carpathians, is known in advance. For instance, within Ukraine, the issue of diagnostics of such vole species as *Chionomys nivalis* or *T. tatricus* exists only in the region of the Carpathian Mountains, while large parts of voluminous diagnostic keys (e. g., couplets leading to steppe species) are irrelevant for the fauna of this region at all.

Moreover, the effectiveness of any identification key depends on the average number of steps leading to the correct species identification, which is usually 3–4 on a regional level, while it could be at least 2–3 times more in large-scale keys (Sviridov, 1994). Therefore, to increase the effectiveness of the identification process it is necessary to develop regional keys (i. e., for natural regions) and exclude taxa that are absent from the local fauna, and even include those that are known from other parts of the studied region. In addition, large-scale keys often do not include characters that could be relevant on local level. Hence, developing of regional identification keys is of great importance for effective and reliable diagnostics of the representatives of the local fauna.

Special publications dealing with diagnostics issues of *Microtus* s. l. are extremely scarce. Some morphological criteria had been considered tangentially in contributions devoted to either morphological variation or paleontological data (e. g., Meulen, 1973; Nadachowski, 1984; Luzi et al., 2016; Rekovets & Kovalchuk, 2017). In case of traditional identification keys, some of them contain incomplete data or incorrect leads in several couplets (e. g., Korneyev, 1952). Besides, there are many examples when leads were included into keys from previous editions without their critical analysis, particularly for a regional fauna.

The aim of the present contribution is searching for diagnostic characters and analysis of their significance in identification of species of *Microtus* s. l. represented in the fauna of the Ukrainian Carpathians, as well as to develop a generalized identification key consisting of approved characters for species diagnostics. In addition, we aim to draw attention on and discuss the problems of diagnostics, which is crucial for working with museum collections.

Material and methods

Data used in this study were obtained by examination of 437 specimens of voles (study skins and skulls) deposited in the following institutions: National Museum of Natural History, NAS of Ukraine (Kyiv), Institute of Zoology, NAS of Ukraine (Kyiv), State Museum of Natural History, NAS of Ukraine (Lviv), Zoological Museum of Taras Shevchenko Kyiv National University, and Zoological Museum of Ivan Franko Lviv National University.

Samples for examination were selected based on biogeographic (specimens only from the Carpathian part of the genus/species range) and general external (body proportions, fur type, eye size, number of *mammae*) criteria. Additionally, standard samples of *T. tatricus* and *T. subterraneus* with chromosomal identification, and

samples of Carpathian 46-chromosome *M. arvalis* were used to examine diagnostic characters. Considering that several important metric cranial characters of voles are largely affected by age-related variation, analyses were restricted only to adult specimens.

Data on external body dimensions were taken from original labels of specimens and collection catalogue cards. Additionally, dimensions of skulls and skull structures of 183 specimens were measured by calliper with an accuracy of 0.1 mm. The following 12 skull measurements were made:

CBL — condylobasal length (from occipital condyles to the front edge of intermaxillary bones); ZYG — zygomatic width (at the front third of zygomatic arches); M1–3 — coronal length of the upper molars; BUL — auditory bulla length; CRH — braincase height (from the lower edge of tympanic bones to the interparietal bone); CRB — braincase width (between ectotympanics); IOR — interorbital constriction width (at its narrowest part); NAL — nasal bones length; NAB — nasal bones width (the largest width at the distal part); ROH — rostral height (from the alveolus of M1 perpendicularly to the distal part of frontal bones); FIL — length of *foramen incisivum*; FIB — width of *foramen incisivum* (the largest width at the distal part).

Metric data were generalized and processed using electronic spreadsheets. The prior aim was to find characters allowing rapid and reliable genus/species identification in museum and field conditions. Therefore, when comparing cranial measurements, Mayr's coefficient of divergence (CD) was used to separate characters with overlapping values, and characters with the least overlaps were tested for their applicability in species diagnostics.

Qualitative characters of the skull, in particular the morphology of bones, sutures, structures of the rostral part, and the dental system, were examined using an MBS-9 microscope. Images of cranial characters and teeth were taken by digital USB microscope. The pictures were used to create drawings that demonstrate the most important cranial and dental diagnostic characters.

In addition, data from a number of special publications, identification keys, and diagnostic descriptions (e. g., Gromov et al., 1963; Pucek, 1984; Rekovets, 1994; Zagorodniuk et al., 1992; Zagorodniuk, 2002) dealing with issues of morphology and diagnostics of voles of *Microtus* s. l. were analysed and revised as well.

Taxonomic reference

Nowadays fauna checklists of certain regions usually are complemented by either adding recently appeared species (e. g., alien species) or due to taxonomic revisions when a 'large' species is divided into a few 'small' ones. For instance, 'new' species appeared because of taxonomic revision of mole rats *Spalax*, birch mice *Sicista*, 'common' voles *Microtus* ex gr. *arvalis*, and 'wood' mice *Sylvaemus*.

Microtus s. l. is considered in this work as a morphological assemblage rather than a currently accepted genus. According to recent taxonomic surveys (Zagorodniuk & Emelyanov, 2012; Barkaszi & Zagorodniuk, 2016), there are nine vole species of the family Arvicolidae in the mammalian fauna of the Ukrainian Carpathians representing six genera, in particular *Terricola* and *Microtus* s. str. (table 1).

Traditionally, *Terricola* Fatio, 1867 is considered as subgenus of the genus *Microtus*, which view has been widely supported (e. g., Pavlinov & Rossolimo, 1987; Gromov & Erbayeva, 1995; Mitchell-Jones et al., 1999; Wilson & Reeder, 2005). Zagorodniuk (1989) in his review on taxonomy and distribution of pine voles in Eastern

Taxon	Note
Genus Terricola	
• <i>Terricola subterraneus</i> European pine vole	It has been recorded in the fauna of the Eastern Carpathians since 1949 (Tatarinov, 1956), however its museum specimens were partly collected as <i>M. arvalis</i> or <i>M. agrestis</i> .
• <i>Terricola tatricus</i> Tatra pine vole	It has been recorded in the fauna of the Eastern Carpathians since 1988 (Zagorodniuk, 1988), mostly due to re-identification of samples of <i>T. subterraneus</i> .
Genus Microtus s. str.	
• <i>Microtus agrestis</i> Field vole	It has been recorded since the first fauna surveys of the region (e. g., in Zawadzki, 1840 as <i>Hypudaeus gregarius</i> Illig.), its specimens were partly collected as <i>M. arvalis</i> or <i>T. subterraneus</i> .
• <i>Microtus arvalis</i> Common vole	It has been recorded since the first fauna surveys; however, a considerable number of specimens were re-identified afterwards according to taxonomic changes of the group (division of <i>'arvalis'</i> into a few species).

Table 1. Species of Microtus s. l. in the fauna of the Ukrainian Carpathians

Europe considered *Terricola* as genus assuming that Palearctic pine voles represent a monophyletic group. Kryštufek et al. (1996) debated the genus status of *Terricola* Fatio, 1867 and stated that it refers only to a morphological assemblage of no systematic validity but not to a monophyletic group. However, a few years later Jaarola et al. (2004) investigating the molecular phylogeny of the genus *Microtus* based on mtDNA sequences revealed that *Terricola* species actually represent a monophyletic group and do share a common ancestor.

Therefore, following the recent taxonomic surveys of East European mammal faunas (Pavlinov & Lissovsky, 2002; Zagorodniuk & Emelyanov, 2012), the taxon *Terricola* Fatio, 1867 is considered in this work in genus status. It is represented in the mammalian fauna of the Carpathians by a pair of sibling species. The high level of morphological variation of a mixed lowland and mountain sample allowed I. Turyanin suggesting about the taxonomic heterogeneity of mountain samples of *Terricola*. He noticed that a part of specimens by morphological characters is similar to *Pitymys subterraneus tatricus* Kratochvíl, 1952 (Turyanin, 1956, 1969). After all, *T. tatricus* as separate vole species in the fauna of the Ukrainian Carpathians was described in 1988 after revision of collection samples (Zagorodniuk, 1988; Zagorodniuk & Zima, 1992). In general, this species is characterized by highly fragmented insular-type range in the entire Carpathians, as well as in separate mountain massifs (Martínková & Dudich, 2003).

The genus *Microtus* s. str. represents in the fauna of the Ukrainian Carpathians a group of morphologically similar species and includes *M. agrestis* and *M. arvalis* (Barkaszi & Zagorodniuk, 2016), the distribution and abundance of which are closely related to the altitudinal zonation. Therefore, the identification of specimens based on external characters (and, accordingly, 'label identification' in collections) could be difficult especially in case of specimens collected in interzonal biotopes (river valleys in mountains, ridges penetrating lowlands). Despite the clear differences between these species, specimens of the field vole are often preserved in collections as '*M. arvalis*' or even as '*T. subterraneus*.'

Basic diagnostic characters

Identification of small mammals is usually based on comparison of a complex of morphological (both metric and non-metric) characters. Difficulties with the diagnostics of similar species, especially sibling ones, are related not only to challenges in searching for relevant characters allowing reliable species identification, but also to the researchers' skills or abilities to see and reveal these characters (Zagorodniuk, 2016).

External characters include linear body dimensions, in particular body length (L), tail length (Ca), hindfoot length (Pl), and auricle length (Au), as well as such non-metric characters as fur and tail coloration, number of *clavi* on the sole, etc. Making measurements of linear characters is a standard procedure when collecting specimens and their value is usually indicated on either labels or catalogue cards.

Character	Terricola	Microtus	Source*
Fur	long, shaggy, dark brown	smooth, dark grey	(2)
Tail	bicoloured: brown on the top, whitish on the underside	rather one-coloured, a little brighter on the underside	(2)
Auricles	barely protruding from the fur	notably protruding from the fur	(1) (2) (3)
Eyes	very small	normal sized	(1) (2) (3)
Clavi	5 on the hind sole	6 on the hind sole	(1) (2) (3)
Mammae	2 pairs of inguinal	2 pairs of both inguinal and pectoral	(2)(3)

Table 2. External non-metric diagnostic characters of the Carpathian voles Microtus s. l.

* (1) Gromov et al., 1963; (2) Pucek, 1984; (3) Zagorodniuk, 2002.

Character, mm	Terr	icola	Microtus						
(M/min-max)	subterraneus	tatricus*	agrestis	<i>arvalis</i> s. str.					
L, body length	<u>89.7</u> 78–107	<u>103.3</u> 93–110	<u>112.2</u> 93–135	<u>98.6</u> 81–120					
Ca, tail length	<u>31.1</u> 25–39	<u>38.9</u> 36-41	$\frac{39.4}{31-49}$	$\frac{34.0}{27-42}$					
Pl, hindfoot length	$\frac{14.5}{12.0-16.0}$	$\frac{16.4}{16.0-17.0}$	<u>18.8</u> 17.0–21.0	14.0-17.0					
Au, auricle length	<u>9.3</u> 7.0–12.0	<u>9.8</u> 8.0–11.0	<u>13.2</u> 11.0–15.0	<u>10.9</u> 9.2–15.0					
Total specimens, N	137	9	107	54					

Table 3. External linear characters of the Carpathian voles Microtus s. l.

*Data taken from Zagorodniuk et al., 1992 due to inaccessibility of primary label information.

A consolidated set of basic external diagnostic characters is presented in tables 2–3. External non-metric characters (table 2) were generalized from several sources and their validity was tested on specimens collected exclusively in the region of the Ukrainian Carpathians. Meanwhile, external metric characters (table 3) were re-estimated in the present study (except for *T. tatricus*).

The hindfoot length is the least variable among linear body dimensions. For instance, the variation coefficient of this character is CV = 4.83 in *M. arvalis* and CV = 5.78 in *M. agrestis*. In addition, each genus is represented by species among which, by the general external dimensions, one is slightly bigger than the other one. It means that juvenile specimens of the larger species by body proportions are similar to the adult specimens of the smaller species, which is often a reason for incorrect identification, especially in field conditions. As an example, fig. 1 demonstrates the relation between body length and hindfoot length in the common vole and field vole.

Considering all sets of external parameters, in case of voles of *Microtus* s. l. of the Carpathians, for genus diagnostics the main attention should be focused on non-metric characters, while the value and variation of linear body dimensions and proportions should be considered for species identification. However, it should be remembered that external characters are usually used only for primary diagnostics in field conditions, and, due to similar morphology, other sets of characters should be involved for reliable species identification, in particular cranial and dental features.



Fig. 1. The relation between body length and hindfoot length in *M. arvalis* and *M. agrestis*.

Contours and proportions of the skull and its structures. Cranial characters traditionally are considered more reliable for species diagnostics than external ones. For identification of *Microtus* s. l. species, the clearest differences between genera *Terricola* and *Microtus* s. str. should be detected first, in particular the general contours of the skull (fig. 2) and the morphology of skullcap and interorbital constriction (figs 3–4). In the first case, attention should be paid to the height of the skull and to the contours of the occipital region. Thus, species of *Terricola* are characterised by a relatively flat skull profile not only in the rostral part, but also in the braincase region. On the contrary, the skull is higher and rounder in species of the genus *Microtus* (it is especially noticeable at the dorsal part of the braincase and occiput).



Fig. 2. General view of the skull of *Terricola* spp. (*a*-*b*) and *Microtus* spp. (*c*-*d*).



Fig. 3. Diagnostic characters of the region of frontal bones in *Terricola* spp., photo (a) and scheme (b).



Fig. 4. Diagnostic characters of the region of frontal bones of *Microtus* s. str. species, photo (a) and scheme (b).

Interspecific differences are also clearly expressed by the structure of some elements of the skullcap. First, it should be mentioned that the bones of the skullcap in *Terricola* are thin and translucent, in older individuals somewhat thickened but still translucent to some extent. Such anatomical feature is probably related to developmental specifics of muscles of mastication and, accordingly, to the absence of bone tissue consolidation in order to form thickenings and crests as it takes place in *Microtus* spp. Therefore, bone septa and 'capsules' of olfactory lobes are clearly visible beneath the frontal bones (fig. 3). The frontoparietal suture is concave backward as a wide arc.

In representatives of the genus *Microtus* s. str., because of developmental features of muscles of mastication, the bones of the skullcap are thicker, not translucent, and thickenings and crests develop with the age for the attachment of muscles. The crests on the surface of frontal bones have important diagnostic value, because these crests are absent in *Terricola* spp. The frontoparietal suture is concave backward as a narrow arc (fig. 4).

Additionally, the general morphological features of the proximal part of intermaxillary and nasal bones, as well as the form of sutures in this region also have significant diagnostic value. However, identification of species using these features is reliable only for the representatives of the genus *Microtus* s. str. In particular, the proximal part of the intermaxillary bones of *Terricola* spp. is wide and aliform, and the fronto-intermaxillary sutures do not go deep in each other. The nasofrontal suture is rather straight, sometimes slightly concave backward (fig. 5, a-b). The intermaxillary bones in *M. agrestis* are connected with the fron-



Fig. 5. General view of the area of frontal sutures: *a–b*) *Terricola* spp., photo and scheme; *c–d*) *M. agrestis*, photo and scheme; *e–f*) *M. arvalis*, photo and scheme.

tal bones by deep digital sutures. The proximal ends of the nasal bones have slight curves (bending) directed forward (fig. 5, c-d). In *M. arvalis*, the proximal part of the intermaxillary bones makes relatively sharp sutures with the frontal bones that deeply go into one another medially. The proximal ends of the nasal bones concave backward (fig. 5, e-f).

The width (CRB) and height of the braincase (CRH) have the highest diagnostic value among craniometrical characters for the pair of sibling species *T. subterraneus–T. tatricus*. Specimens of these two species can be clearly distinguished by these parameters (fig. 6), and the Tatra pine vole is characterized by of their higher values (p < 0.001). Such characters as nasal bones length, condylobasal length, and rostral height can be also involved into diagnostics as additional criteria (table 4).

Regarding the pair of *M. arvalis–M. agrestis*, the three following characters turned out to be the most reliable for identification of museum specimens (table 5, p < 0.001): upper molars length (M1–3), condylobasal length (CBL), and braincase height (CRH). The upper molars length has the least overlap (CD = 4.58), and in the field vole it is the least variable (CV = 3.71) among all of the considered characters. The least variable craniometrical parameters in the common vole are the braincase height (CV = 3.40) and the upper molars length (CV = 3.89).

Such parameters as condylobasal length and braincase width have larger values in the field vole, which should be expected considering that it is a boreal species. Besides, samples of *M. agrestis* and *M. arvalis* also clearly differ by the relation between the upper molars length and *foramen incisivum* length, as well as between the upper molars length and braincase height (figs 7–8).

Among cranial characters having diagnostic value for the genera *Microtus* and *Terricola*, some authors (e. g., Rekovets, 1994; Rekovets & Kovalchuk, 2017) also men-

Character, mm (M/min–max)	T. subterraneus	T. tatricus	CD
CRB, braincase width	<u>10.57</u> 9.8–11.1	<u>11.87</u> 11.3–12.3	3.53
CRH, braincase height	7 <u>.34</u> 7.0-7.3	$\frac{8.46}{8.1-8.8}$	2.52
NAL, nasal bones length	<u>6.03</u> 5.5-6.5	<u>6.75</u> 6.1–7.4	1.60
CBL, condylobasal length	$\frac{21.41}{20.0-22.9}$	<u>23.23</u> 21.0–25.2	1.47
ROH, rostral height	<u>5.41</u> 5.0–5.7	<u>5.95</u> 5.5–6.4	1.33
Total specimens, N	22-37	7-11	_

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Character (M/min–max)	M. arvalis	M. agrestis	CD
M1–3, upper molars length	<u>5.44</u> 5.0–5.9	<u>6.48</u> 6.0–6.9	4.58
CBL, condylobasal length	<u>22.2</u> 20.0–25.0	<u>26.45</u> 25.0–28.6	3.76
CRH, braincase height	<u>8.43</u> 8.0-9.2	<u>9.71</u> 9.0–10.6	3.21
FIL, <i>foramen incisivum</i> length	$\frac{4.28}{3.5-5.0}$	$\frac{5.27}{4.8-5.9}$	3.04
CRB, braincase width	$\frac{11.25}{10.1-13.0}$	$\frac{12.63}{12.0-13.7}$	2.63
Total specimens, N	41-63	15-34	—



Fig. 6. The relation between the braincase height (CRH) and width (CRB) in T. tatricus and T. subterraneus.



Fig. 7. The relation between the upper molars (M1-3) and foramen incisivum length (FIL) in M. arvalis and M. agrestis.

Fig. 8. The relation between the upper molars length (M1-3) and braincase height (CRH) in *M. arvalis* and *M. agrestis*.

Fig. 9. Algorithm of diagnostics of Microtus s. l. voles according to dental characters.

tion the structural features of the posterior part of the hard palate and the relative size of IOR (Gromov et al., 1964). However, these characters in representatives of *Microtus* and *Terricola* from the Ukrainian Carpathians are quite variable and notable differences between the two groups were not revealed. The placement of *foramen mandibulae* in *M. arvalis* and *M. agrestis* considered by Pucek (1984) a diagnostic criteria is turned out to be invalid as well, so did the CBL for *T. tatricus–T. subterraneus*.

Diagnostic dental characters. The morphological structure of the tooth crown is one of the most stable diagnostic characters. For working with collection materials, when both upper and lower molars are well preserved, this method is convenient to be used for species identification. When comparing molars, the main attention should be paid to the number and form of enamel lobes and triangles. For identification of *Microtus* s. l. species, it is handy to use the algorithm showed on fig. 9. A similar scheme was proposed earlier for voles of the fauna of Poland (Pucek, 1984). The structural features of certain teeth are shown on fig. 10.

Fig. 10. General contours of molars: a - M2 in *M. agrestis*; b - M2 in other *Microtus* s. l.; c - m1 in *M. arvalis* and *M. agrestis*; d - m1 in *Terricola* spp.; e - M3 in *T. subterraneus*; f - M3 in *T. tatricus*. A - anteroconid complex.

Discussion

Revised and newly discovered characters

During research, all characters mentioned in formerly published keys were revised for their validity regarding voles of the Ukrainian Carpathians. All of the external non-metric characters described earlier in several sources (see table 2) turned out to be valid on the regional scale suggesting the very low level of geographic variation by these characters. External linear characters were re-estimated (except for *T. tatricus*, see table 3) exclusively on Carpathian samples.

Character	Described for	Source	Validity
Skull contours	Terricola–Microtus	Gromov et al., 1963; Pucek,1984	valid
Frontal crests	Terricola–Microtus	Korneyev, 1952; Gromov et al., 1963	valid
IOR	Terricola–Microtus	Gromov et al., 1963	invalid
Hard palate	Terricola–Microtus	Rekovets, 1994	invalid
Foramen mandibulae	arvalis–agrestis	Pucek, 1984	invalid
CBL	tatricus-subterraneus	Pucek, 1984	invalid
CBL	arvalis–agrestis	Korneyev, 1952	valid*
Occiput	Terricola–Microtus	this paper	valid
Skullcap features	Terricola–Microtus	this paper	valid
Frontal sutures	Terricola – Microtus spp.	this paper	valid
CRB : CHR	tatricus-subterraneus	this paper	valid
M1-3 > CBL > CRH	arvalis–agrestis	this paper	valid
M1-3: FIL	arvalis–agrestis	this paper	valid
M1-3:CRH	arvalis–agrestis	this paper	valid

Table 6. Revised and newly described cranial diagnostic characters in Microtus s. l.

* Mentioned as the only diagnostic character, although M1-3 is more reliable.

On the other hand, among 7 cranial characters proposed earlier 4 turned out to be invalid, and 7 new cranial characters have been proposed in this paper (table 6). Noteworthy that such clearly expressed characters as sutures of the frontal region had been ignored earlier, while CBL proposed in most keys as a leading diagnostic character is invalid for *Terricola* spp., and only relatively valid for *Microtus* spp. In addition, the use of the relation of M1–3 to FIL and/or CRH is promising for reliable identification of *M. arvalis–M. agrestis*, especially when only skull fragments are available.

Dental characters have been considered the most reliable for species diagnostics since the mid-19th century (for example, in the field vole with the additional lobe on M2; Blasius, 1857 and Nehring, 1875 as cited in Méhely, 1908). Here we examined whether the widely accepted dental diagnostic structures are present in voles of the fauna of the Ukrainian Carpathians, and developed an algorithm convenient to use for species identification of Carpathian *Microtus* s. l. Besides that practically each character turned out to be valid, some specimens of T. tatricus do not share the character of M3 considered diagnostic for this species (see fig. 10, f), because some specimens of T. subterraneus with a separated t3 occur quite often. At the same time, the level of merging of t1/t2 dental fields of M3 in T. subterraneus is quite variable (merged completely, separated completely, or a constriction develops), which might be confusing as well. On the other hand, for the species M. agrestis-M. arvalis the dental method is certainly suitable. Since it is known that in certain populations of *M. agrestis* some specimens do not have the fifth lobe and t4 of M2 (Zimmermann, 1956; Reichstein & Reise, 1965), all of the examined field vole specimens were checked for the presence of the additional lobe, and there were no specimens revealed without t4.

It should be noticed that several works have been dedicated to the morphological and morphometric features of the first lower molar of voles belonging to the genus *Microtus*. Differences were found in the tiny morphology of m1 in the pair of *M. arvalis–M. agrestis* as well, in particular in the form and dimensions of triangles and reentrant angles (Kochev, 1986), frequency of different morphotypes by the structure of anteroconid complex (Meulen, 1973; Rekovets, 1994; Ivanov, 2008; Luzi et al., 2016), and relation of m1 length to t4/t5 index (Nadachowski, 1984). These tiny characters might be handy for use when the upper molars are absent in the specimens. Otherwise, in our opinion, the most convenient approach to identify Carpathian *M. arvalis* and *M. agrestis* by dental characters in collections is to compare the structure of M2.

Diagnostic characters and their significance

The experience of studying museum collections of mammals amassed during the 20th century shows not only their heterogeneity, but also indicates the necessity of constant revision, which is an especially actual issue in case of morphologically similar species. Results of such revisions prove that reliable diagnostics of specimens should not be limited to using only a certain set of characters, because different datasets have different diagnostic value, i. e. they do not give equally reliable results. Thus, it is necessary to apply a complex approach including all available diagnostically relevant characters, while the top priority should be given to the most stable characters that are minimally affected by individual, sexual, age-related, or other kinds of variability. To these criteria particularly corresponds the structure of tooth crown, especially the relative spatial placement and form of lobes, triangles, and dentine fields.

Higher level of variability is common for characters that appear, develop, or even change with the individual age. They include, for example, morpho-anatomical features of the skull, its separate bones and sutures. The characteristic structure and form of these parts are important parameters in genus and species diagnostics.

The most plastic, and therefore the most variable among characters studied in this work are the linear body dimensions. The more distant are the species being identified the much significance these characters have (an example of diagnostic value of linear external characters in identification of some mice and vole species is presented in our previous contribution: Barkaszi, 2015). In case of morphologically similar species, in particular of *Microtus* s. 1., these characters are less significant, although their role is important during primary diagnostics of specimens. The hindfoot length (Pl) has the highest diagnostic value among liner dimensions, while non-metric external characters (specifics of fur, tail, sole, etc.) are well applicable for genus diagnostics. Therefore, non-metric characters are important not only for diagnostics in the field, but also for identification of collection specimens being preserved without osteological material (pelts, study-skins).

Nevertheless, during identification of specimens it should be remembered that we are dealing with biological but not physical objects with a significant level of individual variability related to the processes of growth and development throughout their whole lifetime. That is why necessary to apply complex diagnostic approaches that involve the most significant dental, cranial, and external characters.

Generalized key for identification of voles

Based on revised and newly discovered diagnostic characters an identification key was developed. The key contains three groups of characters, such as external features followed by cranial and then dental characters. Such combination of characters is important considering that materials needed to be identified could be of various state and quality, from unprepared skins to tiny bone fragments obtained from pellets or excrements.

 Body length 78–110 mm; tail length 25–41 mm. Bicolored tail: brown on the top, whitish on the underside. Short hindfoot, 12–17 mm. Five *clavi* on the hind sole. Very small eyes. Auricles barely protruding from the fur. Two pairs of inguinal *mammae* in females. Flat profile of the skull. Thin, translucent bones of the skullcap. Intermaxillary bones form an aliform structure joining the frontal bones. Wide arcshaped frontoparietal suture.
Body length 93–135 m One-coloured tail, a l side. Hindfoot length hind sole. Eyes not d protruding from the fur. Rounded skull profile. of the skullcap. Two length bones. Digital or sharp Frontoparietal suture n

Triangles t4 and t5 of m1 with merged dentine fields. genus *Terricola* (2)

- Greyish brown fur with reddish tint on the back. Tail length 31–49 mm. Hindfoot length 17–21 mm. Pigmented same sized *clavi* on the hind sole. M1–3 length 6.0–6.9 mm; condyobasal length 25.0–28.6 mm; braincase height 9.0–10.6 mm. M2 has 5 lobes and 4 triangles (there is an additional

Tail length usually 25–39 mm, 30–35 % of the body length. Hindfoot length 12–16 mm.

Braincase width 9.8–11.1 mm; braincase height 7.0–7.3 mm.

Grey fur with brownish-yellowish tint on the back. Tail length 25–42 mm. Hindfoot length 14–17 mm. Not pigmented differently sized *clavi* on the hind sole. M1–3 length 5.0–5.9 mm; condyobasal length 20.0–25.0 mm; braincase height 8.0–9.2 mm.

M2 has 4 lobes and 3 triangles (two on the lingual and one on the buccal side). *M. arvalis*

Species in collections: the importance of revision and issues of diagnostics

Obviously, the knowledge on the fauna composition in works of the 18th and mid-19th centuries does not correspond to modern views, thus species described in old surveys actually quite often represent genera or even tribes. Nevertheless, old descriptions are extremely important to analyse changes occurred in the fauna composition. They are especially valuable when morphological details and biotopes are also included, and when the described specimens are available in museum collections. However, such specimens are usually preserved with species identification that corresponds to taxonomic views of those times, thus they have to be re-identified. To outline the range of key characters allowing identification of specimens by external and cranial features remains therefore an important task in working with museum collections. In addition, it also allows reassessing and giving value to publications on former states of the fauna.

One of the brightest examples of the importance of collections in species diagnostics is the history of description of the Tatra pine vole. *Terricola tatricus* is an autochthonous species of the Carpathians — it was not introduced, did not appear here due to climate change or because of other factors, and its range is restricted to the Carpathian highlands. Nevertheless, it was identified only in the mid-20th century among specimens of the European pine vole from the Western Carpathians (Kratochvíl, 1952; Kowalski, 1960), and later it was recognised as separate species by the karyotype as well (Kratochvíl, 1964, 1970).

Similarly, the issue of identification of the East European vole (*Microtus levis*¹) among specimens of the common vole collected in Ciscarpathia might appear as well, when records of this species will be reported from neighbouring regions. Such reports should be also expected due to collection revisions of Ciscarpathian (or even Transcarpathian) samples of the common vole. Being a sibling species of *M. arvalis*, practically it is impossible to reliably identify *M. levis* in the field based on external characters, and for its diagnostics it is necessary to involve tiny cranial characters (Masing, 1999; Tovpinets, 2012 a). An example for such revision is the study by Tovpinets (2012 a): it was shown that among 14 specimens of voles collected in the mid-20th century and identified as '*M. arvalis*' four specimens actually were *M. levis* and one specimen was *Myodes glareolus*.

These examples show again the necessity of constant collection revisions, for which a clear system of diagnostic criteria is required; i. e. development of regional keys including the most reliable diagnostic characters is of great importance.

¹ The East European vole or sibling vole (*Microtus levis*) is a 54-chromosome vole species from the group '*arvalis*' s. l. described from the foothills of the Southern Carpathians, Găgeni village, Prahova, Romania (Ellerman & Morrison-Scott, 1954). The closest to the Ukrainian Carpathians records of this species are known from Bukovina and Western Podolia described in the result of re-identification of *M. arvalis* collection specimens (Zagorodniuk, 2005; Tovpinets, 2012 a).

Conclusions

Three groups of morphological characters have been analysed, in particular external (linear dimensions and non-metric characters of the body), cranial (morphology of bones and sutures, dimensions of separate cranial structures), and dental (structure of molars), in samples of *Microtus* s. l. species collected in the Ukrainian Carpathians. The study has showed that for reliable species diagnostics it is necessary to apply a complex approach involving all relevant diagnostic characters.

External non-metric body characters, such as features of the fur, coloration, the number of *clavi* on the hind sole and of *mammae* in females are important for identification of specimens to genus level. Among linear body dimensions, the hindfoot length may allow identification of specimens to species. However, external body parameters are quite variable, thus they can be used only for preliminary diagnostics, usually conducted in field conditions.

Non-metric cranial characters, such as the form of bones and sutures of the skullcap region allow distinguishing from one another the sibling complex *T. subterraneus–T. tatricus*, *M. agrestis*, and *M. arvalis*. Among cranial structures, the braincase height and width have the highest diagnostic value for the sibling complex *T. subterraneus–T. tatricus*, and for the pair of species *M. agrestis–M. arvalis* such value have the upper molars length, structure of M2, condylobasal length, and braincase height.

In case of preservation of integral or partial osteological materials (skull and teeth), the most convenient and reliable diagnostic method is the comparison of molars involving also cranial metric and non-metric characters. Such approach in diagnostics of osteological materials gives reliable results, which is of great importance for using museum specimens in other research areas giving them additional scientific importance.

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References

- Barkaszi, Z. 2015. Muroid rodents of the lowland part of Transcarpathia: state of populations and morphophysiology. *Proceedings of the Theriological School*, **13**, 3–10.
- Barkaszi, Z. & Zagorodniuk, I. 2016. The taxonomy of rodents of the Eastern Carpathians. *Proceedings of the State Natural History Museum*, **32**, 137–154.
- Ellerman, J. R. & Morrison-Scott, T. C. S. 1951. Checklist of Palearctic and Indian Mammals 1758–1946. London, 1–810.
- Gromov, I. M., Gureev, A. A., Novikov, G. A., Sokolov, I. I., Strelkov, P. P. & Chapsky, K. K. 1963. *Mlekopitay-uschiye fauny SSSR. Chast 1.* Izdatelstvo Akademii Nauk SSSR, Moscow, Leningrad, 1–638 [In Russian].
- Gromov, I. M. & Erbayeva, M. A. 1995. The mammals of Russia and adjacent territories. Lagomorphs and rodents. Russian Academy of Sciences, Zoological Institute, St. Petersburg, 1–522 [In Russian].
- Ivanov, D. L. 2008. Identifikatsiya Microtus agrestis L. I Microtus ex gr. arvalis Pall. po morfologii i morfometrii molyarov M, v iskopaemykh faunakh golotsena Belarusi. Vestnik BGU. Ser. 2, 3, 87–93 [In Russian].
- Jaarola, M., Martínková, N., Gündüz, I., Brunhoff, C., Zima, J. et al. 2004. Molecular phylogeny of the speciose vole genus *Microtus* (Arvicolinae, Rodentia) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 33, 647–663.
- Klymyshyn, O. S. 2015. Objectives of the information-analytical function of natural science museums. In: Zagorodniuk, I., ed. Natural History Museums: Role in Education and Science. Part 2. Kyiv, 28–30 [In Ukrainian].
- Kochev, V. A. 1983. Vidovye kriterii molyarov M₁ Microtus agrestis, M. arvalis, M. oeconomus, M. gregalis, M. middendorfi, M. hyperboreus. Vestnik Zoologii, 20 (3), 40–45 [In Russian].
- Korneyev, O. P. 1954. Vyznachnyk zviriv URSR. Radianska Shkola, Kyiv, 1–216 [In Ukrainian].
- Korobchenko, M. 2012. Variability and diagnostic value of cranial characters of Spalax microphthalmus: comparison with other Spalacidae species from Eastern Europe. Proc. Theriol. School, 11, 63-70 [In Ukrainian].

- Kowalski, K. 1960. *Pitymys* McMurtrie (Microtidae, Rodentia) in the Northern Carpathians. *Acta Theriologica*, **4** (6), 81–91.
- Kratochvíl, J. 1952. Hrabosi rodu Pitymys McMurtrie v Ceskoslovensku. Prace Moravske Prirodovedecke Spolecnosti, 24 (8), 155–194.
- Kratochvíl, J. 1964. Die systematische Stellung von Pitymys tatricus Kratochvíl, 1952. Zeitschrift für Säugetierkunde, **29** (4), 230–235.
- Kratochvíl, J. 1970. *Pitymys* Artenaus der HohenTatra (Mammalia, Rodentia). *Acta Scientiarum Naturalium* (Brno), **4** (12), 64.
- Kryštufek, B., Griffiths, H. I. & Vohralik, V. 1996. The status and use of Terricola Fatio, 1867 in the taxonomy of Palearctic 'pine voles' (*Pitymys*) (Rodentia, Arvicolinae). *Bulletin van het Koninlijk Belgisch Instituut voor Natuurwetenschappen — Biologie*, **66**, 237–240.
- Litvaitis, J. A. 2000. Investigating food habits of terrestrial vertebrates. *In:* Boitani, L. & Fuller, T. K., eds. *Research Techniques in Animal Ecology*. Columbia University Press, New York, 165–190.
- Luzi, E., López-García, J. M., Blasco, R., Rivals, F. & Rosell, J. 2016. Variations in *Microtus arvalis* and *Microtus agrestis* (Arvicolinae, Rodentia) dental morphologies in an archaeological context: the case of Teixoneres Cave (Late Pleistocene, North-Eastern Iberia). *Journal of Mammalian Evolution*, 1–9, doi: 10.1007/s10914-016-9355-8.
- Martínková, N. & Dudich, A. 2003. The fragmented distribution range of *Microtus tatricus* and its evolutionary implications. *Folia Zoologica*, **52** (1), 11–22.
- Masing, M. 1999. The skull of Microtus levis (Arvicolinae, Rodentia). Folia Theriologica Estonica, 4, 76-90.

Méhely, L. 1908. Két új poczokfaj a magyar faunában. Állattani közlemények, 7 (1), 3–14.

- Meulen, van der, A. J. 1973. Middle Pleistocene smaller mammals from the Monte Peglia (Orvieto, Italy) with special reference to the phylogeny of *Microtus* (Arvicolidae, Rodentia). *Quaternaria*, **17**, 1–144.
- Mitchell-Jones, A. J., Amori, G., Bogdanowicz, W., Krystufek, B., Reijnders, P J. et al. 1999. *Atlas of European Mammals*. The Academic Press, London, 1–495.
- Nadachowski, A. 1984. Taxonomic value of anteroconid measurements of M₁ in common and field voles. *Acta Theriologica*, **29** (10), 123–143.
- Pavlinov, I. Ya. & Rossolimo, O. L.1987. Sistematika Mlekopitayuschikh SSSR. Izdatelstvo Moskovskogo Universiteta, Moscow, 1–285 [In Russian].
- Pavlinov, I. Ya. & Lissovsky, A. A. (eds). 2002. *The Mammals of Russia: A Taxonomic and Geographic Reference*. KMK Scientific Press Ltd., Moscow, 1–604 [In Russian]
- Pucek, Z. (ed.) 1984. *Klucz do oznaczania ssaków Polski: praca zbiorowa*. Państwowe Wydawnictwo Naukowe, Warszawa, 1–383.
- Reichstein, H. & Reise, D. 1965. Zur Variabilität des Molaren-Schmelzschlingenmusters der Erdmaus, *Microtus agrestis* (L.). Zeitschrift für Säugetierkunde, **30**, 36–47.
- Rekovets, L. I. 1994. *Melkiye Mlekopitayuschiye Antropogena Yuga Vostochnoy Yevropy*. Naukova Dumka, Kyiv, 1–372 [In Russian].
- Rekovets, L. I. & Kovalchuk, O. M. 2017. Phenomenon in the Evolution of Voles (Mammalia, Rodentia, Arvicolidae). Vestnik Zoologii, 51 (2), 99–110.
- Rozhenko, N. 2006. Feeding of some carnivorous mammals in anthropogenic landscape of the Black Sea region. *Proceedings of the Theriological School*, **8**, 191–200 [In Ukrainian].
- Sviridov, A. V. 1994. *Klyuchi v biologicheskoy sistematike: teoriya i praktika*. Izdatelstvo MGU, Moscow, 1–224 [In Russian].
- Tatarinov, K. A. 1956. *Ssavtsi zakhidnykh oblastei Ukrainy*. Vydavnytstvo Akademii Nauk UkrSSR, Kyiv, 1–188 [In Ukrainian].
- Tovpinets, M. 2012 a. Vydy-dviinyky polivok *Microtus arvalis* s. l. v kolektsiiakh zoolohichnykh muzeiv. *In: Stan i bioriznomanittia ecosystem Shatskoho natsionalnoho pryrodnoho parku : Materialy naukovoi konferentsii*, SPOLOM. Lviv, 75–77 [In Ukrainian].
- Tovpinets, M. 2012 b. Theriological collections and aspects of morphological diagnostics of white-toothed shrews, genus Crocidura. *Proceedings of the Theriological School*, **11**, 77–88 [In Russian].
- Turyanin, I. I. 1956. Ekologo-sistematicheskiy obzor podsemeystva polevok (Mammalia, Microtinae) Zakarpatskoy oblasti. *Nauchnye zapiski Uzhgorodskogo Universiteta*, **21**, 81–91 [In Russian].
- Turyanin, I. I. 1969. Ob ekologii podzemnoy polevki *Microtus (Pitymys) subterraneus subterraneus* de Selys Longch. v Ukrainskikh Karpatakh. *In: Voporsy okhrany prirody Karpat.* Karpaty, Uzhgorod, 222–237 [In Russian].
- Wilson, D. E. & Reeder, D. M. (eds) 2005. Mammal Species of the World. A Taxonomic and Geographic Reference (3rd ed.). Johns Hopkins University Press, 1–2142. Accessed from <u>http://www.press.jhu.edu</u> on 30.04.2017.
- Zagorodniuk, I. V. 1988. *Pitymys tatricus* (Rodentia) noviy vid v faune SSSR. *Vestnik Zoologii*, **22** (3), 54 [In Russian].
- Zagorodniuk, I. V. 1989. Taxonomy, distribution and morphological variation of the Terricola voles in East Europe. *Vestnik Zoologii*, **23** (5), 3–14 [In Russian].
- Zagorodniuk, I. V. & Zima, J. 1992. *Microtus tatricus* (Kratochvíl, 1952) in the Eastern Carpathians: cytogenetic evidence. *Folia Zoologica*, **41** (2), 123–126.

- Zagorodniuk, I. V., Vorontsov, N. N. & Peskov, V. N. 1992. Tatra vole (*Terricola tatricus*) in the Eastern Carpathians. *Zoologicheskii Zhurnal*, **71** (6), 96–105 [In Russian].
- Zagorodniuk, I. 2002. Field key to small mammals of Ukraine. *Proceedings of the Theriological School*, **5**, 1–60 [In Ukrainian].
- Zagorodniuk, I. 2005. Biogeography of mammals' cryptic species in the Eastern Europe. *Scientific bulletin of Uzhgorod University. Ser. Biology*, **17**, 5–27 [In Ukrainian].
- Zagorodniuk, I. V. & Atamas', N. S. 2005. A morphological differentiation of hamsters (Cricetidae) from the East Europe: diagnostic weight of signs and pattern of taxonomic relations. *Reports of the National Academy of Sciences of Ukraine*, 4, 153–160 [In Russian].
- Zagorodniuk, I. V. & Emelyanov, I. G. 2012. Taxonomy and nomenclature of mammals of Ukraine. *Proceedings* of the National Museum of Natural History, **10**, 5–30 [In Ukrainian].
- Zagorodniuk, I. 2016. Cryptic diversity of biota, sibling species and researchers' daltonism. *Visnyk of the Lviv* University. Ser. Biology, **71**, 3–30 [In Ukrainian].
- Zawadzki, A. 1840. Fauna der Galizisch-BukowinischenWirbelthiere. E. Schweizerbart Verlagshardlung, Stuttgart, 1–195.
- Zimmermann, K. 1956. Zur Evolution der Molaren-Struktur der Erdmaus, *Microtus agrestis* (L.). Zoologische Jahrbücher, **84**, 269–274.

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