

# Epigenetic Diversity and Similarity of the Voles of “Guentheri” Group (Mammalia: Rodentia) in Anatolian Peninsula and South-Eastern Part of the Balkan Peninsula

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**Abstract:** This study analyzed variations of four vole taxa from Anatolia and Southeastern Balkan Peninsula, i.e. *Microtus guentheri guentheri*, *Microtus lydius lydius*, *Microtus lydius ankaraensis* and *Microtus hartingi strandzensis*, on the basis of 32 qualitative non-metric skull characters. The degree of polymorphism was analyzed by computing the Epigenetic Variability (Vi). The epigenetic divergence among each single studied taxon from the remaining taxa was evaluated by Mean Measure of Divergence (MMD). The recorded epigenetic polymorphism of the studied taxa (i) demonstrated the well expressed similarity between the two subspecies of *M. lydius*, (ii) confirmed the differentiation between *M. h. strandzensis* and *M. g. guentheri* and (iii) revealed the greater similarity between *M. g. guentheri* and the two subspecies of *M. lydius* than between *M. g. guentheri* and *M. h. strandzensis*.

**Keywords:** Epigenetic, guentheri, Anatolian, Balkan peninsula

## Introduction

The voles with diploid number of chromosomes  $2n=54$  are known as the “*guentheri*” group (GOLENISHCHEV *et al.* 2002). According to classical concepts, *Microtus guentheri* (Danford and Alston, 1880), a well-known species in this group, ranges from the south-east Balkans and Turkey through Syria and Israel to Lebanon, with an isolated range segment in northern Libya. Within Europe, it has a discontinuous range in southern Serbia and Montenegro, F. Y. R. of Macedonia, parts of southern and eastern Greece, southern Bulgaria and Turkey (for details, see WILSON, REEDER 2005; AMR *et al.* 2008; KRYŠTUFEK 1999; SHENBROT, KRASNOV 2005).

According to the recent debates on the taxonomic status of the Günther's vole (*M. guentheri sensu lato*) based on the results from morphological, cytogenetic and biochemical examinations, this taxon has a complex structure. Several studies described

taxa of various rank within the classical range of *M. guentheri sensu lato* (for details, see YİĞİT, ÇOLAK 2002; KRYŠTUFEK *et al.* 2009; ZIMA *et al.* 2012). The discussions on these descriptions have been focused on (i) the detachment of *Microtus lydius* as a distinct species and the differentiation of the subspecies *Microtus lydius ankaraensis* described on the basis of biometric differences in Central Anatolia (YİĞİT, ÇOLAK 2002), (ii) the statement by KRYŠTUFEK *et al.* (2009), based on molecular evidence, that the specimens from Western Anatolia and Thrace belonged to *Microtus hartingi* and not to *M. guentheri* and (iii) the statement by YİĞİT *et al.* (2012), based on biometric differences, that the specimens from the group of Günther's vole with  $2n=54$  from South-eastern Thrace and Anatolian Peninsula belonged to different taxonomic categories, i.e. *Microtus hartingi* (Barrett-Hamilton, 1903) in South-eastern Thrace, *M. lydius lydius* in Western Anatolia, *M. lydius an-*

*karaensis* in Central Anatolia and *M. guentheri* in South-eastern Anatolia.

The aim of the present study is (i) to reveal the epigenetic cranial polymorphism of the voles of the “*guentheri*” group in Anatolian Peninsula and south-eastern part of the Balkan Peninsula, i.e. *Microtus guentheri guentheri*, *M. lydius lydius*, *M. lydius ankaraensis* and *M. hartingi strandzensis* (as adopted by Yiğit *et al.* 2012) and (ii) to determine epigenetic divergence among them.

## Material and Methods

The present study examined the epigenetic variation of both Turkish and Bulgarian specimens (n=159) of *M. guentheri (sensu lato)* differentiated into four taxa: *M. g. guentheri*, both subspecies of *M. lydius* (*M. l. ankaraensis* and *M. l. lydius*) and *M. hartingi strandzensis (Microtus guentheri strandzensis)* from their geographical ranges as recognised by Yiğit *et al.* (2012). The classification of specimens of *M. guentheri (sensu lato)* into the studied taxa was based on their karyotype, morphological distinctiveness and geographical delimitation of their populations (for details see Yiğit *et al.* 2012). They have been combined into Operational Taxonomic Units (OUTs) consisting of populations from which the topotype specimens of the studied taxonomic units originated (Fig. 1):

OTU-1 South-eastern Thrace specimens (n=31), topotype specimens of *M. guentheri strandzensis = Microtus hartingi strandzensis* from northern Strandzha Mountain Region in the north part of South-eastern Thrace, Bulgaria and specimens from south part of South-eastern Thrace (southern

Strandzha Mountain Region) in Turkey;

OTU-2: Central Anatolian specimens (n=31), topotype specimens of *M. lydius ankaraensis* from vicinity of Ankara, Turkey;

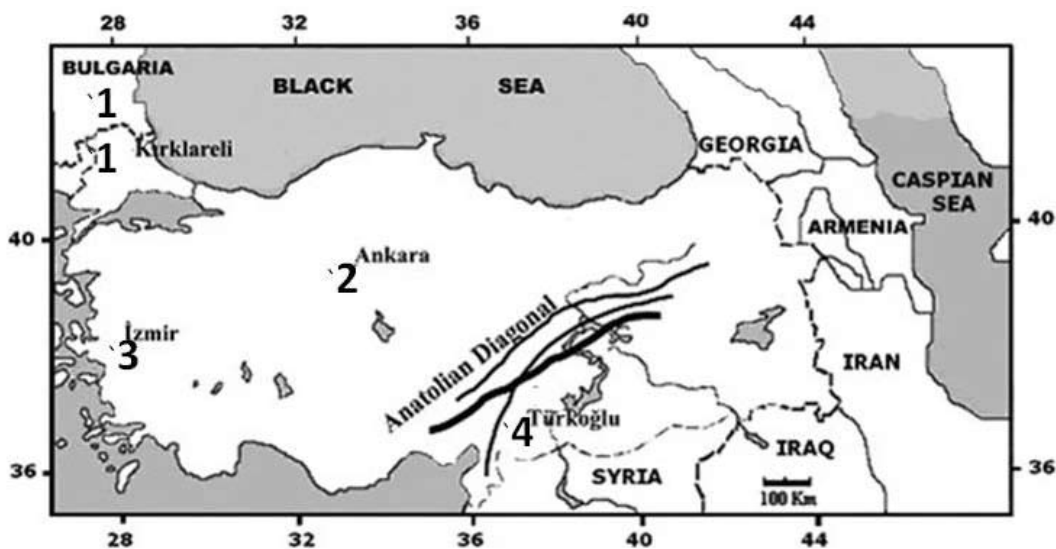
OTU-3: Western Anatolia (n=35), topotype specimens of *M. lydius lydius* from vicinity of İzmir, Turkey;

OTU-4: South-eastern Anatolia (n=31), topotype specimens of *M. guentheri guentheri* from vicinity of Türkoğlu /Kahramanmaraş, Turkey.

Only adult specimens were examined in this study. The age of the specimens was identified on the basis of the skull structure using the criteria described by BASHENINA (1953).

The studied specimens from Anatolia and South-eastern Balkan Peninsula actually were topotype specimens used for descriptions grounding the current nomenclature of voles of “*guentheri*” group (Yiğit *et al.* 2012). These specimens were kept in the Biology Department, Faculty of Science, Ankara University. The major part of the skulls of Bulgarian specimens have been used by MARKOV (1960) to describe the subspecies *M. g. strandzensis* in northern Strandzha Mountain and belonged to the collection of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia. During the period 2005-2010, new specimens from the same region have been added to this sample.

The non-metrical craniological variation of examined operational taxonomic units was evaluated on the basis of a set of qualitative skull traits representing orifices of nerves, blood vessels or shape of sutura. These traits were observed at the left side of the skull under a microscope with magnification of 12X.



**Fig.1.** Geographic location of the studied Operational Taxonomic Units (OTUs) in the area of Günter’s vole (*M. guentheri, sensu lato*) in Anatolian Peninsula and south-eastern part of the Balkan Peninsula: 1 – *M. hartingi strandzensis = M. g. strandzensis*; 2 – *M. lydius ankaraensis*; 3 – *M. lydius lydius*; 4 – *M. guentheri guentheri*

We applied the set of non-metrical craniological traits proposed by UHLÍKOVÁ (2004a) (Fig. 2). The scoring procedures for each trait have been further described in the study of variation of the common vole (*M. arvalis*) in the Czech Republic (UHLÍKOVÁ 2004b). Each specimen was scored for 32 (No 1-21, No 23 and No 25-34) of the proposed 34 non-metric characters; Characters No 22 and No 24 were unclearly expressed on the skull of the Günter’s voles (*M. guentheri sensu lato*) and they were eliminated.

The epigenetic distinctiveness of *M. g. guentheri*, *M. l. lydius*, *M. l. ankaraensis* and *M. h. strandzensis* was statistically estimated by computing: (i) the Epigenetic Variability (Vi) after SMITH (1981); (ii) the Mean Measure of Divergence (MMD) among each single taxon and the remaining taxa (SJOVOLD 1973); and (iii) the Measure of Uniqueness (MU) for each taxon (BERRY 1963). A dendrogram of epigenetic phenetic similarity was constructed on the basis of the observed phenotypic frequencies of variants of the 32 studied traits in the investigated taxa using the cluster (UPGMA) analysis of the statistical package Statistica for Windows (1993).

Although the absence of sexual dimorphism in the studied craniological traits was proven in previous investigations of *Microtus arvalis* (UHLÍKOVÁ 2004 a. b), the independent appearance of the features studied in male and female specimens of *M. guentheri (sensu lato)* was examined by applying the  $\chi^2$ -test. The proved absence of sexual dependence ( $\chi^2 = 0.88124$ ,  $\alpha = 0.05$ ) in the studied characters permitted to pool the specimens of both sexes into one sample.

## Results

The frequency distributions of the examined 32 non-metric traits are given in Table 1. Some traits were monomorphic in all studied taxa. Traits Nos 2, 25 and 30 expressed the maximum value of appearance of the studied state while in traits Nos 11, 15 and 29 it did not appear at all. The frequencies of the remaining 26 traits showed specific distribution for each particular studied taxon.

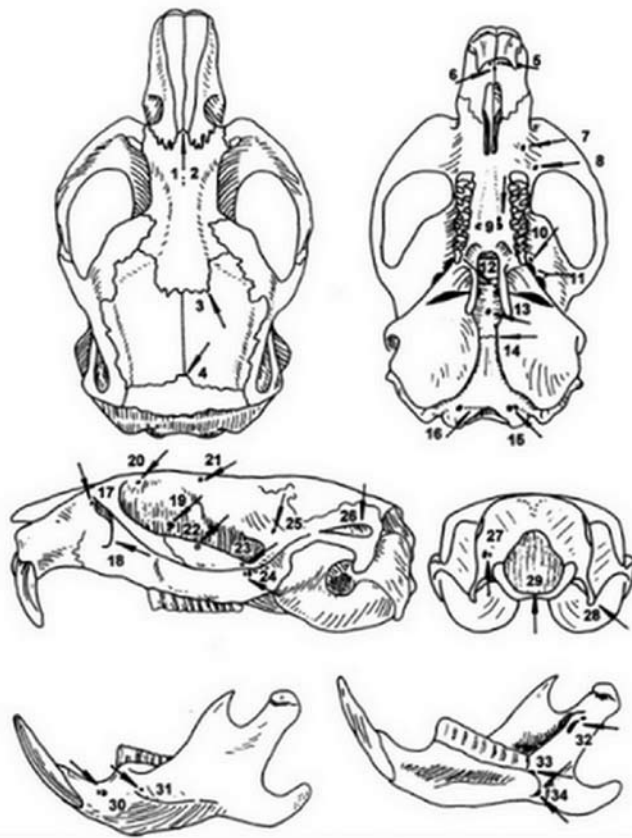
The estimation of the epigenetic variation of the studied voles’ taxa of the “*guentheri*” group showed values with the same range within the interval 0.085-0.110 (Table 2). According to their mean values of Vi (in descending order) studied voles’ taxa could be arranged as follow: *M. g. guentheri* > *M. h. strandzensis* > *M. l. lydius* > *M. l. ankaraensis*. The epigenetic variability compared at subspecies level in the *M. lydius (M. l. lydius and M. l. ankaraensis)* showed a difference of 7.1%. The highest value of Vi

**Table 1.** The number of individuals (n) and frequencies (p) of epigenetic non-metric cranial traits in the taxa evaluated: *Microtus hartingi strandzensis* (M. h. s.); *Microtus guentheri guentheri* (M. g. g.); *M. lydius lydius* (M. l. l.); *M. lydius ankaraensis* (M. l. a). Description and examined status of the scored traits are given in Figure 2

Trait №	Taxa									
	M. g. g.		M. l. l.		M. l. a.		M. l. a.		M. h. s.	
	p	n	p	n	p	n	p	n	p	n
1	0.1429	28	0.0571	35	0.0345	29	0.0345	29	0.0333	30
2	1.0000	29	1.0000	35	1.0000	30	1.0000	30	1.0000	30
3	0.2500	28	0.0294	34	0.0667	30	0.0667	30	0.5217	23
4	0.0000	28	0.0294	34	0.0000	30	0.0000	30	0.1739	23
5	0.9630	27	0.7879	33	0.9667	30	0.9667	30	0.3929	28
6	0.9286	28	0.8857	35	0.9000	30	0.9000	30	0.7143	28
7	0.1481	27	0.0286	35	0.0333	30	0.0333	30	0.0345	29
8	0.6552	29	0.9143	35	0.7333	30	0.7333	30	1.0000	29
9	0.7143	28	0.8485	33	0.8621	29	0.8621	29	0.9231	26
10	0.4643	28	0.6176	34	0.7333	30	0.7333	30	0.4762	21
11	0.0000	28	0.0000	34	0.0000	30	0.0000	30	0.0000	20
12	0.6800	25	0.5556	27	0.6154	26	0.6154	26	0.3333	12
13	0.0000	25	0.0303	33	0.0000	30	0.0000	30	0.0000	22
14	1.0000	25	1.0000	32	0.9667	30	0.9667	30	1.0000	23
15	0.0000	26	0.0000	35	0.0000	30	0.0000	30	0.0000	22
16	0.9600	25	0.9143	35	0.9333	30	0.9333	30	0.6500	20
17	0.0455	22	0.0313	32	0.1538	26	0.1538	26	0.0690	29
18	0.3214	28	0.1176	34	0.1000	30	0.1000	30	0.5556	27
19	0.0370	27	0.0303	33	0.0667	30	0.0667	30	0.1667	24
20	0.1786	28	0.1515	33	0.0690	29	0.0690	29	0.0000	24
21	0.1111	27	0.1515	33	0.1724	29	0.1724	29	0.0417	24
22	-	-	-	-	-	-	-	-	-	-
23	0.8000	25	0.6875	32	0.8667	30	0.8667	30	0.8261	23
24	-	-	-	-	-	-	-	-	-	-
25	0.7857	28	1.0000	33	1.0000	30	1.0000	30	1.0000	21
26	0.5385	26	0.5000	34	0.8667	30	0.8667	30	0.6500	20
27	0.9200	25	0.2941	34	0.4643	28	0.4643	28	0.8750	24
28	0.2800	25	0.2857	35	0.3478	23	0.3478	23	0.7143	14
29	0.0000	25	0.0000	35	0.0000	30	0.0000	30	0.0000	25
30	1.0000	27	1.0000	34	1.0000	30	1.0000	30	1.0000	29
31	0.5714	28	0.9688	32	0.7931	29	0.7931	29	0.7586	29
32	0.0714	28	0.1176	34	0.0357	28	0.0357	28	0.0345	29
33	0.8929	28	0.9706	34	0.9655	29	0.9655	29	1.0000	27
34	0.4815	27	0.5806	31	0.7037	27	0.7037	27	0.8800	25

observed in *M. g. guentheri* exceeded by 22.72% the lowest value found in *M. l. ankaraensis*.

Of all calculated epigenetic distances (MMD), only those among *M. h. strandzensis* and all the other taxa were statistically significant ( $p < 0.05$ ) and thus



**Fig. 2.** Topographic position (dorsal, ventral, lateral and caudal aspects of cranium; lateral and medial aspects of the lower jaw) and examined status (*in italic font*) of the scored non-metric cranial traits in the *Microtus guentheri*. *sensu lato*, according to the set of craniometrical parameters used in the epigenetic studies of *Microtus arvalis* by Uhlíková (2004a)

1: shape of the posterior end of nasal bones – *pointed, bow or straight*; 2: position of the posterior end of nasal bones in regard to sutura incisivofrontalis – *in front of or behind*; 3: shape of sutura coronalis – *bow or rectangular*; 4: shape of sutura lambdoidea – *shape bow, obtuse angle or brace*; 5: foramen – *absent or present*; 6: foramen – *absent or present*; 7: foramen – *absent or present*; 8: foramen – *absent or present*; 9: foramen – *single or double*; 10: foramen – *absent or present*; 11: foramen – *two or four foramens*; 12: foramen – *absent or present*; 13: foramen – *absent or present*; 14: sutura between os basisphenoidale and os basioccipitale – *absent or present*; 15: foramen – *single or double*; 16: position of foramen no. 15 in regard to lower margin of foramen ovale – *above or below*; 17: foramen – *absent or present*; 18: shape of processus zygomaticus maxillae – *bow or straight*; 19: foramen – *single or double*; 20: foramen – *absent or present*; 21: foramen – *absent or present*; 22: foramen – *absent or present*; 23: foramen – *absent or present*; 24: foramen – *single or double*; B 25: foramen – *absent or present*; 26: shape of foramen – *isosceles or rectangular triangle*; 27: foramen – *single or double*; 28: shape of processus paracondylaris – *bow or straight*; 29: shape of foramen ovale – *bow or pointed*; 30: foramen mentale – *absent or present*; 31: foramen – *absent or present*; 32: foramen – *single or double*; 33: foramen – *absent or present*; 34: foramen – *absent or present*

**Table 2.** Epigenetic distances (MMD) (the upper line) and their standard deviation (the lower line in *italic*). Epigenetic cranial uniqueness (MU) and Epigenetic variability (Vi) of the studied taxa of the “*guentheri*” group voles in Anatolian Peninsula and south-eastern part of the Balkan Peninsula: *Microtus hartingi strandzensis* (M. h. s.); *Microtus guentheri guentheri* (M. g. g.); *M. lydius lydius* (M. l. l.); *M. lydius ankaraensis* (M. l. a.). The statistically significant epigenetic distances among the studied populations are marked in bold font

Taxon	M. g. g.	M. l. a.	M. h. s.	MU	Vi
M. l. l.	0.143	0.027	<b>0.235</b>	0.235	0.091
	<i>0.094</i>	<i>0.090</i>	<i>0.102</i>		
M. g. g.		0.088	<b>0.275</b>	0.275	0.110
		<i>0.100</i>	<i>0.112</i>		
M. l. a.			<b>0.250</b>	0.250	0.085
			<i>0.108</i>		
M. h. s.				0.760	0.099

it expressed the highest epigenetic cranial uniqueness (MU) (Table 2).

The cluster analysis of the phenotypic frequencies of the 32 studied traits revealed the epigenetic similarity of the voles from the “*guentheri*” group in the studied regions of Anatolia and Balkan Peninsula (Fig. 3): (i) the highest degree of similarity was found between the both subspecies of the *M. lydius*; (ii) *M. g. guentheri* was closer to them than it is to *M. h. strandzensis*; and (iii) *M. h. strandzensis* differed most in the general picture of the epigenetic similarity among the studied taxa.

## Discussion

The recorded population epigenetic polymorphism in the studied representatives of voles from the “*guentheri*” group suggests the lack of expressed relation among the geographic nearness of the studied taxa and their population epigenetic variation in the studied regions of Anatolia and Balkan Peninsula. The population epigenetic variability of these taxa (*M. g. guentheri* = 0.11; *M. l. lydius* = 0.091; *M. l. ankaraensis* = 0.085; *M. h. strandzensis* = 0.099) shows small differences compared to other rodent species: *Microtus arvalis* – Vi = 0.15 (UHLÍKOVÁ 2004a) and *Mus musculus* – Vi = 0.12 (LAZAROVÁ 1999) from the Czech Republic; *Apodemus sylvaticus* – Vi = 0.13 (MARKOV & CHASSOVNIKAROVA 1999); *Apodemus flavicollis* – Vi = 0.11 (MARKOV & GOSPODINOVA 1999) from Bulgaria and *Clethrionomys glareolus* – Vi = 0.19 from Austria (SPITZENBERGER *et al.* 1999).

The determination of the mean epigenetic measure of divergence among studied taxa clearly

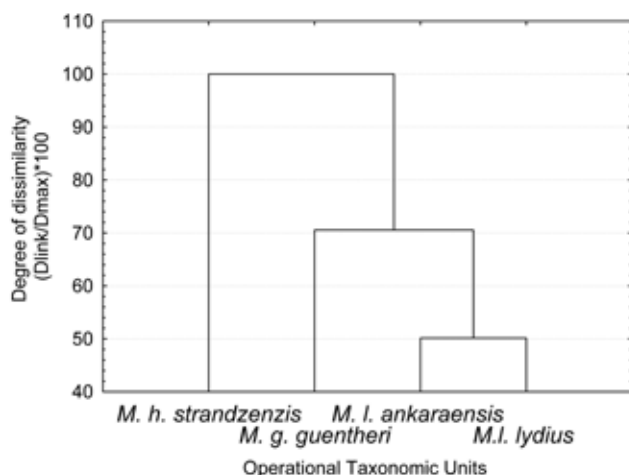


FIG. 3. Phenetic non-metric cranial degree of dissimilarity of the studied taxa of the “guentheri” group voles in Anatolian Peninsula and south-eastern part of the Balkan Peninsula

outlines the absence of statistically significant ( $p < 0.05$ ) epigenetic distances (MMD) at the subspecies level within *M. lydius* (between *M. l. lydius* and *M. l. ankaraensis*) and the evident epigenetic cranial differentiation of the voles in the south-eastern part of the Balkan Peninsula (*M. h. strandzensis*) from all the taxa inhabiting the Anatolian Peninsula.

The probable presence of taxonomic differences among the representatives of the “guentheri” group in South-eastern Thrace, namely *M. guentheri strandzensis* = *Microtus hartingi strandzensis*, and those in the Anatolian Peninsula has been presumed by MARKOV (1960) who has found differences in their biometric and exterior colorimetric parameters. CHASSOVNIKAROVA *et al.* (2008) have also observed differences in the morphology and distribution of heterochromatin in the autosomes and sex chromosomes of their karyotypes. The revealed degree of the phenotypic epigenetic similarity of the studied

taxa determines epigenetic clusters reflecting the presumable taxonomical relationships of the studied geographical populations. It confirms the detachment of *M. h. strandzensis*, the linking of the two subspecies of *M. lydius* in a single group as well as the relative separation of *M. g. guentheri*, which remains more similar to the subspecies of *M. lydius* than to *M. h. strandzensis*.

The established epigenetic diversity and detachment of the presumed taxa from “guentheri” group voles contravenes the idea that all its representatives in Anatolian Peninsula and south-eastern part of the Balkan Peninsula belong to single species, i.e. *M. guentheri*.

We intentionally avoid the nomenclature questions here and use the nomenclatural names of examined populations as classified as applied by YİĞİT *et al.* (2012) because often various authors refer the same specimens to different taxa. Another issue, which has not been addressed yet, is the lack of the detailed morphological, karyotaxonomic and biochemical-genetic data about the differentiation of important from zoogeographical point of view populations within the range of *M. guentheri sensu lato*, which makes its complicated taxonomic structure difficult to be determined. It seems that taxonomic structure of *M. guentheri sensu lato* reflecting the species and subspecies levels of presumptive taxonomic units within the Euro-Asiatic range will be determined in future after more comprehensive analysis. This analysis must necessarily take into account geographic barriers among populations, their isolation by distance, phylogeographic history and genetic status.

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