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Sibling vole species (*Microtus*) in the fragmented landscape of south-eastern part of Thrace, Balkan Peninsula: species presence, habitat selection and craniometry

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ABSTRACT

The presence of sibling vole species (*Microtus*) in the most common forests and open landscapes in south-eastern part of Thrace (Balkan Peninsula) of contemporary territories of Bulgaria and Turkey was studied. Only the southern vole (*Microtus levis*) was found in the investigated region. In his northern part, the Southern vole is associated with semi dry, upland habitats such as deserted and overgrown with wild vegetation vines near sparse forests and large agricultural fields under autumn crops with adjacent non-arable lands with shrub vegetation. In the southern part, it is presented in open landscape related to water areas (meadows near rivers and wetlands). On the basis of trapping the Southern vole is a common species in the north part of Strandzha region. The established craniological characteristics of the Southern vole from investigated region, which can be considered as a zoogeographical crossroads, with a late Pleistocene connection between the Balkan Peninsula and the mammalian fauna of Anatolian peninsula, enriched the knowledge about its craniological variation in Europe.

Key words: *Microtus levis*, southern vole, sibling vole species, south-eastern Thrace, biotopes adherence

Introduction

Population taxonomy of common vole started to change considerably when it was found that the group of the common vole (*Microtus arvalis* sensu lato) in Europe comprises two sibling species: the Common vole (*Microtus arvalis* Pallas, 1778, sensu stricto) with 46 chromosomes and the Southern vole (*Microtus levis* = *rossiaemeridionalis* Ognev, 1924) with 54 chromosomes (Meyer et al., 1969**).** Examination of karyotypes of numerous common vole populations in Europe, aiming to verify to which one of the sibling species do they belong, revealed widespread sympatric distribution of these two species in their European range (Zima & Král, 1984).

The lack of understanding of the systematic status of many sibling voles' populations in the presumable areas of their sympatric distribution was determined by the fact that the main diagnostic features of these morphologically similar species, such as karyotype (Meyer et al., 1969**)** and specific electrophoretic mobility of haemoglobin fractions (Malygin, 1983) were obtained by laborious analyses. The development of craniometrical keys (Markov & Kocheva, 2007, Markov et al., 2009) for species classification of the representatives of *Microtus arvalis* sensu lato, together with the availability of massive craniological material allowed outlining their distribution in many regions in south-east Europe and on the Asiatic part of Turkey.

When it was applied for species craniometric determination of the representatives of *M. arvalis* (sensu lato) on the territory of Bulgaria, it was found that *M. arvalis*, sensu stricto and *M. levis* inhabit together in twenty-three of all studied twenty-five biotopes. Their co-existence is established in North-eastern Bulgaria, Stara Planina foothills, highland fields of Stara Planina, Sredna Gora Mountain and

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Western Bulgaria, sub-Balkan fields and the Black sea coast (Markov & Kocheva, 2008).

At the same time, the distribution and species identity of sibling voles in many other territories of South-eastern Balkan Peninsula, one of which is South-eastern Thrace, have not been clarified and species craniometry was not described. Heretofore no information on species identification of the sibling voles, their habitat distribution and skull shape variation in South-eastern Thrace has ever been reported. Our comprehension of the population taxonomy of the sibling voles (Microtus) in Strandzha Mountain region, situated in South-eastern Thrace on the territory of Bulgaria and Turkey, is still insufficient. They are thus far associated with the classic idea about the distribution of the Common vole *M. arvalis*; namely, that only *M. arvalis* could be found there (Niethammer & Krapp, 1978). Detailed population investigations of species identity and craniometry of the sibling voles in South-eastern Thrace on the territory of Turkey (Yiğit et al., 2003) also need filling out.

Thus, the aim of the present work was: (i) to carry out craniometric species identification of the sibling voles' species (*Microtus*) in the south-eastern part of Thrace, (ii) to assess their biotopic adherence particularly in Strandzha Mountain region; and (iii) to characterize craniometrically the identified species.

Materials and Methods

Study area

Animals were collected during summer season (June and July) during the period 2006-2009 in the South-eastern Thrace on the contemporary territories of Bulgaria and Turkey. This part of Balkan Peninsula was connected to Anatolian peninsula by a land bridge during the late Pleistocene (Lang, 1994) and can be considered as a zoogeographical crossroads, with a late Pleistocene connection to the particularly rich mammalian fauna of Anatolian peninsula (Cheylan, 1991). Its mammalian crossroad status is also indicated by the patterns of the present geographical distribution of several terrestrial mammalian species (Niethammer & Krapp, 1978, 1982; Stubbe & Krapp, 1993a, 1993b).

Detailed study of the biotopic adherence of sibling voles (Microtus) and their relative abundance in the fragmented landscape of south-eastern part of this region is performed in Strandzha Mountain region. It is situated in south-eastern part of Balkan Peninsula and covers much of South-eastern Thrace in Bulgaria and the European part of Turkey and belongs to European Deciduous Forest biome (Tahtadzhyan, 1978). The specific geological, climatic and bio-geographical characteristics of this region have determined the formation of natural ecosystems with great biodiversity of small mammals (Paspalev & Markov, 1961; Peshev & Angelova, 1962; Popov, 1993; Markov et al., 2000; Chassovnikarova et al., 2005; Markov et al., 2008). Nowadays, as a result of development of agriculture and grazing, many semi-natural habitats and agricultural areas have been formed (Strandzha Nature Park site), which can be grouped into Woodlands and Open landscapes by their landscape type.

The set of the investigated habitats covering typical and most common plant associations in this region (Bondev, 1991; Gruev & Kuzmanov, 1994) included much of habitats traditionally studied in biodiversity estimation of small mammals in northern Strandzha region (Markov et al., 2000; Chassovnikarova et al., 2005; Markov et al., 2008) and South-eastern Thrace in the European part of Turkey (Yiğit et al., 2003) in both main types landscapes:

(I) Woodlands, represented by:

(i) Riparian woodlands - periodically flooded riparian mixed deciduous forest (Locations: 11 – Veleka River outfall; 5 – Fakiiska River at Fakiya village);

(ii) Deciduous woodlands of Colchisian-Mediterranean type (Locations: 10 – Kosti village, 3 – Pismenovo village);

(iii) Mixed forests, with artificial coniferous plantations (Locations: 6 A – Tsarevo town, 12 – Malko Tarnovo town, 13 – Demirköy village);

(II) Open landscapes, represented by:

(iv) Meadows near agricultural areas and rivers, covered with steppe-like grass communities, semi-shrub and shrub vegetation (Locations: 6 B – Tsarevo town, 16 - Sarhdere village**,** 17 – Havsa town; 18 – Tekirdag town);

(v) Open wetlands (Locations: 9 A– Gramatikovo village; 18 – Tekirdag town);

(vi) Agricultural lands under alfalfa, autumn cereals or vines (Locations: 8 – Gorno Yabalkovo village, 2 – Iasna Poliana village, 9B – Gramatikovo village; 7 – Ahtopol town; 4 – Novo Panicharevo village);

(vii) Glades in deciduous and mixed forests (Locations: 6C – Tsarevo town; 15 – Kirkiarli town; 14– Dereköy village);

(viii) Pastures with sparse vegetation on drained soil (Locations: 1- Velika village) (Figure 1).

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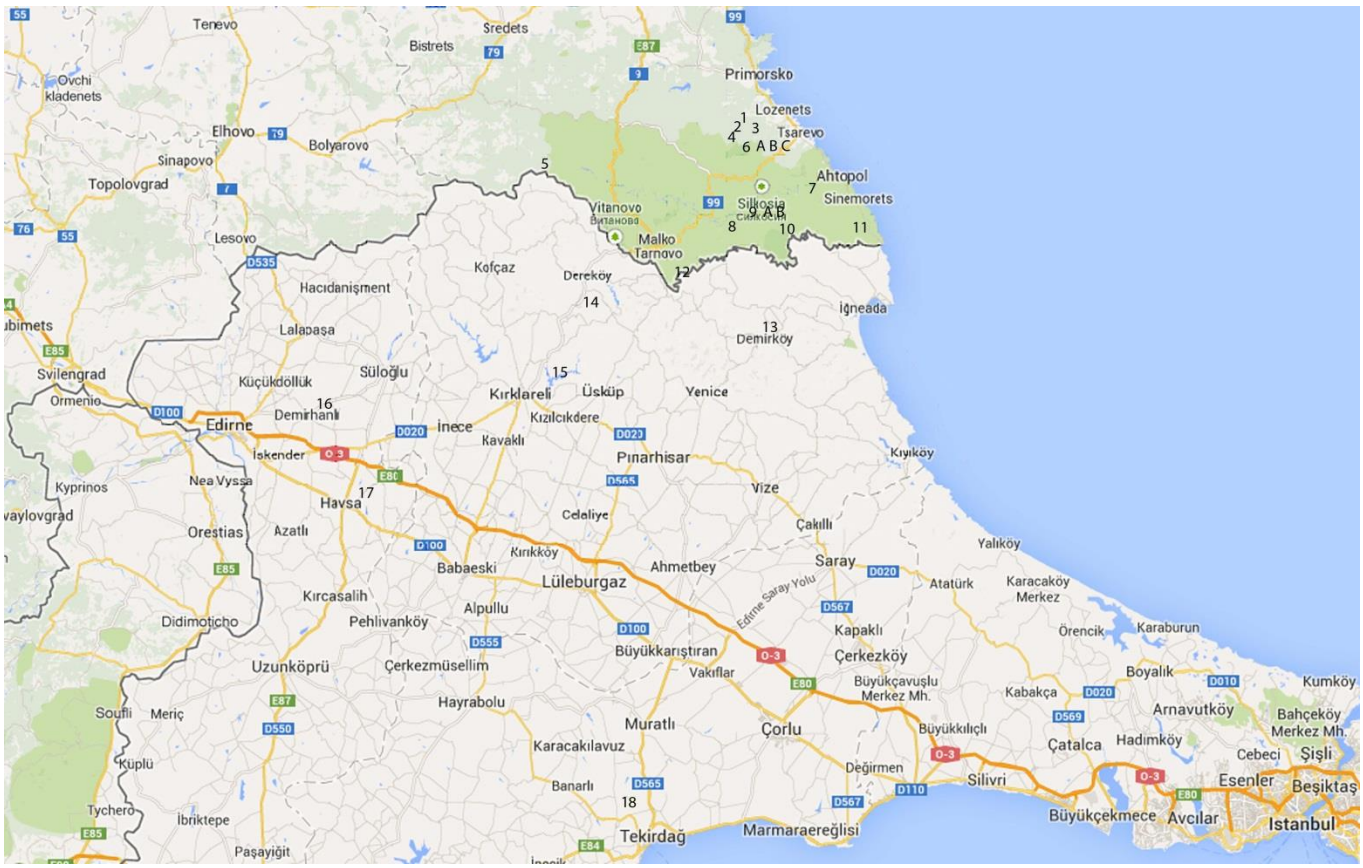

Figure 1. *Geographic origin of the studied samples from south-eastern part of Thrace. The locations are marked by the names of the nearest settlement: 1- Velika village; 2- Iasna Poliana village; 3 – Pismenovo village; 4 – Novo Panicharevo village; 5 – Fakiya village; 6 A, B,C– Tsarevo town; 7 – Ahtopol town, 8 – Gorno Yabalkovo village; 9 A, B – Gramatikovo village; 10 – Kosti village; 11 – Veleka River outfall; 12 – Malko Tarnovo town; 13 – Demirköy village, 14 –Dereköy village; 15 – Kirkiarli town;16 – Sarhdere village;17 – Havsa town;18 – Tekirdag town.*

The studied complex of habitats covers a significant part of heterogeneous natural-geographic pattern of the South-eastern Thrace and comprises most of the biotopes (both natural and man-made) preferred by the studied sibling vole species. The Southern vole prefers biotopes with tree stands and vegetable gardens, undergrowth of large-stalk grass, etc. (Malygin, 1970, 1983; Dobrokhotov et al., 1985; Tikhonov et al., 1998), while the Common vole typically inhabits coenoses of meadow type and agricultural lands (Dobrokhotov et al., 1985; Teslenko & Zagorodnyuk, 1986; Tikhonov et al., 1992; Karaseva et al., 1994), weakly wooded forest biotopes along the river valleys, ravines, forest belts and avoids territories under intensive human impact and transformation (Tikhonov et al., 1992; Tikhonov et al., 1998; Tikhonov & Tikhonova, 1997).

Collecting and age determination

In each of the studied biotopes the presence of small mammals was surveyed by analysing the species composition of the catch carried out for 2 days by three trap lines each consisting of 25 traps, located 25 meters from each other. The age of the specimens belonging to *M. arvalis* sensu lato was determined by craniological criteria (Bashenina, 1953).

Taxonomic classification

All studied adult sibling voles were classified to one of the two sibling species *M. arvalis* or *M. levis* using discriminating craniological function with high classification ability, consisting of four measurements, which did not show sexual dimorphism in the studied species: Alveolar length of upper molars, Zygomatic width, Cranial height between

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bullae ossae and Length of incisive foramen (Markov & Kocheva, 2007). In addition, the method of specific electrophoretic mobility of haemoglobin fractions proposed by Malygin (1983) was applied to verify the species of sibling voles from meadows near agricultural areas and rivers, covered with steppe-like grass communities, semi-shrub and shrub vegetation (Location: Tsarevo town) and agricultural lands under autumn cereals (Location: Achtopol town); the species of the sibling voles caught in the same biotope (Havsa town; Sarhdere village) or in the wetland biotope (Tekirdag) in Turkish Thrace was verified by karyotyping.

Determining the relative numbers

The relative numbers of the sibling voles in the northern region of south-eastern part of Balkan Peninsula was evaluated in two types of biotopes by the method of trap-lines (Novikov 1953). The first one near the village of Novo Panicharevo village represented neglected and overgrown with wild vegetation vines with sparse deciduous forest nearby and the second near the village of Iasna poliana was represented by agricultural lands under autumn crops. In these two biotopes pitfall traps were used, put in places with clear presence of small mammals – burrows, forming colonies. The presence of small mammals was surveyed by 3 trap lines with 25 traps each for 3 days in the vineyards and by 3 trap lines with 25 traps each for 2 days in the agricultural lands under autumn crops.

The relative number of captured small mammals was determined as number of animals caught per 100 Pitfall days and their dominant presence in the studied area was estimated quantitatively by Kouzyakin's dominance gradation scale (Kuzyakin, 1962) as follows: mass species, if its relative presence is ≥30% of the total catch; numerous species, if its relative presence is from 10.0% to 29.9% of the total catch; common species, if its relative presence is from 1.0% to 9.9% of the total catch; rare species, if its relative presence is from 0.2% to 0.9% of the total catch.

Craniometric description

The specimens from Bulgaria and Turkey classified to one of the two species *M. arvalis* or *M. levis* were merged into species' operational taxonomic units for further craniometrical description. Craniometrical analyses were conducted on 42 adult specimens, with established taxonomic affiliation to Southern vole (*M. levis*) from the whole investigated territory of south-eastern part of Thrace. It includes 21specimens established in the northern part of the study region on the territory of Bulgaria (Locations: Tsarevo town; Iasna poliana village; Ahtopol town; Novo Panicharevo) and 21specimens established in its southern part on the territory of Turkey (Locations: Havsa town; Sarhdere village; Tekirdag town).

The set of twenty-six cranial characters (Figure 2), measured with a digital caliper with 0.1 mm accuracy, previously used in obtaining of the craniometric population characteristics of both Microtus sibling species in Europe (Markov & Kocheva, 2007) was applied to obtain population craniometrical characteristics and to evaluate species morphometric variation of the studied sibling voles from south-eastern part of Thrace situated on the territory of the Bulgaria and Turkey.

Statistical analysis

All craniometric data were subject to standard statistic univariate analysis. Data were tested for normality using Kolmogorov-Smirnov D-statistics, and for homogeneity of variances using Levene's test. Although previuos investigations didn't found any sexual dimorphism in studed characters in the European (Markov & Kocheva, 2007) and Asiatic (Markov et al., 2009) range of the sibling voles, the presence of cranial sexual dimorphism in the studied metric characters was tested by Student's t-test. The probability level for decision about statistical significance was $\alpha = 0.05$. For the overall survey of the skull parameters, the descriptive statistics were counted – the mean ($\bar{X}$), the standard deviation (SD) and standard error of mean (SE), and the coefficient of variation (CV). The ± 95 % confidence interval of the mean value of each one variable was also determined. All statisticall analyses were carried out using STATISTICA (Version 8.0).

Results

The sibling voles were found only in open landscapes, represented by:

i. meadows near agricultural areas and rivers, covered with steppe-like grass communities, semi-shrub and shrub vegetation (Locations: Tsarevo town; Havsa town; Sarhdere village);
ii. open wetlands (Locations: Tekirdag town);
iii. agricultural lands under alfalfa, autumn cereals or vines (Locations: Iasna Poliana village; Ahtopol town; Novo Panicharevo village).

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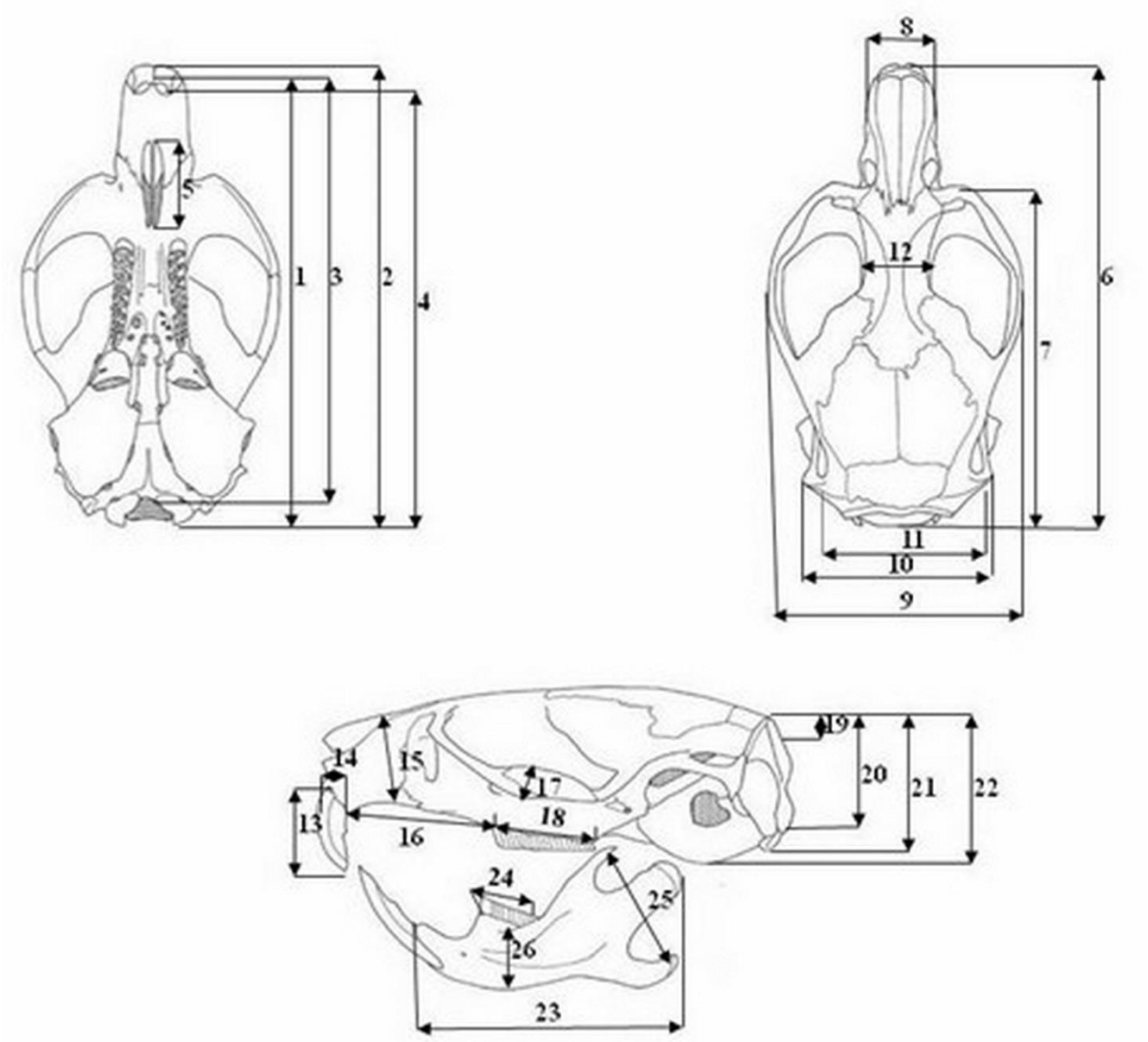

Figure 2. *Skull and mandible measurements: V1 - Condylobasal length I; V2 - Condylobasal length II; V3 - Basal length; V4 - Condylobasilar length; V5- Length of incisive foramen; V6 - Total skull length; V7 - Occipito-maxillar length; V8 -Rostrum width; V9 - Zygomatic width; V10 - Occipital width; V11 - Mastoid width; V12 - Interorbital width; V13 - Length of upper incisives; V14 - Thickness of upper incisives; V15 - Rostrum height; V16 - Length of upper diastema; V17 - Height of zygomatic arch; V18 - Alveolar length of upper molars; V19 - Interparietal-foramen magnum height; V20 - Cranial height between bullae ossae; V21 - Cranial height from mastoids V22 - Cranial height from bullae osseae; V23 - Mandible length; V24 - Alveolar length of lower molars; V25 - Articular height; V26 - Mandible height taken at M.*

All caught adult sibling voles (*M. arvalis* sensu lato) were craniometrical classified to Southern vole (*M. levis*) (Figure 3). This species identity was confirmed for some individuals by a study of specific electrophoretic mobility of their haemoglobin fractions (Location: Tsarevo town; Achtopol town) (Markov & Kocheva, 2008); either by establishing their karyotype (Location: Havsa town; Sarhdere village; Tekirdag town) (Yiğit et al., 2003).

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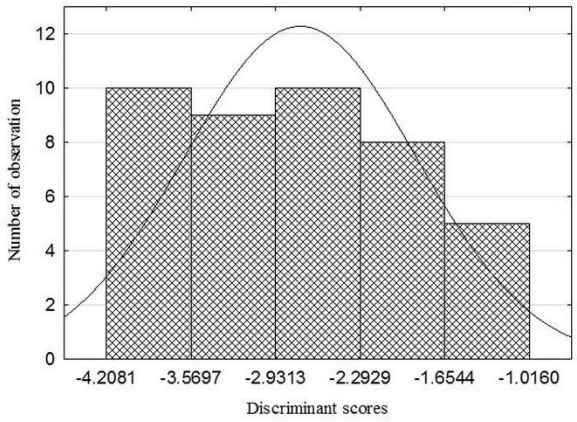

Figure 3. *Classification results for taxonomic identification from craniometrical determination of the investigated common vole (Microtus arvalis, sensu lato) of south-eastern part of Thrace. The values of group centroids are 0.803 for M. arvalis and -2.71 for M. levis.*

The evaluated relative numbers of the sibling voles in the two types of biotopes in the northern region of south-eastern part of Balkan Peninsula showed that:

i. In biotope represented by neglected and overgrown with wild vegetation vines with sparse deciduous forest nearby (near the village of Novo Panicharevo), except the sibling voles, classified craniometrically as *M. levis*, the yellow-necked field mouse (*Apodemus flavicollis*) and bicolored white-toothed shrews (*Crocidura leucodon*) were also found. The relative numbers of the southern vole in this biotop was 2.65 individuals per 100 trap-days, while the relative numbers of the yellow-necked field mouse was 5.31 individuals per 100 trap-days, and the bicolored white-toothed shrew was presented only by 0.44 individuals per 100 trap-days.

ii. In the other biotop, represented by agricultural lands under autumn crops (Iasna poliana village) all captured voles were also classified as *M. levis*. In this biotope, the relative numbers of the southern vole was 4.0 individuals per 100 trap-days. The same relative numbers of 4.0 individuals per 100 trap-days demonstrated the second species, which was found to inhabit this biotope - the yellow-necked field mouse.

The differences between each cranial measurements of adult male and female specimens of Southern vole were not significant ($p>0.05$, Student's t- test) in all studed variables and the obtained varied from $t= 0.20$ to $t = 1.68$. The lack of statistical evidence of differences in the average values of the studied parameters in both sexes of Southern vole allow them to be pooled together for further craniometrical analyses. Based on the classified specimens, a population craniometrical characteristic of the southern vole in south east part of Balkan Peninsula (south-eastern Thrace region) was made (Table 1). All studied craniometrical characters were identified with similar relatively low standard error of the mean and they all showed similar absolute variation. The confidence limits for the means of all investigated craniometrical parameters limited too narrow range of values around the means, where the population means are expected to be located.

Phenotype craniological variation of the southern vole from the south-eastern part of Thrace assessed by comparing the relative variation (CV) of the studied craniometrical characters was within the range from 3.5% to 10.0% (Table 1), which is typical for the most rodents.

Discussion

Taxonomic determinations of the established representatives of *M. arvalis* sensu lato evidently revealed the absence of Common vole (*M. arvalis*, sensu stricto) and confirmed that the Southern vole is presented in the fragmented landscape of south-eastern part of Thrace.

In northen Strandzha mountain region the Southern vole is associated with semi dry, upland habitats such as deserted and overgrown with wild vegetation vines near sparse forests and large agricultural fields under autumn crops with adjacent non-arable lands with shrub vegetation. These biotopes correspond to the ones preffered by the species in other parts of its European range. In this region the sibling species *M. arvalis* (sensu stricto) was not estabnlished by trapping, in the biotopes considered as suitable for its development, such as coenoses of meadow type, agricultural lands under alfalfa or natural biotopes with sparse shrub vegetation along the river valleys. Although the relative numbers of the Southern vole remains near the lower average limit of the grade estimation for presence of common species in particular biotope, the species still could be considered as common in the biotopes, where it was found.

At the same time in the south part of Thrace, Southern vole is presented in open landscapes related to water areas (meadows near rivers and wetlands). This fact suggested an ubiquitous colonization of south-eastern part of Thrace by the Southern vole.

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Table 1. *Descriptive statistic of investigated craniometrical variables of Southern vole (Microtus levis) from south-eastern part of Thrace. Skull and mandible measurements are described on Figure 2.*

	Valid N	Mean $\overline{X}$	Standard error (SE)	Standard deviation (SD)	+95% Confid. interval	-95% Confid. interval	Coef. of Var. (CV)
V1	38	24.59	0.213	1.311	24.16	25.02	5.3
V2	38	25.10	0.202	1.246	24.69	25.51	4.9
V3	35	22.43	0.210	1.241	22.00	22.86	5.5
V4	38	23.93	0.216	1.329	23.49	24.36	5.5
V5	42	4.46	0.046	0.301	4.37	4.56	6.7
V6	41	25.10	0.193	1.233	24.71	25.49	4.9
V7	41	19.68	0.140	0.895	19.40	19.97	4.5
V8	42	4.20	0.049	0.316	4.10	4.30	7.5
V9	42	14.24	0.138	0.891	13.96	14.52	6.2
V10	42	11.49	0.089	0.579	11.31	11.67	5.0
V11	42	10.59	0.085	0.551	10.41	10.76	5.2
V12	42	3.68	0.024	0.156	3.64	3.73	4.2
V13	41	4.49	0.053	0.341	4.39	4.60	7.5
V14	42	1.48	0.020	0.127	1.44	1.52	8.5
V15	42	4.14	0.045	0.289	4.05	4.23	6.9
V16	42	7.46	0.066	0.425	7.32	7.59	5.7
V17	42	1.52	0.023	0.149	1.48	1.57	9.8
V18	42	6.29	0.034	0.223	6.22	6.36	3.5
V19	40	3.30	0.050	0.317	3.20	3.40	9.6
V20	42	7.40	0.068	0.438	7.27	7.54	5.9
V21	42	9.27	0.062	0.400	9.15	9.40	4.3
V22	42	7.54	0.050	0.321	7.44	7.64	4.2
V23	40	15.31	0.112	0.707	15.08	15.53	4.6
V24	42	5.97	0.049	0.320	5.87	6.07	5.3
V25	19	7.98	0.109	0.474	7.75	8.21	5.9
V26	41	4.75	0.036	0.231	4.68	4.83	4.8

The established population craniometrical characters of *M. levis* from Southeastern Balkan Peninsula, a region considered as a zoogeographical crossroads and with key geographic position in the species range, could be used as baseline for estimation of bio-morphological diversity of this species in its Eurasian range. The evaluation of craniological variation and local craniometrical characteristics of *M. levis* under the specific ecological conditions of the south-eastern part of Thrace, particularly in Strandzha Mountain region, affords an opportunity for comparative analysis and revealing of craniological population diversity of this species in main natural and agricultural ecosystems situated on the territory of the Bulgaria and Turkey.

The unambiguously determined relative and absolute variation of craniometrical characters *of M. levis* from this region expands significantly the potential in solving many problems related to assessment of its craniological populations variation. Moreover, such craniological characteristics could be expanded when the species is used as bio-indicator as an integral part of bio-monitoring system of rating environment quality (Peev & Gerasimov, 1990) in south-eastern part of Thrace, both in the two countries and in other specific parts of the species range.

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