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1 **Cytogenetic characteristic of East European vole *Microtus levis* and common pine**  
2 **vole *Microtus subterraneus* (Mammalia: Rodentia) from Turkey: Constitutive**  
3 **Heterochromatin Distribution**

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7 **Abstract:**

8 In this study, the conventionally stained and C - band characteristics of *Microtus levis*  
9 and *Microtus subterraneus* species collected from 3 different localities were determined. The  
10 karyotype of *Microtus levis* is  $2n=54$ ,  $NFa=54$ ,  $NF=56$ . The chromosome set consists of 25  
11 pairs of acrocentric autosomal chromosomes and one pair of metacentric chromosomes.  
12 Acrocentric chromosome pairs of diminishing size prevail in the autosomal set. In the  
13 autosomes, distinct C-heterochromatin regions are located in the centromeric areas. Almost a  
14 half of the X chromosome and the whole Y chromosome consisted of C - heterochromatin  
15 blocks. *Microtus subterraneus* has two different diploid chromosome numbers in Turkey. The  
16 studied karyotype is  $2n=54$ ,  $NFa=56$ ,  $NF=60$ . In the karyotype, besides 24 acrocentric  
17 autosomal chromosomes of continually diminishing size, there is one pair of submetacentrics.  
18 The X chromosome is a submetacentric and there is a wide heterochromatin block on its long  
19 arm. The detailed structure of karyotypes and C - banding patterns same from previously  
20 published results.

21 **Keywords:** Chromosome banding (C - band), Heterochromatin, Karyotype.

## 23 Introduction

24 The *Microtus* genus has a wide distribution in the Holarctic region with 65 extant  
25 species (Jaarola et al. 2004). The karyotypes of the *Microtus* species vary between  $2n=17$  and  
26  $2n=62$  (Zima & Kral 1984, Modi 1987, Zagorodnyuk 1990, Lemskaya et al. 2010). Due to  
27 their different karyotype characteristics, chromosomal data are frequently used in  
28 cytotaxonomy studies (Lemskaya et al. 2010). Nine species of the *Microtus* genus occur in  
29 Turkey (Kryštufek & Vohralík 2005). *Microtus levis* (Miller 1908) is a species of the *arvalis*  
30 group, and it was recognized in Turkey as *M. rossiaemeridionalis* by Dođramacı (1989) and  
31 as *M. epiroticus* by Kefeliođlu (1995). Currently, this species is assessed as *M. levis*  
32 (Synonym: *M. rossiaemeridionalis* and *M. epiroticus*) (Carleton & Musser 2005). Karyotype  
33 of *M. levis* from Turkey was reported by Kefeliođlu (1995). The C-banding pattern was  
34 studied by Zima (2004) and Mitsainas (2010).

35 Two different karyotypes are known in *Microtus subterraneus*, which is included in  
36 the *Terricola* subgenus. *M. subterraneus* has a two alternative diploid numbers  $2n=52$  or  
37  $2n=54$ . Two cytotypes differing in the diploid number, reveal the same fundamental number  
38  $FN=60$ . A single Robertsonian alteration can explain this variation (Bulatova et al. 2007).  
39 Populations with  $2n=52$  occur in central and southern Europe which includes Balkans and the  
40 west of Turkey (Thrace) (Zagorodnyuk 1990, Çolak et al. 1998, Mitsainas et al. 2010).  
41 Specimens from Anatolia have  $2n=54$  chromosomes. A heterozygote inversion was reported  
42 in the  $2n=52$  populations (Sablina et al. 1989). The C-banding pattern was studied by  
43 Macholan et al. (2001) from Turkey.

44 The aim of this study is to understand the C - band characteristics of *M. levis* and *M.*  
45 *subterraneus* to determine the variations in the chromosomal bands of *M. levis* and *M.*  
46 *subterraneus* if there are any.

47

## 48 **Materials and methods**

49 The cytogenetic analyses were performed on four females and two males of *Microtus*  
50 *levis* and two females of *M. subterraneus* collected from Samsun (No on map=1, N=4,  
51 41°25'30 N, 35°44'12 E), Tokat (No on map=2, N=2, 40°14'15 N, 36°30'41 E) and Bolu (No  
52 on map=3, N =2, 40°22'38 N, 31°12'48 E) villages (Figure 1). *M. levis* is a species adapted to  
53 varied habitats. In Samsun, it was collected from deciduous forest where *M. subterraneus*  
54 species was also present. In Tokat, they were collected from agriculture fields. The samples  
55 collected from Bolu were collected from the edges of fields where herbaceous plants were  
56 dominant. Chromosome preparations were obtained from the femoral bone marrow cells of  
57 colchicine-treated animals (Ford & Hamerton 1956). The constitutive heterochromatin  
58 distribution was determined using techniques from Sumner (1972). From each specimen 10 to  
59 20 slides were prepared and at least 10 well spread metaphase plates were analyzed.

60 **Figure 1.** Distribution of *Microtus levis* and *Microtus subterraneus* in Turkey. Circles =  
61 *Microtus levis*, triangles = *Microtus subterraneus*. For numbers see Table 1.

## 62 **Results**

63 **Table 1.** Karyotypic characteristics of *Microtus levis* and *Microtus subterraneus* from Turkey.  
64 N - sample size, 2n – diploid number of chromosomes; NFa – number of autosomal arms; A  
65 and SM/M – acrocentric and submetacentric/metacentric chromosomes.

66 The karyotype of *Microtus levis* is 2n=54, NFa=54, NF=56. The chromosome set  
67 consists of 25 pairs of acrocentric autosomal chromosomes and one pair of metacentric  
68 chromosomes (no:1). The autosomal complement consists of a 25 acrocentric pairs of  
69 diminishing size. The X and Y chromosomes are large-sized acrocentric chromosomes in the  
70 karyotype (Figure 2). The C – banding patterns found in *M. levis* specimens obtained from  
71 different localities were similar. C-band-positive regions were distinct in all chromosomes.  
72 Almost a half of the X chromosome and the whole Y chromosome consisted of C -

73 heterochromatin blocks (Figure 3). Therefore, the sex chromosomes were easily distinguished  
74 in karyotype.

75 **Figure 2.** Conventionally stained chromosomes of the male *M. levis*

76

77 **Figure 3.** C banded karyotypes of female and male *M. levis*

78 The karyotype of *Microtus subterraneus* is  $2n=54$ ,  $NFa=56$ ,  $NF=60$ . In the  
79 karyotype, besides 24 acrocentric autosomal chromosomes of continually diminishing size,  
80 there is one pair of submetacentrics (no. 1) and one pair of small metacentric autosomes  
81 (no. 26). The X chromosome is a large submetacentric (Figure 4). There are C - band  
82 heterochromatin positive and negative band (chromosome no: 1, 2, 4, 6, 12, 15, 21) in *M.*  
83 *subterraneus* karyotype set (Figure 5). The autosomal C- positive bands are situated in the  
84 centromeric regions. The X chromosome is C - band positive. Besides the centromeric  
85 heterochromatin area, there is a wide heterochromatin block on the long arm of the X  
86 chromosome (Figure 5).

87 **Figure 4.** Standard karyotype and sex chromosomes of *M. subterraneus*

88 **Figure 5.** C - banded karyotype of *M. subterraneus* female, arrows indicate heterochromatin  
89 blocks, -/- C - negative band.

## 90 Discussion

91 The karyotypes of individuals from different regions were the same as reported in with  
92 previous studies conducted in Turkey (Kefelioğlu 1995, Yavuz et al. 2011, Aslan & Zima  
93 2014). This species has a conservative diploid chromosome number (Fredga et al. 1990, Zima  
94 2004, Mitsainas et al. 2008, Trifonov et al. 2010, Pavlova & Tchabovsky 2011). While *M.*  
95 *levis* is stable in terms of the number of diploid chromosomes, autosomes in European  
96 populations show variation in morphology. A pericentric inversion which occurs at the first  
97 acrocentric autosome has been reported (Zima et al. 1981, Belcheva et al. 1985, Gavrila et al.  
98 1986). Similarly, variations have been found in the size of the X chromosome and its C-

99 heterochromatin block (Fredga et al. 1990, Yakimenko & Kryukov 1997, Pavlova &  
100 Tchabovsky 2011). The amount of C-heterochromatin can be enormous in the sex  
101 chromosomes of many rodent species (Romanenko & Volobouev 2012). In studies conducted  
102 by Fredga et al. (1990) in Svalbard (Norwegian archipelago) islands and by Pavlova &  
103 Tchabovsky (2011) in Olkhon Island (Western Siberia) the C-heterochromatin block on the X  
104 chromosome reduced in size. The size of the X chromosome was comparable to that of the Y  
105 chromosome. In the present study, almost half of the X chromosome and the whole Y  
106 chromosome is made up of C - heterochromatin block and the X chromosome was distinctly  
107 larger than the Y chromosome.

108 Çolak et al. (1998) recorded two different diploid chromosome sets in *Microtus*  
109 *subterraneus* from Turkey (in Thrace and Anatolia). While Thrace samples have a karyotype  
110 of  $2n=52$ , Anatolia samples have a karyotype of  $2n=54$  (Çolak et al. 1998, Macholan et al.  
111 2001). We confirmed the presence of a population with 54 chromosomes in Anatolia. The  
112 diploid number of chromosomes is similar to that recorded in Boksitogorsk area (Russia) by  
113 Sablina et al. (1989), Tver region (Russia) by Bulatova et al. (2007), in Bolu (Turkey) by  
114 Çolak et al. (1998), in Poland and Bolu, Zonguldak, Amasya (Turkey) by Macholán et al.  
115 (2001). The X chromosome in Turkey populations is commonly reported as metacentric or  
116 submetacentric in *M. subterraneus* (Çolak et al. 1998, Macholan et al. 2001, in this study).

117 Heterochromatin blocks in the sex chromosomes of *Microtus* species can show inter  
118 and intra specific variation (Modi 1987). In this study and a study conducted on *M.*  
119 *subterraneus* samples in Greece by Mitsainas et al. (2010), heterochromatin distribution of C -  
120 band is varied. In the karyotype ( $2n=52$ ) of *M. subterraneus* from Greece, there is a wide  
121 heterochromatin block below centromere in X chromosome. In addition, there is a small  
122 heterochromatin in the telomeric region (Mitsainas et al. 2010). In this study, there is a wide  
123 C-heterochromatin block on the long arm (interstitial) of X chromosome of *M. subterraneus*

124 samples ( $2n=54$ ) from Anatolia in Turkey. The distribution of C-heterochromatin in the  
125 karyotype of *M. subterraneus* is similar to that reported by Macholan et al. (2001).

126 As a conclusion, this study conducted on samples collected from different localities  
127 shows that autosomal and sex chromosomes of *M. levis* samples have similar characteristics  
128 in terms of their morphology. In *M. subterraneus* karyotype, C - band characteristics of sex  
129 chromosomes vary geographically.

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### Figures and Tables

220 **Table 1.** Karyotypic characteristics of *Microtus levis* and *Microtus subterraneus* from Turkey.

221 2n – diploid number of chromosomes; NFa – number of autosomal arms; A and SM/M –

222 acrocentric and submetacentric/metacentric chromosome.

Species	No on map	2n	FNa	X	Y	Method	References
<i>Microtus levis</i>	1 - 3	54	54	A	A	C - band	In this study
"	4 - 6	54	54	A	A	Conventional staining	Aslan & Zima 2014
"	7	54	54	A	A	Conventional staining	Yavuz et al. 2011
"	8 - 10	54	54	A	A	C - band	Zima 2004
"	11 - 30	54	54	A	A	Conventional staining	Kefelioğlu 1995
<i>Microtus subterraneus</i>	31	54	56	SM	-	C - band	In this study
"	32 - 33 34 - 36	52- 54	56	M/SM	A	G and C - band	Macholan et al. 2001
"	37 - 39	52- 54	56	M	A	Conventional staining	Çolak et al. 1989

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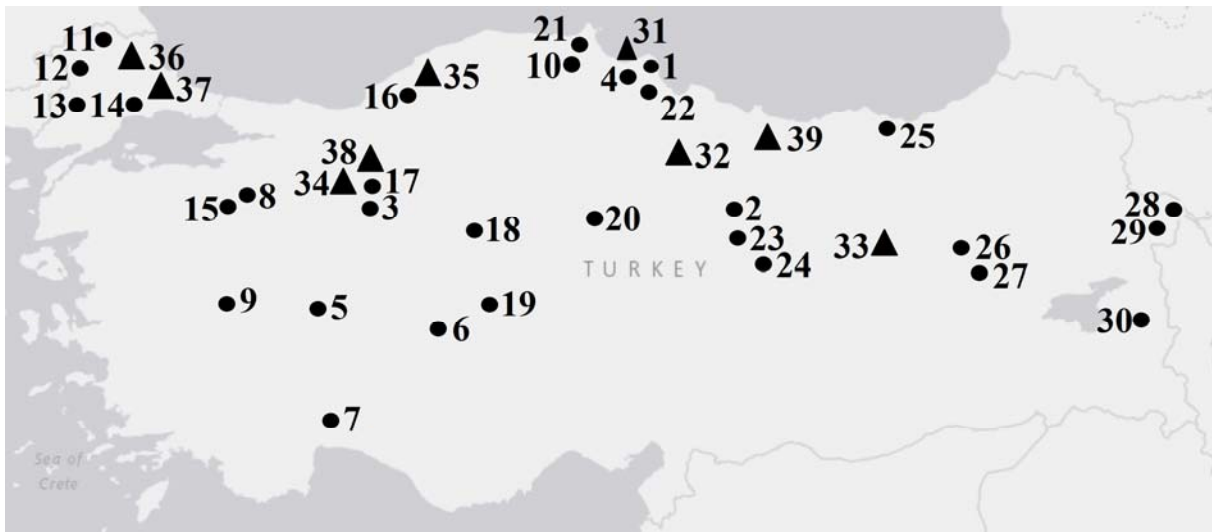
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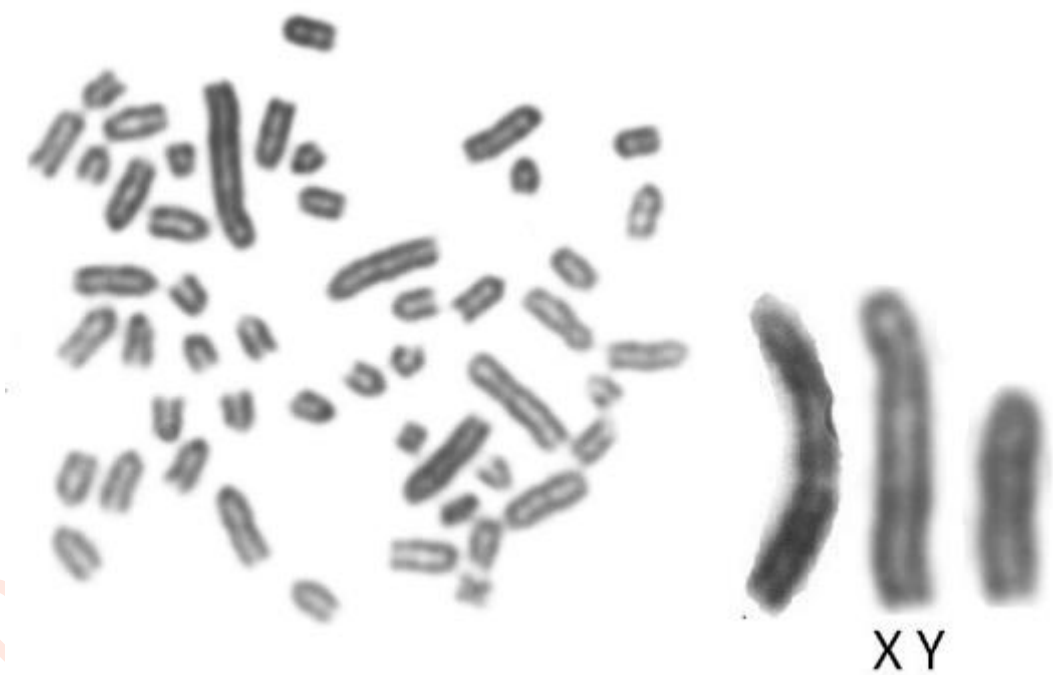
229



230

231 **Figure 1.** Distribution of *Microtus levis* and *Microtus subterraneus* in Turkey. Circles =  
232 *Microtus levis*, triangles = *Microtus subterraneus*. For numbers see Table 1.

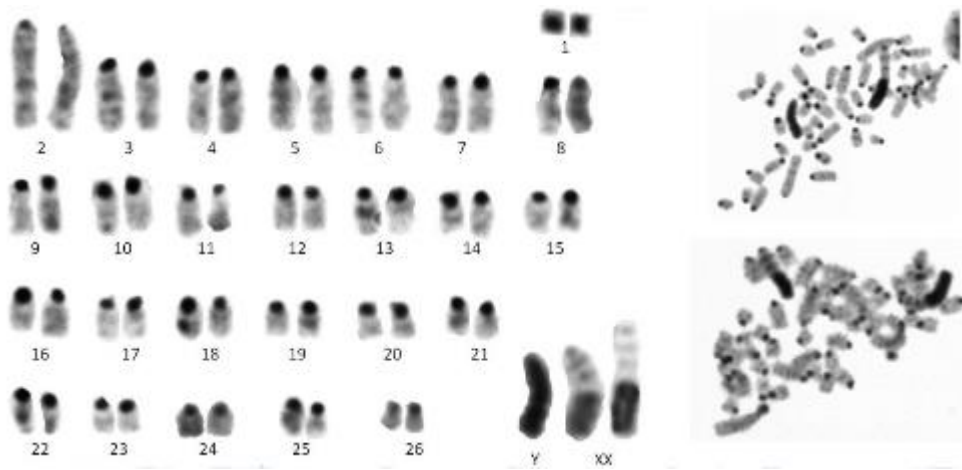
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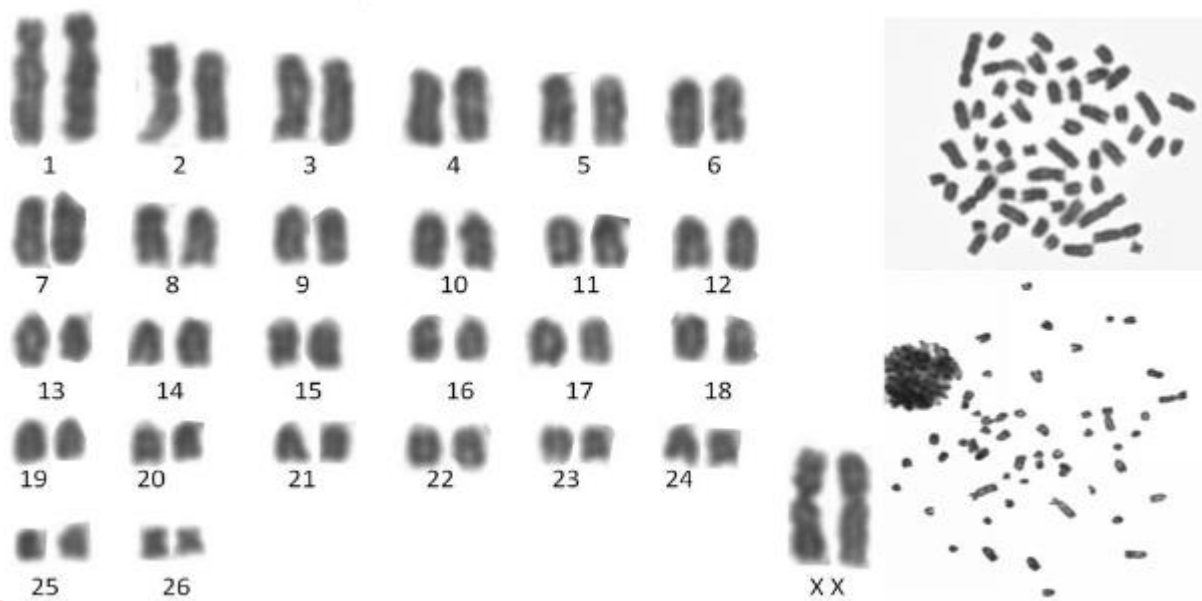
242 **Figure 2.** Conventionally stained chromosomes of the *M. levis* male

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250 **Figure 3.** C - banded karyotype of female and male of *M. levis*

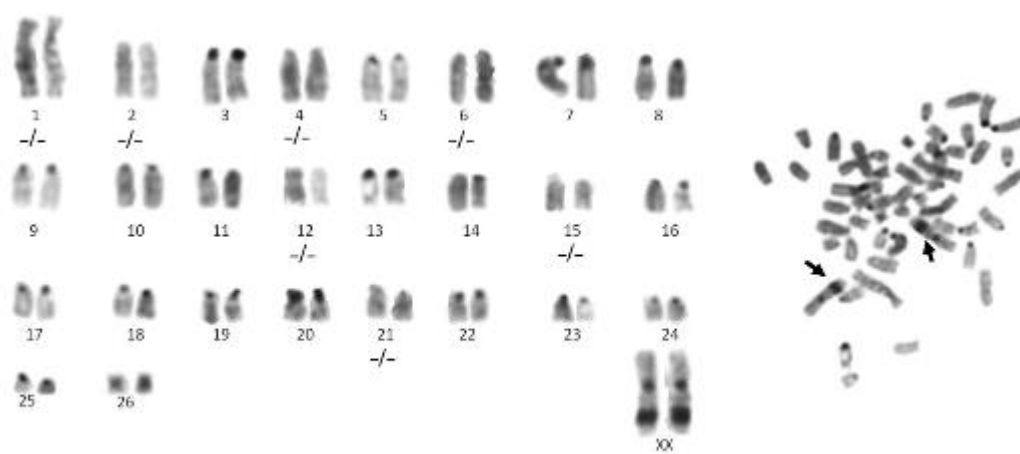


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252 **Figure 4.** Conventional staining and sex chromosomes of *M. subterraneus*

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260 **Figure 5.** C - banded karyotype of *M. subterraneus* female, arrows indicate heterochromatin  
261 blocks in the sex chromosomes, -/- C - negative bands.

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