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Cytogenetic Study on *Microtus guentheri* (Danford and Alston, 1880) (Mammalia: Rodentia) from Turkey: Constitutive Heterochromatin Distribution and Nucleolar Organizer Regions

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Conventionally stained, C- and Ag-NOR banded karyotypes of Guenther's vole, *Microtus guentheri* were studied from Turkey. The species possesses a karyotype of 2n=54, NFa= 52 and NF=54 in specimens from Kahramanmaraş and Gaziantep provinces, whereas NF= 56 in females and NF=55 in males were found in individuals from Kırıkkale and Nevşehir provinces. The X chromosome was a large acrocentric (NF=54) or submetacentric (NF=55, 56) while the Y chromosome was a small telocentric in all specimens examined. Blocks of constitutive heterochromatin were located in the pericentromeric areas of autosomes including the X chromosome. Nucleolar organizer regions (NORs) were located at the telomeric regions of the short arms of five acrocentric pairs and centromeric regions of two telocentric pairs in the Nevşehir and Kırıkkale specimens.

Key words: Guenther's vole, C-banding, Ag-NOR banding, karyotype, Turkey.

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The genus *Microtus* Schrank, 1798 is composed of 15 subgenera and 62 species distributed in the Holarctic region (MUSSER & CARLETON 2005). Nine of the vole species, *Microtus anatolicus*, *M. arvalis*, *M. daghestanicus*, *M. dogramacii*, *M. guentheri*, *M. levis*, *M. majori*, *M. socialis* and *M. subterraneus* have been reported from Turkey (KEFELİOĞLU 1995; ÇOLAK *et al.* 1997; KEFELİOĞLU & KRYŠTUFEK 1999; KRYŠTUFEK & KEFELİOĞLU 2001; YİĞİT & ÇOLAK 2002; MUSSER & CARLETON 2005; MITSAINAS *et al.* 2009). The type locality of Guenther's vole, *M. guentheri* (Danford & Alston, 1880) is situated at Kahramanmaraş in the Taurus Mts., (Turkey). This species is clearly distinguishable from other social voles in Turkey with respect to its karyotype and skull morphology (KEFELİOĞLU & KRYŠTUFEK 1999).

YİĞİT and ÇOLAK (2002) concluded that the distribution range of *Microtus guentheri* in Turkey is restricted to the southeastern parts of the country and that the Anatolian diagonal limited the dispersal of the species into central Anatolia. This region of Turkey is therefore inhabited by another taxon, *M. lydius ankaraensis* (YİĞİT & ÇOLAK 2002). The taxonomical status of *lydius* was also re-examined in a comparative study by KRYŠTUFEK and colleagues (in MUSSER & CARLETON 2005) including holotypes of *hartingi, lydius, philistinus, mustersi* and the topotypes of *martinoi, ankaraensis* and *guentheri*. The authors suggested a continuous morphological variation among these taxa, and recently MUSSER and CARLETON (2005) considered *lydius* as a synonym of *M. guentheri*.

The chromosome set of *Microtus guentheri* was first described by MATTHEY (1952). To date, the conventionally and G- and C- banded karyotypes of this species were reported from Bulgaria (BELCHEVA *et al.* 1980; GOLENISHCHEV *et al.* 2002; CHASSOUNIKARA *et al.* 2008), Greece (MITSAINAS *et al.* 2009), Iran (GOLENISHCHEV *et al.* 2002a) and Turkey (KEFELİOĞLU 1995; ÇOLAK *et al.* 1997; KEFELİOĞLU & KRYŠTUFEK 1999; GÖZÜTOK & ALBAYRAK 2009). All the authors reported the same diploid chromosome number, 2n=54.

The aims of this study are to present the constitutive heterochromatin and NORs distribution of *Microtus guentheri* in Turkey.

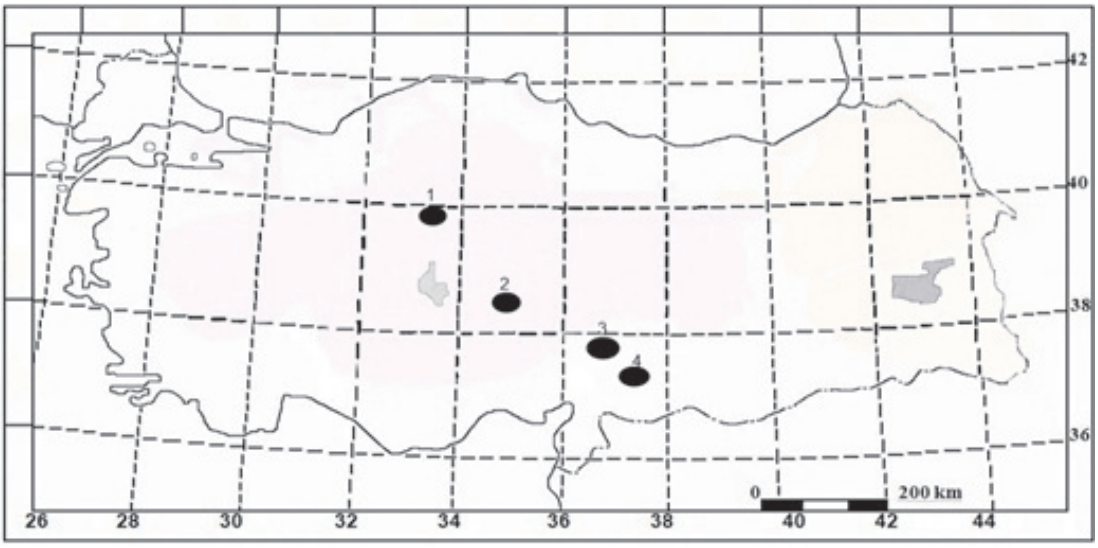


Fig. 1. Collection localities of *Microtus guentheri* in this study (1: Kırıkkale, 2: Nevşehir, 3: Kahramanmaraş, 4: Gaziantep).

Material and Methods

A total of ten specimens were captured by Shermann traps from Kırıkkale (39° 50′ N 33° 30′ E) (n= 1 ♂), Nevşehir (38° 37′ N 34° 42′ E) (n= 3 ♂♂, 1 ♀), Gaziantep (37° 03′ N 37° 22′ E) (n= 2 ♂♂, 1 ♀), and Kahramanmaraş (37° 35′ N 36° 55′ E) (n= 2 ♂♂), from Turkey (Fig. 1).

The specimens were identified as *Microtus guentheri* by examination of the pelage colouration, tail length, skull and baculum structures and molar teeth morphology. Chromosome preparations were obtained from bone marrow cells according to the technique of PATTON (1969). The heterochromatin distribution and location of nucleolar organizer regions (NORs) were determined using the techniques of SUMNER (1972) and HOWELL and BLACK (1980), respectively. The classification of chromosomes were established according to LEVAN *et al.* (1964) and MARTINEZ *et al.* (2009). A total of 12 slides were prepared from each specimen. At least 15 well-spread metaphase plates were photographed and arranged to determine the diploid chromosome number (2n), autosomal fundamental number (NFa) and fundamental number (NF).

All stuffed skins and metaphase slides are deposited at the Department of Biology, University of Kırıkkale.

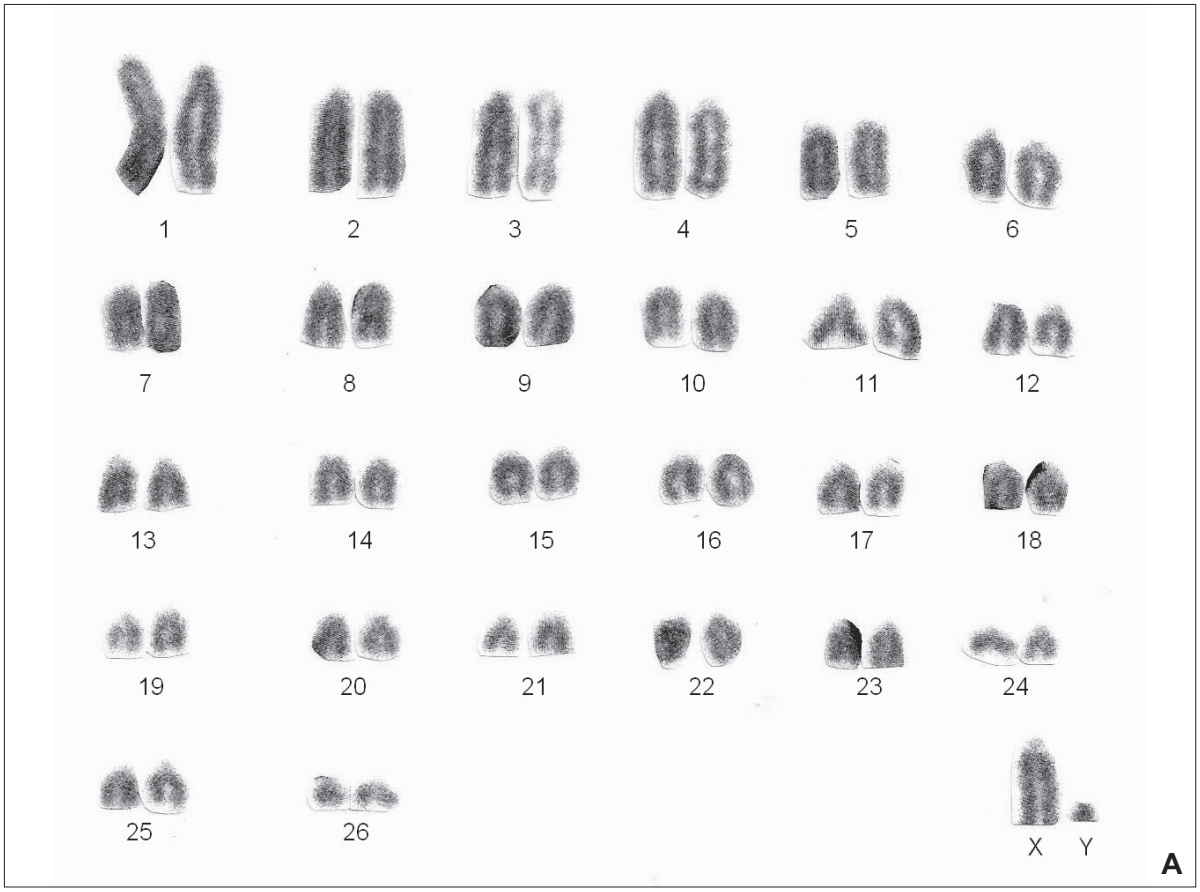
Results

The specimens examined displayed 2n=54, NFa=52 and NF=54 from Kahramanmaraş and Gaziantep provinces, whereas individuals from Kırıkkale and Nevşehir provinces possessed 2n= 54, NFa= 52, NF= 56 in females and NF=55 in males. The karyotype consisted of 26 pairs of acrocentric or telocentric autosomes gradually decreasing in size. The first acrocentric autosome pair was distinctly larger than the others. Nearly all autosomes possessed small heterochromatic arms in the specimens from Nevşehir and Kırıkkale. In addition, one of the medium-sized autosome pairs in one of the specimens from Kırıkkale possessed a secondary constriction in the long arm near the centromere (not shown). The X chromosome was acrocentric in the male and female specimens from Gaziantep and Kahramanmaraş (NF=54) while submetacentric, with a centromere index of 2.3, in all specimens from Kırıkkale and Nevşehir (NF= 56 in females and NF=55 in males). The Y chromosome was a small telocentric element in all specimens examined (Fig. 2).

C-banding revealed that in all autosomes large and clear C-heterochromatin blocks are located in the pericentromeric areas, including the submetacentric X chromosome. The Y chromosome was entirely hetrochromatic in all specimens examined (Fig. 3).

Interstitial C-bands were exceptionally observed in some acrocentric autosomes of the specimens examined (not shown).

Nucleolar organizer regions (NORs) were located in the telomeric regions of the short arms of five medium-sized or small acrocentric autosomes and centromeric regions of two telocentric autosomes in the Nevşehir and Kırıkkale specimens (Fig. 4).



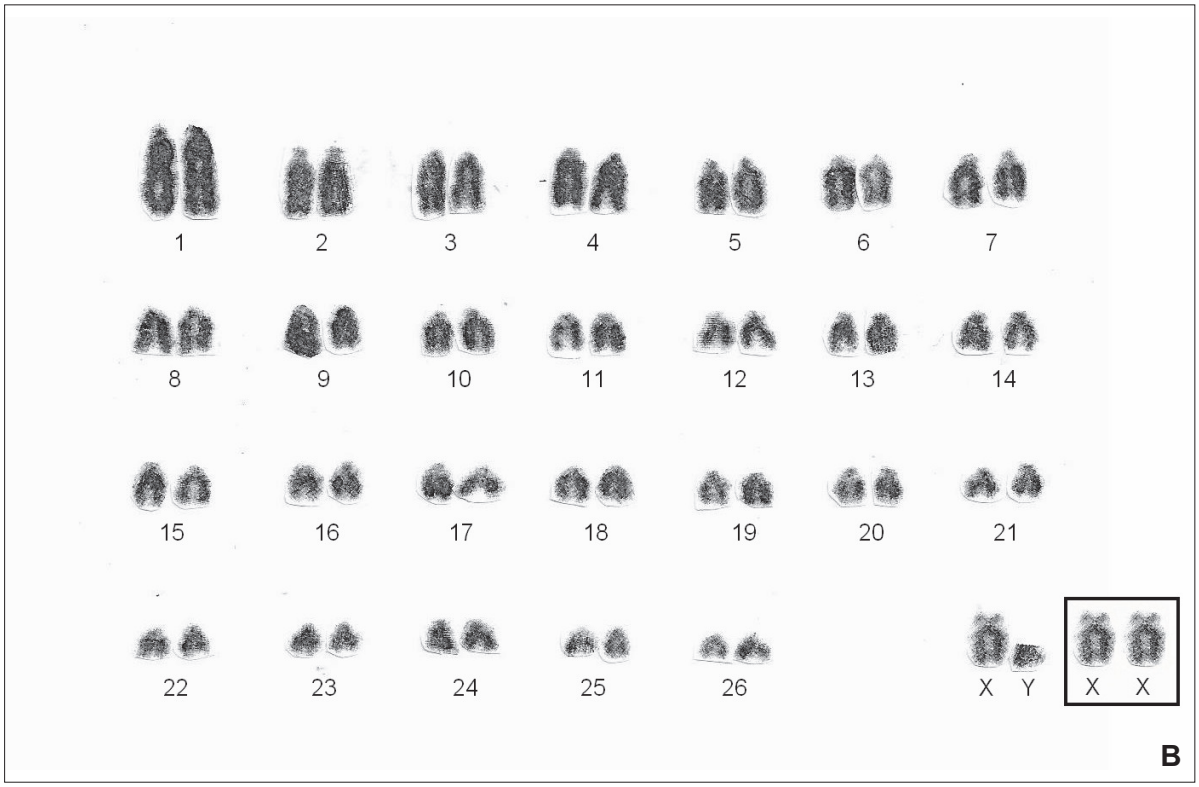


Fig. 2. Conventionally stained karyotype of *Microtus guentheri* from Kahramanmaraş (A) and Nevşehir (B) provinces (The frame in B indicates the X chromosomes of the female specimen studied).

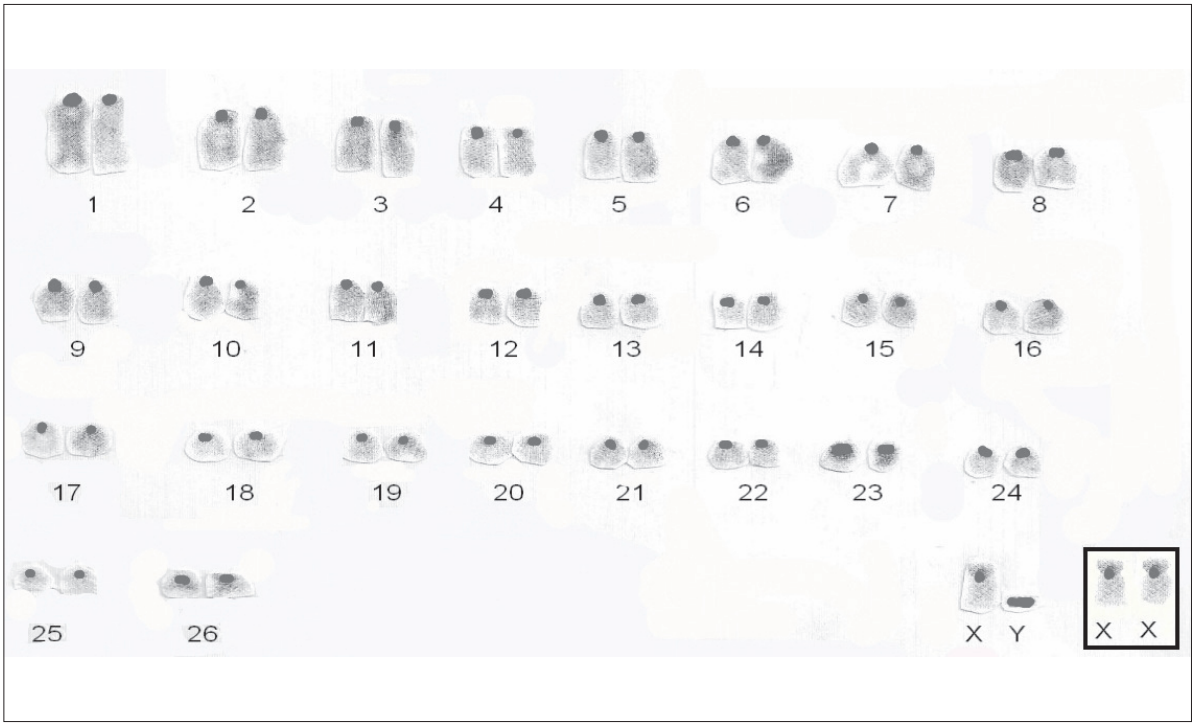
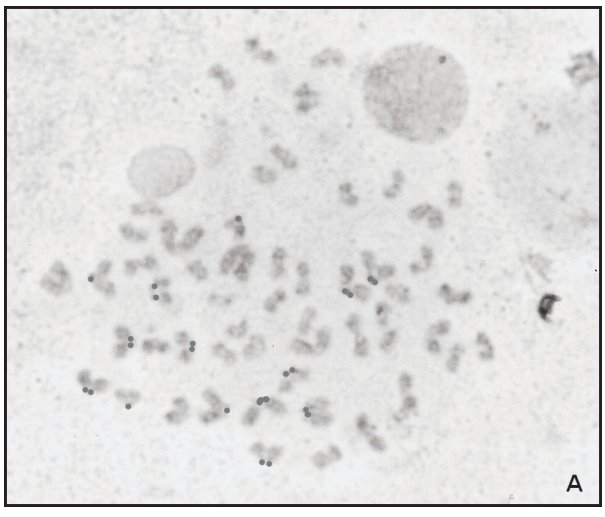


Fig. 3. C-banding karyotype of *Microtus guentheri* from Nevşehir province (The frame indicates the X chromosomes of the female specimen studied).



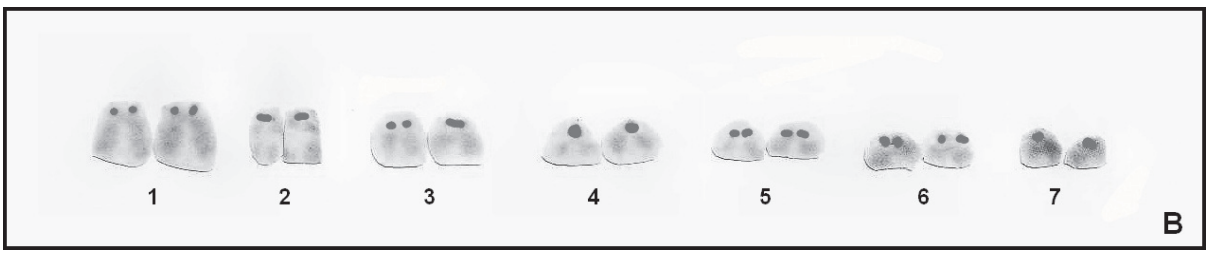


Fig. 4. Ag-NOR banded metaphase plate (A) and NOR-bearing chromosome pairs (B) of *Microtus guentheri* from Nevşehir province.

Discussion

KEFELİOĞLU (1995) and ÇOLAK *et al.* (1997) recorded a small metacentric autosome pair in the chromosome set of *Microtus guentheri* from Turkey. Such a karyotype is typical for *M. levis* (formerly *M. rossiaemeridionalis*) (MITSAINAS *et al.* 2009), therefore species identification performed by the authors might be incorrect.

MODI (1993) and CHASSOVNIKAROVA *et al.* (2008) determined autosomal interstitial C-bands in *Microtus guentheri.* The distribution of constitutive heterochromatin in autosomes and the Y chromosome in Turkish specimens agreed with the results of BELCHEVA *et al.* (1980), MODI (1993), GOLENISHCHEV *et al.* (2002a), CHASSOVNIKAROVA *et al.* (2008) and MITSAINAS *et al.* (2009).

The sex chromosomes of the genus *Microtus* show frequent variation in distribution (CHASSOVNIKAROVA *et al.* 2008). The X chromosome of *M. guentheri* has been described as acrocentric, metacentric, submetacentric and subtelocentric (BELCHEVA *et al.* 1980; KEFELİOĞLU 1995; ÇOLAK *et al.* 1997; GOLENISCHEV *et al.* 2002 a, b; CHASSOVNIKAROVA *et al.* 2008; MITSAINAS *et al.* 2009; GÖZÜTOK & ALBAYRAK 2009). CHASSOVNIKAROVA *et al.* (2008) stated that the shape of the X chromosome of the genus *Microtus* can vary due to the accumulation of constitutive heterochromatin as proposed by various authors. Therefore, the discrepancy found in the shape of the X chromosomes of *M. guentheri* analysed from Turkey could probably be due to the distribution of heterochromatin in the X chromosome.

In addition, CHASSOVNIKAROVA *et al.* (2008) stated that in *Microtus guentheri* the shape of the X chromosome did not only vary between populations but also within a population. GÖZÜTOK and ALBAYRAK (2009) reported acrocentric X chromosomes in the Kırıkkale population of this species, however, one male karyotyped from the same province in this study possessed a submetacentric X chromosome. As a consequence, the difference between the shape of the X chromosomes from the same province could be due to the variation found in a population as proposed by CHASSOVNIKAROVA *et al.* (2008).

NORs have been considered as useful taxonomic and phylogenetic markers in many species (SANCHEZ *et al.* 1990). NOR-bearing chromosomes are reported for *Microtus nivalis, M. cabrerae* and *M. arvalis* by SANCHEZ *et al.* (1990). NORs are located in the centromeres of eight autosome pairs in *M. nivalis* and six autosome pairs in *M. cabrerae* and *M. arvalis*. However, NORs are located in seven pairs in Turkish specimens of *M. guentheri*.

Consequently, the differences in the shape of the X chromosome as well as the location of NORs in *Microtus guentheri* examined from Turkey could be important for the taxonomy of this species. A similar thought was also put forward by CHASSOVNIKAROVA *et al.* (2008) for the Bulgarian population of this species.

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