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Cytogenetic characteristics of *Microtus daghestanicus* (Rodentia: Arvicolinae) from Northeast Anatolia, Türkiye: Conventional Karyotype, C-Banding

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ABSTRACT
In this study, the standard karyotype and the C-band features of chromosomes of the *Microtus daghestanicus* species have been determined, and the karyological characteristics among closely related species have been compared. The diploid chromosome number of *M. daghestanicus* was found as 2n=54, NFa=54. *M. daghestanicus* karyotype has a pair of small metacentric and 25 pairs of different sizes of acrocentric chromosomes. The x chromosome is a large submetacentric. Positive C-bands are in the pericentromeric region of acrocentric autosomal chromosomes. Additionally, there is a heterochromatin block in the pair of small metacentric chromosomes. Moreover, there is a wide interstitial heterochromatin block on the long arm of the X chromosome.

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Microtus daghestanicus (Rodentia: Arvicolinae) türünün sitogenetik özellikleri: Standart ve C bantlı karyotipler

ÖZET
Bu çalışmada, *Microtus daghestanicus* türünün standart karyotipi ve kromozomların C-bant özellikleri belirlenerek, yakın türler arasındaki karyolojik özellikleri karşılaştırılmıştır. *M. daghestanicus* türünün diploid kromozom sayısı 2n=54 ve otozomal kromozomların kol sayısı NFa=54 şeklindedir. *M. daghestanicus* karyotipinde bir çift küçük metasentrik ve 25 çift farklı büyüklüklerde akrosentrik kromozom bulunmaktadır. X kromozomu büyük metasentriktir. Akrosentrik otozomal kromozomlarda pozitif C-bantlar perisentromerik bölgededir. İlaveten küçük metasentrik kromozom çiftinde heterokromatin blok bulunmaktadır. *M. daghestanicus* türünün X kromozomunun uzun kolunda genişlemiş heterokromatin blok bulunmaktadır.

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INTRODUCTION
Microtus Schrank genus which is the group with the most branching among mammals, is a remarkable group in terms of the karyotypic diversity they show (Maruyama & Imai, 1981; Shenbrot & Krasnov, 2005). The karyotypes of *Microtus* species range from 2n=17 to 2n=62 (Zima & Král, 1984; Modi, 1987; Zagorodnyuk, 1990; Lemskaya et al., 2010). *Microtus* species are frequently used in cytotaxonomic studies due to their karyotypic variation (Lemskaya et al., 2010; Romanenko et al., 2020; Orlov et al., 2023). Three *Microtus* species *M. daghestanicus, M. subter,raneus* and *M. majori* classified in the *Terricola* subgenus (Jaarola et al., 2004) are distributed in Turkey (Kryštufek & Vohralik, 2005). *Microtus* (*Terricola*) *dagestanicus* is distributed in a narrow area in the Northeast Anatolia region of Turkey and the Caucasia. Variation has been found in *M. daghestanicus* diploid chromosome number (Ivanov & Tembotov, 1972; Hatuhov, 1982; Zima & Král, 1984; Zagorodnyuk, 1990; Akhverdyan et al., 1992). The karyotypic characteristics of this species (standard karyotype, C banding, G banding) were

found in the samples obtained from the Caucasian region (Baskevich, 1997; Kuliev & Bickham, 2010; Lemskaya et al., 2010). The diploid chromosome number (except M. Macholán, personal communication in Kryštufek & Vohralik, 2005) and chromosome characteristics of the Anatolian population of this species have not been determined so far.

This study aims to compare the standard karyotypes of *Microtus (T.) daghestanicus* and their constitutive heterochromatin regions (C-banding) which is a karyotypic characteristic, with previously conducted studies and contribute to future karyological studies.

MATERIAL and METHOD

Chromosome preparations were obtained from the femoral bone marrow cells of colchicine-treated animals (Ford & Hamerton, 1956). Two specimens (two females) of *M. daghestanicus* species were obtained from the province of Kars village (height: 2100m, Northeast Anatolia) by using live animal traps. Species identification was performed according to chromosome results (current study) and mtDNA cytb gene region (Genbank accession number: MZ198174, Bogdanov et al., 2021). Diploid chromosome number (2n), number of fundamental arms (FN), fundamental number of autosomal arms (NFa), and sex chromosomes of *M. daghestanicus* used in the study were defined as metacentric, acrocentric, and submetacentric. The constitutive heterochromatin distribution was determined using techniques from Summer (1972). Tissue samples (sample no: 209-AYS), museum material, and karyotype preparations of *M. daghestanicus* species are kept in the Artvin-Çoruh University Cytogenetic laboratory for further studies.

RESULTS

Microtus daghestanicus karyotype was diploid chromosome number (2n) = 54, the fundamental number of autosomal arms (NFa)= 54, and the number of fundamental arms (NF)= 58. The karyotype has 25 pairs of different sizes of acrocentric chromosome (chromosome no: 1-25) and a pair of small metacentric (chromosome no: 26). Metacentric chromosome pair is the smallest in the autosomal chromosomes. X chromosome is a large submetacentric (Fig.1).

In *M. daghestanicus* karyotype, constitutive heterochromatin is in the pericentromeric region in acrocentric autosomal chromosomes. In the autosomal small metacentric chromosome (chromosome no: 26), the heterochromatin region is extended towards the long arm. X chromosome shows a C-positive band. Additionally, there is a wide interstitial heterochromatin block on the long arm of the X chromosome (Fig. 2).

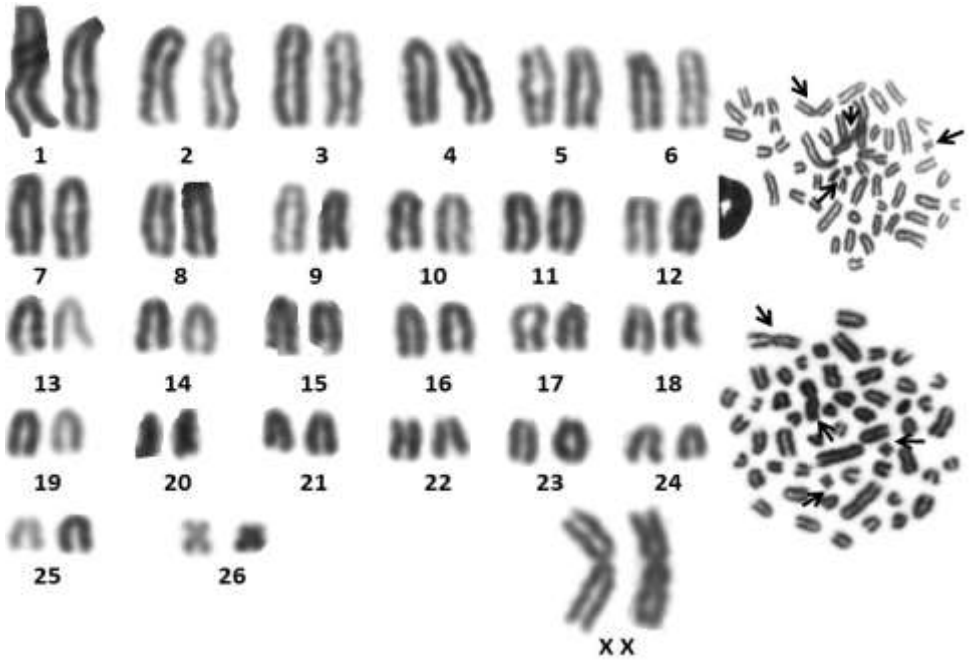

Figure. 1. Metaphase plates and karyotype of *M. daghestanicus* (female) from Kars (Northeast Anatolia). The arrow indicates a bi-armed X chromosome and autosomal chromosome.

Şekil 1. Kars bölgesinden (Kuzey doğu anadolu) elde edilen M. daghestanicus türünün standart karyotipi ve metafaz plakları. Ok: çift kollu X kromozomu ve otozomal kromozomlar

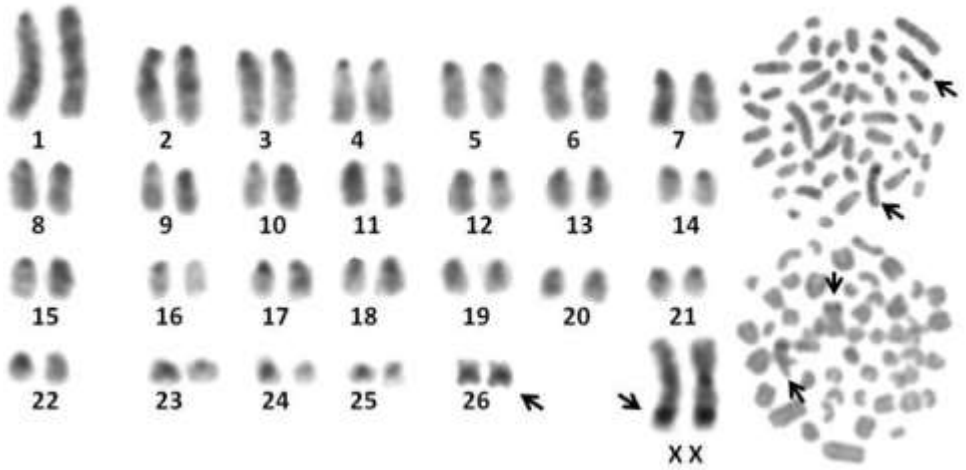

Figure. 2. C-banded karyotype of *M. daghestanicus* (female) from Kars, Arrow: interstitial heterochromatin block on X chromosomes and the heterochromatin area on the long arm of the small metacentric chromosome.

Şekil 2. Kars bölgesinden elde edilen M. daghestanicus türünün C bant yöntemi kullanılarak elde edilen karyotip ve metafaz plakları. Ok: X kromozomundaki kromozom kol içi heterokromatin bölge ve küçük metasentrik kromozom çiftinin uzun kolundaki heterokromatin bölge.

DISCUSSION

Microtus daghestanicus is distributed in a narrow area as Caucasia and Northeast Anatolia. In previously conducted studies in the Caucasia region, the diploid chromosome number (2n=38-54) of *M. daghestanicus* varies (Ivanov & Tembotov, 1972; Hatuhov, 1982; Akhverdyan et al., 1992; Baskevich, 1997; O'Brien et al. 2006; Kuliev & Bickham, 2010; Lemskaya et al., 2010) and some of these Caucasian chromosome races were previously proposed to represent separate species (Kryštufek and Shenbrot, 2022). In a study conducted within the distribution borders of this species in Anatolia, the diploid chromosome number is 2n=54 (Kryštufek & Vohralik, 2005; in the current study). *M.daghestanicus* displays variability in terms of diploid chromosome count, yet it is highly stable regarding the number of fundamental arms (NF=58) (Baskevich et al. 2016). X chromosome is meta/submetacentric in Caucasia (Baskevich, 1997; O'Brien et al., 2006; Kuliev & Bickham, 2010; Lemskaya et al., 2010) and Anatolia (in current study).

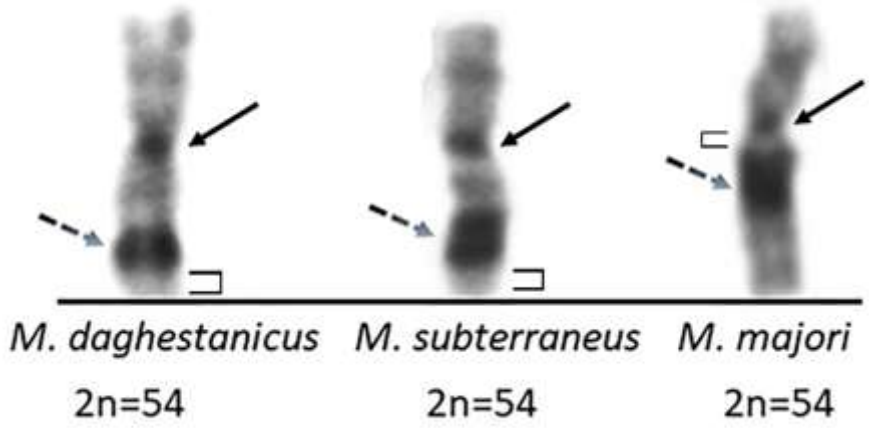

Figure. 3. C-banded of the X chromosome of *M. daghestanicus* (in the current study), *Microtus subterraneus* in Samsun (N41°25', E35°44') village (Selçuk & Kefelioğlu, 2018) and *Microtus major* in Artvin (N41°13' E41°59') village (Selçuk et al., 2019) from Anatolia. Arrow: centromeric heterochromatin, intermittent arrow: interstitial heterochromatin block. Bracket: unstained block

Şekil 3. Bu çalışmada elde edilen M. daghestanicus türünün X kromozomundaki C bant motifi, Samsun bölgesinden elde edilen M.subterraneus türünün X kromozomundaki C bant motifi (Selçuk & Kefelioğlu, 2018), Artvin bölgesinden elde edilen Microtus majori türünün X kromozomundaki C bant motifi (Selçuk et al., 2019). Düz ok: sentromerik C bant, Kesikli ok: Kromozom kol içi heterokromatin blok. Parantez, C-negatif blok

The distribution of heterochromatin is an important part of the karyotype characterization (Dimitri et al., 2009; Lemskaya et al., 2018). *Microtus* species tend to have large heterochromatin blocks or interstitial heterochromatin blocks in sex chromosomes (Modi, 1987, 2003; Burgos et al., 1988, Macholán et al., 2001; Marchal et al., 2003, 2004; Mitsainas et al., 2010; Lamelas et al., 2018). The amplification of repetitive DNA regions on sex chromosomes can lead to the formation of heterochromatin blocks on the chromosomes (Kozlova et al., 2003; Marchal et al., 2003, 2004; Acosta et al., 2008). *M. subterraneus* (2n=54) and *M. major* (2n=54) species classified within the *Terricola* subgenus have interstitial heterochromatin blocks (Borodin et al., 1995; Macholán et al., 2001; Kuliev & Bickham, 2010; Selçuk & Kefelioğlu, 2018,2020; Selçuk et al., 2019). A similar situation is seen in *M. daghestanicus*'s X chromosome (Baskevich et al., 2016, Romanenko et al., 2020, in the current study). In the *M. daghestanicus* karyotype, the heterochromatin block is in the distal region of the long arm of the chromosome, as in its sister taxon (*M. subterraneus*) (Jaarola et al., 2004; Baskevich et al., 2016; Bogdanov et al., 2021). However, variation can be seen in the heterochromatin distribution in sex chromosomes within *Microtus* species (Fredga et al., 1990; Yakimenko & Kryukov, 1997; Acosta et al., 2009; Mitsainas et al., 2009; Pavlova & Tchabovsky, 2011). Contrary to the C banding results obtained in the current study, No interstitial heterochromatin block was found in the X chromosome in Azerbaijan (Caucasia) samples of *M. daghestanicus* (Kuliev & Bickham, 2010).

As a result of the chromosomal rearrangements in the X chromosome of *M. daghestanicus*, *M. subterraneus,* and *M. majori* species (Baskevich et al. 2016; Romanenko et al., 2020), changes may have occurred in the heterochromatin block motif (Fig. 3). The role of constitutive heterochromatin regions containing highly repetitive DNA sequence (Acosta et al., 2008, 2009; Saksouk et al., 2015) in the speciation of arecoline rodents could play a significant role (Modi, 1987; Rubtsov et al. 2002; Romanenko et al., 2020).

Author Contributions

The contribution of the authors is equal

Conflict of Interest

The authors declare that they do not have any competition or any conflicts of interest

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