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A new karyotype and chromosomal banding pattern in *Nannospalax ehrenbergi* (Nehring, 1898) (Rodentia: Spalacidae) from southeast Anatolia, Turkey

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This paper presents new data on chromosomes of the Palestine mole rat, *Nannospalax ehrenbergi*, from southeast Anatolia, Turkey. A new chromosomal form, $2n = 56$, NF = 62 and NFa = 58, is determined from the region. C-banding revealed pericentromeric constitutive heterochromatin in one submetacentric and seven acrocentric autosome pairs. Two acrocentric autosome pairs and an acrocentric chromosome with a secondary constriction showed interstitial C-positive bands on their long arms. A secondary constriction was detected in the Y chromosome. The NORs were observed in one medium-sized metacentric autosome pair, and in four acrocentric pairs of autosomes.

Keywords: Mole rats, C-banding, nucleolus organizer regions, karyosystematic, Turkey

Introduction

The systematics and taxonomic status of mole rats within the family Spalacidae are highly complicated. According to different authors, the family consists of only a single genus, *Spalax* (Nevo et al. 1995; Musser and Carleton 2005; Kryštufek and Vohralik 2009), or two genera, *Spalax* and *Nannospalax* (Lyapunova et al. 1974; Gromov and Baranova 1981; Zima and Kral 1984). Musser and Carleton (2005) treated the family as monogeneric and included the name *Nannospalax* as a synonym of *Spalax*. The authors stated that *Spalax leucodon* Nordmann, 1840, *S. nehringi* (Satunin 1898), and *S. ehrenbergi* (Nehring 1898) are distributed in Turkey. Kryštufek and Vohralik (2009) proposed the name *xanthodon* instead of *nehringi* and indicated that three morphospecies, *Spalax leucodon*, *S. xanthodon* (Nordmann 1840) and *S. ehrenbergi* are distributed in Turkey. Contrary to Musser and Carleton (2005) and Kryštufek and Vohralik (2009), we accepted the genus *Nannospalax*, with mainly acrocentric chromosomes in the chromosome set.

The genus *Nannospalax* underwent a rapid chromosomal evolutionary radiation that involved about 60 chromosomal forms; they are distributed in some parts of the Palearctic region. In Turkey, karyological studies revealed one chromosomal form ($2n = 56$) in *N. leucodon* (Soldatovic and Savic 1978), 11 chromosomal forms ($2n = 36$, 38, 40, 48, 50, 52, 54, 56, 58, 60W, and 60R), in *N. xanthodon* (= *N. nehringi*) (Nevo et al. 1995; Ivanitskaya et al. 2008; Arslan and Bölükbaş 2010), and five chromosomal forms ($2n = 48$, 52, 54, 56 and 58) in *N. ehrenbergi* (Nevo et al. 1995; Coşkun et al. 2006).

Nannospalax ehrenbergi was first described by Nehring in 1898 from Jaffa, Israel (Nehring 1898). This species is distributed in southeastern Turkey, northern Iraq, Syria, Lebanon, Jordan, Israel, Libya and Egypt (Savic and Nevo 1990; Musser and Carleton 2005).

The first karyological studies of *Nannospalax ehrenbergi* were carried out by Wahrman et al. (1969) in Israel. In Turkey, the first karyotypic study was conducted by Yüksel (1984) on the Elazığ population in Turkey. Banded chromosomes of Turkish *Nannospalax nehringi* and *N. ehrenbergi* were described by Ivanitskaya et al. (1997), Gülkaç and Küçükdumlu (1999), Ivanitskaya et al. (2008), Arslan and Bölükbaş (2010), Arslan, Akan, et al. (2011), Arslan, Toyran, et al. (2011), Arslan and Zima (2013), Arslan et al. (2013), Matur et al. (2013), and Aşan Baydemir et al. (2013). However, data on differentially stained karyotypes of mole rats in Turkey are still insufficient.

The aim of this study was to present the karyotype, heterochromatin distribution and nucleolar organizing regions (NORs) of the Palestine mole rat, *Nannospalax ehrenbergi* from Diyarbakır and Batman provinces.

Material and methods

One male and one female specimen of *Nannospalax ehrenbergi* from Özbek village, Kulp, Diyarbakır and Satı village, Sason, Batman were karyotyped, respectively (Figure 1). Mitotic metaphases were obtained from the bone marrow as described by Lee and Elder (1980). The C-banding was achieved according to the method of Sumner (1972). The location of NORs in the chromosomes was determined using the method of Howell and Black (1980). Chromosomes were classified according to Levan et al. (1964). At least 10 well-spread and Ag-NOR banded metaphase plates were

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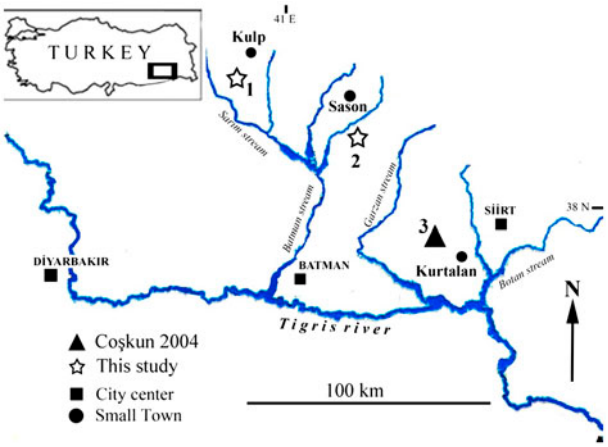

Figure 1. The collection localities (*) of the specimens: (1) Özbek village, Kulp, Diyarbakır; (2) Satı village, Sason, Batman. (3) First description locality of (▲) $2n = 56$, NF = 66 and NFa = 62 chromosomal form from İncirlik village, Kurtalan, Siirt (Coşkun 2004).

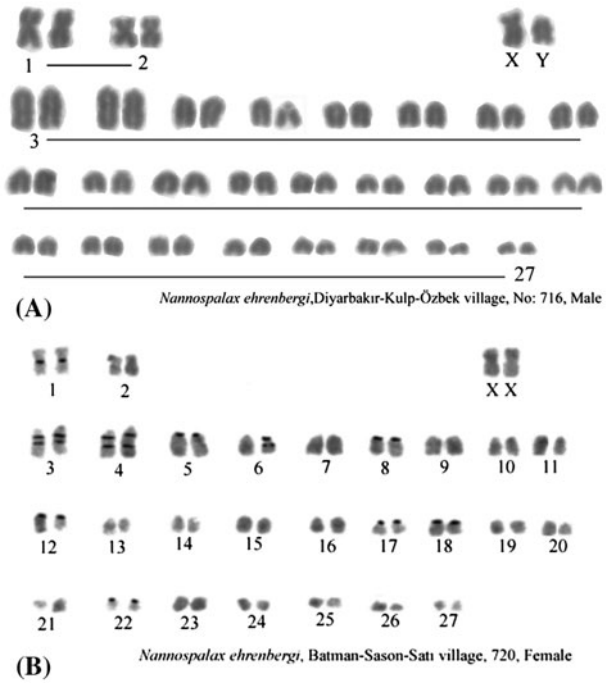

Figure 2. Conventionally stained (A) and C-banded (B) karyotype of *Nannospalax ehrenbergi* with $2n = 56$, NF = 62 and NFa = 58, from southeast Anatolia.

photographed in each specimen and arranged to determine the diploid chromosome number ($2n$), autosomal fundamental number (NFa) and fundamental number (NF). The voucher specimens and the slides are deposited in Department of Biology, Faculty of Science, University of Dicle.

The karyological analysis showed that the diploid number of chromosomes is $2n = 56$, the fundamental number NF = 62, and the number of autosomal arms NFa = 58. The karyotype of the specimens consists of two pairs of meta-submetacentrics and 25 pairs of acrocentrics decreasing in size.

The X chromosome is a large submetacentric while the Y is medium-sized and acrocentric in the Diyarbakır specimen. C-banding of the Batman specimen showed that district centromeric and pericentromeric heterochromatin is located in some of the autosomal pairs (nos. 1, 3, 5, 6, 8, 12, 17, 18, and 22). The two biggest acrocentric autosome pairs (nos. 3 and 4) showed two interstitial bands on their long arms and, in addition, the secondary constriction of the acrocentric pair (no. 6) revealed an interstitial C-band. The majority of the biarmed and acrocentric autosomes as well as the X chromosome possessed no constitutive heterochromatin (Figure 2B).

The active Ag-NORs are located in the telomeric region of one medium-sized metacentric autosome pair (no. 2 in Figure 2A), at the tiny arms of two pairs of medium-sized acrocentric autosomes (nos. 4 and 5 in Figure 2A), in the telomeric region of one pair of small acrocentric autosomes (no. 14 in Figure 2A) and at the secondary constriction of the acrocentric autosome pair (no. 6 in Figure 2A) in the specimen from Batman province (Figure 3).

Discussion

Chromosomal data and chromosomal variation for *Nannospalax ehrenbergi* from Turkey were summarized by Coşkun et al. (2006). The diploid number of chromosomes ranged between $2n = 48$ and 56, while the number of chromosome arms ranged between NF = 66 and 90. The most widespread chromosomal form of the Palestine mole rat has $2n = 52$ and NFa = 72 and it has been hitherto reported by various authors (Yüksel 1984; Yüksel and Gülkaç 1992; Nevo et al. 1995; Ivanitskaya et al. 1997; Coşkun et al. 2006).

The karyotype of the $2n = 56$, NF = 66, and NFa = 62 chromosomal form was first reported from Batman and Siirt provinces by Coşkun (2004) and consisted of four

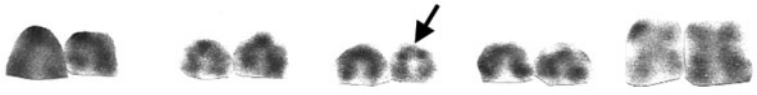

Figure 3. NOR-bearing chromosome pairs of *Nannospalax ehrenbergi* with $2n = 56$, NF = 62 and NFa = 58, from Sason, Batman province. The arrow indicates the heterozygous secondary constriction.

pairs of meta-submetacentric and 23 pairs of acrocentric chromosomes, decreasing in size. The X is a medium-sized submetacentric while the Y is small and acrocentric. However, the secondary constriction in the set was not mentioned by Coşkun (2004). In the present study, the diploid number of the chromosome set is $2n = 56$, as in agreement with Coşkun (2004); however, the complement newly described in the present study has the lowest number of biarmed chromosome pairs (three pairs with autosomes) recorded so far in Turkey.

The heterochromatin distribution of *Nannospalax ehrenbergi* was determined from Tarsus, Gaziantep, and Siverek by Ivanitskaya et al. (1997). According to these authors, the composition and the intensity of the C-bands in the biarmed and acrocentric autosomes as well as the sex chromosomes varied between the populations. In addition, Ivanitskaya et al. (2008), Arslan and Bölükbaş (2010), Arslan, Akan, et al. (2011) and Arslan, Toyran, et al. (2011) examined the C-bands of *N. nehringi/xanthodon* in central and northern Anatolia. The heterochromatin distribution and the intensity in the autosomes and the sex chromosomes also differed between the populations. The authors mentioned above did not detect any interstitial C-bands in the populations examined. With the present study, we add a new heterochromatin distribution to the karyosystematics of mole rats with interstitial C-bands.

The active Ag-NORs were detected in the telomeric regions of subtelocentric chromosomes by Gülkaç and Küçükdumlu (1999) and Ivanitskaya et al. (2008), in the telomeric regions of biarmed chromosomes by Arslan and Bölükbaş (2010), Arslan, Akan, et al. (2011), Arslan, Toyran, et al. (2011), and Aşan Baydemir et al. (2013) in *Nannospalax nehringi* and in the telomeric regions of subtelocentric chromosomes by Ivanitskaya et al. (1997) and Gülkaç and Küçükdumlu (1999) in *N. ehrenbergi*. However, Ivanitskaya et al. (1997) defined an acrocentric autosome pair with NORs localized in the telomeric region. In the Batman specimen, we detected active Ag-NORs in the telomeric regions of the submetacentric and acrocentric chromosomes as stated by those authors. Nevertheless, in contrast to the previous studies, we determined NORs in the tiny arms of acrocentrics and in the secondary constriction of an acrocentric chromosome. Centromeric NOR-bearing acrocentric chromosomes are scarcely rare. In addition, the relationship between the secondary constriction and NORs is also confirmed in the present study.

Apparently, the studied specimens have different karyological peculiarities in terms of NF, NFa values, structure of sex chromosomes, C-banding patterns and NOR-bearing chromosomes. By determining a new chromosomal form with $2n = 56$, NF = 62, and NFa = 58 in contrast to Coşkun (2004) in this study, it is clear that there are two different chromosomal forms of *N. ehrenbergi* in the region.

Consequently, the distribution of constitutive heterochromatin and the number of NOR-bearing chromosomes of mole rats distributed in Turkey vary with regard to the diploid chromosome number and the number of autosomal arms. According to both Kandemir et al. (2012) and Kankılıç et al. (2013) some chromosomal forms of *Nannospalax nehringi* (as stated *N. xanthodon* in Kankılıç et al. 2013) and *N. ehrenbergi* could be considered separate biological species with regard to molecular studies. However, the chromosomal diversity in Turkish spalacids is great, therefore it is difficult to decide whether or not a distinct chromosomal form represents a separate species as stated previously by Coşkun et al. (2006).

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