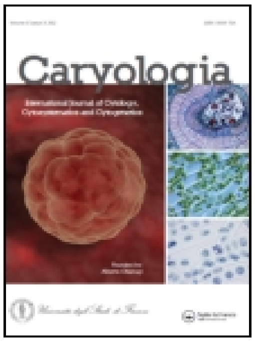

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

ISSN: 0008-7114 (Print) 2165-5391 (Online) Journal homepage: www.tandfonline.com/journals/tcar20

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To cite this article: Nursel Aşan Baydemir, Tuba Yağcı & Şükran Çakır (2013) Karyological studies of the 2*n* = 60 cytotype of *Nannospalax nehringi* from Central Anatolia, Caryologia, 66:1, 49-53, DOI: 10.1080/00087114.2013.780441

To link to this article: https://doi.org/10.1080/00087114.2013.780441

Published online: 17 May 2013.

Caryologia: International Journal of Cytology, Cytosystematics and Cytogenetics, 2013
Vol. 66, No. 1, 49–53, http://dx.doi.org/10.1080/00087114.2013.780441

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Karyological studies of the 2*n* = 60 cytotype of *Nannospalax nehringi* from Central Anatolia

Nursel Aşan Baydemir[a]*, Tuba Yağcı[b] and Şükran Çakır[a]

[a]*University of Kırıkkale, Faculty of Science and Arts, Department of Biology, 71451 Yahşihan, Kırıkkale, Turkey;* [b]*University of Bilecik, Pazaryeri Vocational School, Pazaryeri, Bilecik, Turkey*

In this study, the G-bands and nucleolus organizer regions (NORs) of the 2*n* = 60 chromosomal form of *Nannospalax nehringi* from Central Anatolia (Konya) are presented. The karyotype possesses nine pairs of submetacentric and subtelosentrics and 20 pairs of acrocentric autosomes gradually decreasing in size (fundamental number = 80 and autosomal fundamental number = 76). NORs were localized in the telomeric regions of the short arms of three large pairs and one medium-sized pair of subtelocentric autosomes (nos. 1, 3, 4, 9).

Keywords: Ag-NOR banding; G banding; karyotype; *Nannospalax nehringi*; Turkey

Introduction

The family Spalacidae is composed of fossorial mole rats which are highly adapted to subterranean life. The systematics and taxonomy of extant species of mole rats are still controversial. Gromov and Baranova (1981) stated that the subfamily Spalacinae is represented by two genera: *Nannospalax* (Palmer 1903) and *Spalax* Guldenstaedt, 1770. However, Musser and Carleton (2005) accepted *Nannospalax* as a synonym of the genus *Spalax* and treated the family as monogeneric, including only the nominative genus. 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) and Kryštufek et al. (2012) 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. Both Sözen et al. (2011) and Matur et al. (2011) decided to use *N. nehringi* instead of *S. leucodon*. However, Arslan and Bölükbaş (2010), Arslan et al. (2010), Arslan et al. (2011a) and Arslan et al. (2011b) adopted both *N. nehringi* and *N. xanthodon* for the mole rats distributed in Central Anatolia.

The genus *Nannospalax* is distinguished from *Spalax* with respect to low diploid chromosome number (2*n*) and fundamental number (NF) as well as possessing acrocentrics in the chomosome set (Kryštufek and Vohralik 2009; Németh et al. 2009). Contrary to Musser and Carleton (2005) and Kryštufek and Vohralik (2009), we attributed the mole rats distributed in Turkey to the genus *Nannospalax*, having mainly acrocentric chromosomes (Aşan and Yağcı 2008; Coşkun et al. 2010, Arslan and Bölükbaş 2010; Arslan et al. 2010; Arslan et al. (2011a,b); Matur et al. 2011; Sözen et al. 2011).

To date, more than 50 chromosomal forms have been described for the family Spalacidae distributed in the Palaearctic region, and of them 30 forms were only recorded from Turkey. However, the exact distributional areas of these chromosomal forms are still not known. New cytotypes are being discovered continuously with as more specimens are examined in Turkey (Nevo et al. 1995; Sözen 2004; Sözen et al. 2006).

Recently, Sözen et al. (1999, 2006), Kankılıç et al. (2007), Ivanitskaya et al. (2008), Arslan and Bölükbaş (2010), Arslan et al. (2011a,b) have examined the conventional stained and banded karyotypes of specimens from Central Anatolia. All the authors accepted that the diploid chromosome number of examined specimens was 60 (except the Kırıkkale population with 2*n* = 54) and the autosomal fundamental number ranged between 68 and 80.

The aim of this study is to make a contribution to the karyomorphology of the 2*n* = 60 cytotype of mole rats distributed in Central Anatolia with regard to presenting the G-banding of the chromosomes and nucleolus organizer region (NOR)-bearing chromosome number.

Material and methods

Five specimens (3 ♂♂, 2 ♀♀) were captured from Konya, Cihanbeyli (38°′37′ N, 32°54′ E) and Kulu (39° 05′ N, 33°04′ E) in Central Anatolia. Chromosome preparations were obtained from bone marrow cells according to the technique of Ford and Hamerton (1956). The G-banding was achieved according to technique of

*Corresponding author. Email: nurselasan@yahoo.com

Seabright (1971). 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 20 well-spread and banded metaphase plates were photographed and arranged to determine the diploid chromosome number (2*n*), autosomal fundamental number (NFa) and fundamental number (NF). All stuffed skins and slides are deposited at the Vocational School of Pazaryeri, University of Bilecik and Faculty of Science and Arts, University of Kırıkkale, respectively.

Results

The karyotype had 2*n* = 60, NF = 80 and NFa = 76. The chromosome set consisted of seven pairs of subtelosentric pairs (nos. 1–3, 5–8), two submetacentric pairs (nos. 4, 9) and 20 pairs of acrocentric pairs (nos. 10–29), gradually decreasing in size. The X chromosome was medium sized submetacentric while the Y was small acrocentric. No secondary constriction or heteromorphic chromosome pair are detected in the examined karyotypes (Figure 1).

Chromosome shapes of *Nannospalax nehringi* were determined and are given in Table 1. G-banding karyotype of the 2*n* = 60 is given in Figure 2. NORs are located in the telomeric regions of the short arms of three large pairs (nos. 1, 3, 4) and one medium-sized subtelocentric pair (no. 9). Active Ag-NORs on homologous chromosomes exhibited heteromorphism in some cells (autosome pair nos. 1 and 3) (Figure 3).

Discussion

According to Nevo et al. (1995), the karyotypic evolution in the genus *Spalax* is caused by Robertsonian mutations. Therefore, the spalacids represented a remarkable adaptive ecological speciation, caused by these mutations, in its distributional area.

So far, about 150 populations of mole rats have been karyotyped in Turkey (Coşkun et al. 2010). The diploid chromosome number and fundamental number of the genus *Nannospalax* showed variation from 36 to 62 and 66 to 98, respectively (Sözen et al. 2006; Ivanitskaya et al. 2008; Ulutürk et al. 2009). The 2*n* = 60 chromosomal form is the most common cytotype, although 2*n* = 36 and 2*n* = 62 cytotypes are the extreme ones (Kankılıç et al. 2007; Ivanitskaya et al. 2008; Arslan and Bölükbaş 2010; Arslan et al. (2011b)). Despite the numerous conventionally stained karyotypes prepared for the genus, few banded karyotypes are reported from Turkey (Gülkaç and Küçükdumlu 1999; Ivanitskaya et al. 1997, 2008; Arslan and Bölükbaş 2010; Arslan et al. (2011 a,b). Gülkaç and Küçükdumlu (1999) examined NOR distribution in the 2*n* = 60 chromosomal form from Malatya province and active NORs are recorded on the telomeric regions of three pairs of large subtelocentrics. Ivanitskaya et al. (2008) stated that the 2*n* = 60 cytotype of *Nannospalax nehringi* (attributed as *S. leucodon*) possessed two different kinds of karyotype structure; 2*n* = 60W (wide distribution cytotype with NORs in the telomeric regions of the chromosomes numbered 3, 4, 5, 6 and 13) and 2*n* = 60R (rare distribution cytotype with NORs in the telomeric regions of the chromosomes numbered 6, 8, 12, 13 and 16) in Turkey. Ag-NORs on homologous chromosomes exhibited heteromorphism in the 2*n* = 60W cytotype. Arslan and Bölükbaş (2010) determined active Ag-NORS in the telomeric regions of four biarmed chromosomes. Ag-NORs on one of the homologous chromosomes exhibited heteromorphism. However in addition, Arslan et al. (2011b). recorded Ag-NORs in the telomeric regions of four biarmed autosomal pairs of 2*n* = 54 chromosomal form from Central Anatolia.

Recently, Cihanbeyli (Konya) karyotypes of specimens of the 2*n* = 60 cytotype were examined by Kankılıç et al. (2007) and Arslan et al. (2011a). All authors considered that the karyotype had 2*n* = 60, NF = 80 and NFa = 76 with nine pairs of sub- and

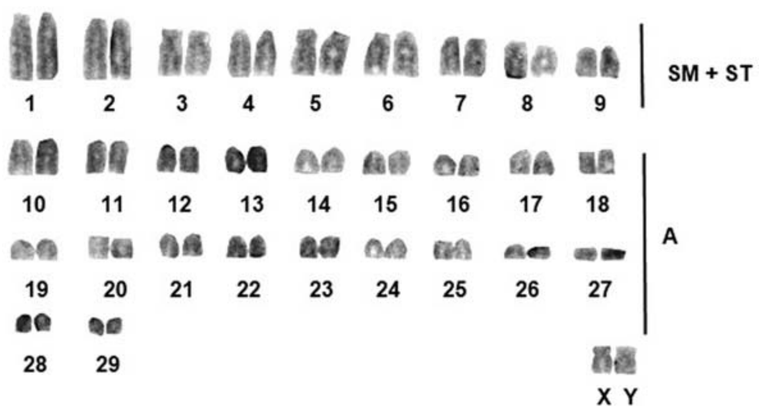

Figure 1. Conventionally stained karyotype of *Nannospalax nehringi*.

Table 1. Chromosome classification in *Nannospalax nehringi* according to Levan et al. (1964).

Chromosome pair	Short arm (S)	Long arm (L)	Total length (S+L)	Arm ratio (L/S)	Relative length	CI	Terminology
1	0.3	2.0	2.3	6.6	6.5	13	ST
2	0.3	2.0	2.3	6.6	6.5	13	ST
3	0.3	1.7	2.0	5.6	5.6	15	ST
4	0.4	1.2	1.6	3.0	4.5	25	SM
5	0.3	1.2	1.5	4.0	4.2	20	ST
6	0.3	1.2	1.5	4.0	4.2	20	ST
7	0.3	1.2	1.5	4.0	4.2	20	ST
8	0.3	1.0	1.3	3.3	3.7	23	ST
9	0.3	0.7	1.0	2.3	2.8	30	SM
10	—	1.4	1.4	—	4.0	—	A
11	—	1.3	1.3	—	3.6	—	A
12	—	1.2	1.2	—	3.4	—	A
13	—	1.0	1.0	—	2.8	—	A
14	—	1.0	1.0	—	2.8	—	A
15	—	1.0	1.0	—	2.8	—	A
16	—	1.0	1.0	—	2.8	—	A
17	—	1.0	1.0	—	2.8	—	A
18	—	1.0	1.0	—	2.8	—	A
19	—	1.0	1.0	—	2.8	—	A
20	—	0.8	0.8	—	2.3	—	A
21	—	0.8	0.8	—	2.3	—	A
22	—	0.8	0.8	—	2.3	—	A
23	—	0.8	0.8	—	2.3	—	A
24	—	0.8	0.8	—	2.3	—	A
25	—	0.8	0.8	—	2.3	—	A
26	—	0.8	0.8	—	2.3	—	A
27	—	0.8	0.8	—	2.3	—	A
28	—	0.5	0.5	—	1.4	—	A
29	—	0.5	0.5	—	1.4	—	A
X	0.5	1.0	1.5	2.0	4.2	33	SM
Y	—	0.5	0.5	—	1.4	—	A

Abbreviations: SM, submetacentric; ST, subtelocentric; A, acrocentric; CI, centromeric index.

subtelocentric autosomes and 20 pairs of acrocentric autosomes. However, discrepancy was found in the shape of the X chromosome in both studies. Kankılıç et al. (2007) stated that the X chromosome was metacentric and the Y subtelocentrics, while Arslan et al. (2011a). recorded the X chromosome as submetacentric. Unfortunately, no male specimen was examined by the latter authors. Arslan et al. (2011a) stated that the $2n$ = 60 chromosomal form differed with respect to the shape and size of the Y chromosome. However, we determined the X chromosome as submetacentric and the Y as acrocentric in this study.

Arslan et al. (2011a) observed Ag-NORs in the telomeric regions of the short arms of four biarmed autosomes (nos. 3, 4, 5 and 6). In addition, we detected an active NOR in the telomeric region of one medium-sized metacentric chromosome (no. 9) in this study. The authors did not mention any active Ag-NORs on this autosome pair. Furthermore, the dissimilarity between NOR-bearing subtelocentric autosomes (no. 6 in Arslan et al. (2011a) and no. 1 in this study) in both studies, was probably due to the different methods used for arrangement of the chromosomes by the authors.

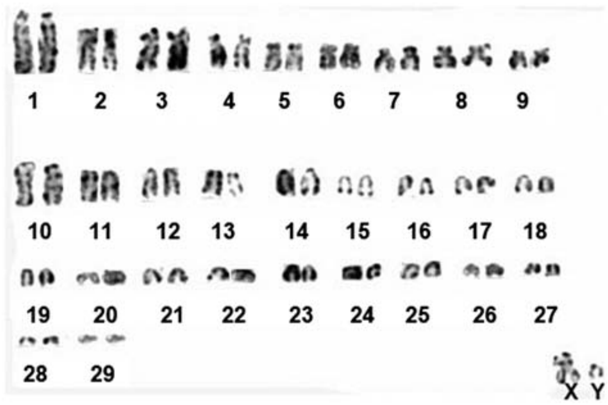

Figure 2. G-banded karyotype of the $2n$ = 60 cytotype of *Nannospalax nehringi*.

Consequently, despite the different shape of NOR-bearing chromosomes, the number of Ag-NORs is recorded as four from Central Anatolia (Arslan and Bölükbaş 2010 a; Arslan et al (2011a,b); this study). For determining intra- and interspecific chromosomal differences the Ag-NOR banding is as useful as the other banding techniques as stated by Arslan et al. (2011a).

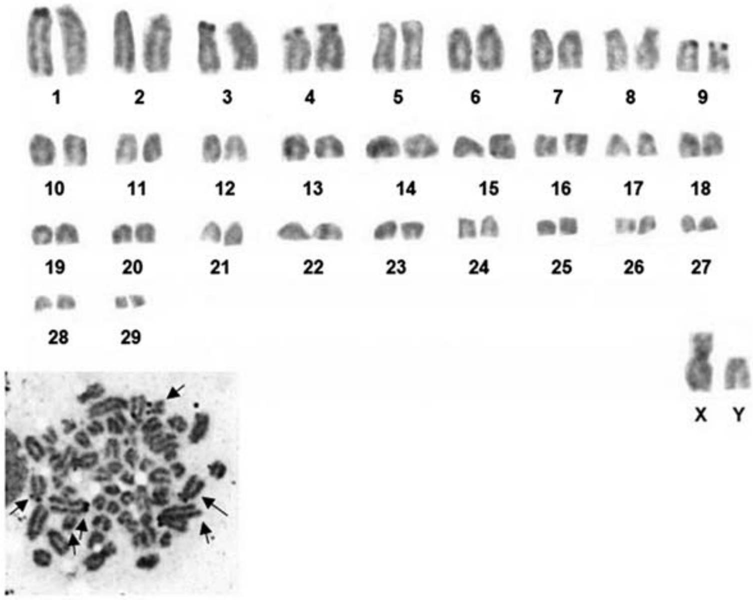

Figure 3. Silver stained metaphase and karyotype of the $2n$ = 60 cytotype of *Nannospalax nehringi* (arrows indicate the NOR-bearing chromosomes).

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