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# The banded karyotype of the 2n = 58 chromosomal race of mole rats from Erzincan, Turkey

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**Abstract.** In this study, the 2n = 58 chromosomal race of blind mole rats, *Nannospalax xanthodon*, from the Erzincan province in Turkey was investigated. Conventional chromosome staining, Ag-NOR staining and C-banding analysis were carried out in the specimens studied. The karyotype included three small or medium-sized meta/submetacentric pairs and twenty-five acrocentric pairs of autosomes of gradually diminishing size (NFa = 62). C-heterochromatin regions were found in centromeric and pericentromeric areas or in short arms of some bi-armed autosomal pairs and in pericentromeric areas of a few acrocentric autosomes. The X chromosome had a centromeric C-positive band and the short arm of the Y chromosome appeared to be C-positively stained. The NORs were localized in distal heterochromatin areas of the short arms of two pairs of biarmed and one pair of acrocentric autosomes. Within the 58 chromosome populations reported from Turkey, two groups can be recognized differing by the presence or absence of a large submetacentric autosomal pair. The populations possessing this marker chromosome occur in central and northern Anatolia, whereas populations form eastern Anatolia have no similar chromosome in their karyotype.

**Key words:** Ag-NOR staining, C-banding, *Nannospalax xanthodon*

## Introduction

Mole rats (Spalacidae) are a small group of subterranean rodents occurring in eastern and south-eastern Europe, the eastern Mediterranean area and the Middle East, and north-western Africa (Savić 1982, Musser & Carleton 2005). They are blind rodents with various morphological, physiological and behavioural particularities that emphasize their adaptation to life in underground (Nevo 1979, Savić & Nevo 1990).

Some authors (Savić & Nevo 1990, Musser & Carleton 2005) treated the family Spalacidae as monogeneric, with the single recognized genus *Spalax*, whereas others distinguished two genera currently named *Spalax* and *Nannospalax* (Topachevski 1969, Savić 1982, Savić & Soldatović 1984, Németh et al. 2009). Most authors recognize three species within the genus *Nannospalax*, i.e. *N. ehrenbergi*, *N. leucodon*, and *N. xanthodon* (Kryštufek & Vohralík 2009 proposed that the name *nehringi* is preoccupied by *xanthodon*). The systematics and phylogenetic relationships of mole rats are not yet definitively resolved, and the currently recognized species are often considered as superspecies presumably including many separate biological species.

The most important reason for the taxonomic uncertainties is remarkable karyotypic variation recorded within and between the *Nannospalax* populations and species. About 50 distinct chromosomal races or cytotypes have been found within the three formally recognized *Nannospalax* species (e.g., Savić 1982, Nevo et al. 1994, 1995), and some authors believe that these cytotypes should be considered as presumptively good biological species (Nevo et al. 2001). Molecular analyses of the cytochrome *b* sequences suggested, however, that associations between genetic and chromosomal variation are not widespread and common in mole rats, and therefore refuted the generalization of a "cytotype-equals-species" approach (Kryštufek et al. 2011, Kandemir et al. 2012).

In Turkey, all three species of *Nannospalax* occur. *N. leucodon* is distributed probably only in Thrace in

European Turkey, *N. xanthodon* in most of Anatolia, and *N. ehrenbergi* in south-eastern parts of Asiatic Turkey. The extent of chromosomal variation in mole rat populations in Turkey is impressive (Nevo et al. 1995, Sözen 2004, Sözen et al. 2006a) and the country can be considered a core area of differentiation processes in chromosomal evolution within this taxon. Nevo et al. (1994, 1995) proposed that chromosomal speciation of mole rats in Turkey is centripetal. The diploid numbers (2n) established in individual populations are assumed to be correlated with aridity stress and climatic unpredictability, and they should increase towards the central Anatolian Plateau from all directions. The mole rat populations with the highest diploid chromosome number (2n = 60) actually occur mainly in central Anatolia, in accordance with the above hypothesis. Populations with lower diploid numbers occur in other areas and/or are interspersed in a mosaic pattern in areas of the 60 chromosome populations. The same diploid number may be found in geographically distant allopatric populations. When such populations at extreme edges of the range display identical diploid numbers, they are designated by their 2n plus a letter such as E for eastern, C for central or W for western (Nevo et al. 1995, Sözen 2004, Sözen et al. 2006a).

The diploid number of 58 chromosomes was reported in several mole rat populations from different parts of Asiatic Turkey. Sözen & Kıvanç (1998) recorded this karyotype for the first time from the type locality of *N. x. cilicicus* in Madenköy, province Niğde. In the same area of south-eastern parts of central Anatolia in the provinces Konya and Niğde, Sözen et al. (2000, 2006a) made further investigations in Ereğli and Ulukışla, and Sözen et al. (2006a) examined a population from a geographically close site in Pozantı (Adana province). In all these sites, populations with 2n = 58 were found. The population from Ereğli was subsequently studied also with the use of C-banding and Ag-NOR staining (Arslan et al. 2011). Karyotypic investigations in northern parts of Anatolia revealed the 58 chromosome populations in a site situated north of Ankara, and in the Kastamonu province (Sözen 2004, Sözen et al. 2006b). In the eastern parts of Anatolia, records of mole rat populations with 58 chromosomes were made by Coşkun (2004) and Coşkun et al. (2010) in two localities in the Tunceli and Erzincan provinces. Both these populations were recognized as a separate species, *N. munzuri* Coşkun, 2004. The diploid number of 58 chromosomes was recorded also in some populations of *N. ehrenbergi* (Nevo et al. 1995, 2001).

Our material originated in eastern Anatolia and the aim of this study is to perform comparisons of chromosomal characteristics, the C-banding pattern and AgNOR distribution among geographically distant populations sharing the same diploid number of 58 chromosomes. We also aim to summarize the available data concerning the 58 chromosome populations in Turkey in order to facilitate taxonomic evaluation of mole rats by providing new data on their karyotypic status and distribution pattern.

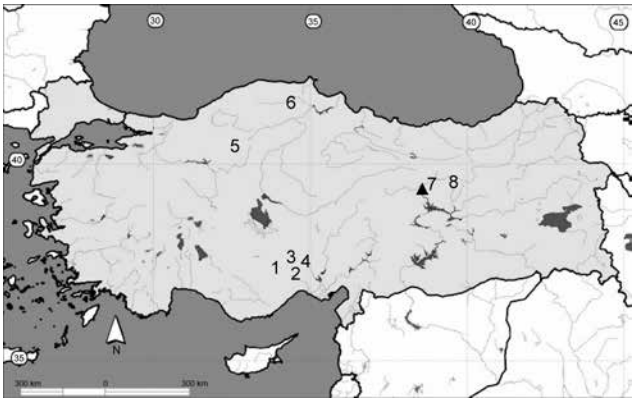


**Fig. 1.** The collecting locality of *Nannospalax xanthodon* (2n = 58) in Başpınar village, Kemaliye, Erzincan (▲), and geographic position of the other studied 2n = 58 populations. For numbering of sites and the references see Table 1.

## Material and Methods

Cytogenetic analyses were performed in two male specimens of mole rats from Başpınar village, Kemaliye, Erzincan (Fig. 1). Karyotype preparations were obtained in the field from bone marrow after colchicine treatment (Ford & Hamerton 1956). Air-

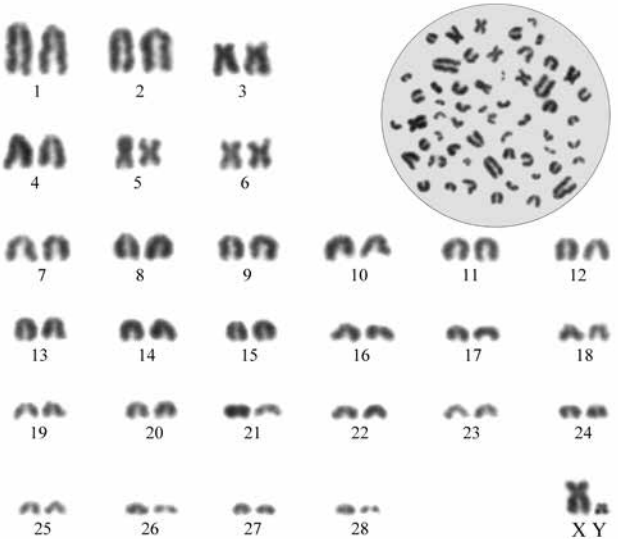


**Fig. 2.** Metaphase spread and karyotype of a specimen from Başpınar village, Kemaliye, Erzincan.

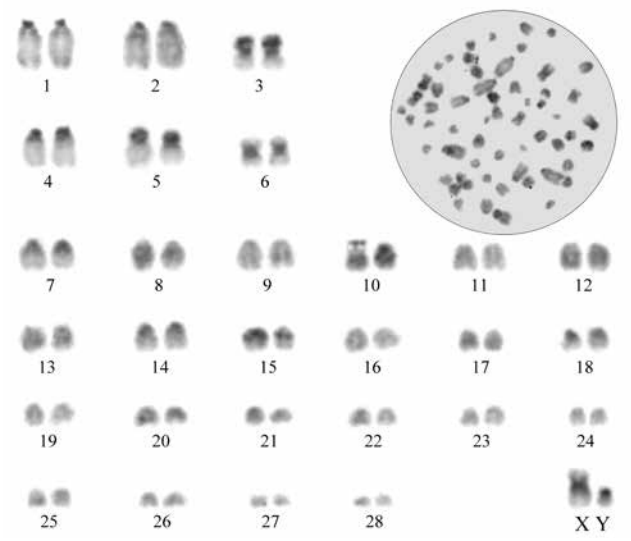


**Fig. 3.** Metaphase spread and C-banded karyotype of a specimen from Başpınar village, Kemaliye, Erzincan.

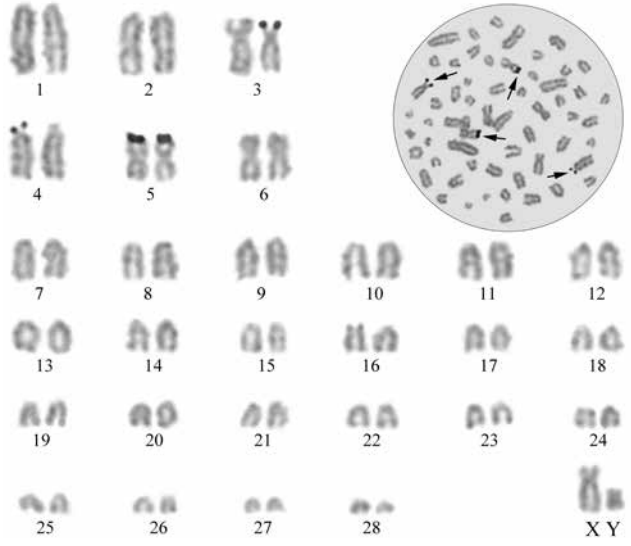


**Fig. 4.** Silver-stained metaphase spread and karyotype of a specimen from Başpınar village, Kemaliye, Erzincan.

dried preparations were stained conventionally by Giemsa. Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected by the techniques of C-banding (Sumner 1972) and Ag-NOR staining (Howell & Black 1980), respectively. From each specimen, 10 to 20 slides were prepared, and at least 20 well-spread metaphase plates were analysed. The nomenclature of chromosomes according to the centromere position was established according to Levan et al. (1964). Standard voucher specimens (skins and skulls) are deposited at Selçuk University, Biology Department, Faculty of Science, Konya, Turkey.

## Results

The karyotype of mole rat from Erzincan consists of 58 chromosomes including three small or medium-sized meta/submetacentric autosomal pair (no. 3, 5, 6) and twenty-five acrocentric autosomal pairs (nos. 1-2, 4, 7-28) of gradually diminishing size (NFa = 62). Distinct short arms are sometimes apparent in the large acrocentric pair no. 4. The X chromosome is medium-sized submetacentric, and the Y chromosome small submetacentric (NF = 66) (Fig. 2).

The C-banded karyotype of mole rat is illustrated in Fig. 3. Some of the autosomes (nos. 1-7, 10, 14, 15, 18) possess distinct C-positive bands. The C-positive regions are usually extensive and include relatively large pericentromeric areas, except of the biarmed pair no. 6 which reveals lower intensity of the dark C-staining. Two biarmed autosomes (nos. 3 and 5) have C-heterochromatic short arms separated from the dark pericentromeric area by a narrow light band. The number of C-positive pericentromeric regions observed in acrocentric autosomes may vary, and intensity of the dark staining is variable between individual pairs. The X chromosome has a centromeric C-positive band and the short arm of the Y chromosome appears to be uniformly C-positively stained.

**Table 1.** Chromosomal records of the 2n = 58 race of *Nannospalax xanthodon* from Turkey NF – fundamental number of chromosomal arms. See Fig. 1 for location of the sites.

| | Site | Province | NF | Reference |
|---|---|---|---|---|
| 1 | Ereğli | Konya | 72<br>75 | Sözen et al. (2006a)<br>Arslan et al. (2011) |
| 2 | Ulukışla | Niğde | 72 | Sözen et al. (2000)<br>Sözen et al. (2006a) |
| 3 | Madenköy | Niğde | 72 | Sözen & Kivanç (1998) |
| 4 | Pozantı | Adana | 72 | Sözen et al. (2006a) |
| 5 | Sarıkavak | Ankara | 78 | Sözen (2004) |
| 6 | Taşköprü | Kastamonu | 74 | Sözen et al. (2006b) |
| 7 | Esentepe-Kemaliye | Erzincan | 68 | Coşkun et al. (2010) |
| 8 | Ovacık | Tunceli | 68 | Coşkun (2004) |
| ▲ | Başpınar-Kemaliye | Erzincan | 66 | This paper |

The Ag-NOR regions were found in two biarmed autosomal pairs (nos. 3, 5) and one acrocentric autosome (no. 4). In all these autosomes, the NORs were observed in telomeric regions of the short arms (Fig. 4).

## Discussion

Our results and published data show that the occurrence of the 58 chromosome populations of mole rats is rather widespread in Anatolia (Table 1, Fig. 1). However, the geographic continuity of the known populations is dubious and improbable. For instance, Sözen (2004) ascertained that the site of collection of a 58 chromosome individual in Sarıkavak (the Ankara province) was completely surrounded by populations with 60 chromosomes. Similarly, the 2n = 58 populations from the Kastamonu province apparently occupy a small area surrounded by populations possessing different diploid numbers of chromosomes (Sözen et al. 2006b).

There are distinct differences between the numbers of chromosomal arms reported from individual populations with 58 chromosomes and it is possible to differentiate two distinct groups among these populations. All the specimens from south-eastern and northern Anatolia (sites 1-6 in Table 1 and Fig. 1) show the presence of a large submetacentric autosomal pair in their karyotypes which can be considered an autapomorphic chromosomal marker. A similar chromosome was recorded also in populations with other diploid numbers (e.g. 50, 52, 54, 56; see Sözen et al. 2006a) but it is absent in the karyotypes with 60 chromosomes. The 58 chromosome populations from eastern Anatolia (sites 7-8 and the finding of this paper in Table 1 and Fig. 1) possess no large submetacentric autosome in their karyotypes, and all the biarmed autosomes are of medium or small size. The differences in the number of chromosomal arms between the 58 chromosome populations can be explained by mechanisms similar to that responsible for autosomal heteromorphism described from population in Ereğli (Arslan et al. 2011). It is also possible that minor differences in the NF values may result from subjective evaluation of the centromere position in individual papers.

The comparison between C-banded karyotypes of the 58 chromosome populations from Ereğli (Arslan et al. 2011) and Başpınar shows that the pattern of distribution of C-heterochromatin is rather similar. The centromeric areas of most of the biarmed autosomes and some of the uniarmed autosomes are stained C-positively. Two of the biarmed autosomes from the karyotype of the Başpınar population possess, however, small C-heterochromatic short arms, whereas the biarmed autosomes from the karyotype of the Ereğli population show only pericentromeric dark C-bands. The C-banding pattern on the sex chromosomes is also largely similar between the two populations but C-positive staining of the short arm of the Y chromosome suggests a heterochromatin amplification event in this submetacentric element. The Y chromosome was determined as subtelocentric in Ereğli, and as acrocentric in the other 58 chromosome populations.

The published reports indicate that the AgNOR sites were observed in telomeric areas of the short arms of three, four or five pairs of chromosomes in complements found in various populations of *N. xanthodon* in Turkey (Ivanitskaya et al. 1997, 2008, Gülkaç & Küçükdumlu 1999, Arslan & Bölükbaş 2010). However, the NORs were recorded in only one or two autosomal pairs in populations of *N. ehrenbergi* from Turkey and Jordan (Ivanitskaya & Nevo 1998, Gülkaç & Küçükdumlu 1999). The position of the AgNORs in the karyotype of the specimens from Başpınar is similar as in the karyotype from Ereğli but the number of nucleolar organizer bearing autosomes is lower. The Başpınar specimens revealed only three NOR-bearing pairs in their karyotype whereas four biarmed pairs possessing NORs were recorded in Ereğli. This finding indicates that distant populations of mole rats with the same diploid chromosome number may differ also in the pattern of the NORs distribution.

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