

## Distribution of chromosomal forms of *Nannospalax nehringi* (Satunin, 1898) (Rodentia: Spalacidae) in Çankırı and Çorum provinces, Turkey

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**Abstract:** Mole rats have adapted to living underground and have a wide range chromosomal variation in Turkey, ranging between  $2n = 36$  and  $2n = 60$ . This study was performed on the subterranean mole rats of the *Nannospalax nehringi* (Nehring, 1898), sampled around Çankırı and Çorum provinces in central Anatolia, and the karyotypes of 91 specimens across 38 localities were analyzed. Different chromosomal forms that have the same  $2n$  values were assigned letters according to their geographic locations in Turkey: C for central forms, N for northern forms, S for southern forms, E for eastern forms, and W for western forms. It was determined that *N. nehringi* has  $2n = 54C$ ,  $NF = 74$ ;  $2n = 56N$ ,  $NF = 72$ ;  $2n = 58N$ ,  $NF = 74$ , and 2 different forms of  $2n = 60$  ( $NF = 78$ ,  $NF = 82$ ) in these areas. This study filled the gaps in distribution of blind mole rat chromosomal forms around Çankırı and Çorum provinces. The distribution areas of  $2n = 54C$ ,  $56N$ ,  $58N$ , and  $60$  forms in the area were brought to light.

**Key words:** Rodentia, Spalacidae, *Nannospalax nehringi*, karyotype, Turkey

### Çankırı ve Çorum illerindeki *Nannospalax nehringi* (Satunin, 1898) (Rodentia: Spalacidae) kromozomal formların dağılımı

**Özet:** Kör fareler toprak altı yaşama uyum sağlamışlardır. Bu canlılar Türkiye'de  $2n = 36$ 'dan  $2n = 60$ 'a kadar değişen geniş bir kromozomal varyasyona sahiptirler. Bu çalışma Çankırı ve Çorum illerinin çevresindeki 38 lokaliteden yakalanan 91 *Nannospalax nehringi* (Nehring, 1898) örneği üzerinde yapıldı. Aynı  $2n$  değerlerine sahip farklı formlar Türkiye'deki coğrafik yayılımlarına göre bir harf ile belirtildi. İç Anadolu formları için C, kuzeydekiler için N, güneydekiler için S, doğudakiler için E ve batıdaki formlar için W kullanıldı. Karyotip analizleri sonucunda *N. nehringi*'nin bu alanlarda  $2n = 54C$ ,  $NF = 74$ ;  $2n = 56N$ ,  $NF = 72$ ;  $2n = 58N$ ,  $NF = 74$  ve  $2n = 60$  için iki forma ( $NF = 78$  ve  $NF = 82$ ) sahip olduğu belirlendi. Bu çalışma Çankırı ve Çorum çevresindeki körfare kromozomal formları ve bunların yayılışlarındaki boşlukları doldurdu.  $2n = 54C$ ,  $56N$ ,  $58N$  ve  $60$  formlarının bölgedeki yayılışları aydınlatıldı.

**Anahtar sözcükler:** Rodentia, Spalacidae, *Nannospalax nehringi*, karyotip, Türkiye

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## Introduction

Chromosomal differentiation among populations can be an initial step in speciation (Zima, 2000). To date, more than 50 chromosomal forms have been determined in blind mole rats and there is still doubt about taxonomy of this taxon. Topachevskii (1969) recognized 2 genera: *Microspalax* and *Spalax*. Later, Gromov and Baranova (1981) recognized 2 genera: *Nannospalax* and *Spalax*, and since *Microspalax* is a homonym of an arachnid species, recent taxonomic books have accepted the generic name *Nannospalax* as valid for blind mole rats in Turkey (Kryštufek and Vohralik, 2001; Yiğit et al., 2006).

The subterranean Spalacinae probably originated from a muroid-cricetoid stock, in or around the vicinity of Asia Minor during Oligocene times and radiated underground in the Balkans, steppic Russia, and the Middle East, extending into North Africa (Savic and Nevo, 1990). There are 3 *Nannospalax* species distributed in Turkey: *N. leucodon*, *N. nehringi*, and *N. ehrenbergi* (Topachevskii, 1969; Wilson and Reder, 1993; Kryštufek and Vohralik, 2005; Yiğit et al., 2006). However, the result of karyological studies showed that more than 30 chromosomal forms were distributed in Turkey (Nevo et al., 1994; Sözen et al., 1999, 2000a, 2000b, 2006a, 2006b; Sözen, 2004). The  $2n$  values ranged between  $2n = 36$  and  $60$ , while NF values ranged between  $66$  and  $92$  (Soldatovic and Savic, 1978; Yüksel, 1984; Gülkac and Yüksel, 1989; Sözen and Kıvanç, 1998a, 1998b; Sözen et al., 1999, 2000a, 2000b, 2006a, 2006b; Tez et al., 2001; Yüksel and Gülkac, 2001; Coşkun, 2003, 2004a, 2004b; Sözen, 2004; Matur and Sözen, 2005; Nevo et al., 1994, 1995; Kankılıç, 2005, 2007; Aşan and Yağcı, 2008; Ivanitskaya et al., 2008). The  $2n = 62$  form was recorded by Nevo et al. (1994, 1995). However, Ivanitskaya et al. (2008) indicated that determination of some chromosomal forms as  $2n = 62$  were because of small B chromosomes; so the  $2n = 62$  forms should be eliminated from the list of Turkish mole rats. Recently, though one revision recognized 3 species (Templeton, 1999), another revision recognized 4 chromosomal forms ( $2n = 52, 54, 58, 60$ ) of blind mole rats in Israel as different species and named them separately (Nevo et al., 2001). For such a process in Turkey, one of the

most important steps is to determine and identify chromosomal forms and their distributions, and, if possible, identify all species. In the last 3 decades, most parts of Turkey have been studied karyologically, with only some small gaps remaining (Sözen et al., 2006a, 2006b; Kankılıç, 2007; Aşan and Yağcı, 2008; Ivanitskaya et al., 2008). Thus, the purpose of this study was to describe the karyotypic characteristics of mole rats from given localities, and to fill the gaps in our knowledge about karyological forms and their distributional areas around Çankırı and Çorum provinces in Turkey.

## Materials and methods

In this study, 91 (39 males, 52 females) blind mole rats were studied from 38 localities around Çankırı and Çorum provinces in Inner Anatolia. The sampled localities, the number of individuals analyzed, and karyological results are presented in the Table and distribution of chromosomal forms and collection sites are shown in Figure 1.

Karyotypes were prepared from bone marrow according to methods by Ford and Hamerton (1956), and about 25-30 metaphase cells, which were well-stained, and whose chromosomes were separate and distinct, were examined from each animal. The diploid number of chromosomes ( $2n$ ), the number of autosomal arms (NFa), the total number of chromosomal arms (NF), and the sex chromosomes were determined from photos of the metaphase plates according to centromere position.

The karyotype preparations and animals examined were deposited in the Department of Biology, the Faculty of Art and Sciences, University of Zonguldak Karaelmas.

## Results

Within our material, 5 different chromosomal forms of *Nannospalax* were recognized ( $2n = 54C, 56N, 58N$ , and  $2n = 60$  NF = 78, 82). Since there are different forms that have the same  $2n$  value, we used here  $2n = 54C$  for central Anatolian 54 form,  $56N$  for northern Anatolian 56 form, and  $58N$  for northern Anatolian 58 form (Figure 1).

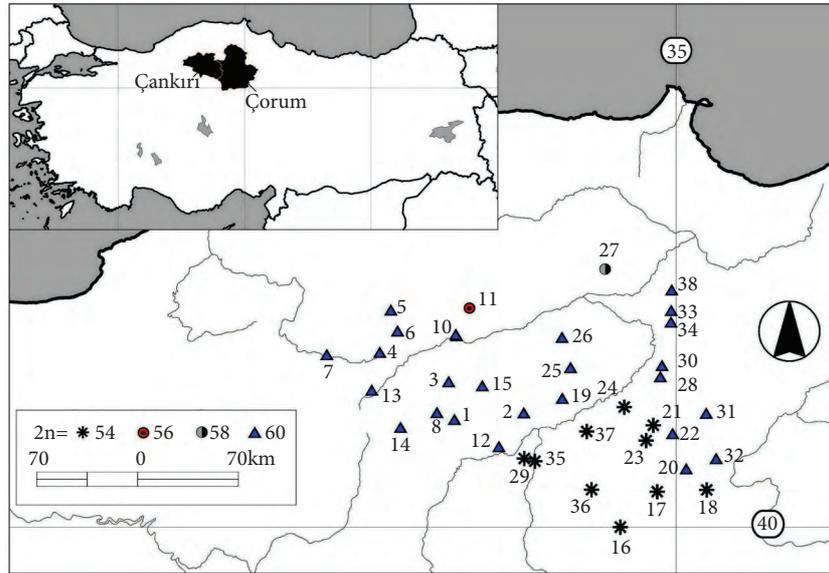


Figure 1. Map of study area in Turkey and distribution of chromosomal forms determined. The numbers of localities are as in the Table.

The  $2n = 54C$  population: Sampling localities of this form are given in the Table and distribution of these localities is shown in Figure 1. The karyotype of this form was  $2n = 54N$ ;  $NF = 74$ ;  $NFa = 70$ , the X chromosome was a medium-sized submetacentric, and the Y chromosome was small-sized acrocentric. The autosomal set contained 9 pairs of biarmed chromosomes, and 17 pairs of acrocentric chromosomes (Table, Figure 2).

The  $2n = 56N$  population: This has a karyotype of  $2n = 56$ ;  $NF = 72$ ;  $NFa = 68$ , the X chromosome was a middle-sized submetacentric, and the Y chromosome was small acrocentric. The autosomal set contained 7 pairs of biarmed chromosomes and 20 pairs of acrocentric chromosomes (Table, Figure 3).

The  $2n = 58N$  population: The karyotypes of the blind mole rats from Kargı Plateau were  $2n = 58$ ;  $NF = 74$ ;  $NFa = 70$ , the X chromosome was a middle-sized submetacentric, and the Y chromosome was small-sized subtelo centric. The autosomal set contained 7 pairs of biarmed chromosomes and 21 pairs of acrocentric chromosomes (Table, Figure 4).

The  $2n = 60$ ,  $NF = 78$  population: The karyotypes of the blind mole rats from the localities 1-10, 12-15,

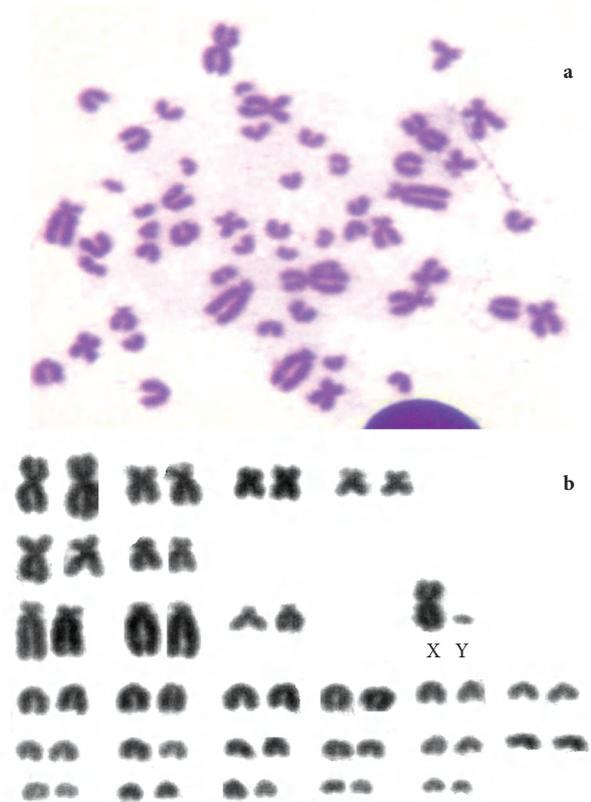


Figure 2. The metaphase plate (a) and karyotype of a male from 8 km east of Kızılırmak town in Çorum (4879 male,  $2n = 54$   $NF = 74$ ,  $NFa = 70$ ).

Table. Localities, sample size, diploid chromosome numbers (2n), and chromosomal arm numbers (NF) of animals examined.

		Male	Female	2n	NF	X	Y
1	15 km S of Çankırı	0	1	60	78	sm	a
2	Ağzıbüyük village, Çankırı	2	0	60	78	sm	a
3	Akçavakıf village, Çankırı	0	2	60	78	sm	a
4	7 km E of Atkaracalar	0	1	60	78	sm	a
5	Harmancık	1	0	60	78	sm	a
6	6 km S of Bayramören	0	3	60	78	sm	a
7	7 km W of Çerkeş	1	0	60	78	sm	a
8	Eldivan village, Çankırı	4	2	60	78	sm	a
9	1 km E of Ilgaz town	1	1	60	78	sm	a
10	Ilgaz Belören road fork	0	1	60	78	sm	a
11	Ilgaz Mountain ski centre	3	5	56	72	sm	a
12	7 km NW of Kızılırmak	1	1	60	78	sm	a
13	3 km N of Orta	0	4	60	78	sm	a
14	Şabanözü town	2	2	60	78	sm	a
15	11 km S of Yapraklı village, Çankırı	3	0	60	78	sm	a
16	8 km S of Boğazkale	1	0	54	74	sm	a
17	4 km E of Alaca	1	2	54	74	sm	a
18	15 km W of Aydıncık	1	1	54	74	sm	a
19	5 km S of Bayat	1	1	60	78	sm	a
20	15 km S of Cemilbey	2	1	60	78	sm	a
21	Çorum Organized industry area	1	1	54	74	sm	a
22	Ortaköy way, 12 km SE of Çorum	3	0	60	82	sm	a
23	19 km SW of Çorum	0	1	54	74	sm	a
24	21 km W of Çorum, İskilip way	0	3	54	74	sm	a
25	Gölköy village, Çorum	1	1	60	82	sm	a
26	34 km N of İskilip	0	1	60	82	sm	a
27	Kargı Plateau	4	4	58	74	sm	a
28	Kırkdilim village	0	2	60	82	sm	a
29	8 km E of Kızılırmak	1	0	54	74	sm	a
30	Laçın town, Çorum	0	3	60	78	sm	a
31	Alören village, Çorum	0	2	60	82	sm	a
32	5 km N of Ortaköy, Çorum	1	0	60	78	sm	a
33	16 km E of Osmancık	0	2	60	78	sm	a
34	14 km E of Osmancık	1	1	60	78	sm	a
35	Çadırhöyük village, Çorum	1	1	54	74	sm	a
36	Sungurlu-Boğazkale road fork	0	1	54	74	sm	a
37	2 km N of Uğurludağ	1	0	54	74	sm	a
38	Öbektaş village, Osmancık	1	1	60	78	sm	a
		39	52				

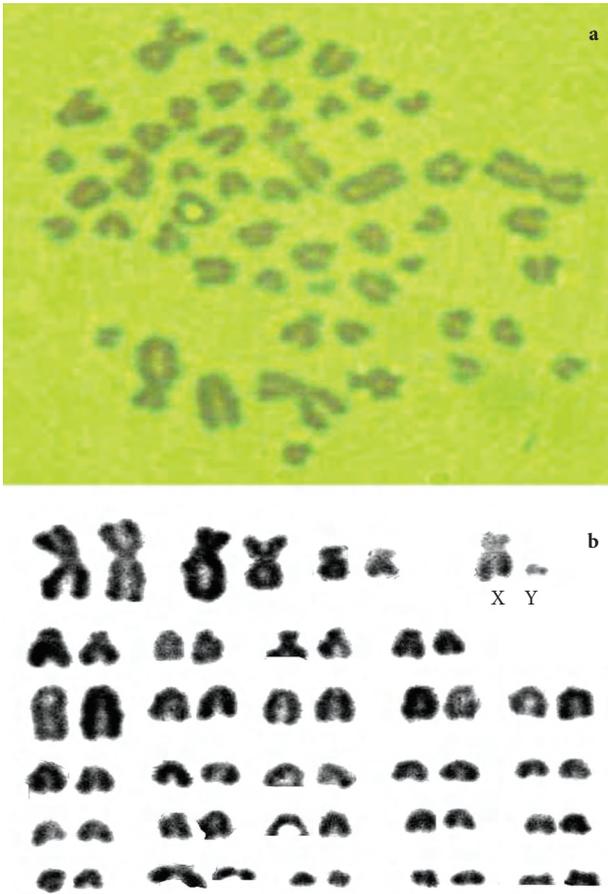


Figure 3. The metaphase plate (a) and karyotype of a male from Ilgaz Mountain in Çankırı (4787 male,  $2n = 56$ ;  $NF = 72$ ;  $NFa = 68$ ).

19, 20, 30, 32-34, and 38 in the Table and Figure 1, were  $2n = 60$ ;  $NF = 78$ ;  $NFa = 74$ , the X chromosome was a middle-sized submetacentric, and the Y chromosome was small subtelocentric. The autosomal set contained 8 pairs of biarmed chromosomes and 21 pairs of acrocentric chromosomes (Table, Figure 5).

The  $2n = 60$ ,  $NF = 82$  population: Çorum-Ortaköy road, Kırkdilim, Alören, Osmançık, İskilip, 34 km north of İskilip were  $2n = 60$ ;  $NF = 82$ , X chromosome was a middle-sized submetacentric, and the Y chromosome was small subtelocentric. The autosomal set contained 10 pairs of biarmed chromosomes and 18 pairs of acrocentric chromosomes (Table, Figure 6).

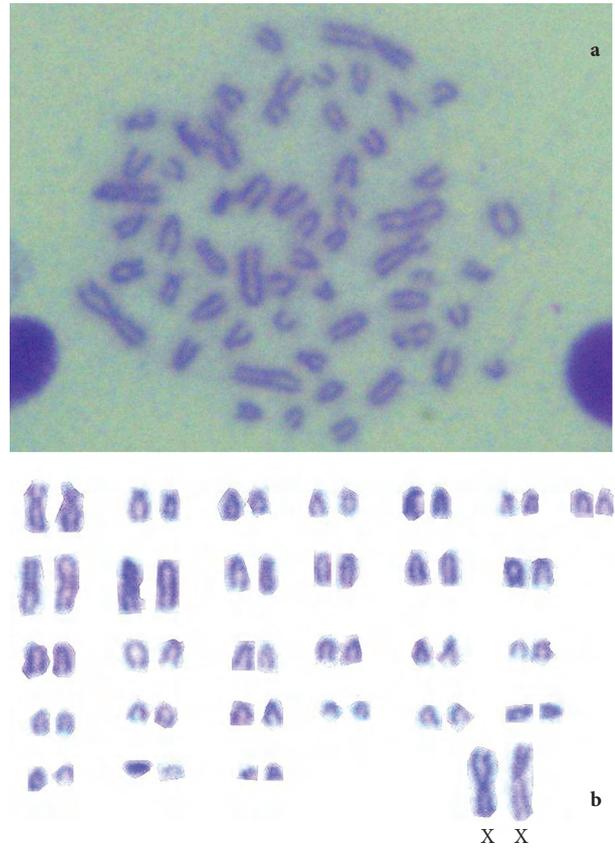


Figure 4. The metaphase plate (a) and karyotype of a female from Kargı plateau in Çorum (5143 female,  $2n = 58N$ ,  $NF = 74$ ).

## Discussion

The additional distribution records for  $2n = 54C$  form from central Anatolia (Yozgat, Nevşehir and Kırşehir) filled the gap in its distribution in the Kızılırmak basin. The distribution records for  $2n = 54C$  and 60 populations have shown that  $2n = 54C$  form in central Anatolia is surrounded by  $2n = 60$  form (see Table 1 in Sözen et al., 2006a for recorded localities).

The  $2n = 56N$  form of *N. nehringi* was formerly recorded from Aşağıçiftlik and Safranbolu (Sözen, 2004), Daday, Kastamonu and Tosya (Sözen et al., 2006a, 2006b). Samples from Ilgaz Mountain expanded the distribution of this form eastward.

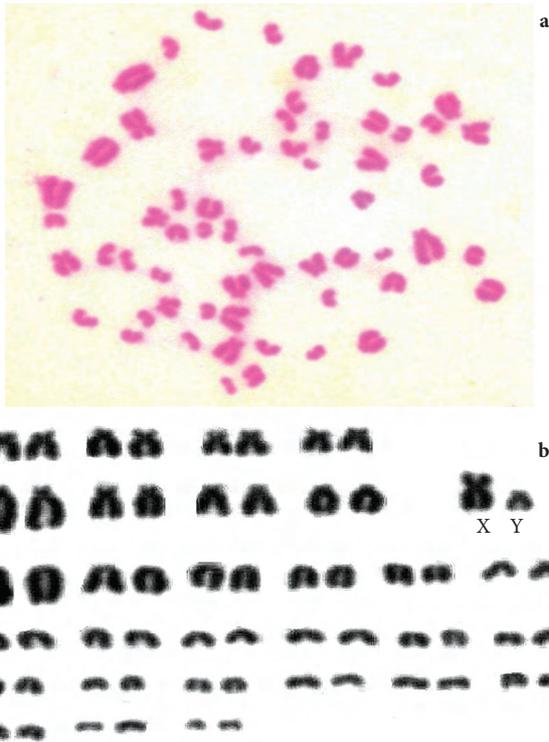


Figure 5. The metaphase plate (a) and karyotype of a male from Harmançık village in Çankırı (5060 male,  $2n = 60$  NF = 78).

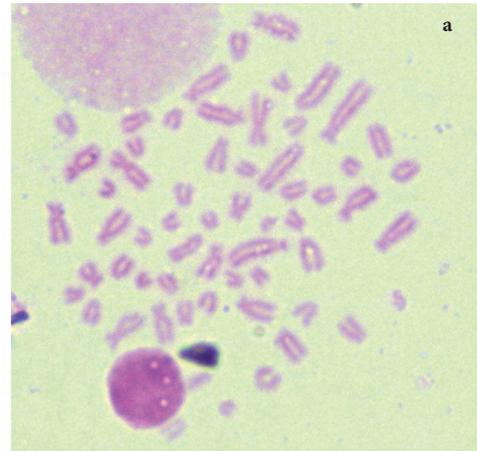


Figure 6. The metaphase plate (a) and karyotype of a male from İskilip in Çorum (5108 female,  $2n = 60$  NF = 82).

The  $2n = 58N$  karyotypic form was previously recorded from Taşköprü (Sözen et al., 2006a) and from Sarıkavak (Sözen, 2004) in northern Turkey. The NF value of the population from Kargı was identical to the Taşköprü population. This result showed that the distribution of  $2n = 58N$  form extended from east of Kastamonu, up to the northern part of Çorum province.

The karyotypes of most populations in central Anatolia were  $2n = 60$ , and the NF value is more diverse in comparison to the  $2n$  value, varying between 72 and 84 for the superspecies *Nannospalax nehringi* (Sözen et al., 2006a, 2006b; Ivanitskaya et al., 2008 and references therein). The diploid karyotype of the Çorum and Çankırı populations is similar on the basis of the  $2n$  value to those reported from most parts of central Anatolia, but there are some

differences in NF values among these populations. The NF values of the Alören village (Çorum), Kırkdilim village, İskilip 34 km N, Gököy village, and Ortaköy were NF = 82, other localities of  $2n = 60$  in Table were NF = 78, their diploid chromosome number is different: 2 submetacentric chromosome pairs caused this difference in NF value. These 2 forms are located close to each other in northern Anatolia (Figure 1), and this common chromosome pair may reflect phylogenetic relationships among them.

This study filled the gaps in distribution of blind mole rat chromosomal forms around Çankırı and Çorum provinces. The distribution areas of  $2n = 54C$ ,  $56N$ ,  $58N$ , and  $60$  forms in the area were brought to light. It is possible that most of the chromosomal forms in Turkey represent different biological

species (Nevo et al., 1994; 1995; Sözen et al., 1999; 2006a, 2006b; Sözen, 2004; Ivanitskaya et al., 2008). Future studies on chromosomal forms may solve such taxonomical problems by using more detailed methods, such as chromosome banding, genetic, molecular, behavioral, and physiological approaches.

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