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Research article

New data on the distribution of cytotypes in the genus *Nannospalax* (Rodentia: Spalacidae) in Turkey

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Abstract: Karyotypes of 131 specimens of blind mole rats from 43 localities in Turkey were analyzed. Examined populations of *N. leucodon* that merely lives in Thrace had 2n = 56 diploid chromosome number with two different NF values (NF = 76 and NF = 78). The karyotype 2n = 38 and NF = 74 was only determined for Western Anatolia and Gökçeada populations of the species *N. xanthodon*. Samples collected from all localities in where *N. tuncelicus* is distributed were karyologically examined and diploid chromosome number of this species found as 2n = 54 and NF = 74. Diploid chromosomal set for populations belonging to the subspecies *N. nehringi nehringi* that is found in Eastern Anatolia was identified to be 2n = 48 and NF = 72. The karyotypes of 2n = 48 and NF = 74, 2n = 52 and NF = 74, 2n = 52 and NF = 76 and 2n = 56 and NF = 72 were detected for blind mole rat samples obtained from distribution area of *N. ehrenbergi*. As to Central Anatolia *N. xanthodon* samples, four different karyotype values, 2n = 54 and NF = 74, 2n = 58 and NF = 73, 2n = 58 and NF = 72 and 2n = 60 and NF = 74, were found. A new karyotype was determined as 2n = 54 and NF = 70 in specimens from Adana (Saimbeyli, İmammoğlu and Karaisalı) as a new cytotype for *N. ehrenbergi* in Turkey. Thus, distribution areas of cytotypes determined for Turkish mole rats were identified by adding new karyological findings in this study.

Keywords: *Nannospalax*, karyotype, cytotype, Turkey.

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Introduction

Nannospalax, which includes more than fifty cytotypes that are differentiated in terms of diploid chromosome number and other karyotype characters, is a karyologically extreme variable genus among rodents (Topachevskii, 1969; Savic and Nevo, 1990; Arslan et al., 2016). Representatives of this genus are distributed in Europe, Asia Minor, Armenia, Syria, Palestine, Iraq, Israel, Jordan and Northeast Africa (Wilson and Reeder, 2005). The most karyological diversity within this genus has been seen in Turkish mole rats that are represented by three species, *N. xanthodon*, *N. ehrenbergi* and *N. leucodon* (Kryštufek and Vohralík, 2009).

Nannospalax xanthodon, which is almost found in the most parts of Anatolia, is a quite intresting species in the sense that this species includes thirteen different cytotypes (2n = 36, 38, 40, 44, 46, 48, 50, 52, 54, 56, 58, 60 and 62) and some of them (2n = 36, 38 and 40) are endemic (Sözen et al., 1999; Kryštufek and Vohralík, 2009; Arslan et al., 2016). Although it shows the same value in terms of diploid chromosome number, when karyotypes with different chromosomal morphology are also evaluated, the number of endemic cytotypes in Turkey reaches very high values (Ivaniskaya et al., 2008; Matur et al., 2011; Arslan and Zima, 2015). The differences in the karyotype of Anatolian mole rats have been defined both in morphologically similar sibling species and in even different populations of a species (Arslan et al., 2011; Arslan et al., 2013; Sözen et al., 2013; Matur et al., 2013). Currently, there are still serious problems related to the naming of the intraspesific karyotype races or cytotypes. Taxonomical uncertainty found in Anatolian mole rats

constitutively arises from lack of data. Description of many species is generally based on information obtained from limited numbers of morphologic features whose genetic backgrounds are not known (Topachevskii, 1969). Detailed morphological studies on Anatolian mole rats that comprise large samples have also been performed; however, diploid chromosome numbers of examined populations have not been taken notice in those studies (Topachevskii, 1969; Kıvanç, 1988). It has been suggested by recent molecular studies that each of chromosomal races has genetic differences at the species level (Nevo et al., 1995; Arslan et al., 2010; Kandemir et al., 2012). Nevertheless, the approach of "cytotype equal species" has been refused by other current studies (Kryštufek et al., 2012; Matur et al., 2019) and that only some cytotypes (2n = 36, 40 and 52) have differences at species level are admitted (Kankılıç et al., 2014a, b).

At present, taxonomic status of numerous taxa which have been defined at species and subspecies level from Anatolia are unresolved. Anatolian mole rats have been classified beneath two species (*N. xanthodon* and *N. ehrenbergi*) and previously defined five species (*N. nehringi, N. labaumei, N. tuncelicus, N. munzuri and N. vasvarii*) heve been considered to be synonyms of *N. xanthodon* by Kryštufek and Vohralík (2009). According to genetic and morphologic examinations, Kankılıç et al. (2014a, b) have contrarily suggested that mole rats in Central Anatolia (*N. labaumei*), and Eastern Anatolia (*N. nehringi*) are not synonyms of *N. xanthodon* and these species should be evaluated as distinct species. Taxonomic status of defined morphospecies from Anatolia and which cytotype are included by these morphospecies are still unaccurate. Purpose of this study is to reveal ultimate distribution borders of cytotypes in Turkey along with new karyological records and to determine relations between known cytotypes and previously described species-subspecies from Turkey.

Material and Methods

A total of 131 (53♂, 78♀) blind mole rat specimens from 43 localities in Anatolia were investigated (Table 1 and Figure 1). Conventional stained chromosomes of specimens were examined with respect to karyological characteristics by using fresh bone marrow obtained from colchicine applied animals and following standard procedures (Ford and Hamerton, 1956). A total of 10-20 slides were prepared for a few animals of each species, and at least 20 well-spread metaphase plates were evaluated. All mitotic preparations were dried in flame and then stained using Giemsa. The stain was prepared by mixing 3 ml of Giemsa with 50 ml phosphate buffer. The preparations were photographed with an Olympus CX31 stereo binocular microscope. Chromosome numbers were determined by counting of well spreading ones for each animal. The most frequent observed chromosome counts were regarded as the valid number. The diploid number of chromosomes (2n), the total numbers of chromosomal arms (NF) and the numbers of autosomal arms (NFa) as well as the X and the Y chromosomes were determined. All chromosomes were arranged from bigger to smaller and considered to be metacentrics, submetacentrics and acrocentrics according to their centromere positions consistent with Levan et al. (1964). Skins, skulls and karyotype preparations of all examined specimens were deposited at the Niğde Ömer Halisdemir University.

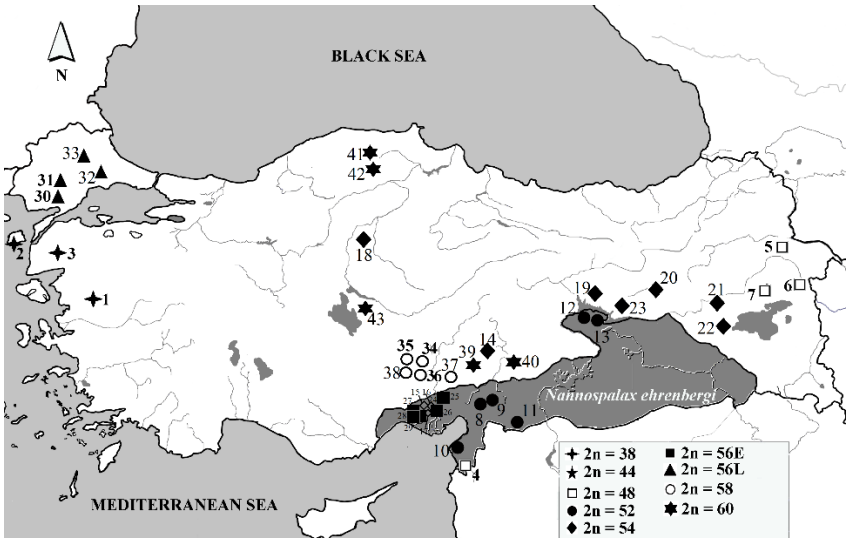

Figure 1. Map of the geographical distribution of the cytotypes studied, the numbers correspond to localities given in Table 1.

Table 1. Localities, sample size (M: males, F: females), diploid chromosome numbers (2n), and chromosomal arm numbers (NF), acrosenric chromosome numbers (a), biarmed chromosome numbers (m / sm / st) of animals examined (MN: Map number in Figure 1).

MN	Localities	Coordinates	M	F	2n	NF	a	m/sm/st	X	Y
1	Manisa (Akhisar - Kırkağaç)	39°05′59″ N / 27°42′38″ E	1	1	38	74	1	17	sm	-
2	Çanakkale (Gökçeada - Lazkoyu)	40°06′19″ N / 25°47′04″ E	1	3	38	74	1	17	sm	-
3	Çanakkale (Ezine - Bayramiç)	39°48′58″ N / 26°34′38″ E	-	1	38	74	1	17	sm	-
4	Hatay (Şenköy - Çatbaşı Place)	36°01′26″ N / 36°10′38″ E	3	3	48	74	12	11	m	a
5	Ağrı (Taşlıçay)	39°38′44″ N / 43°22′11″ E	2	3	48	72	11	12	sm	a
6	Van (Çaldıran - Gönderme)	39°05′15″ N / 43°48′24″ E	-	1	48	72	11	12	sm	-
7	Van (Kocapınar)	39°05′53″ N / 43°13′23″ E	1	2	48	72	11	12	sm	a
8	Osmaniye (Bahçe - Budacık)	37°10′38″ N / 36°30′40″ E	2	2	52	74	15	10	sm	a
9	Osmaniye (Düziçi)	37°15′38″ N / 36°26′40″ E	-	1	52	74	15	10	sm	-
10	Hatay (İskenderun - Arsuz)	36°25′31″ N / 35°55′29″ E	-	3	52	74	15	10	sm	-
11	Kilis (10 km East)	36°42′33″ N / 37°11′12″ E	1	-	52	74	15	10	sm	a
12	Elazığ (Bağdere)	38°36′30″ N / 39°02′48″ E	3	2	52	76	14	11	sm	a
13	Elazığ (Sivrice)	38°26′50″ N / 39°17′30″ E	-	1	52	76	14	11	sm	-
14	Adana (Tufanbeyli - Gezbeli Pass)	38°12′05″ N / 36°03′23″ E	-	3	54	74	17	9	sm	-
15	Adana (Saimbeyli)	37°57′02″ N / 36°04′41″ E	1	1	54	70	19	7	sm	a
16	Adana (Karaisali)	37°15′58″ N / 35°04′24″ E	1	1	54	70	19	7	sm	a
17	Adana (İmammoğlu)	37°17′01″ N / 35°41′33″ E	1	1	54	70	19	7	sm	a
18	Kırıkkale (Sulakyurt)	40°09′06″ N / 33°44′14″ E	1	3	54	74	17	9	sm	a
19	Tunceli (Pertek - Elmakaşı)	38°57′48″ N / 39°21′49″ E	-	1	54	74	17	9	sm	-
20	Bingöl (Yolçatı)	38°56′31″ N / 40°17′44″ E	-	5	54	74	17	9	sm	-
21	Muş (Bozbulut)	38°51′27″ N / 41°26′46″ E	1	5	54	74	17	9	sm	a
22	Bitlis (Rahva)	38°29′02″ N / 42°10′16″ E	1	1	54	74	17	9	sm	a
23	Elazığ (Karabörk)	38°49′10″ N / 39°48′56″ E	-	1	54	74	17	9	sm	-
24	Adana (Yüreğir)	37°01′13″ N / 35°21′33″ E	1	1	56	72	7	20	sm	a
25	Adana (Şeyhmurat)	36°52′01″ N / 35°25′39″ E	-	1	56	72	7	20	sm	-
26	Adana (Çukurova Uni. Campus)	37°02′55″ N / 35°21′02″ E	1	-	56	72	7	20	sm	a
27	Mersin (Tarsus - Mahmutağa)	36°56′43″ N / 34°57′50″ E	3	2	56	72	7	20	sm	a
28	Mersin (Tarsus - Konaklar)	36°56′49″ N / 34°59′36″ E	2	3	56	72	7	20	sm	a
29	Mersin (Tarsus - İncirlikuyu)	36°56′59″ N / 34°51′53″ E	1	2	56	72	7	20	sm	a
30	Tekirdağ (Malkara - Erenler)	40°54′23″ N / 26°53′22″ E	-	3	56	76	9	18	sm	-
31	Tekirdağ (Hayrabolu - Yörükler)	41°08′20″ N / 27°14′34″ E	3	1	56	76	9	18	sm	a
32	Tekirdağ (Çorlu)	41°12′07″ N / 27°48′21″ E	2	-	56	76	9	18	sm	a
33	Kırklareli (Pınarhisar - Evciler)	41°43′04″ N / 27°34′53″ E	3	1	56	78	10	17	sm	a
34	Niğde (Çamardı)	37°47′43″ N / 35°00′10″ E	1	1	58	73	22	6	m	-
35	Niğde (Bor)	37°55′15″ N / 34°35′08″ E	-	1	58	73	21+1	6+1	m	-
36	Adana (Pozantı - Alpu)	37°28′23″ N / 34°52′46″ E	1	1	58	72	22	6	m	-
37	Adana (Aladağlar)	37°33′39″ N / 35°22′13″ E	1	1	58	72	22	6	m	a
38	Niğde (Ulukışla)	37°32′47″ N / 34°30′55″ E	8	9	58	72	22	6	m	-
39	Adana (Feke)	37°48′52″ N / 35°55′19″ E	1	2	60	74	23	6	sm	a
40	Maraş (Göksun - Gölpınar)	37°58′16″ N / 36°29′53″ E	2	1	60	74	23	6	sm	a
41	Kastamonu (Küre - Akmescit)	41°24′52″ N / 33°49′05″ E	2	-	60	74	23	6	sm	a
42	Kastamonu (Küre - Devrekani)	41°47′20″ N / 33°51′03″ E	-	2	60	74	23	6	sm	-
43	Aksaray (Güzelyurt)	38°16′57″ N / 34°21′40″ E	1	1	60	74	23	6	sm	a

Results

Karyotype analyses belonging to five different morphospecies of mole rats (*N. xanthodon*, *N. ehrenbergi*, *N. tuncelicus*, *N. leucodon* and *N. cilicicus*) were performed in this study and eight different diploid chromose values (2n = 38, 48, 52, 54, 56, 58, and 60) were determined for samples from 43 localities (Table 1).

Populations with karyotype of 2n = 38: After karyological examination of *N. xanthodon* populations from Manisa (Loc 1: Akhisar - Kırkağaç) and Çanakkale (Loc 2: Gökçeada - Lazkoyu and Loc 3: Ezine - Bayramiç), 2n = 38, NF = 74 and NFa = 70 karyotype values were obtained. Of the 18 pair autosomal chromosomes, 17 pairs were bi-armed, while 1 pair

autosomal chromosome was an acrocentric and the X chromosome was a large-size metacentric (Figure 2A).

Populations with karyotype of 2n = 48: This diploid chromosome set was observed in the populations of two different species. *Nannospalax nehringi* populations from Ağrı (Loc 5: Taşlıçay) and Van (Loc 6: Çaldıran - Gönderme and Loc 7: Kocapınar) had 2n = 48, NF = 72 and NFa = 68 karyotype values. Autosomal set included 11 pairs of bi-armed and 12 pairs of acrocentric chromosomes. The X chromosome was a submetacentric (Figure 2B). Diploid chromosomal set were formed by 2n = 48, NF = 74 and NFa = 70 karyotype values in the populations of *N. ehrenbergi* from Hatay (Loc 4: Şenköy - Çatbaşı). The autosomal set consisted of 12 pairs of bi-armed and 11 pairs of acrocentric chromosomes. Of the sexual chromosomes, the X chromosome was a metacentric whereas the Y chromosome was a small-size acrocentric (Figure 2C).

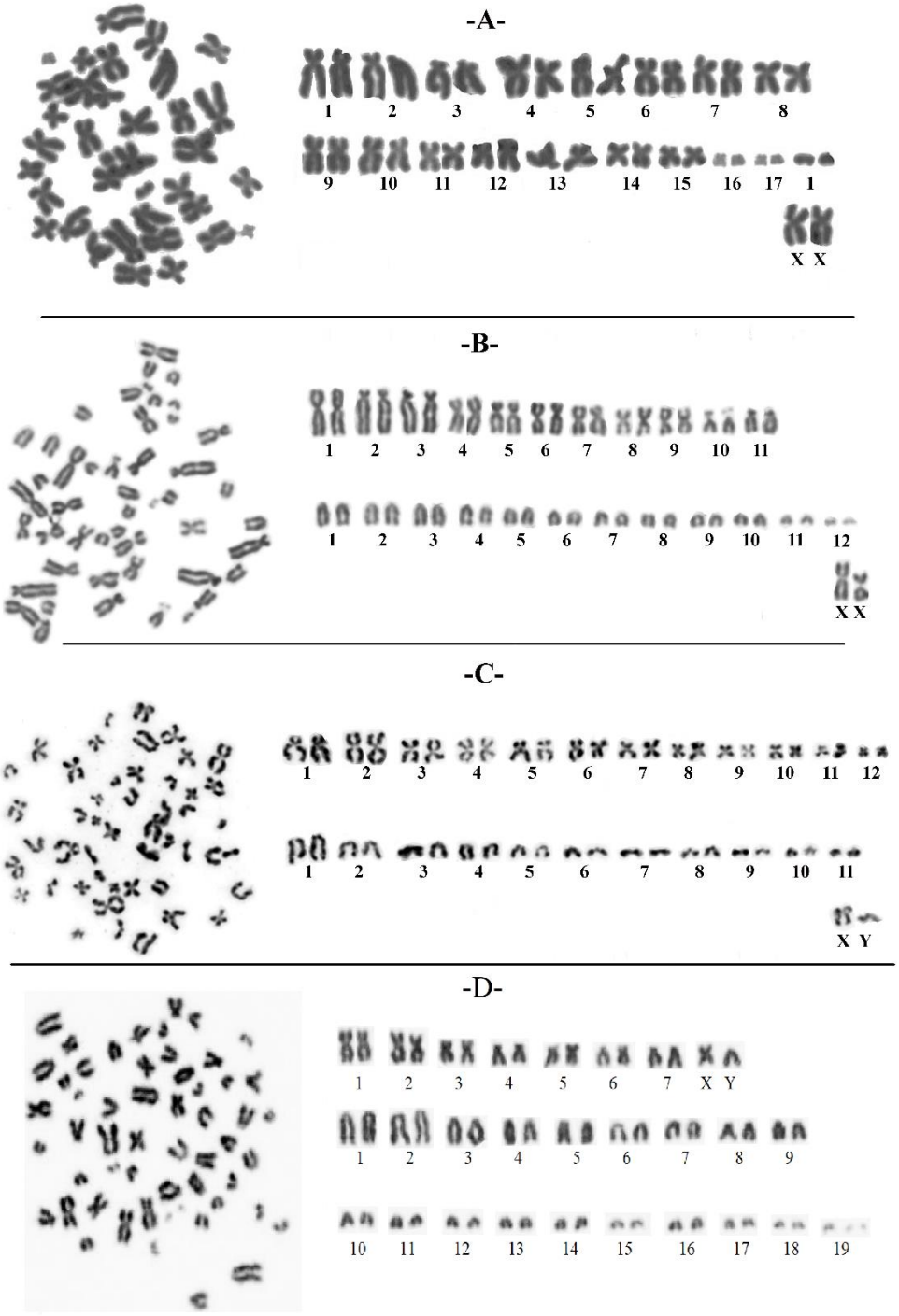

Figure 2. The karyotypes of specimens studied Turkey; A: 2n = 38 NF = 74 from Çanakkale (Loc 2: Gökçeada - Lazkoyu); B: 2n = 48 NF = 72 from Ağrı (Loc 6: Taşlıçay); C: 2n = 48 NF = 74 from Hatay (Loc 5: Şenköy - Çatbaşı); D: 2n = 54 NF = 70 from Adana (Loc16: Karaisalı)

Populations with karyotype of 2n = 52: The karyotype 2n = 52, NF = 74 and NFa = 70 were detected in the populations of *N. ehrenbergi* from Osmaniye (Loc 8: Bahçe - Budacık and Loc 9: Düziçi), Hatay (Loc 10: İskenderun - Arsuz) and Kilis (Loc 11: 10 km East). The X chromosome was a submetacentric and the Y chromosome was a large-size acrocentric. The autosomal set contained 10 pairs of bi-armed and 15 pairs of acrocentric chromosome (Figure 3A). Fundamental number of autosomal arms was different (2n = 52, NF = 76 and NFa = 72) in the populations of *N. ehrenbergi* from two different localities (Loc 12: Bağdere and Loc 13: Sivrice) in Elazığ. This karyotype included 11 pairs of bi-armed and 14 pairs of acrocentric chromosome. The X chromosome was a submetacentric (Figure 3B).

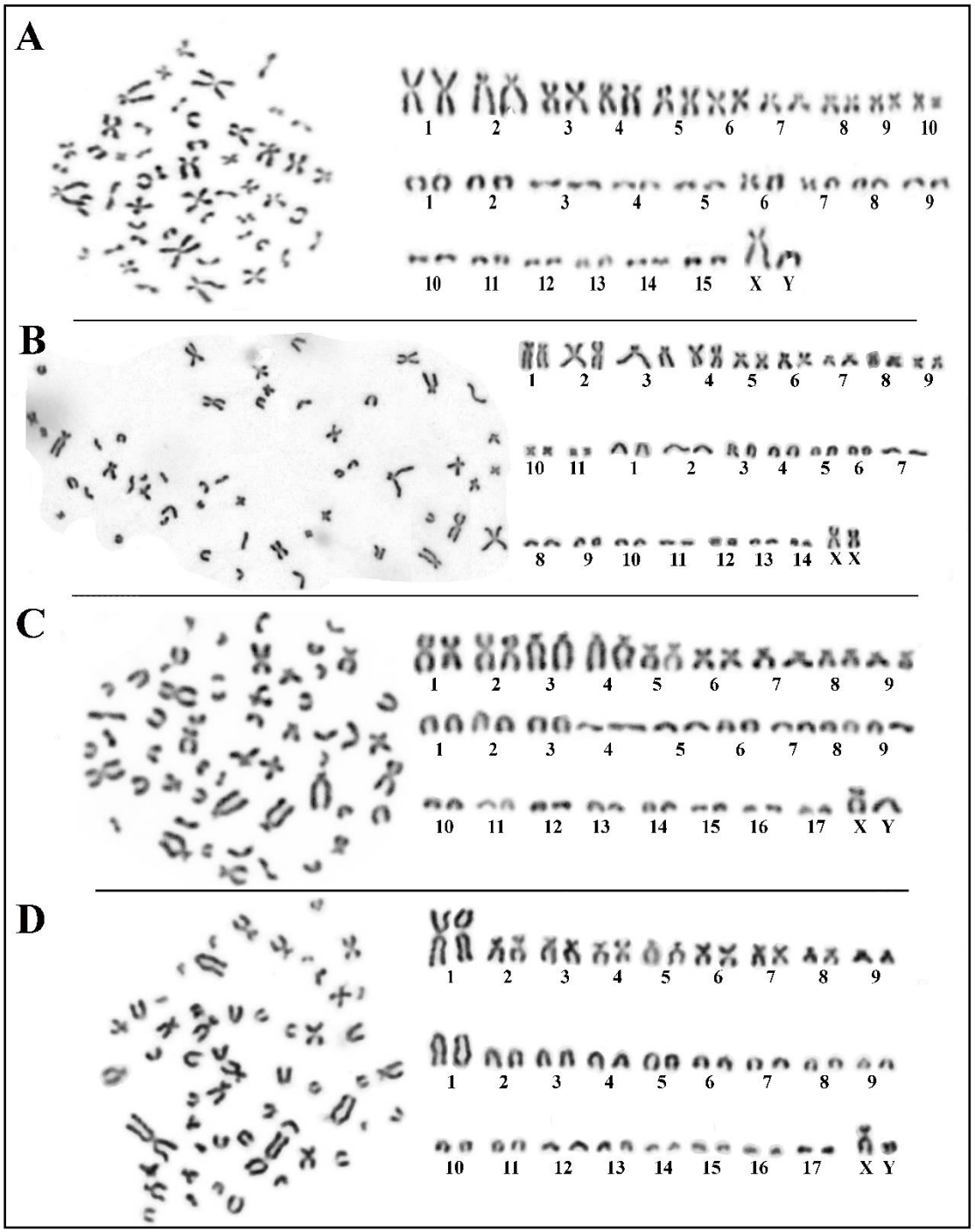

Figure 3. The karyotypes of specimens studied Turkey; A: 2n = 52 NF = 74 from Osmaniye (Loc 9: Bahçe - Budacık); B: 2n = 52 NF = 76 from Elazığ (Loc 13: Bağdere); C: 2n = 54 NF = 74 from Kırıkkale (Loc 16: Sulakyurt); D: 2n = 54 NF = 74 from Bitlis (Loc 20: Rahva).

Populations with karyotype of 2n = 54: This karyotype was observed in the populations of two different species. 2n = 54, NF = 74 and NFa = 70 karyotype values were determined for the populations of *N. cilicicus* from Adana (Loc 14: Tufanbeyli - Gezbeli Pass) and Kırıkkale (Loc 18: Sulakyurt). Chromosomes that create autosomal set involved 9 pairs of bi-armed and 17 pairs of acrocentric in this karyotype. Of the sex chromosomes, the X chromosome was a submetacentric while the Y chromosome was an acrocentric (Figure 3C). The same karyotype values were obtained from populations of *N. tuncelicus* **from** Tunceli (Loc 19: Pertek - Elmakaşı), Bingöl (Loc 20: Yolçatı), Muş (Loc 21: Bozbulut Willage), Bitlis (Loc 22: Rahva) and Elazığ (Loc 23: Karabük) (Figure 3D). For the first time for Adana province, the karyotype 2n = 54, NF = 70 and NFa = 66 from Karaisali (Loc 15), Saimbeyli (Loc 16) and İmamoğlu (Loc 17) determined for *N. ehrenbergi* (Figure 2D).

Populations with karyotype of 2n = 56: This diploid chromosome value was determined for both *N. ehrenbergi* and *N. leucodon*. Karyotype of *N. ehrenbergi* from Adana (Loc 24: Yüreğir; Loc 25: Şeyhmurat and Loc 26: Campus Area of Çukurova University) and Mersin (Loc 27: Tarsus - Mahmutağa; Loc 28: Tarsus - Konaklar and Loc 29: Tarsus - İncirlikuyu) was 2n = 56, NF = 72 and NFa = 68, which includes 7 pairs of bi-armed, 20 pairs of acrocentric, a submetacentric X and a small-size acrocentric Y chromosome (Figure 4A). *Nannospalax leucodon* populations from Tekirdağ (Loc 30: Malkara - Erenler; Loc 31: Hayrabolu - Yörükler and Loc 32: Tekirdağ - Çorlu) had 2n = 56, NF = 76 and NFa = 72 karyotype value. The autosomal set contained 9 pairs of bi-armed and 18 pairs of acrocentric along with the metacentric X chromosomes generated diploid chromosome set. Although Kırklareli (Loc 33: Pınarhisar - Evciler) population of this species had the same diploid chromosome number; however, fundamental number of autosomal arms (NF) showed differnces with the value of 78. The autosomal set contained 10 pairs of bi-armed and 17 pairs of acrocentric chromosome. The X chromosome was a submetacentric and the Y chromosome was a small-size acrocentric in this karyotype (Figure 4B).

Populations with karyotype of 2n = 58: This karyotype was observed as 2n = 58, NF = 73 and NFa = 69 in one population of *N. cilicicus* from Niğde (Loc 35: Bor). Of the autosomal chromosomes, although 6+1 pairs were a bi-armed, 21+1 pairs were an acrocentric. The 7th chromosome pair in the autosomal set was heteromorphic and consisted of a submetacentric and an acrocentric chromosome (Figure 4C). The other populations of the same species from Niğde (Loc 34: Çamardı and Loc 38: Ulukışla) and Adana (Loc 36: Pozantı - Alpu, Loc 37: Aladağ) had 2n = 58, NF = 72 and NFa = 68 karyotype values containing 6 pairs of bi-armed and 22 pairs of acrocentric autosomal chromosomes.

Populations with karyotype of 2n = 60: For *N. cilicicus*, 2n = 60, NF = 74 and NFa = 70 karyotype values found in the populations from Adana (Loc 39: Feke), Maraş (Loc 40: Göksun - Gölpınar), Kastamonu (Loc 41: Küre - Akmescit; Loc 42: Küre - Devrekani) and Aksaray (Loc 43: Güzelyurt). In addition to submetacentric X chromosome and a small-size acrocentric Y chromosome, 6 pairs of bi-armed chromosome and 23 pairs of acrocentric chromosome was existent in the autosomal set (Figure 4D).

Discussion

Proper evaluation of dispersion and taxonomic status of species is a basic need to be addressed biological diversity in any geographic area. This case is a main need to start biodiversity management and conservation planning studies (Braby and Williams, 2016). Revelation of chromosomal structure in blind mole rat populations, for those there is no karyological information and geographical distribution of existent cytotypes in Turkey, will provide properly assessment of taxonomy of those rodents in further studies. When results of current and past studies considered all together, geographic distributions of cytotypes belonging to Turkish blind mole rats is as follows in the Figure 5 (reviwed in Kryštufek and Vohralík, 2009; Arslan et al., 2016).

Two subspecies; one is from near of İzmir, *Spalax typhlus xanthodon*, another is from Bornova, *Spalax monticola anatolicus*, were respectively described by Nordmann (1840) and Mehely (1909). Karyotype of topotype samples belonging to those subspecies from İzmir accompanied by samples from Balıkesir, Manisa, Çanakkale, Bursa, Gökçeada (Imbros) and Bozcaada (Tenedos) was determined as 2n = 38 and NF = 74 (Savic and Soldatovic, 1979; Nevo et al., 1995; Tez et al., 2002; Kankılıç et al., 2009, 2010; Sözen et al., 2013; Arslan et al., 2013). Revealed karyotype for Western Anatolia samples of *N. xanthodon* in present study is similar to previously described karyotypes in terms of both chromosome morphology and diploid chromosome value.

It is determined that mole rat populations with this karyotype value are distributed in the area as from İzmir to the west of Bursa, where is amenable to Aegean and Marmara Seas, and two Turkish islands, Gökçeada (Imbros) and Bozcaada (Tenedos).

Tunceli province is placed in upper Fırat basin and 70% of its territory consists of impenetrable mountains. Since these mountains create insurmountable boundaries, mole rats of Tunceli could not integrate with *N. nehringi* (2n = 48 and 50) distributed in the fertile subsidence field starting from Iğdır plain and extending to Erzincan plain. Extension of the Eastern Taurus Mountains separates northern side of Tunceli province from its southern side by lying in the direction of east to west. Mercan Mountains has led to the formation of *N. munzuri* Coşkun, 2004 (2n = 58) in the north and *N. tuncelicus* Coşkun 2004 (2n = 54) in the south by creating a barrier between the northwestern and southern of the province.

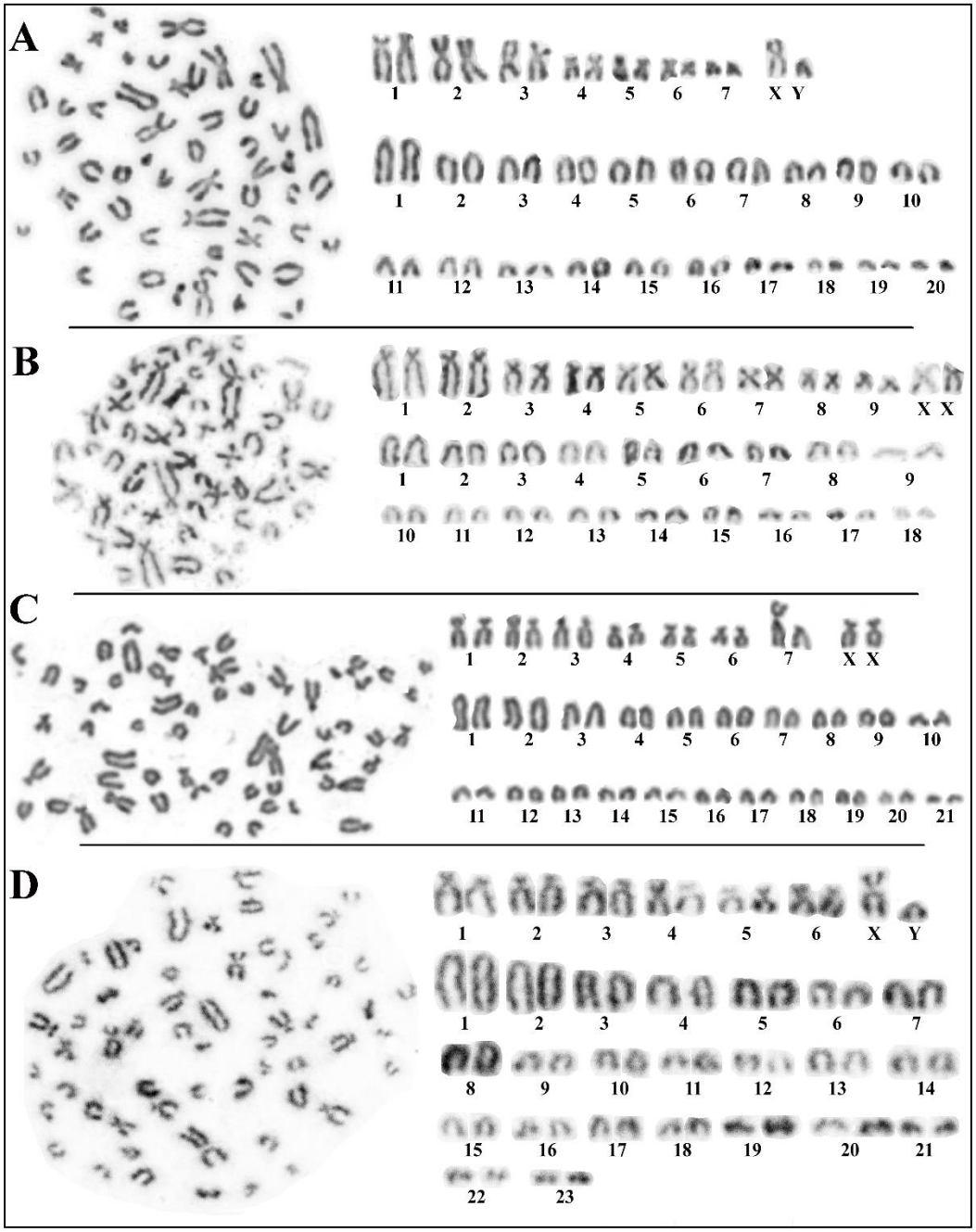

Figure 4. The karyotypes of specimens studied Turkey; A: 2n = 56 NF = 72 from Adana (Loc 22: Yüreğir); B: 2n = 56 NF = 76 from Tekirdağ (Loc 28: Malkara-Erenler); C: 2n = 58 NF = 73 from Niğde (Loc 33: Bor); D: 2n = 60 NF = 74 from Aksaray (Loc 40: Güzelyurt).

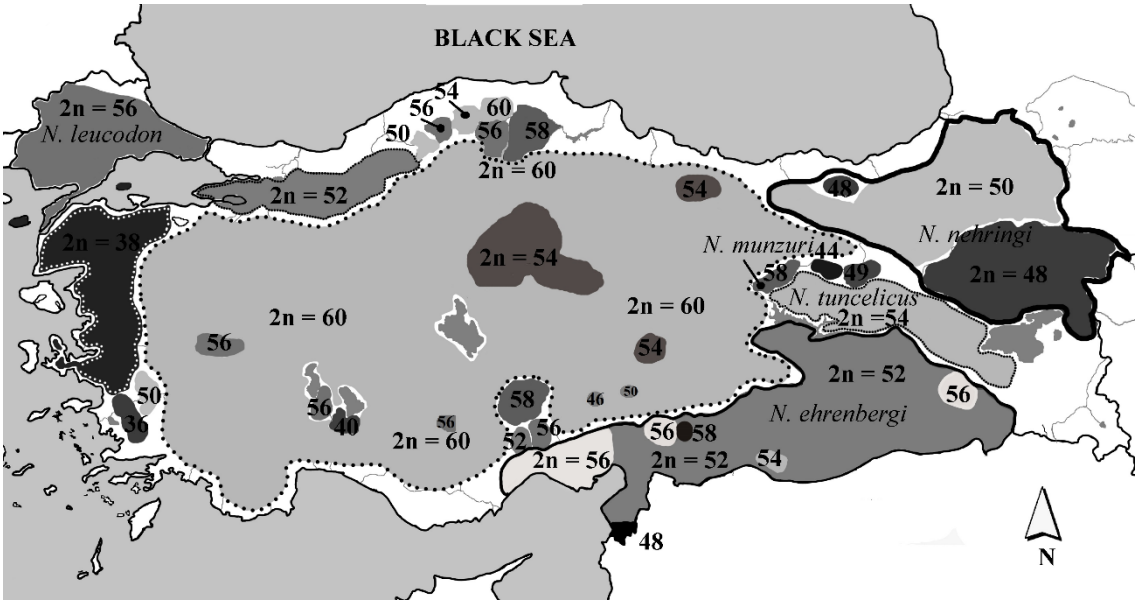

Figure 5. Turkish blind mole rat cytotypes and their geographical distribution.

Nannospalax nehringi was firstly identified by Satunin (1898) based on specimens collected from Kars (Gaziler - Kaskoparan) Ararat region and then Topachevskii (1969) was reported that this species was distributed in Caucaus and Eastern Turkey. Kryštufek and Vohralík (2009) has recently suggested that *N. nehringi* was the name that is synonym of *N. xanthodon*. This taxon is deemed to be a separate species in this study by reason of having some morphological and karyological features such as a spiny process behind of palatin, three roots in upper molars, spesific baculum structure and different number and shape of autosomal chromosomes (Kıvanç, 1988; Coşkun, 2003; Kankılıç et al., 2007, 2014b). A topographical formation known as "Anatolian Diagonal" that includes several mountain chains lying from northeastern part to the southern part of Turkey separates this species from Central Anatolian mole rats. Additionally, one another mountain chain, Souteastern Taurus Mountains prevents connection of this species with the *N. ehrenbergi* in the south. Therefore, this species is distributed in Caucaus geographical region, Eastern Anatolia where is home to the highest plateaus of Turkey and higher slopes in the south of Eastern Black Sea Mountains. Mehely (1909) have determined that two subspecies find in this region. Kıvanç (1988) have classified the specimens from Kars, Ardahan, Erzurum as *N. nehringi armeniacus* Mehely 1909, and those from Iğdır, Ağrı, Muş and Van as *N. nehringi nehringi* Satunin 1898 by morphological analysis of 1100 blind mole rat specimens. Topotype samples that collected from type localities of *N. n. armeniacus* (from Ardahan - Göle) and *N. n. nehringi* (from Igdır - Tuzluca - Kaskoparan) have respectively 2n = 50 and 48 diploid chromosome value (Kankılıç et al., 2007; Coşkun and Kaya, 2013). Results of karyotype studies including *N. nehringi* show interestingly paralelism with those of morphological study performed by Kıvanç (1998). Especially, distribution areas of subspecies determined by Kıvanç (1998) are fully cosistent with those of two cytotypes (2n = 50 and 48) detected by karyotype studies (Sözen et al., 2000). Therefore, it is the right approach to classify populations belonging to *N. nehringi* in Eastern Anatolia beneath the two subspecies with different chromosome number (*N. n. nehringi* - 2n = 48, *N. n. armeniacus* - 2n = 50) in accordance with the classification of Kıvanç (1998). NF = 68 value from Ağrı (Küpkıran, Taşlıçay, Patnos and Tutak), Van (Çaldıran), Iğdır (Tuzluca) and Erzurum (Hınıs) and NF = 72 value from Van (Erciş) and Muş (Malazgirt) of the cytotype 2n = 48 in the *N. n. nehringi* populations were previously reported (Coşkun, 2003; Coşkun et al., 2009; Coşkun et al., 2012 and Coşkun and Kaya, 2013). Karyotype for samples collected from Ağrı (Loc 6: Taşlıçay) and Van (Loc 7: Çaldıran - Gönderme and Loc 8: Kocapınar) is defined as 2n = 48, NF= 72, and NFa = 68 in present study. Thus, this karyotype is different from the first defined karyotype (2n = 48 and NF = 68) of populations from Van (Çaldıran) and Ağrı (Taşlıçay) by Coşkun (2003) due to including different fundamental number of chromosomal arms; however, it is equal to the karyotype revealed for Van ve Muş by the same researcher.

The first description of *N. ehrenbergi* based on four specimens whose gender was unknown was made by

Nehring (1898) from Yafa (Israel). Then, a new species namely *N. intermedius* was defined by the same author from İskenderun (Arsuz - Çengelköy) within the borders of Turkey. Also, Nehring (1898) identified another species (*N. kirgisorum*) from Ryn Peski in the steppes of western Kyrgyzia (now western Kazakhstan; Topachevskii, 1969) by examining only one specimen. However, furher studies admitted that type localty of holotype was incorrect and this holotype may be collected from Syria instead steppes of western Kyrgyzia (Mehely, 1913; Ellerman and Morrison-Scott, 1966; Harrison and Bates, 1991). Populations of *N. ehrenbergi* in Turkey were investigated as two separate subspecies (*N. e. intermedius* and *N. e. kirgisorum*) by Kıvanç (1988). According to Kıvanç (1988), *N. e. kirgisorum* lived in Urfa, which has tended to desertification, in where influence of Mediterranean climate decreases; while, *N. e. intermedius* found in other areas in Southeastern Anatolia apart from Urfa. *Nannospalax kirgisorum* is not a valid species or subspecies for Anatolian mole rats due to uncertainty in the type locality of its first description. For this reason, taxonomy of populations belonging to *N. ehrenbergi* should be revised by paying attention their karyological features. Five different cytotypes (2n = 48, 52, 54, 56 and 58) have been identified from Anatolia till now (Ivanitskaya et al., 1997; Coşkun, 2004b; Coşkun et al., 2006; Sözen et al., 2006b). The karyotype 2n = 48 and NF = 74 in *N. ehrenbergi* were reported from Hatay (Şenköy - Yayladağ) in a previous study (Coşkun et al., 2006). This karyotype is different from other cytotypes of *N. ehrenbergi* in terms of metacentric chromosome number in autosomal set, which may be characteristic cytotaxonomic marker for all representative of the species *N. ehrenbergi*. This cytotype find to be isolated populations in slopes of some mountains located in southern point of Hatay such as Yayladağ, Kel Mountain, Salcan Mountain, Araplar Mountain and Ayvacık Mountain of which height ranges between 450 and 1700 m, valleys and small plains among those mountains.

Two difeerent karyotypes for populations of *N. ehrenbergi*, which have the same diploid chromose number (2n = 52), but two different NF values, (74 and 76) were recorded in current study. NF = 74 value were detected in topotype samples of *N. e. intermedius* and in the populations of Osmaniye and Kilis; whereas, populations from Elazığ have NF = 76 value. These two karyotype separated by the presence of a pair of metacentric/acrocentric in autosomal set. This karyotype has been previously determined in numerous populations of *N. ehrenbergi* and it is the most common cytotype seen in distribution area of this species (Yüksel, 1984; Yüksel and Gülkaç, 1992; Nevo et al., 1994; Ivanitskaya et al., 1997; Coşkun, 1999, 2004b; Sözen et al., 2006b; Coşkun et al., 2006).

The karyotype 2n = 56 and NF = 72 have been given from Adana and Tarsus for *N. ehrenbergi* by most researchers (Coşkun et al., 2006; Ivanitskaya et al., 1997; Nevo et al., 1994; Sözen et al., 2006a). A specimen collected from 50 km east of Adana was described as *N. ehrenbergi* var. *ceyhanus* by Szunyoghy (1941). After karyological and morphological examination of topotype sample of *ceyhanus*, Coşkun et al. (2010) stated that karyotype of this form was 2n = 56 and NF = 72 and the structure known palatal spike or styloid process, which is seen in *N. ehrenbergi* was either uncertain or not presence in this form. Therefore, Coşkun et al. (2010) suggested that *ceyhanus* may be a putative biological species. When regarded the craniums of samples from Adana and Mersin having 2n = 56 and NF = 72 karyotype value, it was determined that the mentioned morphological structure was not observed especially in Adana and some of Tarsus samples, on the contrary, 5 Tarsus samples had indistinct process. However, differences observed in these populations may not adequate to evaluate them as separate taxa. Apart from these records, 2n = 54 NF = 70 NFa =66 karyotype was determined for the first time in this study, within the distribution limits of *N. ehrenbergi* from Adana (Saimbeyli, Karaisali and İmammoğlu).

Specimens from four localities in Thrace were karyotyped and the results showed that populations of Thrace are inhabited by two different forms (2n = 56 and NF = 76, NF = 78). Karyotypes of both forms are similar to previously described karyotypes from different localities in Thrace by Sözen et al. (2006a). When earlier karyotype records and current results for Thracian populations are considered together; it is seen that populations with NF = 76 value are distributed in the south of Thrace in which Marmara Sea has influence; however, populations with NF = 78 value find inner of Thrace which is more arid.

The karyotype 2n = 58 has been reported by numerous authors from Konya (Ereğli), Niğde (Ulukışla and Madenköy), Adana (Pozantı), Kastamonu (Taşköprü), Ankara (Sarıkavak), Erzincan (Kemaliye) and Tunceli

(Ovacık) (reviwed in Arslan & Zima 2013). The 2n = 58 cytotype has several different NF values (NF = 66, 68, 72, 74, 75 and 78) in its distribution area (see Table in Arslan and Zima, 2013). New localities are determined for this cytotype in present study. When these localities are taken into account, it is seen that this cytotype live in the perifer of distribution areas of 2n = 60. Although karyotype of populations from Niğde (Çamardı and Ulukışla) and Adana (Pozantı) show similarity to the previous karyotypes (NF = 72), a difference for described karyotype from Niğde (Bor) cytotype is that 7th pair of autosomal set is heteromorphic (NF = 73).

The cytotype 2n = 60 has the broadest distribution in Anatolia. This cytotype includes 7 different chromosomal forms whose NF values range from 72 and 84 (reviwed in Sözen et al., 2013). It is revealed by current study that samples from Adana (Feke), Maraş (Göksun - Gölpınar), Kastamonu (Küre) and Aksaray (Güzelyurt) have 2n = 60 and NF = 74 karyotype value. This result is the same with previous ones obtained from Niğde, Aksaray, Malatya, Kastamonu, Antalya, Maraş, Burdur, Manisa and Konya (Nevo et al., 1994; Ivanitskaya et al., 1997; Sözen et al., 1999; Sözen et al., 2000; Sözen, 2004; Sözen et al., 2006a, b; Kankılıç et al., 2009; Arslan et al., 2011; Sözen et al., 2013). This karyotype is described for the first time for the populations from Aksaray (Güzelyurt).

Geographical alterations lasting for thousands years, climatic and biotic features which show differences even in close areas of Turkey make mole rat populations, which are well adopted to subterranean life and thus having restricted mobility, into isolated populations in their own localities. This situation has led to decrease of gene flow among mole rat populations and rapid fixation of new chromosomal mutations in cases where geographic isolations (Wahrman, 1969a, b). Existence of fourty different chromosomal forms has been determined by the studies performed on karyological structures of Turkish mole rats in last thirty years (Kryštufek and Vohralík, 2009). New cytotypes has been revealed for Turkish mole rats by karyological studies as it is in this study and thus distribution borders of present cytotypes become clear. Chromosomal studies are considerably useful for descriptions of sibling species like morphologically similar mole rats. To reveal distribution borders of geographically isolated cytotypes and populations belonging to these cytotypes within a nominal species may establish a ground for taxonomic and phylogenetic studies employing different molecular markers in further analysis.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Ethical approval

All applicable national guidelines for the care and use of animals were followed.

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References

Arslan, E., Gülbahçe, E., Arıkoğlu, H., Arslan, A., Bužan, E. V., & Kryštufek, B. (2010). Mitochondrial divergence between three cytotypes of the Anatolian mole rat *Nannospalax xanthodon* (Mammalia: Rodentia). *Zoology in the Middle East,* 50, 27-34.

Arslan, A., Akan, Ş., & Zima, J. (2011). Variation in C-heterochromatin and NOR distribution among chromosomal races of mole rats (Spalacidae) from Central Anatolia, Turkey. *Mammalian Biology,* 76, 28-35.

Arslan, A., Arsoy, A., & Zima, J. (2013). The chromosome banding pattern in two cytotypes (2n = 36 and 38) of blind mole rats from Turkey (Mammalia: Spalacidae). *Zoology in the Middle East,* 59 (2), 95-100.

Arslan, A., & Zima, J. (2013). The banded karyotype of the 2n = 58 chromosomal race of mole rats from Erzincan, Turkey. *Folia Zoologica,* 62, 19-23.

Arslan, A., & Zima, J. (2015). Chromosome banding pattern retrieves an independent origin of 2n= 50 chromosome populations of Nannospalax xanthodon from Turkey. *Mammalian Biology*, 80(5), 440-445.

Arslan, A., Kryštufek, B., Matur, F., & Zima, J. (2016). Review of chromosome races inblind mole rats (*Spalax* and *Nannospalax*). *Folia Zoologica,* 65, 249-301.

Braby, M. F. & Williams, M. R. (2016). Biosystematics and conservation biology: critical scientific disciplines forthe management of insect biological diversity. *Australian Entomological*, 55, 1-17.

Coşkun, Y. (1999). New karyotype of the mole rat *Nannospalax ehrenbergi* from Turkey. *Folia Zoologica,* 48, 313-314.

Coşkun, Y. (2003). A study on the morphology and karyology of *Nannospalax nehringi* (Satunin, 1898) (Rodentia: Spalacidae) from northeast Anatolia, Turkey. *Turkish Journal of Zoology,* 27, 171-176.

Coşkun, Y. (2004a). A new species of mole rat *Nannospalax munzuri* sp. n., and karyotype of *Nannospalax tuncelicus* (Coşkun. 1996) (Rodentia: Spalacidae) in eastern Anatolia. *Zoology in the Middle East,* 33, 153-162.

Coşkun, Y. (2004b). Morphological and karyological characteristics of *Nannospalax ehrenbergi* (Nehring, 1898) (Rodentia: Spalacidae) from Hatay province, Turkey. *Turkish Journal of Zoology,* 28, 205-212.

Coşkun, Y., Ulutürk, S., & Yürümez, G. (2006). Chromosomal diversity in mole-rats of the species *Nannospalax ehrenbergi* (Rodentia: Spalacidae) from South Anatolia, Turkey. *Mammalian Biology*, 71, 244-250.

Coşkun, Y., Kaya, A., & Yürümez, G. (2009). Chromosomal forms of the mole rat, *Nannospalax nehringi* (Satunin. 1898), from the Van Lake Basin in Eastern Turkey. *Zoology in the Middle East,* 48, 17-24.

Coşkun, Y., Nemeth, A., & Csorba, G. (2010). *Ceyhanus* is an available name for a distinct form of *Nannospalax* (superspecies *ehrenbergi*) (Rodentia: Spalacinae). *Mammalian Biology,* 75(5), 463-465.

Coşkun, Y., Kaya, A., Ulutürk, S., Yürümez, G., & Moradi, M. (2012). Karyotypes of the mole rats, genus *Nannospalax* (Palmer, 1903) (Spalacidae: Rodentia) populations in eastern Anatolia, Turkey. *International Journal of Agriculture and Biology (IJAB),* 8(2), 195-202.

Coşkun, Y., & Kaya, A. (2013). Karyological peculiarities of mole rats (*Nannospalax*) (Rodentia: Spalacidae) from Iğdır province. *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi,* 3(2), 119-124.

Ellerman, J. R., & Morrison-Scott T. C. S. (1966). Checklist of Palaearctic and Indian Mammals. 1758-1946, Second edition, *British Museum (Natural History*), London, pp: 1-810.

Ford, C. E., & Hamerton, J. L. (1956). A colchicine hypotonic citrate, squash for mammalian chromosomes. *Stain Tech,* 31, 247-251.

Harrison, D. L., & Bates, P. J. J. (1991). Mammals of Arabia, second edition. *Harr. Zool. Mus. Pub.*, pp: 1-353.

Ivanitskaya, E., Coşkun, Y., & Nevo, E. (1997). Banded karyotypes of mole rats (*Spalax*, Spalacidae, Rodentia) from Turkey. *Journal of Zoological Systematics and Evolutionary Research,* 35, 171-177.

Ivanitskaya, E., Sözen, M., Rashkovetsky, L., Matur, F., & Nevo, E. (2008). Discrimination of 2n= 60 *Spalax leucodon* cytotypes (Spalacidae, Rodentia) in Turkey by means of classical and molecular cytogenetic techniques. *Cytogenet Genome Res.* 122: 139-149.

Kandemir, I., Sözen, M., Matur, F., Martínková, N., Kankılıç, T., Çolak, F., Özkurt, Ş., & Çolak, E. (2012). Phylogeny of species and cytotypes of mole rats (Spalacidae) in Turkey inferred from mitochondrial cytochrome *b* sequences. *Folia Zoologica,* 61, 25-33.

Kankılıç, T., Çolak, R., Kankılıç, T., & Çolak, E. (2007). On the Morphology and Karyology of *Spalax leucodon armeniacus* Mehely, 1909, and *Spalax leucodon cilicicus* Mehely, 1909 (Mammalia: Rodentia) in Turkey. *Acta Zoologica Bulgarica,* 59(1), 41-46.

Kankılıç, T., Çolak, E., Kankılıç, T. (2009). Macro-Anatomical and karyological features of two blind mole rat subspecies (Rodentia: Spalacidae) from Turkey. *Anatomia Histologica Embryologica,* 38, 145-153.

Kankılıç, T., Kankılıç, T., Şeker, P. S., Çolak, R., Selvi, E., & Çolak, E. (2010). Contributions to the karyology and distribution areas of cytotypes of *Nannospalax leucodon* (Rodentia: Spalacidae) in Western Anatolia. *Acta Zoologica Bulgarica,* 62, 161-167.

Kankılıç, T., & Gürpınar, C. (2014a). Revised classification design of the Anatolian species of *Nannospalax* (Rodentia: Spalacidae) using a RFLP analysis. *Turkish Journal of Zoology,* 38, 67-78.

Kankılıç T., Kankılıç, T., Şeker, P. S., & Kıvanç, E. (2014b). Morphological and biometrical comparisons of the baculum in the genus *Nannospalax* Palmer, 1903 (Rodentia: Spalacidae) from Turkey with consideration of the taxonomic importance. *Turkish Journal of Zoology,* 38, 144-157.

Kıvanç, E. (1988). Geographic variations of Turkish *Spalax* (Mammalia: Rodentia). PhD, University of Ankara, Ankara, Turkey.

Kryštufek, B., & Vohralík, V. (2009). Mammals of Turkey and Cyprus. Rodentia II. Cricetinae, Muridae, Spalacidae, Calomyscidae, Capromyidae, Hystricidae, Castoridae. *Knjižnica Annales Majora, Koper.*

Kryštufek, B., Ivanitskaya, E., Arslan, A., Arslan, E., & Bužan, E. (2012). Evolutionary history of mole rats (genus *Nannospalax*) inferred from mitochondrial cytochrome *b* sequences. *Biological Journal of the Linnean Society,* 105, 446-455.

Levan, A., Fredga, K., & Sandberg, A. A. 1964: Nomenclature for centromeric position on chromosomes. *Hereditas,* 52**,** 201-220.

Matur, F., Çolak, F., Sevindik, M., & Sözen. M. (2011). Chromosome differentiation of four 2n= 50 chromosomal forms of Turkish mole rat, *Nannospalax nehringi*. *Zoological Science*, 28: 61-67.

Matur, F., Çolak, F., Ceylan, T., Sevindik, M., & Sözen, M. (2013). Chromosomal evolution of the genus *Nannospalax* (Palmer 1903) (Rodentia, Muridae) from western Turkey. *Turkish Journal of Zoology,* 37, 470-487.

Matur, F., Yanchukov., A, Çolak, F., & Sözen, M. (2019). Two major clades of blind mole rats (*Nannospalax* sp.) revealed by mtDNA and microsatellite genotyping in Western and Central Turkey. *Mammalian Biology*, 94: 38-47.

Méhely, L. (1909). Species generis *Spalax*. A földi kutyák fajai származás-és rendszertani tekintetben, [Species of the genus *Spalax*]. *Magyar Tudományos Akadémia, (in Hungarian)*.

Méhely, L. (1913). Birch mice of Hungary. *Mathematikai és Természettudományi Közlemények,* 32 (1), 1-45.

Nehring, A. (1898). Über mehrere neue *Spalax* Arten. *Schriften der Berlinischen Gesellschaft Naturforschender Freunde*, 171-183.

Nevo, E., Filippucci, M. G., Redi, C., Korol, A., & Beiles, A. (1994). Chromosomal speciation and adaptive radiation of mole rats in Asia Minor correlated with increased ecological stress. *Proceedings of the National Academy of Sciences,* 91, 8160-8164.

Nevo, E., Filippucci, M. G., Redi, C., Simson, S., Heth, G., & Beiles, A. (1995). Karyotype and genetic evolution in speciation of subterranean mole rats of the genus *Spalax* in Turkey. *Biological Journal of the Linnean Society,* 54, 203-229.

Nordmann, A. (1840). Observations sur la Faune Pontique, A. Demidoff Voyage dans la. *Russie Meridion* 3 (35).

Savić, I. R., & Soldatović, B. (1979). Contribution to the knowledge of the genus *Spalax (Microspalax)* karyotype from Asia Minor. *Arhiv Bioloških Nauka,* 31, 1-2.

Satunin, K. A., (1898). *Spalax nehringi* nov. ap. *Zoologischer Anzeiger,* 21, 314-315.

Savić, I., & Nevo, E. (1990). The Spalacidae. Evolutionary history, speciation and population biology. In: Nevo E. & Reig A.O. (eds.), Evolution of subterranean mammals at the organismal and molecular levels. *Alan R. Liss, New York*, 129-153.

Sözen, M., Çolak, E., Yiğit, N., Özkurt, Ş., & Verimli, R. (1999). Contributions to karyology and taxonomy of the genus *Spalax* Güldenstaedt, 1770 (Mammalia; Rodentia) in Turkey. *Mammalian Biology,* 64, 210-219.

Sözen, M., Çolak, E., & Yiğit, N. (2000). A study on karyotypic evolution of the genus *Spalax* Güldenstaedt, 1770 (Mammalia: Rodentia) in Turkey. *Israel Journal of Zoology,* 46, 239-242.

Sözen, M. (2004). A karyological study on subterranean mole rats of the Spalax leucodon Nordmann, 1840 superspecies in Turkey. *Mammalian Biology*, 69: 420-429.

Sözen, M., Matur, F., Çolak, E. Özkurt, Ş., & Karataş, A. (2006a). Karyological records and a new chromosomal form for *Spalax* (Mammalia: Rodentia) in Turkey. *Folia Zoologica,* 55, 247-256.

Sözen, M., Sevindik, M., & Matur, F. (2006b). Karyological and some morphological characteristics of *Spalax leucodon* Nordmann, 1840 (Mammalia: Rodentia) superspecies around Kastamonu province, Turkey. *Turkish Journal of Zoology,* 30, 205-219.

Sözen, M., Çolak, F., Sevindik, M., & Matur, F. (2013). Cytotypes of *Nannospalax xanthodon* (Satunin, 1898) (Rodentia: Spalacidae) from western Anatolia, *Turkish Journal of Zoology,* 37, 462-469.

Szunyoghy, J. (1941). Two new species of mole rats from Asia Minor. *Allattani Kozlemenyek (in Hungarian with German summary),* 38, 78-86.

Tez, C., Gündüz, İ., & Kefelioğlu, H. (2002). New data on the distribution of 2n = 38 *Spalax leucodon* Nordmann, 1840 cytotype in Turkey. *Israel Journal of Zoology,* 48, 155-159.

Topachevski, V. A. (1969). Fauna of the USSR: mammals. Mole rats, Spalacidae. *Smithsonian Institution and the National Science Foundation, Washington.*

Wahrman, J., Gotein, R., & Nevo, E. (1969a). Geographic Variation of Chromosomal Forms in *Spalax*, a Subterranean Mammal of Restricted Mobility. *Comp Mamm Cytogenet ed. K. Benirschke,* 30-48*, New York, Springer Verlag.*

Wahrman, J., Gotein, R., & Nevo, E. (1969b). Mole Rat *Spalax*: Evolutionary Significance of the Chromosome Variation. *Sciences,* 164: 82-84.

Wilson, D. E., & Reeder, D. M. (2005). Mammal species of the world: A taxonomic and geographic reference, 3rd ed. *The Johns Hopkins University Press, Baltimore*.

Yüksel, E. (1984). Cytogenetic study in *Spalax* (Rodentia; Spalacidae) from Turkey. *Comm C Biologie,* 2, 1-12.

Yüksel, E., & Gülkaç, M. D. (1992). On the karyotypes of in some populations of the subterranean mole rats in the lower Euphrates basin, Turkey. *Caryologia,* 45, 175-190.