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Research Article

A New Chromosomal Race and a Hybrid Form of *Nannospalax xanthodon* (Satunin, 1898) (Rodentia: Spalacidae) from Karaman, Turkey

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Abstract: A new race was determined in a population of mole rats *Nannospalax xanthodon* from Karaman. In addition, a hybrid with 2n = 59 chromosomes was detected between the populations of the Uğurlu race. The karyotype of the new race contained 58 chromosomes, including 11 biarmed pairs and 17 acrocentric pairs in the autosomal complement (NFa=78). The X chromosome was submetacentric (NF=82). The karyotype of a hybrid form contained 59 chromosomes, including two heteromorphic pairs in the autosomal complement (NFa=75). The X chromosome was submetacentric (NF=79). The dark centromeric C-bands were observed in some biarmed and acrocentric autosomes in both new race and hybrid form. C-heterochromatic short arms were found in some autosomal pairs in the only new race. The X chromosome possessed centromeric C-positive band in both new race and hybrid form. NORs were detected on the short arms of three pairs of autosomes in the Uğurlu race and two in the hybrid form. The karyotype characteristics observed in the new race are obviously different among hitherto studied 2n= 58 populations of blind mole rats; therefore, it can be evaluated as a new chromosome race of *N. xanthodon*. In this study, finding a new hybrid with interracial hybridisation is important for future studies in terms of the chromosomal evolution of blind mole rats.

Key words: blind mole rat, Uğurlu race, C-banding, Ag-NOR, Anatolia

Introduction

The blind mole rats inhabit the Eastern and South-Eastern Europe, the Eastern Mediterranean, the Middle East and North-West Africa; they prefer settled areas, small forests, steppes and slopes, avoiding densely wooded areas (Musser & Carleton 2005). The species of the genus *Nannospalax* are highly variable in relation to the karyotype structure and have more than 70 different chromosomal races or cytotypes (Arslan et al. 2016). There are three species in this genus: *N. xanthodon, N. ehrenbergi* and *N. leucodon* (see Kryštufek & Vohralík 2009) in Turkey and the chromosome diversity in these species is very high. The karyotype variations in *Nannospalax* is due to various chromosomal rearrangements (Arslan et al. 2016). Differences in the karyotypes between the species of Turkish blind mole rats have also been recorded (Arslan et al. 2011a, 2016, Ivanitskaya et al. 1997, Kankiliç et

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al. 2007a, 2007b, Matur et al. 2013, Nevo et al. 1995, Sözen et al. 2013). *Nannospalax xanthodon* (Satunin, 1898) is distributed throughout most of Anatolia; this taxon contains 28 chromosome races with diploid numbers 2n = 36, 38, 40, 46, 48, 50, 52, 54, 56, 58, 60 and 62 (Arslan et al. 2016). The blind mole rats with 2n = 60 diploid chromosomes are very common in Central Anatolia. Although individuals of the Vasvarii race are widespread in Konya, there are 2n = 58 in Ereğli and its environs and 2n = 40 in a limited area in Beyşehir (Arslan et al. 2011a). While 2n = 60 is known to be widespread in Karaman, blind mole rats belonging to 2n = 56 have been identified in the south of the province (Sözen et al. 2015). So far, four chromosomal races of 2n = 58 from Anatolia have been identified (Arslan et al. 2016). These are Cilicicus race from Adana, Konya and Niğde (Arslan et al. 2011a, Sözen & Kivanç 1998, Sözen et al. 2006a, 2006b); Munziri race from Tunceli and Erzurum (Arslan & Zima 2013, Coşkun 2004, Coşkun et al. 2010); Sarıkavak race from Ankara (Sözen 2004); and Taşköprü race from Kastamonu (Sözen et al. 2006b).

In this study, we present a new chromosomal race of 2n = 58 and the characteristics of a hybrid of 2n=59 found from Karaman. We recommend that the chromosomal race is unique and it should be accepted as a new race.

Materials and Methods

The karyological analyses were done in eight specimens of mole rats from localities in Karaman. The specimens were caught with Permission no. 72784983-488.04-43524 issued by the Ministry of Forest and Water Works of the Republic of Turkey. This permission also replaces the permission of the local ethics committee. Arslan's (2013) metal pipe-type trap was used to catch specimens. This trap ensures that live specimens are obtained without being injured. The number of samples studied and their collection sites are shown in Fig. 1 and Table 1. Kar-

Table 1. Localities of Uğurlu race and the hybrid form from Karaman. The symbols of the sampling sites correspond to the ones in Fig. 1.

Symbol	Species	Locality / Province	Latitude / longitude	no. specimens		2n	NF	NFa	X	Y
				Male	Female					
▲	*N. xanthodon* (Uğurlu race)	Uğurlu / Sarıveliler	36° 38′ N, 32° 41′ E	-	6	58	82	78	SM	-
■	*N. xanthodon* (hybrid form)	Tepebaşı / Ermenek	36° 40′ N, 32° 44′ E	-	2	59	79	75	M	-

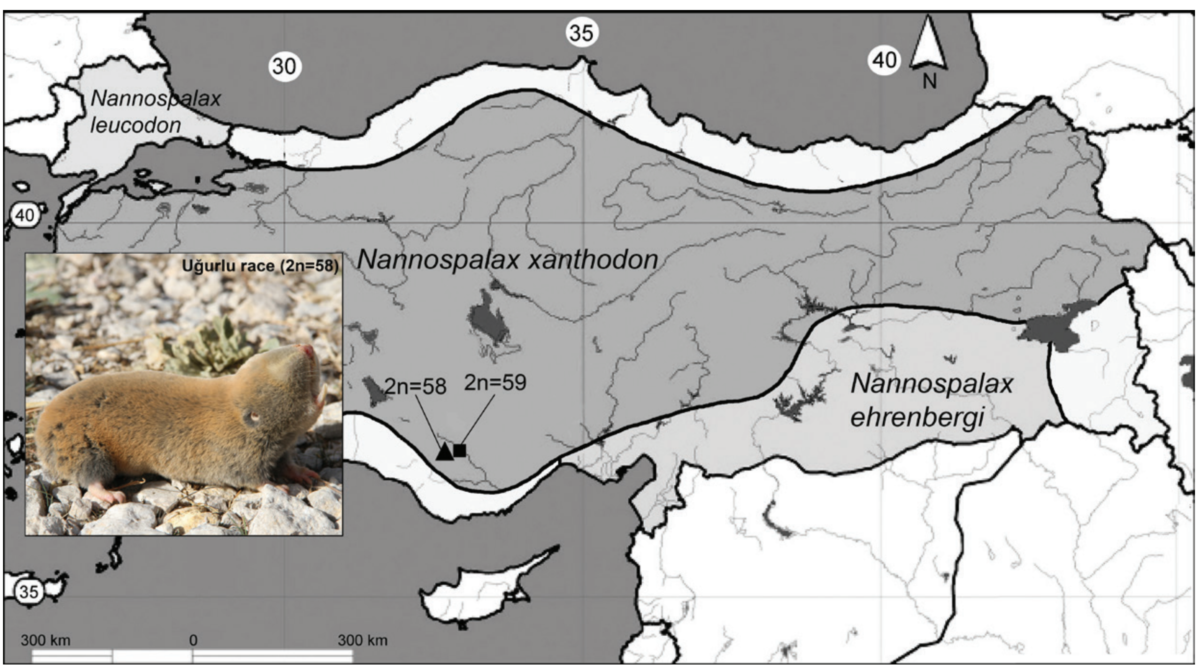

Fig. 1. Collecting sites of Uğurlu race and the hybrid form from Karaman. The symbols of sampling localities correspond to data in Table 1. The approximate ranges of both species are indicated after Kryštufek & Vohralík (2009).

yotype preparations were made according to Ford & Hamerton (1956). Some preparations were stained conventionally with Giemsa. Constitutive heterochromatin bands and nucleolus organizer regions (NORs) were detected according to Sumner (1972) and Howell & Black (1980), respectively. The classification of chromosomes was made according to Hsu & Benirschke (1967-1977). The arrangement of chromosomes was made following Arslan et al. (2011a) and Ivanitskaya et al. (1997, 2008). The large acrocentric autosomal pairs, which could be reliably recognised, were arranged as the first in the complement. The other biarmed and acrocentric autosomes were arranged according to their size.

Results

The karyotype of six females from Uğurlu consisted of 58 chromosomes including a distinctly large acrocentric (no. 1), a large subtelocentric (2), ten medium-sized bi-armed (3-12) and 16 acrocentric autosomal pairs of gradually diminishing size (13-28) (NFa = 78). The X chromosome was a large submetacentric (NF = 82) (Fig. 2.1). The dark centromeric C-bands were observed in three biarmed (4, 9, 12) and some acrocentric autosomes (1, 13-15, 17, 18, 20, 22, 24). C-heterochromatic short arms were found in eight autosomal pairs (2, 3, 5-8, 10, 11). The X chromosome possessed centromeric C-positive band (Fig. 3.1). NORs were observed in the telomeric regions of the short arms of the autosomes 3, 7 and 8 (Fig. 4.1). All the autosomes and both the sex chromosomes can be reliably identified based on their unique G-banding patterns except for some bi-armed and acrocentric chromosomes (Fig. 5.1).

The karyotype of two females from Tepebaşı consisted of 59 chromosomes (hybrid) including a distinctly large acrocentric (no. 1), a large subtelocentric (2), ten medium-sized bi-armed (3-12) and 16 acrocentric autosomal pairs of gradually diminishing size (13-28). The autosomal pairs 2 and 9 were heteromorphic in both studied specimens, consisting of a subtelocentric and an acrocentric chromosome. The autosomal pair 4 had a metacentric and a submetacentric chromosome (NFa = 75). The X chromosome was large submetacentric (NF = 79) (Fig. 2.2). The dark centromeric C-bands were observed in ten biarmed (3-6, 8, 9) and some acrocentric autosomes (1, 12, 13, 15, 22). C-heterochromatic short arm was found in a homolog of two autosomal pair. The X chromosome possessed centromeric C-positive band (Fig. 3.2). NORs were observed in the telomeric regions of the short arms of autosomes 3 and 4 (Fig. 4.2). All autosomes and both the sex chromosomes can be reliably identified based on their unique G-banding patterns except for some bi-armed and acrocentric chromosomes (Fig. 5.2).

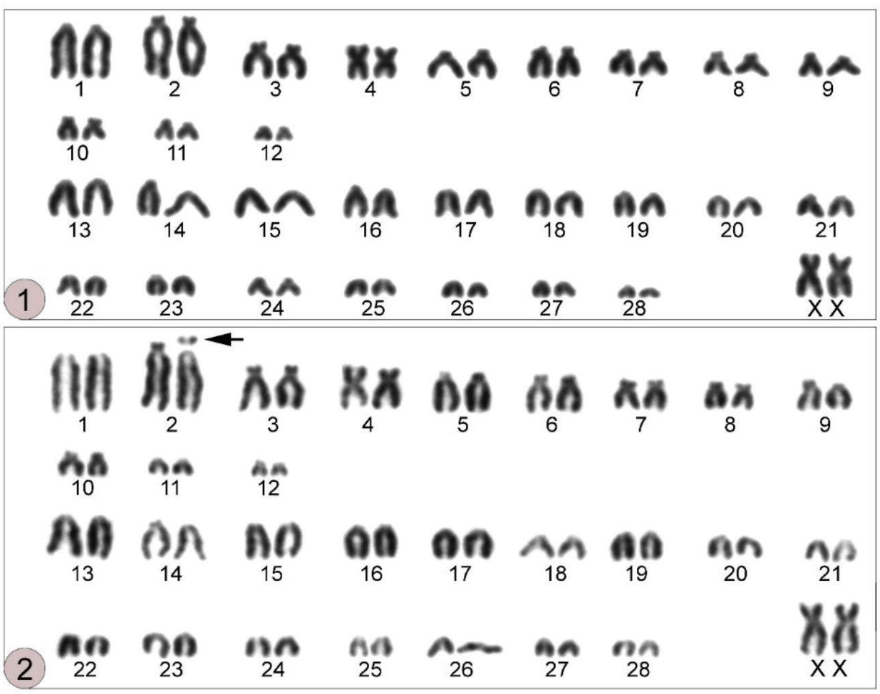

Fig. 2. Standard karyotypes of the Uğurlu race (1) and the hybrid form (2) in Karaman. The arrow indicates the 59th chromosome.

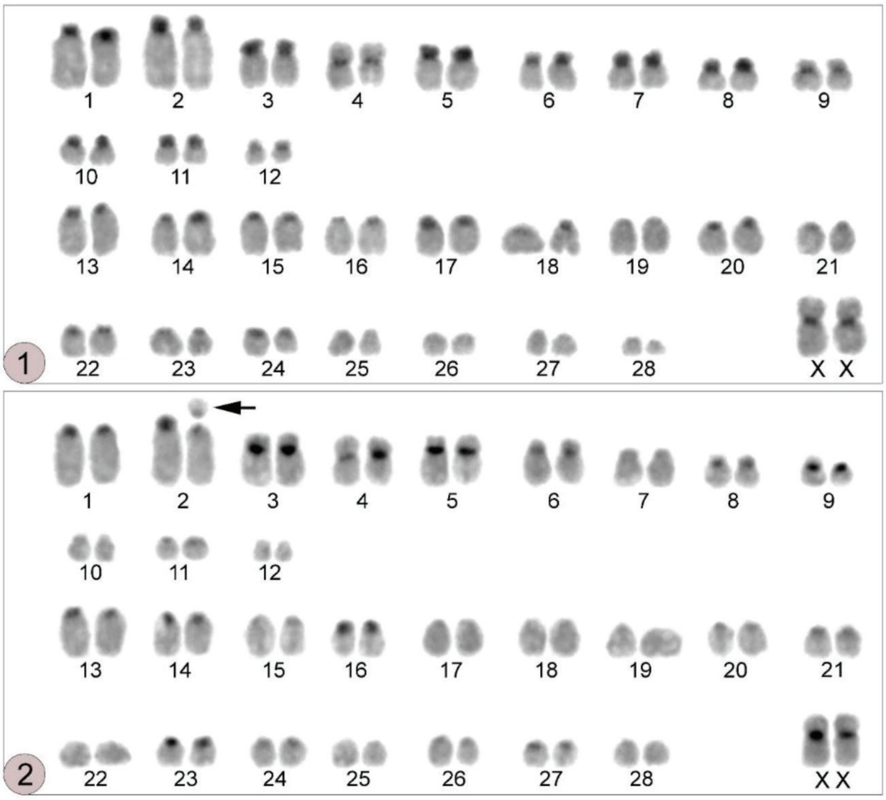

Fig. 3. C-banded karyotypes of the Uğurlu race (1) and the hybrid form (2) in Karaman. The arrow indicates the 59th chromosome.

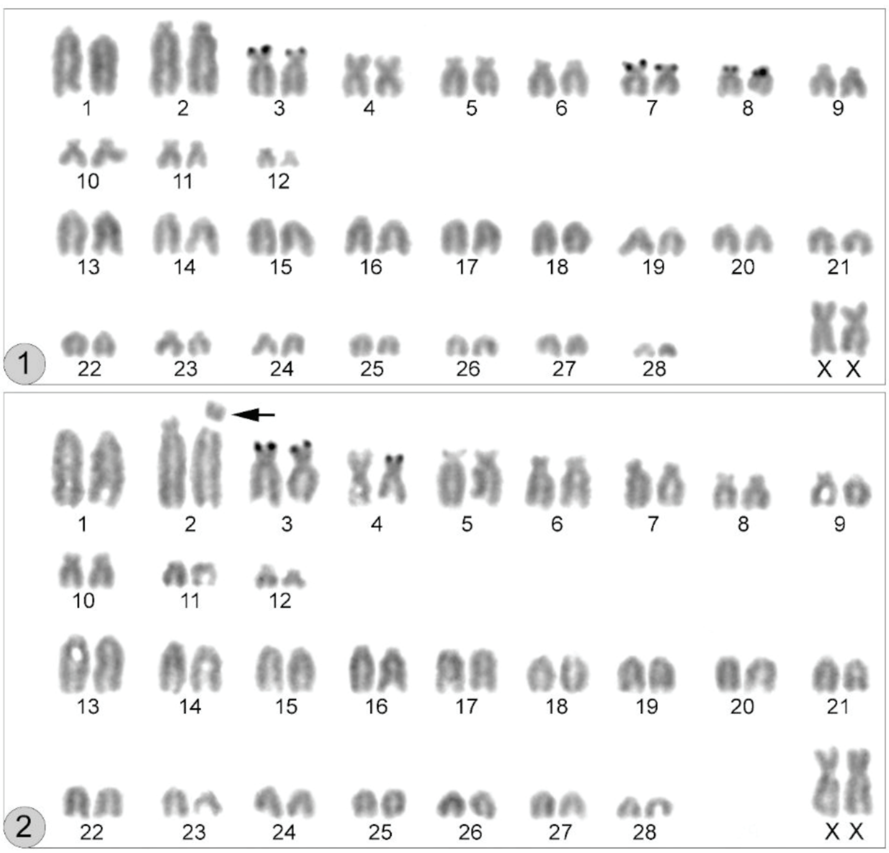

Fig. 4. Silver-stained karyotypes of the Uğurlu race (1) and the hybrid form (2) in Karaman. The arrow indicates the 59th chromosome.

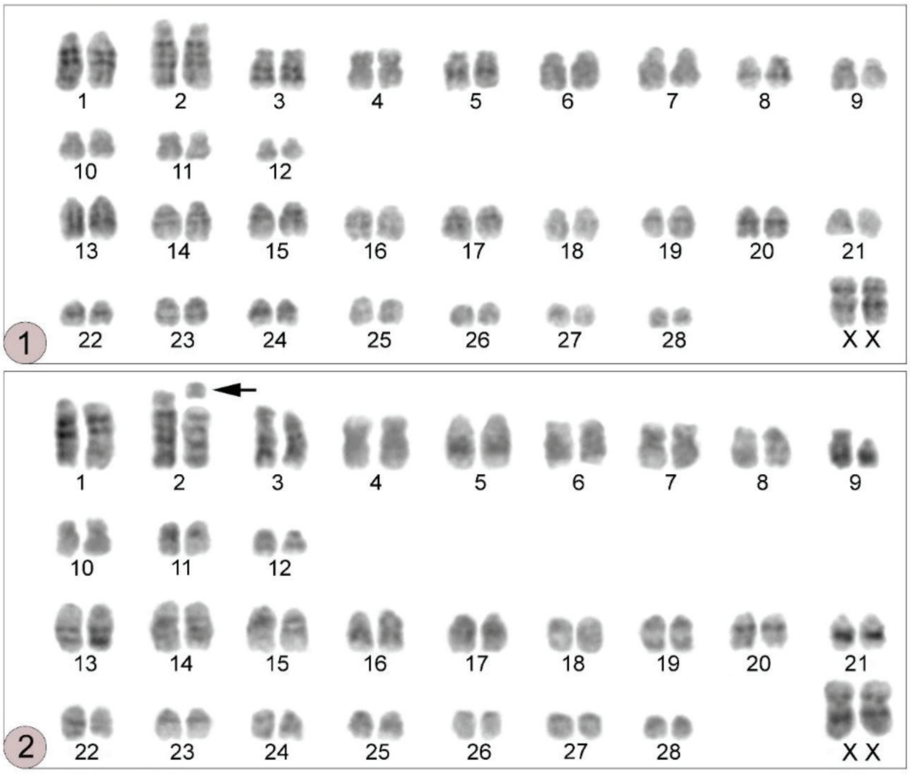

Fig. 5. G-banded karyotypes of the Uğurlu race (1) and the hybrid form (2) in Karaman. The arrow indicates the 59th chromosome.

Discussion

The population with 58 chromosomes from Uğurlu (Sarıveliler) appears to represent a new separate chromosomal race. Its karyotype differs from those of the Cilicicus, Sarıkavak and Taşköprü races (all 2n=58) by the absence of a large submetacentric autosomal pair. Such bi-armed autosome is absent also in the karyotype of the Munzurii race (2n=58) but this race contains only 3 or 4 small bi-armed autosomal pairs. Moreover, the distribution of the new race is apparently disjunct and its range is quite distant, particularly from the Munzurii race. The designation of the new race is thus obviously well substantiated. So far, chromosomal studies on mole rats in Anatolia have shown that there are four different 58 chromosomal races (Table 2). Populations of these races are widespread in Central and Eastern Anatolia. The geographic contact of the populations of the other 2n = 58 chromosomal races, including the Uğurlu race, could not be determined. Populations of these races were surrounded by populations with different diploid chromosomes. As in the Sarıkavak race (Sözen 2004), the Uğurlu race is surrounded by 60 chromosome populations.

All the specimens of 2n=58 races from southeastern and northern Anatolia have a large submetacentric autosomal pair in their karyotypes which can be considered an autapomorphic chromosomal marker. While Cicicicus, Sarıkavak and Taşköprü races in central and southern Anatolia have the marker chromosome, there are no similar chromosomes in the karyotypes of the Munzurii race populations in Eastern Anatolia (Arslan et al. 2016, Arslan & Zima 2013). It was determined that the Uğurlu race was included in the central and southern Anatolian group as it had this marker chromosome. The number of chromosomal arms between 58 chromosome races (NF) is highly variable. So far, the Munzirii race has the lowest NF (66-68), while the Sarıkavak race has the highest NF (78) (Arslan et al. 2016). However, the Uğurlu race identified in this study has 82 NF. Arslan et al. (2011a) argued that the differences in the number of chromosomal arms between the 58 chromosome populations could be explained by mechanisms similar to that responsible for autosomal heteromorphism described in the Ereğli population.

The two females from Tepebaşı (Ermenek) with 2n=59 cannot be recognised as a new race. It may be considered hybrids between the Uğurlu race and surrounding populations of the Vasvarii race with 60 chromosomes. Clearly, the presence of three heterozygous pairs in the hybrid supports this

Table 2. Chromosomal records of 2n = 58 races of *N. xanthodon* from Turkey.

Species	Race	Locality	2n	NF	NFa	X	Y	References
N. xanthodon	Munzurii	Başpınar (Kemaliye, Erzincan)	58	66	62	Sm	Sm	Arslan & Zima (2013)
N. xanthodon	Munzurii	Esentepe (Kemaliye, Erzincan)	58	68	64			Coşkun et al. (2010)
N. xanthodon	Munzurii	Ovacık (Tunceli)	58	68	64			Coşkun (2004)
N. xanthodon	Cilicicus	Ereğli (Konya)	58	72 75	68 71	Sm	A/Sm	Sözen et al. (2006a) Arslan et al. (2011a)
N. xanthodon	Cilicicus	Ulukışla (Niğde)	58	72	68	Sm	A	Sözen et al. (2006b)
N. xanthodon	Cilicicus	Madenköy (Niğde)	58	72	68	Sm	A	Sözen & Kıvanç (1998)
N. xanthodon	Cilicicus	Pozantı (Adana)	58	72	68	Sm	A	Sözen et al. (2006a)
N. xanthodon	Sarıkavak	Sarıkavak (Ankara)	58	78	74	Sm	A	Sözen (2004)
N. xanthodon	Taşköprü	Taşköprü (Kastamonu)	58	74 75	70 71	Sm	A	Sözen et al. (2006b)
N. xanthodon	Uğurlu	Uğurlu (Sarıveliler) Tepebaşı (Ermenek)	58 59	82	78	Sm	-	Present study

hypothesis. Chromosome number two, a trivalent of one submetacentric and two acrocentrics, supposedly may have originated after a centric fusion of one of the largest and the smallest autosome. The G-banding pattern seems to support this interpretation. The submetacentric chromosome in chromosome pair number 4 may be a result of pericentric inversion. This difference in pair 9, consisting of subtelocentric and acrocentric chromosomes, may be due to pericentric inversion and/or the addition of heterochromatin. Unfortunately, the G-banding pattern resolution does not verify the supposed mechanisms of change in pairs 4 and 9. The differences between the individual karyotypes observed are based on the assumption that the Uğurlu race and the geographically-close Vasvarii populations are closely related and originated from a common ancestor. Hybrids seem to be lacking or be extremely rare in Turkey (Ivanitskaya et al. 1997, Nevo et al. 1995, Sözen 2004, Sözen et al. 2013, 2006a). So far, a presumably hybrid karyotype in the blind mole rats was determined from Pülümür-Kangallı in the Tunceli Province, eastern Anatolia (Coşkun et al. 2010). This aberrant karyotype with 2n = 49 is quite distant from the other chromosomal races (Tuncelicus, Nehringi, Gümüşhane). The only geographic area in which hybridisation between races has regularly been reported is Israel (Ivanitskaya et al. 2010, Wahrman et al. 1969).

The C-heterochromatin properties of both the Uğurlu race and hybrid individuals are similar to the nearby Cilicicus race (Arslan et al. 2011a). The new race with C-heterochromatic short arms is similar to the Cilicicus race but differs from the Munziri race from Başpınar (Arslan & Zima 2013). Moreover, the Uğurlu race has more centromeric/pericentromeric heterochromatin acrocentric chromosomes than the other two races. The fact that heterochromatin distribution in the Uğurlu race is more than in the other two races may prove that it is a new race. The C-banding pattern on the X chromosomes is also largely similar among the Uğurlu, Cilicicus and Munziri populations. In the Munziri population, the short arm of the submetacentric Y chromosome is entirely C-positive due to heterochromatin amplification (Arslan & Zima 2013). In the Ereğli population of Cilicicus, the Y chromosome is subtelocentric (Arslan et al. 2011a), whereas, in other populations, it is acrocentric. Since all of the collected Uğurlu individuals are female, Y chromosome morphology is missing.

The position of the Ag-NORs in the karyotype of the new race (2n=58) from Uğurlu is similar to the one in the karyotype of the Munziri race from Başpınar, but the number of nucleolar organizer bearing autosomes in the Uğurlu race is lower than those in the karyotype of the Cilicicus race in Ereğli. The hybrid specimens from Uğurlu revealed only two NOR-bearing pairs in their karyotype, whereas three and four bi-armed pairs possessing NORs were recorded in other 2n=58 races. According to Arslan & Zima (2013), this finding indicates that distant populations of mole rats with the same diploid chromosome number may differ in the NORs distribution pattern. The published reports indicate that the Ag-NOR sites were observed in telomeric areas of the short arms of pairs 3-5 of chromosomes in complements found in various populations of

N. xanthodon and *leucodon* in Turkey (Arslan & Bölükbaş 2010, Arslan et al. 2011a, 2011b, 2013, 2014a, 2014b, Arslan & Zima 2013, 2014, 2015a, 2015b, 2017, Gülkaç & Küçükdumlu 1999, Ivanitskaya et al. 1997, 2008, Kankiliç et al. 2017, in this study). However, the NORs were recorded only in autosomal pairs 1-3 in populations of *N. ehrenbergi* from Turkey and Jordan (Arslan & Zima 2013, Ivanitskaya & Nevo 1998). Some of these NORs localised on acrocentric chromosomes (Arslan & Zima 2013). The published reports show that the morphology of the bi-armed chromosomes where the Ag-NORs are localised is highly variable. While there are also some doubts about the reliability of the NOR distribution as a marker of phylogenetic relationships (Sánchez et al. 1990), in some studies the NOR distribution model is considered an important character to reveal the difference between various races (Ivanitskaya et al. 1997, 2008). The extensive karyotype variation in *Nannospalax* is due to various chromosomal rearrangements that are still not known in nature because banding techniques or molecular and other cytogenetic studies are so rare. Arslan et al. (2016) and Ivanitskaya et al. (1997) stated the main mechanisms of the chromosomal alteration are centromeric fusions and additions/deletions of C-heterochromatin, as well as occasional pericentric inversions, euchromatin deletions, missing chromosomes and centromeric shifts. While there are some doubts about the reliability of the NOR distribution as a marker of phylogenetic relationships (Sánchez et al. 1990), other studies consider that the NOR distribution model is an important character to reveal the difference between various races (Ivanitskaya et al. 1997, 2008).

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