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C- and NOR stained karyotypes of mole rat, *Nannospalax xanthodon* (2n = 54) from Kırıkkale, Turkey

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Abstract: In the present study, the 2n = 54 chromosomal race of blind mole rats, *Nannospalax xanthodon* superspecies, from Kırıkkale Province in Turkey was investigated. Conventional chromosome staining, Ag-NOR (Nucleolus Organizer Region) staining, and C-banding analysis were carried out on specimens of mole rats. The karyotype including 3 metacentric pairs (nos. 1-3), 3 submetacentric pairs (nos. 4-6), 3 subtelocentric pairs (nos. 7-9), and 17 acrocentric pairs (nos. 10-26) of autosomes (NFa = 70). C-heterochromatin regions were found in the centromeric and pericentromeric region and the short arms of some bi-armed autosomal pairs, and C-heterochromatin was localized in pericentromeric areas of a few acrocentric autosomes. The X chromosome has a centromeric C-positive band and the Y chromosome appeared to be uniformly and C-negatively stained. In all of the specimens studied the NORs were localized in distal heterochromatin areas of the short arms of 4 pairs (nos. 4, 5, 8, 9) of biarmed autosomes.

Key words: *Nannospalax xanthodon*, mole rat, karyotype, Kırıkkale, Turkey

Kırıkkale'deki kör fare *Nannospalax xanthodon* (2n = 54)'un C- ve NOR boyalı karyotipleri

Özet: Bu çalışmada, Kırıkkale'deki *Nannospalax xanthodon* üsttürüne ait kör farelerin 2n = 54 kromozomal formu araştırıldı. Kör fare örnekleri üzerine standart kromozom boyama, Ag-NOR (Nükleolar Organizatör Bölge) boyama ve C-bantlama analizi uygulandı. Karyotip üç çift metasentrik (no. 1-3), üç çift submetasentrik (no. 4-6), üç çift subtelosentrik (no. 7-9) ve onyedi çift akrosentrik (no. 10-26) kromozom içerir (NFa = 70). C-heterokromatin bölgeler bazı iki kollu otozomal çiftlerin sentromerik, perisentromerik ve kısa kollarında bulundu ve C-heterokromatin birkaç akrosentrik kromozomun perisentromerik bölgesinde lokalize olmuştu. X kromozom sentromerik bir C-pozitif banda sahiptir ve Y kromozomunun tek tip ve C-negatif boyandığı ortaya çıktı. NOR'lar çalışılan bütün örneklerde dört çift (no. 4, 5, 8, 9) iki kollu otozomların kısa kollarının heterokromatin bölgelerinde lokalize olmuştur.

Anahtar sözcükler: *Nannospalax xanthodon*, kör fare, karyotip, Kırıkkale, Türkiye

Introduction

Blind mole rats are a small group of subterranean rodents occupying the Eastern Mediterranean, Africa, Eastern Europe, and various parts of Asia. The family Spalacidae are strictly fossorial rodents with various specific features that emphasize their adaptation to underground life (1,2). They live in isolated groups and the fragmented distribution pattern is thought to support the speciation events as well as the process of karyotype differentiation. The systematic and phylogenetic relationships of mole rats within the family Spalacidae are not definitively resolved yet. Savić and Nevo (3) and Musser and Carleton (4) treated the family as monogeneric, including only a single genus *Spalax*; however, other authors have preferred to distinguish 2 genera, currently named *Spalax* and *Nannospalax* (2,5-7). The genus *Spalax* includes larger species lacking perforations on sides of the posterior opening to the skull, and possessing karyotypes with higher diploid chromosome numbers (2n = 60 or 62) and no acrocentric autosomes.

According to Musser and Carleton (4), most authors recognize 3 species within *Nannospalax*, i.e. *N. ehrenbergi, N. leucodon*, and *N. nehringi*. *N. nehringi* is found in most of Turkish Anatolia and Transcaucasia. Various authors have lumped *N. nehringi* and *N. leucodon* into a single taxon, *N. leucodon* superspecies (8,9). Kryštufek and Vohralík (10) proposed that the name *nehringi* is preoccupied by *xanthodon*.

The exceptional karyotypic variation within the *Nannospalax* populations is manifested in records of more than 50 chromosomal races (3,8). These races are considered as presumptively good biological species (8,11), and some of them are formally described as separate species (12). Maintaining karyological studies on local populations of blind mole rats are therefore quite important for mapping distribution of chromosome races and better understanding of mechanisms of their karyotype evolution. The conventionally stained karyotype of mole rat (2n = 54) was described by Nevo et al. (8) from Bolu and Bingöl; by Sözen et al. (9,13,14) from Karabük and Yozgat; by Yüksel and Gülkaç (15) from Yozgat; by Coşkun (16) and Coşkun et al. (17) from Bingöl, Elazığ, and Tunceli; and by Kankılıç et al. (18) and Aşan and Yağcı (19) from Kırıkkale. However, information on differentially stained chromosomes and detailed structure of the karyotype is still lacking in this race. The present study provides a detailed description of conventionally stained chromosomes and the distribution pattern of the Ag-NORs and C-heterochromatin regions in the karyotypes of mole rats from Kırıkkale.

Materials and methods

Cytogenetic analyses were performed in 3 specimens of mole rat from Kırıkkale (Figure 1). Karyotype preparations were obtained in the field from bone marrow after colchicine treatment (20). Air-dried preparations were stained conventionally with Giemsa. Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected by the techniques of C-banding (21) and Ag-NOR staining (22), respectively. From each specimen, 10 to 20 slides were prepared, and at least 20 well-spread metaphase plates were analyzed. Definition of the shapes of the chromosomes was established according to Levan et al. (23). Standard voucher specimens (skins and skulls) are deposited at Selçuk University, Biology Department, Faculty of Science, Konya, Turkey.

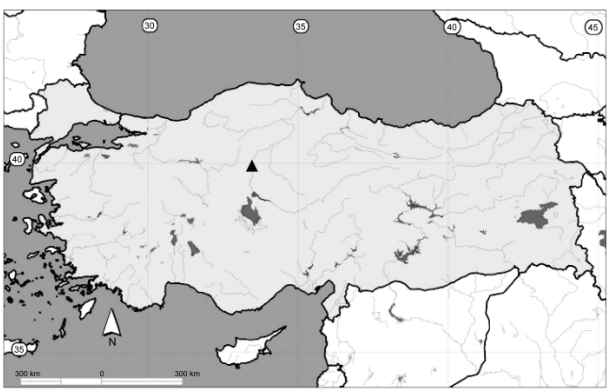

Figure 1. Collecting locality of *Nannospalax xanthodon* (2n = 54) in Kırıkkale (▲).

Results and discussion

The karyotype of mole rat from Kırıkkale consists of 54 chromosomes including 3 metacentric pairs (no. 1-3), 3 submetacentric pairs (nos. 4-6) and 3 subtelocentric pairs (nos. 7-9) and 17 acrocentric pairs (nos. 10-26) of autosomes (NFa = 70). The X chromosome was medium-sized submetacentric, and the Y chromosome small acrocentric (NF = 74) (Figure 2). Chromosome shapes of 2n = 54 race were determined and are given in Table 1.

A similar pattern of the C-heterochromatin distribution was also found in the complements of all specimens. Distinct dark centromeric C-band was observed in a autosome (no. 3). Some autosomes (nos. 4, 5, 7-9) had C-heterochromatic short arms in the complements of all the specimens. The number of C-positive pericentromeric regions observed in some acrocentric autosomes and the intensity of dark staining were variable between individual pairs. The X chromosome had a centromeric C-positive band and the Y chromosome appeared to be uniformly and C-negatively stained (Figure 3).

The active Ag-NOR regions were found in 4 biarmed autosomal pairs (nos. 4, 5, 8, 9) in complements of all the specimens. The NORs were observed in the telomeric region of the short arms of submetacentric and subtelocentric autosomes (Figure 4).

Table 1. Chromosome classification (μm) in 2n = 54 race of *Nannospalax xanthodon* from Turkey according to Levan et al. (23). m: metacentric, sm: submetacentric, st: subtelocentric, a: acrocentric.

Chromosome pair no.	Chromosome arms		Total length	Arm ratio (q/p)	Relative length (%)	Centromer index	Centromeric position
	Short arm (p)	Long arm (q)					
1	1.05	1.52	2.57	1.45	4.32	0.41	m
2	1.62	2.30	3.92	1.42	6.59	0.41	m
3	1.45	2.12	3.57	1.46	6.00	0.41	m
4	0.90	2.14	3.04	2.38	5.11	0.30	sm
5	0.89	1.69	2.58	1.90	4.34	0.34	sm
6	0.74	1.26	2.00	1.44	3.36	0.37	sm
7	0.83	3.42	4.25	4.12	7.14	0.20	st
8	0.81	3.40	4.21	4.20	7.08	0.19	st
9	0.58	1.71	2.29	2.37	3.85	0.25	st
10	-	2.26	2.26	-	3.80	-	a
11	-	2.04	2.04	-	3.43	-	a
12	-	2.02	2.02	-	3.40	-	a
13	-	2.00	2.00	-	3.36	-	a
14	-	1.94	1.94	-	3.25	-	a
15	-	1.73	1.73	-	2.91	-	a
16	-	1.61	1.61	-	2.71	-	a
17	-	1.55	1.55	-	2.61	-	a
18	-	1.51	1.51	-	2.54	-	a
19	-	1.46	1.46	-	2.45	-	a
20	-	1.44	1.44	-	2.41	-	a
21	-	1.42	1.42	-	2.39	-	a
22	-	1.37	1.37	-	2.30	-	a
23	-	1.34	1.34	-	2.25	-	a
24	-	1.23	1.23	-	2.07	-	a
25	-	1.05	1.05	-	1.77	-	a
26	-	0.86	0.86	-	1.45	-	a
X	1.21	1.99	3.20	1.64	5.38	0.37	sm
Y		1.03	1.03	-	1.73	-	a

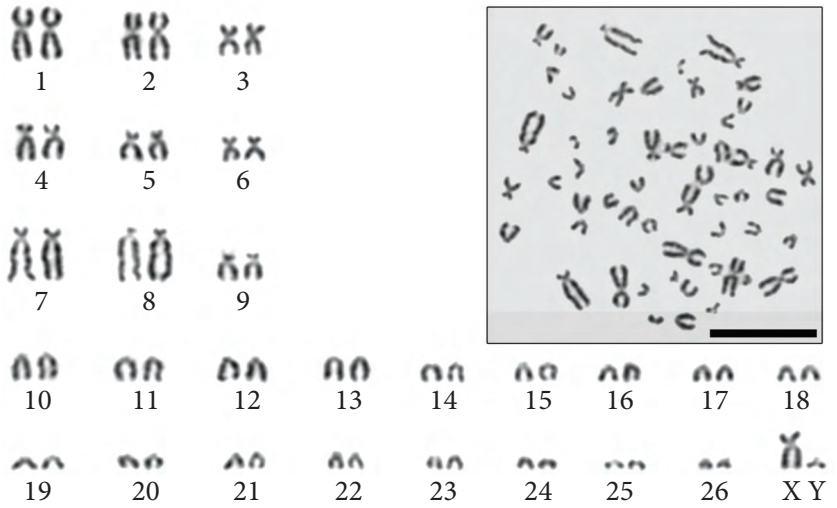

Figure 2. Metaphase spread and karyotype of 2n = 54 from Kırıkkale (scale bar = 10 μm).

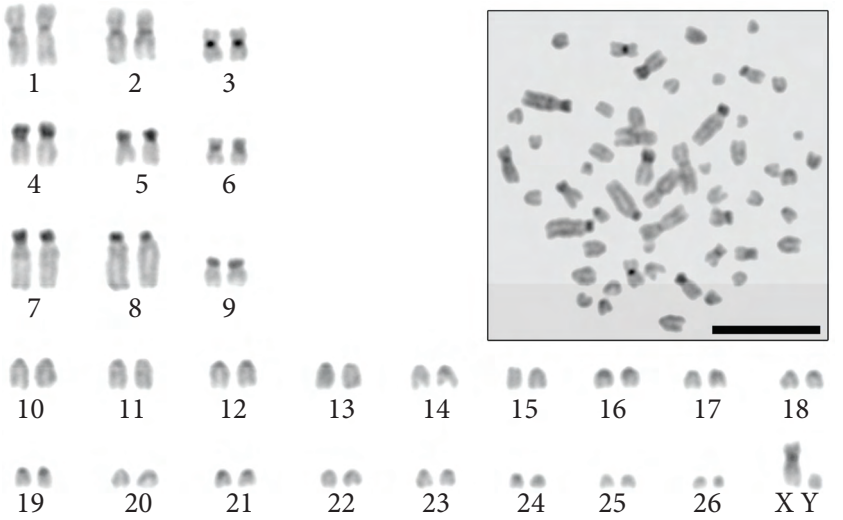

Figure 3. Metaphase spread and C-banded karyotype of 2n = 54 from Kırıkkale (scale bar = 10 μm).

The 2n = 54 race was first identified by Nevo et al (8) from Bolu and Bingöl. Afterwards, many researchers (9,13-19) studied conventionally stained karyotype at different localities in Turkey (Table 2). Results of these studies were also found different only for NF values.

The first study on banding in genus *Nannospalax* in Turkey was done on some chromosomal races of *N. xanthodon* and *N. ehrenbergi* superspecies by Ivanitskaya et al. (24). These researchers determined that some bi-armed autosomes had heterochromatin blocs located on the telomeric regions of short arms of *N. ehrenbergi* in Tarsus, Gaziantep, and Şanlıurfa populations. Arslan et al. (25) also found C-heterochromatin regions in the short arms of 4 biarmed autosomal pairs in the 2n = 40 race from Beyşehir (Konya). However, the same researchers observed dark C-bands in pericentromeric areas of the biarmed autosomes of the 2n = 58 race from Ereğli.

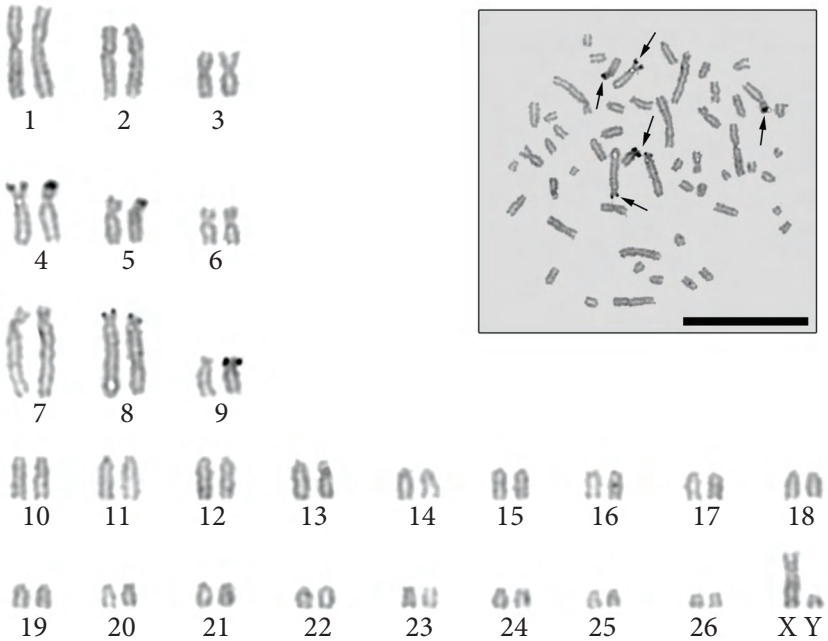

Figure 4. Silver-stained metaphase spread and karyotype of 2n = 54 from Kırıkkale (scale bar = 10 μm).

Table 2. Chromosomal records 2n = 54 race of *Nannospalax xanthodon* from Turkey (2n: diploid chromosome number, NF: fundamental number of chromosomal arms, NFa: number of autosomal arms, sm: submetacentric, st: subtelocentric, a: acrocentric).

Race (2n)	NF	NFa	X	Y	Localities	References
54	-	-	-	-	Bolu, Bingöl	Nevo et al. (8,11)
54	72	68	sm	a	Karabük, Zonguldak, Tokat	Sözen (13), Sözen et al. (16)
54	74	70	sm	st/a	Yozgat	Yüksel and Gülkaç (15)
54	74	70	sm	a	Bigöl, Elazığ, Tunceli	Coşkun (16), Coşkun et al. (17)
54	74	70	sm	a	Kırıkkale	Kankılıç (18), Aşan and Yağcı (19), This study

The NORs on *Nannospalax* were first studied by Ivanitskaya et al. (24) in Turkey. The researchers defined the NOR features of the *N. xanthodon* specimens obtained from Malatya and *N. ehrenbergi* specimens obtained from Gaziantep, İçel (Tarsus), Şanlıurfa (Siverek, Birecik), Elazığ, and Diyarbakır. According to these researchers, in all populations of both species 3 chromosome pairs had NORs, and NORs were localized in the short arms of 3 pairs subtelocentric chromosomes in *N. xanthodon*. The researchers defined NOR in the telomeric region of a long arm of an acrocentric chromosome different from the others in the Gaziantep population. Ivanitskaya and Nevo (26) recorded NORs in 1 or 2 autosomal pairs in the karyotype of Jordan mole rat populations. Gülkaç and Küçükdumlu (27) determined that NORs were localized in 3 pairs of subtelocentric chromosomes in *N. xanthodon* and *N. ehrenbergi* specimens obtained from Malatya and in 2 pairs of chromosomes in the specimens from Elazığ. Ivanitskaya et al. (28) described the NORs in 5 pairs of subtelocentric autosomes in both R (race with

restricted distribution with NOR-bearing) and W (wide distributed race with NOR-bearing) cytotypes of the 2n = 60 race. Arslan et al. (25) determined the NORs in 4 pair autosomes of each 3 races (2n = 40, 58, 60) as done in the present study. However, morphologies of chromosomes that NORs are localized on are different in each race. In the present study the NORs of 2n = 54 chromosomal race were determined to be related to heterochromatin areas as in the 2n = 40 race from the Beyşehir population (25).

Arslan et al. (29) studied mitochondrial divergence between 3 races (2n = 40, 58, 60) of *N. xanthodon* and their results showed that 3 races of *N. xanthodon* from Anatolia, which are characterized by distinct diploid numbers, represent deeply divergent monophyletic lineages. Both molecular and karyological results show that each type of chromosomal race is speciation. According to a recent study by Arslan and Albayrak (30), C- and Ag-NOR banding, used to establish heterochromatin and nucleolar organizer regions on the chromosomes, is frequently used in animals and thus is useful for examining intra- and interspecific chromosomal differences between closely related species.

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References

1. Nevo E. Adaptive convergence and divergence of subterranean mammals. Ann Rev Ecol Syst 10: 269-308, 1979.
2. Savić IR. Familie Spalacidae Gray, 1821 Blindmäuse. In: Niethammer J, Krapp F. eds., Handbuch der Säugetiere Europas 2/I. Akademische Verlagsgesellschaft, Wiesbaden; 1982: pp. 539-584.
3. Savić I, Nevo E. The Spalacidae. Evolutionary history, speciation and population biology. In: Nevo E, Reig AO. eds., Evolution of Subterranean Mammals at the Organismal and Molecular Levels. Alan R. Liss, New York; 1990: pp. 129-153.
4. Musser G, Carleton M. Superfamily Muroidea. In: Wilson DE, Reeder DM. eds., Mammal Species of the World. A Taxonomic and Geographic Reference. The Johns Hopkins University Press, Baltimore; 2005: pp. 894-1531.
5. Topachevski VA. Fauna of the USSR: Mammals. Mole rats, Spalacidae. Smithsonian Institution and the National Science Foundation, Washington; 1969.
6. Savić I, Soldatović B. Karyotype evolution and taxonomy of the genus *Nannospalax* Palmer, 1903, Mammalia, in Europe. Serbian Academy of Sciences and Arts, Separate Editions 59: 1-104, 1984.
7. Németh A, Révay T, Hegyeli Z et al. Chromosomal forms and risk assessment of *Nannospalax* (superspecies *leucodon*) (Mammalia: Rodentia) in the Carpathian Basin. Folia Zool 58: 349-361, 2009.
8. Nevo E, Filippucci MG, Redi C et al. Karyotype and genetic evolution in speciation of subterranean mole rats of the genus *Spalax* in Turkey. Biol J Linn Soc 54: 203-229, 1995.
9. Sözen M, Matur F, Çolak E et al. Some karyological records and a new chromosomal form for *Spalax* (Mammalia: Rodentia) in Turkey. Folia Zool 55: 247-256, 2006.
10. Kryštufek B, Vohralík V. Mammals of Turkey and Cyprus. Rodentia II. Cricetinae, Muridae, Spalacidae, Calomyscidae, Capromyidae, Hystricidae, Castoridae. Knjižnica Annales Majora, Koper; 2009.
11. Nevo E, Filippucci MG, Redi C et al. Chromosomal speciation and adaptive radiation of mole rats in Asia Minor correlated with increased ecological stress. Proc Natl Acad Sci USA 91: 8160-8164, 1994.
12. Nevo E, Ivanitskaya E, Beiles A. Adaptive radiation of blind subterranean mole rats: naming and revisiting the four sibling species of the *Spalax ehrenbergi* superspecies in Israel: *Spalax galili* (2n=52), *S. golani* (2n=54), *S. carmeli* (2n=58) and *S. judaei* (2n=60). Backhuys Publishers, Leiden; 2001.
13. Sözen M. A karyological study on subterranean mole rats of the *Spalax leucodon* Nordmann, 1840 superspecies in Turkey. Mamm Biol 69: 420-429, 2004.
14. Sözen M, Sevindik M, Matur F. Karyological and some morphological characteristics of *Spalax leucodon* Nordmann, 1840 (Mammalia: Rodentia) superspecies around Kastamonu province, Turkey. Turk J Zool 30: 205-219, 2006.
15. Yüksel E, Gülkaç MD. The cytogenetical comparisons of *Spalax* (Rodentia: Spalacidae) populations from middle Kızılırmak Basin, Turkey. Turk J Biol 25: 17-24, 2001.
16. Coşkun Y. A new species of mole rat, *Nannospalax munzuri* sp. n., and karyotype of *Nannospalax tuncelicus* (Coskun, 1996) (Rodentia: Spalacidae) in eastern Anatolia. Zool Middle East 33: 153-162, 2003.

17. Coşkun Y, Ulutürk S, Kaya A. Karyotypes of *Nannospalax* (Palmer 1903) populations (Rodentia: Spalacidae) from Centraleastern Anatolia, Turkey. Hystrix It J Mamm (n.s.) 21(1): 89-96, 2010.

18. Kankılıç TE, Kankılıç TO, Çolak R et al. Karyological comparison of populations of the *Spalax leucodon* Nordmann, 1840 superspecies (Rodentia: *Spalacidae*) in Turkey. Zool Middle East 42: 15-24, 2007.

19. Aşan N, Yağcı T. Karyotype and hair scale structure of *Nannospalax leucodon* (Nordmann, 1840) from Central Anatolia (Rodentia: Spalacidae). Turk J Zool 32: 125-130, 2008.

20. Ford CE, Hamerton JL. A colchicine, hypotonic citrate, squash sequence for mammalian chromosomes. Stain Technol 31: 247-251, 1956.

21. Sumner AT. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75: 304-306, 1972.

22. Howell WM, Black DA. Controlled silver staining of nucleolar organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014-1015, 1980.

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

24. Ivanitskaya E, Coskun Y, Nevo E. Banded karyotypes of mole rats (*Spalax*, Spalacidae, Rodentia) from Turkey. J Zool Syst Evol Res 35: 171-177, 1997.

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

26. Ivanitskaya E, Nevo E. Cytogenetics of mole rats of the *Spalax ehrenbergi* superspecies from Jordan (Spalacidae, Rodentia). Z Säugetierkunde 63: 336-346, 1998.

27. Gülkaç MD, Küçükdumlu İ. Variation in the nucleolus organizer regions (NORs) in two mole rat species (*Spalax leucodon* and *Spalax ehrenbergi*). Turk J Biol 23: 153-158, 1999.

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

29. Arslan E, Gülbahçe E, Arıkoğlu H et al. Mitochondrial divergence between three cytotypes of the Anatolian mole rat *Nannospalax xanthodon*. Zool. Middle East 50: 27-34, 2010.

30. Arslan A, Albayrak İ. C-banded karyotype and nucleolar organizer regions (NORs) of Wild Boar, *Sus scrofa* (Artiodactyla: Suidae) from Anatolia. Turk J Biol 33: 29-33, 2009.