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Authors: Kandemir, İrfan, Sözen, Mustafa, Matur, Ferhat, Kankılıç, Teoman, Martínková, Natália, et al.

Source: Folia Zoologica, 61(1) : 25-33

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: <https://doi.org/10.25225/fozo.v61.i1.a5.2012>

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Phylogeny of species and cytotypes of mole rats (Spalacidae) in Turkey inferred from mitochondrial cytochrome *b* gene sequences

İrfan KANDEMİR¹, Mustafa SÖZEN², Ferhat MATUR^{2*}, Teoman KANKILIÇ³,
Natália MARTÍNKOVÁ⁴, Faruk ÇOLAK², Sakir Ö. ÖZKURT⁵ and Ercument ÇOLAK¹

¹ Department of Biology, Faculty of Science, Ankara University, 06100-Tandoğan, Ankara, Turkey;
e-mail: ikandemir@gmail.com, allactaga@hotmail.com

² Department of Biology, Faculty of Arts and Sciences, Zonguldak Karaelmas University, 67100-Zonguldak, Turkey; e-mail: ferhat.matur@gmail.com, spalaxtr@hotmail.com, farukcolak@gmail.com

³ Department of Biology, Faculty of Arts and Sciences, Nigde University, Niğde, Turkey;
e-mail: spalax06@hotmail.com

⁴ Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Květná 8, 603 65 Brno, Czech Republic; e-mail: martinkova@ivb.cz

⁵ Department of Biology Education, Faculty of Education, Ahi Evran University, Kırşehir, Turkey;
e-mail: onderÖzkurt64@gmail.com

Received 4 October 2010; Accepted 26 August 2011

Abstract. We described the genetic variation of cytochrome *b* gene sequences of blind mole rats in Turkey. We examined 47 individuals belonging to nine cytotypes of three superspecies *Nannospalax leucodon*, *N. xanthodon* and *N. ehrenbergi* in the 402bp gene sequence of cytochrome *b*. Phylogenetic analyses showed that relationships between cytotypes were well supported, but deeper divergence between species showed insignificant relationships. Cytotypes of *N. xanthodon* with low diploid number of chromosomes from western Turkey formed a monophyletic group distinct from the populations with higher number of chromosomes ($2n = 56-60$). The monophyly of *N. xanthodon* was supported with respect to *N. leucodon* ($2n = 56$) in the Bayesian and maximum likelihood phylogenies. The divergence between two analyzed cytotypes of *N. ehrenbergi* ($2n = 52$, $2n = 56$) was 9.4 %, and the Kilis cytotype ($2n = 52$) appeared as the basal branch of the whole analysed dataset. *N. ehrenbergi* cytotypes were paraphyletic and they formed unsupported relationships with previously described *N. galili* ($2n = 52$), *N. golani* ($2n = 54$), *N. carmeli* ($2n = 58$) and *N. judaei* ($2n = 60$) from Israel. The results of this study showed that the *Nannospalax* species complex most likely represents more species than currently recognized, especially in *N. xanthodon*. We suggest that cytotypes of *N. xanthodon* and *N. ehrenbergi* from Turkey should be investigated in detail as possible candidates for being separate species.

Key words: *Nannospalax*, molecular phylogeny, chromosomal form, Anatolia, Thrace

Introduction

The mole rats are adapted for subterranean life. They are distributed in the Palaearctic region, throughout eastern and southeastern Europe, Anatolia, the Caucasus, and the Middle East up to northeastern Africa (Topachevskii 1969, Wilson & Reeder 1993). Their evolutionary history and taxonomic status are difficult to ascertain and molecular, karyological and

morphological studies are needed to determine the phylogenetic relationships of mole rats in Turkey (Nevo et al. 1995, Suziki et al. 1996, Sözen et al. 2000, Kankılıç et al. 2005, Ivanitskaya et al. 2008). For several decades, scientists agree that taxonomy of mole rats (Spalacinae, Rodentia) needs a modern revision based on chromosome and molecular genetic data coupled with morphology, physiology and behavior

(Savić & Nevo 1990, Kryštufek & Vohralík 2009). The genus *Spalax* differs from representatives of the genus *Nannospalax* in having a less variable diploid number ($2n = 60, 62$ in *Spalax* versus $2n = 36-60$ in *Nannospalax*) and a higher number of subtelocentric chromosomes ($NF = 116-124$ in *Spalax* versus $NF = 72-98$ in *Nannospalax* cytotypes) (Lyapunova et al. 1971, Savić & Nevo 1990, Németh et al. 2009), and shows slow chromosomal evolution rate (Kryštufek & Vohralík 2009). Kryštufek & Vohralík (2009) and Németh et al. (2009) ranked *Nannospalax* species as superspecies. Here we use superspecies order for Turkish mole rats instead of species.

According to Wilson & Reeder (1993) and Yiğit et al. (2006), three species, *Nannospalax leucodon*, *N. xanthodon* (senior synonym of *N. nehringi*; Kryštufek & Vohralík 2009) and *N. ehrenbergi*, occur in Turkey. *N. leucodon* is found in the Turkish Thrace, and *N. ehrenbergi* in southeastern Turkey. *N. xanthodon* has a wide distribution area extending over Anatolia.

Karyological studies revealed 17 cytotypes in Turkey: one cytotype ($2n = 56$) in *N. leucodon*, 11 ($2n = 36, 38, 40, 48, 50, 52, 54, 56, 58, 60$ and $60R$) in *N. xanthodon* and five ($2n = 48, 52, 54, 56, 58$) in *N. ehrenbergi* (see reviews in Sözen et al. 1999, 2006a, 2011, Coşkun et al. 2006, Kankılıç et al. 2007, 2010). Additionally, Nevo et al. (1994, 1995) recorded the $2n = 62$ form, but Ivanitskaya et al. (2008) reported that the $2n = 62$ forms should be eliminated from the list of Turkish mole rats. Later Arslan et al. (2011) studied C- and AgNOR banding patterns of three cytotypes ($2n = 40, 58$ and 60) from southern Anatolia. These karyological results show that one of the most complex chromosomal diversity within the distribution range of the genus *Nannospalax* is found in Turkey. These results complicate the taxonomical status of the cytotypes in *Nannospalax* in Turkey.

While taxonomic status of cytotypes of mole rats in Turkey has been under discussion, Nevo et al. (2001) raised four cytotypes ($2n = 52, 54, 58$ and 60) in Israel to species level; *Nannospalax galili* ($2n = 52$), *N. golani* ($2n = 54$), *N. carmeli* ($2n = 58$) and *N. judaei* ($2n = 60$). Arslan et al. (2010) studied mitochondrial DNA (mtDNA) variation between three cytotypes ($2n = 40, 58$ and 60) in southern Anatolia, and they showed well-supported lineages in the phylogenetic tree. But there is no detailed study based on mtDNA sequences on the genetic structure of cytotypes of mole rats in Western Turkey. This study focused on phylogenetic relationships among cytotypes of *N. xanthodon* in Western Turkey, and *N. leucodon* in the Turkish Thrace and *N. ehrenbergi* in southeast

of Turkey inferred from mtDNA cytochrome *b* gene sequences. We aimed to highlight the genetic relationships between and among the cytotypes of the species in Turkey, and also the recently described species of *Nannospalax* from Israel.

Material and Methods

Sampling for molecular studies

A total of 47 mole rat individuals were used in the molecular studies. These samples came from three previously recognized species, namely *N. leucodon* ($N = 3$), *N. xanthodon* ($N = 40$; cytotypes $2n = 36, 38, 40, 50, 56, 60$) and *N. ehrenbergi* ($N = 4$; cytotypes $2n = 52$ and 56) (Fig. 1). Karyotypes were prepared according to Ford & Hamerton (1956). Skins and skulls were deposited to the Department of Biology of Zonguldak Karaelmas and Ankara University, Turkey.

DNA isolation, amplification, and sequencing

We sequenced all 47 samples for a part of the cytochrome *b* locus. Total genomic DNA was isolated from muscle tissues following the techniques reported by Doyle & Doyle (1987) known as CTAB method. Fragment of the cytochrome *b* gene was amplified using polymerase chain reaction in $25 \mu\text{l}$ reaction volume and each reaction included $1 \mu\text{l}$ of each primer (20 pmol) (L14724 and H15154; Irwin et al. 1991,



Fig. 1. Sampled sites in Turkey and respective mole rat cytotypes.

Smith & Patton 1993), $4 \mu\text{l}$ of dNTPs, $2.5 \mu\text{l}$ of $10\times$ Taq Buffer with $(\text{NH}_4)_2\text{SO}_4$, $1.5 \mu\text{l}$ of MgCl_2 (25 mM) and $0.25 \mu\text{l}$ of Taq DNA Polymerase ($5 \text{ units}/\mu\text{l}$, Fermentas, Ontario, Canada). The PCR cycling conditions first included four cycles of 1 min of denaturation at 95°C , 1 min of annealing at 40°C , and 1 min extension at 72°C , followed by 33 cycles using annealing temperature at 50°C . Before the sequencing reaction

Table 1. Samples used in the present study.

Species	Map code	2n	Locality	Genbank accession number	Voucher name	Coordinates		
<i>N. leucodon</i>	1	56Tr	Kırklareli	FJ656299	TR-KIRKLARELİ 5097	41°25'34.05" N	27°7'9.09" E	
	1	56	Kırklareli	FJ656300	TR-KIRKLARELİ 5094	41°25'34.05" N	27°7'9.09" E	
	1	56	Kırklareli	FJ656301	TR-KIRKLARELİ 5095	41°25'34.05" N	27°7'9.09" E	
<i>N. xanthodon</i>	2	36	Aydın	FJ656275	TR-AYDIN 6218	37°51'46.19" N	27°49'27.67" E	
	2	36	Aydın	FJ656276	TR-AYDIN 6256	37°51'46.19" N	27°49'27.67" E	
	2	36	Aydın	FJ656277	TR-AYDIN 6222	37°51'46.19" N	27°49'27.67" E	
	2	36	Aydın	FJ656278	TR-AYDIN 6207	37°51'46.19" N	27°49'27.67" E	
	2	36	Aydın	FJ656279	TR-AYDIN 6250	37°51'46.19" N	27°49'27.67" E	
	2	36	Aydın	FJ656280	TR-AYDIN 6532	37°51'46.19" N	27°49'27.67" E	
	2	36	Aydın	FJ656281	TR-AYDIN 5258	37°51'46.19" N	27°49'27.67" E	
	3	38	Çanakkale	FJ656259	TR-GOKCEADA 4959	40°9'30.61" N	25°50'30.05" E	
	3	38	Çanakkale	FJ656260	TR-GOKCEADA 4957	40°9'30.61" N	25°50'30.05" E	
	3	38	Çanakkale	FJ656261	TR-GOKCEADA 4955	40°1'18.10" N	26°25'26.44" E	
	3	38	Çanakkale	FJ656262	TR-GOKCEADA 4954	40°1'18.10" N	26°25'26.44" E	
	3	38	Çanakkale	FJ656263	TR-GOKCEADA 4958	40°1'18.10" N	26°25'26.44" E	
	3	38	Manisa	FJ656282	TR-MANISA 5214	39°6'41.11" N	27°40'14.72" E	
	3	38	Manisa	FJ656283	TR-MANISA 5211	39°6'41.11" N	27°40'14.72" E	
	3	38	Balıkesir	FJ656284	TR-BALIKESIR 6120	39°22'14.33" N	27°59'25.34" E	
	3	38	Balıkesir	FJ656285	TR-BALIKESIR 6161	39°22'14.33" N	27°59'25.34" E	
	3	38	Manisa	FJ656286	TR-MANISA 5210	39°10'24.88" N	27°51'18.40" E	
	3	38	Manisa	FJ656287	TR-MANISA 6131	39°10'24.88" N	27°51'18.40" E	
	3	38	Manisa	FJ656288	TR-MANISA 6153	39°10'24.88" N	27°51'18.40" E	
	3	38	Izmir	FJ656289	TR-IZMIR 6138	38°27'40.06" N	27°12'51.77" E	
	4	40	Isparta	FJ656264	TR-ISPARTA 6202	37°43'7.76" N	30°56'55.64" E	
	4	40	Isparta	FJ656265	TR-ISPARTA 6225	37°43'7.76" N	30°56'55.64" E	
	4	40	Isparta	FJ656266	TR-ISPARTA 6265	37°43'7.76" N	30°56'55.64" E	
	4	40	Isparta	FJ656267	TR-ISPARTA 6266	37°43'7.76" N	30°56'55.64" E	
	4	40	Konya	FJ656268	TR-KONYA 6268	37°30'42.12" N	31°24'3.10" E	
	4	40	Konya	FJ656269	TR-KONYA 5346	37°30'42.12" N	31°24'3.10" E	
	4	40	Konya	FJ656270	TR-KONYA 6203	37°30'42.12" N	31°24'3.10" E	
	4	40	Konya	FJ656271	TR-KONYA 4734	37°30'42.12" N	31°24'3.10" E	
	4	40	Konya	FJ656272	TR-KONYA 6267	37°30'42.12" N	31°24'3.10" E	
	5	50	Aydın	FJ656273	TR-AYDIN 5255	38°15'15.61" N	28°23'15.30" E	
	5	50	Aydın	FJ656274	TR-AYDIN 5256	38°15'15.61" N	28°23'15.30" E	
	6	56	Uşak	FJ656290	TR-USAK 6224	38°40'28.91" N	29°14'15.28" E	
	6	56	Manisa	FJ656291	TR-MANISA 6141	38°28'1.76" N	28°38'36.98" E	
	6	56	Isparta	FJ656292	TR-ISPARTA 4784	37°47'46.80" N	30°54'23.74" E	
	6	56	Isparta	FJ656294	TR-ISPARTA 6257	37°47'46.80" N	30°54'23.74" E	
	6	56	Karabük	FJ656297	TR-KARABUK 4858	41°13'21.30" N	32°43'16.30" E	
	7	60	Manisa	FJ656293	TR-MANISA 6143	38°43'17.87" N	28°52'13.76" E	
	7	60	Kütahya	FJ656295	TR-KUTAHYA 3683	39°24'5.99" N	29°16'14.63" E	
	7	60	Kütahya	FJ656296	TR-KUTAHYA 6119	39°24'5.99" N	29°16'14.63" E	
	7	60R	Kastamonu	FJ656298	TR-KASTAMONU 5607	41°42'25.42" N	33°35'24.63" E	
	<i>N. ehrenbergi</i>	8	56	Osmaniye	FJ656304	TR-OSMANIYE 5301	37°4'34.75" N	36°14'22.08" E
		8	56	Osmaniye	FJ656305	TR-OSMANIYE 5302	37°4'34.75" N	36°14'22.08" E
		9	52	Kilis	FJ656302	TR-KILIS 5120	36°47'34.56" N	37°15'27.46" E
		9	52	Kilis	FJ656303	TR-KILIS 5121	36°47'34.56" N	37°15'27.46" E

the PCR fragments were cleaned with Nucleospin extract kit (Macherey-Nagel, Düren, Germany) and later sequencing reactions of both DNA strands were commercially performed using Big Dye Terminator

v. 3.1 sequencing chemistry on an ABI 3100 Genetic Analyzer (Applied Biosystems, California, USA). All specimens were deposited to the GenBank under the accession numbers FJ656259-FJ656305 (Table 1).

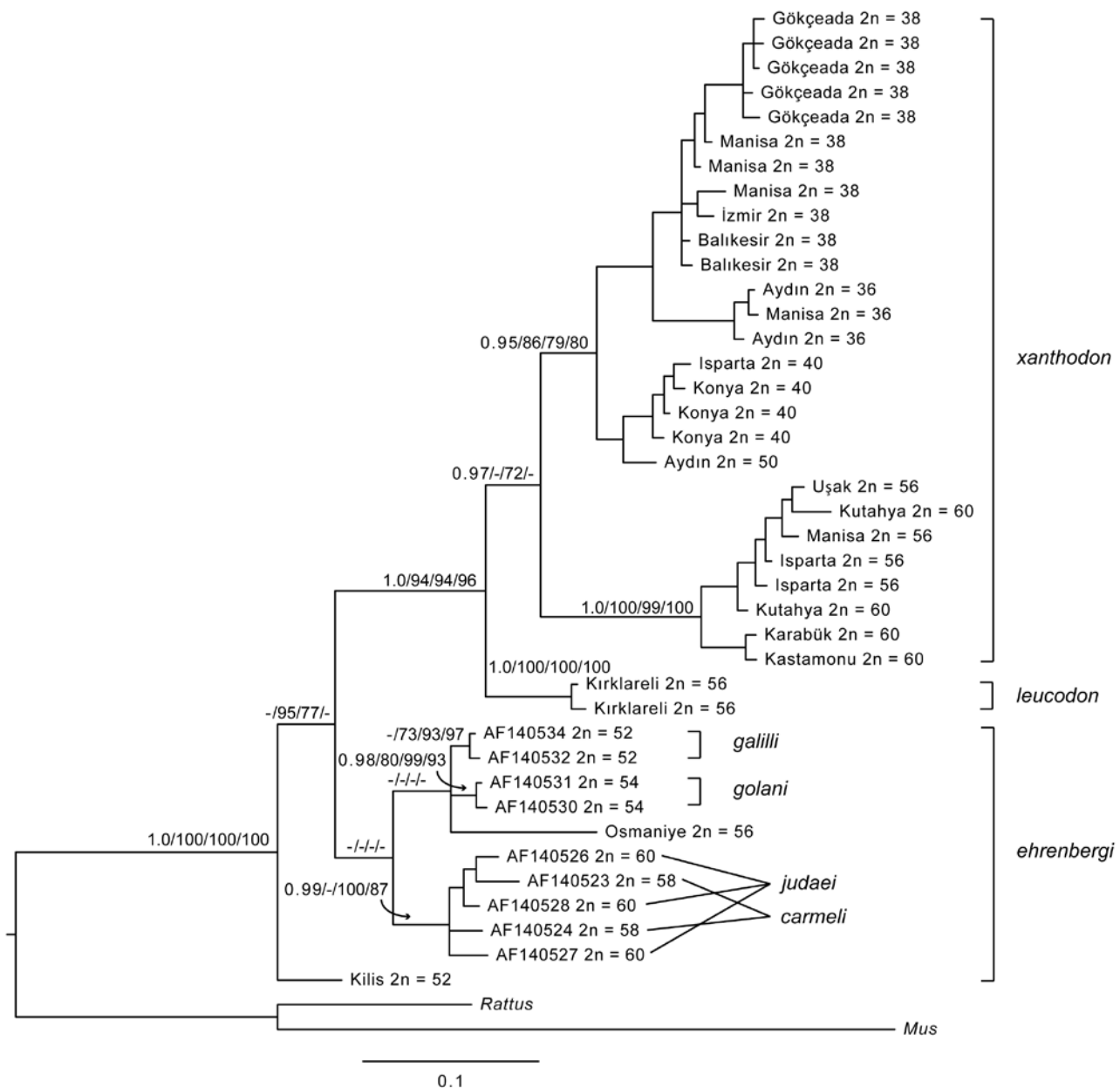


Fig. 2. Bayesian inference phylogenetic tree based on *Nannospalax* partial sequences of the mitochondrial cytochrome b gene (402 bp). Node support from Bayesian posterior probabilities and NJ, ML and MP bootstrapping is shown for main groups. '-' indicates non-significant support, < 70 % for bootstrap analyses and < 0.95 for Bayesian posterior probability.

Phylogenetic analyses

Previously published cytochrome *b* sequences of *Nannospalax* were obtained from GenBank (AF140523-AF140534; Nevo et al. 1999), and two other sequences for *Mus musculus* (NC010339) and *Rattus norvegicus* (EU273707) were added as an outgroup. The dataset was aligned in Clustal X 2.0.9 (Larkin et al. 2007) and had the total length 402 base-pairs.

Genetic divergence was estimated in *MEGA* 4 (Tamura et al. 2007). Neighbour-joining (NJ) and maximum

parsimony (MP) phylogenetic analyses were executed in PAUP 4.0b10 (Swofford 1999), using HKY + Γ substitution model for the NJ analysis selected by Bayesian Information Criterion in Modeltest 3.7 (Posada & Crandall 1998), which was the simplest suitable model, and 10000 bootstrap replicates. Maximum likelihood (ML) tree was obtained from RAxML 7.4 (Stamatakis 2006) with GTR + Γ model and 10000 bootstrap replicates. Bayesian Inference (BI) analysis was run in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) with 12 Markov chains Monte

Carlo for two million generations in two independent runs. The chain swapping was successful in 68-72 % of attempts indicating effective chain mixing. We used default prior settings with the exception of the branch length. That was limited to $\text{brlenspr} = \text{unconstrained: exp}(20)$ to ensure that the posterior 95 % confidence interval of the tree length included the tree length obtained from the maximum likelihood analysis (*cf.* Marshall 2010). The prior setting did not influence tree topology compared to the default setting (data not shown). A median-joining network (Bandelt et al. 1999) was also reconstructed for the sequenced mtDNA region. Finally, tree topologies were compared by using the Shimodaira-Hasegawa test in PAUP 4.0b10 (Swofford 1999).

Results

The 47 sequences represented 42 unique haplotypes, which were used in the phylogenetic analyses. The 402 bp long dataset contained 120 parsimony informative sites and 31 singleton mutations. Support for monophyly of the studied taxa based on partial cytochrome *b* sequences was different for specific analyses (Fig. 2). Total tree length in the MP analysis was 364 steps and the groupings reflected the genetic distances between cytotypes and species.

Nucleotide diversity within cytotypes ranged from 0 to 0.03 substitutions per site (Table 2). Within *N. xanthodon*, the greatest diversity was found in the $2n = 38$ cytotype that was also represented by the highest number of individuals in our dataset.

The genetic distances were calculated between all taxa used in the present study using uncorrected

Table 2. Within group nucleotide diversity for partial sequences of the mitochondrial cytochrome *b* gene (402 bp) for identified *Nannospalax* cytotypes.

Cytotypes	N	Nucleotide diversity	Standard Error
<i>N. galili</i> $2n = 52^1$	2	0.0017	0.0016
<i>N. golani</i> $2n = 54^1$	2	0.0017	0.0016
<i>N. judaei</i> $2n = 60^1$	3	0.0216	0.0058
<i>N. carmeli</i> $2n = 58^1$	2	0.0265	0.0066
<i>N. ehrenbergi</i> $2n = 52^2$	2	0.000	0.000
<i>N. ehrenbergi</i> $2n = 56^2$	2	0.000	0.000
<i>N. xanthodon</i> $2n = 36^2$	7	0.0021	0.0012
<i>N. xanthodon</i> $2n = 38^2$	13	0.0192	0.0044
<i>N. xanthodon</i> $2n = 40^2$	9	0.0072	0.0028
<i>N. xanthodon</i> $2n = 50^2$	2	0.000	0.000
<i>N. xanthodon</i> $2n = 56^2$	5	0.0033	0.0023
<i>N. xanthodon</i> $2n = 60^2$	4	0.0108	0.0038
<i>N. leucodon</i> $2n = 56^2$	3	0.0030	0.0058

¹Nevo et al. 1999, ²this study.

p-distance (Table 3). The distance between four Israeli *Nannospalax* species ranged between 0.019 and 0.058 substitutions per site. The cytotypes attributed to *N. xanthodon* showed deeper divergence ranging between 0.024 and 0.105. The greatest genetic distance between studied cytotypes was between *N. carmeli* and *N. xanthodon* $2n = 40$. Smaller genetic distances tended to be found between the chromosomal cytotypes with the highest diploid chromosome numbers. The genetic distance between the two cytotypes of *N. ehrenbergi* in Turkey was 0.087, which is greater than the genetic distance between the two most distantly related Israeli species (*N. golani* and *N. carmeli*) (Table 3).

Table 3. Pairwise uncorrected *p*-distance for partial sequences of the mitochondrial cytochrome *b* gene (402 bp) for identified *Nannospalax* cytotypes (below diagonal) and their standard errors (above diagonal).

	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>N. galili</i> $2n = 52^1$		0.007	0.010	0.010	0.013	0.012	0.014	0.014	0.015	0.014	0.014	0.015	0.014
<i>N. golani</i> $2n = 54^1$	0.019		0.011	0.010	0.013	0.013	0.015	0.015	0.015	0.014	0.015	0.015	0.014
<i>N. judaei</i> $2n = 60^1$	0.052	0.056		0.005	0.012	0.014	0.014	0.014	0.015	0.014	0.014	0.014	0.014
<i>N. carmeli</i> $2n = 58^1$	0.056	0.058	0.022		0.012	0.014	0.015	0.014	0.015	0.014	0.014	0.014	0.014
<i>N. ehrenbergi</i> $2n = 52^2$	0.080	0.078	0.076	0.076		0.013	0.014	0.015	0.015	0.015	0.015	0.015	0.015
<i>N. ehrenbergi</i> $2n = 56^2$	0.068	0.066	0.096	0.098	0.087		0.014	0.015	0.015	0.014	0.015	0.015	0.015
<i>N. xanthodon</i> $2n = 40^2$	0.119	0.118	0.120	0.125	0.104	0.108		0.010	0.007	0.011	0.012	0.014	0.013
<i>N. xanthodon</i> $2n = 38^2$	0.119	0.118	0.117	0.123	0.113	0.117	0.059		0.010	0.010	0.012	0.014	0.013
<i>N. xanthodon</i> $2n = 50^2$	0.119	0.118	0.117	0.121	0.104	0.112	0.029	0.053		0.012	0.012	0.014	0.013
<i>N. xanthodon</i> $2n = 36^2$	0.100	0.099	0.107	0.107	0.102	0.097	0.062	0.052	0.065		0.013	0.014	0.013
<i>N. leucodon</i> $2n = 56^2$	0.101	0.107	0.106	0.114	0.111	0.116	0.080	0.080	0.076	0.089		0.014	0.013
<i>N. xanthodon</i> $2n = 56^2$	0.123	0.113	0.117	0.120	0.121	0.124	0.104	0.105	0.100	0.102	0.099		0.005
<i>N. xanthodon</i> $2n = 60^2$	0.122	0.116	0.115	0.119	0.121	0.121	0.102	0.104	0.098	0.099	0.098	0.024	

¹Nevo et al. 1999, ²this study.

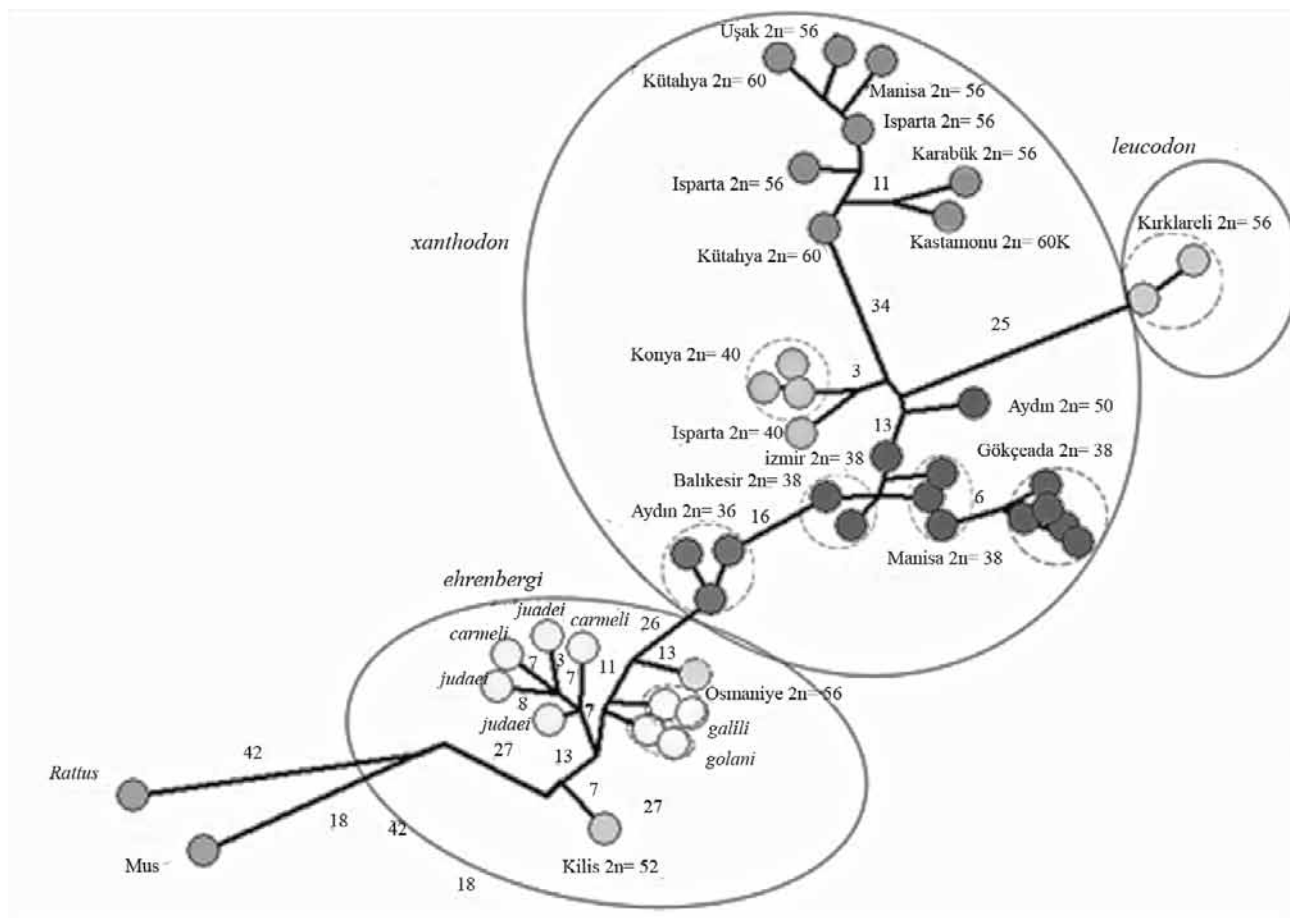


Fig. 3. The 42 sequences appear as three different species and five clades on median joining network congruent with Fig. 2. The samples from identical localities are indicated with small circles. The node size is not proportional to the haplotype frequency. The numbers on branch show differences between two neighboring haplotypes.

We conducted four separate sets of analyses. The data were analyzed using Bayesian inference, neighbour-joining, maximum likelihood and maximum parsimony analyses. The trees showed similar topologies (Fig. 2). The result of the Shimodaira-Hasegawa test showed that only NJ tree topology was significantly different from the other trees (Table 4). This result was caused by the placement of the *N. ehrenbergi* clade. In all trees, cytotypes with low diploid chromosome numbers ($2n \leq 50$) grouped as one supported clade.

Table 4. Results of Shimodaira-Hasegawa (SH) tests for all trees. The NJ tree topology was found different from ML (* $p < 0.05$).

Tree	-ln L	Diff -ln L	P
Bayesian inference	227.988	0.434	0.52
Maximum likelihood	227.553	(best)	
Maximum parsimony	228.626	1.073	0.29
Neighbour-joining	231.095	3.542	0.01*

The other *N. xanthodon* cytotypes ($2n = 56$ and 60) occurred as a separate clade, but monophyly of *N. xanthodon* was supported only in BI and ML analyses (Fig. 2). *N. leucodon* formed a monophyletic group with *N. xanthodon*. The other taxa showed a basal polytomy in most of our analyses. Both cytotypes of *N. ehrenbergi* were paraphyletic with respect to the Israeli taxa. *N. carmeli* and *N. judaei* formed a monophyletic group, but the taxa were not reciprocally monophyletic within this group.

We found similar topology in the network analysis (Fig. 3). The *N. xanthodon* cytotypes formed two putative groups with low ($2n \leq 50$) and high ($2n \geq 56$) chromosome numbers. The former group was more closely related to *N. leucodon* as well as to the group including *N. ehrenbergi* and the Israeli taxa (Fig. 3).

Discussion

Our phylogenetic analysis showed monophyly of *N. xanthodon* and *N. leucodon*, but paraphyletic

relationships of the two *N. ehrenbergi* sequences with Israeli taxa. *N. xanthodon* sequences formed two reciprocally monophyletic groups that included cytotypes with low ($2n \leq 50$) and high ($2n \geq 56$) chromosome numbers. Their sister species was *N. leucodon*. These results are similar to recent findings of Arslan et al. (2010) on a smaller dataset including three cytotypes and Kryštufek et al. (2012) that were based on a longer alignment. The taxa *N. ehrenbergi*, *N. galili*, *N. golani*, *N. carmeli* and *N. judaei* diverged in a basal polytomy wherein only a single group including *N. carmeli* and *N. judaei* was consistently retrieved. While Reyes et al. (2003) also showed an undifferentiated clade of *N. carmeli* and *N. judaei* samples, our analyses did not support their clear relationship of *N. galili* and *N. golani*. This was distorted by position of *N. ehrenbergi* $2n = 56$ as a sister taxon with unresolved relationship to the Israeli species.

Cytotypes of *N. xanthodon* with low diploid number ($2n = 36, 38, 40$ and 50) formed a monophyletic group. This may be a result of specific evolutionary pathways. For example, Matur & Sözen (2005) stated that the River Sakarya separated $2n = 52$ and 60 cytotypes of *N. xanthodon* in Bilecik province, and the river might act as a barrier between these cytotypes. But in our analyses, $2n = 52$ *N. xanthodon* was not analysed and we cannot confirm this scenario. According to our results, $2n = 38$ from Gökçeada (Aegean island) differentiated from the $2n = 38$ populations in the mainland indicating mole rat diversification in island isolation.

Monophyly of specific cytotypes was present in all cytotypes belonging to the *N. xanthodon* $2n \leq 50$ group, but the cytotypes were not likewise differentiated in the $2n \geq 56$ group. Rather, the northern populations of *N. xanthodon*, Karabük ($2n = 56N$) and Kastamonu ($2n = 60R$), were differentiated from the other samples of $2n = 56$ and 60 . This was suggested by Sözen (2004) and Sözen et al. (2006b), who claimed that the cytotypes from Northern Anatolia ($2n = 54N, 56N, 58N$ and $60R$) differentiated from other cytotypes. Ivanitskaya et al. (2008) showed that the $2n = 60$ population in Kastamonu is different from the $2n = 60$ population in central Anatolia using classical and molecular cytogenetic techniques. Matur et al. (2010) studied chromosomal evolution of Turkish mole rats inferred from G- and C- banding of 11 cytotypes. They found that cytotypes from Kastamonu ($2n = 60R$) and Karabük ($2n = 56R$) formed a clade together with $2n$

$= 54$ cytotype not included in our study. This northern clade has independent evolutionary pathway, and $2n = 60R$ might be considered an ancestral karyotype (Matur et al. 2010).

There is a conflict in the ancestral population of Thracian mole rats (*N. leucodon* $2n = 56$). They grouped with *N. xanthodon* in our trees. Recently, Matur et al. (2011) proposed the $2n = 60$ cytotype as an ancestral for all Anatolian cytotypes. Nevertheless, this process needs more detailed studies in order to verify the ancestral karyotype of the *N. xanthodon/leucodon* group.

In our phylogenetic analyses, *N. carmeli* ($2n = 58$) and *N. judaei* ($2n = 60$) did not appear in separate clusters. Reyes et al. (2003) found similar results from the sequence studies of the mitochondrial control region. In their study, *N. golani* and *N. galili* were well separated, whereas *N. judaei* ($2n = 60$) and *N. carmeli* ($2n = 58$) formed a single cluster. Nevo et al. (1999) argued that this is due to the fact that they represent young species. The paraphyly of the two cytotypes of *N. ehrenbergi* ($2n = 52$ and $2n = 56$) from Kilis and Osmaniye further complicate this in our study. Both cytotypes formed unsupported relationships and polychotomies. Tentatively, *N. ehrenbergi* from Osmaniye grouped with *N. galili* and *N. golani*, and *N. ehrenbergi* from Kilis was a basal taxon in the tree (Fig. 2).

Further comprehensive and detailed multidisciplinary research combining morphology, karyology, physiology, behaviour, mtDNA and nuclear DNA phylogeny should be applied to clarify the taxonomic status and phylogenetic relationships of cytotypes of *N. leucodon*, *N. xanthodon*, and *N. ehrenbergi* in Turkey. In addition, research on intra-population variation should also be considered for better understanding of mole rats in Turkey.

Acknowledgements

This study was supported by the Scientific and Technical Research Council of Turkey (TUBITAK 101T084 and 106T225), Zonguldak Karaelmas University (2004-13-06-08, 2008-13-06-01) and Academy of Sciences of the Czech Republic (AV0Z60930519). The authors thank Ahmet Turkyilmaz from REFGEN Biotechnology Inc. for performing the sequencing. Authors thank to Zeynep Yuksel Sezen and Joan E. Johnston for careful editing the manuscript and two anonymous reviewers and the editor for helpful and detailed comments.

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