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# Genetic Variations of Growth Hormone Receptor Exon 10 in Blind Mole-Rat Superspecies (Rodentia: Spalacidae) in Turkey

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**Abstract:** A phylogenetic analysis of 16 cytotypes in a sample of 105 blind mole-rats of the genus *Nannospalax* Palmer, 1903 from the entire Anatolian region has been carried out. Three superspecies (*N. xanthodon*, *N. leucodon* and *N. ehrenbergi*) were found to have undergone monophyletic radiations. We determined that the superspecies *N. ehrenbergi* and *N. xanthodon* contain a mixture of cryptic species distributed in the Anatolian part of Turkey. Our study indicated a fundamental split between two groups, one consisting of populations of *N. ehrenbergi* from the South-eastern Anatolia and the other including the remaining taxa (*N. xanthodon* and *N. leucodon*) from the remaining regions of Anatolia and from Thrace. Phylogenetic analysis indicated that there were two new unnamed cryptic species (2n=48–52 cytotype group and 2n=56 cytotype group) within the superspecies *N. ehrenbergi* that were reciprocally monophyletic. We reviewed species identified by traditional morphological methods to determine their taxonomic validity. We suggest that *N. cilicicus* (stat. n.), which is endemic to the central Anatolia, has to be considered a distinct species and not a synonym of *N. xanthodon*.

**Key words:** *Nannospalax*, nuclear DNA, growth hormone receptor exon 10, phylogeny

## Introduction

The genus *Nannospalax* Palmer, 1903 (blind mole-rats) includes wild rodents of the family Spalacidae; they are descended from a muroid-cricetoid stock widespread in Asia Minor during the Oligocene (Savić & Nevo 1990, 1991). These subterranean rodents are known for their extensive underground lifestyle, primarily characterised by the excavation using teeth and the consumption of tuberous and bulb plants (Sözen 2005). Living in a subterranean environment may expose animals, in comparison with those living above ground, to environmental stress factors such as darkness, low productivity, lack of nutrients, soil and rock barriers, hypoxia, hypercapnia and high pathogen abundance. All these stress factors play a significant role in the evolutionary process of blind mole-rats. Consequently, mole-rats evolved a variety of genetic adaptations to deal with these stressors (Nevo et al. 1994, 1995).

Over the last 30 years, the genus *Nannospalax* has been utilised as a model organism in speciation

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and adaptive radiation research (Nevo 2013). Chromosomal, genetic and biogeographical relationships in blind mole-rat populations have been examined in several studies throughout their distribution range (Savić & Nevo 1990, Kryštufek et al. 2012, Hadid et al. 2012, Németh et al. 2013, Csorba et al. 2015, Matur et al. 2019, Bugarski et al. 2020).

Blind mole-rat species are difficult to classify using conventional methods because they are morphologically identical and exhibit high chromosomal polymorphism. For this reason, recent solutions have been sought through using molecular markers (Kryštufek et al. 2012).

The genus *Nannospalax* is distributed in South-eastern Europe, Turkey, Armenia, Syria, Palestine, Iraq, Israel, Jordan, Egypt and Lebanon (Wilson & Reeder 2005). Recent studies based on morphology have suggested that only three superspecies, *N. ehrenbergi* (Nehring, 1898), *N. leucodon* (Nordmann, 1840) and *N. xanthodon* (Nordmann, 1845), exist in Turkey (Musser & Carleton 2005, Kryštufek & Vohralik 2009). *Nannospalax ehrenbergi* has seven diploid chromosome numbers ranging from 2n = 48 to 62, or 20 cytotypes with different chromosomes (Nevo et al. 2001, Coşkun et al. 2006, Arslan et al. 2016). Seven distinct diploid chromosome numbers have been described in *N. leucodon*, ranging from 2n = 46 to 58, or 25 cytotypes with different chromosome arms (Arslan et al. 2016, Savić et al. 2017, Bugarski et al. 2020). The superspecies with the highest variation in diploid chromosome number is *N. xanthodon*, which is mostly found in Anatolia (Turkey). There are 32 cytotypes of this species, with 13 distinct diploid chromosome numbers (2n = 36 to 62) and chromosome arms (Sözen 2004, Sözen et al. 2006, 2013, Coşkun et al. 2006, Kankiliç et al. 2007, 2009, 2010, Arslan et al. 2016).

On the other hand, there is no precise way to classify Turkey's distinct cytotypes as species (Kryštufek et al. 2012). Especially with the use of molecular techniques to identify genetic interspecific variability, the number of newly-found species gradually increased and cryptic species, which were morphologically identical but genetically distinct, were identified (Adams et al. 2014, Poulin & Pérez-Ponce de León 2016). Methods for detecting cryptic species by using DNA-based methods became more popular (Bugarski-Stanojević et al. 2022).

Although Turkey has a high diversity of blind mole-rat species and superspecies cytotypes, most of the research conducted on has been focused on cytogenetic and morphological comparisons. Few genetic studies aimed at determining the evolutionary relationships between different cytotypes (Kandemir et al. 2012, Kryštufek et al. 2012, Hadid et al. 2012, Matur et al. 2019, Bugarski et al. 2020). Using a small number of specimens, molecular studies in Turkey verified the monophyly of the genus and revealed four primary clades: *vasvarii* (or *cilicicus*), *leucodon*, *xanthodon* and *ehrenbergi*. The clade *vasvarii* (or *cilicicus*) represented the cytotypes (2n = 56, 58, 60) that originated from the Central Anatolian Plateau (Hadid et al. 2012, Kryštufek et al. 2012). These phylogenetic analyses have identified only two blind mole-rat species (*N. xanthodon* and *N. ehrenbergi*) in Turkey's Asian region. The species *N. nehringi, N. labaumei*, *N. tuncelicus, N. munzuri* and *N. vasvarii*, which were previously described using morphological taxonomic techniques, have been considered as synonyms of *N. xanthodon* (Kryštufek & Vohralík 2009).

The purpose of this study on the genus *Nannospalax* was to explore a large sample collection from Turkey and achieve the following objectives: (1) establish a comprehensive molecular phylogeny for the Turkish species of the genus *Nannospalax*, and (2) clarify species boundaries in widely distributed taxa and identify new species. İn this study, we present the DNA sequence of the nuclear gene part, growth hormone receptor exon 10 (*GHR exon 10*) based on phylogeny for the genus *Nannospalax*. We also look into the phylogeography of Turkish species of *Nannospalax*.

# Materials and Methods

### Specimens

We analysed 105 blind mole-rat tissue samples from 71 locations in Turkey. These samples represented 16 chromosomal forms belonging to four species (Kankiliç et al. 2007). The study utilised tissues (such as liver and muscle) that were stored in a deep freezer. These tissues were collected by T. Kankiliç during field work conducted throughout Turkey. These tissue samples were used in accordance with the permission of Niğde Ömer Halisdemir University Animal Experiments Ethics Committee (No. B.30.2.NĞÜ.0.70.00.00/050-99/13 from 04.01.2012) and the permission of the Ministry of Forestry and Water Affairs General Directorate of Nature Conservation and National Parks (No. B.23.0.DMP.0.15.01.510.02-15758 from 14.10.2011).

We studied sequence variability of the nuclear DNA gene segment GHR exon 10 among six blind mole-rat species: 35 individuals of *N. xanthodon* presented with four cytotypes (2n=36, 38, 40, and

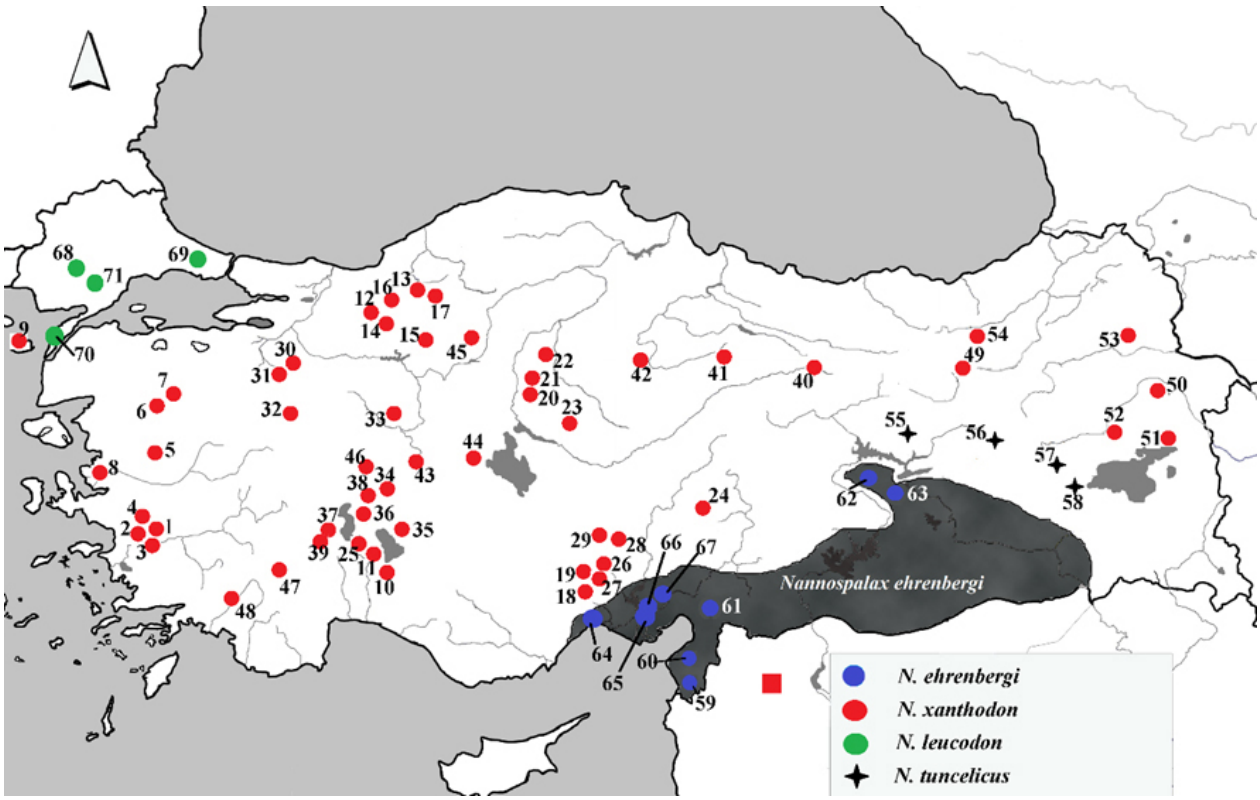


**Fig. 1.** Localities of samples of *Nannospalax* used in this study. Numbers of the localities are given in Supplementary Table 1.

52) in the northern part of Turkey; 34 specimens of *N. cilicicus* presented with five cytotypes (2n=52, 54, 56, 58, and 60) in the southern part of Turkey; six individuals of *N. leucodon* with only one cytotype (2n = 56); 16 individuals of species *N. ehrenbergi* with three cytotypes (2n=48, 52, and 56); eight individuals of species *N. nehringi* with three cytotypes (2n=44, 48, and 50) and six individuals of *N. tuncelicus* with only one cytotype (2n=54). GenBank accession numbers for all data, all sampled species, locations and voucher specimen numbers are listed in the supplementary table, while the localities are presented in Fig. 1. In the phylogenetic analysis, two GHR exon sequences from the genera *Mus* and *Rattus* (Muridae) were utilised as an outgroup.

### Nuclear DNA isolation of samples

In this study, the animals were caught using metal traps and brought to the laboratory alive. DNA was isolated from the liver tissues of the blind mole-rat specimens using CTAB (Cetyltrimethylammonium Bromide) method (Doyle 1991) After DNA isolation, the quality and quantity of DNA were measured using a Shimadzu's Biospec-nano Spectrophotometer. The DNA concentration obtained from the measurements was diluted to a concentration of 50 ng/µl in each tube.

### PCR amplification and sequencing

A segment of 819 bp of the nuclear gene GHR exon 10 was amplified using the primer pairs from Adkins et al. (2001) and Steppan et al. (2004). PCR amplification was carried out in a 50 µl reaction volume using 10 pmol of each primer, 2.5 mM dNTP, 5X buffer, 5U/µl Taq polymerase and distilled water. PCR cycling conditions contained an initial denaturation step at 95 C° for 5 min, followed by 35 cycles of 45 sec at 95 C°, 90 sec at 50 C° and 100 sec at 72 C° and a final extension step at 72 C° for 10 min. Amplification products were separated on a 1.5% agarose gel for 40 min at 100 V and visualised. The PCR products were sent to Macrogen Europe (Netherlands) to determine DNA sequences using the Sanger sequencing method. Bioedit 7.2 (Hall 1999) was used to align chromatograms and trim low-quality nucleotides in the GHR 10 exon for each sample.

### Phylogenetic analyses

The following statistical analyses were performed to calculate the differences and variations between populations. ARLEQUIN ver. 3.5.2.2 software package (Excoffier & Lischer 2010) commonly used in population genetics studies, was used to identify the haplotypes. Haplotypes were deter-

mined individually or based on genus and cytotype, using DnaSP6 (Rozas et al. 2017). The evolutionary history was inferred using the Maximum likelihood (ML), Bayesian Inference (BI) and Neighbour-joining methods (NJ). Prior to the reconstruction of phylogenetic trees, the best nucleotide substitution models for each DNA region were determined using the MEGA-X software (Kumar et al. 2018) and Modelfinder (Chernomor et al. 2016, Kalyaanamoorthy et al. 2017) programs integrated in the IQ-TREE web server (Trifinopoulos et al. 2016, http://iqtree.cibiv.univie.ac.at/). The Kimura 2-parameter (K2, AIC: 4213.48, BIC: 4364.18) model with ML and NJ as well as Hasegawa, Kishino & Yano (HKY AIC: 4205.25, BIC:4370.03) model with BI were used.

An assessment of the reliability of a specific grouping can be made by repeating the process several times (ideally 1000 times) and compiling the results. In BEAST v1.8.0, four separate Markov Chain Monte Carlo inference (Yang & Rannala 1997) runs for 10.000.000 generations with a sampling frequency of every 1000 generations were conducted for the Bayesian analyses to exceed the bottom bound of effective sample size (ESS>200) (Drummond et al. 2012). Mega X was used to estimate genetic distances between populations from each locality using the Kimura 2 parameter (K2P) model (Kumar et al. 2018). Network analysis using Network 5.0.0.1 (Bandelt et al. 1999) was used to further understand how haplotypes were related to each other and sort out blind mole-rat individuals with identical haplotypes.

# Results

### Sequence composition and variation

Sequences of the 819-bp GHR exon 10 gene fragments were obtained for 105 specimens from six "morphological" species of *Nannospalax*: *N. ehrenbergi*, *N. leucodon*, *N. xanthodon*, *N. nehringi*, *N. cilicicus* and *N. tuncelicus*. The most common base is generally A or T (average 51%), and the rarest base is G (average 21%). The aligned GHR data set included 819 characters, of which 26 had the potential to be phylogenetically relevant and 28 were variable sites. The average ratio for transition to transversion was found to be 1.23. The nucleotide frequencies were A = 29.30%, T = 22.48%, C = 26.35%, and G = 21.87%. The species and populations were divided into 15 distinct haplotypes. Except for *N. leucodon*, all of the studied species appear to be polymorphic based on the distribution of these haplotypes within the species. The largest number of haplotypes were observed in *N. cilicicus* (H3, H9, H12 and H14) (Table 1). Nucleotide and haplotype diversities were calculated for all mole-rat populations. The results of these analyses demonstrated that *N. cilicicus* and *N. ehrenbergi* could be qualified by the highest values of nucleotide diversity ($\pi$=0.0018–0.0007). Six samples of *N. leucodon*, all belonging to the same haplotype, exhibited the lowest nucleotide diversity value (Table 1).

### Phylogenetic analysis

Maximum likelihood, BI and NJ methods rendered similar tree topologies. Nevertheless, these trees differed in the number of unresolved nodes: it was higher in the BI than in the ML and NJ trees. The tree presented in Figure 2 represents the consensus topology with confidence values of the interior branch in the following order: NJ, MP and BI analyses.

The genus *Nannospalax* was divided into two major clades with a very high bootstrap support (BS: 100/100/1.00). One clade comprised only a superspecies from South-eastern Anatolia (*N. ehrenbergi*) and the second one the superspecies *N. leucodon* and *N. xanthodon*. Pairwise comparisons among six species of *Nannospalax* are summarized in Table 2. Kimura 2-parameter distances within species of two different major clades ranged from 0.0116–0.0129 (*ehrenbergi* versus *xanthodon*) to 0.019 (*ehrenbergi* versus *leucodon*).

The first major clade I included three distinct clades (A, B and C). All populations of *N. leucodon* (C) and *N. xanthodon* (A-B) formed strongly supported (BS: 95/93/1.00) reciprocal monophyletic groups in major clade I. The two reciprocal monophyletic groups (*xanthodon* and *leucodon*), which ranged in divergence from 0.0141 to 0.0154, could be distinguished clearly due to the relatively large amount of divergence detected across all the sequences studied. The haplotypes of the superspecies *N. xanthodon* (n=11) formed two groups with moderate bootstrap support (BS: 82/62/0.92), clade A (*N. tuncelicus*, *N. xanthodon* and *N. nehringi*) and clade B (*N. cilicicus*), which corresponded completely with the species allocations in Anatolia discussed below. Kimura 2-parameter distances between these groups ranged from 0.0026 to 0.0052. The genetic divergence values within clade A (*N. xanthodon*), the genetic divergence values were low, ranging from 0.0026 (*N. xanthodon* versus *N. tuncelicus*) to 0.0042 (*N. nehringi* versus *N. tuncelicus*).

The second major clade (*N. ehrenbergi*) was clearly monophyletic (100% bootstrap support) and further divided into two groups, supported by a bootstrap value >88%, the first (D) consisted of

**Table 1.** Genetic diversity parameters in six species of *Nannospalax* from Turkey.

| Species | No of sequences | No of haplotypes | Hd | Pi |
|---|---|---|---|---|
| *N. ehrenbergi* | 16 | 3 | 0.4916 | 0.0007 |
| *N. tuncelicus* | 6 | 2 | 0.5333 | 0.0006 |
| *N. xanthodon* | 35 | 3 | 0.4067 | 0.0005 |
| *N. leucodon* | 6 | 1 | 0.0000 | 0.0000 |
| *N. nehringi* | 8 | 2 | 0.4285 | 0.0005 |
| *N. cilicicus* | 34 | 4 | 0.7183 | 0.0018 |

*Hd: haplotype (gene) diversity. Pi: nucleotide diversity.

**Table 2.** Estimates of sequence divergence (Kimura-2 parameter) over sequence pairs.

| | ***N. ehrenbergi*** | ***N. leucodon*** | ***N. cilicicus*** | ***N. tuncelicus*** | ***N. nehringi*** | ***N. xanthodon*** |
|---|---|---|---|---|---|---|
| *N. ehrenbergi* | - | 0.0049 | 0.0035 | 0.0038 | 0.0036 | 0.0036 |
| *N. leucodon* | 0.0198 | - | 0.0041 | 0.0042 | 0.0041 | 0.0041 |
| *N. cilicicus* | 0.0120 | 0.0144 | - | 0.0021 | 0.0018 | 0.0018 |
| *N. tuncelicus* | 0.0129 | 0.0154 | 0.0052 | - | 0.0022 | 0.0014 |
| *N. nehringi* | 0.0116 | 0.0141 | 0.0039 | 0.0042 | - | 0.0020 |
| *N. xanthodon* | 0.0119 | 0.0143 | 0.0041 | 0.0026 | 0.0038 | - |

two cytotypes 2n=48 from Hatay and 2n=52 from İskenderun, Elazığ and Osmaniye, while the second (E) included only 2n=56 cytotype from Mersin and Adana.

The median-joining network using the 15 haplotypes of *Nannospalax* sp. showed a general congruence with the phylogenetic reconstruction, because the same two major clades and subclades of *Nannospalax* sp., as defined above, could be found. Yet the South-eastern Anatolian clade displayed a strong differentiation among other Anatolian clades, while the Anatolian clades (*N. xanthodon*, *N. tuncelicus* and *N. nehringi*) showed the lowest degrees of differentiation (Fig. 3).

# Discussion

In previous studies, the molecular phylogenetic relationships of Anatolian blind mole-rats were never thoroughly examined. Only a small portion of the cytotypes from Anatolia of widely-recognised species of the genus, including *N. leucodon, N. ehrenbergi*, and *N. xanthodon*, had been examined in previous phylogenetic analyses (Kandemir et al. 2012, Kryštufek et al. 2012, Hadid et al. 2012, Németh et al. 2013, Csorba et al. 2015, Matur et al. 2019). In this study, we included additional cytotypes of *Nannospalax*, not used in previous phylogenetic studies. In addition, three methods of phylogenetic reconstruction (ML, BI and NJ), based on the sequences of the nuclear GHR exon 10 gene, were utilised. Three superspecies of *Nannospalax* (*N. xanthodon*, *N. ehrenbergi* and *N. leucodon*) were clearly divided in the preliminary phylogenetic analysis, which was generally consistent with earlier phylogenetic research (Kandemir et al. 2012, Kryštufek et al. 2012, Hadid et al. 2012, Németh et al. 2013, Csorba et al. 2015, Matur et al. 2019, Bugarski et al. 2020). Our analysis revealed a fundamental split between Clade II, which included populations of *N. ehrenbergi*, from the South-eastern Anatolia and Clade I, which included the remaining taxa (*N. xanthodon* and *N. leucodon*) from Thrace, as well as other regions of Anatolia (Fig. 2). The superspecies *N. xanthodon* was represented by a strongly supported clade in the phylogenetic tree (Fig. 2) and was closely related to *N. leucodon*. The close phylogenetic relationships among two previously recognised taxonomic groups in major Clade I are concordant with the cytochrome b studies of Matur et al. (2019), Kandemir et al. (2012), Kryštufek et al. (2012) and Hadid et al. (2012). In addition, the genetic distance estimated between super species *N. xanthodon* and *N. ehrenbergi* was greater than between *N. xanthodon* and *N. leucodon.* These findings confirm the notion proposed by Hadid et al. (2012) that the Anatolian diagonal limits gene flow between *N. xanthodon* and *N. ehrenbergi*. Additionally, the results demonstrate that the South-eastern phylogroup (*N. ehrenbergi*) represents a unique evo-

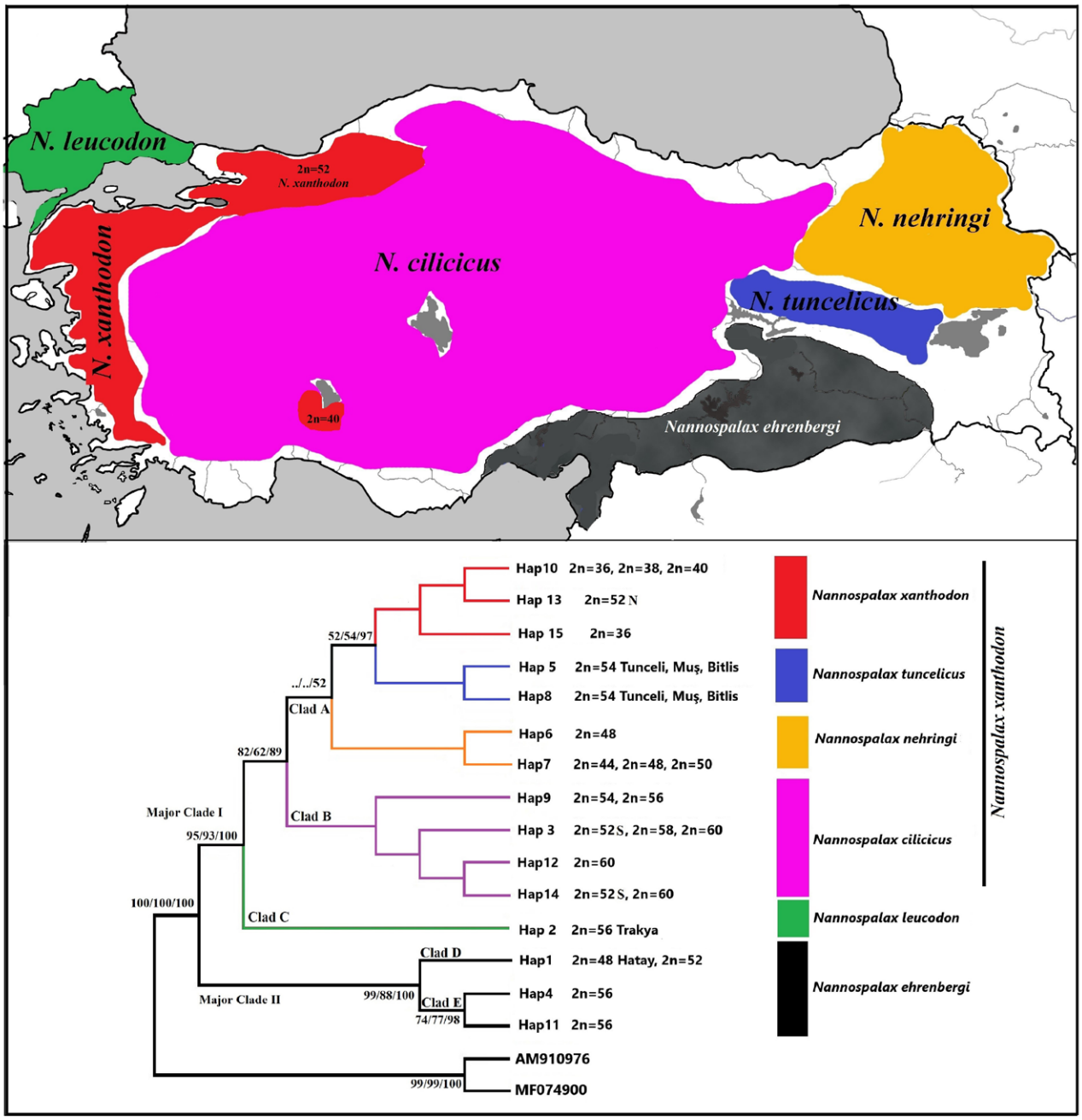


**Fig. 2.** Phylogenetic tree for the 15 haplotypes determined based on the growth hormone receptor (GHR) gene exon 10 sequences. Similar tree topologies were inferred though three methods: Maximum likelihood (ML), Neighbour Joining (NJ) and Bayesian inference (BI). The numbers at nodes presented next to the clade are in order of ML, NJ, and BI. The dash indicates branch scores <50.

lutionary path. According to Kryštufek & Vohralik (2009), there are three species of blind mole-rat in Turkey and if the cytotypes are regarded as different species, about 20 additional species classifications are required. Recent molecular phylogenetic analyses show that each cytotype lacks sufficient genetic diversity to represent a separate species (Kandemir et al. 2012, Kryštufek et al. 2012, Hadid et al. 2012, Németh et al. 2013, Csorba et al. 2015, Matur et al. 2019). On the contrary, phylogenetic studies show that different cytotype groups with allopatric distribution in Anatolia tend to speciate independently of each other (Matur et al. 2019). For example, in this study, sequences of *N. ehrenbergi* formed two reciprocally monophyletic groups that included cytotypes with low (2n=48 and 52) and high (2n=56) chromosome numbers.

Prior research has not fully analysed the molecular phylogenetic relationships of the three cytotypes of *N. ehrenbergi*. In the previously analysed

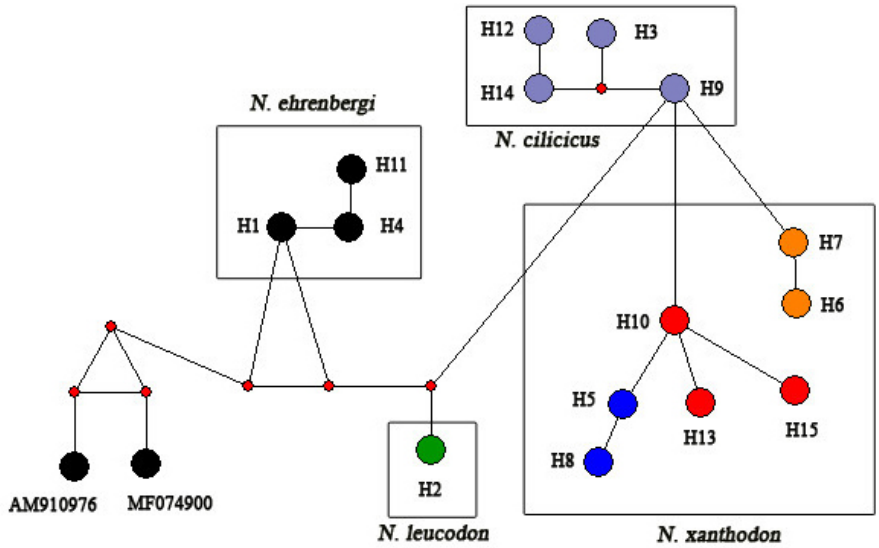


**Fig. 3.** Network analysis of the 15 haplotypes determined based on growth hormone receptor (GHR) gene exon 10 sequences.

Cyt-b and 16s rRNA phylogeny (Kandemir et al. 2012, Kryštufek et al. 2012, Hadid et al. 2012), only 2n=52 of these cytotypes were analysed together with the Israeli populations of *N. ehrenbergi*. In the study that did not include Anatolian *N. ehrenbergi* populations, according to Bugarski et al. (2020), Israel was home to two branches of superspecies of *N. ehrenbergi*, the first with a high 2n (*N. carmeli* / *N. judaei*) and the second with a low 2n (*N. golani* / *N. galili*). In other phylogenetic studies, Kilis population (Kandemir et al. 2012), Şanlıurfa population (Kryštufek et al. 2012) and Diyarbakır population (Hadid et al. 2012) belonging to the 2n=52 cytotype of *N. ehrenbergi* were found to be distinct from the rest of the populations of *N. ehrenbergi* and formed a basal taxon in the phylogenetic trees. Our molecular phylogenetic results are consistent with earlier studies, demonstrating that *N. ehrenbergi* conceals a significant amount of cryptic variety. Additionally, our findings suggest the presence of two new unidentified cryptic species in Anatolia (2n=48–52 cytotypes and 2n=56 cytotypes). The variant 2n=60 was described from Israel. This implies that identical cytotypes (2n=52 Israel and 2n=52 Turkey) might represent different species. Although the phylogenetic locations of the two main clades of *N. ehrenbergi* in this study received strong support (i.e., MLBS = 99%, NJBS = 88%, BIPP = 0.88), more molecular markers and deeper cytotype sampling should be added in subsequent studies to improve taxonomic resolution. Additionally, in order to identify and clarify cryptic species diversity in the superspecies, a more complete systematic analysis of cytotypes of *N. ehrenbergi* in Anatolia should be conducted in conjunction with the existing studies on Levantine mole-rats.

In our phylogenetic analyses, the taxa *N. cilicicus*, *N. nehringi*, *N. tuncelicus* and *N. xanthodon* diverged in basal polytomy. Only a single group, which included *N. cilicicus* populations with high 2n (2n=56, 58, 60), consistently clustered differently from the main group. That differentiation was supported by moderate bootstrap support values (MLBS = 82%, NJBS = 62%, BIPP = 0.92). Two geographically adjacent species, *N. tuncelicus* from Tunceli Province (Coşkun 1996) and *N. nehringi* from Kars-Kaskoparan (Satunin 1898) were described using traditional morphological methods. Kryštufek & Vohralik (2009) advocated that *N. xanthodon* Nordmann, 1840 was the oldest available name for Anatolian mole-rats and should be used as a species name. After this study, *N. nehringi* (Satunin, 1898) was used as a valid species name and the other species identified in Anatolia were accepted as *xanthodon*. Thus, *N. xanthodon* has been accepted as the dominant species spreading in all of Anatolia, except for the South-eastern Anatolian Region (*N. ehrenbergi*). Kryštufek & Vohralik (2009) emphasised that they have no doubt that the taxonomy of *N. xanthodon* would change and many new species could be identified from Anatolia and Transcaucasia with future molecular studies.

The first proof of this prediction was presented in a study of Matur et al. (2019), where Anatolian mole-rats (*N. xanthodon*) grouped into two main clades based on phylogenetic analysis of cyt-b and microsatellite data: Western Anatolian clade (with cytotypes 2n = 38, 2n = 50 and 2n = 52) and Central

Anatolian clade (with cytotypes 2n = 56 and 2n = 60). Our study showed that the mole-rat cytotypes with high 2n (2n=56, 58, 60) found in the interior of Anatolia constitute a monophyletic group different from other populations of *N. xanthodon*. In previous phylogenetic analyses, blind mole-rats in the interior of Anatolia, which were called "clade *vasvari*" (Hadid et al. 2012) or "clade *cilicicus*" (Kandemir et al. 2012, Kryštufek et al. 2012), formed a monophyletic group with cytotypes with high chromosome number. The oldest record representing the population and cytotypes in the interior of Anatolia was by Mehely (1909), with the subspecies name *Spalax monticola cilicicus* from Niğde-Madenköy (2n=58). We hypothesise that it is not correct to classify these cytotypes and populations under the species name *N. xanthodon*, which (in this study and previous phylogenetic analyses) proved to be different from other populations *N. xanthodon*. Therefore, we suggest that this population and cytotypes should be classified under the species name *N. cilicicus* (stat. n.) according to name priority.

Our phylogenetic results show a low bootstrap valuable distinction between western populations (2n=36, 38, 40) and eastern populations (2n=48, 50, 54) from Anatolia. However, previous studies using morphological (Kankiliç et al. 2014) and mitochondrial sequence data (Kryštufek et al. 2012, Matur et al. 2019, Bugarski et al. 2020) provide clear evidence of a deep biogeographical disjunction between western and eastern regions of Anatolia.

# Conclusions

*Nannospalax xanthodon* has not yet been the focus of a cryptic species molecular study. Even though these are early results, the degree of difference among cytotype groups suggests that at least three unique cryptic species can be discovered utilising a small sample size and just one nuclear gene (GHR exon 10). Considering the data, we recommend further molecular analysis and sampling for individuals in the clade A classified as *N. xanthodon* because this branch may detect new species of *Nannospalax*.

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**Conflicts of interest:** The authors declare that they have no conflict of interest.

**Ethics approval:** All blind mole-rat captures and handling were approved by the Turkish Ministry of Agriculture and Forestry in compliance with Turkish laws and regulations. Ethical approval was granted by the Niğde Ömer Halisdemir University Ethics Committee 12.12.2011/ No:8 Niğde, Turkey.

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# Supplementary

**Table 1.** Specimens of the genus *Nannospalax* used in this study. Legend: n: Number of specimens. MN: Number on the map (Fig. 1). HN: Haplotype number.

| MN | HN | Locality | n | Cytotype | GenBank No |
|---|---|---|---|---|---|
| ***Nannospalax xanthodon*** | | | | | |
| 1 | H10 | Aydın-Haydarlı | n=3 | 2n=36 NF=68 | OL740003, OL740001, OL740010 |
| 2 | H10 | Aydın-Yağhanlı | n=3 | 2n=36 NF=68 | OL740004, OL740008, OL740014 |
| 3 | H15 | Aydın- Bıyıklı | n=1 | 2n=36 NF=68 | OL739996 |
| 4 | H10 | Aydın-Ortaklar | n=1 | 2n=36 NF=68 | OL740007 |
| 5 | H10 | Manisa-Akhisar | n=2 | 2n=38 NF=74 | OL739986, OL739991 |
| 6 | H10 | Balıkesir-Çömlekçi | n=2 | 2n=38 NF=74 | OL739994, OL739988 |
| 7 | H10 | Balıkesir-Kepsut | n=2 | 2n=38 NF=74 | OL739993, OL739995 |
| 8 | H10 | İzmir-Foça-Bağarası | n=1 | 2n=38 NF=74 | OL739987 |
| 9 | H10 | Çanakkale-Gökçeada | n=4 | 2n=38 NF=74 | OL739941, OL739944, OL739948, OL739950 |
| 10 | H10 | Konya-Yeşildağ | n=4 | 2n=40 NF=72 | OL740015, OL740016, OL740017, OL740013 |
| 11 | H10 | Isparta-Yenişarbademli | n=4 | 2n=40 NF=72 | OL739999, OL739998, OL740000, OL740012 |
| 12 | H13 | Bolu-Abant | n=1 | 2n=52 NF=70 | OL739982 |
| 13 | H13 | Bolu-Gerede | n=1 | 2n=52 NF=70 | OL739976 |
| 14 | H13 | Bolu-Mudurnu | n=1 | 2n=52 NF=70 | OL739960 |
| 15 | H13 | Ankara-Çeltikli | n=1 | 2n=52 NF=70 | OL739975 |
| 16 | H13 | Bolu | n=1 | 2n=52 NF=70 | OL739984 |
| 17 | H13 | Bolu-Yeniçağ | n=3 | 2n=52 NF=70 | OL739956, OL739957, OL739958 |
| ***Nannospalax cilicicus*** | | | | | |
| 18 | H3 | Mersin-Sebil Plateau | n=2 | 2n=52 NF=72 | OL739928, OL739929 |
| 19 | H14 | Mersin-Çamlıyayla | n=2 | 2n=52 NF=72 | OL739930, OL739931 |
| 20 | H9 | Kırıkkale-Keskin | n=1 | 2n=54 NF=74 | OL739980 |
| 21 | H9 | Kırıkkale-Delice | n=1 | 2n=54 NF=74 | OL739979 |
| 22 | H9 | Kırıkkale-Sulakyurt | n=1 | 2n=54 NF=74 | OL739951 |
| 23 | H9 | Kırşehir-Seyfe lake | n=1 | 2n=54 NF=74 | OL739965 |
| 24 | H9 | Adana-Tufanbeyli | n=2 | 2n=54 NF=74 | OL739945, OL739946 |
| 25 | H9 | Isparta- Aksu | n=1 | 2n=56 NF=72 | OL739973 |
| 26 | H9 | Adana-Pozantı | n=1 | 2n=56 NF=72 | OL739934 |
| 27 | H9 | Mersin-Gülek | n=1 | 2n=56 NF=72 | OL739935 |
| 29 | H3 | Niğde-Campus | n=1 | 2n=58 NF=72 | OL739902 |
| 30 | H3 | Bilecik-Söğüt | n=1 | 2n=60 NF=76 | OL739990 |
| 31 | H14 | Bilecik-Bozüyük | n=1 | 2n=60 NF=76 | OL739992 |
| 32 | H3 | Kütahya | n=2 | 2n=60 NF=76 | OL740021, OL739953 |
| 33 | H3 | Eskişehir-Sivrihisar | n=1 | 2n=60 NF=76 | OL739985 |
| 34 | H3 | Konya-Akşehir | n=1 | 2n=60 NF=76 | OL740022 |
| 35 | H14 | Konya-Beyşehir | n=1 | 2n=60 NF=76 | OL739961 |
| 36 | H3 | Isparta-Gelendost | n=1 | 2n=60 NF=78 | OL739972 |
| 37 | H14 | Isparta-Gönen | n=1 | 2n=60 NF=78 | OL739997 |
| 38 | H14 | Isparta-Yalvaç | n=1 | 2n=60 NF=78 | OL740002 |
| 39 | H12 | Isparta-Atabey | n=1 | 2n=60 NF=78 | OL740006 |
| 40 | H3 | Sivas-İmranlı | n=1 | 2n=60 NF=80 | OL739968 |

| MN | HN | Locality | n | Cytotype | GenBank No |
|---|---|---|---|---|---|
| 41 | H3 | Sivas-Yıldızeli | n=1 | 2n=60 NF=80 | OL739966 |
| 42 | H3 | Yozgat-Saraykent | n=1 | 2n=60 NF=80 | OL740024 |
| 43 | H14 | Konya-Yunak | n=1 | 2n=60 NF=80 | OL739962 |
| 44 | H3 | Konya-Cihanbeyli | n=1 | 2n=60 NF=80 | OL739963 |
| 45 | H3 | Ankara-Kızılcahamam | n=1 | 2n=60 NF=80 | OL739981 |
| 46 | H12 | Afyon-Eber | n=1 | 2n=60 NF=82 | OL740020 |
| 47 | H12 | Burdur-Yeşilova | n=1 | 2n=60 NF=84 | OL740011 |
| 48 | H14 | Denizli-Çameli | n=1 | 2n=60 NF=84 | OL740009 |
| ***Nannospalax nehringi*** | | | | | |
| 49 | H7 | Tunceli | n=1 | 2n=44 NF=74 | OL739916 |
| 50 | H7 | Ağrı-Taşlıçay | n=2 | 2n=48 NF=68 | OL740018, OL739915 |
| 51 | H6 | Van-Gönderme | n=1 | 2n=48 NF=68 | OL739917 |
| 52 | H7 | Van-Kocapınar | n=2 | 2n=48 NF=68 | OL739920, OL739921 |
| 53 | H7 | Kars-Selim | n=1 | 2n=50 NF=70 | OL739978 |
| 54 | H7 | Bayburt-Demirözü | n=1 | 2n=50 NF=72 | OL739969 |
| ***Nannospalax tuncelicus*** | | | | | |
| 55 | H5 | Tunceli-Pertek | n=1 | 2n=54 NF=74 | OL739913 |
| 56 | H8 | Bingöl-Yolçatı | n=2 | 2n=54 NF=74 | OL739918, OL740019 |
| 57 | H5 | Muş-Bozbulut | n=2 | 2n=54 NF=74 | OL739922, OL739923 |
| 58 | H8 | Bitlis-Rahva | n=1 | 2n=54 NF=74 | OL739919 |
| ***Nannospalax ehrenbergi*** | | | | | |
| 59 | H1 | Hatay-Şenköy | n=5 | 2n=48 NF=74 | OL739899, OL739903, -904, OL739907, -908, |
| 60 | H1 | İskenderun-Arsuz | n=2 | 2n=52 NF=74 | OL739905, OL739906 |
| 61 | H1 | Osmaniye-Bahçe | n=1 | 2n=52 NF=74 | OL739910 |
| 62 | H1 | Elazığ-Bağdere | n=2 | 2n=52 NF=76 | OL739914, OL739924 |
| 63 | H1 | Elazığ-Sivrice | n=1 | 2n=52 NF=76 | OL739925 |
| 64 | H4 | Mersin-Tarsus | n=1 | 2n=56 NF=72 | OL739939 |
| 65 | H11 | Adana-Şeyhmurat | n=1 | 2n=56 NF=72 | OL739940 |
| 66 | H4 | Adana-Yüreğir | n=2 | 2n=56 NF=72 | OL739912, OL739911 |
| 67 | H4 | Adana-Campus | n=1 | 2n=56 NF=72 | OL739909 |
| ***Nannospalax leucodon*** | | | | | |
| 68 | H2 | Kırklareli-Hayrabolu | n=1 | 2n=56 NF=78 | OL739938 |
| 69 | H2 | İstanbul-Çatalca | n=1 | 2n=56 NF=78 | OL739900 |
| 70 | H2 | Çanakkale-Eceabat | n=1 | 2n=56 NF=78 | OL739898 |
| 71 | H2 | Tekirdağ-Malkara | n=3 | 2n=56 NF=78 | OL739942, OL739943, OL739947 |