



MORPHOMETRIC ANALYSIS of *Nannospalax leucodon* (Nordmann, 1840) with $2n=60$ DISTRIBUTED in CENTRAL ANATOLIA

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ABSTRACT

In this study, distribution areas of populations with different NF values (76, 78, 80, 82 and 84) belonging to $2n=60$ chromosomal forms of *Nannospalax leucodon* widely distributed in Central Anatolia were examined by using traditional morphometry. When findings from this study and other results obtained from previous karyological studies were evaluated together, it was observed that populations with different NF values were not randomly distributed in Central Anatolia. It was determined that the populations with NF=80 value had the widest distribution and is mostly distributed in the center of Central Anatolia. It was seen that the populations with NF=76 and NF=78 values were found as small populations in northern and southern border of distribution area of the populations with NF=80. It was observed that populations having bigger NF values (NF=82 and 84) present as small populations in western border of the main populations with NF=80. Among populations having different NF values, 8 of the 34 morphometric characters were statistically significant among females of different populations, while 25 of those in male showed significant difference. Statistically, the mean values of 34 morphometric characters were found as highly important by MANOVA. The populations with different NF values distributed in different geographic areas in Central Anatolia were separated in CVA analysis and particularly populations in western parts of Central Anatolia were grouped separately than others.

KEYWORDS: *Nannospalax leucodon*, Morphometry, Central Anatolia, Turkey

1. INTRODUCTION

Distribution of animal and plant species is mainly shaped by changes in climate, geography and geomorphological conglomeration processes which form main geological structure of a certain area. These unstable conditions can also change population genetics and habitats of the species and therefore led to occur both inter- and intra- specific variations. Biogeographically, Turkey is a transition zone between the continental Europe and the near East and has variable geographic, climatic and geological features. Also, Turkey is under the influence of three different phytogeographical regions. The combined effects of its location, all those variable factors and different vegetation types have major impacts on forming and shaping inter- and intra-population species diversity in Turkey.

Topographical features along with the climatic conditions and vegetation of Anatolian peninsula dramatically changed during a period from Paleozoic to Quaternary (1). Conformation of the Western Anatolia Mountains, the Black Sea Mountains, the Taurus Mountains, the Eastern Anatolian Mountains, known as “Anatolian Diagonal”, “Turkish Straits system” (Marmara Sea, Dardanelle and Bosphorus straits) and the Central Anatolia Plateau were gradually realized during this time, which has considerable impacts on Anatolian biodiversity. Anatolian Diagonal, which effects both fauna and flora of Anatolia, divides Anatolia in the north and south directions, continues from the Amanos Mountains to the Erzurum-Kars plateaus. *Nannospalax leucodon* and *Nannospalax ehrenbergi* are separated by the influence of Anatolian Diagonal. Previously, these two species were contacted in Anatolia. However, they had been separated as a result of the ascension and descent activities that occurred since the Oligocene (2, 3, 4, 5, 6, 7, 8). Besides effects

of those major geographical barriers, some geological formations such as Kırşehir block, Menderes-Taurus block, Eastern Taurus block, Pontides, Salt Lake and Sakarya basin (Figure 1) have served as different geological and biogeographical units bringing about differentiation of animal and plant species living in Anatolia (9, 10, 11, 12). Central Anatolia, which is today enclosed by the Black Sea Mountains in the north, the western Anatolia Mountains in the west, the Taurus Mountains in the south and the Anatolian Diagonal in the east, has been shaped by various geological formations during its geological history. Also, this geographical area is mostly comprised Kırşehir block, Menderes-Taurus block and Sakarya continent. These formations also influenced aspects and distributions of vertebrate species living in Anatolia. Phylogenetic relationships of the genus *Aphanius*, distribution patterns of *Pseudophoxinus* species and high genetic diversity in *Mertensiella luschani* populations found in Menderes-Taurus block that is considered to be as a Pleistocene refuge have demonstrated that geological events shaping Central Anatolia is very important power to contribute to diversity of organisms living in Central Anatolia (12, 13, 14).

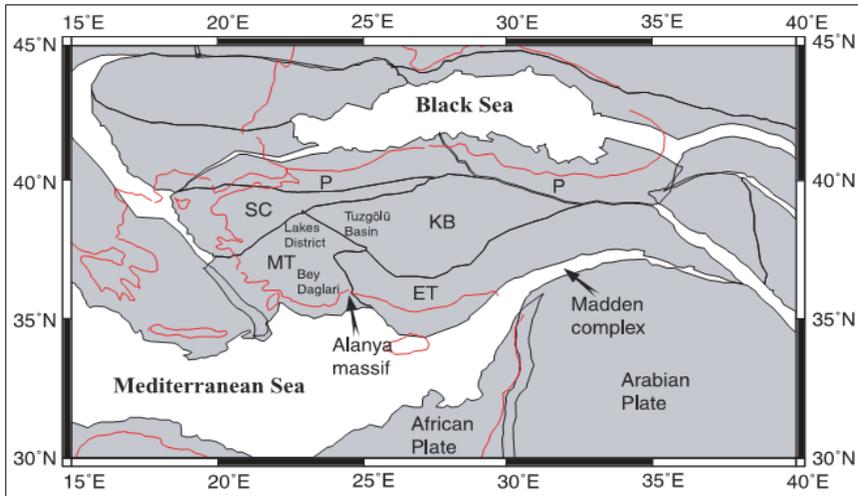


Figure 1. The geological situation of the clusters of Anatolia 15 million years ago (11, 12) SC= Sakarya continent, MT= Menderes-Taurus block, KB= Kırşehir block, ET= East Taurus block, P=Pondites

The level of biodiversity arising from habitat diversity is very high in Turkey, which results from abovementioned factors like geographical and climatic diversities and so on. Approximately more than 160 mammal species, 8 of them are endemic, are hosted by Turkey (15, 16, 17, 18). Rodents adapted to quite different habitats including ground and underground constitute majority among mammal species in Turkey. Of those rodents, the mole voles in the genus *Nannospalax* are the unique and important rodent species specialized to live in subsoil habitat. *Nannospalax leucodon* in this genus has widely distribution in Turkey. This species has 11 chromosomal forms ($2n= 36, 38, 40, 48, 50, 52, 54, 56, 58, 60, 62$) (19). Chromosomal form of $2n=60$ has the widest distribution in Turkey (Figure 2), and has eight different NF values (NF= 72, 74, 76, 77, 78, 80, 82, 84) within all chromosomal forms of this species (20, 21, 22, 23, 24, 25, 26, 27, 28).

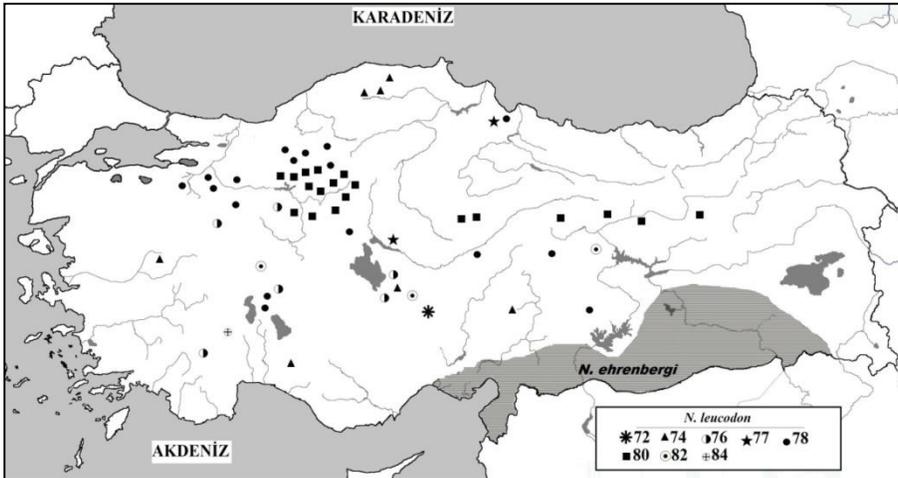


Figure 2. Distribution of populations with different NF values belonging to $2N=60$ chromosomal form in Turkey.

Mole rats have also morphological and morphometric differences in addition to the known high level of chromosomal differentiation. These animals have evolved distinct morphological and morphometric characteristics, resulted from adaptations to the environments in which they live. The most important environmental factors stimulating differentiation in morphological characteristics of mole rats are soil structure, climate and geographical factors. Mole rats in their general life time excavate the soil using incisors and throw out the excavated soil by their feet and head to create subterranean galleries. Therefore, it is possible to observe differentiations in dental and cranial characters of mole rats living in distinct soil structure (dry, arid, soft, gravels) under the influence of the changeable climate and active tectonism. For instance, one of the main dental differentiations used to separate two

Nannospalax species living in different geographies (*N. ehrenbergi* and *N. leucodon*) is groove patterns on anterior side of the upper incisors (29, 30).

Main aims of this study are: 1. to determine if there is morphometric differentiation between populations with different NF values of $2N=60$ chromosomal form, 2. If this is the case, to determine relationships between morphometric differentiations and geological units in Central Anatolia.

2. MATERIAL AND METHODS

A total of 177 specimens (90 males and 87 females) from 49 localities were used in present study (Figure 3). In the measurements, the skull belonging to mature individuals was used. Because of the presence of sexual dimorphism between male and female individuals in mole rats, the sexes were evaluated separately. Measurements of three body (Total length (TL), Hind foot (HF), Weight (gram) (W) and 31 cranial characters were analyzed for morphometric evaluations (Figures 4-7, Table 1).

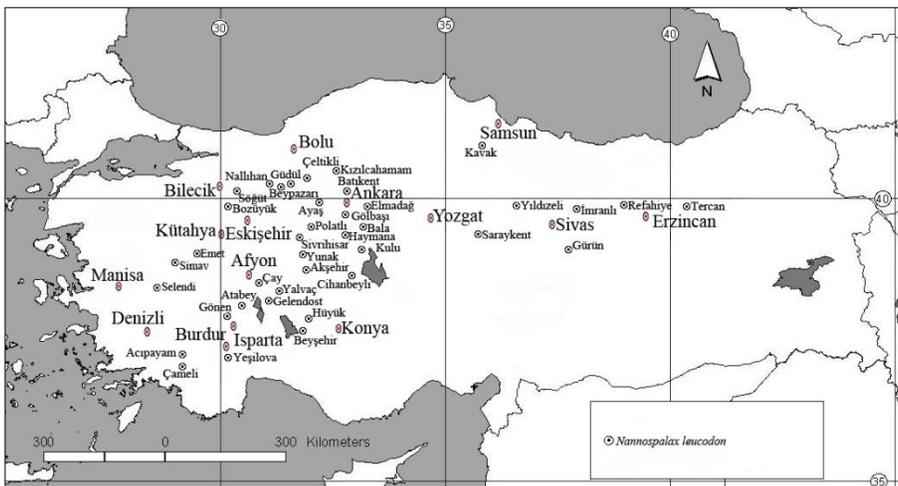


Figure 3. The map showing localities of the collected specimens used in the study

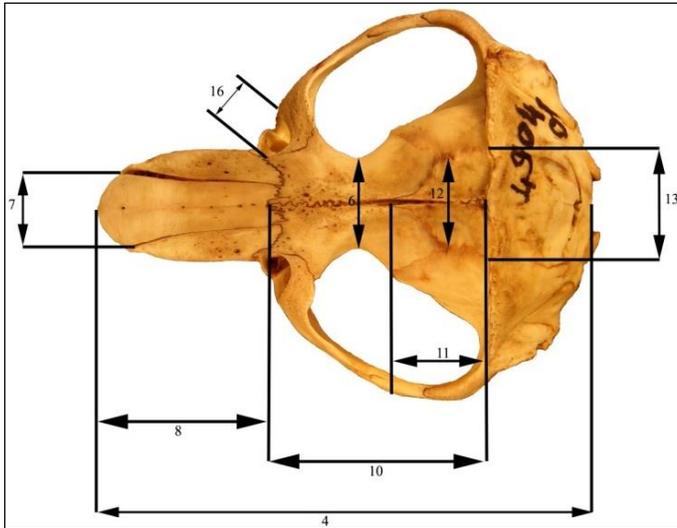


Figure 4. Character measurements of the skull in dorsal view.

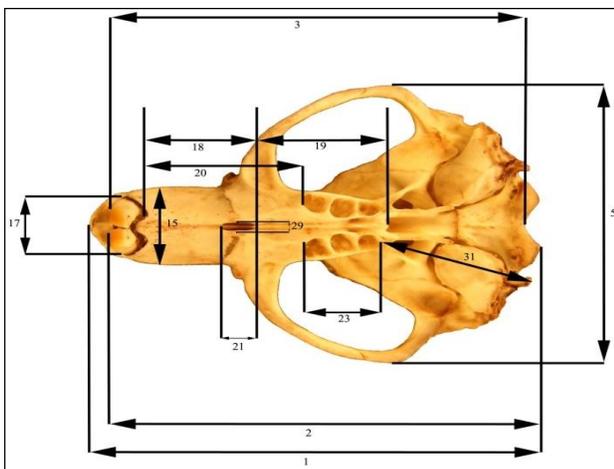


Figure 5. Character measurements of skull in ventral view.

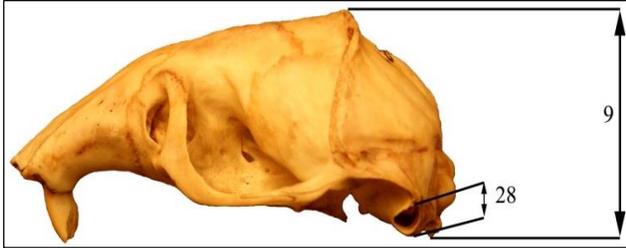


Figure 6. Character measurements of the skull in lateral view.

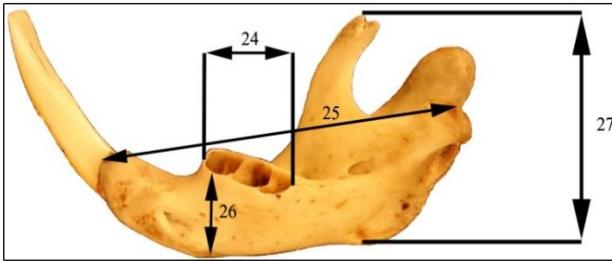


Figure 7. Character measurements taken from mandible

Table 1. Characters of skull measured in present study

Numbers in Figures 4-7	Skull characters	Abbreviation
1	Condylonasal length	CNL
2	Condylobasal length	CBL
3	Basillar length	BL
4	Occipitonasal length	ONL
5	Zygomatic Width	ZW
6	Interorbitale width	IOW
7	Nasal width	NW
8	Nasal length	NL

9	Height of braincase with tympanic bulla	HBT
10	Sagittal crest length	SCL
11	Parietal length	PL
12	Anterior width of Parietal bone	AWPB
13	Width in lambdoid suture of parietal	WLP
14	Supraoccipital length	SOL
15	Rostrum width	RW
16	Width of Foramen infraorbitalia	WFI
17	Alveolar width of upper incisor	AWUI
18	Front palatine length	FPL
19	Hind palatine length	HPL
20	Diastema length	DL
21	Foramen incisive length	FIL
22	Width in mid-part of upper incisor	WMUI
23	Alveolar length of right upper molar	ALRU
24	Alveolar length of right lower molar	ALLM
25	Articular (condylar) length of right lower mandible	CL
26	Mandibula height	MH
27	Coronoid process height	CPH
28	Diameter of left aural hole	DAH
29	Foramen incisive width	FIW
30	Facial length	FL
31	Brain capsule width	BCW

2.1 Statistical Analysis

Five populations with different NF values of the $2n = 60$ chromosomal form of *Nannospalax leucodon* in Central Anatolia were statistically analyzed by using one-way variance analyses, ANOVA (Analysis of Variance) and MANOVA (Multivariate Analysis of Variance). Both analyses were performed by SPSS 15.0 for Windows (31). Male and female specimens in each population of the species were separately analyzed, because mole voles have sexual dimorphism. STATISTICA 7 (32) was used to perform CVA (Canonical Variate Analysis) analysis of morphometric characters. PAST 2008 (33) was used to show relative positions of the populations in the scatter plot, which was formed by CVA scores. Also, NTSYS pc 2.2 (34) was employed to construct UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram based on Mahalanobis distance matrix.

3. RESULTS

Measurements of 3 body and 31 cranial characters were used to determine differences between populations with different NF values. We worked on five NF populations of chromosomal form of $2N=60$, using 34 morphometric characters. Nine characters (IOW, SCL, AWPB, AWUI, HPL, WFI, ALLM, FIW, FL) is statistically insignificant in males, while 26 characters (TL, W, CNL, CBL, ONL, ZW, IOW, NW, NL, SCL, PL, SOL, RW, FIL, AWUI, FPL, DL, WFI, WMUI, ALLM, MH, CPH, DAH, FIW, FL, BCW) are in females ($P>0.05$) Tables 2 - 3). Group mean vectors of NF populations in Central Anatolia were found based on MANOVA analysis.

MANOVA analysis exhibited significant differences between means of NF populations based on morphometric data ($P < 0.001$) (Table 4).

Table 2. ANOVA results of male specimens of five populations with different NF values based on morphometric characters.

Character	Sum of squares between groups	Sum of squares in groups	F Value	Between groups (df1)	In groups (df2)	P
TL	7923,330	18832,575	7,258	4	69	0,000***
HF	112,494	432,006	4,492	4	69	0,003**
W	59400,470	180891,314	5,664	4	69	0,001**
CNL	275,089	920,546	5,155	4	69	0,001**
CBL	272,116	820,191	5,723	4	69	0,000***
BL	372,586	855,028	7,517	4	69	0,000***
ONL	190,125	885,289	3,705	4	69	0,009**
ZW	232,184	876,569	4,569	4	69	0,002**
IOW	1,207	17,945	1,161	4	69	0,336
NW	5,125	23,714	3,728	4	69	0,008**
NL	61,166	231,993	4,548	4	69	0,003**
HBT	70,762	192,182	6,352	4	69	0,000***
SCL	26,376	295,118	1,542	4	69	0,200
PL	20,791	95,587	3,752	4	69	0,008**
AWPB	19,647	201,526	1,682	4	69	0,164
WLP	45,263	149,179	5,234	4	69	0,001**
SOL	27,312	95,049	4,957	4	69	0,001**
RW	7,093	42,819	2,857	4	69	0,030*
FIL	11,011	52,483	3,619	4	69	0,010*
AWUI	3,119	23,589	2,281	4	69	0,069
FPL	20,252	115,710	3,019	4	69	0,024*
HPL	18,938	143,057	2,284	4	69	0,069
DL	72,340	242,369	5,149	4	69	0,001**
WFI	0,641	11,723	0,943	4	69	0,444
WMUI	0,701	4,292	2,816	4	69	0,032*
ALRU	11,372	21,626	9,071	4	69	0,000***
ALLM	5,514	42,015	2,264	4	69	0,071

CL	286,547	346,598	14,261	4	69	0,000***
MH	13,288	43,385	5,283	4	69	0,001**
CPH	45,223	198,680	3,926	4	69	0,006**
DAH	1,596	7,302	3,771	4	69	0,008**
FIW	0,040	1,675	0,412	4	69	0,800
FL	100,217	710,917	2,432	4	69	0,056
BCW	67,031	248,309	4,657	4	69	0,002**

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 3. ANOVA results of female specimens of five populations with different NF values based on morphometric characters.

Character	Sum of squares between groups	Sum of squares in groups	F Value	Between groups (df1)	In groups (df2)	P
TL	599,896	19463,770	0,609	4	79	0,658
HF	21,078	99,244	4,195	4	79	0,004**
W	5313,075	64941,496	1,616	4	79	0,179
CNL	28,454	443,537	1,267	4	79	0,290
CBL	7,481	310,381	0,476	4	79	0,753
BL	75,976	321,073	4,673	4	79	0,002**
ONL	23,345	381,996	1,207	4	79	0,315
ZW	12,097	318,301	0,751	4	79	0,561
IOW	0,208	19,635	0,209	4	79	0,933
NW	0,417	11,682	0,705	4	79	0,591
NL	11,216	103,125	2,148	4	79	0,083
HBT	28,371	108,271	5,175	4	79	0,001**
SCL	11,576	113,139	2,021	4	79	0,100
PL	9,089	74,221	2,418	4	79	0,055
AWPB	40,754	153,302	5,250	4	79	0,001**
WLP	91,355	188,735	9,560	4	79	0,000***
SOL	6,289	54,373	2,284	4	79	0,068
RW	1,131	19,822	1,127	4	79	0,350
FIL	2,771	39,129	1,399	4	79	0,242
AWUI	0,904	17,950	0,995	4	79	0,415
FPL	9,665	90,553	2,108	4	79	0,088

HPL	15,492	87,636	3,491	4	79	0,011*
DL	16,956	137,222	2,440	4	79	0,054
WFI	0,985	15,790	1,232	4	79	0,304
WMUI	0,241	2,803	1,700	4	79	0,158
ALRU	6,656	24,705	5,321	4	79	0,001**
ALLM	0,778	21,607	0,711	4	79	0,587
CL	84,845	198,175	8,456	4	79	0,000***
MH	1,118	32,469	0,680	4	79	0,608
CPH	5,800	94,225	1,216	4	79	0,311
DAH	0,728	7,704	1,867	4	79	0,125
FIW	0,110	2,138	1,013	4	79	0,406
FL	13,785	225,594	1,207	4	79	0,315
BCW	0,940	41,703	0,445	4	79	0,776

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4. MANOVA results of five populations with different NF values based on morphometric characters

Male	Statistical Tests	Test Scores	F	DF1	DF 2	P
	Pillai's trace	2,523	1,958	136,00	156,0	0,000***
	Wilk's lambda	0,013	2,102	136,00	145,966	0,000***
	Hotelling T ²	8,933	2,266	136,00	138,00	0,000***
	Roy's statistic	4,597	5,273	34,00	39,00	0,000***
Female	Pillai's trace	2,608	2,700	136,0	196,0	0,000***
	Wilk's lambda	0,007	3,364	136,0	185,7	0,000***
	Hotelling T ²	13,778	4,508	136,0	178,0	0,000***
	Roy's statistic	9,415	13,569	34,0	49,0	0,000***

*** $P < 0,001$

CVA analysis was used to determine morphometric difference between populations with different NF values in Central Anatolia. The first three vectors clarified 91.1 % of total variation in males, while 92.3 % in females. First vector stated 51.5 % of total variation in males, whereas 68.3 % in females (Table 5). A total of 67 male specimens (90.5 %) of the total 74 specimens were correctly grouped to corresponding population. Three specimens from population of NF=80 were classified to population of NF=78 (Table 5). In males, a total of 39 specimens (52.7 %) of the total 74

specimens were correctly grouped to corresponding group with Cross-validation test (Table 6).

Table 5. Classification results of males of five NF populations with CVA.

Groups	NF=84	NF=82	NF=80	NF=78	NF=76
NF=84	4	0	0	0	0
NF=82	0	8	0	0	0
NF=80	0	0	31	0	1
NF=78	0	0	3	5	0
NF=76	0	0	2	1	19
Total number	4	8	36	6	20
Correctly classified individual	4	8	31	5	19
Ratio of correctly classified individual	% 100	% 100	% 86	% 83	% 95

Table 6. Classification results of males of five NF populations with Cross-validation test

Groups	NF=84	NF=82	NF=80	NF=78	NF=76
NF=84	3	0	3	1	1
NF=82	0	5	0	0	2
NF=80	1	1	20	2	7
NF=78	0	0	6	2	1
NF=76	0	2	7	1	9
Total number	4	8	36	6	20
Correctly classified individual	3	5	20	2	9
Ratio of correctly classified individual	% 75	% 62,5	% 55,6	% 33,3	% 45

In females, 76 specimens (90.5 %) of the 84 specimens were correctly grouped into corresponding population with CVA. One population of NF= 82 was grouped into population on NF=84, one of the population of NF=80 into population of NF=78 (Table 7). In females, 56 ones (% 66.7) of 84 specimens were correctly grouped with corresponding group with Cross-validation test (Table 8).

Table 7. Classification results of females of five NF populations with CVA.

Groups	NF=84	NF=82	NF=80	NF=78	NF=76
NF=84	6	1	0	0	1
NF=82	0	5	0	0	0
NF=80	0	0	33	0	1
NF=78	0	0	1	12	0
NF=76	0	0	4	0	20
Total number	6	6	38	12	22
Correctly classified individual	6	5	33	12	20
Ratio of correctly classified individual	% 100	% 83,3	% 86,8	% 100	% 90,9

Table 8. Classification results of females of five NF populations with Cross-validation test

Groups	NF=84	NF=82	NF=80	NF=78	NF=76
NF=84	4	1	0	1	2
NF=82	0	5	0	0	0
NF=80	0	0	29	2	3
NF=78	0	0	4	5	4
NF=76	2	0	5	4	13
Total number	6	6	38	12	22
Correctly classified individual	4	5	29	5	13
Ratio of correctly classified individual	% 66,7	% 83,3	% 76,3	% 41,7	% 59,1

Populations of NF=84 and 82 distributed in western part of Central Anatolia were grouped into different regions in both males and females in the scatter plot obtained by the CVA scores. When compared to the populations of NF=84 and 82, other populations (NF=80, 78, 76) were grouped more closely to the each other (Figures 8 - 9). Values of Mahalanobis distance matrix (D^2) supported to this result. The highest of Mahalanobis distance estimation was in the populations of NF=84/NF=82 ($D^2=87.8$) and NF=82/NF=78 ($D^2=60.8$). The lowest values were found between populations of NF=80/NF=76 ($D^2=10.08$) and NF=80/NF=78 ($D^2=12.94$).

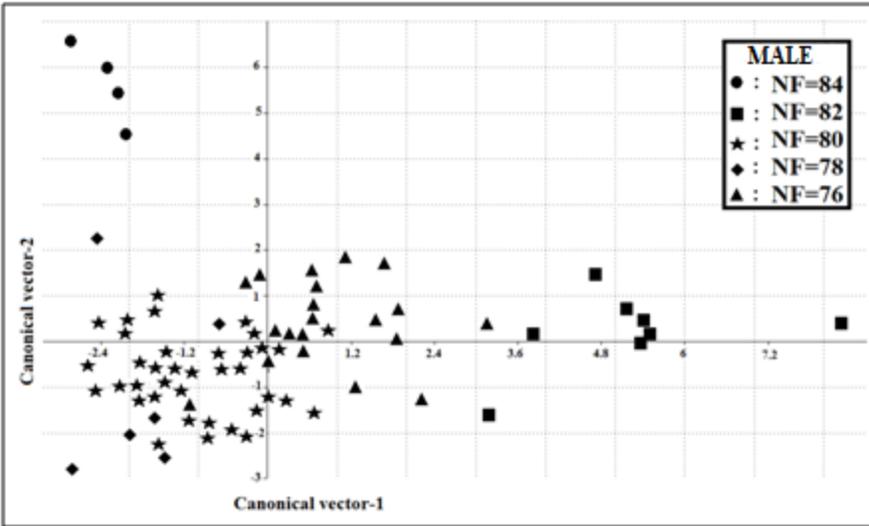


Figure 8. The scatter plot reflects the results of CVA in male specimens of *N. leucodon* of all populations with five different NF values.

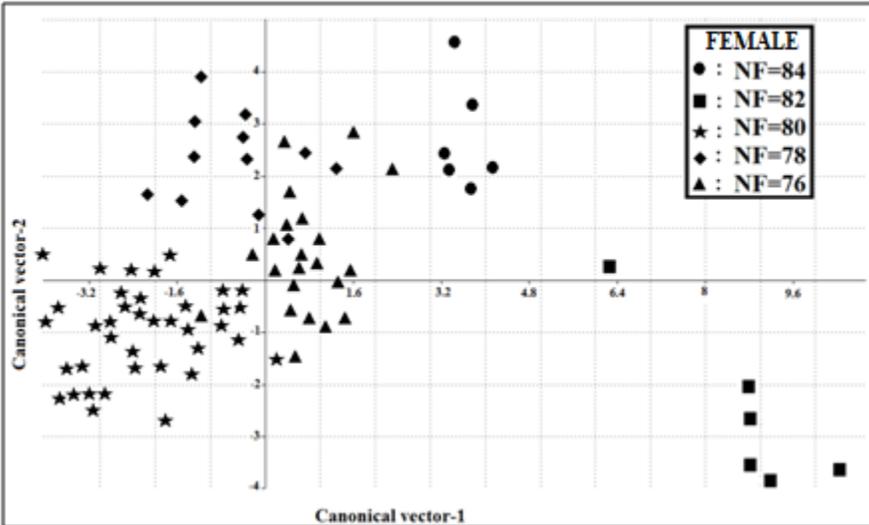


Figure 9. The scatter plot reflects the results of CVA in female specimens of *N. leucodon* of all populations with five different NF values.

Table 9. Mahalanobis distance matrix shows morphometric differentiations in males and females in *N. leucodon* populations with five different NF value.

MALE					
Groups	NF=84	NF=82	NF=80	NF=78	NF=76
NF=84	0,00000				
NF=82	87,8418	0,00000			
NF=80	42,3754	41,6626	0,00000		
NF=78	53,6959	60,8132	12,9433	0,00000	
NF=76	45,0389	28,2776	10,0831	19,3569	0,00000
FEMALE					
Groups	NF=84	NF=82	NF=80	NF=78	NF=76
NF=84	0,00000				
NF=82	66,9339	0,00000			
NF=80	53,6474	125,191	0,00000		
NF=78	35,4315	110,404	17,4440	0,00000	
NF=76	27,5649	80,1643	14,3856	13,3196	0,00000

In the basis of Mahalanobis distance matrix (D^2), UPGMA dendrogram (both males and females) was constructed to clarify morphometric differentiations between *N. leucodon* populations with five different NF values in Central Anatolia. In both males and females, UPGMA dendrogram clustered five populations into three main branches. The first branch includes three different populations with NF=80, 78, 76 which are distributed in areas that are near to the central part of Central Anatolia. In contrast to this, second and third branches contain populations with NF=82 and NF=84 which are distributed in western parts of Central Anatolia (Figures 10-11).

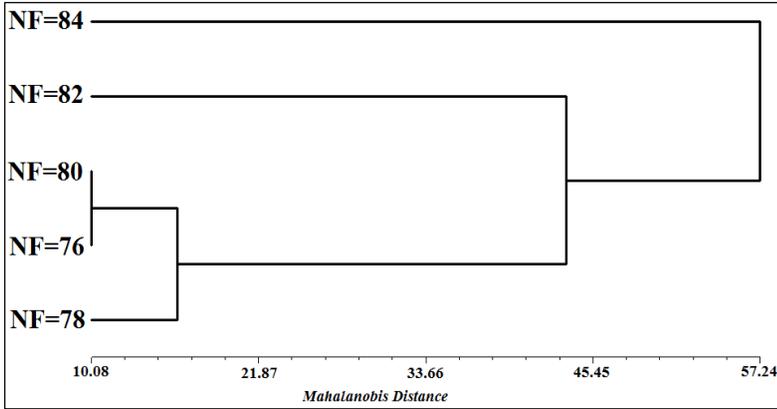


Figure 10. UPGMA dendrogram constructed using Mahalanobis distance matrix in male specimens of *N. leucodon* with five different NF values.

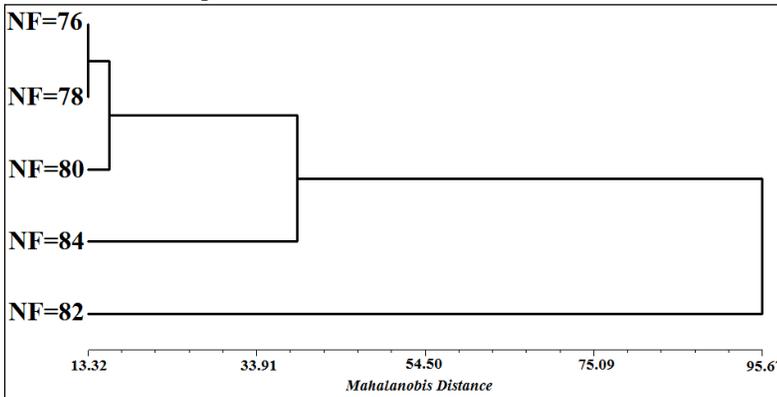


Figure 11. UPGMA dendrogram constructed using Mahalanobis distance matrix in female specimens of *N. leucodon* with five different NF values

4. DISCUSSION

In Turkey, there are some studies on differentiations of mole rats as well as some fish and amphibian, focused on karyological and molecular aspects. Species of the genus *Nannospalax* were divided into different subpopulations, which were described based on their chromosomal aspects, in subsoil conditions; humidity, arid, soil structure and geological history (12, 13, 35). According to Nevo et al. (36), increase in chromosome number provides

adaptation to deal with extensive climatic stress and ecological characterizing central Anatolia. Also, some researchers depended intrapopulational differentiations on geological units in Anatolia (12, 13). All show that mole rats are influenced by changes in both underground ecology and distinct geological units. This study is the first to investigate effects of distinct geological units on mole rats in Turkey as using morphological and biometric methods.

According to the Nevo et al. (35, 36), distributions of six different chromosomal forms ($2n=38, 40, 50, 54, 60$ and 62) of *Spalax leucodon* are not random. Populations, which have high values in frame of diploid number of chromosomes and allozyme heterozygosity, distribute up to central Anatolia with climatically variable and ecologically more aridity and *harsh*. Sözen (19) supported the findings of Nevo et al. (35, 36), based on NF populations (18, 32). In this study, distribution areas of five different NF populations belonging to chromosomal form of $2n=60$ were determined. Our findings are consistent with those of Nevo et al. (35, 36) and Sözen (19).

Morphometric analysis separated different NF populations using 34 morphometric characters (females, Wilks Lambda=0,007, $F=3,364$, $P<0,000$; males, Wilks Lambda=0,013, $F=2,102$, $P<0,000$) (Table 9, Figure 10-11).

There are five geologic units in central Anatolia (9). These units can influence geographic speciation of mole rats. According to Hrbek et al. (12, 13) for the genus *Aphanis* and for the genus *Pseudophoxinus*, distributions of species of two genera are related to the positions of geological units of Anatolia. We have investigated if there is relationship between populations with distinct NF

and geological units. Our results showed that chromosomal form of $2n=60$ divided into distinct NF populations separating in five distinct geological units. In this study, population of NF=80 is in Kırşehir block, NF=78 in Sakarya continent, NF=76 in Kırşehir block, NF=82 in Kırşehir block in at center of Anatolia, in Menderes-Taurus block and areas near to East Taurus, NF=84 in Menderes-Taurus block. Findings of this study are consistent with Hrbek et al. (12, 13). Present study along with Hrbek et al. (12, 13) supported the idea that geographic structure of Anatolia effects on distribution of species in Turkey and biodiversity of Turkey.

As a result, morphometric analysis of populations of $2n=60$ chromosomal form of *Nannospalax leucodon* having distribution in Central Anatolia revealed morphometric differences between populations with different NF values. In the emergence of these differences, it is foreseen that the five geological units (12) in Anatolia are effective.

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