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Geometric Morphometrics Comparation of Two Chromosomal Forms (2n=52 and 56) of *Nannospalax ehrenbergi* (Nehring 1898) from Southeast Region in Turkey.

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ABSTRACT

In this study, 26 (13 ♀♀, 13 ♂♂) specimens of two chromosomal forms of *Nannospalax ehrenbergi* from Mardin and Siirt-Batman provinces were compared with using by geometric morphometrics to evaluate by taxonomically. According to the results of Relative Warp Analysis (RWA), Principal Component Analysis (PCA) and Canonical Variance Analysis (CVA), populations of 2n=52 (Mardin) and 2n=56 (Siirt-Batman) are different from each other by geometric morphometrics. Also according to the CVA, female and male populations' skulls of 2n=56 (Siirt-Batman) showed that sexual dimorphism.
Skulls of specimens are deposited at the Biology Department, Science Faculty, Dicle University, Turkey.

Key words: Rodentia, Spalacidae, *Nannospalax ehrenbergi*, geometric morphometrics, Turkey.

Türkiye'nin Güneydoğu Anadolu Bölgesinden *Nannospalax ehrenbergi* (Nehring 1898)'ye Ait İki Kromozomal Formun (2n=52 and 56) Geometrik Morfometrik Yönden Karşılaştırılması

ÖZET

Bu çalışmada, Mardin ve Siirt-Batman illerinden toplanan *Nannospalax ehrenbergi*'nin 2 kromozomal formuna ait 26 örnek (13 ♀♀, 13 ♂♂) Geometrik Morfometri yöntemi kullanılarak taksonomik olarak değerlendirilmiştir. Relative Warp Analizi (RWA), Principal Component Analizi (PCA) ve Canonical Variance Analizlerinden (CVA) elde edilen sonuçlara göre 2n=52 (Mardin) ve 2n=56 (Siirt-Batman) populasyonlarının geometrik morfometrik yönden birbirinden birbirinden farklı olduğu görülmüştür. Aynı zamanda 2n=56 (Siirt-Batman) populasyonlarının dişi ve erkek örneklerinin kafatasları CVA analizi sonuçlarına göre eşeysel dimorfizm göstermektedir.
Örneklerin kafatasları Dicle Üniversitesi Fen Fakültesi Biyoloji Bölümünde muhafaza edilmektedir.

Anahtar kelimeler: Rodentia, Spalacidae, *Nannospalax ehrenbergi*, geometrik morfometri, Türkiye.

1. Introduction

Mole rats known as Palaearctic subterranean rodents, are widespread in the Turkey, Balkans, Eastern Europe, Caucasus and the Middle East [1, 2, 3, 4]. The phylogeny and systematics of Spalacidae have been largely intractable since the establishment of the family and down to the lower taxa. According to karyological studies so far have been found diploid chromosome numbers (2n) range from 36-62 and fundamental number of chromosomal arms (NF) range from 66-124 [5, 6, 7]. *Nannospalax ehrenbergi* was first described by Nehring (1898) [8] from Yafa-Israel.

Four different chromosomal forms (2n = 52, 54, 58 and 60) of Israel mole rats were recognized by Wahrman et al. in 1969 [9]. The chromosomal diversity among *Nannospalax ehrenbergi* in Turkey was studied in last decades by authors ([Coşkun [10], Sözen et al. [11], Coşkun et al. [12], Sözen et al. [13]). According to Coşkun [14] chromosomal races of Batman and Siirt have found 2n=56, NF=66, NFa= 62. Coşkun and Ulutürk [15] and Coşkun et al. [16] reported that specimens of Mardin have 2n=52, NF=76, NFa=72 and Siirt-Batman have 2n=56, NF=66 NFa=62 chromosomal values. According to Doğan [17] the chromosomal forms of *Nannospalax nehringi* have been differentiated clearly by geometric morphometrics analyses. And also Selvi [18] reported the statistical differences between the different NF values in 2n=60 chromosomal forms in Central Anatolia. We aimed to analyse the morphological variation among the two chromosomal forms of *Nannospalax ehrenbergi* (2n=52 and 2n=56) by geometric morphometrics in this study.

2. Material and Method

Two different chromosomal forms of *Nannospalax ehrenbergi* (Nehring 1898) [8], 15 (8 ♀♀, 7 ♂♂) samples belong to 2n=52 (Mardin population) and 11 (5 ♀♀, 6 ♂♂) samples belong to 2n=56 (Siirt-Batman population), were compared with using geometric morphometrics to evaluate by taxonomically. Sampling locations are showed in figure 1.

TPS programme series (tpsDig2 ver. 2.12 (Rohlf 2008) [19] ; tpsUtil ver. 1.37 (Rohlf 2006a [20]; tpsRelw ver. 1.44 (Rohlf 2006b) [21]), IMP programme series (CVAGen6, PCAGen6 (Sheets 2005) [22]) and NTSYS ver. 2.10 (Rohlf 2000) [23] have been used for geometric morphometric analysis.

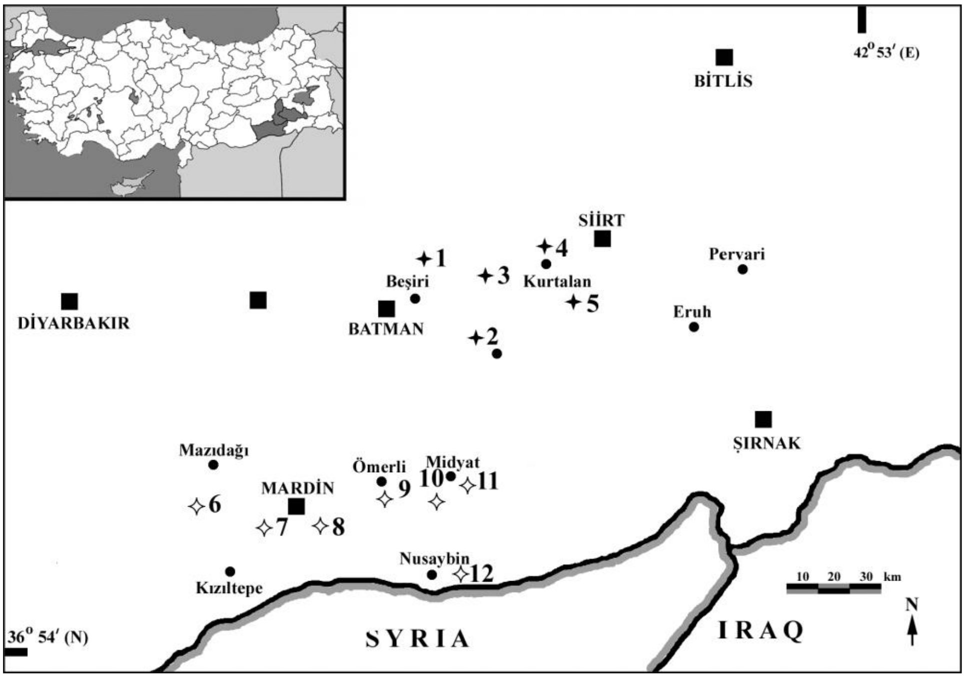

Figure 1. Collecting localities **(2n=56 (✦) :** 1. Batman-Beşiri-Yolkonak village (3 ♂), 2.Batman-Hasankeyf-Suçeken Village (3 ♀, 1 ♂) , 3. Siirt-Kurtalan-İncirlik village (1 ♂), 4. Siirt-Kurtalan road junction (1 ♀, 1 ♂), 5. Siirt-Kurtalan-Bağlıca village (1 ♀); **2n=52 (✧) :** 6. Mardin-Mazıdağı-Evciler village (1 ♀, 2 ♂), 7. Mardin-Center-İstasyon (1 ♀), 8. Mardin-Center-7 km (E) (2 ♀, 2♂), 9. Mardin-Ömerli-4 km (E) (1 ♀), 10. Mardin-Ömerli-Alıçlı village (1 ♂), 11. Mardin-Midyat-2 km (E) (2 ♀), 12. Mardin-Nusaybin-Söğütlü village (1 ♀, 2 ♂)**)**.

Images of dorsal skull were taken using a digital camera with a Nikon. Then landmarks were digitized. A milimetric grid paper was used as reference ruler for each skull.

Images were transferred to the computer and transformed to TPS format for geometric morphometric calculation using TpsUtil software (Rohlf 2006a) [20].

According to Hingst et al. (2000) [24] fourteen landmarks were defined for the dorsal view of skulls. A description of the 14 landmarks are given in figure 2.

Landmarks were digitized on dorsal views of each sample directly from the digital pictures using tpsDig2 software (Rohlf 2008) [19]. The coordinates in milimeters were aligned with Generalized Least-squares Procrustes procedure (GLS), using the software TPSRelw ver.1.44 (Rohlf 2006b) [21]

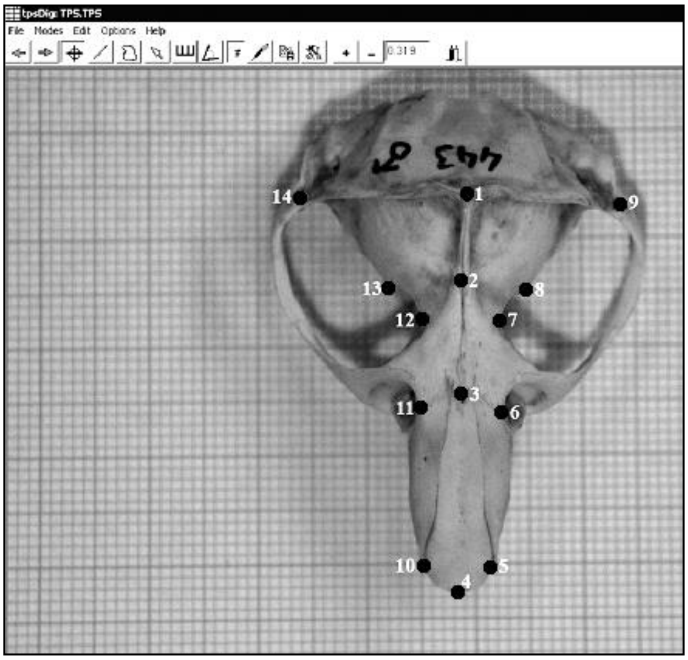

Figure 2. Landmarks for dorsal view of *Nannospalax ehrenbergi* skull (1. Midpoint of parietal-interparietal suture (lambda); 2. Frontal-parietal suture (bregma); 3. Nasal-frontal suture (nasion); 4. Tip of the nasals (rhinon); 5 and 10**.** Most anterior points at nasal-premaxillary suture (Processus nasalis ossis incisivi); 6 and 11. Lateral ends of Nasofrontal suture; 7 and 12. Most narrow point at interorbital constriction; 8 and 13. Sutures between the frontal and parietal bones; 9 and 14. Back of zygomatik notch.

In order to describe the structure of overall shape variation among chromosomal forms, partial warps and uniform component scores of each individual were combined in a data matrix and submitted to Canonical Variate Analyses (CVA) and Principle Component Analyses (PCA). Plots of PCA and CVA were drawn using IMP software (Sheets 2005) [22].

Centroid size (the square root of the sum of the squared distances of each landmark) which is a geometric size of each sample was calculated using TpsRelw version 1.44 (Rohlf 2006b) [21]. ‘Procrustes Distance’ values of sample that are quantification of deviation of each sample from consensus shape configuration of the population was generated using TpsRegr software (Rohlf 1997) [25]. Then, shape deviation from the consensus shape of sample level was illustrated on a digitizing grids using tps-thin-plate spline software (Bookstein, 1991) [26].

3. Results

The consensus configurations variances and the relative contributions of landmarks were determined by Relative Warp (RW) Analysis. Values and vektors of consensus configuration has given the amount and changing direction of each landmark. Consensus configurations of 14 landmarks from the dorsal view of *Nannospalax ehrenbergi* skulls are shown in figure 3.

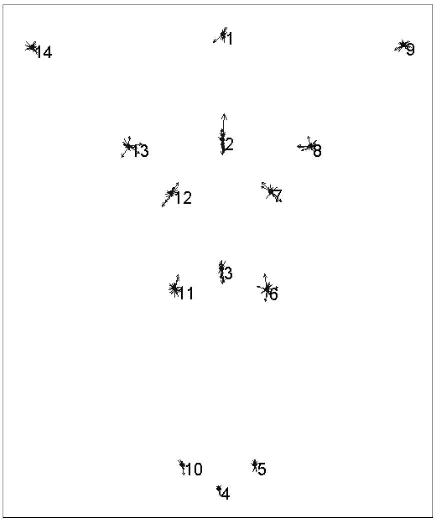

Figure 3. Vectors of *Nannospalax ehrenbergi* skulls.

2, 3, 6, 7, 8, 11, 12 and 13th landmarks have the highest variance values. However, the 4th one has the lowest variance value with S2:0.00003009 (table 1).

Table 1. Variance values of 14 landmarks are taken from the skulls of each chromosomal forms.

LM#	**S^2x**	**S^2y**	**S^2**
1	0.00002142	0.00005458	0.00007601
2	0.00000974	0.00021858	**0.00022832**
3	0.00001080	0.00010031	**0.00011111**
4	0.00000508	0.00002501	**0.00003009**
5	0.00001032	0.00004425	0.00005457
6	0.00005136	0.00008739	**0.00013875**
7	0.00005748	0.00006402	**0.00012151**
8	0.00008176	0.00003853	**0.00012029**
9	0.00006201	0.00003318	0.00009518
10	0.00000985	0.00003996	0.00004981
11	0.00004605	0.00009047	**0.00013652**
12	0.00005382	0.00007571	**0.00012953**
13	0.00007037	0.00004532	**0.00011569**
14	0.00005600	0.00003666	0.00009266

As a result of the PCA, the 2n=52 and 2n=56 populations were occured two different groups. Also, the mean of groups are far from each other.

The ordination for the first and the second Principle Component (PC) axes (Figure 4) represents the partial warps and affine (uniform) shape variation. The first PCA axis explains 34,74%, the second one comprises 20,60%, the third one is 14,26% and the fourth one is 7,21% of the total shape variance between two (2n=52 and 2n=56) populations of *Nannospalax ehrenbergi*. Thus, more than 76% of the variance is included within the first four principle component, which is sufficient for evaluating the shape variation within the studied data.

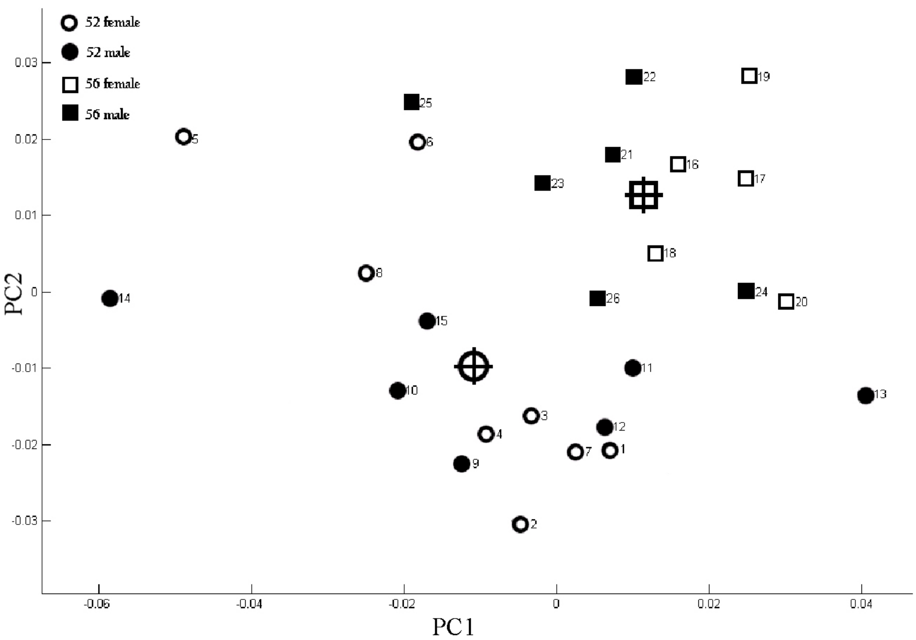

Figure 4. PCA graphic of *Nannospalax ehrenbergi* 2n=52 and 2n=56 populations skulls' (⊕ : the mean of 2n=52 populations, ⊞ : the mean of 2n=56 populations). The first PCA axis explains 34,74%, the second one comprises 20,60%, in PCA Eigen value:1).

Canonical variate analyses (CVA) between two *Nannospalax ehrenbergi* populations were showed in figure 5. The first two canonical variate axis of CVA explain 55,34% of the total variation in shape between two populations and it clearly separates 2n=52 and 2n=56 populations of *N. ehrenbergi*. According to the CVA graphic, female and male populations' skulls of 2n=56 showed sexual dimorphism. But male and female populations' skulls of 2n=52 not show sexual dimorphism (Figure 5).

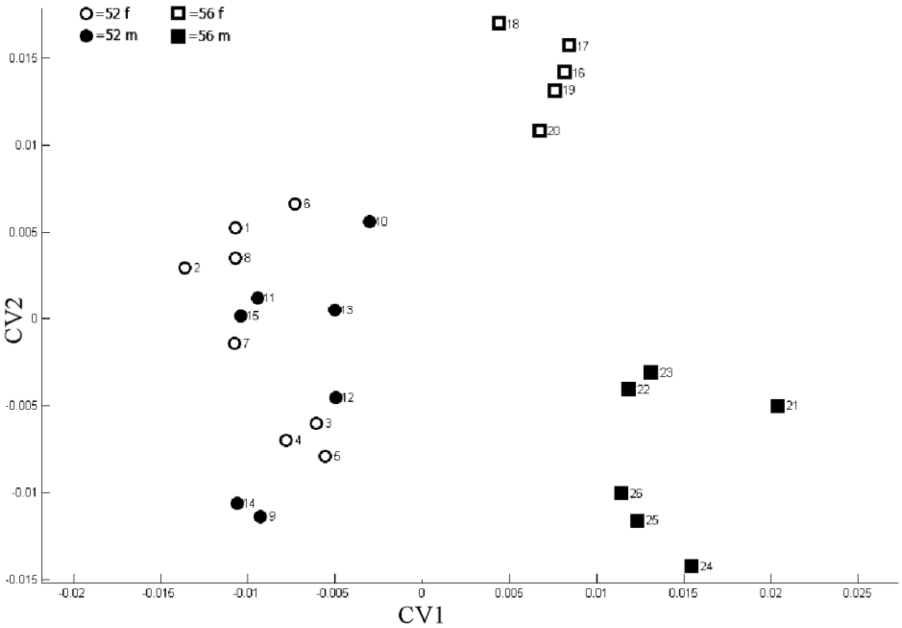

Figure 5. CVA graphic of *Nannospalax ehrenbergi* 2n=52 and 2n=56 populations skulls' (Result of CVA/Manova: Axis1: Wilks' λ = 0.0049, chisq = 63.7122, df = 24 p=1.87677e-005).

4. Discussion

The taxonomic situation of the family Spalacidae is so problematic in Turkey. The previous studies of *Nannospalax ehrenbergi* based on karyological and morphological aspects have been separated this species into chromosomal forms [10, 11, 12, 13, 14]. Coşkun and Ulutürk [15] and Coşkun et al. [16] reported five different chromosomal populations of Turkish *Nannospalax ehrenbergi* and offered each as a putative biological species under the taxon of *Nannospalax ehrenbergi*. And they reported that specimens of Mardin have 2n=52, NF=76, NFa=72 and Siirt-Batman have 2n=56, NF=66, NFa=62. These populations are different cytotypes of *Nannospalax ehrenbergi*. The results that we get from the RWA, PCA and CVA of the geometric morphometrics analysis method supported these distinct chromosomal forms.

The karyological findings alone are inadequate to resolve these taxonomic problems, however need to be supported by other findings such as molecular and geometric morphometric analysis. It's too hard to separate the chromosomal populations by morphologically. But the cranial dimensions of each chromosomal populations are present significantly statistical distinguishes by the result of geometric morphometrics analysis.

According to Doğan [17] the three mole rats species distributed in Turkey and chromosomal populations of these species have been differentiated significantly by geometric morphometric analyses.

According to Selvi [18] there are significant morphometric differences in distinct chromosomal form of *Nannospalax leucodon* species. These differences may be related to environmental factors in different geographical regions.

RW and PCA analysis of this study seperates the 2n=52 and 2n=56 chromosomal forms of *Nannospalax ehrenbergi* exactly. PCA1 and PCA2 described give a 55,34% discriminate ratio between these two populations.

Also, it is seen that 2n=52 and 2n=56 populations are clearly seperated two different groups according to their skull types by CVA graphics. However sexual dimorfism is seen in 2n=56 population.

According to the results, it is determined that geometric morphometric method can separate the *Nannospalax ehrenbergi* 2n=52 and 2n=56 chromosomal forms from each other. When the similarities and differences between Spalacidae family analysed by using geometric morphometric method important results will be determined about systematic of this family.

Consequently, this study supported that 2n=56 Siirt population is a distinct chromosomal form of *Nannospalax ehrenbergi* species defined by Coşkun [14] and Coşkun and Ulutürk [15].

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