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Cytogenetic Characteristics of *Crocidura suaveolens, Crocidura leucodon, Sorex raddei* (Mammalia: Eulipotyphla) from Turkey: Constitutive Heterochromatin Distribution

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Anahtar Kelimeler
Bicolored Shrew,
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Radde's Shrew

Abstract: In this study, heterochromatin distribution in the chromosomes of five small mammals of three different species (*Crocidura suaveolens*, *C. leucodon*, *Sorex raddei*) from four different localities of Turkey were inestigated by using standard karyotype and C-banding method. *Crocidura suaveolens* karyotype included 2n=40 chromosomes, fundamental number of autosomal arms (NFa)= 46, X=submetacentric (SM), Y=acrocentric (A); *Sorex raddei* karyotype was 2n=36, NFa=66, X=A, Y=A; *Crocidura leucodon* karyotype was 2n=28, NFa= 46, X=SM, Y=A. C-banding heterochromatin distribution in *C. suaveolens*, *C.leucodon*, *S. raddei* karyotypes was reported in this study for the first time in Turkey populations.

Türkiye'den *Crocidura suaveolens, Crocidura leucodon, Sorex raddei* (Mammalia: Eulipotyphla) Türlerinin Sitogenetik Özellikleri: Konstitütif Heterokromatin Dağılımı

Keywords
Çift renkli böcekçil,
C-bantlama,
Beyaz dişli böcekçil,
Raddei böcekçili

Özet: Bu çalışmada Türkiye'nin üç faklı lokalitesinden elde edilen üç farklı türe ait (*Crocidura suaveolens*, *Crocidura leucodon*, *Sorex raddei*) 5 küçük memeli hayvanın standart karyotipi ve C band yöntemi kullanılarak kromozomlardaki heterokromatin dağılımı araştırılmıştır. *Crocidura suaveolens* karyotipi 2n=40, temel kromozom kol sayısı (NFa)= 46, X=submetasentrik (SM), Y=akrosentrik (A); *Sorex raddei* karyotipi 2n=36, NFa=66, X=A, Y=A; *Crocidura leucodon* karyotipi 2n=28, NFa= 46, X=SM, Y=A şeklindedir. *C. suaveolens*, *C. leucodon*, *S. raddei* karyotiplerinde C-bant heterokromatin dağılımı Türkiye populasyonlarında ilk defa bu çalışma ile tanımlanmıştır.

1. INTRODUCTION

The family Soricidae includes two subfamilies, Coricidurinae and Soricinae [1]. Altogether, three *Crocidura* species (*Crocidura suaveolens*, *C. leucodon* and *C. arispa*) and five *Sorex* species (*Sorex araneus*, *S. minutus*, *S. raddei*, *S. volnuchini* and *S. satunini*) distribution in Turkey [2]. While *Crocidura* genus shows rare karyotypic variations intraspecifically [3], *Sorex* genus was found to have higher intraspecific chromosome variation rate [4,5]. Karyological studies conducted on *C. suaveolens* (White-toothed Shrew) and *C. leucodon* (Bicolored Shrew) species reported the standard karyotype, which was found in different locations in Turkey by a great number of researchers [6,7,8]. However, karyological studies (Standard karyotype, [4]; G-banding, [5]) conducted on *S. raddei* (Radde's Shrew) a species, which is distributed in Northern Anatolia and Caucasia, are very limited in number. Since *Sorex* species show high levels of variation in terms of diploid chromosome number and chromosome morphology, they are used as models in karyological studies [9].

Although standard karyotypes of *C. suaveolens*, *C. leucodon*, *S. raddei*, have been determined by many researchers, detailed karyotype morphology of these species is usually not known in detail in Turkey, yet. The objective of this study is to compare the standard karyotypes of these species with previous studies and to determine the pattern of constitutive heterochromatin distribution (C-banding) for the first time in Turkish population and to contribute to future karyological studies.

2. MATERIAL AND METHOD

Chromosome preparations were obtained from the femoral bone marrow cells of colchicine treated animals [10]. Two samples (two males) of *Crocidura suaveolens* species and one male from *Crocidura leucodon* species were obtained from the province of Samsun (N41°35'20'' E36°02'14'' and N41°22'53'' E36°00'17'', Northern Anatolia), one sample (male) of *Sorex raddei* species was obtained from the province of Artvin (N41°15'08'' E42°21'10'') and one sample (male) of *Sorex raddei* species was obtained from the province of Ardahan (N41°11'38'' E42°50'59'') Northeastern Anatolia, Caucasia region) by using live animal traps. Diploid chromosome number (2n) and fundamental number of autosomal arms (NFa) and sex chromosomes of small mammals used in the study were defined as metacentric (m), acrocentric (a), submetacentric (sm), and subtelocentric (st). The constitutive heterochromatin distribution was determined using techniques from Summer [11]. From each specimen 10 to 20 slides were prepared and at least10 well-spread metaphase plates were analyzed.
Karyotype preparates and chromosome-fixative solution, which did not undergo diffusion procedure, are being kept at Ondokuz Mayıs University Cytogenetic Laboratory under -20 degrees for future studies.

3. RESULTS

Crocidura suaveolens karyotype was in the form of 2n=40 and NFa= 46. The karyotype has two pairs of subtelocentric (chromosome no: 1-2), one pair of submetacentric (chromosome no:3), one pair of small metacentric (chromosome no: 4) and 15 pairs of different sizes of acrocentric chromosome (chromosome no: 5-19) (Figure 1). X chromosome has large submetacentric and Y chromosome has acrocentric. In *C.suaveolens* karyotype, constitutive heterochromatin is obvious in centromere region in autosomal chromosomes. While X chromosome shows C-positive band, C-heterochromatin region is very obvious and enlarged in Y chromosome (Figure 2).

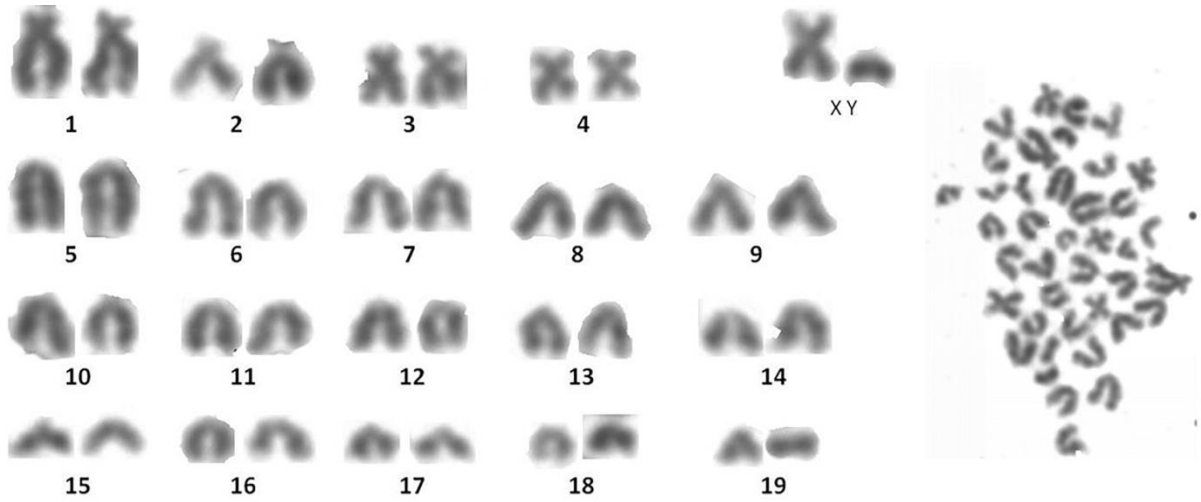

Figure 1. Karyotype of *Crocidura suaveolens* (male) from Samsun (North Anatolia).

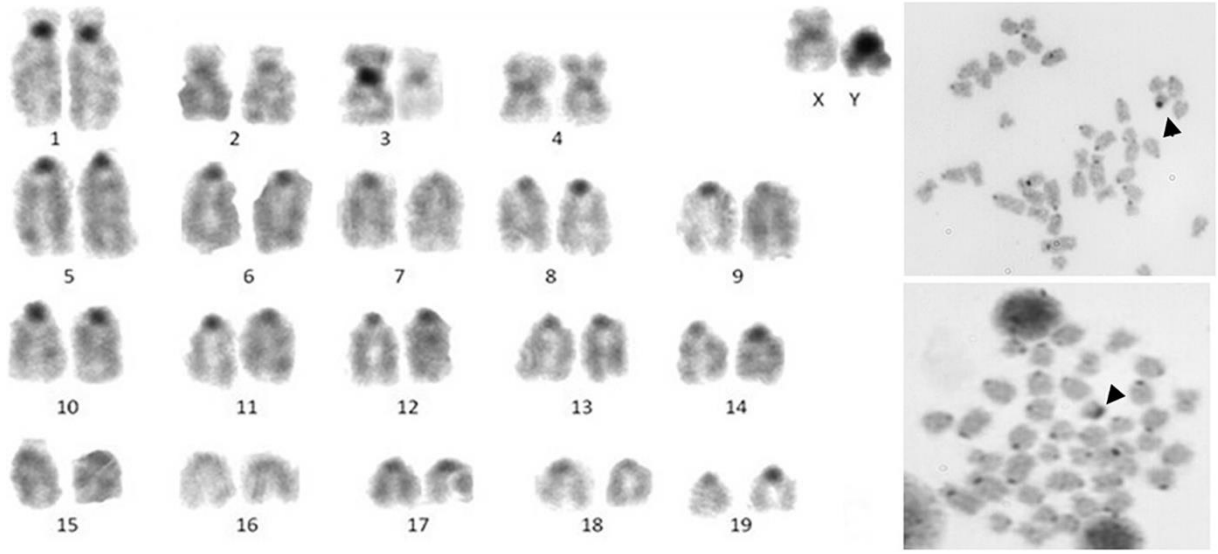

Figure 2. C-banded karyotype of *Crocidura suaveolens* (male) from Samsun, Arrow: C-positive Y chromosome

Crocidura leucodon karyotype is in the form of 2n=28 and NFa= 52. In the karyotype, all autosomal chromosomes have two arms. X chromosome has submetacentric and Y chromosome has acrocentric (Figure 3). In *C. leucodon* karyotype, constitutive heterochromatin is in centromere region in autosomal chromosome (chromosome no: 1,2,3,4,5,8,10,11,12). Since karyotype is not good, C-band structures of some chromosomes cannot be determined. X and Y chromosome have C-positive band characteristic (Figure 4).

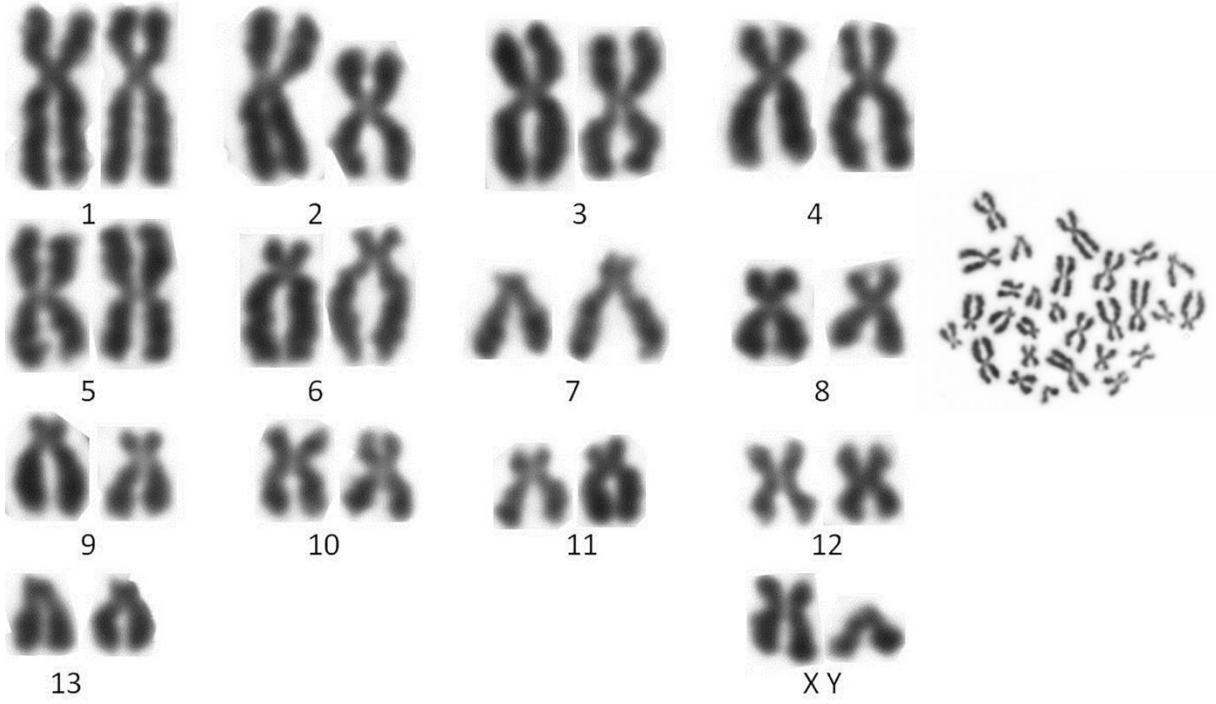

Figure 3. Karyotype of *Crocidura leucodon* (male) from Samsun (North Anatolia).

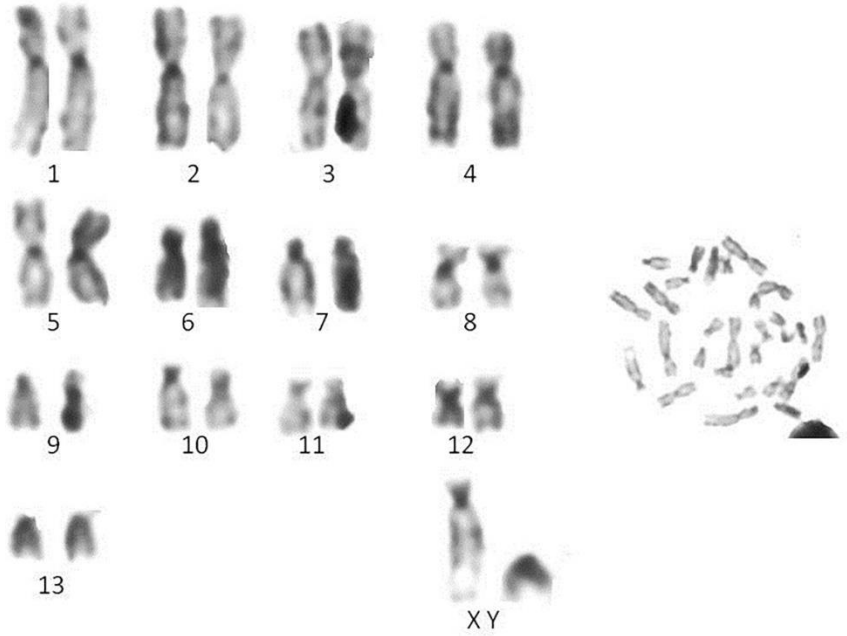

Figure 4. C-banded karyotype of *Crocidura leucodon* (male) from Samsun.

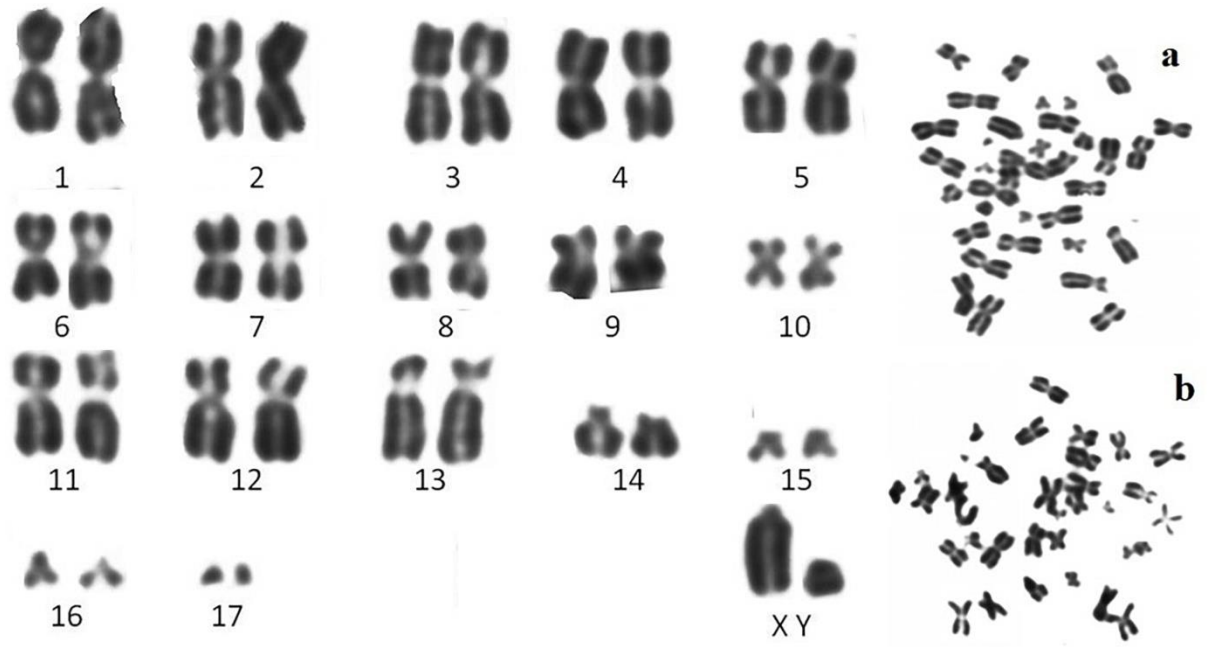

Figure 5. Karyotype of *Sorex raddei* from North east Anatolia, Caucasia region, a: Artvin, b: Ardahan

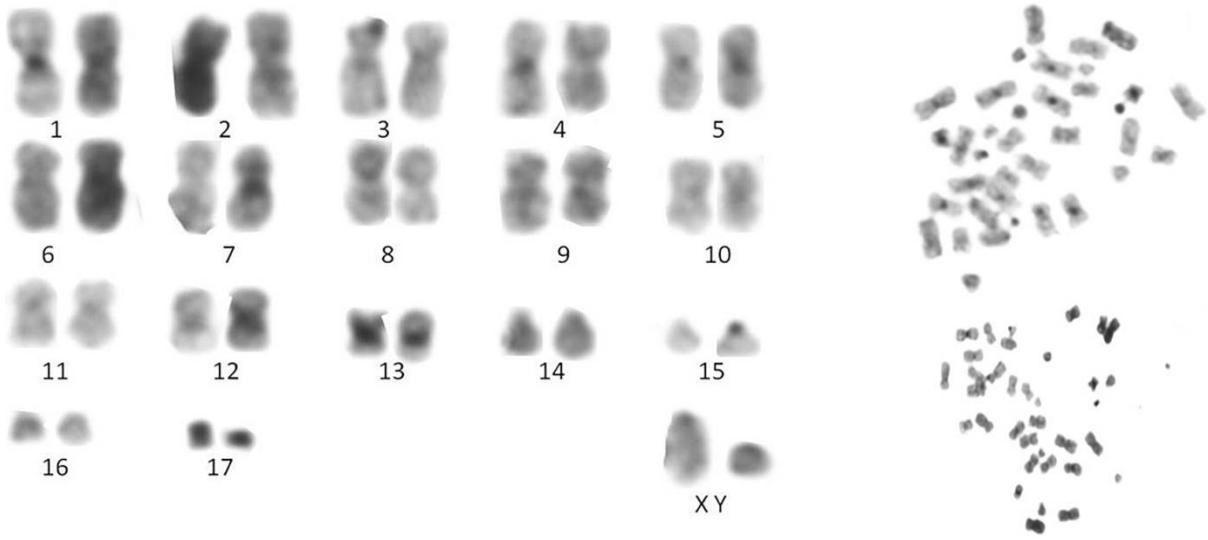

Figure 6. C-banded karyotype of *Sorex raddei* (male) from Ardahan

Karyotypes of Artvin and Ardahan samples of *Sorex raddei* species are in the form of 2n=36 and NFa=66. The chromosome set has 10 pairs of different sizes of metacentric (chromosome no:1-10), three pairs of submetacentric (chromosome no:11-13), three pairs of subtelocentric (chromosome no:14-16) and one pair of acrocentric chromosome (chromosome no:17). X chromosome is large acrocentric and Y chromosome is acrocentric (Figure 5). In *S.raddei* karyotype, positive constitutive heterochromatins are in centromere region in autosomal chromosomes. While X chromosome is obliviously C- positive, Y chromosome has a weakly C-positive (Figure 6).

4. DISCUSSION

Crocidura genus rarely shows polymorphism in autosomal chromosomes [3]. In addition, *Crocidura suaveolens* group, which is also distributed palearctic region, is characterized by stable diploid chromosome number (2n=40) [12]. Diploid chromosome number of *C. suaveolens* and *C. leucodon* has been reported by various researchers from a great number of localities in Turkey as 2n=40 and 2n=28, respectively [6,7,13]. The most extensive of these studies was conducted by Tez [7] in 36 different localities of Turkey and *C. suaveolens* and *C. leucodon* 's diploid chromosome number and chromosome morphology were found to be quite stable. Diploid chromosome number and autosome morphology found in this study are in parallel with the results of studies both conducted on populations in Palearctic region [3,12,14,15,16,17,18,19] and also with studies conducted in Turkey. While *C. suaveolens* is stable in terms of autosomal chromosome set, the morphology of sex chromosomes (X chromosome submetacentric or acrocentric; Y chromosome submetacentric or acrocentric; [3]) can differ. According to Tez [7], while X chromosome of *C. suaveolens* is submetacentric in Turkish population, it is acrocentric in Tajikistan population [20]. In this study, X chromosome is large submetacentric. In addition, karyological studies conducted on Europe populations of *C. suaveolens* and

C. leucodon populations [3,14,19] report B chromosome (Supernumerary chromosome) in the chromosome set. However, both in this study and in previous studies conducted on Turkish populations, B chromosome was not seen. In *C. leucodon* species, as in *C. suaveolens*, variations can be seen in sex chromosomes. Y chromosome was found to be submetacentric, subtelocentric or acrocentric in Bulgaria population [19]. In Turkish population, only acrocentric Y chromosome was found [7,8,in this study]. With this study, constitutive heterochromatin distribution for *C. suaveolens* and *C. leucodon* were found for the first time in Turkish population.

In karyological studies conducted in localities of Caucasia and Trabzon (Turkey) [5]; Trabzon [4]; Caucasia [15,21]; Perelman *et al*. unpublished study, unknown locality [22]; Artvin and Ardahan (Turkey) (in this study), *S.raddei* species has been found to have a quite stable chromosome number (2n=36) and NFa=66. In *S. raddei* karyotype, X chromosome is acrocentric in Turkey and Caucasia samples [5,15,23,in this study]. In a study conducted by Biltueva *et al.* [5], while Y chromosome was found to be acrocentric in Trabzon (Turkey) and Caucasica *S. raddei* sample, as in this study, Y chromosome was found to be submetacentric in samples obtained from Caucasia by Sokolov & Tembotov [21] and from Trabzon (Turkey) by Zima *et al.* [4]. In this study, C band characteristics of *S. raddei* chromosome set have been defined for the first time.

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REFERENCES

[1] Dubey S, Zaitsev M, Cosson JF, Abdukadier A, Vogel P. Pliocene and Pleistocene diversification and multiple refugia in a Eurasian shrew (*Crocidura suaveolens* group). Mol Phylogenet Evol., 38(3):635-47, 2006.

[2] Krystufek B, Vohralík V. Mammals of Turkey and Cyprus, Introduction, Checklist, Insectivora. Koper, Slovenia: Knjižnica Annales Majora; 2001.

[3] Maddalena T, Ruedi M. Chromosomal evolution in the genus *Crocidura* (Insectivora: Soricidae). In: Merritt, J.F., Kirkland, G.L.Jr. & Rose, R.K. (eds): Advances in the biology of Shrews. Carnegie Museum Natural History Special Publication, 18: 335-344, 1994.

[4] Zima J, Lukáčová L, Macholán M. Chromosomal evolution in shrews. In: Wójcik J.M. & Wolsan M. (eds.), The evolution of shrews. Mammal Research Institute, Polish Academy of Sciences, Bialowieza, 175–218, 1998.

[5] Biltueva L, Perelman PL, Polyakov VA, Zima J, Dannelid E, Borodin MP, Graphodatsky SA. Comparative chromosome analysis in three *Sorex* species *S. raddei*, *S. minutus* and *S. caecutiens*. Acta Theriol., 45(1): 119-130, 2000.

[6] Kefelioğlu H, Tez C. The Distribution problem of *Crocidura russula* (Hermann, 1780) (Mammalia: Insectivora) in Turkey. Turk J Zool., 23: 247 – 251, 1999.

[7] Tez C. Taxonomy and Distribution of the White-Toothed Shrews (*Crocidura*) (Soricidae: Insectivora: Mammalia) of Turkey. Turk J Zool., 24: 365-374, 2000.

[8] Arslan A, Zima J. Karyotypes of the mammals of Turkey and neighbouring regions: review. Folia Zool., 63: 1-62, 2014.

[9] Wojcik MJ, Ratkiewicz M, Searle B. Evolution of the common shrew *Sorex araneus*: chromosomal and molecular aspect. Acta Theriol., 47(1): 139-167, 2002.

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

[11] Summer AT. A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res., 75: 304-306, 1972.

[12] Dubey S, Salamin N, Ruedi M, Barrière P, Colyn M, Vogel P. Biogeographic origin and radiation of the Old World crocidurine shrews (Mammalia: Soricidae) inferred from mitochondrial and nuclear genes. Mol Phylogenet Evol., 48: 953–963, 2008.

[13] Catzeflis F, Maddalena T, Hellwing S, Vogel P. Unexpected findings on the taxonomic status of East Mediterranean *Crocidura russula* auct. (Mammalia, Insectivora). Mamm Biol., 50: 185–201, 1985.

[14] Meylan A, Hausser J. Position cytotaxonomique de quelques musaraignes du genre *Crocidura* au Tessin Mammalia, Insectivora). Rev. suisse zool., 81: 701-710, 1974.

[15] Zima, J. & Král, B. Karyotypes of European mammals II. Acta Sc. Nat. Brno., 18(8): 1–62, 1984.

[16] Tembotova FA. On the systematics of white-toothed shrews in the Caucasus. Mammalian fauna and ecology in the Caucasus Nalchik., 163–189 (in Russian), 1987.

[17] Graphodatsky AS, Radjabli SI, Sharshov AV, Zaitsev MV. Karyotypes of five *Crocidura* species of the USSR fauna. Citologiya., 30: 1247–1252. (in Russian with English summary), 1988.

[18] Ivanitskaya E. Constitutitive heterochromatin and nucleolar organizer regions in karyotypes of some shrews (Soricidae, Insectivora). Genetika., 25: 1188-1198 (in Russian), 1989.

[19] Atanasov IN, Chassovnikarova GT. Karyotype Characteristics of *Crocidura leucodon* Herman, 1780 and *Crocidura suaveolens* Pallas, 1811 (Mammalia: Insectivora: Soricidae) in Bulgaria. Acta Zool Bulgar., 61(2):71-78, 2008.

[20] Orlov VN, Radjabli SI, Malygin VM, Chotolchu N, Kovalskaja JuM, Bulatova NS, Baskevic ML. The karyotypes of the mammals of Mongolia: Geografiya i dinamika rastitelnogo i zivotnogo mira MNR, Nauka, Moskva, pp. 149-164 (in Russian), 1978.

[21] Sokolov VE, Tembotov AK. Vertebrates of the Caucasus. Mammals. Insectivores. Nauka, Moskva, (in Russian), 1989.

[22] O'Brien SJ, Menninger JC, Nash WG. Atlas of mammalian chromosomes. Wiley-Liss, Hoboken, NJ., 2006.

[23] Biltueva L, Vorobieva N, Perelman P, Trifonov V, Volobouev V, Panov V, Ilyashenko V, Onischenko S, O'Brien P, Yang F, Ferguson-Smith M, Graphodatsky A. Karyotype Evolution of Eulipotyphla (Insectivora): The Genome Homology of Seven *Sorex* Species Revealed by Comparative Chromosome Painting and Banding Data. Cytogenet Genome Res., 135: 51–64, 2011.