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A Cytogenetic Study on Spermophilus *Xanthoprymnus* (Bennett, 1835) in Isparta Province

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ABSTRACT

This study was conducted to explain the karyological characteristics of *S. xanthoprymnus* from Gencali in Turkey. The chromosomes of the specimens were traditionally stained and examined according to standard procedures. The chromosomes were determined according to centromere positions by examining the photographs of metaphase cells. The karyotype of *S. xanthoprymnus* contained 42 chromosomes (2n=42) and fundamental number of chromosomal arms NF=81 and the number of autosomal arms NFa=78. The karyotype includes four metacentric chromosomes ranging from large to small, three pairs of large and medium-sized submetacentric autosome, twelve ranging from large to medium size subtelocentric, and a pair of acrocentric autosomes. The X chromosome was determined as large subtelocentric and Y chromosome as small acrocentric. Due to there is no karyological information and geographical distribution of cytotypes in Turkey, revelation of chromosomal structure in Anatolian ground squirrel populations will provide properly assessment of taxonomy of those rodents in further studies.

1. Introduction

There are approximately 41 species in the genus *Spermophilus* [1]. *S. citellus* (Linnaeus, 1766), *S. xanthoprymnus* (Bennet, 1835) and *S. taurensis* (Gündüz et al. 2007) are three species found in Turkey.

*Spermophilus xanthoprymnus*, Anatolian ground squirrel, is a group-living, diurnal, obligately hibernating marmotine squirrel (Figure 1). It inhabits the steppes and alpine meadows throughout central lowland and eastern highland Anatolia and adjacent Armenia and northwestern Iran. Its preferred elevation appears to range from about 800 to 2,900 m [2].

Figure 1. *S. xanthoprymnus* from Gencali

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The first karyological investigation of *S. xanthoprymnus* was carried out by Orlov et al. [3] and Voroncov and Lapunova [10] on individuals from Armenia.

In another study, the findings of karyotypes revealed a difference between Anatolian populations (*S. xanthoprymnus*) and Thrace populations (*S. citellus*) [5]. Based on this karyologic difference, they have concluded that the ground squirrel in Central Anatolia is *S. xanthoprymnus* [9].

Krytufek and Vohralík [6] classified the Anatolian ground squirrel as a genus that distributed from Turkey to western Armenia. In the Taurus Mountains of Anatolia, Ozkurt et al. (2002) [7] identified a restricted population with a 2n = 40 karyotype in 2002. Thrace population *S. citellus* is the most closely related to Taurus population *S. taurensis*, according to molecular and karyotype studies [5,7-11].

Ozkurt et al. (2007) [13] and Kalafat [14] determined the taxonomic status of the *S. taurensis* and *S. xanthoprymnus* distribution external and cranial characters, dentition, and karyology in a study performed in Turkey and Iran. According to research on *S. xanthoprymnus*, the species chromosome number in Turkey is 2n=42 [5,7-9,11,13].

Phylogenetic and taxonomic studies include not only taxonomic analyses of morphological characteristics but also karyological similarity. Furthermore, these studies provide very important data and a better understanding of how communities react to disease, fragmentation, global warming, and other ecological and sociological causes. A new locality, Gencali-Isparta was identified for the Anatolian ground squirrel. Since this habitat is impacted by human activities, the Anatolian ground squirrel is at risk of extinction [15].

Accurate assessment of species distribution and their taxonomic status is a fundamental need for addressing biological diversity in any geographic area. This is the most basic requirement to start biodiversity management and conservation planning studies. Revelation of chromosomal structure in Anatolian ground squirrel populations, for those there is no karyological information and geographical distribution of existent cytotypes in Turkey, will provide properly assessment of taxonomy of those rodents in further studies.

The aim of the present study is to investigate the karyological characteristics of *S. xanthoprymnus* in Isparta. The karyotypes of *S. xanthoprymnus* in Gencali were studied before the species became extinct in the area. This study of *S. xanthoprymnus* in Gencali-Isparta can be used to investigate chromosomal evolution and phylogeography [9,16].

### 3. Results and Discussion

*S. xanthoprymnus* (in Gencali Province) has a karyotype of 2n=42, NF=81 and NFa=78. Karyological characteristics show that chromosome 1 is large metacentric, chromosomes 6 and 9 are medium-sized metacentric, chromosome 19 is short metacentric, chromosome 12 is medium-sized submetacentric, chromosomes 2 and 3 are large submetacentric. Subtelocentric chromosomes 4, 5, 7, 8, 10, 11, 13-18 are large to medium-sized. The number of acrocentric chromosomes in one pair is 20. The X chromosome is a large subtelocentric. The smallest acrocentric chromosome was discovered to be the Y chromosome (Figure 2).

### 2. Material and Methods

Habitat of these species are plateau which named “On Kuyular” in Isparta-Gencali province, location altitude at 1592 m. *S. xanthoprymnus* associates with a continental climate and inhabit short-grass steppes. To examine with respect to karyological characteristics, animals (2 males) were collected (in September 2017) from Gencali province. 3 cc of venous blood was taken from the ground squirrels included in the study into tubes containing heparin. For culturing blood samples; the nutrient medium was created by mixing 50 ml RPMI 1640 medium, 1 ml L-Glutamin, 10 cc fetal bovine serum ve 0.5 cc penicillin-streptomycin. 5 ml of this enriched medium was taken into falcon tubes and 0.5 ml blood sample was added. In order to obtain chromosome from lymphocyte cells incubated for 72 hours, it was stopped at the metaphase stage with 0.1 ml colcemid at 71th hour. Then, harvesting steps such as hypotonic solution, fixative application, washing, spreading and drying were applied, respectively. Respectively Trypsin, PBS (Phosphate-buffered saline), Leishman dye and distilled water were applied to the preparations aged by drying and the preparations were prepared for analysis. Using the Akas chromosome analysis system (Argenit, Istanbul, TR), 20 metaphase areas at the 550 band level were examined [17-20].

![Figure 2. Karyotype of a male *S. xanthoprymnus* from Gencali](image-url)
Karyotype of Isparta-Gençali population was found to be similar to those of Polatlı [7], Maden [7], Erzurum [7], and Konya-Selçuklu [10] populations, with a diploid number of 42, a fundamental number of 81, and a number of autosomal arms of 78.

However, karyological study of *S. xanthoprymnus* in Armenia [1,4] (2n=42, NF=70 and NFA=66); Bayburt, Corum, Erzurum, Sivas [5] (2n=42, NF=67 and NFA=64; and Malatya [11] (2n=42, NF=68 and NFA=64) differs from Isparta (Gençali province). Dogubeyazıt-Iğdır, Dogor-Kars and Baskale-Van karyological analysis of *S. xanthoprymnus* revealed that 2n=42, NF=82 and NFA=78 [13].

The karyological study results of Gümüşhane and Bitlis are 2n=42, NF=84 and NFA=80 [21]. In Turkey, Arslan and Zima presented data regarding *S. xanthoprymnus* 2n=42, NFA=76-78, NF=80-82; X=Metacentric, Y=Acrocentric/Metacentric [22]. The karyotype consists of 16 or 17 pairs of submetacentric and subtelocentric, and one or two pairs of acrocentric autosomes. The karyotype of species from Doğor, Ozalp, and Baskale provinces in Turkey, as well as those from Iran, was 2n=42, NF=78, NFA=74 [13]. A karyotype of 2n=42, NF=80 and NFA=76 was found in species from Eregi (Konya) and Sarz (Kayseri) in Turkey. 2n=42, *S. xanthoprymnus* karyotypes were obtained from Trabzon (Altındere), Van (Yatıksırt), Konya (Cihanbeyli, Yesilkoy, İlgın, Cayırbağ), Nigde (Ulusla) [9], Gümüşhane, and Bitlis [21].

To determine chromosomal evolution and phylogeny of *S. xanthoprymnus* isolated populations or species in different locations, extensive comparative studies should be carried out using advanced cytogenetic methods, with the findings being validated by molecular studies. Researchers may clarify the phylogenetic variety of *S. xanthoprymnus* in Turkey using this case.

4. Conclusions

This study was carried out to reveal the karyological characteristics of ground squirrel whose new location was reported for Isparta region. It is the first karyological study performed in the population in Isparta. Since the population in the field is under human pressure and is in danger of extinction, the findings make a significant contribution to the literature. The diploid chromosomes number of *S. xanthoprymnus* was 2n=42 and karyotype of consisted of NF=81 NFA=78. Sex chromosomes characteristics X chromosome is large subtelocentric and Y chromosome is smallest acrocentric were determined. These results are important because it provides basic information for the phylogenetic studies variation of populations living in different locations in Turkey.

References

[1] Toyran, K., Gözütok, S, Yorulmaz, T. (2012) Bitlis İl’inden Anadolu Yer Sincabı, *Spermophilus xanthoprymnus* (Bennett, 1835) (Rodentia: Sciuridae) İçin Yeni Lokalite Kaydı. BEU Journal of Science, 1(2), 107-113.
[2] Gür M., Gür, H. (2010) *Spermophilus xanthoprymnus* (Rodentia: Sciuridae). Mammalian Species, 42(864), 183-194.
[3] Orlov V. N., Rodova M. A., Kotenvoka E. A. (1969) Chromosomal differentiation of the ground squirrels subgenus Citellus. Mat. II. SOV., Pomlekopit., 48-49.
[4] Voroncov N. N., I’apunova E. A. (1969) The chromosomes of Palearctic ground squirrels (Citellus, Marmotinae, Sciuridae, Rodentia). Mat. II. SOV., Pomlekopit., 41-47.
[5] Dogramaci, S., Kefelioglu, H., Gunduz, I. (1994) Karyological analysis of the genus *Spermophilus* (Mammalia: Rodentia) in Turkey. Turkish Journal of Zoology, 18, 167-170.
[6] Krystufek B., Vohralik V. (2005) Mammals of Turkey and Cyprus: Rodentia I: Sciuridae, Dipodidae, Gliridae, Arvicolinae. Knjiznica Annales Majora, Koper, 1-292, Slovenia.
[7] Ozkurt, S., Yigit, N., Colak, E. (2002) (a). Karyotype variation in Turkey populations of *Spermophilus* (Mammalia: Rodentia). Mammalian Biology, 67, 117-120.
[8] Yigit, N., Neumann, K., Ozkurt, S., Colak, E., Çolak, R. (2005) Biometric and genetic evaluation of *Spermophilus* (Mammalia: Rodentia) populations in western Turkey. Israel Journal of Zoology, 51, 191-198.
[9] Gunduz, I., Jaarola, M., Tez, C., Yeniyurt, C., Polly, P.D., Searle, J.B. (2007) Multigenic and morphometric differentiation of ground squirrels (Spermophilus, Sciuridae, Rodentia) in Turkey, with a description of a new species. Molecular Phylogenetics and Evolution., 43, 916-935.
[10] Arslan, A. (2005) Cytogenetic studies on *Spermophilus* xanthoprymnus (Mammalia: Sciuridae) in central Anatolia. Folia Zoologica, 54, 278-284.
[11] Gaffaroglu, M., Yuksel, E. (2006) Karyotype analysis of ground squirrel (Mammalia: Rodentia) in Malatya (Turkey). Journal of Biological Sciences, 6, 754-757.
[12] Yigit, N., Gharkheloo, M.M., Colak, E., Ozkurt, S., Safak, B., Kankılıc¸, T., Colak, R. (2006) The karyotypes of some rodent species (Mammalia: Rodentia) from eastern Turkey and northern Iran with a new record, Microtus schidlovskii (Argyropulo, 1933) from Eastern Turkey. Turkish Journal of Zoology, 30, 459-464.
[13] Ozkurt, S. O., Sozen, M., Yigit, N., Kandemir, I., Colak, R., Gharkheloo, M. M., Colak, E. (2007) (b). Taxonomic Status of The Genus *Spermophilus* (Mammalia: Rodentia) in Turkey and Iran with Description of a New Species. Zootaxa, 1529, 1-15.

[14] Kalafat, A. (2011) Biology, ecology of *Spermophilus* taurensis and the comparison as morfometrie with the other *Spermophilus* species in Turkey. Selçuk University, Graduate School of Natural and Applied Sciences, The Degree of Master of Science in Biology. Konya, 1-36.

[15] Aksan, S. and Cohadar, H. (2018) New Locality Record of The Anatolian Ground Squirrel, *Spermophilus xanthoprymnus* Bennett 1835, in Isparta-Gencali Province. Fresenius Environmental Bulletin, 27(12A), 9172-9178.

[16] Kankılıç T, Kankılıç T. (2017) Chromosomal Evolution and Phylogeography of The Anatolian Mole Rats. Kebikeç İnsan Bilimleri için Kaynak Araştırmaları Dergisi, 0(43), 219-233.

[17] Howe, B., Umrigar, A., Tsien, F. Chromosome Preparation From Cultured Cells. J. Vis. Exp. (83), e50203, doi:10.3791/50203 (2014).

[18] Bulla, L. M. C., Ambrosio E. P., Martins, A. B. T., DellaRosa, V. A. (2014) Viability of lymphocyte culture, at different times after blood collection, for karyotype analysis. J Bras Patol Med Lab, 50(2), 124-130.

[19] Ford, C. E., Hamerton, J. L. A. (1956) Colchicine, hypotonic citrate, squash sequence for mammalian chromosome. Stain Technol, 31(6), 247-251.

[20] Lionel, W., Sian, M. (2009) Shaffer LG, Slovak ML, Campbell LJ (2009): ISCN 2009 an international system for human cytogenetic nomenclature. Human Genetics, 126(4), 603-604.

[21] Arslan, A., Toyran, K., Gözütok, S., (2020) The Karyotype of Anatolian Ground Squirrel *Spermophilus xanthoprymnus* (Bennett, 1835) (Rodentia: Sciuridae) from two localities in Anatolia, Turkey. Acta Zoologica Bulgarica, 72 (3), September 2020: 363-367. http://www.acta-zoologica-bulgarica.eu/002376.

[22] Arslan, A., Zíma, J. (2014) Karyotypes of the mammals of Turkey and neighbouring regions: a review. Folia Zoologica, 63(1), 1-62.