*Acta Biologica Turcica*, 37(3), J4:1-7, 2024 Received 28 Dec. 2023; Accepted 29 Feb. 2024; Published Online 08 Mar. 2024

ACTA BIOLOGICA TURCICA

E-ISSN: 2458-7893, http://www.actabiologicaturcica.com

*Research article*

# Phylogenetic assessment based on the allozyme variations in Turkish ground squirrels (*Spermophilus* spp.) (Mammalia: Rodentia)

**Nuri YİĞİT*, Pınar EROL, Ercüment ÇOLAK**

Department of Biology, Faculty of Science, Ankara University, Ankara, Türkiye

*Corresponding author e-mail: nyigit@science.ankara.edu.tr

**Abstract:** Three ground squirrel species are distributed in Anatolia as *Spermophilus xanthoprymnus, Spermophilus citellus* and *Spermophilus taurensis*. In this study, it is aimed to determine the genetic variation between populations and to contribute to the phylogeny of the species by studying two allozyme variations in three species distributed in Türkiye. Variations in the alleles of isocitrate dehydrogenase (Idh-S) and phosphoglucomutase (Pgm) enzymes were investigated using starch gel electrophoresis technique in the tissues of 37 specimens belonging to the three species of *Spermophilus* from Thrace, Central Anatolia, and Western Mediterranean regions. According to the obtained results, Idh-S enzyme was found to be monomorphic homozygous in the studied taxa. The Pgm enzyme is polymorphic homozygous and is represented by the B allele (B) in *S. citellus* and *S. taurensis*, and (A) allele in the Central Anatolian population of *S. xanthoprymnus*. This finding supports that there is a closer phylogenetic relationship between *S. citellus* and *S. taurensis*.

**Keywords:** Spermophilus, Isocitrate dehydrogenase, Phosphoglucomutase, Allozyme

**Citing:** Yiğit, N., Erol, P., & Çolak, E. (2024). Phylogenetic assessment based on the allozyme variations in Turkish ground squirrels (*Spermophilus* Spp.) (Mammalia: Rodentia). *Acta Biologica Turcica,* 37(3), J4:1-7.

## Introduction

The first taxon from Türkiye belonging to the *Spermophilus* genus was given by Bennet (1835) in 1835 from Erzurum as *Citellus xanthoprymna*. Due to the fact that the type species (*Spermophilus citellus*) is in the *Spermophilus* genus, Euro-asian species are now collected in the *Spermophilus* subgenus and this genus name has been used instead of *Citellus* (Herron et al., 2004).

The first studies to reveal the morphological and biometric characteristics of Turkish *Spermophilus* were made by Mursaloğlu (1964, 1965). Mursaloğlu (1964) compared the populations of *Citellus citellus martinoi*, *Citellus citellus xanthoprymnus* and *Citellus citellus citellus* from Dobruca (Bulgaria) with the Kırklareli population biometrically, and *Citellus xanthoprymnus* from Erzurum as valid taxa, and *Citellus citellus thracius* from Thrace and *Citellus citellus gelengius* from Anatolia for the first time. In addition, Mursaloğlu (1964) noted that in the Turkish population of *Citellus* has at least 4 groups that differ from each other at the subspecies level as given above with the fourth one from Van province.

Zima and Kral (1984) noted that *S. citellus* and *S. xanthoprymnus* are separate taxa based on their karyological characteristics. Later, Doğramacı et al.

(1994) in his taxonomic study on Turkish *Spermophilus*, taking into account the diploid chromosome number (2n), he recorded the population in Thrace as *Spermophilus citellus* with 2n; 42 chromosomes, and the Anatolian population as *Spermophilus xanthoprymnus* with 2n; 40 chromosomes.

On the contrary, Özkurt et al. (2002) noted that the Anatolian population of *Spermophilus* had 2n: 42 chromosomes and showed that the Western Mediterranean samples, unlike Anatolia, had 2n: 40 chromosomes, as in Thrace, and stated that the 2n number was not distinguishing between the species.

In the first genetic study conducted by Yiğit et al. (2005) on Turkish ground squirrels, it was stated that Western Taurus samples with 2n: 40 chromosomes were genetically related to Thrace samples, whereas Central Anatolian samples might have been differentiated by a secondary expansion from Taurus samples.

Two studies published later in the same year identified Ground squirrels in the Western Taurus as a new species (Gündüz et al., 2007; Özkurt et al., 2007). Because researchers defined the same taxon under different species names in the same year, *Spermophilus torosensis*, which was defined by Özkurt et al. (2007), became synonymous of *S. taurensis* (Gündüz et al., 2007). In the light of these findings, it is seen that *S. citellus* is distributed in Thrace, *S. taurensis* in the Western Taurens and *S. xanthoprymnus* in Anatolia.

Under the biochemical and genetic studies carried out to reveal the genetic variation and phylogenic relations in Ground squirrel species in Türkiye; the blood-serum protein profiles of *S. citellus*, *S. xanthoprymnus* in Türkiye and *S. fulvus* in Iran were documented by Çolak & Özkurt (2002), and Çolak et al. (2006). It was reported that the blood-serum protein profiles by using SDS-page distinguished *S. xanthoprymnus* and *S. fulvus* (Çolak et al., 2006). In another study on the phylogeny of ground squirrel performed by Kryštufek et al. (2009), including Thrace samples of Türkiye, using the Cyt-*b* mitochondrial marker, it was stated that the phylogeographic pattern of *S. citellus* was related to the steppe ecosystem and glacial periods. It has been also suggested that the southern lineage, which includes Greece, Macedonia, and Türkiye, diverged ca 0.58 Mya (Cothran et al., 1977). Based on cyt-b sequences, Gündüz et al. (2007) reported that *S. citellus* and *S. taurenis* diverged 2.5 million years ago, and the ancestors of these two species diverged 5 million years ago from *S. xanthopyrmnus*.

Apart from these mitochondrial markers, alloenzyme studies on *Spermophilus* species are scarce, and alloenzyme studies generally focused on North American populations of *Spermophilus* (Nadler et al., 1982; Gill et al., 1992; Gavin et al., 1999; Frisman et al., 1999; Dobson, 1994).

Although the use of mitochondrial and nuclear markers in phylogenetic studies has been popular in recent years, allozyme data were used in the first phylogenetic studies. In this context, it is aimed to determine the genetic variation between populations and to contribute to the phylogeny of the species by studying two allozyme variations in three species distributed in Türkiye.

**Materials and Methods**

In this study, tissue samples of Ground squirrels stored in the Mammalian Research Collection of Ankara University (AUMAC, www.mammalia.ankara.edu.tr) captured before 2003 were used (12 samples in *S. citellus* 12 in *S. xanthopyrmnus* and 13 in *S. taurensis*). The specimens were obtained from Edirne (*S. citellus*) from different locations in Central Anatolia (*S. xanthopyrmnus* and Western mediterranen region (Akseki/Antalya Western Taurus Mountain) (*S. taurensis*)

Protocol of starch gel electrophoresis;

1. Tissue preparation; Frozen muscle tissues were cut into small pieces and placed in ice-cold distilled water. This mixture, taken into the homogenizer tube, was homogenized mechanically. Just before the electrophoresis process, the homogenate was centrifuged at 12 100 rpm for 3 minutes in order to get rid of cellular residues.

2. Electrophoretic procedures were carried out as given by Shaw & Prasad (1970), Harris & Hopkins (1976) with small modifications; the starch gel percentage was 11 % and the samples loaded on gel were run at 12 v / cm for 2.5 hour.

3. Histochemical staining:

For the isocitrate dehydrogenase enzyme;

Dyeing Buffer; 0.1 M Tris – Cl Buffer pH: 8.0 500 ml (Tris was dissolved in 300 ml of water by taking 6.05 g, the pH was adjusted to 8.0 with HCl and made up to 500 ml with distilled water).

Dyeing mixture;

15 ml Tris – Cl pH: 8.0
25 mg isocitric acid
5 mg NADP
20 mg MgCl2
20 mg $MnCl_2$
5 mg MTT
1 mg PMS

Agaroverlay; 300 – 350 mg of agar was added to 35 ml dye buffer and boiled, and after the agar cooled slightly, the dyeing mixture were mixed and poured onto the gel. It was visualized by incubating for 30 - 90 minutes in the dark in an oven at 37 °C (Hills et al., 1996).

For the phosphoglucomutase enzyme;

Dyeing mixture;

25 ml 0.1 M Tris – HCl pH: 8.0
0.1 g MgCl2.6H2O
0.05 g D-Glucose -1- phosphate
25 µl G6pdh (80µ+100µl H2O)
0.02 g NADP
0.02 g NBT
0.005 g PMS

The same protocol was also used for this enzyme.

4. Gel fixation: After the enzyme bands were seen, the reaction was stopped by washing with gel fixation solution [45 parts of 13 methanol, 55 parts of acetic acid solution (1acetic acid: 5 $H_2O$)].

5. Documentation of results: When staining was complete, the gel was photographed by placing it on the light box, and alleles were located by plotting the observed band patterns. Then, the calculation of allele frequencies was done using these zymograms. Allozyme were numbered according to the most common allele. The most common allele was given numbers 100, slow movers from this allele were given numbers less than 100, and fast movers were given numbers greater than 100. Allele frequency was calculated according to the formula (p = f (A) = (2 x number of AA homozygotes) + (number of Aa heterozygotes) / (2 x total number of individuals)).

6. Data analyses; the computer program NTSYS-pc (version 2.1) (Rohlf, 1988) were used in all data analyses. Dendrograms were produced based on the similarity coefficients of Distance. Sahn clustering is the hierarchical method as in UPGMA.

## Results

### *Habitat preferences of ground squirrels*

Ground squirrels are steppe-specific, diurnal and hibernator species. Although Thrace, Central Anatolia and Western Taurus have different climatic and vegatation structures, the distribution areas of Ground squirrels cover these geographies. *S. citellus* ranges on the steppes and plains in the southern parts of Thrace, except for the Istranca, which covers the northern part of Thrace. *S. xanthoprymnus* is distributed from the Central Anatolian steppes with an average altitude of around 1000 m to the east at very high altitudes. In general, the distribution of this species extends from the Anatolian plateau to Iran. The third species, *S. taurensis*, is native to the Western Taurus, which is a part of the Mediterranean region of Türkiye. Although there is no exact information about its exact distribution, it spreads in the high steppes of the Taurus Mountains.

### *Allozyme variations in Turkish ground squirrels*

***Spermophilus citellus* (Lin., 1766)**

Type location: Austria

1857. *Spermophilus citellus* Blasius, Säugethiere Deutschlands, p. 339.

The Idh-S locus of 12 samples belonging to the *S. citellus* species from the Thrace region was analyzed. A single allele of the cytoplasmic enzyme running in the anodal direction was detected in the studied samples of this species.

This was accepted as the most common allele and this allele was recorded as Idh - $S^{100}$ (AA), the allele frequency of the enzyme is 1 and it can be said that the population is homozygous for this monomorphic enzyme. Idh-M, the mitochondrial form of Idh running in the anodal direction but closer to the origin, was not evaluated.

For the Pgm enzyme, 12 samples belonging to the *S. citellus* species were analyzed. It was determined that Pgm has only one allele in this species. This was determined as the most common allele and this allele was recorded as $Pgm^{100}$ (BB) The allele frequency of this enzyme in the population was also 1, and it was determined that all samples examined were homozygous for this enzyme as well.

***Spermophilus xanthoprymnus* (Bennet, 1835)**

Type location: Erzurum

1835. *Citellus xanthoprymna* Bennet. Observations on several Mammalia from Trebizond and Erseroum, Proceed. Zool.Society of London, pp.89, 90.

1877. *Spermophilus xanthoprymnus* Danford and Alston. On the Mammals of Asia Minor, Proceed. Zool.Society of London, pp.277-278.

Idh-S and Pgm loci were analyzed in muscle tissue of 12 samples from Central Anatolia belonging to the *S. xanthoprymnus* species. A single allele was detected in the Idh-S locus of the studied samples, this allele was shown as the common allele as Idh - $S^{100}$ and was determined to be homozygous.

Two different alleles were detected between *S. xanthoprymnus* and *S. taurensis* in the other enzyme Pgm which is polymorphic. Of these two alleles, one which run further from the origin (fast allele), were detected in the Central Anatolian population of *S. xanthoprymnus* and recorded as $PGM^{105}$. Allele frequency is 1 and found to be homozygous (Table 1).

**Table 1.** Frequencies of alleles observed for Idh-S and Pgm in Ground squirrels

| Alleles | *S. citellus* | *S. xanthoprymnus* | *S. taurensis* |
|---|---|---|---|
| Idh (A) | 1 (AA) | 1 (AA) | 1 (AA) |
| Idh (B) | 0 | 0 | 0 |
| Pgm (A) | 0 | 1 (AA) | 0 |
| Pgm (B) | 1 (BB) | 0 | 1 (BB) |

***Spermophilus taurensis* Gündüz et al. 2007**

Type location: Yarpuz, Akseki (Türkiye)

2007. *Spermophilus taurensis* Gündüz et al., Mol. Phylogenet. Evol. 43: 916-935.

Idh-S and Pgm were studied in the muscle tissue of 13 samples captured around Akseki (Antalya). As in the previous species, only one allele was detected in Idh-S and it was shown as Idh - $S^{100}$. The allele frequency is 1 and homozygous. The allele (slow allele) located closer to the origin at the Pgm locus was shown as $PGM^{100}$ and is also homozygous.

***Allele frequency differences and phylogenetic relationships among Turkish ground squirrel species***

In samples of *S. citellus*, *S. xanthoprymnus* (Central Anatolia) and *S. taurensis* species, the Idh - S locus was fixed with only one allele, Idh - $S^{100}$ (AA). In this respect, these three species are homozygous for the Idh-S enzyme and there is no difference between species within species.

In Pgm enzyme, $Pgm^{100}$ allele was detected similarly in *S. citellus* and *S. taurensis* (Taurus Mountain) populations, while Pgm $Pgm^{105}$ allele was detected only in *S. xanthoprymnus* population, unlike these two species. In the phylogeny tree based on allele frequencies, it was determined that *S. citellus* from Turkish Thrace, and *S. taurensis*, which spread in the Western Taurus, were closer to each other (Figure 1).

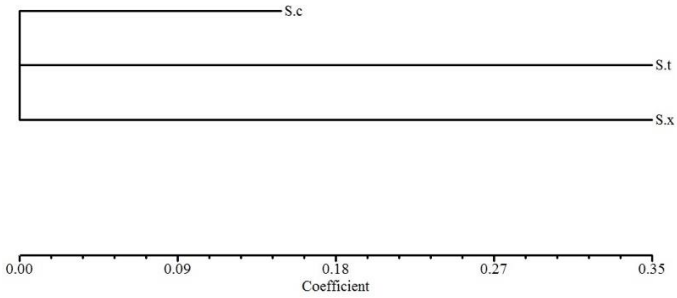


**Figure 1.** UPGMA tree generated from Idh-S and Pgm allele frequencies in three species (S.c: *S. citellus*, S.x: *S. xanthoprymnus*, S.t: *S. taurensis*).

## Discussion

Ground squirrels are species that range in the Thrace and Anatolian steppes. (Yiğit et al., 2003, 2005, 2006; Özkurt et al., 2007; Arslan & Arslan, 2007; Toyran et al. 2012; Topçu & Albayrak, 2013; IUCN, 2023a). Due to the intense anthropogenic impact on the Thrace region, the IUCN (International Union for Conservation of Nature and Natural Resources) listed as endangered (EN) criteria indicating that the *S. citellus* (European Souslik) population is under threat (IUCN, 2023b). *S. xanthoprymnus* (Anatolian ground squirrel) is distributed in the Anatolian steppes to Iran and is a more common species. IUCN

listed this species as near threat (NT) category (IUCN, 2023c). The third species *S. taurensis* (Western Taurus ground squirrel) has been evaluated as IUCN threat category LC (Least concern) due to the absence of a systematic threat on its population, despite its distribution in a constricted area in the western Taurus (Harrison, 2003).

Speciation and evolutionary expansions of ground squirrels have been the focus of attention of many researchers (Mursaloğlu, 1965; Gündüz et al., 2007; Özkurt et al., 2007; Kryštufek et al., 2009; Sinitsa & Pogodina, 2019). The first biochemical studies to reveal genetic variation and phylogenetic relationships in species were performed by Cothran et al. (1977), Nadler et al. (1982), Gill & Yensen (1992), Dobson (1994), Frisman et al. (1999), Gavin et al. (1999).

According to the 28 enzyme loci analyzed by Cothran et al. (1997), *S. tridecemlineatus* and *S. mexicanus* were found to be genetically very identical to each other and the divergence time was estimated to be within 155,000 years. Nadler et al. (1982) analyzed at least 18 enzyme loci in holoartic Ground squirrels, and polymorphic ones among the analyzed enzymes include Pgm. In our study, Pgm was also similarly found to be polymorphic in Turkish populations. In addition, *S. suslicus*, *S. xanthoprymnus*, *S. citellus*, *S. musicus* and *S. pygmaeus* were included in the same clade in the phylogeny tree created according to allozyme data Nadler et al. (1982). Gill & Yensen (1992) studied 31 enzyme locus of Idaho ground squirrels and found 15 loci to be polymorphic. This allozyme data revealed that the genetic distance was found in the range of the subspecies level in the two subpopulations. Dobson's (1994) study on Colombian ground squirrels (*Spermophilus columbianus*) reported increased homozygosity in allele frequencies due to short-distance migrations. Frisman et al. (1999) analyzed 11 enzyme and 4 non-enzyme proteins in *S. suslicus* and emphasized that geographic barriers are effective on the karyological forms of this species. They stated that the Bosphorus separates *S. citellus* and *S. xanthoprymnus* as well as river systems such as Danube, Prut, Dnieper, Donetsk, and the middle Volga on the isolation of *S. citellus*, *S. suslicus* and *S. pygmaeus* populations. In the allozyme study performed by Gavin et al. (1999) provided important information about the genetic structure of subpopulations of *S. brunneus* and interpreted the impact of genetic drift and habitat fragmentation on the subpopulations. These biochemical findings provided by many researchers are consistent with our findings that Thrace population of *S. citellus* and Anatolian species have the close degree of genetic relationships. In addition, these data are consistent with the findings obtained from mitochondrial markers. While Yiğit et al. (2005) suggesting that the origin Anatolian ground squirrels will be of European origin, according to genetic similarity, Gündüz et al. (2007) suggested that *S. citellus* and *S. taurensis* must have originated from *S. xanthoprymnus*, which was distributed in Anatolia (*S. xanthoprymnus* separated from the *S. citellus*–*S. taurensis* lineage about 5 Mya). In contrast, Kryštufek et al. (2009) stated that the southern clade of *S. citellus* which spread in the Balkans, diverged 0.58 Mya. Sinista & Pogodina (2019) stated that the oldest member of the genus, from the late Pliocene and Early Pleistocene of southern Ukraine. Nadler et al. (1982) reported that most of the recent species in these lineages evolved during the late Pleistocene. Kowalski (2001) pointed out that speciation of Ground squirrels took place in the Pleistocene. The close proximity of *S. citellus* and *S. suslicus* in the phylogenetic analyzes by Herron et al. (2004) and Helgen et al. (2009) raises the question that *S. citellus* may have originated from *S xanthoprymnus* in the evaluation of Gündüz et al. (2007).

In the phylogeny trees produced in (Yiğit et al., 2005; Gündüz et al., 2007), and in this allozyme study, *S. citellus* and *S. taurensis* placed closer to each other than *S. xanthoprymnus*. This finding necessitates more comprehensive phylogeographic evaluations regarding the origin of the species.

**Acknowlegements**
The authors would like to thank recreational fisherman Fuat Kasmalar, who caught the fish and share it with us for scientific purpose.

**Ethical Approval**
In this study, tissue samples of ground squirrels stored in the Mammalian Research Collection of Ankara University captured before 2003 were used. Therefore, there is no need to etichal approve.

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

**Funding Statement**
The authors don't declare any fund.

**References**

Arslan, E., & Arslan, A. (2010). Heterochromatin distribution and nucleolar organizer regions (NORs) in chromosomes of the Taurus ground squirrel, *Spermophilus taurensis* Gunduz et al., 2007 (Mammalia: Rodentia), in Turkey. *Turkish Journal of Zoology*, 34(1), 105-110. https://doi.org/10.3906/zoo-0810-5.

Bennet, E. T. (1835). Mammals of the neighbourhood of Trebizond and Erzeroum. In Proc. Zool. Soc. London (Vol. 3, pp. 89-90).

Çolak, R., Gharkheloo, M., & Özkurt, Ş. (2006). Distribution and electrophoretic aspects of blood-serum proteins of the genus *Spermophilus* (Mammalia: Rodentia) in eastern Turkey and Iran. *Pakistan Journal of Biological Sciences*, 9(3). https://doi.org/10.3923/pjbs.2006.519.521

Çolak, R., & Özkurt, S. (2002). Electrophoretic comparison of blood-serum proteins of *Spermophilus citellus* and *Spermophilus xanthoprymnus* (Mammalia: Rodentia) in Turkey. *Zoology in the Middle East*, 25(1), 5-8. https://doi.org/10.1080/09397140.2002.10637897.

Cothran, E. G., Zimmerman, E. G., & Nadler, C. F. (1977). Genic differentiation and evolution in the ground squirrel subgenus *Ictidomys* (genus *Spermophilus*). *Journal of Mammalogy*, 58(4), 610-622. https://doi.org/10.2307/1380009

Dobson, F. S. (1994). Measures of gene flow in the Columbian ground squirrel. *Oecologia*, 100, 190-195. https://doi.org/10.1007/BF00317146.

Doğramacı, S., Kefelioğlu, H., & Gündüz, I. (1994). Türkiye *Spermophilus* (Mammalia: Rodetia) cinsinin karyolojik analizi. *Turkish Journal of Zoology*, 18, 167-170.

Frisman, L. V., Korablev, V. P., Lyapunova, E. A., Vorontsov, N. N., & Brandler, O. V. (1999). Allozyme Differentiation of the Forms of the Spotted Souslik (*Spermophilus suslicus* Güld, 1770, Rodentia) with Different Chromosome Sets. *Russian Journal of Genetics*, 35(3), 307-313.

Gavin, T. A., Sherman, P. W., Yensen, E., & May, B. (1999). Population genetic structure of the northern Idaho ground squirrel (*Spermophilus brunneus brunneus*). *Journal of Mammalogy*, 80(1), 156-168. https://doi.org/10.2307/1383216.

Gill, A. E., & Yensen, E. (1992). Biochemical differentiation in the Idaho ground squirrel, *Spermophilus brunneus* (Rodentia: Sciuridae). *The Great Basin Naturalist*, 155-159.

Gündüz, I., Jaarola, M., Tez, C., Yeniyurt, C., Polly, P. D., & Searle, J. B. (2007). Multigenic and morphometric differentiation of ground squirrels (*Spermophilus*, Scuiridae, Rodentia) in Turkey, with a description of a new species. *Molecular phylogenetics and evolution*, 43(3), 916-935. https://doi.org/10.1016/j.ympev.2007.02.021.

Harris, H., & Hopkinson, D. A. (1976). Handbook of enzyme electrophoresis in human genetics, MRC Human Biochemical Genetics Unit Galton Laboratory Universty Colloge London.

Harrison, R. G., Bogdanowicz, S. M., Hoffmann, R. S., Yensen, E., & Sherman, P. W. (2003). Phylogeny and evolutionary history of the ground squirrels (Rodentia: Marmotinae). *Journal of Mammalian Evolution*, 10, 249-276. https://doi.org/10.1023/B:JOMM.0000015105.96065.f0.

Helgen, K. M., Cole, F. R., Helgen, L. E., & Wilson, D. E. (2009). Generic revision in the Holarctic ground squirrel genus *Spermophilus*. *Journal of Mammalogy*, 90(2), 270-305. https://doi.org/10.1644/07-MAMM-A-309.1.

Herron, M. D., Castoe, T. A., & Parkinson, C. L. (2004). Sciurid phylogeny and the paraphyly of Holarctic ground squirrels (*Spermophilus*). *Molecular Phylogenetics and Evolution*, 31(3), 1015-1030. https://doi.org/10.1016/j.ympev.2003.09.015.

Hillis, D. M., Moritz, C., & Mable, B. K., Eds. (1996). Molecular systematics. Sinauer Associates, Inc., Sunderland, MA.

IUCN, (2023a). https://www.iucnredlist.org/search?query=spermophilus%20citellus&searchType=species.

IUCN, (2023b). https://www.iucnredlist.org/search?query=Spermophilus%20xanthoprymnus%20&searchType=species.

IUCN, (2023c). https://www.iucnredlist.org/species/136590/22266677.

Kowalski, K. (2001). Pleistocene rodents of Europe. *Folia quaternaria*, (72), 3-389.

Kryštufek, B., Bryja, J., & Bužan, E. V. (2009). Mitochondrial phylogeography of the European ground squirrel, *Spermophilus citellus*, yields evidence on refugia for steppic taxa in the southern Balkans. *Heredity*, 103(2), 129-135. https://doi.org/10.1038/hdy.2009.41.

Mursaloğlu, B. (1965). Geographic variation in Citellus citellus (Mammalia: Rodentia) in Turkey. *Communications de la Faculte des Sciences de L'universite D'Ankara Serie C: Sciences naturales*, 10, 79-109.

Mursaloglu, B. (1964). Statistical significance of secondary sexual variations in Citellus citellus (Mammalia: Rodentia) and a new subspecies of *Citellus* from Turkey. *Communications, Faculty of Science, University of Ankara C*, 9, 252-273.

Nadler, C. F., Hoffmann, R. S., Vorontsov, N. N., Koeppl, J. W., Deutsch, L., & Sukernik, R. I. (1982). Evolution in ground squirrels. *Zeitschrift für Säugertierkunde*, 47, 198-215.

Özkurt, S. O., Soezen, M., Yigit, N., Kandemir, I., Colak, R., Gharkheloo, M. M., & Colak, E. (2007). Taxonomic status of the genus *Spermophilus* (Mammalia: Rodentia) in Turkey and Iran with description of a new species. *Zootaxa,* 1529, 1-15.

Özkurt, Ş., Yiğit, N., & Çolak, E. (2002). Karyotype variation in Turkish populations of *Spermophilus* (Mammalia: Rodentia). *Mammalian Biology*, 67(2), 117-119. https://doi.org/10.1078/1616-5047-00017.

Rohlf, J. F. (1988). NTSYS - pc, Numerical Taxonomy and Multivariate Analysis System, version 2.1, Exeter Publishing, LTD, New York.

Shaw, C. R., & Prasad, R. (1970). Starch gel electrophoresis of enzymes—a compilation of recipes. *Biochemical genetics*, 4(2), 297-320. https://doi.org/10.1007/BF00485780.

Sinitsa, M. V., & Pogodina, N. V. (2019). The evolution of early *Spermophilus* in eastern Europe and the antiquity of the Old World ground squirrels. *Acta Palaeontologica Polonica*, 64(3). https://doi.org/10.4202/app.00605.2019.

Topçu, A. A., & Albayrak, İ. (2013). The Bioecology of Spermophilus xanthoprymnus Bennett, 1835 in Kırıkkale Mammalia: Rodentia. *Hacettepe Journal of Biology and Chemistry*, 41(2), 115-124.

Toyran, K., Gözütok, S., & Yorulmaz, T. (2012). Bitlis İli'nden Anadolu Yer Sincabı, *Spermophilus xanthoprymnus* (Bennett, 1835) (Rodentia: Sciuridae) İçin Yeni Lokalite Kaydı. Bitlis Eren Üniversitesi Fen Bilimleri Dergisi, 1(2), 107-113.

Yiğit, N., Gharakheloo, M. M., Çolak, E., Özkurt, Ş., Bulut, Ş., Kankiliç, T., & Çolak, 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(4), 459-464.

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 Ecology and Evolution*, 51(3), 191-198. https://doi.org/10.1560/BQ3N-H0F6-VAXH-MHUQ.

Yiğit, N., Çolak, E., Sözen, M., & Özkurt, Ş. (2003). A study on the geographical distribution along with habitat aspects of rodent species in Turkey. *Bonner Zoologische Beiträge*, 50, 355-368.

Zima, J. (1984). Karyotypes of European mammals III. *Acta Scient Nat Brno*, 18(9), 1-51.