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Heterochromatin distribution and nucleolar organizer regions (NORs) in chromosomes of the Taurus ground squirrel, *Spermophilus taurensis* Gunduz et al., 2007 (Mammalia: Rodentia), in Turkey

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Abstract: The distribution of C-heterochromatin blocks and nucleolar organizer regions (NORs) in the Taurus ground squirrel from Turkey was studied. The karyotype of *Spermophilus taurensis* comprised 40 chromosomes, the fundamental chromosome number (NF) was 80, and there were 76 autosomal arms (NFa). All autosomes and the X chromosome had pericentromeric C-heterochromatin, whereas the Y chromosome appeared to be entirely heterochromatic. NORs were observed in the terminal regions of the short arm of 2 pairs of submetacentric and 2 pairs of subtelocentric chromosomes. All the NORs were homomorphic and of medium size. The *S. taurensis* karyotype differs from that of *S. xanthoprymnus* by a low diploid number, by a C-positive Y chromosome, and by the number and localization of NORs. It was also determined that its Y chromosome resembles that of *S. citellus*, in terms of its heterochromatic nature.

Key words: *Spermophilus taurensis*, C-banding, NOR localization, Turkey

Türkiye'deki Toros yer sincabı, *Spermophilus taurensis* Gunduz et al., 2007 (Mammlia: Rodentia)'in kromozomlarındaki heterokromatin dağılımı ve nükleolar organizatör bölgeler (NORs)

Özet: Türkiye'deki Toros yer sincabında C-heterokromatin blokların ve nükleolar organizatör bölgelerin dağılımları çalışıldı. *Spermophilus taurensis*'in karyotipi 40 kromozomdan oluşmuş, temel kromozom kol sayısı (NF) 80 ve otozomal kromozom kol sayısı (NFa) 76'dır. Y kromozomunun tamamı heterokromatinden oluşmuşken, otozomlarının tamamı ve X kromozom perisentromerik heterokromatine sahiptir. NOR'lar 2 çift submetasentrik ve 2 çift subtelosentrik kromozomların kısa kolunun terminal bölgesinde gözlendi. NOR'ların tamamı benzer şekilde ve orta büyüklüktedir. *S. taurensis* düşük kromozom sayısı, C-pozitif Y kromozomu ve NOR'ların lokalizasyonu ve sayısı açısından *S. xanthoprymnus*'dan farklıdır. Ayrıca onun Y kromozomunun heterokromatin özelliğinin *S. citellus*'a benzediği belirlenmiştir.

Anahtar sözcükler: *Spermophilus taurensis*, C-band, NOR lokalizasyonu, Türkiye

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Introduction

Prior to 1994 it was accepted that only one species of ground squirrel, *Spermophilus citellus*, occurred in Turkey and *S. xanthoprymnus* was recognized as a subspecies (Ellerman and Morrison-Scott, 1951; Corbet, 1978). Doğramacı et al. (1994) found that karyotypes distinguish the Anatolian populations from those distributed in Thrace (European parts of Turkey) (*S. citellus*, 2n = 40), and concluded that based on these differences the ground squirrel in Anatolia is a separate species, *S. xanthoprymnus* (2n = 42). Nonetheless, Özkurt et al. (2002) described a restricted population with an unusual karyotype in the Taurus Mountains of southwest Anatolia. This population has 2n = 40, while the standard chromosome number of *S. xanthoprymnus* is 2n = 42 (Doğramacı et al., 1994; Özkurt, et al., 2002; Arslan, 2005). In 2007 the discovery of a new species of the genus *Spermophilus* in southern Turkey was reported (Gündüz et al., 2007a; Özkurt et al., 2007). The name of the new species is Taurus ground squirrel, *Spermophilus taurensis* or *Spermophilus torosensis*. Gündüz et al. (2007b) proposed that *S. torosensis* was a subjective junior synonym of *S. taurensis*. The new species has restricted distribution in the Taurus Mountains in an area known as a hotspot for biodiversity. Molecular clock analysis suggests that the Taurus species diverged from the European ground squirrel (*S. citellus*) about 2.5 million years ago and that the ancestor of these 2 species diverged from the widespread Anatolian ground squirrel (*S. xanthoprymnus*) about 5 million years ago (Gündüz et al., 2007a).

Belcheva and Peshev (1985) investigated the C-band karyotype of Bulgarian specimens of *S. citellus* and Zima (1987) investigated that of specimens from the Czech Republic. Arslan (2005) observed heterochromatin regions in Anatolian *S. xanthoprymnus* and compared this pattern with that of *S. citellus*.

The present study presents a comparison of C- and Ag-NOR banded karyotypic data for *S. taurensis* from Turkey and they are compared with those from previous studies on this species.

Materials and methods

The 5 male study animals were collected from Konya province (Hadim; Meydacık Yaylası, 36°55′N, 32°25′E) (Figure 1). Karyotype preparations were obtained from the bone marrow of the colchicined animals (Ford and Hamerton, 1956). After this preparation, conventional Giemsa-staining was carried out. Constitutive heterochromatin and nucleolus organizer regions (NORs) were determined to identify pairs of each autosome and sexual chromosomes via C-banding (Sumner, 1972) and Ag-NOR staining (Howell and Black, 1980), respectively. From each specimen, 10-20 slides were prepared and at least 20 well-spread metaphase plates were analyzed. Chromosome morphology was established according to Zima (1978) by calculating centromeric indexes. Standard voucher specimens (skins and skulls) were deposited in the Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey.

Results

The karyotype of *S. taurensis* consists of 40 chromosomes: 3 pairs of metacentric (Figure 2, nos. 1-3), 10 pairs of submetacentric (Figure, nos. 4-13), and 6 pairs of subtelocentric (Figure 2, nos. 14-19) autosomes (NFa = 76). The X chromosome is a medium-sized metacentric and the Y chromosome is a small acrocentric (NF = 80).

The C-banded karyotype of *S. taurensis* is illustrated in Figure 3. All the autosomes have pericentromeric C-bands. Pericentromeric blocks of heterochromatin in *S. taurensis* vary in size and are symmetrical (Figure 3, nos. 2, 3, 5, 9, 10, 11, 12, 13, 14, 18, and 19) or asymmetrical (Figure 3, nos. 1, 4, 6, 7, 8, 15, 16, and 17) with respect to the centromere. The X chromosome has pericentromeric heterochromatin and Y chromosomes appeared to be entirely heterochromatin.

With silver-nitrate staining, NORs were observed in the telomeric region of the short arm of 2 submetacentric pairs (Figure 4, nos. 8 and 12) and 2 pairs of subtelocentric chromosomes (Figure 4, nos. 16 and 17). All the NORs are homomorphic and medium-sized (Figure 4). The number of active NORs ranged from 5 to 8 per cell (mean: 6.3) in the metaphase.

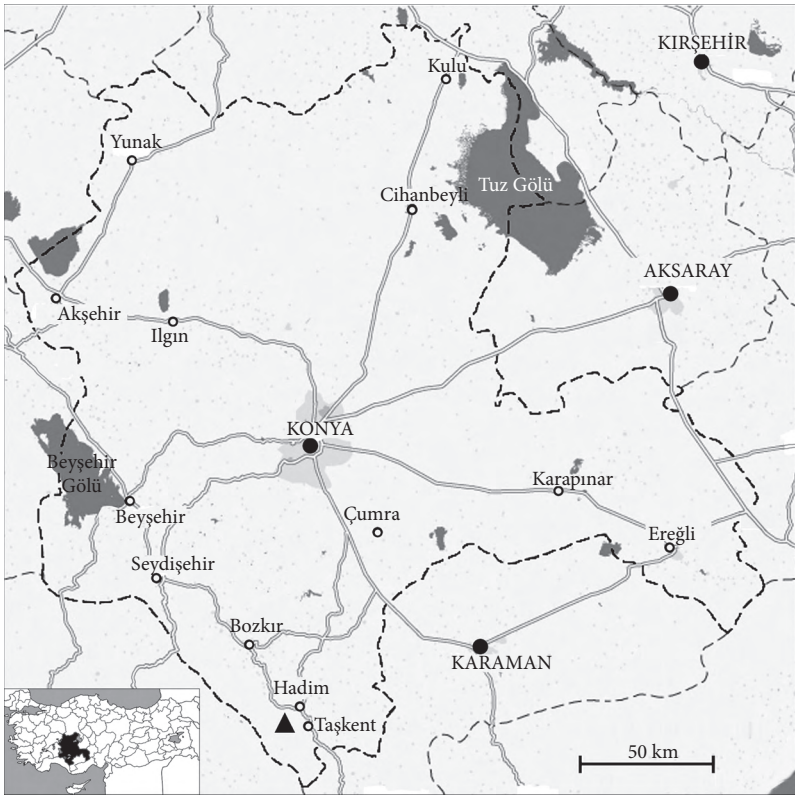

Figure 1. *Spermophilus taurensis* collection site in Konya. ▲: Meydacık Yaylası, Hadim (36°55′N, 32°25′E).

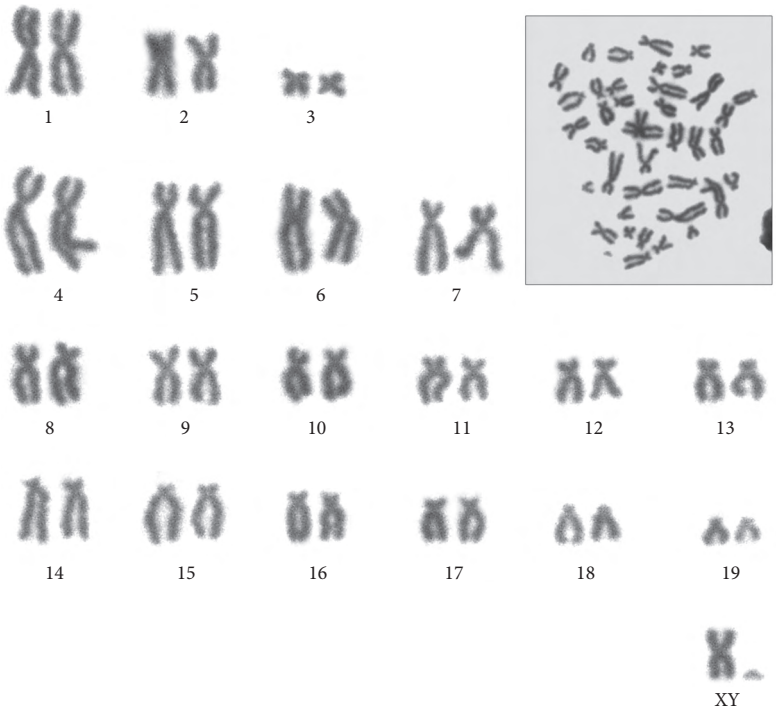

Figure 2. Metaphase spread and karyotype of *Spermophilus taurensis*.

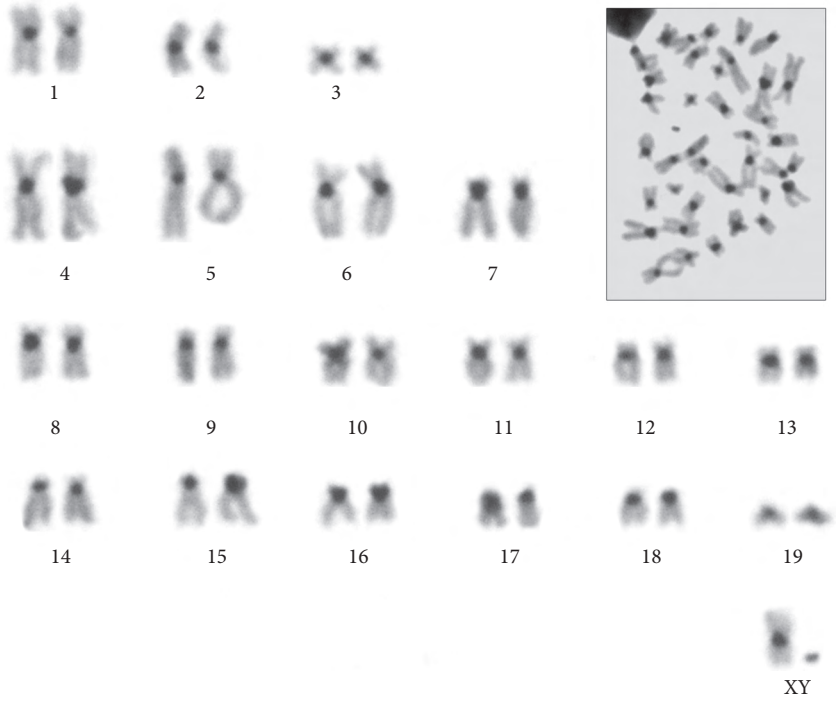

Figure 3. Metaphase spread and C-banded karyotype of *Spermophilus taurensis*.

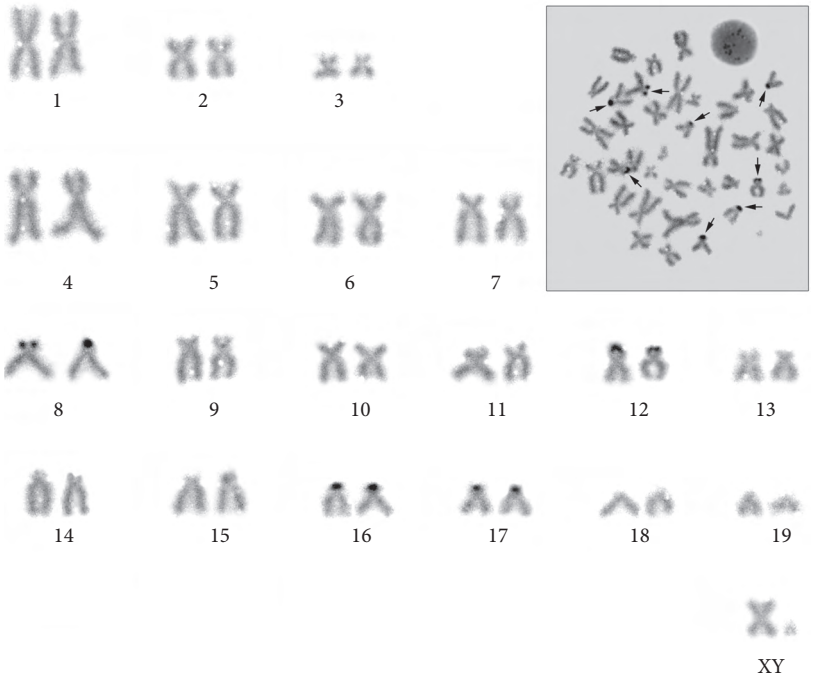

Figure 4. Silver-stained metaphase spread and karyotype of *Spermophilus taurensis*. Arrows indicate the Ag-NOR.

Discussion

The conventional karyotype of *S. taurensis* was determined first by Özkurt et al. (2002) in the Taurus Mountains in southeast Anatolia, before this species was described. The number of chromosomes was subsequently confirmed by Yiğit et al. (2005) and Gündüz et al. (2007a), the authors of this species. According to Özkurt et al. (2002) the autosomes of the Akseki specimens included 2 pairs of acrocentric chromosomes, whereas all the autosomes of the Mut and Hadim specimens were bi-armed. In addition, they determined that the Y chromosome of the Akseki specimens was bi-armed and that of the Mut and Hadim specimens was acrocentric, as in our specimens. In the autosomes of our specimens there were 3 pairs of metacentric, 10 pairs of submetacentric, and 6 pairs of subtelocentric chromosomes, whereas according to Özkurt et al. (2002) there were 2 pairs of metacentric and 15 pairs of submetacentric chromosomes. They determined that only the Mut and Hadim specimens had 2 pairs of subtelocentric chromosomes. These differences in the autosomes of *S. taurensis* specimens are probably the result of methodological inconsistencies in karyotype preparation and the arrangement of the pairs in the diploid complement. Two karyotypes of *S. citellus* have been reported from specimens collected in Turkish Thrace: 2n = 40, NF = 78, and NFa = 74 (Doğramacı et al., 1994), and 2n = 40, NF = 69, and NFa = 66 (Özkurt et al., 2002). The Czech specimens of *S. citellus* (Zima, 1978) had a bi-armed Y chromosome, as did the Trakya specimens reported by Doğramacı et al. (1994). Özkurt et al. (2002) noted that *S. taurensis* had a Y chromosome similar to that of the *S. citellus* specimens mentioned above. In contrast to Doğramacı et al. (1994) the same researchers also determined that the Y chromosome of Trakya specimens was acrocentric.

When the results of the present study and previous studies are compared, the NFa (74-76) and NF (79-80) values of *S. taurensis* populations vary according to researcher—just like for other species—due to both the proportion of acrocentric autosomes and the morphology of the Y chromosome. According to conventional Giemsa staining, *S. taurensis* is karyotypically closer to *S. citellus* than to *S. xanthoprymnus*. This relationship was also confirmed by Yiğit et al. (2005) and Gündüz et al. (2007a).

Belcheva and Peshev (1985) observed variation between the subspecies of Bulgarian *S. citellus*, in terms of C-heterochromatin. Zima (1987) reported that all the chromosomes of a male *S. citellus* from the Czech Republic had centromeric heterochromatin regions. Arslan (2005) reported pericentromeric C-bands in all autosomes and the X chromosome of *S. xanthoprymnus* in Central Anatolia. All the chromosomes of *S. taurensis* have symmetrical or asymmetrical pericentromeric C-blocks. Arslan (2005) emphasized that the Y chromosome of *S. xanthoprymnus* appeared to be entirely euchromatic, which differentiates it from *S. citellus*. In our specimens the entire Y chromosome was heterochromatic, as in *S. citellus*. This supports Yiğit et al. (2005) and Gündüz et al. (2007a) in their claim that *S. taurensis* is phylogenetically closer to *S. citellus*.

Among the ground squirrels distributed in the US, *S. mexicanus* and *S. tridecemlineatus* (2n = 34) have centromeric C-bands (Cothran and Honeycutt, 1984). While *S. pygmaeus* (with 36 diploid chromosomes) has the same bands, terminal bands were observed in some chromosomes of *S. musicus* (Korablev, 1983).

Just as ground squirrels do, some tree squirrels (*Sciurus lis*, *S. vulgaris*, and *S. anomalus*) have centromeric bands (Oshida and Yoshida, 1997; Arslan et al., 2008). These results indicate that most studied ground squirrels have centromeric and pericentromeric C-bands, as do most rodents.

NORs were identified in 3 pairs of chromosomes of *S. xanthoprymnus* in Central Anatolian (Arslan, 2005). Although the NORs of *S. taurensis* are on the ends of short arms, as *S. xanthoprymnus* in Central Anatolian, the number of NORs is higher in the Taurus ground squirrel. Additionally, the morphology of the chromosomes where NORs are localized differs between the 2 species. While the NORs of *S. taurensis* are located on both submetacentric and subtelocentric chromosomes, in *S. xanthoprymnus* NORs are only located on subtelocentric chromosomes. Korablev et al. (1991) and Korablev (1994) reported that the spotted ground squirrel *Spermophilus suslicus* (2n = 36 and 2n = 34) had 1 interstitial and 2 terminal Ag-NORs.

Arslan et al. (2008) reported an active NOR on the short arm of a pair of metacentric chromosomes in the Persian squirrel *S. anomalus*, and noted that Oshida and Yoshida (1997) reported rather different results for *S. lis* and *S. vulgaris*. According to the same researchers, the number of NORs in *S. lis* differs from that in *S. vulgaris*.

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