TÜBİTAK

Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2017) 41: 963-969
© TÜBİTAK
doi:10.3906/zoo-1607-36

Cytogenetic characteristic of the Caucasian pygmy shrew (*Sorex volnuchini*) and Levant mole (*Talpa levantis*) (Mammalia: Eulipotyphla) in northern Anatolia, Turkey

Ahmet Yesari SELÇUK, Haluk KEFELİOĞLU*
Department of Biology, Faculty of Science, Ondokuz Mayıs University, Samsun, Turkey

Received: 20.07.2016 • **Accepted/Published Online:** 01.06.2017 • **Final Version:** 21.11.2017

Abstract: The karyotype of the Caucasian pygmy shrew (*Sorex volnuchini*) consists of 2n = 40 NFa = 54. The X chromosome is metacentric and the Y chromosome is large acrocentric. In the chromosome set, there are six metacentric/submetacentric, one large subtelocentric, one small metacentric, and 11 acrocentric autosomal chromosome pairs. C-negative and C-positive heterochromatin band regions are determined in the karyotype. NORs were localized in the secondary constriction in autosomal pair no. 17 of *Sorex volnuchini*. The diploid number of the Levant mole (*Talpa levantis*) is 2n = 34, NFa = 64. The X chromosome is medium size and metacentric. The Y chromosome is the smallest chromosome in the karyotype. There are 10 pairs of metacentric, three submetacentric, and three subtelocentric chromosomes in the chromosome set and X is metacentric and Y is acrocentric. In the karyotype, NOR is located in the secondary constriction region of chromosome no. 8.

Key words: Chromosome banding (C - G - NORs), *Talpa levantis*, *Sorex volnuchini*, Turkey

1. Introduction

Living Soricidae family members are conventionally represented by two subfamilies: Crocidurinae (white-toothed shrew) and Soricinae (red-toothed shrew) (Reumer, 1987). With 76 species, the genus *Sorex* is dominant in the subfamily Soricinae. The genus *Sorex* in the Palaearctic region is divided into three taxonomic groups: *Sorex araneus* (*S. araneus* and *S. satunini*) *Sorex minutus* (*S. minutus* and *S. volnuchini*), and *S. raddei* (Macholan, 1996; Fumagalli et al., 1999; Hutterer, 2005). In Turkey, 5 species of *Sorex* are common (Krystufek and Vohralik, 2001).

Some members of the genus *Sorex* (*Sorex araneus*) have an XY1Y2 sex chromosome system and show high-level Robertsonian chromosomal polymorphism (Biltueva et al., 2000; Wojcik et al., 2002). Due to these properties, members of the genus *Sorex* are frequently used in studies of mammalian chromosome evolution. Karyological studies of the five *Sorex* species found in Turkey are very scarce (Macholan, 1996; Zima et al., 1997, 1998; Biltueva et al., 2000, 2011); most of them are only standard chromosomal staining studies (except for Biltueva et al., 2000, 2011).

The genus *Talpa* is represented by nine species distributed in the Western Palaearctic (from the Iberian Peninsula to Siberia) (Krystufek and Vohralik, 2001; Hutterer, 2005). Turkey also shows common distribution of four species of *Talpa*, namely *T. europaea*, *T. caucasica*, *T. levantis*, and *T. davidiana* (Krystufek and Vohralik, 2001). Karyological studies of *Talpa* species that show a conservative structure in terms of chromosome number are very limited in Turkey (Colangelo et al., 2010; Sözen et al., 2012). In general, *Talpa* species have 2n = 34 chromosomes. The exception is that *T. caeca* has 2n = 36 and *T. caucasica* has 2n = 38 (Kefelioğlu and Gençoğlu, 1996; Gornung et al., 2008). The karyotype of *T. levantis* was identified by Kefelioğlu and Gençoğlu (1996). However, there is no detailed information about the band structures of chromosomes.

The cytogenetic characteristics of *Sorex volnuchini* and *Talpa levantis* are unknown. With this study, the chromosomal band (G - C - NORs) characteristics of *Sorex volnuchini* and *Talpa levantis* were determined for the first time in the literature.

2. Materials and methods

The cytogenetic analyses were performed with four female and one male *Sorex volnuchini* specimens and two female and one male *Talpa levantis* specimens collected from north of Samsun (Turkey). Chromosome preparations were obtained from the femoral bone marrow cells of colchicine-treated animals (Ford and Hamerton, 1956). The G-banding technique was applied as described by

* Correspondence: halukefe@omu.edu.tr

Seabright (1971). The constitutive heterochromatin distribution and nucleolar organizer regions (NORs) were determined using techniques from Summer (1972) and Howell and Black (1980), respectively. From each specimen 10 to 20 slides were prepared and at least 10 well-spread metaphase plates were analyzed. This study was carried out after permission was obtained from the Ondokuz Mayıs University Local Ethics Committee for Animal Experiments (permit number: B.30.2.ODM.0.20.09.00-050.04-97 for *Sorex volnuchini* and B.30.2.ODM.0.20.09.00-050.04-09 for *Talpa levantis*).

3. Results

The karyotype of the five *Sorex volnuchini* specimens obtained in the study is 2n = 40, NFa = 54. There are six large metacentric/submetacentric (no. 1, 2, 3, 4, 5, 7), one large subtelocentric (no. 6), one small metacentric (no. 8), and 11 acrocentric autosomal chromosome pairs (no. 9–19) in the karyotype. The X chromosome is large metacentric and Y is large acrocentric. Secondary constriction is present in the long arm of acrocentric chromosome pair no. 17 (Figure 1).

The karyotype of *Talpa levantis* is 2n = 34, NFa = 64. There are 10 pairs of metacentric, three pairs of submetacentric, and three pairs of subtelocentric chromosomes. The X chromosome is large metacentric and the Y chromosome is dot shape and the smallest chromosome in the chromosome set. Since these results are consistent with those given by Kefelioğlu and Gençoğlu (1996), standard karyotype staining results are not shown here.

Constitutive heterochromatin regions in the chromosome set were determined via C-band method. C-negative, C-positive, and heteromorphic heterochromatin band structures are present in the karyotype of *S. volnuchini*. While C-bands are significantly pericentromeric in 1, 3, 4, 7, 8, 9, 11, 13, 16 chromosome pairs in the karyotype, the X chromosome is C-negative (Figure 2).

All the autosomes of *T. levantis* possessed distinct C-positive bands in pericentromeric areas (Figure 3). G-band structures of *S. volnuchini* and *T. levantis* autosomes and sex chromosomes were identified for the first time in this study (Figures 4 and 5).

One pair of NORs was determined in the karyotype of *S. volnuchini*. NORs are located in the secondary constriction region and are homomorphic. NORs are also located close to the telomere region in the long arms (Figure 6).

In the karyotype of *T. levantis*, the secondary constriction is located in the large metacentric autosomal chromosome (no. 8) (Figure 7). The number of active NORs varied from 1 to 2 per cell in the metaphase of *T. levantis*. The NOR is in the secondary constriction region, which is located on the short arm of chromosome pairs no. 8.

4. Discussion

While the number of chromosomes in the *Sorex araneus* group shows varying polymorphism, 2n = 20–33 (Yannic et al., 2008), the *Sorex minutus* group shows a more

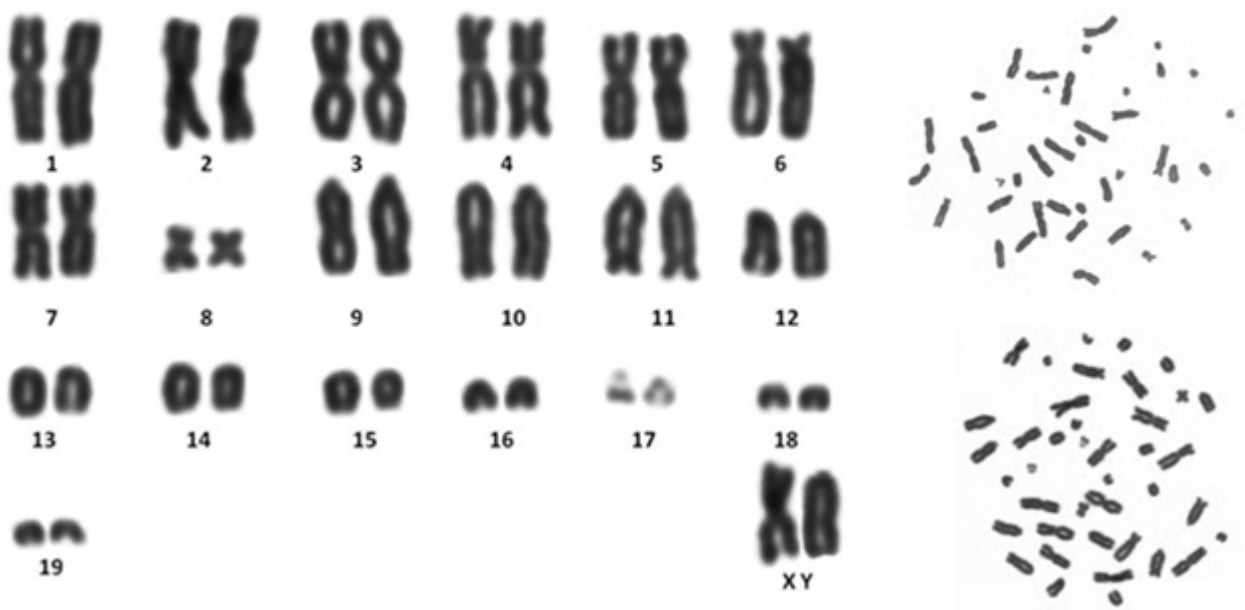

Figure 1. *Sorex volnuchini* standard karyotype (male).

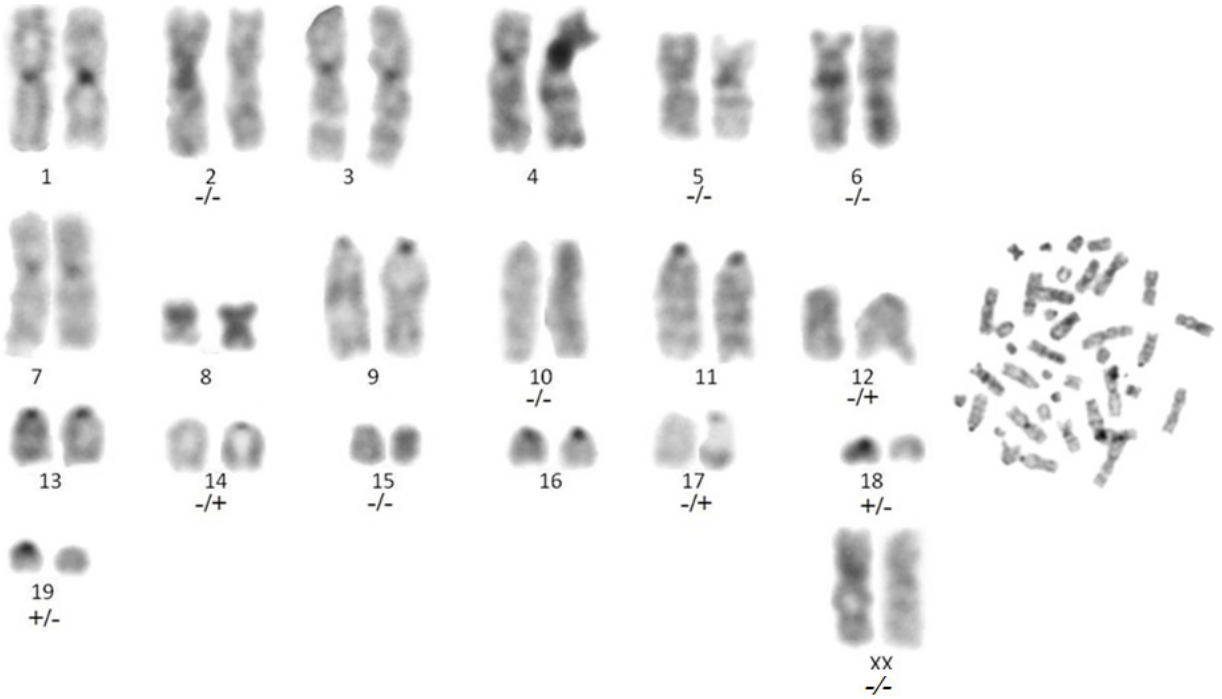

Figure 2. *Sorex volnuchini* C-bands (female), negative C-bands (–/–), heteromorphic C bands (–/+).

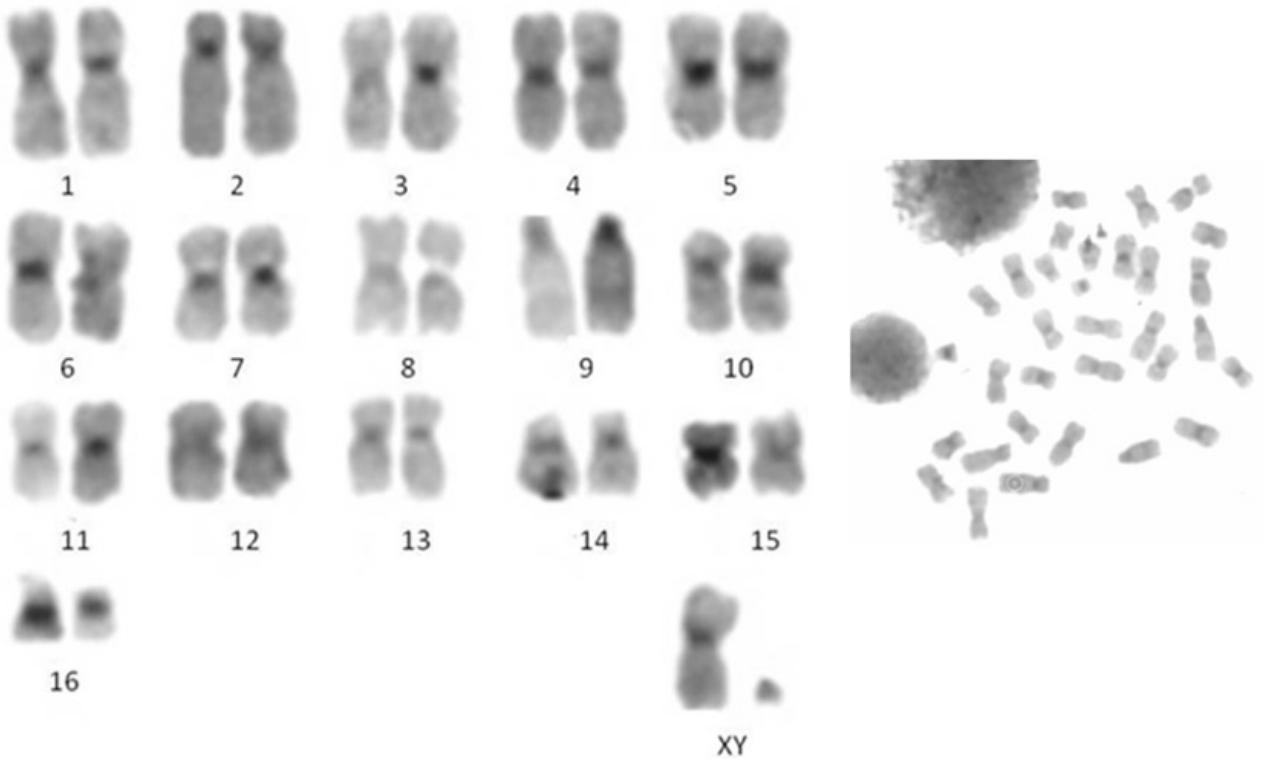

Figure 3. Male C-banded karyotype of *Talpa levantis* (male) from North Anatolia.

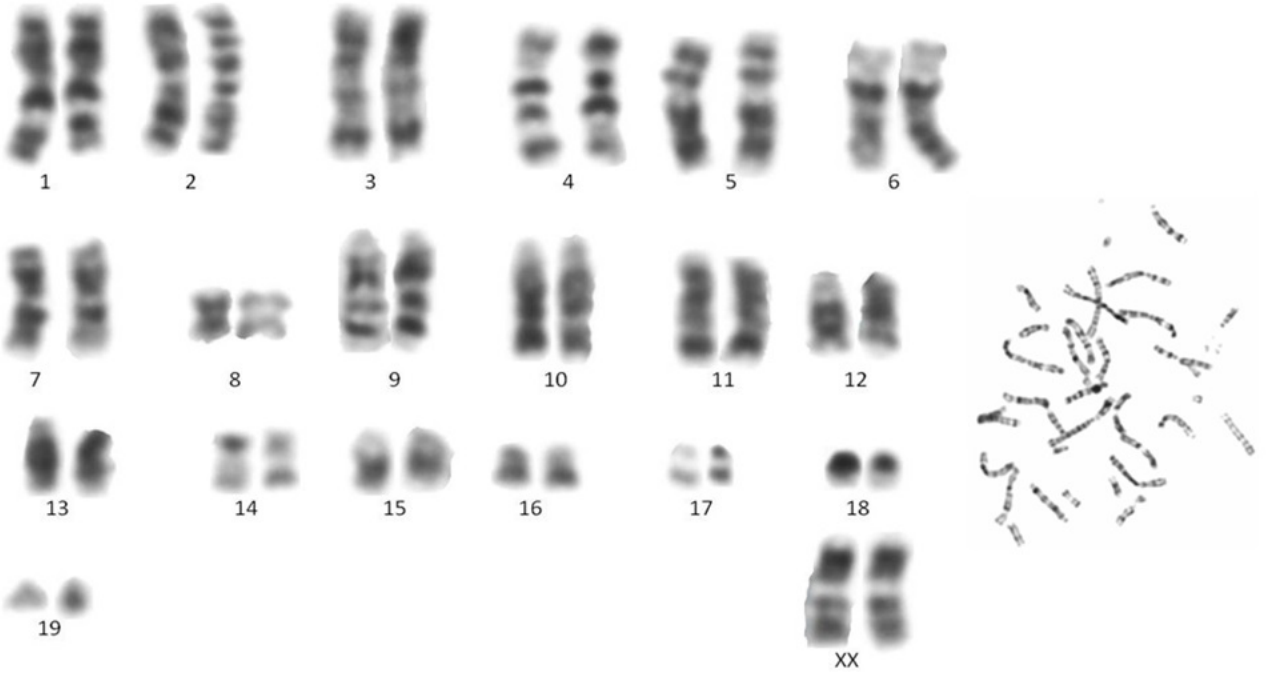

Figure 4. *Sorex volnuchini* G-bands (female).

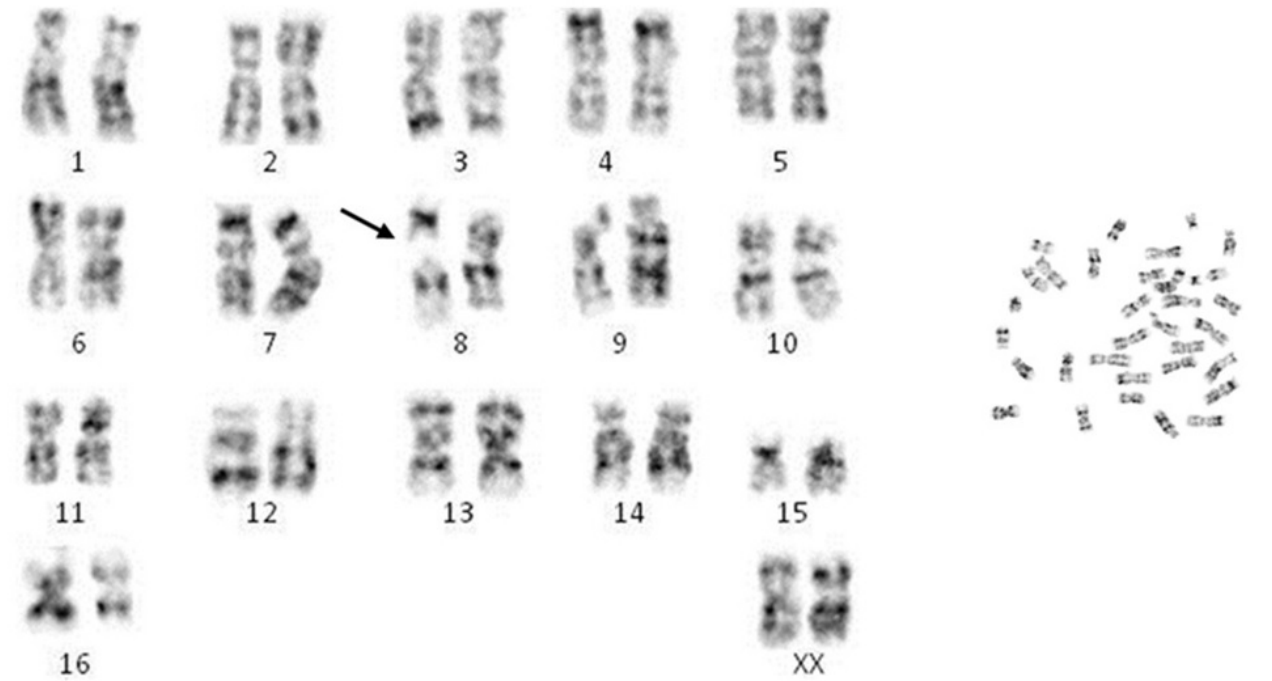

Figure 5. G-banded karyotype of *Talpa levantis* (female); the arrow indicates the secondary constriction.

consistent chromosomal structure in terms of number of chromosomes (Biltueva et al., 2011). The number of diploid chromosomes (2n = 40) in *Sorex volnuchini*, which is included in the *Sorex minutus* group, is consistent with those obtained from previous studies (Zima et al., 1998; Aslan and Zima, 2014). Aslan and Zima (2014) reported two small biarmed autosomal pairs of the karyotype obtained from Meryemana (Trabzon, northern Anatolia).

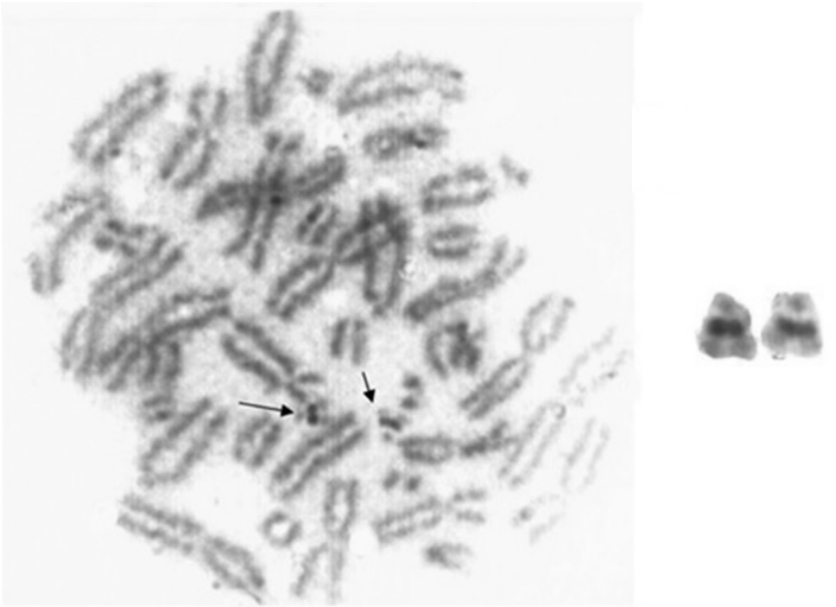

Figure 6. *Sorex volnuchini* NOR bands (female); the arrows show NORs on the chromosomes.

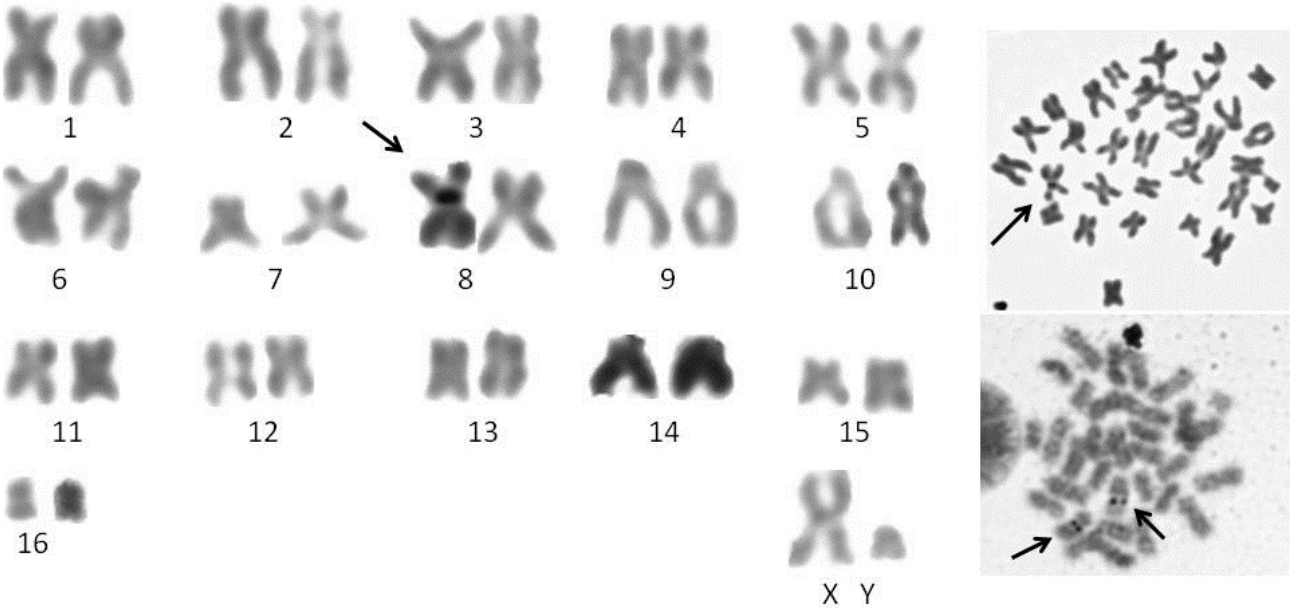

Figure 7. *T. levantis* NOR band; the arrows indicate the NOR bearing chromosome of *Talpa levantis* (male).

Therefore, the karyotype is NFa = 56. However, the number autosomal chromosome arm obtained in this study is NFa = 54. In this study, a pair of small biarmed autosomal chromosomes is present in the karyotype of the samples (four female, one male) obtained from Samsun region (chromosome no. 8). Similarly, the Y chromosome differs from that of previous studies. The Y chromosome of the sample obtained from the Meryemana region of Trabzon is small acrocentric (Zima et al., 1998; Aslan and Zima, 2014). However, the Y chromosome obtained in the present study is large acrocentric in the karyotype set.

The karyotype of *T. levantis* has 2n = 34, NFa = 64. The metacentric, submetacentric, and subtelocentric chromosomes in the chromosome set are consistent with

the result reported by Kefelioğlu and Gençoğlu (1996). In all karyotypes, the structures of C-bands are in the pericentromeric region. In chromosome pair no. 9, the C-heterochromatin region is expanded to include the short arm of the chromosome (Figure 3). C-band positive structures of X and Y chromosomes are apparent. No distal heterochromatin structure was observed in chromosome pairs. According to Gornung et al. (2008), no short arm is present in the chromosome pair no. 9 of *Talpa caeca* and *Talpa stankovici* karyotypes. *Talpa europaea* and *Talpa romana* karyotypes have a heterochromatic short arm in chromosome pair no. 9. A heterochromatic short arm is present in chromosome no. 9 of the karyotype of *Talpa levantis* in this study.

The karyotype of *T. levantis* has a large euchromatin area containing a centromeric region in chromosome no. 1 (Figure 5). The G-band structure of *T. levantis* chromosomes is similar to those of *T. europaea* and *T. romana* (Gornung et al., 2008).

NORs located on the chromosomes are repetitive gene regions consisting of consecutive subunits. In mammals, subunits encoded by rDNA include 18S, 5.8S, and 28S rRNA units (Cazaux et al., 2011). Because species-specific rDNA clusters located on the chromosome are found in different regions and numbers, they are frequently used in phylogenetic and mammalian systematics (Davidian-Britton et al., 2012; Stepinski, 2013). Since silver stains the NOR-associated proteins on the chromosomes, NORs can be determined by silver staining (Rao et al., 2005). Cytogenetic studies of *Taterillus pygargus* (Mammalia: Rodentia) and *Erinaceus algirus* (Mammalia: Erinaceomorpha) (Sanchez et al., 1995; Dobigny et al., 2002) showed that silver can also bind in rDNA-free regions. Because the silver nitrate can also bind to nonspecific regions on the chromosomes, the reliability of the Ag-NOR method is controversial (Dobigny et al., 2002). Similarly, Zurita et al. (1997) reported interchromosomal, intercellular, and interindividual variability of NORs studied with silver staining. However, Ag-NOR is widely used in many taxonomic groups (Dobigny et al., 2002). In addition, although NORs can be determined by silver staining, they can be in the form of secondary constrictions, which are active transcript regions on the metaphase chromosomes (Godpasture and Bloom, 1975; Rao et al., 2005; Caperta et al., 2007; Stepinski, 2013). According to the standard staining results of the karyotype of *S. volnuchini*, the secondary constriction is present on the long arm of chromosome pairs no. 17 (Figure 1). Silver staining shows the presence of an active NOR in this region in the karyotype of *S. volnuchini* (Figure 6). Similarly, in the karyotype of *T. levantis*, an active NOR is found to be close to the centromere and in the secondary constriction region, which is located on the short arm of the metacentric chromosome in the chromosome set. In addition, in the karyotypes of *T. occidentalis* distributed in the Palearctic region (Jimenez et al., 1984; Zurita et al., 1998), *T. europaea*, *T. romana* (Volleth and Müller, 2006; Gornung et al., 2008), and *T. altaica* (Kawada et al., 2002) NORs are located on the short arm of autosomal metacentric chromosomes as in the karyotype of *T. levantis*.

In conclusion, it is necessary to identify the chromosomal band structures of other *Talpa* and *Sorex* species distributed in Turkey to demonstrate the chromosomal evolution of *Talpa* and *Sorex* species. In particular, karyological studies involving samples obtained from different geographical areas are needed to determine the existence of chromosomal evolution of *Sorex* species and different karyotypes.

References

Aslan A, Zima J (2014). Karyotypes of the mammals of Turkey and neighbouring regions: review. Folia Zool 63: 1-62.

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

Biluteva L, Vorobieva N, Perelman P, Trifonov V, Volobouev V, Panov V, Ilyashenko V, Onischenko S, O'Brein P, Yang F et al. (2011). Karyotype evolution of Euliphotypha (Insectivora): the genome homology of seven *Sorex* species revealed by comparative chromosome painting and banding data. Cytogenet Genome Res 135: 51-64.

Caperta AD, Neves N, Viegas W, Pikaard S, C, Preuss S (2007). Relationships between transcription, silver staining, and chromatin organization of nucleolar organizers in Secale cereale. Protoplasma 232: 55-59.

Cazaux B, Catalan J, Veyrunes F, Douzery JPE, Davidian-Britton J (2011). Are ribosomal DNA clusters rearrangement hotspots? A case study in the genus *Mus* (Rodentia, Muridae). Evol Biol 11: 124.

Colangelo P, Bannikova A, Krystufek B, Lebedev VS, Annesi F, Capanna L (2010). Molecular systematics and evolutionary biogeography of the genus *Talpa* (Soricomorpha: Talpidae). Mol Phylogenet Evol 55: 372-380.

Davidian-Britton J, Cazaux B, Catalan J (2012). Chromosomal dynamics of nucleolar organizer regions (NORs) in the house mouse: micro-evolutionary insights. Heredity 108: 68-74.

Dobigny G, Costaz-Ozouf C, Bonillo C, Volobouev V (2002). "Ag-NORs" are not always true NORs: new evidence in mammals. Cytogenet Genome Res 98: 75-77.

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

Fumagalli L, Taberlet P, Stewart D, Ludovic G, Hausser J, Vogel P (1999). Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data. Mol Phylogenet Evol 11: 222-235.

Godpasture C, Bloom SE (1975). Visualisation of molecular organiser regions in mammalians chromosomes using silver staining. Chromosoma 53: 37-50.

Gornung E, Volleth M, Capanna E, Castiglia R (2008). Comparative cytogenetics of moles (Eulipotyphla, Talpidae): chromosomal differences in *Talpa romana* and *T. europaea*. Cytogenet Genome Res 121: 249-254.

Howell WM, Black DA (1980). Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36: 1014-1015.

Hutterer R (2005). Order Soricomorpha. In: Wilson DE, Reeder DM, editors. Mammals Species of the World. A Taxonomic and Geographic Reference, third ed. Baltimore, MD, USA: The Johns Hopkins University Press. pp. 220-311.

Jimenez R, Burgos M, Diaz De La Guardia R (1984). Karyotype and chromosome banding in the mole (*Talpa occidentalis*) from the South-East of the Iberian Peninsula. Implications on its taxonomic position. Caryologia 37: 253-258.

Kawada S, Harada M, Grafodatsky AS, Oda S (2002). Cytogenetic study of the Siberian mole, *Talpa altaica* (Insectivora: Talpidae) and karyological relationships with in the genus *Talpa*. Mammalia 66: 53-62.

Kefelioğlu H, Gençoğlu S (1996). Karadeniz Bölgesi *Talpa*'larının (Mammalia: Insectivora) taksonomisi ve yayılışı. Turk J Zool 20: 57-66.

Krystufek B, Vohralík V (2001). Mammals of Turkey and Cyprus, Introduction, Checklist, Insectivora. Koper, Slovenia: Knjižnica Annales Majora.

Macholan M (1996). Chromosomal and allozyme characterization of the Caucasian shrew, *Sorex satunini*, from north-eastern Turkey. Hereditas 125: 225-231.

Rao VB, Ghosh K, Mohanty D (2005). Nucleolar organising region evaluation using new NOR FISH probe. Indian J Hum Genet 11: 44-46.

Reumer JWF (1987). Redefinition of the Soricidae and the Heterosoricidae (Insectivora, Mammalia), with the description of the Crocidosoricinae, a new subfamily of Soricidae. Rev Paleobiol 6: 189-192.

Sanchez A, Jimenez R, Burgos M, Stitou S, Zurita F, Guardia R (1995). Cytogenetic peculiarities in the Algerian hedgehog: silver stains not only NORs but also heterochromatic blocks. Heredity 75: 10-16.

Seabright M (1971). A rapid banding technique for human chromosomes. Lancet 2: 971-972.

Sözen M, Matur F, Çolak F, Irmak S (2012). Karyological characteristics, morphological peculiarities, and a new distribution locality for *Talpa davidiana* (Mammalia: Soricomorpha) in Turkey. Turk J Zool 36: 806-813.

Stepinski D (2013). Nucleolar chromatin organization at different activities of soybean root meristematic cell nucleoli. Protoplasma 250: 723-730.

Summer AT (1972). A simple technique for demonstrating centromeric heterochromatin. Expl Cell Res 75: 304-306.

Volleth M, Müller S (2006). Zoo-FISH in the European mole (*Talpa europaea*) detects all ancestral Boreo-Eutherian human homologous chromosome associations. Cytogenet Genome Res 115: 154-157.

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

Yannic G, Basset P, Hausser J (2008). A new perspective on the evolutionary history of western European *Sorex araneus* group revealed by paternal and maternal molecular markers. Mol Phylogenet Evol 47: 237-250.

Zima J, Lukáčová L, Macholán M (1998). Chromosomal evolution in shrews. In: Wójcik JM, Wolsan M, editors. The Evolution of Shrews. Mammal Research Institute, Polish Academy of Sciences, Bialowieza 175-218.

Zima J, Slivková L, Andreas M, Benda P, Reiter A (1997). Karyotypic status of shrews (*Sorex*) from Thrace, European Turkey. Z. Säugetierkd 62: 315-317.

Zurita F, Jimenez R, Burgos M, Guardia D (1998). Sequential silver staining and in situ hybridization reveal a direct association between rDNA levels and the expression of homologous nucleolar organizing regions: a hypothesis for NOR structure and function. J Cell Sci 111: 1433-1439.

Zurita F, Sanchez A, Burgos M, Jimenez R, Guardia D (1997). Interchromosomal, intercellular and interindividual variability of NORs studied with silver staining and in situ hybridization. Heredity 78: 229-234.