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Nucleolus organizer regions of *Myotis myotis* (Borkhausen, 1797) and *Miniopterus schreibersii* (Kuhl, 1817) (Mammalia: Chiroptera) from Turkey

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Abstract: In this study, Ag-nucleolar organizing region (NOR) banding patterns of *Myotis myotis* and *Miniopterus schreibersii* were presented. The karyotype of *Myotis myotis* has a diploid number (2n) of 44, a fundamental number (NF) of 54, and an autosomal fundamental number (NFa) of 50. The 2n, NF, and NFa numbers of *Miniopterus schreibersii* were 46, 54, and 56, respectively. In *Myotis myotis*, a homomorphic NOR was localized in 1 of the medium-sized acrocentric pairs; however, NORs were located in 2 pairs on the secondary constriction of *Miniopterus schreibersii*.

Key words: Greater mouse-eared bat, bent-winged bat, karyotype, Ag-NOR staining, Turkey

The second most abundant order in the class Mammalia, bats are represented by 1116 species (Wilson and Reeder, 2005). Bat diversity in Turkey has been presented in various studies (Benda and Horáček, 1998; Benda et al., 2006; Aşan and Albayrak, 2011; Çoraman et al., 2013). Two vespertilionids, the greater mouse-eared bat *Myotis myotis* and the bent-winged bat *Miniopterus schreibersii*, are widely distributed in Turkey (Albayrak and Coşkun, 2000; Karataş and Sözen, 2004; Aşan and Albayrak, 2011; Bilgin et al., 2012). *Myotis myotis* is represented by 2 subspecies: the nominate form, distributed in all regions except the Mediterranean region, and *M. m. macrocephalicus* in the Mediterranean region (Aşan and Albayrak, 2011). *Miniopterus schreibersii* is represented by the nominate form distributed in Turkish Thrace, the Marmara region, and the western Black Sea region, and *M. s. pallidus* in the rest of Anatolia (Albayrak and Coşkun, 2000; Karataş and Sözen, 2004). However, recently Bilgin et al. (2012) proposed that *M. s. schreibersii* and *M. s. pallidus* should be evaluated as separate species rather than as subspecies as stated by Benda et al. (2010) and Furman et al. (2010).

Although the genus *Myotis* is one of the most speciose mammalian genera, it is karyologically conservative with respect to diploid number 2n = 44 and has an autosomal fundamental number (NFa) of 50 in the Palearctic region (Zima and Kral, 1984; Karataş et al., 2004; Wu et al., 2009; Aşan and Albayrak, 2011; Aşan et al., 2011). Volleth and Heller (2012) stated that the NFa of the genus was 52 with respect to the tiny and euchromatic arm found in 1 of the chromosome pairs (no. 7).

The karyotype of *Miniopterus schreibersii* possesses a diploid number (2n) of 46, a fundamental number (NF) of 54, and an NFA of 50 (Karataş and Sözen, 2004; Albayrak, 2006; Karataş et al., 2008). A secondary constriction adjacent to the centromere is generally detected on one of the smallest acrocentric autosomes (Albayrak, 2006; Wu et al., 2009).

This study reports the nucleolar organizing region (NOR)-bearing chromosomes of 2 bat species from Turkey, *Myotis myotis* and *Miniopterus schreibersii,* belonging to the family Vespertilionidae.

One male specimen of *Myotis myotis* from Ardanuç, Artvin Province (41°07′N, 42°03′E) (no. 2024 ♂) and 4 male specimens of *Miniopterus schreibersii* from Kayalı village, Kırklareli Province (41°46′N, 27°05′E) (nos. ZL0036 ♂ and ZL0037 ♂) and Kalecik, Ankara Province (40°15′N, 33°28′E) (nos. ZL0009 ♂ and ZL0010 ♂) were karyotyped. Mitotic metaphases were obtained from the bone marrow as described by Lee and Elder (1980). NORs were detected using the technique of Howell and Black (1980). At least 20 well-spread and Ag-NOR banded metaphase plates were photographed. Chromosomes were classified according to Levan et al. (1964). The voucher specimens and slides were deposited in the Department of Biology, Faculty of Arts and Sciences, Kırıkkale University.

The karyotype of *Myotis myotis* revealed 2n = 44, NF = 54, and NFa = 50. The chromosome set consisted of 3 large pairs (nos. 1–3) and 1 small pair of metacentrics (no. 4) and 17 pairs of acrocentrics ranging in size from

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medium to small (nos. 5–21). The largest acrocentric pair (no. 5) possessed a tiny but distinct heterochromatic arm. It is considered to be an acrocentric autosome in this study. The X was a medium-sized metacentric and the Y a minute acrocentric, as stated by Aşan and Albayrak (2011) and Aşan et al. (2011). No secondary constriction was encountered in the metaphases examined. The NOR was homomorphic and occurred in the telomeric region of one of the medium-sized acrocentric pairs (no. 12) (Figure 1).

The karyotype of *Miniopterus schreibersii* revealed 2n = 46, NF = 54, and NFa = 50. The chromosome set consisted of 2 large pairs and 1 small pair of metacentrics and 19 pairs of acrocentrics ranging in size from medium to small. The X was a medium-sized metacentric and the Y a dot-like acrocentric. A secondary constriction was encountered in a small-sized acrocentric pair (no. 20). NORs were homomorphic and occurred particularly in the telomeric regions of 2 small-sized acrocentric pairs, on the secondary constrictions (nos. 18 and 20) (Figure 2).

In mammalian chromosomes, NORs contain the genes for 18S and 28S rDNA, and determining the NOR sites is accepted as useful for taxonomic and phylogenetic species relationships, although NORs present high interchromosomal mobility (Sanchez et al., 1990; Volleth and Heller, 2012). However, Miller et al. (1976) and Croce et al. (1977) demonstrated that only functional NORs on chromosomes that were active in the preceding interphase could be detected by Ag-NOR banding.

To date, all the *Myotis* species studied in the Palearctic region have been determined to possess a conservative karyotype of 2n = 44, NF = 54, and NFa = 50 by various authors (Bickham and Hafner, 1978; Zima, 1982; Zima and Kral, 1984 and references therein; Karataş et al., 2004; Aşan and Albayrak, 2011; Aşan et al., 2011). However, due to the tiny arms found in the acrocentric chromosomes, NFa ranged from 50 to 54 in the genus (Albayrak and Aşan, 2002; Karataş et al., 2008 and references therein; Volleth and Heller, 2012). The shape of the Y chromosome was determined as subtelocentric by Volleth and Heller (2012), although in the Turkish specimen the Y was a minute acrocentric. The presence of tiny arms on the dot-like chromosomes and differences in the number of NORs are the small chromosomal variations detected in the *Myotis* species (Zima and Kral, 1984; Volleth, 1987; Volleth and Heller, 2012).

Volleth (1987) and Volleth and Heller (2012) described the differences in the number of NOR-bearing chromosomes in the genus *Myotis* in detail. According to those authors, in *M. myotis* all 14 chromosomes from 8 to 23 might possess active NORs; therefore, this species has been recorded among the vertebrate species showing the highest number of NOR-bearing autosomes. Volleth and Heller (2012) determined 7.7 active NOR sites on average in *M. myotis* after studying 4 male and 5 female specimens from Germany, and they stated that examining a single specimen would not allow assessment of the active NORs in the chromosome set. Recently, Arslan (2013) determined active NORs in the pericentromeric region of a biarmed pair and telomeric regions of 4 acrocentric pairs from Konya specimens. In the present study, only one pair with active NORs (no. 12) was determined. The number of examined specimens from 2 provinces was inadequate to describe the NOR-bearing chromosomes of *M. myotis* distributed in Turkey, as stated by Volleth and Heller (2012).

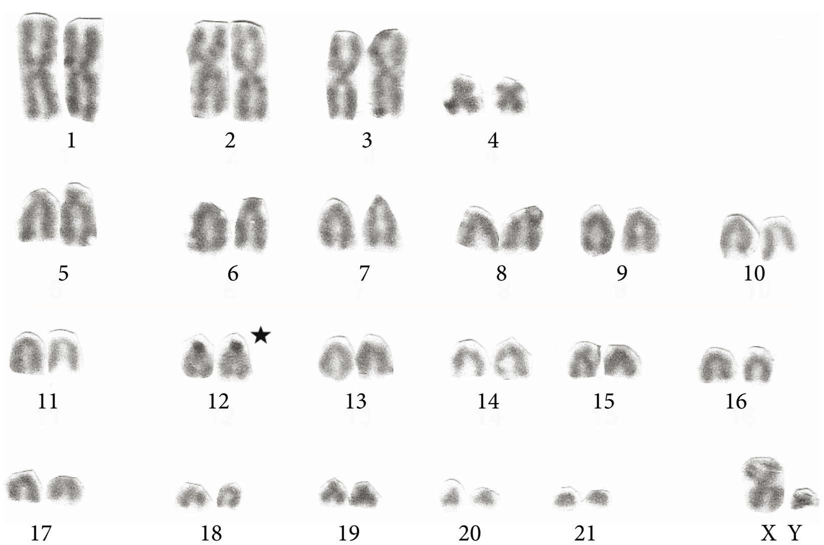

Figure 1. Ag-NOR stained metaphase plate of *Myotis myotis* (asterisk indicates NOR-bearing chromosome pair).

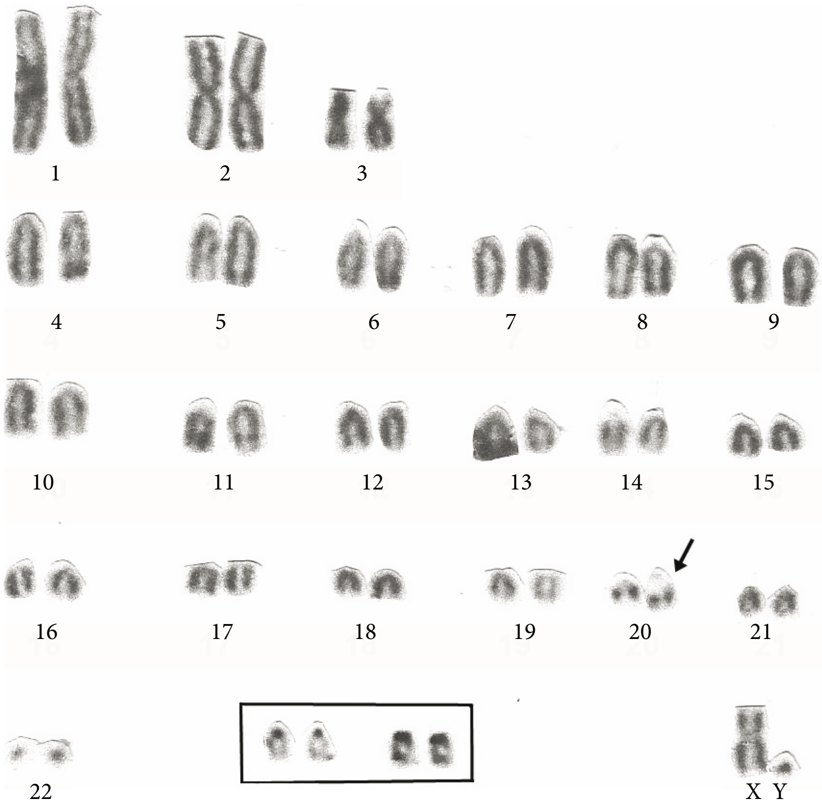

Figure 2. Ag-NOR stained metaphase plate of *Miniopterus schreibersii* (arrow indicates the secondary constriction, inset the NOR-bearing chromosome pairs).

The genus *Miniopterus* is also recorded as a karyologically conservative genus (Zima, 1978; Zima and Kral, 1984; Lin et al., 2002; Albayrak, 2006; Karataş et al., 2008; Wu et al., 2009). However, only Harada et al. (1985) and McBee et al. (1986) described a medium-sized subtelocentric autosome pair from Thailand (NFa = 52). In addition, Karataş and Sözen (2004) recorded the karyotype of the species as 2n = 46, NF=52, and NFa= 48 from Turkey. The dissimilarities in these data were probably due to the different methods used by the authors for classification of the chromosomes. The G-banded and C-banded karyotypes of the species were determined by Bickham and Hafner (1978) and Obara and Tazaki (1980), respectively; however, the Ag-NOR banding karyotype has not yet been given. Zima and Kral (1984) stated that 1 or 2 of the acrocentric autosome pairs possessed secondary constrictions near the centromere. In this study, NORs are located in 2 pairs in particular, on the secondary constriction. Therefore, the relationship between secondary constrictions and NORs as stated by Sato et al. (1980) is supported in Turkey. In addition, no difference was determined in the location of NORs of the specimens from Turkish Thrace and central Anatolia.

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