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C-Heterochromatin and nucleolus organizer region distribution of *Myotis emarginatus* (Chiroptera: Vespertilionidae) from Turkey

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Abstract: In this study, the banded karyotypes (C- and Ag-nucleolus organizer regions (NORs)) of *Myotis emarginatus* in Turkey were determined for the first time. The karyotype contained 44 chromosomes, the number of chromosomal arms (NF) was 54, and the number of autosomal arms (NFa) was 50. Slight C-heterochromatic blocks were observed in the centromeric regions of some autosome pairs, whereas the sex chromosomes were euchromatic. The Ag-NORs were detected in the short arms of a large and a medium-sized acrocentric autosome. The distribution of C-heterochromatin regions in Turkish populations of *M. emarginatus* is similar to those reported in some other *Myotis* species.

Key words: Geoffroy's bat, karyotype, chromosome, C-banding, Ag-NOR banding, Turkey

Bats (Chiroptera) are distributed almost all over the world, and they constitute approximately one-third of the world's mammalian species, playing key roles in many ecosystems (Albayrak and Aşan, 1999). Within the order, 21 families, 227 genera, and 1384 species are currently recognized, and the family Vespertilionidae is the most diverse with 54 genera and 493 species (Burgin et al., 2018). According to the current literature, six families, 14 genera, and 39 species of bats are found in Turkey (Çoraman et al., 2013). There are 11 species of vespertilionid bats belonging to the genus *Myotis* in Turkey (Benda and Horacek, 1998; Benda and Karataş, 2005). The geographic range of *Myotis emarginatus* extends from Anatolia to Southwest Asia, and from the eastern and southern parts of Central and West Europe to the Balkans and Portugal (Piraccini, 2016).

The vespertilionid bats were examined cytogenetically in different regions of the world (Zima and Horacek, 1985) and they show very low karyological diversity at the population, species, and genus levels (Reina et al., 1994). The standard diploid chromosome number is 2n = 44 and the number of chromosomal arms is NF = 50–56 (Arslan and Zima, 2014). The amount of C-heterochromatin in the complement of the *Myotis* species is usually low, whereas the number of nucleolus organizer region (NOR) sites can be relatively high (Volleth, 1987, 1989). Turkish populations of *M. emarginatus* have not yet been examined by karyological methods. The aim of this paper was to perform a chromosomal banding analysis with C-band and Ag-NOR staining for the karyotype of this species from several sites in Turkey.

The cytogenetic analyses were performed for three male of *M. emarginatus* collected in Dupnisa Cave near Kırklareli, one male from the old church in Karaisalı, three females from Adana, and two females from Black Cave near Yozgat (Figure 1). The study was undertaken and the specimens were obtained with the permission of the Republic of Turkey Ministry of Forestry and Water Works (Permit No. 72784983-488.04-188306) and the local ethics committee of Kırıkkale University (Permit No. 16/73). All animals were treated according to the rules of the local ethics committee. The karyotype preparation was carried out according to Ford and Hamerton (1956). Some air-dried slides were stained conventionally by only Giemsa stain. The C- and Ag-NOR bands in the other slides were detected by the techniques of Sumner (1972) and Howell and Black (1980), respectively. A total of 20 well-spread metaphase plates were analyzed.

The diploid chromosome number of all specimens of *M. emarginatus* examined in Turkey was 2n = 44, the fundamental chromosomal arm number (NF) was 54, and the number of arms of autosomal chromosomes (NF) was 50. The chromosome set contained three pairs of large and

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one pair of small metacentric and 17 pairs of acrocentric chromosomes. The X chromosome was a medium-sized metacentric chromosome while the Y chromosome was the smallest acrocentric chromosome in the set (Figure 2). Tiny dark centromeric C-bands were observed in two of the three large biarmed autosomes (nos. 1, 3). At the same time, slight C-bands were detected in the centromeric regions of four acrocentric autosomal pairs (nos. 6, 9, 10, 11). The other autosomal pairs as well as the sex chromosomes stained C-negatively (Figure 3). Homomorphic NOR regions were localized in the telomeric region of the short arm of two pairs (nos. 6, 14) of acrocentric chromosomes (Figure 4).

Figure 1. Collection sites of *Myotis emarginatus* in Dubnisa Cave from Kırklareli (1); in old church from Karaisalı, Adana (2); and in Black Cave from Yozgat (3) in Turkey.

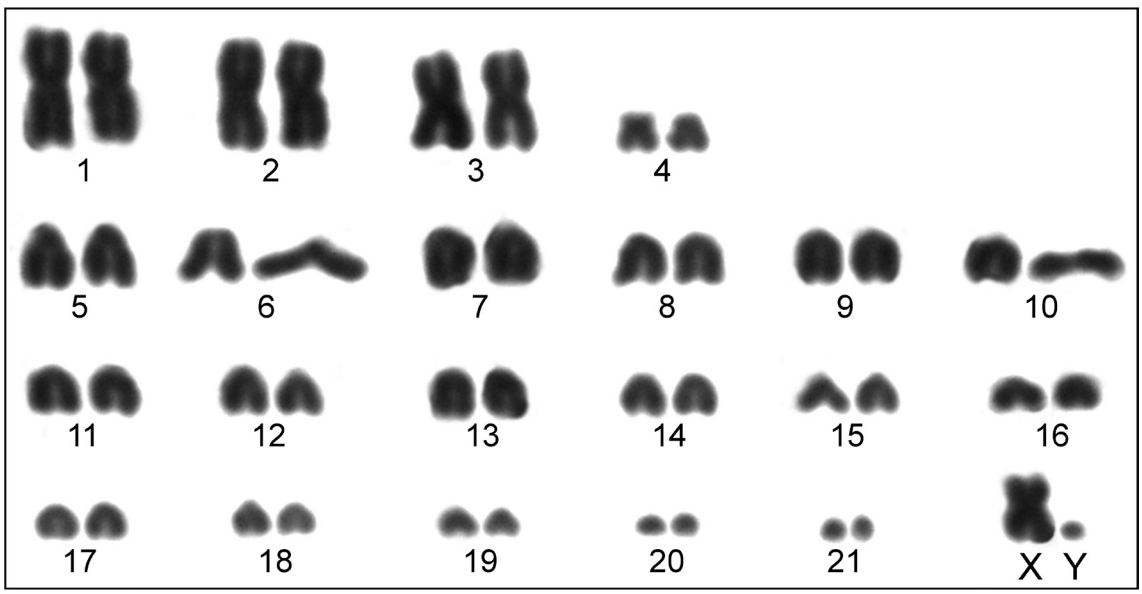

Figure 2. Standard karyotype of *Myotis emarginatus* in Turkey.

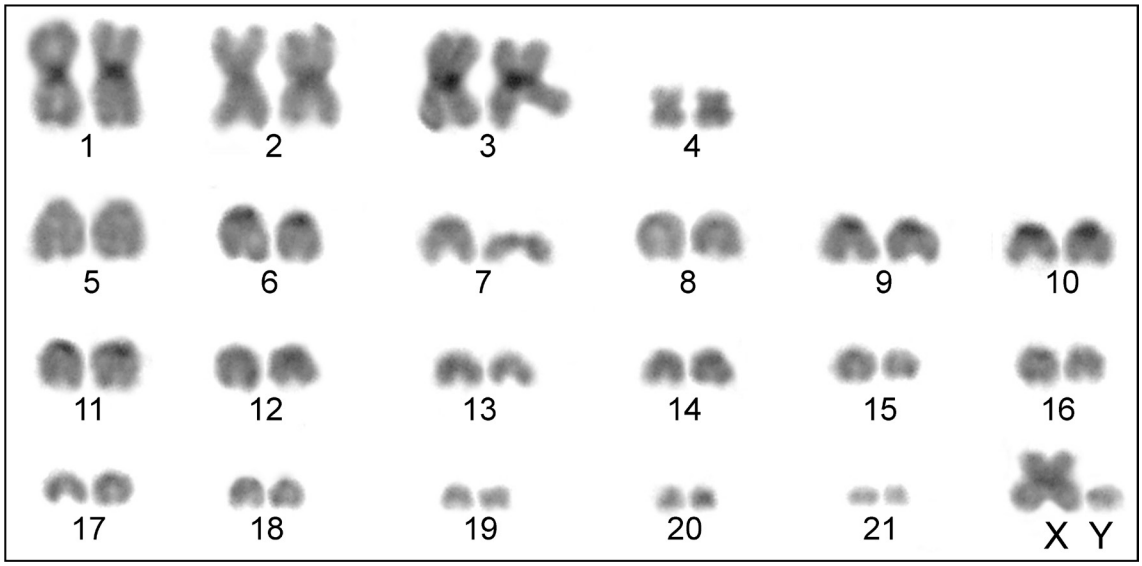

Figure 3. C-banded karyotype of *Myotis emarginatus* in Turkey.

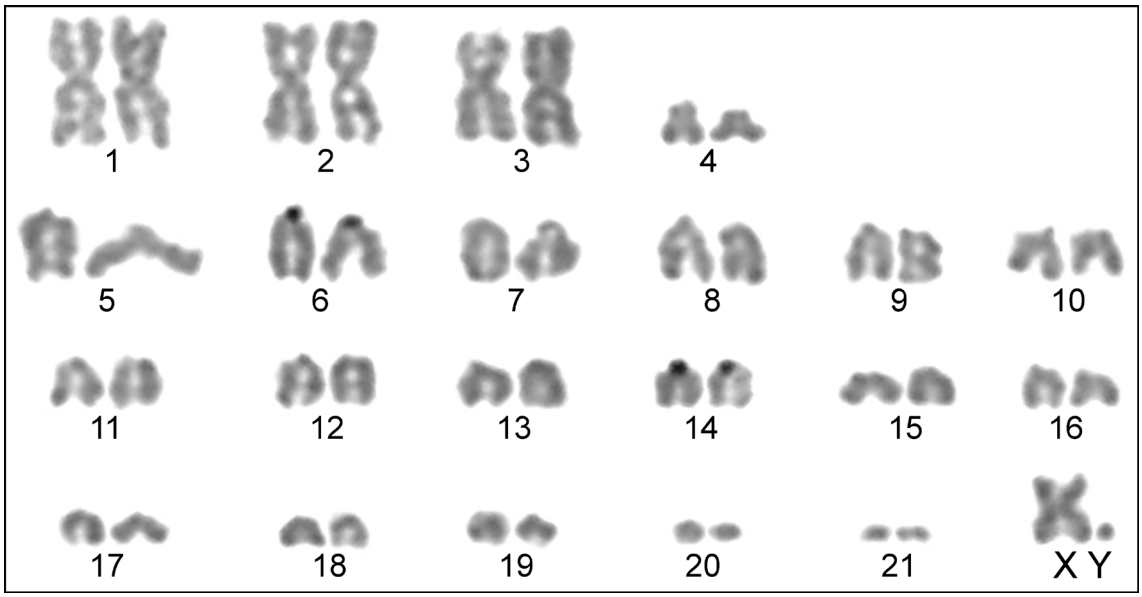

Figure 4. Silver-stained karyotype of *Myotis emarginatus* in Turkey.

The diploid chromosome number (2n = 44) and the fundamental number of autosomal arms (NFa = 50) ascertained in this study in *M. emarginatus* are congruent with the data obtained in most other *Myotis* species (Zima and Horáček, 1985). However, the number of autosomal arms in the complement of the Turkish specimens differs from findings from some other parts of the species' range, wherein one of the smallest autosomal dot-like pairs were identified as biarmed (Zima, 1978; Volleth, 1989).

In this study, only slight C-heterochromatin dark bands were found in some biarmed and acrocentric autosomes of *M. emarginatus* specimens in Turkey, and the sex chromosomes of this species were completely C-negative. However, it was determined to be a heterochromatic Y chromosome in a Greek population by Volleth and Heller (2012). The finding of a small amount of C-heterochromatin in the complement of *M. emarginatus* from Turkey is similar to findings reported in some other *Myotis* species (Bickham and Hafner 1978; Harada and Yosida, 1978; Andō et al., 1980).

Species of the genus *Myotis* usually possess a larger number of NOR-bearing autosomes (Volleth, 1987). The finding of only two NOR sites in *M. emarginatus* from Turkey is rather exceptional in this respect. Volleth (1987, 1989) and Volleth and Heller (2012) recognized several

diffuse NORs in the karyotype of this species. Therefore, our data indicate certain differences in the number of autosomal arms and NOR-bearing pairs between populations of *M. emarginatus* from Turkey and some other parts of the species' range. The potential taxonomic importance of this variation should be considered in the future.

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