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Karyological Variation of *Clethrionomys glareolus* (Mammalia: Rodentia) from Türkiye

Ahmet Yesari SELÇUK[1*], Perinçek Seçkinozan ŞEKER[1]

[1] Department of Forestry, Artvin Vocational School, Artvin Çoruh University, Artvin, Türkiye
Ahmet Yesari SELÇUK ORCID No: 0000-0002-2785-2823
Perinçek Seçkinozan ŞEKER ORCID No: 0000-0002-6103-4840

**Corresponding author: ahmetyesari@gmail.com*

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Abstract: Conventionally stained, C-banded and AgNOR-stained karyotypes of *Clethrionomys glareolus* were studied in Samsun, Türkiye. The autosomal and sexual chromosome pairs were determined by using the conventional, constitutive heterochromatin and nucleolus organizer region (C-, and AgNOR) staining techniques. The diploid chromosome number of a female *C. glareolus* specimen was found as 2n = 56, NFa = 56, and NF = 58. The karyotype consisted of a pair of small metacentric and 26 pairs of acrocentric chromosomes of different sizes. The X chromosomes were large acrocentrics. Similar to the result of past karyotype studies on *C. glareolus*, the C-positive and C-negative heterochromatin blocks were observed in the karyotype. As a difference from the results of a previous study, NORs were localized on the six acrocentric chromosome pairs instead of seven pairs. Thus, a chromosomal variation among *C. glareolus* populations was determined due to variability in the number of NOR-bearing autosomes. In this way, it contributed to the determination of intraspecific variations of this species and to Türkiye's biodiversity at the level of genetic diversity.

Türkiye'den *Clethrionomys glareolus*'un (Mammalia: Rodentia) Karyolojik Varyasyonu

Anahtar Kelimeler
Kromozom, C-bantlama, AgNOR boyama

Öz: Bu çalışmada, Türkiye'den elde edilen *Clethrionomys glareolus* türünün standart, C-bantlı ve AgNOR-bantlı karyotipleri incelendi. Otozomal kromozomları ve eşey kromozomları, standart, C-bantlama ve AgNOR boyama (C- ve AgNOR) teknikleri kullanılarak belirlendi. *C. glareolus* örneğinin diploid kromozom sayısı 2n = 56, NFa = 56 ve NF = 58 şeklindedir. Karyotipte, bir çift küçük metasentrik ve 26 çift farklı boyutlarda akrosentrik kromozomlar bulunmaktadır. X kromozomları büyük akrosentrik şeklindedir. *C. glareolus* üzerinde geçmişte yapılan karyotip çalışmalarının sonucuna benzer şekilde, karyotipte C-pozitif ve C-negatif heterokromatin bölgeler olduğu belirlendi. Bu çalışmanın sonuçlarında, Ag-NOR bölgelerinin altı akrosentrik kromozom çifti üzerinde lokalize olduğu tespit edildi. Böylece, Ag-NOR taşıyan otozomların sayısındaki değişkenliğe bağlı olarak *C. glareolus* populasyonları arasında kromozomal bir varyasyon olduğu belirlendi. Bu çalışma ile *C. glareolus* türünün tür içi varyasyonlarının belirlenmesine ve Türkiye biyoçeşitliliğine genetik çeşitlilik düzeyinde katkı sağlanmış oldu.

1. INTRODUCTION

The bank vole (or red-back vole), *Clethrionomys glareolus* is a rodent species that lives in mixed and deciduous forests consisting of coniferous and broad-leaved trees. This species is widely distributed in the Palearctic region. Its distribution range extends from Europe to Central Asia [1-3]. In Türkiye, this species is widely distributed in the Black Sea Mountains and the Marmara Region across the forests found in the northern line of Türkiye. Also, an isolated population of the species lives in Uludağ, Bursa [15]. The karyotype of *C. glareolus* is characterized by the value of a diploid number of chromosomes 2n = 56, the number of autosomal chromosome arms NFa = 56, and the fundamental number of chromosomal arms NF = 60. The morphology of sex chromosomes is variable [5-8]. The X chromosome is acrocentric, while the Y chromosome is metacentric in Turkish populations or acrocentric in European populations. [4, 5, 9-14]. So far, few studies have been conducted in Türkiye revealing the karyological characteristics of *C. glareolus*. In these studies, both traditional and banded karyotypes (C-

banded and AgNOR stained) of the populations of this species were examined. Accordingly, the standard karyotype of this species included a small metacentric pair and 26 acrocentric pairs in decreasing size. The X chromosome was a large acrocentric; the Y chromosome was a small metacentric [4, 13, 14]. The samples used in these studies were mainly obtained from the localities in the northwestern Anatolia (from Bolu, Kocaeli, Uludağ, and Karabük) and did not represent all populations of the species distributed along the northern line. Therefore, there may be a lack of information about the potential variations in the karyotype of other populations of the species within its distribution range. A few karyotype studies previously performed on the samples from the northwestern Black Sea have not revealed any variation among the species' populations, but according to mitochondrial DNA sequence variations, *C. glareolus* populations are divided into two different geographical lineages in Türkiye, the eastern and the western Black Sea lineages, by the Kızılırmak Valley [15].

Chromosomes are known as the phenotype of the genotype, and although it is not as powerful a molecular marker as DNA sequences, karyotype studies aimed at determining chromosomal variations are still frequently used in rodent systematics to determine intraspecific variations [14]. By detecting variability in the number, morphology, and constitutive heterochromatin, condensed or nucleolus organizer regions (C-, G-, and AgNOR banding) patterns of chromosomes between populations, it may be possible to obtain significant phylogenetic, taxonomic, and even findings that may result in the identification of new taxa [16-18]. Also, the detection of variable karyotypes within populations of any species can help to understand and document the biodiversity at the genetic diversity level. Therefore, this study aimed to compare the conventional karyotypes of *C. glareolus* and their constitutive heterochromatin and nucleolus organizer regions (C- and AgNOR banding) patterns with previously conducted studies and to contribute to the next karyological studies. Thus, potential intraspecific variations will be revealed and a contribution to the biological diversity of our country.

2. MATERIAL AND METHOD

Karyotype analysis was performed on a female specimen of *C. glareolus* collected from Samsun (altitude: 450 m, North Anatolia, 41° 25’ 10.03’’N 36° 03’ 58.54’’E, 12, Apr. 2021). Karyotype preparations were prepared from the captured animals during the field work performed in accordance with the legal permission (no: E-21264211-288.04 -1071462) given by the Ministry of Agriculture and Forestry, General Management of Nature Conservation and National Parks of the Republic of Turkey. The karyotype preparations, including metaphase chromosome plates, were provided from the fresh femoral bone marrow cells of colchicine-treated animals [19]. The autosomal and sexual chromosome pairs were determined by using the conventional, constitutive heterochromatin and nucleolus organizer region (C-, and AgNOR) staining techniques. According to the chromosome staining techniques by Sumner [20] and Howell and Black [21], constitutive heterochromatin (positive C-banded regions) and nucleolus organizer regions (NORs) were detected in the karyotype. In total, 10 slides were prepared and approximately 20 well-spread metaphase plates were investigated. The diploid number of chromosomes (2n), the fundamental number of chromosomal arms (NF), and the fundamental numbers of autosomal arms (NFa), together with the X and the Y chromosomes were organized from largest to smallest. All chromosomes were classified as the metacentric or acrocentric according to their centromere positions, consistent with Levan et al., [22]. The karyotype preparations of the specimen examined were deposited at the Artvin Çoruh University, Artvin, Türkiye.

3. RESULTS

The conventional, C-, and AgNOR-banded karyotype of *C. glareolus* from Samsun (Türkiye) was studied. The karyotype of a female *C. glareolus* specimen was in the form of 2n = 56, NFa = 56, and NF = 58. In the conventional karyotype, the autosomal set comprised a pair of small metacentric (no: 1) and 26 pairs of different sizes of acrocentric chromosomes (nos:2-27).

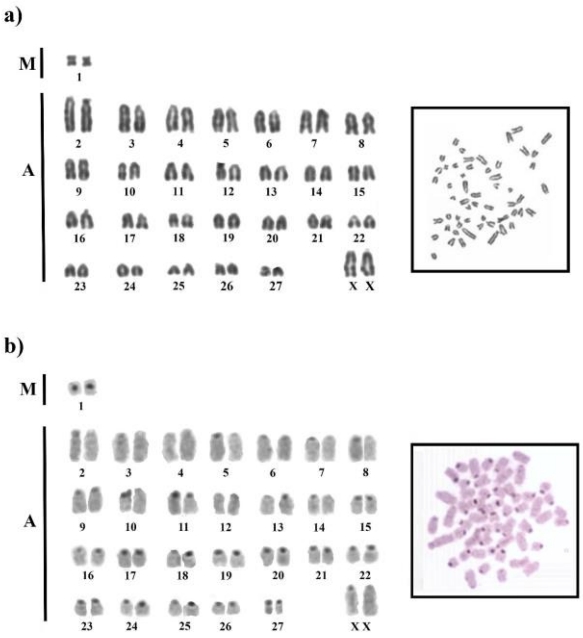

Figure 1. Conventional karyotype (a), C-banded karyotype (b) of a female *C. glareolus* specimen from Samsun, Türkiye, M: metacentric pair, A: acrocentric pairs.

The X chromosomes were large acrocentric. In the C-banded karyotype, acrocentric autosomal chromosomes contained both C-positive (no: 3 and nos: 5-27) and C-negative (no: 2 and 4) bands. The centromere region was clearly visible in the small metacentric chromosome pair (no: 1). X chromosomes showed C-positive bands (Figure 1).

In the AgNOR-stained karyotype of *C. glareolus*, nucleolus organizer regions were localized on the six pairs of acrocentric autosomal chromosomes (nos: 2, 5, 12, 14, 15, and 20). While three chromosome pairs (nos:

2, 5 and 15) had heteromorphic NORs, other NOR-bearing chromosomes were homomorphic (Figure 2).

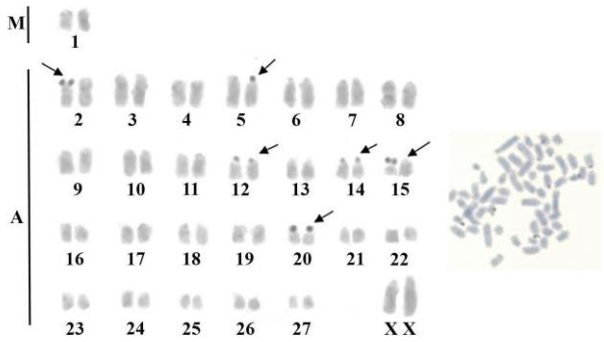

Figure 2. AgNOR-stained karyotype of a female *C. glareolus* specimen from Samsun, Türkiye. Arrows show NOR bearing chromosomes, M: metacentric pair, A: acrocentric pairs.

4. DISCUSSION AND CONCLUSION

This study presents the conventional, C- and AgNOR-stained karyotype of *C. glareolus* from Samsun, Türkiye. The standard karyotype of *C. glareolus* with the values of 2n = 56, NFa = 58, and NF = 58 is compatible with the previously determined karyotypes in the studies carried out within the distribution area of the species [4-6, 8-13, 16]. According to the C-banded patterns revealed by this study, the heterochromatin distribution in the karyotype was observed in most of the chromosome pairs, except for the second and fourth pairs. Supporting this result, Arslan et al. [13], observed a similar situation in their karyotype study including no C-positive band for the fifth pair. A similar case was seen in the karyotypes of specimens from Australia and Greece [10, 12, 23].

AgNOR staining has frequently been used in rodent systematics to determine intraspecific variations, since NORs in the karyotypes may be located on the different chromosome and may be presence in different numbers [24-29]. Variation in the distribution and the number of NORs has been previously reported in *C. glareolus* populations from different regions. In the karyological study conducted on the Bulgarian population, the NORs in the karyotype of *C. glareolus* were reported to be present on three autosomal chromosome pairs [30]. According to the results of the study including specimen from the north-western Black Sea region conducted by Arslan et al. [13], NORs present in seven acrocentric autosomal pairs, consisting of two homologous and five heteromorphic pairs. As a difference from that study, it was determined that NORs were present in six acrocentric chromosome pairs (three homologous and three heteromorphic) in the karyotype of the specimen from Samsun in the current study. Considering this result, it can be stated that there are intraspecific chromosomal variations in *C. glareolus* populations, which are known to have an uninterrupted distribution in the northern part of Türkiye. Analyses based on mitochondrial DNA sequence variations showed that *C. glareolus* populations in Türkiye have two distinct geographical lineages with genetic differences east and west of the Kızılırmak River [16]. Previous karyotype studies on this species include populations distributed west of the Kızılırmak River [4, 13]. The sample examined in this study was obtained from the east of the river and differs from western populations in terms of the number of NORs in its karyotype. It is thought that this is a significant finding that supports intra-specific genetic variations as proposed by mitochondrial DNA variations. In conclusion, karyotype analysis, which is frequently used in systematic studies due to its easy applicability, has enabled the determination of intraspecific variations of *C. glareolus* and contributed to the biodiversity of Türkiye at the level of genetic diversity.

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