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**SHORT COMMUNICATION**

# C-Banded Karyotype and Nucleolar Organizer Regions (NORs) of Marsh Frog, *Rana ridibunda* (Ranidae: Anura) in Central Anatolia

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## Summary

Karyotype, C-banding and nucleolar organizer regions (NORs) characteristics of nine *Rana ridibunda* samples collected from Konya (Meram, Beyşehir, Hadim) province were examined. The diploid number of chromosomes (2n) and the fundamental number of chromosome arms (FN) were determined as 26 and 52, respectively. All of the chromosomes of this species have centromeric constitutive heterochromatin (C-band). Nucleolar organizer regions (secondary constriction) were determined on the long arm of the no. 8 submetacentric chromosome by using silver-nitrate staining technique.

**Keywords:** *Rana ridibunda, Karyotype, C-band, NORs, Central Anatolia*

# Orta Anadolu'daki Bataklık Kurbağası, *Rana ridibunda* (Ranidae: Anura)'nın C-Bantlı Karyotipi ve Nükleolar Organizatör Bölgeleri (NORs)

## Özet

Konya ilinden (Meram, Beyşehir, Hadim) toplanan dokuz *Rana ridibunda* örneğinin karyotip, C-bantlama ve nükleolar organizatör bölgelerinin (NORs) özellikleri incelendi. Diploid kromozom sayısı (2n) ve temel kromozom kol sayısı (FN) sırasıyla 26 ve 52 olduğu belirlendi. Bu türün kromozomlarının tamamı sentromerik konstitutif heterokromatin (C-band)'e sahiptir. Gümüş-nitrat boyama tekniği ile 8 nolu submetasentrik kromozomun uzun kolu üzerinde nükleolar organizatör bölge (ikincil boğum) tespit edildi.

**Anahtar sözcükler:** *Rana ridibunda, Karyotip, C-bandı, NORs, Orta Anadolu*

## INTRODUCTION

*Rana ridibunda* Pallas, 1771, a common frog species of Central Europe and Western Asia, was described in Turkey [1]. Karyological techniques provided a reliable tool for cytotaxonomic analysis, and were effectively used to characterize the *R. ridibunda* karyotypes throughout the world [2,3]. Most *Rana* species are characterized by 26 chromosomes with a variable morphology among populations [4]. C-banding, used to establish hetero-chromatin regions on the chromosomes is frequently used in animals and thus is useful for examining intra and interspesific chromosomal differences between closely related species [5,6]. The first karyotype of this species was described by Alpagut and Falakalı [7] from Turkey. However, there is not any information about C- and Ag-NOR banding of karyotypes. Therefore, this study aims to present conventional, C- and NORs banded karyotypical data on *R. ridibunda* from Central Anatolia.

## MATERIAL and METHODS

Nine animals (three male and six female) studied were collected from Konya province (Meram, Beyşehir

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and Hadim). Karyotype preparations were obtained from the bone marrow of the colchicined animal [8]. In order to identify each autosomal pair and both sex chromosomes, constitutive heterochromatin and nucleolus organizer regions (NORs) were detected with C-banding [9] and Ag-NOR staining [10], respectively.

## RESULTS

The karyotype contains 26 chromosomes and the number of fundamental arms (FN) is 52. All the autosomal pairs are bi-armed. Six autosomal pairs are metacentric (nos. 1, 4, 6, 8, 10, 11) and chromosome no. 1 is larger than others and two pairs (nos. 2, 3) are large and four pairs are small submetacentric (nos. 7, 10, 12, 13) All the chromosomes in our samples have centromeric constitutive heterochromatin (C-band). Telomeric bands were not observed in the long and short arms of the chromosomes *(Fig. 1)*.

Active nucleolar organizer regions (NORs) were determined on the long arm of the number 8 submetacentric chromosome by using silver- nitrate staining technique *(Fig. 2)*.

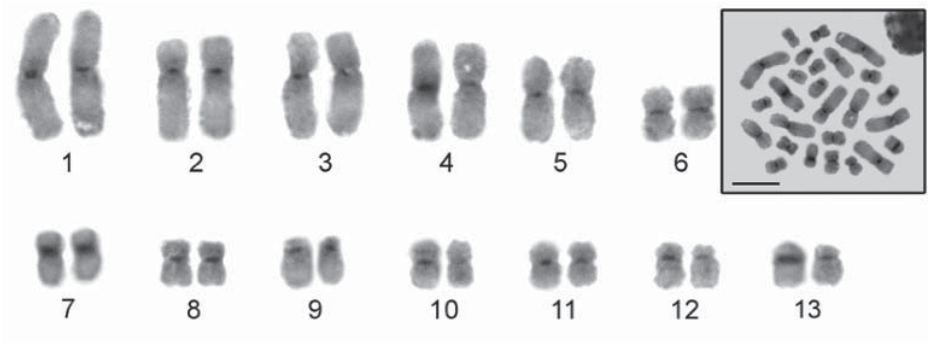


**Fig 1.** Metaphase spread and C-banded karyotype of Rana ridibunda (Scale bar = 10 µm)

**Şekil 1.** Rana ridibunda'nın C-bantlı karyotipi ve metafaz plağı (Scale bar = 10 µm)

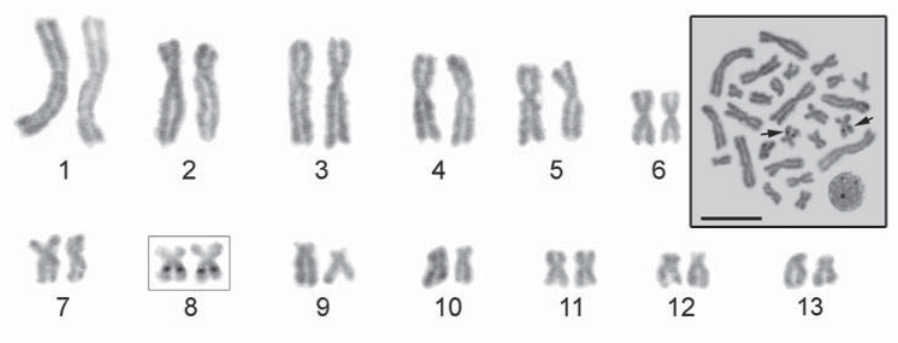


**Fig 2.** Silver-stained metaphase spread and karyotype of Rana ridibunda. Arrows indicate the Ag-NORs (Scale bar = 10 µm)

**Şekil 2.** Rana ridibunda'nın Ag-NOR bantlı karyotipi ve metafaz plağı. Oklar Ag-NOR'ları göstermektedir (Scale bar = 10 µm)

## DISCUSSION

Alpagut and Falakalı [7] studied two populations of this species in Turkey. Unlike our samples, they detected 7 pairs of metacentric, 4 pairs of submetacentric and 2 pairs of subtelocentric at Beyşehir population. These differences may result from the methodology. In the same study polymorphism in a pair of chromosomes at İzmir and Beyşehir populations were observed. However Al-Shehri and Al-Saleh [3], in a study they carried out in Saudi Arabia, reported that sex chromosomes of *R. ridibunda* had different sizes, and X chromosome is a little larger than Y chromosome. Alpagut and Falakalı [7] could not differentiate between the sex chromosomes in the karyotype, but they did for this species. However, these researchers determined that chromosome pair 4 of Beyşehir specimens was heteromorph. Schempp and Schmid [11] described chromosome pair 4 as sex chromosomes in *Rana esculenta,* which is the only *Rana* species for which the sex chromosomes have been identified so far.

In most of ranid species C-band analyses were carried out. As a result of these analyses ranid species were discovered to have centromeric bands [12-14]. In the cytogenetic research done in India on *Rana malabarica, Rana temporalis* and *Rana curtipes* by Joshy et al.[15] noted that all the chromosomes of each three species had centromeric C-bands. But these researchers determined that the telomeric C-bands which did not exist in other ranid species existed in two chromosomes of *R. temporalis* and *R. curtipes* and in three pairs of

chromosomes of *R. malabarica*. Joshy et al.[15] argued that the chromosomes which have telomeric bands differed according to the species and this case was a distinctive feature for these three species. It is inferred from these results that all *Rana* species all over the world have centromeric bands while some of them have also telomeric bands.

Alpagut and Falakalı [7] detected no. 9 secondary constriction in Beyşehir population and no. 10 in İzmir population. Koref-Santibanez [16] found that the *R. ridibunda* in Europe had no. 10 secondary constriction. Al-Shehri and Al-Saleh [3] detected secondary node in a pair of chromosomes (no. 10) in the Arabia population of this species as in Europe and Turkey samples. Joshy et al.[15] determined satellite in two pairs of chromosomes of *R. curtipes* via giemsa staining technique in a study they did in India. But these NORs exist in terminal parts of short arms not long arms unlike *R. ridibunda*. Howell and Black [10] reported that active NORs were exactly detected by silver-nitrate stain. The NORs outside the telomers of chromosomes can be detected by normal Giemsa stain. However Silver-nitrate staining must be implemented in order to exactly detect not only the secondary constructions in this region and also the satellites in telomers. According to the results of our silver-nitrate staining, active NOR existence was detected as a secondary constriction on the long arms of the no. 8 chromosome pair in Konya population.

As a result the chromosome morphology in our samples is similar to the studies on *R. ridibunda* in Turkey and around the world as well. Moreover, the existence NOR, which was detected on no. 8 chromosome of this species by routine giemsa staining several cytogenetic studies, was proved by silver-nitrate staining technique. The centromeric C-bands of *R. ridibunda* samples in Turkey was first detected with this study, as it is the first study to detect the centromeric C-bands of *R. ridibunda,* our samples do not have telomeric bands that are in some species related with *Rana* genus.

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