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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Cytogenetic characteristics of endemic *Squalius cappadocicus* Özuluğ and Freyhof, 2011 in Türkiye

Türkiye'ye endemik *Squalius cappadocicus* Özuluğ and Freyhof, 2011'un sitogenetik özellikleri

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Abstract: In this study, a detailed chromosome analysis of the endemic Cappadocian Chub, *Squalius cappadocicus* in Melendiz Stream (Aksaray) was performed. The standard Giemsa staining, C-banding (CBG and CB-DAPI), and Ag-NOR technique were applied. The diploid chromosome number was 50; its karyotype formula was 14M + 16Sm + 10St + 10A. Heteromorphic sex chromosomes weren't detected in the karyotype of the studied specimens. The number of all chromosomal arms (NF) was 90. In the standard C-banded and CB-DAPI karyotype of the species, dark C-bands were observed in the centromeric region of some bi-armed and acrocentric chromosomes, while slightly centromeric or pericentromeric C-bands were detected in some chromosomes. Three different active Ag-NORs, which were hemizygous, were detected in all samples examined. Two of these active NORs were detected in the bi-armed and the other in the acrocentric chromosome short arm. The Ag-NOR number of this species was evaluated as a feature that distinguishes it from other *Squalius* species in Türkiye.

Keywords: Cappadocian Chub, Chromosome, C-banding, CB-DAPI, Ag-NOR

Öz: Bu çalışmada, Melendiz Çayı'nda (Aksaray) endemik Kapadokya Kefali, *Squalius cappadocicus*'un detaylı kromozom analizi yapıldı. Standart Giemsa boyama, C-bantlama (CBG ve CB-DAPI) ve Ag-NOR tekniği uygulandı. Diploid kromozom sayısı 50'dir; karyotip formülü 14M + 16Sm + 10St + 10A'dır. İncelenen örneklerin karyotipinde heteromorfik cinsiyet kromozomları saptanmadı. Tüm kromozom kollarının (NF) sayısı 90'dı. Türün standart C-bantlı ve CB-DAPI karyotipinde, bazı çift kollu ve akrosentrik kromozomların sentromer bölgesinde koyu renkli C-bantları gözlenirken, bazı kromozomlarda sentromerik veya perisentromerik hafif C-bantlar tespit edildi. İncelenen tüm örneklerde hemizigot olan üç farklı aktif Ag-NOR tespit edildi. Bu aktif NOR'lardan ikisi iki kollu, diğeri akrosentrik kromozom kısa kolunda tespit edildi. Bu türün Ag-NOR sayısı, onu Türkiye'deki diğer *Squalius* türlerinden ayıran bir özellik olarak değerlendirilmiştir.

Anahtar kelimeler: Kapadokya Kefali, Kromozom, C-bandlama, CB-DAPI, Ag-NOR

INTRODUCTION

The Forty-five *Squalius* species in the world are distributed in the Western Palearctic. There are 21 species in total, 15 of which are endemic in Türkiye. These species are distributed in rivers, lakes and dam lakes in different regions of Türkiye (Çiçek et al., 2018). *Squalius cappadocicus* was first described by Özuluğ and Freyhof (2011) in the Melendiz River in the Tuz Lake basin. The karyological studies on *Squalius* species in the world are very few. The cytogenetic features of *S. cephalus* have been investigated by different researchers both in Europe and Türkiye (Wolf et al., 1969; Cataudella et al., 1977; Sofradzija, 1977; Bianco et al., 2004; Boroń et al., 2009; Pekol and Arslan, 2014; Kılıç and Şişman, 2016). The karyological characteristics of four *Squalius* species (*S. lucumonis, S. squalus, S. aradensis* and *S. torgalensis*) in Europe were determined by Rossi et al. (2012) and Nabais et al. (2013). Chromosome structures of only *S. orientalis*, *S. seyhanensis, S. carinus, S. fellowesii, S. anatolicus* and *S. recurvirostris* among 15 endemic species distributed in Türkiye were investigated by different researchers (Kılıç Demirok, 2000; Ünal and Gaffaroğlu, 2016; Karasu Ayata, 2020; Ünal Karakuş and Gaffaroğlu, 2021; Doori and Arslan, 2022). However, until now, no karyological studies have been conducted on *S. cappadocicus*, which spreads in Aksaray and its surroundings. The aim of this article is to perform a chromosomal banding analysis of the karyotype of *S. cappadocicus* using C-banding and Ag-NOR staining, and to compare the findings with those obtained in other previous studies.

MATERIALS AND METHODS

The specimens of *S. cappadocicus* were caught from the Melendiz Stream in Ihlara (Aksaray) (Figure 1) by the appropriate method. The captured samples were brought to the laboratory and rested. The karyology of the samples was done according to the method of Bertollo et al. (2015). Some of the chromosome slides were traditionally stained with Giemsa. Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected by applying C-banding (CBG and CB-DAPI banding) (Sumner, 1972) and Ag-NOR staining (Howell

and Black, 1980). Well-spread metaphases of each staining were photographed under the microscope and karyotyped. Chromosomes were defined according to Levan et al. (1964).

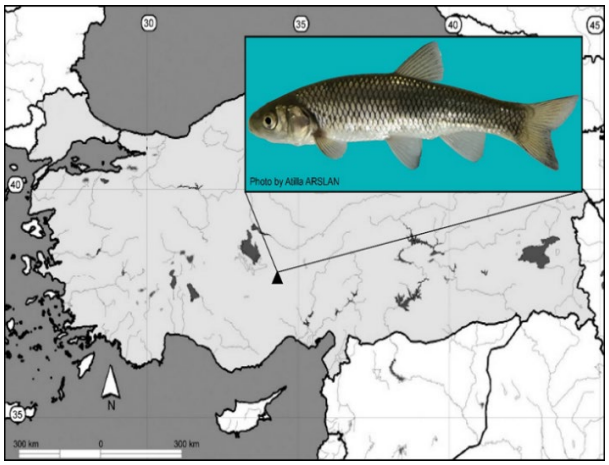

Figure 1. Collecting site in Stream Melendiz at Ihlara from Aksaray

RESULTS

In this study, the diploid chromosome number of *S. cappadocicus* was determined as 2n = 50. The karyotype consists of seven pairs of metacentric (nos. 1-7), six pairs of submetacentric (nos. 8-13), seven pairs of subtelocentric (nos. 14-20) and five pairs of acrocentric (nos. 21-25) autosomal chromosomes (NF = 90) (Figure 2). The heteromorphic sex chromosome of the species was not detected in the karyotype. The standard C-banded and CB-DAPI karyotype of Cappadocian chub was shown in Figure 3, and Figure 4. Dark C-bands in the centromeric region of some bi-armed and acrocentric chromosomes were observed. In addition, slightly centromeric or pericentromeric C-bands were determined in some chromosomes. Three active Ag-NORs were detected in all specimens examined. All three of these NORs are hemizygous. Two of these active NORs were identified in the short arm of one of the homologues of the bi-armed chromosome pair 10 and 11. The other active NOR was found in the short arm of one of the homologues of the acrocentric chromosome pair no. 23 (Figure 5).

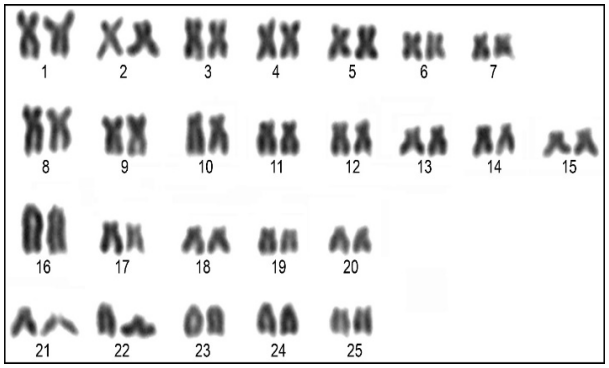

Figure 2. Standard Giemsa staining karyotype of *Squalius cappadocicus*

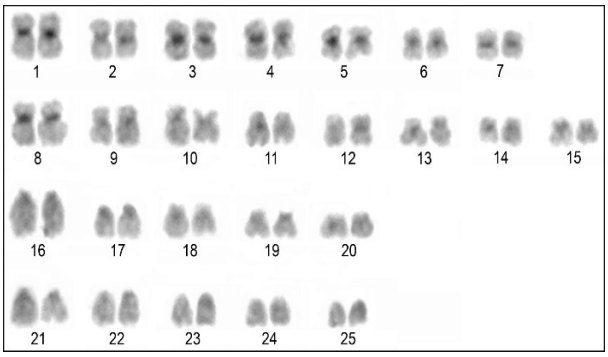

Figure 3. C-banded (CBG) karyotype of *Squalius cappadocicus*

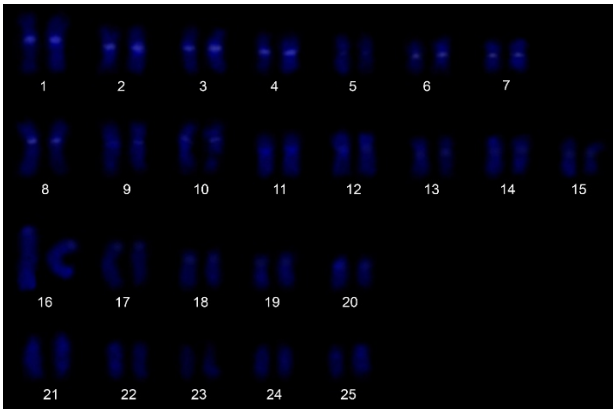

Figure 4. C-banded (CB-DAPI) karyotype of *Squalius cappadocicus*

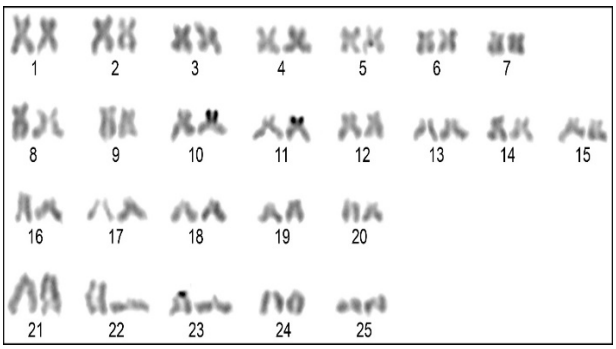

Figure 5. Silver-stained karyotype of *Squalius cappadocicus*

DISCUSSION

After *S. cappadocicus* was described by Özuluğ and Freyhof (2011) from the Melendiz River in the Tuz Lake basin, the cytogenetic features of this species were investigated for the first time in this study. In all examined individuals, the diploid chromosome number (2n) was 50 and the fundamental number of autosomal arms (NF) was 90. Sex chromosomes could not be detected in male and female individuals. The karyological characteristics of eight endemic *Squalius* species in Türkiye have been studied by different researchers and this information is summarized in Table 1.

Among these endemic species, sex chromosomes were detected in *S. recurvirostris*, which is distributed in the Konya

Ilgın basin (Doori and Arslan, 2022). ZZ-ZW sexual chromosome system was detected in Polish samples of *S. cephalus* distributed in Europe Vujosevic et al. (1983). Although very rarely, sex chromosomes have been detected in some cytogenetic studies on fish. It has been determined that some of them have ZZ-ZW and some have XX-XY sex chromosomes system (Doori and Arslan, 2022).

In general, the diploid chromosome number (2n) in leuciscin species (Leuciscidae) is 50. However, some species have different diploid chromosome numbers. In the studies carried out so far, 50 chromosomes have been identified in the chromosome set of all *Squalius* species. However, the number of bi-armed and acrocentric chromosomes in the set of these studied species differs (Table 1).

Table 1. Chromosomal records of *Squalius* species. NF–fundamental number of chromosomal arms, Z and W sex chromosomes

Species	Locality	2n	Karyotype	Z	W	NF	Reference
S. cephalus	Germany	50	38M/Sm + 12St/A	-	-	-	Wolf et al. (1969)
S. cephalus	Italy	50	16M + 12Sm + 12St + 10A	-	-	90	Cataudella (1977)
S. cephalus	Italy	50	16M + 26Sm + 8St/A	-	-	-	Bianco et al. (2004)
S. cephalus	Czechia	50	34M/Sm + 16A	-	-	84	Sofradzija (1977)
S. cephalus	Poland	50	10M + 22Sm + 10St + 10A	-	-	90	Boron et al. (2009)
S. cephalus	Türkiye	50	18M + 12Sm + 20St/A	-	-	-	Pekol and Arslan (2014)
S. cephalus	Türkiye	50	10M + 22Sm + 10St + 8A	-	-	92	Kılıç and Şişman (2016)
S. lucumonis	Italy	50	16M + 26Sm + 8St/A	-	-	-	Rossi et al. (2012)
S. squalus	Italy	50	16M + 26Sm + 8St/A	-	-	-	Rossi et al. (2012)
S. aradensis	Portugal	50	10M + 36Sm + 4St/A	-	-	-	Nabais et al. (2013)
S. torgalensis	Portugal	50	10M + 36Sm + 4St/A	-	-	-	Nabais et al. (2013)
S. orientalis	Türkiye	50	14M + 20Sm + 16St/A	-	-	-	Kılıç Demirok (2000)
S. seyhanensis	Türkiye	50	16M + 28Sm + 6St/A	-	-	-	Ünal and Gaffaroğlu (2016)
S. carinus	Türkiye	50	24M + 20Sm + 6St/A	-	-	-	Karasu Ayata (2020)
S. fellowesii	Türkiye	50	20M + 20Sm + 10St/A	-	-	-	Karasu Ayata (2020)
S. anatolicus	Türkiye	50	14M + 26Sm + 10St/A	-	-	-	Ünal Karakuş and Gaffaroğlu (2021)
S. recurvirostris	Türkiye	50	12M + 18Sm + 10St + 8A	A	St	90	Doori and Arslan (2022)
S. cappadocicus	Türkiye	50	14M + 16Sm + 10St + 10A	-	-	90	This study

Since most of the researchers evaluated submetacentric and acrocentric chromosomes together, the NF value of the studied species was not clear. This is due to the shortness of the chromosomes and the inability to differentiate between submetacentric and acrocentric chromosomes. Leuciscin species have two characteristic features within the chromosome set.

First, there are more metacentric/submetacentric chromosomes, and second, the largest chromosome pair is subtelocentric/acrocentric (Ráb et al., 2008). It was observed that these two features were preserved in both *Squalius* species studied so far and *S. cappadocicus*. The chromosome morphologies of *S. cappadocicus* within the set are quite similar to *S. recurvirostris* distributed in the Ilgın basin. It differs from *S. anatolicus* in the Beyşehir basin in terms of submetacentric, subtelocentric and acrocentric chromosome numbers. The morphology of the chromosomes of *S. cappadocicus* is different from that of *S. orientalis*, *S. seyhanensis*, *S. carinus* and *S. fellowesii* distributed in different regions of Türkiye. This difference can be explained by the different arrangements that occur in the chromosomes.

Heterochromatin blocks (C bands) are used for the comparison of species in karyological studies due to their structural properties. For this reason, the chromosomes of species can be studied in detail with the C-banding method. This method is an important technique in terms of chromosomal identification of species and determination of sex chromosomes of a species (Arslan and Arslan, 2007). Recently, DAPI fluorescent stain is also used instead of Giemsa in the C-banding method. In this study, C-bands of this species were detected using both Giemsa and DAPI. Similar bands were observed in both stainings. The distribution of constitutive heterochromatin (C-band) in the chromosomes of some Leuciscine species has been investigated by different researchers. There are differences in the heterochromatin distribution in the chromosomes of these studied species. According to Boroń et al. (2009), all the chromosomes of *Leuciscus leuciscus*, *L. idus*, and *S. cephalus* have centromeric C-band in different rivers in Poland. The constitutive heterochromatin analysis of some *Squalius* species was performed in Türkiye (Karasu Ayata, 2020; Ünal Karakuş and Gaffaroğlu, 2021; Doori and Arslan, 2022). In these species, C-bands have been found in different regions of some bi-armed and acrocentric chromosomes. Centromeric, pericentromeric and paracentromeric C-bands have been detected in *S. seyhanensis* (Ünal and Gaffaroğlu, 2016). In the centromeric regions of some bi-armed and acrocentric chromosomes of Akşehir Chub *S. recurvirostris* had dark C bands. Also, the some chromosomes of this species had centromeric or pericentromeric slightly C bands, while the sex chromosomes were C-negative (Doori and Arslan, 2022). Similar heterochromatin bands were observed in the chromosomes of *S. carinus*, *S. fellowesii* and *S. anatolicus*, which were distributed in Afyon, Denizli, Beyşehir (Karasu Ayata, 2020; Ünal Karakuş and Gaffaroğlu, 2021). It has been determined that there are terminal bands in some of the chromosomes of *S. anatolicus* (Ünal Karakuş and Gaffaroğlu, 2021). Although

there are some differences, C-heterochromatin amount and distribution of chromosomes of *S. cappadocicus* are similar to that of other *Squalius* species studied in Türkiye.

Ag-NOR technique has been used in some cytotaxonomic studies. Recently, it has been used frequently in the cytogenetic studies of fish. The number of Ag-NORs detected in populations of *L. leuciscus* in Poland varies. Active NORs were localized in the long arm of the largest metacentric pair and the short arm of a submetacentric chromosome pair in some populations. It has been reported to be localized in only one pair of metacentric or one pair of subtelocentrics in different populations (Boroń et al., 2009). Hemizygous three different active Ag-NORs of *S. cappadocicus* were detected in all samples examined. One of these active NORs (no. 10) was considered a characteristic pleisomorphic NOR. The active NOR on acrocentric chromosome 23 was detected for the first time in *Squalius* species in Türkiye. The localization of active NORs detected in two different bi-armed chromosomes in *S. recurvirostris* in Türkiye was determined by Doori and Arslan (2022). The short arms of a pair of submetacentric chromosomes of European specimens of *S. cephalus* have active NOR positively associated with CMA_3 (Bianco et al., 2004). Boroń et al. (2009) reported a 28S rDNA probe localized on a pair of subtelocentric chromosomes and NOR positively associated with CMA_3 in Poland specimens of the same species. One NOR-bearing chromosome pair (with NORs located on the short arms) observed in the karyotype of *S. cephalus* is the most common NOR phenotype among Leuciscinae (Collares-Pereira et al., 1998). This NOR in *S. cephalus* is a highly plesiomorphic character. The active NOR of *S. cephalus* in Kastamonu is also on the short arm of a pair of subtelocentric chromosomes (Pekol and Arslan, 2014), and this NOR phenotype is pleisomorphic for *Squalius* species. NORs in *S. recurvirostris* distributed in the Ilgın basin are on two pairs of submetacentric chromosomes. One of them is localized on the short arm and the other on the long arm. They stated that the NOR on the short arm in this species is pleisomorphic (Doori and Arslan, 2022). Active NOR on the long arm of any chromosome in the chromosome set was detected only in *L. leuciscus* and *S. recurvirostris*. *S. seyhanensis*, *S. carinus*, *S. fellowesii* and *S. anatolicus*, whose cytogenetics have been studied in Türkiye, also have pleisomorphic active NOR on the short arm of a pair of submetacentric chromosomes (Ünal and Gaffaroğlu, 2016; Karasu Ayata, 2020; Ünal Karakuş and Gaffaroğlu, 2021).

CONCLUSION

As a result, the current cytogenetic results of *Squalius* species are examined, it is seen that there are variations in terms of chromosome morphology, heterochromatin distribution and Ag-NOR number. Especially the change in the Ag-NOR number is quite remarkable. Both the high number of NORs and the presence of NOR in the acrocentric chromosome in *S. cappadocicus* can be considered as distinguishing this species from other *Squalius* species in Türkiye.

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AUTHORSHIP CONTRIBUTIONS

The contribution of the authors is equal.

ETHICS APPROVAL

The samples in this study were collected with the permission of the TR Ministry of Forestry and Water Affairs (Permit No: E-21264211-288.04-3435924). This permission replaces the local ethics committee permission in accordance with 8/L of the regulation "On Working Procedures and Principles of Animal Experiments Ethics Committees" prepared by the Ministry of Forestry and Water Affairs and published in the Official Gazette on February 15, 2014.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

DATA AVAILABILITY

All relevant data is inside the article.

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