

## Karyotypes and ZZ/ZW Sex Chromosome System of Endemic *Squalius recurvirostris* (Leuciscinae, Cyprinidae) in Turkey

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### ABSTRACT

In this study, detailed cytogenetic properties of the endemic Akşehir chub, *Squalius recurvirostris*, distributed in the basin of the Ilgın (Çavuşçu) Lake basin was investigated by applying the standard Giemsa staining, C-banding and Ag-NOR technique. Diploid chromosome number is (2n) 50. A pair of heteromorphic chromosomes were detected in the karyotype in females. However, this difference was not observed in males. Therefore, it was determined that the heteromorphic chromosome pair in females are ZZ/ZW sex chromosomes. The Z chromosome in females is large acrocentric, and the W chromosome is medium-sized submeta/subtelocentric. The number of all chromosomal arms (NF) is 90, and the fundamental number of autosomal arms (NFa) is 88 in the karyotyped of the individuals studied. Some bi-armed and acrocentric chromosomes have centromeric C-bands in the chromosome set. The slightly centromeric or pericentromeric C-bands are observed in some chromosomes, while other chromosome pairs are C-negative. In the individuals studied were detected two active NORs. Both of these NORs are hemizygous and associated with a large C-heterochromatin region. One of the NORs was detected on the entire long arm of the submetacentric chromosome pair and the other on the entire short arm of another submetacentric chromosome pair.

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## Türkiye'de Endemik *Squalius recurvirostris*'in (Leuciscinae, Cyprinidae) Karyotipleri ve ZZ/ZW Eşey Kromozom Sistemi

### ÖZET

Bu çalışmada, Ilgın (Çavuşçu) Gölü havzasında yayılış gösteren endemik Akşehir kefali *Squalius recurvirostris*'in ayrıntılı sitogenetik özellikleri, standart Giemsa boyama, C-bantlama ve Ag-NOR tekniği ile araştırıldı. Diploid kromozom sayısı (2n) 50'dir. Dişilerin karyotipinde bir çift heteromorfik kromozom tespit edildi. Ancak erkeklerde bu fark görülmedi. Bu nedenle dişilerdeki heteromorfik kromozom çiftinin ZZ/ZW cinsiyet kromozomları olduğu belirlendi. Dişilerdeki Z kromozomu büyük akrosentriktir ve W kromozomu orta büyüklükte submeta/subtelosentriktir. Çalışılan örneklerin karyotipinde tüm kromozomal kolların (NF) sayısı 90, otozomal kolların (NFa) temel sayısı 88'dir. Kromozom setindeki bazı çift kollu ve akrosentrik kromozomlar sentromerik C bantlarına sahiptir. Bazı kromozomlarda hafif sentromerik veya perisentromerik C-bantları gözlenirken, diğer kromozom çiftleri C-negatiftir. İncelenen bireylerde iki aktif NOR tespit edildi. Bu NOR'lerin her ikisi de hemizigottur ve büyük bir C-heterokromatin bölgesi ile ilişkilidir. NOR'lardan biri submetasentrik kromozom çiftinin tüm uzun kolunda, diğeri ise başka bir submetasentrik kromozom çiftinin tüm kısa kolunda tespit edildi.

### Genetik

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## INTRODUCTION

Chromosomal analysis is very important for cytogenetic and medical studies. Traditional karyological methods (Giemsa staining) and molecular cytogenetic techniques reveal genetic and taxonomic relationships among populations and species (Gill and Kimber, 1974). Heteromorphic sex chromosomes were observed in 72 families classified in Chondrichthyes and Osteichthyes (Devlin and Nagahama, 2002). It is known that approximately 10.4% of the more than 1700 fish species studied cytogenetically have heterogametic sex chromosomes (Arkhipchuk, 1999). In many species, it is necessary to perform the karyotype of male and female individuals to differentiate heteromorphic sex chromosomes. In cytogenetic studies on teleost fishes, it is understood that sexual chromosome systems vary. While XX-XY was detected in some of the fish and ZZ-ZW in some of them, sex chromosomes containing undifferentiated homologues were detected in most fish species. The research on teleost fishes has shown that these animals display a remarkable diversity in the ways that they determine sex. These different mechanisms, including constructive, genetic mechanisms, polygenic constitutive mechanisms, environmental effects, hermaphroditism and monosexuality on the sex chromosomes, have emerged at different times in teleosts (Mank and Avise, 2009). In freshwater species of neotropical fishes, both simple sex chromosome systems (XX-XY and ZZ-ZW) and multiple sex chromosome systems ( $X_1X_2Y-X_1X_1X_2X_2$ ) have been identified (Galetti et al., 1981; de Almeida Toledo and Foresti, 2001; Centofante et al., 2002).

Heterochromatin blocks (C-bands) contain repetitive DNA sequences and are inactive (Balíček et al., 1977). For this reason, these blocks are unlikely to deteriorate and can be preserved for generations (Varley et al., 1980; Sperling et al., 1987). C-bands can be located in any region on the chromosome but are usually located in the centromeric region of the chromosome. The C-banding method is an important technique in studying the chromosomes of the species in detail, identifying the chromosomal species, and identifying the sex chromosomes of a species (Haaf and Schmid, 1984; Arslan and Arslan, 2007). Silver nitrate staining (Ag-NOR) is the reaction where silver is reducing due to the binding of non-histone proteins to ionic silver (Trere, 2000). NORs are usually seen on the short arm end of the chromosome. However, it can also be seen in the long arm, near the centromere, or in the centromere region (Gold et al., 1990).

*Squalius* genus, the chubs in the Leuciscinae subfamily, consists of medium-sized fish represented

by 45 species in Europe and West Asia. After 2011, the genus *Leuciscus* (except *L. aspius*, *L. idus*, *L. vorax* and *L. leuciscus*) was revised as *Squalius* (Özuluğ and Freyhof, 2011; Turan et al., 2013). It is known that 21 *Squalius* species are distributed in freshwater ecosystems in Turkey, and 15 of them are endemic (Çiçek et al., 2015; Çiçek et al., 2018). *Squalius recurvirostris* Özuluğ & Freyhof, 2011, called Akşehir chub, is a local endemic species that live in the basin of Eber and Ilgın lake (Özuluğ and Freyhof, 2011). Although the genus *Squalius* has many species in the world, the karyological studies about them are limited. Different researchers have studied the cytogenetic features of *S. cephalus* (Wolf et al., 1969; Fontana et al., 1970; Cataudella et al., 1977; Sofradzija, 1977; Hafez et al., 1978; Al-Sabti, 1986; Pekol, 1999; Bianco et al., 2004; Boron et al., 2009; Kılıç and Şişman, 2016), *S. anatolicus* (Ünal, 2011), *S. orientalis* (Kılıç Demirok, 2000) and *S. seyhanensis* (Ünal and Gaffaroğlu, 2016). In these studies, it is resulted that the diploid chromosome number (2n) of *Squalius* species is 50, and the chromosomal arm (NF) value varies between species. This study aims to reveal the detailed karyological features and sex chromosome system of *Squalius recurvirostris* and compare the obtained data with the other *Squalius* species.

## MATERIALS and METHODS

Six specimens of *S. recurvirostris* were collected from Ilgın (Çavuşçu) Lake (38 ° 20' N, 31 ° 52' E (Figure 1). The study was carried out with the permission of the Ministry of Forestry and Water Affairs (Permission no. 21264211-288.04-E.1031876) and the local ethics committee. Chromosomal preparation was made according to Bertollo et al. (2015). The some dried slides were traditionally stained with 10% Giemsa. In addition, constitutive heterochromatin and nucleolar organizer regions (NORs) were determined by the Sumner (1972) and Howell and Black (1980) techniques, respectively. The morphology of the chromosomes were defined according to the method of Levan et al. (1964).

## RESULTS and DISCUSSION

In this study, the diploid chromosome number of *Squalius recurvirostris* was determined as  $2n = 50$ . The karyotype contained six pairs of metacentric, nine pairs of submetacentric, five pairs of subtelocentric and four pairs of acrocentric chromosomes. A pair of heteromorphic chromosomes were detected in the karyotypes of females (Figure 2).

However, there is no heteromorphic chromosomes in the karyotype of males (Figure 3).

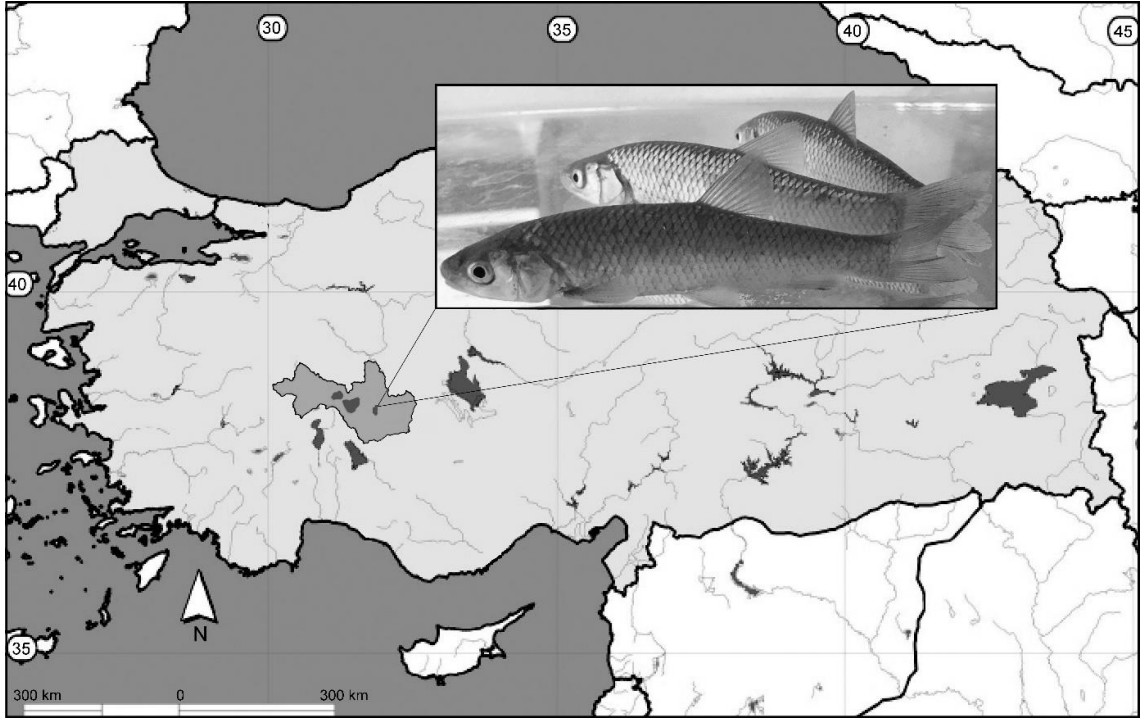


Figure 1. Collecting site in Lake Ilgın (Çavuşçu) from Konya and geographic range of *Squalius recurvirostris* according to IUCN.

Şekil 1. *Squalius recurvirostris*'in Konya Ilgın gölündeki (Çavuşçu) toplama alanı ve IUCN'e göre coğrafik dağılımı

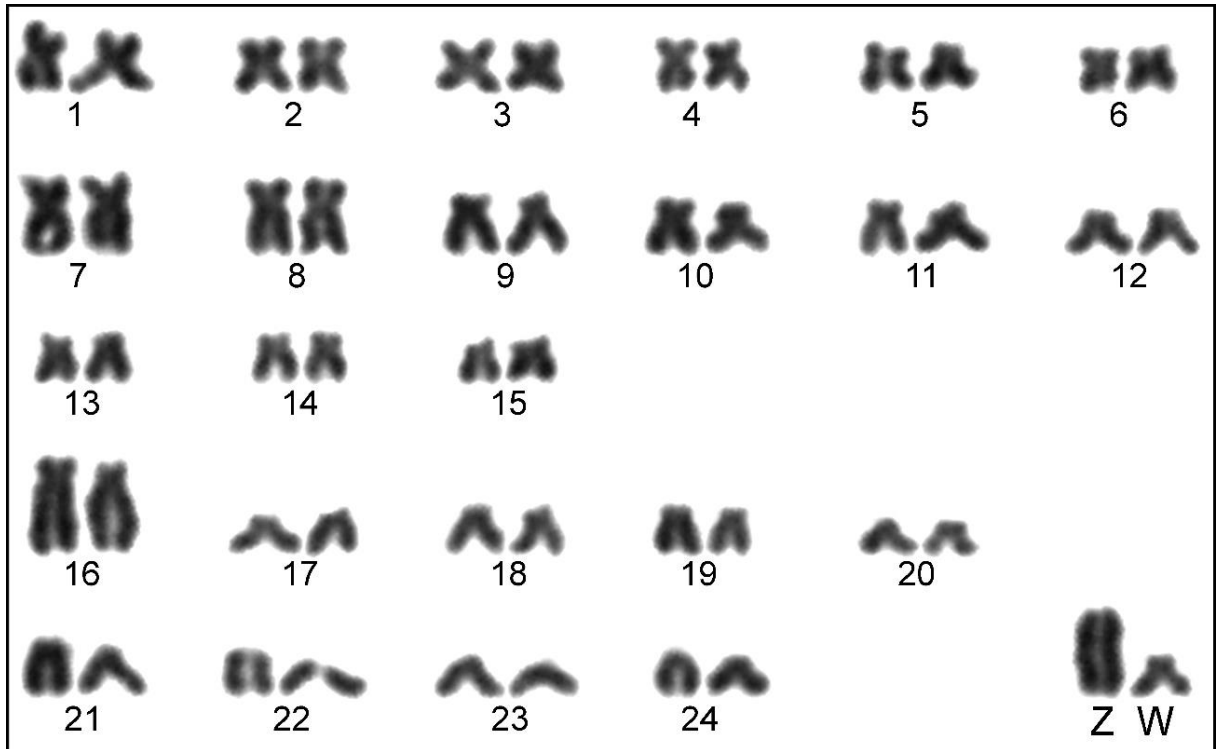


Figure 2. Standard Giemsa staining karyotype of female *Squalius recurvirostris*  
Şekil 2. Dişi *Squalius recurvirostris*'nin Giemsa boyalı standart karyotipi

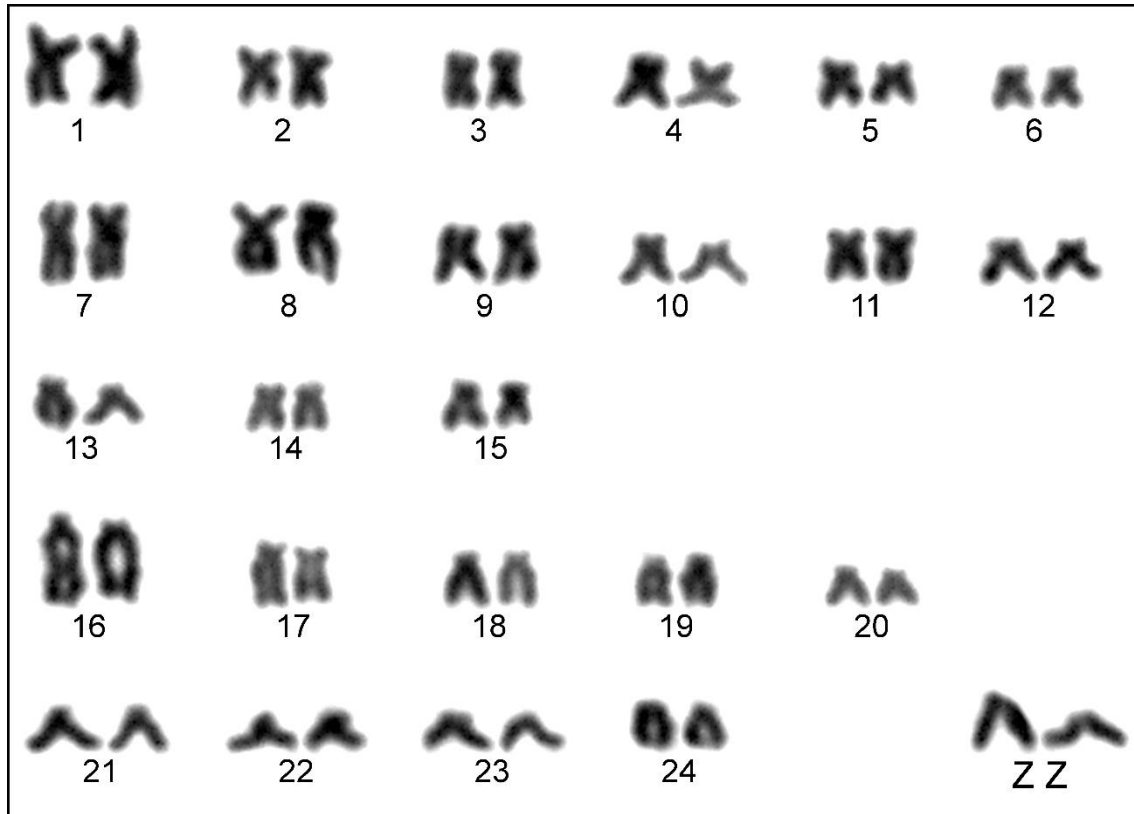


Figure 3. Standard Giemsa staining karyotype of male *Squalius recurvirostris*  
Şekil 3. Erkek *Squalius recurvirostris*'nin Giemsa boyalı standart karyotipi

Therefore, the heteromorphic chromosome pair in females was determined as the sex chromosome. The presence of sex chromosomes with different morphologies in females indicates that this species has ZZ/ZW sexual chromosome system. The Z chromosome is a large acrocentric, and the W chromosome is a medium-sized submeta/subtelocentric in females. The fundamental number of autosomal arms (NFa) and the number of all chromosomal arms (NF) in specimens karyotyped was determined as 88 and 90, respectively.

The C banded karyotype of Akşehir chub was shown in Figure 4. Dark C-bands in the centromere region of some bi-armed and acrocentric chromosomes were observed. In addition, slightly centromeric or pericentromeric C-bands were determined in some chromosomes, while other chromosome pairs and sex chromosomes were C-negative.

Two different active Ag-NORs were detected in studied all populations. Both of these NORs are hemizygous. One of these active NORs is located on the long arm of one of the homologs of the biarmed chromosome pair (no. 9), while the other is on the short arm of one of the homologues of another biarm chromosome pair (no. 12). The hemizygous NORs were localized within the long and short arms, which was C-positively stained (Figure 5).

The Leuciscine species in the Cyprinidae family (Subfam: Cyprininae, Gobioninae, Leuciscinae,

Rasborinae, Schizothoracinae, Barbinae) are distributed in Eurasia and North America (Bogutskaya, 1977; Arai, 2011; Nelson et al., 2016). The species belonging to the Leuciscinae subfamily (*Abramis*, *Acanthobrama*, *Acanthalburnus*, *Alburnoides*, *Alburnus Aspius*, *Blicca*, *Chondrostoma*, *Ladigesocypris*, *Leucaspis*, *Leuciscus*, *Leucalburnus*, *Petroleuciscus*, *Pseudophoxinus*, *Phoxinus*, *Rutilus*, *Squalius*, *Scardinius*) are distributed over a wide area in Anatolia (Çiçek et al., 2015). Despite this wide distribution and species richness, the karyological studies on Anatolian leuciscin are limited (Table 1).

Generally, the diploid chromosome number (2n) is 50 in the Leuciscinae subfamily. However, *Alburnus mossulensis* has 48, *Luciobarbus pectoralis* has 100, *Luciobarbus longiceps* has 148, *Chondrostoma regium*, *Chondrostoma meandrense* and *Leuciscus aspius* (Syn: *Aspius aspius*) have 52, *Scardinius erythrophthalmus* 48 and 50 chromosome pairs (Nygren et al., 1975; Cataudella et al., 1977; Gül et al., 2000; Fişter, 2000; Bianco et al., 2004; Turan et al., 2005; Esmaceli et al., 2010; Luca et al., 2010; Uysal, 2011; Ünal and Gaffaroğlu, 2016). The karyological features of endemic fish *Squalius recurvirostris* were investigated in this study for the first time. The diploid chromosome number of this species is 50, and there are four acrocentric chromosomes in the chromosome set (NF = 90). The Leuciscine species have two characteristic features

within the chromosome set. Firstly, the metacentric/submetacentric chromosomes are more, and the other, the largest chromosome pair is subtelocentric/acrocentric (Ráb et al., 1990; Ráb and

Collares-Pereira, 1995; Ráb et al., 2008; Pereira et al., 2009). It is understood that *S. recurvirostris* has these two characteristics.

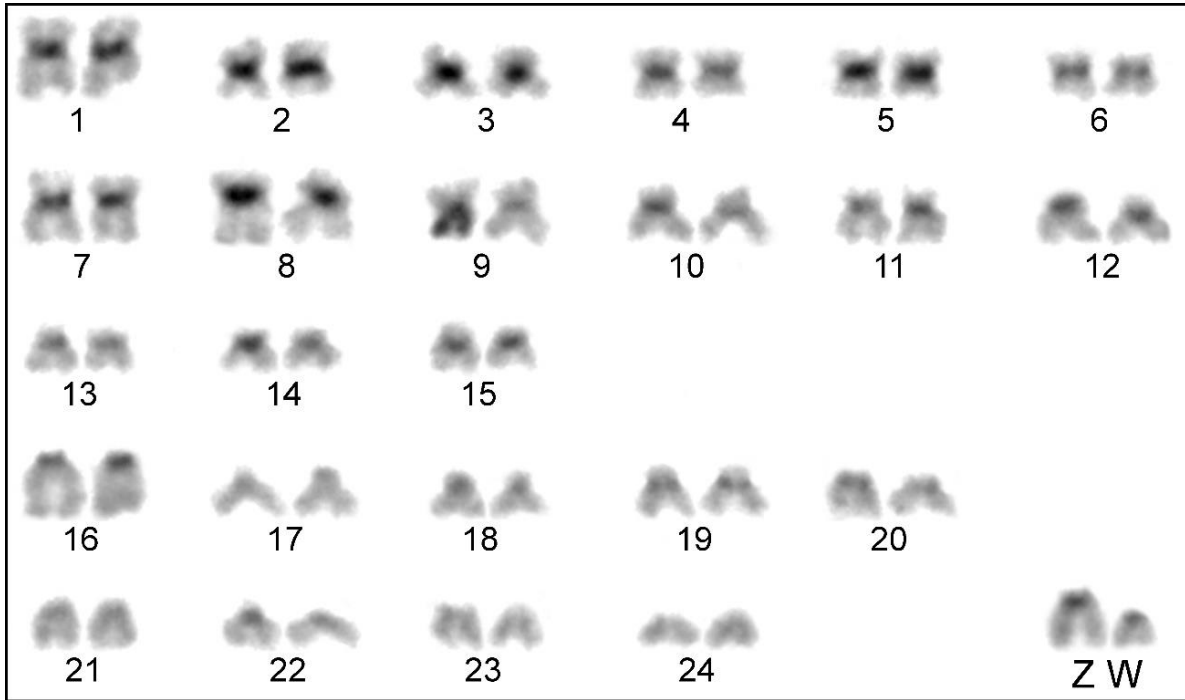


Figure 4. C-banded metaphase spread and arranged karyotype of *Squalius recurvirostris*  
Şekil 4. *Squalius recurvirostris*'in C-bantlı metafaz yayılımı ve karyotipi

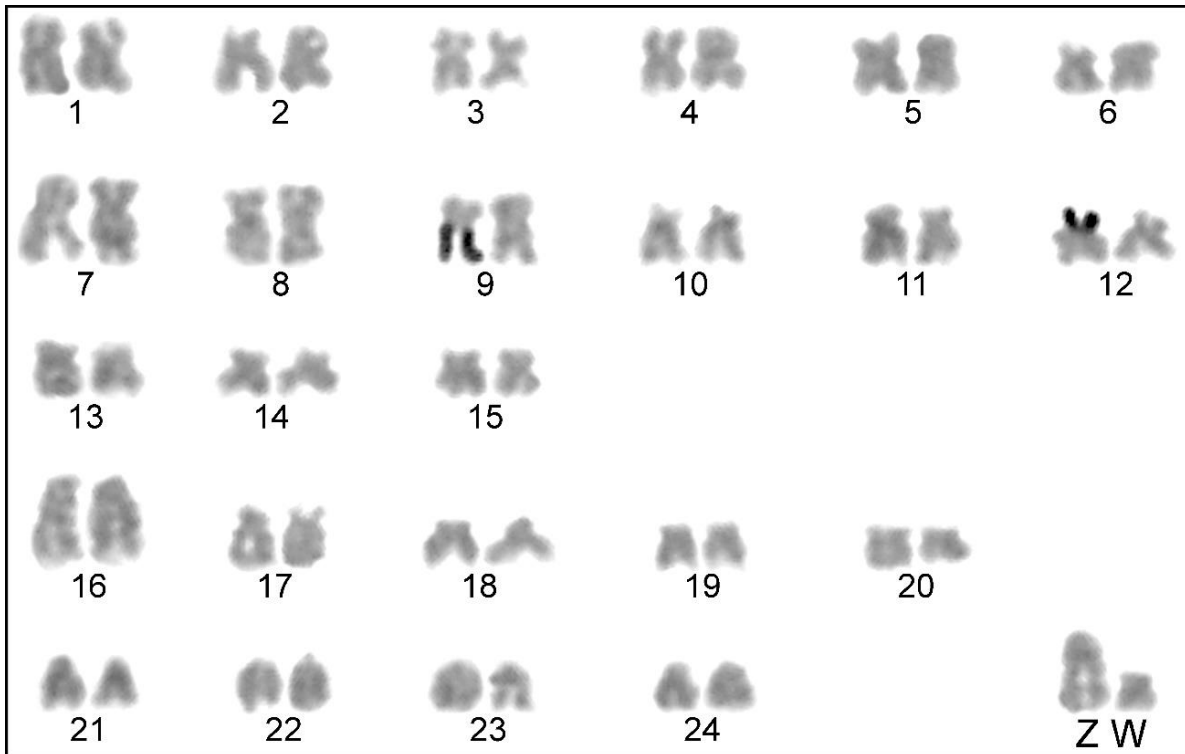


Figure 5. Silver stained metaphase spread and arranged karyotype of *Squalius recurvirostris*  
Şekil 5. *Squalius recurvirostris*'in gümüş boyalı metafaz yayılımı ve karyotipi

Table 1. Chromosomal records of *Squalius* species (Abbreviations; 2n-diploid chromosome number, NF-fundamental number of chromosomal arms, M-metacentric, SM-submetacentric, ST- subtelocentric, A-acrocentric)

Çizelge 1. *Squalius* türlerinin kromozom kayıtları (Kısaltmalar; 2n-diploid kromozom sayısı, NF-temel kromozom kol sayısı, M-metasentrik, SM-submetasentrik, ST-subtelosentrik, A-akrosentrik)

Species	2n	Karyotype	Z	W	NF	Reference
<i>Squalius cephalus</i>	50	38M-SM + 12ST-A	-	-	88	Wolf et al. (1969)
		11M + 29SM-ST + 10A			-	Fontana et al. (1970)
		16M+12SM+12ST+10A	-	-	78	Cataudella (1977)
		34M-SM + 16A	-	-	84	Sofradzija (1977)
		18M+20SM-ST+12A	-	-	88	Hafez et al. (1978)
		34M-SM + 16ST-A	-	-	84	Al-Sabti (1986)
		18M+12SM+20ST-A	-	-	80	Pekol and Arslan (2014)
		16M+26SM+8ST-A	-	-	92	Bianco et al. (2004)
		10M+22SM+10ST+10A	-	-	82	Boron et al. (2009)
		10M+22SM+10ST+8A	-	-	92	Kılıç and Şişman (2016)
<i>Squalius anatolicus</i>	50	10M+22SM+10ST+8A	-	-	82	Ünal (2011)
<i>Squalius orientalis</i>	50	14M+20SM+16ST-A	-	-	84	Kılıç-Demirok (2000)
<i>Squalius seyhanensis</i>	50	16M+28SM+6ST-A	-	-	94	Ünal and Gaffaroğlu (2016)
<i>Squalius recurvirostris</i>	50	12M+18SM+10ST+8A	A	ST	90	<b>This study</b>

*Squalius cephalus* (Syn: *Leuciscus cephalus*) in Poland has the ZZ-ZW sexual chromosome system. The Z chromosome is the largest submetacentric, and the W chromosome is the smallest submetacentric (Vujosevic et al., 1983). The Z chromosome (acrocentric) of *S. recurvirostris* is different from *S. cephalus*, while the W chromosome is similar. The ZZ-ZW sex chromosomes system in fish is important to identify for the first time in this study in Turkey. Due to the detection of sex chromosomes, the fundamental number of autosomal arms (NFa) of *S. recurvirostris* is 88. So far, XX-XY sex chromosomes in *Garra rufa* have been reported by Arzu and Ergene (2009). In chromosome studies on some species in different regions of the world, sexual chromosome systems are also mentioned. In the study in Germany, the sex chromosomes of Rudd, *Scardinius erythrophthalmus* (Cyprinidae) were identified as a pair of large metacentric (ZZ) in males and large metacentric and small acrocentric (ZW) chromosomes in females (Koehler et al., 1995). However, it has been reported that sexual chromosomes cannot be detected in Rudd populations in Romania, Italy and Slovenia (Bianco et al. 2004; Luca et al. 2010). In another cyprinid species, *V. vimba*, XY sex chromosomes were defined as the largest metacentric X and the smallest acrocentric Y (Rudek 1974). In 34 of 55 Neotropical fish species studied, ZZ-ZW system, in 10 of them XY-XX system and 11 of them multiple sex chromosome systems ((X<sub>1</sub>X<sub>1</sub>X<sub>2</sub>X<sub>2</sub>-X<sub>1</sub>X<sub>2</sub>Y) were identified (Centofante et al., 2002).

C-banded karyotypes of *Squalius anatolicus* and *Squalius seyhanensis* in Turkey were investigated (Ünal, 2011; Ünal and Gaffaroğlu, 2016). Most of the

chromosomes in both species have centromeric heterochromatin. In addition, Ünal and Gaffaroğlu (2016) stated that there are pericentromeric and pericentromeric (interstitial) bands in *S. seyhanensis*. Although the C-band feature of *S. recurvirostris* is similar to both species, pericentromeric and pericentromeric bands were not detected. In some European countries (i.e. Italy, Poland and the Czech Republic), all the chromosomes of *Leuciscus idus*, *Squalius leuciscus* and *L. cephalus* have centromeric C-band as in *Squalius* species in Turkey. This situation may be a distinctive feature between *Squalius* and *Leuciscus* species and other Leuciscinae species.

NOR technique is used in taxonomic studies, determination of inter-species and intraspecific relationship and various chromosome variations. The number of Ag-positive NORs varies from 1 to 4 in different populations of *L. leuciscus* in Europe. Signals have been reported to be localized in the long arms of the largest metacentric pair in some populations and in the short arms of a submetacentric chromosome pair, and in only one pair of metacentric or a pair of subtelocentric (Boron et al., 2009). *L. aspius* (Syn: *Aspius aspius*) differs from other *Leuciscus* species in that NORs are localized at the terminal region of the short arm of a small acrocentric pair (Ráb et al., 1990). The active NORs of *S. cephalus* in Kastamonu population are on the short arm of a pair of subtelocentric or acrocentric chromosomes, and the NOR phenotype of these samples is a plesiomorphic character (Pekol and Arslan, 2014). The morphologies of the two chromosomes with NORs are submetacentric in *S.*

*recurvirostris*. The NORs are also localized on the short arm of one of these chromosomes and the long arm of the other. In terms of these two characteristics, *S. recurvirostris* differs from other species studied. Also, *S. recurvirostris* has a plesiomorphic NOR character. Although the phylogenetic studies conducted so far show that *Squalius* species are not closely related to *Leuciscus* (Briolay et al., 1998), in terms of Ag-NOR properties, this species is similar to *S. seyhanensis*, *S. anatolicus* and *L. leuciscus*.

## CONCLUSION

Cytogenetic characteristics and ZZ-ZW sex chromosomes system of endemic *S. recurvirostris* for Turkey have been demonstrated for the first time in this study. The characteristic structure accepted for the Leuciscinae group (the largest chromosome pair is subtelocentric or acrocentric) has been identified in this species. Centromeric heterochromatin regions detected in most chromosomes were found to be compatible with other *Squalius* and *Leuciscus* species. The Ag-NOR-bearing chromosomes of the studied samples are hemizygous. The Ag-NOR regions are related to the large C-heterochromatic blocks on the long and short arms. It is observed that active Ag-NORs are different from other *Squalius* species studied by detecting both on the short arm and the long arm and are similar to *L. leuciscus*. Cytogenetic data on *Squalius* species that have been published in Turkey, although it is not support existing species definitively taxonomic status, these studies may contribute to later molecular cytogenetic or phylogenetic studies of Leuciscinae species to be searched.

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## Statement of Conflict of Interest

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

## Author's Contributions

The contribution of the authors is equal.

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