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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Karyotype, C-band and NOR phenotype of Anatolian endemic fish *Squalius anatolicus* (Bogutskaya, 1997) (Teleostei, Leuciscidae)

Anadolu'ya endemik *Squalius anatolicus* (Bogutskaya, 1997) (Teleostei, Leuciscidae) türünün karyotip, C-bant ve NOR fenotipi

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Abstract: The karyotype and distribution of constitutive heterochromatin and nucleolus organizer regions (NORs) of Anatolian leuciscine endemic to Lake Beysehir, *Squalius anatolicus* (Bogutskaya, 1997) were analyzed respectively using conventional Giemsa-staining, C-banding and Ag-impregnation. Diploid chromosome number was *2n* = 50 and karyotype consisted of 7 pairs of metacentric, 13 pairs of submetacentric, 5 pairs of subtelo- to acrocentric chromosomes, NF value equaled 90. Heteromorphic elements indicating sex chromosomes were not detected. C-banding revealed clear pericentromeric constitutive heterochromatin blocks in several chromosomes. Ag-impregnation revealed the size heteromorphism of NORs that covered almost the entire short arms of the middle-sized submetacentric chromosome pair. The karyotype pattern and simple NOR phenotype of *S. anatolicus* are nearly identical with that found not only in *Squalius* species analyzed to date but also in many other representatives of the Eurasian leuciscine cyprinids, which indicates remarkable chromosome stasis in this leuciscid lineage.

Keywords: Leuciscid cytotaxonomy, fish cytogenetics, chromosome banding, major rDNA sites, *Squalius anatolicus*

Öz: Beyşehir Gölü'ne endemik Anadolu leuciscini *Squalius anatolicus* (Bogutskaya, 1997)'un karyotipi, konstitütif heterokromatin dağılımı ve çekirdekçik organize edici bölgeleri (NOR'lar) sırası ile geleneksel Giemsa-boyama, C-bantlama ve Gümüş emdirme teknikleri kullanılarak analiz edilmiştir. Diploid kromozom sayısı *2n* = 50 olmak üzere; karyotipinin 7 çift metasentrik, 13 çift submetasentrik, 5 çift subtelo – akrosentrik kromozomdan oluştuğu ve toplam kol sayısının 90 olduğu tespit edilmiştir. Cinsiyet kromozomlarını temsil eden heteromorfik yapılar gözlenmemiştir. C-bantlama birçok kromozomda belirgin perisentromerik heterokromatin blokları ortaya çıkarmıştır. Gümüş emdirme ile orta boy submetasentrik kromozom çiftinin neredeyse kısa kolunun tamamını kaplayan NOR'ların büyüklük heteromorfizmi tespit edilmiştir. *S. anatolicus*'un karyotip şekli ve temel NOR fenotipi sadece bugüne kadar analiz edilen *Squalius* türlerinde değil, aynı zamanda bu leuciscid soyunda büyük ölçüde kromozom durumunu gösteren Avrasya leuciscinlerinin diğer birçok temsilcisinde bulunanla hemen hemen aynıdır.

Anahtar kelimeler: Leuciscid sitotaksonomisi, balık sitogenetiği, kromozom bantlama, major rDNA bölgeleri, *Squalius anatolicus*

INTRODUCTION

The genus *Squalius* was recognized within the genus *Leuciscus* for a long time until morphological and molecular data demonstrated that *Leuciscus* represents another leuciscine lineage (Zardoya and Doadrio, 1999). The *Squalius* genus belongs to the Leuciscinae subfamily and comprises at least 45 species that are commonly named chub (Özulug and Freyhof, 2011). The genus represented by 22 species includes *Squalius adanensis, S. anatolicus, S. aristotelis, S. berak, S. cappadocicus, S. carinus, S. cephaloides, S. cephalus, S. cii, S. fellowesii, S. irideus, S. kosswigi, S. kottelati, S. lepidus, S. orientalis, S. orpheus, S. pursakensis, S. recurvirostris, S. semae, S. seyhanensis, S. spurius,* and *S. turcicus* in Anatolia (Çiçek et al., 2020).

Squalius species are present in almost every water body in Anatolia (Stoumboudi et al., 2006) while only a few populations have been defined in sufficient detail in Anatolia and adjacent basins. Some species of *Squalius* were reported from the Tigris, the Euphrates, the Orontes and the Beysehir drainages, respectively. Bogutskaya (1997) identified populations of Lake Beysehir basin as *S. anatolicus* (known as Beysehir dace) (Turan et al., 2009). This species has restricted distribution in central Anatolia, but it exists also in the Manavgat River, draining to the Mediterranean east of Antalya. The current population trend is reported as decreasing and it was known as endangered until 2006 (Özulug and Freyhof, 2011) but recently it is listed as the least concern in the IUCN Red List (Freyhof, 2014).

The subfamily Leuciscinae includes virtually 70 freshwater genera nonetheless approximately 25 leuciscine genera have been cytogenetically investigated (Rab and Collares-Pereira, 1995; Bianco et al., 2004; Rossi et al., 2012). The primary challenge in leuciscines' cytogenetic analysis relevant in

effectively descrying species at the karyotype level, fundamental for advanced chromosomal studies (Pereira et al., 2013). In earlier studies, *S. anatolicus* was described as morphologically and analysed as parasitologically (Turan et al., 2013; Aydogdu et al., 2015) while there are no cytogenetic data reported. The aim of the study is to investigate the karyotype, distribution of constitutive heterochromatin and NORs' phenotype that it means number and position of major rDNA/NOR sites of Anatolian leuciscine endemic to Beysehir Lake, *S. anatolicus* (Bogutskaya, 1997) using conventional Giemsa-staining, C-banding, and Ag-impregnation.

MATERIAL AND METHODS

Eight adult individuals of *S. anatolicus* (four females and four males) were collected from Lake Beyşehir basin, Konya, Turkey (37°52' N, 31°35' E) using electrofishing. The alive individuals were transported to the laboratory and kept in a well-aerated aquarium until analysis. The specimens were deposited in a fish collection of the Genetic Research Laboratory of Kırşehir Ahi Evran University, Kırşehir, Turkey.

The chromosome preparations were obtained according to standard protocol of Collares-Pereira (1992), using direct air drying technique. The slides were stained with 4% Giemsa staining solution (with Sorenson's phosphate buffer, pH 6.8). The metaphase plates were examined and photographed using Leica DMLB. The total sum of metaphases was 411 and metaphase number was not evenly ratio for per each of eight analysed specimens. The C-bands were obtained according to Sumner (1972). The Ag-impregnation method of Howell and Black (1980) was applied to determine NORs. The chromosomes were classified using a modified version of Levan et al. (1964), and the fundamental arm number (NF) was identified by scoring the metacentric (m) and submetacentric (sm) as biarmed and subtelo-acrocentric (st/a) chromosomes as uniarmed chromosomes.

Ethical Statement

Permissions of sampling and laboratory study on fish have issued by respectively the Republic of Turkey Ministry of Agriculture and Forestry (the number and date of fieldwork permission: B.12.0.KKG.0.17/106.01-11-01/3007840-02.02.2010), and the Kırşehir Ahi Evran University Animal Experiments Local Ethics Committee (the number and date of fieldwork permission: 68429034/02-14.02.2019).

RESULTS

All analyzed *S. anatolicus* specimens showed *2n* = 50 chromosomes. Karyotype consisted of 7 pairs of m, 13 pairs of sm, 5 pairs of st/a chromosomes (Table 1). The fundamental number of chromosome arms equaled NF = 90 (Figure 1). The morphologically differentiated sex chromosomes were not detected.

Table 1. Cytogenetic data of *Squalius anatolicus*

Species	Specimen number	Metaphase number	*2n*	NF	Chromosome morphology		
					m	sm	st/a
Squalius anatolicus	8	411	50	90	14	26	10

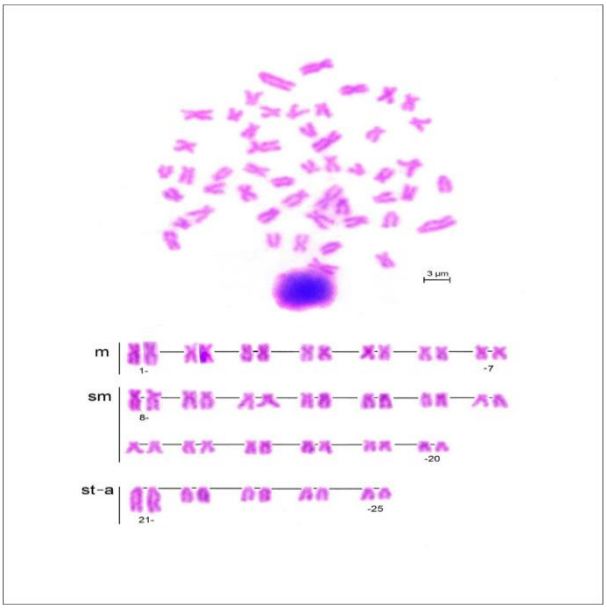

Figure 1. Giemsa stained metaphase and corresponding karyotype of *Squalius anatolicus*. Scale bar = 3 µm

C-banding procedure showed blocks of constitutive heterochromatin (CH) mostly in (peri) centromeric regions of several chromosomes. However, intercalar or terminal C-bands were also visible on some chromosomes (Figure 2).

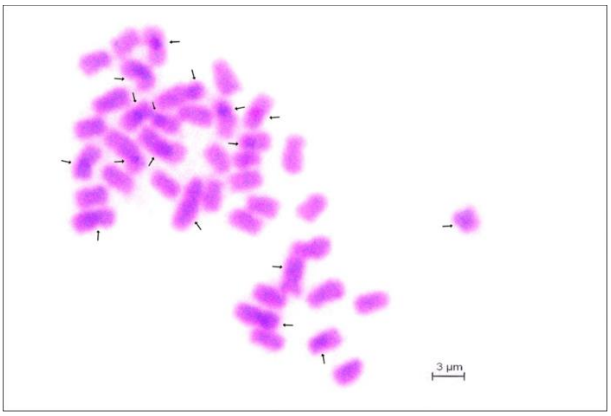

Figure 2. Metaphase chromosomes of *S. anatolicus* after C-banding. Arrows indicate C-bands. Scale bar = 3 µm

The NORs were located terminally on short arms of one sm chromosome pair (Figure 3). Besides, the size heteromorphism of NORs in the homologous chromosomes were identified by Ag-impregnation. NORs covered almost the

entire short arms of one pair of the homologous chromosomes, while the others were identified to be more restricted to the end of the short arm.

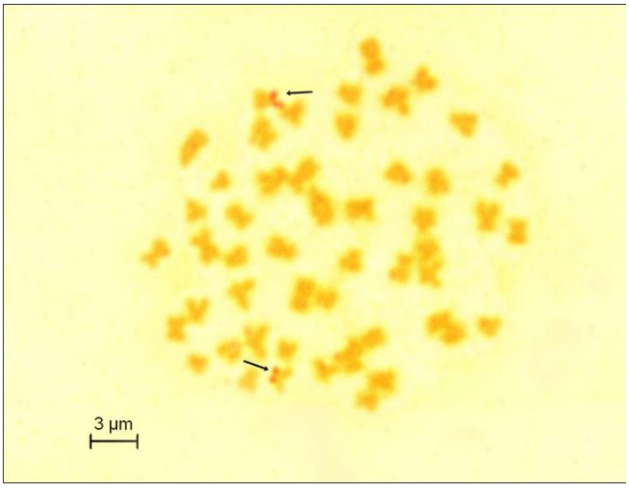

Figure 3. Metaphase chromosomes of *S. anatolicus* after Ag-impregnation. Arrows indicate NORs. Scale bar = 3 µm

DISCUSSION

Bogutskaya (1997) previously recognized that the *Squalius* population in Lake Beyşehir is a different species from *S. cephalus*, and it was identified as endemic species *S. anatolicus*.

The karyotype and chromosomal characteristics of *S. anatolicus* were analyzed in this study for the first time. The diploid chromosome number was *2n* = 50 and NF was 90. Within the individuals examined no karyotype variation was identified among the individuals and their sex. Acrocentric chromosomes were represented by 5 pairs of chromosomes, while the sm chromosomes were represented by 13 pairs, being thus the prevalent morphological chromosome type within the karyotype.

Rab and Collares-Pereira (1995) reported that the diploid chromosome numbers within leuciscins are usually *2n* = (48) 50 (52) and that their karyotype macrostructure is composed of 6 to 8 pairs of m, 12 to 14 pairs of sm, and 2 to 4 pairs of st/t chromosomes. Amemiya and Gold (1990), on the other hand, observed that while 90% of North American cyprinids had a karyotype morphology of *2n* = 50, this *2n* could vary from 48 to 52, and that the NF value could vary from 80 to 100. In terms of *2n* and karyotype macrostructure, results from the present study are in line with above mentioned reports.

Cytogenetic studies of this leuciscine lineage demonstrated that *Squalius carolitertii* (Syn: *Leuciscus carolitertii*) has *2n* = 50 and karyotype with 6 pairs of m, 15 pairs of st, and 4 pairs of a chromosomes, *S. pyrenaicus* (Syn: *Leuciscus pyrenaicus*) *2n* = 50 and karyotype with 6 pairs of m, 16 pairs of sm, and 3 pairs of a chromosomes (while the morphology of the chromosomes of *S. carolitertii* is stable, karyotype of *S. pyrenacius* is variable) (Collares-Pereira et al., 1998) (Table 2). Recent cytogenetic analysis of *S. carinus*, *S. fellowesii* (Karasu Ayata, 2020) and *S. seyhanensis* (Unal and Gaffaroğlu, 2016) have shown the same *2n* as in other *Squalius* and *Leuciscus* species and karyotypes consisted of 12 pairs of m, 10 pairs of sm and 3 pairs of st/a chromosomes (NF = 94); 10 pairs of m, 10 pairs of sm and 5 pairs of st/ chromosomes (NF = 90); 8 pairs of m, 14 pairs of sm and 3 pairs of st/a chromosomes (NF = 94) respectively. Boron et al. (2009) shown that *L. idus* has *2n* = 50 and NF = 86, and karyotype with 5 pairs of m, 13 pairs of sm, 3 pairs of st and 4 pairs of a chromosomes and *L. leuciscus* has *2n* = 50 and NF = 86, karyotype with 6 pairs of m, 12 pairs sm, 4 pairs of st and 3 pairs of a chromosomes. The same study (Boron et al., 2009) have also indicated that *L. leuciscus* (Syn: *Leuciscus leuciscus baicalensis kirgisorum*), *L. schmidti* and *L. bergi* have *2n* = 50 and NF = 90, and karyotype with 9 pairs of m, 11 pairs of sm and 5 pairs a chromosomes (Mazik et al., 1986). In all the previously analyzed species of *Leuciscus*, the number of m chromosomes, in *L. leuciscus* and *L. idus* the number of sm chromosomes higher and, the NF similar to the present study. In terms of NF, results presented in this study are in agreement with above mentioned studies. On the other hand, cytogenetic data of *S. cephalus* (Syn: *Leuciscus cephalus*) vary from one study to another. Al-Sabti (1986) revealed *2n* = 50 and NF = 84, karyotype with a karyotype composed of 17 pairs of m-sm and 8 pairs of st/a chromosomes, while Boron et al. (2009) identified *2n* = 50 and NF = 82, with a karyotype composed of 5 pairs of m, 11 pairs of sm, 5 pairs of st and 4 pairs of a chromosomes. Karyotype characteristics of *S. cephalus* in those studies are different from *S. anatolicus*. In addition, in the karyotypes of *S. alburnoides*, *S. lucumonis*, *S. aradensis* and *S. torgalensis* were determined chromosome structure as distinct from *S. anatolicus* (Table 2) (Gromicho and Collares-Pereira, 2004; Rossi et al., 2012; Nabais et al., 2013).

In *S. cephalus, L. idus*, and *L. leuciscus*, the heterochromatic blocks were identified in the pericentromeric regions of most chromosomes (Boron et al., 2009). Karasu Ayata (2020) and Unal and Gaffaroğlu (2016) found CH blocks in pericentromeric and distal part in chromosomes of *S. carinus*, *S. fellowesii* and *S. seyhanensis*. These studies are fairly similar to results obtained in *S. anatolicus*. While NORs are generally observed at the end of the short arms of st and sm chromosomes, they can sometimes be observed at the end of the long arms of st and sm chromosomes, on the arms of m and a chromosomes; between telomeres and centromeres, and adjacent to centromeres (Galetti et al., 1984). The present study supports the results of Galetti et al. (1984) in that NORs were localized on the short arms of sm chromosomes.

Table 2. Cytogenetic data of some *Squalius* species.

Species	2*n*	Chromosome morphology	NF	References
S. carolitertii	50	10-12m+30-32sm+8st/a	92	Collares-Pereira *et al.* (1998)
S. pyrenaicus	50	12m+32sm+6st/a	94	Collares-Pereira *et al.* (1998)
S. pyrenaicus	50	16m+28sm+6a	-	Gromicho and Collares-Pereira (2004)
S. alburnoides	50	16m+30sm+4st/a	96	Gromicho and Collares-Pereira (2004)
S. lucumonis	50	16m+26sm+8st/a	-	Rossi *et al.* (2012)
S. aradensis	50	10m+36sm+4st/a	96	Nabais *et al.* (2013)
S. torgalensis	50	10m+36sm+4st/a	96	Nabais *et al.* (2013)
S. seyhanensis	50	16m+28sm+6st/a	94	Unal and Gaffaroğlu (2016)
S. carinus	50	24m+20sm+6st/a	94	Karasu Ayata (2020)
S. fellowesii	50	20m+20sm+10st/a	90	Karasu Ayata (2020)
S. anatolicus	50	14m+26sm+10st/a	90	Present study

Karasu Ayata (2020) and Unal and Gaffaroğlu (2016) reported that two NORs were on the short arms of sm chromosomes in *S. carinus*, *S. fellowesii* and *S. seyhanensis* but NORs were reported that on the long arms of second largest sm chromosomes in *L. idus* (Boron et al., 2009). Rossi et al. (2012) observed two heteromorphic in size NORs on the short arms of a medium-sized sm chromosome pair in *S. lucumonis* and this occurrence was explained that by FISH using the 45S rDNA probe. According to this study, it was evident that the 5S signals were proximal and co-localized with the distal NORs and this character should be ancestral within the entire Leuciscinae lineage. The presence of NORs on the sm chromosome pair of *S. anatolicus* is in line with NOR phenotypes and size heteromorphism of NORs presented in these studies.

In conclusion, the karyotype having typically the largest st/a chromosome pair of *S. anatolicus* is characteristic of leuciscines in terms of the chromosome number and morphology, the distribution of CH and NOR phenotype. These findings support that consistency of karyotypes and chromosomal banding patterns in especially leuciscines. In addition, the presented cytotaxonomic characters should be scrutiny by molecular methods for further cytogenetic studies to understand evolutionary processes within the related leuciscid lineages.

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