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Karyotype, NORs, and C-banding analysis of *Pseudophoxinus firati* Bogutskaya, Küçük & Atalay, 2007 (Actinopterygii, Cyprinidae) in the Euphrates River, Turkey

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Abstract: *Pseudophoxinus firati* Bogutskaya, Küçük & Atalay, 2007 (Actinopterygii, Cyprinidae) living in the Euphrates River system were analyzed in terms of their karyotype, C-banding, and nucleolus organizer region properties. Metaphase chromosome spreads were obtained from kidney cells. The diploid chromosome number was found to be $2n = 50$, of which 19 pairs were meta-submetacentric and 6 pairs were subtelocentric, and the fundamental number was found to be 88. No sex chromosome differentiation was observed. C-banding revealed heterochromatin in the pericentromeric regions of 6 pairs of chromosomes. NORs were identified on the short arms of 2 pairs of medium-sized submeta-subtelocentric chromosomes. This study may contribute to other cytogenetic research.

Key words: Chromosome, heterochromatin, NORs, *Pseudophoxinus firati*, Leuciscinae

Fırat Nehri'ndeki *Pseudophoxinus firati* Bogutskaya, Küçük & Atalay, 2007 (Actinopterygii, Cyprinidae)'nin karyotip, NOR ve C-bantlama analizi

Özet: Fırat Nehir sisteminde yaşayan *Pseudophoxinus firati* Bogutskaya, Küçük & Atalay, 2007 (Actinopterygii, Cyprinidae)'nin karyotip, C-bantlama ve nükleolus organizatör bölge analizi yapıldı. Metafaz kromozomları böbrek hücrelerinden elde edildi. Diploid kromozom sayısı 19 çift meta-submetasentrik ve 6 çift subtelosentrik olmak üzere $2n = 50$ ve kol sayısı 88 bulundu. Eşey kromozomu farklılaşması gözlenmedi. C-bantlama ile altı çift kromozomun perisentromerlerinde heterokromatin bölge tespit edildi. Orta büyüklükte 2 çift submeta-subtelosentrik kromozomun kısa kollarında NOR gözlemlendi. Bu çalışmanın, yapılacak olan sitogenetik araştırmalara katkı sağlayacağı düşünülmektedir.

Anahtar sözcükler: Kromozom, heterokromatin, NOR, *Pseudophoxinus firati*, Leuciscinae

Introduction

It was reported that there were 54 species belonging to 17 genera in the subfamily Leuciscinae (Fam: Cyprinidae) in Anatolia, of which 26 species

and subspecies of this subfamily are endemic. It was previously noted that 10 species of the genus *Pseudophoxinus* Bleeker, 1860, which belongs to this subfamily, increased in distribution, while the number of species grew to 15 with the recently

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identified species (Bogutskaya, 1997; Freyhof and Özuluğ, 2006; Bogutskaya et al., 2007; Küçük, 2007). The genus *Pseudophoxinus* is found in a wide range of habitats in central Anatolia (Hrbek et al., 2004). *Pseudophoxinus firati*, which is the first record of the *Pseudophoxinus* species from the Euphrates drainage, is only known from Tohma Creek at Yazyurdu (Bogutskaya et al., 2007).

Methods developed since 1976 for harvesting, preparing, and banding fish chromosomes are now commonly used for taxonomic and phylogenetic studies, genetic control, and chromosome manipulations in fish breeding and the monitoring of aquatic pollutants by examining chromosomal aberrations (Ozouf-Costaz and Foresti, 1992). Moreover, cytogenetic studies have been conducted in Anatolian fishes (Gaffaroglu, 2003; Kılıç-Demirok and Ünlü, 2004).

The present study analyzes endemic *Pseudophoxinus firati* in terms of its karyotype, C-banding, and nucleolus organizer region (NOR) properties.

Materials and methods

Specimens of *Pseudophoxinus firati* (15 females and 5 males) originating from Tohma Creek, Turkey (38°47'N, 36°57'E), 2007-2008, were analyzed. They were transported live to the laboratory and kept in well-aerated aquaria until analysis. Mitotic chromosome slides were prepared according to the methods of Collares-Pereira (1992) from kidney cells. C-banding was performed by the method described by Sumner (1972). Ag-staining to detect NORs followed the method of Howell and Black (1980). Chromosomes were classified using the nomenclatures proposed by Levan et al. (1964). The preparations were observed and photographed digitally using a Leica DMLB research microscope.

Results

The diploid chromosome number of *Pseudophoxinus firati* was determined as $2n = 50$. The karyotype consisted of 19 pairs of meta-submetacentric and 6 pairs of subtelocentric

chromosomes with 88 arms (FN) (Figure 1). There was no sex chromosome differentiation. C-banding revealed heterochromatin in the pericentromeric regions of 6 pairs of chromosomes. Of these, 5 pairs were darkly stained, while 1 was only weakly stained (Figure 2). Ag-staining showed NORs on the short arms of 2 pairs of medium-sized submeta-subtelocentric chromosomes (Figure 3).

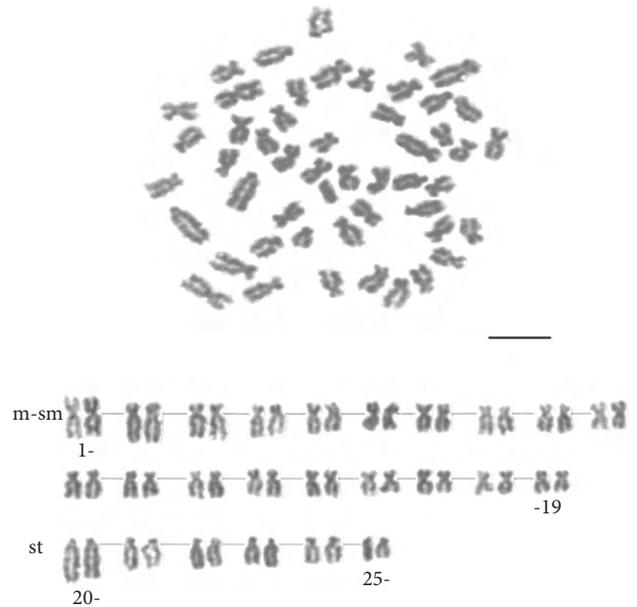


Figure 1. Metaphase and karyotype of *Pseudophoxinus firati*: m-sm: meta-submetacentric, st: subtelocentric chromosomes. Bar represents 5 μ m.

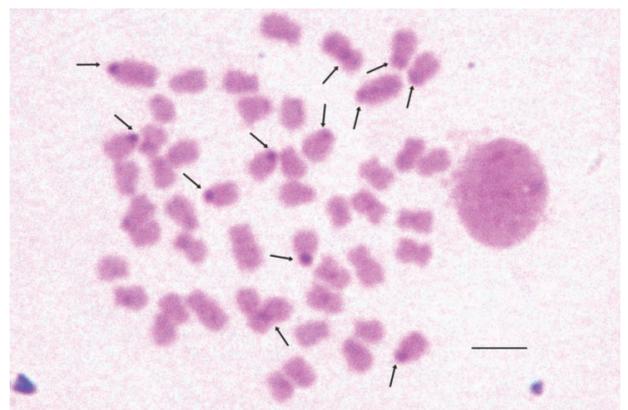


Figure 2. C-band metaphase of *Pseudophoxinus firati*; C-bands bearing chromosomes are indicated by arrows. Bar represents 5 μ m.

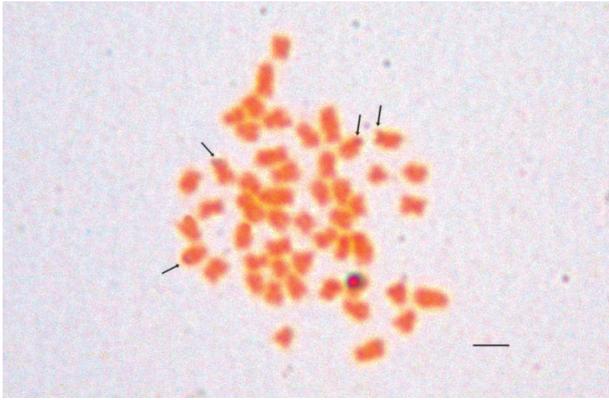


Figure 3. Ag-stained metaphase of *Pseudophoxinus firati*; NORs bearing chromosomes are indicated by arrows. Bar represents 5 μ m.

Discussion

The chromosomes of the family Cyprinidae have been well studied (Rab and Collares-Pereira, 1995). The clear dominant mode of $2n = 50$ chromosomes seems to reflect the plesiomorphic chromosome number for the family. The karyotype of cyprinids is usually characterized by a relatively high number of biarmed (meta- and submetacentrics) compared to uniarmed (subtelo- and acrocentrics) chromosomes (Sola and Gornung, 2001). The largest chromosome pair is characteristically a subtelo-/acrocentric element, which is a cytotaxonomic marker in leuciscine cyprinids (Rab and Collares-Pereira, 1995; Rab et al., 2000). On the whole, cyprinid sex chromosomes appear to have remained morphologically undifferentiated (Sola and Gornung, 2001). *Pseudophoxinus firati* also displays the cyprinid properties mentioned above. Moreover, Anatolian cyprinids *Acanthobrama marmid*, *Chalcalburnus mossulensis*, *Cyprinion macrostomus* (Gaffaroğlu, 2003), and *Alburnoides bipunctatus* (Kılıç-Demirok and Ünlü, 2004) were found to have $2n = 50$ chromosomes, like *P. firati*.

Positive C-bands identify regions of constitutive heterochromatin, which contains transcriptionally inactive highly repetitive DNA sequences (Gold et al., 1990). Most species invariably possess C-bands at or around the centromeres (pericentromeric) and, frequently, at the chromosome end (telomeric). C-bands may also be found along the chromosome arms (interstitial) and as entirely heterochromatic

short arms of acrocentric chromosomes (Gold et al., 1986). Fishes with such characteristic C-band patterns mostly belong to Cyprinidae and other lower teleostean groups. Therefore, C-band distribution in Cyprinidae is of great cytotaxonomic interest (Takai and Ojima, 1988). As is the case with *Pseudophoxinus firati*, distribution of heterochromatin in cyprinids is generally limited to centromeric regions and NOR sites, but some species with a different type of heterochromatin location were also found (Rab and Collares-Pereira, 1995; Boron, 2001; Kirtiklis et al., 2005). It was reported that C-bands were observed in the pericentromeric regions of several chromosome pairs in *Barbus cyclolepis*, and in all chromosome pairs in *Acanthobrama marmid* and *Cyprinion macrostomus*. Furthermore, 1 chromosome pair in *B. cyclolepis* and 2 chromosome pairs in *A. marmid* and *C. macrostomus* were reported to have C-bands on their short arms (Rab et al., 1996; Gaffaroğlu and Yüksel, 2009). However, no C-band was found on the short arm of chromosomes in *P. firati*.

Locations of NORs in the karyotype and NOR phenotypes have proven useful in cyprinid cytotaxonomy (Amemiya and Gold, 1988). *Pseudophoxinus firati* differs from European leuciscine fishes, in which NORs are located on one of the submeta-acrocentric chromosomes (Rab and Collares-Pereira, 1995), and from other species like *Aspius aspius*, in which NORs are located on the tip of the long arm of one subtelo-acrocentric chromosome pair (Rab et al., 1990), and the genus *Vimba*, in which NORs are located on the short arm of the small submetacentric chromosome (Rabova et al., 2003). Furthermore, most North American leuciscine fish bear a single NOR, while there are also species that possess multiple NORs located terminally on the chromosomal short arms, like *P. firati* (Amemiya and Gold, 1990). In terms of its NOR number, *P. firati* resembles leuciscins like *Phoxinus phoxinus*, *Eupallasella perenurus* (Boron, 2001), and *Iberochondrostoma almacai* (Monteiro et al., 2009). It may also be that *P. firati* has a multiple NOR phenotype derived from an ancestral leuciscine NOR phenotype by rearrangements of fission/translocation type, which shows one-to-one similarity to *Acanthobrama marmid* (Gaffaroğlu et al., 2006) in terms of the NOR number and phenotype that was reported in this species.

The obtained results improve the cytogenetic characteristics of *Pseudophoxinus firati*.

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