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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Karyological analysis of endemic *Pseudophoxinus anatolicus* (Hankó 1925) in Türkiye

Türkiye'ye endemik *Pseudophoxinus anatolicus* (Hankó 1925)'un karyolojik analizi

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Abstract: The karyological characteristics of nearly half of the *Pseudophoxinus* species in Türkiye were determined. In this study, it is planned to determine the karyological characteristics of *P. anatolicus*, which is common in Beyşehir Lake, specimens were caught from the coast at Çiftlik village. The captured specimen were karyological analysed and Giemsa staining, C-banding and Ag-NOR staining were applied to the slides that obtained. The chromosome set of this species consists of 12 pairs of metacentric, eight pairs of submetacentric, two pairs of subtelocentric and three pairs of acrocentric chromosomes. Dark and slightly C-bands were observed in the centromeric regions of some chromosomes. Active Ag-NORs were detected in the telomeric region of the short arm of two pairs of chromosomes. Our results are similar to those of other *Pseudophoxinus* species except for some differences and it was determined that Anatolian minnow has a conserved karyotype like other *Pseudophoxinus* species.

Keywords: Anatolian minnow, chromosome, karyotype, C-banding, Ag-NOR

Öz: Türkiye'deki *Pseudophoxinus* türlerin yarısının karyolojik özellikleri belirtilmiştir. Beyşehir gölünde yaygın olan *P. anatolicus*'un karyolojik özelliklerini belirlemek için planlanan bu çalışmada örnekler Çiftlik köyü kıyısından yakalandı. Yakalanan bireylerin karyolojik analizleri yapıldı ve elde edilen slaytlara sırayla giemsa boyama, C-bantlama ve Ag-NOR boyama uygulandı. Bu türün kromozom seti 2 çift metasentrik, sekiz çift submetasentrik, iki çift subtelosentrik ve üç çift akrosentrik kromozomdan oluşmaktadır. Koyu ve açık C-bantlar bazı kromozomların sentromerik bölgelerinde gözlemlendi. Aktif Ag-NOR'lar iki çift kromozomun kısa kolunun telomerik bölgesinde tespit edildi. Sonuçlarımızın diğer *Pseudophoxinus* türlerinkine bazı farklılıklar hariç benzer tarafları vardır ve Anadolu yağ balığının da diğer *Pseudophoxinus* türlerindeki gibi korunmuş karyotipe sahip olduğu belirlendi.

Anahtar kelimeler: Anadolu yağ balığı, kromozom, karyotip, C-bandlama, Ag-NOR

INTRODUCTION

There are 30 known species of *Pseudophoxinus*, a genus of the Leuciscidae family, and they are generally distributed in isolated spring pools and rivers in Central Anatolia and the Levant (Küçük et al., 2012). Türkiye, which is rich in freshwater fishes, has 24 fatfish species (Çiçek et al., 2020). They are mainly distributed in lake basins and rivers in Central and Southwestern Anatolia (Küçük et al., 2016). Although sufficient studies have been carried out on the systematics of fishes, karyological studies are still not advanced. The studies on *Pseudophoxinus* species in Türkiye are not sufficient. So far, the karyological characteristics of 13 species of this genus in Türkiye have been investigated by different researchers (Ergene et al., 2010; Karasu et al., 2011; Ünal et al., 2014; Karasu Ayata et al., 2016; Ünal and Gaffaroğlu, 2016; Gaffaroğlu et al., 2022). In addition to the standard karyological characteristics of *Pseudophoxinus* species found in different rivers and lakes of Anatolia, C-banding and NOR characteristics were also investigated by these researchers. So far, there is no karyological study on *P. anatolicus*. Therefore, the aim of this study was to determine the banded karyological characteristics of *P. anatolicus* and to establish the similarities and differences with other species.

MATERIALS AND METHODS

Four *P. anatolicus* specimens were collected from the shore of Çiftlik village in the east of Beyşehir Lake with the help of an electro-shocker. The captured fish specimens were transported to the laboratory alive under suitable conditions and kept in a well-aerated aquarium until analysis. After this adaptation period, each specimen was performed karyological analysis according to the method of Bertollo et al. (2015). 0.1% colchicine was injected (1ml/100g) into each specimen for which chromosome preparations were to be prepared and kept in the aquarium for 50 minutes. After anaesthetization, the cell suspension from head kidney with 0.075 M KCl was kept in hypotonic solution. Then, fixation steps (methanol: acetic acid, 3:1) were repeated at least three times and at least 10 metaphase slides were prepared from each individual. Some slides were stained with 10% Giemsa for standard karyotype and preserved. The other slides were subjected to C-banding (CBG-banding) (Sumner, 1972) and Ag-NOR staining (Howell and Black, 1980). Well-spread metaphases of each staining were photographed under a microscope. Metaphase chromosomes were identified and karyotyped according to Levan et al. (1964). The fundamental number of autosomal

arms (NF) was calculated considering the number of bidentate and acrocentric chromosomes.

RESULTS

The diploid chromosome number (2n) of four specimens (2 males, 2 females) was 50. According to karyotype analysis, the chromosome set consisted of 12 pairs of metacentric (no. 1-12), eight pairs of submetacentric (no. 13-20), two pairs of subtelocentric (no. 21-22) and three pairs of acrocentric (no. 23-25) chromosomes. The fundamental number of autosomal arms (NF) was 94 (Figure 1). Heteromorphic sex chromosomes were not detected in males and females. The karyotype of the specimens obtained by CBG banding is shown in Figure 2. Constitutive heterochromatin regions (C-bands) observed in centromeric regions of chromosomes were dark in some chromosomes and slightly in others. Active Ag-NORs were detected on two pairs of chromosomes. These were found in the telomeric region of the short arm of the largest metacentric chromosome (no. 1) and the medium-sized submetacentric (no. 16) chromosome in all specimens analyzed. One of these NORs on the largest metacentric chromosome is heterozygous and the other homozygous and neither is associated with the heterochromatin region (Figure. 3).

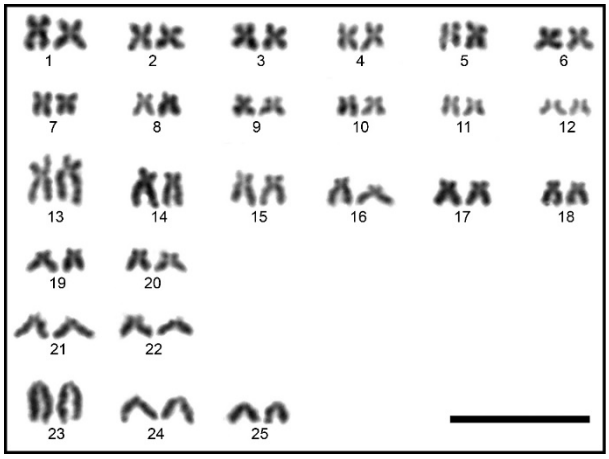

Figure 1. Standard Giemsa staining karyotype of *Pseudophoxinus anatolicus* (Scale bar = 10 µm)

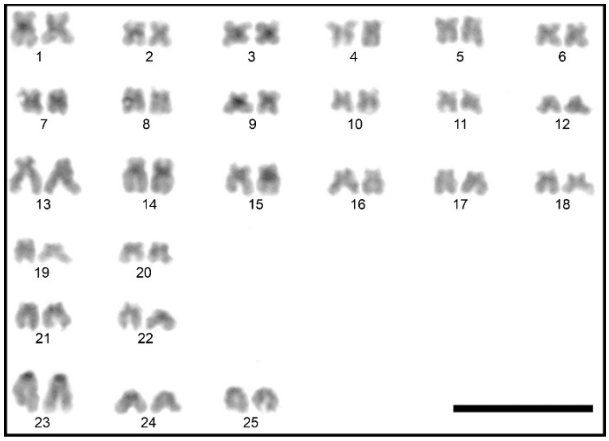

Figure 2. C-banded (CBG) karyotype of *Pseudophoxinus anatolicus* (Scale bar = 10 µm)

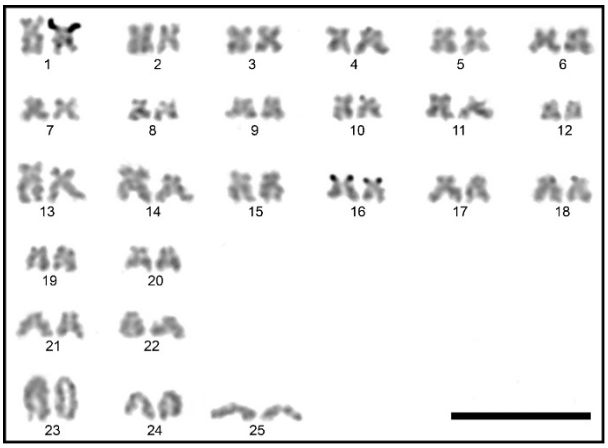

Figure 3. Silver-stained karyotype of *Pseudophoxinus anatolicus* (Scale bar = 10 µm)

DISCUSSION

The karyotype features of *P. anatolicus* were revealed for the first time in this study. The karyotype formula of this species shows a numerical dominance of meta/submetacentric chromosomes as in the basic karyotype model of leuciscines. When the total number of meta/submetacentric chromosomes of *P. anatolicus* is taken into account, it was seen that this species is similar to the majority of some other *Pseudophoxinus* species studied in Türkiye, but different from some others (Table 1). This difference may be as a result of the way the researchers determined the chromosome morphology (numerical variation of meta/submetacentric chromosomes). The fundamental number of autosomal arms (NF) value is not finalised because St and A chromosomes were not evaluated separately. Therefore, the NF value of species may be varied. When these are taken into consideration, the preserved karyotypic evolution in *Pseudophoxinus* species supports the hypothesis. In fact, in a study investigating the phylogenetic and zoogeographic characteristics of *Pseudophoxinus*, which supports this hypothesis, it was found that *Pseudophoxinus* was divided into two clades consisting of species of Anatolian (central and western) and Eastern Mediterranean (Levant) origin (Perea et al., 2010), and Küçük et al. (2012) showed that *Pseudophoxinus* has two main speciation zones, Anatolian and Eastern Mediterranean. Thus, both *P. anatolicus* and other *Pseudophoxinus* species in Anatolia have similar standard karyological characteristics and phylogenetic studies support the hypothesis of conserved karyotypic conservation in the Anatolian line.

In this study, dark and slightly constitutive heterochromatin C-bands were detected in centromeric regions some of chromosomes in *P. anatolicus*. Our C-band results are similar to those of *P. antalyae*, *P. battalgilae*, *P. burduricus* and *P. evliyae* (Ergene et al., 2010; Karasu Ayata et al., 2016). However, our results are partially similar to those of *P. egridiri*, *P. fahrettini*, *P. maeandri* (Karasu Ayata et al, 2016) and *P. zekayi* (Ünal and Gaffaroğlu, 2016), while it is different from the

results of *P. firati*, *P. crassus*, *P. hittitorum*, *P. zekayi*, *P. alii* and *P. elizavetae* which have only pericentromeric C-bands (Karasu et al., 2011; Ünal and Gaffaroğlu, 2016; Gaffaroğlu et al., 2022). The interstitial C-band detected in some cyprinids (Arslan and Gündoğdu, 2016) was not detected in both species and in other *Pseudophoxinus* species.

Table 1. Chromosomal records of Anatolian *Pseudophoxinus* species

Species	Locality	2n	Karyotype	Reference
P. antalyae	Mersin	50	16M + 14Sm + 12St + 8A	Ergene et al. (2010)
P. firati	Malatya	50	38M/Sm + 12St	Karasu et al. (2011)
P. crassus	Konya	50	12M + 30Sm + 8St/A	Ünal et al. (2014)
P. hittitorum	Konya	50	14M + 26Sm + 10St/A	Ünal et al. (2014)
P. battalgilae	Konya	50	6M + 28Sm + 6St/A	Karasu Ayata et al. (2016)
P. burduricus	Burdur	50	18M + 26Sm + 6St/A	Karasu Ayata et al. (2016)
P. egridiri	Isparta	50	14M + 28Sm + 8St/A	Karasu Ayata et al. (2016)
P. evliyae	Antalya	50	14M + 30 Sm + 6St/A	Karasu Ayata et al. (2016)
P. fahrettini	Isparta	50	6M + 26Sm + 8St/A	Karasu Ayata et al. (2016)
P. maeandri	Denizli	50	10M + 32Sm + 8St/A	Karasu Ayata et al. (2016)
P. zekayi	Adana	50	16M + 26Sm + 8St/A	Ünal and Gaffaroğlu (2016)
P. alii	Antalya	50	18M + 24Sm + 8St/A	Gaffaroğlu et al. (2022)
P. elizavetae	Kayseri	50	8M + 34Sm + 8St/A	Gaffaroğlu et al. (2022)
P. anatolicus	Konya	50	24M + 16Sm + 4St + 6A	This study

All individuals of *P. anatolicus* analysed here carried Ag-NOR on metacentric chromosome 1. In addition, except for some metaphases, the submetacentric chromosome 16 was also found to have Ag-NOR. There are similarities and differences between our Ag-NOR results and previously studied *Pseudophoxinus* species, both numerically and in terms of the morphology of the chromosome in which the NOR is localised. Numerically, *P. firati*, *P. zekayi*, *P. evliyae*, *P. fahrettini*, *P. maeandri* and *P. alii* and *P. elizavetae* have active NOR on two pairs of chromosomes (M+Sm, Sm+Sm or Sm+St), while the other species have active NOR on one pair of chromosomes (Sm) (Ergene et al., 2010; Karasu et al., 2011; Ünal et al., 2014; Karasu Ayata et al., 2016; Ünal and Gaffaroğlu, 2016). Gaffaroğlu et al. (2022) also argued that they detected a higher number of Ag-NORs in some metaphases of *P. alii* and *P. elizavetae.* When evaluated in terms of active NOR-bearing chromosome morphology, *P. anatolicus* is close to *P. zekayi*. The variation in active NORs detected by silver staining in *Pseudophoxinus* species in Türkiye needs to be confirmed using molecular cytogenetic techniques. Active NORs detected by silver staining contain 18s rDNA (Diniz et al., 2009). Recently, the presence of active NORs detected by silver staining has been confirmed using 18s rDNA probes. Even inactive NORs are detected with 5s rDNA probes and the results are used to assess the relatedness between species (Bueno et al., 2014).

CONCLUSION

As a result, it was observed that the standard karyological features of this species were similar to those of some of the other *Pseudophoxinus* species studied in Türkiye, but the variations in both standard and C-banding results revealing these differences varied according to the researcher. Therefore, we believe that molecular cytogenetic methods, which are the major deficiency in the researches in Türkiye, can be used to reach more permanent results in the differentiation of species or determination of kinship relationships.

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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 per 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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