

Karyological Analysis of Some Species of *Aphanius* (Osteichthyes: Cyprinodontidae) From Anatolia

Muhammet Gaffaroğlu¹, Muradiye Karasu Ayata^{1*}, Sevgi Ünal² and Mustafa Özkan¹

¹Department of Biology, Faculty of Arts and Sciences, Ahi Evran University, 40100 Kırşehir, Turkey

²Department of Biology, Faculty of Science, Gazi University, 06100 Ankara, Turkey

Abstract.- In this study, chromosomal studies were carried out on four species of the genus *Aphanius* (Osteichthyes, Cyprinodontidae). Metaphase chromosomes were obtained from kidney cells. The diploid chromosome numbers of *Aphanius anatoliae*, *A. danfordii*, *A. splendens* and *A. villwocki* were $2n=48$, and consisted of three pairs of submetacentric and 21 pairs of subtelocentric chromosomes. The arm number (NF) was 54. Constitutive heterochromatin regions were observed on the centromeres of chromosomes in all four species by using C-banding. Nucleolus organizer regions (NOR) were observed on the short arms of two pairs of chromosomes. It is believed that the data obtained will make a contribution to fish cytogenetics.

Key words: *Aphanius*, chromosome, C-banding, NOR

INTRODUCTION

The family Cyprinodontidae is distributed throughout the United States, Africa, Southern Europe and Asia. It is represented by a genus in our country (Geldiay and Balık, 2007). The genus *Aphanius* Nardo, 1827 has 10 species in Anatolia. These species are *Aphanius anatoliae*, *A. asquamatus*, *A. burdurensis*, *A. danfordii*, *A. fasciatus*, *A. mento*, *A. splendens*, *A. sureyanus*, *A. transgrediens* and *A. villwocki*. All of these species are endemic to our country except for *A. fasciatus* and *A. mento* (Fricke *et al.*, 2007).

Fish chromosome studies have been carried out for many years. Chromosome properties of 35 species from a total of 104 species (Fam: Cyprinodontidae) have been reported to date. The diploid chromosome numbers of these species were found to be 48, 50 and 52 (Arai, 2011).

The species of *Aphanius* living in Anatolia has been studied for molecular phylogeny and historical biogeography (Hrbek *et al.*, 2002), genetic relationships (Bardakçı *et al.*, 2004) and population structure (Güçlü *et al.*, 2007). Also molecular analysis of the genetic differentiation among *Aphanius fasciatus* populations has been studied in Tunis (Annabi *et al.*, 2012).

There has been no a detailed study on the cytogenetic features of these species although only haploid chromosome number has been reported in *A. anatoliae* (Öztan, 1954; Wildekamp, 1993).

The purpose of this study is to reveal the chromosomal features (with Giemsa, Ag-NOR staining and C-banding) of four species (*A. anatoliae*, *A. danfordii*, *A. splendens* and *A. villwocki*) from Anatolia.

MATERIALS AND METHODS

Eighteen (10 female, eight male) samples of *Aphanius danfordii* were collected from Sultansazlığı, Develi, Kayseri (38° 22' N, 35° 21' E), 17 (11 female, six male) samples of *A. anatoliae* were collected from Eğirdir Lake, Isparta (37° 51' N, 30° 50' E), 10 (four female, six male) samples of *A. splendens* were collected from Salda Lake, Burdur (37° 31' N, 29° 43' E) and 15 (10 female, five male) samples of *A. villwocki* were collected from Eminekin, Eskişehir (39° 22' N, 31° 06' E). These samples were transported live to the laboratory. Metaphase preparations were prepared according to Collares-Pereira (1992) from anterior kidney cells. The technique of Sumner (1972) was used for C-banding of sample preparations whereas the technique of Howell and Black (1980) was used for silver staining. The metaphase preparations were observed and photographed using a Leica DM3000 microscope. Chromosomes were classified according to Levan *et al.* (1964).

* Corresponding author: muradiye.karasu@gmail.com
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RESULTS

The diploid chromosome numbers of *Aphanius anatoliae*, *A. danfordii*, *A. splendens* and *A. villwocki* were determined to be $2n=48$, and

chromosome morphologies were three pairs of submetacentric (SM) and 21 pairs of subtelocentric (ST) chromosomes (Fig. 1). NF was found to be 54. The differentiations of sex chromosomes were not observed.

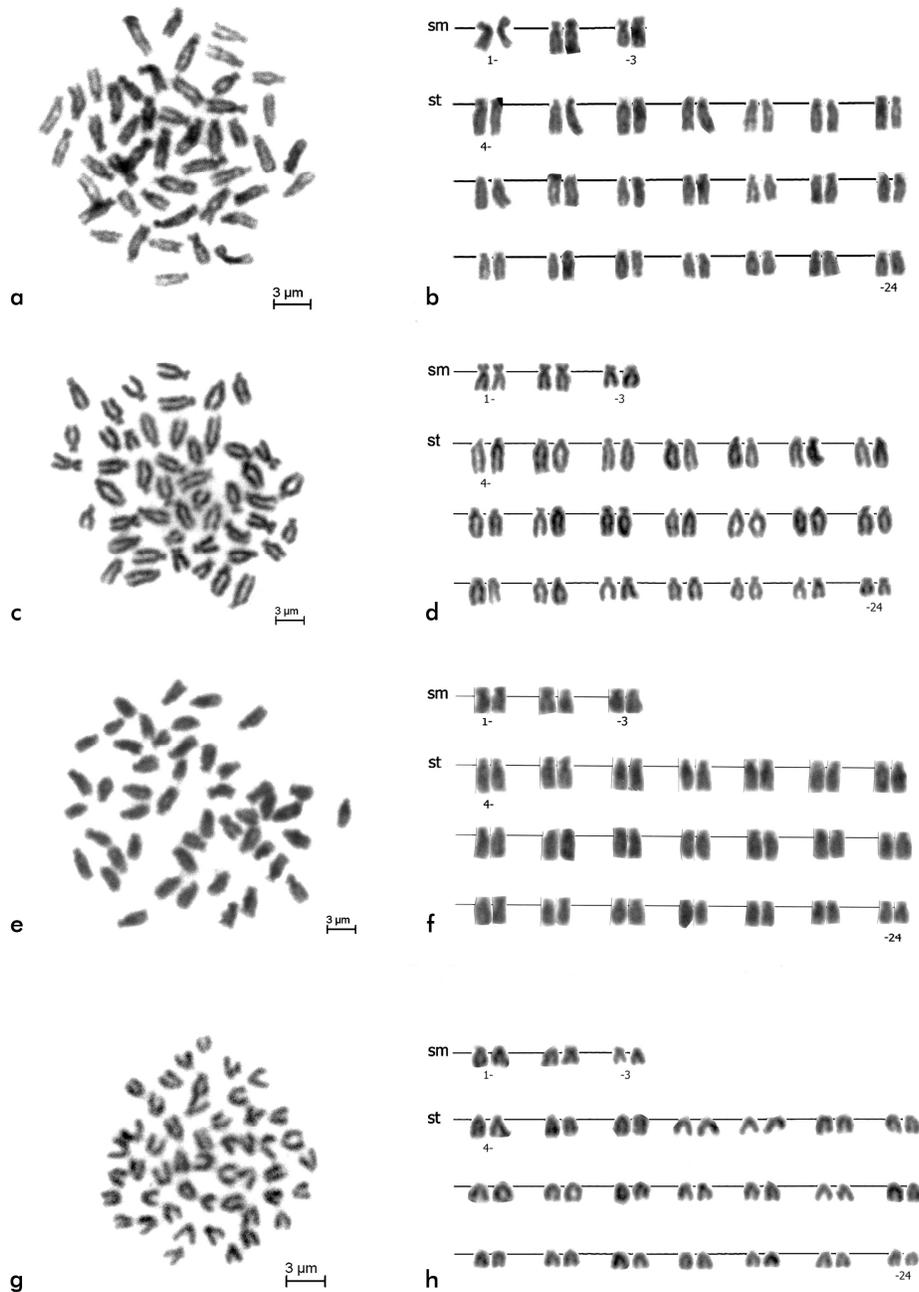


Fig. 1. Giemsa stained metaphase and karyotype of *Aphanius anatoliae* (a-b), *A. danfordii* (c-d), *A. splendens* (e-f) and *A. villwocki* (g-h).

NOR was observed on the telomeric regions of the short arms of two large pairs of subtelocentric chromosomes in each species (Fig. 2a).

By using C-banding, constitutive heterochromatin regions were observed on the centromeres of several chromosomes in all species. Additionally, long arms of some chromosomes had telomeric heterochromatin bands (Fig. 2b).

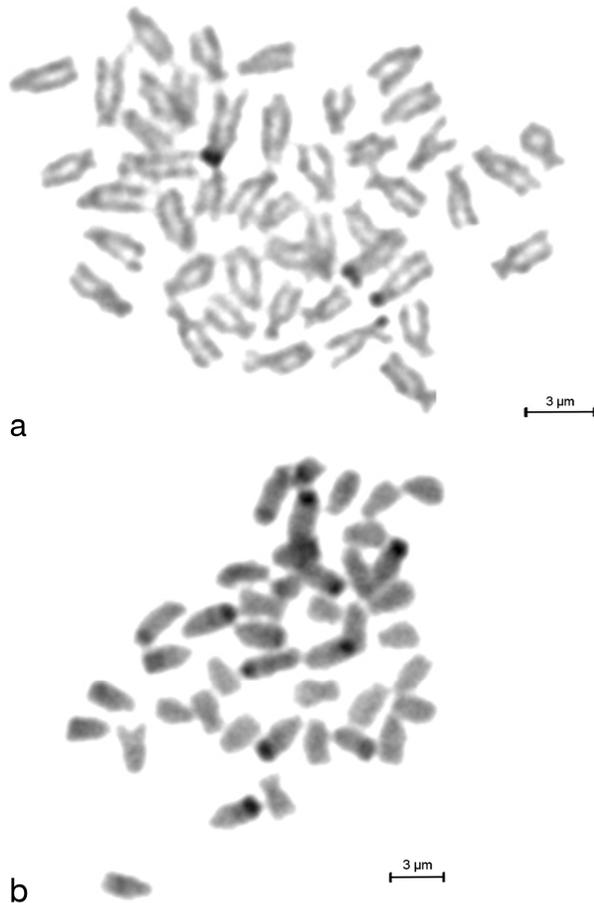


Fig. 2. (a) NORs on the metaphase plate of *Aphanius anatoliae* (b) C-banded metaphase plate of *A. danfordii*

DISCUSSION

According to Öztan (1954), the haploid chromosome number of *Aphanius anatoliae* was $n=24$. This study shares the results that we obtained from *A. anatoliae*. Except for this study, no cytogenetic research has been reported on *A.*

anatoliae, *A. danfordii*, *A. splendens* and *A. villwocki*.

A. anatoliae, *A. danfordii*, *A. splendens* and *A. villwocki* have the same diploid chromosome number as other species of *Aphanius* that were previously studied. However, their chromosome morphologies are different from these species (Table I).

Table I.- Karyotype studies in the genus *Aphanius*.

Species	Diploid chromosome number (2n)	Chromosome morphology	NF	References
<i>A. fasciatus</i>	48	48 ST-A	48	Vitturi <i>et al.</i> (1995)
<i>A. persicus</i>	48	22 SM+6 ST	70	Esmaeili <i>et al.</i> (2007)
<i>A. sophiae</i>	48	28 SM+20 ST	76	Esmaeili <i>et al.</i> (2007)
<i>A. ginaonis</i>	48	14 SM+34 ST	31	Esmaeili <i>et al.</i> (2008a)
<i>A. dispar</i>	48	16 SM+32 ST	32	Esmaeili <i>et al.</i> (2008a)
<i>A. isfahensis</i>	48	12 SM+39 ST	30	Esmaeili <i>et al.</i> (2008b)
<i>A. vladykovi</i>	48	8 SM+40 ST	28	Esmaeili <i>et al.</i> (2009)
<i>A. anatoliae</i>	48	6 SM+42 ST	54	In this study
<i>A. danfordii</i>	48	6 SM+42 ST	54	In this study
<i>A. splendens</i>	48	6 SM+42 ST	54	In this study
<i>A. villwocki</i>	48	6 SM+42 ST	54	In this study

The karyotype of the *Aphanius* species that has been studied in this research is very similar to *A. vladykovi*, which has eight SM and 40 ST chromosomes (Esmaeili *et al.*, 2009).

It has been reported that diploid chromosome numbers of *A. asquamatus* and *A. mento* collected from inland waters of Turkey was $2n=48$. However, there was no information about the chromosome morphologies of these species (Arai, 2011). The similarity of the chromosome number between *Aphanius* species shows that the chromosome number is conservative in this genus. A similar situation has also been reported by other authors (Esmaeili *et al.*, 2009).

It has been shown that some species from the genus *Cyprinodon*, *Garmanella*, *Jordanella*, *Megupsilon* and *Orestias* (Fam: Cyprinodontidae) have $2n=48$ (Arai, 2011). On the other hand, some species of the genus *Fundulus*, *Gambusia* and *Poecilia* (Order: Cyprinodontiformes) also have $2n=48$ (Arai, 2011). The findings from *A. anatoliae*, *A. danfordii*, *A. splendens* and *A. villwocki* are similar to these studies.

According to our observations there was no sex chromosome differentiation in *A. anatoliae*, *A. danfordii*, *A. splendens* and *A. villwocki*. In this regard, these species are similar to *A. persicus*, *A. sophiae*, *A. ginaonis*, *A. dispar*, *A. isfahanensis* and *A. vladkovi* in which sex chromosomes were unreported (Esmaeili *et al.*, 2007, 2008a,b, 2009).

C-band patterns of fish chromosomes have been studied for many years. Constitutive heterochromatin regions are found at or around centromeres and telomeres using the C-banding method. These regions may also be found within chromosomal arms and sometimes the short arms of acrocentric chromosomes may be entirely heterochromatic (Gaffaroğlu and Yüksel, 2009).

There is no difference in the patterns of heterochromatin regions among the species in this study. The findings that C-bands localize in the centromere and telomere regions of the chromosomes in *A. fasciatus* (Vitturi *et al.*, 1995), *A. anatoliae*, *A. danfordii*, *A. splendens* and *A. villwocki* are similar to this study in terms of including centromeric and telomeric C-bands. However, inter-individual polymorphism of telomeric C-bands that have been reported in *A. fasciatus* (Vitturi *et al.*, 1995) were not observed in this study. Moreover, *A. anatoliae*, *A. danfordii*, *A. splendens* and *A. villwocki* are similar to *Gambusia holbrooki* which has centromeric C-bands (Russo *et al.*, 1999). Similar results have also been obtained by other researchers in cyprinid taxa (Gaffaroğlu and Yüksel, 2009).

NOR numbers and locations have proven useful in fish cytotaxonomy (Amemiya and Gold, 1988). In addition to the diploid chromosome numbers, these properties have also been examined in many fish species (Arai, 2011). In this study, no significant differences in the number and location of the NOR have been observed between the species.

It was concluded that the NOR number of *A. fasciatus* varied between one and eight (Vitturi *et al.*, 1995). While polymorphism of NOR number and localization has been reported in *A. fasciatus*, no polymorphism was observed in the *Aphanius* species in this study. They are similar to *G. holbrooki* regarding the existence of NOR in the short arms of two pairs of chromosomes (Russo *et al.*, 1999).

Chromosome banding studies (especially Ag-NOR and C-banding) have not been found on other *Aphanius* species (except *A. fasciatus*) to date. For this reason, a comparison in this regard cannot be made in detail within the genus.

This study may contribute to cytogenetics of the *Aphanius* species.

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