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CHROMOSOME BANDING PROPERTIES OF *Neogobius fluviatilis* (PALLAS, 1814) (PERCIFORMES, GOBIIDAE)

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Abstract: The monkey goby, *Neogobius fluviatilis* (Pallas, 1814) that distributed in Türkiye was studied cytogenetically for the first time. In this context, diploid chromosome number, chromosome morphology and also chromosomal banding properties (C-banding and Ag-NOR staining) of *N. fluviatilis* were revealed out. Chromosome slides were prepared from head kidney cells according to the air-drying technique. Chromosome slides were observed under the microscope and metaphases were photographed. The chromosomes were measured by digital caliper and karyotype was arranged manually. The diploid chromosome number was found as 46. Karyotype was composed with all uniarmed chromosomes. Fundamental arm number was calculated as 46 too. No heteromorphic sex chromosomes were determined in the karyotype. C-bands were detected on the pericentromeres of almost all chromosomes. Otherwise, two Ag-NORs were found in the silver-stained metaphases. This study revealed out chromosomal properties of *N. fluviatilis* from Türkiye with conventional cytogenetic techniques. This report may improve the cytogenetic data of the genus *Neogobius*.

Keywords: Karyotype, C-banding, Nucleolus Organizer Region, Monkey Goby

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1. Introduction

Transboundary waters play an important role in the spreading of fishes to different countries. Many fish species entered into the inland waters of Türkiye from European or other countries. One of the threatened factors in the inland waters of Türkiye is the presence of non-native or invasive fish species. These species should be accelerating the loss of native species (Özuluğ et al., 2023). The family Gobiidae Cuvier, 1816 is one of the largest family that comprises about 200 genera and 2000 species (Karakuş et al., 2018). Gobiids are mostly marine fish but also found in shallow coastal waters (Esmaily and Kalbassi, 2008). The subfamily Gobiinae Cuvier, 1816 belonging to the family Gobiidae contains 18 species that are distributed in the inland waters of Türkiye (Çiçek et al., 2020). From these species, only two *Neogobius* species were listed, *N. fluviatilis* and *N. melanostomus* (Çiçek et al., 2020). An invasive fish is the monkey goby, *N. fluviatilis* distributes in the benthic areas that inhabits nearshore marine to brackish and freshwaters. This species tends high biological plasticity depending on the environment (Karakuş et al., 2018).

Chromosomal studies are important in cytotaxonomy of the gobiids (Ene, 2003). However, fish species have large numbers of relatively small chromosomes that makes cytogenetic studies difficult (Ergene-Gözükara and Çavaş, 2002). Chromosomal reports about non-native fish species from the order Perciformes that are distributed in Türkiye have been reported in limited studies like *Gobius paganellus* (Ergene-Gözükara and Çavaş, 2002) and *Sander lucioperca* (Unal-Karakus et al., 2023). There is no data about the karyological properties of *N. fluviatilis* from Türkiye. In this study it is aimed to reveal out chromosomal properties of *N. fluviatilis* with conventional cytogenetic techniques.

2. Material and Methods

Four individuals (three females and one male) of *N. fluviatilis* were collected from Sakarya Creek, Tozman Village, Bilecik prov., Türkiye (40° 02' N; 30° 28' E). The individuals were transported alive to the laboratory and kept in a well-aerated aquarium until analysis. For karyological studies, air drying technique of Bertollo et al. (2015) was followed. The process was approved by the Local Animal Ethics Committee of Türkiye (Protocol Number: 68429034/09). Each individual was injected intra-peritoneally with a colchicine solution (0.1%; 1 ml/ 100 g body weight). The individuals were kept in a well-aerated aquarium and after two hours head kidney tissue was extracted and placed in a hypotonic solution of 0.56% KCl. After this step, the cellular suspension was centrifuged at 1200 rpm for 10 minutes. The hypotonic solution was discarded, and the pellet was suspended and washed 3 times in methanol: glacial acetic acid (3: 1). After

centrifugation at 1200 rpm for 10 minutes, the drops of cellular suspension were put on a clean slide. The slides were allowed to air dry. Some of them were stained with 10% Giemsa for 20 minutes. At least 10 slide was prepared from each specimen. Otherwise, C-banding was followed by using the method given by Sumner (1972) with slight modifications. For silver staining of the chromosomes, the method given by Howell and Black (1980) was followed. The all prepared slides were observed under a Leica DM3000 microscope. Mitotic metaphase plaques (with Giemsa stained, C-banded and silver stained) were photographed by using AKAS software. Karyotype was prepared by arranging chromosomes in pairs by size. Chromosomes were measured by digital caliper and arranged according to the criteria given by Levan et al. (1964).

3. Results

Karyotype of *N. fluviatilis* with 2n = 46 (Fig. 1A) consisted subtelo-acrocentric chromosomes that gradually decreased in size (Fig. 1A). Fundamental arm number (FN) was 46. No sex chromosomes were observed. C-bands were localized on the pericentromeres of almost all chromosomes (Fig. 1B). Two Ag-NORs were determined on the terminal regions of No. 8 chromosome only pair (Fig. 1C).

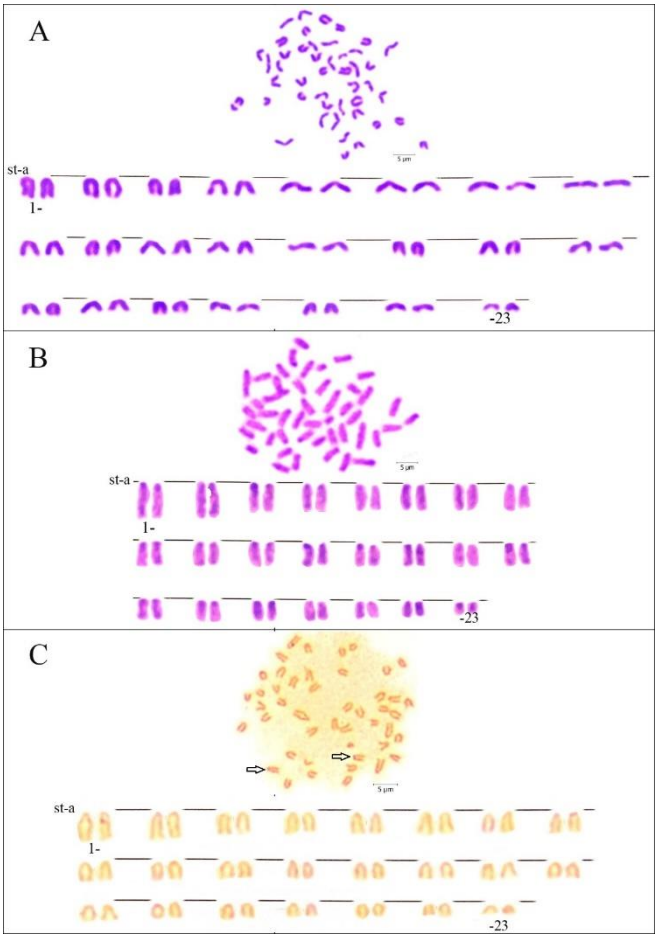

Figure 1. Metaphases and karyotypes of *Neogobius fluviatilis* A. Giemsa-stained metaphase and the arranged karyotype of Giemsa-stained metaphase; B. C-banded metaphase and the arranged karyotype of C-banded metaphase; C. Silver-stained metaphase (arrows indicate the Ag-NORs) and the arranged karyotype of silver-stained metaphase. Scale bar = 5 µm. st-a: subtelo-acrocentric.

4. Discussion and Conclusion

The gobiids are well studied karyologically, with dominant 2n = 44, 46 and 48. Other chromosome numbers appear to be exceptional (Ene, 2003). Karyotype with 46 uniarmed chromosomes is considered to be basis for many gobiids including the genus *Neogobius* (Bigaliev et al., 2017). The ancestral 2n of Teleost fishes is 48 (Thode and Alvares, 1983). Otherwise, the ancestral gobiid karyotype consists of 46 mono-armed chromosomes (Ene, 2003). Thode and Alvares (1983) hypothesized that decreasing in the 2n, deletion should be occurred in the karyotype evolutions of gobiids. Also, it should be occurred after tandem fusion or Robertsonian translocation followed by pericentric inversions (Ocalewicz and Sapota, 2011). This reduction in the 2n should be taken place in the phylogeny of the group (Thode and Alvares, 1983).

In the order Perciformes, species are remarkably conservative in their karyotypes (Thode and Alvares 1983). According to the results of this study *N. fluviatilis*

with 2n = 46 is represented in the diploid set mainly by uniarmed chromosomes like *Neogobius caspius*, *N. pallasi*, *N. melanostomus* (Bigaliev et al., 2017). *N. fluviatilis* from the Russian watersheeds (Arai, 2011) shows similarity to this study in having 46 acrocentric chromosomes. With uniarmed 46 chromosomes, *N. fluviatilis* shows an ancestral karyotype pattern as three different species of the genus *Neogobius* (Arai, 2011), *N. kessleri* (Esmaily and Kalbassi, 2008), *N. caspius*, *N. pallasi* and *N. melanostomus* (Bigaliev et al., 2017). Otherwise, *N. fluviatilis* is different from *N. eurycephalus* (Ene, 2003) that has variable 2n = 30 - 32.

Heteromorphic sex chromosomes were not reported on the other members of *Neogobius* (Ene, 2003; Ocalewicz and Sapota, 2011) or *Gobius* species (Ergene-Gözükara and Çavaş, 2002) like *N. fluviatilis.*

The location and number of NORs have descriptive characters in fish cytotaxonomy (Ene, 2003). Single chromosomal distribution of Ag-NORs also were described in *N. melanostomus* (Ocalewicz and Sapota, 2011) as this study. *N. fluviatilis* is similar to *N. eurycephalus* (Ene, 2003) in terms of Ag-NOR number. However, there are differences about the location of Ag-NORs that were reported in metacentric chromosomes in *N. eurycephalus* (Ene, 2003). Ag-NOR number and location of *Gobius niger* (Mandrioli et al., 2001) and *G. paganellus* (Caputo, 1998) are similar to *N. fluviatilis*. Ag-NOR size polymorphism that was reported in *N. eurycephalus* (Ene, 2003) is not observed in the silver-stained metaphases of *N. fluviatilis*.

Constitutive heterochromatin regions are observed by the application of a C-banding procedure (Sumner, 1972). These regions are an important tool in terms of chromosomal identification of the species. The differences in C-band localization could be used as a cytogenetic marker and reveal the chromosomal evolution between the species (Arslan and Arslan, 2007). C-band pattern of *N. fluviatilis* shows similarity to *N. eurycephalus* (Ene, 2003). Caputo (1998) reported that C-bands were localized at almost all the centromeric and telomeric chromosomal regions in *G. paganellus* and *G. niger*. In this context, *N. fluviatilis* is similar to above mentioned two species in terms of C-band pattern.

The cytogenetic data especially about chromosomal bandings for *Neogobius* species is limited to discuss their relationships in details. Also, 2n and chromosome morphology in the karyotype of *N. fluviatilis* from Russian (Arai, 2011) have been reported however no chromosomal banding features are available.

In conclusion, *N. fluviatilis* from Türkiye is not studied in terms of chromosome banding patterns. We conducted cytogenetic analysis with chromosomal bandings for the first time in Türkiye's population. Chromosomal studies of gobiids, contributed to their knowledge.

Author Contributions

Percentages of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	S.U.K.	M.K.A.	M.G.
C	40	30	30
D		100	
S			100
DCP	50		50
DAI	100		
L	30	40	30
W	30	40	30
CR	40	30	30
SR	40	30	30
PM	30	30	40
FA	40	30	30

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The experimental procedures were approved by the Local Animal Care and Ethics Committee of Kirsehir Ahi Evran University (approval date: March 30, 2023 and protocol code: 68429034/09).

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