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Chromosomal banding properties of *Oxynoemacheilus simavicus* (Balık & Bănărescu, 1978) (Teleostei: Nemacheilidae) from Gediz River (Turkey)

Sevgi Ünal Karakuş

Department of Molecular Biology and Genetics, Faculty of Science, Bartin University, 74100, Bartin, Turkey
Corresponding author: sunal@bartin.edu.tr

Abstract
The chromosomal banding properties of *Oxynoemacheilus simavicus* (Balık & Bănărescu, 1978) were revealed out by C-banding and silver staining. Constitutive heterochromatin regions were determined on the pericentromeres of most chromosomes by C-banding. Otherwise, nucleolus organizer regions (NORs) were observed on the short (p) arms of one middle-sized submetacentric (sm) chromosome pair by silver staining. Also, Ag-NOR size and number polymorphisms were observed. The obtained results shall contribute to the genus *Oxynoemacheilus* chromosomal studies.

Keywords: Chromosome, constitutive heterochromatin region, nucleolus organizer region

Introduction

Chromosomal studies in fish are often limited to the determination of diploid chromosome number (2n), fundamental arm number (FN) and chromosome morphology. C-banding and silver staining are also widely used methods in fish cytogenetics (Rábová et al. 2015). C-band patterns and Ag-NOR phenotypes are useful chromosome markers in karyological studies of fish. The C-banding technique stains the constitutive heterochromatin regions. These regions include highly and moderately repetitive DNA. These bands usually are pericentromeric, telomeric and sometimes intercalary bands are observed (Salvadori et al. 2015). Silver staining, based on the silver impregnation of nucleolar rRNA-protein complexes produces a reversible pattern. This method is identifies transcriptionally active rDNA genes. The number and location of Ag-NORs have been used as a cytotaxonomic character in fish cytotaxonomy (Rábová et al. 2015).

The chromosomal banding properties have been reported in only five out of 41 Anatolian *Oxynoemacheilus* species. These species are *O. argyrogramma*, *O. frenatus* and *O. sp.* (Değer 2011), *O. angorae* (Gaffaroğlu et al. 2014) and *O. atili* (Ayata et al. 2018).

There is no chromosomal banding properties of *O. simavicus*. The aim of this study is to determine chromosomal banding patterns of *O. simavicus* using C-banding and silver staining for the first time.

Material and Methods

Thirteen individuals (six males and seven females) of *O. simavicus* were collected by electrofishing from Tabakdere, Salihli, Manisa, Turkey (38°28′N, 28°03′E) in 2019. The individuals were transported

alive to the laboratory. Metaphase chromosomes were obtained from head kidney cells according to Bertollo et al. (2015)'s air drying protocol. All analysed specimens are deposited in the Genetic Laboratory of Kırşehir Ahi Evran University, Turkey. For C-banding the technique of Sumner (1972) and for silver staining the technique of Howell and Black (1980) were applied to chromosome slides. All stained slides were scanned with a Leica DM 3000 microscope (Leica Microsystems GmbH, Germany) and metaphases were photographed with AKAS software (Argenit Mikrosistem, Turkey).

Results and Discussion

Constitutive heterochromatin regions were determined on the pericentromeres of most chromosomes in *O. simavicus* (Figure 1A). Otherwise, Ag-NORs were observed on the short (p) arms of one middle-sized sm chromosome pair (Figure 1B). Moreover, only one Ag-NOR on the p arms of sm chromosome was observed on some silver stained metaphases (Figure 2A). Also, Ag-NOR size polymorphism was observed on some silver stained metaphases (Figure 2B).

Despite the vast diversity of Anatolian *Oxynoemacheilus* species, only five species chromosomal banding properties have been reported (Değer 2011, Gaffaroğlu et al. 2014, Ayata et al. 2018). *O. simavicus* is similar to this Anatolian *Oxynoemacheilus* species in terms of constitutive heterochromatin region patterns (Table 1). Moreover, *O. simavicus* is similar to other Anatolian nemacheilid loach species - *Seminemacheilus lendlii* (Ünal et al. 2016) and *Turcinoemacheilus kosswigi* (Gaffaroğlu et al. 2012) - in terms of C-band pattern. In addition, *O. simavicus* is like 19 nemacheilid loach species (Sember et al. 2015) in terms of C-band pattern.

Table 1. Chromosomal banding studies in Anatolian nemacheilid species

Species	C-band pattern	Ag-NOR number and location	References
O. argyrogramma	centromeric in most chromosomes	q arms of 2 sm	Değer 2011
O. frenatus	centromeric in most chromosomes	q arms of 4 a	Değer 2011
O. sp.	centromeric in most chromosomes	q arms of 4 a	Değer 2011
T. kosswigi	centromeric in several chromosomes	---	Gaffaroğlu et al. 2012
O. angorae	centromeric in several chromosomes	---	Gaffaroğlu et al. 2014
S. lendlii	centromeric in most chromosomes	p arms of 4 sm	Ünal et al. 2016
O. atili	centromeric in most chromosomes	p arms of 2 sm	Ayata et al. 2018
O. simavicus	pericentromeric in most chromosomes	p arms of 2 sm	This study

p: short; q: long; sm: submetacentric; a: acrocentric

Otherwise, *O. simavicus* is similar to *O. argyrogramma* (Değer 2011) and *O. atili* (Ayata et al. 2018) in terms of Ag-NOR number. In addition, *O. simavicus* is different from *O. argyrogramma* (Değer 2011) about Ag-NOR location. However, *O. simavicus* is different from *O. frenatus*, *Oxynoemacheilus* sp. (Değer 2011) and *S. lendlii* (Ünal et al. 2016) in terms of Ag-NOR number and location. Moreover, *O. simavicus* is similar to other nemacheilid species analyzed by Sember et al. (2015) that showed single Ag-NOR. On the other hand, Ag-NOR polymorphism in *O. simavicus* has been reported in some nemacheilid loaches (Sember et al. 2015) too. Also, Ag-NOR number polymorphism that is reported in *S. lendlii* (Ünal et al. 2016) is similar to this study.

In conclusion, this study reveals the C-banding and Ag-NOR properties of *O. simavicus* for the first time. This study may contribute to Anatolian nemacheilid loach cytogenetics.

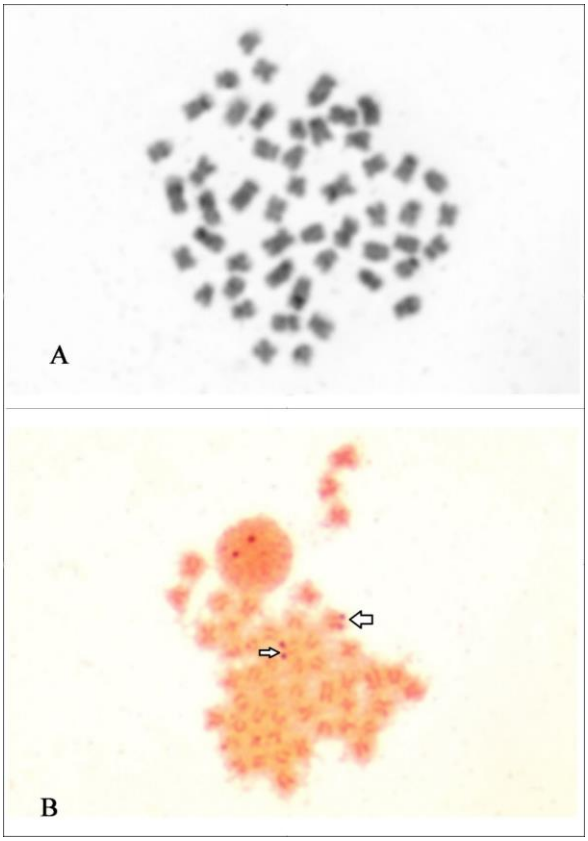

Figure 1. C-banded metaphase (A) and silver stained metaphase of *O. simavicus* (B). Arrows indicates two Ag-NORs.

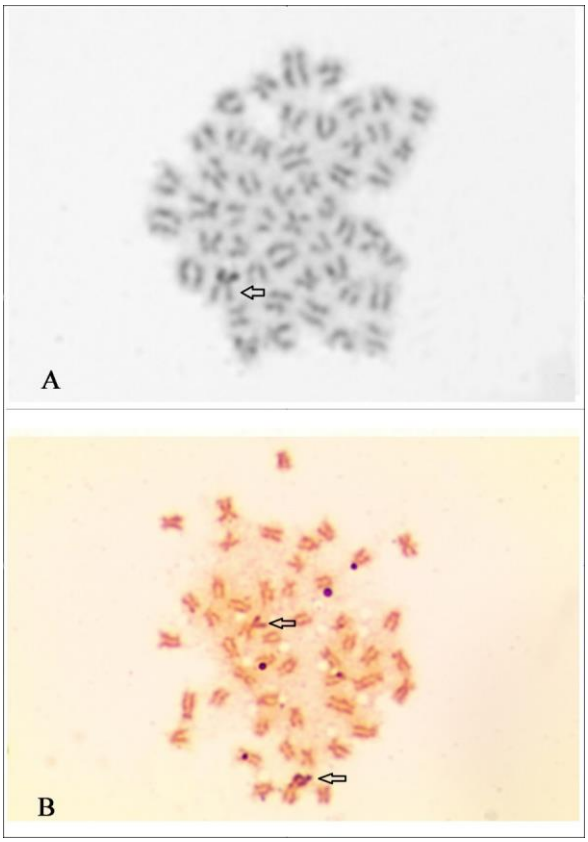

Figure 2. Silver stained metaphases of *O. simavicus*. One Ag-NOR (A), size polymorphism with two Ag-NORs (B). Arrows indicates the Ag-NORs.

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