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PROOF RESEARCH PAPER

Chromosomal Analysis of *Oxynoemacheilus atili* Erk'akan, 2012 (Teleostei, Nemacheilidae)

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Abstract

Diploid chromosome number, chromosome morphologies and chromosomal band properties (with C-banding and Ag-NOR staining) of endemic *Oxynoemacheilus atili* Erk'akan, 2012 were revealed out from Beyşehir Lake Basin, Turkey. Metaphase chromosomes were obtained from kidney cells. Diploid chromosome number was found as 2n = 50 and chromosome morphologies were as follows: five pairs of metacentric, nine pairs of submetacentric and 11 pairs of subtelo-acrocentric chromosomes. Fundamental arm number was calculated as 78. Constitutive heterochromatin regions were determined on the centromeres of chromosomes. NORs were detected on one pair of chromosomes. The obtained results may contribute to nemacheilid chromosomal studies.

Keywords: Beyşehir Lake loach, karyotype, constitutive heterochromatin region, nucleolus organizer region.

Introduction

The loaches of the subfamily Nemacheilinae are mainly small, bottom-dwelling fishes and distributed in the freshwaters. The taxonomy of this loaches is very complicated (Prokofiev, 2009). Most nemacheilid loaches were placed to the genus *Oxynoemacheilus* Bănărescu and Nalbant, 1966 in the recent years, making it a species rich genus of nemacheilid loaches (Freyhof, Erk'akan, Özeren, & Perdices, 2011). The family Nemacheilidae has 40 species in the freshwaters of Turkey and 35 of them belong to the genus *Oxynoemacheilus*. From these species, *Oxynoemacheilus atili*, Beyşehir Lake loach, is an endemic loach and distributes in Beyşehir Lake Basin of Turkey (Kuru, Yerli, Mangıt, Ünlü, & Alp, 2014).

Fish cytogenetics is a unique and useful tool for collecting information that can rarely or never be collected by other genetic methods. Fishes sometimes have very small and numerous chromosomes therefore, obtaining metaphase chromosomes and applying banding procedures to chromosome preparations are very difficult. Also, having seasonal cycles in cell activity, sometimes any dividing cells can be found. According to loach body size, these studies come more difficult. Additionally, the logistical problems in collecting and transporting fish specimens to the laboratory can be encountered. Therefore, fish chromosomal studies are carried out in few research laboratories (Rab, Bohlen, Rabova, Flajhans, & Kalous, 2007).

According to above mentioned problems, chromosomal studies of Anatolian fishes are very limited and have been started in the recent years. However, most of these studies have been conducted in cyprinids compared to loach species (Gaffaroğlu, Yüksel, & Rab, 2006; Karasu, Yüksel, & Gaffaroğlu, 2011; Ünal, Gaffaroğlu, Ayata, & Yüksel, 2014). Despite the being largest genus of Anatolian freshwaters, chromosomal studies have been reported in a few species of the genus *Oxynoemacheilus* (Değer, 2011). Moreover, there is no chromosomal study about endemic *O. atili*. The aim of this study is to reveal chromosomal properties (diploid chromosome number, chromosome morphologies, constitutive heterochromatin regions and Ag-NORs) of *O. atili*.

Materials and Methods

Six specimens (four males and two females) of *O. atili* were collected by electrofishing from Eflatun Pınarı, Beyşehir Lake Basin, Turkey (37°50′N, 31°43′E) in 2013. Specimens were transported alive to the laboratory. Metaphase chromosomes were

obtained from kidney cells according to Collares-Pereira (1992)'s protocol. From each specimen, at least 10 chromosome slides were prepared. All analyzed specimens are deposited in the Genetic Laboratory of Ahi Evran University, Kırşehir, Turkey. Sumner (1972)'s C-banding technique was used for determining the constitutive heterochromatin regions. Besides, Howell and Black (1980)'s technique was used for determining the NORs. All slides (Giemsa and Ag-stained and C-banded) were scanned with a Leica DM 3000 microscope and good metaphases were photographed with AKAS software. Chromosomes were measured by digital caliper and classified according to Levan, Fredga, and Sandberg (1964). For calculating fundamental arm number (FN), meta-submetacentric chromosomes were taken as biarmed, whereas subtelo-acrocentric chromosomes were taken as uniarmed.

Results

Diploid chromosome number of *O. atili* was determined as 2n = 50 (Figure 1a). Karyotype was consisted of five pairs of metacentric, nine pairs of submetacentric and 11 pairs of subtelo-acrocentric chromosomes (Figure 1b). FN was calculated as 78. Constitutive heterochromatin regions were determined on the centromeres of almost all chromosomes (Figure 2a). NORs were detected in the terminal regions of the short arms of one pair of submetacentric chromosomes (Figure 2b).

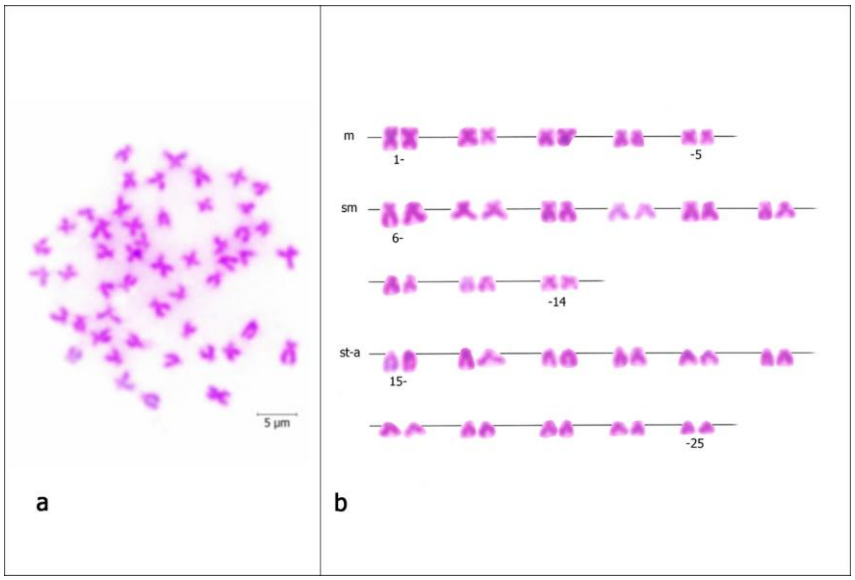

Figure 1. Giemsa stained metaphase (a) and arranged karyotype (b) of *Oxynoemacheilus atili*. Scale bar = 5 µm. (m: metacentric, sm: submetacentric, st-a: subtelo-acrocentric).

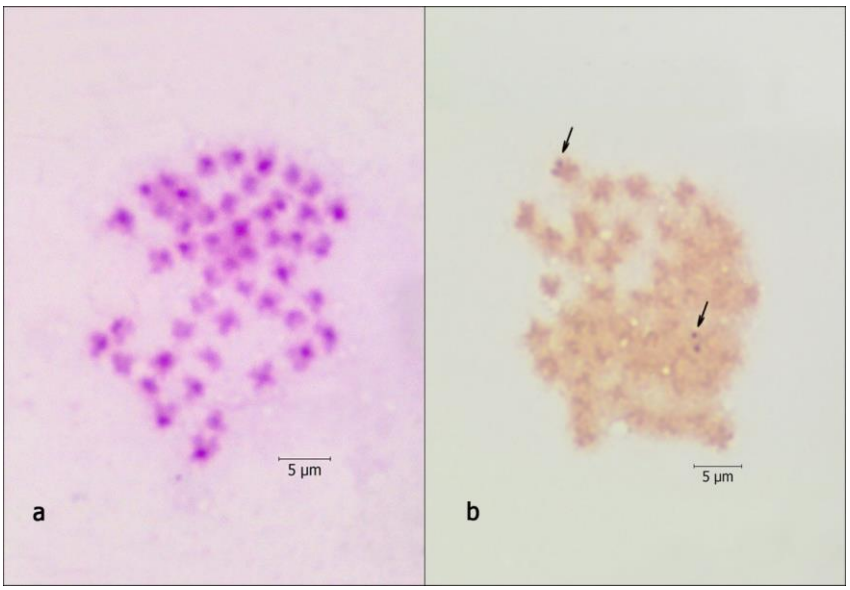

Figure 2. C-banded metaphase (a) and Ag-stained metaphase of *Oxynoemacheilus atili*. Scale bar = 5 µm. Arrows indicate the NORs.

Discussions

Despite the vast diversity of Anatolian nemacheilid loaches only eight species's chromosomal properties have been studied. These loaches retain the same diploid chromosome number as observed in this study. However, some differences on the chromosome morphologies are observed (Table 1). According to this differences, the FNs of the most species are different. It was reported that sharing the same 2n but different FNs in nemacheilid loaches indicates that their chromosomes have evolved by chromosomal rearrangements such as centromeric shifts (Sember et al., 2015). Loach chromosomal studies are very scarce in the other countries too. The number of karyologically studied loaches was reported as 38 (Sember et al., 2015). *O. atili* has the same diploid chromosome number with O*xynoemacheilus persa* and *O. tongiorgii* from Iran (Esmaeili, Pirvar, Ebrahimi, & Geiger, 2015) and *Afronemacheilus abyssinicus* from Ethiopia (Krysanov & Golubtsov, 2014). However, their chromosome morphologies have some differences. A cytogenetic study that was performed in 19 nemacheilid loaches by Sember et al. (2015) showed that 17 species have 2n = 50 as observed in this study. However, their FNs were different (68-90), only one species have the same FN with *O. atili*. It was reported that despite the conservatism of the same 2n, nemacheilid loaches exhibited variability on microstructural level. Pericentric inversions, tandem and centric fusions could be responsible for karyotype differentiation in these loaches (Sember et al., 2015). On the other hand, sex chromosomes are not observed in this study as reported in the other Anatolian nemacheilid loaches (Değer, 2011).

Cytogenetic studies of Anatolian nemacheilid loaches has been limited by determining 2n and chromosome morphologies. So, chromosomal banding studies of these loaches are so limited. Only five of them have been studied by means of C-banding method (Değer, 2011; Gaffaroğlu, Karasu, & Unal, 2012; Ünal, Ayata, & Gaffaroğlu, 2016). This method determines the constitutive heterochromatin regions. These regions are important source of karyotype diversification in fish species (Sember et al., 2015). *O. atili* is similar to *Oxynoemacheilus argyrogramma*, *Oxynoemacheilus frenatus*, *Oxynoemacheilus* sp. (Değer, 2011), *Seminemacheilus lendlii* (Sember et al., 2015; Ünal et al., 2016) and *Turcinoemacheilus kosswigi* (Gaffaroğlu et al., 2012) in terms of C-band patterns. Moreover, *O. atili* resembles with *Barbatula barbatula*, *Lefua costata*, *Mesonoemacheilus guentheri*, *Nemacheilus binotatus*, *Nemachilichthys ruppeli*, *Paracanthocobitis pictilis*, *Paracanthocobitis zonaltemans*, *Petruichthys brevis*, *Physoschistura elongata*, *Physoschistura* sp., *Physoschistura lucidorsum*, *Schistura bolavenensis*, *Schistura fasciolata*, *Schistura hypsiura*, *Schistura notostigma*, *Schistura pridii*, *Schistura savona* that were studied by Sember et al. (2015) from different countries in terms of C-heterochromatin locations. However, heterochromatic blocs that was reported in *T. kosswigi* (Gaffaroğlu et al., 2012), *S. lendlii* (Ünal et al., 2016) and some nemacheilid loaches (Sember et al., 2015) are not observed in this study.

NOR phenotype is another important cytogenetic tool in fish species. Only four Anatolian loaches have been studied about NOR phenotype. *O. atili* is similar to *O. argyrogramma* (Değer, 2011) in terms of NOR number and location. However, it is different from *O. frenatus* and *Oxynoemacheilus* sp. (Değer, 2011) both NOR number and location also *S. lendlii* (Sember et al., 2015; Ünal et al., 2016) has difference in terms of NOR number. Additionally, most of the nemacheilid species analyzed by Sember et al. (2015) showed single NOR like *O. atili*. On the other hand, NOR polymorphism that has been reported for some nemacheilid loaches (Sember et al., 2015) is not observed in this study.

In conclusion, the chromosomal properties of the Anatolian endemic nemacheilid loach, *O. atili*, is determined for the first time. The obtained results may improve the knowledge of nemacheilid cytogenetics.

Table 1. Chromosomal data of Anatolian nemacheilid loaches

Species	2n	Chromosome morphology	FN	References
Oxynoemacheilus angorae	50	14m+14sm+22a	78	Kaya, Gül, & Nur, 2005
Oxynoemacheilus tigris	50	18m+18sm+14a	86	Kılıç, 2006
Oxynoemacheilus panthera	50	14m+18sm+18a	82	Tanrıkulu, 2008
Oxynoemacheilus argyrogramma (Tigris River population)	50	44m-sm+6a	94	Değer, 2011
Oxynoemacheilus argyrogramma (Euphrates River population)	50	42m-sm+8a	92	Değer, 2011
Oxynoemacheilus frenatus	50	32m-sm+18a	82	Değer, 2011
Oxynoemacheilus sp.	50	30m-sm+20a	80	Değer, 2011
Turcinoemacheilus kosswigi	50	8m+14sm-st+28a	72	Gaffaroğlu et al., 2012
Seminemacheilus lendlii	50	16m+20sm+14st	86	Ünal et al., 2016
Oxynoemacheilus atili	50	10m+18sm+22st-a	78	In this study

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