

Ag-NOR Karyotypes of Five Endemic *Pseudophoxinus* Bleeker, 1860 (Teleostei: Leuciscidae) Species from Anatolia

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Abstract

This study presents Ag-NOR (silver-nucleolus organizer region) karyotypes of five endemic *Pseudophoxinus* species from Anatolia. The specimens were collected from central and southern Anatolia. The collected specimens were carried alive to the laboratory. Chromosome slides were prepared and Ag-staining technique was applied. Ag-NOR metaphases were photographed with a camera-equipped light microscope. Karyotypes of this metaphases were arranged. Ag-NORs were distributed between the second and eighth submetacentric chromosome pairs in the studied species. This study reveals the details of the Ag-NOR distributions on the karyotypes and may improve the cytogenetic data of the genus.

Introduction

The cyprinid genus *Pseudophoxinus* belongs to subfamily Leuciscinae and has 22 species –20 of them are endemic– in the inland waters of Anatolia (Güçlü & Küçük, 2017). Chromosomal studies in this genus have been started recently (Karasu, 2009). On the last decade, 12 species in total (*Pseudophoxinus antalyae*, *P. battalgilae*, *P. burduricus*, *P. crassus*, *P. egridiri*, *P. elizavetae*, *P. evliya*, *P. fahrettini*, *P. firati*, *P. hittitorum*, *P. maeandri* and *P. zekayi*) have been studied karyologically (Ergene, Karahan, & Kuru, 2010; Karasu, Yüksel, & Gaffaroğlu, 2011; Gaffaroğlu, Karasu-Ayata, Ünal, & Yüksel, 2014; Unal, Gaffaroğlu, Ayata, & Yüksel, 2014; Ayata, Yüksel, & Gaffaroğlu, 2016; Ünal & Gaffaroğlu, 2016). In these studies, the diploid chromosome numbers (2n), fundamental arm numbers (FNs), chromosome morphologies and chromosome banding properties (with C-banding and silver-nucleolus organizer region (Ag-NOR) staining) have been revealed out. Also, CMA₃ and DAPI staining's of *P. firati* and G-

and Q-banding properties of *P. antalyae* have been reported (Ergene et al., 2010; Gaffaroğlu, Karasu-Ayata, Ünal, & Yüksel, 2012).

Silver staining is a common cytogenetic technique in fish species. This method identifies Ag-NORs. The number and locations of Ag-NORs have been used as a systematic and taxonomic character and these characters' benefit to fish cytotaxonomy (Boron, 1999). Ayata et al. (2016) reported the single Ag-NORs in *P. burduricus*, *P. egridiri* and *P. fahrettini* whereas multiple Ag-NORs in *P. battalgilae* and *P. evliya*. Additionally, they were pointed out that Ag-NOR bearing chromosomes varies from one to five in *P. battalgilae*; one to four in *P. burduricus* and *P. fahrettini*; two to six in *P. evliya*. Also, they were reported that all Ag-NORs were distributed in middle sized submetacentric (sm) chromosomes. However, it is not known which pair of the chromosome in the karyotype is with Ag-NORs.

The aim of this study is to reveal Ag-NOR karyotypes of *P. battalgilae*, *P. burduricus*, *P. egridiri*, *P. evliya* and *P. fahrettini*.

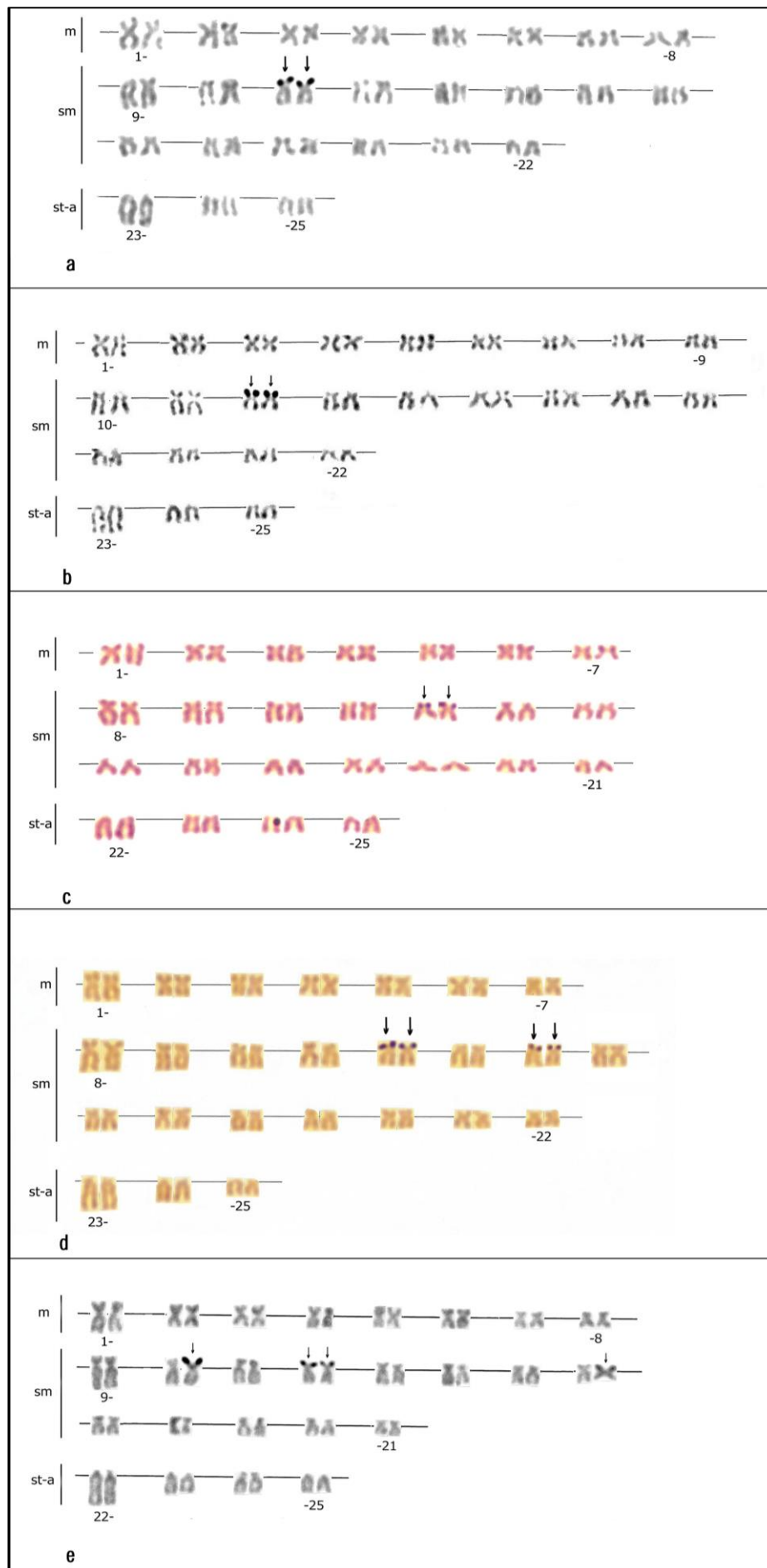


Figure 1. Ag-NOR relative karyotypes of (a) *P. battalgilae*, (b) *P. burduricus*, (c) *P. egridiri*, (d) *P. evliyae*, (e) *P. fahrettini*. Arrows indicate the Ag-NORs.

Materials and Methods

The specimens were collected as follows: *P. battalgilae* (11 specimens) from Seydişehir, Konya; *P. burduricus* (10 specimens) from Düğger, Burdur; *P. egridiri* (8 specimens) from Eğirdir Lake, Isparta; *P. evliya* (11 specimens) from Korkuteli, Antalya; *P. fahrettini* (6 specimens) from Çivril, Denizli. The specimens were carried alive to the laboratory. The chromosomes were obtained from head kidney cells according to the air drying technique of Collares-Pereira (1992). Ag-staining technique of Howell & Black (1980) was applied to chromosome preparations. Details about the modifications of the above mentioned techniques can be seen in Ayata et al. (2016). Ag-stained preparations were scanned in the microscope and photographs of Ag-NOR metaphases were taken. Chromosomes were measured with digital calliper and karyotypes of Ag-NOR metaphases were arranged manually. Chromosomes were classified according to Levan, Fredga, & Sandberg (1964).

Results & Discussion

The Ag-NORs were located in; no. 11 sm in *P. battalgilae* (Figure 1a), no. 12 sm in *P. burduricus* (Figure 1b) and in *P. egridiri* (Figure 1c), no. 12 sm and 14 sm in *P. evliya* (Figure 1d), no. 10 sm, 12 sm and 16 sm chromosomes in *P. fahrettini* (Figure 1e).

The Ag-NORs are located between the second and eighth sm chromosome pairs in this study. Frequently, this Ag-NORs are in the third sm chromosome in *P. battalgilae* and *P. burduricus* whereas in the fifth sm chromosome in *P. egridiri* and *P. evliya*. This obtained results are in concordance with Ayata et al. (2016) about middle sized sm chromosomes with Ag-NORs. Otherwise, Ag-NOR locations in the karyotypes of *P. battalgilae*, *P. burduricus*, *P. egridiri*, *P. evliya* and *P. fahrettini* are different from Anatolian leuciscines like *P. antalyae* which has the Ag-NORs in the second st chromosome (Ergene et al., 2010) and *Chondrostoma beysehirensis* which has the Ag-NORs in the first sm/st chromosome pair (Arslan & Gündoğdu, 2016).

All above mentioned species in this study show differences with *Squalius pyrenaicus* which has the Ag-NORs in the tenth sm chromosome (Gromicho & Collares-Pereira, 2004) and *Petroleuciscus borysthenticus* which has the Ag-NORs in the ninth sm/st chromosome pair (Rab et al., 1996). Otherwise, *P. battalgilae* and *P. burduricus* show similarity to Iberian leuciscines that all have the Ag-NORs in the third sm chromosome pair (Pereira, Neto, & Collares-Pereira, 2009). Furthermore, *P. fahrettini* shows similarity to European leuciscine species of the genera *Leuciscus*, *Scardinius* and *Rutilus* (Bianco et al., 2004) and to *S. lucumonis* (Rossi, Milana, Hett, & Tancioni, 2012) which has the Ag-NORs in the fourth sm chromosome. Also, *P. evliya* shows similarity to *S. aradensis* and *S. torgalensis* that have the Ag-NORs

in the seventh sm chromosome pair (Nabais, Rampin and Collares-Pereira, 2013).

Conclusion

Ag-NOR karyotypes have not often been employed in karyological studies of Anatolian fish. Only Ag-NOR number and the size of the located chromosome have been given. In this study, the Ag-NOR karyotypes of five Anatolian endemic *Pseudophoxinus* species have been revealed out for the first time. The Ag-NOR locations have been determined in Ag-stained metaphases in detail. The importance of the results is species specific Ag-NOR locations have been determined. It should improve the cytogenetic data of the genus. Also, based on the findings of this study, it should contribute the future studies.

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