*Genetics of Aquatic Organisms 5(1), 41-44*
http://doi.org/10.4194/2459-1831-v5_1_05

PROOF
SHORT PAPER

GENAQUA
GENETICS OF AQUATIC ORGANISMS

# Karyology of *Rhodeus amarus* (Block, 1782) (Teleostei, Acheilognathidae) from Turkey

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**Article History**
Received 07 December 2020
Accepted 25 Januray 2021
First Online 09 February 2021

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**Keywords**
Chromosome
Karyotype
C-banding
Ag-NOR

**Abstract**

The aim of this study was to determine chromosomal characteristics of *Rhodeus amarus* (Block, 1782) from Turkey by conventional procedures (Giemsa, C-banding and Ag-NOR staining). Metaphase chromosomes were obtained from the head kidney cells. The diploid number was found as 48 and the fundamental number as 76. Chromosomes were morphologically characterized as metacentric (four pairs), submetacentric (10 pairs) and subtelo-acrocentric (10 pairs). C-bands were found to occur on the pericentromeric regions of most of the chromosomes and a single Ag-NOR was observed on Silver stained metaphases. The results may expand the knowledge on chromosomal features of bitterlings.

## Introduction

The genus *Rhodeus* Agassiz, 1832 belonging to the family Acheilognathidae has 23 valid species in the inland waters of Eurasia (Froese & Pauly, 2020). *R. amarus*, known as European bitterling, is a small freshwater fish inhabiting lakes and slow flowing rivers (Froese & Pauly, 2020), and is also distributed in inland waters of Turkey (İlhan, Sarı, & Ekmekçi, 2014). Although abundant in most of its distribution range, *R. amarus* is threatened by environmental changes like water pollution due to anthropogenic action (Kirtiklis, Ocalewicz, Wiechowska, Boron, & Hliwa, 2014).

The members of *Rhodeus* like other bitterlings show an unusual spawning symbiosis with freshwater mussels. *Rhodeus* females develop long ovipositors that they use to place their eggs onto the gills of a mussel through an exhalant siphon. Males fertilize the eggs by releasing sperm into the inhalant siphon of the mussel and embryos develop inside the mussel about a month. Then, embryos leave the mussel as actively swimming larvae. This reproduction relationship of *Rhodeus* with mussels makes it a very attractive material in different scientific studies (Smith, Reichard, Jurajda, & Przybylski, 2004).

Cytogenetic characters are important tools for many scientific purposes (Kirtiklis et al., 2014). In Turkey, freshwater fish chromosomal studies have been increased after 2003 (Gaffaroğlu, 2003), although often limited to the determination of the diploid number (2n), fundamental arm number (FN) and chromosome morphology (Gaffaroğlu, Yüksel, & Rab, 2006; Ayata, Yüksel, & Gaffaroğlu, 2016; Unal & Gaffaroğlu, 2016), in addition to C-banding and Silver staining methods (Gaffaroğlu et al., 2006; Ayata et al., 2016; Unal & Gaffaroğlu, 2016; Ayata, Yüksel, & Gaffaroğlu, 2019).

Chromosomal studies in *R. amarus* from European localities (Libertini et al., 2008; Kirtiklis et al., 2014) have been reported before. However, there are no chromosomal reports for *R. amarus* from the inland waters of Turkey. Thus, the main goal of the present study was to determine some chromosomal characteristics of this species from Turkey for the first time.

## Material and Methods

Three specimens (1 female, 2 males) were collected from Dibekdere, Ahmetli, Manisa (38°33'N, 27°57'E). The specimens were carried alive to laboratory and kept in well-aerated aquarium until the analysis. Chromosomal preparations were obtained from the head kidney cells according to the air-drying technique of Bertollo, Cioffi, & Moreira-Filho (2015). At least 10 slides were prepared from each individual. Some slides were stained by 5% Giemsa solution. The C-banding technique of Sumner (1972) was used for visualization of constitutive heterochromatin regions, whereas the Silver staining technique of Howell & Black (1980) was followed for determining Ag-NORs. Slides were screened in Leica DM 3000 microscope (Leica Microsystems GmbH, Germany). Photographs of metaphases were taken with AKAS software (Argenit Mikrosistem, Turkey). At least 100 metaphases were examined for determining the 2n number. Karyotypes were manually arranged and chromosomes classified according to Levan, Fredga, & Sandberg, (1964). For calculating the FN, meta- and submetacentric chromosomes were taken as biarmed, whereas subtelo-acrocentric chromosomes were taken as uniarmed. Image processing was performed in Adobe Photoshop CS6.

## Results and Discussion

The diploid number of *R. amarus* was invariably 2n=48 (Figure 1A). Chromosomes were morphologically characterized as metacentric (four pairs), submetacentric (10 pairs) and subtelo-acrocentric (10 pairs) (Figure 1B). The FN was calculated as 76 both in males and female, and heteromorphic sex chromosomes were not detected in this species. C-bands were found to occur in the pericentromeric region of most chromosomes (Figure 2A) and Ag-NORs in the terminal region of short arms of one submetacentric pair (Figure 2B).

Our data confirm the karyological conservativeness of 2n=48, which has been suggested as a basal characteristic for Acheilognathinae (Arai & Akai, 1988). Accordingly, the 2n and FN numbers and the chromosomal morphologies of *R. amarus* from Turkey are the same as those of other European populations (Libertini et al., 2008; Kirtiklis et al., 2014). However, as these are the first data from *R. amarus* from Turkey, other populations deserve to be investigated for a more detailed comparison between European and Anatolian populations.

Otherwise, the diploid number varies from 46 to 48 among *Rhodeus* species (Ueda et al., 2001). In *R. amarus* the chromosomal number and morphology are the same as those found in *R. lighti* (Ueda et al., 1997), *R. sinensis*, *R. ocellatus* (Ueda, Naoi, & Arai, 2001), *R. kurumeus* (Sola et al., 2003), and *R. uyekii* (Gil et al., 2016). Consequently, there is no difference among the karyotypes of the above-mentioned species, pointing to conservatism along the karyotype evolution of the *Rhodeus* genus. Indeed, *R. fangi* is the only divergent species concerning the diploid number (2n=46), FN and chromosomal morphology, suggesting that these

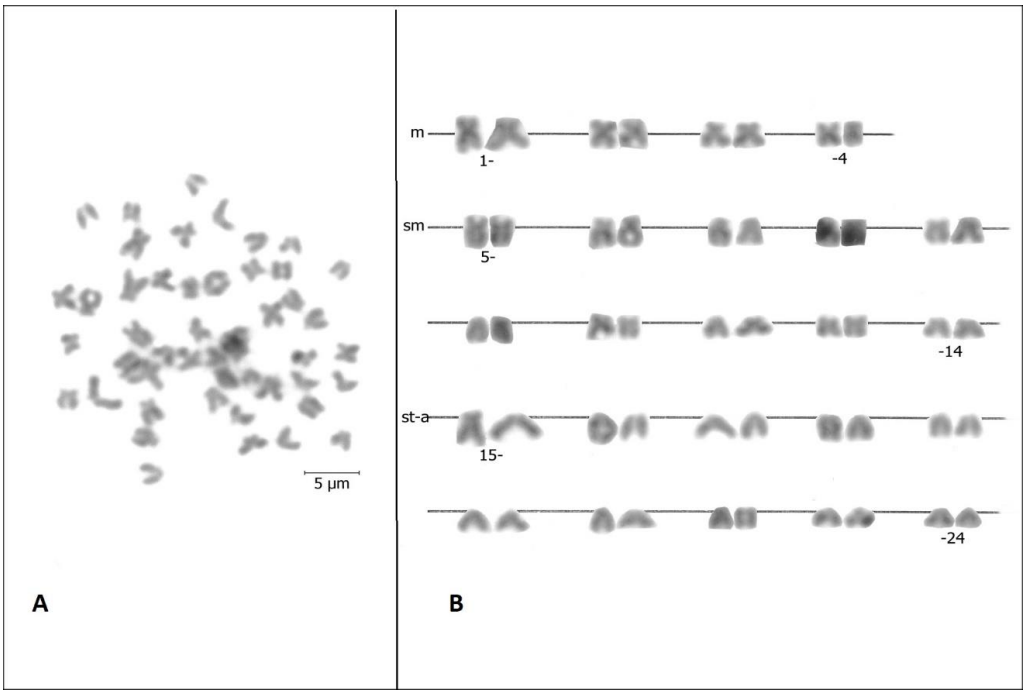


**Figure 1.** Giemsa stained metaphase (A) and the relative karyotype (B) of *Rhodeus amarus*. Scale bar = 5 µm.

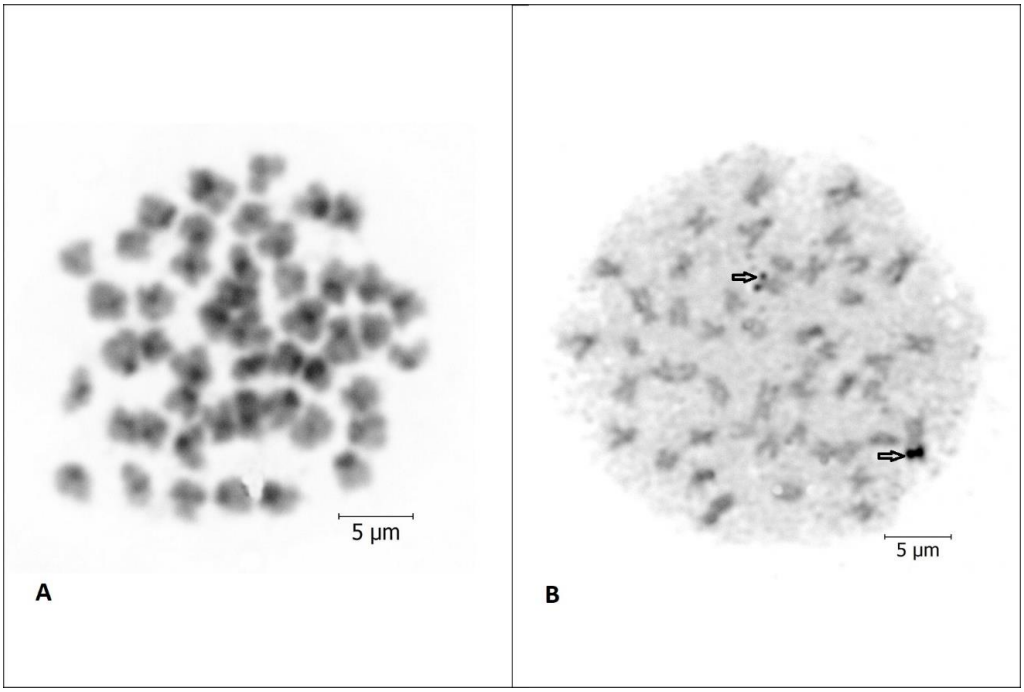


**Figure 2.** C-banded metaphase (A) and Silver stained metaphase (B) of *Rhodeus amarus*. Arrows indicate the Ag-NORs. Note a polymorphism in size between the homologous NORs. Scale bar = 5 µm.

species features are derived by pericentric inversions and tandem fusions (Ueda et al., 2001).

Heteromorphic sex chromosomes are known only in a restricted group of fish species (Arai, 2011). In *R. amarus* they are also not differentiated, both in the present as in other analyzed populations (Libertini et al., 2008; Kirtiklis et al., 2014). Likewise, other *Rhodeus* species, such as *R. lighti* (Ueda et al., 1997), *R. fangi*, *R. sinensis*, *R. ocellatus* (Ueda et al., 2001) and *R. kurumeus* (Sola et al., 2003) do not hold such a characteristic in their karyotypes.

Kirtiklis et al. (2014) reported that the pericentromeric regions of most or even all chromosomes are composed of constitutive heterochromatin in bitterlings, as evidenced by their C-band patterns. The C-band pattern of *R. amarus* now investigated is similar to that of *R amarus* from Poland and other *Rhodeus* species, such as *R. fangi*, *R. sinensis*, *R. ocellatus* (Ueda et al., 2001) and *R. kurumeus* (Sola et al., 2003). Thus, it is likely that constitutive heterochromatin acts as an important genetic component on the karyotype evolution of bitterlings (Ueda et al., 2001).

In turn, the nucleolar organizing regions are not as conservative as the C-bands among *Rhodeus* species. Kirtiklis et al. (2014) report that a single pair of chromosomes carrying Ag-NORs appears to be the main pattern among bitterling species. However, despite this, Libertini et al. (2008) consider that Ag-NORs variability is also a common feature in *Rhodeus*, as a consequence of rDNA rearrangements, including the numerical polymorphism as found in *R. amarus* from northern Italy. The number and location of the Ag-NORs in the sample now investigated are the same as those found in some other European populations (Libertini et al., 2008; Kirtiklis et al., 2014), but differing in chromosomal location with *R. ocellatus* (Ueda et al., 2001) and *R. uyekii* (Gil et al., 2016). In turn, the number of the Ag-NORs of *R. amarus* differs from those in *R. lighti* (Ueda et al., 1997), *R. fangi* and *R. sinensis* (Ueda et al., 2001). In addition, the polymorphism found in *R. amarus* from Poland (Kirtiklis et al., 2014) is also observed between the homologous carrying the Ag-NORs in the present study.

In conclusion, this study reports the chromosomal characteristics of *R. amarus* from Turkey for the first time. Although samples from a single population have so far been investigated, it was characterized that the population now analyzed has similarities, but also disagreements with other *R. amarus* populations, as well as with other *Rhodeus* species. Therefore, the results expand the knowledge of the evolutionary process of this particular species of Acheilognathidae, as well as of the genus *Rhodeus* as a whole.

## Acknowledgements

The authors are grateful to Ali AYATA and Yalçın KARASU for helping in the field. Our special thanks to the anonymous reviewers for their valuable contribution on the manuscript.

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