Karyotype description of three sympatric species of Astyanax (Characiformes, Characidae)

Adriana Magalhães da Silva, Patrícia Cristina Vizzotto, Quizzi Maria Cordova Becker and Reinaldo José de Castro

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Abstract

The genus Astyanax, one of the largest genera in the family Characidae, comprises a large number of similarly shaped fish species. In this study, the karyotypes of three sympatric species of Astyanax were analyzed. Astyanax asuncionensis showed a karyotype of 2n=50 chromosomes (14M+26SM+10ST/A). Astyanax lineatus also had 2n=50 chromosomes, but its karyotype was different (8M+24SM+18ST/A). Astyanax marionae showed a karyotype of 2n=48 chromosomes (8M+24SM+16ST/A). Ag-NORs appeared in two pairs of chromosomes in Astyanax marionae and in only one pair in the other two species. The variation in the karyotype formula and number of nucleolar organizer regions highlights the importance of these markers in distinguishing among similarly shaped species.

Keywords: Fish cytogenetics, karyotype, Ag-NORs, Astyanax

Introduction

Astyanax is one of the largest genera in the family Characidae, and Astyanax species are widely distributed throughout the Americas (Gery 1977). This group comprises fish species with similar shapes and has been recently considered Incertae sedis because the group does not exhibit consistent evidence of monophyly (Lima et al. 2003). Several cytogenetic studies have been conducted on fishes from the Astyanax group (Maistro et al. 2000; Moreira-Filho et al. 2001; Torres-Mariano and Morelli 2006; Ferreira Neto et al. 2009; Kavalco et al. 2011; among others). The cytogenetic study of the genus Astyanax has contributed substantially to the clarification of the systematics and taxonomy of these species. In numerous examples, intraspecific variation among populations indicates that they can form species complexes (Moreira-Filho and Bertollo 1991; Artoni et al. 2006; Vicari et al. 2008a). However, cytogenetic knowledge of Astyanax species from streams in the state of Mato Grosso, Brazil, is still limited.

In the present study, the karyotypes of three Astyanax species were analyzed by conventional Giemsa and Ag-NOR staining. These analyses represent a novel contribution to the cytogenetic literature.

Materials and methods

Three species of Astyanax collected from the Esparramo stream (16º 30.326´ S / 054º 40.526´ W), a small tributary of the Vermelho River in the upper Paraguay River basin, Rondonópolis, Mato Grosso, Brazil, were analyzed. These collections included twelve specimens of Astyanax asuncionensis (Fig. 1A), forty-five specimens of Astyanax lineatus (Fig. 2A) and nine specimens of Astyanax marionae (Fig. 3A). The fishes were identified and deposited in the Laboratório de Zoologia, UFMT, Rondonópolis, MT, Brazil.

Chromosome spreads and staining were performed as described by Foresti et al. (1993). Active nuclear organizer regions (NORs) were detected by silver nitrate staining (Howell and Black 1980). Karyotypes were described based on the arm ratio, as proposed by Levan et al. (1964), and chromosomes were classified as metacentric (M), submetacentric (SM) and subtelocentric/acrocentric (ST/A). For the chromosome arm number (fundamental...
number, NF) calculations, the M and SM chromosomes were considered to have two arms and the ST/A chromosomes were considered to have one arm.

**Results**

The studied samples of *Astyanax asuncionensis* (Fig. 1A) showed a diploid number of $2n=50$ chromosomes, distributed as $14M+26SM+10ST/A$, with a fundamental number (NF) of 90 (Fig. 1B). Ag-NORs appeared at the terminal region of the short arm of submetacentric chromosome pair 9 (Fig. 1B and 1C).

*Astyanax lineatus* (Fig. 2A) exhibited $2n=50$ chromosomes, $8M+24SM+18ST/A$, NF=82, with Ag-NORs located at the terminal region of the short arm of submetacentric chromosome pair 13 (Fig. 2B and 2C).

The species *Astyanax marionae* (Fig. 3A) exhibited a different diploid number and karyotypic formula. This species presented $2n=48$ chromosomes, arranged as $8M+24SM+16ST/A$ and NF=80 (Fig. 3B). The species contained multiple Ag-NORs located at the terminal region of the short arms of submetacentric pair 10 and at the long arms of subtelocentric/acrocentric pair 18 (Fig. 3B and 3C). In all preparations, only one chromosome of pair 18 exhibited Ag-NORs.

No differences were found between the male and female karyotypes in any of the species studied, and none of the species exhibited B chromosomes.

**Discussion**

Morphological and karyotypic variation is common in the genus *Astyanax*, most likely due to the wide geographic distribution of the genus. This variation often makes species identification difficult. However, combining cytogenetics with taxonomy can help overcome this challenge.

The chromosome number $2n=50$ was observed in the majority of the populations belonging to the genus *Astyanax*, although the diploid number in this group can range from $2n=36$ (Moreira-Filho et al. 2001) to $2n=50$ (Maistro et al. 2000; Moreira-Filho et al. 2001; Rosa et al. 2009; Kavalco et al. 2011; among others).
The cytogenetic data from the three Astyanax species studied here are similar to those from other species of this group reported in the literature. In the present study, the diploid chromosome number 2n=50 was the most frequent chromosome number, found in two of the three studied samples. This observation strengthens the hypothesis that 2n=50 is a conserved feature in the Astyanax group. The diploid number 2n=48, observed in Astyanax marionae, was within the limits described in the literature. The occurrence of 2n=48 chromosomes has previously been described for some specimens of Astyanax (Moreira-Filho and Bertollo 1991; Souza 2000; Vicari et al. 2008b; Rosa et al. 2009).

Silver nitrate staining (Ag-NORs) revealed single NOR-bearing chromosome pairs in Astyanax asuncionensis and Astyanax lineatus, both in the short arms of submetacentric chromosomes. Astyanax marionae contained three NOR-bearing chromosomes with one terminal signal in one submetacentric pair and a single homologous element in a subtelocentric/acrocentric pair. The occurrence of three NOR-bearing chromosomes was described previously for Astyanax laticeps (Rosa et al. 2009). Chromosomal rearrangements such as the deletion of one homolog or the transference of ribosomal sites could cause these variations, but transposition events have been suggested as the principal mechanism to explain the majority of cases of genomic NOR variability in these animals (Mantovani et al. 2000; Vicari et al. 2008b; Rosa et al. 2009).

Although cytogenetic data were available for only a few Astyanax species, the similar diploid number and Ag-NOR pattern in Astyanax asuncionensis and Astyanax lineatus indicate a closer phylogenetic relationship between these species, as Astyanax marionae presented cytogenetic characteristics that differentiate it from the two other studied species. Differences in karyotype macrostructure and fundamental number indicate that chromosomal rearrangements were important in the speciation events that have occurred during the evolution of this group.

The cytogenetic variations found among the studied species highlights the importance of these markers in characterizing fish species, especially those with similar morphology. The information available for the genus Astyanax has enabled the inference of evolutionary patterns with respect to chromosomes, reinforcing the importance of basic cytogenetic research in scientifically underdeveloped regions and opening the possibility of further studies.

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