CHROMOSOMAL ANALYSIS OF *Astyanax fasciatus* (PISCES, CHARACIDAE) FROM THE ARAGUARI RIVER, UBERLÂNDIA, MG, BRAZIL

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ABSTRACT

The genus *Astyanax* is one of the most numerous of the family Characidae, comprising a large number of similar-shaped species, but displaying innumerable karyotypic variations in its chromosome number and/or structure. The literature describes *A. fasciatus* populations with diploid chromosome numbers varying from 2n = 45 to 2n = 48. In this study, *A. fasciatus* specimens captured in the Araguari River (Alto Paraná basin) were cytogenetically characterized, revealing a diploid chromosome number of 2n = 46. The nucleolar organizing regions (NORs), detected with silver nitrate staining, showed a multiple system with two pairs of marked chromosomes. These findings are congruent with those of other studies involving populations of the same species.

Keywords: cytogenetics, *Astyanax*, *Astyanax fasciatus.*

RESUMO

Análise cromossômica em *Astyanax fasciatus* (Pisces, Characidae) da população do Rio Araguari, Uberlândia, MG, Brasil

O gênero *Astyanax* é um dos mais numerosos da família Characidae, englobando um grande número de espécies muito semelhantes na forma, mas que apresentam inúmeras variações cariotípicas no número e/ou estrutura cromossômica. Encontram-se descritas na literatura populações de *A. fasciatus* com o número diplóide variando de 2n = 45 a 2n = 48 cromossomos. No presente trabalho, espécimes de *Astyanax fasciatus* da população da calha do rio Araguari (bacia do Alto Paraná), foram caracterizados citogeneticamente. O número diplóide observado foi 2n = 46 cromossomos. As regiões organizadoras de nucléolo (NORs), detectadas com nitrato de prata, apresentaram um sistema múltiplo, com dois pares de cromossomos marcados. Esses dados estão de acordo com os descritos para outras populações da mesma espécie.

Palavras-chave: citogenética, *Astyanax, Astyanax fasciatus*.

INTRODUCTION

The genus *Astyanax* is one of the most numerous of the family Characidae, which comprises species with similar shapes, but displays innumerable karyotypic variations in its chromosome number and/or structure. Various authors have described *A. fasciatus* populations with diploid chromosome numbers ranging from 45 to 48 (Morelli *et al*., 1983; Paganelli, 1990; Justi, 1993; Daniel-Silva, 1996; Vale & Martins-Santos, 1999). According to Malacrida *et al*. (2000), the *A.* cf. *fasciatus* population of the Claro River (Tamarana, PR, Brazil) showed a diploid chromosome number of 48 in some specimens and of 49 or 50 in others. Daniel-Silva (1996) observed 2n = 45 chromosomes in some individuals, and

2n = 46 and 2n = 47 in others captured in the Mogi-Guaçu River. In the same River, Heras (1998, *apud* Néo, 1999) analyzed specimens with diploid numbers varying from 46 to 48 chromosomes. These numbers reveal an interesting feature of the genus *Astyanax*: the high variability among specimens of the same population. In these studies, the nucleolar organizing regions (Ag-NORs) occur in more than one chromosomal pair, characterizing multiple NORs. This paper defines the number of chromosomes and localization of the NORs of an *Astyanax fasciatus* population from the Araguari River (Uberlândia, MG, Brazil).

MATERIAL AND METHODS

Mitotic chromosomes were obtained from anterior kidney cells (Bertollo *et al*., 1978) of *Astyanax fasciatus* (10 females and 7 males) caught at the Pau Furado bridge, which spans the Araguari River 150 Km from its estuary (18° 47' 25" S; 48° 08' 50" W), in Uberlândia, MG, Brazil. Nucleolar organizing regions (NORs) were identified by silver staining, following the method described by Howell & Black (1980). The mitotic chromosomes were photographed and the chromosome morphology was determined on the basis of arm ratios, as proposed by Levan *et al*. (1964).

RESULTS

The determination of the karyotype and nucleolar organizing regions (Ag-NORs) of an *Astyanax fasciatus* population from the Araguari River (MG) involved the cytogenetic analysis of 17 specimens in order to identify their karyotypic macrostructure. Our findings revealed that this fish has 7 metacentric (M), 8 submetacentric (SM), 5 subtelocentric (ST), and 3 acrocentric (A) chromosome pairs (Figs. 1 and 2), characterizing a fundamental number of 86 and a diploid modal number of 2n = 46 chromosomes in both sexes. The chromosome number coincides with that of many other populations described previously, but its chromosomal formula is different, as indicated in Table 1.

One hundred and eleven metaphases were analyzed from 14 specimens of *Astyanax fasciatus*, which were silver-stained to determine the AgNOR system. In this population, this system is multiple and is localized in the terminal region of the short arm in the first submetacentric chromosome pair with a frequent size polymorphism, and in the terminal region of the short arm in the third metacentric pair (Fig. 3).

DISCUSSION

Morphological and karyotypic variations are common in the genera *Astyanax*, probably due to its wide geographic distribution, often making identification in specimens difficult. However, cytogenetics allied with taxonomy (cytotaxonomy) can help overcome such difficulties.

According to Guerra (1988) karyotypic variations can occur among different cells of the same individual, among different individuals of the same population, or among different populations of the same species. These differences can involve not only the number of chromosomes but also the chromosomal structure. Structural variations may be the result of deletions, duplications, inversions (paracentric and pericentric), transpositions, translocations and isochromosomes. Chromosomal polymorphisms occur in almost all natural populations (White, 1973, *apud* Dias & Giuliano - Caetano, 2003) and have been observed in different groups of animals, including fish.

An example of such polymorphism is the *Astyanax fasciatus* population from the Três Bocas Stream (Tiabagi River Bay) in Londrina, studied by Koguissi (1995, *apud* Dias & Giuliano-Caetano, 2003), which displayed 5 different cytotypes, besides a constant chromosome number (2n = 46). Daniel-Silva (1996) and Heras (1998, *apud* Neo, 1999) studied individuals from the Mogi-Guaçu River collected under similar conditions to those employed in the Três Bocas Stream. As can be seen in Table 1, these specimens presented diploid numbers varying from 45 to 48 chromosomes. The specimen examined by Daniel-Silva (1996), which contained 2n = 45 chromosomes, was subjected to RBG-banding, which permitted the pairing of all homologues and resulted in the identification of chromosomes that evolved to form one large metacentric chromosome.

A comparison of the chromosome macrostructure of *Astyanax fasciatus* populations studied previously against that of the population of the Araguari River, shown in Table 1, reveals some variations which may be related to their

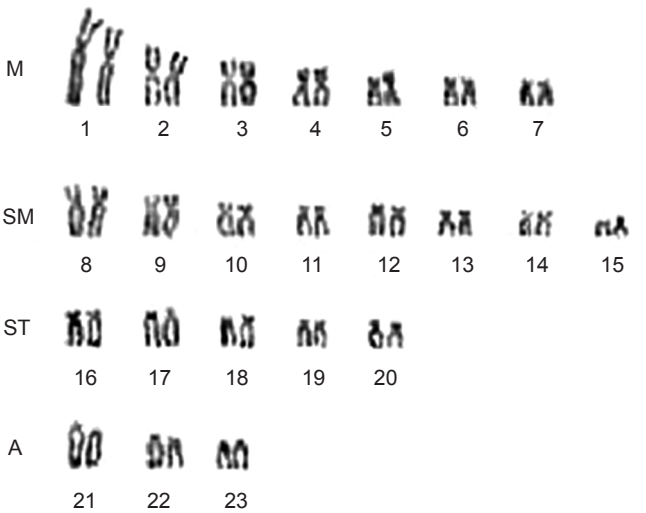

Fig. 1 — Karyotype of a female of the species *Astyanax fasciatus* stained with Giemsa.

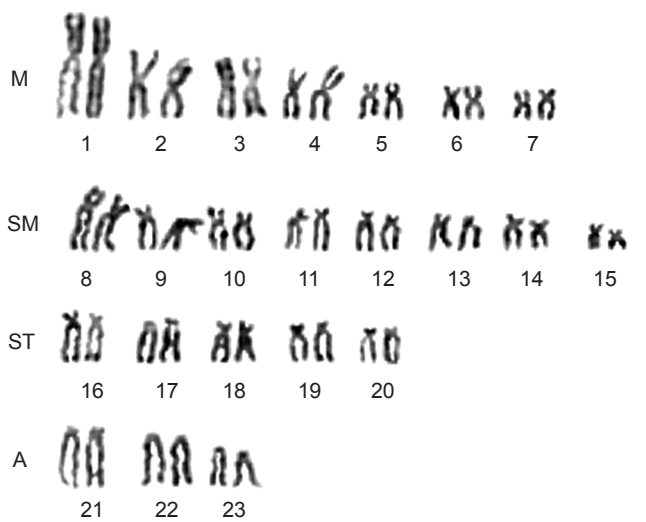

Fig. 2 — Representative karyotype of an *Astyanax fasciatus* male stained with Giemsa.

endemic habits and occupation of several niches, resulting in their geographic isolation. However, some similarities were found in the karyotypes of populations from the Mogi-Guaçu and Araguari Rivers (Table 1), possibly as a result of the proximity of these sites, which allowed for genetic exchanges among these populations.

Differences in chromosome condensation can explain several chromosomal formulas, but does not explain the same pattern observed in many individuals. The karyotype in Fig. 1 exemplifies these differences. An examination of the first metacentric pair reveals that there is a difference in size between the homologues. However, this is not a pattern for the population, since it is present in this metaphase but is rarely observed in other metaphases of the same animal. The chromosomes that comprise the 15th pair present a size variation, which is observed in several metaphases of many animals, making it difficult to do the pairing,

TABLE 1
Chromosome number and types and fundamental numbers found in *Astyanax fasciatus* populations from the Alto Paraná Basin.

	2n	M	SM	ST	A	NF	Ref.
Araguari River (MG)	46	14	16	10	6	86	Present paper
Mogi-Guaçu River (SP)	46	14	20	10	2	90	Morelli *et al*., 1983
Mogi-Guaçu River (SP)	46	12	20	10	4	88	Paganelli, 1990
Mogi-Guaçu River (SP)	45	13	19	10	3	-	Daniel-Silva, 1996
Mogi-Guaçu River (SP)	46	12	20	10	4	-	Daniel-Silva, 1996
Mogi-Guaçu River (SP)	46	12	20	8	6	86	Heras, 1998 (*apud* Néo,1999)
Mogi-Guaçu River (SP)	46	13	20	8	5	87	Heras, 1998 (*apud* Néo,1999)
Piracicaba River (SP)	46	12	20	10	4	88	Justi, 1993
Passa Cinco River (SP)	46	14	18	10	4	88	Heras, 1998 (*apud* Néo,1999)
Jacaré Guaçu River (SP)	46	12	20	16	6	86	Heras, 1998 (*apud* Néo,1999)
Barra Funda Stream (SP)	46	12	20	8	6	86	Heras, 1998 (*apud* Néo,1999)
Mogi-Guaçu River (SP)	47	13	20	8	6	88	Heras, 1998 (*apud* Néo,1999)
Mogi-Guaçu River (SP)	47	12	21	10	4	-	Daniel-Silva, 1996
Mogi-Guaçu River (SP)	48	12	20	8	8	88	Heras, 1998 (*apud* Néo,1999)

Abbreviations: M = metacentric; SM = submetacentric; ST = subtelocentric; A = acrocentric; NF = fundamental number; and 2n = chromosomic diploid number.

Fig. 3 — *Astyanax fasciatus* metaphases treated with silver nitrate. The arrows indicate the chromosomes bearing the nucleolar organizing regions.

probably because of the presence of a major heterochromatic block in one of its homologues. The C-banding technique would have to be employed to confirm this finding and identify the constitutive heterochromatin. In addition, the pair numbers 5 (Fig. 1), 11 and 13 (Fig. 2) also exhibit some differences, probably because of the arrangement in which the chromosomes were found.

Another way to identify a population and, sometimes individuals inside the same population, is by locating the nucleolus organizing regions using silver staining (Ag-NORs). These regions are associated with the nucleolus and are responsible for organizing them at the end of the cell division. They contain the ribosomal DNA (rDNA), which is transcripted to the ribosomal RNA – rRNA.

The multiple Ag-NORs pattern observed in the Araguari River population differs in the maximum number from all the data reported in the literature. The Mogi-Guaçu River population analyzed by Paganelli (1990) presented at the most 12 chromosomes with this region, while

Daniel-Silva (1996), who studied the same population, found a maximum of 7 chromosomes by silver staining, but the frequency in most metaphases was 2 Ag-NOR positive chromosomes. In the present study, a minimum of 1 and a maximum of 4 chromosomes were found, while the majority of metaphases contained 2 stained nonhomologous chromosomes. The variation in the NORs number can be explained by a difference in the activity of ribosomal cistrons, a reduction of this activity, or even the absence of ribosomal RNA.

The size polymorphism observed in the 2nd submetacentric chromosome pair in most metaphases is common in individual groups that present terminal NORs, which can pose some difficulties in pairing and classifying the chromosome type when using Giemsa staining. These polymorphisms can be explained by an alteration in the quantity of ribosomal DNA, its differentiated transcriptional activity in preceding interphases, or even by both.

Comparing the data of the few published papers about the *Astyanax fasciatus* species from the Alto Paraná basin (Paraná River) against that of the population described here (Table 1) revealed some variations in the karyotypic macrostructure and the Ag-NORs system, which was found to be multiple, as has been described for other populations.

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