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Research Article

Chromosomal distribution of the As51 satellite DNA in two species complexes of the genus *Astyanax* (Pisces, Characidae)

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Abstract

The chromosomal localization of the As51 satellite DNA was identified by fluorescent *in situ* hybridization (FISH) in specimens of the characid fish *Astyanax scabripinnis* and *Astyanax fasciatus*, which are considered species complexes because of their extensive karyotypical and morphological variability. A conserved chromosomal distribution of the As51 satellite, coincident with distal C-banded segments was demonstrated. The alternative interstitial localization of this satellite DNA and possible alterations of its structure suggest that this sequence underwent quantitative, positional and structural variations, as the *A. scabripinnis* and *A. fasciatus* complexes diverged.

Key words: As51, *Astyanax scabripinnis*, *Astyanax fasciatus*, fluorescence *in situ* hybridization (FISH), C-banding, satellite DNA.

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Introduction

A large portion of the genome of eukaryotes consists of repetitive sequences, which are dispersed or organized in tandem (Charlesworth *et al.*, 1994). Tandemly organized repetitive DNA is known as satellite DNA and is normally located in heterochromatic regions detectable by C-banding (Brutlag, 1980; Sumner, 1990). In fish, satellite DNAs co-localize with centromeric and pericentromeric heterochromatin (Haaf *et al.*, 1993; Oliveira and Wright, 1998; Phillips and Reed, 2000; Jesus *et al.*, 2003).

Astyanax scabripinnis and *Astyanax fasciatus* are taxonomic entities of the freshwater Neotropical ichthyofauna, and their morphological and karyotypic characteristics indicate that they are species complexes (Moreira-Filho and Bertollo, 1991; Centofante *et al.*, 2003; Pazza *et al.*, 2006). In this context, the extensive interpopulational variation in localization and amount of C-bands allowed the characterization of distinct populations of both the *scabripinnis* and the *fasciatus* complexes (Moreira-Filho and Bertollo, 1991; Souza and Moreira-Filho, 1995; Souza *et al.*, 1995; Maistro *et al.*, 1998; Mizoguchi and Martins-Santos, 1998; Mantovani *et al.*, 2000; Pazza *et al.*, 2006).

In their study of *A. scabripinnis* from the Rio Grande River Basin in southeastern Brazil, Mestriner *et al.* (2000) identified a 59% AT-rich satellite DNA family with monomeric units of 51 bp, called As51, and determined its chromosomal distribution. These authors showed, by Southern blot hybridization, that As51 was also present in other *A. scabripinnis* populations. This satellite DNA was also detected in *A. fasciatus,* but not in *A. altiparanal* and *A. schubarti* (C.A. Mestriner, personal communication). In this work we analyzed the chromosomal distribution of the As51 satellite DNA in the *scabripinnis* complex. The chromosomal localization of this repetitive DNA was determined in specimens from four populations of this species complex and in a sample of *A. fasciatus*, for comparative analysis.

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Materials and Methods

Specimens, chromosome preparations and C-banding

Specimens were collected from five distinct waterways in three river basins in southeastern Brazil (Table 1). Mitotic chromosomes were obtained as described by Bertollo *et al.* (1978), and C-banding was performed according to Sumner (1972).

FISH procedures

We used as probe the As51 satellite DNA sequence inserted in a Promega pGEM4 plasmid (Mestriner *et al.*, 2000) labeled with biotin-14-dATP by nick translation, using the BioNick™ Labeling System (Gibco BRL), according to the manufacturer's instructions. FISH was carried out according to Pinkel *et al.* (1986), with some modifications. Chromosome preparations were pretreated with

Table 1 - Collection sites and number of specimens of *Astyanax* analyzed.

Species	Waterway	River Basin	Location	F	M	2n
A. scabripinnis	Centenário Stream	Paranapanema River	Maringá/Paraná State	4	4	50
A. scabripinnis	Marrecas Stream	Paranapanema River	Londrina/Paraná State	7	3	48
A. scabripinnis	Viveiro de Mudas Stream	São Francisco River	Três Marias/Minas Gerais State	7	4	50
A. scabripinnis	Curral das Éguas Stream	São Francisco River	São Gonçalo do Abaeté/Minas Gerais State	3	2	46
A. fasciatus	Piracicaba River	Tietê River	Piracicaba/São Paulo State	3	4	46

F = females; M = males; 2n = diploid number.

RNAse (40 μg/mL in 2xSSC) in a moist chamber at 37 °C for one hour, followed by dehydration in an alcohol series. Chromosome denaturation was performed with 70% formamide in 2xSSC at 70 °C for 5 min. The hybridization solution [50% formamide, 10% dextran sulfate, 2xSSC and 1 μL of human placenta DNA (10 mg/mL)] containing approximately 125 ηg of the probe, was kept in boiling water for 10 min, and then 50 μL were placed on each slide and covered with a coverslip. Hybridization was performed in a moist chamber containing 60% formamide for 15 h at 37 °C. Then, the slides were rinsed with 50% formamide in 2xSSC at 42 °C for 20 min and with 0.1xSSC at 60 °C for 15 min. Probe detection was carried out with avidin-FITC (fluorescein isothiocyanate) conjugate, biotinylated anti-avidin (Sigma), and avidin-FITC conjugate. The slides were mounted with 25 μL of Vectashield antifade (Vector), and the chromosomes stained with 1 μL of propidium iodide (50 μg/mL). Metaphase chromosomes were examined under an Olympus BX50 fluorescence microscope and photographed with Kodak Gold Ultra 400 ISO film.

Results

In *A. scabripinnis* specimens from the Centenário and Marrecas streams (in the Paranapanema River Basin), As51 satellite DNA was detected by FISH at the ends of the long arms of most subtelocentric and acrocentric chromosomes and of one submetacentric pair, thus corresponding to the non-perincentromeric C-bands (Figure 1).

The *A. scabripinnis* specimens from the Viveiro de Mudas stream showed interindividual numerical variation of the FISH signals observed at the end of the long arm of an acrocentric pair, as well as of the small subterminal signals on the long arms of some subtelocentric and acrocentric chromosomes (Figures 2a, 2b and 2c). C-bands were present in the pericentromeric regions of all chromosomes; small C-banded distal segments were also detected on several pairs of chromosomes, but showed interindividual numerical variation (Figure 2e). The FISH signals of the As51 satellite probe co-localized with the distal C-bands on the long arms of the acrocentric pair, but not with other C-bands.

The As51 satellite probe did not yield signals on the chromosomes of *A. scabripinnis* specimens from the Curral das Éguas stream (Figure 2d). C-bands were pericentromeric or appeared as distal blocks (Figure 2f).

In *A. fasciatus* from the Piracicaba River, the As51 sequence was detected on the ends of the long arms of the subtelocentric (except for pair 17) and acrocentric chromosomes and of the submetacentric pair 11 (Figure 3a). These FISH signals corresponded to C-bands (Figure 3b). No hybridization signals co-localized with the pericentromeric C-bands or with the distal C-band of chromosome pair 8.

Discussion

In the *A. scabripinnis* population from which it was isolated (Córrego das Pedras stream), the As51 satellite DNA was located on the supernumerary chromosome, on the terminal C-bands of the long arms of some acrocentric chromosomes of the A complement and associated with the nucleolar organizer regions (Mestriner *et al.*, 2000). This chromosomal distribution and those reported here, show that this sequence has a variable localization among *A. scabripinnis* populations. It may even be absent, at least at the level of FISH detection, as in the specimens from the Curral das Éguas stream. However, within this variability, the specimens from the Paranapanema River Basin presented the same chromosomal distribution of the satellite As51, which co-localizes with the C-band blocks on the distal long arms of subtelocentric and acrocentric chromosomes. These populations were shown to share some conserved karyotype characteristics within the *scabripinnis* complex (Mantovani *et al.*, 2000), and our data adds a new-shared character. It is noteworthy that the chromosomal localization of the As51 satellite DNA observed in *A. scabripinnis* from the Paranapanema River Basin is very similar to that of *A. fasciatus*.

The *A. scabripinnis* specimens from the Viveiro de Mudas (our data) and Córrego das Pedras (Mestriner *et al.* 2000) streams present fewer acrocentric chromosomes bearing As51 satellite DNA at the ends of the long arms. Therefore, gain or loss of this satellite DNA must have occurred during the differentiation of the populations of the Paranapanema and São Francisco River Basins. Furthermore, unlike the *A. scabripinnis* specimens from the Paranapanema River Basin, in which every nonpericentromeric C-banded segment bears the As51 family, the *A. scabripinnis* specimens from the São Francisco River Ba-

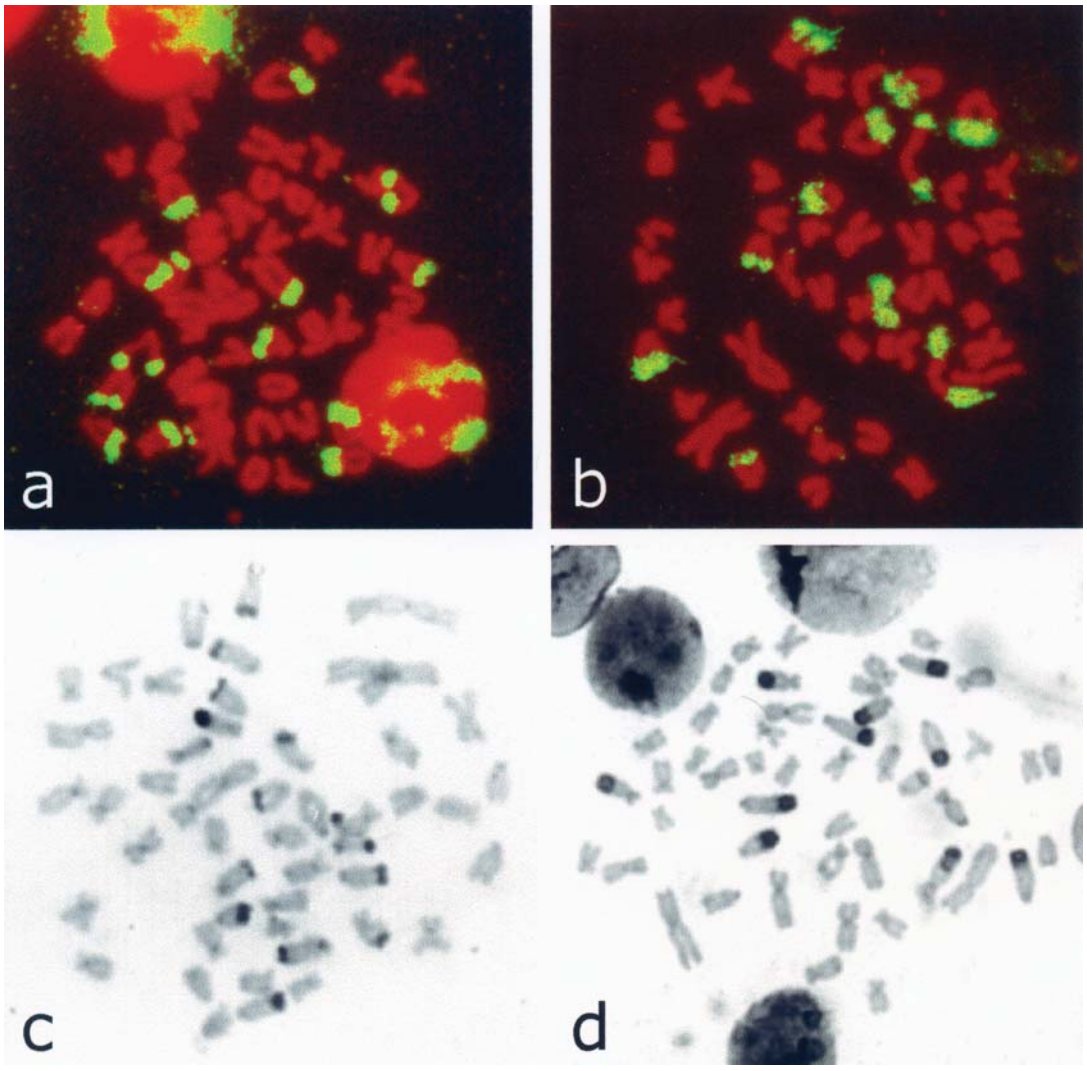

Figure 1 - Chromosomal distribution of As51 satellite DNA after FISH, in specimens of *A. scabripinnis* from the Centenário (a) and Marrecas (b) streams. C-banding pattern in *A. scabripinnis* specimens from the Centenário (c) and Marrecas (d) streams.

sin show distal C-bands which are not coincident with the FISH signals. This is also the case of the short arm of chromosome pair 8 of the *A. fasciatus* specimens analyzed here. These findings indicate that C-band sequences other than the As51 satellite evolved during the diversification of the *A. scabripinnis* and *A. fasciatus* complexes, possibly occupying distinct chromosome territories and being subject to chromosomal dispersion patterns different from those of the As51 satellite.

The interstitial localization of the As51 satellite DNA on some chromosomes of the specimens from the Viveiro de Mudas stream suggests that in this population it probably may have followed an evolutionary path different from that in of the distal segments of acrocentric chromosomes. Mestriner *et al.* (2000) showed that the molecular organization of this sequence is similar to that of mobile elements, suggesting that it may have been transferred to the 45S rDNA spacers of the Córrego das Pedras *A. scabripinnis* population. Analogously, transferences of the As51 family may have caused it to reach the aforementioned interstitial position and to lose its C-band nature, probably as a result of changes in its structural conformation and in the structural chromosomal proteins associated to it.

Based on positive or negative staining by the base-specific fluorochromes mithramycin A and DAPI, Mantovani *et al.* (2004) concluded that the C-band blocks that we showed to be coincident with the As51 satellite DNA localization in *A. scabripinnis* from the Centenário and Marrecas streams possess a different structural organization. These blocks may also differ from those of the Viveiro de Mudas specimens which hybridized with the As51 satellite probe, but were not stained by the base-specific fluorochromes chromomycin A3 and DAPI (our unpublished data). These observations demonstrate that C-banded segments containing As51 satellite might differ structurally.

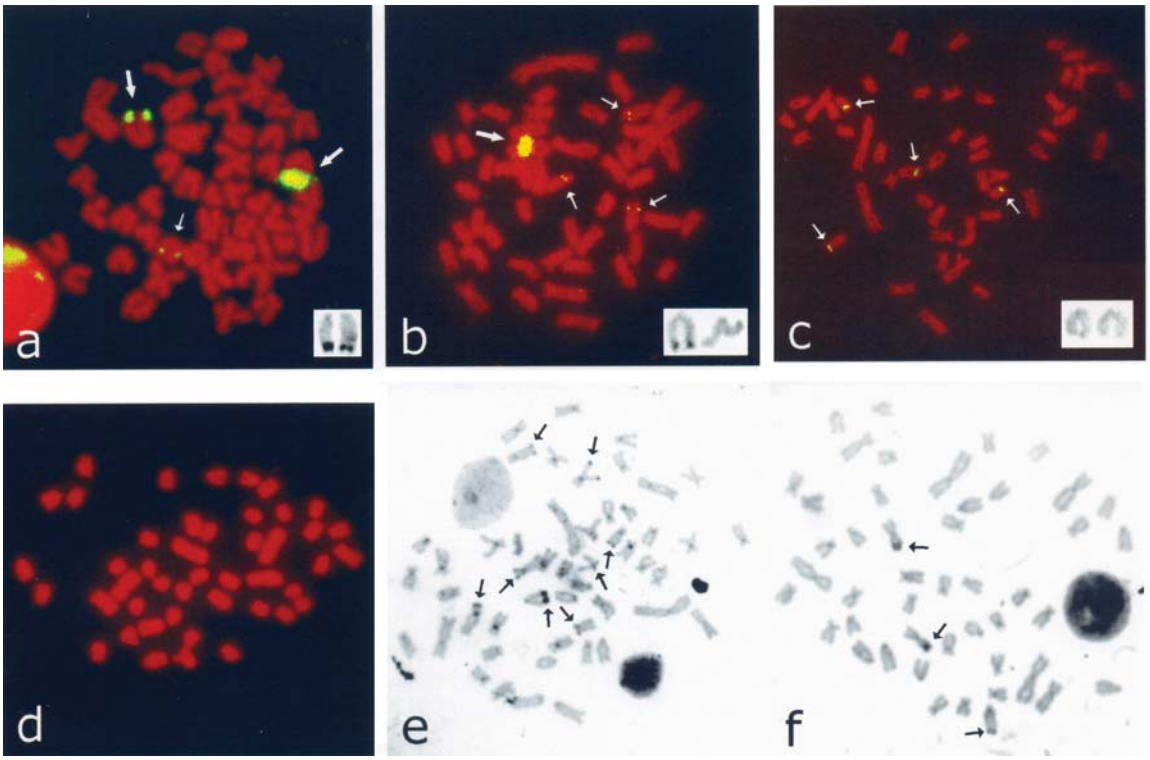

Figure 2 - Chromosomal distribution of FISH signals of As51 satellite DNA in three *A. scabripinnis* individuals from the Viveiro de Mudas stream (a, b and c). Large arrows indicate terminal blocks and small arrows indicate small subterminal signals. The acrocentric chromosomes bearing C-band distal blocks are shown in the inserts. No As51-FISH signals were observed on the chromosomes of *A. scabripinnis* from the Curral das Éguas stream (d). C-banded metaphases from *A. scabripinnis* specimens collected in the Viveiro de Mudas (e) and Curral das Éguas (f) streams (some C-bands are indicated by arrows).

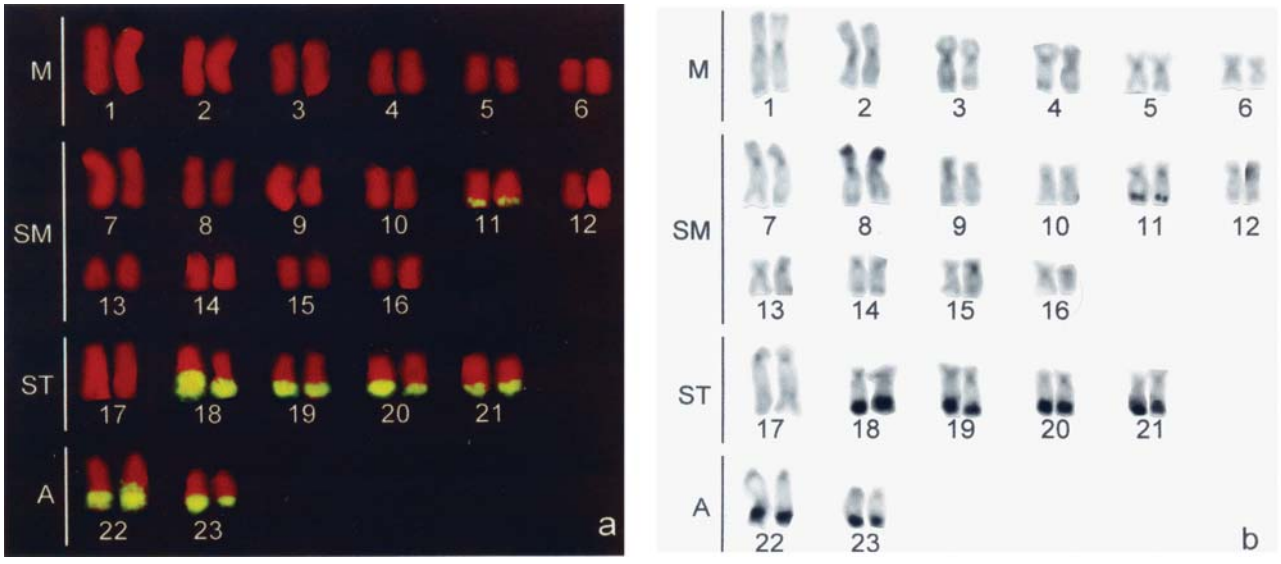

Figure 3 - Chromosomal distribution of As51 satellite DNA after FISH (a), and C-banding (b) in *A. fasciatus* from the Piracicaba River.

The diversification of the As51 satellite DNA in *A. scabripinnis*, in terms of quantity, positioning and structural conformation, reinforces the assumption that this group is a species complex (Moreira-Filho and Bertollo, 1991).

It is well known that heterochromatin can rearrange nuclear architecture and thus interfere in gene activity, depending on the localization effect of heterochromatin blocks on adjacent or distant genes (Henikoff, 1990; Zuckerkandl and Hennig, 1995). Future studies evaluating how the location of the As51 satellite DNA affects molecular, morphological and ecological variants would complement our discussion on the evolution of the As51 satellite DNA in the *scabripinnis* and *fasciatus* species complexes.

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