



## Mapping of the 18S and 5S ribosomal RNA genes in *Astyanax altiparanae* Garutti & Britski, 2000 (Teleostei, Characidae) from the upper Paraná river basin, Brazil

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### Abstract

Fluorescence *in situ* hybridization (FISH) was undertaken in order to determinate the chromosomal distribution pattern of 18S and 5S ribosomal DNAs (rDNA) in four populations of the characid fish *Astyanax altiparanae* from the upper Paraná river basin, Brazil. The 18S rDNA probe FISH revealed numerical and positional variations among specimens from the Keçaba stream compared to specimens of the other populations studied. In contrast to the variable 18S rDNA distribution pattern, highly stable chromosomal positioning of the 5S rDNA sites was observed in the four *A. altiparanae* populations. Divergence in the distribution pattern of 18S and 5S rDNA sites is also discussed.

*Key words:* *Astyanax altiparanae*, fluorescence *in situ* hybridization (FISH), 18S rDNA, 5S rDNA, sequential Ag-NOR.

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### Introduction

Piscine nucleolar organizer regions (NORs) have been extensively analyzed using silver nitrate staining (Ag-NOR) due to the simplicity of this technique. According to Miller *et al.* (1976) this methodology detects only the nucleolar regions that were active in the preceding interphase, and is most suitable for the study of NOR expression. Fluorescence *in situ* hybridization (FISH) is the best method for characterizing NORs for determining the location of both active and inactive ribosomal DNA (rDNA) and almost always allows detection of a larger number of NORs than can be detected using Ag-NOR banding and is also more precise in identifying NORs. In higher eukaryotes the rDNA is organized into two distinct gene classes, the major class (45S rDNA) transcribing 18S, 5.8S, and 28S rRNA genes and the minor class (5S rDNA) that transcribes 5S rRNA genes. The 45S rDNA active sites have shown to have positional coincidence with chromosome NORs but the 5S rDNA sites are unrelated to NORs.

In *Astyanax altiparanae*, previously known as *Astyanax bimaculatus* for the upper Paraná river in Brazil (Garutti and Britski, 2000), cytogenetic studies in different populations have shown a constant diploid number of  $2n = 50$  chromosomes, although with differences in their karyotype formulae and with regard to number and position of NORs (Daniel-Silva and Almeida-Toledo, 2001; Pacheco *et al.*, 2001; Fernandes and Martins-Santos, 2004).

Multiple Ag-NORs have been a common characteristic in *A. altiparanae*, with the number reaching 10 NOR-bearing chromosomes for an *A. altiparanae* specimen from the Índios river in the Brazilian state of Paraná (Fernandes and Martins-Santos, 2004).

In the study described in this paper, FISH was used to determine the chromosomal location of 18S and 5S rDNA sites in four *A. altiparanae* populations with the aim of contributing to the better understanding of the genomic organization of this species.

### Materials and Methods

We collected 31 *Astyanax altiparanae* Garutti & Britski, 2000 (Teleostei, Characidae) specimens from the upper Paraná river basin in the Brazilian state of Paraná, nine from the main Paraná river, ten from Tatupeba stream, four from Keçaba stream and eight from Maringá stream. Mitotic chromosomes were obtained from kidney cells using the methodology described by Bertollo *et al.* (1978). Ribosome cistrons were detected using 18S rDNA probes (18S-FISH) and 5S rDNA probes (5S-FISH) probes as described by Pinkel *et al.* (1986), with slight modifications. The 18S and 5S probes were obtained from *Astyanax scabripinnis* genomic DNA and PCR amplified using the NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS8 (5'-TCCGCAGGTTACCTACGGA-3') primers (White, 1990) and the A (5'-TACGCCCGATCTCGTCCGATC-3') and B (5'-CAGGCTGGTATGGCCGTAAGC-3') primers (Martins and Galetti, 1999; Wasko *et al.*, 2001). Sequential silver nitrate nucleolus organizer region (Ag-

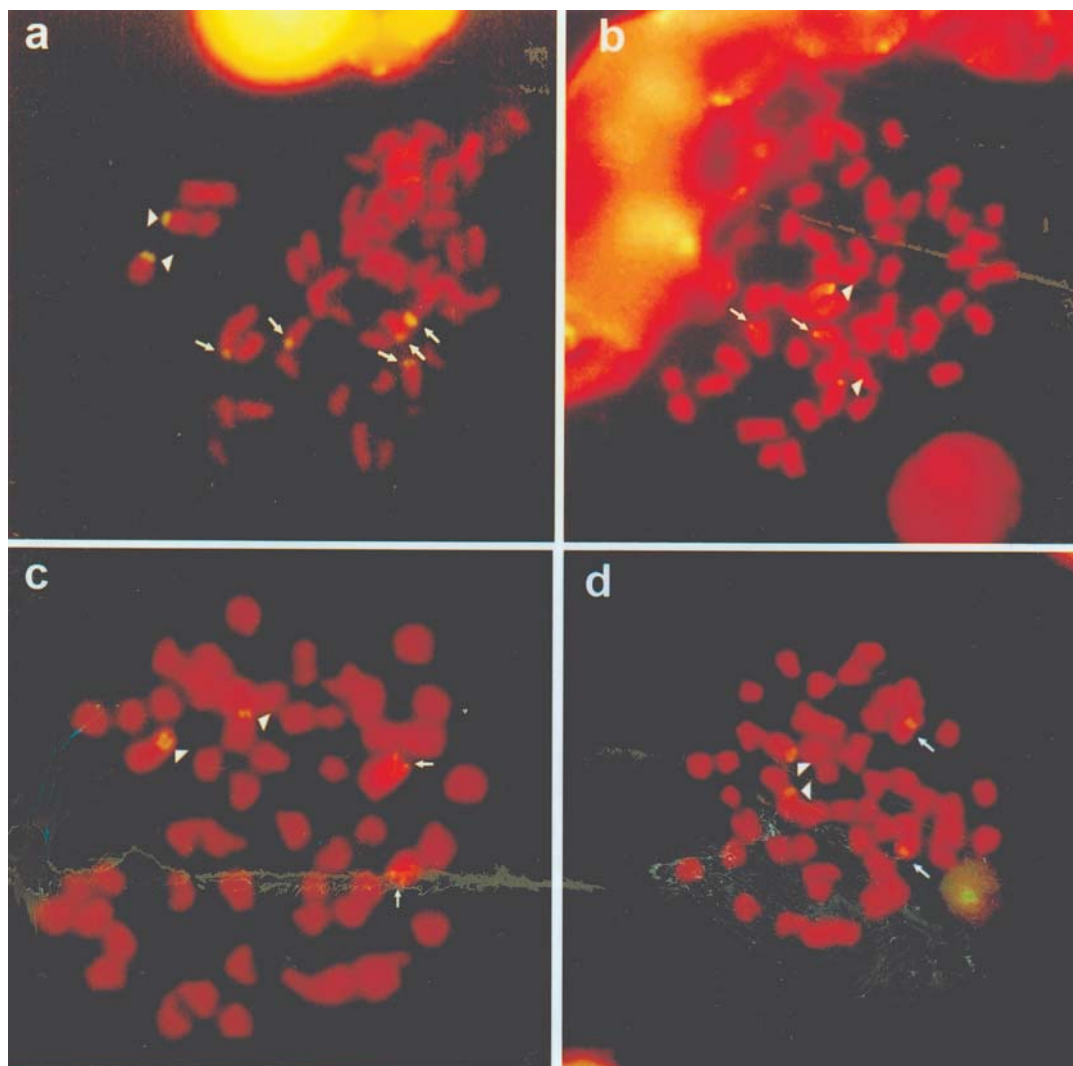
NOR) staining (Howell and Black, 1980) was performed after rinsing the FISH slides in tap water. At least 20 metaphases per specimen were examined in a Carl Zeiss Axioskop 2 Plus fluorescence microscope and digitally photographed using a coupled AxioCam camera and Axiovision Software (Carl Zeiss, Göttingen, Germany).

## Results and Discussion

The four *A. altiparanae* populations revealed a monomorphic macrokaryotype constitution, with  $2n = 50$  chromosomes (6 M, 26 SM, 6 ST and 12A). Thus, specimens of *A. altiparanae* from Tatupeba, Keçaba and Maringá streams presented karyotype formulae identical to the *A. altiparanae* specimens from the Paraná river previously studied by Fernandes and Martins-Santos (2004).

The 18S-FISH technique revealed a bright fluorescence signal spread at the telomeric region of seven chromosomes (the 2A short arm and five other chromosomes)

for Keçaba stream specimens and the telomeric region of four chromosomes (the 2A short arm and two other chromosomes) for Paraná river, Tatupeba and Maringá stream specimens (Figure 1). Almeida-Toledo *et al.* (2002) also reported four chromosomes (2 A and 2 M) were marked with a 28S rDNA probe in *A. altiparanae* specimens, although in two metacentric chromosomes the probes were pericentromeric. The same chromosomal location of 45S rDNA (18S or 28S rDNA) on the short arm of 2 acrocentric *A. altiparanae* chromosomes was seen in our present study and was also observed for specimens from the Mogi-Guaçu river in the Brazilian state of Paraná (Almeida-Toledo *et al.*, 2002), indicating that these are marker chromosomes for this species. On the other hand, the other sites seem not to be conserved among *A. altiparanae* populations, which differ in the position (telomeric or pericentromeric) and type of chromosomes.

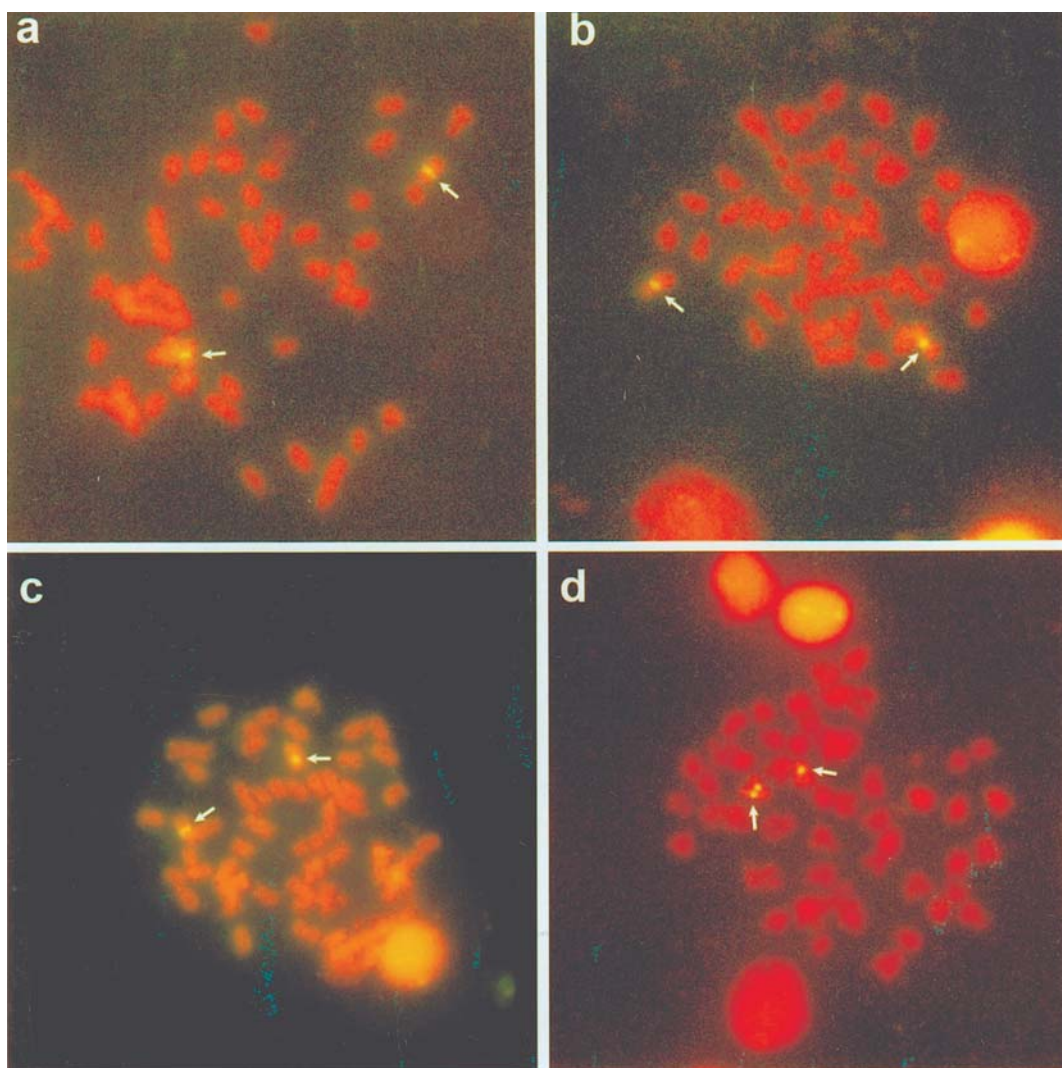


**Figure 1** - Fluorescence *in situ* hybridization (FISH) *Astyanax altiparanae* metaphases showing the chromosomal location of 18S rDNA sites in populations from the Keçaba stream (a), Paraná river (b), Tatupeba stream (c) and Maringá stream (d). The arrows indicate the ribosomal cistrons carrier chromosomes and the arrowheads the ribosomal cistrons in the short arm of 2 acrocentric chromosomes of four populations.

Numerical and positional variations of the 18S rDNA sites reported in specimens of *A. altiparanae* from the Keçaba stream in comparison to the other populations analyzed have also been recorded in other *Astyanax* species, including *A. scabripinnis* (Ferro *et al.*, 2001; Souza *et al.*, 2001; Mantovani *et al.*, 2005; Fernandes and Martins-Santos, in press) and *Prochilodus lineatus* (Jesus and Moreira-Filho, 2003). According to Schweizer and Loidl (1987), the proximity of telomeric regions within interphase nuclei would facilitate genetic material transference as predicted by Rabl's model. In distinct *A. scabripinnis* populations this model has been suggested to explain heterochromatin dispersion in the telomeric regions (Souza *et al.*, 1996; Mantovani *et al.*, 2000; Fernandes and Martins-Santos, 2003). Therefore, the telomeric location of the 18S rDNA sites in the four *A. altiparanae* populations would facilitate transference events, which seems to have

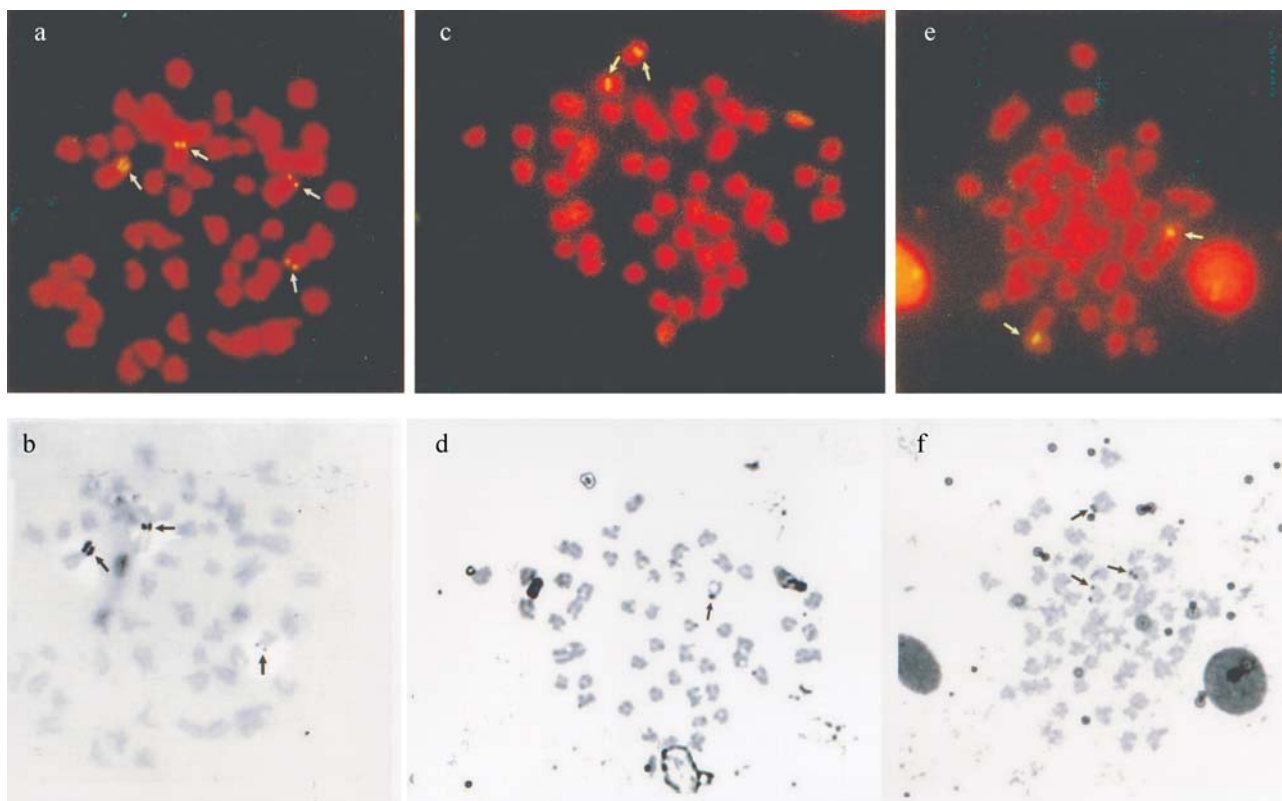
occurred in the case of *A. altiparanae* from the Keçaba stream.

Sequential Ag-staining of an 18S-FISH slide of a specimen from the Tatupeba stream revealed that of the four marked chromosomes three were Ag-NOR positive (Figure 3a, b). Paintner-Marques *et al.* (2002) have pointed out that not all the existing DNAr cistrons are active in multiple NORs systems, so the variation observed in our study and described in other papers (Almeida-Toledo *et al.* 2002, Paintner-Marques *et al.* 2002) probably occurred as a result of the regulation of genetic activity. Moreover, NOR size heteromorphism between homologous chromosomes revealed for the 18S-FISH and sequential Ag-NOR in the short arm of 2 acrocentric chromosomes (Figure 3a, b) indicates variation in the number of copies of this rDNA between homologous chromosomes. This NOR size heteromorphism may have occurred by through transposition



**Figure 2** - Fluorescence *in situ* hybridization (FISH) *Astyanax altiparanae* metaphases showing the chromosomal location of 5S rDNA sites in populations from the Maringá stream (a), Paraná river (b), Keçaba stream (c) and Tatupeba stream (d). The arrows indicate the ribosomal cistrons carrier chromosomes.





**Figure 3** - Sequential Ag-staining of 18S-FISH (a, b) and 5S-FISH (c-f) slides. Specimens from the Tatupeba (a, b), Maringá (c, d) and Keçaba (e, f) streams. Note that the 5S rDNA loci do not lie on the same Ag-NOR chromosome.

events or unequal crossing-over and not the differential expression of NORs.

In contrast to the variability detected regarding the 18S rDNA distribution pattern, we observed a highly conserved chromosomal position of 5S rDNA sites in the four *A. altiparanae* populations. The 5S-FISH method revealed bright fluorescence signal spread over the pericentromeric region of a single, probably submetacentric, chromosomal pair (Figure 2). Considering that 5S rDNA sequences were not localized in the terminal regions of chromosomes the events that dispersed the 18S rDNA may not have been acting upon the 5S rDNA sites. Moreover, the 5S rDNA interstitial position has been found in most species of several orders. For these reasons, the highly conserved chromosomal position of 5S rDNA sites observed in the four *A. altiparanae* populations may have derived from the interstitial localization of these sites in the chromosomes. The 5S rDNA genes situated in a single chromosomal locus have also been identified in *A. altiparanae* and *A. lacustris* (Almeida-Toledo *et al.*, 2002) and other piscine species, including the Atlantic salmon (Pendás *et al.* 1994), *Anguilla anguilla* (Martinez *et al.* 1996), *Prochilodus lineatus* (Jesus and Moreira-Filho, 2003), *Neoplecostomus microps* and *Harttia loricariformis* (Kavalco *et al.* 2004), possibly corresponding to a more ancestral condition in fishes.

Sequential Ag-staining of 5S-FISH slides of *A. altiparanae* specimens from Maringá (Figure 3c, d) and Keçaba (Figure 3e, f) streams revealed that 5S rDNA was not located on the same Ag-NOR chromosomes. Therefore, investigations utilizing double FISH with the two rDNA probes should be carried out in order to prove the different chromosomal location of 18S and 5S rDNA in these specimens. Different chromosomal sites for NOR and 5S rDNA have also been reported for *Anguilla anguilla* (Martinez *et al.* 1996), *Salmo trutta* (Moran *et al.* 1996), *Leporinus elongatus*, *Leporinus obtusidens* and *Leporinus friderici* (Martins and Galetti 1999), *Oreochromis niloticus* (Martins *et al.* 2000) and *A. scabripinnis* (Fernandes and Martins-Santos, in press). According to Lucchini *et al.* (1993) and Suzuki *et al.* (1996), this arrangement is frequently observed in vertebrates. However, Almeida-Toledo *et al.* (2002) detected *in situ* signals for the major rDNA (28S rDNA) co-localized with the 5S rDNA clusters in the pericentromeric region of one marker chromosome in five *Astyanax* species and Mantovani *et al.* (2005) used double FISH to show that the 45S and 5S rDNA loci were syntenic in an *A. scabripinnis* chromosome.

There are still only a few studies which have used FISH to investigate the genus *Astyanax*, and the majority of these studies have been limited to *A. scabripinnis* (Souza *et*

*al.*, 2001; Ferro *et al.*, 2001; Mantovani *et al.*, 2005; Fernandes and Martins-Santos, in press). In *A. altiparanae*, only one Mogi-Guaçu river population (Almeida-Toledo *et al.*, 2002) and the populations analyzed in the present study have been reported as utilizing the FISH technique with rDNA probes. Our results are important for the better characterization of the chromosomal location of *Astyanax altiparanae* 5S, 18S or 28S rDNA and may also aid cytogenetic studies of related species.

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