Genetics and Molecular Biology, 29, 3, 464-468 (2006)

www.sbg.org.br

Research Article

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

Carlos Alexandre Fernandes and Isabel Cristina Martins-Santos

Departamento de Genética e Biologia Celular, Universidade Estadual de Maringá, Maringá, PR, Brazil.

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.

Received: July 25, 2005; Accepted: February 14, 2006.

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.

Send correspondence to Carlos Alexandre Fernandes. Avenida Colombo 5790, 87020-900 Maringá, PR, Brazil. E-mail: fxande@pop.com.br.

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'-TCCGCAGGTTCACCTACGGA-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.

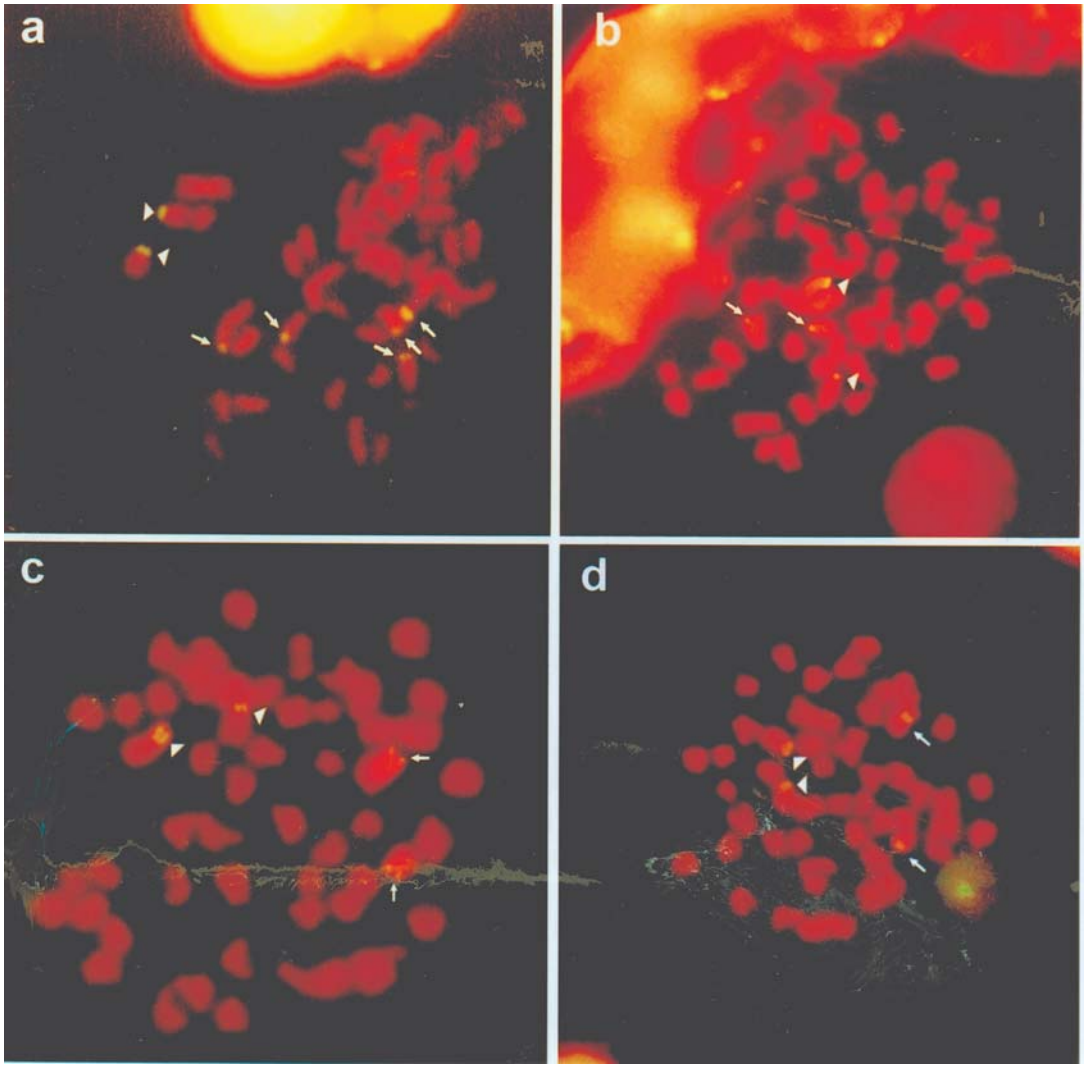

Figure 1 - Fluorescence *in situ* hybridization (FISH) *Astyanax altiparanae* metaphases showing the chromosomal location of 18S rDNA sites in populations from the Keçaba stream (a), Paraná river (b), Tatupeba stream (c) and Maringá stream (d). The arrows indicate the ribosomal cistrons carrier chromosomes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

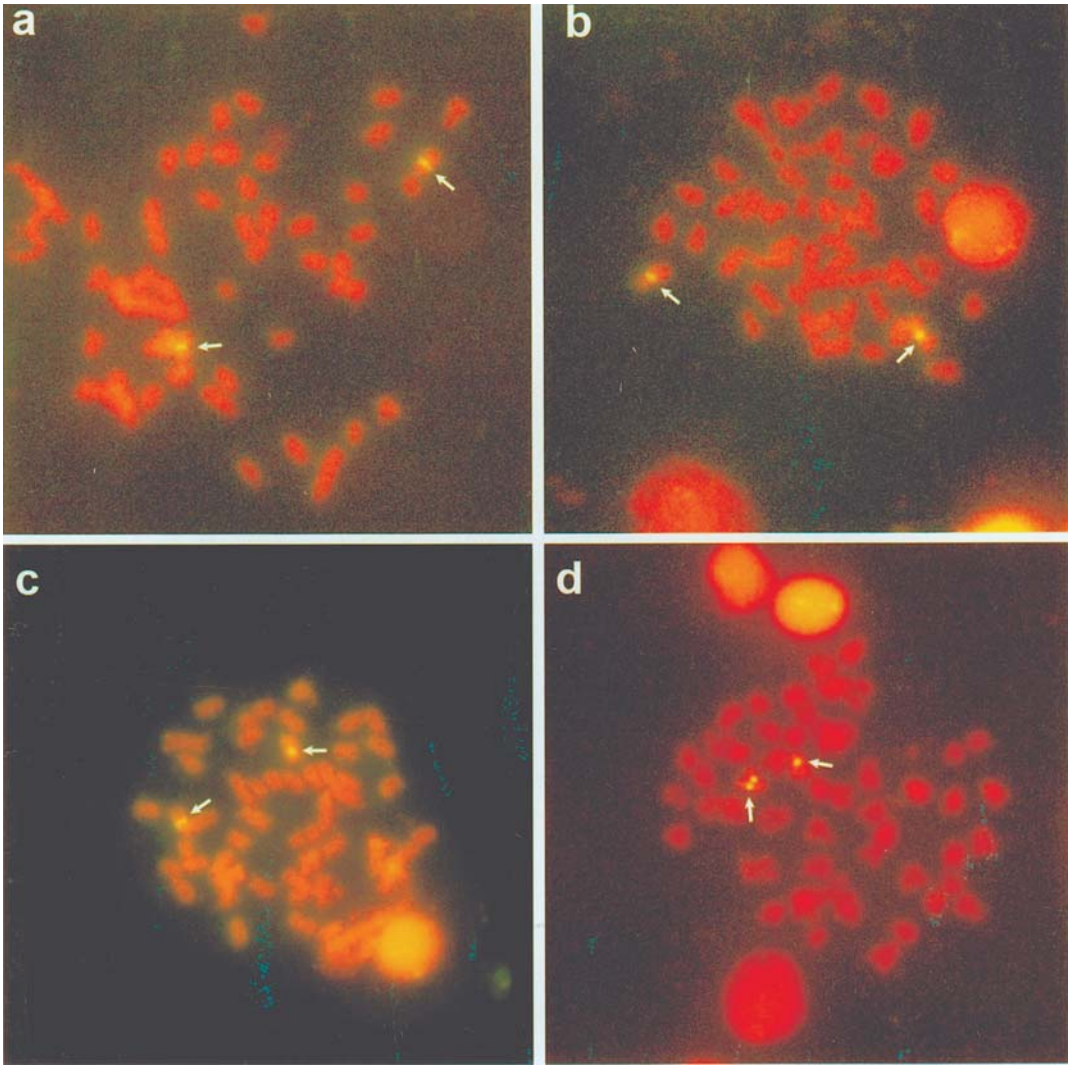

Figure 2 - Fluorescence *in situ* hybridization (FISH) *Astyanax altiparanae* metaphases showing the chromosomal location of 5S rDNA sites in populations from the Maringá stream (a), Paraná river (b), Keçaba stream (c) and Tatupeba stream (d). The arrows indicate the ribosomal cistrons carrier chromosomes.

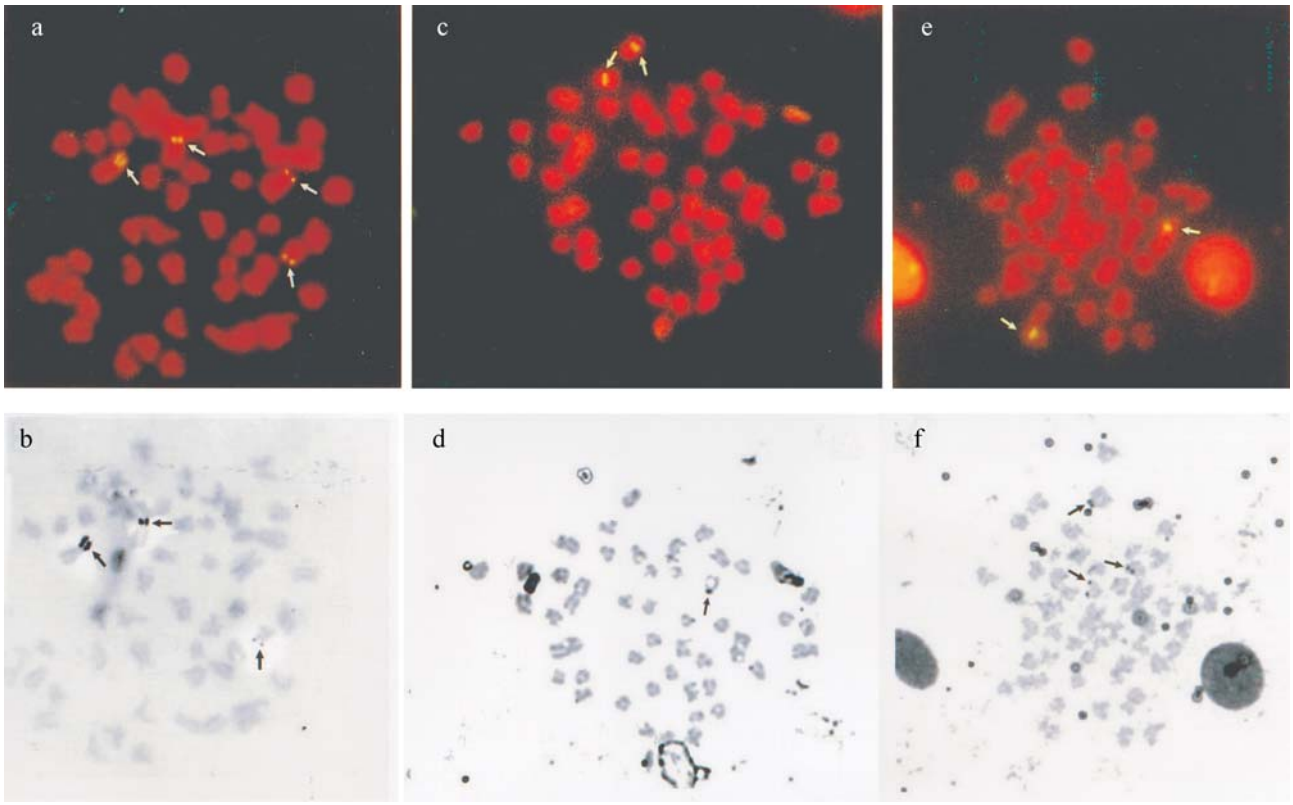

Figure 3 - Sequential Ag-staining of 18S-FISH (a, b) and 5S-FISH (c-f) slides. Specimens from the Tatupeba (a, b), Maringá (c, d) and Keçaba (e, f) streams. Note that the 5S rDNA loci do not lie on the same Ag-NOR chromosome.

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 cytotaxonomic studies of related species.

Acknowledgments

The authors thank the Brazilian agency Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

References

Almeida-Toledo LF, Ozouf-Costaz C, Foresti F, Bonillo C, Porto-Foresti F and Daniel-Silva MFZ (2002) Conservation of the 5S-bearing chromosome pair and co-localization with major rDNA clusters in five species of *Astyanax* (Pisces, Characidae). Cytogenetic Genome Res 97:229-233.

Bertollo LAC, Takahashi CS and Moreira-Filho O (1978) Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae*).* Brazil J Genet 1:103-120.

Daniel-Silva MFZ and Almeida-Toledo LF (2001) Chromosome R-banding pattern and conservation of a marker chromosome in four species, genus *Astyanax* (Characidae, Tetragonopterinae). Caryologia 54:209-215.

Fernandes CA and Martins-Santos IC (2003) Cytogenetic characterization of two populations of *Astyanax scabripinnis* (Pisces, Characiformes) of the Ivaí Basin PR Brazil. Cytologia 68:289-293.

Fernandes CA and Martins-Santos IC (2004) Cytogenetic studies in two populations of the *Astyanax altiparanae* (Pisces, Characiformes). Hereditas 141:328 332.

Ferro DAM, Néo DM, Moreira-Filho O and Bertollo LAC (2001) Nucleolar organizing regions, 18S and 5S rDNA in *Astyanax scabripinnis* (Pisces, Characidae): Populations distribution and functional diversity. Genetica 110:55-62.

Garutti V and Britski HA (2000) Descrição de espécie nova de *Astyanax* (Teleostei, Characidae) da bacia do alto rio Paraná e considerações sobre as demais espécies do gênero na bacia. Comn Mus Ciênc Tecnol 13:65-88.

Howell WM and Black DA (1980) Controlled silver staining of nucleolus organizer regions with a protective colloidal developer: As 1-step method*.* Experientia 36:1014-1015.

Jesus CM and Moreira-Filho O (2003) Chromosomal location of 5S and 18S rRNA genes in Prochilodus lineatus (Characiformes, Prochilodontidae). Caryologia 56:281-287.

Kavalco KF, Pazza R, Bertollo LAC and Moreira-Filho O (2004) Gene mapping of 5S rDNA sites in eight fish species from the Paraíba do Sul river basin, Brazil. Cytogenetic. Genome Res 106:107-110.

Lucchini S, Nardi I, Barsacchi G, Batistoni R and Andronico F (1993) Molecular cytogenetics of the ribosomal (18S + 28S and 5S) DNA loci in primitive and advanced urodele amphibians. Genome 36:762-773.

Mantovani M, Abel LDS, Mestriner CA and Moreira-Filho O (2000) Accentuated polymorphism of heterochromatin and nucleolar organizer regions in *Astyanax scabripinnis* (Pisces, Characidae): Tools for understanding karyotypic evolution. Genetica 109:161-168.

Mantovani M, Abel LDS and Moreira-Filho O (2005) Conserved 5S and variable 45S rDNA chromosomal localisation revealed by FISH in *Astyanax scabripinnis* (Pisces, Characidae). Genetica 123:211-216.

Martinez JL, Moran P, Garcia-Vasquez E and Pendas AM (1996) Chromosomal localization of the major and 5s rRNA genes in the European eel (Anguilla anguilla). Cytogenet Cell Genet 73:149-152.

Martins C and Galetti Jr. PM (1999) Chromosomal localization of 5S rDNA genes in Leporinus fish (Anostomidae, Characiformes). Chrom Res 7:363-367.

Martins C, Wasko AP, Oliveira C and Wright JM (2000) Nucleotide sequence of 5s rDNA and localization of the ribosomal RNA genes to metaphase chromosomes of the Tilapiine cichlid fish, *Oreochromis niloticus.* Hereditas 133:39-46.

Miller DA, Dev VG, Tantravashi R and Miller OJ (1976) Supression of human nucleolus organizer activity in mouse-human somatic hybrid cells. Expl Cell Res 101:235-243.

Moran P, Martinez JL, Garcia-Vasquez E and Pendas AM (1996) Sex linkage of *5s* rDNA in rainbow trout (Oncorhynchus mykiss). Cytogenet Cell Genet 75:145-150.

Pacheco RB, Giuliano-Caetano L and Dias AL (2001) Cytotypes and Multiple NORs in an *Astyanax altiparanae* population (Pisces, Tetragonopterinae). Chromosome Science 5:109-114.

Paintner-Marques TR, Giuliano-Caetano L and Dias AL (2002) Multiple NORs in *Bryconamericus* aff. *exodon* (Osteichthyes, Characidae, Tetragonopterinae). Hereditas 137:107-112.

Pendás AM, Móran P, Freije JP and Garcia-Vásquez E (1994) Chromosomal location and nucleotide sequence of two tandem repeats of the Atlantic salmon 5S rDNA. Cytogenet. Cell Genet 67:31-36.

Pinkel D, Straume T and Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934-2938.

Schweizer D and Loidl J (1987) A model for heterochromatin dispersion and the evolution of C-band pattern. Stahl A, Luciani JM and Vagner-Capodano AM (eds) Chromosome Today. 1st edition, v. 9. Allen & Unwin, New York, pp 61-74.

Souza IL, Moreira-Filho O and Galetti Jr. PM (1996) Heterochromatin differentiation in the characid fish *Astyanax scabripinnis*. Brazil J Genet 19:405-410.

Souza IL, Galian J, De La Ru'a P, Bertollo LAC and Moreira-Filho O (2001) Non-radom distribuition of the GC-rich heterochromatin and nucleolar rDNA sites on *Astyanax scabripinnis* chromosomes. Cytologia 66:85-91.

Suzuki H, Sakurai S and Matsuda Y (1996) Rat rDNA spacer sequences and chromosomal assignment of the genes to the extreme terminal region of chromosome 19. Cytogenet. Cell Genet 72:1-4.

Wasko AP, Martins C, Wright JM and Galetti Jr. PM (2001) Molecular organization of 5S rDNA in fishes of the genus Brycon. Genome 44:893-902.

White TJ, Bruns T, Lee S and Taylor J (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics in PCR Protocols: A Guide to Methods and Applications. Academic Press Inc., San Diego, pp 315-322.

Associate Editor: Fausto Foresti