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Research Article

Satellite DNA sites in four species of the genus *Astyanax* (Teleostei, Characiformes)

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Abstract

Cytogenetic data about satellite DNA distribution in four *Astyanax* species (Characidae) from the Paraitinga river, Paraíba do Sul river basin, Brazil, are presented. In order to characterize the constitutive heterochromatin, C-banding, chromomycin A_3 and DAPI fluorescence staining, as well as fluorescence *in situ* hybridization (FISH) with the satellite DNA As-51 probe were performed. *A. scabripinnis* and *A. parahybae* presented 2n = 50 and 2n = 48 chromosomes, respectively. The heterochromatin was located in the pericentromeric and terminal regions of many chromosomes, corresponding to GC-positive regions and to the As-51 satellite DNA in terminal regions. *A. intermedius* and *A. giton*, both with 2n = 50 chromosomes, showed little heterochromatin, mostly restricted to the terminal and pericentromeric regions of a few chromosomes. No GC-positive regions, neither any correspondence between the scarce heterochromatin of these species and the As-51 satellite DNA was observed. AT-positive blocks were not detected in any of the species studied. Based on these and other available data, the hypothesis that *Astyanax* represents a polyphyletic group is discussed.

Key words: Astyanax, fluorescence *in situ* hybridization (FISH), heterochromatin, karyotypic diversity, satellite DNA.

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Introduction

Astyanax, popularly known as lambaris or piabas, comprises around 74 species distributed from southern United States to northern Argentina (Eigenmann, 1921). In a recent review, about 90 valid species were considered *insertae sedis* in Characidae (Reis *et al.*, 2003). Many of these species inhabit stream headwaters, including highly irregular regions (such as mountain ranges) where the headwaters of the Paraitinga river (Paraíba do Sul river basin, Brazil) are located. Morphological analyses led Weitzman and Malabarba (1998) to propose the genus polyphyly, which was corroborated by the study of 5S rDNA sites distribution (Kavalco *et al.*, 2004a).

Chromosome variability in *Astyanax* species reflects their ecological and genetic characteristics. It comprises variations in diploid numbers (2n = 36 to 2n = 50 chromosomes), karyotypical macrostructure, occurrence of natural polyploidy, presence of B-chromosomes, constitutive heterochromatin polymorphisms, and nucleolar organizer regions (NORs) distribution. Based on these features, at least three "species complexes" can be recognized: *A. scabripinnis* (Moreira-Filho and Bertollo, 1991), *A. altiparanae* (Fernandes and Martins-Santos, 2005) and *A. fasciatus* (Pazza *et al.*, 2006).

Constitutive heterochromatin corresponds to repetitive regions in the genome which are extremely condensed during the cell cycle and have been claimed to have no transcriptional activity. Heterochromatin has played an important role in the study of the diversification of some fish groups, as in the genera *Brycon* (Margarido and Galetti Jr., 1999) and *Leporinus* (Galetti Jr. *et al.*, 1991; Molina *et al.*, 1998; Margarido and Galetti Jr., 2000). In these genera, the karyotypic macrostructure is relatively constant, but the heterochromatin differs between species (Galetti Jr. *et al.*, 1991). In the *Astyanax scabripinnis* species complex (Moreira-Filho and Bertollo, 1991), heterochromatin polymorphisms were useful in the identification of some populations (Mantovani *et al.*, 2000).

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Chromosome banding has been extensively used to study the distribution and nature of constitutive heterochromatin in fishes. The use of base-specific fluorochromes, such as chromomycin A_3 and DAPI, has helped to characterize the composition of the constitutive heterochromatin (GC-, AT-positive or devoid of GC or AT clusters). The occurrence of GC-positive heterochromatin sequences adjacent to or interspersed among NORs has been described for many organisms (Mayr *et al.*, 1985; Schmid and Guttenbach, 1988; Phillips and Hartley, 1988; Sola *et al.*, 1992; Kavalco *et al.*, 2004b). GC-positive heterochromatin not associated with NORs are very rare among fishes (Souza *et al.*, 1996; Artoni *et al.*, 1999; Margarido and Galetti Jr., 2000; Kavalco *et al.*, 2004b). AT-positive regions are also quite uncommon among fishes and have mainly been reported among some Hypostominae species (Artoni and Bertollo, 1999).

Fluorescence *in situ* hybridization (FISH) used to locate DNA sequences has provided more accurate information on the genomic organization of several organisms. In fish, the hybridization of rDNA probes and of satellite DNAs has provided insights into the relationship among species and their karyotypic diversification (Almeida-Toledo *et al.*, 2002; Ferro *et al.*, 2001; Jesus *et al.*, 2003; Kavalco and Moreira-Filho, 2003; Kavalco *et al.*, 2004a; Kavalco *et al.*, 2005; Martins and Galetti Jr., 1999; Mestriner *et al.*, 2000; Pazza *et al.*, 2005; Vicente *et al.*, 2004).

Satellite DNA consists of many tandem repeats of identical or related short basic repeats. The As-51 satellite DNA is a multimeric fragment of 51pb obtained by *Kpn*I restriction enzyme digestion of *Astyanax scabripinnis* samples. The As-51 satellite DNA seems to have similarities with a portion of the *Anopheles gambiae* RT2 retroposon, suggesting it could have arisen from a mobile element (Mestriner *et al.*, 2000). FISH revealed that the As-51 repetitive DNA family was located in a small portion of the *A. scabripinnis* genome, distributed in all the distal C-bands (Mestriner *et al.*, 2000).

Aiming to compare the nature and distribution of constitutive heterochromatin in four *Astyanax* species (*A. scabripinnis*, *A. parahybae*, *A. intermedius* and *A. giton*) from the Paraíba do Sul river basin, C-banding, chromomycin A_3 and DAPI staining, and FISH with the As-51 satellite DNA probe were performed. This is the first report on the nature and distribution of heterochromatin in *A. intermedius* and *A. giton* of the chromomycin A_3 staining in *A. parahybae* and of As-51 satellite DNA distribution in *A. intermedius, A. giton* and *A. parahybae*. Constitutive heterochromatin data for *A. scabripinnis* are compared with previous reports.

Material and Methods

Four *Astyanax* species were analyzed. Six *A. scabripinnis* specimens (all female) were collected from the Macacos river (22°50,050' S, 44°50'940' Wo), nine *A. parahybae* specimens (6 males and 3 females) and 28 *A. intermedius* specimens (2 males and 26 females) from the Paraitinga river (22°52,225' S, 44°51,041' Wo). A total of 21 *A. giton* specimens were collected in two different sites, three males and ten females in the Paraitinga river (22°52,225' S, 44°51,041' Wo), and three males, four females and a sexually undetermined specimen in the Jacuí stream (23°02,436' S, 44°56,103' Wo), both part of the Paraíba do Sul river basin. The samples were identified and deposited in the ichthyological collection of the National Museum of Rio de Janeiro (Museu Nacional do Rio de Janeiro - Brazil), under the registration OMNRJ REG 20020417.

Mitotic chromosomes were obtained according to Bertollo *et al.* (1978). C-banding, chromomycin A_3 and DAPI staining were performed according to Sumner (1972), Schmid (1980) and Schweizer (1980), respectively. Fluorescent in situ hybridization (FISH) with the As-51 satellite DNA probe was performed according to Pinkel *et al.* (1986), with high stringency washes and control experiments. The As-51 probe isolated from *A. scabripinnis* (Mestriner *et al.*, 2000) was labeled with biotinilated uridine (BdUTP) according to the Nick Translation Bionick Labeling System Kit protocol (Invitrogen®). The detection was performed with avidin-FITC and biotin-conjugated-anti-avidin. The slides were mounted with Vectashield Mounting Medium, (Vector®) with propidium iodide (1.5 µg/mL). The preparations were analyzed under a Olympus BX50 epifluorecent microscope and the images were captured (with a 1Mp resolution) with the CoolSnap Pro and the Image Pro Plus softwares (Media Cybernetics). The chromosomes were classified according to their arm ratios, as M-metacentric; SM-submetacentric; ST-subtelocentric; and A-acrocentric (Levan *et al.,* 1964).

Results

All species presented heterochromatin distributed in pericentromeric or distal chromosome regions. *Astyanax scabripinnis* presented 2n = 50 and a karyotypic formula composed of 8M+20SM+8ST+14A. In this species, C-banding revealed constitutive heterochromatin in the pericentromeric regions of several chromosomes, and in the terminal regions of the long arms of pairs 1, 2, 7, 14 and 21; a submetacentric pair had an almost entirely heterochromatic short arm (Figure 1-a). Chromomycin A_3 staining showed three GC-positive sites: two in the terminal region of the short arm of a subtelocentric chromosome pair and one in the long arm of a submetacentric chromosome (Figure 2-a). No AT-positive heterochromatin was observed (data not shown). One subtelocentric and two acrocentric chromosome pairs and a single submetacentric chromosome presented conspicuous terminal signals after fluorescence *in situ* hybridization with the pAs-51 probe (Figure 3-a).

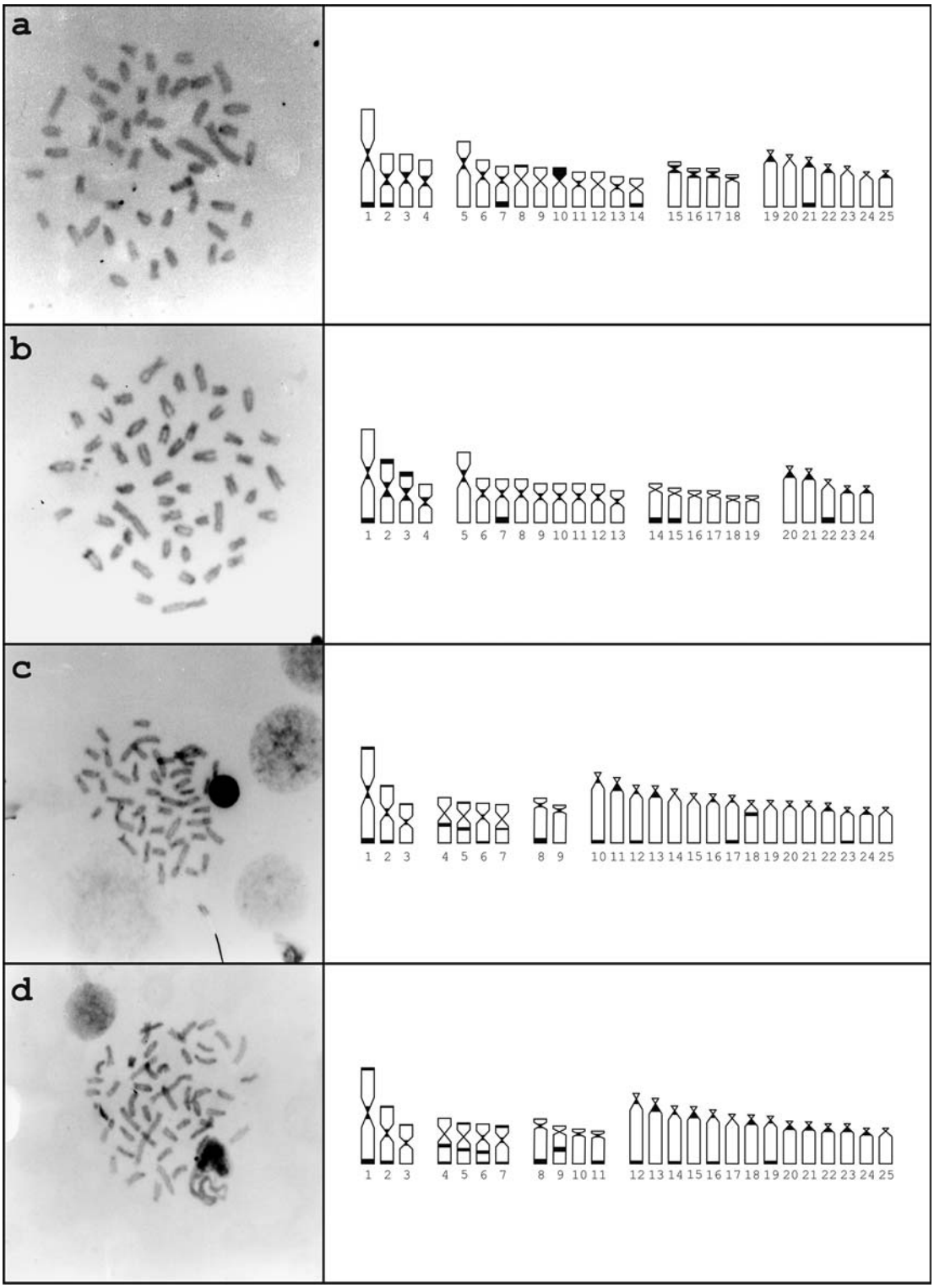

Figure 1 - C-banding metaphases and ideograms of (a) *A. scabripinnis*; (b) *A. parahybae*; (c) *A. intermedius*; and (d) *A. giton*.

Astyanax parahybae showed 2n = 48 chromosomes, with the karyotypic formula M+18SM+12ST+10A. C-banding revealed constitutive heterochromatin in the pericentromeric regions and in the terminal position of the long arms of chromosome pairs 1, 7, 14, 15 and 16, and in the short arms of chromosomes 2 and 3 (Figure 1-b). GC-positive regions were detected in the terminal region of the short arm of a single large metacentric chromosome and of a medium-sized metacentric pair (Figure 2-b). No AT-positive heterochromatin was observed (data not shown).

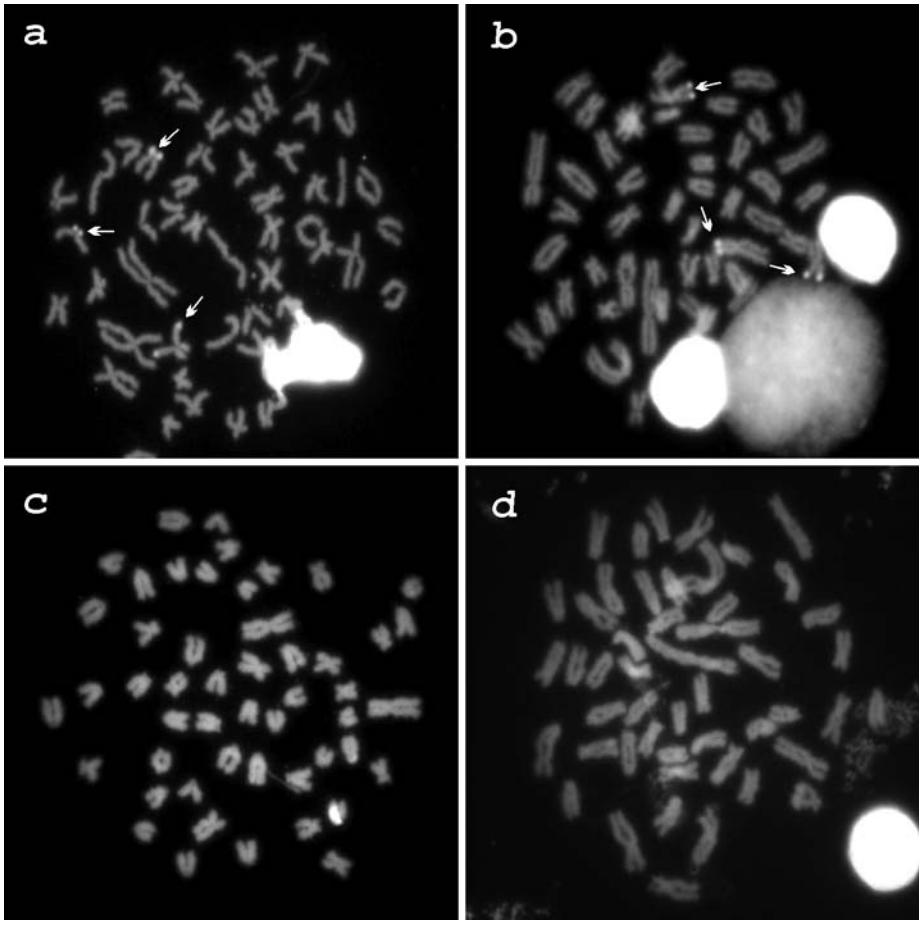

Figure 2 - Chromomycin A_3 stained metaphases evidencing GC-positive regions in (a) *A. scabripinnis*; and (b) *A. parahybae*, and the absence of these sites in (c) *A. intermedius*; and (d) *A. giton*.

The As-51 satellite DNA was terminally located in two small chromosome pairs, one metacentric and one subtelocentric-acrocentric. In a few cells, an additional signal was observed in the interstitial region of the long arm of a single acrocentric chromosome (Figure 3-b).

Astyanax intermedius presented 2n = 50 and a 6M+8SM+4ST+32A karyotypic formula. The heterochromatin was located in the pericentromeric region of many chromosomes and in the terminal region of chromosome pairs 1, 2, 8, 10, 12, 17 and 23 (Figure 1-c). Interstitial positive C-bands were observed in chromosomes 4, 5 and 18. No GC (Figure 2-c), AT- (data not shown) or As-51 satellite DNA sites were observed (Figure 3-c).

Astyanax giton showed 2n = 50 and a karyotype composed of 6M+8SM+8ST+28A in both populations studied. C-banding revealed heterochromatin in the pericentromeric regions of many chromosomes and in interstitial regions of chromosomes 4, 5, 6 and 9 (Figure 1-d). The interstitial band in the ninth pair is more conspicuous than the corresponding band in the *A. intermedius* karyotype. As in *A. intermedius*, no GC- (Figure 2-d), AT (data not shown) or As-51 satellite DNA sites were observed (Figure 3-d).

Discussion

Astyanax is an interesting group for cytogenetic studies, as it comprises species with distinct evolutionary trends leading towards either karyotypic conservation or diversification. The macrokaryotypic diversification that occurs in species from the "*A. scabripinnis*" and "*A. fasciatus*" complexes extends to heterochromatin variation, with frequent heteromorphisms on the nature and location of C-bands among populations, individuals and homologues (Moreira-Filho and Bertollo, 1991; Souza *et al.*, 1996; Mantovani *et al.*, 2000; Fernandes and Martins-Santos, 2005; Abel *et al.*, 2006; among others). No heteromorphisms were observed in the *A. scabripinnis* population herein studied (Figure 1-a), possibly due to the small sample. Its GC-positive heterochromatin distribution was similar to the one reported for other populations from the Tietê river basin (Souza *et al.*, 1996). The exceptions were the single chromosome with a fluorescence signal, an exclusive trait of the population herein analyzed (Figure 2-a), and the distribution of the As-51 satellite DNA (Figure 3-a), less abundant than in the populations from the upper Paraná river basin (Mantovani *et al.*, 2004; Abel *et al.*, 2006).

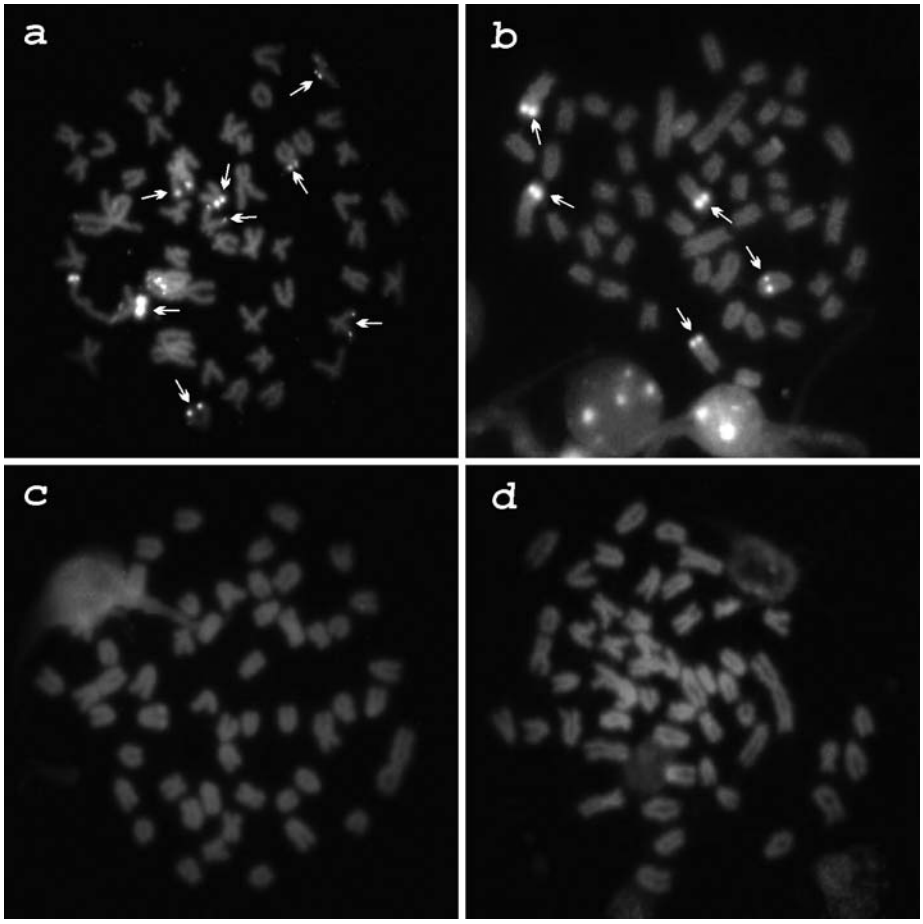

Figure 3 - Fluorescence *in situ* hybridization with As-51 satellite DNA probe. The arrows indicate these sites in: (a) *A. scabripinnis*; (b) *A. parahybae*. In (c) and (d) it is showed lack of signals of the satellite DNA correspondence in *A. intermedius* and *A. giton*, respectively.

In *A. parahybae*, the heterochromatin was distributed in the pericentromeric regions of almost all chromosomes and in the terminal regions of a few ones (Figure 1-b). GC-positive sites were identified in a chromosome pair and in a single homologue of another chromosome pair (Figure 2-b), as in *A. scabripinnis*. This differences between homologues may be due to uneven exchanges during meiosis. The As-51 satellite DNA probe hybridized to the terminal regions of a few chromosomes (Figure 3-b) and to one interstitial site with an apparent size heteromorphism between the homologues. *A. parahybae* has been grouped as a subspecies inside the "*A. fasciatus*" complex until recently. Its taxonomic status was defined by Melo (2001) in a revision of *Astyanax* species from the Serra dos Órgãos, Paraíba do Sul river basin. It is relevant that other *A. fasciatus* populations, from the Piracicaba river (Abel *et al.*, 2006) and from the Mogi-Guaçu river basin (Pazza, 2005) also presented such satellite DNA. Thus, its detection in *A. parahybae* could indicate a closer relationship between both species.

Besides very similar karyotypic formulas and scarce heterochromatin distribution, *A. giton* and *A. intermedius* presented other chromosomal features which indicate a close cytotaxonomical relationship: both of them did not show any GC-positive (Figure 2-c,d) or AT-positive heterochromatin, neither hybridization with the As-51 satellite DNA probe (Figure 3-c,d). Heterochromatin without GC- or AT-positive clusters is uncommon in fish, but has been observed in *Harttia loricariformis* (Kavalco *et al.*, 2004b). Besides the macrokaryotypic differentiation, the presence or absence of the As-51 satellite DNA could corroborate the polyphyletism of the genus *Astyanax*, suggested by Weitzman and Malabarba (1998). Although phylogenetic relationships between these species should be assessed by molecular analyses including several *Astyanax* species, cytogenetic data could support the hypothesis that *A. giton* and *A. intermedius* belong to a clade different from the *A. scabripinnis* and *A. parahybae* clade.

Although cytogenetic data are only available for a small number of *Astyanax* species, the heterochromatin data indicate that at least two distinct *Astyanax* groups occur in the Paraíba do Sul river basin: one group with few acrocentric chromosomes, As-51 satellite DNA and GC-positive sites, and another group with many acrocentric chromosomes, no GC-positive heterochromatin, neither As-51 satellite DNA. Mestriner *et al.* (2000) suggested that

the As-51 DNA distribution pattern found in *A. scabripinnis* and *A. fasciatus* may be an ancestral feature. The two other species possibly occupy a more derived position. Furthermore, *A. scabripinnis* showed different number of clusters and distribution patterns of such satellite DNA (Mestriner *et al.*, 2000; Mantovani *et al.*, 2004), as did *Astyanax fasciatus* (Pazza *et al.*, 2006). The distribution of 5S rDNA sites in *Astyanax* also pointed to the occurrence of two main groups, which is in agreement with the data herein reported. Almeida-Toledo *et al.* (2002) identified a marker metacentric pair in five *Astyanax* species. This pair was also observed in *A. scabripinnis* and *A. parahybae*, and was absent in *A. giton* and *A. intermedius* (Kavalco *et al.*, 2004a). The large number of 5S rDNA sites in the two latter species may reflect their close relationship and their phylogenetic distance from the other species.

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References

Abel LDS, Mantovani M and Moreira-Filho O (2006) Chromosomal distribution of the As51 satellite DNA in two species complexes of the genus *Astyanax* (Pisces, Characidae). Genet Mol Biol 29:448-452.

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). Cytogenet Genome Res 97:229-233.

Artoni RF and Bertollo LAC (1999) Nature and distribution of constitutive heterochromatin in fishes, genus *Hypostomus* (Loricariidae). Genetica 106:209-214.

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

Eigenmann CH (1921) The American Characidae. Mem Mus Comp Zool 23:209-310.

Fernandes CA and Martins-Santos IC (2005) Sympatric occurrence of three cytotypes and four morphological types of B chromosomes of *Astyanax scabripinnis* (Pisces, Characiformes) in the River Ivaí Basin, state of Paraná, Brazil. Genetica 124:301-306.

Ferro DAM, Neo 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.

Galetti Jr. PM, Mestriner CA, Venere PC and Foresti F (1991) Heterochromatin and karyotypic reorganization in fish of family Anostomidae (Characiformes). Cytogenet Cell Genet 56:116-121.

Jesus CM, Galetti Jr. PM, Valentini SR and Moreira-Filho O (2003) Molecular characterization and chromosomal localization of two families of satellite DNA in *Prochilodus lineatus* (Pisces, Prochilodontidae), a species with B chromosomes. Genetica 118:25-32.

Kavalco KF and Moreira-Filho O (2003) Cytogenetical analyses in four species of the genus *Astyanax* (Pisces, Characidae) from Paraíba do Sul river basin. Caryologia 56:453-461.

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

Kavalco KF, Pazza R, Bertollo LAC and Moreira-Filho O (2004b) Heterochromatin characterization of four fish species of the family Loricariidae (Siluriformes). Hereditas 141:237-242.

Kavalco KF, Pazza R, Bertollo LAC and Moreira-Filho O (2005) Molecular cytogenetics of *Oligosarcus hepsetus* (Teleostei, Characiformes) from two Brazilian locations. Genetica 124:85-91.

Levan A, Fredga K and Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. Hereditas 52:201-220.

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, Mestriner CA and Moreira-Filho O (2004) Evidence of the differentiated structural arrangement of constitutive heterochromatin between two populations of *Astyanax scabripinnis* (Pisces, Characidae). Genet Mol Biol 27:536-542.

Margarido VP and Galetti Jr. PM (1999) Heterochromatin patterns and karyotype relationships within and between the genera *Brycon* and *Salminus* (Pisces, Characidae). Genet Mol Biol 22:357-361.

Margarido VP and Galetti Jr. PM (2000) Amplification of a GC-rich heterochromatin in the freshwater fish *Leporinus desmotes* (Characiformes, Anostomidae). Genet Mol Biol 23:569-573.

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

Mayr B, Kalat M and Ràb P (1985) Localization of NORs and counterstain-enhanced fluorescence studies in *Perca fluviatilis* (Pisces, Percidae). Genetica 67:51-56.

Melo FAG (2001) Revisão taxonômica das espécies do gênero *Astyanax* Baird e Girard, 1854 (Teleostei, Characiformes, Characidae) da região da Serra dos Órgãos. Arq Mus Nac RJ 59:1-46 (Abstract in English).

Mestriner CA, Galetti Jr PM, Valentini SR, Ruiz IRG, Abel LDS, Moreira-Filho O and Camacho JPM (2000) Structural and functional evidence that B chromosome in the characid fish *Astyanax scabripinnis* is an isochromosome. Heredity 85:1-9.

Molina W, Schmid M and Galetti Jr. PM (1998) Heterochromatin and sex chromosomes in the Neotropical fish genus *Leporinus* (Characiformes, Anostomidae). Cytobios 94:141-149.

Moreira-Filho O and Bertollo LAC (1991). *Astyanax scabripinnis* (Pisces, Characidae): A species complex. Genet Mol Biol 14:331-357.

Pazza R (2005) Contribuição citogenética à análise da biodiversidade em *Astyanax fasciatus* (Pisces, Characidae). PhD. Thesis, Universidade Federal de São Carlos, São Carlos, 98 pp. Abstract in English. http://www.bdtd.ufscar.br/tde_arquivos/16/TDE-2005-05-16T08:29:20Z-664/Publico/DissCG.pdf.

Pazza R, Kavalco KF, Almeida-Toledo LF and Bertollo LAC (2005) *Hoplosternum littorale* (Teleostei, Callichthyidae) from a Coastal River basin in Brazil - Cytogenetic analysis and gene mapping of 5S and 18S rDNA. Caryologia 58:339-344.

Pazza R, Kavalco KF and Bertollo LAC (2006) Chromosome polymorphism in *Astyanax fasciatus* (Teleostei, Characidae). 1. Karyotype analysis, Ag-NORs and mapping of the 18S and 5S ribosomal genes in sympatric karyotypes and their possible hybrid forms. Cytogenet Genome Res 112:313-319.

Phillips R and Hartley SE (1988) Fluorescent banding patterns of the chromosomes of the genus *Salmo*. Genome 30:193-197.

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

Reis RO, Kullander SO and Ferraris Jr. CJ (2003) Check List of the Freshwater Fishes of South and Central America. EDIPUCRS, Porto Alegre, 742 pp.

Schmid M (1980) Chromosome banding in Amphibia. IV. Differentiation of GC- and AT-rich chromosome region in Anura. Chromosoma 77:83-103.

Schmid M and Guttenbach M (1988) Evolutionary diversity of reverse (R) fluorescent chromosome bands in vertebrates. Chromosoma 97:101-114.

Schweizer D (1980) Simultaneus fluorescent staining of R bands and specific heterochromoatic regions (DA-DAPI bands) in human chromosomes. Cytogenet Cell Genet 27:190-193.

Sola L, Rossi AR, Iaselli V, Rash EM and Mônaco PJ (1992) Cytogenetics of bisexual/unisexual species of *Poecilia*. II. Analysis of heterochromatin and nucleolar organizer regions in *Poecilia mexicana mexicana* by C-banding and DAPI, quinacrine, chromomycin A_3 and silver staining. Cytogenet Cell Genet 60:229-235.

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

Sumner AT (1972) A simple techinique for demonstrating centromeric heterocromatin. Exp Cell Res 75:304-306.

Vicente VE, Bertollo LAC, Valentini SR and Moreira-Filho O (2004) Origin and Differentiation of a sex chromosome system in *Parodon hilarii* (Pisces, Parodontidae). Satellite DNA, G- and C-banding. Genetica 119:115-120.

Weitzman SH and Malabarba LR (1998) Perspectives about the phylogeny and classification of the Characidae (Teleostei, Characiformes). In: Malabarba LR, Reis ER, Vari RP, Lucena ZMS and Lucena CA (eds) Phylogeny and Classification of Neotropical Fishes. EDIPUCRS, Porto Alegre, pp 161-170.

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