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Scientific Note

Cytogenetic data on *Astyanax jacuhiensis* (Characidae) in the lago Guaíba and tributaries, Brazil

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Cytogenetic analyses were performed in *Astyanax jacuhiensis* from lago Guaíba, Brazil. The diploid number was 50, with a karyotype composed of 8m+30sm+4st+8a chromosomes, FN = 92. The AgNORs were observed in 2 to 5 chromosomes, with intra- and interindividual variation. The sm pair 8 observed always carried NORs on the short arms, presenting size heteromorphism between homologous. Fluorescence *in situ* hybridization (FISH) with an 18S rDNA probe only confirmed the location of ribosomal cistrons in the sm pair 8, and heteromorphism of these regions between the homologous chromosomes. C-banding revealed the occurrence of weak C-positive heterochromatin in the pericentromeric regions of several chromosomes, in addition to more evident bands interstitially located on some chromosome pairs and in the terminal region of the short arms in pair 8. C-banding plus CMA_3 revealed light fluorescent signals in different chromosomes of the karyotype, with a strong terminal site in pair 8, indicating the occurrence of several GC-rich heterochromatic regions in this species. Our results provide the first description of the *Astyanax jacuhiensis* karyotype, showing karyotype similarities when compared to various populations of *A. altiparanae* and *A. bimaculatus*, indicating that chromosomal features are very similar for these three species.

Análises citogenéticas foram realizadas em *Astyanax jacuhiensis* do lago Guaíba, Brasil. O número diplóide foi 50, sendo o cariótipo composto por 8m+30sm+4st+8a cromossomos, NF = 92. As regiões organizadoras de nucléolos (AgNORs) foram observadas em 2 a 5 cromossomos, evidenciando uma variação intra e interindividual nesta espécie. O par sm 8 foi constantemente detectado com NORs nos braços curtos, mostrando um heteromorfismo de tamanho entre os homólogos. Entretanto, a hibridação *in situ* fluorescente (FISH) com sonda de DNAr 18S, localizou cístrons ribossômicos apenas no par 8, confirmando o heteromorfismo de tamanho entre os homólogos. O bandamento C revelou a presença de bandas discretas de heterocromatina na região pericentromérica da maioria dos cromossomos, além de algumas bandas mais evidentes intersticiais, bem como na região terminal dos braços curtos do par 8. A associação de BC+CMA_3 evidenciou marcações fluorescentes mais discretas em diferentes cromossomos e uma forte marcação terminal no par 8, confirmando vários sítios de heterocromatina GC-rica nessa espécie. Nossos resultados fornecem a primeira descrição do cariótipo de *Astyanax jacuhiensis*, apresentando semelhanças em relação ao cariótipo de diferentes populações de *A. altiparanae* e *A. bimaculatus*, indicando que as características cromossômicas são muito semelhantes para estas três espécies.

Key words: Chromosomes, Chromomycin A_3,18S rDNA, NOR.

According to Reis *et al*. (2003), the family Characidae comprises 952 validated species and 400 species not yet described, totaling 1352 species. Among characids, the genus *Astyanax* corresponds to a complex group, and its taxonomy has been hampered by several similar forms (Melo, 2001).

Until recently, several Characidae genera were included in the subfamily Tetragonopterinae, encompassing heterogeneous groups from small to large-sized fishes. Due to lack of evidence that this subfamily constitutes a monophyletic group, Lima *et al*. (2003) grouped 88 genera

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as *incertae sedis*, including 620 species, among which 399 (64%) are taxonomically little known and probably made up monophyletic groups: *Hyphessobrycon* (97 species), *Astyanax* (86 species), *Moenkhausia* (58 species), *Bryconamericus* (51 species) and *Hemigrammus* (43 species). Besides, more than 47 genera included in *incertae sedis* are monotypic, and 25 genera contain only two or three species (Lima *et al*., 2003).

Astyanax jacuhiensis was first described as *Tetragonopterus jacuhiensis* (Cope, 1984). It was later transferred to the genus *Astyanax* (Fowler, 1906) and considered synonymous with *A. bimaculatus* (Eigenmann, 1921). This species was recently referred to as *A. jacuhiensis* (Lima *et al*., 2003), for the endemic population of the Jacuí River, RS, Brazil, corresponding to its type-locality.

The present study presents the first karyotype description of *A. jacuhiensis*, using AgNOR conventional and fluorochrome stainings, in addition to C-banding and fluorescence *in situ* hybridization (FISH) with 18S rDNA probe.

Twenty-four specimens of *A. jacuhiensis* were collected from March 2006 to November 2007 from three different localities in the lago Guaíba and tributaries, Rio Grande do Sul State (RS), Brazil, and deposited in the Museu de Zoologia da Universidade Estadual de Londrina (MZUEL): three males and two females from the Gasômetro, 30º02'06.39"S 51º14'31.84"W, (MZUEL 4032); three males and one female from the arroio do Ribeiro, 30º18'39.16"S 51º18'56.33"W, (MZUEL 4819), and nine males and six females from the Barra do Ribeiro, 30º17'29.44"S 51º18'09.27"W, (MZUEL 4034), totalizing fifteen males and nine females.

Mitotic chromosomes were obtained from kidney, according to the conventional air drying method (Bertollo *et al*., 1978). The chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a), according the arm ratio (Levan *et al*., 1964). Metacentrics and submetacentrics were considered biarmed, and subtelocentric-acrocentrics as uniarmed to determine the fundamental number (FN), *i.e*., the number of chromosomal arms. Nucleolar organizer regions (NORs) were identified by silver nitrate staining (Ag-NORs), according to Howell & Black (1980). The location of the C-positive heterochromatin on the chromosomes was detected by C-banding, using barium hydroxide (Sumner, 1972). Fluorochrome staining with GC- specific chromomycin-A_3 (CMA_3) was carried out according to Schweizer (1980). A rDNA 18S probe (1700 pb) obtained from the nuclear DNA of the fish *Oreochromis niloticus* was used for *in situ* hybridization, as described by Swarça *et al*. (2001).

This is the first karyotype description of *A. jacuhiensis*, which has 2n = 50 chromosomes (8m+30sm+4st+8a), and FN = 92. Males and females showed identical karyotypes, without sex heteromorphism (Fig. 1a). The same diploid number has been observed in other *Astyanax* species, including *A. bimaculatus* (Morelli *et al*., 1983; Alberdi & Fenocchio, 1997; Jorge & Moreira-Filho, 2001), *A. altiparanae* (Daniel-Silva & Almeida-Toledo, 2001; Pacheco *et al*., 2001; Fernandes & Martins-Santos, 2004, 2006a) and *A. scabripinnis* (Mantovani *et al*., 2000, 2005; Ferro *et al*., 2001; Fernandes & Martins-Santos, 2005), thus representing a common characteristic in this genus.

Silver nitrate staining showed 2 to 5 chromosomes with probable Ag-NORs sites: on the short arms of sm pair 8 displaying size heteromorphism, on the short arms of st pair 20, and on the long arm of one chromosome of m pair 4, which could correspond to system of multiple NORs. Pair 8 was the most frequent AgNOR-bearing chromosome, being visualized in all metaphases analyzed (Fig. 1b). However, the fluorescence *in situ* hybridization showed that only sm pair 8 displayed 18S rDNA sites, also confirming the size heteromorphism between homologous (Fig. 1c), thereby characterizing a simple NOR system in *A. jacuhiensis*. AgNOR heteromorphism is frequently seen in fish, and can be ascribed to variations in the number of copies of ribosomal cistrons between homologous through unequal crossing-overs, transposition or rearrangements, such as deletions and duplications (Galetti-Jr *et al*., 1995). The other chromosomal sites that were positive for silver nitrate staining, but have proved to be negative after FISH with 18S rDNA probe, may represent only a kind of heterochromatin with some affinity to silver nitrate, irrespective of whether they do or do not carry any site of rDNA.

Although multiple NORs are relatively common in *Astyanax* (Jorge & Moreira-Filho, 2001; Pacheco *et al*., 2001; Souza *et al*., 2001; Almeida-Toledo *et al*., 2002; Mantovani *et al*., 2005; Fernandes & Martins-Santos, 2006a, b), a simple NOR system has also been reported in *A. bimaculatus* from the São Francisco, Doce, and Paraguay Rivers (Paganelli, unpubl. data), and in *A. altiparanae* from the Paraná River (Fernandes & Martins-Santos, 2004) and Monjolinho River (Peres *et al*., 2008).

Heterochromatin has been an important marker for the characterization and differentiation of several species and populations. Its distribution pattern in *A. jacuhiensis* resembled that found in some populations of *A. altiparanae* (Daniel-Silva & Almeida-Toledo, 2001; Fernandes & Martins-Santos, 2004), and *A. bimaculatus* (Paganelli, unpubl. data), where interstitial C-positive bands were also observed. In fact, in addition to discrete pericentromeric bands, *A. jacuhiensis* showed conspicuous interstitial C-positive blocks in several pairs of the karyotype (Fig. 2a), as well as in the terminal region of pair 8, in correspondence with the NOR location (Fig. 2b-c).

Up to four CMA_3 positive signals were found in *A. jacuhiensis* (Fig. 1d), where pair 8 was always seen with a strong terminal staining, and demonstrating the same heteromorphism observed with AgNORs and 18S rDNA sites. C-banding plus CMA_3 staining (CB+CMA_3) evidenced some pericentromeric and interstitial positive sites. However, pair 8 showed strong heteromorphic sites corresponding to the NOR regions (Fig. 2d). Therefore, *A. jacuhiensis* contains a class of GC-rich heterochromatin in some chromosome pairs and

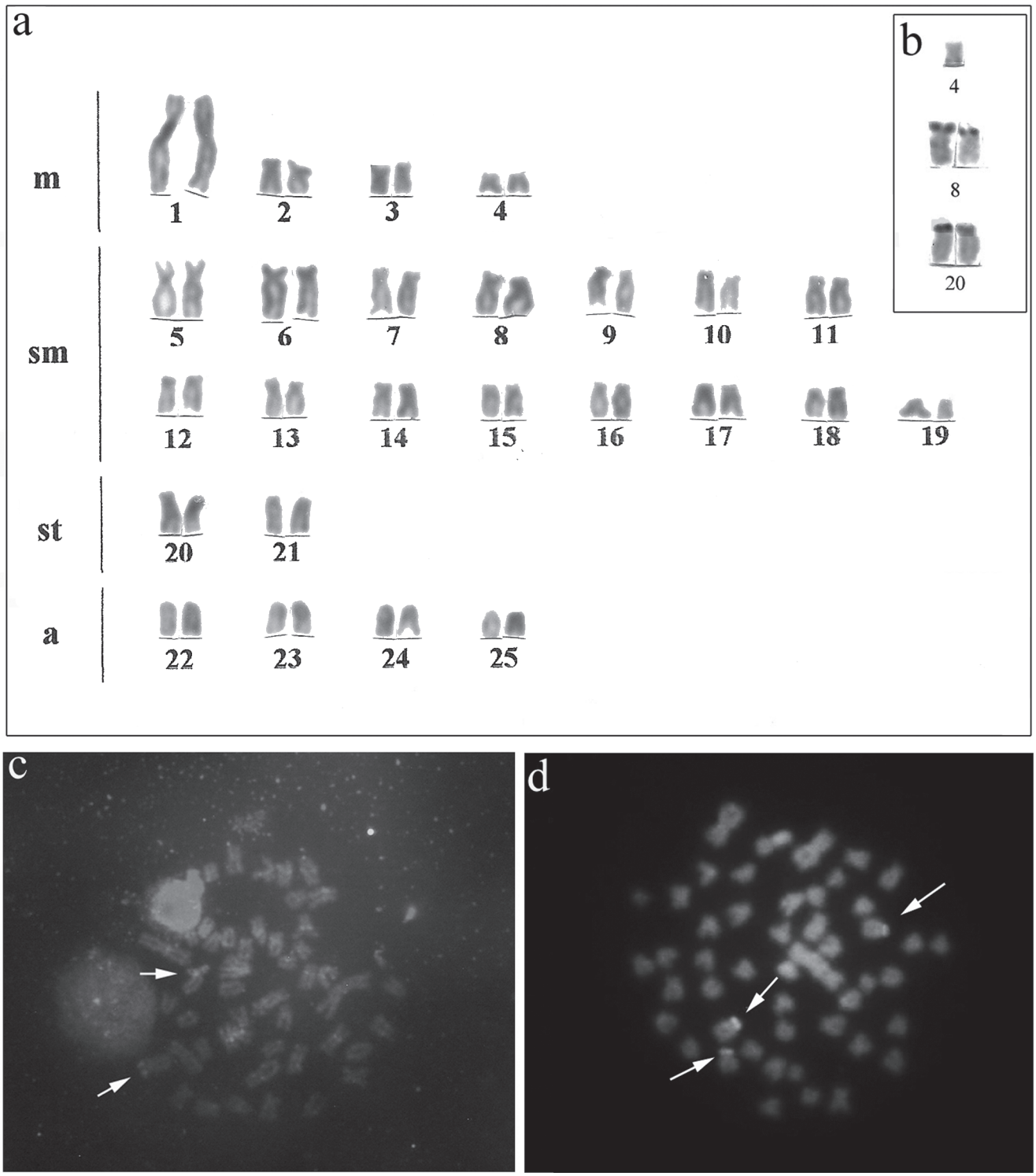

Fig. 1. a) Giemsa-stained karyotype of *Astyanax jacuhiensis*; **b**) Chromosomes with positive sites after silver staining; **c**) Metaphase plate with fluorescence *in situ* hybridization (FISH) showing the 18S rDNA sites (arrows); **d**) Metaphase plate showing CMA_3 positive sites (arrows).

the ribosomal cistrons appear to be interspersed with this kind of heterochromatin.

Astyanax bimaculatus, from the Paraná River, has a karyotypic structure similar to that found in various populations of *A. altiparanae* (Jorge & Moreira Filho, 2001). In addition, *A. jacuhiensis* also showed similarities in the diploid number, karyotype formula, and chromosome banding when compared to various populations of *A. altiparanae* and *A. bimaculatus*, indicating that these three species are very similar in relation to their chromosomal features. It is important to note that these

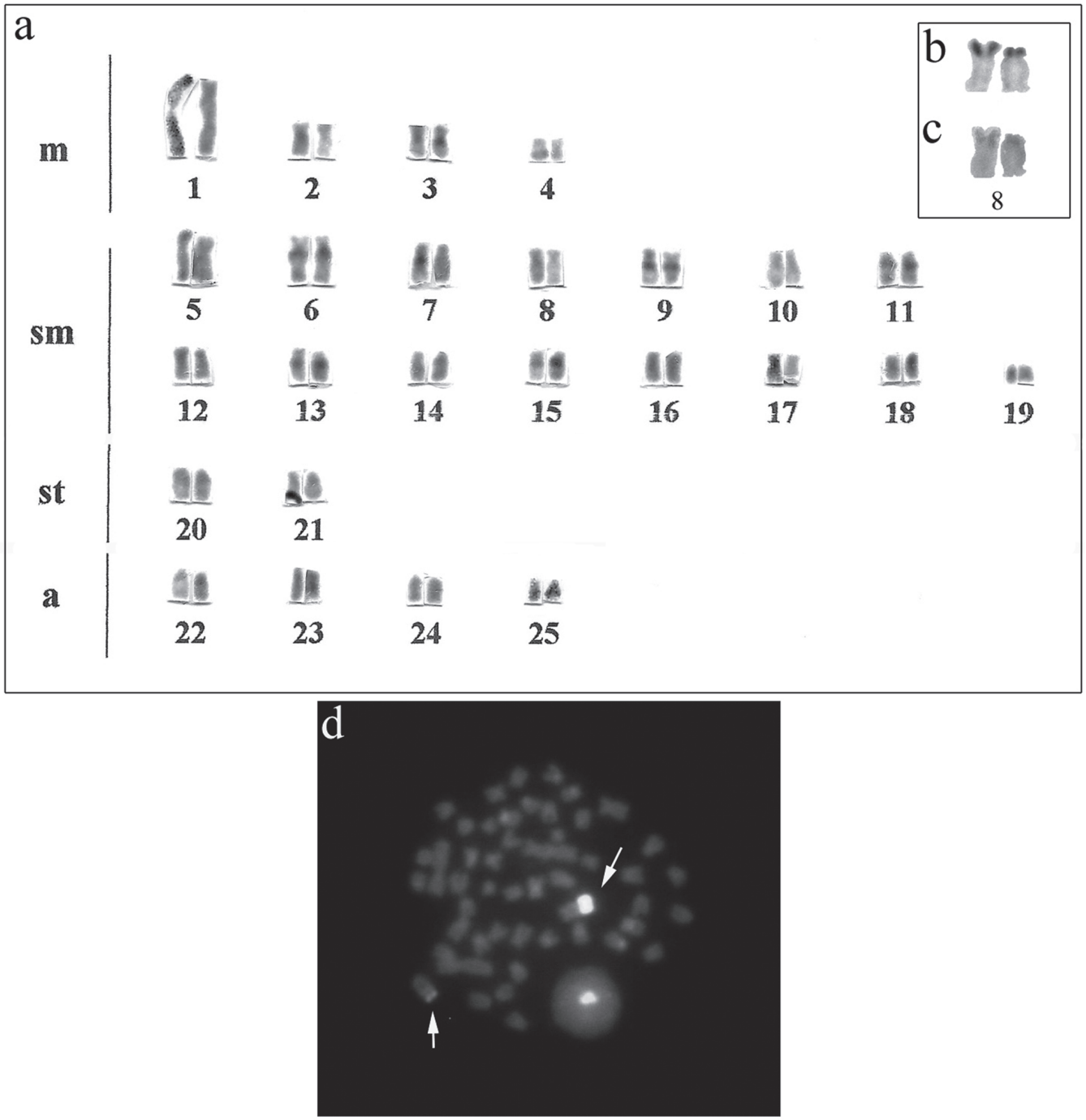

Fig. 2. a) C-banded karyotype of *Astyanax jacuhiensis*; **b**) AgNORs and **c**) C-banding on pair 8; **d**) Metaphase plate with C-banding plus CMA_3 staining (CB + CMA_3) showing size heteromorphism of the NOR regions in pair 8 (arrows).

cytotaxonomic data somehow support Eigenmann's proposal (1921). However, further cytogenetic and taxonomic studies should be conducted to better clarify this question.

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