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Cytogenetic analysis of *Astyanax laticeps* (Cope, 1894) (Ostariophysi: Characidae) from the laguna dos Patos system

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The genus *Astyanax* is prominent among Characiformes, due to the large number of species found and its wide geographic distribution. In this work, *Astyanax laticeps* specimens from the laguna dos Patos system were cytogenetically analyzed. A diploid number of 2n = 50 chromosomes distributed into 6m+16sm+16st+12a (FN = 88) was found, without differences between males and females. A few small heterochromatin blocks were observed, besides three more conspicuous C-bands, corresponding to NORs, as confirmed by silver nitrate and CMA_3 staining, FISH, and DAPI negative staining. These regions were located in a medium-sized subtelocentric and in a large subtelocentric chromosomal pair, probably because of a deletion of this region in one homologous chromosome, or due to a transposition event between them.

O gênero *Astyanax* é destacado entre os Characiformes, pelo grande número de espécies encontradas e a ampla distribuição geográfica. Neste trabalho, foram analisados citogeneticamente espécimes de *Astyanax laticeps* do sistema da laguna do Patos. O número diplóide observado foi de 2n = 50 cromossomos distribuídos em 6m+16sm+16st+12a (NF= 88), sem diferenças entre machos e fêmeas. Foram observados poucos blocos de heterocromatina, além de três bandas-C mais conspícuas, correspondentes às NORs, confirmado pela coloração com nitrado de prata,CMA_3, FISH, e coloração negativa ao DAPI. Estas regiões foram localizadas em um cromossomo subtelocêntrico de tamanho médio e em um par subtelocêntrico grande, provavelmente devido a deleção desta região em um dos cromossomos homólogos, ou por eventos de transposição entre eles.

Key words: *Astyanax scabripinnis* species complex, rDNA, Homologous transposition.

Introduction

Within the family Characidae, the genus *Astyanax*, with 86 species, is considered the second species-richest genus in this family (Lima *et al*., 2003). A remarkable karyotypical diversity is observed in the genus *Astyanax*, involving both chromosomal number and morphology. The diploid number in this genus can range from 2n = 36 in *A. schubarti* Britski 1964 (Moreira-Filho *et al*., 2001) to 2n = 50 in *A. eigenmanniorum* (Cope 1894) (Fauaz *et al*., 1994), *A. scabripinnis* (Jenyns 1842) (Mizoguchi & Martins-Santos, 1997; Kavalco *et al*., 2004; Mantovani *et al*., 2004), *A. altiparanae* Garutti & Britski 2000 (Pacheco *et al*., 2001; Fernandes & Martins-Santos, 2006), among others.

Astyanax scabripinnis, the most cytogenetically studied species, shows a high karyotypical diversity, and is considered a species complex (Moreira-Filho & Bertollo, 1991). In fact, none of the previous studies of the karyotype of *Astyanax scabripinnis* actually seems to have been developed with this species, but with other populations of morphologically similar species. According to Melo (2001) and Bertaco & Lucena (2006), the occurrence of *A. scabripinnis* is restricted to Rio de Janeiro State, and the species may be extinct, since no additional specimens have been collected after its description. Indeed, all published information about the karyotype of *A. scabripinnis* will be referred in our text as to the *A. scabripinnis* species complex and not to a single species. Bertaco & Lucena (2006) have listed fifteen species that form the *A. scabripinnis* complex. *Astyanax laticeps*, formerly regarded as a subspecies of *A.*

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scabripinnis, has been recently recognized as a distinct species by Bertaco & Malabarba (2001), based on morphological characters and color patterns.

There are no reports in the literature on the cytogenetic features of the species *A. laticeps*. In the present work, we report the first cytogenetic analysis in this species, including karyotypical description, heterochromatin distribution pattern, localization of AT- and GC-rich regions, and 45S rDNA sequences.

Material and Methods

Eighteen specimens of *A. laticeps* (MZUEL 4874, ten females and eight males) from rio Forquetinha (52º19'22.7"W 29º16'48.8"S), a tributary of the rio Taquari, laguna dos Patos system, Forquetinha municipality, Rio Grande do Sul State, Brazil, were cytogenetically analyzed. Metaphases were obtained through the air drying method (Bertollo *et al*., 1978). The chromosomes were classified according to Levan *et al*. (1964) into metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a). The heterochromatin distribution pattern was determined using C-banding (Sumner, 1972). Active nucleolar organizer regions (NORs) were detected by silver nitrate staining (Howell & Black, 1980). The AT- and GC-rich blocks were visualized by fluorochrome staining, using 4',6-Diamidino-2-phenylindole (DAPI) and Chromomycin A_3 (CMA_3) respectively (Schweizer, 1976). Fluorescent *in situ* hybridization (FISH) was performed according to Heslop-Harrison *et al*. (1991) and Cuadrado & Jouve (1994), using p*Ta*71 probes carrying the 45S rDNA sequence (Gerlach & Bedbrook, 1979).

Results

The studied samples of *A. laticeps* (Fig. 1a) showed a diploid number of 2n = 50 chromosomes, distributed into 6m+16sm+16st+12a, with a fundamental number (FN) of 88 (Fig. 1b). No chromosomal differences between males and females were detected. The analysis of the constitutive heterochromatin showed weak marks, located on centromeric and telomeric regions of a few chromosomes. Three chromosomes presented small but more evident terminal heterochromatic blocks, which probably corresponded to NORs (Fig. 2d).

All analyzed specimens presented three chromosomes bearing Ag-NORs at the terminal region, comprising a medium-sized subtelocentric and a single homologous pair of a larger subtelocentric chromosme (Fig. 2a). The interphase nuclei stained with silver nitrate had two nucleoli, usually close to each other (Fig. 2b). FISH with 45S rDNA probes also showed fluorescent signals at the telomeric regions of three chromosomes, equivalent to Ag-NOR location. Similarly, the sequential analysis with CMA_3 and DAPI fluorochromes showed positive and negative signals (Figs. 2e and f), respectively, on the three NOR-bearing chromosomes (Fig. 2g).

Discussion

The precise diagnosis of species in the "*A. scabripinnis* species complex" is hindered by their high degree of similarity in the body morphology. Moreira-Filho & Bertollo (1991), studying morphometric and cytogenetic features in five populations formerly identified as *A. scabripinnis*, have reliably discriminated them by both canonical variables analysis and the occurrence of sympatric cytotypes with 48 and 50 chromosomes, without any detectable hybrid forms, thereby suggesting the occurrence of a species complex ("*A. scabripinnis* complex").

The cytogenetic analysis in *A. laticeps* from rio Forquetinha showed a diploid number of 2n = 50 chromosomes, distributed into 6m+16sm+16st+12a (FN = 88), demonstrating a similarity in the chromosome number observed for most of the *A. scabripinnis* species complex populations studied so far (Moreira-filho & Bertollo, 1991; Maistro *et al*., 1992, 2000; Mantovani *et al*., 2000; Mizoguchi & Martins-Santos, 1998), as well as in other species of the genus: *A. mexicanus* (Kirby *et al*., 1977), *Astyanax* aff. *bimaculatus* (Pamponet *et al*., 2008), *A. taeniatus* (Paganelli, pers. comm.), *A. eigenmanniorum* (Fauaz *et al*., 1994), *A. altiparanae* (Pacheco *et al*., 2001), *A. intermedius*, *A. giton* (Kavalco & Moreira-Filho, 2003), and *A. jacuhiensis* (Pacheco, pers. comm.).

Regarding the heterochromatin distribution, *A. laticeps* presented small blocks at pericentromeric and telomeric regions of few chromosomes, besides three more evident heterochromatic blocks equivalent to NORs. Such reduced amount of heterochromatin is also observed in other species of this genus, such as *A. parahybae* (Centofante *et al*., 2003), *Astyanax* sp. B (Fazoli *et al*., 2003), *A. altiparanae* (Fernandes & Martins-Santos, 2004), and *A. jacuhiensis* (Pacheco, pers. comm.). In the *A. scabripinnis* species complex, there is a wide variation in the heterochromatin distribution, since populations with large heterochromatic blocks and populations presenting a reduced heterochromatin content have been reported (Moreira-Filho & Bertollo, 2001; Maistro *et al*., 1994; Mizoguchi & Martins-Santos, 1997).

Analyses of major rRNA genes distribution in *Astyanax laticeps* were performed by Ag-NOR technique, base-specific fluorochromes and FISH with 45S rDNA probes. Silver nitrate staining showed the presence of three NOR-bearing chromosomes with one medium-sized subtelocentric pair and a single homologous element in a large subtelocentric pair. This finding was supported by FISH with 45S rDNA probes, which may indicate the occurrence of a deletion in this region within one of the large subtelocentric homologous, or a transposition between homologues, once the signal on the single chromosome is considerably conspicuous. A similar hypothesis was previously proposed by Mantovani *et al*. (2000) for populations of *A. scabripinnis* species complex from the rios Marrecas and Centenario, based on the chromosomal arrangement in the interphase nucleus corroborated by Swcheizer & Loidl (1987). Considering the observed data, individuals with two chromosomes bearing

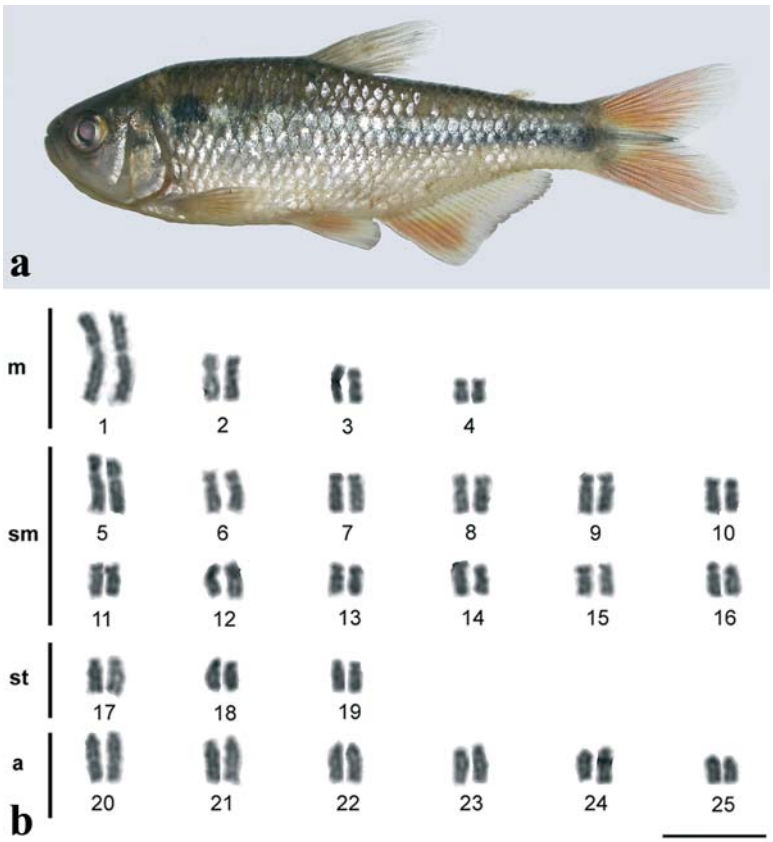

Fig. 1. *Astyanax laticeps* from the laguna dos Patos system. (a) specimen with 67.0 mm standard length; (b) Guiemsa stained karyotype. Bar = 5 μm.

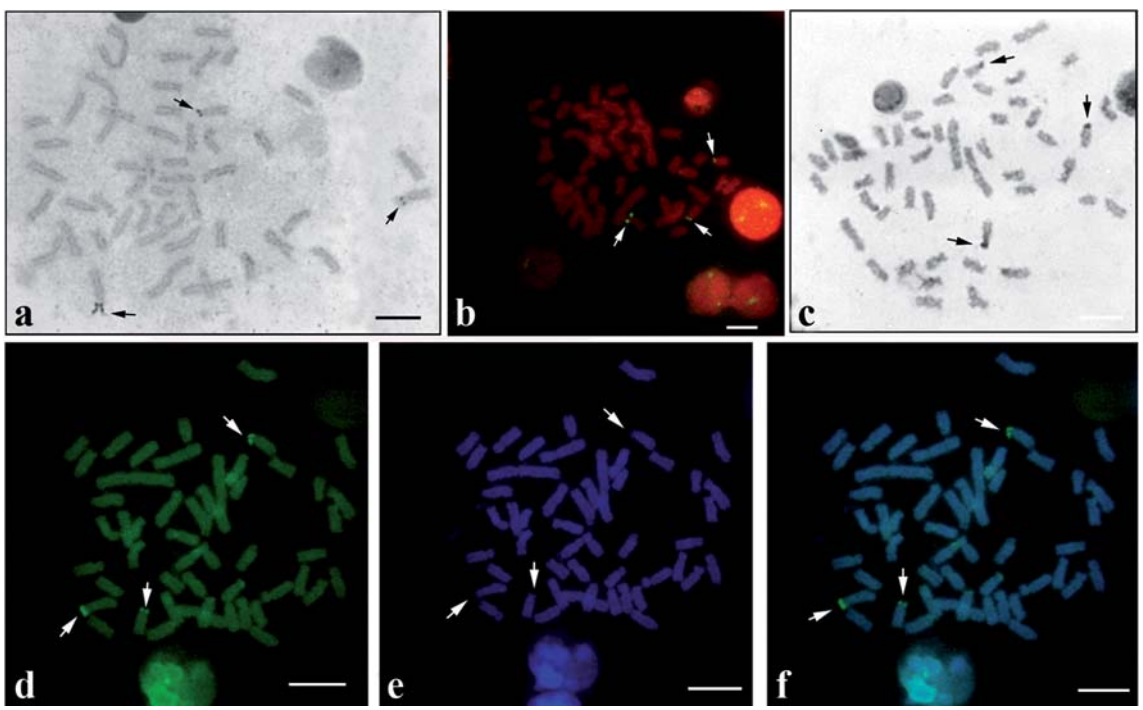

Fig. 2. Metaphases of *Astyanax laticeps*: (a) Ag-NORs stained; (b) Fluorescent *in situ* hybridization with 45S rDNA probe; (c) C-banding; (d) CMA_3 staining; (e) DAPI staining; (f) Overlapping of CMA_3 and DAPI staining images. The arrows indicate the NORs. Bars = 5 μm.

45S rDNA genes - (homozygote 45S rDNA), one homologue with rDNA genes (heterozygote), and two homologues without the 45S rDNA genes (homozygote without 45S rDNA) would be expected. However, only heterozygotes were observed. A possible explanation for this fact may be the lethality of the homozygous lethal condition for these chromosomes, as already observed in *Oncorhynchus mykiss*. In this species, a paracentric inversion involving 18S rDNA - bearing chromosomes is observed only in heterozygosis; the homozygous for the inverted chromosomes results in death (Porto-Foresti *et al*., 2004).

The presence of three 45S rDNA sites is a peculiar feature to *A. laticeps*. Despite having the same chromosome number, the other species of this genus differ in the distribution pattern of ribosomal cistrons. Differential numbers of NORs have already been seen in *A. jacuhiensis* with only two marks (Pacheco, pers. comm.), *A. altiparanae*, with 4 to 7 sites (Fernandes, pers. comm.), *A. giton* with 10, *A. intermedius*, with 12 small sites (Kavalco & Moreira-Filho, 2003) and *A. scabripinnis* species complex, with sites ranging from 4 to 14, the largest variation in number of sites in different populations reported to date (Rocon-Stange & Almeida-Toledo, 1993; Ferro *et al*., 2001).

The three NOR-bearing chromosomes showed CMA_3^+ and $DAPI^-$ blocks, indicating a GC-rich (AT-poor) composition. Similar results on the chemical composition of NORs have been found in *A. scabripinnis* species complex (Ferro *et al*., 2001), *A. altiparanae* (Fernandes & Martins-Santos, 2004), and *A. jacuhiensis* (Pacheco, pers. comm.), among others. This region seems to be linked to GC-rich heterochromatin segments. The presence of nucleolar organizer regions rich in GC is a common finding among fish species, and it is postulated that this feature occurs because heterochromatin would be interspersed with NORs in this group (Pendás *et al*., 1993). The present data, besides reporting the karyotype of this species, corroborates the taxonomic recognition of *A. laticeps* as a valid species, apart from other karyotyped species from the *A. scabripinnis* species complex.

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