

Chromosomal characterization of the bonytongue *Arapaima gigas* (Osteoglossiformes: Arapaimidae)

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The mitotic chromosomes of the pirarucu *Arapaima gigas* inhabiting the middle Araguaia River and collected in the municipality of Araguaiana (MT, Brazil) were studied. The chromosomes were analyzed through Giemsa staining, C-banding, Ag-NOR staining and in situ hybridization using an 18S rRNA gene probe. The karyotype had $2n=56$ comprising 14 banded and 14 unbanded chromosome pairs in both sexes. No cytologically distinguishable sex chromosome was identified. A single NOR-bearing chromosome pair was detected by Ag-NOR staining and confirmed by 18S rDNA – FISH. Faint constitutive heterochromatin was C-banded in the centromeric region of some chromosomes.

Estudamos os cromossomos mitóticos do pirarucu *Arapaima gigas*, proveniente do médio rio Araguaia, município de Araguaiana (MT, Brasil). As preparações mitóticas foram analisadas através de coloração com Giemsa, bandas-C e nitrato de prata (Ag-NORs). Também foram realizadas análises através de hibridização *in situ* utilizando sondas de DNAr 18S. O cariótipo mostrou $2n=56$ cromossomos, sendo 14 pares meta-submetacêntricos e 14 subtelo-acrocêntricos. Não foram observadas diferenças cromossômicas associadas ao sexo. Foi detectado apenas um par de cromossomos portador de Ag-NORs. Essa observação foi confirmada pelo FISH do DNAr 18S. Pequenos blocos de heterocromatina foram observados na região centromérica de alguns pares cromossômicos.

Key words: Pirarucu, Karyotype, rDNA, Heterochromatin.

Introduction

The pirarucu *Arapaima gigas* is among the largest freshwater fish species and is a very important fisheries resource in the Amazonian region. Belonging to the Arapaimidae family (Osteoglossiformes), this fish inhabits the lowland waters of the Amazon and Tocantins-Araguaia basins in the Brazilian territory as well as the rivers of the Guyana (Ferraris, 2003).

The wide geographic distribution of osteoglossiforms and their basal position in the general fish phylogeny make them an important clade for systematic and evolutionary studies (e.g. Lundberg & Chernoff, 1992; Obermiller & Pfeiler, 2003). Their peculiar morphological, biochemical and adaptive characteristics have been extensively studied (e.g. Cavin & Forey, 2001), but little is known of their genetics.

From a cytogenetical point of view, osteoglossiforms have been characterized by several chromosome compositions. *Notopterus chitala* and *Notopterus notopterus* collected in India were first reported having $2n=48$ with 12 metacentric

chromosomes (FN=60) by Nayyar (1965). However, both species were independently reanalyzed by different authors and a karyotype pattern with $2n=42$, comprising only unbanded chromosomes, was reported for *N. chitala* (Uyeno, 1973) and *N. notopterus* (Urushido *et al.*, 1975). Uyeno (1973) also reported the karyotype of *Osteoglossum bicirrhosum* as having $2n=56$, mostly acrocentric chromosomes, while *Pantodon bucholzi*, *Marcusenius brachistius* and *Gnathonema petersii* had $2n=48$, showing different composition of banded and unbanded chromosomes. In addition, *Hiodon alosoides* had $2n=50$, all banded chromosomes, *Xenomystus nigri* had $2n=42$ acrocentrics, and finally *Papyrocranus afer* had $2n=34$, the smaller diploid number reported for this fish group. Urushido *et al.* (1975) also reported $2n=50$ in the karyotype of *Scleropages formosus* and $2n=56$ in *A. gigas*. There is no information concerning chromosome banding in this fish thus far, except the C-banding and Ag-NOR description of *N. chitala* (Akinori & Yoshio, 1998).

In the present paper, the mitotic chromosome complement

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of *A. gigas* was studied using conventional Giemsa staining, C-banding, Ag-NOR staining and 18S rDNA *in situ* hybridization in order to produce more detailed information on the karyotype structure of this fish.

Material and Methods

Sixteen specimens of *A. gigas* (4 males, 7 females and 5 of unknown sex) were analyzed. They were collected from oxbow lagoons at the middle course of the Araguaia River, in the municipality of Araguaiana (MT). Voucher specimens are deposited in the Instituto de Ciências e Letras do Médio Araguaia, Universidade Federal de Mato Grosso, Pontal do Araguaia, MT.

Chromosomes were obtained by direct preparation of anterior kidney cells (Bertollo *et al.*, 1978), short term culture from different tissues (Fenocchio *et al.*, 1991) and conventional cell blood culture (Fenocchio & Bertollo, 1988).

C-banding was obtained using barium hydroxide (Sumner, 1972). Ag-NOR staining was performed as described by Howell & Black (1980) and 18S rDNA fluorescence *in situ* hybridization (FISH) was carried out following Pinkel *et al.* (1986).

Results

The modal diploid number found for *A. gigas* was $2n = 56$ chromosomes in both sexes. The karyotype was composed of 14 biarmed chromosome pairs (meta-submetacentric) and 14 uniarmed pairs (subtelo-acrocentric) (Fig. 1). No cytologically distinguishable sex chromosome was observed. A conspicuous secondary constriction was detected on the short arm of both elements of the chromosome pair 3 and size polymorphism in this chromosome site was identified (Fig. 1).

Ag-NOR sites were observed in an interstitial position on the short arm of a biarmed chromosome pair comparable in size and morphology to pair 3 (Fig. 2a). FISH has also revealed similar results, and two 18S rDNA sites were detected in the chromosome complement, often exhibiting size polymorphisms (Fig. 2b-c) that can indicate structural differences between them. Faint C-banded or not a bit heterochromatin

was detected in the centromeric region of the chromosomes likely due to its reduced amount and the C-banding technical resolution (Fig. 2d).

Discussion

Our results confirm the hypothesis in which an extensive chromosome diversification occurred in parallel to the morphological divergence during the diversification of this fish group (Uyeno, 1973). The *A. gigas* karyotype here studied showed $2n=56$, similar to the previously reported by Urushido *et al.* (1975). However, a significant discordance in the FN values was observed between our results (FN=84) and those reported by the latter authors (FN=72), which could be accounted by population differentiation. The biology and behavior (*e.g.* low vagility) of this fish could contribute to the cytotype divergence between populations. However, information on the specimens collection site was not available in Urushido *et al.* (1975) and methodological artifacts can not be dismissed. Also, the discordance previously reported for the karyotype pattern of *N. chitala* and *N. notopterus* (Nayyar, 1965; Uyeno, 1973; Urushido *et al.*, 1975) is still unclear.

More than 2,700 fish species have been cytogenetically studied thus far (Klinkhardt *et al.*, 1995). An ancestral karyotype pattern for bonefish is still controversial between a diploid number equal to 48 acrocentric chromosomes (Ohno *et al.*, 1968) or a higher value (Brum & Galetti, 1997). The osteoglossiforms have been considered the most basal branch of extant Teleostei (Le *et al.*, 1993; Inoue *et al.*, 2001), and the results of *A. gigas* having $2n=56$ biarmed chromosomes support the idea of a higher $2n$ value for the ancestral Teleostei karyotype (Brum & Galetti, 1997). A complex chain of chromosome rearrangements involving fusions and pericentric inversions could produce chromosome number reductions and structural divergences among karyotypes of this fish group.

The large secondary constriction of pair 3 observed in the Giemsa karyotype of *A. gigas* was detected by both silver nitrate staining (Ag-NOR) and 18S rDNA-FISH. To the best of our knowledge, so far no chromosome banding studies have been carried out on chromosomes of other arapaimatids,

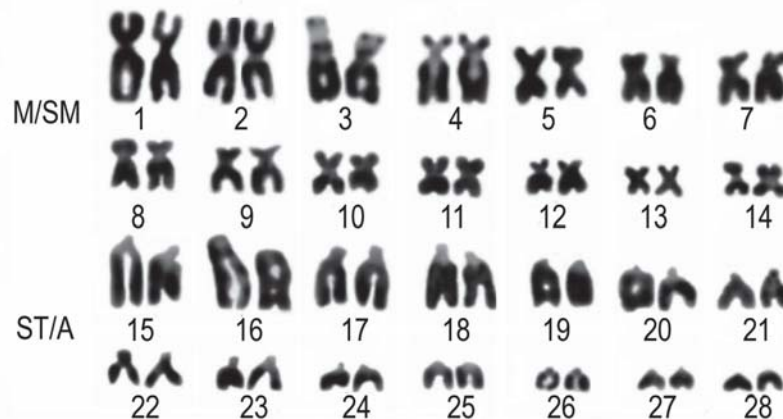


Fig. 1. Karyotype of *Arapaima gigas* showing chromosome constrictions in pair 3. Bar = 5 μ m.

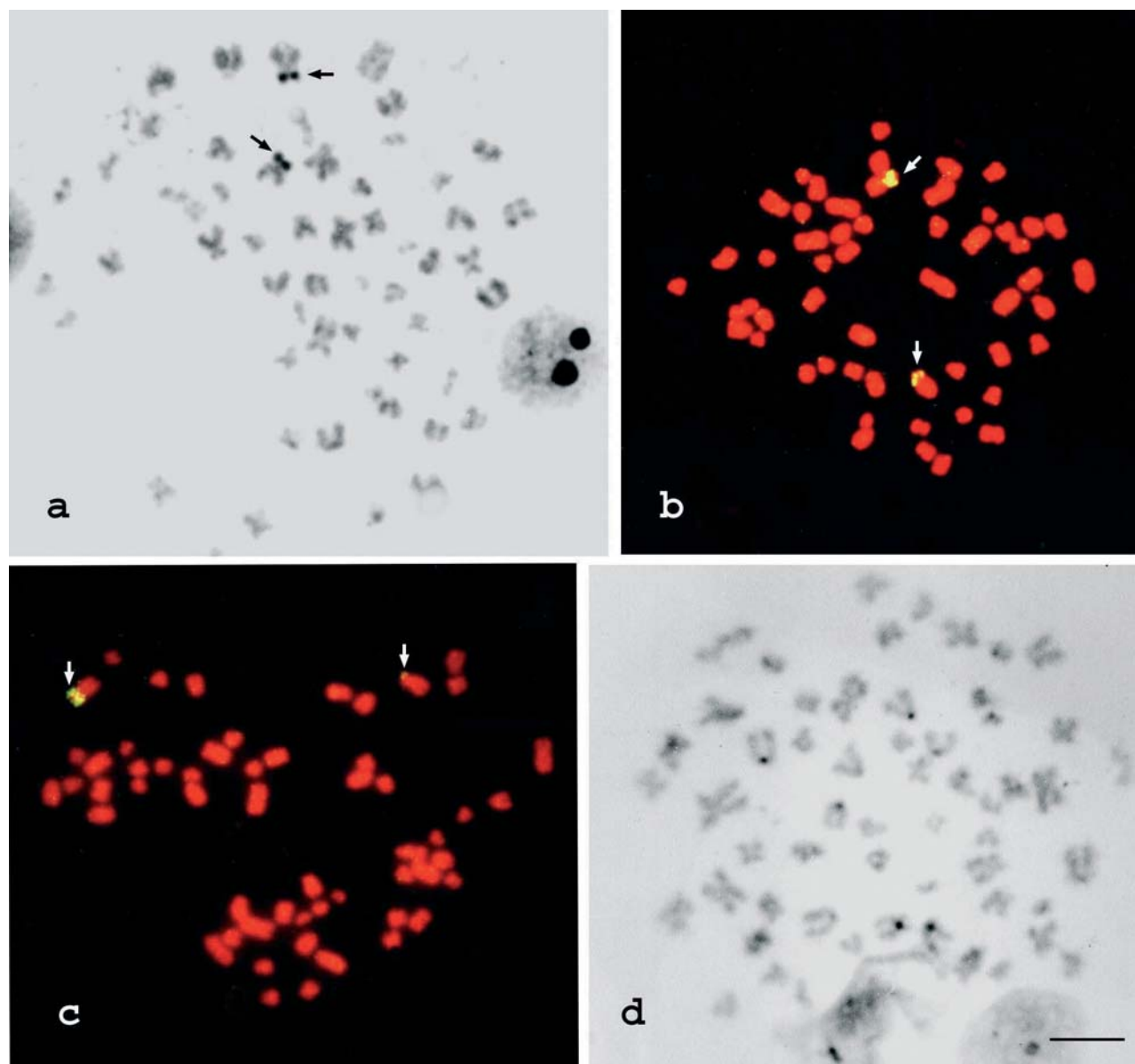


Fig. 2. Metaphases of *Arapaima gigas*. (a) Silver nitrate-stained showing two Ag-NOR sites (arrows); (b) and (c) 18S rDNA-FISH showing a single NOR-bearing chromosome pair and NOR size polymorphism (arrows); (d) C-banded chromosomes showing centromeric heterochromatin. Bar = 5 μ m.

impairing any conclusion on the evolution of the NOR sites within this fish group. However, it is currently believed that the occurrence of NOR sites located in only one chromosome pair in the karyotype, as observed in *A. gigas*, would be the ancestral condition in vertebrates (Hsu *et al.*, 1975).

The faint heterochromatin restricted to the centromeric region of some *A. gigas* chromosomes characterizes them as being quite poor in heterochromatic regions. In contrast, additional interstitial heterochromatic blocks occurred in three chromosome pairs of *N. chilata*, including the NOR-bearing pair, besides the centromeric heterochromatin present in all chromosomes (Akinori & Yoshio, 1998). Such heterochromatin divergence can suggest that this chromosome segment could have an important role in the chromosome diversification of this fish.

The chromosome banding and FISH results obtained here in *A. gigas* support the idea that the Osteoglossiformes seem to have experienced an extensive chromosome diversification and add new findings that show that these changes involved the karyotype macrostructure as well as its microstructure, represented here by the NORs and heterochromatin.

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