Cytogenetic analyses of two Curimatidae species (Pisces; Characiformes) from the Paranapanema and Tietê Rivers

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Abstract

Cytogenetic analyses were performed in two Curimatidae species (*Steindachnerina insculpta* and *Cyphocharax modesta*) from the Paranapanema and Tietê Rivers (São Paulo State, Brazil), showing a karyotype composed of 54 meta-submetacentric chromosomes in both species. Silver- and chromomycyn-staining and fluorescent in situ hybridization (FISH) using a 18S rDNA probe indicated that the nucleolar organizer regions (NORs) of both species are localized in the terminal region of the long arm of two metacentric chromosomes. Although a single NOR system was evidenced in both analyzed species, *S. insculpta* and *C. modesta* presented the nucleolar organizer regions in distinct chromosome pairs, indicating that these cistrons can be considered cytogenetic markers. Variation on the amount and distribution of the constitutive heterochromatin (C-bands) could also be detected between the two species - while *S. insculpta* presented few heterochromatic blocks, intensely stained C-bands were evidenced in *C. modesta* specially in the terminal region of the long arm of the NOR-bearing chromosomes. Although most Curimatidae species have been characterized by homogeneous karyotypes, isolated populations could be established under different environmental conditions leading to karyotype micro-structure variations specially related to the NORs localization and C-banding distribution. The obtained data were useful for the cytogenetic characterization and differentiation of *S. insculpta* and *C. modesta* and could be used in evolutionary inferences in the Curimatidae group.

Keywords: constitutive heterochromatin, *Cyphocharax modestus*, fish, NOR, *Steindachnerina insculpta.*

Análises citogenéticas de duas espécies de Curimatidae (Pisces; Characiformes) dos rios Paranapanema e Tietê

Resumo

Análises citogenéticas foram realizadas em duas espécies de Curimatidae (*Steindachnerina insculpta* e *Cyphocharax modestus*) provenientes dos rios Paranapanema e Tietê (Estado de São Paulo, Brasil), evidenciando um cariótipo composto por 54 cromossomos meta-submetacêntricos em ambas as espécies. Coloração com nitrato de prata e cromomicina e hibridação in situ fluorescente (FISH), utilizando uma sonda de DNAr 18S, mostraram que as regiões organizadoras de nucléolos (RONs) de ambas as espécies estão localizadas na região terminal do braço longo de dois cromossomos metacêntricos. Embora as espécies analisadas tenham apresentado um sistema de RONs simples, *S. insculpta* e *C. modesta* apresentaram as regiões organizadoras de nucléolos em distintos pares de cromossomos, indicando que estes cístrons podem ser considerados marcadores citogenéticos. Variação na quantidade e distribuição de heterocromatina constitutiva (bandas C) também pôde ser detectada entre as duas espécies - enquanto *S. insculpta* apresentou poucos blocos heterocromáticos, bandas C intensamente coradas foram evidenciadas em *C. modesta* especialmente na região terminal do braço longo dos cromossomos portadores de RONs. Embora a maioria das espécies de Curimatidae seja caracterizada por cariótipos homogêneos, populações isoladas podem ter se estabelecido sob condições ambientais distintas, levando à ocorrência de variações na micro-estrutura cariotípica especialmente relacionadas à localização das RONs e à distribuição das bandas C. Os dados obtidos mostraram-se úteis para caracterização e diferenciação citogenética de *S. insculpta* e *C. modesta* e podem ser utilizados em inferências evolutivas no grupo Curimatidae.

Palavras-chave: heterocromatina constitutiva, *Cyphocharax modestus*, peixes, RON, *Steindachnerina insculpta.*

1. Introduction

Neotropical fish fauna can be characterized either by the occurrence of stable karyotypic groups as well as by divergent ones with an extensive chromosome diversity (e.g. Oliveira et al., 1988; Galetti, 1998). In the order Characiformes, these two main general trends of chromosome evolution have been found. Thus, while several taxa show a chromosome evolution relatively divergent concerning to the karyotype macrostructure, other fish groups share a common karyotype structure and equal number of chromosomes (Bertollo et al., 1986; Oliveira et al., 1988; Arefjev, 1990; Galetti et al., 1994).

The family Curimatidae is composed of eight genera and approximately 120 species distributed throughout South and Central America (Weitzman and Vari, 1998) where they are commonly known as "saguirus" or "papa-terras", and represent a fish group found in several freshwater ecosystems including rivers, streams, and lakes (Vari, 1987). Previous cytogenetic data showed a macro-structural karyotype stability of Curimatidae since most studied species present a diploid number of 54 metacentric and submetacentric chromosomes (Scheel, 1973; Galetti et al., 1981; Pauls and Bertollo, 1990; Cestari et al., 1990; Galetti et al., 1991; Feldberg et al., 1992; Galetti et al., 1994; Navarrete and Julio, 1997), supporting a hypothesis of monophyly for the group. The conservation of a basic karyotype formulae composed of 54 biarmed chromosomes is also observed in Prochilodontidae, Anostomidae, and Chilodontidae (Brum and Galetti, 1997). These fish groups and Curimatidae also share osteological and anatomical characters, comprising a monophyletic unit (Vari, 1983, 1989; Vari et al., 1995).

Despite the great karyotype similarity observed among the Curimatidae so far studied, karyotype divergences can also be found in some species, such as *Potamorhina altamazonica* (2n = 102), *P. latior* (2n = 56), *Curimata ocellata* (2n = 56) (Feldberg, 1990), *Cyphocharax* sp. (2n = 58) (Venere, 1991), *C. platanus* (2n = 58) and *Potamorhina squamoralevis* (2n = 102) (Brassesco et al., 2004), and seem to be related to fission events (Scheel, 1973; Venere, 1991; Feldberg et al., 1987, 1992, 1993; Navarrete, 1996). Microstructural changes in chromosome formula, as well as in the C-bands and nucleolar organizer regions (NORs) distribution, have also been described in this fish group (Venere and Galetti, 1989).

Cytogenetic studies can still be considered scarce in Curimatidae, due to its high genera and species diversity. In order to improve cytogenetic data on this group, the karyotype structure of *Steindachnerina insculpta* and *Cyphocharax modestus* from two Brazilian river basins was analyzed by Giemsa-, silver nitrate-, and chomomycyn-staining, 18S rDNA fluorescent in situ hybridization, and C -banding.

2. Material and Methods

2.1. Sample collection

Analyses were performed in the Curimatidae species *Steindachnerina insculpta* and *Cyphocharax modesta*, both from the Paranapanema River (Jurumirim Hydroeletric Reservoir, border municipality of Paranapanema and Angatuba, São Paulo State, Brazil) and the Tietê River (municipality of Botucatu, São Paulo State, Brazil). 23 individuals of *S. insculpta* were collected in the Paranapanema River (5 males and 1 female) and in the Tietê River (5 males and 12 females). A total of 27 specimens (18 males and 9 females) and 12 specimens (3 males and 9 females) of *C. modesta* were caught in the Paranapanema River and Tietê River, respectively. The analyzed animals were deposited in the museum of the Laboratório de Biologia e Genética de Peixes, Instituto de Biociências, UNESP-Botucatu, Brazil.

2.2. Chromosome analysis

Mitotic chromosomes were obtained from kidney cell suspensions based on the method described by Foresti et al. (1993). Analysis of the constitutive heterochromatin (C-banding) was performed as described by Sumner (1972), with some minor modifications. The nucleolar organizer regions (NORs) were identified by silver nitrate (Howell and Black, 1980) and chromomycyn (CMA_3) staining (Schweizer, 1976), and also by florescent in situ hybridization (FISH) (Pinkel et al., 1986; Hamkalo and Elgin, 1991; Martins and Galetti, 1999; Wasko and Galetti, 2000). FISH experiments were performed using a PCR-generated 18S rDNA of *Oreochromis niloticus* (kindly provided by Claudio Oliveira) as a probe labeled by nick translation with biotin-dATP (Bionick™ Labeling System-Gibco. BRL).

3. Results

All analyzed individuals of *Steindachnerina insculpta* and *Cyphocharax modestus*, from the Paranapanema and Tietê Rivers, evidenced a standard karyotype with a diploid number of 2n = 54, consisting exclusively of metacentric and submetacentric chromosomes (Figures 1 and 2). Small supernumerary chromosomes were identified in some individuals of *C. modesta* from the Paranapanema River (Santos et al., in press). No significant differences were observed between the karyotypes of *S. insculpta* and *C. modesta*, as well as between the samples of each species from the Paranapanema and Tietê Rivers. In addition, no chromosome differences were found among males and females of either species.

Silver staining evidenced that the Ag-nucleolar organizer regions (NORs) are localized at the terminal region of the long arm of two large metacentric chromosomes in *S. insculpta* (Figure 1) and *C. modestus* (Figure 2). However, a species-specific NOR location was evidenced - while NORs were seen on the chromosome pair 7 in *S. insculpta*, they were visualized on the chromosome pair 2 in *C. modesta* (Figures 1 and 2;

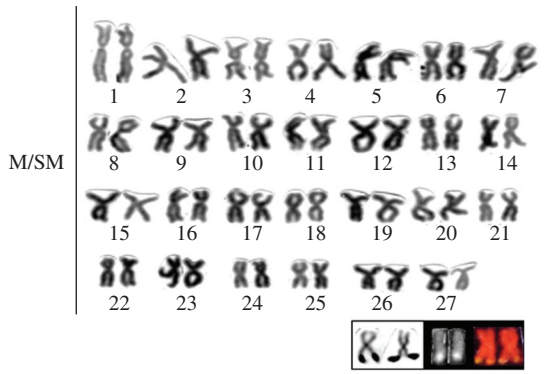

Figure 1. Giemsa-stained karyotype of *Steindachnerina insculpta*. The NOR-bearing chromosome pair 7 is shown in the box by silver nitrate (left), chromomycyn (middle), and 18S rDNA-FISH (right).

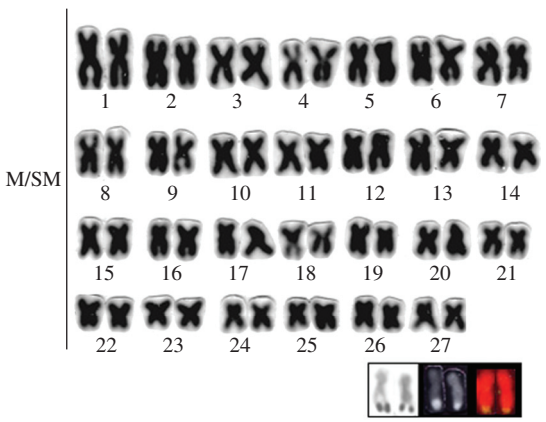

Figure 2. Giemsa-stained karyotype of *Cyphocharax modesta*. The NOR-bearing chromosome pair 2 is shown in the box by silver nitrate (left), chromomycyn (middle), and 18S rDNA-FISH (right).

Table 1). This same location was found in the specimens from the Paranapanema and Tietê Rivers.

Chromomycyn staining and in situ hybridization with a 18S rDNA probe permitted the identification of a single large metacentric chromosome pair of *S. insculpta* and *C. modesta*, with bright signals on the terminal region of the long arm (Figures 1 and 2). No additional minor fluorescent signals were seen and one or two nucleoli were observed in the interphase nuclei cells. Figures 1 and 2 also show the chromosomes of *S. insculpta* and *C. modesta* that bear the 18S rDNA cistrons.

Heterochromatin was seen through several centromeric and a few telomeric regions in the chromosomes of both species (Figure 3). Conspicuous heterochromatic blocks could be easily visualized on the long arm of the chromosome pair 2 of *C. modesta* and the chromosome

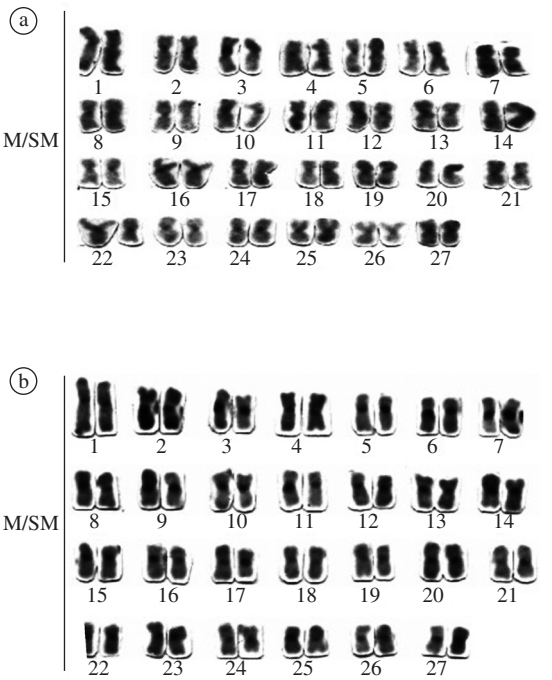

Figure 3. Constitutive heterochromatin patterns of a) *Steindachnerina insculpta*; and b) *Cyphocharax modestus*.

pair 7 of *S. insculpta* (Figure 3). No differences in the C-banding pattern were detected between the two samples of each species.

4. Discussion

The karyotypes of *Steindachnerina insculpta* and *Cyphocharax modestus* from the Paranapanema and Tietê Rivers, comprising 2n = 54 meta and submetacentric chromosomes, a single NOR bearing chromosome pair and heterochromatic segments in the centromeric region of almost all chromosomes, are closely similar to those found in most species of Curimatidae so far studied (Venere and Galetti, 1989; Feldberg et al., 1992; Oliveira and Foresti, 1993; Navarrete and Julio, 1997). The present results are congruent with previous data that indicate a high chromosome stability in Curimatidae and reinforces the idea that this fish group retains the diploid number of 2n = 54 and a gross chromosome structure of a probable ancestral karyotype.

On the other hand, even though the karyotype macrostructure is practically invariable among the curimatids and the presence of a single NOR system is constantly observed in their complement, the NORs can be located on different chromosomes, representing species-specific cytogenetic markers. This fact was clearly evidenced in the present analyzed species - Ag-staining showed that even though *Steindachnerina insculpta* and *Cyphocharax modestus* present a single NOR pair, each species has specific NOR-bearing chromosomes.

Table 1. NOR chromosome characterization in *Steindachnerina insculpta* and *Cyphocharax modestus* from different regions.

Species	NOR chromosome location	Chromosome pair	Reference
Steindachnerina insculpta (Paranapanema River - border municipality of Paranapanema and Angatuba, São Paulo State, Brazil)	long arm large metacentric	7	Present paper
Steindachnerina insculpta (Tietê River - municipality of Botucatu, São Paulo State, Brazil)	long arm large metacentric	7	Present paper
Steindachnerina insculpta (Mogi-Guaçu River - municipality of Pirassununga, São Paulo State, Brazil)	short arm small metacentric	25	Venere and Galetti (1989)
Steindachnerina insculpta (Tibagi River - municipality of Londrina, Paraná State, Brazil)	long arm large metacentric	7	Rodrigo Teribele (personal communication)
Steindachnerina insculpta (Tibagi River - municipality of Londrina, Paraná State, Brazil)	long arm large metacentric	12	Waleska Gravena (personal communication)
Cyphocharax modestus (Paranapanema River - border municipality of Paranapanema and Angatuba, São Paulo State, Brazil)	long arm large metacentric	2	Present paper
Cyphocharax modestus (Tietê River - municipality of Botucatu, São Paulo State, Brazil)	long arm large metacentric	2	Present paper
Cyphocharax modestus (Infernão Lagoon - Mogi-Guaçu River- municipality of Luiz Antonio, São Paulo State, Brazil, and tributary of the Piracicaba River- municipality of Águas de São Pedro, São Paulo State, Brazil	long arm large metacentric	2	Venere and Galetti (1989)
Cyphocharax modestus (Tibagi River, municipality of Londrina, Paraná State, Brazil)	long arm large metacentric	2	Martins et al. (1996)
Cyphocharax modestus (Três Bocas Stream, municipality of Londrina, Paraná State, Brazil)	long arm large metacentric	2	Rodrigo Teribele (personal communication)
Cyphocharax modestus (Três Bocas Stream, municipality of Londrina, Paraná State, Brazil)	long arm large metacentric	2	Waleska Gravena (personal communication)

Ag-NOR location in *C. modesta* is similar to that found for the same species from different Brazilian regions. Although some discrepancies could be observed between the NORs of *S. insculpta* from distinct rivers, most data seem to support the NOR location in a large submetacentric chromosome pair. Table 1 summarizes the NOR data for *C. modesta* and *S. insculpta* from different Brazilian freshwater systems and reinforces the proposition that these nucleolar cistrons can be used as species-specific cytogenetic markers. The distinct NOR location within the Curimatidae species could be due to internal chromosome modifications, such as translocations and/or inversions (Venere and Galetti, 1989).

The Ag-NORs localization was confirmed by chromomycyn-staining on mitotic chromosomes of *S. insculpta* and *C. modesta*. Although Ag-staining detects only transcriptional active NORs in the preceding interphase (Hsu et al., 1975), GC-specific fluorochromes such as CMA_3 may reveal both active and inactive NORs in fish and amphibians (Mayr et al., 1986; Schmid and Guttenbach, 1988; Phillips and Hartley, 1988; Galetti and Rasch, 1993; Mestriner et al., 1995), probably as a consequence of the higher GC content of the rDNA (Schmid and Guttenbach, 1988). The NOR chromosome sites of *S. insculpta* and *C. modesta* have also been identified by fluorescent in situ hybridization using a 18S rDNA probe, confirming the existence of a single locus for this gene family in both analyzed species from the Paranapanema and Tietê Rivers.

Despite the karyotype homogeneity observed in Curimatidae, variation on the amount and distribution of constitutive heterochromatin can be detected among

some species. *S. insculpta* presented few heterochromatic blocks, as observed in the long arm of the NOR-bearing chromosome pair 7, according to previous data on this species (Venere, 1991). On the other hand, highly stained C-bands were evidenced in *C. modesta*, specially in the terminal region of the long arm of the NOR-bearing chromosome pair 2. The agreement of C-bands and NOR sites is a common feature among fish species since the 45S rDNA appears to be interspersed or adjacent to heterochromatin segments in these animals (Pendás et al., 1993; Galetti et al., 1994). The occurrence of constitutive heterochromatin blocks adjacent to nucleolar organizer regions may have facilitated chromosome breaks, thus permitting structural rearrangements concerning these regions (Moreira-Filho et al., 1984). Conspicuous heterochromatin blocks were also visualized on the second pair of *Cyphocharax gilberti*, *C. nagelli*, and *Cyphocharax* sp., suggesting that this could be a basal element in Curimatidae (Venere, 1991).

Although most Curimatidae species have been characterized by nearly homogeneous karyotypes, they can be found in quite diverse ecosystems in the Neotropical region (Vari, 1988). Consequently, isolated populations could be established under different environmental conditions leading to karyotypic microstructural variations, such as NOR localization and C-band distribution.

The present results were useful in the cytogenetic characterization and differentiation of *Steindachnerina insculpta* and *Cyphocharax modestus* and can also be used in evolutionary inferences in the Curimatidae fish group.

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