Cytogenetic Markers in Wild Population of Curimbatá 
(*Prochilodus lineatus*) from Mogi-Guaçu River

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Cytologia 74(3): 281–287, 2009

Summary Within the genus *Prochilodus*, the species *Prochilodus lineatus* is certainly the most studied one from a cytogenetic point of view. In this study, a cytogenetic characterization of specimens of *P. lineatus* from Mogi-Guaçu River was performed in the period from 2003 to 2007, through utilization of cytogenetic markers, such as Giemsa, Ag-NOR, C-banding and cytogenetical-molecular markers (FISH) to detect both 5S and 18S ribosomal genes. All analyzed individuals presented 2n=54 meta/submetacentric chromosomes, besides bearing up to 7 supernumerary microchromosomes. Polymorphic NORs were detected on a single chromosomal pair. The constitutive heterochromatin was distributed at centromeric region of all chromosomes in the A complement, while the microchromosomes were totally heterochromatic. A syntenic distribution of 5S and 18S ribosomal genes was detected, comprising the NOR-bearing chromosomal pair. No additional ribosomal clusters in other chromosomes were observed. Although the karyotype features are commonly conserved within the genus *Prochilodus*, the few differences on the distribution of both ribosomal genes and heterochromatin when compared to specimens of *P. lineatus* from other localities can be associated to the evolutionary changes that these repetitive sequences have undergone through the years.

Key words *Prochilodus lineatus*, Cytogenetic markers, Mogi-Guaçu, Nucleolar organizer regions.

Fishes of the family *Prochilodontidae* are important species for commercial and subsistence fishery in Neotropical environments of South America, excepting Chile, where they are not found (Lowe-McConnell 1975, Goulding 1981, Vari 1983). According to Castro and Vari (2004), this family includes 21 species distributed into 3 genera: *Ichthyoelephas* (2 species), *Prochilodus* (13 species) and (6 species).

The genus *Prochilodus* is composed of widely distributed species throughout South American waters, being considered a fish group of great importance for both trade and subsistence fisheries (Mago-Leccia 1972). Within this genus, the species, *Prochilodus lineatus*, popularly known as curimbatá, is certainly the most abundant and the most studied (Godoy 1975).

Chromosomal information about *Prochilodus* representatives have shown a conserved karyotypic structure of 2n=54 chromosomes (Pauls and Bertollo 1983, 1990). However, few species and/or populations show karyotypic variation because of the presence of supernumerary chromosomes, such as *Prochilodus lineatus* (Pauls and Bertollo 1983, 1990, Oliveira et al. 1997, Dias et al. 1998, Maistro et al. 2000, Cavallaro et al. 2000, Jesus and Moreira-Filho 2003, Artoni et al. 2006). As most karyotypes of Neotropical fish, *P. lineatus* species presents nucleolar organizer
regions (NOR) on a single chromosomal pair (Pauls and Bertollo 1990).

In situ localization of 5S and 18S ribosomal genes showed that both rDNA classes are syntenic in *P. lineatus* and *P. argenteus*, as well as a numerical polymorphism in the 18S rRNA genes located on NORs (Jesus and Moreira-Filho 2003, Hatanaka and Galetti Jr. 2004).

Several C-banding studies have been carried out in representatives of the family Prochilodontidae, including *P. lineatus*. Frequently, the pattern of constitutive heterochromatin distribution is restricted to centromeric blocks in all chromosomes of the standard (A) complement (Pauls and Bertollo 1990, Cavallaro et al. 2000, Jesus and Moreira-Filho 2003, Artoni et al. 2006). On the other hand, the supernumerary chromosomes are generally entirely heterochromatic in this species (Pauls and Bertollo 1990, Maistro et al. 2000, Cavallaro et al. 2000, Jesus and Moreira-Filho, Artoni et al. 2006).

Although the chromosomal features of *P. lineatus* are considered conserved, the goal of the present study was to characterize cytogenetically specimens of this species from Mogi-Guaçu River, Pirassununga, SP, in order to detect similarities and possible cytogenetic changes that may have occurred in this population through their evolutionary history.

Materials and methods

Two hundred and 75 specimens of *Prochilodus lineatus* collected in the Mogi-Guaçu River, Pirassununga, SP from 2003 to 2007 were analyzed. The collected animals were kept in fish ponds at CEPTA/ICMBio, Pirassununga, SP for further cytogenetic procedures.

Mitotic chromosomes were obtained by lymphocyte culture technique, as described by Fenocchio and Bertollo et al. (1988) with some adjustments for the studied species. The karyotype arrangement was performed according to Levan et al. (1964).

Active nucleolar organizer regions (NOR) were identified on chromosomes using silver nitrate staining (Howell and Black 1980) and the constitutive heterochromatin on chromosome preparations was detected following the C-banding method proposed by Sumner (1972).

Fluorescent in situ hybridization was carried out according to Porto-Foresti et al. (2002) using rDNA 5S probes obtained by PCR (Polymerase Chain Reaction) of *Prochilodus lineatus* genome DNA, using the primers A (5’-TACGCCGATCTCG TCCGATC-3’) and B (5’-CAGGCTG-GTATGGCCGTAAGC-3’) (Pendás et al. 1994). The 18S rDNA probe was obtained by PCR using NS1 primer (5’-GTAGTCATATGCTTGTCTC-3’) and NS8 (5’-TCCGCAAGTTCACCTACGGA-3’) (White et al. 1990). Both probes were labeled using the Bionick Labeling System kit (Gibco-BR) according to the manufacturer’s instructions.

In situ hybridization was performed using avidin-fluorescein conjugates and digoxigenin-rhodamine conjugates. The 5S and 18S rDNA probes were denatured in 70% formamide: 2X SSC for 5 min. DNA was hybridized at 37°C overnight in a moist chamber (1 μg of denatured probe, 50% formamide, 10 mg ml of dextran sulfate; 2X SSC, 5 mg/ml of salmon sperm DNA). Hybridized probes were detected by anti-avidin reactions. Afterwards, the slides were counterstained with DAPI (4’6-diamidino-2-phenylindole) propidium iodide and photographed in a photomicroscope (Olympus, BX50 model) equipped with epifluorescence.

Results

The specimens of *Prochilodus lineatus* from Mogi-Guaçu River, Pirassununga, SP, collected from 2003 to 2007 presented a karyotype composed of 2n=54 meta/submetacentric chromosomes, plus the presence of up to 7 supernumerary chromosomes, being 2 the modal number of B-chromosomes for this species (Fig. 1).

By silver nitrate staining, 2 Ag-NOR marks were observed on long arms of the second largest
pair of metacentric chromosomes in the karyotype of *P. lineatus* (Fig. 2a). Moreover, a size polymorphism of this region was found between homologous chromosomes.

Using C-banding technique, conspicuous heterochromatin blocks were observed on centromeric regions of the standard complement and all supernumerary chromosomes proved to be entirely heterochromatic (Fig. 2b).

In situ hybridization technique using fluorescent probes (FISH) was performed in order to provide a better characterization of the 5S and 18S ribosomal genes location in *P. lineatus*. Such genes were observed in synteny, *i.e.*, the 5S ribosomal gene was located subterminally on long arms of the second metacentric pair, adjacent to the 18S rDNA cluster (Fig. 3). No additional clusters of either 5S or 18S rRNA gene was found in chromosomes of this species by FISH.

**Discussion**

Chromosomal features in the studied species have already been reported by several authors (Pauls and Bertollo 1983, 1990, Oliveira *et al.* 1997, Cavallaro *et al.* 2000, Jesus and Moreira-Filho 2003, Artoni *et al.* 2006). Regarding the karyotype structure, the results from the present study are
in agreement with the previous data.

The detection of nucleolar organizer regions (NOR) by silver nitrate staining has widely been investigated in fish, since these regions are considered reliable cytogenetic markers, useful in cytotaxonomic studies (Galetti Jr. 1998). As most of Neotropical fish species, *Prochilodus lineatus* presents a single NOR-bearing pair (Pauls and Bertollo 1990, Maistro et al. 2000, Jesus and Moreira-Filho 2003, Vicari et al. 2006, Artoni et al. 2006).

Maistro et al. (2000), studying the nucleolar organizer regions (NOR) in *P. lineatus* from Mogi-Guaçu River reported marks on long arms of the third chromosomal pair, besides a remarkable size polymorphism. Vicari et al. (2006), studying specimens of *P. lineatus* species from Dourada lake, Tibagi River basin, Ponta Grossa, PR, identified NORs close to centromeres at interstitial region on the long arms of the fourth chromosomal pair and, also, a size polymorphism between homologous.

In some specimens of *P. lineatus* from Mogi-Guaçu River, SP, Jesus and Moreira-Filho (2003), detected major NORs on a single metacentric pair plus a variable number of additional ribosomal sites (one or two minor inactive signals), thereby suggesting an inter-individual numerical polymorphism of 18S rDNA regions.

The fluorescent *in situ* hybridization using 5S and 18S rDNA probes have also corroborated
the previous data reported by Jesus and Moreira-Filho (2003), Hatanaka and Galetti Jr. (2004) and Vicari et al. (2006). No additional cluster was found in relation to either 5S rDNA, as described by Jesus and Moreira-Filho (2003) and Vicari et al. (2006), or 18S rDNA, as observed by Maistro et al. (2000) and Vicari et al. (2006) in studies carried out in Prochilodus lineatus from Dourada Lake and Mogi-Guaçu River, respectively.

A syntenic organization of 5S and 18S ribosomal genes represents a rare feature among vertebrates. Besides P. lineatus, the synteny between both ribosomal genes has been reported in fish, like Salmo solar (Pendás et al. 1994), Oncorhynchus mykiss (Moran et al. 1996), and Astyanax species (Almeida-Toledo et al. 2002) as well as in amphibians (Lucchini et al. 1993). On the other hand, in several other fish species, these loci have been mapped in distinct chromosomes (Martínez et al. 1996, Morán et al. 1996, Martins and Galetti 2001, Born and Bertollo 2000, Ferro et al. 2001, Vicente et al. 2001, Wasko et al. 2001), thus following the frequent condition described for vertebrates (Lucchini et al. 1993, Drouin and Muniz de Sá 1995, Suzuki et al. 1996).

Hatanaka and Galetti Jr. (2004) detected 5S rDNA sites on long arms of the NOR-bearing chromosomes in Prochilodus argenteus, as confirmed by sequential FISH–Ag-NOR analysis, revealing that the 5S rDNA occupies a more terminal and adjacent position when compared to 18S rDNA cluster. Moreover, these authors also observed minor fluorescent signals, occasionally identified on the third chromosomal pair, indicating the presence of additional 5S rDNA sites.

Jesus and Moreira-Filho (2003) carried out FISH experiments in P. lineatus from Mogi-Guaçu River using 18S rDNA probes and showed that the major ribosomal genes were located at interstitial region on long arms of the second chromosomal, equivalent to Ag-NORs. However, inactive additional 18S rDNA sites were also detected in some individuals. A numerical polymorphism of ribosomal genes was also recently reported in P. argenteus from São Francisco River basin by Hatanaka and Galetti Jr. (2004). In situ hybridization analysis in this species revealed three 18S rDNA signals, additional to the two loci commonly detected by silver nitrate staining. In that case, the additional rDNA clusters were restricted to the telomeric regions.

Instead, the major rDNA clusters in P. lineatus were mapped on the pericentromeric regions (Jesus and Moreira-Filho 2003), thereby showing a distinctive distribution of 45S rDNA in the karyotype of both species. Coupled with chromosomal rearrangements, transposition events have also been considered responsible for the NOR dispersal in genomes of different groups (Castro et al. 1996, Almeida-Toledo et al. 1996). So far, both 5S or 18S ribosomal genes have never been identified on the B-microchromosomes of P. lineatus (Jesus and Moreira-Filho 2003), as also shown in the present data. The lack of ribosomal genes on supernumerary chromosomes corresponds to a common condition in organisms bearing such elements.

The C-banding technique has also been useful in fish cytogenetics, allowing the visualization of constitutive heterochromatin segments. Differences in the amount or distribution of heterochromatic regions can be easily identified by C-banding, providing thus important markers to some fish groups. Quantitative or positional dissimilarities on C-bands might be used to differentiate and characterize genera, species and populations (Mantovani et al. 2000).

Several C-banding studies have been performed in the family Prochilodontidae, specially in Prochilodus lineatus. Maistro et al. (2000) reported both centromeric and telomeric distribution of constitutive heterochromatin in the A complement of curimbatás collected in the Mogi-Guaçu River, Pirassununga, São Paulo. However, telomeric C-bands were absent in the chromosomal preparations presently analyzed. Nonetheless, the heterochromatic nature of supernumerary chromosomes was corroborated.

Artoni et al. (2006), characterizing specimens of P. lineatus from Dourada lake in the Tibagi River basin, Ponta Grossa, PR, observed heterochromatin segments at centromeres of most chromosomes as well as at telomeric regions in some pairs from the A complement. The B-chromosomes in these analyzed specimens were frequently heterochromatic. However, the authors
reported a small negative C-band at the pericentromeric region of a metacentric B-chromosome (Artoni et al. 2006).

Based on these reports and the results from the present work, it can be verified that, in spite of the conserved karyotypic features of *P. lineatus*, differences related to the localization and distribution of both ribosomal genes and heterochromatin are observed in this species when distinct localities are compared. Such differences can be associated to the changes these repetitive sequences have undergone during the evolutionary history of each population.

References


