

## Analyses of the Structure of NORs in Two Species of South American Sorubiminae Fishes (Siluriformes) by Means of Several Cytogenetic Techniques

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Accepted October 10, 2007

SWARÇA A. C., FENOCCHIO A. S., CESTARI M. M., DIAS A. L. 2008. Analyses of the structure of NORs in two species of south American Sorubiminae fishes (Siluriformes) by means of several cytogenetic techniques. *Folia biol. (Kraków)* 56: 31-35.

Several cytogenetic techniques (AgNOR, C- G- and RE bandings, DAPI, CMA<sub>3</sub> and FISH) were applied in order to analyze the structure and variability of NORs in the fish species *Steindachneridion melanodermatum* and *S. scripta*. Ag-NORs were observed on the short arm of the first acrocentric chromosome pair, coincidentally with a strong C-positive band on a large secondary constriction. In addition, NORs showed bright fluorescent signals when stained with CMA<sub>3</sub> and treated for FISH with rDNA 18S. However, they showed negative coloration after G- and restriction enzyme banding and DAPI staining. The results evidence a substantial size polymorphism in these regions. The NOR bearing chromosomes in both species may be considered homologues because they maintain conserved characteristics, such as being interspersed with a GC-rich heterochromatin and possessing target sequences for *AluI*, *BamHI* and *EcoRI*.

Key words: NOR polymorphism, Neotropical fishes, *Steindachneridion*.

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In most eukaryotic organisms, tandem arrays of genes for ribosomal RNA are found at one or few chromosomal loci within the genome, termed the nucleolar organizer regions (NORs). These regions can be selectively stained using the silver staining technique (Ag-NORs), however, this methodology is suitable for studying NOR expression because it detects only transcriptionally active sites (HOWELL & BLACK 1980). Another technique frequently used to detect NORs, in fishes and amphibians, is GC specific fluorochrome staining (CMA<sub>3</sub>, mitramycin A), which stains both active and inactive NORs, probably due to their high GC content (SCHMID & GUTTENBACH 1988). The locations of Ag-NORs in these groups have been confirmed by fluorescence *in situ* hybridization (FISH) (LONG & DAVID 1980; GORNUNG *et al.* 1997; WASKO & GALETTI 2000). Polymorphism in size and number of NORs have been reported in several fish genera and species, showing that these regions are generally located on secondary constrictions (FORESTI *et al.* 1981;

HARTLEY 1987; PENDÁS *et al.* 1993a, b). *Steindachneridion* belongs to the Sorubiminae group (Siluriformes) and has a restricted distribution in some South American river basins. Wild populations are greatly diminished: *S. scripta* is found in the Paraná and Uruguay rivers and *S. melanodermatum* is endemic to the upper Iguazu river. Both species show 2n=56 chromosomes (SWARÇA *et al.* 2005, 2006). The aim of the present work was to analyze the structure and characteristics of the NORs in *S. scripta* and *S. melanodermatum* using several cytogenetic techniques (AgNOR, C- G- and RE banding, DAPI, CMA<sub>3</sub> and FISH) in order to obtain new cytogenetic data for these fish species.

### Material and Methods

Six specimens of *Steindachneridion scripta* from the Upper Paraná basin (Parapanema and Tibagi Rivers) (Paraná state, Brazil) and seventeen

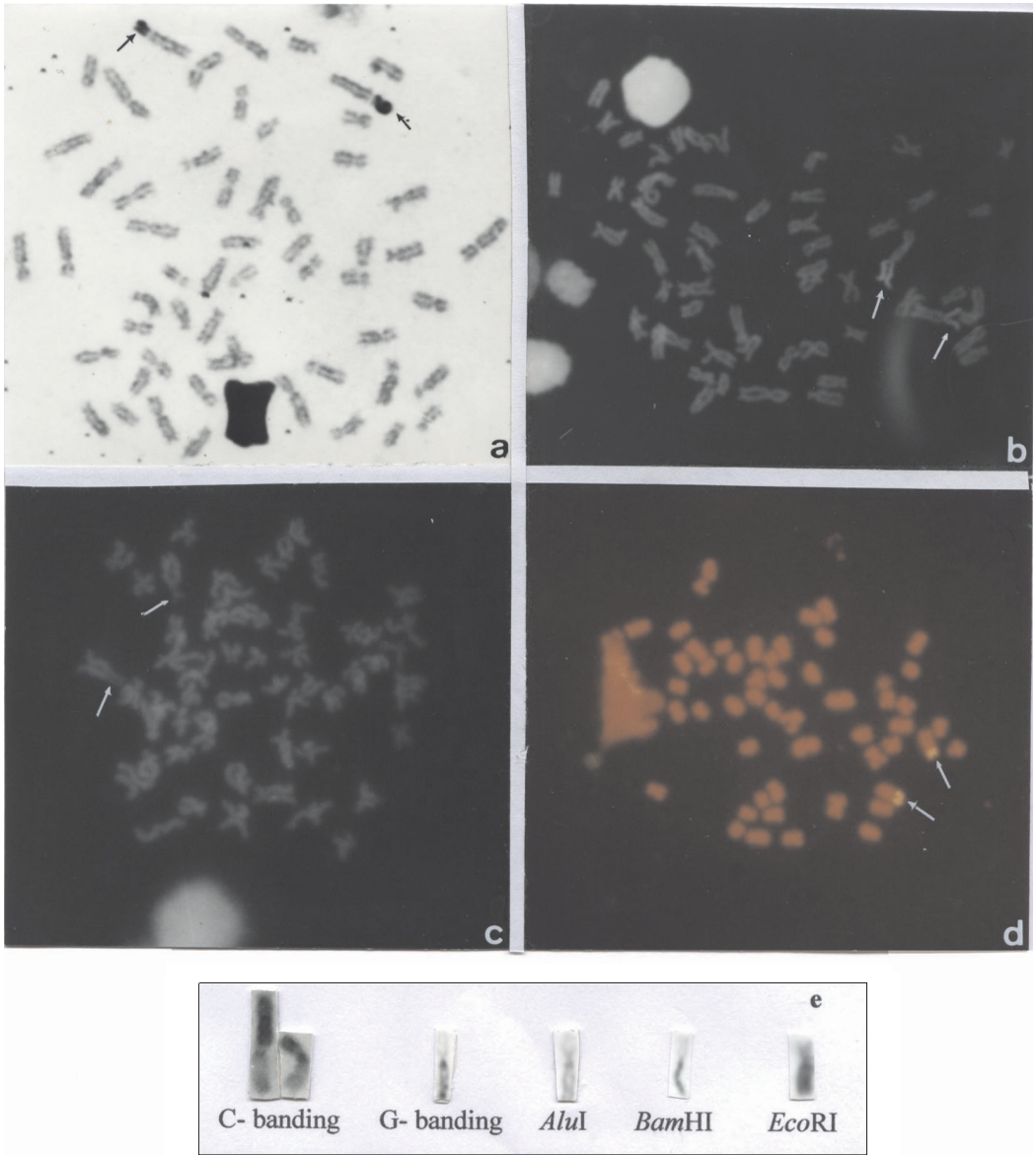


Fig. 1. Somatic metaphase plates of *Steindachneridion scripta* after (a) AgNO<sub>3</sub>, (b) CMA<sub>3</sub> and (c) DAPI staining; (d) FISH with 18S rDNA probe. Arrows indicate the NOR bearing chromosomes; (e) the secondary constrictions after C- and G-banding, *AluI*, *BamHI* and *EcoRI* restriction enzymes treatments.

specimens of *Steindachneridion melanodermatum* from Iguaçú River (Salto Segredo, Paraná state, Brazil) were cytogenetically studied. Mitotic chromosome preparations were obtained from lymphocyte culture (FENOCCHIO & BERTOLLO 1988). NOR silver staining, C- and G- banding were performed using the methods of HOWELL & BLACK (1980), SUMNER (1972) and CANO *et al.* (1996), respectively. Chromomycin A<sub>3</sub> (CMA<sub>3</sub>) and DAPI staining followed VERMA and BABU (1995). Restriction endonucleases (RE) were employed according SÁNCHEZ *et al.* (1990) with some modifications, i.e. *AluI* (0.3 units/ $\mu$ l 37°C/4h), *BamHI* (0.3 ud/ $\mu$ l 37°C/14h) and *EcoRI* (2.0 units/ $\mu$ l 37°C/14h). A rDNA 18S probe (1700 pb) obtained

from the nuclear DNA of the fish *Oreochromis niloticus* was used for *in situ* hybridization, and was carried out as described by SWARÇA *et al.* (2001b).

## Results and Discussion

*Steindachneridion scripta* and *Steindachneridion melanodermatum* have been characterized cytogenetically by SWARÇA *et al.* 2005, 2006 respectively. The latter species was previously referred to by the authors as *Steindachneridion* sp., however, according to a recent taxonomic revision of the genus, the correct specific name is *S. melanodermatum* (GARAVELLO 2005). Both species have

$2n=56$  chromosomes with a very decondensed secondary constriction in the first acrocentric pair.

In the present paper, silver staining permitted the detection of NORs on the short arm of the acrocentric 1<sup>st</sup> chromosome pair coincident with a secondary constriction in both species (Figs 1a, 2a). This may represent a marker feature for the genus since in almost all fishes of the Sorubiminae group the NORs are located on the short arms of submetacentric or subtelocentric chromosomes, i.e. in *Pseudoplatystoma* (FENOCCHIO & BERTOLLO 1992; MARTINS-SANTOS *et al.* 1996; SWARÇA *et al.* 2005), *Zungaro zungaro* (= *Paulicea luetkeni*), *Hemysorubin platyrhynchus* (MARTINS-SANTOS *et al.* 1996; SWARÇA *et al.* 2001b) and *Sorubim lima* (FENOCCHIO & BERTOLLO 1992; MARTINS-SANTOS *et al.* 1996). Differences in size between the homologous, NOR-carrying chromosomes were frequently found (Figs 1a, 2a) and in sizeable chromosomes, separated Ag-NOR blocks were evident,

suggesting a duplication of the region (Fig. 2a). In some species of Sorubiminae, as well as in several pimelodids, the occurrence of size heteromorphism between homologue NOR-bearing chromosomes was frequently reported (FENOCCHIO & BERTOLLO 1992; MARTINS-SANTOS *et al.* 1996; SWARÇA *et al.* 2005).

A strong positive C-band characterizes the secondary constrictions of the NOR bearing chromosomes of *Steindachneridion* species (Figs 1e, 2e) and the size of this heterochromatic region, being highly polymorphic, produces a conspicuous heteromorphism, as observed by means of silver staining. Supportively, in metaphase plates in which the chromosomes were enlarged in size, it was possible to detect ribosomal clusters, each one separated by unstained regions (Fig. 2a). These faint regions are probably constituted by heterochromatin interspersed through the AgNORs regions. These features were also observed in brown

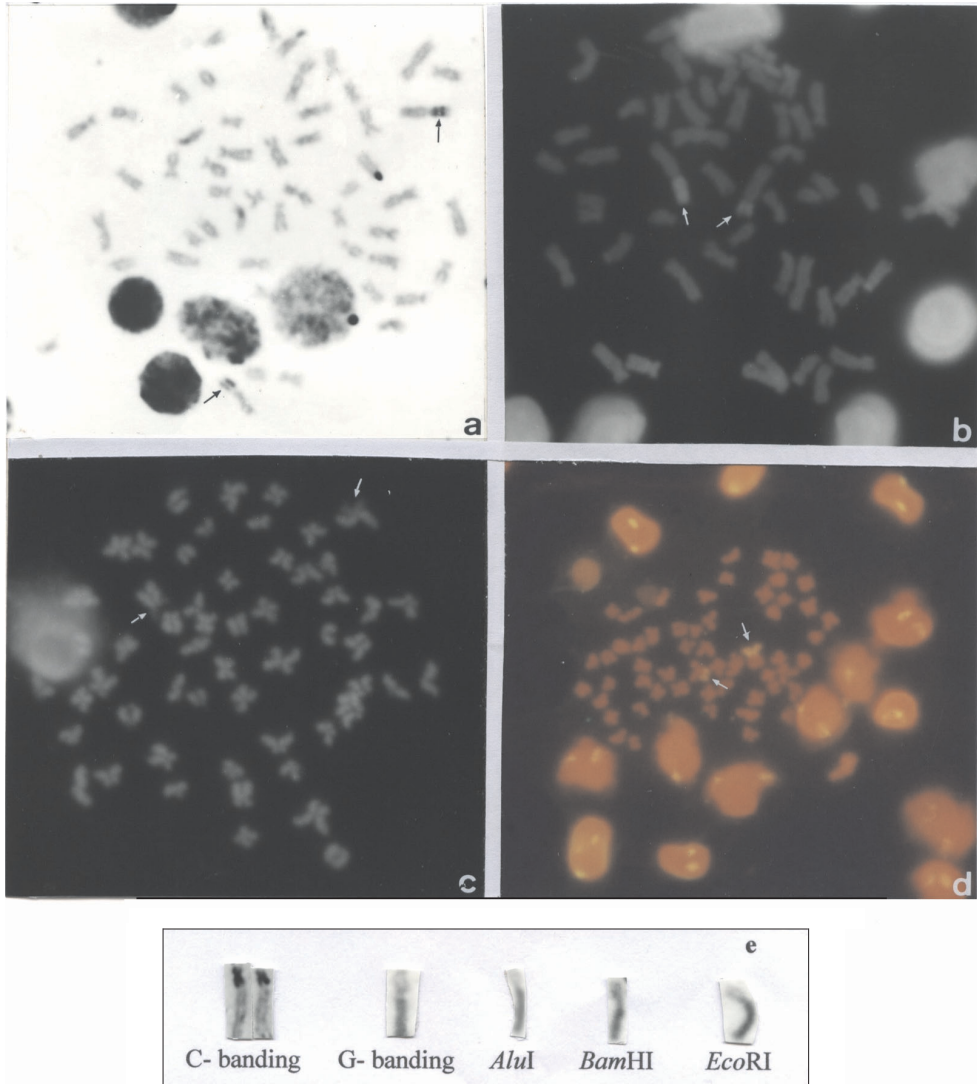


Fig. 2. Somatic metaphase plates of *Steindachneridion melanodermatum* after (a) AgNO<sub>3</sub>, (b) CMA<sub>3</sub> and (c) DAPI staining; (d) FISH with 18S rDNA probe. Arrows indicate the NOR bearing chromosomes; (e) the secondary constrictions after C- and G-banding, AluI, BamHI and EcoRI restriction enzymes treatments.



trout and Atlantic salmon by PENDÁS *et al.* (1993a, b) and in *Sorubim lima* by FENOCCHIO and BERTOLLO (1992).

Chromosomal staining with CMA<sub>3</sub> (GC-specific fluorochrome) evidenced bright signals coincident with the NORs (Figs 1b, 2b). The presence of these regions flanked by GC-rich heterochromatin in *Steindachneridion* species was observed by other authors and seems to be common in Neotropical fishes (GALETTI JR *et al.* 1995; CASTRO *et al.* 1996; SWARÇA *et al.* 1999, 2005).

No AT-rich chromosomal regions were found in either *Steindachneridion* species. This fluorochrome produced pale or negative fluorescence on the secondary constrictions and uniform background staining on the remaining chromosomes (Figs. 1c, 2c). AT-rich regions are not a common feature in fish chromosomes, with few exceptions, as in some salmonids (MAYR *et al.* 1988), *Danio rerio* (GORNUNG *et al.* 1997), and *Hypostomus* sp. (ARTONI & BERTOLLO 1999), in which some chromosomal fluorescent regions were observed after DAPI staining. In some cases these repetitive A-T regions are masked and could only be evidenced clearly after a C-band pre-treatment, as observed by SWARÇA *et al.* 2003 in *Steindachneridion scripta* and *Rhamdia quelen*.

Hybridization with the rDNA 18S probe confirmed the number and location of Ag-NORs. Differences in the size of the hybridization signals among homologues were observed and considered additional evidence for NOR heteromorphism (Figs 1d, 2d). Several authors have suggested that unequal crossing over, transposition or other rearrangements involving homologous segments can be the mechanism related to the structural modification of NORs (GALETTI *et al.* 1995; CASTRO *et al.* 1998).

The results obtained in the present paper could be explained by the presence of NORs flanked by GC-rich heterochromatin, associated and interspersed through rDNA genes that are arranged in tandem.

The NOR regions appear faintly stained when treated with trypsin (G-banding) and restriction enzymes *AluI*, *BamHI* and *EcoRI* (RE-banding) (Figs 1e, 2e). The pale stain after these treatments could be due to a large number of trypsin, *AluI*, *BamHI* and *EcoRI* target sequences in these regions of the *Steindachneridion* chromosomes. In several fish species the NORs are not target sequences for *AluI*, appearing as dark zones after Giemsa staining, as in other species, i.e. *Pinirampus pinirampu* and *Pimelodus maculatus* (SWARÇA *et al.* 1999, 2001a). According to DERENZINI *et al.* (1987), it is probable that the decondensed chromatin of the NORs (i.e. secondary constriction), without a strong nucleosomal struc-

ture, could facilitate the action of the restriction endonucleases, as in *Steindachneridion*.

The present results clearly show the variability in NOR size and suggest that the NOR bearing chromosomes can be considered homologues, maintaining conserved characteristics in both species. Furthermore, the NORs of *Steindachneridion* species are constituted by rDNA copies interspersed with GC-rich heterochromatin, showing target sequences for trypsin, *AluI*, *BamHI* and *EcoRI*.

## Acknowledgements

The authors are grateful to Dr. L. A. C. BERTOLLO for valuable comments and suggestions, to CNPq for the fellows to ACS (Proc.150612/2004-4).

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