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R- AND G-BAND PATTERNS IN *Astyanax scabripinnis paranae* (PISCES, CHARACIFORMES, CHARACIDAE)

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ABSTRACT

The absence of longitudinal bands in fish chromosomes has been associated with technical problems in chromosome preparations or the absence of a structural compartmentalization in the fish genome. In the present study, a R-banding pattern was obtained using a replication banding technique by *in vivo* treatment with 5-bromodeoxyuridine (5-BrdU). G-banding patterns were obtained after trypsin treatment and also after chromosome cleavage by *in situ* treatment with the restriction endonuclease *Bam*HI. A similar G-banding pattern was also obtained after cleavage with the endonuclease *Hin*fI. Presence of a resolute R- and G-banding patterns shows that *Astyanax scabripinnis paranae* chromosomes could present an isochore-like structure similar to that found in other vertebrates.

INTRODUCTION

Although some cytogenetic techniques have been applied successfully to fish, such as Ag-NOR and C-banding, G- or R-bands are usually very laborious to obtain, with frequently poor results. The absence of longitudinal bands in fish chromosomes has frequently been associated with the absence of structural compartmentalization in the fish genome (Medrano *et al.*, 1988; Schmid and Guttenbach, 1988) or with technical problems in chromosome preparations (Gold *et al.*, 1990). The aim of this study was to investigate the possible existence of a resolute chromosome banding pattern in the normal diploid genome and B-chromosomes of *Astyanax scabripinnis paranae*.

MATERIAL AND METHODS

Cytogenetic studies were conducted on eight individuals (four females and four males) of an *A. scabripinnis paranae* population collected from Cascatinha Stream, Botucatu, São Paulo, Brazil, which presents 2n = 50 chromosomes and one macro B-chromosome (Maistro *et al.*, 1994).

Chromosome spreads were obtained as described by Foresti *et al.* (1993). In order to obtain a replication banding pattern (R-band), three animals were injected with 1 ml/100 g body weight of a 0.05% solution of 5-bromodeoxyuridine (5-BrdU) 6.5 h prior to chromosome preparation from a cell suspension (Almeida-Toledo *et al.*, 1988). Slides were analyzed after FPG staining (ISCN, 1978). For G-banding, slides previously stored for about three days at room temperature were incubated in 2 x SSC for 2 h at 60°C, and treated/stained in a trypsin/Giemsa solution for 10 min at room temperature (Gold *et al.*, 1990, modified by Bertollo *et al.*, 1997 for fish chromosomes). For treatment with restriction endonucleases (REs), the enzymes were suspended in appropriate buffer and applied at the following concentrations and times: a) *Bam*HI 2.0 U/µl, for 4 h; b) *Hin*fI 1.0 U/µl, for 4 h. Slides were incubated in a moist chamber at 37°C, washed in distilled water and stained with 5% Giemsa for 7 min. Representative metaphases were karyotyped for all individuals.

RESULTS AND DISCUSSION

Replication banding patterns have been obtained for several fish species (Delany and Bloom, 1984; Almeida-Toledo *et al.*, 1988; Giles *et al.*, 1988; Gold *et al.*, 1990; Hellmer *et al.*, 1991; Sánchez *et al.*, 1993; Bertollo *et al.*, 1997) after brief treatment with BrdU. A replication banding pattern was obtained in chromosome preparations of *A. scabripinnis paranae* using this technique (Figures 1a and 2a).

Consistent G-banding patterns have been described for the chromosomes of *Anguilla anguilla* (Wiberg, 1983), *Opsopoeodus emiliae* (Gold *et al.* 1990), *Oncorhynchus mykiss* and *O. kisutch* (Abuín *et al.*, 1996) and *Hoplias malabaricus* (Bertollo *et al.*, 1997). In the present study, G-banding patterns were obtained for chromosomes of *A. scabripinnis paranae* after trypsin treatment and *Bam*HI treatment (Figures 1b-c, 2b-c). The cleavage with the RE *Hin*fI also resulted in a longitudinal differentiation pattern on the chromosomes which differed from normal G-banding patterns only by the fact that some heterochromatic regions were also digested (Figures 1d, 2d).

Analysis of R- and G-banding patterns revealed a pattern of longitudinal differentiation also in the macro B-chromosomes of *A. scabripinnis paranae* from the Cascatinha Stream. The macro B-chromosome identified in this population is similar in size to the first pair in the karyotype of the individuals (Maistro *et al.*, 1994). Similar ob-

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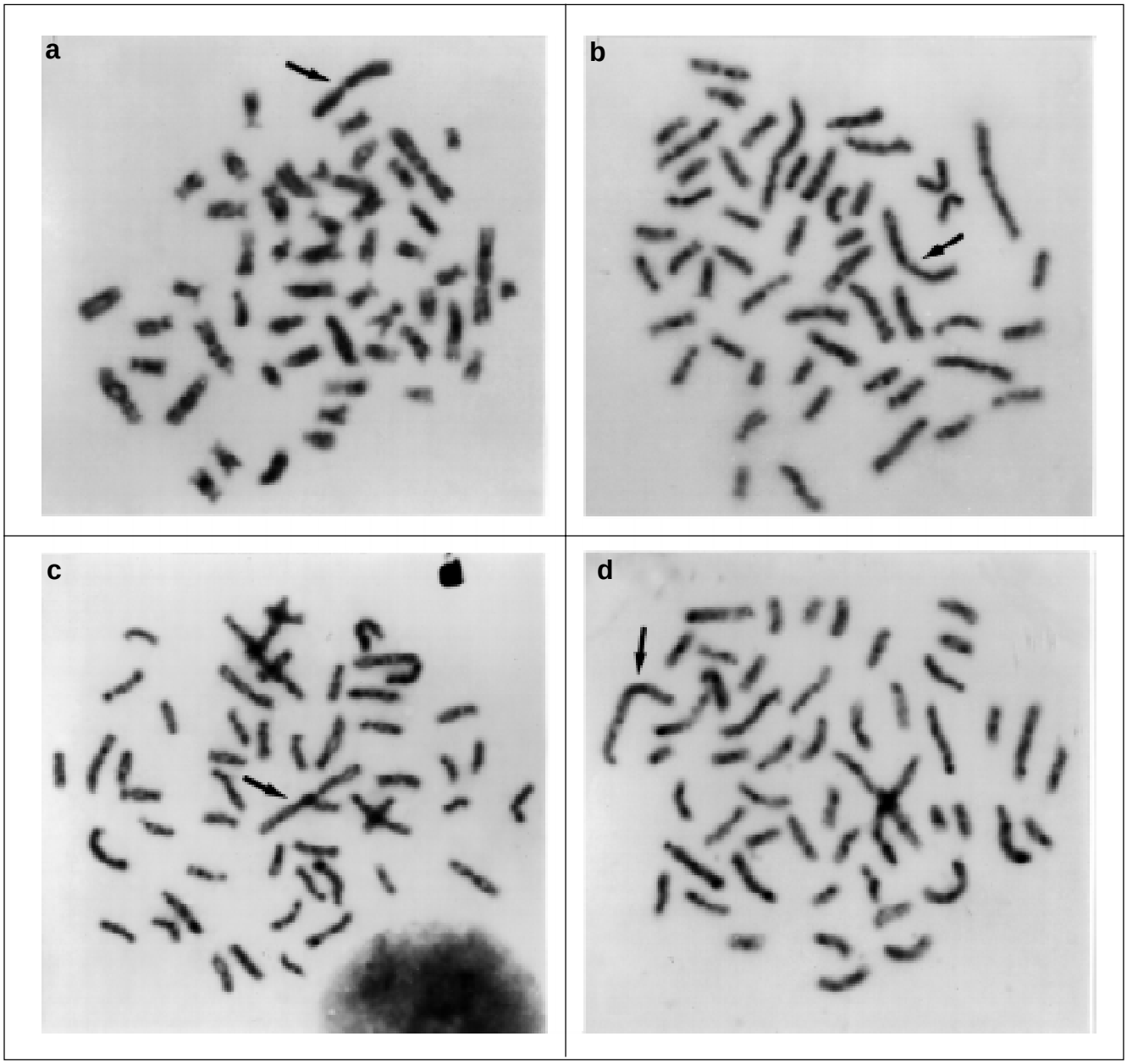

Figure 1 - Somatic metaphase chromosomes of *Astyanax scabripinnis paranae* submitted to different banding procedures: (a) replication banding pattern (R-band) after treatment with 5-BrdU; (b) G-banding pattern obtained after trypsin treatment; (c) G-banding pattern obtained after chromosome cleavage by *in situ* treatment with the restriction endonuclease *Bam*HI, and (d) similar G-banding pattern obtained after cleavage with the restriction endonuclease *Hin*fI. Arrows mean probable B-chromosomes.

servations were made in other populations of *A. scabripinnis* (Salvador and Moreira-Filho, 1992; Maistro *et al.,* 1992; Fauaz *et al.,* 1994, among others). However, the R- and G-banding patterns observed in B-chromosomes in this study were different from those obtained for the first chromosome pair of the A complement. Thus, the B-chromosome of *A. s. paranae* may have originated by chromosomal non-disjunction of the first metacentric pair in the meiotic process (see Beukeboon, 1994), first appearing as a trisomic element, and then following its own evolutionary pathway. Alternatively, the B-chromosome may have originated by amplification of other chromosome segments.

According to Lópes-Fernandez *et al.* (1991), most REs, recognizing 4-5-base pairs (bp), induce C-bands (sometimes associated with G-bands) in chromosomes subsequently stained with Giemsa and/or DNA-specific dyes. On the contrary, G-like bands are produced in chromosomes digested with certain 6-bp cutters and subsequently stained with Giemsa but not DNA-specific dyes. G-band production has been interpreted as the consequence of differential accessibility of REs to different chromosome regions (Gosálvez *et al.*, 1997). The present results

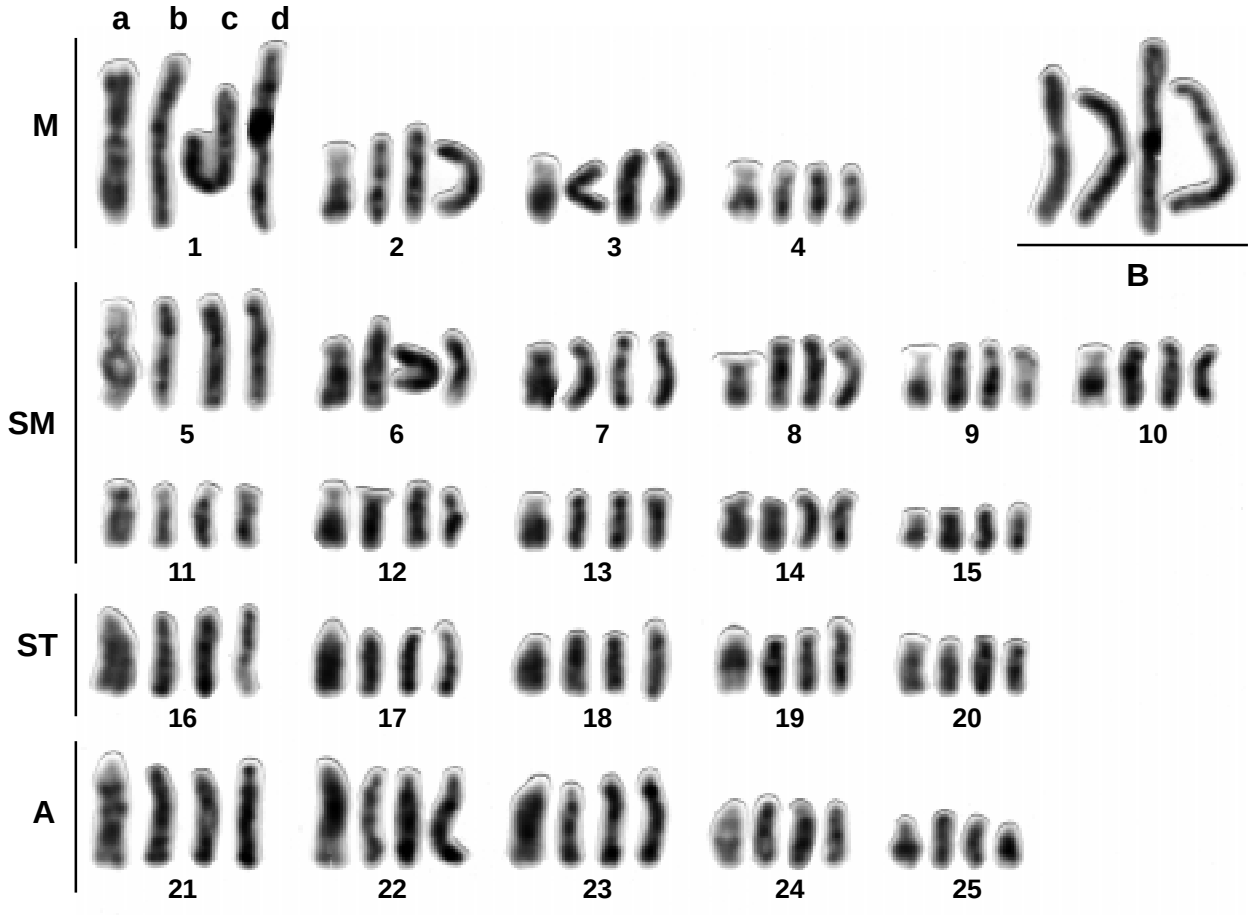

Figure 2 - Haploid chromosome sets of *Astyanax scabripinnis paranae* showing different patterns of longitudinal differentiation after being submitted to banding procedures: (a) R-banding; (b) G-banding with trypsin; (c) G-banding after treatment with RE *Bam*HI, and (d) almost similar G-banding patterns obtained with RE *Hin*fI. B, B-chromosomes. M, Metacentric; SM, submetacentric; ST, subtelocentric; A, acrocentric.

confirm those postulated by the above authors, since RE *Bam*HI recognizes a sequence of 6-bp (5'-G↓GATCC-3') and produces a G-like banding pattern, and the RE *Hin*fI recognizes a sequence of 5-bp (5'-G↓ANTC-3') and produces a G-like banding pattern.

It has been suggested that the chromosomes of many species of cold-blooded vertebrates show only poor or no G-banding, although they may show a replication banding pattern (Cuny *et al.*, 1981; Medrano *et al.*, 1988; Schmid and Guttenbach, 1988; Bernardi, 1989; Bernardi, 1995). In the present study specific and reproducible R-banding and G-banding patterns were obtained in all metaphases of *A. scabripinnis paranae* with decondensed chromosomes; however, they could not be identified in metaphases with condensed chromosomes. These data associated with recent reports of G-banding in cold-blooded vertebrates (Abuín *et al.*, 1996; Bertollo *et al.*, 1997) are in accordance with the hypothesis that the failure to detect chromosome banding in fish chromosomes is due more to technical problems in the use of banding protocols than to the total absence of a structural compartmentalization in the fish genome.

The existence of a correspondence between the results obtained with R-banding and G-banding techniques shows that the longitudinal differentiation bands of the chromosomes correspond to real structural units in the genome of *A. scabripinnis paranae*. Similar results were obtained by Bertollo *et al.* (1997) studying *Hoplias malabaricus* chromosomes, where the G- and replication banding patterns, together with C-banding, permitted not only a good characterization of the X_1 and Y chromosomes but also identification of the rearrangements that occurred in the establishment of the X_1X_2Y sex chromosome system. The presence of replication banding patterns in fish chromosomes indicates that chromosome replication is temporally clustered into early- and late-replicating units, leading to a distinct replication banding pattern on each chromosome (Sánchez *et al.*, 1993), which is comparable to the situation found in warm-blooded vertebrates. The presence of resolutive R- and G-banding patterns shows that the chromosomes of *A. scabripinnis paranae* could present an isochore structure similar to that found in other vertebrates. The use of such methodology in fish cytogenetics could be an important tool for the study of karyo-

type evolution and mechanisms of chromosome diversification in fish, although detection of multiple structural bands in fish chromosomes continues to be a field requiring further studies.

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RESUMO

A ausência de bandas longitudinais em cromossomos de peixes tem sido associada a problemas técnicos nas preparações cromossômicas ou à ausência de uma compartimentalização estrutural no genoma dos peixes. No presente estudo, um padrão de bandas R foi obtido usando a técnica de bandamento de replicação pelo tratamento *in vivo* com 5-BrdU. Padrões de bandamento G foram obtidos após tratamento dos cromossomos com tripsina e também após clivagem *in situ* com a endonuclease de restrição *Bam*HI. Um padrão similar de bandamento G também foi obtido após clivagem dos cromossomos com a endonuclease *Hin*fI. A presença de um resolutível padrão de bandamento R e G em *Astyanax scabripinnis paranae* mostra que seus cromossomos parecem apresentar estrutura "isochore" similar àquela encontrada em outros vertebrados.

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