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Karyotype similarity between two sympatric *Schizodon* fish species (Anostomidae, Characiformes) from the Paraguay River basin

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ABSTRACT

Fish of the neotropical family Anostomidae generally show low karyotype variability. Nevertheless, karyotype variants have been identified within some genera, providing information about their evolutionary history. Species of the genus *Schizodon* show a high degree of morphological and ecological similarity compared to other anostomids. In the present study, karyotype characteristics of *Schizodon borelli* (40 individuals) and *S. isognathum* (one individual), two sympatric species found in the Paraguay River basin, were studied. C-banding, GC-specific fluorochrome Mitramycin (MM) and Ag staining as well as *in situ* hybridization (FISH) with rDNA probes were used. The karyotypes of these species were found to be very similar. Only two NORs were detected in a common chromosome pair of both species under Ag, MM and FISH treatments. Similar heterochromatin distribution patterns were also observed. A parallelism between the small karyotype variation and low morphological and ecological divergence observed for this genus is discussed. Their karyotype homogeneity might be related to populational features or, alternatively, might indicate that the maintenance of a symmetric and conserved karyotype structure represents optimal genomic organization among these fish.

INTRODUCTION

The neotropical freshwater fish family Anostomidae has been considered a monophyletic unit among Characiformes (Vari, 1983), although there is some disagreement regarding interrelationships between genera (Géry, 1977; Winterbottom, 1980). Cytogenetic analyses have set the basis for the phylogenetic studies within and between genera of this family (Galetti Jr. *et al.*, 1984, 1991b, 1995a).

In contrast to other fish groups, the Anostomidae generally have showed a very conserved karyotypic structure. However, variations in the location of NORs (Galetti Jr. *et al*., 1984, 1991b), heterochromatin patterns (Galetti Jr. *et al*., 1991a,b) and sex chromosomes (Galetti Jr. *et al*., 1981b, 1995a; Galetti Jr. and Foresti, 1986, 1987; Koehler *et al.*, 1997) have been observed in *Leporinus* species, the most extensively studied genus of Anostomidae. Chromosome studies are important to address taxonomy and evolutionary biology in these fishes. *Leporinus obtusidens* and *L. elongatus*, for example, are sympatric in the Alto Paraná River system, with negligible morphological differences between species. Although there is high karyotype macrostructure similarity, NOR studies have revealed large differences between them. This fact proves the usefulness of NORs as a decisive cytotaxonomic character (Galetti Jr. *et al*., 1984, 1991b).

Among the anostomid genera, *Schizodon* represents a taxonomically well-defined clade, composed of 14 species residing in all large neotropical hydrographic basins. Cytogenetic data of this genus, however, are restricted to only two species, *Schizodon nasutus*, from the Mogi-Guaçu River/Alto Paraná basin (Galetti Jr. *et al*., 1981a, 1984, 1991a), and *Schizodon fasciatus*, from the Solimões and Madeira Rivers in the Amazon basin (Galetti Jr. *et al.*, 1991a).

In the present study, the chromosomes of *S. borelli* and *S. isognathum*, sympatric species in the Paraguay River basin, were analyzed using Giemsa, C- banding, silver nitrate and fluorochrome staining and rDNA *in situ* hybridization.

MATERIAL AND METHODS

Specimens

Forty *Schizodon borelli* specimens (15 females, 15 males and 10 of unidentified sex) taken from the following four locations along the Paraguay River basin were analyzed: Cuiabá River (Santo Antônio do Laverger/MT), Bento Gomes River (Poconé/MT), Miranda and Vermelho Rivers (Passo do Lontra, Corumbá/MS). Only one young *S. isognathum* specimen of unidentified sex was collected in syntopy with *S. borelli* in the Vermelho River.

Chromosome preparation and banding

Metaphase chromosomes obtained from cephalic kidney cell suspensions were submitted to silver (Ag) staining (Howell and Black, 1980), C-banding using barium hydroxide (Sumner, 1972) and fluorescent staining with the GC-specific fluorochrome Mitramycin (MM), counterstained by Distamycin A (Schmid, 1980).

Non-isotopic *in situ* hybridization (FISH)

Ribosomal DNA probes (HM123 and HM456) which contain 18S and 28S genes of *Xenopus laevis* were labeled with biotin by nick translation according to manufacturer's instructions (Nick Translation kit, Boehringer-Mannheim, Germany). The metaphase chromosome slides were incubated with RNase (40 µg/ml) for 1.5 h in a moist chamber at 37°C. After denaturation of chromosomal DNA in 70% formamide/2 x SSC for 5 min at 70°C, 40 µl of hybridization mixture (1 µg of denatured probe, 50% formamide, 10 mg/ml dextran sulfate, 2 x SSC) was dropped on the slides. Hybridization was performed overnight at 37°C in a moist chamber. The probes were detected by avidin-FITC conjugate (Sigma). The signal was then enhanced by biotinylated anti-avidin and avidin-FITC. The chromosomes were counterstained with 70 µl propidium iodide (100 µg/ml), and the slides were mounted with 25 µl Vectashield antifade (Vector).

RESULTS

The karyotypes of *Schizodon borelli* and *Schizodon isognathum* had 54 meta-submetacentric chromosomes (Figure 1). Ag-NOR$^+$ sites were found in the terminal region of the long arm of one medium-sized metacentric chromosome pair (Figure 2), corresponding to the 20th pair of the complement of both species. C$^+$ band heterochromatin blocks were strongly stained in the centromeres and less intensely stained at the telomeres in the majority of chromosomes in the complement of both species (Figure 3). The Ag-NOR$^+$ regions were found to be heterochromatic (C$^+$ band). Fluorescent MM$^+$ bands were detected in the Ag-NOR sites of chromosome 20 in both species (Figure 4) and interstitially in the short arm of chromosome pair 23. FISH revealed signals corresponding to rDNA units located exclusively in the Ag-NOR$^+$ regions (Figure 5). In *S. borelli*, the homologous NORs, demonstrated by Ag and MM staining and FISH, were found to be heteromorphic. No variation in karyotype was observed for the different *S. borelli* populations analyzed.

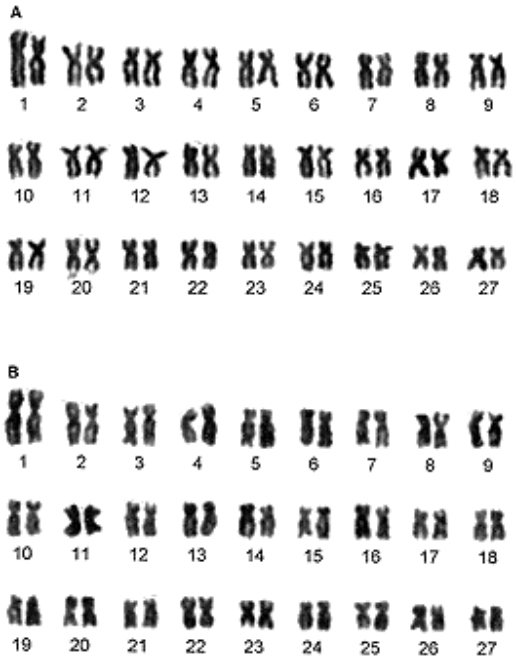

Figure 1 - Giemsa-stained karyotypes of *Schizodon borelli* (A) and *S. isognathum* (B) showing 54 meta-submetacentric chromosomes.

DISCUSSION

Arrangement of nucleolar ribosomal DNA in *Schizodon* chromosomes

Chromosomal NOR phenotypes have been useful to systematic relationships among fish species (Gold, 1984; Venere and Galetti Jr., 1989; Amemiya and Gold, 1990; Gold and Zoch, 1990, among others). In neotropical fishes, NORs appear as a heterogeneous trait, occurring in a single chromosome pair in some groups or in several pairs in others (Bertollo, 1995). In *Leporinus,* the most extensively studied Anostomidae group, NOR phenotypes have demonstrated to be important as systematic markers for cryptic species (Galetti Jr. *et al*., 1984, 1991b). In *Schizodon,* NORs are located in a homologous chromosome pair of different

species and might suggest a close relationship among them.

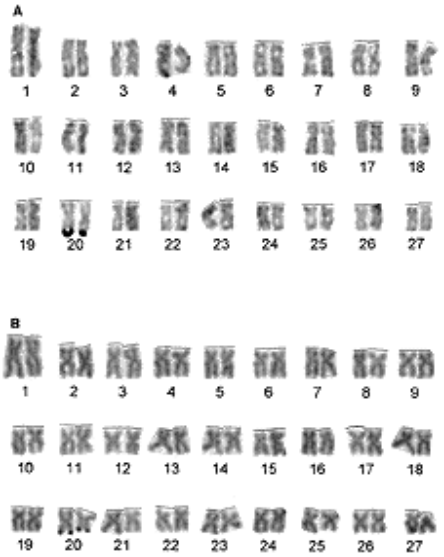

Figure 2 - Karyotypes of *Schizodon borelli* (A) and *S. isognathum* (B) arranged from Ag-stained chromosomes.

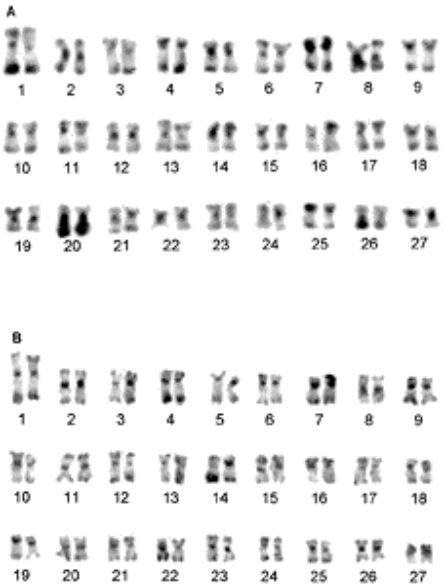

Figure 3 - C-banded karyotypes of *Schizodon borelli* (A) and *S. isognathum* (B).

As often reported for fishes in general (Amemiya and Gold, 1986; Phillips *et al*., 1989; Sola *et al.,* 1997, among others), *S. borelli* and *S. isognathum* had conspicuous MM^+ bands, corresponding to the Ag-NOR^+ regions. MM and other GC-specific fluorochromes have been frequently used in fish due to the role they play in NOR identification (Galetti Jr. and Rasch, 1993a,b; Mestriner *et al.*, 1995; Margarido and Galetti Jr., 1996; Sola *et al.*, 1997, among others) supplying interesting results that have helped to identify polymorphisms and to understand the rDNA structural organization in these organisms (Galetti Jr. *et al.*, 1995b; Castro *et al.,* 1996). However, there are still doubts if fluorochromes stain ribosome cistrons (Schmid *et al.,* 1987) or interspersed heterochromatin (nontranscriber spacer DNA) in rDNA (Pendás *et al.,* 1993). Additional fluorescent MM^+ bands were detected in at least one other chromosome pair (No. 23) of both species. GC-rich bands not corresponding to Ag-NOR^+ segments have already been identified in other fishes (Phillips *et al*., 1988; Mestriner, 1993; Artoni, 1996) and may be associated with inactive ribosomal DNA segments (Amemiya and Gold, 1986). It appears, however, that this is not the case in *Schizodon.* FISH, using rDNA probes (18 and 28S), gave positive signals only in the terminal region of the long arm of the chromosome pair comparable to the Ag-NOR bearing pair, and suggests the lack of rDNA in the additional MM^+ bands observed in chromosome 23. NOR heteromorphism between homologous chromosomes, more clearly visible in *S. borelli* under Ag and MM staining and FISH, indicates that this situation is related to variation in the number of copies of 18S + 28S rDNA genes (Goodpasture and Bloom, 1975) instead of genetic regulation of these cistrons (Miller *et al*., 1976).

Chromosome homogeneity in the genus *Schizodon*

Previous studies have provided information (based on conventional Giemsa and Ag-NOR staining and C-banding) on karyotypes of *S. nasutus* (Alto Paraná) and *S. fasciatus* (Amazônia) (Galetti Jr. *et al*., 1981a, 1984, 1991a). Although these species belong to different hydrographic basins, differences in their karyotype patterns were not found. In fact, cytogenetic data available for *Schizodon* (including the present study) indicate that karyotype homogeneity, already reported for the family Anostomidae in general (Galetti Jr. *et al*., 1981a, among others), is well evidenced in this genus.

Anostomidae, as well as other non-Anostomidae groups (Chilodontidae, Prochilodontidae, Curimatidae and Parodontidae), have a very similar karyotype pattern, consisting of 54 biarmed chromosomes. However, even fish groups with high chromosome homogeneity may experience different chromosome evolutionary trends.

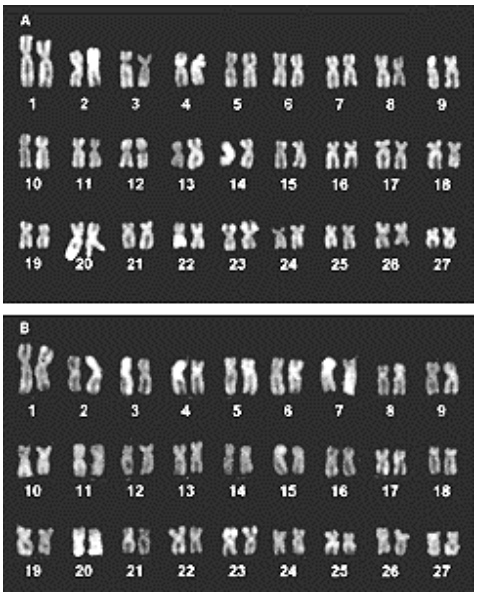

Figure 4 - MM-stained karyotypes of *Schizodon borelli* (A) and *S. isognathum* (B).

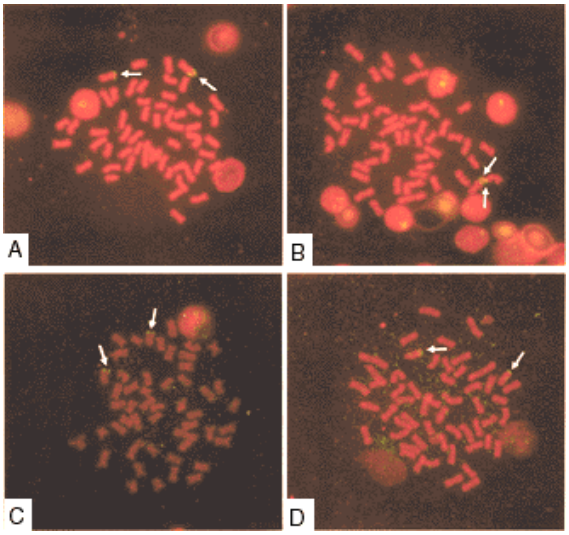

Figure 5 - Metaphases of *Schizodon borelli* (A and B) and *S. isognathum* (C and D) after *in situ* hybridization with 18S + 28S rDNA genes, showing NOR sites (arrows).

Among anostomids, the large genus *Leporinus* shows wide morphological and ecological diversity of forms and considerable chromosome variability associated with the sex chromosomes (Galetti Jr. *et al*., 1981b; Galetti Jr. and Foresti, 1986, 1987), NOR sites (Galetti Jr. *et al*., 1984, 1991b, 1995b) and patterns of constitutive heterochromatin (Galetti Jr. *et al*., 1991a,b). On the other hand, *Schizodon* shows higher ecological and morphological similarity among the species. *S. borelli* and *S. isognathum*, for instance, occur sympatrically and were collected in syntopy in the Vermelho River. They share similar ecological and behavioral characteristics. Therefore, a parallelism between ecological and morphological diversity and the low rate of chromosomal evolution observed in *Schizodon* may exist.

Models of chromosome evolution invoke the primacy of chromosome change in speciation (King, 1993; Sites and Reed, 1994; Qumsiyeh, 1994). White´s principle of karyotype orthoselection might explain the similarity of chromosome in shape and size observed in the karyotype of *Schizodon* (White, 1969). In this case, particular types of rearrangements occurred several times during karyotype differentiation, leading to a very uniform karyotype. Moreover, chromosomal mechanisms of speciation seem to be more prevalent in organisms of restricted mobility (White, 1978), which is not the case for *Schizodon*. Species of this genus are fast swimmers and can spread throughout an entire hydrographic system, and their karyotype homogeneity might be due to these populational features. Karyotypic stability might be reached after a canalization to an optimal karyotypic configuration and further rearrangements are mal-adaptive (Bickham and Baker, 1979). Alternatively, symmetric and homogeneous karyotypes may find a homeostatic balance between selective forces of genome diversity and pressure for cellular constancy during the mitotic process, preventing changes in karyotypes macrostructure, though the minor and cryptic chromosome rearrangements occur (Venere and Galetti Jr., 1989). Thus, once a symmetric and homogeneous karyotype pattern is reached, as observed in *Schizodon,* major changes in the chromosome complement appear to be selected against.

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RESUMO

Peixes neotropicais da família Anostomidae apresentam, de uma forma geral, pouca variação na sua estrutura cariotípica. Mesmo assim, em alguns grupos, foi possível identificar variantes cariotípicas que forneceram informações tanto sobre sua sistemática quanto sobre sua história

evolutiva. As espécies do gênero *Schizodon* apresentam um alto grau de similaridades ecológicas e morfológicas comparado com outros anostomídeos. No presente trabalho, foram estudadas características cariotípicas de *S. borelli* (40 indivíduos analisados) e *S. isognathum* (somente um indivíduo analisado), duas espécies simpátricas do rio Paraguai, utilizando bandamento C, coloração com nitrato de prata, aplicação do fluorocromo GC-específico mitramicina e hibridação *in situ* (FISH) com sonda de DNAr. Os cariótipos destas espécies mostraram-se muito similares. Somente duas NORs foram detectadas sob os tratamentos com Ag, MM e FISH em um par cromossômico comum a ambas as espécies e um padrão similar de distribuição da heterocromatina também foi observado. Estes dados sugerem a existência de um paralelismo entre a pouca variação cromossômica e a baixa divergência ecológica e morfológica observada neste gênero. A homogeneidade cariotípica observada pode estar relacionada a fatores populacionais ou pode indicar que a manutenção de uma estrutura cariotípica conservada e simétrica representa a melhor forma para a organização do genoma destes peixes.

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