Narrow chromosome diversity in fish of the genus *Schizodon* (Characiformes, Anostomidae)

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Key words: fish karyotypes, chromosome banding, *Schizodon* species

Abstract

The anostomid fish have a very similar karyotype, consisting of 54 biarmed chromosomes. Variations in the location of NORs, heterochromatin, and sex chromosomes have been observed in *Leporinus*, the most extensively studied member of the Anostomidae. *Schizodon*, another anostomid genus, composed of only a few species shows a stable karyotype, even in chromosome microstructure. In the present work, chromosomes of *S. altoparanae, S. intermedius, S. nasutus, S. knerii* and *S. vittatus*, from different South American hydrographic basins, were cytogenetically studied and a homogeneous karyotype structure was detected.

Introduction

The family Anostomidae have generally shown a very conservative gross karyotype structure, with 2n = 54 biarmed chromosomes (Galetti *et al.*, 1981a). However, variations in the location of the nucleolar organizer regions (NORs) (Galetti *et al.*, 1984; Galetti *et al.*, 1991b), heterochromatin (Galetti *et al.*, 1991a; Galetti *et al.*, 1991b) and sex chromosomes (Galetti *et al.*, 1981b; Galetti *et al.*, 1995; Galetti and Foresti, 1986, 1987; Koehler *et al.*, 1997), have been observed in *Leporinus*, the most extensively studied member of the Anostomidae. Other genera such as *Schizodon*, which comprise about fourteen species spread through all the main South American hydrographic basins, are little known. Thus far only four species, *S. fasciatus* in the Amazon basin, *S. nasutus* in the Paraná basin, *S. borelli* and *S. isognathum* in the Paraguay basin, have been cytogenetically examined. A stable karyotype, even in chromosome microstructure, as detected by varied chromosome banding (Galetti *et al.*, 1981a; Galetti *et al.*, 1991a; Galetti *et al.*, 1991b; Martins and Galetti, 1998), have been recorded and described for these species.

In the present work, chromosomes of five *Schizodon* species were studied by Giemsa, silver nitrate (Ag-) and mithramycin (MM) staining, and C-banding, and a very homogeneous karyotype structure was detected.

Materials and methods

Samples of five species of the genus *Schizodon* collected in different hydrographic systems were available for this investigation. *Schizodon*

altoparanae, from the Paranapanema river were collected near to the township of Salto Grande (SP), *S. intermedius* was obtained from the Tietê river system, caught in the main water dam of the municipality of Águas de São Pedro (SP), and *S. nasutus* came from two localities, the Mogi-Guaçu river (township of Pirassununga, SP) and the Paraná river (Missiones, Argentina). Thus these species originated from the large Paraná-Paraguay basin. *S. knerii*, from the São Francisco river basin, was collected in the municipality of Três Marias (MG), and finally *S. vittatus*, came from the Amazon basin, and was caught in the Araguaia river near the township of Barra do Garças (MT).

Mitotic chromosomes were obtained from anterior kidney cells following a conventional air drying technique described elsewhere (Bertollo *et al.*, 1978). C-banding (Sumner, 1972), silver nitrate (Ag-NOR) (Howell and Black, 1980) and mythramycin (MM) staining (Schmid, 1980), were carried out.

Results

All five *Schizodon* species investigated had a common karyotype of 2n = 54, with only meta- and submetacentric chromosomes (Figure 1). The Giemsa karyotype, as well as the Ag-staining and C-banding data of *S. nasutus* previously reported by Galetti *et al.* (1991a) are not shown here. Only two Ag-NOR bearing chromosomes, a metacentric pair size-related to chromosome 20 in the karyotype, were detected in all *Schizodon* species (Figure 2).

C-banded chromosomes revealed darker heterochromatin blocks in the centromeres and often lighter heterochromatin ones in the telomeres of most chromosomes (Figure 3). A large heterochromatin segment was apparently coincident with Ag-NOR sites in all these species. A dot-like proximal heterochromatin was detected on the long arm of a small metacentric (chromosome 21) in the *S. vittatus* chromosome complement. Brighter MM fluorescence bands were visible at the end of the long arm of a metacentric pair, comparable with the Ag-NOR sites (Figures 4 and 5). Lesser bright MM bands were detected in the centromeric or pericentromeric region of chromosomes 2 and 4, and were interstitial in chromosome 23 of all the species, except *S. vittatus* in which no MM^+ bands were seen in chromosome 2. Unfortunately C-banding and MM staining of *S. intermedius* could not be carried out.

Figure 1 Giemsa-stained karyotypes of (a) *Schizodon altoparanae*, (b) *S. intermedius*, (c) *S. knerii* and (d) *S. vittatus*.

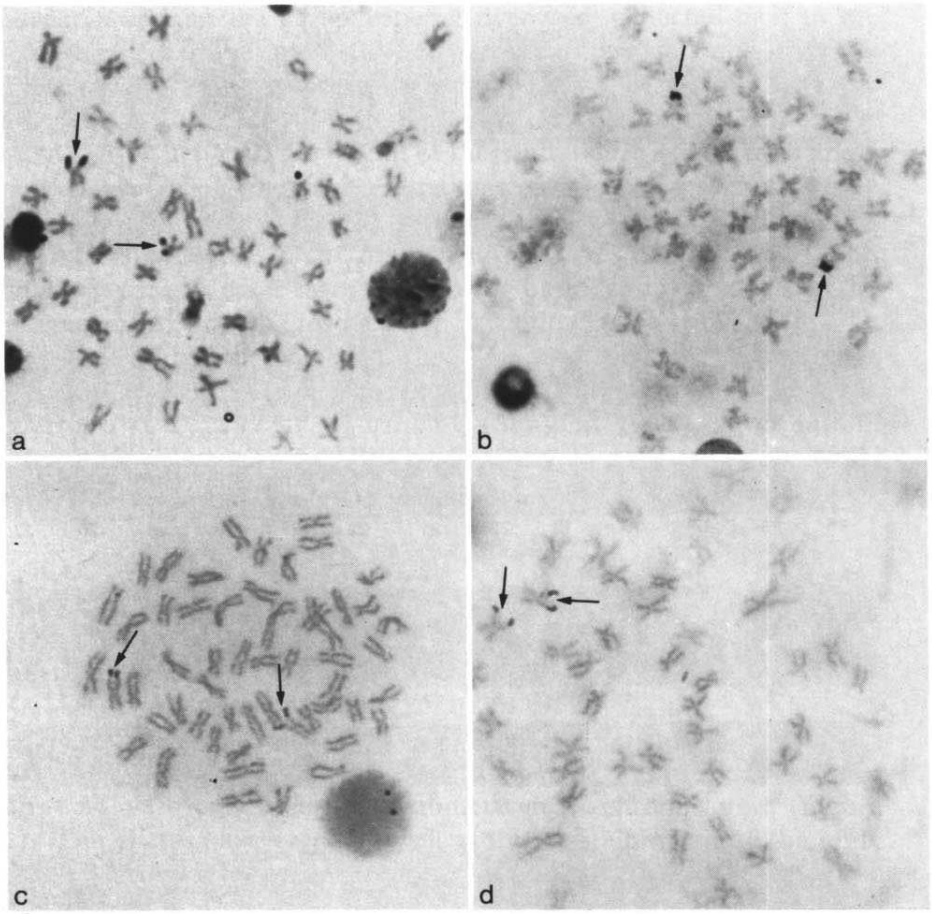

Figure 2 Ag-stained metaphases of (a) *Schizodon altoparanae*, (b) *S. intermedius*, (c) *S. knerii* and (d) *S. vittatus*. Arrows indicate Ag-NOR bearing chromosomes.

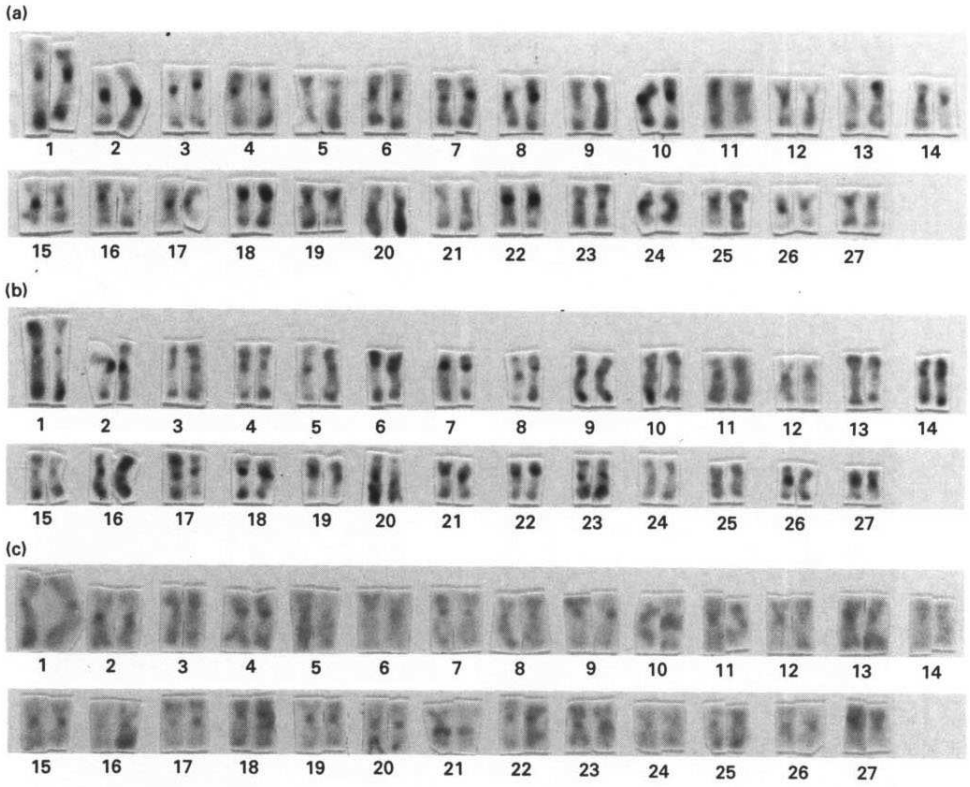

Figure 3 C-banded karyotypes of (a) *Schizodon altoparanae*, (b) *S. knerii*, and (c) *S. vittatus*.

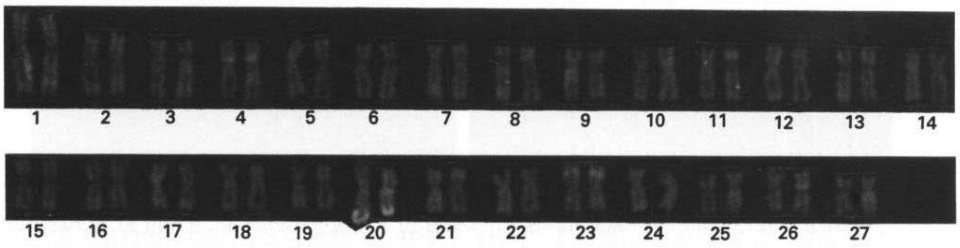

Figure 4 MM stained karyotype of *Schizodon vittatus*.

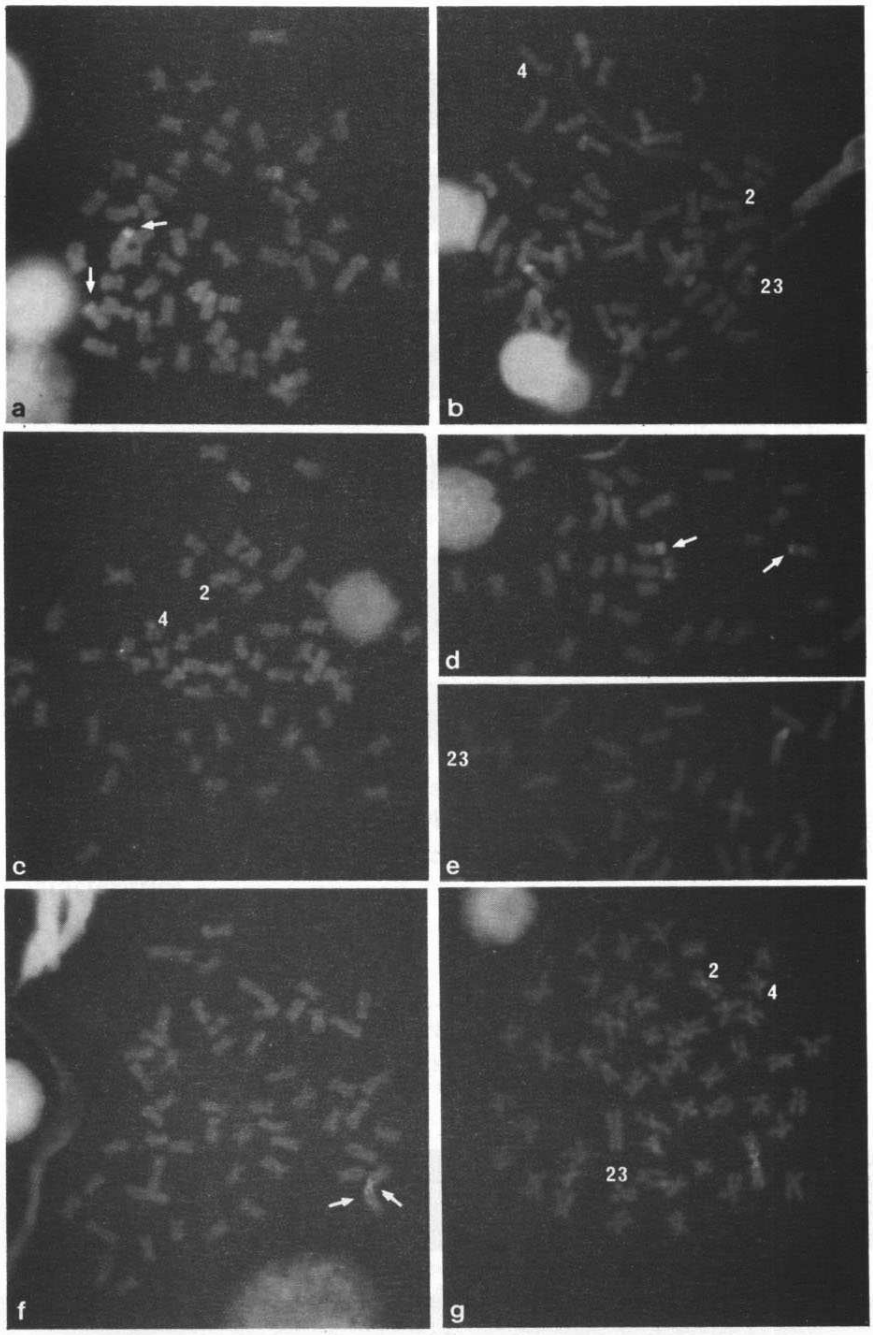

Figure 5 MM stained metaphases of (a and b) *S. altoparanae*, (c, d and e) *S. nasutus* and (f and g) *S. knerii*. MM^+ bands occurred in correspondence to NOR sites (arrows) and in other chromosomes of the complement (2, 4, 23); (d) and (e) are partial metaphases.

Discussion

Except for *S. nasutus*, which was described by Galetti *et al.* (1991a), the remaining four species have been first reported in the present work. This brings to eight the total number of *Schizodon* species cytogenetically known. A common karyotype showing 2n = 54 in all five species suggests a low rate of chromosome evolution among these fish. Only two NOR-bearing chromosomes were detected either by Ag- or MM staining or both in all five species we examined, as well as for those previously reported (Galetti *et al.*, 1984; Galetti *et al.*, 1991a; Martins and Galetti, 1998).

This feature appears to be common among the Anostomidae, although different species of *Leporinus* show NOR sites in diverse chromosomes (Galetti *et al.*, 1984). Thus, *Schizodon* reveals a higher level of chromosome stability, and all species thus far studied seem to share common, small metacentric-bearing NOR sites. Additional and less bright MM^+ bands detected in some centromeric or pericentromeric regions and in the interstitial portion of the short arm of chromosome 23 were never Ag-positive. *S. borelli* and *S. isognathum*, using FISH lacked any rDNA signals in these chromosome regions (Martins and Galetti, 1998).

Population models of chromosome evolution have postulated that migratory and large populations, as observed in *Schizodon* species, may retain higher karyotype stability compared with small and restricted populations (White, 1969; Lande, 1979). However, cryptic chromosome rearrangements occurred involving heterochromatin within the genus *Schizodon*. A small centromeric MM^+ heterochromatin observed in chromosome 2 of *S. altoparanae*, *S. nasutus* (both from the Paraná basin) and *S. knerii* (São Francisco basin), was absent in *S. vittatus* (Amazon basin) and also in *S. borelli* and *S. isognathum*, both from the Paraguay river previously described (Martins and Galetti, 1998). Moreover, dot-like proximal C-banded-heterochromatin was only detected in the long arm of chromosome 21 of *S. vittatus*. It is interesting that a comparable heterochromatin segment was recorded in *S. fasciatus*, another Amazonian species (Galetti *et al.*, 1991a), suggesting a derived condition related to other *Schizodon* species from different hydrographic systems. Symmetric and homogeneous karyotypes may find a homeostatic equilibrium between selective forces of genome diversity and the pressure for cellular constancy during the mitotic process, preventing changes in karyotype macrostructure, despite the occurrence of minor and cryptic chromosome rearrangements (Venere and Galetti, 1989). Once a symmetric and homogeneous karyotype is reached, as in *Schizodon*, major changes appear to be negatively selected along the chromosome complement.

Several fish groups have already been reported as changing the karyotype microstructure without significant changes in the karyotype macrostructure (Venere and Galetti, 1989). In fact, the Anostomidae, as well as other non-anostomids, such as Chilodontidae, Prochilodontidae, Curimatidae, Hemiodontidae and Parodontidae, have very similar karyotypes, consisting of 54 biarmed chromosomes (Galetti *et al.*, 1994). However, these groups may have experienced different chromosome evolutionary trends, especially in their lower taxa. Among anostomids, the species-rich genus *Leporinus* shows wide morphological and ecological diversity of forms and also large chromosome variability related to the presence of sex chromosomes (Galetti *et al.*, 1981b; Galetti and Foresti, 1986, 1987), NOR polymorphisms (Galetti *et al.*, 1984; Galetti *et al.*, 1991b) and heterochromatin differences (Galetti *et al.*, 1991a; Galetti *et al.*, 1991b). On the other hand *Schizodon*, apart from a few chromosome variations, shows little morphological and ecological diversity. In fact, it appears to manifest a parallelism between morphological/ecological diversity and the chromosomal evolution rate among anostomids.

Similar conditions have previously been described in other organisms. Chromosomal evolution seems more rapid in placental mammals than in other vertebrates, such as amphibians. Indeed, the same trend can be seen in morphological and ecological evolution (Wilson *et al.*, 1975). Thus, the Anostomidae may be an interesting model for study, due to the presence of groups which show great morphological/ecological and chromosome diversity, such as *Leporinus*, and others such as *Schizodon*, which are less diverse in such characteristics.

Acknowledgements

The authors thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for assistance and support. The help of A. S. Fenocchio, P. C. Venere and Y. Sato in collecting specimens is appreciated.

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