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Research Article

Physical mapping of the 18S and 5S ribosomal genes in nine Characidae species (Teleostei, Characiformes)

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Abstract

Characidae is one of the largest fish families of the Neotropical region, and presenting a pronounced morphological variability, certainly does not constitute a monophyletic group. The cytogenetical data also show a large chromosomal variation and can provide important information for a better understanding of the relationships between the species of this group. 18S and 5S rDNA probes were used in the present study for the chromosomal mapping in different Characidae species from the São Francisco River (*Astyanax lacustris*, *Astyanax scabripinnis*, *Hasemania nana*, *Piabina argentea*, *Orthospinus franciscensis*, *Serrapinnus heterodon*, *Serrapinnus piaba* and *Myleus micans*) and Alto Paraná (*Astyanax altiparanae*) basins. Species with a single pair of chromosomes bearing the nucleolar organizing regions (NORs) were identified, as well as species with multiple NORs, up to a maximum of seven 18S rDNA sites. The number of 5S rDNA site was also not constant, varying from two to eight. The mapping of the ribosomal genes was useful for the characterization and differentiation of the analyzed species.

Key words: cytogenetics, Neotropical fish, fluorescent *in situ* hybridization (FISH), rDNA probe.

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Introduction

The ribosomal genes are organized into two distinct multigenic families in eukaryotic organisms, one family comprising the 45S rDNA and the other the 5S rDNA. The repetitive 45S rDNA units are separated by non-transcribed external spacers and are composed of the 18S, 5.8S and 28S genes, which constitute the nucleolar organizing regions (NORs). NORs that were active in the preceding interphase are commonly detected by the silver nitrate staining (Ag-NORs). In lower vertebrates, NORs can also be evidenced by GC-specific fluorochromes such as chromomycin A_3 and mithramycin, independent of their activity, due to their GC-rich nature (Schmid, 1980; Schmid and Guttenbach, 1988). Nevertheless, the use of fluorochromes may not be conclusive for NOR studies since GC-rich heterochromatic regions not associated with NORs may be visualized (Souza *et al.*, 2001) and at the same time the few 45S rDNA sites may not appear clearly (Mandrioli *et al.*, 2001; Souza *et al.*, 2001). Thus, the fluorescent *in situ* hybridization (FISH) becomes an important alternative to the study of NORs due to the higher specificity of this methodology.

The 5S ribosomal gene is a smaller DNA sequence that does not participate in the formation of the nucleolus. The repetitive 5S rDNA unit is a 120 bp sequence associated with highly variable non-transcribed spacers (Long and Dawid, 1980) which results in the large evolutionary dynamism of these genes (Williams and Strobeck, 1985). Contrary to the 45S rDNA genes, the 5S rDNA sites can only be mapped in the chromosomes using the FISH method. For this reason, the localization of the 5S rDNA sites can be obtained only for a lower number of species when compared to the localization of the NORs. Since the sequences of the 45S and 5S ribosomal genes remained greatly conserved during the evolution of fish (Fujiwara *et al.*, 1998; Martins and Galetti Jr., 2000), probes prepared from the genome of a given species maybe used in the localization of these regions in other species.

18S and 5S rDNA probes were used in the present study for the physical mapping of these sites in the chromosomes of nine species of the family Characidae, aiming for their characterization and differentiation regarding these features, and a better understanding of the chromosomal evolution in this fish group.

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Material and Methods

Myleus micans, *Astyanax lacustris*, *A. scabripinnis*, *Hasemania nana*, *Piabina argentea*, *Orthospinus franciscensis*, *Serrapinnus piaba* and *S. heterodon* from the São Franscisco River (Três Marias municipality, Minas Gerais State, Brazil), and *Astyanax altiparanae* from the

Monjolinho Stream, Alto Paraná basin (São Carlos municipality, São Paulo State, Brazil) were analyzed.

The mitotic chromosomes were obtained from anterior and posterior kidney cells according to Bertollo *et al.* (1978) and Foresti *et al.* (1993). The chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (a), according to the arm ratio (Levan *et al.*, 1964).

The mapping of the 18S and 5S rDNA sites in the chromosomes was performed through the fluorescent *in situ* hybridization (FISH) following Pinkel *et al.* (1986), with probes obtained from *Prochilodus argenteus* (Hatanaka and Galetti Jr., 2004) and *Leporinus elongatus* (Martins and Galetti Jr., 2001), respectively.

Results

The obtained results are summarized in Table 1. The diploid number, chromosomal formulae and the number of Ag-NORs of each species are based on previous karyotypic analyses of Peres (2005), whose results were not included in the present work. *Astyanax altiparanae*, *A. lacustris*, *Orthospinus franciscensis* and *Serrapinnus heterodon* presented only one pair of 18S rDNA sites located on the short arm of a subtelocentric chromosome pair (Figures 1a, b, f, h). The remaining species presented more than two sites of 18S rDNA. *Astyanax scabripinnis* presented five sites, three on the short arm of subtelo/acrocentric chromosomes and two on the long arm of a subtelocentric pair (Figure 1c). *Hasemania nana* also presented five sites, but all on submetacentric chromosomes, three on the long arms and two on the short arms (Figure 1d). *Piabina argentea* presented six sites on the short arm of subtelo/acrocentric chromosomes (Figure 1e). *Serrapinnus piaba* presented seven sites, two on the long arm of a meta/submetacentroc pair, two on the short arm of another meta/cubmetacentric pair and the rest on the short arm of three subtelo/acrocentric chromosomes (Figure 1g). *Myleus micans* presented seven sites, five on the short arm of subtelo/acrocentric chromosomes and two on the long arm of a metacentric pair (Figure 1i). All of the 18S rDNA sites were preferentially located in the telomeric region of the chromosomes, and only *Myleus micans* presented a chromosome pair with interstitial sites.

The 5S rDNA sites were evidenced in the pericentromeric region of a pair of metacentric chromosomes in *A. altiparanae*, *A. lacustris* and *M. micans* (Figures 2a, b, h). In *A. scabripinnis*, two sites were evidenced on a pair of metacentric chromosomes and two sites on a subtelo/acrocentric pair, all interstitial in the short arm (Figure 2c). In *P. argentea*, four sites were observed in the terminal region of the short arm of two chromosome pairs, one submetacentric and the other subtelo/acrocentric (Figure 2d). Four sites were identified in *O. fransciscensis*, two in an interstitial position on a metacentric pair and the other two in a terminal position on the short arm of a subtelo/acrocentric pair (Figure 2e). Four sites were also observed in *S. heterodon*, two in interstitial positions on metacentric chromosomes and two in terminal positions on the short arm of subtelo/acrocentric chromosomes (Figure 2g). The largest number of 5S rDNA sites was observed in *S. piaba*, which presented eight interstitial markings on six meta/submetacentric chromosomes (Figure 2f), being also the only species to present two sites located in the same chromosome.

Table 1 - Diploid number, karyotypic formula and number of 18S and 5S rDNA sites in nine Characidae species; 2n: diploid number; m: metacentric; sm: submetacentric; st: subtelocentric; a: acrocentric chromosomes.

Species	2n	Karyotypic formula	Number of Ag-NORs	Number and localization of the 18S rDNA sites		Number and localization of the 5S rDNA sites	
				Terminal	Interstitial	Terminal	Interstitial
Astyanax altiparanae	50	8m+20sm+12st+10a	2 (st/a)	2 (st/a)	0	0	2 (m)
Astyanax lacustris	50	8m+20sm+16st+6a	2 (st/a)	2 (st/a)	0	0	2 (m)
Astyanax scabripinnis	50	12m+24sm+8st+6a	2 (st)	5 (st/a)	0	0	2 (m) 2 (st/a)
Hasemania nana	50	8m+42sm	2 (sm)	5 (sm)	0	-	-
Piabina argentea	52	8m+14sm+16st+14a	2 (a)	6 (st/a)	0	2 (sm) 2 (st/a)	0
Orthospinus franciscensis	50	22m+20sm+2st+6a	2 (st)	2 (st)	0	2 (st/a)	2 (m)
Serrapinnus piaba	52	16m+20sm+14st+2a	3 (sm/st)	4 (m/sm) 3 (st/a)	0	0	8 (m/sm)*
Serrapinnus heterodon	52	17m+20sm+14st+1a 16m+20sm+14st+2a 15m+20sm+14st+3a	2 (st)	2 (st)	0	2 (st/a)	2 (m)
Myleus micans	58	26m+18sm+8st+6a	4 st/a	5 (st/a)	2 (m)	0	2 (m)

*two distinct sites present in a given homologous pair, one site in the pericentromeric region and another in the interstitial region of the long arm.

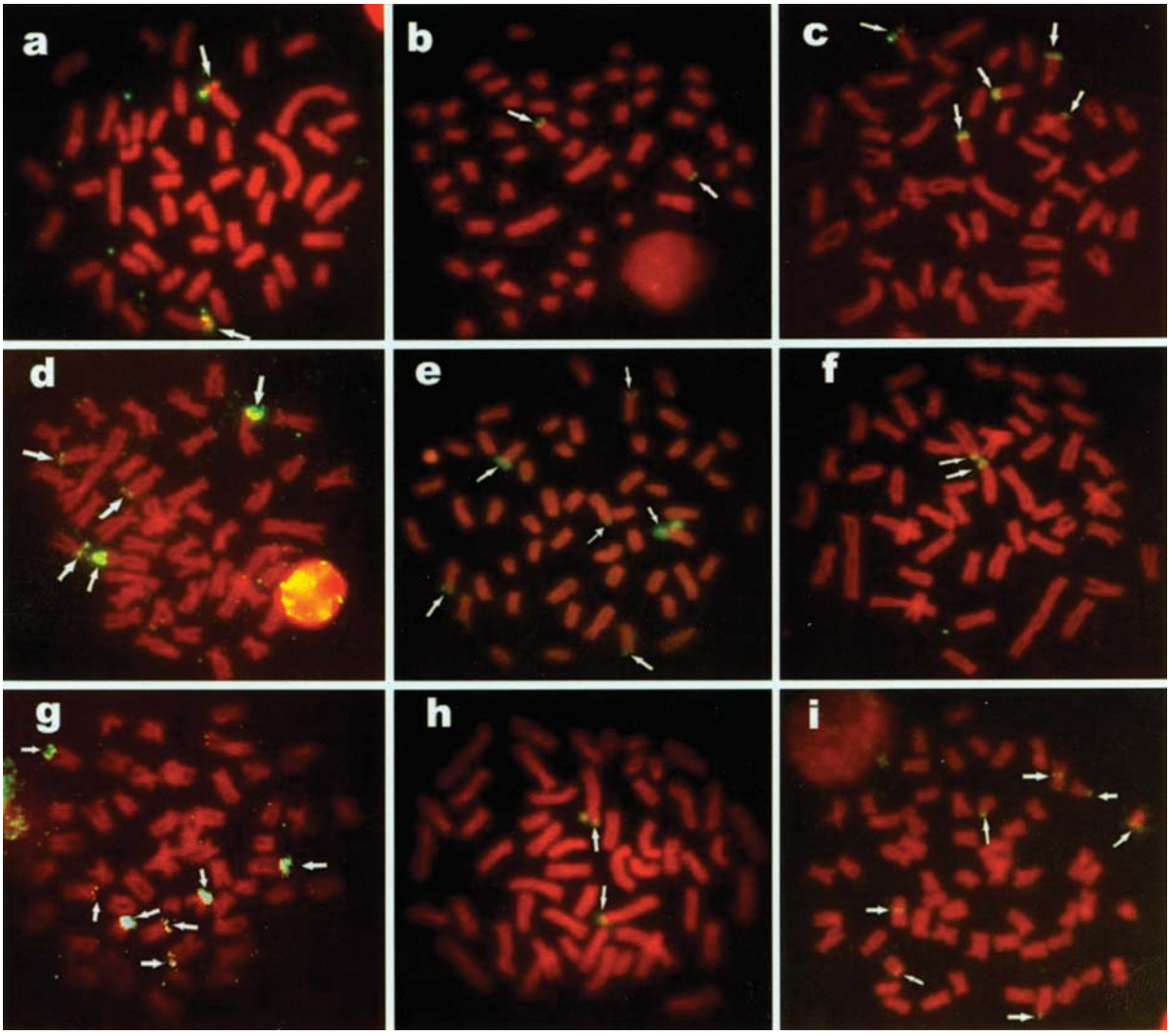

Figure 1 - Localization of 18S rDNA sites (arrows in (a) *Astyanax altiparanae;* (b) *A. lacustris;* (c) *A. scabripinnis*; (d) *Hasemania nana*; (e) *Piabina argentea*; (f) *Orthospinus. franciscensis*; (g) *Serrapinnus. piaba*; (h) *S. heterodon*; (i) *Myleus micans.*

Discussion

Fluorescent *in situ* hybridization has become a much utilized tool for detecting NORs in the metaphasic chromosome complement since it can identify NORs independent of their activity in the previous interphase. This explains the occurrence of a larger number of NOR sites than those identified by the silver staining technique (Ag-NORs), as observed, for example, in *Hoplias malabaricus* (Born and Bertollo, 2000), *A. scabripinnis* (Ferro *et al.*, 2001; Kavalco and Moreira-Filho, 2003), *Prochilodus lineatus* (Jesus and Moreira-Filho, 2003) and in the present work. Concerning the genus *Astyanax*, *A. altiparanae* and *A. lacustris* exhibited only one chromosome pair bearing 18S rDNA sites, while in *A. scabripinnis* five sites were found, confirming variability in NOR number and localization that has previously been observed in this genus. In fact, one of the largest number of NORs described for Characidae was observed in an *A. scabripinnis* population different from the one here analyzed, that had fifteen Ag-NOR sites NORs (Rocon-Stange and Almeida-Toledo, 1993). Similarly in *Serrapinnus* the two analyzed species presented variation in the number of 18S rDNA sites, two in *S. heterodon* and seven in *S. piaba*.

The number and localization of the 5S rDNA sites in *O. franciscensis*, *P. argentea*, *S. heterodon* and *S. piaba* were also analyzed in the present study for the first time, in addition to a reanalysis of *M. micans*, *A. lacustris*, *A. scabripinnis* and *A. altiparanae*. *Hasemania nana* was the only species where mapping of these sites was not possible due to technical problems. Martins and Galetti Jr. (2001) consider that the presence of two interstitial 5S loci could represent a conserved character among the Characiformes. However, additional studies, especially in the family Characidae, have shown a large variability in the number and localization of these sites (Ferro *et al.*, 2001; Almeida-Toledo *et al.*, 2002; Kavalco *et al.*, 2004; Mantovani *et*

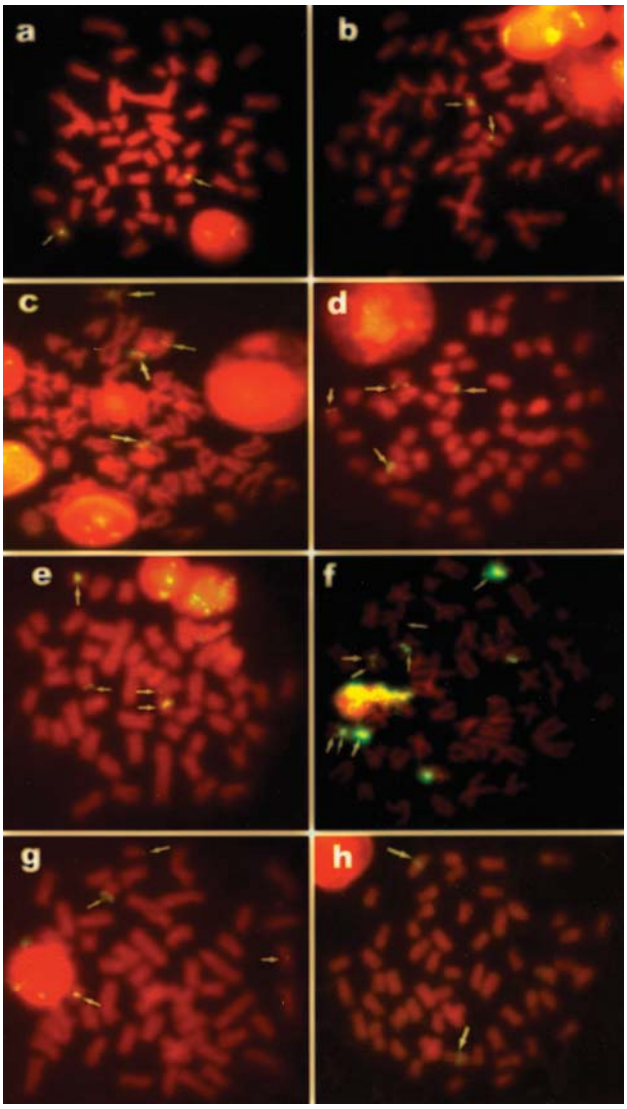

Figure 2 - Localization of 5S rDNA sites (arrows) in (a) *Astyanax altiparanae;* (b) *A. lacustris;* (c) *A. scabripinnis*; (d) *Piabina argentea*; (e) *Orthospinus franciscensis*; (f) *Serrapinnus piaba*; (g) *S. heterodon*; (h) *Myleus micans.*

al., 2005; present study). Thus, the localization and/or position of 5S rDNA sites in the Characidae are as variable as the 18S rDNA sites.

The presence of two pericentromeric 5S rDNA loci in a medium-sized metacentric pair was also suggested as a putative conserved character in *Astyanax* (Almeida-Toledo *et al.*, 2002; Mantovani *et al.*, 2004). In fact, this characteristic was shared by the three species of this genus here studied. However, it is not a diagnostic characteristic for *Astyanax*, since it extends to species of other unrelated groups such as *O. franciscensis*, *S. heterodon S. piaba* and *M. micans.*

Variation in the number and localization of the 5S rDNA loci seems to be common among *Astyanax* species, ranging from two to eight loci in terminal as well as interstitial positions (Ferro *et al.*, 2001; Kavalco *et al.*, 2004; present study). Yet, the presence of syntenic 18S and 5S rDNA sites in a medium-sized metacentric pair of a few *Astyanax* species (Almeida-Toledo *et al.*, 2002) does not occur in the *Astyanax* species here analyzed, since the 18S rDNA sites were not evidenced in any metacentric chromosome of this group. In *A. altiparanae* and *A. lacustris*, the rDNA sites seem to be located on homologous chromosomes, showing that these species are closely related. On the other hand, the larger number of rDNA sites found in *A. scabripinnis* suggests a more ancient evolutionary divergence for this species.

The distribution of the 5S and 18S rDNA sites may be a useful tool for the cytogenetic characterization of some species. *Serrapinnus heterodon* and *S. piaba*, for example, possess similar karyotypic organizations, practically precluding their separation through the conventional karyotype analysis (Peres, 2005). Nevertheless, the localization of the 5S and 18S rDNA sites allow their identification because *S. heterodon* presented a simple NOR system and four 5S rDNA loci, while *S. piaba* presented multiple NORs (seven loci) and eight 5S rDNA loci. Furthermore, the metacentric chromosome pair bearing the 5S rDNA presents a size heteromorphism in *S. heterodon*, with the largest chromosome being almost double the size of the smallest chromosome. The *S. heterodon* population here analyzed also presented a structural chromosome polymorphism, with the differentiation of two other cytotypes in addition to the standard one. These variations indicate that the differences between the three cytotypes must have originated through pericentric inversions related to chromosome pairs 8 and 26 (Peres, 2005). An uncommon characteristic was also observed in *S. piaba*, that is, the presence of two distinct 5S rDNA loci in a single chromosome. It is possible that part of the original 5S rDNA site has been transposed to another chromosome region by a paracentric inversion, as proposed for *Upsilodus* sp. (Kavalco *et al.*, 2004).

The available data show a large variation in the number, localization and position of 18S and 5S rDNA sites in the genome of fishes. However, this variability can be more pronounced within some families, such as Characidae, in comparison to other families, such as Anostomidae and Bryconinae, which coincidently also show a larger karyotypic homogeneity. It is possible that this variability in the Characidae is a reflection of the polyphyletic nature of this fish group.

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