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# Two 5S rDNA arrays in Neotropical fish species: is it a general rule for fishes?

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*Key words:* fish, non-transcribed spacer, NTS, 5S rDNA

**Abstract**

In this paper we describe Southern blot hybridization results probed with 5S rRNA genes for several Neotropical fish species representing different taxonomic groups. All the studied species showed a general trend with the 5S rDNA tandem repeats organized in two distinct size-classes. At the same time, data on 5S rDNA organization in fish genome were summarized. Previous information on the organization and evolution of 5S rRNA gene arrays in the genome of this vertebrate group are in agreement with the Southern results here presented. Sequences obtained for several fish species have revealed the occurrence of two distinct 5S rDNA classes characterized by distinct non-transcribed spacer sequences, which are clustered in different chromosomes in some species. Moreover, the 5S rDNA loci are generally distributed in an interstitial position in the chromosomes and they are usually not syntenic to the 45S rDNA. The presence of two classes of 5S rDNA in several non-related fish species suggests that this could be a common condition for the 5S rRNA gene organization in the fish genome.

## Introduction

The available information concerning the genomic organization of fishes are particularly related to the repetitive portion of their genome and, among these sequences, the ribosomal RNA genes have received a particular attention. In higher eukaryotes, tandem arrays of ribosomal RNA genes are organized in two distinct multigene families composed of hundreds to thousands of copies. One class is represented by the 45S rDNA that codes for the 18S, 5.8S and 26S/28S rRNAs and the other one, represented by the 5S rDNA, codes for the 5S rRNA, an element of the largest subunit of ribosomes. The 5S rDNA repeats consist of 120 base pairs (bp) coding sequences, which are separated from each other by a non-transcribed spacer (NTS) that shows an accentuated length variation (Figure 1). The copy number of the 5S rRNA genes is highly variable among vertebrates. In *Xenopus laevis* the 5S rRNA gene family comprises 27000 copies whereas in human, around 2000 copies (Lewin, 1997).

The genomic organization of the 5S rRNA genes is known on several eukaryote organisms. The accumulating data demonstrate that 5S RNA genes are highly conserved, even among non-related taxa, both with respect to length and nucleotide sequence, whereas the NTS evolves more rapidly. Some fungi species can have 5S genes within the 18–28S rDNA repeat, whereas others present 5S genes dispersed throughout their genome (Belkhiri, Buchko & Klassen, 1992). In some eukaryotes representing non-related taxonomic groups, the 5S rRNA genes can be found interspersed with other multicopy genes, such as histone genes, 45S rDNA (most of the cases) and repeated trans-spliced leader sequences (Drouin & Moniz de Sá, 1995). However, in most eukaryotes, the 5S rRNA genes are normally detected in distinct areas of the genome, organized as one or more tandemly repeated clusters.

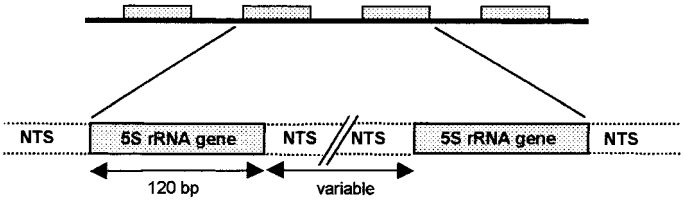


*Figure 1.* Arrangement of higher eukaryotic 5S rRNA genes intercalated with non-transcribed DNA segments (NTS).

*Table 1.* Species analyzed

| Species | Collection sites | Family | Order |
|---|---|---|---|
| *Steindachnerina elegans* | São Francisco River | Curimatidae | Characiformes |
| *Prochilodus affinis* | São Francisco River | Prochilodontidae | Characiformes |
| *Parodon tortuosus* | Mogi-Guaçu River | Parodontidae | Characiformes |
| *Hoplias malabaricus* | Araguaia River | Erythrinidae | Characiformes |
| *Pseudoplatistoma coruscans* | Miranda River | Pimelodidae | Siluriformes |
| *Hypostomus* sp. | São Francisco River | Loricariidae | Siluriformes |
| *Cichla ocelaria* | São Francisco River | Cichlidae | Perciformes |

The structural and functional organization of 5S rRNA genes has been mostly described for plants (Hanson et al., 1996; Amarasinghe & Carson, 1998; among others), mammals (Little & Braaten, 1989; Leah et al., 1990; Suzuki, Moriwaki & Sakurai, 1994) and some amphibian (Korn, 1982; Vitelli et al., 1982; del Pino et al., 1992). In vertebrates, 5S rDNA variants related to either pseudogenes or NTS variations have been reported for several species (Little & Braaten, 1989; Leah et al., 1990; Suzuki, Moriwaki & Sakurai, 1994; Frederiksen et al., 1997). Such NTS variations have been useful on evolutionary studies and can characterize species- or population-specific markers (Suzuki, Moriwaki & Sakurai, 1994, Pendás et al., 1995). A dual 5S rRNA gene system, differently regulated in somatic and oocyte cells, was described for vertebrates, including fish and amphibians (Komiya, Hasegawa & Takemura, 1986). In *Xenopus laevis*, for example, the oocyte unit is about 750 bp and it includes the 120 bp gene, a nontranscribed spacer and a pseudogene (Jacq, Miller & Brownlee, 1977), while the somatic unit has approximately 880 bp and does not contain pseudogenes (Peterson, Doering & Brown, 1980). The 5S rRNA genes and the NTSs seem to have a particular pattern of organization in the genome. Although several authors have considered the usefulness of 5S rDNA sequences as phylogenetic and/or population markers (Suzuki, Moriwaki & Sakurai, 1994; Pendás et al., 1995; Baker, Hedderson & Dransfield, 2000), special attention must be exercised, mainly in phylogenetic interpretations, once the 5S rDNA family might show a complex organization with the presence of paralogous sequences in the genome (Martins and Galetti, 2001).

In order to address the understanding of the dynamics and evolution of 5S rDNA tandemly repetitive sequences, the present paper summarized recent information on 5S rDNA in fish species. Moreover, Southern blot hybridization probed with 5S rRNA gene was carried out in seven non-related Neotropical fish species focusing in the organization of the 5S rDNA. The compilation of previous and present data on 5S rDNA, strongly suggests that this rDNA family is organized in two different classes in the fish genome.

## Materials and methods

Seven non-related Neotropical fish species were analyzed in the present work. The specimens were obtained from different river systems in Brazil, as summarized in Table 1. DNA was extracted from liver cells (fixed in ethanol:methanol – 1 : 1) using a phenol/chloroform extraction method, as described in Sambrook, Fritsch and Maniatis (1989). Genomic DNA (10 μg) was completely digested with the restriction endonuclease *Hin*dIII, that cleave once in the 5S rRNA genes of several non-related fish species (Pendas et al., 1994; Moran et al., 1996; Murakami &

Fujitani 1998; Sajdak, Reed, Phillips, 1998; Martins & Galetti, 2001; Wasko et al., in press), submitted to 1% agarose gel-electrophoresis and Southern-transferred to a Hybon-$N^+$ nylon membrane (Southern, 1975). The hybridization of the filter-immobilized DNA was performed using a 5S rRNA gene sequence of *Leporinus elongatus* (Martins & Galetti, 2001; GenBank accession number: AF284741) as a probe that was labelled and detected by the ECL-direct nucleic acid labelling and detection system (Amersham Life Science).

## Results

Analyses of the filter hybridization patterns showed that the 5S rDNA tandem repeats have distinct organization in the different analyzed Neotropical fish (Figure 2). These differences are due to variations in the structural organization of the 5S rDNA repeats. Although each species has different hybridization patterns, all of them showed two main bands, a large and a small one, characterizing the presence of at least two main arrays of tandem repeats. Additional weak bands were observed in the analyzed species indicating that arrays with few copies of the 5S rRNA gene or pseudogenes may exist. The occurrence of few copies of 5S rDNA tandem repeats can not be eliminated once dispersed 5S rRNA genes have been described in other organisms (Rosenthal & Doeringer, 1983; Belkhiri et al., 1992). Among the analyzed species, *Steindachnerina elegans* showed a small 5S rDNA repeat around 180 bp and, as far we know, the smallest 5S rDNA repeat detected among eukaryotes.

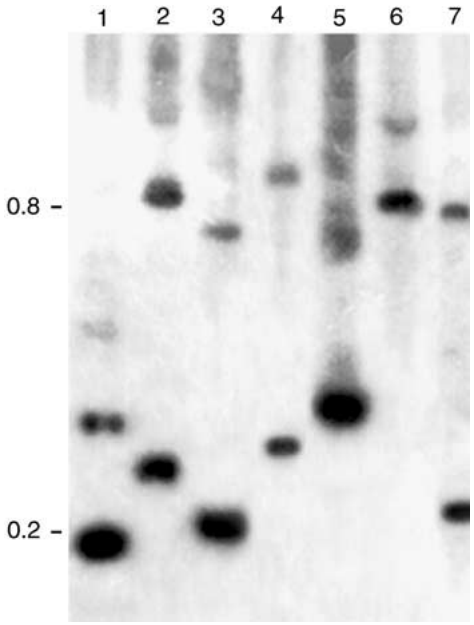


*Figure 2.* 5S rDNA genomic organization in seven representative Neotropical fishes determined by Southern blot hybridization to a 5S rRNA gene probe. Animals: *Steindachnerina elegans* (1), *Prochilodus affinis* (2), *Parodon tortuosus* (3), *Hoplias malabaricus* (4), *Pseudoplatistoma coruscans* (5), *Hipostomus* sp. (6) and *Cichla ocelaris* (7). Molecular weight markers (kb) are shown on the left.

## Discussion

### *Chromosomal distribution of 5S rDNA loci in fish*

The chromosomal localization of the 5S rRNA genes have been described on 43 fish species representing distinct groups, such as Acipenseriformes, Anguilliformes, Characiformes, Perciformes, Salmoniformes and Tetraodontiformes (Table 2). These previous data have shown that the location of 5S rRNA genes occur in an interstitial position in the chromosomes of almost all the analyzed species. The same 5S rRNA gene chromosomal location was also observed in mammals (Mellink et al., 1996; Frederiksen et al., 1997; Mäkinem et al., 1997; among others) and amphibians (Vitelli et al., 1982; Schmid, Vitelli & Batistoni, 1987; Lucchini et al., 1993) suggesting that such pattern seems to be not casual. An interstitially-nested distribution for the 5S rRNA genes could represent some advantage related to the organization of these genes in the vertebrate genome.

Among mammals, the 5S rRNA genes are generally located on a single chromosome pair, while the 45S rRNA genes, that correspond to the nucleolar organizer regions (NORs), are often present on multiple chromosomes (Suzuki, Sakurai & Matsuda, 1996; Mäkinem et al., 1997). In amphibians (Schmid, Vitelli & Batistoni, 1987; Lucchini et al., 1993) and fish species (Fujiwara et al., 1998; Murakami & Fujitani, 1998; Martins & Galetti, 1999), however, the 5S rRNA genes may be located on several chromosomes. Moreover, 45S and 5S rDNA loci may assume a syntenical organization in the chromosome (Pendás et al., 1994; Móran et al., 1996) or can be detected in different chromosome pairs (Martínez et al., 1996; Martins & Galetti, 1999). Such divergent locations of NORs and 5S rDNA loci seem to be the most common situation observed in fish (Table 2) and far the most frequent distribution pattern observed in vertebrates (Lucchini et al., 1993; Suzuki, Sakurai & Matsuda, 1996).

*Table 2.* Compilation of data related to the chromosomal localization of the 5S rDNA in fish chromosomes

| Orders and species | Number of 5S rDNA loci | Interstitial 5S rDNA loci | Syntenic 5S and 45S rDNA | References |
|---|---|---|---|---|
| Acipenseriformes | | | | |
| *Acipenser naccarii* | 4 | +/− | | Fontana et al., 1999 |
| *Acipenser ruthenus* | 2 | − | | Fontana et al., 1999 |
| *Acipenser sturio* | 2 | + | | Tagliavini et al., 1999 |
| *Huso huso* | 2 | | | Fontana et al., 1998 |
| Anguilliformes | | | | |
| *Anguilla anguilla* | 2 | + | − | Martínez et al., 1996 |
| *Anguilla rostrata* | 2 | + | − | Nieddu et al., 1998 |
| Salmoniformes | | | | |
| *Coregonus artedti* | 2 | + | − | Sajdak, Reed and Philips, 1998 |
| *Coregonus zenithicus* | 2 | + | − | Sajdak, Reed and Phillips, 1998 |
| *Hucho perryi* | 8 | + | − | Fujiwara et al., 1998 |
| *Oncorhynchus masou* | 6 | + | + | Fujiwara et al., 1998 |
| *Oncorhynchus mykis* | 3–4 | + | + | Móran et al., 1996 |
| *Salmo salar* | 2 | + | + | Pendás et al., 1994 |
| *Salmo trutta* | 2 | + | − | Móran et al., 1996 |
| *Salvelinus fontinalis* | 8 | + | − | Fujiwara et al., 1998 |
| Cypriniformes | | | | |
| *Acheilognathus tabira* | 4 | +/− | + | Inafuku et al., 2000 |
| *Carassius auratus langsdorfi* | 2− | | | Murakami and Fugitani, 1998 |
| *Cyprinus carpio* | 4 | + | − | Inafuku et al., 2000 |
| *Danio rerio* | 2 | + | + | Phillips and Reed, 2000 |
| *Rhodeos ocellatus* | 2 | + | − | Kikuma et al., 2000 |
| Characiformes | | | | |
| *Brycon lundii* | 4 | + | − | Wasko et al., 2001 |
| *Brycon microlepis* | 4 | + | − | Wasko et al., 2001 |
| *Brycon orbignyanus* | 4 | + | − | Wasko et al., 2001 |
| *Brycon cephalus* | 4 | + | − | Wasko et al., 2001 |
| *Brycon* sp. | 4 | + | − | Wasko et al., 2001 |
| *Brycon brevicauda* | 4 | + | − | Wasko et al., 2001 |
| *Brycon insignis* | 4 | + | − | Wasko et al., 2001 |
| *Hoplias malabaricus* | 2 | | − | Born and Bertollo, 2000 |
| *Leporinus* cf . *elongatus* | 4 | + | − | Martins and Galetti, 2001 |
| *Leporinus elongatus* | 4 | + | − | Martins and Galetti, 1999 |
| *Leporinus friderici* | 4 | + | − | Martins and Galetti, 1999 |
| *Leporinus obtusidens* | 4 | + | − | Martins and Galetti, 1999 |
| *Leporinus reinhardti* | 4 | + | − | Martins and Galetti, 2001 |
| *Schizodon altoparanae* | 4 | + | − | Martins and Galetti, 2000 |
| *Schizodon borelli* | 4 | + | − | Martins and Galetti, 2000 |
| *Schizodon isognathum* | 4 | + | − | Martins and Galetti, 2000 |
| *Schizodon knerii* | 4 | + | − | Martins and Galetti, 2000 |
| *Schizodon nasutus* | 4 | + | − | Martins and Galetti, 2000 |
| *Schizodon vittatus* | 4 | + | − | Martins and Galetti, 2000 |
| Perciformes | | | | |
| *Coris julis* | 4 | − | + | Mandrioli, Colomba and Vitturi, 2000 |

*Table 2.* (*continued*)

| Orders and species | Number of 5S rDNA loci | Interstitial 5S rDNA loci | Syntenic 5S and 45S rDNA | References |
|---|---|---|---|---|
| *Ephinephelus marginatus* | 2 | + | − | Sola et al., 2000 |
| *Micropterus salmoides* | 2 | + | − | Deiana et al., 2000 |
| *Oreochromis niloticus* | 4 | + | − | Martins et al., 2000 |
| Tetraodontiformes | | | | |
| *Tetraodon nigroviridis* | 2 | + | − | Fischer et al., 2000 |

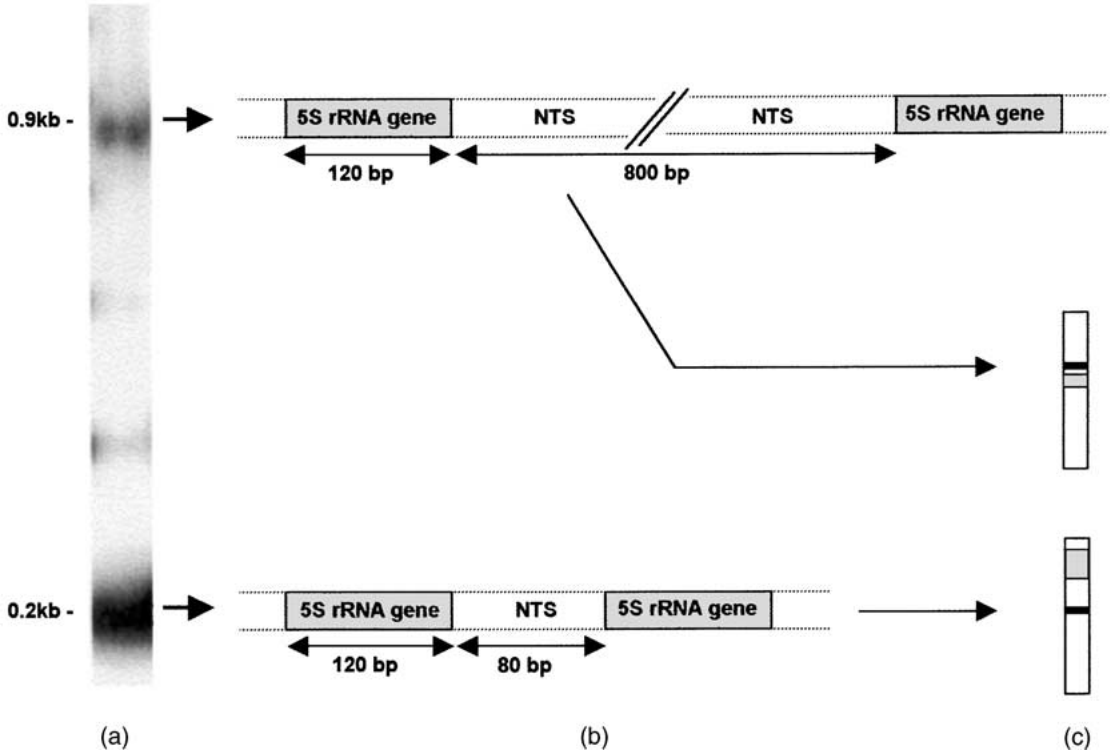


*Figure 3.* Organization of 5S rRNA genes in *Leporinus obtusidens*, *L. elongatus*, *L.* cf. *elongatus* and *L. friderici* according to Martins & Galetti (2001). (a) Southern hybridization results using *Hind*III digested genomic DNA probed with a 5S rRNA gene showing two different-size repeats, one consisting of monomers of 200 bp and another one of 920 bp monomers; (b) scheme of the molecular organization of the 5S rDNA repeats (5S rRNA gene + NTS); (c) idiogram of the chromosomal distribution of the two 5S rDNA different repeats. The chromosomes were probed with the 5S rRNA gene that labelled two chromosome loci. FISH with probes of 5S rRNA gene-free NTS of 200 bp monomer and 5S rRNA gene-free NTS of 920 bp monomer showed that each one of these NTSs was clustered in separated chromosome pairs, corresponding to the two 5S rRNA gene chromosome loci.

In eukaryotes, the large 45S rRNA genes are transcribed by the nucleolar enzyme RNA polymerase I, whereas the 5S genes are transcribed far from the nucleolus by the non-nucleolar RNA polymerase III. It is suggested that such functional divergences would require different physical locations between the large rDNA and the 5S arrays (Amarasinghe & Carlson, 1998). In addition, gene conversion and unequal crossing-over are common mechanisms acting in the evolution processes of the multiple tandem arrays (Dover, 1986). These mechanisms might be more efficient when the 5S and 45S clusters remain separated instead of a linked configuration, avoiding disruptive interference, such as undesired translocation of 5S

sequences inside the 45S arrays (Martins & Galetti, 1999). This could explain why most vertebrates have these clusters on distinct chromosomes.

In the anostomid fishes, the 5S rRNA genes are clustered in two chromosomal loci (Martins & Galetti, 1999, 2000, 2001) and, at least for the genus *Leporinus,* it was demonstrated that each one of these loci has a characteristic repeat unit differentiated by a distinct NTS sequence (Martins & Galetti, in press) (Figure 3). Although the chromosomal sites nesting the 5S rRNA genes have been maintained conserved during the karyotype diversification of anostomids, changes within the array of these genes have occurred. Several organisms have a unique rDNA variant on different chromosomes and, it has been shown, at least for primates, the occurrence of non-homologous chromosome exchange as a mechanism of such homogenization (Williams & Strobeck, 1985). The distinct 5S rDNA arrays detected for *Leporinus* could reflect the absence of non-homologous chromosome exchange between the chromosome pairs bearing 5S rDNA clusters. This scenario is in agreement with the idea that individual chromosomes occupy specific territories in the nucleous (Lamond & Earnshaw, 1998) and the chromosomes bearing 5S rDNA clusters seem to be evolving independently in individual nuclear environments.

*Molecular organization of 5S rDNA in fish genome*

While the 5S rRNA gene is conserved even among non-related taxa, the NTS shows an extensive length variation, which can give an accentuated dynamism to the 5S rRNA genes (Williams & Strobeck, 1985). The 5S rRNA gene is transcribed by the RNA polimerase III and it contains an internal control region (ICR) which functions as a promoter for the gene (Hallenberg, Nederby-Nielsen & Frederiksen, 1994). Although non-transcribed DNA sequences such as the NTSs seem to have no value for the genome, it has been recently demonstrated that a TATA sequence located in the NTS plays an important role in regulation of 5S rRNA gene expression in several mammals (Nederby-Nielsen et al., 1993; Suzuki, Sakurai & Matsuda, 1996). A TATA-like sequence has been observed upstream the 5S rRNA gene in *Salmo salar* (Pendás et al., 1994), *Carassius auratus* (Murakami & Fugitani, 1998), *Coregonus* (Sajdak, Reed, Phillips, 1998), *Gasterosteus aculeatus* (Rocco et al., 1999), *Acheilognathus tabira, Cyprinus carpio* (Inafuku et al., 2000), *Oreochromis niloticus* (Martins et al., 2000), *Brycon* (Wasko et al., in press) and *Leporinus* (Martins & Galetti, 2001), suggesting a possible influence in the transcription level of the 5S rRNA genes. Other short sequences that are present in the NTS could be involved with regulatory functions, acting in the expression/regulation of the 5S rRNA gene.

Different 5S rDNA classes were reported for several mammals (Hallemberg et al., 1994; Frederiksen et al., 1997) and fish species. In the tilapiine cichlid fish, *Oreochromis niloticus,* two distinct 5S rDNA units have been characterized by distinct NTS types and base substitutions in the 5S rRNA gene (Martins et al., 2000). Similar situation was also described for seven species of the genus *Brycon* (Wasko et al., 2001), *Salmo salar* (Pendás et al., 1994), *Oncorhynchus mykiss* (Móran et al., 1996) and the genus *Coregonus* (Sajdak, Reed & Phillips, 1998). In the characiform *Leporinus*, two classes of 5S rDNA, one consisting of monomeric repeat units around 200 bp and another one with monomers of 920 bp were also identified (Martins & Galetti, 2001) (Figure 3). Each of these different-sized 5S rDNA classes was characterized by distinct NTS sequences and was clustered in distinct chromosome pairs. These results were obtained under Southern blot hybridization and FISH condition using probes of 5S rRNA gene and both isolated NTSs of 200 bp and 900 bp monomers (Figure 3). No mosaisism was observed in the organization of these two 5S rDNA classes. It has been believed that multigene families evolve according to homogenization processes governed by molecular drive (Dover, 1986) and concerted evolution (Elder Jr. & Turner, 1995), resulting in a sequence similarity of the repeating units that is greater within than between species. According to the results described for *Leporinus*, the similarity in the repeat units is greater within a specific cluster even among different species than between two clusters in the same species (Martins & Galetti, 2001).

The characterization of 5S rDNA sequences among fish species has identified different types of tandem repeats. The presence of two types of tandem repeats was observed for several fish species belonging to Characiformes (Martins & Galetti, 2001; Wasko et al., 2001), Perciformes (Martins et al., 2000) and Salmoniformes (Pendás et al., 1994; Morán et al., 1996; Sajdak, Reed & Phillips, 1998). At least two types of 5S rDNA tandem repeats, demonstrated by two main bands observed in the 5S rDNA hybridized membranes, were also observed for all fish species Southern blot investigated in the present work. It is

suggested that the presence of two 5S rDNA-arrays could be a common feature in the fish genome. Analyzes of the NTS of several different fish species showed that the minimum NTS length size described for these organisms is 60–80 bp. The characiform *Steindachnerina elegans* showed the shortest 5S rDNA repeat unit (around 180 bp) suggesting a NTS with 60 bp. Martins & Galetti (2001) described NTSs with 80 bp for several *Leporinus* species. This short-NTS pattern seems to be the minimum necessary condition for the maintenance of the array and dynamic of the 5S rRNA genes in the genome, as this may contain required sequences for the expression/regulation of the 5S rRNA genes.

Further studies on several members of different fish orders should be carried out in order to improve the data on 5S rDNA in this vertebrate group. Although the exact number of different tandem repeats remains to be clearly elucidated, the existence of different 5S rDNA classes seems to be a rule for fishes. As demonstrated for *Leporinus*, these different 5S rDNA classes might be clustered in individual chromosome environments. The investigation of the functional role of the genes present in these 5S rDNA classes seems to be an interesting challenge to be reached.

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