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Brief report
5S rDNA organization in the fish *Synbranchus marmoratus* (Synbranchidae, Synbranchiformes)

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Studies on ribosomal RNA genes have gained prominence in a broad range of animals and plants, especially in relation to species or population characterization, evolutionary relationships and genome structuring. The 5S rDNA array consists of multiple copies of a highly conserved 120 base pair (bp) coding sequence, separated by a variable non-transcribed spacer (NTS) (LONG and DAVID 1980). While the 5S rRNA gene is conserved even among non-related taxa, the NTS shows extensive length and sequence variation which can give an accentuated dynamism to these genes (WILLIAMS and STROBECK 1985).

The fish *Synbranchus marmoratus* BLOCH 1795 (Synbranchidae, Synbranchiformes) is widely distributed in freshwater river systems of different hydrographic basins of South America. Previous cytogenetic and biochemical studies have been carried out in several populations of the species and detected extensive levels of variation (NAKAMOTO et al. 1986; FORESTI et al. 1992; MELILLO et al. 1996; TORRES 2000). These data strongly support the hypothesis that *S. marmoratus* represents a complex of species (FORESTI et al. 1992) and that further biological and genetic studies are required for a better characterization of the species. To further understand the genetic structure of *S. marmoratus* and the 5S rDNA organization among fishes, we have studied the nucleotide sequence and genome organization of 5S rDNA tandem repeats in this species.

MATERIAL AND METHODS

Seven wild adult specimens (4 females and 3 males) of *Synbranchus marmoratus* were collected from the Tietê River, municipality of Penápolis, São Paulo State, Brazil. DNA was extracted from the liver according to SAMBROOK and RUSSEL (2001) and PCR amplifications of the 5S rDNA were performed as described by MARTINS and GALETTI JR. (2001a). Primers 5SA (5′-TAC GCC CGA TCT CGT CCG ATC-3′) and 5SB (5′-CAG GCT GGT ATG GCC GTA AGC-3′) were designed to amplify the 5S genes and their NTSs. The PCR-amplified products were cloned in the plasmid pGEM-T (Promega) and used to transform competent cells of the strain DH5α *E. coli* (Invitrogen). Clones were sequenced on an ABI PRISM 377 DNA Sequencer (Applied Biosystems) and the alignment of the sequences was performed using ClustalW (THOMPSON et al. 1994). Nucleic acid sequences were subjected to Blastn (ALTSCHUL et al. 1990) searches at National Center for Biotechnology Information (USA), website (http://www.ncbi.nlm.nih.gov/blast).

The genomic organization of 5S rDNA was determined by Southern-blot hybridization. Genomic DNA (10 μg) was partially to completely digested with 30U of *Hind*III or *Sac*I at 37°C and the digested products were subjected to gel-electrophoresis in 1 % agarose and Southern-transferred to a Hybon-N nylon membrane (Amersham Bioscience) according to SAMBROOK and RUSSEL (2001). Inserts of the 5S clones were labeled, hybridized, and detected by the ECL-Direct Nucleic Acid Labelling and Detection System (Amersham Bioscience), following the instructions of the manufacturer.

RESULTS AND DISCUSSION

PCR amplification of the 5S rDNA in *S. marmoratus* generated one band of approximately 460 bp for the seven analyzed specimens. The 5S rDNA-PCR generated fragments of one of the animals were cloned and the DNA sequence was determined for five positive clones. A consensus sequence was produced based on the alignment of all the obtained sequences (Fig. 1), which enabled us to identify a repeat unit of the 5S rDNA. The used primers were designed to amplify repeat units of the 5S rDNA, including the complete NTS and the incomplete 5S rRNA gene (118 bp) missing the positions +22 and +23 (Fig. 1). Searches at DDBJ-EMBL-GenBank databases iden-

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  +24      5SA→
5′TACGCCCGATCTCGTCTTATCTCGGAAACTAAGCAGGGTCGGGCCTGGTTAGTACTTGGATGGGAGATCGCCTGGGAA-78
              HindIII
TACCAGGTGCTGTAAGCTTTTCACTTCTGTTTAGAAACAGCAGAGGGCGCCCCTGCTGCTTCAATCACCTGCTAATTTAG-158

ATGAGAGGCCGCGGATATTTGAATATCTGTTTTGTTGCTGCTTTAAACCTGTACTTGACTACATGTCCCAGATGAGTCTT-238

TCATATTGTAGTCCGTTCCATTtatataTTGATAAAACACATCAAGGGAtataAATTCATGCAAAATGGGGGTGCGGTTT-318

ATCCAGCAGACACGACCTACACGCAAGAAGAAAAAAAAAAAATTGAGCCCCGGCTCCCCCCTAACCCCTTTTCCCCAATG-398
                        SacI                    +1       ←5SB
ACCATGCATCATGTTTAAAAGAGCTCTGGGTAGACACTGCTGTCGCTTACGGCCATACCAGCCTG-463
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Fig. 1. Consensus sequence of the 5S rDNA repeats of *S. marmoratus*. The 5S rRNA gene coding sequence is in boldface type, the primer (5SA and 5SB) regions are underlined and TATA-like elements are in lower case. The restriction sites for *Hind*III (AAGCTT) and *Sac*I (GAGCTC) are underlined and in italics. The sequence is deposited in GenBank under the accession number AY271269.

tified low divergences between the 5S rRNA gene sequence described here and from those of other fish species previously studied (average similarity of 95 %). On the other hand, no relationship could be established between the NTS of the 5S rDNA of *S. marmoratus* and any sequence of other fish or vertebrate species, suggesting that this spacer region evolves rapidly. The NTS regions are presumed to be free to vary since these variants are selectively neutral or nearly neutral and can become either fixed or lost, thereby causing accentuated differences even between close related species. In contrast, most mutations in the 5S rRNA gene are presumed to be selectively neutral or nearly neutral only when they occur in a subcritical proportion (Cronn et al. 1996). Although little is known about the NTS sequence among fishes, a TATA-like sequence has been observed about 25–30 bp upstream from the 5S rRNA gene of some species (Pendás et al. 1994; Sajdak et al. 1998; Martins and Galetti Jr. 2001a; Wasko et al. 2001). Although the TATA-like elements of *S. marmoratus* were located around 180–200 bp upstream to the 5S rRNA gene (Fig. 1), such sequence may be involved with the transcription control of these genes.

To determine if the genomic organization of the 5S rDNA of *S. marmoratus* was consistent with the PCR products and sequences obtained, Southern-blot hybridization experiments were performed. Complete genomic DNA digestion with *Hind*III or *Sac*I, that cleave once in the 5S rDNA sequence (Fig. 1), showed that most of the 5S rRNA genes are organized in monomers around 460 base pairs in six analyzed specimens (Fig. 2). Faint hybridization bands around 550 bp (samples 1 and 4, Fig. 2a and 2b), 600 bp (samples 1–4, Fig. 2b) and 700 bp (samples 2 and 5, Fig. 2a) give support for the presence of 5S rDNA variant copies in the genome of *S. marmoratus*. Although a complete DNA digestion was carried out with *Hind*III and *Sac*I, some of the samples gave partially digested results showing the presence of multimeric units of the 5S rDNA. The membrane hybridization experiments also detected a polymorphism in the 5S rDNA monomer size among the sampled animals. This is clearly visible in the samples 3 and 4 of Fig. 2a and 2b. Such polymorphism can be useful for population studies in this species.

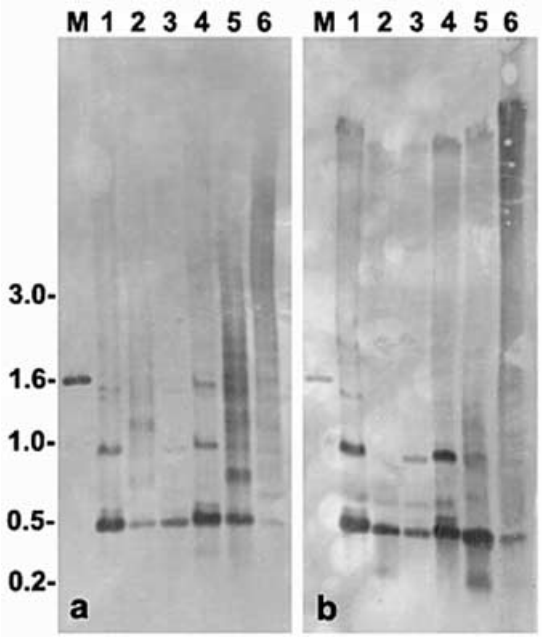

Fig. 2. Genomic organization of 5S rDNA of *S. marmoratus* determined by Southern-blot and hybridization to a 5S rRNA gene probe. Aliquots of 10 μg of genomic DNA were overnight digested with 30U of the restriction endonucleases *Hind*III (a) or *Sac*I (b). Lanes 1–6 are samples of different individuals and M indicates molecular weight markers (kb).

Table 1. *5S rDNA repeat characteristics among fish species.*

Species	Orders	5S rDNA repeat size (bp)	5S rDNA types	Reference
Acipencer sturio	Acipenseriformes	221	1	Tagliavini et al. 1999
Brycon lundii	Characiformes	228, 243	2	Wasko et al. 2001
Brycon orbignyanus	Characiformes	226, 240, 241	2	Wasko et al. 2001
Brycon microlepis	Characiformes	224, 240	2	Wasko et al. 2001
Brycon cephalus	Characiformes	347, 349, 352	1*	Wasko et al. 2001
Brycon sp.	Characiformes	340, 341, 342, 343	1*	Wasko et al. 2001
Brycon brevicauda	Characiformes	237, 238, 239, 797, 798	2	Wasko et al. 2001
Brycon insignis	Characiformes	335, 511	2	Wasko et al. 2001
Leporinus friderici	Characiformes	220, 896	2	Martins and Galetti 2001a
Leporinus elongatus, *L. cf. elongatus*, *L. obtusidens*	Characiformes	200, 900	2	Martins and Galetti 2001a
Danio rerio	Cypriniformes	180, 500	2	Gornung et al. 2000
Gasterosteus aculeatus	Gasterosteiformes	225	1	Rocco et al. 1999
Micropterus salmoides	Perciformes	311, 313, 316, 321	4	Deiana et al. 2000
Oreochromis niloticus	Perciformes	475, 1400	2	Martins et al. 2002
Coregonus artedi, *C. zenithicus*	Salmoniformes	394, 524	2	Sajdak et al. 1998
Salmo salar	Salmoniformes	255, 525	2	Pendás et al. 1994
Oncorhynchus mykiss	Salmoniformes	290, 370	2	Móran et al. 1996
Synbranchus marmoratus	Synbranchiformes	463	1	Present paper

*There are evidences for the presence of a second 5S rDNA type.

5S rDNA variants have been reported for several mammals and amphibians (Komiya et al. 1986; Little and Braaten 1989; Leah et al. 1990; Frederiksen et al. 1997), suggesting that 5S rDNA variant types have originated early in the evolutionary process of vertebrates. Variant types of 5S rDNA also have a common occurrence in fish groups (Table 1). However, *S. marmoratus* (Synbranchiformes), *Gasterosteus aculeatus* (Gasterosteiformes) and *Acipencer sturio* (Acipenseriformes) seem to present just one major 5S rDNA type. Synbranchiformes and Gasterosteiformes represent evolved lineages within the Actinopterygii and Acipenseriformes represents a very ancient group of fishes (Lauder and Liem 1983). Although variant types of 5S rDNA are common among fishes, the evolution might have maintained just one major class of 5S tandem repeats in some groups.

Particularly among fishes, variant classes of 5S rDNA within a single genome that differ extensively in their NTSs, have been identified in *Salmo salar* (Pendás et al. 1994), *Oncorhynchus mykiss* (Móran et al. 1996), species of *Coregonus* (Sajdak et al. 1998), *Leporinus* (Martins and Galetti Jr. 2001a), *Brycon* (Wasko et al. 2001) and *Oreochromis niloticus* (Martins et al. 2002) (Table 1). Although the presence of 5S rDNA variant classes seems to be a common feature for fishes (Martins and Galetti Jr. 2001b) (Table 1), the present results of membrane hybridization show that there is just one major class of 463 bp-tandem repeats of 5S rDNA in the genome of *S. marmoratus*. However, in addition to the 463-bp strong band, faint bands were detected after membrane hybridization, showing that the existence of few copies of other types of 5S rDNA-tandem repeats in the genome of *S. marmoratus* cannot be eliminated.

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