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SHORT COMMUNICATION

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An Intriguing Model for 5S rDNA Sequences Dispersion in the Genome of Freshwater Stingray *Potamotrygon motoro* (Chondrichthyes: Potamotrygonidae)[1]

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Abstract—5S rDNA genes of the stingray *Potamotrygon motoro* were PCR replicated, purified, cloned and sequenced. Two distinct classes of segments of different sizes were obtained. The smallest, with 342 bp units, was classified as class I, and the largest, with 1900 bp units, was designated as class II. Alignment with the consensus sequences for both classes showed changes in a few bases in the 5S rDNA genes. TATA-like sequences were detected in the nontranscribed spacer (NTS) regions of class I and a microsatellite $(GCT)_{10}$ sequence was detected in the NTS region of class II. The results obtained can help to understand the molecular organization of ribosomal genes and the mechanism of gene dispersion.

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Keywords: *Elasmobranchi*, *Potamotrygon*, stingray, 5S rDNA, clone, microsatellite

In eukaryotic organisms, the ribosomal DNA genes (rDNA) exist as two multigenic families, 45S rDNA and 5S rDNA both consisting of tandem repeated units with hundreds of thousands of copies separated in the genome [1]. The 5S rDNA gene repeats consist of both coding sequences of 120 bp and nontranscribed spacers (NTS) that commonly include variations in their nucleotide sequence due to insertions/deletions, mini-replications and pseudogenes [1]. The nucleotide sequence of the 5S rDNA gene coding region is highly conserved, even between unrelated species, while changes in NTSs are very common, generally species-specific [2–4] and have been successfully used in evolutionary studies [5–7].

Considering that the diversity of fish species in the Neotropical region can be examined to expose the mechanisms involved in the dynamics of this genomic segment (NTS), the study of these markes is of great interest. In this study, we extend our analysis of the 5S rDNA in the freshwater stingray *Potamotrygon motoro* to determine the organization of the two segments identified in the NTS region of the 5S site.

We analyzed specimens of the freshwater stingray *P. motoro* collected in the Upper Paraná River (Brazil) and deposited in the museum of Laboratório de Biologia e Genética de Peixes (Botucatu/SP—Brasil) (vouchers, 5202/5203/6716/6717). Genomic DNA from liver tissue samples was extracted and purified [8]. PCR for the isolation of the 5S rDNA was performed [9] using the primers 5SA (5'-TACGCCCGATCTCGTCCGATC-3') and 5SB (5'-CAGGCTGGTATGGCCGTAAGC-3'). The fragments of 5S rDNA generated by PCR were cloned in the pGEM-T plasmid (Promega) and used to transform the host *Escherichia coli* DH5a strain. The clones obtained from the 5S rDNA PCR products were sequenced on the ABI Prism 377 (Perking-Elmer) automatic sequencer with the Dye Terminator Cycle Sequencing Kit (Applied Biosystems Division, Perkin-Elmer), following the manufacturer instructions. Nucleic acid sequences were subjected to Basic Local Alignment Search Tool (BLASTN) [10]. The National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/blast) [11] was used for sequence searches. The sequences were aligned with the software Clustal W [12] implemented in the program Dambe [13]. Consensus sequences were produced manually using the BioEdit software [14].

PCR amplification of *P. motoro* genomic DNA generated two major bands corresponding to sequences of approximately 342 bp and 1900 bp, as well as a bright band of approximately 600 bp, suggesting the existence of at least two 5S rRNA gene families. The main PCR products were eluted from the gel and cloned.

Approximately 10 clones of each band were sequenced, and for the 1900-bp band the sequencing of only the flanker region was performed. Consensus sequences were developed and the GC content of the 342-bp band (class I) was 47.3 and 57% for the 912-bp

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5S  II  CAGGCTGGTATGGCGTAAGCTACGCCCGATCTCGTCCGATCTCGGAAGC  [ 50]
5S  I   .........................................C.....C.  [ 50]
5S  II  TAAGCAGGCTCAGGCCTGGTTAGTACTTGGATGGGAGACCGCCTGGGAAT [100]
5S  I   .GG...A.........C...CA.G.C.CTG.A.....T..........  [100]
5S  II  ACCAGGTGCCGTAGGCTTTTGCTGTTTTGCTGCTGCTGCTGCTGCTGCTG [150]
5S  I   T......T.T...CA..GA.A.ACG.GA.T.A-....T..C.CT..CAAA [150]
5S  II  CTGCTGCTCTGAACAGATGTCCGCAGTAGGAGCTGCTCTCTTCTTTTCAG [200]
5S  I   T...A.T.-....TTC.ATCAT.TTA.GACCA....------..C..A.. [200]
5S  II  TCCGCCTCTGACATACACTGTCGGCACTGCGCTTCTCACTCAGCCCAGAA [250]
5S  I   ---.GT...TTT.C...G.AAG.AA.TTAAA------GA.T.TGGGGA.. [250]
5S  II  ACGCAAGCACCCGGCTCCTGCTGTCAAGGCACAGGAATACAAGAATGTGC [300]
5S  I   .G...G.T.GAT.AA----....--.GCTT..G.CC....C..CC..A-- [300]
5S  II  TTTTGCGAGGCAGCCAGCTGCACCTGCAGCACTAGCGTCCTCATTGCTTC [350]
5S  I   -C..A..GCCATA.....CAGG.T.A.G..CA..C.AG...G-------- [350]
5S  II  CTCCATACCGCCGCGCATCGCCACTCGCCTACTGTCGCCTCTCCCTCAGT [400]
5S  I   ---------------------------------------------------- [400]
5S  II  CTCAGCCAGGCAGCAAGATGCAAGATGCAAGGGGCTCAAGCGTTCTTGCG [450]
5S  I   ---------------------------------------------------- [450]
5S  II  CCTCTCTTCTGCGCTTGCGCAAACCGTCCATTCTCAGTTGAGGCTTTCCT [500]
5S  I   ---------------------------------------------------- [500]
5S  II  TTGCCTTCTGAGCAGGCATCAGCAAAGGCGGCAGCTGCTTCCTGCGGCCA [550]
5S  I   ---------------------------------------------------- [550]
5S  II  AGCGGGCTGGCCGAGTATGGCACAAGCAGCTCYGAATAGCCTCTGATGGC [600]
5S  I   ---------------------------------------------------- [600]
5S  II  TGAGACTCAGCAGCCTGTTCCTGAGCGAGCCGTCGGCCTGGCAGTGGGCA [650]
5S  I   ---------------------------------------------------- [650]
5S  II  GACTCACCCAGGCCCACCCAGGTCTGAGGACGGTTAGCTCAGCTGGTCAG [700]
5S  I   ---------------------------------------------------- [700]
5S  II  AGCGTTGCTAATAACGCCAAGGTCGTGGGTTCGATCCCCATACTGTCCAC [750]
5S  I   ---------------------------------------------------- [750]
5S  II  GCCCCGCATTTCCTTTAATTTCCGCCCACTTTCGTCCAGCAGTCCAGCAC [800]
5S  I   ---------------------------------------------------- [800]
5S  II  TTCTCTGGCTATCCAATGTAGGACATGGCTGGATGGCKGGCGCTTCAGCA [850]
5S  I   ---------------------------------------------------- [850]
5S  II  GAAAAGCAGGCCCCGTGCAGTATGCAGCAGCGTAATTCACGGGAACTGAA [900]
5S  I   ---------------------------------------------------- [900]
5S  II  TGAATGAACGAA [912]
5S  I   ------------ [912]
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Aligned consensus sequences of 5S rDNA gene class I (5S I) and class II (5S II) in the species *Potamotrygon motoro*. The transcription region begins at +1 and ends at +120 (in bold). The TATA-like sequences are highlighted by rectangles. The shaded nucleotide sequence indicates a microsatellite region $(GCT)_{10}$. Dots indicate gaps, and identical nucleotides are indicated by dashes.

band (class II) (figure). Low values of genetic divergence (0.7%) were observed among the class I clones, and a similar result was found for the clones of class II (0.8%) (table).

Although the 5S rDNA gene region is highly conserved throughout evolution, it was possible to identify characteristic nucleotide substitutions for each type of repetition in the analyzed segments. Multiple reports indicate that this gene is characterized by diverse expression levels in animals, with in chickens [see 15], and plants, with in chili pepper [see 16], mainly due to variations in the interspersed NTSs.

Studies in several fish species allowed to identify various types of 5S rDNA genes organized in tandem repeats, which are characterized by marked differences in their NTSs, and the presence of two classes of 5S rDNA gene appears to be a common feature in fish. Both classes have been observed in multiple species of *Characiformes* [17], *Symbranchiformes* [18], *Perciformes* [19], *Carcharhiniformes* [20], *Rajidiformes* [21], *Milyobatiformes* [22], *Gadiformes* [3], and *Polypteri-*

Genetic distance analysis of class I and class II 5S rDNA repeat clones of *Potamotrygon motoro**

	Class I			Class II		
5S rDNA	NC	BP	GD ± SD	NC	BP	GD ± SD
P. motoro	09	342	0.007 ± 0.003	08	912	0.008 ± 0.004

* NC—number of clones; BP—number of base pairs; GD—genetic divergence; SD—standard deviation.

formes [23]. The authors point that 5S rDNA families may evolve through both concerted and/or birth-and-death processes, and that the presence of two classes of 5S rDNA genes in several non-related fish species indicates that this is a common condition for the organization of the 5S rDNA gene in the fish genome.

While NTSs seem to have no specific function, genomic segments identified as TATA-like sequences (modified as AATT and TTAA) are found in several mammals and are generally located near the NTSs. TATA-like sequences have been observed upstream of the 5S gene in the fish genome [17]. Marine rays also have TATA-like sequences along the 5S ribosomal gene [21, 24], as found in *P. motoro*, which presents tetranucleotide TATA-like sequences along its 5S ribosomal gene sequence (figure). The presence of a microsatellite $(GCT)_{10}$, inside the 5S rDNA gene region of *P. motoro* also reinforces the highly dynamic nature of the NTS region. This genomic segment is considered as a potential genetic marker that could be exploited and used for population analysis in stingrays.

The molecular organization of ribosomal genes offers a variety of useful and informative data on evolutionary and taxonomic characteristics of biological groups. The data obtained through this analysis may contribute to the understanding of the mechanisms involved in the dispersion, evolution and diversification of 5S rDNA.

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