In: Focus on Genome Research
Editor: Clyde R. Williams, pp335-363
ISSN 1-59033-960-6


*Chapter X*

# ORGANIZATION AND EVOLUTION OF 5S RIBOSOMAL DNA IN THE FISH GENOME

***Cesar Martins[1] and Adriane Pinto Wasko[2]***
Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, CEP 18618-000, Botucatu, SP, Brazil. Phone/Fax +55 14 38116264,
[1]e-mail cmartins@ibb.unesp.br; [2]e-mail: awasko@ibb.unesp.br

## ABSTRACT

In higher eukaryotes, the 5S ribosomal multigene family (5S rDNA) is tandemly organized in repeat units composed of a coding region (5S rRNA gene) and a non-transcribed spacer sequence (NTS). Although the 5S rDNA organization has been investigated in several vertebrate species, present data are concentrated in mammals and amphibians, whereas other groups, such as fishes, have been poorly studied. To further the understanding on the dynamics and evolution of 5S rDNA arrays in the vertebrate genome, recent studies have focused on the genome organization of these sequences in fish species, which represent the base group of vertebrate evolution. It was evidenced that the chromosome distribution of the 5S rDNA is quite conserved among related fish species occupying an interstitial position in the chromosomes. Although the 5S rDNA clusters have been maintained conserved in the chromosomes, changes in the nucleotide sequences and organization of the repeat units have occurred in fish species, as demonstrated by the presence of 5S rDNA variant types within and between genomes clustered in distinct chromosome environments. These variants are distributed in two major classes, suggesting that such pattern could represent a primitive condition for the fish genome, as well as for vertebrates. The 5S rDNA variant units include the presence of pseudogenes and inverted gene sequences, demonstrating that the organization of the 5S rDNA repeats in fish genome is governed by intense mechanisms of evolution. Data on the 5S rDNA repeat organization can provide interesting insights for the comprehension of (1) the genome evolution, (2) the dynamics of repetitive sequences, and (3) the practical employment of 5S rDNA as genetic markers.

## INTRODUCTION

In general, little is known about the structure and organization of fish genome - most available information in this vertebrate class is related to the structure and evolution of chromosomes. Molecular studies focusing on genes and DNA sequences of this animal group are mainly related to repetitive sequences such as satellite and ribosomal DNAs. Single copy sequences correspond to approximately 2-10% of the vertebrate genomes, while the remainder genome is composed by repetitive sequences (Franck et al. 1991, Wagner et al. 1993, Bernardi 1995). Repetitive DNA includes the tandemly-arrayed satellite, minisatellite and microsatellite sequences, and dispersed repeats such as transposons and retrotransposons (Charlesworth et al. 1994). There are also repetitive multigene families that code several important proteins such as hemoglobins, actins, histones, and ribosomal RNAs (rRNA). While the non-coding tandemly-arrayed sequences are found mainly in chromosome centromeres and telomeres, the dispersed elements are found as isolated units or as clusters distributed throughout the genome. The rRNA genes can be found clustered in one or several regions of the genome.

The ribosomal RNA is the most abundant RNA in the cell, constituting approximately 80% of the total RNA of diving cells. rRNAs represent noncoding final gene products that perform structural and catalytic functions and form the basic structure of the large and small ribosomal subunits that catalyze cellular protein synthesis. In animals, the large ribosome subunit is formed by the association of one copy of each rRNA (28S, 5.8S and 5S) with around 49 ribosomal proteins, and the small subunit is formed by the association of one 18S rRNA copy with around 33 ribosomal proteins. The synthesis of a specific protein requires multiple translation rounds of its messenger RNA molecule providing a large amount of such protein in the cell. For such purpose, adequate quantities of the ribosomal subunits are necessary and high levels of rRNA molecules are required. Large quantities of ribosomal molecules are produced just because the cell contains multiple copies of the rRNA genes that are arranged into several genomic locations.

In higher eukaryotes, tandem arrays of ribosomal RNA genes are organized in multigene families. Multigene families correspond to a common structural element of the eukaryote genomes and are defined as a set of genes derived by duplication of an ancestral gene and that display a similarity of more than 50%. They are frequently found in close linkage with each other, and have similar or overlapping functions. The head-to-tail array of the rRNA multigene families tandem repeats confers a particular dynamic to the evolution of these genome regions, making them important for the comprehension of the genome structure and evolution.

Studies on ribosomal RNA genes have been gaining prominence in a broad range of animal and plants, specially related to species or population characterization, evolutionary relationships and gene expression. In higher eukaryotes, the tandem arrays of rRNA genes are organized in two distinct multigene families (Figure 1) composed of hundreds to thousands of copies. These rRNA molecules are codified by two multigene families that are organized in two distinct repeat classes denominated 45S ribosomal DNA (rDNA) and 5S rDNA. The 45S rDNA contains the genes that code for the 18S, 5.8S and 26S-28S rRNAs (see Long and David 1980). The second ribosomal family codifies the 5S rRNA. The 18S, 5.8S and 26S-28S rRNAs are synthesized, processed and partially assembled to form the ribosome subunits in

the nucleolus. On the other hand, the 5S rRNA is synthesized elsewhere in the genome and enters the nucleolus to participate in the large ribosomal subunit assemblage. The general nucleotide sequence and mapping pattern of these two multigene families have contributed to the comprehension of the structure, organization and evolution of the genomes.

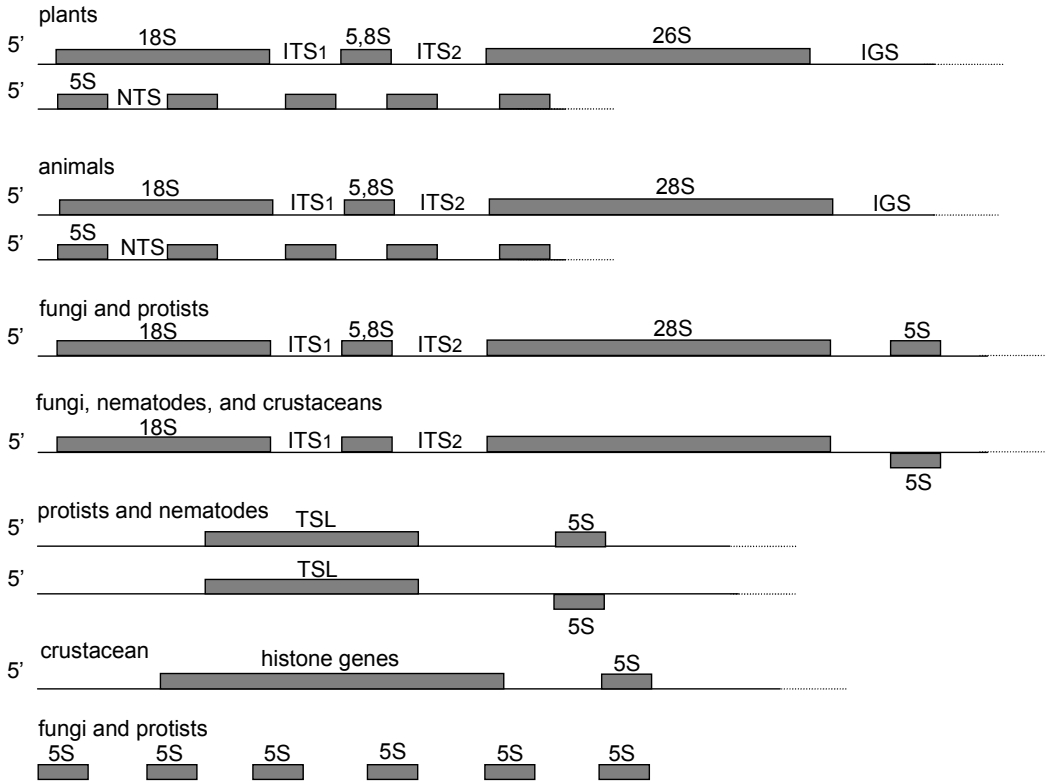


**Figure 1.** Graphical representation of the 5S rRNA genes organization and their relation to the 18S, 5,8S and 28S rRNA genes and other repeat units. The boxes represent the coding region of different genes. Boxes above the line demonstrate that the 5S rRNA gene is transcribed from the opposite strand of the double DNA helix. The 5S rRNA genes are generally organized in a distinct locus from the other rRNA genes as observed for most plant and animals, or dispersed in the genome as observed in fungi and protists. On the other hand, 5S rRNA genes can also be linked to the 18S, 5,8S and 28S rRNAs (protists, fungi, nematodes, and crustaceans), TSL (protists and nematodes) or histone genes (crustacean). ITS, internal transcribed spacer; IGS, intergenic spacer; NTS, non-transcribed spacer; TSL, trans-spliced leader.

A considerable amount of information on the structural and functional organization of the 5S rRNA genes has been gained for plants (Hanson et al. 1996, Adacchi et al. 1997, Amarasinghe and Carlson 1998, among others), mammals (Little and Braaten 1989, Leah et al. 1990, Suzuki et al. 1994) and some amphibian species (Korn 1982, Vitelli et al. 1982, del Pino et al. 1992). In most eukaryotes, the 5S rRNA genes are generally detected in distinct areas of the genome, organized as one or more tandemly repeated clusters. The number of 5S rRNA gene copies ranges from 100 to 300,000, which is usually higher than the number of the 45S rRNA genes (Hadjiolov 1985). While in *Drosophila*, for instance, there is only one cluster of about 160 5S rRNA gene copies, in *Xenopus laevis* the 5S rRNA gene clusters are

located at the telomeric region of most, if not all, chromosomes (Pardue et al. 1973). On the other hand, in the fungi *Neurospora crassa* (Selker et al. 1981) and *Schizosaccharomyces pombe* (Mao et al. 1982) and in the protist *Acanthamoeba castellanii* (Zwick et al. 1991) the 5S rRNA gene copies are dispersed all over the genome. The evolution seems to have lead to a clustered arrangement of the 5S rRNA genes in higher eukaryotes.

The organization and expression of the nuclear rRNA genes have been extensively studied for several organisms from bacteria to animals. The schemes of Figure 1 summarize the organization of the rRNA genes in the genome of eukaryotes. In eubacteria the rRNA genes coding for 16S, 23S, and 5S rRNA are usually organized in operons and are transcribed in this order by a single RNA polymerase. In contrast, eukaryotes have four different rRNA genes that are transcribed by two distinct RNA polymerases. The 18S, 5.8S, and 28S rRNA eukaryote genes are arrayed in such order to form RNA transcript units for the RNA polymerase I, and multiple copies of this units are found clustered in long direct tandem repeats. The 5S rRNA genes are transcribed by the RNA polymerase III and are also found in direct tandem repeats. The eukaryote 18S, 28S, and 5S rRNA genes are homologous to the 16S, 23S, and 5S rRNA genes of prokaryotes, respectively, whereas the 5.8S rRNA eukaryote gene is homologous to the 5' end of the eubacterial 23S rRNA gene (Gerbi 1985) (see Figure 2).

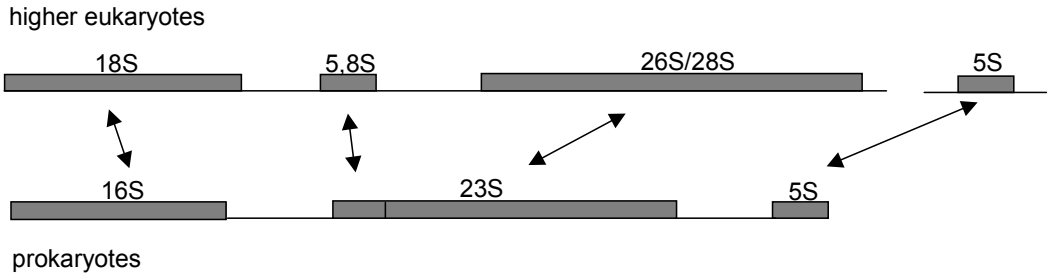


**Figure 2:** Homology of rRNA genes in prokaryotes and eukaryotes. The eukaryotic 18S, 28S, and 5S rRNA genes are homologous to the 16S, 23S, and 5S rRNA genes of prokaryotes, respectively, whereas the 5.8S rRNA gene of eukaryotes is homologous to the 5' end of the eubacterial 23S rRNA gene.

The nuclear 5S rRNA eukaryote genes are relatively independent of the other rRNA genes. Although these genes may be part of the same repeat in some species, in most plants and animals the 5S rRNA genes are generally organized into tandem arrays unlinked to the other rDNA repeat units. Moreover, the 5S rRNA genes and the other ribosomal genes are transcribed separately by distinct RNA polymerases. It seems that the distinct clustering of the 45S and 5S rDNA repeats in most studied animals and plants may facilitate the work of the different RNA polymerases as well as the independent evolution of their repeats. On the other hand, the rDNA multigene families may also assume a linked organization in the genome of several species (Figure 1). In several fungi including species of Ascomycetes, Basidiomycetes, Zygomycetes and a species of Oomycetes, the 5S rRNA genes are embedded in the non-transcribed spacer of the 45S rDNA (Belkhiri et al. 1992), whereas in other fungi such as *Neurospora crassa* (Selker et al. 1981) and *Schizosaccharomyces pombe* (Mao et al. 1982) the 5S rRNA gene copies are dispersed all over the genome and are not linked to the 45S rDNA. Also, the 5S rDNA became linked to other different tandemly repeated multigene

families during the evolution, such trans-spliced leader (TSL) and histone multigene families (Andrews et al. 1987, Drouin and Moniz de Sá 1995, Drouin 1999) (Figure 1). The discovery of 5S and 45S rDNA linkages in lower eukaryotes species has previously been interpreted as a transitional state between the typical eubacterial arrangement and the unlinked arrangement found in higher eukaryote species (Belkhiri et al. 1992). Actually, 5S rRNA genes have also been found linked to repeat units of 45S rDNA of several higher eukaryotes species, such as nematode and arthropod species (reviewed in Drouin and Moniz de Sá 1995). Although the mechanisms involved in the transposition of the 5S rRNA genes to other tandemly repeated families still have to be clearly elucidated, their subsequent spread to all members of the multigene family is believed to be related to concerted evolution by unequal crossing-over (Drouin and Muniz de Sá 1995).

In order to further the understanding on the dynamics and evolution of 5S rRNA gene arrays, a revision on the structure and organization of the 5S rDNA in the fish genome is reported here. The accumulated data have demonstrated that the presence of 5S rDNA variant classes seems to be a general trend for fish species and are a consequence of the intense dynamism of these tandem repeat elements in the genome of these animals. Studies on the 5S rDNA repeat organization can provide interesting insights for the comprehension of the genome evolution, the dynamics of repetitive sequences in the genome and, the practical employment of 5S rDNA as genetic markers.

## 5S rRNA Genes and the Non-Transcribed Spacer Sequences

The 5S rDNA array consists of multiple copies of a highly conserved 120 base pairs (bp) coding sequence, separated from each other by a variable non-transcribed spacer (NTS) (see Long and David 1980) (Figure 3). While the 5S rRNA gene is conserved even among non-related taxa (Figure 4), its NTS shows an extensive length variation, which can give an accentuated dynamism to the 5S rDNA repeats (Williams and Strobeck 1985). Searches at DDBJ-EMBL-GenBank databases identified low divergences between the 5S rRNA gene sequences among related taxa. Fishes, for example, share an average similarity of 95% in their 5S rRNA genes. On the other hand, poor or no relationships are usually established between the NTSs of different species, suggesting that this spacer region evolves rapidly. Even between closely related species (species of a same genus, for example), significant differences in their NTSs can be detected. The NTSs seem to be governed by intense mechanisms of evolution, which makes this region an important source for studies concerning the organization and evolution of multigene families and genomes and also as markers to trace recent events of evolution.

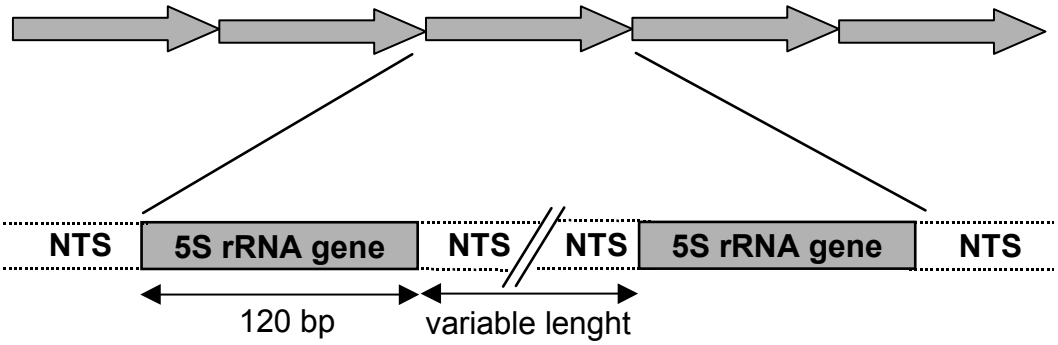


**Figure 3:** Arrangement of higher eukaryotic 5S rRNA genes intercalated with non-transcribed DNA segments (NTS). The 5S rDNA is organized in tandem repeats that form clusters with hundreds to thousands of copies. The 5S rRNA genes can be organized in one to several clusters in the haploid genome.

The 5S rRNA gene is transcribed by the RNA polymerase III that also synthesizes many other types of small RNAs, including t-RNA, 4.5S rRNA, 7SL, 7SK, U6, Alu, B1, H1 RNA, MRP RNA, Y3 RNA, BC1 RNA, VA RNA and EBERs RNA (Nederby-Nielsen et al. 1993). The 5S rRNA gene sequence contains internal control regions (ICRs) that are active as promoters for transcription (Figure 4). The A box is a general ICR sequence for RNA polymerase III. The intermediate element (IE) and the C box are specific to the 5S rRNA transcription and work as binding sites for the transcription factor TFIIIA (Pieler et al. 1987). The binding of the TFIIIA to the 5S rRNA gene is the first step in the formation of the transcription initiation complex (Veldhoen et al. 1994). The second step in the formation of the initiation complex is the binding of the polymerase III general transcription factor TFIIIC to the TFIIIA and DNA sequences in the A box. The final step is the binding of the TFIIIB, which interacts with TFIIIA and TFIIIC (Veldhoen et al. 1994).

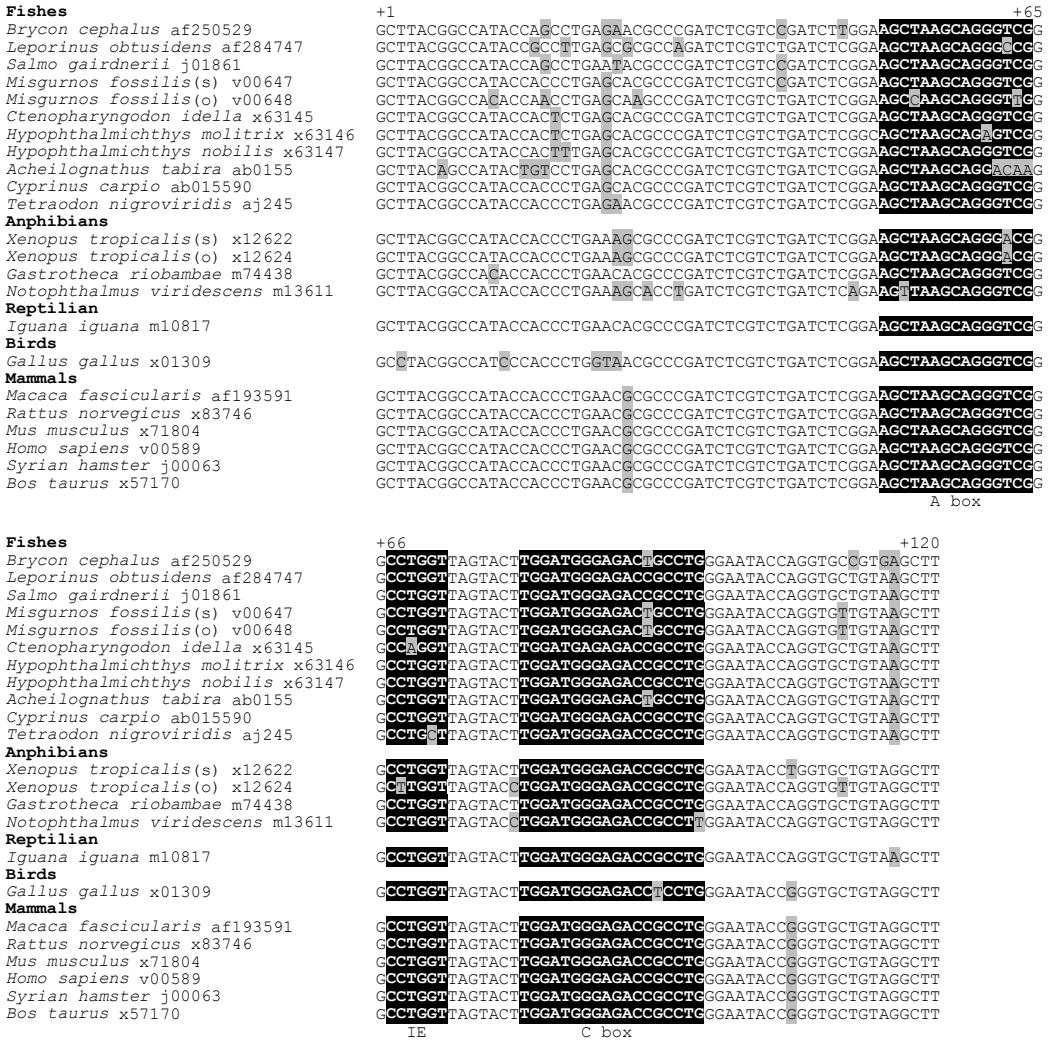


**Figure 4:** Aligned 5S rRNA gene sequences from fishes, amphibians, reptilian, birds and mammals obtained from the nucleotide sequence database available at the web site http://www.ncbi.nlm.nih.gov/. The start point of transcription is indicated by +1, the nucleotide substitutions are indicated in gray shading and the internal control regions (A box, IE and C box) are indicated in black shading. The species names are showed in the right followed by the GenBank accession entries. (s) somatic 5S rRNA gene type, and (o) oocyte 5S rRNA gene type.

Comparisons of aligned nucleotide sequences of the 5S rRNA gene from several vertebrates revealed a total of 38 polymorphic sites that were mainly present outside de ICR region, in the beginning and in the end of the gene (Figure 4). The ICR region was quite conserved among the analyzed sequences with just few base substitutions. Although the polymorphic sites were not informative to distinguish fish, amphibians, reptilian, birds, and mammals, an attempting phylogenetic reconstruction of the vertebrate history was carried out based in the available 5S rRNA gene sequences (Figure 5). However, the relationship among the vertebrate groups was not clear. It seems that the 5S rRNA gene does not represent a good candidate gene for phylogenies due to its highly nucleotide conservation and small size (only

120 bp). Although some studies have considered the usefulness of the nucleotide sequences of the 5S rDNA non-transcribed spacers as phylogenetic or population markers (Suzuki et al. 1994, Pendás et al. 1995, Baker et al. 2000), a special attention must be exercised, mainly in phylogenetic interpretations, as the 5S rDNA family shows a complex organization with the presence of paralogous copies in the genome.

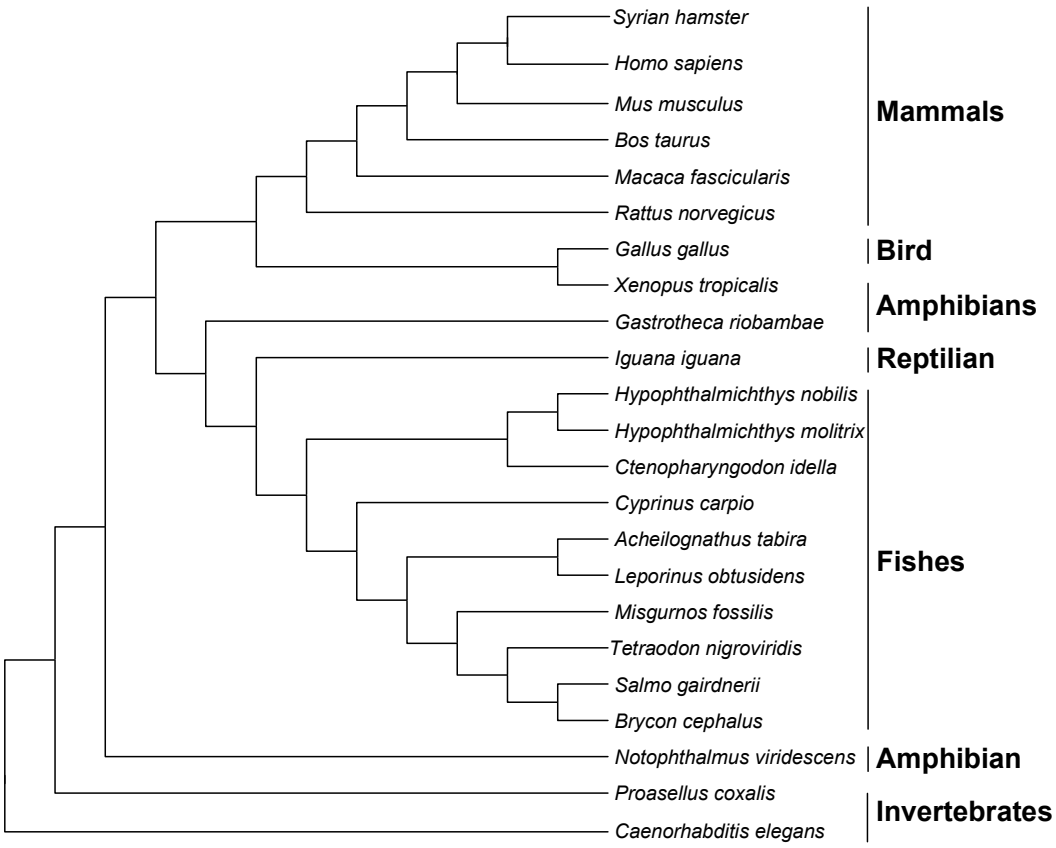


**Figure 5:** An attempting phylogenetic vertebrate reconstruction based in the 5S rRNA gene sequence. The relationship among the different vertebrate groups is not clear, showing that the 5S rRNA gene is not a good sequence for phylogenetic analysis. The crustacean *Proassellus coxallis* and the nematode *Caenorhabditis elegans* were included in the analysis as outgroups.

Although non-transcribed DNA sequences such as the NTSs seem to have no function, it has been recently demonstrated that the presence of conserved elements located inside this non-transcribed spacer also plays an important role in the regulation of the 5S rRNA gene expression in mammals (Nederby-Nielsen et al. 1993, Hallenberg and Frederiksen 2001). Studies of deletion mutants have shown that upstream control elements, i.e. present inside the NTS, are required for the expression of 5S rRNA genes. When the 5' sequence of the 5S rRNA gene is eliminated, the transcription levels are repressed and the initiation of transcription occurs a few nucleotides downstream the +1 site, suggesting that this sequence is also required for the 5S rRNA gene expression and for the correct positioning of RNA polymerase III (Sollner-Webb 1988). Additionally, recent studies have also focused interest

on non-transcribed spacer sequences that exert additional levels of control in a variety of genes transcribed by the RNA polymerase III. Among mammals, a conserved 5'-GGCTCTTGGGGC-3' sequence, denominated D box, located inside the 5S rRNA NTS, was found to stimulate the transcription up to 10-fold (Nederby-Nielsen et al. 1993, Jensen and Frederiksen 2000, Hallenberg and Frederiksen 2001). Another NTS sequences - TATA motifs - act as promoter elements that direct the transcription by RNA polymerase II of a large subset of proteins-encoding genes in eukaryotes. Polymerase III promoters have historically been characterized in three major types: (i) 5S rRNA and (ii) tRNA genes that utilize internal non-TATA promoters, and (iii) U6 snRNA promoters that contain upstream TATA elements (Paule and White 2000).

It was already demonstrated that TATA motifs upstream tRNA and 5S rRNA genes of *Schizosaccharomyces pombe* are obligatory involved in the expression of these genes (Hamada et al. 2001). A conserved TATA-like sequence has been observed upstream the 5S rRNA gene in several fishes, as *Salmo salar* (Pendás et al. 1994), *Carassius auratus* (Murakami and Fugitani 1998), *Coregonus* (Sajdak et al. 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. 2001) and *Leporinus* (Martins and Galetti 2001a), suggesting a possible influence in the transcription level of this gene (Figure 6). The presence of TATA motifs upstream the 5S rRNA genes of fishes and other higher eukaryotes suggests that more organisms than fission yeasts also present NTS promoters for the polymerase III.

```
Leporinus obtusidens (af284747)                GGACTTATATATAGGCAATTGATATA -27
Brycon cephalus (af250529)           CGAAGGTTGAATTTGCTTTATATCTTTTTGCTTTATA -70
Synbranchus marmoratus (ay271269)  CCGTTCCATTTATATATTGATAAAACACATCAAGGGATATA -152
Salmo salar                          ACTTTGACTATATATGTTGTAGCAAGAGATTGTTTCTC -1
Oncorhynchus mykiss                ACAGCTGTTAATCAGGCTCACTTTGACTTTACATGGTGCA -18
```

**Figure 6:** Sequence comparison of upstream regions of the 5S rRNA genes from fish species. TATA-like regulatory elements are indicated in boldface type.

A functional eukaryote 5S rRNA gene also requires a terminator sequence composed of at least four thymidine residues, as evidenced in *Xenopus* (Korn and Brown 1978). A T-rich tail, identified at positions +119 to +122, was also observed in different fish species (Figure 7). Moreover, such termination signal is also found in other genes transcribed by RNA polymerase III. Besides the T-rich region to signalize the end of the 5S rRNA gene, it is possible to detect another conserved region (GAAACAA) downstream the 5S rRNA gene of some fish species (Figure 7). This region could also act as a terminator sequence.


**Figure 7:** Sequence comparison of downstream regions of the 5S rRNA genes from fish species. The 3' end-coding region is indicated in boldface type and the T-rich terminal signal is indicated in gray shadowing. A downstream conserved region (GAAACAA) is also indicated in black shadowing.

## GENOME ORGANIZATION OF 5S RDNA TANDEM REPEATS

A repeated structure consisting of two different repeats of the 5S rDNA was reported for mammals (Hallemberg et al. 1994, Frederiksen et al. 1997, Jensen and Frederiksen 2000). Similar situation was also described for several fish species, such as *Salmo salar* (Pendás et al. 1994), *Oncorhynchus mykiss* (Móran et al. 1996), the genus *Coregonus* (Sajdak et al. 1998), and the genus *Brycon* (Wasko et al. 2001). A more detailed study on the 5S rDNA structure and organization was conducted in several fish species of the South American genus *Leporinus* (Martins and Galetti 2001a) and in the Nile tilapia, *Oreochromis niloticus* (Martins et al. 2002), which clearly evidenced and characterized distinct intraspecific 5S rDNA repeats. In the characiform *Leporinus*, two 5S rDNA classes, one consisting of monomeric repeat units around 200 bp and another one with monomers of 920 bp, were identified (Martins and Galetti 2001a). Each of these different-sized 5S rDNA classes was characterized by distinct NTS sequences and few base substitutions in the 5S rRNA genes and was clustered in distinct chromosome pairs (see details in the topic "Chromosomal distribution of 5S rDNA loci"). Similarly, in the tilapiine cichlid fish *O. niloticus,* two distinct 5S rDNA units (designated 5S rDNA type I with 1400 bp, and 5S rDNA type II with 470 bp) were also identified (Figure 8) (Martins et al. 2002).

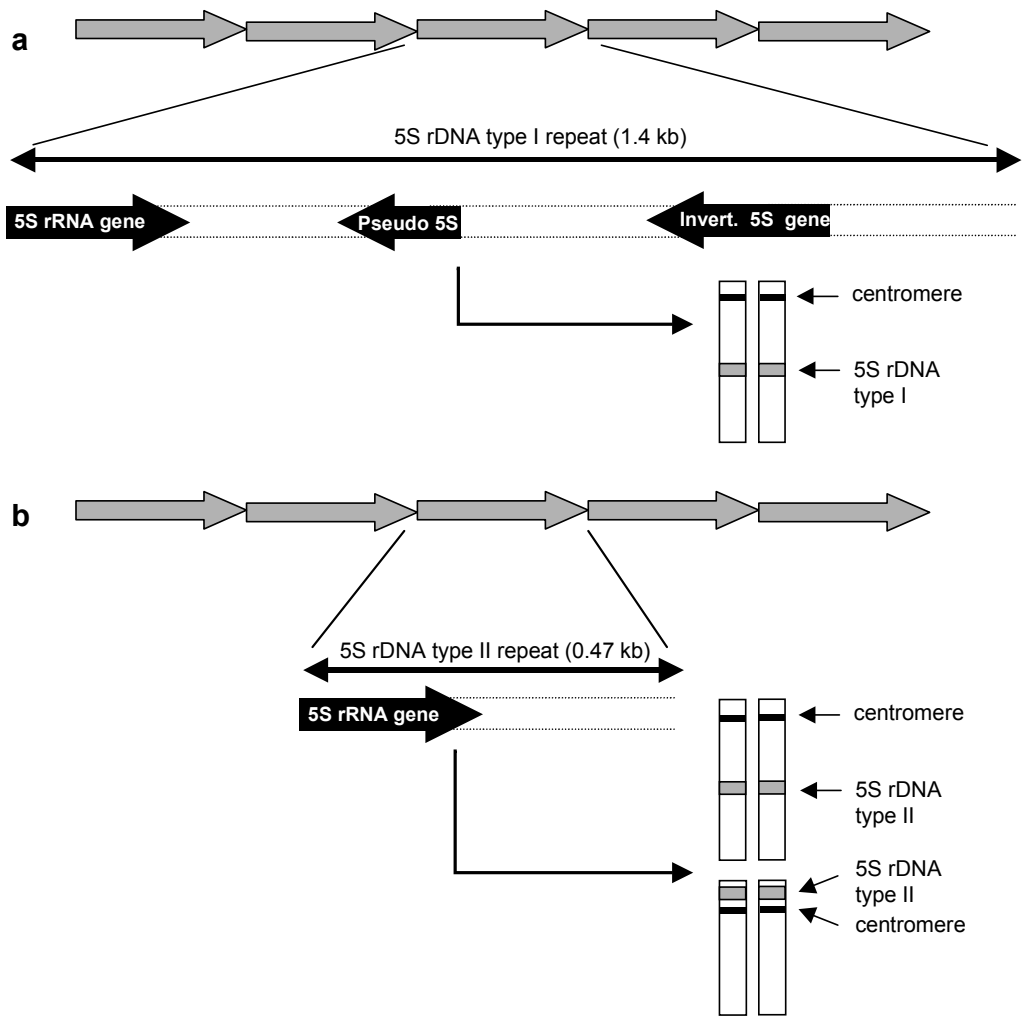


**Figure 8:** Scheme of the tandem repeats organization of distinct 5S rDNA classes in *Oreochromis niloticus*, according to Martins et al. (2002): 5S rDNA type I (a), and 5S rDNA type II (b). Large dark arrows indicate the 5S rRNA gene, an inverted 5S rRNA pseudogene, and an inverted 5S rRNA gene. The 5S rDNA chromosome loci are represented below and on the right of the DNA sequence scheme. Dark bands represent the chromosome centromeres. Light bands indicate the 5S rDNA chromosome localization. The 5S rDNA is located in three chromosome pairs: the NTS type I is localized in the largest subtelocentric-acrocentric pair, and the NTS type II is localized in two other chromosome pairs that correspond to the second and third largest subtelocentric-acrocentric ones.

The 5S rDNA structure in the Nile tilapia genome represents a good example of the complex organization of this repeated element in fishes (Figure 8). Each 5S rDNA class of *O. niloticus* was characterized by a distinct NTS that varied in nucleotide sequence and length between the loci. Moreover, an inverted putative 5S rRNA pseudogene and an inverted 5S rRNA gene sequence were detected within the 5S rDNA type I repeat. As inverted 5S rRNA genes have been reported only within the 45S rDNA array of some non-related eukaryotes (Drouin and Muniz de Sá 1995), the inverted 5S rRNA gene detected in *O. niloticus* corresponds to the first description of such sequence inside a 5S rDNA array. This inverted

gene and the 5S rRNA gene observed in the 5S rDNA type I of *O. niloticus* were very similar, presenting 120 bp and just two nucleotide substitutions (Figure 9). Moreover, a conserved short-sequence was identified at the 3'-end of the 5S rRNA gene and at the 5'-end of the inverted 5S rRNA gene, indicating that the inverted gene seems to have been originated from the 5S rDNA type I through the occurrence of an inversion. The presence of TATA-like elements downstream the 5S rRNA inverted gene of *O. niloticus* also suggests that this inverted segment could be transcriptional active, since similar TATA-like nucleotide regions were also observed upstream the 5S rRNA gene of both 5S rDNA classes of the species and that these sequences seem to play an important role in the regulation of 5S rRNA gene expression.

```
                        +1                                                          +60
Tilapia 5S typeI        GCTTACGGCCATACCAGCCTGAACACGCCCGATCTCGTCCGATCTCGGAAGCTAAGCAGG
Tilapia 5S typeI inv    GCTTACGGCCATACCAGCCTGAATACGCCCGATCTCGTCCGATCTCGGAAGCCAAGCAGG
Tilapia 5S typeII       GCTTACGGCCATACCAGCCTGAATACGCCCGATCTCGTCCGATCTCGGAAGCTAAGCAGG
Tilapia 5S pseudogene   GCTTACGGCCATACCAGCCTG----------ATTTCATCTGATCTCTGAAGCTAAGCGGG
Carp 5S pseudogene      ------------------------------------ATCTCTGAATGTTAGCAGG
Xenopus 5S pseudogene   GCCTATAGCCACACTACCCTGAAAGTGCCTGCTCTCGTCTGATCTGTGAAGTGATACAGG
Mouse 5S Pseudogene     GCCTATGGCCATACCACCCTGAATGAGCCTGATTTCATCTGATCTCATAAGCTAAGCAGG
Human 5S Pseudogene     GCCTATGGCCATACCACCCTGAATGAGCCTGATTTCATCTGATCTCATAAGCTAAGCAGG

                        +61                                                         +120
Tilapia 5S typeI        GTCGGGCCTGGTCAGTACTTGGATGGGAGACCGCCTGGGAATACCAGGTGCTGTAAGCTT
Tilapia 5S typeI inv    GTCGGGCCTGGTCAGTACTTGGACGGGAGACCGCCTGGGAAAACCAGGTCATGTAAGCTT
Tilapia 5S typeII       GTCGGGCCTGGTCAGTACTTGGATGGGAGACCGCCTGGGAATACCAGGTCCTGTAAGCTT
Tilapia 5S pseudogene   GGCGGTCCTAGATAGTA-------------------------------------------
Carp 5S pseudogene      TTTGGGCCTGGTTAGTACATGGATGGGAGACTGCCTGGGAATACCAGGTGCTTTAAACTT
Xenopus 5S pseudogene   GGCAGGCCTGGTTAGTACCTGGATGGGAGACCGCCTGAGAA------GTTTTCAAAGCTT
Mouse 5S pseudogene     GTTGGGCCTGGTTAGTACTTGGATGGGAGACC----------ACCGGGTGCTGAAGGCTT
Human 5S pseudogene     GTTGGGCCTGGTTAGTACTTGGATGGGAGACC----------ACCGGGTGCTGAAGGCTT
```

**Figure 9:** Alignment of the 5S rRNA genes type I and type II, the inverted 5S rRNA pseudogene, and the inverted 5S rRNA gene of the Nile tilapia, the pseudogene of the Japanese silver crucian carp, and the mouse, human and *Xenopus* pseudogenes. The nucleotide substitutions are indicated in gray shadowing and hyphens represent gaps. The start point of transcription is indicated by +1.

5S rDNA variants and pseudogenes seem to be common in mammals (Emerson and Roeder 1984, Doran et al. 1987, Leah et al. 1990, Hallenberg et al. 1994) and have already been isolated from rat, mouse and human cells (Nederby-Nielsen et al. 1993). Among fish, 5S rRNA pseudogenes have only been described for the Japanese silver crucian carp *Carassius auratus langsdorfi* (Murakami and Fujitani 1998) and *Oreochromis niloticus* (Martins et al. 2002). The isolated 5S rRNA pseudogene of *O. niloticus* is very similar to the 5S rRNA gene of the species, differing by some base substitutions and deletion of part of the gene, and also shows a relatively great similarity to mouse and human 5S rDNA pseudogenes (Leah et al. 1990, Hallenberg et al. 1994). However, a lower similarity was observed between the 5S rRNA pseudogene of the Nile tilapia and the silver crucian carp (Figure 9). The reasons concerning the greater similarity among the 5S rRNA pseudogene of tilapia, mouse and human are not clear. Pseudogenes are a consequence of gene duplication that can occur in two fundamentally different ways: by transposition or via genomic DNA duplication (Mighell et al. 2000). Once the *O. niloticus* 5S rRNA pseudogene is in the same direction of the inverted 5S rRNA gene of the species, this element might have arisen as a consequence of the mechanisms involved in the inversion of the 5S rRNA gene. The organization of the 5S

rDNA in tandem repeats probably facilitates the origin of such elements by duplication of genomic segments. As the 5S rRNA pseudogene of *O. niloticus* is maintained in the tandem repeat copies of the 5S rDNA type I, it is suggested that this stretch of DNA might have arisen at the same time as the differentiation of both 5S rDNA types (Martins et al. 2002). Whether the inverted 5S rRNA gene and the pseudogene of *O. niloticus* are transcribed or not remains to be elucidated.

No mosaicism was observed in the organization of the two 5S rDNA classes detected in *Oreochromis niloticus* (Martins et al. 2002). Similar data were also observed in fish species of the genus *Leporinus* (Martins et al. 2002). It has been believed that multigene families evolve according to homogenization processes governed by molecular drive and concerted evolution (Dover 1986, Elder and Turner 1995), resulting in a sequence similarity of the repeat 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 and Galetti 2001a).

Present data on fish species suggest that the presence of two different 5S rDNA arrays could be a common feature in the genome of this vertebrate group. Two types of tandem repeats were observed for several Characiformes (Martins and Galetti, 2001ab, Wasko et al. 2001), Perciformes (Martins et al. 2000, 2002), Salmoniformes (Pendás et al. 1994, Morán et al. 1996, Sajdak et al. 1998), and Cypriniformes (Gornung et al. 2000) (Table 1). Moreover, the NTS data of different fish species showed that the minimum NTS length size described for these organisms is 60-80 bp. The shortest 5S rDNA repeat unit (around 180 bp) was observed in the characiform *Steindachnerina elegans*, suggesting the occurrence of a NTS with 60 bp in this species (Martins and Galetti 2001b). Several *Leporinus* species evidenced a 80 bp NTS (Martins and Galetti 2001a). 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 fish genome, as this may contain required sequences for the expression/regulation of the 5S rRNA genes. The investigation of the nucleotide sequence of the short-NTS pattern and the expression of 5S rRNA genes in fishes such as the genus *Steindachnerina* represents a promising strategy in the understanding of the role of the NTS for the maintenance of the 5S rDNA repeat units.

Two main families of 5S rRNA genes differently expressed in oocytes and in somatic cells have been described for several animals (Komiya et al. 1986). This dual system is well known in amphibians (reviewed by Krämer 1985). Although these 5S rDNAs present a great sequence similarity in the coding region, their NTSs are very distinct. In *Xenopus laevis*, for example, the oocyte unit is about 750 bp and includes the 120 bp 5S rRNA gene, a non-transcribed spacer and a pseudogene (Carrol and Brown 1976, Jacq et al. 1977), while the somatic unit has approximately 880 bp and does not contain pseudogenes (Peterson et al. 1980). Oocyte and somatic 5S rRNAs, that differ in few nucleotide positions, were also identified in the teleost fishes *Tinca tinca* (Dennis and Wegnez 1977) and *Misgurnus fossilis* (Mashkova et al. 1981). Although oocyte and somatic 5S rRNAs were described just for two fish species, the two 5S rDNA classes observed for several fish (Table 1) could be related to this dual system, appearing to represent paralogous copies of the 5S rRNA gene that may have evolved on separate regions of the genome. This dual system could also be organized in different 5S rDNA arrays with distinct chromosome locations as identified in the fishes of the genus *Leporinus* (Martins and Galetti 2001a) and in *Oreochromis niloticus* (Martins et al. 2002).

**Table 1. Characteristics of the 5S rDNA repeats in fish species.**

| Species | Orders | 5S rDNA repeat size (bp) | Number of 5S rDNA types | References |
|---|---|---|---|---|
| *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 |
| *Gobius niger* | Perciformes | 620 | 1 | Mandrioli et al. 2001 |
| *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 | Pendas et al. 1994 |
| *Oncorhynchus mykiss* | Salmoniformes | 290, 370 | 2 | Móran et al. 1996 |
| *Synbranchus marmoratus* | Synbranchiformes | 465 | 1 | Messias et al. submitted |

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

Further studies on several members of different fish orders should be carried out in order to improve 5S rDNA data on 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. The functional role of the genes present in these different 5S rDNA classes also seems to be an interesting point to be investigated.

## CHROMOSOMAL DISTRIBUTION OF 5S RDNA LOCI

The chromosomal localization of the 5S rRNA genes in fishes has shown to be of great importance in the comprehension of the structure and organization of repeated sequences in their chromosomes. Although cytogenetic analyses represent the great portion of 5S rDNA

data in fish, little is known about the chromosomal location of these genes in this animal group, compared to other vertebrate classes (Martins and Galetti 2001b).

In 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 et al. 1996, Mäkinem et al. 1997). In amphibians (Schmid et al. 1987, Lucchini et al. 1993) and fish species (Fujiwara et al. 1998, Murakami and Fujitani 1998, Martins and Galetti 1999), however, both 45S and 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 and Galetti 1999). However, the 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 et al. 1996).

**Table 2. Chromosomal organization of 5S rDNA among fish species.**

| Orders and species | Number of 5S rDNA loci | Chromosome position | Syntenic 5S and 45S rDNA | References |
|---|---|---|---|---|
| **Acipenseriformes** | | | | |
| *Acipenser naccarii* | 4 | interstitial telomeric | | Fontana et al. 1999 |
| *Acipenser ruthenus* | 2 | telomeric | positive | Fontana et al. 1999 |
| *Acipenser sturio* | 2 | interstitial | positive | Tagliavini et al. 1999 |
| *Acipenser stellatus* | 2 | | positive | Fontana et al. 2003 |
| *Acipenser baerii* | 4 | | positive | Fontana et al. 2003 |
| *Acipenser transmontanus* | 4 | | positive | Fontana et al. 2003 |
| *Huso huso* | 2 | | positive | Fontana et al. 1998, 2003 |
| **Anguilliformes** | | | | |
| *Anguilla anguilla* | 2 | interstitial | negative | Martínez et al. 1996 |
| *Anguilla rostrata* | 2 | interstitial | negative | Nieddu et al. 1998 |
| **Salmoniformes** | | | | |
| *Coregonus artedti* | 2 | interstitial | negative | Sajdak et al 1998 |
| *Coregonus zenithicus* | | interstitial | negative | Sajdak et al 1998 |
| *Coregonus lavaretus* | 2 | interstitial | negative | Jankun et al. *in press* b |
| *Coregonus peled* | 2 | interstitial | negative | Jankun et al. *in press* b |
| *Coregonus albula* | 2 | interstitial | negative | Jankun et al. *in press* b |
| *Hucho perryi* | 2 | interstitial | negative | Fujiwara et al. 1998 |
| *Oncorhynchus masou* | 8 | interstitial | positive | Fujiwara et al. 1998 |
| *Oncorhynchus mykis* | 4-6 | interstitial | positive | Móran et al. 1996 |
| *Salmo salar* | 3-4 | interstitial | positive | Pendás et al. 1994 |
| *Salmo trutta* | 2 | interstitial | negative | Móran et al. 1996 |
| *Salvelinus fontinalis* | 2 | interstitial | negative | Fujiwara et al. 1998 |
| *Salvelinus malma* | | | | Phillips et al. 2002 |

| | | | | |
|---|---|---|---|---|
| *Salvelinus confluentes* | | | | Phillips et al. 2002 |
| *Salvelinus alpinus* | | | | Phillips et al. 2002 |
| *Salvelinus namaycush* | | | | Phillips et al. 2002 |
| *Thymallus thymallus* | 6-7 | interstitial | negative | Jankun et al. *in press* a |
| | | | | |
| **Cypriniformes** | | | | |
| *Acheilognathus tabira* | 4 | interstitial telomeric | positive | Inafuku et al. 2000 |
| *Carassius auratus langsdorfi* | 2- | | | Murakami and Fugitani 1998 |
| *Cyprinus carpio* | 4 | interstitial | negative | Inafuku et al. 2000 |
| *Danio rerio* | 2 | interstitial | positive | Phillips and Reed 2000 |
| *Rhodeos ocellatus* | 2 | interstitial | negative | Kikuma et al. 2000 |
| | | | | |
| **Characiformes** | | | | |
| *Astyanax altiparanae* | 2 | interstitial | positive | Almeida-Toledo et al. 2002 |
| *Astyanax lacustris* | 2 | interstitial | positive | Almeida-Toledo et al. 2002 |
| *Astyanax fasciatus* | 4 | interstitial | positive/ negative | Almeida-Toledo et al. 2002 |
| *Astyabax schubarti* | 4 | interstitial | positive/ negative | Almeida-Toledo et al. 2002 |
| *Astyanax scabripinnis* | 4 | interstitial | positive/ negative | Almeida-Toledo et al. 2002 |
| *Astyanax scabripinnis* | 8 | interstitial | negative | Ferro et al. 2001 |
| *Brycon lundii* | 4 | interstitial | negative | Wasko et al. 2001 |
| *Brycon microlepis* | 4 | interstitial | negative | Wasko et al. 2001 |
| *Brycon orbignyanus* | 4 | interstitial | negative | Wasko et al. 2001 |
| *Brycon cephalus* | 4 | interstitial | negative | Wasko et al. 2001 |
| *Brycon* sp. | 4 | interstitial | negative | Wasko et al. 2001 |
| *Brycon brevicauda* | 4 | interstitial | negative | Wasko et al. 2001 |
| *Brycon insignis* | 4 | interstitial | negative | Wasko et al. 2001 |
| *Hoplias malabaricus* | 2 | | negative | Born and Bertollo 2000 |
| *Leporinus* cf. *elongatus* | 4 | interstitial | negative | Martins and Galetti 2001a |
| *Leporinus elongatus* | 4 | interstitial | negative | Martins and Galetti 1999 |
| *Leporinus friderici* | 4 | interstitial | negative | Martins and Galetti 1999 |
| *Leporinus obtusidens* | 4 | interstitial | negative | Martins and Galetti 1999 |
| *Leporinus reinhardti* | 4 | interstitial | negative | Martins and Galetti 2001a |
| *Parodon hilarii* | 4 | interstitial | negative | Vicente et al 2001 |
| *Parodon tortuosus* | 4 | interstitial | negative | Vicente et al 2001 |
| *Parodon* sp. | 4 | interstitial | negative | Vicente et al 2001 |
| *Schizodon altoparanae* | 4 | interstitial | negative | Martins and Galetti 2000 |
| *Schizodon borelli* | 4 | interstitial | negative | Martins and Galetti 2000 |
| *Schizodon isognathum* | 4 | interstitial | negative | Martins and Galetti 2000 |
| *Schizodon knerii* | 4 | interstitial | negative | Martins and Galetti 2000 |
| *Schizodon nasutus* | 4 | interstitial | negative | Martins and Galetti 2000 |
| *Schizodon vittatus* | 4 | interstitial | negative | Martins and Galetti 2000 |

| | | | | |
|---|---|---|---|---|
| **Perciformes** | | | | |
| *Coris julis* | 4 | telomeric | positive | Mandrioli et al. 2000 |
| *Chromis insolata* | 4 | telomeric | negative | Molina and Galetti 2002 |
| *Chromis multilineata* | | | | Molina and Galetti 2002 |
| *Chromis flavicauda* | 4 | interstitial | negative | Molina and Galetti 2002 |
| *Ephinephelus marginatus* | 2 | interstitial | negative | Sola et al. 2000 |
| *Gobius niger* | 2 | interstitial | negative | Mandrioli et al. 2001 |
| *Micropterus salmoides* | 2 | interstitial | negative | Deiana et al. 2000 |
| *Oreochromis niloticus* | 6 | interstitial | negative | Martins et al. 2002 |
| | | | | |
| **Tetraodontiformes** | | | | |
| *Tetraodon fluviatilis* | 2 | telomeric | negative | Mandrioli and Manicardi 2001 |
| *Tetraodon nigroviridis* | 2 | interstitial | negative | Fischer et al. 2000 |

In eukaryotes, the 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 and 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 translocations between the 45S and 5S arrays (Martins and Galetti 1999). This could explain why most vertebrates have these rDNA clusters on distinct chromosomes.

The chromosomal localization of the 5S rRNA genes has been described for 67 fish species representing distinct groups, such as Acipenseriformes, Anguilliformes, Cypriniformes, Characiformes, Salmoniformes, Perciformes, and Tetraodontiformes (Table 2). These previous data have shown that the location of the 5S rRNA genes corresponds to an interstitial position in the chromosomes of almost all the analyzed species (Figure 10). 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 et al. 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.

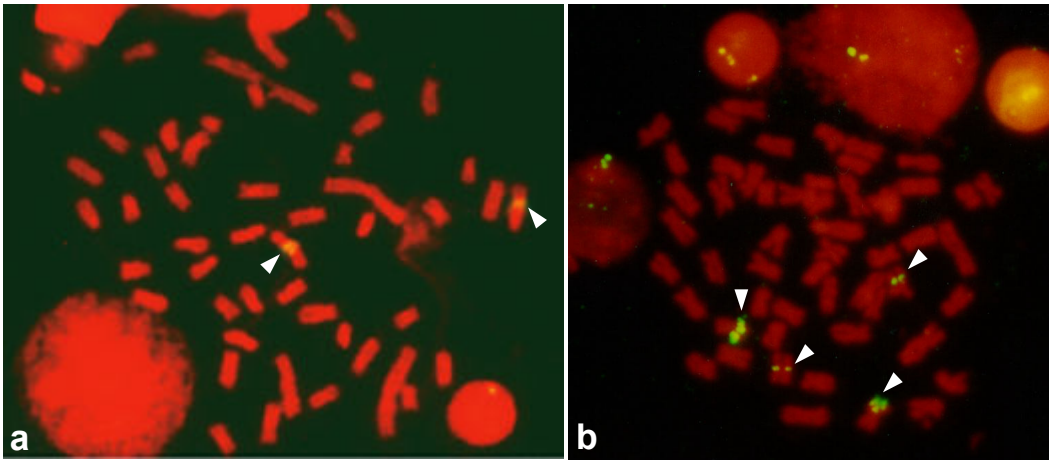


**Figure 10:** Metaphase spreads of *Brycon cephalus* (a) and *Leporinus elongatus* (b) showing 5S rRNA gene sites (arrows). These two species are representatives of the interstitial distribution of the 5S rRNA genes in fish chromosomes. The metaphase chromosomes were hybridized to a 5S rRNA gene probe obtained from *Leporinus* cf. *elongatus* (GenBank accession number AF284741) that was labeled with biotin and detected with FITC (fluorescein isothiocyanate). The chromosomes were counterstained with propidium iodide.

In the anostomid fishes, the 5S rRNA genes are clustered in two chromosomal loci (Martins and Galetti 1999, 2000, 2001a). These 5S rRNA chromosomal sites have been maintained conserved during the karyotype diversification of this fish group, once homeologous chromosomes nesting the 5S rDNA were detected in *Leporinus*, *Schizodon* and *Leporellus* (Martins and Galetti 1999, 2000, Aguilar 2001). Moreover, the two chromosome loci detected in Anostomidae seem to be located at homeologous chromosomes in *Parodon* (Parodontidae) (Vicente et al. 2001, Centofante et al. 2002) and *Prochilodus* (Prochilodontidae) (Hatanaka 2001), showing that events of chromosome evolution have maintained the 5S loci conserved in related fish groups.

Similar situation was observed in the characid fish *Astyanax scabripinnis*. Despite the fact that this species has been characterized by an accentuated chromosome diversity, including variations in the 45S rDNA sites (Mantovani et al. 2000), the 5S rDNA chromosome sites were conserved among several analyzed populations of the species (Mantovani et al. *in preparation*). Conserved 5S rDNA loci were also detected for other species of the genus *Astyanax* (Almeida-Toledo et al. 2002), showing that a conservative pattern for the 5S rDNA chromosome distribution seems to be a general trend for fishes. These results could be related to the chromosome site position of the different rDNA classes - while 5S rDNA sites were detected in an interstitial position in the chromosomes of almost all studied fish, the 45S rDNA was located at a terminal position (Table 2). The terminal chromosome regions containing the 45S rDNA are usually heterochromatic, which facilitate transposition events leading to the dispersion of these segments in the genome. On the other hand, the 5S internal chromosome sites seem to be protected from such events.

The conservation of the 5S rDNA chromosomal sites *versus* the variation in the 45S rDNA observed in fishes also seems to be a general trend for this vertebrate group. Once the 45S rDNA is located in the terminal regions of the chromosomes and the 5S rDNA is interstitially located in most fish species so far studied, the internal chromatid environment

seems to be protect from events such as unequal exchange that could act in the dispersion of sequences that are located in the terminal position of the chromosomes. According to Schweizer and Loidl (1987), telomeric regions are propitious to genetic material transference due to their proximity within interphase nucleus, promoted by the ordering of chromosomes according to Rabl's model. For these reasons, the conservation of the 5S rDNA distribution pattern may derive from the interstitial localization of these sites in the chromosomes.

As previously discussed, different 5S rDNA classes were identified in fish species (Table 1). In the genus *Leporinus*, each of the two 5S rDNA chromosome loci is correlated to an specific repeat unit that is characterized by a distinct NTS sequence (Martins and Galetti 2001a). Two 5S rDNA types were also detected in the Nile tilapia *O. niloticus*. However, this species present three 5S rDNA chromosome loci - one of the 5S rDNA types occupy two chromosome loci, while the other one occupy the third chromosome locus (Martins et al. 2002). Evidences of the presence of distinct 5S rDNA tandem repeats located at different chromosome loci were also observed in seven species of the genus *Brycon* (Wasko et al. 2001). Several organisms have a unique rDNA variant on different chromosomes and, at least for primates, the occurrence of non-homologous chromosome exchange seems to be a mechanism of such homogenization (Williams and Strobeck 1985). The distinct 5S rDNA arrays detected in *Leporinus* and *O. niloticus* 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 and Earnshaw 1998) - chromosomes bearing distinct 5S rDNA clusters seem to be evolving independently in individual nuclear environments.

## EVOLUTION OF THE 5S RDNA TANDEM REPEATS

The repetitive tandem nature of the 5S rDNA is governed by particular patterns of evolution such as unequal exchange, transposition, RNA mediated transposition and gene conversion that lead to a co-evolution of all the members of the multigene family (Figure 11). This phenomenon, known as concerted evolution (Arnheim 1983), prevents an independent evolution of each member of a multigene family, maintaining a high degree of homology between the duplicated copies. The mechanisms that act in the concerted evolution have been termed "molecular drive" (Dover 1986). Although there are several mechanisms that have been proposed to drive homogeneity among gene families, gene conversion and unequal exchange are the major contributors to molecular drive, once they have proved to occur in meiosis as well as in mitosis (Crease and Lynch 1991). Unequal exchange occurs when there is not complete alignment between two chromosomes. One chromosome will gain extra genetic material while the other will lose DNA. When a mutation occurs in one member of a multigene family, the variant can be lost or maintained. If the variant is not lost, unequal exchange can increase the copy number of this variant in the multigene family. This new member of the multigene family can spread through a population by several evolutionary ways such as natural selection, genetic drift, migration and bottleneck effect. The mutation can also be lost or spread by gene conversion, which starts with two slightly different gene copies and ends with identical copies. This process involves an invasion of the double helix of one gene copy by a single strand of the other gene. During DNA elongation and mismatch

repair, the single strand can act as template for the duplication of the strand of the other double helix. In fact, one gene in converted in the other gene.

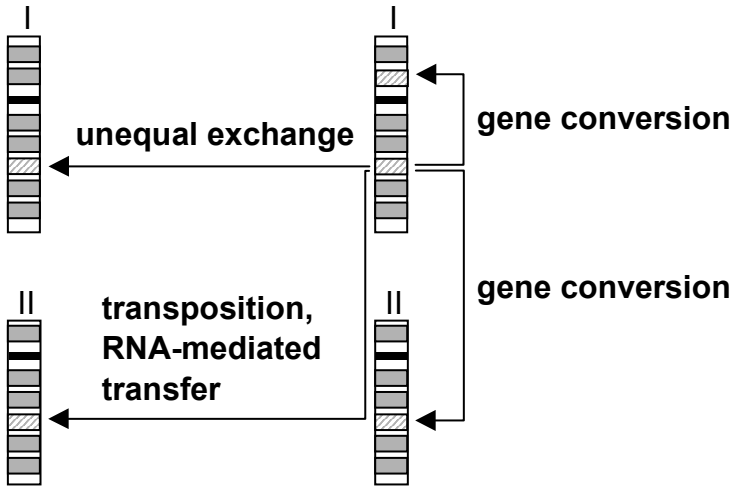


**Figure 11:** Evolutionary dynamics of the repetitive sequences in the genome. I and II represent different chromosome pairs with their homologous representatives.

Recent studies on the 5S rDNA tandem organization in fish genome evidence a partially distinct concept for the concerted evolution of the multigene family members. Whereas the literature had showed that multigene families are governed by particular mechanisms of evolution that homogenize the repeats in the genome, as described above, the results obtained with the 5S rDNA tandem repeats in *Leporinus* and *O. niloticus* evidence that the homogenization can occur just within a specific locus whereas different loci in the same genome can be highly differentiated in the nucleotide sequence and size of the repeat units.

## APPLICATIONS OF 5S RDNA AS GENETIC MARKERS

Several methods based mainly on proteins separation by electrophoresis or high-performance liquid chromatography have been developed for fish species identification (Sotelo et al. 1993). In recent years, new techniques related to DNA analysis are finding their way in the identification of species, subspecies, populations, strains, hybrids and individuals. More recently, DNA amplification using the polymerase chain reaction (PCR) (Mullis and Faloona 1987) has been used as a powerful alternative toll to protein electrophoresis, chromatography and imunological methods, due to its simplicity, specificity and sensitivity. The PCR has become one of the most important technologies in the manipulation of genetic material, mainly for the identification of genetic markers of considerable value for practical and fundamental research. DNA-based genetic markers have been developed for use in aquaculture primarily with the goal of improving fish stocks and strains for important traits such as growth enhancement and viral and bacterial disease resistance. Various DNA markers have been used for the construction of genetic maps that will also be of particular benefit in

aquaculture, specifically for stock identification, breeding selection, analysis of loci segregation and quantitative traits, and accessing genetic variability of species. Genome maps will also find applications in behavioural, morphological, phylogeographic and other evolutionary studies.

The genome organization patterns of the 5S rDNA tandem repeats have been useful as genetic markers not only in evolutionary studies but also in practical approaches for the identification of species, hybrids and strains of fishes, specially farmed animals. The 5S rDNA is a suitable candidate for PCR-based genetic studies for several reasons: (i) head to tail organization of the 5S rDNA multigene family members; (ii) the NTS is flanked by the 5S rRNA gene copies in the 5S rDNA tandem array, thus the PCR technology can be used in the isolation of the NTSs; (iii) the 5S rRNA gene is highly conserved even among non related species and with the use of PCR it is possible to isolate the 5S rDNA repeats of one species based in the available sequence of another non-related species; (iv) repetitive units of the 5S rDNA do not exceed the length of PCR amplification range (see Table 1); (v) the isolation of the repeat units of the 5S rDNA can be obtained from DNA of pour quality and quantity because their reiterated nature and small size.

In the fish rainbow trout (*Oncorhynchus mykiss*), *in situ* hybridization analyses on male and female metaphase spreads revealed a 5S rDNA chromosome sex-specific pattern. Females were characterized by the presence of four fluorescent signals on two metacentric and on two acrocentric chromosomes, whereas males had only three signals, on one metacentric and on two acrocentric chromosomes (Morán et al. 1996). Previous cytogenetic studies in *O. mykiss* had evidenced a female size heteromorphism in the short arm of an acrocentric pair, indicating the occurrence of a XX/XY chromosome sex system where the larger and the minor acrocentric chromosomes correspond to the X and Y elements, respectively (Thorgaard 1977). The results presented by Morán et al. (1996) showed that one of the 5S rDNA loci probably constitutes a genomic segment restricted to the X chromosome. In the chinook salmon (*Oncorhynchus tshawytscha*), the 5S rDNA is present in the short arm of six to seven acrocentric chromosome pairs, including the putative X and Y chromosomes (Stein et al. 2001). The presence of 5S rDNA repeats in the X chromosome of the rainbow trout and on both X and Y chromosomes of the chinook salmon suggests that the sex chromosome may be conserved in both species. Besides the potential use of the 5S rDNA as chromosomal markers for sex identification of species with aquaculture interest, like rainbow trout, such approach is also of high value for studies concerning on evolutionary aspects of sex chromosomes.

Mapping DNA sequences in the chromosomes represents a powerful way to trace useful genetic markers for the improvement of fish production. Chromosome mapping of the 5S rDNA has also found application in the physical mapping of the Nile tilapia (*Oreochromis niloticus*) genome (Martins et al. 2003). In *O. niloticus*, except for the first chromosome pair, the chromosomes are nearly identical in morphology and size, which makes the identification of particular elements difficult. The integration of a physical and a genetic map has been hindered due to the absence of specific chromosome markers for this species and limited information on the structure of the Nile tilapia chromosomes and their identification was available, as molecular cytogenetic information described for the species is mainly related to the distribution of repetitive DNA sequences in the chromosomes. Recently, 5S rDNA repeats were identified as chromosome markers for the species. 5S rDNA sequences exist as two types in the genome of the Nile tilapia: the tandemly-arrayed, type I 5S rDNA composed of

monomers of 1405 bp, and the tandemly-arrayed, type II 5S rDNA composed of monomers of 475 bp (see Figure 8). Both classes were clustered in distinct chromosomes. While the type I 5S rDNA was detected in an interstitial position in the long arm of a subtelo-acrocentric chromosome pair (chromosome 3), the type II 5S rDNA was identified interstitially in the long arm of a different subtelo-acrocentric pair and at the terminal region of the short arm of another subtelo-acrocentric chromosome pair (chromosomes 9 and 13). The integration of 5S rDNA cytogenetic data for the Nile tilapia with the chromosomal location of several other repetitive DNA sequences, such as satellite DNAs, telomeres, 45S rDNA, and the short and long interspersed nucleotide elements (SINEs and LINEs), provide the beginning of a physical genome map for this important teleost fish (Martins et al. 2003).

The application of 5S rDNA sequences as molecular markers was also evidenced in fish species. The Atlantic salmon (*Salmo salar*), the brown trout (*Salmo truta*), and their hybrid (Pendás et al. 1995) are very difficult to be distinguished using morphological characters. However, using specific primers on PCR to amplify the repeat units of the 5S rDNA, the two salmon species and their hybrid were clearly identified as the different sizes in the amplified products gives, by agarose gel analysis, distinct band patterns. Clearly species-specific 5S rDNA-PCR products were also evidenced for fish species of the Neotropical genus *Brycon* (Figure 12) (Wasko et al. 2001).

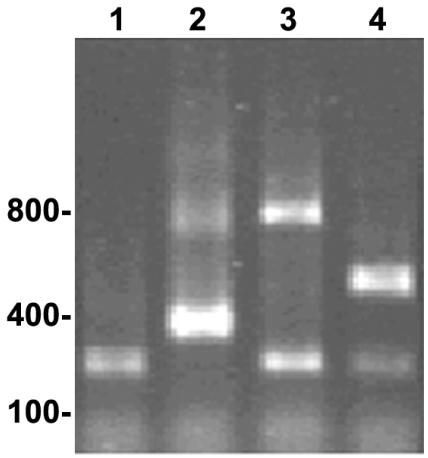


**Figure 12:** Species-specific 5S rDNA-PCR products: *Brycon lundii* (1), *B. cephalus* (2), *Brycon* sp. (3), and *B. insignis* (4). Molecular weight markers (bp) are shown in the left of the figure.

In the aim to assess the species identity of smoked products, Carrera et al. (2002) have also applied the 5S rDNA PCR amplification to differentiate smoked samples of Atlantic salmon, rainbow trout and bream (*Brama raii*). The species identification of smoked fillets becomes a problem because de external characteristics of the animal are removed by the processing of the fillets. Similar results were obtained by Cespedes et al. (1999) for two species of flatfishes, *Solea solea* and *Reinhardtius hippoglossoides.* The results obtained by Pendás et al. (1995), Cespedes et al. (1999), Carrera et al. (2002), and Wasko et al. (2002) evidenced that the 5S rDNA can be an efficient marker for inspection programs intended to access species, hybrids, or smoked products identity.

Polymorphism detected by membrane hybridization analyses of the 5S rDNA repeat units can also be useful in the differentiation of fish species. Nieddu et al. (1998) have successfully applied Southern blot hybridization for the identification of polymorphism between the European eel (*Anguilla anguilla*) and the American eel (*Anguilla rostrata*). Although Southern blot hybridization represents a more expensive and time-consuming technology, when compared to PCR, this approach also allows finding out how the sequence used as probe is organized in the genome.

5S rDNA polymorphisms constitute important nuclear genetic markers for identifying fish species, strains and hybrids of economic importance. Once the NTSs evolve rapidly, the PCR technology can also be easy applied to amplify specific NTS regions that function as species- or strain-specific genetic markers. As discussed in other topics of this chapter, these sequences also have been shown useful for evolutionary studies and have contributed to the knowledge of the biology of fish species in a broad range of aspects.

## CONCLUSION

The conservation of 5S rDNA loci in the chromosomes of related fish species indicates that theses clusters are protected from significant changes that occur in the karyotype macrostructure, probably because of their interstitial location. On the other hand, the existence of different 5S rDNA classes within the same genome, as demonstrated for *Leporinus* and *Oreochromis niloticus*, shows that micro structural changes in the 5S rDNA sequences have occurred during their evolution. As demonstrated for *O. niloticus*, as a consequence of the intense dynamics of the tandem repeat units, the different 5S rDNA classes might present not only extremely distinct NTSs, but also other variants such as pseudogenes and inverted gene sequences. Further investigation on these variants within the 5S rDNA repeats may clarify the role, if any, of such sequences in the genome. The mapping of the 5S rDNA variant types at distinct chromosome loci shows that the tandem repeats coding 5S rRNA can evolve independently at different nuclear territories. The evolution of repetitive sequences is thought to be subjected to the action of several molecular mechanisms such as gene conversion, unequal crossing-over, transposition, slippage-replication and RNA-mediated exchanges, that cause non-Mendelian segregation ratios leading to a particular pattern of evolution. Thus, the tandem repetitive pattern and intense evolution of the 5S rDNA can represent an interesting model to understand the organization and evolution of DNA sequences in the genome. The chromosome mapping, nucleotide sequence and electrophoresis pattern of the tandem repeats of the 5S rDNA have also been useful as genetic markers in a broad range of practical approaches for the identification of species, hybrids and strains of fishes, specially farmed animals. The general aspects of the organization of the 5S rDNA tandem repeats in the fish genome also will be of particular importance in the comprehension of the genome structure of vertebrates.

## ACKNOWLEDGEMENTS

The author thanks FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the financial support.

## REFERENCES

Adacchi J, Watanabe K, Fukui K, Ohmido N, Kosuge K (1997). Chromosomal localization and reorganization of the 45S and 5S rDNA in the *Brachyscome lineariloba* complex (Asteraceae). *J Plant Res 110*:371-377.

Aguilar CT (2001). Estudos citogenéticos e moleculares em populações brasileiras de *Leporellus vittatus* (Characiformes, Anostomidae). Doctoral Thesis. Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ.

Almeida-Toledo LF, Ozouf-Costaz C, Foresti F, Bonillo C, Porto-Foresti F, Daniel-Silva MFZ (2002). Conservation of the 5S bearing chromosome pair and co-localization with major rDNA clusters in five species of *Astyanax* (Pisces, Characidae). *Cytogenet Genome Res 97*:229-233.

Amarasinghe V, Carlson JE (1998). Physical mapping and characterization of 5S rRNA genes in douglas-fir. *Amer Gen Assoc 89*:495-500.

Andrews MT, Vaughn JC, Perry BA, Bagshaw JC (1987). Interspersion of histone and 5S rRNA genes in *Artemia*. *Gene 51*:61-67.

Arnheim N (1983). Concerted evolution of multigene families. In M Nei and RK Koehn, eds. *Evolution of Genes and Proteins*. Sinauer, Sunderland, Mass. pp. 38-61.

Baker WJ, Hedderson TA, Dransfield J (2000). Molecular phylogenetics of *Calamus* (Palmae) and related rattan genera based on 5S nrDNA spacer sequence data. *Mol Phyl Evol 14*:218-231.

Belkhiri A, Buchko J, Klassen GR (1992). The 5S ribosomal RNA gene in *Pythium* species: two different genomic locations. *Mol Biol Evol 9*: 1089-1102.

Bernardi G (1995). The human genome: organization and evolutionary history. *Annu Rev Genet 29*: 445-476.

Born GG, Bertollo LAC (2000). An XX/XY sex chromosome system in a fish species, *Hoplias malabaricus* with a polymorphic NOR bearing X chromosome. *Chromosome Res 8*: 111-118

Carrera E, Garcia T, Céspedes A, Gonzalez I, Fernandez A, Asensio LM, Hernandez PE, Mantin R (2000). Differentiation of smoked *Salmo salar*, *Oncorhynchus mykiss* and *Brama raii* using the nuclear marker 5S rDNA. *Int J Food Sci Tech 35*: 401-406.

Carrol D, Brown DD (1976). Adjacent repeating units of *Xenopus laevis* 5S DNA can be heterogeneous in length. *Cell 7*: 477-486.

Centofante L, Bertollo LAC, Moreira-Filho O (2002). A ZZ/ZW sex chromosome system in a new species of the genus *Parodon* (Pisces, Parodontidae). *Caryologia 55*: 139-150.

Cespedes A, Garcia T, Carrera E, Gonzalez I, Fernandez A, Hernández PE, Martin R (1999). Identification of sole (*Solea solea*) and greenland halibut (*Reinhardtius hippoglossoides*) by PCR amplification of the 5S rDNA gene. *J Agric Food Chem 47*: 1046-1050.

Charlesworth B, Snlegowski P, Stephan W (1994). The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature 371*: 215-220.

Crease TJ, Lynch M (1991). Ribosomal DNA variation in *Daphnia pulex*. *Mol Biol Evol 8*: 620-640.

Deiana AM, Cau A, Salvadori S, Coluccia E, Cannas R, Milia A, Tagliavini J (2000). Major and 5S ribosomal sequences of the largemouth bass *Micropterus salmoides* (Perciformes,

Centrarchidae) are localized in GC-rich regions of the genome. *Chromosome Res 8*: 213-218.

Dennis H, Wegnez M (1977). Oocytes and liver cells of the teleost fish *Tinca tinca* contain different kinds of 5S rRNA. *Devl Biol 59*: 228-236.

Doran JL, Bingle WH, Roy KL (1987). The nucleotide sequence of two human 5S rRNA pseudogenes. *Nucleic Acids Res 15*: 6297.

Dover GA (1986). Linkage disequilibrium and molecular drive in the rDNA gene family. *Genetics 122*: 249-252.

Drouin G (1999). Homogenization of 5S ribosomal genes on the noncoding strand of the rDNA units of two crustacean species. *Genome 42*:150-153.

Drouin G, Moniz de Sá M (1995). The concerted evolution of 5S ribosomal genes linked to the repeat units of other multigene families. *Mol Biol Evol 12*: 481-493.

Elder Jr JF, Turner BJ (1995). Concerted evolution of repetitive DNA sequences in eukaryotes. *Q Rev Biol 70*:277-320.

Emerson BM, Roeder RG (1984). Isolation and genomic arrangement of active and inactive forms of mammalian 5S RNA genes. *J Biol Chem 259*: 7916-7925.

Ferro DA, Neo DM, Moreira Filho O, Bertollo LA (2000). Nucleolar organizing regions, 18S and 5S rDNA in *Astyanaxs scabripinnis* (Pisces, Characidae): populations distribution and functional diversity. *Genetica 110*: 55-62.

Fischer C, Ozouf-Costaz C, Crollius HR, Dasilva C, Jaillon O, Bouneau L, Bonillo C, Weissenbach J, Bernot A (2000). Karyotype and chromosomal location of characteristic tandem repeats in the pufferfish *Tetraodon nigroviridis*. *Cytogenet Cell Genet. 88*: 50-55.

Fontana F, Tagliavini J, Congiu L, Lanfredi M, Chicca M, Laurente C, Rossi R (1998). Karyotypic characterization of the great sturgeon, *Huso huso*, by multiple staining techniques and fluorescent in situ hybridization. *Marine Biol 132*: 495-501.

Fontana F, Lanfredi M, Chicca M, Congiu L, Tagliavini J, Rossi R (1999). Fluorescent in situ hybridization with rDNA probes on chromosomes of *Acipenser ruthenus* and *Acipenser naccarii* (Osteichthyes, Acipenserifomes). *Genome 42*: 1008-1012.

Fontana F, Lanfredi M, Congiu L, Leis M, Chicca M, Rossi R (2003). Chromosomal mapping of 18S-28S and 5S rRNA genes by two-colour fluorescent in situ hybridization in six sturgeon species. *Genome 46*: 473-477.

Franck JPC, Harris AS, Beatzen P, Denovan-Wright EM, Wright JM (1991). Organization and evolution of satellite, minisatellite and microsatellite DNAs in teleost fishes. In: Maclean N, ed. *Oxford Surveys on Eukaryotic Genes*. Oxford: Oxford University Press, pp 51-82.

Frederiksen S, Cao H, Lomholt B, Levan G, Hallemberg C (1997). The rat 5S rRNA bona fide gene repeat maps to chromosome 19q12→qter and the pseudogene repeat maps to 12q12. *Cytogenet Cell Genet 76*:101-106.

Fujiwara A, Abe S, Yamaha E, Yamazaki F, Yoshida MC (1998). Chromosomal localization and heterochromatin association of ribosomal RNA genes loci and silver stained nucleolar organizer regions in salmonid fishes. *Chromosome Res 6*: 463-471

Gerbi SA (1985). Evolution of ribosomal DNA. In: *Molecular evolutionary genetics* (RJ MacIntyre, ed.). Plenum, New York.

Gornung E, De Innocentiis S, Annesi F, Sola L (2000). Zebrafish 5S rRNA genes map to the long arms of chromosome 3. *Chromosome Res 8*: 362-362.

Hadjiolov AA (1985). *The nucleolus and ribosome biogenesis*. Springer-Verlag, New Yorq.

Hallenberg C, Nederby-Nielsen J, Frederiksen S (1994). Characterization of 5S rRNA genes from mouse. *Gene 142*: 291-295.

Hallenberg C, Frederiksen S (2001). Effect of mutations in the upstream promoter on the transcription of human 5S rRNA genes. *Biochim Biophys Acta 91565*: 169-173.

Hamada M, Huang Y, Lowe TM, Maraia RJ (2001). Widespread use of TATA elements in the core promoters for RNA polymerase III, II and I in fission yeast. *Mol Cel Biology 21*: 6870-6881.

Hanson RE, Islam-Faridi MN, Percival EA, Crane CF, Ji Y, McKnight TD, Stelly DM, Price HJ (1996). Distribution of 5S and 18S-28S rDNA loci in a tetraploid cotton (*Gossypium hirsutum* L.) and its putative diploid ancestors. *Chromosoma 105*:55-61.

Hatanaka T (2001). Estudos de marcadores cromossômicos e moleculares no peixe *Prochilodus marggravii* (Prochilodontidae), uma espécie de interesse econômico no rio São Francisco. Doctoral Thesis. Universidade Federal de São Carlos, Brazil.

Inafuku J, Nabeyama M, Kikuma Y, Saitoh J, Kubota S, Kohno S (2000). Chromosomal location and nucleotide sequences of 5S ribosomal DNA of two cyprinid species (Osteichthyes, Pisces). *Chromosome Res 8*:193-199.

Jacq C, Miller JR, Brownlee GG (1977). A pseudogene structure in 5S DNA of *Xenopus laevis*. *Cell 12*:109-120.

Jankun M, Ocalewicz K, Pardo BG, Martinez P, Woznicki P, Sanchez L (2003a). Chromosomal characteristics of rDNA in European grayling *Thymallus thymallus* (Salmonidae). Genetica (*in press*).

Jankun M, Ocalewicz K, Pardo BG, Martinez P, Woznicki P, Sanchez L (2003b). Localization of 5S rRNA loci in three coregonid species (Salmonidae). Genetica (*in press*).

Jensen LR, Frederiksen S (2000). The 5S rRNA genes in *Macaca fascicularis* are organized in two large tandem repeats. *Biochim Biophys Acta 1492*: 537-542.

Kikuma Y, Inafuku J, Kubota S, Kohno S (2000). Banding karyotype and 5S ribosomal DNA loci in the Janapanese bitterling, *Rhodeus ocellatus* (Cyprinidae). *Chromosome Sc 3*: 101-103.

Komiya H, Hasegawa M, Takemura S (1986). Differentiation of oocyte- and somatic-type 5S rRNAs in animals. *J Biochem 100*: 369-374.

Korn LJ (1982). Transcription of *Xenopus* 5S ribosomal RNA genes. Nature 295:101-105.

Korn LJ, Brown DD (1978) Nucleotide sequence of *Xenopus borealis* oocyte 5S DNA: comparison of sequences that flank several eucaryotic genes. *Cell 15*: 1145-1156.

Kramer A (1985). 5S ribosomal gene transcription during *Xenopus* oogenesis. In *Developmental Biology. A comprehensive synthesis*. Vol. 1. (Ed. by Browder LW). Plenum Press, New York. pp. 431-451.

Lamond AL, Earnshaw WC (1998). Structure and function in the nucleous. *Science 280*: 547-553.

Leah R, Frederiksen S, Engberg J, Sorensen PD (1990). Nucleotide sequence of a mouse 5S rRNA variant gene. *Nucleic Acids Res 18*: 7441-7441.

Little R, Braaten D (1989). Genomic organization of human 5S rDNA and sequence of one tandem repeat. *Genomics 4*: 376-383.

Long EO, David ID (1980). Repeated genes in eukaryotes. *Ann Rev Biochem 49*: 727-764

Lucchini S, Nardi I, Barsacchi G, Batistoni R, Andronico F (1993). Molecular cytogenetics of the ribosomal (18S + 28S and 5S) DNA loci in primitive and advanced urodele amphibians. *Genome 36*: 762-773.

Mäkinem A, Zijlstra C, De Haan NA, Mellink CHM, Bosma AA (1997). Localization of 18S plus 28S and 5S ribosomal RNA genes in the dog by fluorescence *in situ* hybridization. *Cytogenet Cell Genet 78*: 231-235.

Mandrioli M, Colomba MS, Vitturi R (2000). Chromosomal analysis of repeated DNAs in the rainbow wrasse *Coris julis* (Pisces, Labridae). *Genetica 108*: 191-195.

Mandrioli M, Manicardi GC (2001). Cytogenetic and molecular analysis of the pufferfish *Tetraodon fluviatilis* (Osteichthyes). *Genetica 111*: 433-438.

Mandrioli M, Manicardi GC, Machella N, Caputo V (2001). Molecular and cytogenetic analysis of the goby *Gobius niger* (Teleostei, Gobiidae). *Genetica 110*: 73-78.

Mantovani M, Abel LDS, Mestriner CA, Moreira-Filho O (2000). Accentuated polymorphism of heterochromatin and nucleolar organizer regions in *Astyanax scabripinnis* (Pisces, Characidae): tools for understanding karyotypic evolution. *Genetica 109*: 161-168.

Mantovani M, Abel LDS, Moreira-Filho O (2003). 5S rDNA conservation versus 45S rDNA variability in *Astyanax scabripinnis* (Pisces, Characidae) as indicative of differentiated evolutionary tendencies in a single genome (*in preparation*).

Mao J, Appel B, Schaack J, Sharp S, Yanada H, Soll D (1982). The 5S rRNA genes of *Schizosaccharomyces pombe*. *Nucleic Acids Res 10*: 487-500.

Martínez JL, Móran P, Garcia-Vásquez E, Pendás AM (1996). Chromosomal localization of the major and minor 5S rRNA genes in the European eel (*Anguilla anguilla*). *Cytogenet Cell Genet 73*:149-152.

Martins C, Galetti Jr PM (1999). Chromosomal localization of 5S rDNA genes in *Leporinus* fish (Anostomidae, Characiformes). *Chromosome Res 7*: 363-367.

Martins C, Galetti Jr. PM (2000). Conservative distribution of 5S rDNA loci in *Schizodon* (Pisces, Anostomidae) chromosomes. *Chromosome Res 8*: 353-355.

Martins C, Wasko AP, Oliveira C, Wright JM (2000). Nucleotide sequence of 5S rDNA and localization of the ribosomal RNA genes to metaphase chromosomes of the tilapiine cichlid fish, *Oreochromis niloticus*. *Hereditas 133*: 39-46.

Martins C, Galetti Jr PM (2001a). Organization of 5S rDNA in species of the fish *Leporinus*: two different genomic locations are characterized by distinct nontranscribed spacers. *Genome 44*: 903-910.

Martins C, Galetti Jr PM (2001b). Two 5S rDNA arrays in Neotropical fish species: Is it a general rule for fishes?. *Genetica 111*: 439-446.

Martins C, Wasko AP, Oliveira C, Porto-Foresti F, Parise-Maltempi PP, Wright JM, Foresti F (2002). Dynamics of 5S rDNA in the tilapia (*Oreochromis niloticus*) genome: repeat units, inverted sequences, pseudogenes and chromosome loci. *Cytogenet Genome Res 98*:78-85.

Martins C, Oliveira C, Wasko AP, Wright JM (2003). Physical mapping of the Nile tilapia (*Oreochromis niloticus*) genome by fluorescent in situ hybridization of repetitive DNAs to metaphase chromosomes - A review. Aquaculture (*in press*).

Mashkova TD, Serenkova TI, Mazo AM, Avdonina TA, Timofeyeva MY, Kisselev LL (1981). The primary structure of oocyte and somatic 5S rRNAs from the loach *Misgurnus fossilis*. *Nucleic Acids Res 9*: 2141-2151.

Mellink CHM, Bosma AA, Haan NA, Zijlstra C (1996). Physical localization of 5S rRNA genes in the pig by fluorescence in situ hybridization. *Hereditas 124*: 95-97.

Messias LHV, Wasko AP, Oliveira C, Foresti F, Martins C. (2003). 5S rDNA organization in the fish *Synbranchus marmoratus*. *Hereditas* (submitted).

Mighell AJ, Smith NR, Robinson PA, Markham AF (2000). Vertebrate pseudogenes. *FEBS Letters 468*:109-114.

Molina W, Galetti Jr. PM (2002). Robertsonian rearrangements in the reef fish *Chromis* (Perciformes, Pomacentridae) involving chromosomes bearing 5S rRNA genes. *Genet Mol Biol 25*: 373-377.

Móran P, Martínez JL, Garcia-Vásquez E, Pendás AM (1996). Sex linkage of 5S rDNA in rainbow trout (*Oncorhynchus mykiss*). *Cytogenet Cell Genet 75*: 145-150.

Mullis KB, Faloona FA (1987). Specific synthesis of DNA in vitro via polymerase-catalyzed chain reaction. *Methods Enzymol 155*: 335-350.

Murakami M, Fujitani H (1998). Characterization of repetitive DNA sequences carrying 5S rDNA of the triploid ginbuna (Japanese silver crucian carp, *Carassius auratus langsdorfi*). *Genes Genet Syst 73*: 9-20.

Nederby-Nielsen J, Hallenberg C, Frederiksen S, Sorensen PD, Lomholt B (1993). Transcription of human 5S rRNA genes is influenced by an upstream DNA sequence. *Nucleic Acids Res 26*: 3631-3636.

Nieddu M, Pichiri G, Coni P, Salvadori S, Deiana AM, Mezzanote R (1998). A comparative analysis of European and American eel (*Anguilla anguilla* and *Anguilla rostrata*) genomic DNA: 5S rDNA polymorphism permits the distinction between the two populations. *Genome 41*: 728-732.

Pardue ML, Brown DD, Birnstiel M (1973). Localization of the genes for 5S ribosomal RNA in *Xenopus laevis*. *Chromosoma 42*: 191-203.

Paule MR, White RJ (2000). Survey and summary: transcription by RNA polymerase I and III. *Nucleic Acids Res. 28*: 1283-1298.

Pendás AM, Móran P, Freije JP, Garcia-Vásquez E (1994). Chromosomal location and nucleotide sequence of two tandem repeats of the Atlantic salmon 5S rDNA. *Cytogenet Cell Genet 67*: 31-36.

Pendás AM, Móran P, Martínez JL, Garcia-Vásquez E (1995). Applications of 5S rDNA in Atlantic salmon, brow trout, and in Atlantic salmon x brown trout hybrid identification. *Molecular Ecol 4*: 275-276.

Peterson RC, Doering JL, Brown DD (1980). Characterization of two *Xenopus* somatic 5S DNAs and one minor oocyte-specific 5S DNA. *Cell 20*: 131-141.

Phillips RB, Reed KM (2000). Localization of repetitive DNAs to zebrafish (*Danio rerio*) chromosomes by fluorescence *in situ* hybridization (FISH). *Chromosome Res 8*: 27-35.

Phillips RB, Matsuoka MP, Reed KM (2002). Characterization of charr chromosomes using fluorescence in situ hybridization. *Environ Biol Fish 64*: 223-228.

Pieler T, Hamm J, Roeder RG (1987). The 5S gene internal control region is composed of three distinct sequence elements, organized as two functional domains with variable spacing. *Cell 48*: 91-100.

Pino EM, Murphy C, Masson PH, Gall JG (1992). 5S rRNA-encoding genes of the marsupial frog *Gastrotheca riobambae*. *Gene 111*: 235-238.

Rocco L, Russo C, Stingo V, Aprea G, Odierna G (1999). Characterization of 5S rDNA in *Gasterosteus aculeatus* (Teleostei, Gasterosteidae). *Ital J Zool 66*: 285-289.

Sajdak SL, Reed KM, Phillips RB (1998). Intraindividual and interspecies variation in the 5S rDNA of coregonid fish. *J Mol Evol 46*: 680-688.

Selker EU, Yanofsky C, Driftmier K, Metzenberg RL, Alznerdeweerd B, Rajbhandary UL (1981). Dispersed 5S rRNA genes in *N-crassa* - structure, expression and evolution. *Cell 24*: 819-828.

Schmid M, Vitelli L, Batistoni R (1987). Chromosome banding in Amphibia. IV. Constitutive heterochromatin, nucleolus organizers, 18S+28S and 5S ribosomal RNA genes in Ascaphidae, Pipidae, Discoglossidae and Pelobatidae. *Chromosoma 95*: 271-284.

Sola L, Innocentiis S, Gornung E, Papalia S, Rossi AR, Marino G, Marco P, Cataudella S (2000). Cytogenetic analysis of *Epinephelus marginatus* (Pisces: Serranidae), with the chromosome localization of the 18S and 5S rRNA genes and of the $(TTAGGG)_n$ telomeric sequence. *Marine Biol 137*: 47-51.

Sollner-Webb B (1988). Surprises in polymerase III transcription. *Cell 52*: 153-154.

Sotelo CG, Pineiro C, Gallardo JM, Perezmartin RI (1993). Fish species identification in seafood products. *Trends Food Sci Tech 4*: 395-401.

Schweizer D, Loidl J (1987). A model for heterochromatin dispersion and the evolution of C band patterns. *Chromosomes Tod 9*: 61-74.

Stein J, Phillips RB, Devlin RH (2001). Identification of the Y chromosome in chinookk salmon (*Oncorhynchus tshawytscha*). *Cytogenet Cell Genet 92*: 108-110.

Suzuki H, Moriwaki K, Sakurai S (1994). Sequences and evolutionary analysis of mouse 5S rDNAs. *Mol Biol Evol 11*: 704-710.

Suzuki H, Sakurai S, Matsuda Y (1996). Rat 5S rDNA spacer sequences and chromosomal assignment of the genes to the extreme terminal region of chromosome 19. *Cytogenet Cell Genet 72*: 1-4.

Tagliavini J, Williot P, Congiu L, Chicca M, Lanfredi M, Rossi R, Fontana F (1999). Molecular cytogenetic analysis of the karyotype of the European Atlantic sturgeon, *Acipenser sturio*. *Heredity 83*: 520-525.

Thorgaard GH (1977). Heteromorphic sex chromosomes in male rainbow trout. *Science 196*: 900-902.

Veldhoen N, You Q, Setzer DR, Romaniuk PJ (1994). Contribution of individual base pair to the interaction of TFIIIA with the *Xenopus* 5S rRNA gene. *Biochemistry 33*: 7568-7575.

Vicente VE, Jesus CM, Moreira-Filho O (2001). Chromosomal localization of 5S and 18S rRNA genes in three *Parodon* species (Pisces, Parodontidae). *Caryologia 54*: 365-369.

Vitelli L, Batistoni R, Andronico F, Nardi I, Barsacchi-Pilone G (1982). Chromosomal localization of 18S + 28S and 5S ribosomal RNA genes in evolutionary divergent anuran amphibians. *Chromosoma 84*: 475-491.

Wagner RP, Maguire MP, Stallings RL (1993). *Chromosomes: a synthesis*. New York: Wiley-Liss, Inc.

Wasko AP, Martins C, Wright JM, Galetti Jr PM (2001). Molecular organization of 5S rDNA in fishes of the genus *Brycon*. *Genome 44*: 893-902.

Williams SM, Strobeck C (1985). Sister chromatid exchange and the evolution of rDNA spacer lenght. *J Theor Biol 116*: 625-636.

Zwick MG, Wiggs M, Paule MR (1991). Sequence and organization of 5S rRNA genes from the eukaryotic protist *Acanthamoeba castellanii*. *Gene 101*: 153-157.