

Chromosomal location of retrotransposable REX 1 in the genomes in five *Prochilodus* (Teleostei: Characiformes: Prochilodontidae) species

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Transposable elements are repetitive DNA sequences comprising a group of segments able to move and carry sequences within the genome. Studies involving comparative genomics have revealed that most vertebrates have different populations of transposable elements with significant differences among species of the same lineage. Few studies have been conducted in fish, the most diverse group of vertebrates, with the objective to locate different types of transposable elements. Therefore, this study proposed to map the retrotransposable element Rex1 applying Fluorescent in situ Hybridization (FISH) in five species of the genus *Prochilodus* (*Prochilodus argenteus*, *Prochilodus brevis*, *Prochilodus costatus*, *Prochilodus lineatus* and *Prochilodus nigricans*). After the application of the Rex1 probe, scattered markings were found throughout the genome of analyzed species, and also the presence of small clusters located in the centromeric and telomeric regions coincident with the heterochromatin distribution pattern. This was the first description of the retrotransposable element Rex1 in *Prochilodus* genome seeking for a better understanding of the distribution pattern of these retrotransposons in the genome of teleost fish.

Introduction

The retrotransposable elements are able to accomplish important roles in the genome evolution.^{1,2} Their copies numbers can rapidly increase by retrotransposition and serve as substrate for homologous recombinations in various categories of DNA rearrangements including deletions, inversions, translocations, duplications and amplifications noted by Ozouf-Costaz et al.³ Knowledge of the origin and function of these retrotransposons and of their role in the structure and organization of chromosomes in the teleost fish genomes is still very scarce and fragmented.⁴

Two classes of transposable elements (TE) are acknowledged among vertebrates: retrotransposons and transposons. The first class, known as retrotransposon, moves through the genome by the action of reverse transcriptase, an enzyme that can promote the synthesis of a DNA strand from a RNA primer, and is divided into autonomous, i.e., LTR (long-terminal repeat) and non-LTR, in which LINEs (long interspersed elements) are part of the genome, and non-autonomous, in which the SINEs (short interspersed elements) are the representatives. The second class includes those sequences which transpose by a “cut and paste” mechanism known as DNA transposons. Fish genome contains

all known types of transposable elements,⁵ and some of these have been mapped at the chromosome level.

The retrotransposable elements of the retrotransposon class (LTR) in which can be highlighted the Ty3/Gypsy, Ty1/copy, DIRS1 retrotransposon and BEL, are the most studied in fish species.⁵⁻⁷ Retrotransposons described above, include 10 elements that display data regarding the location in fish chromosomes. Among these are the elements Rex (Rex1, Rex3 and Rex6) which are retrotransposable elements characterized for the first time in the genome of the fish *Xiphophorus maculatus* and appears to be the most abundant in different teleostei.^{7,8} In the order Characiformes, little is known about the organization of the retrotransposon Rex, and available data are only for *Erythrinus erythrinus*.⁹

The Rex1 element, represents a non-long-terminal-repeat (non-LTR) retrotransposons, related to the group CR1 (Chicken Repeat), comprising a LINEs, and encodes a reverse transcriptase and an endonuclease apurinic / pyrimidine required for cleavage of the target sequence.¹⁰

Thus, the objective of the present research is to isolate and map the retrotransposable element Rex1 in the genome of five species

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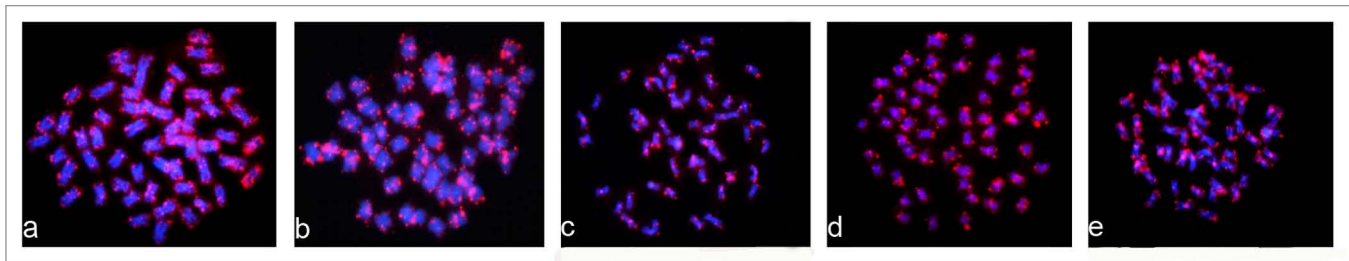


Figure 1. Distribution pattern of the retrotransposable element Rex1 in genome of (a) *P. argenteus*; (b) *P. brevis*; (c) *P. costatus*; (d) *P. nigricans* and (e) *P. lineatus* species.

of the genus *Prochilodus* (*Prochilodus argenteus*, *Prochilodus brevis*, *Prochilodus costatus*, *Prochilodus lineatus* and *Prochilodus nigricans*) by Fluorescent in situ Hybridization (FISH) by studying occurrence of Rex 1 retrotransposon in *Prochilodus* genome and chromosomes and compare the obtained results with others teleost fish groups.

Results

All specimens of *Prochilodus* (*P. argenteus*, *P. brevis*, *P. costatus*, *P. lineatus* and *P. nigricans*) cytogenetically analyzed exhibited a karyotypic constitution of $2n = 54$ chromosomes.

The amplification of the retrotransposable element Rex1 in the genome of the species of *Prochilodus* produced a band of approximately 600 bp for all analyzed species (data not shown). Analysis through the fluorescent in situ hybridization (FISH) technique using the Rex1 retrotransposons probes in chromosome preparations of the five *Prochilodus* species (Fig. 1a–e) showed a dispersed pattern of the retrotransposable element Rex1 throughout the genome of analyzed individuals. Furthermore, the presence of small clusters in the telomeric and centromeric regions was regarded as a common feature in these *Prochilodus*. The presence of these clusters, mainly in the centromeric regions, is coincident with the constitutive heterochromatin distribution pattern previously observed by C-banding for all specimens of these species.

Discussion

Chromosomal information on representatives of the genus *Prochilodus* have revealed a conserved karyotypic structure of $2n = 54$ chromosomes.^{11–20} All *Prochilodus* cytogenetically analyzed in this study exhibited conserved chromosomal characteristics regarding the diploid number of $2n = 54$ in accordance with literature data reported for these species.

The evolutionary dynamics of the transposable elements in various groups, such as insects, fish, birds and mammals are extremely distinct. Mammals genomes contain a large variety of transposable elements lineage types, while fish and *Drosophila* lineages show various strains of these genomic elements, which are typically less abundant, but apparently more deleterious. In many insect and fish species, families of different strains of transposable elements with a relatively low number of copies have remained active for a long period.^{5,21} This variation in diversity

and activity of transposable elements among various animal genomes is caused by the difference of the defense mechanisms of the host genome in opposition to the activities of the transposable elements.

Generally, the transposons have a sufficiently distinct organization among the species and are dispersed across the genome, usually occupying euchromatic regions, as already observed in humans and insects.⁵ This scattered pattern was also observed in this study in the five species of *Prochilodus* analyzed with the Rex1 retrotransposable element, as well as in species of the subfamily Hypoptopominae using Rex1 and Rex3;²² in *Erythrinus erythrinus* with Rex3;⁹ in *Astatotilapia latifasciata* with Rex 1²³ and in some Antarctic Perciformes using the Rex1 and Rex3 elements.² Moreover, fish derived from the group of Tetraodontiformes (*T. nigroviridis*) have an extremely compact genome, and the separation between poor and rich regions of gene segments is much evident according to Fischer et al.²⁴ and da Silva et al.²⁵ Transposable elements in heterochromatin are apparently used as shelter, because the selection pressure is smaller in these regions noted by Lippman, et al.²⁶

As previously mentioned, Rex elements are present in the genome of different species of teleosts.^{2,8} According to a review of retrotransposable use in fish genome mapping performed by Ferreira et al.,⁴ it was observed that the Rex elements exhibit different organizations among fish species. These elements were physically mapped in 28 species of fish. However, in 11 species, Rex elements were organized in heterochromatic regions and in the other remaining 17 species, were scattered across the genome,⁴ as observed in the present study.

A noteworthy example of Rex presence in fish heterochromatic regions is attributed to Cichlidae family, which different Rex elements analysis revealed a compartmentalization in pericentric heterochromatic regions described by Gross et al.^{27,28} similar to that observed in representatives of the Antarctic fish species *Nothothenia coriiceps*.²

Additionally, in Cichlidae family, more specifically in *Oreochromis niloticus* (in Nile tilapia genome), transposable elements were generally found scattered throughout the genome.^{29,30} This pattern, preferably scattered across the euchromatic regions, was also found in five species of the genus *Prochilodus* (*P. argenteus*, *P. brevis*, *P. costatus*, *P. lineatus* and *P. nigricans*), subject of this study, using the retrotransposable element Rex1 (Fig. 1a–e). However, some small clusters were

found in the heterochromatic regions, such as in the centromeres of some chromosome pairs, in the telomeric regions of all samples analyzed.

The distribution pattern of the retrotransposons Rex1 in *Prochilodus* enabled one to observe a scattered pattern common among the five species (Fig. 1a–e), i.e., the Rex 1 retrotransposable element displays a conserved characteristic for this genus, as previously proposed by Pauls and Bertollo¹² using other cytogenetic markers.

Oftentimes, Rex retrotransposable elements were found scattered throughout the genome of the species and more intensely in the chromosome euchromatic regions. However, some blocks of these transposable elements are coincident with heterochromatic regions, suggesting that the distribution pattern of these retroelements may be distinct in different orders of fish,⁴ which corroborates with the observed in *Prochilodus*, where such retrotransposable elements were scattered throughout the genome, but featured a preferential accumulation in the constitutive heterochromatin regions.

Transposable elements are generally more abundant in the heterochromatin of several genomes and their presence in these regions seems to be common among multicellular eukaryotes.^{31,32} Despite some discrepancies, plausible explanations about the relationship between the retrotransposable elements and heterochromatin become reasonable: TEs tend to accumulate in regions with low recombination rates as a consequence of their removal from the region with high recombination rates, whereas ectopic recombination could have more deleterious effects; there are more TEs eliminations in gene-rich regions because of their potential deleterious effects when inserted within genes; the high expression of TE-encoded products might have negative consequences for the genome due to cell cost. Thus, these TEs would be eliminated from the region with high expression levels;³² TEs could accumulate in the heterochromatin as a consequence of their functional involvement in the maintenance of specific genomic regions, such as the pericentromeric and telomeric region.^{31,33}

Although the currently available information on the structural organization, evolution and functional behavior of TEs in the fish genome are still very fragmented and restricted to few species, the data contribute to clarify the understanding of transposable elements distributions in *Prochilodus* genome comparing the results with those obtained in teleostei order fish groups.

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Materials and Methods

It was collected 20 specimens of *P. lineatus* from wild populations of Mogi-Guaçu River, Parana Basin, Pirassununga, SP, Brazil, and also 17 specimens of *P. nigricans* from the Araguaia River (TO), Tocantins-Araguaia basin, Brazil. For the present study, it was also purchased 30 specimens of *P. costatus* from the TROPICAL AQUACULTURE Fish Farm, municipality of Propriá, (SE), Brazil; 6 individuals of *P. argenteus* from the São Francisco River (MG), Brazil; and finally, 5 specimens of *P. brevis* (collected in the DNOCS dam (Departamento de Obras Contra a Seca) (RN), Brazil).

The chromosome preparations were obtained by mitotic stimulation method,^{34,35} direct in vitro preparations of anterior kidney,³⁶ and direct in vivo preparations of anterior kidney.³⁷

Fluorescence in situ Hybridization (FISH) was performed according to Pinkel et al.³⁸ using the retrotransposon probe Rex1 obtained by PCR (Polymerase Chain Reaction) from amplifications of the genomic DNA of *P. lineatus* utilizing the primers 1: RTX1-F1 (5'-TTC TCC AGT GGC CTT CAA CAC C-3') and RTX1-R1 (5'-TTC CTT AAA AAA TAG AGT CTG CTC-3').¹⁰ The Rex1 probe was labeled with Digoxigenin-11-dUTP later detected with an antibody conjugated with rhodamine, providing the red color by the PCR (*Polymerase Chain Reaction*) technique, according to the Roche manufacturer's instructions. The slides were denatured in 70% formamide: 2XSSC for 5 min. DNA was hybridized at 37°C overnight in a moist chamber (0.3 µg of denatured probe, 50% formamide, 10 mg ml of dextran sulfate; 2XSSC, 5 mg/ml of salmon sperm DNA).

Hybridized Rex 1 probe were detected by anti-digoxigenin-rhodamine reactions. Afterwards, slides were counterstained with DAPI (4',6-diamidino-2-phenylindole) and examined under a fluorescence photomicroscope (BX 61, Olympus) equipped with the Olympus DP70 cooled digital camera. Photomicrographs were taken using Pro MC 6.0 software.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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