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A N I N T E R N A T I O N A L J O U R N A L

Chromosome Mapping and Molecular Characterization of the *Tc1/Mariner* Element in *Rineloricaria* (Siluriformes: Loricariidae)

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ABSTRACT

The Tc1/Mariner sequence was isolated and mapped on chromosomes aiming to verify the association of this transposable element (TE) and chromosomal rearrangements in Rineloricaria. Cytogenetic analysis showed that Tc1/Mariner does not co-localize with chromosomal fusion points, in addition the analysis revealed intense molecular degeneration in its DNA sequence.

Key words: chromosome rearrangement; double-FISH; transposon.

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Among repetitive sequence classes, transposable elements (TEs) have been studied for their importance in the modulation of biological events, especially evolutionary processes. They are considered hotspots of mutation, and cytogenetic and molecular analyses have demonstrated the role of TEs in chromosomal rearrangements. TEs can be divided into two categories according to their structural organization and transposition mechanism: class I (retrotransposons - RTEs), which use an intermediate RNA; and class II (DNA transposons), which insert directly into the genome [1]. Among the existing transposon subclasses, the superfamily *Tc1/Mariner* stands out for having the widest distribution in nature. These elements consist of approximately 1000 – 5000 bp and are characterized by containing an open reading frame (ORF) which encodes a transposase flanked by two terminal inverted regions (TIRs) that are flanked by TA nucleotides [2].

In Gymnotiformes, da Silva et al. [3] located *in situ* multiple clusters of 5S rDNA, which was attributed to the *Tc1/Mariner* element present in the non-transcribed spacer sequence (NTS) of the 5S rDNA, contributing to the dispersion of this rDNA in the analyzed species. In Parodontidae, Schemberger et al. [4] found non-autonomous copies of *Tc1/Mariner* with structural variations and different levels of degeneration. Physical mapping of this TE on Parodontidae chromosomes revealed dispersed signals in euchromatins, and some accumulations in terminal regions and sex chromosomes [4].

Loricariidae is the largest family of the Siluriformes order and is subdivided into seven subfamilies [5]. The genus *Rineloricaria* Bleeker, 1862 contains the greatest number of species in the Loricariinae family [6] and shows extensive karyotype variation (2n = 36 – 70), which is attributed to Robertsonian (Rb) events associated with gametic combinations and inversion mechanisms [7-10].

Recently, Glugoski et al. [11] reported the probable involvement of TE *hAT* in the dispersion of 5S rDNA sites in the *Rineloricaria latirostris* genome, which could explain part of the chromosome rearrangements presented in this group. Considering the extensive karyotype variation of Loricariidae, the presence of multiple 5S rDNA sites in some species of *Rineloricaria* [10] and the lack of studies that correlate the involvement of TEs with chromosomal rearrangements, the mapping and molecular analysis of *Tc1/Mariner* could contribute to the understanding of chromosome diversification in this group. The *Tc1/Mariner* element was therefore isolated, characterized and mapped on chromosomes in order to analyze chromosomal diversification in the *Rineloricaria* species.

In this study, fifty-eight specimens of different populations of *Rineloricaria* were genetically analyzed: *R. latirostris* (Laranjinha river - Ventania/PR, Brazil); *R. pentamaculata* (Juruba river - Apucarana/PR, Brazil); *R. stellata* and *R. capitonia* (Uruguai river - São Carlos/SC, Brazil). The procedures were performed according to the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO: 15117-1). The research was approved by the Ethics Committee of Animal Usage (Process CEUA 028/2016) of the Universidade Estadual de Ponta Grossa.

Genomic DNA was extracted from liver using the cetyltrimethylammonium bromide (CTAB) method [12]. The *Tc1/Mariner* element was amplified by polymerase chain reaction (PCR), using a single primer for the TIRs [4]. Approximately 1200-bp-long DNA fragments were isolated, cloned into *Escherichia coli* DH5α and sequenced. The clones obtained were labeled as probes for the physical mapping of *Tc1/Mariner* on chromosomes, according to Pinkel et al. [13].

Mitotic chromosomes were obtained according to Blanco et al. [14]. For fluorescence *in situ* hybridization (FISH), the following probes were used: *i)* 5S rDNA [15] labeled with digoxigenin 11-dUTP (Roche Applied Science) and amplified by PCR; and, *ii) Tc1/Mariner* labeled using the Biotin-Nick Translation Mix (Roche Applied Science). Signal detection was performed using antibodies Streptavidin Alexa Fluor 488 (Molecular Probes) and anti-digoxigenin rhodamine (Roche Applied Science). Chromosomes were counterstained with 4´,6-diamidino-2-phenylindole (DAPI 0.2 µg/mL) in mounting medium Vectashield (Vector).

Cytogenetic data showed 2n = 46 chromosomes for *R. latirostris;* 2n = 56 chromosomes for *R. pentamaculata*; 2n = 54 chromosomes for *R. stellata*; and, 2n = 64 chromosomes for *R. capitonia* [10]. As previously described by Primo et al. [10], hybridization with 5S rDNA probes presented the following chromosomal markers: *R. latirostris* - six pairs marked (m chromosome pair 3 in the centromeric region and five st/a pairs in the terminal region); *R. pentamaculata* - five acrocentric pairs marked in the terminal region; *R. stellata* - five chromosome pairs; *R. capitonia* - four st/a pairs with markers in terminal sites. FISH mapping of the *Tc1/Mariner* sequence revealed

dispersed markers over the chromosomes of all analyzed species, with no accumulations (Fig. 1a, b, c, d). In addition, *Tc1/Mariner* sites did not co-locate with the 5S rDNA sites.

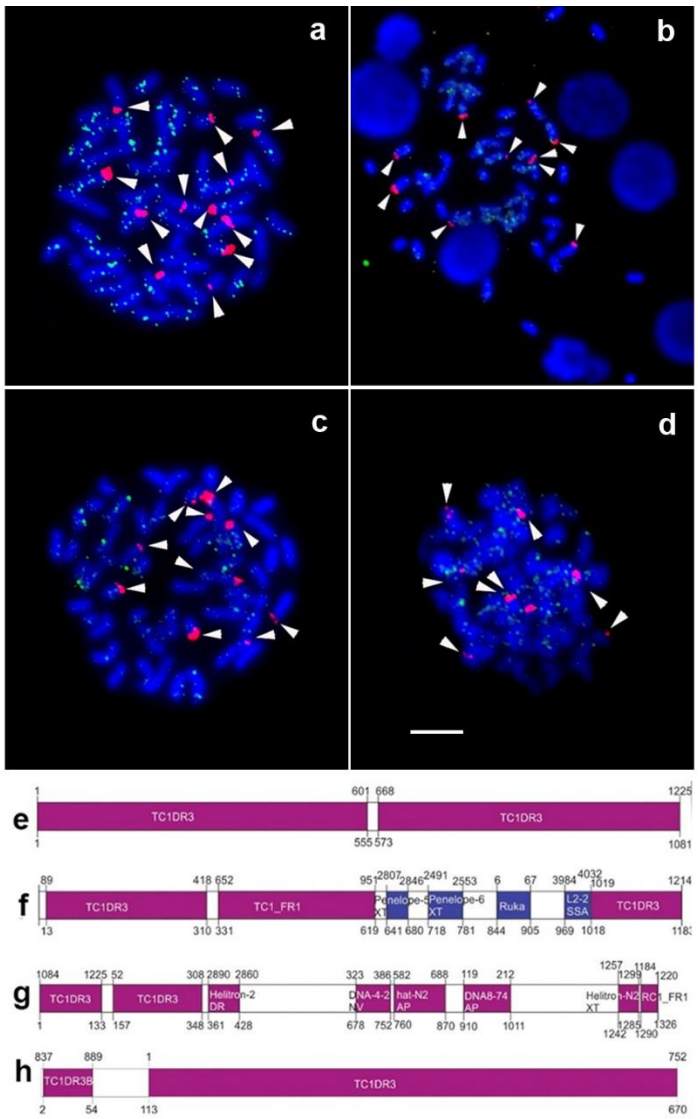

Figure 1. Karyotypes of *Rineloricaria* individuals submitted to FISH using 5S rDNA (arrows, in red) and *R. latirostris Tc1/Mariner* (in green) probes. (a) *R. latirostris*; (b) *R. pentamaculata*; (c) *R. stellata*; and, (d) *R. capitonia*. Schematic figures of *Tc1/Mariner* sequences are shown in (e) *R. latirostris*, (f) *R. pentamaculata*, (g) *R. stellata* and (h) *R. capitonia*. Bar = 10 µm.

Table 1. Characterization of the *Tc1/Mariner* nucleotide sequences obtained from species of *Rineloricaria*, according to CENSOR software.

***Rineloricaria* individuals**	**TE description**	**TE nucleotide sequence position**	**Nucleotide position of the reference TE (CENSOR)**	**Categories**	**Degree of DNA similarity**
R. latirostris	*TC1DR3*	1-555	1-601	DNA	0.8217
	TC1DR3	573-1081	668-1225	DNA	0.8031
R. pentamaculata	*TC1DR3*	13-310	89-418	DNA	0.7855
	TC1 FR1	331-619	652-951	DNA/*Mariner*	0.7840
	Penelope-5 XT	641-680	2807-2846	*NonLTR/Penelope*	0.8780
	Penelope-6 XT	718-781	2491-2553	*NonLTR/Penelope*	0.8000
	Ruka	844-905	6-67	*NonLTR/SINE/SINE2*	0.7258
	L2-2 SSa	969-1017	3984-4032	*NonLTR/L2*	0.8776
	TC1DR3	1018-1183	1019-1214	DNA	0.8070
R. stellata	*TC1DR3*	1-133	1084-1225	DNA	0.8074
	TC1DR3	157-348	52-308	DNA	0.8477
	Helitron-2 DR	361-428	2890-2960	DNA/*Helitron*	0.7183
	DNA-4-2 NV	678-752	323-386	DNA	0.7794
	hat-N2 AP	760-870	582-688	DNA/*hat*	0.7570
	DNA8-74 AP	910-1011	119-212	DNA	0.7753
	Helitron-N2 XT	1242-1285	1257-1299	DNA/*Helitron*	0.8409
	RC1 FR1	1290-1326	1184-1220	DNA/*Mariner*	0.8378
R. capitonia	*TC1DR3B*	2-54	837-889	DNA/*Mariner*	0.8679
	TC1DR3	113-670	1-752	DNA	0.8462

A high degree of deterioration in the *Tc1/Mariner* nucleotide sequences was observed, with truncated regions and absence of intact ORFs (Table 1; Fig. 1e, f, g, h). The analysis of similarity performed using CENSOR online software [16] revealed that the 1081-bp *Tc1/Mariner* sequence from *R. latirostris* (GenBank accession no. MF598590) presented similarities to *Danio rerio Tc1/Mariner* (*TC1DR3*) (Table 1; Fig. 1e). In *R. pentamaculata*, the 1183-bp amplified sequence (GenBank accession no. MF598591) showed similarities to *TC1DR3*, *Takifugu rubripes Tc1/Mariner* (*TC1-FR1*), long interspaced nuclear elements (LINEs) *Penelope-5_XT* and *Penelope-6_XT* from *Xenopus tropicalis*, short interspersed nuclear element (SINE) *Ruka* from *Ixodida* and LINE *L2-2_SSa* from *Salmo salar* (Table 1; Fig. 1f). In *R. stellata*, the 1326-bp amplified sequence (GenBank accession no. MF598592) showed similarities to regions of *TC1DR3*, *Helitron-2 DR* from *D. rerio*, *DNA-4-2 NV* from *Nematostella vectensis*, *hat-N2 AP* and *DNA8-74 AP* from *Acyrthosiphon pisum*, *Helitron-N2 XT* from *Xenopus tropicalis* and *Mariner RC1 FR1* from *Takifugu rubripes* (Table 1; Fig. 1g). The 670-bp TE DNA sequence from *R. capitonia* (GenBank accession no. MF598593) showed similarities to *TC1DR3* and *TC1DR3B* transposon segments from *D. rerio*, without identification of any ORF (Table 1; Fig. 1h).

Repetitive DNA sequences are hotspots for chromosomal breaks and rearrangements in eukaryotes [17] and it is known that fish from the Loricariinae subfamily show a wide karyotype diversity [7,9,10]. Symonová et al. [18] proposed that in some cases, rDNAs use the spread mechanism of TEs to increase the number of its copies and, as a consequence, they affect recombination rates, leading to karyotype differentiation among close genomes. Studies indicate a relation between 5S rDNA dispersion throughout genomes and TEs in fish [19]. RTE fragments of LINE *CR1-79_HM* and the long terminal repeat (LTR) *Gypsy* were found in *Diplodus sargus* 5S rDNA NTS [20]. Da Silva et al. [3] sequenced 5S rDNA from the Gymnotiformes species and found fragments of the *Tc1/Mariner* in the NTS. The dispersion of 5S rDNA in at least 19 chromosome pairs from *Gymnotus paraguensis* was attributed to this TE [3]. Glugoski et al. [11] indicated a possible association between the TE *hAT* and the dispersion of 5S rDNA sites in a population of *R. latirostris*, which could contribute to the karyotypic diversification present in Loricariidae.

The *Rineloricaria* species evaluated in this study presented several chromosome pairs bearing 5S rDNA sites, some of which were located in the centromeric region of metacentric chromosomes as a result of centric fusion [10]. Double FISH data, using 5S rDNA and *Tc1/Mariner* probes, revealed no association between these sequences, moreover, the TE nucleotide sequence did not show any similarity to the 5S rDNA sequence. Therefore, the dispersion of 5S rDNA sites and the mechanisms related to chromosomal fusions/fissions, common in this fish group, could not be correlated to the *Tc1/Mariner* element. It is probable that other TEs, such as the *hAT* element [11], may be involved in the dispersion of 5S rDNA and chromosomal fusions in *Rineloricaria.*

Sequencing data of *Tc1/Mariner* isolated from four *Rineloricaria* species revealed the absence of active ORFs in all analyzed clones and high levels of *Tc1/Mariner* molecular deterioration, which could influence its activity as a TE [21]. Clones obtained from *R. pentamaculata* and *R. stellata* contained insertions of several other TEs, characterized as composite transposons, between the TIRs. Among eukaryotes, TEs are usually present as non-autonomous copies generated through a degradation process [21]; when TEs become inactivated, they accumulate mutations, thereby losing their identity. Nucleotide sequences of *Tc1* families are usually inactivated, including those in fish genomes [4,22]. Some mutated TEs can undergo the evolutionary process of "molecular domestication", losing their TE features and acquiring new functions [23], including genetic regulation at both the transcriptional and post-transcriptional levels, the generation of transcription factor binding sites, and RNA editing and translation processes [24,25]. Thus, due to the intense molecular degeneration of *Tc1/Mariner* elements isolated from *Rineloricaria*, it is probable that they act in other functions or remain as inert material in the genome. The *Tc1/Mariner* results also revealed degenerated sequences in the Parodontidae species. However, transcriptional activity of these degenerated sequences was detected in the gonads of this species, which may indicate the synthesis of functionless truncated proteins or the synthesis of new proteins with novel functions, since the presence of ORFs corresponding to proteins with ligation domains derived from *Tc1/Mariner* of Parodontidae were detected [4].

The combination of transposons and RTEs found in *Tc1/Mariner* from *Rineloricaria* may have been the result of unequal crossover between close TEs, or may indicate the insertion of one TE into another [26]. The sequences obtained in the present study could be correlated to miniature

inverted repeat transposable elements (MITEs), since they contain terminal ends with greater identity to *Mariner* and the absence, or intense degeneration, of the transposase domain [21]. Eukaryotic genomes rarely have complete autonomous TEs sequences, with degenerate non-autonomous TEs being more frequent [22].

In conclusion, the data obtained in this study show no association between the *Tc1/Mariner* element, the dispersion of 5S rDNA sites and Rb events in *Rineloricaria*. The sequences obtained could be characterized as being *Tc1/Mariner*-like, highly degenerate and with no TE function. However, these degenerate sequences could be related to other genomic functions in the species studied here.

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