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Chromosomal organization of retrotransposon *Rex*1 in *Astyanax* species (Characiformes, Characidae)

Gabriela Pessenda[a], Diovani Piscor[a], Edson L. da Silva[a,c], Liano Centofante[b] and Patricia P. Parise-Maltempi[a]

[a]Instituto de Biociências, Departamento de Biologia, Laboratório de Citogenética, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Rio Claro, Brazil; [b]Instituto de Biociências, Departamento de Biologia e Zoologia, Laboratório de Genética Animal, Universidade Federal de Mato Grosso (UFMT), Cuiabá, Brazil; [c]Instituto Federal de Educação, Ciência e Tecnologia do Piauí (IFPI), Laboratório de Biologia, Picos, Brazil

ABSTRACT

The cytogenetic studies of retrotranposons *Rex* have been characterized in several fish groups, including species of the *Astyanax* genus. This group presents an extensive variability in their karyotype, with diploid numbers of 2*n* = 52, 50, 48, 46 and 36 chromosomes. Thus, the aim of this study was to evaluate the distribution of the retrotransposable element *Rex*1, in six *Astyanax* species, with different diploid numbers. The species analyzed were *Astyanax altiparanae* (2*n* = 50), *Astyanax asuncionensis* (2*n* = 50), *Astyanax eigenmanniorum* (2*n* = 50), *Astyanax marionae* (2*n* = 48), *Astyanax fasciatus* (2*n* = 46) and *Astyanax schubarti* (2*n* = 36). *Rex*1 was dispersed in the *Astyanax* species, suggesting that these retrotransposons play important role in genome evolution.

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Introduction

The transposable elements are repetitive sequences (i.e., include the transposons and retrotransposons) that can change position within the genome, resulting in structural alterations, e.g. deletions or duplications. These sequences are also called jumping genes (Kazazian and Moran 1998; Biemont and Vieira 2006) and their importance as sources of genetic variations have been important for the evolution of the genomic structure and gene function in vertebrates and other organisms (Feschotte and Pritham 2007).

The retrotransposons encode a reverse transcriptase that acts on the reverse transcription of RNA transcripts into cDNA, resulting in the production of new copies of these elements. They are classified in two groups, according to the presence or absence of long terminal repeats (LTR) (Bohne et al. 2008). Between the non-LTR retrotransposons are *Rex*1, *Rex*3 and *Rex*6, which are absent from mouse and human genomes, but participated in the evolution of teleost fish (Volff et al. 1999, 2000, 2001, 2003).

Given the important roles of retrotransposable elements on fish evolution, their mapping is very important for understanding the genome structure and evolution process in *Astyanax* (Characiformes, Characidae) genus (Silva et al. 2013, 2014; Daniel et al. 2015;. *Astyanax* is an interesting group because present high karyotype diversity, including species with 2*n* = 52, 50, 48, 46 and 36 chromosomes (Tenório et al. 2013; Piscor et al. 2015; Nishiyama et al. 2016; Piscor et al. 2016; Piscor and Parise-Maltempi 2016a, 2016b).

Thus, considering the chromosomal variability found on *Astyanax* group, the aim of this study was to map the location of retrotransposable element (*Rex*1) in six *Astyanax* species with different diploid numbers, in order to verify the distribution pattern in the genome of this group.

Materials and methods

Sampling

Samples of six species of *Astyanax* genus were employed: *A. altiparanae*, *A. asuncionensis*, *A. eigenmanniorum*, *A. fasciatus*, *A. marionae* and *A. schubarti*. *A. altiparanae* and *A. fasciatus* were collected on the Ribeirão Claro river (São Paulo state (SP), Brazil), *A. schubarti* on Piracicaba river (SP, Brazil), *A. marionae* on Rio Claro stream (Mato Grosso (MT), Brazil), *A. asuncionensis* on Bento Gomes river (MT, Brazil) and *A. eigenmanniorum* was obtained from aquaria in Brazil. The cytogenetic preparations were obtained according to methodology described by Foresti et al. (1993).

DNA extraction and sequence analysis

Genomic DNA was extracted from fin and liver samples of *Astyanax* species, employing the phenol-chloroform-isoamyl alcohol technique (Sambrook and Russell

CONTACT Patricia P. Parise-Maltempi parise@rc.unesp.br

2001). Amplification of *Rex*1 sequences were performed by polymerase chain reaction (PCR), in a reaction containing 12.5 µl of PCR mix (Qiagen, Hilden, Germany), 1 µl of each primer (10 mM), 8.5 µl of Milli-Q water and 2 µl of genomic DNA (400 ng). Specific primers used were: *Rex1As* F – 5′CCT GGA TCA CTG ACT ACC T and *Rex1As* R – 5′CAC ACC AAG GTA TTT GTA GG. The PCR reaction followed the general conditions of initial denaturation at 95°C for 5 min, 34 cycles of denaturation at 95°C for 40 s, annealing at 55°C for 40 s, elongation at 72°C for 5 min, and final extension at 72°C for 5 min, with final temperature maintained at 12°C.

The PCR products were purified using ExoSAP-IT (GE Healthcare™, Chigado, IL, USA), and sequenced by MacroGen (Geumcheon-gu, Korea). The nucleotide sequences were aligned and edited with BioEdit Sequence Alignment Editor software (Hall 1999), sequenced, and deposited in GenBank with the accession numbers: MG793236 to MG793240.

Fluorescence in situ hybridization (FISH)

The FISH experiments were performed according to Pinkel et al. (1986) with modifications described by Cabral-de-Mello et al. (2010). Briefly, *Rex*1 sequences were labeled by PCR with biotin-16-UTP or digoxigenin-11-dUTP (Roche, Basel, Switzerland). Mitotic preparations on glass slides were treated with pepsin (10 µg $^{ml-1}$) for 10 min, and RNAse (100 µg $^{ml-1}$) for 1 h, in a moist chamber at 37°C. The slides were dehydrated in 70, 90 and 100% ethanol for 5 min and air-dried. Subsequently, chromosomes were denatured in 70% formamide (in 2 × SSC) for 2 min at 70°C. The slides were denatured in cold 70, 90 and 100% ethanol and hybridization was performed with *Rex*1 labelled with biotin-16-UTP or digoxigenin-11-dUTP for 45 min in a moist chamber at 37°C. The detection of biotin labeled probes was performed with avidin–FITC conjugate (Sigma, St Louis, MO, USA), and the digoxigenin-labeled probes were detected with anti-digoxigenin-Rhodamine (Roche, Basel, Switzerland). The slides were mounted with DAPI and mounted with Vectashield (Vector, Burlingame, CA, USA), and the metaphases were photographed with the digital capture system Olympus model D71/DP Controller software.

Results

This study is a continuation of previous publications from our group (Piscor et al. 2015, 2016; Piscor and Parise-Maltempi 2016a, 2016b). Amplification of *Rex*1 sequences from *Astyanax* species resulted in fragments of approximately 600 bp for all the samples analyzed.

The *Astyanax* species exhibited $2n = 50$ for *A. altiparanae*, *A. asuncionensis* and *A. eigenmanniorum*, $2n = 48$ for *A. marionae*, $2n = 46$ for *A. fasciatus*, and $2n = 36$ for *A. schubarti* (Figure 1). The FISH experiments demonstrated a dispersed chromosomal distribution of *Rex*1 sequences through the chromosomes of the analyzed species (Figure 1).

Discussion

The repetitive sequences have several distribution patterns among different fish groups. Our results demonstrated that *Rex*1 elements present a dispersed pattern through the chromosomes of all species analyzed in this paper.

Other studies have already reported the distribution of retrotransposable elements in the chromosomes of species from *Astyanax* genus (Pansonato-Alves et al. 2013; Silva et al. 2013, 2014; Daniel et al. 2015) (Table 1). Similar to our data, *Rex*1 presented a dispersed pattern of distribution in *A. paranae* (Silva et al. 2014). On the other hand, *Rex*3 sites had co-location with heterochromatic blocks of this species (Silva et al. 2014). According to Pansonato-Alves et al. (2013), two *A. fasciatus* karyomorphs (A with $2n = 46$ and B with $2n = 48$ chromosomes) exhibited *Rex*3 elements on clusters on terminal positions of long arms (Pansonato-Alves et al. 2013). It was suggested that the variations in the heterochromatin distribution might be directly related to the evolutionary dynamics of mobile sequences, which could explain some different *Rex*3/heterochromatic blocks between karyomorphs A and B (Pansonato-Alves et al. 2013).

*Rex*3 clusters have also been observed co-located with heterochromatic blocks and 18S rDNA sites, in two *Astyanax bockmanni* populations, from Capivara River, Tietê River basin and Água da Madalena Stream, Paranapanema River basin, Brazil (Silva et al. 2013). In this species, the co-location of *Rex*3 and 18S rDNA sites, in some chromosomes, arises as a main mechanism of major rDNA dispersion and could be an alternative to explain the high index of polymorphisms of these regions (Silva et al. 2013).

It was previously demonstrated that *Rex*1 distribution varies according to species in the *Leporinus* genus (also in the Characiformes order, but from the Anostomidae family). It was found dispersed throughout the chromosomes from *L. friderici*, *L. lacustris*, and *L. striatus* species, and in isolated clusters in terminal sites of chromosomes from *L. elongatus*, *L. macrocephalus*, and *L. obtusiden*, with signals in the interstitial region of the W sex chromosome (Borba et al. 2013). On the other hand, *Rex*3 had the same distribution pattern in all species, showing terminal isolated clusters and dispersed signals (Borba et al. 2013).

In species of Antarctic fishes belonging to Notothenioidei suborder, *Rex*1 did not present a clear pattern of distribution, and generally was less abundant than *Rex*3, which showed a homogeneous distribution over the chromosomes, with accumulations in some regions (Ozouf-Costaz et al. 2004). In species of the Hypoptopomatinae subfamily, *Rex*1 and *Rex*3 have been

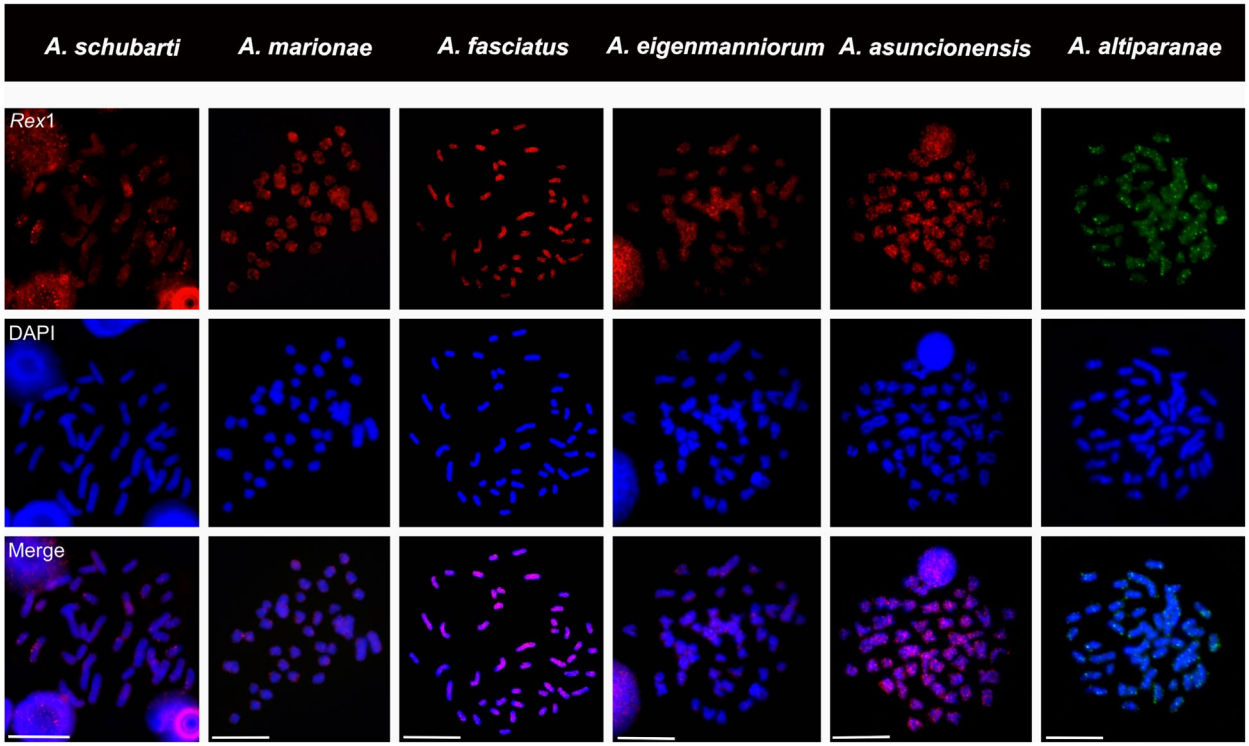

Figure 1. Chromosomal distribution of *Rex*1 in *Astyanax* species with different diploid numbers. Bars = 10 µm.

Table 1. Chromosomal mapping of retrotransposable *Rex* elements distribution in *Astyanax* genus.

Species	Localities/states	2n	*Rex* types	*Rex* distribution	Ref.*
A. fasciatus Kary. A	Tietê river (SP)	46	*Rex*3	Clustered	1
A. fasciatus Kary. B	Tietê river (SP)	48	*Rex* 3	Clustered	1
A. bockmanni	Capivara river (SP)	50	*Rex* 3	Clustered	2
	Água da Madalena stream (SP)	50	*Rex* 3	Clustered	2
A. paranae	Capivara river (SP)	50+B	*Rex*3	Clustered	3
	Capivara river (SP)	50+B	*Rex*1	Scattered	3
A. bockmanni	Alambari stream (SP)	50+B	*Rex*1/ *Rex*3/ *Rex*6	Dispersed	4
	Véu de Noiva waterfall (SP)	50	*Rex*1/ *Rex*6	Dispersed	4
			*Rex*3	Clustered	4
	Batalha river (SP)	50	*Rex*1/ *Rex*6	Dispersed	4
			*Rex*3	Clustered	4
	Campo Novo stream (SP)	50	*Rex*1/ *Rex*3/ *Rex*6	Dispersed	4
	Barra Seca stream (SP)	50	*Rex*1/ *Rex*6	Dispersed	4
			*Rex*3	Clustered	4
	Capivara river (SP)	50	*Rex*1/ *Rex*6	Dispersed	4
			*Rex*3	Clustered	4
A. altiparanae		50	*Rex*1	Scattered	PS
A. asuncionensis		50	*Rex*1	Scattered	PS
A. eigenmanniorum		50	*Rex*1	Scattered	PS
A. marionae		48	*Rex*1	Scattered	PS
A. fasciatus		46	*Rex*1	Scattered	PS
A. schubarti		36	*Rex*1	Scattered	PS

*1 – Pansonato-Alves et al. (2013); 2 – Silva et al. (2013); 3 – Silva et al. (2014); 4 – Daniel et al. (2015). Abbreviations: B, presence of B chromosome; Kary., karyomorph; SP, São Paulo state; PS, present study; Ref., references.

found in small clusters and dispersed, both on heterochromatic and euchromatic regions (Ferreira et al. 2011).

These retrotransposable elements are also found on heterochromatic regions of fish chromosomes (Bouneau et al. 2003; Fischer et al. 2004; Mazzuchelli and Martins 2009; Teixeira et al. 2009; Valente et al. 2011). The explanations for this pattern is that these elements may accumulate on sites of low recombination rates and coding functions, in order to avoid deleterious effects if inserted within genes, or as a consequence of their role in regulating specific regions, such as pericentromeric and telomeric sites (Valente et al. 2011).

The differences between *Rex* elements localizations may reflect distinct evolutionary factors guiding these

sequence distributions. However, it is also not possible to rule out the effects of distinct hybridization conditions employed in the experiments from different studies, such as high-stringency conditions that might mask the signals (Ferreira et al. 2011).

After analyzing our studies and comparing them with others already existing in the literature we can consider that *Rex*1 is dispersed and *Rex*3 is clustered in the *Astyanax* species. These retrotransposons may play an important role in genome evolution.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Biemont C, Vieira C. 2006. Genetics: junk DNA as an evolutionary force. Nature. 443:521–524.

Bohne A, Brunet F, Galiana-Arnoux G, Schultheis C, Volff JN. 2008. Transposable elements as drivers of genomic and biological diversity in vertebrates. Chromosome Res. 16:203–215.

Borba RS, Silva EL, Parise-Maltempi PP. 2013. Chromosome mapping of retrotransposable elements *Rex*1 and *Rex*3 in *Leporinus* Spix, 1829 species (Characiformes: Anostomidae) and its relationships among heterochromatic segments and W sex chromosome. Mob Genet Elements. 3:e27460.

Bouneau L, Fischer C, Ozouf-Costaz C, Froschauer A, Jaillon O, Coutanceau JP, Korting C, Weissenbach J, Bernot A, Volff JN. 2003. An active non-LTR retrotransposon with tandem structure in the compact genome of the pufferfish *Tetraodon nigroviridis*. Genome Res. 13:1686–1695.

Cabral-de-Mello DC, Moura RC, Martins C. 2010. Chromosomal mapping of repetitive DNAs in the beetle *Dichotomius geminatus* provides the first evidence for an association of 5S rRNA and histone H3 genes in insects, and repetitive DNA similarity between the B chromosome and A complement. Heredity. 104:393–400.

Daniel SN, Penitente M, Silva DM, Hashimoto DT, Ferreira DC, Foresti F, Porto-Foresti F. 2015. Organization and Chromosomal Distribution of Histone Genes and Transposable *Rex* Elements in the Genome of *Astyanax bockmanni* (Teleostei, Characiformes). Cytogenet Genome Res. 146:311–318.

Ferreira DC, Oliveira C, Foresti F. 2011. Chromosome mapping of retrotransposable elements *Rex*1 and *Rex*3 in three fish species in the subfamily Hypoptopomatinae (Teleostei, Siluriformes, Loricariidae). Cytogenet Genome Res. 132:64–70.

Feschotte C, Pritham EJ. 2007. DNA transposons and the evolution of eukaryotic genomes. Annu Rev Genet. 41:331–368.

Fischer C, Bouneau L, Coutanceau JP, Weissenbach J, Volff JN, Ozouf-Costaz C. 2004. Global heterochromatic colocalization of transposable elements with minisatellites in the compact genome of the pufferfish *Tetraodon nigroviridis*. Gene. 336:175–183.

Foresti F, Oliveira C, Foresti de Almeida-Toledo L. 1993. A method for chromosome preparations from large fish specimens using *in vitro* short-term treatment with colchicine. Experientia. 49:810–813.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser. 41:95–98.

Kazazian HH, Moran JV. 1998. The impact of L1 retrotransposons on the human genome. Nat Genet. 19:19–24.

Mazzuchelli J, Martins C. 2009. Genomic organization of repetitive DNAs in the cichlid fish *Astronotus ocellatus*. Genetica. 136:461–469.

Nishiyama PB, Vieira MM, Porto FE, Borin LA, Portela-Castro AL, Santos IC. 2016. Karyotypic diversity among three species of the genus *Astyanax* (Characiformes: Characidae). Braz J Biol. 76:360–366.

Ozouf-Costaz C, Brandt J, Körting C, Pisano E, Bonillo C, Coutanceau JP, Volff JN. 2004. Genome dynamics and chromosomal localization of the non-LTR retrotransposons Rex1 and Rex3 in Antarctic fish. Antarct Sci. 16:51–57.

Pansonato-Alves JC, Hilsdorf AW, Utsunomia R, Silva DM, Oliveira C, Foresti F. 2013. Chromosomal mapping of repetitive DNA and cytochrome C oxidase I sequence analysis reveal differentiation among sympatric samples of *Astyanax* fasciatus (Characiformes, Characidae). Cytogenet Genome Res. 141:133–142.

Pinkel D, Straume T, Gray JW. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci U S A. 83:2934–2938.

Piscor D, Parise-Maltempi PP. 2016a. Chromosomal mapping of H3 histone and 5S rRNA genes in eight species of *Astyanax* (Pisces, Characiformes) with different diploid numbers: syntenic conservation of repetitive genes. Genome. 59:167–172.

Piscor D, Parise-Maltempi PP. 2016b. Microsatellite Organization in the B Chromosome and A Chromosome Complement in *Astyanax* (Characiformes, Characidae) Species. Cytogenet Genome Res. 148:44–51.

Piscor D, Alves AL, Parise-Maltempi PP. 2015. Chromosomal microstructure diversity in three *Astyanax* (Characiformes, Characidae) species: comparative analysis of the chromosomal locations of the 18S and 5S rDNAs. Zebrafish. 12:81–90.

Piscor D, Centofante L, Parise-Maltempi PP. 2016. Highly Similar Morphologies Between Chromosomes Bearing U2 snRNA Gene Clusters in the Group *Astyanax* Baird and Girard, 1854 (Characiformes, Characidae): An Evolutionary Approach in Species with 2n = 36, 46, 48, and 50. Zebrafish. 13:565–570.

Sambrook J, Russell DW. 2001. Molecular cloning: a laboratory manual. New York, NY: Cold Spring Harbor Laboratory Press; p. 2100.

Silva DM, Pansonato-Alves JC, Utsunomia R, Daniel SN, Hashimoto DT, Oliveira C, Porto-Foresti F, Foresti F. 2013. Chromosomal organization of repetitive DNA sequences in *Astyanax bockmanni* (Teleostei, Characiformes): dispersive location, association and co-localization in the genome. Genetica. 141:329–336.

Silva DM, Pansonato-Alves JC, Utsunomia R, Araya-Jaime C, Ruiz-Ruano FJ, Daniel SN, Hashimoto DT, Oliveira C, Camacho JP, Porto-Foresti F, Foresti F. 2014. Delimiting the origin of a B chromosome by FISH mapping, chromosome painting and DNA sequence analysis in *Astyanax paranae* (Teleostei, Characiformes). PLoS One. 9: e94896.

Teixeira WG, Ferreira IA, Cabral-de-Mello DC, Mazzuchelli J, Valente GT, Pinhal D, Poletto AB, Venere PC, Martins C. 2009. Organization of repeated DNA elements in the genome of the cichlid fish *Cichla kelberi* and its contributions to the knowledge of fish genomes. Cytogenet Genome Res. 125:224–234.

Tenório RC, Vitorino CA, Souza IL, Oliveira C, Venere PC. 2013. Comparative cytogenetics in *Astyanax* (Characiformes: Characidae) with focus on the cytotaxonomy of the group. Neotrop Ichthyol. 11:553–564.

Valente GT, Mazzuchelli J, Ferreira IA, Poletto AB, Fantinatti BE, Martins C. 2011. Cytogenetic mapping of the retroelements *Rex*1, *Rex*3 and *Rex*6 among cichlid fish: new insights on the chromosomal distribution of transposable elements. Cytogenet Genome Res. 133:34–42.

Volff JN, Korting C, Sweeney K, Schartl M. 1999. The non-LTR retrotransposon *Rex*3 from the fish *Xiphophorus* is widespread among teleosts. Mol Biol Evol. 16:1427–1438.

Volff JN, Korting C, Schartl M. 2000. Multiple lineages of the non-LTR retrotransposon *Rex*1 with varying success in invading fish genomes. Mol Biol Evol. 17:1673–1684.

Volff JN, Korting C, Froschauer A, Sweeney K, Schartl M. 2001. Non-LTR retrotransposons encoding a restriction enzyme-like endonuclease in vertebrates. J Mol Evol. 52:351–360.

Volff JN, Bouneau L, Ozouf-Costaz C, Fischer C. 2003. Diversity of retrotransposable elements in compact pufferfish genomes. Trends Genet. 19:674–678.