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Research Article

Scattered organization of the histone multigene family and transposable elements in *Synbranchus*

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Abstract

The fish species *Synbranchus marmoratus* is widely distributed throughout the Neotropical region and exhibits a significant karyotype differentiation. However, data concerning the organization and location of the repetitive DNA sequences in the genomes of these karyomorphs are still lacking. In this study we made a physical mapping of the H3 and H4 histone multigene family and the transposable elements *Rex1* and *Rex3* in the genome of three known *S. marmoratus* karyomorphs. The results indicated that both histone sequences seem to be linked with one another and are scattered all over the chromosomes of the complement, with a little compartmentalization in one acrocentric pair, which is different from observations in other fish groups. Likewise, the transposable elements *Rex1* and *Rex3* were also dispersed throughout the genome as small clusters. The data also showed that the histone sites are organized in a differentiated manner in the genomes of *S. marmoratus*, while the transposable elements *Rex1* and *Rex3* do not seem to be compartmentalized in this group.

Key words: Synbranchidae, FISH, histone, retrotransposon.

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Introduction

The genomes of eukaryotic organisms are characterized by a large number of repetitive DNA segments. These sequences are identifiable by a high variability in their nucleotide composition, number of copies, function, distribution and organization in the genome (Wagner *et al.*, 1993; Charlesworth *et al.*, 1994). Generally, these sequences may be classified as coding sequences, represented by ribosomal and histone multigene families, and noncoding sequences, repeated *in tandem* or dispersed throughout the genome (Sumner, 2003; Nagoda *et al.*, 2005)

The repetitive nature of these sequences makes them ideal for the development of probes for use in fluorescence *in situ* hybridization (FISH). Studies related to the organization and physical mapping of these types of sequences have enabled a better characterization of the biodiversity and karyoevolution of the ichthyofauna (Vicari *et al.*, 2010). Furthermore, these data have also helped advancing in the knowledge of the organization, diversification, evolution and possible role of repetitive DNA sequences in the genome of vertebrates (Haff *et al.*, 1993; Martins and Galetti Jr, 1999; Gursel *et al.*, 2003). However, the vast majority of mapping studies carried out on Neotropical fishes were focused on the location of ribosomal sites, and there is still very little information regarding other types of sequences, such as histone genes and transposable elements (TEs). Even though studies on physical mapping of histone genes and TEs are few, interesting features about those sequences have been revealed, such as association with other repetitive families (Cioffi *et al.*, 2010; Hashimoto *et al.*, 2011, 2013; Lima-Filho *et al.*, 2012), distinct modes of organization (Valente *et al.*, 2011; Ferreira *et al.*, 2011a), and influences on karyotype diversification (Pansonato-Alves *et al.*, 2013a).

Although known as a single taxonomic entity, *S. marmoratus* (Synbranchiformes, Synbranchidae) displays considerable cytogenetic diversity. As a result, there are distinct karyotype variants (Foresti *et al.*, 1992; Melilo *et al.*, 1996; Sánchez and Fenocchio, 1996; Torres *et al.*, 2005), resulting in five well-differentiated main karyomorphs (unpublished data). Individuals in karyomorph groups A and B have chromosome number 2n = 42. In addition, a pericentric inversion in a submetacentric chromosome of karyomorph A is related to the origin of karyomorph B. In contrast, analyses of chromosome rearrangements cannot definitively explain the origins of karyomorphs C (2n = 46), D and E (2n = 46), because the events responsible for their diploid chromosome numbers seem to originate from undependable and bidirectional events (unpublished data). This study describes the cyto-

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genetic mapping of the histone sequences H3 and H4 and of the transposable elements *Rex1* and *Rex3* in samples from three *S. marmoratus* karyomorphs. The purpose of this study was to investigate the distribution patterns of each element in this group.

Material and Methods

Samples

Mitotic chromosomes were obtained from kidney tissue, as described by Foresti *et al.* (1981), from specimens of karyomorphs A, B and E, collected at different Brazilian locations, as specified in Table 1 and Figure 1. All samples were collected in accordance with the Brazilian Environmental Law (Collection permission MMA/IBAMA/SISBIO - Nr. 3245), and the procedures for fish collection, maintenance and analysis were performed in compliance with the Brazilian College of Animal Experimentation (COBEA) and approved (protocol Nr. 503) by the Bioscience Institute/UNESP Ethics Committee on Use of Animals (CEUA). After the analyses, the fishes were fixed in 10% formalin, conserved in 70% ethanol and deposited in the fish collection of the Fish Biology and Genetics Laboratory (Laboratório de Biologia e Genética de Peixes) - UNESP, Botucatu, São Paulo, Brazil. Voucher information is also presented in Table 1.

Isolation of repetitive DNA sequences and FISH

Genomic DNA of *S. marmoratus* (karyomorph A) was extracted using the Wizard Genomic DNA Purification Kit (Promega). Partial sequences of the histone genes H3 and H4 and the retrotransposable elements *Rex1* and *Rex3* were obtained by polymerase chain reaction (PCR) using previously described primers (White *et al.*, 1990; Colgan *et al.*, 1998; Volff *et al.*, 1999, 2000; Pineau *et al.*, 2005). During the secondary PCR assay, the H3 and H4 histone sequences were labeled with biotin-16-dUTP (Roche), and the *Rex1* and *Rex3* TEs and 18S rDNA with digoxigenin-11-dUTP (Roche), by incorporating these modified nucleotides.

FISH was performed using the method described by Pinkel *et al.* (1986). Slides were incubated with RNase

Table 1 - *Synbranchus marmoratus* specimens analyzed.

Locality	River Basin	Karyomorph (n)	Map	Coordinates	LBP
Bataguassu - MS	Paraná	A (3)	2	S 21°38'49" - W 52°17'52"	11355
Guaíra - PR	Paraná	B (20)	3	S 24°04'13" - W 54°12'08"	11364
Igaraçu do Tietê - SP	Tietê	E (2)	1	S 22°34'43" - W 48°27'48"	17519

n = number of samples; LBP = deposit number in the fish collection of the Fish Biology and Genetics Laboratory (Instituto de Biociências de Botucatu, UNESP).

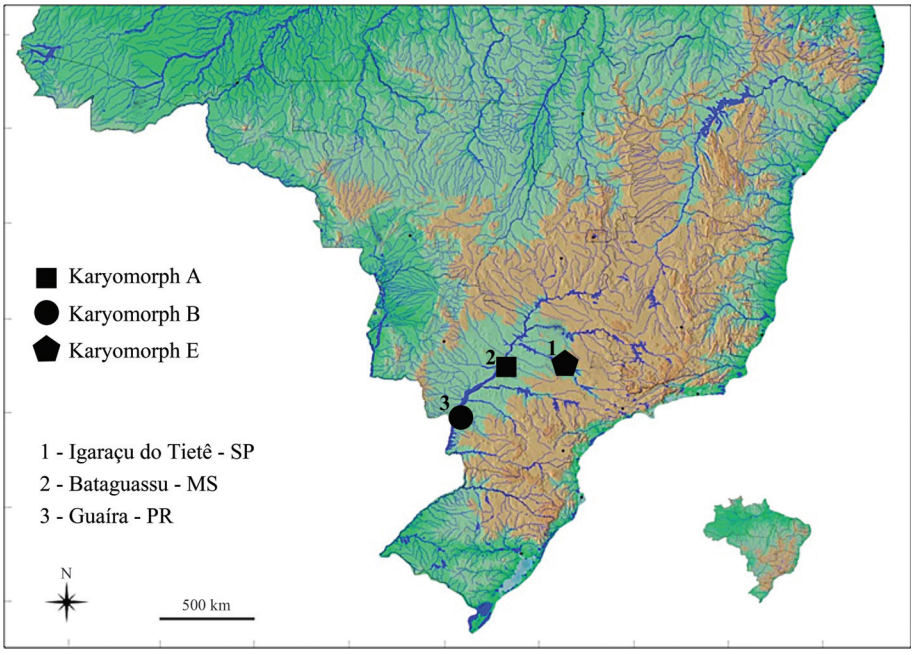

Figure 1 - Map showing the *S. marmoratus* specimen collection sites. The numbers indicate the sample locality, while symbols represent the karyomorphs found in each locality.

(50 µg/mL) for 1 h at 37 °C. Then, the chromosomal DNA was denatured in 70% formamide in 2x SSC for 5 min at 70 °C. For each slide, 30 µL of hybridization solution (containing 200 ng of each labeled probe, 50% formamide, 2x SSC and 10% dextran sulphate) were denatured for 10 min at 95 °C, dropped onto the slides and hybridized overnight at 37 °C in a 2x SSC moist chamber. After hybridization, the slides were washed in a 0.2x SSC solution with 15% formamide for 20 min at 42 °C, followed by a second wash in 0.1x SSC for 15 min at 60 °C, and a final wash in 4x SSC with 0.5% Tween for 10 min at room temperature. Probe detection was carried out with Avidin-FITC (Sigma) or anti-digoxigenin-rhodamine (Roche), the chromosomes were counterstained with DAPI (4',6-diamidino-2-phenylindole, Vector Laboratories), and the FISH images were captured by an optical photomicroscope (Olympus, BX61) with the Image Pro Plus 6.0 software (Media Cybernetics).

Results

Cytogenetic analysis

All repetitive probes used here were clearly visualized in the mitotic chromosomes. Both H3 and H4 histone sequences appeared to be clustered together and were distributed in a general pattern with dispersed signals on all chromosomes. Additionally, both sequences were accumulated in one acrocentric pair (Figure 2a-f).

Since the histone sites mapped until now in fishes were found in conspicuous blocks, we used the double-FISH technique (18S rDNA + H3 histone sequences), in order to compare the hybridization patterns of both probes and check the veracity of the dispersed signal pattern of the histone sequences. Double-FISH confirmed that, as expected, in *Synbranchus* the histone sites are dispersed throughout the genome, while the 18S rDNA sites are only present in one big cluster (Figure 3a-d).

Similarly, the *Rex1* and *Rex3* TEs are arranged in small clusters that are also dispersed throughout the genome (Figure 4a-f). However, in the individuals belonging to karyomorph A collected at Bataguassu, only the *Rex3* elements demonstrated significant accumulation in chromosome pair 3 (Figure 4d).

Discussion

Histone genes constitute a complex multigene family and may show variations in copy number and organization within the genome (Kedes, 1979). Thus, some species may present up to a few thousand histone gene copies, usually

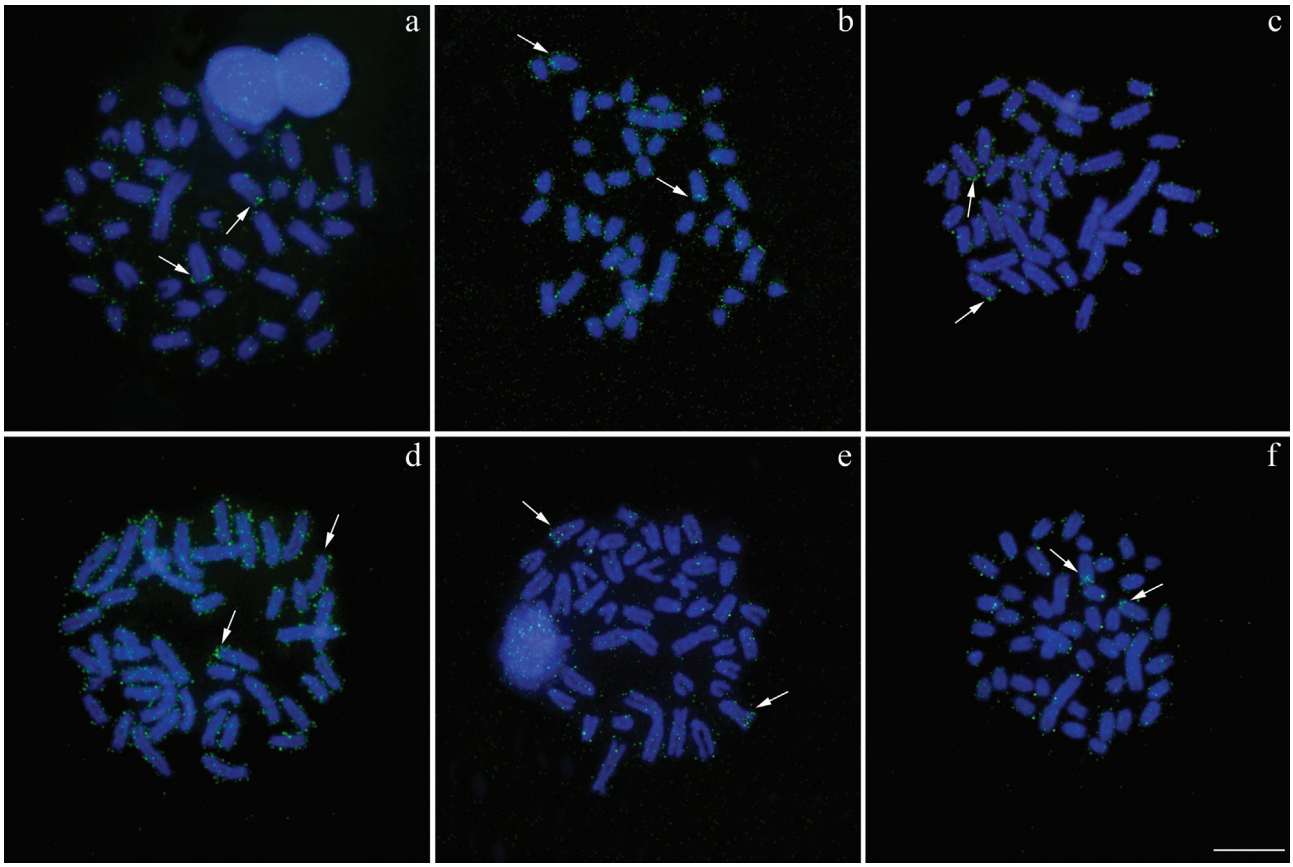

Figure 2 - Metaphases after FISH treatment using probes of H3 and H4 histone sequences, respectively, in karyomorphs A (a, d), B (b, e), and E (c, f). Arrows indicate the histone site clustering in the acrocentric pair. Bar = 10 µm.

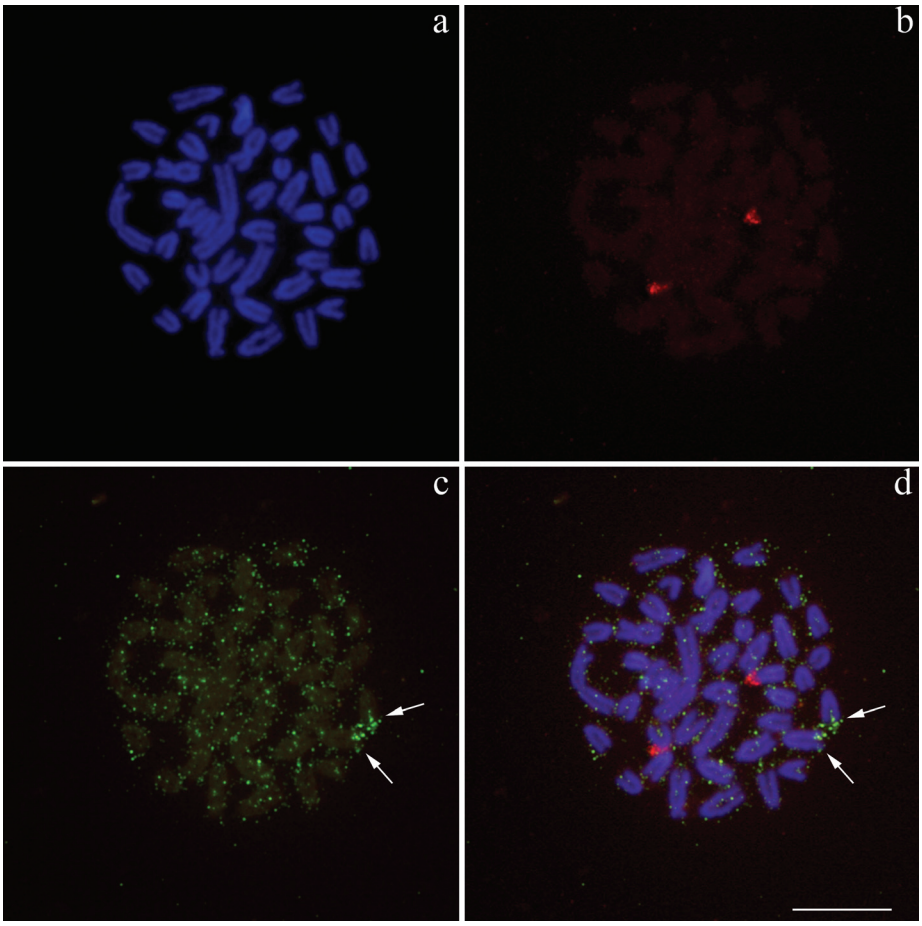

Figure 3 - Karyomorph A metaphase (a) evidencing the 18S rDNA (b) and H3 histone gene (c) hybridization patterns; (d) DAPI-stained metaphase with overlapping images of the 18S rDNA and H3 histone gene. Arrows indicate the histone site clustering in the acrocentric pair. Bar = 10 μm.

organized into tandemly repeated copies, while in others these sequences may be dispersed throughout the genome in small groups of copies (reviewed in Rooney *et al.*, 2002).

In fish species, data about chromosomal location of histone sequences are restricted to eight species mapped for H1 genes (Pendás *et al.*, 1994; Hashimoto *et al.*, 2011, 2013; Lima-Filho *et al.*, 2012) and only five species with mapped H3 sites (Pansonato-Alves *et al.*, 2013a, 2013b; Silva *et al.*, 2013), all of them presenting histone sequences as conspicuous blocks in chromosomes. Despite the restricted sampling, these studies revealed some particular characteristics, such as differential dispersion of sites and association with 5S or 18S rDNA (Hashimoto *et al.*, 2011, 2013; Lima-Filho *et al.*, 2012; Pansonato-Alves *et al.*, 2013a, 2013b). More recent studies also showed that H1, H3 and H4 histone genes are clustered at the same site in fishes of genus *Astyanax* (Pansonato-Alves *et al.*, 2013b; Silva *et al.*, 2013). Our present results show that H3 and H4 histone sequences appear to be linked to one another and that in *Synbranchus* their dispersion occurs in small clusters throughout the genome. Moreover, in some acrocentric chromosome pairs there may be a small accumulation of these sites, which may be considered the major histone clusters, characterizing a distinct mode of organization of these sequences in fish chromosomes.

To date, the mapped sites of histone sequences in fishes of distinct orders such as Characiformes (Hashimoto *et al.*, 2011, Pansonato-Alves *et al.*, 2013a), Siluriformes (Hashimoto *et al.*, 2013; Pansonato-Alves *et al.*, 2013a) and Perciformes (Lima-Filho *et al.*, 2012) are shown as large chromosomal blocks. The distinct organization and distribution of the H3 and H4 histone sites in *S. marmoratus* lead to the conclusion that, in this group, these sites are organised in small and abundant repetitions throughout the genome. This extensive distribution may be attributed to one of the following factors: (*i*) extensive occurrence of orphon genes derived from *in-tandem* repetitive families in eukaryotes, as already demonstrated for histone and ribosomal genes (Childs *et al.*, 1981; Eirín-López *et al.*, 2004) and likely to be related to a birth-and-death evolutionary mechanism (Eirín-López *et al.*, 2004); or (*ii*) associations between histone sequences and transposable elements (TEs) due to the similarity of their distribution patterns mapped in fishes (Ferreira *et al.*, 2011a, 2011b).

Just as for histone sites, the physical mapping of TEs in representatives of the Neotropical ichthyofauna is re-

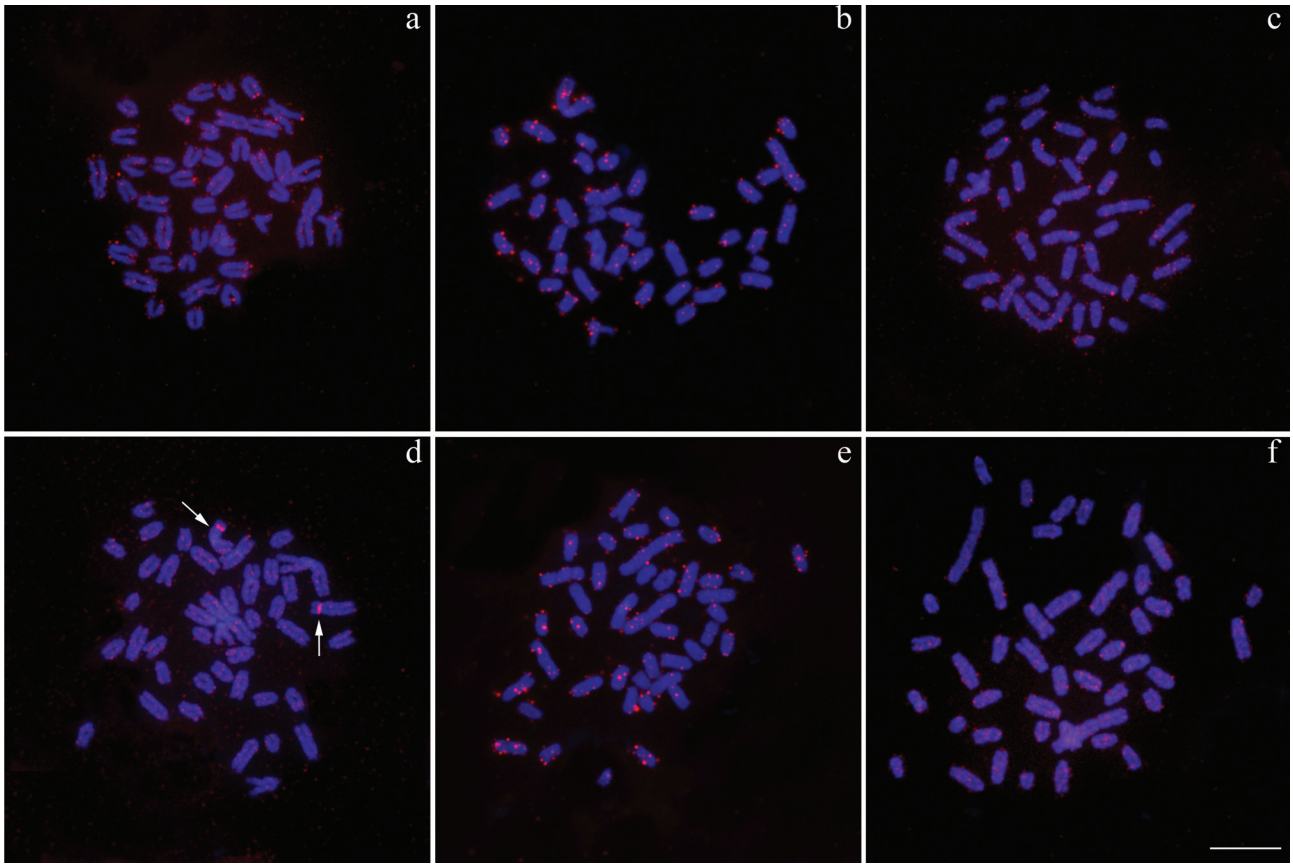

Figure 4 - Metaphases after FISH treatment using *Rex1* and *Rex3* probes, respectively, in karyomorphs A (a, d), B (b, e), and E (c, f). Arrows indicate the clustering of *Rex3* sites in the centromere of a submetacentric pair. Bar = 10 μm.

stricted to a small number of species and to the non-LTR retrotransposons *Rex1*, *Rex3* and *Rex6* (Gross *et al.*, 2009; Cioffi *et al.*, 2010; Valente *et al.*, 2011; Ferreira *et al.*, 2011a,b; Pansonato-Alves *et al.*, 2013a). The overlap of signals generated by FISH among TEs and other repetitive sequences raises questions about their role in the dispersion of repetitive DNA sequences (Mandrioli *et al.*, 2001; Mandrioli and Manicardi, 2001; Cioffi *et al.*, 2010). In *S. marmoratus,* the *Rex1* and *Rex3* elements are found in small clusters dispersed over all chromosomes. Notably, an accentuated accumulation of repetitions in the centromeric region of pair 3 was found in samples from Bataguassu (karyomorph A); however, this seems to be only a local amplification because the individuals of karyomorph B, which is believed to be recently derived from karyomorph A (unpublished data), did not present such blocks in this chromosome pair.

It is further worth noting that these elements may present variable modes of chromosomal distribution in different species, but tend to be distributed in a similar manner in close groups. The *Rex1*, *Rex3* and *Rex6* elements, for example, are primarily compartmentalized in the pericentromeric heterochromatic regions in Cichlid fishes (Teixeira *et al.*, 2009; Valente *et al.*, 2011) and dispersed throughout the genome in Loricariidae, Bathydraconidae and Artedidraconidae species (Ozouf-Costaz *et al.*, 2004; Ferreira *et al.*, 2011a; Pansonato-Alves *et al.*, 2013a). However, in Characiform species, *Rex3* elements may also be compartmentalized (Cioffi *et al.*, 2010; Pansonato-Alves *et al.*, 2013b; Silva *et al.*, 2013), indicating that those elements are highly dynamic and their type of genomic organization does not reflect phylogenetic relationships among species.

Although *S. marmoratus* presents a remarkable variation in karyotype macrostructure, the physical mapping of histone sequences and transposable elements revealed that these sequences are all dispersed in different karyomorphs, and this seems to be a conserved feature. Thus, we stress the importance of further studies regarding the physical mapping of H1, H3 and H4 histone genes and other repetitive DNAs, which may be useful in determining the organization of these genes in eukaryote genomes. Similarly, the mapping of transposable elements can bring new perspectives on the genomic organization, dispersion of genes and speciation driven by those sequences.

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