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Comparative Cytogenetic Analysis of the Genus *Symphysodon* (Discus Fishes, Cichlidae): Chromosomal Characteristics of Retrotransposons and Minor Ribosomal DNA

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Key Words
Fluorescent in situ hybridization · Heterochromatin · Meiosis · Mitosis · 5S rDNA · *Rex3*

Abstract
As part of a genetic screening program for wild Discus fishes, we analyzed karyotypes and cytogenetic characteristics of *Symphysodon aequifasciatus*, *S. discus* and *S. haraldi* using C-banding and fluorescent in situ hybridization (FISH) with the *Rex3* retrotransposon and 5S rDNA probes in mitotic and meiotic chromosomes. In the 3 species, diploid chromosome number was 2n = 60 and karyotypes contained predominantly meta-submetacentric chromosomes. C-banding showed blocks of constitutive heterochromatin mainly in the pericentromeric region. Physical mapping of repetitive 5S rDNA sequences and *Rex3* retrotransposons in mitotic and meiotic chromosomes showed partial colocalization of constitutive heterochromatin and repetitive elements. Correlations among the accumulation of repetitive elements, heterochromatinization and chromosome rearrangements have been hypothesized to explain the karyotype differentiation in the *Symphysodon* genus. The role of repetitive elements in adaptation to highly diverse habitats, as well as in the generation of the phenotypic and genetic variability found in wild Discus populations, needs to be further investigated.

The wild Discus fish constitutes an excellent model for the study of evolution. Endemic to the Amazon Basin, the 3 currently recognized species of the genus *Symphysodon* (*S. aequifasciatus*, *S. discus*, and *S. haraldi*, Cichlidae, Perciformes) [Bleher et al., 2007] have high degrees of genetic variability associated with different types of biotopes [Bleher et al., 2007; Farias and Hrbek, 2008]. These species have long been the subject of taxonomic and phylogenetic studies at both the morphological and molecular levels, mainly due to their wide exploitation as ornamental fish [Kullander, 1996; 1998; Ready et al., 2006; Bleher et al., 2007; Farias and Hrbek, 2008]. Classical cytogenetic analyses revealed that the 3 species of *Symphysodon* have the highest diploid number encountered in

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Table 1. Chromosome dispersion pattern of *Rex3* elements in different species of fishes

Species (order)	Pattern of distribution	Reference
Artedidraco shackletoni (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Astronotus ocellatus[a] (Perciformes)	Compartmentalized	Mazzuchelli and Martins, 2008
Bovichtus angustifrons (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Chionodraco hamatus (Perciformes)	Compartmentalized	Ozouf-Costaz et al., 2004
Dissostichus mawsoni (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Gymnodraco acuticeps (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Gymnodraco victori (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Neopagetopsis ionah (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Notothenia coriiceps (Perciformes)	Compartmentalized	Ozouf-Costaz et al., 2004
Patagonotothen tesselata (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Symphysodon aequifascistus (Perciformes)	Compartmentalized	Present paper
Symphysodon discus (Perciformes)	Compartmentalized	Present paper
Symphysodon haraldi (Perciformes)	Compartmentalized	Present paper
Tetraodon nigroviridis (Tetraodontiformes)	Compartmentalized	Fisher et al., 2004
Trematomus hansoni (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Trematomus newnesi (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Trematomus bernacchii (Perciformes)	Uniform	Ozouf-Costaz et al., 2004
Trematomus pennellii (Perciformes)	Uniform	Ozouf-Costaz et al., 2004

[a] Ao*Rex3* probe.

the family (2n = 60), with predominantly meta-submetacentric chromosomes in the karyotype, an absence of sex heteromorphic chromosomes and the presence of large blocks of constitutive heterochromatin [Ohno and Atkin, 1966; Thompson, 1979; Takai et al., 2002; Mesquita et al., 2008]. All of these characteristics are considered derivative in the Cichlidae family [Feldberg et al., 2003]. Furthermore, *S. aequifasciatus* and *S. haraldi* have the largest meiotic chromosome chains described for vertebrates, comprising as many as 20 elements in males and females, the origin of which may be based on a series of translocations involving heterochromatic regions [Gross et al., 2009].

Heterochromatin in fish genomes is rich in repetitive sequences and these sequences have been the focus of studies on centromeric and telomeric structure [Lanfredi et al., 2001; Vicente et al., 2001], the origin and evolution of sex chromosomes [Devlin et al., 1998; Stein et al., 2001; Venere et al., 2004], supernumerary chromosomes [Mestriner et al., 2000; Jesus et al., 2003] and the evolution of genomes [Dasilva et al., 2002]. Prominent among these repetitive sequences are transposable elements and ribosomal genes. Transposable elements are sequences capable of integrating into new locations of the genome and can mobilize non-autonomous sequences [Volff et al., 2003; Ozouf-Costaz et al., 2004]. Among retrotransposable elements, *Rex* comprises various families of transposable elements that are abundant in teleosts, and *Rex3* has the greatest distribution and a different pattern of organization in the genome for each species (table 1). Other important repetitive sequences are rDNA sequences that code for rRNA. These sequences are divided into 2 multigenic families repeated in tandem. The first class, represented by 45S rDNA, comprises the transcribing regions of the 28S, 18S and 5.8S ribosomal RNAs and an intergenic spacer. The second class, 5S rDNA, consists of a highly conserved coding sequence of the 5S rRNA and a non-transcribed spacer of variable size [Martins, 2007].

To obtain a better understanding of chromosomal organization and karyotype evolution, the present study analyzes the physical chromosome mapping of sequences of the non-LTR retrotransposon *Rex3* and 5S rDNA repeats in the 3 wild species of the genus *Symphysodon*.

Material and Methods

Individuals were collected in the state of Amazonas, Brazil, with the authorization of IBAMA (Brazilian Institute of Environment and Renewable Natural Resources; no. 10609-1/2007) and were identified based on the most recently proposed characteristics for the genus [Bleher et al., 2007]. Eight males and 3 females

of green Discus *(S. aequifasciatus)* were collected from the Tefé River, 15 males and 3 females of Heckel Discus *(S. discus)* were collected from the Negro River (in the proximity of the municipality of Novo Airão) and 15 males and 3 females of blue/brown Discus *(S. haraldi)* were collected from the Manacapuru River. All fishes were anesthetized with ice water and sacrificed. Voucher specimens were deposited in the fish collection of the INPA (National Amazon Research Institute; INPA 28582, INPA 28583 and INPA 28584), and other individuals were deposited in the fish collection of the INPA Animal Genetics Laboratory.

Preparation of Chromosomes and Detection of Heterochromatic Regions

Mitotic and meiotic chromosomes were prepared from kidney and gonadal cells from male and female specimens of *S. aequifasciatus*, *S. discus* and *S. haraldi* following the protocols of Moreira-Filho and Bertollo [1990] and Gross et al. [2009], respectively. The pharmaceutical compound Munolan (Allergan Frumtost) was used to stimulate mitotic cell division following the method of Molina [2001]. Constitutive heterochromatin was detected using the method of Sumner [1972].

DNA Extraction and Amplification of Rex3 *and 5S rDNA Units*

Total genomic DNA was extracted from the muscles of *Symphysodon aequifasciatus* (green Discus)*, Symphysodon discus* (Heckel Discus) and *Symphysodon haraldi* (blue/brown Discus) following the phenol-chloroform protocol detailed by Sambrook and Russell [2001]. Amplification by PCR of the *Rex3* retrotransposon was conducted using the following primers: RTX3-F3 (5′-CGG TGA YAA AGG GCA GCC CTG) and RTX3-R3 (5′-TGG CAG ACN GGG GTG GTG GT) [Volff et al., 1999; 2001]. In order to obtain repetitive units of 5S rDNA through PCR, the primers 5Sa (5′-TAC GCC CGA TCT CGT CCG ATC) and 5Sb (5′-CAG GCT GGT ATG GCC GTA AGC) were used [Komiya and Takemura, 1979]. The PCR reactions were carried out in a final volume of 25 μl consisting of 1 μl of genomic DNA (100 ng), 2.5 μl of 10× buffer with magnesium chloride (1.5 mM), 0.25 μl of Taq DNA Polymerase (5 U/μl), 1.5 μl of dNTP (1 mM), 1.5 μl of each primer (5 mM) and milli-Q water to complete the volume. Cycling conditions were as follows: (a) *Rex3:* 2 min at 95°C (denaturation); 35 cycles of 1 min at 95°C, 40 s at 55°C (annealing) and 2 min at 72°C (extension); 5 min at 72°C (final extension); (b) 5S rDNA: 5 min at 94°C (denaturation); 2 cycles of 1 min at 95°C, 30 s at 61°C and 45 s at 72°C; 2 cycles of 1 min at 95°C, 30 s at 59°C and 45 s at 72°C; 2 cycles of 1 min at 95°C, 30 s at 57°C and 45 s at 72°C; 25 cycles of 1 min at 95°C, 30 s at 61°C and 45 s at 72°C (extension); 7 min at 72°C (final extension).

In situ Fluorescent Hybridization

The *Rex3* and 5S rDNA probes were labeled with biotin-14-dATP by nick translation (BioNick Labeling System, Invitrogen). Homologous and heterologous FISH were carried out based on the protocols described by Pinkel et al. [1986] and Martins and Galetti, Jr. [2001], with modifications. Mitotic chromosomal DNA was denatured for 10 s in 70% formamide in 2× SSC [17.53 g of sodium chloride (0.29 M), 8.82 g of sodium citrate and distilled water in a final volume of 1,000 ml, pH 7.0] at 67°C; meiotic chromosomal DNA was denatured for 15 s. The hybridization solution (100 ng of denatured probe, 10 mg/ml dextran sulfate (2× SSC) and 50% formamide in a final volume of 30 μl) was placed on the slide and hybridized at 37°C overnight in a humid chamber (2× SSC). Slides were washed post hybridization at 72°C in 2× SSC, pH 7.0, for 5 min. The slides were then immersed in PBD buffer (20 ml of 20× SSC, 1 ml of Triton 100, 1 g of powdered skim milk and distilled water in a final volume of 100 ml, pH 7.0). Probe detection was performed using conjugated FITC-Avidin (Sigma) in C buffer (0.1 M sodium bicarbonate, 0.15 M sodium chloride; pH 7.0) for 30 min. The slides were washed 3 times in PBD buffer, pH 7.0, at 45°C for 2 min each. Two signal amplification series were then carried out using conjugated anti-avidin-biotin in PBD buffer, pH 7.0 (2 μl of anti-avidin in 38 μl PBD), in which the slides were incubated for 5 min in a humid chamber at 37°C. Each treatment with conjugated anti-avidin-biotin was followed by incubation with 0.07% FITC-Avidin in C buffer for 8 min in a humid chamber at 37°C. Following each amplification step, the slides were washed 3 times in PBD, pH 7.0, at 45°C for 2 min each. The chromosomes were counterstained with 0.2% propidium iodide diluted in an anti-fade reagent (Vector). Additionally, the *Rex3* probe was labeled by nick translation using digoxigenin (Roche) and used in double hybridization with 5S rDNA, and the chromosomes were counterstained with DAPI.

Microscopy/Image Processing

Hybridized chromosomes were analyzed using an Olympus BX 61 microscope and the images were captured with a digital camera (Olympus DP70), using the Image-Pro MC 6.0 software. Mitotic metaphases were processed on the Adobe Photoshop CS3 program, with the chromosomes measured by the Image J public domain program. The chromosomes were classified as metacentric (m), submetacentric (sm), subtelocentric (st), acrocentric (a) and microchromosome (mi), according to Levan et al. [1964] and Mesquita et al. [2008]. The organization of the karyotypes was carried out following the procedures described by Thompson [1979] and Mesquita et al. [2008].

Results

The karyotype analyses of the 3 species of *Symphysodon* revealed diploid chromosome number 2n = 60 chromosomes, an absence of differentiated sex chromosomes and an absence of interspecific chromosomal polymorphisms. *Symphysodon aequifasciatus* karyotype consists of 2n = 50 m-sm + 6 st-a + 4 mi, *Symphysodon discus* 2n = 50 m-sm + 10 st-a, and *S. haraldi* 2n = 52 m-sm + 4 st-a + 4 mi. Constitutive heterochromatin was detected in the pericentromeric region in the majority of chromosomes in the 3 species (fig. 1). In some pairs, however, constitutive heterochromatin occupied entire chromosome arms, such as the short arms of chromosome pairs 5, 6, 7, 9, 10, 12, 14, 16, 17 and 18 in *S. aequifasciatus* (fig. 1a), pairs 4, 8, 9, 10, 12 to 15, 18, 20, 22 and 23 in *S. discus* (fig. 1b), and pairs 11 and 15 to 20 in *S. haraldi* (fig. 1c). Moreover, only one chromosome of a homolo-

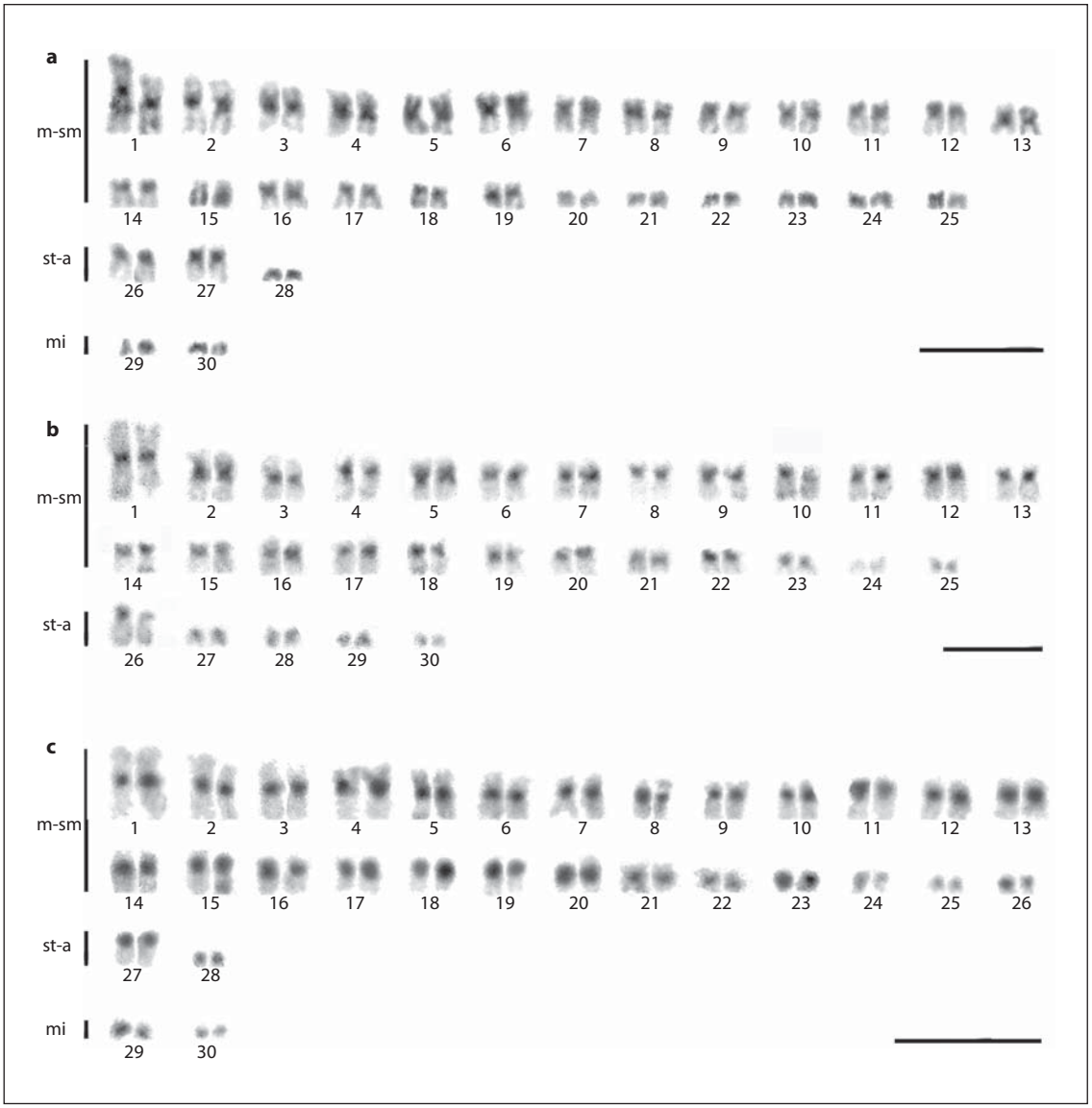

Fig. 1. Karyotypes of *Symphysodon aequifasciatus* (**a**), *S. discus* (**b**), and *S. haraldi* (**c**) after C-banding. Scale bar = 10 μm.

gous pair showed interstitial heterochromatin on the long arm of the first chromosome pair in *S. aequifasciatus* (fig. 1a), and pairs 23 and 29 in *S. haraldi* were completely heterochromatic (fig. 1c).

The homologous (probes from the same species) and heterologous (probe from one species hybridized to the chromosomes of another) hybridizations revealed similar results for both *Rex3* and 5S probes. Regarding the location of the *Rex3* element, the 3 species displayed a compartmentalized distribution pattern in some chromosomes, with weak hybridization signals at the centromeric region of most chromosomes in both males and females (fig. 2). Furthermore, the weak signals were more visible during spermatogonial metaphases (fig. 2d). The most conspicuous labeling of the mitotic chromosomes was found in the pericentromeric regions and occasionally in the short arms of chromosome pairs. *Symphysodon aequifasciatus* exhibited 12 moderately accentuated signals (pericentromeric region with invasion of the short arms of chromosome pairs 7, 9, 10, 13, 15 and 16; fig. 2a). *Symphysodon discus* exhibited 18 evident signals (pericentromeric regions of pairs 19, 21 and 25 and with exten-

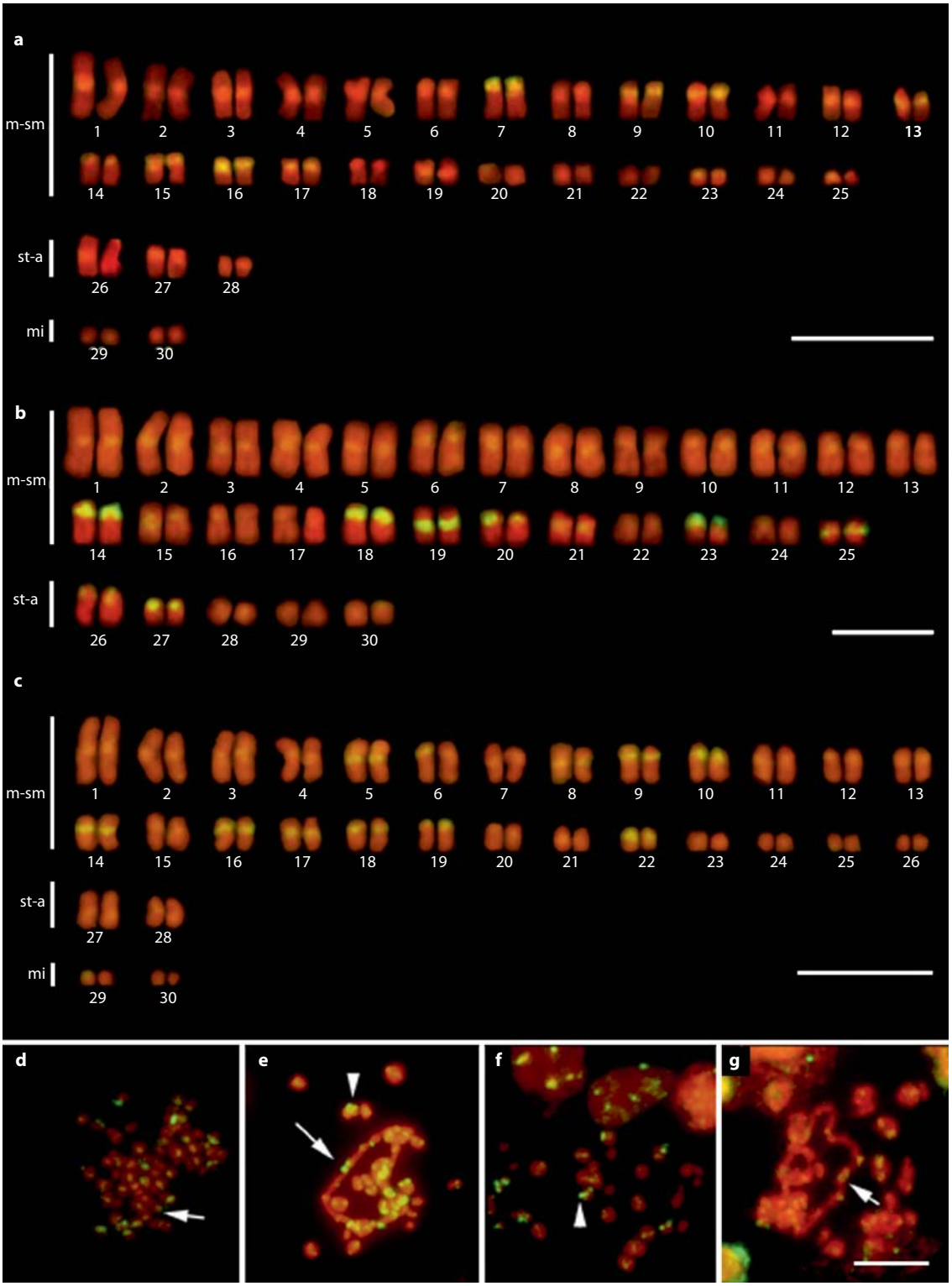

Fig. 2. Physical chromosomal location of *Rex3* retrotransposons (yellow sites) in karyotypes and meiotic testicle cells of *Symphysodon aequifasciatus* (**a**, **d**, **e**), *S. discus* (**b**, **f**) and *S. haraldi* (**c**, **g**). **a–c** Karyotypes. **d** Spermatogonial metaphase chromosomes revealing pericentromeric labeling of *Rex3* (arrow). **e–g** Diplotene revealing *Rex3* elements in the chromosome chain (arrow) and bivalents (arrowheads). Scale bar = 10 μm.

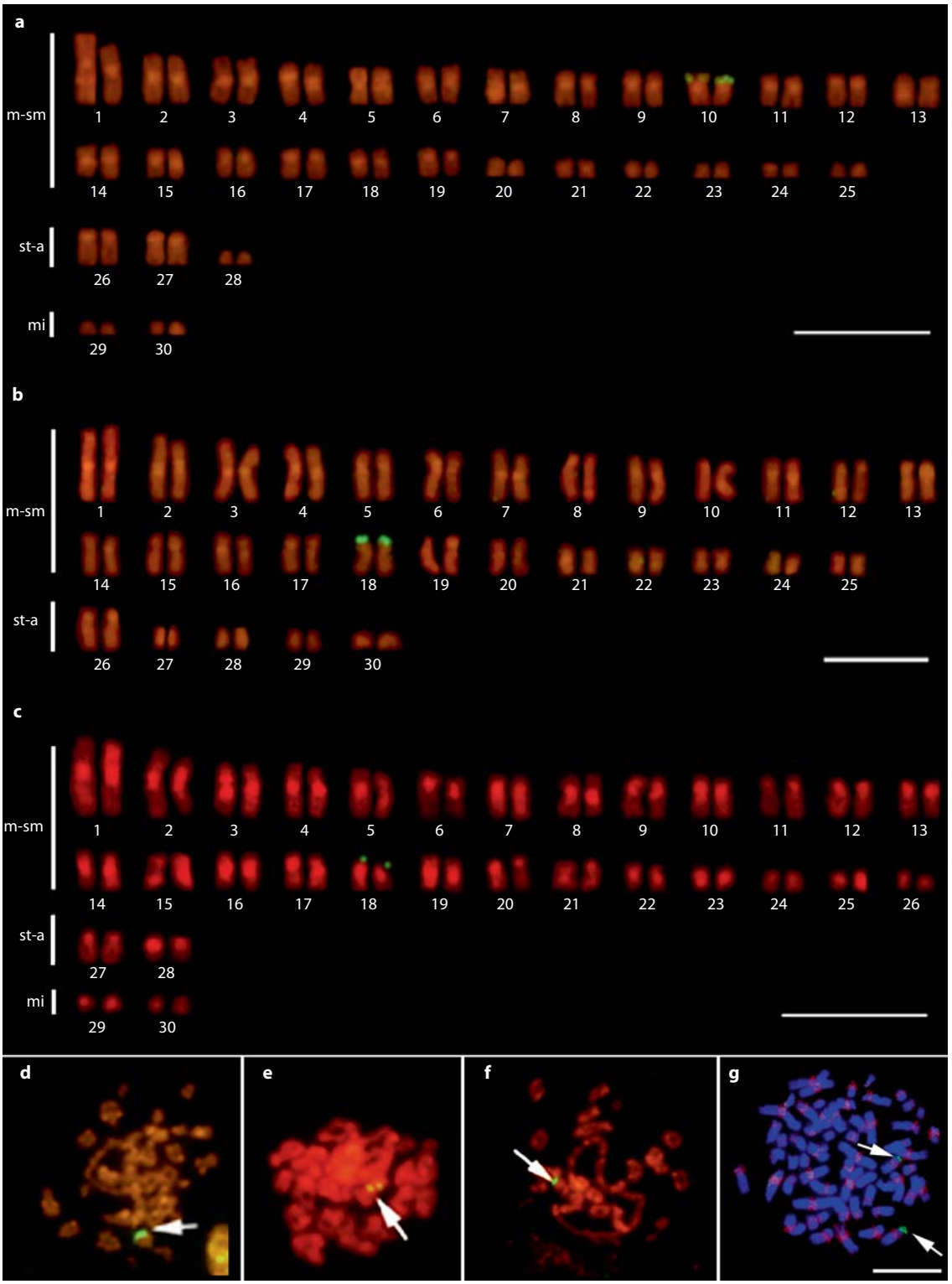

Fig. 3. Physical chromosomal location of 5S rDNA 2 (yellow sites) in karyotypes and meiotic testicle cells of *Symphysodon aequifasciatus* (**a**, **d**, **g**), *S. discus* (**b**, **e**) and *S. haraldi* (**c**, **f**). **a–c** Karyotypes. **d–f** Diplotene revealing 5S rDNA sites in bivalents (arrow). **g** Metaphase of *Symphysodon aequifasciatus* showing the *Rex3* elements (red sites) flanking the 5S rRNA genes (green sites – arrow). The same pattern was found for *S. discus* and *S. aequifasciatus*. Scale bar = 10 μm.

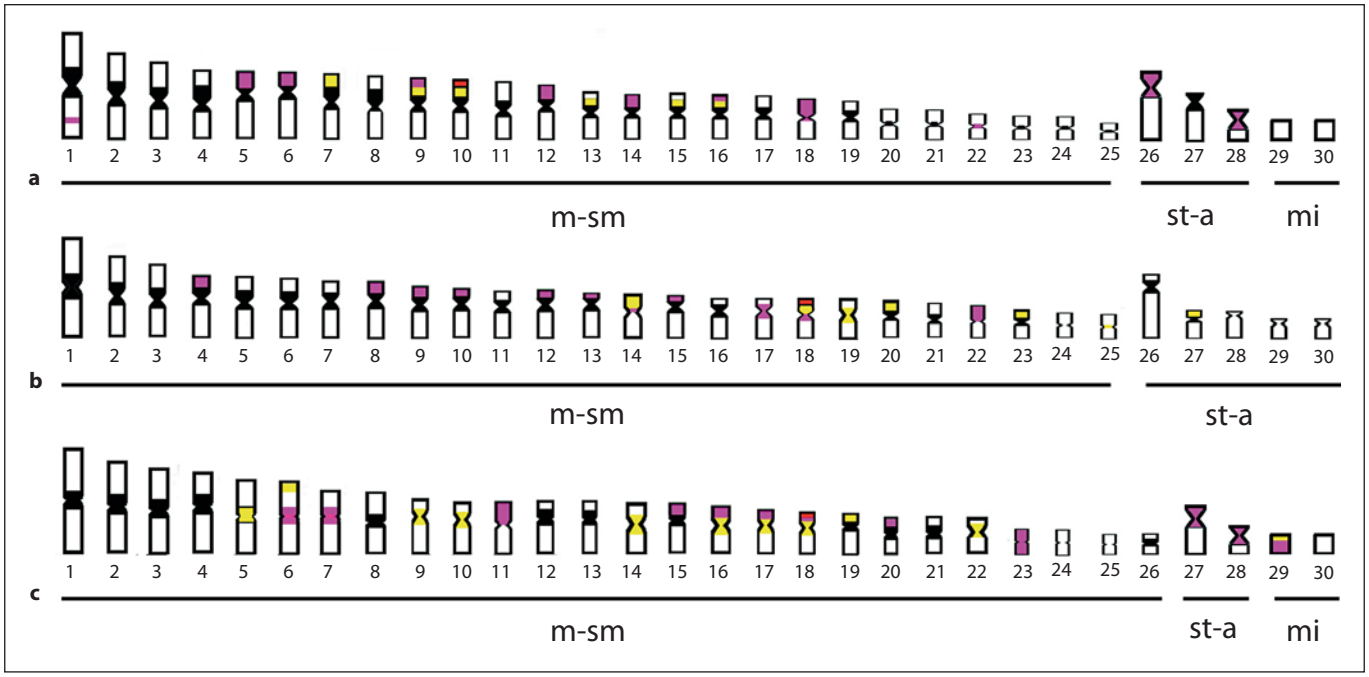

Fig. 4. Representative ideogram of *Symphysodon aequifasciatus* (**a**), *S. discus* (**b**), *S. haraldi* (**c**); in black, the centromeric heterochromatin with tenuous fluorescent labeling of *Rex*3 clearly visualized in the majority of the chromosomes; in yellow, more accentuated conspicuous labeling of *Rex*3; in red, the 5S rRNA gene co-localized in the heterochromatin; in purple, heterochromatic sequences unraveled.

sion of the short arms of pairs 14, 18, 20, 23, 26 and 27; fig. 2b), and *S. haraldi* exhibited 24 tenuous sites (centromeric regions of pairs 5, 8, 14, 16 and 17 with invasion of the short arms of pairs 6, 9, 10, 18, 19, 22 and 29; fig. 2c). In diplotene cells and in diakinesis of the 3 species, the majority of bivalents had 2 conspicuous well-labeled sites, which were most likely located in the pericentromeric region. Moreover, *S. aequifasciatus* (fig. 2e) and *S. haraldi* (fig. 2g) showed a variable number of fluorescent-labeled sites on the chromosomal chain (fig. 2f).

Regarding 5S rDNA, only one pair of labeled chromosomes was observed in the chromosomes of the 3 species. 5S rRNA genes were identified in the sub-terminal region of the short arms of chromosome pair 10 in *S. aequifasciatus* (fig. 3a), and in the sub-terminal region of the short arm of chromosome pair 18 in *S. discus* (fig. 3b) and *S. haraldi* (fig. 3c). In the meiotic chromosomes of the 3 species, the 5S rDNA sites were located in typical bivalents and were not involved with the chromosomal chain (fig. 3d–f). Double FISH revealed that *Rex3* elements appear to flank the 5S rRNA genes in the 3 species (fig. 3g).

Discussion

Rex3 elements may have played an important role in the karyotype evolution of Neotropical freshwater Cichlidae, including the *Symphysodon* species. In *Astronotus ocellatus*, considered to be one of the basal species for Neotropical cichlids [Kullander, 1998; Farias et al., 2001; Smith et al., 2008], the small blocks of heterochromatin are restricted to the pericentromeric region of all chromosomes, with the repetitive Ao*Rex3* elements preferentially located in these regions [Mazzuchelli and Martins, 2008]. *Symphysodon* are considered to be derived cichlids [Kullander, 1998; Farias et al., 2001; Smith et al., 2008], and a large amount of heterochromatin was found in the pericentromeric region of nearly all chromosomes [Mesquita et al., 2008; present paper]. All fluorescent labeling using specific *Rex3* probes resulted in FISH signals that co-localized with heterochromatin (fig. 4a–c), but chromosomes of *S. haraldi* exhibited more tenuous fluorescent labeling in comparison to other species of the genus. In the Discus species, an analysis of the distribution of *Rex3* in meiotic chromosomes has corroborated the mitotic data with regard to the preferential localization of

these sequences to heterochromatin; however, the signal was more intense in testicular cells. C-bands in meiotic cells, such as diplotene cells in *S. aequifasciatus* and *S. haraldi*, indicated that the majority of the bivalents had 2 conspicuous heterochromatic blocks that were most likely located in the pericentromeric region, as well as a variable number of heterochromatic blocks on the chromosomal chain [Gross et al., 2009]. A similar pattern was found with fluorescent in situ hybridization with the *Rex3* probe. Studies involving the physical chromosomal location of retrotransposable *Rex3* elements have revealed different distribution patterns for each species (table 1), most of which exhibit *Rex3* elements dispersed evenly throughout the genome and not associated with heterochromatin. Preferential integration of retrotransposons into heterochromatic regions has been observed in *Notothenia coriiceps*, which has the most derived karyotype among the marine perciform fishes analyzed thus far. This species exhibits a more compartmentalized retrotransposon distribution, with accumulation in pericentromeric regions, suggesting a correlation between karyotype rearrangements and retrotransposon activity [Ozouf-Costaz et al., 2004].

However, in the chromosomes of *Symphysodon,* many heterochromatic regions did not hybridize with the *Rex3* probe, showing that *Symphysodon* many have heterochromatic sequences that are yet to be unraveled (fig. 4a–c).

Heterochromatin is currently recognized as an important part of the genome of eukaryotes. Heterochromatin functions include chromosome segregation, nucleus organization and the regulation of gene expression; it may also affect gene recombination [Grewal and Jia, 2007; Skipper, 2007; Bühler, 2009]. However, evidence shows that heterochromatin has additional functions, including the regulation of mitosis, progression of the cell cycle and cell proliferation as well as being associated with responses to many forms of exogenous stress, such as changes in temperature, thermal shock and hypoxia [Burt and Trivers, 2006; Varriale et al., 2008]. Do the differences found in the heterochromatin pattern and the amount/intensity of *Rex3* signals in the karyotypes of the wild species of *Symphysodon* correlate with adaptation to the environment in which they live? This question cannot be answered with these data, but needs special attention for future work.

The rivers of the Amazon exhibit considerable heterogeneity regarding physiochemical environments, which are easily recognized by differences in water coloration. Amazonian rivers are divided into 3 major categories: (1) white-water rivers, with clay coloration and a large amount of suspended sediment, (2) clear-water rivers, with little suspended matter, and (3) black-water rivers, the coloration of which stems from the partial decomposition of organic matter originating from the forest [Sioli, 1990; Santos and Ferreira, 1999]. The geological characteristics and physiochemical parameters of the waters have a direct effect on the distribution of species of wild Discus fish in the Amazon Basin [Bleher, 2006; Ready et al., 2006; Bleher et al., 2007; Farias and Hrbek, 2008]. The brown/blue Discus *(S. haraldi)* has the widest distribution in the Amazon Basin and is found in tributaries throughout the Amazon-Solimões River axis [Bleher, 2006; Bleher et al., 2007]. The green *(S. aequifasciatus)* and Heckel Discus *(S. discus)* have more restricted distributions. The green Discus is found in the western most portion of the Amazon Basin (Tefé, Juruá and Jutaí region), whereas the Heckel Discus is found in the Negro, Abacaxis and Nhamundá rivers [Ready et al., 2006; Bleher et al., 2007].

The presence of transposable elements may also contribute to genetic variability, which has been observed on both molecular [Ready et al., 2006; Bleher et al., 2007; Farias and Hrbek, 2008] and cytogenetic levels [Mesquita et al., 2008], and in the phenotype variability observed in individuals from both a natural environment and in captivity [Bleher and Göbel, 1992]. Furthermore, the presence of large blocks of repetitive elements also explains the larger genome size of *Symphysodon* species in comparison to other Neotropical cichlids, which is a result of polyploidization as explained by Thompson [1976].

Regarding the physical chromosomal location of the repetitive 5S rRNA genes, the mapping of these sequences in different fish species has revealed that these sites are commonly located on only one pair of chromosomes and in the interstitial segments of the chromosomes [reviewed by Martins and Wasko, 2004; Ferreira et al., 2007; Nakayama et al., 2008]. This same pattern has also been observed for mammals [Frederiksen et al., 1997] as well as amphibians [Schmid et al., 1987; Lucchini et al., 1993]. This chromosomal location pattern appears to correspond to an ancestral condition, especially for fish, as this position is thought to result from the protection of these sequences from transpositions and exchange events [Martins and Galetti Jr., 1999].

Exchanges and transpositions involving terminal regions are more common in species that have the Rabl configuration during cell division, when the chromosomes remain close to each other in the nucleus during interphase, with the centromeres directed toward one

pole and the telomeres directed toward the other [Schweizer and Loidl, 1987; Sumner, 2003]. Although in the *Symphysodon* species only one chromosome pair is labeled in the terminal region of the short arm, divergence occurs between the labeled pairs. The 5S rRNA gene co-localizes with constitutive heterochromatin in the 3 species and is flanked by *Rex3* elements (fig. 4 a–c). The mobile elements may serve as substrates for DNA recombination due to their repetitive nature, especially during the Rabl configuration [Kidwell and Lisch, 1997; Kazazian and Moran, 1998; Rouzic and Capy, 2005]. The repetitive nature of mobile elements may have facilitated the chromosome rearrangements that caused the difference in position of the 5S rDNA between *S. aequifasciatus* and *S. discus*/*S. haraldi*. In plants, the association of rDNA loci with regions of constitutive heterochromatin has been extensively reported [Siljak-Yakovlev et al., 2002], and the number of transposable elements detected in centromeric heterochromatin and nucleolar organizer regions likely favors rDNA mobility as well as affects the evolution and expression of these genes, as suggested for rice and *Arabidopsis* [Dong et al., 1998; Franz et al., 2000].

Despite the evidence that chromosomes with 5S rDNA sites are involved in translocation, the data obtained with these rDNA probes suggest that chromosome pairs 10 and 18 are not involved in the formation of the chromosome chain in *S. aequifasciatus* and *S. haraldi*, respectively.

In the past, repetitive DNA elements were considered to be junk DNA because they had no clearly identified function [Doolittle and Sapienza, 1980; Orgel and Crick, 1980], but accumulated data from eukaryotic species of diverse taxonomic origins have challenged this view over the past few years [Bonaccorsi and Lohe, 1991; Dong et al., 1998; Feschotte and Prithman, 2007; Ferreira and Martins, 2008]. Recent data have supported a major role of repetitive DNA in the structural and functional evolution of genes and genomes in a variety of organisms [Biémont and Vieira, 2006]. Although evolutionary mechanisms have prevented major changes in the karyotypes of different *Symphysodon* species and populations, their genomes are in continuous evolution, demonstrated here by the minor chromosomal variations observed. The repetitive fraction of the genome (exemplified here by 5S rDNA and *Rex3*) can escape from the selective pressure that acts on the non-repetitive segments, thus representing good evolutionary markers to detect recent evolutionary events. In addition, the accumulation of repetitive sequences in specific genomic areas can cause chromosome rearrangements through chromosome breakage, deletions, inversions and amplifications [Lim and Simmons, 1994; Dimitri et al., 1997]. Considering the accumulation of heterochromatin and repeated DNA sequences in *Symphysodon* compared to other cichlids, this genus represents an interesting model to investigate the role of repetitive DNA in chromosomal evolution. Furthermore, the investigation of repetitive DNA families and the copy number of these sequences in the genome of Discus and other Amazon fishes will contribute greatly to the understanding of the basal evolutionary mechanisms involved in the generation of the complex genomic structure of these organisms.

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