Journal of FISH BIOLOGY
Published for the Fisheries Society of the British Isles

Journal of Fish Biology (2010) **76**, 1117–1127
doi:10.1111/j.1095-8649.2010.02550.x, available online at www.interscience.wiley.com

Variability of 18S rDNA locus among *Symphysodon* fishes: chromosomal rearrangements

M. C. Gross*†‡, C. H. Schneider†, G. T. Valente§, C. Martins§ and E. Feldberg†

**Laboratório de Genética Animal, Instituto de Ciências Biológicas, Universidade Federal do Amazonas, Manaus, Amazonas, Brazil, †Laboratório de Genética Animal, Instituto Nacional de Pesquisas da Amazônia, Coordenação de Pesquisas em Biologia Aquática, Manaus, Amazonas, Brazil and §Laboratório de Genômica Integrativa, Departamento de Morfologia, Instituto de Biociências, UNESP-Universidade Estadual Paulista 'Júlio de Mesquita Filho', Botucatu, São Paulo, Brazil*

(Received 9 June 2009, Accepted 13 October 2009)

Three species of cichlids belonging to the genus *Symphysodon* have demonstrated interspecific and intraspecific variation in nucleolus organizer regions (NOR) detected with silver nitrate. In order to understand the evolution of this marker in the genus, the structural variability of these sequences in mitotic chromosomes from *Symphysodon aequifasciatus*, *Symphysodon discus* and *Symphysodon haraldi* was investigated using both silver nitrate impregnation and hybridization of the 18S rRNA gene probe. For the three species, the two markers were intraspecifically and interspecifically variable both in the number and in the size of the sites. This polymorphism may stem from duplications and translocations, which suggests that structural chromosome rearrangements effectively act in the karyoevolution of wild *Symphysodon* species and may have favoured the adaptability of these fishes to diverse aquatic environments in the Amazon.

Key words: Amazon basin; chromosome evolution; discus fish; fluorescent *in situ* hybridization; *Symphysodon* spp.

INTRODUCTION

Ribosomal RNA (rRNA) is the most abundant RNA, accounting for *c*. 80% of the total RNA in cells. The rRNA molecules are the major structural component of the ribosomes and they are involved in catalytic, organizational and regulation activities of protein synthesis in all cells (Doudna & Rath, 2002). The genes for these molecules are organized into two multigenic families that are repeated in tandem, that are situated in independent arrangements and that are normally in distinct chromosome regions in fish (for review, see Martins, 2007). The first class is formed by 5S rDNA, which encodes the 5S rRNA, and is synthesized by RNA Polymerase III. The second class is represented by the 45S rDNA, which codes for the 18S, 5·8S

‡Author to whom correspondence should be addressed. Tel.: +55 92 3643 3242; fax: +55 92 3643 3240; email: gross@ufam.edu.br

and 28S rRNAs, that are synthesized by RNA Polymerase I (Doudna & Rath, 2002). Multiple copies of the second class (45S rDNA) correspond to nucleolus organizer regions (NOR), which, when active, are frequently positive for impregnation with silver nitrate (Ag-NOR) (Howell & Black, 1980).

Cichlid fishes have been the subject of increasing scientific interest because of their rapid adaptive radiation that has led to an extensive ecological diversity and because of their enormous importance to tropical and subtropical aquaculture. Despite the morphological and ecological diversity of cichlid fish, most genetic–genomic information is based on the karyotype details, and is evident as a bimodal distribution of the diploid numbers related to the geographic distribution of the species. African cichlids have a modal diploid number equal to $2n = 44$, whereas Neotropical cichlids have a modal diploid number equal to $2n = 48$ (Feldberg *et al*., 2003). With respect to the Neotropical cichlids, the discus fishes, genus *Symphysodon* Heckel, are the most intriguing and distinctive group due to their body morphology, colours (Ready *et al*., 2006) and chromosomal features (Thompson, 1979; Feldberg *et al*., 2003; Mesquita *et al*., 2008), and are considered to be one of the most derived genera of Cichlidae (for review, see Smith *et al*., 2008). Over the past few years, reports have indicated that *Symphysodon* has the highest diploid number ($2n = 60$) of all cichlids (Ohno & Atkin, 1966; Thompson, 1979; Takai *et al*., 2002), suggesting the occurrence of chromosomal rearrangements during their evolutionary history (Mesquita *et al*., 2008; Gross *et al*., 2009*a*).

In several species of the family Cichlidae, a single locus system of Ag-NORs in the largest chromosome pair of the complement is considered a plesiomorphic characteristic (Feldberg *et al*., 2003). For the wild discus ornamental fish genus *Symphysodon* spp. (Cichlidae, Perciformes), silver nitrate impregnation in mitotic chromosomes from the species *Symphysodon aequifasciatus* Pellegrin, *Symphysodon discus* Heckel and *Symphysodon haraldi* Schultz have demonstrated a multiple NOR system with considerable variability in the number of Ag-NOR sites, both intraspecifically and interspecifically (Mesquita *et al*., 2008). Furthermore, studies on meiotic chromosomes from *S. haraldi* have revealed ribosomal sites in the diplotene meiotic chromosome chain, thereby suggesting the involvement of these sequences in the chromosome rearrangements that originated in this meiotic figure (Gross *et al*., 2009*a*).

Considering the Ag-NOR variability in the three species from the genus *Symphysodon*, the aim of the present study was to investigate the distribution of 45S rDNA sequences in order to determine whether these sequences coincide with Ag-NOR labelling sites in the mitotic chromosomes, and to obtain a better understanding of the chromosomal organization and karyoevolution.

MATERIALS AND METHODS

MATERIALS

Specimens of *Symphysodon* spp. were collected in the state of Amazonas, Brazil, with permission from the Brazilian Environmental Protection Agency, IBAMA (011/2005), and identified according to the most recently proposed characteristics for the genus (Bleher *et al*., 2007). The following specimens were analysed: eight males and eight females of *S. aequifasciatus* from the Tefé River; 15 males and 14 females of *S. discus* from the Negro River (proximities of the municipality of Novo Airão) and 15 males and 15 females of *S. haraldi*

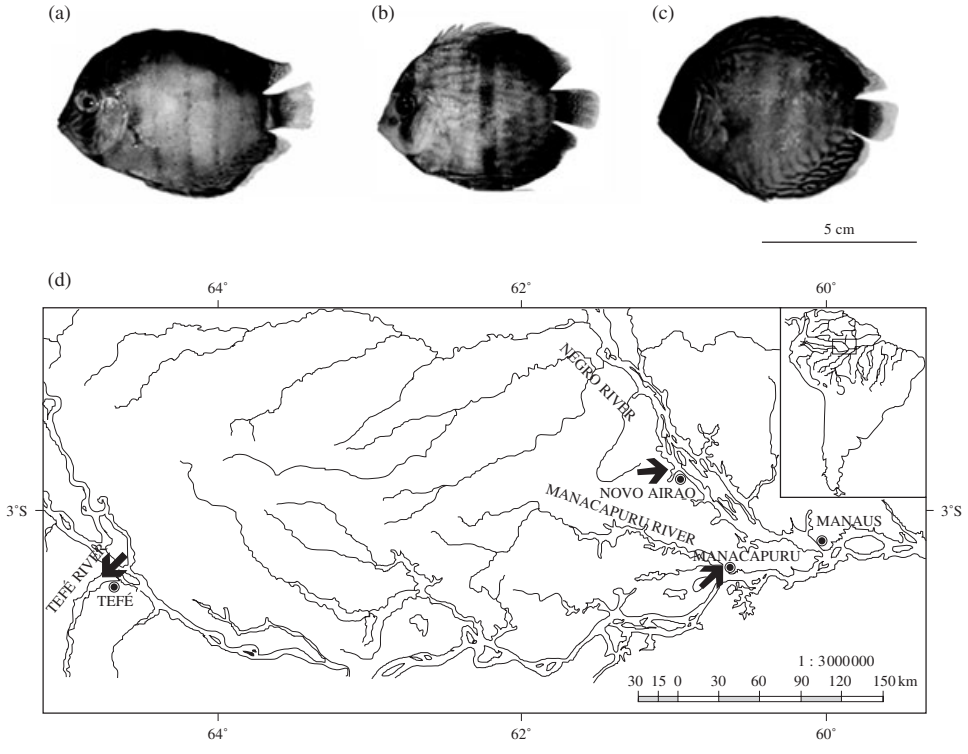

FIG. 1. Amazonian representatives of the *Symphysodon* species [(a) *Symphysodon aequifasciatus*; (b) *Symphysodon discus*; (c) *Symphysodon haraldi*] and (d) map showing the location of *Symphysodon* sampling sites in Amazonas state, Brazil.

from the Manacapuru River (Fig. 1). All the fishes were anaesthetized with ice water and killed. Voucher specimens were deposited in the fish collection of the Instituto Nacional de Pesquisas da Amazônia (INPA 28582, INPA 28583, INPA 28584) and others were deposited in the fish collection of the INPA Animal Genetics Laboratory.

METHODS

DNA extraction and amplification of 18S rDNA units

Total genomic DNA was extracted from the muscles of *S. aequifasciatus, S. discus* and *S. haraldi*, following the phenol–chloroform protocol detailed by Sambrook & Russell (2001). Amplification by polymerase chain reaction (PCR) of the 18S rRNA gene was conducted using the primers 18Sf (5′-CCG CTT TGG TGA CTC TTG AT-3′) and 18Sr (5′-CCG AGG ACC TCA CTA AAC CA-3′), designed from the complete sequence of the 18S rRNA gene from *Ictalurus punctatus* (Rafinesque) available in the databanks of the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) (sequence identification code AF021880). These primers were used to amplify a *c*. 1400 bp DNA segment of the 18S rRNA gene. 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: 2 min at 95° C (denaturation); 35 cycles for 1 min at 95° C, 30 s at 55° C (annealing) and 1 min 40 s at 72° C (extension); 7 min at 72° C (final extension).

Sequencing

The PCR products were sequenced with a MegaBace 1000 automatic sequencer, following the manufacturer's instructions. The DYEnamic ET Terminator Cycle Sequencing kit (GE HealthCare; www.gehealthcare.com) was used for the sequencing reactions (for MegaBace & GE HealthCare, see www.gelifescience.com.br). The generated sequences were compared with sequences deposited in the databanks available through the NCBI (http://www.ncbi.nlm.nih.gov). After verification, all sequences were aligned using the ClustalW multiple alignment programme (Thompson *et al*., 1994), which is included in the BioEdit 7.0 software program (Hall, 1999). The alignment was manually verified and edited with the same programme.

Acquisition of chromosomes and detection of nucleolus organizer regions

Mitotic chromosomes were obtained from kidney cells of *S. aequifasciatus, S. discus* and *S. haraldi*, following the protocols described by Moreira-Filho & Bertollo (1990). The pharmaceutical compound Munolan (Brazilian drugifore Allergan Frumtost) was used to stimulate mitotic cell division, following the method described by Molina (2001). Nucleolus organizer regions were detected based on the method described by Howell & Black (1980).

In situ *fluorescent hybridization*

The PCR products were labelled with biotin-14-dATP by nick translation (Bionick labelling system, Invitrogen; www.invitrogen.com). Homologous and heterologous *in situ* fluorescent hybridizations were carried out based on the protocols described by Pinkel *et al*. (1986), 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 for a final volume of 1000 ml, pH 7·0] at 67° C. The hybridization solution [100 ng of denatured probe, 10 mg ml^{-1} dextran sulphate (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 humidity 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 for a final volume of 100 ml, pH 7·0). Probe detection was performed using conjugated FITC-Avidin (Sigma; www.sigmaaldrich.com) in C buffer (0·1 M sodium bicarbonate, 0·15 M sodium chloride) for 30 min. The slides were washed thrice in phosphate-buffered detergent (PBD) buffer at 45° C for 2 min each. Two signal amplification series were then carried out using conjugated anti-avidin–biotin in PBD buffer (2 μl of anti-avidin in 38 μl PBD), in which the slides were incubated for 5 min in a humidity chamber at 37° C. Each treatment with conjugated anti-avidin–biotin was followed by incubation with 0·07% fluorescein isothiocyanate (FITC)–avidin in C buffer for 8 min in a humidity chamber at 37° C. Following each amplification step, the slides were washed thrice in PBD at 45° C for 2 min each. The chromosomes were counter-stained with 0·2% propidium iodide diluted in antifade mounting media (Vector; www.vectorlabs.com).

Hybridized chromosomes were analysed 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 programme, with the chromosomes measured by the Image J public domain program. The organization of the karyotypes was carried out following the procedures described by Thompson (1979) and Mesquita *et al*. (2008).

RESULTS

Amplification of the 18S rRNA gene resulted in a fragment of *c*. 1400 base pairs (bp) for the three species of *Symphysodon*, which after sequencing and comparative analysis exhibited high similarity with the 18S rRNA gene of other fish species, including the cichlid *Oreochromis esculentus* (Graham) (99%) and the fish considered

a genetic model, *Oryzias latipes* (Temminck & Schlegel) (97%). These sequences are deposited in the NCBI databank. The three species of *Symphysodon* had $2n = 60$ chromosomes and no differentiated sex chromosomes. *Symphysodon aequifasciatus* exhibited 50 m-sm+6st-a+4mi [Fig. 2(a)]; *S. discus* exhibited 50 m-sm+10st-a [Fig. 2(e)] and *S. haraldi* exhibited 52 m-sm+4st-a+4mi [Fig. 2(j)] (m-sm = meta-submetacentric; st-a = subtelo-acrocentric; mi = microchromosome).

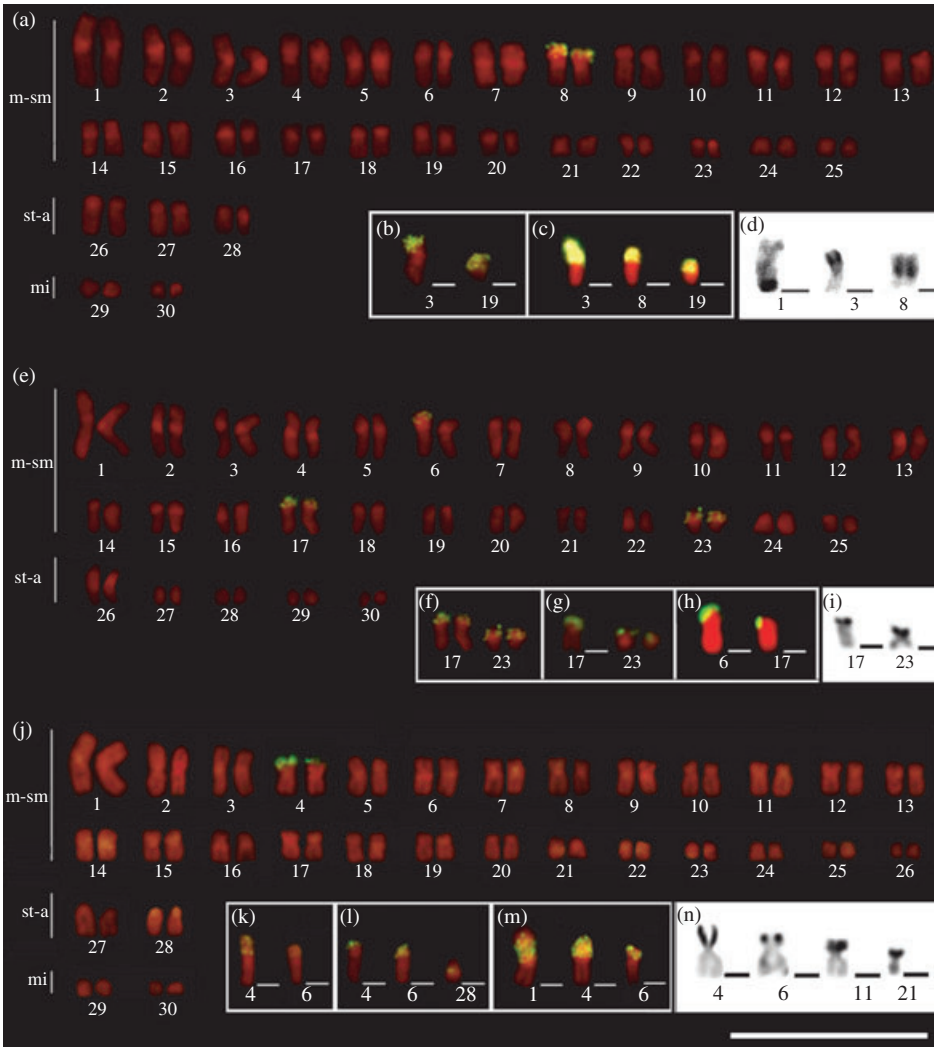

FIG. 2. Distribution patterns of 18S rDNA sites (yellow signals) and nucleolus organizer regions (NOR) revealed by silver nitrate staining in mitotic chromosomes of *Symphysodon aequifasciatus* (a–d); *Symphysodon discus* (e–i); *Symphysodon haraldi* (j–n). Karyotypes (a, e, j). In the box, the variant patterns of 18S rDNA sites (b, c, f, g, h, k, l, m) and the nucleolus organizer regions revealed by silver nitrate staining in each species (d, i, n) are given. As all combinations of NORs involving these chromosome pairs were found, only one of the homologues of each pair is represented (bar = 10 μm) (m-sm = meta-submetacentric; st-a = subtelo-acrocentric; mi = microchromosome).

Regarding the location of the 18S rRNA gene sites, the three species of *Symphysodon* demonstrated intraspecific and interspecific variability in the distribution of these sequences in mitotic chromosomes, but no intra-individual variation was observed. In *S. aequifasciatus,* two to three sites of the 18S rRNA gene were found, which exhibited three karyotype distribution patterns involving terminal regions of the short arms of chromosomal pairs 3, 8 and 19 [Fig. 2(a)–(c)]. In *S. discus,* two to five sites of the 18S rDNA gene were found, which exhibited four karyotype distribution patterns, always involving terminal regions of the short arms of chromosomal pairs 6, 17 and 23 [Fig. 2(e)–(h)]. In *S. haraldi,* two to three sites of the 18S rDNA gene were found, which exhibited four karyotype distribution patterns, involving labelling in the terminal region of chromosomes pairs 4 and 6 as well as interstitial regions in pairs 1 and 28 [Fig. 2(j)–(m)]. The frequency of the different distribution patterns of the 18S rDNA site was similar within *S. discus*, but in *S. aequifasciatus* and *S. haraldi* preferential patterns were found (Table I).

Analysis of the mitotic cells submitted to silver nitrate impregnation revealed a system of multiple NORs in the three species of *Symphysodon*, along with intraindividual, intraspecific and interspecific variation. In *S. aequifasciatus,* the NORs were located in the terminal region of the long arms of chromosome pair 1 and occupying the entire short arm of chromosome pairs 3 and 8 [Fig. 2(d)]. In *S. discus,* the NORs were detected in the terminal region of the short arm of chromosome pairs 17 and 23 [Fig. 2(i)]. In *S. haraldi,* the NORs were detected in the short arm of chromosome pairs 4, 6, 11 and 21 [Fig. 2(n)]. All possible combinations involving these chromosome pairs were found, with NORs active in homologous and non-homologous chromosomes, but never more than two sites per metaphasic cell were detected.

TABLE I. Chromosomal pairs of *Symphysodon aequifasciatus* (▫), *Symphysodon discus* (▪) and *Symphysodon haraldi* (■) showing different patterns of 18S rDNA distribution (A, B – homologous) and frequency of each pattern in the species

	S. aequifasciatus			*S. discus*				*S. haraldi*			
	Pattern			Pattern				Pattern			
Chromosome pair	1	2	3	1	2	3	4	1	2	3	4
1A	–	–	–	–	–	–	–	–	–	–	■
3A	–	▪	▪	–	–	–	–	–	–	–	–
4A	–	–	–	–	–	–	–	■	■	■	■
4B	–	–	–	–	–	–	–	■	–	–	–
6A	–	–	–	▪	–	–	▪	–	■	■	■
8A	▫	–	▫	–	–	–	–	–	–	–	–
8B	▫	–	–	–	–	–	–	–	–	–	–
17A	–	–	–	▪	▪	–	▪	–	–	–	–
17B	–	–	–	▪	▪	▪	–	–	–	–	–
19A	–	▫	▫	–	–	–	–	–	–	–	–
23A	–	–	–	▪	▪	▪	–	–	–	–	–
23B	–	–	–	▪	▪	▪	–	–	–	–	–
28A	–	–	–	–	–	–	–	–	–	■	–
Frequency of pattern (%)	50·0	33·0	17·0	20·0	30·0	30·0	20·0	40·0	20·0	20·0	20·0

DISCUSSION

In fish, the analysis of the number and location of NORs is widely achieved by means of impregnation of these regions with silver nitrate. As silver associates with nucleolar proteins involved in the transcriptional activity of ribosomal genes from the 45S rDNA cistrons (Howell & Black, 1980; Boisvert *et al*., 2007) and can also impregnate heterochromatic regions rich in acidic residuals (Sumner, 1990), however, a large part of the variation that has been detected in fish may be associated with the activity of these cistrons or the presence of acidic heterochromatic blocks.

The three species of *Symphysodon* submitted to silver nitrate impregnation exhibited intraindividual variability as well as intraspecific and interspecific variability. In some *Symphysodon* specimens, the silver impregnated both the NORs and heterochromatic regions without rRNA gene sites, such as chromosome pair 1 in *S. aequifasciatus* [Fig. 2(a)–(d)] as well as pairs 11 and 21 in *S. haraldi* [Fig. 2(j)–(m)]. Therefore, the intraindividual variation in NORs revealed by silver nitrate in the three species of *Symphysodon* may be the result of the activity of ribosomal cistrons. The intraspecific and interspecific variation, however, cannot be credited to epigenetic effects, as the detection of the 18S rDNA sites revealed differences in the number of loci (two to five sites, involving homologues and non-homologues) as well as in size, thereby revealing a complex distribution pattern for these sequences in the mitotic chromosomes of individuals from the same species (Fig. 2; Table I). The variability detected by the 18S rDNA probe also cannot be explained by flaws in the hybridization process, as the probes were hybridized under highly stringent conditions. Moreover, homologous (DNA probes from the same species) and heterologous hybridizations (probe from one species hybridized in the chromosome of another) were performed and revealed the same results.

In *Symphysodon,* chromosomal sites of 18S rDNA and labelling with silver nitrate were mainly located in terminal and proximal interstitial regions of the short arms, which could have facilitated the transposition to other chromosome pairs through translocation events. These rearrangements also appear to have caused a change in the position of 5S rDNA sites, which is not syntenic to 18S rDNA sites in the three species of *Symphysodon*, being present in chromosome pair 10 in *S. aequifasciatus* and in chromosome pair 18 in *S. discus* and *S. haraldi* (Gross *et al*., 2009*a*, *b*). The separate locations of 45S and 5S rDNA sites are a more common situation among fish, probably because the transcription of 45S rRNA genes is carried out by RNA polymerase I and the transcription of 5S rRNA genes is carried out by polymerase III (Martins, 2007).

Along with the variation in the number and position of 18S sites, heteromorphism in the size of the cluster in the homologous chromosomes of the wild discus was also observed, which suggests the duplication of these sequences as a consequence of unequal crossing over, as has been proposed for other fish and mammals (Ashley & Ward, 1993; Boron *et al*., 2006). This supposition is reinforced by the fact that the three species of *Symphysodon* exhibit rDNA cistrons in regions of constitutive heterochromatin (Gross *et al*., 2009*b*), which has been extensively documented in fish (Pendás *et al*., 1993; Artoni *et al*., 2008 and others). Heterochromatin is currently recognized as an important part of the genome of eukaryotes, the functions of which include chromosome segregation, nucleus organization and the regulation of gene expression; it may also affect the gene recombination process (Grewal &

Jia, 2007; Skipper, 2007; Bühler, 2009). Due to the repetitive nature of ribosomal DNA and other sequences found in the constitutive heterochromatin, DNA polymerase errors in the addition of nucleotides during the replication of the genetic material and unequal crossing over may cause rDNA buildup (Pendás *et al*., 1993). Furthermore, the tendency of the association in the nucleolus organization could also facilitate non-reciprocal translocations between NORs (reviewed in Wasko & Galetti Jr., 2000). All these factors, together with the presence of transposable elements in the heterochromatic regions of the three species of *Symphysodon* (Gross *et al*., 2009*b*), may have caused the structural polymorphisms of the 45S rDNA sites found in these species.

A new approach regarding the variability of rDNA has recently emerged, which considers this genomic region unstable and one of the most fragile sites, functioning as a primary sensor of damage caused by diverse factors, including environmental effects (Kobayashi, 2008). Moreover, some transposable elements exhibit preferential insertion in particular nucleotide regions of the genome, as is the case with long interspersed nuclear elements (LINEs), which are specifically inserted in the 28S rRNA genes in *Drosophila melanogaster*, rendering this sequence non-functional (Eickbush, 2002). The maintenance of the number of viable copies of rDNA being expressed is of extreme importance to the cell, and, therefore, either duplications of the sequences or the silencing of these genes may occur, which would explain their association with constitutive heterochromatin that promotes an epigenetic control of the genome by methylation of histones and recruitment of chromodomain proteins (Grewal & Jia, 2007; Kobayashi, 2008). The number of rDNA copies is thought to always fluctuate and thus a large number of these sequences would be found in the genome, thereby ensuring the maintenance of viable copies (Kobayashi, 2008). This hypothesis is supported by the fact that the nucleoli appear to co-ordinate the response of organisms when submitted to stress (Olson, 2004).

Nucleoli are nuclear structures organized around active NORs, which are the chromosomal regions with 45S rDNA sequences repeated in tandem, the primary function of which is the biogenesis of the ribosomes (Hernandez-Verdun *et al*., 2002; Hernandez-Verdun, 2004). Evidence, however, shows that they have additional functions as well, 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 (Boisvert *et al*., 2007; Varriale *et al*., 2008). According to Burt & Trivers (2006), this occurs because repetitive elements (including rDNA) are involved in the machinery of a sophisticated enzyme system, as is the case for the involvement of these elements in the evolution of the immune system in vertebrates.

In addition to its rivers with different physiochemical characteristics (pH, electrical conductivity and temperature), the Amazon basin, the location of endemism of species of *Symphysodon*, exhibits inundation pulses that cause the flooding of forest regions during the rainy season (Junk & Furch, 1993). These fluctuations in water level expose fish to extreme differences in the availability of food sources and shelter, as well as the density of predators and parasites. Changes in the physiochemical properties of the water, such as dissolved oxygen, constitute a further stress factor for fish, as there is a tendency towards the establishment of permanent or transitory hypoxic conditions in areas subject to flooding (Junk, 1997). This environmental pressure has led fish in the region to develop considerable genomic

plasticity during their evolutionary process, and a series of ecological, morphological, physiological, metabolic and molecular adjustments can be seen, which help them to maintain organic homeostasis and allow them to survive during these seasonal changes (Almeida-Val & Farias, 1996). Wild *Symphysodon* species exhibit considerable genetic, molecular and chromosomal variability (Farias & Hrbek, 2008; Mesquita *et al*., 2008), as well as adaptation, which allows them to survive in conditions of moderate hypoxia (Chippari-Gomes *et al*., 2003). As the three species inhabit locations that are susceptible to these environmental variations (Ready *et al*., 2006; Bleher *et al*., 2007), it is possible that, throughout their evolution, these conditions have favoured the movement of transposable elements, leading to translocation events that have promoted the variability of the 18S rDNA and NORs, thereby suggesting that the genome of these species may have been continually modified by the variation in repetitive DNAs. Thus, structural chromosome rearrangements appear to be effectively acting on the karyoevolution of wild *Symphysodon* species and could be favouring their adaptability to adverse environmental conditions common to the Amazon region.

This study was supported by National Council for Scientific and Technological Development (CNPq – 501119/2005-1), National Amazon Research Institute/ Genetic, Conservations and Evolutionary Biology (INPA/GCBEV), The State of Amazonas Research Foundation (FAPEAM), The State of São Paulo Research Foundation (FAPESP 04/14766-6), Ministry of Science and Technology (MCT/CNPq/CT-Amazônia/CT-Energ 13/2006 554057/2006-9) and Centre for Studies of Adaptation to Environmental Changes in the Amazon (INCT ADAPTA, FAPEAM/CNPq 573976/2008-2).

References

Almeida-Val, V. M. F. & Farias, I. P. (1996). Metabolic adjustments to chronic hypoxia. In *Physiology and Biochemistry of the Fishes of the Amazon* (Val, A. L., Almeida-Val, V. M. F. & Randall, D. J., eds), pp. 257–271. Manaus: INPA.

Artoni, R. F., Gross, M. C., Schneider, C. H., Vicari, M. R., Almeida, M. C. & Matoso, D. A. (2008). Epifluorescence and light microscopy evidencing structural and functional polymorphism of ribosomal DNA in fish (Teleostei: *Astyanax fasciatus*). *Micron* **39,** 1156–1159.

Ashley, T. & Ward, D. C. (1993). A hot spot of recombination coincides with interstitial telomeric sequence in the Armenian hamster. *Cytogenetic and Cell Genetics* **62,** 169–171.

Bleher, H., Stölting, K. N., Salzburger, W. & Meyer, A. (2007). Revision of the Genus *Symphysodon* Heckel, 1840 (Teleostei: Perciformes: Cichlidae) based on molecular and morphological characters. *International Journal of Ichthyology* **13,** 133–174.

Boisvert, F. M., Koningsbruggen, S. V., Navascués, J. & Lamond, A. I. (2007). The multifunctional nucleolus. *Molecular Cell Biology* **8,** 574–585.

Boron, A., Ozouf-Costaz, C., Coutanceau, J. P. & Woroniecka, K. (2006). Gene mapping of 28S and 5S rDNA sites in the spined loach *Cobitis taenia* (Pisces, Cobitidae) from a diploid population and a diploid–tetraploid population. *Genetica* **128,** 71–79.

Bühler, M. (2009). RNA turnover and chromatin-dependent gene silencing. *Chromosoma* **118,** 141–151.

Burt, A. & Trivers, R (2006). *Genes in Conflict: The Biology of Selfish Genetic Elements*. Cambridge, MA: Harvard University Press.

Chippari-Gomes, A. R., Lopes, N. P., Paula-Silva, M. N., Oliveira, A. R. & Almeida-Val, V. M. F. (2003). Hypoxia tolerance and adaptations in fishes: the case of Amazon cichlids. In *Fish Adaptation* (Val, A. L. & Kapoor, B. G., eds), pp. 37–54. Enfield, NH: Science Publishers, Inc.

Doudna, J. A. & Rath, V. L. (2002). Structure and function of the eukaryotic ribosome: the next frontier. *Cell* **109,** 153–156.

Eickbush, T. H. (2002). R2 and related site-specific non-long terminal repeat retrotransposons. In *Mobile DNA II* (Craig, N. L., Craigie, R. & Gellert, M., eds), pp. 1111–1144. Washington, DC: ASM Press.

Farias, I. P. & Hrbek, T. (2008). Patterns of diversification in the discus fishes (S*ymphysodon* spp., Cichlidae) of the Amazon basin. *Molecular Phylogenetics and Evolution* **49,** 32–43.

Feldberg, E., Porto, J. I. R. & Bertollo, L. A. C. (2003). Chromosomal changes and adaptation of cichlid fishes during evolution. In *Fish Adaptation* (Val, A. L. & Kapoor, B. G., eds), pp. 285–308. New Delhi & New York: Science Publishers.

Grewal, S. I. S. & Jia, S. (2007). Heterochromatin revised. *Nature Review* **8,** 35–46.

Gross, M. C., Feldberg, E., Cella, D. M., Schneider, M. C., Schneider, C. H. & Porto, J. I. R. (2009*a*). An intriguing evidence of translocations in discus fish (*Symphysodon*, Cichlidae) and report of the biggest meiotic chromosomal chain in vertebrates. *Heredity* **102,** 435–441.

Gross, M. C., Schneider, C. H., Valente, G. T., Martins, C. & Feldberg, E. (2009*b*). Repetitive elements and heterochromatinization can be acting in adaptation and karyoevolution of discus fish: meiotic chromosomal chain and genetic variability. *Cytogenetic and Genome Research* doi:10.1159/0008328.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/96/NT. *Nucleic Acid Symposium Series* **41,** 95–98.

Hernandez-Verdun, D. (2004). The nucleolus: functional organization and assembly. *Journal of Applied Biomedicine* **2,** 57–69.

Hernandez-Verdun, D., Roussel, P. & Gébrane-Younès, J. (2002). Emerging concepts of nucleolar assembly. *Journal of Cell Science* **115,** 2265–2270.

Howell, W. M. & Black, D. A. (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* **36,** 1014–1015.

Junk, W. J. (1997). General aspects of floodplain ecology with special reference to Amazonian floodplains. In *The Central Amazon Floodplain: Ecology of a Pulsing System* (Junk, W. J., ed.), pp. 3–20. Berlin: Springer-Verlag.

Junk, W. J. & Furch, K. (1993). A general review of tropical South American floodplains. *Wetlands Ecology and Management* **4,** 231–238.

Kobayashi, T. (2008). A new role of the rDNA and nucleolus in the nucleus – rDNA instability maintains genome integrity. *BioEssays* **30,** 267–272.

Martins, C. (2007). Chromosomes and repetitive DNA: a contribution to the knowledge of the fish genome. In *Fish Cytogenetics* (Pisano, E., Ozouf-Costaz, C., Foresti, F. & Kappor, B. G., eds), pp. 421–453. New Delhi & New York: Science Publisher.

Mesquita, D. R., Porto, J. I. R. & Feldberg, E. (2008). Chromosomal variability in the wild ornamental fish *Symphysodon* spp. (Perciformes, Cichlidae) from Amazon. *Neotropical Ichthyology* **6,** 181–190.

Molina, W. F. (2001). An alternative method for mitotic stimulation in fish cytogenetics. *Chromosome Science* **5,** 149–152.

Moreira-Filho, O. & Bertollo, L. A. C. (1990). *Astyanax scabripinnis* (Pisces, Characidae): a species complex. *Revista Brasileira de Genética* **14,** 331–357.

Ohno, S. & Atkin, N. B. (1966). Comparative DNA values and chromosome complements of eight species of fishes. *Chromosoma* **18,** 455–466.

Olson, M. O. J. (2004). Sensing cellular stress: another new function for the nucleolus? *Science's STKE* **224,** 1–10.

Pendás, A. M., Moran, P. & Garcia-Vasquez, E. (1993). Ribosomal RNA genes are interspersed throughout a heterochromatic chromosome arm in Atlantic salmon. *Cytogenetic and Cell Genetics* **63,** 128–130.

Pinkel, D., Straume, T. & Gray, J. W. (1986). Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proceedings of the National Academy of Sciences of the United States of America* **83,** 2934–2938.

Ready, J. S., Ferreira, E. J. G. & Kullander, S. O. (2006). Discus fishes: mitochondrial DNA evidence for a phylogeographic barrier in the Amazonian genus *Symphysodon* (Teleostei: Cichlidae). *Journal of Fish Biology* **69,** 200–211.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: A Laboratory Manual*. New York, NY: Cold Spring Harbor Laboratory Press.

Skipper, M. (2007). Mysteries of heterochromatic sequences unraveled. *Nature Review* **8,** 567.

Smith, W. L., Chakrabarty, P. & Sparks, J. S. (2008). Phylogeny, taxonomy, and evolution of Neotropical cichlids (Teleostei: Cichlidae: Cichlinae). *Cladistics* **24,** 625–641.

Sumner, A. T. (1990). *Chromosomes: Organization and Function*. Boston, MA: Blackwell Publishing.

Takai, A., Yoshikawa, T. & Ojima, Y. (2002). C-banded karyotype and nucleolus organizer regions in a discus fish, *Symphysodon aequifasciata axelrodi* (Cichlidae, Perciformes). *Chromosome Science* **6,** 53–55.

Thompson, K. W. (1979). Cytotaxonomy of 41 species of neotropical Cichlidae. *Copeia* **1979,** 679–691.

Thompson, J. D., Higging, D. G. & Gibson, T. J. (1994). ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and matrix choice. *Nucleic Acid Research* **22,** 4673–4680.

Varriale, A., Torelli, G. & Bernardi, G. (2008). Compositional properties and thermal adaptation of 18S rRNA in vertebrates. *RNA* **14,** 1492–1500.

Wasko, A. P. & Galetti, P. M. Jr. (2000). Mapping 18S ribosomal genes in fish of the genus *Brycon* (Characidae) by fluorescence *in situ* hybridization (FISH). *Genetics and Molecular Biology* **23,** 135–138.