ZEBRAFISH
Volume 11, Number 6, 2014

DOI: 10.1089/zeb.2014.1013

Construction and Characterization of a Repetitive DNA Library in Parodontidae (Actinopterygii: Characiformes): A Genomic and Evolutionary Approach to the Degeneration of the W Sex Chromosome

Michelle Orane Schemberger,[1] Jordana Inácio Nascimento Oliveira,[2] Viviane Nogaroto,[2] Mara Cristina Almeida,[2] Roberto Ferreira Artoni,[2] Marta Margarete Cestari,[1] Orlando Moreira-Filho,[3] and Marcelo Ricardo Vicari[1]

Abstract

Repetitive DNA sequences, including tandem and dispersed repeats, comprise a large portion of eukaryotic genomes and are important for gene regulation, sex chromosome differentiation, and karyotype evolution. In Parodontidae, only the repetitive DNAs W*Ap* and pPh2004 and rDNAs were previously studied using fluorescence *in situ* hybridization. This study aimed to build a library of repetitive DNA in Parodontidae. We isolated 40 clones using C_ot-1; 17 of these clones exhibited similarity to repetitive DNA sequences, including satellites, minisatellites, microsatellites, and class I and class II transposable elements (TEs), from *Danio rerio* and other organisms. The physical mapping of the clones to chromosomes revealed the presence of a satellite DNA, a *Helitron* element, and degenerate short interspersed element (SINE), long interspersed element (LINE), and tc1-mariner elements on the sex chromosomes. Some clones exhibited dispersed signals; other sequences were not detected. The 5S rDNA was detected on an autosomal pair. These elements likely function in the molecular degeneration of the W chromosome in Parodontidae. Thus, the location of these elements on the chromosomes is important for understanding the function of these repetitive DNAs and for integrative studies with genome sequencing. The presented data demonstrate that an intensive invasion of TEs occurred during W sex chromosome differentiation in the Parodontidae.

Introduction

Repetitive DNA sequences form a large portion of the genomes of eukaryotes.[1] These sequences are present in multiple copies that appear in tandem repeats (satellite, minisatellite, and microsatellite DNA) and interspersed repeats (transposable elements [TEs]).[2] Two classes of TEs are distinguished by their transposition intermediate: RNA (class I or retrotransposons) or DNA (class II or DNA transposons).[3]

Repetitive elements are important for the genome structure and function.[4–6] For example, satellite DNAs play a role in chromosome segregation during the cell cycle and comprise the centromeres of chromosomes, which bind microtubules.[7] Furthermore, both types of repeats can control gene expression on transcriptional and post-transcriptional regulatory networks.[4–6] Studies demonstrated that satellite DNA plays a role in position-effect variegation and that TEs had been coopted to serve gene regulatory functions for the host, a process that fits under the term ''molecular exaptation.''[8]

Several methods are used to isolate repetitive DNA sequences. The traditional method for isolating repetitive DNA sequences is genomic DNA restriction. However, reassociation kinetics based on C_ot-1 DNA and the microdissection of chromosomes submitted to C-banding and the subsequent amplification of heterochromatic sequences using degenerate oligonucleotide-primed polymerase chain reaction (PCR) have been used successfully.[9–14]

The Parodontidae is a Neotropical fish group that is divided into three genera: *Parodon*, *Apareiodon*, and *Saccodon*.[15] Cytogenetic studies reported a conserved diploid

[1]Programa de Pós-Graduação em Genética, Departamento de Genética, Centro Politécnico, Universidade Federal do Paraná, Curitiba, Paraná State, Brazil.
[2]Programa de Pós-Graduação em Biologia Evolutiva, Departamento de Biologia Estrutural, Molecular e Genética, Universidade Estadual de Ponta Grossa, Ponta Grossa, Paraná State, Brazil.
[3]Departamento de Genética e Evolução, Universidade Federal de São Carlos, São Carlos, São Paulo State, Brazil.

number of 54 chromosomes in Parodontidae with distinctive karyotype formulae and numerical/position variation of 18S and 5S rDNA sites.[16–21] In this group, two probes for repetitive DNAs have been described: pPh2004[16] and W*Ap*,[17] isolated by genomic digestion and chromosome microdissection, respectively. In addition, this Neotropical fish group is interesting for genetics studies because there are species without heteromorphic sex chromosomes, species with protosex chromosomes, species with a ZZ/ZW sex chromosome system, and species with a multiple ZZ/ZW_1W_2 sex chromosome system.[17–21] Furthermore, researchers have proposed that in Parodontidae the spreading of the repeat elements is the major event leading to genome rearrangements.[17–19]

Thus, in this study, we isolated and characterized several classes of repetitive DNAs to gain a greater understanding of the dynamics of these genomic sequences in karyotypes and of the mechanism of W sex chromosome differentiation in Parodontidae.

Materials and Methods

Species analyzed and chromosome preparations

Seven species from two genera of Parodontidae were selected for chromosome analysis: *Apareiodon piracicabae*, *Apareiodon hasemani*, *Apareiodon* sp., *Apareiodon affinis*, *Parodon pongoensis*, *Parodon nasus*, and *Parodon hilarii* (Table 1). Voucher specimens were deposited in the Zoology Museum of the *Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura* of the Maringá State University in Brazil. The procedures for sample collection were performed in compliance with the Ethics Committee on Animal Experimentation (process number: 07/2011) of the Ponta Grossa State University in Brazil. Chromosome preparations were made from anterior kidney cells using an air-drying method.[22]

Construction of a repetitive DNA library using reassociation kinetics (C_ot-1)

Genomic DNA from the *Apareiodon* sp. was extracted using the cetyltrimethylammonium bromide procedure.[23] A library containing DNA enriched for highly and moderately repetitive DNA sequences was constructed based on the renaturation kinetics of C_ot-1 DNA,[24] with modifications.[9] The obtained DNA fragments were inserted into the plasmid vector from the pMOSBlue Blunt-ended kit (Amersham Bioscience, Pittsburgh, PA) and cloned into DH5α *Escherichia coli* competent cells. These sequences were subsequently used as probes for fluorescence *in situ* hybridization (FISH).

Sequencing and characterization of the obtained fragments

The obtained clones were sequenced using an ABI-PRISM Genetic Analyzer (Carlsbad, CA). To identify similarities, the DNA sequences were analyzed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), CENSOR online software,[25] and RepeatMasker (www.repeatmasker.org). The sequences also were analyzed using a tandem repeat finder program (http://tandem.bu.edu/trf/trf.html) to identify the repeat unit. The sequences were deposited into the GenBank database (accession number: KJ809118- KJ809133; Table 2).

Fluorescence in situ *hybridization*

FISH was performed using all of the fragments obtained using C_ot-1 (in this study, called the total C_ot-1 probe), clones of the C_ot-1 library probes, and the pPh2004 probe.[16] The total C_ot-1 probe was labeled with digoxigenin11-dUTP (Roche Applied Science, Mannheim, Germany) through nick translation, and clones of the C_ot-1 library probes were labeled with digoxigenin 11-dUTP through PCR amplification using primers for the vector (T7 promoter and U19). The pPh2004 probe was labeled with biotin through PCR amplification using primers for the vector (T7 promoter and M13 reverse). The PCRs contained 20 ng of template DNA, 1 × reaction buffer, 2 mM $MgCl_2$, 40 μM dTTP, dGTP, and dCTP, 20 μM dATP, 20 μM 14-dATP-biotin (Invitrogen, San Diego, CA), 0.3 μM of each primer, and 1 U of Taq DNA Polymerase (Invitrogen).

FISH was performed under high-stringency conditions (i.e., 2.5 ng/μL of probe, 50% formamide, 2 × SSC, and 10% dextran sulfate for 18 h at 37°C) according to Pinkel *et al.*[26] Signal detection was performed using an anti-streptavidin antibody conjugated to Alexa Fluor 488 (Molecular Probes, Carlsbad, CA) and an anti-digoxigenin antibody conjugated to rhodamine (Roche Applied Science). The chromosomes were counterstained with 0.2 μg/mL of 4′,6-diamidino-2-phenylindole (DAPI) in the Vectashield mounting medium (Vector, Burlingame, CA) and analyzed using an Olympus BX41 epifluorescence microscope equipped with the DP71 digital image capture system (Olympus, Tokyo, Japan). The chromosomes were identified using the system proposed by Levan *et al.*[27] and classified as metacentric (m), submetacentric (sm), or subtelocentric (st).

Table 1. Parodontidae Species Name, Sampling Locality, Hydrographic Basin Name, and Number of Specimens Studied

Species	*River (State)*	*Hydrographic basin*	*GPS localization*	*No.* ♂	*No.* ♀
Apareiodon piracicabae	Rio Piumhi (MG)	São Francisco	−20°31′55″; −46°02′42″	5	3
Apareiodon sp.	Rio Verde (PR)	Alto Paraná	−25°04′35″; −50°04′03″	10	15
Apareiodon hasemani	Rio São Francisco (MG)	São Francisco	−17°21′17″; −44°57′18″	2	2
Apareiodon affinis	Rio Passa-Cinco (SP)	Alto Paraná	−22°25′26″; −47°41′56″	7	5
Parodon pongoensis	Rio Taquaralzinho (MT)	Araguaia	−15°53′28″; −52°14′56″	4	5
Parodon nasus	Rio Paraguai (MT)	Paraguai	−15°34′40″; −56°09′58″	6	3
Parodon hilarii	Córrego do Porta (MG)	São Francisco	−17°21′17″; −44°57′15″	9	12

MG, Minas Gerais State; PR, Paraná State; SP, São Paulo State; MT, Mato Grosso State.

Table 2. Characterization and Chromosomal Mapping of Clones from the Parodontidae C_ot-1 Library

Clone	Size (bp)	Characterization	Similarity (Blastn, Censor, or RepeatMasker (%)/species/accession: cover position)	Accession number
Satellite, minisatellite, and microsatellite DNA				
1	40	Microsatellite (repeating unit: 2 bp)[a]	DNA sequences of the clone DKEYP-10C7 in the linkage group 5 (100%)/*Danio rerio*/emb BX571679.39: 1–40 bp[b] Simple repetitions (100%)/*Danio rerio:* 1–40 bp[c]	—
2	119	Minisatellite (repeating unit: 16 bp)[a]	Simple repetitions (79%)/*Danio rerio*: 23–102 bp[c]	KJ809118
3	85	Minisatellite (repeating unit: 32 bp)[a]	No similarity detected	KJ809119
4	62	Satellite	No similarity detected	KJ809120
5	209	Minisatellite (repeating unit: 61 bp)[a]	No similarity detected	KJ809121
DNA transposon (class II)				
6	112	DNA transposon	DNA sequence of clone CH73-66D24 in the linkage group 25 (88%)/*Danio rerio*/emb FP102951.7: 40–112 bp[b] *Helitron* element (80%)/*Danio rerio*: 12–111 bp[d] *Helitron* element (80%)/*Danio rerio*: 12–111 bp[c]	KJ809122
7	75	DNA transposon	*MuDR* element (70%)/*Arabidopsis lyrata*: 7–40 bp[d] Simple repetitions (75.6%)/*Danio rerio*: 1–63 bp[c]	KJ809123
8	160	DNA transposon	*Polinton* element (77%)/*Danio rerio*: 7–48 bp [d] Tc1-*Mariner* element (77%)/*Takifugu rubripes*: 57–160 bp[d] Simple repetitions (85.3%)/*Danio rerio*: 1–30 bp[c] Tc1-*Mariner* element (76%)/*Danio rerio*: 57–160 bp[c]	KJ809124
9	200	DNA transposon	*HARB-2N1 Mad* element *(71%)/Malus x domestica:* 1–199 bp[d] *HatN2* element (70%)/*Danio rerio*: 80–181 bp[d] Simple repetitions (86%)/*Danio rerio*: 11–59 bp[c] Simple repetitions (72.3%)/*Danio rerio*: 81-181bp[c]	KJ809125
10	156	DNA transposon	Tc1-*Mariner* element (79%)/*Takifugu rubripes*: 1–101 bp[d] *Polinton* element (92%)/*Danio rerio*: 114–154bp[d] *Dada-tL_DR* element (100%)/*Danio rerio*: 122–156 bp[d] Tc1-*Mariner* element (78.2)/*Danio rerio*: 1–101 bp[c] simple repetitions (100%)/*Danio rerio*: 122–154 bp[c]	KJ809126
11	80	DNA transposon	*Polinton* element (76%)/*Strongylocentrotus purpuratus*: 1–80 bp[d]	KJ809127
Retrotransposon (class I)				
12	67	Retrotransposon	*I-2_Deu* element (79%)/*Drosophila eugracilis*: 1–67 bp[d]	KJ809128
13	547	Retrotransposon	B1-dID element (85%)/Rodentia: 302–342 bp[c]	KJ809129
DNA transposon (class II), retrotransposon (class I), and pseudogene				
14	146	DNA transposon and retrotransposon	*EnSpm1_SB* element (84%)/*Sorghum bicolor*: 1–43 bp[d] *Gypsy-12_BD-I* element (76%)/*Brachypodium distachyon*: 79–146 bp[d]	KJ809130

(*continued*)

TABLE 2. (CONTINUED)

Clone	Size (bp)	Characterization	Similarity (Blastn, Censor, or RepeatMasker (%)/species/accession: cover position)	Accession number
15	312	DNA transposon and retrotransposon	DNA sequence from clone CH73-112C8 in the linkage group 15 (97%)/*Danio rerio*/emb CU915286.9: 177–211[b] TC1_TF element (79%)/*Salmo salar:* 781–847 bp[d] SINE_AFC element (68%)/*Lepidiolamprologus elongates*: 103–163 bp[d]; SINE_FR1D element (84%): 172–210 bp[d] L2-2_DRe (85%): 212–264 bp[d] L2-2_DRe (86.8%)/*Danio rerio*: 212–264 bp[c]	KJ809131
16	824	DNA transposon; retrotransposon and pseudogene/ tRNA	tRNAThr_CB (81%)/*Caenorhabditis briggsae*: 15–52 bp[d] SINE2-1_GA (76%)/*Gasterosteus aculeatus*: 383–467 bp[d]; SINE_DR1 (83%)/*Danio rerio*: 468–515 bp[d] TC1_TF (78%)/*Salmo salar:* 517–624 bp[d] TC1_FR1 (85%)/*Takifugu rubripes*: 634–819 bp[d] L2-2_DRe (86.8%)/*Danio rerio*: 468–518 bp[c] TC1DR3 (81.7%)/*Danio rerio*: 63–819 bp[c]	KJ809132
rDNA				
17	59	5S rDNA or retrotransposon	Clone sequence TC-B-6 ribosomal gene 5S RNA[b] (98%)/*Chanodichthys ilishaeformis*/ gbJQ317903.1: 1-30 bp SINE3/5S of *Anolis carolinensis* (94.8%): 1–58 bp[d] 5Sr DNA of *Danio rerio* (94.8%): 1–58 bp[c]	KJ809133

[a]Minisatellite, microsatellite, and satellite DNAs identified using a tandem repeat finder program available at http://tandem.bu.edu/trf/trf.html
[b]Sequences with similarity to repetitive elements present in the database available at http://blast.ncbi.nlm.nih.gov/
[c]Sequences with similarity to repetitive elements present in the database available at www.repeatmasker.org/
[d]Sequences with similarity to repetitive elements present in the database available at www.girinst.org/censor/index.php
SINE, short interspersed elements.

Results

Isolation of repetitive DNA

The fragments obtained through C_ot-1 ranged from 40 to 824 bp in size (Table 2). To evaluate the quality and location pattern of C_ot-1 DNA fragments, the total C_ot-1 probe was used for hybridization onto metaphase specimens of the *Apareiodon* sp. and *P. hilarii* females. The total C_ot-1 probe was detected on the long arm of the W chromosome, on the proximal and terminal regions of the short arm of the Z chromosome, and additional sites on terminal regions of some autosomes in both species. However, a greater accumulation of these sequences was observed on the W chromosome of *P. hilarii* (Fig. 1A, B).

Characterization of the C_ot-1 library

A C_ot-1 library containing 40 clones was constructed and sequenced, which originated 23 clones with DNA inserts. The majority of the obtained sequences represented portions of class II TEs (i.e., nine clones, with three clones also containing retrotransposons), two clones matched retrotransposons, five clones were composed of tandem DNA repeats, one clone exhibited high similarity to rDNAs, one clone contained a pseudogene, and three clones exhibited similarity to two or more transposons each. Six clones exhibited no similarity to any sequences in the database used in this study. The *Polinton*, *Tc1-Mariner*, and *L2-2_DRe* elements were represented by more than one clone (Table 2 and Fig. 2).

Physical chromosome mapping of repetitive DNAs

The clone containing a fragment corresponding to the TE *Helitron* (clone 6, Table 2) was detected in the terminal region on the long arm of the W chromosomes of Parodontidae, and the block of *Apareiodon* sp. was more conspicuous (Fig. 3A, B). In contrast, the Z chromosome of *P. hilarii* exhibited positive signals for this probe in the terminal and proximal regions of the short arm (Fig. 3B). FISH with the clone corresponding to the 5S rDNA (clone 17) was positive in the proximal region of the short arm of autosomal pair 11 of the *Parodon hilarii* (Fig. 3C). Clones 13, 15, and 16 contained several degenerated TE sequences, including short interspersed elements (SINEs), long interspersed elements (LINEs), and *Tc1-Mariner* (Table 2), and exhibited dispersed signals, including signals on the long arm of the W chromosome of *P. hilarii* (Fig. 3D–F).

Clone 4, which contained satellite DNA named in this study sat1WP, possesses 62 bp and exhibits no similarity to any sequence deposited in the genome databases. However,

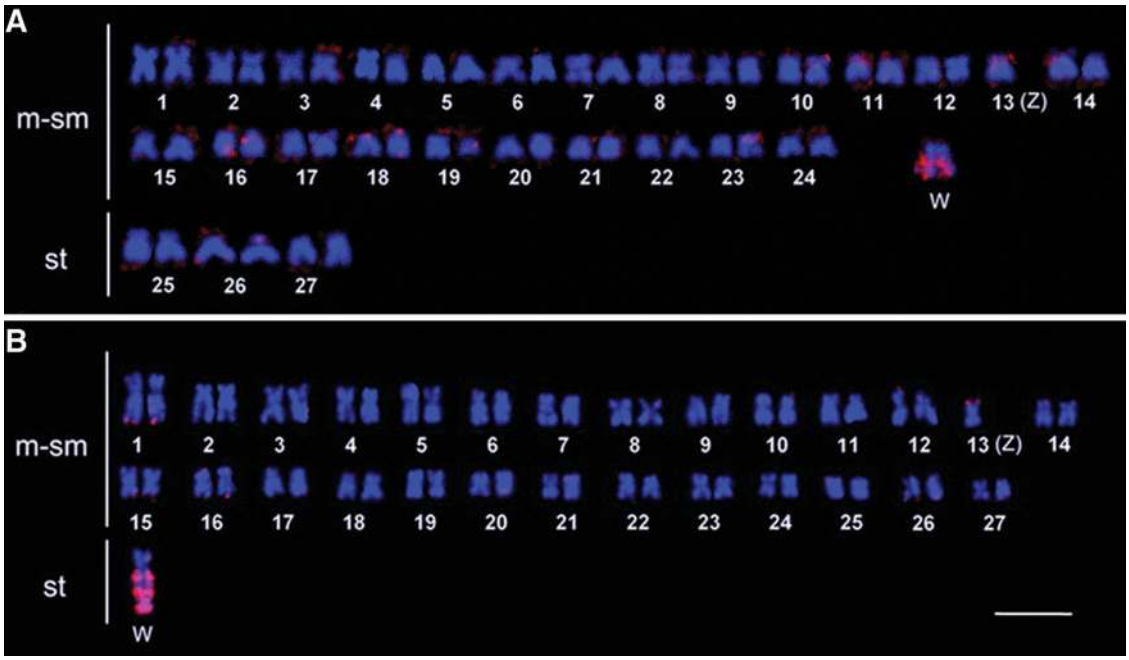

FIG. 1. Karyotypes of females of *Apareiodon* sp. **(A)** and *Parodon hilarii* **(B)** subjected to FISH using the total C_ot-1 probe (*red*). This probe was mapped onto various terminal chromosomes, onto the pericentromeric and terminal regions of the short arm of the Z chromosome, and onto the long arm of the W chromosome of both species, with a greater accumulation in *P. hilarii*. Scale bar = 5 μm. FISH, fluorescence *in situ* hybridization. Color images available online at www.liebertpub.com/zeb

A. piracicabae, which lacks heteromorphic sex chromosomes, contains sites of this sat1WP that are more apparent in the terminal regions of chromosomes 1, 4, 5, 6, 9, 14, 18, 20, 23, 24, and 25 (Fig. 4A). *P. pongoensis* contains sites on the terminal regions of several chromosomes of the karyotype, but the signal was more visible on pairs 3, 4, 6, 8, 11, 13 (i.e., the proto-sex chromosome), 24, and 27 (Fig. 4B). *P. nasus* exhibits terminal signals, including a signal on the pair 13 proto-sex chromosome (Fig. 4C). *A. hasemani* contains terminal sites on pairs 2, Z, 5, 8, 12, 13, 18, 20, 24, 26, 27 and at the terminal region on the short arm of the W chromosome (Fig. 4D). The physical mapping of the sat1WP probe in *P. hilarii* revealed signals in the interstitial region of the long arm of the W sex chromosome and in the terminal regions of the other chromosomes, including pairs 1, 6, 9, 11, 13, and Z (Fig. 4E). This sat1WP repetitive DNA also exhibited signals in the proximal region of the short arm of the Z chromosome in *A. affinis* (Fig. 4F). In addition, terminal sites were observed on the W_1 and W_2 chromosomes and on autosomes, except for pairs 2, 5, 15, and 23. The green pPh2004 probe was only used to facilitate the correct identification of chromosomes, as this probe has been described in other studies.[16,18]

Discussion

The repetitive DNA is important as a key component to shape the genome architecture and led to the emergence of genetic innovations in different eukaryotic lineages.[4,28–32] Furthermore, previous studies reported the participation of these sequences in chromosomal rearrangements associated with karyotype diversification events and the differentiation of sex chromosomes.[33–37] New methods of next-generation genomic sequencing generated a large amount of information, providing extensive data related to the DNA sequences of organisms; however, arranging the position of repetitive sequences is extremely difficult.[38] Thus, numerous studies identified and physically mapped repetitive sequences to assist in the organization of chromosomal regions rich in repetitive DNA, particularly in heteromorphic sex chromosomes.[39–41]

C_ot-1 DNA is a useful tool to isolate highly and moderately repetitive DNAs, according to Ferreira and Martins[42] and Zhang *et al.*[43] In this study, we described different types of repetitive sequences isolated from the Parodontidae C_ot-1 library: satellite DNAs, minisatellite DNAs, microsatellite DNAs, DNA transposons, and retrotransposons. Physical mapping of the total C_ot-1 probe revealed locations in the terminal regions of some autosomes, as well as in the proximal region of the short arm of the Z chromosome and in the long arm of the W chromosome of the *Apareiodon* sp. and *P. hilarii*. These data corroborate the study of Schemberger *et al.*,[17] which reported extensive accumulation of repetitive DNA in the W chromosomes of the Parodontidae.

The most abundant repetitive DNA sequences in the Parodontidae C_ot-1 library were the DNA TEs. Dispersed repeat element degenerates in genomic DNA provide molecular co-option into the host genome, act as new regulatory sites of gene expression, form new genes, generate microRNAs and proteins that are involved in post-transcriptional regulation, and serve as sites for insulator elements.[4,5] Among the repetitive sequences obtained in this study, the *EnSpm1_SB*, *Dada-tL_DR*, *HARB-2N1 Mad*, *Hat N22 Dr*, *MuDR*, Tc1-*Mariner*, and *Polinton*-1 Dr elements were present in dispersed locations on the chromosomes of Parodontidae.

Interestingly, among the TEs obtained in our study, the *MuDR* element isolated of rice originated a TPase protein that

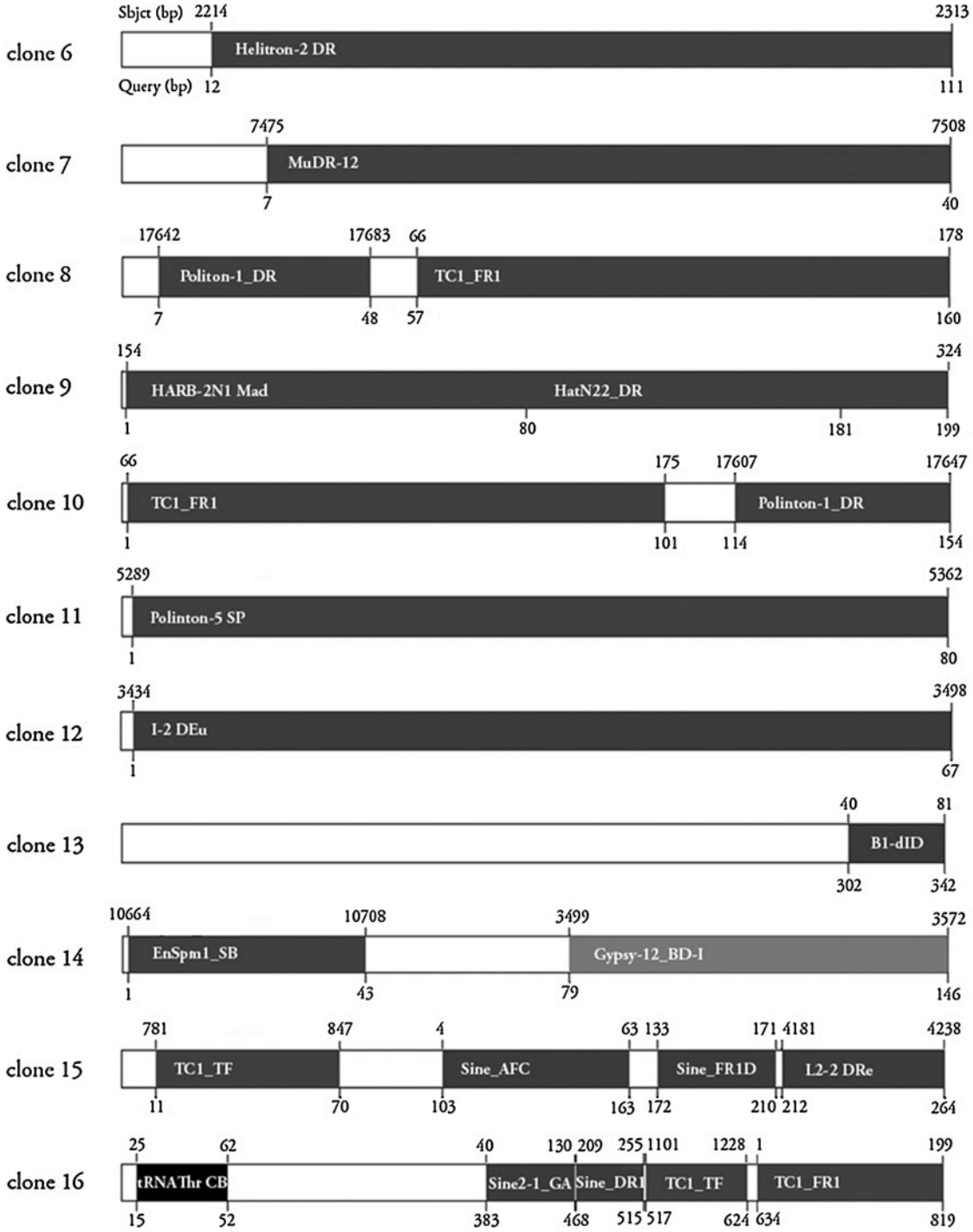

FIG. 2. Identity of the repeat DNA sequences of the TEs of the Parodontidae C_ot-1 library in the Censor repeat DNA database. In the top images, the sequence positions of the TEs are illustrated. Below, the Parodontidae sequence positions are illustrated. TEs, transposable elements.

functions as a transcriptional regulator of its own expression.[44,45] Another TE, the *Hat* element, derived an insulated protein (BEAF-32 of *Drosophila*) that connects the specialized chromatin structure to the adjacent chromatin elements and the nuclear matrix.[46] Other degenerate TE sequences, such as miniature inverted-repeat transposable elements (MITEs), are often transcribed and contain terminal inverted repeats. These MITEs can produce handle RNA, which could theoretically be processed into siRNA.[47] Thus, there are distinct pathways that the degenerated TEs found in this study could act in the modification of gene expression in the Parodontidae genome.

Partial sequences of the retrotransposons *Gypsy-12_BD-I* (i.e., long terminal repeats [LTRs]) and *I-2_Deu* (i.e., non-LTR) were identified in the Parodontidae C_ot-1 library; however, these elements possess dispersed distribution and a

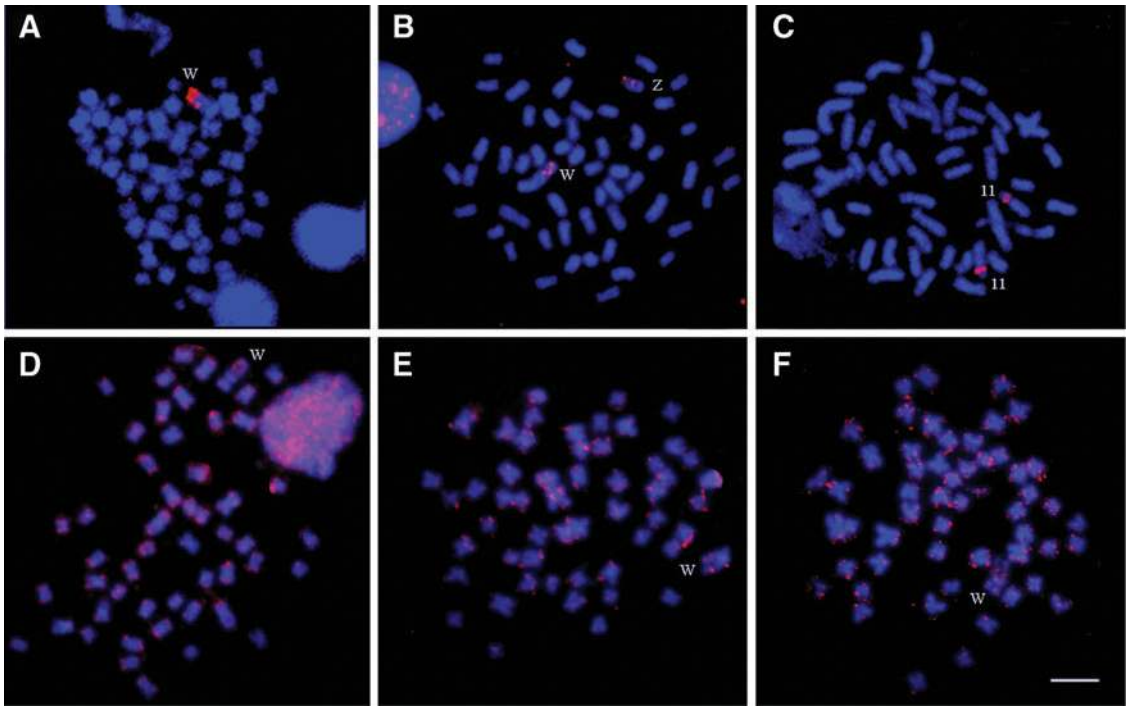

FIG. 3. Chromosomal mapping of the *Helitron* element—clone 6 **(A, B)**, 5S rDNA—clone 17 **(C)**, the TC1_TF element, SINE_FR1D, L2-2 DRe, and the SINE_AFC element—clone 15 **(D)**, tRNAThr_CB, SINE2-1_GA, SINE_DR1, TC1_TF, and TC1_FR1—clone 16 **(E)**, and B1-dID—clone 13 **(F)** through FISH on metaphases *Apareiodon* sp. female **(A)** and *P. hilarii* female **(B–F)**. The *Helitron* element is localized to the terminal region of the long arm of the W chromosome of both species. In addition, proximal and terminal sites were also observed on the short arm of the Z chromosome of *P. hilarii*. The 5S rDNA is present in the pericentromeric region of pair 11, and the clones 13, 15, and 16 exhibited dispersed localization, including locations on the W chromosome of *P. hilarii*. Scale bar = 5 μm. SINE, short interspersed elements. Color images available online at www.liebertpub.com/zeb

small number of the copies, as indicated by FISH (data not shown). The retrotransposons *B1-dID* (i.e., SINE/non-LTR), *SINE_AFC*, *SINE_FR1D*, *L2-2 DRe* (i.e., LINE/non-LTR), *SINE2-1_GA*, and *SINE_DR1* exhibited dispersed signals, including signals on the W chromosome of *P. hilarii* (Fig. 3D–F). These class I elements move in the genome as RNA intermediates, which facilitate the involvement of these elements in the genomic diversification process.[2]

Clones 13, 15, and 16 were localized on the W sex chromosome and organized as composite TEs, with more than one partial sequence of the TE classes I and II juxtaposed. These results reveal that an intensive invasion and accumulation of these repeat elements occurred during W sex chromosome differentiation, promoting gene erosion and heterochromatinization. With respect to the presence of retrotransposons on sex chromosomes, previous studies investigated the accumulation of these elements on the Y chromosome of *Drosophila*.[48] Steinemann and Steinemann[48] reported that the suppression of recombination strongly biases the distribution of retrotransposons in the genome. In addition, their results indicate that retrotransposons represent the major force driving the evolution of Y chromosomes, as these elements remodel euchromatic chromosome structures into heterochromatic structures. Thus, the accumulation of a large number of retrotransposons interferes strongly with the activity of genes.

The repetitive element *Helitron* exhibits an accumulation of repeats on the W and Z chromosomes and can be involved in the sex chromosome differentiation process and gene degeneration on the W chromosome in Parodontidae. The accumulation of dispersed repetitive DNA sequences has been described previously for fish sex chromosomes. The *REX* retrotransposon element and the Tc1-*Mariner* transposon are involved in the differentiation of sex chromosomes in *Chionodraco hamatus*.[49] LTR elements, LINEs, SINEs, and MITEs were also isolated from the sex chromosomes of *Xiphophorus maculatus*.[50] Importantly, the accumulation of the *Helitron* element in the W chromosome can promote gene degeneration and can also cause nucleation sites, promote insulation, generate siRNAs, and exert other effects. Furthermore, a gene named *teximY* (testis expressed in *X. maculatus* on the Y) was identified on the Y, but not on the X chromosome in platyfish; this gene is associated with a *Helitron* transposon in different fishes, suggesting that this gene has been captured and mobilized by these transposons.[51]

An analysis of the physical mapping of W*Ap* repetitive DNA in nine species of Parodontidae by Schemberger *et al.*[17] revealed a potential origin of sex chromosomes in this fish group. In this case, a paracentric inversion event rearranged the terminal W*Ap* sites to the proximal region of the short arms of a metacentric chromosome pair and these sequences subsequently accumulated at the origin of the heteromorphic W chromosome. In the present study, an analysis of the pattern of chromosomal hybridization for sat1WP suggests that this repetitive DNA element was involved in the paracentric inversion of the proto-sex chromosome that resulted in W chromosome differentiation

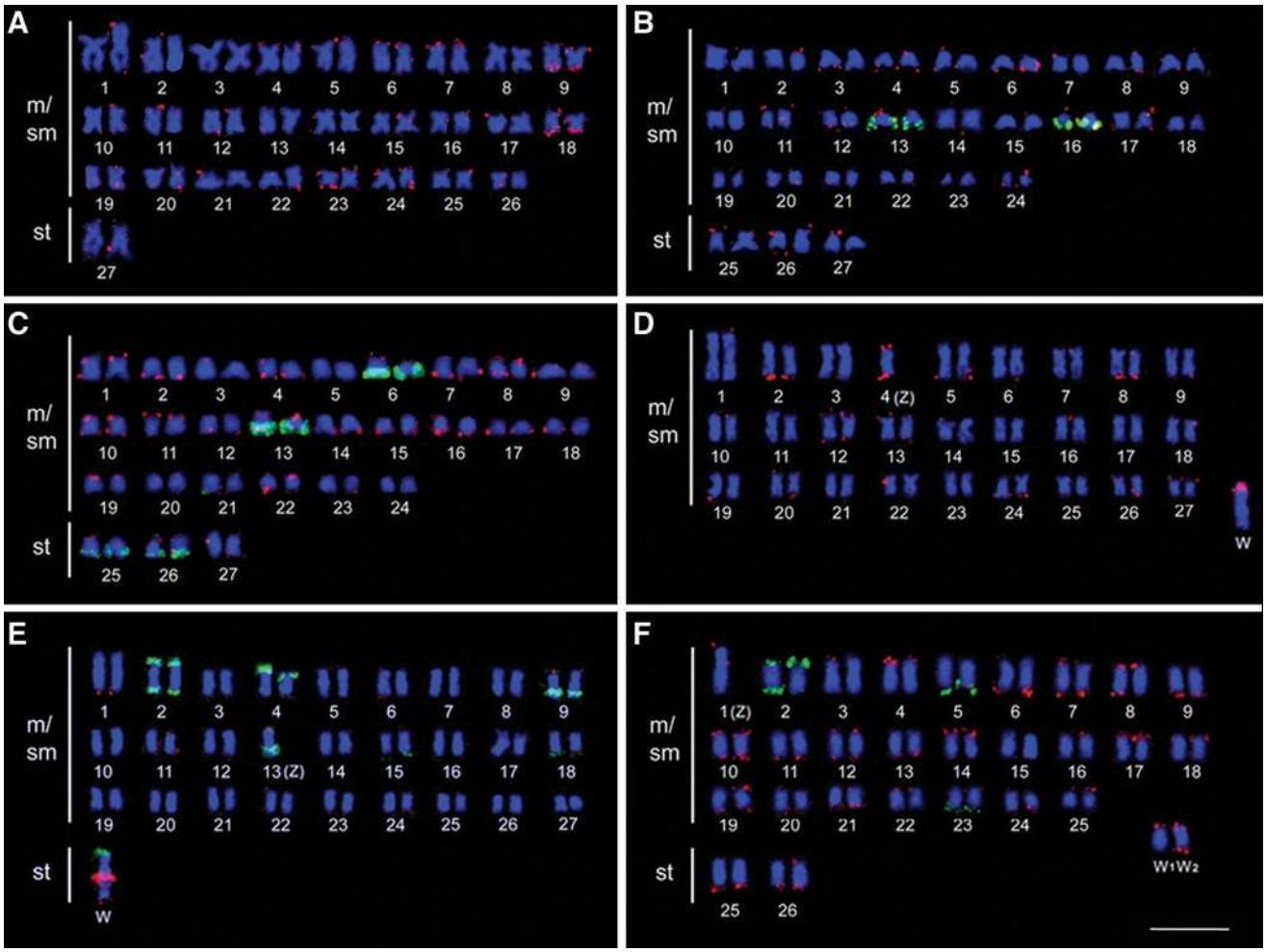

FIG. 4. Karyotypes subjected to FISH using the Sat1WP clone 4 probe (*red signals*) and the satellite DNA pPh2004 (*green signals*). **(A)** *Apareiodon piracicabae* female; **(B)** *Parodon pongoensis* female; **(C)** *Parodon nasus* female; **(D)** *Apareiodon hasemani* female; **(E)** *P. hilarii* female; **(F)** *Apareiodon affinis* female. The sites of clone 4 were located in the terminal regions of various chromosomes in the karyotypes of all species and on the W chromosomes of *A. hasemani* and *P. hilarii* and the Z chromosome of *A. affinis*. Scale bar = 5 μm. Color images available online at www.liebertpub.com/zeb

through the accumulation of repetitive sequences, corroborating the data reported by Schemberger *et al.*[17]

Our data show that different classes and types of repetitive DNAs are involved in the sex chromosome differentiation of Parodontidae. In addition to the satellite DNAs and TEs described in this report, other repetitive DNAs must play a role in gene degeneration and erosion on the W chromosome. Furthermore, these data are important for the molecular characterization of the W chromosome in Parodontidae and for future studies that aim to detect and localize gene sequences and the molecular co-option of TEs in eukaryotic genomes.

Acknowledgments

The authors are grateful to the Ministério do Meio Ambiente/Instituto Brasileiro do Meio ambiente e dos Recursos Naturais Renováveis (IBAMA/MMA/SISBIO No. 15117) for authorization to collect the biological samples. This study was financed by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Secretaria de Ciência e Tecnologia do Estado do Paraná (SETI), and Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná (Fundação Araucária).

Disclosure Statement

No competing financial interests exist.

References

1. Charlesworth B, Sniegowski P, Stephan W. The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 1994;371:215–220.
2. Jurka J, Kapitonov VV, Kohany O, Jurka MV. Repetitive sequences in complex genomes: structure and evolution. Annu Rev Genomics Hum Genet 2007;8:241–259.
3. Wicker T, Sabot F, Hua-Van A, *et al.* A unified classification system for eukaryotic transposable elements. Nat Rev Genet 2007;8:973–982.

4. Feschotte C, Pritham EJ. DNA transposons and the evolution of eukaryotic genomes. Ann Rev Genet 2007;41:331–368.
5. Feschotte C. Transposable elements and the evolution of regulatory networks. Nat Rev Genet 2008;9:397–405.
6. Herpin A, Braasch I, Kraeussling M, Schmidt C, Thoma EC, Nakamura S, *et al.* Transcriptional rewiring of the sex determining dmrt1 gene duplicate by transposable elements. PLoS Genet 2010;6:e1000844.
7. Ekwall K, Olsson T, Turner BM, Cranston G, Allshire RC. Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. Cell 1997;91:1021–1032.
8. Sinzelle L, Izsvak Z, Ivics Z. Molecular domestication of transposable elements: from detrimental parasites to useful host genes. Cell Mol Life Sci 2009;66:1073–1093.
9. Vicari MR, Nogaroto V, Noleto RB, Cestari MM, Cioffi MB, Almeida MC, *et al.* Satellite DNA and chromosomes in Neotropical fishes: methods, applications and perspectives. J Fish Biol 2010;76:1094–1116.
10. Kantek DLZ, Vicari MR, Peres WAM, Cestari MM, Artoni RF, Bertollo LAC, *et al.* Chromosomal location and distribution of As51 satellite DNA in five species of the genus *Astyanax* (Teleostei, Characidae, *Incertae sedis*). J Fish Biol 2009;75:408–421.
11. Blanco DR, Lui RL, Vicari MR, Bertollo LAC, Moreira-Filho O. Comparative cytogenetics of giant trahiras *Hoplias aimara* and *H. intermedius* (Characiformes, Erythrinidae): chromosomal characteristics of minor and major ribosomal DNA and cross-species repetitive centromeric sequences mapping differ among morphologically identical karyotypes. Cytogenet Genome Res 2011;132:171–178.
12. Cabral-de-Mello DC, Cabrero J, López-León MD, Camacho JPM. Evolutionary dynamics of 5S rDNA location in acridid grasshoppers and its relationship with H3 histone gene and 45S rDNA location. Genetica 2011;139:921–931.
13. Valente GT, Mazzuchelli J, Ferreira IA, Poletto AB, Fantinatti BE, Martins C. Cytogenetic mapping of the retroelements Rex1, Rex3 and Rex6 among cichlid fish: new insights on the chromosomal distribution of transposable elements. Cytogenet Genome Res 2011;133:34–42.
14. Pucci MB, Barbosa P, Nogaroto V, Almeida MC, Artoni RF, Pansonato-Alves JC, *et al.* Population differentiation and speciation in the genus *Characidium* (Characiformes: Crenuchidae): effects of reproductive and chromosomal barriers. Biol J Linn Soc 2014;111:541–553.
15. Pavanelli CS. New species of *Apareiodon* (Teleostei: Characiformes: Parodontidae) from the rio Piquiri, upper rio Paraná basin, Brazil. Copeia 2006;1:89–95.
16. Vicente VE, Bertollo LAC, Valentini SR, Moreira Filho O. Origin and differentiation of a sex chromosome system in *Parodon hilarii* (Pisces, Parodontidae). Satellite DNA, G- and C-banding. Genetica 2003;119:115–120.
17. Schemberger MO, Bellafronte E, Nogaroto V, Almeida MC, Schühli GS, Artoni RF, *et al.* Differentiation of repetitive DNA sites and sex chromosome systems reveal closely related group in Parodontidae (Actinopterygii: Characiformes). Genetica 2011;139:1499–1508.
18. Bellafronte E, Schemberger MO, Moreira-Filho O, Almeida MC, Artoni RF, Margarido VP, *et al.* Chromosomal markers in Parodontidae: an analysis of new and reviewed data with phylogenetic inferences. Rev Fish Biol Fisheries 2011;21:559–570.
19. Jesus CM, Moreira-Filho O. Cytogenetic studies in some *Apareiodon* species (Pisces, Parodontidae). Cytologia 2000; 65:397–402.
20. Jesus CM, Moreira-Filho O. Karyotypes of three species of *Parodon* (Teleostei: Parodontidae). Ichthyological Expl Freshw 2000;11:75–80.
21. Bellafronte E, Schemberger MO, Artoni RF, Moreira Filho O, Vicari MR. Sex chromosome system ZZ/ZW in *Apareiodon hasemani* Eigenmann, 1916 (Characiformes, Parodontidae) and a derived chromosomal region. Genet Mol Biol 2012;35:770–776.
22. Bertollo LAC, Takahashi CS, Moreira-Filho O. Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Braz J Genet 1978;1:103–120.
23. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res 1980;8:4321–4326.
24. Zwick MS, Hanson RE, Islam-Faridi MN, Stelly DM, Wing RA, Price HJ, *et al.* A rapid procedure for the isolation of C_0t-1 DNA from plants. Genome 1997;40:138–142.
25. Jurka J, Kapitonov VV, Pavlicek A, Klonowski P, Kohany O, Walichiewicz J. Repbase update, a database of eukaryotic repetitive elements. Cytogenet Genome Res 2005; 110:462–467.
26. Pinkel D, Straume T, Gray JW. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci U S A 1986;83:2934–2938.
27. Levan A, Fredga K, Sandberg AA. Nomenclature for centromeric position on chromosomes. Hereditas 1964;52:201–220.
28. Kuenova E, Champion L, Biessmann H, Mason JM. Directional gene silencing induced by a complex subtelomeric satellite from *Drosophila*. Chromosoma 1998;107:311–320.
29. O'Connell M, Wright JM. Microsatellite DNA in fishes. Rev Fish Biol Fisheries 1997;7:331–363.
30. Monod C, Aulner N, Cuvier O, Käs E. Modification of position-effect variegation by competition for binding to *Drosophila* satellites. EMBO Rep 2002;3:747–752.
31. Hernández-Hernández A, Rincón-Arano H, Recillas-Targa F, Ortiz R, Valdes-Quezada C, Echeverría OM, *et al.* Differential distribution and association of repeat DNA sequences in the lateral element of the synaptonemal complex in rat spermatocytes. Chromosoma 2008;117:77–87.
32. Bussiek M, Hoischen C, Diekmann S, Bennink ML. Sequence-specific physical properties of African green monkey alpha-satellite DNA contribute to centromeric heterochromatin formation. J Struct Biol 2009;167:36–46.
33. Rosa KO, Ziemniczak K, Barros AV, Nogaroto V, Almeida MC, Cestari MM, *et al.* Numeric and structural chromosome polymorphism in *Rineloricaria lima* (Siluriformes: Loricariidae): fusion points carrying 5S rDNA or telomere sequence vestiges. Rev Fish Biol Fisheries 2012;22:739–749.
34. Terencio ML, Schneider CH, Gross MC, Nogaroto V, de Almeida MC, Artoni RF, *et al.* Repetitive sequences associated with differentiation of W chromosome in *Semaprochilodus taeniurus*. Genetica 2012;140:505–512.
35. Terencio ML, Schneider CH, Gross MC, Vicari MR, Farias IP, Passos KB, *et al.* Evolutionary dynamics of repetitive DNA in *Semaprochilodus* (Characiformes, Prochilodontidae): a fish model for sex chromosome differentiation. Sex Dev 2013;7:325–333.
36. Schneider CH, Gross MC, Terencio ML, Artoni RF, Vicari MR, Martins C, *et al.* Chromosomal evolution of neotropical cichlids: the role of repetitive DNA sequences in the organization and structure of karyotype. Rev Fish Biol Fisheries 2013;23:201–214.
37. Palacios-Gimenez OM, Castillo ER, Martí DA, Cabral-de-Mello DC. Tracking the evolution of sex chromosome systems in Melanoplinae grasshoppers through chromo-

somal mapping of repetitive DNA sequences. BMC Evol Biol 2013;13:1–12.
38. Metzker ML. Sequencing technologies—the next generation. Nat Rev Genet 2009;11:31–46.
39. Wang C, Deakin J, Rens W, Zenger K, Belov K, Graves JM, *et al.* A first-generation integrated tammar wallaby map and its use in creating a tammar wallaby first-generation virtual genome map. BMC Genet 2011;12:422.
40. Kai W, Kikuchi K, Tohari S, Chew AK, Tay A, Fujiwara A, *et al.* Integration of the genetic map and genome assembly of fugu facilitates insights into distinct features of genome evolution in teleosts and mammals. Gen Biol Evol 2011;3:424–442.
41. Machado TC, Pansonato-Alves JC, Pucci MB, Nogaroto V, Almeida MC, Oliveira C, *et al.* Chromosomal painting and ZW sex chromosomes differentiation in *Characidium* (Characiformes, Crenuchidae). BMC Genet 2011;12:65.
42. Ferreira IA, Martins C. Physical chromosome mapping of repetitive DNA sequences in Nile tilapia *Oreochromis niloticus*: evidences for a differential distribution of repetitive elements in the sex chromosomes. Micron 2008;39:411–418.
43. Zhang L, Xu C, Yu W. Cloning and characterization of chromosomal markers from a Cot-1 library of peanut (*Arachis hypogaea* L.). Cytogenet Genome Res 2012;137:31–41.
44. Raizada MN, Brewer KV, Walbot V. A maize MuDR transposon promoter shows limited autoregulation. Mol Genet Genomics 2001;265:82–94.
45. Hudson ME, Lisch DR, Quail PH. The FHY3 and FAR1 genes encode transposase-related proteins involved in regulation of gene expression by the phytochrome A-signaling pathway. Plant J 2003;34:453–471.
46. Pathak RU, Rangaraj N, Kallappagoudar S, Mishra K, Mishra RK. Boundary element-associated factor 32B connects chromatin domains to the nuclear matrix. Mol Cell Biol 2007;27:4796–4806.
47. Sijen T, Plasterk RH. Transposon silencing in the *Caenorhabditis elegans* germ line by natural RNAi. Nature 2003;426:310–314.
48. Steinemann S, Steinemann M. Retroelements: tools for sex chromosome evolution. Cytogenet Genome Res 2005;110: 134–143.
49. Ferreira DC, Porto-Foresti F, Oliveira C, Foresti F. Transposable elements as a potential source for understanding the fish genome. Mob Genet Elements 2011;1:112–117.
50. Schultheis C, Zhou Q, Froschauer A, Nanda I, *et al.* Molecular analysis of the sex-determining region of the platyfish *Xiphophorus maculatus*. Zebrafish 2006;3:299–309.
51. Tomaszkiewicz M, Chalopin D, Schartl M, Galiana D, Volff JN. A multicopy Y-chromosomal SGNH hydrolase gene expressed in the testis of the platyfish has been captured and mobilized by a Helitron transposon. BMC Genet 2014;15:44.

Address correspondence to:
Michelle Orane Schemberger
Programa de Pós-Graduação em Genética
Departamento de Genética
Centro Politécnico
Universidade Federal do Paraná
Jardim das Américas, 81531-990, Caixa-Postal
Curitiba 19071
Paraná State
Brazil

E-mail: miorane@hotmail.com