

# Genomic content and new insights on the origin of the B chromosome of the cichlid fish *Astatotilapia latifasciata*

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**Abstract** B chromosomes are additional chromosomes widely studied in a diversity of eukaryotic groups, including fungi, plants and animals, but their origin, evolution and possible functions are not clearly understood. To further understand the genomic content and the evolutionary history of B chromosomes, classical and molecular cytogenetic analyses were conducted in the cichlid fish *Astatotilapia latifasciata*, which harbor 1–2 B chromosomes. Through cytogenetic mapping of several probes, including transposable elements, rRNA genes, a repeated DNA genomic fraction ( $C_{ot} - 1$  DNA), whole genome probes (comparative genomic hybridization), and BAC clones from *Oreochromis niloticus*, we found similarities between the B chromosome and the 1st chromosome pair and chromosomes harboring rRNA genes. Based on the cytogenetic mapping data, we suggest the B chromosome may have evolved from a small chromosomal fragment followed by the invasion of the proto-B chromosome by several repeated DNA families.

**Keywords** Cytogenetics · Evolution · Genome · Repeated DNA · Supernumerary chromosome

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## Introduction

The B chromosomes (Bs), also known as accessory, extra or supernumerary chromosomes, are dispensable chromosomes found in about 15% of eukaryotic species, including animals, plants and fungi (Camacho et al. 2000). It is broadly reported that Bs are heterochromatic and enriched with repetitive elements, such as satellites DNAs, rDNAs and transposable elements (Camacho 2005). The Bs can arise intraspecifically (from the host genome) or interspecifically (from the genome of a related species), from autosomal chromosomes, which is the most described hypothesis, or from sex chromosomes (Sapre and Deshpande 1987; Schartl et al. 1995; McAllister and Werren 1997). The Bs can be found in different numbers and morphologies (Camacho 2005), and the size is also variable, ranging from simple dot-like chromosomes (Trifonov et al. 2002) to large elements, such as the largest chromosome pair of the A complement (Maistro et al. 1992; Ziegler et al. 2003; Poletto et al. 2010a).

Among cichlid fish, Bs were identified in the South American species *Gymnogeophagus balzanii* (Feldberg and Bertollo 1984), *Geophagus brasiliensis*, *Cichlasoma paranaensis*, *Crenicichla niederleinii* (Martins-Santos et al. 1995), *Cichla monoculus*, *Cichla* sp. and *Crenicichla reticulata* (Feldberg et al. 2004). Among African cichlids, Bs were first described in *Astatotilapia latifasciata* (previously identified as *Haplochromis obliquidens* in Poletto et al. 2010a) from Lake Nawampasa, a satellite lake of Lake Kyoga system, and in *Metriaclima lombardoi* from Lake Malawi (Poletto et al. 2010b) in East Africa. Bs were also identified in other 11 African cichlid species from Lake Victoria (Yoshida et al. 2011).

Although the presence of B chromosomes has been extensively described for many species including fish,

several questions concerning its origin, evolutionary mechanisms and function remain unanswered. Here, we investigated the genomic composition of the B chromosome of *A. latifasciata* looking for contributions to understanding the origin and evolutionary pathways of supernumerary chromosomes. Cytogenetic mapping suggests a correlation between the genomic content of the B chromosome, and the largest chromosome pair of the A complement (pair one) and the chromosomes carrying 18S rRNA genes.

## Materials and methods

### Animal samples and conventional cytogenetic analysis

Specimens of *A. latifasciata* were obtained from the fish stock of breeders maintained in the fish room of the Laboratório Genômica Integrativa of UNESP—São Paulo State University. The original stock was obtained in 2008 from the aquarium trade in Botucatu/SP, Brazil. Metaphasic spreads were obtained from 131 *A. latifasciata* animals as described previously (Bertollo et al. 1978). C-banded chromosome preparation was performed according to Sumner (1972), and Chromomycin A<sub>3</sub> (CMA<sub>3</sub>) staining according to Schweizer (1976). To more precisely characterize the karyotype, chromosome measurements were conducted on all chromosomes for nine karyotypes prepared from three different specimens harboring one B chromosome. The data were analyzed according to the centromeric index (ci) that represents the ratio of the length of the short arm ( $p$ ) of the chromosome to that of the total chromosome ( $p + q$ , where  $q$  is the length of the short arm), expressed as a percentage according to the formula  $[100p/(p + q)]$ . The chromosomes were classified as metacentric (ci of 50.00–40.10), submetacentric (ci of 40.00–25.10), telocentric (ci of 25.00–0.01) or acrocentric (ci of 0.00) according to Guerra (1986). The genomic fraction (%) of Bs in relation to the entire genome was calculated based on the chromosome measures.

Based on the measures of Bs, we conducted analysis to verify the morphological divergence between double Bs present in the same cell. To rule out that size differences between double Bs are due to technical artifacts, the same measures were performed with chromosome pairs 1 and 8, which are easily recognizable in the karyotype. The chromosome-size analysis of Bs and members of chromosome pairs 1 and 8 were performed in 26 metaphases. The chromosomes were then separated into a larger or smaller group for each pair 1, pair 8, and double Bs based on their length. The mean length-value of each group (larger and smaller) for each pair 1 and pair 8 chromosomes, and double Bs was calculated. The measures of Bs, 1st and 8th

pairs were subjected to the Student's t-test to determine the statistical significance.

### Cytogenetic mapping

#### *Chromosomal probes obtaining and labeling*

Genomic DNA was extracted using the phenol–chloroform method according to Sambrook and Russel (2001) using liver tissue from 0B (animals with no presence of B chromosomes) and 1B (animals with the presence of one B chromosome) individuals. The probes included the  $C_{0t} - 1$  DNA fraction, the retrotransposons *Rex1*, *Rex3* and *Rex6*, the 5S and 18S rDNAs, and bacterial artificial chromosomes (BACs) from a genomic library of the Nile Tilapia *O. niloticus* (Cichlidae). The whole genome was used as probe for comparative genomic hybridization (CGH) analysis to map metaphasic spreads containing 1B chromosome. All the probes were obtained from 0B individuals, except for the CGH experiments, which included 1B genomic probes, and BACs were from an *O. niloticus* genomic library.

The  $C_{0t} - 1$  DNA fraction was obtained based on the renaturation kinetics of DNA as previously described (Zwick et al. 1997) with modifications (Ferreira and Martins 2008) as follow. DNA samples (200  $\mu$ l of 100–500 ng/ $\mu$ l genomic DNA in 0.3 M NaCl) were autoclaved for 30 min at 1.4 atm and 120°C and the fragmented DNA was sized by electrophoresis in a 1% agarose gel. Expected DNA size fragments should range between 100 and 1,000 base pairs (bp). Samples of 50  $\mu$ l of DNA fragments were denatured at 95°C for 10 min and the single stranded DNA stabilized in lower temperature (ice-cold water bath, 5°C) for 10 s. The single stranded DNA samples were transferred to a 65°C water bath for reannealing. After 1 min of reannealing, the samples were incubated at 37°C for 8 min with 1 U of S1 nuclease to permit digestion of single-stranded sequences. The samples were immediately frozen in liquid nitrogen and submitted to DNA extraction with phenol–chloroform. The DNA fragments obtained ranged in size from 100 to 1,000 bp and were labeled by nick-translation, and used as probes for FISH (100 ng per slide).

The retroelements and rDNAs were obtained by polymerase chain reaction (PCR) using distinct primers as previously described for *Rex1* (Volf et al. 2000), *Rex3* (Volf et al. 1999) and *Rex6* (Volf et al. 2001), the 5S rDNA (Martins and Galetti 1999), and 18S rDNA (Teixeira et al. 2009). The PCR products for *Rex1*, *Rex3*, *Rex6*, 5S rDNA and 18S rDNA were 500, 450, 500, 400 and 1,400 bp, respectively. The DNA fragments were labeled using conventional PCR except with the inclusion of 0.14  $\mu$ M dATP and 0.06  $\mu$ M of biotinylated-dATP (Invitrogen, San Diego, CA, USA). The PCR probes were then

digested with DNase and 100 ng of each probe was used for FISH.

Ten BAC clones selected from a genomic library of *O. niloticus* (Katagiri et al. 2005) were also applied as probes against the 1B metaphasic spreads (Table 1). The BACs selected belong to the linkage groups (LG) 1, 3 and 5. BACs were extracted with the PhasePrep<sup>®</sup>™ BAC DNA Kit (Sigma-Aldrich, St. Louis, MO, USA). The BAC clones were labeled with biotin, digoxigenin coupled nucleotides (Roche Applied Sciences, Indianapolis, IN, USA), CY3 and CY5-avidin (GE, Fairfield, CT, USA) using the whole genome amplification kits WGA2 and 3 (Sigma-Aldrich), according to the supplier's protocol. The DNase-I concentration was then titrated to yield labeled DNA fragments ranging from 100 to 500 bp products. Then, 10 µl of hybridization mixture (62.5% deionized formamide, 12.5% of 20XSSC, 25% of 50% dextran sulfate), 4 µg of blocking DNA (salmon/herring sperm DNA) and 100 ng of each probe were prepared for multicolor FISH. The solution was denatured for 10 min at 95°C, immediately cooled on ice, and then used as probes for FISH.

CGH experiments were carried out to check if the B chromosome of *A. latifasciata* possesses sex specific genomic segments. Genomic DNA from males and females, both with and without Bs, was used as probes in two rounds of experiments against 1B metaphases. Genomic DNA (400 ng) of each male and female (both without B chromosomes) were used to perform the first round of CGH experiments. In the second round, genomic DNA (400 ng) of male and female carrying B chromosomes was used as probes. The male CGH probes (0B male and 1B male genomic DNAs) were labeled with biotin using the BioNick DNA labeling system (Invitrogen) and female CGH probes (0B female and 1B female genomic DNAs)

**Table 1** BAC clones employed as probes for chromosome hybridizations

BAC ID	Probe ID	Linkage group	Marker	Genomic content
b04TI071O04	BAC #1	3	GM204	Repeated DNAs
b04TI010O22	BAC #2	5		Green opsin
b03TI066P02	BAC #3	3	GM354	Repeated DNAs
b03TI086K09	BAC #4	3	UNH115	Repeated DNAs
b04TI071H11	BAC #5	1	UNH995	Repeated DNAs
b04TI053F24	BAC #6	5		ATPase
b03TI073M01	BAC #7	3	Trp1	
b03TI088C14	BAC #8	3	UNH106	
b04TI008J05	BAC #9	1	CYP19A1	Ovarium aromatase
b03TI067N14	BAC #10	3	GM256	

with digoxigenin using the Dig-nick translation mix (Roche Applied Sciences) according to the manufacturer's instructions. Double hybridizations were performed using 400 ng of each sex genomic DNA. In the first part of the experiments, the 0B probes (female [digoxigenin] + male [biotin]) were hybridized on a 1B male spread and 1B female spread. In the second part, the 1B probes (female [digoxigenin] + male [biotin]) were also hybridized on a 1B male spread and 1B female spread.

#### Hybridization procedures

All the hybridizations were conducted against 1B chromosome spreads. Additionally, the retroelements, 5S rDNA and 18S rDNA sequences were hybridized to 2B spreads. The hybridization and detection procedures were performed according to the modifications proposed by Teixeira et al. (2009), except for the BAC probes, which were conducted according to Mazzuchelli et al. (2011).

#### Image processing

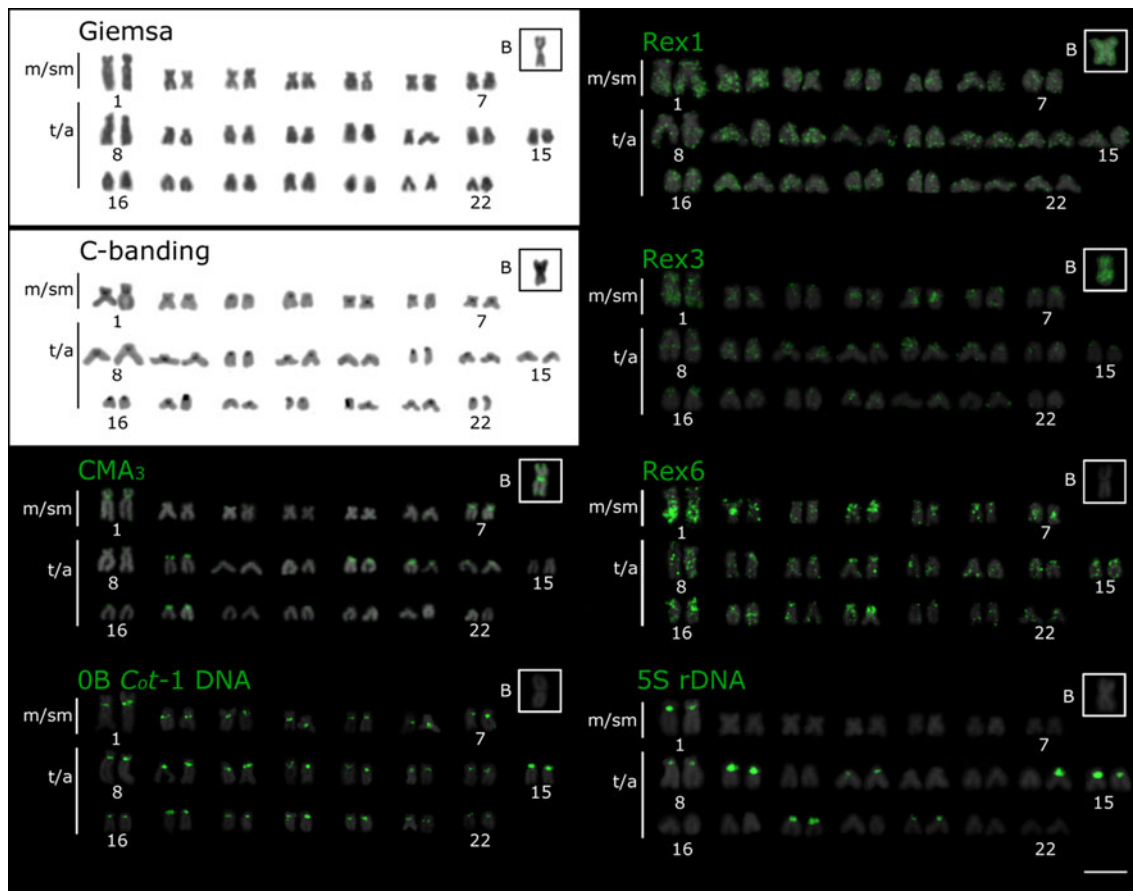
The images were captured with an Olympus DP71 digital camera coupled to a BX61 Olympus microscope and were optimized for brightness and contrast using Adobe Photoshop CS2.

## Results

### Conventional cytogenetic, C-banding and CMA<sub>3</sub> staining analysis

Using chromosomal morphology analysis, it was observed that the karyotype composed of 7 meta/submetacentric (m/sm) and 15 telo/acrocentric (t/a) chromosomes plus one or two large B elements (Fig. 1, Online Resources 1 and 2), which were present in 38 out of 131 sampled animals including both males and females (Table 2). The B chromosome size averaged 3.35 µm corresponding to about 6.48% of the whole chromosomal complement of the species. The mean difference between the largest B and the smallest B was 0.45 µm whereas the difference between members of the 1st pair or 8th pair was 0.23 µm (Online Resources 3, 4 and 5).

Heterochromatic blocks were observed in the centromeric region of most A chromosomes, in the 1p arm and in the whole extension of the B chromosome. CMA<sub>3</sub> staining detected positive sites on the 1p arm, in the pericentromeric region of pairs 1, 7, 13 and 14, in the short arm of chromosomes 8, 9, 12, 16 and 17, and in both pericentromeric and subtelomeric regions of the B chromosome (Fig. 1).



**Fig. 1** Karyotypes of *A. latifasciata* arranged from chromosomes, respectively, Giemsa-stained, C-banded, CMA<sub>3</sub>-stained and hybridized with the *Cot* – 1 DNA fraction, the retrotransposable elements

*Rex1*, *Rex3* and *Rex6*, and 5S rDNA. *m/sm* meta and submetacentric chromosomes; *t/a* telo and acrocentric chromosomes. The B chromosome is highlighted in the *box*. Bar indicates 5 μm

**Table 2** B chromosome distribution in the analyzed *A. latifasciata* sample

Number of Bs	Number of specimens	Males	Females	Unsexed	% of Bs in the sample	% of Bs in males	% of Bs in females
0	93	41	32	20	–	–	–
1	30	10	13	7	22,9	17,8	27
2	8	5	3	–	6,1	8,9	6,2

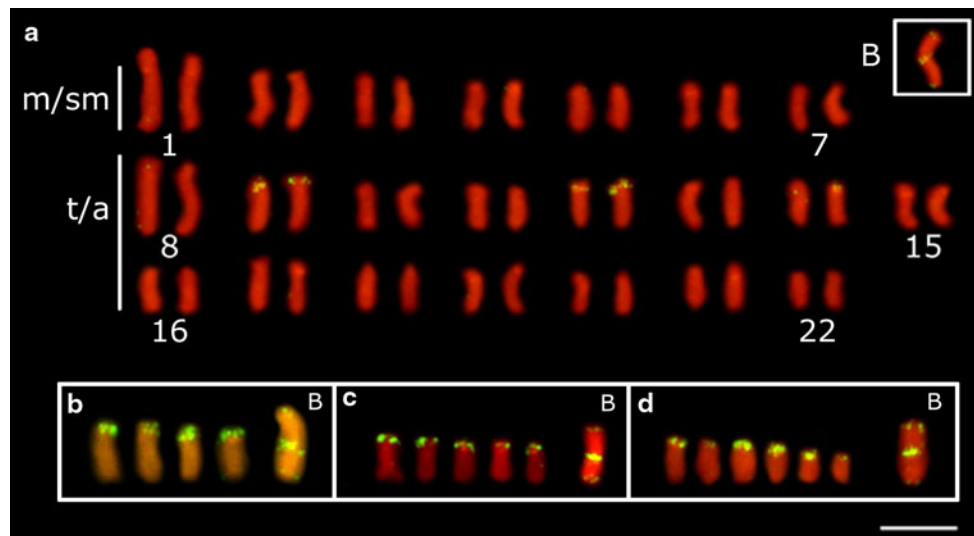
### Molecular cytogenetic mapping

The *Cot* – 1 DNA fraction obtained from a 0B individual generated strong hybridization signals in the pericentromeric region of all chromosomes of the A complement, but no signal was observed in the B chromosomes (Fig. 1).

The transposable elements *Rex1*, *Rex3* and *Rex6* hybridized in several chromosome pairs. Most of the signals were dispersed in the chromosomes with intense enrichment in the pericentromeric regions. *Rex1* presented clusters in most chromosome pairs, with high concentration of signals in most part of 1q and over the pericentromeric areas of most chromosome pairs, and in the whole extension of the B chromosome. *Rex3* was accumulated in the pericentromeric

regions, dispersed on pairs 1, 8p, in the interstitial site of 8q, and dispersed on the entire extension of the B chromosomes. *Rex6* showed dispersed signals in several chromosomes including the long arm of chromosome 1 and was not detected on the B chromosomes (Fig. 1).

The 5S rDNA probe generated signals on the pericentromeric region of 15 chromosomes (pairs 1, 8, 9, 11, 14 [only one homolog member], 15, 18 and 20), and no signal was observed on the B chromosome (Fig. 1). The 18S rDNA probe generated signals on the pericentromeric area of 4–6 chromosomes involving pairs 9, 13 and 14. Positive sites of 18S rDNA were also observed over the pericentromeric and subtelomeric areas of the B chromosome (Fig. 2), as previously published (Poletto et al. 2010a).



**Fig. 2** Mapping of the 18S rDNA in *A. latifasciata* chromosomes. *a* karyotype with the B chromosome in detail (*box*); *b*, *c* and *d*, chromosomes (including B) of different metaphase plates carrying

18S rDNA sites; *m/sm* meta and submetacentric chromosomes; *t/a* telo and acrocentric chromosomes. *Bar* indicates 5  $\mu$ m

Ten selected BAC clones, representing LG 1, 3 and 5 were hybridized against 1B karyotypes of *A. latifasciata* (Fig. 3): LG 1, the BAC #5 yielded a strong interstitial signal on 9q and dispersed small signals on 1q and in other chromosomes; the BAC #9 presented a strong pericentromeric signal on chromosome pair 3 and small signals dispersed in some chromosomes; and no signal was identified on the B chromosomes using both BACs (Fig. 3). For LG 3, the BAC #1 generated dispersed signals on a few chromosomes and pericentromeric signals on most chromosomes. Additionally, strong signals dispersed on 1q and 1p were observed; the BAC #4 showed one interstitial strong signal on 1q and few small signals in some other chromosomes; the BAC #7 presented dispersed signals over 1q and a strong signal close to the centromeric region of 1q; the BACs #8 and #10 showed pericentromeric signals on chromosome 1; and no signal was identified on the B chromosomes using these five BACs. The BAC #3 gave strong hybridization signals in most pericentromeric regions, on the whole extension of 1p and 1q, a interstitial block in 8q, and dispersed signals in the entire length of the B chromosome (Fig. 3). For LG 5, the BAC #2 showed dispersed signals on most chromosomes, an interstitial site in 8q and dispersed signals in 1q; the BAC #6 generated an interstitial signal in 11q; and there were absence of signals in the Bs in both situations (Fig. 3).

The CGH probes were hybridized to 1B metaphasic spreads. All CGH experiments suggest a close relationship between the genomes, with and without Bs, independently of the sex of the probe and target spread. The general hybridization pattern showed very strong signals in the pericentromeric region of all the chromosomes. The B

chromosome exhibits very weak signals in the pericentromeric and subtelomeric regions in both the male and female 0B genomic probes. Similar results were obtained with both 1B male and female genomic probes hybridized against the spreads of both sexes. Additional signals were detected over the entire p arm length and part of the q arm of the chromosome 1 and over the entire length of the B chromosomes (Fig. 4).

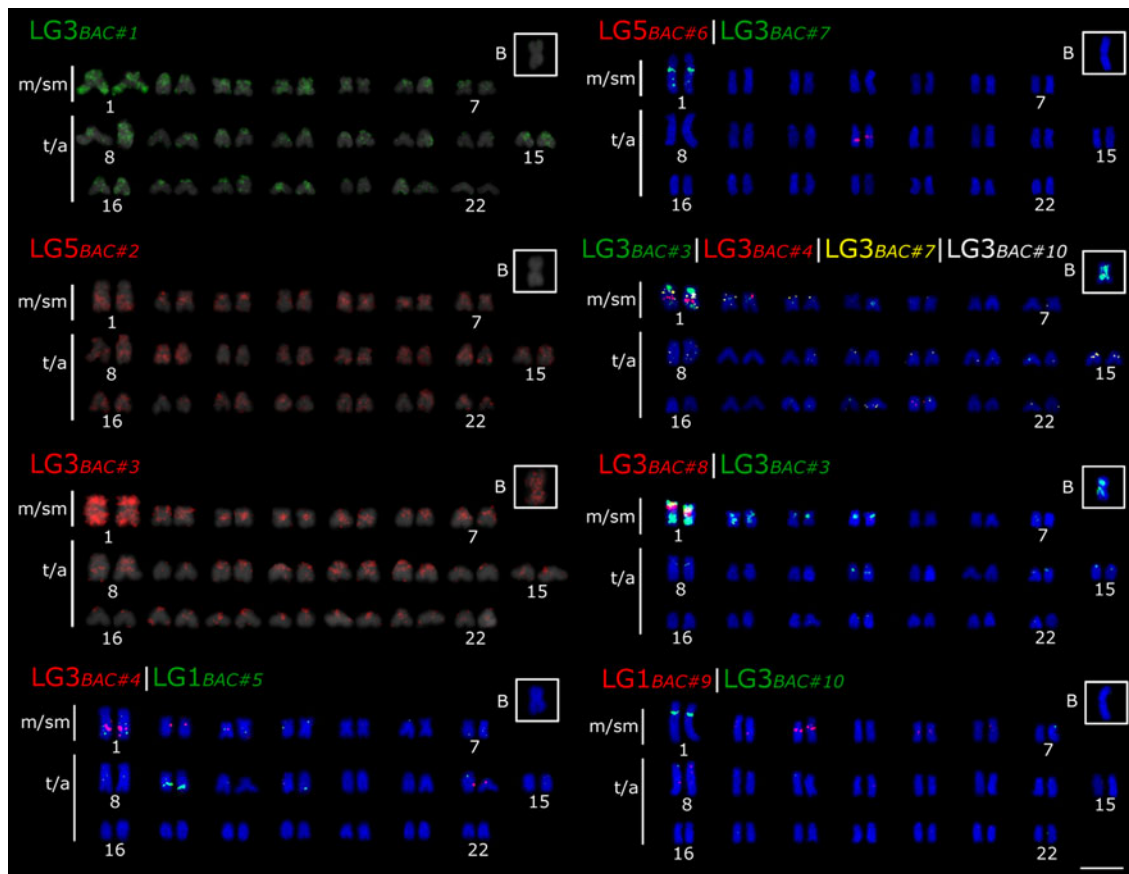
A synthesis of the results obtained is presented in Table 3.

## Discussion

### General features of the B chromosomes of *A. latifasciata*

The chromosomal set of *A. latifasciata* is comprised of 44 chromosomes plus one or two heterochromatic B chromosomes. We must consider that the *A. latifasciata* population was obtained from the aquarium trade and the frequency of individuals with B chromosomes could be a consequence of founder effects from a small population of breeders. The B chromosome of this species corresponds to about 6.48% of the genome and has a similar size to the 1st chromosome pair of the A complement. Among fishes, the presence of large Bs was also observed in *Rutilus rutilus* (Rab and Roth, 1989), *Astyanax scabripinnis* (Maistro et al. 1992), *Microlepidogaster leucofrenatus* (Andreatta et al. 1993), *Metraclima lombardoi* (Poletto et al. 2010b) and *Alburnus alburnus*, which harbors the largest B among vertebrates (Ziegler et al. 2003; Schmid et al. 2006).





**Fig. 3** Karyotypes of *A. latifasciata* hybridized to representative BAC clones of LG 1 (BAC probes #5 and #9), LG 3 (BAC probes #1, #3, #4, #7, #8 and #10) and LG 5 (BAC probes #2 and #6) of *O.*

*niloticus*. The B chromosomes are shown in the box. *m/sm* meta and submetacentric chromosomes; *t/a* telo and acrocentric chromosomes. Bar indicates 5 µm

The comparative analysis of two Bs from the same cell suggests some level of divergence in their sizes. Variation in size among members of a chromosome pair is commonly observed and probably due to different degrees of chromatin condensation, as observed in this study for the 1st and 8th chromosome pairs, or as a consequence of genomic gain or loss between homologs. Compared to the differences among homologous of pairs 1 and 8, the differences between double B chromosomes were statistically significant. Although the probe hybridizations evidenced similar pattern on both Bs, indicating similar genomic content, it could be suggested that both Bs could represent variant forms of Bs in initial stage of differentiation, with putative variation in the copies of repeated DNAs, probably caused by different rates of accumulation of such elements on each B chromosome.

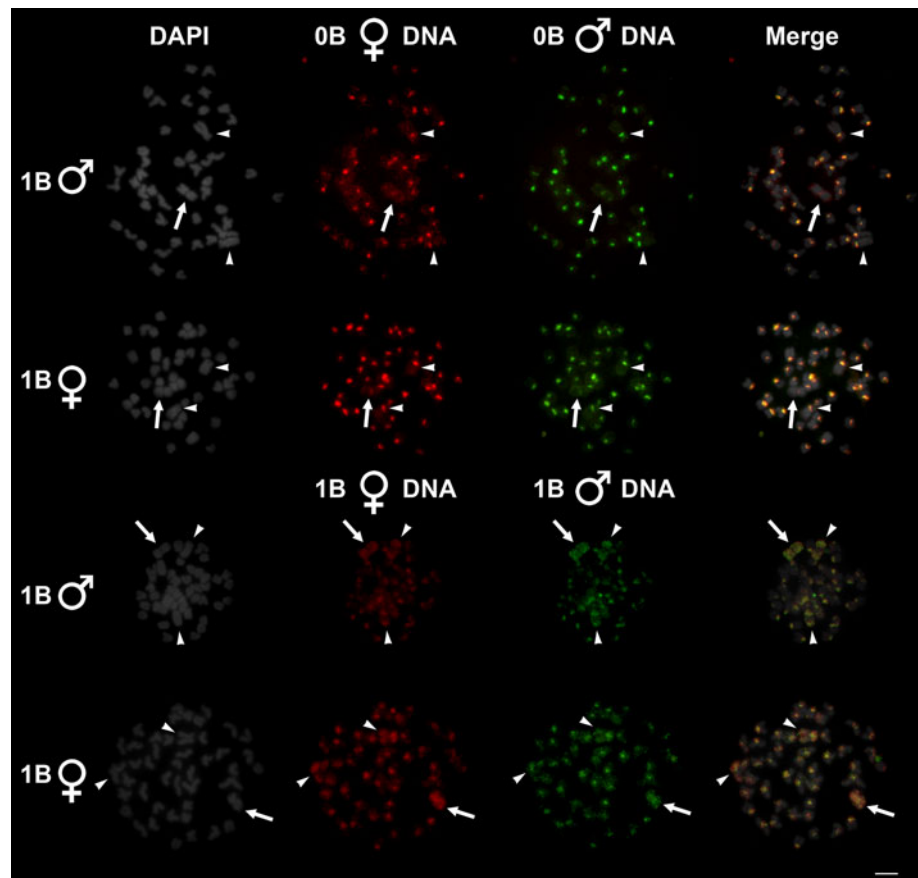
#### Genomic content and the possible origin of the B chromosome

The presence of heterochromatin in the entire extension of the B chromosome as observed in *A. latifasciata* is a

common characteristic among fishes (Pauls and Bertollo 1983; Almeida-Toledo et al. 1992; Venere et al. 1999), although euchromatic Bs were also described (Foresti et al. 1989; Venere et al. 1999). GC rich chromatins were observed both in the pericentromeric and subtelomeric regions of the B and in several chromosomes of the A complement, including the short arms of several pairs. The CMA<sub>3</sub> sites suggest the presence of NORs (nucleolar organizer regions), as observed for several fish karyotypes. The cytogenetic mapping of the 18S rRNA gene in *A. latifasciata* evidenced rDNA sites in four to six chromosomes, supporting the hypothesis that the rDNA carrying chromosomes could have contributed to the origin and/or evolution of the B chromosome.

Using the  $C_{0t} - 1$  DNA of a 0B sample as a probe, the absence of a signal in the B chromosome indicates that the pool of highly and moderately repeated sequences present in the Bs are divergent from that of the A complement. This difference in the pattern of the  $C_{0t} - 1$  DNA fraction is probably a consequence of low selective pressure, leading to a high differentiation rate occurring on Bs compared to the A chromosomes, and also due to the

**Fig. 4** Metaphasic spreads of males and females of *A. latifasciata* submitted to CGH. The 0B and 1B genomic probes were hybridized against 1B metaphasic spreads. *Arrows* indicate the B chromosomes, and *arrowheads* indicate the 1st chromosome pair. *Bar* indicates 5  $\mu$ m



**Table 3** Synthetic view of probes hybridizations and staining procedure over A and B complement

Marker	A complement	B complement
C-banding	Restricted	Pericentromeric/subtelomeric
CMA3	Restricted	Pericentromeric/subtelomeric
<i>Rex1</i>	Widespread	Full length
<i>Rex3</i>	Restricted	Full length
<i>Rex6</i>	Widespread	Absent
$C_{0t} - 1$ DNA	Centromeric	Absent
5S rDNA	Restricted	Absent
18S rDNA	Restricted	Pericentromeric/subtelomeric
LG1—BAC5	Restricted	Absent
LG1—BAC9	Restricted	Absent
LG3—BAC1	Widespread	Absent
LG3—BAC3	Restricted	Full length
LG3—BAC4	Restricted	Absent
LG3—BAC7	Restricted	Absent
LG3—BAC8	Restricted	Absent
LG3—BAC10	Restricted	Absent
LG5—BAC2	Widespread	Absent
LG5—BAC6	Restricted	Absent

absence of recombination between Bs and A chromosomes. On the other hand, the retroelements *Rex1* and *Rex3* hybridized to the entire extension of the B chromosome and also to the A genome. This result suggests that the  $C_{0t} - 1$  DNA fraction contain a low copy number of the *Rex* elements and 18S rDNA that are present on the B chromosome.

The *Rex6* TE was previously detected in various fish species, such as *Xiphophorus maculatus*, *Oryzias latipes*, *Fugu rubripes* (Volf et al. 2001), and cichlids (Teixeira et al. 2009; Valente et al. 2011). It seems to have a restricted distribution and is not detected in various fish families, such as Nototheniidae, Bathydraconidae, Channichthyidae and Tetraodontidae (Ozouf-Costaz et al. 2004), as well as in other species such as *Homo sapiens* (Volf et al. 2001). The *Rex6* is present in the *A. latifasciata* genome (identified previously as *H. obliquidens* in Valente et al. 2011; present work) and it is distributed on the A chromosomes in a manner similar to *Rex1* and *Rex3*, although it is absent in the B chromosomes. The *Rex6* element could even be present on Bs but in a very low copy number, making their detection impossible with the limited resolution of FISH. Considering the absence of *Rex6* in

several genomes and also in the B chromosome investigated here, this TE may have a divergent dynamic behavior compared to the other *Rex* elements and may have been lost or never been transposed to Bs.

The results of the CGH experiments show no differences between the sexes, at least at the FISH resolution level. Although there were no differences, the sharing of hybridization signals between the B and the p arm and distal portion of 1q arm suggests a relationship between these regions. It is possible that the 1p and 1q arms carry segments that have contributed to the B origin or their sequences could have moved to the B during evolution.

One important characteristic of the three *Rex* elements in *A. latifasciata* is their abundance in the 1st chromosome pair (Valente et al. 2011; present work). Although *Rex6* was not present in the B chromosome, the other elements were most enriched in the first chromosome and in the B, suggesting some possible homology between these chromosomal elements. To test this assumption, BAC clones from linkage group 3 (BACs #1, #3, #4, #7, #8, #10), located in the largest chromosome pair of *O. niloticus* (Cnaani et al. 2008), in 1B karyotypes were hybridized. The largest chromosome pair of *O. niloticus* is homologous to chromosome 1 of *A. latifasciata* (identified previously as *H. obliquidens* in Ferreira et al. 2010; Mazzuchelli, unpublished work). Except for BAC #3, which hybridized intensely to the first pair and to the B chromosome, BAC signals were not detected on the B chromosome. The BAC #3 hybridization pattern was similar to the pattern previously observed in *A. latifasciata* chromosomes when BACs enriched with repeated DNAs were applied as probes (Poletto et al. 2010a). BAC #3 (marker GM354) is positioned at the end of 1q in *O. niloticus*, where recombination is suppressed by the presence of numerous repeated DNAs (Cnaani et al. 2008).

We included BACs of LG 1 and 5 as probes because they also contain repeated DNAs and single copy sequences that might provide more specific information about the origin of segments of the B chromosome. The BACs of LG #1 and #5 produced signals mostly in the 1st chromosome pair, but there was not a high concentration of signals on the distal portion of 1q as seen with BAC #3, *Rex1* and *Rex3*. Only BAC #4 showed signals on the 1q arm that was concentrated on an interstitial block and not present in a high concentration on the distal portion. In contrast to the 1B probe used in the CGH experiments, *Rex1* and *Rex3*, and BAC #3 probes gave strong signals on the 1q arm and in the B element. Therefore, we can hypothesize that the genomic segments of 1q are possible sources of B chromosome origin in *A. latifasciata*. On the other hand, the presence of 18S rRNA gene sequences in the B chromosomes suggests a possible origin from the A chromosomes, which carries rRNA genes.

The B chromosomes show some variety of emergence mechanisms, effects and parasitic behavior. It is quite difficult to detect specific possible genomic events involved in its origin. It is known that rearrangements are potential mechanisms involved in species diversification (Raskina et al. 2008). Rearrangements can also lead to emergence of Bs through aneuploidy, which could also result in raw material for the B chromosome formation (Dhar et al. 2002; Camacho 2005). Our results suggest a very close relationship between the B, the 1p arm and the distal portion of the 1q arm, and 18S rDNA sequences. Therefore, we can speculate that the B could have arisen through an aneuploidy event from any chromosome of the A complement followed by euchromatin loss that generated a small, dot-like proto-B chromosome. This proto-B element could have originally contained rDNA and other repeated sequences. In a subsequent step, expansion (duplication) events of existent copies of repeated DNAs could have occurred in the proto-B chromosome, or transposition may also have moved more copies of repeated DNAs, such as rDNA, *Rex* and other sequences, to the initial dot-like proto-B chromosome. It seems most plausible that the B originated from an rDNA-harboring A chromosome. The repeated DNAs accumulated in the interstitial arm positions, resulting in a large chromosome with 18S rDNA on the pericentromeric and subtelomeric regions, which we observed. It is mostly likely that the repeated DNAs invasion occurred in only one arm and the metacentric actual shape of the B originated through an isochromosome formation. In fact, Poletto et al. (2010a) observed that the B chromosomes of this species form a univalent ring structure during meiosis, which is considered a characteristic of isochromosomes. Dhar et al. (2002) presented a similar mode of origin for the B chromosome in the plant *Plantago lagopus*, based on the accumulation of repeated DNA and isochromosome formation.

Considering the absence of recombination between the B chromosomes and the A complement, the sharing of DNA sequences among them could be related to transposition events that does not directly imply homology by origin. In fact, the presence of huge amounts of repeated DNAs in the B chromosome makes it difficult to address conclusive considerations concerning the possible origin of this B element. This is because chromosomal regions harboring repeated DNA are susceptible to a high degree of evolutionary dynamism that can generate thousands of paralog copies in the genome. This often leads to a false sense of homology between the chromosomes.

#### Bs and sex chromosomes

The 1st chromosome pair of *A. latifasciata* is considered homologous to the 1st chromosome pair of *O. niloticus* (the



largest chromosome) (see previous topic of discussion) that is thought to be an ancient state for the sex chromosome in the tilapiine group (Cnaani et al. 2008). Relationships between sex and B chromosomes have been broadly reported among animals and it is evident that these chromosomes share traits such as chromatin distribution, repetitive DNA accumulation and loss of gene activity, which are distinct in relation to the A chromosomes (Camacho et al. 2011). One interesting example of the relationship between the sex and B chromosomes comes from the African cichlid fish of Lake Victoria *Lithochromis rubripinnis* with the presence of female-specific B chromosome that leads to a female-biased sex ratio in the species, although no sex determining genes were identified on the Bs (Yoshida et al. 2011).

The present analysis reveals that there is a shared genomic content between the B chromosome and the 1st pair. Although the Bs of *A. latifasciata* occur in males and females and the CGH experiments did not reveal a sex-specific relationship, we cannot exclude their participation in the sex determination of the cichlid species. However, as previously discussed (Camacho et al. 2011), the similarities between Bs and sex chromosomes may occur by analogy and not homology. Such an assumption will be better addressed though the integration of genomic and cytogenetic analyses for the B chromosome of *A. latifasciata* as well as through advancing studies of sex determination among cichlids.

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