**Supplementary Methods**

Determination of cut-off for coverage regions

To calculate coverage from TE regions, we chose to use the single copy gene hypoxanthine phosphoribosyltransferase (HPRT), the used in qPCR validation, as reference for normalization. Coverage from the different genomes were always normalized, considering the coverage ratio of the HPRT region on those genomes.

To find the HPRT gene in our recently assembled genome, we extracted its sequence from *Metriaclima zebra* available in Sacibase (<http://sacibase.ibb.unesp.br>). With this sequence we performed a BLAST (Altschul et al. 1990) on *A. latifasciata* assembled genome. Region 11,026 to 15,706 from “NODE\_61732” returned high similarity. To confirm that the region belongs to HPRT, a recently assembled transcriptome of the species (Marques, 2016) with gmap 2016-06-09 (Wu & Watanabe 2005). A functional annotation of the mapped transcript with Trinotate 2.0.2 (Haas et al. 2013) was used to confirm the gene annotation. After extraction of per base coverage from the HPRT region, we used R to calculate mean, standard deviation and median for all samples (Table S2). For each alignment, we used the mean coverage value, plus standard deviation as cut-off for transposable elements coverage calculation. Only regions with coverage higher than HPRT gene coverage were to be considered. To summarize, only repeat regions with higher coverage than a typical gene would be used.

We then calculated ratios between HPRT coverage for all alignments, using M1-0B as reference (Table S3). We chose this sample as reference due to its good sequencing quality and previous analysis by our group (Valente et al. 2014). M1-0B coverage values were used to normalize for the differences sequencing coverages, thus creating a common ground for comparing the various samples.

Proportions in Table S3 shows two levels of normalization. First, they are normalized by the sequencing coverage; second, we calculated the ratios between the given sample, and the M1-0B reference. In this regard, table S3 shows the coverage ratio between a given TE, if they have the same coverage value across the comparison. It is expected that, if an element has double the number of copies on the B genome, that number double as well. Therefore, we can calculate how many more copies an element has, in comparison to the M1-0B reference. This approach can indicate repetitive elements with higher number of copies on the B chromosome.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403–10. doi: 10.1016/S0022-2836(05)80360-2.

Haas BJ et al. 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat. Protoc. 8:1494–512. doi: 10.1038/nprot.2013.084.

Marques, D. F. Functional analysis of B chromosome presence using cichlid Astatotilapia latifasciata as model Available online: http://hdl.handle.net/11449/141953.

Valente GT et al. 2014. Origin and Evolution of B Chromosomes in the Cichlid Fish Astatotilapia latifasciata Based on Integrated Genomic Analyses. Mol. Biol. Evol. doi: 10.1093/molbev/msu148.

Wu TD, Watanabe CK. 2005. GMAP: A genomic mapping and alignment program for mRNA and EST sequences. Bioinformatics. 21:1859–1875. doi: 10.1093/bioinformatics/bti310.