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# SATELLITE DNA CHARACTERIZATION AND MOLECULAR CYTOGENETICS ELUCIDATE THE HIGHLY REARRANGED KARYOTYPES OF OWL MONKEYS (*Aotus*, PLATYRRHINI)

Naiara P. Araújo[1], Leonardo G. de Lima[1], Radarane S. Sena[2], Valéria S. Pereira[3], Roscoe Stanyon[4], Gustavo C. S. Kuhn[5], Marta Svartman[5,*]

1. Estudante de Doutorado do Instituto de Ciências Biológicas da UFMG
2. Estudante de Mestrado do Instituto de Ciências Biológicas da UFMG
3. Bióloga da Fundação Zoobotânica de Belo Horizonte
4. Professor do Departamento de Biologia da Universidade de Florença, Itália
5. Professores do Instituto de Ciências Biológicas da UFMG/*Orientadora

**Resumo:**
The New World monkeys of the genus *Aotus* are remarkable by extensive karyotypic variation due to centric fusions/fissions and pericentric inversions. We used DNA and RNA-sequence data from *Aotus nancymaae* to perform a comparative analysis of the content and expression of its satellite DNAs (satDNAs). We identified four satDNA families: the alphoid, OwlRep, CapA_*Aotus*, and an undescribed satDNA. Transcription analysis showed that CapA_*Aotus*, OwlRep and the new satDNA are differentially expressed in several tissues of *A. nancymaae*. Chromosome mapping of these satDNAs in *A. griseimembra, A. infulatus,* and *A. nancymaae* allowed us to correlate their distribution to chromosome rearrangements in *Aotus*. Our data suggest that satDNA chromosomal distribution plays an important role in the structural remodeling of *Aotus* genomes, contributing to the high level of rearrangements found in the karyotypes of owl monkeys.

**Palavras-chave:** Chromosome Evolution; Repetitive sequences; Fluorescent *in situ* hybridization (FISH).

**Apoio financeiro:** CNPq, Fapemig, Capes.

## Introdução:

The New World monkeys of the genus *Aotus* (Platyrrhini, Cebidae) have remarkable chromosomal variation due to centric fusions/fissions and pericentric inversions, evidenced after chromosome banding and painting (Ma 1981; Stanyon et al. 2004, 2011; Ruiz-Herrera et al. 2005; Araújo et al. 2017). Satellite DNAs (satDNAs) appear to be related to genome evolution and karyotype remodeling in eukaryotes (Eichler and Sankoff 2003), so investigating their influence in *Aotus* chromosome variation it of interest. Prakhongcheep et al. (2013a) reported two types of alpha satDNA in *A. azarae*: OwlAlp1, consisting of 185-bp repeat motifs and located at the centromeric regions of all chromosomes, and OwlAlp2, with 344-bp repeat units amplified in the pericentric regions of most chromosomes. Concomitantly, an *Aotus* specific satDNA, OwlRep, was also described (Prakhongcheep et al. 2013b). This satDNA has 187-bp and was mainly mapped to the short arms of the acrocentric chromosomes of *A. azarae* and *A. lemurinus* (Prakhongcheep et al. 2013b; Koga et al. 2017). Fanning et al. (1993), using Southern blot analysis, showed that a 1500-bp satDNA of *Sapajus apella* (CapA) was present in *A. trivirgatus*.
Satellite DNAs usually constitute the heterochromatin located in (peri)centromeric and/or (sub)telomeric regions of chromosomes and often accumulate in non-recombining regions such as the Y chromosome (Charlesworth et al. 1994; Plohl et al. 2014). Due to their potential to promote non-homologous chromosome exchange between homologous sequences, satDNAs have also been considered one of the major drivers of chromosomal rearrangements (Whichman et al. 1991; Sotero-Caio et al. 2015). In fact, cytogenetic mapping of these sequences has shown potential to improve our understanding of karyotype differentiation, since the accumulation of repetitive DNAs in some genomic regions may lead to chromosome breakages, fusions, deletions, inversions and amplifications (Slamovits et al. 2001; Garagna et al. 2014).
Herein, we aimed to identify the satDNAs of *A. nancymaae* by clustering-based repeat analysis, and to map these sequences in the chromosomes of *A. griseimembra, A. infulatus,* and *A. nancymaae*, in order to relate the evolution of these sequences with major chromosomal rearrangements.

## Metodologia:

We performed similarity-based clustering of a female *Aotus nancymaae* (Accession: SRX795828) using RepeatExplorer (Novák et al. 2013) with whole-genome shotgun Illumina reads. We identified four satDNA families: the alpha (OwlAlp1, cluster 2, and OwlAlp2, clusters 4 and 7), OwlRep (cluster 5), CapA (cluster 11) and an undescribed satDNA (clusters 10 and 13). Multiple sequence alignments were performed using the Muscle 4.0 algorithm (Edgar 2004). The MEGA software version 5.05 (Tamura et al. 2011) was used for the calculation of genetic distances and construction of Neighbor-Joining (NJ) tree.
Chromosome preparations and genomic DNAs were obtained by standard procedures from cultured cells of one male each of *A. griseimembra* and *A. nancymaae* and one male and one female *A. infulatus.* The identified satDNAs were amplified by PCR from the genomic DNAs of the three species and their products were purified. The satDNA probes used for fluorescent *in situ* hybridization (FISH) on owl monkey chromosomes were prepared

from the purified PCR products labeled by nick translation with digoxigenin-11-dUTP (OwlAlp1, CapA and the new satDNA cluster 10) or biotin-16-dUTP (OwlAlp2, OwlRep and the new satDNA cluster 13).
We also investigated the transcription of the four satDNA families in several tissues of *A. nancymaae* using the RNA-seq data available at NCBI under the BioProject PRJNA280454. The reads were mapped to a monomer consensus sequence of the satDNAs from *A. nancymaae*, using the "--sensitive-local" preset of Bowtie2 implemented on the Galaxy platform (http://usegalaxy.org; Langmead and Salzberg 2012; Giardine et al. 2005; Goecks et al. 2010).

## Resultados e Discussão:

Our analyses confirmed the presence of four satDNA families, that together comprise about 11.95% of the *A. nancymaae* genome. The most abundant family is composed by the alpha satDNA, that represents 9.05% of the genome and is divided in two subfamilies: OwlAlp1, with 185-bp motif size, and OwlAlp2, with 344-bp. OwlRep, the second most abundant satDNA family, has 187-bp and comprises ~2% of the *A. nancymaae* genome. The third most abundant satDNA family is a yet not characterized tandem repeat, which represents 0.575% of the genome and is composed of 289-bp GC-rich (47.31%) motifs. This satDNA family was retrieved in two distinct clusters (CL10 and CL13). An analysis of the evolutionary relationship between these sequences resulted in a NJ tree evidencing that CL10 and CL13 represent two subfamilies diverging 26.8% from each other. The previously described CapA satDNA of *Sapajus apella* (Fanning et al. 1993) is the fourth most abundant satDNA family of *A. nancymaae*. This satDNA, herein named CapA_*Aotus*, has a monomeric length of ~1.500 bp and comprises 0.347% of this species genome.
In order to investigate the satDNAs distribution in *A. griseimembra* (2n=54), *A. infulatus* (2n=49, male/2n=50, female), and *A. nancymaae* (2n=54) chromosomes, we performed double FISH using OwlAlp1, OwlAlp2, OwlRep, the new satDNA (clusters 10 and 13), and CapA_*Aotus* probes. In *A. infulatus* and *A. griseimembra*, the FISH experiments revealed intense signals of OwlAlp1 at the centromeres of all chromosomes, whereas OwlAlp2 provided signals in the pericentromeric regions of most chromosome pairs. In *A. nancymaae,* on the other hand, OwlAlp1 hybridized to the pericentromeric regions of most chromosomes.
The second satDNA, OwlRep, was detected on the pericentromeric regions of most acrocentric pairs, while CapA-_*Aotus* was detected at pericentromeric and, subtelomeric regions, as well as interstitially along chromosome arms of the three species. The new satDNA hybridized to the pericentromeric regions of some chromosome pairs of the three species. The hybridization sites of the three satDNAs coincided with or were close to chromosome rearrangement breakpoints revealed by chromosome painting (Stanyon et al. 2004, 2011; Ruiz-Hererra et al. 2005; Araújo et al. 2017), suggesting a possible role of these satDNAs with chromosome evolution in *Aotus*.
The transcriptional status of the four satDNAs families in 14 tissues of *A. nancymaae* revealed differential transcription levels for OwlRep, the new satDNA, and CapA_*Aotus* on all tissues. The chromosomal location of these satDNAs may indicate that their transcripts participate in heterochromatin formation.

## Conclusões:

Our study provides a comprehensive description of satDNAs diversity and organization in *Aotus* species. Cytogenetic and bioinformatic data indicate that inversions and centric fusions/fissions are major mechanisms in the karyotypic diversification of *Aotus* species, with the likely occurrence of satDNAs in hotspots of chromosomal rearrangements. Altogether, we suggest that the burst of satDNA sequences in *Aotus* genomes may have played an important role in the chromosomal evolution of the genus, which may have contributed to phenotypic divergence and reproductive isolation among incipient species.

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