UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE BIOLOGIA GERAL
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

TESE DE DOUTORADO

Citogenômica e Evolução Cariotípica em Primatas do Novo Mundo

ORIENTADA: **Naiara Pereira de Araújo**

ORIENTADORA: **Dra. Marta Svartman**

COORIENTADOR: **Dr. Gustavo Campos e Silva Kuhn**

BELO HORIZONTE

Outubro – 2017

Naiara Pereira de Araújo

Citogenômica e Evolução Cariotípica em Primatas do Novo Mundo

Tese apresentada ao Programa de Pós-Graduação em Genética do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, como pré-requisito obrigatório para a obtenção do título de Doutor em Genética, área de concentração "Genética Evolutiva e de Populações".

Orientadora: Dra. Marta Svartman
Coorientador: Dr. Gustavo Campos e Silva Kuhn

BELO HORIZONTE

Outubro – 2017

043 Araújo, Naiara Pereira de.
Citogenômica e evolução cariotípica em primatas do novo mundo [manuscrito] / Naiara Pereira de Araújo. – 2017.

63 f. : il. ; 29,5 cm.

Orientadora: Dra. Marta Svartman. Coorientador: Dr. Gustavo Campos e Silva Kuhn.
Tese (doutorado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas.

1. Genética - Teses. 2. Bandeamento cromossômico. 3. Hibridação genética. 4. Platirrinos. I. Svartman, Marta. II. Kuhn, Gustavo Campos e Silva. III. Universidade Federal de Minas Gerais. Instituto de Ciências Biológicas. IV. Título.

CDU: 575

Ficha elaborada pela Biblioteca do Instituto de Ciências Biológicas da UFMG

Universidade Federal de Minas Gerais
Instituto de Ciências Biológicas
Programa de Pós-Graduação em Genética

ATA DA DEFESA DE TESE

97/2017
entrada
1º/2014
CPF:
072.025.106-00

Naiara Pereira de Araújo

Às quatorze horas do dia **26 de outubro de 2017**, reuniu-se, no Instituto de Ciências Biológicas da UFMG, a Comissão Examinadora de Tese, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho intitulado: **"Citogenômica e Evolução Cariotípica em Primatas do Novo Mundo"**, requisito para obtenção do grau de Doutora em **Genética.** Abrindo a sessão, a Presidente da Comissão, , após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra à candidata, para apresentação de seu trabalho. Seguiu-se a arguição pelos Examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu, sem a presença da candidata e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	Instituição	CPF	Indicação
Gustavo Campos e Silva Kuhn	UFMG	26013664862	APROVADA
Renan Pedra de Souza	UFMG	06448806603	APROVADA
Fernando Araújo Perini	UFMG	03451245612	APROVADA
Cibele Rodrigues Bonvicino	Fiocruz/RJ	43708897404	APROVADA
Gisele Mendes Lessa Del Giudice	UFV	54507642604	Aprovada

Pelas indicações, a candidata foi considerada: _____
O resultado final foi comunicado publicamente à candidata pela Presidente da Comissão.
Nada mais havendo a tratar, a Presidente encerrou a reunião e lavrou a presente ATA, que será assinada por todos os membros participantes da Comissão Examinadora.

Belo Horizonte, 26 de outubro de 2017.

Gustavo Campos e Silva Kuhn _____
Renan Pedra de Souza _____
Fernando Araújo Perini _____
Cibele Rodrigues Bonvicino _____
Gisele Mendes Lessa Del Giudice _____

À minha mãe, à minha irmã e ao meu marido.

AGRADECIMENTOS

À minha querida orientadora, professora Marta Svartman, por todos os anos que convivemos e trabalhamos juntas. Muitíssimo obrigada pelos incentivos, pela credibilidade depositada em mim, pelos ensinamentos e por se alegrar com cada conquista minha. Obrigada pela pequena e nobre mentira que me contou quando eu ainda estava na graduação. Esta mentira fez toda diferença na minha vida acadêmica. Graças a tudo isso, vai ser muito difícil me desligar do LCEv.

Ao meu coorientador, Gustavo Campos e Silva Kuhn, por todas as discussões, paciência, ensinamentos e, principalmente, por trazer consigo um ambiente acadêmico muito mais ousado para o LCEv.

Ao professor Roscoe Stanyon, pelos ensinamentos, disponibilidade em ajudar e por transformar os momentos de trabalho em diversão. Sua alegria e dedicação em cada manhã, durante às visitas ao Brasil, foram motivos de maiores inspiração e disposição para trabalhar com citogenética.

Ao professor Fernando Perini, que sempre esteve disponível em avaliar meus trabalhos, oferecendo valiosas dicas e me incentivando a trabalhar com os equimídeos.

Ao Programa de Pós-Graduação em Genética da Universidade Federal de Minas Gerais.

Às agências de fomento CAPES, pela bolsa, CNPq e Fapemig, pelo financiamento à pesquisa.

Aos atuais e ex-colegas de laboratório, que sempre estão dispostos a ajudar, ensinar, ouvir, pela paciência em trabalhar com uma Naiara extremamente bagunceira e, claro, pelos momentos de descontração. Um agradecimento especial à Alice e Radarane, que me aceitaram com felicidade como coorientadora de seus trabalhos de conclusão de curso.

Ao meu amigo Leonardo Lima, pelas conversas, trabalhos executados juntos, ensinamentos, paciência e pelos ombros sempre disponíveis. Muito obrigada pela credibilidade e por me fazer sentir bem, mesmo naqueles momentos em que eu achava que o meu mundo estava acabando.

Sua amizade me deu muito mais força para viver e acreditar em mim mesma durante a pós-graduação.

Ao técnico Daniel, que sempre está disposto a ajudar. Muitíssimo obrigada pela atenção, respeito e dedicação. Seu trabalho faz muita diferença na nossa rotina do laboratório.

À Valéria Pereira, bióloga da Fundação Zoobotânica de Belo Horizonte, pelos esforços em coletar e nos entregar as inúmeras amostras de primatas que temos disponíveis no laboratório e, principalmente, por entender a necessidade de se estudar os exemplares que tem acesso no zoológico.

Às equipes de outros laboratórios do departamento de Biologia Geral, que sempre estiveram dispostas a ajudar com dúvidas, empréstimo de reagentes, uso de equipamentos e, claro, pelas conversas informais e pelos momentos de descontração.

Às minhas amigas, Daiana, Déborah, Mariana e Rosane, que, mesmo estando longe, sempre estão na torcida por mim. Muito obrigada pelo fato de que, todas as vezes que nos encontramos, ainda sinto que a amizade é a mesma. Muito obrigada também pela confiança e credibilidade.

Ao meu querido esposo, Anézio Oliveira, pelos dez anos de convivência e incentivo aos meus estudos. Muito obrigada pela paciência!!!

À minha querida irmã e "esposa", Samara Araújo, que sempre está na torcida e presente nos momentos difíceis e fáceis da vida. A sua presença constante torna a vida muito mais leve e divertida.

À minha querida mãe, Durce Araújo, por colocar a minha educação e a da minha irmã sempre em primeiro lugar. Obrigada por sempre se desdobrar e fazer o impossível para que pudéssemos estudar e viver com dignidade. O título de doutora também é seu, por todas as dificuldades que enfrentamos juntas para eu chegar até aqui.

ÍNDICE

LISTA DE FIGURAS

Introdução

Resultados e Discussão

Capítulo 1

Artigo "Chromosome painting in *Callicebus nigrifrons* provides insights into the genome evolution of titi monkeys and the ancestral Callicebinae karyotype"

Artigo "Interspecific chromosome painting provides clues to the ancestral karyotype of the New World monkey genus *Aotus*"

Capítulo 2

Artigo "Identification and characterization of a subtelomeric satellite DNA in Callitrichini monkeys"

LISTA DE TABELAS

LISTA DE ABREVIATURAS

2n – Número diploide

ACK – Cariótipo ancestral de Callicebinae

AgRONs (AgNORs) – Marcação das regiões organizadoras de nucléolos pela prata

APLK – Cariótipo ancestral de Platyrrhini

AS – DNA satélite alfa

BAC – Cromossomo artificial de bactéria

BLAST – Basic Local Alignment Search Tool

CBG – Padrões de bandas obtidos por bário e corados com giemsa

CCD – Dispositivo de carga acoplado

CGO – *Callimico goeldii*

CNI – *Callicebus nigrifrons*

CPE – *Callicebus personatus*

DAPI – 4',6-diamidino-2-fenilindole

DMEM – Meio Eagle modificado por Dulbecco

DNA – Ácido desoxirribonucleico

DOP-PCR – Reação em cadeia da polimerase utilizando um primer degenerado

EDTA – Etilenodiaminotetracético

F – Fêmea

FISH – Hibridação *in situ* fluorescente

FITC – Isotiocianato de fluoresceína

Gb – Gigabases

GTG – Padrões de bandas obtidos por tripsina e giemsa

HOR – Higher-order

HSA – *Homo sapiens*

K2P – Kimura 2-Parâmetros

Kb - Quilobases

LINE – Elementos nucleares interdispersos longos

M – Macho

MAR – *Mico argentatus*

Mb - Megabases

MYA – Milhões de anos

NCBI – National Center for Biotechnology Information

ncRNA – Ácido ribonucléico não codificador de proteína

NF (FN) – Número de braços autossômicos

ng - Nanograma

NGS – Sequenciamento de nova geração

NHPRTR – Non-Human Primate Reference Transcriptome Resource

NJ – Neighbor-Joining

nt – Nucleotídeo

NWM – Primatas do Novo Mundo

p – Braço curto do cromossomo

pb (bp) – Pares de base

PBS – Tampão salina-fosfato

PBT – Tampão salina-fosfato com Tween 20

PCR – Reação em cadeia da polimerase

q – Braço longo do cromossomo

RONs (NORs) – Regiões organizadoras de nucléolos

RNA – Ácido ribonucléico

satDNA – DNA satélite

SINE – Elementos nucleares interdispersos curtos

SRA – Sequence Read Archive

TAE – Tris-ácido acético-EDTA

TE – Elementos transponíveis

WGS – Genoma completo por sequenciamento shotgun

RESUMO

Os primatas do Novo Mundo, ou Platyrrhini, compreendem um grupo de mamíferos altamente diversificado, com grande variação morfológica, comportamental e ecológica, o que muitas vezes difículta sua identificação. Platyrrhini também é caracterizado por uma grande variação cromossômica, resultante de inversões, fusões/fissões, reposicionamento centromérico e variação do conteúdo heterocromático. Por serem eventos raros, os rearranjos cromossômicos podem ser considerados marcadores úteis na reconstrução da história evolutiva. Neste trabalho, utilizamos técnicas de bandeamento cromossômico e de pintura com sondas cromossomo-específicas humanas (HSA) para verificar os mecanismos envolvidos na diferenciação cariotípica de *Callicebus nigrifrons* e *Aotus infulatus* em relação a outros taxa relacionados.

Em *Callicebus,* detectamos quatro fusões (HSA 1b/1c, 3c/8b, 13/20 e 14/15/3/21) e uma fissão (HSA 2/22) como sinapormorfias do gênero. A comparação dos resultados obtidos com os previamente publicados para *Callicebus, Cheracebus* e *Plecturocebus* nos permitiu hipotetizar um cariótipo ancestral para Callicebinae com 2n=48. As associações HSA 2/22, 7/15, 10/11 e a inversão de HSA 2/16 estavam presentes nos três gêneros e, portanto, possivelmente presentes no cariótipo ancestral de Callicebinae. Os dados obtidos em *Aotus infulatus* e sua comparação com a literatura sobre as demais espécies do gênero, permitiram identificar sete associações derivadas compartilhadas em *Aotus* (HSA 1/3, 1/16, 2/20, 4/15, 7/11, 10/11, 16/22) e uma inversão da associação HSA 14/15, resultando em HSA 14/15/14/15. Também pudemos inferir um cariótipo ancestral para o gênero *Aotus* com 2n=52 e a detectar os rearranjos associados às variações cariotípicas interespecíficas.

Neste trabalho também investigamos a estrutura, organização e distribuição cromossômica de DNAs satélites em espécies de Callitrichini. Para isto, analisamos o genoma sequenciado de *Callithrix jacchus*, no qual identificamos, além do DNA satélite alfa, um DNA satélite que nomeamos MarmoSAT, com unidades de repetições de 171 pb e localizado em regiões subteloméricas e intersticiais dos cromossomos de *Callithrix, Mico* e *Callimico*. Além da organização monomérica, o MarmoSAT também se mostrou organizado em repetições do tipo higher-order, com 338-pb, em *Callimico*. A análise do perfil de transcrição de MarmoSAT, juntamente com sua localização subtelomérica, nos permitiu sugerir que este DNA satélite pode

estar envolvido com a regulação da telomerase e a modulação da cromatina telomérica nestas espécies.

ABSTRACT

The New World monkeys, or Platyrrhini, comprise a highly diversified group of mammals, with great morphological, behavioral, and ecological variation, which often make their identification difficult. Platyrrhini are also characterized by great chromosomal variation, often resulting from inversions, fusions/fissions, centromeric repositioning, and variation in heterochromatic content. Chromosome rearrangements are rare genomic events and can thus be used as markers for the reconstruction of evolutionary history. We used comparative chromosome banding and painting with human chromosome-specific (HSA) probes to identify the mechanisms involved in the karyotypic differentiating of *Callicebus nigrifrons* and *Aotus infulatus*.

We detected four fusions (HSA 1b/1c, 3c/8b, 13/20, and 14/15/3/21) and one fission (HSA 2/22) as synapomorphies of *Callicebus*. The comparison of our results with those previously published for *Callicebus*, *Cheracebus*, and *Plecturocebus* allowed us to hypothesize an ancestral Callicebinae karyotype with 2n=48. The associations HSA 2/22, 7/15, 10/11, and the inversion of HSA 2/16 were present in all three genera and were thus likely present in the ancestral Callicebinae karyotype. The comparison of the data obtained in *Aotus infulatus* with those previously reported for this genus revealed seven shared derived associations (HSA 1/3, 1/16, 2/20, 4/15, 7/11, 10/11, and 16/22) and an inversion of HSA 14/15, resulting in HSA 14/15/14/15. The chromosome painting results allowed us to infer an ancestral *Aotus* karyotype with 2n=52 and to detect rearrangements associated with interspecific variations.

We also investigated the structure, organization, and chromosomal distribution of satellite DNAs in Callitrichini. The analysis of the sequenced genome of *Callithrix jacchus* led to the identification of, in addition to the alpha satellite DNA, a satellite DNA that we named MarmoSAT, composed by 171-bp motifs and located in the subtelomeric and interstitial regions of *Callithrix*, *Mico*, and *Callimico* chromosomes. Besides its monomeric organization, MarmoSAT also presented higher-order repeats of 338-bp in *Callimico*. The transcription profile and the subtelomeric location of MarmoSAT allowed us to suggest that it may be involved with the regulation of telomerase and the modulation of the telomeric chromatin in these species.

1. INTRODUÇÃO

1.1. Primatas do Novo Mundo (Platyrrhini)

A ordem Primates reúne atualmente cerca de 490 espécies em 77 gêneros (Rylands e Mittermeier 2014) que estão entre os animais mais bem estudados dentre os mamíferos, já que nossa espécie está incluída. As espécies não humanas viventes estão distribuídas em regiões tropicais e subtropicais da África, Madagascar, Ásia e Américas Central e do Sul, e apresentam ecologia, morfologia e taxonomia bastante diversas. Apesar de já terem sido exaustivamente estudados sob diversos aspectos, as relações filogenéticas entre vários gêneros e famílias de primatas ainda são controversas.

Primates é dividida em duas subordens: Strepsirrhini, que inclui as infraordens Lorisiformes (gálagos, potos, lorises), Chiromyiformes (aie-aie) e Lemuriformes (lêmures); e Haplorrhini, que é subdividida nas infraordens Tarsiiformes (társios) e Simiiformes. Os Simiiformes incluem duas parvordens: Platyrrhini (primatas do Novo Mundo) e Catarrhini (Cercopithecoidea – primatas do Velho Mundo, e Hominoidea – humanos, grandes macacos e gibões) (Figura 1; Goodman e col. 1998; Perelman e col. 2011; Springer e col. 2012).

Os primatas do Novo Mundo representam um grupo monofilético que vive exclusivamente nas florestas tropicais das Américas Central e do Sul (Alfaro e col. 2015). Estes primatas apresentam pequeno a médio porte (100 gramas a pouco mais de 10 quilogramas) e são arborícolas, com locomoção predominantemente quadrúpede. O nome Platyrrhini advém do formato do nariz, que é largo e achatado, com narinas dispostas lateralmente, em contraste com os Catarrhini (Bicca-Marques e col. 2006).

Platyrrhini compreende atualmente 145 espécies pertencentes a 20 gêneros, alocados nas famílias Cebidae, Pitheciidae e Atelidae (Figura 1; Tabela 1; Rylands e col. 2012; Schneider e Sampaio 2015; Byrne e col. 2016). Esta diversidade taxonômica é variável, uma vez que novas espécies continuam sendo descritas e as revisões taxonômicas têm aprimorado métodos antigos e/ou empregado novas metodologias.

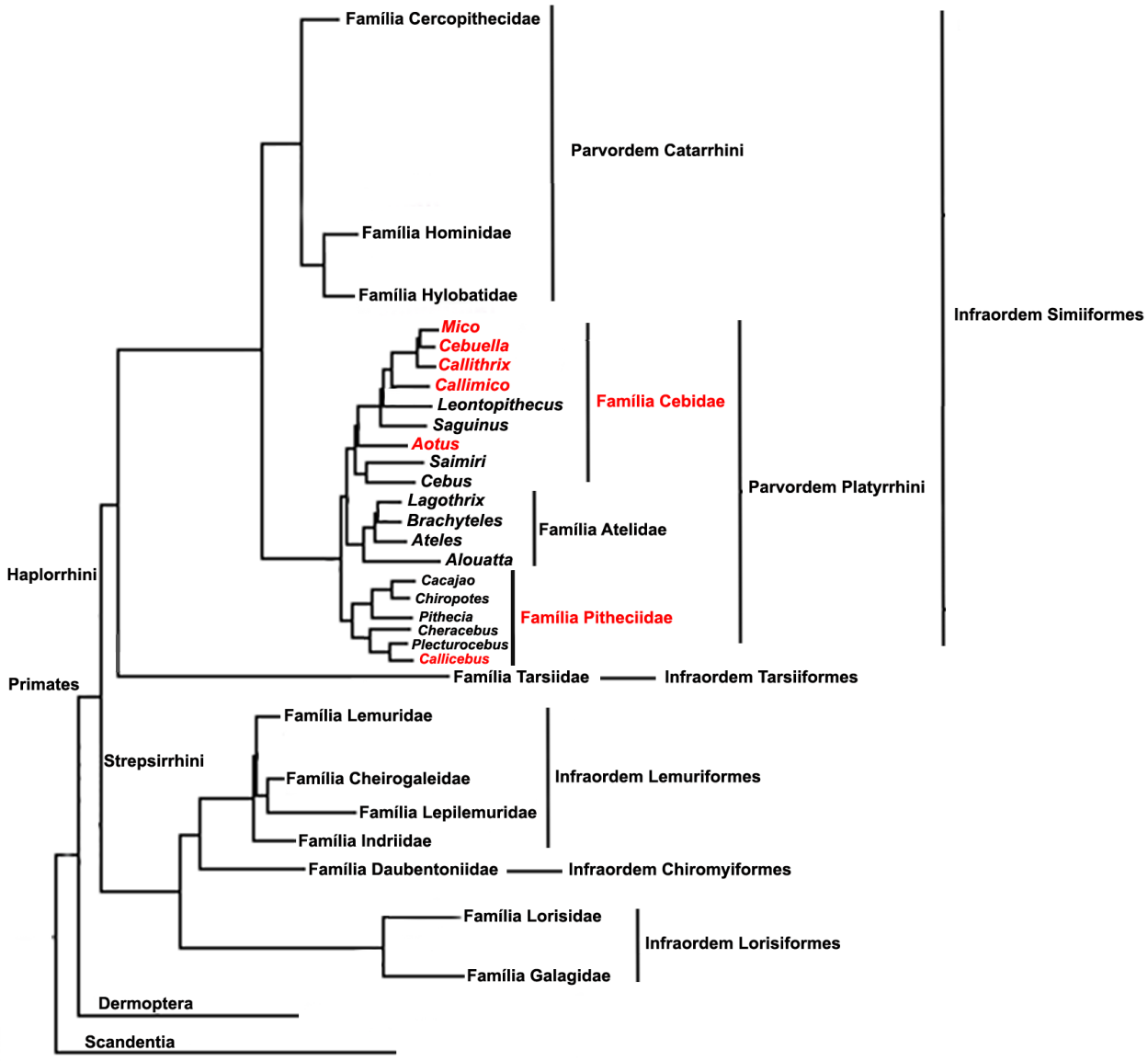


Figura 1. Filogenia de Primates baseada em dados moleculares, adaptada de Perelman e col. (2011). Os gêneros e famílias de Platyrrhini incluídos neste trabalho estão destacados em vermelho.

Após extensa análise filogenética, Perelman e col. (2011) concluíram que Platyrrhini divergiu de Catarrhini há aproximadamente 43,5 milhões de anos (MYA), mas apenas na transição do Oligoceno-Mioceno (25-23,5 MYA) surgiram as famílias atuais. As relações filogenéticas entre várias espécies e gêneros de Platyrrhini ainda são intensamente debatidas, o que se deve em parte à rápida radiação do grupo. Por exemplo, o gênero *Callithrix* era composto por espécies da região Amazônica (grupo *argentata*) e da Mata Atlântica (grupo *jacchus*), mas

com base na análise de sequências de DNAs nuclear e mitocondrial, Tagliaro e col. (2000) sugeriram a sua divisão em dois clados distintos: os da Mata Atlântica e os da Amazônia, com a inclusão de *Cebuella pygmaea* na base do segundo clado. No mesmo ano, Rylands e col. recomendaram a divisão de *Callithrix* nos gêneros *Mico* (originalmente incluídos no grupo *argentata* e distribuídos na região Amazônica) e *Callithrix* (pertencentes ao grupo *jacchus* e localizados na região de Mata Atlântica). Perelman e col. (2011) e Schneider e col. (2012), usando dados moleculares, não só corroboraram essa divisão, como estimaram que a separação de *Callithrix* e *Mico/Cebuella* ocorreu há aproximadamente 5 MYA.

Tabela 1. Gêneros de Platyrrhini reconhecidos atualmente.

Famílias	**Gêneros**	**Nomes populares**
Cebidae	*Aotus* (10)	Macaco-da-noite
	Callimico (1)	Macaco-de-Goeldi
	Callithrix (6)	Sagui, mico
	Cebuella (1)	Sagui-leãozinho, mico-leãozinho, sagui-pigmeu
	Cebus (4)	Macaco-prego, caiarara, mico-preto
	Leontopithecus (4)	Mico-leão
	Mico (15)	Mico, sauim
	Saguinus (15)	Soim, sauim, sagui
	Saimiri (5)	Macaco-de-cheiro, boca-preta
	Sapajus (8)	Macaco-prego
Pitheciidae	*Cacajao* (3)	Uacari, macaco-inglês, carauri
	Callicebus (5)	Titi, Guigó, sauá, zogue-zogue
	Cheracebus (6)	Titi
	Chiropotes (5)	Cuxiú
	Pithecia (5)	Macaco-cabeludo, macaco-velho, parauacu
	Plecturocebus (22)	Titi
Atelidae	*Alouatta* (14)	Bugio, guariba, barbado
	Ateles (7)	Macaco-aranha, coatá
	Brachyteles (2)	Muriqui, mono-carvoeiro
	Lagothrix (5)	Macaco-barrigudo

Os números entre parênteses após o nome de cada gênero representam o número de espécies reconhecidas. Dados baseados em Perelman e col. (2011), Schneider e Sampaio (2015) e Byrne e col. (2016).

A posição dos macacos-da-noite, gênero *Aotus*, dentro de Platyrrhini também é muito discutida na literatura. Alguns autores, como Groves (2005), sugeriram que os macacos-da-noite fossem alocados na sua própria família (Aotidae), enquanto Chatterjee e col. (2009), com base em análises de genes mitocondriais, sugeriu uma maior proximidade de *Aotus* com Atelidae, e Purvis (1995), baseado em dados moleculares publicados, propôs que *Aotus* seria mais próximo

ao gênero *Callicebus*, família Pitheciidae. A proximidade filogenética entre *Aotus* e *Callicebus* também foi sugerida pela análise de dados de pintura cromossômica, que permitiu detectar a associação sintênica HSA 10/11 presente em espécies dos dois gêneros (Stanyon e col. 2004, 2011; Ruiz-Herrera e col. 2005). Mais recentemente, foi proposta uma quarta hipótese, baseada em análises de sequências de DNA nuclear, sugerindo a inclusão de *Aotus* na família Cebidae (Perelman e col. 2011; Springer e col. 2012; Jameson Kiesling e col. 2014). Esta hipótese também foi apoiada por um estudo sobre a organização dos cromossomos HSA 14 e 15, através de experimentos de FISH com BACs em seis espécies deste grupo de primatas. Neste estudo, Capozzi e col. (2015) verificaram que *A. lemurinus* e *C. jacchus* compartilham uma inversão pericêntrica no cromossomo de dois braços que corresponde ao HSA 14/15. Osterholz e col. (2009) analisaram a presença/ausência dos transposons Alu, específicos de Primates, e também sugeriram a inclusão de *Aotus* em Cebidae, uma vez que *Aotus*, *Cebus-Saimiri* e os calitriquídeos compartilhavam dez inserções Alu não encontradas em outros Platyrrhini.

1.2. Evolução Cromossômica em Platyrrhini

Desde meados da década de 1990, diferentes espécies de primatas do Novo Mundo tiveram seus cromossomos comparados após pintura interespecífica com cromossomos individuais humanos ou de outros primatas. Atualmente, há dados de pintura cromossômica para 40 espécies pertencentes aos 20 gêneros reconhecidos de Platyrrhini (revisão em de Oliveira e col. 2012; Araújo e col. 2017a, 2017b). Os dados gerados permitiram estabelecer mapas de homologias cromossômicas entre as espécies e, consequentemente, deduzir o provável cariótipo ancestral de Platyrrhini (APLK), com 2n=54 (Stanyon e col. 2003). O APLK é composto pelos homólogos aos cromossomos humanos HSA 1a, 1b, 1c, 2a, 2b/16a, 3a, 3b, 3c/21, 4, 5/7a, 8a, 8b/18, 9, 10a, 10b/16b, 11, 12, 13, 14/15a, 15b, 17, 19, 20, 22, X e Y (Stanyon e col. 2003; Figura 4 – capítulo 1 artigo 2). O APLK está conservado em *Cebus capucinus* e *Sapajus apella*, indicando uma posição basal de Cebidae em Platyrrhini (Neusser e col. 2001). A partir do APLK, é possível inferir sinapomorfias cromossômicas, que sugerem relações filogenéticas para taxa de diferentes níveis taxonômicos, além de deduzir rearranjos envolvidos na evolução cromossômica destes grupos. Por serem considerados eventos raros, os rearranjos cromossômicos podem ser bons indicadores de origem comum e, consequentemente, ajudar a resolver problemas filogenéticos. Os dados de pintura cromossômica já publicados mostraram que grande parte das

espécies estudadas compartilharam fissões nos cromossomos correspondentes a HSA 1, 3, 8 e 15 e as associações HSA 2/16, 3/21, 5/7, 8/18 e 10/16, dando suporte à relação monofilética de Platyrrhini (Stanyon e col. 2008).

Apesar de haver dados de pintura cromossômica disponíveis para todos os gêneros de Platyrrhini, taxa caracterizados por cariótipos muito rearranjados, como *Aotus* e *Callicebus*, ainda precisam ser melhor investigados para uma melhor compreensão da evolução cromossômica e das relações interespecíficas de Platyrrhini

1.3. DNAs Satélites

O termo DNA satélite tem origem no fato de que, ao serem isolados em gradiente de centrifugação de densidade de cloreto de césio, essas sequências produziam uma fração separada (Szybalski 1968). Outra estratégia utilizada posteriormente para isolar os DNAs satélites é a digestão do DNA genômico com enzimas de restrição e posterior separação em gel de agarose ou poliacrilamida, que produz um padrão de bandas em escada, característico da organização em *tandem* dos monômeros. As bandas de interesse são então eluídas, clonadas, sequenciadas e caracterizadas (Plohl e col. 2008, 2012). As sequências de DNAs satélites estão organizadas em longas cadeias (*arrays*) de repetições em *tandem*, frequentemente ricas em A+T, e não codificam proteínas. O tamanho de cada unidade de repetição (monômero) pode variar em comprimento (de poucos pares de bases até mais de 1 Kb), em número de cópias que compõem as cadeias de repetições e em complexidade. Os monômeros formam cadeias longas e homogêneas, que compõem a heterocromatina constitutiva de regiões centroméricas e pericentroméricas, além de regiões intersticiais e subteloméricas, geralmente coincidentes com os padrões citológicos de bandeamento CBG dos cromossomos (Csink e Henikoff 1998; Plohl e col. 2008, 2012).

Os DNAs satélites representam abundantes frações do genoma de quase todas as espécies de eucariotos (Plohl e col. 2012). Em mamíferos, por exemplo, as sequências satélites podem constituir de 5% a 30% do genoma. Essa variação pode ser atribuída à amplificação e deleção diferenciais das várias famílias de DNAs satélites. Apesar dos avanços no sequenciamento de genomas em larga escala, a caracterização das sequências repetitivas de DNA ainda era muito difícil. Recentemente, foi desenvolvido um *pipeline* computacional (RepeatExplorer) que permite o agrupamento de sequências repetitivas em gráficos para posteriores análises de bioinformática

(Novák e col. 2013). Esta ferramenta tem sido bastante útil, rápida e econômica no estudo de DNAs satélites e foi utilizada neste trabalho.

Evolução dos DNAs satélites

Os DNAs satélites estão entre os componentes do genoma que apresentam as taxas mais rápidas de divergência com relação à abundância, distribuição cromossômica e sequências de nucleotídeos, podendo ser utilizados como marcadores taxonômicos ou até mesmo filogenéticos (Kuhn e col. 2008; Plohl e col. 2012). De fato, essas sequências estão entre os principais elementos responsáveis pela extensa variação na quantidade de heterocromatina observada mesmo entre espécies de eucariotos muito próximas filogeneticamente. Em contraste com a elevada divergência nucleotídica interespecífica, as cópias intraespecíficas dos DNAs satélites são muito homogêneas (baixa variabilidade), devido a um fenômeno conhecido como "Evolução Combinada" (Dover 1986). Neste processo, mutações nas sequências dos monômeros de DNAs satélites podem ser eliminadas ou espalhadas na cadeia, o que leva à homogeneização das repetições, com consequente fixação nos indivíduos da população (Figura 2). A homogeneização de mutações nas repetições de DNAs satélites dentro do genoma ocorre devido a mecanismos moleculares, tais como recombinação desigual e conversão gênica (Dover 1986). Alguns autores sugeriram que a rápida evolução dos DNAs satélites pode gerar uma barreira reprodutiva entre organismos, contribuindo assim para o processo de especiação (Csink e Henikoff 1998; Mestrovic e col. 1998; Henikoff e col. 2001; Schueler e col. 2001; Ferree e Barbash 2009).

Funcionalidade dos DNAs satélites

Os DNAs satélites eram incluídos dentro da fração "inútil" do genoma, juntamente com os chamados "*junk DNAs*" (Doolittle e Sapienza 1980; Orgel e Crick 1980). Por muito tempo acreditou-se que esta hipótese era verdadeira, uma vez que essas sequências altamente diversas não codificam proteínas e, principalmente, porque se acreditava que não desempenhavam qualquer função biológica (Orgel e Crick 1980). Nos últimos anos, no entanto, os DNAs satélites têm ganhado atenção especial, já que um crescente número de estudos vem mostrando que DNAs satélites de vários organismos podem desempenhar papéis importantes em diversas funções biológicas, como a formação da heterocromatina, a formação e manutenção do centrômero, a montagem do cinetócoro, a modulação da cromatina e ainda na regulação gênica, através de

transcritos de RNA (Ugarkovic 2005; Plohl e col. 2012; Kuhn 2015; Biscotti e col. 2015). Também foram descritos exemplos que mostram a relação de transcritos de RNAs derivados de sequências de DNAs satélites com função centromérica. Por exemplo, Rosic e col. (2014) mostraram que o DNA satélite III do cromossomo X de *Drosophila melanogaster* transcreve um RNA não codificante (SAT III RNA) extremamente importante para a correta segregação dos autossomos e do cromossomo X durante a mitose. Neste trabalho, os autores mostraram que a redução de SAT III RNA está associada à perda de componentes do cinetócoro e, consequentemente, falha na interação entre as proteínas do centrômero e as fibras do fuso.

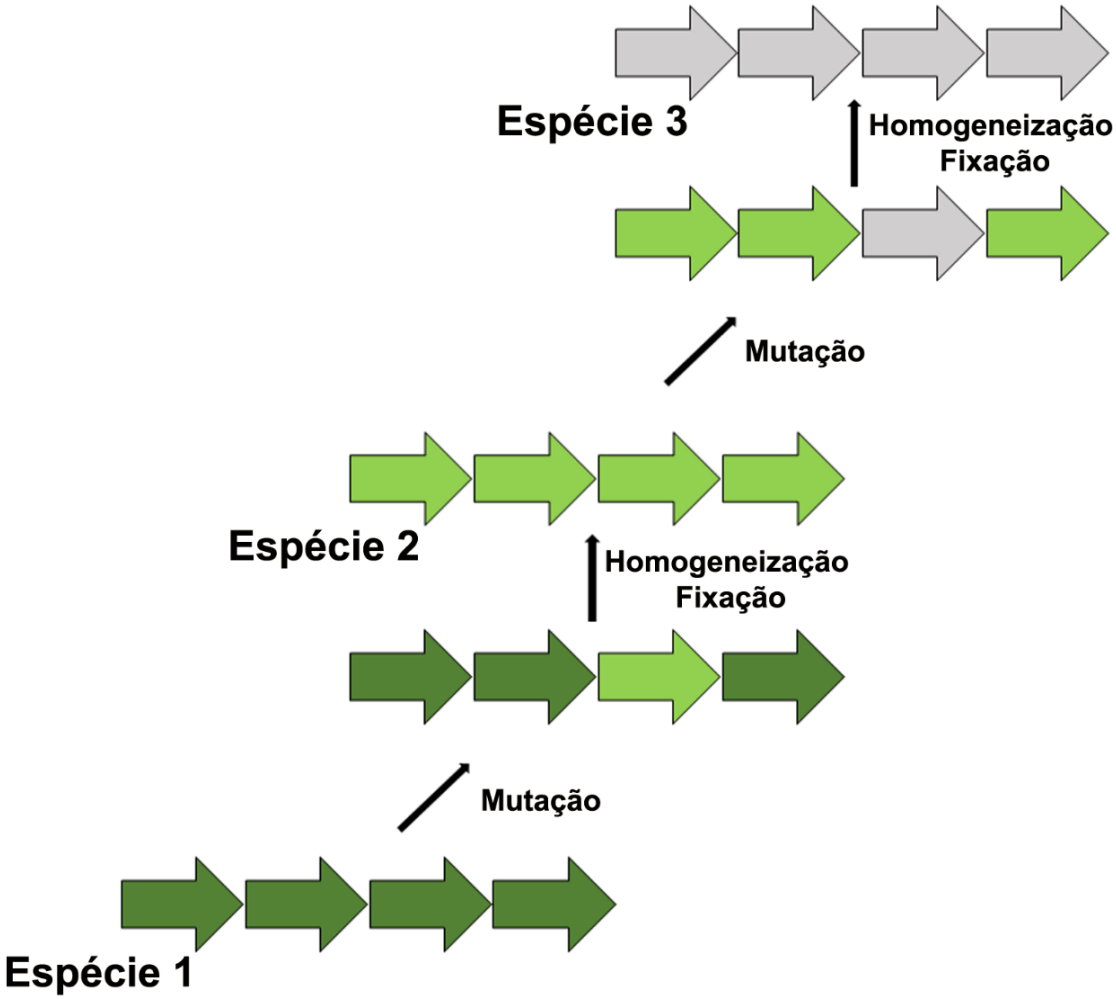


Figura 2. Evolução combinada através da homogeneização de mutações em cópias de um arranjo. Adaptada de Plohl e col. (2012).

Além de sua localização nas regiões pericentroméricas, os DNAs satélites também podem colonizar as regiões subteloméricas. Alguns trabalhos mostraram que DNAs satélites

subteloméricos estão relacionados à estabilidade genômica, replicação cromossômica, pareamento e segregação cromossômica, regulação do ciclo celular, envelhecimento e imortalização celular, localização dos cromossomos dentro do núcleo, regulação da transcrição de genes subteloméricos, entre outras (revisão em Garrido-Ramos 2017).

DNAs satélites em Platyrrhini

Os centrômeros de primatas são compostos principalmente pelo DNA satélite alfa, organizado em unidades de repetições de aproximadamente 171 pb em Catarrhini e de 340 pb em Platyrrhini, com exceção de *Chiropotes satanas*, que apresenta monômeros de 540 bp (Alves e col. 1994; Alkan e col. 2007; Cellamare e col. 2009). De acordo com a organização e propriedades da sequência, o DNA satélite alfa pode ser classificado em estruturas monoméricas ou high-order (HOR; Willard 1991). A estrutura HOR se origina quando alguns monômeros se integram, formando as unidades de repetições (Plohl e col. 2012). A interação dessas unidades de repetições pode se homogeneizar, de forma que as unidades HOR diferem em sequência por uma pequena porcentagem. Por outro lado, a estrutura monomérica é menos homogênea do que as unidades de repetições em HOR. O DNA satélite alfa humano, por exemplo, apresenta repetições monoméricas individuais que compartilham 70-90% de identidade, enquanto que nas unidades em HOR essa identidade é de 95-99% (Figura 3). As unidades em HOR têm sido associadas à função centromérica, uma vez que contêm o CENP-B box, uma sequência de 17 pb incorporada aos monômeros do DNA satélite alfa que se liga à proteína CENP-B. A CENP-B, por sua vez, interage com a proteína CENP-A, estabilizando tanto esta última, quanto as proteínas do cinetócoro. A presença do CENP-B box foi recentemente descrita em primatas do Novo Mundo (Suntronpong e col. 2016; Kugou e col. 2016), mostrando uma grande conservação dessas sequências entre Simiiformes que, até então, era considerada restrita aos hominídeos.

Além das sequências alfa, outras famílias de DNAs satélites foram descritas nos genomas de Platyrrhini: CarB, composto por monômeros de 1528 pb, em *Mico argentatus* e *Callithrix jacchus* (Alves e col. 1995; Canavez e col. 1996; Araújo e col. 2017c); CapA, composto por repetições de 1500 pb, isolado em *Sapajus apella*, mas também presente em outros membros da família Cebidae (Fanning e col. 1993); CgoA e CgoB, com 338 e 916 bp, respectivamente, descritos como restritos ao genoma de *Callimico goeldii* (Fanning e col. 1989); e OwlRep, com 187 pb, descrito em espécies do gênero *Aotus* (Prakhongcheep e col. 2013; Koga e col. 2017).

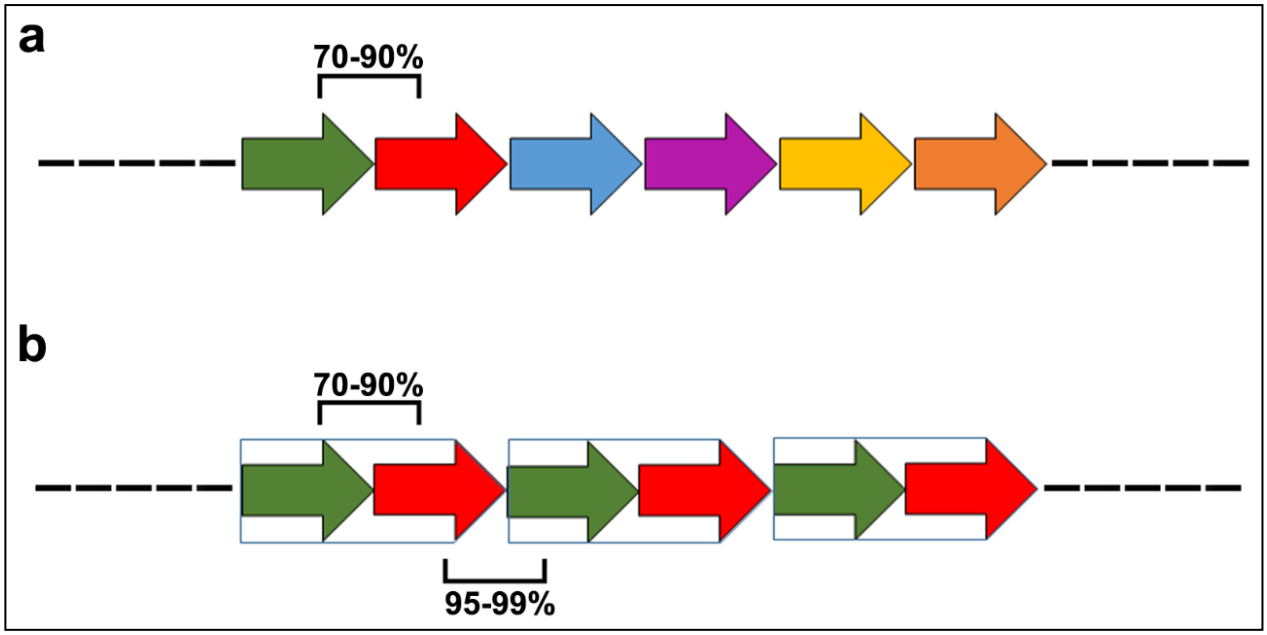


Figura 3. Representação esquemática da estrutura das sequências alfa. As setas indicam monômeros de 171 pb arranjados em *tandem* em estruturas (a) monomérica e (b) higher-order. As porcentagens indicadas referem-se à similaridade entre os monômeros.

2. OBJETIVOS

2.1. Objetivo Geral

Analisar a organização e evolução do genoma de espécies de primatas do Novo Mundo.

2.2. Objetivos Específicos por Capítulo

Capítulo 1

Este capítulo reúne dois artigos baseados na aplicação de pintura cromossômica para o estudo da evolução cromossômica da subfamília Callicebinae (artigo 1) e do gênero *Aotus* (artigo 2). Os objetivos específicos eram:

1. Analisar os cariótipos de *Callicebus nigrifrons* e *Aotus infulatus* após bandeamento GTG, CBG e AgRON e compará-los com dados disponíveis na literatura para outras espécies de cada gênero.
2. Realizar pintura interespecífica com sondas cromossomo-específicas humanas.
3. Contribuir para um melhor entendimento da evolução cromossômica de *Callicebus* e Callicebinae (artigo 1) e de *Aotus* (artigo 2).

Capítulo 2

Este capítulo envolveu a identificação e caracterização de DNAs satélites em Callitrichini, usando o genoma sequenciado de *Callithrix jacchus*. Dessa forma, objetivamos:

1. Identificar e caracterizar DNAs satélites no genoma sequenciado de *Callithrix jacchus*;
2. Verificar a localização dos DNAs satélites MarmoSAT e alfa em cromossomos metafásicos de espécies de *Callithrix*, *Callimico* e *Mico*.
3. Compreender melhor a evolução de MarmoSAT através da sua análise em genomas de outros Platyrrhini.

3. RESULTADOS E DISCUSSÃO

3.1. Capítulo 1: Evolução Cromossômica de Platyrrhini

Neste capítulo estão incluídos dois artigos científicos intitulados: “Chromosome painting in *Callicebus nigrifrons* provides insights into the genome evolution of titi monkeys and the ancestral Callicebinae karyotype” e “Interspecific chromosome painting provides clues to the ancestral karyotype of the New World monkey genus *Aotus*”, publicados nas revistas **Cytogenetic and Genome Research** (doi: 10.1159/000458748) e **Journal of Mammalian Evolution** (doi: 10.1007/s10914-017-9403-z), respectivamente. Nos dois trabalhos foram aplicados os padrões de bandeamento GTG e CBG e a hibridação de sondas cromossomo-específicas humanas aos cromossomos de Platyrrhini com o objetivo de caracterizar os cariótipos estudados e de compreender a evolução cromossômica nos dois gêneros analisados.

Short Report

Cytogenetic and Genome Research

Cytogenet Genome Res
DOI: 10.1159/000458748

Accepted: November 9, 2016
by M. Schmid
Published online: March 10, 2017

Chromosome Painting in *Callicebus nigrifrons* Provides Insights into the Genome Evolution of Titi Monkeys and the Ancestral Callicebinae Karyotype

Naiara Pereira Araújo[a] Alice Alves do Espírito Santo[a]
Valéria do Socorro Pereira[b] Roscoe Stanyon[c] Marta Svartman[a]

[a]Laboratório de Citogenômica Evolutiva, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, and [b]Fundação Zoo-Botânica de Belo Horizonte, Belo Horizonte, Brazil; [c]Department of Evolutionary Biology, University of Florence, Florence, Italy

Keywords
Banding patterns · Chromosome rearrangements · Comparative molecular cytogenetics · Platyrrhini

Abstract
We studied the chromosomes of *Callicebus nigrifrons* with conventional and molecular cytogenetic methods. Our chromosome painting analysis in *C. nigrifrons* together with previous reports allowed us to hypothesize an ancestral Callicebinae karyotype with 2n = 48. The associations of human chromosomes (HSA) 2/22, 7/15, 10/11, and the inverted HSA2/16 would link *Callicebus, Cheracebus*, and *Plecturocebus* and would thus be present in the ancestral Callicebinae karyotype. Four fusions (HSA1b/1c, 3c/8b, 13/20, and 14/15/3/21) and 1 fission (HSA2/22) are synapomorphies of *Callicebus*. The associations HSA3/15 and HSA3/9 are chromosome features linking *Callicebus* and *Cheracebus*, whereas the association HSA13/17 would represent a link between *Callicebus* and the *moloch* group (*Plecturocebus*). Only 6 of the 33 recognized titi monkey species have now been painted with human chromosome-specific probes. Further analyses are needed to clarify the phylogenomic relationships in this species-rich group.

It is now appreciated that titi monkeys are the most speciose group of primates. Their taxonomic history is long and complicated. Modern perspectives on titi monkeys begin with Hershkovitz [1963]. He united 13 Amazonian *Callicebus* taxa in 3 polytypic species (*C. moloch*, *C. torquatus*, and *C. personatus*). Later, after more detailed analyses, Hershkovitz [1988] recognized 13 species resulting in 24 taxa placed into 4 species groups: *modestus*, *donacophilus*, *moloch*, and *torquatus*. Kobayashi [1995] used craniometric measurements supported by karyological data, geographic distribution and pelage coloration to divide the species into 5 groups: *cupreus*, *donacophilus*, *moloch*, *personatus*, and *torquatus*. Subsequently, van Roosmalen et al. [2002] elevated all subspecies to a full species status and identified 28 species. Recently, Byrne et al. [2016] recognized 34 species divided into 4 clades with 3 genera: *Callicebus*, *Cheracebus*, and *Plecturocebus*. In this study, we followed and tested this recent classification. Five species were allocated to the genus *Callicebus*, restricted to the remnants of the Brazilian Atlantic Forest, including *C. nigrifrons*, the species we report on here.

It is well appreciated that titi monkeys have a remarkable chromosomal variation, with diploid numbers ranging from 2n = 16 in *Cheracebus lugens*, the lowest 2n de-

KARGER

E-Mail karger@karger.com
www.karger.com/cgr

1424–8581/17/0000–0000$39.50/0

Marta Svartman
Departamento de Biologia Geral, Instituto de Ciências Biológicas
Universidade Federal de Minas Gerais
Av. Presidente Antônio Carlos, 6627, Belo Horizonte, MG 31270-901 (Brazil)
E-Mail svartmanm@icb.ufmg.br

Table 1. Summary of chromosome painting with human probes in Callicebinae

Species	Associations of human autosomes	Human chromosomes				References
		conserved	2 blocks	3 blocks	4 blocks	
Plecturocebus donacophilus[a] (2n = 50)	2/16, 2/22, 3/21, 5/7, 7/9, 7/15, 8/18, 10/11, 10/16, 12/19 (twice), 14/15	6, 9, 11, 13, 14, 17, 18, 20, 21, X	4, 8, 12, 15, 19, 22	1, 2, 3, 5, 7, 10	16	Barros et al., 2003
Plecturocebus pallescens[a] (2n = 50)	2/16, 2/22, 3/21, 7/15, 8/18, 10/11, 10/16, 12/19, 14/15	4, 6, 7, 11, 13, 14, 17, 18, 19, 20, 21, X	5, 8, 10, 12, 15, 22	1, 2, 3, 16		Stanyon et al., 2000; Dumas et al., 2005
Plecturocebus cupreus[b] (2n = 46)	2/16, 2/22, 3/21, 5/7, 5/10, 7/9, 7/15, 8/18, 10/11, 10/16, 12/19 (twice), 13/17, 13/21, 14/15, 17/20	6, 9, 11, 14, 18, 20, 21, X	4, 8, 12, 13, 15, 17, 19, 22	1, 2, 3, 5, 7, 10	16	Dumas et al., 2005
Cheracebus lugens (2n = 16)	1/3, 1/4, 1/5, 1/13, 1/15, 1/19, 2/12, 2/16 (twice), 2/21, 2/22, 3/9, 3/15, 3/20, 3/21, 4/9, 5/7, 6/8, 7/15, 7/16, 8/10, 10/11 (twice), 10/16 (thrice), 10/19, 11/19, 11/22, 12/13, 14/15, 17/20, 17/22	4, 5, 9, 12, 13, 14, 17, 18, 20, 21, X	6, 7, 8, 11, 15, 19, 22	1, 2, 3	10, 16	Bonvicino et al., 2003; Stanyon et al., 2003
Callicebus personatus (2n = 44)	2/16, 2/22, **3/8**, 3/15, 3/21, 7/15, 8/18, 10/11, 10/16, 12/19 (twice), **13/20**, 14/15	4, 6, 7, 9, 11, 13, 14, 17, 18, 20, 21, 22, X	1, 5, 8, 12, 15, 19	3, 10	2, 16	Rodrigues et al., 2011
Callicebus nigrifrons (2n = 42)	2/16, 2/22 (twice), **3/8**, 3/9, 3/15, 3/21, 7/15, 8/18, 10/11, 10/16, 12/19 (twice), 13/17, **13/20**, 14/15	4, 6, 7, 9, 11, 14, 17, 18, 20, 21, X	1, 5, 8, 12, 13, 15, 19, 22	3, 10	2, 16	present study

[a] *donacophilus* and [b] *moloch* groups. The associations highlighted in bold and with underlines are synapomorphies of *Callicebus* and Callicebinae, respectively.

scribed for primates, to 2n = 50 in *Plecturocebus donacophilus*, *P. pallescens*, and *P. hoffmannsi* [Stanyon et al., 2000; Rodrigues et al., 2001; Barros et al., 2003; Bonvicino et al., 2003]. Although chromosome rearrangements were frequently suggested to have an important evolutionary role in Callicebinae speciation [Schneider et al., 1993; Rodrigues et al., 2001; Dumas et al., 2005], cytogenetic studies in this group are still scarce and most of the described karyotypes do not even include banding patterns.

There are chromosome painting data for only 5 species: *P. cupreus*, *P. donacophilus*, *C. lugens*, *P. pallescens*, and *C. personatus* (Table 1) [Stanyon et al., 2000, 2003; Barros et al., 2003; Bonvicino et al., 2003; Dumas et al., 2005; Rodrigues et al., 2011]. It is certain that cytogenetic studies, especially at the molecular cytogenetic level, could do much to improve our knowledge on titi monkeys' evolutionary biology. Here, we report on the conventional and molecular cytogenetics of *C. nigrifrons*. This is the first report on chromosome painting in this species. Our results make a contribution towards a better understanding of the chromosome evolution of *Callicebus* and Callicebinae in general.

Material and Methods

We analyzed a female *C. nigrifrons* housed at the Fundação Zoo-Botânica de Belo Horizonte, MG, Brazil. Tissue culture and all cytogenetic procedures were performed at the Universidade Federal de Minas Gerais. Cytogenetic analyses were performed on chromosome preparations obtained from peripheral blood, following conventional procedures using concavalin-A as a mitogen [Small et al., 1985]. GTG- and CBG-banding patterns were carried out according to Seabright [1971] and Sumner [1972], respectively. FISH was performed with human chromosome-specific probes previously obtained by flow-sorting at the National Cancer Institute-Frederick. The probes were labeled by DOP-PCR as described in Dumas et al. [2005]. FISH with a synthesized biotinylated telomeric sequence $(TTAGGG)_4$ (Invitrogen) was performed in conditions similar to those described in Araújo et al. [2014]. Alpha satellite sequences were amplified by PCR from *Aotus infulatus* genomic DNA with the following primer sets: forward (5′-ACAGGGAAATATCTGCTTCTAAATC-3′) and reverse (5′-GCTTACTGCTGTTTCTTCCATATG-3′). PCR products were excised from a 1% agarose gel, purified with the Wizard SV Gel and PCR Clean-up System kit (Promega), labeled by nick-translation with digoxigenin-11-dUTP and used as a probe, together with telomeric sequence, in FISH experiments.

Results

The female *Callicebus* analyzed had 2n = 42 and a fundamental number of FN = 68. The GTG-banding pattern (Fig. 1a) was very similar if not identical to that of *C. nigrifrons* [Nagamachi et al., 2003], confirming its taxonomic identification. The results of interspecific FISH with human chromosome-specific probes allowed us to establish complete chromosome correspondence between human and *C. nigrifrons* (Fig. 1a; Table 1). The human probes detected a total of 40 conserved segments in *C. nigrifrons* (Fig. 1a, 2). From the 11 conserved human chromosomes in *C. nigrifrons*, 3 hybridized to entire *C.*

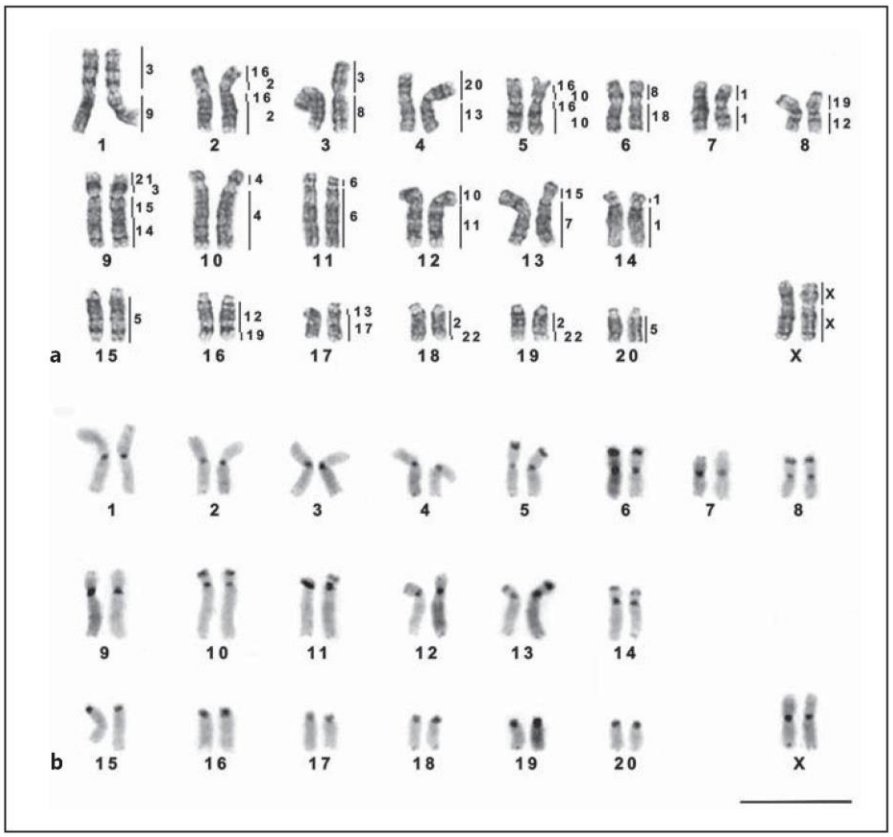


Fig. 1. Karyotype of a female *Callicebus nigrifrons* (2n = 42; FN = 68) after GTG-banding (**a**) and CBG-banding (**b**). The corresponding human chromosomes identified by chromosome painting are shown on the right (**a**). Scale bar, 10 μm.

nigrifrons chromosomes (HSA4, 6, and X) and 8 were found in associations (HSA7, 9, 11, 14, 17, 18, 20, and 21). The remaining 12 human chromosome probes produced 2 (HSA1, 5, 8, 12, 13, 15, 19, and 22), 3 (HSA3 and 10), or 4 (HSA2 and 16) hybridization signals each. Sixteen associations between human chromosomes were detected in the *C. nigrifrons* karyotype: HSA2/16, 2/22 (twice), 3/8, 3/9, 3/15, 3/21, 7/15, 8/18, 10/11, 10/16, 12/19 (twice), 13/17, 13/20, and 14/15. No hybridization signals were present in the constitutive heterochromatin regions revealed after CBG-banding (Fig. 1b).

FISH with a telomeric probe (Fig. 3a) produced signals at the termini of all chromosomes as already reported for *P. hoffmannsi*, *C. lugens*, *C. torquatus*, and *P. moloch* [Barros et al., 2000; Rodrigues et al., 2001; Stanyon et al., 2003; Dumas et al., 2005]. In addition, a strong signal was present in the centromeric region of *C. nigrifrons* (CNI) 18. Double FISH analysis using a telomeric and an alpha satellite probe simultaneously showed that both sequences colocalized in the centromeric region of CNI18 (Fig. 3b). This indicates that the formation of the (peri)centromeric heterochromatin in this chromosome may have occurred by the expansion of internally located telomeric arrays [Ruiz-Herrera et al., 2008] in addition to the alpha satellite.

Discussion

Our chromosome painting results were compared to those from the 5 previously studied Callicebinae species (Table 1) and other New World primates. The chromosome painting pattern in *C. nigrifrons* is closest to that of *C. personatus* (CPE) [Rodrigues et al., 2011]. These 2 species differ only slightly in diploid numbers, CNI (2n = 42) and CPE (2n = 44), due to a fusion of CPE14 (HSA3a) and CPE16 (HSA9) to produce CNI1. We also found the association HSA13/17, which was not detected in *C. personatus* [Rodrigues et al., 2011]. Additionally, we detected the association HSA2/22 twice, whereas Rodrigues et al. [2011] detected it only once. They did map a segment of HSA2 to CPE21 where we found a second signal for HSA22. It is likely that both of these associations were previously missed.

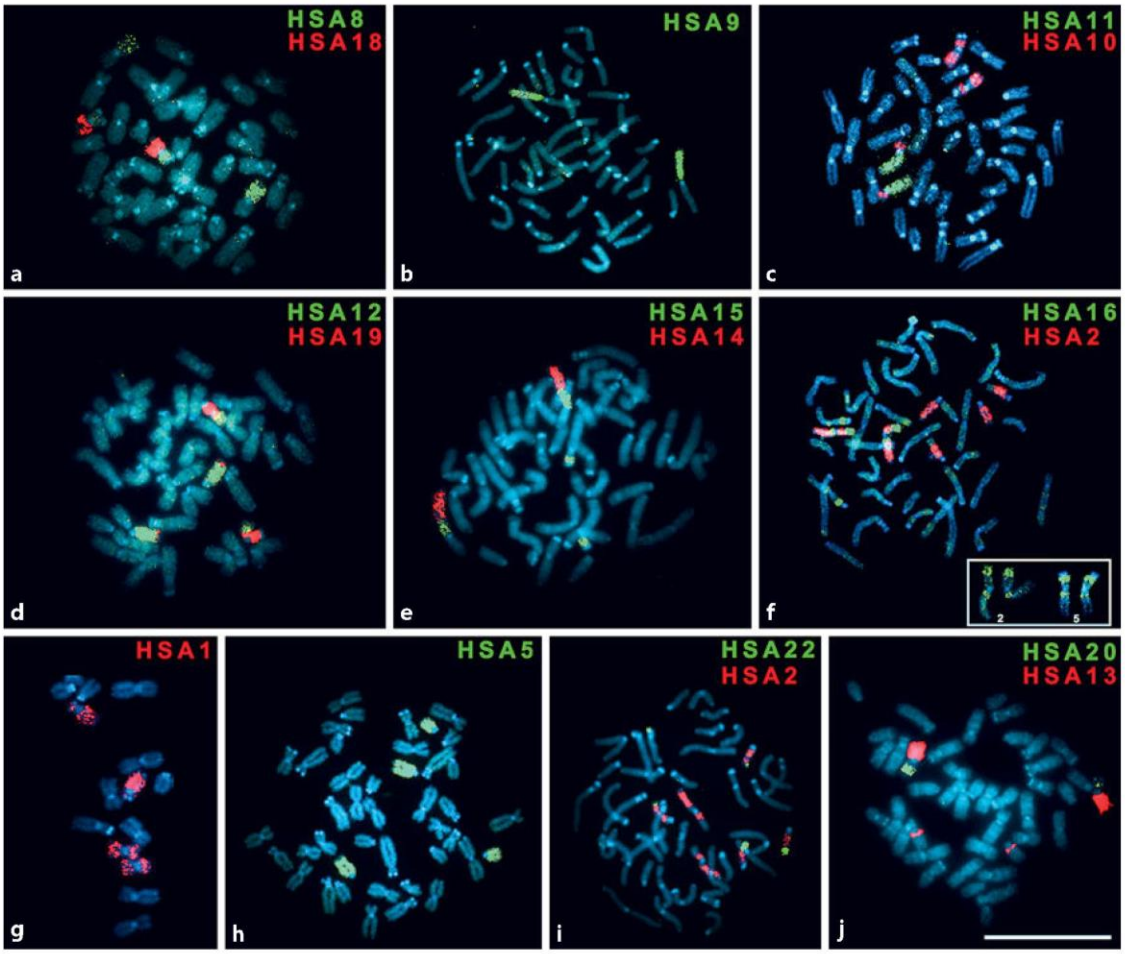


Fig. 2. a–j FISH with human chromosome-specific probes in *Callicebus nigrifrons* cells. Biotin-labeled probes were detected with avidin-FITC (green) and digoxigenin-labeled probes with antidigoxigenin-rhodamine (red). **Inset** HSA16 labeling CNI2 and CNI5. The metaphases were counterstained with DAPI. HSA, human chromosomes. Scale bar, 10 μm.

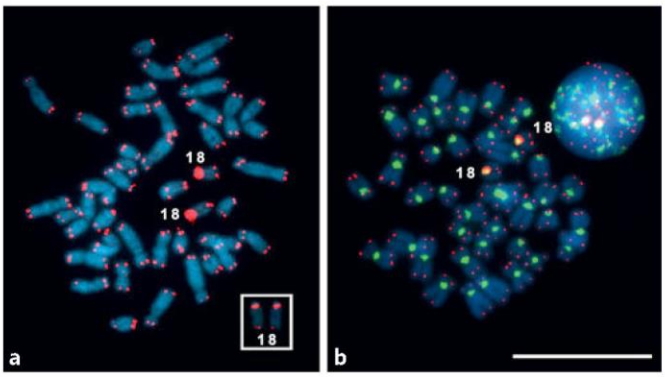


Fig. 3. a FISH with a biotinylated telomeric probe detected with neutravidin conjugated with rhodamine. **b** Double FISH with the same telomeric probe and a digoxigenin-labeled alpha satellite DNA probe detected with antidigoxigenin conjugated with FITC. **Inset** CNI18 interstitial labeling by telomeric sequences. The metaphases were counterstained with DAPI. Scale bar, 10 μm.

Ancestral Callicebinae Karyotype and Test of Evolutionary Scenarios

The comparison of the karyotypes of Callicebinae with the putative ancestral Platyrrhini karyotype (APLK) allows us to hypothesize that the ancestral Callicebinae karyotype (ACK) had 2n = 48 (Fig. 4a). The data of Callicebinae species already analyzed (Table 1) indicate that the associations HSA2/22, 7/15, 10/11, and 22/2/22 as well as the inverted HSA2/16 were found in all Callicebinae and were thus considered present in the common ancestor of all titi monkeys [Stanyon et al., 2000, 2003; Barros et al., 2003; Dumas et al., 2005; Rodrigues et al., 2011]. We used the chromosome painting data in titi monkeys to test the recent taxonomic conclusions and, in particular, the evolutionary scenario presented by Byrne et al. [2016] showing that they were mutually supportive (Fig. 4b).

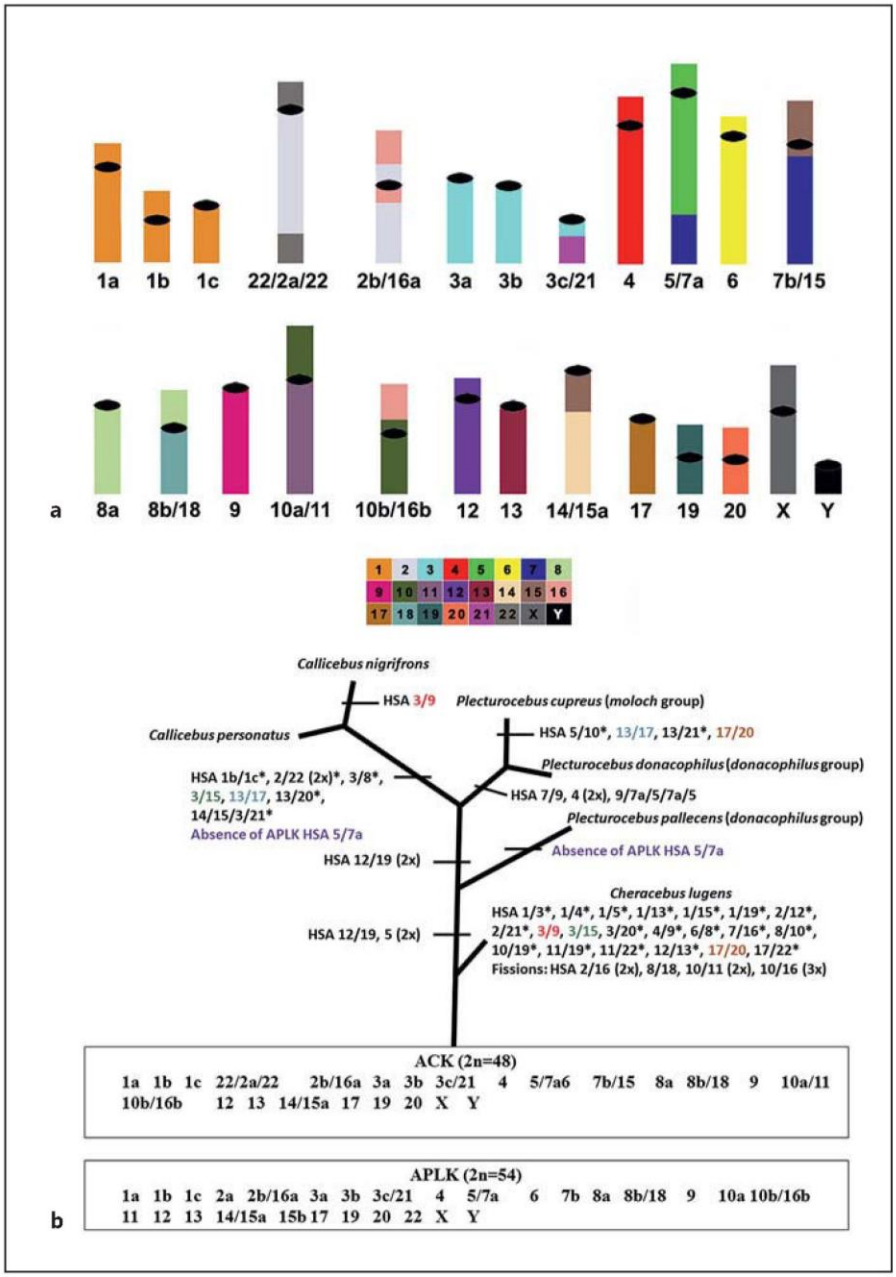


Fig. 4. a Schematic representation of the hypothetical ancestral Callicebinae karyotype (2n = 48) based on the correspondence of human chromosomes. The color of each human chromosome is shown in the color code in the lower middle. The centromere positions are speculative. **b** Series of chromosomal changes in the karyotypes of *Callicebus, Cheracebus*, and *Plecturocebus* from the reconstructed Callicebinae and Platyrrhini ancestral karyotypes, respectively. Different colors indicate shared human associations among taxa. ACK, ancestral Callicebinae karyotype; APLK, ancestral Platyrrhini karyotype, HSA, human chromosomes.

Four fusions (HSA1b/1c, 3c/8b, 13/20, and 14/15a/3a/21) and 1 fission (HSA2a/22) are synapomorphies of *Callicebus*, as they are only present in the 2 species of the genus painted so far. This result supports the phylogenetic separation of *Callicebus* from the other Callicebinae genera, as already proposed based on morphological, biogeographic, molecular, and cytogenetic studies [Kobayashi, 1995; van Roosmalen et al., 2002; Rodrigues et al., 2011; Byrne et al., 2016]. Additional chromosome painting data in the remaining *Callicebus* species are needed, especially in *C. barbarabrownae* and *C. melanochir*, which have not yet been karyotyped, to confirm the chromosome synapomorphies of the genus.

The HSA13/17 association may suggest a phylogenetic link between *Callicebus* and the *moloch* group (*Plecturocebus*). The association HSA3/15 is shared by *C. nigrifrons*, *C. personatus*, and *C. lugens*, whereas HSA3/9 is present in *C. nigrifrons* and *C. lugens*. These results sug-

gest that *Callicebus* is probably closer to *Cheracebus* than to *Plecturocebus*, supporting the phylogenetic arrangement based on morphometry proposed by Kobayashi [1995].

The association HSA22/2/22, reported in species of *Cheracebus* and *Plecturocebus* [Stanyon et al., 2000, 2003; Barros et al., 2003; Dumas et al., 2005], was fissioned in *Callicebus*, giving rise to 2 chromosomes HSA2/22. The derived inversion HSA22/2/22 was probably present in the ancestral of Callicebinae, as it is present in *Cheracebus,* which diverged from the other Callicebinae lineages approximately 11 MYA [Byrne et al., 2016]. HSA2/16/2/16 and HSA10/11 are present in the 3 genera of Callicebinae, but they are found twice in *C. lugens*, indicating fissions in the *Cheracebus* lineage [Stanyon et al., 2003].

The split of HSA1 into 3 blocks was proposed to be present in the APLK. In Callicebinae, 3 blocks of HSA1 are found only in *Plecturocebus*. The segments HSA1b and 1c are fused in *Callicebus* [Stanyon et al., 2003; Rodrigues et al., 2011]. The synteny of HSA4 is conserved in most Callicebinae, but it is split into 2 blocks: in *P. donacophilus* and *P. cupreus* (Table 1).

The fission of HSA5 into 2 chromosomes probably occurred in the common ancestor of *Callicebus* and *Plecturocebus*. Thereafter, a second fission between a segment of HSA5 and 7a, followed by a fusion involving HSA7a and the association HSA7b/15b probably occurred in the ancestral lineages of *Callicebus* and *P. pallescens*. An inverted HSA5/7a (HSA7a/5/7a/5) was then associated with HSA9 in *P. donacophilus* and *P. cupreus* (HSA9/7a/5/7a/5). Furthermore, a segment of HSA5, resulting from the fission of HSA5/7a, fused with HSA10/11 in *P. cupreus*. HSA8a and HSA8b/18, present in APLK and ACK, are found in *Plecturocebus*.

HSA8b/18 is fissioned in *Cheracebus*, and HSA8a is fusioned to a segment of HSA3 in *Callicebus*. The ancestral Callicebinae association HSA10/16 is intact in *P. pallescens*, inverted (HSA10/16/10/16) in *P. donacophilus*, *P. cupreus* and in both *Callicebus* species studied and divided into 3 segments in *C. lugens*. HSA12 and 19 are conserved in APLK and ACK. A fusion between HSA12 and 19 probably took place in the common ancestor of *Plecturocebus* and *Callicebus*. At least 1 inversion and a fission involving the association HSA12/19 gave rise to 2 HSA12/19 segments in *P. donacophilus*, *P. cupreus*, and in both *Callicebus*.

Our comparison of chromosome painting in *C. nigrifrons* with those previously reported for other Callicebinae allowed us to hypothesize an ACK with 2n = 48. This hypothesis is preliminary because from the 33 recognized titi monkey species, only 6 have been painted with human chromosome-specific probes. Moreover, although chromosome painting has been extensively used to study primate chromosomes evolution, it is not effective in detecting intrachromosomal rearrangements, which could influence our conclusion in the reconstruction of ACK. Further molecular cytogenetic analyses will allow unveiling further intra- and intergeneric variation, which will help to make phylogenetic inferences in this still poorly studied species-rich group.

Acknowledgments

This work was supported by a grant from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (process 407262/2013-0) to M.S., and R.S. received a grant from the Progetti di Interesse Nazionale (PRIN). N.P.A. received a doctoral fellowship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

Araújo NP, Loss AC, Cordeiro-Junior DA, da Silva KR, Leite YLR, Svartman M: New karyotypes of Atlantic tree rats, genus *Phyllomys* (Rodentia: Echimyidae). Genome 57:1–8 (2014).

Barros RMS, Pieczarka JC, Brígido MCO, Muniz JAPC, Rodrigues LRR, Nagamachi CY: A new karyotype in *Callicebus torquatus* (Cebidae, Primates). Hereditas 133:55–58 (2000).

Barros RMS, Nagamachi CY, Pieczarka JC, Rodrigues LRR, Neusser M, et al: Chromosomal studies in *Callicebus donacophius pallescens*, with classic and molecular cytogenetic approaches: multicolour FISH using human and *Saguinus oedipus* painting probes. Chromosome Res 11:327–334 (2003).

Bonvicino CR, Penna-Firme V, Nascimento FF, Lemos B, Stanyon R, Seuánez HN: The lowest diploid number (2n = 16) yet found in any Primate: *Callicebus lugens* (Humboldt, 1811). Folia Primatol (Basel) 74:141–149 (2003).

Byrne H, Rylands AB, Carneiro JC, Alfaro JWL, Bertuol F, et al: Phylogenetic relationship of the New World titi monkeys (*Callicebus*): first appraisal of taxonomy based on molecular evidence. Front Zool 13:10 (2016).

Dumas F, Bigoni F, Stone G, Sineo L, Stanyon R: Mapping genomic rearrangements in titi monkeys by chromosome flow sorting and multidirectional in-situ hybridization. Chromosome Res 13:85–96 (2005).

Hershkovitz P: A systematic and zoogeographic account of the monkeys of the genus *Callicebus* (Cebidae) of the Amazonas and Orinoco River basins. Mammalia 27:1–80 (1963).

Hershkovitz P: Origin, speciation, and distribution of South American titi monkeys, genus *Callicebus* (Family Cebidae, Platyrrhini). Proc Acad Nat Sci Philadelphia 140:240–272 (1988).

Kobayashi S: A phylogenetic study of titi monkeys, genus *Callicebus*, based on cranial measurements: I. Phyletic groups of *Callicebus*. Primates 36:101–120 (1995).

Nagamachi CY, Rodrigues LRR, Galetti PM Jr, Mantovani M, Pissinati A, et al: Cytogenetic studies in *Callicebus personatus nigrifrons* (Platyrrhini, Primates). Caryologia 56:47–52 (2003).

Rodrigues LRR, Barros RMS, Pissinati A, Pieczarka JC, Nagamachi CY: Cytogenetic study of *Callicebus hoffmannsii* (Cebidae, Primates) and comparison with *C. m. moloch*. Cytobios 105:137–145 (2001).

Rodrigues LRR, Pieczarka JC, Pissinati A, de Oliveira EHC, Rissino JD, Nagamachi CY: Genomic mapping of human chromosome paints on the threatened masked titi monkey (*Callicebus personatus*). Cytogenet Genome Res 133:1–7 (2011).

Ruiz-Herrera A, Nergadze SG, Santogostino M, Giulotto E: Telomeric repeats far from the end: mechanisms of origin and role in evolution. Cytogenet Genome Res 122:219–228 (2008).

Schneider H, Schneider MPC, Sampaio MIC, Montoya E, Tapia J, et al: Divergence between biochemical and cytogenetic differences in three species of the *Callicebus moloch* group. Am J Phys Anthropol 90:345–350 (1993).

Seabright M: A rapid banding technique for human chromosomes. Lancet 2:971–972 (1971).

Small MF, Stanyon R, Smith DG, Sineo L: High-resolution chromosomes of rhesus macaques (*Macaca mulatta*). Am J Primatol 9:63–67 (1985).

Stanyon R, Consigliere S, Müller S, Morescalchi A, Neusser M, Wienberg J: Fluorescence in situ hybridization (FISH) maps chromosomal homologies between the dusky titi and squirrel monkey. Am J Primatol 50:95–107 (2000).

Stanyon R, Bonvicino CR, Svartman M, Seuánez HN: Chromosome painting in *Callicebus lugens*, the species with the lowest diploid number (2n = 16) known in primates. Chromosoma 112:201–206 (2003).

Sumner AT: A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75:304–306 (1972).

van Roosmalen MGM, van Roosmalen T, Mittermeier RA: A taxonomic review of the titi monkeys, genus *Callicebus* Thomas, 1903, with the description of two new species, *Callicebus bernhardi* and *Callicebus stephennashi*, from Brazilian Amazonia. Neotrop Primates 10:1–52 (2002).

J Mammal Evol
DOI 10.1007/s10914-017-9403-z

ORIGINAL PAPER

Interspecific Chromosome Painting Provides Clues to the Ancestral Karyotype of the New World Monkey Genus *Aotus*

Naiara Pereira Araújo[1] · **Roscoe Stanyon**[2] · **Valéria do Socorro Pereira**[3] · **Marta Svartman**[1]

Abstract The Neotropical monkey genus *Aotus* (owl or night monkeys) are among the most karyological diverse primates of the world. Their diploid numbers range from 2n = 46 to 58, but even owl monkeys with the same diploid number may have radically different karyotypes. This karyotypic variability has provided precious information for taxonomists and has a potential for aiding phylogenetic analysis of these primates. However, up to now only three out of 11 species have been analyzed with molecular cytogenetic methods. Here, we report on a fourth species, *A. infulatus*. Females have a diploid number of 2n = 50 while males, due to a Y/autosome translocation, have 49 chromosomes. We provide a complete map of chromosome homology between humans and *A. infulatus*. Comparisons with previous reports allowed us to propose a putative ancestral karyotype of the genus (2n = 52) and to deduce the rearrangements that were involved in the origin of each species chromosome complement. Integration of chromosome painting and banding analysis suggests at least three chromosomes have evolutionary new centromeres that appeared during the divergence of these four owl monkey species.

Keywords Chromosome evolution · New World monkeys · Phylogenetics · Owl monkeys

Introduction

Cytogenetic data, especially comparative chromosome painting, have proved to be very useful for elucidating essential aspect of genome organization and evolution in New World monkeys (NWM; de Oliveira et al. 2012). These studies, together with morphological and molecular data, can also contribute to conservation programs through species identification, including the recognition of otherwise cryptic species within taxa (Stanyon et al. 2004). An excellent example is the genus *Aotus,* known as owl or night monkeys, originally believed to be a single species, *A. trivirgatus* (Hershkovitz 1949). Based on karyotype, phenotypic characters, and geographical distribution, Hershkovitz (1983) recognized nine species of owl monkeys and more recently, Menezes et al. (2010), using mitochondrial and nuclear DNA sequence data allied with karyotypic and biogeographic data, proposed the division of *Aotus* into 11 species.

Since the 1970's extensive cytogenetic studies have shown that *Aotus* displays high karyotypic variability with at least 18 different karyotypes and diploid numbers ranging from 2n = 46 to 58 (Ma 1981; Ma et al. 1985; Pieczarka et al. 1993; Torres et al. 1998). Centric fusions/fissions and pericentric inversions have been described as the predominant events of chromosomal reorganization in the genus (Ma 1981; Ruiz-Herrera et al. 2005). In addition, uneven diploid numbers due to translocations between the Y chromosome and an autosome have also been found in some species (Ma et al. 1976; Ma 1981; Pieczarka and Nagamachi 1988; Pieczarka et al. 1993). The Y chromosome was found translocated onto the short arm (as in *A. nigriceps*) or interstitially into the long arm (in *A. azarae*) of autosomes (Ma 1981).

✉ Marta Svartman
svartmanm@icb.ufmg.br

[1] Laboratório de Citogenômica Evolutiva, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Avenida Presidente Antônio Carlos, 6627 – Pampulha, Belo Horizonte, MG 31270-901, Brazil

[2] Department of Biology, University of Florence, Via del Proconsolo 12, 50122 Florence, Italy

[3] Fundação Zoo-Botânica de Belo Horizonte, Avenida Otacílio Negrão de Lima, 8000 – Pampulha, Belo Horizonte, MG 31365-450, Brazil

Published online: 14 July 2017

Up until now, only three *Aotus* species were analyzed by molecular cytogenetic methods: an unidentified species *Aotus* sp. (2n = 50), *A. nancymaae* (2n = 54), and *A. griseimembra* (2n = 54) (Stanyon et al. 2004, 2011; Ruiz-Herrera et al. 2005). These species shared the associations of the homologues of human chromosomes (HSA) 1/3, 1/16, 2/20, 3/21, 4/15, 5/7, 7/11, 10/11, 16/22, and the inverted synteny HSA 14/15/14/15 in addition to the disruptions of the syntenic associations HSA 2/16 and 10/16, both present in the supposed ancestral Platyrrhini karyotype (APLK, Table 1). Chromosome paints of *Lagothrix lagothrica* were also hybridized to *A. nancymaae* metaphases and the results showed that this karyotype was highly shuffled with at least 14 fissions and 13 fusions required to derive it from the APLK (Stanyon et al. 2004).

The extensive karyological variability of the genus *Aotus* suggests that more detailed molecular cytogenetic data, including sampling still unstudied species, may yield important data on its phylogeny and taxonomy, both between *Aotus* species and between *Aotus* and other NWM taxa. In this research, we used human chromosome-specific probes to map the karyotype of *A. infulatus*, aiming to contribute to the knowledge of *Aotus* chromosome diversification.

Material and Methods

We analyzed the karyotypes of a male and a female *Aotus infulatus* housed at the Fundação Zoo-Botânica de Belo Horizonte, Minas Gerais state, Brazil. Cytogenetic analyses were performed on chromosome preparations obtained from fibroblast cultures, following standard procedures (Stanyon and Galleni 1991). GTG-, CBG-banding patterns, and silver-staining of the nucleolar organizer regions (Ag-NORs) were carried out according to Seabright (1971), Sumner (1972), and Howell and Black (1980), respectively.

Fluorescence in situ hybridizations (FISH) were performed with human chromosome-specific probes prepared by DOP-PCR from flow sorted chromosomes by PCR amplification and labeling, as previously described by Dumas et al. (2005). FISH using a synthesized biotinylated telomeric sequence $(TTAGGG)_4$ (Invitrogen) was performed in conditions similar to those described in Araújo et al. (2014). Digital images were captured under a Zeiss Axioimager 2 epifluorescence microscope coupled with a CCD camera.

All data generated or analyzed during this study are included in this published article.

Table 1 Associations of human autosomes detected by chromosome painting in *Aotus*

Human Autosomes	*A. nancymaae*[1,2] (2n = 54)	*Aotus* sp.[2] (2n = 50)	*A. griseimembra*[3] (2n = 54)	*A. infulatus*[4] (2n = 50)
1/3	+	+	+	+
1/16	+	+	+	+
2/7				+
2/12	+	+		
2/16				
2/20	+	+	+	+
3/14	+			
3/21	+	+	+	+
4/15	+	+	+	+
5/7	+	+	+	+
5/15	+	+		+
7/11	+	+	+	+
8/18	+	+		+
9/15	+	+		
9/17	+			
10/11	+	+	+	+
10/16				
10/22	+	+		+
11/19			+	
14/15	+	+	+	+
14/15	+	+	+	+
16/22	+	+	+	+

[1] Stanyon et al. (2004); [2] Ruiz-Herrera et al. (2005); [3] Stanyon et al. (2011); [4] Present study

Associations in bold are present in the hypothetical ancestral Platyrrhini karyotype

Results

The diploid numbers of the specimens analyzed were 2n = 49 (male) and 2n = 50 (female). The karyotype was composed of ten pairs of biarmed chromosomes and 14 acrocentric pairs; the X was submetacentric and in the male, the Y chromosome was apparently translocated to the short arm of chromosome 16 (Fig. 1a). After comparing the GTG-banding pattern with the literature, we confirmed that the specimens were *Aotus infulatus*.

CBG-banding revealed pericentromeric constitutive heterochromatin blocks in all chromosomes (Fig. 1b). Additionally, chromosome pairs 10–24 had heterochromatic short arms, pair 7 presented heterochromatin in the telomeric region, and the translocated Y/16 chromosome showed interstitial bands on the short and long arms. Pair 9 had a large AgNOR-bearing interstitial secondary constriction in its long arm (Fig. 2a). The telomeric probe produced signals at the termini of all chromosome arms and additional interstitial signals were also found at the centromeric region of chromosome pairs 6 and 7 (Fig. 2b).

All the human chromosome-specific probes, except the Y, produced bright signals on the *A. infulatus* metaphases. We were able to produce a complete map of homology with human chromosomes (Figs. 1a and 3). A total of 41 conserved segments were found on the haploid set of *A. infulatus*.

Eleven human chromosomes were conserved in *A. infulatus*. Seven painted only one *A. infulatus* counterpart (HSA 6, 9, 12, 13, 17, 19, and X), and four (HSA 18, 20, 21, and 22) were conserved but were associated with other autosomes. The HSA 14 paint hybridized to a single chromosome, in association with HSA 15, but it was divided into two blocks due to an inversion. Multiple hybridization signals were observed with the probes of the other human autosomes: HSA 4, 8, 10, 11, and 16 labeled two pairs of *A. infulatus* each; and HSA 1, 2, 3, 5, 7, and 15 were split into three or more segments. The following syntenic associations of human chromosomes were found: HSA 1/3, 1/16, 2/7, 2/20, 3/21, 4/15, 5/7, 5/15 (twice), 7/11, 8/18, 10/11, 10/22, 15/14/15/14, and 16/22 (Table 1, Fig. 1a). Hybridization signals were not detected in the constitutive heterochromatin regions revealed after CBG-banding (Fig. 1).

Discussion

Centric fusions/fissions and pericentric inversions proposed based primarily on banding were described as important mechanisms of chromosome reorganization in the night monkeys (Ma 1981; Ruiz-Herrera et al. 2005). Our FISH experiment with a telomeric probe showed signals in the centromeric region of *A. infulatus* pairs 6 and 7. The interstitial labeling on pair 6 may indicate a chromosome rearrangement, probably a fusion between HSA 10 and HSA 11. The signal on pair 7 may be related to a pericentric inversion of the conserved segment homologous to HSA 20. The absence of interstitial telomeric sequences in the remaining chromosomes may be due to the loss of these sequences during rearrangements or to the small number of $(TTAGGG)_n$ repetitions, which could not be detected by FISH. Mudry et al. (2007) analyzed the

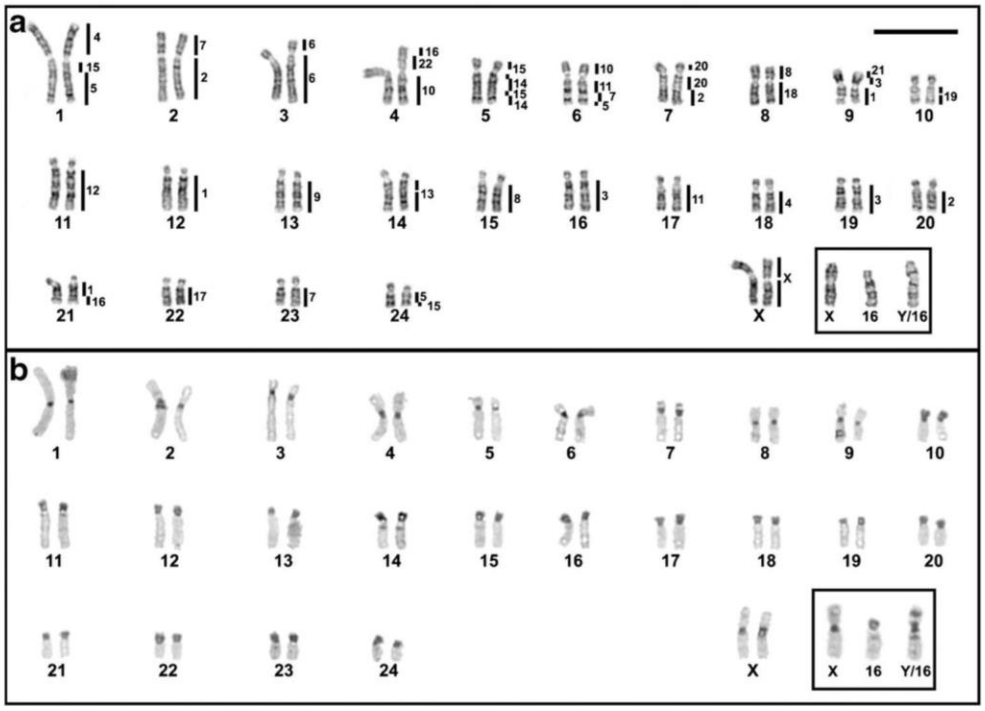


Fig. 1 Karyotype of a female *Aotus infulatus* (2n = 50) after (**a**) GTG- and **b** CBG-banding. The corresponding human chromosomes revealed after chromosome painting are shown on the right in **a**. The male sex chromosomes (2n = 49) are shown in the insets. Bar = 10 μm

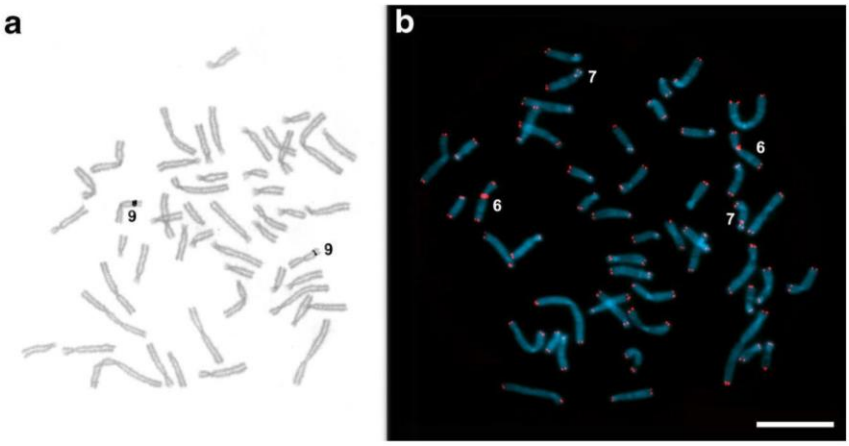


Fig. 2 Cells of a female *Aotus infulatus* (2n = 50) after **a** silver staining of the nucleolus organizer regions (Ag-NORs) and **b** FISH with a telomeric probe. Note the Ag-NORs on pair 9. Bar = 10 μm

karyotype of *A. azarae* after FISH with a telomeric sequence and observed a strong signal at the pericentromeric region of pair 5, which they related to a fusion.

As previously shown for other *Aotus*, our specimens had only one pair bearing NORs in its long arms. This metacentric pair is considered a marker chromosome characteristic of the genus *Aotus* (Torres et al. 1998).

The CBG-banding patterns reported for *Aotus* species, including *A. infulatus*, revealed heterochromatin located at pericentromeric regions of all biarmed pairs and also

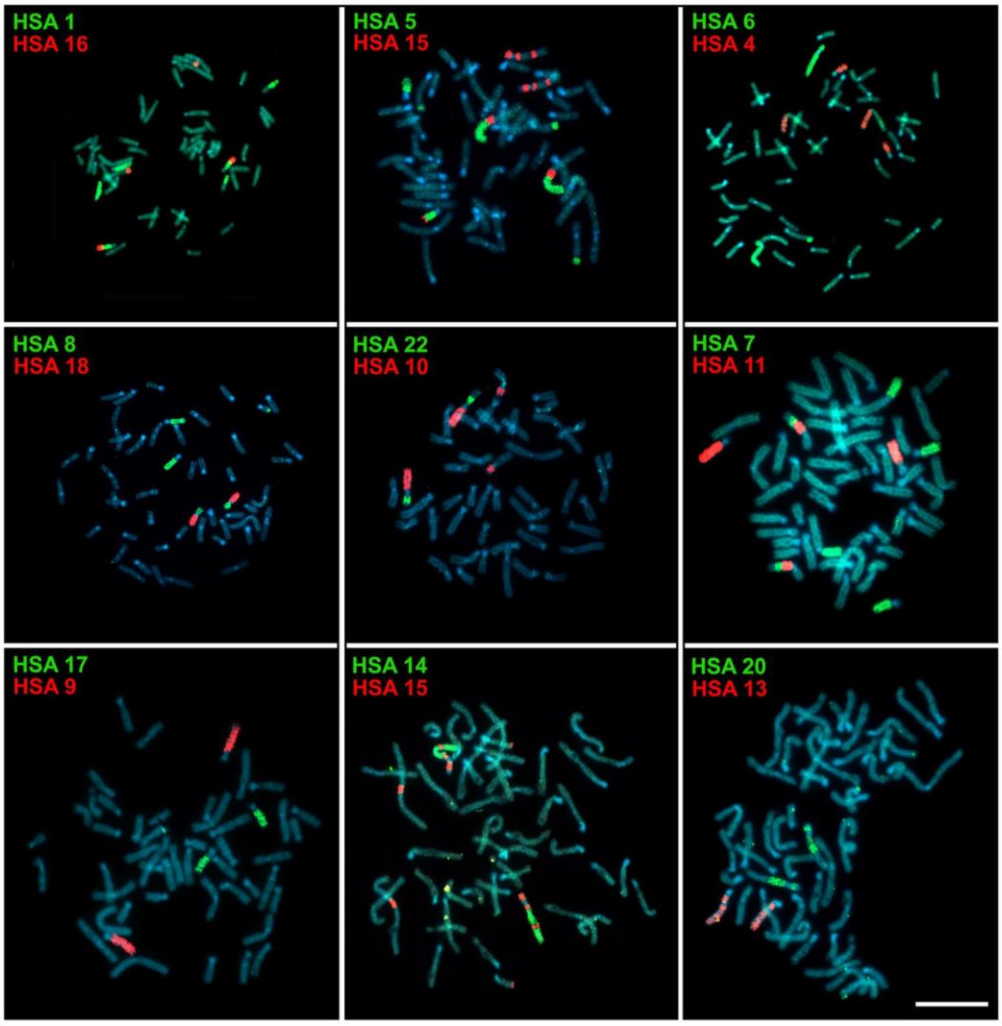


Fig. 3 FISH with human chromosome-specific probes onto *Aotus infulatus* cells. Biotin-labeled probes were detected with avidin-FITC (green signals) and digoxigenin-labeled probes, with antidigoxigenin-rhodamine (red signals). All metaphases were counterstained with DAPI. Bar = 10 μm

composing the short arms of acrocentrics (Torres et al. 1998; Prakhongcheep et al. 2013). These heterochromatic portions are rich in at least four different families of satellite DNAs, which would be involved in *Aotus* chromosome diversification (our unpublished data).

The Y-Translocations in Owl Monkeys

The male analyzed had 2n = 49 due to a translocation of the Y with an autosome, identified as chromosome 16, which resulted in radically different morphologies between homologs in the male. Chromosome 16 is homologous to part of HSA 3q (unpublished data). This segment, called HSA 3a, is believed to be present in the ancestral Platyrrhini karyotype (APLK, Fig. 4). Y-autosome translocations have been previously described based on GTG- and CBG-banding in *A. azarae boliviensis*, *A. A. azarae, A. infulatus,* and an unidentified "*Aotus* from Rondônia" (all with 2n = 49 in males and 2n = 50 in females), and in *A. nigriceps* (2n = 51 M/52F) (Ma et al. 1976; Ma 1981; Pieczarka and Nagamachi 1988; Pieczarka et al. 1993). After comparing the banding patterns of the Y/16 of our *A. infulatus* with these previous accounts, we concluded that the same autosome seems to be involved in the rearrangement, which can thus be hypothesized to have occurred in a common ancestor before the divergence of these species (Pieczarka and Nagamachi 1988; Pieczarka et al. 1993). This hypothesis will need to be tested using other molecular cytogenetic methods and eventually sequencing.

Implications for Chromosome Evolution in *Aotus*

We then compared our banding and hybridization results with those previously published for *Aotus* and other New World monkeys. The proposed APLK has 2n = 54 (Stanyon et al. 2003) and is composed by eleven conserved homologues of human chromosomes (HSA 4, 6, 9, 11, 12, 13, 17, 19, 20, 22, and X), from which only six (HSA 6, 9, 12, 13, 17, and 19) were found undisrupted in *A. infulatus* (Fig. 1a). In this species, HSA 4 and 11 were split into two segments, whereas HSA 20 was found in association with HSA 2, and HSA 22 with HSA 16 and 10. The presumed APLK associations 3/21, 5/7, 8/18, and 14/15 were found in *A. infulatus*, but HSA 2/16 and 10/16 were absent (Table 1, Fig. 1a). Instead, *A. infulatus* has the association HSA 10/22/16, which may indicate an insertion or a fusion of the NWM ancestral HSA 10/16 with HSA 22, followed by an inversion.

After comparisons of the four *Aotus* painted karyotypes, we confirmed that they share seven derived associations, which are absent in the APLK: HSA 1/3, 1/16, 2/20, 4/15, 7/11, 10/11, 16/22, and an inversion of HSA 14/15 resulting in HSA 14/15/14/15 (Table 1). Additionally, *A.* sp., *A. infulatus* and *A. nancymaae* share two derived associations (HSA 5/15 and 10/22), whereas *A.* sp. and *A. nancymaae* both have in common HSA 2/12 and 9/15. These cytogenetic data show that while *A. griseimembra* has the complement that most likely resembles that of the common ancestor of the four analyzed species, the karyotypes of *A.* sp. and *A. nancymaae* are more closely related to each other than either is to that of *A. infulatus*. Thus, the karyotype of an ancestor of these three species probably incorporated further rearrangements after the divergence of *A. griseimembra*. Furthermore, the association HSA 2/7 was found exclusively in *A. infulatus*, whereas HSA 3/14 and 9/17 were restricted to *A. nancymaae* and HSA 11/19 was exclusive to *A. griseimembra* (Table 1; Fig. 4).

The four *Aotus* species analyzed by chromosome painting shared the following features: (a) the conservation of HSA 6, 12, 13, 18, 19, 20, 21, 22, and X; (b) the presence of the APLK associations HSA 3/21, 5/7, 8/18, 14/15, and the fission of HSA 2/16; (c) and the common associations HSA 1/3/21, 1/16, 2/20, 4/15, 5/7/11/10, 10/22/16, and the inverted HSA 14/15/14/15. Based on this comparison among *Aotus* karyotypes and with the APLK, we suggest that the putative ancestral *Aotus* karyotype may have had 2n = 52 chromosomes and would be composed of: (a) the conserved chromosomes 6, 9, 12, 13, 17, 18, 19, 20, 21, 22, and X; (b) the associations HSA 1/3/21, 1/16, 2/20, 4/15, 5/7/11/10, 8/18, 10/22/16, and 14/15/14/15; (c) two pairs homologous to HSA 4, 5, 8, 10, 15, and 16 each; and (d) three pairs homologous to HSA 1, 2, 3 and 7 each (Fig. 4).

Aotus griseimembra apparently had the least derived karyotype. The differences between the *A. griseimembra* karyotype and the putative ancestral karyotype of *Aotus* was a fusion between the homologues of HSA 11 and 19 and two fissions: HSA 8/18, causing a loss of the association, and HSA 10/22/16, producing segments homologous to HSA 10 and HSA 16/22 (Fig. 4).

The other three species of *Aotus* are hypothesized to have karyotypes derived from a common ancestral karyotype after the divergence of *A. griseimembra*. The homologous associations HSA 4/15/5 and 5/15 may be explained by a fusion of the ancestral HSA 4/15 and 5, followed by the disruption of HSA 4/15/5 originating the HSA 5/15 (Fig. 4). Moreover, in the *A. infulatus* karyotype two fusions (HSA 2 and 7 and HSA 3 and Y) explain the main differences detected by chromosome painting, suggesting a possible chromosome marker.

The common ancestor of *A.* sp. and *A. nancymaae* probably had the synteny HSA 2/12. Complex rearrangements involving a segment homologous to HSA 15 and HSA 9 probably occurred, giving rise to the two HSA 9/15 associations. Only *A.* sp. had the HSA 7/11 association, indicating a possible phylogenetic marker. A fission of a segment of HSA 17 followed by a fusion to the presumed association HSA 9/15 apparently gave rise to the association HSA 15/9/17 in *A. nancymaae* (Fig. 4). In addition, fusions/fissions between the homologous segments of HSA 3 and HSA 14/15/14/15 would explain the origin of *A. nancymaae* chromosome 15 (HSA 3/14/15/14), 22 (HSA 3) and 24 (HSA 14/15) (Fig. 4).

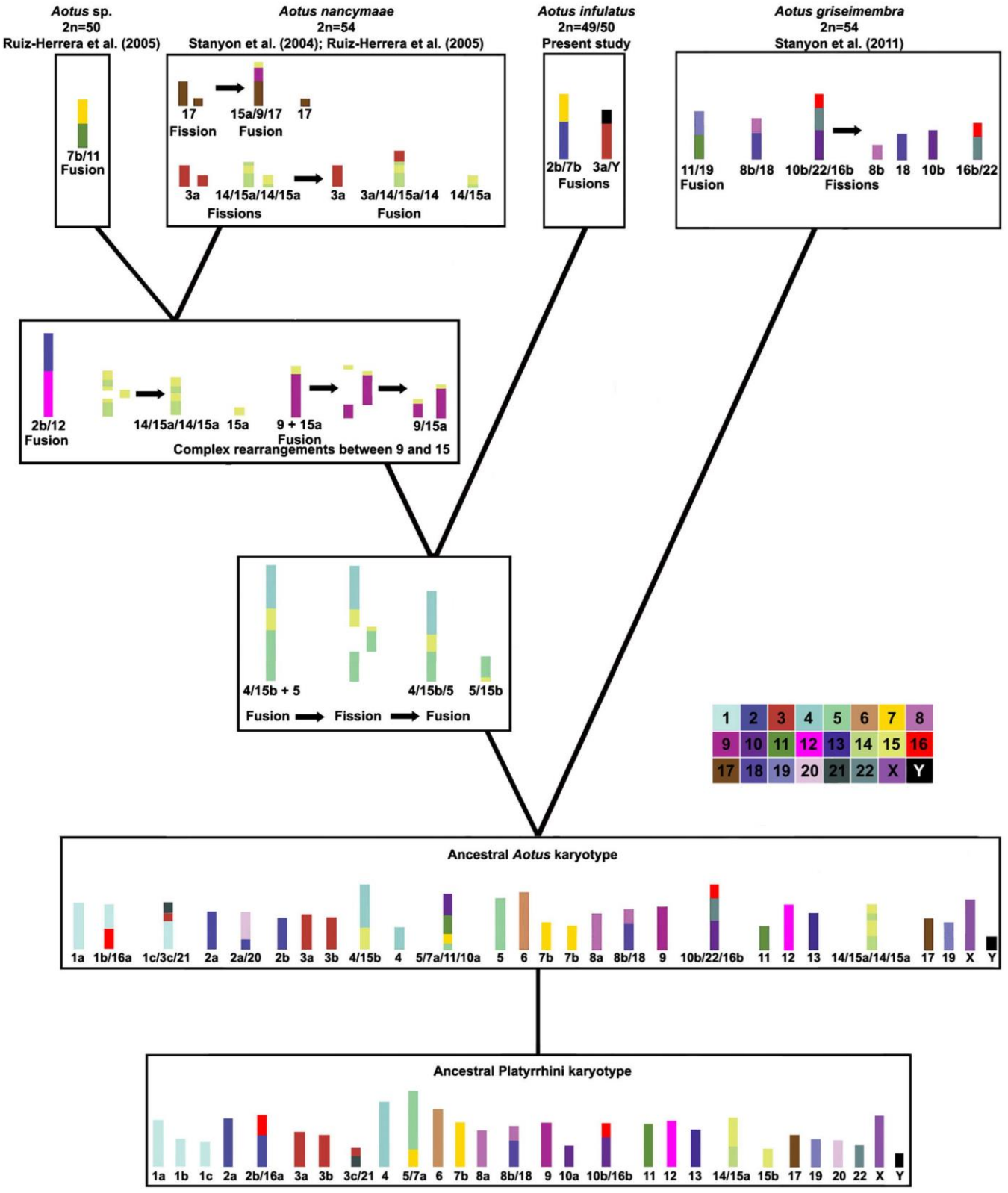


Fig. 4 Hypothetical series of transformations showing the karyotype evolution of *Aotus* sp., *A. nancymaae*, *A. infulatus*, and *A. griseimembra* from the proposed ancestral karyotype of night monkeys (2n = 52) and Platyrrhini (2n = 54), based on the correspondence with human chromosomes. A color code for each human chromosome is shown on the bottom right

Ma (1981) on the basis of banding proposed an ancestral *Aotus* karyotype with 2n = 54, presumably that found in *A. nancymaae*. However, our analysis shows that *A. nancymaae* has the most derived karyotype, with only three

human homologues conserved without disruption or association (HSA 6, 13, and 19) and 17 derived associations absent from the APLK (Stanyon et al. 2004; Ruiz-Herrera et al. 2005). Instead, we hypothesize that of the four species studied with chromosome painting *A. griseimembra* has the karyotype that is closest to the ancestral karyotype.

Chromosomal Link between *Aotus* and Other NWM

The phylogenetic position of *Aotus* has long been controversial. Recently based on Alu insertions and nuclear DNA information, *Aotus* was placed in the family Cebidae (Osterholz et al. 2009; Perelman et al. 2011; Springer et al. 2012; Kiesling et al. 2014). However, our data do not show any derived chromosome characters linking *Aotus* to any Cebidae. Nevertheless, a recent description of the organization of human chromosomes HSA 14 and 15 (found associated in NWM) based on FISH experiments with BACs corroborated this link (Capozzi et al. 2016). The authors of this study showed that *A. lemurinus* and *Callithrix jacchus* share a pericentric inversion, which gave rise to a biarmed chromosome, corresponding to the homologous HSA 14/15. These data provide a weak link between *Aotus* and other Cebidae.

Possible Evolutionary New Centromeres in *Aotus*

From this comparison, it could be hypothesized that in addition to traditional chromosome rearrangements such as fissions, translocations, and inversions, centromere repositioning (neocentromere formation) may have also played an important role in *Aotus* chromosome evolution. For example, the counterpart of the submetacentric HSA 12 is acrocentric in *A. infulatus* and in *A. griseimembra*. The metacentric HSA 19 has an acrocentric correspondent in *A.* sp. and is submetacentric in *A. infulatus* and *A. nancymaae*. Finally, the human syntenic association HSA 1/16 corresponds to a metacentric chromosome in *A.* sp., *A. nancymaae*, and *A. griseimembra*, but is acrocentric in *A. infulatus*. In all these cases the GTG-banding patterns seem conserved between species, suggesting the occurrence of centromere repositioning.

Although chromosome painting provides information about interchromosomal rearrangements, intrachromosomal changes are usually undetected. Therefore, high- resolution molecular cytogenetic approaches using cloned DNA probes, such as BACs, should provide a better understanding of the mechanisms involved in the changes of these chromosomes.

Conclusion

Our study showed that *A. infulatus* has a highly rearranged karyotype when compared to the APLK. It would be of the utmost interest to use molecular cytogenetics in analyses of additional *Aotus* karyotypes, which could provide important information for better understanding the phylogenomics of this complicated New World monkey.

Acknowledgements This work was supported by a grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Process 4072622013-0) to MS and RS and a PRIN (Programmi di Ricerca Scientifica di Rilevante Interesse Nazionale: 2012TZF8HL_003) to RS. NPA was the recipient of a doctoral fellowship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Compliance with Ethical Standards All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

This article does not contain any studies with human participants performed by any of the authors.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Araújo NP, Loss AC, Cordeiro-Junior DA, da Silva KR, Leite YLR, Svartman M (2014) New karyotypes of Atlantic tree rats, genus *Phyllomys* (Rodentia: Echimyidae). Genome 57:1–8

Capozzi O, Archidiacono N, Lorusso N, Stanyon R, Rocchi M (2016) The 14/15 association as a paradigmatic example of tracing karyotype evolution in New World monkeys. Chromosoma 125: 747–756

de Oliveira EHC, Neusser M, Müller S (2012) Chromosome evolution in New World monkeys (Platyrrhini). Cytogenet Genome Res 137: 259–272

Dumas F, Bigoni F, Stone G, Sineo L, Stanyon R (2005) Mapping genomic rearrangements in titi monkeys by chromosome flow sorting and multidirectional *in-situ* hybridization. Chromosome Res 13: 85–96

Hershkovitz P (1949) Mammals of northern Colombia. Preliminary report no. 4: Monkeys (primates) with taxonomic revisions of some forms. Proc US Natl Mus 98:323–427

Hershkovitz P (1983) Two new species of night monkeys, genus *Aotus* (Cebidae, Platyrrhini): a preliminary report on *Aotus* taxonomy. Am J Primatol 4:209–243

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

Kiesling NMJ, Yi SV, Xu K, Sperone FG, Wildman DE (2014) The tempo and mode of New World monkey evolution and biogeography in the context of phylogenomic analysis. Mol Phylogenet Evol 82:386–399

Ma NSF (1981) Chromosome evolution in the owl monkey, *Aotus*. Am J Phys Anthropol 54:293–303

Ma NSF, Aquino R, Collins WE (1985) Two new karyotypes in the Peruvian owl monkey (*Aotus trivirgatus*). Am J Primatol 9:333–341

Ma NSF, Elliott MW, Morgan L, Miller A, Jones TC (1976) Translocation of Y chromosome to an autosome in the Bolivian owl monkey, *Aotus*. Am J Phys Anthropol 45:191–202

Menezes AN, Bonvicino CR, Seuánez HN (2010) Identification, classification and evolution of owl monkeys (*Aotus*, Illiger 1811). BMC Evol Biol 10:248

Mudry MD, Nieves M, Bolzán AD (2007) Chromosomal localization of the telomeric $(TTAGGG)_n$ sequence in eight species of New World

primates (Neotropical primates, Platyrrhini). Cytogenet Genome Res 119:221–224

Osterholz M, Walter L, Roos C (2009) Retropositional events consolidate the branching order among New World monkey genera. Mol Phylogenet Evol 50:507–513

Perelman P, Johnson WE, Roos C, Seuánez HN, Horvath JE, Moreira MAM, Kessin B, Pontius J, Roelke M, Rumpler Y, Schneider MPC, Silva A, O'Brien SJ, Pecon-Slattery J (2011) A molecular phylogeny of living primates. PLoS Genet 7(3): e1001342

Pieczarka JC, Barros RMS, Faria FM Jr, Nagamachi CY (1993) *Aotus* from the southwestern Amazon region is geographically and chromosomally intermediate between *A. azarae boliviensis* and *A. infulatus*. Primates 34:197–204

Pieczarka JC, Nagamachi CY (1988) Cytogenetic studies of *Aotus* from eastern Amazonia: Y/autosome rearrangement. Am J Primatol 14:255–263

Prakhongcheep O, Chaiprasertsri N, Terada S, Hirai Y, Srikulnath K, Hirai H, Kogo A (2013) Heterochromatin blocks constituting the entire short arms of acrocentric chromosomes of Azara's owl monkey: formation processes inferred from chromosomal locations. DNA Res 20:461–470

Ruiz-Herrera A, García F, Aguilera M, Garcia M, Fontanals MP (2005) Comparative chromosome painting in *Aotus* reveals a highly derived evolution. Am J Primatol 65:73–85

Seabright M (1971) A rapid banding technique for human chromosomes. Lancet 2:971–972

Springer MS, Meredith RW, Gatesy J, Emerling CA, Park J, Rabosky DL, Stadler T, Steiner C, Ryder OA, Janecka JE, Fisher CA, Murphy WJ (2012) Macroevolutionary dynamics and historical biogeography of primate diversification inferred from a species supermatrix. PLoS One 7(11): e49521

Stanyon R, Bigoni F, Slaby T, Muller S, Stone G, Bonvicino CR, Neusser M, Seuánez HN (2004) Multi-directional chromosome painting maps homologies between species belonging to three genera of New World monkeys and humans. Chromosoma 113:305–315

Stanyon R, Bonvicino CR, Svartman M, Seuánez HN (2003) Chromosome painting in *Callicebus lugens*, the species with the lowest diploid number (2n=16) known in primates. Chromosoma 112:201–206

Stanyon R, Galleni L (1991) A rapid fibroblast culture technique for high resolution karyotypes. Boll Zool 58:81–83

Stanyon R, Garofalo F, Steinberg ER, Capozzi O, Di Marco S, Nieves M, Archidiacono N, Mudry MD (2011) Chromosome painting in two genera of South American monkeys: species identification, conservation, and management. Cytogenetic Genome Res 134:40–50

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. Exp Cell Res 75:305–306

Torres OM, Enciso S, Ruiz F, Silva E, Yunis I (1998) Chromosome diversity of the genus *Aotus* from Colombia. Am J Primatol 44:255–275

3.2. Capítulo 2: Identificação e Caracterização de um DNA Satélite Subtelomérico em Callitrichini

Este capítulo é composto pelo artigo "Identification and characterization of a subtelomeric satellite DNA in Callitrichini monkeys", publicado no periódico **DNA Research** (doi: 10.1093/dnares/dsx010). O trabalho foi iniciado pela análise do genoma de *Callithrix jacchus*, disponível online, com o RepeatExplorer, implementado na plataforma Galaxy, além de outros programas utilizados para análises de DNAs satélites. Identificamos um DNA satélite com repetições de 171 pb, ainda não descrito em *Callithrix jacchus*. Aprofundamos então a caracterização deste DNA satélite, incluindo seu mapeamento em quatro espécies de Callitrichinae.

DNA Research, 2017, 24(4), 377–385
doi: 10.1093/dnares/dsx010
Advance Access Publication Date: 17 July 2017
Full Paper

OXFORD

Full Paper

Identification and characterization of a subtelomeric satellite DNA in Callitrichini monkeys

Naiara Pereira Araújo[1,†], Leonardo Gomes de Lima[1,†], Guilherme Borges Dias[1], Gustavo Campos Silva Kuhn[1], Alan Lane de Melo[2], Yatiyo Yonenaga-Yassuda[3], Roscoe Stanyon[4], and Marta Svartman[1,*]

[1]Universidade Federal de Minas Gerais, Laboratório de Citogenômica Evolutiva, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Avenida Presidente Antônio Carlos, 6627 - Pampulha, 31270-901, Belo Horizonte, Brazil, [2]Universidade Federal de Minas Gerais, Laboratório de Taxonomia e Biologia de Invertebrados, Departamento de Parasitologia, Instituto de Ciências Biológicas, Belo Horizonte, Brazil, [3]Universidade de São Paulo, Laboratório de Citogenética de Vertebrados, Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, São Paulo, Brazil, and [4]University of Florence, Department of Biology, Florence, Italy

*To whom correspondence should be addressed. Tel. +5531 34092965. Email: svartmanm@icb.ufmg.br

[†]These authors contributed equally to this work.

Edited by Dr. Minoru Yoshida

Received 16 September 2016; Editorial decision 15 February 2017; Accepted 2 March 2017

Abstract

Repetitive DNAs are abundant fast-evolving components of eukaryotic genomes, which often possess important structural and functional roles. Despite their ubiquity, repetitive DNAs are poorly studied when compared with the genic fraction of genomes. Here, we took advantage of the availability of the sequenced genome of the common marmoset *Callithrix jacchus* to assess its satellite DNAs (satDNAs) and their distribution in Callitrichini. After clustering analysis of all reads and comparisons by similarity, we identified a satDNA composed by 171 bp motifs, named MarmoSAT, which composes 1.09% of the *C. jacchus* genome. Fluorescent *in situ* hybridization on chromosomes of species from the genera *Callithrix, Mico* and *Callimico* showed that MarmoSAT had a subtelomeric location. In addition to the common monomeric, we found that MarmoSAT was also organized in higher-order repeats of 338 bp in *Callimico goeldii*. Our phylogenetic analyses showed that MarmoSAT repeats from *C. jacchus* lack chromosome-specific features, suggesting exchange events among subterminal regions of non-homologous chromosomes. MarmoSAT is transcribed in several tissues of *C. jacchus*, with the highest transcription levels in spleen, thymus and heart. The transcription profile and subtelomeric location suggest that MarmoSAT may be involved in the regulation of telomerase and modulation of telomeric chromatin.

Key words: heterochromatin, repetitive DNA, Platyrrhini

1. Introduction

New World monkeys (NWM), infraorder Platyrrhini, represent a diverse group of neotropical primates, which are very important in medical, genomics, and evolutionary studies. Among them, the marmosets (tribe Callitrichini, family Cebidae) comprise a group of 21 species of *Callithrix*, endemic in the Atlantic forest, while *Cebuella* and *Mico* are found in the Amazon rainforest.[1] Recent molecular data support a strong relationship between *Callithrix* and *Cebuella* + *Mico*, which are, in turn, sister groups to *Callimico*.[2,3] In addition to the geographical separation, marmosets have different diploid numbers (2n). *Callithrix* species have 2n = 46, whereas both *Cebuella* and *Mico* species have 2n = 44.[4]

Several studies have shown that NWM genomes are rich in repetitive DNAs, most still uncharacterized. Among them, dispersed repetitive sequences, such as transposable elements (TEs), are major components of primate genomes.[5] For instance, the long interspersed element 1 (LINE-1) and the primate-specific Alu element, a short interspersed element (SINE), were considered as the largest contributors to the genome expansion in primates.[6]

Satellite DNA (satDNA) sequences, which are organized as long arrays of head-to-tail tandem repetitions, are also abundant components of primate genomes.[7] SatDNA monomers (repetitive units) form homogeneous arrays, usually enriched in regions of constitutive heterochromatin, and were hypothesized to be related to the maintenance of centromeric function (reviewed by Plohl *et al.*[8]). Multimers of the same satDNA motif may exhibit high similarity to each other, even when the individual monomers show considerable divergence. This organization is referred to as higher order repeats (HORs). Simian centromeres are mainly composed of α-satellite (AS) consisting of units of ~170 bp in the infraorder Catarrhini and ~340 or ~540 bp in NWM.[9–11] It was assumed that the HOR blocks of AS was a unique attribute of hominoids. However, Sujiwattanarat *et al.*[12] have recently reported HORs in the NWM *Aotus azarae* and *C. jacchus*. They suggested that this type of organization probably occurs in the AS of a wide range of simians.

SatDNAs do not code proteins but their transcription has been reported in many organisms, including vertebrates, invertebrates and plants (reviewed by Pezer *et al.*[13]) where they were shown to participate in the formation of heterochromatin,[14] centromeres[15] and in gene regulation.[16] Chan *et al.*[17] showed that AS transcripts are essential for the localization of mitotic centromere proteins including CENP-C, determining the kinetochore structure on centromeric chromatin during mitosis in humans.

In spite of the great biomedical and evolutionary interest of primates, their satDNAs have only been studied in a few groups.[18–21] In the common marmoset *Callithrix jacchus*, the only satDNA reported to date is the AS DNA, located in the centromeres of all chromosomes.[9,11]

The recent availability of genomic data for *C. jacchus* provides an excellent new opportunity to study how satDNAs are organized and influence NWM genome evolution. In this study, we employed an integrated approach, using whole-genome sequence analysis and molecular cytogenetics, to get an in-depth insight into a new satDNA of *Callithrix*, termed MarmoSAT. Our intention was to better understand its evolution by analyzing an array of NWM genomes.

2. Materials and methods

2.1. Identification of satDNA in *Callithrix jacchus* and sequence analysis

Similarity-based clustering, repeat identification, and classification were performed using RepeatExplorer[22] with whole-genome shotgun (WGS) Illumina reads from a male *C. jacchus* (accession number: SRR957684). This pipeline involves an all-to-all comparison of Illumina reads by MEGABLAST and the grouping of similar reads in clusters that represent unique repetitive DNA families. A minimum of 55 nt overlap is required for clustering different reads. A total of 1540214 100 bp reads, representing ~5% coverage of the *C. jacchus* genome were utilized in the analysis (Supplementary Fig. S1). All clusters with an abundance of at least 0.01% that of the top cluster were analysed in detail (Supplementary Table S1). As the reads utilized represent a random sample of the genome, the abundance of a given repetitive DNA family can be determined by the number of reads present in that specific cluster divided by the total number of reads utilized. The reads from each cluster are further aligned and partially assembled to produce contigs to be used in repeat consensus reconstruction and annotation. All contigs were compared with the mammalian repeat library in Repbase.[23,24] Whenever a significant number of reads from two distinct clusters match the similarity parameters, RepeatExplorer indicates these clusters as 'connected component', pointing to a potential relationship between the repeats.

C. jacchus MarmoSAT repeats were retrieved from this species sequenced genome by BLAST searches on the assembled genome (accession number: ACFV00000000.1) using as query a consensus sequence obtained from the RepeatExplorer analysis. Hits with e-values lower than 1×10^{-5} were considered significant. Furthermore, BLAST searches on *C. jacchus* WGS database present on NCBI were used to retrieve long MarmoSAT arrays on unmapped contigs. In some cases, the Tandem Repeats Finder[25] program was used to help in the delimitation of MarmoSAT monomers. The MarmoSAT arrays analysed in unmapped contigs and in assembled chromosomes files were carefully analysed through dot plots to determine the start and end of each repeat. Dot plots were also used to check for similarity between MarmoSAT and AS. These plots were generated with the Dotlet application with a 15 bp word size and 60% similarity cutoff.[26]

Multiple sequence alignments were performed using Muscle 4.0.[27] The MEGA software version 5.05[28] was used for the calculation of genetic distances and construction of Neighbor-Joining (NJ) trees.

2.2. Samples, DNA extractions, PCR amplifications, cloning and sequencing

Chromosome preparations and genomic DNAs were obtained from fibroblast cultures of one male of each *Callithrix penicillata*, *C. geoffroyi*, *Callimico goeldii* and *Mico argentatus*. Both *Callithrix* specimens are kept by Dr Alan Lane de Melo in animal facilities at Universidade Federal de Minas Gerais (permits 1/31/94/0000-8 and 3106.6995/2012-MG from IBAMA and 167/2006 from CETEA/UFMG, revalidated on 16 March 2012). The *M. argentatus* cells were provided by Dr Yatiyo Yonenaga-Yassuda from the Universidade de São Paulo (Brazil).

AS and MarmoSAT were amplified by polymerase chain reaction (PCR) from genomic DNAs of the three species with the following primer sets: Alpha-F (ACAGGGAAATATCTGCTTCTAAATC) and Alpha-R (GCTTACTGCTGTTTCTTCCATATG); MarmoSAT-F (ACAGAGTAGAATAGGGCATTG) and MarmoSAT-R (CCAACTCAGTATGCTCTCTCATG). The MarmoSAT set of primers were designed from consensus sequences from an unidentified *C. jacchus* satDNA. PCR reactions consisted of an initial denaturation step of 94°C for 3 min, followed by 30 cycles at 94°C for 60 s, 55°C for 60 s and 72°C for 60 s and a final extension at

72°C for 10 min. PCR products were excised from a 1% agarose gel and purified with the Wizard SV Gel and PCR Clean-up System kit (Promega). Selected MarmoSAT repeats were cloned using the pGEM-T-Easy cloning kit (Promega). Recombinant plasmids were sequenced on the ABI3130 platform (Myleus Biotechnology). The sequences generated in this study have GenBank accession numbers KX686899 (MarmoSAT – *Cebuella pygmaea*), KX686900 and KX686901 (MarmoSAT – *Mico argentatus*).

2.3. CBG-banding and fluorescence *in situ* hybridization

CBG-banding was obtained according to Sumner.[29] The *Callithrix*, *Callimico* and *M. argentatus* karyotypes were mounted following Sherlock *et al.*,[30] Neusser *et al.*[31] and Dumas *et al.*,[32] respectively. Fluorescence *in situ* hybridization (FISH) was performed using AS, MarmoSAT and telomeric sequences as probes. The satDNA probes were prepared from PCR purified products labelled by nick translation with digoxigenin-11-dUTP (DIG-Nick Translation mix, Roche Applied Science). A biotinylated telomeric sequence $(TTAGGG)_4$ (Invitrogen) was synthesized and used as probe for FISH. Chromosomes were denatured in 70% formamide/2xSSC at 65% for 1–2 min. The hybridization mix, consisting of 100 ng of labelled probe in 50% formamide/2xSSC, was denatured for 10 min at 98°C and applied to the chromosome preparations. Hybridization was carried out at 37°C for 16–20 hours. Slides were washed in 2xSSC at 37°C for 5 min. Immunodetection was performed with antidigoxigenin conjugated with FITC and neutravidin coupled with rhodamine (Roche Applied Science). The analyses and image acquisition were performed under a Zeiss Axioimager 2 epifluorescence microscope using the AxioVision software (Zeiss).

2.4. Transcription analysis

We investigated the transcription of MarmoSAT in several tissues of *C. jacchus* using the RNA-seq data generated by the Non-Human Primate Reference Transcriptome Resource (NHPRTR; Peng *et al.*[33]). These data are publicly available at NCBI under the BioProject PRJNA271912 and include total Ribo-Zero transcriptomes for bone marrow, the left and right brain hemispheres, the pituitary, colon, heart plus thymus, heart only, kidney, liver, lung, lymph node, muscle and spleen from a female marmoset.

The reads were mapped to a consensus sequence of MarmoSAT using the '–sensitive-local' preset of Bowtie2 implemented on the Galaxy platform (http://usegalaxy.org; Langmead and Salzberg[34]; Giardine *et al.*[35]; Goecks *et al.*[36]). The Neural Network Promoter Prediction tool was used to investigate potential transcription start sites within MarmoSAT (http://www.fruitfly.org/seq_tools/promoter.html; Reese[37]). Transcription of the abundant LINE-1 and Alu elements was also examined as described above and compared with that of MarmoSAT using the Spearman's rank correlation coefficient.

3. Results

3.1. Identification and characterization of MarmoSAT

We identified the most abundant families of repetitive DNAs present in the *C. jacchus* genome using the similarity-based clustering method implemented on RepeatExplorer.[22] We found that highly repetitive elements comprise ~15% of the common marmoset genome, mostly represented by TEs and satDNA families. LINE-like elements represented the most abundant repetitive family, comprising 6.7% of the genome and including 95,112 reads in 21 different clusters. The second most abundant repetitive element, Alu-like/SINE-like, spans 3.6% (54,301 reads) of the common marmoset genome. The AS DNA, the third most abundant repetitive family, represents 1.5% of *C. jacchus* genome (20,308 reads) and is organized on three different clusters inside this species genome. The fourth most abundant cluster of repetitive DNA was a still uncharacterized tandem repeat named herein MarmoSAT, which represents 1.09% of the genome and is composed of 171 bp AT-rich (61%) motifs. Considering an estimated genome size of 3.4 Gb for *C. jacchus*[38] this new satDNA family would account for more than 37 Mb or ~216,000 copies. MarmoSAT arrays were identified in all assembled chromosome files, including the sex chromosomes, and presented an average size of 2,223 bp, ranging from 296 bp on chromosome 13 to 8,188 bp on chromosome 3. The average nucleotide divergence among repeats is 20.18%, ranging from 11.5% (on chromosome 14) to 39.1% (on chromosome 22) (Supplementary Table S2).

The amount of MarmoSAT differed among the assembled chromosome files, but this variation may be related to technical limitations. We also identified arrays present in different loci inside chromosomes 1, 3, 8, 9, 10, 15, 16, 18, 19, and 21, whereas on chromosomes 8, 15, 16 and 21, MarmoSAT is organized as one single array interspersed with several TEs insertions (Supplementary Table S3).

In order to investigate whether MarmoSAT repeats form long-arrays, we performed BLAST searches on unmapped *C. jacchus* contigs and retrieved the five contigs with the highest score values (accession numbers: ACFV01174585.1, ACFV01176989.1, ACFV01177303.1, ACFV01181345.1and ACFV01184555.1). As a result, we obtained 492 copies of MarmoSAT with an average array size of 99.8 copies per contig, suggesting the presence of long arrays of MarmoSAT in the *C. jacchus* unmapped data.

Because MarmoSAT and AS repeat units have similar sizes (~171 bp), we performed extensive sequence comparisons with described AS sequences from several primates, including *C. jacchus* and humans, but dot plot analyses could not find any significant similarity hits between the reads in the MarmoSAT cluster and the ones in the ASor CarB clusters, indicating lack of homology (Supplementary Fig. S2). Moreover, BLAST searches revealed no significant similarity between this sequence and any other deposited in the GenBank or RepBase databases.

In order to investigate the homogenization dynamics of MarmoSAT related to their genomic location (from different arrays or chromosomes), we constructed NJ phylogenetic trees using 458 copies extracted from all chromosome files, three BACs, one from the X (accession number: AC146662.3) and two from the Y chromosome (accession numbers: AC243896.4 and AC243459.3), and 492 copies retrieved from unassembled contigs (Supplementary Fig. S3). The resulting trees showed that MarmoSAT sequences were not clustered in chromosome-specific branches. Similarly, the repeats found in the three BACs did not cluster together with the assembled sequences of the corresponding X and Y chromosomes. We also produced chromosome-specific trees, but still could not find any array specificity for MarmoSAT repeats (data not shown).

3.2. MarmoSAT flanking regions are enriched with Alu-like and L1-Cja-like retrotransposons

Aiming to better understand the genomic distribution and possible association with different genetic elements, we analysed 42 flanking

regions (from 500 bp to 1 kb, when available) of the MarmoSAT arrays found on the assembled chromosome files (Supplementary Table S3). Our analysis revealed that TEs Alu-like and L1-Cja-like were often associated with MarmoSAT arrays. We found 20 insertions of Alu-like elements adjacent to MarmoSAT arrays, whereas 17 L1-Cja-like elements were found neighbouring MarmoSAT sequences. In contrast, we did not find AS sequences associated with MarmoSAT. We found no preferential positions for TE insertion inside MarmoSAT monomers, and no micro-sequence similarities that could indicate any insertion bias (data not shown).

We also found the subtelomeric satDNA family CarB, previously described in *M. argentatus*,[19] flanking the 5' regions of MarmoSAT arrays on chromosomes 14 and 16 (Supplementary Table S3), and one copy flanking MarmoSAT in one BAC mapped on chromosome Y (accession number AC243896.4). Interestingly, we identified CarB as representing only 0.108% of the *C. jacchus* genome. We found low copy numbers of CarB on chromosomes 1, 3–8, 13–20, and it was absent on the other chromosomes.

3.3. Phylogenetic distribution of MarmoSAT

In order to ascertain the distribution of MarmoSAT in *Cebuella pygmaea* and *Mico argentatus* we amplified this sequence by PCR from genomic DNAs of both species. The sequencing of cloned PCR products from both species confirmed the presence of MarmoSAT in their genomes.

The distribution of MarmoSAT in other NWM species was verified by an *in silico* search using a MarmoSAT query against the GenBank non-redundant nucleotide collection (NCBI). BLAST searches retrieved one significant hit with e-value $2e^{-13}$ corresponding to satDNA CgoA (accession number: X52012.1), previously described in *Callimico goeldii*.[18] This 338 bp satDNA family was described as restricted to the *C. goeldii* genome after Southern blot hybridizations with 70% stringency. Dot-plot analysis of the CgoA repeat unit sequences showed that this satDNA is composed of two monomers (Fig. 1), one with 170 bp (CgoA1), and another with 168 bp (CgoA2). Pairwise sequence comparisons between CgoA monomers revealed 32.9% nucleotide divergence, indicating a HOR organization in *C. goeldii*. The comparison of CgoA1 and CgoA2 against MarmoSAT revealed 30.3% and 33.3% of divergence, respectively.

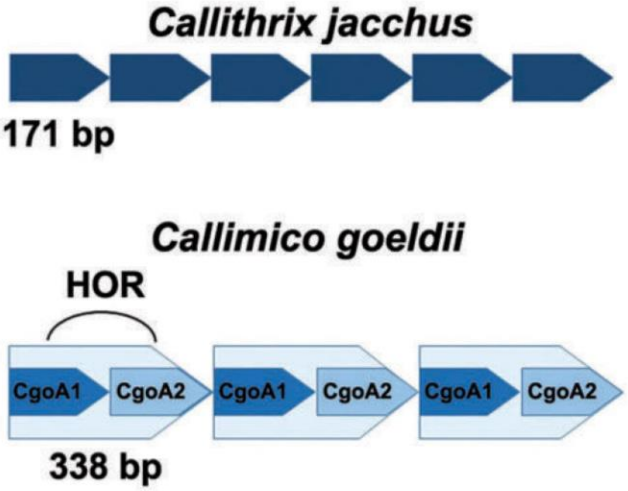


Figure 1. Schematic illustration of MarmoSAT repeat units of 171 bp in *Callithrix jacchus* and 338 bp HOR of CgoA1 and CgoA2 units in *Callimico goeldii*.

We searched for MamoSAT in the outgroup species *Aotus nancymaae* and *Saimiri boliviensis* (accession numbers SRR1692997 and SRR315548), but did not find homologous sequences. The absence of MarmoSAT in the *Aotus* and *Saimiri* lineages suggests that this satDNA family probably amplified after the split of Callitrichinae (Fig. 2).

3.4. Chromosomal location of MarmoSAT on Callitrichini and *Callimico*

The chromosome location of MarmoSAT was investigated after FISH on chromosomes from *Callithrix penicillata, C. geoffroyi, Mico argentatus* and *Callimico goeldii*. CBG-banding was also performed in order to compare the distribution of MarmoSAT in relation to the constitutive heterochromatin in the four species (Figs 3A and C and 4).

The MarmoSAT probe hybridized to both subtelomeric regions of all biarmed chromosomes in *C. penicillata*, with the exception of pair 3 and the sex chromosomes, which had only their short arms labelled (Fig. 3B). Pairs 1 and 18 showed interstitial labelling on their short and long arms, respectively, whereas pairs 16 and 17 had no hybridization signals. *C. geoffroyi* chromosomes presented the same hybridization pattern, with the exception of pair 15, which did not display any signal (Fig. 3D). In *M. argentatus*, MarmoSAT sequences were visualized on both ends of the biarmed pairs 3, 20 and in the sex chromosomes; in the subtelomeric regions of the short arm of pairs 10 and 21 and in the long arms of pairs 2, 5–7, 13 and 14. In addition, interstitial signals were detected in the short arms of pairs 2 and 3 and in the long arms of pairs 5 and 14. Among the acrocentric chromosomes, MarmoSAT sequences hybridized to the long arms of pairs 15, 17 and 19 and to interstitial regions of pairs 15, 16 and 19 (Fig. 4A). In *C. goeldii*, MarmoSAT sequences were located at subtelomeric regions of the short and long arms of biarmed chromosomes, with the exception of pair 11, which showed signals only on its long arms. The acrocentrics had only their long arms labelled (Fig. 4B).

Since MarmoSAT had a telomeric localization, we also performed double FISH with a telomeric probe $(TTAGGG)_4$. Telomeric sequences were detected in all telomeres of the three species (Figs 3B, 3D and 4A) and co-localized with MarmoSAT in most chromosome pairs. Additionally, the Y chromosomes of both *Callithrix* species had their long arms completely labelled with the telomeric probe.

A search for sequencing reads that spanned the interface between MarmoSAT and telomeric sequences on the Trace Archive File of *C. jacchus* (http://www.ncbi.nlm.nih.gov/Traces/home/) revealed two reads composed by MarmoSAT arrays adjacent to telomeric repeats. In both cases, the telomeric repeats were in the terminal 3' position, indicating a subtelomeric location of MarmoSAT (Fig. 5).

Besides the MarmoSAT hybridizations, we also report for the first time the chromosome location of AS in *C. penicillata, C. geoffroyi, M. argentatus* and *C. goeldii* (Fig. 6). This satDNA is present in large amounts in the (peri)centromeric regions of these marmosets' chromosomes, corroborating the bioinformatics analysis and previous cytogenetic studies of *C. jacchus*.[11]

3.5. MarmoSAT is transcribed on several tissues from *C. jacchus*

We used the NHPRTR[33] data to investigate the transcription of MarmoSAT in the tissues of *C. jacchus*. This analysis revealed MarmoSAT transcripts in all tissues surveyed albeit at very different abundances (Fig. 7). The 13 tissues analysed displayed over 11-fold variation in the transcription level of MarmoSAT, with a higher-

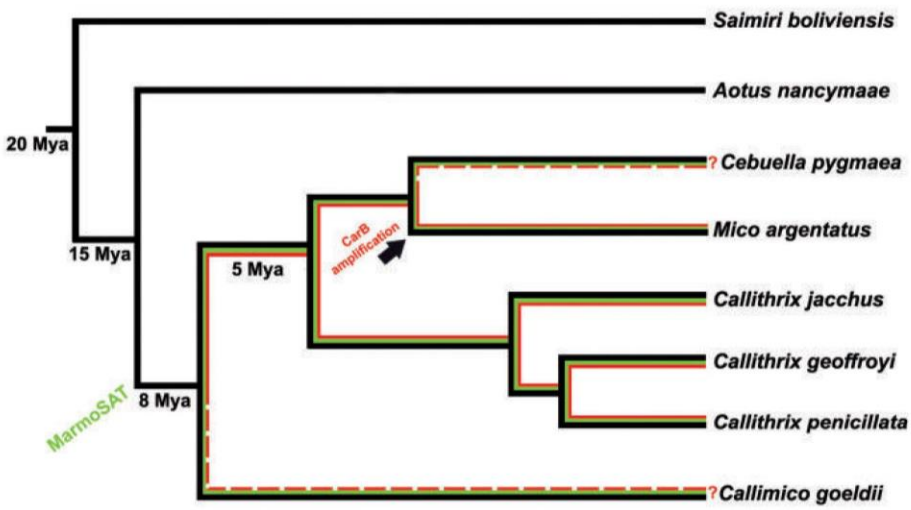


Figure 2. Possible evolutionary pathway of MarmoSAT and CarB amplification in Callitrichini and *Callimico goeldii*. Phylogenetic relationships are based on Perelman et al.[2]. Colored branches indicate the presence of MarmoSAT (green, lighter color) and CarB (red, darker color) satellite DNA families. The traced lines in the *Callimico* and *Cebuella* lineages indicate insufficient data to verify the hypothesis.

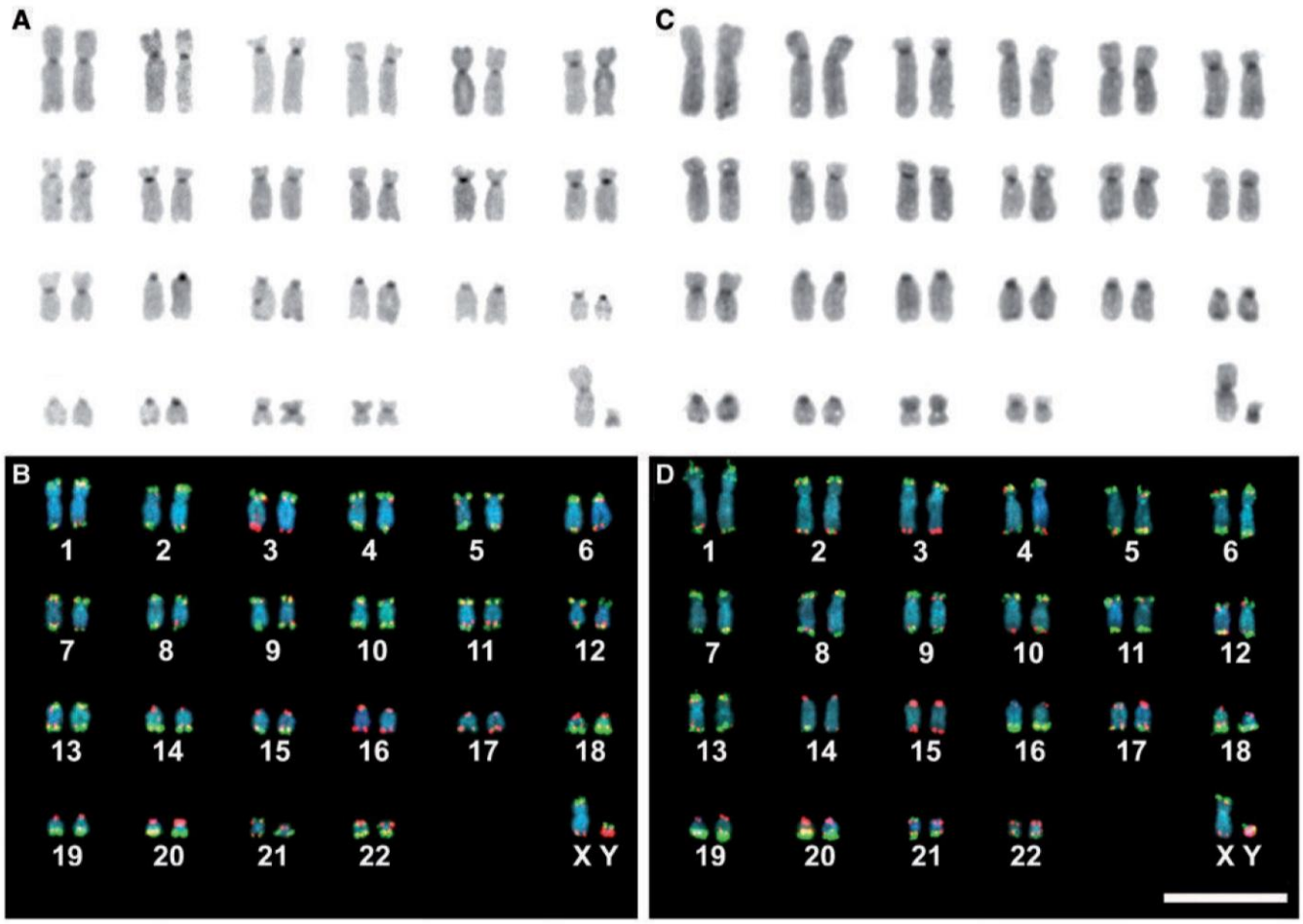


Figure 3. *Callithrix penicillata* metaphases after (A) CBG-banding and (B) FISH with the MarmoSAT (green) and telomeric (red) probes. C-D show the results of the same experiments in *C. geoffroyi*. Bar = 10 μm. Colour visible in online version.

than-average transcript level in brain (left and right hemispheres), heart plus thymus, heart only and spleen (Fig. 7).

Because we found no putative promoter regions in MarmoSAT sequences, since L1 and Alu were the sequences most commonly associated with MarmoSAT arrays, we also analysed their transcripts (Supplementary Table S3). L1 and Alu also displayed ubiquitous transcription, but showed no significant correlation with MarmoSAT transcription levels (Spearman's $R = 0.24692$

35

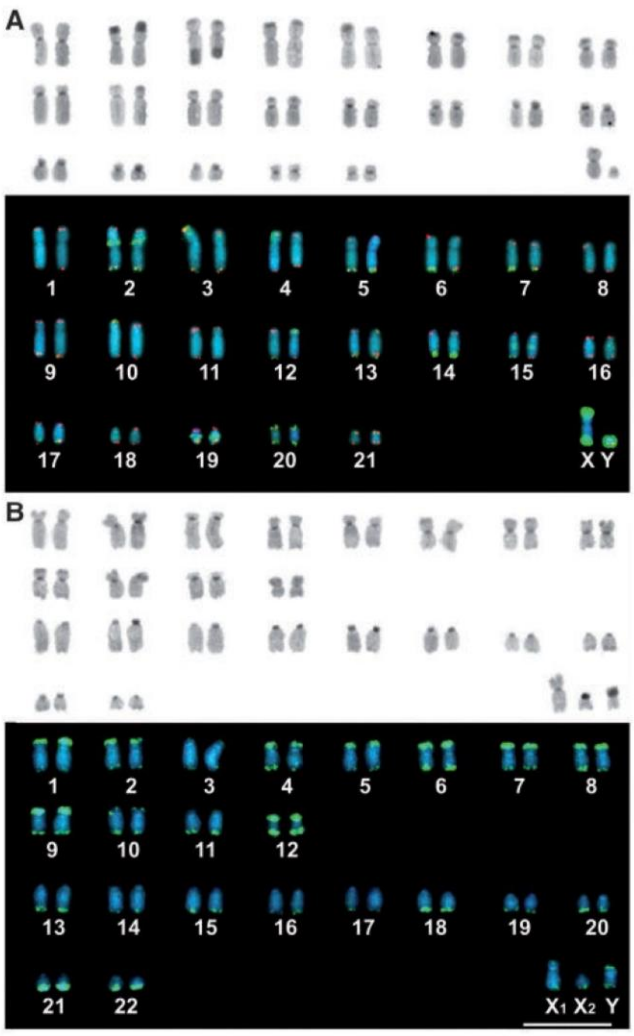


Figure 4. Karyotypes of *Mico argentatus* after (A) CBG-banding and FISH with MarmoSAT (green) and telomeric sequences (red) probes. In (B), CBG-banding and FISH with MarmoSAT sequences probe in *Callimico goeldii*. Bar = 10 μm. Colour visible in online version.

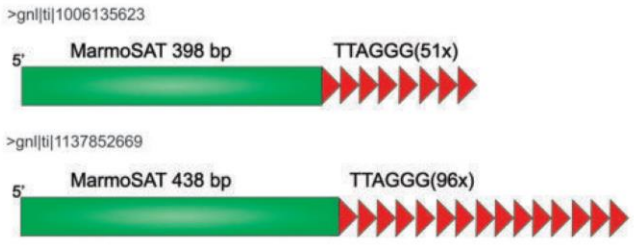


Figure 5. Schematic representation of MarmoSAT repeats adjacent to telomeric repeats on reads *gnl:1006135623* and *gnl:1137852669* found in the NCBI Trace Archive Files database.

and -0.1318, $P = 0.3733$ and 0.6693, respectively), and only moderate correlation with one another ($R = 0.4835$ and $P = 0.097$; Supplementary Fig. S4).

4. Discussion

In this study, we identified a new satDNA with 171 bp monomers in the common marmoset genome, which was named MarmoSAT. Regardless of the same motif size, MarmoSAT and AS do not share any sequence similarity or conserved structure that could suggest a common origin (Supplementary Fig. S2). The presence of two non-homologous complex satDNA families that share the same motif size was not previously reported in primates. It is possible that the convergence to the same motif size reflects the optimal distance for nucleosome positioning, as suggested by Henikoff *et al.*,[39] indicating that despite sequence heterogeneity, different satDNA families may retain structural features important to heterochromatic domains in *C. jacchus*.

4.1. Evolutionary turnover of subtelomeric satDNAs MarmoSAT and CarB in Callitrichini

SatDNAs have been identified in the subtelomeric heterochromatin of most marmoset chromosomes[19, 20, 40]. In the *Callithrix* species analysed herein we found MarmoSAT to be a major component of these regions (Fig. 3). The hybridization of MarmoSAT and telomeric sequences showed very similar patterns in both *C. penicillata* and *C. geoffroyi*, which may be explained by the recent diversification of the clade (2.6 Mya).[3]

Together with the cytogenetic analysis, our searches on assembled genomes and WGS data of NWMs showed that MarmoSAT is apparently restricted to *Callithrix, Mico, Callimico* and *Cebuella*, while CarB is present in *Callithrix* and *Mico* (Fig. 2). Therefore, MarmoSAT and CarB repeats, a satDNA previously described in *Mico* species,[19,20] were already present in the common ancestor of Callitrichini and *C. goeldii*. The FISH experiments revealed that MarmoSAT is more abundant in *Callithrix* and *C. goeldii* than in *Mico* (Figs 3 and 4). Accordingly, Fanning *et al.*[18] CgoA description supports MarmoSAT high abundancy in *C. goeldii*. Altogether, these results support the hypothesis that MarmoSAT sequences were probably abundant in the ancestor of Callitrichini and *C. goeldii* and remained copious in *Callithrix* and *Callimico*. On the other hand, CarB underwent amplification in *Mico*, as shown by Alves *et al.*[19] and Canavez *et al.*,[20] replacing MarmoSAT. This assumption is supported for instance by comparing the homologous *M. argentatus* chromosome 4 and *Callithrix* chromosome 13, *M. argentatus* chromosome 5 short arm and *Callithrix* pair 20, and *M. argentatus* chromosome 18 and *Callithrix* 19, in which the *Callithrix* counterparts showed MarmoSAT hybridization, whereas in *M. argentatus* chromosomes there were large heterochromatic blocks mainly composed of CarB.

The amplification of specific satDNAs was already reported in *Callimico* and *Cebuella*.[18,40] In marmosets, the differential amplification of unrelated satDNAs does not seem to affect karyotype stability, as this group of NWMs has conserved karyotypes.

4.2. MarmoSAT subtelomeric arrays lack chromosome-specificity

SatDNA repeats on the same array or chromosome tend to present a higher level of sequence similarity to each other when compared with those on non-homologous chromosomes.[41,42] An NJ phylogenetic tree with 980 copies of MarmoSAT revealed that these sequences lack chromosome specificity (Supplementary Fig. S3). The absence of differential homogenization for specific variants between MarmoSAT subtelomeric arrays in *C. jacchus* could be the result of exchange events between arrays and chromosomes. This hypothesis is supported by data in chimpanze, in which it has been shown that the subterminal regions of different chromosomes interact with each other to form stable physical contacts in meiosis,[43] which may result

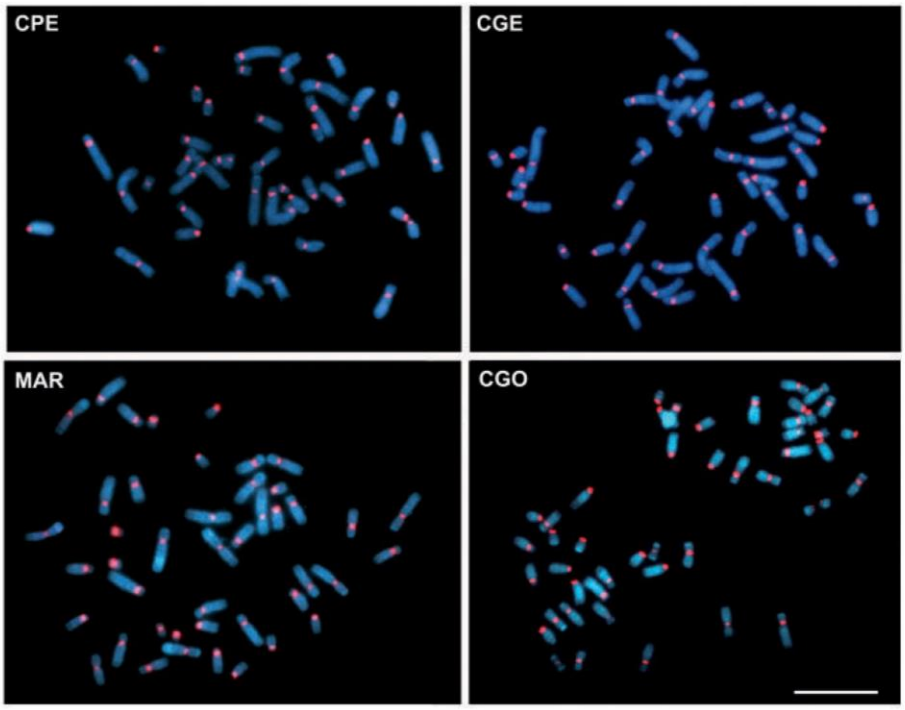


Figure 6. FISH with a digoxigenin-labelled α-satellite DNA in Callitrichini and *Callimico goeldii* cells. CPE = *Callithrix penicillata*, CGE = *C. geoffroyi*, MAR = *Mico argentatus*, CGO = *C. goeldii*. Bar = 10 μm.

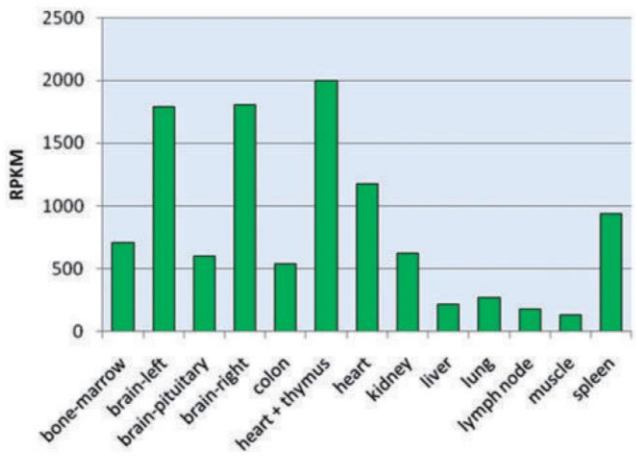


Figure 7. Transcription level of MarmoSAT in several tissues of a female *Callithrix jacchus*. RPKM: *Reads Per Kilobase Million*.

in frequent DNA exchanges between chromatids. Moreover, Rudd *et al.*[44] showed that in humans, 17% of all sister chromatid exchanges occur in the terminal ~100 kb of chromosomes, translating into a recombination rate on subtelomeric regions 160-fold larger than in euchromatic regions.

4.3. MarmoSAT is present as HORs in *Callimico goeldii*

Another significant finding was the identification of MarmoSAT organized in HOR structures in *C. goeldii*. Although HORs are apparently common and widespread in primates,[10,12] they are predominantly located at (peri)centromeric, rather than in subtelomeric regions.[45] Herein we showed that the previously described copies of CgoA satDNA[18] are in fact two highly different monomers of MarmoSAT organized in HORs (Fig. 1). Probably, the establishment of a HOR organization occurred after the divergence of *C. jacchus* and *C. goeldii* ~8 million years ago (Mya) (Fig. 2). Moreover, we observed that the subtelomeric location of MarmoSAT is shared among Callitrichini species and *C. goeldii* (Figs 3 and 4) This is the first report of subtelomeric HORs in primates, indicating that HORs sequences may be found in heterochromatic regions outside of centromeres.

The example of HORs in closely related species such as *C. jacchus* and *C. goeldii* is illuminating since it shows that HORs may develop over a relatively short period of evolutionary time. In contrast with what was found in *C. jacchus* AS,[12] the sequence similarity between *C. goeldii* CgoA1 and CgoA2 repeat units is considerably lower than 70%. This pattern is similar to the observed in previously described HORs from primates and bovids.[21,46]

4.4. Possible role for L1 in MarmoSAT dispersion

The presence of several MarmoSAT arrays adjacent to L1 opens the possibility that their dispersion could have been mediated by these retroelements (Supplementary Table S3). Interestingly, we detected many copies of MarmoSAT located interstitially on *C. penicillata* and *C. geoffroyi* pairs 1 and 18 and *M. argentatus* pairs 2, 3, 5, 14–16 and 19 (Figs 3 and 4). In humans, it has been shown *in vivo* and *in vitro* that the L1 can co-mobilize 3' downstream sequences to other genomic locations as the result of imperfect transcription events[47]. During the transposition event the transcription of an L1-element may bypass its own polyadenylation signal utilizing a second downstream polyadenylation site for 3' end processing, leading to the transcription and later transposition of adjacent flanking sequences. Even though most of L1-elements have truncated sequences, these elements are still capable of retrotransposition,[48] suggesting that even incomplete L1s may play a role in such events.

The finding of Alu-like and L1-Cja-like flanking MarmoSAT may have resulted from a bias in assembling repetitive DNAs with WGS

data, due to the high sequence similarity among repeats.[49] In fact, unmapped sequences have a low frequency of TEs insertions when compared with the sequences present in assembled chromosomes. We also observed that these TEs lack preferential insertion sites in MarmoSAT arrays, either target sites or array positioning, indicating a probable random nature for these events. For example, it has been suggested that L1 elements have a cleavage site preference for sequences rich in AA|TTT,[50] but we did not observe any insertion at these specific sites. Although MarmoSAT sequences have three different AA|TTT regions, the absence of TEs insertions may be affected by the local chromatin structure.[51] Alternatively, TEs loci inside MarmoSAT sequences may have suffered several mutations resulting in different sequences compared with the initial insertion regions.

4.5. Potential functional roles of MarmoSAT

Albeit being devoid of protein-coding capacity, satDNAs may possess structural and/or functional roles, usually via expression of non-coding RNAs (ncRNAs; reviewed by Biscotti *et al.*[52]). Interestingly, we found MarmoSAT-derived transcripts in all tissues analysed (Fig. 7) and because this satDNA does not possess promoter regions we conclude that its transcription is probably initiated in flanking sequences. The most frequent sequences flanking MarmoSAT arrays are L1 and Alu retroelements, but MarmoSAT transcription levels do not correlate with those from these elements (Supplementary Fig. S4). Because of its abundance and presence in almost all chromosomes, MarmoSAT could be transcribed from a number of different loci and have different promoters, inside or outside L1/Alu.

Besides the subtelomeric location determined by FISH, we found Sanger sequencing reads that span both MarmoSAT and the telomeric repeats in *C. jacchus* (Fig. 5). ncRNAs containing telomeric repeats in mammals are known as telomere repeat containing RNAs (TERRAs) and are thought to regulate telomerase and to modulate telomeric chromatin throughout the cell cycle (reviewed in Luke and Lingner[53]). TERRAs transcription has been shown to start at subtelomeric repeats towards the chromosome ends in human, mouse and yeast.[54–56] MarmoSAT abundance and location make it possible to suggest that it could be part of TERRAs. Moreover, MarmoSAT expression profile is similar to the TERRA expression found in mouse, with higher levels of transcripts in spleen, kidney and thymus.[55] Although the results presented herein are suggestive of interesting functional roles for MarmoSAT transcripts, the validity of these hypotheses must be assessed experimentally.

5. Conclusions

In this study, we identified with a combination of bioinformatics and cytogenetics, a subtelomeric satDNA, termed MarmoSAT, in the common marmoset and provided insights into its organization and evolution. Our data suggest that MarmoSAT originated in the common ancestor of Callitrichini and *Callimico goeldii*. Interestingly, the MarmoSAT arrays are organized in HORs in *C. goeldii*. We also propose that MarmoSAT transcripts may play a role in telomeric chromatin.

Supplementary data

Supplementary data are available at *DNARES* Online.

Conflict of interest

None declared.

Accession numbers

Sequencing data generated for this study have been submitted to GenBank under accession numbers KX686899 (MarmoSAT – *Cebuella pygmaea*), KX686900 and KX686901 (MarmoSAT – *Mico argentatus*).

Funding

This work was supported by a grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process 407262/2013-0 to MS and RS); Progetti di Interesse Nazionale 2012 (PRIN) to RS; Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG). NPA, LGL and GBD have doctoral fellowships from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

1. Schneider, H., Sampaio, I. 2015, The systematics and evolution of New World primates – A review. *Mol. Phylogenet. Evol.*, **82**, 348–57.
2. Perelman, P., Johnson, W.E., Roos, C., et al. 2011, A molecular phylogeny of living Primates. *PLoS Genet.*, **7**, e1001342.
3. Schneider, H., Bernardi, J.A.R., da Cunha, D.B., et al. 2012, A molecular analysis of the evolutionary relationships in the Callitrichinae, with emphasis on the position of the dwarf marmoset. *Zool. Scr.*, **41**, 1–10.
4. Nagamachi, C.Y., Pieczarka, J.C., Barros, R.M.S. 1992, Karyotypic comparison among *Cebuella pygmaea, Callithrix jacchus* and *C. emiliae* (Callitrichidae, Primates) and its taxonomic implications. *Genetica*, **85**, 249–57.
5. Sachidanandam, R., Weissman, D., Schmidt, S.C., et al. 2001, A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature*, **409**, 928–33.
6. Cordaux, R., Batzer, M.A. 2009, The impact of retrotransposons on human genome evolution. *Nat. Rev. Genet.*, **10**, 691–703.
7. Melters, D.P., Bradnam, K.R., Young, H.A., et al. 2013, Comparative analysis of tandem repeats from hundreds of species reveals unique insights into centromere evolution. *Genome Biol.*, **14**, R10.
8. Plohl, M., Meštrović, N., Mravinac, B. 2014, Centromere identity from the DNA point of view. *Chromosoma*, **123**, 313–25.
9. Alves, G., Seuánez, H.N., Fanning, T. 1994, Alpha satellite DNA in neotropical primates (Platyrrhini). *Chromosoma*, **103**, 262–7.
10. Alkan, C., Ventura, M., Archidiacono, N., Rocchi, M., Sahinalp, S.C., Eichler, E.E. 2007, Organization and evolution of primate centromeric DNA from Whole-Genome Shotgun sequence data. *PLoS Comput. Biol.*, **3**, e181.
11. Cellamare, A., Catacchio, C.R., Alkan, C., et al. 2009, New insights into centromere organization and evolution from the white-cheeked gibbon and marmoset. *Mol. Biol. Evol.*, **26**, 1889–900.
12. Sujiwattanarat, P., Thapana, W., Srikulnath, K., Hirai, Y., Hirai, H., Koga, A. 2015, Higher-order repeat structure in alpha satellite DNA occurs in New World monkeys and is not confined to hominoids. *Sci. Rep.*, **5**, 10315.
13. Pezer, Ž., Brajković, J., Feliciello, I., Ugarković, Đ. 2012, Satellite DNA-mediated effects on genome regulation. *Genome Dyn.*, **7**, 153–69.
14. Volpe, T.A., Kidner, C., Hall, I.M., Teng, G., Grewal, S.I., Martienssen, R.A. 2002, Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science*, **297**, 1833–7.
15. Rošić, S., Köhler, F., Erhardt, S. 2014, Repetitive centromeric satellite RNA is essential for kinetochore formation and cell division. *J. Cell. Biol.*, **207**, 335–349.
16. Feliciello, I., Akrap, I., Ugarkovic, D. 2015, Satellite DNA modulates gene expression in the beetle *Tribolium castaneum* after heat stress. *PLoS Genet.*, **11**, e1005466.
17. Chan, F.L., Marshall, O.J., Saffery, R., et al. 2012, Active transcription and essential role of RNA polymerase II at the centromere during mitosis. *Proc. Natl. Acad. Sci. USA*, **109**, 1979–84.

18. Fanning, T.G., Seuánez, H.N., Forman, L. 1989, Satellite DNA sequences in the neotropical marmoset *Callimico goeldii* (Primates, Platyrrhini). *Chromosoma*, **98**, 396–401.
19. Alves, G., Canavez, F., Seuánez, H., Fanning, T. 1995, Recently amplified satellite DNA in *Callithrix argentata* (Primates, Platyrrhini). *Chromosome Res*., **3**, 207–13.
20. Canavez, F., Alves, G., Fanning, T.G., Seuánez, H.N. 1996, Comparative karyology and evolution of the Amazonian *Callithrix* (Platyrrhini, Primates). *Chromosoma*, **104**, 348–57.
21. Prakhongcheep, O., Chaiprasertsri, N., Terada, S., et al. 2013, Heterochromatin blocks constituting the entire short arms of acrocentric chromosomes of Azara's owl monkey: formation processes inferred from chromosomal locations. *DNA Res*., **20**, 461–70.
22. Novák, P., Neumann, P., Pech, J., Steinhaisl, J., Macas, J. 2013, RepeatExplorer: a Galaxy-based web server for genome-wide characterization of eukaryotic repetitive elements from next-generation sequence reads. *Bioinformatics*, **29**, 792–3.
23. Jurka, J., Kapitonov, V.V., Pavlicek, A., Klonowski, P., Kohany, O., Walichiewicz, J. 2005, Repbase Update, a database of eukaryotic repetitive elements. *Cytogenet Genome Res*, **110**, 462–7.
24. Bao, W., Kojima, K.K., Kohany, O. 2015, Repbase Update, a database of repetitive elements in eukaryotic genomes. *Mob DNA*, **6**, 11.
25. Benson, G. 1999, Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res*., **27**, 573–80.
26. Junier, T., Pagni, M. 2000, Dotlet: diagonal plots in a web browser. *Bioinformatics*, **16**, 178–9.
27. Edgar, R.C. 2004, MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*., **32**, 1792–7.
28. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011, MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*., **28**, 2731–9.
29. Sumner, A.T. 1972, A simple technique for demonstrating centromeric heterocromatin. *Exp. Cell Res*., **75**, 305–6.
30. Sherlock, J.K., Griffin, D.K., Delhanty, J.D.A., Parrington, J.M. 1996, Homologies between human and marmoset (*Callithrix jacchus*) chromosomes revealed by comparative chromosome painting. *Genomics*, **33**, 214–9.
31. Neusser, M., Stanyon, R., Bigoni, F., Wienberg, J., Müller, S. 2001, Molecular cytotaxonomy of New World monkeys (Platyrrhini) – comparative analysis of five species by multi-color chromosome painting gives evidence for a classification of *Callimico goeldii* within the family of Callitrichidae. *Cytogenet. Cell Genet*., **94**, 206–15.
32. Dumas, F., Stanyon, R., Sineo, L., Stone, G., Bigoni, F. 2007, Phylogenomics of species from four genera of New World monkeys by flow sorting and reciprocal chromosome painting. *BMC Evol. Biol*., **7(Suppl 2)**, S11.
33. Peng, X., Thierry-Mieg, J., Thierry-Mieg, D., et al. 2014, Tissue-specific transcriptome sequencing analysis expands the non-human primate reference transcriptome resource (NHPRTR). *Nucleic Acids Res*., **43** (Database issue), D737–42.
34. Langmead, B., Salzberg, S.L. 2012, Fast gapped-read alignment with Bowtie 2. *Nat. Methods*, **9**, 357–9.
35. Giardine, B., Riemer, C., Hardison, R.C., et al. 2005, Galaxy: a platform for interactive large-scale genome analysis. *Genome Res*., **15**, 1451–5.
36. Goecks, J., Nekrutenko, A., Taylor, J. 2010, Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol*., **11**, R86.
37. Reese, M.G. 2001, Application of a time-delay neural network to promoter annotation in the *Drosophila melanogaster* genome. *Comput. Chem*., **26**, 51–6.
38. Pellicciari, C., Formenti, D., Redi, C.A., Manfredi, M.G. 1982, DNA content variability in primates. *J. Hum. Evol*., **11**, 131–41.
39. Henikoff, S., Ahmad, K., Malik, H.S. 2001, The centromere paradox: stable inheritance with rapidly evolving DNA. *Science*, **293**, 1098–102.
40. Neusser, M., Münch, M., Anzenberger, G., Müller, S. 2005, Investigation of marmoset hybrids (*Cebuella pygmaea* x *Callithrix jacchus*) and related Callitrichinae (Platyrrhini) by cross-species chromosome painting and comparative genomic hybridization. *Cytogenet. Genome Res*., **108**, 191–6.
41. Dover, G. 1982, Molecular drive: a cohesive mode of species evolution. *Nature*, **299**, 111–7.
42. Kuhn, G.C.S., Küttler, H., Moreira-Filho, O., Heslop-Harrison, J.S. 2012, The 1.688 repetitive DNA of *Drosophila*: concerted evolution at different genomic scales and association with genes. *Mol. Biol. Evol*., **29**, 7–11.
43. Hirai, H., Matsubayashi, K., Kumazaki, K., Kato, A., Maeda, N., Kim, H.S. 2004, Chimpanzee chromosomes: retrotransposable compound repeat DNA organization (RCRO) and its influence on meiotic prophase and crossing-over. *Cytogenet. Genome Res*., **108**, 248–54.
44. Rudd, M.K., Friedman, C., Parghi, S.S., Linardopoulou, E.V., Hsu, L., Trask, B.J. 2007, Elevated rates of sister chromatid exchange at chromosome ends. *PLoS Genet*., **3**, e32.
45. Koga, A., Hirai, Y., Terada, S., et al. 2014, Evolutionary origin of higher-order repeat structure in alpha-satellite DNA of primate centromeres. *DNA Res*., **21**, 407–15.
46. Modi, W.S., Ivanov, S., Gallagher, D.S. 2004, Concerted evolution and higher-order repeat structure of the 1.709 (satellite IV) family in bovids. *J. Mol. Evol*., **58**, 460–5.
47. Goodier, J.L., Ostertag, E.M., Kazazian, H.H. Jr 2000, Transduction of 3′-flanking sequences is common in L1 retrotransposition. *Hum. Mol. Genet*., **9**, 653–7.
48. Belancio, V.P., Roy-Engel, A.M., Pochampally, R.R., Deininger, P. 2010, Somatic expression of LINE-1 elements in human tissues. *Nucleic Acids Res*., **38**, 3909–22.
49. Treangen, T.J., Salzberg, S.L. 2011, Repetitive DNA and next-generation sequencing: computational challenges and solutions. *Nat. Rev. Genet*., **13**, 36–46.
50. Cost, G.J., Boeke, J.D. 1998, Targeting of human retrotransposon integration is directed by the specificity of the L1 endonuclease for regions of unusual DNA structure. *Biochemistry*, 18081–93.
51. Cost, G.J., Golding, A., Schlissel, M.S., Boeke, J.D. 2001, Target DNA chromatinization modulates nicking by L1 endonuclease. *Nucleic Acids Res*., **29**, 573–7.
52. Biscotti, M.A., Canapa, A., Forconi, M., Olmo, E., Barucca, M. 2015, Transcription of tandemly repetitive DNA: functional roles. *Chromosome Res*., **23**, 463–77.
53. Luke, B., Lingner, J. 2009, TERRA: telomeric repeat-containing RNA. *EMBO J*., **28**, 2503–10.
54. Azzalin, C.M., Reichenbach, P., Khoriauli, L., Giulotto, E., Lingner, J. 2007, Telomeric repeat-containing RNA and RNA surveillance factors at mammalian chromosome ends. *Science*, **318**, 798–801.
55. Schoeftner, S., Blasco, M.A. 2008, Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II. *Nat. Cell Biol*., **10**, 228–36.
56. Porro, A., Feuerhahn, S., Delafontaine, J., Riethman, H., Rougemont, J., Lingner, J. 2014, Functional characterization of the TERRA transcriptome at damaged telomeres. *Nat. Commun*. **5**, 5379.

Material Suplementar

Supplementary Table S1. Description of the clusters retrieved from a sample of the sequencing reads of *Callithrix jacchus* (NCBI/SRA accession nº: SRR957684) by RepeatExplorer.

Cluster	Read number	Genome Proportion [%][a]	Proportion of Similarity hits to other clusters[b]	Annotation[c]
1	54301	3.53	0.0042	SINE.Alu
2	21125	1.37	2.479	LINE.L1/ Satellite
3[d]	20318	1.32	0.2087	Satellite.centr/ Satellite.Y.chromosome
4[e]	16748	1.09	0.0011	Unclassified
5	14660	0.952	5.071	LINE.L1
6	11087	0.72	4.675	LINE.L1
7	10899	0.708	9.479	LINE.L1/ Satellite
8	10776	0.7	15.66	LINE.L1/ Satellite
9	9725	0.631	5.383	LINE.L1
10	8167	0.53	9.899	LINE.L1
11	7552	0.49	12.73	LINE.L1
12	4010	0.26	6.394	LINE.L1
13	3533	0.229	0.0544	Unclassified
14	3527	0.229	0.8158	LINE.L1
15	2042	0.133	4.498	LINE.L1
16	1916	0.124	0.2371	Unclassified
17	1855	0.12	0.2195	Unclassified
18	1663	0.108	0.3681	Satellite/ LINE.L1/ LTR.ERVL/ Simple_repeat
19	1556	0.101	0.1935	LINE.L1/ SINE.Alu/ LTR.ERV1/ LTR.ERVL.MaLR/ Satellite
20	1423	0.092	0.5938	Unclassified
21	1073	0.07	20.62	LINE.L1
22[d]	1018	0.066	33.87	Satellite.Y.chromosome/ Satellite.centr
23	916	0.059	0	SINE.Alu/ snRNA
24	864	0.056	0	Unclassified
25	829	0.054	0	LTR.ERV.MaLR
26	818	0.053	0	Simple_repeat
27	806	0.052	0.4666	tRNA
28	801	0.052	3.347	LINE.L1
29	701	0.045	3.42	LINE.L1/ SINE.MIR
30	698	0.045	0.1103	SINE.MIR
31	624	0.041	0	LTR.ERV1
32	594	0.039	0.0098	DNA.hAT.Charlie
33	512	0.033	10.68	SINE.Alu
34	507	0.033	0	LTR.ERVL/ LTR.ERV1
35	492	0.032	0.0961	LINE.L1/ Satellite
36	391	0.025	1.071	LINE.L1/ SINE.Alu/ DNA.TcMar.Tigger/ SINE.MIR

37	364	0.024	0	LTR.ERVL.MaLR
38	297	0.019	3.01	LINE.L1
39	276	0.018	0	LTR.ERV1
40	263	0.017	0	LTR.ERV1
41	262	0.017	0	SINE.Alu/ LINE.L1
42	235	0.015	0	LTR.ERVK
43	221	0.014	0	Simple_repeat
44	197	0.013	7.189	SINE.Alu/ LTR.ERV1
45	190	0.012	0	SINE.Alu/ LTR.ERVL/ Simple_repeat
46	171	0.011	0	rRNA
47	158	0.01	0	LTR.ERVL
48	155	0.01	0	srpRNA/ SINE.7SL

[a] Only clusters with an estimated coverage of at least 0.01% of the genome are included.

[b] This proportion indicates whether a significant number of reads from a given cluster also have similarity with reads from outside clusters. It is a measure of uniqueness of the repeat family.

[c] Annotation is given as displayed in the RepeatExplorer output. Clusters with at least 3% of matching similarity hits with known repeats are annotated accordingly (based on the mammalian repeat library of Repbase). Anonymous clusters are marked as "Unclassified".

[d]These clusters represent the AS DNA, a known component of primate centromeres.

[e]This cluster represents the newly described MarmoSAT.

Supplementary Table S2. Intrachromosomal average nucleotide variability between MarmoSAT repeats in the assembled chromosome files of the *Callithrix jacchus* genome.

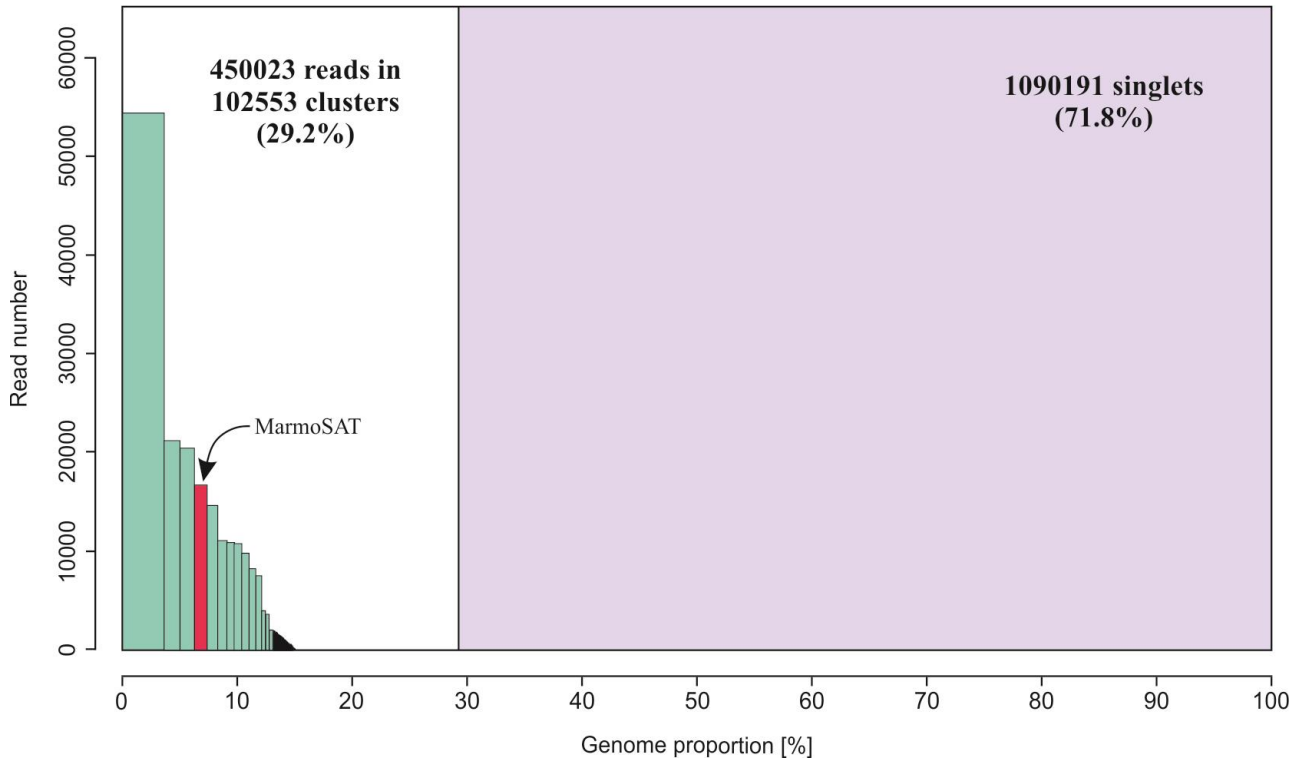
	Nucleotide divergence (sd)
Chromosome 1	0.189 (0.023)
Chromosome 2	0.229 (0.025)
Chromosome 3	0.213 (0.021)
Chromosome 4	0.260 (0.038)
Chromosome 5	0.154 (0.028)
Chromosome 6	0.296 (0.061)
Chromosome 7	0.191 (0.016)
Chromosome 8	0.116 (0.017)
Chromosome 9	0.282 (0.030)
Chromosome 10	0.201 (0.043)
Chromosome 11	0.209 (0.019)
Chromosome 14	0.115 (0.016)
Chromosome 15	0.207 (0.020)
Chromosome 16	0.209 (0.023)
Chromosome 17	0.272 (0.032)
Chromosome 18	0.283 (0.030)
Chromosome 19	0.234 (0.021)
Chromosome 20	0.226 (0.027)
Chromosome 21	0.227 (0.025)
Chromosome 22	0.391 (0.082)
Chromosome X	0.151 (0.018)
Chromosome Y	0.187 (0.030)

Supplementary Table S3. MarmoSAT array size and flanking region sequence analysis according to assembled chromosome files.

Chromosome	Array Size (bp)	Flanking sequence Left	Flanking sequence Right
1	3491	L2-NonLTR	N
1 Bl2	1742	N	MER4CL34/ERV3
2	3270	N	Alu1
3 Bl1	340	N	Alu/L1-cja
3 Bl2	8188	Alu	N
4	838	N	MIR/SINE
5	1085	AluS	L1-cja
6	519	AluSc	AluS
7	4115	Alujb	N
8 Bl1	1303	N	SVA_A/AluS
8 Bl2*	1659	AluSg/AluSc	L1Pa11/L1Prec1
8 Bl3*	400	L1Pa11/L1Prec1	N
9 Bl1	1433	N	N
9 Bl2	2040	MIR	-/Alu
9 Bl3	1145	N	AluSg/AluSc
10 Bl1	418	L1M1 LINE1	L1M1 LINE1
10 Bl2	297	AluSp/L2	N
11 Bl1	5840	-	N
12	304	AluYb3a1	N
13	296	N	L1PA15
14	2294	CarB	N
15 Bl1*	4196	AluS	L1-cja
15 Bl2*	4244	L1-cja	-/MIRb
16 Bl1*	4292	CArB	N
16 Bl2*	784	N	L1ME1
16 Bl3*	1054	L1ME1	L1PREC2
17	1615	N	N
18 Bl1	2879	N	ERV2-1_CJ-I
18 Bl2	3828	L1-1-Cja	LTR78/Alu
18 Bl3	366	N	AluSc/L2
19 Bl1	7802	L1-1-Cja	N
19 Bl2	6095	N	AluJ/L2
19 Bl3	688	AluSx1	L1-Cja
19 Bl4	2862	N	ERV_Cja
19 Bl5	3390	N	N
20	1529	N	L1ME_ORF2
20	557	N	Alu
21 Bl1*	1719	N	L1-1-Cja
21 Bl2*	1353	L1-1-Cja	N
22	550	N	AluSc
X	2206	N	ERV-1_Cja-1
Y	377	N	-/L1MC3

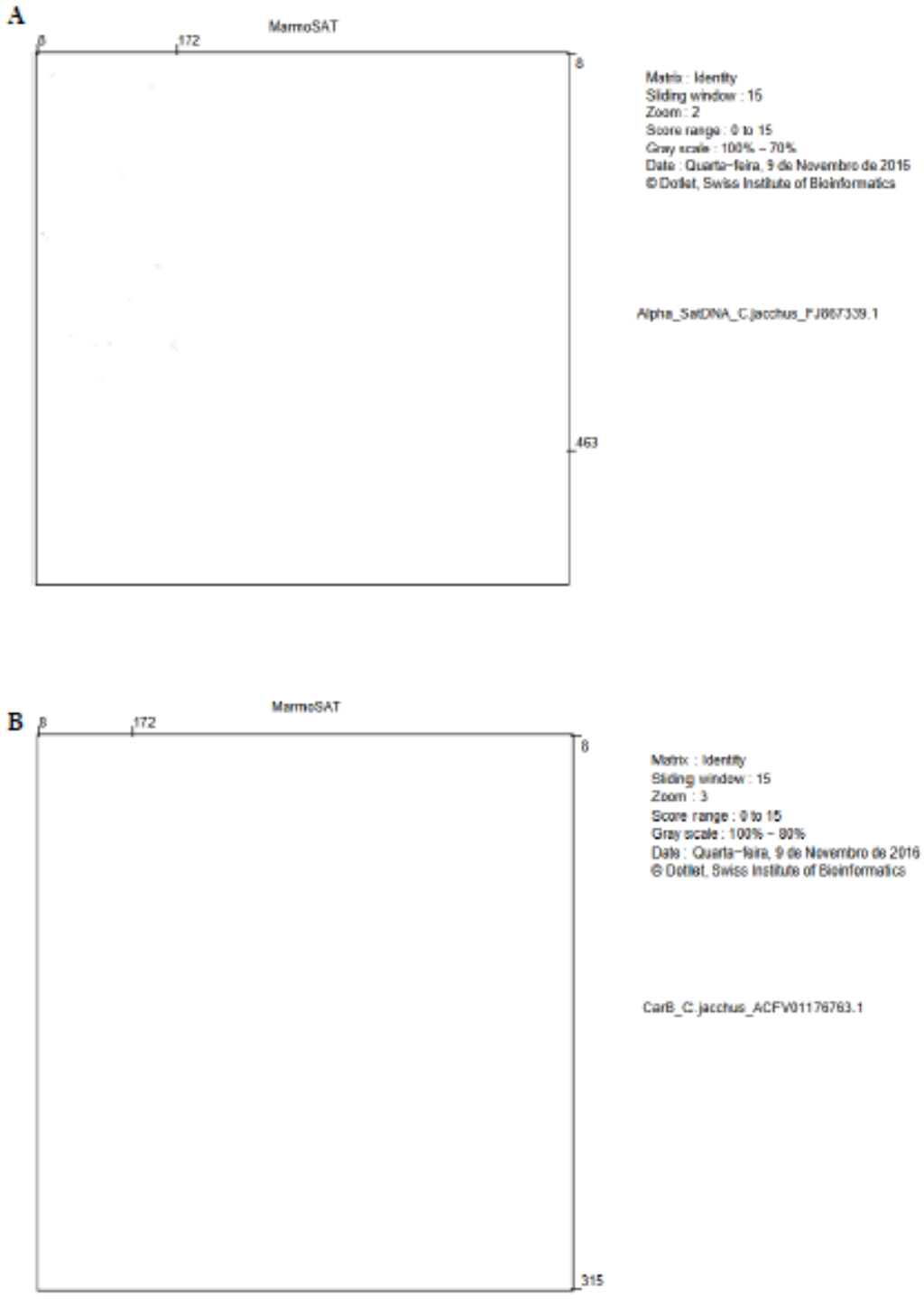
N = Gaps present on genome assemblage; Bl= Different MarmoSAT arrays located at distinct chromosome positions; * MarmoSAT arrays disrupted by different transposable elements

Supplementary Figure S1



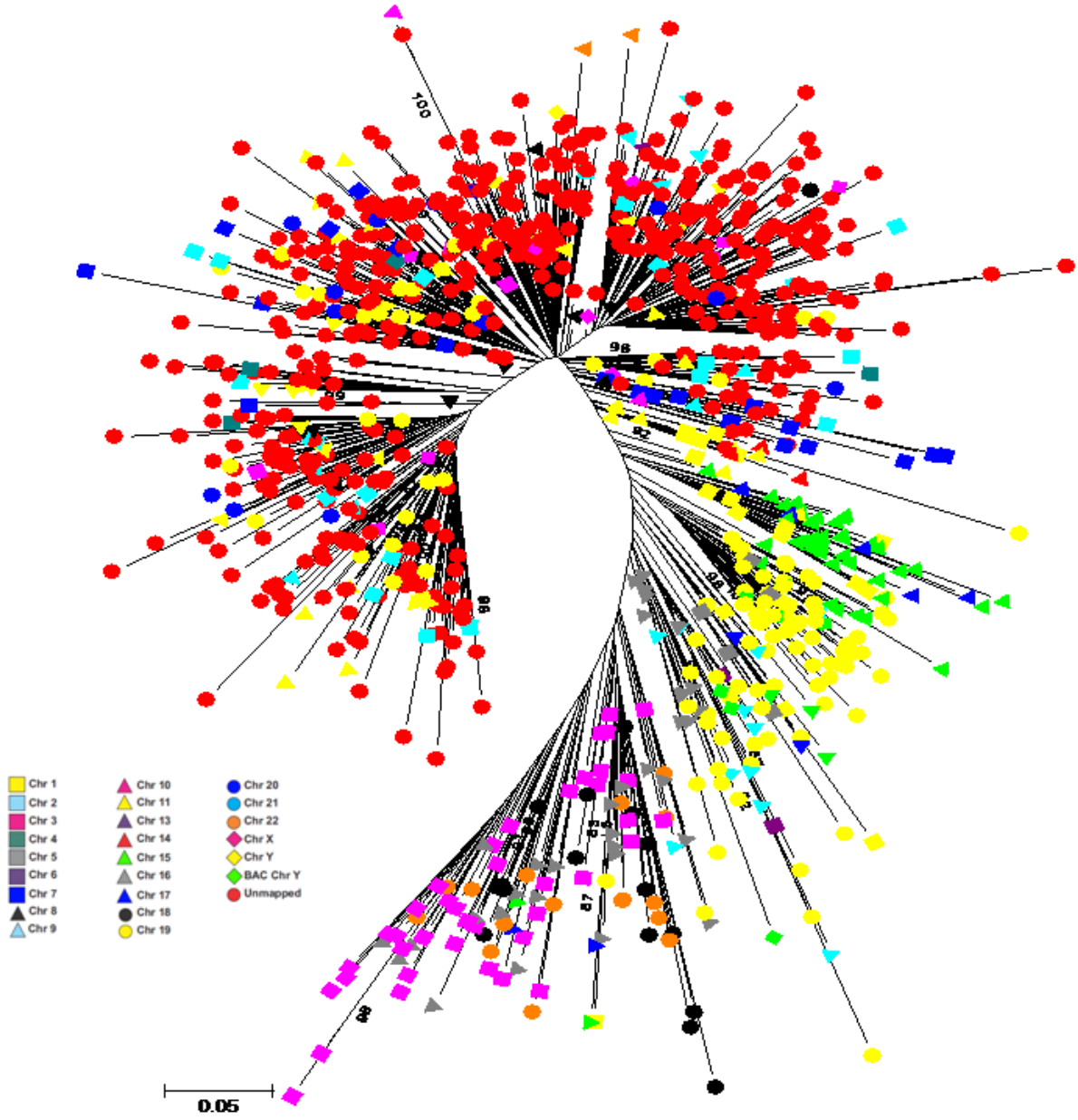
Supplementary Figure S1. Repeat families identified after clusterization of 1540214 Illumina reads with RepeatExplorer. Each bar in the histogram represents a cluster of similar reads. The height of the bar indicates the number of reads placed in that cluster, and the width represents the genomic proportion encompassed by that cluster.

Supplementary Figure S2



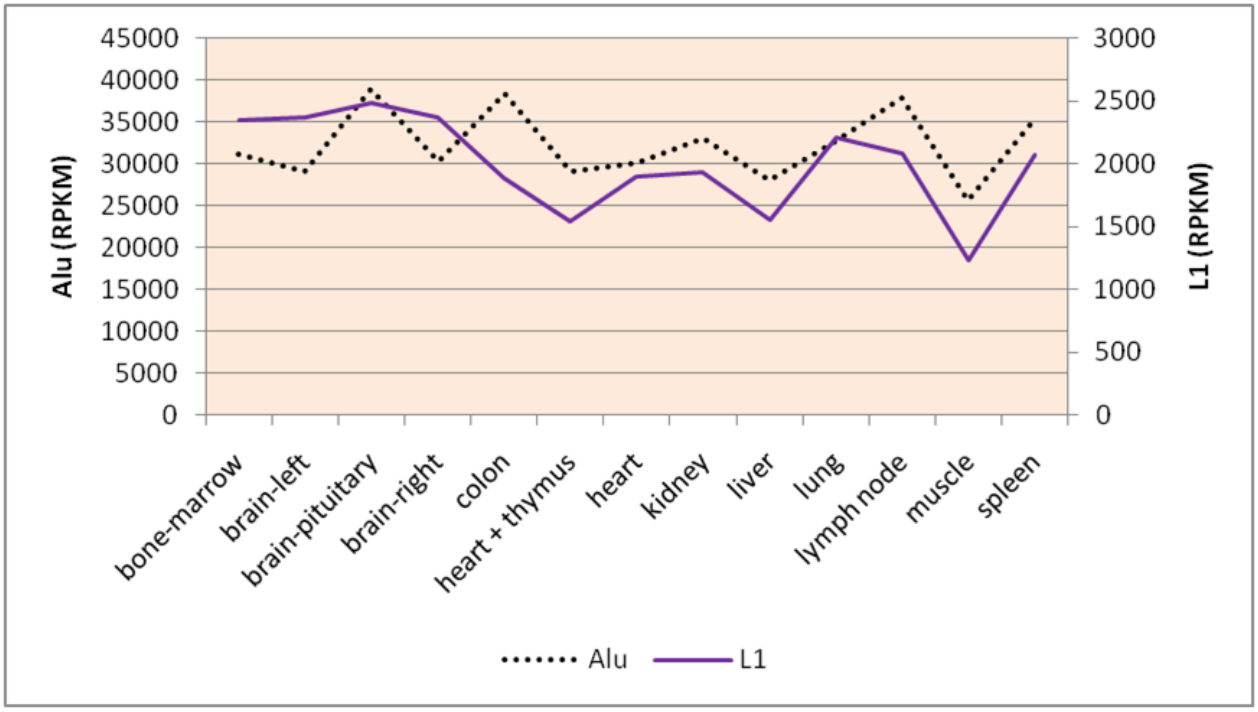
Supplementary Figure S2. Dotplot analysis of MarmoSAT consensus sequence against *C. jacchus* (A) Alpha Satellite DNA array of 462 bp (GenBank: FJ867339) and (B) CarB satellite sequence present on *C. jacchus* unmapped contig ACFV01176763.1 evidencing the absence of sequence similarity between MarmoSAT and previously described repetitive sequences.

Supplementary Figure S3



Supplementary Figure S3. NJ tree built with K2P showing all 980 MarmoSAT monomers extracted from assembled chromosomes and unmapped contigs plus BAC clones with 1000 bootstrap replicates. Bootstrap values higher than 80% are shown to assess support for nodes.

Supplementary Figure S4



Supplementary Figure S4. Transcription levels of Alu and L1 elements. Reference sequences used for RNA-seq mapping with Bowtie2 are available in Repbase (http://www.girinst.org/repbase/) as AluSc and L1-1_Cja. RPKM: *Reads Per Kilobase Million*.

4. CONCLUSÕES

Com o intuito de contribuir para a compreensão da evolução cariotípica em primatas do Novo Mundo, realizamos estudos comparativos, utilizando bandeamento GTG, CBG e pintura cromossômica em *Callicebus nigrifrons* e *Aotus infulatus*. A análise conjunta de nossos dados e os da literatura permitiram identificar ou confirmar sinapomorfias cromossômicas em ambos os grupos, assim como características ancestrais, que são muito úteis para auxiliar na resolução de questões sistemáticas. Eventos de fusão, fissão e inversão estiveram associados à variação cariotípica dentro de cada gênero e entre cada um deles e outros Platyrrhini. Adicionalmente, detectamos possíveis eventos de reposicionamento centromérico em *Aotus*, o que será melhor estudado com experimentos de FISH utilizando como sondas marcadores específicos dos cromossomos envolvidos. Para testar as hipóteses que propusemos de evolução cromossômica em *Aotus* e Callicebinae, será necessário ampliar o número de espécies, já que ainda há espécies de *Callicebus* cujo cariótipo é totalmente desconhecido e das 11 espécies reconhecidas de *Aotus*, apenas quatro tiveram seus cromossomos investigados por pintura cromossômica.

As sinapomorfias cromossômicas observadas em *Callicebus* (quatro fusões – HSA 1b/1c, 3c/8b, 13/20, 14/15a/3a/21 – e uma fissão – HSA 2a/22) apóiam estudos prévios que utilizaram dados morfológicos, biogeográficos e moleculares, que propuseram a separação de *Callicebus* de *Cheracebus* e *Plecturocebus*. Em *Aotus*, por outro lado, as sinapomorfias detectadas devem ser úteis para auxiliar o esclarecimento de questões taxonômicas e filogenéticas envolvendo o gênero. Por exemplo, associações específicas foram observadas para as espécies *A. infulatus* (HSA 2/7), *A. nancymaae* (HSA 3/14 e 9/17), *Aotus* sp. (HSA 7/11) e *A. griseimembra* (HSA 11/19). Além disso, associações derivadas compartilhadas entre as espécies analisadas, sugeriram uma maior proximidade entre *Aotus* sp. e *A. nancymaae*, em relação à *A. infulatus*, e destas três espécies em relação a *A. griseimembra*.

De forma geral, no artigo do capítulo 2 desta tese, identificamos e caracterizamos o DNA satélite MarmoSAT, presente no genoma de espécies da tribo Callitrichini. Como mostrado anteriormente em experimentos de pintura cromossômica (Neusser e col. 2001), espécies de Callitrichini apresentam cariótipos bastante conservados e, ao que parece, a amplificação diferencial de MarmoSAT e CarB parece não afetar a estabilidade cromossômica na tribo. Apesar da diversificação recente de Callitrichini (Schneider e Sampaio 2015) e da conservação

cromossômica, o MarmoSAT mostrou localizações diferentes entre os homólogos das espécies testadas e um padrão de organização estrutural variável (monomérico em *Callithrix*, *Cebuella* e *Mico* e HOR em *Callimico*). As análises de transcrição nos levaram a sugerir que o MarmoSAT pode ter alguma função telomérica.

5. REFERÊNCIAS BIBLIOGRÁFICAS

Alfaro JWL, Cortés-Ortiz L, Fiore A, Boubli JP (2015) Special issue: comparative biogeography of neotropical primates. Mol Phylogenet Evol 82:518-529. doi: 10.1016/j.ympev.2014.09.027

Alkan C, Ventura M, Archidiacono N, Rocchi M, Sahinalp SC, Eichler EE (2007) Organization and evolution of primate centromeric DNA from Whole-Genome Shotgun sequence data. PLoS Comput Biol 3:e181. doi:10.1371/journal.pcbi.0030181

Alves G, Seuánez HN, Fanning T (1994) Alpha satellite DNA in neotropical primates (Platyrrhini). Chromosoma 103:262-267. doi: 10.1007/BF00352250

Alves G, Canavez F, Seuánez H, Fanning T (1995) Recently amplified satellite DNA in *Callithrix argentata* (Primates, Platyrrhini). Chromosome Res 3:207-213. doi: 10.1007/BF00713044

Araújo NP, Espírito Santo AA, Pereira VS, Stanyon R, Svartman M (2017a) Chromosome painting in *Callicebus nigrifrons* provides insights into the genome evolution of titi monkeys and the ancestral Callicebinae karyotype. Cytogenetic Genome Res 151:82-88. doi: 10.1159/000458748

Araújo NP, Stanyon R, Pereira VS, Svartman M (2017b) Interspecific chromosome painting provides clues to the ancestral karyotype of the New World monkey genus *Aotus.* J Mammal Evol 1-8. doi: 10.1007/s10914-017-9403-z

Araújo NP, de Lima LG, Dias GB, Kuhn GCS, Melo AL, Yonenaga-Yassuda Y, Stanyon R, Svartman M (2017c) Identification and characterization of a subtelomeric satellite DNA in Callitrichini monkeys. DNA Res 24:377-385. doi: 10.1093/dnares/dsx010

Bicca-Marques JC, Silva VM, Gomes DF (2006) Ordem Primates. In Reis NR, Peracchi AL, Pedro WA, Lima IP (eds). Mamíferos do Brasil. Curitiba, pp 101-148.

Biscotti MA, Canapa A, Forconi M, Olmo E, Barucca M (2015) Transcription of tandemly repetitive DNA: functional roles. Chromosome Res 23:463-477. doi: 10.1007/s10577-015-9494-4

Byrne H, Rylands AB, Carneiro JC, Alfaro JWL, Bertuol F, da Silva MNF, Messias M, Groves CP, Mittermeier RA, Farias I, Hrbek T, Schneider H, Sampaio I, Boubli JP (2016) Phylogenetic relationships of the New World titi monkeys (*Callicebus*): first appraisal of taxonomy based on molecular evidence. Front Zool 13:1o. doi: 10.1186/s12983-016-0142-4

Canavez F, Alves G, Fanning TG, Seuánez HN (1996) Comparative karyology and evolution of the Amazonian *Callithrix* (Platyrrhini, Primates). Chromosoma 104:348-357. doi: 10.1007/BF00337224

Capozzi O, Archidiacono N, Lorusso N, Stanyon R, Rocchi M (2015) The 14/15 association as a paradigmatic example of tracing karyotype evolution in New World monkeys. Chromosoma 1-10. doi: 10.1007/s00412-015-0565-2

Cellamare A, Catacchio CR, Alkan C, Giannuzzi G, Antonacci F, Cardone MF, Della Valle G, Malig M, Rocchi M, Eichler EE, e col. (2009) New insights into centromere organization and evolution from the white-cheeked gibbon and marmoset. Mol Biol Evol 26:1889-1900. doi: 10.1093/molbev/msp101

Chatterjee HJ, Ho SYW, Barnes I, Groves C (2009) Estimating the phylogeny and divergence times of primates using a supermatrix approach. BMC Evol Biol 9:259. doi: 10.1186/1471-2148-9-259

Csink AK, Henikoff S (1998) Something from nothing: the evolution and utility of satellite repeats. Trends Genet 14:200-204. doi: 10.1016/S0168-9525(98)01444-9

de Oliveira EHC, Neusser M, Müller S (2012) Chromosome evolution in New Work monkeys (Platyrrhini). Cytogenet Genome Res 137:259-272. doi: 10.1159/000339296

Doolittle WF, Sapienza C (1980) Selfish genes, the phenotype paradigm and genome evolution. Nature 284:601-603. doi: 10.1038/284601a0

Dover G (1986) Molecular drive: a cohesive mode of species evolution. Nature 299:111-117. doi: 10.1038/299111a0

Fanning TG, Seuánez HN, Forman L (1989) Satellite DNA sequences in the neotropical marmoset *Callimico goeldii* (Primates, Platyrrhini). Chromosoma 98:396-401. doi: 10.1007/BF00292784

Fanning TG, Seuánez HN, Forman L (1993) Satellite DNA sequences in the New World primate *Cebus apella* (Platyrrhini, Primates). Chromosoma 102:306-311. doi: 10.1007/BF00661273

Ferree PM, Barbash DA (2009) Species-specific heterochromatin prevents mitotic chromosome segregation to cause hybrid lethality in *Drosophila*. PLoS Biol 7(10):e1000234. doi:10.1371/journal.pbio.1000234

Garrido-Ramos MA (2017) Satellite DNA: an evolving topic. Genes 8:230. doi:10.3390/genes8090230

Goodman M, Porter CA, Czelusniak J, Page SL, Schneider H, Shoshani J, Gunnell G, Groves CP (1998) Toward a phylogenetic classification of Primates based on DNA evidence complemented by fossil evidence. Mol Phylogenet Evol 9:585–598. doi: 10.1006/mpev.1998.0495

Groves CP (2005) Order Primates. In: Mammal Species of the World. A Taxonomic and Geographic Reference. Third Edition, D. E. Wilson & D. M. Reeder (eds). Johns Hopkins University Press, Baltimore, MD, pp 111-184.

Henikoff S, Ahmad K, Malik HS (2001) The centromere paradox: stable inheritance with rapidly evolving DNA. Science 293:1098-1102. doi: 10.1126/science.1062939

Howel WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. CMLS 36:1014-1015. doi:10.1007/BF01953855

Jameson Kiesling NM, Yi SV, Xu K, Sperone FG, Wildman DE (2014) The tempo and mode of New World monkey evolution and biogeography in the context of phylogenomic analysis. Mol Phylogenet Evol 82:386-399. doi: 10.1016/j.ympev.2014.03.027

Koga A, Tanabe H, Hirai Y, Imai H, Imamura M, Oishi T, Stanyon R, Hirai H (2017) Co-Opted Megasatellite DNA Drives Evolution of Secondary Night Vision in Azara's Owl Monkey. Genome Biol Evol 9:1963-1970. doi: 10.1093/gbe/evx142

Kugou K, Hirai H, Masumoto H, Koga A (2016) Formation of functional CENP-B boxes at diverse locations in repeat units of centromeric DNA in New World monkeys. Sci Rep 6:27833. doi: 10.1038/srep27833

Kuhn GCS, Sene FM, Moreira-Filho O, Schwarzacher T, Heslop-Harrison JS (2008) Sequence analysis, chromosomal distribution and long-range organization show that rapid turnover of new and old pBuM satellite DNA repeats leads to different patterns of variation in seven species of the *Drosophila buzzatti* cluster. Chromosome Res 16:307-324. doi: 10.1007/s10577-007-1195-1

Kuhn GCS (2015) Satellite DNA transcripts have diverse biological roles in *Drosophila*. Heredity 115:1–2. doi:10.1038/hdy.2015.12

Mestrovic N, Plohl M, Mravinac B, Ugarkovic D (1998) Evolution of satellite DNAs from the genus *Palorus* – experimental evidence for the "library" hypothesis. Mol Biol Evol 15:1062-1068. doi: 10.1093/oxfordjournals.molbev.a026005

Neusser M, Stanyon R, Bigoni F, Wienberg J, Müller S (2001) Molecular cytotaxonomy of New World monkeys (Platyrrhini) – comparative analysis of five species by multi-color chromosome painting gives evidence for a classification of *Callimico goeldii* within the family of Callitrichidae. Cytogenet Cell Genet 94:206-215. doi: 10.1159/000048818

Novák P, Neumann P, Pech J, Steinhaisl J, Macas J (2013) RepeatExplorer: a Galaxy-based web server for genome-wide characterization of eukaryotic repetitive elements from next generation sequence reads. Bioinformatics 29:792-793. doi: 10.1093/bioinformatics/btt054

Orgel LE, Crick FHC (1980) Selfish DNA: the ultimate parasite. Nature 284:604-607. doi: 10.1038/284604a0

Osterholz M, Walter L, Roos C (2009) Retropositional events consolidate the branching order among New World monkey genera. Mol Phylogenet Evol 50:507-513. doi: 10.1016/j.ympev.2008.12.014

Perelman P, Johnson WE, Roos C, Seuánez HN, Horvath JE, Moreira MAM, Kessin B, Pontius J, Roelke M, Rumpler Y, Schneider MPC, Silva A, O'Brien SJ, Pecon-Slattery J (2011) A molecular phylogeny of living Primates. PLoS Genet 7(3): e1001342. doi: 10.1371/journal.pgen.1001342

Plohl M, Luchetti A, Mestrovic N, Mantovani B (2008) Satellite DNAs between selfishness and functionality: structure, genomics and evolution of tandem repeats in centromeric (hetero)chromatin. Gene 409:72-82. doi: 10.1016/j.gene.2007.11.013

Plohl M, Mestrovic N, Mravinac B (2012) Satellite DNA evolution. Genome Dyn Basel 7:126-152. doi: 10.1159/000337122

Prakhongcheep O, Chaiprasertsri N, Terada S, Hirai Y, Srikulnath K, Hirai H, Koga A (2013) Heterochromatin blocks constituting the entire short arms of acrocentric chromosomes of Azara's Owl Monkey: formation processes inferred from chromosomal locations. DNA Res 20:461-470. doi: 10.1093/dnares/dst023

Purvis A (1995) A composite estimate of primate phylogeny. Phil Trans R Soc Lond B 348:405-421. doi: 10.1098/rstb.1995.0078

Rošić S, Köhler F, Erhardt S (2014) Repetitive centromeric satellite RNA is essential for kinetochore formation and cell division. J Cell Biol 207:335-349. doi: 10.1083/jcb.201404097

Ruiz-Herrera A, García F, Aguilera M, Garcia M, Fontanals MP (2005) Comparative chromosome painting in Aotus reveals a highly derived evolution. Am J Primatol 65:73-85. doi: 10.1002/ajp.20098

Rylands AB, Schneider H, Langguth A, Mittermeier RA, Groves CP, Rodríguez-Luna E (2000) An assessment of the diversity of New World Primates. Neotrop Primates 8:61-93.

Rylands AB, Mittermeier RA, Silva Jr JS (2012) Neotropical primates: taxonomy and recently described species and subspecies. Int Zoo Yb 46:11-24. doi: 10.1111/j.1748-1090.2011.00152.x

Rylands AB, Mittermeier RA (2014) Primate taxonomy: species and conservation. Evol Anthr 23:8-10. doi: 10.1002/evan.21387

Schneider H, Bernardi JAR, da Cunha DB, Tagliaro CH, Vallinoto M, Ferrari SF, Sampaio I (2012) A molecular analysis of the evolutionary relationships in the Callitrichinae, with emphasis on the position of the dwarf marmoset. Zool Scr 41:1-10. doi: 10.1111/j.1463-6409.2011.00502.x

Schneider H, Sampaio I (2015) The systematics and evolution of New World primates – A review. Mol Phylogenet Evol 82:348-357. doi: 10.1016/j.ympev.2013.10.017

Schueler MG, Higgins AW, Rudd MK, Gustacha K, Willard HF (2001) Genomic and genetic definition of a functional human centromere. Science 294:109-115. doi: 10.1126/science.1065042

Seabright M (1971) A rapid banding technique for human chromosomes. Lancet 2:971-972. doi:10.1016/S0140-6736(71)90287-X

Springer MS, Meredith RW, Gatesy J, Emerling CA, Park J, Rabosky DL, Stadler T, Steiner C, Ryder OA, Janecka JE, Fisher CA, Murphy WJ (2012) Macroevolutionary dynamics and historical biogeography of primate diversification inferred from a species supermatrix. PLoS ONE 7(11): e49521. doi: 10.1371/journal.pone.0049521

Stanyon R, Galleni L (1991) A rapid fibroblast culture technique for high resolution karyotypes. Boll Zool 58:81-83. doi: 10.1080/11250009109355732

Stanyon R, Bonvicino CR, Svartman M, Seuánez HN (2003) Chromosome painting in *Callicebus lugens*, the species with the lowest diploid number (2n=16) known in primates. Chromosoma 112:201-206. doi: 10.1007/s00412-003-0261-5

Stanyon R, Bigoni F, Slaby T, Muller S, Stone G, Bonvicino CR, Neusser M, Seuánez HN (2004) Multi-directional chromosome painting maps homologies between species belonging to three genera of New World monkeys and humans. Chromosoma 113:305-315. doi: 10.1007/s00412-004-0320-6

Stanyon R, Garofalo F, Steinberg ER, Capozzi O, di Marco S, Nieves M, Archidiacono N, Mudry MD (2011) Chromosome painting in two genera of South American monkeys: species identification, conservation, and management. Cytogenet Genome Res 134:40–50. doi: 10.1159/000324415

Stanyon R, Rocchi M, Capozzi O, Roberto R, Misceo D, Ventura M, Cardone MF, Bigoni F, Archidiacono N (2008) Primate chromosome evolution: ancestral karyotypes, marker order and neocentromeres. Chromosome Res 16:17-39. doi: 10.1007/s10577-007-1209-z

Sumner AT (1972) A simple technique for demonstrating centromeric heterocromatin. Exp Cell Res 75:305-306. doi: 10.1016/0014-4827(72)90558-7

Suntronpong A, Kugou K, Masumoto H, Srikulnath K, Ohshima K, Hirai H, Kogo A (2016) CENP-B box, a nucleotide motif involved in centromere formation, occurs in a New World monkey. Biol Lett 12:20150817. doi: 10.1098/rsbl.2015.0817

Szybalski W (1968) Use of cesium sulfate for equilibrium density gradient centrifugation. Methods Enzymol 12B:330-360. doi: 10.1016/0076-6879(67)12149-6

Tagliaro CH, Schneider MPC, Schneider H, Sampaio IC, Stanhope M (2000) Molecular studies of *Callithrix pygmaea* (Primates, Platyrrhini) based on transferrin intronic and ND1 regions: implications for taxonomy and conservation. Genet Mol Biol 23:729-737. doi: 10.1590/S1415-47572000000400006

Telenius H, Carter NP, Bebb CE, Nordenskjold M, Ponder BA, Tunnacliffe A (1992) Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics 13:718-725. doi: 10.1016/0888-7543(92)90147-K

Ugarkovic D (2005) Functional elements residing within satellite DNAs. EMBO Reports 6:1035-1039. doi: 10.1038/sj.embor.7400558

Willard HF (1991) Evolution of alpha satellite. Curr Opin Genet Dev 1:509-514. doi: 10.1016/S0959-437X(05)80200-X

6. ANEXOS

6.1. Métodos

6.1.1. Técnicas de obtenção de preparações cromossômicas

Obtenção de preparações cromossômicas a partir de cultura de linfócitos de sangue periférico

Este procedimento utiliza o método de cultura *in vitro* para proliferação de linfócitos do sangue periférico. Os linfócitos são induzidos à divisão pelo uso de um mitógeno (concanavalina A ou fitohemaglutinina), que faz com que os linfócitos-T entrem em mitose. O índice mitótico máximo, em geral, é alcançado com 72 horas ou 96 horas. As culturas são colhidas após terem sido tratadas com colchicina ou colcemida, o qual interrompe o ciclo celular em metáfase. Nesta tese, utilizamos esta técnica para obtenção de preparações cromossômicas da fêmea *Callicebus nigrifrons* (Capítulo 1).

1. Preparar 5 mL de meio de cultura em tubo Falcon e adicionar 300 a 500 µL de sangue total.

   O meio de cultura é composto por meio RPM1 (4 mL), 15% de soro fetal bovino (750 µL), 1% de antibióticos penicilina e estreptomicina (100 U/mL) e 25 µL de concanavalina A (10 mg/mL). O pH do meio é ajustado para 7,0, utilizando hepes (ácido) ou bicarbonato de sódio (básico).
2. Homogeneizar os tubos Falcon e mantê-los em uma estufa a 37 ºC por 72/96 horas;
3. Após o período de incubação, segue-se as etapas de colheita das células. Para isso, adicionar 5 µL de colcemida (10 µg/ml) à cultura;
4. Homogeneizar o tubo Falcon e mantê-lo na estufa por uma hora;
5. Centrifugar o tubo Falcon por 10 minutos a 1000 rpm;
6. Retirar o sobrenadante;
7. Ressuspender a amostra em 10 mL de solução hipotônica (KCl 0,075 M), adicionada lentamente;
8. Deixar as células em hipotônica no banho-maria, 37 ºC, por 15 minutos;
9. Adicionar 500 µL de fixador (metanol:ácido acético 3:1) e homogeneizar por inversão;

10. Centrifugar por 10 minutos a 1000 rpm;
11. Fixar o material com 5 mL de metanol:ácido acético (3:1) lentamente;
12. Manter as amostras em temperatura ambiente por 20 a 30 minutos;
13. Centrifugar por 10 minutos a 1000 rpm;
14. Desprezar o sobrenadante e ressuspender o material em fixador fresco. Repetir este passo duas ou mais vezes, até a preparação parecer limpa;
15. Ressuspender em fixador e pingar as preparações em lâminas limpas.

Obtenção de preparações cromossômicas a partir de cultura de fibroblastos

A técnica de cultura de fibroblasto foi descrita em Stanyon e Galleni (1991). Nesta tese, utilizamos esta técnica para obtenção de preparações cromossômicas das espécies *Aotus griseimembra, A. infulatus, A. nancymaae, Callimico goeldii, Callithrix geoffroyi, C. penicillata* e *Mico argentatus.*

Para a coleta do material:

1. Lavar rapidamente o tecido com álcool 70%;
2. Transferir o tecido para um eppendorf contendo 1,5 mL de meio de cultura DMEM com 5% de soro fetal bovino, fungizon (anfotericina B, 2 µg/mL), estreptomicina (100 µg/mL) e penicilina (100 µg/mL);

No laboratório:

3. Lavar o tecido quatro vezes em solução salina estéril (como Hanks);
4. Transferir o tecido para uma pequena placa de petri e picotá-lo com bisturis estéreis;
5. Incubar o tecido picotado em solução de tripsina (0,05%) e EDTA (0,02%) por 30-45 minutos;
6. Adicionar alguns mililitros de meio de cultura com soro;
7. Centrifugar a amostra a 200 rpm por 10 minutos e remover o sobrenadante;
8. Ressuspender a amostra em 1-2 mL de colagenase (1 mg/mL);
9. Manter a amostra em colagenase a 37 °C por 4 horas;
10. Centrifugar a amostra por 10 minutos;
11. Remover o sobrenante;
12. Ressuspender em 2-3 mL de meio de cultura;

O meio de cultura é composto por meio DMEM (4,4 mL) suplementado com 10% de soro fetal bovino (500 µL) e 1% de antibióticos penicilina e estreptomicina (100 U/mL) O pH do meio é ajustado para 7,0, utilizando hepes (ácido) ou bicarbonato de sódio (básico).

13. Colocar a suspensão final em garrafinhas de cultura (pH tem que ser de aproximadamente 7,2);
14. Trocar o meio de cultura em intervalos de 4 dias até crescimento suficiente;
16. Quando o pico mitótico é atingindo, adicionar 5 µL de colcemida (10 µg/ml) à cultura;
17. Manter a garrafinha na estufa por uma hora;
18. Recolher o sobrenadante da garrafinha em um tubo Falcon de 15 mL. Em seguida, lavá-la com PBS e adicionar aproximadamente 500 µL de solução de tripsina (0,05%) e EDTA (0,02%);
19. Esperar as células se desprenderem da garrafinha e transferi-las para o tubo Falcon;
20. Seguir as etapas 5 a 15 do protocolo de obtenção de preparações cromossômicas a partir de cultura de linfócitos de sangue periférico.

6.1.2. Técnicas de coloração e bandeamento cromossômico

Coloração convencional

A coloração convencional é feita com o corante Giemsa e permite a contagem dos números diplóide e de braços autossômicos.

1. Corar as lâminas por 7 minutos, com uma solução de Giemsa 5% (diluído em tampão fosfato);
2. Lavar bem as lâminas com água destilada e deixar secar ao ar.

Padrões de bandeamento GTG

Foi utilizada a técnica descrita por Seabright (1971), com algumas modificações:

1. Mergulhar as lâminas em solução de tripsina (20%, diluída em PBS, pH 7,2, temperatura ambiente) por 60 segundos;
2. Transferir as lâminas para uma solução de Hanks BSS, temperatura ambiente, por 15 segundos;
3. Corar o material com Giemsa 2%, em tampão fosfato por 30 minutos;
4. Lavar com água destilada e deixar secar.

As lâminas devem ser analisadas quanto à correta digestão pela tripsina e o tempo ajustado de acordo com o aspecto dos cromossomos.

Padrões de bandeamento CBG

A heterocromatina constitutiva foi evidenciada pela aplicação da técnica descrita por Sumner (1972), com algumas modificações:

1. Incubar a lâmina com preparações cromossômicas em 2xSSC a 60 ºC por 15 minutos;
2. Incubar a lâmina em HCl 0,2 N à temperatura ambiente por 15 minutos;
3. Incubar a lâmina por 20-25 segundos em uma solução 5% de hidróxido de bário pré-aquecida a 60 °C em banho-maria;
4. Lavar a lâmina com água destilada, incubá-la rapidamente em HCl 1 N 60 ºC e lavar novamente com água destilada;
5. Incubar a lâmina por 30 minutos em 2xSSC pré-aquecido a 60 °C em banho-maria;
6. Lavar com água destilada e deixar secar;
7. Corar com Giemsa 5%, em tampão fosfato, por 50 minutos.

Coloração das regiões organizadoras de nucléolos (Ag-RONs)

A técnica de coloração das regiões organizadoras de nucléolos pela prata foi descrita por Howell e Black (1980):

1. Tratar a lâmina com HCl 0,2 N a 60 °C por 10 minutos;
2. Lavar com água destilada e secar bem;
3. Colocar uma gota de gelatina 0,5% e duas gotas de nitrato de prata 50% sobre a lâmina, misturando rapidamente. Recobrir com uma lamínula de vidro;
4. Incubar a 60 °C em câmara úmida (no banho-maria) por 2 a 5 minutos (até atingir coloração marrom-dourada);
5. Lavar em água destilada;
6. Corar por 30 segundos com Giemsa a 2%.

6.1.3. Hibridação *in situ* fluorescente

Pintura cromossômica

Os cromossomos individuais humanos utilizados como sondas para os experimentos de pintura cromossômica foram marcados por DOP-PCR com dig-11-dUTP ou biotin-16-dUTP (Roche Applied Science) e mantidos a -20°C até o uso.

DOP-PCR, para 50 µL de reação:

X µL de água destilada estéril (até o volume final de 50 µL)

5 µL 10X tampão da Taq

5 µL dNTPs (2:2:2:1; 0,2 mM dos nucleotídeos A, C e G e 0,1 mM do nucleotídeo T)

1,3 µL biotin-16-dUTP ou 1,0 µL dig-11-dUTP

1 µL primer 6MW 100 µM (Telenius e col. 1992)

6 µL $MgCl_2$ 25 mM

0,5 µL Taq polimerase

2 µL DNA

As reações incluíram uma desnaturação inicial de 94 ºC (3 minutos), seguida por 30 ciclos de desnaturação com temperatura de 94 ºC (1 minuto), temperatura de anelamento de 58 ºC (1 minuto) e elongação com temperatura de 72 ºC (1 minuto e 30 segundos) e uma extensão final a 72 ºC (7 minutos). Após a PCR, os fragmentos amplificados foram analisados após eletroforese (70V/100A) em gel de agarose 1%, imerso em tampão TAE 1x (Tris-ácido acético-EDTA) e corado com brometo de etídio.

Para o preparo da sonda, o produto de PCR marcado com biotina ou digoxigenina (10 µL) é precipitado, a -20 ºC, *overnight*, com 5 µL de Cot-1 DNA (1 mg/mL), 1,7 µL de acetato de sódio (3 M) e 56,1 µL de etanol absoluto. Em seguida, a sonda é centrifugada por 15 minutos (velocidade máxima da centrífuga), o sobrenadante é descartado e o DNA é lavado com 300 µL de etanol 70% gelado. O DNA é novamente centrifugado, o sobrenadante descartado, a pellet é seca em temperatura ambiente e ressuspendida em 14 µL de meio de hibridação.

Abaixo, seguem as etapas de hibridação:

1. Preparação das lâminas:
    a. Marcar a área de hibridação;
    b. Incubar as lâminas em um banho de ácido acético 50%, temperatura ambiente, por 10 minutos;
    c. Imergir as lâminas em um banho de etanol 100%, temperatura ambiente, por 3 minutos;

d. Secar as lâminas ao ar;
e. Incubar as lâminas em pepsina (100 µg/mL) diluída em HCL 0,01 N, temperatura ambiente, por 5 minutos;
f. Incubar as lâminas em dois banhos de PBS, temperatura ambiente, 5 minutos cada;
g. Desidratar as lâminas à temperatura ambiente em 3 banhos sucessivos de 3 minutos cada (70% etanol, 90% etanol e 100% etanol);
h. Secar as lâminas em estufa 37 ºC por uma hora.

2. Hibridação:
   a. Preparar 50 ml de solução de desnaturação (35 mL de formamida, 5 mL de 20xSSC e 10 mL de água destilada) e deixar aquecer em um banho a 65 ºC;
   b. Preparar a câmara úmida de hibridação - papel umedecido com água destilada - e deixar na estufa a 37 ºC;
   c. Utilizar lamínulas de vidro, do tamanho da área de hibridação e limpas em álcool;
   d. **Sondas:** Desnaturar as sondas por 20 minutos a 65 ºC e deixá-las por uma hora a duas horas a 37ºC (etapa chamada de *pre-annealing* - necessária para a supressão de sequências repetitivas pela hibridação com o Cot1);
   e. **Desnaturação das lâminas** (deve ser feita durante o *pre-annealing* das sondas):
      - Incubar as lâminas por 1 minuto e 30 segundos na solução de desnaturação a 65 ºC;
      - Imergir as lâminas em banhos de 3 minutos cada em álcool 70%, 90% e 100%, temperatura ambiente;
      - Secar as lâminas ao ar;
      - Pipetar as sondas na área de hibridação, cobrir com a lamínula e aplicar a cola selante;
   f. Manter as lâminas com as sondas na câmara de hibridação a 37 ºC por pelo menos três dias;

3. Lavagem e imunodetecção:
   a. Após a hibridação, lavar a lâmina em dois banhos de 2xSSC a 42 ºC por 10 minutos cada. A temperatura, o número de banhos e a duração de cada um podem ser alterados conforme o resultado observado ao microscópio;

b. Imergir a lâmina em PBT (0,1% de Tween 20 em PBS) por 5 minutos à temperatura ambiente;

c. Colocar 100 µl de antidigoxigenina conjugada com rodamina e/ou avidina acoplada a FITC (Roche Applied Science) (1:100 em PBT) sobre a área de hibridação, recobri-la com uma lamínula de plástico limpa e manter numa câmara úmida em estufa a 37 ºC por 45 minutos;

d. Incubar a lâmina em dois banhos de PBT de 5 minutos cada à 42 ºC;

e. Lavar a lâmina com jatos de PBS e montar a lâmina com 15 µl de uma solução de DAPI (0,8 ng/µL) em Slowfade (Invitrogen).

Hibridação *in situ* fluorescente com sonda telomérica

Sequências teloméricas $(TTAGGG)_4$ foram sintetizadas com uma molécula de biotina na extremidade (Invitrogen). As condições de hibridação foram similares às descritas para os experimentos de pintura cromossômica, com poucas modificações:

1. Aplicar o mix de hibridação, constituído por 1040 ng de sonda em 50% formamida/2xSSC, sem desnaturá-lo, às preparações cromossômicas desnaturadas;
2. Manter as lâminas com a sonda em uma câmara de hibridação a 37 ºC, *overnight*;
3. Proceder à lavagem e imunodetecção com neutravidina conjugada com rodamina (1:100; Roche Applied Science). Após a imunodetecação e os dois banhos de PBT, corar as lâminas com uma solução de DAPI (0,8 ng/µL) em Slowfade (Invitrogen).

Amplificação dos DNAs satélites por PCR (*Polymerase Chain Reaction*)

Os DNAs satélites alfa e MarmoSAT foram amplificados por PCR a partir de DNA genômico extraído de *Aotus infulatus, Callithrix penicillata, Callithrix geoffroyi, Callimico goeldii, Cebuella pygmaea* e *Mico argentatus*. As reações de PCR continham 1µL de cada primer (*forward* e *reverve*, 10µM), 2,5 µL de dNTP (2 mM de cada nucleotídeo), 50 mM de $MgCl_2$, tampão de reação, 1x 0,5 U de Taq DNA polimerase (Promega), 1 µL (aproximadamente 50 ng) de DNA genômico e água ultrapura para um volume final de 25 µL. As reações seguiram as seguintes condições: desnaturação inicial com temperatura de 94 ºC (3 minutos); seguida de 30 ciclos de desnaturação com temperatura de 94 ºC (1 minuto), temperatura de anelamento de 55 ºC (1 minuto), elongação com temperatura de 72ºC (1 minuto); uma extensão final a 72ºC (10

minutos). Os produtos de PCR obtidos foram analisados após eletroforese (70V/100A) em gel de agarose 1%, imerso em tampão TAE 1x (Tris-ácido acético-EDTA) e corado com brometo de etídio. Os produtos de amplificação foram visualizados em transluminador sob luz ultravioleta e os pesos moleculares dos fragmentos amplificados foram estimados através da comparação com o marcador de peso molecular (DNA Ladder, Promega). Posteriormente, os fragmentos de interesse foram eluídos, clonados e sequenciados ou marcados e utilizados como sonda em experimentos de FISH.

Eluição e ligação dos DNAs satélites

Para a eluição dos fragmentos de interesse foi utilizado o kit Wizard SV Gel and PCR Clean-up System (Promega). O DNA das bandas eluídas foi checado através de eletroforese em gel de agarose e posteriormente quantificado no Nanodrop.

A ligação do DNA eluído ao plasmídeo foi feita com o kit pGEM-T Easy Vector (Promega), de acordo com o protocolo do fabricante.

Clonagem dos DNAs satélites

A clonagem dos DNAs satélties foi realizada como descrito abaixo:

1. Centrifugar os tubos contendo as reações de ligação;
2. Adicionar 2 µL da reação de ligação para cada amostra em tubo Falcon de 15 mL;
3. Retirar o tubo com células eletrocompetentes (Phoneutria) do freezer -80ºC e colocar em gelo por 5 minutos. Homogeneizar suavemente;
4. Transferir 25 µL de células eletrocompetentes para cada amostra, utilizando ponteiras refrigeradas;
5. Transferir as amostras para as cubetas e proceder a eletroporação;
6. Adicionar à cubeta 1000 µL de meio LB líquido sem ampicilina e transferir as amostras para um tubo Falcon;
7. Incubar por 60 minutos em termoshaker a 37°C e 230 rpm;
8. Plaquear 100 µL de cada cultura em placa de LB ágar com X-gal (80 µg/mL), IPTG (0,5 mM) e ampicilina (100 µg/mL);
9. Incubar em estufa por 16-24 horas a 37°C;

10. Selecionar as colônias de coloração branca e transferir cada uma delas para diferentes tubos Falcon de 15 mL contendo 10 mL de LB líquido com ampicilina;
11. Incubar as amostras *overnight* em termoshaker a 37°C e 250 rpm.

A presença de insertos de interesse nos plasmídeos recombinantes foi confirmada por PCR (primers M13 *forward* e M13 *reverse*) e os clones positivos foram estocados em glicerina 50% e armazenados a -80ºC. Os plasmídeos foram posteriormente extraídos das bactérias usando o kit PureYield Plasmid Miniprep System (Promega), conforme instruções do fabricante.

Sequenciamento

Os insertos de interesse nos plasmídeos recombinantes foram sequenciados por eletroforese capilar em aparelho ABI3130, utilizando-se os polímeros POP7 e BigDye v3.1 (Myleus Biotechonology). A confirmação de que os clones sequenciados realmente continham sequências dos DNAs satélites desejados foi feita através de buscas por similaridade entre sequências depositadas no GenBank com o programa BLAST (Basic Local Alignment Search Tool), disponível no website http://www.ncbi.nlm.nih.gov/blast/, ou através de alinhamento das sequências obtidas com sequências consensus obtidas durante o estudo.

FISH com sondas de DNAs satélites

Os DNAs satélites utilizados como sondas foram marcados por *nick translation* com digoxigenina- ou biotina-11-dUTP com os kits DIG- ou Biotin-Nick Translation Mix (Roche Applied Science), respectivamente e mantidos a -20°C até o uso. As etapas de preparação das lâminas foram as mesmas descritas acima, para pintura cromossômica. Cem nanogramas de DNA marcado foi ressuspendido em meio de hibridação e desnaturado à 98 ºC, por 10 minutos, e aplicado aos cromossomos desnaturados. As lâminas com as sondas foram mantidas na câmara de hibridação a 37ºC, *overnight*. A lavagem das lâminas foi feita com 2xSCC, 37 ºC, por 5 minutos. Em seguida, as lâminas foram incubadas em PBT, 37 ºC, por 5 minutos, e feita a imunodetecção com antidigoxigenina conjugada com FITC (Roche Applied Science). Após a imunodetecação e os dois banhos de PBT, as lâminas foram coradas com uma solução de DAPI (0,8 ng/µL) em Slowfade (Invitrogen).