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

DISSERTAÇÃO DE MESTRADO

# Caracterização de DNAs Satélites na Preguiça-de-dois-dedos (*Choloepus*, Magalonychidae, Xenarthra)

ORIENTADA: Radarane Santos Sena

ORIENTADORA: Marta Svartman

CO-ORIENTADOR: Gustavo Campos e Silva Kuhn

BELO HORIZONTE

JULHO – 2019

Radarane Santos Sena

# Caracterização de DNAs Satélites na Preguiça-de-dois-dedos (*Choloepus*, Magalonychidae, Xenarthra)

Dissertação apresentada ao programa de Pós-Graduação em Genética da Universidade Federal de Minas Gerais como pré-requisito obrigatório para obtenção do título de Mestre em Genética, área de concentração "Genética Evolutiva e de Populações".

Orientadora: Dra. Marta Svartman

Coorientador: Dr. Gustavo Campos e Silva Kuhn

BELO HORIZONTE

JULHO - 2019

043 Sena, Radarane Santos.
Caracterização de DNAs Satélites na Preguiça-de-dois dedos (Choloepus, Magalonychidae, Xenarthra) [manuscrito] / Radarane Santos Sena. – 2019.

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

Orientadora: Dra. Marta Svartman. Coorientador: Dr. Gustavo Campos e Silva Kuhn.
Dissertação (mestrado) – Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas. Programa de Pós-Graduação em Genética.

1. Genética de populações. 2. Bichos-Preguiça. 3. Sequências Repetitivas de Ácido Nucleico. I. Svartman, Marta. II. Kuhn, Gustavo Campos e Silva. III. Universidade Federal de Minas Gerais. Instituto de Ciências. Biológicas. IV. Título

CDU: 575

Ficha catalográfica elaborada pela Biblioteca do Instituto de Ciências Biológicas da UFMG

Pós-Graduação em Genética
Departamento de Biologia Geral, ICB
Universidade Federal de Minas Gerais
Av. Antônio Carlos, 6627 - C.P. 486 - Pampulha - 31270-901 - Belo Horizonte - MG
e-mail: pg-gen@icb.ufmg.br FAX: (+31) - 3409-2570

# "Caracterização de DNAs Satélites na Preguiça-de-dois-dedos (Choloepus, Megalonychidae, Xenarthra)"

**Radarane Santos Sena**

Dissertação aprovada pela banca examinadora constituída pelos Professores:

Marta Svartman - Orientadora
UFMG

Maria Bernadete Lovato
UFMG

Rafael Félix de Magalhães
UFMG

Belo Horizonte, 31 de julho de 2019.

Dedico

Aos meus pais Denise e Jover que com muito apoio e carinho não mediram esforços para que eu chegasse até mais esta etapa da minha vida.

## AGRADECIMENTOS

Na realização do presente trabalho, contei com o apoio direto ou indireto de muitas pessoas queridas às quais sou imensamente grata:

À minha querida orientadora, professora Marta Svartman, pelos ensinamentos, pela imensa paciência, convivência, amizade e pelos muitos puxões de orelha. Sou muito grata pela oportunidade que você me deu de desenvolver esse projeto sob sua orientação, pela preocupação com a minha formação profissional e com o meu crescimento pessoal.

Ao professor Gustavo Campos e Silva Kuhn, por todos os ensinamentos, paciência, atenção e ajuda.

À Naiara pela amizade e por todos os ensinamentos ao longo desses anos de LCEv.

À Mirela, por todos os ensinamentos, salvamentos, incentivos, pela amizade, carinho e puxões de orelha. Sempre pude contar com sua ajuda e sou imensamente grata.

Ao Pedro pela paciência em compartilhar seus conhecimentos, amizade e incentivos.

Ao Guilherme por seus ensinamentos, carinho, amizade e paciência.

À Alice e seus queridos pais, Jussara e Sinval, pela amizade, carinho e incentivos.

Aos queridos amigos de laboratório que deixam a vida mais leve e divertida, Braúlio, Ana, Rafaella, Erick e Lucas.

Aos queridos Thaís e André pelos ensinamentos, pela amizade e carinho.

À Daniela, minha amiga da Pós-graduação pala amizade e parceria nas disciplinas.

Aos amigos do 2140, em especial Juma, Mariane e Aldo, pelos bons papos e risadas que deixam nossas idas e vidas da UFMG mais divertidas.

Aos técnicos Daniel e Marlene pela convivência, disposição em ajudar, com paciência, bom humor e gentileza.

À equipe dos laboratórios de Genética de Microrganismos, Genética de Populações, de Biodiversidade e Evolução Molecular, de Genética Humana e Médica e do Laboratório de Tecnologia Genética que sempre estiveram de portas abertas para que eu pudesse realizar meus experimentos e tirar dúvidas.

À Fundação de parques municipais e ZooBotânica, em especial à Valéria por nos enviar preciosas amostras.

Às queridas Raíssa e Daniela que com muita competência e gentileza nos ajudam.

Ao programa de Pós-Graduação em Genética da UFMG.

À CAPES pela bolsa de mestrado, à FAMIG e ao CNPq pelo incentivo e investimento em pesquisa.

Aos meus primos e primas, tios e tias, sobrinhos emprestados, meus avós queridos, por todo amor e incentivo.

Às minhas queridas amigas da graduação: Daniela, Letícia e Débora, apesar da distância sempre quando nos encontramos é como se o tempo não tivesse passado. Sempre juntas!!

Ao meu querido irmão, Hassan, pelo amor, amizade e torcida!

Aos meus amados pais que são meus maiores incentivadores, que nunca mediram esforços para que eu pudesse estudar e realizar nossos sonhos, porque sei que os meus sonhos fazem parte dos de vocês. Tudo que conquistei e ainda conquistarei será sempre dedicado a vocês.

# SUMÁRIO

## LISTA DE FIGURAS



**CAPÍTULO 1**



## LISTA DE TABELAS

## LISTA DE ABREVIATURAS

2n – Número diploide

Ag-RONs – Marcações das regiões organizadoras de nucléolos

BLAST – Basic Local Alignment Search Tool

bp – base pair

CBG – Padrões de bandas obtidas por bário e coradas com giemsa

CENP – Proteína centromérica, C*entromeric protein*

Cyt-b – Citocromo-b

DAPI – 4',6-diamidino-2-fenilindole

DNA – Ácido desoxirribonucleico

ECD – Domínio evolutivo conservado, *Evolutionary conserved domain*

Fig – Figura

FISH – Hibridação *in situ* fluorescente, *Fluorescent in situ Hibridization*

FN – Número fundamental

GTG – Padrões de bandas obtidas por tripsina e giemsa

Kb – Kilobases

LINE – Elementos nucleares interdispersos longos, *Long interspersed nuclear element*

Mya – Million years ago

NCBI – National Center for Biotechnology Information

NMDS – Escalonamento multidimensional não métrico, *Non-metric Multimensional Scaling*

pb – Pares de bases

PCR – Reação em cadeia da polimerase, *Polymerase chain reaction*

RNA – Ácido ribonucleico

satDNA – DNA satélite, *satellite DNA*

SD – Desvio padrão, *Standard deviation*

SINE – Elementos nucleares interdispersos curtos, *Short interspersed nuclear element*

**RESUMO**

A família Megalonychidae possui um único gênero de preguiças-de-dois-dedos (*Choloepus*), dividido em duas espécies: *C. didactylus* e *C. hoffmanni*. Neste trabalho identificamos os DNAs satélites (DNAsat) mais abundantes no genoma de *C. hoffmanni*. O DNAsat mais abundante, SATCHO1, corresponde a 2,6% do genoma e é formado por sequências de 117 pb que apresentam baixa divergência entre si (~2,5%). Já o segundo DNAsat mais abundante, SATCHO2, corresponde a 0,23% do genoma de *C. hoffmanii* e se destaca pelo tamanho incomum de suas cópias que têm ~2.292 pb.

Experimentos de hibridação *in situ* fluorescente (FISH) nos cromossomos de *C. didactylus* (2n=51) mostraram que SATCHO1 se localiza nas regiões centroméricas de todos os cromossomos, exceto o X. Já o SATCHO2 foi mapeado nas regiões distais dos braços curtos de 17 autossomos.

A presença de sequências do SATCHO1 foi verificada por PCR em outras duas espécies de Xenarthra: *Myrmecophaga tridactyla* e *Bradypus variegatus*. Entretanto, o mapeamento cromossômico em *M. tridactyla* não revelou marcações nos cromossomos desta espécie, sugerindo que SATCHO1 apresenta padrão de DNAsat restrito ao gênero *Choloepus*.

Nossos dados sugerem que a análise de DNAs repetitivos em Xenarthra devem trazer dados importantes para um melhor conhecimento sobre os genomas de mamíferos.

**ABSTRACT**

*Choloepus*, the single genus of the Megalonychidae family, is divided into two two-toed sloths species: *C. didactylus* and *C. hoffmanni*. In this work we identified the most abundant satellite DNAs (satDNAs) in the sequenced genome of *C. hoffmanni*. SATCHO1, the most abundant satDNA, corresponds to 2.6% of the genome and is composed by 117 pb sequences with low divergence among them (~2.5%). The second satDNA, SATCHO2, corresponds to 0.23% of the *C. hoffmanii* genome and has ~2,292 bp copies, an uncommonly large size. *In situ* fluorescent hibridization (FISH) in the chromosomes of *C. didactylus* (2n=51) allowed us to map SATCHO1 in the centromeric regions of all chromosomes, except the X. SATCHO2 was mapped to the distal regions of the short arms of 17 autosomes. PCR experiments indicated the presence of SATCHO1 sequences in two other Xenarthra species: *Myrmecophaga tridactyla* and *Bradypus variegatus*. Nevertheless, no labeling was produced after FISH in *M. tridactyla* chromosomes, suggesting that SATCHO1 has a satDNA pattern restricted to the genus *Choloepus*.

Our results suggest that the analysis of repetitive DNAs in Xenarthra shall reveal important data for a better understanding of the mammalian genomes.

## 1. INTRODUÇÃO

Os Eutérios representam a grande maioria dos mamíferos, com uma enorme variedade de espécies, nichos ecológicos e morfologias corporais (O'Leary et al., 2013; Meredith et al., 2011). Evidências indicam que esta subclasse se diversificou antes do limite Mesozoico-Cenozoico, há cerca de 66 milhões de anos (Madson et al., 2001; Murphy et al., 2001; O'Leary et al., 2013). Atualmente, é subdividida em 18 ordens e quatro clados supraordinais: Euarchontoglires, Laurasiatheria (juntos formando o clado Boreoeutheria), Afrotheria e Xenarthra (Novacek et al., 2002; Murphy et al., 2007).

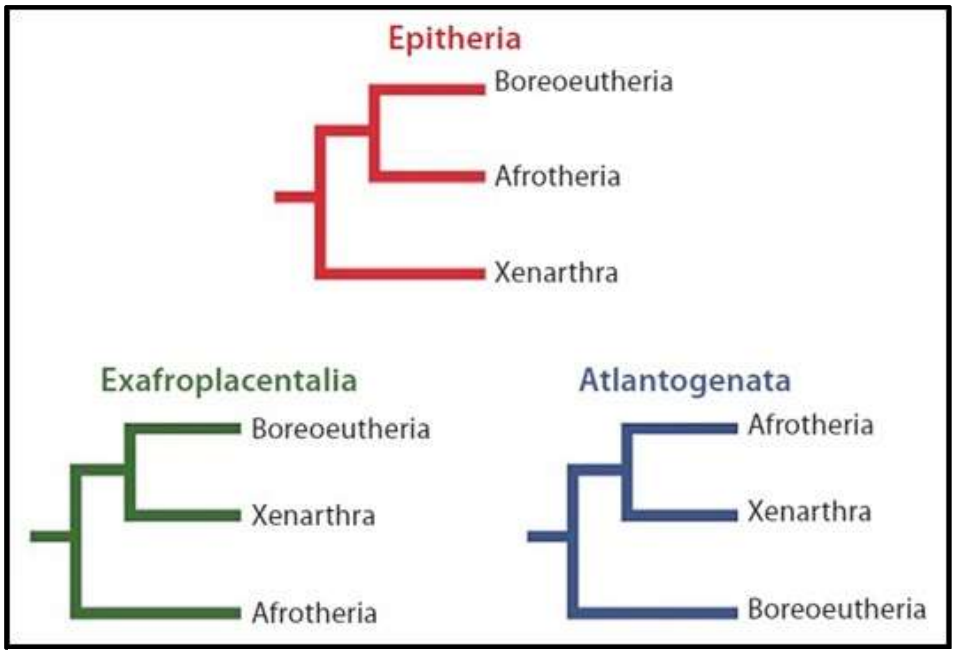



**Figura 1.** Três hipóteses atuais para o grupo basal dos Eutheria. Fonte: Svartman (2012).

Muito se tem discutido sobre a filogenia dos eutérios, especialmente sobre qual seria seu grupo mais basal. Três hipóteses se destacam: Epitheria, que coloca Xenarthra como grupo irmão de todos os outros Eutheria; Exafroplacentalia, que posiciona Afrotheria como o grupo mais basal; e Atlantogenata, em que Afrotheria e Xenarthra juntos formam o grupo mais basal (Svartman, 2012; Foley et al., 2016) (Fig. 1). Estudos

recentes, usando dados moleculares e morfológicos, apontam que Atlantogenata, grupo que reuniria Afrotheria e Xenarthra, é o provável grupo-irmão dos demais clados de Eutheria (Foley et al., 2016).

### 1.1. Xenarthra

Os Xenarthra formam um clado monofilético bem estabelecido com base em análises de caracteres morfológicos e moleculares, com origem e diversificação inteiramente na América do Sul (Delsuc et al., 2004; Superina e Loughry, 2015; Moraes-Barros e Arteaga, 2015). Os padrões filogenéticos encontrados no grupo coincidem com eventos biogeográficos do continente americano (Delsuc et al., 2004; Murphy et al., 2007) e os tempos de divergência calculados por análises moleculares dos principais clados de Xenarthra coincidem com eventos de tectônica de placas e de divergências climáticas ocorridos nesta área (Moraes-Barros e Arteaga, 2015).

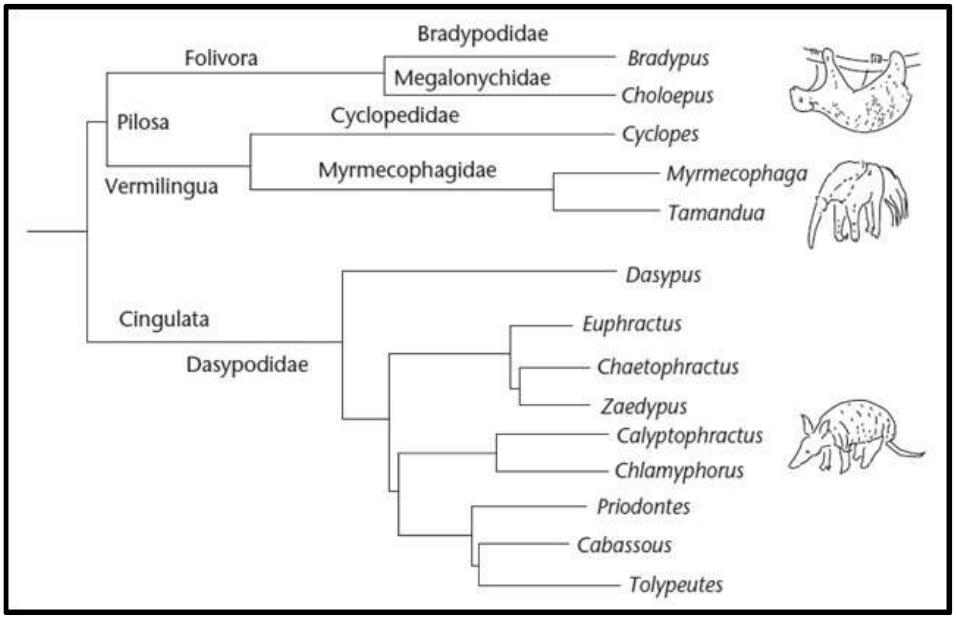



**Figura 2.** Filogenia dos gêneros viventes de Xenarthra. Fonte: Svartman (2012).

Esses animais ocupam diferentes hábitats, compreendendo desde organismos arborícolas a fossoriais, e apresentam especializações morfofisiológicas, como baixa temperatura corporal, dentição reduzida, testículos internalizados, carapaça, pelos e garras (Gaudin e Croft, 2015; Moraes-Barros e Arteaga, 2015). A superordem Xenarthra apresenta 31 espécies viventes divididas entre as ordens Pilosa – composta por tamanduás (Vermilíngua) e preguiças (Folívora) – e Cingulata, representada pelos tatus (Gaudin, 2003; Superina e Loughry, 2015) (Fig. 2).

Apesar de sua importância filogenética, dados sobre os Xenarthra são bastante escassos. A maioria dos trabalhos sobre o grupo foram realizados com tatus e a maioria trata sobre a história paleontológica do grupo. Estudos sobre as características biológicas e ecológicas desta superordem são pouco numerosos em comparação com os realizados com a maioria das outras superordens como, por exemplo, Euarchantoglires e Laurasiatheria, que têm como representantes primatas e morcegos, respectivamente (Superina e Loughry, 2015).

### 1.1.1. Família Megalonychidae

Dentre os Xenarthra, um dos clados menos compreendidos taxonomicamente é a família Megalonychidae (Svartman, 2012). Essa família é composta por um único gênero vivente, dividido entre duas espécies: *Choloepus didactylus* e *C. hoffmanni* (Fig. 3), que têm sido diferenciadas principalmente por características morfológicas. *C. didactylus* apresenta pelagem marrom-acinzentada, com a face mais clara e o topo da cabeça e ombros mais escuros. Já *C. hoffmanni* é caracterizada por uma coloração bem mais clara e tamanho um pouco menor do que o de *C. didactylus* (Meritt, 1985; Wetzel, 1985; Nowak, 1999; Eisenberg e Redford, 1999). As duas espécies apresentam diferentes áreas de distribuição com pequenas regiões de sobreposição na floresta Amazônica (Plese e

Chiarelllo, 2014) (Fig. 3). *C. didactylus* ocorre na América do Sul, em áreas de floresta úmida e quente da região amazônica, bem como em porções de florestas em altitude da região andina (Fig. 3; Adam, 1999; Chiarelllo e Plese, 2014). Já *C. hoffmanni* encontra-se dividida em duas populações distintas distribuídas em áreas de florestas tropicais. Estas populações estão em planícies com altitudes superiores a 1000 metros acima do nível do mar, nas Américas Central e do Sul (Fig. 3; Hayssen, 2011; Plese e Chiarello, 2014).

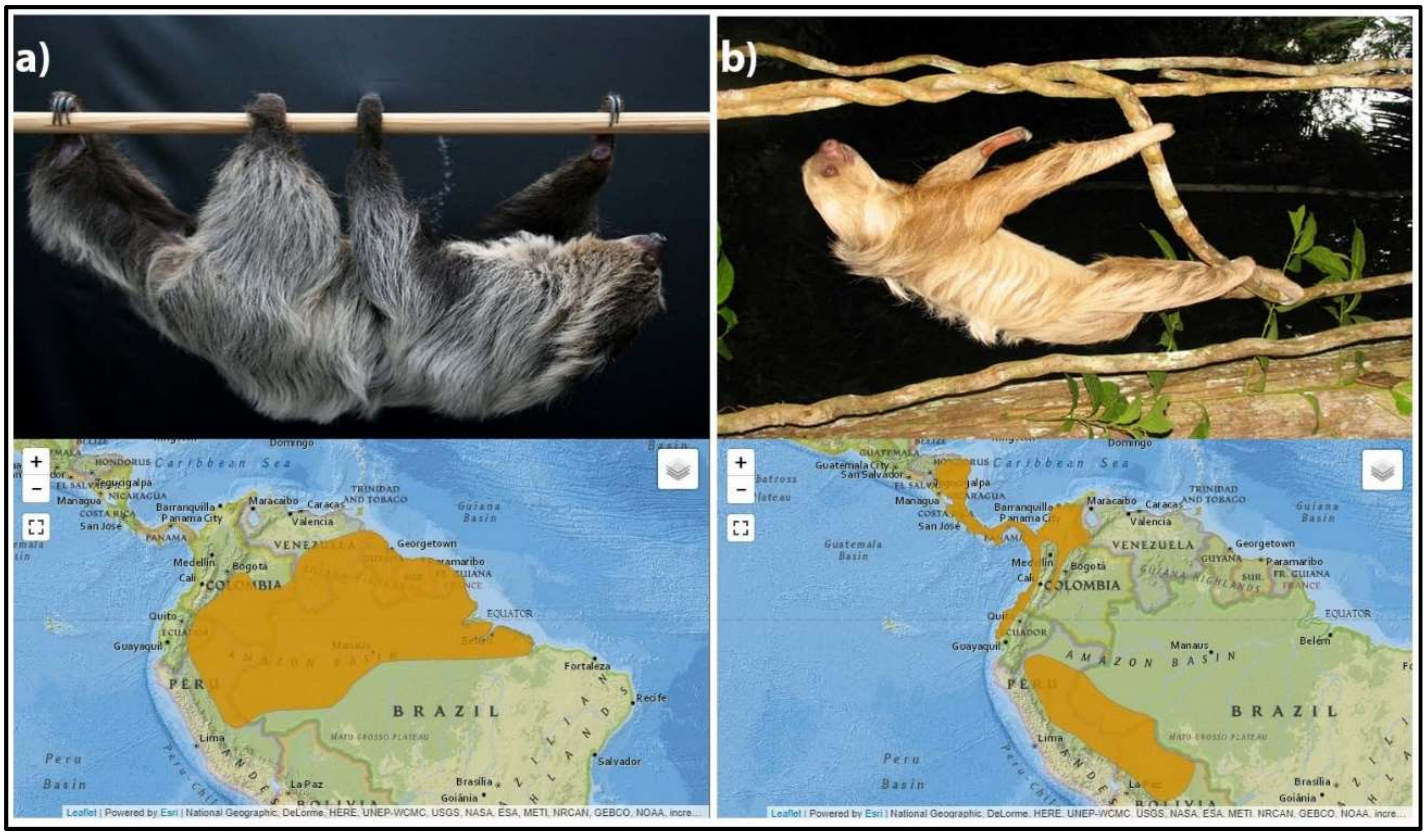



**Figura 3.** Imagens (acima) e distribuição geográfica (abaixo) de: (a) *C. didactylus* e (b) *C. hoffmanni*. Fonte: Adaptado de *The International Union for Conservation of Nature (IUCN) Red List of Threatened Species*, 2019.

## 1.2. Citogenética de Xenarthra.

Outra área bastante negligenciada sobre Xenarthra é a citogenética, já que a maior parte dos trabalhos estão concentrados na descrição básica dos cariótipos de algumas espécies, sem a aplicação de padrões de bandeamento (Svartman, 2012). A análise citogenética permite conhecer o conjunto cromossômico dos organismos e a localização dos genes nestes cromossomos. Através de técnicas citogenéticas clássicas e moleculares, é possível estudar comparativamente os cariótipos de diferentes taxas, levando à compreensão da evolução cariotípica e até mesmo filogenética dos grupos (Graphodatsky et al., 2011).

### 1.2.1. Citogenética de *Choloepus*

Números diploides bastante discrepantes foram encontrados nos poucos espécimes analisados de cada uma das espécies de *Choloepus*: *C. didactylus* apresentou variação de 2n=52-67 e *C. hoffmanni*, de 2n=49-53 (Corin-Frederic, 1969; Sonta et al., 1980; Jorge et al. 1985a; Dobigny et al., 2005; Benirschke, 2006; Svartman et al., 2006). Os poucos estudos citogenéticos realizados neste gênero revelaram um cenário complexo e confuso. Translocações entre o cromossomo Y e autossomos e fêmeas com apenas um cromossomo X foram descritos em indivíduos de ambas as espécies (Corin-Frederic, 1969; Sonta et al., 1980; Jorge et al., 1978, 1981, 1985a; Dobigny et al., 2005; Yu et al., 2012). Também foram descritos cromossomos ímpares, que foram classificados como cromossomos B em três espécimes de *C. hoffmanni* e um de *C. didactylus* (Jorge et al., 1978, 1985a; Benirschke, 2006) (Tabela 1).

**Tabela 1. Dados cromossômicos dos espécimes de *Choloepus* analisadas.**

| Espécies | Espécimes | Número Diploide | Bandeamento | Origem | Referência |
|---|---|---|---|---|---|
| *C. didactylus* | 1M e 1F | 2n=53 | GTG | Zoológico de Tóquio, origem desconhecida | Sonta et al. (1980) |
| | 1F | | | Origem desconhecida | Benirschke (2006) |
| | 1 F | | NA | Lincoln Park Zoo, Illinóis, origem desconhecida | Steiner et al. (2011) |
| | 1 M | 2n=59 | | Animal Kingdom Disney, Flórida, origem desconhecida | |
| | 1 M | 2n=64 | | Memphis Zoological Garden & Aquarium, origem Guiana | |
| | 1 F | 2n=64-66 | | | |
| | 1 F | 2n=66 | | San Diego Zoo, Califórnia, origem Guiana | |
| | 1 M | 2n=65-66 | | Busch Gardens, Flórida, origem desconhecida; | |
| | | | | Zoológico de San Diego, Califórnia, origem Guiana | |
| | 1 F | 2n=67 | | Animal Kingdom Disney, Flórida, origem desconhecida | |
| | 1 F | 2n=52 | NA | Manaus, Brasil | Jorge (1981) |
| | 2 F | 2n=53 | | Belém, Brasil | |
| | 1 M | | | Amazônia brasileira | |
| | 1 M e 1F | 2n=64 | | Manaus, Brasil | |
| | 1 M | 2n=65 | GTG | Zoológico de Budapeste, origem localidade desconhecida, Amazônia brasileira | Dobigny et al. (2005) |
| | 2M e 2F | 2n=65-66 | GTG | Sem informações | Yu et al. (2012) |
| *C. hoffmanni* | 4M e 6F | 2n=49 | NA | Panamá, Costa Rica e Equador | Corin-Frederic (1969) |
| | 3 M | 2n= 50 | NA | Busch Gardens, Flórida, origem desconhecida;<br>Woodland Park Zoo, Washington, origem desconhecida; | Steiner et al. (2011) |
| | 1M | | GTG | Zoológico de San Diego, origem desconhecida | Svartman et al. (2006) |
| | 1 M e 1F | | GTG, CBG | Zoológico de San Diego, pais coletados no Panamá e Colômbia | Jorge et al. (1978) |
| | 1 M | 2n=51 | NA | Lincoln Park Zoo, origem Panamá | Steiner et al. (2011) |
| | 2 F | 2n=51 | NA | Amazonas, Brasil | Jorge (1981) |
| | 2 F | 2n=51 | CBG | | Jorge et al. (1985a) |
| | 2 F | 2n=53 | | | |

M-macho; F-fêmea; 2n-número diploide; GTG-; CBG; NA- sem bandeamento descrito.

Padrões de bandeamento foram descritos em apenas poucos casos. As técnicas de bandeamento cromossômico são importantes por evidenciar detalhes dos cromossomos que permitem o correto pareamento dos homólogos (bandeamento GTG), a localização de blocos de heterocromatina constitutiva ricos em elementos repetitivos (bandeamento CBG) e a identificação de regiões organizadoras de nucléolos ativas no genoma através da impregnação pelo nitrato de prata (Ag-RONs) (Kasahara, 2009). Apenas 15 espécimes de um total de 44 *Choloepus* analisados cariotipicamente tiveram algum padrão de bandas descrito. O bandeamento GTG foi descrito para nove exemplares de *C. didactylus*, cinco fêmeas e quatro machos, e em apenas dois exemplares de *C. hoffmanni*, uma fêmea e um macho. Já o bandeamento CBG foi realizado apenas em exemplares de *C. hoffmanni*, três fêmeas e um macho e nenhum exemplar de *Choloepus* teve suas Ag-RONs estudadas (Tabela 1).

A pintura cromossômica com sondas de cromossomos humanos individuais foi realizada em um espécime de *C. didactylus* com 2n=65 (Dobigny et al., 2005) e em outro de *C. hoffmanni* com 2n=50 (Svartman et al., 2006). Essa técnica de citogenética molecular é bastante útil para a compreensão das relações filogenéticas porque revela regiões cromossômicas conservadas entre espécies (Graphodatsky et al., 2011; Svartman, 2012).

## 1.3. DNAs Repetitivos

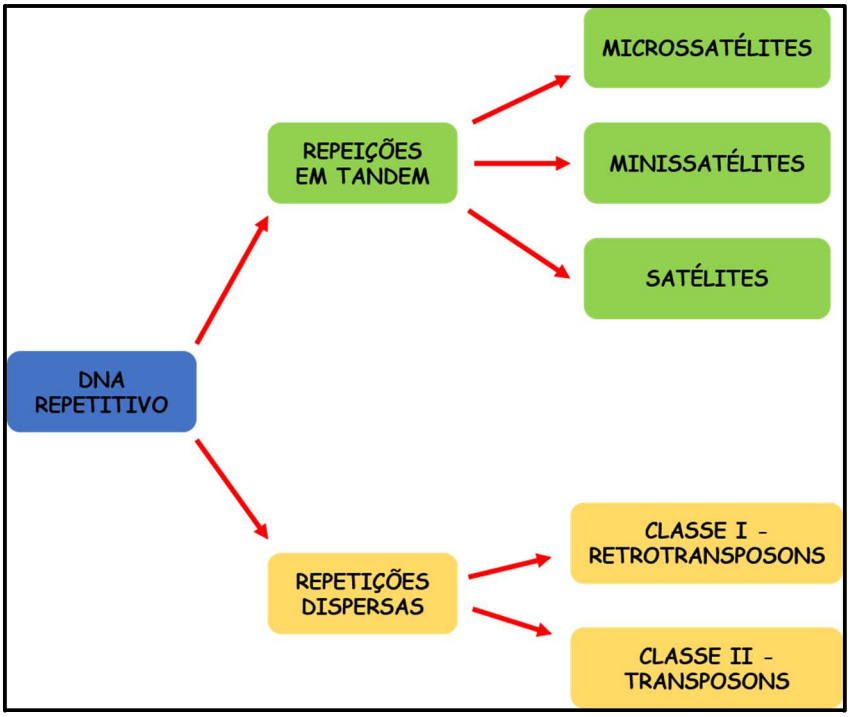



**Figura 4.** Esquema representativo dos principais tipos de sequências repetitivas do genoma dos eucariotos. Adaptação de Biscotti et al. (2015).

Xenarthra e Afrotheria possuem os maiores genomas dentre os diferentes clados de mamíferos, apresentando uma média superior a 4 pg (picogramas), enquanto a média dos demais grupos é de aproximadamente 3pg (Redi et al., 2005, 2007). Esse tamanho acima da média estaria relacionado a um acúmulo de sequências repetitivas ou DNAs repetitivos (Redi et al., 2005).

Os DNAs repetitivos representam uma porção significativa do genoma de eucariotos, algumas sequências correspondem a mais de 50% do genoma em plantas e cerca de 45% do genoma humano (Garrido-Ramos, 2017). Eles são divididos em sequências dispersas no genoma, representados pelos elementos transponíveis, e as repetições em tandem (Fig. 4). As repetições em tandem são divididas em microssatélites, minissatélites e DNAs satélites (DNAsat) e esses três grupos diferem principalmente com relação à sua localização dentro dos genomas hospedeiros. Micro e minissatélites são

comumente encontrados em regiões eucromáticas e podem ser encontrados próximos à centrômeros e telômeros. Enquanto os DNAsat estão presentes normalmente em regiões de heterocromatina e são frequentemente associados à origem e manutenção destas regiões (Tautz, 1993; Charlesworth et al., 1994; Grewal e Jia, 2007). Os DNAsat também fazem parte de estruturas cromossômicas essenciais, como os centrômeros e telômeros (Lower e col., 2018). Sequências de DNAsat já foram descritas na maioria dos grupos de eutérios, sendo as mais abundantes geralmente associadas a regiões centroméricas (Melters e col., 2013). A origem, dispersão e evolução dos DNAsat estão relacionadas a mecanismos de crossing-over desigual, conversão gênica, transposição e inserção de formas extracromossômicas (Dover, 1982, 1986; Garrido-Ramos, 2017; Lower et al., 2018). Esses mecanismos estão relacionados com o processo de *Molecular Drive*, que é o responsável pela homogeneização das cópias nos genomas (Dover, 1982). Após a sua homogeneização nos genomas, essas sequências normalmente ficam bastante divergentes entre espécies e altamente similares dentro das espécies. Ou seja, as sequências de uma mesma família de elementos repetitivos tendem a evoluir em conjunto e esse fenômeno é denominado *Concerted Evolution* (Dover, 1986; Liao, 1999). Algumas sequências de DNAsat evoluem cerca de três vezes mais rapidamente que regiões intergênicas, podendo apresentar diferenças significativas, mesmo entre espécies próximas (Plohl et al., 2008; Lower et al., 2018). Desse modo, os DNAsat têm o potencial de serem utilizados como marcadores espécie-específicos (Garcia-Ramos, 2017; Lower et al., 2018).

### 1.3.1. DNAs Repetitivos em Xenarthra

Além da escassez de estudos cromossômicos, pesquisas sobre os DNAs repetitivos em Xenartra também estão restritas basicamente à identificação de elementos do tipo LINE (long interspersed nuclear element) e SINE (short interspersed nuclear element) em espécies como *Myrmecophaga tridactyla*, *C. hoffmanni*, *Bradypus tridactylus*, *Euphractus sexcinctus* e *Dasypus novemcinctus* (Waters et al., 2004; Nishihara et al., 2007; Bao e Jurka, 2010). Existe apenas um estudo relacionado à identificação e caracterização de um DNAsat em Xenarthra: Alkan et al. (2010), usando o algoritmo RepeatNet, identificaram um DNAsat com 173 pb no tatu *D. novemcinctus*, que mapearam por FISH nos centrômeros.

**Tabela 2. DNAs Repetitivos Identificados em Xenarthra.**

| DNA Repetitivo | Espécies | Referência |
|---|---|---|
| LINEs | *Choloepus hoffmanni* | Bao e Jurka, 2010 |
| | *Euphractus sexcinctus*<br>*Bradypus tridactylus*<br>*Tamandua tetradactyla* | Waters et al., 2004 |
| SINEs | *Myrmecophaga tridactyla* | Nishihara et al., 2007 |
| | *Dasypus novemcinctus* | Churakov et al., 2005 |
| DNASat | *Dasypus novemcinctus* | Alkan et al., 2010 |

Uma das razões para o número restrito de estudos sobre DNAs repetitivos de Xenarthra se deve ao fato de apenas três espécies do grupo terem sido sequenciadas em nível genômico: *D. novemcinctus*, *C. hoffmanni* e, mais recentemente, *C. didactylus*. Análises citogenômicas envolvendo a fração repetitiva em espécies de Xenarthra devem auxiliar na compreensão da sua evolução, fornecendo informações sobre relações filogenéticas entre táxons, incluindo espécies próximas, como é o caso de *C. hoffmanni* e *C. didactylus*.

Com o panorama citogenético confuso e complexo apresentado pelas espécies de *Choloepus*, informações sobre suas sequências repetitivas, em especial os DNAsat, podem ser úteis para solucionar problemas taxonômicos e filogenéticos. Neste trabalho estudamos o cariótipo de um exemplar de *Choloepus didactylus*, incluindo os padrões de bandeamento GTG e CBG, e identificamos e caracterizamos os DNAs satélites mais abundantes do genoma de *C. hoffmanni*, verificando sua presença em outros Xenarthra. Os resultados serão apresentados na forma de artigo.

## 2. OBJETIVOS

### 2.1. Objetivo Geral

Realizar análises citogenômicas, incluindo a identificação e caracterização de DNAsat, em *Choloepus*, a fim de contribuir para o conhecimento da evolução cromossômica e genômica do gênero e de Xenarthra em geral.

### 2.2. Objetivos Específicos

- Caracterizar o cariótipo de *Choloepus didactylus* após a aplicação dos padrões de bandeamento GTG e CBG;
- Identificar e caracterizar sequências de DNA satélite no genoma de *Choloepus hoffmanni*;
- Mapear as sequências dos DNAsat nos cromossomos de *C. didactylus* por hibridação *in situ* fluorescente (FISH);
- Investigar a presença das sequências de DNAsat de *Choloepus hoffmanni* em outras espécies de Xenarthra (*Bradypus variegatus* e *Myrmecophaga tridactyla*), cujo genoma não foi sequenciado.

## 3. CAPÍTULO 1

O seguinte manuscrito será submetido ao periódico Chromosome Research

**Characterization of Satellite DNAs in the *Choloepus* genus (Megalonychidae, Xenarthra)**

Radarane Santo Sena[1]; Pedro Heringer[1]; Mirela Pelizaro Valeri[1]; Valéria do Socorro Pereira[2]; Gustavo Campos e Silva Kuhn[1]; Marta Svartman[1]

[1]Laboratório de Citogenômica Evolutiva, Departamento de Genética, Ecologia e Evolução, Instituto de ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

[2]Fundação de Parques Municipais e Zoobotânica

*Correspondig author
E-mail: svartman@ufmg.br
Telephone number: 5531-34092612
Fax number: 5531-340925

**Abstract**

*Choloepus*, the single genus of the Megalonychidae family, is divided into the two-toed sloths species *C. didactylus* and *C. hoffmanni*. In this work, we identified the main satellite DNAs (satDNAs) in the sequenced genome of *C. hoffmanni*. SATCHO1, the most abundant satDNA, corresponds to ~2.6% of the genome and is composed of 117 pb tandem repeats sequences with low divergence among them (~2.5%). The second satDNA, SATCHO2, corresponds to ~0.23% of the *C. hoffmanii* genome and is composed by ~2,292 bp tandem repeats. *In situ* fluorescent hibridization (FISH) in the chromosomes of *C. didactylus* revelead that SATCHO1 is located in the centromeric region of all chromosomes, except the X. SATCHO2 was mapped to the distal regions of the short arms of 17 autosomes. PCR experiments indicates the presence of SATCHO1 sequences in two other Xenarthra species: *Myrmecophaga tridactyla* and *Bradypus variegatus*. Nevertheless, no labeling was produced after FISH in *M. tridactyla* chromosomes, suggesting that SATCHO1 has a satDNA pattern restricted to the genus *Choloepus*. Our results reveal interesting features of the repeated fraction in the genome of Choloepus and highlight the potential of these analyses to aid phylogenetic studies related to Xenarthra and mammalian genomes in general.

**Keywords:** Repetitive DNAs, Two-toed sloth, RepeatExplorer, FISH, Centromere

## Introduction

Xenarthra is a basal eutherian group which originated and diversified entirely in South America (Delsuc et al., 2004; Superina and Loughry, 2015; Moraes-Barros and Arteaga, 2015). With approximately 31 extant species this superorder is divided into two orders: Cingulata, represented by armadillos, and Pilosa, composed by anteaters (Vermilingua) and sloths (Folivora) (Delsuc and Douzery, 2008). Despite its importance as a placental basal group, Xenarthra is poorly studied. Information about the ecology, behavior, and genetics of Xenarthra species is essential to a better characterization of the group (Superina and Loughry, 2015).

Two-toed sloths are extant representatives of the Megalonychidade family, composed by the single living genus *Choloepus* (Elgelmann, 1985), which comprises two species: *Choloepus didactylus* and *C. hoffmanni*. Both species inhabit the tropical forests in South and Central America with a small overlap area in the Amazon forest. These two species have been differentiated mainly by morphological characters, such as pelage color (Adam, 1999; Heyssen, 2011), osteological differences (Wetzel, 1985), the mitochondrial *COI* and *Cyt-b* genes, and restriction enzyme assay (Moraes et al., 2002; Steiner et al., 2010).

Cytogenetic studies of *Choloepus* are mostly based on simple karyotypic descriptions without banding patterns. These studies revealed a complex and confuse karyotypic scenario. Both species showed diploid numbers (2n) with significant variation, especially *C. didactylus*. Karyotypes reported for this species had 2n varying from 52 to 67 chromosomes (Sonta, 1980; Jorge, 1981; Jorge et al., 1985a; Dobigny et al., 2005; Benirschke, 2006; Steiner et al., 2010; Yu et al., 2012), whereas *C. hoffmanni* showed 2n= 49 to 53 (Corin-Frederic, 1969; Jorge et al., 1977; Sonta, 1980; Jorge, 1981; Jorge et al.1985a; Svartman et al., 2006; Steiner et al., 2010). Both species presented translocations between the Y chromosome and different autosomes, X0 females, and

unpaired chromosomes described as B-chromosomes (Corin-Frederic, 1969; Jorge et al., 1985a; Dobigny et al., 2005; Benirschke, 2006; Yu et al., 2012). Banding patterns, which provide details on the chromosomes, were described for only 17 *Choloepus* specimens: GTG-banding for ten individuals, eight *C. didactylus* (Sonta et al., 1980; Dobigny et al., 2005; Bernirschke, 2006; Yu et al., 2012) and three *C. hoffmanni* (Svartman et al., 2006; Bernirschke, 2006) and CBG-banding for another six *C. hoffmanni* (Jorge, 1985a). Cross-species chromosome painting with human chromosome-specific probes was performed in one specimen of *C. didactylus* (Dobigny et al., 2005) and one *C. hoffmanni* (Svartman et al., 2006). *Choloepus didactylus* showed the two Xenarthran synapomorphic associations HSA 2/8 and 7/10 and *C. hoffmanni* presented a karyotype very similar to the putative ancestral karyotype with 2n=48 (Svartman, 2012).

Repetitive DNAs represent a significant fraction of eukaryote genome. Some elements, such as transposable elements, can correspond to more than 50% of the genome in plants and around 45% in humans (Garrido-Ramos, 2017). Another important class of repetitive DNAs is the satellite DNAs (satDNA), highly repetitive sequences organized *in tandem* and present in different eukaryote groups like plants, arthropods and in all eutherian genomes (Tautz, 1993). SatDNAs are the major components of the constitutive heterochromatin present in fundamental chromosome structures, such as centromeres and telomeres (Lower et al., 2018). SatDNA transcripts have been reported as an important component for the centromeric activity (Garrido-Ramos, 2017; Talbert and Henikoff, 2018; Escudeiro et al., 2019). SatDNAs have a high divergence rate and can evolve three times faster than intergenic regions, resulting in significant differences between sequences from closely related species (Garrido-Ramos, 2017). This rapid evolutionary rate has been explained as a consequence of mechanisms such as unequal crossing-over, gene conversion and slippage replication (Ruiz-Ruano et al., 2016). Due to their high

interspecific variation, satDNA sequences have the potential to be used as species-specific markers.

The repetitive DNA content in Xenarthra genomes is still poorly studied and restricted to the identification of retrotransposons families. LINEs (Long Interspersed Element) and SINEs (Small Interspersed Element) families have been described in six species (the sloths *Choloepus hoffmanni* and *Bradypus tridactylus* (Waters et al., 2004; Bao and Jurka et al., 2010), the armadillos *Dasypus novemcinctus* and *Euphractus sexcinctus* (Waters et al., 2004; Churakov et al., 2005), and the anteaters *Tamandua tetradactyla* and *Myrmecophaga tridactyla* (Waters et al., 2004; Nishihara et al., 2007). The only species to have a satDNA identified is the armadillo *Dasypus novemcinctus* (Alkan et al., 2010). The identification was performed by a computational method (Repeatnet) that found monomeres with ~ 173bp, whose sequence was checked by PCR from the *D. novemcinctus* genome. The same sequence was used as probe in FISH experiments, in which the centromeres of all chromosomes of this species were labeled (Alkan et al., 2010). The reduced number of studies is related to the small number of sequenced Xenarthra genomes, which are restricted to three species: *D. novemcinctus*, *C. hoffmanni*, and very recently *C. didactylus*.

In this work we used *in silico* analysis to identify satDNA sequences in the sequenced genome of *Choloepus hoffmanni*. Two new satDNAs were found, named SATCHO1 and SATCHO2, which were mapped in the chromosomes of *C. didactylus*. In addition, we report a new karyotype for *C. didactylus* (2n=51), including its GTG- and CBG-banding patterns.

## Materials and Methods

### Samples

Chromosome preparations and genomic DNAs were obtained from cultured fibroblasts of male individuals of *Choloepus didactylus* and *Myrmecophaga tridactyla*. Tissue and blood samples from both specimens were obtained from Fundação de Parques Municipais e Zoobotânica de Belo Horizonte. The *C. didactylus* individual came from Rondônia (unknown location) and the *M. tridactyla* was apprehended by IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) in Esmeraldas, Minas Gerais, but its origin is unknown. We also used DNA from a male *Bradypus variegatus* captured by Maria Olímpia Garcia Lopes in Teófilo Otoni, Minas Gerais.

Cell cultures and chromosome spreads were obtained according to Stanyon and Galeni (1991) and the genomic DNAs were obtained with the Wizard® Genomic Purification kit (Promega).

**Identification and Analysis of satDNA Sequences in the *C. hoffmanni* Genome**

In order to identify and characterize satDNA sequences in the *C. hoffmanni* genome we performed a graph-based clustering analysis of sequence reads using the pipeline RepeatExplorer2 (Novak et al., 2013). This pipeline identifies read similarities by comparing pairwise reads all-to-all before grouping them into clusters. The RepeatExplorer analysis was performed in a set of 789160 random sampled reads (~2.6% of genome coverage) from the *C. hoffmanni* genome (accession: SRX282195). The identified satDNA consensus sequences were used as queries in searches conducted on Repbase (Jurka et al., 2005) and GenBank (Benson et al. 2017) in order to detect similarities with previously described sequences. We also retrieved a sample of the satDNA sequences from the *C. hoffmanni* (accession: ABVD00000000.2) and *C. didactylus* (accession: PVKG000000000.1) assembled genomes available on GenBank using Blast searches (Altschul e col., 1990). The software DnaSP 6.12.03 (Rozas et al,

2017) was used to identify DNA polymorphisms and nucleotide diversity along with the satDNA sequences, by applying a window size of 10 bp (SATCHO1 and SATCHO2) and a step size of 1 bp for satcho1 and 5 bp for satcho2. Windows that exhibited more than 2 standard deviation (S.D.) from the average variability were considered.

**Sequence Analysis and Characterization of Centromeric SATCHO1**

We searched on the SATCHO1 monomer sequence for any putative CENP-B like box (CTTCGTTGGAAACGGGA) (Muro et al., 1992) using Muscle (Edgar, 2004) on Mega7 (Kumar et al., 2016). We also searched for dyad symmetries using the EMBOSS palindrome software (Rice et al., 2000) with length palindrome of 3bp and minimum gap between elements of 20 bp. We used the RNAfold web server to search for optimal secondary structure with minimum free energy on the SATCHO1 monomer sequence.

The pairwise evolutionary distance between SATCHO1 sequences from *C. hoffmanni* and *C. didactylus* was estimated using MEGA7. The values were used to obtain non-metric multidimensional scaling (NMDS) ordinations with the R package Vegan (Dixon, 2003), representing Euclidian distances in four dimensions. We used Rstudio1.1.442 (Boston, MA, USA) to conduct NMDS ordinates and plotting. We constructed a phylogeny of the sequences using the Neighbor-Joining method with 1000 replicates on the software MEGA7. The phylogenetic tree was edited using iTOL4.4.1 (Letunic and Bork, 2019).

**Molecular Analysis**

The satDNAs identified in the RepeatExplorer analysis were amplified by polymerase chain reaction (PCR) from genomic DNA from *C. didactylus* with the primers: SATCHO1-F (AGTTGTTTTTCAGCCCAGGG) and SATCHO1-R (CACGTGGGACTCTGCGAAAG); SATCHO2-F (TCTCACCCGGATCTGAACCT)

and SATCHO2-R (GGATACGGGGGTTTGAAGCA). The two sets of primers were designed from the consensus generated on RepeatExplorer. The thermocycling conditions were as follows: 95ºC-5 min, 30 cycles: 95ºC-1 min; 53.4ºC-1 min; 72ºC-1min; final elongation: 72ºC-10 min. The PCR products were extracted from a 1% agarose gel, purified with Wizard SV Gel and PCR Clean-up System kit (Promega) and cloned into a plasmid vector pGEM-T-Easy cloning kit (Promega). The recombinant plasmids were capillary sequenced with the ABI 3730 platform (Applied Biosystems). The sequences obtained have GenBank accession numbers: xxxx.

**Banding Patterns and Fluorescence *in situ* Hybridization (FISH)**

The GTG- and CBG-banding were performed according to Seabright (1971) and Sumner (1972), respectively. Fluorescence *in situ* hybridization (FISH) was performed using the cloned satDNA sequences as probes after they were labeled by nick-translation with digoxigenin-11-dUTP (DIG-Nick Translation mix, Roche Applied Science). The probes (~170ng in 50% formamide/2xSSC) were denatured for 10 min at 98ºC. Chromosomes were dehydrated in an ethanol series (70%, 90%, 100%) and denatured in 70% formamide/2xSSC for 2 min at 75ºC. Immunodetection was performed with anti-digoxigenin conjugated with rhodamine (Roche Applied Science) and the chromosomes were counterstained with DAPI in Slowfade (Invitrogen). Analysis and image acquisition were performed under a Zeiss Axioimager 2 epifluorescence microscope with the AxioVision (Zeiss) software.

**Searching the Identified satDNAs in Other Xenarthra Species**

In order to test the presence of *Choloepus* satDNAs in other Xenarthra species, we ran PCRs with the same set of primers and conditions used to amplify SATCHO1 and SATCHO2 with genomic DNAs from *Bradypus variegatus* and *M. tridactyla* and with the *C. didactylus* sample as positive control. PCR products from *B. variegatus* and *M.*

*tridactyla* were then cloned, sequenced (accession number: xxxx), and used as probes for FISH, under the same condition described above.

## Results

### Identification and *in silico* Analysis of satDNAs

The RepeatExplorer analysis gave an estimated repetitive DNA content of 14.37% in the *C. hoffmanni* genome. Long Interspersed Nuclear Elements (LINEs) represent approximately 10.69% of this species genome, being the most abundant class of repetitive element, followed by satDNAs, which represent ~2.86%. The second most abundant cluster identified is a new satDNA that represents ~2.6% of the *C. hoffmanni* genome, which we named SATCHO1. It has ~117 bp monomers, low levels of inter-repeat nucleotide variability (~3% on average) and AT content of ~59%.

We also identified a second new satDNA, which we named SATCHO2, that corresponds to ~0.23% of the *C. hoffmanni* genome. It has ~2,292 bp monomers, levels of inter-repeat nucleotide variability of ~24% on average, and AT content of ~68%. Although these satDNA sequences are abundant in the *C. hoffmanni* and *C. didactylus* genomes, we did not identify similar sequences in any other species on Repbase or Genbank.

The sliding window analysis of nucleotide variability of both satDNAs reveals the presence of conserved regions within the monomers (Fig.1).

### Phylogenetic and NMDS Analysis

In order to infer the evolutionary history of SATCHO1 copies from the *C. hoffmanni* and *C. didactylus* genomes, we performed a phylogenetic analysis using the Neighbor-Joining method. The phylogenetic tree shows that SATCHO1 copies from the two species are very similar and did not segregate into different branches (Fig.2a). We then used pairwise distance values of the sequences to generate NMDS ordinations

(Fig.2b), which also did not reveal any topological segregation between copies from both species.

**Chromosome Mapping SATCHO1 and SATCHO2**

Our *C. didactylus* individual presented a karyotype with a diploid number 2n=51 and fundamental number of autosome arms FN=62. The biarmed chromosomes correspond to the pairs 3, 15, 19, 21, 24, 25 and 22. This complement was not described before and was mounted according to the karyotype of *C. didactylus* with 2n=53 reported by Benirshke (2006). GTG-banding was essential for the correct pairing of the homologous chromosomes (Fig.3a), especially for the sex chromosomes, because it allowed the identification of a translocation between the Y chromosome and one homologue of pair 22. The CBG-banding revealed the presence of constitutive heterochromatin in the centromeric region of all chromosomes, except the X (Fig.3b).

FISH with the SATCHO1 probe produced labeling in the centromeric regions of all chromosomes, except the X (Fig.4a), overlapping with the constitutive heterochromatin revealed after CBG-banding (Fig.3b). SATCHO2 labeled the distal regions of the short arms of 17 chromosome (pairs 1, 5, 7, 9, 10, 12, 15, 20 and 22) (Fig.4b).

**Characterization of Centromeric SATCHO1**

In order to better characterize the centromeric satDNA SATCHO1 we searched for putative CENP-B box-like motifs within the sequence. We found a motif that shares 10 bp positions with the human CENP-B box (Supplementary Fig. 1) including some bases of the evolutionary conserved domain (ECD) box (NTTCGNNNANNCGGGN) (Stitou et al., 1999). This motif overlaps with the conserved region identified by DnaSP

analysis on the distal portion of SATCHO1 (Fig.1a). We also found some palindrome-like small regions with 3bp – 5bp length in SATCHO1. These dyad symmetries have the potential to form cruciform structures and their abundance tends to form spontaneous and stable non-B-form DNA (Supplementary Fig. 2). The RNAfold also showed that SATCHO1 displays secondary structures with low levels of free energy, also consistent with a stable structure (Supplementary Fig. 3). Spontaneous non-B-form DNA and low energy are characteristic of functional centromeres (Kasinathan and Henikoff, 2018). These results indicate that SATCHO1 contains some of the structural hallmarks of a functional centromeric sequence.

**SATCHO1 and SATCHO2 in Other Xenarthra**

In order to verify if the satDNAs identified in *Choloepus* are present outside the genus we performed PCR reactions with the SATCHO1-F, SATCHO1-R, SATCHO2-F e SATCHO2-R primers with genomic DNAs of the Giant Anteater *Myrmecophaga tridactyla* and the three-toed sloth *Bradypus variegatus*. The SATCHO1 sequence was amplified from both species (Supplementary Fig. 4), which was confirmed by cloning and sequencing. The sequenced copies from *M. tridactyla* presented ~4% of divergence from *Choloepus* SATCHO1, whereas the copies from *B. variegatus* showed a higher divergence (~11%, on average). FISH with the SATCHO1 probe in *M. tridactyla* chromosomes did not produce any signals, suggesting that, although present in this species, this sequence is not abundant and does not display a satDNA pattern. We have no chromosome preparations for *B. variegatus* to perform FISH experiments. The SATCHO2 sequence did not amplify by PCR with the genomic DNAs of *M. tridactyla* and *B. variegatus* (Supplementary Fig. 5).

## Discussion

### Centromeric SATCHO1 Has Features Related to Functional Centromeres

We identified two satDNAs, SATCHO1 and SATCHO2, in the *C. hoffmanni* genome. SATCHO1 is the most abundant satellite in this genome and hybridized in the centromeric regions of all chromosomes, except the X (Fig. 4a), a pattern that coincided with the constitutive heterochromatin revealed by CBG-banding (Fig. 3b). Melters et al. (2013) suggested that the most abundant tandem repeat sequences in the genomes likely correspond to the ones which are highly predominant at the centromeres of plants and animals, a pattern observed for SATCHO1.

Centromere identity may be templated by DNA through the recruiting of sequence-specific DNA binding proteins and/or by recognition of a sequence structure feature such as DNA secondary structure (Kasinathan and Henikoff, 2018). Centromere DNA has been reported adopting non-B-form structures such as R-loops, hairpins, triplex and i-motifs in different organisms, including humans (Zhu et al., 1996; Ohno et al., 2002; Jonstrup et al., 2008; Garavis et al., 2015; Aze et al., 2016; Kabeche et al., 2017). In primates and budding yeast, centromeres with enrichment of DNA dyad symmetries, which may adopt non-B-form conformations (Koch, 2000; Catania et al., 2015), were also observed, suggesting that such palindrome enrichment may represent a centromere identity (Kasinathan and Henikoff, 2018).

Most eukaryotes have their centromere essentially composed by satDNAs (Plohl et al., 2012). SatDNA arrays are comprised of sequences that are very similar to each other and whose variation is caused by mutations and are usually spread by the biased process of molecular drive (Dover, 1986). But some satDNAs have conserved motifs that may be under positive selection. Such conservation may be caused by a functional constraint induced by a possible interaction between these motifs and DNA binding proteins (Pezer et al., 2012; Garrido-Ramos, 2017). SatDNA transcripts have been also

shown to contribute to kinetochore assembly and cell division (Wong et al., 2007; Rosic et al., 2014; Grenfell et al., 2017; Garrido-Ramos, 2017).

The centromeric SATCHO1 exhibited some characteristics that suggest that it may have some function in centromere identity. Analysis of SATCHO1 copies revealed two conserved portions along its sequence. One of them is a motif that shares 10 bp with the human CENP-B box and is present in the in *C. hoffmanni* and *C. didactylus* genomes.

CENP-B and the CENP-B box are quite conserved in mammals. The CENP-B box is present in most mammalian centromeric satDNA sequences and anchors the CENP-B protein, which plays an important role in the kinetochore structuring by helping the assembly of the CENP-A protein (Garrido-Ramos, 2017). Although its sequence is conserved in mammals, the CENP-B box is not essential for CENP-A assembly (Goldberg et al., 1996; Kasinathan and Henikoff, 2018).

The SATCHO1 sequence has an enrichment of dyad symmetries. Such structures have been related with the formation of DNA secondary or tertiary structures at the centromere, or DNA non-B-form, which have been considered necessary for functional interactions of the centromere (Plohl et al., 2012; Kasinathan and Henikoff, 2018). Centromeric proteins like CENP-A tend to better recognize the centromeres that adopt non-B-form DNA (Kasinathan and Henikoff, 2018). We also searched for possible secondary structures with minimum free energy on the SATCHO1 sequence because satellites with such structures also tend to adopt non-B-form DNA (Kasinathan and Henikoff, 2018) and we found a possible stable secondary structure with low minimum free energy (Supplementary Fig.2). Thus, our results suggest that SATCHO1 may contribute to the centromere adoption of non-B-form DNA, a hallmark of functional centromeres, and to the binding of centromeric proteins to the centromere.

**SATCHO2 is an Uncommonly Large SatDNA**

SATCHO2 is located on the distal portion of 17 autosomes (Fig. 4b) and is constituted by a large ~2,292 bp sequence, an uncommon monomer length. Most satDNAs identified in plants and animals show monomer lengths between 150-180 bp and 300-360 bp (Schmidt and Heslop-Harrison, 1998; Henikoff et al., 2001; Plohl et al., 2008). There are a few studies describing satDNAs composed by repetitions with more than 1,000 bp, such as the primate Cap-A with ~1,500 bp (Fanning et al., 1993), the common Cetacean satDNA family composed by monomers with ~1,760 bp (Árnason et al., 1992), the Bovidae sat I and sat II DNA families, whose satellites have monomers with different lengths that varies from ~800 bp to ~1,700 bp (Kopecna et al., 2014), and the banana (*Musa* ssp.) CL18 satellite with a ~2kb monomer unit (Hřibová et al., 2010). Those satDNAs hybridized on distal or centromeric chromosome regions. Each satellite showed a specific location, some in all chromosomes, others in a few specific pairs. Such large satellites still require further analyses in order to better understand their evolution patterns and how they are maintained on their host genomes at such large lengths.

***Choloepus* SatDNAs and Species Differentiation**

Phylogenetic analysis of the *C. hoffmanni* and *C. didactylus* SATCHO1 copies by Neighbor-Joining and NMDS revealed that they did not segregate into different branches. This suggests that these species share a recent common ancestor, they went through a recent process of hybridization, and/or that the SATCHO1 sequences are conserved because they play an important role in these genomes. Molecular data showed that the estimated divergence between the two extant *Choloepus* species has a considerable variation. Using the mitochondrial gene *Cyt-b*, the split of the two *Choloepus* species was estimated at ~18.7 million years ago (Mya) with Bayesian inference and ~5.8 with

Median Joining Network (Ruiz-Garcia et al., 2017). Gibb et al. (2015) estimated a split varying from 3.5 to 16.7 Mya based on mitogenomic shotgun data with Bayesian and maximum likelihood phylogenetic inferences. Such results discard the hypothesis of a recent common ancestor. The two *Choloepus* species live in some overlapping areas of the Amazon forest (Ruiz-Garcia et al., 2017). In addition, there is no precise information about the collecting areas of most *Choloepus* specimens analyzed in these studies, which may compromise the results and lead to incorrect inferences (Ruiz-Garcia et al., 2017). Thus, further molecular analyzes using samples with known collection areas and covering the geographic distribution of the two species are important to understand their taxonomic status. The SATCHO1 sequence identified in *Choloepus* was also found in two additional Xenarthra species, anteater *Myrmecophaga tridactyla* and the two-toed sloth *Bradypus variegatus*, but it only showed a satellite DNA pattern of organization in *Choloepus*. Our analysis of SATCHO1 centromeric hallmarks suggests that this satDNA may play some role in the centromere structure of *Choloepus*.

**The Karyotype for *Choloepus didactylus***

Our specimen presented a karyotype with 2n=51, FN=62 (Fig. 3) not described before for *Choloepus didactylus*. Previous reports documented complements with a wide variation in diploid numbers that ranged from 2n=52 to 2n=67 (Sonta et al., 1980; Jorge et al., 1981; Dobigny et al., 2005; Benirschke, 2006; Stainer et al., 2010; Yu et al., 2012), which led to the suggestion that a taxonomic revision was needed. It appeared that at least two different taxa were represented in these reports, one with lower diploid numbers (2n=52-53) and another with higher diploids numbers, around 60 chromosomes (Svartman, 2012). Ten specimens with lower diploid numbers were studied: six females and three males with 2n=53 and one female with 2n=52 (Sonta et al., 1980; Jorge et al.,

1981; Benirschke, 2006; Stainer et al., 2010). In addition, 14 *C. didactylus* specimens had higher diploid numbers: one male with 2n=59, two females and one male with 2n=64, two males with 2n=65, four females and three males with 2n=66 and one female with 2n=67 (Jorge et al., 1981; Dobigny et al., 2005; Stainer et al., 2010; Yu et al., 2012). Most of these samples came from zoos and their original collection site is unknown, preventing the possibility of correlating diploid numbers with geographic origin (Table 1).

Among the complements described in the literature for *Choloepus* two are similar to the karyotype of our specimen: one of a female *C. didactylus* with 2n=53 (Bernirschke, 2006) and another with 2n=51 found in two *C. hoffmanni* females (Jorge et al., 1985a).

The animal with 2n=53 share 18 autosome pairs and the X chromosome with our specimen, which can be seen comparing their GTG-banding patterns. Five pairs from our specimen (pairs 12, 13, 16, 4 and 7) do not have GTG-banding correspondence with the complement from the animal 2n = 53. And the pair 3, a biarmed autosome, from our sample shares a similar banding pattern with the pair 2 from animal 2n = 53 but this autosome 2 has a different morphology, suggesting the occurrence of centromeric repositioning. The two *C. hoffmanni* females with 2n=51 (Jorge, 1985a) have a karyotype that looks the same as that of our specimen in chromosome number and morphology, but unfortunately no GTG-banding was reported for these individuals, which prevents a more detailed comparison. These two females identified as *C. hoffmanni* were captured in the Belém-Brasília Highway, an area that is considered part of the geographic distribution of *C. didactylus*. *C. hoffmanni* has only been reported in Brazil as living in the state of Acre and in a small area of southwestern of Amazonas (Hayssen, 2011). Our data together with this information strongly suggest that the two females with 2n=51 (Jorge, 1985a) are

actually *C. didactylus* and not *C. hoffmanni* and reinforce the need of a taxonomic revision.

Our specimen had a translocation between the Y chromosome and one homologue of pair 22. The Y/autosome translocation was identified in three male specimens of *C. didactylus*, but the autosome numbering adopted by several authors differed from ours: one male had 2n=53 and a Y/21 translocation (Sonta et al., 1980) and another male presented 2n=65 and a Y/32 translocation (Dobigny et al., 2005; Yu et al., 2012). Corin-Frederick (1969) described four *C. hoffmanni* males (2n=49) that had a Y/23 translocation and six females with 2n=50. Meiosis from both sexes showed a normal behavior, suggesting that they have normal sex determination and gametes, despite the translocation. We cannot determine if the translocated autosomes are the same in all these animals, as GTG-banding was only described in the 2n=65 *C. didactylus* (Dobigny et al., 2005; Yu et al., 2012), in which case the translocated autosome is not the same identified in our specimen.

Our specimen presented constitutive heterochromatin in the centromeric regions of all chromosomes, except the X, as seen after CBG-banding (Fig. 3b). This is the first report of CBG-banding in *C. didactylus*. Four *C. hoffmanni* females (2n=51 and 2n=53) had their CBG-banding described (Jorge et al., 1985a) and the constitutive heterochromatin pattern revealed was restricted to the centromeric regions of all autosomes, similar to what we found in our specimen (Fig. 3b).

In conclusion, in this work we identified and characterized the two most abundant satDNAs in the *Choloepus hoffmanni* genome, SATCHO1 and SATCHO2. SATCHO1 mapped to the centromeres of all autosomes and has characteristics that suggest a possible centromeric function. SATCHO2 has a very large monomer size and was mapped to the short arms of some autosomes. Both satDNAs seem exclusive from Xenarthra, as they

have not been found in any sequenced genomes of other groups. This is the first description of satellite DNAs in the order Pilosa, and our results suggest that further analyzes may provide important data on Xenarthra genomes.

The karyotype of our *C. didactylus* seems to be the same as that described for some *C. hoffmanni* females, which further emphasizes the taxonomic problems of this taxon and the need for a revision.

**Acknowledgments**

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to MS and GCSK. RSS received a Masters fellowship, MPV and PH received doctoral fellowship, all from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

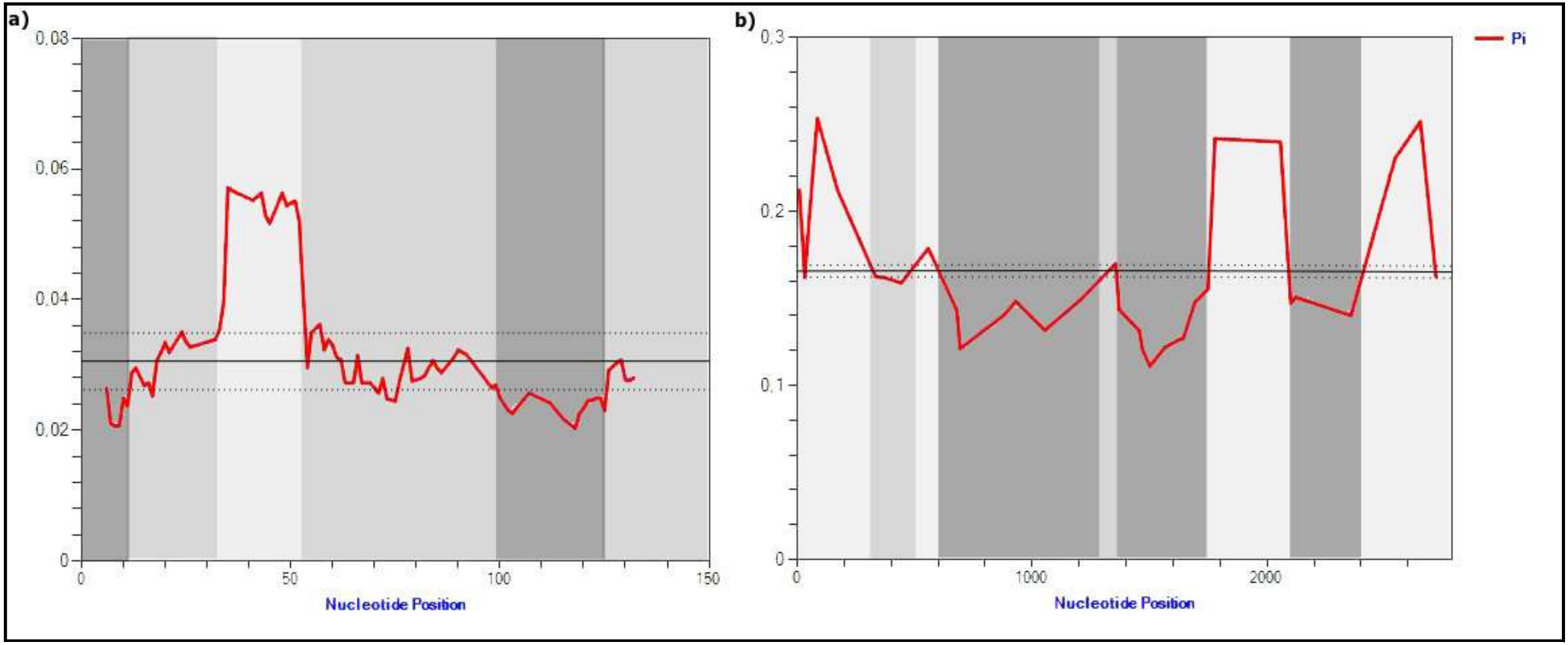



**Figure 1.** Identification of conserved (dark grey) and variable (light grey) satellite DNA segments of *C. hoffmanni* by sliding window analysis. Sliding window of 10 bp for (a) SATCHO1 and (b) SATCHO2. Average nucleotide diversity (Pi) is indicated by the red line, while average diversity ±SD is indicated with the dotted line.

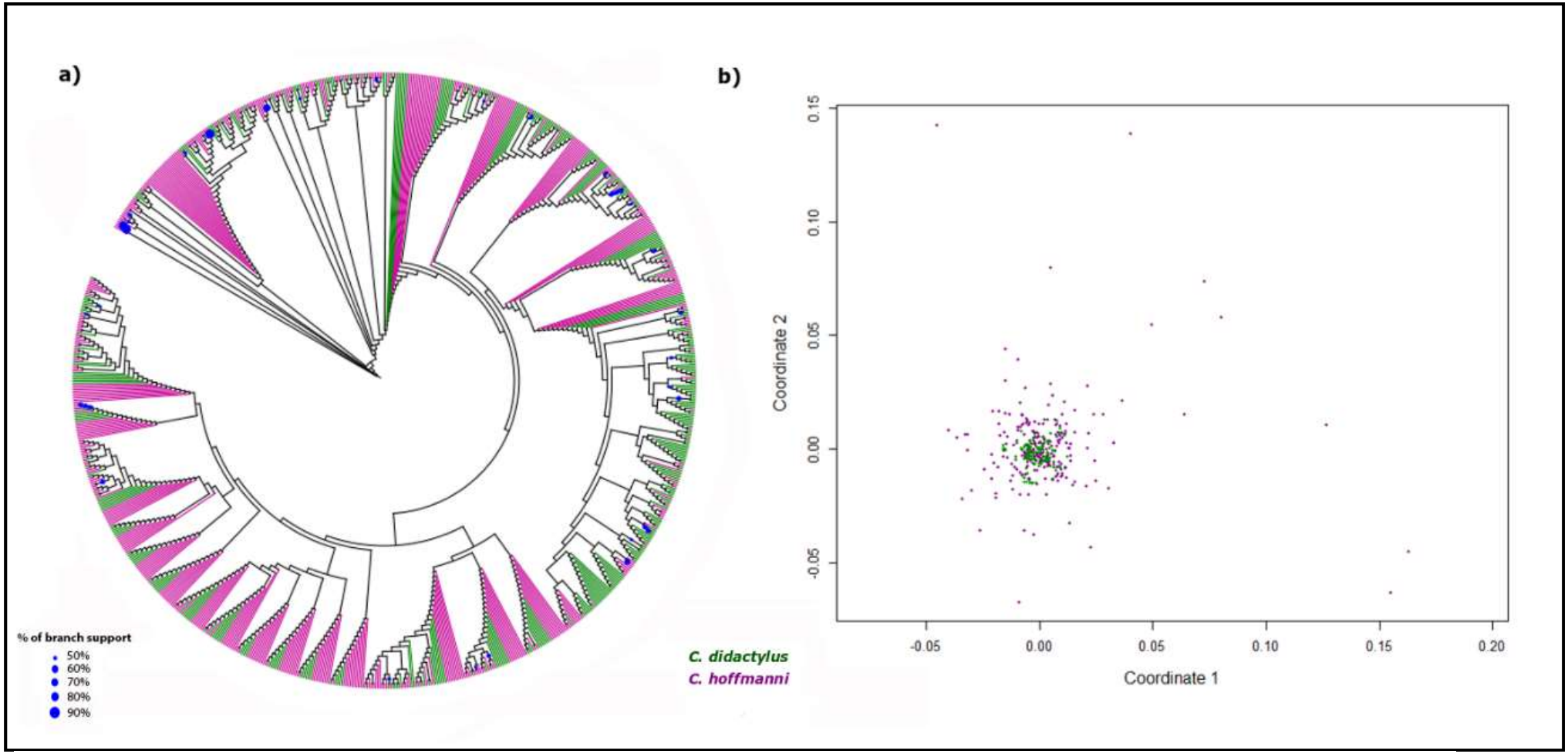



**Figure 2.** (a) Phylogenetic analyses of SATCHO1 sequences from *C. didactylus* and *C. hoffmanni* infered by the Neighbor-Joining method with 1000 bootstraps. Branch color represents the species: *C. didactylus* in green and *C. hoffmanni* in pink. Branch lenghts were ignored. Minimum bootstrap support is 50 %. (b) Non-metric Multidimensional Scaling (NMDS) of evolutionary divergence among the same SATCHO1 sequences used in the phylogenetic analysis. Color ordinations represent the *Choloepus* species. The scaling represents euclidian distances for four dimensions (Stress: 0.08258662).

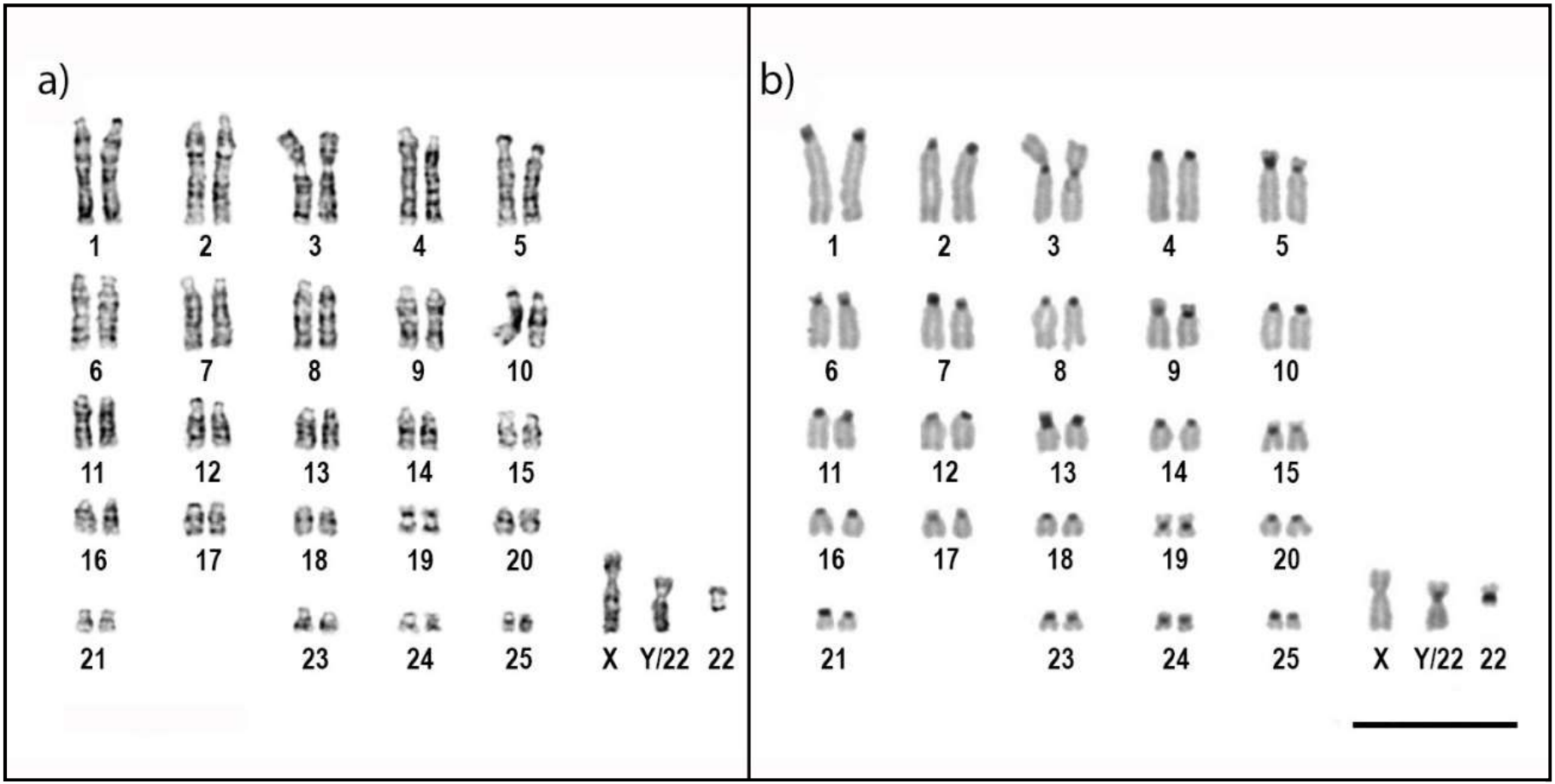



**Figure 3.** Karyotype of *Choloepus didactylus* 2n=51, FN = 62 after: (a) GTG-banding and (b) CBG-banding. Bar = 10µm.

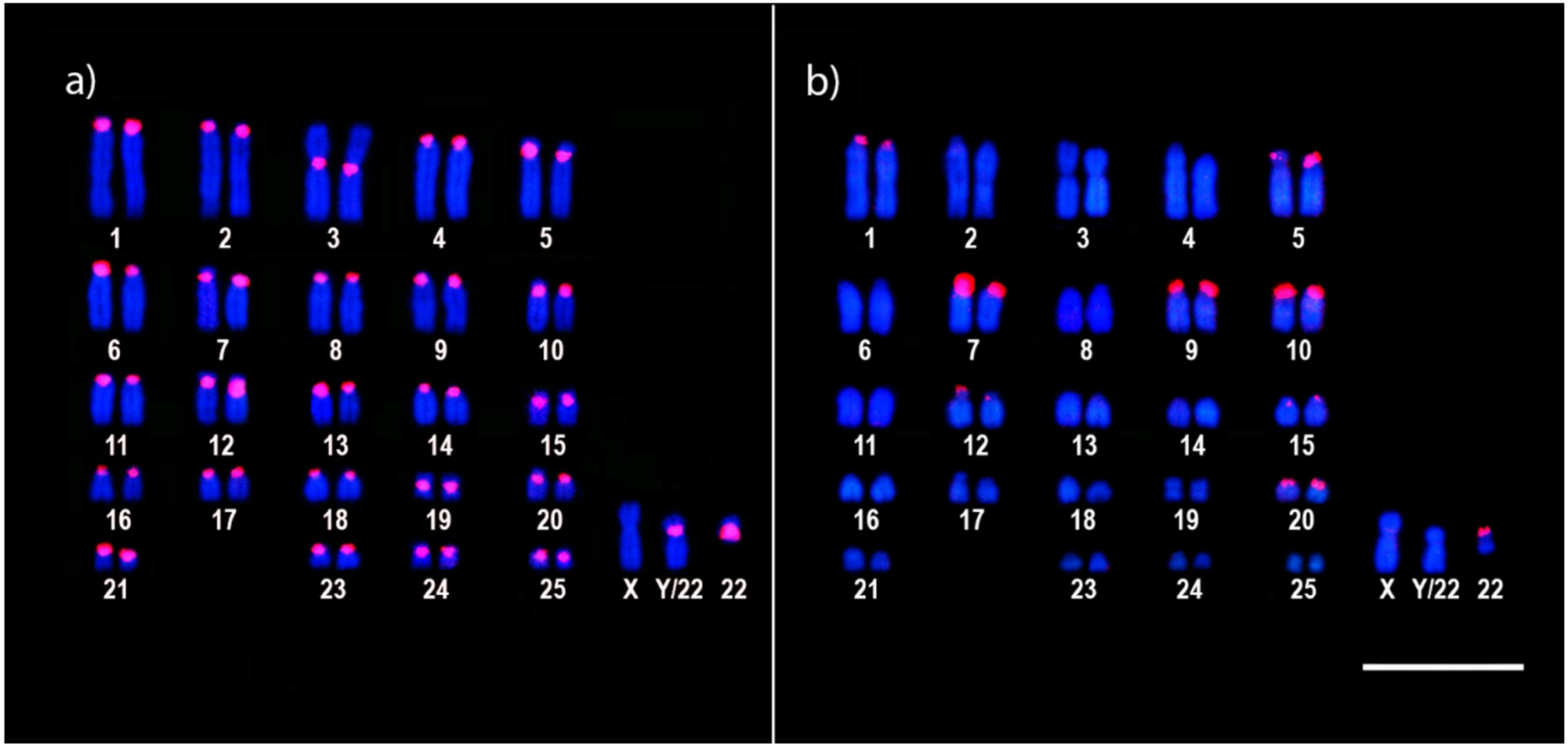
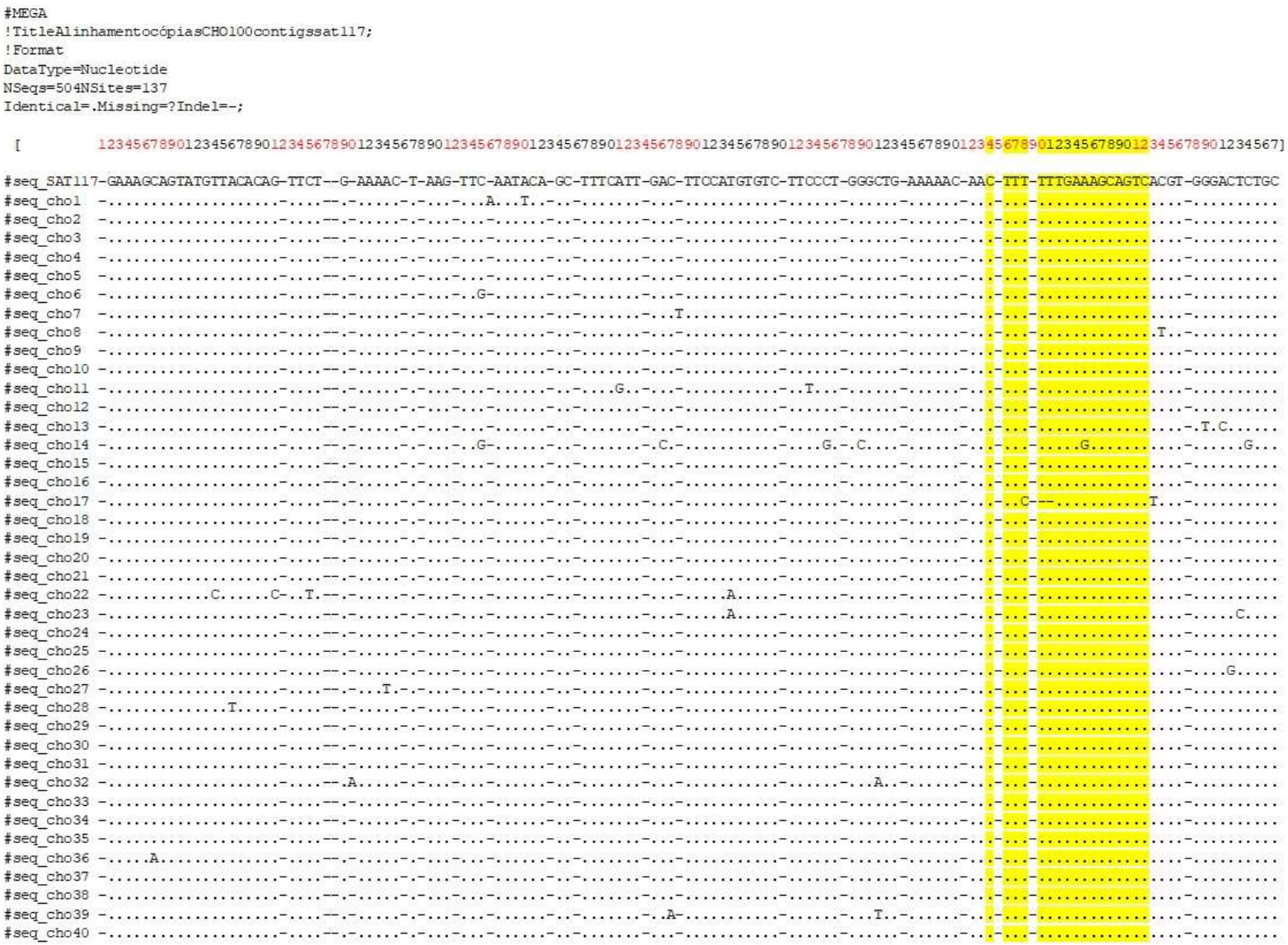


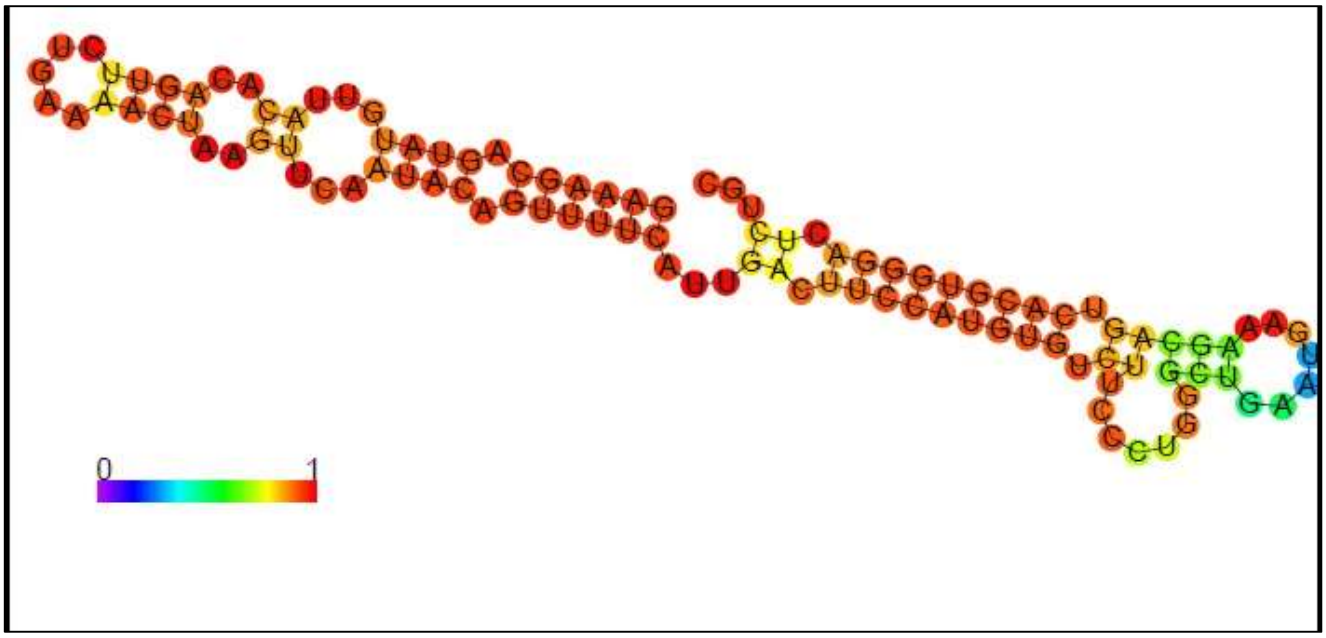

**Figure 4.** Karyotype of *C. didactylus* after FISH using: (a) SATCHO1 and (b) SATCHO2 as probe. In (a) note the signals in the centromeric regions of all chromosomes, except the X. In (b) there are signals in 17 autosomes. Bar = 10µm.

## Supplementary Figures

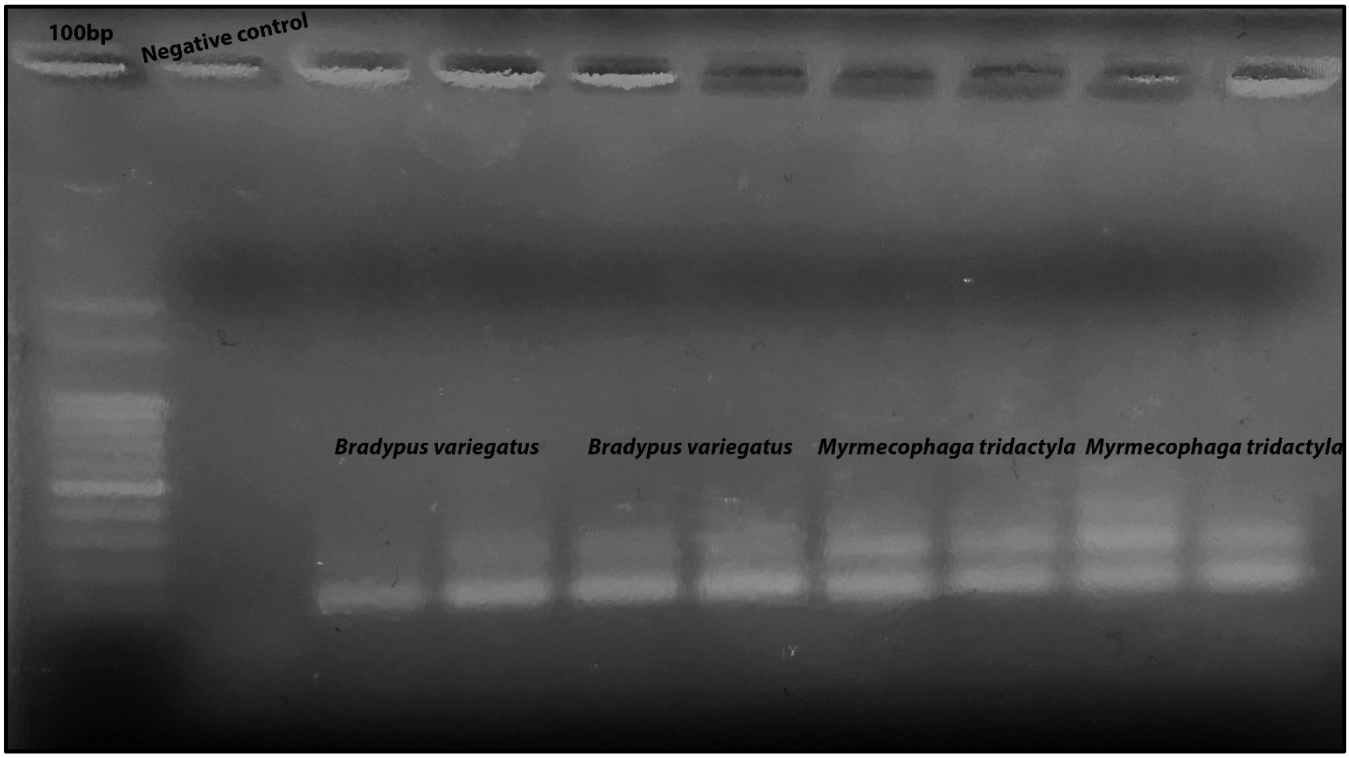



**Supplementary Figure 1.** Alignment of SATCHO1 copies. In yellow: segment correspondent to the CENP-box.

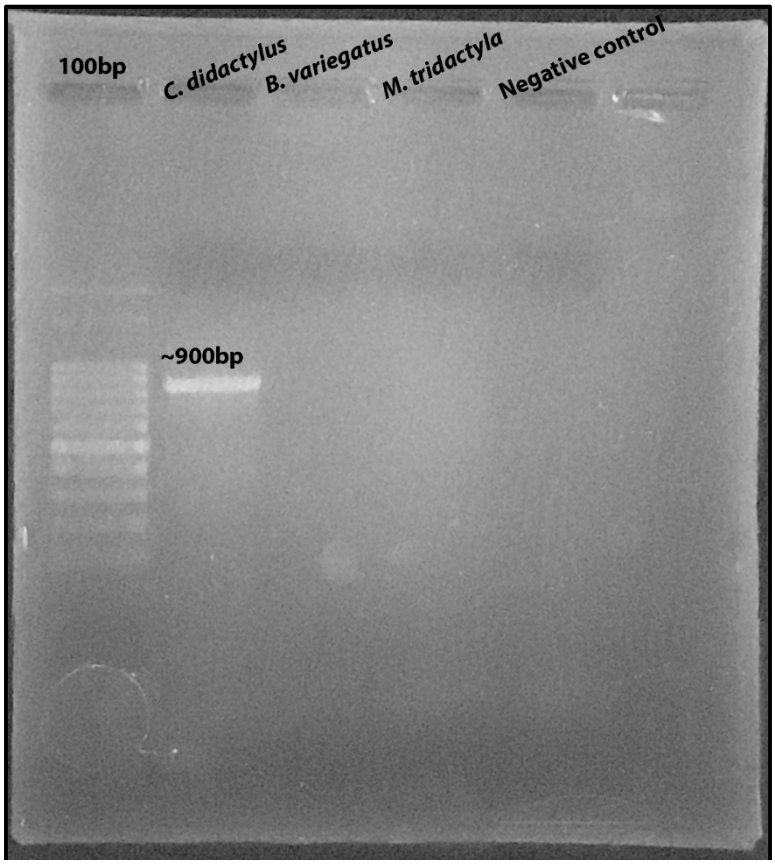

**Supplementary Figure 2.** SATCHO1 sequence with dyad palindrome sequences. Each pair is represented by a color.




**Supplementary Figure 3.** The optimal secondary structure of SATCHO1 predicted by RNAfold. Colored by base-pair probabilities (0 - 1).




**Supplementary Figure 4.** Agarose gel from PCR products of SATCHO1 amplified on genomic DNAs of *Bradypus variegatus* and *Myrmecophaga tridactyla*.




**Supplementary Figure 5.** Agarose gel from PCR products of SATCHO2 amplified on genomic DNAs of *Choloepus didactylus*, *Bradypus variegatus* and *Myrmecophaga tridactyla*.

## References


Adam PJ: *Choloepus didactylus*. Mamm Species 621: 1-8 (1999).

Alkan C, Cardone MF, Catacchio CR, Antonacci F, O'Brien SJ, et al: Genome-wide characterization of centromeric satellites from multiple mammalian genomes. Genome Res 21: 137-145 (2011).

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ: Basic local alignment search tool. J Mol Biol 215: 403-410 (1990).

Arnason U, GRETARSDOTTIR S, WIDEGREN B: *Mysticete* (baleen whale) relationships based upon the sequence of the common cetacean DNA satellite. Mol Biol Evol 9: 1018-1028 (1992).

Aze A, Sannino V, Soffientini P, Bachi A & Costanzo V: Centromeric DNA replication reconstitution reveals DNA loops and ATR checkpoint suppression. Nat Cell Biol 18: 684 (2016).

Bao, W, JURKA J: Origin and evolution of LINE-1 derived "half-L1" retrotransposons (HAL1). Gene 465: 9-16 (2010).

Benirschke K: Xenarthra, in O'Brien SJ, Menninger JC, Nash WG (eds): Atlas of Mammalian Chromosomes, pp 81–93 (John Wiley and Sons, Hoboken 2006).

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD & Sayers EW: Genbank. Nucleic Acids Res 46: D41-D47 (2018).

Catania S, Pidoux AL, Allshire RC: Sequence features and transcriptional stalling within centromere DNA promote establishment of CENP-A chromatin. Plos Genet 11: E1004986 (2015).

Corin-Frederic J: Les formules gonosomiques dites aberrantes chez les Mammiferes Eutheriens. Chromosoma 27: 268-287 (1969).

Churakov G, Smit AF, Brosius J & Schmitz J: A novel abundant family of retroposed elements (DAS-sines) in the nine-banded armadillo (*Dasypus novemcinctus*). Mol Biol Evol 22: 886-893 (2004).

Delsuc F, VIZCAÍNO SF, DOUZERY EJP: Influence of Tertiary paleoenvironmental changes on the diversification of South American mammals: a relaxed molecular clock study within xenarthrans. BMC Evol Biol 4: 11 (2004).

Delsuc F, Douzery EJP: Recent advances and future prospects in xenarthran molecular phylogenetics. The biology of the Xenarthra 11 (2008).

Dixon P: VEGAN, a package of R functions for community ecology. J Veg Sci 14: 927-930 (2003).

Djupedal I, Kos-Braun IC, Mosher RA, Söderholm N, Simmer F et al: Analysis of small RNA in fission yeast; centromeric siRNAs are potentially generated through a structured RNA. EMBO J 28: 3832-3844 (2009).

Dobigny G, Yang F, O'Brien PCM, Volobouev V, Kovacs A, et al: Low rate of genomic repatterning in Xenarthra inferred from chromosome painting data. Chromosome Res 13: 651-663 (2005).

Dover G: Molecular drive: a cohesive mode of species evolution. Nature 299: 111 (1982).

Dover GA: Molecular drive in multigene families: how biological novelties arise, spread and are assimilated. Trends Genet 2: 159-165 (1986).

Edgar RC: MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1792-1797 (2004).

Engelmann GF: The phylogeny of the Xenarthra. Pp. 51–64 in The Evolution and Ecology of Armadillos, Sloths, and Vermilinguas (GG Montgomery, ed.). 1985.

Escudeiro A, Adega F, Robinson TJ, Heslop-Harrison JS & Chaves R: Conservation, Divergence, and Functions of Centromeric Satellite DNA Families in the Bovidae. Genome Biol Evol 11: 1152-1165 (2019).

Fanning TG, Seuánez HN, Forman L: Satellite DNA sequences in the new world primate *Cebus apella* (Platyrrhini, Primates). Chromosoma 102: 306-311 (1993).

Garavis M, Escaja N, Gabelica V, Villasante A, Gonzalez C: Centromeric alpha-satellite DNA adopts dimeric i-motif structures Capped by AT Hoogsteen base pairs. Chemistry 21: 9816–9824 (2015).

Garavis M, Mendez-Lago M, Gabelica V, Whitehead SL, Gonzalez C, Villasante A: The structure of an endogenous *Drosophila* centromere Reveals the prevalence of tandemly repeated sequences able To form i-motifs. Sci Rep. 5: 13307 (2015).

Garrido-Ramos M: Satellite DNA: An evolving topic. Genes 8: 230 (2017).

Gibb GC, Condamine FL, Kuch M, Enk J, Moraes-Barros N, Superina M et al: Shotgun mitogenomics provides a reference phylogenetic framework and timescale for living xenarthrans. Mol Biol Evol 33: 621-642 (2015).

Goldberg IG, Sawhney H, Pluta AF, Warburton PE & Earnshaw WC: Surprising deficiency of CENP-B binding sites in African green monkey alpha-satellite DNA: implications for CENP-B function at centromeres. Mol Cell Biol 16: 5156-5168 (1996).

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

Hayssen, Virginia. *Choloepus hoffmanni* (Pilosa: Megalonychidae). Mamm Species 43: 37-55 (2011).

Hřibová E, Neumann P, Matsumoto T, Roux N, Macas J & Doležel J: Repetitive part of the banana (Musa acuminata) genome investigated by low-depth 454 sequencing. BMC Plant Biol 10: 204 (2010).

Jonstrup AT, Thomsen T, Wang Y, Knudsen BR, Koch J & Andersen AH: Hairpin structures formed by alpha satellite DNA of human centromeres are cleaved by human topoisomerase iiα. Nucleic Acids Res 36: 6165-6174 (2008).

Jorge W, Meritt Jr DA, Benirschke K: Chromosome studies in Edentata. Cytobios 18: 157-172 (1978).

Jorge, W. Estudo cromossômico de algumas espécies da Ordem Edentata. Phd Thesis, Instituto Básico de Biologia Médica e Agrícola, São Paulo, Brasil (1981).

Jorge W, Orsi-Souza AT, Best RC: The somatic chromosomes of Xenarthra, in Montgomery GG (ed): The Evolution and Ecology of Armadillos, Sloths, and Vermilinguas, pp121–129 (Smithsonian Institution Press, Washington 1985a).

Jurka J, Kapitonov VV, Pavlicek A, Klonowski P, Kohany O & Walichiewicz J: Repbase Update, a database of eukaryotic repetitive elements. Cytogenet Genome Res 110: 462-467 (2005).

Kabeche L, Nguyen HD, Buisson R & Zou L: A mitosis-specific and R loop–driven ATR pathway promotes faithful chromosome segregation. Science 359: 108-114 (2018).

Kasinathan S, Henikoff S: Non-B-form DNA is enriched at centromeres. Mol Biology Evol 35: 949-962 (2018).

Koch, J: Neocentromeres and alpha satellite: a proposed structural code for functional human centromere DNA. Hum Mol Genet 9: 149-154 (2000).

Kopecna O, Kubickova S, Cernohorska H, Cabelova K, Vahala J et al: Tribe-specific satellite DNA in non-domestic Bovidae. Chromosome Res 22: 277-291 (2014).

Kumar S, Stecher G, Tamura K: MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870-1874 (2016).

Letunic I, BORK P: Interactive Tree Of Life (itol) v4: recent updates and new developments. Nucleic Acids Res (2019).

Lower SS, McGurk MP, Clark AG & Barbash DA: Satellite DNA evolution: old ideas, new approaches. Curr Opin Genet Dev 49: 70-78 (2018).

Melters DP, Bradnam KR, Young HA, Telis N, May MR et al: Comparative analysis of tandem repeats from hundreds of species reveals unique insights into centromere evolution. Genome Biol 14: R10 (2013).

Moraes-barros N, ARTEAGA MC: Genetic diversity in Xenarthra and its relevance to patterns of neotropical biodiversity. J Mammal 96: 690-702 (2015).

Muro Y, Masumoto H, Yoda K, Nozaki N, Ohashi M & Okazaki T: Centromere protein B assembles human centromeric alpha-satellite DNA at the 17-bp sequence, CENP-B box. J Cell Biol 116: 585-596 (1992).

Nishihara H, Kuno S, Nikaido M & Okada N: Myrsines: a novel SINE family in the anteater genomes. Gene 400: 98-103 (2007).

Novák P, Neumann P, Pech J, Steinhaisl J & Macas J: RepeatExplorer: A Galaxy-based web server for genome-wide characterization of eukaryotic repetitive elements from next-generation sequence reads. Bioinform 29: 792-793 (2013).

Ohno M, Fukagawa T, Lee JS & Ikemura T: Triplex-forming dnas in the human interphase nucleus visualized in situ by polypurine/polypyrimidine DNA probes and antitriplex antibodies. Chromosoma 111: 201-213 (2002).

Pezer Ž, Brajković J, Feliciello I & Ugarković Đ: Satellite DNA-mediated effects on genome regulation. In: Repetitive DNA. Karger Publishers, p. 153-169 (2012).

Plohl M, Luchetti A, Meštrović N & Mantovani B: Satellite DNAs between selfishness and functionality: structure, genomics and evolution of tandem repeats in centromeric (hetero) chromatin. Gene 409: 72-82 (2008).

Plohl M, Meštrović N & Mravinac B: Satellite DNA evolution. In Repetitive DNA Karger Publishers 7: 126-152 (2012).

Rice P, Longden I, Bleasby A: EMBOSS: the European molecular biology open software suite. 2000.

Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P et al: A Dnasp 6: DNA sequence polymorphism analysis of large data sets. Mol Biol Evolution 34: 3299-3302 (2017).

Rstudio Team (2015). Rstudio: Integrated Development for R. Rstudio, Inc., Boston, MA URL http://www.rstudio.com/.

Ruiz-García M, Chacón D, Plese T, Schuler I & Shostell JM: Mitogenomics phylogenetic relationships of the current sloth's genera and species (Bradypodidae and Megalonychidae). Mitochondr DNA 29: 281-299 (2018).

Ruiz-Ruano FJ, López-León MD, Cabrero J & Camacho JPM: High-throughput analysis of the satellitome illuminates satellite DNA evolution. Sci Rep 6: 28333 (2016).

Schmidt T, Heslop-Harrison JS: Genomes, genes and junk: the large-scale organization of plant chromosomes. Trends in Plant Sci 3: 195-199 (1998).

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

Sonta SI, Hayata I, Sasaki M & Kondo N: Karyotype and sex determining in the two-toed sloth*, Choloepus didactylus*. Chromosome Information Service 28: 15-17 (1980).

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

Steiner CC, Houck ML, Ryder OA: Species identification and chromosome variation of captive two-toed sloths. Zoo Biology 30: 623-635 (2011).

Stitou S, de la Guardia RD, Jiménez R & Burgos M: Isolation of a species-specific satellite DNA with a novel CENP-B-like box from the North African rodent *Lemniscomys barbarus*. Exp Cell Res 250: 381-386 (1999).

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

Superina M, Loughry WJ: Why do Xenarthrans matter?. J Mammal 96: 617-621 (2015).

Svartman M, Stone G, Stanyon R: The ancestral eutherian karyotype is present in Xenarthra. Plos Genet 2: E109 (2006).

Svartman M: Chromosome evolution in Xenarthra: new insights from an ancient group. Cytogenet Genome Res 137: 130-143 (2012).

Talbert PB, Henikoff S: Transcribing centromeres: noncoding RNAs and kinetochore assembly. Trends Genet 34: 587-599 (2018).

Waters PD, Dobigny G, Pardini AT & Robinson TJ: LINE-1 distribution in Afrotheria and Xenarthra: implications for understanding the evolution of LINE-1 in eutherian genomes. Chromosoma 113: 137-144 (2004).

Wetzel RM: The identification and distribution of recent Xenarthra (= Edentata). The evolution and ecology of armadillos, sloths, and vermilinguas, 5-21 (1985).

Yu HT, Ma GC, Lee DJ, Chin SC, Chen TL et al: Use of a cytogenetic whole-genome comparison to resolve phylogenetic relationships among three species: implications for mammalian systematics and conservation biology. Theriogenology 77: 1615-1623 (2012).

Zhu L, Chou S, Reid BR: A single G-to-C change causes human centromere TGGAA repeats to fold back into hairpins. Proc Natl Acad Sci 93: 12159-12164 (1996).

## 4. CONCLUSÕES

- Neste trabalho a identificamos e caracterizamos pela primeira vez os DNAs satélites mais abundantes dos genomas de espécies do gênero *Choloepus*. Este também é o primeiro estudo sobre DNAs satélites da ordem Pilosa;
- O DNA satélite mais abundante encontrado (SATCHO1) corresponde a cerca de 2,6% do genoma de *C. hoffmanni*;
- Sua estrutura apresenta algumas características como sequências palindrômicas de <5pb e uma possível estrutura secundária, que têm sido apontadas como marcas estruturais de centrômeros funcionais;
- O segundo DNAsat mais abundante identificado, o SATCHO2, corresponde a 0,23% do genoma de *C. hoffmanni* e chama a atenção por apresentar monômeros com mais de 2.000 pb, um tamanho incomum para um DNAsat. SATCHO2 apresenta duas regiões internas mais conservadas. Análises mais detalhadas serão necessárias para compreender os padrões evolutivos deste DNAsat e explicar como cadeias de monômeros tão grandes se mantêm nos genomas;
- As cópias do SATCHO1 são bastante similares entre *C. hoffmanni* e *C. didactylus* (~3% de divergência) e análises filogenéticas não demonstraram separação das cópias em ramos diferentes. Além disso, estas espécies apresentam sobreposição de áreas de distribuição geográfica, há uma falta de informações precisas sobre as áreas de coletas da maioria dos animais coletados e resultados de estudos de divergência com marcadores moleculares apresentarem uma variação de mais de 10 milhões de anos. Dessa forma, sugerimos análises moleculares adicionais usando amostras com áreas de coleta conhecidas e cobrindo a distribuição geográfica das duas espécies são importantes para entender seu status taxonômico.

- O mapeamento cromossômico em *Choloepus didactylus* mostrou que SATCHO1 está localizado nas regiões centroméricas de todos os autossomos e SATCHO2 foi mapeado em regiões distais dos braços curtos de 17 autossomos;
- O cariótipo do exemplar de *C. didactylus* que analisamos apresentou 2n=51 cromossomos e uma translocação do Y com o cromossomo 22. Este cariótipo parece muito semelhante ao descrito para fêmeas de *C. hoffmanni* (2n=51), que podem ter sido identificadas erroneamente;
- Nossos dados reforçam a necessidade de uma revisão taxonômica de *Choloepus* e indicam que o estudo dos genomas de espécies de Xenarthra podem trazer resultados importantes para entender a evolução de mamíferos.

## 5. REFERÊNCIAS


Adam PJ: *Choloepus didactylus*. Mamm Species 621: 1-8 (1999).

Alkan C, Cardone MF, Catacchio CR, Antonacci F, O'Brien SJ, et al: Genome-wide characterization of centromeric satellites from multiple mammalian genomes. Genome Res 21: 137-145 (2011).

Bao, W, Jurka J: Origin and evolution of LINE-1 derived "half-L1" retrotransposons (HAL1). Gene 465: 9-16 (2010).

Benirschke K: Xenarthra, in O'Brien SJ, Menninger JC, Nash WG (eds): Atlas of Mammalian Chromosomes, pp 81–93 (John Wiley and Sons, Hoboken 2006).

Charlesworth B, Sniegowski P, S W: The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371: 215 (1994).

Chiarello A & Plese T: Choloepus didactylus. The IUCN Red List of Threatened Species 2014: e.T4777A47439542.http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T4777A47439542.en. Downloaded on 9 July 2019.

Corin-Frederic J: Les formules gonosomiques dites aberrantes chez les Mammiferes Eutheriens. Chromosoma 27: 268-287 (1969).

Churakov G, Smit AF, Brosius J & Schmitz J: A novel abundant family of retroposed elements (DAS-sines) in the nine-banded armadillo (*Dasypus novemcinctus*). Mol Biol Evol 22: 886-893 (2004).

Delsuc F, Vizcaíno SF, Douzery EJP: Influence of Tertiary paleoenvironmental changes on the diversification of South American mammals: a relaxed molecular clock study within xenarthrans. BMC Evol Biol 4: 11 (2004).

Dobigny G, Yang F, O'Brien PCM, Volobouev V, Kovacs A, et al: Low rate of genomic repatterning in Xenarthra inferred from chromosome painting data. Chromosome Res 13: 651-663 (2005).

Dover G: Molecular drive: a cohesive mode of species evolution. Nature 299: 111 (1982).

Dover GA: Molecular drive in multigene families: how biological novelties arise, spread and are assimilated. Trends Genet 2: 159-165 (1986).

Eisenberg JF, Redford KH: Order Xenarthra. Mammals of the Neotropics 3: 90-94 (1999).

Foley NM, Springer MS, Teeling EC: Mammal madness: is the mammal tree of life not yet resolved?. Philos Trans R Soc Lond B Biol Sci 371: 20150140 (2016).

Garrido-Ramos M: Satellite DNA: An evolving topic. Genes 8: 230 (2017).

Gaudin TJ: Phylogeny of the Xenarthra. Senckenb Biol 83: 27-40 (2003).

Gaudin TJ, Croft DA: Paleogene Xenarthra and the evolution of South American mammals. J Mammal 96: 622-634 (2015).

Graphodatsky AS, Trifonov VA, Stanyon R: The genome diversity and karyotype evolution of mammals. Mol Cytogenet 4: 22 (2011).

Grewal SIS, JIA S: Heterochromatin revisited. Nat Rev Genet 8: 35 (2007).

Hayssen, Virginia. *Choloepus hoffmanni* (Pilosa: Megalonychidae). Mamm Species 43: 37-55 (2011).

Jorge W, Meritt Jr DA, Benirschke K: Chromosome studies in Edentata. Cytobios 18: 157-172 (1978).

Jorge, W. Estudo cromossômico de algumas espécies da Ordem Edentata. Phd Thesis, Instituto Básico de Biologia Médica e Agrícola, São Paulo, Brasil (1981).

Jorge W, Orsi-Souza AT, Best RC: The somatic chromosomes of Xenarthra, in Montgomery GG (ed): The Evolution and Ecology of Armadillos, Sloths, and Vermilinguas, pp121–129 (Smithsonian Institution Press, Washington 1985a).

Kasahara S: Introdução à pesquisa em citogenética de vertebrados. Sociedade Brasileira de Genética, Ribeirão Preto, p. 160 (2009).

Liao D: Concerted evolution: molecular mechanism and biological implications. Am J Hum Genet 64: 24 (1999).

Lower SS, McGurk MP, Clark AG & Barbash DA: Satellite DNA evolution: old ideas, new approaches. Curr Opin Genet Dev 49: 70-78 (2018).

Mehrotra S, Goyal V: Repetitive sequences in plant nuclear DNA: types, distribution, evolution and function. Genomics Proteomics Bioinformatics 12: 164-171 (2014).

Melters DP, Bradnam KR, Young HA, Telis N, May MR et al: Comparative analysis of tandem repeats from hundreds of species reveals unique insights into centromere evolution. Genome Biol 14: R10 (2013).

Meredith RW, Janečka JE, Gatesy J, Ryder OA, Fisher CA et al: Impacts of the Cretaceous Terrestrial Revolution and KPg extinction on mammal diversification. Science 334: 521-524 (2011).

Meritt DA: The two-toed Hoffmann's sloth, Choloepus hoffmanni Peters. Pp. 333-341. In: Montgomery, G.G. (ed.). The Evolution and Ecology of Armadillos, Sloths, and Vermilinguas. Smithsonian Institution Press, Washington and London. 451p. (1985)

Moraes-barros N, Arteaga MC: Genetic diversity in Xenarthra and its relevance to patterns of neotropical biodiversity. J Mammal 96: 690-702 (2015).

Murphy WJ, Pringle TH, Crider TA, Springer MS & Miller W: Using genomic data to unravel the root of the placental mammal phylogeny. Genome Res 17: 413-421 (2007).

Nishihara H, Kuno S, Nikaido M & Okada N: Myrsines: a novel SINE family in the anteater genomes. Gene 400: 98-103 (2007).

Novacek MJ: Mammalian phytogeny: shaking the tree. Nature 356: 121 (1992).

Nowak RM, Walker EP: Walker's Mammals of the World. JHU press (1999).

O'Leary MA, Bloch JI, Flynn JJ, Gaudin TJ, Giallombardo AG et al: The placental mammal ancestor and the post–K-Pg radiation of placentals. Science 339: 662-667 (2013).

Plese T & Chiarello A: *Choloepus hoffmanni*. The IUCN Red List of Threatened Species 2014: e.T4778A47439751.http://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T4778A47439751.en. Downloaded on 9 July 2019.

Plohl M, Luchetti A, Meštrović N & Mantovani B: Satellite dnas between selfishness and functionality: structure, genomics and evolution of tandem repeats in centromeric (hetero) chromatin. Gene 409: 72-82 (2008).

Redi CA, Zacharias H, Merani S, Oliveira-Miranda M, Aguilera M, et al: Genome sizes in afrotheria, xenarthra, euarchontoglires, and laurasiatheria. J Hered 96: 485-493 (2005).

Redi CA, Garagna S, Zuccotti M & Capanna E: Genome size: a novel genomic signature in support of Afrotheria. J Mol Evol 64: 484-487 (2007).

Sonta SI, Hayata I, Sasaki M & Kondo N: Karyotype and sex determining in the two-toed sloth, *Choloepus didactylus*. Chromosome Information Service 28: 15-17 (1980).

Steiner CC, Houck ML, Ryder OA: Species identification and chromosome variation of captive two-toed sloths. Zoo biology 30: 623-635 (2011).

Superina M, Loughry WJ: Why do Xenarthrans matter?. J Mammal 96: 617-621 (2015).

Svartman M, Stone G, Stanyon R: The ancestral eutherian karyotype is present in Xenarthra. Plos Genet 2: E109 (2006).

Svartman M: Chromosome evolution in Xenarthra: new insights from an ancient group. Cytogenet Genome Res 137: 130-143 (2012).

Tautz D: Notes on the definition and nomenclature of tandemly repetitive DNA sequences. In: DNA fingerprinting: State of the science. Birkhäuser, Basel, p. 21-28 (1993).

The IUCN Red List of Threatened Species. Version 2019-1. Disponível em: <https://www.iucnredlist.org/>. Acesso em 09 de Julho de 2019.

Waters PD, Dobigny G, Pardini AT & Robinson TJ: LINE-1 distribution in Afrotheria and Xenarthra: implications for understanding the evolution of LINE-1 in eutherian genomes. Chromosoma 113: 137-144 (2004).

Wetzel RM: The identification and distribution of recent Xenarthra (= Edentata). The evolution and ecology of armadillos, sloths, and vermilinguas, 5-21 (1985).

Yu HT, Ma GC, Lee DJ, Chin SC, Chen TL et al: Use of a cytogenetic whole-genome comparison to resolve phylogenetic relationships among three species: implications for mammalian systematics and conservation biology. Theriogenology 77: 1615-1623 (2012).