UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO

CENTRO DE CIÊNCIAS HUMANAS E NATURAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

Estrutura genética e filogenia molecular dos primatas mais afetados no surto recente de febre amarela silvestre: *Alouatta guariba* e *Callithrix* spp. (Primates, Platyrrhini)

João Luiz Guedes da Fonseca

Vitória, ES
Junho, 2023

UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO

CENTRO DE CIÊNCIAS HUMANAS E NATURAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

Estrutura genética e filogenia molecular dos primatas mais afetados no surto recente de febre amarela silvestre: *Alouatta guariba* e *Callithrix* spp. (Primates, Platyrrhini)

João Luiz Guedes da Fonseca

Orientador: Prof. Yuri Luiz Reis Leite

Tese submetida ao Programa de Pós-Graduação em Ciências Biológicas da Universidade Federal do Espírito Santo, como requisito parcial para a obtenção do grau de Doutor em Biologia Animal.

Vitória, ES
Junho, 2023

Programa de Pós-Graduação em Ciências Biológicas
UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO

João Luiz Guedes da Fonseca

“Estrutura genética e filogenia molecular dos primatas mais afetados no surto recente de febre amarela silvestre:Alouatta guariba e Callithrix spp. (Primates, Platyrrhini)”

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas do Centro de Ciências Humanas e Naturais, da Universidade Federal do Espírito Santo, como requisito parcial para obtenção do Grau de Doutora em Biologia Animal.

Aprovada em 28 de junho de 2023.

Comissão Examinadora:

Prof. Dr. Yuri Luiz Reis Leite (UFES)
Orientador e Presidente da Comissão

Prof. Dr. Jeronymo Dalapicolla (UFPB)
Examinador Externo

Profª Drª Cibele Rodrigues Bonvicino (Fiocruz)
Examinadora Externa

Prof. Dr. Fabiano Rodrigues de Melo (UFV)
Examinador Externo

Profª Drª Ana Carolina Loss Rodrigues (UFES)
Examinadora Interna

Folha de Aprovação - João Luiz Guedes da Fonseca

Data e Hora de Criação: 23/06/2023 às 19:22:18

Documentos que originaram esse envelope:
- Folha de Aprovação.pdf (Arquivo PDF) - 1 página(s)

Hashs únicas referente à esse envelope de documentos

[SHA256]: e6e9bfb28dd47ea7ff8b95bf9c1c35eeb645449a063e47ad75d855e52cac38d9
[SHA512]: 5f0cda5f5ba932432d147b1ec2d4648c1a6813ca11b267fb95afe390364751c29a3942cf507a6b5e065c77e769c5ae3b574d8c4371bffdf39a272af1f1d12142

Lista de assinaturas solicitadas e associadas à esse envelope

ASSINADO - Ana Carolina Loss Rodrigues (carolloss@gmail.com)
Data/Hora: 28/06/2023 - 11:45:36, IP: 200.137.65.106
[SHA256]: c1ba19ceacb3e1d0374df145d5b35b963c5019162fdfe04272a39c35bb4a4916

ASSINADO - Cibele Rodrigues Bonvicino (cibele.bonvicino@gmail.com)
Data/Hora: 28/06/2023 - 11:45:48, IP: 179.210.180.143, Geolocalização: [-22.926193, -43.203758]
[SHA256]: ecd798c5bce50b625418f07f25e76a334e1232ee49423bd6e11b3a094f2a0e9a

ASSINADO - Fabiano Rodrigues de Melo (frmelo@ufv.br)
Data/Hora: 28/06/2023 - 11:45:27, IP: 200.235.146.70, Geolocalização: [-20.760060, -42.868401]
[SHA256]: d50d20c80fe95b33718631e3e9a95395c116d2c07dcf1ae8463351859f9910a3

ASSINADO - Jeronymo Dalapicolla (jdalapicolla@dse.ufpb.br)
Data/Hora: 28/06/2023 - 11:47:56, IP: 179.102.135.68, Geolocalização: [-20.274359, -40.303580]
[SHA256]: 5851c814f5df9c1fce51d2b2bbbc49c466bef9f3a6fe4d19edd49dd81e6a9d46

ASSINADO - Yuri Luiz Reis Leite (yuri_leite@yahoo.com)
Data/Hora: 28/06/2023 - 11:45:50, IP: 200.137.65.104, Geolocalização: [-20.274465, -40.303537]
[SHA256]: ee852a7e84efce6180674e6922715fa68eb4db378ea80e87d6178a2b507abb98

Histórico de eventos registrados neste envelope

28/06/2023 11:47:56 - Envelope finalizado por jdalapicolla@dse.ufpb.br, IP 179.102.135.68
28/06/2023 11:47:56 - Assinatura realizada por jdalapicolla@dse.ufpb.br, IP 179.102.135.68
28/06/2023 11:47:50 - Envelope visualizado por jdalapicolla@dse.ufpb.br, IP 179.102.135.68
28/06/2023 11:47:50 - Envelope visualizado por jdalapicolla@dse.ufpb.br, IP 179.102.135.68
28/06/2023 11:45:50 - Assinatura realizada por yuri_leite@yahoo.com, IP 200.137.65.104
28/06/2023 11:45:48 - Assinatura realizada por cibele.bonvicino@gmail.com, IP 179.210.180.143
28/06/2023 11:45:36 - Assinatura realizada por carolloss@gmail.com, IP 200.137.65.106
28/06/2023 11:45:27 - Assinatura realizada por frmelo@ufv.br, IP 200.235.146.70
28/06/2023 10:28:05 - Envelope visualizado por cibele.bonvicino@gmail.com, IP 179.210.180.143
28/06/2023 08:01:47 - Envelope registrado na Blockchain por notificacao@astenassinatura.com.br
28/06/2023 08:01:45 - Envelope encaminhado para assinaturas por notificacao@astenassinatura.com.br
23/06/2023 19:22:18 - Envelope criado por victor.prado@ufes.br, IP 200.137.65.102

Ficha catalográfica disponibilizada pelo Sistema Integrado de Bibliotecas - SIBI/UFES e elaborada pelo autor

---

Guedes da Fonseca, João Luiz, 1988-
G924e Estrutura genética e filogenia molecular dos primatas mais afetados no surto recente de febre amarela silvestre: Alouatta guariba e Callithrix spp. (Primates, Platyrrhini) / João Luiz Guedes da Fonseca. - 2023.
108 f. : il.

Orientador: Yuri Luiz Reis Leite.
Tese (Doutorado em Biologia Animal) - Universidade Federal do Espírito Santo, Centro de Ciências Humanas e Naturais.

1. Genética da paisagem. 2. Landscape genetics. 3. Genética de populações. 4. Filogenética. 5. Primatologia. 6. Genética molecular. I. Reis Leite, Yuri Luiz. II. Universidade Federal do Espírito Santo. Centro de Ciências Humanas e Naturais. III. Título.

CDU: 57

UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO

CENTRO DE CIÊNCIAS HUMANAS E NATURAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS

Membros da banca examinadora:

Dr. Yuri Luiz Reis Leite
(Orientador)

Drª. Ana Carolina Loss Rodrigues
(Membro Interno)

Drª. Cibele Rodrigues Bonvicino
(Membro Externo)

Dr. Jeronymo Dalapicolla
(Membro Externo)

Dr. Fabiano Rodrigues de Melo
(Membro Externo)

Drª. Joyce Rodrigues do Prado
(Suplente Externo)

Drª. Sarah Maria Vargas
(Suplente Interno)

Vitória, 29 de junho de 2023

AGRADECIMENTOS

Agradeço ao meu orientador, Yuri Leite, por todos os anos de parceria e aprendizado. Recentemente, ele completou 20 anos de UFES sendo alguém que realmente faz a diferença na universidade com ética e dedicação. Ajudou a formar milhares de alunos em suas excelentes aulas e orientações. Há 20 anos, ele e Leonora Costa chegaram ao Espírito Santo e fundaram a família LAMAB (Laboratório de Mastozoologia e Biogeografia). Além de tudo, um grande amigo que eu espero levar para a vida toda.

Agradeço ao meu grande amigo Felipe Gatti por todo o suporte e amizade. Sem ele essa tese não teria sido escrita. Uma pessoa incrível e brilhante que sempre esteve disposto a me dar ajuda e suporte quando eu mais precisei. Espero poder recompensá-lo por toda a dedicação prestada a mim.

Ich danke Christian Roos who was my co advisor during my stay in Deutschland. Amazing researcher and an outstanding advisor. Christian welcomed me at DPZ and made me feel at home in a foreign country. I'm also thankful to all DPZ staff, especially Christiane Schwarz who showed me all the lab procedures and helped me along the way. I also thank Joanna Malukiewicz and all the co authors from chapter two of this thesis. Joanna is an amazing researcher and a great friend, an inspiring person that helped me a lot during my stay in Germany.

Eu sou grato a todos os meus amigos de longa data e os novos que fiz ao longo desta jornada. Dizem que a felicidade só é real quando compartilhada. Eu posso me considerar bastante afortunado por poder compartilhar tantos momentos felizes com tantos e tantas amadas a quem considero tanto.

A minha família, a quem eu devo tudo nessa vida, merece todo meu agradecimento Obrigado pela confiança, amor e dedicação de todos esses anos de anos à meu pai, José Luiz e à minha mãe, Nice. Agradeço à minha irmã Lorraine. São pessoas nas quais eu me espelho e espero poder contar sempre como um porto seguro nas maiores tempestades. Agradeço em especial à minha incrível e amada esposa Samyra pelo suporte emocional e companheirismo por essa jornada. Não foi fácil para mim e, sem seu apoio, nada disso teria se concretizado.

Agradeço a todos os órgãos de fomento que viabilizaram este projeto, FAPES, CAPES, CNPq e DAAD. Agradeço ao Projeto Sentinelas da Mata, coordenado pelo Sérgio Lucena, e ao Fernando A. Perini por me cederem as amostras utilizadas. Agradeço à UFES por todos os anos de excelente formação profissional e pessoal a mim proporcionados. Em especial às biólogas Juliana Justino do Núcleo de Biodiversidade Genética Luiz Paulo de Souza Pinto (Nubigen) e Monique Nascimento da Coleção de Mamíferos e de Tecidos Animais do Departamento de Ciências Biológicas.

Esses anos foram anos muito difíceis, especialmente para os brasileiros. Portanto agradeço a todos os conterrâneos que ajudaram a nos desviar do caminho tenebroso para o qual estávamos rumando.

RESUMO

Neste estudo, avaliamos a diversidade genética de dois dos gêneros de primatas mais afetados pelo último surto de febre amarela ocorrido no Brasil (2016–2018). No primeiro capítulo usamos a abordagem integrativa entre filogenética, genética de populações e genética de paisagem para testar a hipótese de que a fragmentação da Mata Atlântica poderia ter impedido a rápida disseminação de febre amarela entre as populações de bugios (*Alouatta guariba*). Entretanto, não encontramos evidências para suportar essa hipótese, uma vez que existe conectividade genética entre todos os indivíduos amostrados e não houve padrão geográfico discernível entre os clados recuperados pela análise filogenética. Assim, temos um forte indicativo de que a febre amarela pode ser facilmente transmitida entre populações de bugios aparentemente isoladas. No segundo capítulo, usamos o mitogenoma e a região de controle do mtDNA com o intuito de investigar a diversidade e a ancestralidade de saguis (*Callithrix jacchus*) em instalações internacionais de pesquisa e fornecimento de animais. Nossos resultados sugerem que as análises da variação mitogenômica têm um grande potencial para resolver relações evolutivas entre populações de saguis, definindo limites de espécies e detectando híbridos. Assim, sugerimos que a combinação do mitogenoma com dados fenotípicos e genomas nucleares são essenciais para manter a diversidade genética dos saguis visando planos de reprodução apropriados, auxiliando na seleção adequada do fenótipo para pesquisas biomédicas específicas.

Palavras-chave: Atelidae, Callitrichidae, febre amarela; Mata Atlântica, primatas.

ABSTRACT

In this study, we evaluated the genetic diversity of two primate genera most affected by the last yellow fever outbreak in Brazil (2016–2018). In the first chapter we used an integrative approach combining phylogenetic, population genetics and landscape genetics to test the hypothesis that fragmentation of the Atlantic Forest could have prevented the rapid spread of yellow fever among populations of howler monkeys (*Alouatta guariba*). However, we did not find evidence to support this hypothesis, since there is genetic connectivity among all sampled individuals and there was no discernible geographic pattern among clades retrieved by phylogenetic analysis. Thus, we have a strong indication that yellow fever can be easily transmitted between apparently isolated populations of howler monkeys. In the second chapter, we use the mitogenome and mtDNA control region to investigate the diversity and ancestry of marmosets (*Callithrix jacchus*) at international animal research and supply facilities. Our results suggest that analyzes of mitogenomic variation have great potential for resolving evolutionary relationships among marmoset populations, defining species boundaries and detecting hybrids. Thus, we suggest that the combination of the mitogenome with phenotypic data and nuclear genomes are essential to maintain the genetic diversity of marmosets aiming at appropriate reproduction plans, helping in the adequate selection of the phenotype for specific biomedical research.

Keywords: Atelidae, Callitrichidae, yellow fever; Atlantic Forest, primates.

SUMÁRIO

APRESENTAÇÃO

Das mais de 500 espécies de primatas que existem no mundo, cerca de 170 consistem nos Platyrrhini, os macacos do Novo Mundo, que ocorrem do México à Argentina, e se agrupam em 21 ou 22 gêneros e entre 3 e 5 famílias viventes, dependendo do autor (Rylands & Mittermeier 2014; Beck et al. 2023; Mammal Diversity Database 2023). Dentre estes primatas, podemos destacar os bugios ou barbados do gênero *Alouatta* (família Atelidae) e os saguis do gênero *Callithrix* (família Callitrichidae), que foram os primatas não-humanos mais frequentemente infectados com o vírus da febre amarela durante o surto de 2017–2018 que ocorreu no Brasil (Mares-Guia et al. 2020).

No primeiro capítulo, tratamos do gênero *Alouatta,* que é composto por 11 espécies que ocorrem ao longo da região Neotropical, do sul do México ao norte da Argentina (Crockett 1998; Cortés-Ortiz et al. 2003). Apesar de ser um dos mais bem estudados primatas do novo mundo, a taxonomia deste gênero ainda é problemática (Crockett 1998; Gregorin 2006; Rylands & Mittermeier 2009). Um exemplo disso é a espécie *Alouatta guariba* (Humboldt, 1812), conhecida como bugio marrom. Essa espécie ocorre na Mata Atlântica, com distribuição do sul da Bahia ao norte da Argentina, na província de Misiones. Alguns autores reconhecem duas subespécies, *A. guariba clamitans* ao sul do rio Jequitinhonha (Rylands et al. 1988), ou rio Doce a depender do autor (Kinzey 1982); e *A. guariba guariba* ao norte. Ainda, segundo trabalhos de filogenética que utilizaram DNA mitocondrial (Harris et al. 2005), pode existir uma subdivisão adicional em *A. guariba clamitans* com haplótipos do Rio de Janeiro sendo bastante divergentes de haplótipos do sul do Brasil, com uma área de mistura de haplótipos em São Paulo. Entretanto, outros autores também reconhecem as duas subespécies como espécies separadas com base em dados morfológicos, *Alouatta fusca* e *Alouatta clamitans* (Gregorin 2006). Dentre muitos aspectos ecológicos que permeiam o grupo, vale destacar o fato dos bugios serem extremamente susceptíveis ao vírus da febre amarela (Flaviridae), principalmente se comparados a outros primatas, apresentando alta taxa de mortalidade durante os surtos da doença (Harris et al. 2005; Araújo 2011).

Na América do Sul, são conhecidos dois ciclos de febre amarela, o silvestre e o urbano (Moreno et al. 2015). O ciclo silvestre é endêmico de florestas tropicais,

infectando mosquitos de dossel, principalmente pertencentes aos gêneros *Haemagogus* Linnaeus, 1758 e *Sabethes* Robineau-Desvoidy, 1827, e primatas não humanos (Vasconcelos 2010). Esse ciclo causa epidemias em ondas de 7 a 14 anos, sendo essa periodicidade resultante das dinâmicas populacionais dos animais infectados. Epidemias periódicas podem causar declínio populacional severo, podendo levar à depressão endogâmica ou até mesmo a extinções (Smith et al. 2009, Altizer et al. 2007; Moreno 2015).

Registros de surtos da febre amarela em anos passados mostram que centenas de animais pertencentes ao gênero pereceram (Bica-Marques e Freitas 2010; Almeida et al. 2012). Na Argentina, entre os anos de 2007 e 2008, a febre amarela vitimou muitos bugios. Devido ao surto, *Alouatta guariba* foi classificado como "criticamente em perigo" no país (Agostini et al. 2012).

Em 2016, um novo surto da febre amarela atingiu a Mata Atlântica da região sudeste do Brasil, principalmente nos estados de Minas Gerais e Espírito Santo, dizimando milhares de *A. guariba*. Assim como em eventos passados, também foi relatada a morte desses animais por populações humanas que vivem próximas às áreas dos surtos. Isso ocorre, principalmente, devido à falta de informação, pois essas pessoas acreditavam que os próprios macacos poderiam transmitir a doença, o que agrava ainda mais o declínio populacional da espécie (Holzmann et al. 2010). Esse fato, somado ao estado de fragmentação e degradação em que se encontra a Mata Atlântica (Ribeiro et al. 2009), provavelmente está fazendo com que as populações de *A. guariba* passem por um gargalo genético de difícil recuperação. Atualmente, o status de conservação da espécie pela IUCN está como “pouco preocupante”. Entretanto, quando consideradas as subespécies, *A. g. clamitans* está classificada como “pouco preocupante”, enquanto *A. g. guariba* está como “criticamente em perigo”. Porém, frente ao recente surto de febre amarela, o status da espécie e da subespécie *A. g. clamitans* podem mudar em breve.

Além de ser muito afetada pela febre amarela, *A. guariba*, também sofre com a redução de seu hábitat. O estado atual da Mata Atlântica, um dos mais importantes hotspots da biodiversidade mundial (Myers et al. 2000), é de grande fragmentação. Apesar de alguns trabalhos indicarem que o ritmo de degradação desse bioma diminuiu, a quantidade remanescente de ambientes naturais e o nível de conectividade entre eles são muito baixos (Ribeiro et al. 2009). Os fragmentos remanescentes são geralmente isolados por matrizes de ambiente inóspito para a movimentação de várias espécies, dificultando a movimentação de indivíduos de um fragmento a outro. Ambientes

devidamente conectados, ainda que fragmentados, podem permitir o fluxo gênico relativamente livre. Isso resulta em uma diminuição dos níveis de endogamia e um progressivo aumento da viabilidade das populações de uma espécie. Portanto, estudos que visem esclarecer o estado atual da conectividade efetiva de paisagens são de grande importância, pois trazem informação e objetividade às políticas públicas de conservação (Rayfield et al. 2011).

A estrutura genética de *A. guariba* ainda é bastante desconhecida, principalmente a nível populacional. Isso, somado aos problemas taxonômicos que permeiam o grupo (veja Crockett 1998; Gregorin 2006; Rylands and Mittermeier 2009), configura um desafio para a conservação da espécie. Até o momento, somente um trabalho utilizou amostras de indivíduos ao norte do rio Doce (veja Machado 2011), que seria o limite da distribuição da subespécie *A. g. clamitans* (Rylands et al.1988). Para conservarmos uma espécie, subespécies, ou mesmo populações, devemos conhecer a estrutura das populações em termos de diversidade e divergência genética para que possamos avaliar os efeitos que o surto recente de febre amarela está acarretando nessas populações e, assim, traçarmos planos eficientes de manejo para as populações remanescentes (Kinzey 1984).

A alta susceptibilidade de bugios à febre amarela faz com que esses primatas sejam espécies sentinelas para a detecção de surtos da doença, o que os tornam de extrema importância para a saúde pública (Araújo 2011). O conhecimento antecipado de surtos de febre amarela no ciclo silvestre pode ser crucial para a criação de estratégias de vacinação, ajudando a evitar o ciclo urbano da doença (Araújo 2011).

Assim, este trabalho teve o objetivo de avaliar a estrutura genética das populações de *Alouatta guariba* afetadas pelo recente surto de febre amarela no sudeste do Brasil. Foi caracterizada a diversidade e divergência genética das amostras utilizando marcadores de DNA de regiões de microssatélite e DNA mitocondrial. Além disso, a distribuição geográfica da diversidade genética foi determinada com técnicas de isolamento de resistência, visando implicações para a conservação da espécie na região de estudo.

No segundo capítulo, enfocamos os saguis do gênero *Callithrix* Linnaeus, 1758, que tem seis espécies endêmicas ao Brasil e que apresentam um conjunto único de características biológicas, como gravidez gemelar frequente e taxa reprodutiva rápida (Malukiewicz et al. 2021). Estas características fizeram com que os saguis se tornassem modelos biomédicos valiosos, muito procurados por centros de pesquisa internacionais,

mas cuja oferta é limitada (National Academies of Sciences and Medicine 2019). Estima-se que a população mundial de colônias biomédicas de saguis seja de aproximadamente 6.000 saguis, dos quais 2.500 são encontrados no Japão, 1.900 na América do Norte, 1.000 na Europa e 800 na América do Sul. Nos EUA, os saguis estão alojados em aproximadamente 27 instalações de pesquisa, e os saguis fundadores importados para os EUA durante as décadas de 1960 e 1970 vieram da Europa e do Reino Unido. As informações sobre o pedigree dos saguis inicialmente importados para os EUA são limitadas, mas, em muitos casos, as colônias atuais de saguis são originárias de estoques de algumas dezenas de animais importados (National Academies of Sciences and Medicine 2019).

As instalações de pesquisa geralmente mantêm colônias que consistem em indivíduos com o fenótipo de saguis comuns, *Callithrix jacchus* (Linnaeus, 1758). Esta espécie é caracterizada por tufos brancos e espessos nas orelhas e uma estrela branca na testa, mas as origens biogeográficas ancestrais desses macacos em cativeiro são amplamente desconhecidas. No Brasil, os saguis comuns ocorrem em três grandes biomas brasileiros: a Caatinga arbustiva e semiárida, o Cerrado semiárido e a Mata Atlântica litorânea úmida (Schiel & Souto 2017, Malukiewicz et al. 2021). Sabe-se que os saguis também hibridizam, tanto em condições de vida livre quanto em cativeiro, sendo que a maioria dos casos envolve *C. jacchus* como uma das espécies parentais (Malukiewicz 2018, Malukiewicz et al. 2021). As populações brasileiras de saguis comuns em vida livre estão em declínio e os saguis para uso biomédico são difíceis de importar de acordo com as normas internacionais de comércio de animais silvestres (National Academies of Sciences and Medicine 2019). Dada a oferta limitada de saguis fora do Brasil, descobrir a filogenia, as origens biogeográficas ancestrais e o status híbrido dos saguis em cativeiro é fundamental para seu uso biomédico. Essas informações são úteis na avaliação dos níveis atuais de diversidade genética dos saguis em cativeiro, na identificação de possíveis híbridos e na manutenção da integridade genética das populações biomédicas de saguis em cativeiro.

O DNA mitocondrial (mtDNA) tem sido um marcador genético valioso e informativo na resolução da filogenia, divergência, biogeografia e identificação de híbridos de *Callithrix* (Malukiewicz et al. 2014). Os padrões de divergência interespecífica analisados com o uso da região de controle do mtDNA, que sofre evolução rápida e tem aproximadamente 1.200 pares de bases (pb), são amplamente concordantes com os resultados das análises completas do seu mitogenoma, que é muito

maior, com cerca de aproximadamente 16.500 pb (Malukiewicz et al. 2017). Aqui relatamos uma comparação entre o mitogenoma e a região de controle do mtDNA para determinar a ancestralidade e a diversidade de saguis em instalações internacionais de pesquisa e fornecimento de animais, combinando dados de sequências novas e previamente disponíveis. Utilizamos os dois tipos de dados para descobrir as origens ancestrais e se há híbridos crípticos entre os saguis mantidos em cativeiros internacionais e como a diversidade genética da região de controle do mtDNA e do mitogenoma do *C. jacchus* se compara à de outros táxons de *Callithrix*.

Referências

Altizer, S., Nunn, C. L., & Lindenfors, P. (2007). Do threatened hosts have fewer parasites? A comparative study in primates. Journal of Animal Ecology, 76(2), 304-314. doi: https://doi.org/10.1111/j.1365-2656.2007.01214.x

Agostini I, Holzmann I, Di Bitetti MS 2012. Influence of seasonality, group size and presence of a congener on activity patterns of howler monkeys. J Mammal 93: 645-657

Almeida MAB, Santos E, Cardoso JC, Fonseca DF, Noll CA, Silveira VR, Maeda AY, Souza RP, Kanamura C, Brasil RA (2012) Yellow fever outbreak affecting *Alouatta* populations in southern Brazil (Rio Grande do Sul State), 2008-2009. American Journal of Primatology 74: 68-76.

Araújo FAA, Ramos DG, Santos AL, Passos PHO, Elkhoury ANSM, Costa ZGA, Leal SG, Romano APM 2011. Epizootics in nonhuman primates during reemergence of yellow fever virus in Brazil, 2007 to 2009. Epidemiol Serv Saude 20: 527-536.

Beck R, de Vries D, Janiak M, Goodhead I, Boubli J. 2023. Total evidence phylogeny of platyrrhine primates and a comparison of undated and tip-dating approaches. Journal of Human Evolution. 174. 103293. 10.1016/j.jhevol.2022.103293.

Bicca-Marques JC, Freitas DS 2010. The role of monkeys, mosquitoes and humans in the occurrence of a yellow fever outbreak in a fragmented landscape in South Brazil: protecting howler monkeys is a matter of public health. Tropical Conservation Sci 3: 78-89.

Cortés-Ortiz L, Bermingham E, Rico C, Rodríguez-Luna E, Sampaio I, Ruiz-García M. 2003. Molecular systematics and biogeography of the Neotropical monkey genus, *Alouatta*. Molecular Phylogenetics and Evolution 26:64–81.

Crockett CM. 1998. Conservation biology of the genus *Alouatta*. International Journal of Primatology 19:549–578.

Gregorin R. 2006. Taxonomia e variação geográfica das espécies do gênero *Alouatta* Lacépède (Primates, Atelidae) no Brasil. Revista Brasileira de Zoologia 23:64–144.

Harris EE, Gifalli-Iughetti C, Braga ZH, Koiffmann CP. 2005. Cytochrome B Sequences Show Subdivision between Populations of the Brown Howler Monkey (*Alouatta guariba*) from Rio de Janeiro and Santa Catarina, Brazil. Neotropical Primates 13:16–21.

Holzmann I, Agostini I, Areta JI, Ferreyra H, Beldomenico P, di Bitetti MS. 2010. Impact of yellow fever outbreaks on two howler monkey species (*Alouatta guariba clamitans* and *A. caraya*) in Misiones, Argentina. American Journal of Primatology 72:475–480.

Kinzey, W. G. 1982. Distribution of primates and forest refuges. In: Biological Diversification in the Tropics, G. T. Prance (ed.), pp.455 – 482. Columbia University Press, New York.

Machado S. 2011. Filogeografia do bugio ruivo, *Alouatta guariba* (Primates, Atelidae). Dissertação (Mestrado): 43.

Malukiewicz J. 2018. A Review of Experimental, Natural, and Anthropogenic Hybridization in *Callithrix* Marmosets. International Journal of Primatology, 40 (1), 72–98, doi:10.1007/s10764-018-0068-0.

Malukiewicz J, Boere V, Fuzessy LF et al. 2014. Hybridization effects and genetic diversity of the common and black-tufted marmoset (*Callithrix jacchus* and *Callithrix penicillata*) mitochondrial control region. American Journal of Physical Anthropology, 155 (4), 522–536, doi: https://doi.org/10.1002/ajpa.22605.

Malukiewicz J. Hepp CM, Guschanski K, Stone, AC 2017. Phylogeny of the *jacchus* group of *Callithrix* marmosets based on complete mitochondrial genomes. American Journal of Physical Anthropology, 162 (1), 157–169, doi: https://doi.org/10.1002/ajpa.23105.

Malukiewicz J, Boere V, de Oliveira MAB, et al. 2021. An Introduction to the Callithrix Genus and Overview of Recent Advances in Marmoset Research. ILAR Journal, 61 (2-3), 110–138, doi:10.1093/ilar/ilab027.

Mammal Diversity Database. 2023. Mammal Diversity Database (Version 1.11) [Data set]. Zenodo. https://doi.org/10.5281/zenodo.7830771

Mares-Guia MAM, Horta MA, Romano A. et al. 2020. Yellow fever epizootics in non-human primates, Southeast and Northeast Brazil (2017 and 2018). Parasites Vectors 13, 90 (2020) https://doi.org/10.1186/s13071-020-3966-x

Moreno ES et al. 2015. Yellow fever impact on brown howler monkeys (*Alouatta guariba clamitans*) in Argentina: A metamodelling approach based on population viability analysis and epidemiological dynamics. Memorias do Instituto Oswaldo Cruz 110:865–876.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature, 403(6772), 853-858. doi: https://doi.org/10.1038/35002501

National Academies of Sciences and Medicine. 2019. Care, Use, and Welfare of Marmosets as Animal Models for Gene Editing-Based Biomedical Research: Proceedings of a Workshop. National Academies Press, Washington (DC). doi: 10.17226/25356.

Rayfield, B., Fortin, M. J., & Fall, A. (2011). Connectivity for conservation: a framework to classify network measures. Ecology, 92(4), 847-858. doi: https://doi.org/10.1890/09-2190.1

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM. 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biological Conservation 142:1141–1153.

Rylands, AB, Spironelo, WR, Tornisielo, VL, Sá, RL de, Kierulff, MCM. & Santos, IB. 1988. Primates of the Rio Jequitinhonha valley, Minas Gerais, Brazil. Primate Conservation. 9: 100–109.

Rylands AB, Mittermeier RA. 2009. The Diversity of the NewWorld Primates (Platyrrhini): An Annotated Taxonomy. Page (Garber PA, Estrada A, Bicca-Marques JC, Heymann EW, Strier KB, editors).

Rylands AB, Mittermeier RA. 2014. Primate taxonomy: Species and conservation. Evol. Anthropol., 23: 8-10. https://doi.org/10.1002/evan.21387

Schiel N, Souto A. 2017. The common marmoset: An overview of its natural history, ecology and behavior. Developmental Neurobiology, 77 (3), 244–262, doi: https://doi.org/10.1002/dneu.22458.

Smith, K. F., Acevedo‐Whitehouse, K., & Pedersen, A. B. (2009). The role of infectious diseases in biological conservation. Animal conservation, 12(1), 1-12. doi https://doi.org/10.1111/j.1469-1795.2008.00228.x

Vasconcelos PFC, Rosa APAT, Rodrigues SG, Rosa EST, Monteiro HAO, Cruz ACR 2001. Yellow fever in Pará state, Amazon Region of Brazil, 1998-1999: Entomologic and epidemiologic findings. Emerg Infect Dis 7: 565-569.

Chapter I

Genetic structure of *Alouatta guariba* (Primates, Atelidae) and conservation implications in the face of a recent yellow fever outbreak

Genetic structure of *Alouatta guariba* (Primates, Atelidae) and conservation implications in the face of a recent yellow fever outbreak

João Luiz Guedes da Fonseca, Christian Roos, Yuri Luiz Reis Leite

Abstract

The brown howler monkey, *Alouatta guariba* (Primates, Atelidae), occurs in the Atlantic Forest and is closely linked to forested habitats, being extremely dependent on them. Thus, they suffer directly from fragmentation and loss of habitat from one of the top biodiversity hotspots of the world, leading to inbreeding, loss of genetic diversity and population decline. In addition, they are extremely susceptible to the yellow fever virus, with very high mortality rates during disease outbreaks. Here we use Bayesian phylogenetic trees, population and landscape genetic analyses in brown howler monkey populations affected by yellow fever in southeastern Brazil in 2016, to test the hypothesis that the fragmentation of the Atlantic Forest could prevent the rapid spread of disease among isolated populations. The results suggest there was no discernible geographic pattern among the established clades, suggesting that the disease's spread is not hindered by habitat fragmentation. Additionally, the landscape genetics approach revealed a substantial genetic connectivity among all individuals, indicating that yellow fever transmission can readily take place even among seemingly isolated populations.

Keywords

Atlantic Forest, Landscape Genetics, Microsatellite.

Introduction

Infectious diseases have become a major concern in species conservation, as they can cause a temporary decline in abundance or the extinction of wild populations (Altizer et al. 2007; Smith et al. 2009). Furthermore, the infectious agent can interact with other factors, such as habitat loss and/or climate change, to accelerate this process of population decline or extinction (Smith et al. 2009). Thus, outbreaks in wild populations pose a significant risk to vulnerable species, are difficult to contain, and increase the chance of infection spreading to people (Miller 2022; Sidik 2023; Plewnia et al. 2023).

There are 11 species of howler monkeys [*Alouatta* Lacépède, 1799 (Primates, Atelidae)] distributed along Neotropical region, from southern Mexico to northern Argentina in South America (Crockett 1998; Cortés-Ortiz et al. 2003), being they extremely susceptible to the yellow fever virus with very high mortality rates during disease outbreaks (Araújo 2011; Almeida et al. 2012). The brown howler monkey [*Alouatta guariba* (Humboldt, 1812)] occurs in the Atlantic Forest of eastern South America, from northern Argentina to eastern Brazil, where it is the main *Alouatta* species. Arboreal primates are closely linked to their habitat, being extremely dependent on them (Estrada et al. 2017). Thus, given its distribution of occurrence, the Atlantic Forest hotspots (Myers et al. 2000, Ribeiro et al. 2009, Hudson et al. 2014), Howler monkeys suffer directly with fragmentation and loss of habitat caused by growth cluttered use of areas for agriculture, livestock and logging (Hudson et al. 2014; Estrada et al. 2017; Rezende et al. 2019). This forces primates to live in fragments, usually isolated by inadequate matrices for the exchange of individuals, leading to inbreeding, loss of genetic diversity and population decline, making it difficult to restructure the remaining populations (Segelbacher et al. 2010; Estrada et al. 2017).

In Argentina, researchers estimated that the yellow fever killed 83 howler monkeys during an outbreak that occurred in 2007 and 2008 (Holzmann et al. 2010). Due to the high susceptibility to this disease, *A. guariba* was considered 'critically endangered' (Agostini et al. 2012). Since the 2016 reemergence of the yellow fever virus in southeastern Brazil, hundreds of brown howler monkeys have been found dead. During outbreaks, there have been cases of nearby people killing off monkeys in fear that they were responsible for spreading the yellow fever, which is an aggravating issue

that contributes to problems they face during outbreaks (Holzmann et al. 2010). That summed to the fact that the Atlantic Forest is extremely fragmented (Myers et al. 2000; Fahrig 2003; Ribeiro et al. 2009), is probably causing a genetic bottleneck effect on the populations of *A. guariba*.

Although there have been significant efforts in recent years, scientific data on the majority of primate species is still lacking. The scarcity of such knowledge highlights the urgent need to generate species-specific information regarding population genetics, life history and ecology (Estrada et al. 2017). Only then, one would be able to devise an effective conservation plan for the remaining populations. Here we use Bayesian phylogenetic trees, population and landscape genetic analysis in *A. guariba* populations affected by yellow fever in southeastern Brazil, to test the hypothesis that the fragmentation of the Atlantic Forest could prevent the rapid spread of disease among isolated populations.

Materials and Methods

We collected samples from liver or muscle tissue taken from 160 *Alouatta* individuals (SM Table 1) that died due to the 2016 yellow fever outbreak, collected in the wild, in the states of Espírito Santo and Minas Gerais, Brazil. *Alouatta caraya* (Humboldt, 1812), *Alouatta seniculus* (Linnaeus, 1766) and *Ateles belzebuth* (É. Geoffroy, 1806) were used as outgroups (SM Table 1). Genomic DNA was extracted from the samples through a standard saline protocol (Bruford et al. 1992). We measured the concentration and quality of the DNA with the spectrophotometer Nanodrop. Eleven microsatellite markers (SM Table 2) were genotyped in the *Núcleo de Genética Animal Aplicada à Conservação da Biodiversidade* from the University of Espírito Santo in Brazil (NGACB-UFES). We used the method described by Schuelke (2000) to attach the standardized M13 tail to each primer and the M13 sequence for each fluorescent dye (SM Table 2). The mitochondrial gene D-loop was amplified by PCR and sequenced using the Sanger method in the *Deutsches Primatenzentrum* (DPZ), in Göttingen, Germany.

The D-loop sequences were viewed in MEGA X and aligned with the online platform MAAFT. Then we performed Phylogenetic inference analysis using Bayesian and Maximum Likelihood approaches performed by MrBayes on the online platforms

CIPRES and IQTREE respectively. The chosen evolution model by the AIC and BIC criterion was HKY + F + G4. On MrBayes it was used the evolution model *lset nst* = 2, including gamma and proportion of invariable sites parameter. Eight Markov chain Monte Carlo iterations were run simultaneously with sampling trees every 1000 generations and 25% of burn-in, until the convergence diagnostic reached the stop value (standard deviation of split frequencies <0.01, and monitoring the effective sample size). To estimate the genetic distance among clades, we used the Jukes-Cantor substitution model to generate a Neighbor-Joining phylogenetic reconstruction to generate a pairwise genetic distance matrix.

The microsatellite markers were viewed and genotyped with the software GENEIOUS 9. We performed basic genetic statistical analysis with the PopGenReport package (Adamack and Gruber 2014) in R (R Development Core Team) to assess the characteristics of microsatellite markers. Subsequently, the allele richness, deviations from the Hardy-Weinberg equilibrium by location and locus of the sample were calculated. Genetic divergence, diversity and expected and estimated heterozygosity for each locus and for the whole sample were also estimated for the microsatellite data using population genetic packages in R such as Adegenet (Jombart and Ahmed 2011) and PopGenReport. This analysis provided two different matrices of genetic distances between individual samples, one following methods proposed by Kosman and Leonard, 2005 and another following Smouse and Peakall (1999). Both sets of markers were used separately to evaluate the genetic structure.

For the landscape genetics analyses, an Environmental Niche Model (ENM) was created for the species using occurrence data covering the full range of the known distribution of the species. Species occurrence data were collected from public databases such as Splink, GBIF (Global Biodiversity Information Facility, www.gbif.org) and VertNet (VertNet: Distributed Databases With Backbone, http://vertnet.org/). Occurrence points with many potential location errors were excluded. 19 bioclimatic variables from WorldClim, percentage tree cover variable from Earth Engine Partners, elevation from EROS Center, were used to create a slope layer. Subsequently, the PCA and correlation analysis was performed with all the variables to define which ones to use. After this step, the models were created using the MaxEnt software, the raster maps of the models were edited in R and QGIS (QGIS Development Team 2019). The different models were evaluated and the one with the highest AUC score (area under the curve) was selected to be converted into a resistance surface for

insulation by resistance analysis. The model was transformed into a resistance surface layer in R.

Levels of genetic structure were measured using programs such as the R packages GENELAND (Guillot et al., 2008) and TESS3R (Caye et al., 2017). For GENELAND, the parameters were as follows: the number of HWLE (Hardy-Weinberg equilibrium with linkage equilibrium between loci) populations is uncertain and assumed to be less than 10 in the MCMC (Markov Chain Monte Carlo) simulations. The spatial model was employed in combination with the correlated frequency model. A total of 100,000 MCMC iterations were conducted, with only every 100th iteration being saved on the disk for analysis and further examination. TESS3 employs a Q-matrix to store ancestry coefficients and leverages geographic coordinates for result visualization. This algorithm represents a new iteration of the TESS program (Chen et al., 2007), incorporating geographically constrained matrix factorization and quadratic programming techniques (Caye et al., 2016).

For spatial analyses, we used landscape genetics methods (Manel 2003) based on circuit theory and resistance insulation (McRae and Meier 2007) with the QGis open GIS program and the Circuitscape program (McRae and Meier 2007). Circuitscape is a program that uses circuit theory to model connectivity in landscapes. It can model the movement and gene flow and identify areas that are important for connectivity conservation. Circuit theory is useful because it can evaluate multiple dispersal pathways and relate the calculated current flow, voltages, and effective resistances to ecological processes such as movement and gene flow (McRae et al. 2008). Different possible routes can be used by animals to migrate from one fragment of habitat to another to establish gene flow. Thus, we estimate which parts of the landscape offer the most resistance to gene flow. A matrix of cumulative resistance is generated by this method and we used it as a cost matrix to compare with the genetic distance and geographic distance matrices.

We performed a Mantel Test for each combination of distance matrices generated by Circuitscape and landgenreport function from the popgenreport package to check whether the matrices were significantly different from one another. Then we applied the Partial Mantel Test for each resistance matrix generated by circuitscape with the resistance surfaces of tree cover, altitude and the maxent ENM to evaluate if the environment elements here tested are the ones driving the genetic distances between individuals despite the geographic distances. We also use the same variables to perform

a Multiple Matrix Regression with Randomization analysis (Wang 2013) where the genetic distance matrix was the dependent variable and the resistance (cost) matrices were the independent variables.

Results

Phylogenetic analysis

The Dloop sequences generated 77 unique haplotypes out of 140 total sequences. The total length of the Dloop sequence was 509 bp (410 excluding gaps and missing data) with 118 variable sites. Most haplotypes (51) were composed of only one single sequence. Two haplotypes were represented by 10 sequences, both included in the GREEN clade (Figure 1). The remnant haplotypes had less than 5 sequences.

The Maximum Likelihood and the Bayesian approach generated the same topology (Figure 1) . No clades formed show a significant geographic pattern. One thing to notice is the BLUE clade. All the sequences in this clade were found in the state of Minas Gerais along with one sequence from the North of the Rio Doce. Individuals in the RED clade are found only in the mountainous region of the state Espírito Santo. The closest clade related to the RED clade was composed of one individual (purple dot in Figure 1) collected further west in Minas Gerais but close to the border to Espírito Santo state. Another thing to notice is that individuals located at the far northwestern of the region, close to the distribution range of *A. caraya*, are included in the GREEN clade, which has individuals located everywhere in the sampling region. Genetic distances within and between clades can be seen in SM Table 3.

Samples and Markers

The microsatellite markers were informative (SM Table 2) in the sense that they have enough diversity to differentiate between individuals and do not show signs of null alleles except Apm04. Therefore, we checked if using this marker would make any significant difference for the subsequent analyses. There was no significant difference using this marker or not, so it was not excluded from subsequent analyses. The markers also did not show significant evidence of departure of Hardy-Weinberg equilibrium.

Genetic Structure

The genetic structure analyses using the microsatellites markers with GENELAND did not show evidence of division in subpopulations in the sampling region. Only the TESS approach showed evidence of a division between a north and south cluster, when choosing K=2 (Figure 2 A and B). When we chose K = 3, a west cluster forms with individuals from east Minas Gerais but with individuals also appearing in the mountainous region of Espírito Santo (Figure 2 C and D). However, little evidence of clustering is shown upon inspection of the barplots these two options, K=2 and K=3, generated (Figure 2 A and B).

ENM

For the Environmental Niche Modeling we chose to use the model with the bias layer included in the Maxent algorithm because the samples used here were very concentrated in one region compared to the distribution points we found from other sources. The AUC value was 0.82 with a standard deviation of 0.023.

Landscape Genetics

The Isolation by Resistance approach using Tree Cover, Altitude and ENM generated 3 cumulative current maps (Figure 3), showing the connectivity between each point and the resistance to cross each possible route, and three pairwise cumulative resistance matrices, called here Tree Cover Cost, Altitude Cost and Model Cost respectively. All three variables showed a consistency in possible routes that individuals can be genetically connected to each other (Figure 3). Another matrix was made in PopGenReport containing pairwise geographic distance between points. The Mantel Tests between the matrices showed three pairs of matrices correlated with each other, Model Cost with Altitude Cost, Geographic Distance with Altitude Cost and Geographic Distance with Model Cost (Table 1). This was expected since some of those variables were used to generate the ENM. The series of partial Mantel Tests and Multiple Matrix Regression tests were not significant (Tables 2 and 3). The Tree Cover model was the most correlated to the genetic distance between samples, although also not significant.

Discussion

Based on our results, we can refute the hypothesis that fragmentation of the Atlantic Forest could delay the rapid spread of yellow fever among populations of *A. guariba.* The formed clades did not present a significant geographic pattern, indicating that the spread of the disease is not restricted or limited by habitat fragmentation. Furthermore, the Landscape Genetics approach showed that all individuals are genetically fairly connected, suggesting that yellow fever transmission can easily occur among apparently isolated populations.

Phylogenetics

The D-Loop tree topology did not recover any clearly recognizable biogeographic population division for the region, suggesting that the genetic flow is rather free among them. Thus, we may be facing a phylogenetic signal reflecting ancient events. The complex and dynamic history of landscape changes in the Atlantic Forest during glacial and interglacial periods, with the expansion of the forest in some regions and the retraction in others (Carnaval et al. 2014; Batalha-Filho and Miyaki 2016; Leite et al. 2016; Mascarenhas et al. 2019), may have caused the isolation of populations in forest fragments during cold periods, insufficient time for speciation, but enough to leave marks in the D-Loop marker. Thus, after the temperature increase culminating in the expansion of the Atlantic Forest, the fragments reconnected, leading to a rearrangement in population distribution of howler monkeys. Additionally, due to the high susceptibility of howler monkeys to Yellow Fever, highly connected populations can still maintain high levels of genetic flow and diversity even when facing high loss of individuals when an outbreak hits the populations.

Levels of genetic distance were low within and between clades (Table 4). An interesting thing to notice is that the distance between clades of *A. guariba* were very close to the distance level between *A. seniculus* and *A. caraya,* sister clades treated here as outgroups. This shows that although the sample region is small compared to the occurrence region for the species, the diversity is far greater than expected. We have to take in consideration of course the number of samples for each comparison. For *A. caraya* and *A. seniculus*, we had 3 and 1 sample respectively. This high diversity is also corroborated by the number of unique haplotypes found (77) and haplotype diversity (Hd = 0,9810). In comparison, a recent study from Povil et al. (2023) found 26 unique haplotypes with a haplotype diversity (Hd) of 0.8359 in a study covering a greater

distribution range for the same species although using a different, more conserved mitochondrial marker, Cytochrome B.

Genetic Structure

Comparing the results of both sets of markers, the clusters formed in different regions. This can be due to the different evolution rates of those markers. Microsatellite markers tend to show a higher evolution rate than mitochondrial DNA, so it is expected that the clustering formed by these kinds of markers is showing a more recent effect. The west part of this region is the limit of the species distribution and the contact zone between the *A. caraya* species. In addition, the north part of this region is believed to be the division between the two subspecies (or species according to some authors, eg. Gregorin, 2006) of *A. guariba, A. guariba clamitans* and *A. guariba guariba*.

The clusters formed using the TESS approach also show a significant mix of levels of individual ancestry coefficients for all the two or three clusters analyzed here. Only a few individuals showed a clear membership to one or other clusters but even in those cases there were no geographic components that could justify considering more than one genetic population for the sampled region. So we subsequently considered that we are dealing with only one genetically connected population in the sampled region and treated all the other analyses based on genetic differences between individuals, not populations.

Landscape Genetics e ENM

No tests done here showed evidence of any environmental elements influencing the genetic distances between individuals. What we found instead is that all the populations, if there is more than one in the region, were relatively well genetically connected. That might explain why diseases such as Yellow Fever spread so fast and killed so many individuals during the 2016 outbreak. Neither Tree Cover, Geographic distance, Altitude or Environmental Model correlates significantly with genetic distances between individuals.

That means that the pairwise genetic distances are not well explained in this region by the resistance caused by the negative environmental suitability. Our first hypothesis was that at least Tree Cover or Altitude would show a significant correlation because the individuals located in regions surrounded by areas with low forest cover would have more resistance to move across those areas as shown by the cumulative

current maps generated by Circuitscape. However, we think that, due to most of the samples being clustered in the central mountainous region of Espírito Santo, which is very well forest covered, a bias could have been generated for the matrix correlation tests we did.

The sampled region is relatively uniform in terms of levels of Niche Suitability using climatic, tree cover and altitude variables. This was expected for the mountainous region of Espírito Santo which, although fragmented, is well preserved compared to other regions of the state due to the difficult topographical conditions for agriculture and livestock to thrive (IPEMA 2011, Rezende et al. 2019). The region has also historically been a hotspot for conservation efforts due to high levels of biodiversity and endemism (Moreira et al. 2008; IPEMA 2011).

A small number of individuals were collected from different regions other than along the mountain range of Espírito Santo. We had expected that for these other sample regions, the suitability levels could've been different enough to contribute to hamper genetic flow between other areas. However, we found that, for the state of Espírito Santo, as a whole, the models show intermediate to high levels of niche suitability. In the western part of the state, close to the border of the state of Minas Gerais, we found the highest suitability levels for the whole distribution range of *A.guariba*. This area corresponds to the Caparaó mountainous region where Pico da Bandeira, the second highest mountain of Brazil is located and curiously we even had no sample points there to generate the model. It is necessary to point out that Espírito Santo is where we had most of the distribution points used so it is possible we could've generated a bias toward more suitability levels there. But even for the models generated using a Sample Bias variable we got very similar results.

Tables

Table 1: Pairwise Mantel test matrix. Values above the diagonal are R coefficients. Values below the diagonal represent p-values. * represents significative values

	Altitude Cost	Tree Cover Cost	Model Cost	Geographic Distance	Genetic Distance
Altitude Cost	-	-0.444	0.742	0.625	0.002
Tree Cover Cost	1.000	-	-0.136	-0.013	0.019
Model Cost	***0.000**	0.940	-	0.891	0.014
Geographic Distance	***0.001**	0.692	***0.000**	-	0.010
Genetic Distance	0.452	0.379	0.350	0.376	-

Table 2: Partial Mantel statistic based on Pearson's product-moment correlation. The R values are the correlation coefficients excluding the variance of the geographic distance matrix.

	Mantel statistic R	Significance
Altitude Cost	-0.005	0.509
Tree Cover Cost	0.020	0.375
Model Cost	0.010	0.395

Table 3: Multiple Matrix Regression with randomization analysis results using all resistance surface variables.

	Coefficients	T Statistic	T p-value
Intercept	0.000	0.002	0.754
Altitude Cost	0.000	-0.013	0.998
Tree Cover Cost	0.022	1.506	0.712
Model Cost	0.017	0.903	0.817

Table 4: DNA polymorphism analyses generated by DNAsp.

Hd (Haplotype diversity)	Variance of Haplotype diversity	Standard Deviation of Haplotype diversity	Pi (Nucleotide diversity)
0.981	0.00002	0.004	0.04586

Table 5: Hs and Ht based estimates of differentiation for each microsatellite marker.

	Hs	**Ht**	**Gst**	**Gprime_st**	**D**
ab04	0.469	0.540	0.132	0.266	0.145

ab06	0.835	0.876	0.047	0.313	0.275
ab07	0.758	0.741	-0.023	-0.107	-0.079
ab09	0.616	0.694	0.113	0.314	0.220
ab12	0.673	0.793	0.151	0.497	0.400
ab17	0.794	0.798	0.005	0.028	0.022
ab20	0.408	0.517	0.210	0.377	0.198
apm01	0.718	0.763	0.059	0.225	0.173
apm04	0.512	0.594	0.138	0.302	0.182
apm09	0.635	0.720	0.118	0.348	0.253
ll157	0.415	0.438	0.052	0.097	0.043

Figures

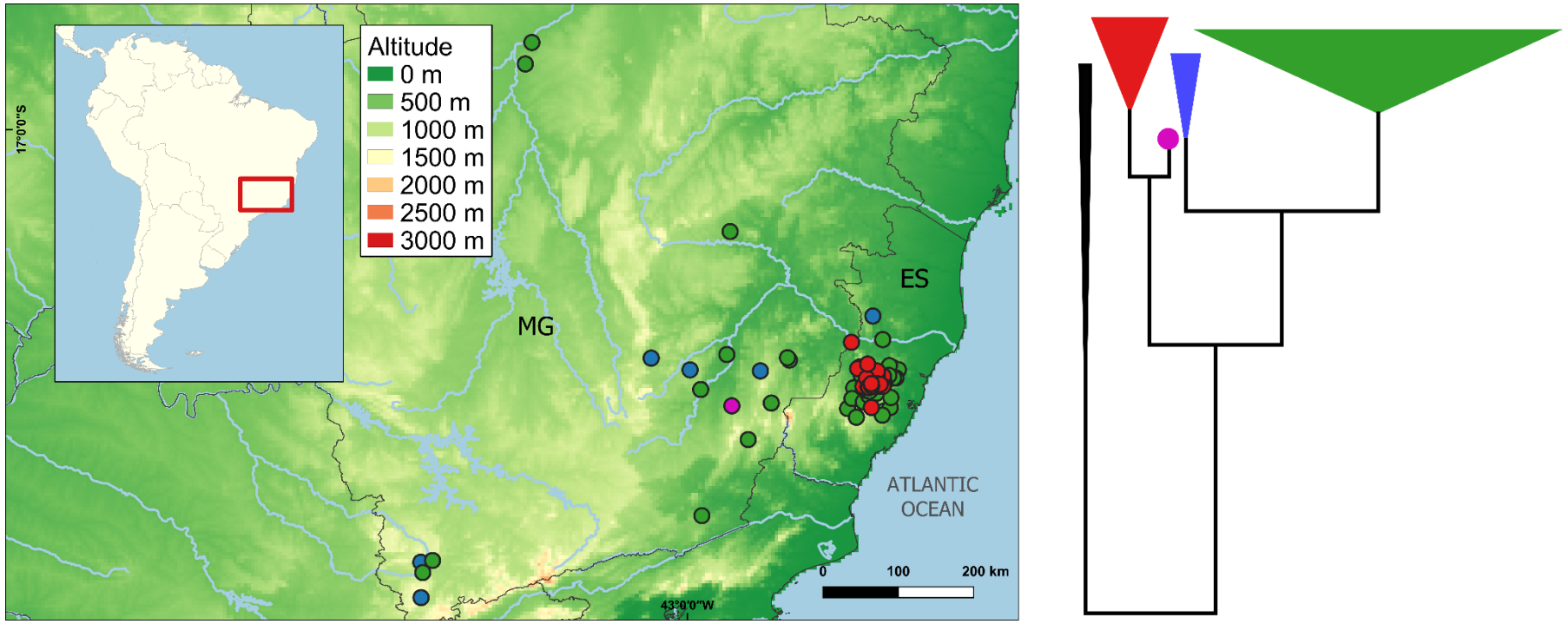

Figure 1. Distribution of *Alouatta guariba* samples and Bayesian/Maximum-likelihood phylogeny. All clades had Posterior Probabilities and Bootstrap > 99. MG: Minas Gerais; ES: Espírito Santo; blues lines: rivers. The colors of the dots match the clades colors: GRENN, BLUE, RED and PURPLE. Outgroup is the black clade.

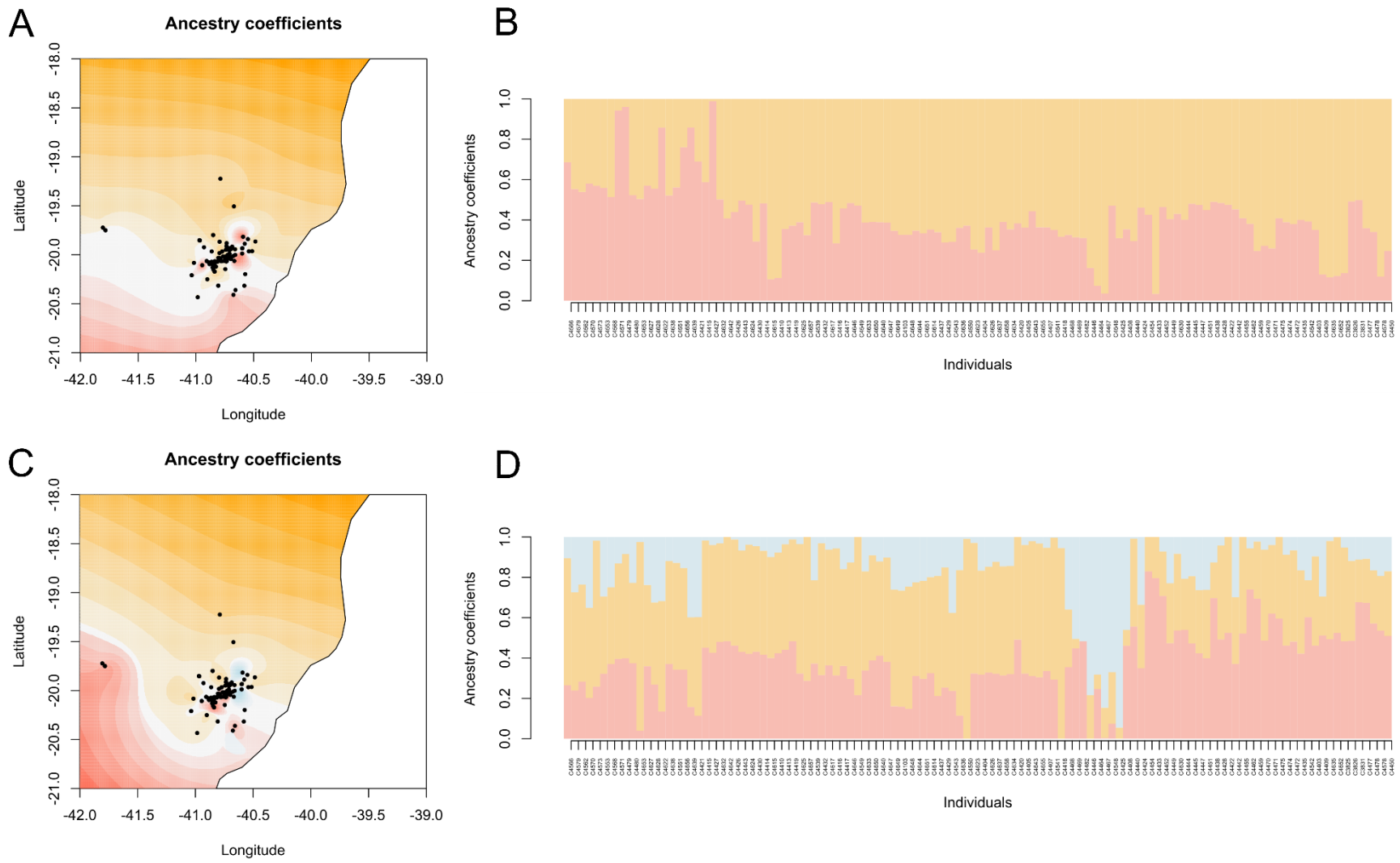

Figure 2. Tessellation map generated in TESS3. **A** and **B** present the result of k = 2 and **C** and **D,** k = 3. Barplots in **B** and **D** show the membership probability of one individual to the clusters (represented by the different colors) The individuals are sorted by Q in the barplots.

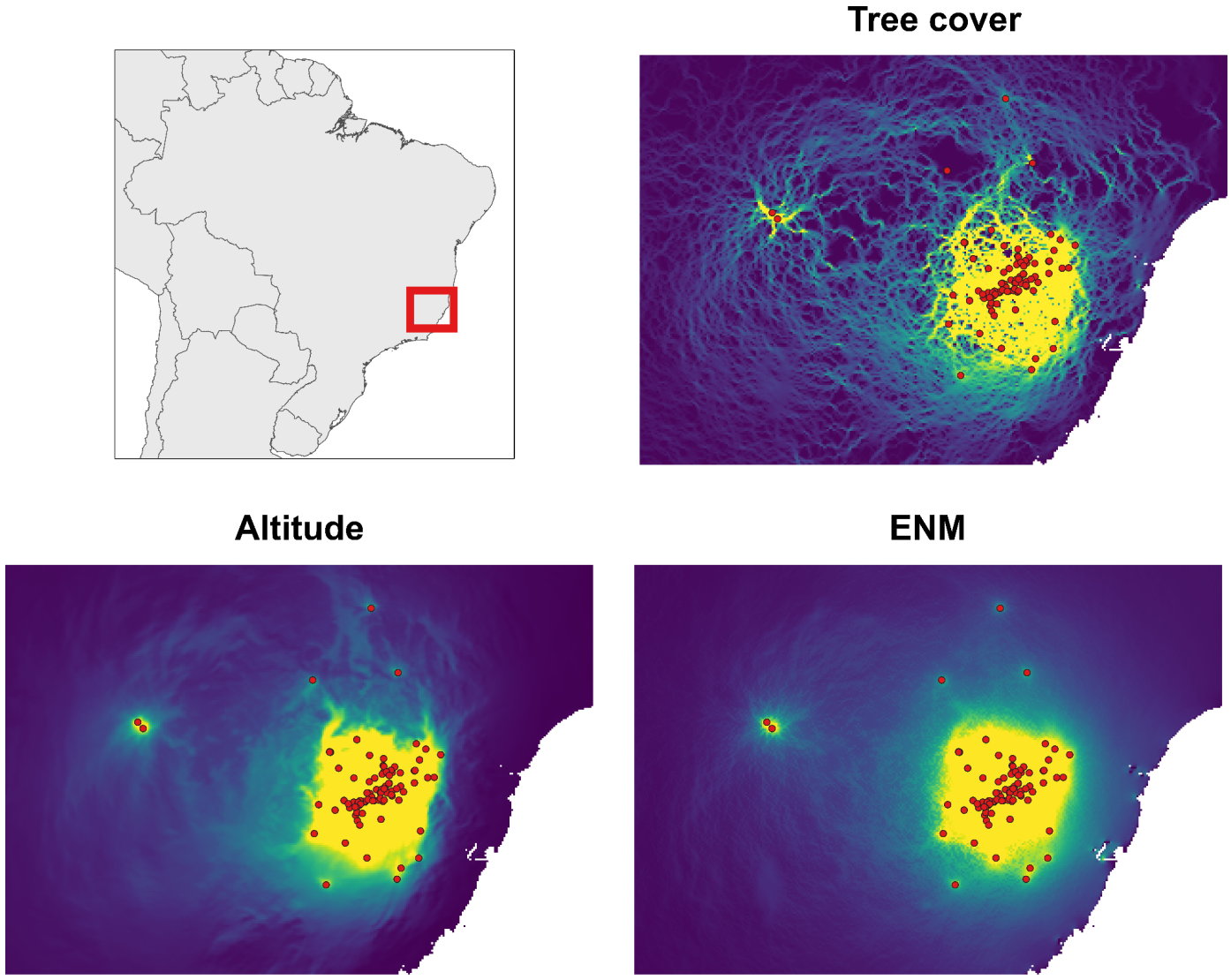

Figure 3. Isolation by Resistance approach using Tree cover, Altitude and ENM (Environmental Niche Model). Red dots are samples; Brighter colors indicate more electric current or a higher connectivity route probability.

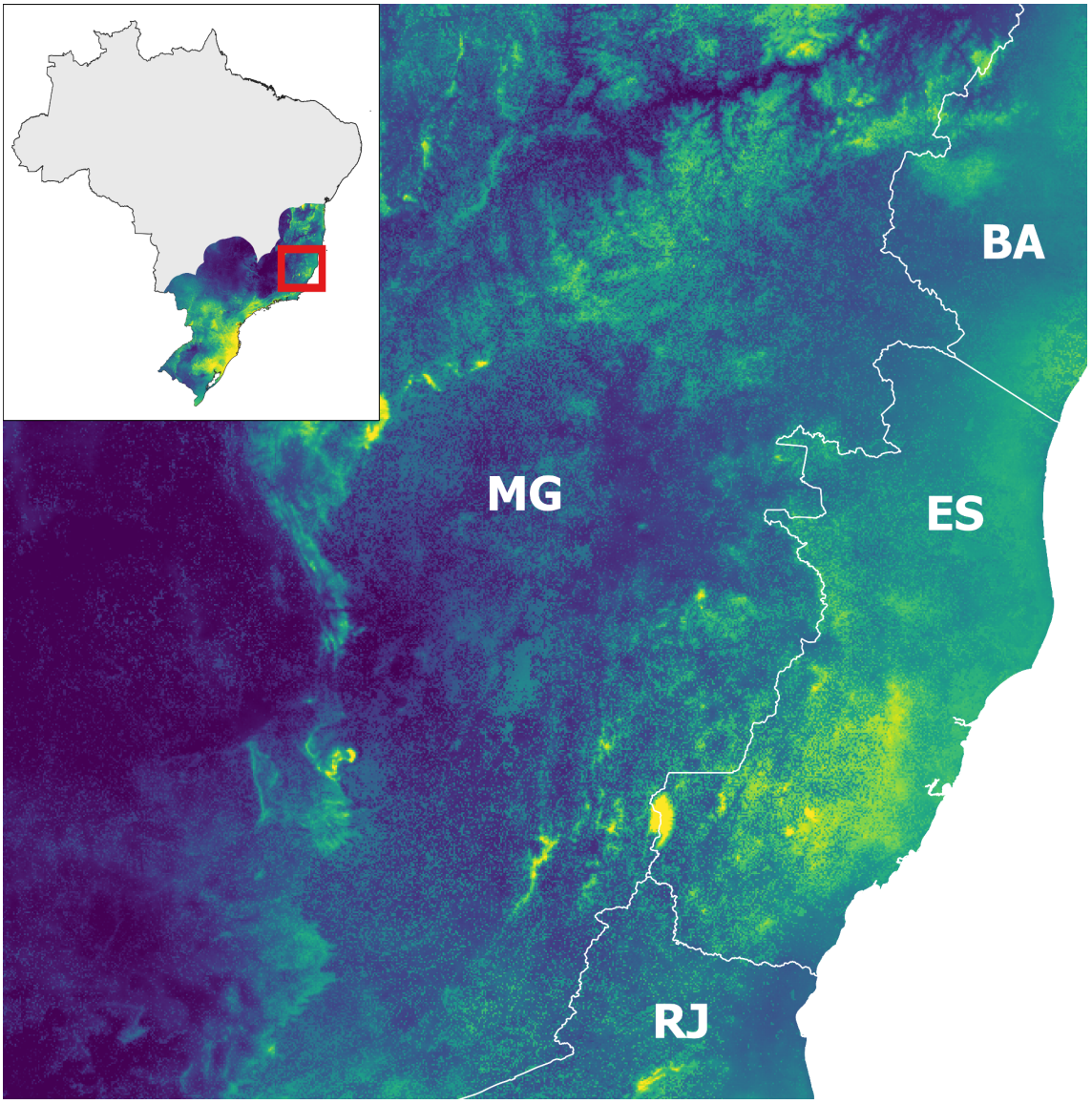

Figure 4. Environmental Niche model generated using Maxent. The model used WorldClim climatic variables, tree cover and altitude. Brighter colors indicated a higher suitability.

Supplementary Material

SM Table 1 - Detailed locations of the *Alouatta* samples. Not all samples included in the analyses had precise collection coordinates and, for the analyses that required this kind of data, we used therefore only those samples with this information. *GenBank Accession Number.

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
MG352A	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG352B	*Alouatta guariba*	Minas Gerais	-		
CTA3825	*Alouatta guariba*	Minas Gerais	Caratinga	-19.7500	-41.7830

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA3826	*Alouatta guariba*	Minas Gerais	Caratinga	-19.7500	-41.7830
CTA3831	*Alouatta guariba*	Minas Gerais	Caratinga	-19.7500	-41.7830
CTA4103	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0440	-40.6970
CTA4403	*Alouatta guariba*	Espírito Santo	Itaguaçu	-19.7990	-40.8510
CTA4404	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0170	-40.7650
CTA4405	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0020	-40.6600
CTA4407	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-19.9890	-40.7100
CTA4408	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9440	-40.6610
CTA4409	*Alouatta guariba*	Espírito Santo	Itaguaçu	-19.7990	-40.8510

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4410	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0710	-40.8150
CTA4413	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0710	-40.8150
CTA4414	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0720	-40.8640
CTA4417	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0640	-40.9070
CTA4418	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-19.9830	-40.7960
CTA4419	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0690	-40.8180
CTA4420	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0020	-40.7250
CTA4421	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.1070	-40.9460
CTA4422	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9120	-40.7360

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4425	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9490	-40.7400
CTA4426	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0860	-40.8410
CTA4427	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0960	-40.8820
CTA4428	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9160	-40.7330
CTA4429	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0300	-40.7480
CTA4430	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0790	-40.8880
CTA4432	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0660	-40.8360
CTA4433A	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9400	-40.7210
CTA4433B	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0940	-40.8390

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4434	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.8400	-40.5500
CTA4435	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0310	-40.8020
CTA4437	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9180	-40.6860
CTA4438	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9420	-40.6970
CTA4440	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.8870	-40.5770
CTA4442	*Alouatta guariba*	Espírito Santo	Afonso Cláudio	-20.0830	-41.0170
CTA4444	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9330	-40.7020
CTA4445	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9330	-40.7020
CTA4446	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9650	-40.5420

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4447	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9330	-40.7020
CTA4449	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9350	-40.6000
CTA4450	*Alouatta guariba*	Espírito Santo	Pancas	-19.2240	-40.7880
CTA4451	*Alouatta guariba*	Espírito Santo	Itarana	-19.9240	-40.9300
CTA4452	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9350	-40.5990
CTA4454	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9490	-40.7210
CTA4455	*Alouatta guariba*	Espírito Santo	Venda Nova	-19.8820	-40.7340
CTA4456	*Alouatta guariba*	Espírito Santo	Venda Nova	-20.3290	-41.0900

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4457	*Alouatta guariba*	Espírito Santo	Fundão	-19.8650	-40.4850
CTA4458	*Alouatta guariba*	Espírito Santo	Venda Nova	-19.8820	-40.7340
CTA4459	*Alouatta guariba*	Espírito Santo	Fundão	-19.8650	-40.4850
CTA4462	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.8670	-40.7950
CTA4463	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.8670	-40.7950
CTA4464	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-19.9650	-40.5420
CTA4467	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9640	-40.5130
CTA4468	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-19.9670	-40.7720
CTA4469	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-19.9660	-40.7730

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4470	*Alouatta guariba*	Espírito Santo	Laranja da Terra	-19.8540	-40.9660
CTA4471	*Alouatta guariba*	Espírito Santo	Laranja da Terra	-19.8540	-40.9660
CTA4472	*Alouatta guariba*	Espírito Santo	Laranja da Terra	-19.8520	-40.9690
CTA4473	*Alouatta guariba*	Espírito Santo	Laranja da Terra	-19.8520	-40.9690
CTA4474	*Alouatta guariba*	Espírito Santo	Laranja da Terra	-19.8520	-40.9690
CTA4475	*Alouatta guariba*	Espírito Santo	Laranja da Terra	-19.8530	-40.9700
CTA4476	*Alouatta guariba*	Espírito Santo	Itaguaçu	-19.8310	-40.9420
CTA4477	*Alouatta guariba*	Minas Gerais	Caratinga	-19.7230	-41.8060
CTA4478	*Alouatta guariba*	Minas Gerais	Caratinga	-19.7230	-41.8060

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4479	*Alouatta guariba*	Espírito Santo	Afonso Cláudio	-20.2090	-41.0370
CTA4480	*Alouatta guariba*	Espírito Santo	Afonso Cláudio	-20.2090	-41.0370
CTA4482	*Alouatta guariba*	Espírito Santo	Itarana	-19.9660	-40.8640
CTA4543	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0300	-40.7180
CTA4548	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9550	-40.7100
CTA4549	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0630	-40.6670
CTA4550	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0260	-40.7230
CTA4551	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.1300	-40.8560
CTA4552	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-19.9650	-40.5410

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4553	*Alouatta guariba*	Espírito Santo	Domingos Martins	-20.3160	-40.8060
CTA4562	*Alouatta guariba*	Espírito Santo	Domingos Martins	-20.3610	-40.6580
CTA4566	*Alouatta guariba*	Espírito Santo	Domingos Martins	-20.4340	-40.9850
CTA4568	*Alouatta guariba*	Espírito Santo	Domingos Martins	-20.3160	-40.8060
CTA4570	*Alouatta guariba*	Espírito Santo	Domingos Martins	-20.3160	-40.5810
CTA4571	*Alouatta guariba*	Espírito Santo	Domingos Martins	-20.2500	-40.9010
CTA4573	*Alouatta guariba*	Espírito Santo	Domingos Martins	-20.3160	-40.8070
CTA4578	*Alouatta guariba*	Espírito Santo	Colatina	-19.5060	-40.6710
CTA4579	*Alouatta guariba*	Espírito Santo	Marechal Floriano	-20.4090	-40.6750

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4614	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0320	-40.7200
CTA4615	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0720	-40.8450
CTA4617	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0650	-40.7750
CTA4622	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.1530	-40.8480
CTA4623	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0230	-40.6980
CTA4624	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0800	-40.8340
CTA4625	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0690	-40.7370
CTA4628	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.1720	-40.8380
CTA4630	*Alouatta guariba*	Espírito Santo	Santa Teresa	-19.9330	-40.5970

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4632	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0960	-40.8520
CTA4633	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0630	-40.7550
CTA4634	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0130	-40.6770
CTA4635	*Alouatta guariba*	Espírito Santo	Itaguaçu	-19.7990	-40.8510
CTA4636	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0300	-40.7450
CTA4637	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0130	-40.7310
CTA4638	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.1470	-40.7450
CTA4639	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.1170	-40.8510
CTA4640	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0550	-40.7490

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4641	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0270	-40.6470
CTA4642	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0930	-40.8580
CTA4643	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-19.9910	-40.7100
CTA4644	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0350	-40.7930
CTA4646	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0640	-40.7320
CTA4647	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0490	-40.7860
CTA4648	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0390	-40.7000
CTA4649	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0490	-40.7860
CTA4650	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0610	-40.8020

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
CTA4651	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0320	-40.8060
CTA4652	*Alouatta guariba*	Espírito Santo	Itaguaçu	-19.7990	-40.8510
CTA4653	*Alouatta guariba*	Espírito Santo	Santa Leopoldina	-20.1980	-40.5730
CTA4656	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.1210	-40.8260
CTA4657	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0660	-40.7810
CTA4658	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0130	-40.7310
CTA4659	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0210	-40.6930
CTA4661	*Alouatta guariba*	Espírito Santo	Baixo Guandu	-19.5390	-41.0430
CTA4662	*Alouatta guariba*	Espírito Santo	Santa Maria de Jetibá	-20.0280	-40.6720

Sample ID	Species	State	County	Latitude	Longitude
MG68	*Alouatta guariba*	Minas Gerais	José Raydan	-18.2160	-42.4910
MG142	*Alouatta caraya*	Minas Gerais	Chapada Gaúcha	-15.2910	-45.6240
MG144	*Alouatta guariba*	Minas Gerais	Manhuaçú	-20.2630	-42.0000
MG227	*Alouatta guariba*	Minas Gerais	Sem Peixe	-20.1020	-42.8420
MG366	*Alouatta guariba*	Minas Gerais	Abre Campo	-20.2970	-42.4700
MG395	*Alouatta guariba*	Minas Gerais	Bom Jesus do Amparo	-19.7270	-43.4320
MG512	*Alouatta caraya*	Minas Gerais			
MG605	*Alouatta guariba*	Minas Gerais	Argirita	-21.6080	-42.8280
MG611	*Alouatta guariba*	Minas Gerais	Senador José Bento	-22.1640	-46.1800
MG654	*Alouatta guariba*	Minas Gerais	Santa Rita de Caldas	-19.8800	-42.1300
MG716	*Alouatta guariba*	Minas Gerais	Marliéria	-19.6850	-42.5300
MG732	*Alouatta guariba*	Minas Gerais	Borda da Mata	-22.2870	-46.1570
MG851	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG871	*Alouatta guariba*	Minas Gerais	Além Paraíba	-21.8740	-42.6920
MG893	*Alouatta caraya*	Minas Gerais	Icaraí de Minas	-16.2160	-44.9340
MG905	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG906	*Alouatta guariba*	Minas Gerais	Congonhal	-22.1430	-46.0400
MG956A	*Alouatta guariba*	Minas Gerais			
MG957	*Alouatta guariba*	Minas Gerais	Senador Amaral	-22.5860	-46.1770
MG1249	*Alouatta guariba*	Minas Gerais			
MG1915	*Alouatta caraya*	Minas Gerais			
MG1940	*Alouatta guariba*	Minas Gerais	São Francisco	-15.9600	-44.8550
MG1374	*Alouatta guariba*	Minas Gerais			
*FJ785422	*Ateles belzebuth*	NA	NA	NA	NA
*KC757386	*Ateles belzebuth*	NA	NA	NA	NA
*HQ644333	*Alouatta seniculus*	NA	NA	NA	NA

SM Table 2 - Details of the microsatellite markers and primers used in this research.Since we used the fluorescent M13 tail, the actual primers used as forward have the 18 bp sequence of the M13 attached to the 5' end.

Loci		Primer sequence (5' - 3')	Repeat unit	N_a	TF (bp)
Ab04[1]	F	AGCGCCTCTCCTGGTTTTTAC	$(GA)_2AA(GA)_{12}AG$	4	169-177
	R	AAAAATTCCCAAACCCCACC			
Ab06[1]	F	GTGATTATTGTGTGGTACTTG	$(CT)_9TTT(CT)_{11}GTCTGTCT$ TAT $(AC)_{16}$	13	261-311
	R	ATGTATTTTTCTGGTTTTACC			
Ab07[1]	F	ACCCCCATCTCTTAAAACACAC	$(AC)_{21}$	7	106-212
	R	CCTACTGCCTAAGTCTCCCAAC			
Ab09[1]	F	AATGAAGACAACAAACGAC	$(GAAA)_{18}$	10	150-226
	R	TGAAGAACACACCTGAGG			
Ab12[1]	F	AAATCAAGGCCCACAGGG	$(GAAA)_{14}$	11	-
	R	CAAAGGCAAGAAAGCAAGAAG			
Ab17[1]	F	GGAAACAGTGGAAGACAAAAGGAG	$(GAAA)_2G(GAAA)_2$ GAGAAAAA$(GAAA)_{14}$	14	193-249
	R	AGATGGCCAAAGATAAAGACATGTAAAA			
Ab20[1]	F	GTGTGGTGGTGGGTGCGC	$(GAAA)_{16}$	7	230-278
	R	TTGCTTTTCCCCTTTTGTGTTTGC			
LL157[2]	F	TGGCAAGTCTGGTTTCAAGC	$(GA)_4(GT)_4(CT)_1(GT)_2(AT)$ $_1(GT)_{13}(GA)_9(GT)_5$	6	233-253
	R	TTCCAGACTGAGCTAGGATGC			
APM01[4]	F	CACGTGTGTCCAGCTTGTCT	$(TG)_{25}$	13	217-243
	R	ATTCTGCTGCCCTTGAGTTC			
APM04[4]	F	TGAGAGTGAGCACCTGCCTA	$(AC)_{21}$	6	232-262
	R	CAGCCCTGATCACAAAGTGT			
APM09[4]	F	CAGGGTTCCTCTTTCACTGG	$(CA)_{16}$	12	180-204
	R	TTGGGATCACAAGTGCTTCA			

The numbers accompanying the loci mean that the markers were published by: 1- Gonçalves *et al.* (2004); 2- Di Fiore; Fleischer (2004); 3- Perez-Sweeney *et al.* (2005); 4- Cortés-Ortiz; Mondragón; Cabotage (2009).
F = direct primer; R = reverse primer; N_a = number of alleles; TF = length of microsatellite fragments.

SM Table 3: Estimates of Evolutionary Divergence over Sequence Pairs between Groups and within groups.

	RED	GREEN	BLUE	*A. seniculus*	*A. caraya*	*Ateles belzebuth*	Genetic Distance within
RED							0.011
GREEN	0.109						0.011
BLUE	0.105	0.053					0.013
A. seniculus	0.186	0.196	0.197				n/c
A. caraya	0.179	0.188	0.186	0.109			0.070
Ateles belzebuth	0.273	0.265	0.262	0.279	0.261		0.029

References

Adamack AT, Gruber B. 2014. PopGenReport: simplifying basic population genetic analyses in R. Methods Ecol Evol 5: 384-387

Agostini I, Holzmann I, Di Bitetti MS 2012. Influence of seasonality, group size and presence of a congener on activity patterns of howler monkeys. J Mammal 93: 645-657

Almeida MAB, Santos E, Cardoso JC, Fonseca DF, Noll CA, Silveira VR, Maeda AY, Souza RP, Kanamura C, Brasil RA (2012) Yellow fever outbreak affecting Alouatta populations in southern Brazil (Rio Grande do Sul State), 2008-2009. American Journal of Primatology 74: 68-76.

Altizer, S, Nunn, CL, Lindenfors, P (2007). Do threatened hosts have fewer parasites? A comparative study in primates. Journal of Animal Ecology, 76(2), 304-314.

Araújo FAA, Ramos DG, Santos AL, Passos PHO, Elkhoury ANSM, Costa ZGA, Leal SG, Romano APM 2011. Epizootics in nonhuman primates during reemergence of yellow fever virus in Brazil, 2007 to 2009. Epidemiol Serv Saude 20: 527-536.
Atlântica no estado do Espírito Santo. Instituto de Pesquisas da Mata Atlântica (IPEMA), Vitória. 91 p.

Batalha-Filho H, Miyaki CY (2016) Late Pleistocene divergence and postglacial expansion in the Brazilian Atlantic Forest: multilocus phylogeography of Rhopias gularis (Aves: Passeriformes). J Zool Syst Evol Res 54:137–147

Bruford MW, Hanotte O, Brookfield JFY, Burke T. 1992. Single-locus and DNA fingerprinting. In Hoelzel AR (ed.), Molecular genetic analyses of populations. A practical approach. Oxford: IRL Press, 225-269

Carnaval AC, Waltari E, Rodrigues MT, Rosauer D, VanDerWal J, Damasceno R et al (2014) Prediction of phylogeographic endemism in an environmentally complex biome. Proc R Soc Lond B Biol Sci 281:20141461

Cortés-Ortiz L, Bermingham E, Rico C, Rodríguez-Luna E, Sampaio I, Ruiz-García M. 2003. Molecular systematics and biogeography of the Neotropical monkey genus, Alouatta. Molecular Phylogenetics and Evolution 26:64–81.

Crockett CM. 1998. Conservation biology of the genus Alouatta. International Journal of Primatology 19:549–578.

Estrada, A et al. 2017. Impending extinction crisis of the world's primates: Why primates matter. Sci. Adv.3, e1600946.

Fahrig L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology and Systematics 34: 487-515

Holzmann I, Agostini I, Areta JI, Ferreyra H, Beldomenico P, di Bitetti MS. 2010. Impact of yellow fever outbreaks on two howler monkey species (Alouatta guariba clamitans and A. caraya) in Misiones, Argentina. American Journal of Primatology 72:475–480.

Hudson LN, Newbold T, Contu S, Hill SL, Lysenko I, De Palma A, Phillips HR, Senior RA, Bennett DJ, Booth H, et al. 2014. The PREDICTS database: a global database of how local terrestrial biodiversity responds to human impacts. Ecol. Evol. 4: 4701–4735.

IPEMA. 2011. Áreas e ações prioritárias para a conservação da biodiversidade da Mata

Jombart T, Ahmed I. 2011. Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 21: 3070-3071

Kosman E, Leonard KJ. 2005. Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. Molecular ecology, 14(2), 415-424.

Kumar S, Stecher G, Li M, Knyaz C, and Tamura K. 2018. Molecular Biology and Evolution 35:1547-1549

Leite YL, Costa LP, Loss AC, Rocha RG, Batalha-Filho H, Bastos AC et al. 2016. Neotropical forest expansion during the last glacial period challenges refuge hypothesis. Proc Natl Acad Sci USA 113:1008–1013

Manel S, Schwartz MK, Luikart G, Taberlet P. 2003. Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18: 189-197

Mascarenhas R., Miyaki CY, Dobrovolski R, Batalha-Filho H. 2019. Late Pleistocene climate change shapes population divergence of an Atlantic Forest passerine: a model-based phylogeographic hypothesis test. Journal of Ornithology, 160, 733-748.

McRae BH, Meier P. 2007. Circuit theory predicts gene flow in plant and animal populations. Proc Natl Acad Sci USA 104: 19885-19890

Miller BJ. 2022. Why unprecedented bird flu outbreaks sweeping the world are concerning scientists. Nature, 606(7912), 18-19.

Moreira DDO, Coutinho BR, Mendes SL. 2008. O status do conhecimento sobre a fauna de mamíferos do Espírito Santo baseado em registros de museus e literatura científica. Biota Neotropica, 8, 163-173.

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature, 403(6772), 853-858.

Plewnia A, Böning P, Lötters S. 2023. Mitigate diseases to protect biodiversity. Science, 379(6637), 1098-1098.

QGIS Development Team. 2019. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://www.qgis.org/

Povill C, Oliveira MB, Abreu FVS, Oliveira RL, Perini FA, Monticelli C, Bueno C, Santos E, Pissinatti A, Bonvicino CR. 2023. Genetic Diversity and Insights about Distribution of Brown Howler Monkeys (Alouatta guariba Group) (Atelidae, Alouattinae). Int J Primatol 44, 517–539.

R Development Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rezende CL, Scarano FR, Assad ED, Joly CA, Metzger JP, Strassburg BBN, Mittermeier RA, et al. 2018. From hotspot to hopespot: An opportunity for the Brazilian Atlantic Forest. Perspectives in ecology and conservation, 16(4), 208-214.

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM. 2009. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biological Conservation 142:1141–1153.

Segelbacher G, Cushman SA, Epperson BK, Fortin M-JJ, Francois O, Hardy OJ, Holderegger R, Taberlet P, Waits LP, Manel S. 2010. Applications of landscape genetics in conservation biology: concepts and challenges. Conservation Genetics 11: 375-385

Sidik SM. 2023. How to stop the bird flu outbreak becoming a pandemic. Nature, 615(7951), 196-197.

Smith KF, Acevedo‐Whitehouse K, Pedersen AB. 2009. The role of infectious diseases in biological conservation. Animal conservation, 12(1), 1-12.

Smouse PE, Peakall ROD. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. Heredity, 82(5), 561-573.

Wang JI. 2013. Examining the full effects of landscape heterogeneity on spatial genetic variation: A multiple matrix regression approach for quantifying geographic and ecological isolation. Evolution, 67: 3403-3411.

Chapter II

Comparison of Mitogenomic and Mitochondrial Markers for the Phylogenetic and Evolutionary Study of Marmosets, with special focus on captive *Callithrix jacchus*

Comparison of Mitogenomic and Mitochondrial Markers for the Phylogenetic and Evolutionary Study of Marmosets, with special focus on captive *Callithrix jacchus*

João Luiz Guedes da Fonseca, Ricardo C. H. del Rosario, Maria Adélia Borstelmann de Oliveira, Guoping Feng, Claudia S. Igayara, Steven A. McCarroll, Patricia A. Nicola, Luiz C. M. Pereira, Jeffery Rogers, Christian Roos and Joanna Malukiewicz

Abstract

Marmosets, especially *Callithrix jacchus*, are becoming valuable, high-demand biomedical models but their supply outside of their native Brazil is limited. Thus, uncovering the ancestry and biogeographic origins of captive marmosets is an essential task to optimize their biomedical use. Such information facilitates assessing standing levels of captive marmoset genetic diversity, identifying hybrids, and maintaining genetic diversity of captive marmoset populations. Here, we present 16 newly sequenced mitochondrial genomes from marmosets with *C. jacchus* phenotypes from international biomedical and animal supply facilities. We combine these new data with publicly available sequences to compare usage of mitogenome and the mtDNA control region for determining the ancestry and diversity of captive marmosets. MtDNA control region and mitogenome haplotypes from all 16 newly sequenced marmosets grouped within *C. jacchus* phylogenetic clades. Overall, phylogenies based on mitogenomes rather than the control region showed better resolved branching patterns with stronger statistical support within *C. jacchus* clades. However, both mtDNA markers show that *C. jacchus* is the sister clade to the phylogenetic *C. penicillata* Caatinga biome clade. *Callithrix jacchus* mtDNA control region haplotypes showed the highest haplotype and nucleotide diversity, and were intermediate in terms of per sequence theta and polymorphic sites. The larger number of available *Callithrix* mtDNA control region sequences relative to mitogenomes were used to determine ancestral mtDNA haplotype biogeographic origins. However, biogeographic origins for many *C. jacchus* haplotypes including those from international captive facilities could not be determined, which we attributed to unknown provenance information from natural *C. jacchus* populations. Given our results, we encourage increased sampling and genomic sequencing efforts for natural *C. jacchus* populations in Brazil, and utilizing the mitogenome over other mtDNA markers for phylogenetic-based analyses. Further, combining marmoset mitogenomic data with phenotypic and nuclear genomic data will greatly enhance the biomedical use of marmosets.

Keywords

mitogenome, d-loop, callitrichids

Introduction

Callithrix Linnaeus, 1758 marmoset species, which are endemic to Brazil (Figure 1), possess a unique set of biological characteristics such as frequent twinning and rapid reproductive rate [1]. Such traits have resulted in marmosets becoming valuable biomedical models that are in high demand among international research facilities, but whose supply is limited [2]. It is estimated that the worldwide population of marmoset biomedical colonies comprises approximately 6,000 marmosets, of which 2,500 are found in Japan, 1,900 in North America, 1,000 in Europe, and 800 in South America [2]. Within the US, marmosets are housed across approximately 27 research facilities, and the founder marmosets imported into the US during the 1960s and 1970s came from Europe and the United Kingdom [2]. Pedigree information on the marmosets initially imported into the US is limited, but in many cases present-day marmoset colonies originate from stocks of a few dozen imported animals [2].

Research facilities generally maintain colonies that consist of individuals with the phenotype of common marmosets [*Callithrix jacchus* (Linnaeus, 1758)]. This species is characterized by white bushy ear tufts and a white forehead star, but the ancestral biogeographic origins of these captive monkeys are largely unknown. In Brazil, common marmosets occur within three major Brazilian biomes: the scrub-brush and semi-arid Caatinga, the semiarid savanna-like Cerrado, and the humid-coastal Atlantic Forest [1, 3]. Marmosets are also known to hybridize, both under free-ranging and captive conditions, with the majority of cases involving *C. jacchus* as one of the parental species [1, 4–6]. Brazilian free-ranging populations of common marmosets are declining [7], and marmosets for biomedical use are difficult to import under international wildlife trade regulations [2]. Given the limited marmoset supply outside of Brazil, uncovering the phylogeny, ancestral biogeographic origins, and hybrid status of captive marmosets is critical for their biomedical use. Such information is helpful in assessing standing levels of captive marmoset genetic diversity, identifying possible hybrids, and for maintaining genetic integrity of captive, biomedical marmoset populations.

Mitochondrial DNA (mtDNA) has been a valuable and informative genetic marker in resolving Callithrix phylogeny, divergence, biogeography, and hybrid identification [4, 6, 8–10]. Patterns of interspecific divergence analyzed using the

approximately 1200 base pair (bp) rapidly-evolving mtDNA control region, which is much smaller than the approximately 16,500 bp *Callithrix* mitogenome, are largely concordant with results from full *Callithrix* mitogenome analyses [4, 6, 8–10]. Here we report on a comparison of the mitogenome and the mtDNA control region for determining the ancestry and diversity of marmosets from international research and animal supply facilities by combining newly and previously available sequence data. We utilized both data types to address the following questions: (1) What are the ancestral origins of marmosets maintained at international captive facilities?; (2) Are cryptic hybrids present among international captive marmoset populations?; (3) How does the genetic diversity of *C. jacchus* mtDNA control region and mitogenome compare to that of other *Callithrix* taxa?

Materials and Methods

For this study, we newly sampled genetic material from a total of 16 marmosets with a *C. jacchus* phenotype from the New England Primate Research Center (NEPRC), a US animal supplier (Company A) and one Japanese animal supplier (CLEA, Tokyo, Japan). We also sequenced the mitogenome of a captive marmoset (BJT23) with a *C. aurita* phenotype whose skin sample was collected in 2015 in Brazil. Furthermore, we obtained publicly available and previously published mtDNA control region and mitogenomic sequences from GenBank from a total of 266 marmosets from five different *Callithrix* species and 4 different hybrid types (Table 1). Detailed information on marmoset mitogenomic and mtDNA control region sequences is given in Supplementary Table S1.

Captive marmosets outside of Brazil were collected under the approval of the Massachusetts Institute of Technology IACUC (protocol number 051705020). Tissue from BJT23 was collected under the approval of the ASU Institutional Animal Care and Use Committee Animals (ASU IACUC, protocols #11-1150R, 15-144R) and Brazilian Environmental Ministry (SISBIO protocols #47964–2 and #28075–2). The Brazilian marmoset sample BJT23 was registered in the Brazilian SISGEN database under number A2E885E. Biological tissue sampling complied with all institutional, national, and international guidelines. This research adhered to the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates Species.

phenotypic classifications of newly sampled marmosets were based on published phenotypic descriptions following [1].

Laboratory and Sequencing Protocol

To obtain the control region sequence of individual BJT23, we sequenced the entire mitogenome following the long-range PCR strategy of [6, 10]. To check for the presence of nuclear copies of mitochondrial genes (numts) in the mitogenone sequence of BJT23, we first checked the sequence electropherogram which showed high quality base calls with no ambiguous nucleotides, indicating absence of different populations of mitochondrial-like sequences [10]. Then, sequences of all 13 mtDNA protein-coding genes were translated into protein sequences to check for premature stop codons and frame shifting insertions or deletions [10, 11]. Next, neighbor-joining trees were constructed with MEGA 11 [12] for each protein-coding gene using BJT23 sequences and those from previously available mitogenome sequences described above to check for unusual phylogenetic placement of species sequences. These analyses did not reveal any unexpected amino acid translation or phylogenetic patterns, therefore mitogenomic sequences from BJT23 were assumed to be free of numts.

For remaining samples, an aliquot of genomic DNA (150ng in 50μL) was used as the input for DNA fragmentation. Fragmentation was performed using a Covaris focused ultrasonicator, targeting 385bp fragments. Following fragmentation, additional size selection was performed using a SPRI cleanup. Library preparation was performed using a commercially available kit provided by KAPA Biosystems (KAPA Hyper Prep with Library Amplification Primer Mix, product KK8504),with palindromic forked adapters using unique 8-base index sequences embedded within the adapter (purchased from Roche). The libraries were then amplified by 10 cycles of PCR. Following sample preparation, libraries were quantified using quantitative PCR (kit purchased from KAPA Biosystems) with probes specific to the ends of the adapters. This assay was automated using Agilent's Bravo liquid handling platform. Based on qPCR quantification, libraries were normalized to 2.2 nM and pooled into 24-plex libraries.

The 24-plex sample pools were combined with NovaSeq Cluster Amp Reagents DPX1, DPX2 and DPX3 and loaded into single lanes of a NovaSeq 6000 S4 flowcell using the Hamilton Starlet Liquid Handling system. Cluster amplification and sequencing were performed on NovaSeq 6000 Instruments utilizing

sequencing-by-synthesis kits to produce 151 bp paired-end reads. Output from Illumina software was processed by the Picard data-processing pipeline to yield CRAM or BAM files containing demultiplexed, aggregated aligned reads.

mtDNA Control Region Alignment and Data Analysis

All mtDNA control region sequences were aligned in MAAFT (https://www.ebi.ac.uk/Tools/msa/mafft/) with default settings and MAFFT alignments were confirmed visually in Mesquite 3.5 [15]. The initial alignment of 1125 bases was trimmed to 881 bases to accommodate for the length of all used sequences. Haplotypes for our dataset were determined with DnaSP 6.12.03 [16], and haplotypes were used for phylogenetic and network reconstruction. The mitogenome and control region sequences of *Cebuella pygmaea* (GenBank accession #MZ747454.1) were used as a phylogenetic outgroup. We reconstructed phylogenetic trees with maximum likelihood (ML) and Bayesian algorithms using IQ-TREE 1.6.12 [17] and MrBayes 3.2.7 [18, 19]. For the ML phylogeny, we used the optimal substitution model "HKY+F+I+G4" as calculated with ModelFinder [20, 21] in IQ-TREE under BIC. We performed the ML analysis two separate times in IQ-TREE with 1000 ultrafast bootstrap (BS) replications [22] per run. The two resulting ML trees were then compared visually for concordance. For the Bayesian tree, we used Modeltest-ng 0.1.6 [23] with the -T flag set to "mrbayes" to determine the optimal substitution model as "HKY + I + G4" under BIC. The Bayesian tree was reconstructed via Markov Chain Monte Carlo (MCMC) runs with 3,000,000 generations and tree and parameter sampling occurring every 100 generations. Upon completion of the two runs, the first 25% of generations were discarded as burn-in. To check convergence of all parameters and the adequacy of the burn-in, we assessed the uncorrected potential scale reduction factor (PSRF) [24] and that all parameter Estimated Sample Size (ESS) values were above 200. Phylogenetic trees were visualized and edited with FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Designations of resulting ML and Bayesian phylogenetic clades followed that of [6].

Due to the large number of *Callithrix* control region sequences available with known sample provenance, we took advantage of this information to reconstruct the biogeographic history of *Callithrix* mtDNA control lineages. Using a user supplied phylogenetic tree, ancestral Callithrix geographic ranges were obtained with the

methodology of [6] using Bayesian Binary Method (BBM) in Reconstruct-Ancestral-States-in-Phylogenies 4.0 (RASP4) [25, 26]. The ML phylogeny obtained with IQ-TREE was used for the BBM analysis, which was conducted as two independent runs of 10 chains that ran for 6,000,000 generations and were sampled every 100 generations. The fixed Jukes-Cantor+Gamma evolutionary model was implemented for each run. DnaSP was used to calculate various statistics of genetic variation, including haplotype diversity, nucleotide diversity, theta based on the number of segregating sites, and number of polymorphic sites for separate mtDNA control region *Callithrix* clades. *Callithrix* clades were those based on the results of the phylogenetic analyses. A NeighborNet network of mtDNA haplotypes of all sampled control region sequences was made with default settings in SplitsTree4 [27].

Mitogenome Alignment and Data Analysis

For mitogenome analysis, we specifically focused on C. jacchus mitogenomes, but used publicly available Cebuella pygmaea (GenBank accession# MZ747454.1), *C. penicillata* (GenBank accession# NC_030788.1), *C. geoffroyi* (GenBank accession# LR745201), and *C. kuhlii* (GenBank accession# KR817257) as phylogenetic outgroups. All mitogenomes were initially aligned in MAFFT with default settings and MAFFT alignments were confirmed visually in Mesquite. The alignment was 16554 bases. Unique mitogenomic haplotypes were determined with DnaSP and then used for ML phylogenetic reconstruction with IQ-TREE and Bayesian phylogenetic reconstruction with MrBayes. For the ML phylogeny, we used the optimal substitution model TIM2+F+I+G as calculated with ModelFinder in IQ-TREE under BIC ,kl. We performed the ML analysis two separate times in IQ-TREE with 1000 ultrafast bootstrap (BS) replications per run. The two resulting ML trees were then compared visually for concordance. For the Bayesian tree, we used Modeltest with the -T flag set to "mrbayes" to determine the optimal substitution model as "HKY+I+G4" with BIC. The Bayesian tree was reconstructed via Markov Chain Monte Carlo (MCMC) runs with 1,000,000 generations and tree and parameter sampling occurring every 100 generations. Upon completion of the two runs, the first 25% of generations were discarded as burn-in. To check convergence of all parameters and the adequacy of the burn-in, we assessed PSRF and that all ESS values were above 200. Phylogenetic trees were visualized and edited with FigTree. We made a MAFFT alignment of C. jacchus

only mitogenomic sequences which was then used to make a NeighborNet haplotype network with default settings in SplitsTree4. DnaSP was used to calculate various statistics of genetic variation, including haplotype diversity, nucleotide diversity, theta based on the number of segregating sites, and number of polymorphic sites for the C. jacchus mitogenomic clade. Due to the lack of known provenance for many of the utilized *Callithrix* mitogenomes, we opted to not perform mitogenomic biogeographic analysis for this data set.

Results

Callithrix Mitochondrial Phylogenetic Analysis

For the mtDNA control region data, branches of the Bayesian and ML phylogenetic trees (Figures 2A-B), respectively, possessed overall strong statistical support with the exception of some weak support towards the tree tips. MtDNA control region haplotypes clustered into eight main species-level clades, with *C. aurita* being the most basal clade. The *C. penicillata* and *C. kuhlii* clades were polyphyletic, with the former forming three clades and the latter forming two clades. Within the *C. penicillata* clades, haplotypes with known provenance clustered together according to similar geographical origin. It was not clear if this is also the case for haplotypes with the *C. kuhlii* clades as their provenance was unknown. *Callithrix jacchus* haplotypes formed the largest and one of the most recent clades of the two phylogenies. Haplotypes from known hybrids clustered within various clades in both phylogenies. There were two instances of discordance between an individual's phenotype and mtDNA control region haplotype within the *C. penicillata* Caatinga clade, (BJT100 haplotype/*C. jacchus* phenotype; BJT22 haplotype/*C. aurita* phenotype). There was also phenotype-haplotype discordance within the *C. jacchus* clade (BJT020 haplotype/*C. aurita* phenotype, BJT79 haplotype/*C. aurita* phenotype, CPE021 haplotype/*C. penicillata* phenotype, and CGE001 haplotype/*C. geoffroyi* phenotype).

The control region ML and Bayesian phylogenies show similar topologies except in respect to the *C. jacchus* clade. The Bayesian phylogeny was not able to resolve relationships between *C. jacchus* clade haplotypes. The ML tree showed better resolution, but lacks strong statistical branch support within the *C. jacchus* clade. The ML phylogeny did possess one well-supported *C. jacchus* subclade of haplotypes

originating from the Nísia Floresta Field Station in Pernambuco state (NISXXX haplotypes in Figure 3). Newly sequenced control region haplotypes from international captive facilities all grouped within the *C. jacchus* clade, thus showing concordance with phenotypes of sampled animals. The included CLEA marmoset control region sequences all shared haplotype CJ154 and those from Company A all shared haplotype CJ134. On the other hand, marmoset control region haplotypes from NEPRC formed two separate clusters, one composed of CJ153 and CJ155 and the other composed of CJ151 and CJ152. No control region haplotypes from across the three facilities clustered together.

The mitogenomic Bayesian and ML trees (Figure 3A-B) show stronger phylogenetic resolution and statistical support among lineages of the *C. jacchus* clade relative to the mtDNA control region phylogenies. Mitogenome haplotypes (BJTXXX) included from marmosets of known provenance within the Caatinga formed their own well supported subgroup within the *C. jacchus* clade. Newly sequenced mitogenomes from marmosets samples from international captive facilities show similar phylogenetic clustering patterns as that for the mtDNA control region, but with better resolution of divergence patterns among mitogenomic haplotypes. In the case of mitogenomes included from CLEA marmosets, we see the lineages collapse into two separate haplotypes. Also, none of the captive facility haplotypes clustered within the Caatinga *C. jacchus* mitogenomic lineages.

Callithrix Mitochondrial Network Analyses

Due to poor phylogenetic resolution of relationships of haplotypes within the *C. jacchus* mtDNA control region clade, we constructed a NeighborNet network (Figure 4A) to try to parse out these relationships. This network resulted in a star-like pattern, which further indicates that the *C. jacchus* clade represents relatively recently Callithrix mtDNA lineages. The Nísia Floresta subclade is also present within the network towards the upper right side. There seems to exist another distinct subclade that stands out on the left side of Figure 4A that includes haplotypes CJ156, CJA002, CGE001, etc. The original provenance of haplotypes forming this second subclade is unknown, so it is not possible to assign them to a possible geographic origin.

In comparison to the mtDNA control region, the *C. jacchus* mitogenome NeighborNet network (Figure 4B) shows relatively better haplotype resolution. The

mitogenome network shows a distinct clade for the BJT Caatinga haplotypes. Haplotypes from NEPRC (CJ023, CJ151, CJ152, CJ153, CJ155, and CJ156) cluster similarly as in the phylogenetic trees. However the network suggests a closer relationship among these haplotypes than in the phylogenetic trees. There were two distinct mitogenomic haplotypes from CLEA marmosets (CJ150 and CJ154), which did not cluster closely.

mtDNA Diversity of Callithrix Taxa

Calculations of *Callithrix* diversity of the mtDNA control region were performed for the major clades defined by the above phylogenetic analysis (Table 2). We made an exception for the two *C. kuhlii* clades, which we collapsed together due to low intraclade sample numbers. Haplotypes from hybrids and haplotypes that were incongruent with individual phenotypes were excluded from the diversity analysis. The *C. jacchus* clade possessed the highest number of haplotypes, haplotype diversity, and number of segregating sites. However, despite highly variable sampling numbers for each clade, we see similar values of inter-clade haplotype diversity. For nucleotide diversity, we see the highest values in the collapsed *C. kuhlii* clade followed by the *C. geoffroyi* clade, and the lowest value in the *C. penicillata* Cerrado clade. We find similar patterns among these three clades for estimates of per sequence theta, but with the positions of *C. kuhlii* and *C. geoffroyi* clades being reversed. The *C. aurita* clade maintains an intermediate position across all diversity measures.

Biogeography of Callithrix mtDNA control region clades

The ancestral origins of *Callithrix* phylogenetic mtDNA control region clades and subclades based on BMM biogeographic analysis were mostly concordant with assigned origins of mtDNA haplotypes (Figure 5). Within the *C. aurita* clade, haplotypes from node 284 in Figure 5 originate in the state of Rio de Janeiro, as expected. However, for the remaining *C. aurita* clade haplotypes, BMM analysis was not able to determine more precise biogeographic origins of haplotypes beyond the general known geographic distribution of *C. aurita*. For the two *C. kuhlii* clades, BMM analysis placed both clades within the Atlantic Forest of Bahia state. While all *C. kuhlii* haplotypes were of unknown provenance, their BMM biogeographic designations are in

line with the known distribution of *C. kuhlii*. Among haplotypes of the *C. geoffroyi* clade, while BMM analysis placed all haplotypes into the known geographic range of *C. geoffroyi* with high posterior probability (PP), the analysis did not show geographic structuring within the clade. However, haplotypes were included with known provenance from the Caatinga biome of Minas Gerais state (BJT143) and Atlantic Forest of Espírito Santo state (BJT 170). These particular haplotypes cluster into two separate subclades that diverge at node 247. BMM biogeographic origins of the three *C. penicillata* clades also received strong PP estimates that were concordant with geographic separation of the clades, as was already apparent in the phylogenetic analysis.

Within the *C. jacchus* clade, BMM analysis placed the nodes of the bottom half of the clade within a combined biogeographic region of the states of Ceará and Pernambuco with high PP. Yet, the biogeography of nodes falling within the upper half of the *C. jacchus* clade could not be determined by BMM analysis. Nodes within the *C. jacchus* clade for which BMM determination agreed with known haplotype provenance included the Atlantic Forest of Pernambuco state at node 189 and 190 and the Caatinga of Pernambuco state for PJ037 and BJT166 at node 193. We were not able to resolve the probable biogeographic origins of newly sequenced control region sequences from *C. jacchus* haplotypes obtained from international captive facilities.

Discussion

With the exception of a single haplotype (BJT100), all haplotypes from marmosets with a *C. jacchus* phenotype, including those sampled at international facilities, clustered within the *C. jacchus* phylogenetic clade. All phylogenies possessed relatively shallow branches within the *C. jacchus* clade compared to the rest of *Callithrix* clades. Previous divergence dating of the *Callithrix* mitogenomic phylogeny shows that the *C. jacchus* clade arose approximately 0.5 million years ago (MYA) [6]. Thus, C. jacchus clade haplotypes from mtDNA are among the youngest within the Callithrix genus, which certainly accounts for the short branch lengths within this clade. Overall, mitogenomic phylogenies provided relatively better resolution of *C. jacchus* haplotype divergence than that of the control region. There were however slight differences in *C. jacchus* haplotype branching topology for the two mitogenome phylogenies. The Bayesian mitogenomic tree showed better support for *C. jacchus*

branching patterns than the ML mitogenomic tree. Due to the shallow branches within the *C. jacchus* mitogenomic and mtDNA control regions clades, networks of *C. jacchus* haplotypes were constructed to complement phylogenetic approaches. The star-like pattern of C. jacchus clade mtDNA control region networks produced in our study as well as that of [4] provide further evidence of the relatively young age of this clade. The mitogenomic *C. jacchus* clade network showed somewhat clearer resolution of network paths among haplotypes. Haplotypes from marmosets originating from the Caatinga biome (those whose names begin with BJT in Figure 4B) form a clear group that is exclusive to the other haplotypes. We also see this grouping within the mitogenomic phylogenies, but neither approach can further resolve relationships among these haplotypes.

Callithrix jacchus genetic diversity based on the mtDNA control region was intermediate relative to other *Callithrix* species. Genetic diversity measures estimated for the mitogenome for *C. jacchus* were similar to that of the mtDNA control region in terms of haplotype diversity but an order of magnitude smaller in terms of nucleotide diversity. Based on differences in haplotype numbers observed for different biomedical facilities included in this study (see Figure 3), it is likely that levels of mitochondrial genetic diversity differ among captive *Callithrix* colonies. Among the international facilities from which we sampled marmosets, it is likely that NEPRC possesses the highest relative level of mtDNA genetic diversity given that marmosets from this facility possessed the highest number of mtDNA and mitogenome haplotypes. It is likely that the CLEA colony would have possessed the lowest relative level of mtDNA genetic diversity given that marmosets from this facility possessed a single haplotype for both mitochondrial datasets. To fully assess the genetic diversity within and among international biomedical facilities, a larger number of animals will need to be sampled and analyzed.

Biogeography of Callithrix jacchus mtDNA Control Region Haplotypes

Unlike other *Callithrix* phylogenetic clades, assignment of biogeographic origin of *C. jacchus* control region haplotypes was not fully successful over the entire clade. Haplotypes from *C. jacchus* in biomedical facilities were assigned to a biogeographic origin within the Atlantic Forest, but a more specific location of origin was not possible. The biogeographic origins for a number of other *C. jacchus* haplotypes could not be

resolved, even when they were associated with marmosets of known provenance. Haplotypes with known provenance whose biogeographic origin was correctly assigned included PJ037 and BJT166 which originate from the Caatinga and TAP10, TAP07, and TAP04 which originated from the Atlantic Forest of Pernambuco state. Previously published *Callithrix* biogeographic analysis based on the complete mitogenome performed better for the *C. jacchus* clade, as biogeographic origins were assigned for all haplotypes [6].

Due to the higher phylogenetic and biogeographic resolution of *Callithrix* haplotypes provided by the mitogenome in comparison to the mtDNA control region, the *C. jacchus* biogeographic results presented here should be interpreted with caution. An increased availability of known-provenance mitogenomes from across the *Callithrix* range would certainly improve the precision of *Callithrix* biogeographic analysis. In the specific case of marmosets from biomedical facilities, we are missing the necessary genetic and geographic information from the natural *C. jacchus* range to more precisely determine their biogeographic origins. Once such information becomes more accessible, based on the previously published results discussed above [6], we suggest that data from the entire mitogenome will provide greater resolution for biogeographic analysis than data from the control region alone.

Based on several published mtDNA phylogenies [4, 6, 9], as well as those presented here, one plausible biogeographic hypothesis is that the *C. jacchus* clade originated in the Caatinga. Although *C. jacchus* occurs naturally in the Caatinga, Cerrado, and northeastern Brazilian Atlantic Forest biomes (Figure 1), mitochondrial phylogenies show that the species diverged from the *C. penicillata* Caatinga clade. Further, biogeographic modeling suggests that the *C. jacchus*/*C. penicillata* ancestor dispersed into the Cerrado, then *C. penicillata* became isolated in either the Cerrado or Caatinga and subsequently *C. jacchus* dispersed across Caatinga, Cerrado, and the Atlantic Forest [28]. It is plausible then that an ancestral *C. penicillata* population may have dispersed from the Cerrado into the Caatinga, and later diverged into the *C. jacchus* and *C. penicillata* Caatinga clade. A possible Caatinga origin for *C. jacchus* carries several implications for the evolutionary biology and biomedical use of this species. Stark environmental differences between the humid Atlantic Forest and semiarid Caatinga and Cerrado biomes may have driven genetic, phenotypic, and behavioral differences in *C. jacchus* subpopulations found within these areas (personal observation, Malukiewicz). Reduced or absent gene flow between subpopulations

spread across a wide geographic range can result in notable genetic differences within the same species [29]. Whether any such genetic differences exist among natural *C. jacchus* subpopulations is currently unknown. We also do not know how any existing genetic and adaptive differences between native *C. jacchus* populations have played into the population genetics of *C. jacchus* in biomedical colonies outside of Brazil. Resolving these questions is important for optimizing biomedical use of marmosets, particularly as genetic differences associated with ancestry and geographic origin of non-human primates can influence results of biomedical experiments dependent on non-human primate models [29–32].

Hybridization within the Callithrix Genus based on Mitogenomic and Mitochondrial Markers

The control region phylogenies showed a number of haplotypes within the *C. jacchus* clade that are discordant with the phenotypes of sampled marmosets (BJT20, BJT79, CPE021, CGE00). This same pattern has been previously observed for BJT20 and BJT79 in a mitogenomic phylogeny and attributed to genetic introgression of *C. jacchus* mtDNA into the genetic background of *C. aurita* as a result of anthropogenic hybridization [6]. In this study and a previous one [4], haplotypes CPE021 and CGE001 grouped within *C. jacchus* mtDNA clades, but were derived with individuals sampled from captive facilities in the US with *C. penicillata* and *C. geoffroyi* phenotypes, respectively. These haplotypes may represent cases of incomplete lineage sorting if the ancestors of the sampled marmosets never interbred with *C. jacchus*, as incomplete lineage sorting should not promote geographical proximity of interspecifically shared alleles under local introgression [33].

The case of haplotype BJT100 represents a cryptic hybrid, and was associated with an individual sampled in a captive facility in Petrolina, Pernambuco, Brazil. This facility is located at the natural border between the natural geographic ranges of *C. jacchus* and *C. penicillata* where a natural hybrid zone exists [4]. However, the two species are also frequently captured illegally, interbred, and kept as pets in Brazil [1, 5].

In many cases hybridization seems to reduce fitness globally, and at the cellular level hybrids may face challenges in reconciling protein interactions [34, 35]. Our current results for marmosets from international biomedical facilities do not indicate the presence of hybrids among our sampled individuals. However, there is phenotypic

evidence of possible *C. jacchus* x *C. penicillat*a hybrids present among marmosets kept in US biomedical facilities (personal observation, Malukiewicz). Additionally, previous mtDNA genetic studies show that cryptic hybridization in the wild does seem to occur regularly within the *Callithrix* genus [4–6, 9].

Accordingly, we would expect that if hybrid marmosets are present in US biomedical facilities, they may not may not show obvious phenotypic signs of interspecific ancestry. Thus, we do not recommend relying solely on phenotype for species identification of marmosets maintained in captivity. It will also be valuable to perform mitogenomic analysis of captive animals as additional support for their ancestry. Eventually, genome-wide autosomal variants will be identified that can provide a more complete picture of ancestry than mitogenomic analysis alone. Such checks could help minimize spurious results in future research looking to utilize marmosets as biomedical models.

Conclusions

The mitogenomic network and phylogenetic results from this and other work [6, 9, 10] show that analyses of the mitogenomic variation holds substantial potential for resolving evolutionary relationships among *C. jacchus* populations, defining species boundaries, assigning individuals to populations, and detecting hybrids. The mitogenome is an especially useful genetic marker for non-model taxa, taxa which are presently deficient in genetic data, or little-studied taxa, as it can be used relatively quickly to access ancestry particularly using approaches such as genomic skimming [9]. Within the US, significant effort and funding are being directed toward assessing whole genome genetic diversity and genotyping among marmosets at biomedical facilities motivated by increasing interest in marmosets as a biomedical model for neurodegenerative and other diseases [1]. Mitogenomes can be mined from whole genome sequencing data [6, 9]. Thus, such mined data can be obtained from marmosets at international captive facilities as whole genomic data becomes increasingly available from these primates. If sampling and genomic sequencing efforts can be also matched for natural *C. jacchus* populations in Brazil, utilization of mitogenomic data from wild *C. jacchus* of known provenance can be harnessed to determine the biogeographic origins of captive *C. jacchus*. Expanding our efforts into understanding the evolutionary history of marmosets will greatly enhance the use of marmosets for biomedical

purposes. Thus, combining marmoset mitogenomic data with phenotypic and nuclear genomic data is crucial for the management of captive marmoset populations with the goal of maintaining genetic diversity, organizing appropriate breeding plans, identifying species and hybrids, and selecting animals with appropriate genetic backgrounds for biomedical research.

Tables

Table 1. Sampling Summary of *Callithrix*.

Classification	Count
C. aurita	13
C. geoffroyi	16
C. jacchus	103
C. kuhlii	9
C. penicillata	55
C. jacchus x C. penicillata	53
C. penicillata x C. geoffroyi	11
C. aurita x Callithrix sp.	5
Callithrix sp. x Callithrix sp.	1
Total	266

Table 2. Summary of *Callithrix* MtDNA Control Region Diversity Statistics.

Taxon	Sequences	Total Number of Sites	Haplotype Number	Haplotype Diversity	Nucleotide Diversity	Theta (per sample)	Polymorphic Sites
C. aurita	9	782	7	0.944	0.021	15.82	43
C. geoffroyi	16	789	12	0.958	0.032	27.42	91
C. jacchus	102	791	62	0.981	0.028	22.51	117
C. kuhlii	9	796	6	0.889	0.043	25.02	68
C. penicillata Caatinga Clade	18	797	10	0.935	0.029	21.51	74
C. penicillata Cerrado Clade	24	795	13	0.924	0.11	8.84	33

C. penicillata Cerrado-Atlantic Forest Clade	11	795	7	0.927	0.023	13.31	39

Table 3. Summary *C. jacchus* Mitogenome Diversity Statistics.

Taxon	Sequences	Total Number of Sites	Haplotype Number	Haplotype Diversity	Nucleotide Diversity	Theta (per sample)	Polymorphic Sites
C. jacchus	1	16262	9	0.892	0.002	27.73	92

Figures

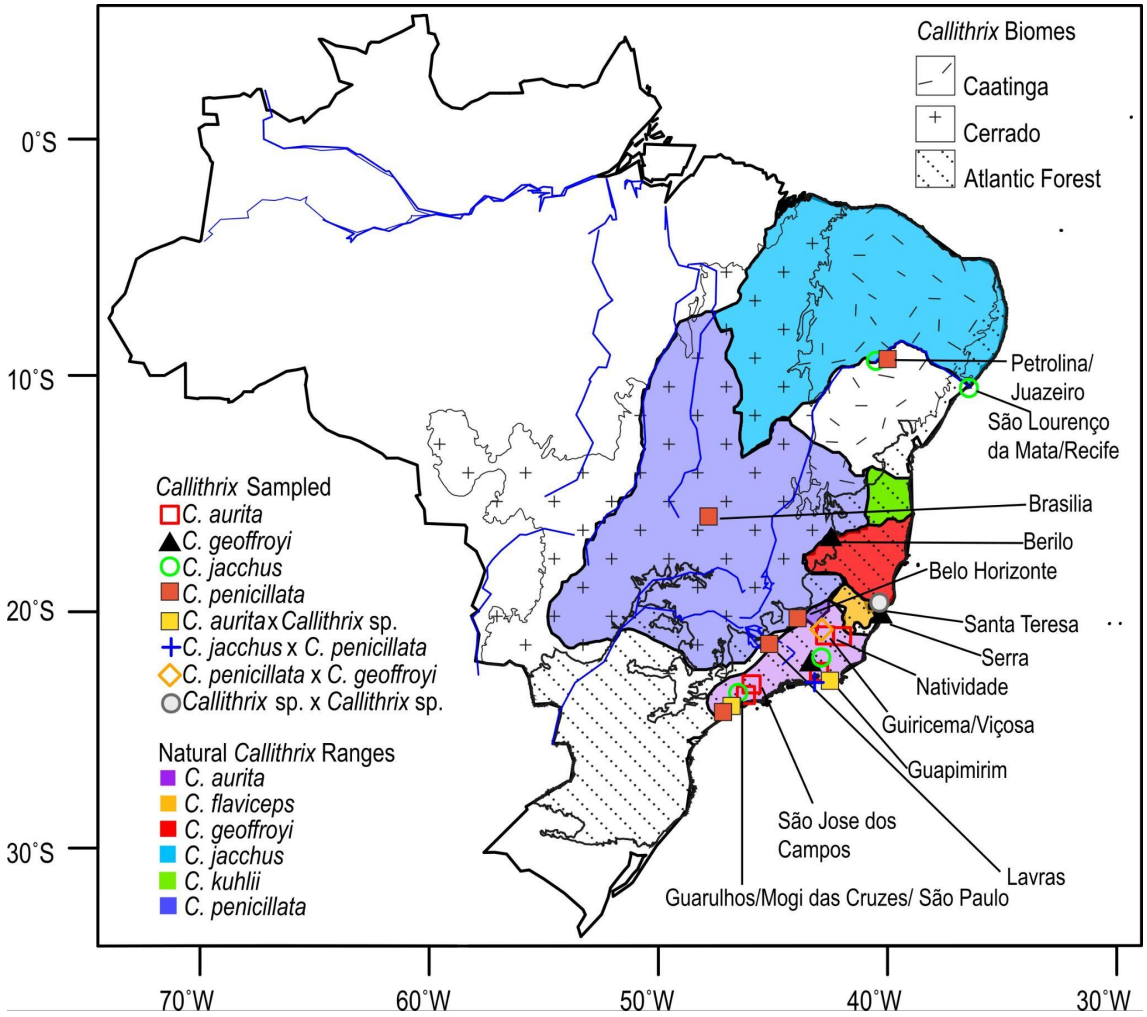

Figure 1. Approximate distribution of Callithrix species in Brazil (2012 IUCN Red List Spatial Data; (http://www.iucnredlist.org/technical-documents/spatial-data) and geographic origins of mtDNA control region sequences available from

previously published studies. Locations of three biomes where Callithrix occur naturally, the Caatinga, Cerrado, and Atlantic Forest, are also indicated.

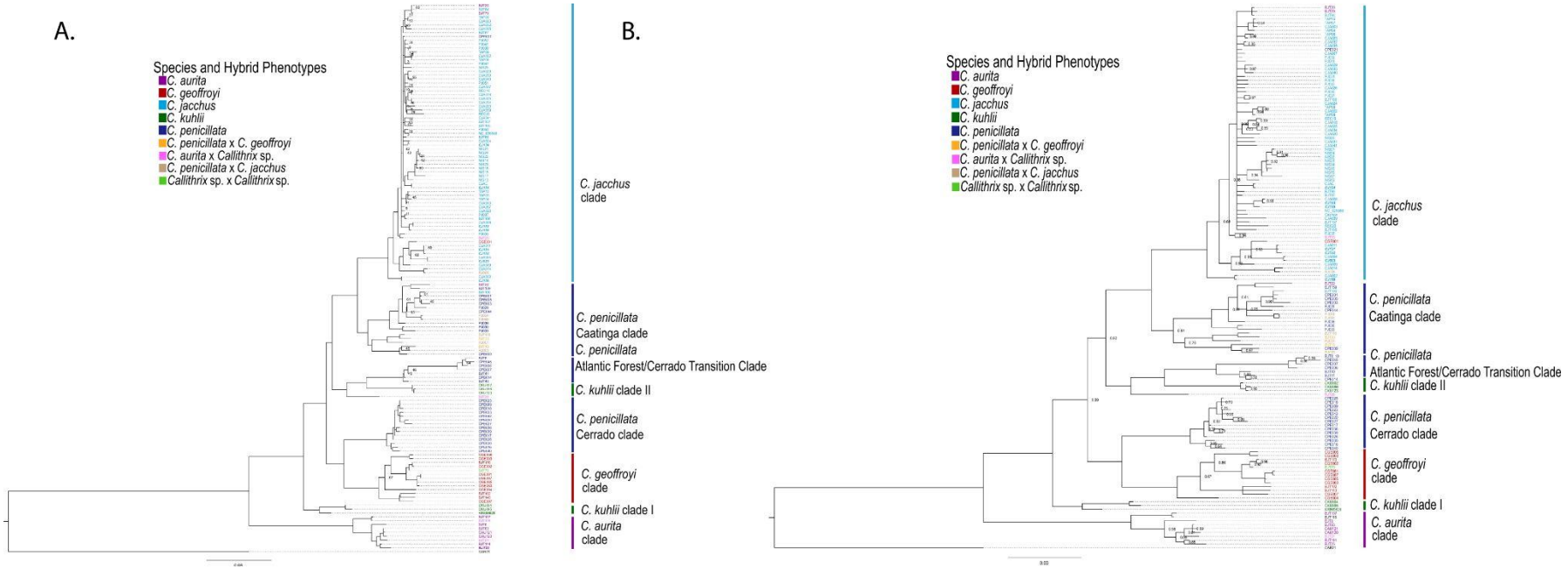

Figure 2. Phylogenetic relationships among Callithrix mtDNA control region. (A) Maximum likelihood phylogenetic relationships among haplotypes for which node support is shown where bootstrap support <70% in the tree. (B) Bayesian phylogenetic tree for which node support is shown where bootstrap support <1.0 in the Bayesian tree. In both plots, newly sequenced mtDNA control region sequences are showed in bold and are italicized. Haplotype colors at tips correspond to the 'Species and Hybrid Phenotypes' legend, and indicate phenotypes associated with each given haplotype.

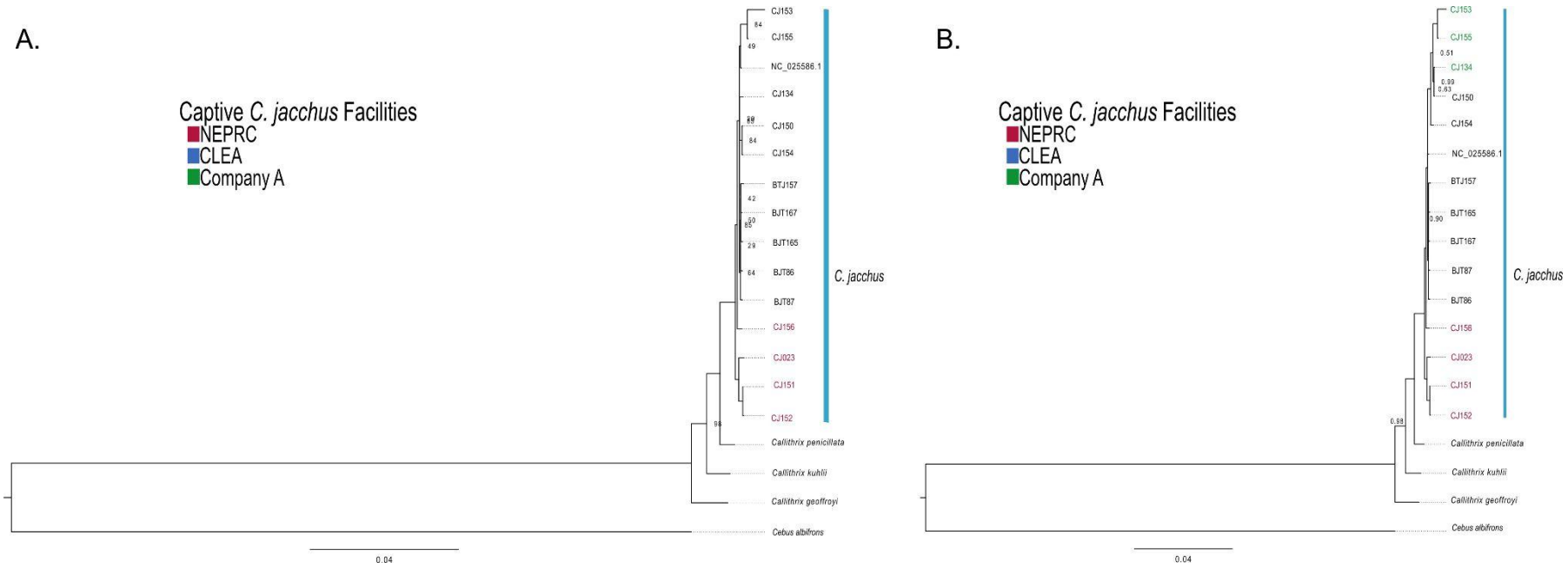

Figure 3. Phylogenetic relationships among mitogenomic haplotypes of *C. jacchus* lineage with focus on marmosets from captive research facilities. (A) Maximum likelihood phylogenetic relationships haplotypes for which node support is shown where bootstrap support <70% in the tree. (B) Bayesian analysis of mitogenomes for which node support is shown where bootstrap support <1.0 in the Bayesian tree. Newly sequenced mitogenome haplotypes are colored. These haplotype colors at tips correspond to the 'Captive *C. jacchus* Facilities' legend, and indicate the captive facility from where a given haplotype originates.

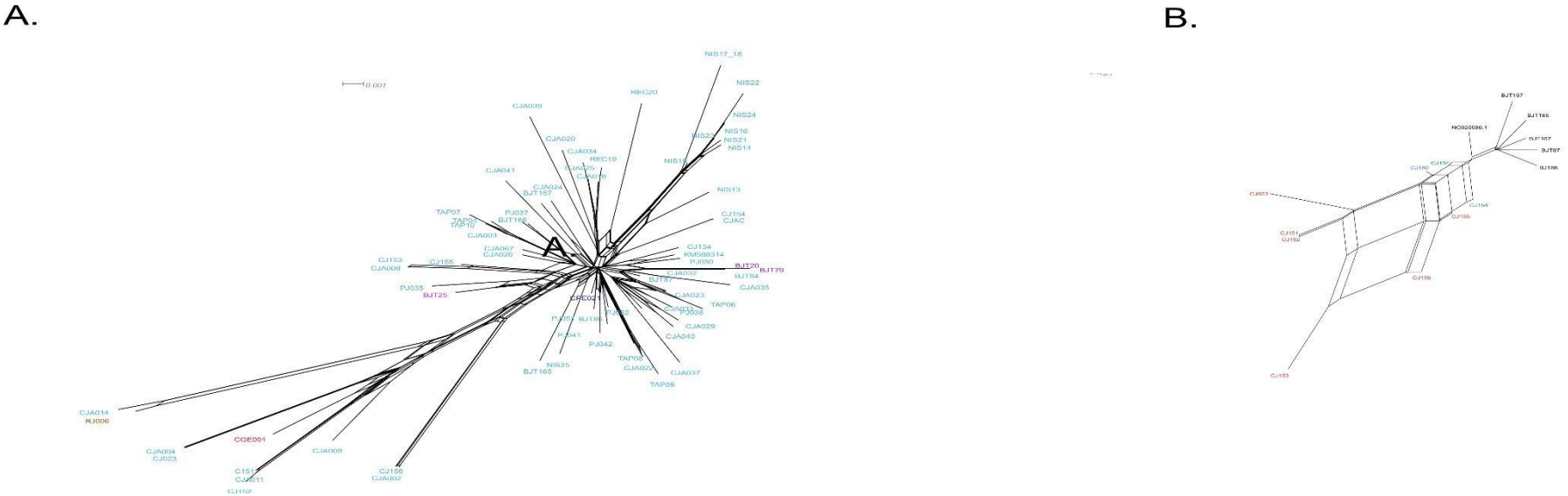

Figure 4. Neighbor network of haplotypes from phylogenetic C. jacchus clade. Haplotype colors at tips follow Fig. 2 ‘Species and Hybrid Phenotypes’ legend, and indicate phenotypes associated with each given haplotype.

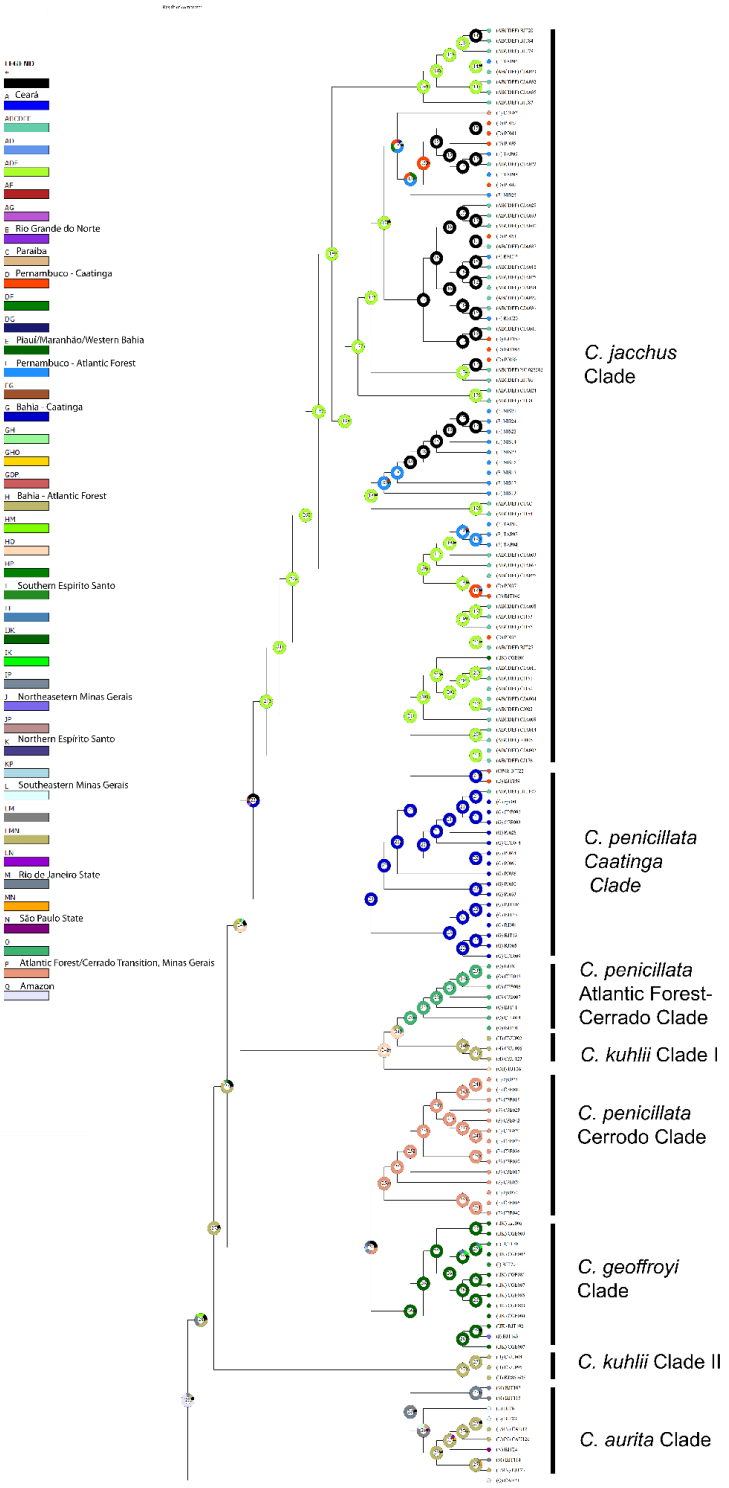

Figure 5. Ancestral state reconstructions performed by the Bayesian Binary MCMC analysis as implemented in RASP using the ML rooted tree. Donut charts at each node represent ancestral host estimations in terms of posterior probability (PP). Each node is internally identified with a number. Localities where species associated with each phylogenetic clade were sampled or known to occur are color coded in the map inset on the left side.

Supplementary Table

Table S1. Metadata for newly collected samples and downloaded sequences of *Callithrix* mitogenomic and mtDNA control region sequences obtained from GenBank.

Sample	Accession Number	Phenotype	mtDNA lineage	Sampling location	Approximate Latitude/Longitude	Reference
BJT10	MN787096	*C. penicillata*	*C. penicillata (Cerrado-AF)*	Lavras, MG	-21.227, -44.980	Malukiewicz et al., 2021
BJT100	MN787090	*C. jacchus*	*C. jacchus*	CPRJ	NA	Malukiewicz et al., 2021
BJT102	MN787083	*C. geoffroyi*	*C. geoffroyi*	CPRJ	NA	Malukiewicz et al., 2021
BJT107	MN787080	*C. aurita*	*C. aurita*	Natividade, RJ (Removed from wild and housed at CPRJ)	-21.043, -41.978	Malukiewicz et al., 2021
BJT109	MN787081	*C. aurita*	*C. aurita*	Natividade, RJ (Removed from wild and housed at CPRJ)	-21.043, -41.978	Malukiewicz et al., 2021
BJT114	MN787082	*C. aurita*	*C. aurita*	Guapirim, RJ (Removed from wild and housed at CPRJ)	-22.488, -42.912	Malukiewicz et al., 2021

BJT115	MN787107	*C. aurita x Callithrix sp.*	*C. aurita*	Guapirim, RJ	-22.488, -42.912	Malukiewicz et al., 2021
BJT116	MN787108	*C. penicillata x C. jacchus*	*C. penicillata*	Guapirim, RJ	-22.488, -42.912	Malukiewicz et al., 2021
BJT13	MN787110	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.755, -42.872	Malukiewicz et al., 2021
BJT14	MN787120	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.755, -42.872	Malukiewicz et al., 2021
BJT143	MN787084	*C. geoffroyi*	*C. geoffroyi*	Berilo, MG	-16.953, -42.466	Malukiewicz et al., 2021
BJT15	MN787117	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.755, -42.872	Malukiewicz et al., 2021
BJT150	MN787118	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.759, -42.866	Malukiewicz et al., 2021
BJT151	MN787119	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.759, -42.866	Malukiewicz et al., 2021
BJT157	MN787091	*C. jacchus*	*C. jacchus*	CEMAFAUNA	NA	Malukiewicz et al., 2021
BJT159	MN787099	*C. penicillata*	*C. penicillata (Caatinga)*	CEMAFAUNA	NA	Malukiewicz et al., 2021
BJT16	MN787114	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.755, -42.872	Malukiewicz et al., 2021

BJT160	MN787100	*C. penicillata*	*C. penicillata (Caatinga)*	CEMAFAUNA	NA	Malukiewicz et al., 2021
BJT165	MN787092	*C. jacchus*	*C. jacchus*	CEMAFAUNA	NA	Malukiewicz et al., 2021
BJT166	MN787093	*C. jacchus*	*C. jacchus*	CEMAFAUNA	NA	Malukiewicz et al., 2021
BJT167	MN787094	*C. jacchus*	*C. jacchus*	CEMAFAUNA	NA	Malukiewicz et al., 2021
BJT169	MN787086	*C. geoffroyi*	*C. geoffroyi*	Serra, ES	-20.213, -40.267	Malukiewicz et al., 2021
BJT170	MN787085	*C. geoffroyi*	*C. geoffroyi*	Serra, ES	-20.213, -40.267	Malukiewicz et al., 2021
BJT171	MN787087	*C. geoffroyi*	*C. geoffroyi*	Serra, ES	-20.213, -40.267	Malukiewicz et al., 2021
BJT20	MN787075	*C. aurita*	*C. jacchus*	Mogi das Cruzes, SP (Removed from wild and housed at Guarulhos Municipal Zoo)	-23.523, -46.179	Malukiewicz et al., 2021
BJT22	MZ027648	*C. aurita*	*C. penicillata*	Guarulhos Municipal Zoo	NA	Malukiewicz et al., 2021

BJT23	MZ394056	*C. aurita*	*C. aurita*	Guarulhos Municipal Zoo	NA	This study
BJT24	MN787103	*C. aurita x Callithrix sp.*	*C. aurita*	Guarulhos Municipal Zoo (apprehended animal)	NA	Malukiewicz et al., 2021
BJT25	MN787104	*C. aurita x Callithrix sp.*	*C. jacchus*	Guarulhos Municipal Zoo (apprehended animal)	NA	Malukiewicz et al., 2021
BJT26	MN787105	*C. aurita x Callithrix sp.*	*C. penicillata*	Guarulhos Municipal Zoo (apprehended animal)	NA	Malukiewicz et al., 2021
BJT27	MN787106	*C. aurita x Callithrix sp.*	*C. geoffroyi*	Guarulhos Municipal Zoo (apprehended animal)	NA	Malukiewicz et al., 2021
BJT31	MN787111	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.758, -42.860	Malukiewicz et al., 2021
BJT33	MN787112	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.778, -42.863	Malukiewicz et al., 2021
BJT38	MN787113	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.755, -42.857	Malukiewicz et al., 2021
BJT39	MN787116	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.755, -42.857	Malukiewicz et al., 2021

BJT40	MN787097	*C. penicillata*	*C. penicillata (Cerrado-AF)*	Belo Horizonte, MG	-19.921, -43.990	Malukiewicz et al., 2021
BJT41	MN787098	*C. penicillata*	*C. penicillata (Cerrado-AF)*	Belo Horizonte, MG	-19.921, -43.990	Malukiewicz et al., 2021
BJT6	MN787074	*C. aurita*	*C. aurita*	Guiricema, MG	-21.010, -42.720	Malukiewicz et al., 2021
BJT65	MT041703	*C. aurita*	*C. aurita*	Guiricema, MG	-21.010, -42.720	Malukiewicz et al., 2021
BJT70	MN787109	*Callithrix sp. x Callithrix sp.*	*C. geoffroyi*	Santa Teresa, ES	-19.935, -40.596	Malukiewicz et al., 2021
BJT75	MN787115	*C. penicillata x C. geoffroyi*	*C. penicillata*	Viçosa, MG	-20.759, -42.866	Malukiewicz et al., 2021
BJT79	MN787077	*C. aurita*	*C. jacchus*	Guarulhos Municipal Zoo	NA	Malukiewicz et al., 2021
BJT8	MN787095	*C. penicillata*	*C. penicillata (Cerrado-AF)*	Lavras, MG	-21.227, -44.980	Malukiewicz et al., 2021
BJT83	MN787078	*C. aurita*	*C. aurita*	Sao Jose dos Campos, SP (Removed from wild and housed at Guarulhos Municipal Zoo)	-23.070, -45.933	Malukiewicz et al., 2021

BJT84	MN787079	*C. aurita*	*C. jacchus*	Guarulhos Municipal Zoo	NA	Malukiewicz et al., 2021
BJT86	MN787088	*C. jacchus*	*C. jacchus*	Guarulhos Municipal Zoo	NA	Malukiewicz et al., 2021
BJT87	MN787089	*C. jacchus*	*C. jacchus*	Guarulhos Municipal Zoo	NA	Malukiewicz et al., 2021
CAU120	U89000	*C. aurita*	*C. aurita*	Iquaquecetuba-Suzano, SP	NA	Tagliaro et al., 1997
CAU121	U89001	*C. aurita*	*C. aurita*	Mogi das Cruzes, SP	NA	Tagliaro et al., 1997
CGE001	KJ020024	*C. geoffroyi*	*C. geoffroyi*	CRC	NA	Malukiewicz et al., 2014
CGE002	KJ020025	*C. geoffroyi*	*C. geoffroyi*	CRC	NA	Malukiewicz et al., 2014
CGE003	KJ020026	*C. geoffroyi*	*C. geoffroyi*	CRC	NA	Malukiewicz et al., 2014
CGE004	KJ020027	*C. geoffroyi*	*C. geoffroyi*	CRC	NA	Malukiewicz et al., 2014
cge006	LR745201	*C. geoffroyi*	*C. geoffroyi*	CRC	NA	GenBank
CGE007	KJ020028	*C. geoffroyi*	*C. geoffroyi*	CRC	NA	Malukiewicz et al., 2014
CGE081	U88993	*C. geoffroyi*	*C. geoffroyi*	Criadouro Barbuse Leal, Brasilia	NA	Tagliaro et al., 1997

CGE083	U88994	*C. geoffroyi*	*C. geoffroyi*	Criadouro Barbuse Leal, Brasilia	NA	Tagliaro et al., 1997
CGE085	U88995	*C. geoffroyi*	*C. geoffroyi*	Criadouro Barbuse Leal, Brasilia	NA	Tagliaro et al., 1997
CGE087	U88996	*C. geoffroyi*	*C. geoffroyi*	Criadouro Barbuse Leal, Brasilia	NA	Tagliaro et al., 1997
CJ005	MZ394062	*C. jacchus*	*C. jacchus*	CLEA, Japan	NA	This study
CJ023	MZ394055	*C. jacchus*	*C. jacchus*	NEPRC, US	NA	This study
CJ134	MZ394057	*C. jacchus*	*C. jacchus*	Company A, US	NA	This study
CJ144	MZ394051	*C. jacchus*	*C. jacchus*	Company A, US	NA	This study
CJ145	MZ394052	*C. jacchus*	*C. jacchus*	Company A, US	NA	This study
CJ146	MZ394053	*C. jacchus*	*C. jacchus*	Company A, US	NA	This study
CJ147	MZ394063	*C. jacchus*	*C. jacchus*	CLEA, Japan	NA	This study
CJ148	MZ394064	*C. jacchus*	*C. jacchus*	CLEA, Japan	NA	This study
CJ149	MZ394058	*C. jacchus*	*C. jacchus*	Company A, US	NA	This study
CJ150	LR745200	*C. jacchus*	*C. jacchus*	CLEA, Japan	NA	This study
CJ151	MZ394050	*C. jacchus*	*C. jacchus*	NEPRC, US	NA	This study
CJ152	MZ394054	*C. jacchus*	*C. jacchus*	NEPRC, US	NA	This study
CJ153	MZ394059	*C. jacchus*	*C. jacchus*	NEPRC, US	NA	This study
CJ154	MZ394061	*C. jacchus*	*C. jacchus*	CLEA, Japan	NA	This study
CJ155	MZ394060	*C. jacchus*	*C. jacchus*	NEPRC, US	NA	This study

CJ156	MZ394065	*C. jacchus*	*C. jacchus*	NEPRC	NA	This study
CJA001	KJ020032	*C. jacchus*	*C. jacchus*	NA	NA	Malukiewicz et al., 2014
CJA002	KJ020033	*C. jacchus*	*C. jacchus*	NEPRC	NA	Malukiewicz et al., 2014
CJA003	KJ020034	*C. jacchus*	*C. jacchus*	NEPRC	NA	Malukiewicz et al., 2014
CJA004	KJ020035	*C. jacchus*	*C. jacchus*	NEPRC	NA	Malukiewicz et al., 2014
CJA006	Same as KJ020033	*C. jacchus*	*C. jacchus*	NEPRC	NA	Malukiewicz et al., 2014
CJA007	Same as AB572419	*C. jacchus*	*C. jacchus*	NEPRC	NA	Malukiewicz et al., 2014
CJA008	KJ020036	*C. jacchus*	*C. jacchus*	NEPRC	NA	Malukiewicz et al., 2014
CJA009	KJ020037	*C. jacchus*	*C. jacchus*	NEPRC	NA	Malukiewicz et al., 2014
CJA010	Same as KJ020036	*C. jacchus*	*C. jacchus*	NEPRC	NA	Malukiewicz et al., 2015
CJA011	KJ020038	*C. jacchus*	*C. jacchus*	NEPRC	NA	Malukiewicz et al., 2014

CJA013	Same as AB572419	*C. jacchus*	*C. jacchus*	CRC	NA	Malukiewicz et al., 2015
CJA014	KJ020039	*C. jacchus*	*C. jacchus*	CRC	NA	Malukiewicz et al., 2014
CJA018	KJ020040	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA019	Same as KJ020040	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2015
CJA020	KJ020041	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA022	KJ020042	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA023	KJ020043	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA024	KJ020044	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA025	KJ020045	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA026	KJ020046	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA028	Same as KJ020043	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014

CJA029	KJ020047	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA030	Same as KJ020043	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA032	KJ020048	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA033	KJ020049	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA034	KJ020050	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA035	KJ020051	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA036	Same as KJ020045	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2015
CJA037	KJ020052	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA039	KJ020053	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA040	KJ020054	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CJA041	KJ020055	*C. jacchus*	*C. jacchus*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014

CJA067	KJ020056	*C. jacchus*	*C. jacchus*	Parque Dois Irmãos, Recife, PE	-8.014090493659184, -34.944594200514557	Malukiewicz et al., 2014
CJAC	AB572419	*C. jacchus*	*C. jacchus*	NA	NA	GenBank
CKU001	KJ020029	*C. kuhlii*	*C. kuhlii*	CRC	NA	Malukiewicz et al., 2014
CKU002	KJ020030	*C. kuhlii*	*C. kuhlii*	CRC	NA	Malukiewicz et al., 2014
CKU004	KR817257	*C. kuhlii*	*C. kuhlii*	CRC	NA	Malukiewicz et al., 2017
CKU094	U88841	*C. kuhlii*	*C. kuhlii*	NA	NA	GenBank
CKU095	U88842	*C. kuhlii*	*C. kuhlii*	NA	NA	GenBank
CKU096	U88843	*C. kuhlii*	*C. kuhlii*	NA	NA	GenBank
CKU122	U88991	*C. kuhlii*	*C. kuhlii*	NA	NA	GenBank
CKU123	U88992	*C. kuhlii*	*C. kuhlii*	NA	NA	GenBank
cpe001	KJ020057	*C. penicillata*	*C. penicillata (Caatinga)*	CRC	NA	Malukiewicz et al., 2014
CPE002	Same as KJ020057	*C. penicillata*	*C. penicillata (Caatinga)*	CRC	NA	Malukiewicz et al., 2014

CPE003	KJ020058	*C. penicillata*	*C. penicillata (Caatinga)*	CRC	NA	Malukiewicz et al., 2014
CPE005	KJ020059	*C. penicillata*	*C. penicillata (Caatinga)*	CRC	NA	Malukiewicz et al., 2014
CPE006	KJ020060	*C. penicillata*	*C. penicillata (Cerrado-AF)*	CRC	NA	Malukiewicz et al., 2014
CPE007	KJ020061	*C. penicillata*	*C. penicillata (Cerrado-AF)*	CRC	NA	Malukiewicz et al., 2014
CPE008	Same as KJ020060	*C. penicillata*	*C. penicillata (Cerrado-AF)*	CRC	NA	Malukiewicz et al., 2014
CPE009	KJ020062	*C. penicillata*	*C. penicillata (Caatinga)*	Muriae, MG	-21.121000, -42.367361	Malukiewicz et al., 2014
CPE010	Same as KJ020062	*C. penicillata*	*C. penicillata (Caatinga)*	Muriae, MG	-21.121000, -42.367361	Malukiewicz et al., 2014
CPE011	Same as KJ020062	*C. penicillata*	*C. penicillata (Caatinga)*	Muriae, MG	-21.121000, -42.367361	Malukiewicz et al., 2014
CPE012	KJ020063	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.770250, -47.833286	Malukiewicz et al., 2014
CPE013	Same as MN787102	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.770250, -47.833286	Malukiewicz et al., 2014
CPE014	KJ020064	*C. penicillata*	*C. penicillata (Cerrado-AF)*	Brasília, DF	-15.803064, -47.799303	Malukiewicz et al., 2014

CPE015	Same as KJ020064	*C. penicillata*	*C. penicillata (Cerrado-AF)*	Brasília, DF	-15.803064, -47.799303	Malukiewicz et al., 2014
CPE016	KJ020065	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.844478, -47.943286	Malukiewicz et al., 2014
CPE017	KJ020066	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.844478, -47.943286	Malukiewicz et al., 2014
CPE018	KJ020067	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.750319, -47.842806	Malukiewicz et al., 2014
CPE020	KJ020068	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.865683, -47.970539	Malukiewicz et al., 2014
CPE021	KJ020069	*C. penicillata*	*C. penicillata (C. jacchus)*	Brasília, DF	-15.865683, -47.970539	Malukiewicz et al., 2014
CPE022	Same as KJ020069	*C. penicillata*	*C. penicillata (C. jacchus)*	Brasília, DF	-15.865683, -47.970539	Malukiewicz et al., 2014
CPE023	KJ020070	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.709292, -47.912444	Malukiewicz et al., 2014
CPE024	KJ020071	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.908753, -47.757294	Malukiewicz et al., 2014
cpe025	MN787101	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.910575, -47.952956	Malukiewicz et al., 2014
CPE026	KJ020073	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.910575, -47.952956	Malukiewicz et al., 2014

CPE027	KJ020074	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.874625, -47.770817	Malukiewicz et al., 2014
CPE029	Same as MN787102	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.861153, -47.828742	Malukiewicz et al., 2014
cpe030	MN787102	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.861, -47.829	Malukiewicz et al., 2014
CPE031	Same as KJ020065	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.861153, -47.828742	Malukiewicz et al., 2014
CPE032	Same as MN787102	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.861153, -47.828742	Malukiewicz et al., 2014
CPE035	Same as MN787102	*C. penicillata*	*C. penicillata (Cerrado)*	Brasília, DF	-15.861153, -47.828742	Malukiewicz et al., 2014
CPE036	KJ020075	*C. penicillata*	*C. penicillata (Cerrado)*	Goiânia, Goiás	-16.679914, -49.208050	Malukiewicz et al., 2014
CPE037	KJ020075	*C. penicillata*	*C. penicillata (Cerrado)*	Goiânia, Goiás	-16.679914, -49.208050	Malukiewicz et al., 2014
CPE039	KJ020076	*C. penicillata*	*C. penicillata (Cerrado)*	IBAMA CETAS, GO	NA	Malukiewicz et al., 2014
CPE040	KJ020077	*C. penicillata*	*C. penicillata (Cerrado)*	IBAMA CETAS, GO	NA	Malukiewicz et al., 2014
CPE042	KJ020078	*C. penicillata*	*C. penicillata (Cerrado)*	IBAMA CETAS, GO	NA	Malukiewicz et al., 2014

CPE043	Same as KJ020076	*C. penicillata*	*C. penicillata (Cerrado)*	IBAMA CETAS, Goias	NA	Malukiewicz et al., 2014
CPE044	KJ020079	*C. penicillata*	*C. penicillata (Caatinga)*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CPE045	KJ020080	*C. penicillata*	*C. penicillata (Cerrado-AF)*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CPE046	Same as KJ020080	*C. penicillata*	*C. penicillata (Cerrado-AF)*	IBAMA CETAS, PE	NA	Malukiewicz et al., 2014
CPE089	U88993	*C. penicillata*	*C. penicillata (Cerrado)*	Brasilia, DF	NA	Tagliaro et al., 1997
CPE41	Same as KJ020076	*C. penicillata*	*C. penicillata (Cerrado)*	IBAMA CETAS, GO	NA	Malukiewicz et al., 2014
KR869628	KR869628	*C. kuhlii*	*C. kuhlii*	NA	NA	GenBank
NC_025586	NC_025586	*C. jacchus*	*C. jacchus*	NA	NA	GenBank
NC021941	NC021941	*C. geoffroyi*	*C. geoffroyi*	NA	NA	Fintermeier et al., 2013
NIS13	AY196762	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
NIS14	AY196763	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
NIS15	AY196764	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003

NIS16	AY196765	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
NIS17	AY196757	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
NIS18	AY196756	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
NIS21	AY196755	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
NIS22	AY196758.	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
NIS23	AY196759	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
NIS24	AY196760.	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
NIS25	AY196761	*C. jacchus*	*C. jacchus*	Nísia Floresta	-6.087670, -35.184983	Faulkes et al., 2003
PJ028	KJ020081	*C. penicillata*	*C. penicillata (Caatinga)*	Juazeiro, BA	-9.421286, -40.482981	Malukiewicz et al., 2014
PJ029	Same as KJ020081	*C. penicillata*	*C. penicillata (Caatinga)*	Juazeiro, BA	-9.421286, -40.482981	Malukiewicz et al., 2014
PJ030	KJ020082	*C. penicillata*	*C. penicillata (Caatinga)*	Juazeiro, BA	-9.466256, -40.558875	Malukiewicz et al., 2014

PJ031	Same as KJ020082	*C. penicillata*	*C. penicillata (Caatinga)*	Juazeiro, BA	-9.466256, -40.558875	Malukiewicz et al., 2014
PJ032	Same as KJ020079	*C. penicillata*	*C. penicillata (Caatinga)*	Juazeiro, BA	-9.466256, -40.558875	Malukiewicz et al., 2014
PJ033	KJ020083	*C. penicillata*	*C. penicillata (Caatinga)*	Juazeiro, BA	-9.409378, -40.513072	Malukiewicz et al., 2014
PJ034	Same as KJ020081	*C. penicillata*	*C. penicillata (Caatinga)*	Juazeiro, BA	-9.421286, -40.482981	Malukiewicz et al., 2014
PJ035	MZ394047	*C. jacchus*	*C. jacchus*	CEMAFAUNA	NA	Malukiewicz et al., 2014, this study
PJ036	MZ394048	*C. penicillata*	*C. penicillata (Caatinga)*	CEMAFAUNA	NA	Malukiewicz et al., 2014, this study
PJ037	MZ394049	*C. jacchus*	*C. jacchus*	CEMAFAUNA	NA	Malukiewicz et al., 2014, this study
PJ038	Same as KJ020086	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.242108, -40.311100	Malukiewicz et al., 2014
PJ041	KJ020084	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.242108, -40.311100	Malukiewicz et al., 2014
PJ042	KJ020085	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.242108, -40.311100	Malukiewicz et al., 2014
PJ043	Same as KJ020086	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.218256, -40.303817	Malukiewicz et al., 2014

PJ044	Same as KJ020086	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.218256, -40.303817	Malukiewicz et al., 2014
PJ045	KJ020086	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.218256, -40.303817	Malukiewicz et al., 2014
PJ046	Same as KJ020086	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.218256, -40.303817	Malukiewicz et al., 2014
PJ047	Same as KJ020086	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.218256, -40.303817	Malukiewicz et al., 2014
PJ048	Same as KJ020084	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.264081, -40.322011	Malukiewicz et al., 2014
PJ049	Same as KJ020084	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.264081, -40.322011	Malukiewicz et al., 2014
PJ050	KJ020087	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.270269, -40.383258	Malukiewicz et al., 2014
PJ051	KJ020088	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.264081, -40.322011	Malukiewicz et al., 2014
PJ052	KJ020089	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.270269, -40.383258	Malukiewicz et al., 2014
PJ053	Same as KJ020087	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.270269, -40.383258	Malukiewicz et al., 2014
PJ054	Same as KJ020087	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.270269, -40.383258	Malukiewicz et al., 2014

PJ055	Same as KJ020087	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.270269, -40.383258	Malukiewicz et al., 2014
PJ056	Same as KJ020087	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.270269, -40.383258	Malukiewicz et al., 2014
PJ057	Same as KJ020084	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.264081, -40.322011	Malukiewicz et al., 2014
PJ058	Same as KJ020087	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.346625, -40.421750	Malukiewicz et al., 2014
PJ059	Same as KJ020088	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.346625, -40.421750	Malukiewicz et al., 2014
PJ060	Same as KJ020088	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.346625, -40.421750	Malukiewicz et al., 2014
PJ061	Same as KJ020088	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.346625, -40.421750	Malukiewicz et al., 2014
PJ062	Same as KJ020088	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.346625, -40.421750	Malukiewicz et al., 2014
PJ063	Same as KJ020088	*C. jacchus*	*C. jacchus*	Petrolina, PE	-9.346625, -40.421750	Malukiewicz et al., 2014
PJ064	KJ020090	*C. penicillata x C. jacchus*	*C. jacchus*	Petrolina, PE	-9.447925, -40.558125	Malukiewicz et al., 2014
PJ065	Same as KJ020090	*C. penicillata x C. jacchus*	*C. jacchus*	Petrolina, PE	-9.447925, -40.558125	Malukiewicz et al., 2014

PJ066	Same as KJ020090	*C. penicillata x C. jacchus*	*C. jacchus*	Petrolina, PE	-9.447925, -40.558125	Malukiewicz et al., 2014
PJ067	Same as KJ020090	*C. penicillata x C. jacchus*	*C. jacchus*	Petrolina, PE	-9.447925, -40.558125	Malukiewicz et al., 2014
PJ068	Same as KJ020090	*C. penicillata x C. jacchus*	*C. jacchus*	Petrolina, PE	-9.447925, -40.558125	Malukiewicz et al., 2014
PJ069	KJ020091	*C. penicillata x C. jacchus*	*C. jacchus*	Petrolina, PE	-9.447925, -40.558125	Malukiewicz et al., 2014
PJ070	Same as KJ020090	*C. penicillata x C. jacchus*	*C. jacchus*	Petrolina, PE	-9.447925, -40.558125	Malukiewicz et al., 2014
REC19	AY196766	*C. jacchus*	*C. jacchus*	Recife, PE	-8.065953,-34.889886	Faulkes et al., 2003
REC20	AY196767	*C. jacchus*	*C. jacchus*	Recife, PE	-8.065953,-34.889886	Faulkes et al., 2003
RJ001	KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.684114, -42.485386	Malukiewicz et al., 2014
RJ002	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.684114, -42.485386	Malukiewicz et al., 2014
RJ003	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.684114, -42.485386	Malukiewicz et al., 2014
RJ004	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.684114, -42.485386	Malukiewicz et al., 2014

RJ005	KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ006	KJ020094	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ007	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ008	Same as KJ020094	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ009	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ010	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ011	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ012	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ013	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ014	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606314, -42.396425	Malukiewicz et al., 2014
RJ015	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606314, -42.396425	Malukiewicz et al., 2014

RJ016	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.601758, -42.392836	Malukiewicz et al., 2014
RJ017	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.601758, -42.392836	Malukiewicz et al., 2014
RJ018	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.601758, -42.392836	Malukiewicz et al., 2014
RJ019	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.601758, -42.392836	Malukiewicz et al., 2014
RJ020	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.601758, -42.392836	Malukiewicz et al., 2014
RJ021	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.601758, -42.392836	Malukiewicz et al., 2014
RJ022	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.601758, -42.392836	Malukiewicz et al., 2014
RJ023	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.601758, -42.392836	Malukiewicz et al., 2014
RJ024	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ025	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606414, -42.403464	Malukiewicz et al., 2014
RJ026	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606414, -42.403464	Malukiewicz et al., 2014

RJ027	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606414, -42.403464	Malukiewicz et al., 2014
RJ028	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ029	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ030	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ031	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ032	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.655550, -42.440044	Malukiewicz et al., 2014
RJ033	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606314, -42.396425	Malukiewicz et al., 2014
RJ034	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606314, -42.396425	Malukiewicz et al., 2014
RJ035	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Rio Vermelho, RJ	-22.716117, -42.564519	Malukiewicz et al., 2014
RJ036	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Rio Vermelho, RJ	-22.716117, -42.564519	Malukiewicz et al., 2014
RJ037	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Rio Vermelho, RJ	-22.716117, -42.564519	Malukiewicz et al., 2014

RJ038	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Rio Vermelho, RJ	-22.716117, -42.564519	Malukiewicz et al., 2014
RJ039	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Rio Vermelho, RJ	-22.716117, -42.564519	Malukiewicz et al., 2014
RJ040	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Rio Vermelho, RJ	-22.716117, -42.564519	Malukiewicz et al., 2014
RJ041	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Rio Vermelho, RJ	-22.716117, -42.564519	Malukiewicz et al., 2014
RJ042	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Rio Vermelho, RJ	-22.716117, -42.564519	Malukiewicz et al., 2014
RJ044	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606314, -42.396425	Malukiewicz et al., 2014
RJ045	Same as KJ020093	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606314, -42.396425	Malukiewicz et al., 2014
RJ046	Same as KJ020092	*C. penicillata x C. jacchus*	*C. penicillata*	Silva Jardim, RJ	-22.606314, -42.396425	Malukiewicz et al., 2014
TAP04	AY196771	*C. jacchus*	*C. jacchus*	Estação Biologica do Tapacurá, PE	-8.037370, -35.198304	Faulkes et al., 2003
TAP06	AY196772	*C. jacchus*	*C. jacchus*	Estação Biologica do Tapacurá, PE	-8.037370, -35.198304	Faulkes et al., 2003
TAP07	AY196775	*C. jacchus*	*C. jacchus*	Estação Biologica do Tapacurá, PE	-8.037370, -35.198304	Faulkes et al., 2003

TAP08	AY196774	*C. jacchus*	*C. jacchus*	Estação Biologica do Tapacurá, PE	-8.037370, -35.198304	Faulkes et al., 2003
TAP09	AY196773	*C. jacchus*	*C. jacchus*	Estação Biologica do Tapacurá, PE	-8.037370, -35.198304	Faulkes et al., 2003
TAP10	Y196768	*C. jacchus*	*C. jacchus*	Estação Biologica do Tapacurá, PE	-8.037370, -35.198304	Faulkes et al., 2003

Acknowledgements

This work was supported by the Stanley Center for Psychiatric Research at the Broad Institute of MIT and Harvard, the James and Patricia Poitras Center for Psychiatric Disorders Research at MIT and the Hock E. Tan and K. Lisa Yang Center for Autism Research at MIT. This work was supported by DAAD doctoral fellowship to JLGF, a Brazilian CNPq Jovens Talentos Postdoctoral Fellowship (302044/2014-0), a Brazilian CNPq DCR grant (300264/2018-6), a Marie-Curie Individual Fellowship (AMD-793641-4), an American Society of Primatologists Conservation Small Grant, and an International Primatological Society Research Grant for JM. We would like to thank Dr. Anne Stone for her methodological assistance and input and Dr. Carlos Ruiz-Miranda for assistance with samples. We thank Vanner Boere, Ita de Oliveira, Daniel S. Silva, the Guarulhos Zoo, the CEMAFAUNA, the CPRJ staff, SERCAS staff, and AMLD staff for assistance with wild and captive populations.

References

1. Malukiewicz, J., Vanner Boere, de Oliveira, M.A.B., D'arc, M., Ferreira, J.V.A., French, J., Housman, G., de Souza, C.I., Jerusalinsky, L., de Melo, F.R., Valença-Montenegro, M.M., Moreira, S.B., de Oliveira e Silva, I., Pacheco, F.S., Rogers, J., Pissinatti, A., del Rosario, R.C.H., Ross, C., Ruiz-Miranda, C.R., Pereira, L.C.M., Schiel, N., de Fátima Rodrigues da Silva, F., Souto, A., Šlipogor, V., and Tardif, S. (2021) An Introduction to the *Callithrix* Genus and Overview of Recent Advances in Marmoset Research. ILAR Journal, 61 (2-3), 110–138, doi:10.1093/ilar/ilab027.

2. National Academies of Sciences, E. and Medicine (2019) Care, Use, and Welfare of Marmosets as Animal Models for Gene Editing-Based Biomedical Research: Proceedings of a Workshop., National Academies Press, Washington (DC); US, doi: 10.17226/25356.

3. Schiel, N. and Souto, A. (2017) The common marmoset: An overview of its natural history, ecology and behavior. Developmental Neurobiology, 77 (3), 244–262, doi: https://doi.org/10.1002/dneu.22458.

4. Malukiewicz, J., Vanner Boere, Fuzessy, L.F., Grativol, A.D., French, J.A., de Oliveira e Silva, I., Pereira, L.C., Ruiz-Miranda, C.R., Valença, Y.M., and Stone, A.C. (2014) Hybridization effects and genetic diversity of the common and blacktufted marmoset (*Callithrix jacchus* and *Callithrix penicillata*) mitochondrial control region. American Journal of Physical Anthropology, 155 (4), 522–536, doi: https://doi.org/10.1002/ajpa.22605.

5. Malukiewicz, J. (2018) A Review of Experimental, Natural, and Anthropogenic Hybridization in Callithrix Marmosets. International Journal of Primatology, 40 (1), 72–98, doi:10.1007/s10764-018-0068-0.

6. Malukiewicz, J., Cartwright, R.A., Curi, N.H.A., Dergam, J.A., Igayara, C.S., Moreira, S.B., Molina, C.V., Nicola, P.A., Noll, A., Passamani, M., Pereira, L.C.M., Pissinatti, A., Ruiz-Miranda, C.R., Silva, D.L., Stone, A.C., Zinner, D., and Roos, C. (2021) Mitogenomic phylogeny of *Callithrix* with special focus on human transferred taxa. BMC Genomics, 22 (1), doi:10.1186/s12864-021-07533-1.

7. Valença-Montenegro, M.M., Bezerra, B.M., Ruiz-Miranda, C.R., Pereira, D.G., Miranda, J.M.D., Bicca-Marques, J.C., da Cruz, M.O.L., Valle, R.R., and RA, M. (2021).

8. Tagliaro, C.H., Schneider, M.P., Schneider, H., Sampaio, I.C., and Stanhope, M.J. (1997) Marmoset phylogenetics, conservation perspectives, and evolution of the mtDNA control region. Molecular Biology and Evolution, 14 (6), 674–684, doi: 10.1093/oxfordjournals.molbev.a025807.

9. Malukiewicz, J., Cartwright, R.A., Dergam, J.A., Igayara, C.S., Nicola, P.A., Pereira, L.M.C., Ruiz-Miranda, C.R., Stone, A.C., Silva, D.L., de Fatima Rodrigues da Silva, F., Varsani, A., Walter, L., Wilson, M.A., Zinner, D., and Roos, C. (2021) Genomic skimming and nanopore sequencing uncover cryptic hybridization in one of world's most threatened primates. Scientific Reports, 11 (1), doi: 10.1038/s41598-021-96404-6.

10. Malukiewicz, J., Hepp, C.M., Guschanski, K., and Stone, A.C. (2017) Phylogeny of the *jacchus* group of *Callithrix* marmosets based on complete mitochondrial genomes.

American Journal of Physical Anthropology, 162 (1), 157–169, doi: https://doi.org/10.1002/ajpa.23105.

11. Pozzi, L., Hodgson, J.A., Burrell, A.S., Sterner, K.N., Raaum, R.L., and Disotell, T.R. (2014) Primate phylogenetic relationships and divergence dates inferred from complete mitochondrial genomes. Molecular Phylogenetics and Evolution, 75, 165–183, doi:10.1016/j.ympev.2014.02.023.

12. Tamura, K., Stecher, G., and Kumar, S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 38 (7), 3022–3027, doi:10.1093/molbev/msab120.

13. Li, H. and Durbin, R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. bioinformatics, 25 (14), 1754–1760.

14. Dierckxsens, N., Mardulyn, P., and Smits, G. (2016) NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Research, pgkw955, doi:10.1093/nar/gkw955.

15. Maddison, W.P. and Maddison, D.R., Mesquite: a modular system for evolutionary analysis. Version 3.61.

16. Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., and Sánchez-Gracia, A. (2017) DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. Molecular Biology and Evolution, 34 (12), 3299–3302, doi:10.1093/molbev/msx248.

17. Nguyen, L.T., Schmidt, H.A., von Haeseler, A., and Minh, B.Q. (2014) IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Molecular Biology and Evolution, 32 (1), 268–274, doi:10.1093/molbev/msu300.

18. Huelsenbeck, J.P. and Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 17 (8), 754–755, doi:10.1093/bioinformatics/17.8.754.

19. Ronquist, F. and Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19 (12), 1572–1574, doi:10.1093/bioinformatics/btg180.

20. Chernomor, O., von Haeseler, A., and Minh, B.Q. (2016) Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. Systematic Biology, 65 (6), 997–1008, doi:10.1093/sysbio/syw037.

21. Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., and Jermiin, L.S. (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods, 14 (6), 587–589, doi:10.1038/nmeth.4285.

22. Minh, B.Q., Nguyen, M.A.T., and von Haeseler, A. (2013) Ultrafast Approximation for Phylogenetic Bootstrap. Molecular Biology and Evolution, 30 (5), 1188–1195, doi:10.1093/molbev/mst024.

23. Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B., and Flouri, T. (2019) ModelTest-NG: A New and Scalable Tool for the Selection of DNA and Protein Evolutionary Models. Molecular Biology and Evolution, 37 (1), 291–294, doi:10.1093/molbev/msz189.

24. Gelman, A. and Rubin, D.B. (1992) Inference from Iterative Simulation Using Multiple Sequences. Statistical Science, 7 (4), doi:10.1214/ss/1177011136.

25. Yu, Y., Harris, A.J., Blair, C., and He, X. (2015) RASP (Reconstruct Ancestral State in Phylogenies): A tool for historical biogeography. Molecular Phylogenetics and Evolution, 87, 46–49, doi:10.1016/j.ympev.2015.03.008.

26. Yu, Y., Blair, C., and He, X. (2019) RASP 4: Ancestral State Reconstruction Tool for Multiple Genes and Characters. Molecular Biology and Evolution, 37 (2), 604–606, doi:10.1093/molbev/msz257.

27. Huson, D.H. and Bryant, D. (2005) Application of Phylogenetic Networks in Evolutionary Studies. Molecular Biology and Evolution, 23 (2), 254–267, doi: 10.1093/molbev/msj030.

28. Buckner, J.C., Alfaro, J.W.L., Rylands, A.B., and Alfaro, M.E. (2015) Biogeography of the marmosets and tamarins (Callitrichidae). Molecular Phylogenetics and Evolution, 82, 413–425, doi:10.1016/j.ympev.2014.04.031.

29. Haus, T., Ferguson, B., Rogers, J., Doxiadis, G., Certa, U., Rose, N.J., Teepe, R., Weinbauer, G.F., and Roos, C. (2014) Genome typing of nonhuman primate models: implications for biomedical research. Trends in Genetics, 30 (11), 482–487, doi: 10.1016/j.tig.2014.05.004.

30. Zhang, X., Meng, Y., Hougton, P., Liu, M., Kanthaswamy, S., Oldt, R., Ng, J., Trask, J.S., Huang, R., Singh, B., Du, H., and Smith, D.G. (2017) Ancestry, *Plasmodium cynomolgi* prevalence and rhesus macaque admixture in cynomolgus macaques (*Macaca fascicularis*) bred for export in Chinese breeding farms. J Med Primatol, 46, 31–41.

31. Andrieu, J.M. and Lu, W. (2017) Evidence of a tolerogenic vaccine against AIDS in the Chinese macaque prefigures a potential human vaccine. Arch Virol., 166, 1273–1282.

32. Williams-Blangero, S. and Rainwater, D.L. (1991) Variation in Lp(a) levels and apo(a) isoform frequencies in five baboon subspecies. Hum Biol, 63, 65–76.

33. Funk, D.J. and Omland, K.E. (2003) Species-Level Paraphyly and Polyphyly: Frequency, Causes, and Consequences, with Insights from Animal Mitochondrial DNA. Annual Review of Ecology, Evolution, and Systematics, 34 (1), 397–423, doi: 10.1146/annurev.ecolsys.34.011802.132421.

34. Moran, B.M., Payne, C., Langdon, Q., Powell, D.L., Brandvain, Y., and Schumer, M. (2021) The genomic consequences of hybridization. eLife, 10, doi:10.7554/elife.69016.

35. Ackermann, R.R., Schroeder, L., Rogers, J., and Cheverud, J.M. (2015) Further evidence for phenotypic signatures of hybridization in descendant baboon populations. J Hum Evol, 76, 54–62.