UNIVERSIDADE FEDERAL DO PARÁ
MUSEU PARAENSE EMÍLIO GOELDI
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA
CURSO DE DOUTORADO EM ZOOLOGIA

Marcela Guimarães Moreira Lima

Filogenia, Biogeografia e História Evolutiva dos Macacos-Prego, Gênero *Sapajus* Kerr, 1792 (Primates: Cebidae)

Tese apresentada ao Programa de Pós-graduação em Zoologia, Curso de Doutorado, do Museu Paraense Emílio Goeldi e Universidade Federal do Pará como requisito parcial para obtenção do grau de doutora em Zoologia.

Orientador: Dr. José de Sousa e Silva Júnior.

Coorientador: Dr. Alexandre Luis Padovan Aleixo

Belém-PA

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“Continue a nadar

Continue a nadar

Continue a nadar, nadar, nadar”

Dori

Ao meu pequeno príncipe (Vínicius),
meu grande amor (Marcos), aos meus
pais (Paulo e Klênia) e
meus irmãos (Fabhyola, Karolina e Caio).

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SUMÁRIO

RESUMO

Em um estudo recente, utilizando dados moleculares, morfológicos e ecológicos, *Cebus* e *Sapajus* foram reconhecidos como gêneros distintos. Apesar de *Sapajus* ser um dos gêneros de primatas neotropicais com maior volume de informação acumulada na literatura, eles têm sido considerados como um dos primatas que possuem a taxonomia mais confusa entre os mamíferos neotropicais. Até pouco tempo atrás, havia poucas informações disponíveis na literatura acerca da origem e diversificação das espécies pertencentes ao gênero *Sapajus*. Apesar dos recentes trabalhos publicados ainda não há uma hipótese robusta sobre a origem e evolução desse grupo. No presente trabalho, nosso primeiro objetivo foi testar a diversificação dos macacos-prego usando o banco de dados moleculares e geográficos mais completo disponível até o momento. Nós reconstruímos uma filogenia molecular datada para esse grupo através de inferência Bayesiana de três genes mitocondriais (D-loop, Cytb e COI). Nossos resultados apoiam uma vicariância entre as populações ancestrais dos Andes e Amazônia versus da Mata Atlântica, e uma invasão na Amazônia durante o Pleistoceno pelos *Sapajus* para explicar a atual simpatria entre *Cebus* e *Sapajus*. Nosso segundo objetivo foi montar o primeiro banco de dados filogenômicos para o gênero *Sapajus* através de elementos ultraconservados do genoma (UCE) e reconstruir a primeira filogenia robusta para o gênero. Foram extraídos os SNPs do conjunto de dados UCE, e foram geradas filogenias por meio de inferência bayesiana e máxima verossimilhança. Nossas análises apoiam fortemente a monofilia recíproca entre *Cebus* e *Sapajus*. Dentro de *Sapajus*, nossas árvores de SNPs recuperam seis espécies: *S. xanthosternos*, *S. robustus*, *S. nigritus*, *S. flavius*, *S. libidinosus*, e *S. apella* (que inclui *S. cay* e *S. macrocephalus*). Como as subdivisões morfológicas e moleculares do grupo amazônico são discordantes, recomendamos colapsar todas as espécies de macaco-prego da Amazônia e savanas do sudeste da Amazônia como *S. apella* sem subespécies.

ABSTRACT

In a recent study, using ecological, morphological and molecular data, *Cebus* and *Sapajus* were recognized as two distinct genera. Although *Sapajus* is one of the most studied genera of Neotropical primates, it has one of the most confusing taxonomic histories among Neotropical mammals. Until recently, there was little information in the literature about the origin and diversification of the species assigned to the genus *Sapajus*. Despite recently published studies on the subject, there is still no robust hypothesis about the origin and evolution of this group. In this study, our first aim was to examine capuchin monkey diversification using the most taxonomically and geographically complete molecular dataset to date for the group. We reconstruct a time-calibrated molecular phylogeny for capuchins under Bayesian inference from three mitochondrial genes (D-loop, Cytb e COI). Our results support vicariance between ancestral populations in the Andes and Amazon (ancestral *Cebus*) *versus* the Atlantic Forest (ancestral *Sapajus*), and a Pleistocene "Amazon invasion" by *Sapajus* that explains the present day sympatry of *Cebus* and *Sapajus*. Our second aim was to assemble the first phylogenomic data set for robust capuchin monkeys using ultra-conserved elements (UCEs) and construct a complete phylogeny for the genus. We extracted SNPs from the UCE data set, and we created phylogenies using Bayesian and Maximum Likelihood methods. Our analyses provide strong support for *Cebus* and *Sapajus* as two reciprocally monophyletic clades. Within Sapajus, our SNPs trees recovered six species: *S. xanthosternos, S. robustus, S. nigritus, S. flavius, S. libidinosus* and *S. apella* (including *S. cay* and *S. macrocephalus*). As morphological and molecular subdivisions of the Amazonian group are discordant, we recommend lumping all Amazonian and southern grassland robust capuchin taxa as *S. apella* without subspecies.

1. Introdução Geral

O gênero *Sapajus* Kerr, 1792 foi recentemente revalidado para abrigar as espécies de macacos-prego, anteriormente incluídas no gênero *Cebus* Erxleben, 1777. Neste último, permaneceram as espécies de caiararas, uma vez que, de acordo com Silva-Júnior (2001), a espécie tipo do mesmo é *Cebus capucinus*. Esta divisão foi proposta por Silva-Júnior (2001, 2002), com base em uma série de diferenças genéticas e da morfologia estrutural, sendo posteriormente apoiada por Lynch Alfaro *et al.* (2012b). Estes gêneros são facilmente identificáveis por apresentarem caracteres conspícuos, especialmente a presença ou ausência de tufos de pêlos na região frontal da cabeça e de crista sagital nos crânios dos machos adultos de *Sapajus* e *Cebus*, respectivamente.

As espécies do gênero *Sapajus* são exclusivas da América do Sul, distribuindo-se desde a bacia Amazônica, passando por todo o Brasil até o Paraguai e nordeste da Argentina (Silva-Júnior, 2001; Lynch Alfaro *et al.*, 2012a; Rylands *et al.*, 2013) (Figura 1). Ao longo de sua distribuição, ocupam diversos tipos de ambientes, que incluem desde os mais secos, como ambiente de Cerrados e Caatingas, até os mais úmidos, como a Amazônia e a Mata Atlântica (Bicca-Marques *et al.*, 2006; Freese & Oppenheimer, 1981).

São mamíferos arbóreos de médio porte, com massa corpórea variando entre 2 e 4 kg e cauda semi-preênsil (Freese & Oppenheimer, 1981). Ocupam tipicamente o estrato médio e sub-bosque da floresta, podendo ocasionalmente descer ao solo para forragear (Freese & Oppenheimer, 1981; Fragaszy *et al.*, 2004; Jack, 2007).

Juntamente com os caiararas, são os únicos platirrinos capazes de utilizar ferramentas e estratégias comparativas bem elaboradas na natureza para facilitar a exploração de recursos (Fragaszy *et al*., 2004; Moura & Lee, 2004). Vivem em grupos sociais numerosos que variam de 6 a 35 indivíduos (Silva-Júnior, 2001; Bicca-Marques *et al*., 2006).

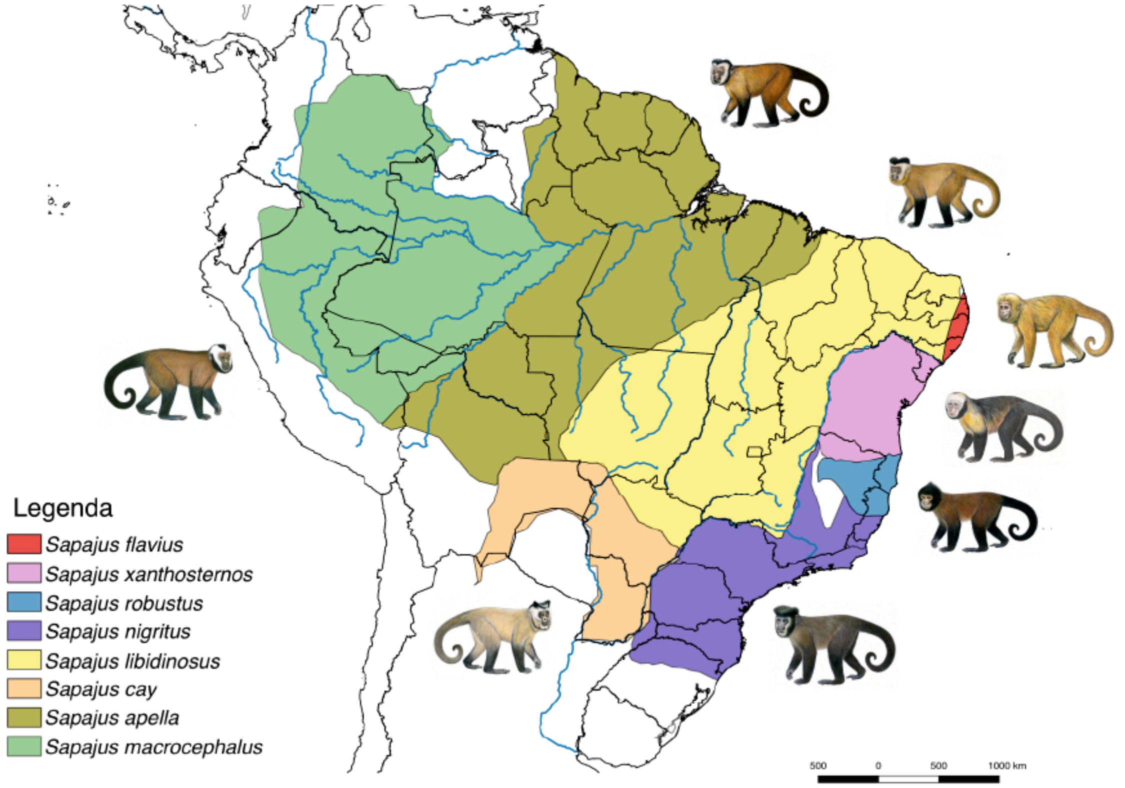

Figura 1: Mapa de distribuição dos táxons agrupados no gênero *Sapajus* segundo Lynch Alfaro *et al.* (2012b).

Sapajus é um dos gêneros de primatas neotropicais com maior volume de informação acumulada na literatura, tanto no papel de modelos experimentais em pesquisas biomédicas (de Palermo *et al.,* 1988; Bergeron *et al.,* 1999; Garcez *et al.*, 2002) como em investigações de campo e laboratório sobre ecologia e comportamento (Oppenheimer & Oppenheimer, 1973; Visalberghi, 1997; Hare *et al.*, 2003). Entretanto, tem sido considerado como um dos primatas que possuem a taxonomia mais confusa entre os mamíferos neotropicais (Silva-Júnior, 2001; Torres de Assumpção, 1983), e pouco se sabe sobre suas relações filogenéticas (Queiroz *et al.*, 2008). As principais causas apontadas para essa confusão são a deficiência de amostragem e a predisposição a apresentar grande polimorfismo, sendo a maioria de natureza individual, aparecendo em membros de uma mesma população (Silva-Júnior, 2001; Torres de Assumpção, 1983).

1.1. Taxonomia de *Sapajus*

O gênero *Cebus* foi proposto por Erxleben em 1777, com o intuito de acomodar várias espécies de primatas neotropicais, incluindo *Simia capucina* Linnaeus, 1758, *Simia apella* Linnaeus, 1758, *Simia trepida* Linnaeus, 1766 e *Simia fatuella* Linnaeus, 1766. Elliot (1913) forneceu uma chave taxonômica que dividia o gênero em dois grupos com base na presença ou não de um conjunto de pêlos na região frontal da cabeça (grupos com ou sem tufo). Entretanto, somente após o trabalho de Hershkovitz (1949), houve um consenso geral sobre essa divisão, sendo reconhecida apenas uma espécie, *Cebus apella* (Linnaeus, 1758), para as formas com tufo (macacos-prego). Contudo, Hershkovitz (1949) não publicou a revisão das formas com tufo, deixando a mesma para Remington Kellogg, que faleceu antes de completar seu estudo.

Revisões subsequentes mantiveram essa divisão, baseada na presença ou ausência de tufos, porém elas não chegaram a um consenso taxonômico (Lynch Alfaro *et al*, 2012a). Dentre os táxons com tufo, Cabrera (1957) reconheceu uma espécie (*Cebus apella*) e 10 subespécies e, posteriormente, Hill (1960) publicou uma revisão taxonômica não crítica baseada em comunicação pessoal de R. Kellogg, reconhecendo 16 subespécies para essa mesma espécie.

Torres de Assumpção (1983, 1988) deu início à, até então, maior revisão sistemática das formas de *Cebus* com tufo, identificando seis áreas onde o grupo demonstrava alguma estabilidade nos caracteres examinados e formulou hipóteses acerca dos possíveis mecanismos de especiação. No entanto, esta autora faleceu antes de publicar os resultados do seu estudo, de modo que a questão taxonômica em si não foi discutida na parte da obra que chegou a ser publicada (Silva-Júnior, 2001; Torres de Assumpção, 1983, 1988). Com base em informações presentes na literatura, Rylands *et al.* (2000) avaliaram a diversidade de táxons de primatas do Novo Mundo, reconhecendo quatro espécies (*C. apella, C. libidinosus, C. nigritus* e *C. xanthosternos*) e 14 subespécies para o grupo de *Cebus* com tufo.

Em um trabalho mais completo, envolvendo dados morfológicos, morfométricos, moleculares, comportamentais e ecológicos, Silva-Júnior (2001) realizou uma nova revisão taxonômica para o gênero *Cebus.* Este autor indicou que as formas com tufo e sem tufo deveriam ser tratadas como subgêneros distintos. O nome subgenérico disponível aplicado ao grupo com tufo foi *Sapajus* Kerr, 1792, para o qual foram reconhecidas sete espécies: *Cebus* (*Sapajus*) *apella* Linnaeus, 1758*, Cebus* (*Sapajus*) *macrocephalus* Spix, 1823*, Cebus* (*Sapajus*) *libidinosus* Spix, 1823, *Cebus* (*Sapajus*) *cay* Illiger, 1815*, Cebus* (*Sapajus*) *xanthosternos* Wied, 1820*, Cebus* (*Sapajus*) *robustus* Kuhl, 1820 e *Cebus* (*Sapajus*) *nigritus* Goldfuss, 1809. No mesmo ano, Groves (2001) propôs um arranjo taxonômico para as formas com tufo, baseado em dados morfológicos, reconhecendo quatro espécies e 14 subespécies, e revalidando vários nomes até então sob sinonímia.

Mendes Pontes *et al*. (2006) descreveram uma nova espécie do gênero *Sapajus* com distribuição restrita à Mata Atlântica nordestina, ao norte do rio São Francisco: *Cebus queirozi* Pontes, Malta & Asfora, 2006. Entretanto, logo após a descrição dessa espécie, Oliveira & Langutth (2006) publicaram um estudo no qual reconheceram que essa “nova espécie” de macacos-prego é, na verdade, uma espécie descrita há 238 anos pelo naturalista alemão Johann Schreber, e chamada originalmente de *Simia Flavia* Schreber, 1774. Eles então revalidaram *Simia flavia*, atualizando a nomenclatura para *Cebus flavius* (atualmente *Sapajus flavius*), e designaram um neótipo para a espécie.

Em um estudo recente, utilizando dados moleculares, morfológicos e ecológicos, Lynch Alfaro *et al.* (2012a, b) corroboraram a decisão de Silva-Júnior (2002), reconhecendo *Cebus* e *Sapajus* como gêneros distintos. Atualmente são reconhecidas oito espécies no gênero *Sapajus*: *S. apella*, *S. macrocephalus*, *S. xanthosternos*, *S. robustus*, *S. nigritus*, *S. cay*, *S. libidinosus* e *S. flavius* (Rylands *et al*, 2013).

1.2. História evolutiva do gênero *Sapajus*

Até pouco tempo atrás, havia poucas informações disponíveis na literatura acerca da origem e diversificação das espécies pertencentes ao gênero *Sapajus*. Em um estudo analisando a divergência molecular entre duas populações de *S. cay* (Brasil e Paraguai), Casado *et al.* (2010) dataram a separação de *Cebus* e *Sapajus* no Plioceno tardio (4,2 Ma). Entretanto, esses autores ainda não os consideraram gêneros válidos e sim subgêneros como proposto por Silva-Júnior (2001).

Casado *et al.* (2010) ainda descrevem de maneira sucinta a relação de parentesco entre algumas espécies de *Sapajus*, utilizando em suas análises apenas três espécies do gênero, onde *S. apella* e *S. cay* formavam um clado, e *S. xanthosternos* seria a espécie irmã desse clado. Segundo esses resultados, a separação entre *S. apella* e *S. cay* seria recente (2,6 Ma), o que explicaria a baixa divergência genética encontrada por esses autores nas análises das duas espécies (1,1%, ±0.2). Entretanto, por não ser o foco do estudo, Casado *et al.* (2010) não se aprofundaram na questão sobre a origem e a evolução dessas espécies, limitando-se a discutir sobre a divergência genética e separação das populações de *S. cay* do Brasil e do Paraguai.

Em estudos recentes, utilizando marcadores mitocondriais (12S e Cytb), morfologia e ecologia, Lynch Alfaro *et al.* (2012a, b) reconheceram *Cebus* e *Sapajus* como gêneros distintos. Segundo esses autores, o ancestral desses dois gêneros seria originário do oeste da Amazônia, e o processo inicial de vicariância teria sido causado pelo estabelecimento do rio Amazonas (7 Ma), de modo que as populações que dariam origem ao ancestral de *Cebus* teriam ficado restritas ao escudo das Guianas, enquanto as populações que dariam origem ao ancestral de *Sapajus* teriam ficado restritas ao escudo Brasileiro.

Desse modo, o ancestral dos macacos-prego teria se originado na Mata Atlântica, ou talvez mais para o interior, onde hoje é o Cerrado, e a partir daí as populações teriam se expandido em direção à Mata Atlântica a cerca de 2,7 Ma. Nesta região, teriam sofrido processos de diversificação e ficado isoladas durante todo o Plioceno (Lynch Alfaro *et al.*, 2012a). Durante esse período, as primeiras espécies a se diversificarem teriam sido aquelas pertencentes ao clado da Mata Atlântica (*S. nigritus*, *S. robustos* e *S. xanthosternos*).

Somente há 700 mil anos, os macacos-prego expandiram sua distribuição novamente em direção ao Cerrado e, há 400 mil anos, eles retornaram à Amazônia. Segundo Lynch Alfaro *et al.* (2012a), houve uma diversificação um pouco mais recente para as espécies que atualmente ocorrem no Cerrado, Pantanal e Amazônia (*S. libidinosus, S. cay, S. apella e S. macrocephalus*, respectivamente). Essa expansão pode ser explicada por duas hipóteses. A primeira seria pela expansão das florestas úmidas no Pleistoceno, havendo evidências de aumento da cobertura florestal em todo o Nordeste, ligando a Amazônia e a Mata Atlântica (Lynch, 1988; Costa, 2003). A segunda seria por meio de uma rota histórica ao longo da bacia do rio Paraná (Por, 1992). Há evidências de que três espécies de marsupiais (*Caluromys lanatus*, *Metachirus nudicaudatus* e *Marmosa murina*) teriam ampliado sua distribuição por meio dessa rota no sentido Mata Atlântica/Amazônia (Costa, 2003) e, segundo Lynch Alfaro *et al.* (2012a), essa seria a rota mais provável para expansão dos macacos-prego para a Amazônia.

Entretanto, apesar de Lynch Alfaro *et al.* (2012a) apresentarem essas hipóteses sobre a origem dos macacos-prego, esses autores reconheceram que contaram com poucas amostras da calha sul do rio Amazonas. Desse modo, eles propuseram que, em uma hipótese alternativa, os macacos-prego poderiam ter se originado na região da calha sul do rio Amazonas, invadindo primeiro a Mata Atlântica e, mais tarde, invadindo novamente o oeste e o norte da Bacia Amazônica. Além disso, esses autores não discutiram de maneira detalhada sobre a diversificação dentro de *Sapajus*. Nesse sentido, ainda não há uma hipótese robusta sobre a origem e evolução desse grupo. Existe a necessidade de estudos mais aprofundados para esclarecer tais questões, incluindo as relações de parentesco entre as espécies pertencentes a esse gênero.

1.3. Sequenciamento de Nova Geração (NGS) e Elementos Ultraconservados (UCE)

O uso de abordagens multilocus para inferir a história de populações e espécies tem se tornado a linha base de estudos filogenéticos e filogeográficos (McCormack *et al.* 2013a). Felizmente, gerar dados para estudos com esse tipo de abordagem tem ficado cada vez menos trabalhoso e mais barato, apesar da grande quantidade de etapas envolvidas nesse processo.

Com o advento do sequenciamento de nova geração (NSG), tornou-se possível realizar o sequenciamento de milhares de pares de bases de marcadores independentes em uma única corrida, de forma eficiente e barata, quando comparada à metodologia tradicional de Sanger (McCormack *et al.*, 2013a). Essa nova tecnologia de sequenciamento promete fornecer estimativas cada vez mais detalhadas da história de espécies e populações através da resolução de radiações rápidas (Wagner *et al.*, 2013). No entanto, a questão de como reduzir os genomas de muitos indivíduos a fragmentos ortólogos continua a ser um obstáculo significativo para incorporar métodos de NGS em estudos filogenéticos e filogeográficos.

A solução para esse problema surgiu com a recente descoberta de marcadores ancorados por elementos ultraconservados do genoma ("Ultraconserved Elements" – UCE). Como o próprio nome sugere, as UCEs são regiões altamente conservadas do genoma e compartilhadas entre táxons evolutivamente distantes, como seres humanos, aves e lagartos (Faircloth *et al.,* 2012, Smith *et al.* 2014). Através das UCEs é possível realizar o sequenciamento de milhares de marcadores ortólogos de diversos táxons, separados por milhões de anos de evolução (Faircloth *et al.,* 2012).

Por possuírem um elevado nível de conservação, as UCEs são fáceis de identificar e alinhar, mesmo em genomas diferentes, sendo dessa forma muito úteis para estudos filogenéticos e filogeográficos (McCormack *et al.,* 2013a). Estudos recentes, utilizando as UCEs em combinação com técnicas de sequenciamento de nova geração, têm conseguido resolver relações filogenéticas em grupos de aves (McCormack *et al.,* 2013b), mamíferos (McCormack *et al.*, 2012), peixes (Faircloth *et al.,* 2013) e himenópteros (Faircloth *et al*, 2015). E por possuírem variação suficiente nas suas regiões flanqueadoras, as UCEs também têm sido utilizadas em estudos filogeográficos com grupos de aves (Smith *et al,* 2014) e peixes (Faircloth *et al.*, 2013), incluindo radiações do Pleistoceno (McCormack *et al.*, 2015).

Nesse sentido, o projeto aqui proposto constitui um esforço para compreender melhor as relações de parentesco do gênero e os processos de diversificação entre as espécies do gênero *Sapajus*, utilizando dados de marcadores mitocondriais e dos UCEs em combinação com a tecnologia de sequenciamento de nova geração, tendo em vista que existem espécies desse gênero distribuídas tanto em biomas florestais como nos de vegetações abertas e, portanto, com histórias evolutivas possivelmente distintas. Assim, uma análise biogeográfica mais abrangente envolvendo todos os táxons pertencentes ao gênero poderá trazer resultados mais conclusivos sobre a história evolutiva desses táxons e suas relações com os eventos históricos que influenciaram na formação dos biomas.

2. General Introduction

The genus *Sapajus* Kerr, 1792 was recently re-validated to encompass the robust capuchin monkeys, which were previously included within the genus *Cebus* Erxleben, 1777. Gracile capuchins remain within *Cebus*, according to Silva-Júnior (2001), with the type species *Cebus capucinus*. This division was proposed by Silva-Júnior (2001, 2002), based on genetic and morphological differences, and later confirmed by Lynch Alfaro *et al.* (2012b). These genera are easily distinguished by conspicuous morphological characters, especially the presence or absence of tufts of hair in the frontal region of the head, as well as a pronounced sagittal crest in the cranium of adult male *Sapajus* and *Cebus*, respectively.

The species in the genus *Sapajus* are found only in South America, both throughout the Amazon Basin, across all Brazil to Paraguay and northeastern Argentina (Silva-Júnior, 2001; Lynch Alfaro *et al.*, 2012a; Rylands *et al.*, 2013) (Figure xx). Across their distribution, they occupy diverse habitats, including the dry Cerrado and Caatinga, and the humid Amazon and Atlantic (Bicca-Marques *et al.*, 2006; Freese & Oppenheimer, 1981).

They are medium-sized mammals, with body masses of about 2 to 4 kg and semi-prehensile tails (Freese & Oppenheimer, 1981). They typically inhabit the middle level of the forest and the understory, occasionally coming to the ground to forage (Freese & Oppenheimer, 1981; Fragaszy *et al*., 2004; Jack, 2007).

Along with the gracile capuchin monkeys, they are the only Neotropical primates capable of using tools and they have comparatively elaborate strategies to extract resources from the environment (Fragaszy *et al*., 2004; Moura & Lee, 2004).

They live in large social group with 6 to 35 individuals (Silva-Júnior, 2001; Bicca-Marques *et al.*, 2006).

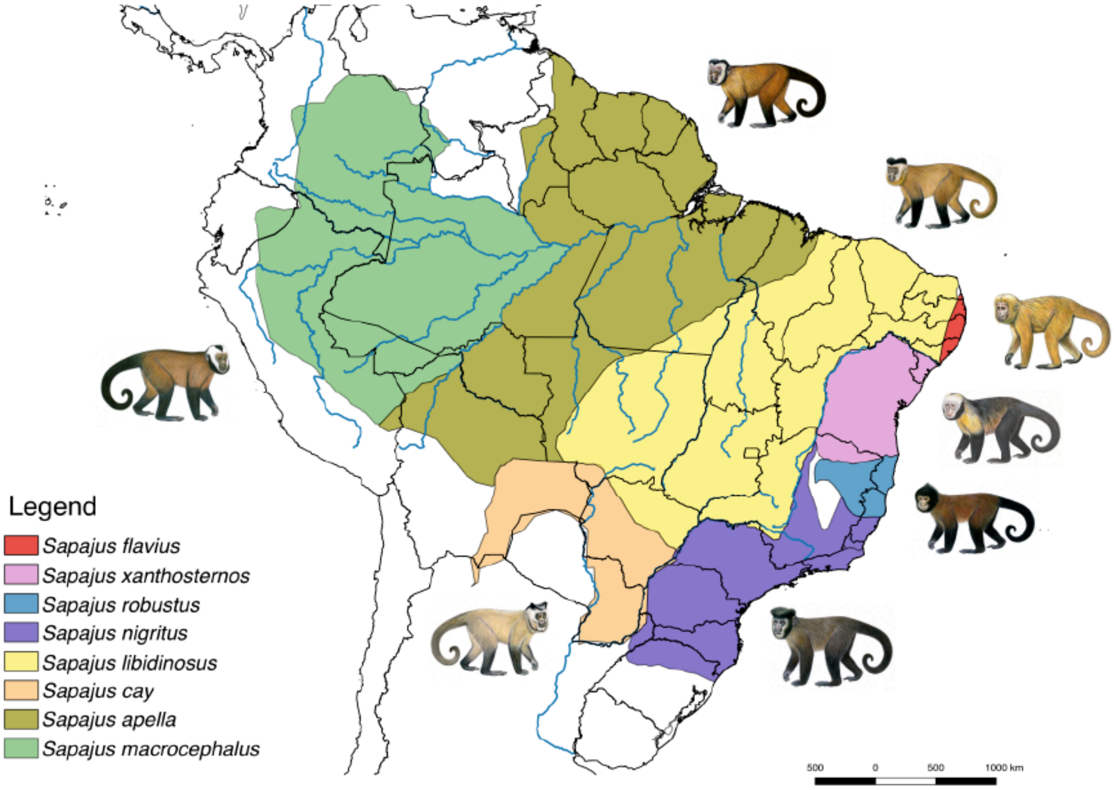

Figure 1: Distribution map of the taxa grouped in the genus *Sapajus* according to Lynch Alfaro *et al.* (2012b).

Sapajus is one of the genera of Neotropical primates with the most information available in the literature, due to the use of capuchins as a model for biomedical research (de Palermo *et al.,* 1988; Bergeron *et al.,* 1999; Garcez *et al.*, 2002) as well as field and laboratory work on ecology and behavior (Oppenheimer & Oppenheimer, 1973; Visalberghi, 1997; Hare *et al.*, 2003). At the same time, it is considered one of the primate groups with the most confusing taxonomy among Neotropical mammals (Silva-Júnior, 2001; Torres de Assumpção, 1983), and little is known about the phylogenetic relationships within the group (Queiroz *et al.*, 2008). The principal reasons for this confusion have been the lack of available samples from many areas that capuchins inhabit, and the fact that capuchins show a high degree of polymorphism, at the individual and group level (Silva-Júnior, 2001; Torres de Assumpção, 1983).

2.1. Sapajus Taxonomy

The genus *Cebus* was proposed by Erxleben in 1777, with the intent to include various Neotropical primates, such as *Simia capucina* Linnaeus, 1758, *Simia apella* Linnaeus, 1758, *Simia trepida* Linnaeus, 1766 and *Simia fatuella* Linnaeus, 1766. Elliot (1913) created a taxonomic key that divided the genus in two groups based on the presence or absence of a grouping of hair in the frontal region of the head (groups with and without tufts). However, it was only after work by Hershkovitz (1949), that there was a general consensus about this division, with only one species, *Cebus apella* (Linnaeus, 1758), included in the tufted group (robust capuchin monkeys). In fact, Hershkovitz (1949) never published a review of tuft shape, leaving this work for Remington Kellogg, who died before completing his work.

Subsequent revisions of the group maintained this division, based on the presence or absence of tufts, although a taxonomic consensus was not reached (Lynch Alfaro *et al*, 2012a). Within the tufted group, Cabrera (1957) recognized one species (*Cebus apella*) and 10 subspecies, and later, Hill (1960) published a taxonomic revision based on the personal communication from R. Kellogg, recognizing 16 subspecies for this same species.

Torres de Assumpção (1983, 1988) pursued the first major taxonomic revision within the tufted group, identifying six areas where the populations showed some stability in the morphological characteristics examined, and she formulated hypotheses about the possible mechanisms for speciation within this group. However, this author died before publishing the results of her study, and the taxonomic evaluation was not included in what was eventually published (Silva-Júnior, 2001; Torres de Assumpção, 1983, 1988). Based on information present in the literature, Rylands *et al.* (2000) evaluated the diversity of Neotropical primate taxa, and recognized four species (*C. apella, C. libidinosus, C. nigritus* and *C. xanthosternos*) and 14 subspecies for the tufted group within *Cebus*.

In a more comprehensive work, using morphological, morphometric, molecular, behavioral and ecological data, Silva-Júnior (2001) performed a new taxonomic revision of the genus *Cebus*. He indicated that the tufted and non-tufted forms should be treated as distinct sub-genera. The available subgeneric name applied to the tufted group was *Sapajus* Kerr, 1792, for which he recognized seven species: *Cebus* (*Sapajus*) *apella* Linnaeus, 1758*, Cebus* (*Sapajus*) *macrocephalus* Spix, 1823*, Cebus* (*Sapajus*) *libidinosus* Spix, 1823, *Cebus* (*Sapajus*) *cay* Illiger, 1815*, Cebus* (*Sapajus*) *xanthosternos* Wied, 1820*, Cebus* (*Sapajus*) *robustus* Kuhl, 1820 and *Cebus* (*Sapajus*) *nigritus* Goldfuss, 1809. In the same year, Groves (2001) proposed a taxonomic arrangement for the tufted forms, based on morphological data, recognizing four species and 14 subspecies, and revalidating several names that had been placed until then as synonyms.

Mendes Pontes *et al*. (2006) described a new species of tufted capuchin with a distribution restricted to the northeastern Atlantic Forest, north of the San Francisco River: *Cebus queirozi* Pontes, Malta & Asfora, 2006. However, immediately after the description of this species, Oliveira & Langutth (2006) published a study that revealed that this “new species” of robust capuchin was, in fact, a species described 238 years earlier by the German Johann Schreber, and originally named *Simia Flavia* Schreber,

1774. They revalidated the name *Simia flavia*, updating the nomenclature to *Cebus flavius* (now *Sapajus flavius*), and designated a neotype for the species.

In a recent study, using ecological, morphological and molecular data, Lynch Alfaro *et al.* (2012a, b) supported the Silva-Júnior's (2001) decision recognizing *Cebus* and *Sapajus* as two distinct genera. Currently eight species are recognized within the genus *Sapajus*: *S. apella*, *S. macrocephalus*, *S. xanthosternos*, *S. robustus*, *S. nigritus*, *S. cay*, *S. libidinosus* and *S. flavius* (Rylands *et al*, 2013).

2.2. Evolutionary History of the genus *Sapajus*

Until recently, there was little available information in the literature about the origin and diversification of the species belonging to the genus *Sapajus*. Casado *et al.*'s (2010) study analyzed the molecular divergence between two populations of *S. cay* (in Brazil and Paraguay), and dated the separation between *Cebus* and *Sapajus* in the late Pliocene (4.2 Ma). These authors considered *Cebus* and *Sapajus* as subgenera, as proposed by Silva-Júnior (2001).

Casado *et al.* (2010) describe the relationships among some species of *Sapajus*, but their analyses only included three species within the genus. *S. apella* and *S. cay* formed a clade together, and *S. xanthosternos* was the sister species to that clade. According to their results, the separation between *S. apella* and *S. cay* was recent (2.6 Ma), which explained the low level of genetic divergence between the two species (1.1% ± 0.2). As it was not a focus of the study, Casado *et al.* (2010) did not say much about the origin and evolution of these species, limiting the discussion to genetic divergence and the separation of populations of *S. cay* in Brazil and Paraguay.

In recent studies, using mitochondrial markers (12S and Cyt b), morphology and ecology, Lynch Alfaro *et al.* (2012a, b) recognized *Cebus* and *Sapajus* as two distinct genera. According to these authors, the ancestor to these genera originated in western Amazon, and the initial process of vicariance may have been caused by the establishment of the Amazon River (7 Ma), so that the population leading to modern *Cebus* was restricted to the Guiana Shield, and the populations that gave rise to *Sapajus* were restricted to the Brazilian Shield.

In this way, the ancestor to robust capuchins originated in Atlantic Forest, or possibly what is today Cerrado, and from there populations expanded in the Atlantic Forest around 2.7 Ma. In this region, populations would have been affected by diversification processes and become isolated during the Pliocene (Lynch Alfaro *et al.*, 2012a). The first robust capuchins to diversify were those belonging to the Atlantic Forest clade (*S. nigritus*, *S. robustos* and *S. xanthosternos*).

Only 700,000 years ago, robust capuchins expanded their distribution toward the Cerrado, and at 400,000 years ago, they expanded into the Amazon. According to Lynch Alfaro *et al.* (2012a), there was a subsequent rapid diversification for the species that currently live in the Cerrado, Pantanal and Amazon (*S. libidinosus, S. cay, S. apella* and *S. macrocephalus*, respectively). This expansion might be explained by two different hypotheses. The first is that there was an expansion of humid forests in the Pleistocene, with evidence of increased forest coverage throughout the Northeast, connecting the Amazon with the Atlantic Forest (Lynch, 1988; Costa, 2003). The second would be by the historic route along the Paraná River basin (Por, 1992). There is evidence that three marsupials (*Caluromys lanatus*, *Metachirus nudicaudatus* and *Marmosa murina*) expanded their distribution by this route from the Atlantic Forest toward the Amazon (Costa, 2003) and according to Lynch Alfaro *et al.* (2012a), this was the most probable route that the robust capuchins used to expand toward the Amazon as well.

Although Lynch Alfaro *et al.* (2012a) present these hypotheses about the origin of robust capuchins, the authors point out that they have few samples in the Southern Amazon basin, south of the Amazon River. For this reason, they propose that an alternative hypothesis could be that the robust capuchins originated south of the Amazon River within the Amazon, and first invaded the Atlantic Forest, and later also spread west and north within the Amazon. The authors did not describe in detail the diversification within *Sapajus*. There is still no robust hypothesis about the origin and evolution of this group. There is a need for more detailed studies to clarify our understanding, including the evolutionary relationships among the species within this genus.

2.3. Next Generation Sequencing (NGS) and Ultraconserved Elements (UCE)

The use of multilocus methods to infer population and species history has become the baseline for phylogenetic and phylogeographic studies (McCormack *et al.* 2013a). Fortunately, it has rapidly become less expensive and less time consuming to do this kind of study, despite the many steps involved in the process.

With the advent of next generation sequencing (NSG), it became possible to sequence millions of base pairs and independent markers in a single run, in an efficient and inexpensive way, compared to traditional Sanger sequencing (McCormack *et al.*, 2013a). This new sequencing technology promises to produce ever more detailed estimates of population and species history because it can resolve topology for rapid radiations (Wagner *et al.*, 2013). However, the problem of reducing the genome of multiple individuals into orthologous fragments continues to be a significant obstacle in incorporating NGS methods into phylogenetic and phylogeographic studies.

The solution to this problem came with the recent discovery of markers anchored by ultraconserved elements in the genome (UCEs). As the name suggests, UCEs are highly conserved regions of the genome and shared among evolutionarily distant taxa, such as humans, birds and lizards (Faircloth *et al.,* 2012, Smith *et al.* 2014). Using UCEs it is possible to sequence thousands of orthologous markers in diverse taxa, separated for millions of years of evolution (Faircloth *et al.,* 2012).

Since they are highly conserved, UCEs are easy to identify and align, even from different genomes, making them useful for phylogenetic and phylogeographic studies (McCormack *et al.,* 2013a). Recent studies, using UCEs in combination with next generation sequencing, have resolved phylogenetic relationships in birds (McCormack *et al.,* 2013b), mammals (McCormack *et al.*, 2012), fish (Faircloth *et al.,* 2013) and Hymenoptera (Faircloth *et al*, 2015). As they have enough variation in the flanking regions, UCEs have also been utilized for phylogeographic studies in birds (Smith *et al,* 2014) and fishes (Faircloth *et al.*, 2013), including Pleistocene radiations (McCormack *et al.*, 2015).

In this sense, the goal of this project is to better understand the evolutionary relationships and diversification processes among the species within the genus *Sapajus*, using mitochondrial markers, as well as UCE markers in combination with next generation sequencing. Species in this genus are distributed in forest biomes as well as in biomes with open vegetation, suggesting distinct evolutionary histories. A more detailed biogeograpic analysis including all the pertinent taxa in the genus will allow for more conclusive results about the evolutionary history of the taxa and its relation to historic events that influenced the formation of the current biomes *Sapajus* inhabits.

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4. CAPÍTULO 1

Biogeografia dos macacos-prego: entendendo a simpatria entre *Cebus* e *Sapajus*

"Capuchin monkey biogeography: understanding sympatry between *Cebus* and *Sapajus*"

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[1] Esse capítulo está no formato de manuscrito e está sob revisão do periódico "*Journal of Biogeography".* Os co-autores revisaram a presente versão.

[1] This chapter is in the manuscript format and is under review of the journal "Journal of Biogeography." The co-authors reviewed the present version.

ORIGINAL ARTICLE

Capuchin monkey biogeography: understanding sympatry between *Cebus* and *Sapajus*

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SHORT RUNNING HEAD: [max. 8 words] Capuchin monkey biogeography

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ABSTRACT

Aim

Our aim was to examine capuchin monkey diversification using the most taxonomically and geographically complete molecular dataset to date for the group. We also wanted to reconstruct the biogeographic history of the clade using statistical methods that model both anagenetic and cladogenetic processes in order to evaluate hypotheses that attempt to explain the extensive geographic overlap between reciprocally monophyletic gracile and robust capuchin monkeys.

Location

Central and South America.

Methods

We reconstruct a time-calibrated molecular phylogeny for capuchins under Bayesian inference from three mitochondrial genes. We then categorized 12 capuchin clades across seven Neotropical centers of endemism and reconstructed the biogeographic history of the capuchin radiation using Bayesian methods. We performed a phylogeographic analysis for a robust capuchin clade that spans the Atlantic Forest, Cerrado, Caatinga, and Amazon Basin.

Results

We found support for a late Miocene vicariant *Cebus*-*Sapajus* divergence and a Pleistocene *Sapajus* invasion of the Amazon from the Atlantic Forest. Our new analyses confirm *Sapajus* diversified first in Atlantic Forest, with subsequent range expansion into widespread sympatry with *Cebus* in Amazonia, as well as multiple expansions into drier savanna-like habitats. We did not find mitochondrial molecular congruence with morphological species distinctions for *Sapajus flavius*, *S. cay*, *S. macrocephalus*, *S. libidinosus*, and *S. apella*; instead, these five morphological types together formed a single widespread clade (Bayesian pp = 1) with shared ancestry during the Pleistocene.

Main conclusions

Our results support vicariance between ancestral populations in the Andes and Amazon *versus* the Atlantic Forest, and a Pleistocene "Amazon invasion" by *Sapajus* to explain the present day sympatry of *Cebus* and *Sapajus*.

Keywords Amazon, Atlantic Forest, BEAST Phylogeography, Caatinga, *Cebus kaapori*, Cerrado, RASP Biogeography, range expansion, *Sapajus flavius*

INTRODUCTION

The study of capuchin monkey biogeography has been confounded by high phenotypic diversity, taxonomic uncertainty and wide geographic distribution (Silva-Júnior, 2001; Torres, 1988; Rylands *et al.*, 2005, 2013). However, recent studies have elucidated major features of this radiation (Ruiz-García *et al.,* 2010, 2012; Boubli *et al.* 2012; Lynch Alfaro *et al.,* 2012a). On the basis of widespread genetic sampling, Lynch Alfaro *et al.* (2012a) tested competing hypotheses for the current distribution of capuchins, including the present day sympatry of gracile and robust capuchins throughout much of the Amazon Basin.

Phylogeographic analyses recovered three key features of capuchin evolution, all concordant with the "Reinvasion of the Amazon" hypothesis: 1) gracile capuchins, *Cebus*, diverged from robust capuchins, *Sapajus* in the early Miocene; 2) crown *Cebus* most likely originated in the western Amazon and crown *Sapajus* in Atlantic Forest; and 3) the presence of *Sapajus* in the Amazon today reflects a recent invasion during the Pleistocene from the Atlantic Forest (Lynch Alfaro *et al.*, 2012a). These results have transformed the interpretation of capuchin taxonomy, conservation biology, morphology and ecology (see Lynch Alfaro *et al.*, 2012b, 2014, 2015b; Matthews, 2012; Rylands *et al.*, 2013; Martins *et al.*, 2014; Oliveira *et al.*, 2014; Boubli *et al.*, 2015; Schneider & Sampaio, 2015; Wright *et al.*, 2015).

Despite progress in understanding capuchin monkey biogeography, important questions remain unanswered due in part to limitations in sampling and methodology in prior studies. In particular, reconstruction of capuchin colonization of the Amazon and Atlantic Forest, that strongly informs the "Reinvasion of the Amazon" *versus* "Out of the Amazon" hypotheses (Lynch Alfaro *et al.*, 2012a), have been based upon pure dispersal models with limited geographic sampling of the Eastern Amazon and Cerrado/Caatinga regions. Increased sampling and statistical biogeographic models provide the opportunity to test more nuanced explanations for capuchin biogeography including:

1. Vicariance between the Amazon and Atlantic forests: Divergence between *Sapajus* and *Cebus* is caused by isolation of ancestral capuchin monkey populations in Atlantic Forest *versus* Amazonian habitats, respectively, with subsequent expansion of *Sapajus* into sympatry with Amazonian *Cebus* ("Reinvasion of the Amazon" hypothesis).
2. Vicariance by the Amazon River: *Cebus* is isolated north of the Amazon River, with *Sapajus* isolated south of the Amazon River and in the Atlantic Forest. Sympatry occurs with crossing of Amazon River in both directions ("Out of the Amazon" hypothesis).

Our overall goal is to test recent hypotheses explaining capuchin biogeography (Boubli *et al.*, 2012; Lynch Alfaro *et al.,* 2012a; Nascimento *et al.*, 2015) in the context of the largest molecular phylogenetic tree for the group. We thus assemble a new cytochrome *b* + D-loop + cytochrome oxidase I matrix with three times the samples and more comprehensive spatial and taxonomic sampling compared to Lynch Alfaro *et al.* (2012a). We produce the most complete phylogeny for capuchin monkeys to date, with all major lineages represented, to confirm the monophyly of *Cebus* and *Sapajus* and to recover major clades within the capuchin radiation. We compare alternative biogeographical scenarios using a Bayesian framework that allows for reconstruction of ancestral areas over a posterior distribution of trees. We also assess whether the employment of a more complete genetic dataset and statistical methods with expanded models increase support for conclusions about capuchin biogeographical evolution from Lynch Alfaro *et al.* (2012a), Nascimento *et al.* (2015) or support new scenarios altogether.

MATERIALS AND METHODS

DNA extraction, amplification, sequencing and alignment

We sequenced three mitochondrial genes, cytochrome *b* (*Cyt b*, 1133 bp), D-loop (1244 bp) and cytochrome oxidase I (COI, 673 bp), for 108 capuchin samples from poorly known or previously unsampled localities and species (Table 1; see Appendix S1for GenBank accession numbers). For *Cyt b* and D-loop we used primers from and followed standard PCR protocols described in Lynch Alfaro *et al.* (2012a) and Boubli *et al.* (2012). We adapted COI primers and protocols from Ward *et al.* (2005) (see Appendix S2). We combined the 108 new samples with 40 samples analyzed previously by Lynch Alfaro *et al*. (2012a) and 13 new samples extracted from GenBank, totaling 161 capuchin samples. We only used sequences over 500 bp long to improve branch support. We used the most recent comprehensive taxonomic review for capuchins (Rylands *et al.*, 2013), in relation to specimen morphology and collection locality, to assign species names to samples. Locality data are provided in Figure 1 and Table 1. Although the limitations of mitochondrial DNA studies are well understood, *Cyt b*, D-loop and COI have performed well in capturing phylogeny and biogeographical history in a wide range of species (Tobe *et al*., 2010; Zhang et al., 2011; Robins *et al.*, 2014). In addition, mtDNA sequence can be reliably generated from museum “crusties” and skins using now standard protocols (e.g., Lynch Alfaro *et al.*, 2012a, 2015a; Mercês *et al.*, 2015). These advantages allowed us to create the largest and most densely sampled alignment to date for capuchins.

Phylogenetic Reconstruction

We used MRBAYES 3.2.6 (Ronquist *et al.*, 2012) to reconstruct the phylogenetic relationships among capuchins, using *Saimiri* as an outgroup. We partitioned *Cyt b* and COI by codon for the analysis and ran the MCMC for 50 million generations. We then used BEAST 1.8.1 (Drummond *et al.*, 2012) to reconstruct a time tree appropriate for our biogeographical analyses by pruning the alignment to include only a single exemplar taxon for each recovered and well-supported clade. We considered branch lengths and recovered polytomies in addition to posterior probabilities at nodes when distinguishing clades. This resulted in a new, pruned alignment with 13 tips, representing 12 capuchin clades and one outgroup. We specified a HKY + G model of nucleotide substitution and partitioned by codon, using *Cyt b* only to avoid branch length bias due to missing data in the other genes. We used *Saimiri* as an outgroup and the fossil *Neosaimiri* (Kay, 2015) to calibrate the split at 12.5 Ma (lognormal prior with offset of 12.5, mean=0, SD=1) between capuchins and squirrel monkeys for the time tree. We ran the Markov chain Monte Carlo (MCMC) analysis for 100 million steps, sampling states every 10,000 generations with a Yule prior on the diversification rate, and an uncorrelated, lognormal relaxed molecular clock.

Biogeographical modelling

Biogeographical scenarios We test for reciprocal monophyly for *Sapajus* in the Amazon (*S. macrocephalus* + *S. apella*); the Cerrado + Caatinga + Central Grasslands

(*S. cay* + *S. libidinosus*); and the Atlantic Forest (*S. flavius* + *S. xanthosternos* + *S. robustus* + *S. nigritus*). Lack of reciprocal monophyly across habitats would suggest multiple independent invasions and convergent adaptations to habitat types by independent *Sapajus* lineages.

Within the Amazon, we test for isolation by river barriers. If gene flow within Amazonian capuchin populations is restricted to the major interfluves, we expect a pattern of closely related clades within, not across, the four major Amazonian regions delineated by the Amazon River and its major tributaries. Evidence for the river barrier hypothesis was found for the sister group to capuchins, the Amazonian squirrel monkeys (Lynch Alfaro *et al.*, 2015a), as well as for marmosets and tamarins (Buckner *et al.*, 2015). Capuchin lineages with clades spanning multiple interfluves would provide evidence against rivers as significant isolating mechanisms.

Under the “Reinvasion of the Amazon” hypothesis we predict that crown *Sapajus* diversified in the Atlantic Forest, and crown *Cebus* within the Amazon basin (Lynch Alfaro *et al.*, 2012a; Nascimento *et al.*, 2015), with *Sapajus* later expanding its range into the Amazon thereby establishing sympatry with *Cebus*. Evidence for the alternate “Out of the Amazon” hypothesis would include the Amazon as the ancestral range for all capuchins; an initial diversification of both robust and gracile capuchins within the Amazon; and subsequent independent and recent invasions of non-Amazonian regions by both gracile and robust capuchins (Lynch Alfaro *et al.*, 2012a). Nascimento *et al.* (2015) suggest an ‘Out of the Atlantic Forest’ hypothesis, with the Atlantic Forest as the ancestral range for all capuchins.

BEAST Phylogeography for Sapajus In order to examine phylogeographic patterns for a widespread capuchin group recovered in the MrBayes tree, we performed both symmetric and asymmetric discrete states phylogeographic analyses in BEAST under a constant size coalescent prior. These analyses allow us to take into consideration the uncertainty in the relationships by simultaneously estimating the topology and the distribution history. We categorized the locality of each tip for the widespread *Sapajus* clade in the MrBayes tree as occurring in one biogeographic 'region' (Figure 2): We divide the Amazon basin into four regions using river boundaries of the Amazon River and two major tributaries, Negro and Madeira, as described by Wallace (1852) and later Cracraft (1985) as significant geographical limits to areas of endemism: Guianas (GU), Negro (NE), Inambari (IN), Rôndonia (RO). We also separate the drier open ecosystems Central Grasslands, Cerrado and Caratinga (CC), from the Atlantic Forest (AF). These Regions are modified from Jameson Kiesling *et al.* (2015), which was derived from centres of endemism proposed by Cracraft (1985). As mtDNA is inherited through the mother only, it tracks the movement of female lineages over time. Capuchin monkeys are female philopatric with male-biased dispersal (Lynch Alfaro *et al.*, 2014), so tracing female lineage is a conservative test for the amount of movement over time in this taxon.

RASP Biogeography for the Capuchin Radiation To test alternative hypotheses of capuchin origins and radiation we used a Bayesian framework implemented in the software RASP 3.0 (Yu *et al.*, 2015). RASP biogeography is a statistical program that estimates ancestral distributions at nodes in a phylogenetic tree. While it allows for the original Bayesian implementation of the BayArea model (Landis *et al.*, 2013), the program reconstructs ancestral states over a posterior distribution of trees for the DIVA (Ronquist *et al.*, 1997) and DEC (Ree *et al.*, 2005) models. Therefore, testing of biogeographical hypotheses takes into account uncertainty in the tree topology that may result from any number of factors (Yu *et al.*, 2015).

We coded presence/absence in seven geographical 'regions' for each representative tip in the RASP analysis based on the locality of the tips within each of the corresponding recovered clades in the MrBayes analysis (Figure 3a). Regions are as described above for the BEAST phylogeographical analysis, and in addition we include Central America/Andes region (CA), as per Jameson Kiesling *et al.* (2015). We used the pruned, time-calibrated tree from BEAST with regional coding to reconstruct biogeographical history in capuchins under the Bayesian DEC (S-DEC), Bayesian DIVA (S-DIVA) and BayArea models.

RESULTS

Capuchin Phylogenetics

We found strong support for reciprocally monophyletic clades of gracile and robust capuchins in the phylogenetic reconstruction from MrBayes (Figure 3a). Our truncated species time tree from BEAST (Figure 4) estimated the mean divergence time between *Sapajus* and *Cebus* at 5.8 Ma [95% HPD = 2.24 - 10.94] (late Miocene).

Within the robust capuchins, we recovered strong support for the clades *S. xanthosternos* (pp = 1) and *S. nigritus* (pp = 1)*,* but little evidence for reciprocally monophyletic 'species' clades for the rest of *Sapajus.* Two *S. robustus* samples formed a weakly supported clade (pp = 0.74). All other robust capuchins formed one large widely distributed clade ('*Sapajus* CLADE 4,' pp = 1), comprising *S. apella*, *S. macrocephalus*, *S. cay*, *S. libidinosus*, and *S. flavius*. Subclades within this group were not always congruent with morphological hypotheses about robust capuchin species.

Most strikingly we found *S. apella sensu stricto* to be extensively paraphyletic with respect to other *Sapajus* species. Within the widespread clade, we recovered several geographical subclades: (1) *S. flavius* (pp = 0.95); (2) *S. libidinosus* together with eastern *S. apella* (pp =0,80); (3) *S. cay* from Paraguay and Rondonia/Mato Grosso plus *S. apella* from Guaporé and Apuí (pp = 0.81); (4) *S. cay* from Chapada dos Guimarães in Mato Grosso and *S. apella* from Alta Floresta in Mato Grosso (pp = 1); (5) *S. apella/macrocephalus* (pp = 1) from north of the Amazon and Solimões rivers, as well as from regions just south of the Amazon River near the Xingu and Tapajos rivers, and just south of Manaus, together reaching all four Amazon quadrants, as far northwest as Colombia; (6) *S. apella/macrocephalus* (pp = 0.95) from the states of Amazonas, Mato Grosso and Rondonia, Brazil and from Peru. Minimum clade ranges for each of the six subclades of the widespread *Sapajus* clade are visualized in Figure 5. The clades are geographically coherent though several do not match current morphological hypotheses about capuchin taxonomy.

Within *Cebus*, we recovered eight strongly supported clades, although the phylogenetic relationships among these clades are not always well supported. *Cebus versicolor* forms a clade (pp = 1) with *C. cesarae* within the Magdalena river valley in the Colombian Andes, as part of a larger clade including *C. capucinus* in Panama and Colombia (pp = 1), and *C. imitator* in Central America (pp = 1). In the eastern Andes, *C. brunneus* (+ *C. a. trinitatis*) (pp = 1) was sister to *C. leucocephalus* (pp = 1). Within Amazonia, there is strong support for a *C. unicolor* +*C. albifrons* clade (pp = 1). *C. yuracus* forms a clade a (pp = 1) in western Brazil and Ecuador, and *C. o. olivaceus* + *C. o. castaneus* + *C. kaapori* form a strongly supported clade (pp = 1). Our *C. kaapori* sample is from the holotype, collected by Queiroz (1992), from the type locality Chega-Tudo, in Carutapera Municipality, Maranhão State, Brazil. Here we provide a correction to the original Queiroz (1992) for the coordinates of that locality, 02°20’S 46°05’W (Table 1).

BEAST Phylogeography: *Reconstruction of widespread Sapajus radiation*

Our phylogeographical reconstruction details the explosive Pleistocene range expansion throughout the Amazon, Cerrado, Caatinga, and southern grasslands for *Sapajus* (Figure 6). Robust capuchins enter RO from AF at around 300-500 ka, with a single expansion to GU at 200 kya (across the Amazon River), and at least two independent expansions from RO to IN (across the Madeira). There is also evidence for expansion from GU to IN, and IN to GU, as well as back-crossing from both IN and GU to RO. No strong geographical pattern demarcates rivers as firm barriers separating the four Amazon regions; instead, there has been significant and recurrent recent movement by *Sapajus* across the Amazon/Solimões, Madeira and Negro rivers. *Sapajus* also spreads from RO multiple times into the Cerrado/Caatinga (CC) region, all within the last 200,000 years.

The addition of a distribution partition to our BEAST analysis provides strong support for the monophyly of *Sapajus robustus* (0.99) and its position as the sister group to a widespread Amazonian-Grasslands-Atlantic Forest clade (pp = 1.0). *S. robustus* is restricted to the Atlantic Forest, making it clear that the explosive radiation of robust capuchin monkeys began with a recent migration into the Amazon basin and grasslands. *S. flavius* is embedded within the widespread clade, suggesting an expansion back into the Atlantic Forest from the Amazon/Grasslands regions.

RASP Biogeography: Reconstruction of Capuchin Biogeographic History

Figure 7 shows the most likely biogeographic regions occupied at each node from the ancestral reconstructions under (a) S-DEC , (b) S-DIVA and (c) BayArea. All models agree in their most likely reconstructions of the CA gracile capuchin clade comprising *C. brunneus*, *C. imitator*, *C. leucocephalus*, *C. cesarae* + *C. versicolor* and *C. capucinus*. Both S-DEC and S-DIVA tended to reconstruct the remaining ancestral nodes for gracile capuchins as having widespread distributions, typical of these models that invoke vicariance frequently to arrive at extant distributions. BayArea consistently reconstructs smaller ancestral ranges, relying mainly on range expansion and range extirpation, without considering vicariance.

S-DEC and S-DIVA differ majorly in their reconstruction of the biogeographic history of the *Sapajus* clade. *Sapajus* ancestors remain widespread until the tips in S-DEC, but are restricted to AF in the S-DIVA reconstruction. The S-DEC reconstruction requires that three independent clades were rapidly extirpated from five areas while maintaining sympatry in the Atlantic Forest with a widespread sister group without gene flow. As we consider the S-DEC scenario biologically unlikely (given the recovered relationships among the clades and their respective geographical distributions), and the support values for the node reconstructions from BayArea were consistently much lower than the other models, we focus our comments below on the S-DIVA reconstruction which is both the best supported on a per node basis and makes biological sense across the tree.

Under the S-DIVA model, the ancestor of all capuchins is widespread across all seven biogeographic regions. The *Cebus* and *Sapajus* clades are then separated by a vicariant event that leaves the *Sapajus* ancestor isolated in the Atlantic Forest and the

Cebus ancestor widespread in the remaining regions. From there, the *Sapajus* clade remains isolated in AF until *Sapajus* CLADE 4 rapidly invades all regions of the Amazon and CC in the last 500,000 years.

Cebus was established in all regions of the Amazon and the Andes by approximately 2.4 Ma, well before the appearance of any *Sapajus* clade in the Amazon basin. It is unclear whether this ancestor also occupied the central grasslands as its presence or absence in this region is equally likely. The distributions of the ancestor of the *C. olivaceus* + *C. kaapori* + *C. yuracus* clade and that of the ancestor to the remaining gracile capuchins are poorly supported ($pp < 0.25$), and therefore uncertain. However, the *C. brunneus* + *C. imitator* + *C. leucocephalus* + *C. cesarae* + *C. versicolor* + *C. capucinus* clade clearly shows a long history in CA region, and possibly as long in GU ($pp = \sim 0.50$).

DISCUSSION

Our expanded sample set with comprehensive representation of capuchin species provided further and complete support for the monophyly of robust and gracile capuchins with a split at 5.8 Ma. A late Miocene *Sapajus*-*Cebus* split has been corroborated with combined nuclear and mitochondrial data in Perelman *et al.* (2011) (6.0 Ma, 95% confidence interval 3.13-9.35 Ma) and Springer *et al.* (2012) (5.7 Ma average, 3.55-8.33 Ma composite 95% min-max). However, previous time trees did not include all capuchin species; here we add *Cebus kaapori, C. versicolor,* and *Sapajus flavius.*

Cebus kaapori has been considered either a subspecies of *C. olivaceus* (Harada & Ferrari, 1996) or its own species (Groves 2001, 2005; Silva-Júnior, 2001), with some morphological characteristics more similar to *C. albifrons sensu lato* (Masterson, 1995).

In our MrBayes analysis, *Cebus kaapori* was recovered within the strongly supported *C. olivaceus* clade. A close relationship between *C. kaapori* and *C. olivaceus* makes sense, as *C. o. castaneus* is from the Guianas and northeastern Amazon, geographically most proximate to *C. kaapori*, found south of the Amazon River in the extreme eastern portion of the Amazon basin.

Cebus versicolor was recovered as sister to *C. cesarae.* This is concordant with geographical distribution as both species are found in isolated pockets of the Magdalena Valley in Colombia. MtDNA COII analysis of *Cebus albifrons sensu lato* by Ruiz-García *et al*. (2010) found *C. cesarae* and *C. pleei* to form the sister clade to *C. versicolor*.

We predicted that *Sapajus flavius* would be recovered either as sister to *S. xanthosternos* because of geographical proximity or as sister to *S. libidinosus* based on a gradation of morphology between the two morphotypes (Silva, 2010); or possibly as sister to all other *Sapajus* species, based on its unusual morphology (Lynch Alfaro *et al.*, 2012a). In our MrBayes and asymmetric BEAST phylogeography analyses, *Sapajus flavius* was recovered as part of the widespread *Sapajus* CLADE 4 (Figure 3b and 6b). However, in the symmetric BEAST phylogeography analysis, *S. flavius* was recovered as the sister group to *Sapajus* CLADE 4 (Figure 6a), suggesting all four Atlantic Forest *Sapajus* taxa may have diverged from each other first prior to *Sapajus* expansion into other regions. Given the difference in topology and the range of support values among analyses, the phylogenetic placement of *S. flavius* remains uncertain, though close phylogenetic affinity to *Sapajus* CLADE 4 is clear.

Sapajus cay has been the subject of controversy within capuchin taxonomy, and two major authorities (Groves, 2001, 2005; Silva-Junior, 2001) disagree significantly about its distribution, in part as a result of differences in the localities of the samples available in each of their studies. *Sapajus cay* (*sensu* Rylands *et al*., 2013) is recovered here as paraphyletic, with evidence for at least two distinct *Sapajus* populations moving independently into more open habitat types. *S. cay* samples from Paraguay, Rondonia, and Mato Grosso cluster with *S. apella* from Rondonia; while *S. cay* from northern central Mato Grosso forms a clade with nearby *S. apella* from Alta Floresta. These two populations have apparently converged morphologically to a lighter pelage phenotype in the drier, open habitats. *Sapajus libidinosus* (*sensu* Rylands *et al*., 2013) marks a third expansion into CC from Amazonian *Sapajus*, with a resultant lighter pelage. All expansions into drier habitats appear to have occurred in the last 200-100 kyr, similar to findings for other Neotropical primates (Lynch Alfaro *et al*., 2015b).

Our biogeographical analyses consistently reconstructed a widespread capuchin ancestral distribution across forested areas of South America, from the Amazon basin to the Atlantic, supporting Amazon *versus* Atlantic Forest vicariant origin for *Cebus* and *Sapajus*. However, the certainty of statistical biogeographical reconstructions is reduced significantly as one approaches the basal node due to a decrease in available information, despite the highly supported root state in the S-DIVA reconstruction. Additionally, the behavior of the DIVA model tends toward widespread ancestral distributions at the root, in part due to the missing information from the remainder of the tree of life and some underlying assumptions that bias the tree against early dispersal (Ronquist, 1997; Nylander *et al.*, 2008). Thus, we believe the root state for the biogeographic distribution of the common ancestor of all capuchins remains unresolved. The distribution of *Sapajus* exclusively in the Atlantic Forest until recently, and *Cebus* in the Amazon and Andes regions from early on, does suggest a cladogenetic event (vicariance) that led to their initial diversification in isolation, though the possibility of a dispersal event prior to the vicariance, from the Atlantic Forest into the Amazon or vice versa, is not excluded. All biogeographical methods used in this study do include at least one Amazonian region in the root state, suggesting the Amazon basin likely formed in part, or wholly, the distribution of the ancestral capuchin.

Throughout capuchin evolution, the Brazilian Shield (within AF, CC, RO Regions) and Guiana Shield (within GU) have been constant highland regions undergoing few physical changes in configuration but perhaps experiencing changes in floral assemblages related to climate (Aleixo & Rossetti, 2007). The divergence between *Sapajus* and *Cebus*, dated consistently at approximately 6 Ma, happened during a time when the flora of the Cerrado was being assembled (4-8 Ma), as indicated by the onset of diversification of many Cerrado plant lineages (Simon *et al.*, 2009). The coincidence in timing of the divergence between robust and gracile capuchins and of the Cerrado assemblage is provocative, especially given the reconstructed distributions of ancestral *Sapajus* (Atlantic Forest) and *Cebus* (Andes and Amazon). The presence or absence of the *Cebus* ancestor in the central grasslands is equally likely in our reconstruction, yet its absence in the region makes sense if the Cerrado and related drier biomes are beginning to expand. At this time, capuchins are likely not adapted to such a biome which they have probably not inhabited previously, making the expansion of the central grasslands a candidate vicariant process for the separation of robust and gracile capuchins. The only extant capuchins distributed in the central grasslands belong to our widespread *Sapajus* CLADE 4 - which invaded those biomes only in the last 500 ky - perhaps aided by an adaptation to durophagy first in the Atlantic Forest which pre-adapted them to inhabit more resource poor areas like the Cerrado. This is not unlike the scenario recently suggested for the biogeography of marmosets: invasion of the central grasslands by Atlantic Forest *Callithrix* species appears to be associated with exploitation of tree exudates enabled by derived dental morphology (Buckner *et al.*, 2015). In both examples, ecomorphological preadaptation in the Atlantic Forest enabled entry into more resource poor biomes less than 1 Ma.

In our analysis, *Cebus* showed strong geographical divisions, suggesting a widespread ancestor diversified in the Amazon and was split by vicariance in the Andes and Central America. The Amazonian group (*C. yuracus, C. unicolor, C. albifrons, C. olivaceus, C. kaapori*) is about the same age as the CA group (*C. capucinus, C. imitator, C. versicolor, C. cesarae, C. leucocephalus, C. brunneus*). The Guiana highlands represent the only area adjacent to the Andes with passable corridors between mountains to the western coast of South America and Central America until the most recent phases of uplift (Hoorn *et al.*, 2010). The ancestor to CA distributed capuchins was probably briefly isolated in that region, and later dispersed into GU. Extirpation of the lineage leading to *C. brunneus* from the CA region would result in the eventual speciation of that taxon in the Guianas. The distributions of the remaining clades of gracile capuchins are weakly delineated by major Amazon River tributaries. *C. olivaceus castaneus* is clearly separated from *C. kaapori* by the Amazon River. *C. olivaceus* + *C. kaapori* are separated from *C. yuracus* by the Negro and Madeira rivers while *C. unicolor* + *C. albifrons* seems only limited by the Andes mountains to the west and the Negro river to the north.

In contrast, the robust capuchins show evidence of multiple moves within and between all regions, except the Central American/Andean region. This may be because the arrival at the foot of the eastern Andes was so recent for *Sapajus* that the mountain range was already fully formed and impassable (Lynch Alfaro *et al.*, 2012a). Species identified through morphology (*S. apella, S. macrocephalus, S. cay, S. libidinosus*) form a large clade that spans all Amazonian and CC regions; within this clade, individuals do not always cluster by morphological species designation; instead we found strong support for clades of some populations in particular geographical regions. While the recent divergence times among all the Amazonian and grasslands capuchins (all under 400 Ka) suggest there probably has not been time enough for speciation among these morphological types, our data do point to geographical clades that deserve further study for their morphological and behavioral population characteristics. For example, morphological differences between *S. macrocephalus* north of the Amazon and those in southern Amazonia have already been described (Rylands *et al.*, 2013); our analysis here provides new hypotheses for geographic boundaries between major population centers within Amazonian *Sapajus*, and suggests the need for new morphological and morphometric analyses for undersampled regions.

Combining information from our biogeographical and phylogeographical analyses, we find strong evidence that all of the Atlantic Forest robust capuchin species are monophyletic clades. Major Amazonian rivers thus don’t seem to be strong barriers for dispersal or gene flow for either *Cebus* or *Sapajus*, especially not in the western Amazon, although mountains seem to be important within the Andes for separating several *Cebus* taxa over the last 1-2 Ma. *Sapajus* shows the most movement across major rivers, with up to twelve examples of dispersal across significant Amazonian rivers demonstrated through our BEAST analysis by just the samples included in this study. Smith *et al.* (2014) point out that speciation rates would decrease in lineages with higher dispersal ability across rivers and mountains in the Neotropics. Note that Boubli et al. (2015) show the northern Negro and the Branco rivers are stopping points for the distribution of *S. apella* (absent directly between the Negro and Branco) and *C. olivaceus* (absent east of the Branco and south of the Negro). The lack of *Sapajus* between the Negro and Branco rivers may be due to the combination of these rivers serving as barriers and to competition with *Cebus olivaceus*, a gracile capuchin species which occupies this area and also has the most convergently robust morphology in comparison to robust capuchins—so may be a stronger competitor for similar resources (Boubli *et al*., 2015).

Rapid Pleistocene expansion throughout the Amazon Basin has been recovered for the sister taxon to capuchin monkeys, the squirrel monkeys (Chiou *et al.*, 2011; Lynch Alfaro *et al.*, 2015a). *Sapajus* presence today in western Amazonia is explained in the BEAST phylogeography analysis as resulting from two distinct pathways: one was expanding from RO west across the Madeira River, into IN, and then spreading north into NE. The second was a move first from RO across the Amazon River into GU, then expansion west across the Negro River into NE, and from there south to IN. The exact distribution of *S. macrocephalus* in western Amazon is contested (Groves, 2001, 2005; Silva-Junior, 2001). Rylands *et al.* (2013) depict *S. macrocephalus* morphological diversity with Brazilian, Peruvian, Bolivian and Colombian forms; one explanation for this variation congruent with our results is that western Amazon has been colonized multiple times from different *Sapajus* seed populations. In contrast, most of *Sapajus* diversity in eastern Amazonia can be explained by a single founder event from AF into RO, and another single founder event from RO into GU, with some later expansions back to RO from GU.

Overall, our data and analyses support the "Reinvasion of the Amazon" hypothesis (Lynch Alfaro *et al.,* 2012) as indicated by the ancestral reconstructions of the crown *Cebus* and crown *Sapajus* clades. The two capuchin genera began diversifying in isolation, with *Sapajus* species only recently invading a *Cebus*-occupied Amazon roughly 500 kya (Table 2). The recovered timing and region(s) of entry into sympatry for gracile and robust capuchins varied by model but most indicate an event in the Amazon less than 1 Ma, as a result of *Sapajus* counter-invasion into regions already occupied by *Cebus*. Southeastern Amazon (RO in Figure 2) is where *Cebus* and *Sapajus* are most frequently reconstructed as first entering into sympatry.

Our new biogeographical and phylogeographic analyses show that the robust capuchins radiate from the Atlantic Forest into the Amazon into sympatry with *Cebus* less than 1 Ma. The robust capuchins were able to colonize an array of divergent habitat types all within a very short time period. Consistent with this recent explosive radiation, we found little support for the internal topology of a morphologically diverse and geographically expansive clade of robust capuchins. Morphological species contained in this group include *S. libidinosus*, *S. cay*, *S. flavius*, *S. macrocephalus*, and *S. apella*. While there is significant individual variation within *Sapajus* populations, these five morphological species each display distinct morphological patterns with clear geographic correspondence (Silva-Júnior, 2001). We suggest nuclear genomic methods will be necessary to provide a complementary perspective to help resolve the relationships within this clade.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 GenBank accession numbers for mitochondrial sequences.

Appendix S2 COI primers and PCR protocol.

BIOSKETCH:

Marcela G. M. Lima is a biologist interested in biogeography, phylogenetics and population genetics, with a focus on Neotropical vertebrates. Author Contributions: M.G.M.L., J.C.B., M.E.A. and J.W.L.A. designed and ran phylogenetic and biogeographic analyses and developed the article; M.G.M.L., J.S.S.J., A.M., A.L., I.P.F., J.P.B., F.R., H.Q., M.N.S., A.D.F. and J.W.L.A. collected and curated sample material; M.G.M.L., A.M. and I.P.F. generated new genetic sequences; K.C., M.G.M.L., J.C.B. and J.L.A. designed maps and figures; and all authors contributed to the writing of the article.

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Tables

Table 1 Sample list with locality data, centres of endemism, and species names according to Rylands *et al.* (2013). Table indicates which samples were used in the BEAST phylogeography analysis, BEAST Timetree and RASP analyses. New sequences will be made publicly available on GenBank upon publication.

Sample N°	Species (Rylands e*t al.,* 2013)	Latitude	Longitude	Regions	BEAST/RASP Analysis	Original Sample N° (Lynch Alfaro *et al.,* 2012)
1	*S. flavius*	-6.56	-35.13	AF		
2	*S. flavius*	-7.01	-34.96	AF		
3	*S. flavius*	-7.02	-35.09	AF		
A	*S. flavius*			AF		
B	*S. xanthosternos*			AF		
4A	*S. xanthosternos*	-14.79	-39.05	AF	X	
4B	*S. xanthosternos*	-14.79	-39.05	AF		
5	*S. xanthosternos*	-15.17	-39.07	AF		
6	*S. xanthosternos*	-15.41	-39.50	AF		
7	*S. robustus*	-21.23	-41.20	AF		
8	*S. robustus*	-18.58	-39.75	AF		50
9	*S. robustus*	-17.85	-41.50	AF	X	51
10	*S. robustus*	-19.95	-43.85	AF		
11	*S. nigritus*	-23.86	-46.14	AF		
12	*S. nigritus*	-21.85	-47.43	AF		
13	*S. nigritus x libidinosus*	-20.27	-50.23	AF		56
14	*S. nigritus*	-22.86	-53.33	AF		
15	*S. nigritus*	-22.85	-53.31	AF	X	
16	*S. nigritus*	-23.94	-54.22	AF		
17	*S. nigritus*	-25.70	-54.44	AF		57
18	*S. libidinosus*	-2.77	-41.81	CC		
19	*S. libidinosus*	-2.8	-41.87	CC		
20	*S. libidinosus*	-2.85	-41.83	CC		
21	*S. libidinosus*	-5.09	-42.43	CC		
22	*S. libidinosus*	-7.93	-44.20	CC		
23	*S. libidinosus*	-5.28	-48.30	RO		
24A	*S. libidinosus*	-14.14	-48.17	CC		
24B	*S. libidinosus*	-14.14	-48.17	CC		
25	*S. libidinosus*	-15.68	-48.20	CC		46
26	*S. libidinosus*	-17.22	-46.87	CC		47

27	*S. libidinosus*	-16.6	-49.26	CC		
28	*S. libidinosus*	-16.11	-50.30	CC		
29A	*S. cay*	-14.88	-55.80	CC		
29B	*S. cay*	-14.88	-55.80	CC		
29C	*S. cay*	-14.88	-55.80	CC		
29D	*S. cay*	-14.88	-55.80	CC		
29E	*S. cay*	-14.88	-55.80	CC		43
30	*S. cay*	-16.06	-57.72	CC		
31	*S. cay*	-13.52	-60.43	CC		
32	*S. cay*	-26.88	-56.88	CC		44
33A	*S. apella*	-6.15	-49.56	RO	X	
33B	*S. apella*	-6.15	-49.56	RO		
34A	*S. apella*	-3.83	-49.64	RO		
34B	*S. apella*	-3.83	-49.64	RO		
34C	*S. apella*	-3.83	-49.64	RO		
34D	*S. apella*	-3.83	-49.64	RO		
34E	*S. apella*	-3.83	-49.64	RO		
35	*S. apella*	-2.61	-51.54	RO		
36	*S. apella*	-3.36	-51.74	RO		
37	*S. apella*	-0.58	-52.33	GU		
38	*S. apella*	-0.94	-53.24	GU		
39	*S. apella*	-0.31	-52.45	GU		
40	*S. apella*	0.47	-52.99	GU		
41	*S. apella*	0.32	-53.23	GU		
42	*S. apella*	3.22	-52.03	GU		
43	*S. apella*	0.83	-53.93	GU		
44	*S. apella*	0.63	-55.73	GU		
45A	*S. apella*	-0.17	-55.19	GU		
45B	*S. apella*	-0.17	-55.19	GU		
46	*S. apella*	-0.96	-55.52	GU		
47	*S. apella*	-1.49	-56.80	GU		
48	*S. apella*	1.29	-58.70	GU		
49	*S. apella*	-1.99	-59.44	GU		
50	*S. apella*	-1.92	-59.47	GU		
51	*S. apella*	4.26	-58.50	GU		
52	*S. apella*	-2.06	-58.38	GU		
53	*S. apella*	-2.47	-58.40	GU		
54	*S. apella*	-2.6	-56.18	RO		
55	*S. apella*	-3.18	-55.80	RO		
56	*S. apella*	-3.88	-56.78	RO		
57	*S. apella*	-4.71	-56.44	RO		
58	*S. apella*	-7.61	-60.79	RO		

59	*S. apella*	-9.2	-59.06	RO		
60A	*S. apella*	-9.6	-56.01	RO		
60B	*S. apella*	-9.6	-56.01	RO		
60C	*S. apella*	-10	-56.04	RO		
60D	*S. apella*	-10	-56.04	RO		
61A	*S. apella*	-11.95	-60.69	RO		
61B	*S. apella*	-11.95	-60.69	RO		
62	*S. apella*	-12.56	-63.44	RO		
63	*S. apella*	-12.5	-63.53	RO		
64	*S. macrocephalus*	-3.37	-60.48	IN		
65	*S. macrocephalus*	-4.44	-60.32	IN		
66	*S. macrocephalus*	-4.75	-61.28	IN		
67	*S. macrocephalus*	-4.86	-61.41	IN		
68	*S. macrocephalus*	-4.99	-62.96	IN		
69	*S. macrocephalus*	-4.99	-62.96	IN		
70	*S. macrocephalus*	-5.69	-63.24	IN		
71	*S. macrocephalus*	-8.19	-64.02	IN		
72	*S. macrocephalus*	-8.8	-63.95	RO		
73A	*S. macrocephalus*	-8.89	-63.24	RO		
73B	*S. macrocephalus*	-8.89	-63.24	RO		
73C	*S. macrocephalus*	-8.89	-63.24	RO		
74	*S. macrocephalus*	-8.67	-62.37	RO		
75	*S. macrocephalus*	-12.45	-62.92	RO		
76	*S. macrocephalus*	-3.89	-64.25	NE		
77	*S. macrocephalus*	-2.47	-64.83	NE		
78	*S. macrocephalus*	-2.59	-64.89	NE		
79A	*S. macrocephalus*	-2.45	-65.36	NE		
79B	*S. macrocephalus*	-2.45	-65.36	NE		
80	*S. macrocephalus*	-1.05	-62.89	NE		
81	*S. macrocephalus*	-0.48	-64.41	NE		
82	*S. macrocephalus*	-0.61	-64.92	NE		
83	*S. macrocephalus*	-0.23	-66.85	NE		
84	*S. macrocephalus*	-1.84	-69.03	NE		
85	*S. macrocephalus*	3.72	-73.48	GU		35
86	*S. macrocephalus*	4.15	-73.63	GU		34
87	*S. macrocephalus*	-4.4	-70.14	IN		
88	*S. macrocephalus*	-5.21	-69.32	IN		
89	*S. macrocephalus*	-4.94	-68.17	IN		
90	*S. macrocephalus*	-7.53	-74.97	IN		37
91	*S. macrocephalus*	-10.00	-71.02	IN		38
92	*C. kaapori*	-2.33	-46.08	RO		
93	*C. o. castaneus*	-0.58	-52.33	GU		

94	*C. o. castaneus*	1.84	-52.74	GU	X	
95	*C. o. castaneus*	3.22	-52.03	GU		
96	*C. o. castaneus*	2.83	-58.95	GU		18
97	*C. o. olivaceus*	9.82	-63.62	GU		13
98	*C. o. olivaceus*	10.66	-62.50	GU		11
99	*C. o. olivaceus*	9.74	-61.42	GU		12
100A	*C. o. olivaceus*	6.28	-61.32	GU		
100B	*C. o. olivaceus*	6.28	-61.32	GU		
100C	*C. o. olivaceus*	6.28	-61.32	GU		15
101	*C. o. olivaceus*	5.03	-60.95	GU		16
102	*C. o. olivaceus*	4.42	-61.58	GU		17
103A	*C. o. olivaceus*	3.62	-65.68	GU		14
103B	*C. o. olivaceus*	3.62	-65.68	GU		
104	*C. a. trinitatis*	10.39	-61.30	GU		21
105	*C. brunneus*	10.90	-68.77	GU	X	10
106A	*C. leucocephalus*	9.20	-72.64	CA	X	24
106B	*C. leucocephalus*	9.20	-72.64	CA		
107	*C. leucocephalus*	7.32	-71.96	GU		25
108	*C. cesarae*	9.14	-73.57	CA		23
109	*C. versicolor*	6.72	-74.15	CA	X	
C	*C. capucinus*			CA	X	
110	*C. capucinus*	9.48	-79.56	CA		7
111	*C. capucinus*	9.07	-79.55	CA		8
112	*C. imitator*	8.38	-83.28	CA		
113	*C. imitator*	9.45	-84.15	CA		5
114	*C. imitator*	10.08	-84.47	CA	X	2
115	*C. imitator*	9.95	-84.55	CA		3
116	*C. imitator*	9.78	-84.93	CA		4
117	*C. imitator*	10.35	-85.35	CA		1
118	*C. albifrons*	2.25	-65.28	GU		27
119A	*C. albifrons*	-0.96	-62.92	NE		
119B	*C. albifrons*	-0.96	-62.92	NE		29
120A	*C. albifrons*	-1.05	-62.89	NE		
120B	*C. albifrons*	-1.05	-62.89	NE		
121	*C. albifrons*	-2.47	-64.83	NE		
122	*C. albifrons*	-2.59	-64.89	NE		
123	*C. unicolor*	-3.14	-55.51	RO	X	
124	*C. unicolor*	-4.66	-56.55	RO		
125	*C. unicolor*	-4.40	-70.14	IN		
126	*C. unicolor*	-4.94	-68.17	IN		
127	*C. unicolor*	-9.22	-66.74	IN		
128	*C. unicolor*	-10.00	-71.02	IN		33

129	*C. yuracus*	-0.70	-76.35	NE	X	30
130	*C. yuracus*	-4.45	-78.27	NE		31
131	*C. yuracus*	-8.67	-72.78	IN		32
132	*Cebus* sp.	10.96	-74.79	CA		22
133	*Cebus* sp.	3.17	-65.82	GU		26
134	*Saimiri boliviensis*	-7.44	-67.44	IN		
135	*Saimiri o. citrinellus*	9.53	-84.50	CA		
136	*Saimiri collinsi*	-6.09	-49.54	RO		

Table 2 Timing and geographic region of entry into sympatry between *Cebus* and *Sapajus*, based on models used in this study.

Model	Area of First Sympatry	Timing (years ago)
RASP S-DIVA	All Amazonian Regions	~500 Ka
RASP S-DEC	All Amazonian Regions	~2.9 Ma
RASP Bayarea	RO	~500 Ka
BEAST Discrete Symmetric	RO	~500 Ka
BEAST Discrete Asymmetric	RO	~500 Ka

Figure Legends

Figure 1 Maps of capuchin monkey sample provenance: (a) *Cebus,* (b) *Sapajus*.

Figure 2 Map delineating Centres of Endemism used for the BEAST phylogeography and RASP analyses: CA: Central America and Andes; GU: Guianas; NE: Negro; RO: Rondonia; IN: Inambari; CC: Caatinga, Cerrado and Central Grasslands; AF: Atlantic Forest.

Figure 3 MrBayes tree for capuchin phylogeny: (a) *Cebus* and *Sapajus* phylogeny and (b) details of *Sapajus* CLADE 4. Branches are colour-coded to regions from polygons map. Posterior probabilities are shown as asterisks for above 0.95 and as circles for between 0.70 and 0.95. Sample species ID and number correspond to Table 1.

Figure 4 BEAST time tree for capuchin monkeys. Posterior probabilities are shown as asterisks for above 0.95 and as circles for between 0.70 and 0.95. Tips used to represent species are listed in Table 1.

Figure 5 Map with minimum convex polygons to show geographic distribution of major subclades within the widespread *Sapajus* clade. Subclades are presented as minimum convex polygons that connect sample localities.

Figure 6 BEAST Phylogeography time tree for widespread clade of robust capuchin monkeys with ancestral state reconstruction for regions of ancestral nodes: (a) Symmetric Discrete States and (b) Asymmetric Discrete States. Branches are colour-coded to regions from inset map. Posterior probabilities are shown as asterisks for above 0.95 and as circles for between 0.70 and 0.95.

Figure 7 RASP visualization of ancestral range reconstruction using the (a) S-DIVA, (b) S-DEC and (c) BayArea models. Colours for different range combinations are depicted in the legend.

Figure 1

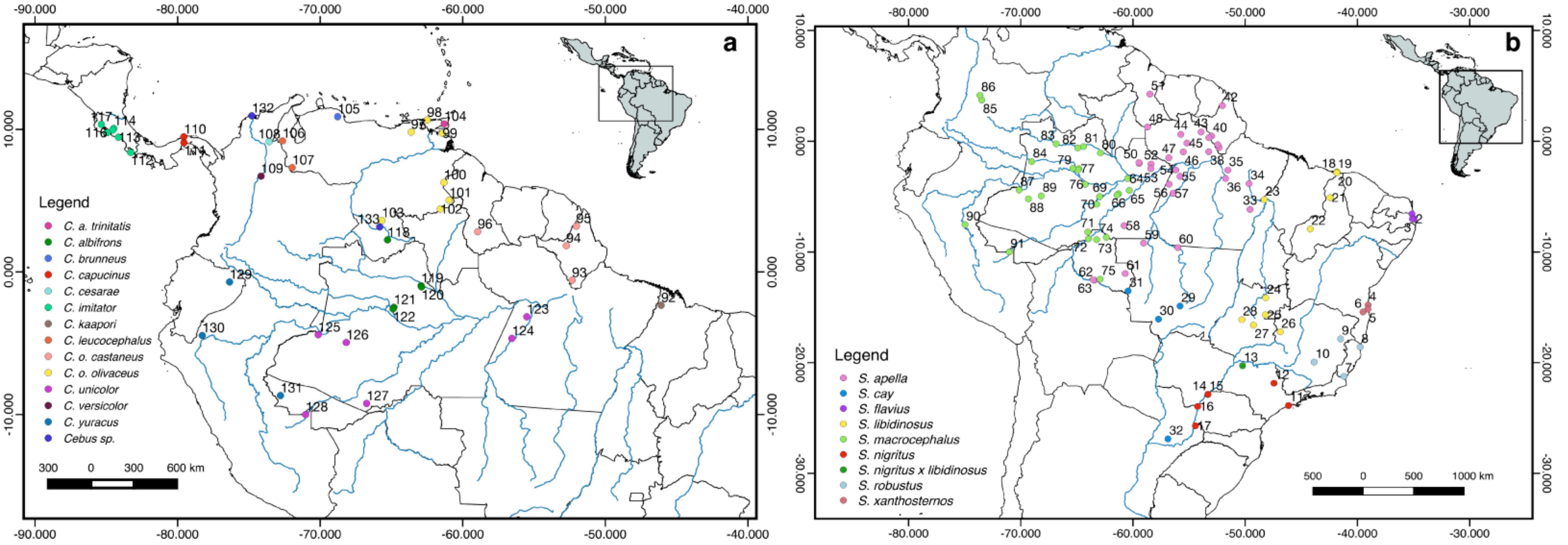

a
-90.000
-80.000
-70.000
-60.000
-50.000
-40.000
10.000
0.000
-10.000
Legend
● C. a. trinitatis
● C. albifrons
● C. brunneus
● C. capucinus
● C. cesarae
● C. imitator
● C. kaapori
● C. leucocephalus
● C. o. castaneus
● C. o. olivaceus
● C. unicolor
● C. versicolor
● C. yuracus
● Cebus sp.
300 0 300 600 km
b
-80.000
-70.000
-60.000
-50.000
-40.000
-30.000
10.000
0.000
-10.000
-20.000
-30.000
Legend
● S. apella
● S. cay
● S. flavius
● S. libidinosus
● S. macrocephalus
● S. nigritus
● S. nigritus x libidinosus
● S. robustus
● S. xanthosternos
500 0 500 1000 km

Figure 2

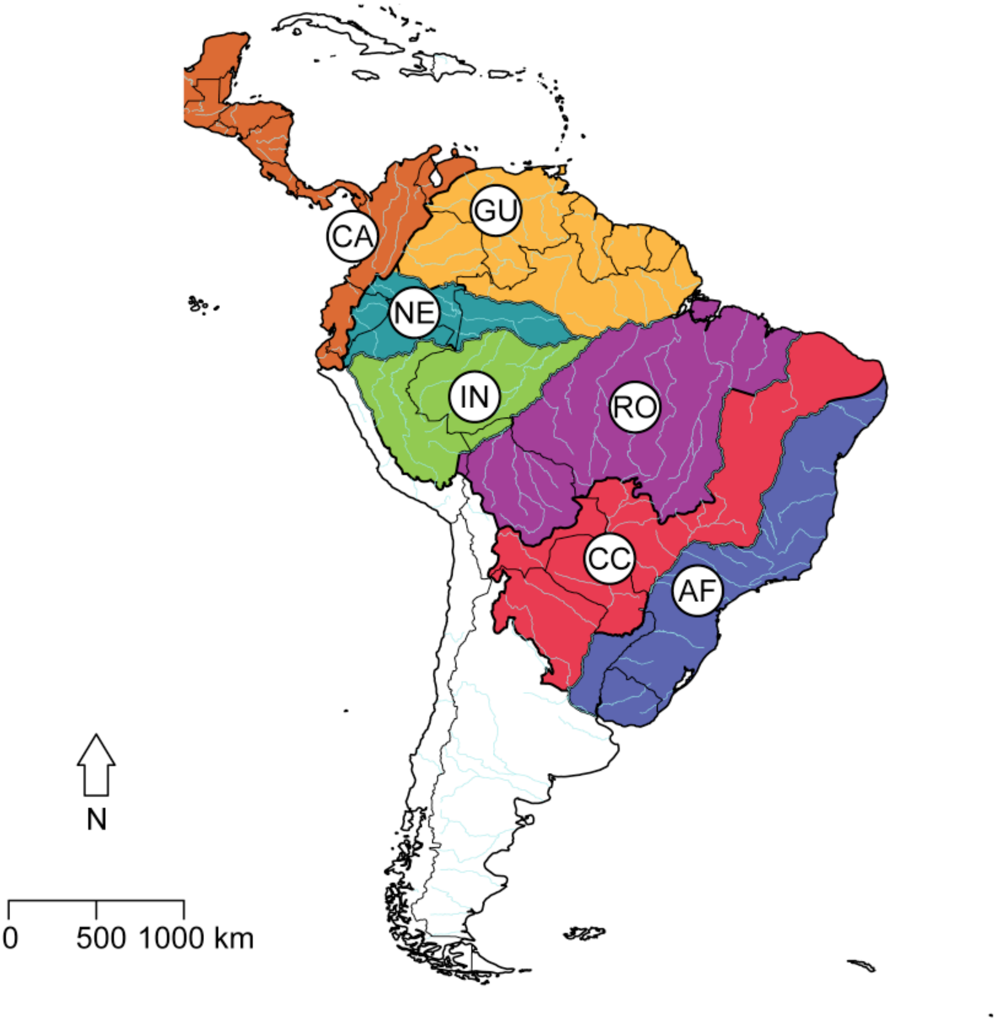


Figure 3

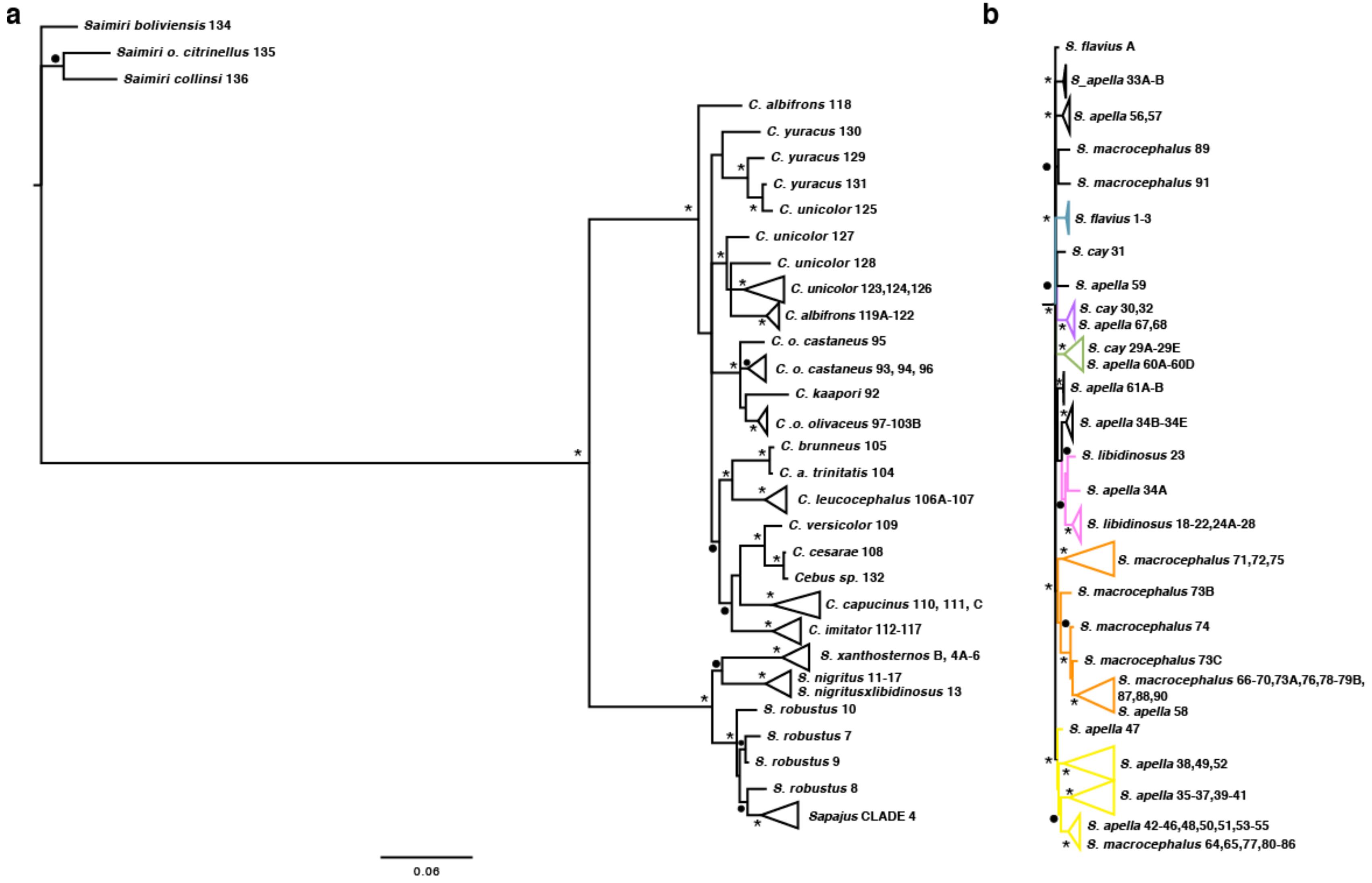

a
Saimiri boliviensis 134
Saimiri o. citrinellus 135
Saimiri collinsi 136
C. albifrons 118
C. yuracus 130
C. yuracus 129
C. yuracus 131
C. unicolor 125
C. unicolor 127
C. unicolor 128
C. unicolor 123,124,126
C. albifrons 119A-122
C. o. castaneus 95
C. o. castaneus 93, 94, 96
C. kaapori 92
C .o. olivaceus 97-103B
C. brunneus 105
C. a. trinitatis 104
C. leucocephalus 106A-107
C. versicolor 109
C. cesarae 108
Cebus sp. 132
C. capucinus 110, 111, C
C. imitator 112-117
S. xanthosternos B, 4A-6
S. nigritus 11-17
S. nigritusxlibidinosus 13
S. robustus 10
S. robustus 7
S. robustus 9
S. robustus 8
Sapajus CLADE 4
0.06
b
S. flavius A
S_apella 33A-B
S. apella 56,57
S. macrocephalus 89
S. macrocephalus 91
S. flavius 1-3
S. cay 31
S. apella 59
S. cay 30,32
S. apella 67,68
S. cay 29A-29E
S. apella 60A-60D
S. apella 61A-B
S. apella 34B-34E
S. libidinosus 23
S. apella 34A
S. libidinosus 18-22,24A-28
S. macrocephalus 71,72,75
S. macrocephalus 73B
S. macrocephalus 74
S. macrocephalus 73C
S. macrocephalus 66-70,73A,76,78-79B, 87,88,90
S. apella 58
S. apella 47
S. apella 38,49,52
S. apella 35-37,39-41
S. apella 42-46,48,50,51,53-55
S. macrocephalus 64,65,77,80-86

Figure 4

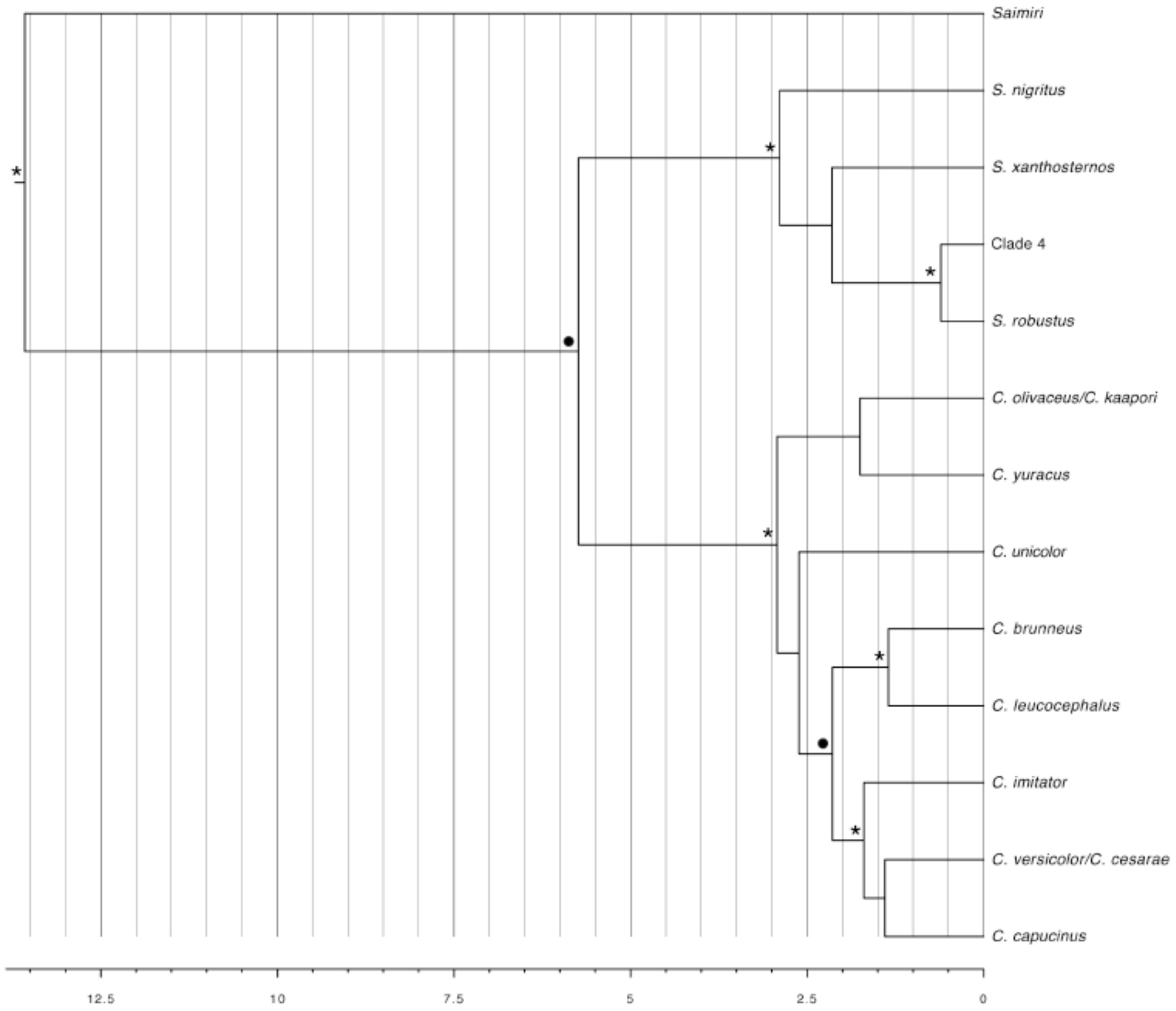

Saimiri
S. nigritus
S. xanthosternos
Clade 4
S. robustus
C. olivaceus/C. kaapori
C. yuracus
C. unicolor
C. brunneus
C. leucocephalus
C. imitator
C. versicolor/C. cesarae
C. capucinus
12.5
10
7.5
5
2.5
0

Figure 5

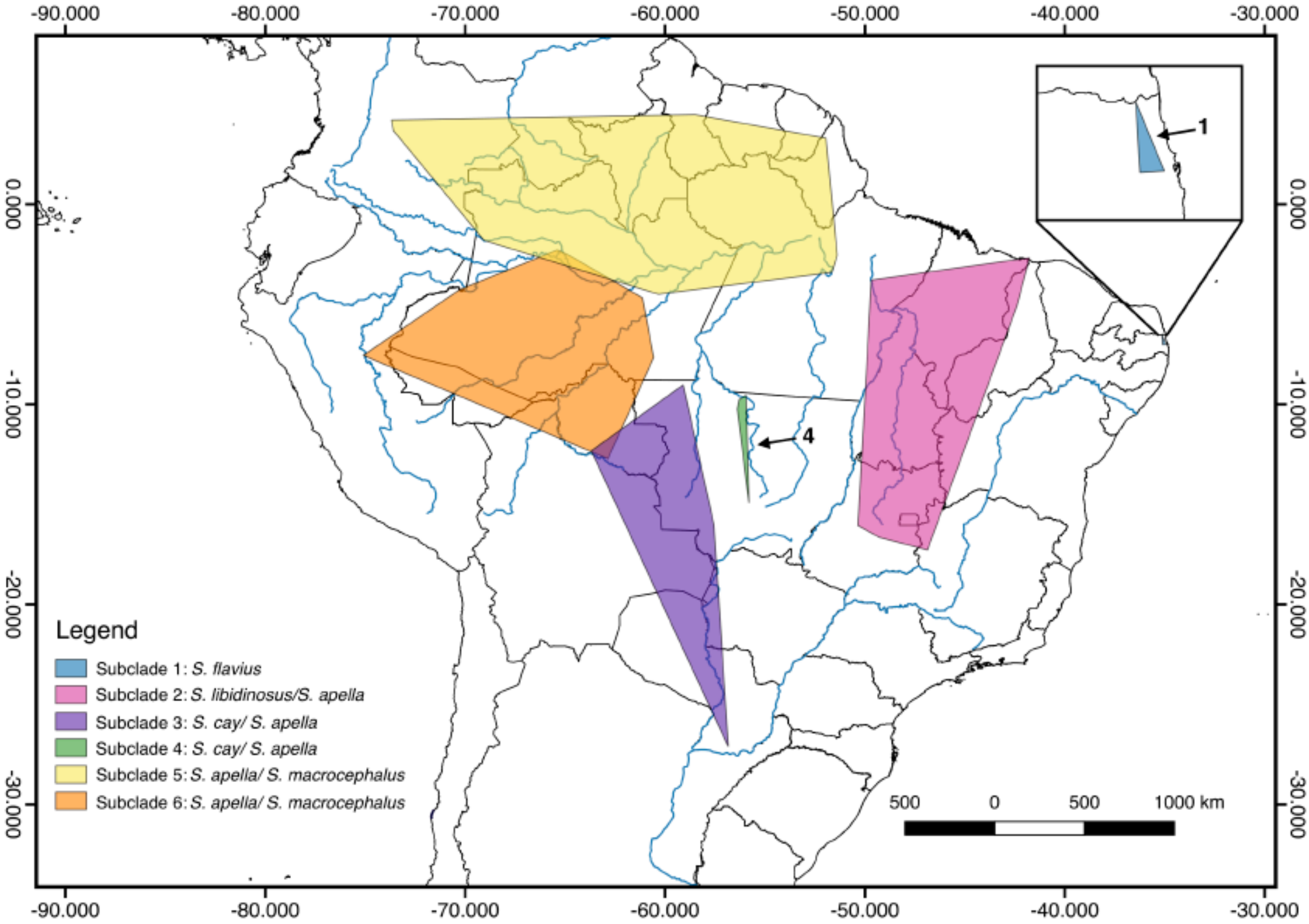

-90.000
-80.000
-70.000
-60.000
-50.000
-40.000
-30.000
0.000
-10.000
-20.000
-30.000
1
4
Legend
Subclade 1: *S. flavius*
Subclade 2: *S. libidinosus/S. apella*
Subclade 3: *S. cay/ S. apella*
Subclade 4: *S. cay/ S. apella*
Subclade 5: *S. apella/ S. macrocephalus*
Subclade 6: *S. apella/ S. macrocephalus*
500
0
500
1000 km

Figure 6

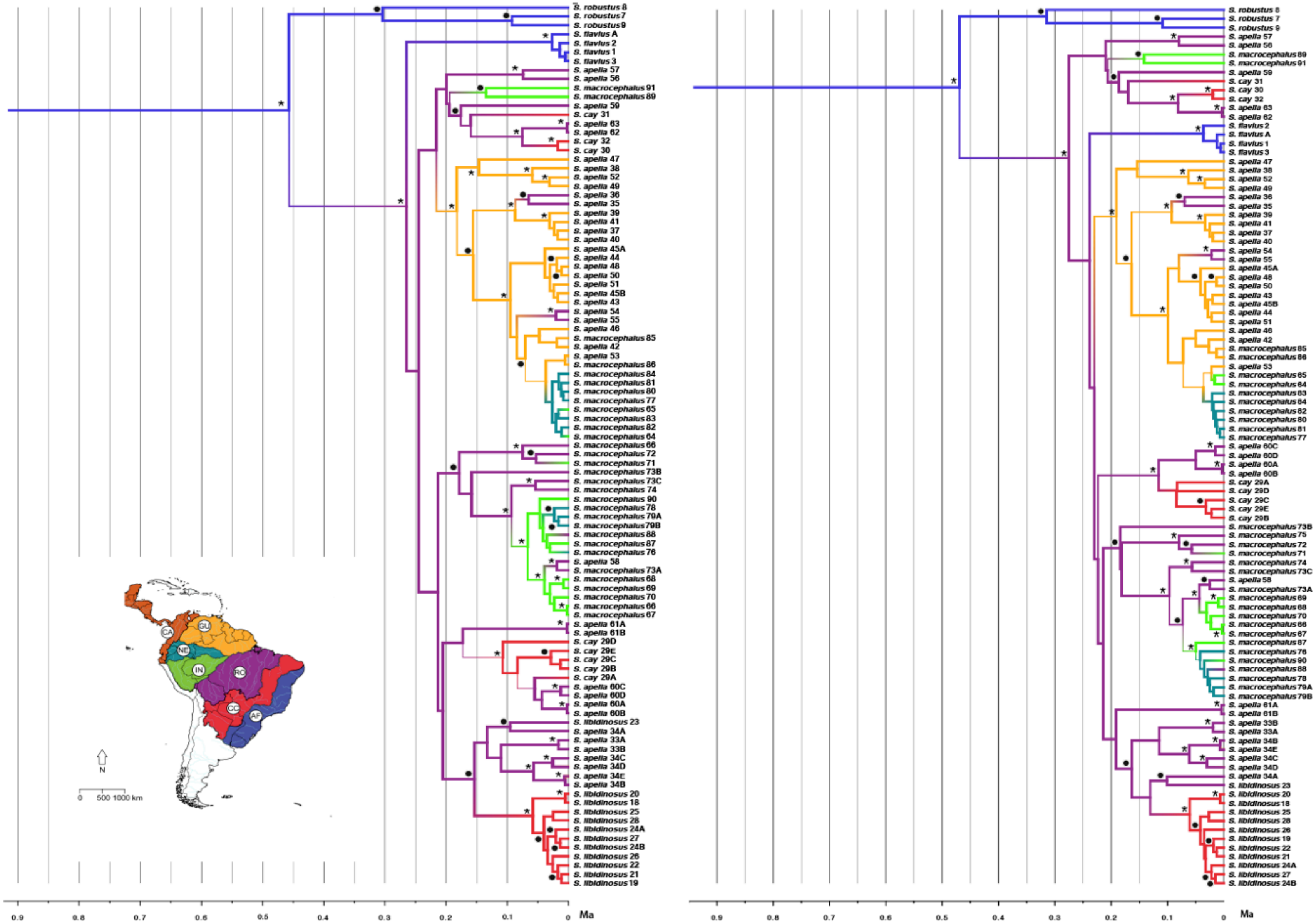
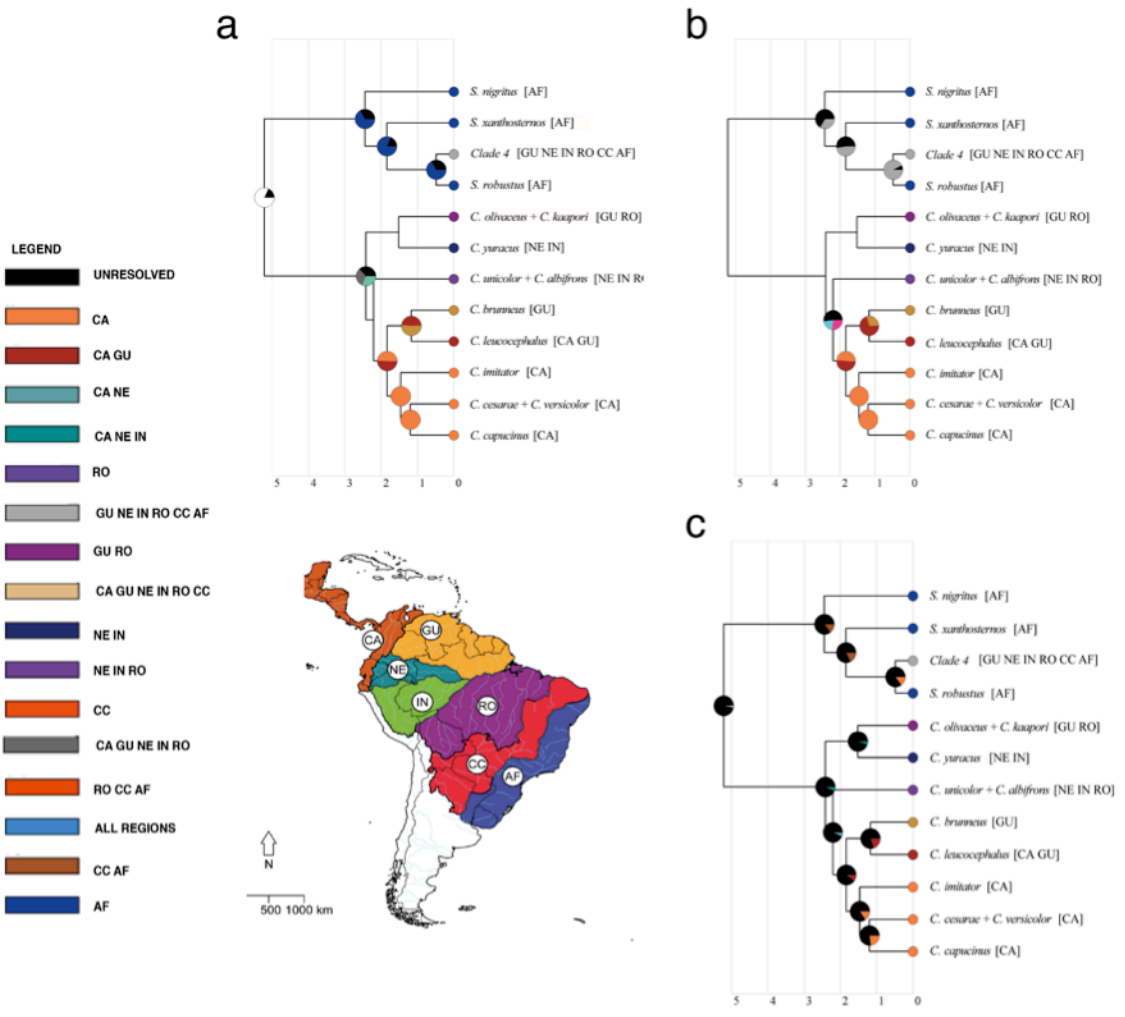

S. robustus 8
S. robustus 7
S. robustus 9
S. flavius A
S. flavius 2
S. flavius 1
S. flavius 3
S. apella 57
S. apella 56
S. macrocephalus 91
S. macrocephalus 89
S. apella 59
S. cay 31
S. apella 63
S. apella 62
S. cay 32
S. cay 30
S. apella 47
S. apella 38
S. apella 52
S. apella 49
S. apella 36
S. apella 35
S. apella 39
S. apella 41
S. apella 37
S. apella 40
S. apella 45A
S. apella 44
S. apella 48
S. apella 50
S. apella 51
S. apella 45B
S. apella 43
S. apella 54
S. apella 55
S. apella 46
S. macrocephalus 85
S. apella 42
S. apella 53
S. macrocephalus 86
S. macrocephalus 84
S. macrocephalus 81
S. macrocephalus 80
S. macrocephalus 77
S. macrocephalus 65
S. macrocephalus 83
S. macrocephalus 82
S. macrocephalus 64
S. macrocephalus 66
S. macrocephalus 72
S. macrocephalus 71
S. macrocephalus 73B
S. macrocephalus 73C
S. macrocephalus 74
S. macrocephalus 90
S. macrocephalus 78
S. macrocephalus 79A
S. macrocephalus 79B
S. macrocephalus 88
S. macrocephalus 87
S. macrocephalus 76
S. apella 58
S. macrocephalus 73A
S. macrocephalus 68
S. macrocephalus 69
S. macrocephalus 70
S. macrocephalus 66
S. macrocephalus 67
S. apella 61A
S. apella 61B
S. cay 29D
S. cay 29E
S. cay 29C
S. cay 29B
S. cay 29A
S. apella 60C
S. apella 60D
S. apella 60A
S. apella 60B
S. libidinosus 23
S. apella 34A
S. apella 33A
S. apella 33B
S. apella 34C
S. apella 34D
S. apella 34E
S. apella 34B
S. libidinosus 20
S. libidinosus 18
S. libidinosus 25
S. libidinosus 28
S. libidinosus 24A
S. libidinosus 27
S. libidinosus 24B
S. libidinosus 26
S. libidinosus 22
S. libidinosus 21
S. libidinosus 19
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Ma
CA
GU
NE
IN
RO
CC
AF
N
0 500 1000 km
S. robustus 8
S. robustus 7
S. robustus 9
S. apella 57
S. apella 56
S. macrocephalus 89
S. macrocephalus 91
S. apella 59
S. cay 31
S. cay 30
S. cay 32
S. apella 63
S. apella 62
S. flavius 2
S. flavius A
S. flavius 1
S. flavius 3
S. apella 47
S. apella 38
S. apella 52
S. apella 49
S. apella 36
S. apella 35
S. apella 39
S. apella 41
S. apella 37
S. apella 40
S. apella 54
S. apella 55
S. apella 45A
S. apella 48
S. apella 50
S. apella 43
S. apella 45B
S. apella 44
S. apella 51
S. apella 46
S. apella 42
S. macrocephalus 85
S. macrocephalus 86
S. apella 53
S. macrocephalus 65
S. macrocephalus 64
S. macrocephalus 83
S. macrocephalus 84
S. macrocephalus 82
S. macrocephalus 80
S. macrocephalus 81
S. macrocephalus 77
S. apella 60C
S. apella 60D
S. apella 60A
S. apella 60B
S. cay 29A
S. cay 29D
S. cay 29C
S. cay 29E
S. cay 29B
S. macrocephalus 73B
S. macrocephalus 75
S. macrocephalus 72
S. macrocephalus 71
S. macrocephalus 74
S. macrocephalus 73C
S. apella 58
S. macrocephalus 73A
S. macrocephalus 69
S. macrocephalus 68
S. macrocephalus 70
S. macrocephalus 66
S. macrocephalus 67
S. macrocephalus 87
S. macrocephalus 76
S. macrocephalus 90
S. macrocephalus 88
S. macrocephalus 78
S. macrocephalus 79A
S. macrocephalus 79B
S. apella 61A
S. apella 61B
S. apella 33B
S. apella 33A
S. apella 34B
S. apella 34E
S. apella 34C
S. apella 34D
S. apella 34A
S. libidinosus 23
S. libidinosus 20
S. libidinosus 18
S. libidinosus 25
S. libidinosus 28
S. libidinosus 26
S. libidinosus 19
S. libidinosus 22
S. libidinosus 21
S. libidinosus 24A
S. libidinosus 27
S. libidinosus 24B
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Ma

Figure 7


a
S. nigritus [AF]
S. xanthosternos [AF]
Clade 4 [GU NE IN RO CC AF]
S. robustus [AF]
C. olivaceus + C. kaapori [GU RO]
C. yuracus [NE IN]
C. unicolor + C. albifrons [NE IN R
C. brunneus [GU]
C. leucocephalus [CA GU]
C. imitator [CA]
C. cesarae + C. versicolor [CA]
C. capucinus [CA]
5 4 3 2 1 0
b
S. nigritus [AF]
S. xanthosternos [AF]
Clade 4 [GU NE IN RO CC AF]
S. robustus [AF]
C. olivaceus + C. kaapori [GU RO]
C. yuracus [NE IN]
C. unicolor + C. albifrons [NE IN RO]
C. brunneus [GU]
C. leucocephalus [CA GU]
C. imitator [CA]
C. cesarae + C. versicolor [CA]
C. capucinus [CA]
5 4 3 2 1 0
c
S. nigritus [AF]
S. xanthosternos [AF]
Clade 4 [GU NE IN RO CC AF]
S. robustus [AF]
C. olivaceus + C. kaapori [GU RO]
C. yuracus [NE IN]
C. unicolor + C. albifrons [NE IN RO]
C. brunneus [GU]
C. leucocephalus [CA GU]
C. imitator [CA]
C. cesarae + C. versicolor [CA]
C. capucinus [CA]
5 4 3 2 1 0
LEGEND
UNRESOLVED
CA
CA GU
CA NE
CA NE IN
RO
GU NE IN RO CC AF
GU RO
CA GU NE IN RO CC
NE IN
NE IN RO
CC
CA GU NE IN RO
RO CC AF
ALL REGIONS
CC AF
AF
CA
GU
NE
IN
RO
CC
AF
N
500 1000 km

Journal of Biogeography

Capuchin monkey biogeography: understanding sympatry between *Cebus* and *Sapajus*

Marcela G. M. Lima, Janet C. Buckner, José de Sousa e Silva-Júnior, Alexandre Aleixo, Amely Martins, Jean P. Boubli, Andrés Link, Izeni P. Farias, Maria Nazareth da Silva, Fabio Röhe, Helder Queiroz, Kenneth L. Chiou, Anthony Di Fiore, Michael E. Alfaro, Jessica W. Lynch Alfaro

Appendix S1 GenBank accession numbers for mitochondrial sequences

Sample N°	**Species (Rylands e*t al.*, 2013)**	**GenBank Accession N° Cyt *b***	**GenBank Accession N° Dloop**	**GenBank Accession N° COI**
1	*S. flavius*	XXXXX	XXXXX	XXXXX
2	*S. flavius*	XXXXX	XXXXX	XXXXX
3	*S. flavius*	XXXXX	XXXXX	XXXXX
A	*S. flavius*	JN835286	XXXXX	XXXXX
B	*S. xanthosternos*	KC757410	XXXXX	XXXXX
4A	*S. xanthosternos*	XXXXX	XXXXX	XXXXX
4B	*S. xanthosternos*	XXXXX	XXXXX	XXXXX
5	*S. xanthosternos*	XXXXX	XXXXX	XXXXX
6	*S. xanthosternos*	XXXXX	XXXXX	XXXXX
7	*S. robustus*	XXXXX	XXXXX	XXXXX
8	*S. robustus*	JN409301	XXXXX	XXXXX
9	*S. robustus*	JN409331	XXXXX	XXXXX
10	*S. robustus*	XXXXX	XXXXX	XXXXX
11	*S. nigritus*	XXXXX	XXXXX	XXXXX
12	*S. nigritus*	XXXXX	XXXXX	XXXXX
13	*S. nigritus x libidinosus*	JN409304	XXXXX	XXXXX
14	*S. nigritus*	XXXXX	XXXXX	XXXXX
15	*S. nigritus*	XXXXX	XXXXX	XXXXX
16	*S. nigritus*	XXXXX	XXXXX	XXXXX
17	*S. nigritus*	JN409334	XXXXX	XXXXX
18	*S. libidinosus*	XXXXX	XXXXX	XXXXX
19	*S. libidinosus*	XXXXX	XXXXX	XXXXX
20	*S. libidinosus*	XXXXX	XXXXX	XXXXX

21	*S. libidinosus*	XXXXX	XXXXX	XXXXX
22	*S. libidinosus*	XXXXX	XXXXX	XXXXX
23A	*S. libidinosus*	XXXXX	XXXXX	XXXXX
23B	*S. libidinosus*	XXXXX	XXXXX	XXXXX
24	*S. libidinosus*	JN409299	XXXXX	XXXXX
25	*S. libidinosus*	JN409300	XXXXX	XXXXX
26	*S. libidinosus*	XXXXX	XXXXX	XXXXX
27	*S. libidinosus*	XXXXX	XXXXX	XXXXX
28A	*S. cay*	FJ529046	XXXXX	XXXXX
28B	*S. cay*	FJ529058	XXXXX	XXXXX
28C	*S. cay*	FJ529050	XXXXX	XXXXX
28D	*S. cay*	FJ529051	XXXXX	XXXXX
28E	*S. cay*	FJ529053	XXXXX	XXXXX
29	*S. cay*	XXXXX	XXXXX	XXXXX
30	*S. cay*	XXXXX	XXXXX	XXXXX
31	*S. cay*	JN409298	XXXXX	XXXXX
32	*S. apella*	XXXXX	XXXXX	XXXXX
33A	*S. apella*	XXXXX	XXXXX	XXXXX
33B	*S. apella*	XXXXX	XXXXX	XXXXX
34A	*S. apella*	XXXXX	XXXXX	XXXXX
34B	*S. apella*	XXXXX	XXXXX	XXXXX
34C	*S. apella*	XXXXX	XXXXX	XXXXX
34D	*S. apella*	XXXXX	XXXXX	XXXXX
34E	*S. apella*	XXXXX	XXXXX	XXXXX
35	*S. apella*	XXXXX	XXXXX	XXXXX
36	*S. apella*	XXXXX	XXXXX	XXXXX
37	*S. apella*	XXXXX	XXXXX	XXXXX
38	*S. apella*	XXXXX	XXXXX	XXXXX
39	*S. apella*	XXXXX	XXXXX	XXXXX
40	*S. apella*	XXXXX	XXXXX	XXXXX
41	*S. apella*	XXXXX	XXXXX	XXXXX
42	*S. apella*	XXXXX	XXXXX	XXXXX
43	*S. apella*	XXXXX	XXXXX	XXXXX
44	*S. apella*	XXXXX	XXXXX	XXXXX
45A	*S. apella*	XXXXX	XXXXX	XXXXX
45B	*S. apella*	XXXXX	XXXXX	XXXXX
46	*S. apella*	XXXXX	XXXXX	XXXXX
47	*S. apella*	XXXXX	XXXXX	XXXXX
48	*S. apella*	XXXXX	XXXXX	XXXXX
49	*S. apella*	XXXXX	XXXXX	XXXXX
50	*S. apella*	XXXXX	XXXXX	XXXXX
51	*S. apella*	XXXXX	XXXXX	XXXXX

52	*S. apella*	XXXXX	XXXXX	XXXXX
53	*S. apella*	XXXXX	XXXXX	XXXXX
54	*S. apella*	XXXXX	XXXXX	XXXXX
55	*S. apella*	XXXXX	XXXXX	XXXXX
56	*S. apella*	XXXXX	XXXXX	XXXXX
57	*S. apella*	XXXXX	XXXXX	XXXXX
58	*S. apella*	XXXXX	XXXXX	XXXXX
59	*S. apella*	XXXXX	XXXXX	XXXXX
60	*S. apella*	XXXXX	XXXXX	XXXXX
61A	*S. apella*	XXXXX	XXXXX	XXXXX
61B	*S. apella*	XXXXX	XXXXX	XXXXX
61C	*S. apella*	XXXXX	XXXXX	XXXXX
62	*S. apella*	XXXXX	XXXXX	XXXXX
63	*S. apella*	XXXXX	XXXXX	XXXXX
64A	*S. apella*	XXXXX	XXXXX	XXXXX
64B	*S. apella*	XXXXX	XXXXX	XXXXX
64C	*S. apella*	XXXXX	XXXXX	XXXXX
64D	*S. apella*	XXXXX	XXXXX	XXXXX
65A	*S. apella*	XXXXX	XXXXX	XXXXX
65B	*S. apella*	XXXXX	XXXXX	XXXXX
66	*S. apella*	XXXXX	XXXXX	XXXXX
67	*S. apella*	XXXXX	XXXXX	XXXXX
68	*S. apella*	XXXXX	XXXXX	XXXXX
69	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
70	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
71	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
72	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
73	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
74	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
75	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
76	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
77	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
78	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
79A	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
79B	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
80	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
81	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
82	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
83	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
84	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
85	*S. macrocephalus*	JN409296	XXXXX	XXXXX
86	*S. macrocephalus*	JN409324	XXXXX	XXXXX

87	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
88	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
89	*S. macrocephalus*	XXXXX	XXXXX	XXXXX
90	*S. macrocephalus*	JN409326	XXXXX	XXXXX
91	*S. macrocephalus*	JN409327	XXXXX	XXXXX
92	*C. kaapori*	XXXXX	XXXXX	XXXXX
93	*C. o. castaneus*	XXXXX	XXXXX	XXXXX
94	*C. o. castaneus*	XXXXX	XXXXX	XXXXX
95	*C. o. castaneus*	XXXXX	XXXXX	XXXXX
96	*C. o. castaneus*	JN409316	XXXXX	XXXXX
97	*C. o. olivaceus*	JN409312	XXXXX	XXXXX
98	*C. o. olivaceus*	JN409311	XXXXX	XXXXX
99	*C. o. olivaceus*	JN409290	XXXXX	XXXXX
100A	*C. o. olivaceus*	JQ317661	XXXXX	XXXXX
100B	*C. o. olivaceus*	JQ317660	XXXXX	XXXXX
100C	*C. o. olivaceus*	JN409291	XXXXX	XXXXX
101	*C. o. olivaceus*	JN409314	XXXXX	XXXXX
102	*C. o. olivaceus*	JN409315	XXXXX	XXXXX
103A	*C. o. olivaceus*	JN409313	XXXXX	XXXXX
103B	*C. o. olivaceus*	JQ317659	XXXXX	XXXXX
104	*C. a. trinitatis*	JN409317	XXXXX	XXXXX
105	*C. brunneus*	JN409310	XXXXX	XXXXX
106A	*C. leucocephalus*	JN409319	XXXXX	XXXXX
106B	*C. leucocephalus*	JQ317662	XXXXX	XXXXX
107	*C. leucocephalus*	JN409293	XXXXX	XXXXX
108	*C. cesarae*	JN409292	XXXXX	XXXXX
109	*C. versicolor*	XXXXX	XXXXX	XXXXX
C	*C. capucinus*	FJ529110	XXXXX	XXXXX
110	*C. capucinus*	JN409309	XXXXX	XXXXX
111	*C. capucinus*	AY065907	XXXXX	XXXXX
112	*C. imitator*	JQ317658	XXXXX	XXXXX
113	*C. imitator*	JN409307	XXXXX	XXXXX
114	*C. imitator*	JN409287	XXXXX	XXXXX
115	*C. imitator*	JN409288	XXXXX	XXXXX
116	*C. imitator*	JN409306	XXXXX	XXXXX
117	*C. imitator*	JN409305	XXXXX	XXXXX
118	*C. albifrons*	JN409321	XXXXX	XXXXX
119A	*C. albifrons*	FJ529108	XXXXX	XXXXX
119B	*C. albifrons*	FJ529109	XXXXX	XXXXX
120A	*C. albifrons*	XXXXX	XXXXX	XXXXX
120B	*C. albifrons*	XXXXX	XXXXX	XXXXX
121	*C. albifrons*	XXXXX	XXXXX	XXXXX

122	*C. albifrons*	XXXXX	XXXXX	XXXXX
123	*C. unicolor*	XXXXX	XXXXX	XXXXX
124	*C. unicolor*	XXXXX	XXXXX	XXXXX
125	*C. unicolor*	XXXXX	XXXXX	XXXXX
126	*C. unicolor*	XXXXX	XXXXX	XXXXX
127	*C. unicolor*	XXXXX	XXXXX	XXXXX
128	*C. unicolor*	JN409295	XXXXX	XXXXX
129	*C. yuracus*	JN409322	XXXXX	XXXXX
130	*C. yuracus*	JN409294	XXXXX	XXXXX
131	*C. yuracus*	JN409323	XXXXX	XXXXX
132	*Cebus* sp.	JN409318	XXXXX	XXXXX
133	*Cebus* sp.	JN409320	XXXXX	XXXXX
134	*Saimiri boliviensis*	KM234530	XXXXX	XXXXX
135	*Saimiri o. citrinellus*	HQ644335	XXXXX	XXXXX
136	*Saimiri collinsi*	KJ128100	XXXXX	XXXXX

Appendix S2 COI primers and PCR protocol.

The amplification of COI was performed in 25 µl of final volume reaction, with 1 µl of DNA template (approximately 50 ng), 12 µl of Master Mix (Madison, WI, USA), 0.5 µl (10 mm) each of forward and reverse primers (Fish1SapF: 5' TCAACTAACCATAAGGATATTGGTAC 3' and Fish1SapR: 5' TATACCTCTGGGTGACCAAAAAATCA 3') and 11.5 µl of ultra pure water. The reactions were performed with an initial denaturation step at 95° C for 5 min, followed by 30 cycles of 94° C for 45 sec, 60° C for 45 sec, 72° C for 1 min and 72° C for 5 min for the extension. PCR products were purified with the Polyethylene glycol protocol (PEG) and both strands of each sample were sequenced on an Applied Biosystems Automated 3130 with the ABI Prism Big Dye Terminator Kit.

5. CAPÍTULO 2

Uma perspectiva filogenômica da radiação dos macacos-prego (*Sapajus*)

“A phylogenomic perspective on the robust capuchin monkey (*Sapajus*) radiation”

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[1] Esse capítulo está no formato de manuscrito e será submetido ao periódico “Molecular Phylogenetics and Evolution”. Os co-autores revisaram a presente versão.

[1] This chapter is in the manuscript format and will be submitt to the journal “Molecular Phylogenetics and Evolution”. The co-authors reviewed the present version.

A phylogenomic perspective on the robust capuchin monkey (*Sapajus*) radiation

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Graphical Abstract

Highlights

- Phylogenomic analyses support *Sapajus* and *Cebus* clades within capuchin monkeys
- Molecular data support *Sapajus nigritus, S. robustus* and *S. xanthosternos* as species
- UCE phylogeny lumps *Sapajus* Amazonian and grassland morphospecies
- SNP data separate *S. flavius* and *S. libidinosus* as sister species
- We recommend collapsing *S. apella*, *S. macrocephalus* and *S. cay* as one species

Abstract

Phylogenetic relationships among robust capuchin monkeys (*Sapajus*) are poorly understood. Taxonomies for this group based on morphology have considered from one to twelve different species. Current IUCN classification lists eight robust capuchins: *S. xanthosternos*, *S. nigritus*, *S. robustus*, *S. flavius*, *S. libidinosus*, *S. cay*, *S. apella* and *S. macrocephalus*. Here we assembled the first phylogenomic data set for robust capuchin monkeys using ultra-conserved elements (UCEs) to construct a robust capuchin phylogeny using RAxML. We extracted SNPs from the UCE data set, and created SNP phylogenies using Bayesian and Maximum Likelihood methods. We estimated a species tree using SVDquartets analyses. All phylogenomic analyses strongly supported *Sapajus* and *Cebus* clades within capuchin monkeys, and *Sapajus nigritus, S. robustus* and *S. xanthosternos* as species. However, the UCE phylogeny lumped morphospecies *S. cay, flavius, libidinosus, apella, macrocephalus,* and *flavius* together as a single widespread evolutionary lineage. The Bayesian SNP phylogeny was better resolved, and recovered *S. flavius* and *S. libidinosus* as sister species, together as sister to an *S. apella* + *macrocephalus* + *cay* clade; *S. apella*, *S. cay,* and *S. apella* individuals were interspersed together in the topology with no evidence for monophyly for any of these three morphological species. The species tree topology differed from the UCE and SNP topologies in that it reconstructed two major clades for robust capuchin monkeys: one Atlantic Forest clade (*S. robustus*, *S. xanthosternos*, and *S. nigritus*) and one widely distributed clade (*S. flavius*, *S. libidinosus*, plus north and south Amazonian robust capuchins). As morphological and molecular subdivisions of the Amazonian group + southern grasslands group (currently recognized as *S. cay*, *S. apella* and *S. macrocephalus*) are discordant, we recommend lumping all Amazonian plus southern grassland robust capuchin taxa as *S. apella* without subspecies.

Keywords

Neotropical primates, phylogeny, single nucleotide polymorphisms (SNPs), species tree, Ultraconserved elements (UCEs)

1. Introduction

Robust capuchin monkeys (*Sapajus*) comprise a widespread Neotropical primate genus found across cis-Andean Latin America, from the Colombian Llanos to the Guianas and throughout the Amazon basin as well as in the Atlantic Forest, Cerrado, Caatinga and Central Grasslands of South America, as far south as northern Argentina (Rylands et al., 2013). These primates as a group are true habitat generalists, with an incredible diet breadth compared to other Neotropical primates. While fruit and insects form the bulk of their diets, their robust jaw morphology coupled with behavioral adaptations for tool use and manipulative and extractive foraging together allow for the exploitation of encased and hidden foods unavailable to most other non-human animals (Fragaszy et al., 2004; Lynch Alfaro et al., 2012b).

Taxonomists have disagreed about the proximity of the relationship of robust capuchins to gracile capuchins. Elliot (1913) created a taxonomic key that divided the genus *Cebus* into tufted and non-tufted groups on the basis of the presence or absence of hair tufts on the frontal region of the head. However, only after Hershkovitz (1949) was there a general consensus about this division, with just one species (*Cebus apella* Linnaeus, 1758) recognized among the tufted group. Hill (1960) also considered all robust capuchins as one cosmopolitan species, *Cebus apella*, placed within the gracile capuchin genus, *Cebus*. Groves (2001, 2005) considered capuchins to form two species groups: (1) *C. capucinus* group with *C. capucinus*, *C. albifrons*, *C. olivaceus*, and *C. kaapori*; and (2) *C. apella* group with *C. apella*, *C. libidinosus*, *C. nigritus*, and *C. xanthosternos* (Table 1). Silva-Júnior (2001) separated robust capuchins as a different subgenus (*Sapajus*) from gracile capuchins (*Cebus*) on the basis of distinct cranial, postcranial and pelage morphology. Subsequently, genetic research validated the separation of robust and gracile capuchins as two distinct and equally diverse clades using mitochondrial (Lynch Alfaro et al., 2012a; Lima et al., in review) and a combination of mtDNA and nuclear (Perelman et al., 2011; Springer et al., 2012) markers. Two Alu elements provide strong evidence for the monophyly of robust *versus* gracile capuchins: Alu element S49P is present in *Sapajus* but not *Cebus* (Viana et al., 2015) and the AluSc8 insertion is found in *Cebus* but not *Sapajus* (Martins Jr. et al., 2015). A recent review justified the splitting of robust and gracile capuchins into two genera (*Cebus* for gracile capuchins and Sapajus for robust capuchins) based on the distinct morphology, biogeographic history, behavior, and ecology of each type (Lynch Alfaro et al., 2012b).

Taxonomists have also disagreed about the number of species encompassed by extant robust capuchins based on morphology (Table 1). Elliot (1913) recognized twelve species of robust capuchins, but Cabrera (1957) and Hill (1960) placed all robust forms into one species, *Cebus apella*, while retaining 11 and 16 subspecies, respectively. For the four decades between 1960 and 2000, most researchers lumped all robust capuchins as one species irrespective of place of origin, usually without regard for subspecies designations (e.g. Cole, 1992; Daegling, 1992; Ford and Hobbs, 1996; Masterson, 1997; Wright, 2005a; 2005b, 2007), leading to obfuscation of species or population differences within the robust capuchin literature (see Lynch Alfaro et al., 2014 for discussion). However, Torres de Assumpção (1983) pointed to distinct geographical variation in morphology among robust capuchin populations within Brazil, and especially within the Atlantic Forest. More recent morphological analyses have provided evidence for multiple *Sapajus* species (Groves, 2001, 2005; Silva-Júnior, 2001, 2002, 2005; Rylands et al., 2005, 2012, 2013; Rylands and Mittermeier, 2009). The robust capuchin group is now considered by most taxonomists to be comprised of four to eight species (Silva-Júnior., 2001; Groves, 2001; Rylands and Mittermeier, 2009; Rylands et al., 2005, 2012, 2013). The IUCN (2015) currently recognizes eight species: *Sapajus flavius*, the blonde capuchin; *S. xanthosternos*, the yellow-breasted capuchin; *S. robustus*, the robust tufted capuchin; *S. nigritus*, the black-horned capuchin; *S. apella*, the brown capuchin; *S. macrocephalus*, the large-headed capuchin; *S. cay*, Azara's capuchin; and *S. libidinosus*, the bearded capuchin.

Recent biogeographic analyses based on mitochondrial DNA suggest that the time depth of the radiation of extant robust capuchins is about 2.5 My of diversification, with diversity accumulating first in the Atlantic Coastal Forest of Brazil, and a recent expansion of robust capuchins throughout the Amazon Basin and Cerrado, Caatinga and Central Grasslands in the last 500,000 years (Lynch Alfaro et al., 2012a; Lima et al., in review). These analyses suggest that while the Atlantic Forest populations are relatively old and distinct, and can be separated as up to four different species, the Amazon/Grasslands radiation is better considered a highly polymorphic single species or species complex (Lima et al., in review). If our current nuclear data set is congruent with the mtDNA data, we would expect to see evidence for four to five species: *S. nigritus*, *S. robustus*, and *S. xanthosternos* each as reciprocally monophyletic clades, with *S. flavius* either nested within or as the sister group to a single clade that extends across the Amazon and grasslands habitats in South America (and encompasses *S. apella*, *S. libidinosus*, *S. macrocephalus* and *S. cay* morphospecies) (Lima et al., in review).

Here we use phylogenomic markers, ultraconserved elements (UCEs), to infer the phylogeny for robust capuchin monkeys, and to assess the evidence for congruence with species assignment by morphology and by mitochondrial and Alu markers. The UCE-based approach enriches DNA libraries for hundreds or thousands of UCEs and their flanking regions; then employs massively parallel sequencing for these libraries, and informatic tools to assemble, align and analyze the data (Faircloth et al., 2013). The UCE approach has been used successfully to resolve historically contentious taxonomical questions (McCormack et al., 2012; Crawford et al., 2012) including Pleistocene radiations (McCormack et al., 2015). Previous studies using nuclear markers for capuchin phylogeny have utilized a limited number of taxa and used captive individuals from unknown provenance as species exemplars (i.e. Perelman et al., 2011, Springer et al., 2012). The present study marks the first test of robust capuchin phylogeny using phylogenomic markers to analyze genetic relationships across species-representative individuals from known provenance and assigned morphologically to each of the eight currently recognized *Sapajus* species. Based on the most comprehensive mtDNA analysis for the capuchin monkey radiation (Lima et al., in review) we expect that much of the diversification within the *Sapajus* genus has occurred relatively recently, within the Pleistocene. We use SNP (Single Nucleotide Polymorphisms) data recovered within the UCE results in order to refine our understanding of robust capuchin diversification, as this technique was successful recently in elucidating the scrub-jay phylogeny across a similar geologic time frame (McCormack et al., 2015).

2. Material and methods

2.1. Samples, DNA extraction and sequencing

We sampled 67 individuals from 8 species of the genus *Sapajus* and 4 species of the genus *Cebus* from 62 localities distributed throughout the Atlantic Forest, Amazon, and Central Grasslands habitats (Figure 1 and Table 2). The total genomic DNA was extracted from muscle and blood samples using the Qiagen DNeasy Blood & Tissue Kit, according to the manufacturer's protocol. Library preparation, sequence capture and sequencing of ultraconserved elements were performed by RAPiD Genomics (Gainesville, FL, USA). Samples were quantified, normalized and sheared to an average fragment length of 350 base pairs (bp) for library preparation. Samples were dual-indexed with unique i5 and i7 8bp indexes. Libraries were then pooled with equimolar concentrations and the target sequence was captured using a custom set of 4715 probes targeting approximately 2300 UCE loci and 46 exons. Capture libraries were then pooled with equimolar concentrations for multiplexed dual-end (2x100bp) sequencing on an Illumina HiSeq 2500 v4 machine.

2.2. Sequence read quality control, assembly and UCE identification

We performed quality control using the trimming tool Trimmomatic 0.32.1 (Bolger et al., 2014) which trimmed sequences for adapter contamination, barcodes and low-quality regions using the parallel wrapper script in Illumiprocessor 2.0.6 (Faircloth, 2013) (https://github.com/faircloth-lab/illumiprocessor). We assembled the contigs for each sample using Trinity software package (vers. 2-25-2013) with default parameters using Phyluce 1.5.0 (Faircloth, 2016). We matched our assembled contigs to 4715 UCE loci custom-designed probe set using phyluce_assembly_match_contigs_to_probes integrating LASTZ 1.02.00 (Harris, 2007) from the Phyluce 1.5.0 (Faircloth, 2016) to remove any contigs that did not match probes or that matched multiple probes designed from different UCE loci. We performed in Phyluce 1.5.0 (Faircloth, 2016) the alignment of the contigs using the program phyluce_align_seqcap_align with MAFFT 7.271 (Katoh and Standley, 2013).

2.3. Phylogenetic analyses

For the phylogenetic analysis we used a concatenated data set in a single alignment constructed in Phyluce 1.5.0 (Faircloth et al., 2012; Faircloth, 2016). We used two data sets of UCE alignments that included greater than 95% of taxa present for each UCE locus (5% missing) and greater than 75% of taxa present for each UCE locus (25% missing), totaling 1838 UCEs with five exons (RAPGEF1, NAT15, GRIA21, CLOCK e BDNF) and 1388 UCEs with two exons (NAT15, GRIA21) respectively. We performed phylogenetic tree reconstruction under maximum likelihood (ML) in RAxML 8.0.19 (Stamatakis, 2014), using a GTRCAT model of nucleotide substitution, 1000 replicate searches to identify the optimal tree and we generated non-parametric bootstrap replicates using the autoMRE option of RAxML. To find the best partitioning scheme, we used PartitionFinder (Lanfear et al., 2012). We considered each UCE as a data block and enabled hcluster (Lanfear et al., 2014) with equal weights. To evaluate the fit of each model we used the Bayesian information criterion (BIC).

2.4. SNPs Analyses

Upon identifying the target UCE loci, we computed the coverage at each base of each contig using a python wrapper included in Phyluce (phyluce_assembly_get_trinity_coverage_for_uce_loci). We then employed a de novo SNPs calling approach by aligning all raw reads against our sample of the *S. robustus*, the reference sample with the highest coverage across all UCE loci enriched. This method integrated BWA (v 0.7.7-1) and PICARD (v 1.106-0) to output de novo aligned alignments in BAM format, repair any formatting violations, add read group header information, and mark duplicates in each BAM. We then merged all resulting BAMs into one file, realigning the data and calling SNPs and indels using GATK (v 3.5-0-g36282e4). To ensure high-quality SNPs in downstream analyses, we hierarchically filtered the data according to stringent quality and validation parameters, excluding SNPs with QUAL under 25, low variant confidence, and poor validation. Finally, the resulting VCF was passed through VCFTOOLS (v 0.1.14) to remove all loci that missed SNP calls for over 25% of all 67 samples.

On a parallel track, we passed our SNP data through a recently developed automatic pipeline called SNPhylo (Lee et al. 2014), designed to efficiently reconstruct trees based on genome wide SNPs. We modified our filtered VCF file by manually filling in autosomal chromosome positions for each SNP call, a necessary condition in order to run the program. We then set the Minor Allele Frequency threshold to 0.04 and negated the LD threshold to enable a more inclusive dataset for phylogenetic inference. We also bypassed the default low-quality data removal step, because the dataset had already undergone quality filtration with GATK. As a final step, the SNPhylo pipeline employs DNAML to generate a maximum likelihood hypothesis and passes the tree through PHANGORN, which generates 1000 bootstrap replicates for the final result.

Additionally, in ExaBayes 1.4.1 (Aberer et al., 2014), we performed two independent runs, each with four chains (three heated and one cold), from random starting topologies for 10 million generations with a sampling frequency of 500 generations. Posterior distributions of trees were summarized with the consensus script and combined with the postProcParam script. Convergence and stationarity of parameter estimates were verified using Tracer 1.6.0 (Rambaut et al., 2013).

We estimated a species tree using SVDquartets analyses (Singular Value Decomposition Scores for Species Quartets; Chifman and Kubatko, 2014) implemented in PAUP* v4.0a147 (Swofford, 2002). This method infers quartets based on summaries of SNPs in a concatenated sequence matrix species using a coalescent model. We randomly sampled 10 million quartets from the data matrix to infer a species tree and we measured uncertainty in relationships using nonparametric bootstrapping with 1000 replicates. For this analysis we did not include the samples from the widely distributed clade that did not form a part of the Northern Amazon or Southern Amazon subclades in the Bayesian (Exabayes) and maximum likelihood (SNPhylo) trees.

3. Results

3.1. Quality control

We sequenced a total of 178 million read pairs (mean = 2,661,695.4) for all samples. An average of 3309 contigs per sample (min = 1162, max = 6170) were assembled from 67 individuals (Table 2). After alignment and trimming as described above, we got an average of 1882 unique contigs matching UCE loci from each sample. We produced a 75% complete data matrix containing 1843 alignments of UCE loci, which produced a concatenated matrix of 550,515 bp (average length: 298.70 bp per alignment) and a 95% complete data matrix containing 1390 alignments of UCE loci, which produced a concatenated matrix of 439,190 bp (average length: 315.96 bp per alignment).

3.2. Phylogenomic analyses

We recovered strong support in the tree topology from our RAxML (75% and 95%) analyses for reciprocal monophyly between the *Sapajus* and *Cebus* clades (Figure

2 and Supplementary 1). Our analyses show strong molecular support for three of the morphological species within the genus *Sapajus*: *S. robustus, S. xanthosternos* and *S. nigritus*, all within the Atlantic Forest of Brazil. All other morphologically defined species within the genus (*S. flavius*, *S. libidinosus*, *S. apella*, *S. cay,* and *S. macrocephalus*) group together with high support in a widely distributed clade (from the Atlantic Forest to the Amazon), but there is no support for any subclades within this group in either the 75% or 95% taxa sets. Thus, the RAxML tree suggests four species of *Sapajus*: *S. robustus, S. xanthosternos* and *S. nigritus* from the Atlantic Forest of Brazil, and a widespread species that encompasses morphotypes *S. flavius*, *S. libidinosus*, *S. apella*, *S. cay,* and *S. macrocephalus*.

3.3. SNPs Analyses

After filtering out low quality SNPs, we retained a total of 19,583 SNPs across all samples. We then filtered for missing data and included only the SNPs that were parsimony-informative sites, generating a 75% complete matrix with a total of 11,462 informative high quality SNPs.

Similar to the RAxML analyses, our Maximum Likelihood and Bayesian trees using SNPs from the UCE data recover *S. xanthosternos* and *S. nigritus* as monophyletic clades, with the single *S. robustus* sample as the sister group to *S. xanthosternos* (Figure 3). However, within the widely distributed clade in the SNP trees, there are two distinct subclades. One subclade recovers monophyly of the species *Sapajus flavius* and also contains all *S. libidinosus* samples in a clade with *S. apella* specimens from Tucurui. The other subclade contains *S. cay*, *S. apella*, and *S. macrocephalus*; clusters within this subclade are geographically coherent but do not correspond to the current morphological taxonomy of the genus *Sapajus*. There is a clear division between Amazonian *Sapajus* north and south of the Amazon River, with some exceptions. Thus our phylogenomic SNP data provides some support for six distinct species within *Sapajus*: *S. nigritus*, *S. robustus*, *S. xanthosternos*, *S. flavius, S. libidinosus* and a widespread Amazonian and southern grasslands species.

While the Exabayes and SNPhylo had similar topologies, the two trees differed in the strength of their support for particular clades. For example, the SNPhylo tree resolved *S. nigritus* as the sister group to the widespread *Sapajus* clade (98), and *S. robustus* as sister to *S. xanthosternos* (96). SNPhylo also resolved *S. flavius* + (*S. libidinosus* + Tucuruí *S. apella*) clade as the sister group to *S. apella* + *S. macrocephalus* + *S. cay* (100). On the other hand, the Exabayes tree provided higher support for the *S. flavius* + (*S. libidinosus* + Tucuruí *S. apella*) clade (0.99) and for the *S. cay* + Rondonia *S. apella* clade (0.99). Within the widespread Amazonian *S. apella* + *S. macrocephalus* + *S. cay* clade, Exabayes recovered a northwestern *S. macrocephalus* subclade (0.99) and a northeastern *S. apella* subclade (0.99) that were strongly supported as sister to each other (0.97). Exabayes also supported the sister relationship (0.95) between the *S. cay* + Rondonia *S. apella* subclade and a south-central Amazonian *S. macrocephalus* clade (Atalaia, Purus, Jirau, Canutama, Cujubim, Mamiraua, Japura, Jamari; 0.91). In contrast, the internal topology for the subclades of the *S. apella* + *S. macrocephalus* + *S. cay* clade was less well-supported in SNPhylo.

In the species tree recovered using SVDquartets analyses (Figure 4), we found strong support (100) in the tree topology for reciprocal monophyly between *Sapajus* and *Cebus.* The internal topology differed in some regards for *Sapajus* when compared to our RAxML, ML and Bayesian trees using SNPs from the UCE data. As in other analyses, *Sapajus xanthosternos* and *S. robustus* were strongly supported as sister taxa

(100), but here *S. nigritus* was weakly supported (77) as sister to *S. xanthosternos* + *S. robustus.* While in the the other trees, *S. apella*, *S. macrocephalus*, *S. cay*, *S. flavius*, and *S. libidinosus* formed a subclade nested within the Atlantic forest robust capuchin clade and sister to *S. nigritus*, here this widespread group forms a second and well-supported (100) clade distinct from the Atlantic forest clade, with *S. flavius* supported (90) as sister to *S. libidinosus*, and Northern Amazonian and Southern Amazonian robust capuchins together forming a clade (100).

4. Discussion

Together our analyses provide genetic support for six distinct species within *Sapajus*: five morphological species (strong support for *S. robustus, S. xanthosternos, S. nigritus,* and more equivocal support for *S. libidinosus* and *S. flavius*) and one morphologically diverse Amazonian + Central Grasslands species that contains two major clades separated by distributions in Northern *versus* Southern Amazonia. Recent mitochondrial studies provide some additional support for the species status of *S. robustus*, *S. xanthosternos* and *S. nigritus* though the exact relationships among species varies (Lima et al., in review; Ruiz-Garcia et al., 2012). *S. flavius* is recovered as a monophyletic group with mitochondrial data, but is embedded within the widespread clade, or positioned as sister to the widespread clade (Lima et al., in review), whereas the nuclear results here place *S. flavius* and *S. libidinosus* as sister taxa. Both the mtDNA and the nuclear DNA topologies are discordant with Groves' (2001) taxonomic hypothesis that *S. robustus* is a subspecies of *S. nigritus*, because *S. nigritus* and *S. robustus* do not group together as sister taxa within *Sapajus.* In the previous studies employing large numbers of concatenated loci to elucidate primate relationships

(Perelman et al., 2011; Springer et al., 2012), *S. robustus* and *S. xanthosternos* are recovered as sister taxa to the exclusion of *S. apella*. In Springer et al. (2012) *S. apella* is recovered as sister to *S. libidinosus*, consistent with our present phylogeny.

While all *S. libidinosus* samples with light yellow pelage phenotype found across *S. libidinosus* distribution in the relatively dry biomes of Caatinga and Cerrado cluster together in one clade, that clade also includes samples that present standard *S. apella* pelage at the border of the two species distributions, near Tucuruí, Pará, on the eastern side of the lake that was formed by the damming of the Tocantins River for a Hydroelectric Plant (Figure 5b). These same individuals with *S. apella* morphotypes from Tucuruí cluster genetically with all sampled individuals with *S. libidinosus* pelage from within *S. libidinosus* distribution when using mitochondrial markers as well (Lima et al., in review). Tucuruí capuchins have darker pelage and live in tropical forest habitat, while nearby *S. libidinosus* are adapted to open Cerrado and Caatinga habitats, and have lighter pelage. *S. libidinosus* has also been shown to have cranial and post-cranial adaptations to increased ground use and encased fruit extraction (Wright et al., 2015). Morphometric data are not available for the Tucurui specimens, to determine if their cranial and post-cranial characteristics cluster with *S. libidinosus* or *S. apella*. Their external coloration should also be studied in detail to compare with other *Sapajus* specimens. The unexpected topology leaves us with various possibilities; it may be that the *S. libidinosus* lineage has expanded from the Cerrado biome to make inroads into the Amazon, and that *S. libidinosus* populations living in forested areas evolve darker pelage, so that they converge in appearance with *S. apella*. This could be a result of genetic adaptation, or it could be that capuchins have a developmental response with coat color adjusting to habitat conditions. Either way, this suggests ecological forces may be driving coat color and morphological characteristics. A second possibility is that

S. apella east of the Tocantins River became isolated from other robust Amazonian capuchins, and over time gave rise to the Caatinga and Cerrado populations of *S. libidinosus*. A third possibility is that *S. apella* and *S. libidinosus* have come into secondary contact at the borders of their distribution, and that despite significant gene flow, the two populations maintain their pelage characteristics. More morphological, genetic and ecological data will need to be collected in the Cerrado-Amazon transition zone in order to better understand relationships among capuchin populations here.

Note that *S. libidinosus* + Tucurui samples formed a clade with *S. flavius*. For this study, we sampled across western Caatinga and Cerrado for *S. libidinosus*, but we do not have samples here for eastern Caatinga where *S. libidinosus* is found close to *S. flavius* in northeastern Brazil (Figure 5b). More data from the Cerrado-Amazon transition zone and the Caatinga-Atlantic Forest transition zone could resolve if *S. flavius* and *S. libidinosus* are geographical variants of the same species, two distinct species, or are best lumped within the widespread *S. apella* group described below.

The molecular distinctiveness of the other morphological species currently assigned to *Sapajus* is not supported. Within the widespread *Sapajus* clade recovered in the SNP tree, there were strong indications for shared evolutionary history among morphotypes *S. cay*, *S. apella* and *S. macrocephalus*. There was no reciprocal monophyly between any of these morphologically defined species; instead, we observed geographic coherence for recovered lineages that did not correspond to current species hypotheses for Amazonian and grassland *Sapajus*. The pattern is more concordant with an isolation by distance model across the entire 'widespread *Sapajus'* clade, and morphological variation driven by habitat type. The samples designated as *S. cay* formed a clade with geographically proximate *S. apella* samples, indicating either a high index of gene flow between the two, or that the two types actually are within the same species and have evolved phenotypic variation related to habitat type. Another possibility is that there is more than one taxon encompassed within the current taxonomic classification of *S. cay*. Some studies have already indicated that *S. cay* from the Brazilian Pantanal and from Paraguay may not be a monophyletic group (Casado et al., 2010; Lima et al., in review), but in this study we do not have samples from both areas. *S. macrocephalus* as defined by Rylands et al. (2013) is also paraphyletic in our study, with two distinct lineages, one found north of the Solimões and Japurá rivers and south of the Rio Negro (recovered as sister to *S. apella* north of the Amazon River: Figure 5c) and the other in south-central Amazon south of the Amazon/Solimoes (recovered as the sister group to south Amazonian *S. apella* and *S. cay*: Figure 5d). Note that our study extends the *S. macrocephalus* morphotype east of the Madeira River, into the Brazilian state of Rondonia. *S. apella* appears in multiple places across the topology of both the RAxML and SNP trees, divided among various lineages which do not form a monophyletic group, but instead are interspersed with clades of *S. libidinosus*, *S. macrocephalus*, and *S. cay*.

It is important to note that the geographic boundaries and taxonomic affinities for *S. apella, S. cay*, *S. libidinosus* and *S. macrocephalus* are disputed by the two predominant morphological authorities (Groves 2001, 2005; Silva-Júnior, 2001, 2002). For example, Groves (2001) considers *S. cay* as two distinct subspecies of *S. libidinosus* (called *Cebus libidinosus paraguayanus* and *Cebus libidinosus pallidus*), and *S. macrocephalus* as a subspecies of *S. apella* (*Cebus apella macrocephalus*). Neither mitochondrial (Lynch Alfaro et al., 2012a; Lima et al., in review) nor nuclear data from the present study recovered reciprocal monophyly for *S. cay*, *S. apella*, or *S. macrocephalus.* Combining genetic and morphological data, we interpret that these morphotypes are not clearly defined and discrete species, but instead form one morphologically diverse, recently evolved pan-Amazonian plus grassland clade of robust capuchins. If we collapse these three taxa into one species, the taxonomic name would be *Sapajus apella*, which has priority over the other names because it was given first by Linnaeus in 1758. We do not recommend the use of subspecies within this cosmopolitan species, because molecular and morphological subdivisions are discordant with one another suggesting a high index of morphological plasticity and convergence within the species.

We also note that while the two major *Sapajus* clades within the Amazon are divided roughly by the Amazon River (see Figures 5c and d), that some samples within the Northern clade were from individuals south of the Amazon, and vice versa. In most cases these were individuals that were very close geographically to the Amazon River itself, and may be the result of human-mediated transport across the rivers in recent or modern times. It is also possible that capuchins cross the Amazon at low frequency in areas where there are many seasonal islands. Squirrel monkeys show a similar pattern in the eastern Amazon basin, where the Amazon River forms the border for the distributions of *Saimiri sciureus* and *S. collinsi*, with some cases of limited dispersal to the opposite bank of the Amazon River for each species in the Juruti and Faro regions of Pará State, Brazil (Merces et al., 2015).

5. Conclusions

Our phylogenomic data provided strong support for *Cebus* and *Sapajus* as two reciprocally monophyletic clades. This is concordant with morphological evaluations of distinctiveness between robust and gracile capuchins (Elliott, 1913; Hershkovitz, 1949; Groves, 2001, 2005; Silva-Júnior, 2001, 2002; Lynch Alfaro et al., 2012b), and mitochondrial and Alu element data that also point to this split (Lynch Alfaro et al., 2012a; Lima et al., in review; Martins Jr. et al., 2015**;** Viana et al., 2015**).**

In general, our phylogenies based on ultraconserved elements were congruent with mitochondrial phylogenies for robust capuchins (Lynch Alfaro et al., 2012; Lima et al., in review), although the placement of *S. robustus* as sister to *S. xanthosternos* was unique to the nuclear phylogenomic data, as was the recovery of a sister relationship between *S. flavius* and *S. libidinosus*. Our UCE tree distinguished only four *Sapajus* species, but the Exobayes SNP tree provided more support for six robust capuchin species, *S. xanthosternos*, *S. robustus*, *S. nigritus*, *S. flavius*, *S. libidinosus*, and *S. apella* (which subsumes *S. cay* and *S. macrocephalus*), although *S. apella* morphotypes from Tucurui were found within the *S. libidinosus* clade. The major division for Amazonian capuchins according to molecular data is a North-South division (both in the present work and from mitochondrial data in Lima et al., in review), whereas the morphological division of *S. macrocephalus* and *S. apella* is more of an East-West division, with the Madeira and Negro rivers as the suggested dividing line (Groves, 2001, 2005; Silva-Júnior, 2001, 2002). As morphological and molecular subdivisions of the Amazonian group are discordant, we recommend lumping all Amazonian plus southern grassland robust capuchin taxa as *S. apella* without subspecies. However, this does not discount the importance of populational differences in behavior, morphology and ecology in *S. apella* across the Amazon and southern grasslands; these populational differences may serve as a model for understanding the rapid evolution of populational differences across diverse habitats in other highly polymorphic species, such as humans.

The taxonomic relationship of *S. nigritus* to other capuchins is not well supported, with the species tree placing it as the sister group to *S. xanthosternos* + *S. robustus*, but the gene trees placing it as the sister group to the widespread clade of robust capuchins (*S. flavius*, *S. libidinosus*, *S. apella* as above). In contrast, mitochondrial phylogenetic reconstructions have placed *S. nigritus* as the sister to all other *Sapajus* (Lima et al., in review). More work needs to be done delineating the relationship and geographical boundaries between *S. nigritus nigritus* from Minas Gerais to Sao Paulo, Brazil and *S. n. cucullatus* from southern Brazil and Argentina, and their relationships to other capuchins. Future work is also needed to determine the relationship of Critically Endangered *S. apella margaritae* endemic to Margarita Island, Venezuela to the other Amazonian and Guianan robust capuchins.

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Tables

Table 1: Taxonomies of robust capuchins.

Elliot (1913)	Hershkovitz (1949)	Cabrera (1957)	Hill (1960)	Groves (2001, 2005)	Silva Jr (2001, 2005)	Rylands et al. (2013)
Cebus apella	*Cebus apella*	*Cebus apella*	*Cebus apella*	*Cebus apella*	*Cebus (Sapajus) apella*	*Sapajus apella*
Cebus fatuellus		*C. a. apella*	*C. a. apella*	*C. a. apella*	*Cebus (Sapajus) macrocephalus*	*Sapajus macrocephalus*
C. f. fatuellus		*C. a. margaritae*	*C. a. margaritae*	*C. a. fatuellus*	*Cebus (Sapajus) libidinosus*	*Sapajus libidinosus*
C. f. peruanus		*C. a. macrocephalus*	*C. a. fatuellus*	*C. a. macrocephalus*	*Cebus (Sapajus) cay*	*Sapajus cay*
Cebus macrocephalus		*C. a. libidinosus*	*C. a. peruanus*	*C. a. peruanus*	*Cebus (Sapajus) nigritus*	*Sapajus nigritus*
Cebus libidinosus		*C. a. paraguayanus*	*C. a. tocantinus*	*C. a. tocantinus*	*Cebus (Sapajus) robustus*	*S. n. nigritus*
Cebus azarae		*C. a. pallidus*	*C. a. macrocephalus*	*C. a. margaritae*	*Cebus (Sapajus) xanthosternos*	*S. n. cucullatus*
C. a. azarae		*C. a. xanthosternos*	*C .a. libidinosus*	*Cebus libidinosus*		*Sapajus robustus*
C. a. pallidus		*C. a. versutus*	*C. a. cay*	*C. l. libidinosus*		*Sapajus xanthosternos*
Cebus frontatus		*C. a. nigritus*	*C. a. pallidus*	*C. l. pallidus*		*Sapajus flavius*
Cebus variegatus		*C. a. vellerosus*	*C. a. frontatus*	*C. l. paraguayanus*		
Cebus versuta		*C. a. robustus*	*C. a. xanthosternos*	*C. l. juruanus*		
Cebus cirrifer			*C. a. nigritus*	*Cebus nigritus*		
Cebus crassiceps			*C. a. robustus*	*C. n. nigritus*		
Cebus caliginosus			*C. a. magnus*	*C. n. robustus*		
Cebus vellerosus			*C. a. juruanus*	*C. n. cucullatus*		
			C. a. maranonis	*Cebus xanthosternos*		

Table 2: List of samples, locality data and resulting for UCE data.

Code	Species	Latituide	Longitude	Trimmed reads	Contigs Assembled	Avg Len	UCE contigs	Avg Len
1	S. xanthosternos	-15.17	-39.07	2681597	3274	388.5	1970	408
2	S. xanthosternos	-15.41	-39.5	2843593	3661	382.9	1995	413.9
3A	S. xanthosternos	-14.79	-39.05	3196673	3802	392.4	1998	441.5
3B	S. xanthosternos	-14.79	-39.05	3521726	4275	389	2003	459
4	S. robustus	-19.95	-43.85	4538948	5198	373.5	2044	466.4
5	S. nigritus	-23.86	-46.14	2762021	3471	389.6	1825	409
6	S. nigritus	-23	-49.32	946881	1937	328	1450	284.3
7	S. flavius	-6.56	-35.13	2713906	3096	402.9	1971	421.9
8	S. flavius	-7.01	-34.96	4787966	5150	363.5	2031	457.3
9	S. flavius	-7.02	-35.09	2877922	3601	397.1	2000	435.4
10	S. libidinosus	-2.77	-41.81	2764451	3430	381.4	1941	402.1
11	S. libidinosus	-2.8	-41.87	4348317	5094	357.8	2025	435.7
12	S. libidinosus	-5.09	-42.43	2612178	3208	417.7	1890	357.4
13	S. libidinosus	-7.93	-44.2	3068523	3551	395.7	1986	421.6
14	S. libidinosus	-5.28	-48.3	3303530	3885	372.6	1966	401.2
15	S. libidinosus	-14.14	-48.17	3381894	3603	377.5	1965	399.5
16	S. libidinosus	-16.6	-49.26	3301692	3884	372.2	1989	410.1
17A	S. apella	-3.83	-49.64	3541159	3793	380.3	1991	423.3
17B	S. apella	-3.83	-49.64	2980533	3534	379.2	1961	408.5
18	S. apella	-6.15	-49.56	1908769	2828	416.6	1920	418.8
19	S. apella	-3.36	-51.74	3391742	3723	382.9	1996	418.8
20	S. apella	-2.61	-51.54	5485708	6170	355.6	2034	487.9

21	S. apella	-0.58	-52.33	1311929	2137	373.2	1621	326.1
22	S. apella	3.22	-52.03	1757726	2338	384	1728	356
23	S. apella	0.83	-53.93	2781762	2805	352.7	1754	338.4
24	S. apella	1.29	-58.7	2130450	2604	384.5	1839	366.7
25	S. apella	-1.49	-56.8	1572934	2413	385.4	1773	360.6
26	S. apella	-2.47	-58.4	3571090	3780	385.6	1999	420.9
27	S. apella	-2.6	-56.18	2394355	3227	394.6	1966	412
28	S. apella	-3.18	-55.8	1890413	2709	391.7	1884	383
29	S. apella	-3.88	-56.78	1276241	2039	363.8	1520	325.7
30	S. apella	-4.71	-56.44	1746336	2515	379	1812	359.1
31	S. apella	-10	-56.04	1791793	2450	394.9	1741	352.4
32	S. apella	-9.2	-59.06	2103015	2895	365.5	1886	359.4
33	S. apella	-12.03	-60.67	2339872	3027	382.9	1898	377.2
34	S. apella	-12.56	-63.44	3883141	4558	380.2	2024	447.3
35	S. cay	-16.06	-57.72	1624662	2588	373.8	1765	350.1
36	S. cay	-13.52	-60.43	2361492	2991	384.1	1933	388
37	S. macrocephalus	-12.45	-62.92	2986344	3335	381.3	1967	399.7
38	S. macrocephalus	-8.67	-62.37	2962283	3477	370.5	1952	392.7
39	S. macrocephalus	-9.1	-62.88	2222218	2882	376.6	1900	371.4
40	S. macrocephalus	-8.89	-63.24	3054313	3411	372.6	1963	391.9
41	S. macrocephalus	-8.8	-63.95	1459387	2148	361.4	1570	324.7
42	S. macrocephalus	-8.19	-64.02	2196025	2741	375.9	1881	365.5
43	S. macrocephalus	-5.69	-63.24	3840307	4395	363	2009	422.6
44A	S. macrocephalus	-4.99	-62.96	3199632	3780	383.4	1994	433.9
44B	S. macrocephalus	-4.99	-62.96	1163783	2218	355.6	1650	326.1
45	S. macrocephalus	-4.75	-61.28	2351064	3072	394.7	1932	379.2

46	S. macrocephalus	-4.44	-60.32	2219015	2938	374.6	1922	366.9
47	S. macrocephalus	-3.37	-60.48	1876035	2707	367.5	1841	343.6
48	S. macrocephalus	-1.05	-62.89	2044899	2699	387.1	1871	372.3
49	S. macrocephalus	-0.48	-64.41	2723327	3234	385	1922	398.4
50	S. macrocephalus	-0.61	-64.92	3169376	3983	350	1980	379.2
51	S. macrocephalus	-0.23	-66.85	2105443	2681	383	1868	368.8
52	S. macrocephalus	-2.47	-64.83	3117247	3756	419.3	2015	484.5
53	S. macrocephalus	-2.59	-64.89	2484843	2946	408.9	1937	424.5
54	S. macrocephalus	-2.45	-65.36	1918138	2692	401.6	1869	401.9
55	S. macrocephalus	-1.84	-69.03	2085573	2716	394.7	1878	391.5
56	S. macrocephalus	-4.4	-70.14	3522837	4000	369.4	1992	422.5
57	S. macrocephalus	-4.94	-68.17	4107017	4659	370.7	2003	453.9
-	C. unicolor	-9.22	-66.74	2057387	3279	394.2	1902	371.2
-	C. o. castaneus	-0.58	-52.33	2107696	3145	402	1836	376.3
-	C. o. castaneus	1.84	-52.74	1401630	2151	373.9	1483	316.4
-	C. kaapori	-2.33	-46.08	2885841	3593	443.1	1983	425.9
-	C. capucinus			3954729	4702	419.8	2026	450.1
-	C. capucinus			508807	1162	288.9	891	267.2
-	C. albifrons	-2.59	-64.89	3111458	3951	391	1995	428.9

Figure Captions

Graphical Abstract. (a) Maximum likelihood and **(b)** Bayesian inference for robust capuchin phylogeny based on SNP data.

Figure 1. Map showing the sampled localities for *Sapajus*

Figure 2. Maximum likelihood **(**RAxML) 75% phylogeny for UCE data.

Figure 3. (a) Maximum likelihood and **(b)** Bayesian inference for robust capuchin phylogeny based on SNP data.

Figure 4. Species tree for robust capuchins using SNP quartets.

Figure 5. (a) Map with minimum convex polygons to show geographic distribution of major subclades within the widespread *Sapajus* clade, **(b)** Minimum convex polygon for range distribution for *S. flavius* and *S. libidinosus* clades within the Exabayes phylogeny, **(c)** Minimum convex polygon for range distribution for the Northern Amazonian *Sapajus* clade within the Exabayes phylogeny and **(d).**Minimum convex polygon for range distribution for the Southern Amazonian *Sapajus* clade within the Exabayes phylogeny. Larger map depicts subclades of south central Amazonian *S. macrocephalus* and southern Amazonian + grasslands *S. apella* + *cay.*

Supplementary 1. Maximum likelihood (RaxML) 95% phylogeny for UCE data.

Graphical Abstract.

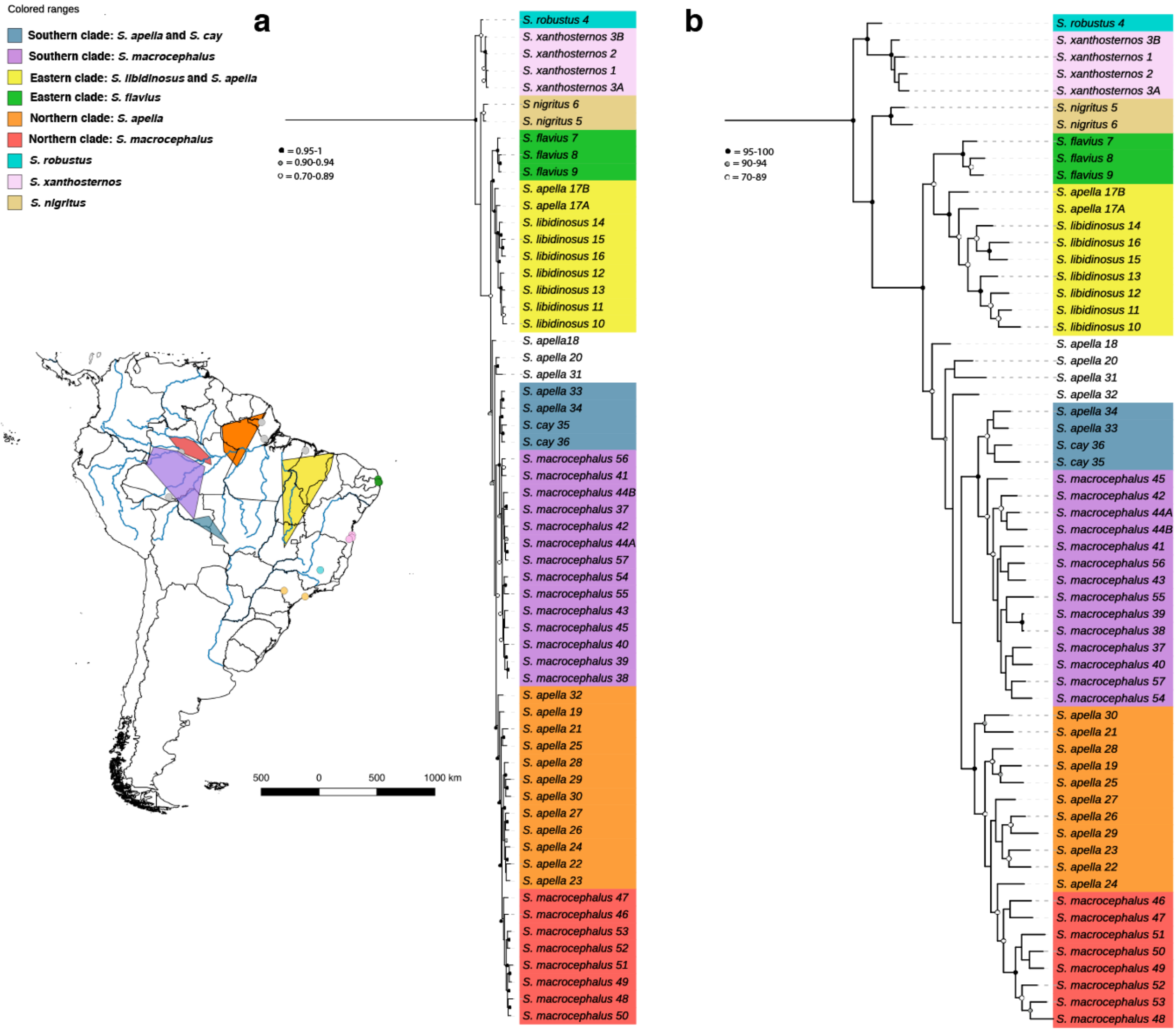
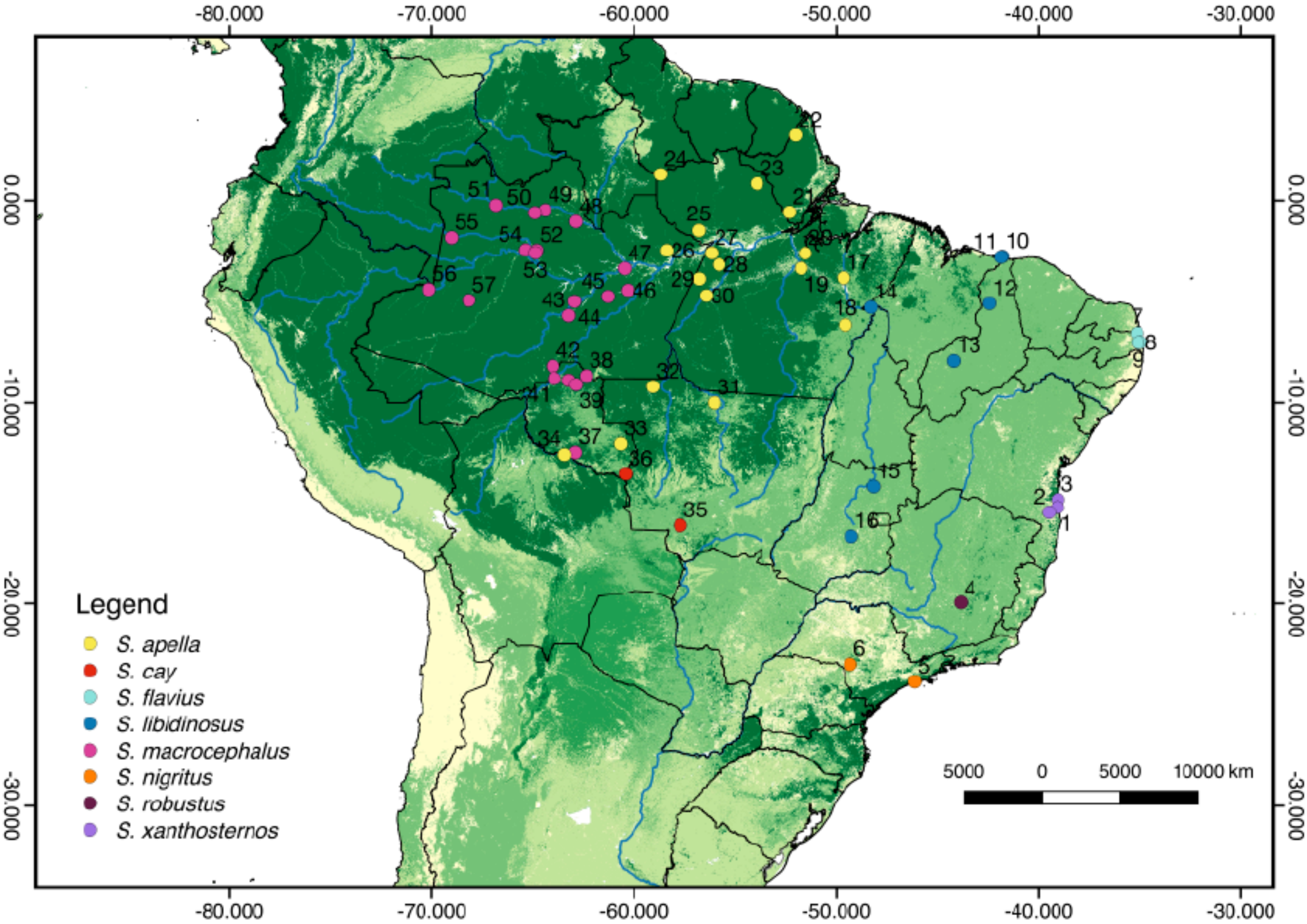


Figure 1.


-80.000
-70.000
-60.000
-50.000
-40.000
-30.000
0.000
-10.000
-20.000
-30.000
Legend
● S. apella
● S. cay
● S. flavius
● S. libidinosus
● S. macrocephalus
● S. nigritus
● S. robustus
● S. xanthosternos
5000
0
5000
10000 km
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
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44
45
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51
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56
57

Figure 2.

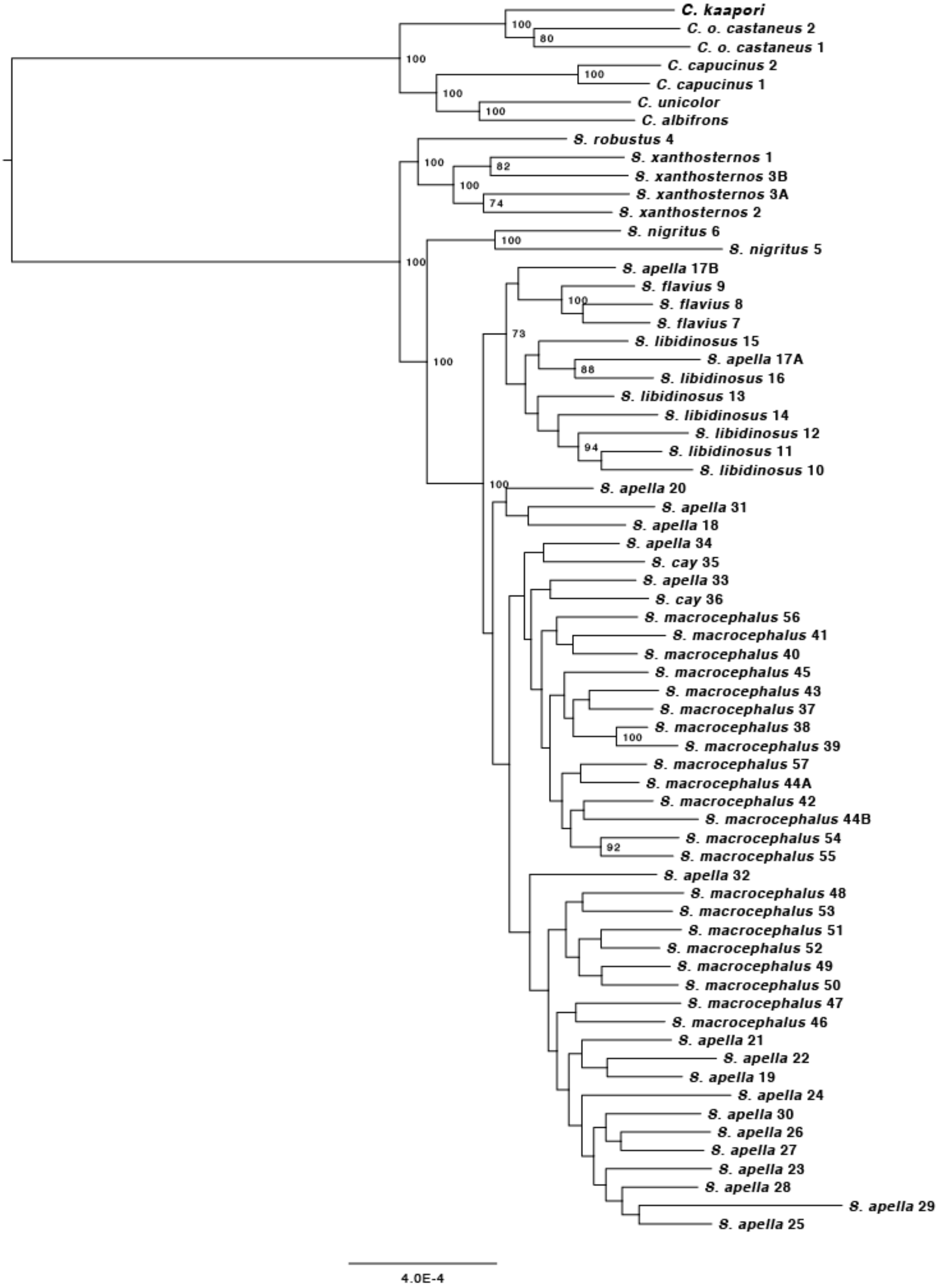

C. kaapori
C. o. castaneus 2
C. o. castaneus 1
C. capucinus 2
C. capucinus 1
C. unicolor
C. albifrons
S. robustus 4
S. xanthosternos 1
S. xanthosternos 3B
S. xanthosternos 3A
S. xanthosternos 2
S. nigritus 6
S. nigritus 5
S. apella 17B
S. flavius 9
S. flavius 8
S. flavius 7
S. libidinosus 15
S. apella 17A
S. libidinosus 16
S. libidinosus 13
S. libidinosus 14
S. libidinosus 12
S. libidinosus 11
S. libidinosus 10
S. apella 20
S. apella 31
S. apella 18
S. apella 34
S. cay 35
S. apella 33
S. cay 36
S. macrocephalus 56
S. macrocephalus 41
S. macrocephalus 40
S. macrocephalus 45
S. macrocephalus 43
S. macrocephalus 37
S. macrocephalus 38
S. macrocephalus 39
S. macrocephalus 57
S. macrocephalus 44A
S. macrocephalus 42
S. macrocephalus 44B
S. macrocephalus 54
S. macrocephalus 55
S. apella 32
S. macrocephalus 48
S. macrocephalus 53
S. macrocephalus 51
S. macrocephalus 52
S. macrocephalus 49
S. macrocephalus 50
S. macrocephalus 47
S. macrocephalus 46
S. apella 21
S. apella 22
S. apella 19
S. apella 24
S. apella 30
S. apella 26
S. apella 27
S. apella 23
S. apella 28
S. apella 29
S. apella 25
100
80
100
100
100
100
100
82
100
74
100
100
100
73
100
88
94
100
100
92
4.0E-4

Figure 3.

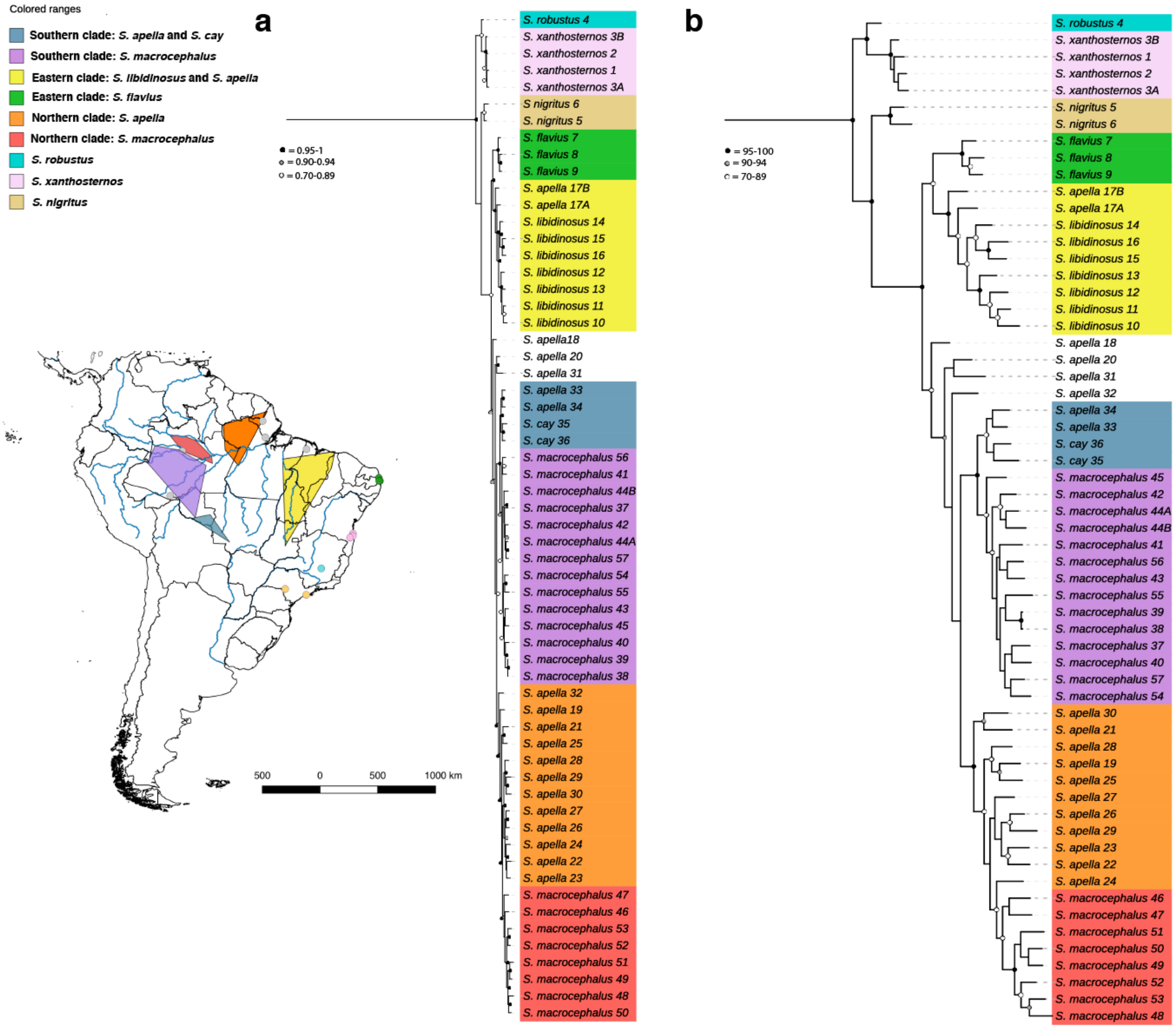

Colored ranges
🟦 Southern clade: S. apella and S. cay
🟪 Southern clade: S. macrocephalus
🟨 Eastern clade: S. libidinosus and S. apella
🟩 Eastern clade: S. flavius
🟧 Northern clade: S. apella
🟥 Northern clade: S. macrocephalus
S. robustus
S. xanthosternos
S. nigritus
a
● = 0.95-1
● = 0.90-0.94
○ = 0.70-0.89
500
0
500
1000 km
S. robustus 4
S. xanthosternos 3B
S. xanthosternos 2
S. xanthosternos 1
S. xanthosternos 3A
S nigritus 6
S. nigritus 5
S. flavius 7
S. flavius 8
S. flavius 9
S. apella 17B
S. apella 17A
S. libidinosus 14
S. libidinosus 15
S. libidinosus 16
S. libidinosus 12
S. libidinosus 13
S. libidinosus 11
S. libidinosus 10
S. apella18
S. apella 20
S. apella 31
S. apella 33
S. apella 34
S. cay 35
S. cay 36
S. macrocephalus 56
S. macrocephalus 41
S. macrocephalus 44B
S. macrocephalus 37
S. macrocephalus 42
S. macrocephalus 44A
S. macrocephalus 57
S. macrocephalus 54
S. macrocephalus 55
S. macrocephalus 43
S. macrocephalus 45
S. macrocephalus 40
S. macrocephalus 39
S. macrocephalus 38
S. apella 32
S. apella 19
S. apella 21
S. apella 25
S. apella 28
S. apella 29
S. apella 30
S. apella 27
S. apella 26
S. apella 24
S. apella 22
S. apella 23
S. macrocephalus 47
S. macrocephalus 46
S. macrocephalus 53
S. macrocephalus 52
S. macrocephalus 51
S. macrocephalus 49
S. macrocephalus 48
S. macrocephalus 50
b
● = 95-100
● = 90-94
○ = 70-89
S. robustus 4
S. xanthosternos 3B
S. xanthosternos 1
S. xanthosternos 2
S. xanthosternos 3A
S. nigritus 5
S. nigritus 6
S. flavius 7
S. flavius 8
S. flavius 9
S. apella 17B
S. apella 17A
S. libidinosus 14
S. libidinosus 16
S. libidinosus 15
S. libidinosus 13
S. libidinosus 12
S. libidinosus 11
S. libidinosus 10
S. apella 18
S. apella 20
S. apella 31
S. apella 32
S. apella 34
S. apella 33
S. cay 36
S. cay 35
S. macrocephalus 45
S. macrocephalus 42
S. macrocephalus 44A
S. macrocephalus 44B
S. macrocephalus 41
S. macrocephalus 56
S. macrocephalus 43
S. macrocephalus 55
S. macrocephalus 39
S. macrocephalus 38
S. macrocephalus 37
S. macrocephalus 40
S. macrocephalus 57
S. macrocephalus 54
S. apella 30
S. apella 21
S. apella 28
S. apella 19
S. apella 25
S. apella 27
S. apella 26
S. apella 29
S. apella 23
S. apella 22
S. apella 24
S. macrocephalus 46
S. macrocephalus 47
S. macrocephalus 51
S. macrocephalus 50
S. macrocephalus 49
S. macrocephalus 52
S. macrocephalus 53
S. macrocephalus 48

Figure 4.

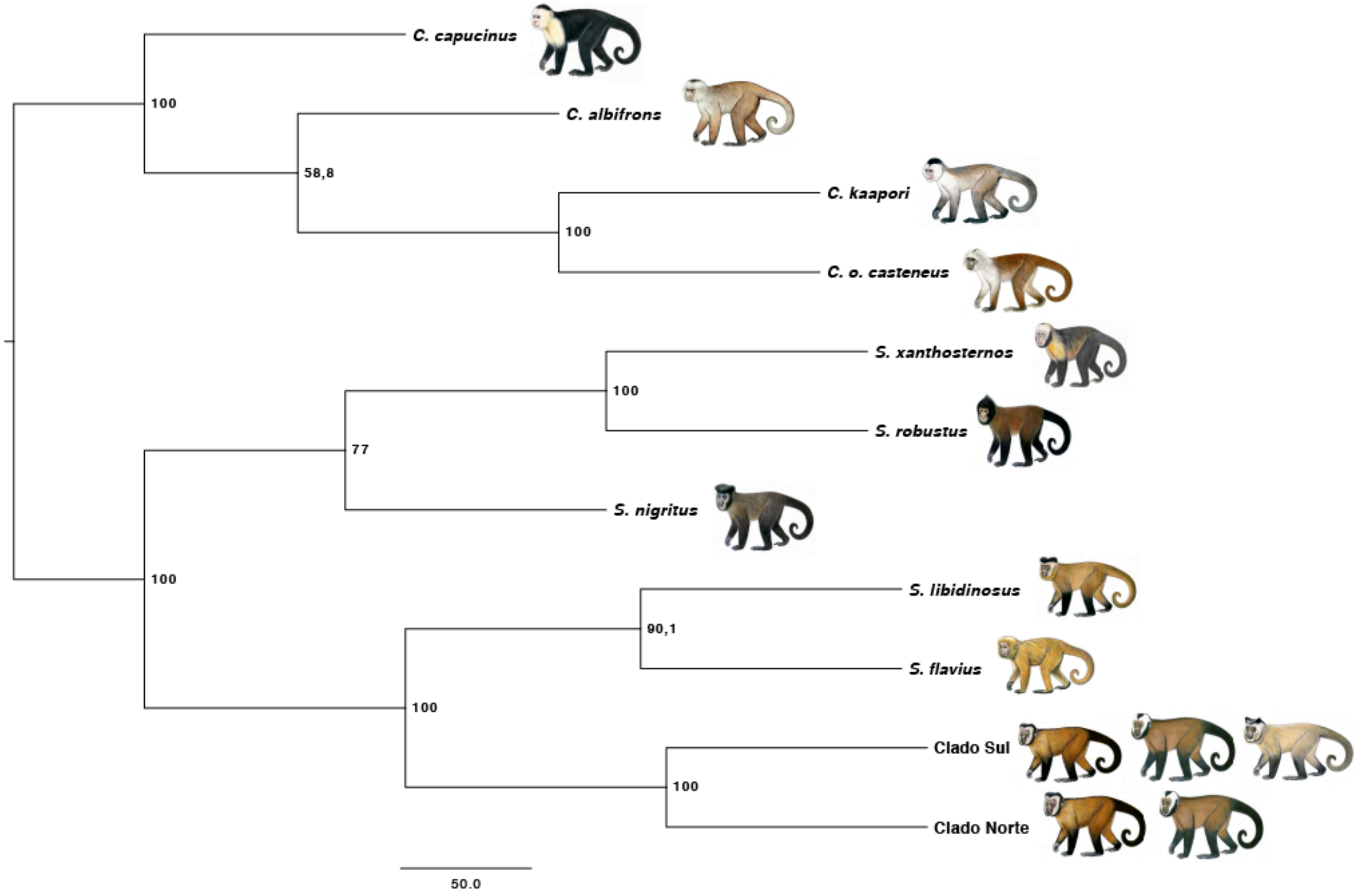
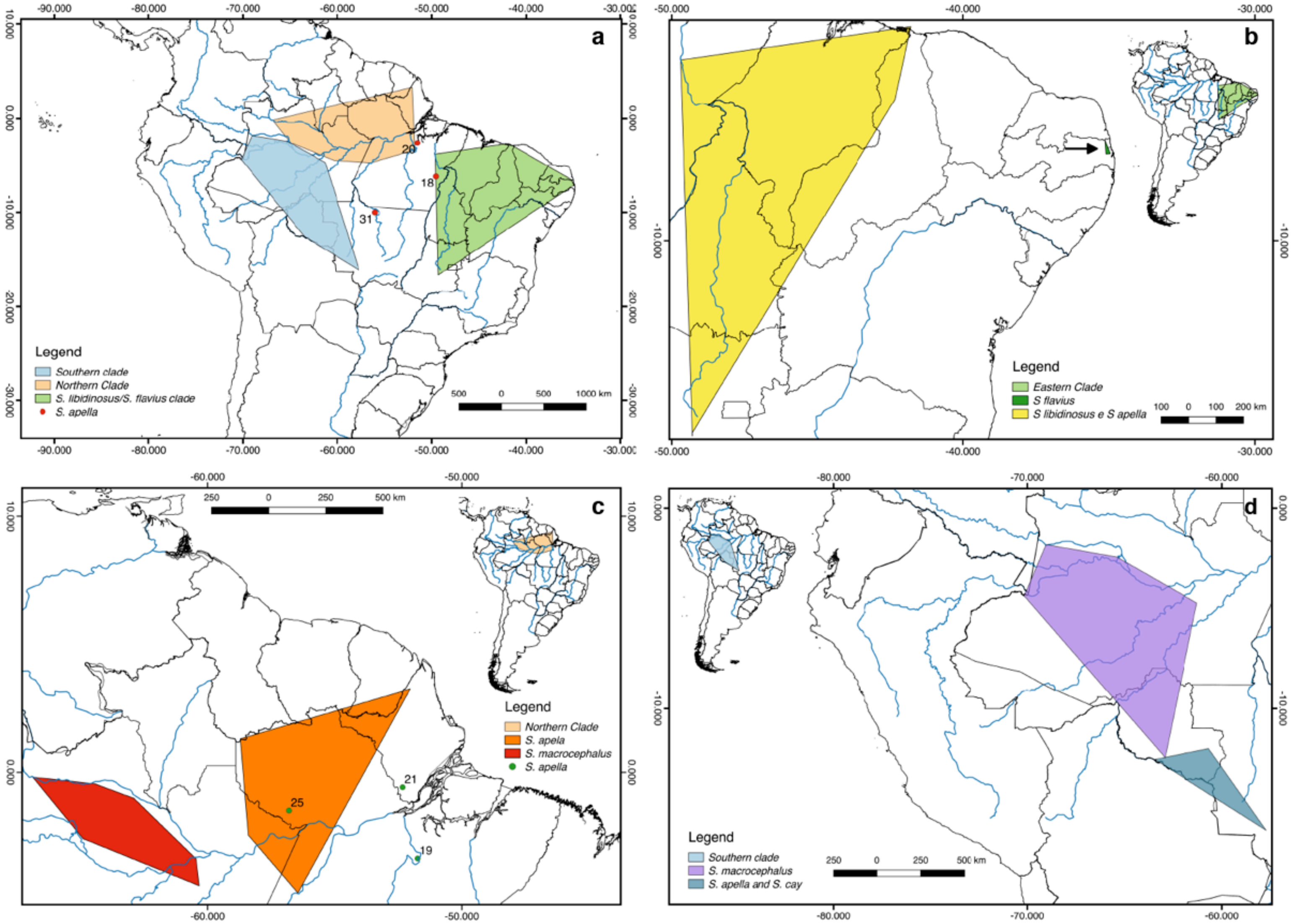

C. capucinus
100
C. albifrons
58,8
C. kaapori
100
C. o. casteneus
S. xanthosternos
100
S. robustus
77
S. nigritus
100
S. libidinosus
90,1
S. flavius
100
Clado Sul
100
Clado Norte
50.0

Figure 5.


a
-90.000
-80.000
-70.000
-60.000
-50.000
-40.000
-30.000
10.000
0.000
-10.000
-20.000
-30.000
20
18
31
Legend
Southern clade
Northern Clade
S. libidinosus/S. flavius clade
S. apella
500
0
500
1000 km
b
-50.000
-40.000
-30.000
-10.000
Legend
Eastern Clade
S flavius
S libidinosus e S apella
100
0
100
200 km
c
-60.000
-50.000
10.000
0.000
250
0
250
500 km
21
25
19
Legend
Northern Clade
S. apela
S. macrocephalus
S. apella
d
-80.000
-70.000
-60.000
0.000
-10.000
Legend
Southern clade
S. macrocephalus
S. apella and S. cay
250
0
250
500 km

Supplementary 1.