UNIVESIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Tese de Doutorado

Biogeografia de mamíferos e as rotas de conexão entre florestas da Amazônia e Mata Atlântica

Arielli Fabrício Machado

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia da Universidade Federal do Rio Grande do Sul como um dos pré-requisitos para obtenção do título de Doutora em Ciências – ênfase em Ecologia.

Orientador: Dr. Leandro da Silva Duarte

Porto Alegre, outubro de 2020.

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“Among the scenes which are deeply impressed on my mind, none exceed in sublimity the primeval forests undefaced by the hand of man; whether those of Brazil, where the powers of Life are predominant, or those of Tierra del Fuego, where Death and decay prevail. Both are temples filled with the varied productions of the God of Nature”

Charles Darwin, Voyage of the Beagle.

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RESUMO

Muitas evidências mostram conexões históricas entre florestas da Amazônia e Mata Atlântica por pelo menos três rotas pelas florestas: 1) do Nordeste do Brasil (ligando o Leste da Amazônia e o Nordeste da Mata Atlântica), 2) do interior do Cerrado (entre Sudeste da Amazônia e Sudeste da Mata Atlântica) e 3) da Bacia do Paraná (entre Oeste da Amazônia e Sudeste da Mata Atlântica). Estudos sugerem que esta última rota seria a mais antiga e frequente ao longo do tempo. Essas conexões foram reveladas pelo padrão de distribuição de espécies e/ou evidências moleculares, porém estudos que testem essas hipóteses ainda são raros, principalmente para mamíferos e considerando múltiplas espécies ou abordagens integrativas. O objetivo desta tese foi investigar as conexões florestais entre Amazônia e Mata Atlântica através da biogeografia de mamíferos e avaliando hipóteses propostas anteriormente através de diferentes abordagens: 1) compilando espécies de mamíferos potenciais para investigar essas conexões e dados genéticos disponíveis, avaliando as principais rotas evidenciadas e a relação com o tipo de habitat das espécies; 2) avaliando a filogeografia comparada e mudanças na paleodistribuição de três espécies de marsupiais desses biomas para entender quando e onde conexões teriam ocorrido; 3) avaliando os padrões espaço-temporais dessas conexões para múltiplas espécies de mamíferos testando a hipótese de variação temporal entre as rotas. Diferente do proposto, encontramos mais espécies com distribuição para a rota do Nordeste e Sudeste. Encontramos uma relação com o uso do habitat das espécies e uma alta disponibilidade de dados genéticos. Através da filogeografia comparada de marsupiais com tempos mais antigos, dentro de uma escala mais recente de tempo, assim como recentes para ambas as rotas do Sudeste e Nordeste. Quando avaliamos esse padrão para múltiplas espécies, encontramos tanto conexões antigas como recentes para cada rota e grupo taxonômico. Devido à história biogeográfica de cada grupo, essas conexões não podem ser explicadas por um único padrão espaço-temporal. Aqui, exploramos desde conexões históricas recentes até a história biogeográfica das florestas e mamíferos do passado remoto, trazendo novos insights mais dinâmicos sobre os padrões das conexões entre Amazônia e Mata Atlântica. Além disso, nossos resultados destacam o potencial dos mamíferos para estudos biogeográficos sobre essas conexões apoiando estudos futuros.

Palavras-chave: Florestas Neotropicais, conexões florestais, filogeografia, modelos de distribuição de espécies, macroecologia.

SUMÁRIO

INTRODUÇÃO GERAL

A biogeografia é a ciência que estuda os padrões espaciais da biodiversidade através da distribuição geográfica dos organismos ao longo do tempo e sua relação com as mudanças na Terra buscando entender os processos que levaram a esses padrões (Lomolino et al., 2010; Cox et al., 2016). Um exemplo clássico de uma teoria sobre conexões biogeográficas é o paradigma da deriva continental de Alfred Wegener (Wegener, 1915), a qual atualmente abrange, devido ao avanço da Panbiogeografia de Leon Croizat (Croizat, 1958), dois dos principais processos associados aos padrões de distribuição dos organismos: dispersão e vicariância (Parenti & Ebach, 2009; Cox et al., 2016). Essa teoria foi proposta e corroborada por distintas fontes de evidências (registros paleoclimáticos, geológicos, fósseis e atualmente moleculares) mostrando que os continentes atuais foram parte de um supercontinente há cerca de 200 milhões de anos atrás (Ma), a Pangeia, a qual subdividiu-se ao longo do tempo devido aos movimentos tectônicos (Lomolino et al., 2010; Cox et al., 2016). Nesse período plantas e animais dispersaram por esse supercontinente, isolando-se posteriormente (há cerca de 100 Ma) por eventos vicariantes que dividiram a Pangeia em Laurásia (ao Norte) e Gondwana (ao Sul) (Lomolino et al., 2010; Cox et al., 2016). Foi durante esse processo de separação dos continentes que as florestas tropicais teriam surgido (Morley, 2000; Couvreur et al., 2011; Lavina & Fauth, 2011) e os primeiros mamíferos teriam ocupado a América do Sul (Goin et al., 2012).

As florestas tropicais teriam se originado na Laurásia há pelo menos 100 Ma (Couvreur et al., 2011) e ocupado a América do Sul há pelo menos 65 Ma (Morley, 2000; Jaramillo et al., 2010; Wing et al., 2009). Nesse período elas teriam expandido para o sul desse continente, onde o clima era mais úmido, diferente do norte do continente onde o clima era árido (Hallam, 1985; Lavina & Fauth, 2011). Desde então, essas florestas passaram por diversas mudanças, como por exemplo, ciclos de expansões e retrações associados às mudanças climáticas (Morley, 2000). Acredita-se que as florestas da Amazônia e da Mata Atlântica, as maiores florestas tropicais da América do Sul, se distribuíam continuamente no passado (Morley, 2000; Lavina & Fauth, 2011). Desde o Oligoceno (há cerca de 23 Ma) até o Plioceno (cerca de 12 Ma), sucessivos eventos tectônicos levaram ao soerguimento dos Andes, causando mudanças no clima da América do Sul e, consequentemente, na vegetação (Morley, 2000; Barreda & Palazzesi, 2007;

Hoorn et al., 2010). Esses eventos tornaram o clima mais seco na região central da América do Sul, onde a redução das florestas que ocupavam essa região deram lugar à expansão das savanas, o que levou a separação das florestas da Amazônia e da Mata Atlântica (Bigarella & Andrade-Lima, 1982; Costa, 2003; Hoorn et al., 2010).

As florestas da Amazônia e da Mata Atlântica representam as maiores florestas tropicais da América, estando entre as mais diversas do mundo (Mittermeier et al., 1998; Jenkins et al., 2013). Apesar da aparente distribuição disjunta entre a Amazônia e a Mata Atlântica, existe um mosaico de florestas (incluindo florestas montanas, submontanas, ripárias, semidecíduas e decíduas) dentro da chamada 'diagonal seca' (composta pelos biomas da Caatinga, do Cerrado e do Chaco árido) que pode ser considerado como remanescentes de uma ponte histórica de conexão biogeográfica entre elas (Oliveira-Filho & Ratter, 1995). Diversas espécies arbóreas de florestas do Cerrado apresentam distribuição comum entre a Amazônia e a Mata Atlântica, assim como algumas da Caatinga (Oliveira-Filho & Ratter, 1995). Ademais, são encontradas conexões filogeográficas entre a fauna desses biomas, como para aves (Batalha-Filho et al., 2013; Batalha-Filho et al., 2014; Carvalho et al., 2017; Capurucho et al., 2018; da Silva et al., 2020; Moura et al., 2020), mamíferos (Cortés-Ortiz et al., 2003; Costa, 2003; Nascimento et al., 2008; Pavan et al., 2011), répteis (Pellegrino et al., 2011; Prates et al., 2016), anfíbios (Fouquet et al., 2012; Thomé et al., 2016) e inclusive insetos (Peres et al., 2017).

Para explicar essas conexões, foram propostas três principais rotas de conexão histórica entre a Amazônia e a Mata Atlântica (Figura 1): (1) a rota do Nordeste através das florestas do Nordeste do Brasil; (2) pelas florestas de galeria do nordeste do Cerrado e (3) a rota Sudeste-Noroeste através das florestas da Bacia do Paraná, Chaco úmido e Pantanal passando por florestas do sudoeste do Cerrado (Por, 1992; Oliveira-Filho & Ratter 1995; Costa, 2003). Também foi proposta variação temporal entre as rotas de conexão, sendo a rota do Nordeste mais recente e a rota do Sudeste-Noroeste mais antiga e estabelecida com maior frequência (Por, 1992). Recentemente, alguns estudos buscaram testar essa hipótese, corroborando-a através da biogeografia de aves (Batalha-Filho et al., 2013) e através da revisão de evidências moleculares para diferentes espécies animais (Ledo & Colli, 2017). Ainda assim, pouco se sabe sobre a totalidade das espécies que podem evidenciar as conexões entre esses biomas para confirmação dessa hipótese.

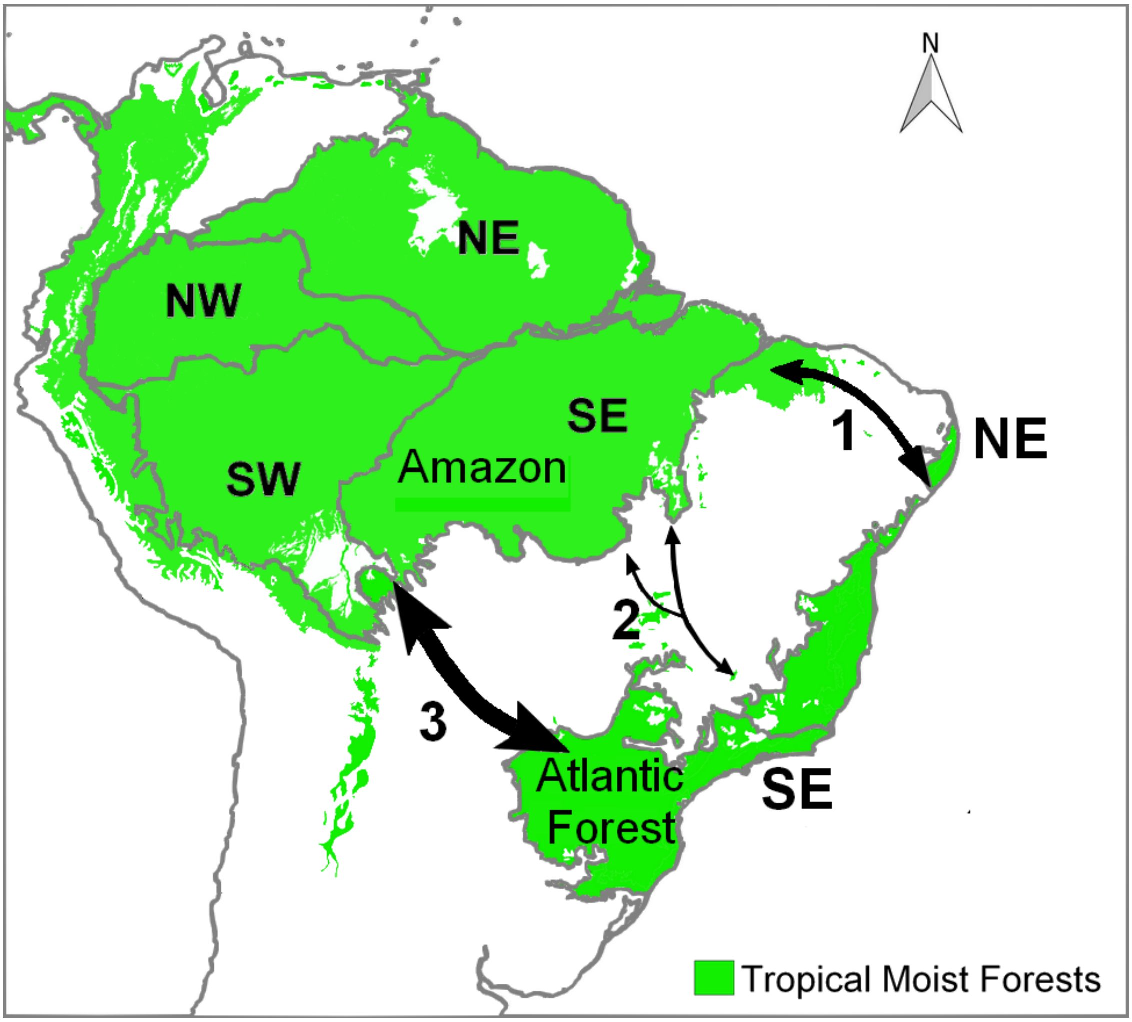

Figura 1. Rotas de conexão entre a Amazônia e a Mata Atlântica: 1) através da rota Nordeste (NE); 2) através da rota do Sudeste da Amazônia e Sudeste da Mata Atlântica Sudeste (SE-SE) (2); 3) através do Sudoeste da Mata Atlântica - Noroeste da Amazônia (SE-NW). A largura das setas representa a frequência das rotas propostas anteriormente na literatura (Por, 1992). Editado de Costa 2003.

As conexões biogeográficas entre a Amazônia e a Mata Atlântica foram recentemente foco de alguns estudos, porém poucos deles usaram os mamíferos como sistema de estudo e menos ainda considerando múltiplas espécies. Costa (2003) foi o principal estudo para mamíferos com esse foco, mais especificamente para pequenos mamíferos. Estudos que investigam essas conexões têm utilizado apenas a similaridade florística destes biomas (Oliveira-Filho & Ratter, 1995) ou relações filogenéticas e filogeográficas das espécies animais (Costa, 2003; Cortés-Ortiz et al., 2003; Batalha-Filho et al., 2014). Porém, diversos estudos recentes mostram que a integração entre a filogenética e ecologia tem auxiliado na compreensão dos eventos biogeográficos e processos evolutivos (ver revisão Alvarado-Serrano & Knowles, 2014).

Com o avanço dos sistemas de informação geográfica, acessando inúmeras variáveis ambientais em diversas escalas (Hijmans et al., 2005), tornou-se possível investigar os padrões de distribuição das espécies e avaliar o nicho ecológico delas (Phillips, 2006). Inserindo o contexto filogeográfico é possível investigar corredores de dispersão históricos entre grupos de espécies, como por exemplo, através de modelos de distribuição de espécies integrados aos dados genéticos (Kozak et al., 2008; Chan et al., 2011). Tais ferramentas, aplicadas em estudos que investiguem as conexões biogeográficas entre Amazônia e Mata Atlântica, podem auxiliar na compreensão das mudanças das florestas tropicais ao longo do tempo, e no teste de hipóteses sugeridas sobre as rotas de conexão, através de estudos comparativos entre espécies buscando compreender onde e quando as conexões entre esses biomas teriam ocorrido. Além disso, ferramentas utilizadas em macroecologia, a qual busca entender os mecanismos que geram os padrões encontrados em grandes escalas geográficas e temporais (Gaston & Blackburn, 2000), tem sido utilizadas com muito sucesso para a compressão dos padrões biogeográficos no Neotrópico (Maestri et al., 2016; Diniz-Filho et al., 2019; Villalobos et al., 2020), podendo ser extremamente útil para entender os padrões históricos dessas conexões.

Sendo assim, o objetivo desta tese foi investigar as conexões biogeográficas entre as florestas da Amazônia e Mata Atlântica através da biogeografia de espécies de mamíferos buscando compilar evidências dessas conexões para este grupo avaliando as hipóteses propostas para as rotas de conexão anteriormente sugeridas: 1) As conexões florestais entre esses biomas através da rota do Nordeste são mais recentes? 2) A rota do Sudoeste é a mais importante entre elas, sendo a mais antiga e mais frequente ao longo do tempo? Além disso, buscamos investigar as conexões históricas entre a Amazônia e a Mata Atlântica através da biogeografia de mamíferos testando hipóteses sugeridas por estudos anteriores em diferentes escalas espaciais e temporais (micro e macroecológicas) utilizando dados sobre a distribuição geográfica das espécies disponíveis e simulados, assim como dados genéticos intra e interespecíficos. Através de diferentes abordagens, buscamos reunir dados sobre espécies de mamíferos potenciais para investigar essas conexões, avaliando as principais rotas de conexão evidenciadas por elas. Avaliamos a filogeografia comparada de algumas espécies, estimando a distribuição atual assim como ao longo do passado recente e avaliamos os padrões espaço-temporais dessas conexões, conforme proposto por estudos prévios. Para isso, dividimos esta tese em três capítulos, sendo estes:

Capítulo 1: **Espécies de mamíferos potenciais para avaliar as rotas de conexão entre a Amazônia e a Mata Atlântica.**

Muitas evidências mostram que a Amazônia e a Mata Atlântica foram conectadas no passado por três rotas de conexão: pelas florestas do Nordeste do Brasil (conhecia como rota do Nordeste, conectando o Leste da Amazônia e o Nordeste da Mata Atlântica), pelo interior do Cerrado brasileiro (conectando o Sudeste da Amazônia e o Sudeste da Mata Atlântica) e pelas florestas da Bacia do Paraná (conectando o Oeste da Amazônia e o Sudeste da Mata Atlântica). A rota de conexão entre o oeste da Amazônia e o Sudeste da Mata Atlântica foi sugerida como a mais importante delas, mas essa hipótese ainda continua pouco testada. Neste capítulo realizamos um levantamento dos dados disponíveis sobre a distribuição geográfica das espécies de mamíferos com distribuição comum entre a Amazônia e a Mata Atlântica, a fim de explorar quais seriam as espécies potenciais para avaliar essas conexões. Este capítulo teve colaboração dos mastozoólogos: Dra. Maria João Ramos Pereira (PPG BAN/UFRGS) e Dr. Cleuton Lima Miranda (UFPA, UEMA, UFPI). Também avaliamos a hipótese sugerida anteriormente sobre a frequência dessas rotas e buscamos responder algumas perguntas, as quais serão detalhadas a seguir. Assim, apresentamos aqui uma lista de espécies de mamíferos potenciais para investigar essas conexões, avaliando as principais rotas de conexão e a sua relação com o uso do habitat. Para isso, compilamos dados de distribuição geográfica de espécies de mamíferos da Amazônia e Mata Atlântica, identificando suas prováveis rotas de conexão. O tipo de habitat de cada espécie foi classificado e utilizado para testar sua relação com o uso de cada uma das rotas de conexão. O número de dados genéticos disponíveis para essas espécies também foi compilado para verificar o potencial de cada espécie para estudos filogeográficos sobre as conexões biogeográficas entre essas florestas. Encontramos 127 espécies de mamíferos potenciais para investigar essas conexões e, diferentemente do proposto anteriormente, a rota do Nordeste foi a mais frequente. Também encontramos uma relação entre uso de habitat e os tipos de rotas de conexão. Além disso, encontramos uma quantidade moderada a alta de dados genéticos disponíveis para muitas dessas espécies (97), destacando seu potencial para estudos biogeográficos sobre as conexões entre a Amazônia e a Mata Atlântica, apoiando estudos futuros sobre as conexões entre a Amazônia e a Mata Atlântica.

Capítulo 2: **Conexões passadas recentes entre a Amazônia e a Mata Atlântica através de filogeografia comparativa e paleodistribuição de mamíferos didelfídeos.**

Apesar da aparente distribuição disjunta entre a Amazônia e a Mata Atlântica, as evidências sugerem rotas históricas de conexão entre essas florestas até o passado recente. Aqui, investigamos as conexões históricas entre populações de três espécies de mamíferos didelfídeos da Amazônia e da Mata Atlântica (*Caluromys philander*, *Marmosa murina* e *Marmosa demerarae*), a fim de descobrir as conexões históricas recentes entre essas florestas com uma abordagem filogeográfica comparada e de paleodistribuição dessas espécies. Este capítulo teve colaboração dos pesquisadores do INPA e da UFAM: Dra. Marina Anciães, Dra. Maria Nazareth da Silva, Dra. Izeni Pires Farias e do Msc. Mario Nunes; assim como do Doutor em genética da UFRGS: Willian Thomaz Peçanha. Esse estudo representa a primeira investigação sobre as conexões entre a Amazônia e a Mata Atlântica através da filogeografia e da paleodistribuição simultaneamente. Utilizando registros de ocorrência dessas espécies e variáveis bioclimáticas atuais, geramos modelos de distribuição de espécies (SDM), que foram projetados para o passado para prever as paleodistribuições dessas espécies desde o Holoceno até o Plioceno. Inferimos tempos de divergência entre populações de cada espécie por meio de sequências mitocondriais do gene do citocromo b, calibração fóssil e taxas de substituição. Alterações na paleodistribuição em relação ao presente foram avaliadas. Os resultados revelaram que todas as espécies mostram populações da Amazônia e Mata Atlântica divergindo entre 1 e 2 milhões de anos atrás, mas algumas também em períodos mais recentes. A paleodistribuição para a maioria dessas espécies foi maior para períodos anteriores aos tempos de divergência estimados, tornando-se menor após eles. Sugerimos que as oscilações climáticas durante o início do Pleistoceno influenciaram amplamente a estruturação filogenética dessas espécies que habitam a floresta, levando à separação de populações atualmente distribuídas na Amazônia e na Mata Atlântica. Encontramos uma congruência entre os resultados filogenéticos e de paleodistribuição, nunca avaliados simultaneamente anteriormente. Também mostramos que essas conexões ocorreram mais de uma vez ao longo do tempo. Encontramos conexões mais recentes para as rotas Nordeste e Sudeste. Este estudo também mostra que características biológicas e históricas específicas de cada grupo são fatores importantes a serem considerados na avaliação de respostas a mudanças climáticas passadas.

Capítulo 3: **Padrões espaço-temporais das conexões históricas entre a Amazônia e a Mata Atlântica.**

Apesar da aparente distribuição disjunta entre a Amazônia (AM) e a Mata Atlântica (AF), foram levantadas três hipóteses de conexão histórica entre essas florestas: pelas florestas do Nordeste do Brasil (rota NE), pelas florestas de galeria do Cerrado do Brasil Central e pelas florestas da Bacia do Paraná conectando o Sudeste da Mata Atlântica com o Oeste da Amazônia (rota SE-NW). Foi sugerido também que essas conexões teriam ocorrido em diferentes períodos, com conexões mais recentes para a rota NE e conexões mais antigas para a rota SE-NW. Neste capítulo, testamos a hipótese de variação temporal para as conexões AM-AF usando mamíferos neotropicais como sistema modelo. Este capítulo teve colaboração dos pesquisadores Dr. Gabriel Nakamura (PPG Ecologia/UFRGS) e Dra. Camila Duarte (Doutora em Ecologia pelo INPA e atualmente Pós-doutoranda na Universidade de Duisburg-Essen). Dados filogenéticos e espaciais foram combinados para gerar mapas espaço-temporais de conexões entre mamíferos AM e AF. Os tempos mínimos de divergência entre os táxons filogeneticamente mais próximos nas famílias de cada bioma foram extraídos de filogenias datadas e projetados no espaço geográfico usando distribuições de espécies para gerar tempos médios de divergência (ADT) para redes de 1º grau. A relação entre a rota NE e os tempos de rota SE-NW e ADT por grade entre AM e AF foi testada usando regressão linear considerando a autocorrelação espacial. Os resultados revelam tempos de divergência mais altos para redes considerando espécies da AM do que da AF, mas o padrão espaço-temporal sugerido não foi corroborado (exceto para a família Didelphidae na AM). No entanto, um padrão espaço-temporal oposto ao sugerido anteriormente foi encontrado para a AF, com tempos de divergência recentes em relação à rota SE-NW para a maioria das famílias. Além disso, cada família revelou conexões específicas entre o AM e o AF em momentos diferentes. Os tempos de divergência para os mamíferos AM revelaram-se mais antigos que os dos mamíferos AF, evidenciando o AM como o centro de origem dos mamíferos neotropicais. O padrão espaço-temporal oposto para a AF, no entanto, revela conexões recentes entre a AF do sudoeste e a Amazônia andina, destacando um papel importante da AF como fonte de dispersão. Devido à história biogeográfica de cada grupo nos neotrópicos, diferentes conexões foram encontradas em diferentes momentos. Portanto, as conexões entre a AM e a AF não podem ser explicadas por um único padrão espaço-temporal.

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CAPÍTULO 1

Potential mammal species to evaluate connection routes between the Amazonia and the Atlantic Forest

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Artigo preparado para submissão na revista *Mammalia*

Abstract

Much evidence shows that the Amazonia and the Atlantic Forest have been connected in the past through three connection routes: by the forests of Northeast Brazil (NE route) connecting Eastern Amazonia and Northeastern Atlantic Forest, by the interior of the Brazilian Cerrado (SE-SE route) and the forests of the Paraná Basin connection (SE-NW route). The SE-NW route was hypothesized as the most important of them, but it remains poorly evaluated. Here we present a list of potential mammal species to investigate these connections, evaluating the main connections routes and the relationship with habitat use. For this, we compiled geographic distribution data of mammal species occurring both in the Amazonia and the Atlantic Forest identifying their likely connection routes. The habitat type of each species was classified and used to evaluate its relationship with the use of connection routes. The number of genetic data available for these species has been compiled to verify the potential of each species for phylogeographic studies on the biogeographic connections between these forests. We found 128 species of potential mammals to investigate these connections and differently from what was proposed previously, the NE route was the most frequent route. We also found a relationship between habitat use and connection routes, with most routes are associated with species with more generalist habits. In addition, we found a large amount of genetic data available for many of these species, highlighting their potential for biogeographic studies on the connections between the Amazonia and the Atlantic Forest. Our results support future studies on the connections between the Amazonia and the Atlantic Forest for mammal species and bring new insights about the previously proposed on their connection routes.

Keywords: Biogeography, connection routes, Amazonia, Atlantic Forest, Neotropical Forests, Moist Tropical Rainforests, mammals.

Introduction

The Amazonia is among the largest tropical forests in the world, and such as the Atlantic Forest, it is also among the most diverse regions in the world (Mittermeier et al., 1998; Jenkins et al., 2013). The Amazonia and Atlantic Forest forests are apparently currently separated, but several studies show that they have been connected in the past, either by biogeographical studies (Por, 1992; Costa, 2003; Fouquet et al., 2012; Batalha-Filho et al., 2013; Sobral-Souza et al., 2015; Ledo & Coli, 2017), as well as geological (Wang et al., 2004) or palynological records (de Oliveira et al., 1999; Behling et al., 2000;

Auler et al., 2004). Several tree species from forests of the Brazilian Cerrado biome have a common distribution between the Amazonia and the Atlantic Forest (Oliveira-Filho & Ratter, 1995; Méio et al., 2003), and the same can be found for tree species from the Caatinga, the biome of northeastern Brazil (Oliveira-Filho & Ratter, 1995, Melo Santos, 2007), as well as from the deciduous forests from the Pantanal and the Chaco regions (Oliveira-Filho & Ratter, 1995).

The historical connections between the Amazonia and the Atlantic Forest allowed the dispersion and diversification of several animal species, which are evidenced through phylogeographic studies of mammals (Costa, 2003; Cortés-Ortiz et al., 2003; Nascimento et al., 2008; Pavan et al., 2011; Machado et al., 2018), birds (Batalha-Filho et al., 2013; Carvalho et al., 2017; da Silva et al., 2020; Moura et al., 2020), reptiles (Pellegrino et al., 2011; Prates et al., 2016), amphibians (de Sá et al., 2018) (for a literature revision see Ledo & Colli, 2017) and recently also for insects (Peres et al., 2017). Recently, plant researchers can also be found investigating these connections for vegetal species under an evolutionary approach (Thode et al., 2019). Still, few current studies have using multiple species to assess the suggested biogeographic patterns for these connection routes (Batalha-Filho et al., 2013; Ledo & Colli, 2017), and even less for mammals (Costa, 2003).

There were three suggested connecting routes between these forests, one through the forests of the Northeastern Brazil (previously called Northeast route [NE route] for connect the Eastern Amazonia and Northeastern Atlantic Forest), another through the gallery forest of the Brazilian Cerrado biome (which we will named here as the SE-SE route, since it would connected the Southeastern Amazonia with the Southeastern Atlantic Forest) and another through the forests of the Paraná Basin, Moist Chaco and Pantanal (called the Southeastern Atlantic Forest – Northwestern Amazonia route [SE-NW route]) (Por, 1992; Oliveira-Filho & Ratter, 1995; Costa, 2003) (Figure 1). According to Por (1992), the SE-NW connection route between the Amazonia and the Atlantic Forest would be the main one among the routes, being the oldest and consequently would have occurred more times over time, while the connection by the NE route would be the second most frequent, and the route through riverside forests in the interior of the Cerrado representing the SE-SE route would be the least frequent.

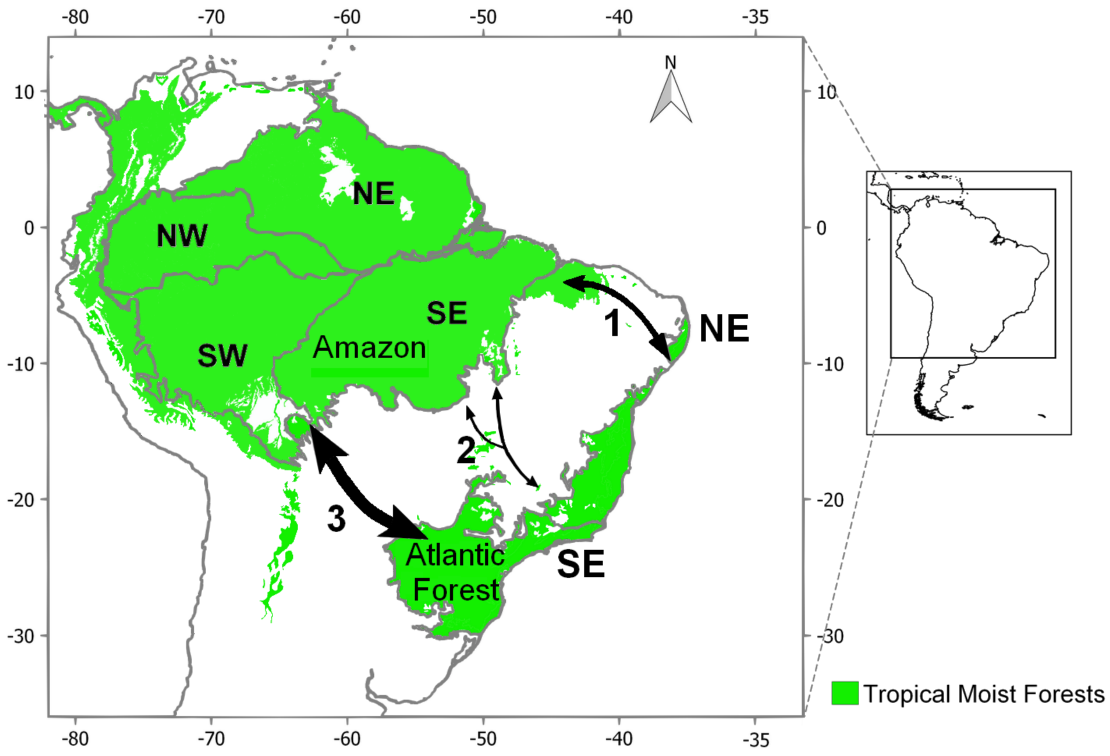

Figure 1. Distribution of tropical forests in South America. Tropical Moist Forests are shown in green and emphasize the Amazonia and the Atlantic Forest. Internal arrows represent connection routes between the Amazonia Forest and the Atlantic Forest through the Northeast/NE route (1), Southeast Amazonia – Southeast Atlantic Forest/SE-SE route (2) and the Southeast Atlantic Forest - Northwest Amazonia/SE-NW route (3). The width of the arrows represents the frequency of the routes proposed by Por (1992). Edited from Costa 2003.

Recently, a study aimed to test this hypothesis by reviewing the literature on the molecular evidence of connections between these forests for vertebrates, finding more studies that evidence connections by the SE-NW route compared to the NE route (Ledo & Coli, 2017). However, this result can be a sampling artifact due to poor sampling in the Northeast region (Carmignotto & Astúa, 2017) and then remains uncertain whether the SE-NW route was the most frequent. Batalha-Filho et al. (2013), for example, found a greater number of recent sister species of birds between the Amazonia and the Atlantic Forest for the NE route. However, these studies did not consider the SE-SE route as an independent route, including it as the Northeast route. Nevertheless, the SE-SE route has been well documented in the literature for both animals and plants (Por, 1992; Oliveira-Filho & Ratter, 1995; Silva, 1996; Ledru, 2002; Costa, 2003). For mammals, Costa (2003) found a larger number of small mammals related between the Amazonia and the Atlantic

Forest through the SE-SE route. Thus, it remains unclear which of these routes would have played a major role.

Here we aim to investigate the connections between the Amazonian and Atlantic forests through the geographical distribution patterns of forest mammal species with occurrence in both regions: 1) identifying the potential species to investigate these connections and the number of genetic data available for these species, 2) the main connection routes evidenced by these species, and 3) evaluating species-specific relationships regarding the use of the habitat of these species with the different routes evidenced by them. The central region of Brazil comprises the vast ecoregion of the Cerrado and the northeast region of Brazil is the ecoregion of the Caatinga, and it is where connections are found two of the three connection routes between the Amazonia and the Atlantic Forest. The Cerrado and the Caatinga are regions with high environmental heterogeneity (de Oliveira & Marquis, 2002; Nogueira Ferraz et al., 2003; Queiroz et al., 2017). The diversity of species is related to environmental heterogeneity (Schimidt et al., 2008), thus, regions considered to be of greater environmental heterogeneity may be associated with a greater diversity of species habits.

Based on that, here we seek to answer questions such as: What is the main connection route between the Amazonia and the Atlantic Forest for species of forest mammals? We also aim to evaluate the quantity of genetic data available for these species, quantifying the potential of each taxonomic group within mammals to assist future studies. What is the potential of these mammal species and within each taxonomic group to investigate these connections based on the availability of available genetic data? Is there a relationship between the type of habitat of the species and these connection routes? Since connection between the Amazonia and the Atlantic Forest via SE-SE route was considered the most important for small mammals (Costa, 2003), we would except to corroborate it considering all mammalian species. We assume that the habitat type of the mammal species will be related to the connection routes evidenced by them, with the generalist species showing all the connection routes together, as well as more present in each route separately; and the NE and SE-SE routes associated with different habitat types, assuming that the greater environmental heterogeneity of the ecoregions found in these routes allowed a greater diversity of species using these connection routes. We hope that the results of this study will assist future studies on the connections between the Amazonia and the Atlantic Forest, serving as the basis for specific biogeographic studies considering different mammalian taxonomic groups.

Material and Methods

Sampling species data

Geographical distribution maps of forest mammalian species from the Amazonia and the Atlantic Forest were compiled to evaluate the potential species to investigate the connection routes between these regions. We considered a potential mammal species to investigate these connection routes, delimiting the following criteria: species of forest mammals occurring in both the Amazonia and the Atlantic Forest regions. For this, distribution maps for all mammalian species from South America were downloaded from the IUCN - International Union for Conservation of Nature (IUCN, 2019).

To identify the mammalian species that occur in either the Amazonia and the Atlantic Forest, the IUCN maps were overlaid on the Ecoregions maps (Dinerstein et al. 2017), identifying the overlap with the Amazonia and Atlantic Forest limits using the *gIntersection* function of the R package 'rgeos' (Bivand & Rundel, 2013) in the R (R Core Team, 2019). Subsequently, only species with occurrence between these biomes were selected. The predefined identifications based on IUCN maps on the occurrence in both biomes have been revised using the Annotated List of Mammals in Brazil (Paglia et al., 2012). As our goal was to access forest connections between the Amazonia and the Atlantic Forest, we selected only forest species for this study accessing the IUCN habitat type of species using the function *rl_habitats* of the R package ‘rredlist’ v. 0.6.0 (Scott, 2020). Then, spatialized species exclusively in open areas and/or aquatic were not considered.

Connection routes

To identify the connection routes evidenced by each species of mammal selected on this study (Table 1), first the geographical areas of these connections were delimited using the ecoregion polygons (Dinerstein et al., 2017). The Northeast route (NE route) area was selected using the boundaries of the Caatinga ecoregions, transition areas between the Caatinga, North of the Cerrado and the East Amazonia, the Babaçu forests and adjacent dry forests, which represent transition areas between the Amazonia and the Atlantic Forest. The area of the SE-SE route was selected using the limits of the Cerrado ecoregion (excluding the North of the Cerrado area which were selected for the NE route), and the area of the SE-NW route was delimited using the boundaries of the Pantanal, humid Chaco ecoregions and transition areas between the Amazonia and the Pantanal (such as Chiquitano Dry Forests), South of the Cerrado and Southwest of the Atlantic Forest.

To evaluate the frequency of connection routes evidenced by the known distribution of forest mammal species from the Amazonia and the Atlantic Forest, the previously delimited areas for each connection route (NE route, SE-SE route and SE-NW route) were intersected with the species maps using the function *gIntersection* of the R package 'rgeos' (Bivand & Rundel, 2013), calculating the total number of species for each route and more than one route simultaneously. To visualize this result in the geographic space, we calculate the sum of rasters using the R package 'raster' (Hijmans, 2017).

Habitat classification

Habitat type categories were classified for each selected species generating a habitat preference scale based on the detailed text on species' Habitat and Ecology available in the IUCN database and additional reviews about habitat preference from the literature (e.g. Emmons & Feer, 1997; Eisenberg & Redford, 1999; Gardner, 2008; Patton et al., 2015; Nowak & Walker, 1999; Wilson & Mittermeyer, 2009; Luza et al., 2019), when the IUCN texts were not conclusive. The criterion used for the classification of the habitat type categories of the species was defined considering the following levels: forestry specialist (FS), preferably forestry (PF) or generalist (G). Forest specialists were considered species that use only forests, mainly forest species that use not only forest environments but prefer this environment, and generalist's species that use both forests and open environments (Table 1). We used a Pearson's Chi-squared test to assess the relationship among the different connection routes and the types of habitat of the species that occur in each route which tests the which is applied to categorical data to assess how likely it is that any difference observed between the data sets is random .

Genetic data

Genetic data available in the Genbank database (http://www.ncbi.nlm.nih.gov/GenBank/) were compiled for each species. For this, a search was performed for nucleotide sequences available by each taxon in this database. The compilation of these data was carried out during the month of January 2020, recording the number of molecular data available (sequences of DNA) for each species and the specific search access link for each taxon listed. These data were used in order to assess the taxonomic representativeness (it is, which taxonomic groups represent the highest availability of published genetic data) and, consequently, potentialities for studies evidencing on the connections between the Amazonia and the Atlantic Forest. For that, we divided the data (sequence numbers available by species) into four categories across the quartiles of that data, representing within the limits of the amount of genetic data available, limits to represent low, regular, satisfactory and large availability of genetic data.

Results

We compiled geographical distribution maps and habitat type for 127 species of mammals distributed between the forests of the Amazonia (AM) and the Atlantic Forest (AF), covering the Orders Didelphimorphia (7), Pilosa (9), Perissodactyla (1), Artiodactyla (1), Cetartiodactyla (2), Primates (3), Carnivora (12), Chiroptera (86) and Rodentia (9) (Table 1). According to the geographic distribution of these species by IUCN, 114 appear to be continuously distributed between the Amazonia and the Atlantic Forest and 14 have a disjunct distribution.

Table 1. Potential mammal species to investigate connections between the Amazonia and the Atlantic Forest based on IUCN expert species distribution maps. H = type of habitat (FS = forestry specialist; PF = preferably forestry; G = generalist); Routes = Connection routes between Amazonia and the Atlantic Forest based on these maps (0 = Unidentified route; 1 = Northeastern route [NE]; 2 = Southeastern route [SE-SE]; 3 = Southwestern route [SE-NW]); N = Total number of nucleotide DNA sequences available on Genbank online database.

Species	**Family**	**Order**	**H**	**Routes**	**N**
Tapirus terrestris	Tapiriidae	Perissodactyla	G	1, 2, 3	199
Mazama americana	Cervidae	Cetartiodactyla	PF	2, 3	79
Pecari tajacu	Tayassuidae	Cetartiodactyla	G	1, 2, 3	522
Tayassu pecari	Tayassuidae	Cetartiodactyla	G	1, 2, 3	57
Bradypus variegatus	Bradypodidae	Pilosa	SF	1, 2	580
Tamandua tetradactyla	Myrmecophagidae	Pilosa	G	1, 2, 3	195
Cyclopes didactylus	Cyclopedidae	Pilosa	PF	1	210
Cabassous unicinctus	Chlamyphoridae	Cingulata	G	1, 2, 3	60
Dasypus novemcinctus	Chlamyphoridae	Cingulata	G	1, 2, 3	118219
Dasypus septemcinctus	Chlamyphoridae	Cingulata	G	1, 2, 3	22
Myrmecophaga tridactyla	Myrmecophagidae	Cingulata	G	1, 2, 3	206
Alouatta belzebul	Atelidae	Primates	SF	1, 2	255
Alouatta caraya	Atelidae	Primates	PF	2, 3	555

Sapajus libidinosus	Cebidae	Primates	G	1, 2	92
Cerdocyon thous	Canidae	Carnivora	G	1, 2, 3	160
Panthera onca	Felidae	Carnivora	G	1, 2, 3	472
Eira barbara	Mustelidae	Carnivora	PF	2, 3	41
Galictis vittata	Mustelidae	Carnivora	G	1, 2	52
Leopardus pardalis	Felidae	Carnivora	G	1, 2, 3	356
Leopardus tigrinus	Felidae	Carnivora	G	1, 2	348
Leopardus wiedii	Felidae	Carnivora	PF	2, 3	310
Nasua nasua	Procyonidae	Carnivora	G	2, 3	84
Potos flavus	Procyonidae	Carnivora	SF	1, 2	102
Procyon cancrivorus	Procyonidae	Carnivora	G	1, 2, 3	30
Puma yagouaroundi	Felidae	Carnivora	G	1, 2, 3	213
Speothos venaticus	Canidae	Carnivora	G	2, 3	74
Anoura caudifer	Phyllostomidae	Chiroptera	G	2, 3	81
Anoura geoffroyi	Phyllostomidae	Chiroptera	G	1, 2, 3	1068
Artibeus concolor	Phyllostomidae	Chiroptera	G	1, 2	118
Artibeus lituratus	Phyllostomidae	Chiroptera	G	1, 2, 3	948
Artibeus obscurus	Phyllostomidae	Chiroptera	G	1, 2, 3	689
Artibeus planirostris	Phyllostomidae	Chiroptera	G	1, 3	861
Carollia brevicauda	Phyllostomidae	Chiroptera	G	1, 2	305
Carollia perspicillata	Phyllostomidae	Chiroptera	G	1, 2, 3	1697
Centronycteris maximiliani	Emballonuridae	Chiroptera	SF	1, 2	11
Choeroniscus minor	Phyllostomidae	Chiroptera	G	1	22
Chrotopterus auritus	Phyllostomidae	Chiroptera	PF	1, 2, 3	128
Cynomops abrasus	Molossidae	Chiroptera	SF	2	58
Cynomops greenhalli	Molossidae	Chiroptera	PF	1	3
Cynomops planirostris	Molossidae	Chiroptera	PF	1, 2, 3	62
Dermanura cinerea	Phyllostomidae	Chiroptera	G	1, 2	236
Dermanura gnoma	Phyllostomidae	Chiroptera	G	1, 2, 3	213
Diaemus youngi	Phyllostomidae	Chiroptera	G	1, 2, 3	42
Diclidurus albus	Emballonuridae	Chiroptera	G	1, 2, 3	6
Diphylla ecaudata	Phyllostomidae	Chiroptera	G	1, 2	44
Eptesicus brasiliensis	Vespertilionidae	Chiroptera	G	1, 2, 3	15
Eptesicus furinalis	Vespertilionidae	Chiroptera	G	1, 2, 3	74
Eumops auripendulus	Molossidae	Chiroptera	G	1, 2, 3	317
Eumops delticus	Molossidae	Chiroptera	G	1, 2	0
Eumops glaucinus	Molossidae	Chiroptera	G	2, 3	88
Eumops perotis	Molossidae	Chiroptera	G	1, 2, 3	29
Furipterus horrens	Furipteridae	Chiroptera	PF	1, 2	70
Gardnerycteris crenulatum	Phyllostomidae	Chiroptera	G	1, 2	107
Glossophaga soricina	Phyllostomidae	Chiroptera	G	1, 2	457
Histiotus velatus	Vespertilionidae	Chiroptera	G	1, 2, 3	0
Lasiurus blossevillii	Vespertilionidae	Chiroptera	G	1, 2, 3	68
Lasiurus cinereus	Vespertilionidae	Chiroptera	G	2, 3	588
Lasiurus ega	Vespertilionidae	Chiroptera	G	1, 2, 3	50
Diclidurus scutatus	Emballonuridae	Chiroptera	PF	0	5
Eptesicus diminutus	Vespertilionidae	Chiroptera	G	1, 2, 3	17
Lichonycteris degener	Phyllostomidae	Chiroptera	SF	1	0
Lonchorhina aurita	Phyllostomidae	Chiroptera	PF	1, 2	19
Lophostoma brasiliense	Phyllostomidae	Chiroptera	G	1	35

Lophostoma silvicolum	Phyllostomidae	Chiroptera	G	1, 3	444
Macrophyllum macrophyllum	Phyllostomidae	Chiroptera	G	1, 2, 3	30
Glyphonycteris sylvestris	Phyllostomidae	Chiroptera	PF	2	20
Micronycteris megalotis	Phyllostomidae	Chiroptera	G	1, 2, 3	140
Micronycteris minuta	Phyllostomidae	Chiroptera	G	1, 2, 3	50
Micronycteris schmidtorum	Phyllostomidae	Chiroptera	G	1	15
Lasiurus egregius	Vespertilionidae	Chiroptera	SF	3	17
Mimon bennettii	Phyllostomidae	Chiroptera	G	1, 2	10
Molossops mattogrossensis	Molossidae	Chiroptera	G	1, 2	3
Molossops neglectus	Molossidae	Chiroptera	G	3	12
Molossops temminckii	Molossidae	Chiroptera	G	1, 2, 3	12
Molossus currentium	Molossidae	Chiroptera	G	3	15
Micronycteris hirsuta	Phyllostomidae	Chiroptera	G	0	50
Molossus molossus	Molossidae	Chiroptera	G	1, 2, 3	710
Molossus rufus	Molossidae	Chiroptera	G	1, 2, 3	127
Myotis albescens	Vespertilionidae	Chiroptera	G	1, 2, 3	71
Myotis nigricans	Vespertilionidae	Chiroptera	G	1, 2, 3	72
Myotis riparius	Vespertilionidae	Chiroptera	G	1, 2, 3	89
Molossus pretiosus	Molossidae	Chiroptera	G	2, 3	13
Noctilio albiventris	Noctilionidae	Chiroptera	G	1, 2, 3	365
Noctilio leporinus	Noctilionidae	Chiroptera	G	1, 2, 3	336
Nyctinomops laticaudatus	Molossidae	Chiroptera	G	1, 2, 3	22
Nyctinomops macrotis	Molossidae	Chiroptera	G	1, 2, 3	30
Myotis simus	Vespertilionidae	Chiroptera	G	1, 3	6
Nyctinomops aurispinosus	Molossidae	Chiroptera	G	1, 2	25
Peropteryx kappleri	Emballonuridae	Chiroptera	G	1, 2	18
Peropteryx leucoptera	Emballonuridae	Chiroptera	G	1	13
Peropteryx macrotis	Emballonuridae	Chiroptera	G	1, 2, 3	34
Phylloderma stenops	Phyllostomidae	Chiroptera	G	1, 2	40
Phyllostomus discolor	Phyllostomidae	Chiroptera	G	1, 2, 3	35825
Phyllostomus elongatus	Phyllostomidae	Chiroptera	SF	1, 2	217
Phyllostomus hastatus	Phyllostomidae	Chiroptera	G	1, 2, 3	90
Platyrrhinus lineatus	Phyllostomidae	Chiroptera	G	1, 2, 3	68
Promops centralis	Molossidae	Chiroptera	G	3	26
Promops nasutus	Molossidae	Chiroptera	G	2, 3	2
Pteronotus personatus	Mormoopidae	Chiroptera	G	1, 2	276
Pygoderma bilabiatum	Phyllostomidae	Chiroptera	SF	2, 3	15
Rhinophylla pumilio	Phyllostomidae	Chiroptera	SF	1, 2	430
Rhogeessa hussoni	Vespertilionidae	Chiroptera	SF	1, 2	0
Rhogeessa io	Vespertilionidae	Chiroptera	G	1, 2	44
Rhynchonycteris naso	Emballonuridae	Chiroptera	PF	1, 2, 3	179
Saccopteryx bilineata	Emballonuridae	Chiroptera	G	1, 2	317
Saccopteryx leptura	Emballonuridae	Chiroptera	G	1, 2, 3	94
Sturnira tildae	Phyllostomidae	Chiroptera	PF	1, 2	558
Thyroptera tricolor	Thyropteridae	Chiroptera	SF	1	234
Trachops cirrhosus	Phyllostomidae	Chiroptera	PF	1, 2	425
Uroderma bilobatum	Phyllostomidae	Chiroptera	G	1, 2	304
Uroderma magnirostrum	Phyllostomidae	Chiroptera	PF	1, 2	20
Caluromys philander	Didelphidae	Didelphimorphia	PF	1, 2	89
Chironectes minimus	Didelphidae	Didelphimorphia	PF	2	24

Caluromys lanatus	Didelphidae	Didelphimorphia	PF	2, 3	34
Marmosa demerarae	Didelphidae	Didelphimorphia	PF	1, 2	133
Marmosa murina	Didelphidae	Didelphimorphia	PF	1	203
Metachirus nudicaudatus	Didelphidae	Didelphimorphia	PF	2, 3	123
Monodelphis americana	Didelphidae	Didelphimorphia	SF	1, 2	201
Coendou prehensilis	Erethizontidae	Rodentia	PF	1, 2, 3	41
Cuniculus paca	Ctenomyidae	Rodentia	SF	1, 2, 3	103
Dasyprocta leporina	Dasyproctidae	Rodentia	PF	2	80
Dasyprocta prymnolopha	Dasyproctidae	Rodentia	G	1, 2	0
Hydrochoerus hydrochaeris	Caviidae	Rodentia	G	1, 2, 3	140
Hylaeamys megacephalus	Cricetidae	Rodentia	SF	2	428
Nectomys rattus	Cricetidae	Rodentia	G	1, 2	16
Oecomys trinitatis	Cricetidae	Rodentia	SF	1, 2	7
Guerlinguetus aestuans	Sciuridae	Rodentia	SF	1, 2	21

Regarding the connection routes evidenced by the IUCN geographical distribution maps of these mammalian species to investigate the forest connections between the Amazonia and the Atlantic Forest, 54 of the total species are distributed through the previously connection routes simultaneously (NE, SE-SE and SE-NW routes), 37 of them between both NE and SE-SE routes, 14 between the SE-SE and SE-NW routes, nine between the NE route, five between the SE-SE route, four between the SE-NW, three between the NE and SE-NW routes. The most frequent connection routes were the NE route followed by the SE-SE route (Figure 2). Two species had their connection route unidentified such as chiropterans (bats) of the species *Diclidurus scutatus* and *Micronycteris hirsuta* that present an extremely disjunct distribution between Amazonia and Atlantic Forest (Table 1).

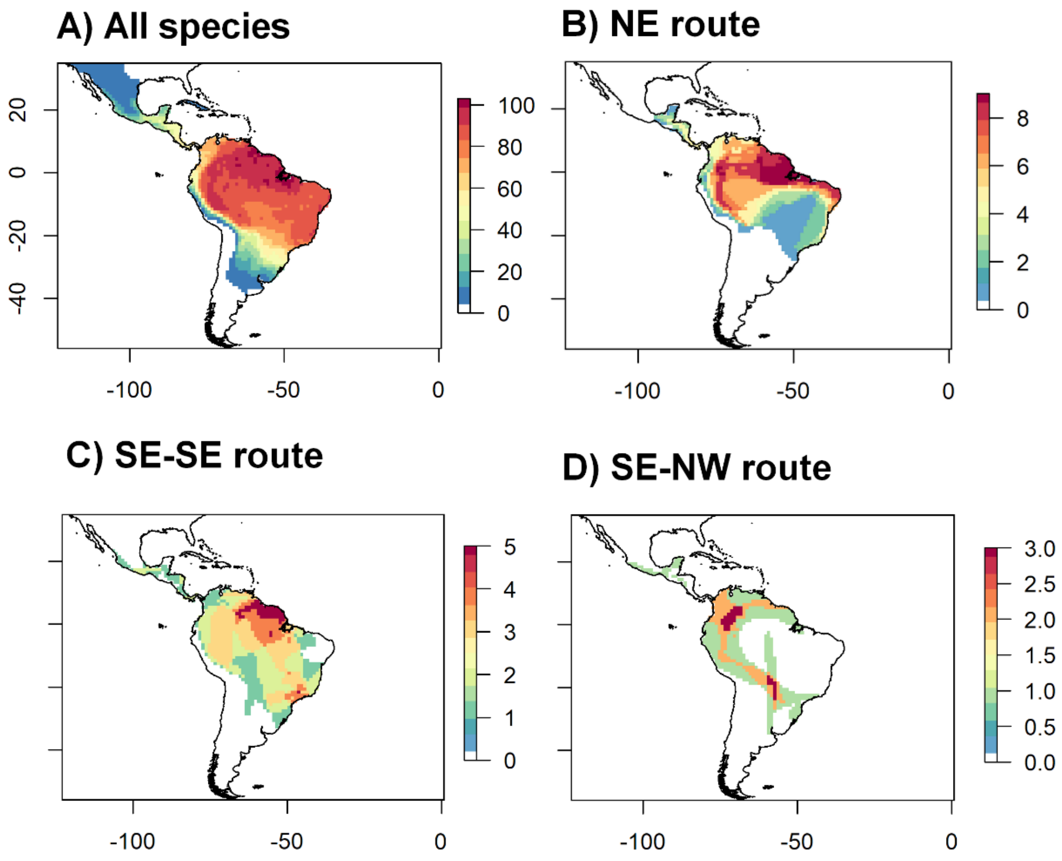

Figure 2. Overlaying the distribution maps of potential mammal species to investigate the connections between the Amazonia and the Atlantic Forest showing more connections for the Northeast route (NE route), followed by the Southeast route (SE-SE) and the smallest number of species for the Southwest route (SE-NW). The color scale represents the number of species per pixel on the map. A) Overlapping the species distribution maps of all mammal species sampled in this study; B) Overlapping species distribution maps of species that show connections by the NE route; C) Overlapping species distribution maps of species that show connections by the SE-NW route.

About the habitat type, 17 species were classified as Strictly Forestry (SF), 23 of them as Predominantly Forestry (PF) and 88 as Generalist (G) (Figure 3). For the NE route, two species were SF, three PF and four G. For the SE-SE route, two species were SF, two PF and no one G. For the SE-NW route, two species were G, one SF and no one PF. For NE and SE-SE routes together, 10 species were SF, seven PF and 20 G. For the three connection routes together, one species was SF, four PF and 49 G. For the NE and SE-NW routes together, three species were G and no one SF or PF. For the SE-SE and SE-NW routes together, one species was SF, five PF and eight G (Figure 3 and Table 1).

The result of the chi-square test for the habitat type was significant (X-squared = 35.904, df = 12, p-value = 0.0003357) rejecting the null hypothesis that the habitat type is independent of the type of connection route.

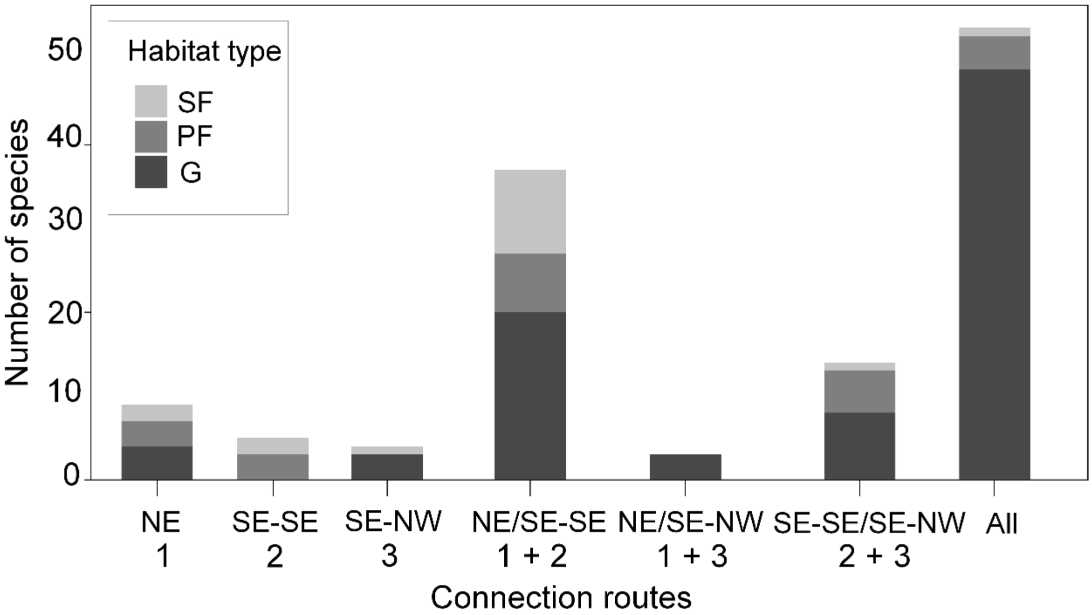

Figure 3. Frequency of connection routes between the Amazonia and the Atlantic Forest evidenced by mammalian species geographical distribution. Routes are represented in x axis: NE = Northeast route, SE-SE = Southeast – Southeast route, SE-NW = Southeast - Northwest route, and combinations of routes with bar. Colors fill represent the habitat type of species in each route or combinations: FS = Forestry Specialist; PF = Preferably Forest and G = Generalist.

Most of the species identified in this study as potential to assess the connections between AM and AF show a large amount of genetic data available in the investigated database including different molecular markers. Most species have sufficient genetic data, with only 30 out of 127 species with little genetic data available (Figure 4). Cingulata was the Order with greater available genetic data, following by Carnivora and Chiroptera (Figure 5). The orders of Cetartiodactyla, Primates, Perissodactyla, Perissodactyla and Didelphimorphia also showed a considerable number of available genetic data (with at least 100 sequences in proportion to the number of species), as well as other order sampled here such as Rodentia (with about 90 sequences by species) and Artiodactyla (with 79 for the only species sampled) (Figure 5).

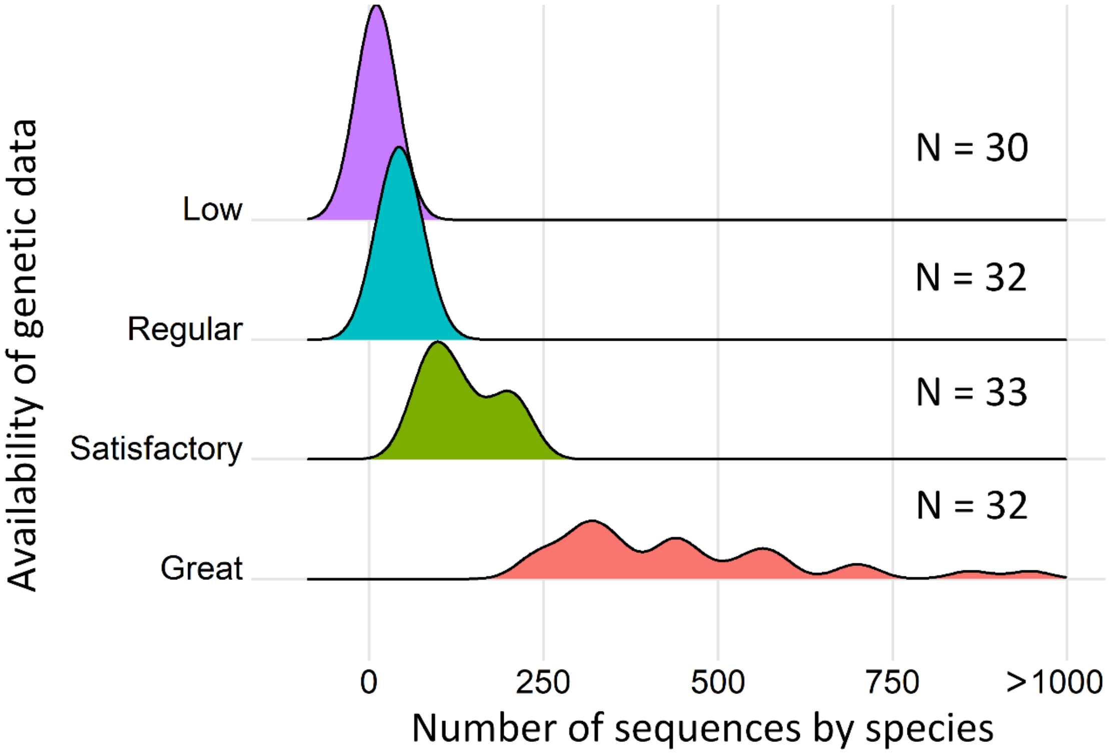

Figure 4. Availability of genetic data by mammalian species with both occurrence between Amazonia and Atlantic Forest divided between availability categories: Low = species with low availability of genetic data (from 0 to 22 available DNA sequences), Regular = species with regular availability of genetic data (from 23 to 74), Satisfactory = species with availability of genetic data satisfactory (from 75 to 225), and Great = species with high availability of genetic data (from 226 to more than 1000 available DNA sequences).

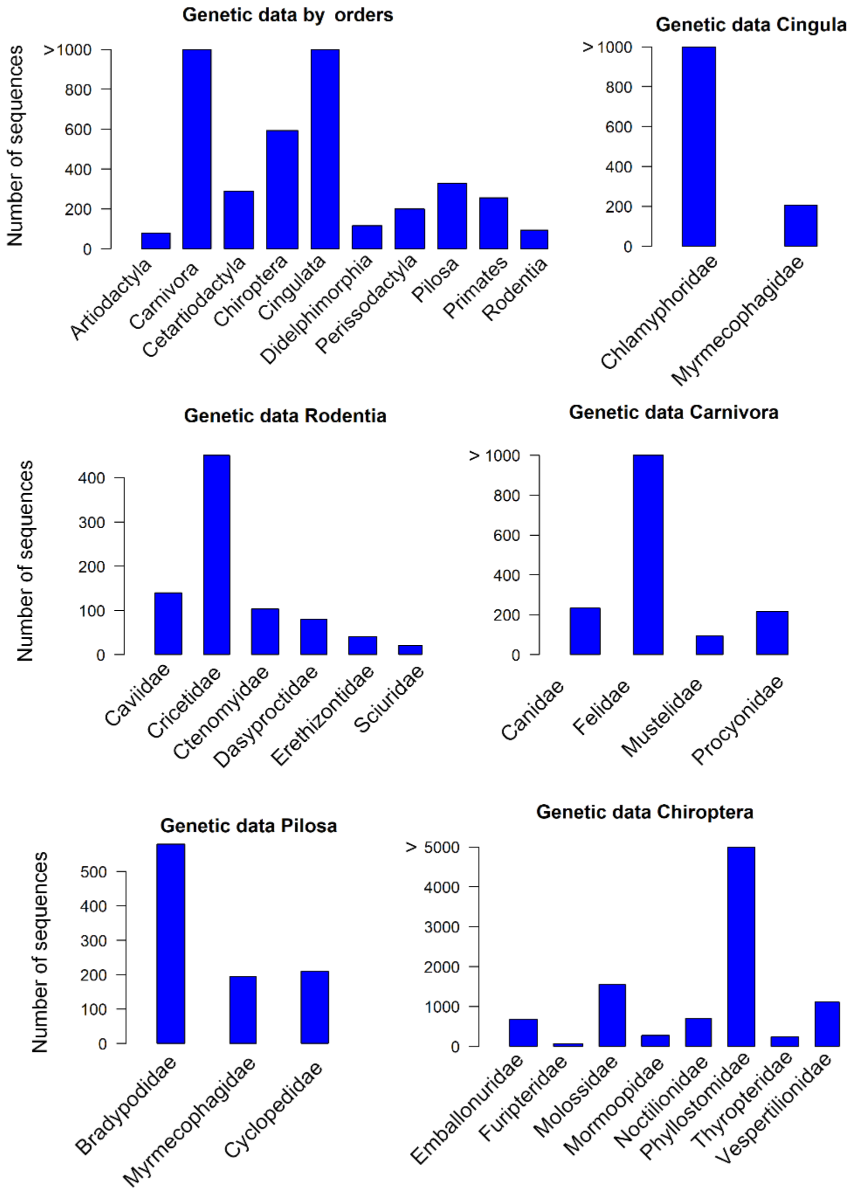

Figure 5. Availability of genetic data by mammalian orders and families of each order considering species with both occurrence between Amazonia and Atlantic Forest.

Discussion

Here we present a unprecedented list of potential mammal species to investigate the forest connections between the Amazonia and the Atlantic Forest, evaluating the routes evidenced by them, the association between the habitat use of the species and the use of the routes, including also a compilation of the quantity of genetic data available to further investigate these connections for these species. We evaluated the previously suggested hypothesis about the representativeness of the connection routes between these biomes. Unlike what was previously proposed, we found a greater number of connections for the NE route.

According to Por (1992), the SE-NW connection route between the Amazonia and the Atlantic Forest is the main one, while the connection through the NE route is the second, and the route through the riverine forests in the interior of the Cerrado biome (SE-SE route) is the minor. This hypothesis would have been confirmed by Ledo & Coli (2017). The only previous study that compiled data for multiple mammal species evaluating these connection routes was Costa (2003) for the phylogeography of small mammals, having found the SE-SE route as the most frequent. However, our results show that the NE route is the most frequent compared to the SE-NW and SE-SE routes. In fact, paleo vegetation, pollen data and biogeographic approaches have shown evidences of past connections between northern Atlantic Forest and eastern Amazonia (Andrade-Lima, 1982; Behling et al., 2000; Auler & Smart, 2001; Auler et al., 2004; Wang et al., 2004; Melo Santos, 2007; Pellegrino et al., 2011; Fouquet et al., 2012), corroborating the results of the present study for mammals. When evaluated together, that is, many species show more than one of these routes at the same time, the NE and SE-SE routes are more frequent, as well as for all routes together. Sampling artifacts in the dry diagonal in the Northeast, in addition to the non-inclusion of points of records published for the region or available from specimens deposited in mammal collections (Carmignotto & Astúa, 2017) would be the main factors for underestimating potential taxa that would suit the NE route evidenced in the present study. Therefore, with increased sampling and systematic and biogeographic studies that include this region, the number of species presented here may still increase (e.g. Gregorin, 2006; Pavan et al., 2011; Viana et al., 2015).

We found the number of potential mammalian species to investigate the connections between Amazonia and Atlantic Forest is slightly underestimated given the current knowledge about species distribution, especially for the NE route. Besides that, this number may still increase, since the hypotheses of disjunct distribution for various mammalian species between these regions (e.g. Emmons & Feer, 1997; Eisenberg & Redford, 1999; Gardner, 2008; Patton et al., 2015) have been refuted or new records has raised the possibility of reassessment. Species with possibility of insertion in the NE route in the future, such as the anteater *Cyclopes didactylus*, the Kinkajou *Potos flavus* and the squirrel *Guerlinguetus brasiliensis* still need to be reevaluated (Coimbra et al., 2017; de Vivo & Carmignotto, 2015; respectively). The taxonomic status of the guariba *Alouatta* *ululata*, previously a subspecies of *A. belzebul* and elevated to the species category by Gregorin (2006) based on morphological data, needs to be reevaluated as suggested by Viana et al. (2015) through molecular and cytogenetic approach. Miranda et al. (2005, 2009) performed unprecedented records of typically forest species with distribution to the Amazonia and Atlantic Forest or predominantly to the Amazonia in the northern state of Piauí, such as the marsupials *Marmosa demerarae*, *Metachirus nudicaudatus* and *Didelphis marsupialis* (personal obs. C.L. Miranda). The rodent *Oecomys catherinae*, considered typical from Atlantic Forest, has been recorded in the NE route (Gurgel-Filho et al., 2015; Malcher et al., 2017). Even so these species and probably others were not included in this study because the IUCN has not yet updated its data according to these studies. The Black-eared opossum *D. marsupialis* has a disjunct distribution, but recent records point to no disjunction in NE route (e.g. Gurgel-Filho et al., 2010; Miranda et al., 2009; Vieira & Oliveira, 2020). The Three-striped Short-tailed opossum, *Monodelphis americana*, is also present in the eastern Amazonia and in the Northeastern Atlantic Forest, with the other populations to the south of the São Francisco River being other species (Pavan et al., 2011).

We also found that many mammalian species seem to be distributed continuously between the Amazonia and the Atlantic Forest through deciduous and semi-deciduous forests in the interior of the dry diagonal (composed by the Caatinga, Cerrado and Chaco arid) according to the species distribution maps from IUCN. However, many phylogeographic studies reveal that some of these species considered to be continuously distributed among these biomes, are populations that are currently isolated (e.g. Moraes-Barros & Arteaga, 2015) or have a considerable degree of genetic divergence requiring their taxonomic status to be reviewed, and represent species to describe and/or revalidate or even evolutionary lineages with a well-structured and phylogeographically well-structured degree of genetic divergence (e.g. Costa, 2003; Machado et al., 2015; Patton et al., 2000; Voss et al., 2019). However, phylogeographic studies are necessary to reveal whether the populations of these species are currently connected or isolated due to the current level of forest fragmentation which continues to increase due to the habitat loss and failures in the environmental protection system for the connecting ecoregions between the Amazonia and the Atlantic Forest (Gonçalves-Souza et al., 2020).

We found a relationship between the type of habitat of the species and the connection routes between the Amazonia and the Atlantic Forest, with most routes are associated with species with more generalist habits, except for the SE-SE route, which is represented only by species of forest habit. This result differs from what we had expected considering the high environmental heterogeneity for the region of this route, which comprises the Cerrado region (de Oliveira & Marquis, 2002). We expected to find a greater variety of species habitat use on routes considered to be of greater environmental heterogeneity. However, this was corroborated for the NE route, located in the Caatinga, a region of high environmental heterogeneity (Nogueira Ferraz et al., 2003; Queiroz et al., 2017), where we find a greater variety of species habitat use. Farther, it would be very interesting to make use of this list of species disclosed here to evaluate these forest connections by comparing different sampling methods and evaluating the differences in the patterns observed considering the specific ecological characteristics of each group.

As highlighted by Costa (2003), the forest environments in the Cerrado of central Brazil would function as ecological (Fonseca & Redford, 1982) and historical corridors, allowing typically forest species from forest environments to be present in the region, forming a connection route that until then had not been named (which we called SE-SE route), but considered as an independent route by several studies (Por, 1992; Oliveira-Filho & Ratter, 1995; Silva et al., 1996; Costa, 2003). It is important to highlight that in addition to the riparian forests in the northeastern open diagonal, on the NE route, there is a marked presence of other types of forest habitats that could also perform the same function as babassu forests, semi-deciduous forests, mangroves and Brejos de Altitude (Miranda et al., 2009), requiring specific studies for the species that show the NE route to better evaluate this hypothesis.

The results of the availability of genetic data for these species reveal many of them showing enough data to assess the connections between the Amazonia and the Atlantic Forest. Although we show here only the total sequences available for each species, requiring a more detailed evaluation for the selection of these species for future studies, this initial compilation can be extremely useful as it facilitates the process of reviewing potential species to assess these connections, stimulating phylogeographic studies for mammals focusing on these connections. The presented list of potential mammalian species to investigate connections between the Amazonia and the Atlantic Forest allow us to evaluate these connections through a comparative phylogeography approach and paleodistribution models of didelphid marsupials (Machado et al., *in prep*). However, it is important to highlight that, although many of these species have a large amount of available genetic data, unfortunately it is common for many of the sequences to contain missing information, which can make the use of many of them unfeasible. Even so, many recent studies provide complete genetic data for mammals favoring the development of these studies (Gutiérrez et al., 2010; Faria et al., 2017; Machado et al., 2019; Voss et al., 2019). Therefore, the results presented here, not only help in the knowledge about the mammal species that show the connections between the humid Neotropical forests, but also bring new insights about the previously proposed about the connection routes between the Amazonia and the Atlantic Forest.

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CAPÍTULO 2

Recent past connections between Amazonia and Atlantic Forest seen through comparative phylogeography and paleodistribution of didelphid mammals

Artigo preparado para submissão na revista *Journal of Biogeography*

Abstract

Despite the apparent disjunct distribution between the Amazonia and the Atlantic Forest, evidence suggests historical connection routes between these forests until the recent past. Here we investigate the historical connections between populations of three didelphid mammal species from the Amazonia and the Atlantic Forest (*Caluromys philander*, *Marmosa murina* and *Marmosa demerarae*) in order to uncover the recent past connections between these Neotropical rainforests with a comparative phylogeography approach and evaluated the paleodistribution for the three species. This represents the first simultaneous investigation on the connections between the Amazonia and the Atlantic Forest through phylogeography and paleodistribution. Using occurrence records of these species and current bioclimatic variables, we generated species distribution models (SDM), which were projected to predicted paleoclimates from the Holocene to the Pliocene. We inferred divergence times between populations of each species via mitochondrial sequences of the cytochrome b gene, fossil calibration and substitution rates. Changes in paleodistribution in relation to the present were evaluated. The results revealed that all species shows populations from the Amazonia and Atlantic Forest diverging between 1 and 2 million years ago, but some also in more recent periods. Paleodistribution projected for most of these species were wider for periods before the estimated divergence times, becoming narrower in more recent times. We suggest that the climatic oscillations during the onset of the Pleistocene largely influenced the phylogenetic structuring of these forest dwelling species, leading to the separation of populations of taxa currently distributed in the Amazonia and the Atlantic Forest. We found a congruence between the phylogenetic and paleodistribution results, which had not been evaluated simultaneously before. We also show that these connections have occurred more than once over time. We found more old connections for both the Northeastern and Southeastern routes, but younger connections only for the Southeastern route. This study also shows that specific characteristics and history of each group are important factors to be considered when assessing responses to past climate changes.

Keywords: Historical biogeography, connection routes, Amazonia, Atlantic Forest, Neotropical rainforests, species distribution modeling, divergence times, mammals.

Introduction

Amazonian and Atlantic forests represent the largest tropical forests in the Americas, being among the most diverse regions in the world (Mittermeier et al., 1998; Jenkins et al., 2013). Despite the apparent disjunct distribution between them, the mosaic of forests in the interior of the Cerrado, Caatinga and Chaco arid biomes, which form the so-called 'dry diagonal', is considered a bridge of historical connection between these forests (Por, 1992; Oliveira-Filho & Ratter, 1995). Several tree species from the Cerrado forests have a common geographical distribution between the Amazonia and Atlantic Forest, and the same is true for some Caatinga species (Oliveira-Filho & Ratter, 1995; Melo Santos et al., 2007). Recently, some biogeographical studies have also shown these connections through phylogenetic relationships and reconstruction of ancestral area between plants (Terra-Araujo et al., 2015; Thode et al., 2019). Accordingly, connections are also evidenced through the intra and interspecific relationships between the fauna of the Amazonia and Atlantic Forest, such as found in birds (Batalha-Filho et al., 2013; Batalha-Filho et al., 2014; Carvalho et al., 2017; da Silva et al., 2020; Moura et al., 2020), mammals (Cortés-Ortiz et al., 2003; Costa, 2003; Nascimento et al., 2008; Pavan et al., 2011), reptiles (Pellegrino et al., 2011; Prates et al., 2016), amphibians (Fouquet et al., 2012; de Sá et al., 2018) and including insects (Peres et al., 2017). These evidences showed connections occurring in different periods over time, including both older connections as before the Miocene (Pellegrino et al., 2011; Fouquet et al., 2012; Batalha-Filho et al., 2013; Machado et al., *in review*) and recent connections during the Pleistocene (Costa, 2003; Nascimento et al., 2008; Batalha-Filho et al., 2013; Prates et al., 2016). Phylogeographic studies for most mammal species tend to show more recent connections, associated with forest expansions and retractions caused by interglacial and glacial cycles during the Quaternary (Costa, 2003; Nascimento et al., 2008; Pavan et al., 2011).

To explain the connections observed in fauna and flora between the Amazonia and Atlantic Forests, three historical routes have been suggested: one through the forests of the Paraná Basin passing to the southwest of the Brazilian Cerrado, Pantanal and Moist Chaco biomes named Southeastern-Northwestern route (SE-NW); a second through gallery forests of the Cerrado; and a third through Northeast Brazil via Caatinga forests named Northeastern route (NE) (Por, 1992; Oliveira-Filho & Ratter, 1995; Silva et al., 1996; Costa, 2003). These last two corridors would be related to the expansion of the gallery forests and wetlands during the Pleistocene climate oscillations (Batalha-Filho et al., 2013). Palynological and speleological data reveal short but recurring periods of rainfall intensification for the current northeastern semi-arid region of Caatinga during the Pleistocene and Holocene (de Oliveira et al., 1999; Behling et al., 2000; Auler et al., 2004; Wang et al., 2004). Some of these evidences suggest that during periods of strong rainfall in the Caatinga, two connecting routes between the Amazonia and the Atlantic Forest would have been established: one near to the coast of northeastern Brazil, probably through the highland swamps and another in a transition area of the Cerrado biome, through the seasonal forests of Chapada Diamantina (Oliveira et al., 1999). Paleogeographic studies indicate an increase in savannas during the late Miocene and early Pleistocene in the Cerrado due to the marked reduction in rainfall (Hoorn et al., 2011; Ledru, 2002). For Chaco/Pantanal, there is evidence of range expansion for forest plant species in at least two periods: during the Last Interglacial (ca. 130-150 kya), for plants adapted to warm climate and during the Last Glacial Maximum (ca. 18-21 kya) for plants adapted to cold climate (Ledo & Coli, 2017).

Studies focusing on the biogeographic connection between the Amazonia and the Atlantic Forest have used the floristic similarity of these biomes (Oliveira-Filho & Ratter, 1995; Méio et al., 2003), phylogeographic patterns and phylogenetic relationships of animal species (Costa, 2003; Batalha-Filho et al., 2014), or species distribution models (SDMs) (Sobral-Souza et al., 2015; Ledo & Coli, 2017) independently. However, several studies show that the integration between ecology and evolution has helped to understand biogeographic patterns and processes (Graham et al., 2004; Anciães & Peterson, 2009; Carnaval et al., 2009; Carnaval et al., 2014; Gutiérrez et al., 2014; Machado et al., 2019). With the advance of geographic information systems and increased availability of molecular data, it becomes possible to access numerous environmental variables at many scales and investigate the geographic distribution patterns of species, estimating their ecological niches and inserting the phylogenetic context (Kozak et al., 2008; Chan et al., 2011; Alvarado-Serrano & Knowles, 2014; Hoban et al., 2019). Such tools, applied in studies on the historical connections between the Amazonia and the Atlantic Forest, can help to understand the changes incurring in tropical forests and the biogeographic routes between these biomes over time.

Thereby, the aim of this study is to investigate recent past connections between the Amazonia and the Atlantic rainforests through reconstructing the historical connections between populations of didelphid mammal species from these regions using comparative phylogeography coupled with paleodistribution modeling that allow predicting changes in geographical distributions over time. Previously, studies about connections between these biomes used either molecular data, geographic distribution data or paleodistributions rather independently, but never integrated into the same framework (but see Batalha-Filho et al., 2013 for a macroevolutionary scale focus on these connections; and also see da Silva et al. 2020 for an integrative framework although without a direct focus on these connections). Thus, we propose for the first time to evaluate historical connecting routes between the Amazonia and the Atlantic Forest through a comparative approach integrating molecular data and SDMs for mammals. Previous studies suggested recent connection routes through the Caatinga forests (Northeast) and older routes to the Chaco/Pantanal region (Southwest) (Batalha-Filho et al., 2013; Sobral-Souza et al., 2015; Ledo & Coli, 2017). However, recent studies revealed conflicting results with those that previously predicted retraction of forests for the Last Glacial Maximum (LGM) in South America (Haffer, 1969), such as expansion of dry forests or even wet forests on this continent (Leite et al., 2016; Costa et al., 2017). As such, we also test for scenarios of forest expansion during the LGM, besides the Holocene (ca. 6 kya) and previous periods in the history of forest mammal species.

The specific questions we address in this study were: 1) When did these connections and disruptions between the Amazonia and the Atlantic Forest occur for the populations of the species investigated here? 2) In which past periods did expansions or retractions in the distribution of specialist forest species occurred, revealing connections and disruptions between the Amazonia and the Atlantic Forest? 3) Are the answers to these questions congruent when evaluated by different approaches, such as phylogenetic and ecological niche modelling? We are also interested in assessing in which regions these connections may have occurred in view of the alternative previously suggested connection routes between the Amazonia and the Atlantic Forest. The present study aims to answer these questions by evaluating the comparative phylogeography and paleodistribution between species of forest mammals that occur between the Amazonia and the Atlantic Forest. Previous studies on the historical connection routes between these biomes indicate that the Northeast route (through the Caatinga forests) would be more recent than the South route (through the Paraná Basin forests) (Por, 1992; Batalha-Filho et al., 2013; Ledo & Coli, 2017), then we expect to find more recent connections to the Northeast. We then expect to find congruence between the results revealed by the phylogeographic and paleoecological approaches, revealing expansions in the paleodistributions for periods prior to the phylogenetic divergences, followed by retractions, which would be evidence of periods of connection and separation between the Amazonia and Atlantic Forest.

Materials and Methods

Study area

The study area covers the area of distribution of Neotropical forests, encompassing the humid tropical forests of this region, focusing on the Amazonia and the Atlantic Forest , as well as on Dry Seasonal Forests that occur between these inland these biomes: Cerrado, Caatinga and Chaco/Pantanal in South America. The Amazonia and the Atlantic Forest represent the main moist tropical forests of South America distributed mainly in Brazil. Currently, these forests have disjointed distribution in the region, isolated by the 'dry diagonal' which is composed of the open area biomes of the Cerrado, Caatinga and Chaco arid. Although the dry diagonal restricts the current spread between the Amazonia and the Atlantic Forest for much of the typical biodiversity of rainforests, Seasonally dry forests located inland (such as Deciduous and Semideciduous Forests; and riparian forests or gallery forests) are considered bridges of historical connection between these biomes (Por, 1992; Costa, 2003; Batalha-Filho et al., 2013).

Species sampled

We selected three forest species of didelphid marsupials occurring between the Amazonia and the Atlantic Forest to investigate recent past connections between these forests through phylogeography and paleodistribution. These species are *Caluromys philander*, *Marmosa murina* and *Marmosa demerarae,* which are mammals of the order Didelphimorphia, family Didelphidae, representing the marsupials of the new world known as opossums. They occur in the humid forests of the Amazonia and the Atlantic Forest, as well as in seasonal deciduous, semi-deciduous forests and forest of galleries in the interior of the Caatinga, Cerrado and Chaco in South America (Emmons & Feer, 1997; Gardner, 2008). Although these species are typically from forest habitats, they show differences in the use of the forest strata. *C. philander* is the most arboreal among these species using rather the canopy, *M. demerarae* is the second arboreal species, both of them use more frequently the upper to the middle levels of the forest (canopy and sub-canopy), while *M. murina* is usually seen on the ground to the understory (Vieira & Camargo, 2012). Regarding the type of diet of these species, all of them are omnivorous, but *C. philander* is the most fruitful while *M. murina* consumes more invertebrates and

M. demerarae more arthropods and fruits (Santori et al., 2012). *M. murina* is the most tolerant species in terms of forest disturbances (Pardini, 2004; Passamani et al., 2005), while the other species are mostly associated with richer forests (Emmons & Feer, 1997). Even so, all species can vary in the use of vertical space, and consequently in the diet during the dry season depending on the scarcity of resources (Santori et al., 2012; Vieira & Camargo, 2012).

Sampling data

Genetic data

This study used sequences of mitochondrial DNA of the Cytochrome b (*Cytb*) region for the three didelphid mammal species to evaluated genetic relationships between Amazonian and Atlantic Forests populations. The majority *Cytb* sequences analyzed for *Caluromys philander* (27) were generated for this work. Hence, the minority sampled sequences for *C. philander* (13) were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/GenBank/) and incorporated in the analyses (Supporting Information, Table S1). The *C. philander Cytb* sequences were made available by the Mammal Collection of the National Research Institute of the Amazonia (INPA) and sequenced at the Laboratory of Animal Evolution and Genetics (LEGAL) of the Federal University of Amazonas (UFAM). Samples from *Marmosa murina* and *Marmosa demerarae* were gathered from the Genbank (77 and 47, respectively).

The total genomic DNA was extracted following the phenol–chloroform extraction (Longmire et al., 1997) and amplified the partial *Cytb* gene sequences using the polymerase chain reaction (PCR) using the set of primers OryzCytbF1 and OryzCytbR1 following the instructions of the manufacturer (Smith and Patton 1993). The PCR was performed with a final volume of 13 µl, containing 5.2 µl of H_2O; 1.5 µl of $MgCl_2$ (25 mM); 1.25 µl of dNTP (2.5 mM); 1.25 µl of 10x buffer (100mM Tris-HCl, 500mM KCl); 1 µl of each primer (0.2 µM); 0.8 µl of Taq DNA Polymerase (5 U/µl) and 1µl of the extracted DNA (concentration varied between 10 ng and 30 ng). PCR consisted of an initial denaturation at 5 minutes for 94°C; followed by 30 cycles at 1 minute for 94°C, 1 minute and 30 seconds for 57°C and 1 minute for 72°C; and a final extension at 10 minutes for 72°C. The PCR products were checked in 1% agarose gel stained with GelRed$^{®}$ (Biotium) and purified using ExoSAP following the protocol described by Werle et al. (1994) as suggested by the manufacturer. The sequencing reaction followed the protocol recommended by the manufacturer of the BigDye Sequencing Kit (Life

Technologies), while the products were sequenced on an ABI 3130XL (Life Technologies) automatic sequencer. Sequences were deposited in the GenBank database (Supporting Information, Table S1).

Occurrence records

Georeferenced occurrence records for the three didelphid mammalian species distributed between the Amazonia and the Atlantic Forest (*Caluromys philander*, *Marmosa murina* and *Marmosa demerarae*) were collected from the online databases of SpeciesLink (http://splink.cria.org.br/), GBIF - Global Biodiversity Information Facility (www.gbif.org/) and VertNet (http://vertnet.org/). Furthermore, geographical coordinates of sampled locations with available genetic data (from GenBank, see below), as well as records in others literature was employed to complete the entire known distribution area for each didelphid species (Supporting information, Table S2). All occurrence records were checked using the geographic information system program QGIS 3.6 (QGIS, 2019). A total of 293 occurrence records were compiled for *C. philander*, 568 for *M. murina* and 199 for *M. demerarae*.

Environmental data

To generate the Species Distribution Model (SDMs) for each species, we used digital layers of selected continuous bioclimatic variables extracted from the PaleoClim database (Brown et al., 2018; paleoclim.org), in ca. 5 km of resolution, which were cut considering the limits of the Neotropical region. To project SDMs to the past digital layers of continuous bioclimatic variables simulated for different past periods (Holocene to Pliocene) were also extracted from the PaleoClim database (Brown et al., 2018) also cutting considering the limits of the Neotropical region. This database provides current bioclimatic variables using the original data from CHELSA model simulations (Karger et al., 2017) and the most paleoclimatic variables from PaleoClim were simulated by the CCSM model (Fordham et al., 2017), or by the HadCM3 model for the Pliocene (Brown et al., 2018; Hill 2015; Dolan et al., 2015). The paleoclimatic variables made available by this database are simulated using the CCSM model for most periods and the HadCM3 model for the Pliocene.

Phylogeography

The *Cytb* sequences were aligned using the ClustalW method and edited in Geneious software Trial Version (http://www.geneious.com, Kearse et al., 2012). In general, partial sequences of the mitochondrial *Cytb* gene were obtained for *Marmosa murina* and *Marmosa demerarae* with 801 pb (with 135 and 153 informative sites, respectively), and a less fragment with 500 pb for *Caluromys philander* (with 68 informative sites). These sequences represent 39, 29 and 15 localities (Table S1 in Supporting Information). We estimated gene trees using Bayesian inference (BI) based on three didelphid mammalian species datasets, originally generated from samples housed in scientific collections, with additional sequences retrieved from GenBank (Supporting information, Table S1). Sequences from four species of didelphids were used as outgroup: *Didelphis marsupialis* [JF280998.1], *Monodelphis emiliae* [KM071602.1], *Marmosa paraguayanus* [HM106372.1] and *Caluromys derbianus* [MK817302.1] (Supporting information, Table S1). The substitution models used were the HKY$_{+}$G for *C. philander*, HKY$_{+}$I$_{+}$G for *M. murina* and *M. demerarae*, selected as the best models for each species based on the Akaike Information Criterion (AIC) as implemented in jModelTest v2.1 (Darriba et al., 2012). We estimated BI trees using BEAST v2.6.0 (Bouckaert et al., 2019), assuming a Birth-Death speciation model, with estimated base frequencies, four gamma rate categories, and an uncorrelated lognormal relaxed-clock model distribution, as tree priors. Runs were carried out under an uncorrelated lognormal relaxed-clock model with calibration.

The phylogenetic tree was dated using calibration points based on a fossil record, incorporating a substitution rate of 0.04 that represents the average of didelphid substitution rates simulated by Leite et al. (2016). We incorporated information from two fossils to calibrate the nodes. The calibrations used were 15.9 to 16.3 mya for the separation time between Caluromyinae and Didelphidae (Marshall, 1976), and between 12.1 to 12.6 mya for the separation between *Monodelphis* and *Marmosa* following Jansa et al. (2014). We used three independent Markov Chain Monte Carlo (MCMC) runs, each one with four streams per 15 million steps of the MCMC for *C. philander*, 10 million steps for *M. demerarae*, and 30 million steps of the MCMC for *M. murina*, sampling every 1000 generations and discarding the initial about 20% generations as burn-in, starting the initial trees with randomness, without restriction. MCMC convergence also was checked in Tracer v.1.6 (Rambaut et al., 2014), where we confirmed if effective sample sizes (ESS) were above 200. After discarding the initial 20% generations, remaining trees were compiled using TreeAnnotator (Bouckaert et al., 2019) into a maximum clade credibility (MCC) tree obtaining posterior probability (PP) values for all nodes. The tree consensus generated was visualized and edited in Figtree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

In addition, summary diversity index, such as the number of variable sites (S), number of haplotypes (H), haplotype diversity (*h*), nucleotide diversity (π), and average number of nucleotide differences (k) were identified computationally using the software DnaSP v5.10 (Librado & Rozas, 2009) and Arlequin v3.5 (Excoffier & Lischer, 2010). The evolutionary relationships between the haplotypes for each dataset (i.e. *C. philander*, *M. murina* and *M. demerarae*) were estimated using the median-joining method implemented in the Network v10.0 (http://www.fluxus-engineering.com).

Species Distribution Models

Data treatment

To avoid the effect of sampling bias on the final Species Distribution Models (SDMs), a polarization file was used, which generates a grid of sampling probabilities, where cell values reflect the variation in sampling effort between pixels to weight background points models used for modeling (Elith et al., 2010). The bias file was generated for species occurrence records using the kernel density estimation method with the *kernelUD* function of the adehabitatHR 0.4.16 R package (Calenge, 2006). The digital layers of the environmental variables used to calibrate and test the SDMs were crop based on the known geographical distribution area of each species using the International Union for Conservation of Nature - IUCN polygons (IUCN, 2019) including a buffer of five pixels, considering that the distribution area may be larger than the currently known. To project the results of the final SDMs were crop to the same geographical extent covering the study area representing the Neotropics (x minimum: -95; x maximum: -25; y minimum: -40; y maximum: 24). All treatment of environmental variables was performed in the R program (R Core Team, 2019) using the raster R package (Hijmans, 2017).

The level of correlation between all cropped variables was assessed by Pearson's correlation test using the *cor* function of the stats 3.6.2 R package (R Core Team, 2019). The selection of uncorrelated variables followed the same procedure used by Rissler & Apodaca (2007), considering the correlation threshold of 0.75. Only the variables with greater biological relevance for each species were used to generate the final models, which was assessed in the results of the Jackknife graphs generated in preliminary models in which each variable is tested in isolation and by excluding each other variable, revealing the gain and loss of models containing or missing each variable (Phillips, 2006). Thus, the variables selected for *Caluromys philander* were Temperature Seasonality [standard deviation*100] (bio4), Annual Precipitation [mm/year] (bio12), Precipitation of Driest Month [mm/month] (bio14), Precipitation Seasonality [coefficient of variation] (bio15), Precipitation of Warmest Quarter [mm/quarter] (bio18) and Precipitation of Coldest Quarter [mm/quarter] (bio19); for *Marmosa murina* were bio4, Mean Temperature of Wettest Quarter [°C*10] (bio8), bio12, bio14, bio15, bio18 and bio19; and for *Marmosa demerarae* were bio4, Mean Temperature of Driest Quarter [°C*10] (bio9), bio12, bio15, bio18 and bio19.

Models construction

The final Species Distribution Models (SDMs) for each species were built using all the non-duplicated occurrence records for each species, the bias file and the previously uncorrelated environmental variables selected based on the relevance and biological contribution of each species (as detailed above). SDMs were built using the MaxEnt v.3.3.3 (Phillips et al., 2007), which is a prediction algorithm for incomplete data sets (presence data only) based on the principle of maximum entropy, assuming that the best approximation for an unknown probability distribution is one that satisfies any constraint on its distribution (Phillips et al., 2006; Elith et al., 2011). Due to the scarcity of long-term studies that collect reliable absence data for the species, MaxEnt becomes a suitable and widely used algorithm as it has also been more successful in generating models from a small number of samples (Elith et al., 2011). A total of 114 not duplicated occurrence records were used construct the SDM for *Caluromys philander*, 137 for *Marmosa murina* and 70 for *Marmosa demerarae*.

The final models were generated using 10 independent replicates, MaxEnt default parameters and cross-validate method to evaluate models. We chose the logistic output for the presentation of ENMs in geographic space (potential distributions), with each pixel representing suitability from 0 (representing inadequate conditions) to 1 (maximum suitability) (Phillips, 2006). The performance of the models was evaluated using the AUC (Area Under the Curve) method, with the threshold value of AUC > 0.7 to accept models (Phillips 2006 suggest values greater than 0.5). The SDMs were projected for 11 past times representing: Late-Holocene ~4 thousand years ago (kya), Mid-Holocene ~8 kya, Early-Holocene ~11 kya, Pleistocene Younger Dryas Stadial ~13 kya, Pleistocene Bølling-Allerød ~15 kya, Pleistocene Heinrich Stadial 1 ~17 kya, Pleistocene Last Glacial Maximum (LGM) ~21 kya, Pleistocene Last Interglacial (LIG) ~130 kya, Pleistocene MIS19 ~787 kya, Mid-Pliocene warm ~3 million years ago (mya) and Pliocene M2 ~3.3 mya. These paleodistribution were used to evaluate the historic connecting routes between the Amazonia and the Atlantic Forest through the past.

Paleodistributions

Paleodistribution models were used to evaluate the connection routes between mammalian didelphid species in the Amazonia and the Atlantic Forest through the recent past times towards the present, measuring the expansion or retraction of the potential distribution over time. For this, the SDMs generated with continuous environmental suitability (present and past) were transformed into binary models using a threshold value that maximizes the sensitivity and specificity in the model tests (Liu et al., 2013). The threshold value that maximizes the sensitivity and specificity for *C. philander* model was 0.3214, 0.1665 for *M. murina* model and 0.296 for the *M. demerarae* model. These analyses were performed using the R package 'raster' (Hijmans, 2017). Then, the number of pixels for the region of each route (i.e. Northeastern route, NE route; Southeastern-Southeastern route, SE-SE route; and Southeastern-Northwestern route, SE-NW route) in the models of each period was summed. We delimited the areas of each connection route corresponding to three regions in the 'dry diagonal' according to the literature (Por, 1992; Oliveira-Filho & Ratter, 1995; Silva et al., 1996; Costa, 2003; Machado et al., *in review*) using the ecoregion polygons (Dinerstein et al., 2017; https://ecoregions2017.appspot.com/), considering the boundaries of the Caatinga, Babaçu forests and adjacent semidecidual forests for NE route (which represent transition areas between Caatinga and the Amazonia), the Cerrado ecoregion boundaries (excluding areas from Northern Cerrado (which correspond to the NE route and areas from Southern Cerrado which correspond to the SE-NW route) and the limits of the ecoregions of the Pantanal, wet Chaco and adjacent semidecidual forests for the SE-NW route.

Results

Genetic diversity

A total of 40 *Cytb* sequences were obtained for *Caluromys philander* (479 bp), 77 for *Marmosa murina* (801 bp) and 47 for *Marmosa demerarae* (801 bp), sampled in 15, 39 and 29 sites, respectively (Table S1 in Supporting Information). The number of segregating sites (S) between species ranged from 68 for *C. philander* and 153 for *M. demerarae* (Table 1), and the number of haplotypes (H) varied between 25 in *C. philander* and 54 in *M. murina*. Haplotype diversity (*h*) and nucleotide diversity (π) were much higher in *M. demerarae* samples than anywhere else (Table 1), while the average number of nucleotide differences (k) was also far higher, despite the *M. murina* presents large amount of specimens analyzed.

Table 1. Molecular diversity indices of *Cytb* (mtDNA) of three didelphid mammalian species in South America. N = Number of sequences; H = Number of haplotypes; S = number of variable sites; *h* ± SD = haplotype diversity ± standard deviation; π ± SD = nucleotide diversity ± Standard deviation; k = average number of nucleotide differences.

Species	**N**	**H**	**S**	***h* ± SD**	**π ± SD**	**k**
C. philander	40	25	68	0.9026 ± 0.0331	0.01757 ± 0.00333	7.87051
M. murina	77	54	135	0.9836 ± 0.0063	0.02895 ± 0.00106	22.66576
M. demerarae	47	42	153	0.9944 ± 0.0060	0.03313 ± 0.00224	26.24144

Phylogeography and divergence time

The divergence time estimate for *Caluromys philander* sequences shows that the separation of the populations of the Atlantic Forest in relation to the others (from the Cerrado, Southeastern Amazonia, Northeastern Amazonia, Northwestern Amazonia and Antilles) occurred about 1.84 million years ago (mya) with 97% of support (Fig. 1 and Table 2). The divergence between populations of the Cerrado and those of the Southeastern Amazonia for *C. philander* were not recovered with support (Fig. 1). For *Marmosa murina*, divergence between most of the populations from the Northern Atlantic Forest were all well supported and dated for about 1.33 mya (Fig. 2 and Table 2), although for one of the locations the sequence was grouped into the Cerrado and Southeastern Amazonia clade diverging within this about 0.04 mya (Fig. 2). In addition, populations in the Southwestern Atlantic Forest diverged from populations in the Cerrado about 0.02 mya (Fig. 2 and Table 2). For *Marmosa demerarae* the phylogenetic results were well supported for all major clades, showing populations from the Caatinga and the Northern Atlantic Forest grouped in the same clade and diverging from the populations of the Cerrado, Northeastern Atlantic Forest and Southeastern Amazonia about 2 mya (Fig. 3

and Table 2). The populations from the Northeastern Atlantic Forest would have diverged from those of the Cerrado and some locations in the Southeastern Amazonia about 1.4 mya (Fig. 3 and Table 2).

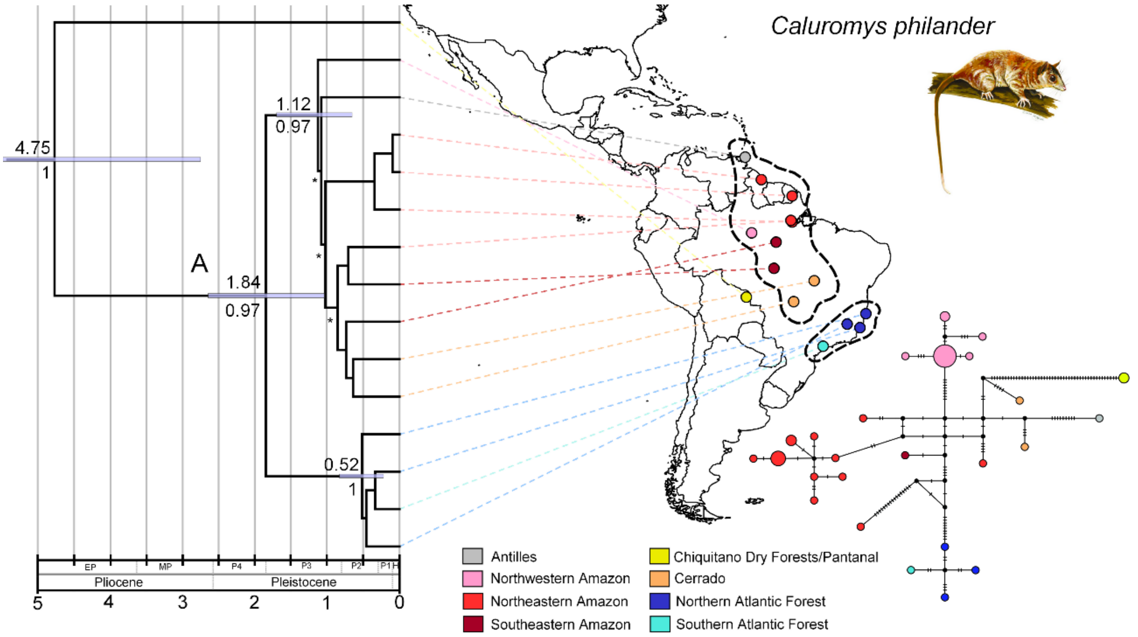

Figure 1. Time-calibrated phylogenetic tree for *Caluromys philander* based on cytochrome b gene (*Cytb*) plotted in geographical space. The axis represents the time scale in millions of years indicating the respective geological ages (EP = Early Pliocene, MP = Late Pliocene, P4 = First period of Early Pleistocene, P3 = Second period of Early Pleistocene, P2 = Middle Pleistocene, P2 = Late Pleistocene, H = Holocene). Node values represents the node supports and range of the HPD%, and asterisks nodes without support. Letters show divergence times between populations from the Amazonia and the Atlantic Forest detailed in the Table 1. The colors of the occurrence records represent biogeographic regions: gray = Antilles, pink = Northwest Amazonia, light red = Northeast Amazonia, dark red = Southeast Amazonia, yellow = Chiquitano Dry Forests/Pantanal, orange = Cerrado, dark blue = North Atlantic Forest and light blue = South Atlantic Forest. Down right, is shown the haplotype networks of *Cytb*: the circles represent shared haplotypes; the size circle represents de number of sharing haplotypes and colors the biogeographical regions corresponding to the map. Species illustration by Eisenberg & Redford (2000).

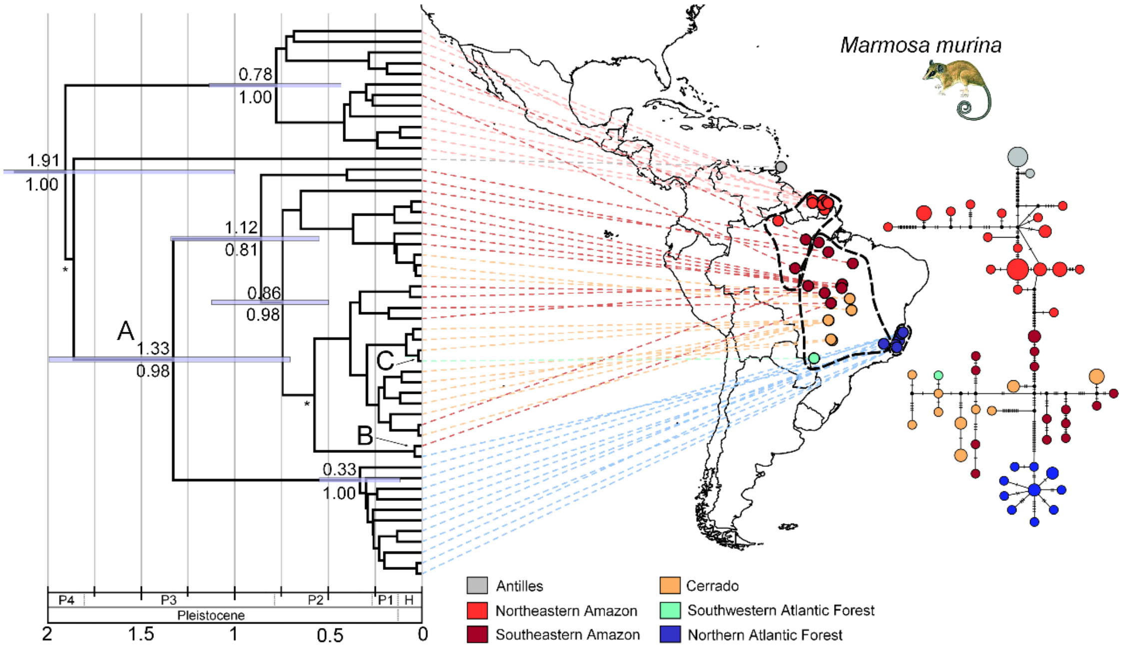

Figure 2. Time-calibrated phylogenetic tree for *Marmosa murina* based on cytochrome b gene (*Cytb*) plotted in the geographical space. The axis represents the time scale in millions of years indicating the respective geological ages (EP = Early Pliocene, MP = Late Pliocene, P4 = First period of Early Pleistocene, P3 = Second period of Early Pleistocene, P2 = Middle Pleistocene, P2 = Late Pleistocene, H = Holocene). Node values represents the node supports and HPD%, and asterisks nodes without support. Letters highlight divergences between Amazonia and Atlantic Forest. The colors of the occurrence records represent biogeographic regions: dark blue = Northern Atlantic Forest, orange = Cerrado, dark red = Southeastern Amazonia, light red = Northern Amazonia and gray = Antilles. Haplotype networks of *Cytb*: the circles represent shared haplotypes; the size circle represents the number of sharing haplotypes and colors the biogeographical regions corresponding to the map. Species illustration by Eisenberg & Redford (2000).

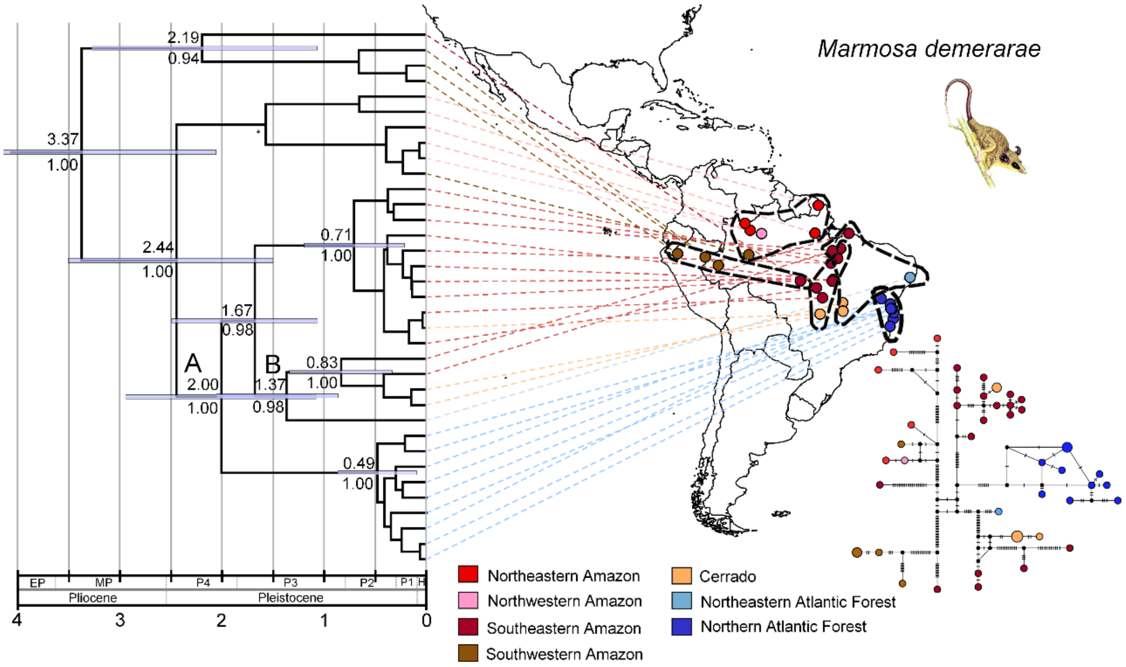

Figure 3. Time-calibrated phylogenetic tree for *Marmosa demerarae* based on cytochrome b gene (*Cytb*) plotted in geographical space. The axis represents the time scale in millions of years indicating the respective geological ages (EP = Early Pliocene, MP = Late Pliocene, P4 = First period of Early Pleistocene, P3 = Second period of Early Pleistocene, P2 = Middle Pleistocene, P2 = Late Pleistocene, H = Holocene). Node values represents the node supports and range of the HPD%, and asterisks nodes without support. Letters highlight divergences between Amazonia and Atlantic Forest. The colors of the occurrence records represent biogeographic regions: light red = Northeastern Amazonia, pink = Northwestern Amazonia, dark red = Southeastern Amazonia, brown = Southwestern Amazonia, orange = Cerrado, violet = Caatinga, dark blue = Northern Atlantic Forest and light blue = Northeastern Atlantic Forest. Haplotype networks of *Cytb*: the circles represent shared haplotypes; the size circle represents the number of sharing haplotypes and colors the biogeographical regions corresponding to the map. Species illustration by Eisenberg & Redford (2000).

Table 2. Divergence time estimates between nodes that separate populations from the Amazonia and the Atlantic Forest for three species of didelphid mammals investigated in this study. The letters represent the nodes highlighted in the figures of the trees dated for each species. Age = estimated divergence time in million years, HPD% = 95% confidence intervals of Highest Posterior Density (HPD) and PP = Posterior probability values. Regions show the connection regions for each population of each species.

Species	**Node**	**Age**	**HPD%**	**PP**	**Connection regions**
Caluromys philander	A	1.84	1.04 – 2.71	0.97	Cerrado or Caatinga
Marmosa murina	A	1.33	0.73 – 2.03	0.98	Cerrado
Marmosa demerarae	A	2.00	1.14 – 2.91	1.00	Cerrado
	B	1.37	0.71 – 2.08	0.98	Caatinga

The median-joining haplotype network based on the mtDNA showed agreement with the Bayesian consensus trees for all three species studied. However, in *C. philander* despite the several mutational steps between biogeographic populations, the presence of several median vectors suggests non-sampled or extinct ancestral sequences (Fig. 1). None widely distributed biogeographic population was found, although the Amazonia (Northwestern, Northeastern and Southeastern Amazonia) shelter most of the haplotypes found. The populations of Atlantic Forest and Pantanal appear as the most distant from each other with many mutational steps, when compared to other biogeographic populations. Although the samples from the Pantanal appear to be more related to the Cerrado (and these with Antilles). In general, all populations were found in restricted areas (distribution) for this species, without sharing haplotypes.

Considering *M. murina,* we found a structure similarity revealed by *C. philander* haplotype network, with several mutational steps and median vectors between biogeographic populations. Nonetheless, samples from the Atlantic Forest appears closer related with Cerrado and Southeastern Amazonia, even presents many mutational steps, than other populations towards to north (i.e. Northeastern Amazonia and Antilles). The pattern of *M. murina* network show that Cerrado samples emerging as intermediaries between the Atlantic Forest and the Southeast of the Amazonia. Further, the samples from the Northeast of the Amazonia (the most diverse) are related to some samples from the Southeast of the Amazonia, with massive mutational steps. Thus, acting like a direct link of Northeast-Southeast Amazonia populations and other populations towards the South. While samples from the Antilles are only related to the Northeast of the Amazonia (Fig. 2).

According to the haplotype network for *M. demerarae*, despite following a similar haplotype network structure, showed the most complex relationship when compared to the other species studied. Although revealed low frequency haplotypic variants, the network presents a high number of median vectors (i.e. non-sampled or extinct ancestral sequences) and mutational steps (Fig. 3). The network was divided into a reticulated portion consisting of Northern Atlantic Forest and Caatinga haplotypes and a complex structure composed of haplotypes from all other locations. In addition, Southeastern Amazonia populations was most diversified and widespread cluster, with three main connections: Northern Atlantic Forest and Caatinga, Northeastern Atlantic Forest, and Cerrado populations. The other biogeographic populations were verified haplotypes isolated and covered smaller areas when compared to Northern Atlantic Forest or Southeastern Amazonia biogeographic populations, except for Southwestern Amazonia with the majority haplotype occurrence grouped.

Species Distribution Models

All SDMs showed high AUC value: 0.899 for the *C. philander* SDM, 0.956 for the *M. murina* SDM and 0.888 for the *M. demerarae* SDM. The variables of greatest contribution to the *C. philander* SDM were Temperature Seasonality [standard deviation*100] (bio4) with 22.4%, followed by Annual Precipitation [mm/year] (bio12) with 18.1% and Precipitation of Driest Month [mm/month] (bio14) with 17.7%. The variables of greatest contribution to the *M. murina* SDM were bio14 with 31.7%, followed by Mean Temperature of Wettest Quarter [°C*10] (bio8) with 18.7%, Precipitation of Coldest Quarter [mm/quarter (bio19) with 18% and bio4 with 17.7%. The variables of greatest contribution to the *M. demerarae* SDM were bio19 with 26.9%, followed by Mean Temperature of Driest Quarter [° C*10] (bio9) with 25.4% and bio4 with 25.3%.

Current SDMs for all species predicted disjunct areas of greater environmental suitability between the Amazonia and the Atlantic Forest (Fig. 4A, Fig. 5A, and Fig. 6A), mainly for *M. murina* (Fig. 5A). The SDM for *C. philander* predicted disjunct areas of greater environmental suitability between the Atlantic Forest, the Cerrado and the

Amazonia (Fig. 4A). The result of this model also reveals areas of high environmental suitability for the occurrence of the species in regions where there is not record, such as regions of the Brejos de Altitude and Babaçu Forests in the Caatinga, as well as for the slope of the Andes (Fig. 4A). The SDM for *M. murina* predicted considerable disjunct areas of greater environmental suitability between the Amazonia and the Atlantic Forest (Fig. 5). The result of this model also reveals areas of high environmental suitability for the occurrence of the species in regions where there is not record, such as regions of the Southwestern Amazonia (Fig. 5A). The SDM for *M. demerarae* also predicted disjunct areas of greater environmental suitability between the Atlantic Forest, the Cerrado and the Amazonia (Fig. 6A). The result of this model also reveals areas of high environmental suitability for the occurrence of the species in regions where there is not record, such as regions of the Brejos de Altitude and Babaçu Forests in the Caatinga (Fig. 6A).

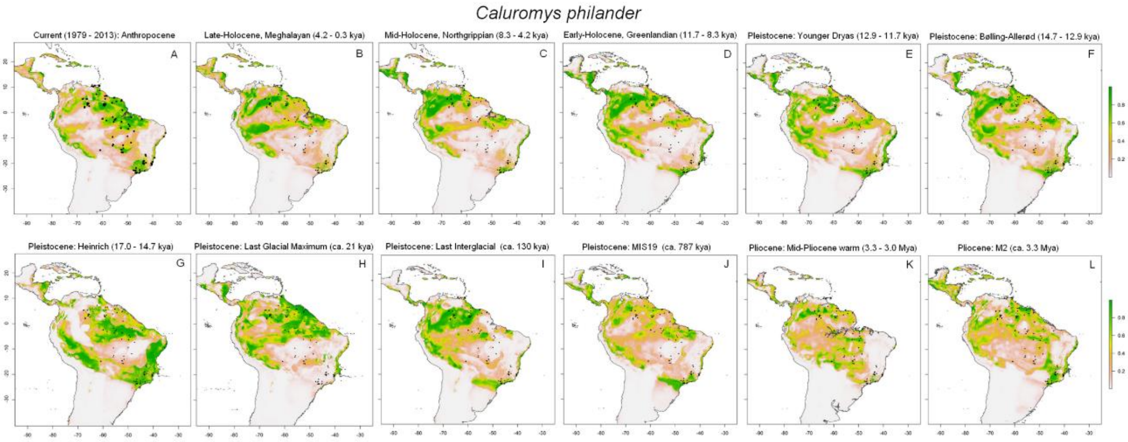

Figure 4. Species distribution models for *Caluromys philander* from top-left to bottom-right for the present and 11 past times. Color scales represent environmental suitability values ranging from 0 (in gray) to 1 (in green).

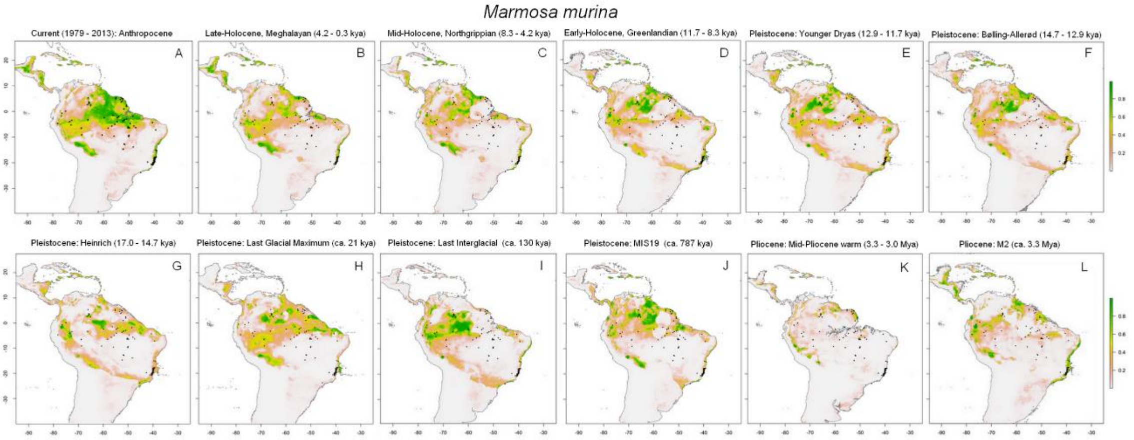

Figure 5. Species distribution models for *Marmosa murina* from top-left to bottom-right for the present and 11 past times. Color scales represent environmental suitability values ranging from 0 (in gray) to 1 (in green).

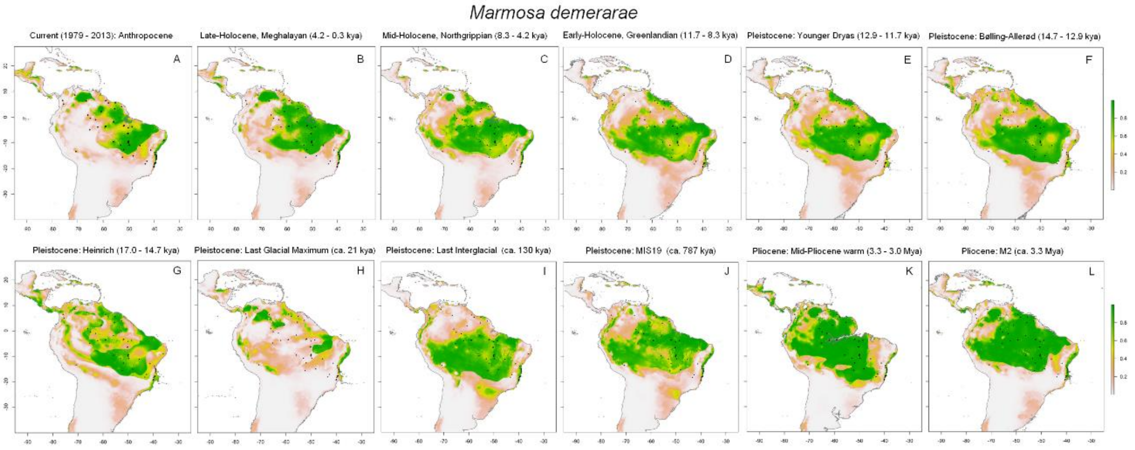

Figure 6. Species distribution models for *Marmosa demerarae* from top-left to bottom-right for the present and 11 past times. Color scales represent environmental suitability values ranging from 0 (in gray) to 1 (in green).

Paleodistribution changes

The analysis of the change in paleodistribution models over time revealed an increase of suitable areas for all species and all regions (Caatinga, Cerrado and Chaco/Pantanal) for about 17 kya in relation to the present (Fig. 7). During the LGM (~ 21 kya) in relation to the present, the models show a reduction in paleodistribution for all species for Cerrado regions, but for the other regions the paleodistributions have not changed much in relation to the present, except for *M. demerarae* in the Caatinga (Fig. 7). During LIG (~ 130 kya), paleodistribution models show an increase in areas suitable for the occurrence of most species in most regions, except for *C. philander* which decreased in the Cerrado and remained reasonably stable for the other regions, for *M. murina* that has not changed much in the Caatinga and Chaco/Pantanal regions and for *M. demerarae* for the Caatinga where it has reduced slightly (Fig. 7). For about 3 mya, the paleodistribution models reveal an increase in the areas suitable for the occurrence of *C. philander* and *M. demerarae* in the Cerrado and Chaco-Pantanal but decrease in the Caatinga. For *M. murina*, there was a slightly reduction for the Caatinga and the Cerrado route and stability for the Chaco-Pantanal (Fig. 7).

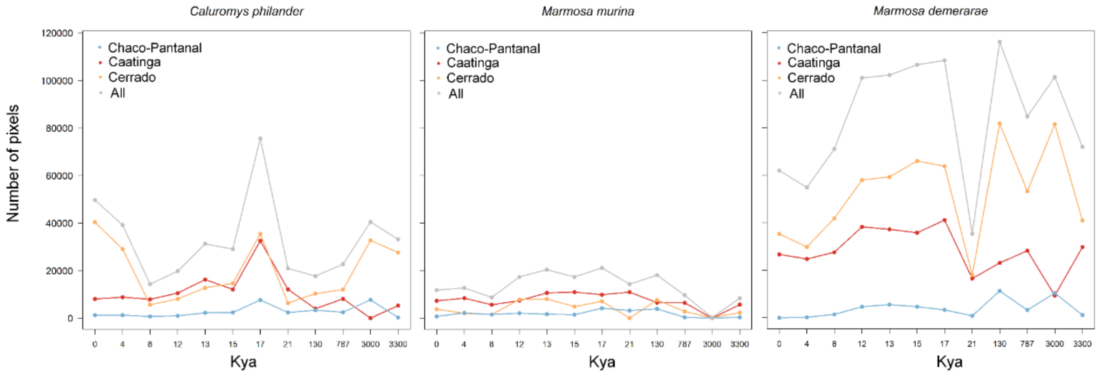

Figure 7. Change in paleodistribution models over time of each didelphid mammalian species for connection routes between Amazonia and Atlantic Forest. The y-axis represents the number of pixels in the models; The x-axis represents time on a scale of a thousand years ago (Kya). The Caatinga region is represented in red, the Cerrado in yellow, the Chaco-Pantanal in blue and all regions in grey.

Discussion

Many studies have investigated the connections between the Amazonia and the Atlantic Forest through the phylogeography of species from these regions (Costa 2003; Nascimento et al., 2008; Pavan et al., 2011; Fouquet et al., 2012; Terra-Araujo et al., 2015; Prates et al., 2016; Peres et al., 2017; Capurucho et al., 2018; and also see Ledo & Colli, 2017 for a review), some also through paleodistribution models (Sobral-Souza et al., 2015; Ledo & Colli, 2017; Maciel et al., 2017), but none through both approaches simultaneously focus on these connections. The research presented here is the first to investigate the connections between the Amazonia and the Atlantic Forest through comparative phylogeography and paleodistribution simultaneously. Thus, although those studies corroborated, through different approaches, the connections between the

Amazonia and the Atlantic Forest proposed by Por (1992), the present study brings a different proposal when evaluating these connections through both phylogeographic and paleogeographic approaches simultaneously.

Among previous studies on molecular evidence of connections between the Amazonia and the Atlantic Forest for animals, few have been recent (for example, Costa, 2003; Nascimento et al., 2008; Pavan et al. 2011; see review in Ledo & Coli, 2017), with only one covering didelphid mammals (Costa, 2003). Although this study has brought a lot of information and data for species of didelphid mammals, many years have passed since its publication and, with this, there has been a considerable increase in the number of genetic data available. Although the results revealed by Costa (2003) for the time of divergence between the populations of the Amazonia and the Atlantic Forest are in accordance with those presented here, we bring additional results for *Marmosa demerarae*. In addition, after recent reviews of phylogenetic relationships between didelphid mammal species, some groups that were previously considered to be the same taxonomic group have been separated, for example, for *Marmosa murina* (Gutiérrez et al., 2010; Faria et al., 2013). Therefore, the data used here for the species investigated are up to date and the inferences we made considered these revisions.

Our study brings new evidence about the historical connections between the Amazonia and the Atlantic Forest, revealing two times of connections and isolation between populations from these forests, one during the Pleistocene from about 2 and 1.3 million years ago (mya), and other during the Holocene (from about 0.02 to 0.1 mya), the most recent being for the Northeast region of Brazil for *M. demerarae*. Evaluating these molecular results together with changes in paleodistribution of species, the paleodistribution of two species was greater for periods prior to those of isolation (such as about 3 mya), becoming narrower in younger periods (787 thousand years ago [kya]), except *M. murina* and for the northeast route. The beginning of the Pleistocene was a period of declining temperature in relation to previous periods and of greater climatic instability (Hansen et al., 2013). Therefore, we suggest that climatic changes during the onset of the Pleistocene had great influence on the phylogenetic structure of these species, leading to the separation of the populations of the Amazonia and the Atlantic Forest. Furthermore, we demonstrated for the first time a congruence between molecular and ecological evidence for the connections between these regions.

According to our results on the connection regions between the Amazonia and the Atlantic Forest, there are at least two routes of recent past connections, which had also been previously suggested, the Northeastern and Southeastern routes (Por, 1992; Oliveira-Filho & Ratter, 1995; Silva et al., 1996; Costa, 2003). *M. murina* shows connections between Amazonia and the Atlantic Forest through the Cerrado and Southeastern Amazonia with Northern Atlantic Forest representing a connection via the Southeastern route. Interestingly, we found connections between Amazonia and the Atlantic Forest in two different times for *M. demerarae*, the oldest dispersal event through Southeastern Amazonia and Cerrado to the Northern Atlantic Forest and the younger also through Southeastern Amazonia and Cerrado to the Northeastern Atlantic Forest. The first event probably representing connections through the SE-SE route, while the second and most recent event probably representing connections through the Northeastern route. Unfortunately, it is not clear whether *C. philander* shows connections between these biomes through the Northeastern or Southeastern Amazonia due to many unsampled relationships or extinct ancestors being difficult to assess the role of intermediate regions as connecting routes between the Amazonia and the Atlantic Forest for this species. Batalha-Filho et al. (2013) suggests connections across the Northeast to more recent periods compared to the Southwest (Chaco and Pantanal regions). However, in general our results also revealed more recent connections to the Southeast route in agreement with other previous studies (Por, 1992; Ledo & Coli, 2017), although within a limited time scale. Our results corroborate Costa's (2003) proposal that the forests of central Brazil play an important role as a present and past habitat for tropical forest species.

Unlike what was found in previous studies on the connections between the Amazonia and the Atlantic Forest through paleodistribution (Sobral-Souza et al., 2015; Leite et al., 2016; Costa et al., 2017; Ledo & Colli, 2017), we have not found a considerable expansion in the paleodistribution of the species investigated here during the Last Glacial Maximum (LGM). For two species there was a significant decrease in paleodistribution during LGM compared to the present, except for *M. murina* where there was a slight expansion mainly for Caatinga. This species is the most resilient species among those investigated here, occupying disturbed forest areas (Pardini, 2004; Passamani et al., 2005). Given this peculiarity of *M. murina*, it becomes evident that species respond to climate change accordingly to its characteristics, which will pose great influence on the outcome this response (Graham et al., 1996). As such, species-specific traits, such as environmental tolerance, will play different roles on the biogeographic history of each group (Papadopoulou & Knowles, 2016; Machado et al., *in review*). For example, through paleodistribution models Ledo & Coli (2017) found expansion of species adapted to the cold in the LGM, while in the Last Inter-Glacial (LIG), the expansion involved species adapted to warmer climates. Neotropical marsupials, that is didelphid mammals, being better adapted to warmer climates (Hunsaker, 2012), which explains why they do not follow this expansion observed for other groups during the LGM, but during the LIG. In addition, we emphasize that each species can respond in a different way to the climatic changes of each past time, according to their biological and/or ecological characteristics, in addition to their biogeographic history. These findings can be particularly important for the conservation of species through studies on the responses of species to future climate changes (da Silva et al., 2020). Another important point to highlight is that the genetic data used came mainly from online databases, showing that the increase in the availability of molecular data in recent years favors biogeographic studies. Thus, we encourage the molecular data available in the databases to be used to answer questions of biogeographic and evolutionary ecology.

Conclusion

The present study shows a congruence between the phylogeography and paleodistribution models, something never evaluated simultaneously before the present study for the historical connections between the Amazonia and the Atlantic Forest. We show that the connections between these forests have occurred more than once over recent time through phylogeography and paleodistribution models. We found more recent connections for both the Northeastern and Southwestern routes. The present study also shows that the different biological and ecological characteristics of each group, as well as biogeographic history, are important factors to be considered when assessing responses to past climate changes and, consequently, for future climate changes. We also highlight that the increase in the availability of molecular data in recent years favors biogeographic studies and we encourage that these data be used to answer biogeographic and ecological-evolutionary questions.

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CAPÍTULO 3

Spatiotemporal patterns of historical connections between the Amazonia and the Atlantic Forest

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Abstract

Aim: Despite the apparent disjunct distributions of the Amazonia (AM) and the Atlantic Forest (AF), three historical connections between these biomes have been hypothesized: through the forests of Northeast Brazil (NE route), through the gallery forests of the Central Brazilian Cerrado and through forests of the Parana Basin in the Southeast-Northwest (SE-NW route). Temporal variation in these connections has been suggested, with more recent connections for the NE route and older connections for the SE-NW route. We tested the hypothesis of temporal variation for the AM-AF connections using Neotropical mammals as a model system.

Location: South America.

Taxon: Mammalia.

Methods: Phylogenetic and spatial data were combined to generate spatiotemporal maps of connections between AM and AF mammals. Minimum divergence times between phylogenetically nearest taxa within families from each biome were extracted from dated phylogenies and projected into geographic space using species distributions to generate average divergence times (ADT) for grids of 1° degree. The relationship between the NE route and SE-NW route times and ADT per grid between the AM and the AF was tested using linear regression considering spatial autocorrelation.

Results: The results reveal the previously suggested spatiotemporal pattern was not corroborated (except for the family Didelphidae in the AM grids), showing old connections for the NE route. Nonetheless, a spatiotemporal pattern opposite to that previously suggested was found for the AF, with recent divergence times towards the SE-NW route for most families. In addition, each family revealed specific connections between the AM and the AF at different times.

Main conclusion: Our study refutes the previously proposed absence of old biogeographic connections through the NE route. This temporal variation differs among the investigated mammalian families according to their evolutionary histories, so the same can be expected for the other routes with both recent and older connections, depending on the evolutionary history and biological traits of each group. Due to the biogeographic history of each group in the Neotropics, different connections were found at different times. Therefore, connections between the AM and the AF cannot be explained by a single spatiotemporal pattern.

Keywords: Biogeography, biogeographical connections, connection routes, biogeographical patterns, historical biogeography, macroecology, forests, Neotropical forests, Tropical Rainforests, mammals.

Introduction

Patterns of species diversity are strongly structured in space and time because ecological and evolutionary processes occur in a geographical context through adaptation, selection, genetic drift, and dispersal (Diniz-Filho et al., 2008). Nonetheless, understanding how species diversity is structured in space and time remains one of the main challenges of the fields of ecology and evolution, and is considered one of the 100 fundamental ecological questions (Sutherland et al., 2013). One way of elucidating factors that structure ecological communities is to integrate species distributions with their evolutionary history. This integration between ecology and evolution has provided new insights for biogeographic, macroevolutionary and macroecological studies (Wiens & Donoghue, 2004; Graham et al., 2017).

The Amazonia (AM) and the Atlantic Forest (AF) represent the largest tropical rainforests of the Neotropics and are among the most diverse regions of the world (Mittermeyer et al., 1998; Jenkins et al., 2013). The current distributions of the forests of these two biomes are disjunct, being separated by the 'dry diagonal' comprising the Caatinga, Cerrado and arid Chaco. However, there is a mosaic of forests within the ‘dry diagonal’ (including montane, submontane, riparian, semideciduous and deciduous forests) that can be considered to comprise remnants of a historical bridge of biogeographical connection between these forests (Oliveira-Filho & Ratter, 1995). The composition of biotas is in agreement with this interpretation, with the Eastern Amazonia (AM) being more similar to the Northern Atlantic Forest (AF) than to the Western Amazonia and the Western AM being more similar to the Southern and Southeastern AF than to the Eastern AM (Por, 1992). Based on these patterns of similarity, historical connections between the AM and the AF have been proposed by many biogeographers over recent decades, both with regard to floral (Oliveira-Filho & Ratter, 1995; Melo Santos et al., 2007) and faunal (Willis, 1992; Silva, 1996; Costa, 2003; Pellegrino et al., 2011; Fouquet et al., 2012; Batalha-Filho et al., 2013) connections. As a result, three main connection routes have been proposed between the AM and the AF for mammal species (Fig. 1): (1) the Northeast route (NE route) through the forests of Northeast Brazil, (2) by the gallery forests of northeastern Cerrado and (3) the Southeast and Northwest (SE-NW) route through the forests of the Paraná Basin, moist Chaco and Pantanal passing through southwestern Cerrado forests (Por, 1992; Oliveira-Filho & Ratter 1995; Costa, 2003). Although some authors consider only two major regions of connection between the AM and the AF (NE and SE-NW routes), based on the climatic similarity portions of these regions (Bigarella, 1975; Batalha-Filho et al., 2013; Ledo & Colli, 2017), the gallery forests of the interior of the Brazilian

Cerrado indicate historical connections between the Southeast Amazonia and the Southeast Atlantic Forest (Costa, 2003).

These historical connections have been corroborated for different groups of animals (mammals, birds, reptiles and frogs) based on a review of molecular dating evidence (Ledo & Colli, 2017). The spatiotemporal pattern of these connection routes has also been evaluated for birds (Batalha-Filho et al., 2013), suggesting older connections through the SE-NW (during the Miocene) and recent connections through the NE (during the Plio-Pleistocene). Additionally, temporal variation among these connection routes has been shown for vertebrates, with both old and recent connections for the SE-NW, but no older connections for the NE route (Ledo & Colli, 2017). Although many biogeographic studies have elucidated historical connection routes between the AM and the AF, they have rarely been evaluated in a macroevolutionary context (but see Batalha-Filho et al., 2013). However, tools used in macroecology, which seeks to understand the mechanisms that generate the patterns found in large geographic and temporal scales (Gaston & Blackburn, 2000), have been used with great success for the compression of biogeographic patterns in the Neotropics (Maestri et al., 2016; Diniz-Filho et al., 2019; Villalobos et al., 2020). Batalha-Filho et al. (2013) investigated the correlation between divergence times for these connections on a biogeographical and macroevolutionary scale, suggesting variation in the timing of routes for birds; however, evidence is still lacking for other groups. Mammals represent a very promising model for assessing historical connection routes between the AM and the AF on a biogeographical and macroevolutionary scale since they represent a truly diverse group with both endemic and widely distributed species with different modes of dispersal (i.e., flying and non-flying) (Emmons & Feer, 1997), which may reveal not only biogeographic patterns but also the processes responsible, being also an ideal group to test the hypotheses previously suggested.

The integrative use of species distributions and phylogenetic information might help to elucidate the open debate regarding the ages of the three connection routes between the AF and the AM. Therefore, our aim was to investigate the spatiotemporal pattern of historical connection routes between the AM and the AF using mammals. We tested the hypothesis of temporal variation in the NE route and the SE-NW route as connections between the AM and the AF. In this hypothesis we assume recent divergence times between AM and AF taxa near the NE route, and older divergence times restricted to the SE-NW route (Fig. 1) (Por, 1992; Batalha-Filho et al., 2013; Ledo & Colli, 2017). To test this hypothesis, we applied an integrative biogeographical and evolutionary approach using Mammalia as a model for evaluating the spatiotemporal pattern of connection routes between the AM and the AF over time.

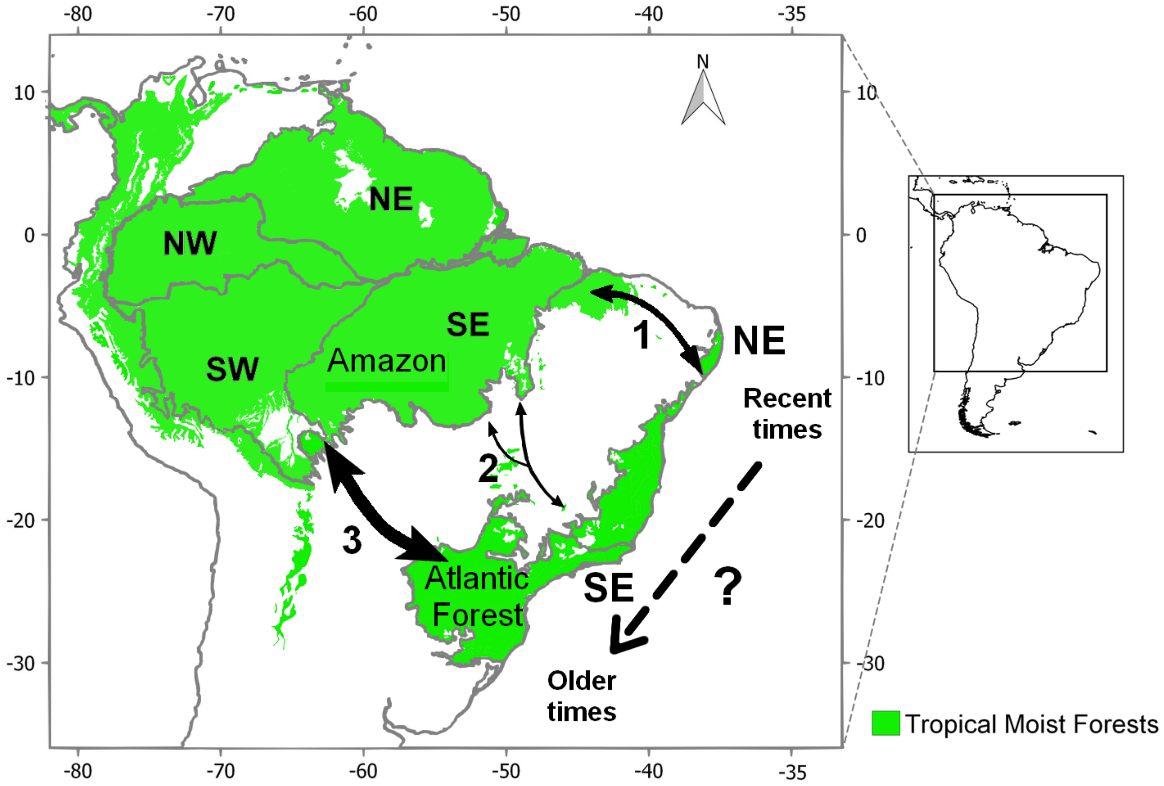

Figure 1. Distribution of tropical forests in South America. Tropical Moist Forests are shown in green and emphasize the Amazonia and the Atlantic Forest. Internal arrows represent connection routes between the Amazonia Forest and the Atlantic Forest through the Northeast/NE route (1), Southeast Amazonia – Southeast Atlantic Forest/SE-SE route (2) and the Southeast Atlantic Forest - Northwest Amazonia/SE-NW route (3). Dashed external arrow indicates suggested temporal gradient for the connections with recent divergence times for the NE route and older divergence times for the SE-NW route (Batalha-Filho et al., 2013). Edited from Costa 2003.

Materials and Methods

Sampling data

Data for mammal species distributions for the AM and the AF were obtained from IUCN Red List (2019). We used the IUCN distribution data since these maps are created and verified by specialists based on occurrence records, and restrict species occurrences to areas with presumably suitable habitat where the species is known, following a precautionary principle to guide conservation efforts (IUCN Red List Technical Working Group, 2019; IUCN

Standards and Petitions Committee, 2019). Although these maps were designed for conservation purposes, they have been shown to be an important source of information in macroecological studies (Sandom et al., 2013; Belmaker & Jetz, 2015; Mouchet et al., 2015; Maestri et al., 2016; Gonçalves-Souza et al., 2020 Vilela & Villalobos, 2015). Mammalian species distributions were overlapped with ecoregion maps (Dinerstein et al., 2017) to identify species that occur in Tropical and Subtropical Moist Broadleaf Forests using the function *gIntersection* of the R package ‘rgeos’ (Bivand & Rundel, 2013), selecting species that occur only in either the AM or the AF. We did not consider species with disjunct or continuous distributions between the AM and the AF and species with occurrence in open areas, because it is not possible to extract the time of divergence between populations of these species with the phylogeny used, and because we are interested in forest species to reveal the connections between AM and AF.

Phylogenetic data for species were extracted from dated phylogenies of mammals available in the PHYLACINE 1.2 database (Faurby et al., 2018), which is one of the most recent and inclusive set of data on mammal phylogenies. These phylogenies were generated by Bayesian inference including several molecular markers and encompasses phylogenetic relationships and divergence times estimated for 5,831 species in 1,000 resolved trees. This database review and update the taxonomy of the species contained in these trees to correspond with the most recent IUCN taxonomy (Faurby & Svenning, 2015), which favors the integration of phylogenetic and geographical data from both databases.

A total of 296 forest mammal species were identified between the AM and the AF based on geographic data, of which 214 were from the AM and 82 from the AF; 19 of these species, however, did not contain genetic data in the PHYLACINE 1.2 database (14 for the AM and five for the AF). Of the species with available genetic and geographical data (277 species), only those from taxonomic families with at least one representative species in each area were considered for this study, resulting in 249 forest mammalian species, 173 from the AM and 76 from the AF (Appendix 1 Tables S1.1 and S1.2 in Supporting Information). A maximum clade credibility tree was calculated for the Bayesian phylogenetic inferences using the R package ‘phangorn’ (Schliep et al., 2017) to assemble a summarized tree for the sampled mammalian species (Fig. 2).

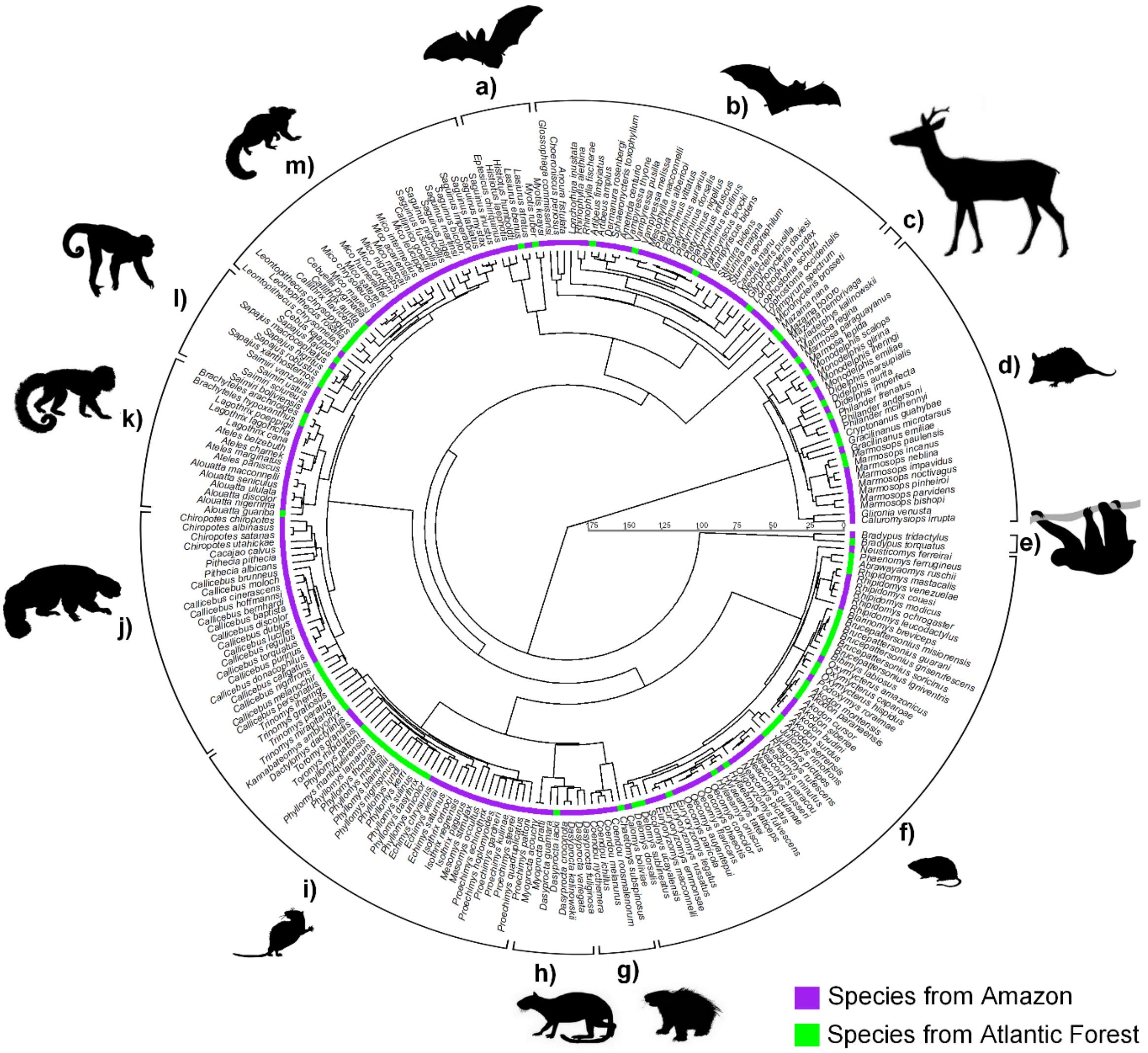

Figure 2. Maximum clade credibility tree for the forest mammal species from the Amazonia (in purple) and the Atlantic Forest (in green) sampled in this study. The axis represents the time scale in millions of years. The lettered images are silhouettes of species representing each taxonomic family (silhouettes available at http://phylopic.org/): a) Vespertilionidae; b) Phyllostomidae; c) Cervidae; d) Didelphidae; e) Bradypodidae; f) Cricetidae; g) Erethizontidae; h) Dasyproctidae; i) Echimyidae; j) Pitheciidae; k) Atelidae; l) Cebidae; m) Callitrichidae.

The species used in the study represent six orders of the subclass Theria (Mammalia Class), including marsupials (infraclass Marsupialia) and placental mammals (infraclass Placentalia): Didelphimorphia (family Didelphidae with 18 species from the AM and nine from the AF); Artiodactyla (family Cervidae with one AM and two AF); Pilosa (family Bradypodidae with one AM and one AF); Chiroptera (family Phyllostomidae with 31 AM and four AF; family Vespertilionidae with five AM and two AF); Primates (family Atelidae with 12 AM and three AF; family Callitrichidae with 21 AM and five AF; family Cebidae with six

AM and four AF; family Pitheciidae with 21 AM and three AF); and Rodentia (family Cricetidae with 28 AM and 24 AF; family Dasyproctidae with seven AM and one AF; family Echimyidae with 19 AM and 17 AF; family Erethizontidae with four AM and one AF) (Appendix 1 Tables S1.1 and S1.2 in Supporting Information).

Data analysis

Minimum divergence times between species or groups of mammal species from the AM and the AF within families were extracted from the dated phylogenetic trees (Faurby et al., 2018). In each dated phylogenetic tree, half of the phylogenetic distance between a pair of groups from each biome (one from the AM and other from the AF) was considered as their divergence time. The divergence time for pairs of mammal species between the AM and the AF were computed for each phylogenetic tree using the *cophenetic* function of the R package 'stats' (R Core Team, 2018), selecting among them the minimum divergence time for pairs of taxa, one in each biome, representing the time that links a specific species from one biome with its most closely related species (or taxa) in the other. The mean divergence time between AM and AF taxa was calculated based on all phylogenies and used as a single measure of groups pairwise minimum divergence time.

We opted to use minimum divergence times delimited to within each family to avoid deep phylogenetic patterns that may not be related to the historical routes considered in this work. Other strategies were adopted in other works to avoid this problem, for example the use of only pairs of sister species (e.g. Batalha-Filho et al., 2013). Despite this being a more conservative approach, the restriction to sister species would constrain our data to only five species since many sister species can occur in open areas, and thus are not considered in our analysis. As our goal is to test temporal variation of forest connections, species adapted to open areas, that is, that use indiscriminately both open and forest environments, are not good predictors for forest connections.

Minimum divergence times between each pair of species and taxa, one from the AM and one from the AF, were projected onto a species-by-plot matrix, which was obtained by converting distribution polygons for the species into raster layers containing grids of cells of 1 x 1° (~ 110 x 110 km) throughout South America. Minimum divergence times between species and taxa occurring between the AM and the AF were then projected into geographic space using the *matrix.t* function of the R package SYNCSA 1.3.3 (Debastiani & Pillar, 2012; updated in 2018). The procedure generated a matrix describing each plot (grid-cell) by average divergence time (ADT) between the phylogenetically closest species occurring between the AM and the

AF, which enabled the evaluation of the age of connection routes between the AM and the AF based on the evolutionary history of forest mammals of these biomes.

To evaluate temporal variation for the biogeographical connection routes between the AM and the AF, and to test the hypothesis of whether the NE route is more recent than the SE-NW route, the geographical area for these connections was delimited from ecoregion polygons (Dinerstein et al., 2017) considering the ‘dry diagonal’ in order to extract the ADT value from each grid (response variable). The area of the ‘dry diagonal’ was selected using the boundaries of the Caatinga, Babaçu Forests and adjacent Dry Forests, which represent transition areas between Caatinga and the AM, the Cerrado ecoregion boundaries, and the limits of the ecoregions of the Pantanal and wet Chaco. Using these previously delimited areas for the connection between the AM and the AF, a distance gradient from the NE route to the SE-NW route was generated to be used as a predictor variable by calculating the distance from each plot to the NE route centroid. The centroid of this connection route was calculated using the function *gCentroid* in the R package 'rgeos' (Bivand & Rundel, 2013), while the distance of each grid from the centroid was calculated using the function *distanceFromPoints* in the R package 'raster’ (Hijmans, 2017).

To test the hypothesis about temporal variation of the connection routes between the AM and the AF, we evaluated the association of ADT between AM and AF mammals per plot (response variable) and the distance of these grids to the centroid of the Northeast, representing the distance gradient from NE route to SE-NW route (predictor variable). For this, ADT per plot between AM and AF mammals was modeled as a function of the distance gradient from NE to SE-NW routes using generalized least squares (GLS) models. Through this method, it is possible to test suggested biogeographic patterns, in this case we test the hypothesis that connections between AM and AF through the NE are more recent compared to the SE-SW connections. The models were generated considering all species together and separately for each mammalian family with at least four species from the AM and four species from the AF (Didelphidae, Phyllostomidae and Cricetidae). GLS models were built to consider spatial autocorrelation between grids, including a spatial covariate to reduce correlation in model residues. This analysis was performed using the *gls* function in the R package 'nlme' (Pinheiro et al., 2018). We chose to use a GLS including spatial autocorrelation because the residuals of previously generated linear models pointed to autocorrelation.

Results

The regression model considering all mammal species for the AM did not reveal a strong spatiotemporal pattern for the tested hypotheses (Fig. 3a and Table 1). Although the result showed a trend of a positive relationship between ADT and distance to the NE route, all the connection routes (NE route, interior route of the Cerrado forests which we call Southeast-Southeast Amazonia route or SE-SE and SE-NW route) had both recent and old ADT values (Fig. 3a). Nevertheless, the regression model for the AM grids within mammalian families confirmed the tested pattern for Didelphidae (Fig. 3c and Table 1), revealing recent divergence times for the NE route and older divergence times for the SE-NW route; this pattern, however, was not corroborated for the other families investigated, namely Phyllostomidae (Fig. 3e and Table 1) and Cricetidae (Fig. 3g and Table 1).

Table 1. Estimated parameters (Estimate), standard error (Std. Error), Student's t-test value (*t*-value) and calculated probability (*P*-value) of linear regression models considering spatial autocorrelation (GLS) between average divergence time (ADT) for mammals from the Amazonia and the Atlantic Forest per plot and distance gradient from NE route to SE-NW route. AM = Amazonia; AF = Atlantic Forest; Dist NE = distance gradient from northeastern to southern connection routes.

Coefficients	**Estimate**	**Std. Error**	***t*-value**	***P*-value**
All species AM				
Intercept	7.794	3.791	2.06	0.041
Dist NE	0.004	0.002	1.93	0.055
All species AF				
Intercept	10.248	1.124	9.11	< 0.001
Dist NE	-0.002	0.001	-2.52	0.013*
Didelphidae AM				
Intercept	2.187	3.198	0.68	0.496
Dist NE	0.005	0.002	3.02	0.003**
Didelphidae AF				
Intercept	6.234	2.589	2.41	0.019
Dist NE	< -0.001	0.001	-0.12	0.904
Phyllostomidae AM				
Intercept	7.832	7.320	1.07	0.287
Dist NE	0.002	0.003	0.50	0.617
Phyllostomidae AF				
Intercept	5.183	0.557	9.30	< 0.001
Dist NE	-0.001	< 0.001	-4.08	< 0.001***
Cricetidae AM				
Intercept	3.540	3.450	1.01	0.321
Dist NE	0.002	0.001	1.15	0.258
Cricetidae AF				
Intercept	4.904	0.702	6.98	< 0.001
Dist NE	< -0.001	< 0.001	-0.16	0.876

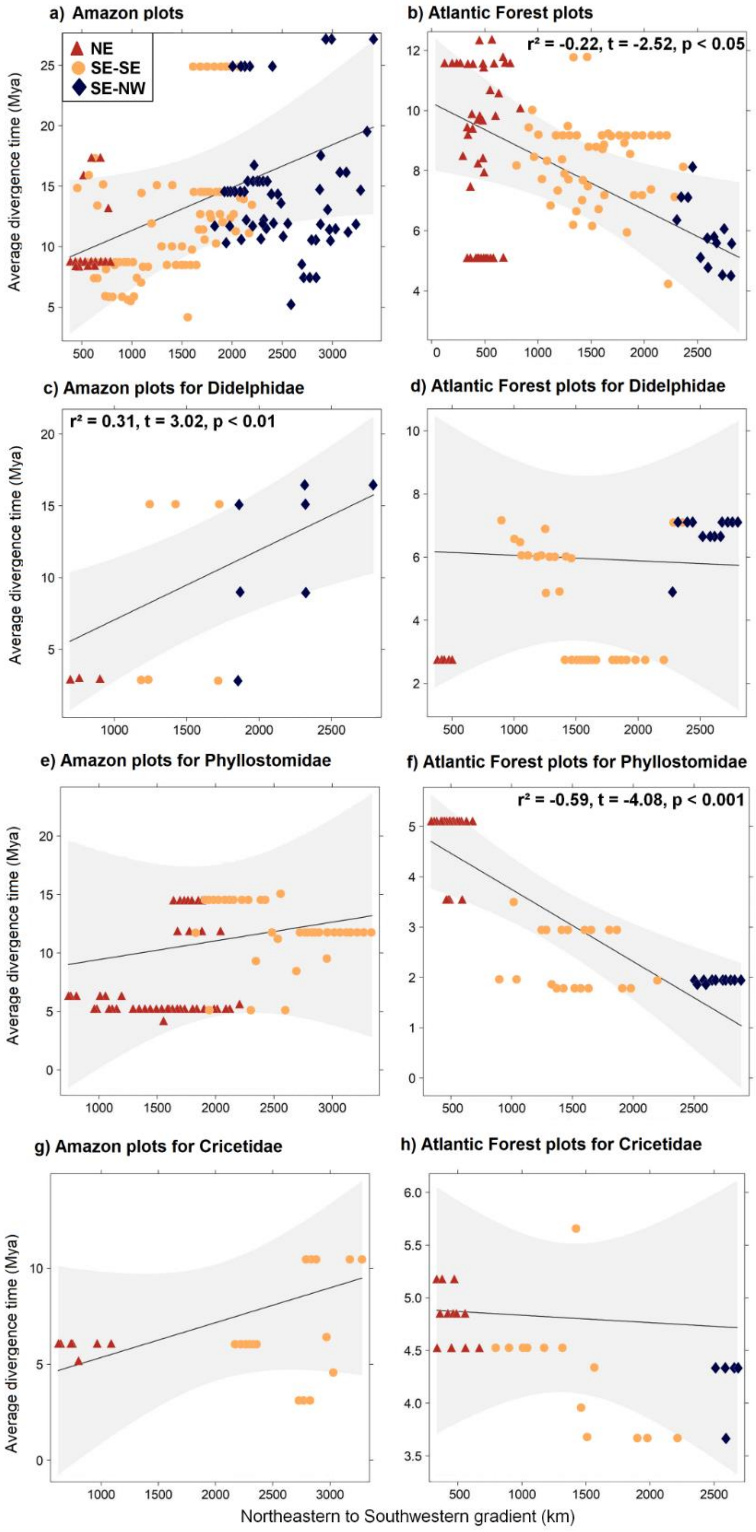

Figure 3. Scatter plot and predictive regression analysis of average divergence time per grid in millions of years ago (Mya) for connection routes for mammals between the Amazonia (AM) and the Atlantic Forest (AF) along the Northeastern to Southwestern distance gradient (km). The Y axis shows the average divergence time (ADT) for species with occurrence in each ‘dry diagonal’ grid and the X axis shows the distance between these grids and the centroid of the Northeastern route. Colored symbols represent connection regions across the ‘dry-diagonal’ with red triangles for the Northeastern route (NE), orange circles for the Southeast- Southeast route (SE-SE) and blue diamonds for the Southeast – Northwestern route (SE-NW).

The results for the AF grids considering all the mammalian families revealed a strong spatiotemporal pattern with older divergence times for the NE route and more recent divergence times for the SE-NW route (Fig. 3b and Table 1). The regression models for AF grids within mammalian families revealed the same strong spatiotemporal pattern for Phyllostomidae (Fig. 3f and Table 1), with older divergence times for the NE route and more recent divergence times for the SE-NW route; this spatiotemporal pattern was not, however, corroborated for Didelphidae (Fig. 3d and Table 1) and Cricetidae (Fig. 3h and Table 1). Still, for most of the AF families investigated, the relationship between ADT and distance from the NE route was negative, with older divergence times for this route and more recent divergence times for the SE-NW route (except for Didelphidae, for which divergence times were more recent for the NE route than for the SE-NW route).

The three previous-established connection routes between the AM and the AF were found to be used by mammals based on spatial projections of ADT. These routes are: (1) through forests of Northeast Brazil (NE route) near the coast; (2) through forests of northeastern Cerrado; and (3) through forests of the Southeast-Northwest (SE-NW route) together with southwestern Cerrado (Fig. 4a and 4b). However, when mammalian families were evaluated separately, some families revealed specific routes between the AM and the AF. Phyllostomidae showed a connection between the AM and AF through the SE-NW route, and a current non-connectivity between the AM and the AF through the NE route, which is currently interrupted (Fig. 5b). This family also revealed a spatiotemporal pattern delimited by the Northern and Southern AF and a recent incomplete connection between the Southern AF and the Andean Amazonia. Erethizontidae, Echimyidae and Cervidae a possible connection route through the Cerrado or through the SE-NW route (Fig. 6e, g and j) revealed. In addition, the maps reveal that connections through the NE route for the Echimyidae may also have occurred. Cricetidae and Vespertilionidae revealed connections through the SE-NW route (Fig. 5c and Fig. 6h); and Dasyproctidae through the NE route (Fig. 6f). Phyllostomidae, Cricetidae (Fig. 5b and 5c) and Vespertilionidae (Fig. 6h) showed an extended distribution through the southern Andean Yungas forests in the Andean Amazonia.

Average divergence time (ADT) between pairs of mammalian species for the AM ranged from 34 to 1.9 million years ago (Mya) (Appendix 1 Table S1.1 in Supporting Information), while ADT for the AF ranged from 26 to 1.8 Mya (Appendix 1 Table S1.2 in Supporting Information). Spatial projection of ADT values showed older times of divergence for AM grids than for AF grids, with ADT values varying from about 27 to 4 Mya for AM grids (Fig. 4a) and from about 14 to 1 Mya for AF grids (Fig. 4b). Spatial projections of ADT revealed incongruent values between AM and AF grids for the NE and the SE-NW route, but mainly for the SE-NW route in which divergence times ranged from 27 to 5 Mya for AM grids and from 8 to 2 for AF grids. The same pattern was found when mammalian families were evaluated separately (Fig. 5 and Fig. 6), except for Cricetidae and Echimyidae (Fig. 5c and Fig. 6g) and other families with only a few species or clades for each biome (Fig. 6c, e, i and j). Spatial projections of ADT for the interior Cerrado region revealed similar values between AM and AF (Fig. 4a and 4b).

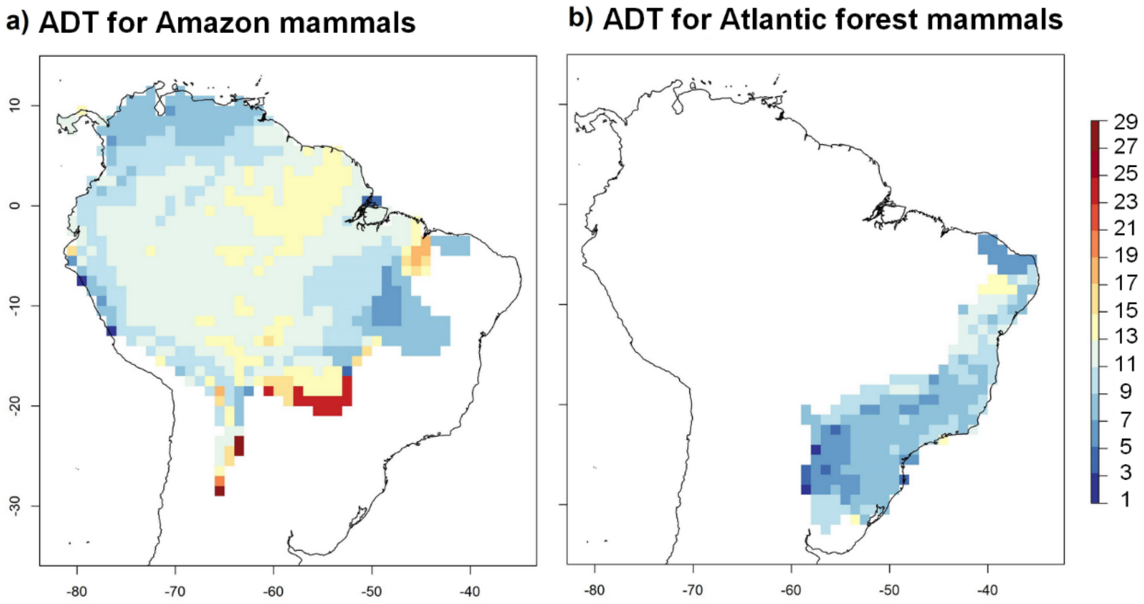

Figure 4. Average divergence times per plot between a) Amazonian and b) Atlantic Forest mammalian species showing spatiotemporal patterns of connections between species from these regions. Color scale indicates intervals of divergence time in millions of years ago (Mya).

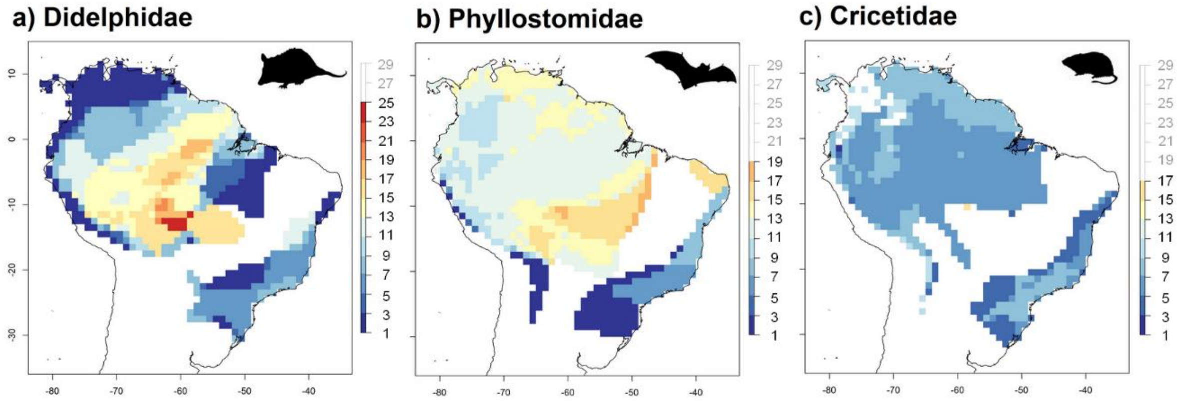

Figure 5. Average divergence time per plot between Amazonian and Atlantic Forest mammals according to family showing spatiotemporal patterns of connections between species from these regions. Color scale indicates intervals of divergence times in millions of years ago (Mya).

The time scale for ADT between AM and AF mammalian species varied among families. The rodent family Cricetidae had more recent divergence times per plot, dated from Early Miocene to Early Pleistocene (ranging from about 16 to 2 Mya, Fig. 4d) compared to the other families, with the exception of the primate families, Atelidae and Callitrichidae, and the artiodactyl family Cervidae, for which divergence times per plot dated from Late Miocene (Fig. 6a, c and j), and Cebidae, for which divergence times per plot dated from Late to Early Miocene (Fig. 6b). Echimyidae had divergence times per plot dated from Early to Middle Miocene (~22 to 14 Mya, Fig. 6g), as did Pithecidae, Dasyproctidae and Bradypodidae (represented by *Bradypus torquatus* and *Bradypus tridactylus*) (Fig. 6d, f and i). Didelphidae, as well as Phyllostomidae and Vespertilionidae, had a wide range of divergence times per plot dated from Late Oligocene to Early Pleistocene (~24 to 2 Mya, Fig. 4a, 4b and Fig. 6h). Rodents of the family Erethizontidae were represented by the genus *Coendou* versus *Chaetomys*, for which species diverged between the AM and the AF at about 27 Mya in the Late Oligocene (Fig. 6e).

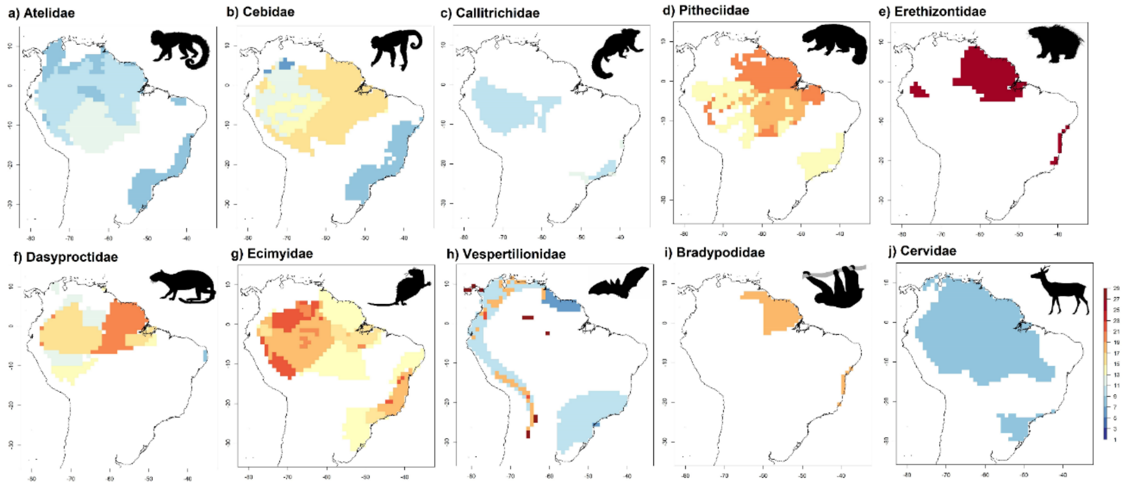

Figure 6. Average divergence time per plot between Amazonian and Atlantic Forest mammals by family showing spatiotemporal patterns of connections between species from these regions. Color scale indicates intervals of divergence times in millions of years ago (Mya).

Discussion

This is the first study to directly test the hypothesis of temporal variation in the connection routes between the AM and the AF (Ledo & Coli, 2017; Batalha-Filho et al., 2013) and the first to evaluate these connections by integrating phylogenetic and geographic data using multiple mammalian taxa. Our results showed asymmetry in the divergence times between the AM and the AF for the tested temporal variation hypothesis (Por, 1992; Oliveira-Filho & Ratter, 1995; Costa, 2003; Batalha-Filho et al., 2013). The previous hypothesis about the spatiotemporal pattern of connections between the AM and the AF proposed old connections through the SE-NW route (Batalha-Filho et al., 2013) and no older connections through the NE route (Por, 1992; Batalha-Filho et al., 2013; Ledo & Coli, 2017). Our results, however, show recent divergence times for the SE-NW route in the AF and older divergence times for the NE route in the AF, except for some families as discussed below. The discrepancy between our results and results from previous studies can be explained by the approach used here. Assessing not only the general pattern, but also within taxonomic groups can reveal, as we see here, different patterns from those previously suggested. Even though we did not test the connection routes themselves, our results show three previously suggested connection routes — NE route, gallery forests of Central Brazilian Cerrado route and SE-NW route (Por, 1992; Oliveira-Filho & Ratter, 1995; Silva et al., 1996; Costa, 2003) — and not just two routes

— (NE and SE-NW routes) — as suggested by other studies (Bigarella, 1975; Batalha-Filho et al., 2013; Ledo & Colli, 2017). This third route, through the gallery forests of Central Brazilian Cerrado, should be further tested to establish their identity as an independent route for other groups. This connection route has been previously considered for birds and mammals (Silva et al., 1999; Costa, 2003), and shows connections between the Southeast AF and the Southeast and/or Northeast AM. We suggest that this connection be referred to as the Southeast AF-Southeast AM (SE-SE) route, and now discuss spatiotemporal patterns found throughout all these areas of connection between the AM and the AF and for each of the connection regions specifically.

Spatiotemporal patterns throughout connection routes:

The spatiotemporal patterns of the connections between the AM and the AF have only been evaluated symmetrically (Ledo & Coli, 2017; Batalha-Filho et al., 2013), under the assumption of temporal reciprocity within each route. We, however, have found spatiotemporal patterns within each region for some mammal families opposite to those reported in the literature Although we found older divergence times for AM region closer to the SE-NW route, we also found recent and old times for the NE route. However, when evaluating the spatiotemporal pattern for the AF, we found younger divergence times for the SE-NW route and both old and recent times for the NE route, differing from what has been reported in the literature (Ledo & Coli, 2017; Batalha-Filho et al., 2013). We also found both older and more recent divergence times for the NE route compared to the SE-NW route for the AF region. When we evaluated these spatiotemporal patterns for each family of mammals, we found older times for the SE-NW route, such as for Didelphidae in both the AM and the AF, and for Cricetidae in the AM. However, for the AF, most of the assessed families showed more recent divergence times in the SE-NW route. Therefore, the present results for the AF were, in the vast majority of cases, contrary to what was previously proposed (Por, 1992; Batalha-Filho et al., 2013; Ledo & Coli, 2017), with more recent times for the SE-NW route and older times for the NE route.

Our results reveal that these routes appear to have occurred concurrently over different times, which prevents the observation of general spatiotemporal patterns. Therefore, historical connections between the AM and the AF cannot be explained by a single spatiotemporal pattern as previously shown for the connections between these regions for small mammals (Costa, 2003). In addition, our results differed among families and between biomes, being congruent with that previously described in the literature for only one of the evaluated families [family Didelphidae, one of the oldest mammal families in the Neotropics (Goin et al., 2012), as discussed further below], revealing the importance of also evaluating patterns for specific groups. Indeed, many studies have found incongruent patterns in biogeography, suggesting that the search for temporal patterns should not always be generalized, but rather investigated based on specific studies of multiple taxa (Cho & Shank, 2010; Vale & Jenkins, 2012; Serra-Diaz, 2013; Martín-Regalado et al., 2019). Furthermore, species-specific biological traits are crucial when looking for congruencies in biogeographical scenarios and for assessing the dissimilarity of patterns (Papadopoulou & Knowles, 2016). In each topic that follows we discuss the divergence times of the present results for each region according to the connection routes previously suggested in the literature (Por, 1992; Oliveira-Filho & Ratter, 1995; Silva, 1996; Costa, 2003) and defined a priori in this study, both considering all species and families separately.

1. Northeast route (NE route):

Different from what was expected, the results of the regression revealed old divergence times for connections between the AM and the AF through the NE in AF. Palynological evidence suggests recent connection routes through ‘Brejos de Altitude’ and transition areas with Cerrado (Oliveira et al., 1999), in association with short and recurrent periods of intensified precipitation during the Pleistocene and Holocene (Oliveira et al., 1999; Behling et al., 2000; Auler et al., 2004; Wang et al., 2004). However, since our present results are based on divergences between species or taxa, and not of populations, no recent phylogenetic relationships were recovered as predicted by these available palynological records for the region and also by some phylogeographic studies (Thomé et al., 2016; Gehara et al., 2017; see more in Ledo & Coli, 2017). Besides that, most Neotropical species are older than the Pleistocene corridors (Rull, 2008), making it difficult for multitaxon studies to reveal such recent connections. The most recent divergence times that we recovered (~ 2 Mya) were between the Southwest AF and the Andean Amazonia (e.g. genus *Vampyressa*). The present results reveal that divergence times for the NE ranged between from about 13 to 5 Mya. The results of older divergences between the AM and the AF through the NE route are scarce in the literature (but see Fouquet et al., 2012 for a possible NE connection). On the other hand, evidence of more recent divergence times (~ 3 Mya) was also found for this route between disjunct populations of *Cyclopes didactylus* (Coimbra et al., 2017). Thus, we argue that both early and late connections between the AM and the AF occurred through the NE route. Indeed, our results corroborate this argument, not just for the NE route, but also by showing both recent and old connections through the SE-NW as found in previous studies (Ledo & Coli, 2017), as discussed below.

2. Southeast Atlantic Forest - Southeast Amazonia route (SE-SE route):

Here, we discuss the divergence times revealed in our results for central connections in the 'dry diagonal' located in the Cerrado, corresponding to what we call the SE-SE route. Divergence times for the northern Cerrado were reasonably comparable for both the AM and the AF, which would have occurred between about 15 and 5 Mya. However, there were also connections in southwest Cerrado near the SE-NW route, which would have been disrupted between about 25 and 15 Mya. These divergence times coincide with evidence of past environmental changes in the Cerrado. The Cerrado, as well as other Neotropical biomes, underwent several environmental changes over time that are largely associated with climatic changes defined by periods of greater or lesser rainfall. Palynological evidence indicates that savannas (typical vegetation of the Cerrado) originated recently in relation to rainforests, and diversified during the middle Eocene (Pennington et al., 2006). Paleogeographic studies also indicate an increase in savannas during the late Miocene and early Pleistocene due to marked reduction of rainfall regimes (Ledru, 2002; Azevedo et al., 2020). The results presented here coincide mostly with the last savanna expansion event in the Cerrado. Nevertheless, we note that, as for the other connecting routes, each family investigated here reveals connections between the AM and the AF through this biome at different times. Even so, we highlight that this region seems to represent different temporal patterns in each region of the distance gradient from the NE route to the SE-NW route, as observed by Batalha-Filho et al. (2013) (even though these authors considered this route to be part of the SE-NW). These issues can be resolved with comparative phylogeographic studies of the taxa that evidence these connections (such as Costa, 2003 and the previous chapter of this thesis) presented here and in other multitaxon studies (Batalha-Filho et al., 2013).

3. Southeast Atlantic Forest - Northwest Amazonia route (SE-NW route):

Our results revealed both old and recent divergence times for the SE-NW route considering AM mammals, which agree with the compilation of Ledo & Colli (2017). However, considering AF mammals, our results showed only young divergence times, differing from what was expected based on previous studies that show evidence when restricted to some region, such as the NE route and not the SE-NW route (Batalha-Filho et al., 2013; Ledo & Coli, 2017). These recent connections through the SE-NW route for AF mammals reveal currently interrupted connections with the Andean Amazonia, as well as the role of the AF as a source of dispersion. Since older divergence times were found for the AM than for the AF, evidencing the role of the AM as center of origin for Neotropical mammals (see also Antonelli et al., 2018), what would be the role of the AF as a source of diversity? Recent connections between the AF and the Andean Amazonia highlight the important role of the AF as a source of dispersion (e.g. Maestri et al., 2019). In fact, other studies have shown recent connections between these regions for rodents (Percequillo et al., 2011; Upham et al., 2013), some didelphids (Pavan et al., 2016) and birds (Batalha-Filho et al., 2013; Trujillo-Arias et al., 2017). The results presented here point to the southern Andean Yungas forests as an important element in understanding these connections (as also pointed out by Hueck, 1972). Thus, if these regions were connected in the past, what would have caused this isolation? Many marine and freshwater incursions occurred in South America during the Miocene, such as Lake Pebas in western Amazonia (Wesselingh & Salo, 2006; Hoorn et al., 2010; Roddaz et al., 2010) and the Paraná Sea (Marshall et al., 1993; Marshall & Lundberg, 1996; Hernández et al., 2005; Uba, 2005). Although there is little evidence of a connection between these paleo-seas, they may have provided forest corridors that were subsequently disrupted due to drastic climate changes caused by intensified Andean elevation (Rohrmann et al., 2016), which progressively changed vegetation to arid-adapted floras to the detriment of Paleocene and early Eocene rainforest (Barreda & Palazzesi, 2007).

The old connections revealed for the SE-NW in the AM are corroborated by the climatic context of ancient South America. The climate in northern South America was dry during its process of isolation from Antarctica, Africa and Australia, while the climate in the south was hot and humid, favoring rainforests (Lavina & Fauth, 2011; Goin et al., 2012). Indeed, the biogeographical history of ancient mammals coincides with this scenario. The results for Didelphidae, one of the most ancient mammalian taxa in South America (since Paleocene, see Goin et al., 2012), reveals old divergence times between the AM and the AF for the SE-NW route. However, for caviomorph rodents (families Dasyproctidae, Echimyidae and Erethizontidae) an ancient separation was found between northern AF and eastern AM, which agrees with their biogeographic history of dispersal from Africa at the end of Eocene and early Oligocene (Ortiz-Jaureguizar & Cladera, 2006;

Antoine et al., 2012; Upham & Paterson, 2015). Phyllostomidae, artiodactyls, carnivores and cricetid rodents invaded South America in the Miocene via the Isthmus of Panama (Lim, 2009; Jansa et al., 2014). Therefore, each family reveals specific routes between the AM and the AF for specific periods, which explains why the tested spatiotemporal pattern was not confirmed for most families and even considering all families together. This is because some groups colonized South America in older periods (during the separation of the continents of Africa and America) while others, in more recent periods (during the connection of the Isthmus of Panama). Other interesting biogeographic pattern was also found for the family Phyllostomidae delimiting divergence times between the Northern and Southern AF near the Rio Doce, corroborating previous studies (Carnaval et al., 2014). This pattern does not cover the focus of this study, but it is interesting to highlight it as it is widely discussed and shown in the literature for other groups (Camelier & Zanata, 2014; Neto et al., 2016), including mammals (Costa & Leite, 2000).

Conclusion:

Due to the biogeographic history of each taxonomic group in the Neotropics, they do not present a general congruent spatiotemporal pattern for connections between the AM and the AF. Therefore, historical connections between the AM and the AF cannot be explained by a single spatiotemporal pattern. Thus, our study refutes the previously proposed absence of old biogeographic connections through the NE route (Ledo & Coli, 2017; Batalha-Filho et al., 2013), as well as the absence of recent connections through the SE-NW route (Batalha-Filho et al., 2013) for mammals. This temporal variation differs among the investigated mammalian families according to their evolutionary histories, so the same can be expected for the other routes with both recent and older connections, depending on the evolutionary history and biological traits of each group. Our findings may aid future studies of the connection routes between the AM and the AF, and present new perspectives for these connections and their biogeographic implications. In addition, we warn that biogeographical studies should focus on, and evaluate more carefully, the role of the AF as a source of dispersion in South America for each taxon for which this pattern is being tested.

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Appendices

Appendix 1

Table S1.1. List of mammalian species from the Amazonia with corresponding data for average minimum time of divergence from Atlantic Forest mammals for all phylogenetic trees.

Amazonia species	Family	Order	Divergence time	Standard deviation
Caluromysiops irrupta	Didelphidae	Didelphimorphia	31.26206232	0.971594857
Didelphis marsupialis	Didelphidae	Didelphimorphia	2.87695482	2.388369236
Didelphis imperfecta	Didelphidae	Didelphimorphia	4.4228319	3.046182787
Glironia venusta	Didelphidae	Didelphimorphia	31.36486912	0.517258512
Gracilinanus emiliae	Didelphidae	Didelphimorphia	9.14814537	4.076509775
Hyladelphys kalinowskii	Didelphidae	Didelphimorphia	27.53296175	3.30253989
Marmosa lepida	Didelphidae	Didelphimorphia	11.12905692	4.285678844
Marmosops bishopi	Didelphidae	Didelphimorphia	15.09754534	4.48285701
Marmosops impavidus	Didelphidae	Didelphimorphia	11.60318043	4.330970353
Marmosops neblina	Didelphidae	Didelphimorphia	11.60318043	4.330970353
Marmosops noctivagus	Didelphidae	Didelphimorphia	11.60318043	4.330970353
Marmosops parvidens	Didelphidae	Didelphimorphia	15.09754534	4.48285701
Marmosops pinheiroi	Didelphidae	Didelphimorphia	15.09754534	4.48285701
Marmosa regina	Didelphidae	Didelphimorphia	6.20126932	3.343818737
Monodelphis emiliae	Didelphidae	Didelphimorphia	11.20741282	4.799469421
Monodelphis glirina	Didelphidae	Didelphimorphia	4.85112473	2.966355096

Philander andersoni	Didelphidae	Didelphimorphia	7.1021383	3.556345743
Philander mcilhennyi	Didelphidae	Didelphimorphia	7.1021383	3.556345743
Bradypus tridactylus	Bradypodidae	Pilosa	18.80508729	1.99451772
Mazama nemorivaga	Cervidae	Artiodactyla	8.66389697	2.873436393
Alouatta discolor	Atelidae	Primates	8.35854515	2.849644872
Alouatta macconnelli	Atelidae	Primates	8.66891517	2.751985131
Alouatta nigerrima	Atelidae	Primates	8.35854515	2.849644872
Alouatta seniculus	Atelidae	Primates	8.66891517	2.751985131
Alouatta ululata	Atelidae	Primates	8.35854515	2.849644872
Ateles belzebuth	Atelidae	Primates	12.98967469	2.03725305
Ateles chamek	Atelidae	Primates	12.98967469	2.03725305
Ateles marginatus	Atelidae	Primates	12.98967469	2.03725305
Ateles paniscus	Atelidae	Primates	12.98967469	2.03725305
Lagothrix cana	Atelidae	Primates	9.89804939	2.505168956
Lagothrix lagotricha	Atelidae	Primates	9.89804939	2.505168956
Lagothrix poeppigii	Atelidae	Primates	9.89804939	2.505168956
Callimico goeldii	Callitrichidae	Primates	10.22562737	1.793575567
Cebuella pygmaea	Callitrichidae	Primates	9.81921456	1.860560962
Mico acariensis	Callitrichidae	Primates	9.81921456	1.860560962
Mico chrysoleucos	Callitrichidae	Primates	9.81921456	1.860560962
Mico humeralifer	Callitrichidae	Primates	9.81921456	1.860560962
Mico intermedius	Callitrichidae	Primates	9.81921456	1.860560962
Mico leucippe	Callitrichidae	Primates	9.81921456	1.860560962
Mico marcai	Callitrichidae	Primates	9.81921456	1.860560962
Mico mauesi	Callitrichidae	Primates	9.81921456	1.860560962
Mico nigriceps	Callitrichidae	Primates	9.81921456	1.860560962
Mico rondoni	Callitrichidae	Primates	9.81921456	1.860560962
Mico saterei	Callitrichidae	Primates	9.81921456	1.860560962
Saguinus bicolor	Callitrichidae	Primates	14.89	1.51E-15
Saguinus fuscicollis	Callitrichidae	Primates	14.89	1.30E-15
Saguinus imperator	Callitrichidae	Primates	14.89	1.36E-15
Saguinus inustus	Callitrichidae	Primates	14.89	1.48E-15
Saguinus labiatus	Callitrichidae	Primates	14.89	1.56E-15
Saguinus martinsi	Callitrichidae	Primates	14.89	1.51E-15
Saguinus mystax	Callitrichidae	Primates	14.89	1.54E-15
Saguinus niger	Callitrichidae	Primates	14.89	1.39E-15

Saguinus nigricollis	Callitrichidae	Primates	14.89	1.30E-15
Cebus kaapori	Cebidae	Primates	13.70785428	2.352775796
Sapajus macrocephalus	Cebidae	Primates	5.46896709	3.504191864
Saimiri boliviensis	Cebidae	Primates	16.8	2.24E-15
Saimiri sciureus	Cebidae	Primates	16.8	2.28E-15
Saimiri ustus	Cebidae	Primates	16.8	2.22E-15
Saimiri vanzolinii	Cebidae	Primates	16.8	2.20E-15
Cacajao calvus	Pitheciidae	Primates	20.24	2.56E-15
Callicebus baptista	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus bernhardi	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus brunneus	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus caligatus	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus cinerascens	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus discolor	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus donacophilus	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus dubius	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus hoffmannsi	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus lucifer	Pitheciidae	Primates	14.25001279	3.497355101
Callicebus moloch	Pitheciidae	Primates	14.78894435	2.933895952
Callicebus purinus	Pitheciidae	Primates	14.25001279	3.497355101
Callicebus torquatus	Pitheciidae	Primates	14.25001279	3.497355101
Callicebus regulus	Pitheciidae	Primates	14.25001279	3.497355101
Chiropotes albinasus	Pitheciidae	Primates	20.24	2.56E-15
Chiropotes chiropotes	Pitheciidae	Primates	20.24	2.52E-15
Chiropotes satanas	Pitheciidae	Primates	20.24	2.50E-15
Chiropotes utahickae	Pitheciidae	Primates	20.24	2.56E-15
Pithecia albicans	Pitheciidae	Primates	20.24	2.43E-15
Pithecia pithecia	Pitheciidae	Primates	20.24	2.47E-15
Ametrida centurio	Phyllostomidae	Chiroptera	6.23770165	2.923897082
Anoura fistulata	Phyllostomidae	Chiroptera	21.99963233	4.638584783
Dermanura rosenbergi	Phyllostomidae	Chiroptera	4.59154703	2.462476749
Artibeus amplus	Phyllostomidae	Chiroptera	1.93800668	1.493590115
Carollia manu	Phyllostomidae	Chiroptera	15.86190094	4.48524345
Choeroniscus periosus	Phyllostomidae	Chiroptera	21.99963233	4.638584783
Glossophaga commissarisi	Phyllostomidae	Chiroptera	21.99963233	4.638584783
Glyphonycteris daviesi	Phyllostomidae	Chiroptera	15.75484837	4.49664954

Lonchorhina inusitata	Phyllostomidae	Chiroptera	17.63058131	5.095366642
Lophostoma occidentalis	Phyllostomidae	Chiroptera	24.84615146	4.41201373
Lophostoma schulzi	Phyllostomidae	Chiroptera	24.84615146	4.41201373
Mesophylla macconnelli	Phyllostomidae	Chiroptera	4.10149598	2.632329538
Neonycteris pusilla	Phyllostomidae	Chiroptera	15.75484837	4.49664954
Platyrrhinus albericoi	Phyllostomidae	Chiroptera	5.0969435	2.664213434
Platyrrhinus aurarius	Phyllostomidae	Chiroptera	5.09548434	2.664109203
Platyrrhinus dorsalis	Phyllostomidae	Chiroptera	5.09548434	2.664109203
Platyrrhinus infuscus	Phyllostomidae	Chiroptera	5.09548434	2.664109203
Platyrrhinus nigellus	Phyllostomidae	Chiroptera	5.09548434	2.664109203
Platyrrhinus vittatus	Phyllostomidae	Chiroptera	5.0969435	2.664213434
Rhinophylla alethina	Phyllostomidae	Chiroptera	13.67847429	4.238644643
Rhinophylla fischerae	Phyllostomidae	Chiroptera	13.67847429	4.238644643
Sphaeronycteris toxophyllum	Phyllostomidae	Chiroptera	6.23770165	2.923897082
Sturnira magna	Phyllostomidae	Chiroptera	11.81315514	3.99233759
Sturnira bidens	Phyllostomidae	Chiroptera	11.81315514	3.99233759
Sturnira oporaphilum	Phyllostomidae	Chiroptera	11.81315514	3.99233759
Vampyressa melissa	Phyllostomidae	Chiroptera	2.05607064	1.889644668
Vampyressa thyone	Phyllostomidae	Chiroptera	2.13635918	1.92307918
Vampyriscus bidens	Phyllostomidae	Chiroptera	7.83681634	3.372411749
Vampyriscus brocki	Phyllostomidae	Chiroptera	7.83681634	3.372411749
Vampyrum spectrum	Phyllostomidae	Chiroptera	24.88735566	4.407160293
Eptesicus chiriquinus	Vespertilionidae	Chiroptera	27.38621409	5.357551092
Histiotus laephotis	Vespertilionidae	Chiroptera	27.38621409	5.357551092
Histiotus humboldti	Vespertilionidae	Chiroptera	27.38621409	5.357551092
Lasiurus atratus	Vespertilionidae	Chiroptera	6.82852324	5.090089493
Myotis keaysi	Vespertilionidae	Chiroptera	9.16726481	4.871539324
Akodon budini	Cricetidae	Rodentia	5.87696484	2.397695264
Akodon siberiae	Cricetidae	Rodentia	5.87696484	2.397695264
Akodon surdus	Cricetidae	Rodentia	3.92404797	2.564651998
Calomys boliviae	Cricetidae	Rodentia	10.48767726	3.132788096
Euryoryzomys emmonsae	Cricetidae	Rodentia	4.03664928	2.456705146
Euryoryzomys legatus	Cricetidae	Rodentia	3.09823584	2.361374692
Euryoryzomys macconnelli	Cricetidae	Rodentia	4.22896521	2.450841651
Hylaeamys tatei	Cricetidae	Rodentia	1.97208795	1.855957834
Neacomys guianae	Cricetidae	Rodentia	9.58920543	2.949464017

Neacomys minutus	Cricetidae	Rodentia	9.58920543	2.949464017
Neacomys musseri	Cricetidae	Rodentia	9.58920543	2.949464017
Neacomys paracou	Cricetidae	Rodentia	9.58920543	2.949464017
Neacomys pictus	Cricetidae	Rodentia	9.58920543	2.949464017
Neusticomys ferreirai	Cricetidae	Rodentia	16.37076431	3.553380406
Oecomys auyantepui	Cricetidae	Rodentia	6.01779506	2.423295718
Oecomys concolor	Cricetidae	Rodentia	6.01779506	2.423295718
Oecomys flavicans	Cricetidae	Rodentia	6.01779506	2.423295718
Oecomys paricola	Cricetidae	Rodentia	6.01779506	2.423295718
Oecomys phaeotis	Cricetidae	Rodentia	6.01779506	2.423295718
Oligoryzomys fulvescens	Cricetidae	Rodentia	9.58920543	2.949464017
Oxymycterus amazonicus	Cricetidae	Rodentia	6.61557326	3.143412379
Podoxymys roraimae	Cricetidae	Rodentia	7.29761565	3.399604453
Rhipidomys couesi	Cricetidae	Rodentia	7.36508215	3.608741286
Rhipidomys leucodactylus	Cricetidae	Rodentia	7.36583542	3.589927202
Rhipidomys modicus	Cricetidae	Rodentia	7.36508215	3.608741286
Rhipidomys ochrogaster	Cricetidae	Rodentia	7.17681925	3.535918266
Rhipidomys venezuelae	Cricetidae	Rodentia	7.30030797	3.58394281
Scolomys ucayalensis	Cricetidae	Rodentia	10.73249271	3.079989925
Dasyprocta croconota	Dasyproctidae	Rodentia	13.34826974	5.913073866
Dasyprocta fuliginosa	Dasyproctidae	Rodentia	12.85022121	6.072187527
Dasyprocta guamara	Dasyproctidae	Rodentia	13.24237882	5.962297487
Dasyprocta kalinowskii	Dasyproctidae	Rodentia	13.0951757	5.85880163
Dasyprocta variegata	Dasyproctidae	Rodentia	13.23000053	5.954547395
Myoprocta acouchy	Dasyproctidae	Rodentia	19.56196453	5.387142361
Myoprocta pratti	Dasyproctidae	Rodentia	19.56196453	5.387142361
Dactylomys dactylinus	Echimyidae	Rodentia	14.44509158	1.753078371
Echimys chrysurus	Echimyidae	Rodentia	14.55266086	1.622717542
Echimys saturnus	Echimyidae	Rodentia	14.55266086	1.622717542
Echimys vieirai	Echimyidae	Rodentia	14.55266086	1.622717542
Isothrix negrensis	Echimyidae	Rodentia	19.27586172	2.176139445
Isothrix orinoci	Echimyidae	Rodentia	19.27586172	2.176139445
Isothrix pagurus	Echimyidae	Rodentia	19.27586172	2.176139445
Mesomys occultus	Echimyidae	Rodentia	19.3386864	2.189657764
Mesomys stimulax	Echimyidae	Rodentia	19.3386864	2.189657764
Proechimys echinothrix	Echimyidae	Rodentia	21.03414049	2.190637691

Proechimys gardneri	Echimyidae	Rodentia	21.03414049	2.190637691
Proechimys hoplomyoides	Echimyidae	Rodentia	21.03414049	2.190637691
Proechimys kulinae	Echimyidae	Rodentia	21.03414049	2.190637691
Proechimys pattoni	Echimyidae	Rodentia	21.03414049	2.190637691
Proechimys quadruplicatus	Echimyidae	Rodentia	21.03414049	2.190637691
Proechimys steerei	Echimyidae	Rodentia	21.03414049	2.190637691
Toromys grandis	Echimyidae	Rodentia	16.42830401	1.999017373
Toromys rhipidurus	Echimyidae	Rodentia	16.42830401	1.999017373
Coendou ichillus	Erethizontidae	Rodentia	26.3645882	2.470082679
Coendou melanurus	Erethizontidae	Rodentia	26.3645882	2.470082679
Coendou nycthemera	Erethizontidae	Rodentia	26.3645882	2.470082679
Coendou roosmalenorum	Erethizontidae	Rodentia	26.3645882	2.470082679

Table S1.2. List of mammalian species from the Atlantic Forest with corresponding data for average minimum time of divergence from Amazonian forest mammals for all phylogenetic trees.

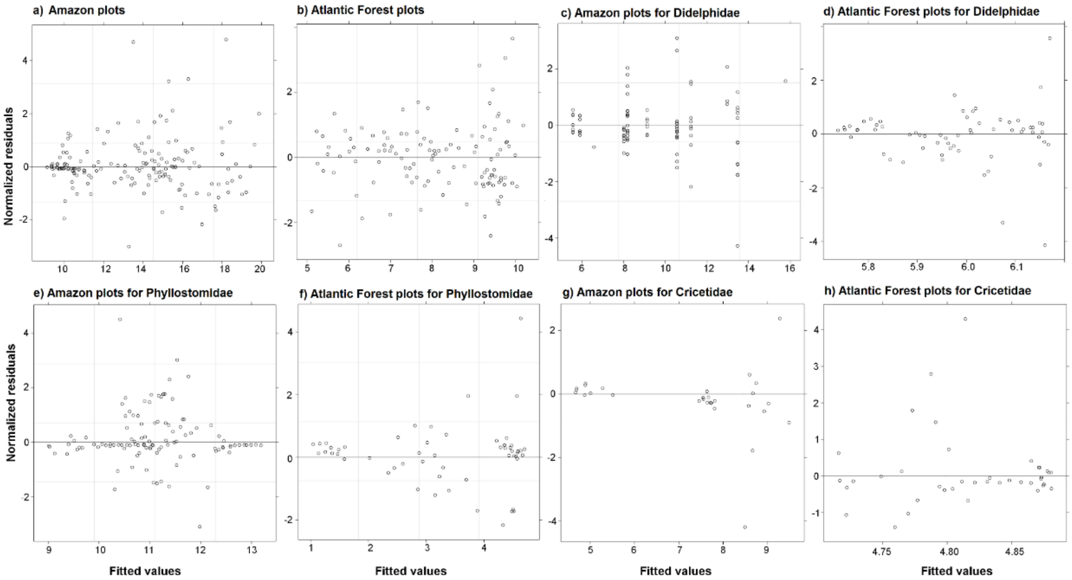
Atlantic Forest species	Family	Order	Divergence time	Standard deviation
Cryptonanus guahybae	Didelphidae	Didelphimorphia	12.48582494	4.393553155
Didelphis aurita	Didelphidae	Didelphimorphia	2.72971637	2.33004173
Gracilinanus microtarsus	Didelphidae	Didelphimorphia	9.14814537	4.076509775
Marmosops incanus	Didelphidae	Didelphimorphia	11.60318043	4.330970353
Marmosops paulensis	Didelphidae	Didelphimorphia	11.60318043	4.330970353
Marmosa paraguayanus	Didelphidae	Didelphimorphia	6.20126932	3.343818737
Monodelphis iheringi	Didelphidae	Didelphimorphia	4.84447706	2.961617833
Monodelphis scalops	Didelphidae	Didelphimorphia	8.58423808	3.884076527
Philander frenatus	Didelphidae	Didelphimorphia	7.1021383	3.556345743
Bradypus torquatus	Bradypodidae	Pilosa	18.80508729	1.99451772
Mazama bororo	Cervidae	Artiodactyla	8.66389697	2.873436393
Mazama nana	Cervidae	Artiodactyla	8.66389697	2.873436393
Alouatta guariba	Atelidae	Primates	7.86721024	2.89245201
Brachyteles arachnoides	Atelidae	Primates	9.89804939	2.505168956
Brachyteles hypoxanthus	Atelidae	Primates	9.89804939	2.505168956
Callithrix aurita	Callitrichidae	Primates	9.81921456	1.860560962
Callithrix flaviceps	Callitrichidae	Primates	9.81921456	1.860560962
Leontopithecus chrysomelas	Callitrichidae	Primates	12.2117869	1.6551566
Leontopithecus chrysopygus	Callitrichidae	Primates	12.2117869	1.6551566
Leontopithecus rosalia	Callitrichidae	Primates	12.2117869	1.6551566
Sapajus flavius	Cebidae	Primates	7.1089477	3.768666122
Sapajus nigritus	Cebidae	Primates	7.92643265	3.328723102
Sapajus robustus	Cebidae	Primates	7.92643265	3.328723102
Sapajus xanthosternos	Cebidae	Primates	8.46096548	3.735426288
Callicebus melanochir	Pitheciidae	Primates	14.41683034	3.281137581
Callicebus nigrifrons	Pitheciidae	Primates	14.42825015	3.274985275
Callicebus personatus	Pitheciidae	Primates	14.39325598	3.327792333
Artibeus fimbriatus	Phyllostomidae	Chiroptera	1.93800668	1.493590115
Lonchophylla mordax	Phyllostomidae	Chiroptera	16.82474189	4.771138839
Platyrrhinus recifinus	Phyllostomidae	Chiroptera	5.09548434	2.664109203
Vampyressa pusilla	Phyllostomidae	Chiroptera	1.77647195	1.795547

Lasiurus ebenus	Vespertilionidae	Chiroptera	6.82852324	5.090089493
Myotis ruber	Vespertilionidae	Chiroptera	9.16726481	4.871539324
Abrawayaomys ruschii	Cricetidae	Rodentia	12.07222589	3.564935079
Akodon cursor	Cricetidae	Rodentia	4.52351726	2.340210144
Akodon montensis	Cricetidae	Rodentia	4.32958093	2.41469187
Akodon paranaensis	Cricetidae	Rodentia	4.16666133	2.540020112
Akodon serrensis	Cricetidae	Rodentia	7.11004062	2.877220455
Bibimys labiosus	Cricetidae	Rodentia	10.78315433	3.667354672
Blarinomys breviceps	Cricetidae	Rodentia	9.97069933	4.421439033
Brucepattersonius guarani	Cricetidae	Rodentia	10.18099349	4.135499511
Brucepattersonius griserufescens	Cricetidae	Rodentia	10.18099349	4.135499511
Brucepattersonius igniventris	Cricetidae	Rodentia	10.18099349	4.135499511
Brucepattersonius misionensis	Cricetidae	Rodentia	10.18099349	4.135499511
Brucepattersonius soricinus	Cricetidae	Rodentia	10.18099349	4.135499511
Delomys dorsalis	Cricetidae	Rodentia	10.93081518	3.270981476
Delomys sublineatus	Cricetidae	Rodentia	10.93081518	3.270981476
Euryoryzomys russatus	Cricetidae	Rodentia	3.02227519	2.359449383
Hylaeamys laticeps	Cricetidae	Rodentia	2.33495633	1.904020126
Hylaeamys oniscus	Cricetidae	Rodentia	2.4780177	2.016470984
Juliomys pictipes	Cricetidae	Rodentia	13.69898736	3.578344777
Juliomys rimofrons	Cricetidae	Rodentia	13.69898736	3.578344777
Oxymycterus caparoae	Cricetidae	Rodentia	6.71421063	3.182531967
Oxymycterus hispidus	Cricetidae	Rodentia	7.15518897	2.885863477
Phaenomys ferrugineus	Cricetidae	Rodentia	12.26468941	3.529832762
Rhagomys rufescens	Cricetidae	Rodentia	14.15766504	3.29825243
Rhipidomys mastacalis	Cricetidae	Rodentia	5.16420598	3.236043582
Dasyprocta iacki	Dasyproctidae	Rodentia	8.03934679	5.234653906
Kannabateomys amblyonyx	Echimyidae	Rodentia	14.44509158	1.753078371
Phyllomys blainvillii	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys dasythrix	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys kerri	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys lamarum	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys lundi	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys mantiqueirensis	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys medius	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys nigrispinus	Echimyidae	Rodentia	14.55266086	1.622717542

Phyllomys pattoni	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys thomasi	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys sulinus	Echimyidae	Rodentia	14.55266086	1.622717542
Phyllomys unicolor	Echimyidae	Rodentia	14.55266086	1.622717542
Trinomys gratiosus	Echimyidae	Rodentia	22.38174173	2.133988273
Trinomys iheringi	Echimyidae	Rodentia	22.38174173	2.133988273
Trinomys mirapitanga	Echimyidae	Rodentia	22.38174173	2.133988273
Trinomys paratus	Echimyidae	Rodentia	22.38174173	2.133988273
Chaetomys subspinosus	Erethizontidae	Rodentia	26.3645882	2.470082679

Appendix 3

Figure S3.1. Model validation graphs.



CONSIDERAÇÕES FINAIS E CONCLUSÃO GERAL

As conexões biogeográficas entre a Amazônia e a Mata Atlântica foram recentemente foco de alguns estudos (Costa, 2003; Batalha-Filho et al., 2013; Sobral-Souza et al., 2015; Ledo & Colli, 2017), porém poucos antes do aqui apresentado usaram os mamíferos como sistema modelo (Cortés-Ortiz et al., 2003; Nascimento et al., 2008; Pavan et al., 2011) e menos ainda considerando múltiplas espécies de mamíferos (Costa, 2003). Estudos anteriores propuseram três principais rotas de conexão histórica entre a Amazônia e a Mata Atlântica: (1) a rota do Nordeste através das florestas do Nordeste do Brasil; (2) pelas florestas de galeria do nordeste do Cerrado e (3) a rota Sudeste-Noroeste através das florestas da Bacia do Paraná, Chaco úmido e Pantanal passando por florestas do sudoeste do Cerrado (Por, 1992; Oliveira-Filho & Ratter 1995; Costa, 2003). A rota do Nordeste seria a mais recente e a rota do Sudeste-Noroeste mais antiga e estabelecida com maior frequência (Por, 1992). Recentemente, alguns estudos buscaram testar essa hipótese, corroborando-a através da biogeografia de aves (Batalha-Filho et al., 2013) e através da revisão de evidências moleculares para diferentes espécies animais (Ledo & Colli, 2017). Ainda assim, pouco se sabia sobre a totalidade das espécies que poderiam evidenciar as conexões entre esses biomas para confirmação dessa hipótese.

O estudo aqui apresentado trouxe amplas informações sobre as espécies de mamíferos potenciais para investigar as conexões biogeográficas entre a Amazônia e a Mata Atlântica. Além disso, testamos as hipóteses sugeridas para as rotas de conexões entre esses biomas, sua frequência, padrões espaço-temporais e variações ao longo do tempo. Encontramos um grande número de espécies de mamíferos potenciais para investigar as conexões entre esses biomas. Diferente do proposto anteriormente (Por, 1992; Batalha-Filho et al., 2013; Ledo & Colli, 2017), encontramos que a rota do Nordeste foi a mais frequente quando avaliada para populações de espécies de mamíferos em detrimento da rota do Sudoeste sugerida como a mais importante delas. De acordo com Costa (2003), a rota que passa pelas florestas de galeria do bioma do Cerrado também foi muito importante para os mamíferos aqui investigados. Também encontramos uma relação entre uso de habitat e os tipos de rotas de conexão. Além disso, encontramos uma grande quantidade de dados genéticos disponíveis para muitas dessas espécies, destacando seu potencial para estudos biogeográficos sobre as conexões entre a Amazônia e a Mata Atlântica. Nossos resultados apoiam estudos futuros sobre as conexões entre a Amazônia e a Mata Atlântica.

Um ponto importante que alcançamos com os estudos apresentados nesta tese é esclarecer a definição das rotas de conexão entre a Amazônia e a Mata Atlântica. Não havia um consenso na literatura recente sobre essas rotas de conexões. Com base na proposta de conexões biogeográficas associadas a similaridades climáticas atuais e passadas da América do Sul proposta por Bigarella & Andrade-Lima (1982), estudos recentes consideraram apenas duas rotas de conexão (Batalha-Fiho et al., 2013; Ledo & Coli, 2017). Sendo elas a rota do Nordeste (rota NE), a qual representa conexões entre o Leste da Amazônia e o Nordeste da Mata Atlântica (incluindo aparentemente as conexões através das florestas do Cerrado como sendo desta rota), e a rota do Sudoeste, a qual representa conexões entre o Oeste da Amazônia e o Sudeste da Mata Atlântica. Ainda assim, muitos estudos já haviam descrito pelo menos três rotas de conexão, avaliadas através da distribuição das espécies destacando a importância da rota através das florestas de galeria do Cerado (Por, 1992; Oliveira-Filho & Ratter, 1995; Silva, 1996; Costa, 2003). Além disso, mesmo considerando condições de similaridade climática da América do Sul entre Leste e Oeste da Amazônia e Norte/Sul da Mata Atlântica, foram encontradas três rotas de conexão (Sobral-Souza et al., 2015). Desta forma, sugerimos aqui considerar a rota através das florestas do Cerrado como uma rota independente, a qual definimos como rota de conexão entre o Sudeste da Amazônia e o Sudeste da Mata Atlântica (rota SE-SE) usando como base as regiões biogeográficas de ambos os biomas, os resultados aqui apresentados e corroborados pela literatura (Por, 1992; Oliveira-Filho & Ratter, 1995; Silva, 1996; Costa, 2003).

Através da filogeografia comparada e da paleodistribuição de espécies de marsupiais, encontramos mais de uma rota de conexão para dois períodos do passado recente. Além disso, sugerimos que características biológicas e históricas específicas de cada grupo são fatores importantes a serem considerados na avaliação de respostas a mudanças climáticas passadas. Diferentemente do que foi encontrado em estudos anteriores sobre as conexões entre a Amazônia e a Mata Atlântica por meio da paleodistribuição (Sobral-Souza et al., 2015; Leite et al., 2016; Costa et al., 2017; Ledo & Colli, 2017), não encontramos uma expansão considerável na distribuição pálida das espécies investigadas aqui durante o Último Máximo Glacial (LGM). Para a maioria das espécies, houve uma diminuição significativa da distribuição de paleodistribuição durante o LGM em relação ao presente, exceto para a espécie mais resiliente entre as investigadas que ocupa áreas florestais perturbadas (Pardini, 2004; Passamani et al., 2005). Os achados desse capítulo são particularmente importantes para a conservação de espécies por meio de estudos sobre as respostas das espécies às futuras mudanças climáticas (da Silva et al., 2020). Além disso, destacamos que os dados genéticos utilizados vieram principalmente de bancos de dados online, mostrando que o aumento da disponibilidade de dados moleculares nos últimos anos favorece os estudos biogeográficos. Assim, encorajamos os dados moleculares disponíveis nas bases de dados a serem utilizados para responder a questões de ecologia biogeográfica e evolutiva.

Ao avaliarmos os padrões espaço-temporais das conexões entre a Amazônia e a Mata Atlântica para múltiplas espécies de mamíferos, corroboramos que a Amazônia foi o principal centro de origem dos mamíferos neotropicais (Antonelli et al., 2018). Através desse estudo com múltiplas espécies, refutamos a ausência proposta anteriormente de conexões biogeográficas antigas pela rota do Nordeste (Ledo & Coli, 2017; Batalha-Filho et al., 2013), bem como a ausência de conexões recentes pela rota do Sudeste-Noroeste (Batalha-Filho et al., 2013) para mamíferos. Outro ponto importante que encontramos através desse estudo dos padrões espaço-temporais dessas conexões para múltiplas espécies é que a história biogeográfica de cada grupo influenciou nas diferenças desses padrões e quando estudos multiespécies não consideram essas relações independentes entre grupos taxonômicos, a incongruência entre padrões pode ser subestimada. Essas descobertas podem auxiliar estudos futuros sobre as rotas de conexão entre a Amazônia e a Mata Atlântica e apresentar novas perspectivas para essas conexões e suas implicações biogeográficas. Além disso, alertamos que os estudos biogeográficos devem focar e avaliar com mais cuidado o papel da Mata Atlântica como fonte de dispersão na América do Sul para cada táxon para o qual esse padrão está sendo testado.

O estudo aqui apresentado trouxe novos insights sobre as conexões biogeográficas entre a Amazônia e a Mata Atlântica avaliadas através da biogeografia de diversas espécies de mamíferos, de diferentes grupos taxonômicos, através de diferentes abordagens biogeográficas compilando um grande número de espécies potenciais para estudos futuros sobre o tema e a disponibilidade de dados permitindo a inclusão do contexto evolutivo nessas análises. Além de abordagens de filogeografia comparada e modelos de paleodistribuição, também inovamos utilizando métricas geralmente usadas em análises de comunidades para avaliar o padrão espaço-temporal dessas rotas de conexão para múltiplas espécies. Através de dados disponibilizados sobre a distribuição atual das espécies de mamíferos florestais considerando desde o contexto intraespecífico até o contexto interespecífico, exploramos desde conexões históricas recentes até a história biogeográfica das florestas e os mamíferos do passado remoto. Como perspectivas futuras, será muito importante avaliar as respostas dessas florestas e das populações dos mamíferos florestais, assim como de outros grupos que ocorram nessas florestas, frente às mudanças climáticas futuras, tanto em nível ecológico quanto evolutivo, para compreender como as mudanças climáticas poderão alterar os padrões de distribuição das espécies, como suas populações poderão sobreviver frente a essas mudanças considerando as conexões florestais como importante viabilizador da manutenção dessas populações.

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