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Universidade de Brasília

Instituto de Ciências Biológicas

Programa de Pós-Graduação em Ecologia

História das paisagens florestais mésicas dentro da diagonal de formações abertas: contribuições de paleomodelagem, filogeografia de espécies associadas e de conservação

Roger Maia Dias Ledo

Brasília – DF

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Orientador: Dr. Guarino Rinaldi Colli

Co-orientadora: Dra. Lilian G. Giugliano

Tese apresentada ao Programa de Pós-Graduação em Ecologia da Universidade de Brasília como parte dos requisitos necessários para a obtenção do título de Doutor em Ecologia.

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Tese realizada com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/SISBIOTA) e aprovada junto ao Programa de Pós-Graduação em Ecologia da Universidade de Brasília como requisito para obtenção do título de Doutor em Ecologia.

Comissão Examinadora:

Prof. Dr. Guarino R. Colli
Presidente/Orientador
UnB

Prof. Dra. Lilian G. Giugliano
Co-orientadora
UnB

Profa. Dra. Leonora P. Costa
Membro Externo não vinculado ao Programa
UFES

Prof. Dr. Adrian A. Garda
Membro Externo não vinculado ao Programa
UFRN

Prof. Dra. Rosane Collevatti Membro
Externo não vinculado ao Programa
UFG

Prof. Dr. Ricardo Bomfim Machado
Membro Interno vinculado ao
Programa UnB

Prof. Dr. Renato Caparroz
Membro suplente vinculado ao Programa
UnB

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Introdução Geral

Os principais mecanismos que operam na distribuição e diversificação dos organismos na região Neotropical é assunto de grande interesse há mais de um século (Wallace, 1869; Wallace, 1876; Haffer, 1969). A região Neotropical apresenta uma alta biodiversidade (Myers *et al.*, 2000; Rull, 2008; Hoorn *et al.*, 2010) e também uma alta variedade de formações vegetais (Pennington *et al.*, 2000), muitas vezes encontradas em mosaicos e áreas de transição (Bigarella & Andrade-Lima, 1982; Oliveira-Filho & Ratter, 1995, 2000), o que sugere um passado dinâmico, com inúmeros eventos de conexão entre as fisionomias que compõem os principais biomas da região. De forma oposta, o ritmo acelerado de desmatamento que várias dessas formações vegetais têm sofrido (Myers *et al.*, 2000; Klink & Machado, 2005; Mittermeier *et al.*, 2005) faz com que sejam urgentes esforços de compreensão dos principais eventos biogeográficos envolvidos da diversificação das espécies da América do Sul para fins de conservação.

Os domínios da Amazônia e da Floresta Atlântica (Ab' Saber, 1977) representam as florestas tropicais mais diversas do mundo. Entre elas ocorre um corredor de vegetações mais abertas (Werneck, 2011), formado pela Caatinga, no nordeste do Brasil, pelo Cerrado, no Brasil Central e Bolívia, e pelo Chaco argentino e paraguaio. Esse corredor é chamado de "corredor de savanas" (Schmidt & Inger, 1951), "diagonal de formações abertas" (Vanzolini, 1963), "corredor de vegetações xéricas" (Bucher, 1982; Oliveira-Filho & Ratter, 1995) ou ainda de "a maior disjunção da América do Sul" (Brieger, 1969), e tem sido considerado um importante obstáculo para a migração de espécies entre essas duas regiões de florestas tropicais (Por, 1992). Entretanto, analisando mais profundamente, padrões de distribuição disjunta de um considerável número de táxons, ocorrendo tanto no litoral Atlântico quanto na floresta Amazônica (Ducke, 1953; Rizzini, 1963, 1967; Vanzolini, 1970; Bigarella & Andrade-Lima, 1982), e ainda a existência de uma rede ampla e interconectada de vegetações mésicas imersas na diagonal seca (Oliveira-Filho & Ratter, 1995; Vivo, 1997; Oliveira-Filho & Ratter, 2000) sugerem ligações passadas entre esses domínios (Fig. 1).

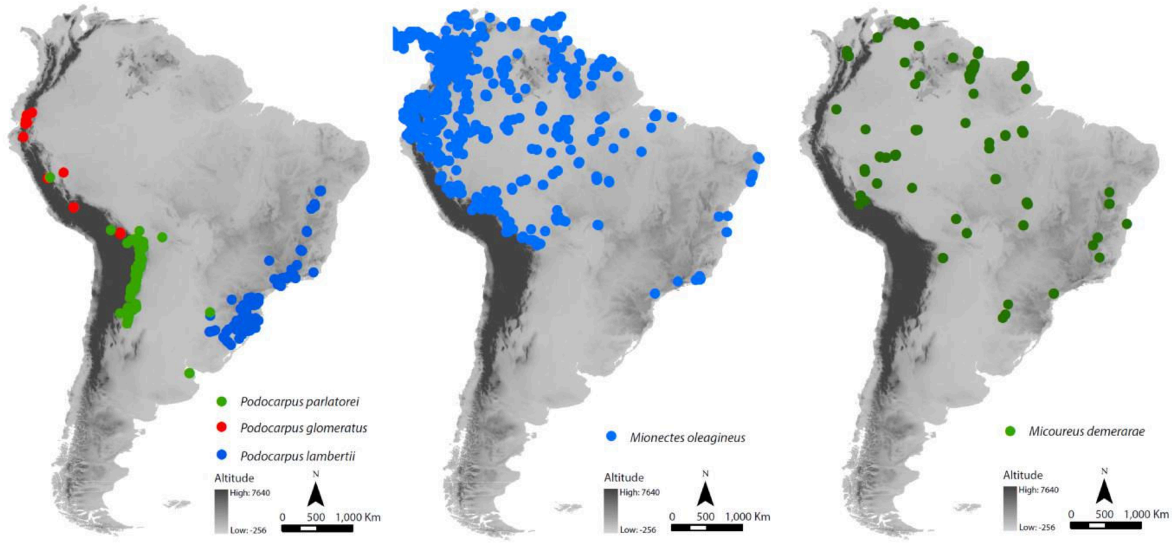

Fig. 1. Padrões de distribuição disjunta de alguns taxons ocorrendo tanto na Amazônia quanto na Floresta Atlântica. Da esquerda para a direita, espécies irmãs de plantas dentro do gênero *Podocarpus* (sensu Quiroga *et al.* 2016), uma espécie de ave do gênero *Mionectes* e uma espécie de marsupial do gênero *Micoureus*. Pontos de ocorrência de cada espécie foram obtidos no site www.gbif.org.

Diversas rotas de conexão entre a Amazônia e Floresta Atlântica já foram postuladas, baseadas na distribuição atual das espécies e no registro fóssil (Bigarella & Andrade-Lima, 1982; Por, 1992; Costa, 2003; Auler *et al.*, 2004; Wang *et al.*, 2004). Uma das rotas poderia ter atravessado a atual distribuição da Caatinga ao longo da região costeira do nordeste do Brasil (Bigarella & Andrade-Lima, 1982), possivelmente associadas à mudanças no deslocamento médio de longo prazo da Zona de Convergência Intertropical e do Verão Monsônico Sul Americano mais ao sul e/ou no aumento da intensidade delas associados a uma maior insolação

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no verão do Hemisfério Sul (Wang *et al.*, 2004; Cruz jr. *et al.*, 2005). Andrade-Lima (1982) considerou ainda as manchas de florestas montanas e submontanas que se encontram isoladas no interior da Caatinga (chamados "brejos de altitude", ou simplesmente "brejos") como possíveis remanescentes de uma cobertura vegetacional mésica mais ampla no passado, relacionada à Amazônia e Floresta Atlântica. Essa hipótese de rota foi sustentada por estudos biogeográficos e moleculares com plantas (Melo Santos *et al.*, 2007), anfíbios (Carnaval & Bates, 2007; Thomé *et al.*, 2016), mamíferos (Costa, 2003) e aves (Batalha-Filho *et al.*, 2013).

Uma segunda rota de intercâmbio biótico entre a Amazônia e Floresta Atlântica na região nordeste, entretanto em nível continental, foi proposta com base em estudos de geomorfologia e datação de rochas calcárias (Auler & Smart, 2001), espeleotemas (Auler & Smart, 2001; Auler *et al.*, 2004), paleopalinologia (De Oliveira *et al.*, 1999), paleontologia (Czaplewski & Cartelle, 1998; Lessa *et al.*, 1998; Ribeiro & Carvalho, 2009) e paleobotânica (Auler *et al.*, 2004), em regiões que no passado suportaram matas de galeria e plantas de florestas úmidas e de altitude (De Oliveira *et al.*, 1999). Essa rota teria cruzado regiões que atualmente são cobertas pela Caatinga e Cerrado na região nordeste (De Oliveira *et al.*, 1999).

Uma outra hipótese de ligação entre a Amazônia e Floresta Atlântica foi proposta (Bigarella *et al.*, 1975), chamada de ponte Sudeste-Noroeste (SE-NW), alternativa às rotas através da Caatinga. Ela foi proposta com base em várias espécies de plantas e animais ocorrendo tanto no oeste da Amazônia quanto no sudeste do Brasil, contudo sem ocorrências na região nordeste. Oliveira-Filho e Ratter (1995) concluíram que essa ponte poderia perfeitamente ter ocorrido no Brasil Central, através das matas de galeria e outras formações florestais do Cerrado. Nesse estudo, os autores apontaram 135 espécies vegetais que ocorrem tanto na Amazônia quanto na Floresta Atlântica e nas matas de galeria do Cerrado. Estudos no Cerrado indicam que as matas de galeria representam unidades distintas, com uma fauna e flora relacionada diretamente com a Amazônia e Floresta Atlântica (Cabrera & Willink, 1973; Rizzini, 1979; Redford & Fonseca, 1986; Oliveira Filho & Ratter, 1995), contribuindo para o aumento da diversidade local e regional do Cerrado (Redford & Fonseca, 1986; Colli et al., 2002; Brandão & Araújo, 2002; Silva, 1995; Silva & Bates, 2002; Oliveira-Filho & Ratter, 1995; Nogueira et al., 2009). Contudo, até o presente momento, essa hipótese ainda não foi efetivamente testada para nenhum táxon, com métodos biogeográficos históricos.

Andrade-Lima (1969) sugere ainda uma rota de intercâmbio em forma de "lua crescente", ligando a Floresta Atlântica e o oeste da Amazônia, com base na distribuição de espécies de pteridófitas. Esse corredor seria amplo e periférico em relação à região nordeste da América do Sul (Salgado-Labouriau *et al.*, 1992), ocorrendo do Sul da Floresta Atlântica (nos Planaltos de Minas Gerais e São Paulo), passando pelas florestas do médio rio Paraná e do rio Paraguai, alcançando as florestas submontanas do oeste da Amazônia (nas cabeceiras do rio Madeira) e atingindo ainda as florestas de altitude das Guianas. Muitas espécies de pteridófitas apresentaram esse formato de distribuição em arco (Tryon & Tryon, 1982). Ainda, a grande maioria das espécies de vespas solitárias dos gêneros *Montezuma* e *Monobia* estão diversificadas e distribuídas ao longo desse arco, chamado por Willink (1988) de arco periamazônico. Por (1992) ainda apresenta essa rota como contínua ou quase contínua, oferecendo um alto grau de conexões biogeográficas entre a Amazônia e Floresta Atlântica e, provavelmente, como a mais importante para trocas bióticas entre os dois domínios (Fig. 2).

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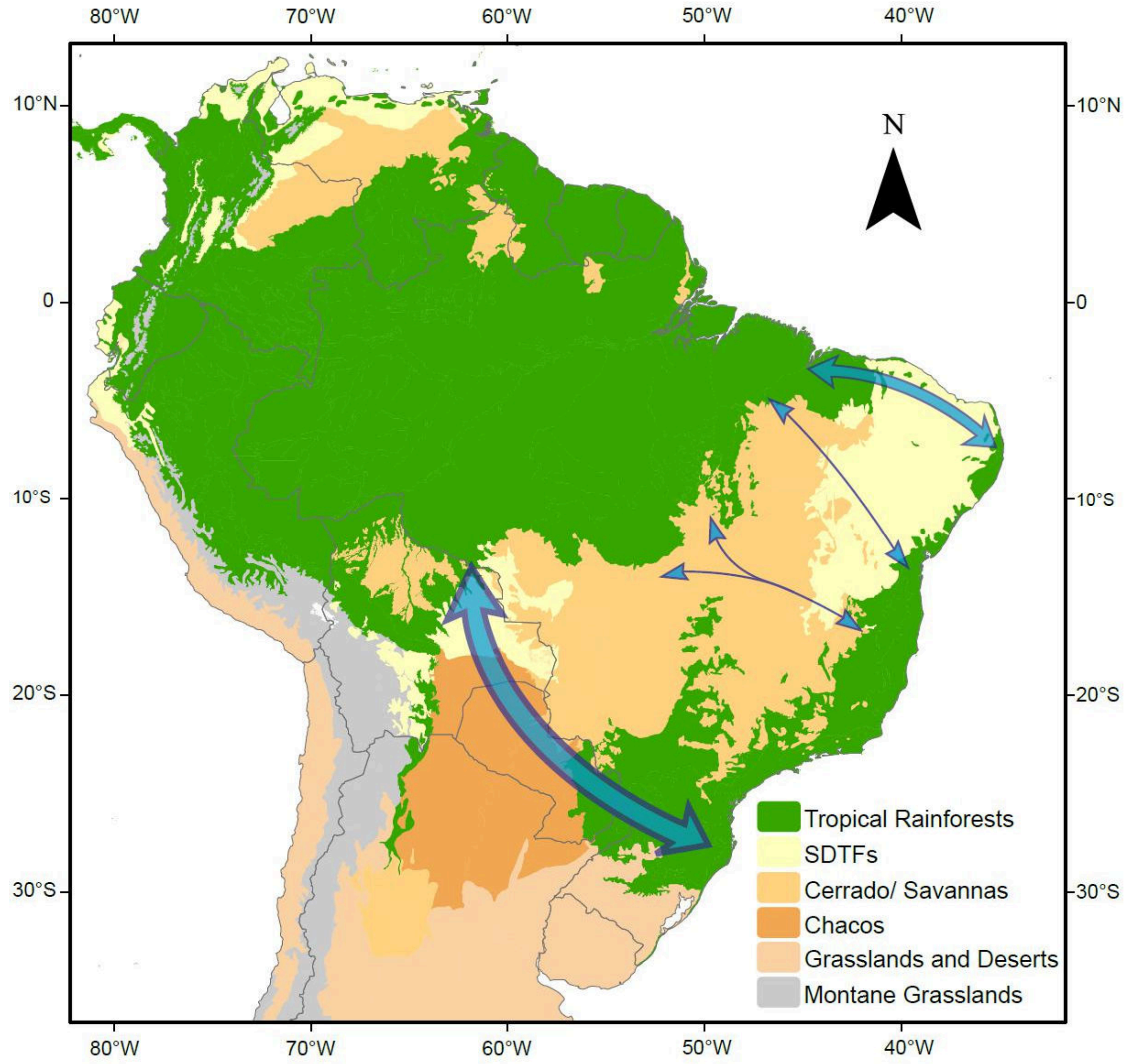

Fig. 2. Principais rotas de intercâmbio biótico entre a Amazônia e a Floresta Atlântica apresentados por Por (1992) (adaptado). A importância de cada rota em termos de recorrência e duração são evidenciadas pela largura das setas.

As formações mésicas (matas de galeria, brejos de altitude e florestas estacionais semideciduais) inseridas na diagonal de formações abertas têm sido pouco valorizadas no contexto biogeográfico da América do Sul (Werneck *et al.*, 2011). Pouco se sabe sobre as origens, estabilidade e dinâmica das matas de galeria no Cerrado e Caatinga, bem com suas relações com a Amazônia e Floresta Atlântica (Oliveira-Filho & Ratter, 1995; De Oliveira *et al.*, 1999; Oliveira-Filho & Ratter, 2000; Auler *et al.*, 2004). Alguns estudos sugerem uma longa história de coexistência entre as matas de galeria e a vegetação savânica do Cerrado (Laboriau, 1963; Innocencio, 1989; Sousa-Neto *et al.*, 2016). Outros estudos afirmam ainda que as flutuações climáticas do Pleistoceno tiveram importância fundamental na formação de espécies e na configuração dessas formações florestais na diagonal de formações abertas da América do Sul (Haffer, 1969; Vanzolini & Williams, 1970; Oliveira-Filho & Ratter, 1995; Silva, 1995; De Oliveira *et al.*, 1999; Rodrigues, 2005; Werneck, 2011). Pouco também se sabe sobre a dinâmica das bacias hidrográficas e seus efeitos na estruturação das populações e comunidades animais distribuídos ao longo delas (Rocha *et al.*, 2011; Rocha *et al.*, 2014). Isso parece ser importante, visto que, por exemplo, no Cerrado são encontradas nascentes e uma ampla rede de rios relacionados às quatro maiores bacias hidrográficas do Brasil. Por fim, o Brasil ainda pode sofrer grande perda de diversidade florestal de forma silenciosa, haja vista que o código florestal brasileiro foi recentemente modificado para uma lei mais branda (BRASIL, 2012), mesmo com esforços de parte do Congresso Nacional e da sociedade civil em não permitir a mudança. Nota-se que, apesar de todo o embate no Congresso acerca do tema no passado, há uma falta de estudos científicos testando a eficiência dos textos legais do Direito Ambiental para conservação (Metzger, 2010).

Filogeografia é uma disciplina bastante indicada para se estudar a dinâmica de conexão entre a Amazônia e Floresta Atlântica, pois é altamente eficiente em explicar como a distribuição de animais e plantas tem sido influenciada por eventos históricos durante o Terciário e Quaternário (Soltis *et al.*, 2006; Carnaval & Bates, 2007; Leaché *et al.*, 2007; Beebee & Rowe, 2008; Carnaval, 2009; Thomé *et al.*, 2010). A filogeografia também tem sido amplamente

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utilizada em estimativas de parâmetros populacionais (diversidade genética, variações no tamanho populacional, expansões e gargalos populacionais, e migração) (Knowles & Maddison, 2002; Knowles, 2009) ao longo das gerações no passado, na informação de momentos de separação de linhagens (Leaché *et al.*, 2007; Leaché & Fujita, 2010) e ainda na localização de áreas estáveis de endemismo (Carnaval, 2009; Thomé *et al.*, 2010; May *et al.*, 2011), o que é importante para políticas conservacionistas. De uma maneira interessante, estudos moleculares de organismos da América do Sul têm sido crescentes (Turchetto-Zolet *et al.*, 2013), revelando a história da região por meio de datações moleculares e por inferências de parâmetros populacionais.

Associadas à filogeografia, ferramentas de modelagem de nicho também são utilizadas amplamente com populações e ecossistemas (Richards *et al.*, 2007; Richardson *et al.*, 2007), respondendo perguntas acerca da dinâmica de biomas ao longo do tempo, mudanças climáticas e conexões entre formações (Carnaval & Moritz, 2008; Werneck *et al.*, 2011; Werneck *et al.*, 2012), podendo também trazer informações sobre possíveis rotas de intercâmbio de fauna entre a Amazônia e Floresta Atlântica. Estudos independentes também são utilizados para a compreensão de condições climáticas do passado e muitas vezes são utilizados na validação de modelagens, como palinologia e plaeocronologia de espeleotemas (De Oliveira *et al.*, 1999; Auler & Smart, 2001; Auler *et al.*, 2004; Cheng *et al.*, 2013).

Répteis (e em específico, lagartos) são considerados animais modelo para diversos estudos, incluindo filogeografia (Camargo *et al.*, 2010), pois são altamente relacionados ao ambiente onde vivem (Colli et al., 2002; Nogueira et al., 2005; Bastazini et al., 2007; Vitt et al., 2007; Nogueira et al., 2009), além de ocorrerem em alta abundância nas comunidades. Assim, a filogeografia de répteis, além de informar as relações e tempos de divergência entre as linhagens

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ou espécies próximas, também pode informar indiretamente a origem e a dinâmica das paisagens na qual essas espécies estão inseridas, bem como possíveis rotas de trânsito entre áreas.

Considerando o expresso acima, essa tese representa um conjunto de esforços no objetivo de compreender a dinâmica das paisagens florestais através da diagonal de formações abertas. Para isso utilizaremos abordagens de modelagem de nicho, validação de modelos com estudos paleoecológicos, além do uso de dados moleculares para elucidar perguntas específicas acerca de conexões antigas entre a Amazônia e a Floresta Atlântica. Além disso, utilizamos dados de ecologia para fazer uma avaliação da eficiência da legislação ambiental brasileira (em específico, o código florestal) na manutenção de espécies de lagartos em matas de galeria. Essa tese está organizada em três capítulos (excetuando a introdução). Nela, testo questões específicas acerca da natureza das rotas de conexão propostas na literatura (capítulo 1), testo especificamente questões acerca da rota proposta por Oliveira-Filho e Ratter (1995) através do Brasil Central (capítulo 2), a rota menos estudada na literatura até o presente momento e, por fim, faço uma avaliação da eficiência do código florestal na conservação de espécies de lagartos em matas de galeria (capítulo 3), considerando que essa lei traz a falsa impressão de conservação. Para cada capítulo apresento previamente um resumo estendido (em português) e, na forma de anexo, trago o manuscrito produzido (em inglês) bem como para qual revista ele foi submetido.

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Capítulo 1 - The historical connections between the Amazon and the Atlantic Forest revisited

(Submetido à Journal of Biogeography)

Resumo

A Amazônia e a Floresta Atlântica estão entre as florestas tropicais mais ricas do mundo. Entre elas há um corredor de vegetações mais abertas formado pela Caatinga, Cerrado e Chaco, considerado obstáculo para o trânsito de espécies florestais. Contudo, distribuições disjuntas de vários taxons ocorrendo tanto na Amazônia quanto na Floresta Atlântica sugerem eventos passados de conexões entre os dois biomas. Diversas rotas foram propostas na literatura, podendo ser resumidamente classificadas em rotas através do nordeste do Brasil, conectando o leste da Amazônia e o norte da Floresta Atlântica; e rotas que conectam o oeste da Amazônia e o sudeste da Floresta Atlântica. Das diversas rotas propostas na literatura, a ponte SE-NW foi considerada contínua ou quase-contínua por Por (1992). Apesar de avanços sobre o tema, perguntas básicas sobre quando as rotas ocorreram, por onde passaram, com que frequência ocorreram e sobre quais condições climáticas existiram permanecem contenciosas. Estudos com modelagem de nicho para o nível ecossistêmico são interessantes alternativas, se associadas com estudos independentes (ex. palinologia e dados moleculares). O presente estudo revisitou as conexões antigas entre a Amazônia e a Floresta Atlântica por meio de modelagem de nicho ecológico e dados paleobiológicos (paleopalinologia e cronologia de espeleotemas) e evidencias moleculares disponíveis na literatura. Além disso, este estudo testou a hipótese de Por (1992) sobre a rota SE-NW sendo a mais importante em termos de frequência e duração.

4000 pontos foram extraídos de um *shapefile* da Amazônia e Floresta Atlântica por meio do aplicativo hawth’s tools→ para Arc Gis 9.3→ e sobre eles foram associadas informações bioclimáticas atuais. Esses pontos foram submetidos a uma análise de componentes principais e posterior análise de agrupamento do tipo K-means, originando dois grupos distintos separados basicamente por diferenças de temperatura e umidade nos meses mais frios. Esses grupos foram ainda suportados por modelos generalizados, considerando o efeito espacial. Em seguida esses grupos de pontos foram utilizados separadamente na modelagem de condições climáticas no presente e projetados para o passado (Holoceno médio, último máximo glacial e último interglacial) e validados com 62 artigos sobre palinologia e cronologia de espeleotemas, buscando informações acerca de condições climáticas e espécies que pudessem estar associadas a cada um dos grupos. Por fim, informações moleculares sobre vertebrados foram utilizadas para avaliar o tempo de ocorrência de cada uma das rotas presentes na literatura, de forma a perceber se a ponte SE-NW teve maior recorrência que as demais rotas propostas.

A modelagem dos dois grupos de pontos mostrou comportamentos distintos em projeções para o Holoceno, último máximo glacial e último interglacial, e esses comportamentos foram muito similares a resultados palinológicos para espécies amazônicas e atlânticas adaptadas a climas quentes e frios. Os resultados da modelagem sugerem que a rota SE-NW ocorreu mais de uma vez no passado e envolveu diferentes comunidades vegetais (adaptadas a condições quentes e adaptadas a condições frias). Informações moleculares de vertebrados disponíveis na literatura corroboram a rota SE-NW como a mais antiga e como contínua ou quase-contínua, suportando a hipótese de Por (1992). Além disso, os dados moleculares sugerem que a rota SE-NW deve ter ocorrido desde o soerguimento dos Andes e o estabelecimento do sistema de monções sul americano. Provavelmente a diversidade Amazônica e da Floresta Atlântica sejam altas também

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por causa do fluxo de espécies entre os biomas pela rota SE-NW ao longo do Terciário e Quaternário.

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Apêndice - manuscrito submetido à revista Journal of Biogeography

Original Article

The historical connections between the Amazon and the Atlantic Forest revisited

Roger Maia Dias Ledo[1, 2], Guarino Rinaldi Colli[2]

[1] Instituto Federal de Educação, Ciência e Tecnologia de Brasília, Campus Samambaia, Área de Meio Ambiente, 72.304-300, Samambaia, DF, Brazil

[2] Universidade de Brasília, Departamento de Zoologia, 70.910-900, Brasília, DF, Brazil

Correspondence author's name (e-mail): Roger Maia Dias Ledo (roger.ledo@ifb.edu.br)

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ABSTRACT

Aim: To assess ancient connections between the Amazon Forest (AM) and the Atlantic Forest (AT) based on environmental niche modelling, palaeobiological, and molecular evidences; to test Por (1992)'s hypothesis of the SE-NW route as the most important route in terms of frequency and duration; to evaluate past climatic conditions associated with the routes.

Location: South America.

Methods: We generated random points from a merged AM and AT shapefile, obtained bioclimatic data for each group, and identified two climatic groups (colder and warmer) with a K-means cluster analysis. We modelled each climatic group with MaxEnt, projecting the results until 120 ka. We used published data on South American palaeopalynology, speleothem, and travertine chronology for model validation. We also accessed vertebrate molecular studies and extracted the split timing of lineages distributed both in the AM and the AT to test Por (1992)'s hypothesis in more ancient times.

Results: Palynological and speleothem studies validated our MaxEnt model, indicating expansion of cold-adapted species along the SE-NW and NE routes in the LGM. The SE-NW route was also supported in the LIG, however involving the expansion of warm-adapted species. Our results support Por (1992)'s hypothesis and suggest that the SE-NW was established more than once in the past, involving different plant communities each time, adapted to different temperature conditions. Vertebrate molecular studies also support Por (1992)'s hypothesis and suggest that the SE-NW route was established since the Andean uplift.

Main Conclusions: The SE-NW route is the most ancient connection route between the AM and the AF and probably was established since the Andean uplift and the formation of the South

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American Monsoon System. This route was established many times in the past, alternating between cold-adapted and warm-adapted plant communities.

Keywords: Amazon Forest, Atlantic Forest, dispersal routes, LIG, LGM, palynology, biotic interchange, species distribution modelling.

INTRODUCTION

Understanding biogeographical patterns and evolutionary processes in the Neotropics has been a subject of great interest for centuries (Wallace, 1852; Bates, 1862; Haffer, 1969). The Amazon Forest (AM) and the Atlantic Forest (AT) are among the most diverse rainforests in the world (Myers *et al.*, 2000; Mittermeier *et al.*, 2005; Hoorn *et al.*, 2010) and between them there is corridor of drier and more open vegetation (Brieger, 1969; Bucher, 1982), formed by the Caatinga, Cerrado, and Chaco (Werneck, 2011). This corridor is an important obstacle for biotic exchange between AM and AT (Raven & Axelrod, 1974; Oliveira-Filho & Ratter, 1995), however several lines of evidence support ancient connections between them.

One route would have occurred along the coastal region of northeastern Brazil in suitable niches (Rizzini, 1963; Bigarella & Andrade-Lima, 1982), supported by species distributions (Ducke, 1953; Rizzini, 1963; Santos *et al.*, 2007), by montane and submontane forest enclaves within the Caatinga (“brejos de altitude”) (Tabarelli & Santos, 2004; Santos *et al.*, 2007), and by biogeographical, systematic, and phylogeographical studies (Costa, 2003; Carnaval & Bates, 2007; Batalha-Filho *et al.*, 2013; Thomé *et al.*, 2016). A second route would also have crossed the interior of the present-day Caatinga, supported by paleopalynological (De Oliveira *et al.*, 1999), travertine and speleothem data (Auler & Smart, 2001; Auler *et al.*, 2004; Wang *et al.*,

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2004), and mammal fossil records (Hartwig & Cartelle, 1996; Czaplewski & Cartelle, 1998; Lessa *et al.*, 1998).

Alternatively to the northeastern routes, Bigarella *et al.* (1975) proposed the southeastern-northwestern bridge (SE-NW) based on the disjunct distribution of several taxa (ferns, insects, mammals) occurring in western AM and southeastern Brazil, but not in northeastern AT (Andrade-Lima, 1969; Willink, 1988; Costa, 2003). Por (1992) conceived this corridor linking southeastern AT (uplands in Minas Gerais and São Paulo), the forests from the medium Paraná and Paraguay rivers, montane and submontane forests of western AM in the Madeira river headwaters, and montane forests of the Guianas. Oliveira-Filho and Ratter (1995) also advocated the SE-NW route occurring through central Brazil, given the vast interconnected net of riparian forests and other forest formations in the Cerrado. Por (1992) suggested this route as continuous or quasi-continuous in terms of frequency and persistence, being the most important between the two biomes (Fig. 1). Recently, Batalha-Filho *et al.* (2013) suggested that the SE-NW and the NE routes occurred in distinct times in the past: the SE-NW route during the Middle and Late Miocene, whereas the NE route during the Pliocene and Pleistocene.

Many studies showed that AM and AT do not behave as distinct units themselves, considering northern AT more related to eastern AM than to southeastern AT itself (Ducke, 1953; Rizzini, 1963; Santos *et al.*, 2007), and southeastern AT more related to western AM (Costa, 2003; Patton & Costa, 2003; Fouquet *et al.*, 2012; Batalha-Filho *et al.*, 2013). However, in spite of the evidence for ancient connections between these two rainforests, basic questions about when, where, how frequently, and under what climatic conditions they occurred remain contentious (Bigarella *et al.*, 1975; Bigarella & Andrade-Lima, 1982; Por, 1992; Oliveira-Filho & Ratter, 1995; Cheng *et al.*, 2013). In this context, ecological niche modelling (ENM) approaches applied to the ecosystem level have brought interesting insights about the evolution of tropical rainforests (Carnaval & Moritz, 2008; Carnaval, 2009; Sobral-Souza *et al.*, 2015), seasonally dry tropical forests (Werneck *et al.*, 2011), and open vegetation biomes of the dry diagonal in South America (Werneck *et al.*, 2012).

Sobral-Souza *et al.* (2015) conducted the only study evaluating past connections between the AM and the AT using ENM so far, reconstructing models for the Last Glacial Maximum (LGM). Their findings supported connections during the LGM between (1) western AM and northern AT along the northern coast of Brazil; (2) southern AT, northern AT and western AM along the southern limits of Caatinga; and (3) southern AT and eastern AM, along the southern limits of the latter. However, we find two important shortcomings in their approach. First, the division of the AM into western and eastern portions based on the course of the Madeira and Amazon rivers is equivocal (e.g., Cheng *et al.*, 2013; Morrone, 2014), not being supported by the very works they cited (Costa, 2003; Fiaschi & Pirani, 2009; Turchetto-Zolet *et al.*, 2013). Second, they made no use of molecular studies or fossil/palaeopalynological records for ENM validation and hypothesis testing (Svenning *et al.*, 2011; Werneck *et al.*, 2011; Collevatti *et al.*, 2013). Palaeobiological information has contributed to understanding climate and vegetation changes in the past (Colinvaux *et al.*, 1996; Colinvaux *et al.*, 2000; Ledru *et al.*, 2009) and is also useful for ENM validation, for targeting new palaeobiological field sites, and for investigating the history of a region (Svenning *et al.*, 2011). Moreover, molecular data are useful for determining areas of vegetation and genetic stability, for constraining geological hypotheses (Baker *et al.*, 2014), and for contrasting ENM projections with molecular signatures (i.e. population expansion, and species divergences) (Carnaval, 2009; Fouquet *et al.*, 2012; Carnaval *et al.*, 2014).

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Here we revisit the ancient connections between the AM and AT using ENM, independent palaeobiological (palaeopalynology and speleothem chronology), and molecular evidences. Specifically, we reconstruct ancient connections between these rainforests with ENM from the present until the Last Interglacial (LIG, 120 ka) and validated our results with palaeobiological information. Moreover, we tested Por (1992)'s hypothesis of the SE-NW route being the most important in terms of frequency and duration using ENM, palaeopalynological, and molecular (vertebrate systematics and phylogeography) data. We predict that, if the connection routes between AM and AT were established during climatic changes in the past, we should be able to reconstruct them through ENM projections until 120 ka. However, these reconstructions must make biological sense, also being supported by palaeobiological data. Further, if Por (1992)'s hypothesis is correct, ENM will suggest the SE-NW route occurring more often than other routes, and the same pattern will be corroborated by palaeobiological and molecular information. Finally, we attempt to determine under what climatic conditions these routes would have been established in the past.

MATERIALS AND METHODS

Environmental niche modelling (ENM)

We downloaded high resolution bioclimatic layers (30 arc-seconds, ~ 1 km) for the Current, Mid-Holocene (6 ka; MIROC-ESM), Last Glacial Maximum (LGM – 21 ka, MIROC-ESM), and Last Interglacial (LIG, 120-140 ka) (Otto-Bliesner *et al.*, 2006) from the WorldClim project (http://www.worldclim.org/). These bioclimatic layers were cropped based on a South America shapefile. We delimited AM and AT based on WWF Ecoregions (available at http://www.worldwildlife.org/) and Oliveira-Filho and Fontes (2000), respectively. However,

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differently from the WWF delimitation, we did not consider the Caatinga forest enclaves as part of these biomes, in a more conservative approach.

We generated 4000 random points from a merged AM and AT shapefile and extracted for them climatic information from the current envelope of bioclimatic variables. Assuming that AM and AT do not behave as independent units, but that part of one biome can be biogeographically (and climatically) more related to part of the other biome and vice-versa, we subjected the bioclimatic data to principal component analysis (PCA) followed by a K-means cluster analysis of PCA scores to identify two distinct climatic spaces. This resulted in two groups, mainly separated by temperature (first principal component axis) and precipitation (second principal component axis) differences (see Appendix S1 in Supporting Information). The first group included points with lower temperature in colder seasons (BIO6), and lower precipitation in drier seasons (BIO17), mainly in southern and central AT, but also in western AM; the second included points with opposite characteristics, including the central and eastern AM, and northern AT (Fig. 2; Table S1.1). These groups were further supported by a generalized linear model analysis with Poisson errors, considering the effect of spatial autocorrelation (Table S1.2; Fig. S.1.1). Considering that these climatic groups also have biogeographical correspondence with the literature (Costa, 2003; Patton & Costa, 2003; Batalha-Filho *et al.*, 2013), we decided to use them into ENM. For clearness and brevity hereafter we will refer to these groups as colder (first group) and warmer (second group).

To avoid redundancy in our ENM, we performed pairwise correlation analyses with the current climatic variables in R (R Core Team, 2013). Highly correlated variables ($r > 0.9$) were excluded from the model, based on their biological relevance and ease of interpretation, following a procedure described by Rissler and Apodaca (2007), and used in Werneck et al.

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(2011; 2012). Based on this, we excluded 8 of the 19 bioclimatic variables, and used the following in ENM: BIO1, BIO2, BIO3, BIO4, BIO7, BIO12, BIO15, BIO16, BIO17, BIO18, BIO19, and altitude.

We modelled the current distribution of the colder and warmer groups of points and projected the results to the Holocene, LGM, and LIG with MaxEnt (Phillips & Dudík, 2008), considering the favourable performance of this algorithm in comparisons (Elith *et al.*, 2006; Pearson *et al.*, 2007; Elith, 2010). To assess model performance, we adopted the area under the curve (AUC) value from the receiver operating characteristic (ROC) curve (Fielding & Bell, 1997).

Palaeobiological and molecular data

We used data from 62 different articles on South American rainforests palaeopalynology, speleothem, and travertine chronology of the Quaternary (see Appendix S1 in Supporting Information) to validate ENM results. These articles included both sites within the current range of the AM and AT and in their predicted ranges. As we used two climatic groups for ENM (colder and warmer), we also separated the pollen record into two groups, based on the pollen spectra characteristics of cold-adapted and warm-adapted forest species already mentioned in the literature (Colinvaux *et al.*, 1996; Marchant *et al.*, 2002; Ledru *et al.*, 2007), and also based in the present-day species distribution and if its distribution is embraced in the colder or warmer groups.

To test Por (1992)'s hypothesis about the importance of the SE-NW route, we accessed molecular systematic and phylogeographic studies of vertebrates with lineages distributed both in the AM and the AT, and we accessed the split timing between these lineages, considering it as

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a “signature” of past connection routes. We decided to use vertebrate data for this purpose due to the growing number of molecular studies on this group during the last 15 years (Costa, 2003; Fouquet *et al.*, 2012; Batalha-Filho *et al.*, 2013; Turchetto-Zolet *et al.*, 2013). To facilitate comparisons, we separated this information into two classes: lineages associated with NE routes, and lineages associated with SE-NW routes.

RESULTS

Environmental Niche Modelling

ENMs of the colder and warmer groups showed AUC values of 0.91 and 0.75, respectively. As expected, ENMs of the colder and warmer groups presented opposite behaviours in our projections of past scenarios. When the colder group showed climatic suitability expansions towards the SE-NW and NE route during the LGM, the warmer group showed retractions and fragmentation in the central and eastern AM; and when the warmer group showed climatic suitability expansions towards the SE-NW route during the LIG, the colder group showed retractions (Fig. 3).

Projections for the Mid-Holocene, LGM and LIG of the colder group indicated expansion of suitable conditions through eastern Paraguay and across Bolivia, in regions of the hypothesized SE-NW route. However, this expansion was most pronounced during the LGM (Fig. 3). All cold group projections recovered high suitability areas in regions of the present-day Caatinga forest enclaves, and projections of the LGM showed larger areas of suitable conditions passing through the “brejos de altitude”, suggesting higher climatic similarity to this group than to the warmer group.

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Projections of the warmer group for the Mid-Holocene and LGM did not indicate any route of forest connection, but reduction and fragmentation of the present-day AM distribution, in regions of the proposed AM dry corridor. However, LIG projections indicated suitable climate expansions through Bolivia, areas of the current Cerrado, and AT, very similar to the SE-NW route.

Palaeopalynological and speleothem studies validation

The majority of the palynological and speleothem studies contained information about the LGM and Holocene, whereas some had plant and/or climate data for 120 ka (Table S2.3, S2.4, and S2.5, respectively). In general, the palynological literature indicates the existence of two different rainforest communities in the AM and AT, and with different requirements and responses in the past, similar to ENM results. One of them includes cold/wet adapted species, i.e. *Podocarpus*, *Weinmannia*, *Hedyosmum*, *Ilex*, *Drimys*, *Myrsine* and *Alnus*, whereas the other is composed by warm/wet adapted species, i.e. Melastomataceae, Combretaceae, Moraceae, Urticaceae, *Copaifera*, *Tapirira*, *Alchornea* and *Aparisthium*. In present-day conditions, these communities occupy different habitats in the AM and AT. *Podocarpus*, *Weinmannia*, *Hedyosmum*, *Ilex*, *Myrsine* and *Alnus* occur in Andean high elevations, in southeastern Brazil highlands, and in mountain refuges of the Caatinga (Ledru *et al.*, 2007), which correspond to the colder group distribution of the ENM. Nevertheless, Melastomataceae, Combretaceae, Moraceae, Urticaceae, *Copaifera*, *Tapirira*, *Alchornea* and *Aparisthium* are typical lowland species present in the central/eastern AM and AT, with records in Cerrado riparian forests (Colinvaux *et al.*, 1996; Mayle *et al.*, 2000; Burbridge *et al.*, 2004; Pessenda *et al.*, 2009), similar to the warmer group distribution of the ENM.

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All palynological studies applied to the LGM suggested temperature reductions of at least 5 C below present (Table S2.4). Nevertheless, contrary to previously thought, LGM temperature reductions of South America were not directly associated with precipitation reductions at the same localities. From 53 studies, 42 suggested moist/wet conditions in at least part of the LGM, and some studies indicated even wetter conditions in the LGM and Holocene than in the present (Fig. 4; Table S2.4). Records of cold-adapted forest elements in lowlands suggest distribution expansion of these species during the LGM and also support conclusions of cold and wet conditions during at least part of this period (Fig. 4; Table S2.4). These results validate our ENM projections to the LGM and support cold and moist conditions during this period.

From 32 studies located in the SE-NW route during the LGM, 26 suggested that the western AM and the southeastern AT persisted wet during the LGM (Fig. 4; Table S2.4). These studies suggested wet conditions during the LGM in the Bolivian Altiplano and nearby (eight of ten studies; Fig. 4; Table S2.4), and in central Brazil (all six studies), along the SE-NW route. Pollen frequency increases of *Podocarpus* and *Ilex* in Laguna Chaplin, near Noel Kempff Mercado National Park, and in central Brazil (Fig 4, Table S2.4) also support this hypothesis. During the same period, regions in northeastern Brazil experienced moist conditions at least in part of the LGM (9 of 11 studies), and experienced the expansion of montane cold adapted forest species (*Podocarpus*, *Rapanea*, *Hedyosmum*, *Humiria*, and *Ilex*) as well (Fig. 4; Table S2.4). However, most of these studies suggest shorter periods of wet conditions along the NE route compared to the SE-NW route. These results validate our model projections for the LGM and corroborate forest expansions through the SE-NW and NE routes through cold and moist conditions (Fig. 4). This scenario changed in the Holocene, with cold-adapted forest distribution

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retraction, and with warm-adapted forests and savanna elements expansion into lowlands (Table S2.3).

Studies suggest warmer and wet conditions than the present for the LIG on the Bolivian Altiplano and southeastern Brazil (Table S2.5; Fig. 5). The SE Brazil experienced high rates of Flacourtiaceae, Melastomataceae/Combretaceae, and total arboreal pollen increase in Colônia (Ledru *et al.*, 2009), and the Bolivian Altiplano experienced high pollen abundance of *Polylepis*/*Acaena* and of Chenopodiaceae/Amaranthaceae, species adapted to warmer conditions and that nowadays are not found in this region (Gosling *et al.*, 2008). Speleothem studies also support wet conditions for the LIG in western AM (Cheng *et al.*, 2013) (Table S2. 5; Fig. 5). These results support warm and wet conditions in western AM and southeastern Brazil, validating our ENM, and supporting the SE-NW route in this period. Based on this, we can suggest that the SE-NW route might have occurred during the LIG and during the LGM, but under different climatic conditions (warm/wet and cold/wet, respectively), and involving different plant communities (cold-adapted and warm-adapted forests). These results support Por (1992)'s hypothesis and also suggest a mechanism by how the SE-NW route could have occurred many times in the past. Considering that the western AM maintained wetter conditions for longer periods (Colinvaux *et al.*, 1996; Colinvaux *et al.*, 2000; Cheng *et al.*, 2013), and the persistence of the South American monsoon system (SAMS) during the Quaternary and more anciently (Cheng *et al.*, 2013), probably this route would have been established many times in the past, for longer times, and involved the expansion of different plant communities, adapted to different temperature conditions.

Vertebrate Molecular Information

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Vertebrate lineages associated with the SE-NW route exhibited older split times compared to the NE route, supporting Por (1992)'s hypothesis (Fig. 6). The SE-NW route contained lineage splits in Eocene, Oligocene, Miocene, Pliocene and Pleistocene, suggesting a continuous occurrence of this route (Fig. 6). NE route lineage splits in the literature were associated with the Pliocene and Pleistocene. The lineage splits timing supports the hypothesis of the SE-NW route as more ancient than NE routes, and that both routes occurred during the Pliocene and Pleistocene. Nevertheless, we also noticed a taxonomic influence in the lineage splitting time (e.g. mammals are associated with younger split times than bords and amphibians, considered older taxa), so we consider a multitaxa approach a better approach for the comprehension of ancient connection routes connecting the Amazon and the Atlantic Forest biota.

DISCUSSION

We identified two climatic clusters associated with AM and AT, diagnosable mainly by temperature differences, and with biogeographical correspondence with Vertebrate (Costa, 2003; Patton & Costa, 2003; Batalha-Filho *et al.*, 2013) and plant (Andrade-Lima, 1969; Santos *et al.*, 2007) data. Our ENM recovered both the SE-NW and NE routes. However we found support for the NE route only during the LGM. Accordingly, when the colder group ENM indicated climatic suitability expansion towards the SE-NW and NE routes in the LGM, the warmer group showed retractions and fragmentation in AM; however, during the LIG the opposite occurred. Associated to this, palaeopalynological studies showed a similar correspondence, with cold-adapted pollen frequency increase and expansion towards the SE-NW and NE routes during the LGM, and warm-adapted species retraction (Ledru *et al.*, 2001; Sifeddine *et al.*, 2003; Ledru *et al.*, 2006).

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Similarly, during the LIG warmer-adapted plants expanded their distributions and cold-adapted plants retracted their distributions to highlands.

Podocarpus is one of the most studied genus for understanding South American LGM conditions. Nowadays, it has a disjunct distribution in South America, with several species occurring in Andean rainforests, whereas *P. brasiliensis* and *P. selowii* occur in riparian forests and highlands of southeastern Brazil (Marchant *et al.*, 2002; Ledru *et al.*, 2007), and in mountain refuges of Caatinga, maintained by moisture brought with Caribbean trade winds (Ledru *et al.*, 2007). In all cases, the presence of *Podocarpus* is associated with moisture availability throughout the year and with relatively cold conditions. *Weinmannia*, *Hedyosmum*, *Ilex*, and *Myrsine* show similar disjoint distributions; along with *Alnus*, *Drimys*, and other co-distributed species, they have been used as pollen spectra for LGM reconstructions (Marchant *et al.*, 2002; Ledru *et al.*, 2007). During the LGM, there were signs of *Podocarpus*, *Ilex*, *Myrsine*, and *Hedyosmum* expansions in central Brazil (Ferraz-vicentini & Salgado-Labouriau, 1996; Salgado-Labouriau, 1997; Salgado-Labouriau *et al.*, 1998), and *Podocarpus*, *Myrsine*, and *Ilex* frequency increases in Laguna Chaplin (Bolivia) (Burbridge *et al.*, 2004), along the proposed SE-NW route. *Podocarpus*, *Weinmannia* and *Alnus* were also recorded in the Bolivian Altiplano during the LGM (Paduano *et al.*, 2003). Even with total arboreal pollen frequency decreasing, there were records of *Podocarpus*, *Ilex*, *Hedyosmum*, and *Myrsine* increases in eastern AM (Hermanowski *et al.*, 2012), high frequency of *Podocarpus* in Barreirinhas (Maranhão) (Pessenda *et al.*, 2004), Lagoa do Caçó (Maranhão) (Ledru *et al.*, 2001; Sifeddine *et al.*, 2003; Ledru *et al.*, 2006), and in the Icatu River (De Oliveira *et al.*, 1999), supporting both the occurrence of the SE-NW and NE routes in the LGM, under cold and moist conditions. Conversely, the ENM of the warmer group and the palaeopalynological data indicated reduction of suitable areas, fragmentation, arboreal pollen reduction, and frequency increase of savanna species in eastern AM (Absy *et al.*, 1991; van der Hammen & Absy, 1994; Hermanowski *et al.*, 2012). This is in accordance with ENM applied to savannas in South America (Werneck *et al.*, 2012), the AM dry corridor during the LGM (van der Hammen & Hooghiemstra, 2000; Anhuf *et al.*, 2006), and the western AM as more resilient than the eastern AM (Cheng *et al.*, 2013).

ENMs of the warmer group indicated expansion of suitable areas along the SE-NW during the LIG, being supported by speleothem (Cruz Jr *et al.*, 2007; Cheng *et al.*, 2013) and palaeopalynological studies in the Bolivian Altiplano (Gosling *et al.*, 2008; Hanselman *et al.*, 2011) and southeastern Brazil (Ledru *et al.*, 2009). These results corroborate the SE-NW route as the most important route of biotic interchange between the AM and the AT, suggesting it might have been established many times in the past compared to other routes, under different temperature conditions. This might explain why vertebrates with so different climatic tolerances were associated with this route in the past, such as *Dendrophryniscus*, composed by small-ranged species associated with hills or mountain ridges in AT (relatively colder habitats) (Fouquet *et al.*, 2012), and *Enyalius*, with a broader distribution in the AT, such as rainforests, semideciduous forests and riparian forests (Rodrigues *et al.*, 2014), in a larger gradient of climatic conditions. These patterns can also be noticed in mammals (Patton & Costa, 2003) and birds (http://neotropical.birds.cornell.edu/portal/home).

Interestingly, the proposed SE-NW route has correspondence with the current South American monsoon system (SAMS) (Zhou & Lau, 1998) and precipitation pattern (Cheng *et al.*, 2013). During the austral summer, when the Intertropical Convergence Zone (ITCZ) is placed southward, low-level trade winds enter tropical South America from the Atlantic Ocean and transport moisture, which is recycled over the AM westward towards the Andes (Zhou & Lau,

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1998; Gan *et al.*, 2004). Then it is deflected southward, being transported by the Andean low-level jet towards southern and southeastern Brazil, contributing to the South Atlantic Convergence Zone (SACZ) (Zhou & Lau, 1998; Gan *et al.*, 2004). The warm season precipitation pattern follows a NW-SE path (Cheng *et al.*, 2013), similar to the proposed SE-NW route. During the austral winter, the SAMS is weaker (Gan *et al.*, 2004), nevertheless the dry season is often attenuated in southeastern Brazil by extratropical circulation and by the presence of the SACZ (Ledru *et al.*, 2009), promoting high winter rainfalls (Ledru *et al.*, 2009). All these patterns support the SE-NW route. Considering the Andean uplift as an important element for the SAMS (Zhou & Lau, 1998), and considering the split times of vertebrate lineages occurring both in AM and AT, we suggest a starting point for the SE-NW route. Probably this route emerged with the Andean uplift in the Paleogene, and became more pronounced in the Neogene (Hoorn *et al.*, 2010), as supported by the vertebrate molecular data.

The Andean uplift is considered important for AM biodiversity (Hoorn *et al.*, 2010), by generating species turnover (Hoorn *et al.*, 2010), changing the South America regional climate (Zhou & Lau, 1998; Gan *et al.*, 2004), generating a wider climatic gradient, and allowing the evolution of montane species inside AM. However, it also allowed the entrance and persistence of alien lineages, such the southeastern AT, but also Austral-Antartic and Holartic (van der Hammen & Hooghiemstra, 2000). These results corroborate the Andean uplift as crucial in the evolution of Amazonian biodiversity. However, our results support the western AM diversity as function of the southeastern AT diversity and vice-versa, given their biogeographical correspondence. Our results also indicate that current biodiversity patterns in South America predate the Quaternary (Hoorn *et al.*, 2010) and that geologic events were crucial in their genesis.

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SUPPORTING INFORMATION

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

Appendix S1: Statistics associated with the K-means cluster analysis for 4000 random points extracted with bioclimatic information from Amazon and Atlantic Forest shapefiles.

Appendix S2: Tables with palaeopalynological and speleothem studies depicting main species associated and climatic conditions for localities in the Amazon, Atlantic Forests, and along the proposed connection routes between the two biomes. These studies were used for ENM validation for the Mid-Holocene, Last Glacial Maximum, and Last Interglacial.

BIOSKETCH

Roger Maia D. Ledo is a Brazilian PhD student at the University of Brasilia. He is interested in biogeography, ecology, phylogeography, conservation, and niche modelling applied to forested

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habitats inside the South American dry diagonal, with emphasis in reptiles. He is also professor at the Instituto Federal de Educação, Ciência e Tecnologia de Brasília. This work is the result of his doctoral dissertation and is part of the efforts of the research group led by **Guarino R. Colli** to uncover patterns and processes associated with the diversification of the herpetofauna of South American open biomes, especially the Cerrado.

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Figure legends

Figure 1. Main routes of biotic interchange between the Amazon and the Atlantic Forest summarized in Por (1992). Arrow thickness is related to route importance (times of occurrence and duration), acoording to Por (1992) . Therefore, the SE-NW route is considered the most important, followed by the NE route.

Figure 2. 3D plot of the first three components of a principal component analysis from 4000 random points representing the environmental space of the actual distribution of the Amazon and Atlantic Forest (A). This points were organized in two clusters, colder (red) and warmer (blue), mainly based of temperature differences between them, as represented in part B.

Figure 3. ENM projections to the present, mid-Holocene, Pleistocene, and Last Interglacial of the colder and warmer groups. Notice suitable condition expansions of the colder group (above) in all projections, however more pronounced in the LGM. Otherwise, projections of the warmer group indicated suitable condition expansions towards the SE-NW route in the LIG.

Figure 4. South America palynological information associated with rainforests during the LGM. In the left, sites that suggest moist/wett conditions in at least part of the LGM. In the right, sites with cold-adapted pollen records from the literature.

Figure 5. South America palynological information associated with rainforests during the LIG (˜120 ka). In the left, ENM projection of the colder group. In the right, ENM projection of the

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warmer group. Circles represent sites with information of moist/wett conditions (blue) and dry conditions (yellow) during the LIG.

Figure 6. Molecular dating splits of lineages inhabiting both the Amazon and the Atlantic Forest. Notice that lineage splits in the SE-NW route seems continuous and older than those from the NE route.

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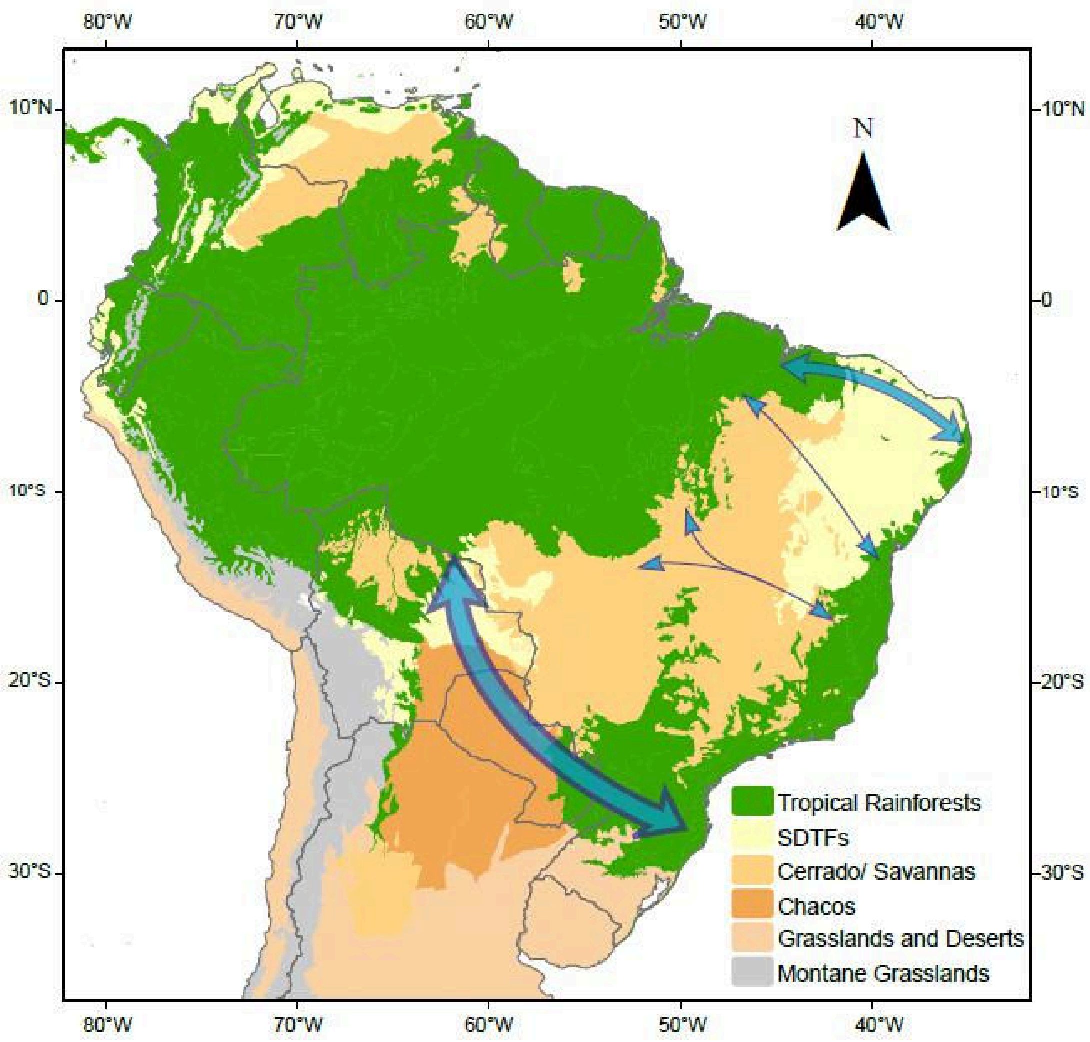
80°W
70°W
60°W
50°W
40°W
10°N
0
10°S
20°S
30°S
N
Tropical Rainforests
SDTFs
Cerrado/ Savannas
Chacos
Grasslands and Deserts
Montane Grasslands

Fig. 1

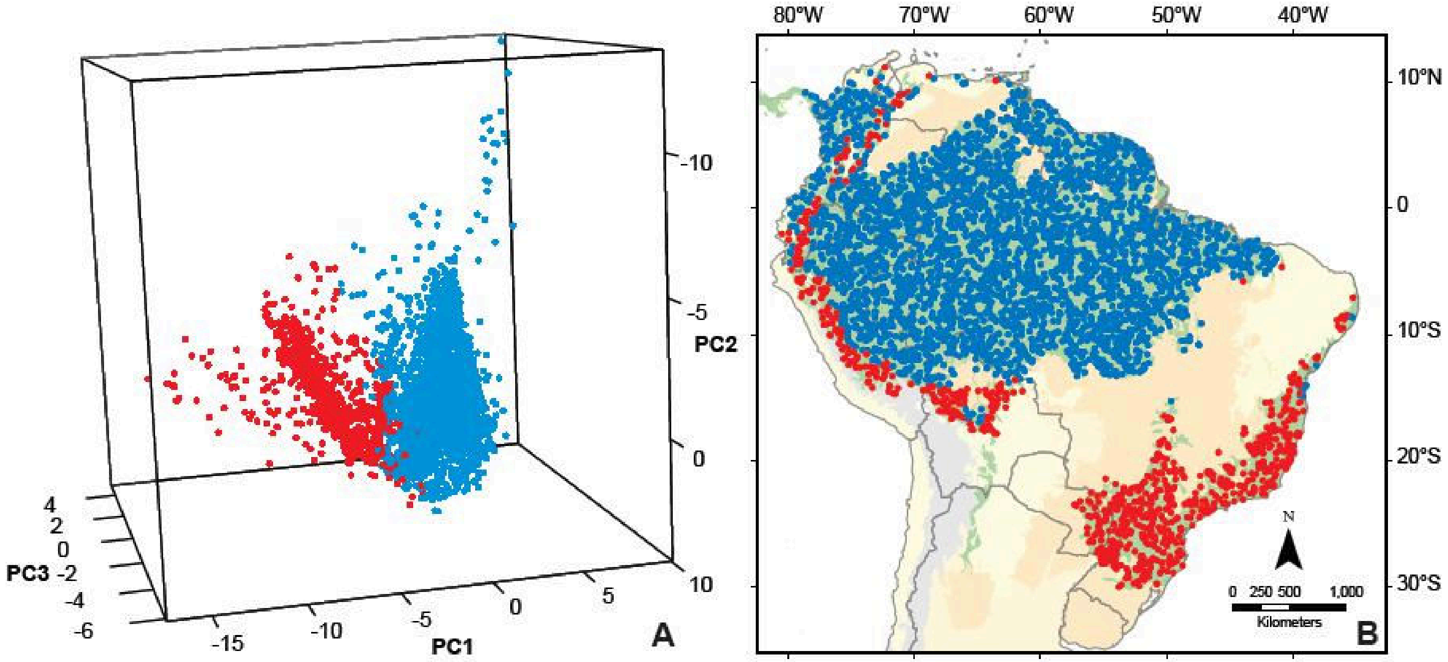
-10
-5
PC2
0
10
4
2
0
PC3 -2
-4
-6
-15
-10
-5
PC1
0
5
A
80°W
70°W
60°W
50°W
40°W
10°N
0
10°S
20°S
30°S
N
0 250 500 1,000
Kilometers
B

Fig. 2

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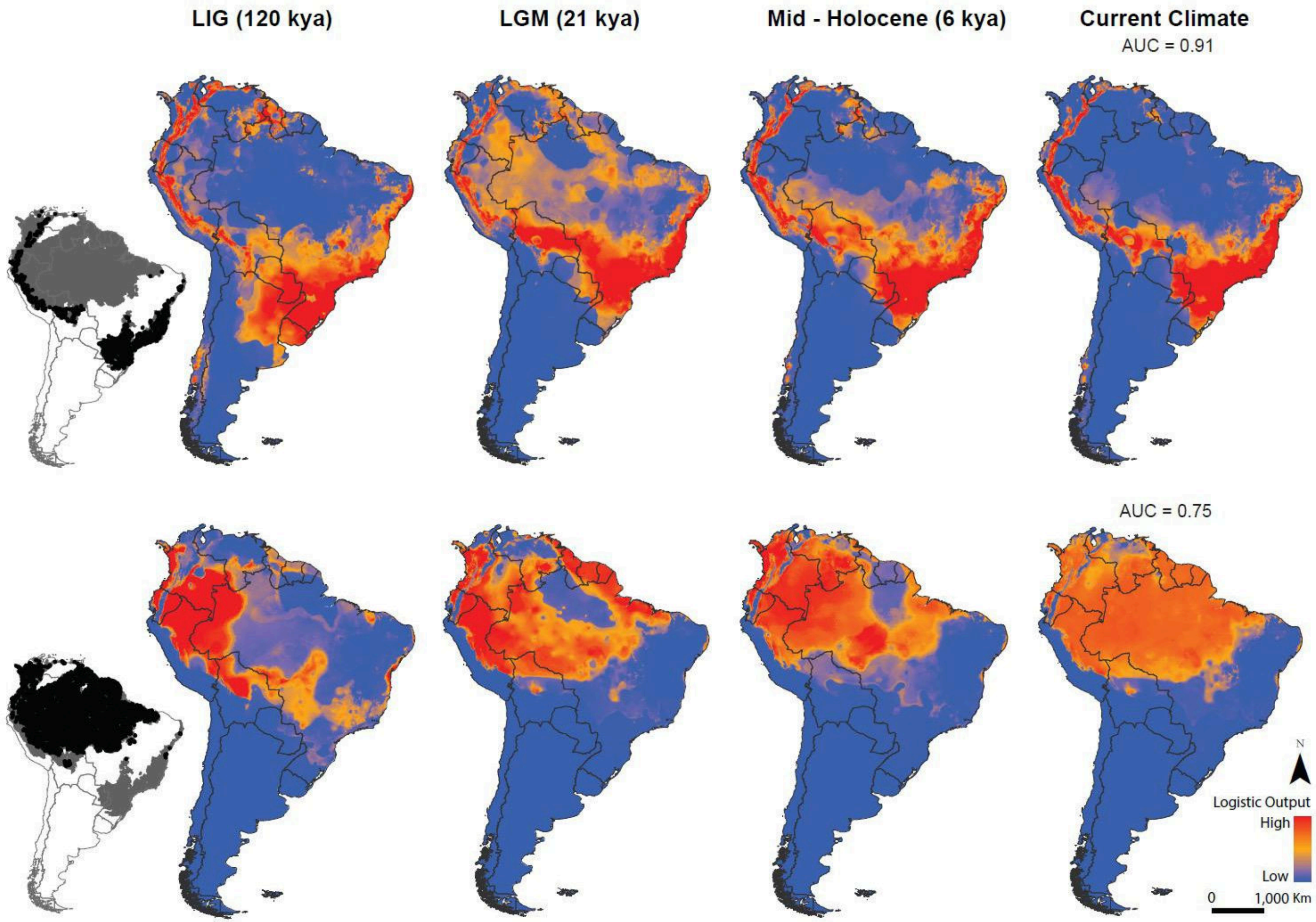

LIG (120 kya)
LGM (21 kya)
Mid - Holocene (6 kya)
Current Climate
AUC = 0.91
AUC = 0.75
N
Logistic Output
High
Low
0
1,000 Km

Fig. 3

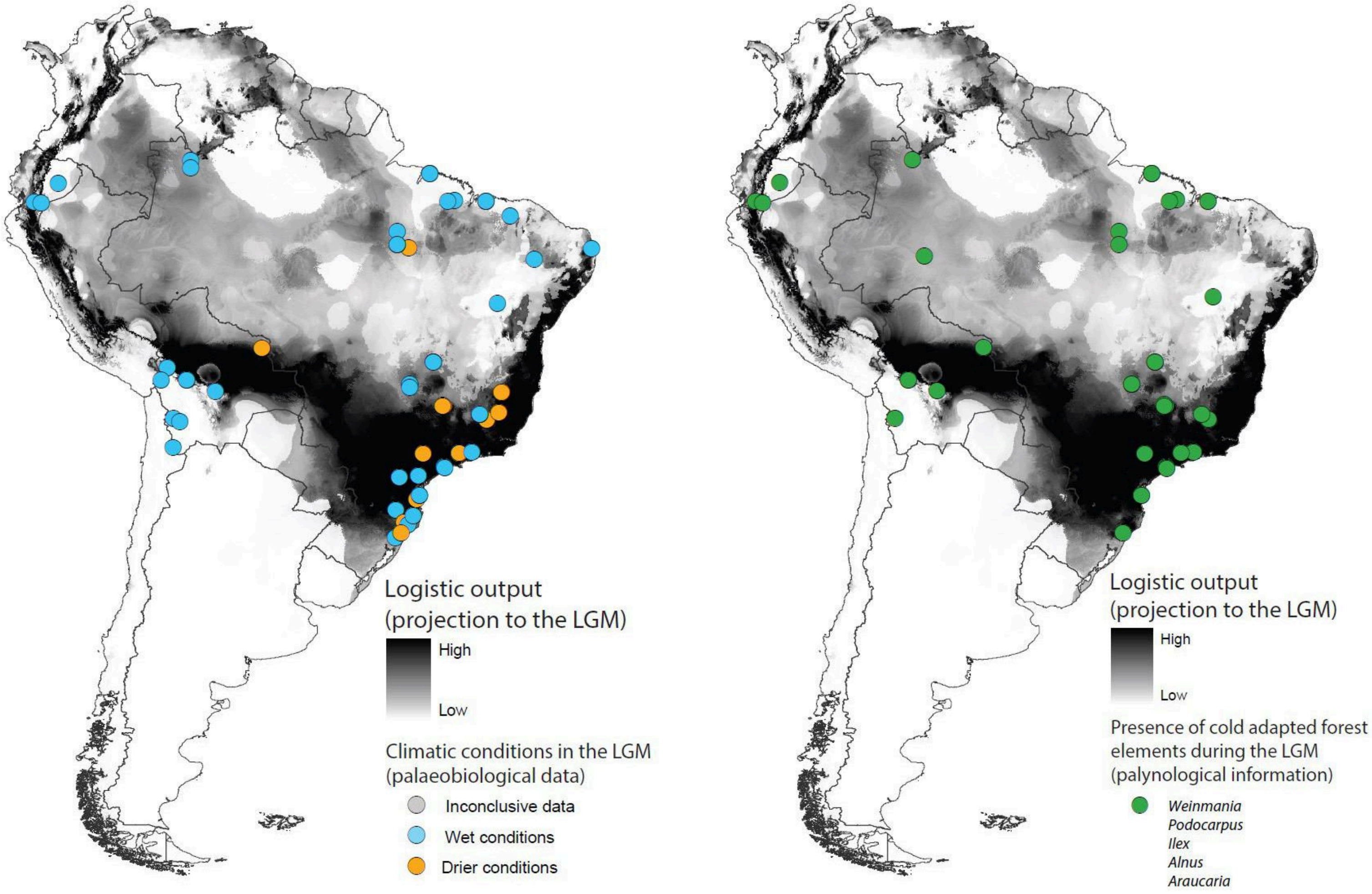
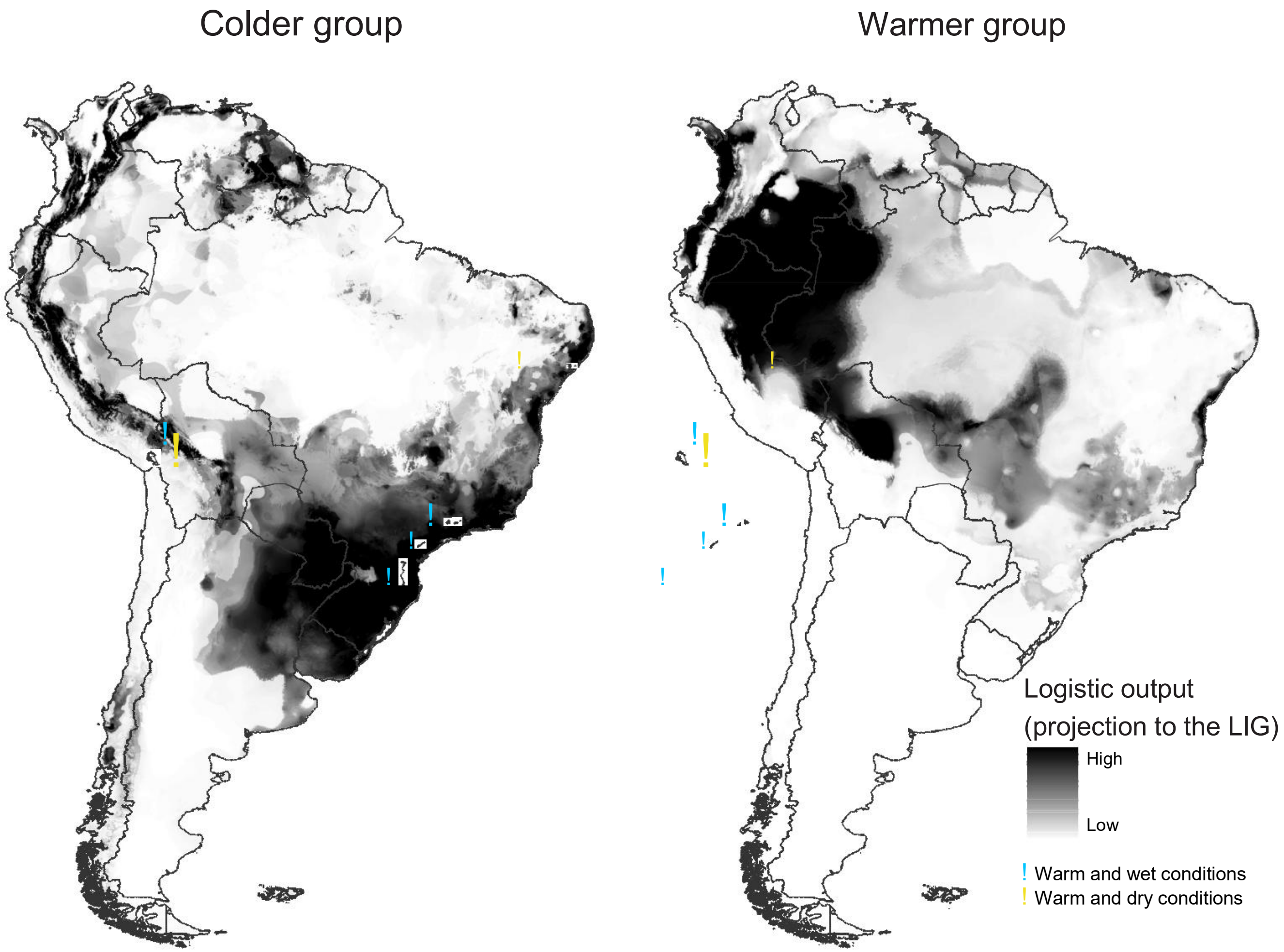

Logistic output
(projection to the LGM)
High
Low
Climatic conditions in the LGM
(palaeobiological data)
Inconclusive data
Wet conditions
Drier conditions
Logistic output
(projection to the LGM)
High
Low
Presence of cold adapted forest
elements during the LGM
(palynological information)
Weinmania
Podocarpus
Ilex
Alnus
Araucaria

Fig. 4

LIG projections



Colder group

Warmer group

Fig. 5

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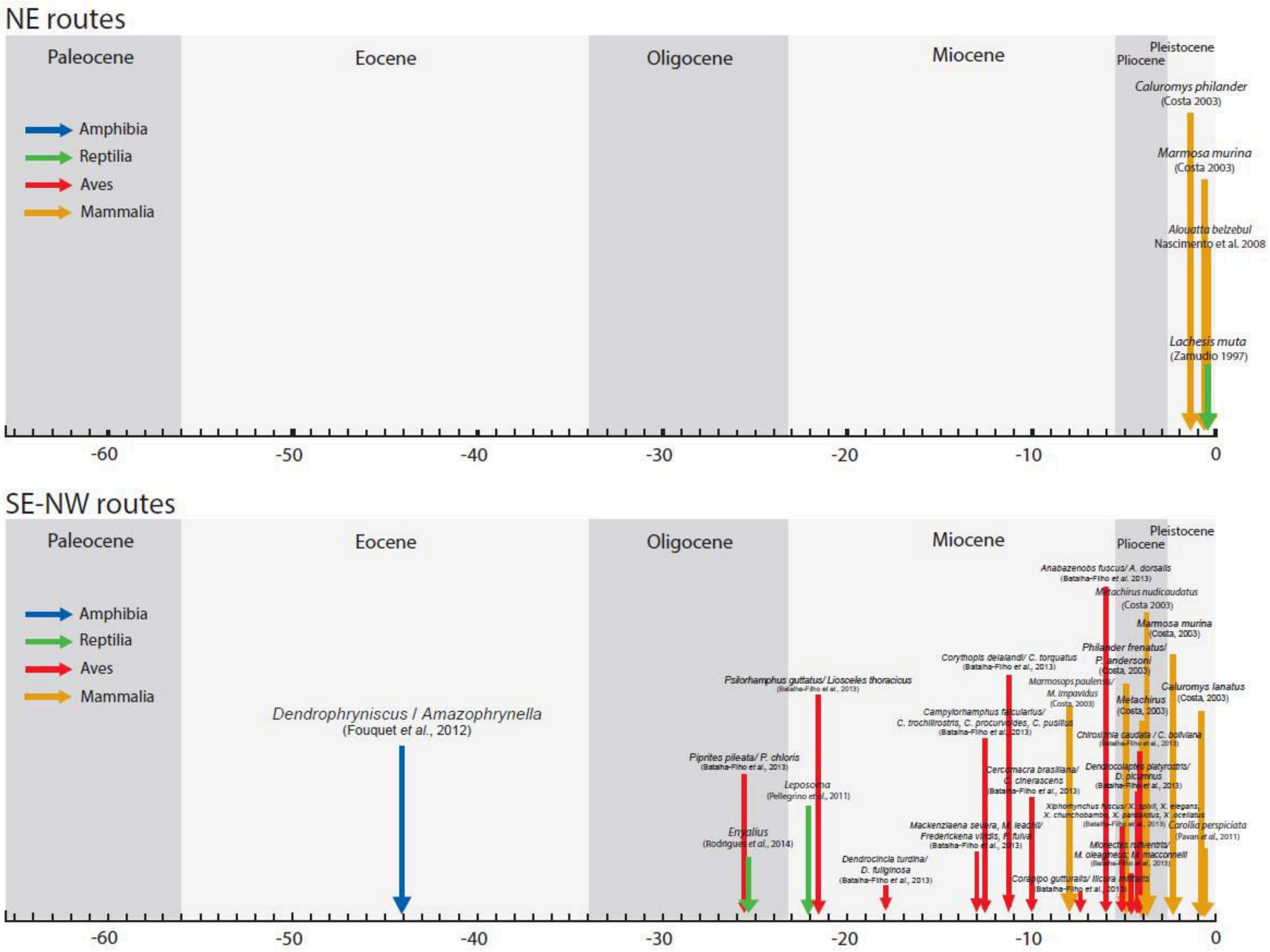
NE routes
Paleocene
Eocene
Oligocene
Miocene
Pleistocene
Pliocene
Amphibia
Reptilia
Aves
Mammalia
Caluromys philander
(Costa 2003)
Marmosa murina
(Costa 2003)
Alouatta belzebul
Nascimento et al. 2008
Lachesis muta
(Zamudio 1997)
-60
-50
-40
-30
-20
-10
0
SE-NW routes
Paleocene
Eocene
Oligocene
Miocene
Pleistocene
Pliocene
Amphibia
Reptilia
Aves
Mammalia
Dendrophryniscus / Amazophrynella
(Fouquet et al., 2012)
Piprites pileata/ P. chloris
(Batalha-Filho et al., 2013)
Enyalius
(Rodrigues et al., 2014)
Psilorhamphus guttatus/ Liosceles thoracicus
(Batalha-Filho et al., 2013)
Leposoma
(Pellegrino et al., 2011)
Dendrocincla turdina/
D. fuliginosa
(Batalha-Filho et al., 2013)
Mackenziaena severa, M. leachii/
Frederickena viridis, F. fulva
(Batalha-Filho et al., 2013)
Campylorhamphus falcularius/
C. trochilirostris, C. procurvoides, C. pusillus
(Batalha-Filho et al., 2013)
Corythopis delalandi/ C. torquatus
(Batalha-Filho et al., 2013)
Cercomacra brasiliana/
C. cinerascens
(Batalha-Filho et al., 2013)
Marmosops paulensis/
M. impavidus
(Costa, 2003)
Anabazenops fuscus/ A. dorsalis
(Batalha-Filho et al. 2013)
Metachirus nudicaudatus
(Costa 2003)
Marmosa murina
(Costa, 2003)
Philander frenatus/
P. andersoni
(Costa, 2003)
Metachirus
(Costa, 2003)
Caluromys lanatus
(Costa, 2003)
Chiroxiphia caudata / C. boliviana
(Batalha-Filho et al., 2013)
Dendrocolaptes platyrostris/
D. picumnus
(Batalha-Filho et al., 2013)
Xiphorhynchus fuscus/ X. spixii, X. elegans,
X. chunchotambo, X. pardalotus, X .ocellatus
(Batalha-Filho et al., 2013)
Carollia perspiciata
(Pavan et al., 2011)
Mionectes rufiventris/
M. oleagineus; M. macconnelli
(Batalha-Filho et al., 2013)
Corapipo gutturalis/ Ilicura militaris
(Batalha-Filho et al., 2013)
-60
-50
-40
-30
-20
-10
0

Fig. 6

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Journal of Biogeography

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Supporting Information (appendix S1)

Table S1.1. Loadings of a principal component analysis (PC1 and PC2) involving 4000 random points extracted with bioclimatic information from Amazon and Atlantic Forest shapefiles. In bold are the two variables with highest loading values in each principal component. In parenthesis are the explanation percentage for each principal component.

Selected bioclimatic variables	PC1 (49.2%)	PC2 (21.0%)
BIO1 (annual mean temperature)	0.295	0.162
BIO2 (mean diurnal range)	-0.182	0.177
BIO3 (Isothermality = 100*BIO2/BIO7)	0.177	-0.193
BIO4 (temperature seasonality = sd*100)	-0.196	-
BIO5 (Max temperature of warmest month)	0.234	0.273
BIO6 (Min temperature of coldest month)	**0.308**	
BIO7 (temperature annual range = BIO5-BIO6)	-0.218	0.196
BIO8 (mean temperature of wettest quarter)	0.27	0.184
BIO9 (mean temperature of driest quarter)	**0.303**	0.121
BIO10 (mean temperature of warmest quarter)	0.273	0.187
BIO11 (mean temperature of of coldest quarter)	0.301	0.132
BIO12 (annual precipitation)	0.223	-0.262
BIO13 (precipitation of wettest month)	0.226	-
BIO14 (precipitation of driest month)	0.112	**-0.412**
BIO15 (precipitation seasonality)	-	0.374
BIO16 (precipitation of wettest quarter)	0.23	-
BIO17 (precipitation of driest quarter)	0.118	**-0.414**
BIO18 (precipitation of warmest quarter)	-	-0.27
BIO19 (precipitation of coldest quarter)	0.2	-0.185
Altitude	-0.249	-0.163

*sd = standard deviation.

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Table S1.2. Coefficients of a generalized linear model (autoPoisson family) of two bioclimatic groups involving 4000 random points extracted with bioclimatic information from a Amazon and Atlantic Forest shapefile. Bold lines indicate significant coefficients (alpha = 0.005). P = probability associated with the Z value; ac = autocovariate distance.

Coefficients	Estimate	Standard error	Z value	P (>|Z|)
Intercept	-8.99E-01	1.64E+00	-0.549	0.583133
BIO1	**7.70E-02**	**2.40E-02**	**3.203**	**0.001359**
BIO2	1.00E-02	1.29E-02	0.78	0.435376
BIO3	-2.86E-02	1.74E-02	-1.639	0.101311
BIO4	-1.15E-03	6.72E-04	-1.714	0.086579
BIO5	-5.56E-03	1.10E-02	-0.506	0.61256
BIO6	7.83E-03	1.06E-02	0.738	0.460546
BIO7	NA	NA	NA	NA
BIO8	-1.50E-02	8.44E-03	-1.775	0.07591
BIO9	**2.69E-02**	**7.77E-03**	**3.467**	**0.000526**
BIO10	-3.96E-02	2.92E-02	-1.358	0.174389
BIO11	-3.92E-02	2.83E-02	-1.382	0.166957
BIO12	-3.21E-04	1.73E-04	-1.856	0.063501
BIO13	9.22E-04	1.21E-03	0.76	0.447078
BIO14	-1.63E-03	2.71E-03	-0.601	0.547562
BIO15	-2.42E-03	3.33E-03	-0.728	0.466432
BIO16	6.15E-04	5.72E-04	1.074	0.282673
BIO17	4.89E-04	1.03E-03	0.474	0.635852
BIO18	**7.70E-04**	**1.92E-04**	**4.013**	**6.00E-05**
BIO19	**-2.62E-04**	**8.73E-05**	**-2.996**	**0.002734**
Altitude	7.04E-05	1.76E-04	0.401	0.688678
ac	2.26E-03	1.34E-03	1.686	0.091803

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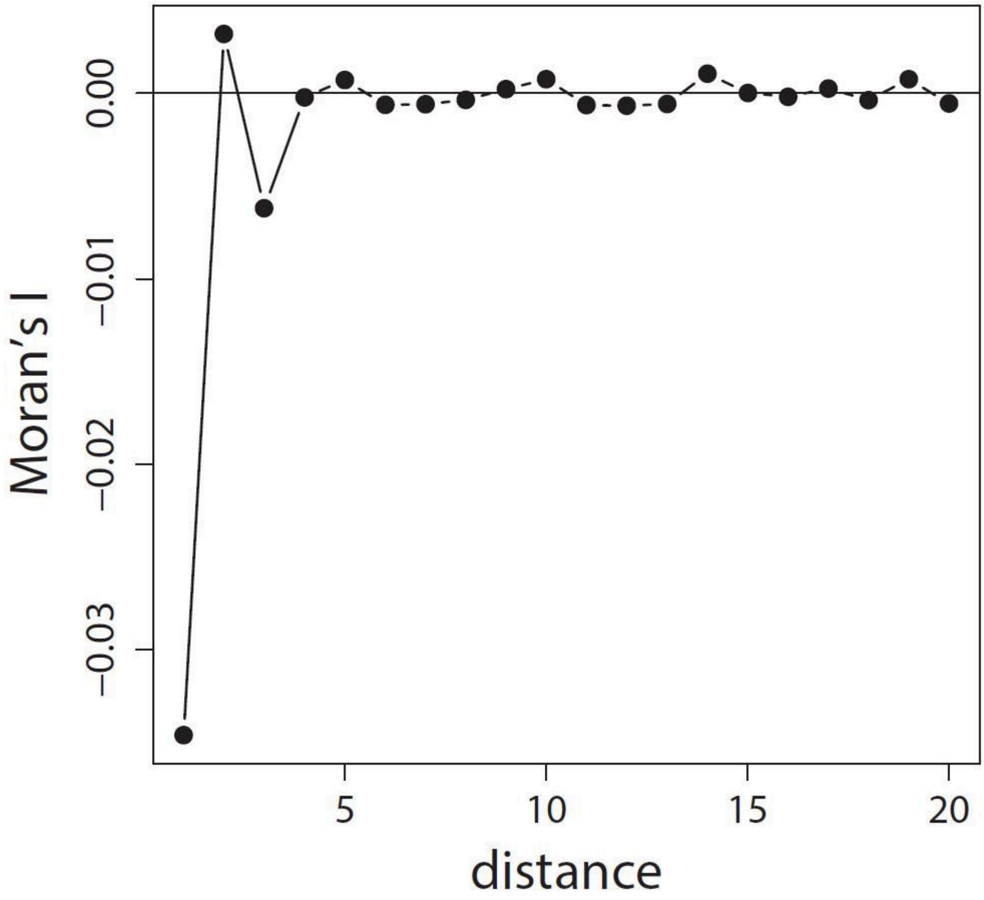

Fig. S1.1. Correlogram of geographic distance and Moran's I coefficient of spatial autocorrelation of 4000 random points extracted with bioclimatic information from a Amazon and Atlantic Forest shapefile.

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Journal of Biogeography

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Supporting Information (appendix S2)

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Table S2.3. Palaeopalynological and speleothem studies depicting main species associated and climatic conditions during the Holocene for localities in the Amazon, Atlantic Forests, and along the proposed connection routes between the two biomes. These studies were used for ENM validation in projection to the Mid-Holocene. dd = decimal degrees.

Author	**Locality**	**Lat (dd)**	**Long (dd)**	**Altitude**	**Period**	**Route**	**Main results of this paper**	**Conditions (Holocene)**
Garcia *et al.* (2004).	Jacareí, SP	-23.2833	-45.9667	550 m	9.72 to 1.95 ka	SE-NW	Moist conditions during all the period, changing from cool and warm climates. Return of the cool climate in the late Holocene was inferred by the presence of *Araucaria*, *Drimys*, *Daphnopsis*, Ericaceae, *Podocarpus* and *Myrsine* in the upper sections of the pollen diagram.	Moist and cool conditions from 9.72 to 8.24 ka. Moist and warm conditions from 8.24 to 3.5 ka. Cooler and moister climate from 3.5 to 1.95 ka.
Ledru *et al.* (2009).	Colônia, SP	-23.8667	-46.7056	900 m	130 ka to present (here: just from 9 ka to present)	SE-NW	High presence of arboreal pollen, with the dominance of *Alchornea*, Melastomataceae/ Combretaceae, Myrtaceae, *Ilex*, *Weinmannia*, and Asteraceae from 9 ka to 3.7 ka. Slight reduction of arboreal pollen from 6.6 to 3.7 ka (from 34-74% to 39-	Seasonal climate with a summer precipitation regime and dry winters.

							54%).	
Pessenda *et al.* (2009).	Curucutu, SP	-23.9333	-46.65	750 m to 850 m	28 ka to present (here: from 15 ka to present)	SE-NW	High concentrations of arboreal pollen during all the period. After ~12 ka the reduction of algal spores, terrestrial, and aquatic herbaceous pollen may suggest a decrease in humidity at the region. However, a higher frequency of arboreal pollen and pteridophytes indicate rainforest expansion during the Holocene.	Moist conditions during the period.
Cruz Jr *et al.* (2007).	Botuverá (S) and Santana (SE) caves	-27.2233 -24.5308	-50.5056 -48.7267	varies from 230 to 700 m a.s.l.	116 ka to present (here: from 10 ka to present).	SE-NW SE-NW	Higher values of Sr/Ca and Mg/Ca ratios (indicating drier conditions) during most part of the Holocene.	Dry conditions during most of the Holocene.

De Oliveira *et al.* (1999).	Icatu River (BA).	-11.0000 to -10.0000	-42.5000 to -43.3333		10.99 ka to present (Here: from 10.54 ka to present).	NE	10.54 ka to 6.8 ka - Presence of Cerrado and Caatinga species dominating the pollen record (high pollen records of Anacardiaceae). *Mauritia* and *Ilex* were also abundant between 8.92 and 8,91 ka, suggesting wetter and warm conditions; 6.23 ka to 4.535 ka - marked by the increase of tropical forest and decline of Caatinga and Cerrado taxa (decrease of *Cuphea* from 8 to 1%). The arboreal maximum in this zone is related to another significant increase in *Mauritia* pollen; 4.535 ka to present - progressive decline of *Mauritia*, *Mimosa* and stable percentages of *Cuphea*. *Cassia*, *Ilex*, Melastomataceae, Myrtaceae, *Mimosa* and *Byrsonima*, clearly suggesting a pronounced tendency towards a Caatinga and Cerrado expansion at the expense of gallery forest.	Dry and warm conditions during most of the Holocene, but there were some wet and warm conditions between 8.92 ka and 8.91 ka and between 6.23 and 4.535 ka.
Behling (2001).	Lagoa da Curuça, Belém (PA)	-0.7667	-47.84		11 ka to present (here: the Holocene).	NE	Savanna elements were not found during the study. Persistence of rainforest elements. Evidence of early Holocene *Rhizophora* pollen suggests early and rapid Atlantic sea-level rise. *Ilex* was also well recorded during the period.	Wet and warm conditions.

Colinvaux *et al.* (1996).	Lake Pata. Western Amazonia (AM)	0.2667	-66.6833	40 ka to present (here: the Holocene).	NE-SW	Persistence of Amazonian lowland elements. Absence of *Podocarpus* pollens and expressive reduction of other cold adapted species pollens. Absence of savanna and grasslands elements.	Wet and warm conditions.
Behling (1998).	Southern (Serra do Rio Rastro; Morro da Igreja; Serra da Boa Vista; Poço Grande, Serra dos Campos Gerais)	-28.3833 -28.1833 -27.7 -26.4167 -24.6667	-49.55 -49.8667 -49.15 -48.8667 -50.2167	14 ka to present.	NE-SW	Southern Brazil. 14 ka to 10 ka: Predominance of grasslands (Serra do Rio Rastro, Serra da Boa Vista - SC). From 10 ka to 3 ka: tropical climate (Serra do Rio Rastro, Morro da Igreja, Serra da Boa Vista). Grasslands were progressively changed to Atlantic Forest elements in the early Holocene. 3 ka to present: tropical climate and tropical vegetation. Cool and moist climate. *Araucaria* forest, probably placed on Highlands.	Dry and cold conditions in the early Holocene, and progressive shift to warm and wet conditions in the mid Holocene.
	Southeastern (Morro de Itapeva; Catas Altas; Lago do Pires)	-22.7833 -20.0833 -17.95	-45.5333 -43.3667 -42.2167	48 ka to present.	NE-SW	Southeastern Brazil -10 ka to 0.970 ka: riparian forest expansion and campo/Cerrado elements (Lago do Pires). Warm and moist conditions on the slopes and warm and dry conditions on the highlands (Morro de Itapeva, 10 ka to 3 ka). From 0.970 to present: Closed semideciduous forest under the current climatic conditions (Lago do Pires). Cloud forest records on slopes, high records of *Araucaria* and *Podocarpus.* Holocene (Lago do Pires -lowland). Moisture	Seasonal influence of precipitation in Lago do Pires during most of the Holocene; Moist conditions during the Holocene at Morro do Itapeva.

						increases on highlands.	
	Northern (Lagoa da Curuça)	-0.7667 -0.7667	-47.84 -47.84		NE	Savanna elements were not found during the study. Persistence of rainforest elements. Evidence of early Holocene *Rhizophora* pollen suggests an early and rapid Atlantic sea-level rise. *Ilex* was also well recorded during the period.	Wet and warm conditions.
Behling (2002).	Southern Brazil (Fazenda do Pinto (RS); Serra do Rio do Rastro (SC); Morro da Igreja (SC); Serra da Boa Vista (SC); Volta Velha (PR); Serra dos Campos Gerais (PR)	-29.4 -28.3833 -28.1833 -27.7 -26.0667 -24.6667	-50.5667 -49.55 49.86666667 -49.15 -48.6333 -50.2167	26 ka to present (Here: from 10 ka to present).	SE-NW	Volta Velha (PR/SC) from 12.3 ka to present: changes in species compositions, with the return of tropical Atlantic species. From 3 ka to present: Araucaria forest expansion. Somewhat wetter climates.	Warm and progressively wet conditions.
	Southeastern Brazil (Botucatu (SP), Morro de Itapeva (SP); Catas Altas (MG); Lago dos Olhos (MG);	-22.8 -22.7833 -20.0833 -19.6333 -19.6333 -19.5167 -17.9667	-48.3833 -45.5333 -43.3667 -43.9 -43.9 -42.4167 -42.2	48 ka to present (Here: from 10 ka to present).	SE-NW	10 ka to present: forest expansion, with *Araucaria* elements. During 12.3 ka tropical forests replaced the community. Lago do Pires (MG): Cerrado persisted until 5.5 ka. Then, semidecidual forests replaced the savanna landscape. This results are similar to results from Lagoa Santa (MG), Lago	Progressive warming and moist conditions.

	Lagoa Santa (MG); Lagoa Silvana (MG); Lagoa Nova (MG), Lagoa do Pires (MG).	-17.95	-42.2167				dos Olhos (MG), Lagoa Silvana (MG) and Lagoa Nova (MG). From 1 ka to present (Lagoa do Pires): Only semidecidual forests dominating the region.	
Pessenda *et al.* (2004).	Barreirinhas (MA)	-2.8667	-45.9167	100-120 m a.s.l.	15 ka to present (Here: just the Holocene)	NE	9 ka to 4 ka- Cerrado elements expanded on the region. Drier period. 4 ka to present: Forest expansion replacing savanna formations. The lake sediment record spans at least the past 18 ka before present. The arrival of *Picramnia* and Mimosaceae and the decline of *Podocarpus* in ca. 12 ka suggest an increase in temperature. Expansion of the forest vegetation is indicative of a more humid phase during the last ~3 ka.	Warm and dry conditions during most of the Holocene. Return of wet conditions after 3 ka.
Pessenda *et al.* (2010).	Floresta Nacional do Araripe (FLONA); Parque Nacional das Sete Cidades (PARNA) , Reserva Biológica do Guaribas (REBIO).	-7.4772 -4.0833 -6.6667	-39.6091 -41.5 -35.1167		18 ka to present (here: 10 ka to present)	NE	Woody savanna expansion between 10 ka to 4.5/3.2 ka. Forest expansion after 3.2 ka. This vegetation dynamic is similar to that observed in some regions of Maranhão (northeastern Brazil), the Amazonas and Rondônia states (northern Brazil), where forests were predominant during the late glacial period and savanna expansion was observed during the dry (less humid) and warm early/middle Holocene.	drier conditions during most of the Holocene. 3.3 ka to present: return of wet conditions.

Sifeddine *et al.* (2001).	Carajás area	-6.5833	-49.5		30 ka to present	NE	10 ka to 8 ka: wet climate and presence of dense forest; 8 to 4 ka: dry climate alternating with moist conditions, and presence of savanna species. 4 ka to 1.5 ka (changing climate. Moist and dry climates during the period).	Wet conditions during most of the Holocene.
Absy *et al.* (1991).	Carajás area	-6.3333	-50.4167	700-800 m	60 a to present (here: from 10 ka to present)	NE	Relatively conditions during the late Holocene. Dominance of Gramineae and reduction of arboreal pollen. From 6 ka to present: increase of arboreal pollen and progressive reduction of Gramineae.	Dry conditions during the last Holocene and progressive increase of wet conditions from the mid Holocene until present days.
Argollo and Mourguiart (2000).	Bolivian Altiplano	66.00 - 71.00 S	14.00 - 22.00 W	3650 and 3900 m	30 ka to present (Here: from 10 ka to present).	SE-NW	From 10 ka to 3.9 ka: progressive dryness in the region. From 8 ka to 3.9 ka: lake level: 3760 m a.s.l. (dry climate, but unstable). 3.9 ka to present: Wetter conditions.	Conditions drier than present during the early and mid Holocene.
Argollo and Mourguiart (1995).	Bolivian Altiplano	66.00 - 71.00 S	14.00 - 22.00 W	3500 and 3900 m	25 ka to present (Here: from 25 ka to present)	SE-NW	From 15 ka to 8 ka: higher lake levels, indicative of wetter conditions; From 8 ka to 3.9 ka: drier climate; From 3.9 ka to present: wetter climates.	Conditions drier than present during the early and mid Holocene.

Behling and da Costa (2001).	Lago Crispim (PA)	-0.7667	-47.85	7 ka to present	NE	Dense, diverse and tall Amazon rain forest and Restingas covered the region in 7 ka. *Mauritia*/ *Mauritiella* palm swamp at 6.62 ka. Sea-level transgression around 3.63 ka, development of mangroves near the site, replacement of the local palm swamp by a Cyperaceae swamp, substitution of the surrounding former Amazon rain forest and some Restingas mainly by salt marshes.	Wet conditions during the early and mid Holocene.
Ledru *et al.* (2002).	Lagoa do Caçó (MA)	-2.9667	-43.4167	18 ka to present (here: 18 ka to 10 ka)	NE	10 ka to 7.5 ka (forest reduction, dry forests and savanna vegetation in the region; 7.5 ka to present: more forest elements: *Byrsonima*, *Curatella*, Mimosaceae).	Wet conditions since the mid Holocene.
Sifeddine *et al.* (2003).	Lagoa do Caçó (MA)	-2.9667	-43.4167	21 ka to present (here: 21 ka to 10 ka)	NE	11.5k-7 ka: the Holocene was characterized by lower moisture availability and a distinct dry period until 7 ka, in response to South American insolation conditions. Nowadays the modern vegetation ranges from littoral Restinga to sandy savanna (Cerrado with Restinga species admixed), and is governed by dune dynamics.	dry conditions during the mid Holocene.

Hermanowski *et al.* (2012b).	Pântano da *Maurítia*. the Serra Sul dos Carajás (PA)	-5.35172	-50.3935	740 m	73 ka to present (Here: from 11.4 ka to present)	NE	11.4-10.2 ka - tropical forest expanded, reflecting stable and very moist conditions. Moraceae/Urticaceae (36%) and Melastomataceae/Combretaceae are characteristic of this zone. 10.2 to 3.4 ka - marked reduction of tropical forest area, expansion of savanna with abundant Poaceae, with overall low occurrence of typical arboreal savanna taxa. 3.4 ka - wet conditions. Marked increase of the tropical forest taxa (moist rainforest in this region).	11.4-10.2 ka: wetter and possibly slightly warmer conditions. 10.2-3.4 ka: dry conditions. 3.4 ka to present: wet conditions.
Behling *et al.* (2001).	Lago Calado (AM)	-3.2667	-60.5833	23m a.s.l.	8.33 ka to present	Eastern Amazon	8.28k-7.7 ka: moist conditions with *Mauritia* types. 7.7- 4 ka: expansion of Várzea/Igapó elements in the region (abundant Poaceae and Cyperaceae), suggesting the local colonization of unflooded mud banks around the lake margin and the formation of flooding meadows. Probably related to seasonal Amazonian high and low water stands. From 4 to 2 ka: wet and inundated conditions. Highest proportion of Várzea/Igapó elements. 2 ka to present: moist conditions.	Wet conditions during most of the Holocene.

Behling *et al.* (1999).	Pântano de Monica (COL)	-0.7	-72.0667	160 m	11.15 ka to present.	SE-NW	Wet, with records of *Podocarpus* in the end of the LGM and beginning of Holocene. Cooling in the tropical lowlands during the period. The main differences during the Holocene occurred in terms of water drainage, reflecting changes in abundances and species compositions. Records of *Protium* and *Caryocar* are indicative of well drained terrains.	Relatively wet during all the period. From wet and cold during the end of the LGM to wet and warm in the Holocene.
Behling and da Costa (2000).	Caxiuanã (PA)	-1.73527	-51.46305		From 8 ka to present		Wet conditions during the period. The vegetation changed during the period, mainly from inundated (Várzea) to Terra Firme vegetation.	Wet during most of the Holocene.
Ledru (1993).	Lagoa Campestre de Salitre	-19	-46.7667	970 m	From 50 ka to present (here: from 10 ka to present)	SE-NW	9.5-5 ka (seasonal period). High records of *Araucaria*. 4 ka-3 ka (return of moisture).	Seasonal conditions during the early and mid Holocene. Moist conditions during the late Holocene.
Ledru *et al.* (2006).	Lagoa do Caçó. Northeastern Brazil	-2.9667	-43.4167		From 19 ka to present	NE	From 11 ka until 8.5 ka: arboreal pollen records between 41% and 63%. Fire were frequent. Poaceae records increase and became dominant. The conditions were probably seasonal. From 8.5 ka to present: seasonal conditions. Similar to the Cerrado conditions.	From wet to Seasonal conditions.

Baker *et al.* (2001b).	Lake Titicaca. Bolivian and Peruvian Altiplano.	-16	-68.5	3810 m	25 ka to present	SE-NW	From 8 ka to 5.5 ka: dry conditions. From 5.5 ka to present: wet conditions.	Dry Early Holocene and Wet Late Holocene.
Baker *et al.* (2001a).	Salar de Uyuni. Bolivian Altiplano	-20.2495	-67.5005	3653m	50 ka to present (here: just the Holocene)	SE-NW	Warm conditions. However the Holocene had also relatively wet conditions.	Wetter conditions for the LGM.
Burbridge *et al.* (2004).	Noel Kempff Mercado National Park. Bolivia	-14.4667	-61.0667	600 to 900 m	50 ka to present (Here: just the Holocene)	SE-NW	From 6 ka to present: expansion of moist forests. (high records of Moraceae/ Urticaceae and reductions of Poaceae). The region was marked by the presence of tropical rainforests and dry forests.	Warm and dry conditions during the early Holocene. Wet and warm conditions during the late Holocene.
Colinvaux *et al.* (1997).	Lake Sucurucho. Equador.	-3	-79	3180 m	From LGM to present (Here: From 10 ka to present).	SE-NW	Cold adapted elements returned to the region. Atlantic/Amazon elements appear in the pollen record: *Cecropia*, *Podocarpus*, *Weinmannia* and *Alnus*.	Cold (warmer relative to LGM conditions) and moist conditions.
Salgado-Laboriau (2005).	Central Brazilian and Venezuelan Savanna.						7 ka to present: return of Cerrado elements in the Central Brazil (Águas Emendadas, Cromínia, Lagoa Bonita, Lagoa dos Olhos, and Lagoa Santa).	Seasonal wet conditions.
Salgado-Labouriau *et al.* (1998).	Águas Emendadas (DF)	-15.5667	-47.5833		32.4 ka to present.	SE-NW	From 19-7 ka: drier and cold conditions. 7 ka - moist conditions returned to the region again. 5 ka - the savanna region reached the present values of moisture.	Moist and warm conditions in the region since the Mid Holocene.
	Cromínia (GO)	-17.2833	-49.41667					

Salgado-Labouriau *et al.* (1997).	Cromínia (GO)	-17.2833	-49.41667	710 m	32.4 ka to present (Here: the Later Pleistocene and Holocene).	SE-NW	From 18.5 to 11 ka the climate was dry, and sparse vegetation was growing in the region at that time. From 6.5 ka to present, moister conditions returned to the place again.	Dry conditions during the early Holocene and Moist conditions during the mid and Late Holocene.
Barberi *et al.* (2000).	Águas Emendadas (DF)	-15.5667	-47.5833		30.5 ka to present (Here: the Later Pleistocene and Holocene).	SE-NW	From 21 ka to 7 ka: drier conditions. Since 7 ka to present days: Higher records of *Mauritia*. Warmer and seasonal precipitations, similar to present conditions.	Drier early Holocene and seasonal conditions during the Mid and Late Holocene.
Haberle and Maslin (1999).	Amazon				50 ka to present (Here: Holocene)	SE-NW/NE	From 11 to 9 ka - warm conditions returned to the region. Records of species with secondary growth (*Cecropia*) at that region. 9 ka to present - vegetation similar to present.	Wet and warm conditions.

Hermanowski *et al.* (2012a).	Pântano da *Mauritia* (SE Amazon). Serra Sul dos Carajás (PA)	-6.35172	-50.3935	740 m	25 ka to present (Here: just the Holocene)	NE	11.4 - 10.2 ka: Forest elements dominating the pollen record (Moraceae, Urticaceae, Melastomataceae and Combretaceae). 10.2- 3.4 ka: decrease to generally low values of main tropical forest taxa, and slight higher values of Anacardiaceae, Bignoniaceae, *Zanthoxylum*, and higher values of Fabaceae. 3.4 ka to present: increase of tropical forest taxa: *Alchornea*/*Aparisthmium*, *Celtis*-type, Melastomataceae/ Combretaceae and Moraceae/Urticaceae.	Drier and warm conditions during Early and Mid Holocene. Wet conditions since 3.4 ka.
Behling (2006).	Serra do Araçatuba (PR)	-25.91667	-48.9833	1500 m	14.88 ka to present	SE-NW	From 14.88 ka to 2 ka: Grasslands dominated the region. From 2 ka to present: *Araucaria* forest taxa dominated the region, reflecting moister conditions. From 2 ka to 0.7 ka: *Araucaria* forest taxa increased markedly, specially *Ilex*. Some other taxa, such as *Symplocos lanceolata*-type, *S. tenuifolia*-type, *Araucaria angustifolia* and *Drimys brasiliensis* also increased in number. Probably this data is related to a return of this species to the region, due to warming.	Colder conditions than today during Early Holocene and progressive warming after Mid Holocene.

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Behling and Negrelle (2001).	Volta Velha (Itapoá) - SC	-26.0667	-48.6333		37.5 ka to present	SE-NW	From 14.4 to 6.7 ka: Ferns, including some tree ferns, became frequent in the rain forest. *Myrsine*, Arecaceae, *Weinmannia*, *Hedyosmum*, Solanea, and *Clusia* became more frequent in the rain forest. Marine incursions occurred during the Early Holocene. A closed Atlantic rain forest developed at the study site after 6.1 ka.	Wet conditions during almost all the Holocene.

Behling *et al.* (2007).	Serra da Bocaina (SP)	-22.675	-44.5567	1500 and 1650 m	18.57 ka to present	SE-NW	10.84-8.03 ka: higher records of montane forest groups (*Weinmannia, Symplocos lanceolata*-type, and *Ilex*. Reductions of Poaceae. 10.38-6.39 ka (Serra da Bocaina 2): High records of Poaceae. Also pollen of the upper montane forest group (*Myrsine*, with some *Weinmannia*, *Symplocos tenuifolia*-type, *S. lanceolata*-type, *Ilex*, *Drimys*, and also *Podocarpus*). 7.2-4.1 ka: higher percentages of the upper montane forest group than in the previous zone, mainly due to *Weinmannia* and the *S. lanceolata*-type in the lower part of the zone. Pollen of *Cecropia* and the *Annona speciosa*-type begins to appear in this zone. 5-1.36 ka: Increase of herb pollen, mainly in the upper part of the zone. Slight decrease of the upper montane forest group, while the group of other shrubs and trees increases slightly. *Weinmannia* and Myrtaceae pollen percentages decrease, while *Alchornea* and *Euterpe*/*Geonoma* increase.	From cold and moist conditions during the Early Holocene to warmer conditions in the mid Holocene.
Ledru *et al.* (2001).	Lagoa do Caçó (Maranhão). Northeastern Brazil	-2.9667	-46.4167	120 m	14 ka to present	NE	Reduction of cold adapted forest taxa during the Holocene. Arboreal pollen and non-arboreal pollen frequencies were kept similar during the Holocene.	Warmer conditions.

Enters *et al.* (2009).	Lago Aleixo (MG)	-17.9878	-42.1189	390 m	9.3 kato present	SE-NW	9.3-6.9 ka: Records of open savanna elements and species from riparian forests. From 6.9 ka to 0.8 ka: increased rainfall and shorter annual dry period allowed riparian forests and semideciduous forests to expand, leading to a more closed Cerrado vegetation. From 0.8 ka to present: closed, semideciduous forest developed under present-day climate conditions.	Warm conditions during the Holocene. Wet conditions during the mid Holocene.
Behling (2003).	Lagoa Nova (MG)	-17.9667	-42.2	390 m	10 ka to present	SE-NW	10-8.5 ka: the landscape was dominated by savanna of the campo-Cerrado type, with frequent trees of *Curatella americana,* and areas of riparian forests. Fires were frequent. From 8.5 to 7.56 ka: riparian forests expanded (wetter period). 7.5-6 ka: savanna expanded and riparian forests retracted (drier conditions); 6-2.1 ka: valleys were covered by semideciduous forest, but Cerrado persisted on the hills. 2.8-0.6 ka: hills changed to closed Cerrado, reflecting wetter conditions. After 0.6 ka a dense semi-deciduous forest expanded throughout the study region, indicating the beginning of modern wet conditions.	Warm and seasonal conditions. Wet conditions occurred during a small period in mid Holocene and at the late Holocene.

Mourguiart and Ledru (2003).	Near the village of Siberia, Bolivia.	-17.83333333	-64.7188889	2920 m	40 ka to present (Here: just the Holocene).	SE-NW	From 10 to 4 ka: Poaceae dominated the vegetation community. High frequency of microcharcoal particles. From 4 ka to present: a more forested vegetation.	Dry conditions during the early and mid Holocene. Wet conditions during the Late Holocene.
Mayle *et al.* (2000).	Noel Kempff Mercado National Park. Bolivia	-14.4667	-61.0667	600 to 900 m	50 ka to present	SE-NW	Evidence for early to middle Holocene savanna in the Bolivian Amazonia. Holocene aridity did not affect the vegetation at the southern margin of Amazonia. Expansion of Amazon forest elements after 3 ka.	Warm and dry conditions during the early and Mid Holocene. Wet conditions since 3 ka.
Paduano *et al.* (2003).	Lake Titicaca. Bolivian and Peruvian Altiplano.	-17	-67	3810 m	27.5 ka to present (here: just the Holocene).	SE-NW	At the period of 13.7 ka, elements similar to present plant communities occurred at the region. A possible dry event occurred from 9 to 3 ka, caused by records of some aquatic elements, and also by the increase of Poaceae, Apiaceae, *Plantago*, and *Polylepis* pollen. Fire is evident after 3.1 ka with the appearance of weed species.	Dry event during almost all the Holocene.
Ferraz-vicentini and Salgado-Labouriau (1996).	Close to Cromínia (GO)	-17.2833	-49.4167		32.4 ka to present	SE-NW	From 10.5 to 7.7 ka: a dry but not so cold condition occurred at the region. The region warmed up during this period. From 6.7 to 3.5 ka: veredas, riparian forests, and an arboreal Cerrado formations returned to the region (possibly warm and moist conditions). From 3.5 ka to present: frequent microcharcoal records.	Dry conditions during the early Holocene and moist conditions during the mid and Late Holocene.

Salgado-Labouriau (1997).	Carajás (PA)	-6.3333	-50.4167	between 700 and 800 m	From LGM to present.	SE-NW	In general, moister than the present from 36 ka to 22–18 ka.	For most localities, wetter or drier conditions during the early Holocene, semi-humid conditions during the mid Holocene, wetter conditions after mid Holocene and, then, return to semi-humid conditions.
	Águas Emendadas (DF)	-15.5667	-47.5833	between 1040 and 1170 m			In general, moister than the present from 36 ka to 22–18 ka. Águas Emendadas: From 32.4-21.4 ka - arboreal pollen dominated the region (moister and colder conditions than present, with cold adapted forests in the pollen record. From 21.4-7.3 ka: dry period. From 7.3 ka to present: moister conditions.	
	Cromínia (GO)	-17.5667	-49.4167	710 m			In general, moister conditions from 36 ka to 22–18 ka. From 32.4 to 28.3 ka: moist conditions; 28 ka to 19 ka: humidity was high and the climate was cooler than present. Natural fires occurred during this time. 18.5-11.3 ka: dry and cold period. The dry (but not cold phase) persisted from 10.5 to 7.7 ka. From 6.7 ka to present warm and moist conditions persisted, with palm swamps at the region.	

Serra Negra (MG)	-18.9167	-46.8333		In general, moister than present from 36 ka to 22–18 ka. Records of *Podocarpus*, *Araucaria*, *Ilex, Alchornea* and *Raphanea*. Cold elements coexisting with elements from Tropical forests. At 14.34 ka *Podocarpus*, *Araucaria*, *Ilex* and Ericaceae were also present (in low abundance) with tropical elements. 14.34 ka to present: temperate climate taxa were absent. From 5 ka to present: savanna elements and Tropical semi-deciduous forest were well represented, suggesting reduction of precipitation and higher temperature. A riparian forest occurred then at the region. From 12.5 ka to present the vegetation was very similar to present vegetation.
Salitre (MG)	-19	-46.8333	1050 m	32 kato 28.7 ka - presence of a flooded marshy forest (Igapó) in a cold climate, but represented by Cerrado species. From 13 ka to 8 ka: *Araucaria* and *Araucaria* forest elements in the pollen record, interpreted as a northwestern expansion of the *Araucaria* forest, which today grows around 24°S. Genera such as *Araucaria*, *Drimys*, *Ilex*, *Podocarpus* were present. From 5.5 to 4.5 ka: a dry episode. From 4.3 ka to 3.06 ka: a semi-deciduous forest grew up at the

				region.
Lagoa Santa (MG)	-19.6333	-43.9	740 m	Dry conditions during the Mid Holocene, but with transitions to wet conditions around 3 ka and, then, changes to present conditions (semi-humid).

Lagoa dos Olhos (MG)	-19.6333	-43.9	730 m	Records of *Podocarpus* at low concentrations in the region, however riparian forests and high levels of *Caryocar*, cerradão and arboreal savanna elements. A marsh occupied the site of the present lake from 19 kato 13.7 ka. The climate was cold and semi-humid with a short dry season. 13.7 ka: moisture decrease and temperature increased, associated with fire records in the region. 6.8 ka: a marsh returned to the region. At 4 ka a lake was formed at the site, suggesting increase in moisture. Between 4 ka to 1.5 ka moisture was higher than present. 1.3 ka to present: moisture levels returned to present conditions.
Lago Valencia (Venezuela)	-10.1833	-67.7167	400 m	Dry conditions between 12.9 ka to 11.5 ka. Then the lake was replaced by a marsh or an intermittent lake during this time. The climate was semi-arid. The beginning of the Holocene a saline endorheic lake was formed. Open forest elements were present (*Bursera*, *Spondias* and *Tribulus*). From 8.6 ka to present arboreal elements increased at the region.

Parizzi *et al.* (1998).	Lagoa Santa (MG)	-19.6333	-43.9	740 m	6.1 ka to present	SE-NW	6.1 ka to 5.4 ka: gradual moisture increase at the ancient valley. From 5.4 to 4.6 ka: presence of an intermittent marsh on the valley floor and climate drier than the present. 4.6 ka: moist environment. The marsh was replaced by a permanent lake. A mosaic of forest and Cerrado covered the region and the climate was warm and semi-humid, similar to present.	Some signs of wetter conditions during the mid Holocene. Then, return to present conditions.
Salgado-Labouriau *et al.* (1992)	Macubaji Lake (Paramo region). Venezuela.	8.7833	-70.8167	3540 m	8.3 ka to present		Low changes in the vegetation during the period. Records of *Podocarpus* in lowlands probably were reduced due to human impact. There were less 'espelitas' (Compositae) than at Present. The vegetation was denser between 6.07 ka to 5.4 ka. Lake level decreased from 2.2 ka to present.	Probably moister conditions during the mid Holocene.

Behling *et al.* (2004).	Cambará do Sul (RS)	-29.0525	-50.1011	1040 m	42.8 ka to present	SE-NW	Somewhat wetter from 42.8 to 41.47 ka and not so cold. From 41.47 to 26.9 ka: 'campos' vegetation suggest a cold climate with repeated frost events and minimum austral winter temperatures below -10°C. From 26.9 to 10.12 ka pollen percentages continue to be high and *Araucaria* pollen, low. During the Holocene Poaceae pollen were high and the frequency of *Araucaria* pollen were slightly higher than previously. Fires were frequent after 7.4 ka. *Araucaria* forest probably was present in refuges until 4.3 ka, where this species expanded its distribution through riparian forests in the region. *Weinmannia* was common at 1.52 and 1.77 ka, suggesting a shift for wetter conditions.	Dry conditions during most of the Holocene. Return of moist conditions at that altitude after 4.3 ka.

Hanselman *et al.* (2011).	Lake Titicaca	-17	-67	3810 m	370 ka to present	SE-NW	370-330 ka: Puna vegetation dominating the record: cold and wet conditions); 330-301 ka: Puna vegetation (50%), *Alnus* (20%) and *Podocarpus* (7%) - warmer and relatively wet conditions. From 301-288 ka: Mainly Puna vegetation, with *Alnus* records and *Podocarpus*. warmer and wet conditions. 288 to 260 ka: cold and moist conditions. 238 to 211 ka: *Polylepis* dominating the community record - warm and moist conditions. 211 to 196 ka: Puna vegetation (80%), Polylepis (34%) - warm and wet conditions. 196 to 150 ka: High levels of *Polylepis*, *Podocarpus* also present (less than 10%) - warm and wet conditions. From 150 to 139 ka: Puna vegetation, records of *Polylepis* (15%), *Podocarpus* (15%) -warmer and moist conditions. From 139 to 115 ka: Records of Amaranthaceae (84%). Low aquatic pollen, but *Pediastrum* concentrations were extremely high - probably warm and dry conditions. From 115 to 63 ka: records of *Polylepis*, with - cold and moist conditions. 63 to 55 ka: high concentrations of *Isoetes*, and taxa from Puna vegetation - cold and moist conditions. 55 to 42 ka: (high *Isoetes* concentration - cold and	Warm and wet conditions for the Holocene.

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moist conditions; From 42 to 28 ka: colder and moist conditions. 28 kato 20 ka: Caryophyllaceae pollen was high from 25-24 ka: *Podocarpus* occupied 17% of the pollen record at 23 ka. Cold and moist conditions. From 20 to 3 ka: Low pollen concentration - warm and wet conditions High *Pediastrum* and
Isoetes concentrations.

Behling (1997).	Morro de Itapeva, SP	-22.7833	-45.5333	1850 m	35 ka to present	SE-NW	From 35 ka to 17 ka: dry and cold conditions predominating on high elevations. From 17.6 to 10 ka: cool and moist conditions. Records of *Podocarpus*, *Araucaria*, *Weinmannia*, *Ilex* at the region, probably in lowlands or in spaced groups. Atlantic forest elements were also present. Conditions were probably a little warmer than previously. From 10.9 to 9.9 ka: variations between grassland elements and cloud forests elements. From 9.9 to 2.6 ka: *Weinmannia*, *Araucaria*, *Ilex*, *Drimys* and *Clethra* dominated the pollen data at the region. Probably the climate was warmer and moist, because of the expansion of cloud forest on high elevations. From 2.6 kato 0.43 ka: *Araucaria* and *Podocarpus* on higher elevations, suggesting moist conditions at high elevations. From 0.43 to 0.07 ka: decrease of *Weinmannia* pollen data.	Cold and moist conditions in the region during the Holocene.

Pessenda *et al.* (2009).	Curucutu, 20 km de Itanhaém, SP. Serra do Mar	-23.9333	-46.65	750-850 m	28.46 ka to present	SE-NW	From 28.46 to 20 ka: forest and herbs (20-32%; 82-67%, respectively). Among arboreal elements the most common taxa are *Alchornea*, *Araucaria*, Melastomataceae, Myrtaceae, *Podocarpus* and *Symplocos* with percentages ranging from 2% to 5%. Herbs: Poaceae and Asteraceae; 23 ka to present: sharp increase of arboreal elements, reaching 55% of the total pollen sum. *Cybianthus* reached a 15% peak, *Ilex* (5%–12%) and *Symplocos* (1%–5%) also increased abundance in the pollen record. The concentration of *Araucaria* and *Podocarpus* pollen found in the Curucutu core reach values between 125,000 and 17,000, and 4,000 and 1,500 grains/cm^3, respectively. This comparison allows us to infer a physiognomy similar to that of an *Araucaria* forest for Curucutu from 28.46 to 22.78 ka. The high *Araucaria* pollen concentration and the presence of *Podocarpus*, *Myrsine*, *Symplocos*, *Weinmannia*, Ericaceae, Melastomataceae, and Myrtaceae suggest a cold and moist forest. From 22 ka to 12 ka: forested landscape around the peat area, whereas the increase of algal spores suggests higher moisture.	Moist and relatively cold conditions during the Holocene.

The presence of botanical elements such as *Araucaria*, *Podocarpus*, and *Weinmannia,* clearly associated with cool and moist climates, suggests a significant temperature depression during the Late Glacial at Curucutu. After 12 ka until the present, the reduction in algal spores, and terrestrial and aquatic herbaceous pollen as well, suggest a decrease in moisture, although a higher frequency of arboreal elements and pteridophytes indicates the expansion of rainforest during the Holocene.

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Williams *et al.* (2011).	Laguna Khomer Kocha Upper	-7.27523	-65.7324167	4153m	18 ka to present	SE-NW	Before 14.5 ka, warm and relatively moist conditions free from fire, facilitated the expansion of high Andean *Polylepis* woodland. Fire at 14.5 ka quickly transformed the local vegetation, and the woodlands became restricted to areas protected from fire. 10.1 to 6.4 ka: dry event in the Holocene. From 6.4 to present: wetter conditions, sustaining the same community as before the Holocene dry event.	Drier conditions during the early Holocene. Wet conditions in the mid and late Holocene.

Table S2.4. Palaeopalynological and speleothem studies depicting main species associated and climatic conditions during the Last Glacial Maximum (LGM) for localities in the Amazon, Atlantic Forests, and along the proposed connection routes between the two biomes. These studies were used for ENM validation in projection to the LGM. Latitude (lat*) and longitude (Long*) are expressed in decimal degrees or in DMS format.

Study	**Site (Lat*, Long*, altitude)**	**Period**	**route**	**Main results**	**Conditions**	**Cold-adapted species**	**Warm-adapted species**	**Validate ENM?**
Ledru *et al.* (2009)	Colônia, SP (-23.8667; -46.7056, 900m)	130ka to the present (here: the LGM)	SE-NW	From 28.5 to 23.5 ka - sharp increase of arboreal pollen at the region, high frequency of cold adapted forests (*Weinmannia*, *Hedyosmum*, *Podocarpus,* and *Ilex*). These records mark the development of a tropical cloud forest under cool temperatures; From 23.5 to 9.9 ka - Progressive reduction of arboreal pollen, appearance of *Araucaria* pollen and progressive expansion of Poaceae. Probably cold adapted forests persisted in the region in refuge areas (see also Fig. 2, Ledru et al. 2009).	cold and moist during the LGM	*Weinmania, Hedyosmun, Podocarpus, and Ilex.*		yes
Pessenda *et al.* (2009)	Curucutu, SP (-23.9333; -46.65, 750m to 850m)	28ka to the present (here: the LGM)	SE-NW	From 28 to 12 ka: Presence of arboreal elements and herbs. Among arboreal elements, the most common taxa are *Alchornea*, *Araucaria*, Melastomataceae, Myrtaceae, *Podocarpus, Symplocos* and *Weinmannia*. Among herbs, Poaceae and Asteraceae were common. This results suggest high moisture levels and low temperature during this period (see Fig. 2 and appendix, Pessenda et al. 2009).	low temperature and high moisture	*Araucaria, Podocarpus, Myrsine, Symplocos, Weinmania,* Poaceae*.		yes
Cruz Jr. *et al.*	Botuverá (S)	116kyr BP	SE-NW	Wet conditions persisted throughout	LGM	stalagmite data	stalagmite data	yes

(2007)	cave (-27.2233; -50.5056, from 230 to 700m a.s.l.)	to the present		most of the Last Glacial period from approximately 70 ka to 17 ka. The glacial period was predominantly wet.	predominantly wet			
Cruz Jr. *et al.* (2007)	Santana (SE) cave (-24.5308; -48.7267, from 230 to 700m a.s.l.)	116kyr BP to the present	SE-NW	Wet conditions persisted throughout most of the Last Glacial period from approximately 70 ka to 17 ka. The glacial period was predominantly wet.	LGM predominantly wet	stalagmite data	stalagmite data	yes
Behling (2001)	Lagoa da Curuça, Belém (PA) (-0.7667; -47.84)	11ka to present (here: the Late-Glacial)	NE	Dense and high diverse Amazon rain forest in the coastal area. Absence of savanna elements during the Late-Glacial period. Presence of *Podocarpus* in the region, suggesting wet and cold conditions.	Cold and wet conditions	*Podocarpus*		yes
Behling (2002)	Serra da Boa Vista (SC) (27°42'S 49°09' W; 1160m)	14ka to present	SE-NW	Average temperature reduction during the LGM (3-5 C). Low frequency of fires.	Cold and wet conditions	grasslands and reduction of *Araucaria* records.	none	yes
Behling (2002)	Volta Velha (PR) (26°04' S; 48°38' W, 5m)	25.65 ka to present	SE-NW	Grasslands and cold-adapted forests persistance during the LGM. *Alchornea* (palm tree) were almost absent.	Cold and wet conditions	Grasslands and cold-adapted elements.	none	yes
Behling (2002)	Botucatú (SP) (22° 48' S; 48° 23' W, 770m)	30ka to 18ka	SE-NW	Almost treeless. Average temperature 5-7°C lower than today.	Cold and dry conditions	absent	none	no
Behling (2002)	Morro do Itapeva (SP) (22° 47' S; 45° 32' W, 1850)	35ka to present	SE-NW	From 35ka to 17ka: dry and cold conditions on high elevations. From 17.6 to 10ka: cool and moist conditions. Records of *Podocarpus*, *Araucaria*, *Weinmania*, and *Ilex* at the region, probably in lowlands or in	Cold and dry climate until 17ka, and progressive warm conditions from 17ka to	high-elevation grasslands, *Araucaria*, cloud forest elements. From 17ka to 10ka progressive growth of	Progressive expansion of Atlantic forest elemets between 17ka and 10ka.	yes

				spaced groups. Altantic forest elementes were also present. Conditions were probably a little more warmer than previously. From 10.9 to 9.9kyr BP: variations between grassland elements and cloud forests elements. From 9.9 to 2.6Kyr BP: *Weinmania*, *Araucaria*, *Ilex*, *Drymis* and *Clethra* dominated the polen data at the region. Probably the climate was warmer and moist, because of the expansion of cloud forest on high elevations. From 2.6k to 0.43kyr BP: *Araucaria* and *Podocarpus* on higher elevations, suggesting moist conditions at high elevations. From 0.43 to 0.07kyr BP: decrease of *Weinmania* pollen data.	10ka (Late Glacial).	cloud-forest elements.		
Behling (2002)	Catas Altas (MG) (20° 05' S; 43° 22' W, 755m)	48ka to 18ka	SE-NW	Almost treeless. Moisture possibly present only in riparian areas. Average temperature 5-7°C lower than today.	Cold and dry conditions	*Araucaria* and *Podocarpus*	none	yes
Argollo and Mourguiart (2000)	Bolivian Altiplano (66.00 - 71.00 S; 14.00 - 22.00 W, 3650 and 3900 m)	30Ka to present (Here: from 30kyr to 10kyr BP).	SE-NW	From 30 to 26ka: lake levels were clearly much higher than at the present-day. Cool and dry conditions from 26ka to 14ka. Before and latter this period, conditions were warm and wet.	Cool and dry conditions from 26ka to 14ka.		none	no
Argollo and Mourguiart (1995)	Lake Titicaca and Pocoyu, Bolivian Altiplano (66.00 - 71.00 S; 14.00 - 22.00	25ka to present		Similar water levels from 25kyr BP to 21kyr BP. Low temperatures (3.5-4°C below present conditions) and lower lake levels from 21 to 18Kyr BP. The climate was colder, and problably drier than the present. From 15kyrBP to 8Kyr BP: higher lake levels, indicative	Dry conditions during early and mid LGM. Wet conditions at Late Glacial.			no

	W, 3500 and 3900 m)			of wetter conditions.			
Ledru *et al.* (1996)	Lagoa campestre de Salitre (-19; -46.7667, 980 m)	50kyr BP to the present (here: from 50kyr to 10Kyr BP)	SE-NW	50ka to 40ka (dry phase). 40ka to 27ka (high mosture levels). Gap in sedimentation during the LGM. 16k-11ka (moisture increasing). Records of *Podocarpus* and *Araucaria* are indicative of cooler conditions. High levels of Myrtaceae (see fig. 4). 9.5ka-5ka (seasonal period). 4ka-3ka (return of moisture).	Gap of sedimentation during the LGM. Cold conditions during the period.	*Podocarpus*, *Myrsine*, *Simplocos*, *Solanum, and Drimys.*	inconclusive.
Colinvaux *et al*. (1996)	Lake Pata, western Amazonia (AM) (0.2667; -66.6833)	40 kyr to present (here: LGM).	SE-NW	The tropical rainforest persisted in the region, savannas and grasslands were absent. *Podocarpus* were present in the region (10%) with other cold adapted species (*Weinmania*, *Ilex*, *Humiria*, *Hedyosmum*, *Rapanea*) during the Pleistocene. Marked expansion of Amazonian Andine species (above 1,000m) to Amazonian lowlands.	Cold and wet conditions. The western Amazon remainend not fragmented.	*Podocarpus*, *Humiria*, *Hedyosmum*, *Rapanea*, *Weinmania*, and *Ilex*.	yes
Colinvaux *et al*. (2000)	Amazonian lowlands.		NE/SE-NW	Acording to them, the Amazon vegetation was not fragmented in its lowlands during the LGM, and also not replaced by savanna elements, as proposed by the refugia hypothesis. Parts in the NE Brazil were moister than nowadays during late-glacial times.	Cold and wet during the LGM.	Cold-adapted elements from Andes (i.e. *Podocarpus*).	yes.
Gosling 2008.	Huiñaimarca sub-basin of Lake Titicaca (Bolivian and Peruvian altiplano) (-16; -68.5, 3812m)	151ka to present (Here: LGM)	SE-NW	Probably wet conditions during the LGM. Between 7 and 4.5 mblf (c. 28,000–14,200 cal yr BP), *Isoëtes* concentrations > 10,000 grains/cm3 suggest that the basin contained water, though *Pediastrum* is absent. Results from this work suggests that the LGM (21,000 cal yr BP) would have been a	wet conditions during the LGM	Records of *Isoëtes*.	yes

				relatively wet time. After 14.2ka aquatic concentrations decline. However, the extreme cold conditions of the glacial maximum may have inhibited biological productivity and hence masked the “wet” signal associated with this time.				
Chepstow-Lusty et al. 2005	Bolivian Altiplano. Salar de Uyuni (-20; -68, 3653 m)	108 to 18ka (Here: the LGM)	SE-NW	Cold and wet conditions during the LGM. The Altiplano sustained a lake continuously throughout the LGM.	Cold and wet conditions in the Altiplano.	*Polylepis, Isoëtes.*		yes
Baker *et al.* (2001a)	Lake Titicaca. Bolivian and Peruvian Altiplano. (-16; -68.5, 3810m)	25ka to present	SE-NW	25 ka to 15 ka: Lake Titicaca was deep, fresh and a continuously overflowing lake during the LGM. This period was wetter than today.	The LGM in Lake Titicaca was wetter than today.			yes
Baker *et al.* (2001b)	Salar de Uyuni. Bolivian Altiplano (-20.2495; -67.5005, 3653m)	50 ka to present (here: 50ka to 10ka)	SE-NW	From 50 ka to 38.1 ka the climate was wetter than today. In a similar way, from 26.1 ka to 14.9 ka the conditions were wetter than today.	LGM conditions for the LGM.			yes
Burbridge *et al.* (2004)	Noel Kempff Mercado National Park. Bolivia (-14.4667; -61.0667, 600 to 900m)	50ka to present (here: 50ka to 10ka)	SE-NW	There were moments where precipitation in Bolivian Altiplano were 20 to 75% higher than present days. However, this region was more stable from 50 ka to the LGM. Pollen records from the region suggest presence of savanna elements during the LGM. There were records of moist forests species in the region, however maybe restricted to some areas, e.g.	Cold and dry conditions.	Peak of *Podocarpus* during the LGM.	Moraceae/Urticaceae, *Alchornea* (<10%).	yes (partialy. The author suggest dry conditions).

				associated with river courses (records of Moraceae and *Alchornea*). From 29.9-10.7 ka there were a peak of *Podocarpus* records, suggesting also cold and relatively moist conditions, due to species requirements. Absence of *Anadenanthera*, a typical Seasonaly Dry Tropical Forest elemnt.				
Colinvaux *et al.* (1997)	Lake Sucurucho. Equador. (-3; -79, 3180m)	From LGM to the present.	SE-NW	Typical plants in the region during pre LGM and in present times: *Weinmannia*, *Podocarpus, Hedyosmum* and *Alnus*. During the LGM this region was treeless, suggesting even colder conditions. During the end of the LGM warm conditions permitted the return of cold adapted elements.	Colder and moist conditions during the LGM.	*Podocarpus, Weinmania, Hedyosmum, Polypelis*, and *Alnus.*		yes
Salgado-Laboriau (2005)	Cromínia (GO) (-17.2833; -49.41667; 710m)	LGM to present (Here: the LGM)		Cromínia and Águas Emendadas were cold and wet during the LGM. Even with colder conditions, moist conditions were present and arboreal pollen were abundant, more abundant at that time than in present times. During the LGM, the Cerrado arboreal pollen occurred in conjoint with cold elements (*Rapanea*, *Hedyosmum*, *Ilex*, *Celtis*, *Salacia*, *Symplocos*, *Podocarpus*, Moraceae, Cunoniaceae and others), indicating colder climates in the region. *Mauritia* were absent in pollen records during the LGM, supporting cold conditions during that time. From 15 ka to 10 ka: dry conditions in Águas Emendadas and Serra dos Carajás.	Cold and somehow wet conditions during the LGM. Key-species such as *Podocarpus* and other cold adapted species support a SE-NW route during the LGM.	*Rapanea*, *Hedyosmum*, *Ilex*, *Celtis*, *Salacia*, *Symplocos*, *Podocarpus*, Moraceae, Cunoniaceae.	Description of some elements occurring in conjoint with cold-adapted species.	yes
	Aguas emendadas (DF) (-15.5667; -47.5833)	LGM to present (Here: the LGM)						
	Lagoa Bonita (MG)	LGM to present (Here: the LGM)						
	Lagoa dos Olhos (MG) (19° 38' S; 43°54' W, 739 m)	LGM to present (Here: the LGM)						
	Lagoa Santa	LGM to						

	(MG) (19° 38' S; 43°54' W, 739 m) Serra dos Carajás (PA)	present (Here: the LGM) LGM to present (Here: the LGM)						
Salgado-Laboriau *et al.* (1998)	Aguas emendadas (DF) (-15.5667; -47.5833)	32.4ka to present	SE-NW	From 32.4 ka to 30 ka - moist and warm climate. From 30-26 ka: cold and moist climate. Records of *Podocarpus*, *Hedyosmum* and *Ilex* pollens in Águas Emendadas from 25 ka to 21 ka, and the absence of *Mauritia* pollens are indicative of cold and wet conditions during the LGM. The Tardiglacial were drier in the region, though. Cromínia also presented a relative similar pattern.	Colder and moist conditions during the LGM.	*Podocarpus*, *Hedyosmum*, *Ilex*.	Absence of *Mauritia* pollen records.	yes
Salgado-Laboriau *et al.* (1998)	Cromínia (GO) (-17.2833; -49.41667; 710m)	32.4ka to present	SE-NW	From 32.4 ka to 30 ka - moist and warm climate. From 30-26 ka: cold and moist climate. Records of *Podocarpus*, *Hedyosmum* and *Ilex* pollens in Águas Emendadas from 25 ka to 21 ka, and the absence of *Mauritia* pollens are indicative of cold and wet conditions during the LGM. The Tardiglacial were drier in the region, though. Cromínia also presented a relative similar pattern.	Colder and moist conditions during the LGM.	*Hedyosmum*, *Ilex*.	Absence of *Mauritia* pollen records.	yes
Salgado-Labouriau et al. (1997)	Cromínia (GO) (-17.2833; -49.41667; 710m)	32.4ka to present		Prior to 32.4 ka the climate was semi-humid and warm. From 32.4-20 ka grasslands and riparian forests with cold adapted species dominated the records, suggesting colder and wet conditions. There were records of *Hedyosmum* and *Ilex, Cuphea*,	Colder and moist conditions during the LGM.	*Hedyosmum* and *Ilex*, *Cuphea*, *Plantago*, Cyperaceae, and Melastomataceae	Absence of *Mauritia* pollen records.	yes

				Plantago, Cyperaceae, and Melastomataceae during the LGM. From 18.5 ka to 11 ka the climate was dry, and sparse vegetation was growing in the region at that time. From 6.5 ka to present, moister conditions returned to the place again.			
Barberi *et al.* (2000)	Aguas emendadas (DF) (-15.5667; -47.5833)	30.5 ka to present	SE-NW	Between 24 ka an 21 ka the vegetation was denser than today, with aquatic plants and cold adapted species, suggesting a cold and wet period. Records of Hedyosmum, Ilex and Podocarpus in the region at that time. From 21 ka to 7 ka the climate was probably drier.	Wetter and and colder conditions during the early LGM and drier conditions during the Tardiglacial.	*Podocarpus*, *Hedyosmum*, *Ilex*.	yes
Haberle and Maslin (1999).	Amazon	50 ka to present	SE-NW/NE	From 50 ka to 42 ka - wet and cold conditions. From 42 ka to 19.8 ka - wet and warm conditions dominated the system; From 19.8-11 ka - wet and cool conditions (abundance increase of *Hedyosmum* and *Podocarpus*). From 11-9 ka - warm conditions returned to the region. Records of species with secondary growth (Cecropia) at that region. 9 ka to present - vegetation similar to present.	Wet conditions until the end of the Pleistocene. Wet and cold conditions during the LGM.	*Podocarpus*, *Hedyosmum*, *Alnus*.	yes
Bush *et al.* (2002)	Lakes Pata, Verde and Dragão (Amazon) (-0.26667; -66.6833, 320m)	170 ka to present (Robust chronology from 43 ka to present).	SE-NW	The LGM was a wet period (from 22 ka to 18 ka). Drier conditions occurred from 35 ka to 27 ka. The precipitations distribution is a better condition indicator (more sensitive) than temperature itself. The correspondence of insolation and precipitation is more complex than the correspondence of insolation and temperature (see p. 343). Probably even with precipitation	cold and wet		yes

				changes, the vegetation community did not changed substantially, keeping a forest appearance.				
Colinvaux and Oliveira (2000)	Amazonia	LGM to present		According to their interpretations, records from Lake Pata, Carajás and the Amazon fan suggest Amazon vegetation stability instead of fragmentation. According to them, savannas did not replace forest in the Amazon lowlands at any time in the Glacial Cycle. Pollen information from the fan gives generality to their conclusion. The main differences through time in Amazonian species composition was related to the expansion of cool-adapted populations in glacial times (*Podocarpus, Drimys, Alnus, Hedyosmum, Rapanea, Humiria, Ilex, Symplocos, Magnolia*, Ericaceae, and *Weinmannia*-type). According to them, the Carajás region did not suffered drastic changes in community composition during the LGM.	Wet and cold conditions during the LGM.	*Podocarpus, Drimys, Alnus, Hedyosmum, Rapanea, Humiria, Ilex, Symplocos, Magnolia,* Ericaceae, and *Weinmannia*-type		yes
Bush *et al.* (1990)	Mera (Ecuador) (-1.4833; -77.1)	30 ka to present	SE-NW	Regional cooling, suggesting 7.5°C depression at low latitudes near 0° during 33 ka to 30 ka. From 30 ka to 26 ka the region warmed up, and *Alnus* and Gramineae reduced their abundances, whereas some lowland elements become established with montane elements (*Podocarpus*, *Weinmannia*, *Drimys* and *Hedyosmum*) (but see Heine 1994 for other discussions).	Cooling of 7.5°C at the Andean foothills.	*Podocarpus, Weinmannia, Drimys* and *Hedyosmum.*	Yes (mixed with cold-adapted elements).	yes
Bush *et al.* (1990)	San Juan Bosco	30 ka to present	SE-NW					yes

	(Ecuador) (-3.0625; -78.4583)							
Hermanoswki *et al.* (2012a)	Pantano da Mauritia (SE Amazon). Serra Sul dos Carajás (PA) (-6.35172; -50.3935, 740m)	25k to present	NE	The southeastern part of Amazonia is of particular interest because it is especially sensitive to shifts of the ITCZ and to moisture input from the Atlantic Ocean. 25-11.4 ka - Cerrado species dominating the pollen record, associated with forest species. Also, records of cold-adapted species. There were also cold adapted species (*Myrsine*, 11%, *Ilex, Hedyosmum*, above 5%). Low evidence of fire. Probably forest elements were placed on mountain slopes. 11.4 - 10.2 ka: Forest elements dominating the record (Moraceae, Urticaceae, Melastomataceae and Combretaceae). 10.2- 3.4 ka: decrease to generally low values of main tropical forest taxa, and slightly higher values of Anacardiaceae, Bignoniaceae, and Zanthoxylum, and higher values of Fabaceae. 3.4 ka to present: increase of tropical forest taxa: Alchornea/Aparisthmium, Celtis-type, Melastomataceae/ Combretaceae and Moraceae/Urticaceae.	Wet and cold conditions during the LGM.	*Myrsine*, *Ilex*, *Hedyosmum*.	Presence of Cerrado elements.	yes
Behling and Negrelle (2001)	Volta Velha (Itapoá, SC) (-26.0667; -48.6333)	37.5 ka to present	SE-NW	From 37.5 to 14.5 ka: high frequencies of Poaceae, Asteraceae, *Euricaulon*/*Paepalanthus*. A cold adapted forest was also present. The average temperature was about 5.4 to 7.4°C lower than today at the region. The relatively frequent occurrence of	Wet and cold conditions during the LGM. The rainforest was replaced by grasslands and			yes

				Myrtaceae and Melastomataceae and of rarer trees, such as *Podocarpus*, *Symplocos*, and *Ilex*, suggests the existence of cold-adapted subtropical forests in the lowland during the pre-LGM and the LGM. *Symplocos tenuifolia*-type and then *Ilex* reached maximum pollen percentages. This change happened somewhat before most of the tropical taxa reached higher values, indicating progressive change toward a warmer climate. From 14.4 to 6.7 ka: Ferns, including some tree ferns, became frequent in the rain forest. Myrsine, Arecaceae, *Weinmannia*, *Hedyosmum*, *Solanea*, and *Clusia* became more frequent in the rain forest.	cold adapted forest.		
Behling et al. (2007)	Serra da Bocaina (SP) (-22.675; -44.5567, 1500 and 1650m)	18.57ka to present	SE-NW	18.5-12.5 ka: High levels of *Poaceae.* Presence of *Myrsine*, *Weinmannia*, *Podocarpus*, *Symplocos tenuifolia*-type, *S. lanceolata*- type, *Ilex* and *Drimys*with low abundance; 10.84-8.03 ka: higher records of montane forest groups (*Weinmannia*, *Symplocos lanceolata*-type, and *Ilex*. Reductions of Poaceae.	Cold and drier conditions during the LGM. Cold and Moister conditions in the Pleistocene/Holocene transition.	*Myrsine*, *Weinmannia*, *Podocarpus*, *Symplocos tenuifolia*-type, *S. lanceolata*-type, *Ilex* and *Drimys*	no
Ledru et al. (2001).	Lagoa do Caçó (Maranhão). Northeastern Brazil (-2.9667; -46.4167, 120m)	14ka to present	NE	High frequencies of *Podocarpus* during the LGM. From 14 to 13.5 ka - *Byrsonima* and *Didymopanax* are the dominant taxa. From 13.5 to 13 ka: high frequencies of Melastomataceae. From 13 to 12.8 ka: High frequencies of Moraceae and *Cecropia*. High levels od *Podocarpus*. From 12.5 to 11.5 ka: High records of Myrtaceae.	Cold and Moist conditions during the LGM.	*Podocarpus* and other cold-adapted species.	yes

				Podocarpus disappeared in the fossil record. The presence of *Myrsine*, *Byrsonima,* and *Ilex* indicates that this forest was similar to the *Podocarpus sellowii* forests found today at high elevations. Lake Pata (Amazon) record from the central Amazon lowland shows high *Podocarpus* percentages along with *Myrsine* immediately following the Last Glacial Maximum as well.				
Kull *et al.* (2003)	Sierra de Santa Victoria. Northwestern Argentina (-22.25; -68.0833, 3800 a 5000 m)	LGM	SE-NW	Their model indicates that both a massive temperature reduction and precipitation increase are necessary to explain the observed maximum glaciation. In combination with a precipitation enhancement of a factor of 2–4, temperatures must have been lowered by 4.5–8.01° C to explain maximum glacier extent. These results indicate that a massive temperature depression in combination with a moderate precipitation increase are necessary to explain the glacier advances during the LGM.	Cold and Moist conditions during the LGM.			yes
Mourguiart and Ledru (2003)	Near the village of Siberia, Bolivia. (-17.8333; -64.7189, 2920m)	40ka to present	SE-NW	Before the full glacial period (40 ka to 29 ka), the vegetation was dominated by forest with cloud forest elements (see Fig. 2) (*Alnus*, Melastomataceae-Combretaceae, Myrtaceae, Podocarpaceae, and *Polylepis-Acaena* (cold and humid climate conditions). From 28 to 17 ka: Increase of *Alnus* at lower altitudes, and also high records of Poaceae. This suggests cold and, possibly, dry condition (however,	Cold and dry conditions with a peak of moisture at 20ka.	*Polylepis-Acaena*, *Alnus*, Podocarpaceae.		yes

				microcharcoal particles were low in the period). The authors suggest that the region was drier than today during that time. A peak of *Polylepis-Acaena* and *Botryococcus* was also evident in the region (suggesting a short and abrupt increase of moisture at 20ka). From 16 to 11 ka: reforestation and return to moist conditions.		
Fritz et al. (2007)	Lake Titicaca (-17; -69, 3810m)	370 ka to present	SE-NW	High lake levels and expanded cordilleran glaciers of the last glacial stage and prior glacial stages were brought about by a combination of lower temperatures and higher regional precipitation. Thus, in the southern tropical Andes, climate conditions during global glacial stages are inferred to be cold and wet. Also, regional water balance and glacial mass balance are strongly influenced by global-scale temperature changes, as well as by precessional forcing of the SASM.	Cold and Wet conditions during the LGM.	yes
Mayle et al. (2000)	Noel Kempff Mercado National Park. Bolivia (-14.4667; -61.0667, 600 to 900m)		SE-NW	44 ka to 38.6 ka: Alchornea, Leguminosae (Papilionoideae), and Talisia-type pollen. They are indicative of plant communities that are very different from those of the Holocene (possibly semideciduous dry forests). The absence or negligible abundance of Moraceae pollen show that these forest communities were not rain forest. High levels of Mauritia at Laguna Chaplin. Occurrence of savanna species during the LGM in the fossil record. No too much change during this period in the records.	Cold and seasonal conditions at the region during the LGM.	no

Paduano et al. (2003)	Lake Titicaca. Bolivian and Peruvian Altiplano. (-17; -67, 3810m)	27.5Kyr BP to the present	SE-NW	From 27.5 to 21 ka: dry conditions. From 21 to 17.5 ka: wet and warmer conditions. Isoetes were also very frequent between 21 and 17.5 ka. Podocarpus, Weinmannia, and Alnus were also found between 21 and 17.5 ka, and between 17.7 and 13.7 ka; From 17.7 to 17.3 ka Puna brava was replaced by Puna and sub-Puna elements. At the period of 13.7 ka elements similar to present plant communities occurred at the region. A possible dry event occurred from 9 to 3 ka, caused by some aquatic elements records, and also by the increase of Poaceae, Apiaceae, Plantago, and Polylepis pollen. Fire is evident after 3.1 ka with the appearance of weed species. During the LGM the lake water level was higher than its past and present conditions.	Warm and possibly wetter conditions during the LGM at the Lake Titicaca.	Records of *Podocarpus*, *Weinmania*, *Polylepis-Acaena*, and other cold-adapted elements.		yes
Ferraz-Vicentini and Salgado-Labouriau (1996)	Close to Cromínia (GO) (-17.2833; -49.4167,)	32.4 ka to present	SE-NW	Before 32.4 ka palynological data support the hypothesis of existence of a Vereda vegetation and also a riparian forest at the region, and an arboreal Cerrado possibly occurred around this vegetation, suggesting warmer and semi-humid conditions. From 32.4 to 28.3 ka the arboreal elements decreased at the region. *Hedyosmum* and *Ilex* were frequent during this period. From 27.4 to 20 ka the Vereda vegetation was replaced by a shallow lake and open grasslands. Conditions were wet and cold. From 18.5 to 11.3 ka dry and cold conditions occurred at	Cold and moist conditions during the LGM.			yes

				the region. From 10.5 to 7.7 ka a dry but not so cold condition occurred at the region. The region was warming up during this period. From 6.7 to 3.5 ka Veredas, riparian forests, and an arboreal Cerrado formations returned to the region (possibly warm and moist conditions). From 3.5 ka to present there were frequent microcharcoal records.			
Salgado-Labouriau (1997).	Carajás (PA) (-6.3333; -50.4167, between 700 and 800m)	From LGM to present.	NE	In general, moister than the present from 36 ka to 22-18 ka.	Four of the sites with informations about the LGM showed moister conditions than at present from 36 ka to 22-18 ka (Carajás, Águas Emendadas, Cromínia, Serra Negra). Salitre had a flooded forest followed by a hiatus of sedimentation during the LGM, due to local conditions. Also, some of them contained records of cold forest expansion, during the	*Ilex*, *Weinmania*, *Salacia*, *Symplocos, Podocarpus,* and *Araucaria* (Carajás, Aguas Emendadas, Serra Negra, and Salitre).	yes

					LGM.	
Salgado-Labouriau (1997).	Aguas emendadas (DF) (-15.5667; -47.5833, between 1040 and 1170 m)	From LGM to present.	SE-NW	In general, moister than at present from 36 ka to 22-18 ka. Águas Emendadas: From 32.4-21.4 ka - arboreal pollen dominated the region (moister and colder conditions than present, with cold adapted forests in the pollen record. From 21.4-7.3 ka: dry period. From 7.3 ka to present: moister conditions.		yes
Salgado-Labouriau (1997).	Cromínia (GO) (-17.5667; -49.4167, 710m)	From LGM to present.	SE-NW	In general, moister conditions from 36 ka to 22-18 ka. From 32.4 to 28.3 ka: moist conditions; 28 ka to 19 ka: moisture was high and the climate was cooler than present. Natural fires occurred during this time. 18.5-11.3 ka: dry and cold period. The dry (but not cold phase) persisted from 10.5 to 7.7 ka. From 6.7 ka to present warm and moist conditions persisted, with palm swamps at the region.		yes
Salgado-Labouriau (1997).	Serra Negra (MG) (-18.9167; -46.8333,)	From LGM to present.	SE-NW	in general, more humid than at present from 36 ka to 22-18 ka. Records of *Podocarpus*, *Araucaria*, *Ilex*, *Alchornea* and *Raphanea*. Cold elements coexisting with elements from Tropical forests. At 14.34 ka *Podocarpus*, *Araucaria*, *Ilex* and Ericaceae were also present (in low concentrations) with tropical elements. 14.34 ka to present: temperate climate taxa were absent. From 5 ka to present: savanna elements and Tropical semi-deciduous forest were well represented, suggesting reduction of precipitation and higher temperature. A riparian		yes

				forest occurred then in the region. From 12.5 ka to present the vegetation was very similar to present vegetation.	
Salgado-Labouriau (1997).	Salitre (MG) (-19; -46.8333, 1050m)	From LGM to present.	SE-NW	32 ka to 28.7 ka - presence of a flooded marshy forest (Igapó) in a cold climate, but represented by Cerrado species, according to Salgado-Laboriau. Gap of sedimentation until 13 ka. From 13 ka to 8 ka: *Araucaria* and Araucaria forest elements present in the pollen record, interpreted as a northwestern expansion of the Araucaria forest, which today grows around 24° S. Genera such as *Araucaria*, *Drimys*, *Ilex*, *Podocarpus* were present. From 5.5 to 4.5 ka: a dry episode. From 4.3 ka to 3.06 ka: a semi-deciduous forest grew up at the region.	yes
Salgado-Labouriau (1997).	Lagoa Santa (MG) (-19.6333; -43.9,)	From LGM to present.	SE-NW	Records just since Mid Holocene.	inconclusive
Salgado-Labouriau (1997).	Lagoa dos Olhos (MG) (-19.6333; -43.9, 730m)	From LGM to present.	SE-NW	Records of *Podocarpus* at low concentrations in the region, however riparian forests and high levels of *Caryocar*, cerradão and arboreal savannas. A marsh occupied the site of the present lake from 19 ka to 13.7 ka. The climate was cold and semi-humid with a short dry season. 13.7 ka: humidity decreased and the temperature increased. Fire records in the region during this moment. 6.8 ka: a marsh returned to the region. At 4 ka a lake was formed at the site, suggesting increase in moisture.	yes

				Between 4 ka to 1.5 ka humidity was higher than present. 1.3 ka to present: moisture levels returned to present conditions.				
Salgado-Labouriau (1997).	Lago Valencia (Venezuela) (-10.1833; -67.7167, 400m)	From LGM to present.		Dry conditions between 12.9 ka to 11.5 ka. Then the lake was replaced by a marsh or an intermittent lake during this time. The climate was semi-arid. The beginning of the Holocene a saline endorheic lake was formed. Open forest elements were present (Bursera, Spondias and Tribulus). From 8.6 ka to present arboreal elements increased at the region.				no
Behling *et al*. (2004).	Cambará do Sul (RS) (-29.0525; -50.1011, 1040m)	42.8k to the Present	SE-NW	Somewhat wetter from 42.8 to 41.47 ka and not so cold. From 41.47 to 26.9 ka: Campos vegetation suggest a cold climate with repeated frost events and minimum austral winter temperatures below -10°C. From 26.9 to 10.12 ka pollen percentages continue to be high and *Araucaria* pollen, low. During the Holocene Poaceae pollen were high and the frequency of Araucaria pollen were slightly higher than previously. Fires were frequent after 7.4 ka. *Araucaria* forest probably was present in refugia until 4.3 ka, from where this species expanded its distribution through riparian forests in the region. *Weinmannia* was common at 1.52 and 1.77 ka, suggesting a shift for wetter conditions.	Cold and dry conditions during the LGM.	Reduction of cold-adapted species, suggesting very cold conditions. Just grasslands were present in abundance.	no	Yes for cold conditions. No for moist conditions. Also, it is incloncusive. There is no information about pollen records in lowlands.
Hanselman et al. (2011)	Lake Titicaca (-17; -67, 3810m)	370 ka to present	SE-NW	370-330 ka: Puna vegetation dominating the record: cold and wet conditions); 330-301 ka: Puna	Apparently cold and wet conditions	*Podocarpus*.	no	yes

vegetation (50%), *Alnus* (20%) and *Podocarpus* (7%) - warmer and relatively wet conditions. From 301-288 ka: Mainly Puna vegetation, with *Alnus* records and *Podocarpus*. warmer and wet conditions. 288 to 260 ka: cold and moist conditions. 238 to 211 ka: *Polylepis* dominating the community record - warm and moist conditions. 211 to 196 ka: Puna vegetation (80%), *Polylepis* (34%) - warm and wet conditions. 196 to 150 ka: High levels of *Polylepis*, *Podocarpus* also present (less than 10%) - warm and wet conditions. From 150 to 139 ka: Puna vegetation, records of *Polylepis* (15%), *Podocarpus* (15%) -warmer and moist conditions. From 139 to 115 ka: Total pollen concentration was extremely high, higher than in any other portion of this record. Records of Amaranthaceae (84%). *Polylepis* was more abundant than it is today. Low aquatic pollen, but *Pediastrum* concentrations were extremely high - probably warm and dry conditions. From 115 to 63 ka: records of *Polylepis*, with cold and moist conditions. 63 to 55 ka: high concentrations of *Isoetes*, and taxa from Puna vegetation - cold and moist conditions. 55 to 42 ka: (high *Isoetes* concentration - cold and moist conditions; from 42 to 28 ka: colder and moist conditions. 28 to 20 ka: Caryophyllaceae pollen was high from	during the LGM.

				25-24 ka. *Podocarpus* occupied 17% of the pollen record at 23 ka. Cold and moist conditions. From 20 to 3 ka: Low pollen concentration - warm and wet conditions High *Pediastrum* and *Isoetes* concentrations.				
Behling (1997)	Morro de Itapeva, SP (-22.7833; -45.5333, 1850m)	35Kyr BP to the Present	SE-NW	From 35 ka to 17 ka: dry and cold conditions predominating on high elevations. From 17.6 to 10 ka: cool and moist conditions. Records of *Podocarpus*, *Araucaria*, *Weinmannia*, *Ilex* at the region, probably in lowlands or in spaced groups. Atlantic forest elements were also present. Conditions were probably a little warmer than previously. From 10.9 to 9.9 ka: variations between grassland elements and cloud forests elements. From 9.9 to 2.6 ka: *Weinmannia*, *Araucaria*, *Ilex*, *Drimys* and *Clethra* dominated the pollen data at the region. Probably the climate was warmer and moist, because of the expansion of cloud forest on high elevations. From 2.6 to 0.43 ka: Araucaria and Podocarpus on higher elevations, suggesting moist conditions at high elevations. From 0.43 to 0.07 ka: decrease of Weinmannia pollen data.	Cold and dry conditions during the LGM. Change to cool and moist conditions on part of the LGM (late Glacial). Considering the altitude, it is likely that cold-adapted forests migrated to lowlands.	*Podocarpus, Araucaria, Weinmannia, Ilex*		yes (considering the high altitude and records of cold-adapted forests in the Late Glacial at the region). Inconclusive about climatic conditions on lowlands. The study suggest dry conditions in lowlands, not supported by other studies

								(i.e. see below).
Pessenda *et al*. (2009)	Curucutu, 20km de Itanhahem, SP. Serra do Mar (-23.93; -46.65, 750-850m)	28.46 ka to present	SE-NW	From 28.46 to 20 ka: forest and herbs (20-32%; 82-67%, respectively). Among arboreal elements the most common taxa are *Alchornea*, *Araucaria*, Melastomataceae, Myrtaceae, *Podocarpus* and *Symplocos* with percentages ranging from 2% to 5%. Herbs: Poaceae and Asteraceae; 23 ka to present: sharp increase of arboreal elements, reaching 55% of the total pollen sum. Arboreal increase of *Cybianthus*, reaching a 15% peak, *Ilex* (5%–12%) and *Symplocos* (1%–5%). The concentration of *Araucaria* and *Podocarpus* pollen found in the Curucutu core reach values between 125,000 and 17,000 and 4000 and 1500 grains/cm^3, respectively. Physiognomy similar to that of an *Araucaria* forest for Curucutu from 28.46 to 22.78 ka. The high *Araucaria* pollen concentration and the presence of *Podocarpus*, *Myrsine*, *Symplocos*, *Weinmannia*, Ericaceae, Melastomataceae, and Myrtaceae suggest a cold and humid forest. From 22 ka to 12 ka: forested landscape, whereas the increase of algal spores suggests higher moisture levels. The presence of botanical elements such as *Araucaria*, *Podocarpus*, and *Weinmannia,* clearly associated with cool and moist climates, suggests a significant temperature depression	Wet and cold conditions during the LGM.	*Podocarpus*, *Myrsine*, *Simplocos, Weinmannia.*		yes

				during the Late Glacial at Curucutu. After 12 ka until the present, the reduction in algal spores, as well as in terrestrial and aquatic herbaceous pollen, may suggest a decrease in moisture, although a higher frequency of arboreal elements and pteridophytes indicates the expansion of rainforest during the Holocene.				
Williams *et al*. (2011)	Laguna Khomer Kocha Upper, Bolivia (-7.27523; -65.7324167, 4153m)	18 ka to present	SE-NW	Before 14.5 ka, warm and relatively moist conditions free from fire, facilitated the expansion of high Andean *Polylepis* woodland. Fire at 14.5 ka quickly transformed the local vegetation, and the woodlands became restricted to areas protected from fire. 10.1 to 6.4 ka: dry event in the Holocene. From 6.4 to present: wetter conditions, sustaining the same community as before the Holocene dry event.	Moist and warm conditions in the LGM.	*Polylepis*.		yes
Auler and Smart (2001)	Laje dos Negros and Abreus, NE Brazil (-10.1817; -40.9577)	Here: the LGM	NE	Travertine information support wetter conditions from 21 ka to 10 ka compared to present.	Wet conditions during the LGM.			yes
Wang et al. (2004)	Northern Bahia (-10.1667; -40.8333)	210 ka to Present (Here: just the LGM)	NE	Wet conditions during the Mid and Late LGM, probably associated with a southward displacement of the ITCZ.	Wet conditions in part of the LGM.			yes
De Oliveira *et al.* (1999)	Icatu River (BA) (-11 to -10; -42.5 to -43.33)	10.99ka to present (here: from 10.99ka to	NE	Presence of forest taxa and montane taxa *(Rapanea*, *Hedyosmum*, *Humiria*, and *Ilex*) between 10,990 years ago and 10,540 years ago. Some species from Amazon lowlands were also present, at	The late Pleistocene conditions were wetter than today. Probably,	*Rapanea, Hedyosmum, Humiria, and Ilex*	Low concentration of *Cecropia, Cedrela, Pouteria,*	yes

		10.54ka)		low concentrations (*Cecropia*, *Cedrela*, *Pouteria*, *Protium*, *Simarouba*, *Symphonia* and *Trichilia*). *Humiria* is found at altitudes over 1000 m in northwestern Brazilian Amazonia, while Ericaceae, *Rapanea* and *Hedyosmum* are distinctly montane taxa in the Atlantic rainforests of southeastern Brazil. *Rapanea* and *Hedyosmum* is also documented in western Amazonia.	the temperature was lower than today, because of cold adapted forest records.		*Protium, Simarouba, Symphonia and Trichilia.*	
Behling (2001)	Lagoa do Curuça (PA) (-0.7667; -47.84)	11 ka to present (here: the Late-Glacial)	NE	Dense and high diverse Amazon rain forest in the coastal area. Absence of savanna elements during the Late-Glacial period. Presence of *Podocarpus* in the region, suggesting wet and cold conditions.	Wet and cold conditions during the Late-Glacial, 5°C below today.	*Podocarpus*		yes
Behling (1998)	Serra do Rio Rastro (SC) (-28.3833; -49.55,) Morro da Igreja (SC) (-28.1833; -49.8667,) Serra da Boa Vista (SC) (-27.7; -49.15,) Poço Grande (SC) (-26.4167; -48.8667,) Serra dos Campos Gerais (PR)	14ka to present.	NE-SW	Southern Brazil. 14 ka to 10 ka: predominance of grasslands (Serra do Rio Rastro, Serra da Boa Vista - SC). From 10 ka to 3 ka: tropical climate (Serra do Rio Rastro, Morro da Igreja, Serra da Boa Vista). Grasslands were progressively substituted by Atlantic Forest elements in the early Holocene. 3 ka to present: tropical climate and tropical vegetation. Cool and moist climate. *Araucaria* forest, probably placed on Highlands.	Dry and cold conditions in the early late Pleistocene.			no

	(-24.6667; -50.2167,)						
	Morro de Itapeva (SP) (-22.7833; -45.5333,)	48ka to present (here: from 48ka to 10ka)	SW-NE	Southeastern Brazil: 48 ka to 17 ka - Grasslands dominating the region, with *Araucaria* forests along rivers (Catas Altas). Markedly cooler and drier than today (Catas Altas and Morro do Itapeva). However cold and moist conditions returned to Morro de Itapeva during the LGM (records of *Araucaria* forest and cloud forest elements, probably at lower elevations).	Cold and dry in the Early LGM. In some places, cold and moister conditions took place in the Mid and Late LGM.		yes
	Catas Altas (MG) (-20.0833; -43.3667,)		SW-NE				
	Lago do Pires (MG) (-17.95; -42.2167,)		SW-NE				
	Lagoa da Curuça A (PA) (-0.7667; -47.84,)	11ka to present (here: the Late-Glacial)	NE	Northern Brazil Dense and high diverse Amazon rain forest in the coastal area. Absence of savanna elements during the Late-Glacial period. Presence of *Podocarpus* in the region, suggesting wet and cold conditions.	Wet and cold conditions during the Late-Glacial, 5°C below today.		yes
	Lagoa do Curuça B (PA) (-0.7667; -47.84,)		NE				
Pessenda *et al*. (2004)	Barreirinhas (MA) (-2.8667; -45.9167, 100-120 m a.s.l.)	15ka to present (here: from 15ka to 9 ka)	NE	15 ka-9 ka: arboreal vegetation. Forest and woody savanna covered most of the ecosystem transect of 78-km (that is constituted actually of a Forest–Cerrado–Restinga ecotone in the Barreirinhas region). 14 ka: based on the presence of *Didymopanax*, Myrtaceae, Moraceae, and *Podocarpus*, there was an expansion of	Wet and cold conditions. Warm conditions beginning at 12 ka.	*Podocarpus*	yes

				the forest. 12 ka: arrival of *Picramnia* and Mimosaceae, and the decline of *Podocarpus,* suggesting an increase in temperature.			
Pessenda *et al*. (2010)	Floresta Nacional do Araripe (FLONA) (-7.4772; -39.6091)	18 ka to present (here: 18 ka to 10 ka)	NE	Forested period between 18 ka to 10 ka, probably due to wet conditions.	wet conditions		yes
Pessenda *et al*. (2010)	Parque Nacional das Sete Cidades (PARNA) (-4.0833; -41.5)						
Pessenda *et al*. (2010)	Reserva Biológica do Guaribas (REBIO) (-6.6667; -35.1167)						
Sifeddine *et al*. (2001)	Carajás area (-6.5833;-49.5)	30ka to present	NE	30 ka to 22 ka: tropical rainforest dominating the pollen record; 22 ka to 13 ka: dry climate; 13 ka to 10 ka: wet climate and presence of forest elements in the region.	Wet conditions until the beginning of the LGM. Dry conditions during the LGM.		yes (wam group)
Absy *et al.* (1991)	Carajás area (-6.5333;-50.4167, 700-800m)	60ka to present (here: 60ka to 10ka)	NE	Dry conditions in ~60 ka, ~40 ka and from 21 ka to 11 ka. Local rainfall reduction in the order of 500 mm (from 1,500-2,000 to 1,000-1,500 mm). High records of *Ilex* around 50 ka and 28 ka.	Dry conditions during the LGM. Probably cold conditions too. Similar results to Sifeddine et al		yes (warm group)

					(2001).		
Ledru *et al.* (2002)	Lagoa do Caçó (MA) (-2.9667; -43.4167,)	18 ka to present (here: 18 ka to 10 ka)	NE	18 ka to 14 ka - open vegetation species dominating the pollen record (grasses, halophytes, herbaceous plants); 14 ka to 10 ka - forest dominating the pollen record (Myrtaceae and Moraceae).	Wet conditions at the end of the LGM.		yes (warm group)
Sifeddine *et al.* (2003)	Lagoa do Caçó (MA) (-2.9667; -43.4167,)	21 ka to present (here: 21 ka to 10 ka)	NE	21-17.4 ka - dry climate. However, this does not imply that conditions were extremely dry all over, but the dry climate was interrupted by moist events; 17.4-16.2 ka - humid conditions; 16.2-14.7 ka - humid and cold conditions (*Podocarpus* records). 14.7-12.9 ka- moist and warmer conditions. 13-12 ka - dry period. 12k-11.5 ka - moist period. 11.5k-7 ka: moist period. The Late Glacial humid phase was marked by three extreme events which caused lake-level rises at 17.4 ka, 14.9 ka and 12 ka.	Dry LGM with three peaks of wet climates along the LGM.	*Podocarpus*	yes
Hermanoswki *et al.* (2012b)	Pántano da Maurítia. the Serra Sul dos Carajás (PA) (-5.35172; -50.3935, 740m)	73 ka to present	NE	73 ka - the dominance of savanna vegetation reflects drier conditions, however there were also moist conditions before 50 ka. 50-30 ka - the conditions were alternating from wet to dry phases, probably under generally cool conditions. Relatively high records of cold adapted elements (*Ilex* and *Myrsine*). 30-25 ka: moist conditions with cold adapted species (mainly *Myrsine*). High records of Melastomataceae/ Combretaceae and comparatively lower records of Poaceae. 25-11.4 ka: relatively dry conditions. There were records of	Wet conditions during the pre-LGM (30 ka - 25 ka). Higher diversity of cold adapted taxa with *Hedyosmum*, *Podocarpus*, *Styrax*, and *Weinmannia*-type indicates the transition to colder conditions. Dry		yes (warm group)

				tropical taxa (10-15%) and Cerrado species occurring together (Poaceae - 40%; *Spermacoce* - 15%). 11.4-10.2 ka - tropical forest expanded, reflecting stable and very humid conditions. Moraceae/Urticaceae (36%) and Melastomataceae/Combretaceae are characteristic of this zone.	conditions during the LGM. Tropical taxa and cerrado species. 11.4-10.2 ka: wetter and possibly slightly warmer conditions.		
Ledru *et al.*(2006)	Lagoa do Caçó (MA) (-2.9667; -43.4167,)	19 ka to present	NE	From 19 ka to 17.5 ka: dry conditions. From 17.5 to 12.8 ka: moist conditions. Presence of forests and occurrence of *Podocarpus* and *Ilex* in the region. From 12.8 to 11 ka: forest reduction.	Dry and cold conditions during the first part of the LGM. Wet and cold conditions during the late LGM (Tardiglacial). Expansion of forests elements in regions where Restinga and Cerrado vegetation currently dominate the landscape.	*Podocarpus* and *Ilex*	yes

Table S2.5. Palaeopalynological and speleothem studies depicting main species associated and climatic conditions during the Last Interglacial (LIG) for localities in the Amazon, Atlantic Forests, and along the proposed connection routes between the two biomes. These studies were used for ENM validation in projections to LIG. dd = decimal degrees.

Autor	**Site (Lat dd, Long dd, altitude)**	**Period**	**Route**	**Main results of this paper**	**Warm-adapted elements**	**Cold-adapted elements**	**Validate ENM?**
Ledru *et al.* (2009)	Colônia, SP (-23.8667; -46.7056, 900 m)	130 ka to present (here: 130 to 50 ka)	SE-NW	130 ka to 116 ka - moist conditions with extratropical winter rains in some moments. Tropical forest species during almost all the period (Flacourtiaceae and Melastomataceae/ Combretaceae), and presence of some cold adapted species (e.g.: Araucaria and *Podocarpus*). Higher percentage of arboreal pollen and higer pollen diversity. From 116 ka to 50 ka: moist conditions on average.	Flacourticeae and Melastomataceae/Combretaceae	*Podocarpus*	yes
Cruz Jr et al. (2007).	Botuverá (S) and (-27.2233; -50.5056, varies from 230 to 700 m a.s.l.)	116 ka to present	SE-NW	Lower values of Sr/Ca and Mg/Ca ratios (indicating wetter conditions) before 116 ka and between 96 ka and 85 ka. Wet conditions persisted throughout most of the last glacial period from approximately 70 to 17 ka.			yes
	Santana (SE) caves (-24.5308; -48.7267,)	116 ka to present	SE-NW				yes
Gosling et al. (2008).	Huiñaimarca sub-basin of Lake Titicaca (Bolivian and Peruvian altiplano) (-16; -68.5, 3812m)	151 ka to present (Here: data related just to the LIG)	SE-NW	High lake levels in Huiñaimarca are revealed to occur during the transition into, and out of, the last interglacial period (c. 134, 114 and 92 ka), and during full glacial conditions (c. 70 and 45 ka). Records of Chenopodiaceae/ Amaranthaceae during the last interglacial indicate that there were communities in the past that have no modern analogue in the Andes. Peaks in *Polylepis*/*Acaena* pollen is probably indicative of expanded *Polylepis* woodlands, indicative of moist/ warmer periods in the transition from full interglacial to full glacial conditions (118–70 ka).	In this context, *Polylepis*/*Acaena*		yes
Hanselman et al. (2011).	Lake Titicaca (-17; -67, 3810 m)	370 ka to present	SE-NW	370-330 ka: Puna vegetation dominating the record: cold and wet conditions); 330-301 ka: Puna vegetation (50%), *Alnus* (20%) and *Podocarpus* (7%) - warmer and relatively	In this context, *Polylepis*		yes

wet conditions. From 301-288 ka: Mainly Puna vegetation, with *Alnus* records and *Podocarpus*. warmer and wet conditions. 288 to 260 ka: cold and moist conditions. 238 to 211 ka: *Polylepis* dominating the community record - warm and moist conditions. 211 to 196 ka: Puna vegetation (80%), *Polylepis* (34%) - warm and wet conditions. 196 to 150 ka: High levels of *Polylepis*, *Podocarpus* also present (less than 10%) - warm and wet conditions. From 150 to 139 ka: Puna vegetation, records of *Polylepis* (15%), *Podocarpus* (15%) - warmer and moist conditions. From 139 to 115 ka: High total pollen concentration. Records of Amaranthaceae (84%). Low aquatic pollen, but *Pediastrum* concentrations were extremely high. *Polylepis* pollen representation reached 30% between 125 and 123ka, higher levels than today - probably warm and dry conditions. From 115 to 63 ka: records of *Polylepis*, with cold and moist conditions. 63 to 55 ka: high concentrations of *Isoetes*, and taxa from Puna vegetation - cold and moist conditions. 55 to 42 ka: (high *Isoetes* concentration - cold and moist conditions; From 42 to 28 ka: colder and moist conditions. 28 to 20 ka: Caryophyllaceae pollen was high from 25-24k. *Podocarpus* occupied 17% of the pollen record at 23 ka. Cold and moist conditions. From 20 to 3 ka: Low pollen concentration - warm and wet conditions High *Pediastrum* and *Isoetes* concentrations.

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Captítulo 2 - Crossing the dry diagonal: shallow divergence and recent expansion of a Neotropical lizard support ancient connections between the Amazon and Altantic Forest.

Resumo

A Amazônia e a Floresta Atlântica estão entre as florestas tropicais mais ricas do mundo. Entre elas há um corredor de vegetações mais abertas formado pela Caatinga, Cerrado e Chaco, considerado obstáculo para o trânsito de espécies florestais. Contudo, distribuições disjuntas de vários taxons ocorrendo tanto na Amazônia quanto na Floresta Atlântica sugerem eventos passados de conexões entre os dois biomas. Diversas rotas foram propostas na literatura, podendo ser resumidamente classificadas em rotas através do nordeste do Brasil, conectando o leste da Amazônia e o norte da Floresta Atlântica; e rotas que conectam o oeste da Amazônia e o sudeste da Floresta Atlântica. Das diversas rotas propostas na literatura, a rota de conexão utilizando a rede de matas de galeria do Cerrado, proposta por Oliveira-Filho e Ratter (1995), é a menos estudada e, aparentemente, não há nenhum estudo testando essa hipótese com dados moleculares. Werneck (2011) afirma que as matas de galeria do Cerrado possuem uma história recente por terem sido formadas a partir do Pleistoceno, por meio do desgaste das áreas de platô formando as depressões do Cerrado. Assim, as linhagens presentes nas matas de galeria apresentariam baixa diversidade genética, baixa resolução filogenética, comprimentos de ramos curtos e assinaturas genéticas de expansão, sugerindo forte influência das mudanças climáticas do Quaternário nos seus padrões de diversidade genética delas. Contrapondo a hipótese de Oliveira-Filho e Ratter (1995), trabalhos sugerem que as áreas de platô do Cerrado também podem funcionar como barreiras para o fluxo gênico e de espécies de matas de galeria. Assim, este estudo testou as hipóteses de Werneck (2011) acerca do

padrão filogenético/filogeográfico em organismos presentes de matas de galeria com *Colobosaura modesta*, um organismo típico de matas de galeria, mas com ocorrência marginal na Amazônia, Floresta Atlântica e brejos de altitude nordestinos. Também testamos a hipótese de Oliveira-Filho e Ratter (1995) afirmando que as matas de galeria se comportam como uma rede interconectada permitindo o fluxo gênico e de espécies contra a hipótese das áreas de platô atuando como barreiras para o fluxo gênico de espécies de matas.

Realizamos reconstruções filogenéticas com inferência Bayesiana e Máxima Verossimilhança, bem como cronogramas a partir de inferência Bayesiana. Populações foram agrupadas com o programa BAPS 6.0 e testadas sobre a possibilidade de serem espécies diferentes com o BPP 3.2. Padrões de isolamento por distância e do efeito do relevo na estruturação genética de *Colobosaura modesta* foram avaliados com testes de Mantel parcial e uso do algoritmo de Monmonier implementado no Barrier 2.2. A expansão populacional em *Colobosaura modesta* foi avaliada com Bayesian Skyline Plots e Computações Bayesianas Aproximadas (ABC), contrastando diferentes cenários que sugerem ou não a influência de mudanças climática do Pleistoceno na história da espécie. Por fim, modelagens de distribuição potencial da espécie foram realizadas a fim de observar o surgimento de rotas de conexão através do Cerrado, bem como outras possíveis rotas de conexão.

Os resultados corroboram que as matas de galeria se comportam como uma rede interconectada permitindo fluxo gênico e de espécies através do Cerrado e corroboram que essas regiões tenham sido intensamente influenciadas pelas flutuações climáticas do Pleistoceno. A única divergência antiga em *Colobosaura modesta* ocorreu no Plioceno, separando duas linhagens: uma linhagem da Serra do Cachimbo de todas as demais linhagens. Essas linhagens apresentam baixa resolução filogenética e baixos comprimentos de ramos. De modo semelhante, a estrutura genética de *Colobosaura modesta* suportou modelos de expansão populacional recente, associados ao Pleistoceno.

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Apêndice - Manuscrito a ser submetido à revista Molecular Ecology

Original Article

Crossing the dry diagonal: shallow divergence and recent expansion of a Neotropical lizard support ancient connections between the Amazon and Altantic Forest.

Roger Maia D. Ledo[1,2], Fabricius M. C. B. Domingos[2], Lilian G. Giugliano[3], Jack W. Sites Jr[4], Fernanda P. Werneck[5], Guarino R. Colli[2].

[1] Instituto Federal de Educação, Ciência e Tecnologia de Brasília, Campus Samambaia, Área de Meio Ambiente, 70860-100, Samambaia, DF, Brazil

[2] Universidade de Brasília, Departamento de Zoologia, 70.910-900, Brasília, DF, Brazil

[3] Universidade de Brasília, Departamento de Genética e Morfologia, 70.910-900, Brasília, DF, Brazil.

[4] Department of Biology and Bean Life Sciences Museum, Brigham Young University, Provo, Utah 84602, USA.

[5] Instituto Nacional de Pesquisas da Amazônia, Programa de Coleções Científicas Biológicas, Coordenação de Biodiversidade, 69060-000, Manaus, AM, Brazil.

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Corresponding author: Roger Maia D. Ledo (roger.ledo@ifb.edu.br)

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Abstract

The hypothesis of ancient connections between the Amazon and the Atlantic forest through the central Brazil is the one with less attention in the literature so far. Studies suggest that riparian forests, mainly placed in Central Brazilian valleys, would contain species with Pleistocenic lineages, shallow divergences and with signatures of population expansion, contrasting to patterns of more ancient routes proposed in the literature. Moreover, studies also suggest that the Central Brazilian plateaus act as barriers to gene flow for riparian forest-dwelling species. We tested the hypothesis of recent events shaping the genetic structure of a riparian forest species, and also tested the hypothesis of riparian forests acting as an interconnected network for gene and species flow with *Colobosaura modesta*. We conducted phylogenetic and spatial analysis with a multi-locus approach, and also employed species delimitation tests to our data. We also tested contrasting past demographic scenarios with Approximate Bayesian Computation to describe the history of *Colobosaura modesta* history. Our results support a recent history of colonization and expansion along the riparian forests of the Brazilian Cerrado and the dry diagonal, associated with the Pleistocene. Our study also supports the existence of two species within *Colobosaura*, one associated with Serra do Cachimbo (PA) and the other comprising the remaining distribution of the species. This study corroborates that riparian forests act as an interconnected network in the Cerrado context, and also supports the hypothesis of recent events shaping riparian forest organisms.

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Introduction

The most important mechanisms of species diversification in the Neotropics have intrigued biogeographers for centuries (Bates 1862; Haffer 1969; Vanzolini & Williams 1981; Wallace 1852). The Amazon and the Atlantic Forest are among the most diverse rainforests in the world (Hoorn *et al.* 2010; Mittermeier *et al.* 2005; Myers *et al.* 2000) and these two forest biomes are separated by a corridor of drier and more open vegetations (Brieger 1969; Bucher 1982), formed by the Caatinga, Cerrado, and Chaco (Werneck 2011), considered an important obstacle for biotic exchange between both rainforests (Oliveira-Filho & Ratter 1995; Raven & Axelrod 1974). However, the disjunct distribution of several taxa occuring in both the Amazon and the Atlantic Forest support ancient connections between these forests through the dry diagonal (Batalha-Filho *et al.* 2013; Costa 2003; Ducke 1953; Patton & Costa 2003; Santos *et al.* 2007).

Several distinct connection routes between the Amazon and the Atlantic Forest were proposed in the literature. They can be arranged in two main groups: routes passing through the northeastern Brazil (NE routes) (De Oliveira *et al.* 1999; Por 1992; Thomé *et al.* 2016), and routes connecting the western Amazon and the southeastern Atlantic Forest (SE-NW routes) (Batalha-Filho *et al.* 2013; Bigarella *et al.* 1975; Oliveira-Filho & Ratter 1995).

Bigarella *et al.* (1975) were the first to propose a SE-NW bridge, supported by the disjunct distribution of several taxa (ferns, insects, mammals) occurring in western Amazon and southeastern Brazil, but not in northeastern Atlantic Forest (Andrade-Lima 1969; Costa 2003; Willink 1988). Por (1992) conceived this corridor linking the southeastern Atlantic Forest (uplands in Minas Gerais and São Paulo), the forests from the medium Paraná and Paraguay rivers, montane and submontane forests of western Amazon in the Madeira River headwaters, and montane forests of the Guianas. This route was also supported by several other taxonomic groups (Batalha-Filho *et al.* 2013; Costa 2003; Fouquet *et al.* 2012; Patton &

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Costa 2003; Rodrigues *et al.* 2014), and was considered the most ancient ammong all proposed routes (Batalha-Filho *et al.* 2013). Oliveira-Filho and Ratter (1995) proposed a SE-NW route occurring through central Brazil, given the vast interconnected net of riparian forests and other forest formations in the Cerrado. Considering all the proposed routes, Oliveira-Filho and Ratter (1995)'s received less attention in the literature, and there is no molecular study evaluating details about when and how these connections routes occurred might have occurred.

Riparian forests are frequently associated with depressions and steep-sided hills in central Brazil (Silva 1997), following river basin water courses. The highest proximity among river basins occurs in their headwaters, usually placed on plateaus, where savanna vegetations are widespread (Silva 1997; Silva & Bates 2002). Besides currently embracing about 10-20% of the Cerrado distribution (UNESCO 2002), riparian forests contains a high diversity of vascular plants (Ribeiro *et al.* 2001), and also increase the Cerrado richness by harboring a flora and fauna more related to the Amazon and Atlantic Forest (Oliveira-Filho & Ratter 1995; Redford & Fonseca 1986; Silva 1996). Rodrigues (2005) also suggested that riparian forests could also have increased forest species richness by capturing savanna species in wetter conditions during Pleistocene, through an asymmetric vanishing refuge model (Vanzolini & Williams 1981).

Werneck (2011) hypothesized that riparian forest-dwelling species would depict a recent history of population expansion, low phylogenetic resolution and shallow branches, considering that this physiognomy is associated with depressions in the Central Brazilian plateaus, and probably originated during Pleistocene erosive processes and climatic changes (Colli 2005; Silva & Bates 2002). This phenomenon would result in older lineages bein found in species associated with the plateaus (savannas) and recent lineages in species associated with depressions (riparian forests) (Werneck 2011). Although there are evidences corroborating old lineages and deep divergences associated with species inhabiting plateaus (Domingos *et al.* 2014; Guarnizo *et al.* 2016; Maciel *et al.* 2010; Santos *et al.* 2014), molecular studies investigating species inhabiting depressions are still scarce.

It can also be derived from Werneck (2011) and other studies that the Central Brazilian plateaus and its open savanna formations could also act as barriers to gene flow for typical forest-dwelling species in the Cerrado, usually distributed along depressions (Silva 1997; Silva & Bates 2002). Some studies considered the altitude from the Brazilian plateau as important in explaining frog (Valdujo *et al.* 2013), bird (Silva 1996), and lizard (Nogueira *et al.* 2009) species turnover within the Central Brazilian plateau. However, some studies suggest other factors, such as the ecological gradient (Cabanne *et al.* 2011), niche conservatism (Valdujo *et al.* 2012), and isolation by distance (i.e. the core/periphery hypothesis) (Santos *et al.* 2014), as more important. In a similar fashion, previous studies suggest that the central Brazilian riparian forests act as a network permitting gene flow and species transit across the entire Cerrado (Mares & Ernest 1995; Oliveira-Filho & Fontes 2000; Oliveira-Filho & Ratter 1995) and the Central Brazilian plateaus would not act as barriers to forest-dwelling species.

Statistical phylogeography is a powerful approach to test temporal and spatial contrasting hypotheses, due to coalescent information and estimation of demographic parameters through time (Knowles 2009; Knowles & Maddison 2002; Lacey Knowles & Alvarado-Serrano 2010). Phylogeographic studies also provide an important chronological component, essential for evolutionary investigations, and increased explanatory power compared to classic diversification studies (i.e. biogeographical, or paleoecological evidence) (Rull 2011). Associated with modern model-based statistics, such as Approximate Bayesian Computation (ABC), phylogeography has developed into a explicit hypothesis testing discipline (Beaumont *et al.* 2010), contrasting with customary descriptive approaches from its inception. The publication of South American phylogeographic studies haa also increased during the last decade (Turchetto-Zolet *et al.* 2013) compared to previous years (Beheregaray 2008). However, interpretations of the main mechanisms of diversification in the Neotropics might be biased, as the majority of phylogeography studies concerned the biota of the Andes, Amazon, and Atlantic Forest (Turchetto-Zolet *et al.* 2013). Inside the Cerrado, there are also some publications concerning species mainly distributed in plateaus (Domingos *et al.* 2014; Guarnizo *et al.* 2016; Santos *et al.* 2014), whereas phylogeographic studies of species from depressions are neglected (Rocha *et al.* 2014; Rocha *et al.* 2011).

Lizards are considered model organisms for several studies (Huey *et al.* 1983), including phylogeography and speciation, due to its abundance and specific habitat requirements in local communities (Camargo *et al.* 2010). Phylogeographic studies applied to the lizards from the dry diagonal have frequently revealed cryptic species (D'Angiolella *et al.* 2011; Domingos *et al.* 2014; Werneck *et al.* 2012a) and helped to elucidate diversification patterns in the Neotropical region (Carnaval 2009; Werneck *et al.* 2012a). *Colobosaura modesta* is a leaf litter-dwelling lizard, widely distributed in forested habitats of the Cerrado, such as riparian forests and "cerradão" (Colli *et al.* 2002; Nogueira *et al.* 2009), and also in eastern Amazon (Ávila-Pires 1995; Cunha 1977). Isolated populations of *C. modesta* were also collected in restinga habitats from the Atlantic Forest of northern Bahia (Couto-Ferreira *et al.* 2011), and also in Chapada do Araripe, Cratéus, and Planalto do Ibiapaba forest enclaves (Freire *et al.* 2012), landscapes sustained by orographic rainfall in the Caatinga (Tabarelli & Santos 2004). The patchy distribution of this species in the Caatinga and Atlantic Forest suggest that the present-day distribution of this species could be the result of a larger distribution in northeastern Brazil in the past, since several independent data, such as molecular (Batalha-Filho *et al.* 2013; Carnaval & Bates 2007; Thomé *et al.* 2016), paleoecological data (De Oliveira *et al.* 1999; Pessenda *et al.* 2010; Pessenda *et al.* 2004), and fossil record (Czaplewski & Cartelle 1998; Hartwig & Cartelle 1996) suggest Pleistocene moister conditions at that region. The distribution of this species in Cerrado riparian forests could also be useful for testing hypothesis of the riparian forests acting as an interconnected network promoting gene and species flow across the Cerrado. Moreover, besides being considered a monotypic genus, the validity of some other taxa within *Colobosaura* are still in debate. *C. kraepelini* (Werner 1910), was described based on only one young female from Puerto Max, Paraguay, but later synonymized to *Colobosaura modesta* (Amaral, 1932). Similarly, *Colobosaura landii* (Cunha 1977) was described based on some specimens collected in primary forests from the eastern Amazon, but later recognized as synonyms of *Colobosaura modesta* (Cunha & Nascimento 1982, 1983; Nascimento *et al.* 1987).

Herein, we studied the phylogeography of *Colobosaura modesta* and tested several hypotheses concerning the evolution of this widespread species. Given that *C. modesta* is mainly associated with riparian vegetation in central Brazil and eastern Amazon, and considering the isolated populations in the Caatinga forest enclaves and in the Atlantic Forest, we specifically tested Werneck (2011)'s hypothesis of Pleistocene climatic events shaping the diversity and population structure of this species. If this hypothesis is correct, we would expect that (a) *C. modesta* would present shallow divergence among populations, depicting a pattern of recent colonization history and population expansion; (b) recent divergence times among lineages within the species, indicating a history more associated to Pleistocene climatic changes than to pre-Pleistocene geological events; and (c) presence of genetic signature of population expansion, associated with forest expansions in the Cerrado valleys during the Quaternary. Moreover, we also tested Oliveira-Filho and Ratter (1995)'s hypothesis of riparian forests acting as an interconnected network permitting gene and species flow across the Cerrado vs. the hypothesis of the Cerrado plateaus acting as barriers for gene flow for this species. If riparian forests are interconnected, populations of *C. modesta* will show (d)

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genetic similarity in the headwaters of the main river basins of the Cerrado, and (e) patterns of isolation by distance will be important in explaining genetic differences among populations. However, if the Cerrado plateaus act as barriers to gene flow, genetic breaks will be probably associated with river basin limits, and other features, i.e. altitude, will be important in explaining genetic structure.

Material and Methods

Collection of specimens and laboratory protocols

We obtained 76 tissue samples of *Colobosaura modesta* from 20 different localities, comprising populations in the Cerrado, Amazon, Atlantic Forest and Caatinga (Chapada do Araripe, CE) (Table 1; Fig.1A). Tissue samples were obtained primarily from specimens deposited at Coleção Herpetológica da Universidade de Brasília - CHUNB, but we also obtained tissue samples through fieldwork led by the authors, and through loans from colleagues and other museums (Zoological collections at Universidade Federal de Goiás - ZUFG, Universidade Federal do Rio Grande do Norte - UFRN, and Universidade Católica de Salvador - UCSAL). We also obtained specimens of *Acratosaura mentalis* (AAGARDA 4227, 5689, 5690, 6874, and 8631) and *Vanzosaura rubricauda* (CHUNB 58600) to use as outgroups in phylogenetic reconstructions.

We extracted genomic DNA with the DNAeasy Qiagen® and Invitrogen® extraction kits, following the manufacturers' protocol. We sequenced all individuals for a fragment of the mitochondrial cytochrome B (cytb). We complemented the mitochondrial information by sequencing a subset of the individuals for fragments of five nuclear genes: the prolactin receptor (PRLR), beta-fibrinogen (B-FIB), matrix remodeling associated 5 (MXRA5), alpha-cardiac actin gene - intron 3 and exon 4 (ACA), and synuclein alpha interacting protein gene (SINCAIP). PCR protocols varied for each locus (Table 2). We vacuum-purified PCR products using MANU 30 PCR plates Millipore and subsequently resuspended the DNA with ultra-pure water. Sequencing reactions used the ABI Big-Dye Terminator v3.1 Cycle Sequencing Kit in an ABI GeneAmp PCR 9700 thermal cycler. Sequencing products were purified with Sephadex G-50 Fine (GE Healthcare) and sequenced on an ABI 3730xl DNA Analyzer at the Brigham Young University DNA Sequencing Center (http://dnasc.byu.edu/). Some samples were also sent to Macrogen® for sequencing. Chromatograms were assembled, aligned using MUSCLE (Edgar 2004), concatenated (when necessary), and inspected by eye using GENEIOUS R 8.1 (Geneious Co., Wellington, New Zealand).

Nuclear genetic data of all subsampled individuals were phased using PHASE (Stephens *et al.* 2003) from within DNASP 5 (Librado & Rozas 2009). Haplotype and nucleotide diversity, and other general genetic statistics were also obtained with DNASP 5. To analyze cytb haplotype relationships among populations, we employed a median joining network (Bradlet *et al.* 1999) with POPART 1.0 (http://popart.otago.ac.nz.), using the default epsilon = 0 to avoid excessive median vectors.

Phylogenetic relationships and divergence times in Colobosaura modesta

To reconstruct the phylogeographic structure in *Colobosaura modesta*, and also to look for shallow divergences and small branch lengths in riparian forest-dwelling species, as proposed by Werneck (2011), we estimated gene genealogies, and divergence times based on single-genes and partitioned concatenated alignments (mitochondrial and nuclear DNA). Gene genealogies were based on Bayesian Inference (BI) and Maximum Likelihood (ML). We estimated the optimal nucleotide substitution model for each gene for both BI and ML analyses using PARTITION FINDER 1.1.1 (Lanfear *et al.* 2012). We implemented the Bayesian Information Criterion (BIC) in PARTITION FINDER 1.1.1 to select the optimal nucleotide substitution model for each gene for BI (cytb: HKY+G; PRLR: HKY+I+G; BFIB:

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HKY+I+G; MXRA5: HKY+G; ACA: HKY+I+G; SINCAIP: HKY+I+G). We used MrBayes 3.2.5 (Ronquist *et al.* 2012) to generate 10 million generations with the Metropolis-coupled Markov chain Monte Carlo (MCMC) algorithm. Each run contained four incrementally heated Markov chains, sampled every 1000 generations. Convergence of the two runs was assumed when the average standard deviation of the split frequencies was less than 0.01. We contrasted our BI analysis with a partitioned maximum likelihood inference (ML) using RAxML version 8.0 (Stamatakis 2014), using the GTRGAMMAI model of evolution and a standard heuristic search with 1000 pseudoreplicates (bootstrap).

Divergence times within specific level in *Colobosaura modesta* were based on the mitochondrial and the five phased loci under a coalescent exponential model implemented in Beast v.2.3.1 (Bouckaert *et al.* 2014). We used a coalescent exponential prior, uncorrelated lognormal relaxed clocks, and calibrated the time estimates using a normal prior distribution on the mtDNA global substitution rate (mean = 0.0065 substitutions/my), following the estimate for lizards of 0.65% changes/my (Macey *et al.* 1998). Substitution rates for the five nuclear markers were estimated relative to the mtDNA rate using a uniform prior for *ucld.mean* with default values, and uniform prior for *ucld.stev*, with a mean of 0.5. We performed five independent runs of 100 million generations each, sampled at every 2,000 steps, totaling a posterior distribution of 50,000 trees/run. We accessed stationary posterior distributions, effective sample sizes (ESS above 200), and convergence between runs with Tracer v. 1.6 (Bouckaert *et al.* 2014). We combined trees after removing a burn-in of 25% with Log Combiner v.1.7.5 (Bouckaert *et al.* 2014) and subsequently annotated the combined tree file with TreeAnnotator v.1.7.5 (Bouckaert *et al.* 2014) to calculate the maximum clade credibility (MCC) species tree. We accessed the tree and divergence times with FigTree 1.4.2 (Bouckaert *et al.* 2014).

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We investigated the genetic structure within *Colobosaura modesta* with a Bayesian mixture and admixture analysis implemented in BAPS 6.0 (Corander *et al.* 2013), aiming to identify divergent genetic groups that could correspond to potential populations/cryptic species and guide the following phylogeographic reconstructions. We conducted the mixture analyses using a phased concatenated alignment of the six genes, and used individuals and localities as priors. We ran BAPS with the maximal number of groups (K) set as a vector ranging from one to 10, running five times for each group (total of 50 runs). After the mixture analysis of individuals, we conducted the admixture analysis with the same data, implementing 1,000 simulations from posterior allele frequencies.

We used the software BPP 3.2 (Yang & Rannala 2010, 2014) to test species delimitation hypotheses within *C. modesta*, based on the genetic groups obtained in BAPS 6.0. Different from previous versions, BPP 3.2 estimates a species tree while running the reversible-jump MCMC species delimitation algorithm, eliminating the concern on over-estimating species limits (Caviedis-Solis *et al.* 2015; Leaché & Fujita 2010). Briefly, the software estimates a species tree using a Subtree Pruning and Regrafting algorithm (Rannala & Yang 2015), while species hypotheses are tested by collapsing branches of the different possible phylogenetic hypotheses (species trees) and comparing their posterior probabilities.

We separated *Colobosaura modesta* individuals into candidate species based on the groups generated in BAPS 6.0. First, we ran initial trials using different parameters and checking for convergence among repeated runs, which denote prior quality and run consistency in BPP. Later, we used a gamma prior of ~G (1,1000) for population size (theta, θ), ~G (1,100) for the age of the root in the species tree (tau, τ_0), and the Dirichlet prior (Yang & Rannala 2010: Equation 2) for other divergence time parameters. The gamma prior G (α, β) has mean α/β, so the theta prior ~G (1,1000) corresponds to one difference per kilobase (0.001), while the tau prior ~G (1,100) corresponds to 1% sequence divergence (0.01).

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Therefore, these priors indicated large population size and a relatively shallow divergence time. They returned similar results after employing different runs (indicating consistency), while other priors delivered different results in each run. Moreover, these priors are in accordance with other results in this study, which indicate large population sizes and shallow divergence for the species (see Results). We ran analyses for 500,000 MCMC generations, taking samples every five generations, and using 10,000 burn-in generations. We used both available reversible-jump MCMC species delimitation algorithms (algorithms 0 and 1', Yang & Rannala 2010), excluding or not alignment gaps (cleandata = 1, the program remove all columns that have gaps or ambiguity characters, and cleandata = 0 means that those will be used in the likelihood calculation). To check for consistency of results, we conducted at least two independent runs starting at random tree models for each analyses type.

Geographic structure and population history

We evaluated patterns of isolation by distance within *Colobosaura modesta* conducting a Mantel test with pairwise genetic and geographic distances among cytb haplotypes. We conducted this analysis with *dist.gene* (package *ape*) and *mantel.rtest* (*ad4*) functions in R 2.3.1 (R Development Core Team 2015), employing 9,999 Monte Carlo permutations. To evaluate hypothesis of riparian forests acting as a interconnected net vs. hypothesis of central Brazilian plateaus acting as barriers to gene flow among riparian forest-dwelling species, we used circuit theory and calculated the environmental cost of all possible routes connecting pairs of localities, and identified the corridor with the lowest resistance using CIRCUITSCAPE 4.0 (McRae & Beier 2007). We used an altitude raster (2.5 arc-minutes resolution) as resistance map and calculated a resistance matrix among localities, and then we conducted a partial Mantel test using genetic distance, resistance matrix, and isolation by distance as a covariate. We conducted this analysis using the *mantel.partial* (*vegan*) function in R.2.3.1, employing 9,999 Monte Carlo permutations.

We also used Monmonier's (1973) maximum difference algorithm implemented in Barrier 2.2 (Manni *et al.* 2004) to identify genetic barriers among *Colobosaura modesta* haplotypes. If riparian forests act as a interconnected network for gene and species flow, genetic breaks will not be associated with the central distribution the species, which correspond to the river basin limits, but they will show a different pattern. However, if the Central Brazilian plateau act as a barrier for gene and species flow, maximum genetic breaks will probably be associated with the river basins limits, as each river basin is positioned and flows in different sides of the plateau. We conducted this analysis subsampling one haplotype per locality and setting the algorithm to identify three barriers, considering the three most important river basins inside the Cerrado domain: Tocantins/Araguaia, São Francisco, and Paraná/Paraguay.

To identify genetic signatures of past demographic processes within *Colobosaura modesta*, we ran an analysis of mismatch distributions of pairwise differences between mtDNA haplotypes, as it can provide some information about the historical demography of populations. Populations with historically stable demography have multimodal mismatch distributions, whereas a unimodal distribution suggests recent demographic expansion (Prado *et al.* 2012). We conducted a mismatch distribution test using DNASP 5.0, and compared the observed data with the expected data, in a case of population expansion, with the parameters *tau*, *theta1* e *theta0* estimated by the software. The fit and significance of the mismatch distributions to the expansion model was estimated with a chi-square test.

To test the influence of recent events shaping the genetic structure within *Colobosaura modesta*, as suggested in (Werneck 2011), we conducted a Bayesian Skyline Plot (BSP, Drummond *et al.* 2005) implemented in BEAST 2.3.1 (Bouckaert *et al.* 2014). Considering the inherent sample-effect already reported for BSP analyses (Heller *et al.* 2013), we randomly sampled one haplotype per locality prior analysis and estimated the optimal nucleotide substitution model for each gene using PARTITION FINDER. Five independent runs were obtained using the following parameters: cytb (GTR+I+G), PRLR and SINCAIP (HKY+I+G), BFIB and MXRA5 (HKY+I+G), and ACA (GTR+I+G) evolution models, gamma category count = 4; relaxed log normal Clock model with a clock rate of 0.0065 for cytb, estimating the nuclear clock rates based on cytb; priors: coalescent Bayesian skyline, starting with a UPGMA Tree, MarkovChainedPopSizes.t = "Jeffreys" and Shape equal to 1 (default). GammaShape.s = log normal (inicial =1); Kappa.s = log normal (inicial = 2); proportion invariant.s = normal (inicial 0.1); *ucldstdev.c* = gamma (inicial 0.1); chain length of 100 million generations, sampled every 10,000 steps. We checked parameters convergence among runs and performance (ESS values above 200) with Tracer 1.6 (Rambaut *et al.* 2014).

To test the hypothesis that *Colobosaura modesta* populations were subject to a recent expansion due to Pleistocene climatic fluctuations, we generated six competing population history scenarios and submitted them to Approximate Bayesian Computation (ABC), implemented in DIY-ABC 2.0 (Cornuet *et al.* 2014). The six population history scenarios were: constant population size (two models), population expansion following the end of the Pliocene and rise of Pleistocene, population expansion during the Last Interglacial (LIG), bottleneck following the Pliocene, and bottleneck after LIG. These scenarios were designed to evaluate if the Pleistocene influenced the demography of *C. modesta*, indicating population expansion signature, as expected for riparian forest-dwelling species (Werneck 2011), or if the Period influenced negatively the demography of the species, or if this period did not influenced the species. The time of effective population size changes in the models involved the Pliocene (5 Ma to 2.6 Ma), the entire Pleistocene (2.6 Ma to 10 Ka), and also the Last interglacial (LIG) (50 to 150 Ka) (Fig. 2). We considered the LIG in these models, and not only the entire Pleistocene period, because the former contains explicit information of warm and wet conditions, and signs of lowland rainforest expansion towards the South America dry diagonal (Cheng *et al.* 2013; Gosling *et al.* 2008; Ledru *et al.* 2009). Moreover, this approach can also improve our understanding of the evolutionary process that took place in *C. modesta,* providing information of population size changes in the later Pleistocene, since the Pleistocene itself is more than two-million years long, and associated with variable climatic conditions during its whole extension (Hanselman *et al.* 2011; Hermanowski *et al.* 2012; Pessenda *et al.* 2010). We included all sequence data into the analysis, used a HKY evolution model for each marker, selected all within-population summary statistics in the program, and a prior of equal probability to each scenario (16.67%). We considered a generation time of one year for *C. modesta*, which is consistent with studies from closely related gymnophthalmids (Garda *et al.* 2014; Sousa *et al.* 2015). Six million datasets were simulated for each model. Logistic regression was used to estimate the posterior probability of each model based on 1% of simulated datasets for each scenario, producing scenario summary statistics closest to the observed summary statistics.

In order to access suitable climatic conditions that might correspond to distribution expansion in *Colobosaura modesta* and also signs of past connection routes between the Amazon and the Altantic Forest used by the species, we modelled the current distribution of *Colobosaura modesta* and projected the results to the Holocene, Last Glacial Maximum (LGM), and Last Interglacial (LIG) with MaxEnt (Phillips & Dudík 2008), considering the favourable performance of this algorithm in comparisons (Elith 2010; Elith *et al.* 2006; Pearson *et al.* 2007). We downloaded high-resolution bioclimatic layers (30 arc-seconds, ~ 1 km) for the Current, Mid-Holocene (6 ka; MIROC-ESM), Last Glacial Maximum (LGM – 21 ka, MIROC-ESM), and Last Interglacial (LIG, 120-140 ka) (Otto-Bliesner *et al.*, 2006) from the WorldClim project (http://www.worldclim.org/), and delimited them based on a South

America shapefile. To assess model performance, we adopted the area under the curve (AUC) value from the receiver operating characteristic (ROC) curve (Fielding & Bell 1997) (Fielding & Bell, 1997) and used 25% of the species distribution as test data. Moreover, we used palynological information from the literature to access the South America climatic conditions (temperature and humidity) in each bioclimatic scenario, discuss climatic requirements for *C. modesta*, and also discuss in which climatic conditions the species expanded its distribution.

Results

Divergences within Colobosaura modesta

Our BI and ML concatenated gene tree inference methods recovered similar results. *Colobosaura modesta* was monophyletic in both reconstructions. For brevity, we present only the Bayesian phylogeny obtained from the concatenated analysis (Fig. 1B). Overall, our phylogenetic tree inference indicated shallow divergences and unresolved topologies within *C. modesta*. Nevertheless, the divergence of the lineage from Novo Progresso (CHUNB34981) and from the remaining populations of *C. modesta* from the Cerrado, Caatinga, and Atlantic Forest is well supported (Fig. 1B). Clades from Chapada do Araripe , Santo Antônio do Leverger, and in central Brasil are also well supported; however, their relationships are unclear (Fig. 1B). The haplotype network for cytb indicated high divergence of the lineage of Novo Progresso and the remaining lineages (Fig. 3).

Our cluster analysis from BAPS recovered five groups within *Colobosaura modesta* (Fig. 1A and 1B). Group I was composed by the population from Novo Progresso (PA); group II, by individuals from Santo Antônio do Leverger; group III, by individuals from northeastern Brazil, except from Chapada do Araripe (CE); group IV, by individuals from Mateiros (TO); and group V, by individuals from central Cerrado plus Chapada do Araripe (CE) (Fig. 1A and 1B). Besides having some geographic correspondence, most groups were not assigned as different species in BPP, except the population from Novo Progresso - PA. Using both algorithms (algorithms 0 and 1, Yang & Rannala 2010), and excluding gaps or not, BPP consistently returned the same result, corroborating the hypothesis of the population from Novo Progresso – PA belonging to a different species. Different BPP runs recovered four species with a posterior probability of 1: the outgroups (*Vanzosaura rubricauda* and *Acratosaura mentalis*), the population from Novo Progresso - PA, and the last comprising all remaining *C. modesta* populations. The best species tree estimated by BPP (higher posterior probability in all runs) had the same topology estimated by all concatenated phylogenetic analyses.

The Bayesian Inference implemented in BEAST 3.2.5 recovered more resolved relationships within *Colobosaura modesta* than in MRBAYES. The lineage from Novo Progresso (PA) is the only one that diverged from the remaining during the Pliocene; all other divergences occurred in the Pleistocene (Fig. 4). This supports the BPP results and also suggests a recent history of colonization and distribution expansion of *Colobosaura modesta* within the dry diagonal.

Geographic structure and population history of Colobosaura modesta

The Mantel test indicated association of genetic structure and geographic distance among sites in *Colobosaura modesta* ($r = 0.41$; $p < 0.001$). However, the partial Mantel test indicated no significant association of genetic distance and pairwise altitude resistance, controlling the effects of geographic distance ($r = -0.13$; $p = 0.83$). This suggests that the central Brazilian plateaus do not promote genetic breaks within *C. modesta*. The Monmonier's maximum difference algorithm implemented in Barrier 2.2 indicated three main barriers for gene flow, all of them at the extremes of *C. modesta* distribution (Fig. 5). This also indicates that the central Brazilian plateaus do not play an important role in shaping genetic structure

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within *C. modesta* and suggests that riparian forests act as a interconnected network allowing gene and species flow across the Cerrado domain, as populations in the core distribution are more similar to each other than the peripheral populations.

The mismatch distribution analysis in *Colobosaura modesta* indicated no difference from a unimodal distribution model (χ^2 = 2280; P = 1.00), suggesting recent population expansion. The Bayesian Skyline Plot of *Colobosaura modesta* (excluding the population from Novo Progresso - PA) indicated significant increase in population size since the mid-Pleistocene (Fig. 6). The Approximate Bayesian Computation implemented in DIY-ABC indicated the scenarios of population expansion during the Pleistocene (scenarios 1 and 3) as the best in explaining *C. modesta* demography (75 % explanation together), and also suggested the scenario of recent population expansion during the LIG as the most likely among the six competing scenarios (52% explanation) (Fig. 7). Scenario 2 had 13% of explanation, and the remaining scenarios had probabilities below 7%. All these results suggest that *Colobosaura modesta* had a recent history of population expansion within the dry diagonal, associated with the Pleistocene.

The environmental niche modeling (ENM) of *Colobosaura modesta* indicated AUC values of 0.94 and 0.92 for training and testing data, respectively. Projections of the ENM indicated suitable climatic conditions for population expansion in a western (LIG) to eastern/southeastern (LGM and Mid-Holocene) direction (Fig. 8). Suitable climatic conditions for population expansion in LGM and Mid-Holocene projections occurred both in northeastern Brazil, in regions of the known forest enclaves in the Caatinga, and also in southeastern Brazil (Fig. 8). Palynological data suggest progressively warm and moist conditions during the Late Glacial and the Holocene in northeastern Brazil (Barrerinhas - BA, Middle São Francisco River, PARNA das Sete Cidades - PI, REBIO Guaribas - PB, FLONA do Araripe-CE), and also warm and wet conditions in southeastern Brazil (Jacareí - SP,

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Colônia - SP, Curucutu - SP, and Morro de Itapeva - SP), supporting results of the ENM for the Holocene. These results suggest the expansion of suitable conditions for *Colobosaura modesta* in warm and wet conditions.

Discussion

Pre-Pleistocene geologic (Colli 2005; Hoorn *et al.* 2010) and Pleistocene climatic changes (Haffer 1969; Vanzolini & Williams 1981) are among the most important hypotheses of diversification in the Neotropics and, in some cases, both are evoked together explaining diversification patterns (Rull 2008; Rull 2011). Werneck (2011) suggested that riparian forests are more recent than plateaus in the Central Brazil context, and would present species with lower genetic structure, lower phylogenetic resolution, and with signs of population expansion, due to recent erosion and Pleistocene climatic fluctuations effects. Our study supported this hypothesis and, as far as we know, this is the first study evaluating this hypothesis with a forest-dwelling species, given that previous herpetological studies testing this hypothesis were applied to organisms from plateaus (Domingos *et al.* 2014; Guarnizo *et al.* 2016; Maciel *et al.* 2010).

Molecular studies suggested that Pleistocene climatic fluctuations are important in shaping frogs (Carnaval & Bates 2007; Thomé *et al.* 2016), reptiles (Oliveira *et al.* 2015; Zamudio & Greene 1997), and birds (Batalha-Filho *et al.* 2013) demography and distribution patterns in northeastern Brazil. Studies also suggest that most of the biotic interchange between the Amazon and the Atlantic Forest across the northeastern Brazil took place in the Pleistocene (Batalha-Filho *et al.* 2013), and not in pre-Pleistocenic times, being also supported by paleoecological studies in the same regions (De Oliveira *et al.* 1999; Pessenda *et al.* 2010; Pessenda *et al.* 2004). Our study corroborates this hypothesis, even with a with a riparian forest-dwelling organism. Moreover, our results also suggest that the SE-NW route passing through the central Brazil (Oliveira-Filho & Ratter 1995) also occurred in the Pleistocene, and probably not in more ancient times. Therefore, routes passing through northeastern Brazil and also along the Central Brazil are more recent than the SE-NW route proposed by Bigarella *et al.* (1975) and Por (1992). However, some studies with plants (Caesalpinoidae) suggest old stories associated with the evolution of forest-dwelling species (Sousa-Neto *et al.*, 2016), contrasting our results, and suggesting a complex and lineage-dependent history associated with the forested habitats within the dry diagonal.

Some studies suggested the Central Brazilian plateaus as important barriers for gene flow for riparian forest-dwelling species (Silva 1996; Valdujo *et al.* 2013). Our partial Mantel test indicated that altitude is not an important variable explaining genetic diversity divergence within *Colobosaura modesta*. Moreover, regions with highest genetic divergence are associated with the edge of the species limits, and not with the river basin limits. These results are similar to other studies applied to lizards (Santos *et al.* 2014) suggesting that isolation by distance mechanisms are more important than geologic barriers in shaping genetic structure within the Cerrado. One premise of the core/periphery hypothesis is lower differentiation among populations in the core distribution of a species, due to higher population density, and higher differentiation in marginal populations, leading to low N_e in peripheral areas and low gene flow caused by isolation (Hardie & Hutchings 2010). The observed genetic structure of *C. modesta* also supports this hypothesis and also supports the hypothesis of riparian forests acting as a interconnected network promoting species and gene flow across the entire Cerrado (Mares & Ernest 1995; Oliveira-Filho & Ratter 1995). Probably, the genetic structure within *C. modesta* may be the result of recurrent forest expansions and retractions during the Pliocene and Pleistocene, isolating peripheral populations in relation to core populations, promoting differences between them. This scenario can explain the maximum genetic differences associated with peripheral in relation to core populations observed in *C. modesta*.

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Paleoecological studies suggest significant vegetation shifts in the central Brazil during the Late Pleistocene, showing wetter conditions than present (Barberi *et al.* 2000; Parizzi *et al.* 1998; Salgado-Laboriau 2005; Salgado-Labouriau 1997), and similar results were also suggested in northeastern Brazil (Auler & Smart 2001; De Oliveira *et al.* 1999; Pessenda *et al.* 2010). Our study supports the hypothesis that Pleistocene climatic changes also played an important role in the Neotropical diversity, including the Cerrado domain. Nevertheless, deep divergences of typical plateau taxa (Domingos *et al.* 2014; Guarnizo *et al.* 2016; Werneck *et al.* 2012a) and environmental niche modeling studies (Werneck *et al.* 2012b) suggest that climatic changes were not so expressive and ubiquitous in the entire Cerrado. Considering all these studies together, we might conclude the existence of vegetation changes driven by climatic shifts in the Cerrado during the Pleistocene, however these changes did not modify widely the savanna landscape. As plateau areas contain different edaphic characteristics compared to valleys, it is possible that typical savanna elements had their distribution reduced but persisted in plateaus even in unfavorable conditions of the Pleistocene, suggesting stability areas. Some studies suggest that portions in the western Cerrado maintained a stable vegetation during glacial times (Burbridge *et al.* 2004), and that these regions also contained genetic stability and deep divergences for amphibians (Prado *et al.* 2012) and reptiles (Guarnizo *et al.* 2016; Santos *et al.* 2014). Moreover, wide climatically stable regions were also suggested for central-eastern Cerrado (Werneck *et al.* 2012b). Currently, riparian forests account for 10-20% of the Cerrado vegetation cover (Ribeiro & Walter 2008). Probably this vegetation was larger in favorable moments in the past and was connected along the headwaters of different river basins, but it did not dramatically replace savanna vegetation communities, as some can imagine. Palynological evidence suggest that even with arboreal vegetation changes, grassland vegetation, very common in savanna landscapes, persisted with high densities in the pollen record (Salgado-Labouriau *et al.* 1998).

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Rodrigues (2005) proposed that riparian forests could increase forest biodiversity by asymmetrically capturing savanna species during favorable moments of forest expansion and promoting ecological adaptation to forest habitats through a vanishing refuge model (Vanzolini & Williams 1981). Moreover, the author also proposed that this hypothesis would occur with *Colobosaura modesta*, considering the occurrence of this species in eastern Amazon as a result of a directional evolution and adaptation from a previous savanna-adapted lineage (Rodrigues 2005). We did not formally test this hypothesis in this study. However, considering that all sister species of *C. modesta* are distributed in forested habitats in the Amazon and Atlantic Forest, such as *Rondonops*, *Iphisa*, and *Alexandresaurus* (Colli *et al.* 2015; Rodrigues *et al.* 2008), and also considering that the most ancient divergence in *C. modesta* separated populations in Novo Progresso, currently in the Amazon, from the remaining populations, we suggest that the evolution in this group occurred from a forest to a riparian forest habitat, and not the opposite, as Rodrigues (2005) suggests. Moreover, this hypothesis was also refuted both with ecological (Nogueira *et al.* 2009) and molecular (Werneck *et al.* 2009) data. Probably this hypothesis is equivocal in its assumptions, considering that open landscapes species can use riparian habitats. Besides occurring for mammals (Redford & Fonseca 1986), this is not supported for reptiles (Nogueira *et al.* 2009). The Pleistocene refuge hypothesis (Haffer 1969) and the vanishing refuge hypothesis (Vanzolini & Williams 1981) are considered the most important mechanisms of diversification during the Pleistocene. Probably, the evolution of *Colobosaura modesta* occurred under these mechanisms, considering that the sister generas *Iphisa* and *Rondonops* also occurs in eastern Amazon, but apparently not in sympatry with *Colobosaura modesta*. Also considering that the eastern Amazon suffered more expressive climatic changes than the western Amazon (Cheng *et al.* 2013), *Colobosaura modesta* could have originated through evolution in an ecological gradient model and, in moments of forest reduction in eastern

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Amazon due to drier and/or cold conditions, ancestral lineages could have been progressively selected from wet habitats to progressive mesic habitats in riparian forests since the Pliocene and Pleistocene.

Colobosaura modesta (Reinhardt & Lütken 1862) is currently considered the only valid species within the genus *Colobosaura*. It was formerly described as *Perodactylus modestus* based on one specimen collected at an old farmland nearby the mountain Morro da Garça, some miles north of the city of Curvelo - MG, Brazil (Reinhardt & Lütken 1862). Considering the fact that *Perdodactylus* was preoccupied in *Peropus* (*Perodactylus*) *oceanicus* (Fitzinger 1843), the generic name was changed to *Colobosaura* by Boulenger (1887). A new species, *Perodactylus kraepelini* (Werner 1910), was described based on only one young female (SVL = 40 mm) from Puerto Max, Paraguay, but later synonymized to *Colobosaura modesta* (Amaral, 1932), and the diagnosis (interparietals as wide as parietals, instead of interparietal narrower than parietals from *C. modesta*) was considered a variation inside *C. modesta* (Amaral 1932; Cunha 1977). Unfortunately, the type specimen was lost from the Hamburg Zoological Museum (Rodrigues *et al.* 2008). Similarly, *Colobosaura landii* (Cunha 1977) was described based on some specimens collected in primary forests from the eastern Amazon (Vila do Curupati, close to Viseu, northeastern Pará) in a different habitat from previously known records of *Colobosaura*, only for the "Cerrado" at that time. These specimens and other individuals from eastern Pará were later recognized as synonyms of *Colobosaura modesta* (Cunha & Nascimento 1982, 1983; Nascimento *et al.* 1987).

The information of the above mentioned studies associated with the results in this study support the idea of *Colobosaura modesta* as a recent lineage composed by only one species and that the previous described species may be all of them part of only one species. Nevertheless, the high genetic divergence of *C. modesta* from Novo Progresso compared to the other populations inside the Cerrado and the results from BPP support the idea of at least

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two species in this genera, one occurring in Serra do Cachimbo (Novo Progresso - PA), and the other occupying the remaining distribution of *C. modesta* (eastern Pará, Cerrado riparian forests, montane and submontane forest enclaves in the Caatinga, and restinga habitats in northeastern Bahia). There are already endemic species in Serra do Cachimbo, such as *Tropidurus insulanus* (Rodrigues 1987) and *Dendropsophus cachimbo* (Napoli & Caramaschi 1999), also indicating biogeographic importance to this region. Interestingly, this lineage of *C. modesta* occurs in the well known "arc of deforestation" (Aldrich *et al.* 2012; Ferreira *et al.* 2014), a region with high diversity and intense habitat loss. Recently a new genus of gymnophthalmid was described for the region (Colli *et al.* 2015), indicating knowledge scarcity for this region. Considering the rate of deforestation associated with the "arc of deforestation", probably many species will go extinct before being discovered. Therefore, conservation effort is needed for this region.

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Author Contributions

R.M.D.L., G.R.C., and L.G.G. conceived the initial idea of the study. R. M. D. L. generated the sequence data and performed the analyses. F. M. C. B. D. and R. M. D. L. performed the BPP 3.2 analysis. R. M. D. L. led the manuscript writing. All authors commented and improved this first version of the manuscript.

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Table 1. Material used in this study with origin, ID, and locality. Species ID acronyms: AAGARDA = Adrian Antonio Garda field series deposited at the coleção coleção do laboratório de Anfíbios e Répteis da Universidade Federal do Rio Grande do Norte; DS = Diego Santana field series deposited at the coleção do laboratório de Anfíbios e Répteis da Universidade Federal do Rio Grande do Norte; GRCOLLI = Guarino Rinaldi Colli field series deposited at the Coleção Herpetológica da Universidade de Brasília; MARA = Mara Sousa Albuquerque-e-Silva field series deposited at the Coleção Herpetológica da Universidade de Brasília. CHUNB = Coleção Herpetológica da Universidade de Brasília. Brazilian states: PA = Pará; MT = Mato Grosso; TO = Tocantins; MA = Maranhão; BA = Bahia; GO = Goiás; CE = Ceará; MG = Minas Gerais; PE = Pernambuco; RN = Rio Grande do Norte. A numeric code is associated with each site for geographic location purposes (see Fig. 1).

ID	Species	Site (numeric code)	Subsampled for nuclear markers?
AAGARDA4227	*Acratosaura mentalis*	Paulo Afonso – BA	yes
AAGARDA5689	*Acratosaura mentalis*	João Câmara – RN	yes
AAGARDA5690	*Acratosaura mentalis*	João Câmara – RN	yes
AAGARDA6874	*Acratosaura mentalis*	Palmeiras – BA	yes
AAGARDA8631	*Acratosaura mentalis*	Buíque – PE	yes
CHUNB11387	*Colobosaura modesta*	Palmas – TO (1)	yes
CHUNB11388	*Colobosaura modesta*	Palmas – TO (1)	yes

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CHUNB11393	*Colobosaura modesta*	Palmas – TO (1)	no
CHUNB11394	*Colobosaura modesta*	Palmas – TO (1)	yes
CHUNB12344	*Colobosaura modesta*	Palmas – TO (1)	yes
CHUNB12349	*Colobosaura modesta*	Palmas – TO (1)	yes
CHUNB12352	*Colobosaura modesta*	Palmas – TO (1)	yes
CHUNB12566	*Colobosaura modesta*	Palmas – TO (1)	yes
CHUNB14552	*Colobosaura modesta*	Palmas – TO (1)	no
CHUNB26057	*Colobosaura modesta*	Paracatu – MG (2)	no
CHUNB26058	*Colobosaura modesta*	Paracatu – MG (2)	yes
CHUNB26059	*Colobosaura modesta*	Paracatu – MG (2)	no
CHUNB26060	*Colobosaura modesta*	Paracatu – MG (2)	no
CHUNB26061	*Colobosaura modesta*	Paracatu – MG (2)	yes
CHUNB26065	*Colobosaura modesta*	Paracatu – MG (2)	yes
CHUNB26066	*Colobosaura modesta*	Paracatu – MG (2)	no
CHUNB26068	*Colobosaura modesta*	Paracatu – MG (2)	no
CHUNB26069	*Colobosaura modesta*	Paracatu – MG (2)	no

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GRCOLLI06616	*Colobosaura modesta*	Paracatu – MG (2)	no
CHUNB27020	*Colobosaura modesta*	Mateiros – TO (3)	no
CHUNB27023	*Colobosaura modesta*	Mateiros – TO (3)	no
CHUNB27024	*Colobosaura modesta*	Mateiros – TO (3)	no
CHUNB27027	*Colobosaura modesta*	Mateiros – TO (3)	yes
CHUNB27028	*Colobosaura modesta*	Mateiros – TO (3)	yes
CHUNB27029	*Colobosaura modesta*	Mateiros – TO (3)	no
CHUNB27030	*Colobosaura modesta*	Mateiros – TO (3)	no
CHUNB27032	*Colobosaura modesta*	Mateiros – TO (3)	yes
CHNB27033	*Colobosaura modesta*	Mateiros – TO (3)	no
CHUNB27034	*Colobosaura modesta*	Mateiros – TO (3)	yes
CHUNB27035	*Colobosaura modesta*	Mateiros – TO (3)	no
CHUNB27037	*Colobosaura modesta*	Mateiros – TO (3)	no
LJVITT09153	*Colobosaura modesta*	Mateiros – TO (3)	no
CHUNB33061	*Colobosaura modesta*	São Domingos – GO (4)	no
CHUNB33062	*Colobosaura modesta*	São Domingos – GO (4)	yes

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CHUNB33063	*Colobosaura modesta*	São Domingos – GO (4)	yes
CHUNB33064	*Colobosaura modesta*	São Domingos – GO (4)	no
CHUNB34981	*Colobosaura modesta*	Novo Progresso – PA (5)	yes
CHUNB35332	*Colobosaura modesta*	São Domingos – GO (4)	yes
CHUNB37535	*Colobosaura modesta*	Alvorada do Norte – GO (6)	yes
CHUNB38407	*Colobosaura modesta*	Flores de Goiás – GO (7)	yes
CHUNB44689	*Colobosaura modesta*	Colinas do Sul – GO (8)	yes
CHUNB45248	*Colobosaura modesta*	Caseara – TO (9)	yes
CHUNB45258	*Colobosaura modesta*	Caseara – TO (9)	yes
CHUNB45262	*Colobosaura modesta*	Caseara – TO (9)	yes
CHUNB51319	*Colobosaura modesta*	Cocos – BA (10)	yes
CHUNB51321	*Colobosaura modesta*	Cocos – BA (10)	yes
CHUNB52009	*Colobosaura modesta*	Carolina – MA (11)	yes
CHUNB52010	*Colobosaura modesta*	Carolina – MA (11)	no
CHUNB52011	*Colobosaura modesta*	Carolina – MA (11)	yes
CHUNB52012	*Colobosaura modesta*	Carolina – MA (11)	yes

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CHUNB52015	*Colobosaura modesta*	Carolina – MA (11)	no
CHUNB52016	*Colobosaura modesta*	Carolina – MA (11)	yes
CHUNB52017	*Colobosaura modesta*	Carolina – MA (11)	yes
CHUNB52018	*Colobosaura modesta*	Carolina – MA (11)	yes
CHUNB52019	*Colobosaura modesta*	Carolina – MA (11)	yes
CHUNB52619	*Colobosaura modesta*	Carolina – MA (11)	yes
CHUNB52631	*Colobosaura modesta*	Peixe – TO (12)	yes
CHUNB52632	*Colobosaura modesta*	Peixe – TO (12)	no
CHUNB58600	*Vanzosaura rubricaura*	Serranópolis – GO	yes
CHUNB61035	*Colobosaura modesta*	Piripiri – PI (13)	yes
CHUNB63208	*Colobosaura modesta*	Nova Xavantina – MT (14)	yes
CHUNB63209	*Colobosaura modesta*	Nova Xavantina – MT (14)	yes
CHUNB63213	*Colobosaura modesta*	Nova Xavantina – MT (14)	no
CHUNB63218	*Colobosaura modesta*	Nova Xavantina – MT (14)	yes
CHUNB63224	*Colobosaura modesta*	Nova Xavantina – MT (14)	Yes
CHUNB69378	*Colobosaura modesta*	Caiapônia – GO (15)	No

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CHUNB69379	*Colobosaura modesta*	Caiapônia – GO (15)	Yes
CHUNB69380	*Colobosaura modesta*	Caiapônia – GO (15)	Yes
CHUNB71775	*Colobosaura modesta*	Mata de São João – BA (16)	Yes
CHUNB72566	*Colobosaura modesta*	Jardim – CE (17)	Yes
CHUNB72567	*Colobosaura modesta*	Jardim – CE (17)	yes
CHUNB96415	*Colobosaura modesta*	Crato – CE (18)	Yes
DS078	*Colobosaura modesta*	Bonito de Minas – MG (19)	yes
DS087	*Colobosaura modesta*	Bonito de Minas – MG (19)	yes
GRCOLLI22165	*Colobosaura modesta*	Crato – CE (18)	yes
MARA271	*Colobosaura modesta*	Santo Antonio do Leverger –MS (20)	yes
MARA272	*Colobosaura modesta*	Santo Antonio do Leverger –MS (20)	yes

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Table 2. Mitochondrial and nuclear markers and primers used in this study, with details of PCR protocols (changing from I to V, depending on the marker and primer used). npcl = nuclear protein coding locus.

Gene	Primer	Primer sequence 5'-3'	Source	nDNA/mtDNA	PCR Protocols*
cytB	CB1-5	CCATCCAACATCTCAGCATGATGAAA	Palumbi, 1996	mtDNA	Protocol I; *Ta* = 46 to 48°C.
	CB3-3	GGCAAATAGGAARTATCATTC	Palumbi, 1996		
PRLR	PRLR_f1	GACARYGARGACCAGCAACTRATGCC	Townsend et al., 2008	nDNA (npcl)	Protcol II.
	PRLR_r3	GACYTTGTGRACTTCYACRTAATCCAT	Townsend et al., 2008		
bFIB	FIB-BI7U	GGAGAAAACAGGACAATGACAATTCAC	Prychitko & Morre, 1997	nDNA (npcl)	Protcol II.
	FIB-BI7L	TCCCCAGTAGTATCTGCCATTAGGGTT	Prychitko & Morre, 1997		
SINCAIP	F10	CGCCAGYTGYTGGGRAARGAWAT	Townsend et al., 2008	nDNA (npcl)	Protocol IV. Ta = 4 degrees below the average Tm.
	R13	GGWGAYTTGAGDGCACTCTTRGGRCT	Townsend et al., 2008		
MXRA5	MXRA5 - F2	KGCTGAGCCTKCCTGGGTGA	Portik et al., 2011	nDNA (npcl)	Protocol V. *Ta* = 66°C (MXRA5-F2 and MXRA5-R2).
	MXRA5 - R2	YCTMCGGCCYTCTGCAACATTK	Portik et al., 2011		Protocol V. *Ta* = 58°C (MXRA5-F2 and RN1).
ACA	F	GAGCGTGGCTAYTCCTTTGT	Waltari and Edwards, 2002	nDNA	Protcol III;
	R	GTGGCCATTTCATTCTCAAA	Waltari and Edwards, 2002		

* **PCR Protocols**: **I**. . 95°C -3 min; 40X(95°C-1 min; *Ta*-1 min; 72°C-1min); 72°C-5min; 4°C-infinite; **II**. 95°C -3 min; (95°C- 30 sec; 60°C-20 sec; 72°C- 1min 30 sec); (95°C- 30 sec; 58°C-20 sec; 72°C- 1min 30 sec); (95°C- 30 sec; 56°C-20 sec; 72°C- 1min 30 sec); 30X(95°C- 30 sec; 54°C-20 sec; 72°C- 1min 30 sec); 72°C-

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7min; 4°C-infinite; **III.** 95°C -1min30sec; 10X[95°C-35sec,63°C-35sec(-0.5°C/cycle),72°C-1min]; 10X(95°C-35sec, 58°C-35sec,72°C-1min);15X(95°C-35sec, 52°C-35sec,72°C-1min), 72°C-10min, 4°C-infinite; **IV**. 95°C -3 min; 40X(95°C-30 sec; Ta-30 sec; 72°C-1min); 72°C-5min; 4°C-infinite; **V**. 94°C-5min; 38x(94°C-40sec,*Ta*°C-20sec,72°C-1min);72°C-7min;4°C-infinite.

Figure legends

Fig. 1. Known localities for *C. modesta* (gray dots) and distribution of the samples used for the present study, with colors based on the groups from BAPS (on the left, letter A). Numbers represent sites with tissue samples: 1. Palmas (TO), 2. Paracatu (MG), 3. Mateiros (TO), 4. São Domingos (GO), 5. Novo Progresso (PA), 6. Alvorada do Norte (GO), 7. Flores de Goiás (GO), 8. Colinas do Sul (GO), 9. Caseara (TO), 10. Cocos (BA), 11. Carolina (MA), 12. Peixe (TO), 13. Piripiri (PI), 14. Nova Xavantina (MT), 15. Caiapônia (GO), 16. Mata de São João (BA), 17. Jardim (CE), 18. Crato (CE), 19. Bonito de Minas (MG), 20. Santo Antônio do Lerverger (MT). Concatenated gene genealogy of *Colobosaura modesta* reconstructed with Bayesian Inference (BI) in MrBayes 3.2.5 (on the right, letter B), and genetic structure within *C. modesta* (colored bar) accessed with BAPS 6.0 (right part of letter B). Each color (five in total) is associated with one different group generated in BAPS (group I – black; group II – yellow; group III - red; group IV – blue; group 5 – green). One of the outgroups (*Vanzosaura rubricauda*) was omitted in the figure for aesthetic purposes. On the upper left, a specimen of *Colobosaura modesta* (photo: Carlos Cândido).

Fig. 2. Different scenarios for approximate Bayesian computation (ABC) generated in DIY-ABC. Time variations (minimum - maximum) used in each scenario: t_{LIG} (50ka - 150 Ka), t_{PLEI} (10Ka - 2.6 Ma), and t_{PLIO} (2.6 Ma - 5.0 Ma); Variations in population size: 100,000 to 1,000,000. Scenario conditions: $t_{LIG} < t_{PLEI}$; $Nsmall < Nlarge$.

Fig. 3. Median-joining haplotype network of mitochondrial (CytB) and nuclear genes (PRLR, BFIB, MXRA5, ACA, and SINCAIP) for *Colobosaura modesta*. Black spots (median

vectors) represent not sampled haplotypes. Colors are associated with the different groups generated in BAPS 6.0 (Fig. 1). Circle size is sample size dependent.

Fig. 4. Bayesian coalescent reconstruction with divergence times of *Colobosaura modesta* generated in BEAST 3.2.5. Horizontal bars represent 95% posterior credibility intervals of the time divergence estimates. Numbers bellow the gray box indicate estimated ages (Ma). One of the outgroups (*Vanzosaura rubricauda*) was omitted in the figure with aesthetic purpose. Bellow in the figure, a specimen of *Colobosaura modesta* (photo: Carlos Cândido).

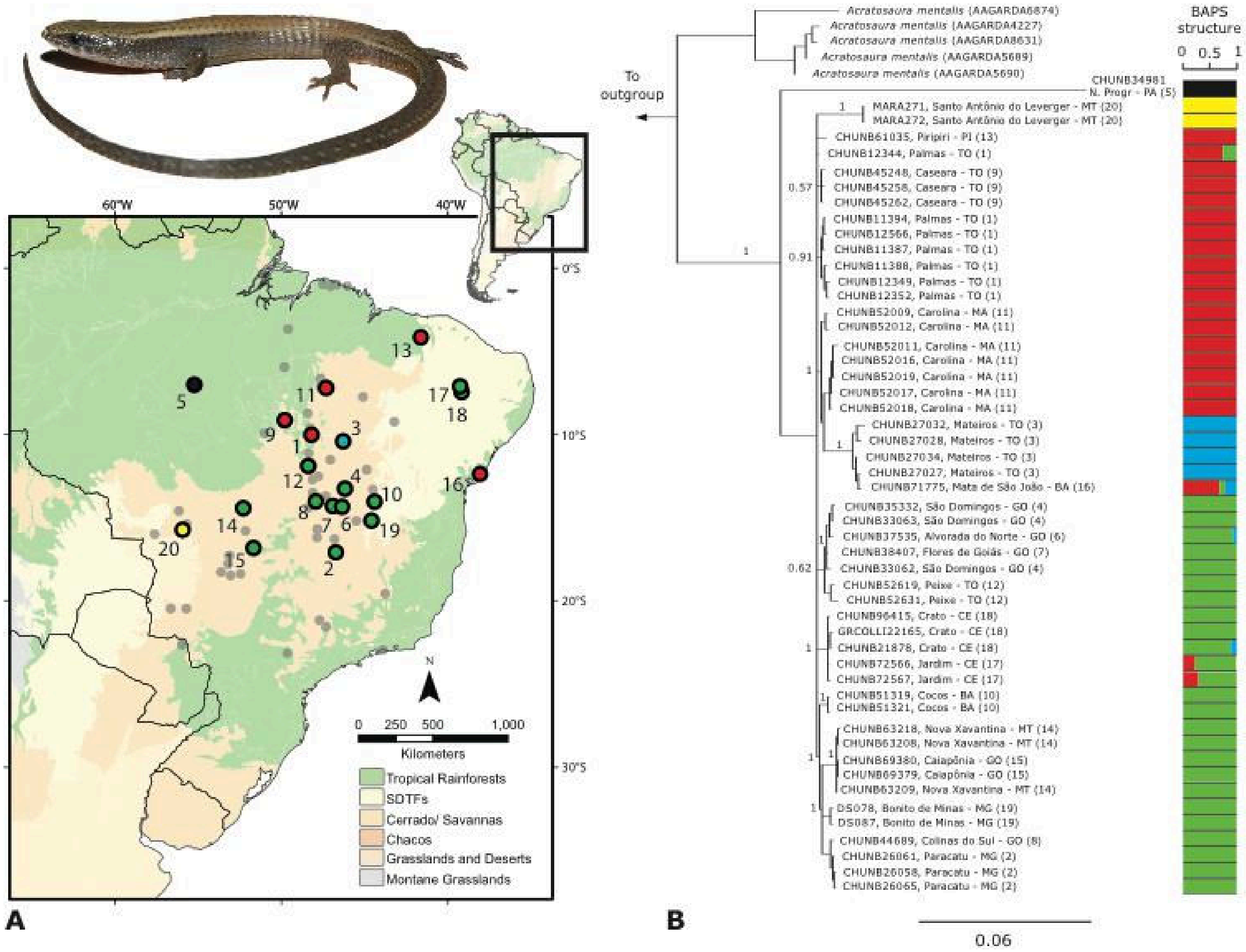
Fig. 5. Barriers generated by Monmonier's (1973) maximum difference algorithm applied to genetic data (cytb) for *Colobosaura modesta* in Barrier 2.2. Barrier codes A, B, and C represent maximum differences, from highest to lowest. Colored circles represent BAPS 6.0 assignments (group I – black; group II – yellow; group III - red; group IV – blue; group 5 – green). Numbers represent sites with tissue samples: 1. Palmas (TO), 2. Paracatu (MG), 3. Mateiros (TO), 4. São Domingos (GO), 5. Novo Progresso (PA), 6. Alvorada do Norte (GO), 7. Flores de Goiás (GO), 8. Colinas do Sul (GO), 9. Caseara (TO), 10. Cocos (BA), 11. Carolina (MA), 12. Peixe (TO), 13. Piripiri (PI), 14. Nova Xavantina (MT), 15. Caiapônia (GO), 16. Mata de São João (BA), 17. Jardim (CE), 18. Crato (CE), 19. Bonito de Minas (MG), 20. Santo Antônio do Lerverger (MT). Notice that all barriers were placed in the boundaries of the species distribution limit, refuting hypothesis of the Central Brazil plateau promoting genetic structure within *C. modesta*.

Fig. 6. Bayesian skyline plot illustrating effective population size (Ne) variation through time for *Colobosaura modesta*, excluding the population from Novo Progresso (PA). The black

line represent the media population size, and the blue area represent 95% higher and lower confidence interval of posterior probabilities.

Fig. 7. Principal component analysis of the posterior probabilities for each of the six scenarios from the Approximate Bayesian computation (ABC) implemented in DIY-ABC (to acess each of the six scenarios, see Fig. 2). Hollow spots represent priors. Spots with black circles are the simulated data. The yellow larger spot is the observed data. Notice similarity among the observed and simulated data in the third scenario (green). Scenarios in this figure correspond to models present in Fig 2.

Fig. 8. Environmental niche modeling (ENM) of *Colobosaura modesta* to current conditions (A) and projections to the Holocene (B), Last Glacial Maximum (LGM) (C), and Last Interglacial (LIG) (D), using MaxEnt. Black dots are occurrence points of *C. modesta* used in ENM. Signs of population expansion were notice mainly in Holocene projections, supporting both a northeastern route and a SE-NW route through Central Brazil.


60°W
50°W
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10°S
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250
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Kilometers
🟩 Tropical Rainforests
SDTFs
Cerrado/ Savannas
Chacos
Grasslands and Deserts
Montane Grasslands
A
To outgroup
Acratosaura mentalis (AAGARDA6874)
Acratosaura mentalis (AAGARDA4227)
Acratosaura mentalis (AAGARDA8631)
Acratosaura mentalis (AAGARDA5689)
Acratosaura mentalis (AAGARDA5690)
CHUNB34981 N. Progr - PA (5)
1
MARA271, Santo Antônio do Leverger - MT (20)
MARA272, Santo Antônio do Leverger - MT (20)
CHUNB61035, Piripiri - PI (13)
CHUNB12344, Palmas - TO (1)
0.57
CHUNB45248, Caseara - TO (9)
CHUNB45258, Caseara - TO (9)
CHUNB45262, Caseara - TO (9)
CHUNB11394, Palmas - TO (1)
CHUNB12566, Palmas - TO (1)
CHUNB11387, Palmas - TO (1)
1
0.91
CHUNB11388, Palmas - TO (1)
CHUNB12349, Palmas - TO (1)
CHUNB12352, Palmas - TO (1)
CHUNB52009, Carolina - MA (11)
CHUNB52012, Carolina - MA (11)
CHUNB52011, Carolina - MA (11)
CHUNB52016, Carolina - MA (11)
1
CHUNB52019, Carolina - MA (11)
CHUNB52017, Carolina - MA (11)
CHUNB52018, Carolina - MA (11)
CHUNB27032, Mateiros - TO (3)
1
CHUNB27028, Mateiros - TO (3)
CHUNB27034, Mateiros - TO (3)
CHUNB27027, Mateiros - TO (3)
CHUNB71775, Mata de São João - BA (16)
CHUNB35332, São Domingos - GO (4)
CHUNB33063, São Domingos - GO (4)
1
CHUNB37535, Alvorada do Norte - GO (6)
CHUNB38407, Flores de Goiás - GO (7)
0.62
CHUNB33062, São Domingos - GO (4)
CHUNB52619, Peixe - TO (12)
CHUNB52631, Peixe - TO (12)
CHUNB96415, Crato - CE (18)
GRCOLLI22165, Crato - CE (18)
1
CHUNB21878, Crato - CE (18)
CHUNB72566, Jardim - CE (17)
CHUNB72567, Jardim - CE (17)
1
CHUNB51319, Cocos - BA (10)
CHUNB51321, Cocos - BA (10)
CHUNB63218, Nova Xavantina - MT (14)
CHUNB63208, Nova Xavantina - MT (14)
1
1
CHUNB69380, Caiapônia - GO (15)
CHUNB69379, Caiapônia - GO (15)
CHUNB63209, Nova Xavantina - MT (14)
1
DS078, Bonito de Minas - MG (19)
DS087, Bonito de Minas - MG (19)
CHUNB44689, Colinas do Sul - GO (8)
CHUNB26061, Paracatu - MG (2)
CHUNB26058, Paracatu - MG (2)
CHUNB26065, Paracatu - MG (2)
BAPS structure
0
0.5
1
0.06
B

$

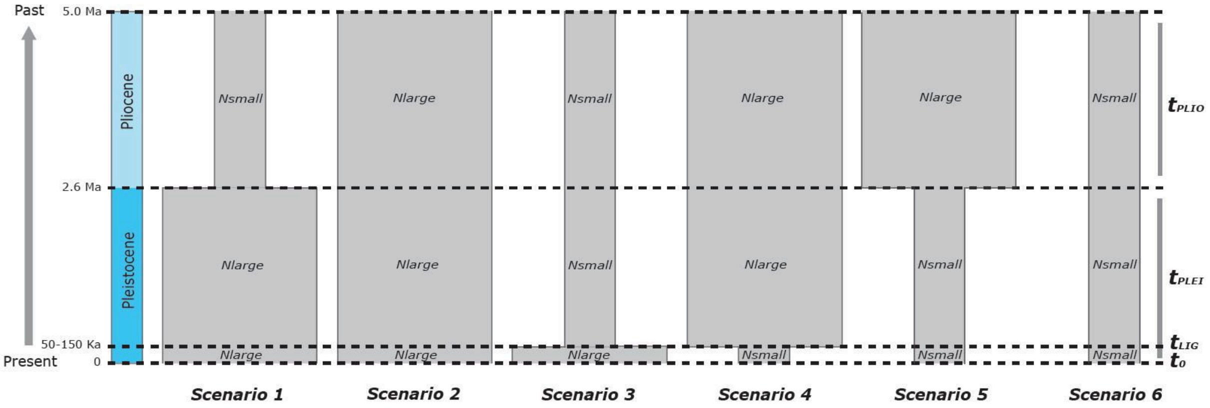
Past
5.0 Ma
2.6 Ma
50-150 Ka
Present
0
Pliocene
Pleistocene
Nsmall
Nlarge
Nlarge
Nlarge
Nlarge
Nlarge
Nsmall
Nsmall
Nlarge
Nlarge
Nlarge
Nsmall
Nlarge
Nsmall
Nsmall
Nsmall
Nsmall
Nsmall
Nsmall
tPLIO
tPLEI
tLIG
t0
Scenario 1
Scenario 2
Scenario 3
Scenario 4
Scenario 5
Scenario 6

Fig.2.

$

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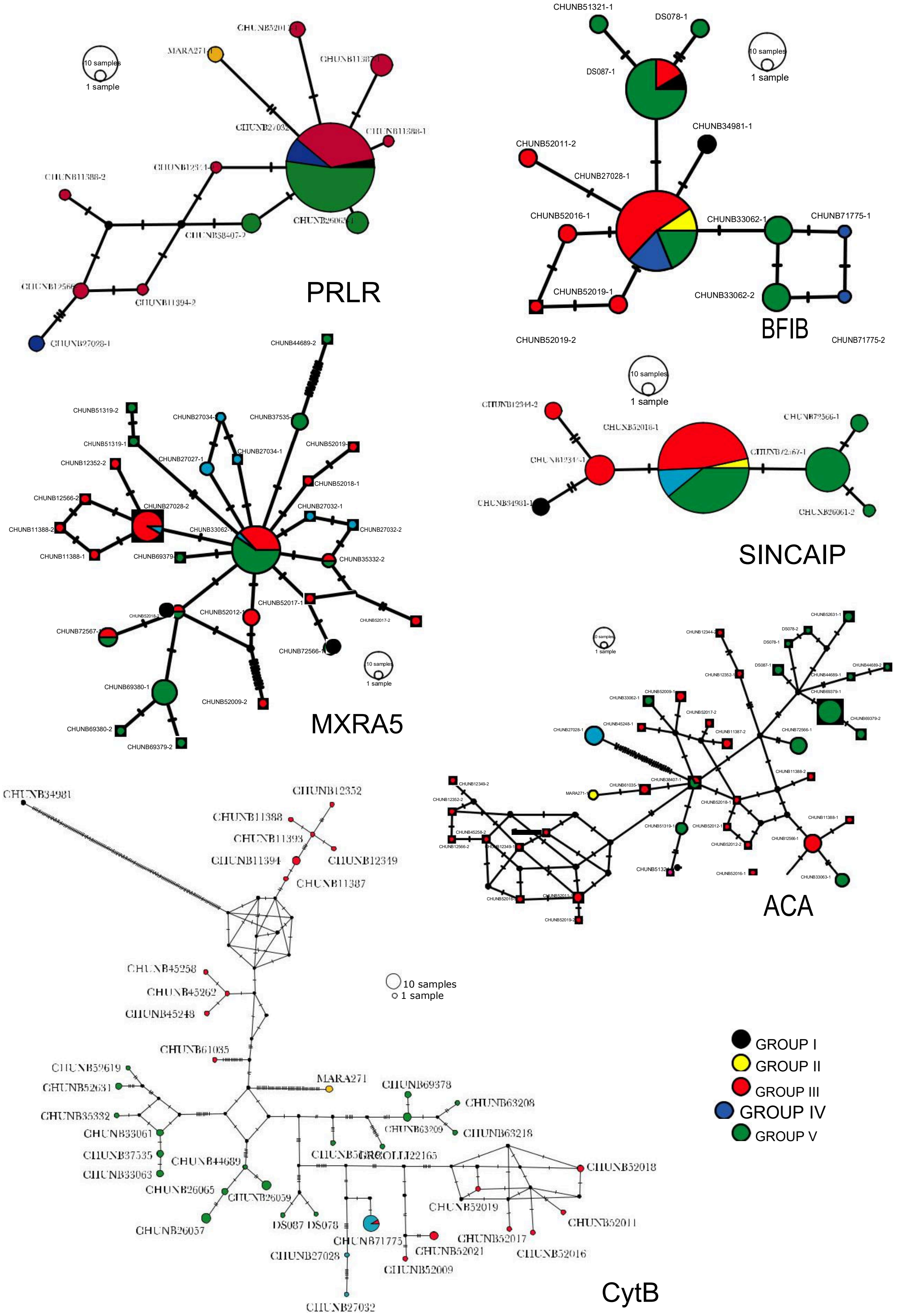
PRLR
10 samples
1 sample
MARA271
CHUNB27032
CHUNB11388-1
CHUNB11388-2
CHUNB12566
CHUNB11394-2
CHUNB27028-1
BFIB
CHUNB51321-1
DS078-1
DS087-1
10 samples
1 sample
CHUNB34981-1
CHUNB52011-2
CHUNB27028-1
CHUNB52016-1
CHUNB33062-1
CHUNB71775-1
CHUNB52019-1
CHUNB33062-2
CHUNB52019-2
CHUNB71775-2
MXRA5
CHUNB44689-2
CHUNB51319-2
CHUNB27034-2
CHUNB37535
CHUNB51319-1
CHUNB27034-1
CHUNB52019
CHUNB27027-1
CHUNB12352-2
CHUNB52018-1
CHUNB12566-2
CHUNB27028-2
CHUNB27032-1
CHUNB11388-2
CHUNB33062
CHUNB27032-2
CHUNB11388-1
CHUNB69379
CHUNB35332-2
CHUNB52017-1
CHUNB52018-2
CHUNB52012-1
CHUNB52017-2
CHUNB72567-1
CHUNB72566-1
10 samples
1 sample
CHUNB69380-1
CHUNB52009-2
CHUNB69380-2
CHUNB69379-2
SINCAIP
10 samples
1 sample
CHUNB12344-2
CHUNB52018-1
CHUNB72566-1
CHUNB72567-1
CHUNB12344-1
CHUNB34981-1
CHUNB26061-2
ACA
10 samples
1 sample
MARA271
CHUNB27028-1
CHUNB12566-1
CHUNB33063-1
CHUNB52016-1
CHUNB52019-2
CytB
CHUNB34981
CHUNB12352
CHUNB11388
CHUNB11393
CHUNB12349
CHUNB11394
CHUNB11387
CHUNB45258
CHUNB45262
CHUNB45248
10 samples
1 sample
CHUNB61035
CHUNB59619
CHUNB52631
MARA271
CHUNB69378
CHUNB63208
CHUNB35332
CHUNB63209
CHUNB63218
CHUNB33061
CHUNB37535
CHUNB44689
CHUNB33063
CHUNB26065
CHUNB26059
CHUNB26057
DS087
DS078
CHUNB71775
CHUNB27028
CHUNB27032
CHUNB52018
CHUNB52019
CHUNB52011
CHUNB52017
CHUNB52021
CHUNB52016
CHUNB52009
GROUP I
GROUP II
GROUP III
GROUP IV
GROUP V

Fig. 3.

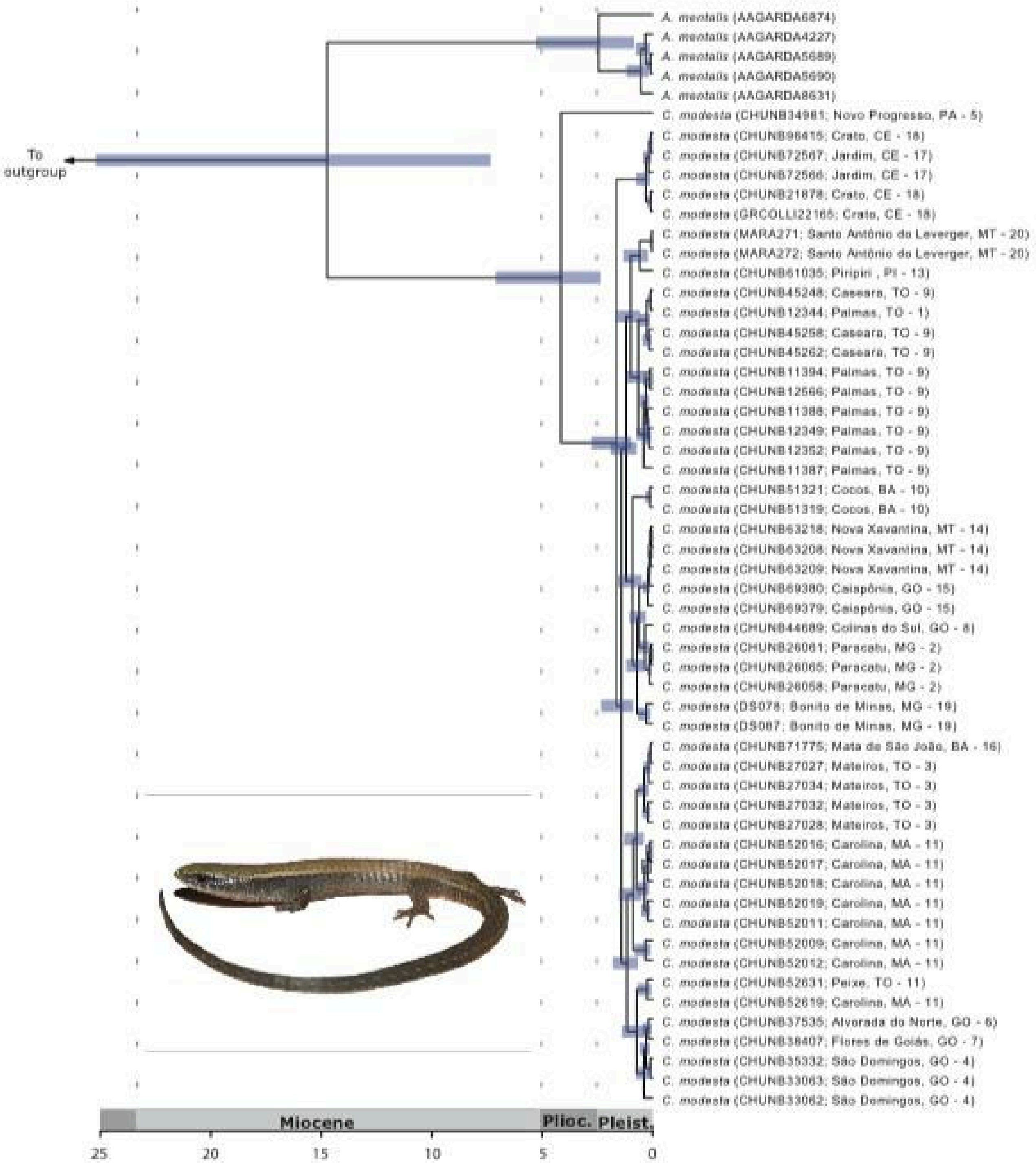
A. mentalis (AAGARDA6874)
A. mentalis (AAGARDA4227)
A. mentalis (AAGARDA5689)
A. mentalis (AAGARDA5690)
A. mentalis (AAGARDA8631)
C. modesta (CHUNB34981; Novo Progresso, PA - 5)
C. modesta (CHUNB96415; Crato, CE - 18)
C. modesta (CHUNB72567; Jardim, CE - 17)
C. modesta (CHUNB72566; Jardim, CE - 17)
C. modesta (CHUNB21878; Crato, CE - 18)
C. modesta (GRCOLLI22165; Crato, CE - 18)
C. modesta (MARA271; Santo Antônio do Leverger, MT - 20)
C. modesta (MARA272; Santo Antônio do Leverger, MT - 20)
C. modesta (CHUNB61035; Piripiri , PI - 13)
C. modesta (CHUNB45248; Caseara, TO - 9)
C. modesta (CHUNB12344; Palmas, TO - 1)
C. modesta (CHUNB45258; Caseara, TO - 9)
C. modesta (CHUNB45262; Caseara, TO - 9)
C. modesta (CHUNB11394; Palmas, TO - 9)
C. modesta (CHUNB12566; Palmas, TO - 9)
C. modesta (CHUNB11388; Palmas, TO - 9)
C. modesta (CHUNB12349; Palmas, TO - 9)
C. modesta (CHUNB12352; Palmas, TO - 9)
C. modesta (CHUNB11387; Palmas, TO - 9)
C. modesta (CHUNB51321; Cocos, BA - 10)
C. modesta (CHUNB51319; Cocos, BA - 10)
C. modesta (CHUNB63218; Nova Xavantina, MT - 14)
C. modesta (CHUNB63208; Nova Xavantina, MT - 14)
C. modesta (CHUNB63209; Nova Xavantina, MT - 14)
C. modesta (CHUNB69380; Caiapônia, GO - 15)
C. modesta (CHUNB69379; Caiapônia, GO - 15)
C. modesta (CHUNB44689; Colinas do Sul, GO - 8)
C. modesta (CHUNB26061; Paracatu, MG - 2)
C. modesta (CHUNB26065; Paracatu, MG - 2)
C. modesta (CHUNB26058; Paracatu, MG - 2)
C. modesta (DS078; Bonito de Minas, MG - 19)
C. modesta (DS087; Bonito de Minas, MG - 19)
C. modesta (CHUNB71775; Mata de São João, BA - 16)
C. modesta (CHUNB27027; Mateiros, TO - 3)
C. modesta (CHUNB27034; Mateiros, TO - 3)
C. modesta (CHUNB27032; Mateiros, TO - 3)
C. modesta (CHUNB27028; Mateiros, TO - 3)
C. modesta (CHUNB52016; Carolina, MA - 11)
C. modesta (CHUNB52017; Carolina, MA - 11)
C. modesta (CHUNB52018; Carolina, MA - 11)
C. modesta (CHUNB52019; Carolina, MA - 11)
C. modesta (CHUNB52011; Carolina, MA - 11)
C. modesta (CHUNB52009; Carolina, MA - 11)
C. modesta (CHUNB52012; Carolina, MA - 11)
C. modesta (CHUNB52631; Peixe, TO - 11)
C. modesta (CHUNB52619; Carolina, MA - 11)
C. modesta (CHUNB37535; Alvorada do Norte, GO - 6)
C. modesta (CHUNB38407; Flores de Goiás, GO - 7)
C. modesta (CHUNB35332; São Domingos, GO - 4)
C. modesta (CHUNB33063; São Domingos, GO - 4)
C. modesta (CHUNB33062; São Domingos, GO - 4)
To outgroup
Miocene
Plioc.
Pleist.
25
20
15
10
5
0

Fig.4.

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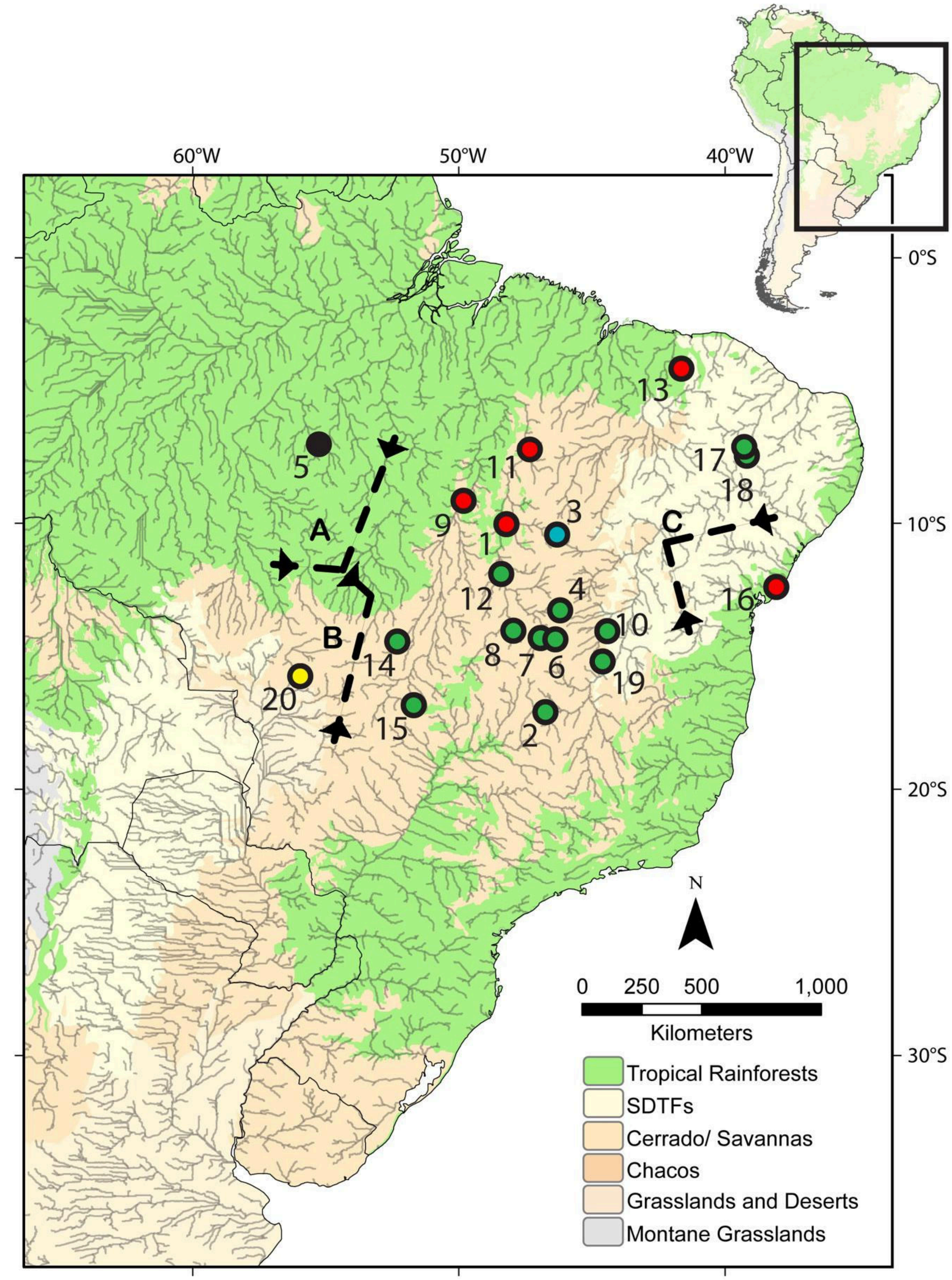
60°W
50°W
40°W
0°S
10°S
20°S
30°S
13
5
11
17
18
9
3
A
1
C
12
4
16
B
10
14
8
7
6
19
20
15
2
N
0
250
500
1,000
Kilometers
Tropical Rainforests
SDTFs
Cerrado/ Savannas
Chacos
Grasslands and Deserts
Montane Grasslands

Fig.5.

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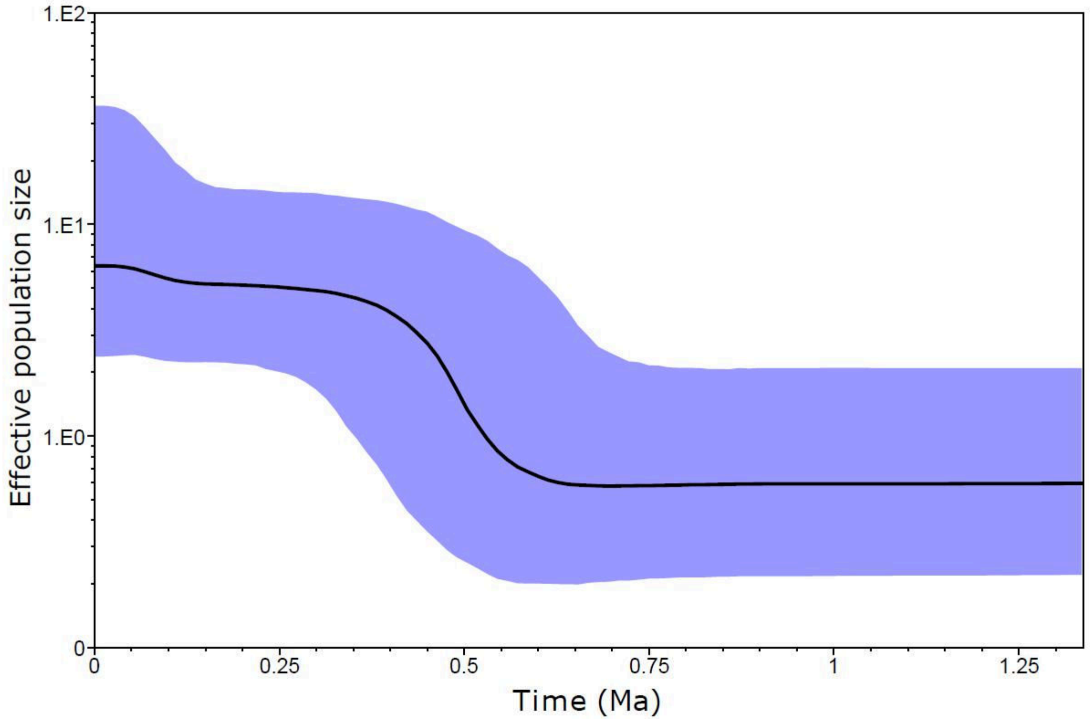
1.E2
1.E1
1.E0
0
Effective population size
0
0.25
0.5
0.75
1
1.25
Time (Ma)

Fig. 6.

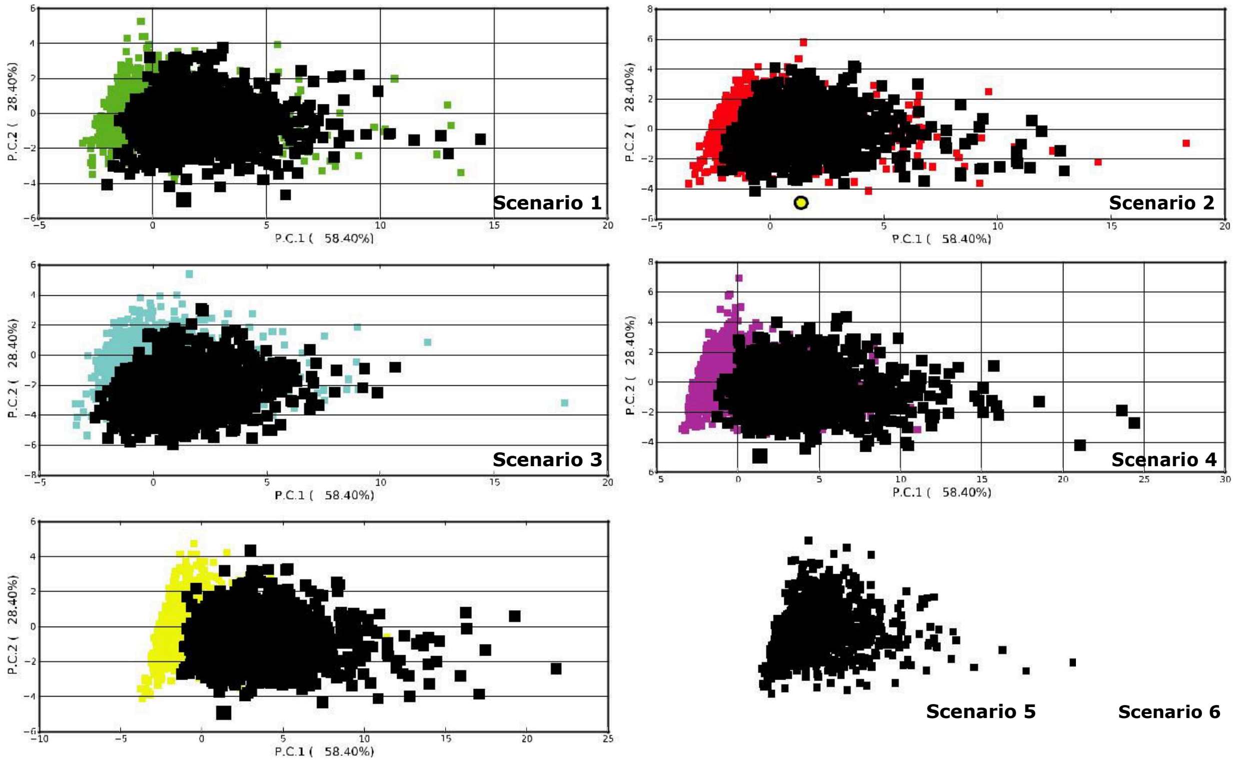
P.C.2 (28.40%)
P.C.1 (58.40%)
Scenario 1
Scenario 2
Scenario 3
Scenario 4
Scenario 5
Scenario 6

Fig. 7.

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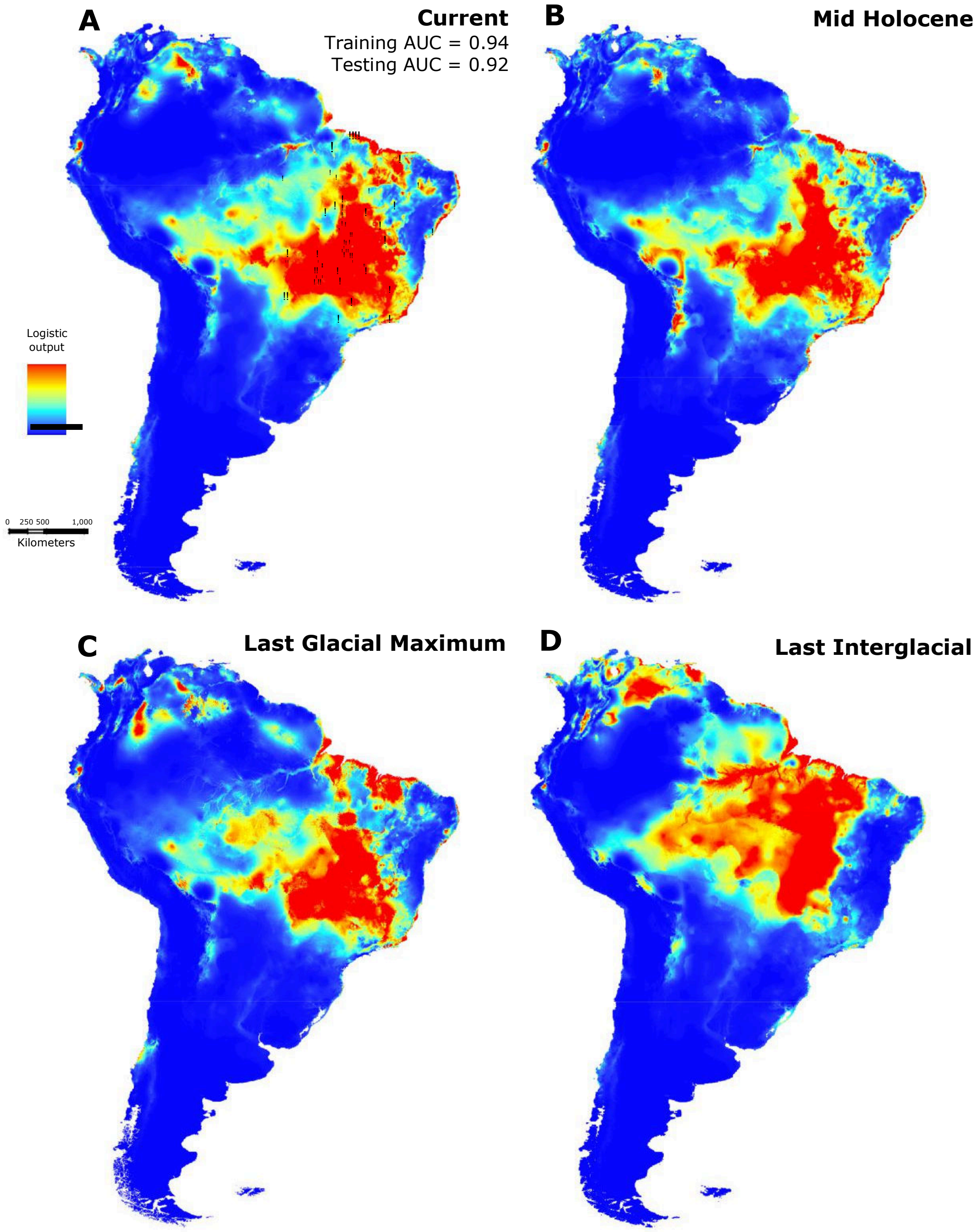
A
Current
Training AUC = 0.94
Testing AUC = 0.92
B
Mid Holocene
C
Last Glacial Maximum
D
Last Interglacial
Logistic output
0 250 500 1,000
Kilometers

Fig. 8

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Capítulo 3 - Silent death: the new Brazilian Forest Code does not protect lizard assemblages in Cerrado riparian forests

(submetido à Herpetological Conservation and Biology)

Resumo

A nova versão do código florestal brasileiro atribui apenas 30 metros de mata de galeria em torno de rios menores que dez metros como Áreas de Preservação Permanente, com o objetivo de proteção de diversos serviços e produtos ecossistêmicos, dentre eles a conservação biológica. Contudo, o limite imposto pela lei é arbitrário e não possui amparo científico. Considerando a alta quantidade de córregos menores que 10m no Brasil central e que essa lei possa trazer uma falsa ilusão de conservação, nós testamos a efetividade do limite imposto pela lei na conservação de lagartos típicos de matas de galeria, comparando duas áreas protegidas e uma área deflorestada até o limite imposto pela lei. Instalamos armadilhas de queda com cercas-guia (tipo *pitfall*) em cada uma das matas e monitoramos, indo ao campo duas vezes na semana pelo menos, a cada uma delas. Cada mata foi monitorada por pelo menos um ano. Lagartos foram identificados até o nível de espécie e devolvidos à natureza, próximo ao local de coleta. A riqueza, equidade, diversidade filogenética e abundância das assembéias de lagartos foram comparadas entre os dois grupos de matas de galeria.

Nós coletamos 11 espécies diferentes durante o período de amostragem. A mata de galeria reduzida ao nível da lei apresentou baixa abundância, riqueza, equidade e diversidade filogenética comparada às matas preservadas. Da mesma forma, espécies especialistas (ex.: *Enyalius bilineatus*) não estiveram presentes na floresta reduzida, enquanto que apenas

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espécies generalistas ocorreram nessa área (*Tropidurus torquatus* e *Ameiva ameiva*). Esse estudo sugere que o código florestal não protege espécies de lagartos em matas de galeria e que o limite de floresta de 30m para córregos menores que 10m de largura imposto pela lei deve ser repensado. Da mesma forma, sugerimos que outros fatores também sejam considerados no texto legal, como a qualidade do ambiente em volta das áreas de proteção ao longo das matas de galeria.

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Apêndice: manuscrito submentido à revista Herpetological Conservation and Biology

Short Title.—the Brazilian Forest Code and lizard assemblages protection

Title.—Silent death: the new Brazilian Forest Code does not protect lizard assemblages in Cerrado riparian forests

ROGER MAIA DIAS LEDO[1], GUARINO RINALDI COLLI[2]

[1] *Instituto Federal de Educação, Ciência e Tecnologia de Brasília, Campus Samambaia, Área de Meio Ambiente, 70860-100, Samambaia, DF, Brazil.*

[2] *Departamento de Zoologia, Universidade de Brasília, 70.910-900, Brasília, DF, Brazil. Corresponding author: roger.ledo@ifb.edu.br*

Abstract.—The new version of the Brazilian Forest Code prescribes a 30 m forest buffer around small width streams (less than 10 m width) as Areas of Permanent Preservation (APPs), to ensure water supply, erosion control, and biodiversity conservation. We tested the effectiveness of the prescribed buffer in maintaining lizard assemblages in riparian forests associated with small width streams in the Cerrado of central Brazil. We used a capture-recapture study in three riparian forests: two in protected areas, and one deforested until the limit imposed by law. We captured lizards using pitfall traps with drift fences during a period of 12-14 months in each forest. We recorded 11 lizard species during the sampling period. The reduced riparian forest had lower abundance, richness, evenness, and phylogenetic diversity compared to the preserved habitats. This forest also lacked forest specialist species (e.g., *Enyalius bilineatus*), bearing an assemblage composed exclusively by two generalist, synanthropic species (*Tropidurus*

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***torquatus* and *Ameiva ameiva*). Our results indicate that the new Brazilian Forest Code is inadequate to ensure the protection of lizard assemblages in Cerrado riparian forests, and should take into account larger riparian buffers associated with small rivers, and also consider other factors such as the environmental quality around areas of permanent protection along riparian forests.**

Key Words.—communities; reptile conservation; environmental law; empty forest; forest buffer; gallery forest; habitat loss; riparian forest; squamates

Introduction

Habitat loss can profoundly affect riparian forests, leading to reduced water quality and increased erosion (Kauffman and Krueger 1984; Jansen and Robertson 2001; Kauffman et al. 2004), restriction of animal movements (Cecala et al. 2014), reduction in population abundance and species richness (Metzger et al. 1997; Lees and Peres 2008), and invasion of exotic or generalist species (Hood and Naiman 2000; Moraes et al. 2007; Richardson et al. 2007; Almeida-Gomes et al. 2015). Roughly 53% of Brazil's native vegetation occurs in private properties (Soares-Filho et al. 2014) and the Brazilian Forest Code (Brasil 2012) is the only law that requires the protection of native vegetation in private areas. The first Brazilian Forest Code was created in 1934 to control deforestation on mountain slopes and rivers, excessive use of fire to prepare land for farming, riparian forests removal for charcoal production, and excessive deforestation and soil exploitation (Brasil 1934), practices reminiscent of the Brazilian colonial times (D´Andrada e Silva 1825). This code was replaced in 1965 and received increments in the 1990s-2000s via Presidential Decrees, requiring landowners to conserve fixed values of native vegetation on their properties, maintaining Areas of Permanent Preservation (APPs) and Legal Reserves (LRs).

APPs are protected areas provided by law, covered or not by native vegetation, with the purpose of protecting water resources, the landscape, geological stability, and biodiversity, facilitating the gene flow of flora and fauna, protecting the soil, and ensuring the welfare of human populations (Brasil 2012). APPs consist mostly of riparian habitats, but also include sandbanks, mangroves, and hilltop areas (hilltops, high elevations, steep slopes, and plateau edges). LRs are areas with native vegetation cover (without considering the application of APPs) inside private properties, kept intact to ensure sustainability in the economic use of natural resources, promoting the conservation of biodiversity and ecosystem processes, and providing shelter and protection for wildlife. LRs range from 20% to 80% of the property, depending on the forest physiognomy considered and on which biome they are located (Brasil 2012). In response to efforts to strengthen law enforcement since the last decade, the Brazilian Forest Code was recently changed due to increased pressure from the agribusiness (Metzger 2010a; Soares-Filho et al. 2014). In a fierce debate between environmentalists vs. ruralists in the Congress, the agribusiness lobby proposed a more lenient Forest Code, which was approved in late 2012 (Brasil 2012). However, none of the proposed changes related to APPs and LRs in the new Forest Code took into account scientific assessments of the efficiency of riparian forest buffers.

In fact, the limits of APPs imposed by the previous and the current Forest Code are arbitrary and lack a scientific basis (Metzger 2010b). According to the new Forest Code, small width streams (less than 10 m), very common in the plateaus of central Brazil (Souza et al. 2012), must have 30 m of protected vegetation buffer as APP (Brasil 2012). This buffer is insufficient for the maintenance of some ecosystem services, such as soil and water resources protection (Tundisi and Matsumura-Tundisi 2010), and biodiversity conservation (Galetti et al. 2010; Toledo et al. 2010), included in the legal text as APP functions (Metzger et al. 1997; Metzger 2010b). There are few *in situ* studies evaluating the effectiveness of APPs for conserving terrestrial assemblages in Brazil. Metzger et al. (1997) recommend at least 100 m of riparian forest buffer for the maintenance of 80% of plant diversity in southeastern Brazil. Lima and Gascon (1999) suggest at least 140 m of forest corridors to maintain small mammals and litter-frogs in Amazon forest, while Lees and Peres (2008) suggest at least 200 m to control edge effects and maintain specialist species of mammals and birds. In other countries, forest buffer recommendations are of 92.6 m to retain 95% of amphibian assemblages and control edge effects in southern Appalachian streams (Crawford and Semlitsch 2007), 40-100 m of forest buffer for riparian-dependent species in the U.S. Pacific Northwest (Olson et al. 2007), and 30-40 m of forest corridors to maintain arboreal mammals or 200 m of forest corridors to protect vulnerable species in Queensland, Australia (Laurance and Laurance 1999). Metzger (2010b) suggested the riparian forest distance to be maintained by law should not just consider river width as a parameter, but its most demanding role, which is biodiversity conservation. Therefore, by only preserving water quality parameters (Pinay and Décamps 1988) but not allowing riparian forest terrestrial assemblages to persist (Crawford and Semlitsch 2007; Metzger 2010b), narrow corridors would lose part of their usefulness. In addition, the habitat surrounding riparian forests may hold key resources for species maintenance (Tubelis et al. 2004; Martin et al. 2006). For instance, a study evaluating the importance of the land use around a riparian habitat in the Cerrado suggests at least 120 m of savanna habitats along riparian forests for adequate bird conservation (Tubelis et al. 2004).

Riparian forest associated with narrow rivers are very common in the Cerrado of central Brazil, as this region contains numerous headwaters that supply the major river basins in the country (Lima and Silva 2005; Lima and Silva 2008). These linear forests play important ecosystem functions (Barling and Moore 1994; Hood and Naiman 2000; Richardson et al. 2007) and have high biodiversity value in relation to their size (Redford and Fonseca 1986; Naiman et al. 1993; Silva 1996, 1997). Nevertheless, they are also being modified or lost at an alarming rate (Kauffman et al. 1997; Brandão and Araújo 2002; Machado et al. 2004; Klink and Machado 2005). Lizards are model organisms for numerous studies in ecology (Huey et al. 1983; Vitt and Pianka 1994) and Cerrado lizards are ubiquitous in terrestrial communities, easy to collect and monitor, and strongly associated with their environments (Colli et al. 2002; Nogueira et al. 2005; Nogueira et al. 2009). Cerrado riparian forests harbor many specialist (Colli et al. 2002; Nogueira et al. 2009), endemic (Manzani and Abe 1997; D'Angiolella et al. 2011; Freire et al. 2012), and cryptic lizard species (D'Angiolella et al. 2011), justifying efforts towards their conservation. Considering the importance of the Brazilian Forest Code for species conservation in private areas and the lack of studies on this subject applied to lizards, herein we assessed the effectiveness of APPs in Cerrado riparian forests, by comparing the structure of lizard assemblages in preserved and reduced riparian forests in central Brazil. If APPs are effective in protecting terrestrial lizard assemblages, we predict that reduced riparian forests in central Brazil will show no differences in richness, abundance, evenness, and phylogenetic diversity compared to preserved habitats; and that specialist species will be equally abundant in reduced riparian forests compared to natural areas.

Materials and Methods

Study Sites.—The Cerrado originally covered approximately 2,000,000 km^2 in central South America, is the second largest biome in Brazil, and also a global biodiversity hotspot

(Myers et al. 2000; Colli et al. 2002). Climate is of the Aw (equatorial savannah with dry winter) type in Köppen-Geiger's classification (Kottek et al. 2006; Alvares et al. 2013), characterized by marked wet/dry seasonality, with a rainy season from October to April and a dry season from April to September (Nimer 1989). The vegetation varies from savannas and open fields to forest formations (Ribeiro and Walter 2008). Riparian forests represent 10-20% of the Cerrado cover, contain 30% of all Cerrado vascular plants including many endemics, and are crucial to watershed protection (Ribeiro et al. 2001; UNESCO 2002; Rezende 2006). Riparian forests associated with small width streams are also very common in central Brazil, and often represent around 20% of the natural vegetation cover (UNESCO 2002).

The Distrito Federal (DF) was placed in central Brazil in mid 1900s, containing Brasília as the new capital of the country. Present estimates suggest a population of nearly three million people in DF (IBGE 2015. **Estimativas populacionais para os municípios e para as Unidades da Federação brasileiros em 01.07.2015.** Available from: http://ibge.gov.br/home/estatistica/populacao/estimativa2015/default.shtm [Acessed 31 December 2015]) and, since the transfer of DF to central Brazil until 2001, more than 47% of the riparian forest area was suppressed by urban and rural expansion (UNESCO 2002).

We conducted this study in three riparian forests in DF (Fig. 1). Two of them are well-preserved, more than 150 m wide (forest width), surrounded by natural savanna habitats, and located in two protected areas: Reserva Ecológica do IBGE (RECOR) and Estação Ecológica do Jardim Botânico de Brasília (EEJBB). They belong to Área de Proteção Ambiental (APA) das Bacias Gama e Cabeça de Veado, a 25,000 ha core protected area, part of the Cerrado Biosphere Reserve (IBGE-RECOR, R.E.D. 2002. Reserva Ecológica do IBGE. Available from http://www.recor.org.br/ [Accessed 16 April 2015]; UNESCO 2003). The third riparian forest is located in Parque Ecológico Saburo Onoyama (PESO) (Brasil 1996), a sustainable use protected area that was deforested until the limit of 40 m around the Taguatinga stream, a little more than the limits in the Brazilian Forest Code. Prior to its creation, PESO was a farm and the matrix surrounding the riparian forest is composed of *Eucalyptus* plantations, urban areas, recreation areas and small natural wet fields (summary information about the studied forests are located in Table 1). The climate is highly predictable (Nimer 1989), with a mean annual precipitation of 1,540.6 mm and a mean annual air temperature of 20.6 ºC (Ramos et al. 2009).

Data Collection.—We installed arrays of pitfall-traps with drift-fences in each riparian forest. Each array consisted of a group of four 25 cm diameter PVC pitfall-traps (~30 l) buried in the ground forming a "Y" shape, where three tubes were placed on the edges of the array, and one in the center. Pitfall-traps at the edges were connected to the central one by 5m-long and 50cm-high plastic fences, forming 120º angles (Cechin and Martins 2000) (Fig. 2; Fig. 3A). Pitfall-traps arrays were spaced between each other by at least 20 m intervals. Considering the inherent size differences among the riparian forests in this study, we installed 20 arrays of pitfall-traps with drift-fences in RECOR, 20 in EEJBB, and ten in the PESO riparian forest. All riparian forests were sampled uninterruptedly, 2-3 times a week, during a period of at least 12 months to include the Cerrado wet and dry seasons (Table 1). All captured lizards were identified to the species level, individually marked by toe clipping (Duham et al. 1994), and immediately released at the site of capture.

Statistical analyses.—To evaluate the sampling effort and to estimate lizard richness by extrapolation for each study site, we constructed sample-based species accumulation curves and used the individual-based Chao 1 richness estimator (Chao 1987, 2005), respectively, with EstimateS 9.1 (Colwell, 2006). For each analysis, we employed 1,000 randomizations without replacement (Colwell et al., 2004; Mao et al., 2005). Considering that richness and evenness are sample-size dependent, we also conducted individual-based rarefaction analyses (Gotelli and Colwell 2001; Gotelli and Colwell 2011) to compare species richness and evenness among assemblages. For richness, we used the *rarefaction* function (Jacobs, J. 2011. Individual-based rarefaction using R-package. Available from: http://www.jennajacobs.org/R/rarefaction.html [Accessed 10 December 2014]) in R 3.0.1 (R Core Team 2013), and compared the richness among sites with a Z-test (Zar 1999). For evenness, we used the Probability of Interspecific Encounter (PIE) (Hurlbert 1971), which is the probability of two individuals in one assemblage, sampled randomly, represent two different species (Olszewski 2004):

$$!"\#\$\%\&\#'()*+,- = * \frac{/}{/01}\left(1 - * \sum_{781^6}\left(\frac{/_4{}^5}{/}\right)\right),$$

where S is the total number of species in a sample, n_i is the number of individuals from the species i and n is the number of individuals of the entire sample. We conducted the rarefaction analysis employing 1,000 randomizations without replacement in EcoSim 7.0 (Gotelli and Entsminger 2004) and compared richness or evenness values with Z-tests.

For each study site we also calculated the phylogenetic diversity (PD) (Faith 1992), defined as the sum of branch lengths along the minimum spanning path connecting all species (Faith 1992; Smith et al. 2000; Faith and Baker 2006). For this calculation, we used the *picante* package (Kembel et al. 2010) in R 3.0.1 (R Core Team 2013), and the squamate tree from Pyron et al. (2013), and we did not prune the tree before analysis. As PD is also sensible to sampling effort like many other measures of diversity, we also conducted individual-based rarefaction analysis to compare PD between the studied communities, with the *phylorare*

(Nipperess and Matsen 2013; Nipperess, D.A. 2014. Phylorare: an R function for calculating the rarefied Phylogenetic Diversity of ecological samples. Available from: davidnipperess.blogspot.com.au/ [Accessed 10 December 2014]) and *phyocurve.perm* (Nipperess, D.A. 2012. Phylocurve.perm: an R function for generating a rarefaction curve of Phylogenetic Diversity by randomisation. Available at: http://davidnipperess.blogspot.com.au/ [Accessed 10 December 2014]) functions in R 3.0.1 (R Core Team 2013). For all statistical tests, we used a significance level of 0.05.

Results

In fourteen months of sampling and with a sampling effort of 4,600 trap.days in the riparian forests from RECOR and EEJBB (2,300 trap.days each), we obtained 322 lizard captures, belonging to 11 different species (Figs. 3-4). Both riparian forests showed the same richness of nine lizard species. Total lizard abundance (184 individuals) was higher at RECOR. *Notomabuya frenata* was the most abundant species in both assemblages, followed by *Tropidurus torquatus* in RECOR and by *Enyalius bilineatus* in EEJBB (Fig. 4). *Tropidurus itambere* and *Aspronema dorsivittatum* were collected only in EEJBB, whereas *Cercosaura schreibersii* and *Anolis meridionalis* were captured only in RECOR. In twelve months of sampling and with a sampling effort of 1,030 trap.days in the PESO riparian forests, we collected only two lizard species: *Ameiva ameiva* (8 individuals) and *T. torquatus* (7) (Figs. 3-4). Individuals of Leiosauridae, Gymnophthalmidae, Scincidae, and Polychrotidae were not captured in PESO (Fig. 4).

The species accumulation curves from RECOR and EEJBB riparian forests showed similar patterns, suggesting the existence of species still uncollected in each forest. However, the species accumulation curve from PESO approached an asymptote, suggesting the lack of uncollected species (Fig. 5). The species richness estimator Chao1 showed similar results for RECOR and EEJBB riparian forests (10.5 ± 2.6 species for each forest), but not for PESO (2.0 ± 0.04). The accumulation curves from preserved riparian forests differed greatly from the PESO riparian forests. Rarefaction analyses (rarefied to n = 15, the total abundance in PESO) showed significant differences in species richness ($Z_{RECOR\text{-}PESO} = 86.34$, $P < 0.001$; $Z_{EEJBB\text{-}PESO} = 95.42$; $P < 0.001$) and evenness ($Z_{RECOR\text{-}PESO} = 87.85$, $P < 0.001$; $Z_{EEJBB\text{-}PESO} = 69.68$; $P < 0.001$) between the preserved riparian forests and PESO (Table 2). PD values were higher in RECOR and EEJBB relative to the PESO riparian forest ($PD_{RECOR} = 4.01$; $PD_{EEJBB} = 3.67$; $PD_{PESO} = 1.86$). Rarefaction analyses of PD (n = 15) indicated significant differences between RECOR and PESO, but not between EEJBB and PESO (Table 2).

Discussion

Less than 20% of the species that inhabit riparian forests in protected areas were collected in APPs at the PESO (*Ameiva ameiva* and *Tropidurus torquatus*). Similarly, the lizard assemblage at PESO showed lower richness, evenness and phylogenetic diversity in almost all comparisons, indicating that the proposed APP limit for narrow rivers (30 m of forest buffers) in the Brazilian Forest Code is inefficient to protect lizard communities in Cerrado riparian forests. Moreover, the only two species collected in the PESO riparian forest are generalist, synanthropic species in the Cerrado.

Tropidurus torquatus is broadly distributed in central Brazil, and ranges from northern Argentina and Uruguay to the northern Cerrado, and from the Brazilian coast of southern Brazil from São Paulo until Bahia to eastern Paraguay (Rodrigues 1987). This species also occurs in Brazilian Restingas and in the Abrolhos Archipelago (Rodrigues 1987; Kiefer et al. 2007). Besides occurring naturally in Cerrado riparian forests (Colli et al. 2002; Nogueira et al. 2009), this species is heliophilous and omnivore, being also frequent in urban areas (Wiederhecker et al. 2003). In a similar way, *Ameiva ameiva* uses riparian forests borders (Colli 1991; Vitt and Colli 1994; Nogueira et al. 2005), but also occurs in open fields, savanna formations, edges of forest gaps, and is abundant in the extensive forest-savanna ecotones (Colli 1991; Nogueira 2006). Both species are broadly distributed in South America and apparently are less demanding than other riparian lizards in terms of habitat quality.

Enyalius bilineatus was only found in the preserved riparian forests from EEJJB and IBGE. *Enyalius* lizards are arboreal to semiarboreal and associated with tropical and subtropical Brazilian rainforests (Jackson 1978; Rodrigues et al. 2006; Rodrigues et al. 2014). *Enyalius bilineatus* ranges in the southeastern Brazilian Atlantic Forest (Jackson 1978), but with apparently disjunct populations in central Brazil (Colli et al. 2002), probably representing an undescribed species (Nogueira et al. 2005). This species is often locally abundant (Ledo 2009), but has very restrictive habitat requirements (Nogueira et al. 2005), being primarily associated with undisturbed riparian forests (Ledo 2009). Our results suggest a local extinction of *E. bilineatus* in the PESO riparian forest, given that all study sites are relatively close to each other and part of a same river basin. Further, our results indicate that the APP limits in the Brazilian Forest Code may lead to the local extinction of forest-specialist species in riparian forests, in agreement with other studies in Brazil (Linares and Eterovick 2013) and abroad (Laurance and Laurance 1999; Olson et al. 2007).

Other species not found in the PESO riparian forest were members of Scincidae (3 species), Gymnophthalmidae (3 species), and Polychrotidae (1 species). *Notomabuya frenata*, *Copeoglossum nigropunctatum*, *Cercosaura ocellata*, and *Ce. schreibersii* occur in riparian forests, but also in savanna and grasslands in the Cerrado (Nogueira et al. 2009). Their absence from PESO riparian forests is probably not only related to riparian forest size, but also with the absence of preserved savanna habitats around the forest. Studies with birds have stressed the importance of preserved habitats adjacent to riparian forests in the Cerrado (Tubelis et al. 2004) or other biomes (Martin et al. 2006). *Anolis meridionalis, Tropidurus itambere*, and *Micrablepharus atticolus* are typical from areas of savanna (Nogueira et al. 2009), and not from Cerrado riparian forests, suggesting their occurrence in the latter is sporadic. However, these species were found in EEJBB and IBGE riparian forests, but not in the PESO riparian forest, suggesting the low quality of the matrix in the latter.

Our results suggest that APP limits prescribed by the new Brazilian Forest Code can lead to local extinction of lizard species in riparian forests associated with small-width streams. Similar patterns were recorded in Cerrado, with the depletion of medium and large-sized mammals and the persistence of only small-sized species, generally tolerant to urbanization, such as small tamarins and rats (Diniz and Brito 2013). This defaunation is mainly caused by habitat loss (Dirzo and Miranda 1991; Kurten 2013), but also by hunting (Harrison et al. 2013; Stokstad 2014), burning (Silveira et al. 1999), and feral animals (Clavero and Garcia-Berthou 2005). For instance, in Brazil it is a common practice to kill snakes, lizards, and amphibians due to misinformation and by considering them all disgusting, poisonous, or potentially harmful (de Moura et al. 2010; Alves et al. 2012). Riparian forests in Distrito Federal have suffered reduction since 1954, when the Brazilian capital moved to the center of the country, and about half of its riparian forests had already been lost by 2001 (UNESCO 2002). Riparian corridors are known to enhance movement of forest specialist species in fragmented forests (Gillies and Clair 2008), promoting gene flow and keeping species in terrestrial communities (Naiman et al. 1993). The PESO forest is disconnected from any other riparian forest due to its matrix, and the single corridor considered in the DF urban planning involves the connection of three large protected areas (APA GAma-Cabeça-de-Veado, Estação Ecológica de Águas Emendadas e Parque Nacional de Brasília) in the eastern part of DF (UNESCO 2002, 2003). There is no similar plan for the western portion of DF, where the PESO and other protected areas are placed. This proposed corridor has not materialized yet, and it is unlikely it will ever be due to increasing urbanization pressure around these protected areas. If the forest corridor is not established, it is probable that no specialist lizard species will be found outside large protected areas.

Besides being intrinsically associated with the Cerrado evolution (Ferraz-vicentini and Salgado-Labouriau 1996; Salgado-Laboriau 2005; Simon et al. 2009), wildfires have negative effects in riparian forests (Dantas et al. 2013). Moreover, the fire regime has become more severe than usual around cities, due to anthropogenic activities (Silveira et al. 1999; Diniz and Brito 2013). Frequent fires promote the death of arboreal seedlings (Hoffmann 1996), reduced plant growth (Hoffmann 2002), reduced soil biomass (leaf litter) (Silva et al. 2011), and also topkill (complete death of the aerial biomass) in Cerrado riparian habitats (Hoffmann et al. 2009). Large and medium sized mammals can use riparian forests as immediate refuge from fires and also for food and water supply after fires (Redford and Fonseca 1986; Silveira et al. 1999), highlighting the importance of the riparian forests in the context of Cerrado conservation. No studies have addressed the effects of fires on lizard populations in Cerrado riparian forests, but frequent fires negatively affect lizard populations in Cerrado savanna habitats (Costa et al. 2013; Sousa et al. 2015). Probably, only 30 m of riparian forest buffer is insufficient to maintain intact/unburned areas inside the forest, and the limits imposed by law need to be rethought considering also the edge effects on it.

Besides the recent review of the Brazilian Forest Code, it still lacks a scientific basis (Metzger 2010b). Our results suggest that APPs limits of 30 m are insufficient to protect lizard species in small width streams (less than 10 m); therefore, they will not meet their expected function of biodiversity conservation. Therefore, we suggest that the APPs limits for small width streams be rethought, considering wider forest buffers. Moreover, other studies have brought to light important factors for species conservation, such as the quality of the matrix around forest fragments (Tubelis et al. 2004; Martin et al. 2006), and the presence of corridors for gene flow (Naiman et al. 1993; Laurance and Laurance 1999; Gillies and Clair 2008; Lees and Peres 2008), which are not part of the Brazilian Forest Code discussions. Therefore, we also suggest that these other factors be also included in Brazilian laws discussions. Further studies are necessary to test the efficiency of the Brazilian Forest Code and to assess the ideal riparian buffer size for effective conservation of terrestrial assemblages in this region.

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Table 1. Characteristics of three riparian forests sampled in this study: Reserva Ecológica do IBGE (RECOR), Estação Ecológica do Jardim Botânico de Brasília (EEJBB), and Parque Ecológico Saburo Onoyama (PESO).

	RECOR	EEJBB	PESO
Coordinates	15°55'49"S 47°53'W	15°53'09"S 47°50'33.7W	15°50'52"S 48°03'17.5"W
Forest width (m)	170	200	40
Pitfall arrays	20	20	10
Sampling period	Aug 2007–Sep 2008	Aug 2007–Sep 2008	Apr 2013–Mar 2014
Sampling effort	2,300 trap*days	2,300 trap*days	1,200 trap*days
Category	Preserved	Preserved	Reduced

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Table 2. Individual-based rarefaction analyses of richness, evenness, and phylogenetic diversity for lizard assemblages in intact (Reserva Ecológica do IBGE - RECOR, and Estação Ecológica do Jardim Botânico de Brasília - EEJBB) and reduced riparian forests (Saburo Onoyama Ecological Park - PESO). Values represent parameter estimates for a sample size of 15 individuals, the number of individuals recorded at PESO, and standard deviation (SD).

	Richness		Evenness		Phylogenetic Diversity	
Site	Mean ± SD	95% C.I.	Mean ± SD	95% C.I.	Mean ± SD	95% C.I.
RECOR	4.44 ± 0.89*	3–6	0.725 ± 0.069*	0.552–0.827	1.99 ± 0.48*	1.42–2.76
EEJBB	4.79 ± 0.92*	3–7	0.713 ± 0.084*	0.533–0.848	1.72 ± 0.44	1.12–2.62
PESO	2	–	0.53	–	1.29	–

* Significant differences ($P < 0.05$) between the community and the PESO community.

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Figure Legends

Fig. 1. Top left: South America with the Cerrado limits and the Brazilian Distrito Federal (DF); Top right: Distrito Federal (medium gray) indicating the studied riparian forests. (1): riparian forest from Reserva Ecológica do IBGE (RECOR), (2): riparian forest from Estação Ecológica do Jardim Botânico de Brasília (EEJBB), (3): riparian forest from Parque Ecológico Saburo Onoyama (PESO), and (4): riparian forest from Reserva Particular do Patrimônio Natural Chapada Imperial. Stars indicate the centroid were arrays of pitfall-traps with drift-fences were installed.

Fig. 2. Schematic representation of a pitfall trap array used in this study, indicating drift-fences extension, angle, and minimum distance between pitfall traps.

Fig. 3. Pitfall trap and species collected in riparian forests in Distrito Federal, Brazil (A: Pitfall trap; B: *Notomabuya frenata*, C: *Copeoglossum nigropunctatum*, D: *Aspronema dorsivittatum* (photo: Davi Pantoja), E: *Cercosaura ocellata*, F: *Cercosaura schreibersii* (photo: Daniel Velho), G: *Micrablepharus atticolus*, H: *Ameiva ameiva* (photo: Davi Pantoja), I: *Anolis meridionalis* (photo: Davi Pantoja), J: *Enyalius bilineatus*, K: *Tropidurus torquatus*, L: *Tropidurus itambere* (photo: Davi Pantoja).

Fig. 4. Abundance of lizard species sampled in each riparian forest studied (Reserva Ecológica do IBGE, Estação Ecológica do Jardim Botânico de Brasília, and Parque Ecológico Saburo Onoyama).

Fig. 5. Sample-based and individual-based species accumulation curves from Estação Ecológica do Jardim Botânico de Brasília (EEJBB), Reserva Ecológica do IBGE

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(RECOR), and Parque Ecológico Saburo Onoyama (SOEP). Curves were obtained with EstimateS 9.1 (Colwell, 2006), employing 1,000 randomizations without replacement and inferring the expected richness S (Colwell et al., 2004; Mao et al., 2005).

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Figures.

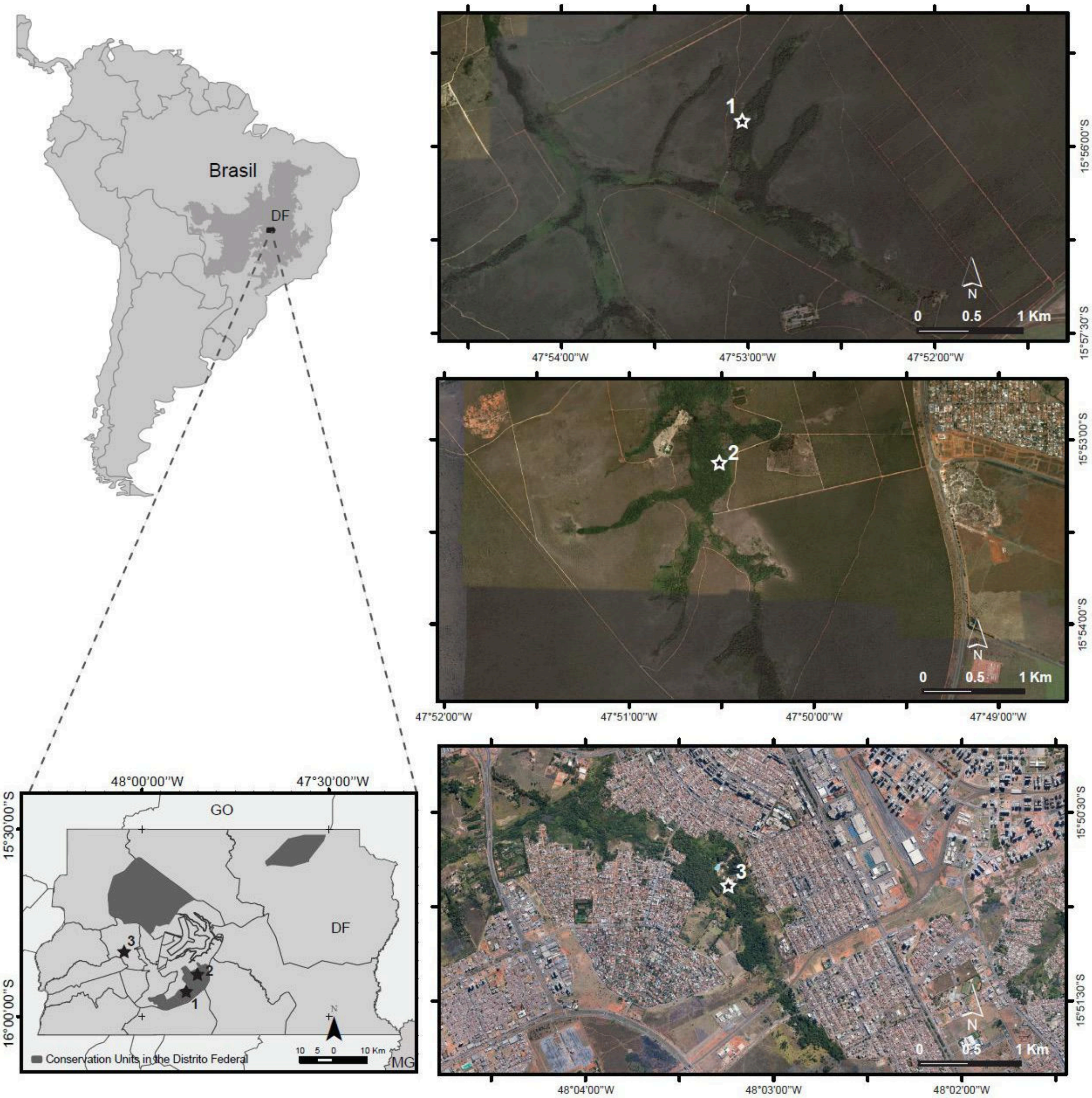

Fig. 1.

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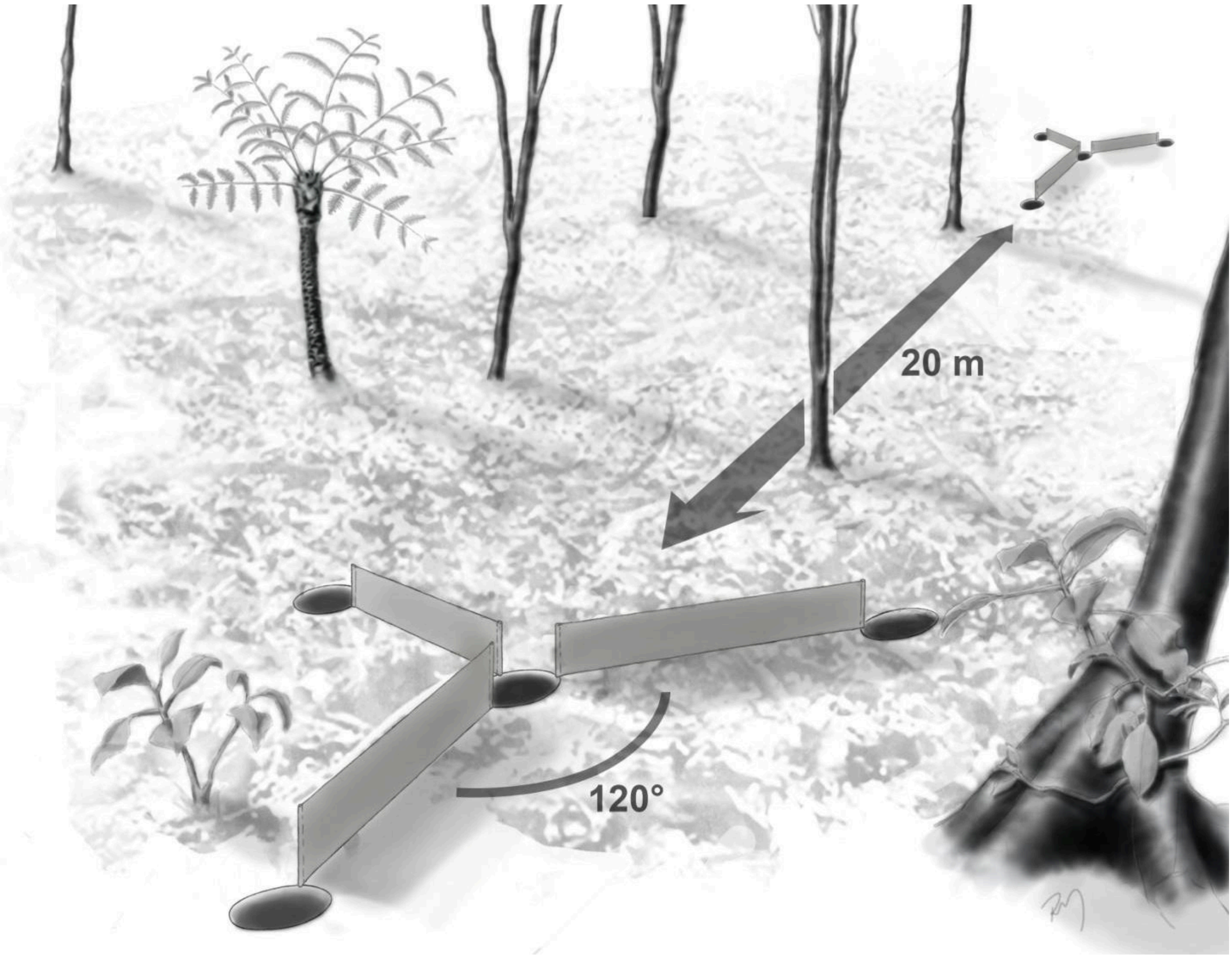

Fig.2

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Fig. 3 (plate 1).

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Fig. 3 (plate 2).

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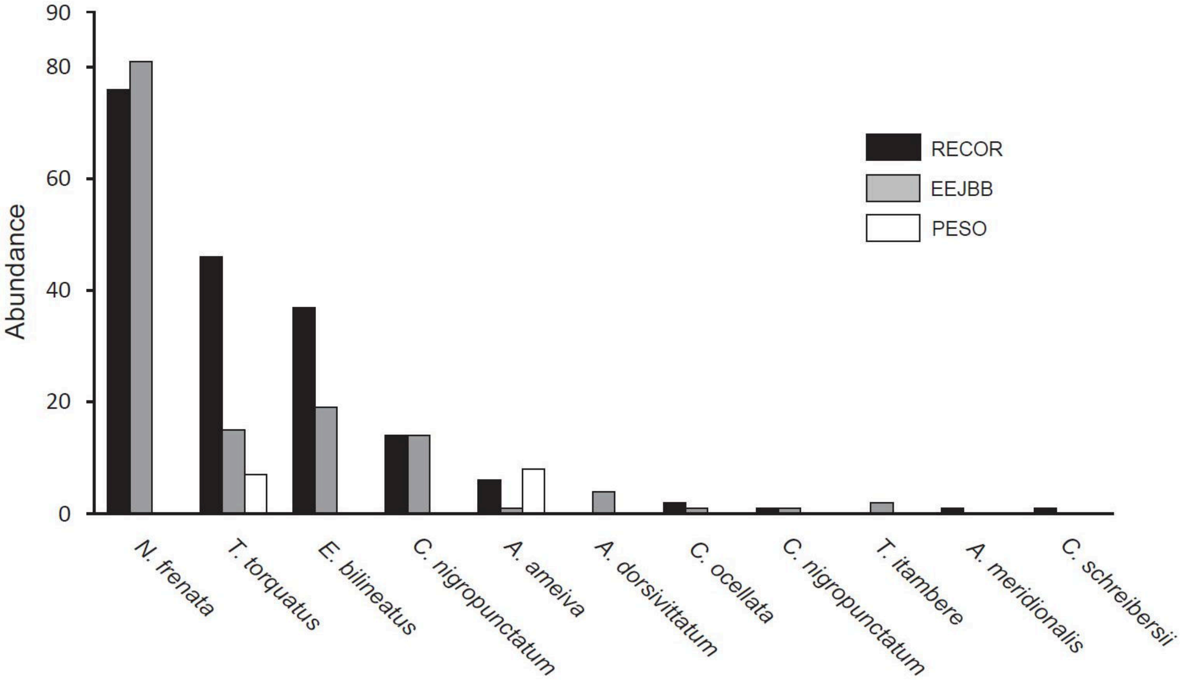
90
80
60
40
20
0
Abundance
RECOR
EEJBB
PESO
N. frenata
T. torquatus
E. bilineatus
C. nigropunctatum
A. ameiva
A. dorsivittatum
C. ocellata
C. nigropunctatum
T. itambere
A. meridionalis
C. schreibersii

Fig. 4

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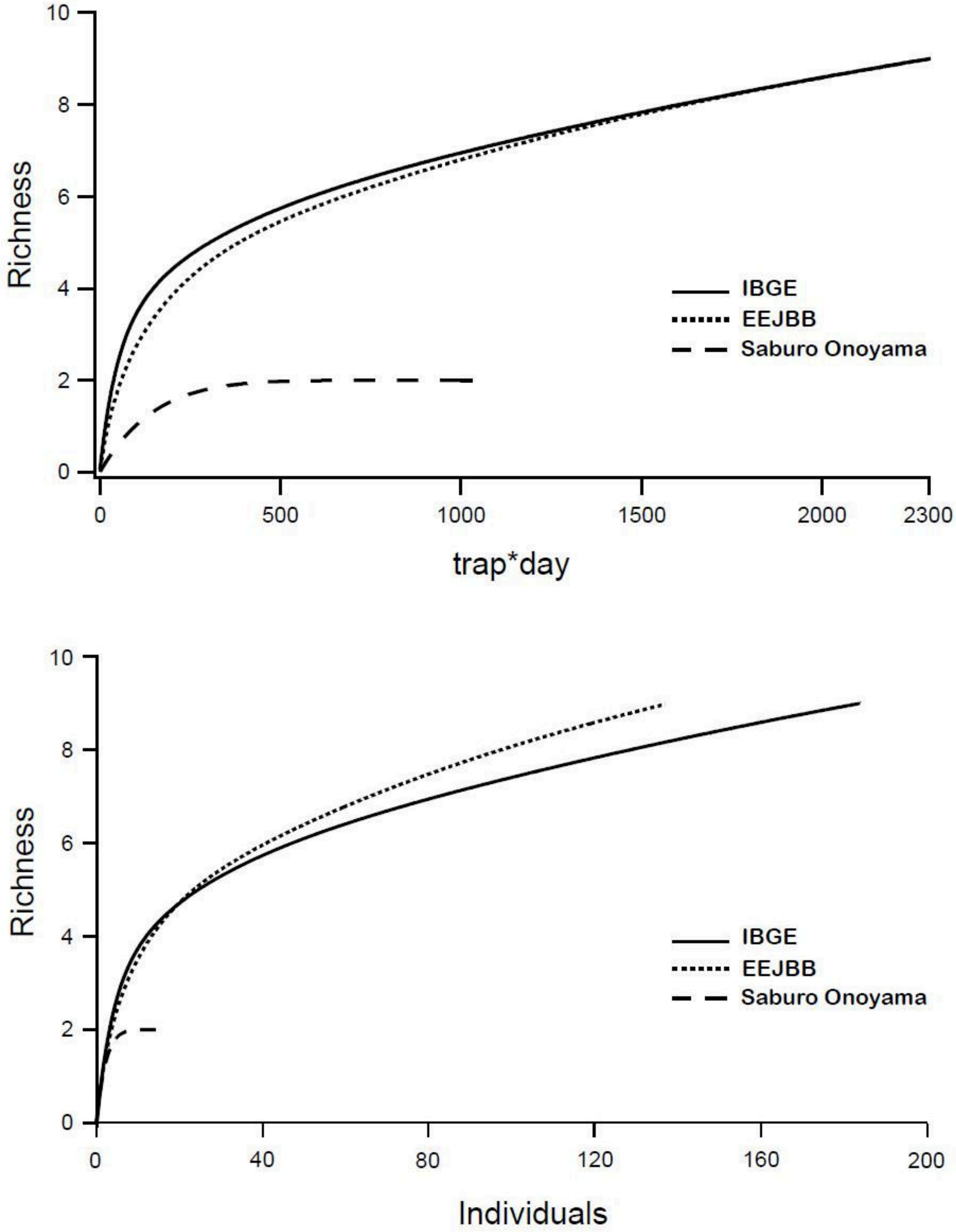
Richness
10
8
6
4
2
0
IBGE
EEJBB
Saburo Onoyama
0
500
1000
1500
2000
2300
trap*day
Richness
10
8
6
4
2
0
IBGE
EEJBB
Saburo Onoyama
0
40
80
120
160
200
Individuals

Fig. 5.