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A dinâmica filogeográfica explica o isolamento entre *Pithecopus ayeaye* e *Pithecopus megachephalus*, duas pererecas endêmicas de ilhas de altitude no domínio dos campos rupestres?

Belo Horizonte – MG
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"Ninguém solta a mão de ninguém".

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RESUMO

No Brasil, duas grandes unidades fisiográficas estendem-se ao longo da porção leste do país, cortando os estados da Bahia e Minas Gerais: a Serra do Espinhaço (SE) e o Quadrilátero Ferrífero (QF). Embora a história destas formações seja diferente, ambas possuem em comum o ecossistema dos campos rupestres, que se restringe a altitudes acima de 900m. Apesar de ocupar menos de um por cento do território nacional, este ecossistema detém uma alta taxa de endemismos. Entre as espécies endêmicas desta fitofisionomia, estão duas pererecas-macaco: *Pithecopus ayeaye* (Lutz, 1966) e *P. megacephalus* (Miranda-Ribeiro, 1926) (Hylidae: Phyllomedusinae) que embora não sejam espécies irmãs, possuem aspectos ecológicos, morfológicos e reprodutivos convergentes, tais como o padrão de coloração reticulado, comportamento territorial dos machos e uso de corpos hídricos cristalinos de primeira ordem, permanentes ou temporários, para a reprodução. Apesar de possuírem distribuição alopátrica, com limite de distribuição entre o QF e o Espinhaço Meridional (SdEM), uma área de alta adequabilidade climática entre essas unidades fisiográficas foi sugerida em um trabalho prévio. Diante disso, objetivamos testar as seguintes hipóteses: (1) as oscilações climáticas do Pleistoceno propiciaram o contato entre *P. ayeaye* e *P. megacephalus*, o que resultaria em assinaturas históricas de fluxo gênico interespecífico e altas taxas de difusão em momentos de expansão das áreas climaticamente adequadas (i.e., períodos glaciais). Alternativamente, (2) essas espécies permaneceram isoladas ao longo de suas histórias evolutivas, o que implicaria em ausência de assinatura de fluxo gênico e taxas de difusão não correlacionadas com os períodos glaciais. Para testar essas hipóteses, aplicamos modelos de isolamento com migração, nicho ecológico e difusão filogeográfica.

ABSTRACT

The Serra do Espinhaço (SE) and the Quadrilátero Ferrífero (QF) are two large physiographic units that extend lengthways the eastern portion of Brazil along the states of Bahia and Minas Gerais. Although the history of these formations is different, both have in common the ecosystem of the *campos rupestres*, that are generally restricted to altitudes above 900m. Despite occupying less than one percent of the national territory, this ecosystem has a high rate of endemism. Among the endemic species of this phytophysiognomy are two monkey-frogs, *Pithecopus ayeaye* Lutz, 1966 and *P. megacephalus* (Miranda-Ribeiro, 1926) (Hylidae: Phyllomedusinae), that show several similarities, such the reticulated pattern on flanks, the territorial behaviour of males and the use of first order crystalline water bodies, permanent or temporary, for reproduction even though they are not sister lineages. Although they have an allopatric distribution, with a distribution limit between the QF and the Southern Espinhaço (SdEM), an area of high climatic suitability between these physiographic units was evidenced in a previous study. In this sense, we aimed to test the following hypotheses: (1) the climatic oscillations of Pleistocene propitiated the contact between *P. ayeaye* and *P. megacephalus*, which would be evidenced by historical signatures of interspecific gene flow and the increase of diffusion rates in moments of expansion of suitable climatic areas (i.e., glacial periods), if they occurred. Alternatively, (2) those species remaining isolated throughout their evolutionary history, which would imply in strong genetic isolation resulting from barriers to gene flow and lack of correlation between diffusion acceleration and glacial periods. In addition, we do not expect to find a relation between phylogeographic diffusion rates and past changes in suitable areas for the species. To test these hypotheses, we applied models on gene flow, ecological niche, and phylogeographic diffusion in a statistical phylogeographic way.

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INTRODUÇÃO GERAL

A história das formações e soerguimentos das cadeias montanhosas fazem parte de um longo debate entre os geólogos (Li et al., 2016), principalmente, por constituírem importantes fenômenos geológicos que podem ter efeitos duradouros sobre a diversificação da biota (Luebert & Muller, 2015). As cadeias montanhosas representam paisagens acidentadas que se elevam a ponto de afetar o clima local (Martin & Wiebe 2004; Fjeldsa et al., 2012) e consequentemente a fauna e a flora endêmicas (Fjeldsa et al., 2012; Bitencourt & Rapini, 2013; Steinbauer et al., 2016; Neves et al., 2017). Por este motivo, elas são zonas onde a evolução adaptativa costumeiramente ocorre devido a uma alta heterogeneidade espacial da paisagem, afetando a migração de organismos e a distribuição de espécies, já que as montanhas podem agir como corredores em alguns casos e barreiras ecológicas em outros (Luebert & Muller, 2015).

No Brasil, o mais extenso e contínuo cinturão orogênico Pré-cambriano, datado do Neoproterozóico (1000 - 545 Mya) (Plumb, 1991), é a Serra do Espinhaço (Von Eschwege, 1822), que possui uma complexa fisiografia em formato arqueado (Saadi, 1995) e se alonga por mais de 1200 km latitudinais na região leste do país, entre os centros dos estados da Bahia e de Minas Gerais. Saadi (1995) propôs que o termo planalto seria a melhor definição para a Serra do Espinhaço, uma vez que a extensão longitudinal é mais expressiva que o alinhamento das cordilheiras, promovendo a configuração especial de um platô (Saadi, 1995; Magalhães Junior et al., 2015). Esta morfologia é o resultado de um longo período de dissecação associado ao desnudamento geoquímico diferencial, refletindo a heterogeneidade na resistência das rochas e o importante papel geomorfológico das drenagens fluviais (Saadi, 1995; Magalhães Junior et al., 2015). Tal morfologia irregular, moldada durante o Cenozoico, contribuiu significativamente para a diversidade geomorfológica da região, destacando duas subunidades morfotectônicas que compõem a Serra do Espinhaço: a região nordeste, também conhecida como Espinhaço Setentrional (SdES; estado da Bahia, nordeste brasileiro) e o Espinhaço Meridional (SdEM; localizado entre o centro do estado de Minas Gerais, sudeste do Brasil e o extremo sul da Bahia) (Saadi 1995; Knauer, 2007).

A Serra do Espinhaço marca a significativa diferença altitudinal entre as superfícies mais elevadas do interior continental e as superfícies mais baixas da costa do Atlântico (Valadão, 2009). Sua complexidade topográfica reitera a importância da região como um dos principais centros de endemismos do Brasil

(Silveira et al., 2016). Este complexo de altitude atua como um grande divisor de importantes bacias hidrográficas (i.e., bacias do São Francisco e do Atlântico Leste) e domínios fitogeográficos (i.e., Cerrado, Caatinga e Mata Atlântica) (Fiaschi & Pirani, 2009; Valadão, 2009). Dois destes domínios, a Floresta Tropical Atlântica na porção leste, e o Cerrado na porção oeste, são considerados importantes *hotspots* da biodiversidade Sul-Americana (Meyer et al., 2000). Adicionalmente, a biota destes domínios possui grande similaridade com várias das espécies endêmicas da Serra do Espinhaço, o que indica que além de centro de endemismo, esta cadeia de montanhas também funciona como uma zona ecotonal (Silveira et al., 2016), o que ajuda a explicar a grande riqueza de espécies observada na região (Spector, 2002). Devido à alta riqueza e grau de endemismos de sua biota (e.g., Vasconcelos & D'Angelo-Neto 2007; Vasconcelos 2008; Versieux et al., 2008), a Organização das Nações Unidas para a Educação, a Ciência e a Cultura (UNESCO) reconheceu, em 2005, a Serra do Espinhaço como uma Reserva da Biosfera (Gontijo, 2008) (Figura 1).

Margeando a porção centro-sul do SdEM, o Quadrilátero Ferrífero (QF) é uma formação datada entre 3.28 e 2.61 bilhões de anos (i.e., Eras Meso- e Paleoarqueanas) (Machado & Carneiro, 1992). O QF é caracterizado por sua paisagem acidentada devido aos intemperismos físicos e químicos durante a sua pedogênese. A formação geotectônica do QF ocorreu durante dois principais eventos de compressão: o primeiro foi o Transamazônico (2,2 e 2 bilhões anos; Era Paleoproterozóica); já o segundo foi o Brasiliano (700 e 450 milhões de anos; entre a Eras Neoproterozóica do Éon Proterozóico e o início da Era Terreneuviana), ocasionando em diferentes graus de dobramentos que resultaram em três unidades fisiográficas: (a) Supergrupo Rio das Velhas, (b) Supergrupo Minas e (c) Grupo Itacolomi (Chemale et al., 1994; Alkimin & Marshak, 1998; Cunningham et al., 1998).

Com sua localização estratégica na região centro-sul do estado de Minas Gerais, o QF moldou ao longo dos anos importantes fatos históricos ocorridos no Brasil entre os séculos XVII – XXI (Salgado & Carmo, 2015). Com uma área aproximada de 7200 km^2, o QF é considerado uma das regiões mais importantes do país devido às suas grandes reservas minerais, que incluem jazidas de alumínio, ferro, manganês e ouro, dentre outros (Spier, Oliveira & Rosière, 2003; Roeser & Roeser, 2010), contabilizando cerca de 80% da extração de ferro do Brasil e 65% de toda a produção mineral do país (Carmo & Campos, 2012). Tamanha riqueza despertou o interesse econômico global, impactando negativamente a paisagem local com o crescimento urbano e a mineração

(Jacobi & Carmo, 2008). A extração exacerbada e o desenvolvimento citadino desordenado chamaram a atenção dos conservacionistas para a área, especialmente pelo alto índice de endemismos de sua fauna e flora (Giullieti & Pirani, 1997; Jacobi & Carmo, 2008; Echternacht, 2010, Silveira et al., 2016). Não menos importante que o fator econômico, a beleza cênica do QF chama atenção principalmente por suas encostas íngremes, cachoeiras, vales e pelo seu diversificado geosistema (Salgado & Carmo, 2015).
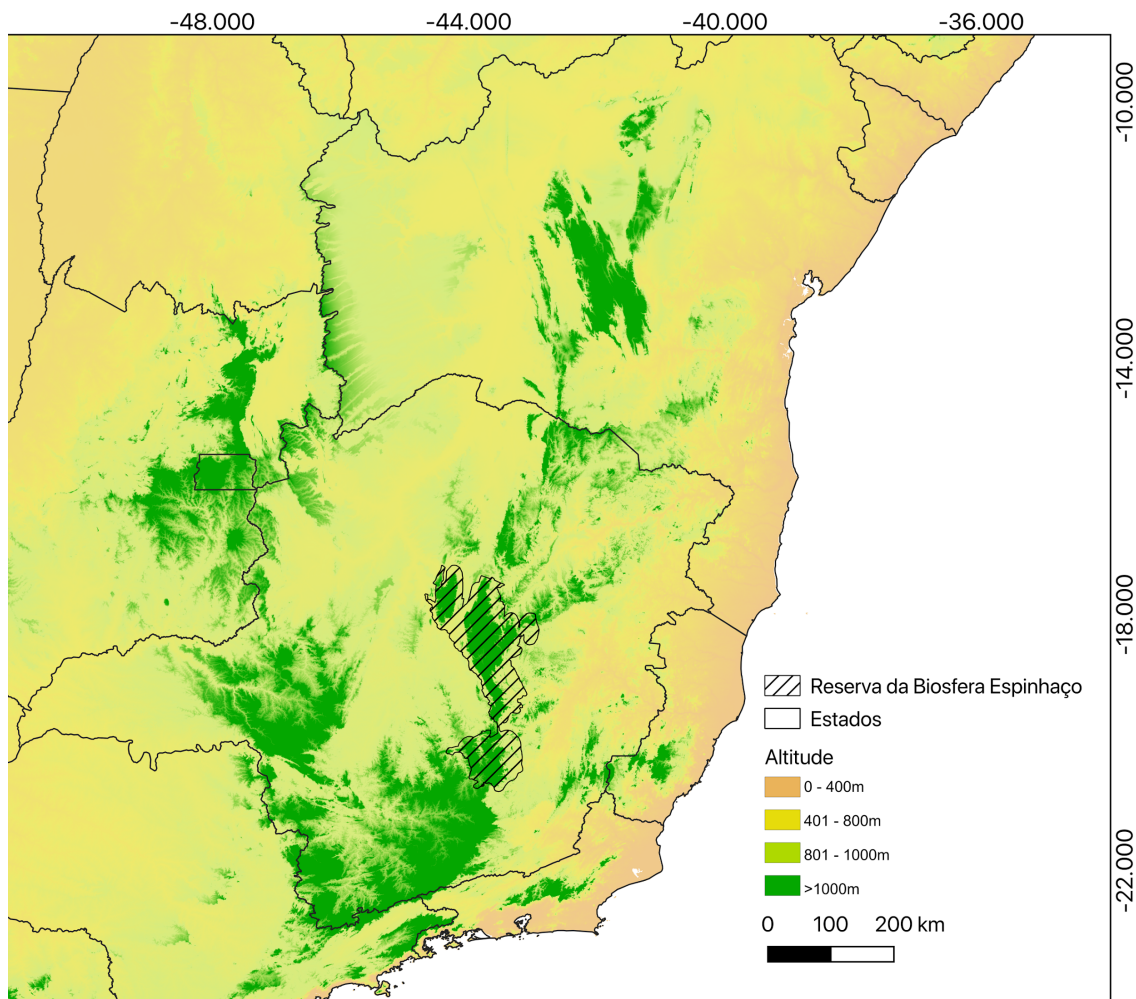
Figura 2: Mapa das regiões do QF e SdEM, com destaque para a Reserva da Biosfera do Espinhaço e áreas de altitude.

Apesar das diferenças entre os processos de formação e características geomorfológicas de ambas as regiões, seus processos de formação geológica são muito antigos e não influenciaram diretamente na diversificação da biota atual. Uma forte evidência disso é que, apesar da Serra do Espinhaço e o QF possuírem os campos rupestres como um ecossistema em comum (Fernandes, 2016), a flora desta fitofisionomia apresenta pouca distinção filogenética em relação às encontrada no Cerrado adjacente (Neves et al., 2018), com muitas espécies originadas recentemente (e.g., Collevatti et al., 2009; Bonatelli et al.,

2014; Barres et al., 2019). Os campos rupestres estão intimamente associados às formações quartzíticas e ferruginosas, são azonais e estão localizados em altitudes acima de 900m, abrangendo um mosaico de diferentes comunidades florísticas dependentes da profundidade do solo (Conceição & Pirani, 2005) e da disponibilidade de água (Assis et al., 2011). Apesar de ocorrerem em outras áreas de altitude do Escudo Brasileiro, o núcleo dos campos rupestres está distribuído ao longo do Espinhaço e do QF (Silveira et al., 2016; Fernandes, 2016). Este ecossistema é considerado o tipo fitofisionomia campestre mais abundante nas zonas de altitude do Escudo Brasileiro (Ribeiro et al., 2014; Fernandes, 2016). Endêmicos do Brasil, os campos rupestres ocorrem em regiões cuja média de precipitação anual é acima de 800 - 1500 mm, com uma estação árida que dura entre dois e cinco meses (Alves et al., 2014). Outros fatores como variação do vento, temperatura, estresse hídrico e ocorrência de incêndios, favoreceram a composição florística esclerófila do ecossistema (Giulietti et al., 1997), assim como sua alta taxa de endemismo (Menezes & Giulietti, 2000; Silveira et al., 2016; Morellato & Silveira, 2018; Neves et al., 2018).

Silveira et al. (2016) postularam a ocorrência de 14,7% de todas as plantas vasculares brasileiras para os campos rupestres *sensu stricto*, em uma área correspondente a apenas 0,78% do território nacional. Dentre os elementos da flora vascular mais comuns nos campos rupestres, destacam-se as seguintes famílias: Asteraceae, Bromeliaceae, Cyperaceae, Fabaceae, Eriocaulaceae, Lamiaceae, Melastomataceae, Myrtaceae, Orchidaceae, Poaceae, Rubiaceae, Velloziacea e Xyridaceae (Mello Silva et al., 2011; Jacobi & Carmo, 2012; Silveira et al., 2016). Em relação à fauna, Chaves e colaboradores (2015) identificaram sete espécies de aves endêmicas da Serra do Espinhaço, sendo duas exclusivas de campos rupestres. Neves e colaboradores (2017), por sua vez, encontraram 23 espécies de anfíbios anuros endêmicos da Serra do Espinhaço, dentre elas uma está em um dos únicos gêneros endêmicos de tetrápodes da região (i.e., *Rupirana cardosoi*, Heyer, 1999). Ao avaliar o padrão de endemicidade na distribuição de aves nas montanhas do sudeste do Brasil, Chaves e colaboradores (2015) revelaram barreiras geográficas ao fluxo gênico, reforçando a separação das áreas de endemismo mostradas pelo padrão de distribuição dos anfíbios encontrados por Neves et al. (2017).

A origem da biota dos campos rupestres pode estar relacionada com a hipótese dos OCBILs (paisagens antigas, inférteis e climaticamente tamponadas) proposta por Hopper (2009) ou com a hipótese de refúgios

climáticos do Pleistoceno (Haffer, 1969). Estudos recentes apontam para uma complexidade no surgimento e diversificação das espécies endêmicas, com origem geralmente no Neógeno e subsequente radiação no Quaternário (Gustafsson et al., 2010; Bonatelli et al., 2014; Chaves et al., 2014; Nascimento et al., 2018), portanto estas hipóteses não são mutuamente exclusivas. De acordo com a teoria proposta por Hopper (2009), o solo infértil de formação lenta e a estabilidade climática a longo prazo são os principais fatores para a diversificação e manutenção dos OCBILs, já que estes influenciam nas complexas e contínuas adaptações biológicas das linhagens capazes de persistirem nos mesmos por longos períodos de tempo. Espécies de OCBILs apresentam conservadorismo de nicho filogenético e tendência a manterem características ecológicas ancestrais (Crisp et al., 2009; Sales et al., 2017). Por estes motivos, a hipótese de OCBILs prevê que a biota endêmica destes ambientes possua tempos de divergência antigos (Hopper, 2009), apesar de não especificar o que é antigo e o que é recente em sua formulação.

Em contrapartida, a hipótese proposta por Haffer (1969) pressupõe as oscilações cíclicas do clima no Quaternário como os principais fatores responsáveis pelo isolamento de linhagens em determinadas regiões. Tais processos propiciaram a expansão e retração de paisagens, gerando refúgios ecológicos fragmentados durante os períodos de contração das mesmas. Haffer (1969) postula sobre três possíveis consequências das oscilações climáticas do Pleistoceno sobre a distribuição das espécies, sendo elas: (a) especiação alopátrica seguida de contato secundário. Neste caso, barreiras reprodutivas e incompatibilidades ecológicas evoluem durante o isolamento geográfico das populações, resultando em simpatria e sobreposição mais ou menos extensa dos intervalos ocupados quando as paisagens se expandem e as novas espécies entram em contato; (b) Exclusão geográfica, na qual as linhagens apresentam incompatibilidade reprodutiva mas possuem nichos ecológicos similares, levando a uma exclusão mútua como resultado de competição ecológica; (c) Hibridação, na qual o processo de especiação não foi concluído e as espécies geram híbridos ao longo de uma zona de contato. A hibridação pode ocorrer ao longo de uma faixa geográfica estreita, geralmente com características ecológicas intermediárias àquelas cujas espécies parentais estão submetidas, indicando um alto valor adaptativo dos híbridos apenas nesta zona. Quando a zona de hibridização é extensa, indicando alto valor adaptativo dos híbridos (i.e., vigor do híbrido), o contato entre as espécies pode levar à completa fusão das mesmas (Canestrelli et al., 2016; Barker et al., 2017).

Em geral, topos de montanhas tendem a ser berçários de novas espécies, por apresentarem um conjunto de variáveis específicas que selecionam espécies adaptadas às condições edáficas e climáticas específicas destas regiões (Fjeldsa et al., 2012; Neves et al., 2018). Neste contexto, anfíbios anuros tornam-se excelentes modelos de estudos sobre dinâmicas geográficas associadas à diferenciação de linhagens, já que geralmente apresentam baixas capacidades de dispersão, levando à uma alta estruturação genética (Zeisset & Beebee, 2008). Embora os vales possam agir como fortes barreiras biogeográficas, a ocorrência de contato secundário por populações previamente isoladas de anfíbios nestas áreas pode ocorrer em caso de expansão das mesmas, o que é favorecido durante períodos glaciais (Baker, 2008). Tendo isso em vista, não é incomum a detecção de sinais de fluxo gênico histórico entre populações ou espécies que atualmente se encontram isoladas entre diferentes ilhas de altitude (e.g., Magalhães et al., 2017; Ramos et al., 2018). Diante disso, nós investigamos se, e quais processos intrínsecos (i.e., capacidade de dispersão, isolamento ecológico e isolamento reprodutivo) e extrínsecos (i.e., barreiras geográficas e ecológicas) estão relacionados ao isolamento geográfico observado entre duas espécies de pererecas-macaco endêmicas das ilhas de altitudes do QF e do SdEM.

As pererecas-macaco, como são conhecidas as espécies do gênero *Pithecopus* (Hylidae: Phyllomedusinae), estão distribuídas ao longo da América do Sul (Frost, 2018) e apresentam características morfológicas e comportamentais marcantes que auxiliam no reconhecimento do grupo. Dentre estas características estão a coloração esverdeada na região dorsal, cores púrpura ou laranja sob um fundo negro nos membros e flancos, pupila em fenda vertical, hálux e pólex opositores, caminhada lenta ao invés de saltos, comportamento territorial de machos por combate físico, com emissão de vocalizações agressivas, além da presença de glândulas paratóides em algumas espécies (Haddad & Prado, 2005; Álvares, 2008; Faivovich et al., 2010; Duellman et al., 2016; Oliveira, 2017). As espécies do gênero *Pithecopus* depositam seus ovos junto com capsulas gelatinosas em uma única folha de pequenos arbustos ao redor de riachos, drenagens temporárias ou pequenas lagoas. Esta folha é fechada durante a oviposição com esforços combinados do casal e os ninhos ficam pendentes sobre os corpos d'água (modos reprodutivos 24 e 25 *sensu* Haddad & Prado, 2005; Álvares, 2008; Oliveira, 2017).

O gênero conta atualmente com 11 espécies descritas (Haga et al., 2017), das quais quatro, *P. ayeaye*, *P. centralis*, *P. megacephalus* e *P. oreades*,

constituem um complexo de espécies com flancos reticulados, todas ocupantes de paisagens rupestres e que utilizam riachos com águas cristalinas como sítios reprodutivos (Brandão, 2002). As *Pithecopus* estão separadas em duas linhagens monofiléticas, a primeira delas com espécies ocupantes de áreas de baixada do interior do subcontinente sul-americano (i.e., *P. araguaius, P. azureus, P. hypochondrialis, P. nordestinus* e *P. palliatus*) e a segunda, com a maior parte das espécies descritas, ocupantes da Mata Atlântica e das ilhas de altitudes do Escudo Brasileiro (i.e., *P. ayeaye, P. centralis, P. megacephalus, P. oreades, P. rohdei* e *P. rusticus*) (Faivovich et al., 2010; Duellman et al., 2016; Haga et al., 2017). Apesar de pertencerem a um mesmo clado, as pererecas de flancos reticulados não constituem um grupo monofilético, com *P. megacephalus* sendo mais aparentada a *P. rohdei* do que com as espécies morfológica e ecologicamente similares a ela (Faivovich et al., 2010; Bruschi et al., 2014; Duellman et al., 2016; Haga et al., 2017). Neste trabalho, as duas espécies investigadas fazem parte deste grupo fenético de pererecas de flancos reticulados, sendo elas *Pithecopus ayeaye* (B. Lutz, 1966) e *P. megacephalus* (Miranda-Ribeiro, 1926), ambas com distribuição restrita a campos em ilhas de altitude do Escudo Brasileiro, sobretudo aos *campos rupestres*.

Descrita da região de Poços de Caldas (MG) em 1966 pela ilustre herpetóloga brasileira Bertha Lutz, *Pithecopus ayeaye* (Figura 2) recebeu este nome em referência ao grupo dos prossímios – que incluem os lêmures, lóris e os extintos adapiformes – após a descrição *Phyllomedusa loris, P. tarsius* e *Agalychnis lemur*, ambos filomedusíneos com nomes específicos associados aos mencionados primatas. Estes epítetos aludem à forma similar de locomoção entre estas pererecas e os prossímios (Lutz, 1966). Além do Planalto de Poços de Caldas, a espécie também é encontrada em serras dos Campos das Vertentes, no Planalto da Canastra e, em sua porção mais setentrional, no QF. Estas populações estão divididas em três unidades evolutivamente significativas, cujas distribuições coincidem com unidades fisiográficas do relevo (Magalhães et al., 2017). Devido à distribuição disjunta da espécie e as pressões antropogênicas sofridas por suas populações, tais como perda de habitat por atividades agropecuárias, incêndios induzidos e mineração (Caramaschi et al., 2010; Magalhães et al., 2017), a espécie foi classificada como Criticamente Ameaçada de Extinção (CR) pela União Internacional para a Conservação da Natureza (IUCN) (2014). Entretanto, após a sinonimização de *P. itacolomi* com *P. ayeaye*, o que aumentou a sua extensão de ocorrência (Araujo et al., 2007; Baêta et al., 2009), a espécie foi removida das categorias de ameaça da Lista

Vermelha de Espécies Ameaçadas de Extinção do Brasil em 2014 (ICMBio, 2014; Nali et al., 2015; Magalhães et al., 2017).

Figura 3: Indivíduo de *Pithecopus ayeaye*. Imagem: Tiago Pezzuti.

Pithecopus megacephalus (Figura 3), por sua vez, é endêmica das ilhas de altitude do SdEM com registro mais meridional na Serra do Cipó em Minas Gerais e o mais setentrional no distrito de Igaporã, no estado da Bahia. A espécie foi descrita por Alípio de Miranda-Ribeiro em 1926 e seu nome remete ao grande tamanho da cabeça em relação ao corpo (Miranda-Ribeiro, 1926), um importante caractere utilizado para diferenciá-la das demais *Pithecopus* de flancos reticulados (Caramaschi, 2006). Atualmente a espécie encontra-se categorizada como Deficiente em Dados (DD) pela IUCN (2016), devido à falta de conhecimento sobre a sua ecologia e distribuição geográfica (Angulo, 2016). Contudo, estas carências de dados foram supridas por Brandão et al., (2012), Oliveira et al., (2012) e Ramos et al., (2018), que revelaram dados sobre a sua distribuição geográfica, ecologia reprodutiva e estruturação populacional, respectivamente. Alguns estudos demonstraram a necessidade da mudança deste *status* para a categoria de Em Perigo de Extinção (EM), principalmente pela perda e fragmentação do habitat (Morais et al. 2013; Barata et al., 2016). Contudo, mais de 30% dos registros conhecidos para a espécie estão em áreas protegidas por unidades de conservação de proteção integral (Ramos et al., 2018), o que pode indicar menores preocupações sobre o seu risco de extinção.

Figura 4: Indivíduo de *Pithecopus megacephalus.* Imagem: Antônio Cruz.

Embora *P. ayeaye* e *P. megacephalus* não sejam espécies irmãs (Faivovich et al., 2010; Bruschi et al., 2014; Duellman et al., 2016), ambas são muito semelhantes quanto a aspectos da morfologia, ecologia e biologia reprodutiva. Ambas apresentam um padrão de coloração reticulado (Figuras 2 e 3) (Caramaschi, 2006), distribuição geográfica restrita aos campos rupestres, em regiões acima de 900m de altitude (Brandão et al., 2012; Magalhães et al., 2017; Ramos et al., 2018; 2019) e uso de riachos de primeira ordem – permanentes ou temporários – e com água cristalina para a reprodução (Oliveira et al., 2012; Oliveira, 2017). Ademais, apesar de estarem distribuídas alopatricamente entre as serras do sudeste e sul de Minas e o SdEM, Magalhães et al. (2017) encontraram uma área de alta adequabilidade climática compartilhada entre elas nos extremos de suas distribuições geográficas (i.e., entre o QF e o Planalto de Diamantina). Considerando este potencial de coocorrência, neste estudo objetivamos testar as seguintes hipóteses: (1) as oscilações climáticas do Pleistoceno propiciaram o contato entre *P. ayeaye* e *P. megacephalus*, o que seria evidenciado por assinaturas históricas de fluxo gênico interespecífico e altas taxas de difusão em momentos de expansão de áreas climáticas convenientes. Alternativamente, (2) essas espécies permaneceram isoladas ao longo de sua história evolutiva, o que implicaria em fortes barreiras geográficas e/ou reprodutivas ao fluxo gênico. A primeira hipótese concorda com as

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expectativas da hipótese de refúgios de Haffer (1969). Por outro lado, a segunda hipótese pode concordar tanto com a hipótese de refúgios quanto com a de OCBILs (Hopper, 2009), a depender dos tempos de coalescência das espécies. Para testar essas hipóteses, aplicamos modelos de barreira, isolamento com migração, nicho ecológico e difusão de forma filogeográfica estatística.

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CAPÍTULO I

**Phylogeographic and past climatic dynamics explain the current isolation
between two endemic species of the Brazilian Shield sky islands***

**Manuscrito preparado para submissão no periódico Journal of Biogeography, com alguns detalhes de
formatação adaptados para a dissertação.*

Phylogeographic and past climatic dynamics explain the current isolation between two endemic species of the Brazilian Shield sky islands

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ABSTRACT

Aim: Elucidate the processes involved in the allopatry between two endemic species of monkey-frogs (Phyllomedusinae, Hylidae) of the *campos rupestres*, the mostly threatened phytophysiognomy in the Brazilian sky islands. Here, we tested if the climatic cycles of Pleistocene are related with the distributional pattern of the species.

Location: The Brazilian Shield sky islands complex.

Methods: We used 143 specimens from 26 localities of *Pithecopus ayeaye* and *P. megacephalus*, encompassing most of the known distribution of the species. One mitochondrial and six nuclear genes were used for phylogeographical analyses. We evaluated the haplotype sharing using haplotype networks. Subsequently, we tested between gene flow or incomplete lineage sorting to explain this sharing through tests of hybridization and historical gene flow, using multigenic networks and two island migration model, respectively. Complementarly, we used spatial-temporal diffusion models and ecological niche

modelling with past projection to search the possible processes related with the genetic patterns observed.

Results: We found evidence of historical gene flow between *P. ayeaye* and *P. megacephalus* and discarded current hybridization between them. Both species presented a diffusion pattern of centre-periphery, with increases in diffusion rates coinciding with past glacial cycles. Climatic oscillations of the Pleistocene influenced directly phylogeographic patterns of these species, evidencing the Depression of Belo Horizonte as a transient barrier between them.

Main conclusions: Our results are congruent with previous studies in which the climatic fluctuations of the Pleistocene directly influenced in the distribution of the biota in the Brazilian Shield sky islands complex. Moreover, the current isolation between these species is supported by limited dispersion capacity and an apparent niche divergence between them. In short, this study supports the hypothesis of ecological refugia as a process related to the diversification of the *campos rupestres* fauna.

KEYWORDS

Quadrilátero Ferrífero, Espinhaço mountain range, *campos rupestres*, *Pithecopus ayeaye*, *Pithecopus megacephalus*, phylogeography.

1. INTRODUCTION

The Brazilian Shield sky islands complex (*sensu* Warshall, 1995) is among the most diverse places in the country, with high rates of endemism of the fauna and flora due to soil and rocky substrates, as well as microclimatic particularities (e.g., Silveira et al., 2016; Silva et al., 2018). Among the archipelagos in this complex, the Quadrilátero Ferrífero (QF) region and the Serra do Espinhaço mountain range (SE) are two of the oldest geological formations in the world, dated back from the Archean Eon (3.28 - 2.5 Gya) (Machado & Carneiro, 1992), and from the Neoproterozoic age (1000 - 545 Mya) (Plumb, 1991), respectively. Together, these areas extend over 8400 km^2 in a latitudinal direction, from central Bahia State to the centre of Minas Gerais State (Magalhães-Júnior, de Paula Barros & Felippe, 2015). The remarkable differences between the geomorphological features of these regions, associated with complex climatic regimes, shaped the habitat heterogeneity, favouring the formation of several climatic refugia through

Pleistocene, in addition to areas of endemism (Bonatelli, et al., 2014; Barbosa, Fernandes, & Sanchez-Azofeita, 2015; Barres et al., 2019).

The typical mountaintop environments in QF and SE are named *campos rupestres* (Fernandes et al., 2016). Naturally fragmented by altitude, sequences of ridges, and erosive scarps, the *campos rupestres* occur on quartzite, sandstone and ironstone formations mostly above 900 m a.s.l., and are characterised as the most common azonal phytophysiognomy in the QF and in the Meridional portion of the SE (SdEM), despite the disjoint areas that occur along these and other mountain ranges across central Brazil (Almeida-Abreu & Renger, 2002; Fernandes et al., 2016). A geological depression, videnced among the QF and SE, becoming a natural barrier to a varied range of organisms (Giulietti et al., 1987; Menezes & Giulietti, 2000; Jacobi & Carmo, 2012; Arias et al., 2014; Chaves et al., 2015; Hamdan & Fernandes, 2015; Neves et al., 2018), leading to geographically structured endemism(Hughes, Pennington, & Antonelli, 2013; Silveira et al., 2016). Although the origins of QF and SE are ancient, several studies show a recent origin of its biota, occurring mainly from the Neogene and Pleistocene periods (Collevatti, Rabelo & Vieira, 2009; Antonelli et al., 2010; Santos et al., 2010; Bonatelli et al., 2014; Chaves et al., 2015; Magalhães et al., 2017; Nascimento et al., 2018; Sabbag et al., 2018; Ramos et al., 2019).

Pithecopus ayeaye (B. Lutz, 1966) and *P. megacephalus* (Miranda-Ribeiro, 1926) (Hylidae: Phyllomedusinae), known as monkey frogs, are endemic species of *campos rupestres*, adapted to the ecological singular conditions found in this environment (Magalhães et al., 2017; Ramos et al., 2018). These species appeared in Pleistocene (Magalhães et al., 2017; Ramos et al., 2019) and they are strongly dependent on streams and rivulets with crystal clear water for reproduction (Oliveira, Nogueira, & Eterovick, 2012; Oliveira, 2017; Borges, Nali, Fiorillo, & Prado, 2018). This type of microenvironment is commonly found in *campos rupestres* due to the bedrocks of the streams and low amount of leached organic matter dissolved in them (Fernandes, 2016). Around those water bodies, the eggs are deposited together with gelatinous capsules in a single leaf of small shrubs of riparian vegetation, which is closed during fecundation with combined efforts of the couple, hanging over a temporary static water habitat formed after a precipitation period during rainy seasons in the warm and wet period from October to January (Haddad & Prado, 2005; Oliveira et al., 2012; Oliveira, 2017; Borges et al., 2018). In addition, those species show similarities in morphology and behaviour, such the reticulated pattern on flanks, the male territory defence by physical combat, besides the type of microhabitat used for reproduction (Nali,

Borges, & Prado, 2015; Oliveira, 2017, Borges, et al., 2018). Beyond those similarities, these species have never been recorded in sympatry, but there is a current area of high climatic suitability for both, located in a plateau between SE and the QF (Magalhães et al., 2017). Therefore, the resemblances between *P. ayeaye* and *P. megacephalus* make them good models for the study of the factors related to the geographic isolation of *campos rupestres*' endemic species (Magalhães et al., 2017).

Several evidences suggest that *campos rupestres* have suffered few changes due to the Pleistocene climatic variations, being considered as long-term stable areas (Bonatelli et al., 2014; Barbosa et al., 2015). Despite this, the patterns of historical gene flow observed for populations of *P. ayeaye* (Magalhães et al., 2017) and *P. megacephalus* (Ramos et al., 2018) suggest that the contemporaneously isolated demes must have been connected in the past by the favourable ecological conditions in the surround lowland areas. If this hypothesis is corroborated, these moments may have created conditions for the secondary contact between *P. ayeaye* and *P. megacephalus.* These evidences led us to question whether historical processes have played a role in the observable allopatry between these species. Thus, in this study, we aimed to test if the following hypotheses: (1) the climatic oscillation of Pleistocene propitiated the contact between *P. ayeaye* and *P. megacephalus*, which would be evidenced by historical signatures of interspecific gene flow and the increase of diffusion rates in moments of expansion of suitable climatic areas, if they occurred. Alternatively, (2) those species remained isolated throughout their evolutionary history, which would imply in strong genetic isolation, resulting from geographic and/or reproductive barriers to gene flow. Additionally, we do not expect to find a relation between phylogeographic diffusion rates and past changes in suitable areas for the species. To test these hypotheses, we applied gene flow, phylogeographic diffusion, and ecological niche modelling in a statistical phylogeographic way.

2. MATERIALS AND METHODS

2.1 Population data

We used the same 88 individuals of *P. ayeaye* which were sampled by Magalhães et al. (2017), and the 55 individuals of *P. megacephalus* sampled by Ramos et al. (2018), distributed, in total, across 26 localities which encompass most of the known distribution of each species.

2.2 Genetic data

Genetic data were obtained from GenBank for cytochrome b (cyt-b), proopiomelanocortin (POMC), and intron 5 of the ribosomal protein L3 (RPL$_3$) for *P. ayeaye* (accession numbers: MF158349-MF158608); and cyt-b, POMC, RPL$_3$, intron 7 of beta-fibrinogen (®Fib$_7$), tensin 3 (TNS$_3$) and intron 1 of alpha-fibrinogen (⟨Fib$_1$) for *P. megacephalus* (accession numbers: MF171732–MF171764, MF171765-MF171838, MF171790-MF171822, MF171823-MF171838, MF171839-MF171845) (Magalhães et al., 2017; Ramos et al., 2018; Ramos et al., 2019). Additionally, we have sequenced three other fragments ⟨Fib$_1$, ®Fib$_7$, and TNS$_3$) for *P. ayeaye* following the protocols of Ramos et al. (2019) and the protooncogene c-myc for *P. ayeaye* and *P. megacephalus* following the protocols of Magalhães et al. (2017). We used the primers cmyc1U (Crawford, 2003) and cmyc-ex2R (Wiens et al., 2005), with temperature and time melting of 54°C and 60 sec, respectively. All sequences were aligned in MAFFT v.7, using the algorithm G-INS-i (Katoh, Rozexicki, & Yamada, 2017), with gap opening penalised in 1.75 for the introns.

2.3 Phylogeographic analyses

2.3.1. Haplotype sharing, hybridization and gene flow

Hybridization is a common process occurring in evolution of phyllomedusines (Haddad, Pombal Jr., & Batistic, 1994; Gray, 2011; Brunes et al., 2014). Since *Pithecopus* species have conserved karyotype (2n = 26) (e.g., Barth, Souza, Solé, & Costa, 2013; Bruschi, Lucas, Garcia & Recco-Pimentel, 2014), which could facilitate interspecific gene flow and introgression, we tested for the evidence of gene flow between *P. ayeaye* and *P. megacephalus*. This was made in three steps, including two exploratory and one model-based analysis. In order to visualise if there are haplotypes sharing between the species, we firstly inferred the haplotype phases of nuclear fragments using the algorithm implemented in software PHASE 2.1.1 (Stephens, Smith, & Donnelly, 2001) under a solving cut-off of posterior probability (PP) ≥ 0.7. Thereafter we inferred haplotypes networks in PopART for each fragment using the algorithm TCS (Clement et al., 2002; Leigh & Bryant, 2015).

To estimate the genetic relatedness between individuals and check for hybrids between the species, we calculated a matrix of pairwise multigenic distances through the genpofad algorithm, implemented in pofad 1.07 (Joly, Bryant, & Lockhart, 2015). That is computed taking intraindividual allelic variation into account. This matrix allows identifying hybrid individuals between the species through the graphic representation of the multigenic distance matrix in a network (Joly & Bruneau, 2006; Joly et al., 2015). This distance network was represented by the algorithm Neighbor-Net in software SplitsTree 4 (Huson & Bryant, 2006).

Finally, we used isolation with migration and two population island models to distinguish between gene flow and incomplete lineage sorting in the haplotype sharing observed between *P. ayeaye* and *P. megacephalus* (see Results). These models were implemented in the software IMa (Hey & Nielsen, 2007), where three replicates were made. In the first one, we set a broad parameters values *a priori*. From this exploratory result, we set maximum values for integrated parameters being θ_1 (*P. megacephalus*): 1.5, θ_2 (*P. ayeaye*): 1.5, θ_A (ancestor): 12, m_1 (*P. ayeaye* → *P. megacephalus*): 2, m_2 (*P. megacephalus* → *P. ayeaye*): 2, and τ: 1 for the isolation with migration model and θ_1: 3, θ_2: 1.5, m_1: 2, and m_2: 2 for the two islands model. The substitution models and rates for each fragment were set as HKY and relative, respectively. Each model was run in two replicates, where the runs had a burn-in period of 1×10^6 and a sampling period of 5×10^6 (thinning 1,000). As suggested in IMa manual, the Metropolis-Hastings MCMC was implemented using 40 chains, with the geometric increment of term 1 and 2 being 0.8 and 0.9, respectively. In IMa we also estimated more simplistic submodels (e.g., lacking some gene flow route or with equal θ between lineages) and selected the best model between them using the Akaike Information Criterion (AIC) scores (Carstens, Stoute, & Reid, 2009, 2010).

2.3.2. Ecological Niche modelling (ENM)

For the ENM we used the geographic data available from Magalhães et al. (2017) and Ramos et al. (2018), supplied by some points from literature (Appendix S1). The climatic layers were downloaded from WorldClim (Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) for characterising the environmental space for ENMs. We selected four of 19 bioclimatic variables: temperature annual range, the mean temperature of warmest quarter, precipitation of wettest quarter and precipitation of driest quarter, using a factorial analysis with varimax rotation (a package implemented in PSYCH in R; available at https://CRAN.R-

project.org/pacage=psych) to minimise collinearity problems. In order to improve the accuracy of ensemble forecasting, we utilised four modelling methods to build the ENMs: Bioclim (Busby, 1991) and Gower Distance (Gower, 1971) from the Dismo package, Support Vector Machine (SVM) from the Kernlab package, and Maxent (Steven, Dudík, & Schapire, 2004) from the rJava package, all of them are available in R (https://www.R-project.org/). We utilised present climatic conditions (1960-1990) to calibrate with 31 and 55 occurrence records for *P. ayeaye* and *P. megacephalus,* respectively. We maintained the prevalence between presence/pseudo-absence to evaluate the predictive performance of each algorithm through the areas under the curves (AUC) of ROC plots. Since the models were generated, we projected them to the past for the Last Glacial Maximum (LGM) and to the Mid-Holocene (Mid) using the CCSM4 (Gent et al., 2004), MPI-ESM-P (Giorgetta et al., 2013), and MIROC-ESM (Watanabe et al., 2011) to accommodate uncertainties about past climates.

2.4. Diffusion models

To estimate the centres of origin, the colonization routes, and the rates of diffusion through time to *P. ayeaye* and *P. megacephalus*, we used Bayesian implementations of the spatial diffusion process that take phylogenetic uncertainties into account (Lemey, Rambaut, Drummond, & Suchard, 2009; Lemey, Rambaut, Welch, & Suchard, 2010). These models were developed for application in gene trees, so we implemented them only for the Cyt-b fragment. For this, we first selected the best-fit model of substitution within an 88-models scheme as implemented in JModelTest2 package (Darriba, Taboada, Doallo, & Posada, 2012). The best model was selected through Bayesian information criterion and the analyses were run in the Cipres Science Gateway (Miller, Pfeifer, & Schwartz, 2010). To select between strict or relaxed lognormal clock, we ran two analyses for each fragment. For both clocks, we set the substitution model as selected by JModelTest2 as HKY to *P. ayeaye* and HKY+I to *P. megacephalus*. The tree model set (i.e., Bayesian Skyline plot – Drummond, Rambaut, Shapiro, & Pybus, 2005) was the same used in subsequent analyses, with group size estimated in 16 to *P. ayeyaye* and 10 to *P. megacephalus* taking the differences of sample sizes between the species into account. The MCMC length of $5x10^7$ and thinning of $5x10^3$. The clock rate of both species was set with a mean (min - max) of 0.0131 (0.01 – 0.0161) substitutions per lineage per million years, following Johns and Avise (1998) and Stock et al. (2012). Further, we checked the stationarity of the analyses in Tracer v.1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018) and ran a second round to check convergence

between runs. Then, to select the clock model of the better fit to the data, each analysis was run to estimate its log marginal likelihood. The method implemented was path-sampling/stepping-stone (PS/SS) (Baele et al., 2012). Each analysis was run with 100 path steps where each MCMC was set with a length of 5×10^7 for each power posterior. Finally, the best model was selected using Bayes Factors (i.e., $2ln$BF; Kass & Raftery, 1995).

Subsequently, we estimated the Time Homogeneous Continuous Diffusion under Brownian Motion (Lemey et al., 2010) to estimate the diffusion routes, rates, and the ancestral areas of *P. ayeaye* and *P. megacephalus*. Additionally, we also estimated Relaxed Random Walks (i.e., that relax the assumptions of Brownian Motion) under Gamma, Cauchy, and Lognormal distributions (Lemey et al., 2010). Each analysis was first implemented in an exploratory way to better define the prior limits of each settable parameters since subsequent analyses are sensitive to improper priors (see Baele, Li, Drummond, Suchard, & Lemey, 2013). As proper priors were defined, the analyses were time-calibrated using the same range of substitution rates that were set in clock model selection. We used Bayesian Skyline plot model (Drummond et al., 2005) for tree estimation and the clock for each species was set according to the result of the previous analyses. We set a jitter = 0.1 for individuals sampled in the same geographical coordinate. Each analysis was made separately for each species and replicated twice to check convergence. For each replicate, we set the MCMC with 5×10^7 generations and thinning 5×10^3. The stationarity (i.e., proper mixing of posterior estimations of each parameter and ESS > 200) and convergence of analyses were checked with Tracer 1.7 (Rambaut, et al., 2018). Furthermore, to select the diffusion model of a better fit to the data, we estimated the log marginal likelihood and selected the best model as was made for the selection of the best clock model.

For the reconstruction of the better diffusion model of each species, the maximum clade credibility (MCC) trees were annotated through TreeAnnotator (Drummond & Rambaut, 2007). These MCC trees were processed in the SpreaD3 program (Bielejec et al., 2016) to generate the spatialized diffusion routes in a Keyhole Markup Language (.kml) archive, which presents polygons in animation form that represents the possible ancestral area of each node of the phylogenetic tree with its respective spatial confidence intervals (Bielejec et al., 2016). In addition, we exported the KML files to Quantum GIS (QGIS) v. 3.4 and extracted the vectors, which were latter plotted in the stability map from the ENM.

3. RESULTS

3.1. Haplotype sharing, hybridization and gene flow

The mitochondrial gene Cyt-b is separated by 113 mutational steps from haplotypes 42 of *P. ayeaye* to the nearest matriline found in *P. megacephalus* (Fig. 1a). For nuclear fragments, the haplotype richness ranged between 16 and 48 haplotypes, being Tns_3 the less rich and Rpl_3 the richest markers. Five of six DNA markers have shown haplotype sharing between the species (Fig. 1b, c, e, f, g). Despite this, the multigenic network has shown no signs of recent hybridization between the *P. ayeaye* and *P. megacephalus* (Fig. 2).

The model selection of the two population islands model shows the full model (i.e., all parameters with independent values, all different to zero) as the best to explain the data (Table 1). In this model, the migration was 41 times higher, on average, from *P. megacephalus* to *P. ayeaye* than the opposite (Table 2). Additionally, the value of m_1 approaches to zero, indicating a possible historical unidirectional gene flow between the species. The theta for *P. megacephalus* was also much larger – 10.5 times on average – than the theta of *P. ayeaye.* In short, this model selection supports the hypothesis that the haplotype sharing between the species is better explained by gene flow than incomplete lineage sorting. However, the two island model implementation do not test isolation nor unidirectional gene flow models. Even though the isolation with migration model is inadequate for our species due to the breach of assumptions (i.e., the two lineages are sister), we implemented it to check if the isolation model is accepted or discarded. Again, the full model was the best to explain the data, and it was 3.838×10^{111} times better than the best isolation model (Table S2). However, the estimated parameters (Table S3) are biased for the violation of the assumption that species share an immediate and recent common ancestor.
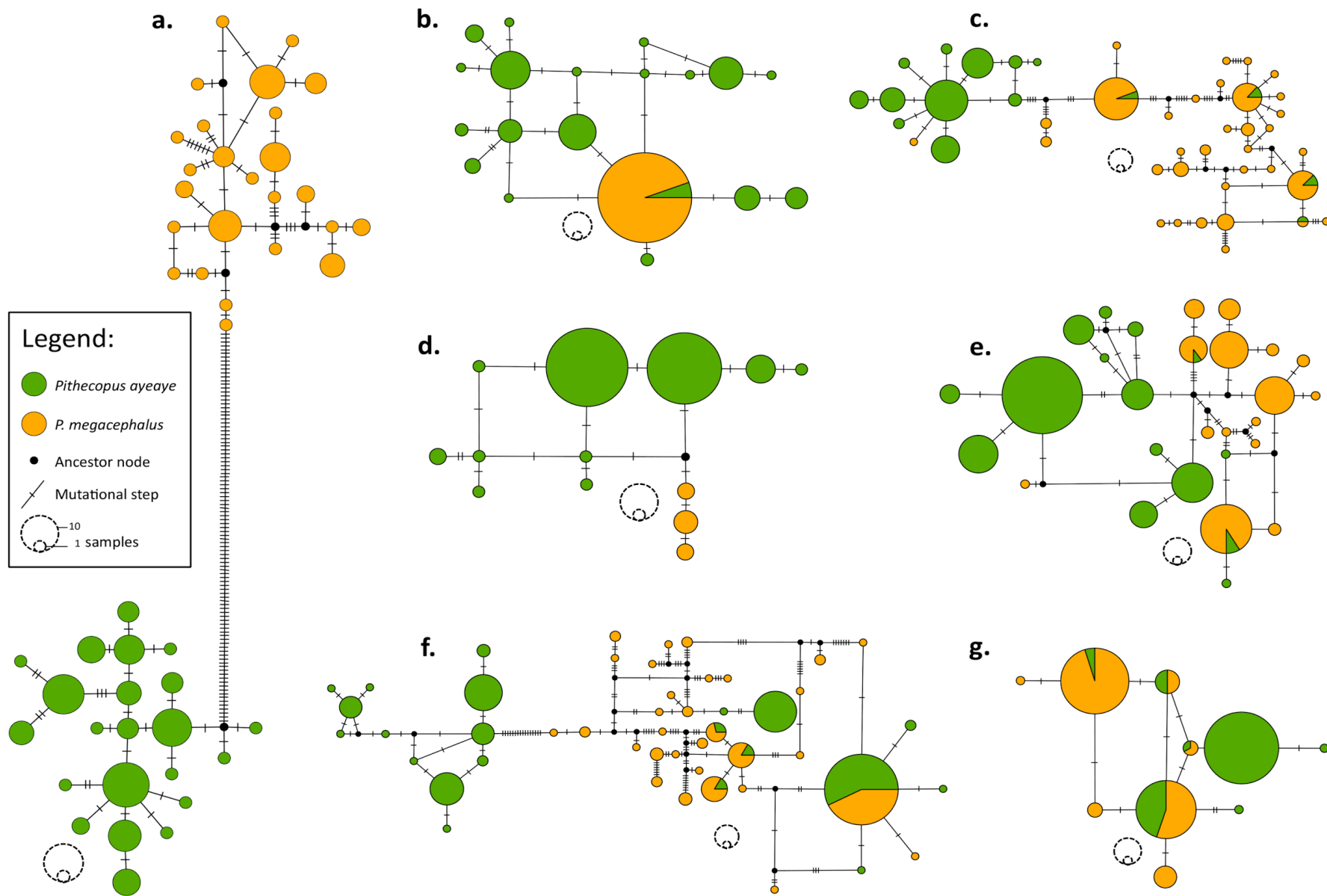
FIGURE 1 Haplotype relationships among *Pithecopus ayeaye* and *P. megacephalus* represented as networks. In (a) Cyt-b, (b) Fib$_1$, (c) Fib7, (d) C-myc, (e) POMC, (f) Rpl$_3$, and (g) Tns$_3$.
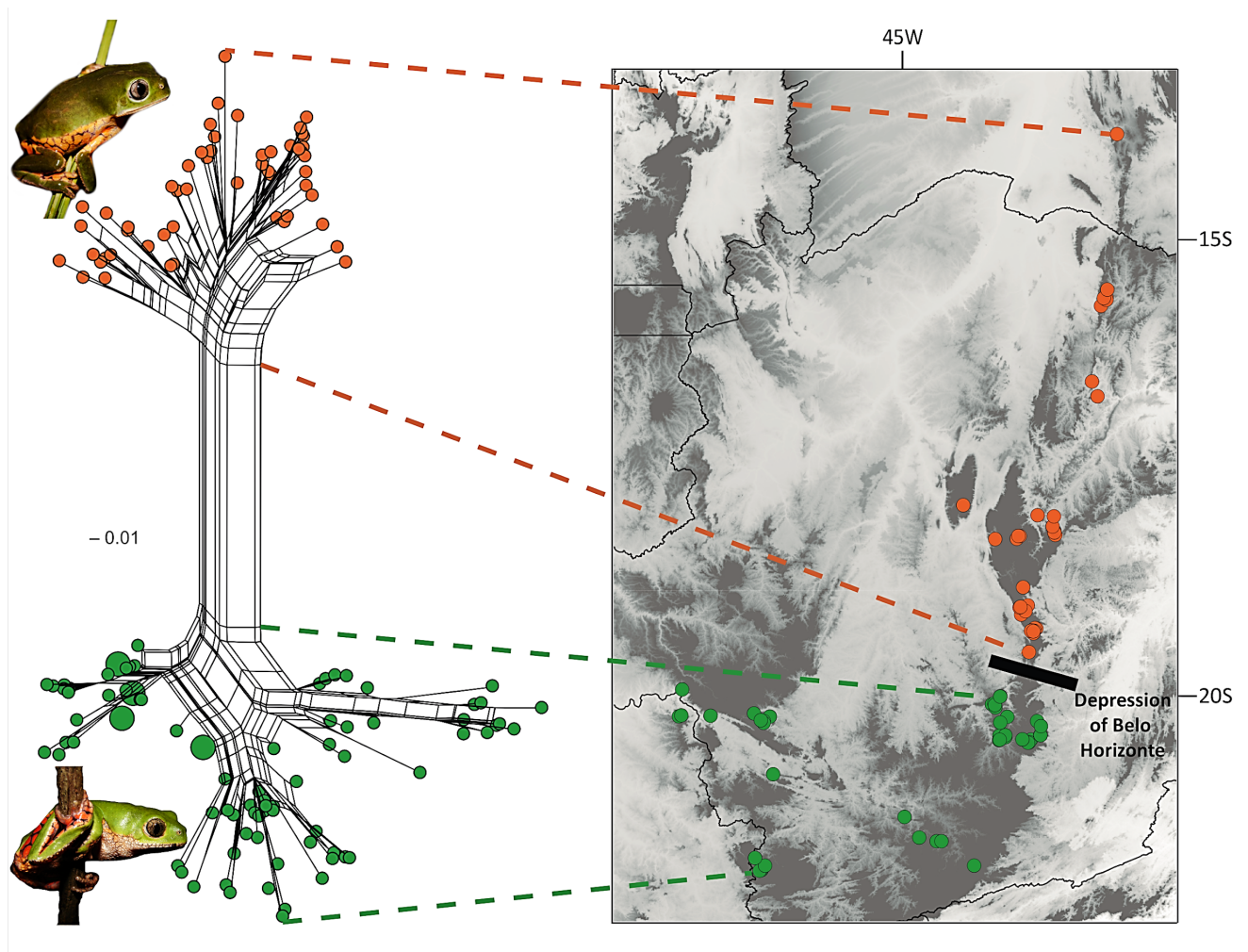
FIGURE 2 Neighbor-net network of genpofad distances between individuals of *Pithecopus ayeaye* (green) and *P. megacephalus* (orange) and the geographical distribution of each species. The location of the Depression of Belo Horizonte barrier is evidenced in the map. Note that are no signs of recent hybridization between the species in the torso of the network.

TABLE 1 Information-theoretic statistics of each of the two islands IMa models.

Two population island model					
Model	**k**	**AIC**	**Δi**	**ωᵢ**	**Emin/i**
FULL	4	-3.088	0	1.000	
AB_DD	3	37.566	40.654	1.486E-09	6.7E+08
AA_DE	3	903.732	906.821	1.22E-197	8.2E+196
AA_DD	2	908.730	911.818	1.002E-198	1E+198

Models considered by IMa, the number of parameters of each model (K), its AIC score, AIC differences (Δi), model probabilities (ωi) and evidence ratio (Emin/i).

TABLE 2 Parameter estimation of IMa.

Two population island model				
	ϑ_1	ϑ_2	m_1	m_2
HiPt				
Run1	12.184	1.132	0.001	0.261
Run2	12.069	1.132	0.001	0.261
Mean				
Run1	12.371	1.173	0.008	0.333
Run2	12.336	1.169	0.008	0.334
HPD_{90Lo}				
Run1	10.076	0.813	0.001	0.131
Run2	10.043	0.805	0.001	0.129
HPD_{90Hi}				
Run1	14.652	1.516	0.021	0.535
Run2	14.635	1.516	0.023	0.537

The first row indicates the summaries of each run, where HiPt is the value of the bin with the highest count, Mean is the mean value of the parameter, HPD_{90Lo} is the lower bound of the estimated 90% highest posterior density (HPD), and the HPD_{90Hi} is the upper bound of the estimated 90% HPD interval. The other parameters are θ_1 is the population of *Pithecopus megacephalus* and θ_2 is the population of *P. ayeaye*, m_1 and m_2 the migration rates *P. ayeaye* → *P. megacephalus* and *P.megacephalus* → *P.* ayeaye, respectively.

3.2. ENM

Model performance was generally high for two species with an AUC for *P. ayeaye* ranging from 0.8 to 1.0 (mean = 0.954, sd = 0.056) and for *P. megacephalus* ranging from 0.845 to 0.984 (mean = 0.955, sd = 0.034). Evaluation metrics indicated a similar performance of single-algorithm models across model sets (Appendix S5). Yet, our ENMs results demonstrate an expansion in habitat suitability area of both species from the Last Glacial Maximum to the Mid-Holocene, mostly in the northern region of the QF fronting with the SdEM. From the Mid-Holocene to current, the suitability area of *P. ayeaye* has suffered a retraction in some parts of the east region of its distribution. *P. megacephalus* has also experienced a habitat suitability expansion in the Mid-Holocene in comparison to the LGM, mostly to the northern region of the QF. Both species had not a significant habitat retraction in their southern portion distributions. However, as proposed by Magalhães et al. (2017), our models recovered a high suitability area of co-adequability in their distribution extremes, between the Diamantina plateau and QF (Figure 3; Appendix S4).
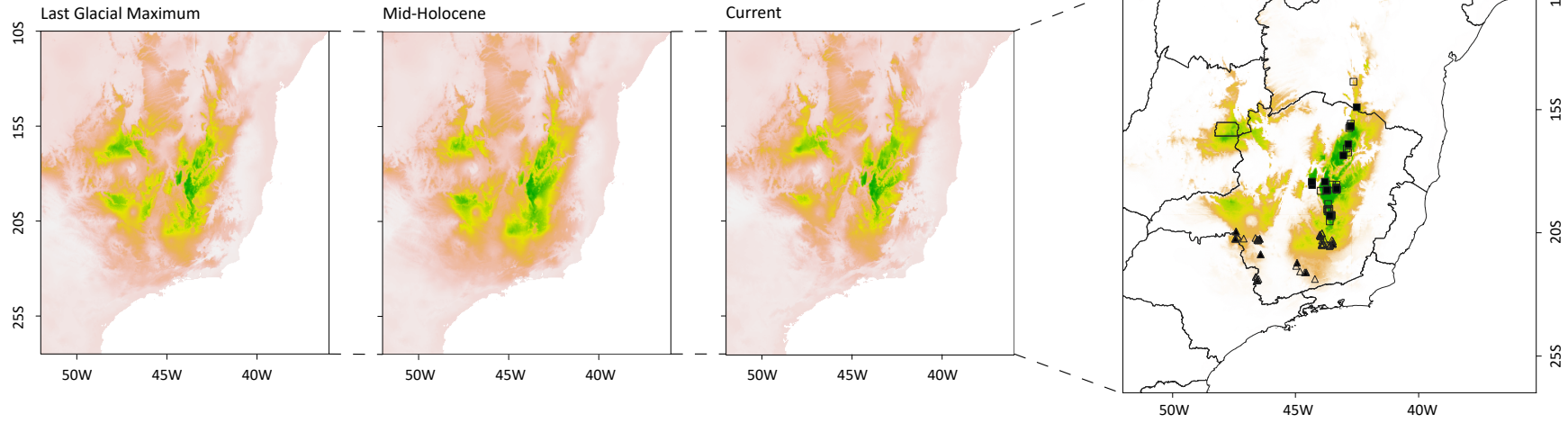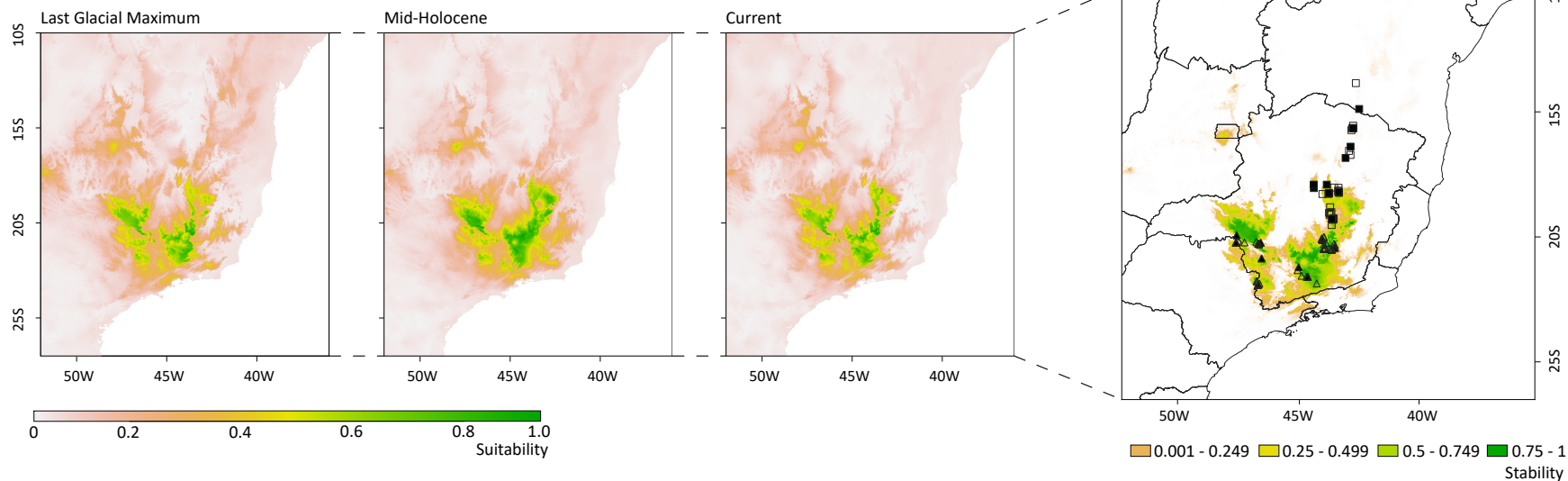
FIGURE 3 Potential geographic distribution for *Pithecopus megacephalus* and *P. ayeaye* for both current time, Mid-Holocene (7-5 Ky), and Last Glacial Maximum (21ky). Climate suitability and stability areas are in left and right, respectively. In stability map, squares and triangles represent the geographic distributions of *P. megacephalus* e *P. ayeaye* respectively, with open symbols representing geographical points from the literature with no genetic data and black symbols representing the genetic sampling.

3.3. Diffusion models

The best clock models selected to *Pithecopus ayeaye* and *P. megacephalus* were the relaxed log-normal and the strict, respectively (Table 3), while the best diffusion models to *P. ayeaye* and *P. megacephalus* were the RRW under gamma and lognormal distributions, respectively (Table 4). The mean of diffusion rates was 449.68 (95%-HPD: 193.50 – 760.72) and 411.67 (95%-HPD: 224.14 – 635.24) kilometres per million years (km/My) to *P. ayeaye* and *P. megacephalus*, respectively.

TABLE 3 Model selection of the clock model for the Cyt-b fragment for *P. ayeaye* and *P. megacephalus*.

Pithecopus ayeaye			
Model	**lnMLE**	**lnBF**	**Probability**
Relaxed Log-Normal	-13.88.484	-586.360	1
Strict	-1681.666	0	4.710E-128
Pithecopus megacephalus			
Model	**lnMLE**	**lnBF**	**Probability**
Strict	-1405.072	0	1
Relaxed Log-Normal	-1703.763	-597.383	1.910E-130

The first row indicates the clock models for the Cyt-b fragment of the species. lnMLE is the log value of the Marginal likelihood estimation, lnBF means the log of the Bayes Factor analysis, and Probability is the value of the best clock model selected to the Cyt-b fragment.

TABLE 4 Model selection of the diffusion model for *P. ayeaye* and *P. megacephalus*.

Pithecopus ayeaye			
Model	**lnMLE**	**lnBF**	**Probability**
RRW Gamma	-1544.333	-84.489	1
RRW Lognormal	-1578.340	-16.474	1.702E-15
RRW Cauchy	-1579.207	-14.741	7.150E-16
Brownian	-1586.577	0	4.502E-19
Pithecopus megacephalus			
Model	**lnMLE**	**lnBF**	**Probability**
RRW Lognormal	-1667.720	-278.231	1
RRW Gamma	-1693.159	-227.354	8.959E-12
RRW Cauchy	-1706.152	-201.368	2.039E-17
Brownian	-1806.836	0	3.828E-61

The first row indicates the tested models of the diffusion model selction of the species. lnMLE is the log value of the Marginal likelihood estimation, lnBF means the log of the Bayes Factor analysis, and Probability is the value of the best clock model selected to the Cyt-b fragment.
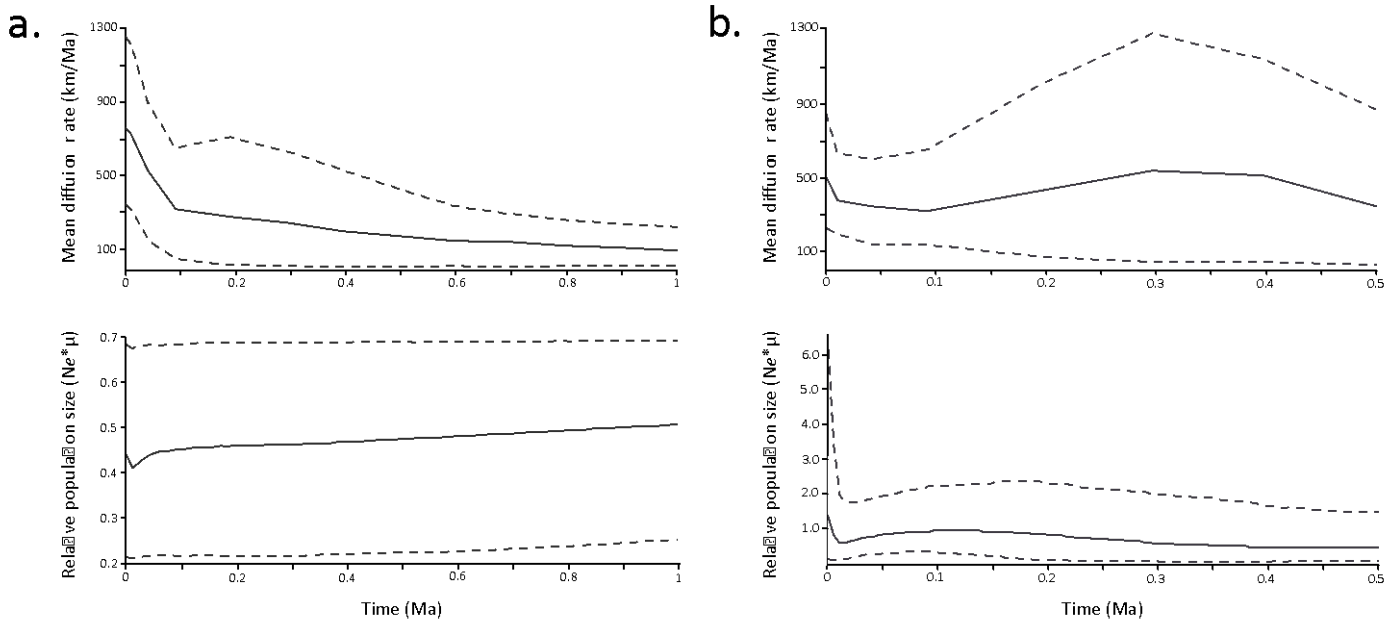
FIGURE 4 Diffusion rates and population sizes of *Pithecopus ayeaye* (a) and *P. megacephalus* (b).

The diffusion rate of *P. ayeaye* have an increase from approximately 330 km/My to 750 km/My since the last 100ky, but this was not related to changes in population size of the species (Fig. 4a). On the other hand, *P. megacephalus* had an increase in population size in the last 20 ky, which was congruent with the increment of the diffusion rate of the species (Fig. 4b). The most recent common ancestor (MRCA) of *P. ayeaye*'s matrilineages coalesced during the Pleistocene 1.06 Mya, close to the region of Campos das Vertentes (Fig. 5a). From this area, two diffusion events, one to southeast and one to northeast have been completed to approximately 266Kya, establishing the populations of Serra da Canastra (SC) and CV, respectively (Fig. 5b). From CV, a new diffusion event began and established the population from eastern QF to about 58Kya (Fig. 5c). 68Kya a diffusion event from SC to began, ending 35Kya on average (Fig. 5d). Again from SC, a diffusion event to started approximately 42Kya and ended 20Kya (Fig. 5e). Two distinct events, one beginning from SC 100Kya and one from eastern QF 29Kya, established the populations of Pedregulho and western QF about 7Kya, respectively (Fig. 5f). Finally, a diffusion event started 25Kya gave rise the Alpinópolis population, shaping the actual distribution currently observed for *P. ayeaye* (Fig. 5g).

The origin of the matrilineages of *P. megacephalus* is much more recent than that of *P. ayeaye*, having coalesced in Pleistocene approximately in 460 kya (Fig. 6a). The first major diffusion event occurred from origin area, going to northern Serra do Espinhaço Meridional (NEM) up to 300 Kya (Fig. 6b). Concomitantly

from the NEM, a diffusion event from origin area towards the south to northern Planalto de Diamantina (NPD) occurred until 288 Kya (Fig. 6c). From NPD, two main diffusion events occurred. The first of them beginning 205Kya in a westerly direction to Serra do Cabral and finished 65Kya (Fig. 6d). The second beginning approximately 130 Kya toward the south southern Planalto de Diamantina, in the region of Serra do Cipó, and finished 59 Kya (Fig. 6 e). Finally, a diffusion event from NEM started 22 Kya gave rise the Jacaraci population in southern Bahia state, shaping the actual current distribution observed for the species (Fig. 6 f). It is important to note that most of the diffusion events for both species beginning and/or ending in glacial cycles (Figs. 5 and 6).
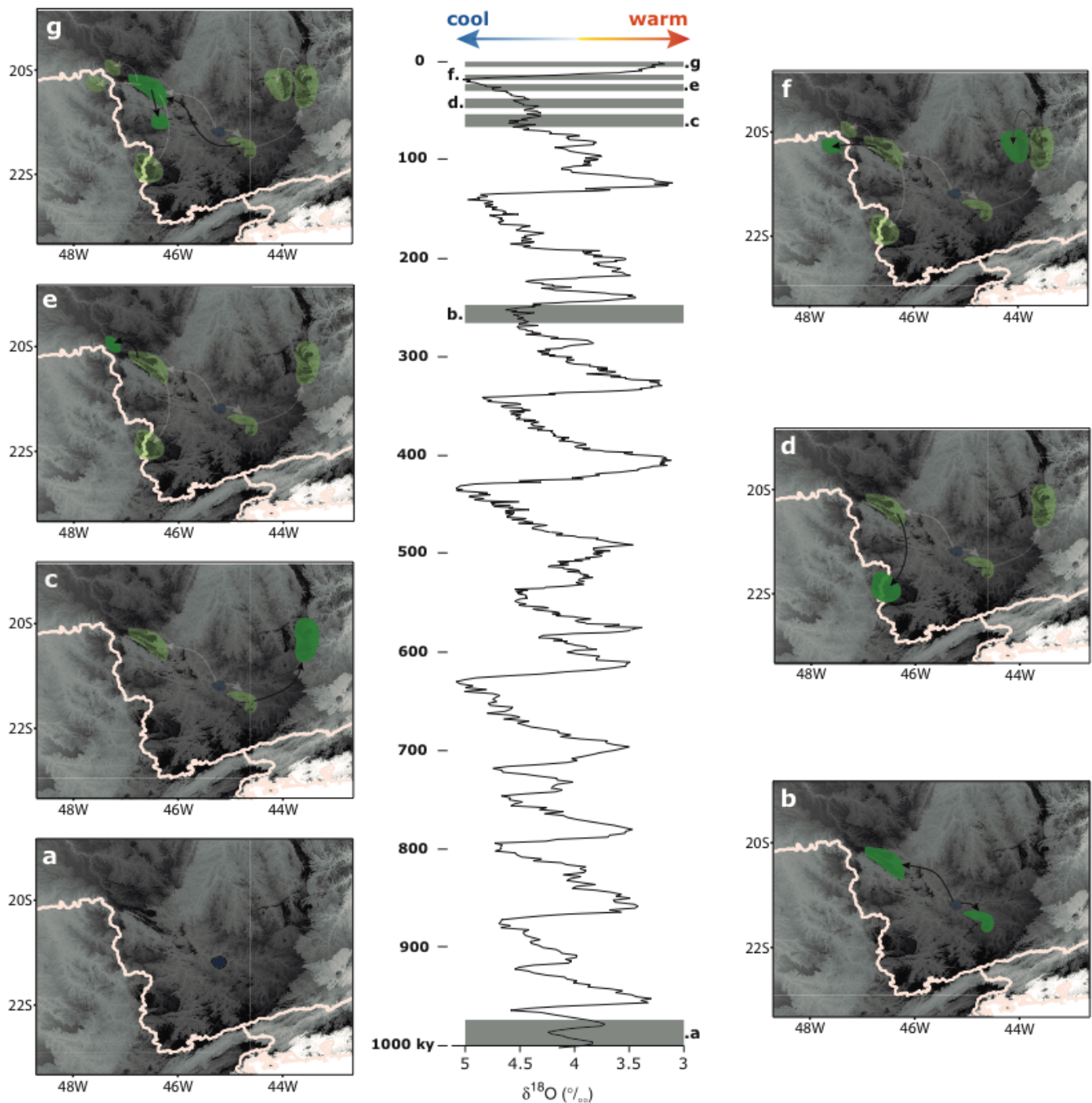
Figure 5 Spatial projection of the diffusion analyses of *P. ayeaye* based on the maximum clade credibility tree estimated with a Bayesian phylogeographic analysis (RRW Gamma model) at seven major spatial diffusion events. The arrows represent diffusion events, temporally identified in the $\delta^{18}O$ curve of the composite benthic stable oxygen isotope ratio (adapted from Lisiecki and Raymo, 2005), with grey bars indicating the approximate moments of the establishment of new geographic distributions. (a) Dark blue area indicates the estimated origin of *P. ayeaye* diversification, to about 1.06Mya. (b) From origin area, going to northwest Serra da Canastra (SC) and southeast Campos das Vertentes (CV) up to 266kya. (c) From CV to eastern Quadrilatero Ferrífero (QF) up to 58kya. (d) From SC to Poços de Caldas, up to 35kya. (e) From SC to Sacramento, up to 20kya. (f) Two distinct events, one from SC to Pedregulho and another from eastern to western QF up to 7kya. (g) Finally, a diffusion event from SC started 25kya gave rise the Alpinópolis population, shaping the actual current distribution observed for the species. Dark green areas and black arrows indicate the establishment of new populations through diffusion events and the source population, respectively. Light green areas and grey arrows indicate previous diffusion events.
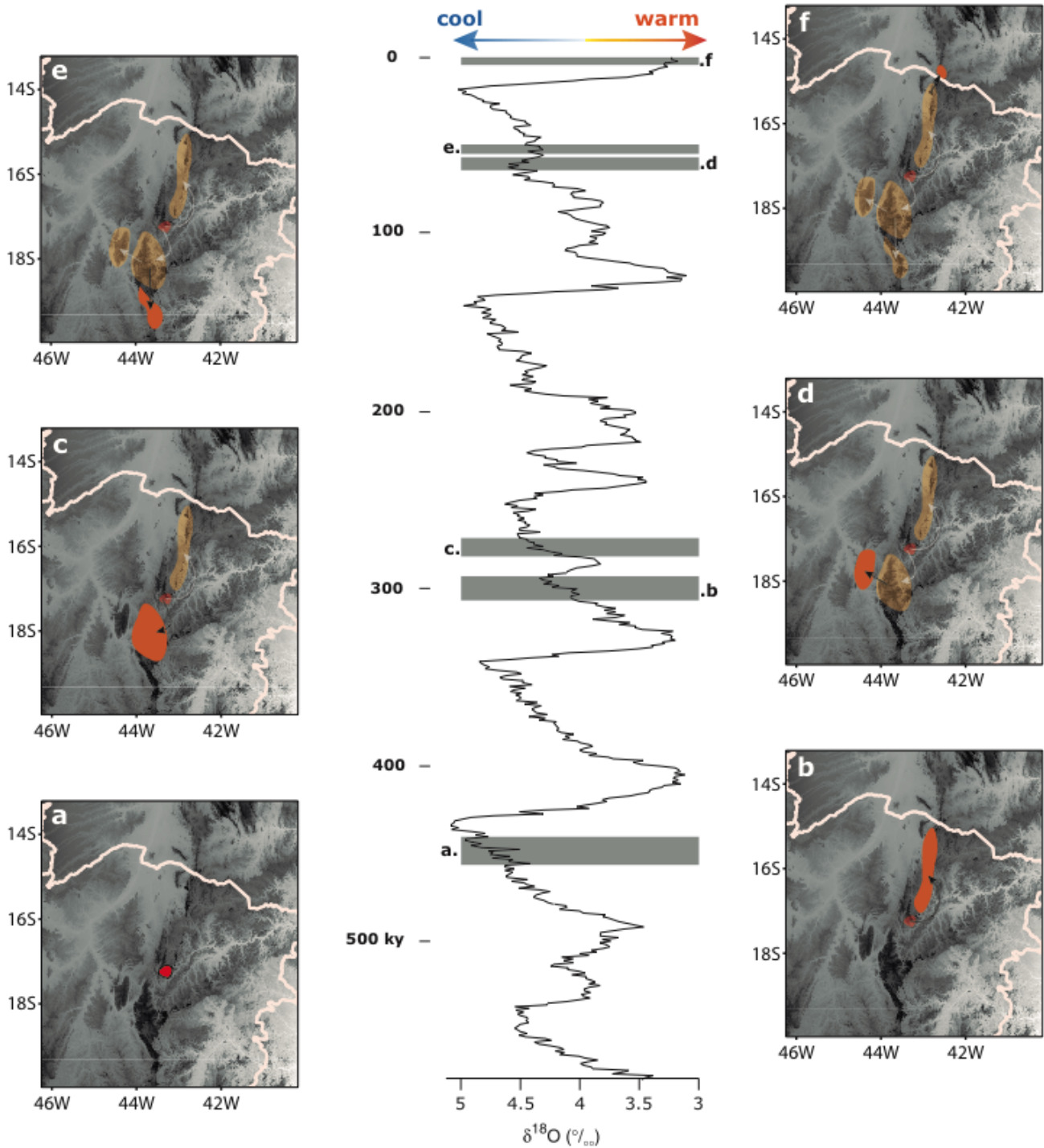
Figure 6 Spatial projection of the diffusion analyses of *P. megacephalus* based on the maximum clade credibility tree estimated with a Bayesian phylogeographic analysis (RRW Lognormal model) at six major spatial diffusion events. The arrows represent diffusion events, temporally identified in the δ¹⁸O curve of the composite benthic stable oxygen isotope ratio (adapted from Lisiecki and Raymo, 2005), with grey bars indicating the approximate moments of the establishment of new geographic distributions. (a) Red area indicates the estimated origin of *P. megacephalus* diversification, beginning at approximately 460kya. (b) From origin area, going to northern Serra do Espinhaço Meridional (NEM) up to 300kya. (c) From origin area to northern Planalto de Diamantina (NPD) up to 288kya. (d) From NPD to Serra do Cabral, up to 65kya. (e) From NPD to southern Planalto de Diamantina, up to 59kya. (f) Finally, a diffusion event from NEM started 22kya gave rise the Jacaraci population in southern Bahia state, shaping the actual current distribution observed for the species. Dark orange areas and black arrows indicate the establishment of new populations through diffusion events and the source population, respectively. Light orange areas and grey arrows indicate previous diffusion events.

DISCUSSION

Despite the extremely old geologic history of the QF and the SE, as well as the agreement of the *campos rupestres* should be an ancient landscape (see Silveira et al., 2016), *P. ayeaye* and *P. megacephalus* are as young (Magalhães et al., 2017; Ramos et al., 2019) as another species found in this phytophysiognomy, specially of the flora (Collevati et al., 2009; Bonatelli et al., 2014; Barres et al., 2019). Although these evidences supports the hypothesis that cycles of expansion and retraction of species ranges during Quaternary climatic changes drove the current levels of population structure and diversity in *campos rupestres*, there is no only explanation. For example, Nascimento et al. (2018) found four deep conspecific lineages in waterfall frog *Bokermannohyla saxicola*, each of them distributed in distinct mountains from the SdEM. These lineages diverged between 6.1 and 2.6 Mya, among the late Miocene and the end of Pliocene. Based on these results, the authors suggested past vicariance as the process responsible by the cladogenesis of these lineages, although they did not suggest which paleogeological processes led to the observed pattern. However, regardless the dating, Nascimento et al. (2018) found several common barriers for *B. saxicola* lineages than that found *P. megacephalus* populations (Ramos et al., 2018), a species much younger (Ramos et al., 2019). In short, the endemic biota from *campos rupestres* must be the result of a mosaic of complex climatic and geological events, all resulting in isolation in sky islands. In spite of that, a geographical barrier amid the boundaries of the QF and the SdEM could be thriving the current allopatry between *P. ayeaye* and *P. megacephalus* (Fig. 2), as well as of several other endemic species from *campos rupestres*. This barrier coincides with the Depression of Belo Horizonte, a lowland transitional area between Cerrado and the Atlantic Forest.

In order to evaluate the consequences of the Pleistocene climatic oscillations in species distributions, Haffer (1969) postulated about the possible consequences of refugia formation. One of them is allopatric speciation followed by geographic contact, where reproductive and ecological incompatibilities evolve during the geographic isolation of populations, resulting in geographic overlap when landscapes expand, and new species come into contact. On the other hand, when the ecological incompatibility does not completely evolve, a mutual exclusion as a result of ecological competition in the contact zones could occur. Finally, when neither ecological nor reproductive incompatibilities completely evolve, the species could hybridize in the contact zone. The extension of the hybrid zone depends on the adaptive value of the hybrids, which may lead to

complete fusion of species in case of outbreeding enhancement (Canestrelli et al., 2016; Barker et al., 2017). Our results indicate that climate oscillations in Pleistocene have propitiated the contact between *P. ayeaye* and *P. megacephalus*, showing a historical signature of interspecific gene flow and increased diffusion rates in moments of expansion of suitable climatic areas, as expected by the Refugial hypothesis when the zygotic isolation is not evolved (Haffer, 1969). Hybrid zones formed during range expansions made gene flow an important process in the evolution of several recent species (Hewitt, 2011), especially by introgressive hybridization, a common phenomenon observed in adaptive radiations (Brawand et al., 2014; Lamichhaney et al., 2015).

The Cyt-b network shows a great number of mutational steps between the species, supporting the hypothesis that *P. ayeaye* and *P. megacephalus* are phylogenetically unrelated (Faivovich et al., 2010; Bruschi et al., 2014; Duellman et al., 2016; Haga et al., 2017). On the other hand, almost all nuclear DNA markers show haplotype sharing between the species. As the gene flow between *P. ayeaye* and *P. megacephalus* existed but is not contemporary, it is possible to conclude that the actual interglacial period is propitiating the current isolation of the species in distinct sky island, as was observed for other cold and dry-adapted species in *campos rupestres*, for example the bush *Lychnophora ericoides*, the herb *Richterago discoidea*, and cactus from the genus *Pilosocereus* (Collevati et al., 2009; Bonatelli et al., 2014; Barres et al., 2019).

The areas of higher suitability found in our ENM analyses, as well as the areas of stability, are similar to those found for the ecosystem as all (Barbosa, & Fernandes, 2016) and for endemic plants of it (Bonatelli et al., 2014; Barres et al., 2019), indicating that past climate conditions had a major impact in present distributions. In all those researches, there is evidence for greater climatic stability in latitude greater than 20S. This historical stability is also projected to the future (Fernandes et al., 2014; Magalhães et al., 2017), corroborating a long-term persistence in the southern distribution of *campos rupestres*. This result is in accordance with the climatic and demographic stability found for *P. ayeaye.* Antonelli et al. (2010) postulated that during these climatic cycles, the limits of *campos rupestres* had decreased by 200m. Thus, the formation of ecological corridors in Depression of Belo Horizonte must be permitted the past contact of *P. ayeaye* and *P. megacephalus*, between the south of Diamantina plateau and the QF. This mountain pass must be formed in glacial periods and disappeared in interglacial ones, where the increase of warm and humidity forced the species to retract again into the mountain tops. Thus, our paleo-distribution models go in

accordance with Haffer's hypothesis, with the Pleistocene climate variation propitiating isolation followed by contact between *P. ayeaye* and *P. megacephalus*.

In previous studies regarding conservation strategies of *P. ayeaye*, Magalhães et al. (2017) demonstrated that the climatic stability in southern *campos rupestres* makes the coming global warming a minor concern on conservation of the species, and our long-term past stability results reinforces this interpretation. However, the species suffers with several other types of threats, mainly habitat loss. Magalhães et al. (2017) affirm that the species have low genetic variability, but they did not demonstrate this in a comparative way. Here, we demonstrate, through the parameter theta, that the genetic diversity of *P. megacephalus* is 10.55 times greater than that of *P. ayeaye*. Additionally, both species show similarities in patterns of genetic-spatial structure (Ramos et al., 2018) besides all the ecological resemblances already mentioned. While *P. megacephalus* presents more than 30% of its distribution inside of protected areas, contemplating all genetic clusters (Ramos et al., 2018), *P. ayeaye* has only 4% of its distribution effectivelly protected, with one evolutionarily significant unit out of any protected area (Magalhães et al., 2017). Even so, since 2014 the species is no longer part of the Brazilian conservation priorities. Our results confirm the conclusions of Magalhães et al. (2017) and reinforces the need for the reassessment of extinction risk of *P. ayeaye* taking these new pieces of evidence into account. Additionally, we also confirm the conclusion of Ramos et al. (2018) that the extinction risk of *P. megacephalus* is from least concern. Although our study is mainly focused on the evolutionary history of species, we show that phylogeographic results can assist in assessing the conservation status of the species*.*

With respect to the diffusion processes, *P. ayeaye* and *P. megacephalus* presented low diffusion rates, on the order of half a kilometre per year. Camargo et al., (2013) compared the diffusion rates of the lizard *Liolaemus darwinii* with dispersion rates registered for the congener *L. koslowskyi*. They found equivalence between the rates, indicating that Bayesian diffusion rates could be a realistic approximation of dispersion rates. While Camargo et al. (2013) found a mean diffusion rate of 1.1m/year to the reptile, we recovered means of 0.45 and 0.41m/year for *P. megacephalus* and *P. ayeaye*, respectively. These differences are within the expectations, since *L. darwinii* is a species with a much broader and continuous distribution than *P. ayeaye* and *P. megacephalus*, both with distribution restricted to sky islands. Additionally, monitoring studies by marking

and recapture indicate that *P. ayeaye* and *P. megacephalus* show high site fidelity (Oliveira et al., 2012; Oliveira, 2017; Borges et al., 2018), which in accordance with the low diffusion rates found. However, it is important to note that the diffusion rates through time for the species seem to be an unrealistic simplification. At least one individual of *P. oreades*, another similar monkey frog with reticulated pattern on flanks, was observed walking two kilometres to the reproductive site (R. A. Brandão, pers. inf.), indicating considerably great home range and locomotive capacity in these frogs. In other words, it is possible that these frogs do not expand their distribution more rapidly only due to the ecological constraints of the valleys between the sky islands. Thus, we hypothesize that there may have been more than one event of acceleration in dispersion rates when and if mountain passes were formed more than one time.

Due to the climatic instability found in the sky islands, mid- and high-elevation amphibians usually exhibit ecological strategies as thermoregulatory behaviour such as basking and physiological adaptations in order to optimise thermoregulation and avoid dehydration, since these environments tend to be colder, drier and unstable (Navas, 1997; Centeno et al., 2015). As *P. ayeaye* and *P. megacepalus* are restricted to these environments, it is expected that they show some behavioural, physiological, or both adaptations. Blaylock, Ruibal, & Platt-Aloia (1976) demonstrated that *P. hypochondrialis* has a very regular arrangement of dorsal glands responsible for production of lipids that diminish skin permeability, avoiding water loss by evaporation (Shoemaker, Balding, Ruibal, & Mc-Clanahan, 1972; Blaylock et al., 1976). Once *P. hypochondrialis* is widely distributed through South America (Bruschi et al., 2014; Frost, 2018), especially in areas with high temperature and precipitation. Therefore, we think that sky island lineages can present, for example, higher mid-dorsal glands and production of lipids than the lowland sister lineage, as a result of the driest climatic conditions of the occupied areas. About behavioural adaptations, there is no knowledge related to *Pithecopus*. As *P. megacephalus* is distributed in a more unstable area than *P. ayeaye*, we hypothesize that this species is more ecologically tolerant to environmental changes than *P. ayeaye*, as indicated by the greatest instability through time in northern *campos rupestres.* This is reinforced by the evidence that *P. megacephalus* was much more capable to disperse to the areas of *P. ayeaye* than the opposite, as can be observed in the differences in gene flow rates between the species. Additionally, as the hybrid zone no longer exists, the bred hybrids should have a lower adaptive value leading to the collapse of the zone in unfavourable interglacial climates. Thereby, future researches related to the physiological adaptations in this group would

provide a more detailed picture of the causes related to the isolation of *Pithecopus* in sky islands.

Our results are not free of caveats. For example, we did not observe haplotype sharing in C-myc, and we cannot exclude the possibility of a sampling randomness in this fragment, since only three individuals were sampled for *P. megacephalus*. This missing data may also have biased the gene flow estimates. Still on these estimates, we are aware that IMa is not the best software to implement gene flow models taking the particularities of our data into account. While the paraphyly of *P. ayeaye* and *P. megacephalus* breaks the assumption that the species should share an immediate and recent common ancestor (Hey, & Nielsen, 2007), the more adequate two island model implemented in IMa does not include isolation or unidirectional gene flow in the estimation of nested sub models. Best implementations of the two island model selection could be made in Migrate-N software (Beerli, & Palczewski, 2010) or through genetic simulations coupled with approximate Bayesian computation (e.g., Bertorelle et al., 2010). The ENM clearly perpetrated commission errors, with both *P. ayeaye* and *P. megacephalus* showing high suitability areas in west of the São Francisco River, where only *P. oreades* is found. Finally, the diffusion model was estimated only for the Cyt-b gene, and the diffusion routes and rates may not reflect the species' colonization patterns, but the history of only matrilineages (Maddison, 1997).

Niche disparities seem to drive the allopatric distribution of *P. ayeaye* and *P. megacephalus* in the sky islands of the QF and the SdEM, as can be seen in the ENM. However, niche equivalence test should be made to test it quantitatively (Warren et al., 2008). Our study is congruent with previous findings where the climatic fluctuations of the Pleistocene directly influenced in the distribution of the biota in the sky islands of the Brazilian Shield, propitiating gene introgression mainly from *P. megacephalus* to *P. ayeaye* in the past. Moreover, the isolation between these species is supported by limited dispersion capacity and an apparent niche divergence between them, with *P. ayeaye* occurring in colder and more humid areas than *P. megacephalus*. Finally, if the species really are adapted to distinct climates, it is possible that the northern distribution of *P. ayeaye* and the southern distribution of *P. megacephalus* are the least appropriate areas in the geographical distribution of each species. If this is true, these areas are expected to be more resistant to the dispersion of the species, and this should be investigated (Chan, Brown, & Yoder, 2011). In short, our study provides important insights on the influence of Pleistocene climate changes on the diversification of endemic biota from Brazilian Shield sky islands, and we

recommend a deepening in the study of this theme to improve the understanding of the processes associated with the diversification of this biota.

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SUPLEMENTARY MATERIAL

TABLES

TABLE S1: *Pithecopus ayeaye* and *P. megacephalus,* geographical coordinates of ENMS and ESUs populations, based on Magalhães et al., (2017) and Ramos et al., (2018); (-) points extracted from: Eterovick et al., (2005); Caramaschi et al., (2006); Brandão et al., (2012); Oliveira et al., (2012).

Species	Locality	Lat.	Long.
Pithecopus ayeaye	Alpinópolis – MG	-20.8597	-46.4203
Pithecopus ayeaye	Arantina – MG	-21.8609	-44.2156
Pithecopus ayeaye	Brumadinho – MG	-20.1014	-43.9888
Pithecopus ayeaye	Brumadinho – MG	-20.094	-44.021
Pithecopus ayeaye	Brumadinho – MG	-20.0837	-43.9951
Pithecopus ayeaye	Brumadinho – MG	-20.1016	-43.9884
Pithecopus ayeaye	Carrancas - MG	-21.5939	-44.6272
Pithecopus ayeaye	Congonhas do Campo - MG	-20.4536	-43.8742
Pithecopus ayeaye	Congonhas do Campo - MG	-20.1278	-43.9835
Pithecopus ayeaye	Congonhas do Campo - MG	-20.4381	-43.9398
Pithecopus ayeaye	Congonhas do Campo - MG	-20.4867	-43.9431
Pithecopus ayeaye	Congonhas do Campo - MG	-20.4334	-43.874
Pithecopus ayeaye	Congonhas do Campo - MG	-20.4827	-43.9368
Pithecopus ayeaye	Itabirito – MG	-20.2309	-43.8524
Pithecopus ayeaye	Itabirito – MG	-20.2934	-43.9306
Pithecopus ayeaye	Lavras – MG	-21.2013	-44.9413
Pithecopus ayeaye	Lavras – MG	-21.3277	-44.9797
Pithecopus ayeaye	Luminárias – MG	-21.5535	-44.8187
Pithecopus ayeaye	Minduri – MG	-21.5934	-44.5719
Pithecopus ayeaye	Minduri – MG	-21.5941	-44.5739
Pithecopus ayeaye	Nova Lima – MG	-20.0552	-44.0135
Pithecopus ayeaye	Nova Lima – MG	-20.1015	-43.9886
Pithecopus ayeaye	Nova Lima – MG	-20.0051	-43.9281
Pithecopus ayeaye	Nova Lima – MG	-20.0083	-43.9309
Pithecopus ayeaye	Ouro Branco – MG	-20.5143	-43.6197
Pithecopus ayeaye	Ouro Branco – MG	-20.5089	-43.6155
Pithecopus ayeaye	Ouro Branco – MG	-20.4797	-43.5931
Pithecopus ayeaye	Ouro Branco – MG	-20.5086	-43.6131
Pithecopus ayeaye	Ouro Branco – MG	-20.5149	-43.6262
Pithecopus ayeaye	Ouro Preto – MG	-20.4309	-43.4904
Pithecopus ayeaye	Ouro Preto – MG	-20.4323	-43.4877
Pithecopus ayeaye	Ouro Preto – MG	-20.4315	-43.487
Pithecopus ayeaye	Ouro Preto – MG	-20.4321	-43.4877
Pithecopus ayeaye	Ouro Preto – MG	-20.2277	-43.5257
Pithecopus ayeaye	Ouro Preto – MG	-20.333	-43.4833
Pithecopus ayeaye	Ouro Preto – MG	-20.4777	-43.6874
Pithecopus ayeaye	Pedregulho – SP	-20.2256	-47.4539
Pithecopus ayeaye	Pedregulho – SP	-20.2148	-47.4264
Pithecopus ayeaye	Poços de Caldas – MG	-21.9172	-46.5686
Pithecopus ayeaye	Poços de Caldas – MG	-21.8612	-46.5086
Pithecopus ayeaye	Poços de Caldas – MG	-21.7772	-46.6192
Pithecopus ayeaye	Poços de Caldas – MG	-21.7766	-46.6178
Pithecopus ayeaye	Poços de Caldas – MG	-21.9088	-46.5467
Pithecopus ayeaye	Poços de Caldas – MG	-21.8977	-46.547
Pithecopus ayeaye	Sacramento – MG	-19.9316	-46.5553
Pithecopus ayeaye	Sacramento – MG	-20.2197	-47.1062
Pithecopus ayeaye	São Roque de Minas – MG	-20.2669	-46.5553
Pithecopus ayeaye	São Roque de Minas – MG	-20.1939	-46.6306
Pithecopus ayeaye	São Roque de Minas – MG	-20.2792	-46.5208
Pithecopus ayeaye	São Roque de Minas – MG	-20.2682	-46.5549
Pithecopus ayeaye	São Roque de Minas – MG	-20.2283	-46.4564
Pithecopus ayeaye	São Roque de Minas – MG	-20.2778	-46.5221
Pithecopus ayeaye	São Roque de Minas – MG	-20.2977	-46.5244
Pithecopus ayeaye	São Roque de Minas – MG	-20.2682	-46.5549

Pithecopus megacephalus	Augusto de Lima – MG	-18.045280	-44.329722
Pithecopus megacephalus	Botumirim – MG	-16.837903	-43.061100
Pithecopus megacephalus	Buenópolis – MG	-17.908900	-43.814500
Pithecopus megacephalus	Conceição do Mato Dentro – MG	-19.009100	-43.627800
Pithecopus megacephalus	Congonhas do Norte – MG	-18.811340	-43.678957
Pithecopus megacephalus	Cristália – MG	-16.718840	-42.863769
Pithecopus megacephalus	Diamantina – MG	-18.249.722	-43.716667
Pithecopus megacephalus	Diamantina – MG	-18.248611	-43.731389
Pithecopus megacephalus	Diamantina – MG	-18.278889	-43.748917
Pithecopus megacephalus	Diamantina – MG	-18.283000	-43.986100
Pithecopus megacephalus	Diamantina – MG	-18.249900	-43.716800
Pithecopus megacephalus	Diamantina – MG	-18.248700	-43.731400
Pithecopus megacephalus	Grão Mogol – MG	-16.388611	-42.861389
Pithecopus megacephalus	Grão Mogol – MG	-16.556400	-42.924200
Pithecopus megacephalus	Igaporã – BA	-13.845800	-42.649700
Pithecopus megacephalus	Jaboticatubas – MG	-19.521700	-43.615600
Pithecopus megacephalus	Jacaraci – BA	-14.893400	-42.516900
Pithecopus megacephalus	Jacaraci – BA	-14.880700	-42.517200
Pithecopus megacephalus	Lassance – MG	-17.837778	-44.467500
Pithecopus megacephalus	Lassance – MG	-17.911389	-44.336250
Pithecopus megacephalus	Morro do pilar – MG	-19.262500	-43.533900
Pithecopus megacephalus	Porteirinha – MG	-15.727500	-42.826100
Pithecopus megacephalus	Rio Pardo de Minas – MG	-15.656000	-42.758300
Pithecopus megacephalus	Rio Pardo de Minas – MG	-15.656048	-42.758251
Pithecopus megacephalus	Rio Pardo de Minas – MG	-15.645000	-42.762778
Pithecopus megacephalus	Rio Pardo de Minas – MG	-15.661400	-42.765600
Pithecopus megacephalus	Rio Pardo de Minas – MG	-15.642300	-42.794600
Pithecopus megacephalus	Rio Pardo de Minas – MG	-15.548600	-42.756700
Pithecopus megacephalus	Santana do Riacho – MG	-19.280417	-43.609222
Pithecopus megacephalus	Santana do Riacho – MG	-19.286667	-43.563889
Pithecopus megacephalus	Santana do Riacho – MG	-19.291900	-43.557500
Pithecopus megacephalus	Santana do Riacho – MG	-19.293611	-43.566389
Pithecopus megacephalus	Santana do Riacho – MG	-19.258700	-43.551300
Pithecopus megacephalus	Santana do Riacho – MG	-19.110600	-43.701700
Pithecopus megacephalus	Santana do Riacho – MG	-19.072600	-43.653700
Pithecopus megacephalus	Santana do Riacho – MG	-19.032300	-43.718200
Pithecopus megacephalus	Santana do Riacho – MG	-19.025700	-43.708100
Pithecopus megacephalus	Santana do Riacho – MG	-19.290200	-43.561900
Pithecopus megacephalus	Santana do Riacho – MG	-19.289100	-43.556000
Pithecopus megacephalus	Santana do Riacho – MG	-19.285400	-43.576400
Pithecopus megacephalus	Santana do Riacho – MG	-19.284900	-43.582300
Pithecopus megacephalus	Santana do Riacho – MG	-19.283300	-43.583300
Pithecopus megacephalus	Santana do Riacho – MG	-19.315600	-43.567200
Pithecopus megacephalus	Santana do Riacho – MG	-19.289722	-43.593889
Pithecopus megacephalus	Santana do Riacho – MG	-19.294722	-43.568056
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.199722	-43.336667
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.210870	-43.335347
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.194146	-43.335411
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.211729	-43.335376
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.237301	-43.326802
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.199167	-43.339444
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.210000	-43.336667
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.199722	-43.336667
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.210000	-43.336667
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.144100	-43.356500
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.033900	-43.341700
Pithecopus megacephalus	São Gonçalo do Rio Preto – MG	-18.021944	-43.521111

TABLE S2 Information-theoretic statistics for each of the Isolation with Migration IMa models.

Isolation with migration model					
Model	**k**	**AIC**	**Δi**	**ωi**	**Emin/i**
FULL	5	2.830	0	1	
ABC00	3	511.314	508.484	3.8387E-111	2.605E+110
ABCD0	4	513.318	510.488	1.4088E-111	7.0982E+110
ABC0D	4	513.319	510.489	1.4087E-111	7.0989E+110
ABCDD	4	513.319	510.489	1.4081E-111	7.1017E+110
AAC00	2	578.228	575.398	1.1323E-125	8.8317E+124
AACDD	3	580.228	577.398	4.1654E-126	2.4007E+125
AACDE	4	582.237	579.407	1.5253E-126	6.5559E+125
AAA00	1	923.034	920.204	1.5144E-200	6.6033E+199
AAADD	2	925.034	922.204	5.5712E-201	1.795E+200
ABA00	2	925.034	922.204	5.5712E-201	1.795E+200
ABB00	2	925.034	922.204	5.5712E-201	1.795E+200
AAADE	3	927.034	924.204	2.0495E-201	4.8792E+200
ABADD	3	927.034	924.204	2.0495E-201	4.8792E+200
ABBDD	3	927.034	924.204	2.0495E-201	4.8792E+200
ABADE	4	929.034	926.204	7.5397E-202	1.3263E+201
ABBDE	4	929.034	926.204	7.5397E-202	1.3263E+201

Models considered by IMa, the number of parameters of each model (K), its AIC score, AIC differences (Δi), model probabilities (ω_i) and evidence ratio ($E_{min/i}$).

TABLE S3 Parameter estimates of IMa models.

Isolation with migration model						
	ϑ_1	ϑ_2	ϑ_A	m_1	m_2	τ
HiPt						
Run1	0.094	0.053	38.537	0.001	0.001	0.003
Run2	0.094	0.053	38.668	0.001	0.001	0.003
Mean						
Run1	0.103	0.058	39.356	0.852	0.924	0.003
Run2	0.100	0.057	39.409	0.855	0.933	0.003
HPD90Lo						
Run1	0.053	0.029	31.935	0.001	0.001	0.002
Run2	0.045	0.029	31.935	0.001	0.001	0.002
HPD90Hi						
Run1	0.176	0.110	46.578	1.695	1.751	0.009
Run2	0.168	0.110	46.774	1.699	1.757	0.009

The first row indicates the summaries of each run, where HiPt is the value of the bin with the highest count, Mean is the mean value of the parameter, HPD_{90Lo} is the lower bound of the estimated 90% highest posterior density (HPD), and the HPD_{90Hi} is the upper bound of the estimated 90% HPD interval. The other parameters are θ_1 is the population of *Pithecopus megacephalus* and θ_2 is the population of *P. ayeaye*, m_1 and m_2 the migration rates *P. ayeaye* → *P. megacephalus* and *P. megacephalus* → *P.* ayeaye, respectively.

TABLE S4 AUC values of four models (mean ±SD) for *Pithecopus ayeaye* and *P. megacephalus*.

ENM	Time	GCMs	*Pithecopus ayeaye*		*Pithecopus megacephalus*	
			AUC	Sensit./Specif.mean threshold value	AUC	Sensit./Specif.mean threshold value
Bioclim			0.858 ± 0.104	0.125 ± 0. 035	0.896 ± 0.107	0.111 ± 0.063
Gower		Current	1.000 ± 0	0.183 ± 0.222	0.979 ± 0.026	0.411 ± 0.149
MaxEnt			1.000 ± 0	0.440 ± 0.140	0.981 ± 0.020	0.449 ± 0.132
SVM			1.000 ± 0	0.623 ± 0.184	0.993 ± 0.011	0.598 ± 0.255
Bioclim			0.816 ± 0.094	0.129 ± 0.048	0.907 ± 0.890	0.092 ± 0.032
Gower		CCSM	1.000 ± 0	0.306 ± 0.076	0.968 ± 0.046	0.410 ± 0.115
MaxEnt			1.000 ± 0	0.502 ± 0.071	0.973 ± 0.026	0.422 ± 0.103
SVM			1.000 ± 0	0.706 ± 0.119	0.965 ± 0.034	0.627 ± 0.168
Bioclim			0.866 ± 0.097	0.109 ± 0.031	0.861 ± 0.118	0.134 ± 0.109
Gower	MID	MIROC-ESM	0.991 ± 0.026	0.340 ± 0.136	0.953 ± 0.052	0.294 ± 0.115
MaxEnt			0.997 ± 0.008	0.458 ± 0.080	0.968 ± 0.035	0485 ± 0.195
SVM			0.997 ± 0.008	0.732 ± 0.150	0.960 ± 0.041	0.529 ± 0.268
Bioclim			0.900 ± 0.086	0.099 ± 0	0.928 ± 0.082	0.080 ± 0.028
Gower		MPI-ESM-P	1.000 ± 0	0.311 ± 0.151	0.968 ± 0.032	0.397 ± 0.112
MaxEnt			0.994 ± 0.017	0.457 ± 0.076	0.970 ± 0.027	0.524 ± 0.078
SVM			1.000 ± 0	0.762 ± 0.105	0.973 ± 0.041	0.664 ± 0.149
Bioclim			0.833 ± 0.103	0.219 ± 0.133	0.850 ± 0.106	0.072 ± 0.058
Gower		CCSM	0.983 ± 0.035	0.290 ± 0.140	0.973 ± 0.033	0.378 ± 0.106
MaxEnt			0.991 ± 0.026	0.414 ± 0.097	0.979 ± 0.018	0.457 ± 0.090
SVM			0.986 ± 0.035	0.533 ± 0.209	0.984 ± 0.022	0.596 ± 0.143
Bioclim			0.850 ± 0.094	0.099 ± 0.057	0.918 ± 0.051	0.092 ± 0.077
Gower	LGM	MIROC-ESM	1.000 ± 0	0.314 ± 0.166	0.985 ± 0.020	0.345 ± 0.149
MaxEnt			0.997 ± 0.008	0.462 ± 0.120	0.996 ± 0.009	0.510 ± 0.094
SVM			1.000 ± 0	0.692 ± 0.212	0.995 ± 0.007	0.752 ± 0.126
Bioclim			0.800 ± 0.119	0.179 ± 0.161	0.845 ± 0.075	0.072 ± 0.038
Gower		MPI-ESM-P	0.980 ± 0.034	0.364 ± 0.136	0.971 ± 0.047	0.340 ± 0.157
MaxEnt			0.991 ± 0.018	0.462 ± 0.067	0.993 ± 0.010	0.433 ± 0.070
SVM			0.986 ± 0.023	0.697 ± 0.216	0.993 ± 0.015	0.582 ± 0.148

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Na busca da resposta à nossa pergunta inicial, se as dinâmicas filogeográficas explicariam a alopatria entre as espécies de pererecas-macaco das ilhas de altitude do Escudo Brasileiro, levantamos também importantes considerações referentes aos processos ecológicos e morfodinâmicos entre o Quadrilátero Ferrífero e o Espinhaço, como a influência direta da barreira da Depressão de Belo Horizonte na distribuição das espécies que, juntamente a outros fatores extrínsecos, influenciaram nos altos índices de endemismos presentes em ambas as formações. No que tange *P. ayeaye* e *P. megacephalus* nossos resultados demonstram parte da complexidade das relações filogenéticas no grupo, assim como na história biogeográfica da região e das interferências climáticas na biologia das espécies. Embora nosso trabalho agregue consideráveis contribuições para o grupo e comunidade científica, entendemos as limitações e possíveis consequências destas em relação aos nossos resultados, como por exemplo a quebra de premissa do modelo de fluxo gênico selecionado, o qual não inclui isolamento ou fluxo gênico unidirecional, erros de comissão do ENM relacionado à seleção de área de ocorrência de uma terceira espécie de pererecas-macaco (*P, oreades*), assim como a inferência matrilineal nos padrões de colonização das espécies.

Pensando na distância geográfica encontrada entre o QF e o SdEM, nas condições levantadas por nossos resultados, assim como na biologia evolutiva das espécies, futuras metodologias estatísticas serão utilizadas, a saber: o caminho de menor custo (least cost pathway), que utiliza de superfícies de distância e direção ponderadas de custo de uma área para determinar uma rota mais curta/econômica entre uma origem e um destino, a qual poderá indicar a(s) possível(is) rota(s) utilizada(s) pelas espécies referentes à zona de contato, como por exemplo a Depressão de Belo Horizonte, ou por uma área alternativa. Outra análise a ser utilizada será o compartilhamento de nicho (niche sharing/overlap) que se refere ao compartilhamento parcial ou total de recursos ou outros fatores ecológicos (predadores, área de forrageio, tipo de solo, etc.), por duas ou mais espécies, determinando se estas podem coexistir em uma comunidade. Como indicam nossos resultados, *P. ayeaye* e *P. megacephalus* aparentam possuir compartilhamento de nicho parcial, sendo assim, tal análise poderá quantificar o quão equivalente o nicho destas espécies podem ou não se sobrepor.